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(54) **Title**: POLYPEPTIDES AND USES THEREOF

FIG. 1A

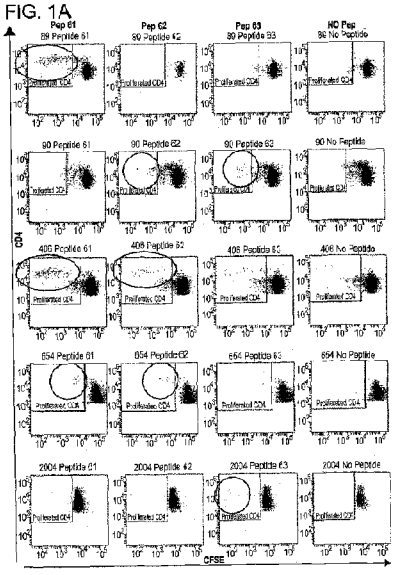
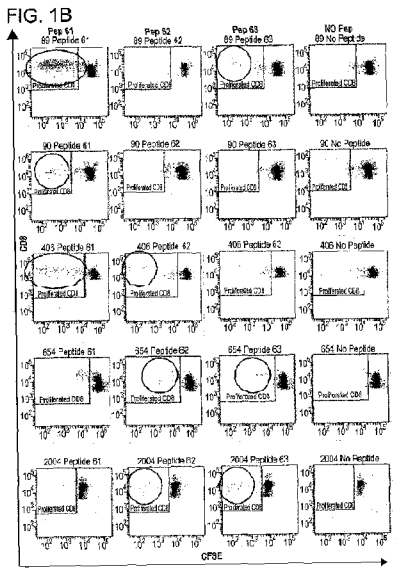


FIG. 1B



(57) **Abstract**: The invention provides a polypeptide having a sequence comprising from 10 to 24 consecutive amino acids from residues 215-233 of the sequence of human cyclin B1 protein or homolog thereof, as well as a nucleic acid molecule encoding the polypeptide, and compositions thereof. The invention also provides a method for using such polypeptides, nucleic acid molecules, or combinations thereof for treatment or prophylaxis of cancer in patients.

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POLYPEPTIDES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 61/083,800, filed July 25, 2008, which is incorporated by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with Government support under grant number Pittsburgh Lung Cancer SPORE (NCI) 5P50 CA90440-07 and Immunology Predoctoral Training Grant #5T32CA82084-08, both awarded by the National Cancer Institute. The Government has certain rights in the invention.

SEQUENCE LISTING

[0003] Incorporated by reference in its entirety herein is a nucleotide/amino acid sequence listing submitted concurrently herewith.

BACKGROUND OF THE INVENTION

[0004] United States Patent Application 11/366,196 (published as US 20060147460 A1), the entire contents of which are incorporated herein by reference, notes the desire for additional tumor antigens. While that application provides cyclin molecules and fragments derived from cyclin molecules as tumor antigens, additional tumor antigens are desired.

BRIEF SUMMARY OF THE INVENTION

[0005] In one embodiment, the invention provides a polypeptide having a sequence comprising from 10 to 24 consecutive amino acids from residues 215-233 of the sequence of human cyclin B1 protein or homolog thereof.

[0006] In another embodiment, the invention provides a nucleic acid molecule encoding the polypeptide.

[0007] The invention also provides a method for using such polypeptides and nucleic acid molecules or combinations thereof for treatment or prophylaxis of cancer in patients.

[0008] These and other aspects of the invention will become apparent upon reading the following detailed description in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0009] Figure 1: Cyclin B1 amino acids #215-233 contain an immunogenic region of cyclin B1 that stimulate T cell responses in the peripheral blood of heavy smokers who are negative for lung cancer by computed tomography (CT) scan. CD4+ T cells from 5 heavy smokers show proliferation in response to one or more of the inventive polypeptides: Pep61 (SEQ ID NO:1), Pep62 (SEQ ID NO:2); and Pep63 (SEQ ID NO:3). Similarly, CD8+ T cells from the same 5 individuals also are capable of proliferation in response to epitopes within amino acids 215-233. Cells considered to have proliferated above background (no peptide, on right) are circled.

[0010] Figure 2: Cyclin B1 DNA prime-protein boost vaccination elicits cyclin B1-specific cellular and humoral responses and delays tumor growth. For (A) and(B), mice primed with either pcDNA 3.1 empty vector (group 1), mouse cyclin B1 (mCB1, group 2), or human cyclin B1 (hCB1, group 3) cDNA were boosted with human cyclin B1 recombinant protein and the LT/IS patch two times in 3 week intervals. (A) ELISPOT performed on mouse splenocytes. Error bars indicate standard deviation. (B) ELISA for anti-human (left) and anti-mouse (right) cyclin B1 IgG. Bars indicate geometric mean. For (C) and (D), mice from groups 1, 2, and 3 with the addition of untreated and pcDNA3.1 empty vector and LT/IS patch controls were challenged with LO2 tumor cells. (C) Tumor growth on day 28 after tumor challenge. Bars indicate mean tumor size. (D) Survival after tumor challenge (Logrank test, $p<0.0001$).

