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(54) Titre : CONSTRUCTIONS AMELIOREES DE LAMP COMPRENANT DES ANTIGENES DU CANCER
(54) Title: IMPROVED LAMP CONSTRUCTS COMPRISING CANCER ANTIGENS

(57) **Abrégé/Abstract:**

The present invention provides improved LAMP Constructs comprising specific fragments of the LAMP luminal domain to deliver cancer antigens to immune cells for enhanced processing. These LAMP Constructs can be used for the treatment of disease and in particular hyperproliferative disorders and/or cancer. The improved LAMP Constructs allow for presentation of properly configured three dimensional epitopes for production of an immune response when administered to a subject. The improved LAMP Constructs can be multivalent molecules, and/or can be provided as part of a multivalent vaccine containing two or more LAMP Constructs. The improved LAMP Constructs as described herein can also be used to generate antibodies when administered to a non-human vertebrate.

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Abstract:

The present invention provides improved LAMP Constructs comprising specific fragments of the LAMP luminal domain to deliver cancer antigens to immune cells for enhanced processing. These LAMP Constructs can be used for the treatment of disease and in particular hyperproliferative disorders and/or cancer. The improved LAMP Constructs allow for presentation of properly configured three dimensional epitopes for production of an immune response when administered to a subject. The improved LAMP Constructs can be multivalent molecules, and/or can be provided as part of a multivalent vaccine containing two or more LAMP Constructs. The improved LAMP Constructs as described herein can also be used to generate antibodies when administered to a non-human vertebrate.

IMPROVED LAMP CONSTRUCTS COMPRISING CANCER ANTIGENS

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The invention relates to improved LAMP Constructs comprising cancer antigens and their use in treating subjects suffering from hyperproliferative disorders and/or cancer. Prime boost protocols utilizing the improved LAMP Constructs described herein are also described.

Discussion of the Related Art

[0002] In the following discussion, certain articles and methods will be described for background and introductory purposes. Nothing contained herein is to be construed as an "admission" of prior art. Applicant expressly reserves the right to demonstrate, where appropriate, that the articles and methods referenced herein do not constitute prior art under the applicable statutory provisions.

[0003] DNA vaccines are new and promising candidates for the development of both prophylactic and therapeutic vaccines. They are proven to be safe and the lack of immune responses to a vector backbone may be a definitive advantage if repetitive cycles of vaccination are required to achieve clinical benefits. However, one perceived disadvantage of conventional DNA vaccines is their low immunogenicity in humans. A key limiting step in the immunogenicity of epitope-based DNA vaccines may be the access of epitopes to the major histocompatibility (MHC) class II presentation pathway to T cells, which is likely a stochastic process in the case of a vaccine without targeting technology.

[0004] U.S. Pat. No. 5,633,234 describes chimeric proteins comprising an antigenic domain of modified influenza hemagglutinin (HA) and a cytoplasmic endosomal/lysosomal targeting signal which effectively target antigens to that compartment. The antigenic domain was processed and peptides from it presented on the cell surface in association with MHC class II molecules. The cytoplasmic tail of LAMP-1 was used to form the endosomal/lysosomal targeting domain of the chimeric protein.

[0005] U.S. Pat. No. 8,318,173 extended these initial observations to describe chimeric proteins (and the corresponding DNAs that encode these proteins) comprising the HIV-1 Gag protein inserted between the full luminal domain and a transmembrane domain of LAMP-1. This construct was introduced into dendritic cells which were then reported to target the MHC II pathway.

[0006] This approach has proved useful in increasing cellular and humoral responses to several virus antigens, human papillomavirus E7, dengue virus membrane protein, HIV-1 gp160

membrane protein, HIV-1 p55 Gag, West Nile membrane protein, hepatitis C virus NS3 protein and cytomegalovirus pp65 (see, e.g., Bonini, et al., J. Immunol. 166: 5250-5257, 2001). The enhanced immune response can be attributed to co-localization of LAMP with MHC II and the more efficient processing and delivery of antigenic peptides. In addition, LAMP-targeting is reported to result in the presentation of an increased number of immunogenic epitopes, thus inducing a qualitatively broadened immune response compared to untargeted antigen. For example, Fernandes et al., 2000, Eur. J. Immunol. 30(8): 2333-43, demonstrated an increase in the number of presented peptides of a LAMP-trafficked OVA antigen encoded in a vaccinia vector. Of 12 peptides generated from exogenously supplied OVA, 9 were presented by an OVA/LAMP chimera, as compared to only 2 by the construct without LAMP.

[0007] While it has been determined that the cytoplasmic domain of LAMP is necessary (in conjunction with a signal sequence and transmembrane domain), it is not always sufficient for endosomal/lysosomal trafficking of all antigens. Instead, the full luminal domain of LAMP has been shown to be also required for the trafficking of proteins to the lysosomal vesicular pathway.

[0008] However, even with the presence of the complete luminal domain and the complete transmembrane/cytoplasmic tail of LAMP ("complete LAMP Constructs"), it has increasingly been found that the efficacy of a particular antigen to raise an immune response is highly dependent on the particular sequence used in these constructs. In fact, different antigenic fragments of the same protein when inserted into the complete LAMP constructs have been found to not elicit the same immune response. Sometimes the antigen fragment generates an immune response and other times it does not. These observations make the ability to predict ahead of time which particular antigenic sequence from a protein of interest will raise an immune response difficult with the complete LAMP Constructs.

[0009] Moreover, in generating the complete LAMP Constructs, it has been repeatedly observed that the full luminal domain is required to properly express and process an antigen. For example, in Godinho et al., PLoS ONE 9(6): 9(6): e99887. doi:10.1371/journal.pone.0099887, the authors reported that the complete and intact luminal domain was the necessary minimal region needed to target an antigen to the lysosomes and that fragments of the luminal domain did not work. See, *id.* at page 6.

[0010] Specifically, the Godinho authors showed that by completely removing the first luminal domain and some of the second luminal domain (i.e., T1-Lum/gag construct), both protein expression and antibody response is decreased. Similarly, removing 25% of first luminal domain but having an intact second luminal domain (i.e., T2-lum/gag), both protein expression and

antibody response comparatively increased but still less than the results obtained with the complete LAMP construct.

[0011] Moreover, the authors acknowledged that the ability to raise an immune response is dependent upon the particular antigen and the epitopes used in these complete LAMP Constructs. For example, on page 9, column 2, the authors state “accordingly, previous studies demonstrated that DNA vaccines that generate Gag secreted as VLP, or in a soluble form, induce different levels of T and B cell activation, which were also different from the response induced by cytoplasmic Gag.” However, insertion of an antigenic sequence between the full luminal domain of LAMP and the full transmembrane/cytoplasmic domain of LAMP as has been described in the literature results in such large polynucleotide sequences that it becomes either too costly to produce at commercial levels or impractical from a scientific perspective.

[0012] Previously, the inventors discovered that while it has been widely reported in the literature that fragments smaller than the full luminal domain of LAMP-1 were not effective in generating a robust immune response (*see, e.g.* Godinho et al.), specific fragments, in certain arrangements, do in fact effectively present antigens to the immune system, generating a robust immune response, including the generation of a different repertoire of antibodies. For example, the inventors have identified that the minimal LAMP luminal domain fragment that is effective for generating a robust immune response is not the full luminal domain (as widely reported in the literature) but rather a single homology domain of the luminal domain of a LAMP protein. Accordingly, constructs can comprise, not the full luminal domain, but instead a single homology domain of the luminal domain of a LAMP protein. Nevertheless, the inventors have found that a preferred construct, described in co-pending Pat. Applicn. 16/607,082 named ILC-4 (depicted in Figure 1 herein), comprises at least one antigen of interest fused in between a first homology domain of a LAMP protein and a second homology domain of a LAMP protein (or at least between two Cysteine Conserved Fragments), for example the at least one antigen of interest may be placed in the LAMP hinge region. In preferred embodiments, this construct also comprises a transmembrane domain of a LAMP protein, and/or the cytosolic tail of a LAMP protein. The two homology domains may be derived from, for example, LAMP-1, LAMP-2, LAMP-3, or an Endolyn protein. The inventors unexpectedly found that improved LAMP Constructs such as ILC-4 can, for example, elicit strong T-cell and antibody responses against the antigen(s) of interest, making them viable candidates for use as DNA vaccines.

[0013] Notwithstanding the above, there is further need to design new and improved LAMP Constructs that can be used as vaccines to effectively treat, for example, hyperproliferative

disorders and/or cancer. Moreover, once improved, these new LAMP Constructs can be used to generate antibodies.

SUMMARY OF THE INVENTION

[0014] This Summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This Summary is not intended to identify key or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter. Other features, details, utilities, and advantages of the claimed subject matter will be apparent from the following written Detailed Description including those aspects illustrated in the accompanying drawings and defined in the appended claims.

[0015] It is an object of this invention to provide novel constructs ("improved LAMP Constructs") comprising specific fragments and/or variants of LAMP domains that effectively present the cancer antigens specified herein to the immune system to generate an enhanced immune response. These improved LAMP Constructs effectively direct the antigens to the lysosomal/endosomal compartment where they are processed and presented to major histocompatibility complex (MHC) class II molecules so that helper T cells are preferentially stimulated and/or antibodies are generated.

[0016] The improved LAMP Constructs and methods described herein may elicit an immune response in a subject. The immune response may be an immune response to the epitopes of the antigens in the improved LAMP Construct (e.g., vaccine). Vaccines arm the immune system of the subject such that the immune system may detect and destroy that which contains the antigens of the vaccines in the subject. The improved LAMP Constructs and methods described herein may elicit a Th1 immune response in the subject. Th1 immune responses may include secretion of inflammatory cytokines (e.g., IFN γ , TNF α) by a subset of immune cells (e.g., antigen specific T-cells). In some cases, the inflammatory cytokines activate another subtype of immune cells (e.g., cytotoxic T-cells) which may destroy that which contains the antigen in the subject.

[0017] In some cases, an epitope and/or antigen used in the improved LAMP Constructs and methods described herein may be recognized by the immune system of a subject to elicit a Th1 immune response and release Type I cytokines. The Th1 response may be initiated by the interaction between the epitope and the T-cell, more specifically, the major histocompatibility complex (MHC) expressed by the T-cell. For example, high affinity binding of an epitope to an MHC receptor may stimulate a Th1 response. MHC receptors may be at least one of a plurality of types of MHC receptors. The MHC receptors engaged on a T-cell may vary across individuals in a population.

[0018] In some cases, the immune response is a Type 1 immune response. In some cases, the immune response is characterized by a ratio of Type I cytokine production to Type II cytokine production that is greater than 1. In some cases, the immune response is characterized by a ratio of Type I cytokine production to Type II cytokine production that is less than 1. In some cases, the immune response is characterized by a ratio of IFN γ production to IL-10 production that is greater than 1. In some cases, the immune response is characterized by a ratio of IFN γ production to IL-10 production that is less than 1.

[0019] Prime boost protocols are also contemplated. For example, the invention further provides a method for generating an immune response in a subject to a cancer antigen, comprising priming the subject with an improved LAMP Construct comprising a cancer antigen as described herein followed by at least one boosting of the subject with the antigen or a related antigen (e.g., a second antigen derived from the same or highly similar protein sequence). Mixtures of antigens can be used in either or both the priming and the boosting step. Use of an improved LAMP Construct for the prime step followed by an antigen boost step has been shown to significantly produce higher titers, indicating the power of LAMP in enhancing antibody response.

[0020] In further preferred embodiments, a nucleic acid molecules encoding the improved LAMP Constructs described herein can be used in a manner to provide a prime boost to elicit an enhanced immune response in a subject to a cancer antigen (e.g. an immune response comprising a significantly higher antibody titer). For example, an improved LAMP Construct comprising an epitope and/or cancer antigen described herein can be used for priming an immune response (i.e. where the epitope and/or antigen is processed and presented to MHC class II molecules so that helper T cells are preferentially stimulated, memory cells are initiated and/or antibodies are generated), as well as providing for the expression of a further cancer epitope and/or antigen for boosting the immune response by, for example, expanding the memory response, wherein the boosting epitope and/or antigen is secreted into the circulation of the subject. The cancer epitope and/or antigen for priming the immune response may be the same or different to the epitope and/or antigen for boosting the immune response.

[0021] It is yet another object of this invention to provide improved methods of treatment for cancer and/or hyperproliferative disorders (e.g. by eliciting an anti-tumor immune response through stimulation of helper T cells).

[0022] The invention further provides a nucleic acid molecule encoding any of the improved LAMP Constructs comprising the cancer antigens described herein, and specifically, those cancer antigens listed in Table 1, which include any one fo the following cancer antigens: Large T antigen, Small T antigen, MAGE-A10, MAGE-A12, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A4, MAGE-

A4, MAGE-A6, MAGE-A6, MAGE-A6, MAGE-A9, MAGE-B10, MAGE-B16, MAGE-B17, MAGE-B1, MAGE-B2, MAGE-B3, MAGE-B4, MAGE-B5, MAGE-B6, MAGE-C1, MAGE-C2, MAGE-C3, MAGE-D1, MAGE-D2, MAGE-D4, MAGE-E1, MAGE-E1_(MAGE1), MAGE-E2, MAGE-F1, MAGE-H1, or MAGEL2. The invention also provides an improved LAMP Construct described herein, wherein the nucleic acid molecule can, for example, comprise a nucleic acid sequence encoding an improved LAMP construct comprising at least one antigen for priming and also a nucleic acid sequence encoding at least one antigen for boosting, wherein either or both of the nucleic acid sequences are operably linked to expression control sequence(s). In one preferred aspect, the nucleic acid molecule encoding the improved LAMP Construct is a vaccine vector, suitable for vaccinating a subject. In another aspect, the invention provides a delivery vehicle for facilitating the introduction of the improved LAMP Construct, epitopes and/or antigens into a cell. The delivery vehicle may be lipid-based (e.g., a liposome formulation), viral-based (e.g., comprising viral proteins encapsulating the nucleic acid molecule), or cell-based.

[0023] In preferred embodiments, the invention provides an injectable composition comprising an improved LAMP Construct comprising a cancer antigen of interest for eliciting an immune response (e.g., generation of antibodies) in a mammal to the cancer antigen. In preferred embodiments, this vaccine generates a preferential Th1 response to a Th2 response. The improved LAMP Constructs comprise at least one epitope of a cancer antigen as described herein.

[0024] The invention also provides a cell comprising any of the improved LAMP Constructs described herein which can be used to generate an immune response. In one aspect, the cell is an antigen presenting cell. The antigen presenting cell may be a professional antigen presenting cell (e.g., a dendritic cell, macrophage, B cell, and the like) or an engineered antigen presenting cell (e.g., a non-professional antigen presenting cell engineered to express molecules required for antigen presentation, such as MHC class II molecules). The molecules required for antigen presentation may be derived from other cells, e.g., naturally occurring, or may themselves be engineered (e.g. mutated or modified to express desired properties, such as higher or lower affinity for an antigenic epitope). In one aspect, the antigen presenting cell does not express any co-stimulatory signals and/or the optional priming and boosting antigen is an auto-antigen.

[0025] The invention additionally provides a kit comprising a plurality of cells comprising any of the improved LAMP Constructs described herein. At least two of the cells express different MHC class II molecules, and each cell comprises the same LAMP Construct. In one aspect, a kit is provided comprising an improved LAMP Construct and a cell for receiving the vector.

[0026] The invention also provides a transgenic animal comprising at least one of the cells and/or at least one of the improved LAMP Construct as described herein. The invention also provides a transgenic animal comprising at least one of the cells described herein.

[0027] The invention further provides a method for generating an enhanced immune response in a subject (e.g., a human or a non-human vertebrate) to an antigen, comprising: administering to the subject a cell as described above, wherein the cell expresses, or can be induced to express, the improved LAMP Construct in the subject. In one aspect, the cell comprises an MHC class II molecule compatible with MHC proteins of the subject, such that the subject does not generate an immune response against the MHC class II molecule. In one preferred aspect, the subject is a human.

[0028] In one further aspect, the invention provides a method for eliciting an enhanced immune response to a cancer antigen, comprising administering to a subject, such as a human or a non-human vertebrate, any of the improved LAMP Constructs as described herein. Preferably, the improved LAMP Construct is infectious for a cell of the subject. For example, the improved LAMP Construct may be a viral vector, such as a vaccinia vector.

[0029] For example, the invention further provides a method for generating an immune response in an animal to a cancer antigen, comprising priming the animal with an improved LAMP Construct comprising an antigen as described herein followed by at least one boosting of the animal. Use of an improved LAMP Construct for the prime step followed by an antigen boost step has been shown to significantly produce higher titers, indicating the power of LAMP in enhancing antibody response.

[0030] In a further aspect, a cell is obtained from a subject, the improved LAMP Construct (and optionally along with a boosting epitope and/or antigen) described herein is introduced into the cell and the cell or progeny of the cell is reintroduced into the subject. In one aspect, the cell is a stem cell-capable of differentiating into an antigen presenting cell. Treatments of human patients as well as veterinary use are specifically contemplated.

[0031] The present invention also comprises methods of generating antibodies in a non-human vertebrate wherein the non-human vertebrate is injected with an improved LAMP Construct comprising an antigen of interest as described herein. The cancer antigen of interest is then efficiently presented to the immune system with the help of LAMP in the non-human vertebrate to initiate memory cells and raise antibodies against the antigen.

[0032] Specifically, by combining presentation of the antigen of interest with LAMP, the antigen is then effectively transported to the cytoplasmic endosomal/lysosomal compartments, where the

antigen can be processed and peptides from it presented on the cell surface in association with major histocompatibility (MHC) class II molecules.

[0033] These generated antibodies can be isolated from the blood of the vertebrate (as polyclonals) and then further isolated to generate monoclonal antibodies using standard techniques.

[0034] In preferred embodiments, the genome of the non-human vertebrate comprises an introduced partially human immunoglobulin region, said introduced region comprising human immunoglobulin variable region locus coding sequences and non-coding sequences based on the endogenous immunoglobulin variable region locus of the non-human vertebrate. Preferably, the non-human vertebrate's genome has at least part or all of the endogenous immunoglobulin region removed.

[0035] In further preferred embodiments, the production of human monoclonal antibodies in the non-human vertebrate requires that the host have at least one locus that will express human heavy chain immunoglobulin proteins and one locus that will express human light chain immunoglobulin proteins.

[0036] In some aspects, the partially human immunoglobulin variable region locus comprises human V_H coding sequences and non-coding V_H sequences based on the endogenous V_H region of the non-human vertebrate. In these aspects, the partially human immunoglobulin variable region locus further comprises human D and J gene coding sequences and non-coding D and J gene sequences based on the endogenous genome of the non-human vertebrate host.

[0037] In other aspects, the immunoglobulin region comprises an introduced region comprising human V_L coding sequences and non-coding V_L sequences based on the endogenous V_L region of the non-human vertebrate. More preferably, the introduced partially human immunoglobulin region comprising human V_L coding sequences further comprises human J gene coding sequences and non-coding J gene sequences based on the endogenous genome of the non-human vertebrate host.

[0038] In certain aspects, the vertebrate is a mammal, and preferably the mammal is a rodent, e.g., a mouse or rat. In other aspects, the vertebrate is avian, e.g., a chicken. Other non-human vertebrates include rabbits, llamas, camels, a cow, a guinea pig, a hamster, a dog, a cat, a horse, a non-human primate, a simian (e.g. a monkey or ape), a monkey (e.g. marmoset, baboon, rhesus macaque), or an ape (e.g. gorilla, chimpanzee, orangutan, gibbon).

[0039] In further embodiments, the partially human immunoglobulin region comprises human V_H gene coding regions, and further comprises i) human D and J gene coding sequences and ii) non-coding D and J gene and pre-DJ sequences based on the endogenous genome of the non-human

vertebrate host. In other aspects, the V_H gene coding regions derive (at least partially) from other sources, e.g., they could be rationally or otherwise designed sequences, sequences that are a combination of human and other designed sequences, or sequences from other species, such as nonhuman primates.

[0040] In yet another specific aspect, the partially human immunoglobulin region comprises human V_L gene coding regions, and further comprises i) human J gene coding sequences and ii) non-coding J gene sequences based on the endogenous genome of the non-human vertebrate host. In a specific aspect, the partially human immunoglobulin region comprises human V_H coding regions, human D and J gene coding sequences, and non-coding D and J gene and pre-DJ sequences based on the endogenous genome of the non-human vertebrate host.

[0041] The methods described herein can be used in the production and/or optimization of antibodies, including fully human antibodies, humanized antibodies, chimeric antibodies, for diagnostic and therapeutic uses. Hybridomas producing such antibodies are also a further object of the invention.

[0042] These and other aspects, objects and features are described in more detail below.

BRIEF DESCRIPTION OF THE FIGURES

[0043] The objects and features of the invention can be better understood with reference to the following detailed description and accompanying drawings.

[0044] **Figure 1** illustrates the general scheme of different types of improved LAMP Constructs (identified as ILC-1, ILC-2, ILC-3, ILC-4, ILC-5 and ILC-6) that can be used as described herein.

[0045] **Figure 2B** illustrates the domains of the LAMP proteins defined herein while **Figure 2A** defines the specific amino acid boundaries of these domains for human LAMP-1 (SEQ ID NO:1), human LAMP-2 (SEQ ID NO:2), human LAMP-3 (SEQ ID NO:3), human LAMP-4 (SEQ ID NO:4), human Endolyn (SEQ ID NO:5), human Macrosialin (SEQ ID NO:80), human LAMP-5 (SEQ ID NO:93) and human LIMBIC (SEQ ID NO:67). As described herein the LAMP luminal domains, Homology Domains, transmembrane domains, the cytoplasmic tail and the signal sequences can be used to generate the improved LAMP Constructs ILC-1, ILC-2, ILC-3, ILC-4, ILC-5 and ILC-6 as described herein.

[0046] **Figure 3** provides alignment of LAMP-1 proteins found in other species as compared to human LAMP-1 (SEQ ID NO:1). The equivalent domains of these other species can be used to generate the improved LAMP Constructs described herein and are readily identifiable by comparing the domains identified for human LAMP-1 in Figures 2 and Figure 3 to the alignments shown in Figure 3.

[0047] **Figure 4** provides alignment of LAMP-2 proteins found in other species as compared to human LAMP-2 (SEQ ID NO:2). The equivalent domains of these other species can be used to generate the improved LAMP Constructs described herein and are readily identifiable by comparing the domains identified for human LAMP-2 in Figures 2 and Figure 4 to the alignments shown in Figure 4.

[0048] **Figure 5** provides alignment of LAMP-3 proteins found in other species as compared to human LAMP-3 (SEQ ID NO:3). The equivalent domains of these other species can be used to generate the improved LAMP Constructs described herein and are readily identifiable by comparing the domains identified for human LAMP-3 in Figures 2 and Figure 5 to the alignments shown in Figure 5.

[0049] **Figure 6** provides alignment of LIMP-2 proteins found in other species as compared to human LIMP-2 (SEQ ID NO:4). The equivalent domains of these other species can be used to generate the improved LAMP Constructs described herein and are readily identifiable by comparing the domains identified for human LIMP-2 in Figures 2 and Figure 6 to the alignments shown in Figure 6.

[0050] **Figure 7** provides alignment of LIMBIC proteins found in other species as compared to human LIMBIC (SEQ ID NO:67). The equivalent domains of these other species can be used to generate the improved LAMP Constructs described herein and are readily identifiable by comparing the domains identified for human LIMBIC in Figures 2 and Figure 7 to the alignments shown in Figure 7.

[0051] **Figure 8** provides alignment of Endolyn proteins found in other species as compared to human Endolyn (SEQ ID NO:5). The equivalent domains of these other species can be used to generate the improved LAMP Constructs described herein and are readily identifiable by comparing the domains identified for human Endolyn in Figures 2 and Figure 8 to the alignments shown in Figure 8.

[0052] **Figure 9** provides alignment of Macrosailin proteins found in other species as compared to human Macrosailin (SEQ ID NO:80). The equivalent domains of these other species can be used to generate the improved LAMP Constructs described herein and are readily identifiable by comparing the domains identified for human Macrosailin in Figures 2 and Figure 9 to the alignments shown in Figure 9.

[0053] **Figure 10** provides alignment of LAMP-5 proteins found in other species as compared to human LAMP-5 (SEQ ID NO:93). The equivalent domains of these other species can be used to generate the improved LAMP Constructs described herein and are readily identifiable by

comparing the domains identified for human LAMP-5 in Figures 2 and Figure 10 to the alignments shown in Figure 10.

[0054] **Figure 11A** provides the amino acid sequence of the LT^{S220A} cloned in the Improved LAMP Constructs described herein. Preferably, ILC-4 is used and is exemplified. **Figure 11B** illustrates the study design. **Figure 11C** shows the results of LT^{S220A} in ILC-4 induces IFN γ peptide recall responses in whole splenocytes. **Figure 11D** shows that LT^{S220A} in ILC-4 induces IFN γ and TNF α production in peptide recall responses in splenic CD4 T cells, but not splenic CD8 T cells. **Figure 11E** shows that peptide pool 2 (aa141-160 of SEQ ID NO:114), peptide 13 (aa145-157 of SEQ ID NO:114), and to a lesser extent, peptides 6 (aa117-129 of SEQ ID NO:114), 12 (aa141-153 of SEQ ID NO:114), and 17 (aa161-173 of SEQ ID NO:114) induce the observed IFN γ peptide recall responses in whole splenocytes.

[0055] **Figure 12A** shows LT^{S220A}-LAMP delays tumor onset in vaccinated mice given B16-LTS220A tumors. **Figure 12B** reports that blood ELISPOT responses may predict eventual tumor burden in mice vaccinated with LT^{S220A}-LAMP.

[0056] **Figure 13A** shows that LT^{S220A}-LAMP significantly extends survival in the B16-LTS220A model. **Figure 13B** shows that LT^{S220A}-LAMP induces tumor infiltration of CD45+ immune cells, including CD4 and CD8 T cells. **Figure 13C** demonstrates that LT^{S220A}-LAMP induces tumor infiltration of both NK and NKT cells. **Figure 13D** shows that LT^{S220A}-LAMP alters myeloid cells within the tumor. **Figure 13E** shows that LT^{S220A}-LAMP enhances the number of regulatory T cells within the TME, but increased numbers of Tregs does not correlate with tumor burden. **Figure 13F** suggests value in combination therapy with LT^{S220A}-LAMP.

DETAILED DESCRIPTION

[0057] The invention provides improved LAMP Constructs which can be used to generate vaccines and/or used to raise antibodies. The improved LAMP Constructs can be used to modulate or enhance an immune response. In one preferred aspect, the invention provides a method for treating a patient with cancer or a hyperproliferative disorder by providing an improved LAMP Construct comprising one or more of the cancer antigens as described herein. The improved LAMP Constructs can also be used to raise antibodies in non-human vertebrates, and in preferably, non-human mammals.

DEFINITIONS

[0058] The following definitions are provided for specific terms which are used in the following written description.

[0059] As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof. The term "a nucleic acid molecule" includes a plurality of nucleic acid molecules.

[0060] As used herein, the term "comprising" is intended to mean that the improved LAMP Constructs and methods include the recited elements, but do not exclude other elements. "Consisting essentially of", when used to define improved LAMP Constructs and methods, shall mean excluding other elements of any essential significance to the combination. Thus, an improved LAMP Construct consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the improved LAMP Constructs of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

[0061] The term "about" or "approximately" means within an acceptable range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, e.g., the limitations of the measurement system. For example, "about" can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5 fold, and more preferably within 2 fold, of a value. Unless otherwise stated, the term "about" means within an acceptable error range for the particular value, such as $\pm 1-20\%$, preferably $\pm 1-10\%$ and more preferably $\pm 1-5\%$.

[0062] Where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

[0063] As used herein, "the lysosomal/endosomal compartment" refers to membrane-bound acidic vacuoles containing LAMP molecules in the membrane, hydrolytic enzymes that function in antigen processing, and MHC class II molecules for antigen recognition and presentation. This compartment functions as a site for degradation of foreign materials internalized from the cell

described herein, as well as orthologs (such as, for example, the LAMP proteins shown in Figures 3-10), and allelic variants.

[0069] As used herein, "expression" refers to the process by which polynucleotides are transcribed into mRNA and/or translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA transcribed from the genomic DNA.

[0070] As used herein, "under transcriptional control" or "operably linked" refers to expression (e.g., transcription or translation) of a polynucleotide sequence which is controlled by an appropriate juxtaposition of an expression control element and a coding sequence. In one aspect, a nucleic acid sequence is "operably linked" to an expression control sequence when the expression control sequence controls and regulates the transcription of that sequence. In another context, the term "operably linked" refers to the linkage of a peptide, polypeptide or proteins such as an epitope or antigen with a signal sequence, such as a secretion signal sequence to bring about the secretion of the peptide, polypeptide or protein from a host cell.

[0071] As used herein, "signal sequence" denotes an endoplasmic reticulum translocation sequence. This sequence encodes a signal peptide that communicates to a cell to direct a polypeptide to which it is linked (e.g., via a chemical bond) to an endoplasmic reticulum vesicular compartment, to enter an exocytic/endocytic organelle, to be delivered either to a cellular vesicular compartment, the cell surface or to secrete the polypeptide. This signal sequence is sometimes clipped off by the cell in the maturation of a polypeptide. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

[0072] As used herein, "coding sequence" is a sequence which is transcribed and translated into a peptide, polypeptide or protein when placed under the control of appropriate expression control sequences. The boundaries of a coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, a prokaryotic sequence, cDNA from eukaryotic mRNA, a genomic DNA sequence from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0073] As used herein, two coding sequences "correspond" to each other if the sequences or their complementary sequences encode the same amino acid sequences.

[0074] As used herein, "trafficking" denotes movement or progression of the polypeptide encoded by the improved LAMP Construct through cellular organelles or compartments in the pathway

from the rough endoplasmic reticulum to the endosomal/lysosomal compartment or related organelles where antigen processing and binding to MHC II occurs.

[0075] As used herein, an "improved LAMP Construct" and an "improved LAMP Construct comprising an antigen" and an "improved LAMP Construct comprising an antigen of interest" are used interchangeably. The different arrangements of the improved LAMP Constructs are illustrated in Figure 1 as ILC1-ILC6. Moreover, the use of an "improved LAMP Construct" encompasses both the polynucleotide sequence of the improved LAMP Construct as well as the protein encoded by the polynucleotide sequence of the improved LAMP Construct. In preferred embodiments, the improved LAMP Construct comprises the amino acid sequence of SEQ ID NO:151.

[0076] As used herein, "cancer antigen" or "antigen" is defined as one of the cancer antigens listed in Table 1, and in particular, at least one Large T antigen, Small T antigen, MAGE-A10, MAGE-A12, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A4, MAGE-A4, MAGE-A6, MAGE-A6, MAGE-A6, MAGE-A9, MAGE-B10, MAGE-B16, MAGE-B17, MAGE-B1, MAGE-B2, MAGE-B3, MAGE-B4, MAGE-B5, MAGE-B6, MAGE-C1, MAGE-C2, MAGE-C3, MAGE-D1, MAGE-D2, MAGE-D4, MAGE-E1, MAGE-E1_(MAGE1), MAGE-E2, MAGE-F1, MAGE-H1, or MAGEL2. In preferred embodiments, the antigen comprises SEQ ID NO:115 or SEQ ID NO:116.

[0077] As used herein, an "improved LAMP Construct delivery vehicle" is defined as any molecule or group of molecules or macromolecules that can carry an improved LAMP Construct into a host cell (e.g., such as genes or gene fragments, antisense molecules, ribozymes, aptamers, and the like) and which occurs in association with an improved LAMP Construct as described herein.

[0078] As used herein, "improved LAMP Construct delivery," or "improved LAMP Construct transfer," refers to the introduction of the improved LAMP Construct into a host cell, irrespective of the method used for the introduction. The introduced improved LAMP Constructs may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced improved LAMP Construct either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome.

[0079] As used herein, a "viral improved LAMP Construct" refers to a virus or viral particle that comprises the improved LAMP Construct to be delivered into a host cell, either *in vivo*, *ex vivo* or *in vitro*. Examples of viral improved LAMP Constructs include, but are not limited to, adenovirus vectors, adeno-associated virus vectors, retroviral vectors, and the like. In aspects where gene transfer is mediated by an adenoviral vector, an improved LAMP Construct includes the

adenovirus genome or part thereof, and a selected, non-adenoviral gene, in association with adenoviral capsid proteins.

[0080] As used herein, "adenoviral-mediated gene transfer" or "adenoviral transduction" refers to the process by which an improved LAMP Construct is transferred into a host cell by virtue of the adenovirus entering the cell. Preferably, the improved LAMP Construct is able to replicate and/or integrate and be transcribed within the cell.

[0081] As used herein, "adenovirus particles" are individual adenovirus virions comprised of an external capsid and an improved LAMP Construct, where the capsid is further comprised of adenovirus envelope proteins. The adenovirus envelope proteins may be modified to comprise a fusion polypeptide which contains a polypeptide ligand covalently attached to the viral protein, e.g., for targeting the adenoviral particle to a particular cell and/or tissue type.

[0082] As used herein, the term "administering" or "immunizing" or "injecting" an improved LAMP Construct refers to transducing, transfecting, microinjecting, electroporating, or shooting the cell with the improved LAMP Construct. In some aspects, improved LAMP Constructs are introduced into a target cell by contacting the target cell with a delivery cell (e.g., by cell fusion or by lysing the delivery cell when it is in proximity to the target cell).

[0083] As used herein, the phrase "prime boost" describes the use of an improved LAMP Construct described herein used to prime a T-cell response followed by the use of a second improved LAMP Construct comprising an antigen, a DNA vaccine comprising an antigen or a recombinant antigen to boost the response (or visa versa). These heterologous prime-boost immunizations elicit immune responses of greater height and breadth than can be achieved by priming and boosting with the same vector. The priming with an improved LAMP Construct comprising an antigen initiates memory cells; the boost step expands the memory response. Preferably, two different agents that do not raise responses against each other are used and thus do not interfere with each other's activity. Mixtures of antigens are specifically contemplated in the prime and/or boost step. Boosting can occur once or multiple times.

[0084] In further embodiments, a nucleic acid molecule encoding an improved LAMP Construct can be used to provide a prime boost to elicit an enhanced immune response in a subject (e.g. an immune response comprising a significantly higher antibody titer). For example, the nucleic acid molecule (e.g. a plasmid or vector) may provide for: the expression of an improved LAMP construct comprising a cancer epitope and/or antigen for priming an immune response (referred to herein as a "priming epitope" or "priming antigen") wherein the priming epitope and/or antigen is processed and presented to MHC class II molecules so that helper T cells are preferentially stimulated, memory cells are initiated and/or antibodies are generated; and for the expression of

an epitope and/or antigen for boosting the immune response (referred to herein as a "boosting epitope" or "boosting antigen") by expanding the memory response, wherein the boosting epitope and/or antigen is secreted into the circulation of the subject. The priming epitope and/or antigen for priming the immune response may be the same or different to the boosting epitope and/or antigen for boosting the immune response, however mixtures of epitopes and antigens are also specifically contemplated.

[0085] The term "secreted" as used herein refers to processes and pathways within cells which result in a peptide, polypeptide or protein being transported through the cell wall such that the peptide, polypeptide or protein is released into the extracellular environment and may, for example, enter the circulation of a subject. A peptide, polypeptide or protein destined for the extracellular environment (i.e. to be secreted) will typically be provided with a secretion signal sequence, generally located at the N-terminus, which directs the ribosomes translating the peptide, polypeptide or protein to the rough endoplasmic reticulum (rough ER), from where newly made peptide, polypeptide or protein may be incorporated into small transport or secretory vesicles which transport the peptide, polypeptide or protein to the cell surface for release.

[0086] As used herein, "hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

[0087] As used herein, a polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) which has a certain percentage (for example, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%) of "sequence identity" to another sequence means that, when maximally aligned, using software programs routine in the art, that percentage of bases (or amino acids) are the same in comparing the two sequences.

[0088] Two sequences are "substantially homologous" or "substantially similar" when at least about 50%, at least about 60%, at least about 70%, at least about 75%, and preferably at least about 80%, and most preferably at least about 90 or 95% of the nucleotides match over the defined length of the DNA sequences. Similarly, two polypeptide sequences are "substantially homologous" or "substantially similar" when at least about 50%, at least about 60%, at least about

66%, at least about 70%, at least about 75%, and preferably at least about 80%, and most preferably at least about 90 or 95% of the amino acid residues of the polypeptide match over a defined length of the polypeptide sequence. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks. Substantially homologous nucleic acid sequences also can be identified in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. For example, stringent conditions can be: hybridization at 5xSSC and 50% formamide at 42°C, and washing at 0.1xSSC and 0.1% sodium dodecyl sulfate at 60°C. Further examples of stringent hybridization conditions include: incubation temperatures of about 25 degrees C to about 37 degrees C; hybridization buffer concentrations of about 6xSSC to about 10xSSC; formamide concentrations of about 0% to about 25%; and wash solutions of about 6xSSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40 degrees C to about 50 degrees C.; buffer concentrations of about 9xSSC to about 2xSSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5xSSC to about 2xSSC. Examples of high stringency conditions include: incubation temperatures of about 55 degrees C to about 68 degrees C.; buffer concentrations of about 1xSSC to about 0.1xSSC; formamide concentrations of about 55% to about 75%; and wash solutions of about 1xSSC, 0.1xSSC, or deionized water. In general, hybridization incubation times are from 5 minutes to 24 hours, with 1, 2, or more washing steps, and wash incubation times are about 1, 2, or 15 minutes. SSC is 0.15 M NaCl and 15 mM citrate buffer. It is understood that equivalents of SSC using other buffer systems can be employed. Similarity can be verified by sequencing, but preferably, is also or alternatively, verified by function (e.g., ability to traffic to an endosomal compartment, and the like), using assays suitable for the particular domain in question.

[0089] The terms "percent (%) sequence similarity", "percent (%) sequence identity", and the like, generally refer to the degree of identity or correspondence between different nucleotide sequences of nucleic acid molecules or amino acid sequences of polypeptides that may or may not share a common evolutionary origin (see Reeck et al., *supra*). Sequence identity can be determined using any of a number of publicly available sequence comparison algorithms, such as BLAST, FASTA, DNA Strider, GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin), etc.

[0090] To determine the percent identity between two amino acid sequences or two nucleic acid molecules, the sequences are aligned for optimal comparison purposes. The percent identity between the two sequences is a function of the number of identical positions shared by the

sequences (i.e., percent identity = number of identical positions/total number of positions (e.g., overlapping positions) x 100). In one embodiment, the two sequences are, or are about, of the same length. The percent identity between two sequences can be determined using techniques similar to those described below, with or without allowing gaps. In calculating percent sequence identity, typically exact matches are counted.

[0091] The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, Proc. Natl. Acad. Sci. USA 1990, 87:2264, modified as in Karlin and Altschul, Proc. Natl. Acad. Sci. USA 1993, 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al, J. Mol. Biol. 1990; 215: 403. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to sequences of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to protein sequences of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al, Nucleic Acids Res. 1997, 25:3389. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationship between molecules. See Altschul et al. (1997) *supra*. When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See ncbi.nlm.nih.gov/BLAST/ on the WorldWideWeb.

[0092] Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS 1988; 4: 1 1-17. Such an algorithm is incorporated into the ALIGN program (version 2.0), which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

[0093] In a preferred embodiment, the percent identity between two amino acid sequences is determined using the algorithm of Needleman and Wunsch (J. Mol. Biol. 1970, 48:444-453), which has been incorporated into the GAP program in the GCG software package (Accelrys, Burlington, MA; available at accelrys.com on the WorldWideWeb), using either a Blossum 62 matrix or a PAM250 matrix, a gap weight of 16, 14, 12, 10, 8, 6, or 4, and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package using a NWSgapdna.CMP matrix, a gap weight of 40, 50, 60, 70, or 80, and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that can be used if the practitioner is uncertain about

what parameters should be applied to determine if a molecule is a sequence identity or homology limitation of the invention) is using a Blossum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

[0094] Another non-limiting example of how percent identity can be determined is by using software programs such as those described in Current Protocols In Molecular Biology (F. M. Ausubel et al., eds., 1987) Supplement 30, section 7.7.18, Table 7.7.1. Preferably, default parameters are used for alignment. A preferred alignment program is BLAST, using default parameters. In particular, preferred programs are BLASTN and BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+SwissProtein+SPupdate+PIR. Details of these programs can be found at the following Internet address: <http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST>.

[0095] Statistical analysis of the properties described herein may be carried out by standard tests, for example, t-tests, ANOVA, or Chi squared tests. Typically, statistical significance will be measured to a level of $p=0.05$ (5%), more preferably $p=0.01$, $p=0.001$, $p=0.0001$, $p=0.000001$

[0096] "Conservatively modified variants" of domain sequences also can be provided. With respect to particular nucleic acid sequences, conservatively modified variants refer to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Specifically, degenerate codon substitutions can be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer, et al., 1991, Nucleic Acid Res. 19: 5081; Ohtsuka, et al., 1985, J. Biol. Chem. 260: 2605-2608; Rossolini et al., 1994, Mol. Cell. Probes 8: 91-98).

[0097] The term "biologically active fragment", "biologically active form", "biologically active equivalent" of and "functional derivative" of a wild-type protein, possesses a biological activity that is at least substantially equal (e.g., not significantly different from) the biological activity of the wild type protein as measured using an assay suitable for detecting the activity.

[0098] As used herein, "*in vivo*" nucleic acid delivery, nucleic acid transfer, nucleic acid therapy" and the like, refer to the introduction of an improved LAMP Construct (and optionally along with the boosting cancer epitope and/or antigen) as described herein directly into the body of a subject, such as a human or non-human mammal, whereby the improved LAMP Construct is introduced to a cell of such organism *in vivo*.

[0099] As used herein, the term "*in situ*" refers to a type of *in vivo* nucleic acid delivery in which the improved LAMP Construct is brought into proximity with a target cell (e.g., the nucleic acid is not administered systemically). For example, *in situ* delivery methods include, but are not limited to, injecting an improved LAMP Construct directly at a site (e.g., into a tissue, such as a tumor or heart muscle), contacting the improved LAMP Construct with cell(s) or tissue through an open surgical field, or delivering the improved LAMP Constructs to a site using a medical access device such as a catheter.

[0100] As used herein, the term "isolated" or "purified" means separated (or substantially free) from constituents, cellular and otherwise, in which the polynucleotide, peptide, polypeptide, protein, antibody, or fragments thereof, are normally associated with in nature. For example, with respect to an improved LAMP Construct as described herein, an isolated polynucleotide is one that is separated from the 5' and 3' sequences with which it is normally associated in the chromosome. As is apparent to those of skill in the art, a non-naturally occurring polynucleotide, peptide, polypeptide, protein, antibody, or fragments thereof, does not require "isolation" to distinguish it from its naturally occurring counterpart. By substantially free or substantially purified, it is meant at least 50% of the population, preferably at least 70%, more preferably at least 80%, and even more preferably at least 90%, are free of the components with which they are associated in nature.

[0101] As used herein, a "target cell" or "recipient cell" refers to an individual cell or cell which is desired to be, or has been, a recipient of the improved LAMP Construct described herein. The term is also intended to include progeny of a single cell, and the progeny may not necessarily be completely identical (in morphology or in genomic or total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A target cell may be in contact with other cells (e.g., as in a tissue) or may be found circulating within the body of an organism.

[0102] As used herein, a "subject" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. In other preferred embodiments, the "subject" is a rodent (e.g., a rat, a mouse or rabbit), a llama, camel, a cow, a guinea pig, a hamster, a dog, a cat, a horse, a non-human primate, a simian (e.g. a monkey or ape), a monkey (e.g. marmoset, baboon, rhesus macaque), or an ape (e.g. gorilla, chimpanzee, orangutan, gibbon). In other embodiments, non-human mammals, especially mammals that are conventionally used as models for demonstrating therapeutic efficacy in humans (e.g. murine, primate, porcine, canine, or rabbit animals) may be employed.

[0103] The terms "cancer," "neoplasm," and "tumor," are used interchangeably and in either the singular or plural form, refer to cells that have undergone a malignant transformation that makes them pathological to the host organism. Primary cancer cells transformation that makes them pathological to the host organism. Primary cancer cells (that is, cells obtained from near the site of malignant transformation) can be readily distinguished from non-cancerous cells by well-established techniques, particularly histological examination. The definition of a cancer cell, as used herein, includes not only a primary cancer cell, but any cell derived from a cancer cell ancestor. This includes metastasized cancer cells, and in vitro cultures and cell lines derived from cancer cells. When referring to a type of cancer that normally manifests as a solid tumor, a "clinically detectable" tumor is one that is detectable on the basis of tumor mass, e.g., by procedures such as CAT scan, MR imaging, X-ray, ultrasound or palpation, and/or which is detectable because of the expression of one or more cancer-specific antigens in a sample obtainable from a patient.

