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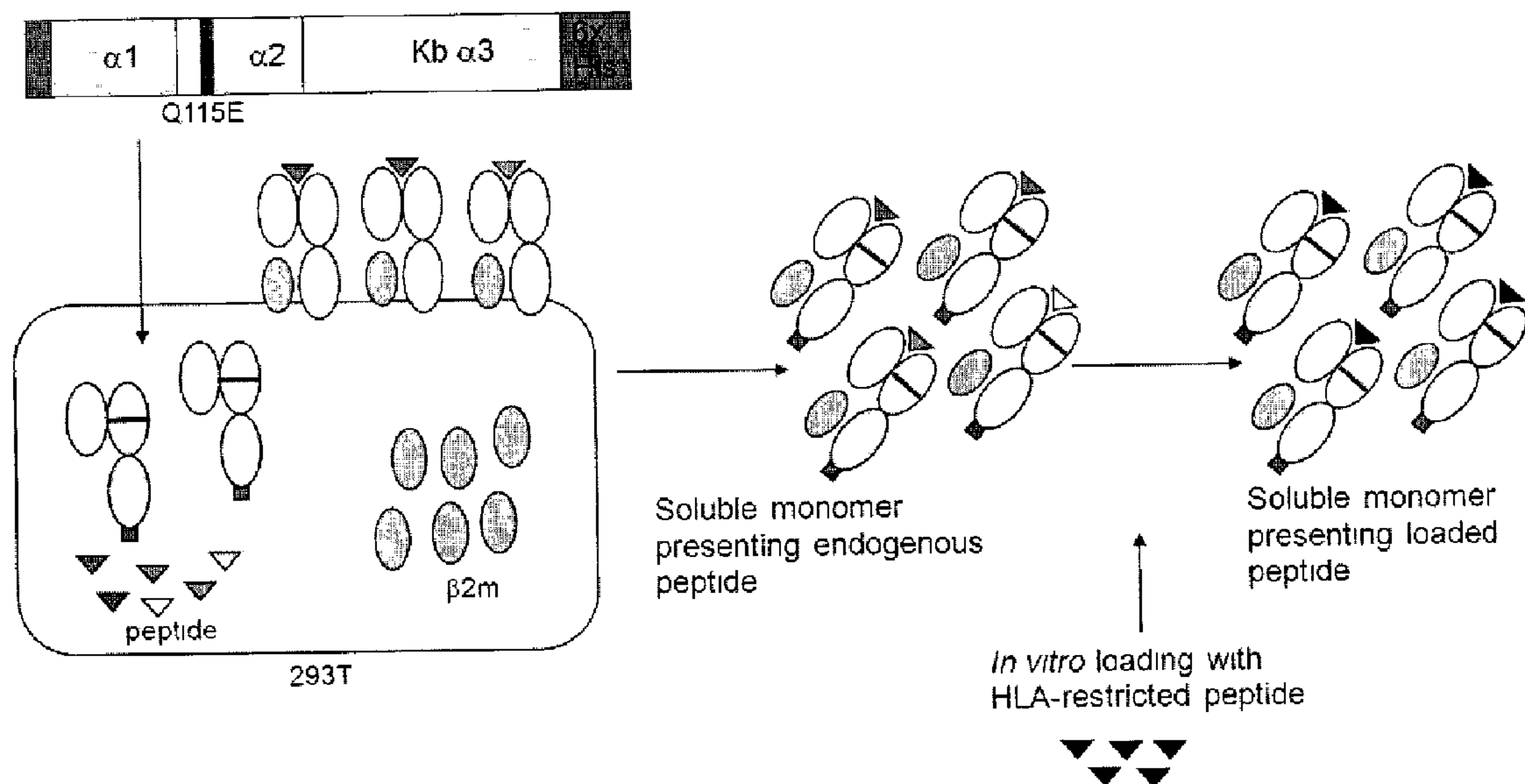


Figure 2

(57) Abrégé/Abstract:

There is provided herein, the use of mammalian derived HLA class I molecule for in vitro peptide exchange. For example, there is provided a method of producing an HLA class I molecule complexed to a pre-selected peptide comprising: (a) providing a mammalian derived HLA class I molecule complexed to an existing peptide; (b) incubating, in vitro, the HLA class I molecule complexed to the existing peptide with the pre-selected peptide, wherein the pre-selected peptide is at a concentration sufficient to replace the existing peptide to produce the HLA class I molecule complexed to the pre-selected peptide; and the HLA class I molecule comprises $\alpha 1$, $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\beta 2m$ domains.

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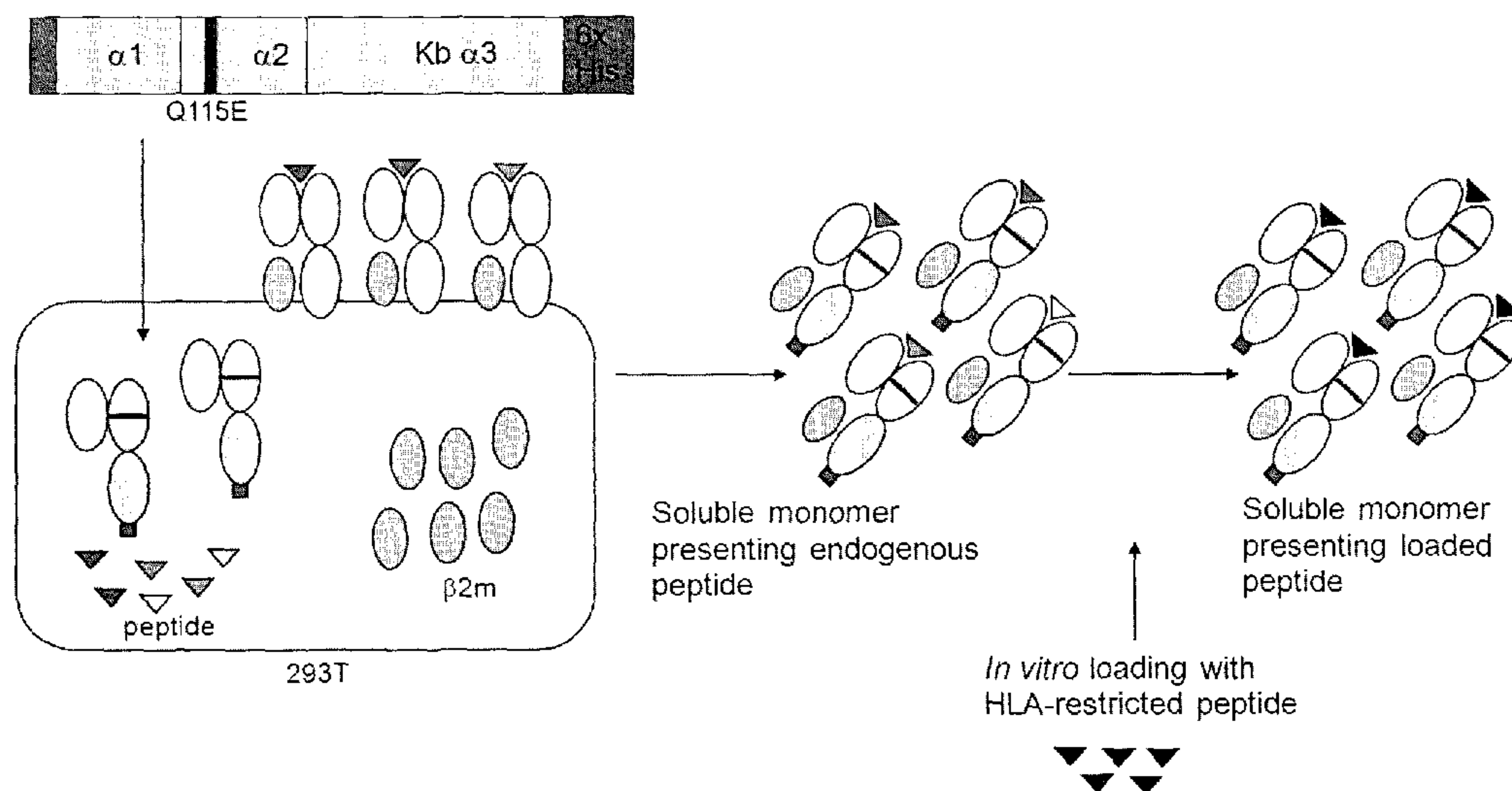


Figure 2

(57) Abstract: There is provided herein, the use of mammalian derived HLA class I molecule for *in vitro* peptide exchange. For example, there is provided a method of producing an HLA class I molecule complexed to a pre-selected peptide comprising: (a) providing a mammalian derived HLA class I molecule complexed to an existing peptide; (b) incubating, *in vitro*, the HLA class I molecule complexed to the existing peptide with the pre-selected peptide, wherein the pre-selected peptide is at a concentration sufficient to replace the existing peptide to produce the HLA class I molecule complexed to the pre-selected peptide; and the HLA class I molecule comprises $\alpha 1$, $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\beta 2m$ domains.

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PEPTIDE-HLA COMPLEXES AND METHODS OF PRODUCING SAME

FIELD OF THE INVENTION

The invention relates to the field of human leukocyte antigen (HLA) class I molecules
5 and, more specifically, to methods of producing HLA class I molecules complexed with
a peptide.

BACKGROUND OF THE INVENTION

Analysis of antigen-specific T-cells using flow cytometry with peptide-MHC (pMHC)
10 multimers has been established as a standard technique in immunology^{1,2}. These
reagents enable the tracking and phenotypic analysis of antigen-specific T cells during
immune responses associated with infection, autoimmunity, GVHD, and cancer.

$\alpha\beta$ T-cell antigen receptors (TCRs) in T cells recognize peptide antigens presented by
MHC class I or II molecules on the cell surface^{3,4}. The interaction between TCR and
15 pMHC is so weak that monomeric soluble pMHC in general cannot stably associate
with the cell surface of T cells bearing a cognate TCR. pMHC multimers in the form of
avidin-biotin-based pMHC tetramers were first introduced by Mark Davis' group in
1996 and immediately transformed the analysis of antigen-specific T cells⁵. pMHC
multimers have been used in numerous studies and several commercial vendors, such
20 as BD BioSciencesTM, ProImmuneTM, ImmudexTM, and TC matrixTM, sell pMHC
multimers in various forms. pMHC multimers can be used in association with a
combination of antibodies specific for other cell surface molecules⁶. Accordingly,
simultaneous staining of TCR and immunoaccessory molecules allows the
classification of antigen-specific T cells into various phenotypically distinct subsets.
25 Such phenotyping can be used to characterize antigen-specific T cells in terms of their
antigen exposure, effector function, and status.

Escherichia coli expression is the preferred method for production of MHC class I
proteins and can provide large quantities of highly purified protein
(<http://tetramer.yerkes.emory.edu/support/protocols>). Unlike class II molecules, most
30 class I molecules are unstable as empty without peptide in the groove⁷. Therefore, in
virtually all cases, MHC class I molecules are loaded with synthetic peptide of interest,

where the class I expression process is coupled to a peptide-loading process to produce complete pMHC complex. There are some known issues with the bacterial system. For some HLA class I genes, such as HLA-B alleles, pHLA production using bacteria is difficult partly because of poor refolding^{8,9}. Although glycosylation on class I protein is not necessary for the interaction between pMHC and cognate TCR, lack of sugar moieties on bacterially expressed MHC class I proteins may have a negative impact on their stability. Furthermore, bacterially expressed and *in vitro* refolded pMHC proteins may not have exactly the same higher structure as those produced in mammals and refolded *in vivo*. Although *in vitro* peptide exchange of generated complete pMHC proteins is possible, it requires multiple complicated steps¹⁰⁻¹². Therefore, high-throughput production of pMHC proteins is labor-intensive, cumbersome, and not widely available. Finally, it has been shown that the pMHC – TCR affinity required for pMHC multimer binding exceeds that required for T cell activation¹³. The observed difference in affinity threshold means that current pMHC tetramer staining cannot detect all antigen-specific T cells, especially with those with low affinity. Failure to stain all cognate T cells expressing TCR with a broad range of affinity is likely to be a serious issue when pMHC multimers are used to stain self antigen-specific T associated with immune responses in autoimmunity and cancer, which tend to express lower affinity TCRs.

20

SUMMARY OF INVENTION

According to one aspect, there is provided a method of producing an HLA class I molecule complexed to a pre-selected peptide by providing a mammalian derived HLA class I molecule complexed first to an existing peptide. The HLA class I molecule complexed to the existing peptide is then incubated, *in vitro*, with the pre-selected peptide at a concentration sufficient to replace the existing peptide, thereby producing the HLA class I molecule complexed to the pre-selected peptide. The HLA class I molecule comprises $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\beta 2m$ domains.

According to a further aspect, there is provided a kit for producing an HLA class I molecule complexed to a pre-selected peptide, comprising a mammalian derived HLA class I molecule complexed to an existing peptide and instructions corresponding to the method described above. In some embodiments, the kit further comprises the pre-selected peptide.

30

According to a further aspect, there is provided a polypeptide comprising the $\alpha 1$, $\alpha 2$ and $\alpha 3$ domain of an HLA class I molecule, a signal peptide at the N terminus and a 6xHis tag joined by a GS linker at the C terminus.

5 According to a further aspect, there is provided a nucleic acid encoding the polypeptide described above.

According to a further aspect, there is provided a vector comprising the nucleic acid described above.

According to a further aspect, there is provided a mammalian cell transfected with the vector described above.

10 According to a further aspect, there is provided a compound comprising the polypeptide described above complexed with a $\beta 2m$ domain.

According to a further aspect, there is provided a multimer of at least two of the compounds described above.

15 In an aspect, the method of screening/selecting in a population of T-cells for antigen specific T-cells that recognize pre-selected peptide antigens, the method comprising: providing a mammalian-derived HLA class I molecule complexed to the pre-selected peptides; screening the population of T-cells for antigen specific T-cells that bind the mammalian-derived HLA class I molecule complexed to the pre-selected peptides.

20 BRIEF DESCRIPTION OF FIGURES

Embodiments of the invention may best be understood by referring to the following description and accompanying drawings. In the drawings:

Figure 1 shows a schematic representation of the general structure of an HLA class I molecule complexed with a peptide.

25 Figure 2 shows production of monomeric pHLA presenting peptide of interest by *in vitro* peptide exchange.

Figure 3 shows a timeline chart of harvest, peptide loading, and dimerization of pHLA to stain antigen-specific T cells.

Figure 4 shows staining data showing peptide exchange occurring in a supernatant sample.

Figure 5 shows staining data showing A2/MART1 monomer staining high avidity A2/MART1 T cells but not A2/NY-ESO-1 T cells.

- 5 Figure 6 shows staining data showing A2/NY-ESO-1 monomer staining high avidity A2/NY-ESO-1 T cells but not A2/MART1 T cells.

Figure 7 shows staining data showing A2/MART1 dimer staining A2/MART1 T cells but not A2/NY-ESO-1 T cells.

- 10 Figure 8 shows staining data showing A2/NY-ESO-1 dimer staining A2/NY-ESO-1 T cells but not A2/MART1 T cells.

Figure 9 shows staining data showing a dimer embodiment staining low affinity A2/MART1 TCRs better than a known Pentamer.

Figure 10 shows staining data showing A24Q115E-Kb dimer staining low affinity A24/WT1 TCRs better than a prior art A24/WT1 tetramer.

- 15 Figure 11 shows staining data showing B35Q115E-Kb dimer stains B*35:01/EBNA-1₄₀₇₋₄₁₇ TCR (Clone TK3).

Figure 12 shows staining data showing high throughput A2 dimer staining of TILs.

Figure 13 shows B*44:05 dimer stains B*44:05/EBNA-6₂₈₁₋₂₉₀ TCR.

- 20 Figure 14 shows C*07:02/MAGE-A1₂₈₉₋₂₉₇ and C*07:02/MAGE-A12₁₇₀₋₁₇₈ dimers stain respective TCRs.

Figure 15 shows A2* melanoma TILs.

Figure 16 shows high throughput A2 dimer staining of TILs (TIL : M25 TIL16 REP1 2E7 2016-9-15).

- 25 Figure 17 shows high throughput A2 dimer staining of TILs (TIL : M31 TIL3 REP1A 2E7 2015-06-03).

Figure 18 shows high throughput A2 dimer staining of TILs (TIL : M37 TIL3 REP1B 2E7 2015-06-03).

Figure 19 shows high throughput A2 dimer staining of TILs (TIL : M40 TIL3 REP1A 2E7 2015-06-04).

Figure 20 shows high throughput A2 dimer staining of TILs (TIL : M66 YT REP1A D14 2E7 2012-02-01).

- 5 Figure 21 shows high throughput A2 dimer staining of TILs (TIL : M96 YT REP1A 2E7 2015-06-04).

Figure 22 shows IFN- γ ELISPOT assay (TIL : M25 TIL16 REP1 2E7 2016-9-15).

Figure 23 shows IFN- γ ELISPOT assay (TIL : M31 TIL3 REP1A 2E7 2015-06-03).

Figure 24 shows IFN- γ ELISPOT assay (TIL : M37 TIL3 REP1B 2E7 2015-06-03).

- 10 Figure 25 shows IFN- γ ELISPOT assay (TIL : M40 TIL3 REP1A 2E7 2015-06-04).

Figure 26 shows IFN- γ ELISPOT assay (TIL : M66 YT REP1A D14 2E7 2012-02-01).

Figure 27 shows IFN- γ ELISPOT assay (TIL : M96 YT REP1A 2E7 2015-06-04).

Figure 28 shows an A2 dimer staining summary.

Figure 29 shows Enrichment of dimer positive TILs.

15

DETAILED DESCRIPTION

- We have developed a novel technology which enables high throughput production of mammalian-derived peptide/HLA class I (pHLA) multimers that can stain low affinity TCRs. One example application of this technology is the generation of personalized
20 pHLA reagents which enables high-throughput measurement of antitumor T cell responses in cancer patients.

- According to one aspect, there is provided a method of producing an HLA class I molecule complexed to a pre-selected peptide by providing a mammalian derived HLA class I molecule complexed first to an existing peptide. The HLA class I molecule
25 complexed to the existing peptide is then incubated, *in vitro*, with the pre-selected peptide at a concentration sufficient to replace the existing peptide, thereby producing the HLA class I molecule complexed to the pre-selected peptide. The HLA class I

molecule comprises $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\beta 2m$ domains. In some embodiments, the HLA class I molecule is soluble.

Human Leukocyte Antigen

The HLA system is a gene complex encoding the major histocompatibility complex (MHC) proteins in humans. These cell-surface proteins are responsible for the regulation of the immune system in humans. HLA genes are highly polymorphic, and different classes have different functions. HLA class I genes encoding MHC class I molecules function to display or present peptide fragments of non-self or self proteins from within the cell to cytotoxic T cells.

As used herein, the expression "HLA class I molecule" refers to a protein molecule derived from the expression of wild type or variant HLA class I genes encoding MHC class I molecules. A schematic representation of the general structure of an HLA class I molecule, including its $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\beta 2m$ domains, is depicted in Figure 1.

The schematic representation also illustrates a peptide complexed to the HLA class I molecule. As used herein, the expression "peptide" refers to peptide fragments that are capable of complexing with the HLA class I molecule and are displayed or presented by the HLA class I molecule. Such peptides have been well described in the art. In general, these particular peptides are about 8-15 amino acids in length but can also vary from between 8-10, 7-11, or 6-12 amino acids in length.

For some HLA class I genes, pHLA production using bacteria is difficult partly because of poor refolding. Furthermore, bacterially expressed and *in vitro* refolded pMHC proteins may not have exactly the same higher structure as those produced in mammals and refolded *in vivo*. As used herein, the expression "mammalian derived" refers to production of molecules utilizing mammalian cell systems which are well known in the art, such as human cell lines (for example, HeLa, HEK293, HEK293T and their derivatives), monkey cell lines (for example, CV-1, COS and their derivatives), mouse cell line (for example, NIH3T3 and their derivatives, NS-1 and their derivatives), hamster cell lines (for example, BHK, CHO and their derivatives). In one embodiment, human cell lines are used. In one example, HEK 293T cell lines can be used. The HLA class I molecule complexed to the existing peptide is produced by a mammalian cell transfected with a soluble HLA class I molecule, wherein the $\beta 2m$ domain may be endogenous or exogenous. In preferred embodiments, the $\beta 2m$ domain is exogenous and encoded on a second vector.

In some embodiments, the soluble HLA class I molecule comprises a signal peptide directing secretion of the HLA class I molecule outside of the mammalian cell. In other embodiments, the soluble HLA class I molecule complexed to the existing peptide is provided in the supernatant of a culture of the mammalian cells.

5 HLA Class I Genes

The HLA class I genes is a family of genes. The HLA class I molecule can be HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G.

As used herein, "HLA-A" refers to a protein molecule derived from the expression of an HLA-A gene. "HLA-B" refers to a protein molecule derived from the expression of an HLA-B gene. "HLA-C" refers to a protein molecule derived from the expression of an HLA-C gene. "HLA-D" refers to a protein molecule derived from the expression of an HLA-D gene. "HLA-E" refers to a protein molecule derived from the expression of an HLA-E gene. "HLA-F" refers to a protein molecule derived from the expression of an HLA-F gene. "HLA-G" refers to a protein molecule derived from the expression of an HLA-G gene. All of the genes HLA-A to HLA-G are part of the HLA class I family of genes.

Amino Acid Sequences of the HLA Class I Molecule

The HLA class I molecule may have a number of amino acid sequence variants.

In some embodiments, the $\alpha 3$ domain of the HLA class I molecule is the mouse Kb $\alpha 3$ domain (designated Kb)). In other embodiments, in the $\alpha 2$ domain of the HLA class I molecule, Gln has been replaced with Glu at position 115 (designated Q115E).

Exemplary HLA class I molecules include but are not limited to the following.

The HLA class I molecule may be HLA-A and comprises the $\alpha 1$, $\alpha 2$ and $\alpha 3$ domains of any of SEQ ID NOs. 6 or 12. In other embodiments, the HLA-A $\alpha 1$, $\alpha 2$ and $\alpha 3$ domains may be wildtype as in SEQ ID NOs. 2 or 14 respectively. Additionally, in yet other embodiments, the $\alpha 1$ and $\alpha 2$ domains are wildtype and the $\alpha 3$ domain of the HLA class I molecule is the mouse Kb $\alpha 3$ domain as in SEQ ID NOs. 4 or 10 respectively. Any combination of the foregoing is also possible.

The HLA class I molecule can be HLA-B and comprises the $\alpha 1$, $\alpha 2$ and $\alpha 3$ domains of any of SEQ ID NOs. 14, 16, 18, 20, or 22. As with exemplary HLA-A molecules, the

$\alpha 1$, $\alpha 2$ and $\alpha 3$ domains may be wildtype, or be select variants, such as Kb and Q115E, or any combinations thereof.

The HLA class I molecule can be HLA-C and comprises the $\alpha 1$, $\alpha 2$ and $\alpha 3$ domains of any of SEQ ID NOs. 24, 26, 28, or 30. As with exemplary HLA-A molecules, the $\alpha 1$, $\alpha 2$ and $\alpha 3$ domains may be wildtype, or be select variants, such as Kb and Q115E, or any combinations thereof.

In yet other embodiments, the HLA class I molecule comprises the $\alpha 1$, $\alpha 2$ and $\alpha 3$ domains described herein with a $\beta 2m$ domain.

10 Multimers

The HLA class I molecule may also be multimerized. According to a further aspect, the method described above further comprises multimerizing the HLA class I molecules, preferably into one of dimers, trimers, tetramers and pentamers.

In some embodiments, the HLA class I molecules are dimerized using an antibody that recognizes a corresponding tag on HLA class I molecule. In further embodiments, the tag is a 6xHis tag at the C' end of the $\alpha 3$ domain, preferably connected by a flexible linker, more preferably a GS linker. Other suitable tags for antibody binding are known in the art. Examples of acceptable tags are numerous and include AviTag, Calmodulin-tag, polyglutamate tag, His-tag, Myc-tag, and VSV-tag. Examples of acceptable flexible linkers are numerous; see for example Chen et al, Adv Drug Deliv Rev. 2013 Oct 15; 65(10): 1357-1369.

Kits and Reagents

According to a further aspect, there is provided a kit for producing an HLA class I molecule complexed to a pre-selected peptide, comprising a mammalian derived HLA class I molecule complexed to an existing peptide and instructions corresponding to the method described above. In some embodiments, the kit further comprises the pre-selected peptide.

According to a further aspect, there is provided a polypeptide comprising the $\alpha 1$, $\alpha 2$ and $\alpha 3$ domain of an HLA class I molecule, a signal peptide at the N terminus and a 6xHis tag joined by a GS linker at the C terminus. In some embodiments, the polypeptide is SEQ ID NO. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

According to a further aspect, there is provided a nucleic acid encoding the polypeptide described above.

According to a further aspect, there is provided a vector comprising the nucleic acid described above.

- 5 According to a further aspect, there is provided a mammalian cell transfected with the vector described above. In some embodiments, the mammalian cell further comprises a second vector encoding $\beta 2m$.

According to a further aspect, there is provided a compound comprising the polypeptide described above complexed with a $\beta 2m$ domain.

- 10 According to a further aspect, there is provided a multimer of at least two of the compounds described above. In some embodiments, the at least two compounds are dimerized by an antibody recognizing the 6xHis tag.

The following examples are illustrative of various aspects of the invention, and do not limit the broad aspects of the invention as disclosed herein.

15 T-Cell Screening and Selection, including Tumor-Infiltrating Lymphocytes

- It is known that HLA class I molecules complexed to a pre-selected peptide can be used to screen/select for T-cells that recognize said peptide antigen through its T-cell receptor. Advantageously, the mammalian derived HLA class I molecules described herein allow the skilled person to swap out an existing (or holder) peptide with a pre-selected peptide of interest. This was not possible with existing bacteria-derived HLA class I molecules. Rather, the existing bacteria-derived HLA class I molecules had to be produced, denatured and then re-folded with the peptide antigen of interest.
- 20

- The present mammalian-derived HLA class I molecules therefore represent a streamlined and more flexible procedure to easily produce molecules that can present a peptide antigen. For example, the present mammalian-derived HLA class I molecules can be pre-made, the holder peptide being swapped before use. Further, the present mammalian-derived HLA class I molecules are likely more representative of a natural HLA class I molecules as they do not have to be refolded and are glycosylated.
- 25

- 30 Accordingly, in an aspect, the method of screening/selecting in a population of T-cells for antigen specific T-cells that recognize pre-selected peptide antigens, the method

comprising: providing a mammalian-derived HLA class I molecule complexed to the pre-selected peptides; screening the population of T-cells for antigen specific T-cells that bind the mammalian-derived HLA class I molecule complexed to the pre-selected peptides.

- 5 In some embodiments, the method further comprises first providing a mammalian-derived HLA class I molecule complexed to a holder peptide; incubating, in vitro, the HLA class I molecule complexed to the holder peptide with the pre-selected peptide, wherein the pre-selected peptide is at a concentration sufficient to replace the existing peptide to produce the HLA class I molecule complexed to the pre-selected peptide.
- 10 In some embodiments, the mammalian-derived HLA class I molecule complexed to the pre-selected peptide is prepared using the method of described herein.