DETAILED DESCRIPTION OF THE INVENTION

[0011] In one embodiment, the invention provides a polypeptide having a sequence comprising, consisting of, or consisting essentially of from 10 to 24 (such as 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24) consecutive amino acids from residues 215-233 of the sequence of human cyclin B1 protein. The inventive polypeptide alternatively comprises, consists of, or consists essentially of from 10 to about 24 amino acids from the cyclin B1 protein from another animal, such as mouse, chimpanzee, or other homolog. The sequence of human cyclin B1 is known (GenBank Accession No. NM_031966), as is that of other animals (e.g., GenBank Accession No. NM_172301). Accordingly, ordinary skilled

artisans are able to select suitable sequences of from about 10 to about 24 consecutive amino acids from residues 215-233 of the human cyclin B1 polypeptide or its homologs. Exemplary polypeptides of the present invention have sequences consisting of KFRLLQETMYMTVSI (SEQ ID NO:1), LQETMYMTVSIIDRF (SEQ ID NO:2), or MYMTVSIIDRFM (SEQ ID NO:3).

[0012] The inventive polypeptide can be produced by any suitable method. For example, it can be synthesized using standard direct peptide synthesizing techniques (Bodanszky, Principles of Peptide Synthesis (Springer-Verlag, Heidelberg: 1984)), such as via solid-phase synthesis (see, e.g., *Merrifield, J. Am. Chem. Soc.*, 85, 2149-54 (1963); Barany et al., *Int. J. Peptide Protein Res.*, 30, 705-739 (1987); and U.S. Pat. No. 5,424,398). Alternatively, a gene encoding the desired protein or peptide can be subcloned into an appropriate expression vector using well-known molecular genetic techniques. The protein or peptide can then be produced by a host cell and isolated from the cell. Any appropriate expression vector (see, e.g., Pouwels et al., Cloning Vectors: A Laboratory Manual (Elsevior, N.Y.: 1985)) and corresponding suitable host cells can be employed for production of the desired protein or peptide. Expression hosts include, but are not limited to, bacterial species, mammalian or insect host cell systems including baculovirus systems (see, e.g., Luckow et al., *Bio/Technology, 6*, 47 (1988)), and established cell lines such 293, COS-7, C127, 3T3, CHO, HeLa, BHK, etc.

[0013] Once it is manufactured and suitably isolated, the inventive polypeptide can be substantially purified by standard methods and formulated into a composition (e.g., including a pharmacologically- or physiologically-compatible carrier), lyophilized, or otherwise employed or preserved. Accordingly, the invention further comprises a composition comprising one or more of the inventive polypeptides and a carrier. For lyophilizing the composition, a carrier can include a lyoprotectant, such as sucrose. A pharmaceutically-acceptable composition can be formulated as a cream, wafer, dermal patch, spray, drop, suspension, emulsion, lozenge, tablet, capsule, or otherwise, as desired. Of course, in such compositions, suitable buffers, binders, glidants, preservatives, and other standard excipients can be included as desired, employing the ordinary skill of pharmaceutical compounding. For administration as a vaccine to patients, the composition typically will be formulated with carriers and buffers suitable for injection (e.g., parentarally or intratumorally). Many synthetic forms of the vaccine can be used, for example, a branched polymer can be synthesized to have a different peptide on each branch.

[0014] The invention also provides nucleic acid molecules encoding one or more of the inventive polypeptide sequences. The nucleic acid molecule can be administered to a patient as naked DNA or as a vector (e.g., bacterial or viral vectors) for use as a vaccine. Any suitable vector can be used. Examples of viral vectors include pox viruses, adenovirus, and HPV. Examples of bacterial vectors include recombinant salmonella and Listeria.

[0015] The polypeptide sequences can also be engineered into antibodies that target receptors on dendritic cells (example: anti-DEC-205 antibody) and these antibodies used as vaccines (see, e.g., Bozzacco et al., *Proc Nat. Acad. Sci. USA.* 2007 Jan 23;104(4):1289-94).

[0016] The inventive polypeptides are not restricted to a single HLA-Class I or HLA-Class II molecule as they elicit both CD8+ and CD4+ T cells in multiple individuals with multiple different HLA Class I and Class II types. Thus, the inventive cyclin B1 polypeptides can be mixed with HLA-A2 restricted cyclin B1 polypeptides (e.g., as described in U.S. patent application publication US 20060147460 A1) to provide help by stimulating CD4+ T cells as well as additional CD8+ T cells.

[0017] Using the inventive polypeptides, nucleic acid molecules, and antibodies, the invention provides a method for vaccinating a patient against cancer. In accordance with the method, a pharmaceutically-acceptable composition comprising one or more of the inventive polypeptides, nucleic acid molecules, and antibodies is administered to a patient in an amount and at a location sufficient to treat cancer in a patient having cancer or for prophylaxis of cancer in a patient at risk for developing the cancer. Typically, the patient will be a human who either has cancer or is determined to be at risk for developing cancer (e.g., having a genetic predisposition or family history of cancer). However, the patient alternatively can be a non-human animal, such as a common pets (cats, dogs, etc.), livestock (swine, cattle, sheep, goats, etc.), beasts of burden (horses, donkeys, elephants, camels, etc.), laboratory animals (mice, rats, and the like), or zoologically-important animals (e.g., endangered or threatened species or animals in captivity).