[0104] In preferred embodiments, the cancer (including all stages of progression, including hyperplasia) is an adenocarcinoma, sarcoma, skin cancer (e.g., merkel cell carcinoma), melanoma, bladder cancer, brain cancer (including glioblastoma multiforme), breast cancer, uterine cancer, ovarian cancer, prostate cancer, lung cancer (including, but not limited to NSCLC, SCLC, squamous cell cancer), colorectal cancer, anal cancer, rectal cancer, cervical cancer, liver cancer, head and neck cancer, oral cancer, salivary gland cancer, esophageal cancer, pancreas cancer, pancreatic ductal adenocarcinoma (PDA), renal cancer, stomach cancer, kidney cancer, multiple myeloma or cerebral cancer. Also, preferred diseases to be treated with the LAMP Constructs described herein include those hyperproliferative disorders or cancer caused by cytomegalovirus (CMV).

[0105] As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. Compositions comprising the improved LAMP Constructs also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Martin Remington's Pharm. Sci., 15th Ed. (Mack Publ. Co., Easton (1975)).

[0106] A cell has been "transformed", "transduced", or "transfected" by a nucleic acid molecule of an improved LAMP Construct when such a nucleic acid molecule has been introduced inside the cell. Transforming DNA may or may not be integrated (covalently linked) with chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the nucleic acid molecule of an improved LAMP Construct may be maintained on an episomal

element, such as a plasmid. In a eukaryotic cell, a stably transformed cell is one in which the nucleic acid molecule of an improved LAMP Construct has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the nucleic acid molecule. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations (e.g., at least about 10).

[0107] As used herein, an "effective amount" is an amount sufficient to affect beneficial or desired results, e.g., such as an effective amount of the improved LAMP Construct transfer and/or expression, and/or the attainment of a desired therapeutic endpoint. An effective amount can be administered in one or more administrations, applications or dosages. In one aspect, an effective amount of an improved LAMP Construct is an amount sufficient to transform/transduce/transfect at least one cell in a population of cells comprising at least two cells.

[0108] As used herein, a "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, correct and/or normalize an abnormal physiological response. In one aspect, a "therapeutically effective amount" is an amount sufficient to reduce by at least about 30 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant feature of pathology, such as for example, size of a tumor mass, antibody production, cytokine production, fever or white cell count, etc.

[0109] An "antibody" is any immunoglobulin, including antibodies and fragments thereof, that binds a specific antigen. The term encompasses polyclonal, monoclonal, and chimeric antibodies (e.g., bispecific antibodies). An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically binds antigen. Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules, and those portions of an immunoglobulin molecule that contains the paratope, including Fab, Fab', F(ab')₂ and F(v) portions, which portions are preferred for use in the therapeutic methods described herein. Thus, the term antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives such as fusion proteins) of antibodies and antibody fragments. Examples of molecules which are described by the term "antibody" in this application include, but are not limited to: single chain Fvs (scFvs), Fab fragments, Fab' fragments, F(ab')₂, disulfide linked Fvs (sdFvs), Fvs, and fragments comprising or alternatively consisting of, either a VL or a VH domain. The term "single chain Fv" or "scFv" as used herein refers to a polypeptide comprising a VL domain of an antibody linked to a VH domain of an antibody. See Carter (2006) Nature Rev. Immunol. 6:243.

[0110] Additionally, antibodies that may be generated using the improved LAMP Construct described herein include, but are not limited to, monoclonal, multi-specific, bi-specific, human, humanized, mouse, or chimeric antibodies, single chain antibodies, camelid antibodies, Fab fragments, F(ab') fragments, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), domain antibodies and epitope-binding fragments of any of the above. The immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0111] Most preferably, the antibodies are human antibodies. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries and xenomice or other organisms that have been genetically engineered to produce human antibodies. The improved LAMP Construct described herein can be used in combination with known techniques for generating human antibodies and human monoclonal antibodies as described in the exemplified protocols, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598; and Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995).

[0112] Human antibodies or "humanized" chimeric monoclonal antibodies can be produced using the improved LAMP Constructs in combination with techniques described herein or otherwise known in the art. For example, standard methods for producing chimeric antibodies are known in the art. See, for review the following references: Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., *Nature* 312:643 (1984); Neuberger et al., *Nature* 314:268 (1985).

[0113] Antibodies that may be generated using an improved LAMP Construct described herein may be monovalent, bivalent, trivalent or multivalent. For example, monovalent scFvs can be multimerized either chemically or by association with another protein or substance. A scFv that is fused to a hexahistidine tag or a Flag tag can be multimerized using Ni-NTA agarose (Qiagen) or using anti-Flag antibodies (Stratagene, Inc.). Additionally, the improved LAMP Construct can be used to generate monospecific, bispecific, trispecific or of greater multispecificity for the encoded antigen(s) contained in the improved LAMP Construct. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., *J. Immunol.* 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., *J. Immunol.* 148:1547-1553 (1992).

[0114] An "epitope" is a structure, usually made up of a short peptide sequence or oligosaccharide, that is specifically recognized or specifically bound by a component of the immune system. T-cell epitopes have generally been shown to be linear oligopeptides. Two epitopes correspond to each other if they can be specifically bound by the same antibody. Two epitopes correspond to each other if both are capable of binding to the same B cell receptor or to the same T cell receptor, and binding of one antibody to its epitope substantially prevents binding by the other epitope (e.g., less than about 30%, preferably, less than about 20%, and more preferably, less than about 10%, 5%, 1%, or about 0.1% of the other epitope binds). It will be understood that multiple epitopes can make up an antigen.

[0115] The term "antigen" or "antigen of interest" as used herein covers any polypeptide sequence encoded by a polynucleotide sequence cloned into the improved LAMP Construct which is used to elicit an innate or adaptive immune response as shown in Table 1 and/or Figure 11A. An "antigen" encompasses both a single antigen as well as multiple antigenic sequences (derived from the same or different proteins) cloned into the improved LAMP Construct.

[0116] The term "antigen presenting cell" as used herein includes any cell which presents on its surface an antigen in association with a major histocompatibility complex molecule, or portion thereof, or, alternatively, one or more non-classical MHC molecules, or a portion thereof. Examples of suitable APCs are discussed in detail below and include, but are not limited to, whole cells such as macrophages, dendritic cells, B cells, hybrid APCs, and foster antigen presenting cells.

[0117] As used herein an "engineered antigen-presenting cell" refers to an antigen-presenting cell that has a non-natural molecular moiety on its surface. For example, such a cell may not naturally have a costimulator on its surface or may have an additional artificial costimulator in addition to a natural costimulator on its surface, or may express a non-natural class II molecule on its surface. In preferred embodiments, the engineered antigen-presenting cell has the antigen expressed from the improved LAMP Construct on its surface.

[0118] As used herein, "immune effector cells" refers to cells capable of binding an antigen and which mediate an immune response. These cells include, but are not limited to, T cells, B cells, monocytes, macrophages, NK cells and cytotoxic T lymphocytes (CTLs), for example CTL lines, CTL clones, and CTLs from tumor, inflammatory, or other infiltrates.

[0119] As used herein, "partially human" refers to a nucleic acid having sequences from both a human and a non-human vertebrate. In the context of partially human sequences, the partially human nucleic acids have sequences of human immunoglobulin coding regions and sequences based on the non-coding sequences of the endogenous immunoglobulin region of the non-human

vertebrate. The term "based on" when used with reference to endogenous non-coding sequences from a non-human vertebrate refers to sequences that correspond to the non-coding sequence and share a relatively high degree of homology with the non-coding sequences of the endogenous loci of the host vertebrate, e.g., the non-human vertebrate from which the ES cell is derived. Preferably, the non-coding sequences share at least an 80%, more preferably 90% homology with the corresponding non-coding sequences found in the endogenous loci of the non-human vertebrate host cell into which a partially human molecule comprising the non-coding sequences has been introduced.

[0120] The term "immunoglobulin variable region" as used herein refers to a nucleotide sequence that encodes all or a portion of a variable region of an antibody molecule or all or a portion of a regulatory nucleotide sequence that controls expression of an antibody molecule. Immunoglobulin regions for heavy chains may include but are not limited to all or a portion of the V, D, J, and switch regions, including introns. Immunoglobulin region for light chains may include but are not limited to the V and J regions, their upstream flanking sequences, introns, associated with or adjacent to the light chain constant region gene.

[0121] By "transgenic animal" is meant a non-human animal, usually a mammal, having an exogenous nucleic acid sequence present as an extrachromosomal element in a portion of its cells or stably integrated into its germ line DNA (i.e., in the genomic sequence of most or all of its cells). In generating a transgenic animal comprising human sequences, a partially human nucleic acid is introduced into the germ line of such transgenic animals by genetic manipulation of, for example, embryos or embryonic stem cells of the host animal according to methods well known in the art.

[0122] A "vector" includes plasmids and viruses and any DNA or RNA molecule, whether self-replicating or not, which can be used to transform or transfect a cell.

[0123] An "isolated" or "purified" population of cells is substantially free of cells and materials with which it is associated in nature. By substantially free or substantially purified APCs it is meant at least 50% of the population are APCs, preferably at least 70%, more preferably at least 80%, and even more preferably at least 90%, are free of non-APCs cells with which they are associated in nature.

[0124] As used herein, a "genetic modification" refers to any addition, deletion or disruption to a cell's normal nucleotides. Any method which can achieve the genetic modification of APCs are within the spirit and scope of this invention. Art recognized methods include viral mediated gene transfer, liposome mediated transfer, transformation, transfection and transduction, e.g., viral-

mediated gene transfer such as the use of the improved LAMP Constructs based on DNA viruses such as adenovirus, adeno-associated virus and herpes virus, as well as retroviral based vectors.

[0125] The practice of the present invention employs, unless otherwise indicated, conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Maniatis, Fritsch & Sambrook, In Molecular Cloning: A Laboratory Manual (1982); DNA Cloning: A Practical Approach, Volumes I and II (D. N. Glover, ed., 1985); Oligonucleotide Synthesis (M. J. Gait, ed., 1984); Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins, eds., 1985); Transcription and Translation (B. D. Hames & S. I. Higgins, eds., 1984); Animal Cell Culture (R. I. Freshney, ed., 1986); Immobilized Cells and Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide to Molecular Cloning (1984).

[0126] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications mentioned herein are incorporated by reference for the purpose of describing and disclosing devices, formulations and methodologies that may be used in connection with the presently described invention.

Exemplified Embodiments

[0127] The following are exemplified preferred embodiments:

1. An improved LAMP Construct comprising:
 - a. a Cysteine Conserved Fragment of a LAMP Protein; and
 - b. at least one cancer antigen, wherein said cancer antigen is selected from Large T antigen, Small T antigen, MAGE-A10, MAGE-A12, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A4, MAGE-A4, MAGE-A6, MAGE-A6, MAGE-A6, MAGE-A9, MAGE-B10, MAGE-B16, MAGE-B17, MAGE-B1, MAGE-B2, MAGE-B3, MAGE-B4, MAGE-B5, MAGE-B6, MAGE-C1, MAGE-C2, MAGE-C3, MAGE-D1, MAGE-D2, MAGE-D4, MAGE-E1, MAGE-E1_(MAGE1), MAGE-E2, MAGE-F1, MAGE-H1, or MAGE-L2.
2. The improved LAMP Construct of claim 1, wherein:
 - a. the antigen is placed at the N-terminus of the Cysteine Conserved Fragment;
 - b. the antigen is placed at the C-terminus of a single Cysteine Conserved Fragment; or
 - c. the antigen is placed in between two Cysteine Conserved Fragments.

3. The improved LAMP Construct of either claim 1 or claim 2, wherein the improved LAMP Construct comprises at least one fragment/epitope described in Column 1 or Column 4 of Table 1, and preferably is constructed as depicted in ILC-1, ILC-2, ILC-3, ILC-4, ILC-5 or ILC-6.
4. The improved LAMP Construct of claim 3, wherein each antigen is separated by a linker, preferably wherein said linker is the amino acid sequence GP GPG (SEQ ID NO: 152) or PMGLP (SEQ ID NO:153).
5. The improved LAMP Construct of of any one of the preceding claims, wherein the improved LAMP Construct comprises more than one Cysteine Conserved Fragment.
6. The improved LAMP Construct of any one of the preceding claims, wherein the Cysteine Conserved Fragment comprises a Homology Domain of a LAMP Protein.
7. The improved LAMP Construct of any one of the preceding claims, wherein the improved LAMP Construct further comprises a Transmembrane Domain of a LAMP Protein.
8. The improved LAMP Construct of any one of the preceding claims, wherein the improved LAMP Construct further comprises a signal sequence, preferably wherein the signal sequence is derived from a LAMP Protein.
9. The improved LAMP Construct of any one of the preceding claims, wherein the LAMP protein is selected from LAMP-1, LAMP2, LAMP-3, LIMP 2, Macrosailin, Endolyn, LAMP5 or LIMBIC.
10. The improved LAMP Construct of any one of the preceding claims, wherein the improved LAMP Construct comprises:
 - A) the LAMP Protein is selected from any one of SEQ ID NO:1-113 and the cancer antigen is selected from any one of SEQ ID NO:114-116;
 - B) the amino acid sequence of LT^{S220A}-LAMP (SEQ ID NO:151); or
 - C) either (A) or (B) in combination with any one of SEQ ID NO:117-150.
11. The improved LAMP Construct of claim 12, wherein the LAMP Protein is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least

about 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO:1-113 and/or the cancer antigen is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO:114-151.

12. The improved LAMP Construct of any one of the preceding claims, wherein said improved LAMP Construct comprises the amino acid sequence of SEQ ID NO:116, and preferably wherein said LAMP protein is Human LAMP1, and preferably, wherein said LAMP Construct is ILC-4.
13. A polynucleotide encoding the improved LAMP Construct of any one of claims 1-12.
14. The polynucleotide of claim 13, wherein the polynucleotide is a DNA vaccine.
15. The polynucleotide of claim 14, wherein the polynucleotide is a self-replicating RNA vaccine.
16. A host cell comprising the polynucleotide of any one of claims 13-15.
17. A composition comprising the improved LAMP Construct of any one of claims 1-12, the polynucleotide of any one of claims 13-15, or the host cell of claim 16.
18. A method of treating a subject having a disease or a disorder, wherein the method comprises administering to a subject in need thereof the improved LAMP Construct of any one of claims 1-12, the polynucleotide of any one of claims 13-15, the host cell of claim 16, or the composition of claim 17 in an amount sufficient to reduce or treat the disease or disorder.
19. The method of claim 18, wherein the method comprises a priming step and at least one boosting step.
20. The method of claim 19, wherein the improved LAMP Construct of any one of claims 1-12, the polynucleotide of any one of claims 13-15, the host cell of claim 16, or the composition of claim 17 is used in the priming step.
21. The method of either claim 19 or 20, wherein the boosting step comprises administration of an antigen, an improved LAMP Construct, a polypeptide encoded by an improved LAMP Construct, or a cell comprising the improved LAMP Construct.
22. The method of any one of claims 18-21, wherein:
 - a) the antigen used to prime is the same that is used to boost;

- b) the antigen used to prime is derived from the same protein as a second antigen used to boost;
 - c) more than one antigen is used to prime and/or boost.
23. The method of any one of claims 18-22, wherein the subject has a hyperproliferative disorder.
24. The method of claim 23, wherein the hyperproliferative disorder is selected from an adenocarcinoma, sarcoma, skin cancer (e.g., merkel cell carcinoma), melanoma, bladder cancer, brain cancer (including glioblastoma multiforme), breast cancer, uterine cancer, ovarian cancer, prostate cancer, lung cancer (including, but not limited to NSCLC, SCLC, squamous cell cancer), colorectal cancer, anal cancer, rectal cancer, cervical cancer, liver cancer, head and neck cancer, oral cancer, salivary gland cancer, esophageal cancer, pancreas cancer, pancreatic ductal adenocarcinoma (PDA), renal cancer, stomach cancer, kidney cancer, multiple myeloma, or cerebral cancer.
25. The method of either claim 23 or 24, wherein the hyperproliferative disorder is caused by cytomegalovirus (CMV).
26. The method of any one of claims 18-25, wherein said subject is also administered a checkpoint inhibitor, preferably, wherein said checkpoint inhibitor is a PD-1/PD-L1 inhibitor or a CTLA inhibitor.
27. The method of claim 26, wherein said checkpoint inhibitor is selected from Pembrolizumab (Keytruda™), Nivolumab (Opdivo™), Cemiplimab (Libtayo™), Atezolizumab (Tecentriq™), Avelumab (Bavencio™), Durvalumab (Imfinzi™), Ipilimumab (Yervoy™).

LAMP Constructs

[0128] LAMP-1, as deduced from a cDNA clone (Chen, et al., J. Biol. Chem. 263: 8754, 1988) consists of a polypeptide core of about 382 amino acids with a large (346-residue) luminal amino-terminal domain followed by a 24-residue hydrophobic transmembrane region and short (12-residue) carboxyl-terminal cytoplasmic tail. See, Figure 2A and 2B. The luminal domain is highly glycosylated, being substituted with about 20 asparagine linked complex-type oligosaccharides and consists of two approximately 160-residue "homology domains" that are separated by a proline/serine-rich 22-residue "hinge" region. Each of these "homology domains" contains 4 uniformly spaced cysteine residues, disulfide bonded to form four 36-38-residue loops

symmetrically placed within the two halves of the luminal domain (Arterburn, et al., J. Biol. Chem. 265: 7419, 1990; see, also Chen, et al., J. Biol. Chem. 25: 263(18): 8754-8, 1988). Figure 2A schematically shows the conserved domains between LAMP-1, LAMP-2, LAMP-3, Endolyn, LIMBIC, LAMP5, or Macrosialin.

[0129] Previously reported LAMP constructs comprise the following elements in this specific arrangement:

- (a) a full luminal domain of LAMP-1 protein, the antigen and then the full transmembrane/cytoplasmic tail of LAMP-1 protein; or
- (b) the antigen and the full transmembrane/cytoplasmic tail of a LAMP-1 protein.

[0130] In example (a), the antigenic sequence is inserted in between the full luminal domain of a LAMP-1 protein and the LAMP-1 full transmembrane domain/cytoplasmic tail. Both constructs have been shown to successfully target an antigenic sequence to the lysosome/endosome and will be referred to as “complete LAMP Constructs” as shown in Figure 1 as compared to the improved LAMP Constructs ILC-1-ILC-6 described herein. The improved LAMP Constructs described herein do not include the complete LAMP Constructs described in the prior art.

[0131] Additionally, the improved LAMP construct described herein can comprise at least one priming epitope and/or antigen of interest fused to the N-terminus of the luminal domain of a LAMP protein, the N- or C- terminus of at least one homology domain of a LAMP protein, or the N- or C-terminus of at least one Cysteine Conserved Fragment of a LAMP protein (see, for example ILC-1-ILC-6 of Figure 1). However, some preferred improved LAMP constructs comprise at least one priming epitope and/or antigen of interest fused between a first homology domain of a LAMP protein and a second homology domain of a LAMP protein (or at least between two Cysteine Conserved Fragments) (see, for example, ILC-4 of Figure 1). For example, at least one antigen of interest may be placed in, or may replace, the LAMP hinge region. In some preferred embodiments, this construct also comprises a transmembrane domain of a LAMP protein, and/or the cytosolic tail of a LAMP protein.

[0132] Although it has been widely reported in the literature that fragments smaller than the full luminal domain of LAMP-1 were not effective in generating a robust immune response (*see, e.g.* Godinho et al.). In contrast, the inventors unexpectedly discovered that specific fragments, in certain arrangements, did in fact effectively present antigens to the immune system, generating in many cases a more robust immune response, including the generation of a different repertoire of antibodies. For example, the inventors have identified that the minimal LAMP luminal domain fragment that is effective for generating a robust immune response is not the full luminal domain (as

widely reported in the literature) but rather a single Homology Domain of the Luminal Domain of a LAMP Protein.

[0133] For example, constructs can comprise, not the full luminal domain, but instead a single Homology Domain of the Luminal Domain of a LAMP Protein. As used herein, the “Homology Domain” comprises at least the 4 uniformly spaced cysteine residues shown in Figures 3-10. These cysteine residues are labeled 1, 2, 3, and 4 (and in LIMP-2 and Macrosailin – five cysteines are identified, LIMBIC – six cysteines are identified and Endolyn – eight cysteines are identified) in each Homology Domain as shown in Figures 3-10 and are defined herein as the “Cysteine Conserved Fragment.” Additional amino acids can be included to either the N-terminus end and/or the C-terminus end of the Cysteine Conserved Fragment to generate, up to and including a full Homology Domain of a LAMP protein. These additional added amino acids can be derived from the Homology Domain from which the Cysteine Conserved Fragment is derived or from other LAMP Protein Homology Domains. Thus, as used herein, a LAMP Homology Domain comprises and/or consists of one Cysteine Conserved Fragment. At least two LAMP Homology Domains make up the Luminal Domain of LAMP-1, LAMP-2, LAMP-3, or Endolyn.

[0134] Specifically, in one preferred embodiment, the improved LAMP Construct comprises at least one cancer antigen described in Table 1 (preferably the sequences/fragments/epitopes described in column 4 of Table 1) and/or the combinations described in paragraph [134] fused to the N-terminus of the luminal domain of a LAMP protein, at least one homology domain of a LAMP protein, or at least one Cysteine Conserved Fragment of a LAMP protein. See, for example ILC-2 and ILC-6 of Figure 1. In preferred embodiments, these constructs also comprise a transmembrane domain of a LAMP protein, and/or the cytosolic tail of a LAMP protein. In other preferred embodiments, when an antigen contains a transmembrane domain, the transmembrane domain of a LAMP protein and/or the cytosolic tail of a LAMP protein is unnecessary. In preferred embodiments, two homology domains are included in the improved LAMP Construct (e.g., ILC-1 of Figure 1). In further preferred embodiments, the two homology domains are derived from a LAMP-1, LAMP-2, LAMP-3, or an Endolyn protein. Alternatively, the two homology domains are derived from different LAMP proteins. In these constructs comprising two homology domains, a LAMP hinge domain may also be included. The improved LAMP Constructs described in this paragraph are unexpected in view of the prior art as the antigen has always been placed in between the full luminal LAMP-1 domain and the full LAMP-1 transmembrane/cytoplasmic tail, as fragments of the luminal domain have not been reported to be effective in generating a robust immune response.

[0135] In another preferred embodiment, the improved LAMP Construct comprises at least one cancer antigen described in Table 1 (preferably the sequences/fragments/epitopes described in column 4 of Table 1) and/or the combinations described in paragraph [134] fused to the C-terminus of a single homology domain of a LAMP protein or a single Cysteine Conserved Fragment of a LAMP protein (e.g., ILC-5 of Figure 1). In preferred embodiments, these constructs also comprise a transmembrane domain of a LAMP protein, and/or the cytosolic tail of a LAMP protein (e.g., ILC-3 of Figure 1). In other preferred embodiments, when an antigen contains a transmembrane domain, the transmembrane domain of a LAMP protein and/or the cytosolic tail of a LAMP protein is unnecessary. Cancer antigens described in Table 1 can also be inserted into the complete LAMP constructs as depicted in Figure 1. Alternatively, two homology domains from two different LAMP proteins may be used. The improved LAMP Constructs described in this paragraph are unexpected in view of the prior art as the antigen has always been placed in between the full luminal LAMP-1 domain and the full LAMP-1 transmembrane/cytoplasmic tail, as fragments of the luminal domain have not been reported to be effective in generating a robust immune response.

[0136] Thus, the improved LAMP Construct comprises at least one cancer antigen described in Table 1 (preferably the sequences/fragments/epitopes described in column 4 of Table 1) fused to the C-terminus of a single homology domain of a LAMP protein or a single Cysteine Conserved Fragment of a LAMP protein. See, for example, ILC-3 and ILC-5 of Figure 1. In preferred embodiments, these constructs also comprise a transmembrane domain of a LAMP protein, and/or the cytosolic tail of a LAMP protein. In other preferred embodiments, when an antigen contains a transmembrane domain, the transmembrane domain of a LAMP protein and/or the cytosolic tail of a LAMP protein is unnecessary. The improved LAMP Constructs described in this paragraph are unexpected in view of the prior art as described above.

[0137] In another preferred embodiment, the improved LAMP Construct comprises at least one antigen of interest fused in between a first homology domain of a LAMP protein and a second homology domain of a LAMP protein (or at least between two Cysteine Conserved Fragments). See, for example, ILC-4 of Figure 1. In preferred embodiments, the two homology domains are derived from LAMP-1, LAMP-2, LAMP-3, or an Endolyn protein. In these constructs, the antigen may be placed in the LAMP hinge region. Alternatively, two homology domains from two different LAMP proteins may be used. This arrangement of at least one cancer antigen described in Table 1 (preferably the sequences/fragments/epitopes described in column 4 of Table 1) fused in between two LAMP homology domains (including Cysteine Conserved Fragments) is unexpected in view of the prior art as described above.

[0138] The improved LAMP Constructs described above can be generated using the domains defined in the Figures. For example, it is specifically contemplated that the domains included in the improved LAMP Constructs illustrated in Figure 1, for example, can originate from sequences derived from orthologous sequences. See, Figures 3-10 for example. It is expressly contemplated that the equivalent domains defined in Figures 2A and 2B be used to generate the improved LAMP Constructs illustrated in Figure 1 for orthologous sequences. Moreover, the orthologous sequences shown in Figures 3-10 are representative of the sequences that can be used to generate the domains. It is well within the skill in the art to identify other orthologous sequences and/or isotypes and comparing them to the alignments shown in Figures 3-10. Thus, by identifying the equivalent boundaries defined in Figure 2A and 2B for a human LAMP protein with the alignments shown in Figures 3-10, one can generate the improved LAMP Constructs illustrated in Figure 1.

[0139] As would be well understood by the skilled artisan, the boundaries of each domain are an approximation and may be adjusted at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids based on cloning considerations and restriction enzyme placement. Therefore, when a particular domain (e.g., a LAMP Homology Domain) is included in the improved LAMP Construct, the amino acids beginning and ending of the domain may be adjust by at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids as those boundaries defined in Figure 2A.

[0140] Each of the improved LAMP Constructs described herein can additionally comprise a signal sequence and/or additional amino acids in between each domain for cloning purposes as is well known in the art. Additionally, the LAMP homologous domains, the LAMP luminal domain, the LAMP transmembrane domain, and/or the LAMP cytosolic tail domain can originate from the same LAMP protein (e.g., human LAMP-1) or different LAMP proteins (e.g., luminal domain from human LAMP-1 and transmembrane domain from human LAMP-2, and/or mixing of orthologous domains in the same gene family (e.g., LAMP-1) or different gene family (LAMP-1 and LAMP-2).

[0141] Polypeptide variants of the described LAMP Constructs are contemplated. For example, polypeptides at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identity to any of the improved LAMP Constructs described herein as well as polynucleotides encoding these variants. Variants of the improved LAMP Constructs retain the ability to function by targeting the antigenic sequence to the lysosome. For example, a modified luminal sequence must retain the ability to traffic both membrane and non-membrane antigenic materials to an endosomal compartment with at least about 50%, at least about 60%, at least 70%, at least about 80%, at least about 90%, or at least about 100% efficacy compared to the original domain sequence, i.e.,

an efficacy that results in sufficient antigen presentation by a cell comprising the chimeric sequence for it to mount an immune response. In one aspect, sequences containing a suitable trafficking signal may be identified by constructing an improved LAMP Construct containing the well-characterized antigenic domain of ovalbumin, a transmembrane domain, and the cytoplasmic domain of a protein containing a putative lysosomal/endosomal targeting signal. Efficiency of targeting can be measured by determining the ability of antigen presenting cells, expressing the improved LAMP Construct, to stimulate HA epitope-specific, MHC class II restricted T-cells (see, e.g., Example 5 of U.S. Pat. No. 5,633,234).

[0142] Polynucleotides encoding any of the described improved LAMP Constructs are preferred embodiments of the invention, along with polynucleotides at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identity to any of the improved LAMP Construct polynucleotides described herein. Variants of the improved LAMP Constructs retain the ability to function by targeting the antigenic sequence to the lysosome. For example, a modified luminal sequence must retain the ability to traffic both membrane and non-membrane antigenic materials to an endosomal compartment with at least about 50%, at least about 60%, at least 70%, at least about 80%, at least about 90%, or at least about 100% efficacy compared to the original domain sequence, i.e., an efficacy that results in sufficient antigen presentation by a cell comprising the chimeric sequence for it to mount an immune response. In one aspect, sequences containing a suitable trafficking signal may be identified by constructing an improved LAMP Construct containing the well-characterized antigenic domain of ovalbumin, a transmembrane domain, and the cytoplasmic domain of a protein containing a putative lysosomal/endosomal targeting signal. Efficiency of targeting can be measured by determining the ability of antigen presenting cells, expressing the improved LAMP Construct, to stimulate HA epitope-specific, MHC class II restricted T-cells (see, e.g., Example 5 of U.S. Pat. No. 5,633,234).

Cancer Antigens

[0143] The following antigens shown in Table 1 can be cloned into each of the LAMP Constructs described herein using techniques well known to the skilled artisan. The sequences/fragments/epitopes described in the fourth column can be also cloned into the improved LAMP Constructs as described herein. Moreover, it is specifically contemplated that any one of the cancer antigen listed in Table 1 can be combined with any other antigen listed in Table 1 (including the sequences/fragments/epitopes described in the fourth column) and inserted into the improved LAMP Constructs as described herein.

SEQ ID NO:	Protein Name of Antigen	Accession/ Derived From	Preferred Epitopes/Fragments that can be cloned into the LAMP Constructs described herein, comprise at least
114	Large T antigen	YP_009111421	Amino acids 1-327 of SEQ ID NO:114, with or without mutations at amino acid 220, wherein the wild type serine is preferably replaced with an alanine (or other inactivating amino acid) (e.g., SEQ ID NO:115-116) so that phosphorylation of the Rb binding motif is inhibited as described in Sharma et al. 38, 1153-1162 (JUC 2016). Fragments of amino acids 1-327 of SEQ ID NO:114 are also contemplated, such as those exemplified as SEQ ID NO:115-116 so long as these fragments retain the ability to raise an antibody response to the Large T Antigen of Merkel cell polyomavirus (MCPyV). One example of a preferred embodiment comprises the amino acid sequence of SEQ ID NO:51.
117	Small T antigen	YP_009111422	MCPyVgp4 small T antigen, full length and fragments.
118	MAGE-A10	UniProtKB: A0A024RC14	SEQ ID NO:118
119	MAGE-A12	UniProtKB: P43365	SEQ ID NO:119
120	MAGE-A1	UniProtKB: P43355	SEQ ID NO:120
121	MAGE-A2	UniProtKB: P43356	SEQ ID NO:121
122	MAGE-A3	UniProtKB: P43357	SEQ ID NO:122
123	MAGE-A4	UniProtKB: A0A024RC12	SEQ ID NO:123
124	MAGE-A4	UniProtKB: P43358	SEQ ID NO:124
125	MAGE-A4	UniProtKB: Q1RN33	SEQ ID NO:125
126	MAGE-A6	UniProtKB: A8K072	SEQ ID NO:126
127	MAGE-A6	UniProtKB: P43360	SEQ ID NO:127
128	MAGE-A6	UniProtKB: Q6FHI5	SEQ ID NO:128
129	MAGE-A9	UniProtKB: P43362	SEQ ID NO:129

130	MAGE-B10	UniProtKB: Q96LZ2	SEQ ID NO:130
131	MAGE-B16	UniProtKB: A2A368	SEQ ID NO:131
132	MAGE-B17	UniProtKB: A8MXT2	SEQ ID NO:132
133	MAGE- B1	UniProtKB: Q96TG1	SEQ ID NO:133
134	MAGE-B2	UniProtKB: O15479	SEQ ID NO:134
135	MAGE-B3	UniProtKB: O15480	SEQ ID NO:135
136	MAGE-B4	UniProtKB: O15481	SEQ ID NO:136
137	MAGE-B5	UniProtKB: Q9BZ81	SEQ ID NO:137
138	MAGE-B6	UniProtKB: Q8N7X4	SEQ ID NO:138
139	MAGE-C1	UniProtKB: O60732	SEQ ID NO:139
140	MAGE-C2	UniProtKB: Q9UBF1	SEQ ID NO:140
141	MAGE-C3	UniProtKB: Q8TD91	SEQ ID NO:141
142	MAGE-D1	UniProtKB: Q9Y5V3	SEQ ID NO:142
143	MAGE-D2	UniProtKB: Q9UNF1	SEQ ID NO:143
144	MAGE-D4	UniProtKB: Q96JG8	SEQ ID NO:144
145	MAGE- E1	UniProtKB: Q6IAI7	SEQ ID NO:145
146	MAGE- E1_(MAGE1)	UniProtKB: Q9HCl5	SEQ ID NO:146
147	MAGE-E2	UniProtKB: Q8TD90	SEQ ID NO:147
148	MAGE-F1	UniProtKB: Q9HAY2	SEQ ID NO:148
149	MAGE-H1	UniProtKB: Q9H213	SEQ ID NO:149
150	MAGEL2	UniProtKB: Q9UJ55	SEQ ID NO:150

[0144] Additionally, the antigens (including the sequences/fragments/epitopes shown in Column 4) described in Table 1 can be cloned into the LAMP Constructs described herein either individually, or in combination with one another. Thus, each one of the sequences shown in Column 1 of Table 1, including the epitopes/fragments described in Column 4 of Table 1 can be used to generate a LAMP Construct in combination with another sequence also selected from Column 1 or Column 4 of Table 1. To illustrate different possible combinations, but in no way limiting the disclosure, the combinations of antigens (including the sequences shown in Column 1 of Table 1 and/or the sequences/fragments/epitopes described in Column 4 of Table 1) can be cloned into the LAMP Constructs as described herein. The order of the combination of antigens in a particular LAMP construct can vary. Moreover, it is specifically envisioned that these antigens can be combined within a single LAMP Construct or can be delivered in a composition comprising multiple LAMP Constructs.

Assembly of Sequences Encoding Improved LAMP Constructs

[0145] Procedures for constructing improved LAMP Constructs comprising the antigen of interest are well known in the art (see e.g., Williams, et al., J. Cell Biol. 111: 955, 1990). DNA sequences encoding the desired segments can be obtained from readily available recombinant DNA materials such as those available from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852, U.S.A., or from DNA libraries that contain the desired DNA.

[0146] For example, the DNA segments corresponding to the desired domain sequences can be assembled with appropriate control and signal sequences using routine procedures of recombinant DNA methodology. See, e.g., as described in U.S. Pat. No. 4,593,002, and Langford, et al., Molec. Cell. Biol. 6: 3191, 1986.

[0147] A DNA sequence encoding a protein or polypeptide can be synthesized chemically or isolated by one of several approaches. The DNA sequence to be synthesized can be designed with the appropriate codons for the desired amino acid sequence. In general, one will select preferred codons for the intended host in which the sequence will be used for expression. The complete sequence may be assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, Nature 292: 756, 1981; Nambair, et al. Science 223: 1299, 1984; Jay, et al., J. Biol. Chem. 259: 6311, 1984.

[0148] In one aspect, one or more of the nucleic acids encoding the domain sequences of the improved LAMP Construct are isolated individually using the polymerase chain reaction (M. A. Innis, et al., In PCR Protocols: A Guide to Methods and Applications, Academic Press, 1990). The domains are preferably isolated from publicly available clones known to contain them, but they may also be isolated from genomic DNA or cDNA libraries. Preferably, isolated fragments are bordered by compatible restriction endonuclease sites which allow an improved LAMP Construct encoding the cancer antigen sequence to be constructed. This technique is well known to those of skill in the art. Domain sequences may be fused directly to each other (e.g., with no intervening sequences), or inserted into one another (e.g., where domain sequences are discontinuous), or may be separated by intervening sequences (e.g., such as linker sequences).

[0149] The basic strategies for preparing oligonucleotide primers, probes and DNA libraries, as well as their screening by nucleic acid hybridization, are well known to those of ordinary skill in the art. See, e.g., Sambrook, et al., 1989, *supra*; Perbal, 1984, *supra*. The construction of an appropriate genomic DNA or cDNA library is within the skill of the art. See, e.g., Perbal, 1984, *supra*. Alternatively, suitable DNA libraries or publicly available clones are available from suppliers of biological research materials, such as Clontech and Stratagene, as well as from public depositories such as the American Type Culture Collection.

[0150] Selection may be accomplished by expressing sequences from an expression library of DNA and detecting the expressed peptides immunologically. Clones which express peptides that bind to MHC II molecules and to the desired antibodies/T cell receptors are selected. These selection procedures are well known to those of ordinary skill in the art (see, e.g., Sambrook, et al., 1989, *supra*).

[0151] Once a clone containing the coding sequence for the desired polypeptide sequence has been prepared or isolated, the sequence can be cloned into any suitable vector, preferably comprising an origin of replication for maintaining the sequence in a host cell.

Nucleic Acid Delivery Vehicles

[0152] In one aspect, a vaccine composition comprising an improved LAMP Construct is introduced into a cell. The cell may be a host cell for replicating the nucleic acid or for expressing the improved LAMP Construct. Preferably, the host cell for expressing the improved LAMP Construct is an antigen presenting cell (described further below).

[0153] In preferred embodiments, the improved LAMP Construct further comprises a polynucleotide sequence for insertion into a target cell and an expression control sequence operably linked thereto to control expression of the polynucleotide sequence (e.g., transcription and/or translation) in the cell. Examples include plasmids, phages, autonomously replicating sequences (ARS), centromeres, and other sequences which are able to replicate or be replicated *in vitro* or in a host cell (e.g., such as a bacterial, yeast, or insect cell) and/or target cell (e.g., such as a mammalian cell, preferably an antigen presenting cell) and/or to convey the sequences encoding the improved LAMP Construct to a desired location within the target cell.

[0154] In another aspect, the invention provides a nucleic acid molecule (e.g. a plasmid or vector) comprising (i) a first polynucleotide sequence encoding an improved LAMP Construct as described herein (e.g., in some preferred embodiments, an improved LAMP Construct wherein the Construct comprises at least one priming epitope and/or antigen of interest fused in between a first homology domain of a LAMP protein and a second homology domain of a LAMP protein (or at least between two Cysteine Conserved Fragments), for example at least one epitope and/or antigen of interest may be placed in, or may replace, the LAMP hinge region); and (ii) a second polynucleotide sequence encoding at least one boosting epitope and/or antigen operably linked to a secretion signal sequence, wherein the boosting epitope and/or antigen is secreted into the circulation of the subject. The epitope and/or antigen for priming the immune response may be the same or different to the epitope and/or antigen for boosting the immune response and are preferably selected from Table 1.

[0155] The nucleic acid molecule can be provided as a vaccine composition and introduced into a cell. The cell may be a host cell for replicating the nucleic acid molecule or for expressing the improved LAMP Construct (providing the priming epitope and/or antigen) and the boosting epitope and/or antigen operably linked to a secretion signal sequence (such that the boosting epitope and/or antigen is secreted from the cell). Preferably, the host cell is an antigen presenting cell (described further below).

[0156] In preferred embodiments, the first polynucleotide sequence encoding the improved LAMP Construct further comprises a polynucleotide sequence for insertion into a target cell and an expression control sequence operably linked thereto to control expression of the first polynucleotide sequence (e.g., transcription and/or translation) in the cell. Similarly, in preferred embodiments, the second polynucleotide sequence encoding the boosting epitope and/or antigen further comprises a polynucleotide sequence for insertion into a target cell and

an expression control sequence operably linked thereto to control expression of the second polynucleotide sequence (e.g., transcription and/or translation) in the cell. The nucleic acid molecule comprising the first and second polynucleotide sequences may be provided as, for example, a plasmid, phage, autonomously replicating sequence (ARS), centromeres, and other sequences which are able to replicate or be replicated *in vitro* or in a host cell (e.g., such as a bacterial, yeast, or insect cell) and/or target cell (e.g., such as a mammalian cell, preferably an antigen presenting cell) and/or to convey the sequences encoding the improved LAMP Construct and/or the boosting epitope and/or antigen to a desired location within the target cell.

[0157] Recombinant expression vectors may be derived from micro-organisms which readily infect animals, including man, horses, cows, pigs, llamas, giraffes, dogs, cats or chickens. Preferred vectors include those which have already been used as live vaccines, such as vaccinia. These recombinants can be directly inoculated into a host, conferring immunity not only to the microbial vector, but also to express foreign antigens. Preferred vectors contemplated herein as live recombinant vaccines include RNA viruses, adenovirus, herpesviruses, poliovirus, and vaccinia and other pox viruses, as taught in Flexner, *Adv. Pharmacol.* 21: 51, 1990, for example.

[0158] Expression control sequences include, but are not limited to, promoter sequences to bind RNA polymerase, enhancer sequences or negative regulatory elements to bind to transcriptional activators and repressors, respectively, and/or translation initiation sequences for ribosome binding. For example, a bacterial expression vector can include a promoter such as the lac promoter and for transcription initiation, the Shine-Dalgarno sequence and the start codon AUG (Sambrook, et al., 1989, *supra*). Similarly, a eukaryotic expression vector preferably includes a heterologous, homologous, or chimeric promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of a ribosome.

[0159] Expression control sequences may be obtained from naturally occurring genes or may be designed. Designed expression control sequences include, but are not limited to, mutated and/or chimeric expression control sequences or synthetic or cloned consensus sequences. Vectors that contain both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA *in vitro* or *in vivo*, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.).

[0160] In order to optimize expression and/or transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the vectors to eliminate extra, or alternative translation initiation codons or other sequences that may interfere with, or reduce, expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. A wide variety of expression control sequences--sequences that control the expression of a polynucleotide sequence operatively linked to it--may be used in these vectors to express the polynucleotide sequences of this invention. Such useful expression control sequences include, for example, the early or late promoters of SV40, CMV, vaccinia, polyoma, adenovirus, herpes virus and other sequences known to control the expression of genes of mammalian cells, and various combinations thereof.

[0161] The first and second polynucleotide sequences (encoding the improved LAMP construct and boosting epitope and/or antigen) may be expressed from the same or different expression control sequences. For example, a single promoter may be used for transcription of a bicistronic mRNA molecule encoding both the improved LAMP construct and boosting epitope and/or antigen. Those skilled in the art will be well aware that translation of the "second" of the encoded proteins may be achieved by the inclusion of a translation-enhancing element such as an internal ribosome entry site (IRES) (Plank et al., Wiley Interdiscip. Rev. RNA 3:195-212, 2012) or an unstructured junction sequence to achieve post-termination re-initiation of translation (Onishi et al., G3 (Bethesda) 6(12):4115-4125, 2016). However, in some preferred embodiments, the polynucleotide sequences encoding the improved LAMP construct and boosting epitope and/or antigen are expressed from different expression control sequences (e.g., different promoters).

[0162] In order to achieve secretion of the at least one boosting epitope and/or antigen, the second polynucleotide sequence may include a polynucleotide sequence encoding a secretion signal sequence (also known as a leader sequence) typically 16-30 amino acids in length, so that expression of the second polynucleotide sequence results in the boosting epitope and/or antigen being operably linked to the secretion signal sequence. Those skilled in the art are well aware of suitable secretion signal sequences and include, for example, the signal sequence of interleukin-2, CD5, the Immunoglobulin Kappa light chain (hereinafter referred to as the Igkappa leader), trypsinogen, serum albumin, and prolactin (Stern et al., Trends Cell Mol. Biol. 2:1-17, 2007; Kober et al., Biotechnol. Bioengin. 110:1164-1173, 2013).

The secretion signal sequence may, in some cases, be a secretion signal sequence that is "native" to the boosting antigen.

[0163] In one aspect, the nucleic acid molecule comprises an origin of replication for replication. Preferably, the origin functions in at least one type of host cell which can be used to generate sufficient numbers of copies of the sequence for use in delivery to a target cell. Suitable origins therefore include, but are not limited to, those which function in bacterial cells (e.g., such as *Escherichia* sp., *Salmonella* sp., *Proteus* sp., *Clostridium* sp., *Klebsiella* sp., *Bacillus* sp., *Streptomyces* sp., and *Pseudomonas* sp.), yeast (e.g., such as *Saccharomyces* sp. or *Pichia* sp.), insect cells, and mammalian cells. In one preferred aspect, an origin of replication is provided which functions in the target cell into which the nucleic acid delivery vehicle is introduced (e.g., a mammalian cell, such as a human cell). In another aspect, at least two origins of replication are provided, one that functions in a host cell and one that functions in a target cell.