In some embodiments, the screening comprises flow cytometry.

- In some embodiments, the HLA class I molecule complexed to the holder peptide comprises any one of SEQ ID NO. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or
15 30, preferably with a β 2m domain.

In some embodiments, the HLA class I molecule complexed to the holder peptide comprises the polypeptide described herein, preferably with a β 2m domain.

- In some embodiments, the method may be used to screen/select for T-Cell populations associated with a cancer. Cancer may include adrenal cancer, anal
20 cancer, bile duct cancer, bladder cancer, bone cancer, brain/cns tumors, breast cancer, castleman disease, cervical cancer, colon/rectum cancer, endometrial cancer, esophagus cancer, ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumor (gist), gestational trophoblastic disease, hodgkin disease, kaposi sarcoma, kidney cancer, laryngeal and
25 hypopharyngeal cancer, leukemia (acute lymphocytic, acute myeloid, chronic lymphocytic, chronic myeloid, chronic myelomonocytic), liver cancer, lung cancer (non-small cell, small cell, lung carcinoid tumor), lymphoma, lymphoma of the skin, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-hodgkin
30 lymphoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma - adult soft tissue cancer, skin

cancer (basal and squamous cell, melanoma, merkel cell), small intestine cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, waldenstrom macroglobulinemia, or wilms tumor.

In some embodiments, the antigen specific T-cells that recognize pre-selected peptide
5 antigens are tumor-infiltrating lymphocytes.

In some embodiments, the pre-selected peptide antigens are associated with cancer.

EXAMPLES

Materials and Methods

10 *Peptides*

Synthetic peptides were purchased from Prolimmune, Genway Biotech, and GenScript. Peptides used were A2-restricted heteroclitic MART1₂₆₋₃₅ (ELAGIGILTV), heteroclitic NY-ESO-1₁₅₇₋₁₆₅ (SLLMWITQV), A24-restricted heteroclitic WT1₂₃₅₋₂₄₃ (CYTWNQMNL), B35-restricted wild-type EBNA-1₄₀₇₋₄₁₇ (HPVG EADYFEY) peptides, B44-restricted wild-
15 type EBNA-6₂₈₁₋₂₉₀ (EENLLDFVRF), C7-restricted wild-type MAGE-A1₂₈₉₋₂₉₇ (RVRFFFPSL), and C7-restricted wild-type MAGE-A12₁₇₀₋₁₇₈ (VRIGHLYIL) peptides. A2 peptides used to stain TILs are listed in Table 1 below.

Table 1: A2 peptides tested

	Name	Sequence	SEQ ID NO.
1	WT1 (37-)	VLDFAPPGA	35
2	WT1 (126-)	RMFPNAPYL	36
3	WT1 (87-)	SLGEQQYSV	37
4	WT1 (235-)	CMTWNQMNL	38
5	MIA (54-)	YMAPDCRFL	39
6	MIA (99-)	RLGYFPSSI	40
7	ALX1 (142-)	LQLEELEKV	41
8	ALX1 (170-)	ELTEARVQV	42
9	GAPDHS (358-)	FLGDTHSSI	43
10	GAPDH2 (345-)	ILAYTEDEV	44
11	S100B (44-)	FLEEIKEQEV	45

12	S100B (74-)	FMAFVAMVT	46
13	ABCB5 (1078-)	LLDEATSAL	47
14	ABCB5 (700-)	VLNGTVHPV	48
15	EXTL1 (249-)	VLLSPRWEL	49
16	EXTL1 (13-)	FLWDAYFSS	50
17	EXTL1 (330-)	WLALSASWL	51
18	CPN1 (379-)	LLLPGIYTV	52
19	CPN1 (249-)	KLFQKLAKV	53
20	CPN1 (297-)	YLHTNCFEI	54
21	TSPAN10 (81-)	FLSNFPFSL	55
22	TSPAN10 (94-)	ALAIGLWGL	56
23	TSPAN10 (142-)	ALCENTCLL	57
24	GJB1 (155-)	LLYPGYAMV	58
25	GJB1 (5-)	GLYTLLSGV	59
26	GJB1 (147-)	AVFMYVFYL	60
27	MITF (378-)	LMDDTLSPV	61
28	MITF (142-)	LQMANTLPV	62
29	MITF (392-)	LLSSVSPGA	63
30	DUSP4 (362-)	SQFVFSFPV	64
31	DUSP4 (326-)	QLLQFESQV	65
32	DUSP4 (53-)	FLAHSAGYI	66
33	cyclin-A1 (227-)	FLDRFLSCM	67
34	cyclin-A1 (341-)	SLIAAAAFCLA	68
35	HERV-K-MEL (1-)	MLAVISCAV	69
36	LAGE-1 (1-)	MLMAQEALAFI	70
37	MAGE-A1 (278-)	KVLEYVIKV	71
38	MAGE-A2 (157-)	YLQLVFGIEV	72
39	MAGE-A3 (271-)	FLWGPRALV	73
40	MAGE-A3 (112-)	KVAELVHFL	74
41	MAGE-A4 (230-)	GVYDGREHTV	75
42	MAGE-A9 (223-)	ALSVMGVYV	76
43	MAGE-A10 (254-)	GLYDGMEHL	77
44	MAGE-A12 (271-)	FLWGPRALV	78

45	MAGE-C1 (959-)	ILFGISLREV	79
46	MAGE-C1 (1083-)	KVVEFLAML	80
47	LAGE-2 (1-)	MLMAQEALAF	81
48	SSX-2 (41-)	KASEKIFYV	82
49	XAGE-1b (21-)	RQKKIRIQL	83
50	CEA (691-)	IMIGVLVGV	84
51	gp100 (154-)	KTWGQYWQV	85
52	gp100 (177-)	AMLGTHTMEV	86
53	gp100 (178-)	MLGTHTMEV	87
54	gp100 (209-)	ITDQVPFSV	88
55	gp100 (280-)	YLEPGPVTA	89
56	gp100 (457-)	LLDGTATLRL	90
57	gp100 (476-)	VLYRYGSFSV	91
58	gp100 (570-)	SLADTNSLAV	92
59	gp100 (619-)	RLMKQDFSV	93
60	gp100 (639-)	RLPRIFCSC	94
61	NY-BR-1 (904-)	SLSKILDTV	95
62	TRP-2 (180-)	SVYDFFVWL	96
63	TRP-2 (360-)	TLDSQVMSL	97
64	tyrosinase (1-)	MLLAVLYCL	98
65	tyrosinase (8-)	CLLWSFQTSA	99
66	tyrosinase (369-)	YMDGTMSQV	100
67	CD274 (15-)	LLNAFTVTV	101
68	CPSF (250-)	KVHPVIWSL	102
69	CPSF (1360-)	LMLQNALTTM	103
70	cyclin D1 (101-)	LLGATCMFV	104
71	IDO1 (199-)	ALLEIASCL	105
72	mdm-2 (53-)	VLFYLGQY	106
73	p53 (264-)	LLGRNSFEV	107
74	p53 (65-)	RMPEAAPPV	108
75	PRAME (100-)	VLDGLDVLL	109
76	PRAME (142-)	SLYSFPEPEA	110
77	PRAME (300-)	ALYVDSLFFL	111

78	PRAME (425-)	SLLQHLIGL	112
79	SOX10 (332-)	AWISKPPGV	113
80	SOX10 (331-)	SAWISKPPGV	114
81	survivin (95-)	ELTLGEFLKL	115
82	Telomerase (865-)	RLVDDFLLV	116
83	Wild type MART1 (27-35)	AAGIGILTV	117
84	Heteroclitic MART1 (26-35)	ELAGIGILTV	118
85	Wild type NY-ESO-1 (157-165)	SLLMWITQC	119
86	Heteroclitic NY-ESO-1 (157-165)	SLLMWITQV	120
87	HIV pol (476-484)	ILKEPVHGV	121
88	HTLV-1 tax (11-19)	LLFGYPVYV	122
89	No peptide exchange		

Cells and cDNAs

HEK293T cells were obtained from American Type Culture Collection. TILs isolated from an HLA-A2⁺ patient with metastatic melanoma were grown *in vitro* as reported previously¹⁴. Appropriate informed consent and institutional review board approval were obtained. All clonotypic TCR genes were reconstituted in Jurkat 76/CD8 cells or primary T cells as previously described. cDNAs were fused with puromycin resistance gene via internal ribosome entry site^{15,16}. Transduced cells were isolated by puromycin selection. All cDNAs were cloned into pMX vector and transduced using 293GPG cell-based retrovirus system¹⁶⁻¹⁹.

Flow cytometry analysis

mAbs recognizing the following surface antigens were used: β 2m (551337, BD BioSciences), His (ab72467, Abcam). Mouse isotype controls were from BD BioSciences. Surface molecular staining was carried out as described elsewhere^{16,20}.

Immunoblotting

For immunoblotting, cells were extracted in ice-cold Nonidet P-40 (NP-40) extraction buffer (20 mM Tris-HCl, pH 7.5, containing 1 mM EDTA, 150 mM NaCl, 2.5mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1% NP-40, 1 mM PMSF, and 1 μ g/ml Aprotinin). Cell extracts were centrifuged at 10,000 g for 10 min at 4°C and separated by Tris-Glycine SDS-PAGE followed by electrophoretic transfer to Immobilon-P

membrane (Millipore). After blocking with 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20, the membranes were incubated with the indicated mouse anti-His mAb (sc-53073, Santa Cruz Biotechnology) at 4°C overnight, washed and incubated with HRP-conjugated goat anti-mouse IgG (H+L) secondary antibody
 5 (Promega) at room temperature for 1 hr. The signal was detected by enhanced chemiluminescence (GE Healthcare).

Results and Discussion

Structure of soluble monomeric peptide/HLA class I (pHLA) complexes

10 HLA class I molecules are heterodimers consisting of two polypeptide chains, α and β 2-microglobulin (β 2m), which are non-covalently linked. While the α chain is highly polymorphic, the β 2m subunit is monomorphic. The HLA class I α 1 and α 2 domains constitute a groove for peptides of 8-10 amino acids in length. The α 3 domain, which contains a transmembrane domain, binds β 2m. While TCR on the surface of cytotoxic
 15 T cells recognizes the peptides presented by the HLA class I α 1 and α 2 domains to check antigenicity, the CD8 co-receptor binds the α 2 and α 3 domains to stabilize the interaction between the TCR and pHLA. Therefore, enhancement of the CD8 and HLA class I interaction leads to the improvement in the strength of the interaction between pHLA and cognate TCR.

20 It has been demonstrated that replacement of HLA class I α 3 domain with mouse K^b α 3 domain, named hereafter class I- K^b , enhances the interaction between the class I and CD8 by 10 times. Substitution of the Gln (Q) residue at position 115 of the α 2 domain with a Glu (E) residue, named hereafter class I^{Q115E}, further improves the interaction by 1.5 times^{21,22}. By fusing the extracellular domain of wild-type (wt) HLA
 25 class I with a Gly-Ser (GS) flexible linker followed by a 6xHis tag, we have generated soluble class I-wt. Soluble class I- K^b and class I^{Q115E}- K^b were similarly produced. Nucleotide and amino acid sequences of soluble class I-wt, class I- K^b , and class I^{Q115E}- K^b genes used in this study are listed below.

Production of soluble monomeric pHLA complexes using mammalian cells

30 HEK293T cells were initially transfected with β 2m gene and subsequently with soluble HLA class I- K^b or HLA class I^{Q115E}- K^b gene using the pMX vector and 293GPG cell-based retrovirus system¹⁶⁻¹⁹.

Enhanced β 2m expression by gene transduction

Flow cytometry analysis following β 2m-specific mAb staining demonstrated enhanced β 2m expression in HEK293T cells stably transfected with β 2m gene along with a soluble form of HLA-A2-K^b or A2^{Q115E}-K^b. HLA-A*02:01 (A2) gene, which is one of the most frequent HLA class I alleles, was used as a representative HLA class I gene. The same strategy was applied to generate HEK293T-derived cell lines stably expressing a soluble form of other class I genes.

Cellular expression of soluble monomeric peptide/HLA (pHLA) in HEK293T transfectants

Total cell lysates of HEK293T cells stably expressing soluble HLA-A2-K^b or A2^{Q115E}-K^b gene in conjunction with or without β 2m gene were blotted with anti-His mAb as reported previously²³⁻²⁵. Cellular expression of soluble HLA-A2-K^b and A2^{Q115E}-K^b was demonstrated at the protein level.

Secretion of soluble monomeric pHLA complexes into the supernatant.

Supernatant of HEK293T cells transfected with soluble HLA-A2-K^b or A2^{Q115E}-K^b gene along with or without β 2m gene was harvested and blotted with His-specific mAb. Indicated amounts of bacterially-expressed and 6xHis-tagged HLA-A2/heteroclitic MART1₂₆₋₃₅ monomer (NIH tetramer core facility) were loaded as controls. Ten μ l of each supernatant was loaded per lane without any concentration. Secretion of monomeric HLA-A2-K^b and A2^{Q115E}-K^b into the medium was confirmed.

Monomeric pHLA complexes were secreted only when β 2m was overexpressed.

When HEK293T cells were transduced with soluble HLA-A2-K^b or A2^{Q115E}-K^b gene alone without β 2m gene, secretion of soluble A2-K^b and A2^{Q115E}-K^b into the medium was not detectable. This suggests that the endogenous β 2m expression level was not sufficient to enable the secretion of ectopically expressed soluble A2-K^b and A2^{Q115E}-K^b.

Production of monomeric pHLA loaded with peptide of interest by in vitro peptide exchange.

Soluble HLA-A2-K^b and A2^{Q115E}-K^b-containing supernatant produced by the HEK293T transfectants were simply mixed with the indicated concentration of A2-restricted peptide of interest at room temperature for in vitro peptide exchange (see Figure 2).

Dimerization of monomeric pHLA complexes.

Soluble HLA class I^{Q115E}-K^b monomer in the HEK293T conditioned medium was dimerized using anti-His mAb conjugated with fluorochrome such as phycoerythrin (PE) at at 2:1 molar ratio. Note that the soluble proteins were fused with a 6xHis tag at
 5 the C-terminus.

Overall protocol for production of dimeric pHLA complexes to stain antigen-specific T cells.

Stable HEK293T cell lines ectopically expressing soluble monomeric class I^{Q115E}-K^b and β 2m were established as described above. The stable cell lines were grown until
 10 confluent and medium was changed. After 48 hrs, the conditioned medium was harvested and immediately used or frozen until use. The supernatant was loaded with class I-restricted peptide of interest for 24 hrs at 37°C for *in vitro* peptide exchange. The soluble monomeric class I^{Q115E}-K^b loaded with the peptide was dimerized using fluorochrome-conjugated anti-His mAb for 24 hrs at 4°C (see Figure 3).

15 *Peptide exchange occurs in the supernatant by simple mixing.*

Soluble A2^{Q115E}-K^b monomer was loaded with A2/MART1₂₆₋₃₅ (ELAGIGILTV) or A2/NY-ESO-1₁₅₇₋₁₆₅ (SLLMWITQV) peptide by simple mixing, dimerized with PE-conjugated anti-His mAb, and used to stain human Jurkat 76/CD8 T cells expressing clonotypic cognate TCR (see Figure 4). Jurkat 76/CD8 cells, lacking the endogenous TCR
 20 expression, stably express CD8 α / β genes^{26,27}.

Soluble monomeric A2^{Q115E}-K^b stains high avidity antigen-specific T cells.

Soluble A2^{Q115E}-K^b monomer was loaded with A2/MART1₂₆₋₃₅ or A2/NY-ESO-1₁₅₇₋₁₆₅ peptide by simple mixing and, without dimerization, directly used to stain Jurkat 76/CD8 T cells expressing clonotypic cognate TCR. Jurkat 76/CD8 cells expressing
 25 high but not low affinity TCRs were stained by monomeric soluble A2^{Q115E}-K^b loaded with cognate peptide (see Figures 5 and 6)²⁷.

Soluble dimeric A2^{Q115E}-K^b stains both high and low avidity antigen-specific T cells.

Soluble monomeric A2^{Q115E}-K^b containing supernatant was loaded with A2/MART1₂₆₋₃₅ or A2/NY-ESO-1₁₅₇₋₁₆₅ peptide by simple mixing, dimerized with PE-conjugated anti-His
 30 mAb, and utilized to stain Jurkat 76/CD8 T cells expressing clonotypic cognate TCR

(see Figures 7 and 8). Both high and low affinity TCRs expressed in Jurkat 76/CD8 cells were stained by soluble dimeric A2^{Q115E}-K^b loaded with respective peptide²⁷.

Soluble dimeric class I^{Q115E}-K^b stains low affinity TCRs better than Pentamer (ProImmune) or tetramer (NIH)

- 5 PE-conjugated soluble dimeric A2^{Q115E}-K^b and A24^{Q115E}-K^b were loaded with A2/MART1₂₈₋₃₅ and A24/WT1₂₃₅₋₂₄₃ (CYTWNQMNL) peptides, respectively. The loaded dimers were employed to stain Jurkat 76/CD8 T cells expressing clonotypic cognate TCRs with various affinities^{26,27}. Our dimer stained low affinity TCRs better than Pentamer (ProImmune) and NIH's tetramer (see Figures 9 and 10). Pentamer
10 was used according to the protocol provided by the vendor (<https://www.proimmune.com/ecommerce/page.php?page=protocols>). Tetramer staining was performed according to the standard protocol as published elsewhere^{28,27}.

Soluble dimeric HLA-B^{Q115E}-K^b works as well.

- Soluble monomeric HLA-B35^{Q115E}-K^b was loaded with B35/EBNA-1₄₀₇₋₄₁₇
15 (HPVGEADYFEY) peptide, dimerized with PE-conjugated anti-His mAb, and used to stain Jurkat 76/CD8 T cells expressing clonotypic cognate TCR (see Figure 11).

Soluble monomeric HLA-B44^{Q115E}-K^b was loaded with B44/EBNA-6₂₈₁₋₂₉₀ (EENLLDFVRF), dimerized with PE-conjugated anti-His mAb, and used to stain Jurkat 76/CD8 T cells expressing clonotypic cognate TCR (Figure 13).

- 20 *Soluble dimeric HLA-C^{Q115E}-K^b works as well.*

Soluble monomeric HLA-C7^{Q115E}-K^b was loaded with C7/MAGE-A1₂₈₉₋₂₉₇ (RVRFFFPSTL) peptide and C7/MAGE-A12₁₇₀₋₁₇₈ (VRIGHLYIL) peptide, dimerized with PE-conjugated anti-His mAb, and used to stain Jurkat 76/CD8 T cells expressing clonotypic cognate TCR (Figure 14).

- 25 *Staining of in vitro expanded tumor-infiltrating lymphocytes with a panel of soluble A2 dimers.*

- Peripheral T cells do not always reflect the immune response to the tumor taking place in cancer patients and antitumor cellular immunity in the periphery does not often correlate with prognosis. In contrast, tumor infiltrating lymphocytes (TILs) interact
30 more closely with the tumor cells and are likely to reflect the tumor host interaction with higher fidelity. The use of TILs as a graft for adoptive cell transfer therapy to treat

cancer has been pioneered by Rosenberg's group at the National Cancer Institute in the US²⁸.

It is believed that TILs are a polyclonal population of T cells with various antigen specificities²⁹. To investigate the tumor specificity of TILs using our soluble dimer pHLA technology, TILs were isolated from nine HLA-A2* patients with metastatic melanoma and grown *in vitro* as reported previously¹⁴. A large panel of 8-11 mer peptides derived from proteins highly expressed by autologous tumor cells were predicted using publicly available algorithms as reported previously (see Table 1)^{19,23,30}. A library of soluble dimeric A2^{Q115E}-K^b loaded with the predicted A2 peptides were produced as described above and used to stain the TILs (see Figures 12 and Figure 15-21). A2/HIV pol₄₇₆₋₄₈₄ and A2/ MART1₂₈₋₃₅ Pentamers from ProImmune were utilized as a negative and positive control, respectively. The result showed that the *in vitro* grown TILs possessed reactivity to MART1, which is one of the well-established melanoma-associated antigen (<http://www.uniprot.org/uniprot/Q16655>).

15 *Functional assays of dimer* T cells*

Using ELISPOT assays, A2-restricted peptide-specific IFN- γ secretion was confirmed for all the 6 TIL samples for which dimer staining was positive. PVDF plates (Millipore, Bedford, MA) were coated with capture mAb (1D1K; MABTECH, Mariemont, OH). TILs were incubated with 2×10^4 per well of T2 cells in the presence of each peptide for 20-24 hours at 37°C. The plates were washed and incubated with biotin-conjugated detection mAb (7-B6-1; MABTECH). HRP-conjugated SA (Jackson ImmunoResearch) was then added, and IFN- γ spots were developed. The reaction was stopped by rinsing thoroughly with cold tap water. ELISPOT plates were scanned and counted using an ImmunoSpot plate reader and ImmunoSpot version 5.0 software (Cellular Technology Limited, Shaker Heights, OH) (Figure 22-27).

A summary of dimer staining and ELISPOT assays of TILs is shown in Figure 28.

Enrichment of dimer positive TILs

Two TIL samples (M37 TIL3 REP1B 2E7 2015-06-03 and M40 TIL3 REP1A 2E7 2015-06-04) were stained with A2/SSX-2₄₁₋₄₉ dimer and A2/SSX-2₄₁₋₄₉ T cells were purified using flow-cytometry-guided sorting (Figure 29).

Possible Advantages.

There are a number of possible advantages of the present methods. The present HLA class I molecules may represent a more natural folding and/or glycosylation of the protein. The present HLA molecules might be produced in relatively quick fashion (~ 2 days vs. 4-10 days using conventional methods). Peptides might be exchanged
 5 relatively simply *in vitro*. A simpler protocol resulting in a more natural product might also result in significant cost savings.

Sequences

Soluble A*02:01-wt, nucleotide sequence (SEQ ID NO.1) and amino acid sequence (SEQ ID NO.2)

10 Sequences are listed in the following order:

Signal peptide derived from Fibroin-L (in regular Arial font below)

HLA-A*02:01 α 1 domain (underlined below)

HLA-A*02:01 α 2 domain (in bold below)

*HLA-A*02:01 α 3 domain* (in italics below)

15 **Flexible GS linker** (in bold and underlined below)

6xHis tag (in bold and italics below)

SEQ ID NO.1:

20 ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCGGA
 AGCCACAGCATGCGGTACTTTTTACCAGCGTGTCCAGACCCGGCAGAGGCGAG
 CCCAGATTCATTGCCGTGGGCTACGTGGACGACACCCAGTTCGTCAGATTCGAC
 AGCGACGCCGCCAGCCAGCGGATGGAACCTAGAGCCCCTTGGATCGAGCAGGA
 AGGCCCGAGTACTGGGACGGCGAGACACGGAAAGTGAAGGCCACAGCCAGA
 CCCACAGAGTGGATCTGGGCACCCTGCGGGGCTACTACAATCAGTCTGAGGCCG
 25 GCTCCACACCGTGCAGAGGATGTACGGCTGTGACGTGGGCAGCGACTGGCG
 GTTCCTGAGAGGCTACCACAGTACGCCTACGACGGCAAGGACTATATCGCCC
 TGAAAGAGGACCTGCGGAGCTGGACAGCCGCCGATATGGCCGCCAGACCAC
 CAAGCACAAATGGGAAGCCGCCACGTGGCCGAGCAGCTGAGAGCTTATCTG
 GAAGGCACCTGTGTGGAATGGCTGCGGAGATACCTGGAAAACGGCAAAGAGA
 30 CACTGCAGCGCAGCGGACGCCCCCAAACGCATATGACTCACCACGCTGTCTCTG
 ACCATGAAGCCACCCTGAGGTGCTGGGCCCTGAGCTTCTACCCTGCGGAGATCA
 CACTGACCTGGCAGCGGGATGGGGAGGACCAGACCCAGGACACGGAGCTCGTG
 GAGACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCGGCTGTGGTGGT
 GCCTTCTGGACAGGAGCAGAGATACACCTGCCATGTGCAGCATGAGGGTTTGCC
 35 CAAGCCCTCACCTGAGATGGGAGCCGGGCGAGCCACCACCACCATCACCAATT
 GA

SEQ ID NO.2:

MMRPIVLVLLFATSALAGSHSMRYFFTSVSRPGRGEPFIAVGYVDDTQFVRFDSDA
 ASQRMEPRAPWIEQEGPEYWDGETRKYKAHSQTHRVDLGLRGYYNQSEAGSHTV
 QRMYGCDVGSDWRFLRGYHQYAYDGKDIALKEDLRSWTAADMAAQTTKHKWE
 AAHVAEQLRAYLEGTCVEWLRRLYLENGKETLQRTDAPKTHMTHHAVSDHEATLRC
 5 WALSFPAPAEITLTWQRDGEDQTQDTELVEPTRPAGDGTQKWAADVVPSPGQEQRYT
 CHVQHEGLPKPLTLRWEPSHHHHHHZ

Soluble A*02:01-K^b, nucleotide sequence (SEQ ID NO.3) and amino acid sequence (SEQ ID NO.4)

10 Sequences are listed in the following order:

Signal peptide derived from Fibroin-L (in regular Arial font below)

HLA-A*02:01 α 1 domain (underlined below)

HLA-A*02:01 α 2 domain (in bold below)

Mouse K^b α 3 domain (in italics below)

15 **Flexible GS linker** (in bold and underlined below)

6xHis tag (in bold and italics below)

SEQ ID NO.3

20 ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCGGA
 AGCCACAGCATGCGGTACTTTTTACCAGCGTGTCAGACCCGGCAGAGGCGAG
 CCCAGATTCATTGCCGTGGGCTACGTGGACGACACCCAGTTCGTCAGATTCGAC
 AGCGACGCCGCCAGCCAGCGGATGGAACCTAGAGCCCCTTGGATCGAGCAGGA
 AGGCCCGAGTACTGGGACGGCGAGACACGGAAAGTGAAGGCCACAGCCAGA
 CCCACAGAGTGGATCTGGGCACCCCTGCGGGGCTACTACAATCAGTCTGAGGCCG
 25 GCTCCCACACCGTGACAGAGGATGTACGGCTGTGACGTGGGCAGCGACTGGCG
 GTTCCTGAGAGGCTACCACCAGTACGCCTACGACGGCAAGGACTATATCGCCC
 TGAAAGAGGACCTGCGGAGCTGGACAGCCGCCGATATGGCCGCCAGACCAC
 CAAGCACAAATGGGAAGCCGCCACGTGGCCGAGCAGCTGAGAGCTTATCTG
 GAAGGCACCTGTGTGGAATGGCTGCGGAGATACCTGGAAAACGGCAAAGAGA
 30 CACTGCAGCGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACCTG
 AAGATAAAGTCACCCTGAGGTGCTGGGGCCCTGGGCTTCTACCCTGCTGACATCA
 CCCTGACCTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGTGG
 AGACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCATCTGTGGTGGTG
 CCTCTTGGGAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGCCT
 35 GAGCCCCTCACCCCTGAGATGGGAGCCG**GGCAGCCACCACCACCATCACCATTG**
 A

