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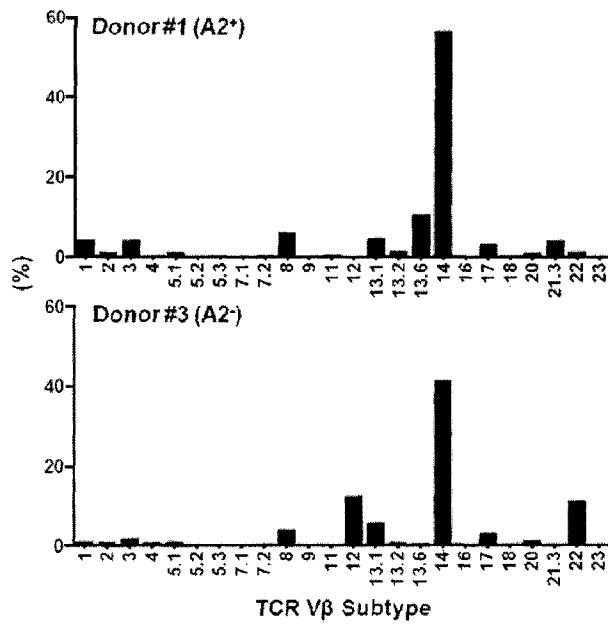
(57) Abrégé/Abstract:
Provided is a method for determining a TCR polypeptide chain that can form a TCR specific for a peptide of interest. Also provided are methods and compositions for producing a cell expressing a T cell receptor (TCR) specific for a peptide of interest, methods and compositions for producing a TCR chain nucleic acid and/or pair of TCR chain polypeptides and/or nucleic acids encoding a TCR, a cell population comprising the cell harboring the nucleic acids encoding a TCR obtained by said method, and a method for treating a disorder comprising administering to the subject said cell population.

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Fig. 3

(57) Abstract: Provided is a method for determining a TCR polypeptide chain that can form a TCR specific for a peptide of interest. Also provided are methods and compositions for producing a cell expressing a T cell receptor (TCR) specific for a peptide of interest, methods and compositions for producing a TCR chain nucleic acid and/or pair of TCR chain polypeptides and/or nucleic acids encoding a TCR, a cell population comprising the cell harboring the nucleic acids encoding a TCR obtained by said method, and a method for treating a disorder comprising administering to the subject said cell population.

TITLE: METHODS AND COMPOSITIONS FOR PRODUCING A CELL EXPRESSING A T CELL RECEPTOR

[0001]

TECHNICAL FIELD

[0002]   The present disclosure relates to methods and compositions for producing a recombinant cell expressing a T cell receptor (TCR) specific for a peptide of interest, methods and compositions for obtaining a nucleic acid or pair of TCR chain polypeptides and/or nucleic acids encoding a TCR, a cell population comprising the recombinant cell harboring the one or more nucleic acids encoding a TCR or TCR chain obtained by said method, and a method for treating a disorder comprising administering to the subject said cell population.

INTRODUCTION

[0003]   Gene transfer of tumor reactive TCR has been shown to confer both high avidity and tumor reactivity to non-reactive peripheral blood mononuclear cells (PBMCs) and tumor infiltrating lymphocytes (TILs) (Johnson et al. 2006). These authors reported that generating TIL clones directly from melanoma patient tumor digests reveals a diversity of MART-1 reactive T cells with varying cellular avidities and that TCR transfer is sufficient to confer overall cellular avidity to donor PBMCs in an antigen specific manner. Further, nonreactive TIL can be made tumor reactive upon RNA electroporation with a high avidity MART-1 TCR. Recent clinical trials have demonstrated that adoptive transfer of T cells transduced with anti-tumor TCR can induce sustained objective clinical responses in patients with cancer (Kunert A et al., 2013, Hinrichs CS, et al., 2014).

[0004]   Most of the tumor associated-antigens identified so far are self-antigens. Because of central and peripheral T cell tolerance, a majority of TCRs cloned from peripheral T cells possess low affinity, which is not sufficient to recognize tumor cells expressing self-antigens as tumor-associated antigens. Methods for identifying or generating high affinity TCRs include bacteriophage display mutation and selection technology (Li et al., 2005) and amino acid substitution in TCR CDRs (Robbins et al., 2008). TCR variants identified using these methods can possess affinities that range 1 million fold between the wild-type receptor and that of the tightest binding TCR.

SUMMARY OF THE DISCLOSURE

[0005]   The present disclosure relates to in an aspect a method for producing a recombinant cell expressing a T cell receptor (TCR) specific for a peptide of interest, which comprises the step of transducing a cell population with a nucleic acid which encodes either one of two polypeptide chains constituting a TCR expressed and previously isolated from a T cell recognizing said peptide of interest, wherein said cell population comprises a cell which is able to express a TCR or differentiate into a cell expressing a TCR. In an embodiment, the method comprises the step of culturing the transduced cell population with an antigen presenting cell presenting the peptide of interest. In another embodiment, the method further comprises the step of selecting a cell expressing a TCR specific for a peptide of interest from the transduced cell population. In yet another embodiment; said cell population is a population of PBMCs or PBMCs activated with a CD3 ligand. In another embodiment, said nucleic acid transduced into said cell population encodes a TCR alpha chain or a TCR beta chain. In yet another embodiment, said nucleic acid transduced into said cell population encodes a TCR chain which predominantly contributes to peptide recognition by a TCR.

[0006]   An aspect includes a method for generating a high affinity TCR specific for a peptide of interest comprising:

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a)   transducing a cell population comprising cells able to express a TCR and/or differentiate into a cell expressing a TCR with a bait nucleic acid encoding a bait TCR polypeptide chain, wherein the bait TCR polypeptide chain can constitute a parent TCR with a counterchain TCR polypeptide chain that specifically binds said peptide of interest; and

b)   culturing under conditions that permit the bait TCR to be expressed.

[0007]   In an embodiment, the method further comprises selecting a cell expressing a TCR comprising the bait TCR polypeptide chain and a prey TCR polypeptide chain that selectively binds said peptide of interest from the transduced cell population obtained in step (a) or (b).

[0008]   In another embodiment, the method further comprises isolating a prey nucleic acid encoding the prey TCR polypeptide chain from the selected cell.

[0009]   Another aspect includes a method for obtaining a TCR polypeptide chain that can form a TCR specific for a peptide of interest comprising:

a)   transducing a cell population comprising cells able to express a TCR and/or differentiate into cells expressing a TCR with a bait nucleic acid encoding a bait TCR polypeptide chain, wherein the bait TCR polypeptide chain can constitute a parent TCR with a counterchain TCR polypeptide chain that specifically binds said peptide of interest;

b)   optionally selecting a cell expressing a TCR comprising the bait TCR polypeptide chain and a prey TCR polypeptide chain that selectively binds said peptide of interest from the transduced cell population obtained in step (a); and

c)   isolating a prey nucleic acid encoding the prey TCR polypeptide chain from the selected cell.

[00010]   In an embodiment, the bait TCR polypeptide chain is selected from a bait TCRalpha and/or bait TCRbeta polypeptide chain.

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[00011]   In an embodiment, the step of selecting the cell expressing a TCR comprising the bait

TCR polypeptide chain and a prey TCR polypeptide chain that selectively binds said peptide of interest

from the transduced cell population obtained in step (a) comprises isolating cells that express the

transduced bait polypeptide and which bind the peptide of interest.

[00012]   In an embodiment, the prey nucleic acid is isolated by cloning the prey nucleic acid.

[00013]   TCRs comprise a CDR1, CDR2 and CDR3 region. In an embodiment, the prey TCR

polypeptide CDR3 region comprises at least one amino acid modification relative to the CDR3 region in

a control TCR polypeptide CDR3 region, optionally the CDR3 region of the cognate polypeptide chain

(e.g. same chain type) in the parent TCR.

[00014]   In an embodiment, the method is for determining a TCR polypeptide chain that forms a

TCR with increased avidity and/or high affinity for a peptide of interest, wherein the method further

comprises: i) introducing the isolated prey nucleic acid and the bait nucleic acid into a cell able to

express a TCR or differentiate into a cell expressing a TCR; ii) measuring the avidity and/or affinity of

the TCR comprising the prey TCR polypeptide chain and the bait TCR polypeptide chain; and iii)

isolating a prey nucleic acid clone wherein the bait polypeptide chain and the prey TCR polypeptide

chain constitute a TCR having increased avidity and/or affinity for the peptide of interest compared to a

control TCR optionally the parent TCR.

[00015]   In an embodiment, the bait polypeptide, optionally TCRalpha and/or bait TCRbeta

polypeptide chain, was expressed in and previously isolated from a T cell recognizing said peptide of

interest.

[00016]   Also provided in another aspect is a method for producing a pair of nucleic acids

encoding a TCR specific for a peptide of interest, which comprises the steps of:

(a) transducing a cell population with a nucleic acid which encodes either one of two

polypeptide chains constituting a TCR expressed and previously isolated from a T cell recognizing said

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peptide of interest, wherein said cell population comprising cells which are able to express a TCR or differentiate into cells expressing a TCR,

(b) selecting a recombinant cell expressing the TCR specific for a peptide of interest from the transduced cell population obtained in step (a),

5   (c) isolating a nucleic acid encoding a polypeptide which constitutes TCR with the polypeptide encoded by the nucleic acid transduced into said cell population from the cell selected in step (b), and

(d) pairing the nucleic acid transduced into said cell population in step (a) with the nucleic acid isolated in step (c).

10   [00017]   Another embodiment, also provides a method for producing a nucleic acid encoding a TCR polypeptide chain which in combination with a counterchain TCR polypeptide constitutes a TCR specific for a peptide of interest, the method comprising the steps of:

a)   transducing a cell population comprising cells which cells can express a TCR or differentiate into cells expressing a TCR with a bait nucleic acid which encodes a TCR

15   polypeptide, optionally selected from a TCRalpha or a TCRbeta polypeptide chain, which in combination with a counterchain TCR polypeptide chain constitutes an expressed TCR, wherein the bait polypeptide was previously isolated from a T cell recognizing said peptide of interest,

b)   selecting a recombinant cell expressing the TCR specific for a peptide of interest from

20   the transduced cell population obtained in step (a), and

c)   isolating a nucleic acid encoding a prey polypeptide which constitutes a TCR with the polypeptide encoded by the bait nucleic acid transduced into said cell population from the cell selected in step (b).

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[00018] Another embodiment includes a method for producing a recombinant cell expressing a T cell receptor (TCR) specific for a peptide of interest, which comprises the step of transducing a cell population with a nucleic acid which encodes either TCR polypeptide chain (e.g. either TCR alpha or TCR beta or TCR gamma or TCR delta) constituting a TCR expressed and previously isolated from T cell recognizing said peptide of interest, wherein said cell population comprises a cell which is able to express a TCR or differentiate into cells expressing a TCR, and culturing said transduced cell population under conditions that permit the bait TCR polypeptide to be expressed (e.g. and a TCR to be formed).

[00019]   In an embodiment, step (a) further comprises the step of culturing the transduced cell population with an antigen presenting cell presenting the peptide of interest. In another embodiment, the cell population is a population of peripheral blood mononuclear cells (PBMCs) or PBMCs activated with a CD3 ligand. In an embodiment, the nucleic acid transduced into said cell population in step (a) encodes a TCR alpha chain or a TCR beta chain.

[00020]   In an embodiment, the method further comprises the step of selecting the cell expressing the TCR specific for a peptide of interest from the transduced cell population.

[00021]   In an embodiment, said nucleic acid transduced into said cell population encodes a TCR alpha chain or a TCR beta chain.

[00022]   In an embodiment, said nucleic acid transduced into said cell population encodes a TCR chain which predominantly contributes to peptide recognition by a TCR.

[00023]   In an embodiment, the transduction is repeated a second, third, fourth, fifth or sixth time.

[00024]   In an embodiment, the prey TCR can for example be isolated from a T cell, e.g. an endogenously expressed TCR chain.

[00025]   In an embodiment, the isolated prey nucleic acid encoding the prey TCR polypeptide is transduced into a population of cells comprising a cell which is able to express a TCR or can differentiate into a cell expressing a TCR, optionally wherein the isolated prey nucleic acid is

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transduced in combination with a nucleic acid encoding a TCR polypeptide chain that in combination

with the prey TCR polypeptide chain constitutes a TCR, optionally the bait TCR nucleic acid, to produce

a transduced cell population comprising cells expressing a TCR specific for a peptide of interest.

[00026]   In an embodiment, the cell population is transduced with an antisense molecule for

suppressing expression of an endogenous TCR chain.

[00027]   In an embodiment, the nucleic acid being transduced, optionally the bait nucleic acid, is

codon optimized.

[00028]   Also provided is a recombinant cell comprising a high affinity TCR specific for a peptide

of interest, comprising a TCR prey nucleic acid described herein and/or obtained as described herein.

[00029]   Also provided in another aspect is a cell population comprising a cell expressing a TCR

specific for a peptide of interest comprising TCR prey polypeptide or nucleic acid described herein or

obtained by a method described herein; optionally a cell population comprising a cell expressing a TCR

specific for a peptide of interest which comprises a pair of nucleic acids described herein and/or

obtained by a method described herein.

[00030]   A further aspect includes a method for treating a disorder comprising the step of

administering to the subject a therapeutically effective amount of a cell population or recombinant

obtained as described herein.

[00031]   In an embodiment, the method further comprises the step of activating the cell

population with a cytokine and/or an antigen peptide prior to the administering step.

[00032]   Another aspect is a nucleic acid encoding a TCR beta chain having an amino acid

sequence represented by SEQ ID NO: 4, 6, 94, 96 or 98.

[00033]   Yet another aspect is a nucleic acid encoding a TCR alpha chain having an amino acid

sequence selected from the group consisting of SEQ ID NOs: 8, 10, 12 and 14.

[00034] Also provided in an embodiment, is an isolated and/or recombinantly engineered polypeptide

comprising a sequence selected from SEQ ID NO: 4, 6, 8, 10, 12, 14, 52, 54, 56, 58, 60, 62, 81-86, 88-

91, 94, 96, 98, 112, 114, 116-122 and/or a sequence having at least 85%, at least 90%, at least 95%,

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at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a sequence selected from to a sequence selected from SEQ ID NOs: 4, 6, 8, 10, 12, 14, 52, 54, 56, 58, 60, 62, 81-86, 88-91, 94, 96, 98, 112, 114, 116-122 or a portion thereof such as a CDR region or a non-CDR region. In an embodiment the polypeptide comprises a sequence of any one of SEQ ID NOs:4, 6, 8, 10, 12, 14, 52, 54, 56, 58, 60, 62, 81-86, 88-91, 94, 96, 98, 112, 114, 116-122.

[00035] In an embodiment, the isolated and/or recombinantly engineered polypeptide is encoded by any one of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 51, 53, 55, 57, 59, 61, 93, 95, 97, 111, 113, 115 and/or a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a sequence selected from to a sequence selected from SEQ ID NOs: 3, 5, 7, 9, 11, 13, 51, 53, 55, 57, 59, 61, 93, 95, 97, 111, 113, 115 and for example encoding a polypeptide selected from 4, 6, 8, 10, 12, 14, 52, 54, 56, 58, 60, 62, 81-91, 112, 114 and 116-122 or a portion thereof such as a CDR region or a non-CDR region.

[00036] In an embodiment, the isolated and/or recombinantly engineered nucleic acid comprises a sequence as shown in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 51, 53, 55, 57, 59, 61, 93, 95, 97, 111, 113 and 115.

[00037] Also provided in an embodiment, is an isolated and/or recombinantly engineered nucleic acid encoding a TCR chain comprising a CDR3 region amino acid sequence selected from SEQ ID NO: 52, 54, 56, 58, 60 62,81 to 91, 112, 114 and 116-122.

[00038] In an embodiment, the isolated and/or recombinantly engineered nucleic acid described herein wherein the TCR beta chain comprises an amino acid sequence represented by SEQ ID NO: 4, 6, 94, 96 or 98.

[00039] Also provided in an embodiment is an isolated and/or recombinantly engineered TCRbeta chain wherein the CDR3 region comprises any one of SEQ ID NOs: 52, 54, 81 to 91, 112, 114, and 116 to122.

[00040] Another embodiment includes an isolated TCRalpha chain wherein the CDR3 region comprises any one of SEQ ID NO: 56, 58, 60 and 62.

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[00041] Another aspect includes an isolated and/or recombinantly engineered nucleic acid encoding a TCR alpha chain comprising a CDR3 region amino acid sequence selected from the group consisting of SEQ ID NOs: 56, 58, 60 and 62.

[00042] In an embodiment, the isolated and/or recombinantly engineered nucleic acid encodes a TCR alpha chain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 10, 12 and 14.

[00043] Another embodiment includes an isolated and/or recombinantly engineered TCR comprising a TCRalpha chain and a TCRbeta chain wherein the TCRbeta chain comprises a CDR3 region comprising the sequence of any one of SEQ ID NO: 52, 54, 81 to 91, 112, 114 and 116 to 122 and/or the TCR alpha chain a CDR3 region comprising the sequence of any one of SEQ ID NO: 56, 58, 60 and 62.

[00044] Another embodiment includes an isolated and/or recombinantly engineered cell comprising the isolated nucleic acid and/or polypeptide described herein, the TCRbeta chain described herein the TCRalpha chain described herein, and/or the TCR described herein.

[00045] Another aspect includes a nucleic acid, polypeptide composition, optionally a pharmaceutical composition, recombinant cell or cell population comprising a prey nucleic acid or prey polypeptide described herein and/or produced using a method described herein for treating a disorder.

[00046] A further aspect includes a use of a nucleic acid, composition and/or cell population producing using a method described herein for treating a disorder.

BRIEF DESCRIPTION OF THE DRAWINGS

[00047] **Fig. 1** is a flow cytometric analysis demonstrating that when paired with SupT1 TCRβ, SIG35α but not DMF5α recognizes A2/MART1. SupT1 cells, a TCRα-deficient human T cell line, were transduced with SIG35α, DMF5α, and DMF5αβ. Transfectants were stained with A2/MART1 or A2/Flu multimer and with α-human CD3 mAb.

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[00048]   **Fig. 2** is a flow cytometric analysis and ELISPOT assay demonstrating that thymic selection does not appear to affect TCRβ repertoire that can constitute A2/MART1 TCR with SIG35α. A) Peripheral T cells freshly isolated from 2 HLA-A2+ donors and 2 A2- donors were stimulated with 50 ng/mL α-human CD3 mAb (OKT3) in the presence of 100 IU/mL IL-2 and retrovirally transduced with truncated NGFR (ΔNGFR) gene (Mock), SIG35α/ΔNGFR gene. SIG35α and ΔNGFR gene was intervened by furin, sgsg and F2A sequence derived from foot and mouth disease virus. After 6 times of transduction, 2.0×10^5 transfectants were stained with 8 μg/mL A2/MART1 multimer or A2/HIV multimer in conjunction with α-human CD8 mAb and α-human NGFR mAb. ΔNGFR-positive cells were gated and multimer/CD8 positivity was analyzed; B) SIG35α-transduced A2+ peripheral CD8+ T cells recognize A2/MART1. HLA-A2+ peripheral CD8+ T cells were transduced with SIG35α or Mock. Before aAPC stimulation, A2/MART1 specificity was analyzed by multimer staining using A2/MART1 and A2/HIV multimer. A2/HIV multimer was used as a negative control. SIG35α-transduced A2- peripheral CD8+ T cells recognize A2/MART1. HLA-A2- peripheral CD8+ T cells were transduced with SIG35α or Mock. Before aAPC stimulation, A2/MART1 specificity was analyzed by multimer staining using A2/MART1 and A2/HIV multimer. A2/HIV multimer was used as a negative control; C) SIG35α-transduced peripheral T cells are highly avid for A2/MART1 recognition. SIG35α or Mock-transduced CD8+ T cells derived from an A2+ or A2- donor were subjected to IFN-γ ELISPOT analysis. SIG35α or Mock-transduced CD8+ T cells after first stimulation with 10 μg/mL MART1$_{27\text{-}35}$ peptide-pulsed wtA2-aAPC were used as responder cells. T2 cells pulsed with 10 μg/mL MART1$_{27\text{-}35}$ peptide or HIV pol476-484 peptide were used as stimulator cells (left). HIV pol476-484 peptide was used as a negative control peptide. The A2+/MART1+ melanoma line, Malme-3M and the A2+/MART1- melanoma line, A375 were used as stimulator cells (right). All of experiments were carried out in triplicate and error bars show SD; D) SIG35α-transduced A2/MART1 CD8+ T cells expand upon wtA2-aAPC-based stimulation. SIG35α-transduced CD8+ T cells in an A2+ or A2- donor were stimulated with 10 μg/mL MART1$_{27\text{-}35}$ peptide-pulsed wtA2-aAPC once a week. Between stimulations, T cells were supplemented with IL-2 (10 IU/mL) and IL-15 (10 ng/mL) every 3 days. A2/MART1 multimer staining done after 1st

and 2nd stimulation is shown; E) SIG35α-transduced A2/MART1 CD8+ T cells expand upon mutA2-aAPC-based stimulation. SIG35α-transduced CD8+ T cells in an A2+ or A2- donor were stimulated with 10 μg/mL MART1$_{27-35}$ peptide-pulsed IL-21-secreting mutA2-aAPC once a week. Between stimulations, T cells were supplemented with IL-2 (10 IU/mL) and IL-15 (10 ng/mL) every 3 days. A2/MART1 multimer staining done after second and third stimulation is shown.

[00049]   **Fig. 3** is a bar chart and flow cytometric analysis demonstrating that SIG35α predominantly pairs with TCR Vβ14 to recognize A2/MART1. A) SIG35α-transduced CD8+ T cells before aAPC stimulation in an A2+ or A2- donor were co-stained with A2/MART1 multimer, monoclonal antibodies (mAbs) for TCR Vβ subtypes and α-human CD8 mAb. SIG35α predominantly pairs with TCR Vβ14 to recognize A2/MART1 in both A2+ and A2- donors. The percentage of A2/MART1 multimer+ CD8+ T cells expressing each subtype is shown. B) "Many" TCR Vβ14 clonotypes can recognize A2/MART1 when paired with SIG35α. The percentage of A2/MART1 multimer+ cells in CD8+ Vβ14+ T cells transduced with SIG35α is shown. The nomenclature used is the one from Wei et al., 1994.