[0164] The nucleic acid molecule may alternatively, or additionally, comprise a polynucleotide sequence(s) to facilitate integration of at least a portion of the nucleic acid molecule (e.g. delivery vector) into a target cell chromosome. For example, the nucleic acid molecule may comprise regions of homology to target cell chromosomal DNA. In one aspect, the nucleic acid molecule is provided as a delivery vector which comprises two or more recombination sites which flank a nucleic acid sequence encoding the improved LAMP Construct and the boosting epitope and/or antigen.

[0165] The vector may additionally comprise a detectable and/or selectable marker to verify that the vector has been successfully introduced in a target cell and/or can be expressed by the target cell. These markers can encode an activity, such as, but not limited to, production of RNA, peptide, or protein, or can provide a binding site for RNA, peptides, proteins, inorganic and organic compounds or compositions and the like.

[0166] Examples of detectable/selectable markers genes include, but are not limited to: DNA segments that encode products which provide resistance against otherwise toxic compounds (e.g., antibiotics); DNA segments that encode products which are otherwise lacking in the recipient cell (e.g., tRNA genes, auxotrophic markers); DNA segments that encode products which suppress the activity of a gene product; DNA segments that encode products which can be readily identified (e.g., phenotypic markers such as beta-galactosidase, a fluorescent protein (GFP, CFP, YFP, BFP, RFP, EGFP, EYFP, EBFP, dsRed, mutated, modified, or enhanced forms thereof, and the like), and cell surface proteins); DNA segments that bind

products which are otherwise detrimental to cell survival and/or function; DNA segments that otherwise inhibit the activity of other nucleic acid segments (e.g., antisense oligonucleotides); DNA segments that bind products that modify a substrate (e.g., restriction endonucleases); DNA segments that can be used to isolate or identify a desired molecule (e.g., segments encoding specific protein binding sites); primer sequences; DNA segments, which when absent, directly or indirectly confer resistance or sensitivity to particular compounds; and/or DNA segments that encode products which are toxic in recipient cells.

[0167] The marker gene can be used as a marker for conformation of successful gene transfer and/or to isolate cells expressing transferred genes and/or to recover transferred genes from a cell. For example, in one aspect, the marker gene is used to isolate and purify antigen presenting cells expressing an improved LAMP Construct described herein.

[0168] Substantially similar genes may be provided, e.g., genes with greater than about 50%, greater than about 70%, greater than 80%, greater than about 90%, and preferably, greater than about 95% identity to a known gene. Substantially similar domain sequences may initially be identified by selecting a sequence which specifically hybridizes to a domain sequence of interest under stringent hybridization conditions. Performing assays to determine the suitability of homologous, variant, or modified domain sequences is merely a matter of screening for sequences which express the appropriate activity. Such screening is routine in the art.

[0169] The nucleic acid molecule encoding the improved LAMP Construct optionally with a boosting epitope may be provided as a naked nucleic acid molecule or in a delivery vehicle associated with one or more molecules for facilitating entry of a nucleic acid into a cell. Suitable delivery vehicles include, but are not limited to: liposomal formulations, polypeptides, polysaccharides, lipopolysaccharides, viral formulations (e.g., including viruses, viral particles, artificial viral envelopes and the like), cell delivery vehicles, and the like.

Lipid-Based Formulations

[0170] Delivery vehicles designed to facilitate intracellular delivery of the improved LAMP Constructs must interact with both non-polar and polar environments (in or on, for example, the plasma membrane, tissue fluids, compartments within the cell, and the like). Therefore, preferably, delivery vehicles are designed to contain both polar and non-polar domains or a translocating sequence for translocating an improved LAMP Construct into a cell.

[0171] Compounds having polar and non-polar domains are termed amphiphiles. Cationic amphiphiles have polar groups that are capable of being positively charged at, or around, physiological pH for interacting with negatively charged polynucleotides such as DNA.

[0172] The improved LAMP Constructs described herein optionally along with a boosting antigen can be provided in formulations comprising lipid monolayers or bilayers to facilitate transfer of the vectors across a cell membrane. Liposomes or any form of lipid membrane, such as planar lipid membranes or the cell membrane of an intact cell, e.g., a red blood cell, can be used. Liposomal formulations can be administered by any means, including administration intravenously or orally.

[0173] Liposomes and liposomal formulations can be prepared according to standard methods and are well known in the art, see, e.g., Remington's; Akimaru, 1995, Cytokines Mol. Ther. 1: 197-210; Alving, 1995, Immunol. Rev. 145: 5-31; Szoka, 1980, Ann. Rev. Biophys. Bioeng. 9: 467; U.S. Pat. No. 4,235,871; U.S. Pat. No. 4,501,728; and U.S. Pat. No. 4,837,028. In one aspect, the liposome comprises a targeting molecule for targeting a liposome:nucleic acid molecule (encoding an improved LAMP Construct and optionally along with a boosting epitope and/or antigen) complex to a particular cell type. In a particularly preferred aspect, a targeting molecule comprises a binding partner (e.g., a ligand or receptor) for a biomolecule (e.g., a receptor or ligand) on the surface of a blood vessel or a cell found in a target tissue.

[0174] Liposome charge is an important determinant in liposome clearance from the blood, with negatively charged liposomes being taken up more rapidly by the reticuloendothelial system (Juliano, 1975, Biochem. Biophys. Res. Commun. 63: 651) and thus having shorter half-lives in the bloodstream. Incorporating phosphatidylethanolamine derivatives enhances the circulation time by preventing liposomal aggregation. For example, incorporation of N-(omega-carboxy)acylamidophosphatidylethanolamines into large unilamellar vesicles of L-alpha-distearoylphosphatidylcholine dramatically increases the in vivo liposomal circulation lifetime (see, e.g., Ahl, 1997, Biochim. Biophys. Acta 1329: 370-382). Liposomes with prolonged circulation half-lives are typically desirable for therapeutic and diagnostic uses. For a general discussion of pharmacokinetics, see, e.g., Remington's, Chapters 37-39, Lee, et al., In Pharmacokinetic Analysis: A Practical Approach (Technomic Publishing AG, Basel, Switzerland 1996).

[0175] Typically, liposomes are prepared with about 5 to 15 mole percent negatively charged phospholipids, such as phosphatidylglycerol, phosphatidylserine or phosphatidyl-inositol.

Added negatively charged phospholipids, such as phosphatidylglycerol, also serve to prevent spontaneous liposome aggregation, and thus minimize the risk of undersized liposomal aggregate formation. Membrane-rigidifying agents, such as sphingomyelin or a saturated neutral phospholipid, at a concentration of at least about 50 mole percent, and 5 to 15 mole percent of monosialylganglioside can also impart desirably liposome properties, such as rigidity (see, e.g., U.S. Pat. No. 4,837,028).

[0176] Additionally, the liposome suspension can include lipid-protective agents which protect lipids against free-radical and lipid-peroxidative damages on storage. Lipophilic free-radical quenchers, such as alpha-tocopherol and water-soluble iron-specific chelators, such as ferrioxianine, are preferred.

[0177] The improved LAMP Constructs of the invention can include multilamellar vesicles of heterogeneous sizes. For example, vesicle-forming lipids can be dissolved in a suitable organic solvent or solvent system and dried under vacuum or an inert gas to form a thin lipid film. If desired, the film can be redissolved in a suitable solvent, such as tertiary butanol, and then lyophilized to form a more homogeneous lipid mixture which is in a more easily hydrated powderlike form. This film is covered with an aqueous solution of the peptide or polypeptide complex and allowed to hydrate, typically over a 15 to 60 minute period with agitation. The size distribution of the resulting multilamellar vesicles can be shifted toward smaller sizes by hydrating the lipids under more vigorous agitation conditions or by adding solubilizing detergents such as deoxycholate. The hydration medium preferably comprises the nucleic acid at a concentration which is desired in the interior volume of the liposomes in the final liposome suspension.

[0178] Following liposome preparation, the liposomes can be sized to achieve a desired size range and relatively narrow distribution of liposome sizes. One preferred size range is about 0.2 to 0.4 microns, which allows the liposome suspension to be sterilized by filtration through a conventional filter, typically a 0.22 micron filter. Filter sterilization can be carried out on a high throughput basis if the liposomes have been sized down to about 0.2 to 0.4 microns. Several techniques are available for sizing liposome to a desired size (see, e.g., U.S. Pat. No. 4,737,323).

[0179] Suitable lipids include, but are not limited to, DOTMA (Felgner, et al., 1987, Proc. Natl. Acad. Sci. USA 84: 7413-7417), DOGS or Transfectain™ (Behr, et al., 1989, Proc. Natl. Acad. Sci. USA 86: 6982-6986), DNERIE or DORIE (Felgner, et al., Methods 5: 67-75), DC-CHOL (Gao and Huang, 1991, BBRC 179: 280-285), DOTAP™ (McLachlan, et al., 1995, Gene

Therapy 2: 674-622), Lipofectamine™. and glycerolipid compounds (see, e.g., EP901463 and WO98/37916).

[0180] Other molecules suitable for complexing with the improved LAMP Constructs include cationic molecules, such as, polyamidoamine (Haensler and Szoka, 1993, Bioconjugate Chem. 4: 372-379), dendritic polysine (WO 95/24221), polyethylene irinine or polypropylene h-nine (WO 96/02655), polylysine (U.S. Pat. No. 5,595,897; FR 2 719 316), chitosan (U.S. Pat. No. 5,744,166), DNA-gelatin coacervates (see, e.g., U.S. Pat. No. 6,207,195; U.S. Pat. No. 6,025,337; U.S. Pat. No. 5,972,707) or DEAE dextran (Lopata, et al., 1984, Nucleic Acid Res. 12: 5707-5717).

Viral-Based Gene Delivery Vehicles

[0181] In one aspect, the improved LAMP Construct and/or boosting epitope and/or antigen is provided as a delivery vehicle comprising a virus or viral particle. In this aspect, preferably, the improved LAMP Construct comprises a viral vector. Viral vectors, such as retroviruses, adenoviruses, adeno-associated viruses and herpes viruses, are often made up of two components, a modified viral genome and a coat structure surrounding it (see, e.g., Smith et al., 1995, Ann. Rev. Microbiol. 49: 807-838), although sometimes viral vectors are introduced in naked form or coated with proteins other than viral proteins. Most current vectors have coat structures similar to a wild-type virus. This structure packages and protects the viral nucleic acid and provides the means to bind and enter target cells.

[0182] Preferably, viral vectors comprising the improved LAMP Constructs described herein are modified from wild-type viral genomes to disable the growth of the virus in a target cell while enabling the virus to grow in a host cell (e.g., such as a packaging or helper cell) used to prepare infectious particles. Vector nucleic acids generally essential cis-acting viral sequences for replication and packaging in a helper line and expression control sequences for regulating the expression of a polynucleotide being delivered to a target cell. Other viral functions are expressed in trans in specific packaging or helper cell lines as are known in the art.

[0183] Preferred viral vectors may be derived from a virus selected from the group consisting of herpes viruses, cytomegaloviruses, foamy viruses, lentiviruses, Semliki forrest virus, AAV (adeno-associated virus), poxviruses, adenovirases and retroviruses. Such viral vectors are well known in the art.

[0184] In one preferred aspect, a viral vector used is an adenoviral vector. The adenoviral genome consists of a linear double-stranded DNA molecule of approximately 36 kb carrying more than about thirty genes necessary to complete the viral replication cycle. The early genes are divided into 4 regions (E1 to E4) that are essential for viral replication with the exception of the E3 region, which is believed to modulate the anti-viral host immune response. The E1 region (E1A and E1B) encodes proteins responsible for the regulation of transcription of the viral genome. Expression of the E2 region genes (E2A and E2B) leads to the synthesis of the polypeptides needed for viral replication. The proteins encoded by the E3 region prevent cytolysis by cytotoxic T cells and tumor necrosis factor (Wold and Gooding, 1991, *Virology* 184: 1-8). The proteins encoded by the E4 region are involved in DNA replication, late gene expression and splicing and host cell shut off (Halbert, et al., 1985, *J. Virol.* 56: 250-257). The late genes generally encode structural proteins contributing to the viral capsid. In addition, the adenoviral genome carries at cis-acting 5' and 3' ITRs (Inverted Terminal Repeat) and packaging sequences essential for DNA replication. The ITRs harbor origins of DNA replication while the encapsidation region is required for the packaging of adenoviral DNA into infectious particles.

[0185] Adenoviral vectors can be engineered to be conditionally replicative (CRAAd vectors) in order to replicate selectively in specific cells (e.g., such as proliferative cells) as described in Heise and Kim (2000, *J. Clin. Invest.* 105: 847-85 1). In another aspect, an adenoviral vector is replication-defective for the E1 function (e.g., by total or partial deletion or mutagenesis of E1). The adenoviral backbone of the vector may comprise additional modifications (deletions, insertions or mutations in one or more viral genes). An example of an E2 modification is illustrated by the thermosensitive mutation localized on the DBP (DNA Binding Protein) encoding gene (Ensinger et al., 1972, *J. Virol.* 10: 328-339). The adenoviral sequence may also be deleted of all or part of the E4 region (see, e.g., EP 974 668; Christ, et al., 2000, *Human Gene Ther.* 11: 415-427; Lusky, et al., 1999, *J. Virol.* 73: 8308-8319). Additional deletions within the non-essential E3 region may allow the size of the polynucleotide being delivered to be increased (Yeh, et al., 1997, *FASEB Journal* 11: 615 623). However, it may be advantageous to retain all or part of the E3 sequences coding for polypeptides (e.g., such as gp19k) allowing the virus to escape the immune system (Gooding, et al., 1990, *Critical Review of Immunology* 10: 53-71) or inflammatory reactions (EP 00440267.3).

[0186] Second generation vectors retaining the ITRs and packaging sequences and comprising substantial genetic modifications to abolish the residual synthesis of the viral antigens also may be used in order to improve long-term expression of the expressed gene in the transduced cells (see, e.g., WO 94/28152; Lusky, et al., 1998, J. Virol 72: 2022-2032).

[0187] The improved LAMP Constructs being introduced into the cell may be inserted in any location of the viral genome, with the exception of the cis-acting sequences. Preferably, it is inserted in replacement of a deleted region (E1, E3 and/or E4), preferably, within a deleted E1 region.

[0188] Adenoviruses can be derived from any human or animal source, in particular canine (e.g. CAV-1 or CAV-2 Genbank ref. CAVIGENOM and CAV77082, respectively), avian (Genbank ref. AAVEDSDNA), bovine (such as BAV3; Reddy, et al., 1998, J. Virol. 72: 1394-1402), murine (Genbank ref. ADRMUSMAVI), ovine, feline, porcine or simian sources or alternatively, may be a hybrid virus. Any serotype can be employed. However, the human adenoviruses of the C sub-group are preferred, especially adenoviruses 2 (Ad2) and 5 (Ad5). Such viruses are available, for example, from the ATCC.

[0189] Adenoviral particles or empty adenoviral capsids also can be used to transfer improved LAMP Constructs by a virus-mediated cointernalization process as described in U.S. Pat. No. 5,928,944. This process can be accomplished in the presence of cationic agent(s) such as polycarbones or lipid vesicles comprising one or more lipid layers.

[0190] Adenoviral particles may be prepared and propagated according to any conventional technique in the field of the art (e.g., WO 96/17070) using a complementation cell line or a helper virus, which supplies in trans the missing viral genes necessary for viral replication. The cell lines 293 (Graham et al., 1977, J. Gen. Virol. 36: 59-72) and PERC6 (Fallaux et al., 1998, Human Gene Therapy 9: 1909-1917) are commonly used to complement E1 deletions. Other cell lines have been engineered to complement defective vectors (Yeh, et al., 1996, J. Virol. 70: 559-565; Kroughak and Graham, 1995, Human Gene Ther. 6: 1575-1586; Wang, et al., 1995, Gene Ther. 2: 775-783; Lusky, et al., 1998, J. Virol. 72: 2022-203; EP 919627 and WO 97/04119). The adenoviral particles can be recovered from the culture supernatant but also from the cells after lysis and optionally further purified according to standard techniques (e.g., chromatography, ultracentrifugation, as described in WO 96/27677, WO 98/00524 WO 98/26048 and WO 00/50573).

[0191] Cell-type specific targeting may be achieved with vectors derived from adenoviruses having a broad host range by the modification of viral surface proteins. For example, the

specificity of infection of adenoviruses is determined by the attachment to cellular receptors present at the surface of permissive cells. In this regard, the fiber and penton present at the surface of the adenoviral capsid play a critical role in cellular attachment (Defer, et al., 1990, J. Virol. 64: 3661-3673). Thus, cell targeting of adenoviruses can be carried out by genetic modification of the viral gene encoding fiber and/or penton, to generate modified fiber and/or penton capable of specific interaction with unique cell surface receptors. Examples of such modifications are described in Wickarn, et al., 1997, J. Virol. 71: 8221-8229; Arriberg, et al., 1997, Virol. Chem 268: 6866-6869; Roux, et al., 1989, Proc. Natl. Acad. Sci. USA 86: 9079-9083; Miller and Vile, 1995, FASEB J. 9: 190-199; WO 93/09221, and in WO 95/28494.

[0192] In a particularly preferred aspect, adeno-associated viral sequences are used as vectors. Vectors derived from the human parvovirus AAV-2 (adeno-associated virus type 2) are among the most promising gene delivery vehicles currently being developed. Several of the features of this system for packaging a single-stranded DNA suggest it as a possible alternative to naked DNA for delivery. A primary attractive feature, in contrast to other viral vectors such as vaccinia or adenovirus, is that AAV vectors do not express any viral genes. The only viral DNA sequences included in the vaccine construct are the 145 bp inverted terminal repeats (ITR). Thus, as in immunization with naked DNA, the only gene expressed is that of the antigen, or antigen chimera. Additionally, AAV vectors are known to transduce both dividing and non-dividing cells, such as human peripheral blood monocyte-derived dendritic cells, with persistent transgene expression, and with the possibility of oral and intranasal delivery for generation of mucosal immunity. Moreover, the amount of DNA required appears to be much less by several orders of magnitude, with maximum responses at doses of 10^{10} to 10^{11} particles or copies of DNA in contrast to naked DNA doses of 50 ug or about 10^{15} copies.

[0193] In one aspect, AAV vectors are packaged by co-transfection of a suitable cell line (e.g., human 293 cells) with the DNA contained in the AAV ITR chimeric protein encoding constructs and an AAV helper plasmid ACG2 containing the AAV coding region (AAV rep and cap genes) without the ITRs. The cells are subsequently infected with the adenovirus Ad5. Vectors can be purified from cell lysates using methods known in the art (e.g., such as cesium chloride density gradient ultracentrifugation) and are validated to ensure that they are free of detectable replication-competent AAV or adenovirus (e.g., by a cytopathic effect bioassay). AAV titer may be determined by quantitative PCR with virus DNA samples prepared after

digestion with proteinase K. Preferably, vector titers produced by such a method are approximately 5×10^{12} to 1×10^{13} DNase resistant particles per ml.

[0194] In other aspects, retroviral vectors are used. Retroviruses are a class of integrative viruses which replicate using a virus-encoded reverse transcriptase, to replicate the viral RNA genome into double stranded DNA which is integrated into chromosomal DNA of the infected cells (e.g., target cells). Such vectors include those derived from murine leukemia viruses, especially Moloney (Gilboa, et al., 1988, Adv. Exp. Med. Biol. 241: 29) or Friend's FB29 strains (WO 95/01447). Generally, a retroviral vector is deleted of all or part of the viral genes gag, pol and env and retains 5' and 3' LTRs and an encapsidation sequence. These elements may be modified to increase expression level or stability of the retroviral vector. Such modifications include the replacement of the retroviral encapsidation sequence by one of a retrotransposon such as VL30 (see, e.g., U.S. Pat. No. 5,747,323). Preferably, the improved LAMP Construct is inserted downstream of the encapsidation sequence, preferably in opposite direction relative to the retroviral genome. Cell specific targeting may be achieved by the conjugation of antibodies or antibody fragments to the retroviral envelope protein as is known in the art.

[0195] Retroviral particles are prepared in the presence of a helper virus or in an appropriate complementation (packaging) cell line which contains integrated into its genome the retroviral genes for which the retroviral vector is defective (e.g. gag/pol and env). Such cell lines are described in the prior art (Miller and Rosman, 1989, BioTechniques 7: 980; Danos and Mulligan, 1988, Proc. Natl. Acad. Sci. USA 85: 6460; Markowitz, et al., 1988, Virol. 167: 400). The product of the env gene is responsible for the binding of the viral particle to the viral receptors present on the surface of the target cell and, therefore determines the host range of the retroviral particle. In the context of the invention, it is advantageous to use a packaging cell line, such as the PA317 cells (ATCC CRL 9078) or 293E16 (WO97/35996) containing an amphotropic envelope protein, to allow infection of human and other species' target cells. The retroviral particles are preferably recovered from the culture supernatant and may optionally be further purified according to standard techniques (e.g. chromatography, ultracentrifugation).

[0196] Other suitable viruses include poxviruses. The genome of several members of poxyviridae has been mapped and sequenced. A poxyviral vector may be obtained from any member of the poxyiridae, in particular canarypox, fowlpox and vaccinia virus. Suitable vaccinia viruses include, but are not limited to, the Copenhagen strain (Goebel, et al., 1990,

Virol. 179: 247-266; Johnson, et al., 1993, Virol. 196: 381-401), the Wyeth strain and the modified Ankara (MVA) strain (Antoine, et al., 1998, Virol. 244: 365-396). The general conditions for constructing a vaccinia virus vector are known in the art (see, e.g., EP 83 286 and EP 206 920; Mayr et al., 1975, Infection 3: 6-14; Sutter and Moss, 1992, Proc. Natl. Acad. Sci. USA 89: 10847-10851). Preferably, the polynucleotide of interest is inserted within a non-essential locus such as the noncoding intergenic regions or any gene for which inactivation or deletion does not significantly impair viral growth and replication.

[0197] Poxviral particles are prepared as described in the art (Piccini, et al., 1987, Methods of Enzymology 153: 545-563; U.S. Pat. No. 4,769,330; U.S. Pat. No. 4,772,848; U.S. Pat. No. 4,603,112; U.S. Pat. No. 5,100,587 and U.S. Pat. No. 5,179,993). Generally, a donor plasmid is constructed, amplified by growth in *E. coli* and isolated by conventional procedures. Then, it is introduced into a suitable cell culture (e.g. chicken embryo fibroblasts) together with a poxvirus genome, to produce, by homologous recombination, poxyviral particles. These can be recovered from the culture supernatant or from the cultured cells after a lysis step (e.g., chemical lysis, freezing/thawing, osmotic shock, sonication and the like). Consecutive rounds of plaque purification can be used to remove contaminating wild type virus. Viral particles can then be purified using the techniques known in the art (e.g., chromatographic methods or ultracentrifugation on cesium chloride or sucrose gradients).

[0198] The use of vaccinia as a live virus vaccine in the global campaign to eradicate smallpox made vaccinia an obvious choice for development as a live recombinant vaccine vector. Live recombinant vaccinia viruses expressing close to 100 different foreign proteins have been reported, and a number of these are effective experimental vaccines (reviewed by Moss and Flexner, 1987). Vaccinia is particularly versatile as an expression vector because of its large genomic size, capability of accepting at least 25,000 base pairs of foreign DNA, and its ability to infect most eukaryotic cell types, including insect cells (*ibid.*). Unlike other DNA viruses, poxviruses replicate exclusively in the cytoplasm of infected cells, reducing the possibility of genetic exchange of recombinant viral DNA with the host chromosome. Recombinant vaccinia vectors have been shown to properly process and express proteins from a variety of sources including man, other mammals, parasites, RNA and DNA viruses, bacteria and bacteriophage.

[0199] The expression of DNA encoding a foreign protein is controlled by host virus regulatory elements, including upstream promoter sequences and, where necessary, RNA processing signals. Insertion of foreign DNA into nonessential regions of the vaccinia virus genome has

been carried out by homologous recombination (Panicali, et al., Proc. Nat'l. Acad. Sci, USA, 79: 4927, 1982; Mackett, et al., Proc. Nat'l. Acad. Sci. USA, 79: 7415, 1982).

[0200] Expression of cancer antigens by the improved LAMP Construct and optionally along with a boosting epitope and/or antigen may occur because of transcriptional regulatory elements at or near the site of insertion or by more precise genetic engineering. Plasmid vectors that greatly facilitate insertion and expression of foreign genes have been constructed (Mackett, et al., J. Virol, 49: 857, 1982). These vectors contain an expression site, composed of a vaccinia transcriptional promoter and one or more unique restriction endonuclease sites for insertion of the foreign coding sequence flanked by DNA from a nonessential region of the vaccinia genome. The choice of promoter determines both the time (e.g., early or late) and level of expression, whereas the flanking DNA sequence determines the site of homologous recombination.

[0201] Only about one in a thousand virus particles produced by this procedure is a recombinant. Although recombinant virus plaques can be identified by DNA hybridization, efficient selection procedures have been developed. By using segments of nonessential vaccinia virus thymidine kinase (TK) gene as flanking sequences, the foreign gene recombines into the TK locus and by insertion inactivates the TK gene. Selection of TK virus is achieved by carrying out the virus plaque assay in TK cells in the presents of 5-bromodeoxyuridine. Phosphorylation of the nucleoside analogue and consequent lethal incorporation into viral DNA occurs only in cells infected with TK+ parental virus. Depending on the efficiency of the transfection and recombination, up to 80 of the plaques are desired recombinants, and the rest are spontaneous TK mutants.

[0202] Plasmid vectors that contain the *E. coli* beta-galactosidase gene, as well as an expression site for a second gene, permit an alternative method of distinguishing recombinant from parental virus (Chakrabarti, et al., Mol. Cell. Biol., 5: 3403, 1985). Plaques formed by such recombinants can be positively identified by the blue color that forms upon addition of an appropriate indicator. By combining both TK selection and beta-galactosidase expression, recombinant virus is readily and quickly isolated. The recombinants are then amplified by propagation in suitable cell lines and expression of the inserted gene is checked by appropriate enzymological, immunological or physical procedures.

[0203] An upper limit to the amount of genetic information that can be added to the vaccinia virus genome is not yet known. However, the addition of nearly 25,000 base pairs of foreign DNA had no apparent deleterious effect on virus yield (Smith, et al., Gene, 25:21, 1983).

Were it necessary, large segments of the vaccinia virus genome could be deleted to provide additional capacity (Moss, et al., J. Virol. 40: 387, 1981).

[0204] Viral capsid molecules may include targeting moieties to facilitate targeting and/or entry into cells. Suitable targeting molecules, include, but are not limited to: chemical conjugates, lipids, glycolipids, hormones, sugars, polymers (e.g. PEG, polylysine, PEI and the like), peptides, polypeptides (see, e.g., WO 94/40958), vitamins, antigens, lectins, antibodies and fragments thereof. Preferably, such targeting molecules recognize and bind to cell-specific markers, tissue-specific markers, cellular receptors, viral antigens, antigenic epitopes or tumor-associated markers.

[0205] Compositions comprising an improved LAMP Construct optionally along with a boosting epitope and/or antigen, based on viral particles may be formulated in the form of doses of between 10 and 10^{14} i.u. (infectious units), and preferably, between 10 and 10^{11} i.u. The titer may be determined by conventional techniques. The doses of LAMP Constructs are preferably comprised between 0.01 and 10 mg/kg, more especially between 0.1 and 2 mg/kg.

Self-Replicating RNA

[0206] Self-replicating RNA virus vectors can also be constructed using the improved LAMP Constructs as described herein. For example, alphaviruses, flaviviruses, measles virus and rhabdoviruses can be used to generate self-replicating RNA virus vaccines. Preferred strains of self-replicating RNA viruses include, but are not limited to rabies virus (RABV), vesicular stomatitis virus (VSV), West Nile virus, Kunjin virus, Semliki Forest virus (SFV), Sindbis virus (SIN) and/or Venezuelan equine encephalitis virus (VEE).

[0207] Self-replicating RNA viruses express the native antigen upon delivery into tissue, thus mimicking live attenuated vaccines without having the risk of reversion to pathogenicity. They also stimulate the innate immune system, thus potentiating responses. See, e.g., Ljungberg, K. "Self-replicating alphavirus RNA vaccines," Expert Rev Vaccines (2):177-94 (2015); Lundstrom, K., "Oncolytic Alphaviruses in Cancer Immunotherapy", Vaccines 5:9 (2017); Lundstrom, K. "Replicon RNA Viral Vectors as Vaccines," Vaccines 4:39 (2016) (hereby incorporated by reference in their entirety). Use of self-replicating vaccines comprising the improved LAMP Constructs described herein can also be used in prime-boost protocols.

[0208] Moreover, self-replicating RNA viruses can also be encapsulated by liposomes, as described herein, to improve delivery and targeting. Immunization with self-replicating RNA viruses comprising the improved LAMP Constructs described herein may provide higher

transient expression levels of antigens resulting in generation of neutralizing antibody responses and protection against lethal challenges under safe conditions.

Cell-Based Delivery Vehicles

[0209] The improved LAMP Constructs according to the invention can be delivered to target cells by means of other cells ("delivery cells") which comprise the constructs. Methods for introducing improved LAMP constructs into cells are known in the art and include microinjection of DNA into the nucleus of a cell (Capechi, et al., 1980, Cell 22: 479-488); transfection with CaP0₄ (Chen and Okayama, 1987, Mol. Cell Biol. 7: 2745-2752), electroporation (Chu, et al., 1987, Nucleic Acid Res. 15: 1311-1326); lipofection/liposome fusion (Feigner, et al., 1987, Proc. Natl. Acad. Sci. USA 84: 7413-7417) and particle bombardment (Yang, et al., 1990, Proc. Natl. Acad. Sci. USA 87: 9568-9572). Suitable cells include autologous and non-autologous cells, and may include xenogenic cells. Delivery cells may be induced to deliver their contents to the target cells by inducing their death (e.g., by providing inducible suicide genes to these cells).

Accessory Molecules

[0210] The compositions comprising the improved LAMP Constructs according to the invention may comprise one or more accessory molecules for facilitating the introduction of an improved LAMP Construct into a cell and/or for enhancing a particular therapeutic effect and/or enhancing antibody production.

[0211] In addition, the composition comprising the improved LAMP Construct according to the present invention may include one or more stabilizing substance(s), such as lipids, nuclease inhibitors, hydrogels, hyaluronidase (WO 98/53853), collagenase, polymers, chelating agents (EP 890362), in order to inhibit degradation within the animal/human body and/or improve transfection/infection of the vector into a target cell. Such substances may be used alone or in combination (e.g., cationic and neutral lipids).

[0212] It has also been shown that adenovirus proteins are capable of destabilizing endosomes and enhancing the uptake of DNA into cells. The mixture of adenoviruses to solutions containing a lipid-complexed DNA vector or the binding of DNA to polylysine covalently attached to adenoviruses using protein cross-linking agents may substantially improve the uptake and expression of an improved LAMP Construct optionally along with a

boosting epitope and/or antigen (see, e.g., Curiel, et al., 1992, Am. J. Respir. Cell. Mol. Biol. 6: 247-252).

Host Cells

[0213] Improved LAMP Constructs according to the invention can be expressed in a variety of host cells, including, but not limited to: prokaryotic cells (e.g., *E. coli*, *Staphylococcus* sp., *Bacillus* sp.); yeast cells (e.g., *Saccharomyces* sp.); insect cells; nematode cells; plant cells; amphibian cells (e.g., *Xenopus*); avian cells; and mammalian cells (e.g., human cells, mouse cells, mammalian cell lines, primary cultured mammalian cells, such as from dissected tissues).

[0214] The molecules can be expressed in host cells isolated from an organism, host cells which are part of an organism, or host cells which are introduced into an organism. In one aspect, improved LAMP Constructs are expressed in host cells *in vitro*, e.g., in culture. In another aspect, improved LAMP Constructs are expressed in a transgenic organism (e.g., a transgenic mouse, rat, rabbit, pig, primate, etc.) that comprises somatic and/or germline cells comprising nucleic acids encoding the improved LAMP Construct along with a boosting epitope and/or antigen. Methods for constructing transgenic animals are well known in the art and are routine.

[0215] Improved LAMP Constructs also can be introduced into cells *in vitro*, and the cells (e.g., such as stem cells, hematopoietic cells, lymphocytes, and the like) can be introduced into the host organism. The cells may be heterologous or autologous with respect to the host organism. For example, cells can be obtained from the host organism, improved LAMP Constructs can be (optionally along with a boosting epitope and/or antigen) introduced into the cells *in vitro*, and then reintroduced into the host organism.

Antigen Presenting Cells

[0216] In a preferred aspect of the invention, an improved LAMP Construct as described herein is introduced into a natural or engineered antigen presenting cell.

[0217] The term "antigen presenting cell" (APC) as used herein intends any cell which presents on its surface an antigen in association with a major histocompatibility complex molecule, preferably a MHC class II molecule, or portion thereof. Examples of suitable APCs are discussed in detail below and include, but are not limited to, whole cells such as

macrophages, dendritic cells, B cells, hybrid APCs, and foster antigen presenting cells. Methods of making hybrid APCs are described and known in the art.

[0218] Dendritic cells (DCs) are potent antigen-presenting cells. It has been shown that DCs provide all the signals required for T cell activation and proliferation. These signals can be categorized into two types. The first type, which gives specificity to the immune response, is mediated through interaction between the T-cell receptor/CD3 ("TCR/CD3") complex and an antigenic peptide presented by a major histocompatibility complex ("MHC" defined above) class I or II protein on the surface of APCs. This interaction is necessary, but not sufficient, for T cell activation to occur. In fact, without the second type of signals, the first type of signals can result in T cell anergy. The second type of signals, called co-stimulatory signals, is neither antigen-specific nor MHC-restricted, and can lead to a full proliferation response of T cells and induction of T cell effector functions in the presence of the first type of signals.

[0219] Several molecules have been shown to enhance co-stimulatory activity. These include, but are not limited to, heat stable antigen (HSA), chondroitin sulfate-modified MHC invariant chain (Ii-CS), intracellular adhesion molecule I (ICAM-1), and B7 co-stimulatory molecule on the surface of APCs and its counter-receptor CD28 or CTLA-4 on T cells.

[0220] Other important co-stimulatory molecules are CD40, CD54, CD80, CD86. As used herein, the term "co-stimulatory molecule" encompasses any single molecule or combination of molecules which, when acting together with a peptide/MHC complex bound by a TCR on the surface of a T cell, provides a co-stimulatory effect which achieves activation of the T cell that binds the peptide. The term thus encompasses B7, or other co-stimulatory molecule(s) on an APC, fragments thereof (alone, complexed with another molecule(s), or as part of a fusion protein) which, together with peptide/MHC complex, binds to a cognate ligand and result in activation of the T cell when the TCR on the surface of the T cell specifically binds the peptide. Co-stimulatory molecules are commercially available from a variety of sources, including, for example, Beckman Coulter.

[0221] In one aspect of the invention, the method described in Romani et al., J. Immunol. Methods 196: 135-151, 1996, and Bender et al, J. Immunol. Methods 196: 121-135, 1996, are used to generate both immature and mature dendritic cells from the peripheral blood mononuclear cells (PBMCs) of a mammal, such as a murine, simian or human. Briefly, isolated PBMCs are pre-treated to deplete T- and B-cells by means of an immunomagnetic technique. Lymphocyte-depleted PBMC are then cultured for in RPMI medium 9 e.g., about 7 days), supplemented with human plasma (preferably autologous plasma) and GM-CSF/IL-

4, to generate dendritic cells. Dendritic cells are nonadherent when compared to their monocyte progenitors. Thus, on approximately day 7, non-adherent cells are harvested for further processing.

[0222] The dendritic cells derived from PBMC in the presence of GM-CSF and IL-4 are immature, in that they can lose the nonadherence property and revert back to macrophage cell fate if the cytokine stimuli are removed from the culture. The dendritic cells in an immature state are very effective in processing native protein antigens for the MHC class II restricted pathway (Romani, et al., J. Exp. Med. 169:1169, 1989). Further maturation of cultured dendritic cells is accomplished by culturing for 3 days in a macrophage-conditioned medium (CM), which contains the necessary maturation factors. Mature dendritic cells are less able to capture new proteins for presentation but are much better at stimulating resting T cells (both CD4 and CD8) to grow and differentiate.

[0223] Mature dendritic cells can be identified by their change in morphology, such as the formation of more motile cytoplasmic processes; by their nonadherence; by the presence of at least one of the following markers: CD83, CD68, HLA-DR or CD86; or by the loss of Fc receptors such as CD 115 (reviewed in Steinman, Annu. Rev. Immunol. 9: 271, 1991). Mature dendritic cells can be collected and analyzed using typical cytofluorography and cell sorting techniques and devices, such as FACScan and FACStar. Primary antibodies used for flow cytometry are those specific to cell surface antigens of mature dendritic cells and are commercially available. Secondary antibodies can be biotinylated Igs followed by FITC- or PE-conjugated streptavidin.

[0224] Alternatively, others have reported that a method for upregulating (activating) dendritic cells and converting monocytes to an activated dendritic cell phenotype. This method involves the addition of calcium ionophore to the culture media convert monocytes into activated dendritic cells. Adding the calcium 21 ionophore A23187, for example, at the beginning of a 24-48 hour culture period resulted in uniform activation and dendritic cell phenotypic conversion of the pooled "monocyte plus DC" fractions: characteristically, the activated population becomes uniformly CD 14 (Leu M3) negative, and upregulates HLA-DR, HLA-DQ, ICAM-1, 137.1, and 137.2. Furthermore, this activated bulk population functions as well on a small numbers basis as a further purified. Specific combination(s) of cytokines have been used successfully to amplify (or partially substitute) for the activation/conversion achieved with calcium ionophore: these cytokines include but are not limited to G-CSF, GM-CSF, IL-2, and IL-4. Each cytokine when given alone is inadequate for optimal upregulation.

[0225] The second approach for isolating APCs is to collect the relatively large numbers of precommitted APCs already circulating in the blood. Previous techniques for isolating committed APCs from human peripheral blood have involved combinations of physical procedures such as metrizamide gradients and adherence/nonadherence steps (Freudenthal et al. PNAS 87: 7698-7702, 1990); Percoll gradient separations (Mehta-Damani, et al., J. Immunol. 153: 996-1003, 1994); and fluorescence activated cell sorting techniques (Thomas et al., J. Immunol. 151: 6840-52, 1993).

[0226] There are many other methods routine in the art for isolating professional antigen presenting cells (or their precursors) and that such methods and others which may be developed are not limiting and are encompassed within the scope of the invention.

[0227] In one embodiment, the APCs and therefore the cells presenting one or more antigens are autologous. In another embodiment, the APCs presenting the antigen are allogeneic, i.e., derived from a different subject.

[0228] As discussed herein, improved LAMP Constructs can be introduced into APCs using the methods described above or others known in the art, including, but not limited to, transfection, electroporation, fusion, microinjection, viral-based delivery, or cell based delivery. Arthur et al., Cancer Gene Therapy 4(l): 17-25, 1997, reports a comparison of gene transfer methods in human dendritic cells.

[0229] Known, partial and putative human leukocyte antigen (HLA), the genetic designation for the human MHC, amino acid and nucleotide sequences, including the consensus sequence, are published (see, e.g., Zemmour and Parham, Immunogenetics 33: 310-320, 1991), and cell lines expressing HLA variants are known and generally available as well, many from the American Type Culture Collection ("ATCC"). Therefore, using PCR, MHC class II-encoding nucleotide sequences are readily operatively linked to an expression vector of this invention that is then used to transform an appropriate cell for expression therein.

[0230] Professional APCs can be used, such as macrophages, B cells, monocytes, dendritic cells, and Langerhans cells. These are collected from the blood or tissue of 1) an autologous donor; 2) a heterologous donor having a different HLA specificity than the host to be treated; or 3) from a xenogeneic donor of a different species using standard procedures (Coligan, et. al., Current Protocols in Immunology, sections 3 and 14, 1994). The cells may be isolated from a normal host or a patient having an infectious disease, cancer, autoimmune disease, or allergy.

[0231] Professional APCs may be obtained from the peripheral blood using leukopheresis and "FICOLL/HYPAQUE" density gradient centrifugation (stepwise centrifugation through Ficoll and discontinuous Percoll density gradients). Procedures are utilized which avoid the exposure of the APCs to antigens which could be internalized by the APCs, leading to activation of T cells not specific for the antigens of interest.

[0232] Cells which are not naturally antigen presenting can be engineered to be antigen presenting by introducing sequences encoding appropriate molecules. For example, nucleic acid sequences encoding MHC class II molecules, accessory molecules, co-stimulatory molecules and antigen processing assisting molecules can be introduced after direct synthesis, cloning, purification of DNA from cells containing such genes, and the like. One expedient means to obtain genes for encoding the molecules used in the improved LAMP Constructs and methods described herein is by polymerase chain reaction (PCR) amplification on selected nucleic acid templates with selected oligonucleotide primer pairs. For example, epithelial cells, endothelial cells, tumor cells, fibroblasts, activated T cells, eosinophils, keratinocytes, astrocytes, microglial cells, thymic cortical epithelial cells, Schwann cells, retinal pigment epithelial cells, myoblasts, vascular smooth muscle cells, chondrocytes, enterocytes, thyrocytes and kidney tubule cells can be used. These may be primary cells recently explanted from a host and not extensively passaged in cell culture to form an established cell line, or established cell lines that are relatively homogeneous and capable of proliferating for many generations or indefinitely.

[0233] Cells that are not professional APCs are isolated from any tissue of an autologous donor; a heterologous donor or a xenogeneic donor, where they reside using a variety of known separation methods (Darling, *Animal Cells: Culture and Media*. J. Wiley, New York, 1994; Freshney, *Culture of Animal Cells*. Alan R. Liss, Inc., New York, 1987). Non-autologous cells, e.g., heterologous or xenogeneic cells, can be engineered *ex vivo* to express HLA class I and class II molecules that match known human HLA specificities. These cells can then be introduced into a human subject matching the HLA specificity of the engineered cells. The cells are further engineered *ex vivo* to express one or more LAMP Constructs according to the invention.

[0234] The engineered cells are maintained in cell culture by standard cell culture methods (Darling, *Animal Cells: Culture and Media*". J. Wiley, New York, 1994; Freshney, *Culture of Animal Cells*". Alan R. Liss, Inc., New York, 1987). Cell lines for use in the present invention are obtained from a variety of sources (e.g., ATCC Catalogue of Cell Lines & Hybridomas,

American Type Culture Collection, 8th edition, 1995), or are produced using standard methods (Freshney, Culture of Immortalized Cells, Wiley-Liss, New York, 1996). Non-transformed cell lines are preferred for use in human subjects.

[0235] In one aspect, CD34+ precursors that are differentiating under the influence of GM-CSF into dendritic cells are obtained from the body of a subject and nucleic acid molecules encoding an improved LAMP Construct are introduced into the cells, which are then injected into the subject. Use of the improved LAMP Constructs as described herein will enhance the association of peptides derived from a particular antigen with MHC class II molecules on the transduced antigen presenting cells, resulting in significantly more potent systemic T cell dependent immune responses and/or antibody production. While the antigen presenting cells transfected in this strategy are preferably autologous cells, any MHC class II cells that effectively present antigen in the host may be used as described above.

Peptide Vaccines

[0236] Also within the scope of this invention are peptide vaccines encoded by the improved LAMP Construct. Preferably, the antigen is processed within the compartment/organelle (or subsequent compartment/organelle to which it is delivered) to generate an epitope bound to an MHC class II molecule capable of modulating an immune response.

[0237] The peptide vaccines encoded by the improved LAMP Constructs may also may be bound in a membranous structure to facilitate its administration to the body of an organism. For example, the peptide vaccine encoded by the improved LAMP Construct may be incorporated into liposomes, as described in U.S. Pat. No. 4,448,765.

[0238] When a protein or polypeptide is to be used as an immunogen, it may be produced by expression of any one or more of the improved LAMP Constructs described herein in a recombinant cell or it may be prepared by chemical synthesis. For example, the Merrifield technique (Journal of American Chemical Society, vol. 85, pp. 2149-2154, 1968), can be used.