SEQ ID NO.4

40 MMRPIVLVLLFATSALAGSHSMRYFFTSVSRPGRGEPFIAVGYVDDTQFVRFDSDA
 ASQRMEPRAPWIEQEGPEYWDGETRKYKAHSQTHRVDLGLRGYYNQSEAGSHTV
 QRMYGCDVGSDWRFLRGYHQYAYDGKDIALKEDLRSWTAADMAAQTTKHKWE
 AAHVAEQLRAYLEGTCVEWLRRLYLENGKETLQRTDSPKAHVTHHSRPEDKVTLRC
 WALGFYPADITLTWQLNGEELIQDMELVEPTRPAGDGTQKWASVVVPLGKEQYYTC
 HVYHQGLPEPLTLRWEPSHHHHHHZ

22

6xHis tag (in bold and italics below)

10 ATGATGCGGCCCATCGTGCTGGTGGTGGTGGTTTGCCACATCTGCCCTGGCCGGC
TCCCACCTCCATGAGGTATTTCTCCACATCCGTGTCCCGGCCCGGCCGCGGGGAG
CCCCGCTTCATCGCCGTGGGCTACGTGGACGACACGCAGTTCTGTGCGGTTTCGAC
AGCGACGCCGCGAGCCAGAGGATGGAGCCGCGGGCGCCGTGGATAGAGCAGG
AGGGGCCGGAGTATTGGGACGAGGAGACAGGGAAAGTGAAGGCCCACTCACAG
15 ACTGACCGAGAGAACCCTGCGGATCGCGCTCCGCTACTACAACCAGAGCGAGGCC
GGTTCTCACACCCTCCAGATGATGTTTGGCTGCGACGTGGGGTTCGGACGGGCG
CTTCCTCCGCGGGTACCACCAGTACGCCTACGACGGCAAGGATTACATCGCCC
TGAAAGAGGACCTGCGCTCTTGGACCGCGGCGGACATGGCGGCTCAGATCAC
AAGCGCAAGTGGGAGGCGGCCCATGTGGCGGAGCAGCAGAGAGCCTACCTGG
20 AGGGCACGTGCGTGGACGGGCTCCGCGAGATACCTGGAGAACGGGAAGGAGAC
GCTGCAGCGCACGGACCCCCCAAGACACATATGACCCACCACCCCATCTCTGA
CCATGAGGCCACTCTGAGATGCTGGGCCCTGGGCTTCTACCCTGCGGAGATCAC
ACTGACCTGGCAGCGGGATGGGGAGGACCAGACCCAGGACACGGAGCTTGTGG
AGACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCAGCTGTGGTGGTA
CCTTCTGGAGAGGAGCAGAGATACACCTGCCATGTGCAGCATGAGGGTCTGCCC
25 AAGCCCTCACCTGAGATGGGAGCCGGGCAGCCACCACCACCATCACCATTA
A

30 MMRPVIVLLFATSALAGSHSMRYFSTSVSRPGRGEPRIAVGYVDDTQFVRFDSDA
ASQRM EPRAPWIEQEGPEYWDEETGKVK AHSQTDRENLRALRYYNQSEAGSHTLQ
MMFGCDVGSDGRFLRGYHQYAYDGKD YIALKEDLRSWTAADMAAQITKRKWEAA
HVAEQQRAYLEGTCVDGLRRYLENGKETLQRTDPPKTHMTHHPISDHEATLRCWAL
GFYP AEITLTWQRDGEDQTQDTEL VETRPAGDGT FQKWAAVVVPSGEEQRYTCHV
QHEGLPKPLTLRWEFGSHHHHHH

Mouse K^b α3 domain (in italics below)

Flexible GS linker (in bold and underlined below)

6xHis tag (in bold and italics below)

SEQ ID NO.9

5 ATGATGCGGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCGGC
 TCCCACTCCATGAGGTATTTCTCCACATCCGTGTCCCGGCCCGGCCGCGGGGAG
 CCGCGCTTCATCGCCGTGGGCTACGTGGACGACACGCAGTTCTGTGCGGTTTCGAC
 AGCGACGCCGCGAGCCAGAGGATGGAGCCGCGGGCGCCGTGGATAGAGCAGG
 AGGGGCCGGAGTATTGGGACGAGGAGACAGGGAAAGTGAAGGCCCACTCACAG
 10 ACTGACCGAGAGAACCTGCGGATCGCGCTCCGCTACTACAACCAGAGCGAGGCC
 GGTTCACACCCCTCCAGATGATGTTTGGCTGCGACGTGGGGTTCGGACGGGGC
 CTTCTCCGCGGGTACCACCAGTACGCCTACGACGGCAAGGATTACATCGCCC
 TGAAAGAGGACCTGCGCTCTTGGACCGCGCGGACATGGCGGCTCAGATCACC
 AAGCGCAAGTGGGAGGCGGGCCCATGTGGCGGAGCAGCAGAGAGCCTACCTGG
 15 AGGGCACGTGCGTGGACGGGCTCCGCAGATACCTGGAGAACGGGAAGGAGAC
 GCTGCAGCGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACCTGA
 AGATAAAGTCACCTGAGGTGCTGGGGCCCTGGGCTTCTACCCTGCTGACATCAC
 CCTGACCTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGTGGA
 GACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCATCTGTGGTGGTGC
 20 CTCTTGGGAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGCCTG
 AGCCCTCACCTGAGATGGGAGCCG**GGCAGCCACCACCACCATCACCATTGA**

SEQ ID NO.10

MMRPIVLVLLFATSALAGSHSMRYFSTSVSRPGRGEPFIAVGYVDDTQFVRFDSDA
 25 ASQRMEPRAPWIEQEGPEYWDEETGKVKASHQTDRENLRALRYYNQSEAGSHTLQ
 MMFGCDVGS DGRFLRGYHQYAYDGKDIALKEDLRSWTAADMAAQITKRKWEAA
 HVAEQQRAYLEGTCVDGLRRYLENGKETLQRTDSPKAHVTHHSRPEDKVTLCWA
 LGFYPADITLTWQLNGEELIQDMELVETRPAGDGTQKQWASVVVPLGKEQYYTCHVY
 HQGLPEPLTLRWE**PGSHHHHHH**Z

30 Soluble A*24:02^{Q115E}-K^b, nucleotide sequence (SEQ ID NO.11) and amino acid
 sequence (SEQ ID NO.12)

Sequences are listed in the following order:

Signal peptide derived from Fibroin-L (in regular Arial font below)

35 HLA-A*24:02 α1 domain (underlined below)

HLA-A*24:02 α2 domain with Q115E mutation (in bold below)

Mouse K^b α3 domain (in italics below)

Flexible GS linker (in bold and underlined below)

6xHis tag (in bold and italics below)

40

SEQ ID NO.11

ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCGGC
 TCCCACTCCATGAGGTATTTCTCCACATCCGTGTCCCGGCCCGGCCGCGGGGAG
 CCCCCTTCATCGCCGTGGGCTACGTGGACGACACGCAGTTTCGTGCGGTTTCGAC
 AGCGACGCCGCGAGCCAGAGGATGGAGCCGCGGGCGCCGTGGATAGAGCAGG
 5 AGGGGCCGGAGTATTGGGACGAGGAGACAGGGAAAGTGAAGGCCCACTCACAG
 ACTGACCGAGAGAACCTGCGGATCGCGCTCCGCTACTACAACCAGAGCGAGGCC
 GGTTCACACCCCTCCAGATGATGTTTGGCTGCGACGTGGGGTCCGACGGGGC
 CTTCTCCGCGGGTACCACGAGTACGCCTACGACGGCAAGGATTACATCGCCC
 TGAAAGAGGACCTGCGCTCTTGGACCGCGCGGACATGGCGGCTCAGATCACC
 10 AAGCGCAAGTGGGAGGCGGCCCATGTGGCGGAGCAGCAGAGAGCCTACCTGG
 AGGGCACGTGCGTGGACGGGCTCCGCAGATACCTGGAGAACGGGAAGGAGAC
 GCTGCAGCGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACCTGA
 AGATAAAGTCACCCTGAGGTGCTGGGGCCCTGGGCTTCTACCCTGCTGACATCAC
 CCTGACCTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGTGGA
 15 GACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCATCTGTGGTGGTGC
 CTCTTGGGAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGCCTG
 AGCCCCTCACCTGAGATGGGAGCCGGG**CAGCCACCACCACCATCACCATTGA**

SEQ ID NO.12

20 MMRPIVLVLLFATSALAGSHSMRYFSTSVSRPGRGEPRIAVGYVDDTQFVRFDSDA
 ASQRMPEPRAPWIEQEGPEYWDEETGKVKAHSQTDRENLRALRYYNQSEAGSHTLQ
 MMFGCDVGS DGRFLRGYHEYAYDGKDIALKEDLRSWTAADMAAQITKRKWEAA
 HVAEQQRAYLEGTCVDGLRRYLENGKETLQRTDSPKAHVTHHSRPEDKVTLCRWA
 LGFYPADITLTWQLNGEELIQDMELVETRPAGDGTQKQWASVVVPLGKEQYYTCHVY
 25 HQGLPEPLTLRWE**PGSHHHHHH**Z

Soluble B*35:01^{Q115E}-K^b, nucleotide sequence (SEQ ID NO.13) and amino acid
 sequence (SEQ ID NO.14)

Sequences are listed in the following order:

- 30 Signal peptide derived from Fibroin-L (in regular Arial font below)
HLA-B*35:01 α 1 domain (underlined below)
 HLA-B*35:01 α 2 domain with Q115E mutation (in bold below)
Mouse K^b α 3 domain (in italics below)
Flexible GS linker (in bold and underlined below)
 35 ***6xHis tag*** (in bold and italics below)

SEQ ID NO.13

40 ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCGGC
 TCCCACTCCATGAGGTATTTCTACACCGCCATGTCCCGGCCCGGCCGCGGGGAG
 CCCCCTTCATCGCAGTGGGCTACGTGGACGACACCCAGTTTCGTGAGGTTTCGAC
 AGCGACGCCGCGAGTCCGAGGACGGAGCCCCGGGCGCCATGGATAGAGCAGG
 AGGGGCCGGAGTATTGGGACCGGAACACACAGATCTTCAAGACCAACACACAGA
 CTTACCGAGAGAGCCTGCGGAACCTGCGCGGCTACTACAACCAGAGCGAGGCC
 GGGTCTCACATCATCCAGAGGATGTATGGCTGCGACCTGGGGCCCGACGGGGC

CCTCCTCCGCGGGGCATGACGAGTCCGCCTACGACGGCAAGGATTACATCGCCC
 TGAACGAGGACCTGAGCTCCTGGACCGCGCGGACACCGCGGCTCAGATCAC
 CCAGCGCAAGTGGGAGGCGGCCCCGTGTGGCGGAGCAGCTGAGAGCCTACCTG
 GAGGGCCTGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAGGAGA
 5 CGCTGCAGCGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACCTG
 AAGATAAAGTCACCCTGAGGTGCTGGGCCCTGGGCTTCTACCCTGCTGACATCA
 CCCTGACCTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGTGG
 AGACCAGGCCTGCAGGGGATGGAACCTTCCAGAAAGTGGGCATCTGTGGTGGTG
 CCTCTTGGGAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGCCT
 10 GAGCCCCCTACCCTGAGATGGGAGCCGGG**CAGCCACCACCACCATCACCATTG**
 A

SEQ ID NO.14

MMRPIVLVLLFATSALAGSHSMRYFYTAMSRPGRGEPRIAVGYVDDTQFVRFDSDA
 15 ASPRTEPRAPWIEQEGPEYWDRNTQIFKTNTQTYRESLRNLRGYYNQSEAGSHIIQR
MYGCDLGPDGRLLRGHDESAIDGKDIALNEDLSSWTAADTAAQITQRKWEAARV
AEQLRAYLEGLCWEWLRRYLENGKETLQRTDSPKAHVTHHSRPEDKVTLRQWALG
FYPADITLTWQLNGEELIQDMELVETRPAGDGTFOKWASVVVPLGKEQYYTCHVYH
 20 QGLPEPLTLRWEPSHHHHHHZ

Soluble B*40:02^{Q115E}-Kb, nucleotide sequence (SEQ ID NO. 15) and amino acid
 sequence (SEQ ID NO. 16)

Sequences are listed in the following order:

Signal peptide derived from Fibroin-L (in regular Arial font below)

25 HLA-B*40:02 α 1 domain (underlined below)

HLA-B*40:02 α 2 domain with Q115E mutation (in bold below)

Mouse K^b α 3 domain (in italics below)

Flexible GS linker (in bold and underlined below)

6xHis tag (in bold and italics below)

30

SEQ ID NO. 15

ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCGGC
 AGCCACAGCATGCGGTACTTCCACACCAGCGTGTCCAGACCCGGAAGAGGGCA
 GCCCAGATTCATCACCGTGGGCTACGTGGACGACACCCTGTTCTGTCAGATTCTGA
 35 CAGCGACGCCACCAGCCCCCGGAAAGAACCTAGAGCCCCCTTGGATCGAGCAGG
AAGGCCCGAGTACTGGGACAGAGAGACACAGATCAGCAAGACCAACACCCAGA
CCTACAGAGAGAGCCTGCGGAACCTGCGGGGCTACTACAATCAGAGCGAGGCC
GGCTCTCACACCCTGCAGTCTATGTACGGCTGCGACGTGGGCCCCGATGGCAG
 ACTGCTGAGAGGCCACAACGAGTACGCCTACGACGGCAAGGACTATATCGCCC
 40 TGAACGAGGACCTGCGGAGCTGGACAGCCGCCGATACAGCCGCCCAGATCAC
 CCAGAGAAAGTGGGAGGCCGCCAGAGTGGCCGAACAGCTGAGAGCCTATCTG
 GAAGGCCGAGTGCGTGGAAATGGCTGCGGAGATACCTGGAAAACGGCAAAGAGA
 CACTGCAGCGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACCTG

AAGATAAAGTCACCCTGAGGTGCTGGGCCCCTGGGCTTCTACCCTGCTGACATCA
 CCCTGACCTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGTGG
 AGACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCATCTGTGGTGGTG
 CCTCTTGGGAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGCCT
 5 GAGCCCCTCACCCTGAGATGGGAGCCGGG**CAGCCACCACCACCATCACCATTG**
 A

SEQ ID NO. 16

10 MMRPIVLVLLFATSALAGSHSMRYFHTSVSRPGRGEPRFITVGYVDDTLFVRFDSDAT
 SPRKEPRAPWIEQEGPEYWDRETQISKNTNTQTYRESLRNLRGYYNQSEAGSHTLOS
 MYGCDVGPDGRLLRGHNEYAYDGKDYIALNEDLRSWTAADTAAQITQRKWEAARV
 AEQLRAYLEGECEVWLRRLRYLENGKETLQRTDSPKAHVTHHSRPEDKVTLCRWALG
 FYPADITLTWQLNGEELIQDMELVETRPAGDGTQKQWASVVVPLGKEQYYTCHVYH
 15 QGLPEPLTLRWE**PGSHHHHHH**Z

Soluble B*44:05^{Q115E}-Kb, nucleotide sequence (SEQ ID NO. 17) and amino acid
 sequence (SEQ ID NO. 18)

Sequences are listed in the following order:

Signal peptide derived from Fibroin-L (in regular Arial font below)

20 HLA-B*44:05 α 1 domain (underlined below)

HLA-B*44:05 α 2 domain with Q115E mutation (in bold below)

Mouse K^b α 3 domain (in italics below)

Flexible GS linker (in bold and underlined below)

6xHis tag (in bold and italics below)

25

SEQ ID NO. 17

ATGATGAGGCCCATCGTGCTGGTGCTGCTGTTCCGCCACATCTGCCCTGGCCGGC
 AGCCACAGCATGCGGTACTTTTACACCGCCATGAGCAGACCCGGCAGAGGCGAG
 30 CCCAGATTCATCACCGTGGGCTACGTGGACGACACCCTGTTCTGTCAGATTGAC
 AGCGACGCCACCAGCCCCCGGAAAGAACCTAGAGCCCCTTGGATCGAGCAGGA
 AGGCCCCGAGTACTGGGACAGAGAGACACAGATCAGCAAGACCAACACCCAGAC
 CTACAGAGAGAACCTGCGGACCGCCCTGCGGTACTACAATCAGTCTGAGGCCGG
 CTCCACATCATCCAGCGGATGTACGGCTGTGACGTGGGCCCCGATGGCAGAC
 35 TGCTGAGAGGCTACGACGAGTACGCCTACGACGGCAAGGACTATATCGCCCTG
 AACGAGGACCTGAGCAGCTGGACAGCCGCCGATACAGCCGCCCAGATCACCC
 AGAGAAAGTGGGAGGCCGCCAGAGTGGCCGAGCAGGATAGAGCCTATCTGGA
 AGGCCTGTGCGTGGAAGCCTGCGGAGATACCTGGAAAACGGCAAAGAGACA
 CTGCAGCGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACCTGAA
 40 GATAAAGTCACCCTGAGGTGCTGGGCCCTGGGCTTCTACCCTGCTGACATCACC
 CTGACCTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGTGGAG
 ACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCATCTGTGGTGGTGCCT
 CTTGGGAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGCCTGAG
 CCCCTCACCCTGAGATGGGAGCCGG**CAGCCACCACCACCATCACCATTGA**
 45

SEQ ID NO. 18

MMRPVLLVLLFATSALAGSHSMRYFYTAMSRPGRGEPFRFITVGYVDDTLFVRFDSDA
 TSPRKEPRAPWIEQEGPEYWDRETQISKNTNTQTYRENLRRTALRYYNQSEAGSHIIQR
 MYGCDVGPDGRLLRGYDEYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARV
 5 AEQDRAYLEGLCVESLRRYLENGKETLQRTDSPKAHVTHHSRPEDKVTLCWALGF
 YPADITLTWQLNGEELIQDMELVETRPAGDGTFOKWASVVVPLGKEQYYTCHVYHQ
 GLPEPLTLRWE**GS**HHHHHHZ

10 Soluble B*07:02^{Q115E}-Kb, nucleotide sequence (SEQ ID NO. 19) and amino acid
 sequence (SEQ ID NO. 20)

Sequences are listed in the following order:

Signal peptide derived from Fibroin-L (in regular Arial font below)

HLA-B*07:02 α 1 domain (underlined below)

HLA-B*07:02 α 2 domain with Q115E mutation (in bold below)

15 *Mouse K^b α 3 domain* (in italics below)

Flexible GS linker (in bold and underlined below)

6xHis tag (in bold and italics below)

SEQ ID NO. 19

20 ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCGGC
AGCCACAGCATGCGGTACTTTTACACCAGCGTGTCCAGACCCGGCAGAGGCGAG
CCCAGATTCATCAGCGTGGGCTACGTGGACGACACCCAGTTCGTCAGATTCGAC
AGCGACGCCGCCAGCCCCAGAGAGGAACCTAGAGCCCCCTTGGATCGAGCAGGA
 25 AGGCCCCGAGTACTGGGACCGGAACACCCAGATCTACAAGGCCCGAGGCCCAGA
CCGACAGAGAGAGCCTGAGAAACCTGCGGGGCTACTACAACCAGAGCGAGGCC
GGCTCTCACACCCTGCAGTCTATGTACGGCTGCGACGTGGGCCCCGATGGCAG
ACTGCTGAGAGGCCACGATGAGTACGCCTACGACGGCAAGGACTATATCGCCC
TGAACGAGGACCTGCGGAGCTGGACAGCCGCCGATACAGCCGCCCAGATCAC
 30 CCAGAGAAAGTGGGAGGCCGCCAGAGAGGCCGAACAGAGAAGGGCCTATCTG
GAAGGCGAGTGCGTGGAATGGCTGCGGAGATACCTGGAAAATGGCAAGGACA
AGCTGGAACGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACCTG
AAGATAAAGTCACCCTGAGGTGCTGGGGCCCTGGGCTTCTACCCTGCTGACATCA
CCCTGACCTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGTGG
 35 AGACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCATCTGTGGTGGTG
CCTCTTGGGAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGCCT
GAGCCCCTCACCCCTGAGATGGGAGCCGGG**CAGCCACCACCACCATCACCATTG**
 A

40

SEQ ID NO. 20

5 MMRPIVLVLLFATSALAGSHSMRYFYTSVSRPGRGEPFISVGYVDDTQFVRFDSDA
 ASPREEPRAPWIEQEGPEYWDRNTQIYKAAQTDRESLRNLRGYYNQSEAGSHTLQ
 SMYGCDVGPDGRLLRGHDEYAYDGKDIALNEDLRSWTAADTAAQITQRKWEAAR
 EAEQRRAYLEGECEVWLRRLRYLENGKDKLERTDSPKAHVTHHSRPEDKVTLCWAL
 GFYPADITLTWQLNGEELIQDMELVETRPAGDGTQKQWASVVVPLGKEQYYTCHVY
 HQGLPEPLTLRWEPE**GS**HHHHHHZ

10 Soluble B*08:01^{Q115E}-Kb, nucleotide sequence (SEQ ID NO. 21) and amino acid sequence (SEQ ID NO. 22)

Sequences are listed in the following order:

Signal peptide derived from Fibroin-L (in regular Arial font below)

HLA-B*08:01 α 1 domain (underlined below)

15 **HLA-B*08:01 α 2 domain with Q115E mutation** (in bold below)

Mouse K^b α 3 domain (in italics below)

Flexible GS linker (in bold and underlined below)

6xHis tag (in bold and italics below)

20 SEQ ID NO. 21

ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCGGC
 AGCCACAGCATGCGGTACTTTGACACCGCCATGAGCAGACCCGGCAGAGGGCGA
 GCCCAGATTCATCAGCGTGGGCTACGTGGACGACACCCAGTTCGTCAGATTCTGA
 25 CAGCGACGCGCCAGCCCCAGAGAGGAACCTAGAGCCCCTTGGATCGAGCAGG
 AAGGCCCCGAGTACTGGGACCGGAACACCCAGATCTTCAAGACCAATACCCAGA
 CCGACAGAGAGAGCCTGCGGAACCTGCGGGGCTACTACAATCAGAGCGAGGCC
 GGCTCTCACACCCTGCAGTCTATGTACGGCTGCGACGTGGGCCCCGATGGCAG
 ACTGCTGAGAGGGCCACAACGAGTACGCCTACGACGGCAAGGACTATATCGCCC
 30 TGAACGAGGACCTGCGGAGCTGGACAGCCGCCGATACAGCCGCCCAGATCAC
 CCAGAGAAAGTGGGAGGGCCGCCAGAGTGGCCGAGCAGGATAGAGCCTACCTG
 GAAGGCACCTGTGTGGAATGGCTGCGGAGATACCTGGAAAATGGCAAGGACA
 CCCTGGAACGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACCTG
 AAGATAAAGTCACCCTGAGGTGCTGGGCCCCTGGGCTTCTACCCTGCTGACATCA
 35 CCCTGACCTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGTGG
 AGACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCATCTGTGGTGGTG
 CCTCTTGGGAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGCCT
 GAGCCCCTCACCTGAGATGGGAGCCGGG**CAGCC**ACCACCACCATCACCATTG
 A
 40

SEQ ID NO. 22

MMRPIVLVLLFATSALAGSHSMRYFDTAMSRPGRGEPFRFISVGYVDDTQFVRFDSDA
 ASPREEPRAPWIEQEGPEYWDRNTQIFKTNTQTDRESLRNLRGYYNQSEAGSHTLQ
 5 SMYGCDVGPDGRLLRGHNEYAYDGKDIALNEDLRSWTAADTAAQITQRKWEAAR
 VAEQDRAYLEGTCVEWLRRYLENGKDTLERTDSPKAHVTHHSRPEDKVTLCWAL
 GFYPADITLTWQLNGEELIQDMELVETRPAGDGTQKQWASVVVPLGKEQYYTCHVY
 HQGLPEPLTLRWEPE**GS**HHHHHHZ

10 Soluble C*05:01^{Q115E}-Kb, nucleotide sequence (SEQ ID NO. 23) and amino acid
 sequence (SEQ ID NO. 24)

Sequences are listed in the following order:

Signal peptide derived from Fibroin-L (in regular Arial font below)

HLA-C*05:01 α 1 domain (underlined below)

15 **HLA-C*05:01 α 2 domain with Q115E mutation** (in bold below)

Mouse K^b α 3 domain (in italics below)

Flexible GS linker (in bold and underlined below)

6xHis tag (in bold and italics below)

20 SEQ ID NO. 23

ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACAAGCGCCCTGGCCTGC
 TCTCACAGCATGCGCTATTTTTACACGGCAGTTAGTCGGCCTGGGAGGGGTGAG
 CCGAGATTCATTGCTGTAGGCTACGTAGACGACACTCAATTTGTACAGTTCGACT
 25 CAGACGCTGCTTCACCGCGAGGAGAGCCCAGGGCACCCCTGGGTAGAACAAGAA
 GGGCCCGAATACTGGGATCGAGAAACCCAGAAGTATAAGAGGCAAGCACAACT
 GATCGGGTCAACTTGAGAAAACCTGCGAGGCTACTATAATCAAAGTGAGGCAGGAT
 CCCATACACTTCAGAGGATGTATGGCTGCGACCTTGGTCCAGATGGCCGGCTC
 CTCAGAGGGTATAACGAATTTGCATACGACGGGAAGGATTACATAGCTCTCAAT
 30 GAGGACCTTAGATCATGGACGGCAGCGGATAAGGCAGCCCAAATTAATCAAAG
 GAAATGGGAGGCGGGCCCGAGAAGCAGAGCAGAGAAGAGCCTACCTGGAAGGT
 ACATGCGTGGAGTGGCTTCGGCGCTATCTCGAAAACGGTAAAAAGACATTGCA
 ACGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACCTGAAGATAA
 AGTCACCCTGAGGTGCTGGGCCCCTGGGCTTCTACCCTGCTGACATCACCCTGAC
 35 CTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGTGGAGACCAG
 GCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCATCTGTGGTGGTGCCTCTTGG
 GAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGCCTGAGCCCCT
 CACCCTGAGATGGGAGCCGG**GCGAGCC**ACCACCACCATCACCATTGA

40 SEQ ID NO. 24

MMRPIVLVLLFATSALACSHSMRYFYTAVSRPGRGEPFRFIAVGYVDDTQFVQFDSDA
 ASPRGEPRAPWVEQEGPEYWDRETQKYKRAQTDRVNLRLRGYYNQSEAGSHT
 LQRMYGCDLGPDGRLLRGYNEFAYDGKDIALNEDLRSWTAADKAAQITQRKWEA