[00050]   **Fig. 4** is a bar chart identifying TCR beta variable gene 27 (TRBV27) clonotypes that can compose A2/MART1 TCR in conjunction with SIG35α are highly heterogeneous and unique. SIG35α-transduced CD8+ T cells in an A2+ or A2- donor were stimulated with 10 μg/mL MART1$_{27-35}$ peptide-pulsed wtA2-aAPC. A2/MART1 multimer+ CD8+ T cells were sorted by flow cytometry cell sorting. TCR TRBV27 chains isolated from sorted T cells were sequenced. Jβ gene segments and CDR3 length of isolated TCR TRBV27 chains are shown.

[00051]   **Fig. 5** is a cytometric analysis showing that the structural avidity range of A2/MART1 TCR consisting of SIG35α can be very broad in the absence or presence of CD8. A) Jurkat76 cells, which lack the expression of CD8αβ and intrinsic TCR, were retrovirally transduced with CD8αβ. Jurkat76 or Jurkat76/CD8αβ cells were stably transduced with individual TCRβ chains (clone: 413, 523, 788, 1086, 830, or 794) and TCRα SIG35α chain or DMF5 TCR. All transfectants were >95% positive for CD3. A2/HIV multimer was used as a negative control. Reconstituted A2/MART1 TCRs on Jurkat76 possess various structural avidities. Jurkat76 transfectants were stained with 2 μg/mL A2/MART1

multimer or A2/HIV multimer and α-human CD3 mAb. B) Reconstituted A2/MART1 TCRs on

Jurkat76/CD8αβ possess various structural avidities. Jurkat76/CD8αβ transfectants were stained with 2

μg/mL A2/MART1 multimer or A2/HIV multimer and α-human CD8 mAb. C) Reconstituted A2/MART1

TCRs show a broad range of structural avidities. The structural avidity of Jurkat76 transfectants (left)

5   and Jurkat76/CD8αβ transfectants (right) were assessed by multimer staining with graded

concentrations of A2/MART1 multimer.

[00052]   **Fig. 6** is an ELISPOT assay showing that the functional avidity window of A2/MART1

TCR consisting of SIG35α can be very wide in the absence or presence of CD8. A) Jurkat76 or B)

Jurkat76/CD8αβ cells were stably transduced with individual TCRβ chains (clone: 413, 523, 788, 1086,

10   830, or 794) and SIG35α chain or DMF5 TCR. All transfectants were >95% positive for CD3.

Transfectants were subjected to IL-2 ELISPOT analysis. T2 cells pulsed with 10 μg/mL $MART1_{27-35}$

peptide or HIV pol476-484 peptide were used as stimulator cells (left). HIV pol476-484 peptide was

used as a negative control peptide. The A2+/MART1+ melanoma line, Malme-3M and the A2+/MART1-

melanoma line, A375 were used as stimulator cells (right). (A)   Reconstituted A2/MART1 TCRs on

15   Jurkat76 are highly avid for A2/MART1 recognition. IL-2 ELISPOT was performed in Jurkat76

transfectants using peptide-pulsed T2 cells (left) and tumor cell line targets (right). All experiments

were carried out in triplicate and error bars show SD; (B) Reconstituted A2/MART1 TCRs on

Jurkat76/CD8αβ are highly avid for A2/MART1 recognition. IL-2 ELISPOT was performed in

Jurkat76/CD8αβ transfectants using peptide-pulsed T2 cells (left) and tumor cell line targets (right). All

20   experiments were carried out in triplicate and error bars show SD; (C) Reconstituted A2/MART1 TCRs

show various functional avidities. The functional avidity of Jurkat76 transfectants (left) and

Jurkat76/CD8αβ transfectants (right) were assessed by IL-2 secreting using T2 cells pulsed with graded

concentrations of $MART1_{27-35}$ peptide as stimulator cells.

[00053]   **Fig. 7** is a flow cytometric analysis demonstrating TAK1β-centric recognition of

25   A24/WT1. Peripheral T cells freshly isolated from 5 HLA-A*24:02 (A24)+ donors and 2 A24- donors

were stimulated with 50 ng/mL α-human CD3 mAb (OKT3) in the presence of 100 IU/mL IL-2 and

retrovirally transduced with truncated NGFR (ΔNGFR) gene (Mock), TAK1α/ΔNGFR or TAK1β/ΔNGFR gene. TAK1α or TAK1β gene and ΔNGFR gene was intervened by furin, sgsg and F2A sequence derived from foot and mouth disease virus. After 6 rounds of transduction, 2.0×10^5 transfectants were stained with 50 μg/mL A24/WT1 heteroclitic peptide tetramer or A24/Survivin tetramer in conjunction with α-human CD8 mAb and α-human NGFR mAb. ΔNGFR-positive cells were gated and tetramer/CD8 positivity was analyzed. Representative tetramer-staining data of Mock (top), TAK1α (middle) and TAK1β (bottom)-transduced T cells obtained from one A24+ and one A24- donors out of 7 donors is shown.

[00054]   **Fig. 8** is an ELISPOT assay demonstrating that TAK1β-transduced T cells stimulated with A24-aAPC expand and recognize A24/WT1 with reproducibility. CD8+ T cells derived from Mock, TAK1α and TAK1β-transduced T cells were isolated and stimulated with A24-aAPC cells loaded with 1 μg/mL $WT1_{235-243}$ heteroclitic peptide. Transduction efficiency in each gene-modified T cells was approximately 70%. Following 2 stimulations, TAK1β-transduced T cells derived from 7 out of 7 donors expanded. IFN-γ ELISPOT was conducted where 2.0×104 gene-modified CD8+ T cells were co-cultured with HLA null-aAPC, A24-aAPC, A24-aAPC loaded with 1 μg/mL HIV-1 env584-592 peptide, A24-aAPC loaded with 1 μg/mL $WT1_{235-243}$ heteroclitic peptide or none of aAPC cells (indicated as each bar). TAK1β-transduced T cells derived from all 7 donors strongly produced IFN-γ against WT1 peptide pulsed A24-aAPC. Representative results from two A24+ and one A24- donors out of 7 donors are shown. All of experiments were carried out in triplicate and error bars show SD.

[00055]   **Fig. 9** is a flow cytometric analysis and ELISPOT assay showing that A24-aAPC stimulation of TAK1β-transduced T cells can enrich A24/WT1 T cells with high avidity. A representative result from 2 out of 7 donors is shown. TAK1β-transduced T cells were stained with 50 μg/mL A24/WT1 heteroclitic peptide tetramer or A24/Survivin tetramer in conjunction with α-human CD8 mAb and α-human NGFR mAb. ΔNGFR+ cells were gated and tetramer/CD8 positivity was analyzed. Tetramer-staining data of TAK1β-transduced T cells derived from one A24+ (top left) and one A24- (bottom left) donors following 2 stimulations with A24-aAPC cells loaded with $WT1_{235-243}$ heteroclitic peptide is

shown. IFN-γ ELISPOT using 2.0×10^4 CD8+ gene-modified T cells following 2 stimulations was performed in the presence or absence of indicated aAPC cells as target cells. ELISPOT assay was carried out in triplicate. These TAK1β-transduced CD8+ T cells derived from one A24+ (top right) and one A24- (bottom right) donors following 2 stimulations both produced IFN-γ against A24-aAPC cells that naturally processed and presented A24/WT1 on the cell surface. A24-aAPC cells loaded with 1 µg/mL HIV-1 env584-592 peptide or 1 µg/mL WT1235-243 heteroclitic peptide were used as negative and positive controls for WT1235-243 peptide specificity. All of experiments were carried out in triplicate and error bars show SD.

[00056] **Fig. 10** is a bar chart showing a repertoire of TCRα clonotypes which can recognize A24/WT1 when paired with TAK1β. The Variable region and J region of a TCRα clonotype was denoted by IMGT nomenclature. The result is an aggregate of 3 HLA-A24+ and 1 HLA-A24- donors and 41 different TCRα clonotypes are shown. The J region (A) and the length of CDR3α amino acid sequences (B) in each TRAVs are summarized by stacked bar graph.

[00057]   **Fig. 11** is an ELISPOT assay showing that novel TCRα/TAK1β TCRs recognize WT1-derived peptide endogenously processed and presented by A24 with different avidity. Jurkat76 cells, which lack the expression of CD8αβ and intrinsic TCR, were retrovirally transduced with CD8αβ and TAK1β gene. Following transduction of these genes, Jurkat76/CD8αβ/TAK1β transfectant was additionally transduced with TCRα gene (clone: T53, A262, T243, T262) or parent TAK1α gene. All transfectants were >95% positive for CD3. IL-2 ELISPOT was done by incubating 4.0x104 Jurkat76/CD8αβ/TAK1β/TCRα cells in the presence or absence of aAPC cells. Jurkat76 transfectants (clone: T53, A262, T243, T262) produced IL-2 against A24-aAPC cells that naturally processed and presented A24/WT1 on the cell surface (left). A24-aAPC cells loaded with 1 µg/mL HIV-1 env584-592 peptide or WT1235-243 heteroclitic peptide were used as negative and positive controls for WT1235-243 peptide specificity (right). All of experiments were carried out in triplicate and error bars show SD.

[00058]   **Fig. 12** shows the sequences of nucleotide (top, SEQ ID NO: 1) and amino acid (bottom, SEQ ID NO: 2) of clone SIG35α TCR alpha chain. The CDR1, CDR2 and CDR 3 nucleotide

and amino acid sequences of the cloned SIG35α alpha chain are represented by SEQ ID NOs: 17, 33, 49, 18, 34 and 50, respectively.

[00059]   **Fig. 13** shows the sequences of nucleotide (top, SEQ ID NO: 3) and amino acid (bottom, SEQ ID NO: 4) of clone 794 TCR beta chain. The CDR regions of the nucleotide and the amino acid are underlined. The CDR1, CDR2 and CDR 3 nucleotide and amino acid sequences of the cloned 794 TCR beta chain are represented by SEQ ID NOs: 19, 35, 51, 20, 36 and 52, respectively.

[00060]   **Fig. 14** shows the sequences of nucleotide (top, SEQ ID NO: 5) and amino acid (bottom, SEQ ID NO: 6) of clone 830 TCR beta chain. The CDR regions of the nucleotide and the amino acid are underlined. The CDR1, CDR2 and CDR3 nucleotide and amino acid sequences of the cloned 830 TCR beta chain are represented by SEQ ID NOs: 21, 37, 53, 22, 38 and 54, respectively.

[00061]   **Fig. 15** shows the sequences of nucleotide (top, SEQ ID NO: 7) and amino acid (bottom, SEQ ID NO: 8) of clone T53 TCR alpha chain. The CDR regions of the nucleotide and the amino acid are underlined. The CDR1, CDR2 and CDR 3 nucleotide and amino acid sequences of the cloned T53 TCR alpha chain are represented by SEQ ID NOs: 23, 39, 55, 24, 40 and 56, respectively.

[00062]   **Fig. 16** shows the sequences of nucleotide (top, SEQ ID NO: 9) and amino acid (bottom, SEQ ID NO: 10) of clone A262 TCR alpha chain. The CDR regions of the nucleotide and the amino acid are underlined. The CDR1, CDR2 and CDR 3 nucleotide and amino acid sequences of the cloned A262 TCR alpha chain are represented by SEQ ID NOs: 25, 41, 57, 26, 42 and 58, respectively.

[00063]   **Fig. 17** shows the sequences of nucleotide (top, SEQ ID NO: 11) and amino acid (bottom, SEQ ID NO: 12) of clone T243 TCR alpha chain. The CDR regions of the nucleotide and the amino acid are underlined. The CDR1, CDR2 and CDR 3 nucleotide and amino acid sequences of the cloned T243 TCR alpha chain are represented by SEQ ID NOs: 27, 43, 59, 28, 44 and 60, respectively.

[00064]   **Fig. 18** shows the sequences of nucleotide (top, SEQ ID NO: 13) and amino acid (bottom, SEQ ID NO: 14) of clone T262 TCR alpha chain are shown. The CDR regions of the nucleotide and the amino acid are underlined. The CDR1, CDR2 and CDR 3 nucleotide and amino acid

sequences of the cloned T262 TCR alpha chain are represented by SEQ ID NOs: 29, 45, 61, 30, 46 and 62, respectively.

[00065]   **Fig. 19** shows the sequences of nucleotide (top, SEQ ID NO: 15) and amino acid (bottom, SEQ ID NO: 16) of clone TAK1 TCR beta chain. The CDR1, CDR2 and CDR 3 nucleotide and amino acid sequences of the cloned TAK1 TCR beta chain are represented by SEQ ID NOs: 31, 47, 63, 32, 48 and 64, respectively.

[00066]   **Fig. 20** illustrates that de novo TCRs generated by TCR single chain transduction are unselected by Thymus.

[00067]   **Fig. 21** is a schematic of a bait TCR construct.

[00068]   **Fig. 22** is a chart describing anti-tumor TCR gene therapy.

[00069]   **Fig. 23** is a flow cytometric analysis showing that both HLA-A2$^+$ and A2$^-$ peripheral CD4$^+$ T cells can recognize A2/MART1 when transduced with chain-centric SIG35α. Both HLA-A2$^+$ and A2$^-$ peripheral CD4$^+$ T cells become A2/MART1-reactive upon transduction of chain-centric SIG35α. Peripheral CD4$^+$ T cells freshly isolated from one HLA-A2$^+$ donors #7 and one A2$^-$ donors #3 and were retrovirally transduced with SIG35α or Mock. The transfectants were stimulated with IL-21-secreting mutA2-aAPC pulsed with 10 µg/ml MART1$_{27-35}$ peptide once a week. Between stimulations, IL-2 (10 IU/ml) and IL-15 (10 ng/ml) were added every 3 days. Data for A2/MART1 or A2/HIV multimer staining conducted after second stimulation are shown.

[00070]   **Fig. 24** is a bar chart demonstrating that SIG35α predominantly selects with TRBV5-1, 27 and 2 in CD4$^+$ T cells to recognize A2/MART1. SIG35α-transduced CD4$^+$ T cells and CD8$^+$ T cells after second stimulation with mutA2-aAPC in an A2$^+$ or an A2$^-$ donor were co-stained with A2/MART1 multimer, monoclonal antibodies (mAbs) for TCR Vβ subtypes and α-human CD4 mAb or α-human CD8 mAb. The percentage of A2/MART1 multimer$^+$ CD4$^+$ T cells or A2/MART1 multimer$^+$ CD8$^+$ T cells expressing each subtype is shown.

[00071]   **Fig. 25** is a bar chart demonstrating that TRBV2, 5-1 and 27 clonotypes that can compose A2/MART1 TCRs in conjunction with SIG35α are highly heterogeneous and unique. SIG35α-

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transduced CD4$^+$ T cells in an A2$^+$ or A2$^-$ donor were stimulated with 10 µg/ml MART1$_{27-35}$ peptide-pulsed mutA2-aAPC. A2/MART1 multimer$^+$ CD4$^+$ T cells were collected by flow cytometry cell sorting. TRBV2, 5-1 and 27 TCRβ chains isolated from the A2/MART1 multimer$^+$ T cells were sequenced. Jβ gene segments and CDR3 lengths of isolated each TRBV chain are shown.

[00072]　　　　**Fig. 26** is a flow cytometric analysis demonstrating that reconstituted CD4$^+$ A2/MART1 TCRs recognize A2/MART1 in a CD8-independent manner. Jurkat 76 cells, which lack the expression of CD8αβ and endogenous TCRs, were retrovirally transduced with CD8αβ to produce Jurkat 76/CD8αβ. Jurkat 76 or Jurkat 76/CD8αβ cells were individually transduced with TRBV2, 5-1, or 27 TCRβ chains (clone: 6B, 6X, 11C, 9I, 9J, 4K, 7E, 7Q, or 8H), which was isolated from CD4$^+$ A2/MART1 T cells, along with SIG35α chain. All Jurkat 76 or Jurkat 76/CD8αβ transfectants were stained with 2 µg/ml A2/MART1 or A2/HIV multimer along with anti-CD3 mAb or anti-CD8 mAb. Data for multimer staining of Jurkat 76 (top) or Jurkat 76/CD8αβ transfectants (bottom) are shown.

[00073]　　　　**Fig. 27** is a series of charts demonstrating that reconstituted CD4$^+$ A2/MART1 TCRs possess a broad range of functional and structural avidities. Functional avidities of Jurkat 76 or Jurkat 76/CD8αβ cells expressing 9 different A2/MART1 TCRβ chains paired with SIG35α and DMF5 are depicted as % IL-2 secreting abilities determined by IL-2 ELISPOT assays using T2 cells pulsed with graded concentrations of MART1$_{27-35}$ peptide as stimulator cells (left). Structural avidities of the same transfectants are shown as multimer staining percentages determined by staining with graded concentrations of A2/MART1 multimer (right).

[00074]　　　　**Fig. 28** shows the sequences of nucleotide (top, SEQ ID NO: 93) and amino acid (bottom, SEQ ID NO: 94) of clone 8H TCR beta chain. The CDR regions of the nucleotide and the amino acid are underlined. The CDR1, CDR2 and CDR 3 nucleotide and amino acid sequences of the cloned 8H TCR beta chain are represented by SEQ ID NOs: 99, 105, 111, 100, 106 and 114, respectively.

[00075]　　　　**Fig. 29** shows the sequences of nucleotide (top, SEQ ID NO: 95) and amino acid (bottom, SEQ ID NO: 96) of clone 7Q TCR beta chain. The CDR regions of the nucleotide and the

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amino acid are underlined. The CDR1, CDR2 and CDR 3 nucleotide and amino acid sequences of the

cloned 7Q TCR beta chain are represented by SEQ ID NOs: 101, 107, 113, 102, 108 and 114,

respectively.

[00076]   **Fig. 30** shows the sequences of nucleotide (top, SEQ ID NO: 97) and amino acid

5   (bottom, SEQ ID NO: 98) of clone 9J TCR beta chain. The CDR regions of the nucleotide and the

amino acid are underlined. The CDR1, CDR2 and CDR 3 nucleotide and amino acid sequences of the

cloned 9J TCR beta chain are represented by SEQ ID NOs: 103, 109, 115, 104, 110 and 116,

respectively.

DETAILED DESCRIPTION

10   [00077]   In understanding the scope of the present disclosure, the term "comprising" and its

derivatives, as used herein, are intended to be open ended terms that specify the presence of the

stated features, elements, components, groups, integers, and/or steps, but do not exclude the presence

of other unstated features, elements, components, groups, integers and/or steps. The foregoing also

applies to words having similar meanings such as the terms, "including", "having" and their derivatives.

15   Finally, terms of degree such as "substantially", "about" and "approximately" as used herein mean a

reasonable amount of deviation of the modified term such that the end result is not significantly

changed. These terms of degree should be construed as including a deviation of at least ±5% of the

modified term if this deviation would not negate the meaning of the word it modifies. In understanding

the scope of the present disclosure, the term "consisting" and its derivatives, as used herein, are

20   intended to be close ended terms that specify the presence of stated features, elements, components,

groups, integers, and/or steps, and also exclude the presence of other unstated features, elements,

components, groups, integers and/or steps. The recitation of numerical ranges by endpoints herein

includes all numbers and fractions subsumed within that range (e.g. 1 to 5 includes 1, 1.5, 2, 2.75, 3,

3.90, 4, and 5). It is also to be understood that all numbers and fractions thereof are presumed to be

25   modified by the term "about." Further, it is to be understood that "a," "an," and "the" include plural

referents unless the content clearly dictates otherwise. The term "about" means plus or minus 0.1 to 50%, 5-50%, or 10-40%, preferably 10-20%, more preferably 10% or 15%, of the number to which reference is being made. Further, the definitions and embodiments described in particular sections are intended to be applicable to other embodiments herein described for which they are suitable as would

5   be understood by a person skilled in the art. For example, in the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

10   **(1) Methods for identifying a TCR polypeptide chain that can constitute a TCR specific for a peptide of interest and for producing one or more nucleic acids encoding a TCR specific for a peptide of interest**

[00078]   An aspect includes a method for generating a high affinity TCR specific for a peptide of interest comprising:

15   a)   transducing a cell population comprising cells able to express a TCR and/or differentiate into a cell expressing a TCR with a bait nucleic acid encoding a bait TCR polypeptide chain, wherein the bait TCR polypeptide chain can constitute a parent TCR with a counterchain TCR polypeptide chain that specifically binds said peptide of interest; and

20   b)   culturing under conditions that permit the bait TCR to be expressed.

[00079]   In an embodiment, the method further comprises selecting a cell expressing a TCR comprising the bait TCR polypeptide chain and a prey TCR polypeptide chain that selectively binds said peptide of interest from the transduced cell population obtained in step (a) or (b).

[00080]   In another embodiment, the method further comprises isolating a prey nucleic acid encoding the prey TCR polypeptide chain from the selected cell.

[00081]   In another aspect, the disclosure includes a method for identifying and/or obtaining a TCR polypeptide chain that can constitute a TCR specific for a peptide of interest or a cell comprising said TCR polypeptide further comprising :

c)   obtaining a recombinant cell expressing a TCR comprising the bait TCR polypeptide chain and a prey TCR polypeptide chain that selectively binds said peptide of interest from the transduced cell population obtained in step (a) or (b); and

d)   isolating a prey nucleic acid encoding the prey TCR polypeptide chain from the selected cell.

[00082]   In another embodiment, the method further comprises step e) pairing the isolated prey nucleic acid isolated with the bait nucleic acid which when expressed provide a high affinity TCR.

[00083]   As used herein, the term "TCR" refers to a molecule comprising a single fused TCR (e.g. fusing the TCR chains comprising the variable regions (CDR1/2/3) of TCRalpha and TCRbeta chains or the variable regions of TCRdelta and TCRgamma chains), including TCRalpha:TCRbeta, TCRbeta:TCRalpha, TCRdelta:TCRgamma and TCRgamma:TCRdelta fusions)   and/or complex minimally comprising two molecules each comprising a TCR chain, each TCR chain minimally comprising a variable region, wherein each TCR chain is the counterchain of the other (e.g. TCRalpha and TCRbeta chains or TCRgamma and TCRdelta chains). As used herein "TCR" can refer to nucleic acid molecules that can encode the TCR polypeptide chains, e.g. TCR alpha and beta chain polypeptides, or single fused TCR and/or the TCR polypeptide chains e.g. TCR alpha and beta chain comprising polypeptides, fused or separate.