Methods of Producing Antibodies using LAMP Constructs

[0239] The nucleic acid molecules as described herein and/or cells into which the nucleic acid molecules have been introduced (such as antigen presenting cells which express the improved LAMP Constructs described herein) can be used to generate antibodies by methods well known by the skilled artisan, such as, for example, methods described in the art. See,

for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., *Proc. Natl. Acad. Sci. USA* 82:910-914 (1985); and Bittle et al., *J. Gen. Virol.* 66:2347-2354 (1985). If *in vivo* immunization is used, animals may be immunized with a protein encoded by the improved LAMP Construct and/or a polynucleotide comprising the improved LAMP Construct comprising an antigen, and optionally along with a boosting epitope and/or antigen, as described herein. Priming with improved LAMP Constructs as polynucleotides, the encoded proteins of the improved LAMP Constructs, and/or cells (such as antigen presenting cells which express the improved LAMP Constructs described herein) followed by boosting with an antigen is a preferred embodiment of the invention. In further preferred embodiments, priming with an improved LAMP Construct as described herein followed by boosting with an antigen is specifically contemplated and can be used to generate an even more robust immune response, especially in view of antibody repertoire diversity and titer.

[0240] The improved LAMP Construct comprising the antigen may be injected into the non-human vertebrate to raise antibodies. Preparation and injection of LAMP Constructs into non-human vertebrates can be accomplished according to principles of immunization of animals that are well known to those skilled in the art.

[0241] The use of an improved LAMP Construct to effectively present the antigen involves, in one aspect, the antigen being processed by LAMP in Antigen Presenting Cells after endocytosis and fusion of the endosome with a lysosome. The endosome then merges with an exocytic vesicle from the Golgi apparatus containing class II MHC molecules, to which the resultant peptides bind. The MHC-peptide complex then trafficks to the plasma membrane where the antigen is available for display to CD4⁺ T cells.

[0242] Animals such as rabbits, rats, mice, llamas, camels, and/or cows can be immunized with the improved LAMP Construct comprising an antigen and/or a polynucleotide encoding the improved LAMP Construct comprising an antigen. Additional animals suitable for immunization include, non-human mammals, such as a rodent (e.g. a guinea pig, a hamster, a rat, a mouse), murine (e.g. a mouse), canine (e.g. a dog), feline (e.g. a cat), equine (e.g. a horse), a primate, simian (e.g. a monkey or ape), a monkey (e.g. marmoset, baboon, rhesus macaque), an ape (e.g. gorilla, chimpanzee, orangutan, gibbon).

[0243] For instance, intraperitoneal and/or intradermal injection of emulsions containing about 100 micrograms of an improved LAMP Construct comprising an antigen or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response may be used. Several booster injections (such as with the recombinant antigen

protein) may be needed, for instance, at intervals of about two weeks, to provide a useful titer of an anti-antigen antibody which can be detected, for example, by ELISA assay using free antigen adsorbed, directly or indirectly (e.g., via a biotinylated AviTag), to a solid surface. The titer of anti-antigen antibodies in serum from an immunized animal may be increased by selection of anti-antibodies, for instance, by adsorption to the antigen on a solid support and elution of the selected antibodies according to methods well known in the art.

[0244] Also, a nucleic acid molecule encoding an improved LAMP Construct comprising an antigen (and optionally with a boosting epitope and/or antigen) can also be directly introduced into animals. See, for example, U.S. patent numbers 5,676,954; 6,875,748; 5,661,133; Sahin et al., Nat Rev Drug Discov, 2014 Oct;13(10):759-80; Kariko et al., Mol Ther, 2008 Nov;16(11):1833-40; Kariko et al., Nucleic Acid Res, 2011, Nov;39(21):e142; US Patent number 6,511,832. In one example, an improved LAMP Construct comprising an antigen is directly injected into a non-human vertebrate. Injection into the animals can occur via intramuscular, intradermal, intranasal, subcutaneous, intravenous, intratracheal, and intrathecal deliveries. Follow-on boosting with a recombinant antigen can also be include in generating the antibodies.

[0245] Additionally, antibodies generated by the disclosed methods can be affinity matured using display technology, such as for example, phage display, yeast display or ribosome display. In one example, single chain antibody molecules ("scFvs") displayed on the surface of phage particles are screened to identify those scFvs that immunospecifically bind to the antigen and/or the starting protein. The present invention encompasses both scFvs and portions thereof that are identified to immunospecifically bind to the antigen and/or the starting protein. Such scFvs can routinely be "converted" to immunoglobulin molecules by inserting, for example, the nucleotide sequences encoding the VH and/or VL domains of the scFv into an expression vector containing the constant domain sequences and engineered to direct the expression of the immunoglobulin molecule.

[0246] Recombinant expression of the raised antibodies (including scFvs and other molecules comprising, or alternatively consisting of, antibody fragments or variants thereof (e.g., a heavy or light chain of an antibody of the invention or a portion thereof or a single chain antibody of the invention)) using the improved LAMP Construct comprising an antigen and/or a polynucleotide encoding the improved LAMP Construct comprising an antigen of the invention, requires construction of an expression vector(s) containing a polynucleotide that encodes the antibody or fragment or variant thereof. Once a polynucleotide encoding an

antibody molecule (e.g., a whole antibody, a heavy or light chain of an antibody, or variant or portion thereof (preferably, but not necessarily, containing the heavy or light chain variable domain)), of the invention has been obtained, the vector(s) for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing an antibody by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination and are described herein. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding the anti-antigen antibody obtained and isolated as described herein (e.g., a whole antibody, a heavy or light chain of an antibody, a heavy or light chain variable domain of an antibody, or a portion thereof, or a heavy or light chain CDR, a single chain Fv, or fragments or variants thereof), operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy chain, the entire light chain, or both the entire heavy and light chains.

[0247] The expression vector(s) can be transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce either the anti-antigen antibody. Thus, the invention includes host cells containing polynucleotide(s) encoding the anti-antigen antibody (e.g., whole antibody, a heavy or light chain thereof, or portion thereof, or a single chain antibody of the invention, or a fragment or variant thereof), operably linked to a heterologous promoter. In preferred embodiments, for the expression of entire antibody molecules, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0248] A variety of host-expression vector systems may be utilized to express anti-antigen antibody. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected, with the appropriate nucleotide coding sequences, express the anti-antigen antibody. These include, but are not limited to, microorganisms such as

bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, are used for the expression of the anti-antigen antibody. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

[0249] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the intended use. For example, when a large quantity of a protein is to be produced (for either antibody production or encoded polypeptides of the improved LAMP Construct), vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther et al., *EMBO* 1. 2:1791 (1983)), in which the coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or Factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0250] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) may be used as a vector to express an anti-antigen antibody or the encoded polypeptides of the

improved LAMP Construct. The virus grows in *Spodoptera frugiperda* cells. Coding sequences may be cloned individually into non-essential regions (for example, the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example, the polyhedrin promoter).

[0251] In mammalian host cells, a number of viral-based expression systems may be utilized express an anti-antigen antibody or the encoded polypeptides of the improved LAMP Construct. In cases where an adenovirus is used as an expression vector, the coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination.

[0252] Insertion in a non-essential region of the viral genome (e.g., region EI or E3) will result in a recombinant virus that is viable and capable of expressing the anti-antigen antibody or the encoded polypeptides of the improved LAMP Construct in infected hosts (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)).

[0253] Specific initiation signals may also be required for efficient translation of inserted coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, e.g., Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

[0254] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed, to this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include, but are not limited to, CHO, VERO, BHK, HeLa, COS, NSO, MDCK, 293, 3T3, W138, and in particular, breast cancer cell lines such as, for example,

BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and HsS78Bst.

[0255] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the express an anti-antigen antibody or the encoded polypeptides of the improved LAMP Construct may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with a polynucleotide controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign polynucleotide, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the anti-antigen antibody or the encoded polypeptides of the improved LAMP Construct.

[0256] A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthineguanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:8 17 (1980)) genes can be employed in tk-, hgprt- or apt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 (Goldspiel et al., Clinical Pharmacy, 12: 488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, AnnPharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62: 191-217 (1993); TIB TECH 11(5):155-2 15 (May; 1993)); and hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example; in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13,

Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981).

[0257] The expression levels of either an anti-antigen antibody or the encoded polypeptides of the improved LAMP Construct can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The Use Of Vectors Based On Gene Amplification For The Expression Of Cloned Genes In Mammalian Cells In DNA Cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing an anti-antigen antibody or the encoded polypeptides of the improved LAMP Construct is amplifiable, an increase in the level of inhibitor present in the host cell culture will increase the number of copies of the marker gene. Since the amplified region is associated with the coding sequence, production of the anti-antigen antibody express or the encoded polypeptides of the improved LAMP Construct will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

[0258] Other elements that can be included in vector sequences include heterologous signal peptides (secretion signals), membrane anchoring sequences, introns, alternative splice sites, translation start and stop signals, inteins, biotinylation sites and other sites promoting post-translational modifications, purification tags, sequences encoding fusions to other proteins or peptides, separate coding regions separated by internal ribosome reentry sites, sequences encoding "marker" proteins that, for example, confer selectability (e.g., antibiotic resistance) or sortability (e.g., fluorescence), modified nucleotides, and other known polynucleotide cis-acting features not limited to these examples.

[0259] The host cell may be co-transfected with two expression vectors of the invention, for example, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain is preferably placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2 197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA or synthetic DNA sequences.

[0260] Once an anti-antigen antibody or the encoded polypeptides of the improved LAMP Construct has been produced by recombinant expression, it may be purified by any method known in the art for purification of a protein, for example, by chromatography (e.g., ion exchange, affinity (particularly by Protein A affinity and immunoaffinity for the specific

antigen), and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, an anti-antigen antibody or the encoded polypeptides of the improved LAMP Construct may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification. [0261] In one example, the anti-antigen antibody or the encoded polypeptides of the improved LAMP Construct may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998), resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life *in vivo*. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the anti-antigen antibody or the encoded polypeptides of the improved LAMP Construct described herein can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix-binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni²⁺ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Administration

[0262] Vaccine material according to this invention may contain the nucleic acid molecules encoding immune stimulatory improved LAMP Constructs described herein (and the boosting epitope and/or antigen) or may be recombinant microorganisms, or antigen presenting cells which express the immune stimulatory improved LAMP Constructs (and the boosting epitope and/or antigen). Preparation and administration of such nucleic acid molecules for immunization of individuals are accomplished according to principles of immunization that are well known to those skilled in the art.

[0263] Large quantities of these materials may be obtained by culturing recombinant or transformed cells containing the nucleic acid molecules. Culturing methods are well-known to those skilled in the art and are taught in one or more of the documents cited above. The vaccines comprising nucleic acid molecules as described herein are generally produced by culture of recombinant or transformed cells and formulated in a pharmacologically acceptable solution or suspension, which is usually a physiologically-compatible aqueous solution, or in coated tablets, tablets, capsules, suppositories or ampules, as described in the art, for example in U.S. Pat. No. 4,446,128, incorporated herein by reference. Administration may be any suitable route, including oral, rectal, intranasal or by injection where injection may be, for example, transdermal, subcutaneous, intramuscular or intravenous.

[0264] The nucleic acid molecules as described herein may be administered to a mammal in an amount sufficient to induce an immune response in the mammal. A minimum preferred amount for administration is the amount required to elicit antibody formation to a concentration at least 4 times that which existed prior to administration. A typical initial dose for administration would be 10-5000 micrograms when administered intravenously, intramuscularly or subcutaneously, or 10^5 to 10^{11} plaque forming units of a recombinant vector, although this amount may be adjusted by a clinician doing the administration as commonly occurs in the administration of vaccines and other agents which induce immune responses. A single administration may usually be sufficient to induce immunity, but multiple administrations may be carried out to assure or boost the response.

[0265] The improved LAMP Construct vaccines may be tested initially in a non-human mammal (e.g., a mouse or primate). For example, assays of the immune responses of inoculated mice can be used to demonstrate greater antibody, T cell proliferation, and cytotoxic T cell responses to the improved LAMP Constructs than to wildtype antigen. Improved LAMP Constructs can be evaluated in Rhesus monkeys to determine whether the vaccine

formulation that is highly effective in mice will also elicit an appropriate monkey immune response. In one aspect, each monkey receives a total of 5 mg nucleic acid molecules per immunization, delivered IM and divided between 2 sites, with immunizations at day 0 and at weeks 4, 8, and 20, with an additional doses optional. Antibody responses, ADCC, CD4+ and CD8+ T-cell cytokine production, CD4+ and CD8+ T-cell antigen-specific cytokine staining can be measured to monitor immune responses to the vaccine.

[0266] Further description of suitable methods of formulation and administration according to this invention may be found in U.S. Pat. No. 4,454,116 (constructs), U.S. Pat. No. 4,681,762 (recombinant bacteria), and U.S. Pat. 4,592,002 and 4,920,209 (recombinant viruses).

Cancer Immunotherapy: Candidates for Prevention and Treatment

[0267] The improved LAMP Constructs described herein comprising at least one cancer antigen described in Table 1 can be used to treat patients who have cancer and/or a hyperproliferative disorder. Examples include patients with documented Epstein-Barr virus associated lymphomas, patients with HPV associated cervical carcinomas, patients with chronic HCV, or patients with a defined re-arrangement or mutation in an oncogene or tumor suppressor gene. Further preferred embodiments include patients having hyperproliferative disorders, including cancer, resulting from CMV infection.

[0268] In preferred embodiments, cancers that can be treated using the improved LAMP Constructs described herein include, but are not limited to all stages of progression, including hyperplasia of an adenocarcinoma, sarcoma, skin cancer (e.g., merkel cell carcinoma), melanoma, bladder cancer, brain cancer (including glioblastoma multiforme), breast cancer, uterine cancer, ovarian cancer, prostate cancer, lung cancer (including, but not limited to NSCLC, SCLC, squamous cell cancer), colorectal cancer, anal cancer, rectal cancer, cervical cancer, liver cancer, head and neck cancer, oral cancer, salivary gland cancer, esophageal cancer, pancreas cancer, pancreatic ductal adenocarcinoma (PDA), renal cancer, stomach cancer, kidney cancer, multiple myeloma or cerebral cancer.

[0269] It is envisioned that therapy with an improved LAMP Construct, such as in the case of a vaccine composition comprising the improved LAMP Construct, could be utilized at any period during the course of the individual's cancer, once it is identified. It is also possible that in high risk patients, vaccination in order to prevent the subsequent emergence of a cancer. Protein based LAMP Construct vaccines as well as cell therapy (e.g, dendritic cell therapy comprising

LAMP Constructs) and self-replicating constructs comprising the cancer antigen(s) of Table 1 are also envisioned.

Procedure for Therapy

[0270] In one embodiment, the improved LAMP Constructs (optionally along with a boosting epitope and/or antigen) could be injected into the patient at any suitable time during the course of their malignancy. For example, the improved LAMP Construct comprising at least one cancer antigen described in Table 1 would be injected at a stage when the tumor burden was low. In an alternative embodiment in which the improved LAMP Construct is introduced into the individual's antigen presenting cells (such as, for example, dendritic cells), precursors to the antigen presenting cells or mature antigen presenting cells are drawn either from the individual's bone marrow or peripheral blood by vena puncture. These cells are established in culture followed by transduction with the improved LAMP Construct. Once transduction had occurred, these antigen presenting cells are injected back into the patient.

[0271] In a particularly preferred embodiment, the invention provides a method of treatment for a cancer patient having low tumor burden, such as early in the disease, after resection of a neoplastic tumor, or when the burden of tumor cells is otherwise reduced. In this method, a cell population containing autologous stem cells capable of differentiation into antigen presenting cells which will express MHC class II molecules is obtained from the patient. These cells are cultured and transformed by introducing an improved LAMP Construct with or without a boosting epitope and/or antigen to deliver the cancer antigen to be associated with an MHC class II molecule either within the compartment/organelle or within another compartment/organelle to which the antigen is delivered, and secrete the boosting antigen into the circulation.

[0272] The transfected stem cell population is then reintroduced into the patient, where the stem cells differentiate into antigen presenting cells which express MHC class II molecules complexed with T_h epitopes from the antigen. The immune response to the antigen will be enhanced by enhanced stimulation of the helper T cell population. The secreted boosting antigen boosts the immune response by, for example, expanding the memory response.

[0273] More generally, in one embodiment, this invention provides a vaccine composition comprising the improved LAMP Construct (optionally along with a boosting epitope and/or antigen) for modulating an immune response in a mammal to an antigen (i.e., stimulating, enhancing, or reducing such a response).

Kits

[0274] The invention further comprises kits to facilitate performing the methods described herein. In one aspect, a kit comprises an improved LAMP Construct and optionally a boosting epitope and/or antigen as described herein and a cell for receiving the improved LAMP Construct. The kit may additionally comprise one or more nucleic acids for engineering the cell into a professional APC. In one aspect, the cell is a professional APC. The cell may or may not express co-stimulatory molecules. In a preferred aspect, when the cell does not express co-stimulatory molecules, the antigen encoded by the improved LAMP Construct is an auto-antigen. In another aspect, a panel of cells is provided expressing different MHC molecules (e.g., known to be expressed in human beings). In a further aspect, the kit comprises reagents to facilitate entry of the improved LAMP Constructs into a cell (e.g., lipid-based formulations, viral packaging materials, cells, and the like). In still a further aspect, one or more T cell lines specific for the antigen encoded by the improved LAMP Construct is provided, to verify the ability of the improved LAMP Construct to elicit, modulate, or enhance an immune response.

EXAMPLES

[0275] The invention will now be further illustrated with reference to the following examples. It will be appreciated that what follows is by way of example only and that modifications to detail may be made while still falling within the scope of the invention.

Example 1 – Construction of LAMP Constructs

[0276] The improved LAMP Constructs illustrated in Figure 1 can be constructed using standard molecular biology techniques well known to the skilled artisan. For example, plasmids comprising the polynucleotides can be designed to generate the different structures ILC-1 to ILC-6 shown in Figure 1. The LAMP domains illustrated in Figure 1 can be derived from the amino acid sequences shown in Figures 3-10. Preferably the LAMP domains are derived from the human LAMP proteins shown in Figures 3-10. The boundaries of each domain can be derived from Figure 2A and Figure 2B. It is envisioned that the corresponding domains can also be cloned from the orthologous sequences by identifying the equivalent domains when compared to the human sequence. The cancer antigens can be cloned into the described LAMP Constructs either individually or in combination as described in Table 1.

Example 2 - Immune Response Evaluation of Mice to LAMP Constructs

[0277] The ability of the improved LAMP Constructs as described in Example 1 can be tested for their ability to modulate an immune response. For example, Female BALB/c mice can be immunized i.d with 50ug of the improved LAMP Constructs and 5ug of GMCSF in 100ul PBS using nanopass on day 0, 14 and 28. Experiment will then be terminated 4 weeks after the last dose.

[0278] Splenocytes (3×10^5 /well) are stimulated with antigenic protein (10ug/ml) in T cell media (RPMI with 10% heat inactivated FBS, 1% penicillin/streptomycin, and 1X 2-ME), supernatants are collected 72h after. Supernatants are diluted (400ul supernatant + 200ul T cell media) and cytokines are evaluated by ELISA. IL-10 or IL-4 production can be measured via ELISPOT assay.

Example 3 – Improved Antigen Presentation using LAMP Constructs

[0279] Cancer is a heterogeneous group of diseases where abnormal cell growth with potential to invade other body parts takes control of normal homeostasis and becomes fatal if not timely and rightly treated. Immunotherapy specifically targets tumor cells thereby avoiding collateral damage to non-tumor cells and inducing anti-tumor response. This anti-tumor response also has the potential to eradicate tumor at distant sites in the body which may not be possible by surgical resection. Induction or enhancement of anti-tumor immune response is a formidable challenge in cancer because tumor cells use multiple evasion strategies and avoid being detected or eliminated by immune cells.

[0280] The aim of this project is to evaluate *in vivo* immune response of all new generation of LAMP Constructs injected by I.D. in BALB/c mice. Specifically, mice are immunized with 50 µg of the tested constructs by intradermal injection. No adjuvants are added to this experiment. Six mice per group are administrated with vaccines every 7 days with total three dose in one month. Immune response is monitored 14 days after the last immunization.

[0281] The tested cancer antigen LAMP constructs can be generated as described herein and the sequence of each tested construct is shown in **Figure 11**. ELISPOT antibody pairs for IFN γ were obtained from Biolegend. Fluorescently coupled CD3, CD4, CD8, CD44, CD62L, IFN γ , TNF α , granzyme B, CD69 monoclonal antibodies and Zombie aqua fixable viability kit were purchased from BioLegend (San Diego, CA). Streptavidin-HRP was

purchased from Thermo Fisher (Waltham, MA). SureBlue TMB microwell peroxidase substrate and TMB stop solution were purchased from KPL (Gaithersburg, MD).

[0282] In preferred embodiments, 50 µg of each cancer antigen LAMP construct is used in a total volume of 100 µl per mouse per dose for Pharmajet. Mice are immunized with the vaccine by *i.d.* delivery on days 0, 7, and 14. Mice are bled on days 28 for serum collection. Serum is collected and stored in -30°C. Spleens are collected on day 28 at the termination of experiment and processed for ELISPOT and FACS to evaluate T cell responses.

[0283] **Measurement of plasma antigen-specific total IgG by ELISA.** The murine antibody response to a cancer antigen can be assessed by indirect ELISA. ELISA plates (MaxiSorp) are coated with 2 µg/ml the cancer antigen in carbonate-bicarbonate buffer overnight and then blocked with 2% BSA in PBS. Plasma samples are diluted 1:100 in blocking buffer. Samples are detected with goat anti-mouse IgG-HRP (Southern Biotech, Birmingham, AL). Reaction is developed with SureBlue TMB Substrate and stopped with TMB Stop Solution from KPL (Gaithersburg, MD). Plates are read (OD450) by using Epoch ELISA reader (BioTek, Winooski, VT).

[0284] **Evaluation of antigen-specific T cell response.** To assess antigen-specific T cell response in the vaccinated mice with a cancer antigen LAMP construct described herein, splenocytes from vaccinated mice are evaluated for antigen-specific IFN γ production by Enzyme-linked immunospot (ELISPOT). For ELISPOT assays, 96-well nitrocellulose plates (Millipore) are coated overnight at 4°C with 100 µl/well of capture monoclonal antibody in PBS. The plates are washed three times with 200 µl/well PBS and blocked with 200 µl/well T cell media for at least 2 hrs at room temperature. Splenocytes are plated at 3×10^5 cells/well and co-cultured with 2 µg/ml pooled peptides of cancer antigen (Table 2) or concavalin A (0.125 µg/ml) or medium alone in a total volume of 200 µl/well T cell media (RPMI-1640 with L-Glutamine and HEPES (ATCC), 1% penicillin, 1% streptomycin, and 5×10^{-5} M β -ME) at 3×10^5 cells/well for 48h at 37°C in 5% CO $_2$. The plates are washed two times with 200 µl/well PBS and two times with 200 µl/well PBS-T (0.05% Tween/PBS). Diluted detection antibodies (50 µl/well in PBS-T/0.5%BSA) are added and plates are incubated for 2 hrs with shaking at room temperature. Plates are washed four times with PBS. Streptavidin-alkaline phosphatase diluted in PBS (50 µl/well) are added and incubated for 2 h. Plates are washed with PBS four times and developed with 50 µl/well of 3-Amino-9-Ethylcarbazole (AEC, BD Bioscience) substrate for 10 min. Color development is stopped by washing under running tap water. After drying 72h at room temperature in dark, colored spots are counted using an AID ELISPOT

High-Resolution Reader System and AID ELISPOT Software version 3.5 (Autoimmun Diagnostika GmbH).

[0285] **Western blots.** 293T cells are transfected with the tested constructs using lipofectamine 2000 reagents (Invitrogen). Transfected cells are washed with PBS and suspended in 200 µl of RIPA lysis buffer with halt proteinase inhibitors (Thermo Scientific, Waltham, MA). Lysates are centrifuged (700 g for 15 minutes at 4°C), followed by measurement of protein concentration in the clarified supernatants using Pierce BCA protein Assay kit (ThermoFisher Scientific, Waltham, MA). 10 µg of protein is electrophoresed in pre-cast (4-20%) SDS-PAGE gels (BioRad, Hercules, California), and transferred onto nitrocellulose membranes (BioRad) and immunoblotted with mAbs to hLAMP. Membranes are blocked with Detection™ block buffer (KPL) and probed with rabbit anti-human LAMP (Sino Biological Inc., Beijing, China) or anti-cancer antigen antibody and goat anti-rabbit-HRP antibody, and then developed with TMB (KPL).

[0286] **Flow cytometry.** Cells are first labelled with Zombie aqua fixable viability dye in PBS (1:500 dilution), followed by surface antibodies (1:100 dilution) in staining buffer (4% FBS, 2% rat serum, 2% mouse serum in PBS). For intracellular staining cells are stained with Zombie aqua, followed by surface staining, fixation with 4% paraformaldehyde, and stained with intracellular antibody in permeabilization buffer (PBS with 1% FCS 0.1% saponin). Samples can be analyzed on a CytoFlex flow cytometer (Beckman Coulter) and analyzed using Kaluza software (Beckman Coulter).

[0287] **Statistics.** Two-Way ANOVA test is performed using GraphPad Prism 6.0 software or R file to evaluate the statistical significance. Each mouse's RPMI result is deducted from the results of the antigen activation.

[0288] **Vaccines, and immunizations.** Control, cancer antigen+Complete LAMP, cancer antigen-ILC-1 Construct, cancer antigen-ILC-2, cancer antigen-ILC-3, and cancer antigen-ILC-4 Construct vaccines are used in a total volume of 25µl per mouse per dose. Mice are immunized with the vaccine by ID delivery on days 0, 7, and 14. Mice are bled on days -13, and 28 for serum collection. Serum is collected and stored in -30°C. Spleens are collected on day 28 and processed for ELISPOT/ELISA assays.

Example 4: Therapeutic Treatment of LAMP Constructs

[0289] Female BALB/c mice can be inoculated s.c with syngeneic 7000 4T1 mammary carcinoma cells on day 0. Vaccine 50ug and 5ug of GMCSF in 100ul PBS is given i.d using

nanopass once the tumors are palpable. Primary tumors are measured with a caliper and tumor volume is calculated using the formula $p/6 \text{ (length} \times \text{width)}^{3/2}$. Average tumor volume as a function of days after tumor inoculation can be measured. A Kaplan-Meier plot can be used to show overall survival at the point of termination.

Example 5 – Prime/Boost Protocol

[0290] A DNA vaccine encoding a cancer antigen construct described herein can be used for therapeutic applications. In this setting, goat anti-mouse IgG-HRP can be purchased from Southern Biotechnologies (Birmingham, AL). SureBlue TMB microwell peroxidase substrate and TMB stop solution can be purchased from KPL (Gaithersburg, MD). ELISPOT plates are ordered from EMD Millipore (Billerica, MA, Cat. No. MAIPS4510). IFN- γ antibody pair used in ELISPOT is purchased from BioLegend (San Diego, CA) and clones AN18 and R46A2 were used as coating and detection, respectively. Streptavidin-HRP and AEC substrate are purchased from BD Biosciences (San Jose, CA).

[0291] Six to eight-week-old female Balb/c mice are purchased from Harlan Laboratories (Frederick, MA) and can be maintained at animal facility in Immunomic Therapeutics, Inc. (Rockville, MA). Mice (n=6) are treated with 10 μ g/dose of the cancer antigen improved LAMP Constructions described herein (with appropriate controls) by electroporation IM delivery at days 0, 7, and 14. On day 35, mice are boosted with 5 μ g cancer antigen protein, for example, in the presence of Alum by i.p. injection. On day 28 and 49, mice are bled and sera is isolated for antibody detection. Mice are sacrificed on day 56 and splenocytes are tested for IFN- γ production by ELISPOT.

[0292] ELISA procedure is followed by Su et al., J of Immunol Res; (10):1-15 (2016). Plates are coated with 5 μ g/ml cancer antigen protein. Data is analyzed by using Microsoft Excel and Prism 6 software.

Example 6 – Validation of LT^{S220A} in C57BL/6

[0293] Merkel cell carcinoma (MCC) is rare type of skin cancer, affecting approximately 2500 people annually in the United States. MCC presents as a fast-growing flesh-colored or bluish-red nodule, typically on the face, head, or neck. Incidence of MCC increases with age and correlates with long-term sun exposure and immunosuppression. MCC is very aggressive and highly metastatic, with limited treatment options, demonstrating a need for new, targeted therapies for this disease. Approximately 80% of MCC cases are associated with infection

with Merkel cell polyomavirus (MCPyV), a circular, double-stranded DNA virus that clonally integrates into Merkel cells within the skin. Infection with MCPyV is thought to be common, with the virus ubiquitously expressed in the human skin microbiome. As MCC is very rare, disease development is clearly multifactorial.

[0294] MCC cells express mutated viral proteins, chief among them a truncated form of the large T (LT) antigen of MCPyV4. CD4 T cells from both healthy persons and MCC patients recognize LT, making it an attractive potential tumor-associated antigen for vaccine development. LT functions as an oncoprotein, primarily through N-terminus-mediated sequestration of retinoblastoma protein (pRB). Mutating serine to alanine at position 220 eliminates LT binding to pRB, abolishing its pro-oncogenic properties. Sharma et al. 38, 1153-1162 (IJC 2016). CD4 T cells recognize both LTS220A and wild-type LT, suggesting that vaccinating with LTS220A could induce immune responses capable of recognizing endogenous LT in tumors.

[0295] We generated a DNA vaccine encoding LT^{S220A} (SEQ ID NO:151) in addition to lysosomal-associated membrane protein 1 (LAMP1) as described herein. Specifically, SEQ ID NO:116 having a mutation at position 220 (serine replaced with an alanine) was constructed and inserted into hinge region of LAMP as described for ILC-4. See, Figure 11A and SEQ ID NO:151.

[0296] We report that LT^{S220A}-LAMP induces potent systemic antigen-specific CD4 T cell responses, as measured by production of both IFN γ and TNF α . This study suggests that LT^{S220A} is functional and can induce systemic antigen-specific CD4 T cell responses to LT-MCPyV.

[0297] LT^{S220A}-LAMP was manufactured by Nature Technology Corporation (Lincoln, NE). Control vector was generated by ITI. The overlapping peptide library spanning LT-MCPyV was synthesized by GenScript. Peptides were resuspended at 50mg/ml in DMSO, and five peptide pools containing 25 peptides each were created at a final concentration of 2mg/ml. Working concentration of all peptides was 2ug/ml. Concanavalin A (Sigma C5275) was used at 0.125ug/ml. "Media" used was "T cell media" (RPMI 1640, 10% FBS, 1% penicillin/streptomycin, 0.1% beta-mercaptoethanol). RBC lysis buffer was purchased from Tonbo. ELISPOT plates (Millipore MSIPS4510) were coated with purified anti-mouse IFN γ (Biolegend 517902) and developed with biotin-conjugated anti-mouse IFN γ (Biolegend 505704), streptavidin-HRP (BD 557630), and AEC substrate kit (BD 551015). For intracellular flow cytometry, cells were incubated with brefeldin A (BD 555029), monensin (BD 554724),

and/or Cell Stimulation Cocktail (eBioscience 00-4970-03) per the Working Instructions. Cells were washed with FACS buffer (PBS, 2% FBS), and stained with fluorochrome-conjugated antibodies against surface targets: CD4 FITC (Biolegend 100406), CD8 PerCPy5.5 (Biolegend 100734), CD44 PE-Cy7 (Biolegend 103030), CD3 A700 (Biolegend 100216), CD62L BV605 (Biolegend 104438). Cells were then fixed (BD 554714) and stained with fluorochrome-conjugated antibodies against intracellular targets: granzyme B PE (eBioscience 12-8898-82), TNF α APC (Biolegend 506308), and IFN γ e450 (eBioscience 48-7311-82).

[0298] **Immunization and cytokine measurement.** 7-8 week old female C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, MA) and maintained at ITI animal facility (Rockville, MD). Four weekly doses of vaccine were given in the ear (ID/EP) weekly starting on day 0. Mice were euthanized two weeks following the final vaccination, and serum and splenocytes were collected and RBC lysed. Splenocytes were frozen in 10% DMSO prior to analysis. Upon thaw, viable splenocytes were used for determination of cytokine production by ELISPOT and intracellular cytokine staining. Data were analyzed using a CytoFLEX flow cytometer (Beckman Coulter), AID plate reader, FlowJo, Microsoft Excel, and Prism 6 software.

[0299] **Results.** As shown in Figure 11C, LT^{S220A}-LAMP induces IFN γ peptide recall responses in whole splenocytes. Mice were vaccinated with LT^{S220A}-LAMP as shown in Figure 11B. Splenocytes were frozen in 10% DMSO at the time of euthanization. After thawing, 3×10^5 viable splenocytes were plated per well in ELISPOT plates coated with purified anti-mouse IFN γ . Appropriate stimuli were added, including five separate peptide pools (2 μ g/ml) containing segments of the overlapping peptide library spanning LT-MCPyV, and concanavalin A as a positive control. After 48 hours of incubation, the ELISPOT plate was washed and developed using biotin-conjugated anti-mouse IFN γ , streptavidin-HRP, and AEC substrate. The plate was allowed to dry and imaged with an AID ELISPOT plate reader.

[0300] Peptide pools 2 and 5 produced the strongest recall responses in splenocytes from mice vaccinated with LT^{S220A}-LAMP. In a corroborating experiment, IL-10 production was not detectable in any group under any condition (data not shown). Of note, peptide pool 5 contains additional, non-overlapping peptides, including those previously identified by other groups as being immunodominant epitopes for both CD8 and CD4 T cells. The immunodominant CD8 epitope is also found in pool 1, while the immunodominant CD4 epitopes are also found in pool 2. These ELISPOT data suggest that CD4 T cells are likely to

be the dominant producers of IFN γ in this model, in agreement with previous studies. Additionally, as the recall responses were stronger to peptide pool 2 as compared with pool 5, pool 2 may contain an additional peptide and/or a further optimized peptide that serves as the immunodominant epitope in the context of LT^{S220A}-LAMP. Further studies should characterize the specific peptides within peptide pool 2 that induce IFN γ production.

[0301] Figure 11D: LT^{S220A}-LAMP induces IFN γ and TNF α production in peptide recall responses in splenic CD4 T cells, but not splenic CD8 T cells. Mice were vaccinated with LT^{S220A}-LAMP as shown in Figure 11B. Splenocytes were frozen in 10% DMSO at the time of euthanization. After thawing, approximately 2E6 splenocytes were plated per well in the presence of brefeldin A, monensin, and five separate peptide pools (2ug/ml) containing segments of the overlapping peptide library spanning LT-MCPyV. Two wells were incubated with PMA/ionomycin for use as positive and isotype controls. Each splenocyte sample was also plated as a no-peptide control. After five hours of incubation, cells were washed, stained with fluorochrome-conjugated antibodies, and analyzed on a CytoFLEX flow cytometer.

[0302] The intracellular cytokine staining data is in agreement with the ELISPOT data, with peptide pools 2 and 5 producing the strongest recall responses in splenocytes from mice vaccinated with LT^{S220A}-LAMP. Additionally, as predicted, CD4 T cells were the dominant producers of both IFN γ and TNF α in this model, while effects on CD8 T cells were negligible.

[0303] Figure 11E shows that peptide pool 2 (aa141-160 of SEQ ID NO:114), peptide 13 (aa145-157 of SEQ ID NO:114), and to a lesser extent, peptides 6 (aa117-129 of SEQ ID NO:114), 12 (aa141-153 of SEQ ID NO:114), and 17 (aa161-173 of SEQ ID NO:114) induce the observed IFN γ peptide recall responses in whole splenocytes. Splenocytes as described above were frozen on the day of termination, thawed 15 days later, and individual mice were pooled within groups. 3x10⁵ live splenocytes were plated per well in ELISPOT plates coated with purified anti-mouse IFN γ . Each of the 25 peptides from peptide pool 2 were tested at a final concentration of 2ug/ml. Known CD4 and CD8 epitopes were also tested for reference, with media alone and concanavalin A as a positive control. After 48 hours of incubation, the ELISPOT plates were washed and developed using biotin-conjugated anti-mouse IFN γ , streptavidin-HRP, and AEC substrate. The plate was allowed to dry and imaged with an AID ELISPOT plate reader.

[0304] Peptide 13 from pool 2 induced the strongest IFN γ peptide recall responses, even stronger than those observed using known, previously identified CD4 immunodominant epitopes. Peptide 13 shows overlap with both known epitopes, but is unique. This peptide

may be optimal when the antigen is processed in the context of LAMP. Additional peptides showed IFN γ peptide recall responses, including peptides 6 and 17 which do not overlap with the known epitopes.

Example 8: Preventative B16-LT^{S220A} study in C57BL/6

[0305] This study tested LT^{S220A}-LAMP's ability at preventing tumor growth in a mouse model of B16. C57BL/6 mice were vaccinated three times, weekly, by intradermal administration without electroporation. One week following the final vaccination, peripheral blood was analyzed for IFN γ peptide recall responses to assess immune responses to vaccination with LT^{S220A}-LAMP's. Two weeks following the last vaccination, mice were given 2E5 B16-LT^{S220A} tumor cells subcutaneously in the right flank. Tumor growth and overall survival were measured over time. Unexpectedly, prophylactic vaccination with LT^{S220A}-LAMP's 3000 significantly delayed the onset of tumor growth by approximately 30 days. Once tumor growth was observed in vaccinated mice, additional doses of LT^{S220A}-LAMP's were given weekly for a total of five doses. Approximately 50% of vaccinated mice showed further delayed tumor kinetics after the onset of tumor growth. 1 of 10 (10%) of the vaccinated mice remained tumor free at 70 days after initial injection of tumor cells. This study demonstrates that LT^{S220A}-LAMP's is capable of significantly delaying and/or preventing growth of B16-LT^{S220A} tumors when given in a prophylactic setting.

[0306] Specifically, Figure 12A shows LT^{S220A}-LAMP delays tumor onset in vaccinated mice given B16-LT^{S220A} tumors. Mice were vaccinated with LT^{S220A}-LAMP. 2E5 B16-LT^{S220A} tumor cells were injected subcutaneously into the right flank and tumor growth and kinetics were measured over time. Mice were euthanized when tumors reached a size of 20mm in any direction or 2000mm³, when tumors had ulcerations greater than 2mm, or when mice showed distress or signs of peritoneal tumors, indicating that the tumor had grown through the peritoneal wall.

[0307] As expected, mice vaccinated with control vector experienced rapid, uncontrolled tumor growth, with 100% of animals euthanized by 20 days after tumor injection. 100% of vaccinated mice showed significant delay in B16-LT^{S220A} tumor growth, with 10% of animals remaining tumor free at 70 days after tumor injection. Of the vaccinated mice that went on to develop tumors, 50% of these exhibited partial response to repeated vaccination, with reduced tumor growth for a period of approximately three weeks.

[0308] Figure 12B reports that blood ELISPOT responses may predict eventual tumor burden in mice vaccinated with LT^{S220A}-LAMP. Data from the blood ELISPOT as described above was correlated with tumor kinetics as shown in Figure 11A. Blood ELISPOT data was normalized to concanavalin A signal and compared with survival after tumor initiation. Mice that responded to further vaccination with LT^{S220A}-LAMP (Figure 12A) were designated as “responders.” Although these data are not statistically significant, they are trending towards significance ($p = 0.059$).

Example 9: Therapeutic study of LTS220A DNA vaccine against B16-LTS220A in C57BL/6

[0309] This study tested the therapeutic efficacy of LT^{S220A}-LAMP at preventing or delaying tumor growth in the mouse model of B16-LTS220A described above. C57BL/6 mice were vaccinated four times, weekly, by intradermal administration with electroporation starting three days after injection of 5×10^4 B16-LTS220A tumor cells subcutaneously in the right flank. Tumor growth and overall survival were measured over time. On day 17 after tumor initiation, the cellular composition of the tumor microenvironment (TME) was examined in eight vaccinated and eight unvaccinated mice by flow cytometry. As shown in Figure 13, vaccination with LT^{S220A}-LAMP unexpectedly and significantly enhanced survival in the therapeutic setting ($p = 0.003$), with a median survival seven days greater than that seen in unvaccinated mice (31 vs 24 days). In agreement with a previous study, LT^{S220A}-LAMP induced remarkable cellular changes within the TME. LT^{S220A}-LAMP-vaccinated mice showed a large influx of immune cells within tumors, including CD4 and CD8 T cells and NK cells.

[0310] Specifically, **Figure 13A** shows that LT^{S220A}-LAMP significantly extends survival in the B16-LTS220A model. Overall survival was significantly enhanced in vaccinated mice, with a median survival of 31 days in LT^{S220A}-LAMP-vaccinated mice and 24 days in control-vaccinated mice. 50% (5/10) of the vaccinated animals showed the strongest responses to the vaccine.

[0311] **Figure 13B** shows that LT^{S220A}-LAMP induces tumor infiltration of CD45+ immune cells, including CD4 and CD8 T cells. On day 17 after tumor initiation, eight mice in each treatment group were injected intraperitoneally with brefeldin A. Four hours after injection, mice were euthanized and spleens and tumors were collected, processed, and analyzed by flow cytometry. Therapeutic vaccination with LT^{S220A}-LAMP resulted in a significant increase in CD45+ immune cells within the TME, including increased numbers of total T cells as well

as increased frequency of T cells of the total CD45+ cell population. Both CD4 and CD8 T cells were increased per gram tumor in vaccinated mice. Of these CD4 T cells, a higher frequency were PD-1+ in vaccinated mice.

[0312] **Figure 13C** demonstrates that LT^{S220A}-LAMP induces tumor infiltration of both NK and NKT cells. Therapeutic vaccination with LT^{S220A}-LAMP resulted in a significant increase in NK cells within the TME, both in total numbers per gram tumor and in the frequency of total CD45+ CD3- cells. NK cells in tumors from vaccinated mice also showed higher expression of CD11b, suggestive of a more mature phenotype. Vaccinated mice also had increased total NKT cells within the TME.

[0313] **Figure 13D** shows that LT^{S220A}-LAMP alters myeloid cells within the tumor. Therapeutic vaccination with LT^{S220A}-LAMP resulted in a decreased frequency of bulk CD11b+ myeloid cells within the TME. Additionally, vaccinated mice showed a trend toward enhanced iNOS production in tumor-associated macrophages.

[0314] **Figure 13E** shows that LT^{S220A}-LAMP enhances the number of regulatory T cells within the TME, but increased numbers of Tregs does not correlate with tumor burden. Therapeutic vaccination with LT^{S220A}-LAMP resulted in increased numbers of regulatory T cells (Tregs) within the TME of vaccinated animals. However, the frequency of Tregs was unchanged, and neither the absolute number of Tregs nor the frequency of Tregs of total CD4 T cells correlated with tumor burden.

[0315] **Figure 13F** suggests potential value in combination therapy with LT^{S220A}-LAMP. As demonstrated above, therapeutic vaccination with LT^{S220A}-LAMP resulted in an increased relative frequency of T cells infiltrating the TME, with a concomitant decrease in the frequency of myeloid cells infiltrating the TME. Increased PD-1 on CD8 T cells correlated with enhanced tumor burden, regardless of vaccination status, suggesting that combining LT^{S220A}-LAMP with PD-1 blockade may be an effective treatment strategy. PD-1 blockade is an FDA-approved treatment for Merkel cell carcinoma. The frequency of myeloid cells in the TME also correlated with enhanced tumor burden, regardless of vaccination status, suggesting that targeting the recruitment and/or polarization of myeloid cells within the TME may enhance the effectiveness of LT^{S220A}-LAMP. Thus, these results support the combination therapy of an improved LAMP Construct described herein, and preferably LT^{S220A}-LAMP, with a checkpoint inhibitor. Examples of checkpoint inhibitors include, but are not limited to a PD-1/PD-L1 inhibitor or a CTLA inhibitor, such as Pembrolizumab (Keytruda™), Nivolumab (Opdivo™),

Cemiplimab (Libtayo™), Atezolizumab (Tecentriq™), Avelumab (Bavencio™), Durvalumab (Imfinzi), or Ipilimumab (Yervoy™).

Example 10: Production of an Antibody from a Polypeptide

[0316] Anti-antigen antibodies can be prepared by a variety of standard methods of raising antibodies using animal injection. (See, Current Protocols, Chapter 2.) For example, cells expressing an improved LAMP Construct comprising a cancer antigen described herein is administered to a non-human vertebrate to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of the LAMP/antigen protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into the non-human vertebrate to produce polyclonal antisera of greater specific activity.