[0018] The inventive method can be used prophylactically or as a treatment for many types of cancer (e.g., cancers of bladder, bone, brain, breast, cervix, colon, epithelium, esophagus, head and neck, kidney, liver, lung, ovary, pancreas, prostate, skin, stomach, testicle, uterus, etc., and the various leukemias and lymphomas). Because Cyclin B1 appears to be overexpressed in cells that turn off the function of the tumor suppressor protein p53 (Yu et al., *Mol. Immunol.*, 38(12-13), 981-87 (2002)), tumors associated with disruption of p53 would be particularly attractive candidates for treatment in accordance with the present

invention. Moreover, the polypeptides, nucleic acid molecules, and antibodies have the potential or capability to prevent or reduce the risk of developing cancer in individuals without cancer but who are or may become at risk of developing cancer. It will be observed that successful use of the inventive method does not require curing or complete remission of the cancer. It is sufficient if the method retards the progression of the cancer. Moreover, the inventive method can be used adjunctively with other methods of cancer treatment, such as through chemotherapy or radiotherapy.

[0019] The administration to a patient of a vaccine in accordance with this invention for prophylaxis and/or treatment of cancer can take place before or after a surgical procedure to remove the cancer, before or after a chemotherapeutic procedure for the treatment of cancer, or before or after radiation therapy for the treatment of cancer and any combination thereof. In addition, the vaccine can be given together with adjuvants and/or immuno-modulators to boost the activity of the vaccine and the patient's response. Moreover, additional inoculations of the vaccine (e.g., "booster" inoculations) can be employed as desired.

[0020] In one embodiment, a priming inoculation of the inventive nucleic acid molecule (e.g., formulated as a composition) can be administered to the patient, followed by one or more (e.g., one, two, three, four, or more) subsequent boosting inoculations of the inventive polypeptide (e.g., formulated as a composition), which is referred to as a DNA prime-protein boost vaccination protocol.

[0021] The boosting inoculation can be administered at any suitable time period after the priming inoculation (e.g., 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year, 2 years, 3 years, 4 years, 5 years, or 10 years). Similarly, if additional boosting inoculations are desired, the additional boosting inoculations (e.g., of the inventive polypeptide or nucleic acid molecules or compositions thereof) can be administered at any suitable time period after the initial boosting inoculation (e.g., 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year, 2 years, 3 years, 4 years, 5 years, or 10 years).

[0022] Following the inoculation(s), the patient's immune response to the antigen can be monitored, for example, by drawing blood from the patient and assessing the presence of immunoglobulins reactive against cyclin B1 or fragments thereof or for the presence of lymphocytes reactive against such polypeptides.

[0023] While, as mentioned, a pharmaceutically-acceptable composition according to the present invention can be formulated via standard methods as desired, typically the

composition is formulated for injection for use in the inventive method. Desirably, the composition also can comprise an adjuvant. Preferred adjuvants are agonists of Toll-like receptors, and desirably a human Toll-like receptor (TLR). For use in human patients, preferably the composition includes one or more agonists of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, or TLR10. However, the agonist can additionally target TLR11, TLR12, or TLR13. While any suitable TLR agonists can be employed, exemplary agonists suitable for use in the present invention include unmethylated CpG-containing polypeptides, Lipopolysacharide A (LPS), Monophosphoryl LipidA (MPL), and Poly-ICLC. Polypeptides also can be precipitated on Alum (aluminum salt), which is an FDA approved adjuvant for human vaccination.

[0024] Another composition suitable for use in the inventive method includes dendritic cells loaded with one or more of the inventive polypeptides or combination thereof. Methods for loading dendritic cells are known to persons or ordinary skill (see, e.g., U.S. patent application publication US 20060147460 A1 and Fey et al., *Cancer Immunol. Immunother.* 2006 Oct;55(10):1209-1).

[0025] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0026] This example demonstrates the identification of three Cyclin B1 (CB1) polypeptides that carry immunogenic epitopes.

[0027] The amino acid sequence of the whole CB1 protein was synthesized as a series of peptides (generally 12-15 amino acids in length) that overlap by 11 amino acids (each peptide is offset by 4 amino acids). This approach is used when HLA of the subjects being tested is unknown and when there is a potential for multiple reactive peptides restricted by a variety of HLA molecules. It is also useful when the blood collection volumes are too small for dendritic cell generation, and other antigen presenting cells in the peripheral blood are counted on to present peptides without the need for further antigen processing.

[0028] Peripheral blood was collected into vaccutainers containing heparin sulfate and processed to obtain peripheral blood mononuclear cells (PBMC) by density centrifugation. PBMC were washed in PBS and labeled with 5 μM CFSE (Invitrogen) in warmed 0.1% FBS in PBS for 10 minutes at 37 °C. The CFSE was quenched with 3 volumes of ice cold complete RPMI and incubated for 5 minutes before washes with complete RPMI. PBMC

were then resuspended in media containing anti-CD28 and anti-CD49d antibodies for additional costimualtion. Wells coated with anti-CD3 were used as positive controls. Peptides were added at approximately 2 μg/ml. No peptide was added to negative control wells. After 6 days, cells were stained for cell surface markers and tested for proliferation by CFSE dilution using flow cytometry. Peptides that induced proliferation above that seen in the wells receiving no peptides were considered to be stimulatory.

