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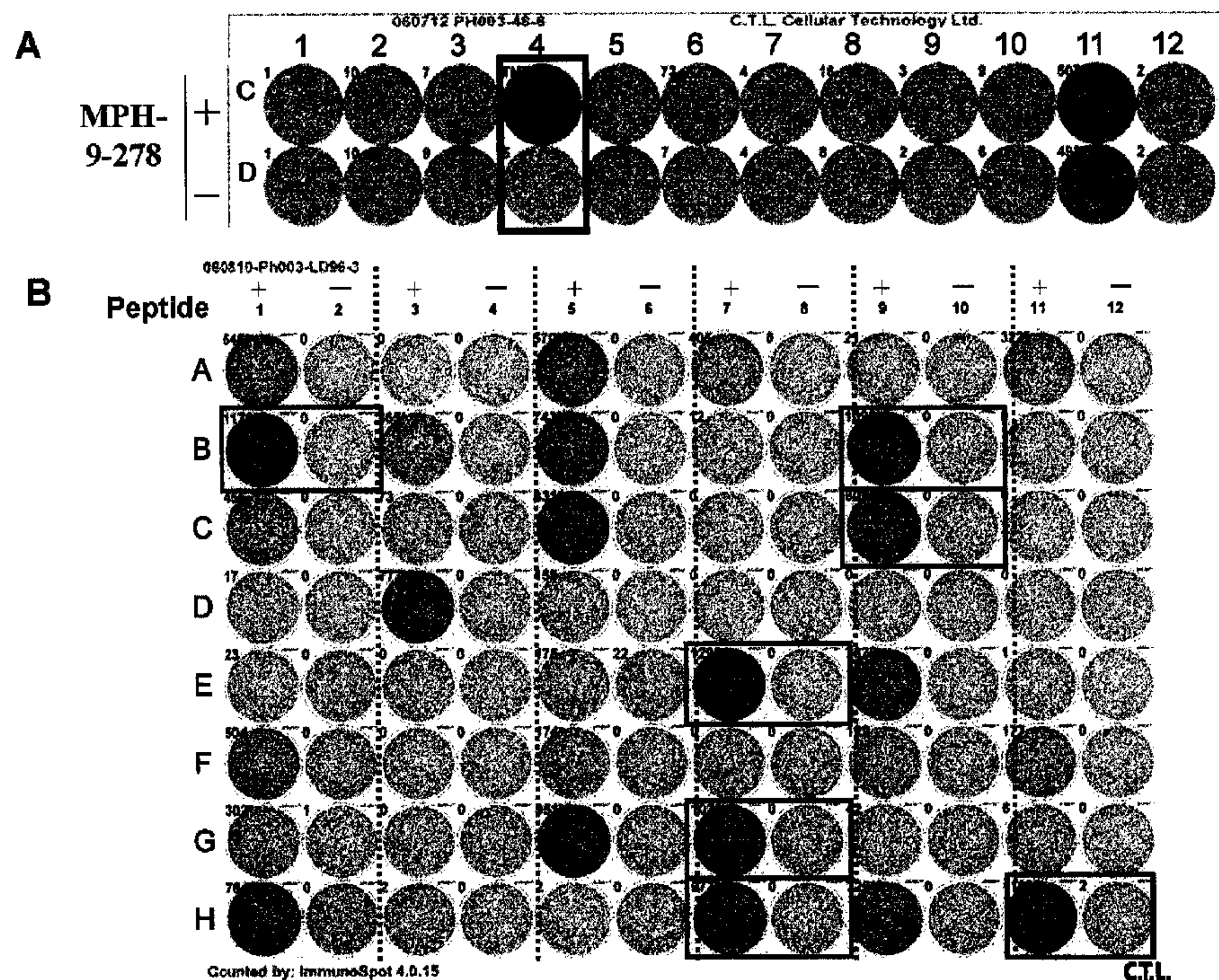
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(54) **Titre : VACCINS PEPTIDIQUES POUR DES CANCERS EXPRIMANT LES POLYPEPTIDES MPHOSPH1 OU DEPDC1**

(54) **Title: PEPTIDE VACCINES FOR CANCERS EXPRESSING MPHOSPH1 OR DEPDC1 POLYPEPTIDES**



Description

PEPTIDE VACCINES FOR CANCERS EXPRESSING MPHOSPH1 OR DEPDC1 POLYPEPTIDES

Technical Field

- [0001] The present application claims the benefit of U.S. Provisional Application No. 60/852,575, filed October 17, 2006, the entire contents of which are incorporated by reference herein.
- [0002] The present invention relates to the field of biological science, more specifically to the field of cancer therapy. In particular, the present invention relates to novel peptides that serve as extremely effective cancer vaccines, and drugs for treating and preventing tumors containing such peptides.

Background Art

- [0003] It has been demonstrated that CD8⁺ cytotoxic T lymphocytes (CTLs) recognize epitope peptides derived from tumor-associated antigens (TAAs) presented on MHC class I molecules, and lyse the tumor cells. Since the discovery of the MAGE family as the first example of TAAs, many other TAAs have been discovered using immunological approaches (Boon T. (1993) Int J Cancer 54: 177-80.; Boon T. et al., (1996) J Exp Med 183: 725-9.; van der Bruggen P et al., (1991) Science 254: 1643-7.; Brichard V et al., (1993) J Exp Med 178: 489-95.; Kawakami Y et al., (1994) J Exp Med 180: 347-52.). Some of them are now in clinical development as targets of immunotherapy. TAAs discovered so far include MAGE (van der Bruggen P et al., (1991) Science 254: 1643-7.), gp100 (Kawakami Y et al., (1994) J Exp Med 180: 347-52.), SART (Shichijo S et al., (1998) J Exp Med 187:277-88.), and NY-ESO-1 (Chen Y.T. et al., (1997) Proc. Natl. Acd. Sci. USA, 94: 1914-8.). On the other hand, certain gene products demonstrated to be somewhat specifically over-expressed in tumor cells have been shown to be recognized as targets for inducing cellular immune responses. Such gene products include p53 (Umano Y et al., (2001) Br J Cancer, 84:1052-7.), HER2/neu (Tanaka H et al., (2001) Br J Cancer, 84: 94-9.), CEA (Nukaya I et al., (1999) Int. J. Cancer 80, 92-7.) and the like.
- [0004] Despite significant progress in basic and clinical research concerning TAAs (Rosenberg SA et al., (1998) Nature Med, 4: 321-7.; Mukherji B. et al., (1995) Proc Natl Acad Sci USA, 92: 8078-82.; Hu X et al., (1996) Cancer Res, 56: 2479-83.), only a very limited number of candidate TAAs suitable for treatment of cancers are presently available. TAAs that are abundantly expressed in cancer cells, and whose expression is restricted to cancer cells, would be promising candidates as immunotherapeutic targets.

- [0005] Both HLA-A24 and HLA-A0201 are common HLA alleles in the Japanese and Caucasian populations (Date Y et al., (1996) *Tissue Antigens* 47: 93-101.; Kondo A et al., (1995) *J Immunol* 155: 4307-12.; Kubo RT et al., (1994) *J Immunol* 152: 3913-24.; Imanishi et al., *Proceeding of the eleventh International Histocompatibility Workshop and Conference* Oxford University Press, Oxford, 1065 (1992); Williams F et al., (1997) *Tissue Antigen* 49: 129-33.). Thus, antigenic peptides of cancers presented by these HLA alleles may find particular utility in the treatment of cancers among Japanese and Caucasian patients. Further, it is known that the induction of low-affinity CTL in vitro usually results from exposure to high concentrations of peptide, generating a high level of specific peptide/MHC complexes on antigen-presenting cells (APCs), which can effectively activate these CTL (Alexander-Miller et al., (1996) *Proc Natl Acad Sci USA* 93: 4102-7.).
- [0006] Recent developments in cDNA microarray technologies have enabled the construction of comprehensive profiles of gene expression of malignant cells as compared to normal cells (Okabe, H. et al., (2001) *Cancer Res.*, 61, 2129-37.; Lin YM. et al., (2002) *Oncogene*, 21;4120-8.; Hasegawa S. et al., (2002) *Cancer Res* 62:7012-7.). This approach enables an understanding of the complex nature of cancer cells and the mechanisms of carcinogenesis and facilitates the identification of genes whose expression is deregulated in tumors (Bienz M. et al., (2000) *Cell* 103, 311-20.). Among the transcripts identified as up-regulated in cancers, MPHOSPH1 (M-phase phosphoprotein 1; GenBank Accession No. NM_016195; SEQ ID Nos.1, 2), and DEPDC1 (DEP domain containing 1; GenBank Accession No. BM683578) have been recently discovered. See WO 2004/031413, WO 2006/085684 and WO 2007/013,665, the entire contents of which are incorporated by reference herein. DEPDC1 has been described in the context of two different transcriptional variants - DEPDC1 V1 (SEQ ID Nos.3, 4) and DEPDC1 V2 (SEQ ID Nos: 5, 6). These genes have been shown to be specifically up-regulated in tumor cells of the various cancer tissues of the cases analyzed (see below); however, Northern blot analyses demonstrate that these gene products are not found in normal vital organs (see PCT/JP2006/302684). In that immunogenic peptides derived from MPHOSPH1, and DEPDC1 may find utility in killing tumor cells expressing those antigens, these genes are of particular interest to the present inventors.
- [0007] Since cytotoxic drugs, such as M-VAC, often cause severe adverse reactions, it is clear that thoughtful selection of novel target molecules on the basis of well-characterized mechanisms of action is important in the development of effective anti-cancer drugs having a minimized risk of negative side effects. Toward this goal, the inventors previously performed expression profile analysis on various cancers and normal human tissue, and discovered multiple genes that are specifically over-

expressed in cancer (Lin YM, et al., *Oncogene*. 2002 Jun 13;21:4120-8.; Kitahara O, et al., *Cancer Res*. 2001 May 1;61:3544-9.; Suzuki C, et al., *Cancer Res*. 2003 Nov 1;63:7038-41.; Ashida S, *Cancer Res*. 2004 Sep 1;64:5963-72.; Ochi K, et al., *Int J Oncol*. 2004 Mar;24(3):647-55.; Kaneta Y, et al., *Int J Oncol*. 2003 Sep;23:681-91.; Obama K, *Hepatology*. 2005 Jun;41:1339-48.; Kato T, et al., *Cancer Res*. 2005 Jul 1;65:5638-46.; Kitahara O, et al., *Neoplasia*. 2002 Jul-Aug;4:295-303.; Saito-Hisaminato A et al., *DNA Res* 2002, 9: 35-45.). Of these, MPHOSPH1 (in house No. C2093) and DEPDC1 (in house No. B5860N) were identified genes over-expressed in various cancers. In particular, MPHOSPH1 was identified as over-expressed in bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, soft tissue tumor. Similarly, DEPDC1 was identified as over-expressed in bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, NSCLC, lymphoma, osteosarcoma, prostate cancer, SCLC, soft tissue tumor

[0008] MPHOSPH1 was previously identified as one of the proteins specifically phosphorylated at the G2/M transition and characterized as a plus-end-directed kinesin related protein (Abaza A et al., *J Biol Chem* 2003, 278: 27844-52.). More particularly, MPHOSPH1 has been previously documented to be a plus-end-directed molecular motor that plays a crucial role in cytokinesis, and accumulates in the midzone of the spindle during anaphase to telophase in HeLa cells (Abaza A et al., *J Biol Chem* 2003, 278: 27844-52; Kamimoto T et al., *J Biol Chem* 2001, 276: 37520-8). The MPHOSPH1 cDNA encodes a 1780-amino acid protein that is composed of three domains: an NH2-kinasin motor domain, a central coiled coil-stalk domain, and a C-globular tail domain. Together, this data suggests that MPHOSPH1 is an NH2-type kinesin-related protein.

[0009] As for DEPDC1, its function remains unclear. The DEP domain contained in this protein is also found in Dishevelled, Egl-10, and Pleckstrin. The DEP domain in *Drosophila* dishevelled plays an essential role in rescue planar polarity defects and induces JNK signaling; nevertheless, its function in Humans has not yet been clarified. However, as disclosed in PCT/JP2006/302684, DEPDC1 siRNAs can suppress the growth of cancer cells. These results demonstrate that DEPDC1 plays an important role in growth of most cancer cells.

[0010] Summary of the Invention

As noted above, MPHOSPH1 (M-phase phosphoprotein 1), and DEPDC1 (DEP domain containing 1) have been identified as up-regulated in various cancers. More particularly, the genes were identified using gene expression profiling with a genome-wide cDNA microarray. As discussed above, expression of MPHOSPH1 and DEPDC1 has been shown to be specifically up-regulated in various tumor cells, including lung

cancer and bladder cancer. As described in Table 1, MPHOSPH1 expression was shown to be validly elevated in 30 out of 31 bladder cancers, 8 out of 36 breast cancers, 18 out of 18 cervical cancers, 5 out of 17 cholangiocellular carcinomas, 25 out of 31 CMLs, 6 out of 11 colorectal cancers, 6 out of 14 gastric cancers, 5 out of 5 NSCLCs, 7 out of 7 lymphomas, 6 out of 10 osteosarcomas, 7 out of 22 prostate cancers, 10 out of 18 renal carcinomas and 15 out of 21 soft tissue tumors. At the same time, DEPDC1 expression was shown to be validly elevated in 23 out of 25 bladder cancers, 6 out of 13 breast cancers, 12 out of 12 cervical cancers, 6 out of 6 cholangiocellular carcinomas, 3 out of 4 CMLs, 2 out of 4 colorectal cancers, 6 out of 6 NSCLCs, 7 out of 7 lymphomas, 10 out of 14 osteosarcomas, 11 out of 24 prostate cancers, 14 out of 14 SCLCs and 22 out of 31 soft tissue tumors as described in Table 1.

[0011] The present invention is based, at least in part, on the identification of specific epitope peptides of the gene products of these genes (MPHOSPH1 and DEPDC1) which possess the ability to induce cytotoxic T lymphocytes (CTLs) specific to the corresponding molecules. As discussed in detail below, Peripheral Blood Mononuclear Cells (PBMC) of healthy donor were stimulated using HLA-A*2402 and HLA-A*0201 binding candidate peptides derived from MPHOSPH1 or DEPDC1. CTL clones and/or lines were then established with specific cytotoxicity against the HLA-A24 or HLA-A2 positive target cells pulsed with each of the candidate peptides. These results demonstrate that these peptides are HLA-A24 or HLA-A2 restricted epitope peptides that can induce potent and specific immune responses against cells expressing MPHOSPH1 or DEPDC1.

[0012] Accordingly, the present invention provides methods for treating or preventing a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancer. Such methods involves the step of administering to a subject in need thereof a MPHOSPH1 and/or DEPDC1 polypeptide of the invention. Administration of such peptide(s) results in the induction of anti-tumor immunity. Thus, the present invention provides methods for inducing anti-tumor immunity in a subject, such methods involving the step of administering to the subject a MPHOSPH1 and/or DEPDC1 polypeptide, as well as pharmaceutical compositions for treating or preventing a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancer, that include the MPHOSPH1 and/or DEPDC1 polypeptides. Exemplary cancers include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, and soft tissue tumor.

[0013] That is, the present application includes following embodiments, and any combinations thereof.

- [1] An isolated peptide having cytotoxic T cell inducibility, wherein said peptide derived from amino acid sequence of SEQ ID NO: 2, 4, or 6.
- [2] An isolated peptide of less than about 15 amino acids selected from the group consisting of peptides comprising the amino acid sequences of SEQ ID NO: 7, 8 and 12, or a peptide having cytotoxic T cell inducibility, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 7, 8 and 12, wherein 1, 2, or several amino acids are substituted, deleted, or added.
- [3] The peptide having cytotoxic T cell inducibility of [2], wherein the second amino acid from the N-terminus is phenylalanine, tyrosine, methionine, or tryptophan.
- [4] The peptide having cytotoxic T cell inducibility of [2], wherein the C-terminal amino acid is phenylalanine, leucine, isoleucine, tryptophan, or methionine.
- [5] An isolated peptide of less than about 15 amino acids selected from the group consisting of peptides comprising the amino acid sequences of SEQ ID NO: 9, 10, 11, 192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 253, 254 and 255, or a peptide having cytotoxic T cell inducibility, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, 10, 11, 192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 253, 254 and 255, wherein 1, 2, or several amino acids are substituted, deleted, or added.
- [6] The peptide having cytotoxic T cell inducibility of [5], wherein the second amino acid from the N-terminus is leucine or methionine.
- [7] The peptide having cytotoxic T cell inducibility of [5], wherein the C-terminal amino acid is valine or leucine.
- [8] A vector in which the DNA encodes peptides of any one of [1] to [7].
- [9] A pharmaceutical composition for treating or preventing a disease associated with over-expression of the genes of SEQ ID NO: 1, 3 and/or 5, said composition comprising one or more peptides of any one of [1] to [7].
- [10] The pharmaceutical composition of [9], wherein the disease is cancer.
- [11] The pharmaceutical composition of [10], wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC and soft tissue tumor.
- [12]. An exosome that presents on its surface a complex comprising a peptide of any one of [1] to [7] and an HLA antigen.
- [13] The exosome of [12], wherein the HLA antigen is HLA-A24.
- [14] The exosome of [13], wherein the HLA antigen is HLA-A2402.
- [15]. The exosome of [12], wherein the HLA antigen is HLA-A2.
- [16]. The exosome of [13], wherein the HLA antigen is HLA-A0201.
- [17] A method of inducing antigen-presenting cells having a high cytotoxic T cell in-

ducibility comprising the step of contacting an antigen-presenting cell with a peptide of any one of [1] to [7].

[18] A method of inducing cytotoxic T cells by contacting a T cell with a peptide of any one of [1] to [7].

[19] A method of inducing antigen-presenting cells having high cytotoxic T cell inducibility, said method comprising the step of transferring a gene comprising a polynucleotide encoding a peptide of any one of [1] to [7] to an antigen-presenting cell.

[20] An isolated cytotoxic T cell, which is induced by contacting a T cell with a peptide of any one of claims 1 to 7 or which is transduced with the nucleic acids encoding the TCR subunits polypeptides binding with a peptide of any one claims 1 to 7 in the context of HLA-A24 or HLA-A2.

[21] An antigen-presenting cell, which comprises a complex formed between an HLA antigen and a peptide of any one of [1] to [7].

[22] The antigen-presenting cell of [21], induced by the method of [17].

[23] A vaccine for inhibiting proliferation of cells expressing genes of SEQ ID NO: 1, 3 and/or 5, wherein the vaccine comprises a peptide of any one of [1] to [7] as the active ingredient.

[24] The vaccine of [23], wherein the cell is a cancer cell.

[25] The vaccine of [24], wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC and soft tissue tumor.

[26] The vaccine of [23], formulated for administration to a subject whose HLA antigen is HLA-A24 or HLA-A2.

[27] A method of treating or preventing a disease associated with the over-expression of the genes of SEQ ID NO: 1, 3 and/or 5 in a subject comprising administering to said subject a vaccine comprising one or more peptide of any one of [1] to [7], an immunologically active fragment thereof, or a polynucleotide encoding said peptide or immunologically active fragment.

[28] The method of [27], wherein the disease is cancer.

[29] The method of [28], wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC and soft tissue tumor.

[0014] Alternatively, the present invention also relates to a method of inducing cytotoxic T cells comprising the step of contacting a T-cell with the antigen-presenting cell produced by the method of [19].

[0015] These and other objects and features of the invention will become more fully

apparent when the following detailed description is read in conjunction with the accompanying figures and examples. However, it is to be understood that both the foregoing summary of the invention and the following detailed description are of preferred embodiments, and not restrictive of the invention or other alternate embodiments of the invention.

Brief Description of the Drawings

[0016] [fig.1]Figure 1A depicts the results of an IFN-gamma ELISPOT assay for the screening of epitope peptides which, in turn, demonstrate that MPHOSPH1-A24-9-278 (SEQ ID NO: 7) is a potent producer of IFN-gamma. CTLs for those peptides derived from MPHOSHP1 were generated according to the protocols described in "Materials and Methods" section of the examples below. Resulting CTLs having detectable specific CTL activity are shown. In particular, the cells in the well number #4 stimulated with MPHOSPH1-A24-9-278 showed potent IFN-gamma production to recognize peptide pulsed target cells, as compared to the control. Figure 1B depicts the results of the IFN-gamma ELISPOT assay for the screening of CTL clones after limiting dilution (MPHOSPH1-A24-9-278 CTL clone). The cells in the positive well were expanded and limiting dilution was performed. As the depicted results demonstrate, CTL clones having higher specific CTL activities against the peptide-pulsed target as compared to the activities against target without peptide pulse were established.

[fig.2]Figure 2A depicts the results of an IFN-gamma ELISPOT assay for the screening of epitope peptides cytotoxicity, which, in turn, demonstrate that MPHOSPH1-A24-10-278 (SEQ ID NO: 8) is a potent producer of IFN-gamma. CTLs for those peptides derived from MPHOSHP1 were generated according to the protocols described in "Materials and Methods" section of the examples below. Resulting CTLs having detectable specific CTL activity are shown. In particular, the cells in the well number #8 stimulated with MPHOSPH1-A24-10-278 showed potent IFN-gamma production as compared to the control. Figure 2B depicts the results of an IFN-gamma ELISPOT assay for the screening of CTL clones after limiting dilution (MPHOSPH1-A24-10-278 CTL clone). The cells in the positive well were expanded and limiting dilution was performed. As the depicted results demonstrate, CTL clones having higher specific CTL activities against the MPHOSPH1-A24-10-278-pulsed target as compared to the activities against target without peptide pulse as shown were established.

[fig.3]Figure 3A depicts the establishment of CTL clones stimulated with MPHOSPH1-A24-9-278. (SEQ ID NO: 7). This CTL clone demonstrated high specific CTL activity against target cells (A24LCL) pulsed with MPHOSPH1-A24-9-278, but

did not show significant CTL activity against the same target cells (A24LCL) pulsed with no peptides. Figure 3B depicts the establishment of CTL clones stimulated with MPHOSPH1-A24-10-278 (SEQ ID NO: 8). This CTL clone demonstrated high specific CTL activity against target cells (A24LCL) pulsed with MPHOSPH1-A24-10-278, whereas it did not show significant CTL activity against the same target cells (A24LCL) pulsed with no peptides. R means Responder: CTL clone. S means Stimulator: peptide-pulsed A24-LCL (1×10^4 /well).

[fig.4]Figure 4 depicts the expression of MPHOSPH1-A24-9-278 (SEQ ID NO: 7) on the target cell surface with HLA-A24. Specific CTL activity against COS7 transfected both with the full length MPHOSPH1 gene and the HLA-A*2402 molecule was assayed using as effector cells the CTL clone raised by MPHOSPH1-A24-9-278. COS7 transfected with full length MPHOSPH1 but not HLA-A*2402 and COS7 transfected with HLA-A*2402 but not full length MPHOSPH1 were prepared as controls. The CTL clone demonstrated high specific CTL activity against COS7 transfected with both MPHOSPH1 and HLA-A24. However, it did not show significant specific CTL activity against COS7 transfected neither MPHOSPH1 nor HLA-A24. R means Responder: CTL clone. S means Stimulator: COS7 transfectant (1×10^4 / well).

[fig.5]Figure 5 depicts the CTL activity against bladder cancer cell lines endogenously expressing MPHOSPH1. The established CTL clone induced with MPHOSPH1-A24-9-278 peptide recognized tumor cells endogenously expressing MPHOSPH1. HT1376, RT-4 and J82 cells expressed MPHOSPH1 endogenously, respectively. CTL clone showed IFN-gamma production against HT1376 which have HLA-A*2402 genotype, but no showing response against RT-4 and J82, does not have HLA-A*2402 genotype.

[fig.6]Figure 6 depicts in vivo immunogenicity analysis using MPHOSPH1-A24-9-278 peptide. IFA-conjugated peptide was injected subcutaneously into BALB/c mice on days 0 and 7. On day 14, splenocytes of vaccinated mice were harvested and used as responder cells, and 1×10^4 RLmale1 cells pulsed MPHOSPH1-A24-9-278 peptide were used as stimulator cells for IFN-gamma ELISPOT assay. Spot forming counts (SFC) were indicated in cases of each mice; five mice (Ani1~Ani5) were vaccinated epitope peptide and three mice (nega1~nega3) were injected Mock IFA emulsion as a negative control.

[fig.7]Figure 7 depicts the results of an IFN-gamma ELISPOT assay for the screening of epitope peptides, which, in turn, demonstrate that MPHOSPH1-A2-9-282 (SEQ ID NO: 9), MPHOSPH1-A2-9-638 (SEQ ID NO: 10) and MPHOSPH1-A2-10-1714 (SEQ ID NO: 11) possess potent IFN-gamma production activity. CTLs for those peptides derived from MPHOSHP1 were generated according to the protocols described in

"Materials and Methods" section of the examples set forth below. Resulting CTLs having detectable specific CTL activity are shown. In particular, Figure 7A demonstrates that the cells in the well number #1 and #5, stimulated with MPHOSPH1-A2-9-282, showed potent IFN-gamma production sufficient to recognize peptide pulsed target cells, as compared to the control. Figure 7B demonstrates that the cells in the well number #8 stimulated with MPHOSPH1-A2-9-638 showed potent IFN-gamma production sufficient to recognize peptide pulsed target cells, as compared to the control. Figure 7C demonstrates that the cells in the well number #4 stimulated with MPHOSPH1-A2-10-1714 showed potent IFN-gamma production to recognize peptide pulsed target cells, as compared to the control.

[fig.8]Figure 8 depicts the establishment for CTL lines stimulated with MPHOSPH1-A02-9-282, (SEQ ID NO: 9) MPHOSPH1-A02-9-638 (SEQ ID NO: 10) and MPHOSPH1-A02-10-1714 (SEQ ID NO: 11). The cells in the positive well were expanded, and, as the depicted results demonstrate, CTL lines having higher specific CTL activities against the MPHOSPH1-A02-9-282-pulsed target (A), MPHOSPH1-A02-9-638-pulsed target (B) or MPHOSPH1-A02-10-1714-pulsed target (C) compared to the activities against target without peptide pulse were established. R means Responder: CTL lines. S means Stimulator: peptide-pulsed T2 (1×10^4 /well).

[fig.9]Figure 9A depicts the results of an IFN-gamma ELISPOT assay for the screening of CTL clones after limiting dilution (MPHOSPH1-A2-9-282 CTL clone). The cells in the positive well were expanded and limiting dilution was performed. As the depicted results demonstrate CTL clones having higher specific CTL activities against the MPHOSPH1-A2-9-282 (SEQ ID NO: 9) pulsed target as compared to the activities against target without peptide pulse were established. Figure 9B depicts the establishment of CTL clones stimulated with MPHOSPH1-A02-9-282. The CTL clone demonstrated high specific CTL activity against target cells (T2) pulsed with MPHOSPH1-A2-9-282, but did not possess significant CTL activity against the same target cells (T2) pulsed with no peptides. R means Responder: CTL clone. S means Stimulator: peptide-pulsed T2 (1×10^4 /well).

[fig.10]Figure 10A depicts the results of an IFN-gamma ELISPOT assay for the screening of epitope peptides, which, in turn, demonstrate that DEPDC1-A24-9-294 (SEQ ID NO: 12) is a potent producer of IFN-gamma. CTLs for those peptides derived from DEPDC1 were generated according to the protocols described in "Materials and Methods" section of the examples set forth below. Resulting CTLs showing detectable specific CTL activity are shown. The cells in the well number #10 stimulated with DEPDC1-A24-9-294 showed potent IFN-gamma production to recognize peptide pulsed target cells, compared with the control. Figure 10B depicts the results of an IFN-gamma ELISPOT assay for the screening of CTL clones after

limiting dilution (DEPDC1-A24-9-294 CTL clone). The cells in the positive well were expanded and limiting dilution performed. As the depicted results demonstrate, CTL clones having higher specific CTL activities against the DEPDC1-A24-9-294-pulsed target compared to the activities against target without peptide pulse were established. [fig.11]Figure 11 depicts the establishment for CTL clones stimulated with DEPDC1-A24-9-294 (SEQ ID NO: 12). The CTL clone showed high specific CTL activity against target cells (A24LCL) pulsed with DEPDC1-A24-9-294, whereas it did not show significant CTL activity against the same target cells (A24LCL) pulsed with no peptides. R means Responder: DEPDC-A24-9-294 CTL clone. S means Stimulator: peptide-pulsed A24-LCL (1×10^4 /well).

[fig.12]Figure 12 depicts the expression of DEPDC1-A24-9-294 (SEQ ID NO: 12) on the target cell surface with HLA-A24. Specific CTL activity against COS7 transfected with both the full length DEPDC1 gene and the HLA-A*2402 molecule was assayed using as effector cells the CTL clone raised by DEPDC1-A24-9-294. COS7 transfected with full length DEPDC1 but not HLA-A*2402 and COS7 transfected HLA-A*2402 but not full length DEPDC1 were prepared as controls. The CTL clone established demonstrated high specific CTL activity against COS7 transfected with both DEPDC1 and HLA-A24. However, it did not show significant specific CTL activity against COS7 transfected with neither DEPDC1 nor HLA-A24. R means Responder: DEP-A24-9-294 CTL clone. S means Stimulator: COS7 transfectant (1×10^4 /well).