[00084]   As used herein, the term "TCR chain" refers to 1) a nucleic acid encoding a TCR chain polypeptide and/or encoding a functional fragment thereof and/or 2) a TCR chain polypeptide and/or

functional fragment thereof, selected for example from TCRα, TCRβ, TCRdelta and TCRgamma which

can with a counterchain TCR chain constitute a TCR, the functional fragment minimally comprising

CDR1, CDR2 and CDR3 regions. The TCR can comprise one or more mammalian, optionally human

TCR chains, and/or hybrid TCR chains, for example a mouse:human hybrid chain, optionally wherein

5   the TCR chain comprises human CDR1, CDR2 and CDR3 region and a mouse constant region and/or

mouse CDR1, CDR2 and CDR3 regions and a human TCR constant region. A hybrid TCR comprising

mouse CDR1/2/3 regions and a human constant region has been reported (Parkhurst et al. 2009,

Theoret et al. 2008).

[00085]   Peptides, typically about 9 amino acids long, are recognized by TCRs in the context of

10   presentation by a human leukocyte antigen (HLA) (which is the human version of the major

histocompatibility complex (MHC) (e.g. in a HLA: peptide complex). Some peptides are promiscuous

and can be presented by more than one HLA type. Antigen presenting cells (APCs) (both authentic and

artificial) present for example a peptide of interest to an effector cell, such as a T cell comprising a TCR

that recognizes the peptide of interest, in the context of a HLA molecule. Artificial APCs (aAPCs) which

15   can be used in methods for identifying a counterchain TCR, can express a single allele of HLA. For

example as shown herein, aAPC used in the experiments described below comprise HLA-A2 for the

MART1 peptide of interest and HLA-A24 for the WT1 peptide of interest.

[00086]   The term "functional fragment thereof" in reference to a TCR chain means a molecule at

least comprising a variable CDR3 region, optionally comprising CDR1, CDR2 and CDR3 regions that

20   together can function to confer peptide of interest specificity. The functional fragment optionally

comprises the extracellular portion (EC portion), optionally in combination with the membrane spanning

portion of the TCR chain (e.g. intracellular portion is deleted). The functional fragment can for example

be comprised in an agent such as a therapeutic agent. For example, the EC portion, optionally in

combination with the membrane spanning portion of each TCR counterchain (e.g. TCRalpha and

25   TCRbeta), can be fused to make a single chain TCR fusion which is then conjugated to an effector

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such as an antibody, optionally comprising a cytotoxic moiety. A TCR effector conjugate can be administered to a subject in need thereof.

[00087]   In an embodiment, the bait TCR polypeptide chain is selected from a bait TCRalpha or bait TCRbeta polypeptide chain.

[00088]   In another embodiment, the bait TCR polypeptide chain is selected from a bait TCRgamma or bait TCRdelta polypeptide chain.

[00089]   The term "bait nucleic acid" as used herein means a nucleic acid encoding a TCR polypeptide chain –optionally alpha or beta, or delta or gamma– that has, in an embodiment, been previously isolated (e.g. identified and cloned) and/or for which the sequence of the CDR regions (e.g. CDR1, CDR2 and CDR3) have been previously determined. For example, for TCR chains wherein the sequence of the CDR regions have been determined, the bait nucleic acid can be cloned and/or constructed, optionally by combining recombinantly produced CDR region nucleic acids with a known constant region, and/or replacing CDR regions in a known TCR chain (e.g. known sequence).

[00090]   The term "prey nucleic acid" as used herein means a nucleic acid that encodes a prey TCR polypeptide chain, optionally a TCRalpha or TCRbeta polypeptide chain, that can constitute a TCR with the bait TCR polypeptide chain, and is in some embodiments advantageously one that encodes a TCR polypeptide chain that in combination with a second TCR polypeptide chain optionally the bait TCR polypeptide chain, constitutes a TCR which has increased or decreased avidity and/or affinity for the peptide of interest, compared to a control TCR such as a parent TCR.

[00091]   The transduced bait nucleic acid is expressed in the transduced or recombinant cell as a bait polypeptide TCR chain and pairs with a prey polypeptide TCR chain to make a TCR. Prey nucleic acids encoding the prey TCR polypeptide chains that in combination with the bait TCR polypeptide chain constitute a TCR that can recognize the peptide of interest (in the context of an HLA/peptide complex) can be selected e.g. they can be isolated for example in the context of the cell and/or the prey nucleic acid cloned.

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[00092]   The term "nucleic acid" as used herein refers to a sequence of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars, and intersugar (backbone) linkages, and includes single-stranded and double-stranded molecules, RNA and DNA, optionally wherein the DNA is non-naturally occurring cDNA, optionally codon optimized DNA. The term also includes modified or substituted oligomers comprising non-naturally occurring monomers or portions thereof, which function similarly. Such modified or substituted nucleic acids may be preferred over naturally occurring forms because of properties such as enhanced cellular uptake or increased stability in the presence of nucleases. The term also includes chimeric nucleic acids that contain two or more chemically distinct regions. For example, chimeric nucleic acids may contain at least one region of modified nucleotides that confer beneficial properties (e.g., increased nuclease resistance, increased uptake into cells), or two or more nucleic acids of the disclosure may be joined to form a chimeric nucleic acid.

[00093]   The term "isolated nucleic acid" as used herein refers to a nucleic acid substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors, or other chemicals when chemically synthesized. An isolated nucleic acid is also substantially free of sequences which naturally flank the nucleic acid (i.e. sequences located at the 5' and 3' ends of the nucleic acid) from which the nucleic acid is derived.

[00094]   The isolated and/or recombinant nucleic acids and/or polypeptides can comprise one or more conservative substitutions.

[00095]   The term "conservative substitutions" as used herein include nucleotide substitutions that do not result in changes in the amino acid sequence, as well as nucleotide substitutions that result in conservative amino acid substitutions, or amino acid substitutions which do not substantially affect the character of the polypeptide translated from said nucleotides.

[00096]   Conservative substitutions of amino acid sequences include amino acid substitutions or deletions that do not substantially affect the character of the variant polypeptide relative to the starting peptide. For example, polypeptide character is not substantially affected if the substitutions or deletions do not preclude specific binding of the variant peptide to a specific binding partner of the starting

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peptide. Included in this definition are glycosylated and other variants and derivatives that will be apparent to those skilled in the art and are considered to fall within the scope of this invention. Also included in this definition are amino acid insertions, substitutions, deletions and truncations that do not substantially affect the polypeptide character relative to the starting peptide.

[00097]   In an embodiment, the substitution includes a molecule in the following list:

Alanine A D--Ala, Gly, beta-Ala, L--Cys, D—Cys

Arginine R D--Arg, Lys, D--Lys, homo-Arg, D-homo-Arg, Met, Ile, D--Met, D--Ile, Orn, D—Orn

Asparagine N D--Asn, Asp, D--Asp, Glu, D--Glu, Gln, D--Gln

Aspartic Acid D D--Asp, D--Asn, Asn, Glu, D--Glu, Gln, D--Gln

Cysteine C D--Cys, S--Me--Cys, Met, D--Met, Thr, D--Thr

Glutamine Q D--Gln, Asn, D--Asn, Glu, D--Glu, Asp, D--Asp

Glutamic E D--Glu, D--Asp, Asp, Asn, D--Asn, Gln, Acid D--Gln

Glycine G Ala, D--Ala, Pro, D--Pro, β-Ala Acp

Isoleucine I D--Ile, Val, D--Val, Leu, D--Leu, Met D--Met

Leucine L D--Leu, Val, D--Val, Leu, D--Leu, Met, D--Met

Lysine K D--Lys, Arg, D--Arg, homo-Arg, D-homo-Arg, Met, D--Met, Ile, D--Ile, Orn, D--Orn

Methionine M D--Met, S--Me--Cys, Ile, D--Ile, Leu, D--Leu, Val, D--Val

Phenylalanine F D--Phe, Tyr, D--Thr, L-Dopa, His, D--His, Trp, D--Trp, Trans-3,4, or 5- phenylproline, cis-3,4, or 5-phenylproline

Proline P D--Pro, L--I-thioazolidine-4- carboxylic acid, D-- or L-1 oxazolidine-4-carboxylic acid

Serine S D--Ser, Thr, D--Thr, allo-Thr, Met, D--Met, Met(O), D--Met(O), L--Cys, D--Cys

Threonine T D--Thr, Ser, D--Ser, allo-Thr, Met, D--Met, Met(O), D--Met(O), Val, D--Val

Tyrosine Y D--Tyr, Phe, D--Phe, L-Dopa, His, D--His

Valine V D--Val, Leu, D--Ieu Ile, D--Ile, Met, D—Met

[00098] In an embodiment the following substitutions can be made: S and T; I, V, and L; and/or F and Y.

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[00099] In an embodiment, the conservative substitution is selected from the following six groups which each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);

2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

[000100] The step of obtaining the recombinant cell expressing a TCR comprising the bait TCR polypeptide chain and a prey TCR polypeptide chain that selectively binds said peptide of interest from the transduced cell population can comprise isolating one or more cells (e.g. one or more clones) that express the transduced bait polypeptide and which bind the peptide of interest.

[000101] As used herein "counterchain TCR chain" relates to a TCR chain that can associate with a stipulated TCR chain type to constitute a TCR. For example, in the context of a bait TCRalpha chain, the counterchain prey TCR chain would be a prey TCRbeta chain and vice versa. Similarly, in the context of a bait TCRgamma chain, the counterchain prey TCR chain would be a prey TCRdelta chain and vice versa.

[000102] In an embodiment, the obtaining step comprises removing non-transduced cells, cloning the prey nucleic acid, for example into a vector, and determining the avidity and/or affinity of reconstituted TCRs comprising the bait and prey nucleic acids for the peptide of interest. The avidity and/or affinity can be relative, such as relative to other cloned nucleic acids and/or a control or be absolute. For example, prey nucleic acids that in combination with a bait nucleic acid (or other same type TCR chain) constitute a TCR with a desired or preselected affinity/avidity can be isolated. For example the prey nucleic acid can be amplified and/or the previously selected clone can be replicated, using a method appropriate for the type of vector employed.

[000103]   Alternatively, the recombinant cell expressing the prey nucleic acid can be propagated, and/or packaged.

[000104]   In an embodiment, the prey nucleic acid is isolated, for example by cloning. The method of cloning the prey nucleic acid is not particularly limited and can comprise determining the TCR Vbeta repertoire of the selected cell and amplifying the prey nucleic acid to make a cDNA using a primer that is specific for the identified TCR Vbeta chain and a TCR beta constant region primer, as further described below. If the prey nucleic acid is a TCRalpha chain, the TCR Valpha repertoire of the selected cell can be determined as above. In addition, 5'RACE based methods can be employed. In an embodiment, two constant region reverse primers are used as described in the examples to enhance the specificity of PCR and specifically clone TCR genes.

[000105]   It is demonstrated in Fig. 2, that thymic selection does not appear to affect the TCRβ repertoire that can constitute a peptide specific TCR with a prey TCRalpha nucleic acid (e.g. as demonstrated with SIG35α).

[000106]   Fig. 20 shows a schematic of TCRs that can be obtained with single chain transduction (e.g. transduction of either the TCRalpha or the TCRbeta chain or a functional portion thereof. Single chain transduction (e.g. transduction of one TCR chain) produces a thymically unselected TCR repertoire with an unphysiological range of affinity, including TCRs with greater or lesser affinity/avidity for a specific peptide.

[000107]   As shown in Fig. 22, single chain applications require transduction of for example an alpha or beta TCR polypeptide chain, which result in highly polyclonal TCR clonality, and highly broad TCR affinity/avidity.

[000108]   For example, single chain gene transfer can generate high avidity antitumor T cells. As shown in Example 2, high affinity tumor-reactive TCRs from peripheral T cells were identified by generating a thymically unselected T cell repertoire. CD8+ T cells were transduced with a TCRalpha chain, SIG35alpha, and were able to recognize A2/MART1 peptide. Clonotypic TRBV27 TCRbeta

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chains were reconstituted with SIG35alpha on human TCRαβ-deficient T cells in the presence or absence of the CD8 co-receptor. Six transfectants, clones 8H, 7Q, 9J, 4K, 7E, 9I and 6X, presented higher avidity than the one expressing A2/MART1 TCR, DMF5. The transfectants also recognized A2+ MART1+ tumor cells in a CD8-independent manner (see Table 7).

[000109]   Accordingly, the method allows for a variety of prey TCR chains to be identified which pair with the bait TCR chain to constitute a TCR with increased or decreased avidity and/or affinity compared to a control, optionally the parent TCR, or a preselected avidity and/or affinity.

[000110]   In an embodiment, the prey TCR chain polypeptide which comprises a CDR3 region comprises at least one amino acid modification relative to the CDR3 region in a control TCR chain polypeptide CDR3 region, optionally the CDR3 region of the parent TCR chain. CDR3, which is the most hypervariable region of the TCR, can comprise from 6 to 20 amino acids and is a major determinant of peptide specificity. The at least one amino acid modification can for example be an amino acid change and or an increase or decrease in the number of amino acid residues in the region that constitutes the CDR3 region.

[000111]   The isolated prey nucleic acid is in an embodiment, modified. Mutations in CDR3 can be introduced in an embodiment, to increase and/or decrease peptide affinity/avidity.

[000112]   CDR1 and CDR2 are also determinates of peptide recognition (e.g. sequence and length) although to a lesser extent typically than CDR3. CDR1 for example can be swapped with other CDR1 regions. Other regions such as the constant region which are not directly involved in peptide recognition may tolerate changes that do not affect or only minimally affect peptide binding avidity and/or affinity. In an embodiment, the isolated prey nucleic acid is modified and comprises a prey CDR3 region in combination with a CDR2 and CDR1 region and a constant region, wherein the CDR1 and/or CDR2 region, and/or the constant region comprise one or more amino acid changes, optionally wherein the CDR1 region is swapped and/or wherein a portion of the constant region or other non-CDR region is deleted producing for example a truncated functional fragment.

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[000113]   In an embodiment, once a bait nucleic acid is isolated, a fused TCR and/or heterogeneous TCR can be constituted, optionally with the isolated prey TCR chain or a modified TCR prey chain and the bait TCR chain.

[000114]   An isolated prey nucleic acid can also in an embodiment be used as a bait nucleic acid to identify a counterchain to the isolated prey nucleic acid, for example to provide a constituted TCR that comprises neither chain of the parent TCR.

[000115]   In an embodiment, the bait polypeptide chain and the prey TCR polypeptide chain constitute a TCR with i) a high avidity and/or affinity (e.g. higher than a preselected standard); and/or ii) an increased avidity and/or affinity for the peptide of interest compared to the parent TCR.

[000116]   In an embodiment, the obtaining step comprises testing, e.g. determining, the avidity and/or affinity of a constituted TCR (e.g. bait and cloned prey nucleic acid) for peptide binding avidity and/or affinity.

[000117]   In an embodiment, the obtaining step comprises enriching for high avidity T cells, for example prior to cloning the prey TCR nucleic acids, optionally by stimulating T cells with an aAPC comprising a mutated HLA, optionally mutated HLA-A2. For example, mutated HLA-A2 abrogates binding to CD8 molecules without affecting affinity of the TCR/HLA interaction can be used to enrich for high avidity and/or affinity TCRs. It has been previously shown that artificial APC expressing mutated HLA-A2 can selectively expand high avidity antigen-specific T cells (Imataki et al., 2012).

[000118]   In another embodiment, the obtaining step comprises one or more of isolating transduced/recombinant cells, cloning one or more prey TCR chain nucleic acids and expressing one of the cloned prey TCR chain nucleic acid in a cell to provide a heterogeneous TCR and testing the avidity and/or affinity of the heterogeneous TCR to the peptide of interest. In an embodiment a prey TCR chain nucleic acid which when constituted in a TCR imparts high avidity and/or affinity is isolated.

[000119]   In an embodiment, the method is for identifying a TCR polypeptide chain that constitutes a TCR with a high avidity and/or affinity and/or an increased avidity and/or affinity for a peptide of interest, wherein the method further comprises introducing the cloned prey nucleic acid and the bait

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nucleic acid into a cell able to express a TCR or to differentiate into a cell able to express a TCR and measuring the avidity and/or affinity of the TCR comprising the prey TCR polypeptide chain and the bait TCR polypeptide chain; identifying a clone wherein the bait TCRalpha or TCRbeta polypeptide chain and the prey TCR polypeptide chain constitute a TCR having increased (or alternatively decreased)

5   avidity and/or affinity for the peptide of interest compared to a control optionally the parent TCR.

[000120]   In an embodiment, a cell expressing the heterogeneous TCR has an avidity sufficient to recognize a peptide of interest presented on a cell in the absence of exogenously pulsing the cells with peptide. For example in the case where the peptide of interest is a tumor antigen peptidethe heterogeneous TCR can recognize a tumor cell presenting the tumor antigen in the absence of

10   modification, e.g. without exogenously pulsing the tumor cell with the peptide of interest.

[000121]   In an embodiment, the avidity and/or affinity of the heterogeneous TCR in increased at least 25%, at least 50%, at least 75%, at least 100%, at least 200%, at least 500% or more compared to a control such as the parent TCR.

[000122]   In an embodiment, the avidity and/or affinity of the heterogeneous TCR in decreased at

15   least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or more compared to a control such as the parent TCR.

[000123]   In an embodiment, the increased avidity is an increased structural avidity and/or an increased functional avidity.

[000124]   As used herein, the term "avidity" as used herein refers to a measure off multiple

20   affinities, optionally the overall strength of binding, between an antigen with antigenic determinants (such as a peptide of interest), optionally presented by an antigen presenting cell, and a binding protein such an a TCR or antibody, optionally in the contest of a cell expressing the TCR or antibody, for example the overall strength of binding between a displayed peptide such as a tumor associated antigen and a TCR or the overall strength of binding between an aAPC displayed peptide such as a

25   tumor associated antigen and a cell expressing a TCR. The term "functional avidity" as used herein is the concentration of an antigen (e.g. peptide) required to achieve 50% of maximal response in a

functional assay. For each functional assay, the maximal response is determined. Functions measured include for example abilities to secrete cytokines such as IL-2 and IFN-gamma and to cytolyze. The maximal response is obtained when T cells are maximally stimulated. Therefore, maximal responses depend on the functional capabilities of given T cell(s) and can for example be expressed as EC50 in µM or other molar units. The term "structural avidity" as used herein and which can be expressed as EC50 in µg/mL or other concentration units, is the concentration of an antigen (e.g. a peptide multimer) required to achieve half maximal antigen staining (e.g. multimer staining). Functional and structural avidity can for example be calculated with GraphPad prism 6 software.

[000125] The term "affinity" as used herein means the strength of a single interaction between for example a peptide (presented in the context of HLA) and a TCR. Affinity is measured for example where the peptide of interest is presented in a cell free context, for example where the HLA presented peptide is bound to a surface or bead.

[000126] As used herein "high avidity" means avidity sufficiently high to recognize target cells presenting a peptide of interest without any modification such as pulsing of exogenous peptides.

[000127] Functional avidity can be measured for example using an enzyme-linked immunospot (ELISPOT) assay and measuring for example, interferon-gamma (IFNgamma) and/or IL-2 production as described in the Examples, and/or a combination thereof. Structural avidity can be measured by staining, TCR expressing cells, optionally T cells using graded concentrations of HLA/peptide multimers. In some embodiments, avidity is assessed by flow cytometric analysis for specific TCR binding to fluorochrome-labeled multimeric synthetic HLA/peptide complexes, and/or functional assessment via transduction of peripheral blood lymphocytes and stimulating in vitro with peptide-pulsed, HLA-matched APC and screening for IFN-gamma production using standard enzyme-linked immunosorbent assay (ELISA) or ELLISPOT assays. In some embodiments, T cell avidity is detected via a dual stain cell sorting protocol that detects cells bound to the fluorescently labeled multimer in conjunction with an intracellular stain for IFN-gamma, indicating that the cell was activated by the recognition of the TCR-multimer complex.

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[000128]   In an embodiment, the bait TCR polypeptide chain, optionally the bait TCRalpha and/or bait TCRbeta polypeptide chain was expressed and previously isolated from a T cell recognizing said peptide of interest. In another embodiment, the bait polypeptide is constructed using known CDR1, CDR2 and CDR3 sequences.

[000129]   An embodiment includes a method for producing a nucleic acid encoding a TCR polypeptide chain which in combination with a counterchain TCR polypeptide constitutes a TCR specific for a peptide of interest, the method comprising the steps of:

   (a)   transducing a cell population comprising cells able to express a TCR or differentiate into cells expressing a TCR with a bait nucleic acid which encodes a TCR polypeptide chain selected from TCRalpha or TCRbeta polypeptide chains, which TCR polypeptide chain in combination with a counterchain TCR polypeptide chain constituted an expressed TCR, wherein the bait TCR polypeptide chain was previously isolated from a T cell recognizing said peptide of interest,

   (b)   culturing the transduced cell population under conditions that permit the bait TCR polypeptide chain to be expressed;

   (c)   obtaining one or more cells expressing the TCR specific for a peptide of interest from the transduced cell population obtained in step (b), and

   (d)   isolating a prey nucleic acid encoding a prey TCR polypeptide chain which constitutes a TCR with the TCR polypeptide chain encoded by the bait nucleic acid transduced into said cell population from the cell obtainedd in step (c).

[000130]   In another aspect, the present disclosure relates to a method for producing a pair of nucleic acids encoding a TCR specific for a peptide of interest, which comprises the steps of:

   (a)   transducing a cell population with a bait nucleic acid which encodes either one of TCRalpha or TCRbeta polypeptide chains constituting a TCR expressed and previously isolated from a T cell recognizing said peptide of interest, wherein said cell population comprising cells which are able to express a TCR or differentiate into cells expressing a TCR,

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(b)   culturing the transduced cell population under conditions that permit the bait TCR polypeptide chain to be expressed;

(c)   selecting one or more cells expressing a TCR specific for a peptide of interest from the transduced cell population obtained in step (b),

(d)   isolating a prey nucleic acid encoding a TCR polypeptide chain which can constitute a TCR with the TCR polypeptide chain encoded by the bait nucleic acid from the one or more cells selected in step (c), and

(e)   pairing the bait nucleic acid transduced into said cell population in step (a) or a nucleic acid encoding a same chain TCR polypeptide chain with the prey nucleic acid isolated in step (d).