[0317] In the most preferred method, the anti-antigen antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing a non-human vertebrate animal (preferably a rabbit, mouse, cow, camel, llama) with an improved LAMP Construct comprising an antigen, the encoded polypeptide of an improved LAMP Construct comprising an antigen or, more preferably, with an improved LAMP Construct-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

[0318] The splenocytes of such non-human vertebrate host (e.g, mice) are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP20), available from the ATCC™. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the antigen.

[0319] It will be appreciated that Fab and F(ab')₂ and other fragments of the anti-antigen antibodies may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

[0320] For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Pat. No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

Example 11: Use of Polynucleotides to Generate Polyclonal and Monoclonal Antibodies

[0321] Methods of directly injecting polynucleotides into animals are well described in the art. See, for example, U.S. patent numbers 5,676,954; 6,875,748; 5,661,133. For example, a polynucleotide encoding an improved LAMP Construct comprising an antigen (and optionally with a boosting epitope and/or antigen) can be injected into the quadriceps muscles of restrained awake mice (female 6-12 week old BALB/c or Nude, nu/nu, from Harlan Sprague Dawley, Indianapolis, Ind.). In one embodiment, 50 µg of a polynucleotide in 50 µl solution using a disposable sterile, plastic insulin syringe and 28G ½ needle (Becton-Dickinson, Franklin Lakes, N.J., Cat. No. 329430) fitted with a plastic collar cut from a micropipette tip can be used to inject the mice, as described in Hartikka, J., et al., Hum. Gene Ther. 7:1205-1217 (1996)).

[0322] Alternatively, 6-week old Sprague Dawley female mice (body weight 20-25 grams) can be given 5000 ppm ZnOSO₄ in their drinking water beginning 24 hours prior to injection. This amount of zinc has been shown to be able to activate the metallothionein promoter. Each mouse is then injected intravenously through a tail vein puncture with a 25 gauge needle with 30 µg of a polynucleotide encoding an improved LAMP Construct comprising an antigen (and optionally with a boosting epitope and/or antigen) complexed with 150 µg liposome

(Lipofection™) in a total volume of 30 µl. Animal care should be maintained throughout the study and should be performed in compliance with the “Guide for the Use and Care of Laboratory Animals”, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press.

[0323] After the injected polynucleotide encoding the improved LAMP Construct comprising an antigen is delivered into the cells in the animal, the antigen is delivered to the endosome/lysosome, processed and presented to the immune system as the priming antigen, while the boosting epitope and/or antigen is secreted from the cell. The improved LAMP Construct comprising an antigen can then stimulate the production of antibodies specific to the antigen, and the boosting antigen may enhance this production of antibodies specific to the antigen. These antibodies can be isolated and used as a polyclonal mixture or further isolated into single species or monoclonals. The process of the immune response and production of antibodies against foreign antigens *in vivo* are well known in the art.

[0324] In a third animal model, Balb/c 3T3 A31 cells are transfected by electroporation with a polynucleotide encoding an improved LAMP Construct comprising an antigen. G418 resistant clones expressing LAMP Construct comprising an antigen are identified by their ability to bind human RBC. To generate polyclonal antibodies, Balb/c mice are immunized twice intraperitoneally, at an interval of 14 days, with 10^7 cells comprising the improved LAMP Construct comprising an antigen. After a final boost, the immune serum is collected, IgG is purified by protein G Sepharose and passed over an antigen column prepared by coupling 1.0 mg purified antigen to cyanogen bromide activated Sepharose CL-4B. Bound IgG can be eluted with 0.1 M glycine buffer pH 2.5 and neutralized with 0.1 volumes of 0.1 M Tris pH 8.0. To generate a monoclonal antibody (mAb), Balb/c mice are immunized with LAMP Construct comprising an antigen and hybridomas are generated by fusing immune spleen cells with the SP2 myeloma following standard methods (28). A positive well reacting specifically with an antigen can be identified by enzyme-linked immunosorbent assays as described in the art. The hybridoma is cloned three times by limiting dilution to produce an antibody.

Example 12: Immunization of an Improved LAMP Construct Comprising a Cancer Antigen

[0325] Methods of raising antibodies in mammals are well known in the art. In one example, polyclonal antiserum against a LAMP Construct comprising an antigen is raised by immunization of pathogen free rabbits with a total of 500 µg of an improved LAMP Construct

comprising an antigen over a period of two months. For example, the improved LAMP Construct comprising an antigen can be dissolved in PBS and emulsified with an equal volume of Freund's adjuvant. After the final booster, the serum of the rabbits can be separated to determine the titer of the polyclonal antiserum.

[0326] In an additional animal model, groups of 5 mice (C57BL/6J; Jackson Labs) can be subcutaneously immunized with 5 µg of endotoxin-free LAMP Construct comprising an antigen emulsified in alum. Three weeks later, mice are bled and the presence of anti-antigen specific antibodies can be determined by titering the sera by ELISA (direct binding of antibodies in sera to wild type BPTI or APP-KI coated, directly or indirectly (via a biotinylated tag and streptavidin), on the wells).

[0327] To obtain monoclonal antibodies, 4-6 week old Balb/c mice can be immunized with an improved LAMP Construct comprising an antigen (for example 4 times with 2 week intervals with 10-100 µg/injection dissolved in Freund's complete adjuvant for the first injection, and Freund's incomplete adjuvant for subsequent immunizations). Splenocytes are isolated and fused with a fusion cell line such as Sp2/0 myeloma cells, followed by limiting dilution. Growing clones are screened using for example an enzyme-linked immunosorbent assay (ELISA). 96 cells plates are coated with an improved LAMP Construct comprising an antigen or with a control protein. The culture supernatant is added, followed by washing and addition of a labeled anti-mouse antibody for detection. After limited dilution cloning of the anti-antigen antibody producing stable hybridomas are obtained. From each cell, supernatant is collected and by affinity chromatography using protein A sepharose columns monoclonal antibodies can be purified.

[0328] Variations, modifications, and other implementations of what is described herein will occur to those of ordinary skill in the art without departing from the spirit and scope of the invention and the claims. All of the patents, patent applications, international applications, and references identified are expressly incorporated herein by reference in their entireties.

CLAIMS

What is Claimed

1. An improved LAMP Construct comprising:
 - a. a Cysteine Conserved Fragment of a LAMP Protein; and
 - b. at least one cancer antigen, wherein said cancer antigen is selected from Large T antigen, Small T antigen, MAGE-A10, MAGE-A12, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A4, MAGE-A4, MAGE-A6, MAGE-A6, MAGE-A6, MAGE-A9, MAGE-B10, MAGE-B16, MAGE-B17, MAGE-B1, MAGE-B2, MAGE-B3, MAGE-B4, MAGE-B5, MAGE-B6, MAGE-C1, MAGE-C2, MAGE-C3, MAGE-D1, MAGE-D2, MAGE-D4, MAGE-E1, MAGE-E1_(MAGE1), MAGE-E2, MAGE-F1, MAGE-H1, or MAGEL2.
3. The improved LAMP Construct of claim 1, wherein:
 - a. the antigen is placed at the N-terminus of the Cysteine Conserved Fragment;
 - b. the antigen is place at the C-terminus of a single Cysteine Conserved Fragment; or
 - c. the antigen is placed in between two Cysteine Conserved Fragments.
3. The improved LAMP Construct of either claim 1 or claim 2, wherein the improved LAMP Construct comprises at least one fragment/epitope described in Column 1 or Column 4 of Table 1, and preferably is constructed as depicted in ILC-1, ILC-2, ILC-3, ILC-4, ILC-5 or ILC-6.
4. The improved LAMP Construct of claim 3, wherein each antigen is separated by a linker, preferably wherein said linker is the amino acid sequence GPGPG (SEQ ID NO: 152) or PMGLP (SEQ ID NO:153).
5. The improved LAMP Construct of of any one of the preceding claims, wherein the improved LAMP Construct comprises more than one Cysteine Conserved Fragment.
6. The improved LAMP Construct of any one of the preceding claims, wherein the Cysteine Conserved Fragment comprises a Homology Domain of a LAMP Protein.

7. The improved LAMP Construct of any one of the preceding claims, wherein the improved LAMP Construct further comprises a Transmembrane Domain of a LAMP Protein.
8. The improved LAMP Construct of any one of the preceding claims, wherein the improved LAMP Construct further comprises a signal sequence, preferably wherein the signal sequence is derived from a LAMP Protein.
9. The improved LAMP Construct of any one of the preceding claims, wherein the LAMP protein is selected from LAMP-1, LAMP2, LAMP-3, LIMP 2, Macrosailin, Endolyn, LAMP5 or LIMBIC.
10. The improved LAMP Construct of any one of the preceding claims, wherein the improved LAMP Construct comprises:
 - A) the LAMP Protein is selected from any one of SEQ ID NO:1-113 and the cancer antigen is selected from any one of SEQ ID NO:114-116;
 - B) the amino acid sequence of LT^{S220A}-LAMP (SEQ ID NO:151); or
 - C) either (A) or (B) in combination with any one of SEQ ID NO:117-150.
11. The improved LAMP Construct of claim 12, wherein the LAMP Protein is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO:1-113 and/or the cancer antigen is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO:114-151.
12. The improved LAMP Construct of any one of the preceding claims, wherein said improved LAMP Construct comprises the amino acid sequence of SEQ ID NO:116, and preferably wherein said LAMP protein is Human LAMP1, and preferably, wherein said LAMP Construct is ILC-4.
13. A polynucleotide encoding the improved LAMP Construct of any one of claims 1-12.

14. The polynucleotide of claim 13, wherein the polynucleotide is a DNA vaccine.
15. The polynucleotide of claim 14, wherein the polynucleotide is a self-replicating RNA vaccine.
16. A host cell comprising the polynucleotide of any one of claims 13-15.
17. A composition comprising the improved LAMP Construct of any one of claims 1-12, the polynucleotide of any one of claims 13-15, or the host cell of claim 16.
18. A method of treating a subject having a disease or a disorder, wherein the method comprises administering to a subject in need thereof the improved LAMP Construct of any one of claims 1-12, the polynucleotide of any one of claims 13-15, the host cell of claim 16, or the composition of claim 17 in an amount sufficient to reduce or treat the disease or disorder.
19. The method of claim 18, wherein the method comprises a priming step and at least one boosting step.
20. The method of claim 19, wherein the improved LAMP Construct of any one of claims 1-12, the polynucleotide of any one of claims 13-15, the host cell of claim 16, or the composition of claim 17 is used in the priming step.
21. The method of either claim 19 or 20, wherein the boosting step comprises administration of an antigen, an improved LAMP Construct, a polypeptide encoded by an improved LAMP Construct, or a cell comprising the improved LAMP Construct.
22. The method of any one of claims 18-21, wherein:
 - a) the antigen used to prime is the same that is used to boost;
 - b) the antigen used to prime is derived from the same protein as a second antigen used to boost;
 - c) more than one antigen is used to prime and/or boost.

23. The method of any one of claims 18-22, wherein the subject has a hyperproliferative disorder.
24. The method of claim 23, wherein the hyperproliferative disorder is selected from an adenocarcinoma, sarcoma, skin cancer (e.g., merkel cell carcinoma), melanoma, bladder cancer, brain cancer (including glioblastoma multiforme), breast cancer, uterine cancer, ovarian cancer, prostate cancer, lung cancer (including, but not limited to NSCLC, SCLC, squamous cell cancer), colorectal cancer, anal cancer, rectal cancer, cervical cancer, liver cancer, head and neck cancer, oral cancer, salivary gland cancer, esophageal cancer, pancreas cancer, pancreatic ductal adenocarcinoma (PDA), renal cancer, stomach cancer, kidney cancer, multiple myeloma, or cerebral cancer.
25. The method of either claim 23 or 24, wherein the hyperproliferative disorder is caused by cytomegalovirus (CMV).
26. The method of any one of claims 18-25, wherein said subject is also administered a checkpoint inhibitor, preferably, wherein said checkpoint inhibitor is a PD-1/PD-L1 inhibitor or a CTLA inhibitor.
27. The method of claim 26, wherein said checkpoint inhibitor is selected from Pembrolizumab (Keytruda™), Nivolumab (Opdivo™), Cemiplimab (Libtayo™), Atezolizumab (Tecentriq™), Avelumab (Bavencio™), Durvalumab (Imfinzi™), or Ipilimumab (Yervoy™).

Figure 1

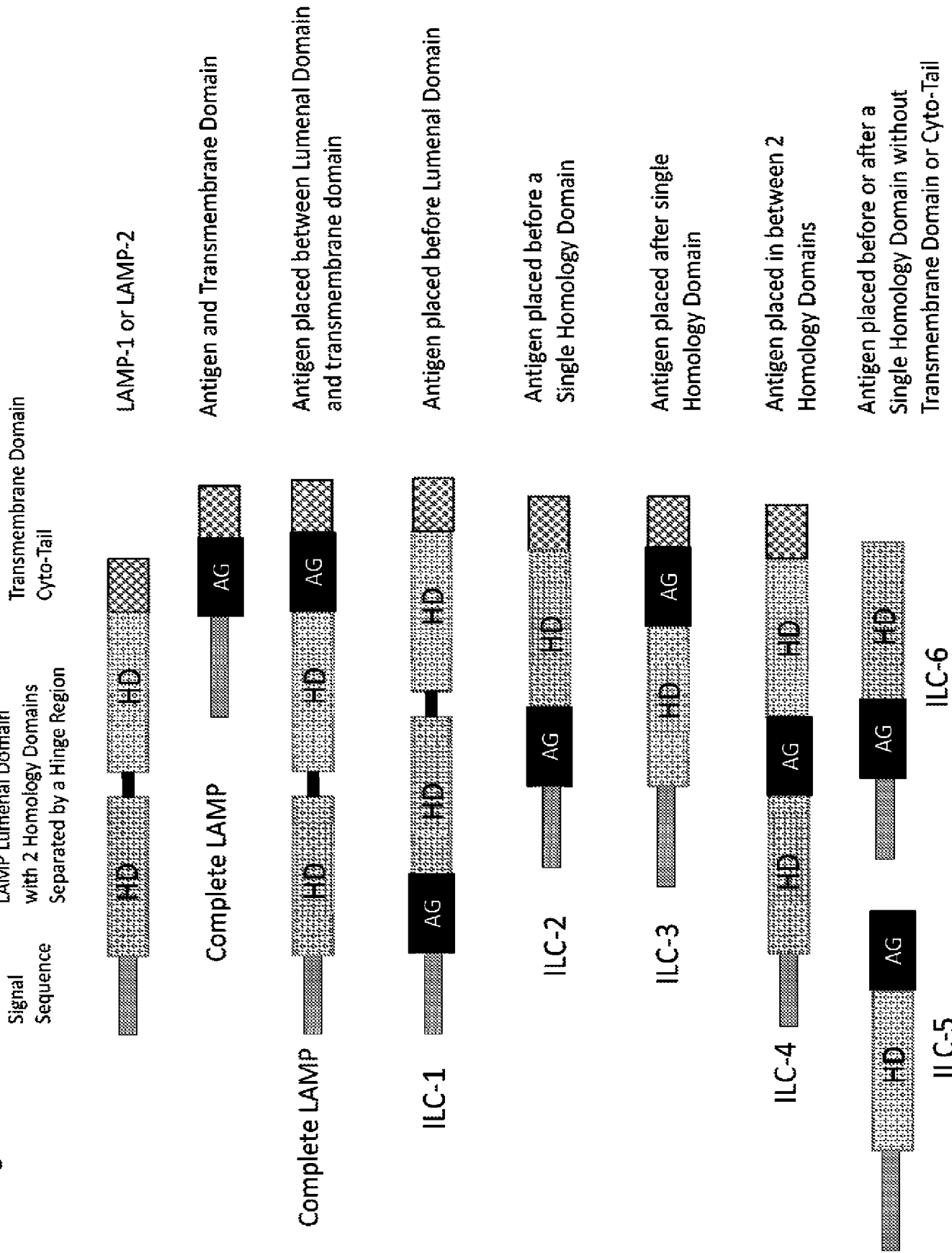


Figure 2A

Gene Name Accession No.	Alternative Names	SEQ ID NO.	Orthologs	Signal Seq.	Luminal Domain			Transmembrane Domain	Cytoplasmic Tail
					First Homologous domain	Hinge Region	Second Homologous Domain		
h. LAMP-1 NP_005552.3	CD107a; LAMPA; LGP120	1	SEQ ID NO: 6-24	1-28	29-194	195-227	228 to 381 or 382	382 or 383 to 405	406-417
h. LAMP-2 NP_002285.1	CD107b; LAMPB; LGP110	2	SEQ ID NO:25-43	1-28	29-192	193-228	229-375	376-399	400-410
h. LAMP-3 NP_055213.2	CD208; DC LAMP; DC- LAMP; DCLAMP; TSC403	3	SEQ ID NO:44-55	1-27	28-219	220-234	235-381	382-402	403-416
LIMP-2 Q14108	AMRF; EPM4; LGP85; CD36L2; HLGP85; LIMPII; SR-BII; SCARB2	4	SEQ ID NO:56-66	*5-27 Transm em. *Unclea vable	28-433			434-459	460-478
h. Endolyn NP_006007.2	Sialomucin CD164 MUC-24	5	SEQ ID NO:73-79	1-23	24-162			163-183	184-197
Macrosialin NP_001242.2	CD68	80	SEQ ID NO: 81-92	1-21	22-319			320-344	345-354

Figure 2A cont.

LAMP5 NP_036393	BD-LAMP	93	SEQ ID NO: 94-101	1-29	30-235	236-256	257-280
h. LIMBIC NP_002329.2	LSAMP IGLON3	67	SEQ ID NO: 68-72 and 102-113	1-28	29-315	316-338	No tail

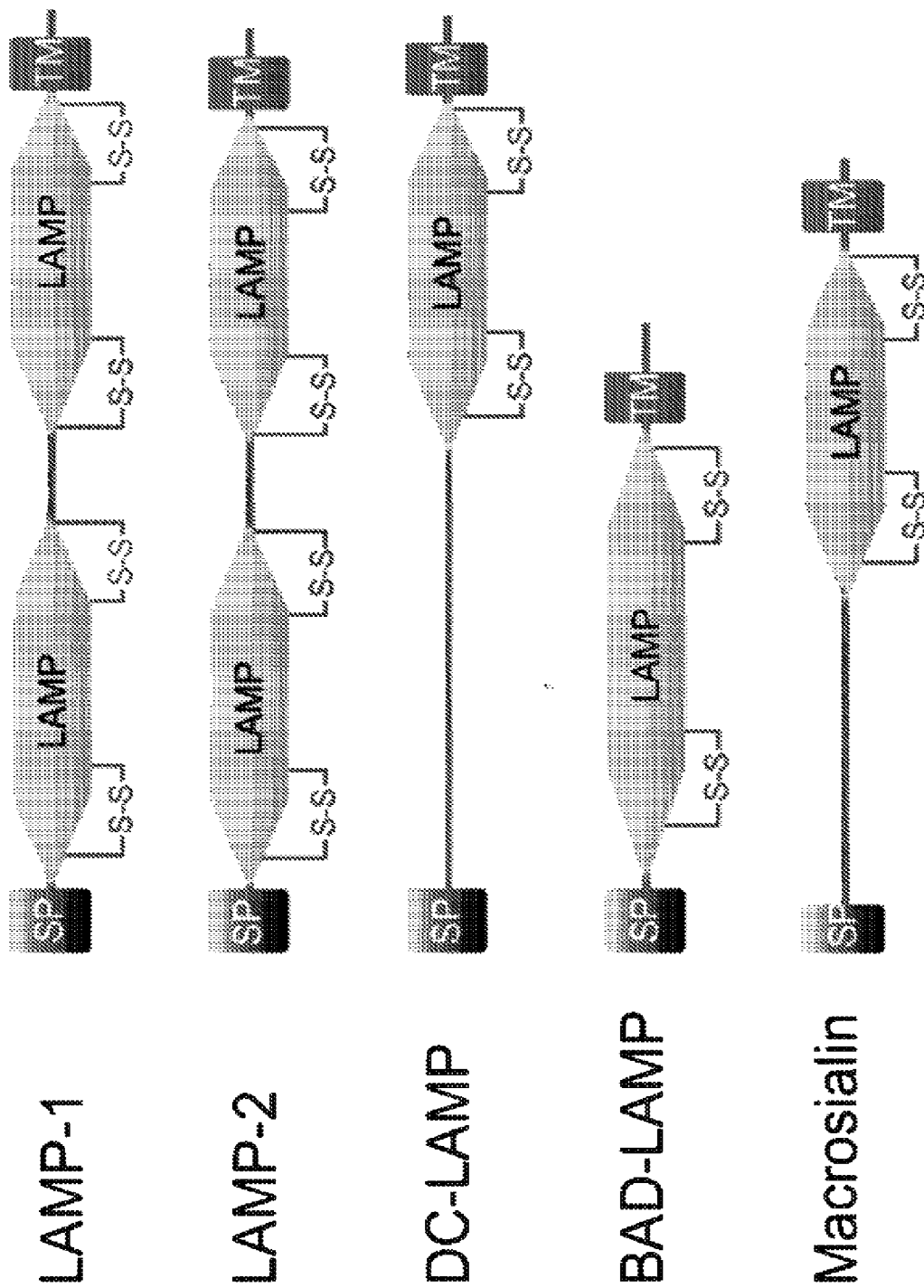


FIGURE 2B

FIGURE 3: HUMAN LAMP-1 ALIGNMENT WITH ORTHOLOGOUS SEQUENCES

SIGNAL SEQUENCE		LAMP HOMOLGY DOMAIN 1	
		1	
SEQIDNO:1	MAAPGSARRPFLLLLLLLLLLGLMH-CASA	MAAPGVKNGN-GTACIMANFSAAFSVNYDTKS	58
SEQIDNO:6	MAAPGAR-RPEL---LLLLAGLAH--GASALFEVK--NN--GTTTCIMASFSASFLTYYETAN		52
SEQIDNO:7	-----MARAAG-VCWTLLMGCVFA-AHAVTTFEVTDCN--STCIKGEINASFISISYNTTN		50
SEQIDNO:8	-MSWRQVKMPVYWMAMVLLIGVVQ-VATAVQFEVKDGKTNITCILADLSINFVSYNVSS		58
SEQIDNO:9	MAAPGSARRPFLLLLLLLLLLGLVH-CASAAMFMVKNGN-GTACIMANFSAAFSVNYDTKS		58
SEQIDNO:10	MAAPGSARRSLLL-LLLLLLGLPH-CASAAMFVKNGN-GTACIMANFSAAFSVNYDTKS		57
SEQIDNO:11	MAAPGGARFRPL--LLLLLAGLVH--GAAAVFVVKDAN-GTACIMANFSAAFSLASYETRS		55
SEQIDNO:12	MEAPGGARRPFLLL---LLLGLVH--GASAVFVVRNSN-GTACIMANFSAFVSIVYESKS		54
SEQIDNO:13	MAAPGGARRRPLLL--LLFAGLVH--GASAVFVVKNGN-GTACIMADFSATFLTYSYDTRS		55
SEQIDNO:14	MAAPGAR-RPEL---LLLLAGLAH--SAPALFEVKDNN-GTACIMASFSASFLTYYDAGH		53
SEQIDNO:15	-----MGGAA--RAVLLGFL---QASSFVDVRDST-GKVCIIANLTVAFSVEYKSSG		46
SEQIDNO:16	MAAPGGAWRRPLLLL-LLLLGLAR--GASAVFVVDGN-GTACIMADFAAAFEISYDSRS		56
SEQIDNO:17	MAEPGGARTPQRLL--LLLLGLIH--VASSIFVVKNGT-GTACIMANFSATFSMNYTTKS		55
SEQIDNO:18	-----MARALL--AAVLLGFL---QASSFVDVRDST-GKVCIIANLTVAFSVEYKSSG		47
SEQIDNO:19	-----MARGLLA--AAALLGFL---QASSFVKDSS-GKVCILADLTVAFSVEYKTNV		48
SEQIDNO:20	MVSSSSCRGELL--AAVLLGFL---QASSFVEVRDST-GKICILANFSAEFTVDYSTKA		54
SEQIDNO:21	MKSFPSPFVAFI-VCSAVLADT---QAVVTLEVKEGN--STCIKAEPFSAFVSITYNTTN		53
SEQIDNO:22	MKRSHALVVL-I-IAWFSLSGC---IQAVSLEVKEGN--STCIKANLSAYFSITYNTSS		52
SEQIDNO:23	MTRTCPFVVG-I-AC-FAILGCVTVVQSQVTLVTEGN--STCIKAELSASFISITYDTAN		55
SEQIDNO:24	-----		0
		LAMP HOMOLGY DOMAIN 1	
		2	
SEQIDNO:1	GPKNMTFDLPDAIVVLNRSSCGKENTSDPSLVIAFGRGH--TLTLNFTNRNATRYSVQLM		116
SEQIDNO:6	GSQIVNISLPASAEVLKNGSSCGKENVSDPSLTITFGRGY--LLTLNFTKNTRYSVQHM		110
SEQIDNO:7	GTSVSVFALPASASVSE-RSSCGS-AAVPPPELALVFGDTHHTLSLLFSRDQRLYRVSN		108
SEQIDNO:8	KMELATFVLPS EAVTNINKSSCGVENTTAPVLAIQFGSNH--SLSIHFARNTRYEVAEL		116
SEQIDNO:9	GPKNMTFDLPDSDATVVLNRSSCGKENTSDPSLVIAFGRGH--TLTLNFTNRNATRYSVQLM		116
SEQIDNO:10	GPKNMTFDLPDAKVLNRSSCGKENTSDPSLVIAFGRGQ--TLTLNFTNRNATRYSVQLM		115
SEQIDNO:11	GPKNMTFDLPDA-VVLNRSSCGKENTSDPSLVIAFGRGH--GLTLNFTNRNATRYSVQLM		112
SEQIDNO:12	GYKNASFELPATA-EVQNTSSCGRENTSNPQLQIAFGRGH--VLALNFTNRNATLYSVPLL		111
SEQIDNO:13	GPQNKSFELPAGA-EVSNSSSCGKENASDSSLVITFGRGH--TLTLNFTNRNATRYEVQLM		112
SEQIDNO:14	VSKVSNMTLPASAEVLKNSSSCGKENASEPTLAITFEGGY--LLKLTFTKNTRYSVQHM		111
SEQIDNO:15	QKQFAHFFLPQNATSQ-SHSSCGEGNTSHPILALSFGAGH--LISLNFSTLDKYQVEEL		103
SEQIDNO:16	GAKNTTFLPASA-QVLNRSSCGKENTSDSSLVIAFGRGH--TLTLNFTNRNATRYSVQLM		113
SEQIDNO:17	GLESTTFRLPQNA-SVMNSSSCGKENTSNPILEIGFGGGH--TLTMNFSSTTSYQVELL		112
SEQIDNO:18	QKQFAHFFLPQNATSQ-SHSSCGEGNTSHPILALSFGAGH--LISLNFSTLDKYQVEEL		104
SEQIDNO:19	QKEFVHFFLPQNASVD-SQSSCGKDNASHPILVLDFFGGH--SLSLNFSESADKYQVEEL		105
SEQIDNO:20	KVERKTFLPSSAHINKESSSCGKEKETSQVLVVEFGTGN--SLTFTFEKSNDFYHVSNL		112
SEQIDNO:21	DTRTVSVFLPNSTTVDSANSSCGS-NGSTPGLMAKFGPGH--YFGMNFSTNGSLYSVDTL		110
SEQIDNO:22	STRTAQFILLPSATVDPDSSTCGG-NGSSPWLVAVFGAGH--ALGLGFSTNGSFYSVANL		109
SEQIDNO:23	GTRTMVPLPGSAVVG-ASSCGG-DGRSPWLVALFGDGH--ALGLGFSSNDSLYSAKL		111
SEQIDNO:24	-----		0

FIGURE 3 cont.

LAMP HOMOLGY DOMAIN 1

	3	
SEQIDNO:1	SEFVYNLSDETHLFPNASSK-EIKT-VESITDIRADIDKKYRCVSGTQVHMNN-VTVTLHDA	173
SEQIDNO:6	YFTYNLSDTEHFPNAISK-EIYT-MDSTTDIKADINKAYRCVSDIRVYMKN-VTVVLRDA	167
SEQIDNO:7	SLQYNLSDGDI FPQSSSAGVQSVMASVSELMASRLNSTYRCVSSSSISLSAAVNLTLSGV	168
SEQIDNO:8	VMSYNLSDKRIIFPNASENGTKTV-STNKTAVLAENDTVYKCMNPHLRMDN-ANATFHD	174
SEQIDNO:9	SEFVYNLSDTHLFPNASSK-EIKT-VESITDIRADIDKKYRCVSGTQVHMNN-VTVTLHDA	173
SEQIDNO:10	SEFVYNLSDTHLFPNASSK-EIKT-VESITDIRADIDKKYRCVSGTQVHMNN-VTVTLHDA	172
SEQIDNO:11	SFIYNLSDTQIFPNASSK-ETKT-VESATDIRADINKKYRCVSNQIHMNN-VTVTFHDV	169
SEQIDNO:12	SEFVYNLSDSDLFPNASSK-DIKT-VGSTTDIKADIDKKYRCVSDSKVPMGN-VTVTLQDA	168
SEQIDNO:13	RFAYNLSDTDTFPNSSST-GVKT-VESATDIKADINKTYRCVSETQVNMDN-VTVTLRDA	169
SEQIDNO:14	YFTYNLSDTQFFPNASSK-GPDT-VGSTTDIKADINKTYRCVSDIRVYMKN-VTIVLWDA	168
SEQIDNO:15	TFHYNLSDETLFPNATEG-KVMV-ATQKSVIQAIRIGTEYRCINSKYVRMKH-VNITFSNV	160
SEQIDNO:16	TLVYNLSDAEFFFSASSK-GTKT-VAASTDIRADLNTKYRCVSNQVHLLN-VTVTLGNA	170
SEQIDNO:17	SFSYNLSDATLFPNASKGSEESS-VKSKTDIQADIHKKYRCVSSNRITMSN-VTIVLSDV	170
SEQIDNO:18	TFHYNLSDETLFPNASEG-KVME-VTQKSVIQAIRIGTEYRCINSKYIYIRH-VNITFSNV	161
SEQIDNO:19	VFHYNLSDATLFPNSSSTG-GMKT-VSHKSLIQAHMGTYRCINSKHINMKN-VNVTFSNV	162
SEQIDNO:20	TFSYNLSDSSFFPNSSG--GQRE-VSRAGDIQANINTTYRCRSNHRVNMN-VTVLFSNV	168
SEQIDNO:21	FLRYNLSDASLFPEANSSGPVDFELSASVGIWAPTNTTYRCLSPPTITITR-PSVTFSEM	169
SEQIDNO:22	TLQYNLSDASVFPDANSSGVTVF-VSSSVGIWAAVNTTYRCLSSVLFQVGG-ATVTFSDM	167
SEQIDNO:23	TLQYNLSDVSNFPEANSTDVVTVE-TTSVGMVARVNTTYRCISASPVIVGG-ATVTFSDM	169
SEQIDNO:24	-----	0

LAMP HOMOLGY DOMAIN 1

Hinge Region

	4	
SEQIDNO:1	TIQAYLSSNSSFSGETRCEQDRPSPPTTAPPAP-----PSP-SP---SPVPKSPS	218
SEQIDNO:6	TIQAYLSSGNFSKEETHCTQDGPSPPTTGP-----PSP-SP---PLVPTNPT	209
SEQIDNO:7	QMEAYMSSANLSADESVCSAQSPSTTVAPPPSTT-----TSFPPIPPVPE	213
SEQIDNO:8	RLEAYLKQSNFSQKVSTCSSEDTPTSPAPA-PV--T-----T-----TAPVPAP-VPDPP	219
SEQIDNO:9	TIQAYLSSNSSFSGETRCEQDRPSPPTTAPPAP-----PSP-SP---SPVPESPS	218
SEQIDNO:10	TIQAYLSSNSSFSGETRCEQDRPSPPTTAPPAP-----PSP-SP---SPVPESPS	217
SEQIDNO:11	TIQAYLSSNFSKEETRCEQDGPFPPTTAPPPP-----PHP-SP---SPAPESPS	214
SEQIDNO:12	TIQAYLWNNFSQAESRCQDMPSPTTAPPAPPVP-----PSPPSP-SP---PPKPESPS	219
SEQIDNO:13	AIQAYLSSSNFSREETRCEQDLPT-----P-----TTPPQP-AP---TPAPASPA	210
SEQIDNO:14	TIQAYLPSSNFSKEETRCPQDQPSPTTGP-----PSP-SP---PLVPTNPS	210
SEQIDNO:15	TLEAYPTNDTFSANKTECREDMVSTTTVAPTTPKH-----ATSQVPTTSPAPTAAPSSPA	215
SEQIDNO:16	TIQAYLANNSFSQETRCEQDKPSP-----PTP-----TAPPTP-TP---TPAPTSEV	214
SEQIDNO:17	TIQAYLSSNNTFSKEETRCSQDTPSPSPVPTTHPTT-----IPVPTP-TPTRPPTPAEIPP	224
SEQIDNO:18	TLEAYPTNGTFTSNKTECEGDMVSTTTVAPTTPKH-----ITSQVPATSPAPTAAPSNEA	216
SEQIDNO:19	TLEAYLTNGTSLVNKTECAEDRVSTTTMVPTTPKQ-----TTSQSPTTGPAPTS-PPNPT	216
SEQIDNO:20	TLEAYLPNNAFKNDSVCAEDKTSTVA--PPITTH-----IPTTTSLAPPT-PPPTDTPK	220
SEQIDNO:21	KLEAYMPGNDLSPFAERVCAADQTTTGAPTITT-----SAATP-TT-PSPTPAGTPE	218
SEQIDNO:22	RLEAYMPGNDLSPRESFCAADQTTTAPPTTTAAP-----TTTAATTM-AP-PAPTTPGTPEV	221
SEQIDNO:23	TMEAFMTGEDLSPNESVCTADQSFPTTAPPPPPS-----TTTAA-PA-PVPTPPGTPE	219
SEQIDNO:24	-----MVQICRVQSWFVGVTPLLI FATVLHQGFATVAP-PTPAPHKEPGRPE	46

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Hinge LAMP HOMOLOGY DOMAIN 2

	1	2
SEQIDNO:1	VDKYNVSGTNGT-CLLASMGQLQLNLTYE-RKDNTTVTRLLNINPNKTSASGSCGAHLVTL	276
SEQIDNO:6	VSKYNTVTGNNGT-CLLASMGQLQLNLTYL-KKDNKTVTRAENISPNDT-SSGSGCINLVTL	266
SEQIDNO:7	RGNYSVTDGNGTVCVLALMGLQLNLTHT-TTONQSVSELMNLQPNQTTVSGSCGVTESSL	272
SEQIDNO:8	VVQYSVNRSSSEP-CLLAKVGLQMNITYT-TKDGKNGSVYFNIESKGVTVDGNCTNITAYL	277
SEQIDNO:9	VDKYNVSGTNGT-CLLASMGQLQLNLTYE-RKDNTTVTRLLNINPNKTSASGSCGAHLVTL	276
SEQIDNO:10	VDKYNVSGTNGT-CLLASMGQLQLNLTYE-RKDNTTVTRLLNINPNKTLASGSCGAHLVTL	275
SEQIDNO:11	VHKYNVSGANGT-CLLASMGQLQLNVTYK-KKDNTTVVKVVSINPNKTTAGGSCGAQLVTL	272
SEQIDNO:12	VSRYNVSDGNAT-CLLASMGQLQLNLTIV-HRDNATVTRVFNINPNKTKPSGHCGAQQVTL	277
SEQIDNO:13	VFRYNVSGSNGT-CLLASMGQLQLNVTYR-RVDNKTVTREFNVPNKTTFGGNCSATLATL	268
SEQIDNO:14	VSKYNVTGDNGT-CLLASMGQLQLNITYM-KKDNTTVTRAENINPSDK-YSGTCGAQLVTL	267
SEQIDNO:15	VGKYNVTGANGT-CVLASMGQLQLNITYV-KKDEKMGDLNFI PHNTSASGMCESTSAFL	273
SEQIDNO:16	VSRYNVSGANGT-CLLASMGQLQLNVTYR-TKDNTTVTRGLNINPNKTTFGGSCSAQLVTL	272
SEQIDNO:17	IFKYNVSDANGT-CLLASMGQLQLNITYA-KKDNSSARI IWNINPNKTVAGGSCSPQVAIL	282
SEQIDNO:18	VGKYNVTGANGT-CVLASMGQLQLNITYL-KKDGTGLDNLNLFVPHNTNASGTCENTSACL	274
SEQIDNO:19	VGKYNVTGPNGT-CVLAYMGLQLNITYQ-QKDEKMGDLNLFVPHNTSSGRCDNTSALL	274
SEQIDNO:20	IGRYNVTGLHGI-CLLATMGLQVNVVTYS-TKNKTSKSELLNLPP-TAEVSGTCENSSITL	277
SEQIDNO:21	QGSYSVKNASGTVCLMAKMGVQLNVSYFSQSQNKTVOELLNLTPNLTSSSGLCGGTNATL	278
SEQIDNO:22	RGTYSVVNGNETTCLLAQMGLQLNVSYFSRSQNKTQSLVNLTPLNLTNSTGSCCKGSATL	281
SEQIDNO:23	QGSYSVSNNGTVCLLARMALQLNISHFSASQNKTIQEVVNLLPNQTTSSGSCDPTSATL	279
SEQIDNO:24	RGYYNVTNHNGTICLMAVMGLQLNII SYNSTSQKKVVDVMNLQPNLTKRHSLGLCDSDIASL	106

SEQIDNO:1	ELRS-EGSTVLLFLFGMNASSSRFFLQGIQLNTTLP-DARDPAFKAANGSLRALQATVGN	334
SEQIDNO:6	KVEN-K-NRALELQFGMNASSSLFFLQGVRLNMTLP-DALVPTFSISNHSIKALQATVGN	323
SEQIDNO:7	RLSD--ETTNLTFSTFTMNSTTQKYVLSAVSVSALWFDMS-VVFEAGNTSLSALQCSVGR	328
SEQIDNO:8	SLST-GS-IDLRFNFTLNSSLEVPYLDGVSLSSTGLPADANDTHFEAANSSSLNYMQTNVHK	335
SEQIDNO:9	ELHS-EGSTVLLFLFGMNASSSRFFLQGIQLNTTLP-DARDPAFKAANGSLRALQATVGN	334
SEQIDNO:10	ELHS-EGSTVLLFLFGMNASSSRFFLQGIQLNTTLP-DARDPAFKAANGSLRALQATVGN	333
SEQIDNO:11	ELRS-ESVTLLAFQFGMNASTRFFLQGIQLNMTLP-DARDPFFKAGNSSLRALQATIGN	330
SEQIDNO:12	ELQS-ERSTVLVFPFGMNASSGQYFLQGVLLNTTLP-DAREPAFSASNSSLRALQATLGN	335
SEQIDNO:13	ELHS-ENLLLLALQFVMNESSSRVFLQGVQLNLTLP-DAKEGSFTATNSSLRALQATAGN	326
SEQIDNO:14	KVGN-K-SRVLELQFGMNATSSLFFLQGVQLNMTLP-DAIEPTFSTSNYSIKALQASVGN	324
SEQIDNO:15	NLAF-EK-TKITFHFVLNASSEKFFLQGVNVSTTLPSEAKAPTFEASNDMSSES RATVGN	331
SEQIDNO:16	ELQG-ESLRLLLALQFALNTSSSRVFLQGVQLNMTLP-DARDPFSFAANSSSLRALQATAGN	330
SEQIDNO:17	ELQT-EN-STLAFSGFMNATSSKFFLREIRFHKFFP-DAKDPAGFAGVNSSLKLQATVGN	339
SEQIDNO:18	NLAF-EK-TKITFHFVLNASSEKFFLQGVNVSTTLPSEAKAPMFEASNDMSSEL RATVGN	332
SEQIDNO:19	NLTF-EK-TRVIFQFALNATAEKFFLQGVSVSTTLPSEAKNPKFEATNNSMSSELRASVGN	332
SEQIDNO:20	NLTS-ES-TSLSFQFSQNTSTEKYFLQGIIVTANLPPEATEKNISYNHNTLNALKTSVGK	335
SEQIDNO:21	VLAQ-EETTVLSFLFTVNSTSNKYHLSGITLQANWT-DMX-SPFSASNTSLDYLRSSLGH	335
SEQIDNO:22	ILTQ-Q-TTILIFTFSLNSTSSKYHLSGLSLQANWS-DMA-AAFSASNASLSYLRSTFGH	337
SEQIDNO:23	VLTQ-ANATNLSFLFTLNSTSNRYHLTGLSVVAAWS-DMT-APFNSTSNSSLDYQRGSLGR	336
SEQIDNO:24	NLTVDAVKTNLTFVFTMNSTSNKYHLSSEVTVSAAWP-EMK-EPVSVHNSSLDYLRGTVG	164

FIGURE 3 cont.

	LAMP HOMOLOGY DOMAIN 2	Trans. Domain	
	3	4	
SEQIDNO:1	SYKCNAAEEHVDTFAFSVNIKVVWQAFKVEGGQFGSVVEECQLDENSM	LIPIAVGGALAG	394
SEQIDNO:6	SYKCNTEEHIFVSKMLSLNVFSVQVQAFKVDSDRFGSVVEECVQDGNMMLIPIAVGGALAG		383
SEQIDNO:7	SYVCSAQQMLSVTPVFSINTFRLQLQPFNITANRFSTAEECRVQDENMMLIPIIVGAALAG		388
SEQIDNO:8	SFKCNSKQTLQITDPFTVNTYHLQVQAFNSD-NTFASAVECSLDENGMLVPIVVGALAG		394
SEQIDNO:9	SYKCNAAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVVEECVLDENNMLIPIAVGGALAG		394
SEQIDNO:10	SYKCNAAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVVEECQLDENNMLIPIAVGGALAG		393
SEQIDNO:11	SYKCNAGEHVQVTEAFSVNIIKVVWQAFQVQGDGKFGSVVEECQLDENSMMLIPIAVGGALAG		390
SEQIDNO:12	SYKCNSEEHVRVTPAFSLSIKVVWQAFQVKGDKFGSVVEECQLDQDSMLIPIAVGGALAG		395
SEQIDNO:13	SYKCNAEQRLRVTSFSLNMFVWLQAFRVDGDKFGFVEECQLDENSMMLIPIAVGGALAG		386
SEQIDNO:14	SYKCNSEEHIFVSKALALNVFSVQVQAFRVESDRFGSVVEECVQDGNMMLIPIAVGGALAG		384
SEQIDNO:15	SYKCSAEENFQVTDKALNVFNQVQAFKVDGDKFGAMEECQLDENNMLIPIIVGAALAG		391
SEQIDNO:16	SYKCRSEQRLQVTEAFALNVFQVRVQAFRVDGDKFGPAEECQLDENSMMLIPIAVGGALAG		390
SEQIDNO:17	SYKCNAAEENVHVTGGFSVNI FRVRVQAFKVEGDKFGSVVEECQLDENNMLIPIAVGGALAG		399
SEQIDNO:18	SYKCSAEENLQVTDKALNVFNQVQAFKVDGDKFGAVEECQLDENNMLIPIIVGAALAG		392
SEQIDNO:19	SYKCSSEENLQVTDQALNVFNQVQVQFKIDGDKFGFVEECQLDENNMLIPIIVGAALAG		392
SEQIDNO:20	SYKCIAEESIWIISGKAAVNIFNIQLQAFKI PGDKFGAVEECQLDENNMLIPIIVGAALAG		395
SEQIDNO:21	SYMCNAEQTLFVVSTFSLNMFELQVQPFVGTSTQFASAEEVCQIDQDQMLIPIIVGAALAG		395
SEQIDNO:22	SYMCNAEQILAVTPVFSLNTFSLQIQPFVGTNNQFAAAEECQMDQDQMLIPIIVGASLAG		397
SEQIDNO:23	SYMCISEQTLVVDQNFSLNTFQLQVQVQPFGITRGQFAQAEECQLDQDNMLIPIVVGALAG		396
SEQIDNO:24	SYFCRDEQTLNVAQNLSINTFQLQVQVQFAVKGQDQFGAAEECQLDEDDMLIPIVVGALAG		224
	* * * * *	* * * * *	

Cytoplasmic Tail

SEQIDNO:1	<u>LVLIVLIAAYLVGRKRSHAGYQTI</u>	417
SEQIDNO:6	LVLIVLIAAYLI GRKRSHAGYQTI	406
SEQIDNO:7	LVLIVLVAYLI GRKRTHAGYQTI	411
SEQIDNO:8	LVLIVLIAAYLI GRKRSHAGYQTI	417
SEQIDNO:9	LVLIVLIAAYLVGRKRSHAGYQTI	417
SEQIDNO:10	LVLIVLIAAYLVGRKRSHAGYQT-	415
SEQIDNO:11	LVLIVLIAAYLI GRKRSHAGYQTI	413
SEQIDNO:12	LVLVVLIAAYLI GRKRSHAGYQT-	417
SEQIDNO:13	LVLIVLLAYLI GRKRSHAGYQTI	409
SEQIDNO:14	LVLIVLIAAYLI GRKRSHAGYQTI	407
SEQIDNO:15	LVLIVLIAAYLI GRKRSHAGYQTI	414
SEQIDNO:16	LVLVVLMAAYLVGRKRSHAGYQTI	413
SEQIDNO:17	LVLIVLIAAYLI GRKRSHAGYQTI	422
SEQIDNO:18	LVLIVLIAAYLI GRKRSHAGYQTI	415
SEQIDNO:19	LVLIVLIAAYLI GRKRSHAGYQTI	415
SEQIDNO:20	LVLIVLIAAYLI GRKRSHAGYQTI	418
SEQIDNO:21	LVLIVLIAAYLI GRKRSHAGYQTI	418
SEQIDNO:22	LVLIVLIAAYLI GRKKSHAGYQTI	420
SEQIDNO:23	LVLIVLIAAYLI GRKRSHAGYQTI	419
SEQIDNO:24	LVLIVLLAYLI GRKRSHAGYQSI	247
	** : ** : ** : ** : ** : ** :	

FIGURE 3 cont.