[fig.13]Figure 13 depicts the CTL activity against bladder cancer cell lines endogenously expressing DEPDC1. The established CTL clone induced with DEPDC1-A24-9-294 peptide recognized tumor cells endogenously expressing DEPDC1. HT1376, RT-4 and J82 cells expressed DEPDC1 endogenously, respectively. CTL clone showed IFN-gamma production against HT1376 which have HLA-A*2402 genotype, but no showing response against RT-4 and J82, does not have HLA-A*2402 genotype.

[fig.14]Figure 14 depicts the in vivo immunogenicity analysis using DEPDC1-A24-9-294 peptide. IFA-conjugated peptide was injected subcutaneously into BALB/c mice on days 0 and 7. On day 14, splenocytes of vaccinated mice were harvested and used as responder cells, and 1×10^4 RLmale1 cells pulsed DEPDC1-A24-9-294 peptide were used as stimulator cells for IFN-gamma ELISPOT assay. Spot forming counts (SFC) were indicated in cases of each mice; five mice (Ani1~Ani5) were vaccinated epitope peptide and two mice (nega1 and nega2) were injected Mock IFA emulsion as a negative control.

[fig.15]Figure 15 depicts potent IFN-gamma production of DEPDC1-A02-10-644, -10-575, -10-506, -10-765, -10-395, -10-224, -9-297, -10-296 and -10-302 by IFN-gamma ELISPOT assay for the screening of epitope peptides. CTLs for those peptides

derived from DEPDC1 were generated in the way described in "Materials and Methods". The cells in the well number #4 and #7 stimulated with DEPDC1-A02-10-644, #2 with DEPDC1-A02-10-575, #7 with DEPDC1-A02-10-506, #1 with DEPDC1-A02-10-765 and #1 with DEPDC1-A02-10-395, #1 and #2 with DEPDC1-A02-10-224, #4 with DEPDC1-A02-9-297, #3 and #4 with DEPDC1-A02-10-296 and #2, #3, #5 and #7 with DEPDC1-A02-10-302 showed potent IFN-gamma production compared with the control.

[fig.16]Figure 16 depicts IFN-gamma production of CTL line generated with DEPDC1-A02-10-296 peptide. The established CTL lines raised by DEPDC1-A02-10-296 peptide have potent IFN-gamma production activity. It was shown IFN-gamma production against peptide-pulsed target cells, but not shown that against target cells without peptide pulse. Target cells were used T2 cells, expressed HLA-A2 molecule at cell surface.

[fig.17]Figure 17 depicts CTL activity against targets endogenously expressing DEPDC1 and HLA-A2 molecule. It was shown in upper panel that the established CTL line generated with DEPDC1-A02-10-296 peptide possessed IFN-gamma production activity against target cells which endogenously expressed DEPDC1V2 and HLA-A2. The case of using DEPDC1-A02-10-296 peptide was shown in lower panel. The target cells expressing only DEPDC1V2 and expressing only HLA-A2 with treatment of DEPDC1V1-9-674 or DEP-9-462 peptide pulse were prepared as the negative control. The target cells were prepared from HEK293 transfectant which stable expressed HLA-A2 or mock.

[fig.18]Figure 18 depicts antigen expression in Case 2. In Case 2, both MPHOSPH1 and DEPDC1 were expressed strongly. Therefore, two kinds of epitope peptides derived from MPHOSPH1 and DEPDC1 have been vaccinated.

[fig.19]Figure 19 depicts the clinical evaluation for local recurrence of bladder cancer in Case 2. Case 2 were evaluated SD in accordance with RECIST criteria.

[fig.20]Figure 20 depicts antigen expression in Case 3. In Case 3, DEPDC1 was expressed strongly. Therefore, we have vaccinated the epitope peptide derived from DEPDC1 alone.

[fig.21]Figure 21 depicts clinical evaluation for right lobe of metastatic lung in Case 3. The progression rate was decreased after vaccination. Especially, the size of the tumor was decreased after 3rd courses.

[fig.22]Figure 22 depicts clinical evaluation for left lobe of metastatic lung in Case 3. The progression rate was decreased after vaccination. Especially, the size of the tumor was decreased after 3rd courses.

[fig.23]Figure 23 depicts the Anti-tumor effect in Case 3. The progression rate of metastatic tumor was decreased after vaccination.

[fig.24]Figure 24 depicts specific CTL response in Case 3. Specific CTL response was strongly shown after vaccination.

[fig.25]Figure 25 depicts antigen expression in Case 4. In Case 4, MPHOSPH1 and DEPDC1 were expressed. Therefore, two kinds of epitope peptides derived from MPHOSPH1 and DEPDC1 have been vaccinated.

[fig.26]Figure 26 depicts the clinical evaluation for local recurrence of bladder cancer in Case 4. The size of the tumor was reduced 20% in accordance with RECIST criteria after 1st course vaccination.

[0017] Detailed Description of the Invention

The words "a", "an", and "the" as used herein mean "at least one" unless otherwise specifically indicated.

[0018] Unless otherwise defined, 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.

[0019] Identification of new TAAs, particularly those that induce potent and specific anti-tumor immune responses, warrants further development of the clinical application of the peptide vaccination strategy in various types of cancer (Boon T et al., (1996) J Exp Med 183: 725-9.; van der Bruggen P et al., (1991) Science 254: 1643-7.; Brichard V et al., (1993) J Exp Med 178: 489-95.; Kawakami Y et al., (1994) J Exp Med 180: 347-52.; Shichijo S et al., (1998) J Exp Med 187:277-88.; Chen YT et al., (1997) Proc.Natl.Acd. Sci.USA, 94: 1914-8.; Harris CC, (1996) J Natl Cancer Inst 88:1442-55.; Butterfield LH et al., (1999) Cancer Res 59:3134-42.; Vissers JL et al., (1999) Cancer Res 59: 5554-9.; van der Burg SH et al., (1996) J. Immunol 156:3308-14.; Tanaka F et al., (1997) Cancer Res 57:4465-8.; Fujie T et al., (1999) Int J Cancer 80:169-72.; Kikuchi M et al., (1999) Int J Cancer 81 : 459-66.; Oiso M et al., (1999) Int J Cancer 81:387-94.). As noted above, MPHOSPH1 (M-phase phosphoprotein 1; GenBank Accession No. NM_016195; SEQ ID Nos.1, 2) and DEPDC1 (DEP domain containing 1; GenBank Accession No. BM683578), more particularly its two variants, DEPDC1V1 (SEQ ID Nos.3, 4) and DEPDC1V2 (SEQ ID No. 5, 6), were previously identified using cDNA microarray technologies as over-expressed in various cancers. MPHOSPH1 was previously identified as one of the proteins specifically phosphorylated at the G2/M transition, and characterized as a plus-end-directed kinesin related protein (Abaza A et al., J Biol Chem 2003, 278: 27844-52.). More particularly, MPHOSPH1 was previously documented to be plus-end-directed molecular motor that plays a crucial role in cytokinesis, and accumulates in the midzone of the spindle during anaphase to telophase in HeLa cells (Abaza A et al., J Biol Chem 2003, 278: 27844-52; Kamimoto T et al., J Biol Chem 2001, 276: 37520-8.).The MPHOSPH1 cDNA encodes a 1780-amino acid protein that is

composed of three domains: an NH2-kinasin motor domain, a central coiled coil-stalk domain, and a C-globular tail domain. These data suggest that MPHOSPH1 is an NH2-type kinesin-related protein.

- [0020] The function of DEPDC1 protein remains unclear. The DEP domain included this protein is found in Dishevelled, Egl-10, and Pleckstrin. In particular, the DEP domain in *Drosophila* dishevelled is essential to rescue planar polarity defects and induces JNK signaling; nevertheless, its function in Human has not yet been clarified. However, as disclosed in PCT/JP2006/302684, DEPDC1 (in house No. B5860N) has two different transcriptional variants consisting of 12 and 11 exons, corresponding to DEPDC1 V1 and V2, respectively. Alternative variations in exon 8 of V1 were noted, and the other remaining exons were found to be common to both variants. V2 variant has no exon 8 of the V1, but generates the same stop codon within last exon. The full-length cDNA sequences of the B5860NV1 and B5860NV2 variants consist of 5318 and 4466 nucleotides, respectively. The ORF of these variants start at within each exon 1. Eventually, V1 and V2 transcripts encode 811 and 527 amino acids, respectively. siRNAs suppressed the growth of cancer cells. These results demonstrate that DEPDC1 plays important roles in growth of most cancer cells.
- [0021] As disclosed in PCT/JP2006/302684, MPHOSPH1 and DEPDC1 are over-expressed in bladder cancer but show minimal expression in normal tissues. In addition, these genes were found to have a significant function related to cell proliferation.
- [0022] In the present invention, peptides derived from MPHOSPH1 or DEPDC1 are shown to be TAA epitopes restricted by HLA-A24 and HLA-A2, an HLA allele commonly found in the Japanese and Caucasian populations. Specifically, using their binding affinities to HLA-A24 and HLA-A2, candidates of HLA-A24 and HLA-A2 binding peptides derived from MPHOSPH1 or DEPDC1 were identified. After the in vitro stimulation of T-cells by dendritic cells (DCs) loaded with these peptides, CTLs were successfully established using MPHOSPH1-A24-9-278 (IYNEYIYDL (SEQ ID NO: 7)), MPHOSPH1-A24-10-278 (IYNEyIYDLF (SEQ ID NO: 8)), MPHOSPH1-A2-9-282 (YIYDLFVPV (SEQ ID NO: 9)), MPHOSPH1-A2-9-638 (RLAIFKDLV (SEQ ID NO: 10)), MPHOSPH1-A2-10-1714 (TMSSsKLSNV (SEQ ID NO: 11)), DEPDC1-A24-9-294 (EYYELFVNI (SEQ ID NO: 12)), DEPDC1-A02-10-644 (SLMIhTFSRC (SEQ ID NO: 240)), DEPDC1-A02-10-575 (SLLPaSSMLT (SEQ ID NO: 241)), DEPDC1-A02-10-506 (QLCRsQSLLL (SEQ ID NO: 243)), DEPDC1-A02-10-765 (KQFQkEYPLI (SEQ ID NO: 244)), DEPDC1-A02-10-395 (IMGGSCHNLI (SEQ ID NO: 249)), DEPDC1-A02-10-224 (NMANtSKRGV (SEQ ID NO: 253)), DEPDC1-A02-9-297 (ELFVNILGL (SEQ ID NO: 226)), DEPDC1-A02-10-296 (YELFvNILGL (SEQ ID NO: 254)), DEPDC1-A02-10-301 (NILGILQPHL (SEQ ID NO: 255)), DEPDC1-A2-9-589

(LLQPHLERV (SEQ ID NO: 192)), DEPDC1-A2-9-619 (LLMRMISRM (SEQ ID NO: 195)), DEPDC1-A2-9-290 (LLTFEYYEL (SEQ ID NO: 197)), DEPDC1-A2-9-563 (RLCKSTIEL (SEQ ID NO: 209)), DEPDC1-A2-9-653 (CVLCCAEEV (SEQ ID NO: 225)), DEPDC1-A2-10-674 (FLMDhHQEIL (SEQ ID NO: 228)) and DEPDC1-A2-10-302 (ILVVcGYITV (SEQ ID NO: 230)). These CTLs demonstrated potent cytotoxic activity against the peptide-pulsed A24LCL and T2 cells. Furthermore, CTL clones derived from these cells also demonstrated specific cytotoxicity against HLA-A24 or HLA-A2 positive cells expressing MPHOSPH1 or DEPDC1, respectively.. However, these CTL clones did not express cytotoxic activity against cells having expression of only one of peptides, including HLA-A24, HLA-A2, MPHOSPH1 and DEPDC1. Together these results suggest the utility of MPHOSPH1 and DEPDC1 as TAAs for cancer cells and that MPHOSPH1-A24-9-278 (IYNEYIYDL (SEQ ID NO: 7)), MPHOSPH1-A24-10-278 (IYNEyIYDLF (SEQ ID NO: 8)), MPHOSPH1-A2-9-282 (YIYDLFVPV (SEQ ID NO: 9)), MPHOSPH1-A2-9-638 (RLAIFKDLV (SEQ ID NO: 10)), MPHOSPH1-A2-10-1714 (TMSSsKLSNV (SEQ ID NO: 11)), DEPDC1-A24-9-294 (EYYELFVNI (SEQ ID NO: 12)), DEPDC1-A02-10-644 (SLMIhTFSRC (SEQ ID NO: 240)), DEPDC1-A02-10-575 (SLLPaSSMLT (SEQ ID NO: 241)), DEPDC1-A02-10-506 (QLCRsQSLLL (SEQ ID NO: 243)), DEPDC1-A02-10-765 (KQFQKEYPLI (SEQ ID NO: 244)), DEPDC1-A02-10-395 (IMGGSCHNLI (SEQ ID NO: 249)), DEPDC1-A02-10-224 (NMANtSKRGV (SEQ ID NO: 253)), DEPDC1-A02-9-297 (ELFVNILGL (SEQ ID NO: 226)), DEPDC1-A02-10-296 (YELFvNILGL (SEQ ID NO: 254)), DEPDC1-A02-10-301 (NILGILQPHL (SEQ ID NO: 255)), DEPDC1-A2-9-589 (LLQPHLERV (SEQ ID NO: 192)), DEPDC1-A2-9-619 (LLMRMISRM (SEQ ID NO: 195)), DEPDC1-A2-9-290 (LLTFEYYEL (SEQ ID NO: 197)), DEPDC1-A2-9-563 (RLCKSTIEL (SEQ ID NO: 209)), DEPDC1-A2-9-653 (CVLCCAEEV (SEQ ID NO: 225)), DEPDC1-A2-10-674 (FLMDhHQEIL (SEQ ID NO: 228)) and DEPDC1-A2-10-302 (ILVVcGYITV (SEQ ID NO: 230)) are epitope peptides of each TAA restricted by HLA-A24 or HLA-A2. Since these antigens are over-expressed in most cancers and are associated with tumor cell proliferation, they find utility as immunotherapeutic targets against cancers. Exemplary cancers include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, soft tissue tumor.

[0023] Accordingly, the present invention further provides methods of treating or preventing a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancers, in a subject, such methods including the steps of administering to a subject in

need thereof an immunogenic peptide of less than about 40 amino acids, often less than about 20 amino acids, usually less than about 15 amino acids and having the amino acid sequence of SEQ ID NOs: 7, 8, 9, 10, 11, 12, 192, 195, 197, 209, 225, 226 228, 230, 240, 241, 243, 244, 249, 253, 254 or 255. Alternatively, the immunogenic peptide may be composed of a sequence of SEQ ID NOs: 7, 8, 9, 10, 11, 12, 192, 195, 197, 209, 225, 226 228, 230, 240, 241, 243, 244, 249, 253, 254 or 255 in which 1, 2, or several (e.g., up to 5) amino acids are substituted, deleted or added, provided the resulting variant peptide retains the immunogenic activity (i.e., the ability to induce CTLs specific to cells expressing MPHOSPH1 and/or DEPDC1, e.g. cancers). The number of residues to be substituted, deleted, or added is generally 5 amino acids or less, preferably 4 amino acids or less, more preferably 3 amino acids or less, even more preferably one or two amino acids. The cancers contemplated include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, soft tissue tumor.

- [0024] Variant peptides (i.e., peptides having an amino acid sequence modified by substituting, deleting, or adding one, two or several amino acid residues to an original amino acid sequence) are known to retain the original biological activity (Mark DF et al., (1984) Proc Natl Acad Sci USA 81: 5662-6.; Zoller MJ and Smith M, (1982) Nucleic Acids Res 10:6487-500.; Dalbadie-McFarland G et al., (1982) Proc Natl Acad Sci USA 79: 6409-13.). In the context of the present invention, it is preferable that the amino acid modification results in conservation of the properties of the original amino acid side-chain (a process known as conservative amino acid substitution). Examples of properties of amino acid side chains include hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), and side chains having the following functional groups or characteristics in common: an aliphatic side-chain (G, A, V, L, I, P); a hydroxyl group containing side-chain (S, T, Y); a sulfur atom containing side-chain (C, M); a carboxylic acid and amide containing side-chain (D, N, E, Q); a base containing side-chain (R, K, H); and an aromatic containing side-chain (H, F, Y, W). Note, the parenthetic letters indicate the one-letter codes of amino acids.
- [0025] In preferred embodiments, the immunogenic peptide is a nonapeptide (9-mer) or a decapeptide (10-mer).
- [0026] The present invention further provides a method of inducing anti-tumor immunity for a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancers, in a subject, such a method including the steps of administering to a subject in need thereof an immunogenic peptide of the present invention, namely one having the amino acid sequence of S SEQ ID NOs: 7, 8, 9, 10, 11, 12, 192, 195, 197, 209, 225,

226 228, 230, 240, 241, 243, 244, 249, 253, 254 or 255 or a variant thereof (i.e., including 1, 2, or several amino acid substitutions, deletions, or additions). The cancers contemplated include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, soft tissue tumor.

- [0027] In the context of the present invention, the subject is preferably a mammal. Exemplary mammals include, but are not limited to, e.g., a human, non-human primate, mouse, rat, dog, cat, horse, or cow.
- [0028] In the present invention, the peptide can be administered to a subject via an in vivo or ex vivo protocol. Furthermore, the present invention also provides use of nonapeptide or decapeptide selected from peptides having the amino acid sequence of SEQ ID NOs: 7, 8, 9, 10, 11, 12, 192, 195, 197, 209, 225, 226 228, 230, 240, 241, 243, 244, 249, 253, 254 and 255 (and variants thereof) for manufacturing an immunogenic composition for treating or preventing a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancers. The cancers contemplated include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, soft tissue tumor.
- [0029] Homology analyses of MPHOSPH1-A24-9-278 (IYNEYTYDL (SEQ ID NO: 7)), MPHOSPH1-A24-10-278 (IYNEyIYDLF (SEQ ID NO: 8)), MPHOSPH1-A2-9-282 (YIYDLFVPV (SEQ ID NO: 9)), MPHOSPH1-A2-9-638 (RLAIFKDLV (SEQ ID NO: 10)), MPHOSPH1-A2-10-1714 (TMSSsKLSNV (SEQ ID NO: 11)), DEPDC1-A24-9-294 (EYYELFVNI (SEQ ID NO: 12)), DEPDC1-A2-9-589 (LLQPHLERV (SEQ ID NO: 192)), DEPDC1-A2-9-619 (LLMRMISRM (SEQ ID NO: 195)), DEPDC1-A2-9-290 (LLTFEYYEL (SEQ ID NO: 197)), DEPDC1-A2-9-563 (RLCKSTIEL (SEQ ID NO: 209)), DEPDC1-A2-9-653 (CVLCCAEEV (SEQ ID NO: 225)), DEPDC1-A2-10-674 (FLMDhHQEIL (SEQ ID NO: 228)), DEPDC1-A2-10-302 (ILVVcGYITV (SEQ ID NO: 230)), DEPDC1-A02-10-644 (SLMIhTFSRC (SEQ ID NO: 240)), DEPDC1-A02-10-575 (SLLPaSSMLT (SEQ ID NO: 241)), DEPDC1-A02-10-506 (QLCRsQSLLL (SEQ ID NO: 243)), DEPDC1-A02-10-765 (KQFQkEYPLI (SEQ ID NO: 244)), DEPDC1-A02-10-395 (IMGGSCHNLI (SEQ ID NO: 249)), DEPDC1-A02-10-224 (NMANtSKRGV (SEQ ID NO: 253)), DEPDC1-A02-9-297 (ELFVNILGL (SEQ ID NO: 226)), DEPDC1-A02-10-296 (YELFvNILGL (SEQ ID NO: 254)) and DEPDC1-A02-10-301 (NILGILQPHL (SEQ ID NO: 255)) demonstrate that they do not have significant homology with the peptides derived from any known human gene products. Accordingly, the possibility of unknown or undesirable immune responses with immunotherapy against these molecules is significantly reduced.

- [0030] Regarding HLA antigens, the data presented here demonstrate that the uses of A-24 type or A-2 type antigens (which are said to be highly expressed among the Japanese) are favorable for obtaining effective results. The uses of subtypes such as A-2402 and A-0201 are even more preferable. Typically, in the clinic, the type of HLA antigen of the patient requiring treatment is investigated in advance, which, in turn, enables the selection of appropriate peptides having high levels of binding affinity to the patient antigen, or having cytotoxic T cell (CTL) inducibility by antigen presentation. Furthermore, in order to obtain peptides having high binding affinity and CTL inducibility, substitution, deletion, or addition of 1, 2, or several amino acids may be performed based on the amino acid sequence of the naturally occurring MPHOSPH1 and DEPDC1 partial peptide. Herein, the term "several" means refers to 5 or less, more preferably 3 or less. Furthermore, in addition to peptides that are naturally displayed, since the regularity of the sequences of peptides displayed by binding to HLA antigens is already known (Kubo RT, et al., (1994) J. Immunol., 152, 3913-24.; Rammensee HG, et al., (1995) Immunogenetics. 41:178-228.; Kondo A, et al., (1995) J. Immunol. 155:4307-12.), modifications based on such regularity can be performed on the immunogenic peptides of the invention. For example, peptides possessing high HLA-24 binding affinity in which the second amino acid from the N terminus substituted with phenylalanine, tyrosine, methionine, or tryptophan may be favorably used. Likewise, peptides whose C-terminal amino acid is substituted with phenylalanine, leucine, isoleucine, tryptophan, or methionine may also be used favorably. On the other hand, peptides possessing high HLA-A2 binding affinity having their second amino acid from the N terminus substituted with leucine or methionine, and peptides whose C-terminal amino acid is substituted with valine or leucine may be used favorably. Furthermore, 1 to 2 amino acids may be added to the N terminus and/or C terminus of the peptide.
- [0031] However, when the peptide sequence is identical to a portion of the amino acid sequence of an endogenous or exogenous protein having a different function, side effects such as autoimmune disorders or allergic symptoms against specific substances may be induced. Therefore, it is preferable to avoid the situation wherein the immunogenic sequence matches the amino acid sequence of a known protein. This situation may be avoided by performing a homology search using available databases. If homology searches confirm that peptides in which 1, 2 or several different amino acids do not exist in nature, then the danger that modifications of the above-mentioned amino acid sequence that, for example, increase the binding affinity with HLA antigens, and/or increase the CTL inducibility can be avoided.
- [0032] Although peptides having high binding affinity to the HLA antigens as described above are expected to be highly effective as cancer vaccines, the candidate peptides,

which are selected according to the presence of high binding affinity as an indicator, must be examined for the actual presence of CTL inducibility. CTL inducibility may be routinely confirmed by inducing antigen-presenting cells carrying human MHC antigens (for example, B-lymphocytes, macrophages, and dendritic cells), or more specifically dendritic cells derived from human peripheral blood mononuclear leukocytes, and, after stimulation with the peptide of interest, mixing with CD8-positive cells and measuring the cytotoxic activity against the target cells. As the reaction system, transgenic animals produced to express a human HLA antigen (for example, those described in BenMohamed L, et al., (2000) Hum. Immunol.; 61(8):764-79 Related Articles, Books, Linkout.) may be used. For example, the target cells can be radio-labeled with ^{51}Cr and such, and cytotoxic activity can be calculated from radioactivity released from the target cells. Alternatively, it can be examined by measuring IFN-gamma produced and released by CTL in the presence of antigen-presenting cells that carry immobilized peptides, and visualizing the inhibition zone on the media using anti-IFN-gamma monoclonal antibodies.

[0033] As a result of examining the CTL inducibility of peptides as described above, it was discovered that those peptides having high binding affinity to an HLA antigen did not necessarily have high inducibility. However, nonapeptides or decapeptides selected from the group of peptides having the amino acid sequences indicated by IYNEYIYDL (SEQ ID NO: 7), IYNEyIYDLF (SEQ ID NO: 8), YIYDLFVPV (SEQ ID NO: 9), RLAIKFKDLV (SEQ ID NO: 10), TMSSsKLSNV (SEQ ID NO: 11), EYYELFVNI (SEQ ID NO: 12), LLQPHLERV (SEQ ID NO: 192), LLMRMISRM (SEQ ID NO: 195), LLTFEYYEL (SEQ ID NO: 197), RLCKSTIEL (SEQ ID NO: 209), CVLCCAEEV (SEQ ID NO: 225), FLMDhHQEIL (SEQ ID NO: 228), ILVVcGYITV (SEQ ID NO: 230) DEPDC1-A02-10-644 (SLMIhTFSRC (SEQ ID NO: 240)), DEPDC1-A02-10-575 (SLLPaSSMLT (SEQ ID NO: 241)), DEPDC1-A02-10-506 (QLCRsQSLLL (SEQ ID NO: 243)), DEPDC1-A02-10-765 (KQFQkEYPLI (SEQ ID NO: 244)), DEPDC1-A02-10-395 (IMGGSCHNLI (SEQ ID NO: 249)), DEPDC1-A02-10-224 (NMANtSKRGV (SEQ ID NO: 253)), DEPDC1-A02-9-297 (ELFVNILGL (SEQ ID NO: 226)), DEPDC1-A02-10-296 (YELFvNILGL (SEQ ID NO: 254)) and DEPDC1-A02-10-301 (NILGILQPHL (SEQ ID NO: 255)) showed particularly high CTL inducibility.

[0034] As noted above, the present invention provides peptides having cytotoxic T cell inducibility, namely those having the amino acid sequence of SEQ ID NOs: 7, 8, 9, 10, 11, 12, 192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 249, 253, 254 or 255 or a variant thereof (i.e., those in which 1, 2, or several amino acids are substituted, deleted, or added). It is preferable that the amino acid sequences composed of 9 or 10 amino acids indicated in SEQ ID NOs: 7, 8, 9, 10, 11, 12, 192, 195, 197, 209, 225, 226

228, 230, 240, 241, 243, 244, 249, 253, 254 or 255 or a variant thereof do not match an amino acid sequence associated with another endogenous protein. In particular, amino acid substitution to leucine or methionine at the second amino acid from the N terminus, amino acid substitution to valine or leucine at the C-terminal amino acid, and amino acid addition of 1 to 2 amino acids at the N terminus and/or C terminus are examples of preferred variants. One of skill in the art will recognize that in addition to amino acid substitutions and additions, immunologically active fragments of the peptides may also be used in the methods of the invention. Methods for determining active fragments are well known in the art. CTL clones obtained by stimulation by these modified peptides can recognize the original peptides and cause damage for cells expressing the original peptides.

[0035] Peptides of the present invention can be prepared using well known techniques. For example, the peptides can be prepared synthetically, using either recombinant DNA technology or chemical synthesis. Peptides of the present invention may be synthesized individually or as longer polypeptides comprising two or more peptides. The peptides of the present invention are preferably isolated, i.e., substantially free of other naturally occurring host cell proteins and fragments thereof.

[0036] The peptides of the present invention may contain modifications, such as glycosylation, side chain oxidation, or phosphorylation; so long as the modifications do not destroy the biological activity of the peptides as described herein, namely the ability to binding to an HLA antigen and induce CTL. Other modifications include incorporation of D-amino acids or other amino acid mimetics that can be used, for example, to increase the serum half life of the peptides.

[0037] The peptides of this invention can be prepared as a combination, which includes two or more of peptides of the invention, for use as a vaccine for a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancers, such a vaccine inducing CTL in vivo. The cancers contemplated include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, soft tissue tumor. The peptides may be in a cocktail or may be conjugated to each other using standard techniques. For example, the peptides can be expressed as a single polypeptide sequence. The peptides in the combination may be the same or different. By administering the peptides of this invention, the peptides are presented at a high density on the HLA antigens of antigen-presenting cells, which, in turn, induces CTLs that specifically react toward the complex formed between the displayed peptide and the HLA antigen. Alternatively, antigen-presenting cells having immobilized the peptides of this invention on their cell surface, obtained by removing dendritic cells from the subjects, may be stimulated by the peptides of this invention.

Re-administration of these cells to the respective subjects induces CTL, and, as a result, aggressiveness towards the target cells can be increased.

- [0038] More specifically, the present invention provides drugs for treating and/or preventing proliferation, metastasis, and such of a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancers, which include one or more of peptides of this invention. The peptides of this invention find particular utility in the treatment of a disease associating MPHOSPH1 and/or DEPDC1, e.g. cancers. The cancers contemplated include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, soft tissue tumor.
- [0039] The peptides of this invention can be administered to a subject directly, as a pharmaceutical composition that has been formulated by conventional formulation methods. In such cases, in addition to the peptides of this invention, carriers, excipients, and such that are ordinarily used for drugs can be included as appropriate, without particular limitations. The immunogenic compositions of this invention may be used for treatment and prevention of a disease associating MPHOSPH1 and/or DEPDC1, e.g. cancers. The cancers contemplated include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, soft tissue tumor.
- [0040] The immunogenic compositions for treatment and/or prevention of a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancers, which comprise as the active ingredient one or more peptides of the present invention, can further include an adjuvant so that cellular immunity will be established effectively. Alternatively, they may be administered with other active ingredients, such as anti-cancer agents. The cancers contemplated include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, soft tissue tumor. Suitable formulations include granules. Suitable adjuvants are described in the literature (Johnson AG. (1994) Clin. Microbiol. Rev., 7:277-89.). Exemplary adjuvants include, but are not limited to, aluminum phosphate, aluminum hydroxide, and alum. Furthermore, liposome formulations, granular formulations in which the drug is bound to few-micrometer diameter beads, and formulations in which a lipid is bound to the peptide may be conveniently used. The method of administration may be oral, intradermal, subcutaneous, intravenous injection, or such, and may include systemic administration or local administration to the vicinity of the targeted tumor. The dose of the peptide(s) of this invention can be adjusted appropriately according to the disease to be treated, age of the patient, weight,

method of administration, and such. Though the dosage is ordinarily 0.001 mg to 1000 mg, preferably 0.01 mg to 100 mg, more preferably 0.1 mg to 10 mg, preferably administered once in a few days to few months, one skilled in the art can readily select the appropriate dose and method of administration, as, the selection and optimization of these parameters is well within routine skill.