AREAEQRRAYLEGTCVEWLRRLRYLENGKKTLLQRTDSPKAHVTHHSRPEDKVTLCRW
 ALGFYPADITLTWQLNGEELIQDMELVETRPAGDGTFFQKWASVVVPLGKEQYYTCHV
 YHQGLPEPLTLRWEPEGSHHHHHHZ

- 5 Soluble C*07:01^{Q115E}-Kb, nucleotide sequence (SEQ ID NO. 25) and amino acid sequence (SEQ ID NO. 26)

Sequences are listed in the following order:

Signal peptide derived from Fibroin-L (in regular Arial font below)

HLA-C*07:01 α 1 domain (underlined below)

- 10 **HLA-C*07:01 α 2 domain with Q115E mutation** (in bold below)

Mouse K^b α 3 domain (in italics below)

Flexible GS linker (in bold and underlined below)

6xHis tag (in bold and italics below)

- 15 SEQ ID NO. 25

ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACAAGCGCCCTGGCCTGC
 AGCCACAGCATGCGGTACTTTGACACCGCCGTGTCCAGACCCGGAAGAGGCGA
 GCCCAGATTCATCAGCGTGGGCTACGTGGACGACACCCAGTTTCGTCAGATTCGA
 CAGCGACGCCGCCAGCCCCAGAGGCGAACCTAGAGCACCTTGGGTGGAACAGG
 20 AAGGCCCGAGTACTGGGACAGAGACACAGAACTACAAGCGGCAGGCCAG
 GCCGACAGAGTGTCCCTGAGAAACCTGCGGGGCTACTACAACCAGAGCGAGGA
 CGGCAGCCACACCTGCGAGAGAATGTACGGCTGTGACCTGGGCCCCGATGGC
 AGACTGCTGAGAGGCTACGATGAGAGCGCCTACGACGGCAAGGACTATATCGC
 CCTGAACGAGGACCTGCGGAGCTGGACAGCCGCCGATACAGCCGCCAGATC
 25 ACCCAGAGAAAACCTGGAAGCCGCCAGAGCCGCCGAGCAGCTGAGAGCTTATC
 TGGAAGGCACCTGTGTGGAATGGCTGCGGAGATACCTGGAAAACGGCAAAGA
 GACACTGCAGCGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACC
 TGAAGATAAAGTCACCCTGAGGTGCTGGGGCCCTGGGCTTCTACCCTGCTGACAT
 CACCCTGACCTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGT
 30 GGAGACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCATCTGTGGTGG
 TGCCTCTTGGGAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGC
 CTGAGCCCCTCACCCTGAGATGGGAGCCGGGCAGCCACCACCACCATCACCAT
 TGA

- 35 SEQ ID NO. 26

MMRPIVLVLLFATSALACSHSMRYFDTAVSRPGRGEPFISVGYVDDTQFVRFDSDA
 ASPRGEPRAPWVEQEGPEYWDRETQNYKRQAQADRVSLRNLRGYYNQSEDGSHT
 LQRMYGCDLGPDRLLRGYDESAYDGKDIALNEDLRSWTAADTAAQITQRKLEA
 ARAAEQLRAYLEGTCVEWLRRLRYLENGKETLQRTDSPKAHVTHHSRPEDKVTLCRW
 40 ALGFYPADITLTWQLNGEELIQDMELVETRPAGDGTFFQKWASVVVPLGKEQYYTCHV
 YHQGLPEPLTLRWEPEGSHHHHHHZ

Soluble C*07:02^{Q115E}-Kb, nucleotide sequence (SEQ ID NO. 27) and amino acid sequence (SEQ ID NO. 28)

Sequences are listed in the following order:

Signal peptide derived from Fibroin-L (in regular Arial font below)

5 HLA-C*07:02 α 1 domain (underlined below)

HLA-C*07:02 α 2 domain with Q115E mutation (in bold below)

Mouse K^b α 3 domain (in italics below)

Flexible GS linker (in bold and underlined below)

6xHis tag (in bold and italics below)

10

SEQ ID NO. 27

ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCTGC
 AGCCACAGCATGCGGTACTTTGACACCGCCGTGTCCAGACCCGGAAGAGGCCGA
 GCCCAGATTCATCAGCGTGGGCTACGTGGACGACACCCAGTTCGTCAGATTCGA
 15 CAGCGACGCCGCCAGCCCCAGAGGCCGAACCTAGAGCACCTTGGGTGGAACAGG
AAGGCCCGAGTACTGGGACAGAGAGACACAGAAGTACAAGCGGCAGGCCCAG
GCCGACAGAGTGTCCCTGAGAAACCTGCGGGGCTACTACAACCAGAGCGAGGA
CGGCAGCCACACCCTGCAGAGAATGAGCGGCTGTGACCTGGGCCCCGATGGC
 AGACTGCTGAGAGGCTACGATGAGAGCGCCTACGACGGCAAGGACTATATCGC
 20 CCTGAACGAGGACCTGCGGAGCTGGACAGCCGCCGATACAGCCGCCCAGATC
 ACCCAGAGAAAACCTGGAAGCCGCCAGAGCCGCCGAGCAGCTGAGAGCTTATC
 TGGAAAGGCACCTGTGTGGAATGGCTGCGGAGATACCTGGAAAACGGCAAAGA
 GACACTGCAGCGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACC
 TGAAGATAAAGTCACCCTGAGGTGCTGGGGCCCTGGGCTTCTACCCCTGCTGACAT
 25 CACCCTGACCTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGT
 GGAGACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCATCTGTGGTGG
 TGCCTCTTGGGAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGC
 CTGAGCCCCTCACCCTGAGATGGGAGCCGGGCAGCCACCACCACCATCACCAT
 TGA

30

SEQ ID NO. 28

MMRPVLVLLFATSALACSHSMRYFDTAVSRPGRGEPREISVGYVDDTQFVREDSDA
 ASPRGEPRAPWVEQEGPEYWDRETQKYKROAQADRVSLRNLRGYYNQSEDGSH
 35 LQRMSGCDLGPDGRLLRGYDESAYDGKDIALNEDLRSWTAADTAAQITQRKLEA
 ARAAEQLRAYLEGTCVEWLRRYLENGKETLQRTDSPKAHVTHHSRPEDKVTLCRW
 ALGFYPADITLTWQLNGEELIQDMELVETRPA GDGTFQKWASVVVPLGKEQYYTCHV
 YHQGLPEPLTLRWEFGSHHHHHHZ

Soluble C*16:01^{Q115E}-Kb, nucleotide sequence (SEQ ID NO. 29) and amino acid sequence (SEQ ID NO. 30)

40

Sequences are listed in the following order:

Signal peptide derived from Fibroin-L (in regular Arial font below)

HLA-C*16:01 α 1 domain (underlined below)

HLA-C*16:01 α 2 domain with Q115E mutation (in bold below)

Mouse K^b α 3 domain (in italics below)

Flexible GS linker (in bold and underlined below)

5 ***6xHis tag*** (in bold and italics below)

SEQ ID NO. 29

ATGATGAGGCCCATCGTGCTGGTGGTGGTGGTTCGCCACATCTGCCCTGGCCTGC
 AGCCACAGCATGCCGTACTTTTACACCGCCGTGTCCAGACCCGGCAGAGGCGAG
 10 CCTAGATTCATTGCCGTGGGCTACGTGGACGACACCCAGTTCGTGAGATTGACA
 GCGACGCCGCCAGCCCCAGAGGGGAACCTAGAGCACCTTGGGTGGAACAGGAA
 GGCCCCGAGTACTGGGACAGAGAGACACAGAAGTACAAGCGGCAGGCCCAGAC
 CGACCGGGTGTCCCTGAGAAACCTGCGGGGGCTACTACAACCAGAGCGAGGCCG
 GCTCTCACACCCTGCAGTGGATGTACGGCTGCGACCTGGGCCCTGATGGCAGA
 15 CTGCTGAGAGGCTACGACGAGTCCGCCTACGACGGCAAGGACTATATCGCCCT
 GAACGAGGACCTGCGGAGCTGGACAGCCGCCGATACAGCCGCCCAGATCACC
 CAGAGAAAGTGGGAAGCCGCCAGAGCCGCCGAGCAGCAGAGAGCTTATCTGG
 AAGGCACCTGTGTGGAATGGCTGCGGAGATACCTGGAAAACGGCAAAGAGAC
 ACTGCAGCGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACCTGA
 20 AGATAAAGTCACCCTGAGGTGCTGGGCCCTGGGCTTCTACCCTGCTGACATCAC
 CCTGACCTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGTGGA
 GACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCATCTGTGGTGGTGC
 CTCTTGGGAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGCCTG
 AGCCCCTCACCTGAGATGGGAGCCGGGCAGCCACCACCACCATCACCATTGA

25

SEQ ID NO. 30

MMRPIVLVLLFATSALACSHSMRYFYTAVSRPGRGEPRFIAVGYVDDTQFVRFDSDA
 ASPRGEPRAPWVEQEGPEYWDRETQKYKRQAQTDRVSLRNLRGYYNQSEAGSHT
 LQWMYGCDLGPDRLLRGYDESAYDGKDYIALNEDLRSWTAADTAAQITQRKWE
 30 AARAAEQQRAYLEGTCVEWLRRYLENGKETLQRTDSPKAHVTHHSRPEDKVTLC
 WALGFYPADITLTWQLNGEELIQDMELVETRPAGDGTGFKWASVVVPLGKEQYYTC
 HVYHQGLPEPLTLRWEFGSHHHHHHZ

As with the sequences noted above, the present application may similarly be directed
 35 to the following sequences:

Soluble B*35:01-wt, nucleotide sequence (SEQ ID NO.31) and amino acid sequence (SEQ ID NO.32)

Sequences are listed in the following order:

Signal peptide derived from Fibroin-L (in regular Arial font below)

40 HLA-B*35:01 α 1 domain (underlined below)

HLA-B*35:01 α 2 domain (in bold below)

*HLA-B*35:01 α 3 domain* (in italics below)

Flexible GS linker (in bold and underlined below)

6xHis tag (in bold and italics below)

SEQ ID NO.31

5 ATGATGCGGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCGGC
 TCCCACTCCATGAGGTATTTCTACACCGCCATGTCCCGGCCCGGCCGCGGGGAG
 CCGCGCTTCATCGCAGTGGGCTACGTGGACGACACCCAGTTCGTGAGGTTCGAC
 AGCGACGCCGCGAGTCCGAGGACGGAGCCCCGGGCGCCATGGATAGAGCAGG
 AGGGGCCGGAGTATTGGGACCGGAACACACAGATCTTCAAGACCAACACACAGA
 10 CTTACCGAGAGAGCCTGCGGAACCTGCGCGGCTACTACAACCAGAGCGAGGCC
 GGGTCTCACATCATCCAGAGGATGTATGGCTGCGACCTGGGGCCCGACGGGCG
 CCTCCTCCGCGGGCATGACCAGTCCGCCTACGACGGCAAGGATTACATCGCCC
 TGAACGAGGACCTGAGCTCCTGGACCGCGGCGGACACCGCGGCTCAGATCAC
 CCAGCGCAAGTGGGAGGCGGGCCCGTGTGGCGGAGCAGCTGAGAGCCTACCTG
 15 GAGGGCCTGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAGGAGA
 CGCTGCAGCGCGCGGACCCCCCAAAGACACACAGTGACCCACCACCCCGTCTCT
 GACCATGAGGCCACCCTGAGGTGCTGGGCCCTGGGCTTCTACCCTGCGGAGAT
 CACACTGACCTGGCAGCGGGATGGCGAGGACCAAACTCAGGACACTGAGCTTGT
 GGAGACCAGACCAGCAGGAGATAGAACCCTTCCAGAAGTGGGCAGCTGTGGTGGT
 20 GCCTTCTGGAGAAGAGCAGAGATACACATGCCATGTACAGCATGAGGGGCTGCC
 CAAGCCCTCACCTGAGATGGGAGCCGGG**CAGCCACCACCACCATCACCAT**
 GA

SEQ ID NO.32

25 MMRPIVLVLLFATSALAGSHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDA
 ASPRTEPRAPWIEQEGPEYWDRNTQIFKTNQTYRESLRNLRGYYNQSEAGSHIIQR
 MYGCDLGPDRLLRGHDQSAYDGKDYLALNEDLSSWTAADTAAQITQRKWEAARV
 AEQLRAYLEGLCWEVLRRYLENGKETLQRADPPKTHVTHHPVSDHEATLRCWALG
 FYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQH
 30 EGLPKPLTLRWE**PSHHHHHHZ**

Soluble B*35:01-Kb, nucleotide sequence (SEQ ID NO.33) and amino acid sequence (SEQ ID NO.34)

Sequences are listed in the following order:

35 Signal peptide derived from Fibroin-L (in regular Arial font below)

HLA-B*35:01 α 1 domain (underlined below)

HLA-B*35:01 α 2 domain (in bold below)

Mouse K^b α 3 domain (in italics below)

Flexible GS linker (in bold and underlined below)

40 ***6xHis tag*** (in bold and italics below)

SEQ ID NO.33

ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCGGC
 TCCCACTCCATGAGGTATTTCTACACCGCCATGTCCCGGCCCGGCCGCGGGGAG
 CCCCCTTCATCGCAGTGGGCTACGTGGACGACACCCAGTTCGTGAGGTTGAC
 5 AGCGACGCCGCGAGTCCGAGGACGGAGCCCCGGGCGCCATGGATAGAGCAGG
 AGGGGCCGGAGTATTGGGACCGGAACACACAGATCTTCAAGACCAACACACAGA
 CTTACCGAGAGAGCCTGCGGAACCTGCGCGGCTACTAGAACCAGAGCGAGGCC
 GGGTCTCACATCATCCAGAGGATGTATGGCTGCGACCTGGGGCCCCGACGGGCG
 CCTCCTCCGCGGGCATGACCAGTCCGCCTACGACGGCAAGGATTACATCGCCC
 10 TGAACGAGGACCTGAGCTCCTGGACCGCGGCGGACACCGCGGCTCAGATCAC
 CCAGCGCAAGTGGGAGGCGGGCCCGTGTGGCGGAGCAGCTGAGAGCCTACCTG
 GAGGGCCTGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAGGAGA
 CGCTGCAGCGCACAGATTCCCCAAAGGCCCATGTGACCCATCACAGCAGACCTG
 AAGATAAAGTCACCCTGAGGTGCTGGGGCCCTGGGCTTCTACCCTGCTGACATCA
 15 CCCTGACCTGGCAGTTGAATGGGGAGGAGCTGATCCAGGACATGGAGCTTGTGG
 AGACCAGGCCTGCAGGGGATGGAACCTTCAGAAAGTGGGCATCTGTGGTGGTG
 CCTCTTGGGAAGGAGCAGTATTACACATGCCATGTGTACCATCAGGGGCTGCCT
 GAGCCCCCTCACCCTGAGATGGGAGCCGGGCGAGCCACCACCACCATCACCATTG
 A

20

SEQ ID NO.34

MMRPIVLVLLFATSALAGSHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDA
 ASPRTEPRAPWIEQEGPEYWDRNTQIFKTNTQTYRESLRNLRGYYNQSEAGSHIIQR
 MYGCDLGPDGRLLRGHDQSAYDGKDIALNEDLSSWTAADTAAQITQRKWEAARV
 25 AEQLRAYLEGLCWEVLRRYLENGKETLQRTDSPKAHVTHHSRPEDKVTLRQWALG
 FYPADITLTWQLNGEELIQDMELVETRPAGDGTQKQWASVVVPLGKEQYYTCHVYH
 QGLPEPLTLRWEPSHHHHHHZ

Although preferred embodiments of the invention have been described herein, it will be
 30 understood by those skilled in the art that variations may be made thereto without
 departing from the spirit of the invention or the scope of the appended claims. All
 documents disclosed herein, including those in the following reference list, are
 incorporated by reference.

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CLAIMS:

1. A method of producing an HLA class I molecule complexed to a pre-selected peptide comprising:
 - (a) providing a mammalian derived HLA class I molecule complexed to an existing peptide;
 - (b) incubating, *in vitro*, the HLA class I molecule complexed to the existing peptide with the pre-selected peptide,

wherein the pre-selected peptide is at a concentration sufficient to replace the existing peptide to produce the HLA class I molecule complexed to the pre-selected peptide; and

the HLA class I molecule comprises $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\beta 2m$ domains.
2. The method of claim 1, wherein the HLA class I molecule is soluble.
3. The method of claim 1 or 2, wherein the HLA class I molecule complexed to the existing peptide is produced by a mammalian cell transfected with a soluble HLA class I molecule, wherein the $\beta 2m$ domain may be endogenous or exogenous, preferably exogenous and encoded on a second vector.
4. The method of claim 3, wherein the soluble HLA class I molecule comprises a signal peptide directing secretion of the HLA class I molecule outside of the mammalian cell.
5. The method of claim 4, wherein the soluble HLA class I molecule complexed to the existing peptide is provided in the supernatant of a culture of the mammalian cells.
6. The method of any one of claims 1-5, wherein the HLA class I molecule is HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G.
7. The method of claim 6, wherein the HLA class I molecule is HLA-A and comprises the $\alpha 1$, $\alpha 2$ and $\alpha 3$ domains of any of SEQ ID NOs. 2, 4, 6, 8, 10, or 12.

8. The method of claim 6, wherein the HLA class I molecule is HLA-B and comprises the $\alpha 1$, $\alpha 2$ and $\alpha 3$ domains of any of SEQ ID NOs. 14, 16, 18, 20, or 22.
9. The method of claim 6, wherein the HLA class I molecule is HLA-C and comprises the $\alpha 1$, $\alpha 2$ and $\alpha 3$ domains of any of SEQ ID NOs. 24, 26, 28, or 30.
10. The method of claim 6, wherein the $\alpha 3$ domain of the HLA class I molecule is the mouse K^b $\alpha 3$ domain.
11. The method of claim 6, wherein in the $\alpha 2$ domain of the HLA class I molecule, Gln has been replaced with Glu at position 115.
12. The method of claim 6, wherein the HLA class I molecule comprises SEQ ID NO. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30 with a $\beta 2m$ domain.
13. The method of any one of claims 1-12, further comprising multimerizing the HLA class I molecules, preferably into one of dimers, trimers, tetramers and pentamers.
14. The method of claim 13, wherein the HLA class I molecules are dimerized using an antibody that recognizes a corresponding tag on HLA class I molecule.
15. The method of claim 14, wherein the tag is a 6xHis tag the at the C' end of the $\alpha 3$ domain, preferably connected by a flexible linker, such as a GS linker.
16. A kit for producing an HLA class I molecule complexed to a pre-selected peptide, comprising a mammalian derived HLA class I molecule complexed to an existing peptide and instructions corresponding to the method of claim 1.
17. The kit of claim 16, further comprising the pre-selected peptide.
18. A polypeptide comprising the $\alpha 1$, $\alpha 2$ and $\alpha 3$ domain of an HLA class I molecule, a signal peptide at the N terminus and a 6xHis tag joined by a GS linker at the C terminus.
19. The polypeptide of claim 18, being SEQ ID NO. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

20. A nucleic acid encoding the polypeptide of claim 18 or 19.
21. A vector comprising the nucleic acid of claim 20.
22. A mammalian cell transfected with the vector of claim 21.
23. The mammalian cell of claim 22, further comprising a second vector encoding $\beta 2m$.
5
24. A compound comprising the polypeptide of claim 18 or 19 complexed with a $\beta 2m$ domain.
25. A multimer of at least two compounds according to claim 24.
26. The multimer of claim 25, wherein an antibody recognizing the 6xHis tag dimerizes the at least two compounds.
10
27. A method of screening/selecting in a population of T-cells for antigen specific T-cells that recognize pre-selected peptide antigens, the method comprising:
providing a mammalian-derived HLA class I molecule complexed to the pre-selected peptides;
15 screening the population of T-cells for antigen specific T-cells that bind the mammalian-derived HLA class I molecule complexed to the pre-selected peptides.
28. The method of claim 27, further comprising
first providing a mammalian-derived HLA class I molecule complexed to a
20 holder peptide;
incubating, in vitro, the HLA class I molecule complexed to the holder peptide with the pre-selected peptide,
wherein the pre-selected peptide is at a concentration sufficient to replace the existing peptide to produce the HLA class I molecule complexed to the pre-
25 selected peptide.

29. The method of claim 27, wherein the mammalian-derived HLA class I molecule complexed to the pre-selected peptide is prepared using the method of any one of claims 1-15.
- 5 30. The method of any one of claims 27-29, wherein the screening comprises flow cytometry.
31. The method of any one of claims 27-30, wherein the HLA class I molecule complexed to the holder peptide comprises any one of SEQ ID NO. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30, preferably with a β 2m domain.
- 10 32. The method of any one of claims 27-30, wherein the HLA class I molecule complexed to the holder peptide comprises the polypeptide of claim 18 or 19, preferably with a β 2m domain.
33. The method of any one of claims 27-32, wherein the antigen specific T-cells that recognize pre-selected peptide antigens are tumor-infiltrating lymphocytes.
- 15 34. The method of claim 33, wherein the pre-selected peptide antigens are associated with cancer.

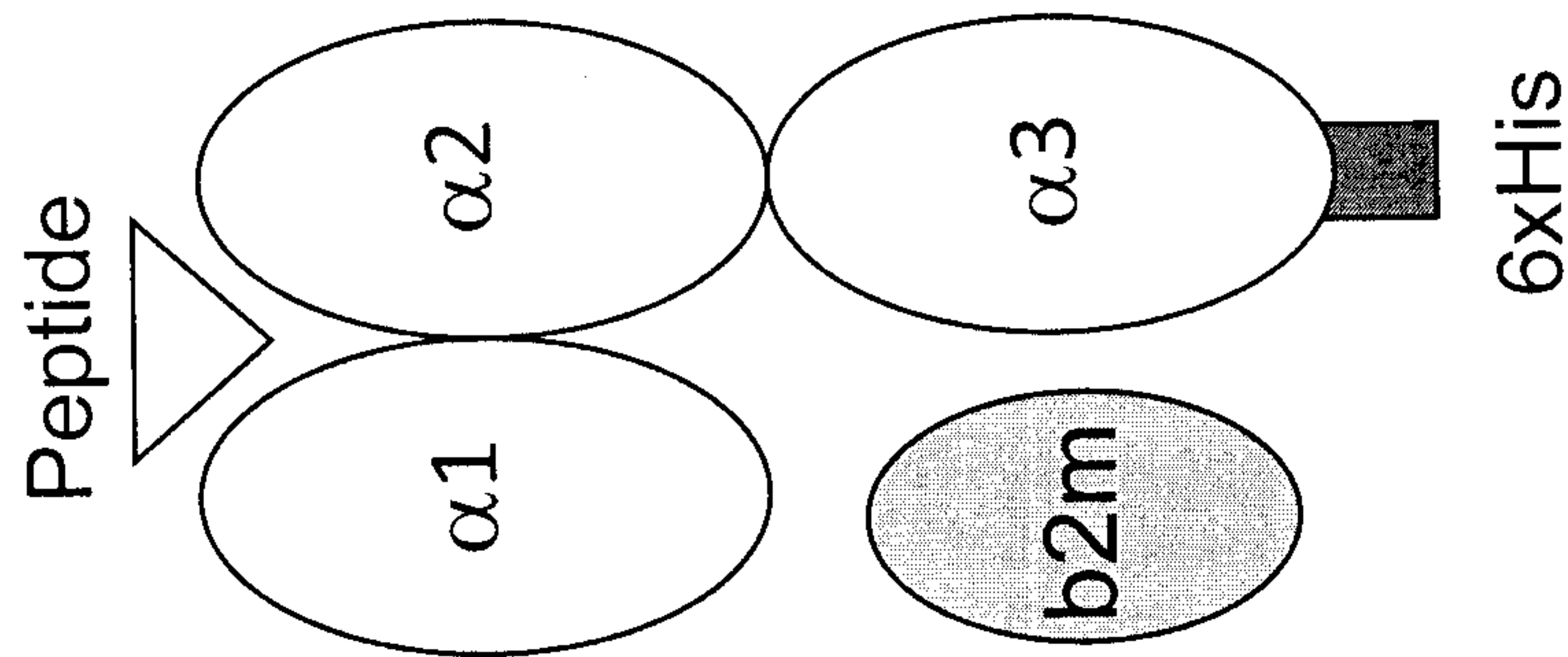


Figure 1 (Prior Art)

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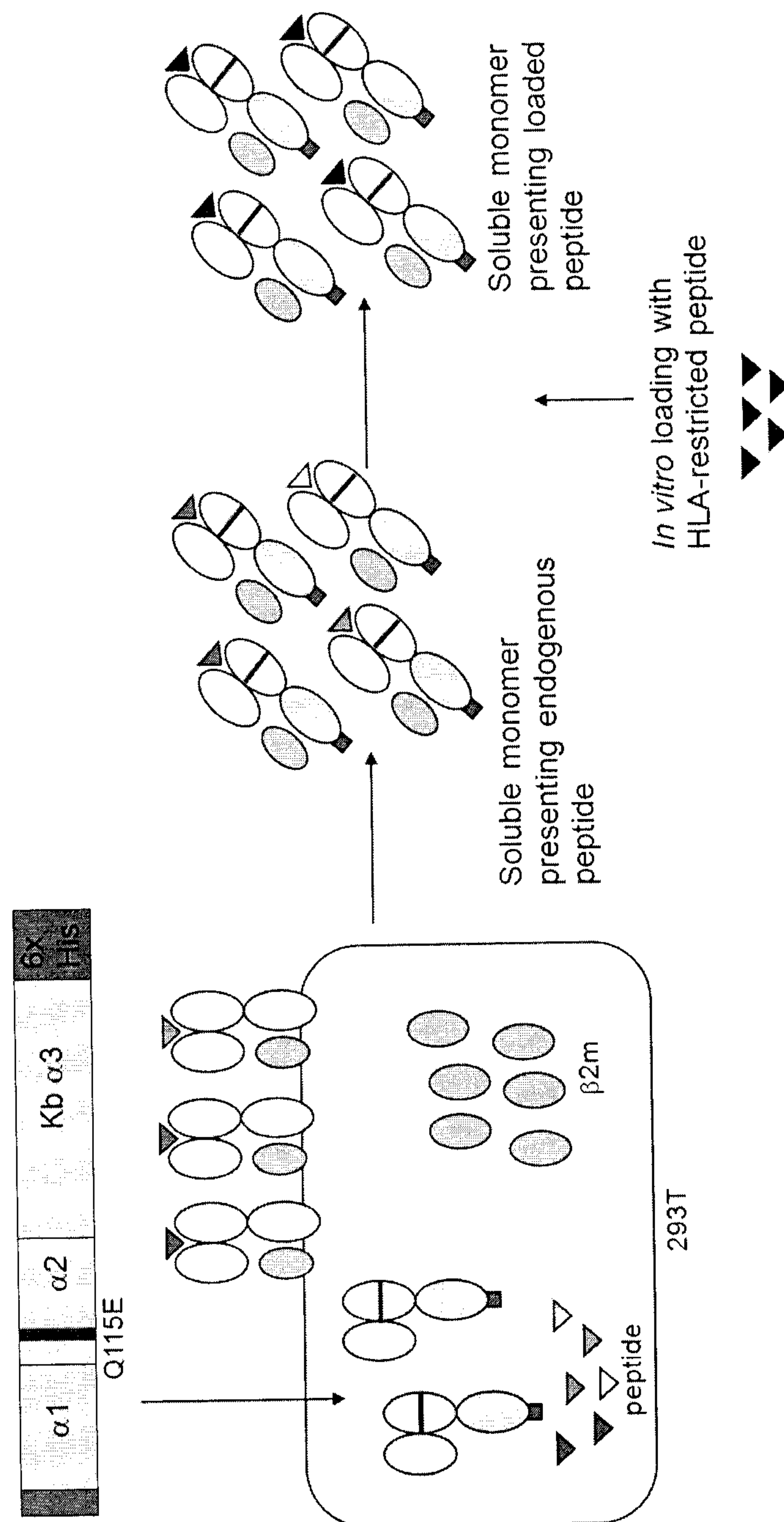