LAMP-1					
Accession No.	Species	SEQ ID NO:	Accession No.	Species	SEQ ID NO:
NP_005552.3	<i>H. sapiens</i>	1	NP_990614.1	<i>G. gallus</i>	15
NP_034814.2	<i>M. musculus</i>	6	NP_001011507.1	<i>S. scrofa</i>	16
NP_955996.1	<i>D. rerio</i>	7	XP_001374132.1	<i>M. domestica</i>	17
NP_001087042.1	<i>X. laevis</i>	8	XP_003203252.1	<i>M. gallopavo</i>	18
NP_001233491.1	<i>P. troglodytes</i>	9	XP_002191607.2	<i>T. guttate</i>	19
XP_001087801.1	<i>M. mulatta</i>	10	XP_003218797.1	<i>A. carolinensis</i>	20
XP_534193.2	<i>C. lupus familiaris</i>	11	XP_004067118.1	<i>O. latipes</i>	21
XP_002723509.1	<i>O. cuniculus</i>	12	XP_003969941.1	<i>T. rubripes</i>	22
NP_001068592.1	<i>B. taurus</i>	13	NP_001158846.1	<i>S. salar</i>	23
NP_036989.1	<i>R. norvegicus</i>	14	XP_003452974.1	<i>O. niloticus</i>	24

FIGURE 4: HUMAN LAMP-2 ALIGNMENT WITH ORTHOLOGOUS SEQUENCES

SEQIDNO:2	-----	0
SEQIDNO:25	-----	0
SEQIDNO:26	-----	0
SEQIDNO:27	-----	0
SEQIDNO:28	-----	0
SEQIDNO:29	-----	0
SEQIDNO:30	-----	0
SEQIDNO:31	-----	0
SEQIDNO:32	-----	0
SEQIDNO:33	-----	0
SEQIDNO:34	-----	0
SEQIDNO:35	-----	0
SEQIDNO:36	-----	0
SEQIDNO:37	MAMKNFTLQQERDTSVALIIRTYVRAFLKVYTKVPKPQRCHNQW--QSLNIEGIEGIEI	57
SEQIDNO:38	-----	0
SEQIDNO:39	-----MECREGEVTRCKQKNNLFSGIN-DDISGAKQ	30
SEQIDNO:40	-----	0
SEQIDNO:41	-----	0
SEQIDNO:42	-----	0
SEQIDNO:43	-----	0

SEQIDNO:2	-----	0
SEQIDNO:25	-----	0
SEQIDNO:26	-----	0
SEQIDNO:27	-----	0
SEQIDNO:28	-----	0
SEQIDNO:29	-----	0
SEQIDNO:30	-----	0
SEQIDNO:31	-----	0
SEQIDNO:32	-----	0
SEQIDNO:33	-----	0
SEQIDNO:34	-----	0
SEQIDNO:35	-----	0
SEQIDNO:36	-----	0
SEQIDNO:37	VKGSKWR--SALETIITIQVKRK-----SQVQKYHPFSLHSECQKTNQE	99
SEQIDNO:38	-----	0
SEQIDNO:39	AKQRQCTPQKPPKRATATLPLQRFPFGIPGPAPAAVAAVAADRITPSGSHQTRPPEAAR	90
SEQIDNO:40	-----	0
SEQIDNO:41	-----	0
SEQIDNO:42	-----	0
SEQIDNO:43	-----	0

FIGURE 4 cont.

SEQIDNO:2	-----MVCF	4
SEQIDNO:25	-----M	1
SEQIDNO:26	-----MGDT	4
SEQIDNO:27	-----	0
SEQIDNO:28	-----MVCF	4
SEQIDNO:29	-----MVCF	4
SEQIDNO:30	-----MVCF	4
SEQIDNO:31	-----MVCF	4
SEQIDNO:32	-----MVCF	4
SEQIDNO:33	-----MVCF	4
SEQIDNO:34	-----MVCF	4
SEQIDNO:35	-----MVCF	4
SEQIDNO:36	-----MR	2
SEQIDNO:37	G---TGGVATVIADECLLWPSIPFSTLAQKVNLGSCAFSIIIGYSVFALFIYLPNMLDF	156
SEQIDNO:38	-----	0
SEQIDNO:39	DERPVRDPRNRAAAPSGHWRRAGGPQRHR-----HHR-----HRRHGPAPLRR	133
SEQIDNO:40	-----	0
SEQIDNO:41	-----	0
SEQIDNO:42	-----	0
SEQIDNO:43	-----	0

SIGNAL SEQUENCE

LAMP HOMOMOLOGY DOMAIN 1

1

SEQIDNO:2	-RL---FPVPGSGLVLVCLVLGAVR---SYA LELNLTDS ENATCLYAKWQMNFTVRYETT	57
SEQIDNO:25	-CL---SPVKGAKLILIFLFLGAVQ---SNALIVNLTDS-KGTCLYAEWEMNFTITYETT	53
SEQIDNO:26	GAM---ERCACPAAVLLLSVL---MG---ATAFEVEIKDDKNATCIYAKLSVNITVQYETD	57
SEQIDNO:27	-----MAVRGFLPLLFIILLSGIVHADMMTSPLPS-----TAEK	35
SEQIDNO:28	-RL---FPVPGSGLVLVCLVLGAVQ---SYALELNLTDSGKATCLYAKWQMNFTVRYETT	57
SEQIDNO:29	-RL---FPVPGSGLVLVCLVLGAVQ---SYALELNLTDSGKATCLYAKWQMNFTVRYETT	57
SEQIDNO:30	-RL---FPVPGSGLVLVCLVLGAVR---SHALELNEADSAIN-----	39
SEQIDNO:31	-RL---SPAPGSGLVLLCLVLGAVS---SYALEVNVTDSKATCLYAKWQMNFTIQYNTT	57
SEQIDNO:32	-RL---SPVPGSGLVLLCLVLGAVS---SYALELNLTDSKALCLYAKWQMNFTIPYETT	57
SEQIDNO:33	-RL---APVPGSGFLLLCLVLGAVS---SYALELNLTSSNATCLYAKWQMNFTIRYETT	57
SEQIDNO:34	-RL---SPVPGSGLMLLCLVLGAVS---SYALELNLTNSEKATCLYAKWQMNFTIRYETT	57
SEQIDNO:35	-RL---APVPGCGFLLFCLVLGTVS---SYALELNLTSSKATCLYAKWQMNFTIRYETT	57
SEQIDNO:36	-LL---SFVTGSKLVLLFLFLGAVR---SDALKNLNTDS-KGTCLYAEWEMNFTITYEAL	54
SEQIDNO:37	IELAELMLSTETQLLEPTRVCCGICQ---SYALELNLTDSKNATCLYSKWQMTFTINYETT	214
SEQIDNO:38	--MAPPRCPAGLALLLLLLGACGFFQ---SYAVEVDVKDASNFTCLYAQWMMKFLIKYETN	56
SEQIDNO:39	LLLRPPP-PAA-----AAARFLGFFQ---SYAVEVDIKDASNATCLYADWMMRFLIKYESN	185
SEQIDNO:40	--M---ERCACPAALLLSVL---MG---AMAFDVEIKDDKNATCIYAKLSVNVTVQYETN	51
SEQIDNO:41	-----MF-RCAFLILFLALGNELHLSHGTEVSVNNTENKLCLYANLMVNFSTVYEVG	51
SEQIDNO:42	-----MKVSHATAGLVVWFVVLGCIDAVT---L-EVKESNTTCIKADLSASFSTIYNTT	50
SEQIDNO:43	-----	0

FIGURE 4 cont.

LAMP HOMOLGY DOMAIN 1

2

SEQIDNO:2	NKTYKTVTISDH--GTVTYNGSICGDDQNGPKIAVQFGPGFS-WIANFTKAA---STYSI	111
SEQIDNO:25	NQTNKTITIAVP--DKATHDGSSCGDDRNNSAKIMIQFGFAVS-WAVNFTKEA---SHYSI	107
SEQIDNO:26	TSSSKNITFPVP--SDVTNGSSCGSDGKAPLLVINFGNSQS-WSLNFTRRN---STYSG	111
SEQIDNO:27	---T---ANLP--LVIQTTSSTSTTTT-SRP--SSTSTHSTLTTEPAA-----	73
SEQIDNO:28	NKTYKTVTISDR--GTVTYNGSICGDDQNGPKIAVQFGPGFS-WIANFTKAA---STYSI	111
SEQIDNO:29	NKTYKTVTISDR--GTVTYNGSICGDDQNGPKIAVQFGPGFS-WIANFTKAA---STYSI	111
SEQIDNO:30	CSKCKTVTISDH--GTVTYNGSICGDDQNGPKIAVQFGPGFS-WIANFTKAA---STYSI	93
SEQIDNO:31	SKNFKTATISDF--STATYNGSVCGNDQNNPKIVVQFGSGFS-WIVNFTKKE---SAYLI	111
SEQIDNO:32	SKSYKTVTISNF--GTPYNGSICGDNQNGSRIAVQFGSGFS-WIVNFTKSV---SVYSI	111
SEQIDNO:33	DKHNKTVPISDL--GAATYNGSFCGDDQNGPKIAVQFGSGFS-WIVNFTKEASPSTYLV	114
SEQIDNO:34	NNSHRTVISDF--GAATYNGSFCGDDHNDPQIVMQFGSGFS-WIVNFAKES---SSYLI	111
SEQIDNO:35	DKHNKTVTISDF--DAAAYNGSVCGDDQNGPKIAVQFGSGFS-WIVNFTKEASSTSTYLV	114
SEQIDNO:36	K-VNETVTITVP--DKVTYNGSSCGDDKNGAKIMIQYGSTLS-WAVNFTKEA---SQYFI	107
SEQIDNO:37	GNETKNVTITVP--ENVTYDGSSCGDNQTVQIAVQFGLGYS-WHLNFTKKEN--NSYSF	269
SEQIDNO:38	SSDYKNASLDLT--STVTHNGSICGSDTQAALLAVQFGDGHs-WSINFTKNN---ETYRA	110
SEQIDNO:39	SGDYKTTTLNLS--SSVTHNGSVCGNDTQAALLAVQFGEGHS-WSINFTKNN---ETYQG	239
SEQIDNO:40	TSSTKNVTFVSP--SEVTNGSSCGSNGKAPILVINFGNGHS-WSLNFTPRD---SMYSG	105
SEQIDNO:41	VNKNETVIFVLP--ENVTEGSTCDNTTSTLKL--SFGHGHs-WTVEFTKKN---KTYQV	103
SEQIDNO:42	HAER-TVQVLLPNSTTVDTANSTCGKDGSSPRLVAVFGSGY-TLGLNFSTNG---TLYQV	105
SEQIDNO:43	-----	0

LAMP HOMOLGY DOMAIN 1

3

SEQIDNO:2	DSVFSFSYNTGDNTTFPDAEDKGI-LTVDELLAIKIPLNDFRCNSLSTLEKNDVVQHYWD	170
SEQIDNO:25	HDIVLSYNTSDSTVFPGAVAKGV-HTVKNPENFKVPLDVIFKCNsvLTYNLTpvvQKYWG	166
SEQIDNO:26	SALIFTYNTNDTILFPDALRKGLIS-STAMFLGPVPLNSTYKCIISREVVSENVTOIYD	170
SEQIDNO:27	-----KTTTARTTVTTSA---PTSTQSTSSSSTSATVTTLAP	107
SEQIDNO:28	DSISFSYNTGDNTTFPDAEDKGI-ITVDELLAIKIPLNDFRCNSLSTLEKNDVVQNYWD	170
SEQIDNO:29	DSISFSYNTGDNTTFPDAEDKGI-ITVDELLAIKIPLNDFRCNSLSTLEKNDVVQNYWD	170
SEQIDNO:30	DSISFSYNTGDNTTFPDAEDKGI-LTVDELLAIKIPLNDFRCNSLSTLEKNDVVQHYWD	152
SEQIDNO:31	DSISFSYNLSDNATFPDAKEKGI-LTVHDLVGFRIPLNDFRCNSLSTLEKNGVVQYYWD	170
SEQIDNO:32	DSISFSYNTGDNTTFPDAEDKGI-LTVNESVAFKIPLNDFRCNSLSSLVKNGVVQNYWD	170
SEQIDNO:33	DTISFSYNTNDNKTFPDAKEKEV-FTVNNRVALKIPLNDFRCNSLSTLENRDVVQHYWD	173
SEQIDNO:34	NSISFSYNTSDTTTFPDAKKGV-LTVNDSVGFQVPLNDIFRCNSLSTLEKNDVVQHYWD	170
SEQIDNO:35	DSISFSYNTNDNATFPDAKEKGV-FTVNNRVALKIPLNDFRCNSLSTLEKSDVVQHYWD	173
SEQIDNO:36	NNITLSYNTNDTKTFPGAVPKGI-LTVIIPVGSQPLPLGVIFKCSSVLTFNLSPVVQHYWG	166
SEQIDNO:37	DTIVFTYNTSDNETFPDAKEKGVLSVFEFYARIPLNKIFRCHSEESLIGDKATHHYWE	329
SEQIDNO:38	EFITFTYNTNDTAVFPDARRQGPVTIVVKDAMHPIQLNNVFVCHHTTSLEAENVTOIFWN	170
SEQIDNO:39	DFITLTYNTNDTAVFPDAKRGPITVLVRDPSRPIQLNTVFVCHNSFVIEAENTTOIFWN	299
SEQIDNO:40	GALIFTYNTNDSTLFPDALREGLIS-STAAFLGPIPLNSTYKCIISSEVVVSENVTOIISD	164
SEQIDNO:41	DTIVFSYNLNDSSVFPNSTSKETKEFVTVKSIITNVSVDTYYCKSENVLTVESVIQTLVD	163
SEQIDNO:42	SSLTLQYNLSDTSVFPNATISGVVTLVSASVGLIENAVNTTYKASPTVIDVATAKVNFTD	165
SEQIDNO:43	-----MTQIGGVQPVFLA	13

FIGURE 4 cont.

	LAMP HOMOLGY DOMAIN 1	Hinge Region	
	4		
SEQIDNO:2	VLVQAFVQNGTVSKNEFLCDKDKTS---TVAPTIHTTVPS-----PTTTPT--PKEKPE		219
SEQIDNO:25	IHLQAFVQNGTVSKNEQVCEEDQTP--TTVAPIIHTTAPSTTTTLTPTSTPTPTPTPTPT		224
SEQIDNO:26	VKLEAFMANGTLGK-EIICDADKPS--PVPSPTQFST-----TASTAIPAPTSKPLDKPT		222
SEQIDNO:27	TTTGHNTTNSTTEFPPTTGHNTTNS--TTDAPTTHTNAT---VAPTEPPPTTPSVPKPT		161
SEQIDNO:28	VLVQAFVQNGTVSTNEFLCDEDKTS---TVAPTIHTTVPS-----PTTTPT--PKEKPE		219
SEQIDNO:29	VLVQAFVQNGTVSTNEFLCDEDKTS---TVAPTIHTTVPS-----PTTTPT--PKEKPE		219
SEQIDNO:30	VLVQAFVQNGTVSTNEFLCDKDKTS---TVAPTIVHTTVPS-----PTTTPT--RIP---		198
SEQIDNO:31	VHVQAFVQNGTVSTKEFLCEKDKTS---TTVVPTISTTTTPS-----PTTTPT--PKEKPE		220
SEQIDNO:32	VHVQAFVQNGTVSTNEFLCEKDKNTT--TTVAPIVPTTVPSPTTTSSPTTTPS--PKEKPD		226
SEQIDNO:33	VHVQAFVQNGTVSTTEFLCDKDKTV--TTAVPIVPTTLPS-----PT-----KPV		216
SEQIDNO:34	VHVQAFVQNGTVSTKEFLCDKDKTL--TTTVPIVPTSVPS-----PTTTPT--PKEKPE		220
SEQIDNO:35	VHVQAFVQNGTVSTTEFLCDKDKTV--TTAMPIVPTTAPS-----PT-----KPV		216
SEQIDNO:36	IHLQAFVQNGTVSKNEQVCKEDKTA--TTVAPIIHTTVPSPTTTLTPTSI----PVPTPT		220
SEQIDNO:37	TVVQAFIQNGTISKKEFICSKDRAS--TTVAPVTTQVVPs-----TTATPVPQDKPY		379
SEQIDNO:38	VTMQAFVQNGTISKKESRCYADPTAAPTVLPTVANVTAS--TTISPAPTTAPKPAENFV		229
SEQIDNO:39	VTMQAFVQNGTVSKKESRCADPTPTSEPTVPPTIANVTASTTTLSAPPTTAPKPFVENFV		359
SEQIDNO:40	VKLEAFMQNGTLGK-EVSCDADKPS--PTPT-TNPST----TASTTTPTPTSKPLDNPT		215
SEQIDNO:41	VALQAFVINGSKSDTDTVCSADMTS--TVAPT---TV---TSTAAPTSTPTLTPTPT		213
SEQIDNO:42	MRLEAYMPGNELSPNETVCFADQTS---TTPSPTTVSTTAV---PTQT---P--PGTRQ		213
SEQIDNO:43	VTVHLIL-----ATV--LHQTF--AT---VTFPVTTA---VPHK---E--PGRPD		48

	Hinge	LAMP HOMOLGY DOMAIN 2	
	1	2	
SEQIDNO:2	AGTYSVNNG--NETCLLATMGLQLNITQ-----DKVASVININPNTHSTGSCRSHTAL		271
SEQIDNO:25	VGNYSIRNG--NTTCLLATMGLQLNITE-----EKVPFIFNINPATTNFTGSCQPQSAQ		276
SEQIDNO:26	MGNYSIVSDA--SGICLLASMGLOINTSLL--SEGKNIWRFFNIDPLGIKNGTCTNQTGT		278
SEQIDNO:27	VGNYSVKTD--NVSDCLLAKMGLQFSFKIS----GNASLQTVNLDPNVTKVNGTCGSGGSD		216
SEQIDNO:28	AGTYSVNNG--NETCLLATMGLQLNITQ-----DKVASVININPNTHSTGSCRSHTAL		271
SEQIDNO:29	AGTYSVNNG--NETCLLATMGLQLNITQ-----DKVASVININPNTHSTGSCRSHTAL		271
SEQIDNO:30	-----PKVASVININPNTHSTGSCRSHTAL		224
SEQIDNO:31	VGSYSVNNS--NGTCLLATMGLQLNITH-----NKVASVININPNTHSTGSCQPQTAL		272
SEQIDNO:32	VGSYLVKNG--SDTCLLATMGLQLNVTH-----DKVASVININPNVTGYSGSCHPQTAL		278
SEQIDNO:33	VGSYSVNNS--NGTCLLATMGLQLNITH-----DKVASVININPNTHSTGSCQPQTAL		268
SEQIDNO:34	TGSYSVTSS--NGTCLLANMGLQLNITQ-----DKVASVININPNTHSTGSCQPQTAL		272
SEQIDNO:35	VGSYSVNNS--NGTCLLATMGLQLNITH-----DKVASVININPNTHSTGSCQPQTAL		268
SEQIDNO:36	VGNYSISNG--NATCLLATMGLQLNITE-----EKVPFIFNINPATTNFTGSCQPQTAQ		272
SEQIDNO:37	PGKYAVKNG--NETCLLATMGLQLNITQ-----NKVNSVININPNVTDFGSCSNETAE		431
SEQIDNO:38	TGNYSLKSG--NKTCLLATVGLQLNISQ-----DK-PLLINIDPKTTHADGTGNTSAT		280
SEQIDNO:39	TGNYSVSDV--NKTCLLATVGLQLNVSQ-----EK-PLLININPKTTVADGACGNTTAT		410
SEQIDNO:40	TGNYSVSDV--NGTCLLASMGLOINTSLL--SEGKNIWTAFFNIDPTAMSKNGTCSNQTGT		271
SEQIDNO:41	TGKYSIAPDVNSTACLMATFGLQIGYKQG---D--KEETINLVPNITEVGGACGANSS-		266
SEQIDNO:42	QGNYSVKDA--NDTICLLAKMGLQLNVSYT--SQNKTVQDVLNLPNVNTNSTGSCGASSAT		270
SEQIDNO:43	QGDYQVTSS--NGTVCFLLASMGLOINIFNSTSQNKTLQEVINIQPNRTKSSGSCDTSSAL		107

. *: * * *

FIGURE 4 cont.

LAMP HOMOMOLOGY DOMAIN 2

SEQIDNO:2	LRLNS-STIKYLDVFVAVKNE-----NRFYLKEVNVSMY-LVNGSV-FSIANNNLSYWDAP	324
SEQIDNO:25	LRLNN-SQIKYLDVFI FAVKNE----KRFYLKEVNVVYMY-LANGSA-FNISNKNLSFWDAP	329
SEQIDNO:26	LILTE--NRTIIEFTFALKNK----NHFLKEEVNITLI---NGSAFSSRQONQLSTWEAS	329
SEQIDNO:27	SSLFLTS--KDIVHFVFTNDS---QKFLHALTLTVD-LGNG-NIFNDSNTNLSLWEAS	269
SEQIDNO:28	LRLNS-STIKYLDVFVAVKNE----NRFYLKEVNVSMY-LVNGSV-FSIANNNLSYWDAP	324
SEQIDNO:29	LRLNS-STIKYLDVFVAVKNE----NRFYLKEVNVSMY-LVNGSV-FSIANNNLSYWDAP	324
SEQIDNO:30	LRLNS-STIKYLDVFVAVKNE----NRFYLKEVNVSMY-LVNGSV-FSIANNNLSYWDAP	277
SEQIDNO:31	LRLNS-SNIKYLDVFVAVKNE----NRFYLKEVNVSMY-LVNGSV-FSIANNNLSYWDAP	325
SEQIDNO:32	LRLNS-SNIKYLDVFVAVKNE----NRFYLKEVNVSMY-LVNGSV-FSIANNNLSYWDAP	331
SEQIDNO:33	LRLSS-SNIKYLDVFVAVKNE----NRFYLKEVNVSMI-LVNGSV-YSISNTNLSYWDAP	321
SEQIDNO:34	LRLSG-SNIKYLDVFVAVKND----NRFYLKEVNVSVY-LVNGSV-FSIANNNLSYWDAP	325
SEQIDNO:35	LRLSS-SNIKYLDVFVAVKNE----NRFYLKEVNVSMI-LVNGSV-YSISNTNLSYWDAP	321
SEQIDNO:36	LRLNN-SQIKYLDVFI FAVKNE----KRFYLKEVNVVYMY-LANGSA-FHVSNNNLSFWDAP	325
SEQIDNO:37	LRLSG-SNVKYIDFI FAVKNG----NRFYLKEVNVVIS-FVNASD-LNVANNNLSYWDAP	484
SEQIDNO:38	LKLND-GNRTLIDFTFIV-NASASVQKFLREVNVTLNLYQNGSVILSADNNNLSKWDAS	338
SEQIDNO:39	LKLND-GNSTLIGFTFAVKNTSASVQKFLREVNVTLNRLNGSVISSADNSNLSKWDAS	469
SEQIDNO:40	LILTD--NSTVIEFTLALKNK----NHFLKEVNVVALI---NGSASSTRQONQLSAWEAS	322
SEQIDNO:41	-DLILTS--DTITIMFTFSNDG---KKFHLHALKVTVK-PATG-DPVIANNNNMSIWAAS	318
SEQIDNO:42	LVLTD--TQSTILTFTNFTLNSTT----NKYHLSGVTLIAN-WFDS-AHFSMSNNLSNLYLRST	324
SEQIDNO:43	LTLTTDAEKTNLTFVFAFNLTTS---NKYHLSEVSLSAA-LSDMKETFFVAQNHSLDYLRGT	163
	* : : : * : : *	

LAMP HOMOMOLOGY DOMAIN 2

Trans. Domain

	3	4	
SEQIDNO:2	LGSSYMCNKEQTVSVSGAFQINTFDLRVQPFNVVTQGGKYSTAQDCSADD-NFLVPIAVGA		383
SEQIDNO:25	LGSSYMCNKEQVLSVSRAVQINTFNLKVQPFNVTKGQYSTAQECSLDDD-TILIPPIIVGA		388
SEQIDNO:26	VDSSYMCHKEQQIKVSEDLFINAFDVRVQPFVGNNGTFATAEDCFAD-Q-NFIVPIIVGA		387
SEQIDNO:27	VGSSYMCNKEQSYNISDKLTNLTFFELQVQPFVVKNSPSTAHECSLDDT-SLLIPPIIVGA		328
SEQIDNO:28	LGSSYMCNKEQTVSVSGAFQINTFDLRVQPFNVVTQGGKYSTAECSSADSDNLFIPVAVGV		384
SEQIDNO:29	LGSSYMCNKEQTVSVSGAFQINTFDLRVQPFNVVTQGGKYSTAQDCSADD-DNFLVPIAVGA		383
SEQIDNO:30	LGSSYMCNKEQTVSVSGAFQINTFDLRVQPFNVVTQGGKYSTAECSSADSDNLFIPVAVGV		337
SEQIDNO:31	LGSSYMCNKEQTVSVSGAFQINTFDLRVQPFNVMEGKYSTAQECSLDDD-TILIPPIIVGA		384
SEQIDNO:32	LGSSYMCNKEQTVSVSGAFQINTFDLRVQPFNVKDGKYSTAQDCRADD-DNFLVPIAVGA		390
SEQIDNO:33	LGSSYMCNKEQTVSVSGAFQINTFDLRVQPFVTEGKYSTAQECSLDDD-TILIPPIIVGA		380
SEQIDNO:34	LGSSYMCNKEQTVSVSGAFQINTFNLRVQPFVMEGKYSTAQDCSADD-DNFIVPIAVGA		384
SEQIDNO:35	LGSSYMCNKEQTVSVSGAFQINTFDLRVQPFVTEGKYSTAECSSADSDNLFIPVAVGV		381
SEQIDNO:36	LGSSYMCNKEQTVSVSRTFQINTFNLKVQPFNVTKGEYSTAQDCSADD-NFLVPIAVGA		384
SEQIDNO:37	LGSSYMCNKEQTLALADSLQINTFNLRVQPFVVGKYSTAEDCSADDD-NFIVPIAVGA		543
SEQIDNO:38	LGNSYMCNKEQTLQINENVQVHTFNLVWQPFVVKENKFSIAEBCFADSDNLFIPVAVGM		398
SEQIDNO:39	LGSSYMCNKEQTLQINENVQVHTFNLVWQPFVVEANKFATAEBCIADSDNLFIPVAVGV		529
SEQIDNO:40	VGSSYMCNKEQQIKVSEDLVINSPFVRFVQLFVGKNETFATAQDCSLDDD-SIVIPPIIVGA		381
SEQIDNO:41	VGSSYMCNKEQTLNVTDTLTLYTFELRVQPFVVKGEFATAHECSLDDT-SILIPPIIVGA		377
SEQIDNO:42	LGSSYMCNKEQTLFVTPSFSLNTFDLQVQPFVVKSGRFATAEBCQMDQN-QMIIPPIIVGA		383
SEQIDNO:43	LGSSYMCNKEQTLGVTPDFAINTFQVQVQPFVGTGKQFAAAEBCQLDKD-DMLIPPIIVGA		222
	: . * . . . * : : : : : * * * : : * . : * : : : * *		

FIGURE 4 cont.

Cytoplasmic Tail

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SEQIDNO:2      ALAGVLILVLLAYFIGLKRHH-AGYEQF      410
SEQIDNO:25     GLSGLIIVIVIAIYLIIGRRKTY-AGYQTL      415
SEQIDNO:26     ALGVLVVLVMVAYFIGRRKQSSAGYEQM      415
SEQIDNO:27     ALAGLIFIVVIAIYVIGRRRTY-VGYQTL      355
SEQIDNO:28     ALGFLIIVVFISYMIGRRKSR-TGYQSV      411
SEQIDNO:29     ALAGVLILVLLAYFIGLKRHH-AGYEQF      410
SEQIDNO:30     ALGFLIIVVFISYMIGRRKSR-TGYQSV      364
SEQIDNO:31     GLSGLIIVIVIAIYLIIGRRKSY-AGYQTL      411
SEQIDNO:32     ALAGVLILVLLAYFIGLKRHH-AGYEQF      417
SEQIDNO:33     GLSGLIIVIVIAIYLIIGRRKSY-AGYQTL      407
SEQIDNO:34     ALAGVLILVLLAYFIGLKRHH-AGYEQF      411
SEQIDNO:35     ALGFLIIVVFISYMIGRRKSR-TGYQSV      408
SEQIDNO:36     ALGGVLILVLLAYFIGLKRHH-TGYEQF      411
SEQIDNO:37     ALGGLVILVLMAYFVGRKRRA-TGYEQF      570
SEQIDNO:38     ALGFLIILVFISYIIGRRKSR-TGYQSV      425
SEQIDNO:39     ALGFLIILVFISYIIGRRKSR-TGYQSV      556
SEQIDNO:40     ALAGLIVIVIAIYLIIGRRKGY-SGYQTL      408
SEQIDNO:41     ALAGLILIVVIAIYVIGRRKTY-VGYQTL      404
SEQIDNO:42     ALAGLVLTITLIAYLIGRRSH-AGYQAI      410
SEQIDNO:43     ALAALVLIVLSAYLIGRRSH-AGYQSI      249
      *.  :::.  .  :*.:*  ::  **:  .

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LAMP-2					
Accession No.	Species	SEQ ID NO:	Accession No.	Species	SEQ ID NO:
NP_002285.1	<i>H. sapiens</i>	2	NP_001231184.1	<i>S. scrofa</i>	34
NP_034815.2	<i>M. musculus</i>	25	XP_004022401.1	<i>O. aries</i>	35
NP_001087881.1	<i>X. laevis</i>	26	NP_058764.2	<i>R. norvegicus</i>	36
NP_001013551.1	<i>D. rerio</i>	27	XP_001510101.2	<i>O. anatinus</i>	37
XP_003918270.1	<i>P. Anubis</i>	28	NP_001001749.1	<i>G. gallus</i>	38
XP_001084005.2	<i>M. mulatta</i>	29	XP_002191794.1	<i>T. guttata</i>	39
XP_003317709.1	<i>P. troglodytes</i>	30	NP_001116192.2	<i>X. tropicalis</i>	40
XP_005641822.1	<i>C. lupus familiaris</i>	31	NP_001133282.1	<i>S. salar</i>	41
XP_001493687.3	<i>E. caballus</i>	32	XP_003445830.1	<i>O. niloticus</i>	42
NP_001029742.1	<i>B. Taurus</i>	33	XP_003961835.1	<i>T. rubripes</i>	43

FIGURE 5: HUMAN LAMP-3 ALIGNMENT WITH ORTHOLOGOUS SEQUENCES

	SIGNAL SEQUENCE	LAMP HOMOLOGY DOMAIN 1		
SEQIDNO:3	MPRQLSAAAAALFASLAVILHD----	GSQMRKAKAFPETRDYSQPTAAATVQDIKKPV-QQP	55	
SEQIDNO:44	MPRQLSAAAAALFASLAVILHD----	GSQMRKAKAFPETRDYSQPSAAATVQDIKKPV-QQP	55	
SEQIDNO:45	MPRQLSAAAVLFASLAVILHD----	GSQMRKAKAFPETRDYSQPTAAATGQDIKKPV-QQP	55	
SEQIDNO:46	MPRQLSAAAVLFASLAVILHD----	GSQMRKAKAFPETRDYSQPTAAATGQDIKKPV-QQP	55	
SEQIDNO:47	MSWQLSAAVALFVSLALILHY----	GSQIRAKMFPETVDFQ-PTTAATVRATAKPF-LHL	54	
SEQIDNO:48	MSWRLSAVLVSFVSLAVFLHY----	GHHMKAKVFPEITDSSSPTTAATVQATAEPSLWKP	56	
SEQIDNO:49	-----	-----	0	
SEQIDNO:50	MAWQLSAVVVLFVSLAVILYY----	GSHVRANVFPEITDYSQPTTAATIQTQAQPSLSQP	56	
SEQIDNO:51	MSWQIPAVVMSFMALVAIWYDSDHYNHMQAKVFPEITGYSSPTTG---	QATVKPSLLQP	57	
SEQIDNO:52	MSWQISAVVLFVSLAVIWIYDS----	HMKANVFPEITGYSSPTTG---	QATVKPSLLQP	53
SEQIDNO:53	MPGQTSAAVAL-LCLAVILH----	GYQIREKEFPEARGYLQYTATTTEQITAKPFL-PL	53	
SEQIDNO:54	MPGQISAAVALFLSLTVILH----	GYQIREKEFPKARGYLQYTATSAEQITTKPLL-QL	54	
SEQIDNO:55	-----	-----	0	
LAMP HOMOLOGY DOMAIN 1				
SEQIDNO:3	AKQAPHQTLAARFMDGHITFQTAATVKIP-----	TTTPATTKNTATTSPITY	102	
SEQIDNO:44	AKQAPHQTLAARFMDGHITFQTAATVKIP-----	TTTPATTKNTATTSPITY	102	
SEQIDNO:45	ANQAPHQTLAARLMDGHITFQTAATIKTP-----	TTTPVTTKNTPTTSPIIY	102	
SEQIDNO:46	ANQAPHQTLAARLMDGHITFQTAATIKTP-----	TTTPVTTKNTPTTSPIIY	102	
SEQIDNO:47	TNQVPSQTLAARSMDGHILASQRAATSSSEPPTHTTVKTLVTTSLVTANSTPSSSPIIY		114	
SEQIDNO:48	TNHTPHKTLAAKSTDGHVTSQIATTVDSETLTHTTTITTLAATSLAATNSTPSTSPHTH		116	
SEQIDNO:49	-----	-----	0	
SEQIDNO:50	TNQVPHKTLATRSMDGQVTSQTAATVNPETPVTHTTIKTAAATSLVTTNSTLSTSPITN		116	
SEQIDNO:51	TNYVPHKTAAARSTDGHVTSQTVAKTSSSETLTNTTTIDVLATTSPVTTKSTLPTTPHTH		117	
SEQIDNO:52	TNHVPCNTAAAKSTDGHVTSQTVAKTSSPETLTNTTTIEVLVTTSPVTTQSTLPTTPHTH		113	
SEQIDNO:53	TNQTSHATLASRSKDDYIQTAETS--TFE----	DTAHITMKTAIPVTTKSLPISSTSY	107	
SEQIDNO:54	INQRSHITLASRFKDDYIQMAAETS--AIE----	NTAHITMKTVTPVTTKSLPPISSASY	108	
SEQIDNO:55	-----	-----MDRVSLSTILLY	14	
LAMP HOMOLOGY DOMAIN 1				
SEQIDNO:3	TLVT-----TQATPNNSETAPPVTEVTVGPSLAPYSLPPTI--	TPPAHTTGTSSSTV	152	
SEQIDNO:44	TLVT-----TQATPNNSETAPPVTEVTVGPSLAPYSLPPTI--	TPPAHTTGTSSSTV	152	
SEQIDNO:45	TLVT-----TQATSNNSHTAPPLTKVTVGPSLAPYSLPPTI--	TPPAHTTGTSSSTV	152	
SEQIDNO:46	TLVT-----TQATSNNSHTAPPLTKVTVGPSLAPYSLPPTI--	TPPAHTTGTSSSTV	152	
SEQIDNO:47	TLVT-----TIVTPNNSTAAAPVTEATIGPSADFGSLPTTS--	TPLAHTTTRTNPSTL	164	
SEQIDNO:48	TLFT-----TLATPNTSHMAAPVTEAAISPSAGLSSLLPTI--	IPPAHTTGTSSSTL	166	
SEQIDNO:49	-----	-----	0	
SEQIDNO:50	TLLT-----TLATPDNTHTTTTPVTEATIGPSAGPGSPPTTIITTTSSAYTTGTRSSSTV		168	
SEQIDNO:51	TLVT-----TLATPNKSHVTFPVTEAKVGLSVGPSSEPPVTV--	NPTAHTTGNRPSTA	167	
SEQIDNO:52	TLVT-----TLATPSKSHVTFPVTEAKAGLSIGPSSPPVTI--	NPAHTTGNRPSTA	163	
SEQIDNO:53	TFV-----RTNNSHMTASSTEDTIGSGSITHL-----	PFPTTRASLAIV	146	
SEQIDNO:54	TFV-----RSNNAHMTASSTDDTIGSGSIAHL-----	FVPTTRASLAIV	147	
SEQIDNO:55	GLLYINDAYSSENTFAQPSNTTTTPAINTTTHVTSNTTTLAP-----	NTTT	59	

FIGURE 5 cont.

		LAMP HOMOLOGY DOMAIN 2	
		3	4
SEQIDNO:3		QGIKHAIVVMFQTAVGHSHFKCVSEQSLQLSAHLQLKTTDVQLQAFDFEDDHFGNVDECSSD	379
SEQIDNO:44		QGIKHAIVVMFQTAVGHSHFKCVSEQSLQLSAHLQLKTTDVQLQAFDFEDDHFGNVDECSSD	381
SEQIDNO:45		QGMKHAIVVMFQTAVGHSHFKCVSEQSLQLSAHLQLKTTNVQLQAFDFEDDHFGNVDECSSD	379
SEQIDNO:46		QGMKHAIVVMFQTAVGHSHFKCVSEQSLQLSAHLQLKTTNVQLQAFDFEDDHFGNVDECSSD	379
SEQIDNO:47		QGMKSSVVMFETGVGHSHFKCVSEQSLQLSTHLQLKTMNVQFQAFDFEDDHFGNVDECSSD	391
SEQIDNO:48		QGMKNAVVMFETMIGHSHFKCVSEQSLQLSPHLQLNTMNVQLQAFDFEDDHFGNVDECSSD	393
SEQIDNO:49		VGLVDNEKLFKAKNGLSFNCKSETMLILADYFRKLKLVPLQIQAFDLVNGAFGKEVEECWAD	220
SEQIDNO:50		QGMKSPVVMFETVIGHSHFKCVSEQSLQLSTHLQLKTTNVQLQAFDFEDDHFGNVDECSSD	395
SEQIDNO:51		QGLKHAMMMFETVVGHSFKCVSEQSLQLSTYLQLKTMNVQLQAFDFEDDHFGNADECISD	394
SEQIDNO:52		QGMKYAMMMFETVVGHSFKCVSEQSLQLSNHLQLKTVNVQLQAFDFEDDHFGNADECISD	390
SEQIDNO:53		QGKS-TMMMFETVVGHSFKCVSEQSLQLSAQLQMKTMNIHLQAFDFEGDSFGIVDECLSD	371
SEQIDNO:54		QGKKNLTMMMFETVVGHSFKCVSEQSLQLSAQLQMKTMNIHLQAFDFEGDSFGVNECLSD	374
SEQIDNO:55		GNAT-NQKLLKTENGYSVKCKNTPKIQLGDTMNLVMTNVKLQVFNFKDNSFGKETTCKYD	277
		. : : : * * . : * . : : : : * * : : * *	

Trns Memb. Cyto. Tail

SEQIDNO:3	YTIV-LPV-IGAIVVGLCLMGMGVYKIRLRQSSGYQRI	416
SEQIDNO:44	YTIV-LPV-IGAIVVGLCLMGMGVYKIRLRQSSGYQRI	418
SEQIDNO:45	YTIV-LPV-IGAIVVGLCLVGIGVYKIRLRQSSGYQRI	416
SEQIDNO:46	YTIV-LPV-IGAIVVGLCLVGMGVYKIRLRQSSGYQRI	416
SEQIDNO:47	YTVV-LPV-IGAIVLGLCAVGLIVYGIHLRRESSGYQRI	428
SEQIDNO:48	YTIV-LPV-IGAIVLGLCAVGLIVYGIHLKRESSEYQRI	430
SEQIDNO:49	YNKRMIPIILGAVAAAICLIAILTYVLVREHRNQGYEQL	259
SEQIDNO:50	YTVV-LPV-IGAIVLGLFAVGLIVYGVVRREASGYQRI	432
SEQIDNO:51	RNRREIPVAVGLSIAVLLAVLLTACLVTTRKRPSRGYERM	433
SEQIDNO:52	RNRREIPVAVGLSIAVLLAVLLTACLVTTRKRPSRGYERM	429
SEQIDNO:53	YTVV-LPV-VGIIVVLCVVGGLGIYKIRQRQSSAYQRI	408
SEQIDNO:54	YTVV-LPM-VAIIVVICVVGGLSVYKIRQRQSSAYQRI	411
SEQIDNO:55	HNFG-LMI-AGIVIVVIVLGVIIYFIWHKRKSSGYQRI	314
	. : : . : : : : * : :	

LAMP-3					
Accession No.	Species	SEQ ID NO:	Accession No.	Species	SEQ ID NO:
NP_055213.2	<i>H. sapiens</i>	3	XP_001496333.1	<i>E. caballus</i>	50
XP_001155195.3	<i>P. troglodytes</i>	44	NP_001095605.1	<i>B. Taurus</i>	51
XP_003894825.1	<i>P. Anubis</i>	45	XP_004003158.1	<i>O. aries</i>	52
NP_001028044.1	<i>M. mulatta</i>	46	NP_001012015.1	<i>R. norvegicus</i>	53
XP_848889.2	<i>C. lupus familiaris</i>	47	NP_796330.2	<i>M. musculus</i>	54
XP_003358746.1	<i>S. scrofa</i>	48	XP_002936919.2	<i>X. tropicalis</i>	55
XP_001342688.2	<i>D. rerio</i>	49			

FIGURE 6: HUMAN LIMP-2 ALIGNMENT WITH ORTHOLOGOUS SEQUENCES

		SIGNAL SEQUENCE
SEQIDNO: 4	-----	MGRCCFYTAGTLS 13
SEQIDNO: 56	-----	MGRCCFYTAGTLS 13
SEQIDNO: 57	-----	MGRCCFYTAGTLS 13
SEQIDNO: 58	-----	MGRCCFYTVGTLS 13
SEQIDNO: 59	-----	MGRCCFYAVGTLS 13
SEQIDNO: 60	-----	MGRCCFYTAGTLS 13
SEQIDNO: 61	-----	MPRRSCTIYATGIVC 15
SEQIDNO: 62	-----	MARCCFYTAGTLS 13
SEQIDNO: 63	-----	MRSCLCLVTGVLA 13
SEQIDNO: 64	-----	MVKWAVFGTAAVS 13
SEQIDNO: 65	MQLDDILHINNCKADCSSLSTTPNPKTDLVNMNGPKHKFCTKLSSTYLKRWKITIV--VA 58	
SEQIDNO: 66	-----	MYGRSNRLCAKLSSAFLRKWWFVIA--FA 27
		. . .