[000131]   The steps for example of (a) (b) and (c) shown above of the method for producing a pair of nucleic acids of present disclosure can be carried out by the methodology described herein. The isolating step can be performed by common method for isolating TCR chain gene, for example, PCR using the primer complementary to a nucleic acid sequence encoding constant region of TCR peptide. If the prey nucleic acid has been cloned for example in step (c), the isolating step can comprise, selecting a clone prey nucleic acid with the desired avidity compared to the parental TCR, for example by propagating the vector comprising the prey nucleic acid and/or amplifying the prey nucleic acid from the selected cells.

[000132]   The bait nucleic acid encoding the TCR polypeptide chain can be previously isolated from a cytotoxic T lymphocyte (CTL) and/or tumor infiltrating lymphocyte (TIL). In an embodiment, the CTL is isolated from a patient with a disorder such as cancers (e.g. leukemia, solid tumors and the like), hepatitis, infectious diseases caused by a virus (e.g. influenza virus, HIV or the like), a bacteria (e.g. Mycobacterium tuberculosis, MRSA, VRE or the like), a fungus (e.g. Aspergillus, Candida, Cryptococcus and the like) are indicated as examples.

[000133]   Pairing can include cloning the bait and prey nucleic acids in a vector or separate vector and transducing the cell with the cloned prey and bait nucleic acids. It can also include combining the

nucleic acids to encode a fused single chain TCR. The vector can be any kind of vector, for example for producing quantities of the cloned nucleic acids or for expression in a host cell.

[000134]   Nucleic acids described herein including the nucleic acids paired may be used in various embodiments. Each of the nucleic acids may be combined and/or operatively linked with one or more elements controlling transcription or translation, or inserted into the vector, for example as described in (2). Particularly preferred embodiments include (i) a vector in which one or both of the isolated and/or paired nucleic acids are inserted, and (ii) a combination of vectors in which each of the paired nucleic acids is inserted. In the aspect of (i), the nucleic acids encoding the TCR polypeptide chains or other polypeptides may be transcribed and translated by separate promoters, respectively, or may be transcribed and translated by one promoter using an internal ribosome entry site (IRES) or a cleavage site between the polypeptide chains such as a furin cleavage site or a self-digesting foot and mouth disease virus F2A peptide (F2A).

[000135]   The term "obtaining" and "producing" as used herein include at least one physical step and can include a selection assay, optionally FACS, chemical selection for example using a selection marker, TCR selection using antigen loaded antigen presenting cells and the like .

[000136]   The term "operatively linked to" refers to the functional relationship of a nucleic acid with another nucleic acid sequence. Promoters, enhancers, transcriptional and translational stop sites, and other signal sequences are examples of nucleic acid sequences operatively linked to other sequences. For example, operative linkage of DNA to a transcriptional control element refers to the physical and functional relationship between the DNA and promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA.

[000137]   As used herein, the term "F2A" refers to the foot-and-mouth virus peptide and F2A like sequences that have been optimized that can mediate protein cleavage between to adjoined sequences. For example F2A peptide sequences can be cloned between nucleic acid molecules encoding a polypeptide allowing expression of multiple proteins from a single open reading frame. The

F2A peptide is represented by SEQ ID NO: 70 and is encoded for example by a nucleic acid comprising SEQ ID NO: 69.

[000138]   The term "furin cleavage site sequence" or "furin cleavage site" as used herein means a nucleic acid that encodes an amino acid sequence (or the encoded amino acid sequence) that is cleaved by a furin enzyme, including for example, amino acid RX(K/R)R (SEQ ID NO: 123), optionally RAKR (SEQ ID NO:66) which is encoded for example by the nucleic acid sequence comprising SEQ ID NO:65.

[000139]   The term "furin" as used herein means a member of the subtilisin-like proprotein convertase family, having typically RX(K/R)R consensus motif (SEQ ID NO: 123) and includes without limitation all known furin molecules including naturally occurring variants and for example those deposited in Genbank with the accession numbers CAA27860   CAA27860.1, CAA37988.1, NP_062204.1, NP_003782.1, NP_001161382.1. Furin is also known as Pace and PC1, PCSK3 SPC1.

[000140]   In addition linker sequences can be added between discrete entities, e.g. the TCR chain and the marker, such as a sgsg sequence represented for example by SEQ ID NO: 68 which is encoded for example by a nucleic acid comprising SEQ ID NO:67.

[000141]   As used herein, the term "sgsg" refers to a sequence of "Glycine" and "Serine" amino acids that can be used as a flexible spacer/linker, including for example Short Linkers such as (Gly-Gly-Ser-Gly) (SEQ ID NO: 124), Middle Linkers such as (Gly-Gly-Ser-Gly)x2, Long Linkers such as (Gly-Gly-Ser-Gly)x3. Other linkers include linkers described in the International Genetically Engineered Machine (iGEM) repository of Standard Biological parts.

[000142]   In an embodiment the nucleic acid encoding the TCR polypeptide chain is combined with a nucleic acid encoding a selection marker polypeptide such as a nucleic acid encoding truncated NGFR polypeptide (ΔNGFR) represented by SEQ ID NO: 72 (e.g. encoded by a nucleic acid comprising SEQ ID NO:71) or any other useful marker e.g. for embodiments wherein the nucleic acid encoding the TCR polypeptide chain is transduced into a cell, suitable selectable markers include

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fluorescent proteins, such as EGFP and related molecules, and cell surface proteins not found in the cell to be transduced and preferably deleted of signaling activity, such as ΔNGFR, truncated EGFR, truncated CD19. The selection marker can for example be fused to the TCR in a single ORF and/or comprised as a separate ORF in a vector. For example, the nucleic acid encoding the TCR polypeptide can be comprised in a vector further comprising the selectable marker.

[000143]   Nucleic acid sequences encoding one or more cleavage sites, such as a furin cleavage site or F2A peptide, can be introduced between the nucleic acid encoding the TCR polypeptide chain and the nucleic acid encoding the selectable marker polypeptide, allowing for marker cleavage if desired.

[000144]   A person skilled in the art in making a fusion construct, would recognize that the stop codons, for example, as found in the TCR chain sequences, would be deleted as shown for example in Fig. 21.

[000145]   In an embodiment, the cell population transduction is repeated a second, third, fourth, fifth or sixth time, for example to increase the diversity of the prey TCR chain that can be baited.

(2) Cells expressing a TCR specific for a peptide of interest

[000146]   In an aspect, the present disclosure relates to a method for producing a cell expressing a TCR specific for a peptide of interest, which comprises the step of transducing a cell population with a nucleic acid which encodes either one of two counterchain polypeptide chains (e.g. TCRalpha or beta) constituting a TCR, optionally where the nucleic acid was expressed and previously isolated from a T cell recognizing said peptide of interest, and wherein said cell population comprises a cell which is able to express a TCR or differentiate into a cell expressing a TCR. See for example Fig. 22. In an embodiment, the method further comprises introducing a nucleic acid encoding an additional TCR polypeptide chain, optionally the counterchain of an introduced or co-introduced TCR polypeptide chain (e.g. TCRalpha where TCR beta has been or is being co-introduced) or a different TCR polypeptide chain (e.g. TCRalpha and TCRdelta).

[000147]   In an embodiment, the isolated prey nucleic acid encoding the prey TCR polypeptide chain is transduced into a population of cells comprising a cell which is able to express a TCR or can differentiate into a cell expressing a TCR, optionally wherein the isolated prey nucleic acid is transduced in combination with a nucleic acid encoding a TCR polypeptide chain that in combination with the prey TCR polypeptide chain constitutes a TCR, optionally the bait TCR nucleic acid, to produce a transduced cell population comprising cells expressing a TCR specific for a peptide of interest.

[000148]   In an embodiment, an isolated bait nucleic acid encoding the bait TCR polypeptide chain is transduced into a population of cells comprising a cell which is able to express a TCR or can differentiate into a cell expressing a TCR, optionally wherein the isolated bait nucleic acid is transduced in combination with a nucleic acid encoding a TCR polypeptide chain that in combination with the bait TCR polypeptide chain constitutes a TCR, optionally the prey TCR nucleic acid, to produce a transduced cell population comprising cells expressing a TCR specific for a peptide of interest. The bait nucleic acid can in addition to encoding the bait polypeptide comprise one or more elements herein described. For example, the bait nucleic acid (and/or the prey nucleic acid for example when used to produce a TCR or recombinant cell) can comprise one or more elements described in [00155]. In the examples where TCR SIG35alpha and TAK1beta genes are used as bait, the bait nucleic acids comprise   a   sequence   that   encodes   an   insect-derived Fibroin   light   chain   signal   sequence   at   the   N-terminus,   which   is efficiently cleaved.   Accordingly in an embodiment, the bait nucleic acid (and/or a prey nucleic acid) comprises a non-native signal sequence such as an insect fibrion light chain signal sequence.

[000149]   The cell population is in an embodiment selected for cells expressing the prey and/or bait nucleic acids.

[000150]   The term "cell that can express a TCR" means as used herein any T cell including for example CD4+, CD8+ and double positive CD4+CD8+ cells and/or any cell that comprises the cellular machinery to express a TCR, either where the TCR is typically present endogenously in said cell type or where it is not endogenous and is introduced into the cell (e.g. by viral infection).

[000151] The term "cell that can differentiate into a cell expressing a TCR" means as used herein a cell can differentiate into a T cell such as a T cell precursor cell, a thymocyte, a hematopoietic stem cell, a bone-marrow cell, an embryonic stem cell or an induced pluripotent stem cell.

[000152] The bait nucleic acids which encode respective polypeptide chains (TCR chains) constituting the TCR specific for a peptide of interest can be identified and/or previously isolated, for example, as described above. As an example, an RNA is prepared from a T cell, for example, a CTL recognizing the peptide of interest by a conventional method, and then a cDNA is synthesized. Using the cDNA as a template, 5'-rapid amplification of cDNA end (RACE) is performed using an antisense primer complementary to a nucleic acid encoding the TCR constant region. 5'-RACE may be performed by a known method. For example, 5'-RACE can be performed using a commercially available kit such as SMART PCR cDNA Synthesis Kit (manufactured by Clontech). The nucleotide sequence of the DNA amplified by the aforementioned procedure is determined, the DNA encoding a TCR chain is selected. When the amino acid sequence of a polypeptide chain constituting a TCR is known, nucleic acids which encode the polypeptide chain can be synthesized chemically.

[000153] A "cDNA" is defined as copy-DNA or complementary-DNA, and is a product of a reverse transcription reaction from an mRNA transcript. "RT-PCR" refers to reverse transcription polymerase chain reaction and results in production of cDNAs that are complementary to the mRNA template(s).

[000154] In an aspect of the present disclosure, a cell is transduced with the nucleic acid encoding either a TCR alpha chain and/or a TCR beta chain. In the case that one TCR chain predominantly contributes to peptide recognition by the TCR, the nucleic acid encoding such TCR chain is preferably used in the present disclosure. A TCR centricity of the peptide recognition may be determined by crystal structure analysis.

[000155] Examples of the nucleic acid which encodes a TCR polypeptide chain include, but are not limited to, a nucleic acid including a variety of elements which enables the translation of a polypeptide encoded by the nucleic acid when said nucleic acid is introduced into a cell are added. For example, the nucleic acid which encodes the TCR polypeptide chain may include a promoter sequence

(e.g., mammal-derived promoters such as phosphoglycerate kinase promoter, Xist promoter, β-actin promoter, RNA polymerase II promoter, etc., virus-derived promoters such as SV40 early promoter, cytomegalovirus promoter, thymidine kinase promoter of herpes simplex virus, LTR promoters of various retroviruses, etc.), a terminator sequence, an enhancer sequence, or other transcription control regions. Further, the nucleic acid may have a sequence which contributes to the translation of the TCR chain (Kozak sequence, etc.). Of course, the aforementioned elements are placed at functionally associated positions with each other so as to be suitable for the transcription of the nucleic acid to an RNA or the translation of a polypeptide. In the case where the nucleic acid is an RNA, elements relating to transcription control may not be added to the nucleic acid.

[000156]   The nucleic acid which encodes a TCR polypeptide chain can be incorporated into a vector as described herein. In addition, the nucleic acid which is a DNA or an RNA can be also transduced directly into a cell to express the TCR polypeptide chain or chains.

[000157]   The term "vector" as used herein is a nucleic acid molecule that is able to replicate autonomously in a host cell and can accept foreign DNA. A vector carries its own origin of replication, one or more unique recognition sites for restriction endonucleases which can be used for the insertion of foreign DNA, and usually selectable markers such as genes coding for antibiotic resistance, and often recognition sequences (e.g. promoter) for the expression of the inserted DNA. Common vectors include plasmid vectors, viral vectors such as retroviral vectors, lentiviral vectors, adeno-associated virus vectors, and adenoviral vectors.

[000158]   A method of transducing a nucleic acid into a cell is not particularly limited, and can be a known method. For example, a method using an electroporation, a calcium phosphate, a cationic lipid or a liposome can be used. The nucleic acid can be easily introduced into a cell with high efficiency by using a commercially available transfection reagent.

[000159]   As used herein, the term "transducing" or alternatively "transforming" which can be used interchangeably, refers to the introduction of a nucleic acid into a cell optionally via introduction into a cell's genome (e.g. using a retroviral method). The method of transducing a nucleic acid into a cell can

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include viral or non-viral methods such as electroporation, calcium phosphate, cationic lipid or liposome based methods, as well as gene gun, sonoporation, magnetofection, etc.) For example, the nucleic acid can be introduced into a cell with high efficiency by using a commercially available transfection reagent. A nucleic acid can be incorporated into a vector. The vector is not particularly limited, and a suitable vector may be selected and then used from known vectors such as a plasmid vector and a virus vector depending on the purpose.

[000160]   A virus vector having the ability to infect a cell to introduce a foreign DNA into the cell is suitable in the present disclosure. In the present disclosure, known virus vectors such as a retrovirus vector (including lentivirus vector, pseudo-typed vector, etc.), an adenovirus vector, an adeno-associated virus vector, a herpesvirus vector, etc., can be used. A virus vector in which a nucleic acid encoding a TCR chain is inserted makes it possible to infect a target cell under the conditions suitable for each virus, and to transduce the nucleic acid into the cell. A retrovirus vector having the ability to incorporate an inserted foreign nucleic acid onto a chromosome is suitable for the present disclosure.

[000161]   In an embodiment, the population of cells, such as a population of PBMC cells, is transduced 2X, 3X, 4X, 5X, 6X or more to increase the heterogeneity of endogenous TCR chains interacting with the transduced nucleic acid encoding a TCR polypeptide chain. A method of obtaining a T cell expressing a transduced TCR polypeptide chain at a high ratio to the endogenous TCR is known. In this method, the expression of the endogenous TCR chain which the T cell originally expresses is suppressed by antisense technology such as siRNA specifically targeted to the endogenous TCR chain (see WO 2008/153029). When the aforementioned method is applied to the present disclosure, a cell expressing a transduced TCR polypeptide chain or chains at a high ratio can be obtained by targeting an endogenous TCR RNA with a siRNA (or other means) that is different in sequence from the nucleic acid to be transduced. The nucleotide sequence to be transduced can be made based on the degeneracy of the genetic code so as it would not be knocked down by siRNA used. In an embodiment, the nucleotide sequence to be transduced (including for example the bait nucleic acid or a nucleic acid to be transduced in combination with a counterchain TCR chain nucleic acid) can be codon optimized.

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[000162]   Since a TCR plays an important role in recognition of an antigen by a T cell, a T cell transduced with a nucleic acid which encodes a TCR chain is one of the preferable aspect of the present disclosure. In this aspect, the nucleic acid may be transduced into a cell capable of differentiating into a cell which can express a TCR, thereafter, the cell may be differentiated into a T cell. Examples of the cell capable of differentiating into a cell which can express a TCR include a hematopoietic stem cell, a common lymphoid progenitor, and a T cell progenitor. In addition, it is not necessary that a cell used in an above transduction be fractionated into a single cell species. A cell population containing the cell, for example, a peripheral blood mononuclear cell (PBMC) population can be obtained from a subject into which a nucleic acid is transduced. In an embodiment, the transduction is repeated a second, third, fourth, fifth or sixth time. In addition, such cell population can be stimulated with CD3 ligand, lectin and the like to enhance the proliferation of T cells, for example prior to transduction, for example to increase the transduction efficiency.

[000163]   The term "subject" includes all members of the animal kingdom, including human. In one embodiment, the subject is an animal. In another embodiment, the subject is a human.

[000164]   In the present disclosure, CD3 ligand is not limited in particular as long as it is a substance having the feature of binding to CD3, but, for instance, it may be an anti-CD3 antibody, particularly preferably an anti-CD3 monoclonal antibody may be used. For instance, the monoclonal antibody OKT3 (Kuneg et al., 1979) is indicated as an example. There is no particular limitation on the concentration of CD3 ligand in the culture medium, but, for instance, when using an anti-CD3 monoclonal antibody, for instance, 0.001 to 100 µg/mL, and in particular 0.01 to 100 µg/mL concentrations are preferred.

[000165]   Additionally, in the present disclosure, cells can also be co-stimulated by adding other co-stimulating factors such as CD28 ligand, as necessary.

[000166]   The cell population containing the subject cell into which a nucleic acid is transduced may be collected from, for example, peripheral blood, bone marrow or umbilical blood of a human or a non-human mammal. If necessary, a T cell and/or a cell capable of differentiating into a T cell can be

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fractionated or enriched and then used in the present disclosure. When a nucleic acid is introduced in a cell for use for example in treating a cancer, the cell population can be collected from a patient to be treated, or a donor having an HLA type matched with or similar to that of the patient. Non-HLA matched donor cells can also be used as long as the transduced TCR recognizes the HLA/peptide complexes on recipient's cells, for example cancer cells when the peptide of interest is from a tumor associated antigen.

[000167]   In the present disclosure, a cell population which has been transduced with a nucleic acid encoding a TCR chain may contain a cell expressing a T cell receptor (TCR) specific for a peptide of interest. The TCR expressed in such T cell consists of the TCR chain expressed from the transduced nucleic acid and another TCR chain endogenously expressed in the T cell. The cell population thus obtained can be stimulated with at least one stimulating factor selected from the group consisting of an antigen presenting cell presenting a peptide of interest, a peptide of interest, a CD3 ligand, a CD28 ligand, a cytokine, a chemokine and a cell having the capability of producing a cytokine or a chemokine.

[000168]   Any cell having the capability of presenting a peptide (such as dendritic cells, macrophages, monocytes, B cells and the like) to which the appropriate peptide of interest has been added, a cell in which a gene is introduced to express the peptide of interest, or a cell collected from an organism, which presents the peptide of interest, can be used as an antigen presenting cell. In addition, an artificial antigen presenting cell (see WO 2003/065977) can be used in the present disclosure.

[000169]   As used herein, the term "aAPC" refers to an artificial antigen-presenting cell. The aAPC can be any cell, including a fibroblastic cell, or antigen displaying fragment thereof, that is modified to display an antigenic peptide (e.g. the peptide of interest in the context of HLA) and optionally costimulatory molecules on a surface such that the peptide and/or costimulatory molecules is/are accessible for TCR activation. The aAPC can also be a bead or carrier comprising the peptide of interest (e.g. in the context of HLA) or any agent that can stimulate T cells in a peptide specific manner. Cytokine antibodies such as anti-CD3 antibodies and other TCR signaling effectors can be added for example to promote aAPC function, e.g. in assays presenting antigen to for example a T cell

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expressing a heterogeneous TCR, when costimulatory molecules are absent in the aAPC used. aAPC can comprise a single allele of HLA, for example aAPC comprising HLA-A2 for MART1 and HLA-A24 for WT1 are described herein.

[000170]   As described in the Examples, an aAPC includes for example HLA null cells, such as K562 cells, transduced with costimulatory ligands such as CD80 and CD83, as well as HLA molecules and/or IL-21, anti-CD3 antibodies, for example wherein the stimulatory molecules and HLA complexed peptide are conjugated to a bead.

[000171]   In the present specification, a peptide of interest is a peptide or a glycopeptide derived from tumor antigen, bacterial antigen or viral antigen. The peptide of interest may be a purified or isolated peptide. The peptide of interest may be a peptide obtained from tumor cell extracts, or tumor cell sonicates and tumor cell hot water extracts containing an antigen peptide, or processed materials of bacteria or virus. Peptides of interest include tumor antigen peptides such as any T cell defined tumor antigen, including for example peptides from cancer-germline genes, differentiation antigens expressed in malignancies, antigens overexpressed in tumors, including for example antigens described in Schultz et al., 2000; Vigneron et al., 2005; Tomita et al., 2011; Vigneron et al., 2012; Ma et al., 2011; Corbiere et al., 2011; Dalet, Stroobant et al., 2011; Charpiro et al., 2006; Guillaume et al., 2010; Dalet, Robbins et al., 2011; Vigneron et al., 2004; Skipper et al., 1996; Hanada et al., 2004; Chaux et al., 1999; and Zarour et al., 2000. A peptide from any antigen that is for example expressed by cancer cells but not by normal cells and that can be presented to T cells can be used.

[000172]   In the method of the present disclosure for producing the cell, the well-known culture media prepared by mixing constituents that are necessary for cell culture, for example, suitable for the lymphocyte culture can be used. For instance, commercially available culture media can be selected and used appropriately. These culture media may contain cytokines, appropriate proteins and other constituents in addition to the original ingredients thereof. Preferably, a culture medium containing IL-2 cytokine is used. There is no particular limitation on the concentration of IL-2 in the culture medium, but

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it may be for instance, preferably 0.01 to 1×10^5 U/mL, more preferably 1 to 1×10^4 U/mL. In addition, for instance, fibronectin, fibronectin fragment or anti-IL-4 antibody can be used as an appropriate protein.