[0029] The peptide library contained over 200 peptides divided into 10 pools. One of these pools repeatedly showed stimulation of T cells and when further analyzed, focused on 3 peptides that spanned amino acids #215-223 of the CB1 sequence. They are identified below according to their order in the peptide library as well as in the amino acid sequence:

p61: amino acids 215-229: KFRLLQETMYMTVSI (SEQ ID NO:1)
p62: amino acids 219-233: LQETMYMTVSIIDRF (SEQ ID NO:2)
p63: amino acids 223-234: MYMTVSIIDRFM (SEQ ID NO:3)

[0030] Memory T cell responses specific for these peptides were observed in the blood samples obtained from heavy smokers who were enrolled in a lung cancer screening study and determined to be negative for lung cancer by CT. See Figure 1.

EXAMPLE 2

[0031] This example demonstrates that CB1-specific immune responses can prevent tumor growth.

[0032] Given that healthy individuals have both humoral and cellular immune responses that are specific for the self and tumor antigen CB1, the potential significance of having anti-CB1 immunity prior to the onset of cancer was explored. A transplantable mouse tumor model, a CB1 overexpressing lymphoma cell line (LO2) that was derived from a $p53^{-/-}$ mouse and spontaneously overexpresses CB1, was used. LO2 cells (maintained *in vitro* in RPMI-1640 medium (Gibco, Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Cellgro; Media Tech Inc.), penicillin (100 U/ml), streptomycin (100 μg/ml), 0.3% glutamine (Gibco, Invitrogen), 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 10 μM β-mercaptoethanol (Gibco, Invitrogen)) were subcutaneously inoculated into syngeneic C57Bl/6 mice to establish the transplantable tumor model.

[0033] Because overexpressed CB1 in tumors is neither mutated nor altered postranslationally, it was expected that immune responses in healthy mice might be subject to self-tolerance and therefore difficult to elicit. For that reason, both mouse (self) and human (85% homologous but could be considered non-self) CB1 in two different forms, recombinant protein and cDNA, were chosen to test as immunogens. Transdermal delivery at the site of antigen injection of heat labile enterotoxin (LT) applied via an immunostimulatory (IS) patch was chosen as an adjuvant.

[0034] For the groups administered recombinant CB1 protein (i.e., the inventive polypeptide), C57Bl/6 mice were immunized subcutaneously with 25 μg/100 μl/mouse recombinant human cyclin B1 (hCB1) protein, mouse cyclin B1 protein (mCB1), or 100 μl PBS as a control. At the time of immunization or PBS treatment, an IS patch containing 20 μg heat-labile enterotoxin (LT) (IOMAI Corporation) was applied to the immunization site. Repeat injections and LT/IS patch application were repeated twice in 3 week intervals. Sera were collected to measure antibody response. Seventeen days after the last immunization, 3 mice per group were sacrificed to study T cell responses. The remaining mice from the hCB1 and PBS groups, as well untreated, age-matched mice, were challenged with $1x10^6$ LO2 cells subcutaneously. The protein plus adjuvant vaccine elicited CB1-specific $CD4^+$ T cells and IgG.

[0035] For the groups administered CB1 cDNA (i.e., a nucleic acid molecule encoding the inventive polypeptide), hCB1 cDNA derived from a Hela cell line (from Dr. Qimin Zhan at the University of Pittsburgh) or mCB1 cDNA (an RT-PCR product derived from the mouse $p53^{-/-}$ LO2 cell line) was used. Briefly, RT-PCR was performed using forward primer ATGGCGCTCAGGGTCACTAG (SEQ ID NO:4) and reverse primer CAGTCTATTGGAGTTATGCCTTTG (SEQ ID NO:5). A band at approximately 1.3 kbp migrated on a 1.2% E-Gel Agarose gel (Invitrogen). The mCB1 band was eluted using a MiniElute Kit (Qiagen, Valencia, CA) and subcloned into PCR2.1-TOPO vector (Invitrogen) and used to transform One-Shot TOP10 competent cells (Invitrogen) as described by the manufacturer. Colonies were picked for culture, and plasmids were isolated and identified positively by an EcoRI digest. Both cDNAs were then subcloned into the BamHI-XhoI site of the pcDNA3.1 expression vector (Invitrogen). All inserts were verified by DNA sequencing.

[0036] Fifteen mice per group were immunized with either pcDNA3.1 control vector (group 1) or gene expression vectors encoding either mCB1 (group 2) or hCB1 (group 3)

cDNA. Three weeks and 6 weeks later, mice were boosted with recombinant hCB1 protein followed by the application of the LT/IS patch. Untreated mice and mice primed with empty pcDNA3.1 vector and boosted with LT/IS patch only were used as controls. Seventeen days after the last immunization, three mice per group were sacrificed for assessment of *in vitro* T cell responses and the remaining mice were challenged with LO2 tumor.

[0037] The DNA prime-protein boost vaccination induces CB1-specific T cell responses that can only partially be blocked by anti-CD4 antibody (groups 2 and 3). These results implied successful priming of $CD8^+$ T cells, as well. The same results were obtained by boosting with mouse CB1 protein, and in those experiments it was confirmed that CB1 specific T cell responses can also be blocked by anti-CD8 antibody. CB1 DNA prime-protein boost vaccination also successfully elicited both anti-human and anti-mouse CB1 antibodies.