- [0041] The present invention further provides intracellular vesicles called exosomes, which present complexes formed between the peptides of this invention and HLA antigens on their surface. Exosomes can be prepared, for example, by using the methods described in detail in Published Japanese Translation of International Publication Nos. Hei 11-510507 and 2000-512161, and are preferably prepared using antigen-presenting cells obtained from subjects who are targets of treatment and/or prevention. The exosomes of this invention can be inoculated as cancer vaccines, similarly to the peptides of this invention.
- [0042] The type of HLA antigens used must match that of the subject requiring treatment and/or prevention. For example, in the Japanese population, HLA-A24 or HLA-A2, particularly HLA-A2402 or HLA-A0201, is often appropriate.
- [0043] In some embodiments, the vaccine compositions of the present invention include a component which primes cytotoxic T lymphocytes. Lipids have been identified as agents capable of priming CTL in vivo against viral antigens. For example, palmitic acid residues can be attached to the epsilon-and alpha-amino groups of a lysine residue and then linked to an immunogenic peptide of the invention. The lipidated peptide can then be administered either directly, in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant. As another example of a lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinylserine (P3CSS), can be used to prime CTL when covalently attached to an appropriate peptide (see, e.g., Deres K, et al., (1989) *Nature* 342:561-4.).
- [0044] The immunogenic compositions of the present invention may also include nucleic acids encoding one or more of the immunogenic peptides disclosed here. See, e.g., Wolff JA et al., (1990) *Science* 247:1465-8; U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; and WO 98/04720. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivacaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (see, e.g., U.S. Patent No. 5,922,687).
- [0045] The immunogenic peptides of the invention can also be expressed by viral or bacterial vectors. Examples of suitable expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus, e.g., as a vector to express nucleotide sequences that encode the peptide. Upon introduction into a host, the recombinant vaccinia virus expresses the immunogenic

peptide, and thereby elicits an immune response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Patent No. 4,722,848. Another suitable vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover CK, et al., (1991) *Nature* 351:456-60. A wide variety of other vectors useful for therapeutic administration or immunization e.g., adeno and adeno-associated virus vectors, retroviral vectors, *Salmonella typhi* vectors, detoxified anthrax toxin vectors, and the like, are known in the art. See, e.g., Shata MT, et al., (2000) *Mol. Med. Today* 6:66-71; Shedlock DJ and Weiner DB., et al., (2000) *J. Leukoc. Biol.* 68:793-806; and Hipp JD, et al., (2000) *In Vivo* 14:571-85.

[0046] The present invention also provides methods of inducing antigen-presenting cells using one or more peptides of this invention. The antigen-presenting cells can be induced by inducing dendritic cells from the peripheral blood monocytes and then contacting (stimulating) them with one or more peptides of this invention in vitro, ex vivo or in vivo. When peptides of the present invention are administered to the subjects, antigen-presenting cells that have the peptides of this invention immobilized to them are induced in the body of the subject. Alternatively, after immobilizing the peptides of this invention to the antigen-presenting cells, the cells can be administered to the subject as a vaccine. For example, the ex vivo administration may include the steps of:

a: collecting antigen-presenting cells from a subject, and

b: contacting the antigen-presenting cells of step a with a peptide of the present invention.

[0047] The antigen-presenting cells obtained by step b can be administered to the subject as a vaccine.

[0048] This invention also provides a method for inducing antigen-presenting cells having a high level of cytotoxic T cell inducibility, in which the method includes the step of transferring genes composed of polynucleotide(s) encoding one or more peptides of this invention to antigen-presenting cells in vitro. The introduced genes may be in the form of DNAs or RNAs. For the method of introduction, without particular limitations, various methods conventionally performed in this field, such as lipofection, electroporation, and calcium phosphate method may be suitably used. More specifically, transfection may be performed as described in Reeves ME, et al., (1996) *Cancer Res.*, 56:5672-7.; Butterfield LH, et al., (1998) *J. Immunol.*, 161:5607-13.; Boczkowski D, et al., (1996) *J. Exp. Med.*, 184:465-72.; Published Japanese Translation of International Publication No. 2000-509281. By transferring the gene into antigen-presenting cells, the gene undergoes transcription, translation, and such in the cell, and then the obtained protein is processed by MHC Class I or Class II, and proceeds through a presentation pathway to present partial peptides.

[0049] The present invention further provides methods for inducing CTL using one or more peptides of this invention. When the peptides of this invention are administered to a subject, CTL are induced in the body of the subject, and the strength of the immune system targeting the cells expressing MPHOSPH1 and/or DEPDC1, e.g. cancer cells in the tumor tissues is thereby enhanced. The cancers contemplated include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, soft tissue tumor. Alternatively, the peptides of the present invention may be used in the context of an ex vivo therapeutic method, in which subject-derived antigen-presenting cells and CD8-positive cells or peripheral blood mononuclear leukocytes are contacted (stimulated) with one or more peptides of this invention in vitro, and, after inducing CTL, the cells are returned to the subject. For example, the method may include the steps of:

- a: collecting antigen-presenting cells from a subject,
- b: contacting the antigen-presenting cells of step a with a peptide of the present invention,
- c: mixing the antigen-presenting cells of step b with CD⁸⁺ T cells and co-culturing so as to induce cytotoxic T-cells, and
- d: collecting CD⁸⁺ T cells from the co-culture of step c.

The CD⁸⁺ T cells having cytotoxic activity obtained by step d can be administered to the subject as a vaccine.

[0050] The present invention further provides methods for producing activated cytotoxic T cell using the peptides of this invention. For example, the method may include the following steps of:

- a: collecting T cells from a subject, and
- b: contacting T cells with following peptides.

(1) An isolated peptide of less than about 15 amino acids selected from the group consisting of peptides having the amino acid sequences of SEQ ID NOs: 7, 8, 9, 10, 11, 12, 192, 195, 197, 209, 225, 226 228, 230, 240, 241, 243, 244, 249, 253, 254 and 255.

(2) A peptide having cytotoxic T cell inducibility, wherein said peptide has an amino acid sequence selected from the group consisting of SEQ ID NOs: 7, 8, 9, 10, 11, 12, 192, 195, 197, 209, 225, 226 228, 230, 240, 241, 243, 244, 249, 253, 254 and 255, wherein 1, 2, or several amino acids are substituted, deleted, or added.

[0051] The present invention also provides method for producing APC having activated T-cell inducibility using the peptides of the present invention. For instance, the method may include the step of contacting antigen presenting cells with the peptides to produce antigen presenting cells presenting the peptide and HLA antigen on the

surface.

In the context of the present invention, "activated cytotoxic T cell" induces IFN-gamma producing, IFN-gamma releasing, and death of tumor cells.

[0052] The present invention further provides isolated cytotoxic T cells induced using the peptides of this invention. The cytotoxic T cells, induced by stimulation with an antigen-presenting cell presenting one or more peptides of this invention, are preferably derived from subjects who are the target of treatment and/or prevention, and can be administered alone or in combination with other drugs, including one or more peptides of this invention or exosomes having anti-tumor activity. The obtained cytotoxic T cells act specifically against target cells presenting the peptides of this invention, or preferably the same peptide(s) used for induction. The target cells may be cells that express MPHOSPH1 and/or DEPDC1 endogenously, or cells that are transfected with MPHOSPH1 and/or DEPDC1 genes. Cells that present the peptides of this invention on the cell surface, due to stimulation with these peptides, can also become targets of attack.

[0053] The present invention also provides antigen-presenting cells presenting complexes formed between HLA antigens and one or more peptides of this invention. The antigen-presenting cells, obtained through contact with the peptides of this invention or the nucleotides encoding such peptides, are preferably derived from subjects who are the target of treatment and/or prevention, and can be administered as vaccines, alone or in combination with other drugs, including the peptides, exosomes, or cytotoxic T cells of the present invention.

[0054] The present invention also provides a composition comprising nucleic acids encoding polypeptides that are capable of forming a subunit of a T cell receptor (TCR), and methods of using the same. The TCR subunits have the ability to form TCRs that confer specificity to T cells for tumor cells presenting MPHOSPH1 or DEPDC1. By using the known method in the art, the nucleic acids of alpha- and beta-chain as the TCR subunits of the CTL induced with one or more peptides of this invention may be identified (WO2007/032255 and Morgan et al., J Immunol, 171, 3288 (2003)). The derivative TCRs preferably bind target cells displaying the MPHOSPH1 or DEPDC1 peptide with high avidity, and optionally mediate efficient killing of target cells presenting the MPHOSPH1 or DEPDC1 peptide in vivo and in vitro.

[0055] The nucleic acids encoding the TCR subunits can be incorporated into suitable vectors e.g. retroviral vectors. These vectors are well known in the art. The nucleic acids or the vectors comprising them usefully can be transferred into a T cell, which T cell is preferably from a patient. Advantageously, the invention provides an off-the-shelf composition allowing rapid modification of a patient's own T cells (or those of another mammal) to rapidly and easily produce modified T cells having excellent

cancer cell killing properties.

[0056] Also, the present invention provides CTLs which are prepared by transduction with the nucleic acids encoding the TCR subunits polypeptides binding with MPHOSPH1 or DEPDC1 peptide e.g. SEQ ID NOs: 7, 8, 9, 10, 11, 12, 192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 249, 253, 254 or 255 in the context of HLA-A24 or HLA-A2. The transduced CTLs are capable of homing to cancer cells in vivo, and expanded by well known culturing method in vitro (e.g., Kawakami et al., J Immunol., 142, 3452-3461 (1989)). The T cells of the invention can be used to form an immunogenic composition useful in treating or preventing cancer in a patient in need of therapy or protection (WO2006/031221).

[0057] In the context of the present invention, the term "vaccine" (also referred to as an immunogenic composition) refers to a substance that induces anti-tumor immunity or suppresses cancers upon inoculation into animals. According to the present invention, polypeptides having the amino acid sequence of SEQ ID NO: 7, 8 or 12 were suggested to be HLA-A24 restricted epitope peptides and those of SEQ ID NO: 9, 10, 11, 192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 249, 253, 254 or 255 were suggested to be HLA-A2 restricted epitope peptides that may induce potent and specific immune response against cells expressing MPHOSPH1 and/or DEPDC1, e.g. cancer cells expressing MPHOSPH1 and/or DEPDC1. The cancers contemplated include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, soft tissue tumor. Thus, the present invention also encompasses a method of inducing anti-tumor immunity using polypeptides having the amino acid sequence of SEQ ID NO: 7, 8, 9, 10, 11, 12, 192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 249, 253, 254 or 255 or a variant thereof (i.e., including 1, 2, or several amino acid substitutions, deletions, or additions). In general, anti-tumor immunity includes immune responses such as follows:

- an induction of cytotoxic lymphocytes against tumors containing cells expressing MPHOSPH1 and/or DEPDC1,
- an induction of antibodies that recognize tumors containing cells expressing MPHOSPH1 and/or DEPDC1, and
- an induction of anti-tumor cytokine production.

[0058] Therefore, when a certain peptide induces any one of these immune responses upon inoculation into an animal, the peptide is decided to have anti-tumor immunity inducing effect. The induction of the anti-tumor immunity by a peptide can be detected by observing in vivo or in vitro the response of the immune system in the host against the peptide.

- [0059] For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. A foreign substance that enters the living body is presented to T cells and B cells by the action of antigen-presenting cells (APCs). T cells that respond to the antigen presented by APC in antigen specific manner differentiate into cytotoxic T cells (also referred to as cytotoxic T lymphocytes or CTLs) due to stimulation by the antigen, and then proliferate; this process is referred to herein as "activation" of T cells. Therefore, CTL induction by a certain peptide can be evaluated by presenting the peptide to a T cell by APC, and detecting the induction of CTL. Furthermore, APCs have the effect of activating CD4+ T cells, CD8+ T cells, macrophages, eosinophils and NK cells. Since CD4+ T cells are also important in anti-tumor immunity, the anti-tumor immunity inducing action of the peptide can be evaluated using the activation effect of these cells as indicators.
- [0060] A method for evaluating the inducing action of CTL using dendritic cells (DCs) as APC is well known in the art. DC is a representative APC having the strongest CTL inducing action among APCs. In this method, the test polypeptide is initially contacted with DC and then this DC is contacted with T cells. Detection of T cells having cytotoxic effects against the cells of interest after the contact with DC shows that the test polypeptide has an activity of inducing the cytotoxic T cells. Activity of CTL against tumors can be detected, for example, using the lysis of ^{51}Cr -labeled tumor cells as the indicator. Alternatively, it is well known to evaluate the degree of tumor cell damage using ^3H -thymidine uptake activity or LDH (lactose dehydrogenase)-release as the indicator. Furthermore, it can be also examined by measuring IFN-gamma produced and released by CTL in the presence of antigen-presenting cells that carry immobilized peptides by visualizing using anti-IFN-gamma antibodies, such as an ELISPOT assay.
- [0061] Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL is reported to be enhanced by culturing PBMC in the presence of GM-CSF and IL-4. Similarly, CTL has been shown to be induced by culturing PBMC in the presence of keyhole limpet hemocyanin (KLH) and IL-7.
- [0062] The test polypeptides confirmed to possess CTL inducing activity by these methods are polypeptides having DC activation effect and subsequent CTL inducing activity. Therefore, polypeptides that induce CTL against tumor cells are useful as vaccines against diseases associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancers. Furthermore, APC that have acquired the ability to induce CTL against a disease associating MPHOSPH1 and/or DEPDC1, e.g. cancers, by contacting with the polypeptides are useful as vaccines against the disease. Furthermore, CTL that have acquired cytotoxicity due to presentation of the polypeptide antigens by APC can be also used as vaccines against a disease associating MPHOSPH1 and/or DEPDC1, e.g.

cancers. Such therapeutic methods for a disease associating MPHOSPH1 and/or DEPDC1, e.g. cancers, using anti-tumor immunity due to APC and CTL, are referred to as cellular immunotherapy. The cancers contemplated include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, soft tissue tumor.

- [0063] Generally, when using a polypeptide for cellular immunotherapy, efficiency of the CTL-induction can be increased by combining a plurality of polypeptides having different structures and contacting them with DC. Therefore, when stimulating DC with protein fragments, it is advantageous to use a mixture of multiple types of fragments.
- [0064] The induction of anti-tumor immunity by a polypeptide can be further confirmed by observing the induction of antibody production against tumors. For example, when antibodies against a polypeptide are induced in a laboratory animal immunized with the polypeptide, and when growth, proliferation and/or metastasis of tumor cells is suppressed by those antibodies, the polypeptide is determined to induce anti-tumor immunity.
- [0065] Anti-tumor immunity can be induced by administering a vaccine of this invention, and the induction of anti-tumor immunity enables treatment and prevention of a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancers. Therapy against or prevention of the onset of a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancers, may include inhibition of the growth of cells expressing MPHOSPH1 and/or DEPDC1, e.g. cancer cells, involution of these cells and suppression of occurrence of these cells, e.g. cancer cells. Decrease in mortality of individuals having a disease associating MPHOSPH1 and/or DEPDC1, e.g. cancers, decrease of the disease markers in the blood, alleviation of detectable symptoms accompanying the disease and such are also included in the therapy or prevention of the disease, e.g. cancers. Such therapeutic and preventive effects are preferably statistically significant, for example, observed at a significance level of 5% or less, wherein the therapeutic or preventive effect of a vaccine against a disease associating MPHOSPH1 and/or DEPDC1, e.g. cancers, is compared to a control without vaccine administration. For example, Student's t-test, the Mann-Whitney U-test or ANOVA may be used for determining statistical significance.
- [0066] In that the present invention provides a method for treating, or preventing a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancers, the therapeutic compounds or compositions may be administered prophylactically or therapeutically to subjects suffering from or at risk of (or susceptible to) developing the disease. Such subjects may be identified using standard clinical methods. In the context

of the present invention, prophylactic administration occurs prior to the manifestation of overt clinical symptoms of disease, such that a disease or disorder is prevented or alternatively delayed in its progression. In the context of the field of medicine, the term "prevent" encompasses any activity which reduces the burden of mortality or morbidity from disease. Prevention can occur at primary, secondary and tertiary prevention levels. While primary prevention avoids the development of a disease, secondary and tertiary levels of prevention encompass activities aimed at preventing the progression of a disease and the emergence of symptoms as well as reducing the negative impact of an already established disease by restoring function and reducing disease-related complications.

- [0067] In the context of cancer treatment, the term "efficacious" refers to a treatment that leads to a decrease in size, prevalence or metastatic potential of cancer in a subject. When a treatment is applied prophylactically, "efficacious" means that the treatment retards or prevents occurrence of non cancer or alleviates a clinical symptom of cancer. The assessment of cancer can be made using standard clinical protocols. Furthermore, the efficaciousness of a treatment may be determined in association with any known method for diagnosing or treating cancer. For example, cancer can be diagnosed histopathologically or by identifying symptomatic anomalies.
- [0068] The above-mentioned peptide, having immunological activity, or a polynucleotide or vector encoding such a peptide, may be combined with an adjuvant. An adjuvant refers to a compound that enhances the immune response against the peptide when administered together (or successively) with the peptide having immunological activity. Examples of suitable adjuvants include cholera toxin, salmonella toxin, alum and such, but are not limited thereto. Furthermore, a vaccine of this invention may be combined appropriately with a pharmaceutically acceptable carrier. Examples of such carriers are sterilized water, physiological saline, phosphate buffer, culture fluid and such. Furthermore, the vaccine may contain as necessary, stabilizers, suspensions, preservatives, surfactants and such. The vaccine is administered systemically or locally. Vaccine administration may be performed by single administration or boosted by multiple administrations.
- [0069] When using APC or CTL as the vaccine of this invention, a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancers, can be treated or prevented, for example, by the ex vivo method. More specifically, PBMCs of the subject receiving treatment or prevention are collected, contacted ex vivo with a peptide of the present invention. Following the induction of APC or CTL, the cells may be administered to the subject. APC can be also induced by introducing a vector encoding the peptide into PBMCs ex vivo. APC or CTL induced in vitro can be cloned prior to administration. By cloning and growing cells having high activity of damaging

target cells, cellular immunotherapy can be performed more effectively. Furthermore, APC and CTL isolated in this manner may be used for cellular immunotherapy not only against individuals from whom the cells are derived, but also against similar types of diseases in other individuals.

[0070] Aspects of the present invention are described in the following examples, which are presented only to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

[0071] Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below.

[0072] EXAMPLES

Hereinafter, the present invention is exemplified, but not restricted, by the following Examples. However, materials, methods and such described herein only illustrate aspects of the invention and in no way are intended to limit the scope of the present invention. As such, materials, methods and such similar or equivalent to those described therein may be used in the practice or testing of the present invention.

[0073] EXAMPLE 1

MATERIALS AND METHODS

Cell lines

A24LCL cells (HLA-A24/24), human B-lymphoblastoid cell lines, T2 cell and COS7 were purchased from ATCC.

[0074] Candidate selection of peptide derived from MPHOSOH1 and DEPDC1

9-mer and 10-mer peptides derived from MPHOSOH1 or DEPDC1 that bind to HLA-A*2402 and HLA-A*0201 molecule were predicted using binding prediction software "BIMAS" (bimas.dcrt.nih.gov/cgi-bin/molbio/ken_parker_comboform) (Parker KC, et al., (1994) J Immunol.;152(1):163-75.; Kuzushima K, et al., (2001) Blood.;98(6):1872-81.). These peptides were synthesized by Sigma (Sapporo, Japan) according to the standard solid phase synthesis method and purified by reversed phase HPLC. The purity (>90%) and the identity of the peptides were determined by analytical HPLC and mass spectrometry analysis, respectively. Peptides were dissolved in dimethylsulfoxide (DMSO) at 20 mg/ml and stored at -80 degrees C.

[0075] In vitro CTL Induction

Monocyte-derived dendritic cells (DCs) were used as antigen-presenting cells (APCs) to induce CTL responses against peptides presented on HLA. DCs were generated in vitro as described elsewhere (Nukaya I et al., (1999) Int. J. Cancer 80, 92-7., Tsai V et al., (1997) J. Immunol 158:1796-802.). Briefly, peripheral blood mononuclear cells (PBMCs) isolated from a normal volunteer (HLA-A*2402 and/or

HLA-A*0201) by Ficoll-Paque (Pharmacia) solution were separated by adherence to a plastic tissue culture flask (Becton Dickinson) so as to enrich them for the monocyte fraction. The monocyte-enriched population was cultured in the presence of 1000 U/ml of GM-CSF (Genzyme) and 1000 U/ml of IL-4 (Genzyme) in AIM-V (Invitrogen) containing 2% heat-inactivated autologous serum (AS). After 7 days in the culture, the cytokine-generated DCs were pulsed with 20 mcg/ml of the synthesized peptides in the presence of 3 mcg/ml of beta 2-microglobulin for 4 hrs at 20 degrees C in AIM-V. These peptide-pulsed DCs were then inactivated by MMC (30 mcg/ml for 30 mins) and mixed at a 1:20 ratio with autologous CD8⁺ T cells, obtained by positive selection with Dynabeads M-450 CD8 (Dyna) and DETACHa BEAD™ (Dyna). These cultures were set up in 48-well plates (Corning); each well contained 1.5x10⁴ peptide-pulsed DCs, 3x10⁵ CD8⁺ T cells and 10 ng/ml of IL-7 (Genzyme) in 0.5 ml of AIM-V/2% AS. Three days later, these cultures were supplemented with IL-2 (CHIRON) to a final concentration of 20 IU/ml. On day 7 and 14, the T cells were further restimulated with the autologous peptide-pulsed DCs. The DCs were prepared each time by the same way described above. CTL was tested against peptide-pulsed A24LCL cells or T2 cells after the 3rd round of peptide stimulation on day 21.

[0076] CTL Expansion Procedure

CTLs were expanded in culture using the method similar to that described by Riddell SR, et al., (Walter EA et al., (1995) N Engl J Med 333:1038-44.; Riddell SR, et al., (1996) Nature Med. 2:216-23.). A total 5x10⁴ of CTLs were resuspended in 25 ml of AIM-V/5% AS with 2 kinds of human B-lymphoblastoid cell lines, inactivated by MMC, in the presence of 40 ng/ml of anti-CD3 monoclonal antibody (Pharmingen). One day after initiating the cultures, 120 IU/ml of IL-2 were added to the cultures. The cultures were fed with fresh AIM-V/5% AS containing 30 IU/ml of IL-2 on days 5, 8 and 11.

[0077] Establishment of CTL clones

The dilutions were made to have 0.3, 1, and 3 CTLs/well in 96 round-bottomed micro titer plate (Nalge Nunc International). CTLs were cultured with 7x10⁴ cells/well of 2 kinds of human B-lymphoblastoid cell lines, 30ng/ml of anti-CD3 antibody, and 125 U/ml of IL-2 in total of 150 mcl/well of AIM-V containing 5%AS. 50 mcl /well of IL-2 was added to the medium 10 days later so that IL-2 became 125 U/ml in the final concentration. CTL activity of CTLs was tested on the 14th day, and CTL clones were expanded using the same method above.

[0078] Specific CTL activity

To examine the specific CTL activity, IFN-gamma ELISPOT assay and IFN-gamma ELISA were performed.

Briefly, peptide-pulsed A24-LCL or T2 cell (1x10⁴/well) was prepared as a

stimulator cell. Cultured Cells in 48 wells or CTL clones after limiting dilution were used as a responder cells. IFN-gamma ELISPOT assay and ELISA were performed under manufacture procedure.

[0079] Cell Culture and Transfection

HLA-A24 B-LCLs (A24LCL), Epstein Bar virus-transformed, was established. Jiyoye, EB-3, COS7, HT1376, RT-4 and J82 were purchased from American Type Culture Collection (Rockville, MD). A24LCL, Jiyoye and EB-3 were maintained in RPMI1640 containing 10% fetal bovine serum (GEMINI Bio-Products) and 1% antibiotic solution (Sigma). COS7, HT1376, RT-4 and J82 were maintained in appropriate medium and antibiotics. Transfection of COS7 and HEK were performed using FUGENE6 (Roche). HEK-A2 cell, HLA-A*0201 molecule expressing stable clone, was established by transfection of pcDNA6.2-HLA-A2 plasmid and isolated by limiting dilution method in the presence of 5 mcg/ml Blastcidin S.

[0080] Immunogenicity of epitope peptides in BALB/c mice

For induction of the peptide-specific CTLs, immunization was given using 100 ml of vaccine mixture, which contains 50 mcl (100 mcg) of HLA-A24 restricted peptide and 50 mcl of IFA per mouse. The vaccine was subcutaneously injected in the right flank for the first immunization on day 0 and in the left flank for the second on the day 7. On day 14, splenocytes of the vaccinated mice, without any in vitro stimulation, were used responder cells, and RLmale1 cells pulsed with or without peptides were used as the stimulator cells for IFN-gamma ELISPOT assay.

[0081] RESULTS

Enhanced MPHOSPH1 and DEPDC1 expression in cancers

The global gene expression profile data obtained from various cancers using cDNA-microarray revealed that MPHOSPH1 (GenBank Accession No. NM_016195; SEQ ID No.1) and DEPDC1 (GenBank Accession No. BM683578) which had two variants; DEPDC1 V1 (SEQ ID Nos.3) and DEPDC1 V2 (SEQ ID No. 5) expression was elevated. MPHOSPH1 expression was validly elevated in 30 out of 31 bladder cancers, 8 out of 36 breast cancers, 18 out of 18 cervical cancers, 5 out of 17 cholangiocellular carcinomas, 25 out of 31 CMLs, 6 out of 11 colorectal cancers, 6 out of 14 gastric cancers, 5 out of 5 NSCLCs, 7 out of 7 lymphomas, 6 out of 10 osteosarcomas, 7 out of 22 prostate cancers, 10 out of 18 renal carcinomas and 15 out of 21 soft tissue tumors as compared with corresponding normal tissue. DEPDC1 expression was validly elevated in 23 out of 25 bladder cancers, 6 out of 13 breast cancers, 12 out of 12 cervical cancers, 6 out of 6 cholangiocellular carcinomas, 3 out of 4 CMLs, 2 out of 4 colorectal cancers, 6 out of 6 NSCLCs, 7 out of 7 lymphomas, 10 out of 14 osteosarcomas, 11 out of 24 prostate cancers, 14 out of 14 SCLCs and 22 out of 31 soft tissue tumors as compared with corresponding normal tissue (Table 1).

[0082] [Table 1]

Ratio of cases observed up-regulation of *MPHOSPH1* or *DEPDC1* in cancerous tissue as compared with normal corresponding tissue

	Bladder cancer	Breast cancer	Cervical cancer	Cholangiocellular Carcinoma	CML	Colorectal cancer	Gastric cancer
MPHOSPH1	30/31	8/36	18/18	5/17	25/31	6/11	6/14
DEPDC1	23/25	6/13	12/12	6/6	3/4	2/4	-

	NSCLC	Lymphoma	Osteosarcoma	Prostate cancer	Renal cancer	SCLC	Soft Tissue Tumor
MPHOSPH1	5/5	7/7	6/10	7/22	10/18	-	15/21
DEPDC1	6/6	7/7	10/14	11/24	-	14/14	22/31

[0083] Prediction of HLA-A*24 and HLA-A*2 binding peptides derived from MPHOSPH1 or DEPDC1

Table 2 sets forth the HLA-A*2402 binding peptides for MPHOSPH1 in order of binding affinity. Table 2A sets forth 9-mer peptides derived from MPHOSPH1 and Table 2B sets forth 10-mer peptides derived from MPHOSPH1.

Table 3 sets forth the HLA-A*0201 binding peptides for MPHOSPH1 in order of binding affinity. Table 3A sets forth 9-mer peptides derived from MPHOSPH1 and Table 3B sets forth 10-mer peptides derived from MPHOSPH1.

Table 4 sets forth the HLA-A*2402 binding peptides for DEPDC1 V1 and V2 in order of binding affinity. Table 4A sets forth 9-mer peptides derived from DEPDC1 V1 and V2 and Table 4B sets forth 10-mer peptides derived from DEPDC1 V1.

Table 5 sets forth the HLA-A*0201 binding peptides for DEPDC1 V1 and V2, Table 5A sets forth 9-mer peptides derived from DEPDC1 V1 and V2 and Table 5B sets forth 10-mer peptides derived from DEPDC1 V1 and V2.