Figure 2

Grow confluent mammalian cells stably secreting monomer HLA

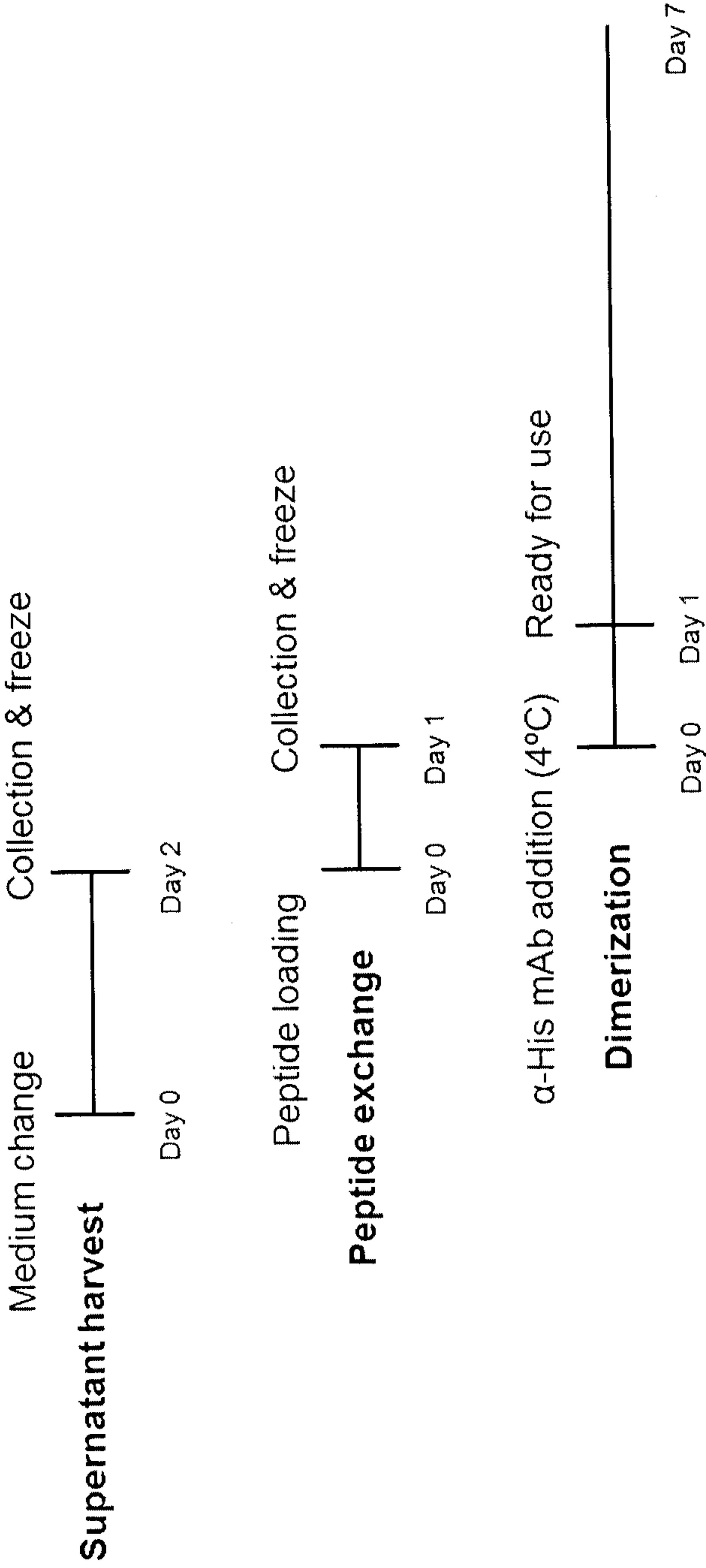


Figure 3

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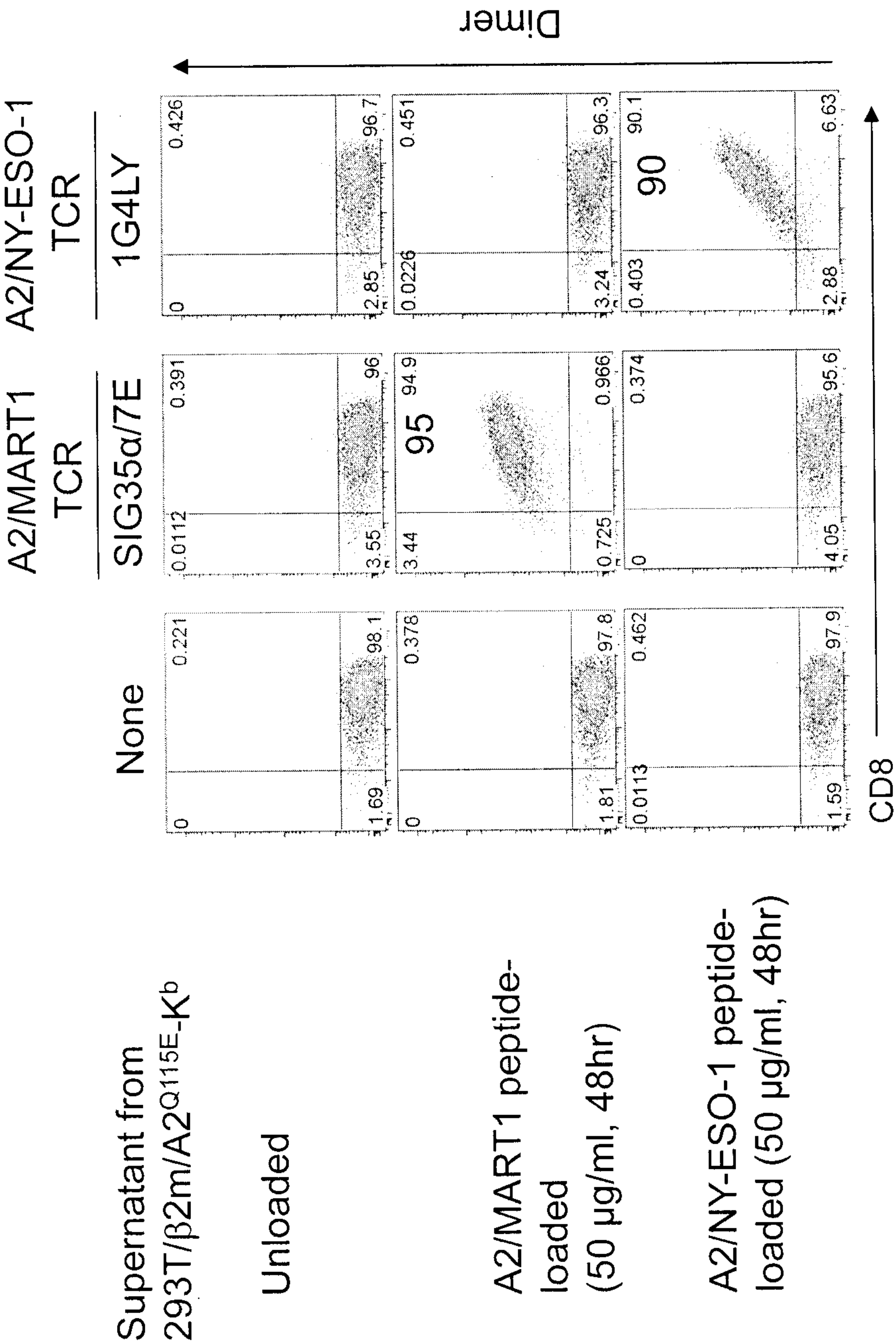


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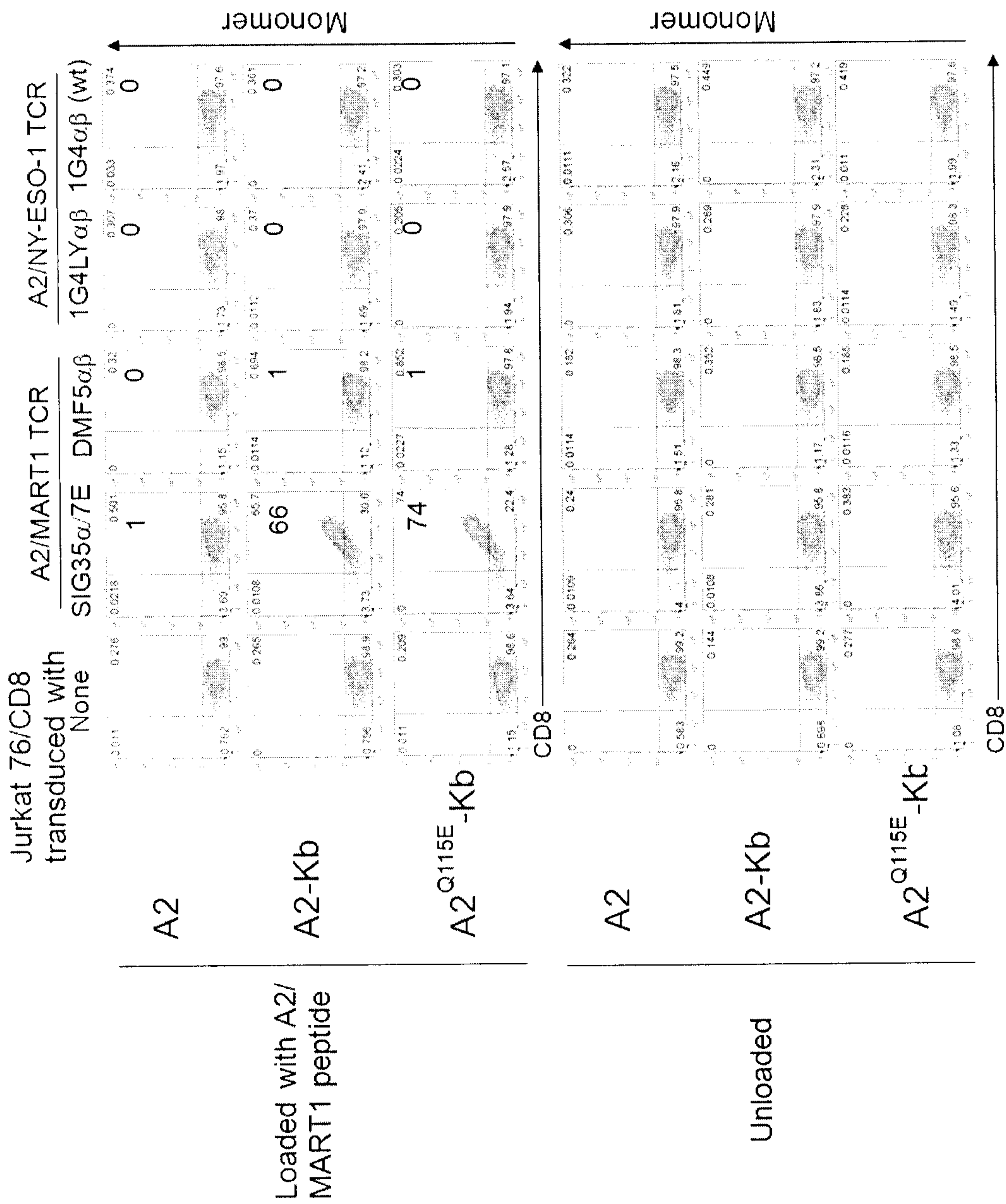


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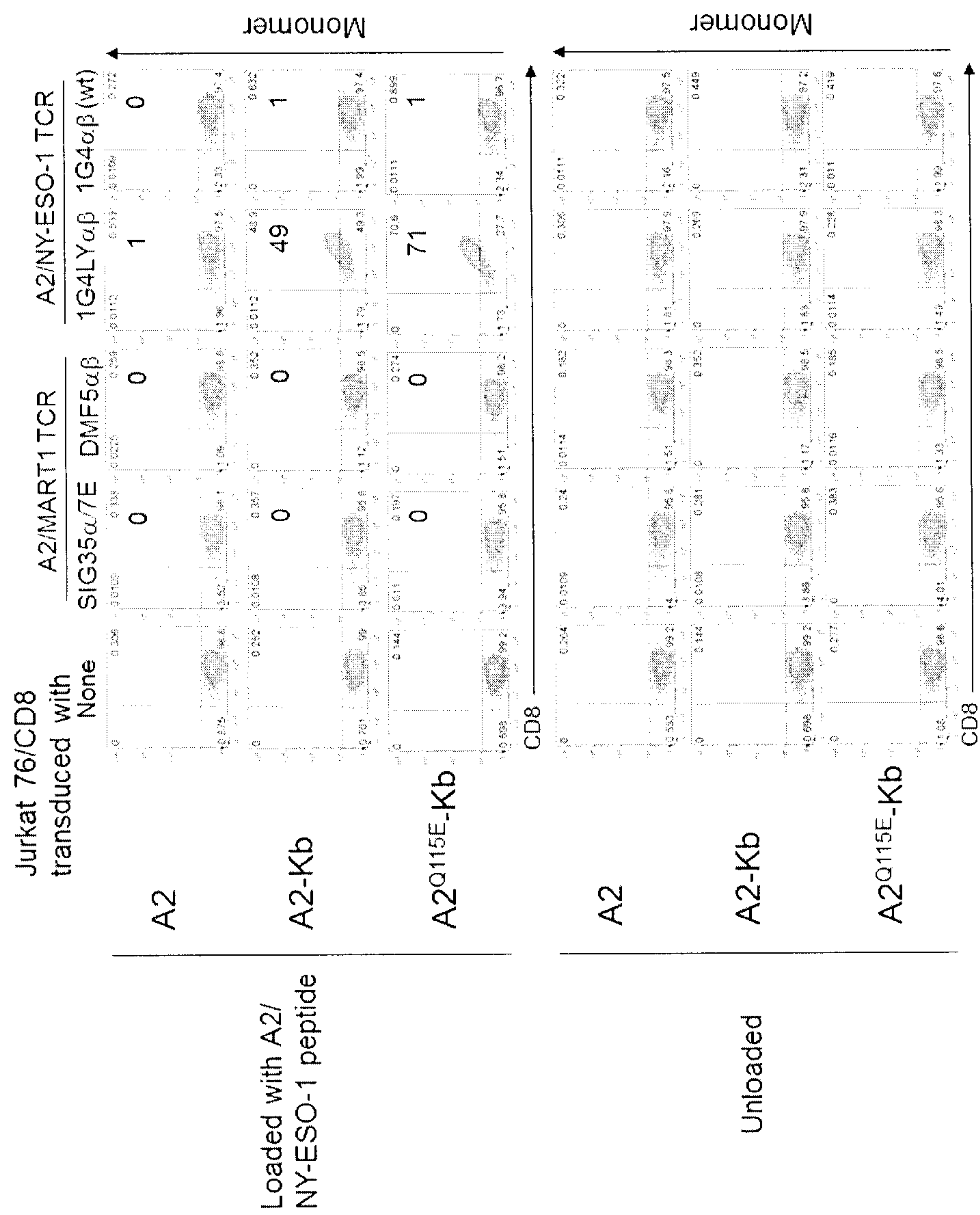


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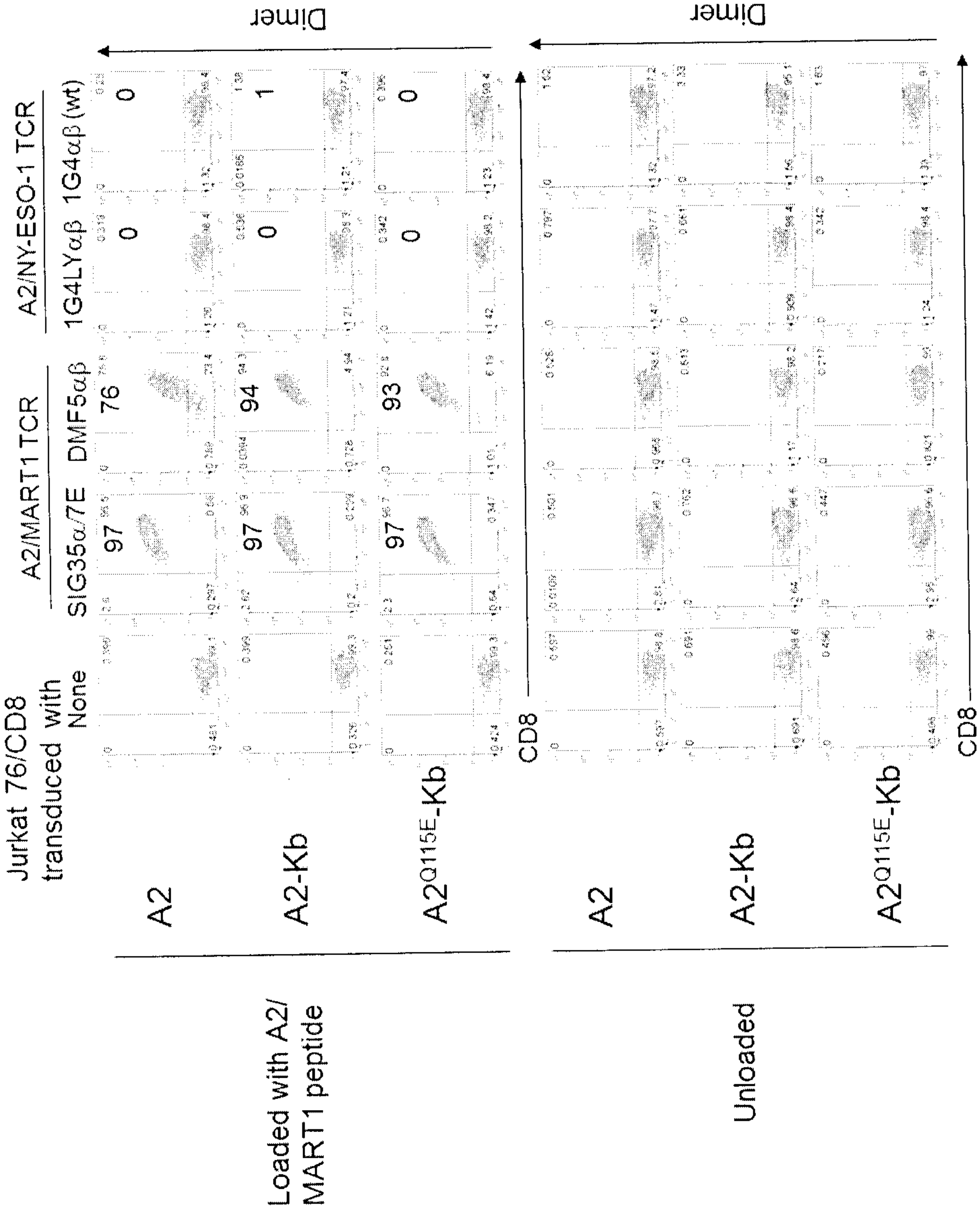


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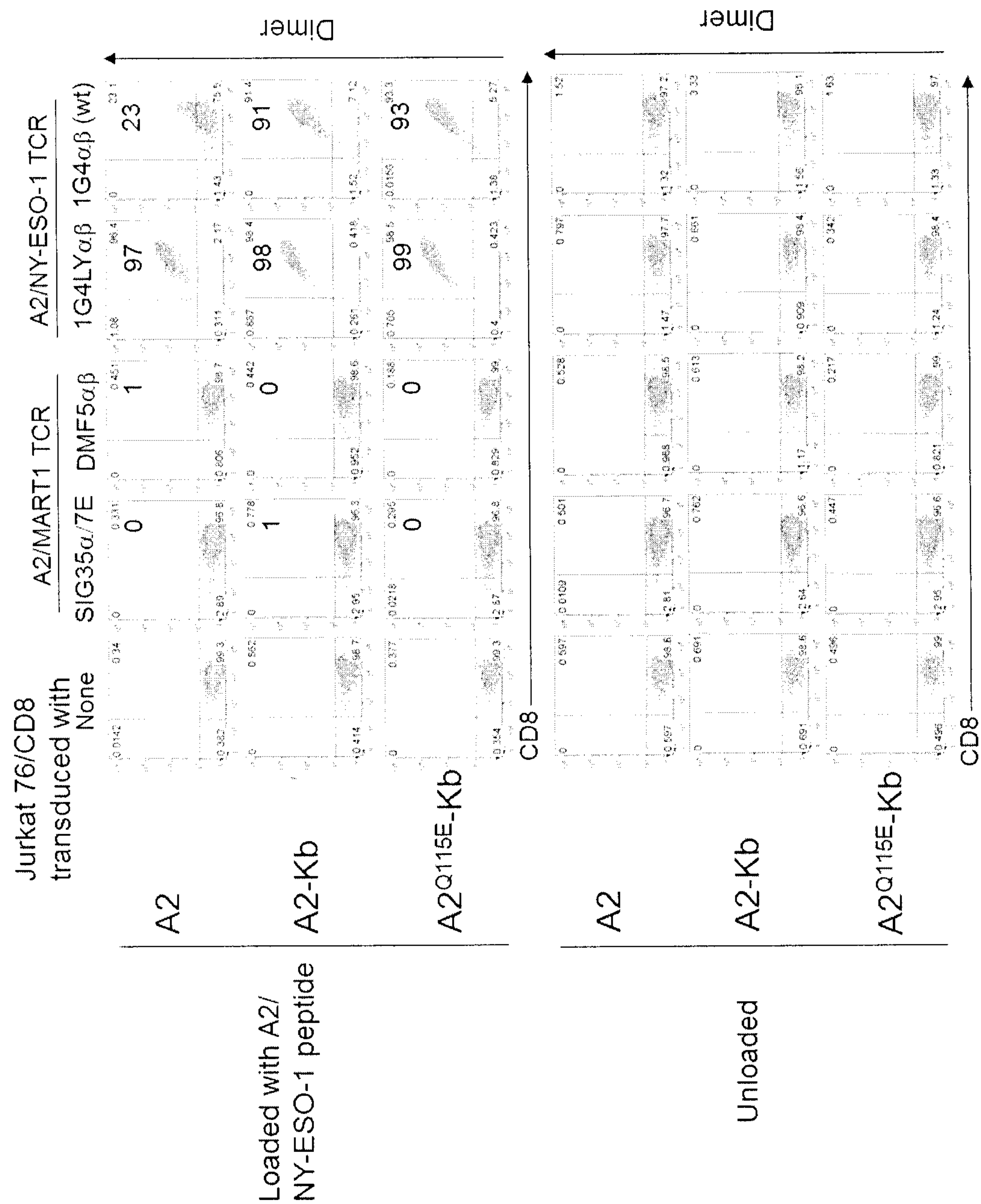


Figure 8

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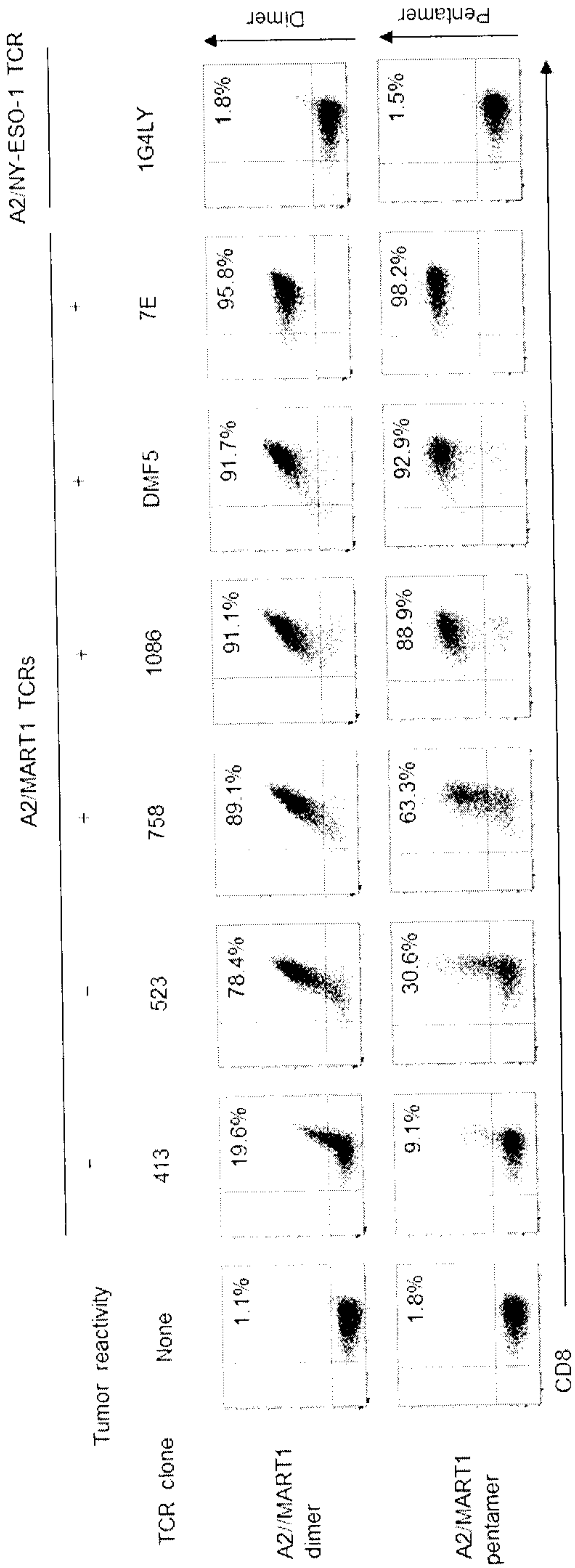
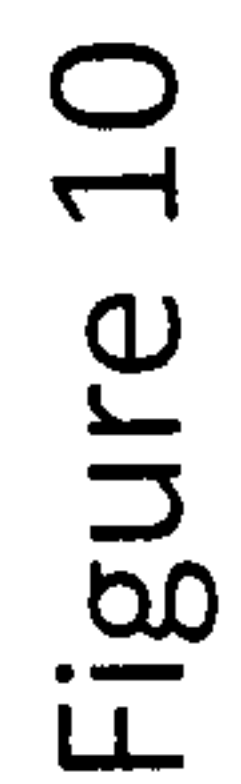


Figure 9

SUBSTITUTE SHEET (RULE 26)