[0038] The presence of anti-CB1 immune responses prior to tumor challenge significantly delayed or completely prevented tumor growth (see Figure 2). By day 28 after tumor challenge, groups that received the DNA prime-protein boost vaccine had significantly lower mean tumor volume ($p<0.0001$) and significantly higher number of tumor-free mice ($p=0.0013$) than no treatment and adjuvant only controls. By day 42, all mice in the control groups were sacrificed due to excessive tumor burden, while 2 mice in group 1, 6 mice in group 2 and 5 mice in group 3 remained tumor free. Vaccination significantly enhanced survival. No evidence of self-tolerance to mCB1 was observed, since priming with mouse cDNA protected equally or slightly better than the hCB1 DNA vaccine. Similar protection was observed when the mCB1 DNA vaccine was boosted with mCB1 protein.

[0039] This data supports that the inventive polypeptides and nucleic acid molecules can be used to treat and prevent tumor growth.

[0040] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein. The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not

limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein.

[0041] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention. Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention.

[0042] Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. A composition comprising a polypeptide having a sequence comprising from 10 to 24 consecutive amino acids from residues 215-233 of the sequence of human cyclin B1 protein or homolog thereof.

2. A composition comprising a nucleic acid molecule encoding a polypeptide having a sequence comprising from 10 to 24 consecutive amino acids from residues 215-233 of the sequence of human cyclin B1 protein or homolog thereof.

3. The composition of claim 1 or 2, wherein the polypeptide has a sequence consisting of KFRLLQETMYMTVSI (SEQ ID NO:1), LQETMYMTVSIIDRF (SEQ ID NO:2), MYMTVSIIDRFM (SEQ ID NO:3), or a combination of two or three thereof and a carrier.

4. The composition of claim 3, wherein the carrier is a pharmaceutically-acceptable carrier.

5. The composition of claim 3 or 4, wherein said carrier is a lyoprotectant.

6. The composition of any of claims 1-5 in lyophilized form.

7. The composition of claim 6, which is a vaccine.

8. The composition of any claims 1-7, further comprising an adjuvant.

9. The composition of claim 8, wherein the adjuvant comprises an agonist of a Toll-like receptor (TLR).

10. The composition of claim 9, wherein the TLR is a human TLR.

11. The composition of claim 9 or 10, wherein the TLR is TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, or TLR10.

12. The composition of claim 8, wherein the adjuvant is an unmethylated CpG-containing polypeptide, Lipopolysacharide A (LPS), Monophosphoryl LipidA (MPL), Poly-ICLC, or a combination of two or more of these.

13. The composition of claim 8, wherein the adjuvant comprises Alum, onto which the polypeptide has been precipitated.

14. The composition of any of claims 1-13, wherein the composition comprises dendritic cells loaded with said polypeptide or combination of polypeptides.

15. The composition of any of claims 4-14 formulated for injection.

16. A method of vaccinating a patient against cancer comprising administering to a patient having cancer or at risk for developing cancer a composition according to any of claims 4-13 at a location and in an amount suitable for treatment or prophylaxis of cancer.

17. The method of claim 16, wherein said cancer is associated with disrupted expression of p53.

18. The method of claim 16, wherein said cancer is bladder, bone, brain, breast, cervix, colon, epithelium, esophagus, head and neck, kidney, liver, lung, ovary, pancreatic, prostate, skin, stomach, testicle, uterus cancers, and the various leukemias and lymphomas.

19. A method of vaccinating a patient against cancer comprising

(a) administering to a patient having cancer or at risk for developing cancer a composition comprising a nucleic acid molecule encoding a polypeptide having a sequence comprising from 10 to 24 consecutive amino acids from residues 215-233 of the sequence of human cyclin B1 protein or homolog thereof, and

(b) subsequently administering to the patient a composition comprising the polypeptide, thereby vaccinating the patient against cancer.

20. The method of claim 19, wherein the polypeptide has a sequence consisting of KFRLLQETMYMTVSI (SEQ ID NO:1), LQETMYMTVSIIDRF (SEQ ID NO:2), MYMTVSIIDRFM (SEQ ID NO:3), or a combination of two or three thereof.