[0084]

[Table 2A]

HLA-A*2402 binding 9-mer peptides derived from *MPHOSPH1*

Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score	Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score
278	IYNEYIYDL	7	360	179	LFDSLQERL	29	24
1244	DYADLKEKL	13	316.8	268	KFSVWVSFF	30	20
1319	QYERACKDL	14	300	575	KLLDLIEDL	31	17.28
459	CYLAYDETL	15	300	1577	RFPKPELEI	32	16.5
462	AYDETLNVL	16	288	1414	KYNADRKKW	33	16.5
1054	GYKDENNRL	17	288	1230	RTQNLKADL	34	14.4
236	LYGSLTNSL	18	288	1421	KWLEEKMML	35	14.4
1446	KYAEDRERF	19	240	1617	KSNEEEDL	36	14.4
899	NYDIAIAEL	20	220	1555	KIEDGSVVL	37	14.4
1118	CYKAKIKEL	21	220	1456	KQQNEMEIL	38	12
57	DYLQVCLRI	22	105	389	KTQNEGERL	39	12
676	KFNQIKAEL	23	92.4	1371	KWKEKCNDL	40	11.52
14	SYVFSADPI	24	75	1122	KIKELETIL	41	11.52
326	AYRLCLKGI	25	60	850	FLLTENEL	42	11.088
255	DYEQANLNM	26	37.5	763	SSLINNKL	43	11.088
29	NFDGIKLDL	27	28	1400	KLTLNQDEL	44	10.56
286	LFVPVSSKF	28	27.72	133	IMQPVKDLL	45	10.08

Start position indicates the number of amino acid from N-terminal of MPHOSPH1.

Binding score is derived from "BIMAS" described in Materials and Methods.

[0085] [Table 2B]

HLA-A*2402 binding 10-mer peptides derived from *MPHOSPH1*

Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score	Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score
1414	KYNAdRKKWL	46	600	1274	KLLRiKINEL	56	15.84
278	IYNEyIYDLF	8	252	1332	KIIEdMRMTL	57	14.4
1446	KYAEdRERFF	47	240	1299	RTIQqLKEQL	58	14.4
611	QYWAqREADF	48	100	1134	KVECsHSAKL	59	13.2
1740	LYTSeISSPI	49	70	859	KNEKeEKAEL	60	13.2
293	KFQKrKMLRL	50	60	586	KLINeKKEKL	61	13.2
849	AFLLaENEL	51	55.44	943	KLMHtKIDEL	62	13.2
1667	TYSLrSQASI	52	50	838	RVLQeNNEGL	63	12
1695	DFLQhSPSIL	53	30	369	RVIRvSELSL	64	12
174	RTLNVLFDSL	54	17.28	1159	RNLKeFQEHL	65	12
870	KQIVhFQQEL	55	15.84	281	EYTYdLFVPV	66	10.8

Start position indicates the number of amino acid from N-terminal of MPHOSPH1.

Binding score is derived from "BIMAS" described in Materials and Methods.

[0086]

[Table 3A]

HLA-A*0201 binding 9-mer peptides derived from *MPHOSPH1*

Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score	Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score
575	KLLDLIEDL	31	1278.29	1184	KLKEEITQL	93	24.677
282	YIYDLFVPV	9	1096.62	888	TLSKEVQQI	94	23.995
298	KMLRLSQDV	67	650.504	280	NEYTYDLFV	95	23.802
218	ALLRQIKEV	68	591.888	552	LLDEDLDKT	96	23.415
850	FLLTIENEL	42	363.588	461	LAYDETLNV	97	21.546
1108	ALSELTQGV	69	285.163	980	NLPNTQLDL	98	21.362
331	KLGIKHQSV	70	243.432	409	TLGKCINVL	99	20.145
1689	TLQKFGDFL	71	218.772	175	TLNVLFDSL	100	19.888
1251	KLTDAAKKQI	72	149.711	923	KLSNEIETA	101	19.596
638	RLAIFKDLV	10	129.506	1389	KEHENNTDV	102	19.407
1467	QLTEKDSDL	73	87.586	987	DLLGNDYLV	103	19.301
1195	NLQDMKHLL	74	87.586	920	KIMKLSNEI	104	18.577
270	SVWVSFFEI	75	83.497	1703	ILQSKAKKI	105	17.736
129	FQGCIMQPV	76	74.608	512	ILNVKRATI	106	17.736
839	VLQENNEGL	77	72.959	1124	KELETILET	107	17.695
1094	TLDVQIQHV	78	63.988	453	IVNISQCYL	108	17.477
1019	AIWEECKEI	79	48.816	771	LICNETVEV	109	16.258
1696	FLQHSPSIL	80	40.289	623	TLLQEREIL	110	15.879
528	DLMEDEDLV	81	38.775	560	TLEENKAFI	111	15.057
406	SLLTLGKCI	82	38.601	1415	YNADRKKWL	112	14.465
1400	KLTNLQDEL	44	36.637	307	KGYSFIKDL	113	13.65
170	GILPRTLNV	83	35.385	133	IMQPVKDLL	45	12.852
171	ILPRTLNVL	84	34.246	1594	KMAVKHPGC	114	12.558
786	KICSERKRV	85	33.472	365	SEMSRVIRV	115	11.509
880	SLSEKKNLT	86	30.553	1191	QLTNNLQDM	116	11.426
944	LMHTKIDEL	87	29.559	871	QIVHFQQEL	117	11.162
1422	WLEEKMLLI	88	28.963	245	NISEFEESI	118	10.951
466	TLNVLFKFA	89	28.814	484	TLNSSQEKL	119	10.468
1539	KLQTEPLST	90	26.082	764	SLIINNCLI	120	10.433
132	CIMQPVKDL	91	24.997	587	LINEKKEKL	121	10.032
1260	KQVQKEVSV	92	24.681				

Start position indicates the number of amino acid from N-terminal of MPHOSPH1.

Binding score is derived from "BIMAS" described in Materials and Methods.

[0087]

[Table 3B]

HLA-A*0201 binding 10-mer peptides derived from *MPHOSPH1*

Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score	Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score
1274	KLLRiKINEL	56	626.279	1318	QQYErACKDL	140	28.417
551	KLLDeDLDKT	122	445.913	452	MIVNiSQCYL	141	27.464
460	YLAYdETLNV	123	319.939	923	KLSNeIETAT	142	26.082
943	KLMHtKIDEL	62	311.777	1257	KQIKqVQKEV	143	24.681
262	NMANsIKFSV	124	291.346	980	NLPNtQLDLL	144	24.075
178	VLFDsLQERL	125	269.948	985	QLDLIGNDYL	145	23.029
770	KLICnETVEV	126	243.432	1427	MMLItQAKEA	146	22.569
34	KLDLsHEFSL	127	173.463	1523	QIMDiKPKRI	147	21.762
407	LLTLgKCINV	128	118.238	1484	QLVAaLEIQL	148	21.362
1714	TMSSsKLSNV	11	115.534	466	TLNVIKFSAl	149	19.822
1353	QVLEaKLEEV	129	104.046	511	KILNvKRATI	150	18.577
880	SLSEkKNLTL	130	87.586	1340	TLEEqEQTQV	151	18.25
235	TLYGsLTNSL	131	68.36	372	RVSEISLCDL	152	17.627
1019	AIWEeCKEIV	132	65.381	1561	VVLDsCEVST	153	16.816
552	LLDEdLDKTL	133	59.558	309	YSFIkDLQWI	154	14.663
1093	VTLDvQIQHV	134	57.298	353	SIFTvKILQI	155	12.208
559	KTLEeNKAFl	135	42.314	1094	TLDVqIQHVV	156	11.407
1332	KIIEdMRMTL	57	42.151	1688	GTLQkFGDFL	157	11.242
152	GLTNsGKTYT	136	40.986	311	FIKDIQWIV	158	10.732
830	NIAEiEDIRV	137	39.21	1079	TLIQqLKEEL	159	10.468
586	KLINeKKEKL	61	36.637	1128	TILEtQKVEC	160	10.363
182	SLQErLYTKM	138	30.553	1487	AALEiQLKAL	161	10.352
1043	QQIEKLQAEV	139	28.912	170	GILPrTLNVL	162	10.249
870	KQIVhFQQEL	55	28.807				

Start position indicates the number of amino acid from N-terminal of MPHOSPH1.

Binding score is derived from "BIMAS" described as Materials and Methods.

[0088] [Table 4A]

HLA-A*2402 binding 9-mer peptides derived from *DEPDC1*

Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score	Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score
295	YYELFVNIL	163	360	505	KQLCRSQSL	167	14.4
294	EYYELFVNI	12	86.4	275	VFRTIADYF	168	14
282	YFLDLPEPL	164	43.2	36	HFKKYGNCf	169	12
118	RYPELRKNN	165	21.6	307	GYTVSDRS	170	10.5
338	SFKSTECLL	166	20	298	LFVNILGLL	171	42

Start position indicates the number of amino acid from N-terminal of DEPDC1.

Binding score is derived from "BIMAS" described in Materials and Methods.

[0089]

[Table 4B]

HLA-A*2402 binding 10-mer peptides derived from *DEPDC1*

Start Position	Amino Acid Sequence	SRQ ID NO.	Binding Score	Start Position	Amino Acid Sequence	SRQ ID NO.	Binding Score
294	EYYEIFVNIL	172	288	275	VFRTiADYFL	182	20
281	DYFLdLPEPL	173	240	113	KTLPPrYPEL	183	15.84
118	RYPEIRKNNI	174	216	277	RTIAdYFLDL	184	14.4
770	EYPLiYQKRF	175	150	270	GFERdVFRTI	185	12.6
267	TYVGfERDVF	176	150	146	RTPKrhGLHL	186	12
523	SYINtPVAEI	177	82.5	505	KQLCrSQSLL	187	12
282	YFLDIPEPLL	178	36	340	KSTEcLLLSL	188	11.52
191	RYViiYLQT	179	21	295	YYELfVNILV	189	10.5
338	SFKStECLLL	180	20	129	NFSKdKDSIF	190	10
103	LFRFpATSPL	181	20				

Start position indicates the number of amino acid from N-terminal of DEPDC1.

Binding score is derived from "BIMAS" described in Materials and Methods.

[0090] [Table 5A]

HLA-A*0201 binding 9-mer peptides derived from *DEPDC1*

Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score	Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score
674	FLMDHHQEI	191	728.022	563	RLCKSTIEL	209	21.362
589	LLQPHLERV	192	133.255	506	QLCRSQSLL	210	21.362
575	SLLPASSML	193	79.041	193	VILIYLQTI	211	20.753
246	WVLSAMKCL	194	73.172	297	ELFVNILVV	212	18.201
619	LLMRMISRM	195	71.872	235	ILQNKSDDL	213	17.795
581	SMLTGTQSL	196	57.085	616	KLQLLMRMI	214	16.797
290	LLTFEYYEL	197	54.474	623	MISRMSQNV	215	16.258
220	YIMYNMANT	198	40.111	72	TIQLLRKFL	216	16.155
283	FLDLPEPLL	199	39.307	421	CSLEGIVDV	217	15.841
787	ALFGDKPTI	200	38.601	303	LVVCGYITV	218	15.519
582	MLTGTQSLL	201	36.316	524	YINTPVAEI	219	15.177
773	LIYQKRFTPT	202	32.33	194	ILYILQTIL	220	14.89
114	TLPRRYPEL	203	32.044	239	KSDDLPHWV	221	14.333
505	KQLCRSQSL	167	28.049	576	LLPASSMLT	222	12.668
765	KQFQKEYPL	204	28.049	646	MIHTFSRCV	223	12.356
395	IMGGSCHNL	205	26.228	645	LMIHTFSRC	224	11.589
296	YELFVNILV	206	23.018	653	CVLCCAEEV	225	11.034
278	TIADYFLDL	207	22.882	297	ELFVNILGL	226	13.635
601	ALQLCCLLL	208	21.362				

Start position indicates the number of amino acid from N-terminal of DEPDC1.

Binding score is derived from "BIMAS" described in Materials and Methods.

[0091]

[Table 5B]

HLA-A*0201 binding 10-mer peptides derived from *DEPDC1*

Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score	Start Position	Amino Acid Sequence	SEQ ID NO.	Binding Score
666	LLAGrLVSFL	227	459.398	575	SLLPaSSMLT	241	27.572
674	FLMDhHQEIL	228	299.484	296	YELFvNILVV	242	21.706
588	SLLQpHLERV	229	290.025	506	QLCRsQSLLL	243	21.362
302	ILVVcGYTV	230	177.358	765	KQFQKEYPLI	244	20.547
291	LTFEYELFV	231	137.017	682	ILQVpSYLQT	245	19.003
201	ILGVpSLEEV	232	133.255	269	VGFErDVFRT	246	16.735
195	LIYLqTILGV	233	119.657	381	QLVNIRNRRV	247	13.91
688	YLQTaVEKHL	234	98.267	283	FLDLpEPLLT	248	13.712
645	LMIHtFSRCV	235	64.9	395	IMGGsCHNLI	249	12.809
581	SMLTgTQSLL	236	57.085	403	LIGLsNMHDL	250	11.485
622	RMISrMSQNV	237	50.232	773	LIYQkRFPTT	251	10.591
618	QLLMrMISRM	238	42.278	488	TLTVqDQEEL	252	10.468
654	VLCCaEEVDL	239	36.316	224	NMANtSKRGV	253	10.046
644	SLMIhTFSRC	240	34.925	296	YELFvNILGL	254	16.26
505	KQLCrSQSLL	187	28.049	301	NILGILQPHL	255	10.868

Start position indicates the number of amino acid from N-terminal of DEPDC1.

Binding score is derived from "BIMAS" described in Materials and Methods.

[0092] Stimulation of the T cells using the predicted peptides from MPHOSPH1 restricted with HLA-A*2402

CTLs for those peptides derived from MPHOSHP1 (SEQ ID No: 2) were generated according to the protocols set forth in "Materials and Methods" section above. Resulting CTLs having detectable specific CTL activity, as assessed by IFN-gamma ELISPOT assay, are shown in Figure 1A and Figure 2A. In Figure 1A, the cells in the well number #4 stimulated with MPHOSPH1-A24-9-278 (SEQ ID NO: 7) demonstrated potent IFN-gamma production as compared with the control. In Figure 2A, the cells in the well number #8 stimulated with MPHOSPH1-A24-10-278 (SEQ ID NO: 8) demonstrated potent IFN-gamma production as compared with the control. Next, these cells in the positive well were expanded and limiting dilution was performed. As shown in Figure 1B (MPHOSPH1-A24-9-278 (SEQ ID NO: 7)) and Figure 2B (MPHOSPH1-A24-10-278 (SEQ ID NO: 8)), CTL clones having higher specific CTL activities against the peptide-pulsed target as compared to the activities against target without peptide pulse were established

[0093] The CTL clones stimulated by the MPHOSPH1-A24-9-278 (IYNEYIYDL (SEQ ID NO: 7)) (Figure 3A) and MPHOSPH1-A24-10-278 (IYNEYIYDLF (SEQ ID NO: 8)) (Figure 3B) demonstrated potent specific CTL activity against the peptide-pulsed target without showing any significant specific CTL activity against targets not pulsed with any peptide. This suggests that the CTL clone has the peptide-specific cytotoxicity.

[0094] Specific CTL activity against the target cells expressing MPHOSPH1

The established CTL clones raised against these peptides were examined for their ability to recognize the target cells endogenously expressing MPHOSPH1 and HLA-A*2402. Specific CTL activity against COS7 transfected with both the full length MPHOSPH1 gene and the HLA-A*2402 molecule, which is a specific model for the target cells endogenously express MPHOSPH1 and HLA-A*2402, was tested using as effector cells the CTL clone raised by MPHOSPH1-A24-9-278 (SEQ ID NO: 7). COS7 transfected with full length MPHOSPH1 but not HLA-A*2402 and COS7 transfected HLA-A*2402 but not full length MPHOSPH1 were prepared as controls. The CTL Clone having the highest specific CTL activity against COS7 was that transfected with both MPHOSPH1 and HLA-A24. However, it did not show significant specific CTL activity against COS7 transfected with neither MPHOSPH1 nor HLA-A24 (Figure 4).

[0095] These results clearly demonstrate that MPHOSPH1-A24-9-278 (SEQ ID NO: 7) was naturally expressed to the target cell surface with HLA-A24 molecule and recognized CTL.

[0096] CTL activity against bladder cancer cell lines endogenously expressing MPHOSPH1

The established CTL clone raised against MPHOSPH1-A24-9-278 (SEQ ID NO: 7) peptide was examined for their ability to recognize the tumor cells endogenously expressing MPHOSPH1. CTL activity against HT1376 cells, which endogenously express MPHOSPH1 and HLA-A24, was tested using the CTL clone raised by MPHOSPH1-A24-9-278 (SEQ ID NO: 7) as effector cells. J82 cells and RT-4 cells were used as the target cells which endogenously express MPHOSPH1 but do not express HLA-A24. The established CTL clone showed high IFN-gamma production against HT1376 cells that express both MPHOSPH1 and HLA-A24. On the other hand, The CTL did not show significant CTL activity against J82 and RT-4 cells which express MPHOSPH1 but not HLA-A24 (Figure 5). It clearly demonstrated that MPHOSPH1-A24-9-278 (SEQ ID NO: 7) peptide was naturally processed to the tumor cell surface with HLA-A24 molecule and recognized by CTL.

[0097] In vivo CTL induction with MPHOSPH1-A24-9-278 peptide in BALB/c mice

It has been known that H-2Kd molecule, one of the mouse MHC class I, has resemble peptide anchor motif for HLA-A24 molecule and partially cross-react HLA-A24 restricted peptide. The present inventors then examined whether MPHOSPH1-A24-9-278 peptide induce the CTL in vivo by vaccination with this peptide using BALB/c mice (H-2Kd). IFA-conjugated peptide was subcutaneously injected into BALB/c mice on the day 0 and 7. On day 14, splenocytes were harvested and used as the responder cells for ELISPOT assay. Splenocytes of all mice injected peptide (Ani1~5) showed potent IFN-gamma production compared with control mice, which were injected IFA alone (nega1~3) (Figure 6). This data indicated that

MPHOSPH1-A24-9-278 peptide could elicit CTL response even in vivo.

[0098] Stimulation of the T cells using the predicted peptides from MPHOSPH1 restricted with HLA-A*0201

Resulting CTLs having detectable specific CTL activity, as assessed by IFN-gamma ELISPOT assay, are shown in Figure 7. As shown in Figure 7A, the cells in the well number #1 and #5, stimulated with MPHOSPH1-A2-9-282 (YIYDLFVPV (SEQ ID NO: 9)) demonstrated potent IFN-gamma production as compared with the control. As shown in Figure 7B, the cells in the well number #8 stimulated with MPHOSPH1-A2-9-638 (RLAIFKDLV (SEQ ID NO: 10)) demonstrated potent IFN-gamma production as compared with the control. As shown in Figure 7C, the cells in the well number #4 stimulated with MPHOSPH1-A2-10-1714 (TMSSsKLSNV (SEQ ID NO: 11)) demonstrated potent IFN-gamma production as compared with the control.

[0099] As shown in figure 8A (MPHOSPH1-A2-9-282 (SEQ ID NO: 9)), figure 8B (MPHOSPH1-A2-9-638 (SEQ ID NO: 10)), and figure 8C (MPHOSPH1-A2-10-1714 (SEQ ID NO: 9)), these cells in the positive well were expanded, and CTL lines having higher specific CTL activities against the peptide-pulsed target as compared to the activities against target without peptide pulse were established.

[0100] The CTL clones stimulated by the MPHOSPH1-A2-9-282 (YIYDLFVPV (SEQ ID NO: 9)) (Figure 9A, and 9B) demonstrated potent specific CTL activity against the peptide-pulsed target without any significant specific CTL activity against targets not pulsed with any peptide.

[0101] Stimulation of the T cells using the predicted peptides from DEPDC1 restricted with HLA-A*2402

CTLs for those peptides derived from DEPDC1 were generated according to the protocol described in "Materials and Methods" section above. Resulting CTLs having detectable specific CTL activity, as assessed by IFN-gamma ELISPOT assay, are shown in Figure 10. As shown in Figure 10A, the cells in the well number #10 stimulated with DEPDC1-A24-9-294 (EYYELFVNI (SEQ ID NO: 12)) demonstrated potent IFN-gamma production as compared with the control. Accordingly, these cells in the positive well were expanded and limiting dilution was performed. As shown in Figure 10B (DEPDC1-A24-9-294 (SEQ ID NO: 12)), CTL clones having higher specific CTL activities against the peptide-pulsed target compared to the activities against target without peptide pulse were established. The CTL clones stimulated by the DEPDC1-A24-9-294 (EYYELFVNI (SEQ ID NO: 12)) (Figure 11) demonstrated potent specific CTL activity against the peptide-pulsed target without showing any significant specific CTL activity against targets not pulsed with any peptide. The results

suggest that the CTL clone has the peptide-specific cytotoxicity.

[0102] Specific CTL activity against the target cells expressing DEPDC1 and HLA-A*2402

The established CTL clones raised against these peptides were examined for their ability to recognize the target cells endogenously expressing DEPDC1 and HLA-A*2402. Specific CTL activity against COS7 transfected both with the full length DEPDC1 gene and the HLA-A*2402 molecule, which serves as a specific model for the target cells endogenously express DEPDC1 and HLA-A*2402, was tested using as effector cells the CTL clone raised by DEPDC1-A24-9-294 (EYYELFVNI (SEQ ID NO: 12)). COS7 transfected with full length DEPDC1 but not HLA-A*2402 and COS7 transfected with HLA-A*2402 but not full length DEPDC1 were prepared as controls. The CTL Clone demonstrated high specific CTL activity against COS7 transfected both DEPDC1 and HLA-A24. However, it did not demonstrate significant specific CTL activity against COS7 transfected neither DEPDC1 nor HLA-A24 (Figure 12).

[0103] These results clearly demonstrate that DEPDC1-A24-9-294 (EYYELFVNI (SEQ ID NO: 12)) is naturally expressed to the target cell surface with HLA-A24 molecule and recognized CTL.

[0104] CTL activity against bladder cancer cell lines endogenously expressing DEPDC1

The established CTL clone raised against DEPDC1-A24-9-294 peptide was examined for their ability to recognize the tumor cells endogenously expressing DEPDC1. CTL activity against HT1376 cells, which endogenously express DEPDC1 and HLA-A24, was tested using the CTL clone raised by DEPDC1-A24-9-294 as effector cells. J82 cells and RT-4 cells were used as the target cells which endogenously express DEPDC1 but do not express HLA-A24. The established CTL clone showed high IFN-gamma production against HT1376 cells that express both DEPDC1 and HLA-A24. On the other hand, it did not show significant CTL activity against J82 and RT-4 cells which express DEPDC1 but not HLA-A24 (Figure 13). It clearly demonstrated that DEPDC1-A24-9-294 was naturally processed to the tumor cell surface with HLA-A24 molecule and recognized by CTL.

[0105] In vivo CTL induction with DEPDC1-A24-9-294 peptide in BALB/c mice

It has been known that H-2Kd molecule, one of the mouse MHC class I, has resemble peptide anchor motif for HLA-A24 molecule and partially cross-react HLA-A24 restricted peptide. The present inventors then examined whether DEPDC1-A24-9-294 peptide induce the CTL in vivo by vaccination of this peptide using BALB/c mice (H-2Kd). IFA-conjugated peptide was subcutaneously injected into BALB/c mice on the day 0 and 7. On day 14, splenocytes were harvested and used as the responder cells for ELISPOT assay. Splenocytes of all mice injected peptide (Ani1~5) showed potent IFN-gamma production compared with control mice, which were injected IFA alone (nega1, 2) (Figure 14). This data indicated that

DEPDC1-A24-9-294 peptide could elicit CTL response even in vivo.

[0106] Stimulation of the T cells using the predicted peptides from DEPDC1 restricted with HLA-A*0201

Resulting CTLs having detectable specific CTL activity when screened by IFN-gamma ELISPOT assay are shown in Figure 15 and Table 6. The cells in the well number #4 and #7 stimulated with DEPDC1- A02-10-644 ((SLMIHTFSRC SEQ ID NO: 240)) showed potent IFN-gamma production compared with the control. The cells in the well number #2 stimulated with DEPDC1- A02-10-575 (SLLPASSMLT (SEQ ID NO: 241)) showed potent IFN-gamma production compared with the control. The cells in the well number #7 stimulated with DEPDC1- A02-10-506 (QLCRSQSLLL (SEQ ID NO: 243)) showed potent IFN-gamma production compared with the control. The cells in the well number #1 stimulated with DEPDC1- A02-10-765 (KQFQKEYPLI (SEQ ID NO: 244)) showed potent IFN-gamma production compared with the control. The cells in the well number #1 stimulated with DEPDC1- A02-10-395 (IMGGSCHNLI (SEQ ID NO: 249)) showed potent IFN-gamma production compared with the control. The cells in the well number #1 and #2 stimulated with DEPDC1- A02-10-224 (NMANTSKRGV (SEQ ID NO: 253)) showed potent IFN-gamma production compared with the control. The cells in the well number #4 stimulated with DEPDC1- A02-9-297 (ELFVNILGL (SEQ ID NO: 226)) showed potent IFN-gamma production compared with the control. The cells in the well number #3 and #4 stimulated with DEPDC1- A02-10-296 (YELFVNILGL (SEQ ID NO: 254)) showed potent IFN-gamma production compared with the control. The cells in the well number #2, #3, #5 and #7 stimulated with DEPDC1- A02-10-301 (NILGLLQPHL (SEQ ID NO: 255)) showed potent IFN-gamma production compared with the control. The cells in the well number #6 stimulated with DEPDC1-A02-9-598 (LLQPHLERV (SEQ ID NO: 192)) demonstrated potent IFN-gamma production as compared with the control. The cells in the well number #6 stimulated with DEPDC1-A02-9-619 (LLMRMISRM (SEQ ID NO: 195)) demonstrated potent IFN-gamma production as compared with the control. The cells in the well number #2 stimulated with DEPDC1-A02-9-290 (LLTFEYYEL (SEQ ID NO: 197)) demonstrated potent IFN-gamma production as compared with the control. The cells in the well number #5 stimulated with DEPDC1-A02-9-563 (RLCKSTIEL (SEQ ID NO: 209)) demonstrated potent IFN-gamma production as compared with the control. The cells in the well number #1 and #3, stimulated with DEPDC1-A02-9-653 (CVLCCAEEV (SEQ ID NO: 225)), demonstrated potent IFN-gamma production as compared with the control. The cells in the well number #1 stimulated with DEPDC1-A02-10-674 (FLMDhHQEIL (SEQ ID NO: 228)) demonstrated potent IFN-gamma production as compared with the control. Finally, the cells in the well number #2 and #6, stimulated

with DEPDC1-A02-10-302 (ILVVcGYITV (SEQ ID NO: 230)), demonstrated potent IFN-gamma production as compared with the control.

[0107] The CTL lines stimulated by the DEPDC1-A02-10-296 (YELFVNILGL (SEQ ID NO: 254)) and DEPDC1-A02-9-653 (CVLCCAEEV (SEQ ID NO: 225)) (Figure 16) showed potent specific CTL activity against the peptide-pulsed target without showing any significant specific CTL activity against targets not pulsed with any peptide. It demonstrates that the CTL clone has the peptide-specific cytotoxicity.

[0108] [Table 6]

The candidate peptides from DEPDC1 restricted with HLA-A*0201

peptide name	SEQ ID No.	Well No.
DEPDC1-A02-9-589	192	#6
DEPDC1-A02-9-619	195	#6
DEPDC1-A02-9-290	197	#2
DEPDC1-A02-9-563	209	#5
DEPDC1-A02-9-653	225	#1
DEPDC1-A02-9-653	225	#3
DEPDC1-A02-10-674	228	#1
DEPDC1-A02-10-302	230	#2
DEPDC1-A02-10-302	230	#6

[0109] Specific CTL activity against the target cells expressing DEPDC1 and HLA-A*0201

The established CTL lines raised against DEPDC1-A02-10-296 peptide (YELFVNILGL (SEQ ID NO: 254)) and DEPDC1-A02-9-653 (CVLCCAEEV (SEQ ID NO: 225)) were examined for their ability to recognize the target cells endogenously expressing DEPDC1 and HLA-A2. At first, we established HEK293 cell line constitutively expressed HLA-A*0201 (HEK-A2) to efficiently determine specific CTL response. Specific CTL activity against HEK-A2 cells transfected full length of DEPDC1 gene, which is specific model for the target cells expressed DEPDC1 and HLA-A2, was tested using the established CTL lines raised by DEPDC1-A02-10-296 (YELFVNILGL (SEQ ID NO: 254)) or DEPDC1-A02-9-653 (CVLCCAEEV (SEQ ID NO: 225)) as effector cells. HEK-A2 transfected Mock expressed vector and HEK-A2 pulsed with no corresponding peptide derived from DEPDC1 were prepared for the negative control. The established CTL lines showed specific CTL activity against HEK-A2 transfected DEPDC1. On the other hand, the CTL lines did not show significant specific CTL activity against HEK-A2 transfected Mock expressed vector and which pulsed DEPDC1-A02-9-674 peptide or DEPDC1-A02-9-462 peptide (Figure 17). It clearly demonstrated that DEPDC1-A02-10-296 and DEPDC1-A02-9-653

peptide was naturally processed to the target cell surface with HLA-A2 molecule and recognized by CTL.