[000173]   There is no particular limitation on the cell culture instrument used in the method for producing the cell selected in the present disclosure. For instance, plates, flasks, bags, large culture containers, bioreactors or the like can be used. For example, a CO_2 gas-permeable cell culture bag can be used as bag. In addition, although the cultivation can be carried out in an open system or a closed system, it is preferred to carry out the cultivation in a closed system from the point of view of safety of the selected cell.

[000174]   In addition to being dissolved to coexist in the culture medium, co-stimulating factors such as CD3 ligand or fibronectin fragment, appropriate protein, cytokines or other constituents contained in the culture medium may also be immobilized on an appropriate solid phase, for instance, cell culture instrument such as plates, flasks and bags, cell culture supports such as beads, membrane or slide glass. There is no particular limitation on the material of these solid phases as long as it can be used for cell cultivation.

[000175]   In the present specification, a cytokine is not limited in particular as long as it can act on and activate a lymphocyte, however, for instance, IL-2, IFN-γ, TGF-β, IL-15, IL-7, IFN-α, IL-12, CD40L, IL-27 and the like are indicated as examples, and from the point of view of enhancing cellular immunity, IL-2, IL-15 and IL-21 are particularly preferably indicated as examples.

[000176]   In the present specification, there is no particular limitation on a cell capable of producing a cytokine, but, for instance, from the point of view of enhancing cellular immunity, Th1 cell is indicated as an example.

[000177]   By adding antigen stimulation to a cell obtained by the method of the present disclosure, induction of a useful antigen-specific lymphocyte is allowed, with extremely high cytotoxic activity and high antigen recognition capability. The cell produced in the present disclosure can survive in an organism over a long period, being an extremely useful cell having high therapeutic effects.

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[000178]   In the present specification, production of a cell or a cell population is synonymous to cultivation of a cell or a cell population, and means a process that comprises each step of induction (activation), maintenance, expansion of the cell or cell population, and/or steps combining these.

[000179]   The method for producing a cell in the present disclosure may further comprise the step of selecting, separating or isolating a cell expressing a TCR specific for a peptide of interest from a cell population transduced with a nucleic acid. This step can be carried out by using a complex (tetramer or multimer) of the peptide of interest and major histocompatibility complex (MHC) (e.g. using a MHC tetramer assay) or by the method based on the property of a cell, for example, a response against a cell presenting the peptide of interest (cytotoxicity or release of cytokine, etc.). In addition, the present disclosure comprises the step of selecting, separating or isolating a cell expressing TCR which has low allogeneic response.

[000180]   For example, as shown in Example 3, the antigen reactivity and allogeneic reactivity in human T cells can be separated at the molecular level by modulating the primary structure of TCRs. TAK1, a WT1-specific TCR clone, recognizes the HLA-A*24:02/WT1235-243 (A24/WT1) while possessing allo-reactivity for HLA-B*57:01 (B57). T cells transduced with a TAK1β chain reconstituted with TRAV36 TCRα chains were shown to be reactive to both A24/WT1 and B57, however TAK1β chains reconstituted with non-TRAV36 TCRα chains showed reactivity to A24/WT1, but not to B57, thus demonstrating that antigen-specific reactivity and allo-reactivity of clonotypic TCRs are separable by modulating the primary structure of TCRs.

[000181]   For example T cells that are less allogeneically reactive can be selected based based on the decreased reactivity to antigen-presenting cells expressing specific allogeneic (non-self) HLA molecules in in vitro assays such as a cytokine ELISPOT assay.To determine alloreactivity, one can screen against a panel of antigen-presenting cells expressing different allogeneic HLA molecules, to identify which specific HLA molecules are associated with alloreactivity. As demonstrated in the Examples, B57 confers alloreactivity to the A24-resrtricted WT1 (235-243) peptide-specific TCR, clone

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TAK1. B57 can confer alloreactivity to other clonotic TCRs with unknown HLA-restriction and antigen specificity.

(3) The cell population comprising the cell expressing a TCR, and the method for treating a disorder

5   [000182]   The present disclosure relates to the cell population comprising the cell expressing a TCR specific for a peptide of interest obtained by a method described in (2). In addition, the present disclosure relates to the cell population comprising the cell expressing a TCR encoded by the pair of nucleic acids obtained by the method described in (1).

[000183]   Furthermore, the present disclosure relates to the method for treating a disorder

10   comprising the step of administering to the subject a therapeutically effective amount of said cell population.

[000184]   Also provided is use of a nucleic acid, composition and/or cell population produced using a method described herein for treating a disorder. Another aspect is a nucleic acid, composition or cell population produced using a method described herein for treating a disorder.

15   [000185]   The cell population produced by the method of the present disclosure is a cell population comprising a cell that recognizes a peptide of interest. For instance, such a cell population is useful for the treatment of a variety of disorders because it provides a cytotoxic activity against a cell presenting the peptide recognized by the TCR. Although there is no particular limitation on the disorder for which the cell population or composition comprising the cell population is administered, for instance,

20   cancers (leukemia, solid tumors and the like), hepatitis, infectious diseases caused by a virus (influenza virus, HIV or the like), a bacteria (Mycobacterium tuberculosis, MRSA, VRE or the like), a fungus (Aspergillus, Candida, Cryptococcus and the like) are indicated as examples. In addition, the cell population produced by the method of the present disclosure can also be used for donor lymphocyte infusion for the purpose of achieving remission of relapsed leukemia, or the like.

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[000186]   In an example, Epstein-Barr virus(EBV)-specific cytotoxic T lymphocytes generated from EBV-seropositive blood donors have been used to successfully treat patients with EBV-positive post transplantation lymphoproliferative disease on the basis of the best HLA match and specific in vitro cytotoxicity (Barker et al., 2010, Haque et al., 2007). Haque et al., 2007 reported that out of 33 patients, 14 achieved a complete remission, 3 showed a partial remission and 16 had no response. Patients receiving CTLs with closer HLA matching responded better.

[000187]   Infused cells, for example recombinant T cells transduced and expressing a heterogeneous TCR, may not have to be recipient-derived and HLA-matched. However, the transduced TCR must recognize the HLA/peptide complexes on the recipient's cells, for example cancer cells when the peptide of interest is a cancer peptide. Hence, the subject cell (e.g. cancer cell) must express an HLA type that is utilized by the heterogeneous TCR to recognize the peptide of interest. Depending on the peptide and the HLA, the donor cell can comprise for example 6, 7, 8 or 9 matching HLA.

[000188]   The population of cells infused or to be infused can comprise cells expressing different heterogeneous TCRS, optionally directed to the same or different peptides of interest.

[000189]   Furthermore, the present disclosure provides a pharmaceutical composition (therapeutic agent) comprising the above cell population and/or isolated and/or recombinantly engineered nucleic acid as an active ingredient, optionally in combination with a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

[000190]   The therapeutic agent containing the cell population is appropriate for use in immunotherapy. In immunotherapy, a cell appropriate for the treatment in a patient is administered, for instance, via injection or drip infusion transvenously, transarterially, subdermally, intraperitoneally or the like. The therapeutic agent is can be used for the above-mentioned disorder or donor lymphocyte

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infusion. The therapeutic agent can be prepared, for instance, as a drip infusion agent or an injectable

agent according to a well-known method in the pharmaceutical field, by mixing the cell population

prepared by the method of the present disclosure as an active ingredient, with a well-known organic or

inorganic carrier, diluent, stabilizer or the like, which is appropriate for parenteral administration. The

5   content of cell population of the present disclosure in the therapeutic agent, the dosage of the

therapeutic agent and the conditions for the therapeutic agent can be determined appropriately

according to well-known immunotherapies. For instance, although there is no particular limitation on the

content of cell population of the present disclosure in a medicine, the cell concentration may be

preferably 1×10^3 to 1×10^{11} cells/mL, more preferably 1×10^4 to 1×10^{10} cells/mL and even more

10   preferably 1×10^5 to 1×10^9 cells/mL. In addition, although there is no particular limitation on the dosage

of the medicine of the present disclosure, for instance, the adult dosage may be preferably 1×10^6 to

1×10^{12} cells/day, more preferably 1×10^7 to 5×10^{11} cells/day and even more preferably 1×10^8 to

2×10^{11} cells/day. In addition, combinations of the immunotherapy with the therapeutic agent and a

medicinal therapy by administration of well-known medicines, or a treatment by radiotherapy or surgical

15   operation can be used.

[000191]   The present disclosure further provides a therapeutic or prophylactic method for a

disorder comprising administering to a subject an effective amount of the cell population obtained by

the above-mentioned method. Although there is no particular limitation on the subject herein,

preferably, an organism (for instance a human patient, or a non-human animal) with the above-

20   mentioned disorder for which the cell population prepared by the method of the present disclosure is

administered, is indicated. The therapeutic agent of the present disclosure containing as active

ingredient a cell population into which a nucleic acid encoding the TCR is introduced, is administered to

a subject expressing an HLA molecule that is identical to or has up to three locus mismatches with the

HLA molecule expressed by the cell population.

25   [000192]   In an embodiment, the cell population is purified prior to administration.

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[000193]   As used herein the term "purified cell population" as used herein refers to a population

of cells that has been removed and separated (e.g. isolated) from a mixed or heterogeneous population

of cells and/or other components such as culture medium. In some embodiments, a purified cell

population is a substantially pure population of transduced cells as compared to the heterogeneous

5   population from which the cells were isolated or enriched from.

[000194]   The term "substantially pure", with respect to a particular cell population, refers to a

population of cells that is at least about 65%, preferably at least about 75%, at least about 85%, more

preferably at least about 90%, and most preferably at least about 95% pure, with respect to the cells

making up a total cell population. Similarly, with regard to a "substantially pure" population, refers to a

10   population of cells that contain fewer than about 30%, fewer than about 20%, more preferably fewer

than about 15%, 10%, 8%, 7%, most preferably fewer than about 5%, 4%, 3%, 2%, 1%, or less than

1%, of cells that are not transduced.

[000195]   The term "treatment" as used herein as applied to a subject, refers to an approach

aimed at obtaining beneficial or desired results, including clinical results and includes medical

15   procedures and applications including for example pharmaceutical interventions, surgery, radiotherapy

and naturopathic interventions as well as test treatments. Beneficial or desired clinical results can

include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions,

diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of

disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and

20   remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean

prolonging survival as compared to expected survival if not receiving treatment.

[000196]   In addition, the term "effective amount" as used herein is the amount of the cell

population, exerting therapeutic or prophylactic effects when the cell population is administered to the

subject, compared to a subject into which the cell population has not been administered. While a

25   specific effective amount can vary depending on the dosage form, administration method, purpose of

use, age and body weight of the subject, symptoms and the like, preferably, it is similar to the above-

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mentioned medicines. The administration method is also not limited, but, for instance, administration by drip infusion, injection or the like is preferred, similarly to the above medicines.

[000197] Provided herein in another aspect is a use of a cell comprising a heterogeneous TCR, a population of cells comprising said cell, a therapeutic agent, an agent comprising a heterogeneous TCR

5 conjugated to an agent such as an antibody and/or a toxic moiety, a nucleic acid encoding a single chain heterogeneous TCR and/or a pair of nucleic acids which together encode a heterogeneous TCR, or a pharmaceutical composition comprising one of the aforementioned products for alleviating a symptom and/or treating a subject, optionally a subject afflicted by a cancer or other disease expressing a peptide recognized by the heterogeneous TCR.

10 [000198] For example, adoptive transfer of TCR gene-modified T cells may be used for cancer immunotherapy. As shown in Example 2, adoptive transfer of SIG35alpha transduced T cells was also shown to inhibit *in vivo* growth of A2+ MART1+ tumor cells. This strategy was also used to in generate CD8+ T cells specific for peptides expressed in other types of malignancies, including A2/NYESO-1 and A2/Her2.

15 **(4) Isolated molecules**

[000199] The present disclosure also provides novel TCR chains and CDR3 region polypeptides and nucleic acids encoding such TCR chains and CDR3 regions including for example, one or more of the sequences described herein. A cell expressing a TCR specific for the MART1$_{27-35}$ peptide, represented by SEQ ID NO: 73, can be obtained by using the nucleic acid encoding SIG35α chain

20 which has an amino acid sequence of SEQ ID NO:2. The TCR expressed in obtained T cell is formed by SIG35α chain and a β chain endogenously expressed in the transduced cell. For example, Clone 794 and Clone 830 each express a TCR constituted by the SIG35α chain and the endogenously expressed TCR β chain, and having a high avidity to the MART1$_{27-35}$ peptide. An isolated and/or recombinantly engineered nucleic acid encoding the TCR β chains of Clone 794 (SEQ ID NO:4) and

25 clone 830 (SEQ ID NO:6) are included in the present disclosure. Similarly, a cell expressing a TCR

specific for the WT1$_{235-243}$ peptide, represented by SEQ ID NO: 74, can be obtained by using the nucleic acid encoding TAK1β chain which has an amino acid sequence of SEQ ID NO:16. Clone T53, Clone A262, Clone T243 and Clone T262 each express a TCR constituted by the TAK1β chain and the endogenously expressed TCR α chain, and having a high avidity to the WT1$_{235-243}$ peptide. A nucleic acid encoding the TCR α chains of Clone T53 (SEQ ID NO:8), Clone A262 (SEQ ID NO:10), Clone T243 (SEQ ID NO:12) and Clone T262 (SEQ ID NO:14) are included in the present disclosure. In addition, Clone 8H, Clone 7Q and Clone 9J each express a TCR constituted by the SIG35α chain and the endogenously expressed TCR β chain, and having a high avidity to the MART1$_{27-35}$ peptide. An isolated and/or recombinantly engineered nucleic acid encoding the TCR β chains of Clone 8H (SEQ ID NO:94), Clone 7Q (SEQ ID NO:96) and Clone 9J (SEQ ID NO:98) are included in the present disclosure

[000200]   The term "recombinantly engineered" as used herein means an entity prepared using recombinant technology and not existing in nature, for example such as cDNA, a transduced cell, labelled nucleic acids (e.g. labelled with a fluorescent or radioactive label), chimeric nucleic acids and polypeptides and the like.

[000201]   In another aspect, the disclosure includes an isolated and/or recombinantly engineered polypeptide comprising a sequence selected from SEQ ID NOs: 4, 6, 8, 10, 12, 14, 52, 54, 56, 58, 60, 62, 81-91, 94, 96, 98, 112, 114, 116-122 and/or a sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a sequence selected from to a sequence selected from SEQ ID NOs: 4, 6, 8, 10, 12, 14, 52, 54, 56, 58, 60, 62, 81-91, 94, 96, 98, 112, 114, 116-122 or a portion thereof such as a CDR region or a non-CDR region. In an embodiment, the polypeptide is encoded by a nucleic acid selected from any one of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 51, 53, 55, 57, 59, 61, 93, 95, 97, 111, 113, 115 and/or a sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a sequence selected from SEQ ID NOs: 3, 5, 7, 9, 11, 13, 51, 53, 55, 57, 59, 61, 93, 95, 97, 111, 113, 115 and/or a portion thereof that encodes for example a CDR region or a non-CDR region. In an

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embodiment the polypeptide comprises a sequence of any one of SEQ ID NOs:4, 6, 8, 10, 12, 14, 52, 54, 56, 58, 60, 62, 81-91, 94, 96, 98, 112, 114, 116-122.

[000202] A further aspect includes an antibody or binding fragment thereof that is specific for any one of SEQ ID NOs: 52, 54, 56, 58, 60, 62, 81-91, 94, 96, 98, 112, 114 and 116-122. Methods for making antibodies are known in the art. In an embodiment, the antibody is monoclonal antibody. In an embodiment the antibody is a polyclonal antibody.

[000203] In yet another aspect, the disclosure includes an isolated and/or recombinantly engineered nucleic acid comprising a sequence as shown in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 51, 53, 55, 57, 59, 61, 93, 95, 97, 111, 113 and 115.

[000204] Another aspect includes an isolated and/or recombinant TCR polypeptide chain comprising a CDR3 sequence selected from SEQ ID NO: 52, 54, 56, 58, 60 62 and 81 to 91, 94, 96, 98, 112, 114 and 116-122.

[000205] A further aspect includes an isolated and/or recombinantly engineered TCRbeta chain polypeptide wherein the CDR3 region comprises any one of SEQ ID NOs: 52, 54, 81 to 91, 112, 114 and 116 to 122.

[000206] A further aspect includes an isolated and/or recombinantly engineered TCR comprising a TCRbeta chain polypeptide wherein the CDR3 region comprises any one of SEQ ID NOs: 52, 54, 81 to 9, 112, 114 and 116 to 122.

[000207] Also provided is an isolated and/or recombinantly engineered TCRalpha chain polypeptide wherein the CDR3 region comprises any one of SEQ ID NOs: 56, 58, 60 and 62.

[000208] Another aspect includes an isolated and/or recombinantly engineered TCR comprising a TCRalpha chain polypeptide wherein the CDR3 region comprises any one of SEQ ID NOs: 56, 58, 60 and 62.

[000209] In an embodiment, the isolated TCR polypeptide chain comprises and/or is selected from any amino acid sequence shown in Table 1.

[000210]   In another embodiment, the TCR polypeptide chain comprises a CDR1 region having a sequence selected from any one of the amino acid sequences shown in Table 1, for example SEQ ID NO: 18, 20, 22, 24, 26, 28, 30, 32, 100, 102 or 104; a CDR2 region having a sequence selected from any one of the amino acid sequences shown in Table 1, for example SEQ ID NO: 34, 36, 38, 40, 42, 44, 46, 48, 106, 108 or 110; and a CDR3 region having a sequence selected from any one of the amino acid sequences shown in Table 1, for example SEQ ID NO: 50, 52, 54, 56, 58, 60, 62, 64, 87-92 and 111-122.

[000211]   In an embodiment, the TCRalpha polypeptide chain comprises a CDR1 region comprising the sequence of SEQ ID NO: 20, a CDR2 region comprising the sequence of SEQ ID NO: 36 and a CDR3 region comprising the sequence of SEQ ID NO: 52. In another embodiment, the TCRbeta polypeptide chain comprises a CDR1 region comprising the sequence of SEQ ID NO: 22, a CDR2 region comprising the sequence of SEQ ID NO: 38 and a CDR3 region comprising the sequence of SEQ ID NO: 54. In yet another embodiment, the TCRalpha polypeptide chain comprises a CDR1 region comprising the sequence of SEQ ID NO: 24, a CDR2 region comprising the sequence of SEQ ID NO: 40 and a CDR3 region comprising the sequence of SEQ ID NO: 56. In another embodiment, the TCRalpha polypeptide chain comprises a CDR1 region comprising the sequence of SEQ ID NO: 26, a CDR2 region comprising the sequence of SEQ ID NO: 42 and a CDR3 region comprising the sequence of SEQ ID NO: 58. In an embodiment, the TCRalpha polypeptide chain comprises a CDR1 region comprising the sequence of SEQ ID NO: 28, a CDR2 region comprising the sequence of SEQ ID NO: 44 and a CDR3 region comprising the sequence of SEQ ID NO: 60. In yet another embodiment, the TCRalpha polypeptide chain comprises a CDR1 region comprising the sequence of SEQ ID NO: 30, a CDR2 region comprising the sequence of SEQ ID NO: 46 and a CDR3 region comprising the sequence of SEQ ID NO: 62.

[000212]   In an embodiment, the isolated TCR polypeptide chain is a recombinant TCR comprising a label or tag. In an embodiment, the polypeptide comprises at least one amino acid difference from a sequence described herein.

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[000213]   In an embodiment, the isolated TCR polypeptide chain comprises a sequence selected from SEQ ID NO: 4, 6, 8, 10, 12, 14, 94, 96 and 98.

[000214]   Also provided is an isolated nucleic acid molecule encoding one of the TCR chain polypeptides, optionally comprising a tag or label. In an embodiment, the nucleic acid comprises at least one nucleotide difference from a sequence described herein optionally wherein said nucleotide difference encodes at least one amino acid chain.

[000215]   In an embodiment, the isolated nucleic acid encoding a TCR polypeptide chain comprises and/or is selected from any nucleic acid sequence shown in Table 1.

[000216]   Another aspect includes an isolated and/or recombinantly engineered cell comprising a TCR comprising a TCR alpha polypeptide and a TCR beta polypeptide wherein the TCRbeta chain polypeptide comprises a CDR3 region having a sequence selected from any one of SEQ ID NOs: 52, 54, 81 to 91, 112, 114 and 116 to 122.

[000217]   Another aspect of the disclosure is an isolated and/or recombinantly engineered cell comprising a TCR comprising a TCR alpha polypeptide and a TCR beta polypeptide wherein the TCRalpha polypeptide chain comprises a CDR3 region having a sequence selected from any one of SEQ ID NOs: 56, 58, 60 and 62.

[000218]   In an embodiment, the isolated and/or recombinantly engineered cell comprises a nucleic acid encoding a TCR chain comprising any one of SEQ ID NOs: 52, 54, 56, 58, 60, 62, 81 to 91, 112, 114 and 116 to 122.

[000219]   In an embodiment, the nucleic acid is comprised in a composition, optionally in combination with a diluent, such as water and/or buffer.

[000220]   In an embodiment, the polypeptide is comprised in a composition, optionally comprising a lipid membrane. In an embodiment, the polypeptide is comprised in a complex with CD3. In an embodiment, the complex comprises a TCR (optionally TCRalpha and TCRbeta) and CD3 consisting of a CD3γ chain, a CD3δ chain, and two CD3ε chains.

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[000221]   In an embodiment, the cell is comprised in a composition, optionally comprising an isotonic buffer.

[000222]   In another aspect the paired nucleic acids are used to provide an isolated recombinantly engineered TCR.