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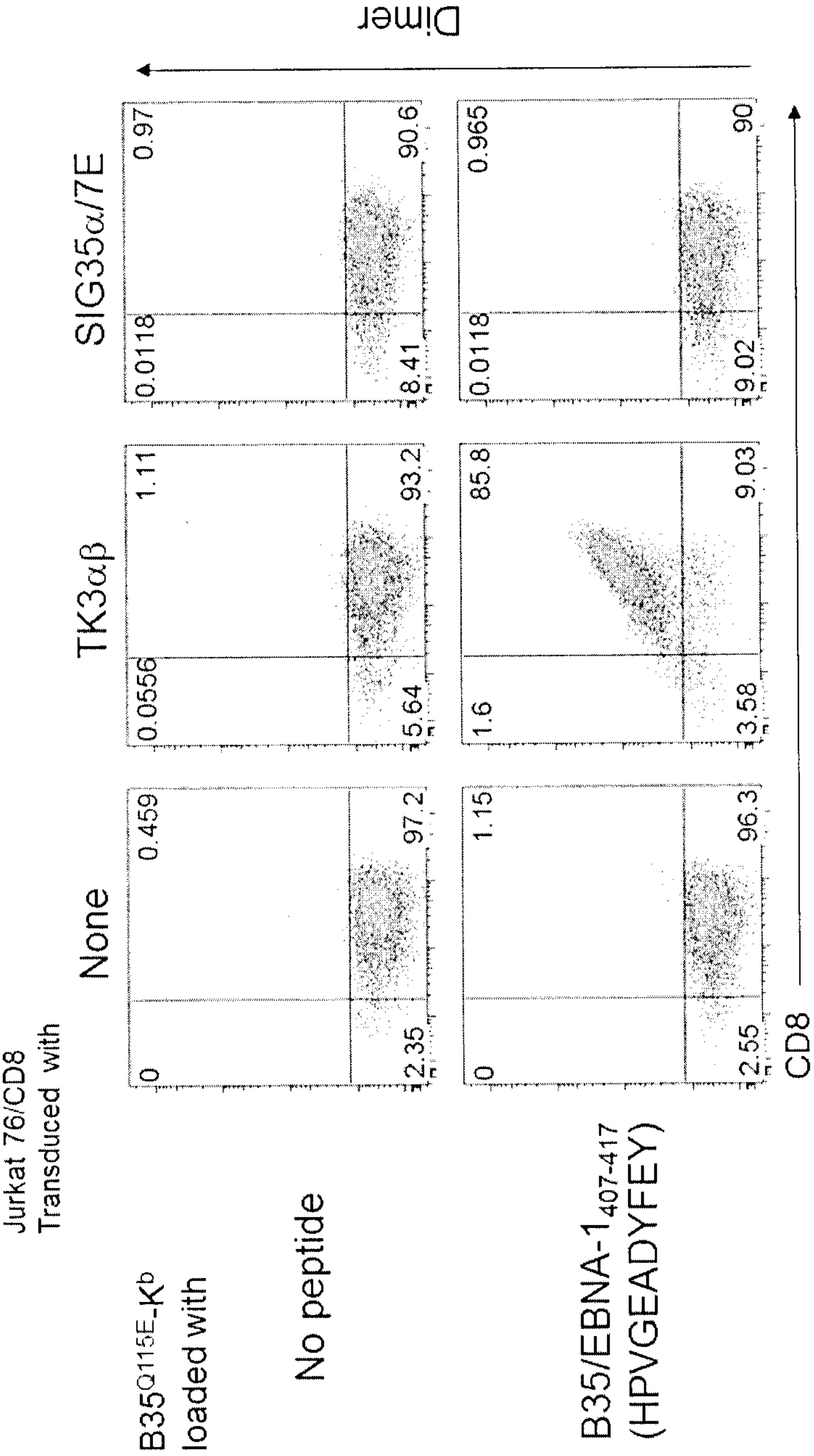


Figure 11

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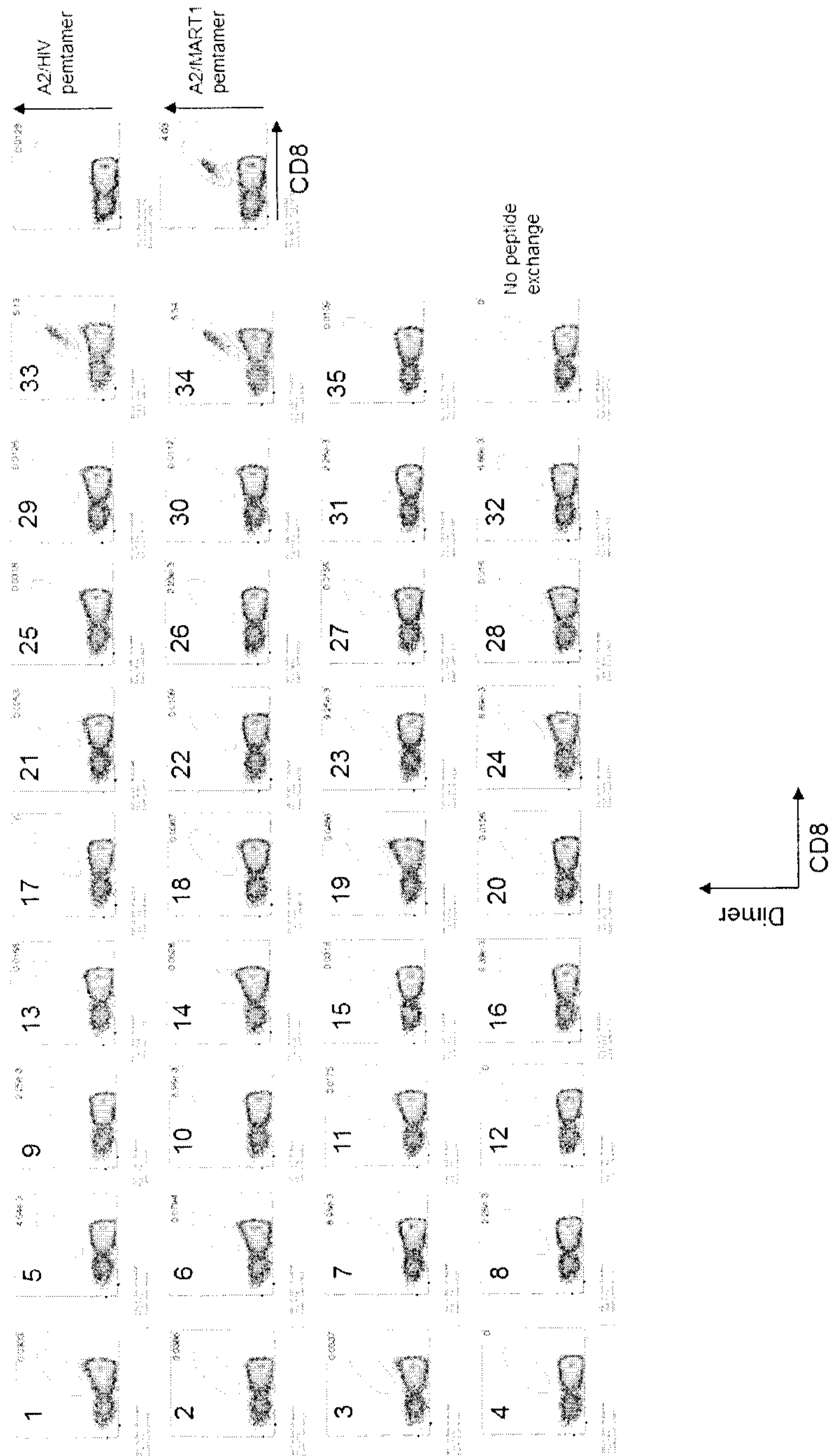


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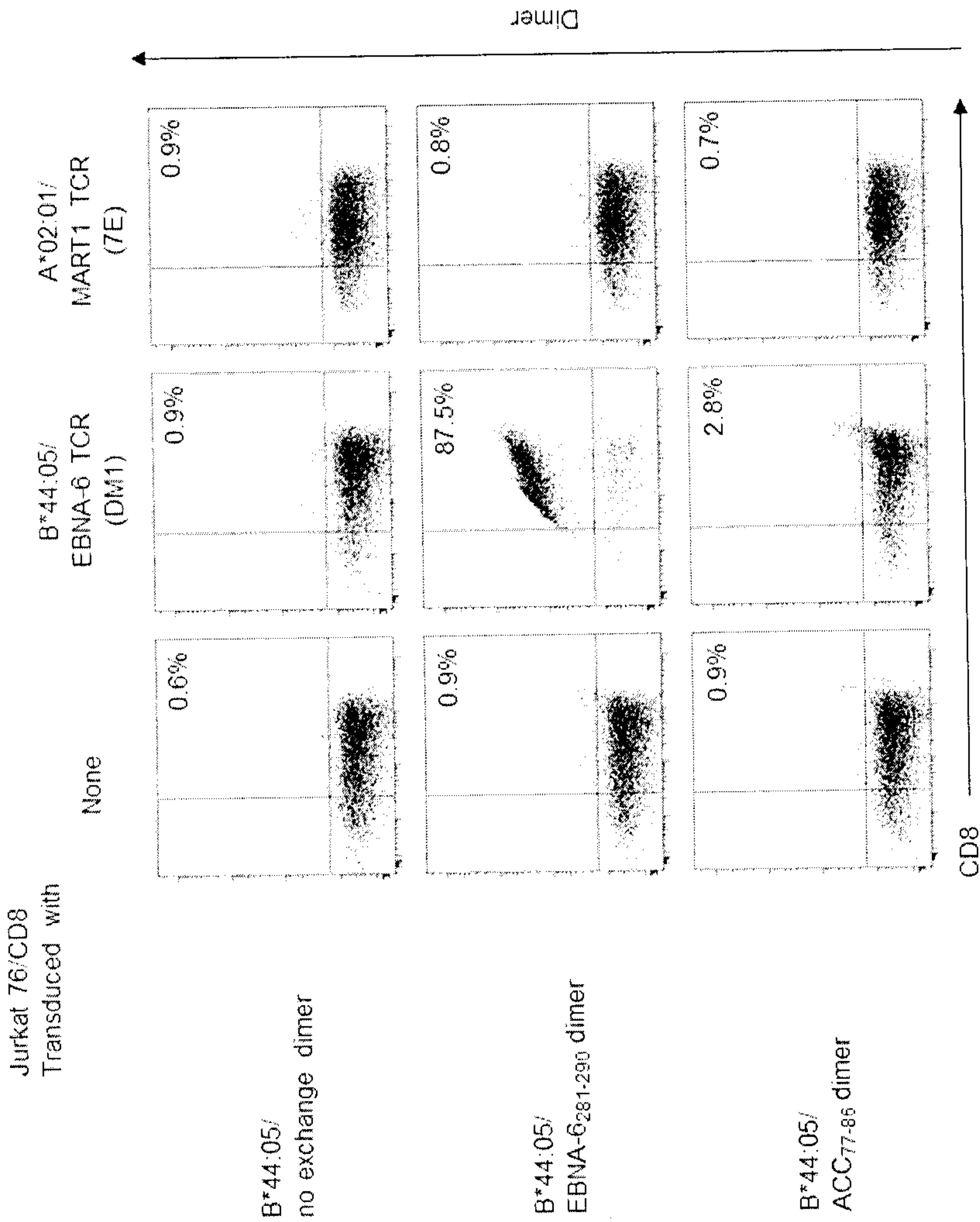


Figure 13

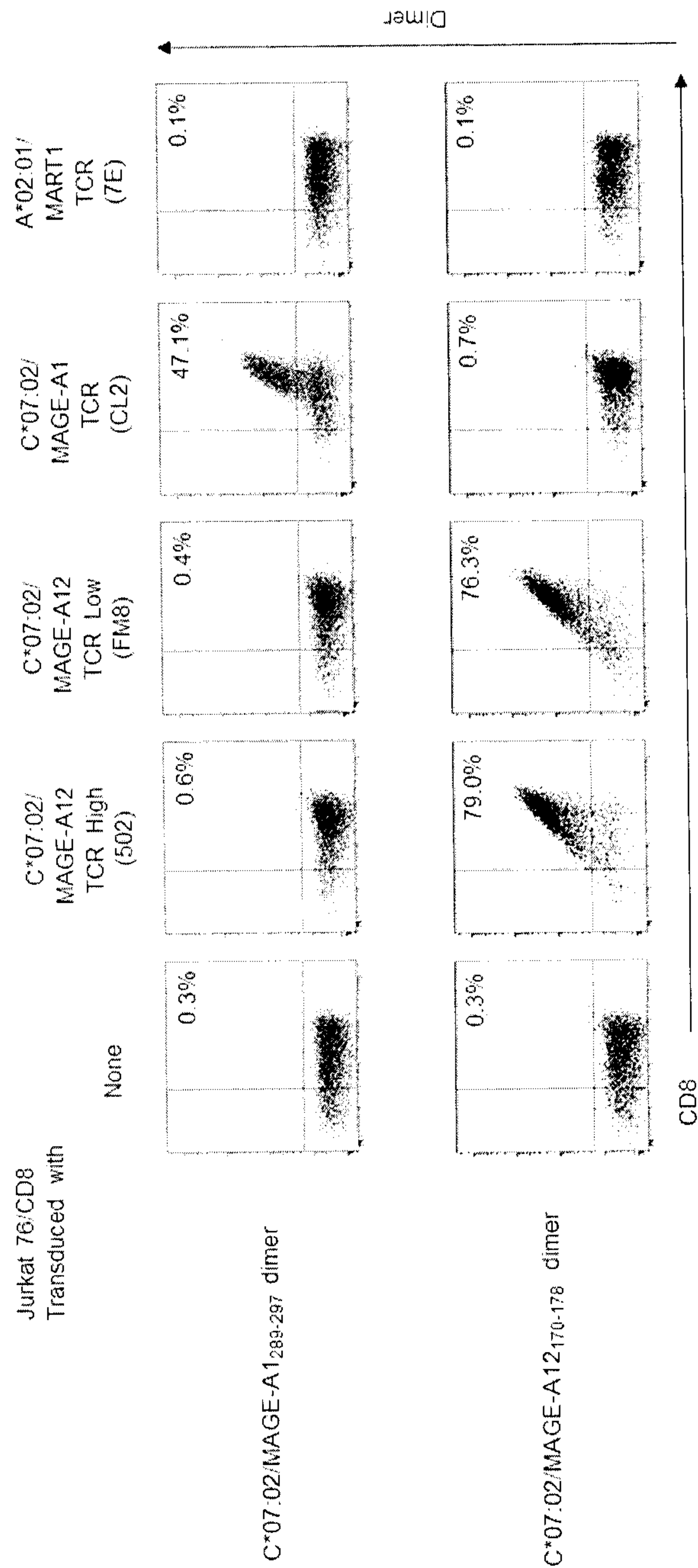


Figure 14

No.	Specimen code	HLA-A	HLA-B	HLA-C
1	M25	01:01/02:01	07:02/08:01	07:01/07:02
2	M29	02:01/11:01	07:02/27:02	02:02/07:02
3	M31	02:01/24:02	40:01/44:02	03:04/05:01
4	M37	02:01/24:02	14:02/48:01	08:02/08:03
5	M38	02:01/-	15:01/44:02	03:03/05:01
6	M40	02:01/30:02	18:01/45:01	05:01/06:02
7	M66	02:01/32:01	07:02/27:05	02:02/07:02
8	M69	02:01/03:01	08:01/14:02	07:01/08:02
9	M96	01:01/02:01	08:01/51:01	07:01/15:02

Figure 15

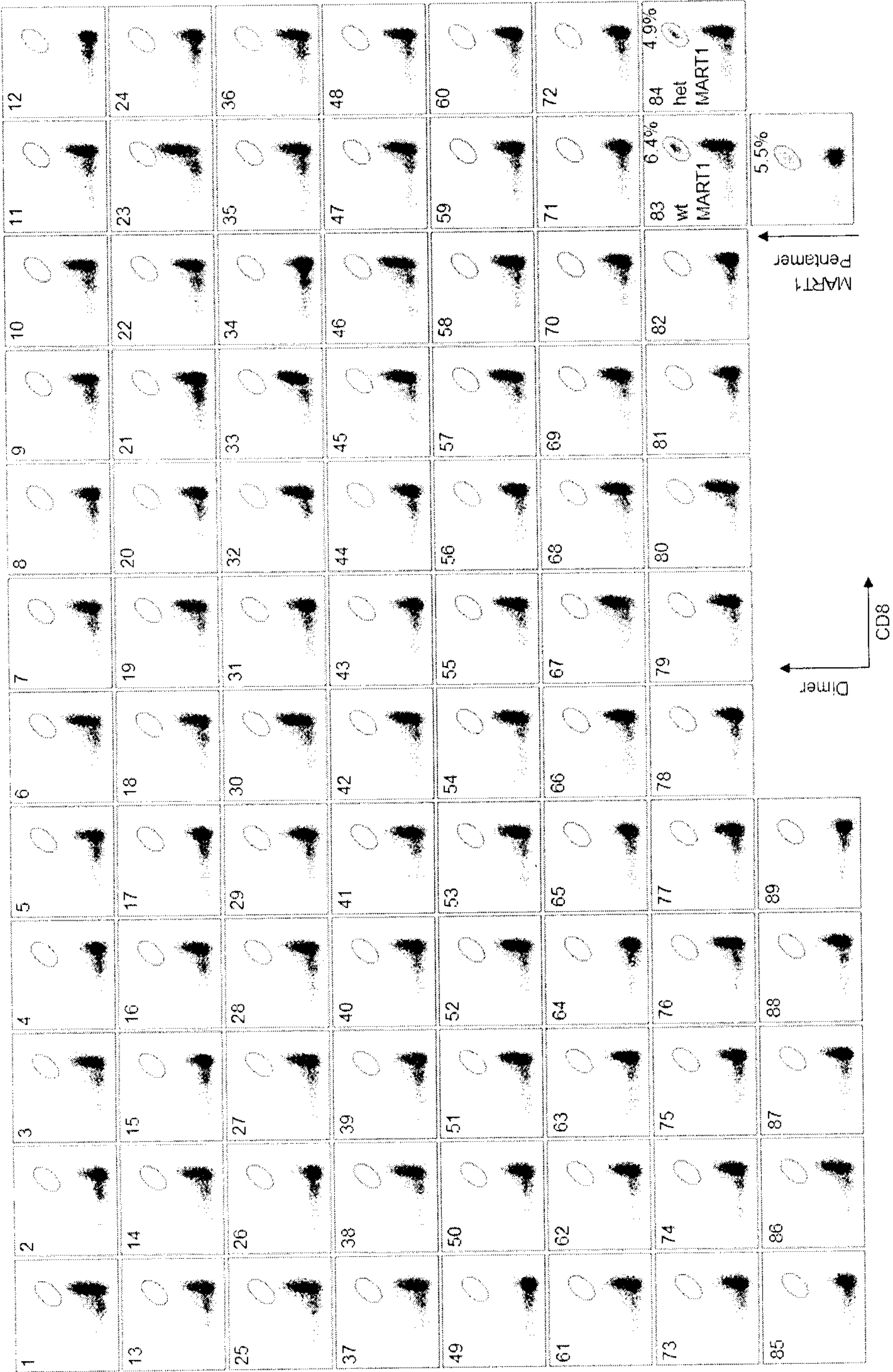


Figure 16

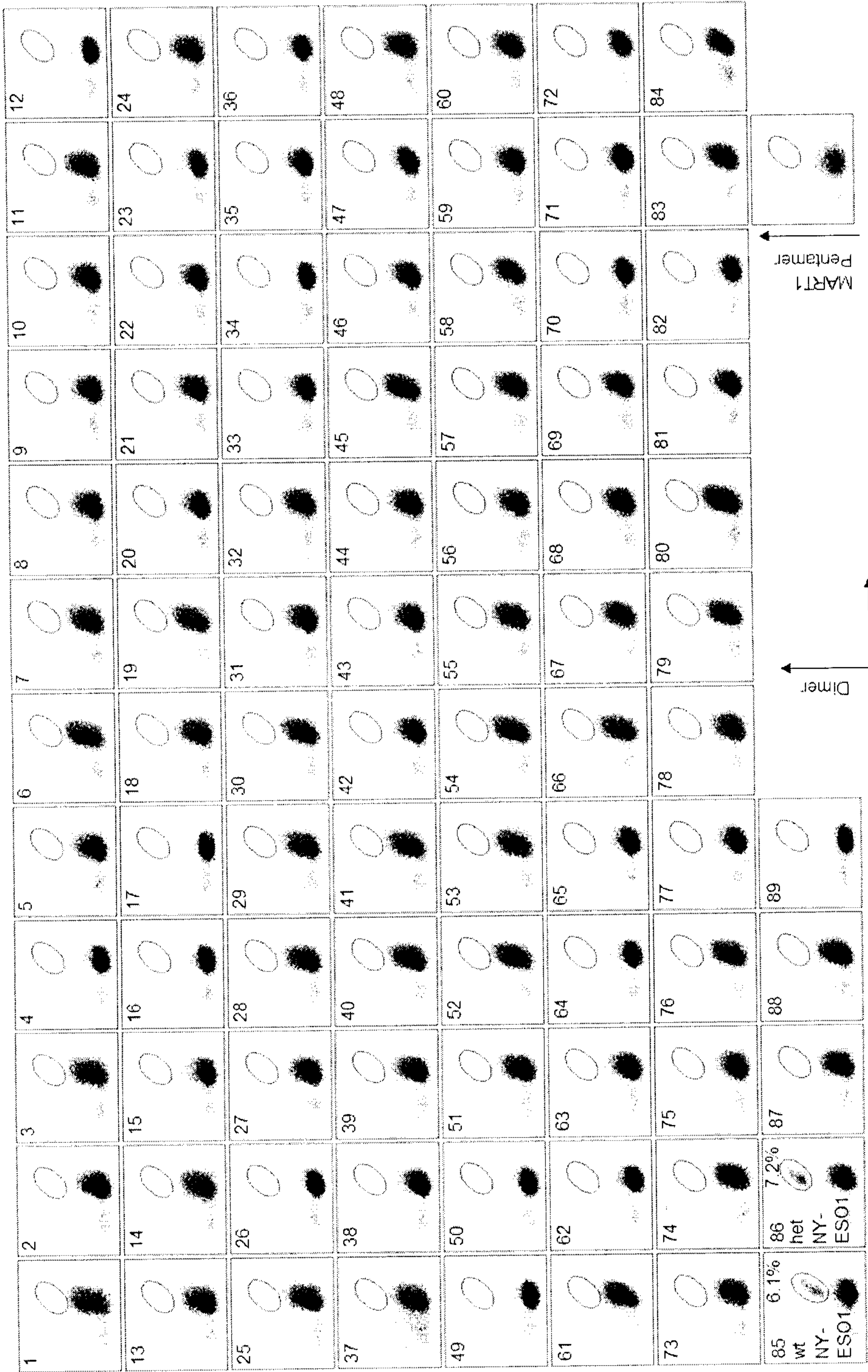


Figure 17

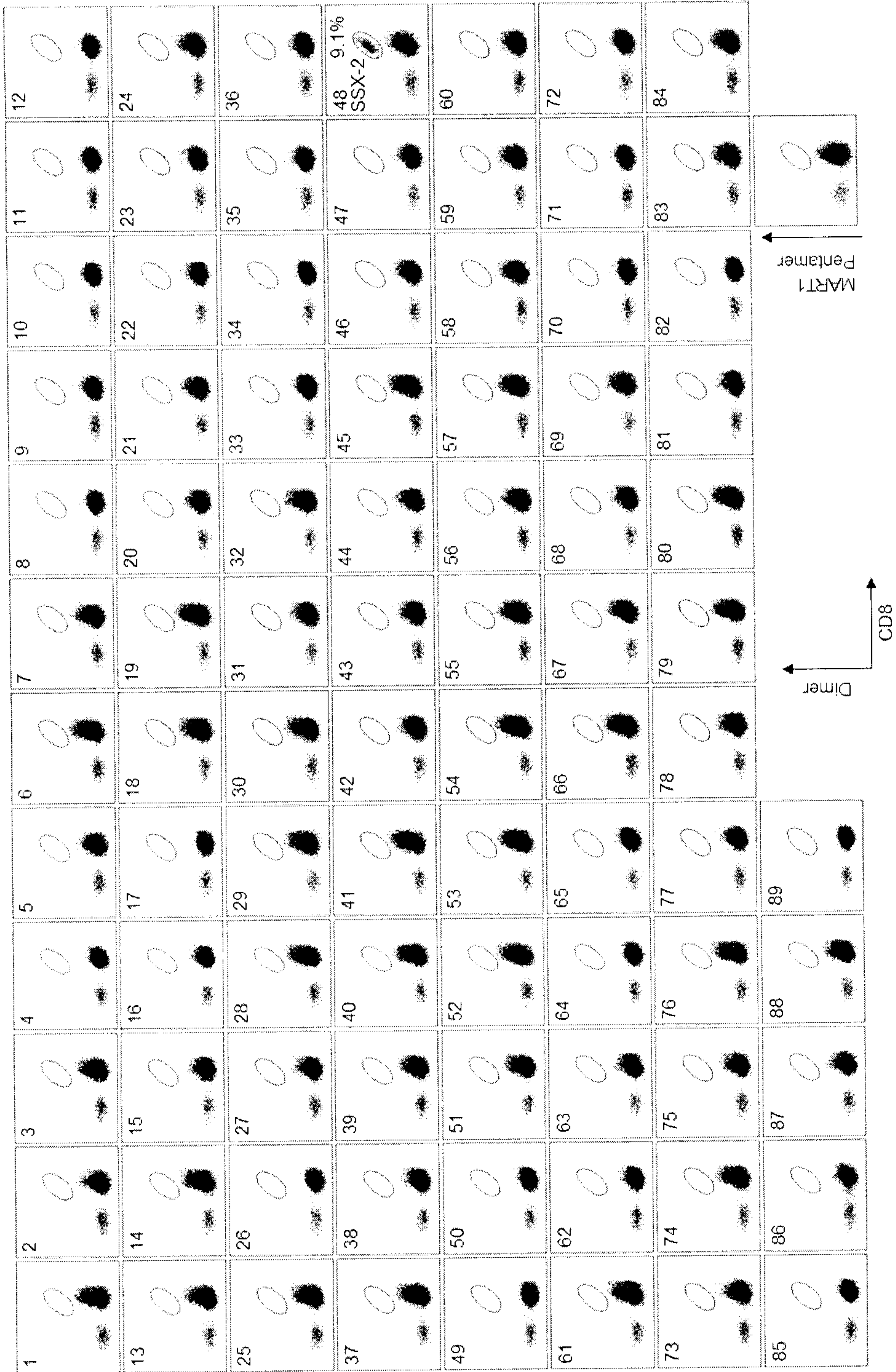


Figure 18

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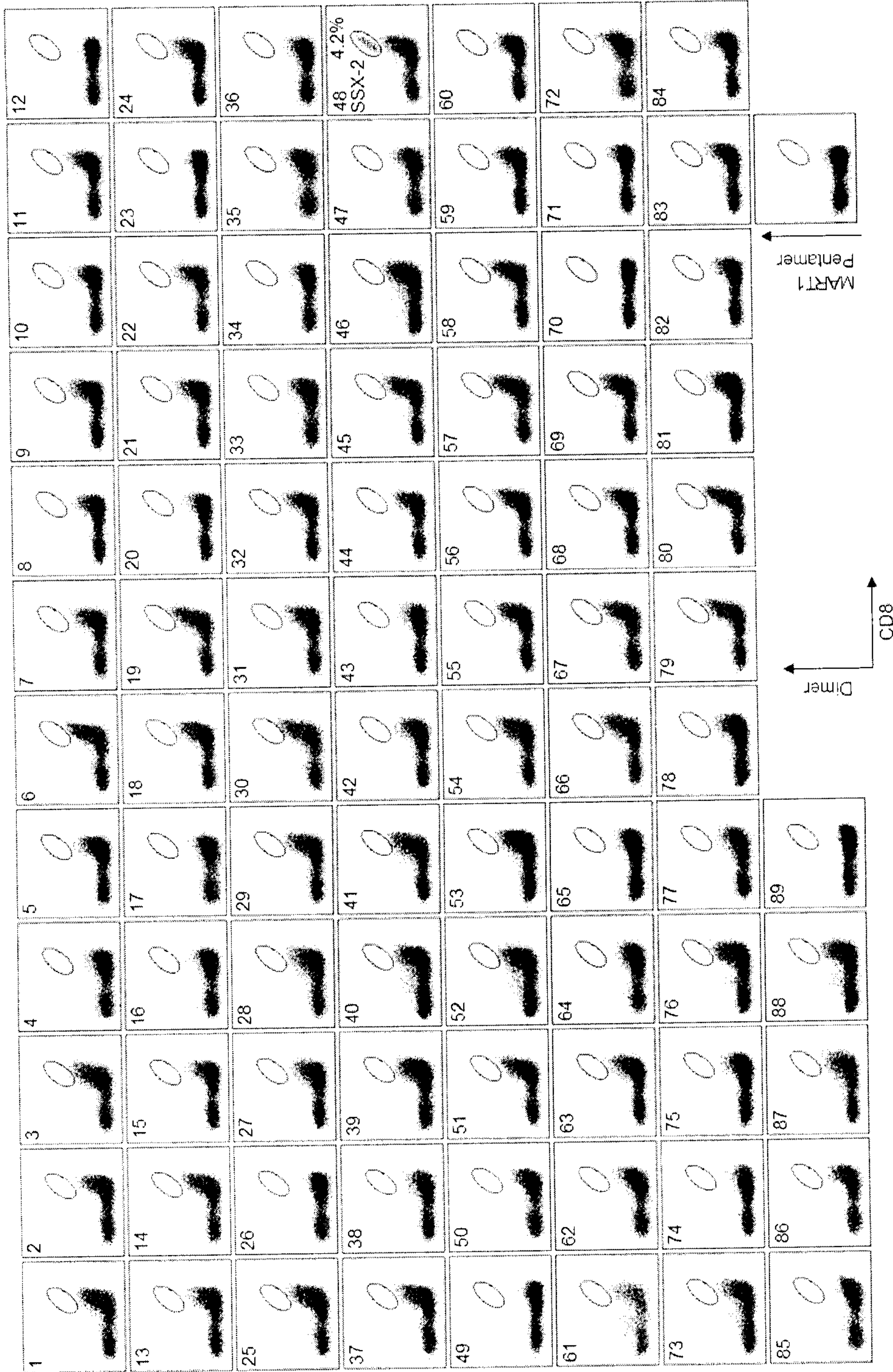