LAMP HOMOLGY DOMAIN

SEQIDNO: 4	LLLLVTSVTLVARVTFQKAVDQSIKKIVLRNGTEAFDSWEKPPLPVYTQFYFFNVTNPE 73
SEQIDNO: 56	LLLLVTSVTLVARVTFQKAVDQSIKKIVLRNGTEAFDSWEKPPLPVYTQFYFFNVTNPE 73
SEQIDNO: 57	LLLLVTSVTLVARVTFQKAVDQSIKKIVLRNGTEAFDSWEKPPLPVYTQFYFFNVTNPE 73
SEQIDNO: 58	LLLLVTSIALVARVTFQKAVDQTIKNI VLRNGSETFDSWKKPPLPVYAQFYFFNVTNPE 73
SEQIDNO: 59	LLLLVTSITLLVARVTFQKAVDQTIKNI VLRNGSETFDSWKKPPLPVYTQFYFFNVTNPE 73
SEQIDNO: 60	LLLLVTSVTLVARVTFQKAVDQTIKNI VLRNGSETFDSWKKPPLPVYTQFYFFNVTNPE 73
SEQIDNO: 61	AHLLIILGIALLLAQVFQTMIOERIKKEITLAENSRVLDGWINPPPPVYMQYFFNVTNPD 75
SEQIDNO: 62	LLLLVTSVTLVARVTFQKAVDQTIKNI VLRNGSETFDSWKKPPLPVYTQFYFFNVTNPE 73
SEQIDNO: 63	LTLLIASISLLVAHVFTVVDLQVKGQTVLNGTETFEAWEDPPPPVYMQYFFNVTNPE 73
SEQIDNO: 64	VTLLIVSIVLLLTHTFMDIVEGOVQQAIVLKNSEVFEDWANPPPPVYMQYFFNVTNPE 73
SEQIDNO: 65	AALIIG--GIVVACEFTVLIDAVVDRMVALRPGAKTFGWAAKPPVEPRISLYIYNVTNAD 116
SEQIDNO: 66	LSLLVL--GALVTFGFTAFIRTIIDHQVALRVGGQSPGWWSRPPVEPIIRIFVYNVTNAD 85
	*:: : : * : : : . * . : * ** : : : ****

LAMP HOMOLGY DOMAIN

SEQIDNO: 4	EILRGE-TPRVEEVGPYTYRELRNKANIQFGDNGTTISAVSNKAYVFERDQSVGDPKIDL 132
SEQIDNO: 56	EILRGE-TPRVEEVGPYTYRELRNKANIQFGDNGTTISAVSNKAYVFERDQSVGDPKIDL 132
SEQIDNO: 57	EILRGE-TPRVEEVGPYTYRELRNKANVQFGDNGTTISAVSNKAYVFERDQSVGDPKIDL 132
SEQIDNO: 58	EILRGE-IPRLEEVGPHYTYRELRDKADIQFGDNGTTISAVSNKAYVFERDQSVGDPKIDL 132
SEQIDNO: 59	EILNGE-TPRLEEVGPHYTYRELRNKDDIQFGDNGTTISAVSNKAYVFERDQSVGDPKIDL 132
SEQIDNO: 60	EILQGE-IPLLEEVGPHYTYRELRNKANIQFGENGTTISAVTNKAYVFERDQSVGDPNVDL 132
SEQIDNO: 61	EFLAGKEKAKVTQMGPHYTYREYRPRENVTYLENGTKIFATNPKSFVFLRNMSAGDPFVDL 135
SEQIDNO: 62	EILQGE-IPLLEEVGPHYTYRELRNKANVQFGENGTTISAVTNKAYIFERNQSVGDPTVDL 132
SEQIDNO: 63	EVLQGA-TPLVEEKGPHYTYREYRPVHVQFLDNGTKVSALNPKTYVFEPEKSVGNPEVDL 132
SEQIDNO: 64	EVLSGE-KPFVDEIGPHYTYREYRPRENITFSVNGTEVSAVTPKTYVFEPEKSIGDPKVDL 132
SEQIDNO: 65	DFLSNGSKAIVDEVGPYVYSETEWVKVNIENDNGTL-SYNLRKIYSFREDLSVG-PEDDV 174
SEQIDNO: 66	EFLNNGTKPILDELGPYVYVQTEWVKVNIENPNGTI-SYNQKRVYIFNEDLSGG-LEDDV 143
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FIGURE 6 cont.

LAMP HOMOLOGY DOMAIN

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SEQIDNO:4      IRTLNIPVLTVIEWSQ-V-HFLREIIEAMLKAYQQKLFVTHTVDELLWGYKDEILSLIHV 190
SEQIDNO:56     IRTLNIPVLTVIEWSQ-V-RFLREIIEAMLKAYQQKLFVTHTVDELLWGYKDEILSLIHV 190
SEQIDNO:57     IRTLNIPVLTVIEWSQ-V-HFLREIIEAMLKAYQQKLFVTHTVDELLWGYKDEILSLIHV 190
SEQIDNO:58     IRTLNIPAVTAMEWAN-L-HFFREIIEALLKAYQQTLFVTHTVDELLWGYKDEILSLINV 190
SEQIDNO:59     LRTLNIPLALTAMEWTQ-L-PLLRDIEEALLKAYRQKLFVTHTVDELLWGYKDEILSLINT 190
SEQIDNO:60     IRTINIPLLTVVDLAQ-L-TLLRELIIEAMLKAYQQKLFVIHTVHELLWGYKDEILSLVHI 190
SEQIDNO:61     VTTVNIPMIAMNELNSYSFFVRTAVSMYMGSMGMLFMNRTVHEILWGFKDPLLTKLHA 195
SEQIDNO:62     IRTINIPLLTVVEMAQ-Q-PFLREIIEAMLKAYQQTLFVTHTVHELLWGYKDEVLSLVHI 190
SEQIDNO:63     IRTINVPVAVTAMEWTR-A-TSLQFATEVLLLLLYQESLFTVRTVHELLWGYKDKLLSTIHV 190
SEQIDNO:64     IRTVNIPLVITILEMTK-DSSLLRPFIIAALKTYKEGMFVTRTVDELLWGYKDAVLSILHP 191
SEQIDNO:65     VIVFNIPMLSATSQS KHAARFLRLAMASIMDI LKIKPFVQVSVGQLLWGYEDPLLKLAKD 234
SEQIDNO:66     VIVFNIPMLSATSQS KHAARFLRLAMASIMDI LKIKPFVEVSVGQLLWGYEDPLLKLAKD 203
                : . * : * : . . . : . : * : * : * : * : * : * :

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LAMP HOMOLOGY DOMAIN

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SEQIDNO:4      FRPDI-----SPYFGLFYKNGTNDGDYVFLTGEDSYLNFTKIVEWNGKTSLDWWTIDKCN 246
SEQIDNO:56     FRPDI-----SPYFGLFYKNGTNDGDYVFLTGEDSYLNFTKIVEWNGKTSLDWWTIDKCN 246
SEQIDNO:57     FRPDI-----SPYFGLFYKNGTNDGDYVFLTGEDSYLNFTKIVEWNGKTSLDWWTIDKCN 246
SEQIDNO:58     FKPEI-----SPYFGLFYKNGTNDGDYVFLTGEDSYLNFTKIVEWNGKTSLDWWTIDKCN 246
SEQIDNO:59     FKHDV-----SPYFGLFYKNGTNDGDYVFLTGEDSYLNFTKIVEWNGKTSLDWWTIDKCN 246
SEQIDNO:60     FKPDV-----SPNFGFLFYERNGTNDGEYVFLTGEDSYLNFTKIVEWNGKTSLDWWTIDKCN 246
SEQIDNO:61     MRPEV-----DEHFGLMYNKNGTHEGEFVFHTGEKNYMNKGKIDTWNGISQMNWWSNQS 251
SEQIDNO:62     FRPDV-----SPNFGFLFYERNGTNDGEYVFLTGEDSYLNFTKIVEWNGKTSLDWWTIDKCN 246
SEQIDNO:63     LHPEI-----DPVFGFFNKMNGTDDGEYVFLSGEMNYLNFTSRIVEWKGKESLNWWTIDKCN 246
SEQIDNO:64     FKKNI-----SDTFGLFYKMNTTDDGEYIFLSGKDYLEFTQIAEWKGQKALNWWTIDKCN 247
SEQIDNO:65     VVPKEQKLPYEEFGLLYGKNGTSSDRVTVNTGVDDIRRYGIIDNFNGRTHLPHWTTIDACN 294
SEQIDNO:66     VVPKEQKLPYEEFGLMYGKNSTSKDTFTVWTVGDDITQYGIIDKYNGRSHQTHWLSEQC 263
                . . . ** : * * . . . : * . . : * : * : * : * : * : *

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LAMP HOMOLOGY DOMAIN

2

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SEQIDNO:4      MINGTDGDSFHPLITKDEVLYVFPSDFCRSVYITFSDYES-VQGLPAFRYKVP AEILANT 305
SEQIDNO:56     MINGTDGDSFHPLITKDEVLYVFPSDFCRSVYITFSDYES-VQGLPAFRYKVP AEILANT 305
SEQIDNO:57     MINGTDGDSFHPLITKDEVLYVFPSDFCRSVYITFSDYES-VQGLPAFRYKVP AEILANT 305
SEQIDNO:58     MINGTDGDSFHPLIDKDEILYVFPSDFCRSVYITFSDYES-VQGLPAFRYKVP AEILANT 305
SEQIDNO:59     MINGTDGDTFHPLITRDEVLYVFPSDFCRSVYITFSDYES-VQGLPALRYKVP AEILANT 305
SEQIDNO:60     MINGTDGDSFHPLISKDEVLYVFPSDFCRSVYITFSSFEN-VEGLPAFRYKVP AEILANT 305
SEQIDNO:61     MINGTDGDSVFHTFLSRKELLYIFAADLCRSIHLGYVRDME-VKGIPAFRFP APPSDVLAPP 310
SEQIDNO:62     MINGTDGDSFHPLISKDETLYIFPSDFCRSVYITFSSFEN-VEGLPAFRYKVP AEILANT 305
SEQIDNO:63     MINGTDGTSFHPLISKDENIYIFSSDFCRSLYLVYDSSGS-VAGVPTYRFVPS PMVFANT 305
SEQIDNO:64     MINGTDGTSFHPLLNKDDTIYMFSSDLCRSIYAVYESSSEN- IKDISVFRFSP PASVFANV 306
SEQIDNO:65     TLAGTDGSIFFPHIDHDRI LHVYDKDLCRLLPLVFKEKVTMSNEVPGYRFT PPEWVFALV 354
SEQIDNO:66     RLNGTDGSIFFPRITKNSTLHVYDKDLCRLLPLSFEKEVTVRGGVKGYRFT PPSDFVASV 323
                : **** * : . . : : : : : * : : * : : *

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FIGURE 6 cont.

LAMP HOMOLOGY DOMAIN

	3	4	5	
SEQIDNO: 4	---SDNAGFCIPE-GNCLGSGVLNVSICKNGAPIIMSFPHFYQADERFVSAIEGMHP-NQ			360
SEQIDNO: 56	---SDNAGFCIPE-GNCLGSGVLNVSICKNGAPIIMSFPHFYQADERFVSAIEGMHP-NK			360
SEQIDNO: 57	---SDNAGFCIPE-GNCLGSGVLNVSICKNGAPIIMSFPHFYQADERFVSAIEGMHP-NK			360
SEQIDNO: 58	---SDNAGFCVPE-GNCLGSGVLNVSICKNGAPIIISFPHFYQADERFVSAIDGMRP-NK			360
SEQIDNO: 59	---SDNAGFCIPK-GNCLGSGVLNVSICKNGAPIIMSFPHFYQADERFVSAIGGMHP-NK			360
SEQIDNO: 60	---SENAGFCIPE-GNCMDSGVLNVSICKNGAPIIMSFPHFYQADERFVSAIKGMHP-NK			360
SEQIDNO: 61	DENPANAGFCVPE-GDCLGKGVLVSVCRQGAPIVVSFPHFYQADERYINAIEGMNP-NE			368
SEQIDNO: 62	---SENAGFCIPE-GNCMDAGVLNVSICKNGAPIIMSFPHFYQADERFVSAIKGMRP-NK			360
SEQIDNO: 63	TVNPDNAGFCVPE-GNCPGAGVLNVSICKQGAPIFLSAPHFYQADQKFVSDIEGMHP-TK			363
SEQIDNO: 64	SVNPQNKGFVPE-GNCLPSGLLVNVSICKQGAPIVLSSPHFYQADENVINSIRGMKP-VK			364
SEQIDNO: 65	DSHPDNMCFPCAGKPCS PNGLFNVSLCQYDSPIMLSFPHFYLADES LRQVEGISPPMK			414
SEQIDNO: 66	DKNPNMNCYCEAG-PPCAPHGLFNVSLCQYDSPILLSFPHFYMAQT LR TAVEGISPPEK			382
	*	:	*	*:::***: .:***: *****: .:***: *

LAMP HOMOLOGY DOMAIN

SEQIDNO: 4	EDHETFVDINPLTGIILKAAKRFQINIIYVKLLDDFVETGDIRTMVFPVMYLNESVHIDKE	420	
SEQIDNO: 56	EDHETFVDINPLTGIILKAAKRFQINIIYVKLLDDFVETGDIRTMVFPVMYLNESVHIDKE	420	
SEQIDNO: 57	EDHETFVDINPLTGIILKAAKRFQINIIYVKLLDDFVETGDIRTMVFPVMYLNESVHIDKE	420	
SEQIDNO: 58	DYHETFVDINPLTGIILRAAKRFQINIIYVKLLDDFIETGNI RTMVFPVMYINESV LIDKD	420	
SEQIDNO: 59	EYHETFVDINPLTGIILRAAKRFQINIIYVKLLDDFVETGNI QTLVFPVMYINESV LIDKE	420	
SEQIDNO: 60	EEHESFVDINPLTGIILRGAKRFQINTYVRKLLDDFVETGDIRTMVFPVMYLNESV LIDKE	420	
SEQIDNO: 61	EEHETYLDINPTIGVPIRACKRAQLNII LKRVRGFPNTKFLNETIIFPIMYVNETATIDDE	428	
SEQIDNO: 62	EEHESFVDINPLTGIILRGAKRFQINTYVRKLLDDFVETGNI RTMVFPVMYLNESV LIDKE	420	
SEQIDNO: 63	EYHETFVDINPLTGLVLQAAKRMQINIIHVRKLPEFFETGNI RTLIFPVMYINESV LIDEA	423	
SEQIDNO: 64	EHMTFLDLNPLTGTLIQAAKRIQVNVYVRKINVYLITQDIQTLFFPVMHNLNESV LIDDK	424	
SEQIDNO: 65	EKKQFFFDVQPKMGTTLRVARIQINLAVSQVVDIKQVANFPDII FPIILWFEEGIDNLPD	474	
SEQIDNO: 66	DKHQLFIDVQPDMGTTALRARIQINLAVSQVVDIKQVANFPDII FPIILWFEEGIDSLPD	442	
	: *	: .:***: *	: : *

	Trans. Domain	Cytoplasmic Tail
SEQIDNO: 4	TASRLKSMINTTLLIITNI	PYIIMALGVFFGLVFTWLACKQGQSMDEGTADERAPLI
SEQIDNO: 56	TASRLKSMINTTLLIITNI----	PYIIMALGVFFGLVFTWLACKQGQSMDEGTADERAPLI
SEQIDNO: 57	TASRLKSVINTTLLIITNI----	PYIIMALGVFFGLVFTWLACKQGQSMDEGTADERAPLI
SEQIDNO: 58	TASRLKSVINTTLLIITNI----	PYIIMALGVFFGLVFTWLACKQGQSMDEGTADERAPLI
SEQIDNO: 59	TASRLKSVINTTLLIVTNI----	PYIIMALGVFFGLVFTWLACKQGQSMDEGTADERAPLI
SEQIDNO: 60	TANQLKSVINTTLLIVTNI----	PYIIMALGVFFGLVFTWLACKQGQSMDEGTADERAPLI
SEQIDNO: 61	SAAQMRMLLLIVTVSNF----	PVIILALGVILLVLI FLVCRNRQRKNEVKRIDFTEAF
SEQIDNO: 62	TASQLKSVINTTLLIVTNI----	PYIIMALGVFFGLVFTWLACKQGQSMDEGTADERAPLI
SEQIDNO: 63	SANKLKHVLEASVVTGI----	PEVIMAIGIVFGIVFSVLVCRAQGAREESTEEERSFLI
SEQIDNO: 64	SAGRLRSILFQGRVVANI----	PFIMGLGII LAFLFTTSLCQKRSRDEGTEEERGFLI
SEQIDNO: 65	EVTDL--MRFAEQVPPKIRVALIVGLCALGVILLLLSTF--	CLIRNSHRQSTLHLEGSNY
SEQIDNO: 66	EILD--MKVATNIPRAKFI LTALFGLGGFLFVVAVI--	CLVRKSHRQSTLHLEGSNY
	: :	: .:***: *

FIGURE 6 cont.

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SEQIDNO:4      RT----- 478
SEQIDNO:56     RT----- 478
SEQIDNO:57     RT----- 476
SEQIDNO:58     RT----- 478
SEQIDNO:59     RT----- 478
SEQIDNO:60     RT----- 478
SEQIDNO:61     HSFATTKDETAYTQVSNQAEDSPENRNNQPLRNGSYIAMS PVEAQKC 531
SEQIDNO:62     RT----- 478
SEQIDNO:63     RT----- 481
SEQIDNO:64     RAS----- 483
SEQIDNO:65     LATA-----QVDMNKKQNKDNQPARY----- 551
SEQIDNO:66     LATA-----SVDAQAKKKAKMDNGMSSKSN----- 522

```

1

LIMP-2					
Accession No.	Species	SEQ ID NO:	Accession No.	Species	SEQ ID NO:
NP_005497.1	<i>H. sapiens</i>	4	NP_775366.1	<i>D. rerio</i>	61
XP_517214.2	<i>P. troglodytes</i>	56	NP_446453.1	<i>R. norvegicus</i>	62
XP_001096458.1	<i>M. mulatta</i>	57	XP_420593.1	<i>G. gallus</i>	63
XP_005639134.1	<i>C. lupus familiaris</i>	58	NP_001016557.1	<i>X. tropicalis</i>	64
NP_001095623.1	<i>B. Taurus</i>	59	NP_726504.2	<i>D. Melanogaster</i>	65
NP_031670.1	<i>M. musculus</i>	60	XP_314345.2	<i>A. gambiae</i>	66

FIGURE 7: HUMAN LIMBIC/SLAMP ALIGNMENT WITH ORTHOLOGOUS SEQUENCES

SIGNAL SEQUENCE		LAMP HOMOLGY DOMAIN	
		1	
SEQIDNO: 67	-MVRRVQPDNRKQLPLV-LLRLLCL-LPTGLPVRSDENRGTDNITVRQGDTAILRCVVED	57	
SEQIDNO: 68	-MVRRVQPDNRKQLPLV-LLRLLCL-LPTGLPVRSDENRGTDNITVRQGDTAILRCVVED	57	
SEQIDNO: 69	-MGARVQPDNRKQLPLV-LLRLLCL-LPTGLPVRSDENRGTDNITVRQGDTAILRCVVED	57	
SEQIDNO: 70	-MVGRVQPDNRKQLPLV-LLRLLCL-LPTGLPVRSDENRGTDNITVRQGDTAILRCVVED	57	
SEQIDNO: 71	-MVGRSQSDNRKQLPLF-LLRLLCL-LPTGLPVRSGDENRSTDNMTVRQGDTAILRCFVED	57	
SEQIDNO: 72	----MSCLWIHSVFIPGFF--LLF-GFEGFPVISVESQRSTDNITVRQGDFTVIRCYVDD	53	
SEQIDNO: 102	-MVGRVQPDNRKQLPLV-LLRLLCL-LPTGLPVRSDENRGTDNITVRQGDTAILRCVVED	57	
SEQIDNO: 103	-MVGRVQPDNRKQLPLV-LLRLLCL-LPTGLPVRSDENRGTDNITVRQGDTAILRCVVED	57	
SEQIDNO: 104	-MVARVQPDNRKQLPLV-LLRLLCL-LPTGLPVRSDENRGTDNITVRQGDTAILRCVVED	57	
SEQIDNO: 105	-MVARVQPDNRKQLPLV-LLRLLCL-LPTGLPVRSDENRGTDNITVRQGDTAILRCVVED	57	
SEQIDNO: 106	-----MSFAGEAA-----SQILNKAEPLFISRSEAFKFAVGDTITLPCEVAS	42	
SEQIDNO: 107	-----MRPCLLHSIWMLGFVLCCLLSLQGLPVRSGDENRSTDNITVRQGDTAILRCFVED	54	
SEQIDNO: 108	-MLGARRPPRSQPLV-LLRLLCL-LPTGLPVRSDENRGTDNITVRQGDTAILRCVVED	57	
SEQIDNO: 109	-MVGRVHPDRKQLPLV-LLRLLCL-LPTGLPVRGVDFTRGTDNITVRQGDTAILRCYVED	57	
SEQIDNO: 110	-MVARAQPDNRKQLPLV-LLRLLCL-LPTGLPVRSDFTRGTDNITVRQGDTAILRCFVED	57	
SEQIDNO: 111	---MRTYW-LHSIWVL-GFFLSLF-SLQGLPVRSDFTRGTDNITVRQGDTAILRCYVED	54	
SEQIDNO: 112	-----MRPCLLHSIWMLGFVLCCLLSLQGLPVRSGDENRSTDNITVRQGDTAILRCFVED	54	
SEQIDNO: 113	MQVGRKSCWRQ--LQASFFRLLCL-IPFGFPVRSDMQRATDNITVRQGDTAILRCYVDD	57	
		:	:: :.. *** : * * .
		LAMP HOMOLGY DOMAIN	
		2	
SEQIDNO: 67	KN-SKVAWLNRSGIIFAGHDKWSLDPVELEKRHSLEYSRLRIQKVDVYDEGSYTCVQTQ	116	
SEQIDNO: 68	KN-SKVAWLNRSGIIFAGHDKWSLDPVELEKRHSLEYSRLRIQKVDVYDEGSYTCVQTQ	116	
SEQIDNO: 69	KN-SKVAWLNRSGIIFAGHDKWSLDPVELEKRHSLEYSRLRIQKVDVYDEGSYTCVQTQ	116	
SEQIDNO: 70	KN-SKVAWLNRSGIIFAGHDKWSLDPVELEKRHALEYSLRIQKVDVYDEGSYTCVQTQ	116	
SEQIDNO: 71	KS-SKVAWLNRSGIIFAVDDKWSLDPVELEKRSPFEYSRLRIQKVDVSDDEGPYICSVQTN	116	
SEQIDNO: 72	KV-SKVAWLNRSGIIFAGEDKWSLDPVELVTQGLEYSRLRIQKVDVDFDEGPYTCSIQTK	112	
SEQIDNO: 102	KN-SKVAWLNRSGIIFAGHDKWSLDPVELEKRHALEYSLRIQKVDVYDEGSYTCVQTQ	116	
SEQIDNO: 103	KN-SKVAWLNRSGIIFAGHDKWSLDPVELEKRHSLEYSRLRIQKVDVYDEGSYTCVQTQ	116	
SEQIDNO: 104	KN-SKVAWLNRSGIIFAGHDKWSLDPVELEKRHSLEYSRLRIQKVDVYDEGSYTCVQTQ	116	
SEQIDNO: 105	KN-SKVAWLNRSGIIFAGHDKWSLDPVELEKRHSLEYSRLRIQKVDVYDEGSYTCVQTQ	116	
SEQIDNO: 106	PGTYVLAWKRGIATLTAAGSVKVPDPVRIVN---GYSLQIRDAVPQDAGDYICQIAML	98	
SEQIDNO: 107	RS-SRVAWLNRSGIIFAGDDKWSLDPVELEKRSLLEYSRLRIQKVDVSDDEGPYTCSIQTK	113	
SEQIDNO: 108	KN-SKVAWLNRSGIIFAGHDKWSLDPVELEKRHSLEYSRLRIQKVDVYDEGSYTCVQTQ	116	
SEQIDNO: 109	KS-SKVAWLNRSGIIFAGHDKWSLDPVELEKRRTALEYSRLRIQKVDVYDEGSYTCVQTQ	116	
SEQIDNO: 110	RS-SKVAWLNRSGIIFAGEDKWSLDPVELEKRSPLEYSRLRIQKVDVYDEGSYTCVQTQ	116	
SEQIDNO: 111	RS-SKVAWLNRSGIIFAGEDKWSLDPVELEKRNPLEYSLRIQKVDVYDEGSYTCVQTQ	113	
SEQIDNO: 112	RS-SRVAWLNRSGIIFAGDDKWSLDPVELEKRSLLEYSRLRIQKVDVSDDEGPYTCSIQTK	113	
SEQIDNO: 113	KV-SKVAWLNRSGIIFAGQDKWSLDPVLDVTKGLEYSRLRIQKVDVYDEGSYTCVQTQ	116	
		;	** . * : * * : **** * . ***: :.. * * * * .;

FIGURE 7 cont.

		LAMP HOMOLGY DOMAIN	
		5	6
SEQIDNO:67	TGRQASLKCEASAVPAPDFEWYRDDTRINSANGLEIKSTEGQSSSLTVTNVTEEHYGNITC	290	
SEQIDNO:68	TGRQASLKCEASAVPAPDFEWYRDDTRINSANGLEIKSTEGQSSSLTVTNVTEEHYGNITC	290	
SEQIDNO:69	TGRQASLKCEASAVPAPDFEWYRDDTRINSANGLEIKSTEGQSSSLTVTNVTEEHYGNITC	290	
SEQIDNO:70	TGRQASLKCEASAVPAPDFEWYRDDTRINSANGLEIKSTEGQSSSLTVTNVTEEHYGNITC	290	
SEQIDNO:71	TGKQAILRCEASAVPAPDFEWYKDDTRINSAQGLEIRNTGSRSVLMVANVTEEHYGNITC	290	
SEQIDNO:72	VGQAGVLHCEASAVPQPEFEWYRDERRLSSSSQSLTIQVSGSRTVLVVANVTEEDYGNITC	285	
SEQIDNO:102	TGRQASLKCEASAVPAPDFEWYRDDTRINSANGLEIKSTEGQSSSLTVTNVTEEHYGNITC	290	
SEQIDNO:103	TGRQASLKCEASAVPAPDFEWYRDDTRITSANGLEIKSTEGQSSSLTVANVTEEHYGNITC	290	
SEQIDNO:104	TGRKASLKCEASAVPAPDFEWYRDDTRINSANGLEIKSTEGQSSSLTVTNVTEEHYGNITC	290	
SEQIDNO:105	TGRKASLKCEASAVPAPDFEWYRDDTRINSANGLEIKSTEGQSSSLTVTNVTEEHYGNITC	290	
SEQIDNO:106	EGQEAMLVCIHGESQPEVLWHKDTMQIDQTERHVIENRGARHTLIIRKVHPQDFGNYS	276	
SEQIDNO:107	TGKQAILRCEASAVPAPDFEWYKDDTRINSAQGLEIRNTGSRSVLMVANVTEEHYGNITC	287	
SEQIDNO:108	TGRKASLKCEASAVPAPDFEWYRDDTRINSANGLEIKSTEGQSSSLTVTNVTEEHYGNITC	290	
SEQIDNO:109	TGRQASLKCEASAVPAPDFEWYRDDTRINSANGLEIKSTEGQSSSLMTVNTEEHYGNITC	290	
SEQIDNO:110	TGRQALLRCEASAVPTPDFEWYRDDTRINSANGLEIKSTGQSLLMVANVTEEHYGNITC	290	
SEQIDNO:111	TGRQALLRCEASAVPTPDFEWYRDDTRINSANGLEIKSTGQSLLMVANVTEEHYGNITC	287	
SEQIDNO:112	TGKQAILRCEASAVPAPDFEWYKDDTRINSAQGLEIRNTGSRSVLMVANVTEEHYGNITC	287	
SEQIDNO:113	VGRNGTLRCEVTAVPTPEFEWYRDDKRLANTQSITIQTSGTTTSLTIANITEEDYGNITC	289	
		* : . * * . . * : . * : * : : . : * . * : : : . : * : * :	

Trns Memb.

SEQIDNO:67	VAANKLGVTNASLVLFPRGSGVRGINGSISLAVPLWLLAASLLCLL-SKC-----	338
SEQIDNO:68	VAANKLGVTNASLVLFPRGSGVRGINGSISLAVPLWLLAASLLCLL-SKC-----	338
SEQIDNO:69	VAANKLGVTNASLVLFPRGSGVRGINGSISLAVPLWLLAASLLCLL-SKC-----	338
SEQIDNO:70	VAANKLGVTNASLVLFPSKYAKTEPDSMQVIE-FLHIDLKSIKHPK-KVNPIQK-----	341
SEQIDNO:71	VAANKLGITNTSLYLYI-GPGTPIDNATSLAASLWLMANILLCLF-CTC-----	337
SEQIDNO:72	VATNRLGVHNASVFLYKPGMGRDINSAGCICQSLWLLLLCVSSAL-LQC-----	333
SEQIDNO:102	VAANKLGVTNASLVLFPRGSGVRGINGSISLAVPLWLLAASLFCCLL-SKC-----	338
SEQIDNO:103	VAANNLGVTNASLVLFPRGSGVRGINGSISLAVPLWLLAASLLCLL-SKC-----	338
SEQIDNO:104	VAANNLGVTNASLVLFPRGSGVRGINGSISLAVPLWLLAASLLCLL-SKC-----	338
SEQIDNO:105	VAANNLGMTNASLVLFPRGSGVRGINGSISLAVPLWLLAASLLCLL-SKC-----	338
SEQIDNO:106	IADNQLGKTRKTVTLTGKPKTAVF---RSVPNSQWKDKYNI SWIVDSHSPIEEFKLYYRQ	333
SEQIDNO:107	VAANKLGITNTSLYLYI-GPGTPIDSATSLAASLWLMANILLCLF-CTC-----	334
SEQIDNO:108	VAANNLGVTNASLVLFPRGSGVRGINGSISLAVPLWLLAASLLCLL-SKC-----	338
SEQIDNO:109	VAANKLGVTNASLILFRPGSGVRGINGSISLAVPLWLLAASLFCCLL-SKC-----	338
SEQIDNO:110	VAANKLGVTNASLYLYRPGTGRVDNGSVSLAVPLWLLAASLLCLL-SKC-----	338
SEQIDNO:111	VAANKLGVTNASLYLYRPGTGRVDNGSMSLAVPLWLLAASLLCLL-SKC-----	335
SEQIDNO:112	VAANKLGITNTSLYLYI-GPGTPIDSATSLAASLWLMANILLCLF-CTC-----	334
SEQIDNO:113	VASNRLGVQNASLFLYRPGTGRDINGSACVSQSLWLLLASFACLF-LKC-----	337
	: * * . * . : : * : : . : . : .	

FIGURE 7 cont.

SEQIDNO:67	-----	338
SEQIDNO:68	-----	338
SEQIDNO:69	-----	338
SEQIDNO:70	-----	341
SEQIDNO:71	-----	337
SEQIDNO:72	-----	333
SEQIDNO:102	-----	338
SEQIDNO:103	-----	338
SEQIDNO:104	-----	338
SEQIDNO:105	-----	338
SEQIDNO:106	MTFSIGQLQLQTDWRDIVLPAPFPYSHHYTQGMSYLRIGLEPDQOYEARVQSRNRYGWS	393
SEQIDNO:107	-----	334
SEQIDNO:108	-----	338
SEQIDNO:109	-----	338
SEQIDNO:110	-----	338
SEQIDNO:111	-----	335
SEQIDNO:112	-----	334
SEQIDNO:113	-----	337

SEQIDNO:67	-----	338
SEQIDNO:68	-----	338
SEQIDNO:69	-----	338
SEQIDNO:70	-----	341
SEQIDNO:71	-----	337
SEQIDNO:72	-----	333
SEQIDNO:102	-----	338
SEQIDNO:103	-----	338
SEQIDNO:104	-----	338
SEQIDNO:105	-----	338
SEQIDNO:106	FSESFLFTTSNTGKWMGQCCTNPG	417
SEQIDNO:107	-----	334
SEQIDNO:108	-----	338
SEQIDNO:109	-----	338
SEQIDNO:110	-----	338
SEQIDNO:111	-----	335
SEQIDNO:112	-----	334
SEQIDNO:113	-----	337

LIMBIC/LSAMP					
Accession No.	Species	SEQ ID NO:	Accession No.	Species	SEQ ID NO:
NP_002329.2	<i>H. sapiens</i>	67	NP_001192297.1	<i>B. Taurus</i>	105
XP_516662.2	<i>P. troglodytes</i>	68	XP_312298.5	<i>A.gambiae</i>	106
XP_002716722.1	<i>O. cuniculus</i>	69	NP_001096385.1	<i>X.tropicalis</i>	107
NP_780757.1	<i>M. musculus</i>	70	XP_003434117.1	<i>C. lupus familiaris</i>	108
NP_001086181.1	<i>X. laevis</i>	71	XP_001362972.1	<i>M. domestica</i>	109
NP_001034921.1	<i>D. rerio</i>	72	NP_990205.1	<i>G. gallus</i>	110
NP_058938.1	<i>R. norvegicus</i>	102	XP_002190582.1	<i>T. guttate</i>	111
XP_001502710.1	<i>E. caballus</i>	103	NP_001096385.1	<i>X. tropicalis</i>	112
NP_001231626.1	<i>S. scrofa</i>	104	XP_003449349.1	<i>O. niloticus</i>	113

Endolyn					
Accession No.	Species	SEQ ID NO:	Accession No.	Species	SEQ ID NO:
NP_006007.2	<i>H. sapiens</i>	5	NP_001039506.1	<i>B. taurus</i>	76
NP_058594.1	<i>M. musculus</i>	73	XP_004011265.1	<i>O. aries</i>	77
XP_001091286.1	<i>M. mulatta</i>	74	XP_532256.2	<i>C. lupus familiaris</i>	78
XP_001924661.2	<i>S. scrofa</i>	75	NP_114000.1	<i>R. norvegicus</i>	79

FIGURE 9: HUMAN MACROSAILIN ALIGNMENT WITH ORTHOLOGOUS SEQUENCES

	SIGNAL SEQUENCE	LAMP HOMOLOGY DOMAIN 1
	1	
SEQIDNO: 80	MRLAVLFSGALLGL-LAAQGTGNDCPHKKSATLLPSETVTPVTEST----	GTTSHRTTK 55
SEQIDNO: 81	MRLAVLFSGALLGL-LAAQGTGNDCPHKKSATLLPSETVTPVTEST----	GTTSHRTTK 55
SEQIDNO: 82	MRLAVFFSGALLGL-LAAQGTGNDCPHKKSATLLPSETVTPVTEST----	GTTSHRTTK 58
SEQIDNO: 83	MRLPVCL--ILLGP-LIAQGTEDCPHKKAATLLPSETVTPVTEST----	GTTSHRTTK 56
SEQIDNO: 84	MRLAVLFSGALLGL-LAAQGTGNDCPHKKSATLLPSETVTPVTEST----	GTTSHRTTK 55
SEQIDNO: 85	MRFPVCL--TLLVL-LVAQGTGKDCPHKKAATLLPSETVTPVTEST----	GTTSHRTTK 56
SEQIDNO: 86	MRLAVFFSGALLGL-LAAQGTGNDCPHKKSATLLPSETVTPVTEST----	GTTSHRTTK 58
SEQIDNO: 87	MRLAVLFSGALLGL-LAAQGTGNDCPHKKSATLLPSETVTPVTEST----	GTTSHRTTK 58
SEQIDNO: 88	MRLPVFLA-LLGL-HAA-----	S-GTTSHRTTK 27
SEQIDNO: 89	MTLAVLFSGALLGL-LAESTTSH-----	22
SEQIDNO: 90	MRLAVLFSGALLGL-LAAQGTGNDCPHKKSATLLPSETVTPVTEST----	GTTSHRTTK 55
SEQIDNO: 91	MRLSLLSGILLGL-LAEQAGBKCPQEKSVTLVPSFTVTIATERSTTSPETTTSSGS-	58
SEQIDNO: 92	-----MGLTLFLPAQGSQCRANCPHKKSATLVPSFTVTPTATSG----	PTTAAHQT 49
	:	
	LAMP HOMOLOGY DOMAIN 1	
SEQIDNO: 80	SHKTTTHRTTT-----TGTTSHGPTTATHNPPTTSHGNVTVHPTSNNSTATSQGPSTA	108
SEQIDNO: 81	SHKTTTHRTTT-----TGTTSHGPTTATHNPPTTSHGNVTVHPTSNNSTATSQGPSTA	108
SEQIDNO: 82	SHRTTWRISTTTHTTNTTGTSSSESPTATHSPA-----	92
SEQIDNO: 83	SHGNVTVH-----T-----SSGPTTVTHNPA-----	77
SEQIDNO: 84	SHKTTTHRTTT-----TGTTSHRPTTATHNPPTTSHRNATVHPTSNNSTATSQGPSTA	108
SEQIDNO: 85	SHRPTTTS-----HRPTTTSRPTTTSRPT-----TTS	85
SEQIDNO: 86	AETT-----SHAENTTHQ-----	72
SEQIDNO: 87	SHRTTTT-----GTTSDHPTTATHNP-----	79
SEQIDNO: 88	NPHTT-----SHSTATPGTTSRPTTAT-----	50
SEQIDNO: 89	--RTTTPR-----TTTTGTTSHGPTTVTHNPA-----	47
SEQIDNO: 90	SHKTTTHRTTT-----TGTTSHRPTTATHNPPTTSHRNATVHPTSNNSTATSQGPSTA	108
SEQIDNO: 91	--TATTYRTS-----TAATTPHS-----NSTATS-----	80
SEQIDNO: 92	DHGTTTSHETTTSQGTSTHGTSPTHTTTTGHGTT-TGHQN-----TSH-----	91
	LAMP HOMOLOGY DOMAIN 1	
SEQIDNO: 80	HSPATTSHGNATVHPT-SNSTATSPG-----F-T--SSAHPEPPPPSPSPSPSTSK	154
SEQIDNO: 81	HSPATTSHGNATVHPT-SNSTATSPG-----F-T--SSAHPEPPPPSPSPSPSTSK	154
SEQIDNO: 82	---TTTSHQNTTVHPT-SNITATSPG-----PST--RSPHPEP-PPSPSPSPGSK	135
SEQIDNO: 83	---TTTSHGNATISHATVSPTT-----NG-TATSPRSST--VGPHPGPPPPSP--SPRSK	124
SEQIDNO: 84	HRPATTSHGNATVHPT-SNSTATSPG-----L-T--SSAHPEPPPPSPSPSPASK	154
SEQIDNO: 85	HRPTTTSNATV-----SP-----TTNSPGFST--VGPHPGPPPPSPSPSPST	128
SEQIDNO: 86	-APTTPGHRNTTIHPTTSNSTSNTTGTGTGKPHSTSY--TQPGPGPPPPSPPGPGQ	129
SEQIDNO: 87	---TTTSHGNATVHPTTSNSTVTSFG-----SAS--SSPHPRPPPPSPSPSPGSK	124
SEQIDNO: 88	---PTTGHGNVTVHPTTSNTTSTTTTGTGTS---PGFST--STPHPGPPPPSPSPGSR	102
SEQIDNO: 89	---TTTSHGNATVHPT-SSSTATSPG-----SST--RPPHPGPPPPSPSPSPGQ	91
SEQIDNO: 90	HRPATTSHGNATVHPT-SNSTATSPG-----L-T--SSAHPEPPPPSPSPSPASK	154
SEQIDNO: 91	--YSTTSEGTAVTHGTTTSPRN-----TSTT--STSQSVFVPPSPQPTSSPS	123
SEQIDNO: 92	--STTTSHGTSTPHKTTTRHPTTSHGTTTSHGTSTGHWTARPTIRPGPPPPPP--SPG	145
	*	* * * *

FIGURE 9 cont.

LAMP HOMOLOGY DOMAIN

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SEQIDNO:80  ETIGDYTWTNGSQPCVHLQAQIQIRVMYTTQGGG-----EAWGISVLNPNK-TKVQ 204
SEQIDNO:81  ETIGDYTWTNGSQPCVHLQAQIQIRVMYTTQGGG-----EAWGISVLNPNK-TKVQ 204
SEQIDNO:82  EAIGDYTWSNGSQPCVRLQAQIQIRVLYPTQGGG-----EAWGISVLNPNR-TKAQ 185
SEQIDNO:83  GALGNYTWANGSQPCVQLQAQIQIRILYPIQGGGRVKLKWGLKRAWGISVLNPNK-TKVQ 183
SEQIDNO:84  ETIGDYMWTNGSQPCVHLQAQIQIRVMYTTQGGG-----EAWGISVLNPNK-TKVQ 204
SEQIDNO:85  GALGNYTWTNGSQPCVQLQAQIQIRILYLTQGGK-----KAWGLSVLNPNK-TKVQ 178
SEQIDNO:86  DAIGDYTWTNGSQPCARLQARIQIGVVYPTQAGG-----QAWGISVLNPNK-TKPW 179
SEQIDNO:87  EAIGDYIWTNGSQPCVRLQAQIQIRVLYPTLGGG-----KAWGISVLNPNK-TKAQ 174
SEQIDNO:88  EAVGNYTWTNGSQPCVQLQAQIQIRVLYPTQGGG-----QAWGMSVLNPNR-TKAQ 152
SEQIDNO:89  EAIGDYTWTNGSQPCVQLQAQIQIRVLYPTQGGG-----EAWGISVLNPNK-TKAL 141
SEQIDNO:90  ETIGDYMWTNGSQPCVHLQAQIQIRVMYTTQGGG-----EAWGISVLNPNK-TKVQ 204
SEQIDNO:91  GAVGDYIGANGSQLCVHLRAQIQMRVLYQASGGG-----KLWGI FVLNPNR-TMAQ 173
SEQIDNO:92  KAVGNYTVFNGSQPCRLRLRAEIRLWVLYQAQEEGEAPPVSG-----AASFPPPRPRPVA 199
      :*: * .*** * :*:*.*: :*: : *

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LAMP HOMOLOGY DOMAIN

3

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SEQIDNO:80  GSCEGAHPHLLLSF----PYGHLSFGFMQDLQQ--KVVYLSYMAVEYNVSFPHAAQWTF 258
SEQIDNO:81  GSCEGAHPHLLLSF----PYGHLSFGFMQDLQQ--KAVYLSYMAVEYNVSFPHAAQWTF 258
SEQIDNO:82  GGCEGTHSHLLLSF-----PSGQLSFGFKQDPLQ--SAVYLNMAVEYNVSFPQAVQWTF 239
SEQIDNO:83  GGCDGTHPHLSLSF-----PYGQLTFGFKQDLHQSPSTVYLDYMAVEYNVSFPQAAQWTF 239
SEQIDNO:84  GSCEGAHPHLLLSF----PYGHLSFGFMQDLQQ--RVVYLSYMAVEYNVSFPHAAQWTF 258
SEQIDNO:85  GGCDSAHPHLALSF----PYGQLTFGFKQDRHQSHSTVYLNMAVEYNVSFPQAAQWTF 234
SEQIDNO:86  GDCDGARPHLLLSF----PFGQLSFGFTQEPQQ--GSVYLDYLALQYNVSFPQAAQWTF 233
SEQIDNO:87  GGCA--HPHLLLSF----PYGQLSFGFKQEPQLQ--STVYLN YIAVEYNVSFPQAAQWTF 226
SEQIDNO:88  GGCEGPRPHLLLSF----PYGQLSFGFKQDPGQGQSAVYLSYLA VEYNVSFPQAARWTF 208
SEQIDNO:89  GGCEGAHPHVRLSF----PYGQLTFGFKQQPQE--STVYLNMAVEYNVSFPRAAQWTF 195
SEQIDNO:90  GSCEGAHPHLLLSF----PYGHLSFGFMQDLQQ--RVVYLSYMAVEYNVSFPHAAQWTF 258
SEQIDNO:91  GNCEANHSSLLLSF----PNGKLI FGFKQDSIK--KIVYLSHLATEFNVSFP SATRWIF 227
SEQIDNO:92  GEGDGRSRVTPVASAMTVEGGSRAEFAM----- 228
      *      ;      *      **

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LAMP HOMOLOGY DOMAIN

4

5

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SEQIDNO:80  AQNASLRDLQAPLGQSFSCSNSSIILSPAVHLDLLSLRLQAAQLPHTGVFGQSFSCPSDR 318
SEQIDNO:81  AQNASLRDLQAPLGRSFSCSNSSIILSPAVHLDLLSLRLQAAQLPHTGVFGQSFSCPSDR 318
SEQIDNO:82  VQNSSLRDLQTPLGHSFSCRNASIIIVSPALHLDLLSLKLQAAQLSPSGAFGPFSCPNDR 299
SEQIDNO:83  AQNSSLRDLQAPLGQSFCCGNASIVLSPAVHLDLLSLRLQAAQLPDKGHFGPCFSCNRDQ 299
SEQIDNO:84  AQNASLRDLQAPLGQSFSCSNSSIILSPAVHLDLLSLRLQAAQLPHTGVFGQSFSCPSDR 318
SEQIDNO:85  AQNSSLQELQAPLGQSFCCGNTSIVLSPAHLHLDLLSLRLQAAQLPDKGHFGPCFSCASDQ 294
SEQIDNO:86  GQNASLRDLQAPLGQSFSCRNASIIILTPALRLDHLHLKLQAAQLPFGSGAFGPFSCPSEH 293
SEQIDNO:87  VQNSSLRDLQAPLGQSFSCRNASIALSPAFHLDLLSLKLQAAQLTPTGAFGPFSCPSDQ 286
SEQIDNO:88  AQNASLRDLQAPLGQSFSCRNASIAVSPALHLDLLSLRVQAAQLPRTGIFGPFSCPADH 268
SEQIDNO:89  VQNSSLRDLQTPVGRSYSCRNASIIILSTAFHLDLLSLKLQAAQLPPTGNFGPFSCPSDQ 255
SEQIDNO:90  AQNASLRDLQAPLGQSFSCSNSSIILSPAVHLDLLSLRLQAAQLPHTGVFGQSFSCPSDR 318
SEQIDNO:91  VENSLLQDLQTPLGHSFSCRNRSIALSPDIHLDLLSLQLQAAQLSSSGAFGA AFSCSADL 287
SEQIDNO:92  -LGAEVRSRAPSLGRAGKTRL--RIHQPVVVLQ-----HTYYV----- 263
      .: : : :      *:      . *:      :

```

FIGURE 9 cont.