FIG. 1A

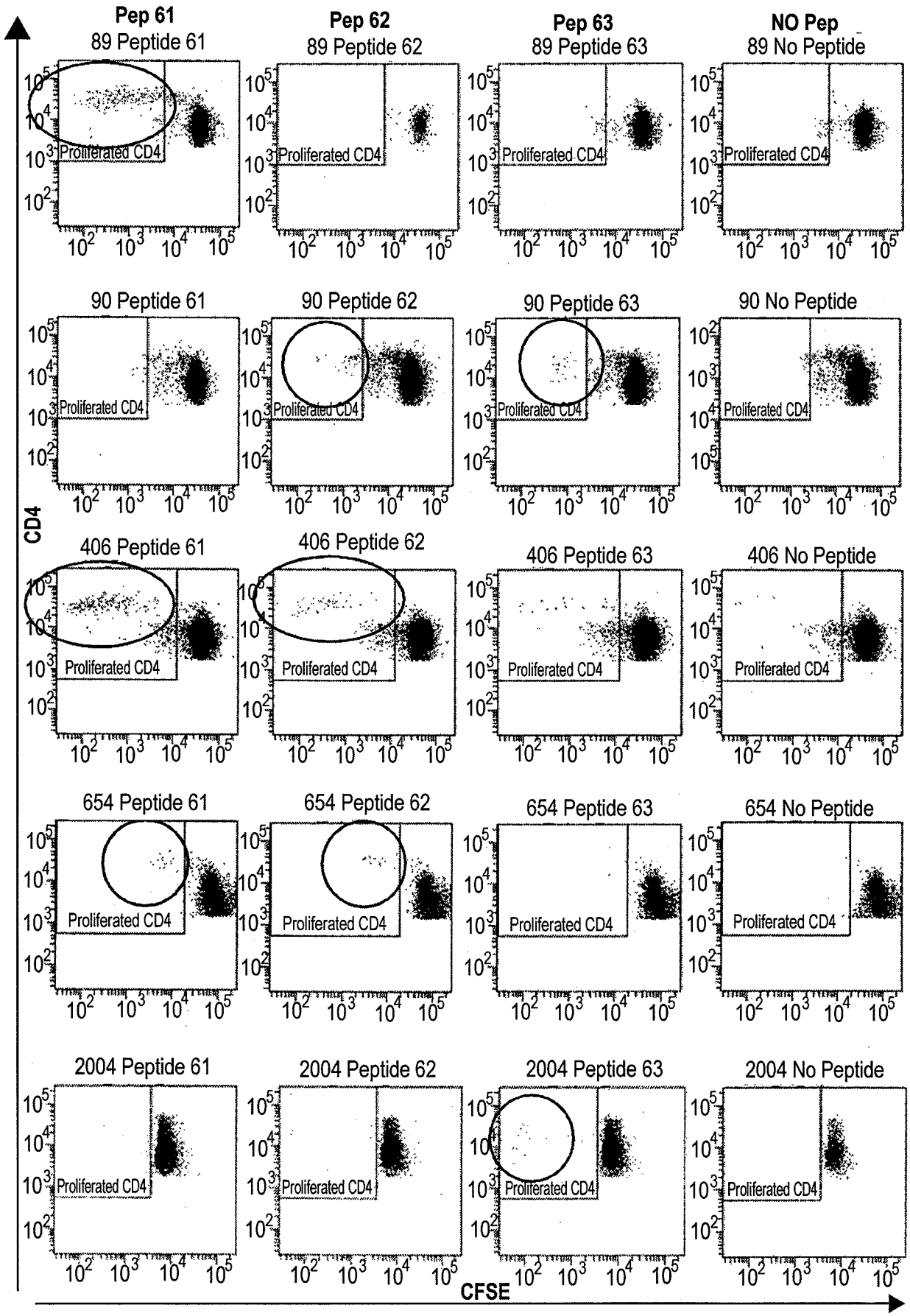
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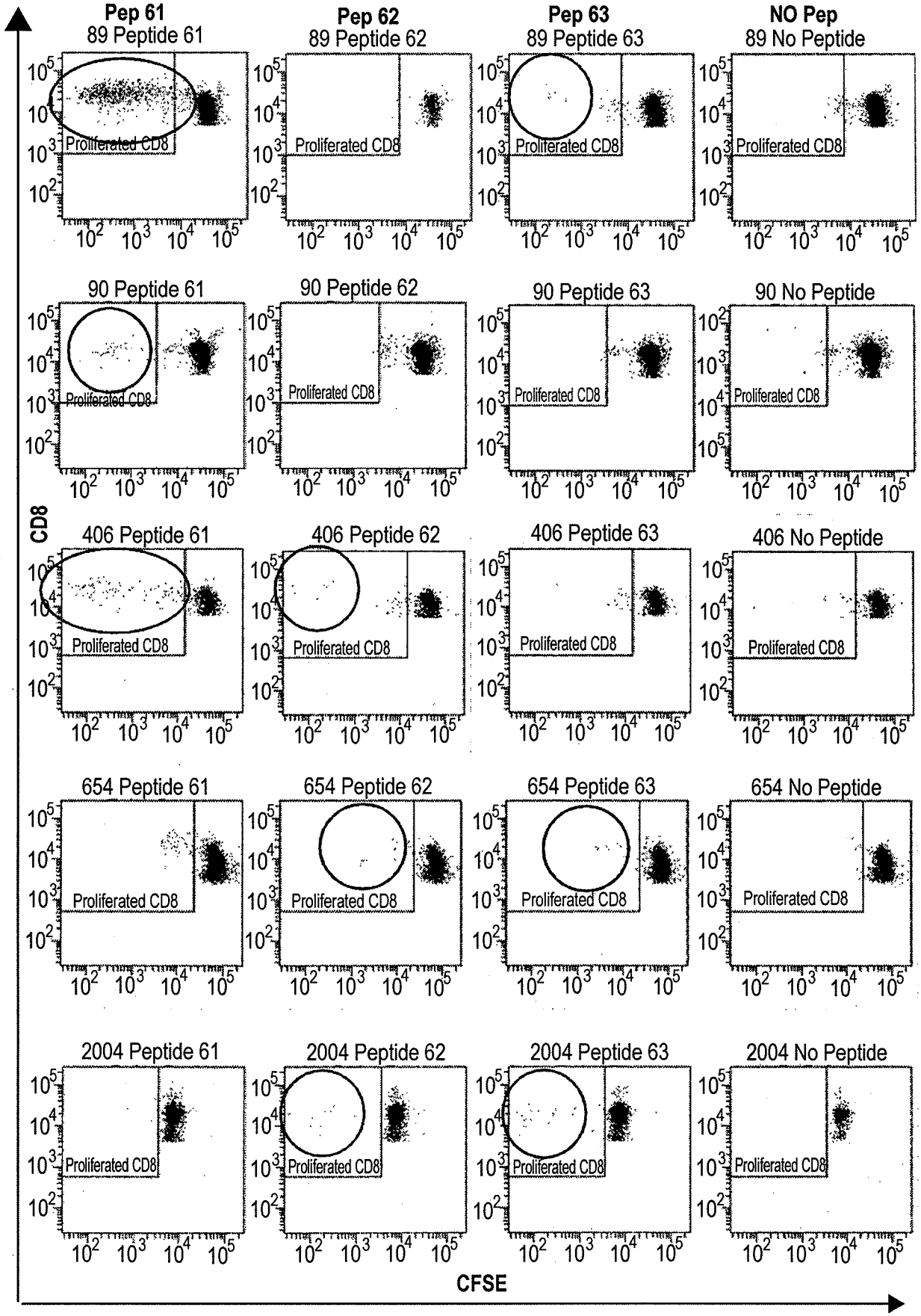
FIG. 1B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654 Peptide 61
654 Peptide 62
654 Peptide 63
654 No Peptide
2004 Peptide 61
2004 Peptide 62
2004 Peptide 63
2004 No Peptide
Proliferated CD8
CD8
CFSE