Figure 19

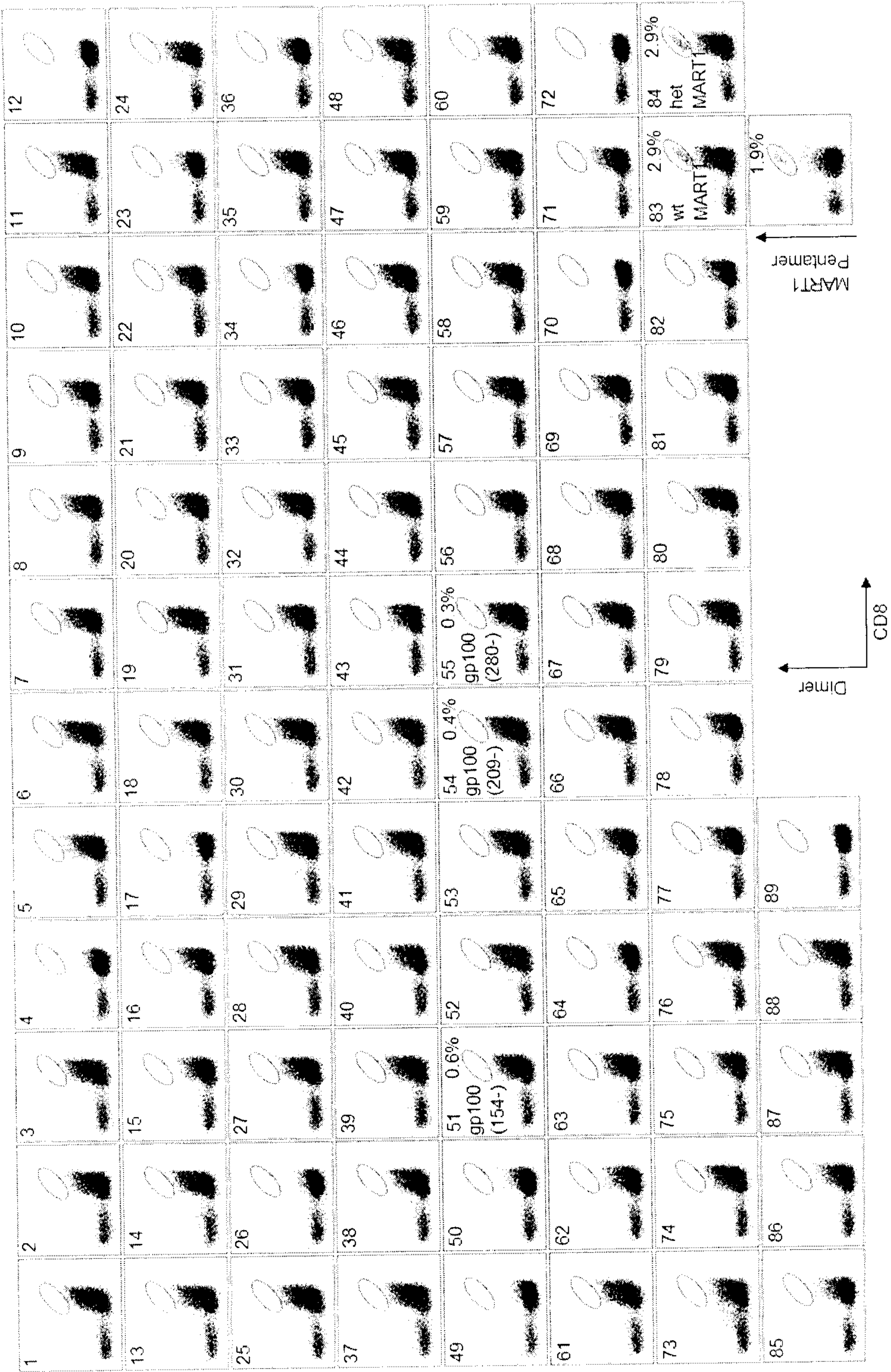


Figure 20

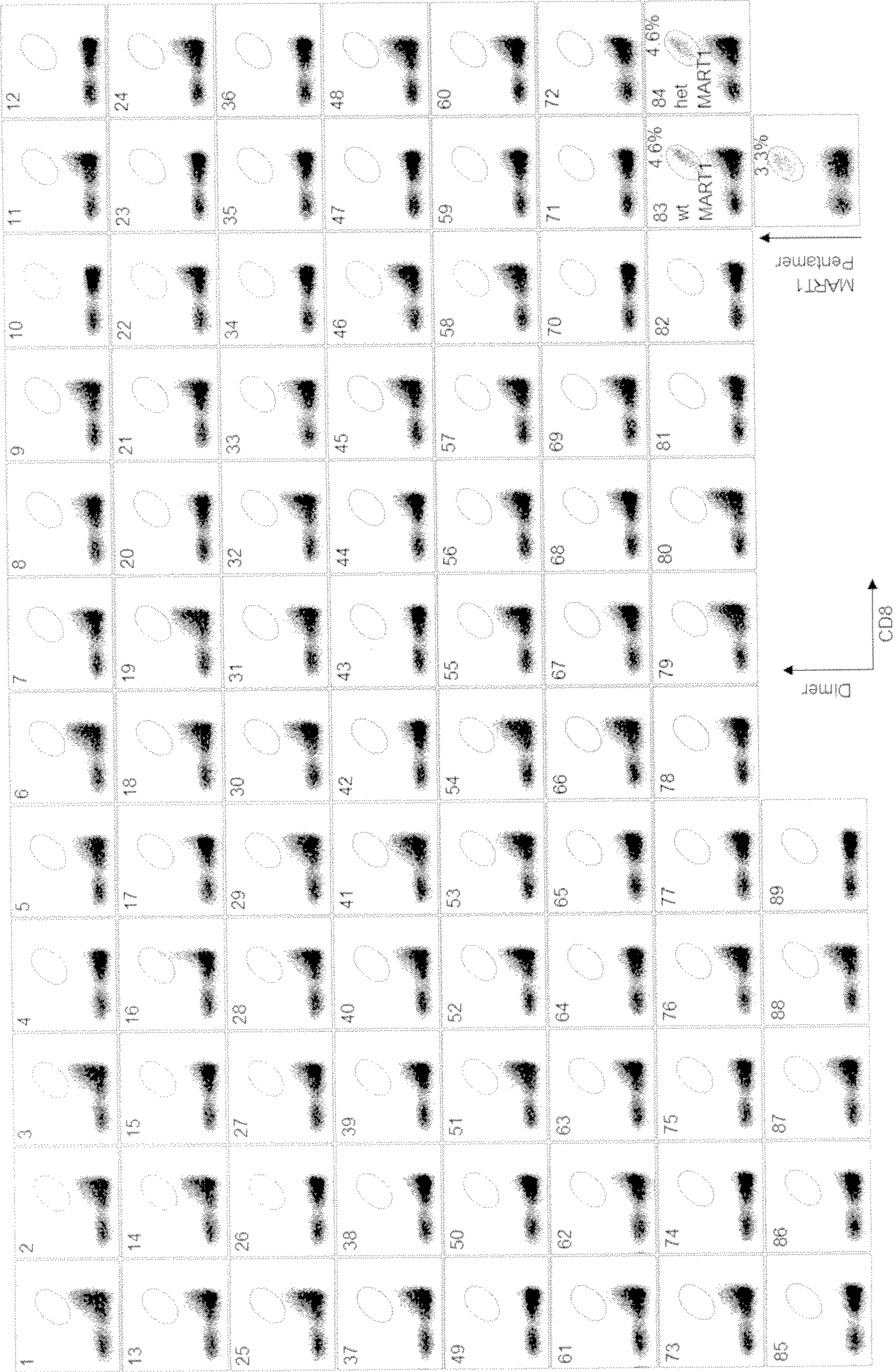
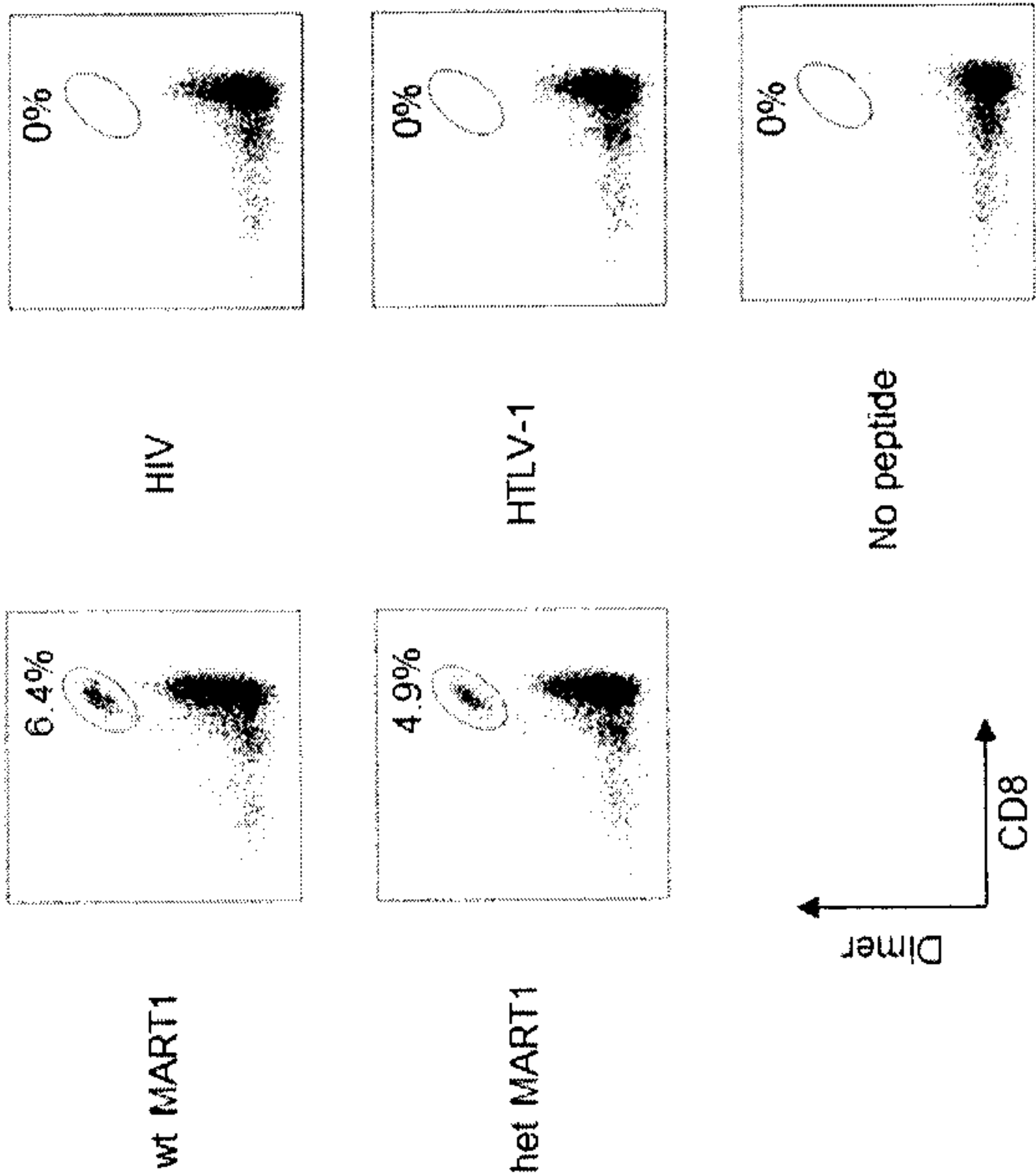
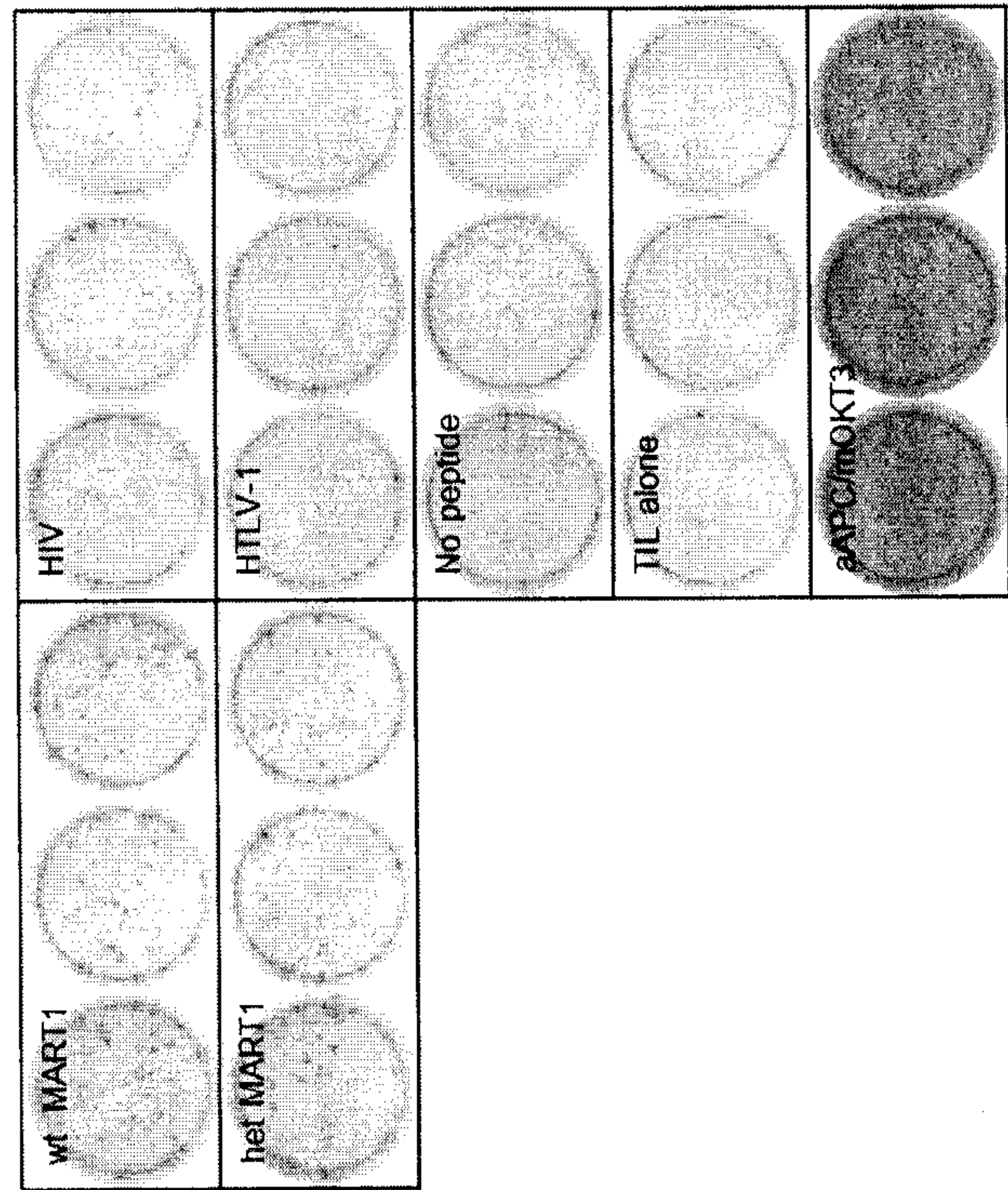
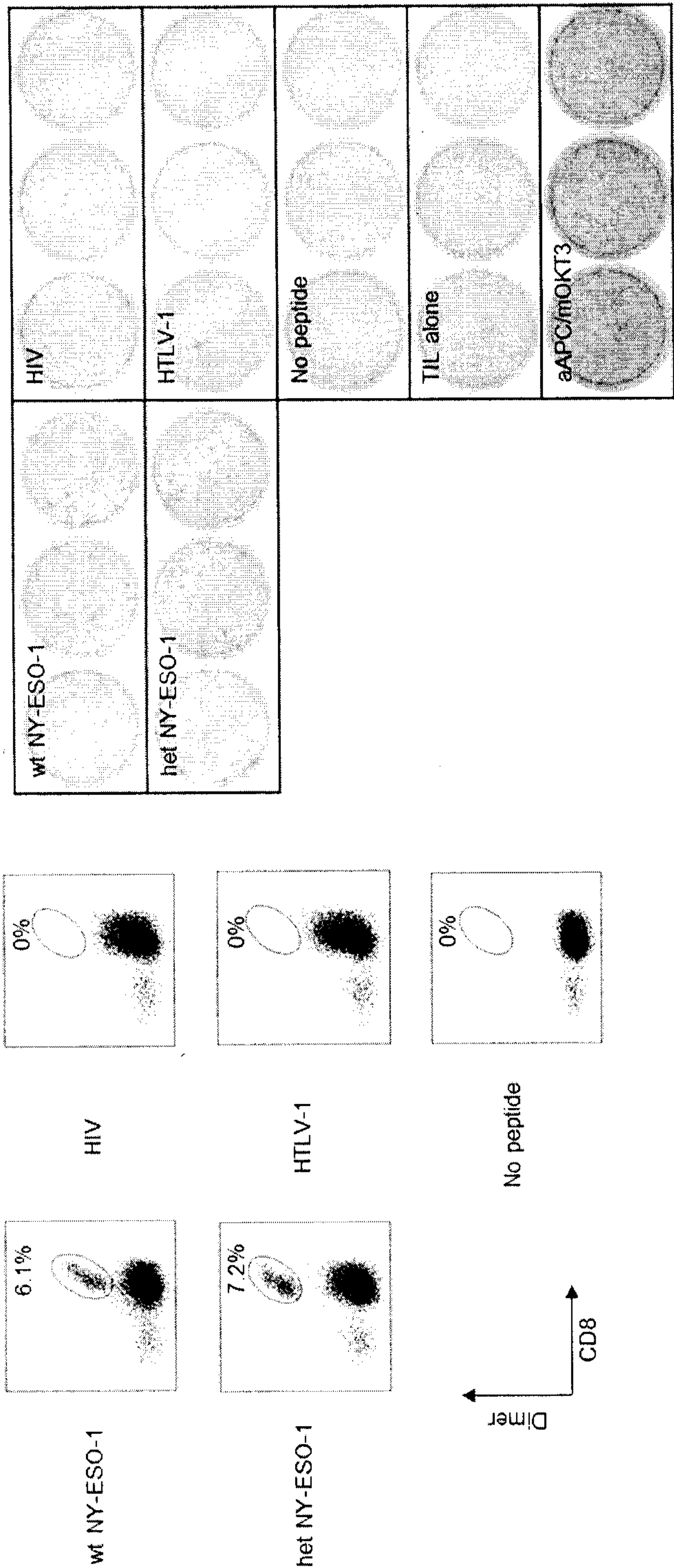


Figure 21



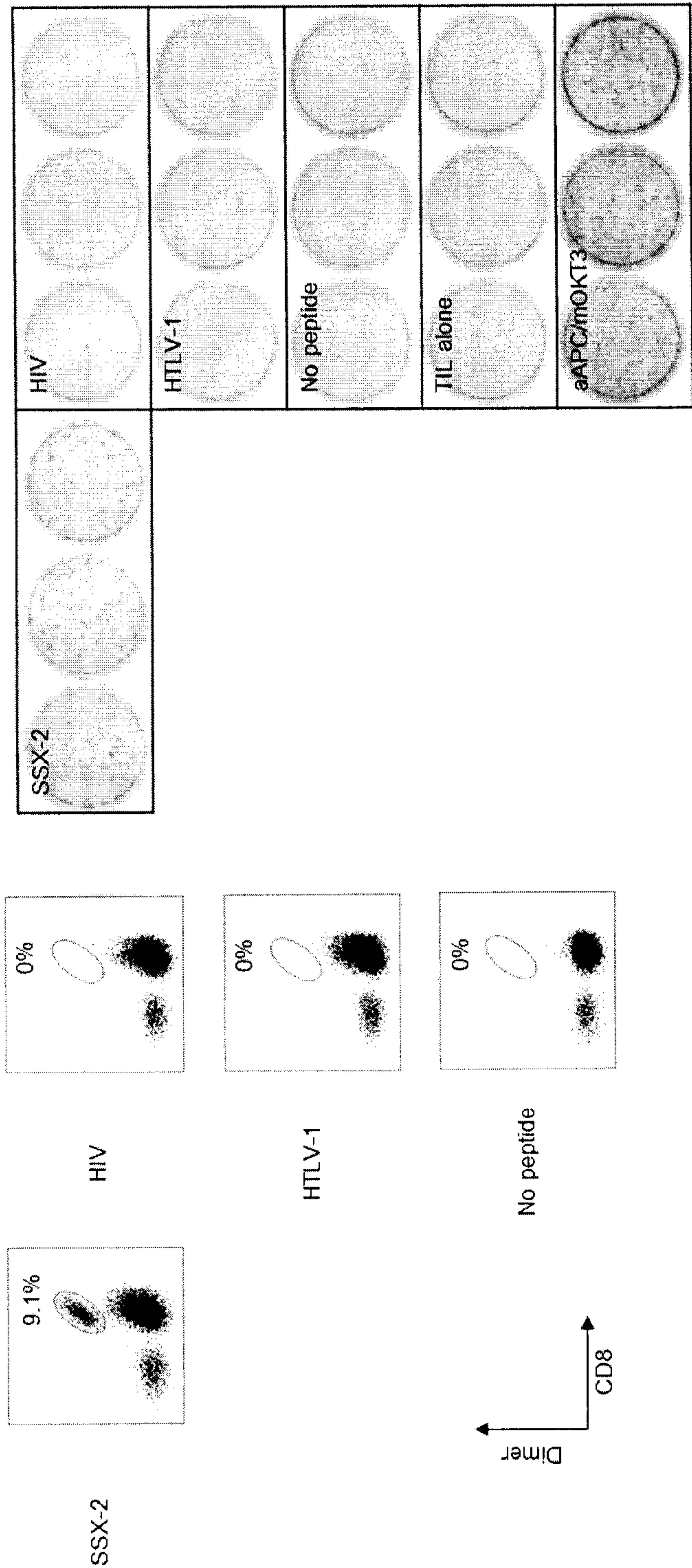
Stimulator cells : 2x10⁴ T2 + peptide / well
Responder cells : 1x10⁵ TILs / well