Trans. Domain Cyto Tail

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SEQIDNO:80  -SILLPLIIGLILLGLLLALVLIAPFCIIRRRPSAYQAL 354
SEQIDNO:81  -SILLPLIIGLILLGLLLALVLIAPFCIIRRRPSAYQAL 354
SEQIDNO:82  -SILLPLIIGLILLGLLLTLVLVTFCCIRRRPPTYQPL 335
SEQIDNO:83  -SLLLPLIIGLVLLGLLLTLVLIAPFCITRRRQSTYQPL 335
SEQIDNO:84  -SILLPLIIGLVLLGLLLALVLIAPFCIVRRRPSAYQAL 354
SEQIDNO:85  -SLLLPLIIGLVLLGLLLTLVLIAPFCVTRRRQSTYQPL 330
SEQIDNO:86  -FNLLPLIVGVISLGLLLALVTFCCIRRRPPTYQPL 329
SEQIDNO:87  -SILLPLIIGLILLGLLFAVLITFCVIRRRRPPTYQAL 322
SEQIDNO:88  PSILVPLIIGLILVGLLLALVLVAFCIARRRPSAYQAL 305
SEQIDNO:89  -TILLPLIIGLIFLGLLLTLVLVTFCCIRRRPPTYQPL 291
SEQIDNO:90  -SILLPLIIGLVLLGLLLALVLIAPFCIVRRRPSAYQAL 354
SEQIDNO:91  -NILVPLVVGVLVLLTLLILVLSAFCISRRPPTYQPL 323
SEQIDNO:92  ----- 263

```

Macrosallin					
Accession No.	Species	SEQ ID NO:	Accession No.	Species	SEQ ID NO:
NP_001242.2	<i>H. sapiens</i>	80	XP_849733.1	<i>C. lupus familiaris</i>	86
XP_003315403.1	<i>P. troglodytes</i>	81	NP_001093232.1	<i>E. caballus</i>	87
NP_001039367.1	<i>B. taurus</i>	82	XP_002719034.1	<i>O. aries</i>	88
BAA23738.1	<i>M. musculus</i>	83	XP_003131995.1	<i>S. scrofa</i>	89
XP_014974003.1	<i>M. mulatta</i>	84	XP_003912313.1	<i>P. anubis</i>	90
NP_001026808.1	<i>R. norvegicus</i>	85	XP_001369761.1	<i>M. domestica</i>	91
			XP_001517723.2	<i>O. anatinus</i>	92

FIGURE 10 cont.

LAMP HOMOMOLOGY DOMAIN

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```

SEQIDNO:93  KDAVSAGKHTANSHHLSALVTPAGKSYECQAQQTISLASSDPQKTVTMILSAVHIQPFDI 213
SEQIDNO:94  KDAVSAGKHTANSHHLSALVTPAGKSYECQAQQTISLASSDLQKTVTMILSAVHIQPFDI 213
SEQIDNO:95  KDAVSAGKHTANSHHLSALVTPAGKSYECQAQQTISLASSDPQKMTMILSAVHIQPFDI 213
SEQIDNO:96  KDAVSAGKHTANSHRLSALVTPAGKSYECQAQQTISLASSDPQKTVTMILSAVHIQPFDI 213
SEQIDNO:97  KDAVSAGKHTANSHHLSALVTPAGKSYECQAQQTISLASSDPQKTVTMILSAVHIQPFDI 213
SEQIDNO:98  KAPVKVNKYIASSHHLSALVTPAGMSYECQAQQTISLASSDPQKTVTMILSAVHIQPFDI 213
SEQIDNO:99  KDAVSAGKHTANSHHLSALVTPAGMSYECQAQQTISLASSDPQKTVTMILSAVHIQPFDI 213
SEQIDNO:100 KDAVSPGKHTASSHRLSALVTPAGKSYECQAQQTISLISSDHQKSVQLLLSEVRIQPFDI 232
SEQIDNO:101 KSGARPGRHTANSHHLSLMVTPAGMSYECQATQRISLTSTDHQKIVVLYLSEVHLQPFDI 209
*      .      .:: *.*:* :***** :***** : * * * * : * * * : * * :*****

```

Trans. Domain

Cyto Tail

4

```

SEQIDNO:93  ISDFVFSEEHKCPVDEREQLEETLPLILGLILGLVIMVTLAIYHVHHKMTANQVQIPRDR 273
SEQIDNO:94  ISDFVFSEEHKCPVDEREQLEETLPLILGLILGLVIMVTLAIYHVHHKMTANQVQIPRDR 273
SEQIDNO:95  ISDFVFSEEHKCPVDEREQLEETLPLILGLILGLVIVVTLTIYHVHHKMTANQVQIPRDR 273
SEQIDNO:96  ISDFVFSEEHKCPVDEREQLEETLPLILGLILGLVIVVTLAIYHIIHHKMTANQVQIPRDR 273
SEQIDNO:97  ISDFVFSEEHKCPVDEREQLEETLPLILGLILGLVIVVTLVIYHIIHHKMTANQVQIPRDR 273
SEQIDNO:98  ISDFVFSEEHKCPVDEREQLEETLPLILGLILGLVIVITLVIYHIIHHKMTANQVQIPRDR 273
SEQIDNO:99  ISDFVFSEEHKCPVDEREQLEETLPLILGLILGLVIVITLVIYHIIHHKMTANQVQIPRDR 273
SEQIDNO:100 TADFVFSEEHKCPVDQREQLEETLPLILGLILGLVIVITLCVYHIIHHKMTANQVQIPRDR 292
SEQIDNO:101 KSDFVYSEYKCPDQKQLEETLPLILGLITLGVAILIIVAVYHIIHHKMTANQVQIPRDR 269
:***:***:***.*:::***** :***:***: : :***:***:*****

```

```

SEQIDNO:93  SQYKHMG 280
SEQIDNO:94  SQYKHM 280
SEQIDNO:95  SQYKHM 280
SEQIDNO:96  SQYKHM 280
SEQIDNO:97  SQYKHM 280
SEQIDNO:98  SQYKHM 280
SEQIDNO:99  SQYKHM 280
SEQIDNO:100 SQYKHM 299
SEQIDNO:101 SLYKHM 276
* *****

```

LAMP5

Accession No.	Species	SEQ ID NO:	Accession No.	Species	SEQ ID NO:
NP_036393.1	<i>H. sapiens</i>	93	NP_001076887.1	<i>B. taurus</i>	97
XP_514512.3	<i>P. troglodytes</i>	94	NP_083806.2	<i>M. musculus</i>	98
NP_001181627.1	<i>M. mulatta</i>	95	NP_001014205.1	<i>R. norvegicus</i>	99
XP_850634.1	<i>C. lupus familiaris</i>	96	XP_004935300.1	<i>G. gallus</i>	100
			NP_001090781.1	<i>X. tropicalis</i>	101

Figure 11A**SEQ ID NO:114**

LOCUS YP_009111421 817 aa linear VRL 13-AUG-2018
 DEFINITION large T antigen [Merkel cell polyomavirus].
 ACCESSION YP_009111421
 VERSION YP_009111421.1

```

1   mdlvlnrker ealcklleia pncygniplm kaafkrsclk hhpdkggnpv immelnltlws
61  kfqqnihklr sdfsmfdevd eapiygttkf kewwrsqgfs fgkayeygpn phgtnsrsrk
121 pssnasrgap sgsspphsqs sssgygsfsa sqasdsqrg pdippehhee ptsssgsssr
181 eettnsgres stpnqtsvpr nssrtdgtwe dlfcdeslss peppssseep eeppssrssp
241 rqpsssaase asssqftdee yrsssfthtpk tpppfsrkrk fggsrassass assasftstp
301 pkpkknretp vptdfpidls dylshavysn ktvscfaiyt tsdkaielyd kiekfkvdfk
361 srhacelgci llfitlskhr vsaiknfest fctisflick gvnkmpemyn nckkppykll
421 qenkpllnye fqekekeasc nwnlvaeafac eyelddhfii lahyldfakp fpcqkcenrs
481 rlkphkahea hhsnaklfye sksqkticqg aadtvlakrr lemlemtrte mlckkfkckhl
541 erlrdldtid llyymggvaw yccfleekek klqkiiqlt enipkyrniw fkgpingsgt
601 sfaaalidll egkalnincp sdklpfelgc aldkfmvffe dvkgqnslnk dlqpgqgin
661 ldnlrhdldg avavslekkh vnkkhqifpp civtandyfi pktliarfsy tlhfskpanl
721 rdsldqnmei rkrrilqsgt tlllcliwcl pdttfkpcql eeiknwkqil qseisygkfc
781 qmienveagq dpllniliee egpeeteetq dsgrtfsq

```

Truncated Large T (LT) 220A (SEQ ID NO:115 and 116)

```

MDLVLNKEREALCKLLEIAPNICYGNIPLMKAFAKRSCLKHHPDKGGNPVIMMELNLTLSKFEQQNIHK
LRSDFSMFDEVDEAPIYGTITKFKEWNRSGGTFEGKAYEYGNPHGTNSRSRKPSSNASRGAPSGSSPE
HSQSSSSSGYGSFSASQASDSQSRGPDIPPEHHEEPTSSSSGSSSREETTNSGRESSTPNQTSVPRNSSR
TDGTWEDLEFCDESLSXPEPPSSSEEPPEPPSSSRSSPRQPPSSSAEEASSSQFTDEEYRSSSFTHPKTP
PPFS[REDACTED]FGGSRSSASSASSASFTSTPPKPKKNRETPVPTDFPIDLSDYLSHAV

```

X can be any amino acid. In preferred embodiments, X is a alanine (S220A).

Red text is RB binding motif necessary for cell proliferation activity

Purple highlight is nuclear localization signal

Regions known not to be relevant for protein function shown in grey

Figure 11A – continued

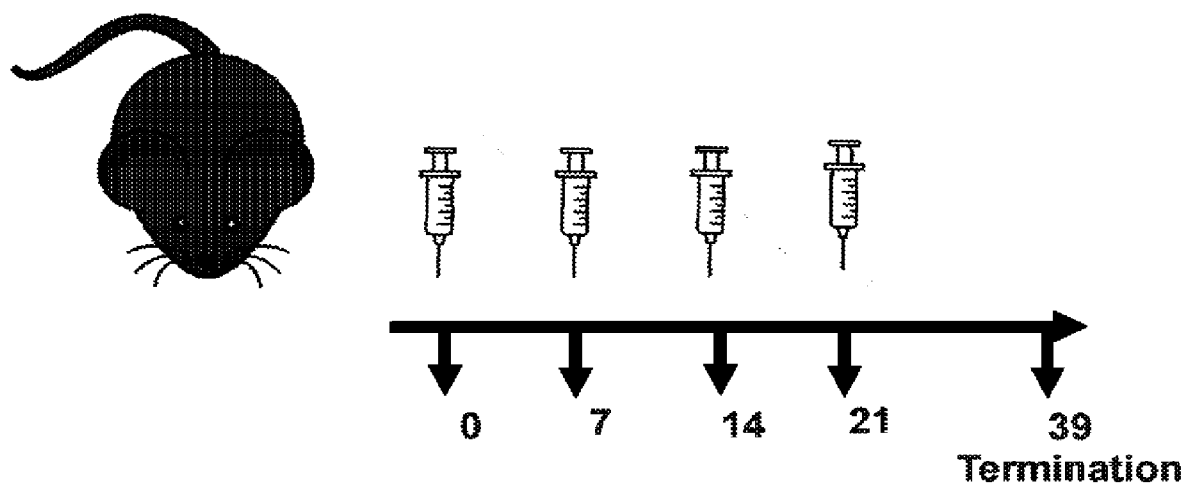
MAPRSARRPLLLLLLLLLGLMHCAAMFMVKNGNGTACIMANFSAAFSVNYDTKSGPKNMTLDLPSDATVV
ENRSSCGKENTSDPSLVIAFGRGHTLTNFTRNATRYSVQLMSFVYNISDTHLPNAASSKEIKTVESITDIRADIDK
KYRCVSGTQVHMNNVTVTLHDATIQAYLSNSSFSRGETRCEQDLEM**MDLV****LNRKEREALCKLLEIAPNCYGNIPLM**
KAAFKRSCLKHHPDKGGNPVIMMELNTLWSKFQQNIHKLRSDFSMFDEVDEAPIYGTTFKEWWRSGGFSFGKA
YEYGNPHGTNSRSRKPPSSNASRGAPSGSSPPHSQSSSSGYGSFSASQASDSQSRGPDIPPEHHEEPTSSSGSSSREE
TTNSGRESSTPNGTSVPRNSSRTDGTWEDLFCDELSAPEPPSSSEEPEEPPSSRSPRQPPSSSAEEASSSQFTDEEY
RSSSFTTPKTPPPFSRKRKEGGSRSSASSASSASFTSTPPKPKKNRETPVPTDFPIDLSDYLSHAVEFTCLASMGLOL
NLTVERKDNTTVTRLLNINPNKTSASGSCGAHLVTLHLHSEGTTVLLFQFGMINASSSRFFLQGIQNTILPDARDP
AFKAANGSIRALQATVGNSYKCAEEHVVRTKAFSVNIFKVVVQAFKVEGGQFGSVVEECLLDENSM**LPIAVGG**
ALAGLVLIYLVGRKRSHAGYQT

SEQ ID NO: 151

Grey: Luminel Domain 1 and 2

Underlined: LT^{S220A}

Black and italics: LAMP TM/Cytoplasmic

Figure 11B

Groups: A – Control; B – LTS220A DNA Vaccine, 40 ug;
N=7 for each group; ID/ear route of administration

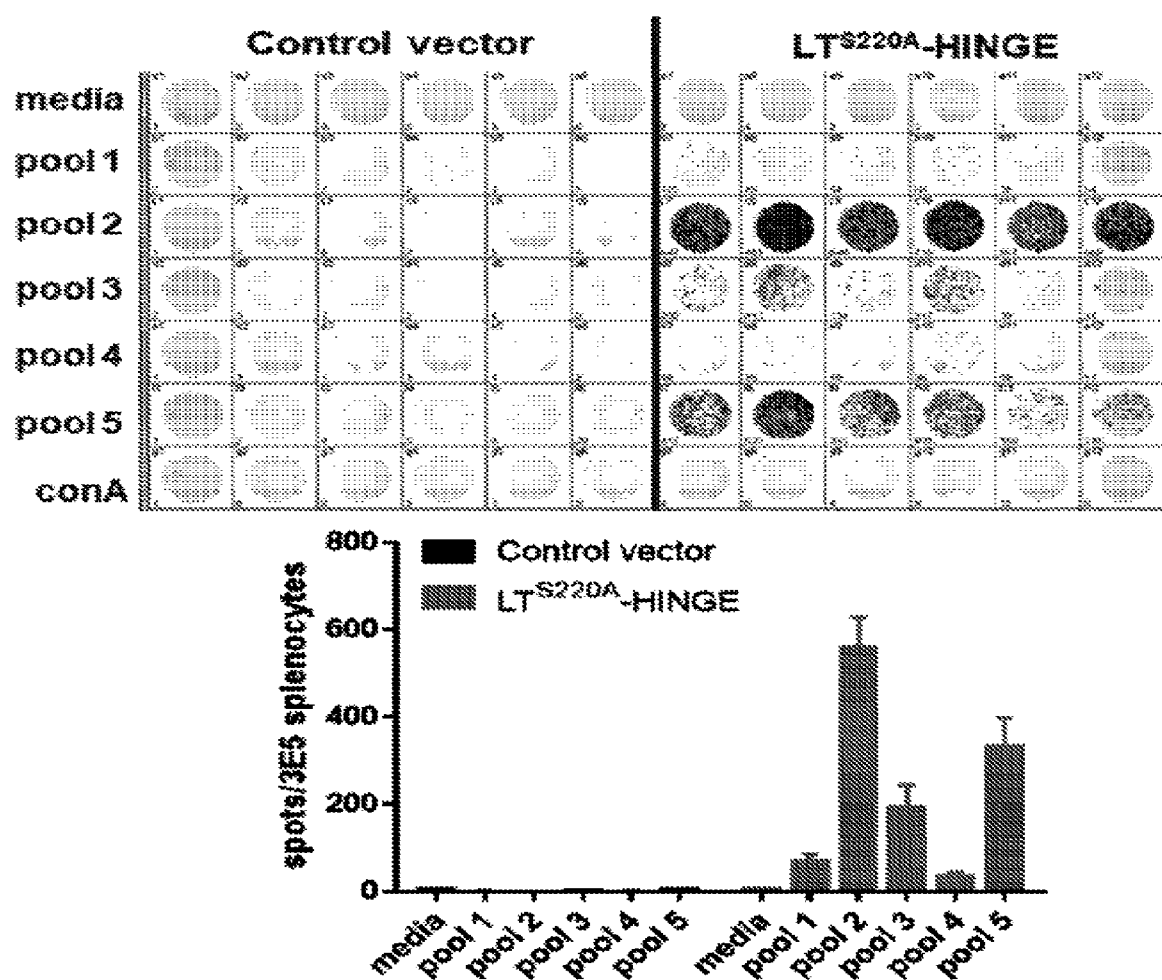
Figure 11C

Figure 11D

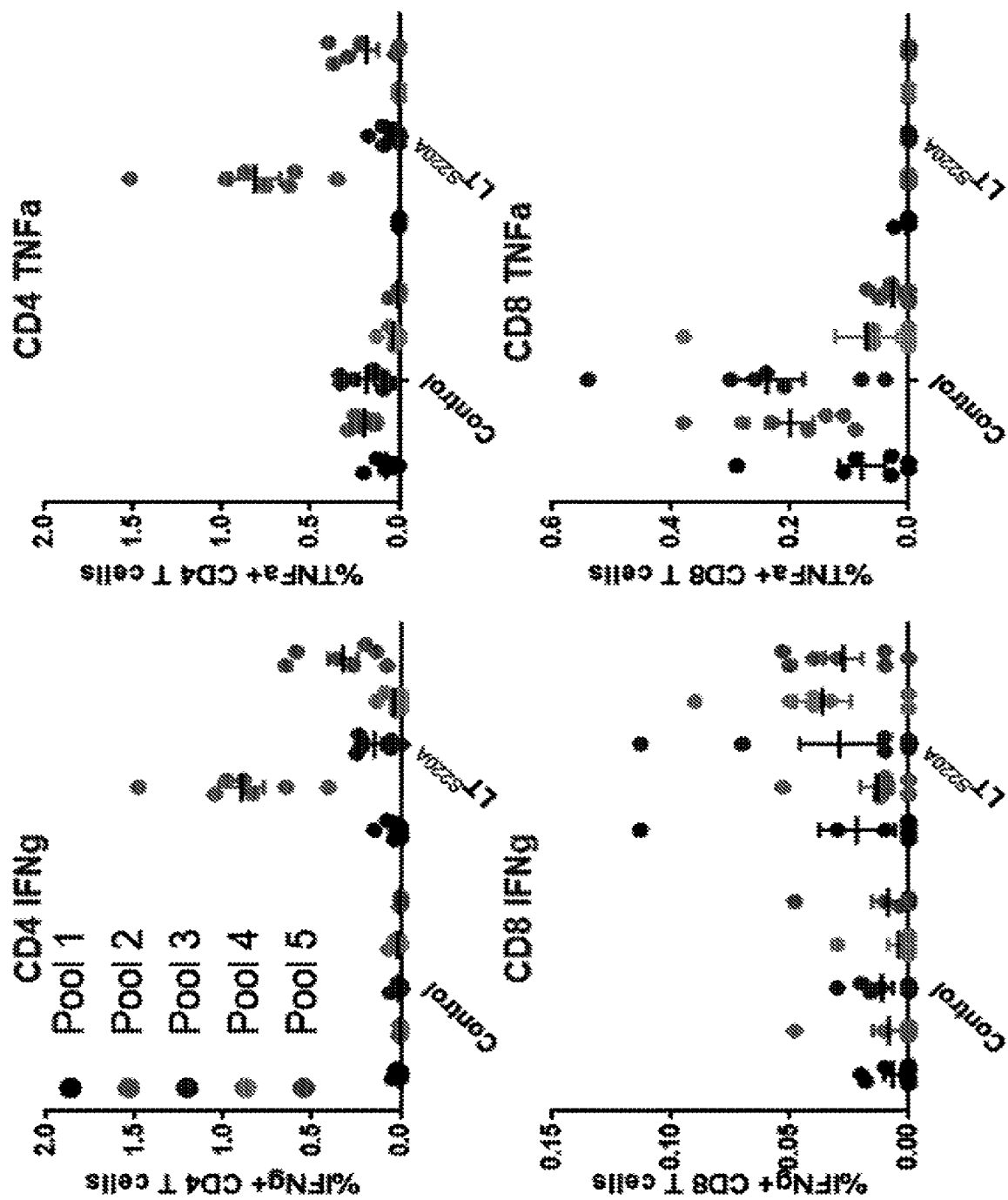


Figure 11E

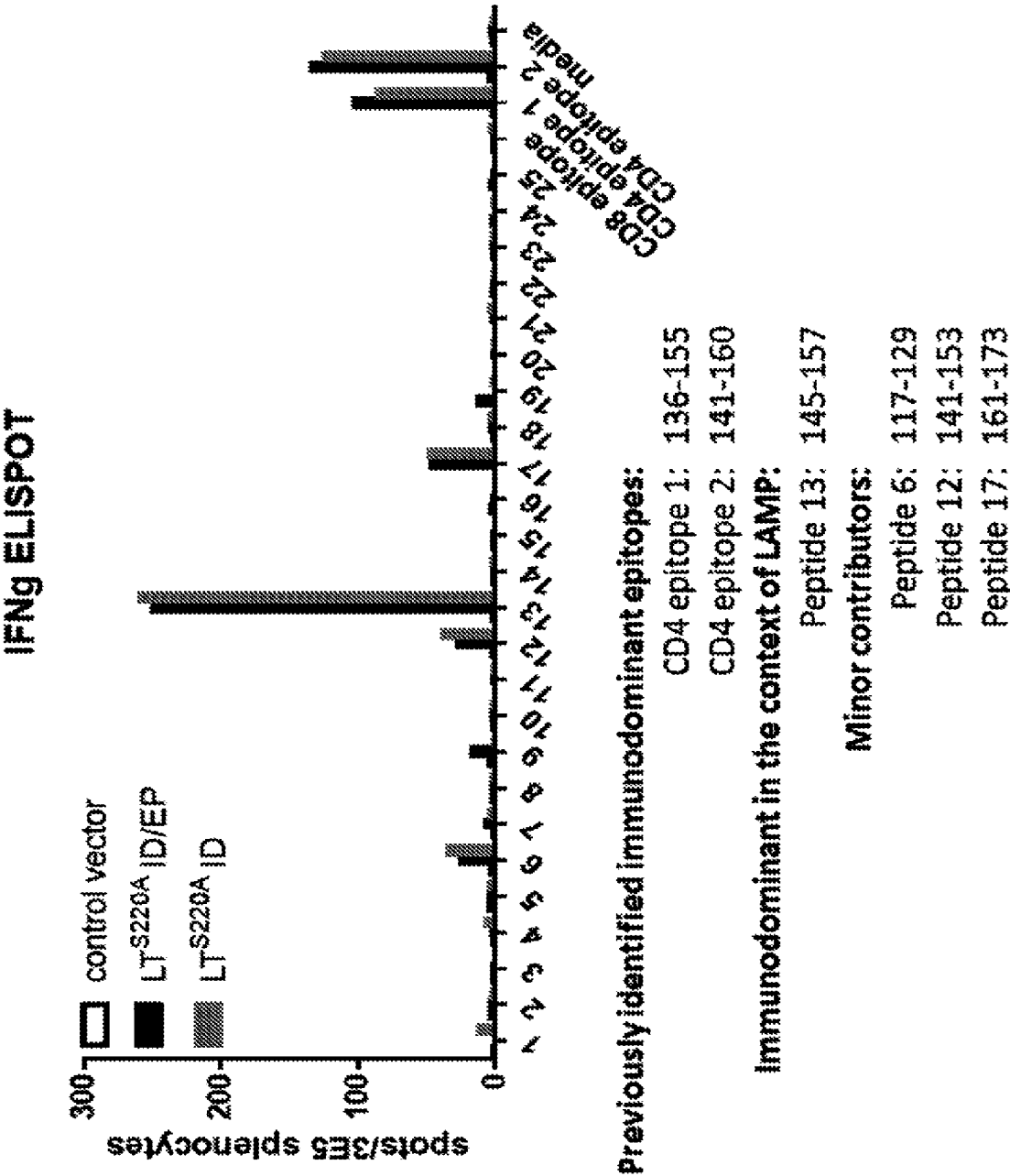


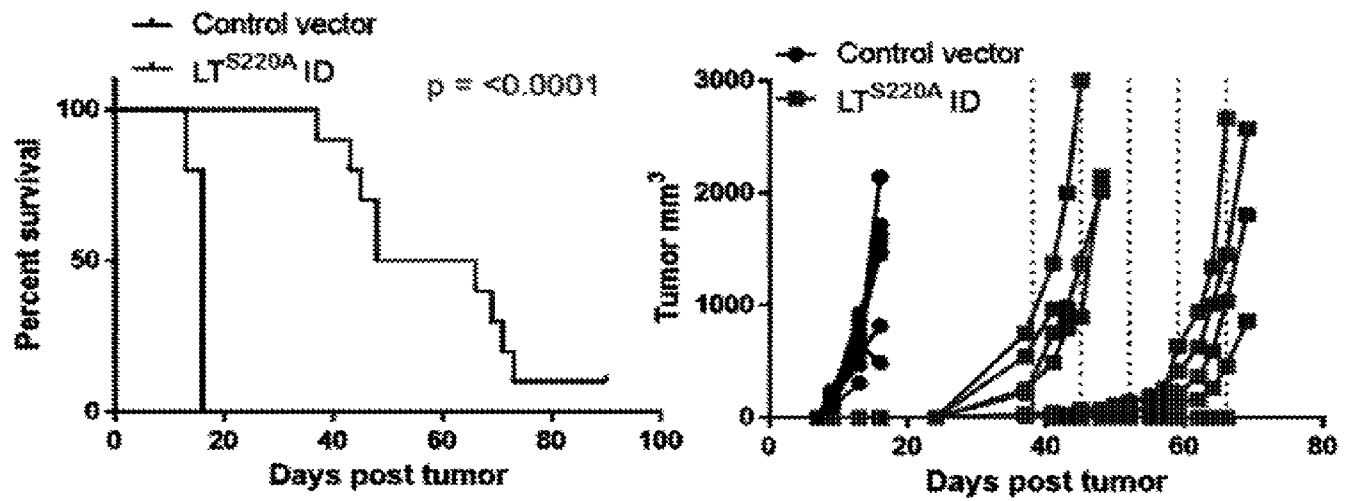
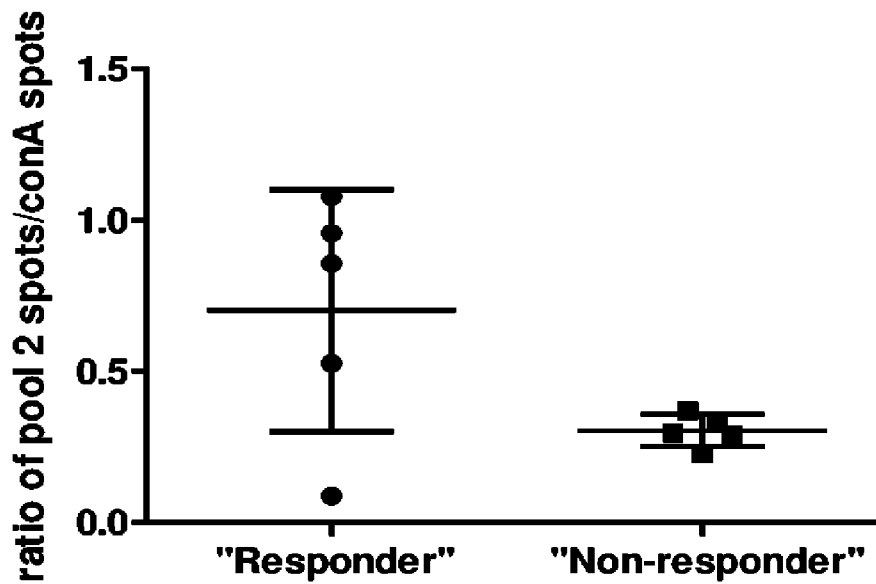
Figure 12A**Figure 12B**

Figure 13A

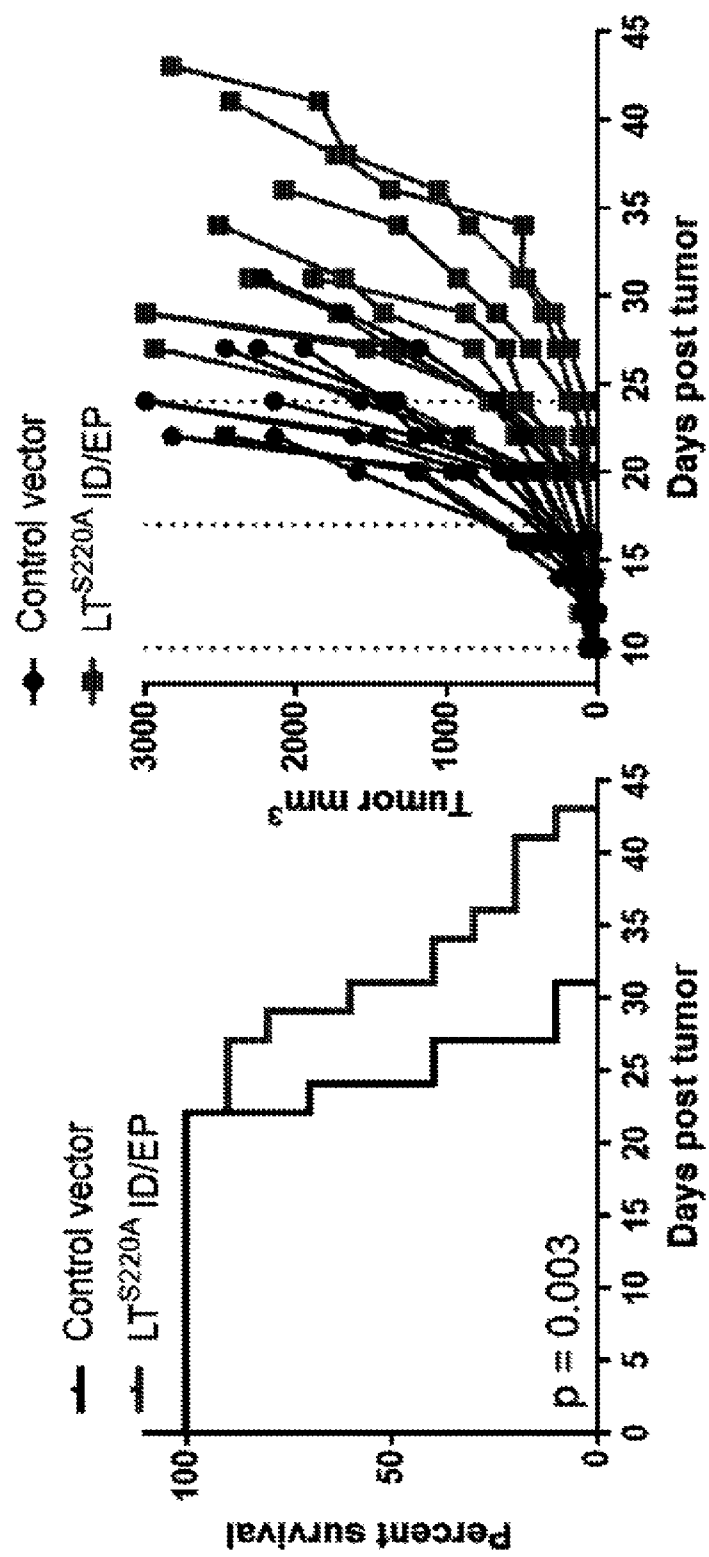


Figure 13B

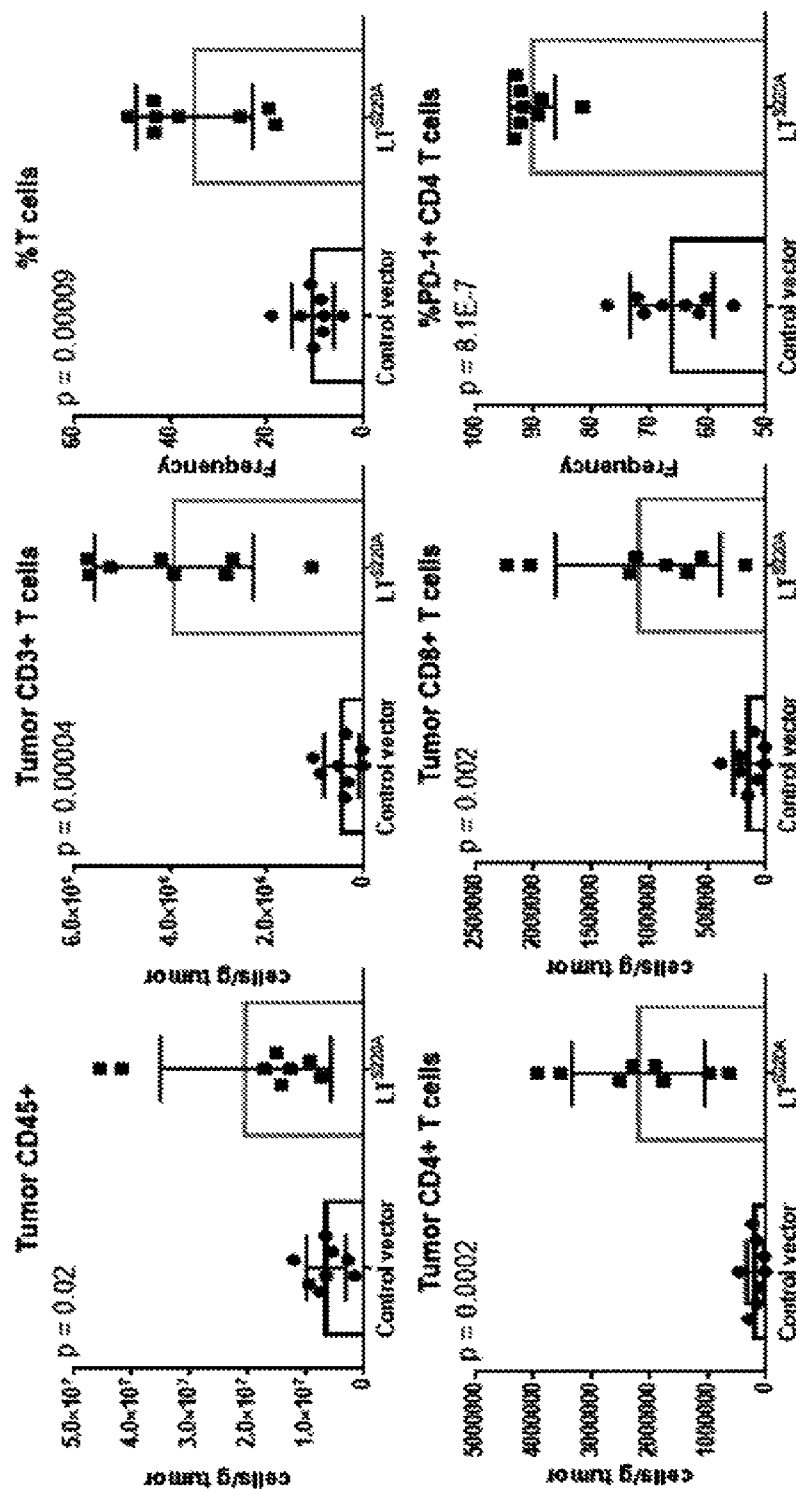


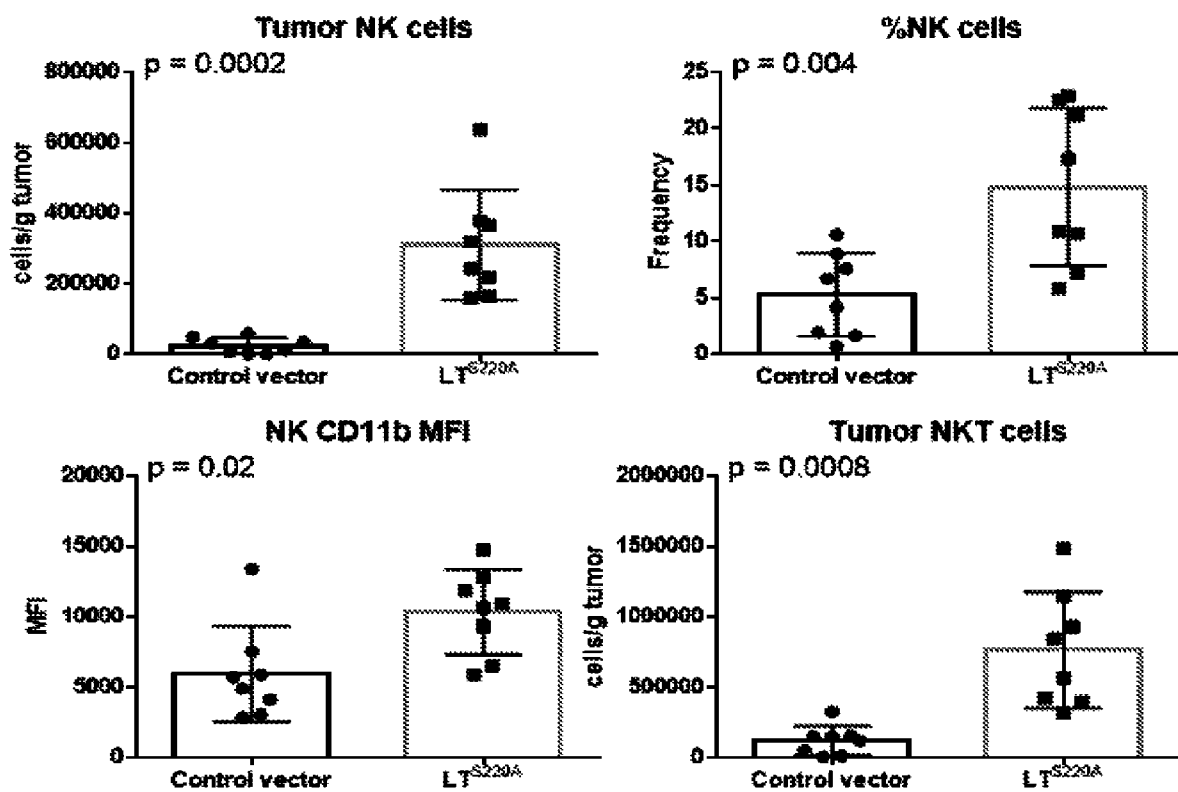
FIGURE 13C

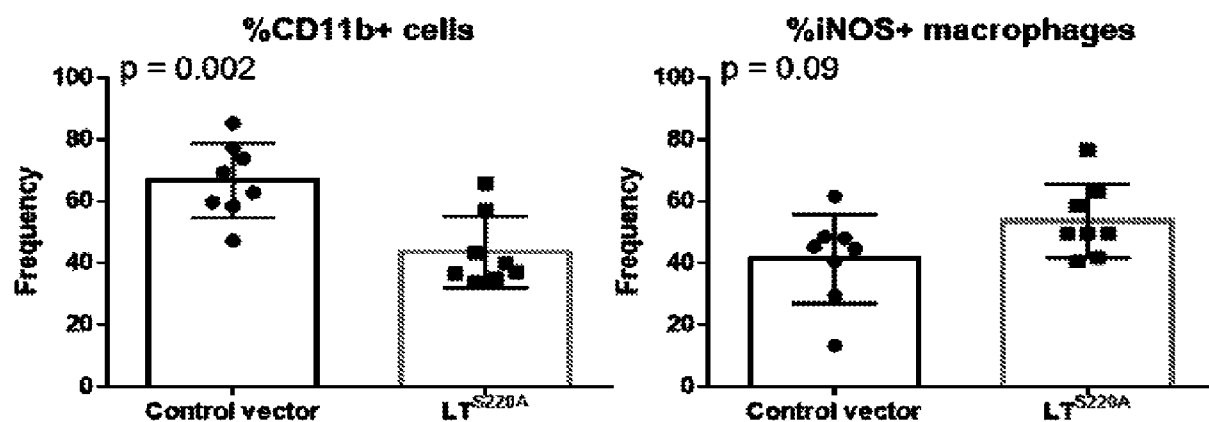
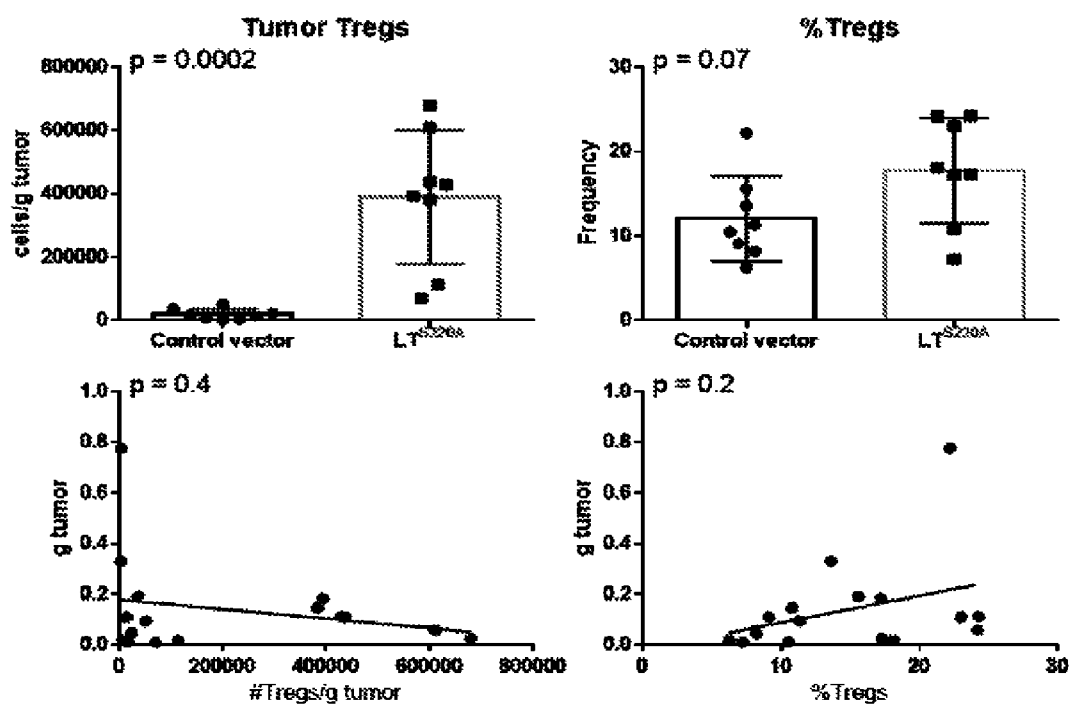
FIGURE 13D**FIGURE 13E**

FIGURE 13F