[000223]   Reference is made herein to the "IMGT nomenclature". The IMGT nomenclature as used herein refers to the nomenclature used for naming immunoglobulins, TCR, MHC including HLA, by the ImMunoGeneTics information system and for HL follows the following rule. The first three letters indicate the locus. The fourth letter indicates the V,D,J, or C region. The next couple of numbers or letters allow for the unambiguous identification of the gene. Alleles are denoted by an asterisk followed by a two-figure number (D1222–D1227 Nucleic Acids Research, 2013, Vol. 41, Database issue, The IMGT/HLA database, James Robinson). The above disclosure generally describes the present application. A more complete understanding can be obtained by reference to the following specific examples. These examples are described solely for the purpose of illustration and are not intended to limit the scope of the application. Changes in form and substitution of equivalents are contemplated as circumstances might suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

[000224]   The following non-limiting examples are illustrative of the present disclosure:

EXAMPLES

[000225]   The present disclosure will be further explained more in more detail by way of Examples, which the present disclosure is not limited to.

Example 1

Cells

[000226]   The PG13 cell line and the phoenix-eco cell line were cultured in DMEM medium supplemented with 10% fetal calf serum (FCS) and 50 µg/mL gentamicin (Gibco). The Jurkat76 cell

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line, which is a Jurkat cell subclone that lacks the expression of CD8αβ and intrinsic TCR molecule, was cultured in RPMI 1640 medium supplemented with 10% FCS and 50 μg/mL gentamicin. HLA-null K562 cells that were transduced with CD80 and CD83 genes were additionally transduced with HLA-A*02:01 or HLA-A*24:02 gene and used as an aAPC. In some experiments, mutated-aAPC cells that were transduced with a mutated HLA-A*02:01 gene bearing two amino acid substitutions at positions 227 and 228 (D227K/T228A) and human IL-21 gene in lieu of wild-type HLA-A*02:01 gene were used. aAPC cells were also cultured in RPMI 1640 medium supplemented with 10% FCS and 50 μg/mL gentamicin. Particularly, mOKT3-aAPC cells, which were transduced with the heavy and light chains of a membranous form of α-human CD3 mAb (clone OKT3) instead of HLA class I molecule, were cultured in RPMI 1640 medium supplemented with 1 mg/mL G418 sulfate (Cellgro), 2.5 μg/mL puromycin (InvivoGen) and 50 μg/mL gentamicin. SupT1 and T2 cells obtained from American Type Culture Collection (ATCC) were cultured in RPMI 1640 supplemented with 10% FCS and 50 μg/mL gentamicin. Melanoma cell lines, A375 (HLA-A*02:01^{+}, MART1^{-}) and Malme-3M (HLA-A*02:01^{+}, MART1^{+}) obtained from ATCC were cultured in DMEM supplemented with 10% FCS and 50 μg/mL gentamicin. Peripheral blood mononuclear cells from healthy volunteers were isolated and stored in liquid nitrogen until use.

Construction of SIG35α, TAK1α and TAK1β retroviral vector

[000227]   A TCRα chain, cloned from the HLA-A*02:01-restricted and MART1$_{27-35}$ (A2/MART1)-specific TCR and designated as SIG35α, was reported as a public TCRα chain (Dietrich et al., 2003, Trautmann et al., 2002, Li et al., 2010). The HLA-A*24:02-restricted and WT1$_{235-243}$ (A24/WT1)-specific TCRα and TCRβ genes were cloned from an established CTL clone, TAK1, using the 5' RACE method (Clontech). The SIG35α gene is TRAV12-2/TRAJ35/Cα, and the TCRα and TCRβ genes of TAK1 are TRAV20/TRAJ33/Cα and TRBV5.1/TRBD2/TRBJ2-1/Cβ2, respectively. SIG35α, TAK1α and TAK1β genes were codon-optimized and each gene was linked with furin cleavage sequence, sgsg linker and foot-and-mouth disease virus (F2A) peptide followed by truncated NGFR gene (ΔNGFR) and the

construct was integrated into a pMX retroviral vector. Ecotropic retroviral vectors were obtained by transient transfection of SIG35α/ΔNGFR, TAK1α/ΔNGFR or TAK1β/ΔNGFR retroviral plasmid with TransIT293 (Mirus Bio) to the phoenix-eco cell line; subsequently, PG13 cell lines were transduced with the ecotropic retroviral vectors and cloned. High-titer GaLV-pseudotyped retroviral vectors were obtained from a stable PG13 cell line and used for transduction into human peripheral T cells.

Establishment of TCR gene-transduced T cells

[000228]   Peripheral blood mononuclear cells (PBMCs) were isolated from healthy volunteers and stimulated with 50 ng/mL α-human CD3 mAb (clone OKT3) in the presence of 100 IU/mL human IL-2 (Proleukin; Novartis) 3 days before transduction.   Then, T cells were transduced with the SIG35α/ΔNGFR retroviral vector by centrifuging 1 hour at 1000g at 32°C for following 6 days. To measure the frequency of A2/MART1-specific T cells after transduction, the cells were labeled with α-human CD8 mAb (clone B9.11; Beckman Coulter), α-human NGFR mAb (clone ME20.4; Biolegend) and PE-conjugated HLA-A*02:01/MART1$_{26-35}$ heteroclitic multimer (Proimmune), HLA-A*02:01/HIV pol$_{476-484}$ multimer (Proimmune) or HLA-A*02:01/Flu$_{58-66}$ multimer.   In other experiments, the TAK1α/ΔNGFR or TAK1β/ΔNGFR gene was also transduced into stimulated T cells as described above to see which TCR chain has a dominant role in dictating A24/WT1 specificity. To evaluate the positivity of WT1-specific T cells after transduction, the cells were labeled with antibodies described above and PE-conjugated HLA-A*24:02/WT1$_{235-243}$ heteroclitic tetramer or HLA-A*24:02/Survivin-2B$_{80-88}$ tetramer. The labeled cells were analyzed using a CANTO™ II flow cytometer (Becton Dickinson) and FlowJo Version 7.6.4 software (TreeStar). The frequency of tetramer and CD8$^+$ gene-modified T cells was analyzed by gating of ΔNGFR$^+$ cells.

Stimulation of gene-modified T cells with aAPC cells

[000229]   To establish antigen-specific T cell lines, CD8$^+$ gene-modified T cells were obtained by CD8$^+$ T cell isolation kit (MACS® beads; Miltenyi Biotec). For expansion of A2/MART1-specific CD8$^+$

gene-modified T cells, wtA2-aAPC cells which express wild-type HLA-A*02:01 molecule or mutA2-aAPC cells which express mutated HLA-A*02:01 molecule and human IL-21 described above instead of wild-type HLA-A*02:01 was pulsed with 10 μg/mL MART1$_{27-35}$ peptide (AAGIGILTV) (SEQ ID NO: 73). For expansion of A24/WT1-specific CD8$^+$ gene-modified T cells, A24-aAPC cells which express HLA-A*24:02 molecule was pulsed with 1 μg/mL WT1$_{235-243}$ heteroclitic peptide (CYTWNQMNL) (SEQ ID NO: 74). aAPC cells were pulsed with peptides in serum-free RPMI 1640 medium for 6 hour at room temperature. Then, aAPC cells were irradiated with 20,000 rads, washed, and added to purified CD8$^+$ gene-modified T cells at a ratio of 1:20 in 24-well plates in RPMI supplemented with 50 μg/mL gentamicin and 10% human AB serum (Gemini Bio-products). The following day, redirected CD8$^+$ T cell cultures were supplemented with low dose of human IL-2 (10 IU/mL) and human IL-15 (10 ng/mL) (PeproTech) every 3 to 4 days. Repeat stimulations were done every 7 days. Following 2 rounds of stimulation, lines were evaluated for each tetramer positivity and IFN-γ secretion. The frequency of tetramer and CD8$^+$ gene-modified T cells was analyzed by gating of ΔNGFR$^+$ cells.

Analysis of TRBV usage in A2/MART1-specific gene-modified T cells

[000230] As shown in Tables 1 and 2 TCR Vβ subtype analysis was performed on A2/MART1 multimer$^+$ CD8$^+$ T cells with the Beta Mark TCR Vβ Repertoire kit (Beckman-Coulter). The nomenclature used for the TCR Vβ subtype analysis is the one from Wei et al., 1994.

Cloning of TCRβ chains paired with SIG35α and TCRα chains paired with TAK1β

[000231] Total RNA was extracted from the SIG35α/ΔNGFR or TAK1β/ΔNGFR transduced T cells using the TRIzol (Ambion) according to the manufacturer's instructions. Full-length TCRβ genes that contain TRBV27 and paired with SIG35α were amplified by RT-PCR using a TRBV27 specific forward primer, 5'-TRBV27 (5'-ATCCCAGTGTGGTGGTACGGGAATTCTGCCATGGGCCCCCAGCTCCTTGGC-3'), (SEQ ID NO: 75) and β constant region specific reverse primers, 3'-Cβ-1 (5'-

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ATCGTCGACCACTGTGCTGGCGGCCGCTCGAGTTCCAGGGCTGCCTTCAGAAATCC-3') (SEQ ID

NO: 76) and 3'-Cβ-2 primer (5'-

GACCACTGTGCTGGCGGCCGCTCGAGCTAGCCTCTGGAATCCTTTCTCTTGACCATTGC-3') (SEQ

ID NO: 77). Full-length TCRα genes paired with TAK1β were cloned as follows. Briefly, cDNA was

prepared by SMART RACE cDNA Amplification Kit (Clontech). For the first PCR, cDNA was amplified

using a 5'-RACE primer and a 3'-TCRα UTR region primer; 5'-GGAGAGTTCCCTCTGTTTGGAGAG-3'

(SEQ ID NO: 78). The second-round semi-nested PCR was performed by using a first PCR product as

a template, a modified 5'-RACE primer; 5'-

GTGTGGTGGTACGGGAATTCAAGCAGTGGTATCAACGCAGAGT-3' (SEQ ID NO: 79) and a 3'-

TCRa constant region primer; 5'-ACCACTGTGCTGGCGGCCGCTCAGCTGGACCACAGCCGCAGCG-

3' (SEQ ID NO: 80). Each TCRβ or TCRα chain amplicon was cloned into the pMX retroviral vector by

Gibson Assembly reaction and sequenced. TCRβ or TCRα gene names are in accordance with IMGT

unique gene nomenclatures. Cloned pMX/TCRβ or pMX/TCRα plasmids were directly used for

transduction.

ELISPOT assay

[000232]   96-well flat-bottom polyvinylidene difluoride plates (Millipore) were coated with capture

α-human interferon-gamma (IFN-γ) mAb (clone 1D1K; MABTECH) or α-human Interleukin-2 (IL-2) mAb

(R&D Systems) and incubated overnight at 4°C. After being washed with PBS supplemented with 2%

FCS, T cells were incubated with 2.0×10^4 per well of indicated APC in the presence or absence of

peptides in RPMI 1640 medium supplemented with 10% FCS for 20-24 hour at 37°C. Peptides used

were 10 µg/mL MART1$_{27-35}$ or 10 µg/mL HIV pol$_{476-484}$ peptide for A2/MART1 and 1 µg/mL WT1$_{235-243}$

heteroclitic peptide or HIV-1 env$_{584-592}$ peptide for A24/WT1. After incubation, plates were washed and

incubated with biotin-conjugated detection α-human IFN-γ mAb (7-B6-1; MABTECH) or α-human IL-2

mAb (R&D system) overnight at 4°C, followed by exposure to HRP-conjugated streptavidin (SA) for

IFN-γ or ALP-conjugated SA for IL-2. To reveal specific spots, 100 µL of 0.1 M acetate buffer

containing 3-amino-9-ethylcarbazole (Sigma-Aldrich) and 0.015% H_2O_2 for IFN-γ or 100 mM Tris-HCl

(pH9.5) buffer containing 100 mM NaCl and 0.5 mM $MgCl_2$ and nitro blue tetrazolium / 5-bromo-4-

chloro-3-indolyl-phosphate (Promega) for IL-2 was added to each well. After 40 minutes, the color

reaction was interrupted by washing with water, and the plates were dried. Diffuse large spots were

5   counted using ImmunoSpot® Version 5.0.2 software (Cellular Technology Limited).

Evaluation of A2/MART1-specific TCR reactivity and A24/WT1-specific TCR reactivity

[000233]   To investigate A2/MART1 reactivity, Jurkat76 cell lines were retrovirally transduced with

SIG35α gene with or without CD8αβ gene. Following transduction of these genes, Jurkat76/SIG35α or

Jurkat76/CD8αβ/SIG35α transfectants were additionally transduced with TCRβ gene (clone: 413, 523,

10   788, 1086, 830, or 794) and sorted with human CD3 MACS® beads system (Miltenyi Biotec). Jurkat76

cell lines were also transduced with an A2/MART1-specific TCR, designated as DMF5, with or without

CD8αβ gene.. Jurkat76/DMF5 TCR transfectants and Jurkat76/CD8αβ/DMF5 TCR transfectants were

also established to compare A2/MART1 reactivity of DMF5 TCR with that of SIG35α/TCRβ TCRs.

These Jurkat76 transfectants were stained with HLA-A*02:01/MART1$_{27-35}$ heteroclitic multimer with

15   graded concentrations to evaluate structural avidity of TCR for A2/MART1. Functional avidity was

tested using T2 cells pulsed with graded concentrations of MART1$_{27-35}$ peptide as stimulators in an IL-2

ELISPOT assay. To assess A24/WT1 reactivity, Jurkat76/CD8αβ transfectants were transduced with

TAK1β gene. Jurkat76/CD8αβ/TAK1β transfectants were additionally transduced with TCRα gene

(clone: T53, A262, T243, T262) or parent TAK1α gene and sorted with FITC-conjugated α-human

20   Vβ5.1 mAb (Beckman Coulter) and an α-FITC-MACS® beads system (Miltenyi Biotec). The established

Jurkat76/CD8αβ/TAK1β/TCRα cell lines were evaluated for A24/WT1 reactivity in an IL-2 ELISPOT

assay. In some experiments, these Jurkat76 transfectants were used to see the reactivity for

A2/MART1 or A24/WT1 that naturally processed and presented on tumor cell surface. Malme-3M cells

were used for A2/MART1-specific TCRs and A24-aAPC cells were used for A24/WT1-specific TCRs.

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Example 2: TCR single chain gene transfer generates high avidity antitumor T cells

[000234]   Adoptive transfer of TCR gene-modified T cells is technically feasible and a promising treatment for cancer immunotherapy. However, thymic selection and peripheral tolerance make it difficult to identify high affinity tumor-reactive TCRs from peripheral T cells. To efficiently isolate high affinity TCRs, a thymically unselected T cell repertoire was generated by introducing peripheral T cells with a single TCR chain gene (e.g. also referred to herein as a hemichain), which alone can dictate HLA-restricted peptide specificity. A shared TCRα gene (clone SIG35α) has been isolated from multiple HLAA*02:01(A2)/MART1 CD8+ T cell clones expressing different clonotypic TCRβ chains. When transduced with SIG35α, peripheral CD8+ T cells, from both A2+ and A2- donors, recognized A2/MART1 and expanded in an A2/MART1-specific manner. In all donors tested, A2/MART1 multimer+ cells predominantly expressed TRBV27 TCRβ chains and their CDR3β sequences were highly diverse (see Fig. 24 and 25)]. Eleven clonotypic TRBV27 TCRβ chains were individually reconstituted with SIG35α on human TCRαβ-deficient T cells in the presence or absence of the CD8 co-receptor. These transfectants possessed a broad range of structural and functional avidities (>2 orders of magnitude). As shown in Table 6, six out of 11 transfectants demonstrated higher avidity than the one expressing A2/MART1 TCR, clone DMF5, and recognized A2+ MART1+ tumor cells in a CD8-independent manner. Adoptive transfer of polyclonal SIG35α chain-transduced CD8+ T cells inhibited the growth of A2+ MART1+ tumor cells *in vivo*. Importantly, the single chain TCR gene transfer strategy was successfully extended to generating CD8+ T cells specific for A2/NYESO-1 and A2/Her2.

Example 3: Molecular separation of antigen reactivity and allogeneic reactivity in human T cells

[000235]   A routine strategy to separate graft versus leukemia (GVL) effect from graft versus host disease (GVHD) in allogeneic hematopoietic stem cell transplantation (HSCT) is still lacking. It has been shown that a clonotypic T-cell receptor (TCR) can possess not only MHC-restricted antigen-specific reactivity but also allogeneic MHC-restricted reactivity (allo-reactivity). It was investigated

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whether GVL and GVHD caused by T cells are separable at a molecular level by modulating the primary structure of TCRs. Wilms tumor 1 (WT1) is a tumor-associated antigen overexpressed in many tumors but not normal cells. A WT1-specific TCR, clone TAK1, which recognizes the HLA-A*24:02/WT1235-243 (A24/WT1) while possessing allo-reactivity for HLA-B*57:01 (B57) was previously isolated. Peripheral T cells transduced with the TAK1β chain but not with the TAK1α chain recognized both A24/WT1 and B57 in all 6 donors tested including two A24-negative donors. Importantly, the A24/WT1 reactivity shown by polyclonal TAK1β-transduced T cells did not correlate with the B57 allo-reactivity. Forty three A24/WT1 and/or B57 reactive TCRs composed of different TCRα chains along with TAK1β chain on CD8α/β+ TCR- T cell line were reconstituted. It was found that A24/WT1 reactivity and B57 allo-reactivity of TRAV36 TCRα chains paired with TAK1β chain did correlate (R2=0.904, *p*<0.0001). In contrast, A24/WT1 and B57 reactivities of non-TRAV36 TCRα chains reconstituted with TAK1β chain did not (R2=0.031, *p*<0.471). A clonotypic non-TRAV36 TCRα chain reactive for A24/WT1 but not B57 was successfully identified. These results suggest that antigen-specific reactivity and allo-reactivity of clonotypic TCRs are separable by modulating the primary structure of TCRs.

Table 1. Sequences of A2/MART1 TCRα (clone SIG35α) and paired TCRβ

Donor	Clone	TRAV	CDR3α	TRAJ	TRBV	CDR3β	TRBJ
Healthy	5H9	12-2	CAVSIGFGNVLHC (SEQ ID NO: 50)	35	27	CASSLLGGSTDTQYF (SEQ ID NO: 81)	2-3
Patient A	4C8	12-2	CAVSIGFGNVLHC (SEQ ID NO: 50)	35	27	CASSPIDGLNTEAFF (SEQ ID NO: 82)	1-1
Patient B	31	12-2	CAVSIGFGNVLHC (SEQ ID NO: 50)	35	27	CASSFNDEQFF (SEQ ID NO: 83)	2-1
Patient C	31	12-2	CAVSIGFGNVLHC (SEQ ID NO: 50)	35	27	CASSPSQGGNTEAFF (SEQ ID NO: 84)	2-1
Patient C	16	12-2	CAVSIGFGNVLHC (SEQ ID NO: 50)	35	27	CASSDSTASSEQFF (SEQ ID NO: 85)	2-1
Patient D	29	12-2	CAVSIGFGNVLHC (SEQ ID NO: 50)	35	5.1	CASSLSGSGDEQFF (SEQ ID NO: 86)	2-1

Clone SIG35alpha can pair with multiple distinct clonotypic TCRβ chains to recognize A2/MART1.

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Table 2. Sequencing results of TCR TRBV27 chains isolated from A2/MART1 multimer^{+} CD8^{+} T cells

Donor	wtA2-aAPC stimulation		mutA2-aAPC/IL-21 stimulation	
	Number of unique clonotypes	Number of isolates sequenced	Number of unique clonotypes	Number of isolates sequenced
#1	56	190	12	19
#3	83	122	26	89
Total	139	312	38	108

Table 3. Functional and structural avidities of the A2/MART1 TCRs

Clone	Donor	Stimulation	TRBV	CDR3β	TRBJ	Functional avidity w/o CD8	Functional avidity w/CD8	Structural avidity w/o CD8	Structural avidity w/CD8
						EC50 (µM)	EC50 (µM)	EC50 (µg/mL)	EC50 (µg/mL)
Cl.794	#3 (A2-)	mutA2-aAPC/IL-21	27	CASSLLGDYGYTF (SEQ ID NO: 52)	1-2	0.12	0.16	0.06	0.02
Cl.830	#3 (A2-)	mutA2-aAPC/IL-21	27	CASSLGGAYEQYF (SEQ ID NO: 54)	2-7	0.13	0.14	0.01	0.006
DMF5	--	--	6-4	CASSLSFGTEAFF (SEQ ID NO: 87)	1-1	1.4	0.33	0.03	0.02
Cl.1086	#3 (A2-)	wtA2-aAPC	27	CASSLHGPGGYTF (SEQ ID NO: 88)	1-2	2.4	0.63	0.04	0.01
Cl.788	#3 (A2-)	wtA2-aAPC	27	CASGPSYEQYF (SEQ ID NO: 89)	2-7	2.9	0.57	0.05	0.01
Cl.523	#3 (A2-)	wtA2-aAPC	27	CASGSYEQYF (SEQ ID NO: 90)	2-7	-	2.7	-	0.3
Cl.413	#1 (A2+)	wtA2-aAPC	27	CASSVFGGDMGEKLFF (SEQ ID NO: 91)	1-4	-	10	-	-

Functional avidity, expressed as EC50 in µM, was defined as the concentration of peptide required to achieve 50% of maximal response. Structural avidity, expressed as EC50 in µg/mL, was defined as the concentration of A2/MART1 multimer required to achieve half maximal multimer staining. They were calculated with GraphPad prism 6 software.