[0110] Homology analysis of the antigen peptides

The CTLs established against peptides of this invention demonstrated potent specific CTL activity. This suggests that the sequences of MPHOSPH1-A24-9-278 (SEQ ID NO: 7), MPHOSPH1-A24-10-278 (SEQ ID NO: 8), MPHOSPH1-A2-9-282 (SEQ ID NO: 9), MPHOSPH1-A2-9-638 (SEQ ID NO: 10), MPHOSPH1-A2-10-1714 (SEQ ID NO: 11), DEPDC1-A24-9-294 (SEQ ID NO: 12), DEPDC1-A2-9-589 (SEQ ID NO: 192), DEPDC1-A2-9-619 (SEQ ID NO: 195), DEPDC1-A2-9-290 (SEQ ID NO: 197), DEPDC1-A2-9-563 (SEQ ID NO: 209), DEPDC1-A2-9-653 (SEQ ID NO: 225), DEPDC1-A2-10-674 (SEQ ID NO: 228), DEPDC1-A2-10-302 (SEQ ID NO: 230), DEPDC1-A02-10-644 (SEQ ID NO: 240), DEPDC1-A02-10-575 (SEQ ID NO: 241), DEPDC1-A02-10-506 (SEQ ID NO: 243), DEPDC1-A02-10-765 (SEQ ID NO: 244), DEPDC1-A02-10-395 (SEQ ID NO: 249), DEPDC1-A02-10-224 (SEQ ID NO: 253), DEPDC1-A02-9-297 (SEQ ID NO: 226), DEPDC1-A02-10-296 (SEQ ID NO: 254) and DEPDC1-A02-10-301 (SEQ ID NO: 255) are homologous to the peptides derived from other molecules, which are known to sensitize human immune system. To exclude this possibility, homology analysis was performed with the peptide sequences as queries using BLAST algorithm (<http://www.ncbi.nlm.nih.gov/blast/blast.cgi>) No significant sequence homology was revealed.

[0111] These results suggest that the sequences of MPHOSPH1-A24-9-278 (SEQ ID NO: 7), MPHOSPH1-A24-10-278 (SEQ ID NO: 8), MPHOSPH1-A2-9-282 (SEQ ID NO: 9), MPHOSPH1-A2-9-638 (SEQ ID NO: 10), MPHOSPH1-A2-10-1714 (SEQ ID NO: 11), DEPDC1-A24-9-294 (SEQ ID NO: 12), DEPDC1-A2-9-598 (SEQ ID NO: 192), DEPDC1-A2-9-619 (SEQ ID NO: 195), DEPDC1-A2-9-290 (SEQ ID NO: 197), DEPDC1-A2-9-563 (SEQ ID NO: 209), DEPDC1-A2-9-653 (SEQ ID NO: 225), DEPDC1-A2-10-674 (SEQ ID NO: 228), DEPDC1-A2-10-302 (SEQ ID NO: 230), DEPDC1-A02-10-644 (SEQ ID NO: 240), DEPDC1-A02-10-575 (SEQ ID NO: 241), DEPDC1-A02-10-506 (SEQ ID NO: 243), DEPDC1-A02-10-765 (SEQ ID NO: 244), DEPDC1-A02-10-395 (SEQ ID NO: 249), DEPDC1-A02-10-224 (SEQ ID NO: 253), DEPDC1-A02-9-297 (SEQ ID NO: 226), DEPDC1-A02-10-296 (SEQ ID NO: 254) and DEPDC1-A02-10-301 (SEQ ID NO: 255) are unique and thus possess a low risk of raising unintended immunologic response to any unrelated molecule.

[0112] DISCUSSION

Identification of new TAAs, particularly those that induce potent and specific anti-tumor immune responses, warrants further development of the clinical application of peptide vaccination strategies in various types of cancer (Boon T. et al., (1996) J Exp Med 183: 725-9.; van der Bruggen P et al., (1991) Science 254: 1643-7.; Brichard V et

al., (1993) J Exp Med 178: 489-95.; Kawakami Y et al., (1994) J Exp Med 180: 347-52.; Shichijo S et al., (1998) J Exp Med 187:277-88.; Chen YT et al., (1997) Proc.Natl.Acd. Sci. USA, 94: 1914-8.; Harris CC., (1996) J Natl Cancer Inst 88:1442-5.; Butterfield LH et al., (1999) Cancer Res 59:3134-42.; Vissers JL et al., (1999) Cancer Res 59: 5554-9.; van der Burg SH et al., (1996) J. Immunol 156:3308-14.; Tanaka F et al., (1997) Cancer Res 57:4465-8.; Fujie T et al., (1999) Int J Cancer 80:169-72.; Kikuchi M et al., (1999) Int J Cancer 81 : 459-66.; Oiso M et al., (1999) Int J Cancer 81:387-94.).

- [0113] cDNA microarray technologies can disclose comprehensive profiles of gene expression of malignant cells (Lin YM, et al., Oncogene. 2002 Jun 13;21:4120-8.; Kitahara O, et al., Cancer Res. 2001 May 1;61:3544-9.; Suzuki C, et al., Cancer Res. 2003 Nov 1;63:7038-41.; Ashida S, Cancer Res. 2004 Sep 1;64:5963-72.; Ochi K, et al., Int J Oncol. 2004 Mar;24(3):647-55.; Kaneta Y, et al., Int J Oncol. 2003 Sep;23:681-91.; Obama K, Hepatology. 2005 Jun;41:1339-48.; Kato T, et al., Cancer Res. 2005 Jul 1;65:5638-46.; Kitahara O, et al., Neoplasia. 2002 Jul-Aug;4:295-303.; Saito-Hisaminato A et al., DNA Res 2002, 9: 35-45.) and, find utility in the identification of potential TAAs. Among the transcripts that are up-regulated in various cancers, two novel human genes, termed MPHOSPH1 and DEPDC1, respectively, were identified using these technologies.
- [0114] As demonstrated above, MPHOSPH1 and DEPDC1, are over-expressed in various cancers but show minimal expression in normal tissues. In addition, these genes have been shown to have a significant function related to cell proliferation (See PCT/JP2006/302684). Thus, peptides derived from MPHOSPH1 and DEPDC1 can serve as TAA epitopes, which, in turn, can be used to induce significant and specific immune responses against cancer cells.
- [0115] Thus, as MPHOSPH1 and DEPDC1 are novel TAAs, vaccines using these epitope peptides find utility as immunotherapeutics against various carcinomas or other disease expressing these molecules.
- [0116] EXAMPLE 2
MATERIALS AND METHODS
Peptides and adjuvant
 The synthesized GMP grade peptides were purchased from Neo Multi Peptide System (MPS) (San Diego, CA). As an adjuvant, incomplete Freund's adjuvant (IFA) (MONTANIDE *ISA51) were used. 1mg of the appropriate peptide was emulsioned with 1mg of IFA.
- [0117] Antigen Expression
 The present inventors performed immunohistochemical analysis. Tumor cells or tumor tissues from bladder cancers which was obtained from surgery or biopsy was

stained by each MPHOSPH1 and DEPDC1-specific polyclonal antibody. Protocol of staining was established in Human Genome Center, Institute for Medical Science, the University of Tokyo as described previously (Kanehira M et al. Cancer Res.;67(7):3276-3285, 2007., Kanehira M et al. Oncogene. 2007 Apr 23; [Epub ahead of print]). HLA-A*2402 expression was tested to performed at SRL (Tachikawa, Japan)

[0118] Enrolled patients

Enrolled criteria were as follows;

1. Patients with inoperable recurrent bladder cancer with previously treated with standard chemotherapy and turned to be failure.
2. Patients with performance status 0 or 1 in Japanese Criteria.
3. Patients from 20 years old to 80 years old
4. Patients with primary tumor or metastasis which can be recognized by image inspection (CT/MRI) before treatment, regardless of RECIST guideline
5. Patients with more than 4 weeks after prior treatment (surgery, chemotherapy, radiotherapy, thermotherapy, other immunotherapy etc.)
6. Patients expected more than 3 months prognosis
7. Patients with bone marrow function (WBC more than 2000, 15000 less than, plate more than 50000), liver function (GOT less than 150, GPT less than 150, T-bil less than 3.0), renal function (Cr less than 3.0)
- 8 Patients with HLA-A*2402
- 9 Tumor of the patients with expression of MPHOSPH1 and/or DEPDC1

[0119] Exclusion criteria were as follows;

1. Patients with pregnant
2. Patients with breast-feeding
3. Patients willing to be made pregnant
4. Patients with uncontrollable infection
5. Patients with necessity of following medicine in the period of clinical trial
systemic administration of steroid
systemic administration of immunosuppressant
6. Patients who are not thought to be enrolled this trial by doctor or principal investigator

[0120] Protocol

Enrolled bladder cancer patients with HLA-A*2402, whose tumors express M phase phosphoprotein 1 (MPHOSPH1) and/or DEP domain containing 1 (DEPDC1) were immunized with HLA-A*2402-restricted epitope peptides, MPHOSPH1-9-278 (IYNEYIYDL (SEQ ID NO: 7)) and/or DEPDC1-9-294 (EYYELFVNI (SEQ ID NO: 12)). Each peptide was combined with 1mL of incomplete Freund's adjuvant (IFA,

MONTANIDE *ISA51) and was subcutaneously injected into axillary or inguinal lesion once a week. Four times injection is defined as one course, then after 1 course for immunological and clinical evaluation, blood was drawn and CT/MRI was performed.

[0121] Evaluation of Safety

Evaluation of adverse effect was performed along with National Cancer Institute-Common Toxicity Criteria version 3, (NCI-CTC ver.3).

[0122] Immunological evaluation

This is one of secondary endpoint in this study and we confirm whether peptide-specific CTL response occurred or not. Specific CTL response was measure as follows; Peripheral blood mononuclear cells were collected, and re-stimulated by the appropriate peptides. CTL response was tested on the 14th day by IFN-g ELISPOT assay.

[0123] Evaluation of anti-tumor effects

Evaluation of clinical response was performed in accordance with RECIST criteria.

[0124] RESULTS

Table 7 showed the summary of this clinical trial. There were no severe adverse effects, except Grade 2 of exanthema of Case 3. One minor response (Case 3) and one mixed response (Case 4) were obtained. The expression of MPHOSPH1 was 4 of 5 cases, whereas that of DEPDC1 was 5 of 5 cases, respectively.

[0125] [Table 7]

The summary of this clinical trial

Case	Age/ Gender	Vaccination	Adv. Effect	DTH	Eva. Lesion	Eva.	Present Status	Ag expression		CTL
								MPHOSPH1	DEPDC1	
1	79/M	1 course	No	No	LN's, Brain	PD	1.8mo, dead	○	○	No
2	72/F	in 3 course	No	No	Local Rec	SD (4.5mo)	5.0mo, alive	○	○	NT
3	49/M	in 4 course	exanthema	No	Lung Mets	Minor Response	3.7mo, alive	x	○	Yes
4	74/M	in 2 course	No	No	Local Rec	Minor Response	1.4mo, alive	○	○	NT
5	78/M	in 2 course	No	No	Local Rec	SD	1.4mo, alive	○	○	NT

NT: not tested

[0126] Case 2

In case 2, 72 years old female with far advanced bladder cancer in standard chemotherapy failure was enrolled this clinical trial. In figure 18, the antigen expression of her tumor revealed both MPHOSPH1 and DEPDC1 were expressed strongly. Therefore, we have vaccinated two kinds of epitope peptides derived from MPHOSPH1 and DEPDC1. Case 2 had local recurrence of the bladder cancer. It was evaluated stable disease (SD) in accordance with RECIST criteria (Figure 19).

[0127] Case 3

In case 3, 49 years old male with far advanced bladder cancer in standard

chemotherapy failure was enrolled this clinical trial. Only DEPDC1 was expressed strongly (Figure 20). Therefore, we have vaccinated the epitope peptide derived from DEPDC1 alone. Case 3 had multiple lung metastases of the bladder cancer. In right (Figure 21) and left (Figure 22) lobes of lung metastases, the progression rate was decreased after vaccination. Especially, the size of the tumor was decreased after 3rd courses. Figure 23 showed the anti-tumor effect in accordance with RECIST criteria. It was clarified that the progression rate of metastatic tumor was decreased after vaccination. It indicated that minor response was obtained by vaccination using epitope peptide derived from DEPDC1. In terms of immunological evaluation in case 3, specific CTL response was measured before and after vaccination. Specific CTL response was strongly shown after vaccination (Figure 24). It clearly indicated that CTL induced by epitope peptide derived from DEPDC1 may show the anti-tumor effect.

[0128] Case 4

In case 4, 74 years old male with far advanced bladder cancer in standard chemotherapy failure was enrolled this clinical trial. MPHOSPH1 and DEPDC1 were expressed from his tumor (Figure 25). Therefore, we have vaccinated two kinds of epitope peptides derived from MPHOSPH1 and DEPDC1. Case 4 had local recurrence of the bladder cancer. After 1 course vaccination, the size of the tumor was reduced 20% in accordance with RECIST criteria (Figure 26). However, new metastatic lesions in the lung were appeared. It indicated that mixed response was obtained by vaccination using two kinds of epitope peptides derived from MPHOSPH1 and DEPDC1.

[0129] DISCUSSION

Rationale of this clinical trial is described blow;

1. Since MPHOSPH1 and DEPDC1 are not expressed in normal tissues except testis, both antigens are highly tumor-specific.
2. These peptides are considered to have strong immunogenicity, since potent and specific CTLs were established by these epitope peptides.
3. There is 60% of Japanese population with HLA-A*2402.
4. These peptides are chemically stable enough to apply to the clinical trial.

The purpose of this study is to obtain clinical information of its toxicity, immunological response and anti-tumor activity.

- [0130] Previously reported adverse effects of vaccine clinical trial using peptides are fur-like symptom, such as fever, headache and discomfort. In rare cases, radical skin reaction with blisters, considered as transient cross reactivity at injected site, was reported. In this study, there were no severe adverse effects, except Grade 2 of exanthema of Case 3. This patient had clinical history to show exanthema during chemotherapy. It indicated that this adverse effect did not come from this vaccination, and therefore this

protocol may be safe.

- [0131] Immunological analysis was performed by specific CTL induction after vaccination. In case 1, specific CTL response was not obtained after vaccination (data not shown). In case 3, specific CTL response against DEPDC1 derived peptide was clearly shown after 1st and 2nd course of vaccination. In case 3, anti-tumor effect was obtained by vaccination. It clearly demonstrated that this DEPDC1 derived peptide showed anti-tumor effect against bladder cancer by induction of the specific CTL.
- [0132] In case 4, after only 1st course of vaccination, anti-tumor effect was clearly obtained against the local recurrence of the bladder cancer. This evidence strongly supports that these epitope peptides show anti-tumor effect against bladder cancer.
- [0133] In conclusion, it was clarified that this epitope therapy was safe, and furthermore showed strong anti-tumor effect without severe adverse effects.

Industrial Applicability

- [0134] The present invention identifies new TAAs, particularly those which induce potent and specific anti-tumor immune responses. Such TAAs warrant further development as peptide vaccines against diseases associated with MPHOSPH1 and/or DEPDC1, e.g. cancers.
- [0135] All patents, patent applications, and publications cited herein are incorporated by reference.
- [0136] While the invention has been described in detail and with reference to specific embodiments thereof, it is to be understood that the foregoing description is exemplary and explanatory in nature and is intended to illustrate the invention and its preferred embodiments. Through routine experimentation, one skilled in the art will readily recognize that various changes and modifications can be made therein without departing from the spirit and scope of the invention. Thus, the invention is intended to be defined not by the above description, but by the following claims and their equivalents.

CLAIMS:

1. An isolated peptide of less than about 15 amino acids, wherein the peptide is selected from the group consisting of:
 - 5 (a) a peptide comprising the amino acid sequence of SEQ ID NO: 7 or 8, and
 - (b) a peptide having cytotoxic T cell inducibility, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 7, and 8, in which 1, 2, or several amino acids are substituted, deleted, or added.
- 10 2. The peptide having cytotoxic T cell inducibility of claim 1, wherein the second amino acid from the N-terminus is phenylalanine, tyrosine, methionine, or tryptophan.
3. The peptide having cytotoxic T cell inducibility of claim 1 or 2, wherein the
15 C-terminal amino acid is phenylalanine, leucine, isoleucine, tryptophan, or methionine.
4. An isolated peptide of less than about 15 amino acids, wherein the peptide is selected from the group consisting of:
 - 20 (a) a peptide comprising the amino acid sequence of SEQ ID NO: 9, 10 or 11 and
 - (b) a peptide having cytotoxic T cell inducibility, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 9, 10 and 11, in which 1, 2, or several amino acids are substituted, deleted, or added.
- 25 5. The peptide having cytotoxic T cell inducibility of claim 4, wherein the second amino acid from the N-terminus is leucine or methionine.
6. The peptide having cytotoxic T cell inducibility of claim 4 or 5, wherein the C-terminal amino acid is valine or leucine.
- 30 7. The peptide of claim 1 or 4, wherein the peptide consists of the amino acid sequence selected from the group consisting of SEQ ID NOs: 7, 8, 9, 10 and 11.
8. A vector comprising DNA which encodes the peptide of any one of claims 1 to
35 7.
9. A pharmaceutical composition for treating or preventing a disease associated with over-expression of the gene of SEQ ID NO: 1, said composition comprising one or

more peptides of any one of claims 1 to 7 and a carrier.

10. The pharmaceutical composition of claim 9, wherein the disease is cancer.
- 5 11. The pharmaceutical composition of claim 10, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC and soft tissue tumor.
- 10 12. An exosome that presents on its surface a complex comprising a peptide of any one of claims 1 to 7 and an HLA antigen.
13. The exosome of claim 12, wherein the HLA antigen is HLA-A24.
- 15 14. The exosome of claim 13, wherein the HLA antigen is HLA-A2402.
15. The exosome of claim 12, wherein the HLA antigen is HLA-A2.
- 20 16. The exosome of claim 15, wherein the HLA antigen is HLA-A0201.
17. An *in vitro* method of inducing antigen-presenting cells having a high cytotoxic T cell inducibility, said method comprising the step of contacting an antigen-presenting cell with the peptide of any one of claims 1 to 7.
- 25 18. An *in vitro* method of inducing cytotoxic T cells by contacting a T cell with the peptide of any one of claims 1 to 7.
19. An *in vitro* method of inducing antigen-presenting cells having high cytotoxic
30 T cell inducibility, said method comprising the step of transferring a gene comprising a polynucleotide encoding a peptide of any one of claims 1 to 7 to an antigen-presenting cell.
20. An *in vitro* method of inducing cytotoxic T cells, said method comprising the
35 steps of:
 - (i) contacting an antigen-presenting cell with a peptide of any one of claims 1 to 7, and
 - (ii) mixing the antigen-presenting cell of step (i) with a CD8+ T cell and

co-culturing them.

21. An isolated cytotoxic T cell, which is induced by the method of claim 18 or 20,
or which is transduced with the nucleic acids encoding the TCR subunits polypeptides
5 binding with a peptide of any one claims 1 to 7 in the context of HLA-A24 or HLA-A2.
22. An antigen-presenting cell, which comprises a complex formed between an
HLA antigen and a peptide of any one of claims 1 to 7.
- 10 23. The antigen-presenting cell of claim 22, which is induced by the method of
claim 17 or 19.
24. A vaccine for inhibiting proliferation of cells expressing gene of SEQ ID NO:
1, wherein the vaccine comprises the peptide of any one of claims 1 to 7 and a carrier.
15
25. The vaccine of claim 24, wherein the cell is a cancer cell.
26. The vaccine of claim 25, wherein the cancer is selected from the group
consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular
20 carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma,
prostate cancer, renal carcinoma, SCLC and soft tissue tumor.
27. The vaccine of any one of claims 24 to 26, formulated for use in a subject
whose HLA antigen is HLA-A24 or HLA-A2.
25
28. A use of a vaccine comprising one or more peptide(s) of any one of claims 1 to
7, an immunologically active fragment thereof, or a polynucleotide encoding said
peptide or immunologically active fragment for treating or preventing a disease
associated with the over-expression of the gene of SEQ ID NO: 1, in a subject.
30
29. The use of claim 28, wherein the disease is cancer.
30. The use of claim 29, wherein the cancer is selected from the group consisting
of bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML,
35 colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer,
renal carcinoma, SCLC and soft tissue tumor.
31. A composition for inducing a CTL, said composition comprising one or more

peptide(s) of any one of claims 1 to 7 and a carrier.

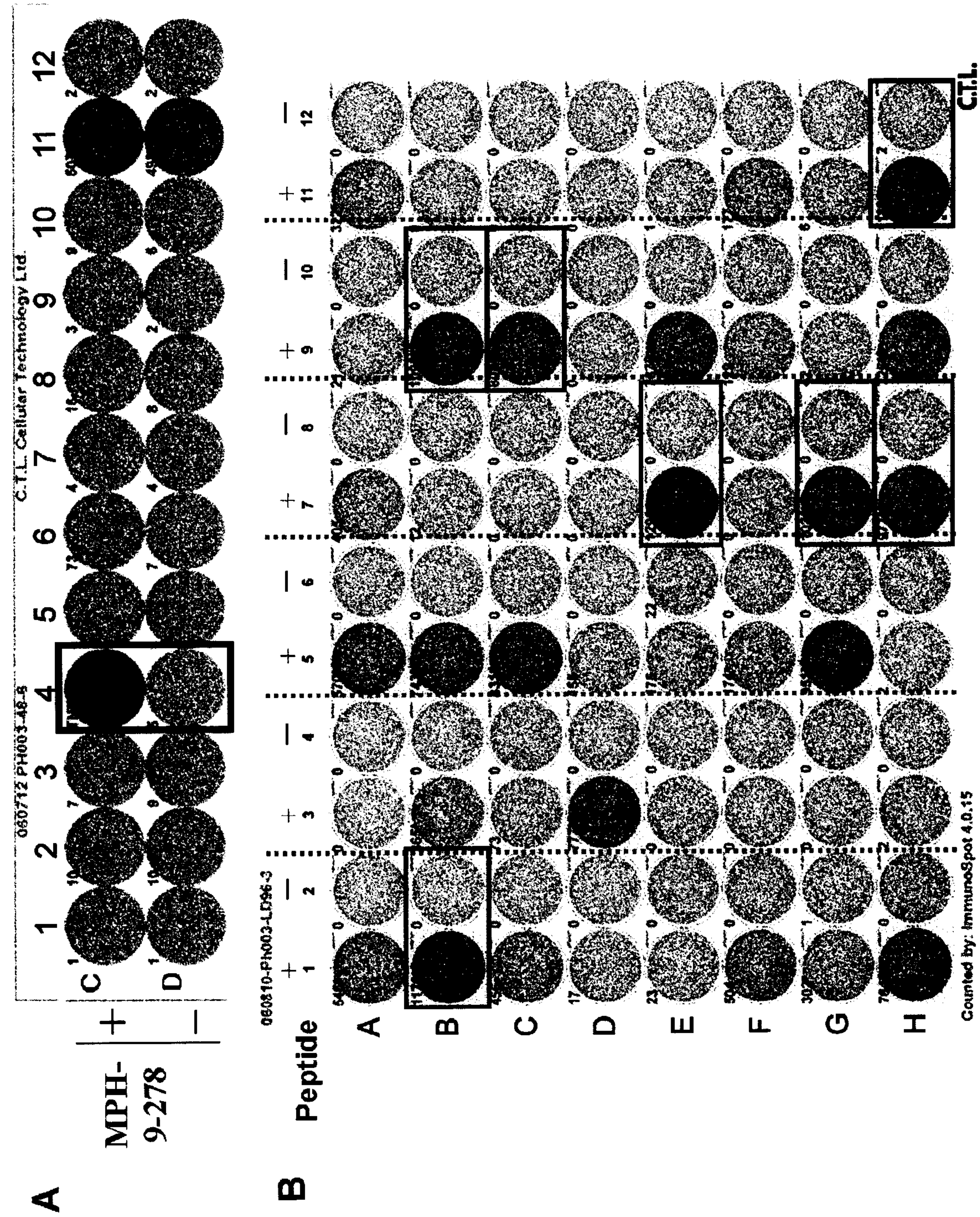
32. A use of an antigen-presenting cell contacted with the peptide of any one of claims 1 to 7 for inducing antigen-presenting cells having a high cytotoxic T cell
5 inducibility.

33. A use of a T cell contacted with the peptide of any one of claims 1 to 7 for inducing cytotoxic T cells.

10 34. A use of an antigen-presenting cell comprising a gene comprising a polynucleotide encoding a peptide of any one of claims 1 to 7 for inducing antigen-presenting cells having high cytotoxic T cell inducibility.

35. A use of an antigen-presenting cell contacted with a peptide of any one of
15 claims 1 to 7, and mixed with a CD8⁺ T cell for inducing cytotoxic T cells.

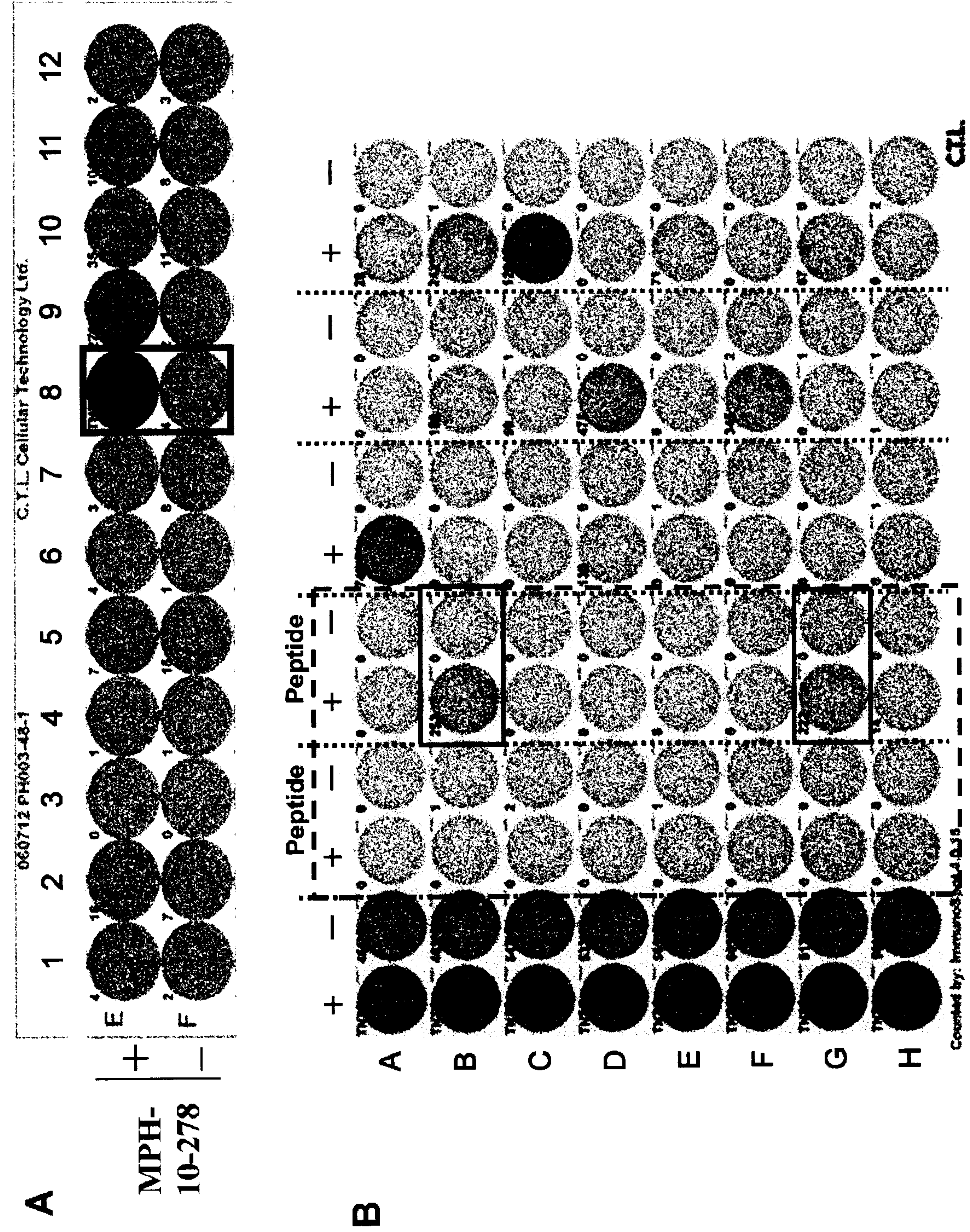
[Fig. 1]