FIG. 2A

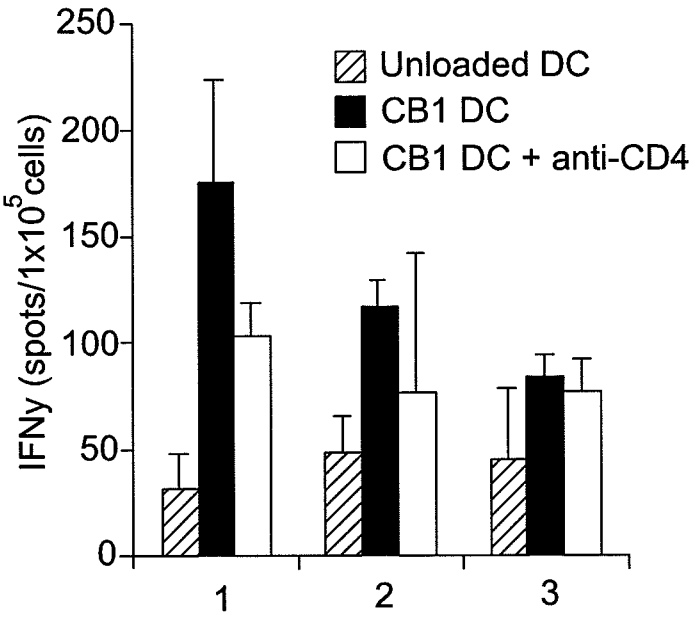

250
200
150
100
50
0
IFNy (spots/1x10^5cells)
Unloaded DC
CB1 DC
CB1 DC + anti-CD4
1
2
3