Table 4. Sequences of A24/WT1TCRα

Clone	TRAV	CDR3α	TRAJ
T53	36	CAVITGGTSYGKLTF (SEQ ID NO: 56)	52
A262	36	CAVQNAGGTSYGKLTF (SEQ ID NO: 58)	52
T243	36	CAVLTQTGANNLFF (SEQ ID NO: 60)	36
T262	20	CAVQALRNNAGNNRKLIW (SEQ ID NO: 62)	38
TAK1a	20	CAVQAVDSNYQLIW (SEQ ID NO: 92)	33

5　## Table 5. List of Sequences

SEQ ID NO	NAME OF SEQUENCE	SEQUENCE

SEQ ID NO	NAME OF SEQUENCE	SEQUENCE
1	TCRα chain, Clone SIG35α nucleotide sequence	See Fig. 12
2	TCRα chain, Clone SIG35α amino acid sequence	See Fig.12
3	TCRβ chain, Clone 794 nucleotide sequence	See Fig.13
4	TCRβ chain, Clone 794 amino acid sequence	See Fig.13
5	TCRβ chain, Clone 830 nucleotide sequence	See Fig.14
6	TCRβ chain, Clone 830 amino acid sequence	See Fig.14
7	TCRα chain, Clone T53 nucleotide sequence	See Fig.15
8	TCRα chain, Clone T53 amino acid sequence	See Fig.15
9	TCRα chain, Clone A262 nucleotide sequence	See Fig.16
10	TCRα chain, Clone A262 amino acid sequence	See Fig.16
11	TCRα chain, Clone T243 nucleotide sequence	See Fig.17
12	TCRα chain, Clone T243 amino acid sequence	See Fig.17
13	TCRα chain, Clone T262 nucleotide sequence	See Fig.18
14	TCRα chain, Clone T262 amino acid sequence	See Fig.18
15	TCRβ chain, Clone TAK1 nucleotide sequence	See Fig.19
16	TCRβ chain, Clone TAK1 amino acid sequence	See Fig.19
17	CDR1 sequence of TCRα chain, Clone SIG35α nucleotide sequence	GACCGGGGCTCCCAGAGC
18	CDR1 sequence of TCRα chain, Clone SIG35α amino acid sequence	DRGSQS
19	CDR1 sequence of TCRβ chain, Clone 794 nucleotide sequence	ATGAACCATGAGTAT
20	CDR1 sequence of TCRβ chain, Clone 794 amino acid sequence	MNHEY
21	CDR1 sequence of TCRβ chain, Clone 830 nucleotide sequence	ATGAACCATGAGTAT
22	CDR 1 sequence of TCRβ chain, Clone 830 amino acid sequence	MNHEY
23	CDR1 sequence of TCRα chain, Clone T53 nucleotide sequence	GTGACTAACTTTCGAAGC
24	CDR1 sequence of TCRα chain, Clone T53 amino acid sequence	VTNFRS
25	CDR1 sequence of TCRα chain, Clone A262 nucleotide sequence	GTGACTAACTTTCGAAGC

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SEQ ID NO	NAME OF SEQUENCE	SEQUENCE
26	CDR1 sequence of TCRα chain, Clone A262 amino acid sequence	VTNFRS
27	CDR1 sequence of TCRα chain, Clone T243 nucleotide sequence	GTGACTAACTTTCGAAGC
28	CDR1 sequence of TCRα chain, Clone T243 amino acid sequence	VTNFRS
29	CDR1 sequence of TCRα chain, Clone T262 nucleotide sequence	GTCAGCGGTTTAAGAGGG
30	CDR1 sequence of TCRα chain, Clone T262 amino acid sequence	VSGLRG
31	CDR1 sequence of TCRβ chain, Clone TAK1 nucleotide sequence	AGCGGCCACAGAAGC
32	CDR1 sequence of TCRβ chain, Clone TAK1 amino acid sequence	SGHRS
33	CDR2 sequence of TCRα chain, Clone SIG35α nucleotide sequence	ATCTACAGCAACGGCGAC
34	CDR2 sequence of TCRα chain, Clone SIG35α amino acid sequence	IYSNGD
35	CDR2 sequence of TCRβ chain, Clone 794 nucleotide sequence	TCAATGAATGTTGAGGTG
36	CDR2 sequence of TCRβ chain, Clone 794 amino acid sequence	SMNVEV
37	CDR2 sequence of TCRβ chain, Clone 830 nucleotide sequence	TCAATGAATGTTGAGGTG
38	CDR 2 sequence of TCRβ chain, Clone 830 amino acid sequence	SMNVEV
39	CDR2 sequence of TCRα chain, Clone T53 nucleotide sequence	CTAACTTCAAGTGGAATTGAA
40	CDR2 sequence of TCRα chain, Clone T53 amino acid sequence	LTSSGIE
41	CDR2 sequence of TCRα chain, Clone A262 nucleotide sequence	CTAACTTCAAGTGGAATTGAA
42	CDR2 sequence of TCRα chain, Clone A262 amino acid sequence	LTSSGIE
43	CDR2 sequence of TCRα chain, Clone T243 nucleotide sequence	CTAACTTCAAGTGGAATTGAA
44	CDR2 sequence of TCRα chain, Clone T243 amino acid sequence	LTSSGIE
45	CDR2 sequence of TCRα chain, Clone T262 nucleotide sequence	CTGTATTCAGCTGGGGAAGAA
46	CDR2 sequence of TCRα chain, Clone T262 amino acid sequence	LYSAGEE
47	CDR2 sequence of TCRβ chain, Clone TAK1 nucleotide sequence	TACTTCAGCGAGACACAG

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SEQ ID NO	NAME OF SEQUENCE	SEQUENCE
48	CDR2 sequence of TCRβ chain, Clone TAK1 amino acid sequence	YFSETQ
49	CDR3 sequence of TCRα chain, Clone SIG35α nucleotide sequence	TGTGCCGTGTCCATCGGCTTCGGCAACGTGCT GCACTGC
50	CDR3 sequence of TCRα chain, Clone SIG35α amino acid sequence	CAVSIGFGNVLHC
51	CDR3 sequence of TCRβ chain, Clone 794 nucleotide sequence	TGTGCCAGCAGTCTACTCGGGGACTATGGCTA CACCTTC
52	CDR3 sequence of TCRβ chain, Clone 794 amino acid sequence	CASSLLGDYGYTF
53	CDR3 sequence of TCRβ chain, Clone 830 nucleotide sequence	TGTGCCAGCAGTTTAGGGGGTGCCTACGAGCA GTACTTC
54	CDR 3 sequence of TCRβ chain, Clone 830 amino acid sequence	CASSLGGAYEQYF
55	CDR3 sequence of TCRα chain, Clone T53 nucleotide sequence	TGTGCTGTGATAACTGGTGGTACTAGCTATGG AAAGCTGACATTT
56	CDR3 sequence of TCRα chain, Clone T53 amino acid sequence	CAVITGGTSYGKLTF
57	CDR3 sequence of TCRα chain, Clone A262 nucleotide sequence	TGTGCTGTGCAGAATGCTGGTGGTACTAGCTA TGGAAAGCTGACATTT
58	CDR3 sequence of TCRα chain, Clone A262 amino acid sequence	CAVQNAGGTSYGKLTF
59	CDR3 sequence of TCRα chain, Clone T243 nucleotide sequence	TGTGCTGTGCTTACCCAAACTGGGGCAAACAA CCTCTTCTTT
60	CDR3 sequence of TCRα chain, Clone T243 amino acid sequence	CAVLTQTGANNLFF
61	CDR3 sequence of TCRα chain, Clone T262 nucleotide sequence	TGTGCTGTGCAGGCCTTAAGGAATAATGCTGG CAACAACCGTAAGCTGATTTGG
62	CDR3 sequence of TCRα chain, Clone T262 amino acid sequence	CAVQALRNNAGNNRKLIW
63	CDR3 sequence of TCRβ chain, Clone TAK1 nucleotide sequence	TGTGCCTCTTCTCTGGGCTGGCGGGAAACCTA CAACGAGCAGTTCTTC
64	CDR3 sequence of TCRβ chain, Clone TAK1 amino acid sequence	CASSLGWRETYNEQFF
65	Furin recognition nucleotide sequence	AGGGCCAAGAGA
66	Furin recognition amino acid sequence	RAKR
67	SGSG linker nucleotide sequence	TCTGGATCTGGC
68	SGSG linker amino acid sequence example	SGSG
69	F2A nucleotide sequence	GCCCCTGTGAAGCAGACCCTGAACTTCGACCT GCTGAAGCTGGCCGGCGACGTGGAAAGCAAC CCTGGCCCC
70	F2A amino acid sequence	APVKQTLNFDLLKLAGDVESNPGP

SEQ ID NO	NAME OF SEQUENCE	SEQUENCE
71	Δ NGFR nucleotide sequence	ATGGACGGGCCGCGCCTGCTGCTGTTGCTGC TTCTGGGGGTGTCCCTTGGAGGTGCCAAGGA GGCATGCCCCACAGGCCTGTACACACACAGC GGTGAGTGCTGCAAAGCCTGCAACCTGGGCG AGGGTGTGGCCCAGCCTTGTGGAGCCAACCA GACCGTGTGTGAGCCCTGCCTGGACAGCGTG ACGTTCTCCGACGTGGTGAGCGCGACCGAGC CGTGCAAGCCGTGCACCGAGTGCGTGGGGCT CCAGAGCATGTCGGCGCCCATGCGTGGAGGCC GACGACGCCGTGTGCCGCTGCGCCTACGGCT ACTACCAGGATGAGACGACTGGGCGCTGCGA GGCGTGCCGCGTGTGCGAGGCGGGCTCGGG CCTCGTGTTCTCCTGCCAGGACAAGCAGAACA CCGTGTGCGAGGAGTGCCCCGACGGCACGTA TTCCGACGAGGCCAACCACGTGGACCCGTGC CTGCCCTGCACCGTGTGCGAGGACACCGAGC GCCAGCTCCGCGAGTGCACACGCTGGGCCGA CGCCGAGTGCGAGGAGATCCCTGGCCGTTGG ATTACACGGTCCACACCCCCAGAGGGCTCGGA CAGCACAGCCCCCAGCACCCAGGAGCCTGAG GCACCTCCAGAACAAGACCTCATAGCCAGCAC GGTGGCAGGTGTGGTGACCACAGTGATGGGC AGCTCCCAGCCCGTGGTGACCCGAGGCACCA CCGACAACCTCATCCCTGTCTATTGCTCCATCC TGGCTGCTGTGGTTGTGGGTCTTGTGGCCTAC ATAGCCTTCAAGAGGTGGAACAGC
72	Δ NGFR amino acid sequence	MDGPRLLLLLLLGVSLGGAKEACPTGLYTHSGEC CKACNLGEGVAQPCGANQTVCEPCLDSVTFSDV VSATEPCKPCTECVGLQSMSAPCVEADDAVCRC AYGYYQDETTGRCEACRVCEAGSGLVFSCQDK QNTVCEECPDGTYSDEANHVDPCLPCTVCEDTE RQLRECTRWADAECEEIPGRWITRSTPPEGSDS TAPSTQEPEAPPEQDLIASTVAGVVTTVMGSSQP VVTRGTTDNLIPVYCSILAAVVVGLVAYIAFKRWN S
73	MART1$_{27-35}$ peptide	AAGIGILTV
74	WT1$_{235-243}$ heteroclitic peptide	CYTWNQMNL
75	TRBV27 specific forward primer	5'-ATCCCAGTGTGGTGGTACGGGAATTCTGCCAT GGGCCCCAGCTCCTTGGC-3'
76	β constant region specific reverse primers, 3'-Cβ-1	5'-ATCGTCGACCACTGTGCTGGCGGCCGCTCGA GTTCCAGGGCTGCCTTCAGAAATCC-3'
77	β constant region specific reverse primers, 3'-Cβ-2	5'-GACCACTGTGCTGGCGGCCGCTCGAGCTAGC CTCTGGAATCCTTTCTCTTGACCATTGC-3'
78	3'-TCRα UTR region primer	5'-GGAGAGTTCCCTCTGTTTGGAGAG-3'
79	modified 5'-RACE primer	5'-GTGTGGTGGTACGGGAATTCAAGCAGTGGTAT CAACGCAGAGT-3'
80	3'-TCRa constant region primer	5'-ACCACTGTGCTGGCGGCCGCTCAGCTGGACC ACAGCCGCAGCG-3'

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SEQ ID NO	NAME OF SEQUENCE	SEQUENCE
81	Paired TCRβ chain of CR3 sequence of SIG35 α amino acid sequence from healthy donor, clone 5H9	CASSLLGGSTDTQYF
82	Paired TCRβ chain of CR3 sequence of SIG35 α amino acid sequence from Patient A, clone 4C8	CASSPIDGLNTEAFF
83	Paired TCRβ chain of CR3 sequence of SIG35 α amino acid sequence from Patient B, clone 31	CASSFNDEQFF
84	Paired TCRβ chain of CR3 sequence of SIG35 α amino acid sequence from Patient C, clone 31	CASSPSQGGNTEAFF
85	Paired TCRβ chain of CR3 sequence of SIG35 α amino acid sequence from Patient C, clone 16	CASSDSTASSEQFF
86	Paired TCRβ chain of CR3 sequence of SIG35 α amino acid sequence from Patient D, clone 29	CASSLSGSGDEQFF
87	CDR3 sequence of TCRβ chain, DMF5 amino acid	CASSLSFGTEAFF
88	CDR3 sequence of TCRβ chain, Clone 1086 amino acid	CASSLHGPGGYTF
89	CDR3 sequence of TCRβ chain, Clone 788 amino acid	CASGPSYEQYF
90	CDR3 sequence of TCRβ chain, Clone 523 amino acid	CASGSYEQYF
91	CDR3 sequence of TCRβ chain, Clone 413 amino acid	CASSVFGGDMGEKLFF
92	CDR3 sequence of TCRα chain, Clone TAK1 amino acid sequence	CAVQAVDSNYQLIW
93	TCRβ chain, Clone 8H nucleotide sequence	See Fig.28
94	TCRβ chain, Clone 8H amino acid sequence	See Fig.28
95	TCRβ chain, Clone 7Q nucleotide sequence	See Fig.29
96	TCRβ chain, Clone 7Q amino acid sequence	See Fig.29
97	TCRβ chain, Clone 9J nucleotide sequence	See Fig.30
98	TCRβ chain, Clone 9J amino acid sequence	See Fig.30
99	CDR1 sequence of TCRβ chain, Clone 8H nucleotide sequence	ATGAACCATGAGTAT

SEQ ID NO	NAME OF SEQUENCE	SEQUENCE
100	CDR1 sequence of TCRβ chain, Clone 8H amino acid sequence	MNHEY
101	CDR1 sequence of TCRβ chain, Clone 7Q nucleotide sequence	ATGAACCATGAGTAT
102	CDR1 sequence of TCRβ chain, Clone 7Q amino acid sequence	MNHEY
103	CDR1 sequence of TCRβ chain, Clone 9J nucleotide sequence	TCTGGGCATAGGAGT
104	CDR1 sequence of TCRβ chain, Clone 9J amino acid sequence	SGHRS
105	CDR2 sequence of TCRβ chain, Clone 8H nucleotide sequence	TCAATGAATGTTGAGGTG
106	CDR2 sequence of TCRβ chain, Clone 8H amino acid sequence	SMNVEV
107	CDR2 sequence of TCRβ chain, Clone 7Q nucleotide sequence	TCAATGAATGTTGAGGTG
108	CDR2 sequence of TCRβ chain, Clone 7Q amino acid sequence	SMNVEV
109	CDR2 sequence of TCRβ chain, Clone 9J nucleotide sequence	TACTTCAGTGAGACACAG
110	CDR2 sequence of TCRβ chain, Clone 9J amino acid sequence	YFSETQ
111	CDR3 sequence of TCRβ chain, Clone 8H nucleotide sequence	TGTGCCAGCAGTCCCCTGGGGGCCATGGAGCAGTACTTC
112	CDR3 sequence of TCRβ chain, Clone 8H amino acid sequence	CASSPLGAMEQYF
113	CDR3 sequence of TCRβ chain, Clone 7Q nucleotide sequence	TGTGCCAGCAGTCCCTACATGATGAACACTGAAGCTTTCTTT
114	CDR3 sequence of TCRβ chain, Clone 7Q amino acid sequence	CASSPYMMNTEAFF
115	CDR3 sequence of TCRβ chain, Clone 9J nucleotide sequence	TGCGCCAGCAGCTGGACAGGGGATGGCTACACCTTC
116	CDR3 sequence of TCRβ chain, Clone 9J amino acid sequence	CASSWTGDGYTF
117	Paired TCRβ chain of CR3 sequence of SIG35 α amino acid sequence from Clone 4K	CASSHGGNEQYF
118	Paired TCRβ chain of CR3 sequence of SIG35 α amino acid sequence from Clone 7E	CASSRDFGNTIYF
119	Paired TCRβ chain of CR3 sequence of SIG35 α amino acid sequence from Clone 9I	CASSLAMGATEAFF
120	Paired TCRβ chain of CR3 sequence of SIG35 α amino acid sequence from Clone 6X	CATGVTDTQYF
121	Paired TCRβ chain of CR3 sequence of SIG35 α amino acid sequence from Clone 6B	CASSEVAWQFF

SEQ ID NO	NAME OF SEQUENCE	SEQUENCE
122	Paired TCRβ chain of CR3 sequence of SIG35 α amino acid sequence from Clone 11C	CASDEGFGYTF
123	Furin concensus motif	RX(K/R)R
124	Glycine serine linker	GGSG

Table 6. Sequencing results of TCRβ chains isolated from A2/MART1 multimer^{+} CD4^{+} T cells

	Donor #7 (A2+)		Donor #3 (A2-)	
Vb subtype	Number of unique clonotypes	Number of isolates sequenced	Number of unique clonotypes	Number of isolates sequenced
TRBV2	7	42	7	36
TRBV5-1	11	19	11	31
TRBV27	13	29	13	30

Table 7. Functional and structural avidities of Jurkat 76 cells reconstituted with A2/MART1 TCRs

Clone	Donor	aAPC used for stimulation	TRBV	CDR3b	TRBJ	Functional avidity* without CD8 EC50 (mg/ml)	Functional avidity with CD8 EC50 (mg/ml)	Structural avidity† without CD8 EC50 (mg/ml)	Structural avidity with CD8 EC50 (mg/ml)
Cl.8H	#3 (A2-)	mutA2-aAPC/IL-21	27	CASSPLGAMEQYF (SEQ ID NO: 112)	2-7	0.063	0.040	0.014	0.008
Cl.7Q	#3 (A2-)	mutA2-aAPC/IL-21	27	CASSPYMMNTEAFF (SEQ ID NO: 114)	1-1	0.065	0.043	0.016	0.009
Cl.9J	#3 (A2-)	mutA2-aAPC/IL-21	5-1	CASSWTGDGYTF (SEQ ID NO: 116)	1-2	0.075	0.071	0.018	0.010
Cl.4K	#7 (A2+)	mutA2-aAPC/IL-21	5-1	CASSHGGNEQYF (SEQ ID NO: 117)	2-7	0.083	0.080	0.018	0.011
Cl.7E	#3 (A2-)	mutA2-aAPC/IL-21	27	CASSRDFGNTIYF (SEQ ID NO: 118)	1-3	0.102	0.071	0.010	0.006
Cl.9I	#3 (A2-)	mutA2-aAPC/IL-21	5-1	CASSLAMGATEAFF (SEQ ID NO: 119)	1-1	0.140	0.097	0.025	0.013
Cl.6X	#7 (A2+)	mutA2-aAPC/IL-21	2	CATGVTDTQYF (SEQ ID NO: 120)	2-3	0.150	0.083	0.039	0.018
DMF5	-	-	6-4	CASSLSFGTEAFF (SEQ ID NO: 87)	1-1	0.363	0.102	0.022	0.013
Cl.6B	#7 (A2+)	mutA2-aAPC/IL-21	2	CASSEVAWQFF (SEQ ID NO: 121)	2-1	0.600	0.120	0.044	0.013
Cl.11C	#3 (A2-)	mutA2-aAPC/IL-21	2	CASDEGFGYTF (SEQ ID NO: 122)	1-2	2.460	0.292	0.108	0.018

* Functional avidity, expressed as EC50 in mg/ml, was defined as the concentration of peptide required to achieve 50% of maximal response.

† Structural avidity, expressed as EC50 in mg/ml, was defined as the concentration of A2/MART1 multimer required to achieve half maximal multimer staining.

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WHAT IS CLAIMED IS:

1.   A method for obtaining a high affinity T cell receptor (TCR) specific for a peptide of
        interest, a recombinant cell expressing a TCR specific for a peptide of interest or a
        nucleic acid encoding a TCR polypeptide chain that forms a TCR specific for a peptide of
        interest and wherein the high affinity TCR has a higher affinity than a preselected
        standard or is increased for the peptide of interest compared to a parent TCR, the
        method comprising:
        a. transducing a cell population comprising cells expressing a TCR or cells that when
            differentiated express a TCR with a bait nucleic acid encoding a bait human TCR
            polypeptide chain, wherein the bait human TCR polypeptide chain constitutes the
            parent TCR with a counterchain TCR polypeptide chain that specifically binds said
            peptide of interest;
        b. culturing the transduced cell population under conditions that permit the bait
            human TCR polypeptide chain to be expressed; and
        c.

            i) obtaining a recombinant cell from the transduced cell population in step (b), the
            recombinant cell expressing a TCR comprising the bait human TCR polypeptide
            chain and a prey TCR polypeptide chain that selectively binds said peptide of
            interest, said obtaining step comprising testing the avidity of the recombinant cell
            expressing the TCR for peptide binding avidity and enriching for high avidity T
            cells, and wherein the high avidity is higher than a preselected avidity or is
            increased for the peptide of interest compared to the parent TCR and said
            enriching step comprising culturing the transduced cell population with an antigen
            presenting cell presenting the peptide of interest;

            ii) isolating a prey nucleic acid encoding the prey TCR polypeptide chain from
            the recombinant cell obtained in step (c)(i); and

            iii) pairing the prey nucleic acid isolated in step (c)(ii) with the bait nucleic acid,
            wherein the prey TCR polypeptide chain paired with the bait human TCR
            polypeptide chain constitutes the high affinity TCR.

2.   The method of claim 1, wherein the step of obtaining the recombinant cell expressing a
        TCR comprising the bait human TCR polypeptide chain and the prey TCR polypeptide
        chain that selectively binds said peptide of interest from the transduced cell population

obtained in step (b) comprises isolating one or more cells of the cell population that express the transduced bait human TCR polypeptide chain and which bind the peptide of interest.

3.   The method of claim 1 or 2, wherein the prey nucleic acid is isolated by cloning the prey nucleic acid.

4.   The method of any one of claims 1 to 3, wherein the prey TCR polypeptide chain comprises a CDR3 region comprising at least one amino acid modification relative to the CDR3 region of a control TCR polypeptide CDR3 region.

5.   The method of any one of claims 1 to 3, wherein the prey TCR polypeptide chain comprises a CDR3 region comprising at least one amino acid modification relative to the CDR3 region of the cognate polypeptide chain in the parent TCR.