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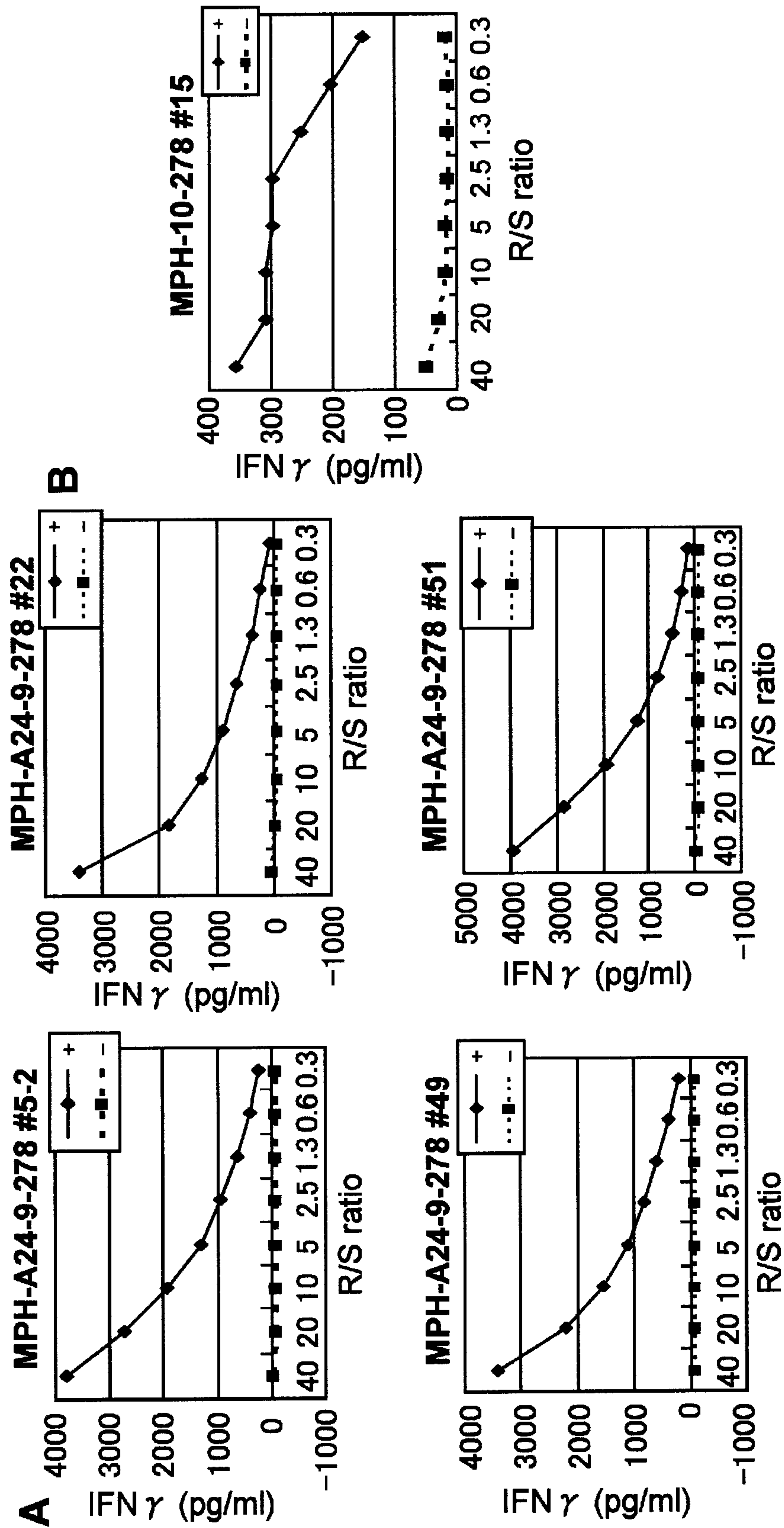
[Fig. 2]



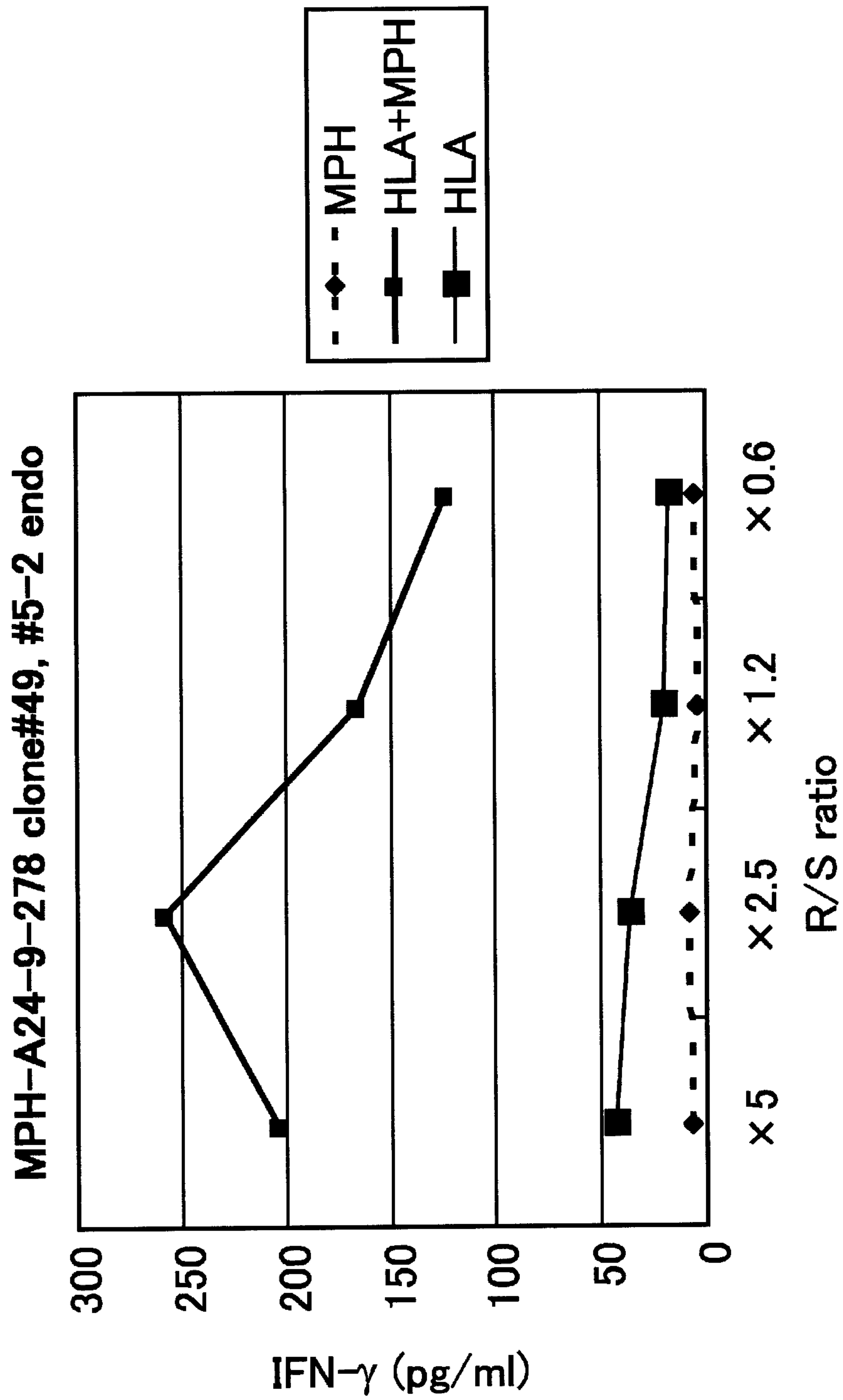
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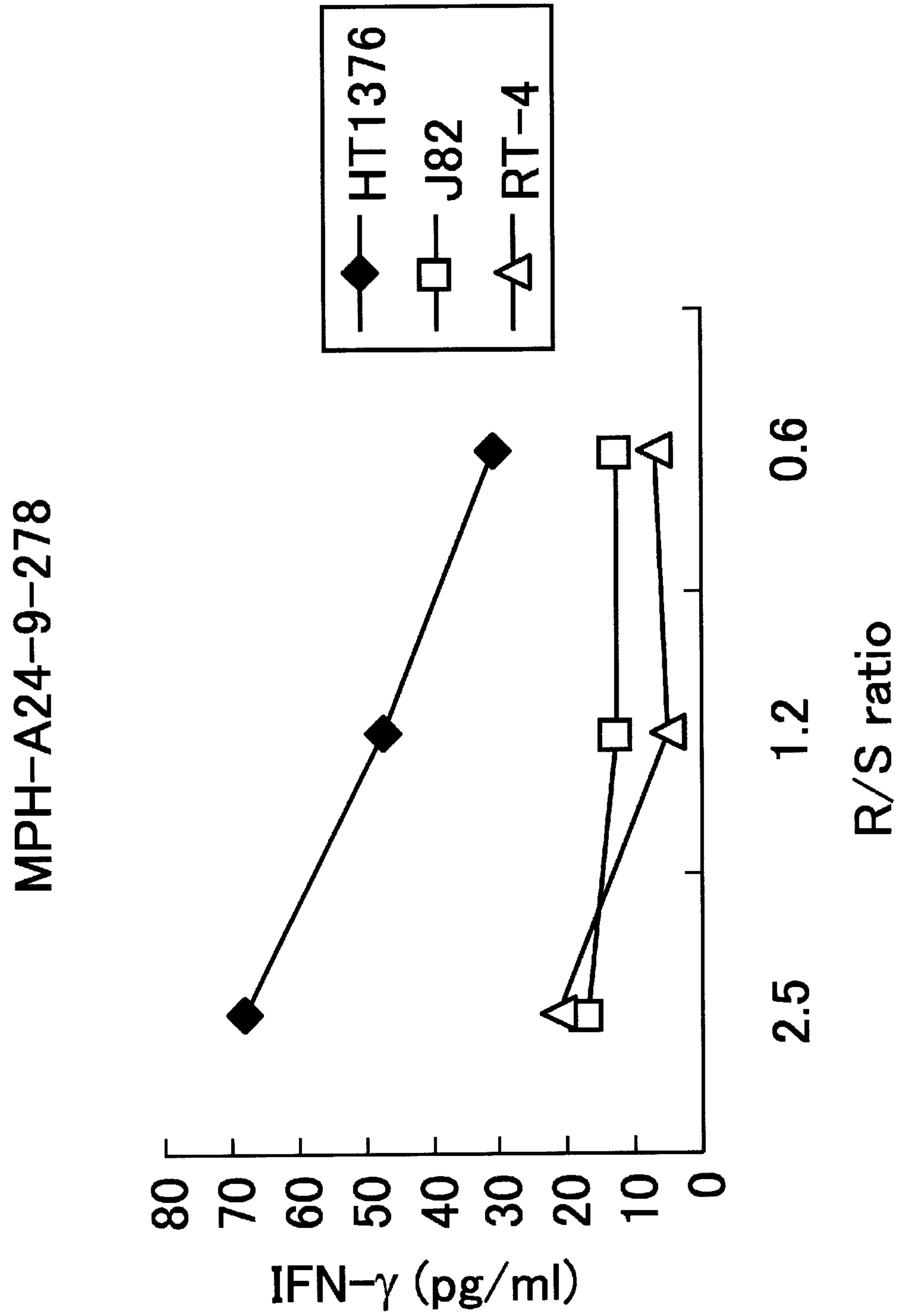
[Fig. 3]



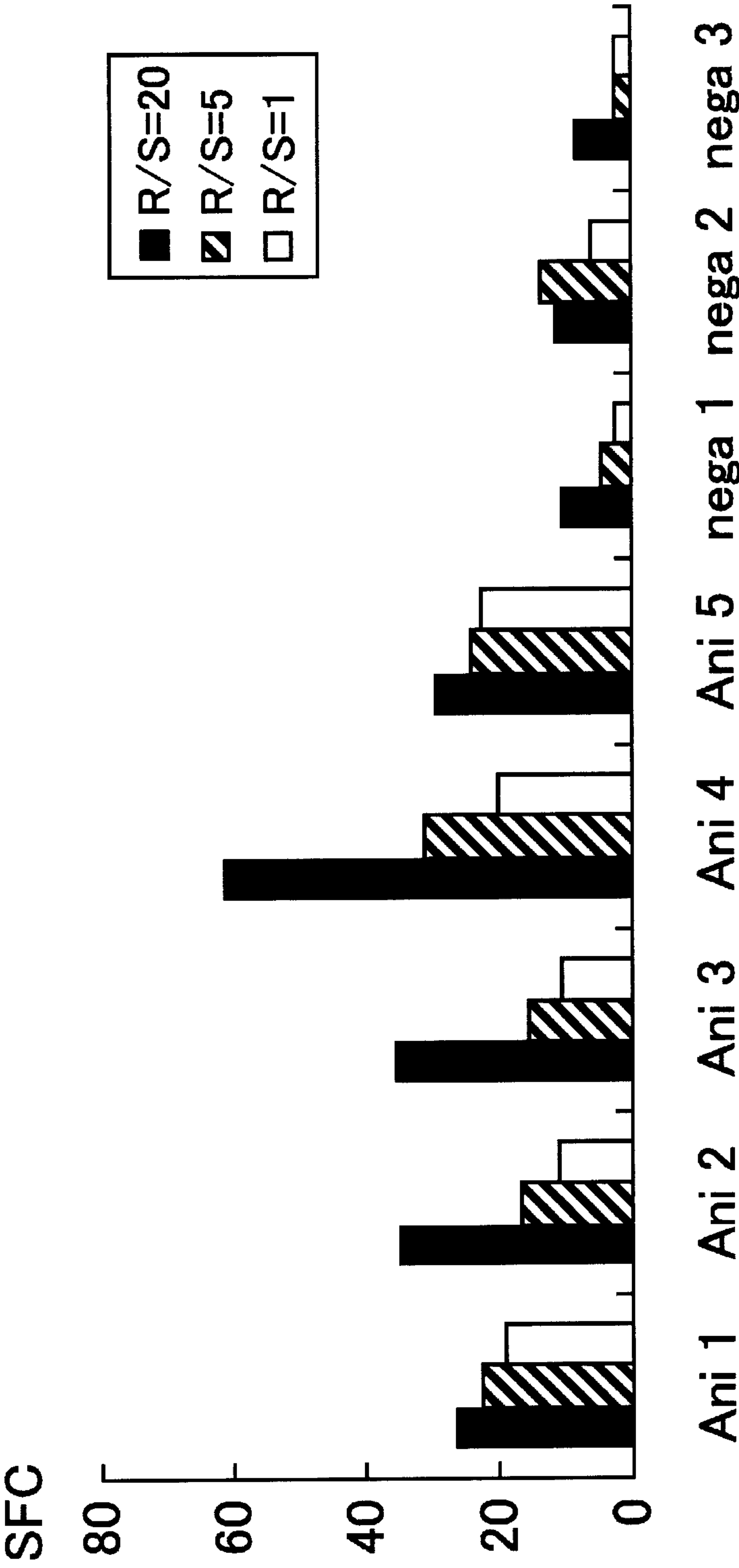
[Fig. 4]



[Fig. 5]



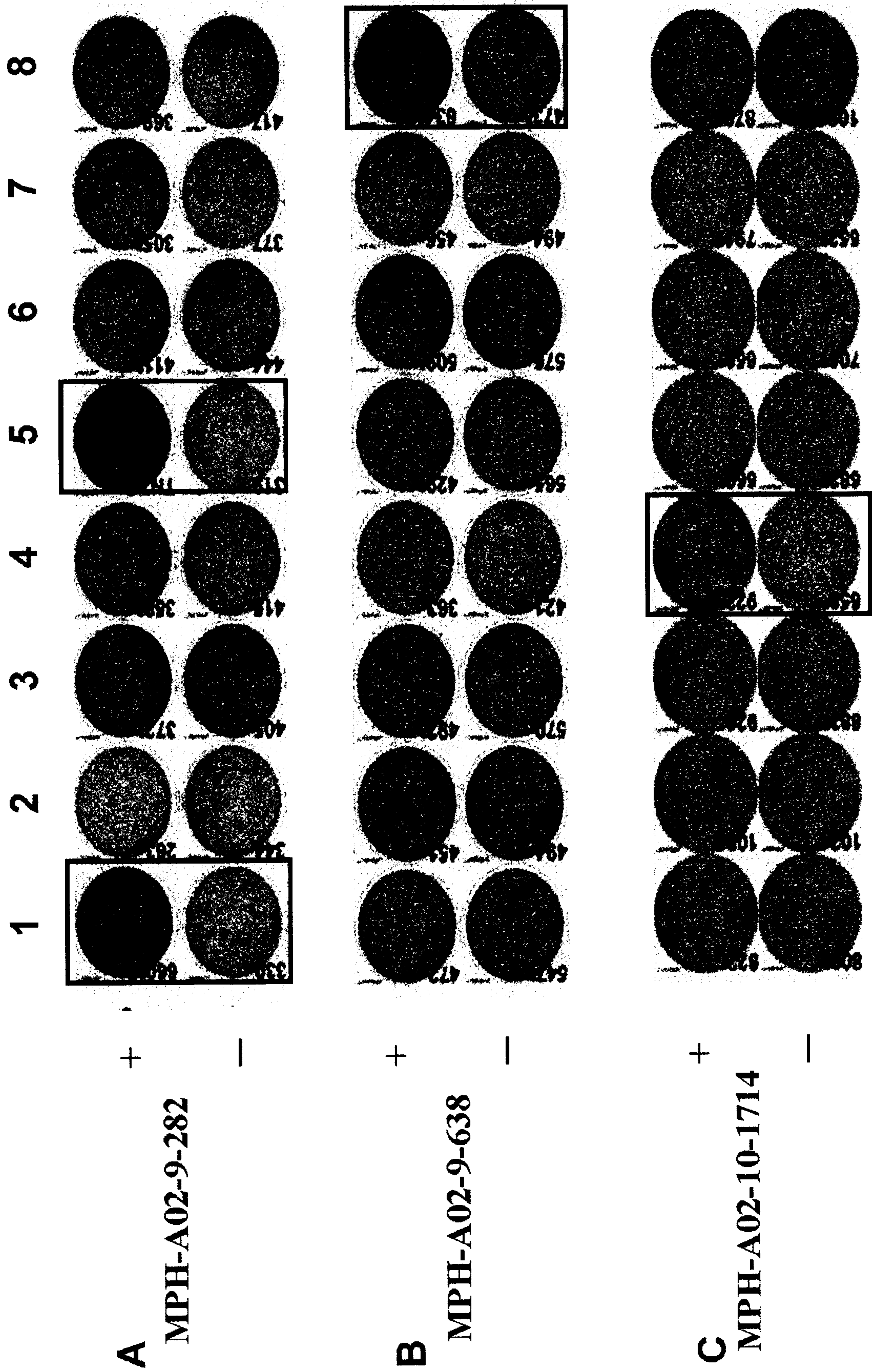
[Fig. 6]



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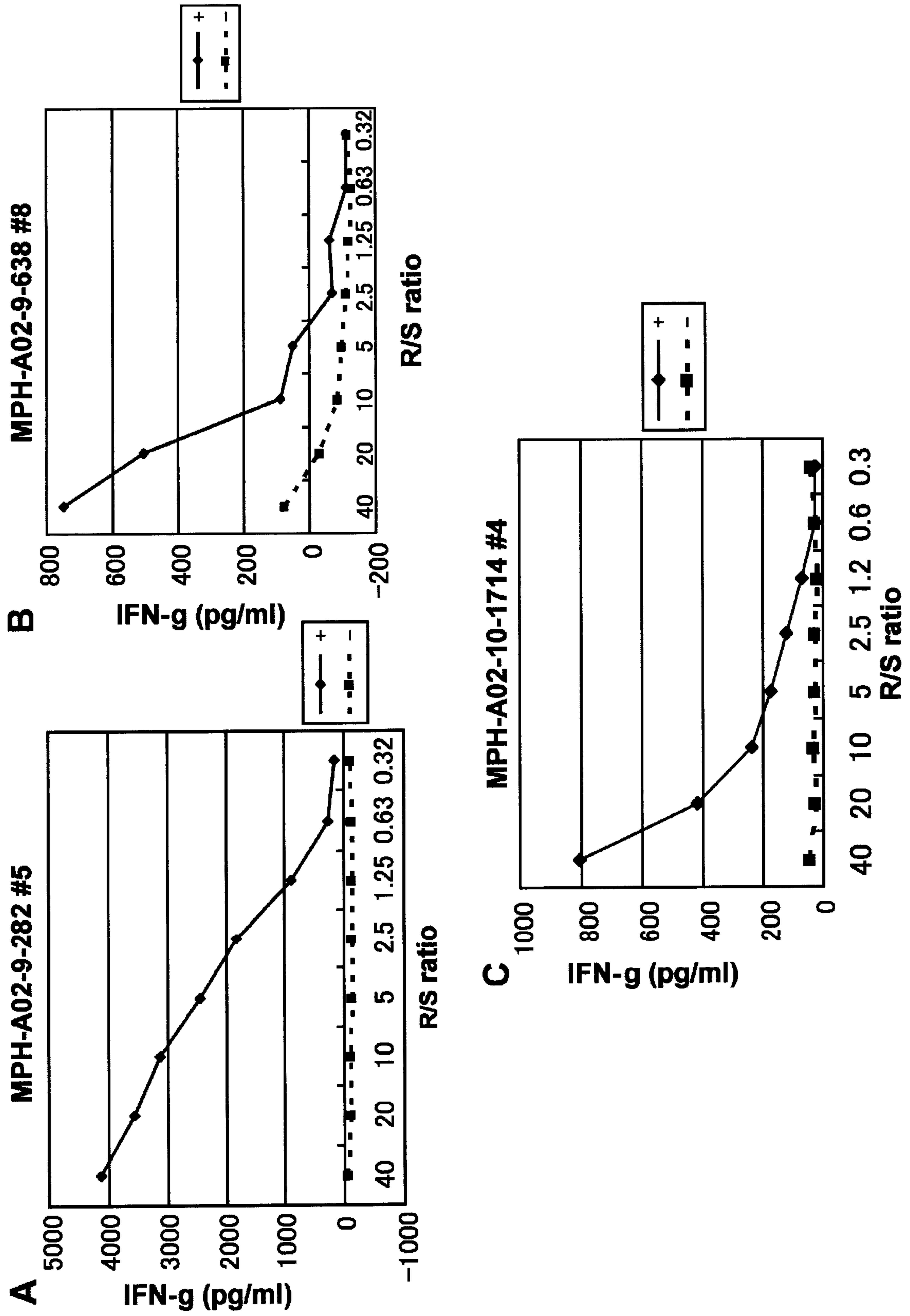
[Fig. 7]



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[Fig. 8]



[illegible]

A

DEPDC1-
A24-9-294

060712 PH003.4B-5 C.T.L. Cellular Technology Ltd.

1 2 3 4 5 6 7 8 9 10 11 12

A B

+

-

B

Peptide

A B C D E F G H

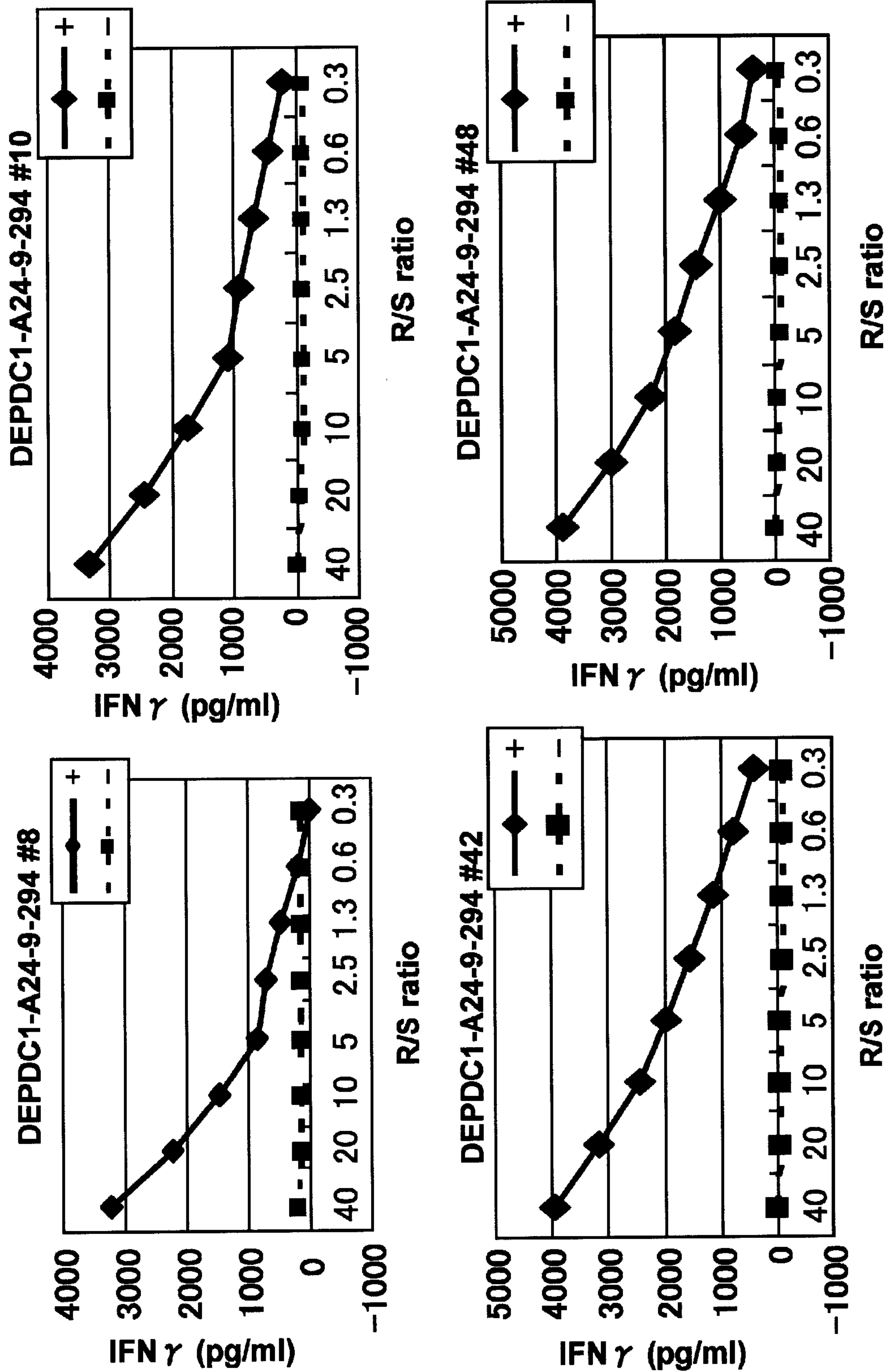
+

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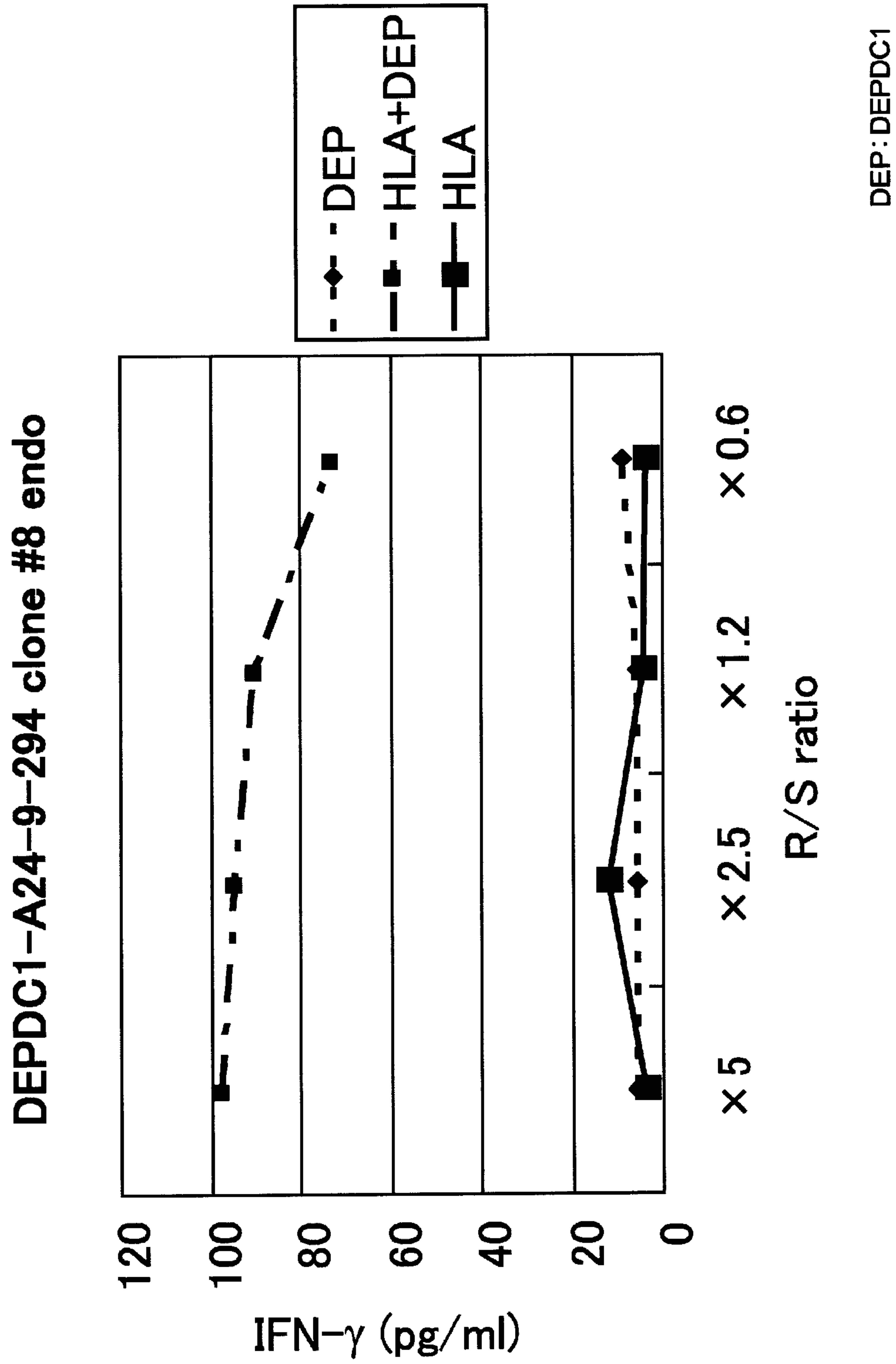
C.L.