Figure 22



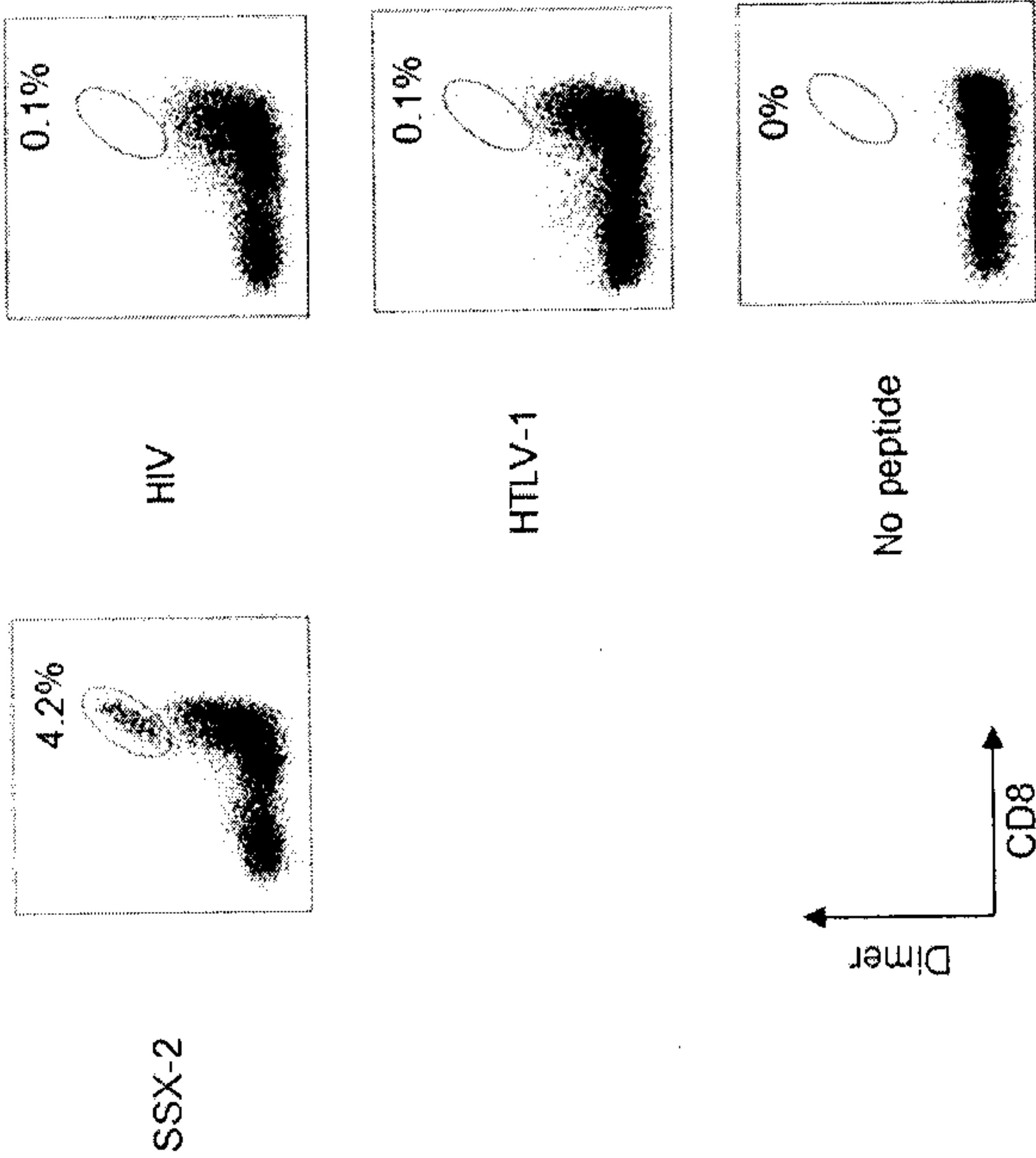
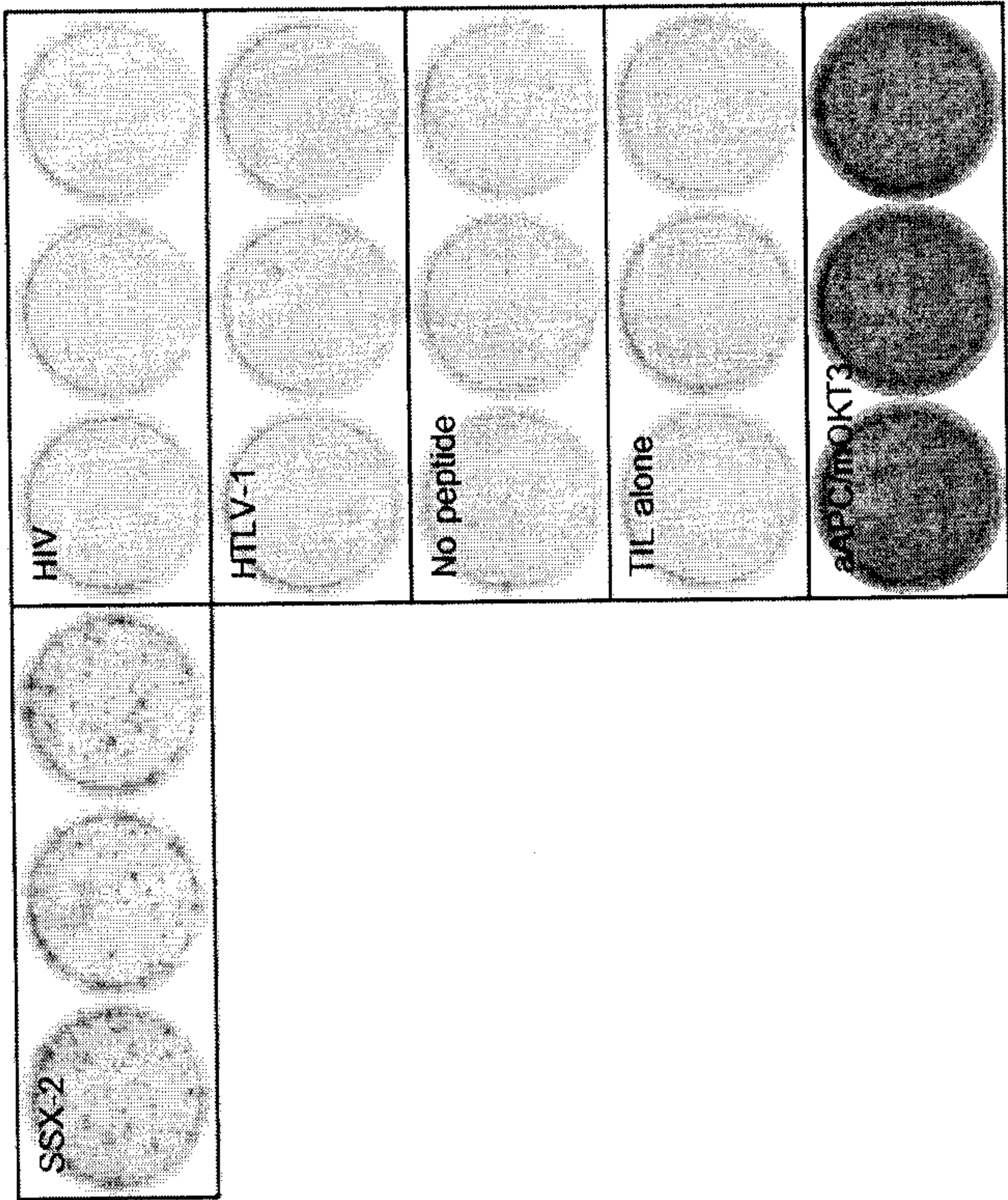
Stimulator cells : 2x10⁴ T2 + peptide / well
Responder cells : 1x10⁵ TILs / well

Figure 23



Stimulator cells : 2x10⁴ T2 + peptide / well
Responder cells : 1x10⁵ TILs / well

Figure 24



Stimulator cells : 2x10⁴ T2 + peptide / well
Responder cells : 1x10⁵ TILs / well

Figure 25

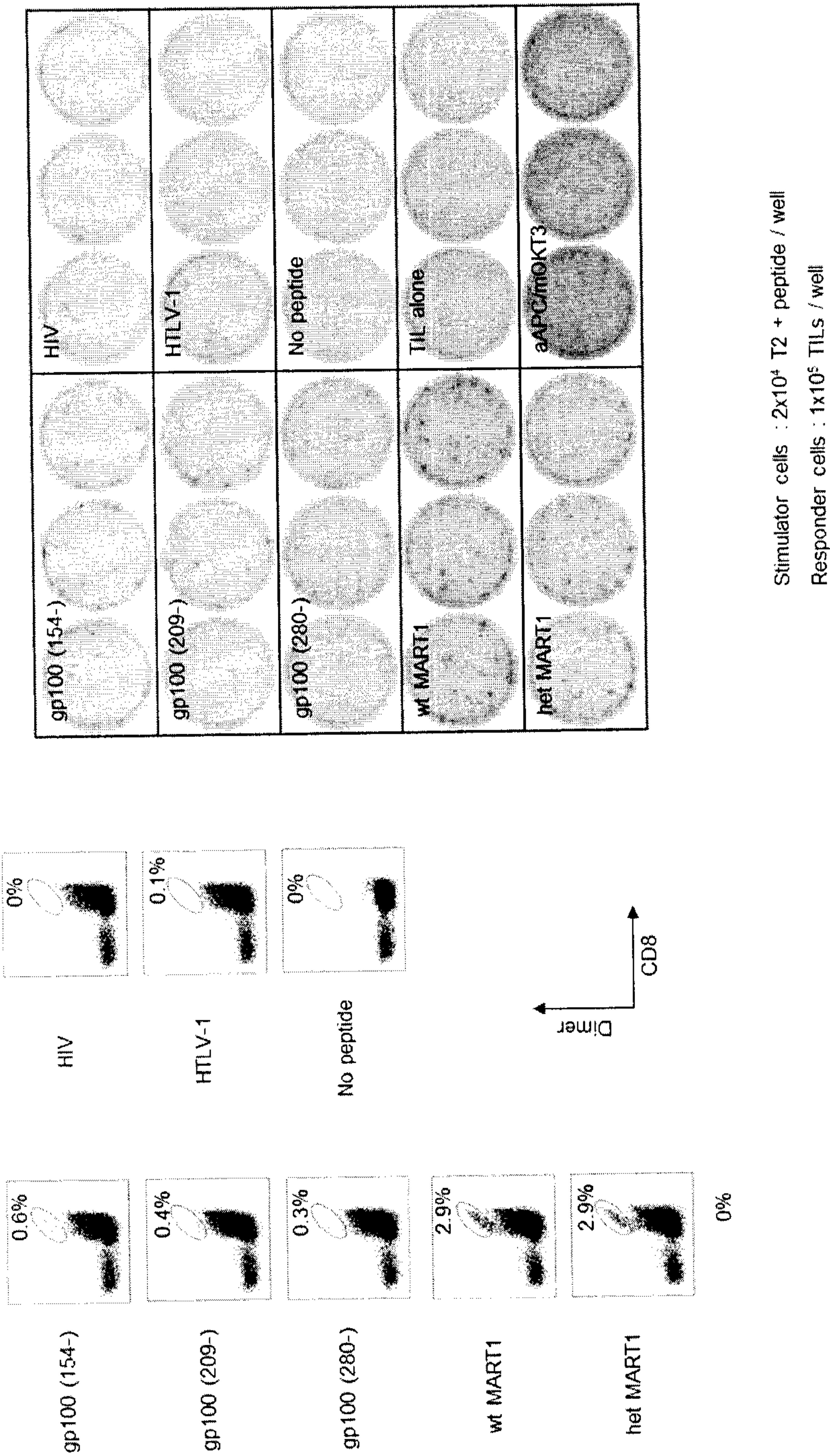
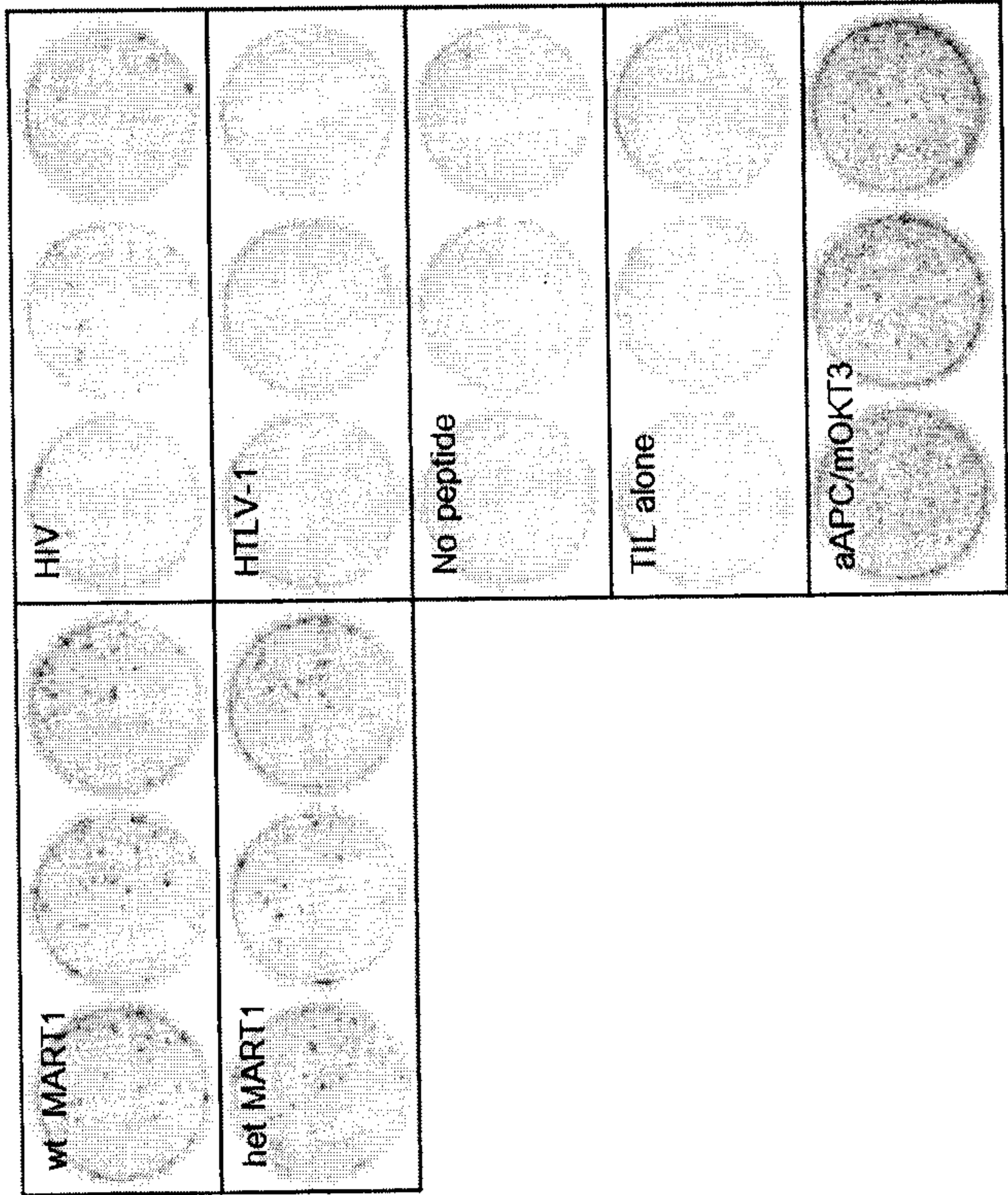
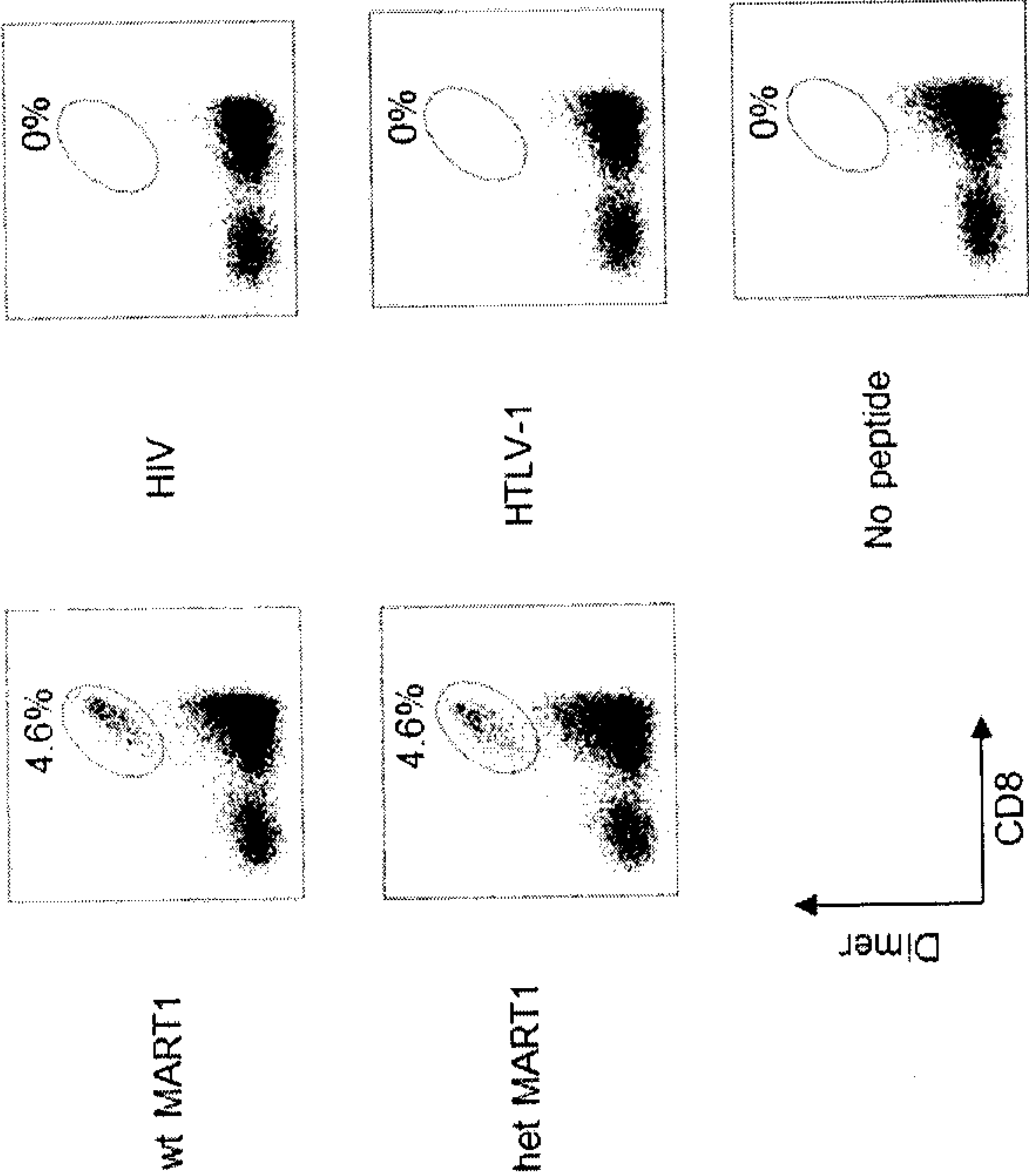


Figure 26



Stimulator cells : 2x10⁴ T2 + peptide / well
Responder cells : 1x10⁵ TILs / well

Figure 27



No.	Specimen code	HLA-A	HLA-B	HLA-C	Positive A2 dimer staining (> 0.3%)	ELISPOT
1	M25	01:01/02:01	07:02/08:01	07:01/07:02	Wild type MART-1 Heteroclitic MART-1	+ +
2	M29	02:01/11:01	07:02/27:02	02:02/07:02	None	NT
3	M31	02:01/24:02	40:01/44:02	03:04/05:01	Wild type NY-ESO-1 Heteroclitic NY-ESO-1	+ +
4	M37	02:01/24:02	14:02/48:01	08:02/08:03	SSX-2 (41-)	+
5	M38	02:01/-	15:01/44:02	03:03/05:01	None	NT
6	M40	02:01/30:02	18:01/45:01	05:01/06:02	SSX-2 (41-)	+
7	M66	02:01/32:01	07:02/27:05	02:02/07:02	gp-100 (154-) gp-100 (209-) gp-100 (280-) Wild type MART-1 Heteroclitic MART-1	+ + + + +
8	M69	02:01/03:01	08:01/14:02	07:01/08:02	None	NT
9	M96	01:01/02:01	08:01/51:01	07:01/15:02	Wild type MART-1 Heteroclitic MART-1	+ +

Figure 28

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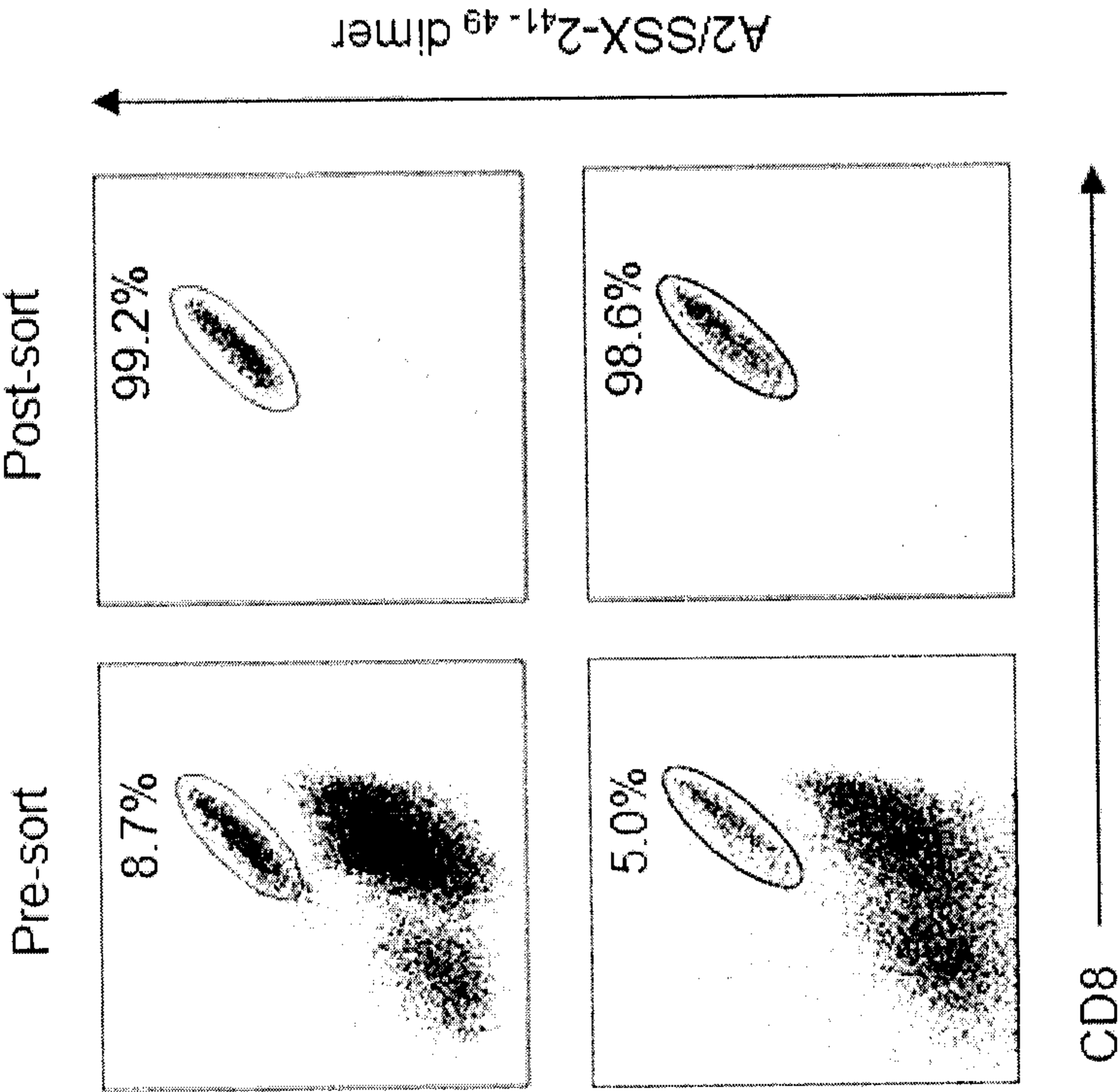


Figure 29

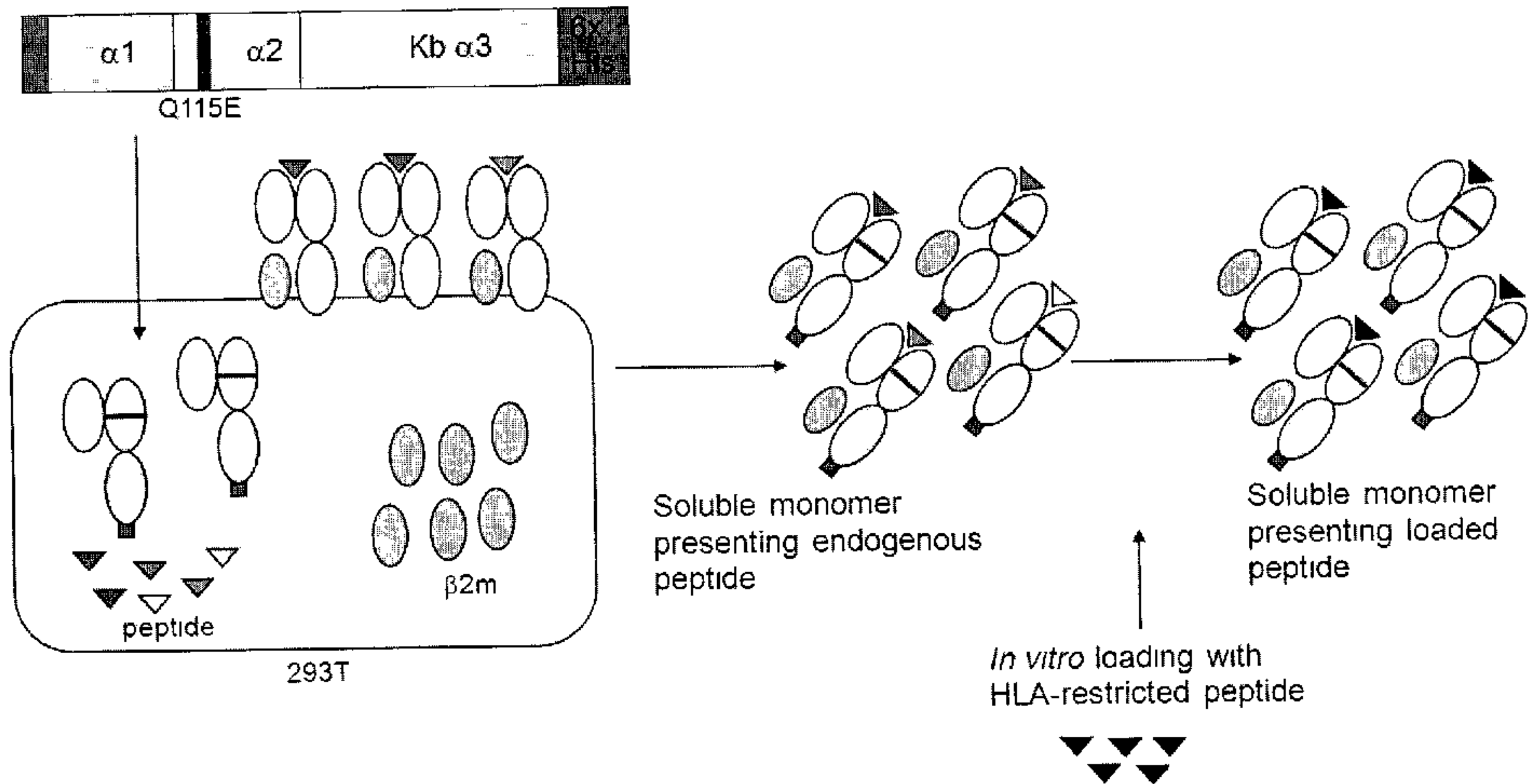


Figure 2