FIG. 2B

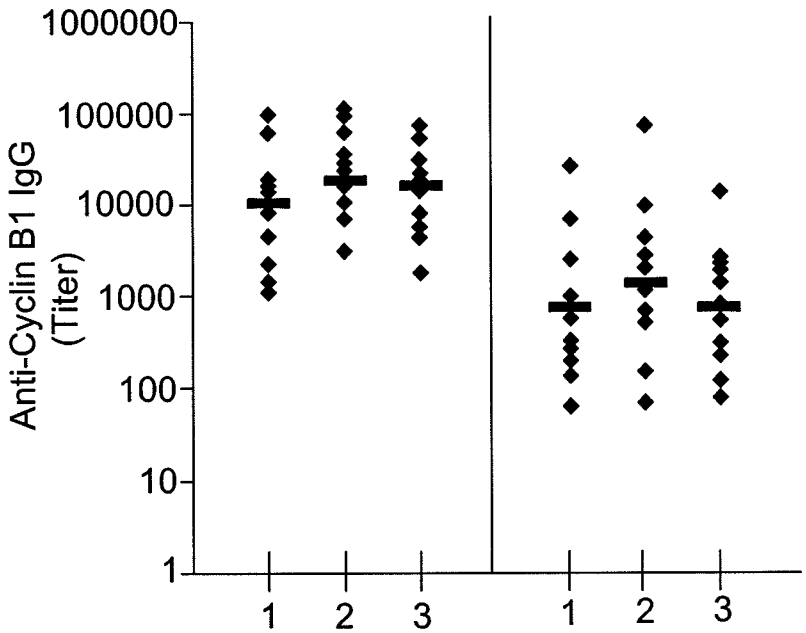

1000000
100000
10000
1000
100
10
1
Anti-Cyclin B1 IgG (Titer)
1
2
3
1
2
3

FIG. 2C

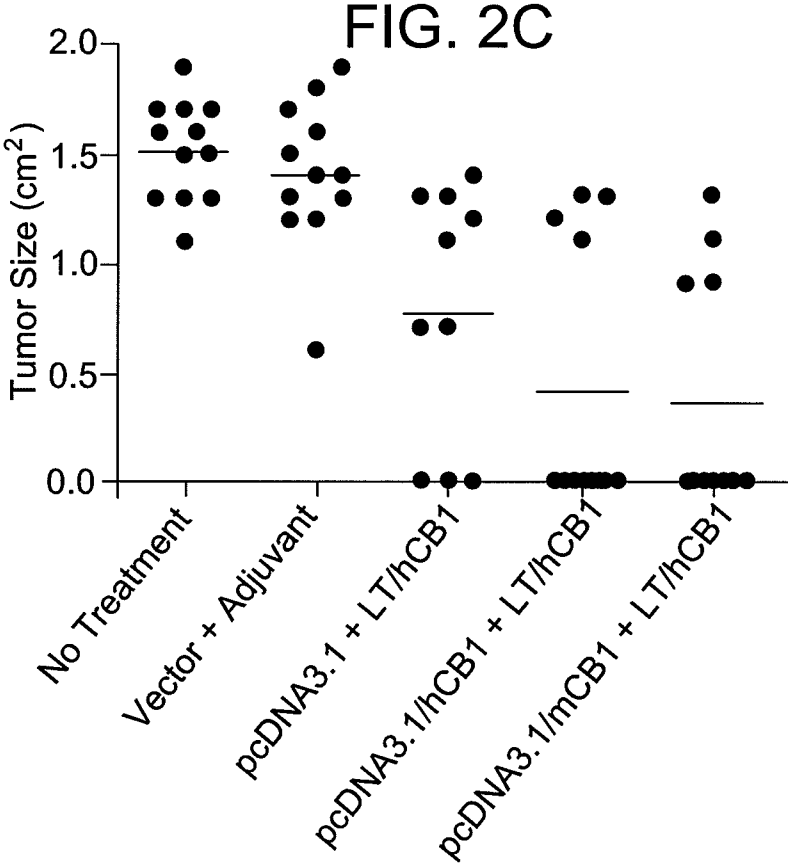
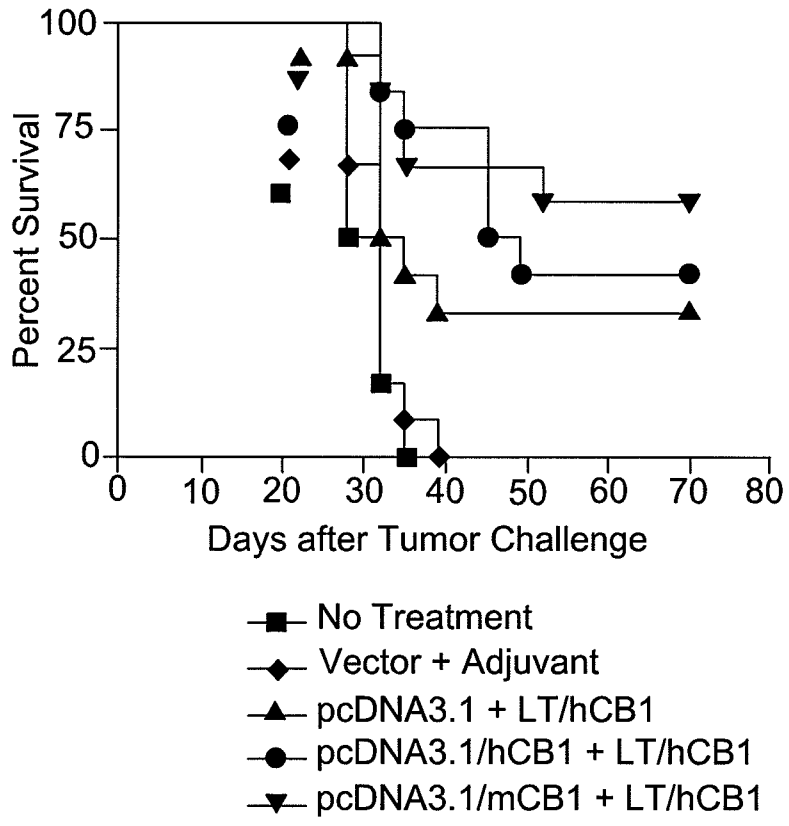

Tumor Size (cm^2)
2.0
1.5
1.0
0.5
0.0
No Treatment
Vector + Adjuvant
pcDNA3.1 + LT/hCB1
pcDNA3.1/hCB1 + LT/hCB1
pcDNA3.1/mCB1 + LT/hCB1

FIG. 2D


Percent Survival
100
75
50
25
0
0
10
20
30
40
50
60
70
80
Days after Tumor Challenge
No Treatment
Vector + Adjuvant
pcDNA3.1 + LT/hCB1
pcDNA3.1/hCB1 + LT/hCB1
pcDNA3.1/mCB1 + LT/hCB1