Counted by: ImmunoSpot 4.0.15

[Fig. 11]



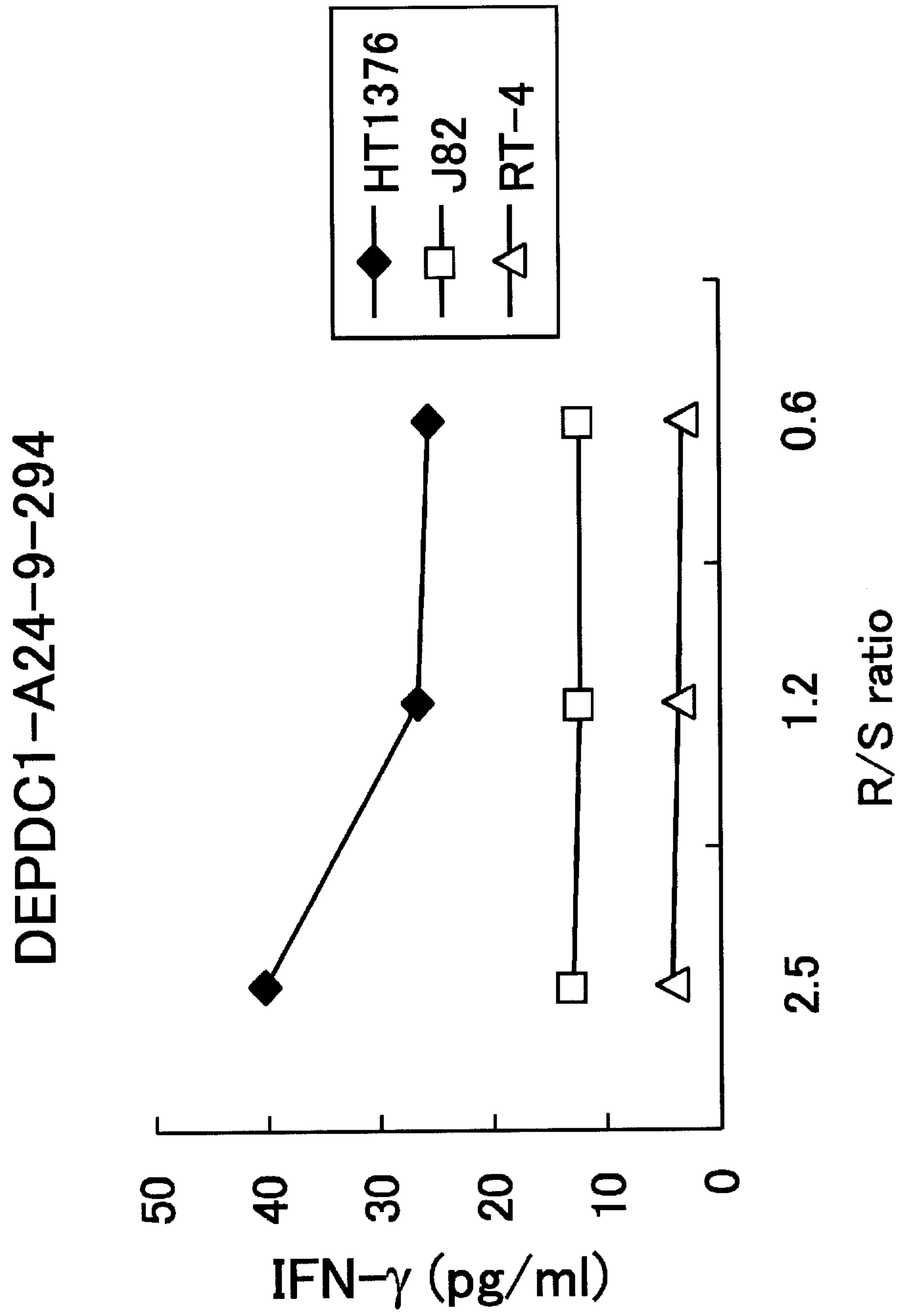
[Fig. 12]



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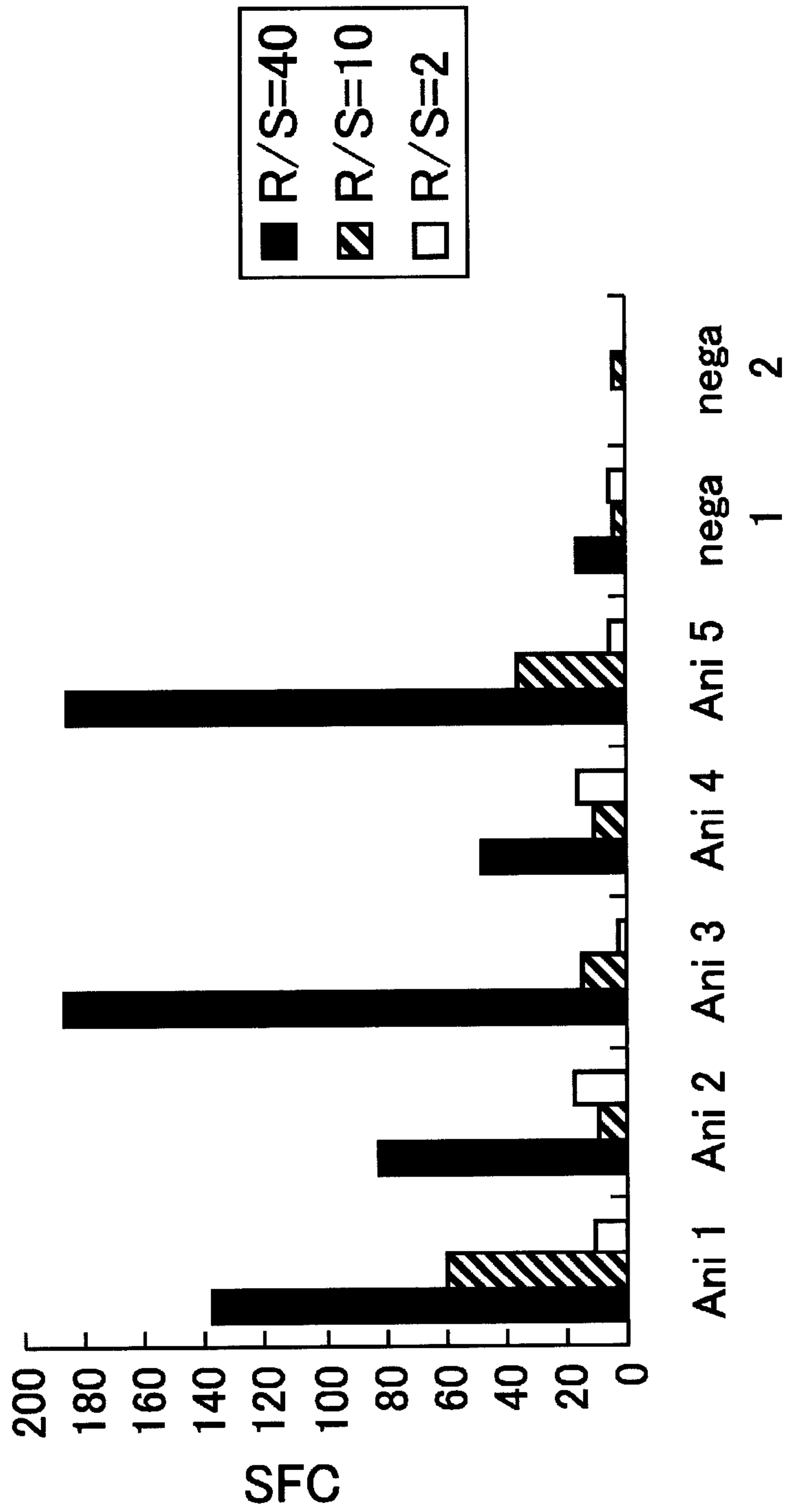
[Fig. 13]



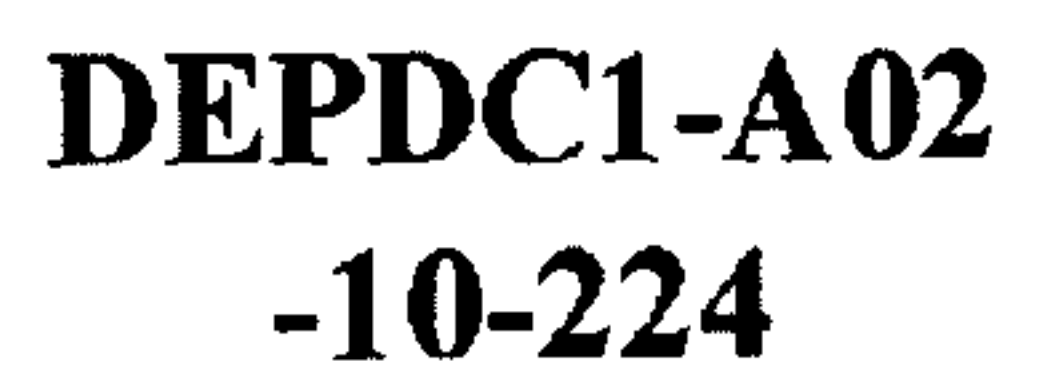
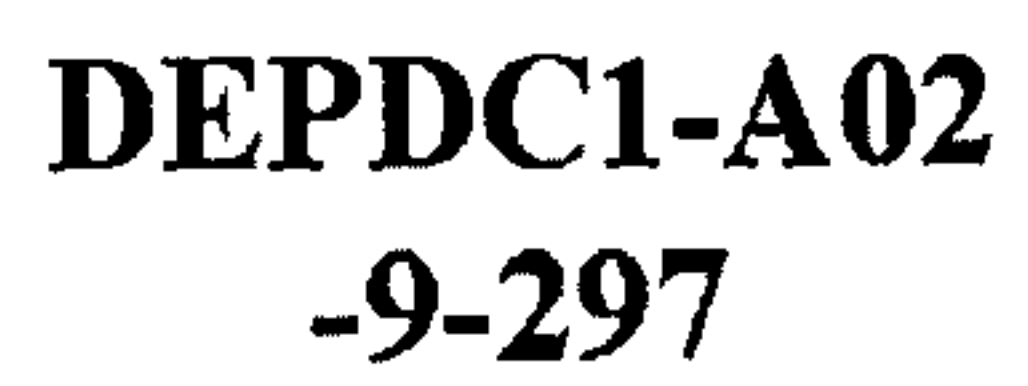
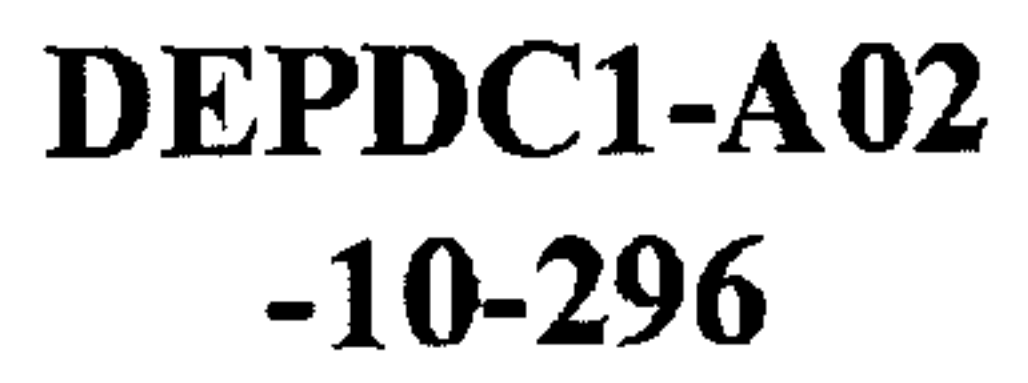
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[Fig. 14]

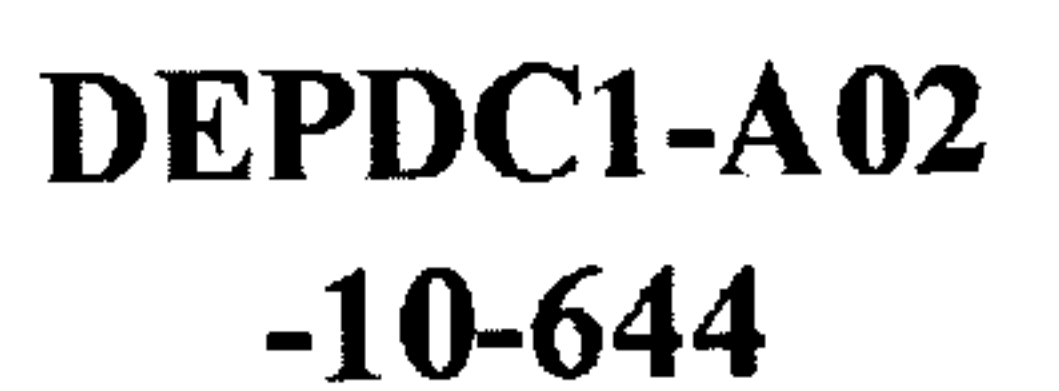
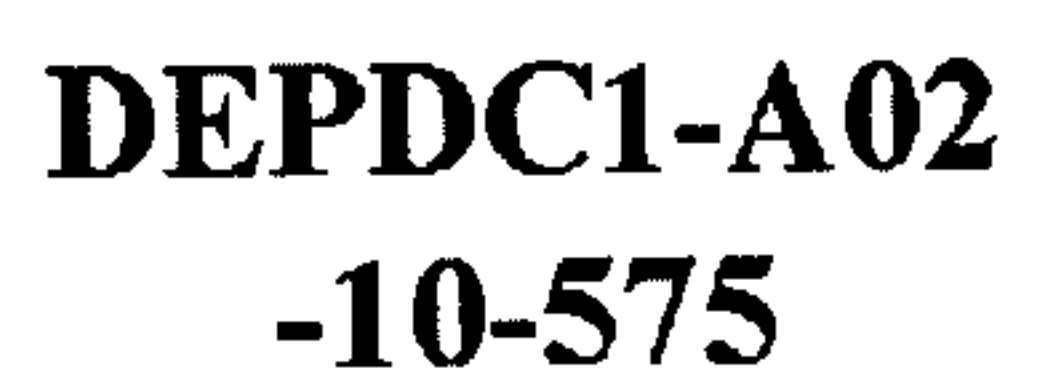
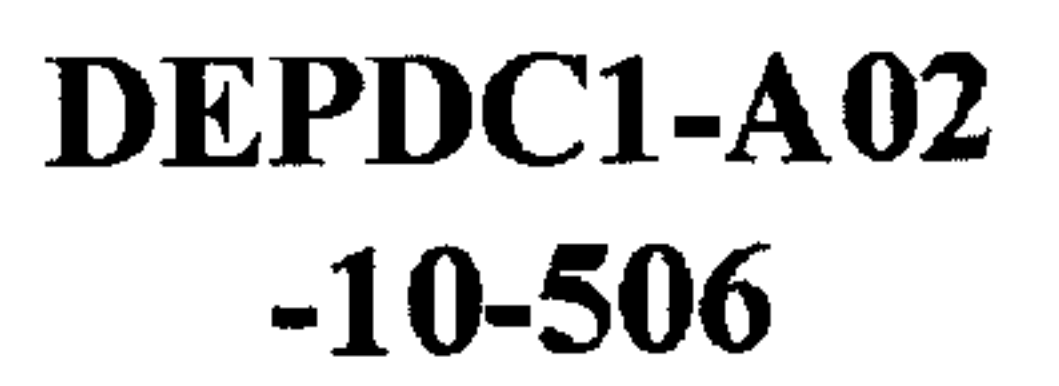
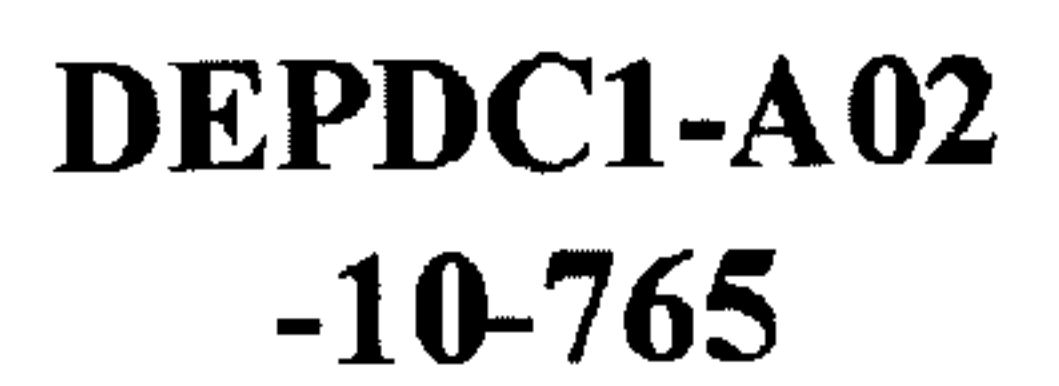
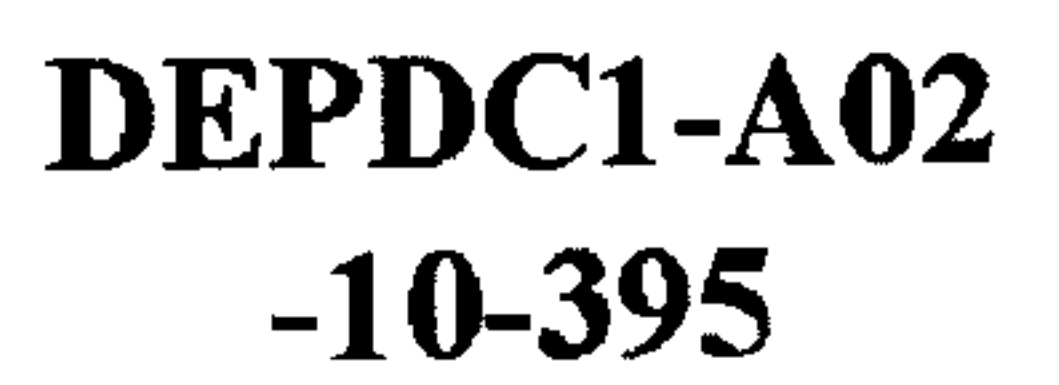


DEPDC1-A02
-10-301



Peptide

1 2 3 4 5 6 7 8



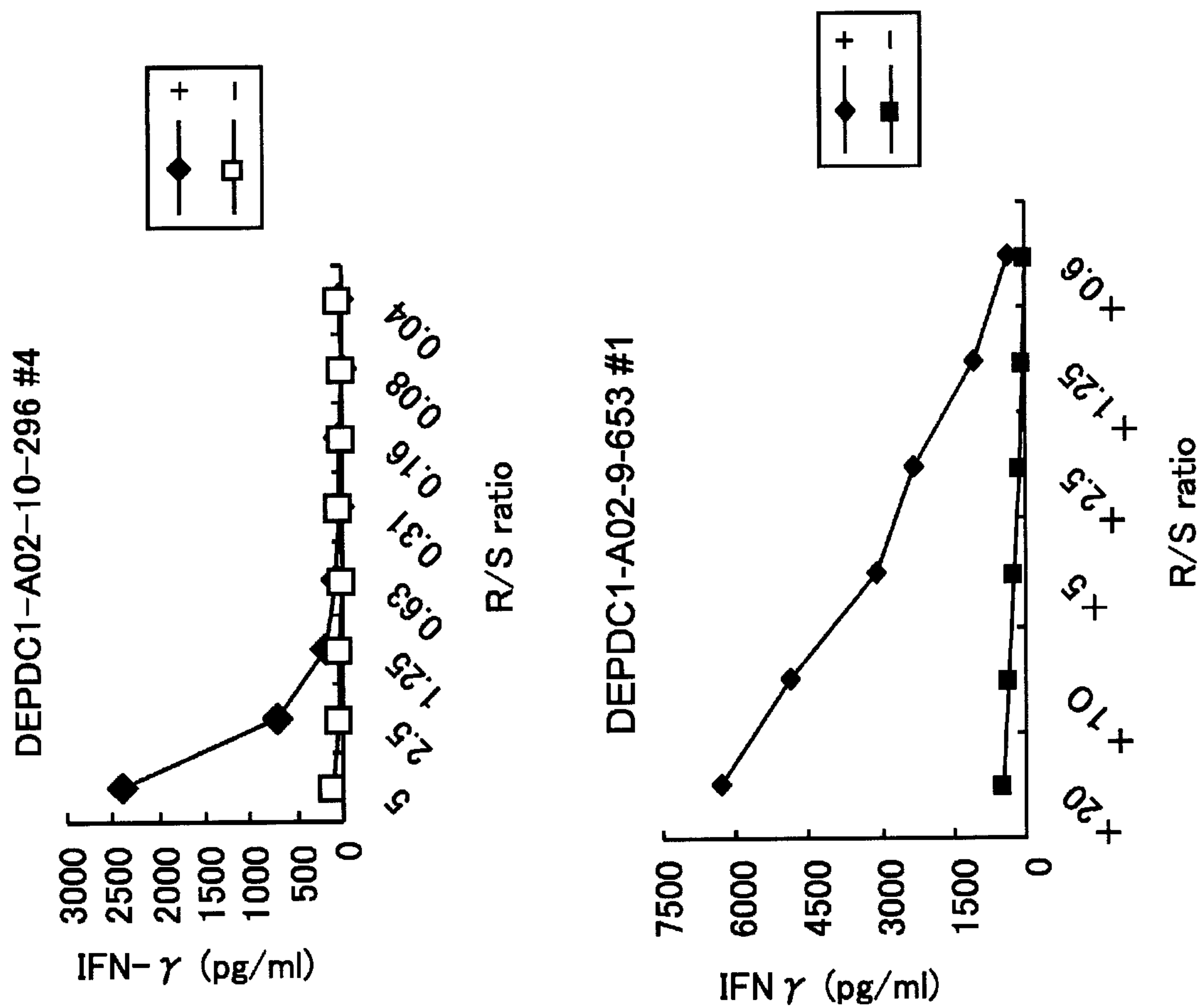
Peptide

1 2 3 4 5 6 7 8

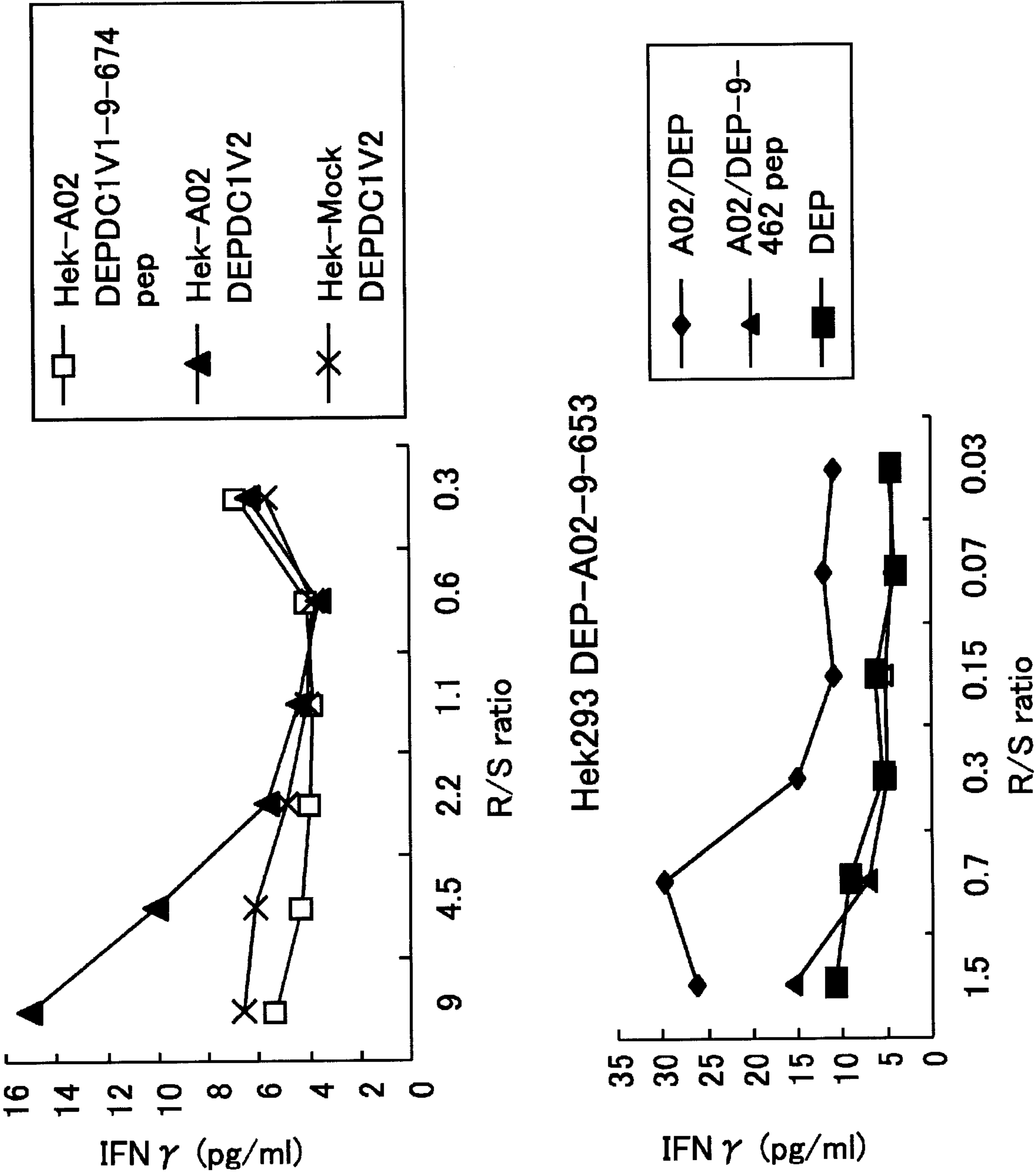
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[Fig. 16]



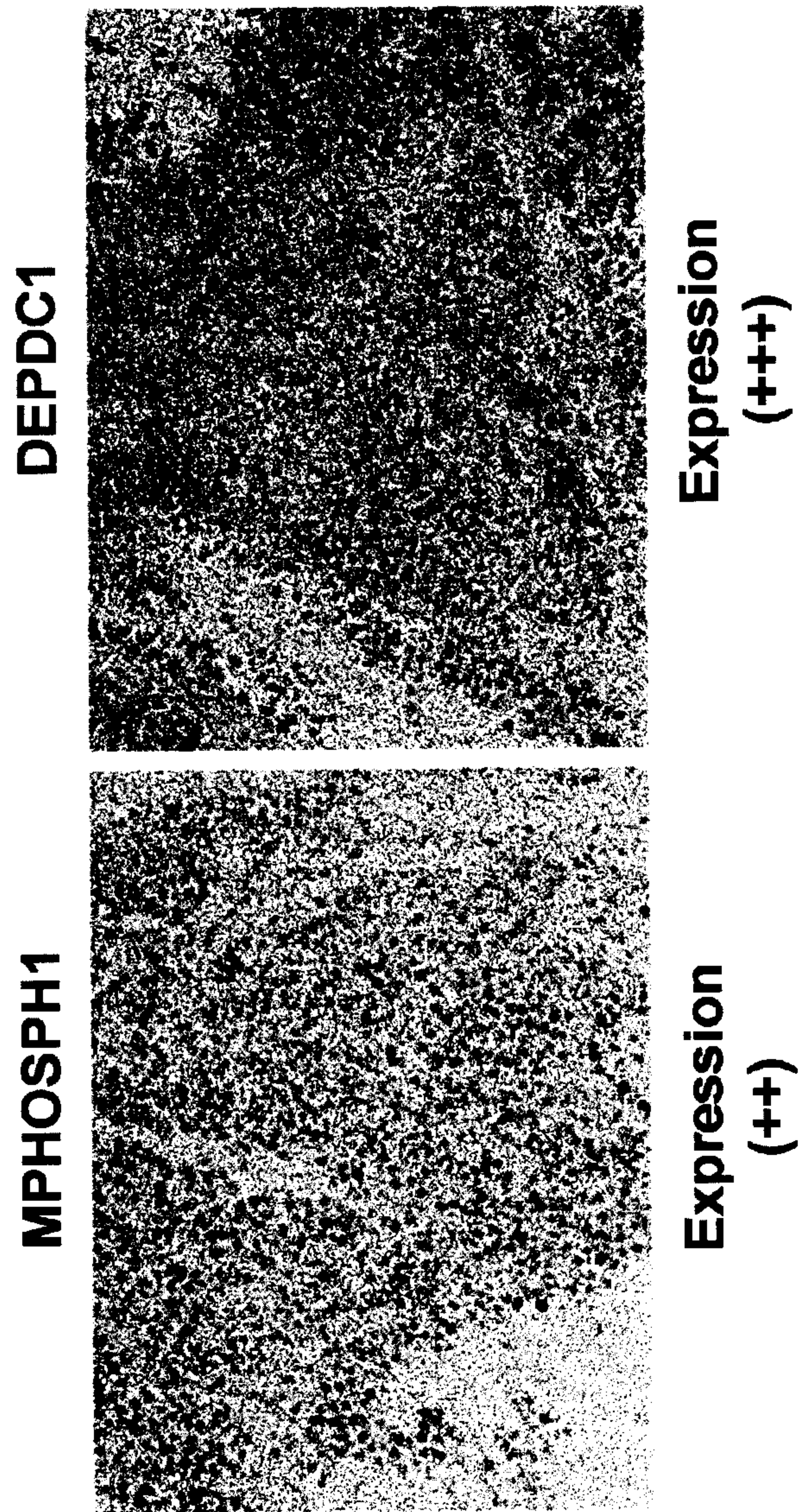
[Fig. 17]



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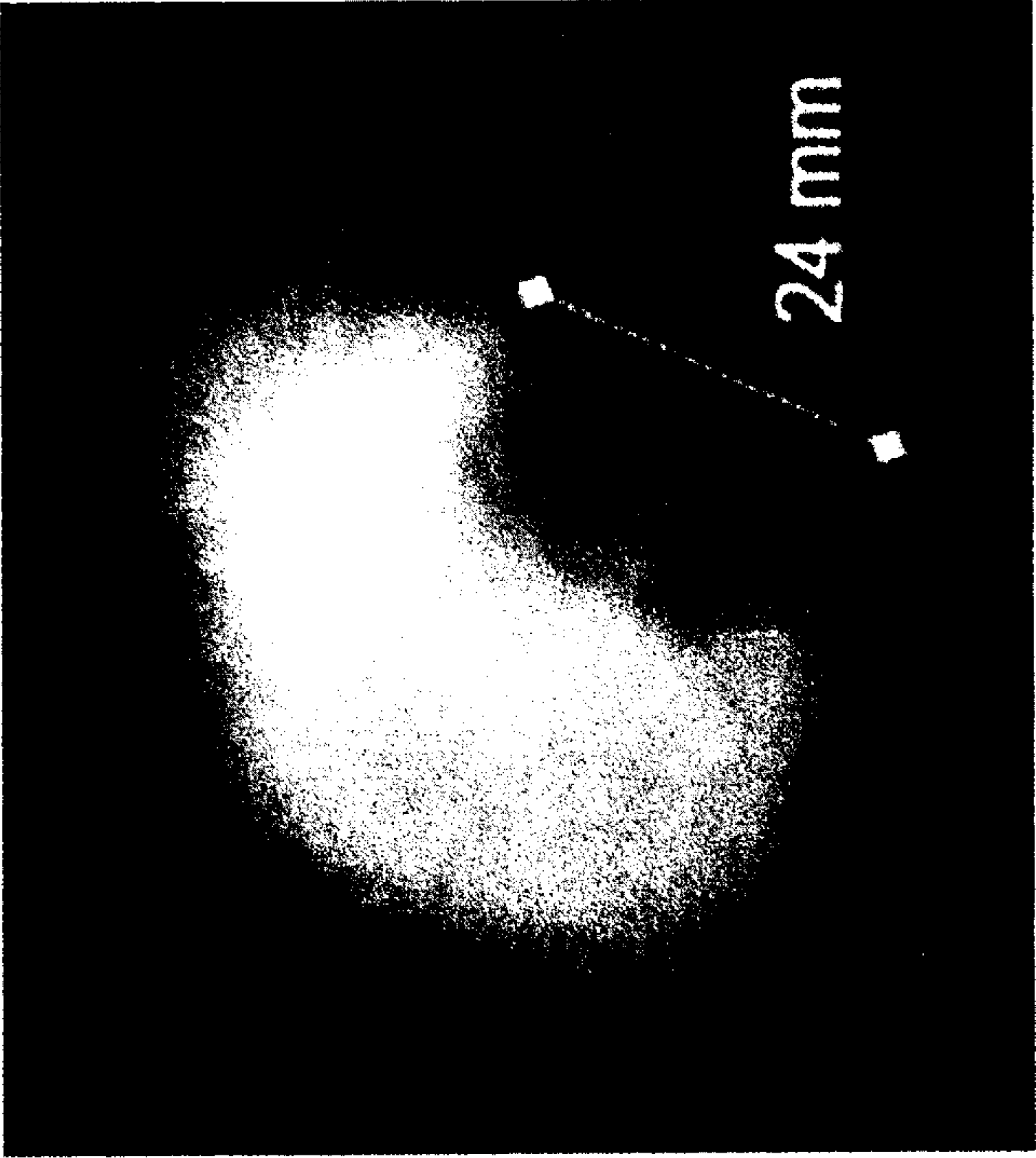
PCT/JP2007/001122

[Fig. 18]



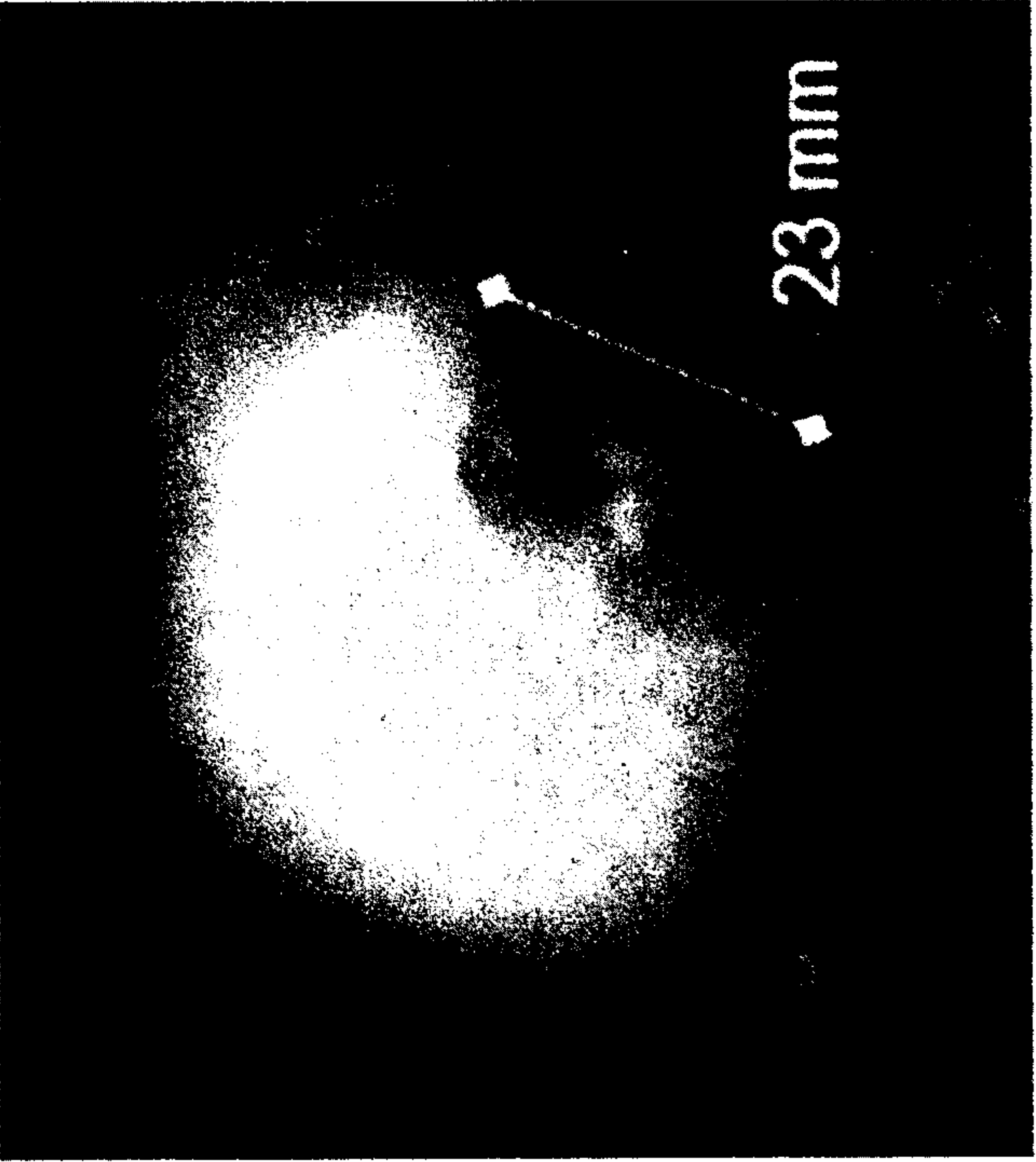
[Fig. 19]

Bladder



Before Vaccination

Bladder

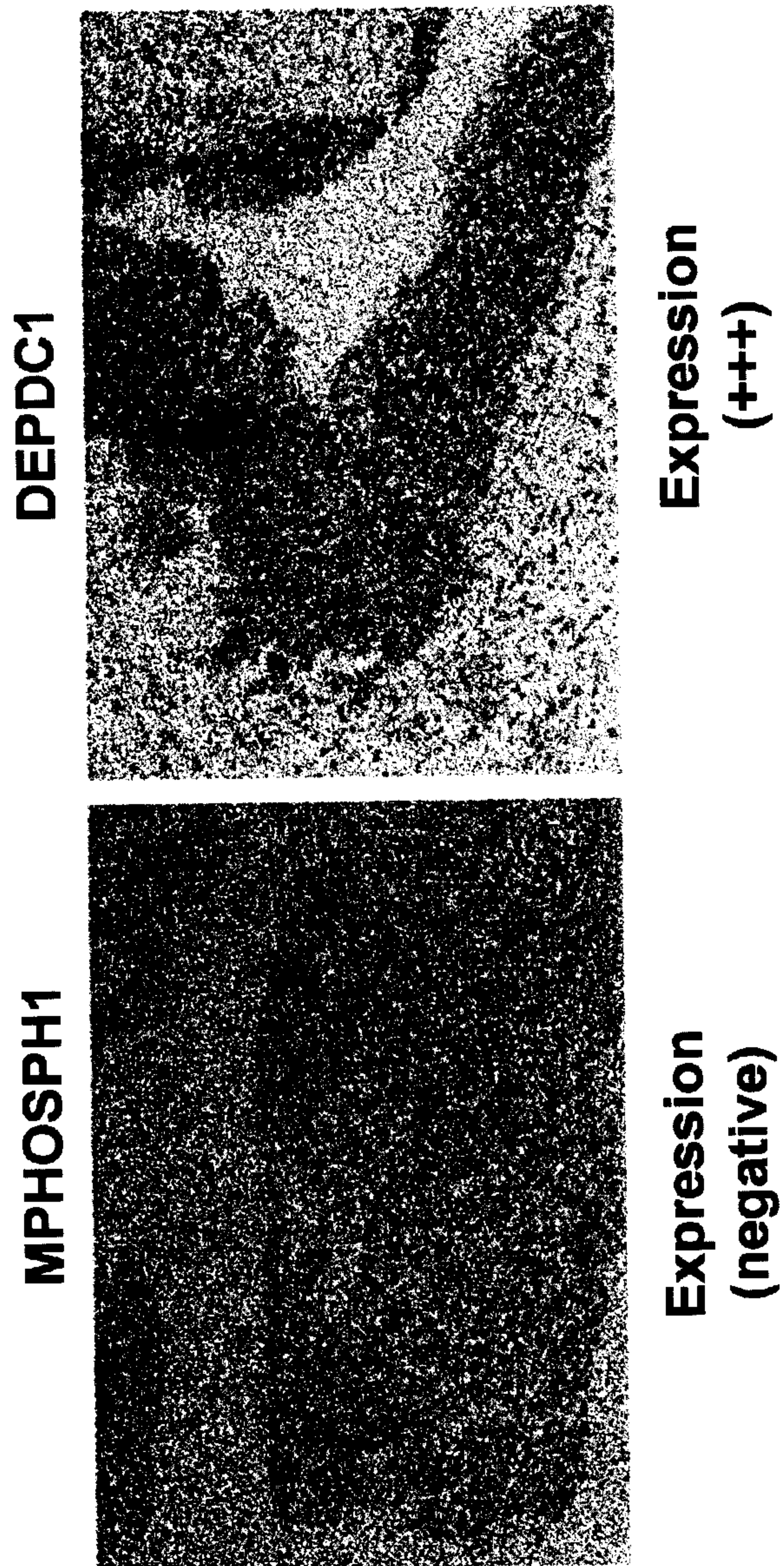


After Vaccination

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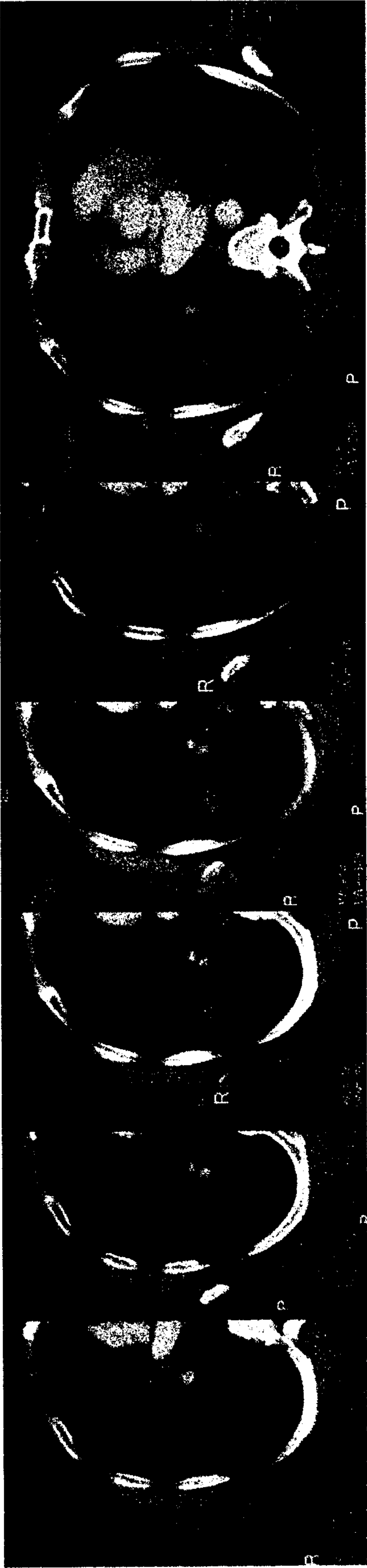
[Fig. 20]



[Fig. 21]



2006.12.12	2007.02.06	2007.05.08	2007.06.04	2007.07.06	2007.08.04
		Before	After 1 co.	After 2 co.	After 3 co.

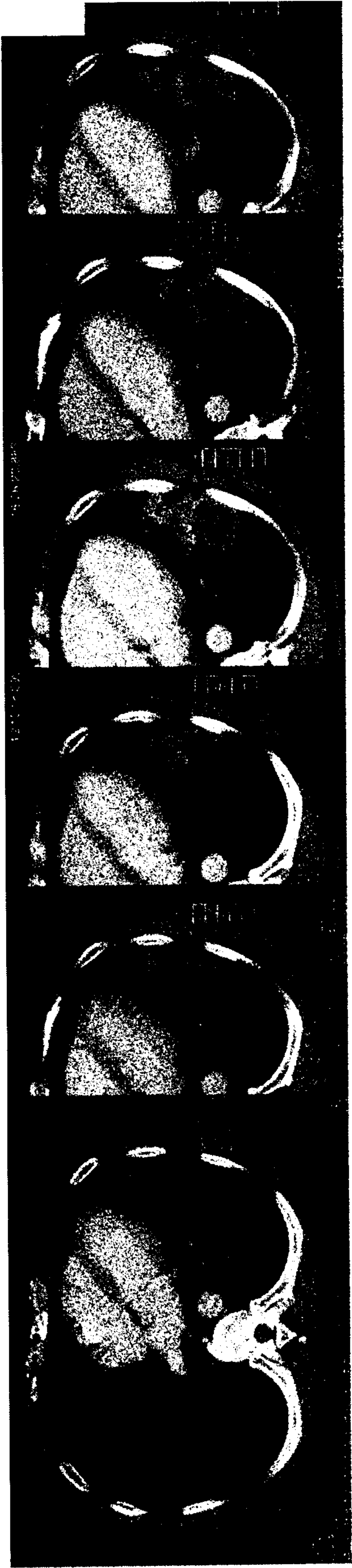


2006.12.12	2007.02.06	2007.05.08	2007.06.04	2007.07.06	2007.08.04
		Before	After 1 co.	After 2 co.	After 3 co.

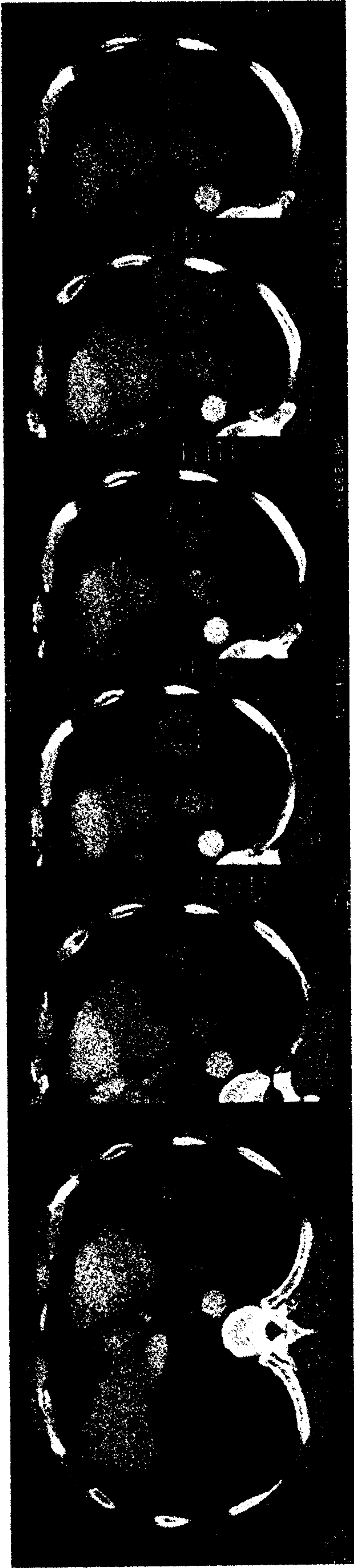
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[Fig. 22]



2006.12.12	2007.02.06	2007.05.08	2007.06.04	2007.07.06	2007.08.04
		Before	After 1 co.	After 2 co.	After 3 co.

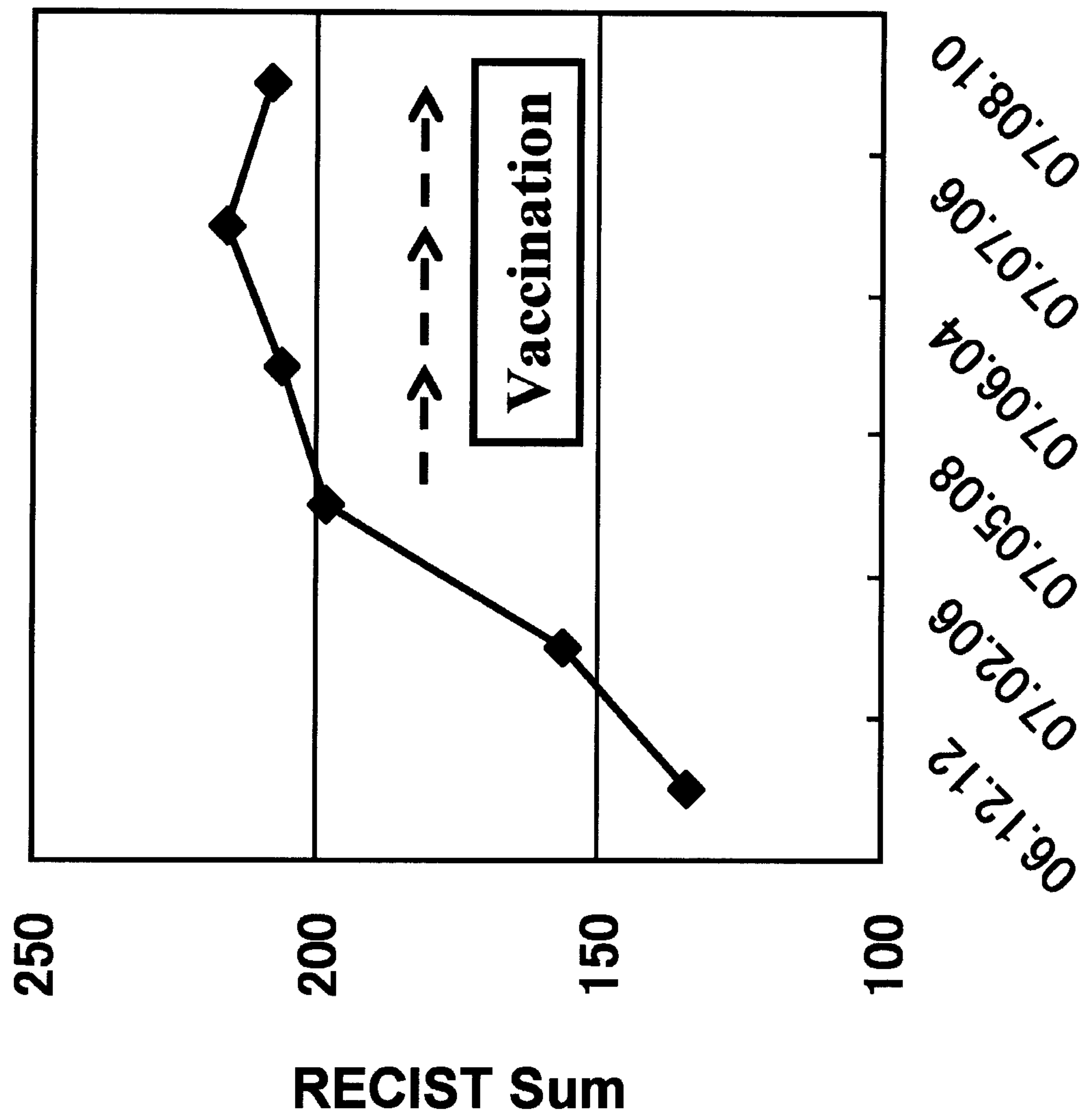


2006.12.12	2007.02.06	2007.05.08	2007.06.04	2007.07.06	2007.08.04
		Before	After 1 co.	After 2 co.	After 3 co.

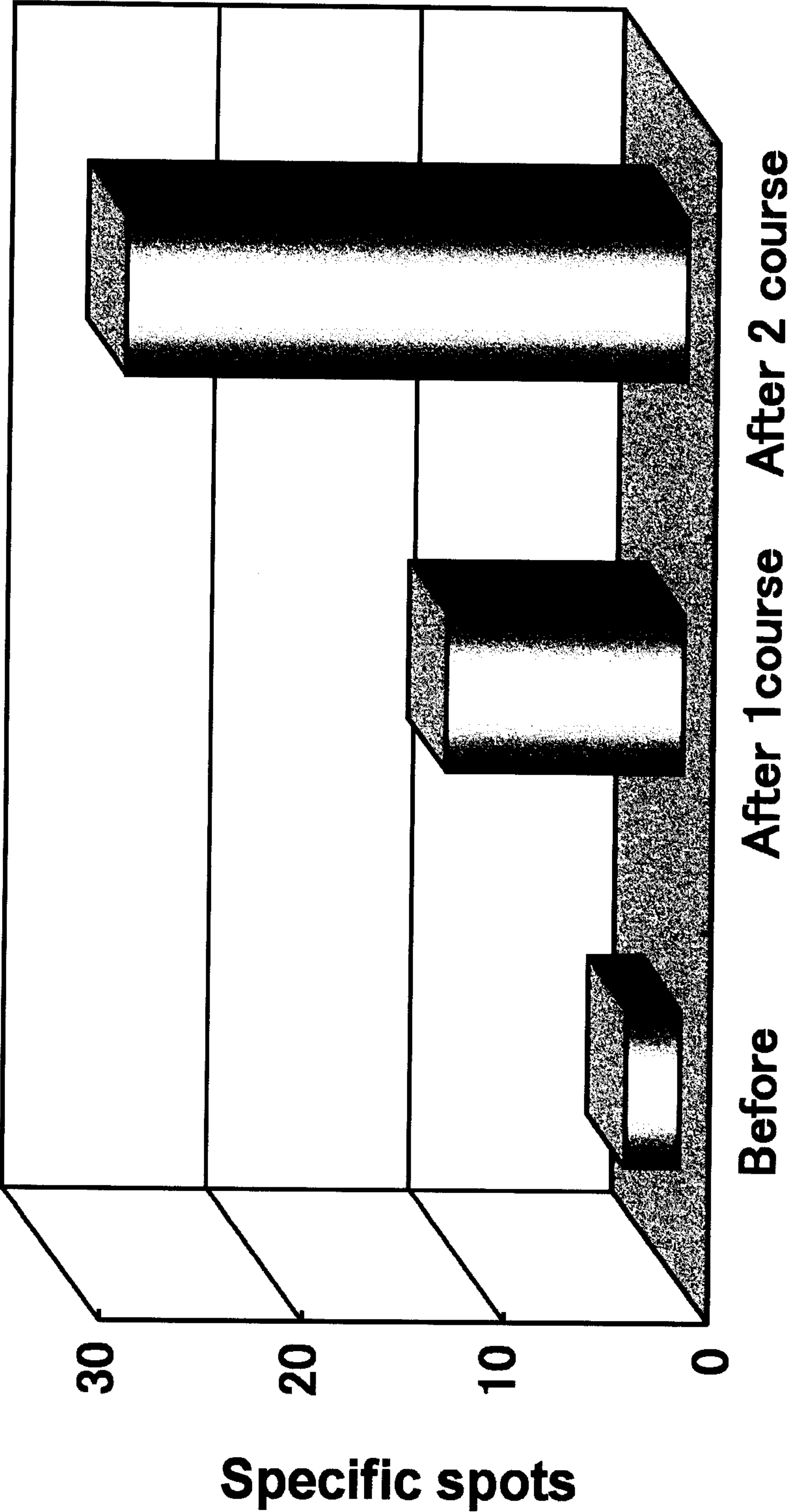
WO 2008/047473

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[Fig. 23]



[Fig. 24]

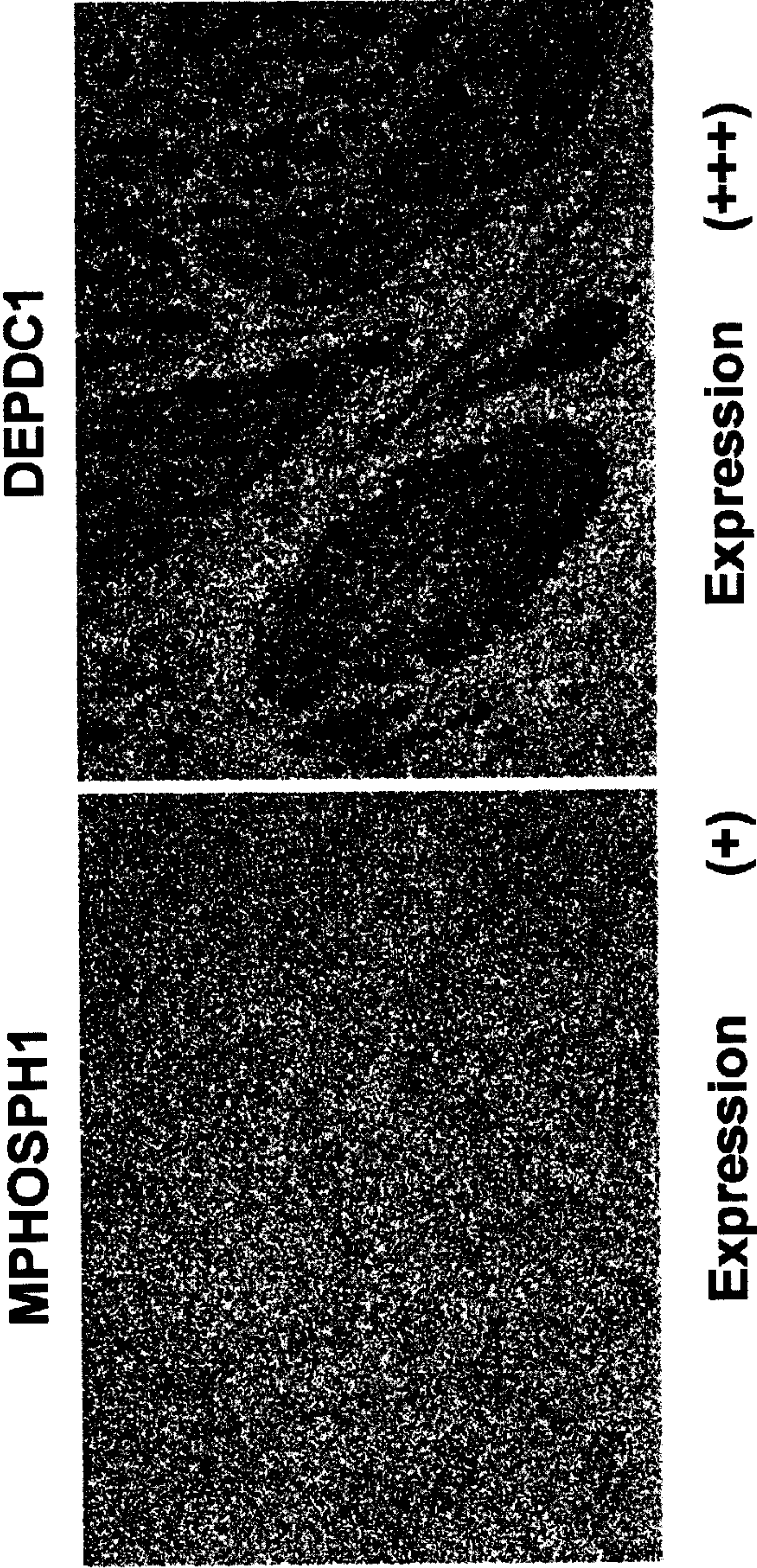


Specific spots = Spots(DEPDC1 pep) – Spots(HIV pep)

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[Fig. 25]



[Fig. 26]