6.   The method of any one of claims 1 to 5, wherein the method further comprises: i) introducing the isolated prey nucleic acid and the bait nucleic acid into a cell expressing a TCR or a cell that when differentiated expresses a TCR under conditions that permit the bait human TCR polypeptide chain to be expressed; ii) measuring the affinity of the TCR comprising the prey TCR polypeptide chain and the bait TCR polypeptide chain; and iii) isolating a prey nucleic acid clone encoding the prey TCR polypeptide chain, wherein the bait human TCR polypeptide chain and the prey TCR polypeptide chain constitute a TCR having increased affinity for the peptide of interest compared to a control TCR.

7.   The method of any one of claims 1 to 5, wherein the method further comprises: i) introducing the isolated prey nucleic acid and the bait nucleic acid into a cell expressing a TCR or a cell that when differentiated expresses a TCR under conditions that permit the bait human TCR polypeptide chain to be expressed; ii) measuring the affinity of the TCR comprising the prey TCR polypeptide chain and the bait human TCR polypeptide chain; and iii) isolating a prey nucleic acid clone encoding the prey TCR polypeptide chain, wherein the bait human TCR polypeptide chain and the prey TCR polypeptide chain constitute a TCR having increased affinity for the peptide of interest compared to the parent TCR.

8.   The method according to any one of claims 1 to 7, wherein the step of obtaining the recombinant cell expressing the TCR specific for a peptide of interest from the transduced cell population comprises using cell sorting.

9.   The method according to any one of claims 1 to 7, wherein the step of obtaining the recombinant cell expressing the TCR specific for a peptide of interest from the transduced cell population comprises using fluorescence-activated cell sorting (FACS).

10.   The method according to any one of claims 1 to 9, wherein the cell population is a population of peripheral blood mononuclear cells (PBMCs).

11.   The method according to any one of claims 1 to 9, wherein the cell population is a population of PBMCs activated with a CD3 ligand.

12.   The method according to any one of claims 1 to 11, wherein the bait nucleic acid transduced into said cell population in step (a) encodes a TCR alpha chain or a TCR beta chain.

13.   The method according to any one of claims 1 to 12, wherein the bait nucleic acid transduced into said cell population in step (a) encodes a TCR chain which predominantly contributes to peptide recognition by a TCR.

14.   The method of any one of claims 1 to 13, wherein the transduction is repeated a second, third, fourth, fifth or sixth time.

15.   The method of any one of claims 1 to 14, wherein the isolated prey nucleic acid encoding the prey TCR polypeptide chain is transduced into a cell expressing a TCR or a cell that when differentiated expresses a TCR to produce a transduced cell population comprising the recombinant cell.

16.   The method of claim 15, wherein the isolated prey nucleic acid is transduced in combination with a nucleic acid encoding a TCR polypeptide chain that in combination with the prey TCR polypeptide chain constitutes a TCR.

17. The method of claim 16, wherein the nucleic acid encoding a TCR polypeptide chain is the bait TCR nucleic acid.

18. The method of any one of claims 1 to 17, wherein the cell population or cell is also transduced with an antisense molecule for suppressing expression of an endogenous TCR chain.

19. The method of any one of claims 1 to 18, wherein one or more of the nucleic acids being transduced is codon optimized.

20. The method of any one of claims 1 to 18, wherein the bait nucleic acid is codon optimized.

Fig. 1
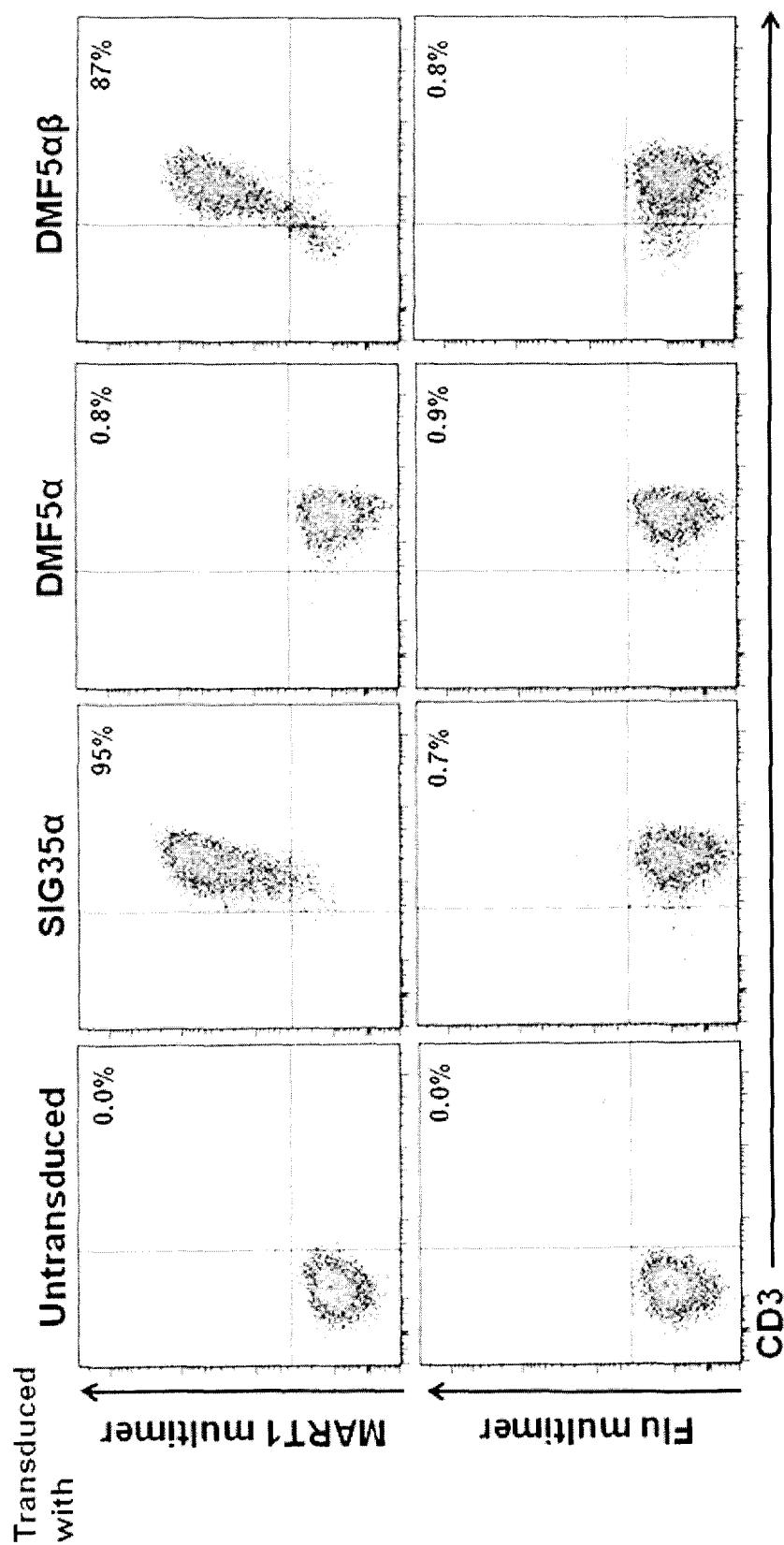
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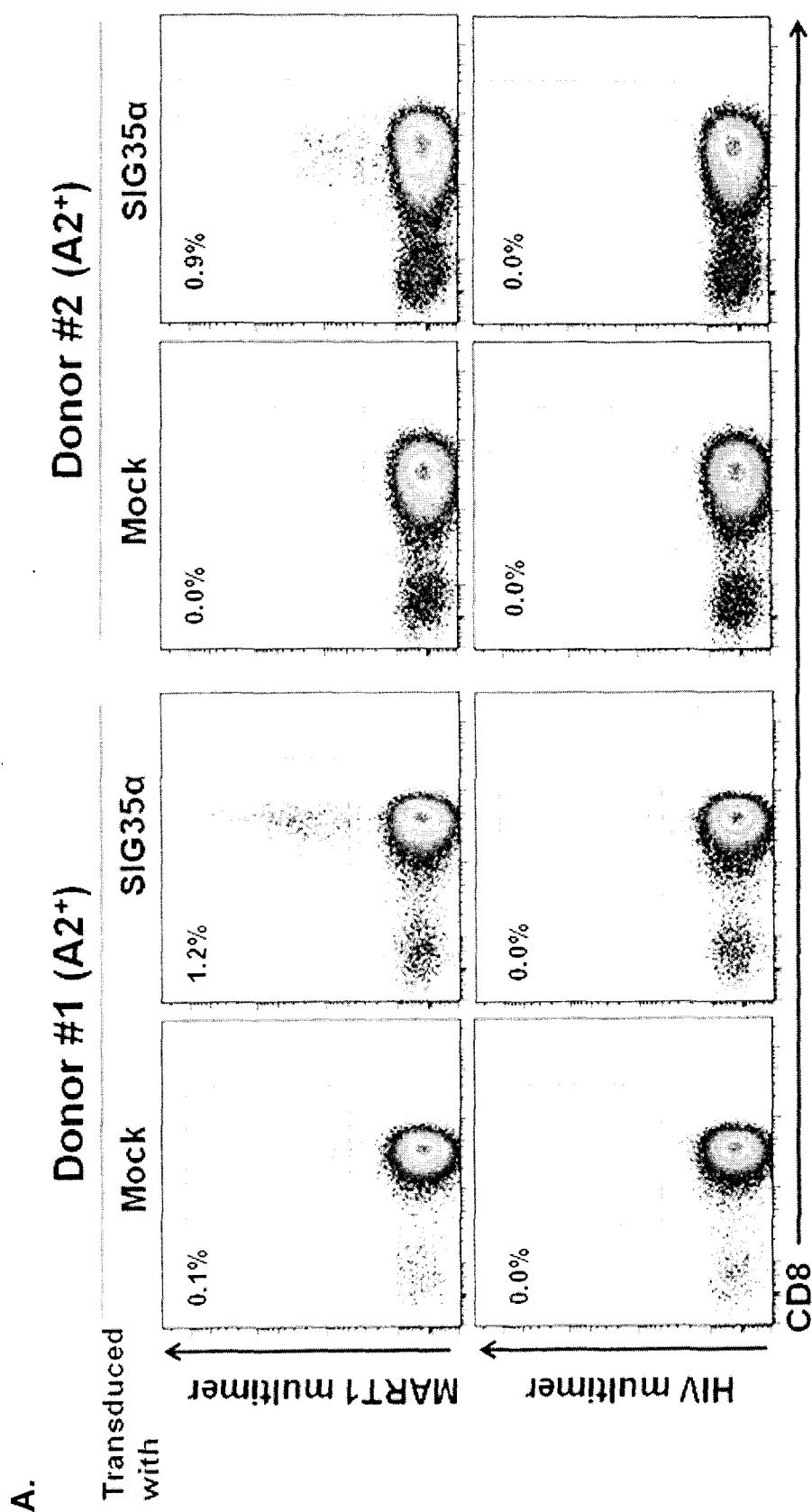
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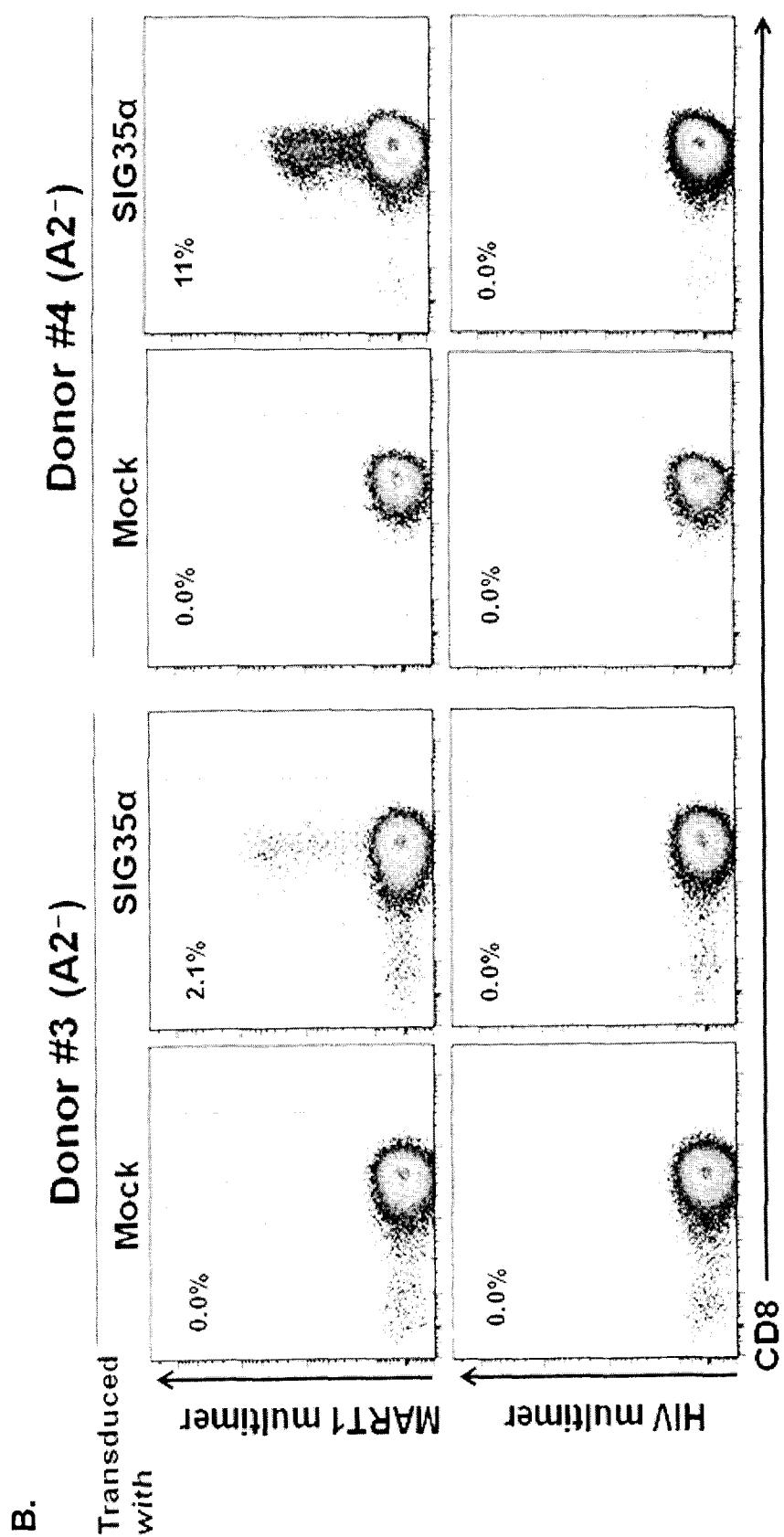
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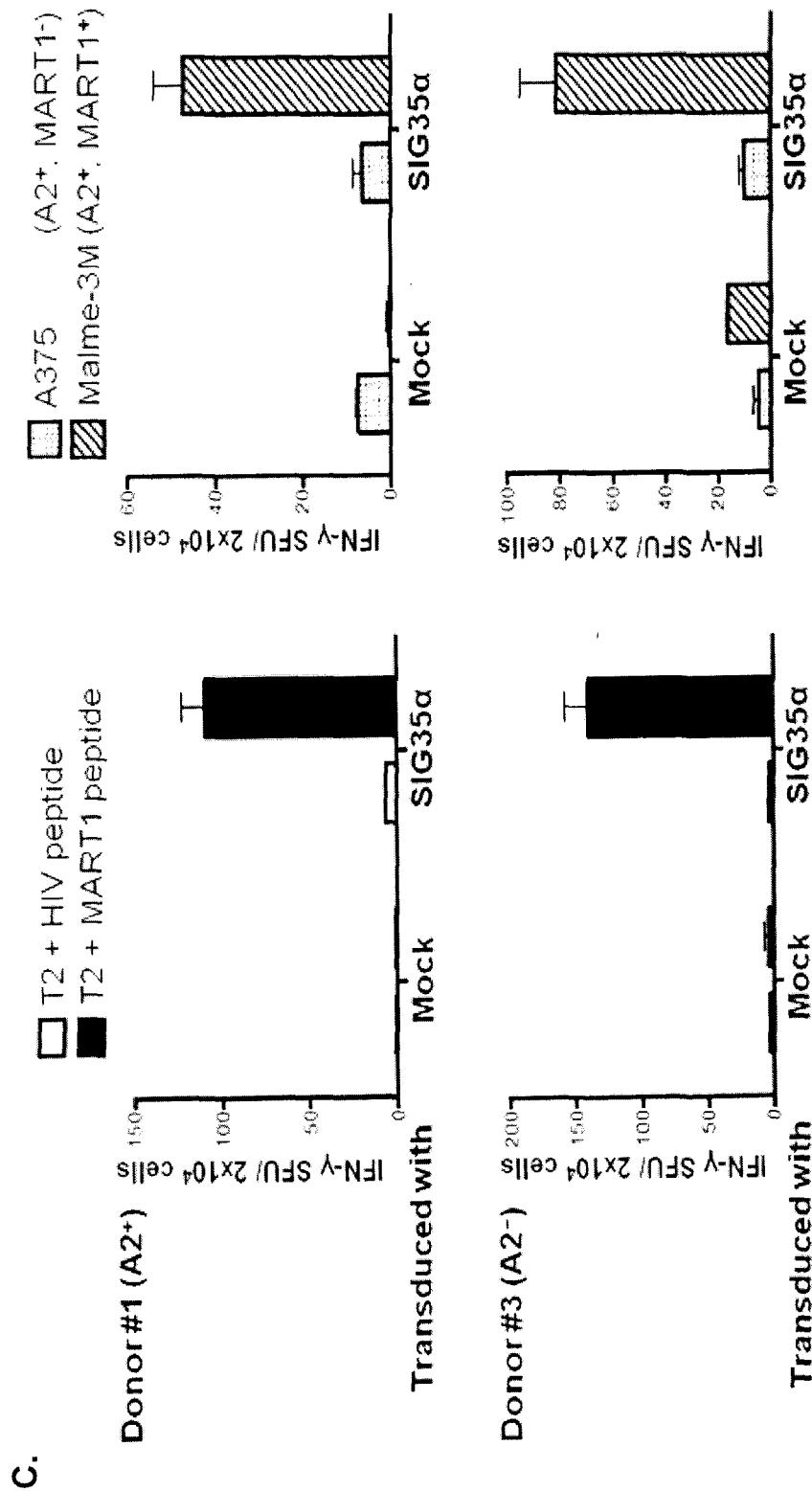
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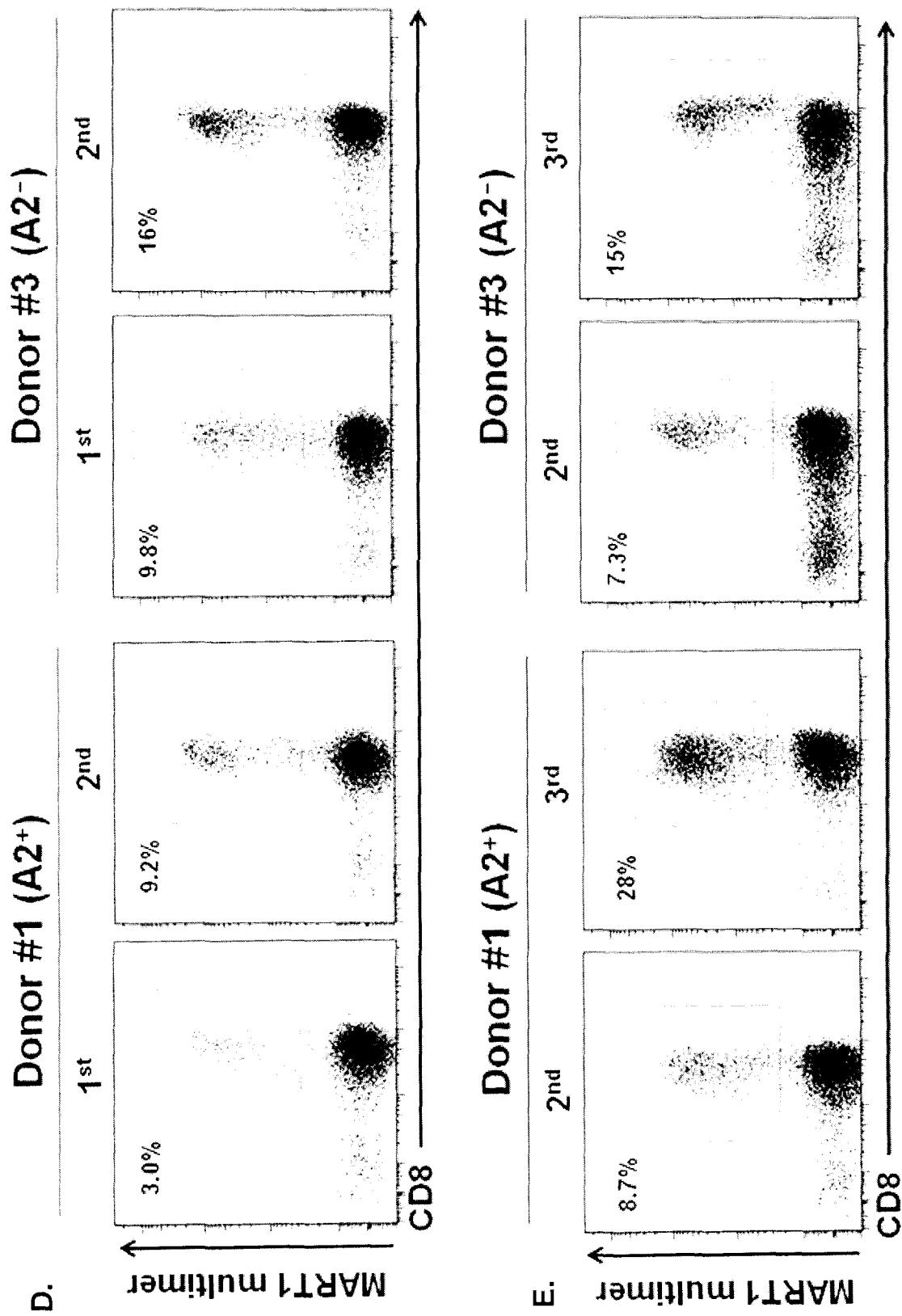
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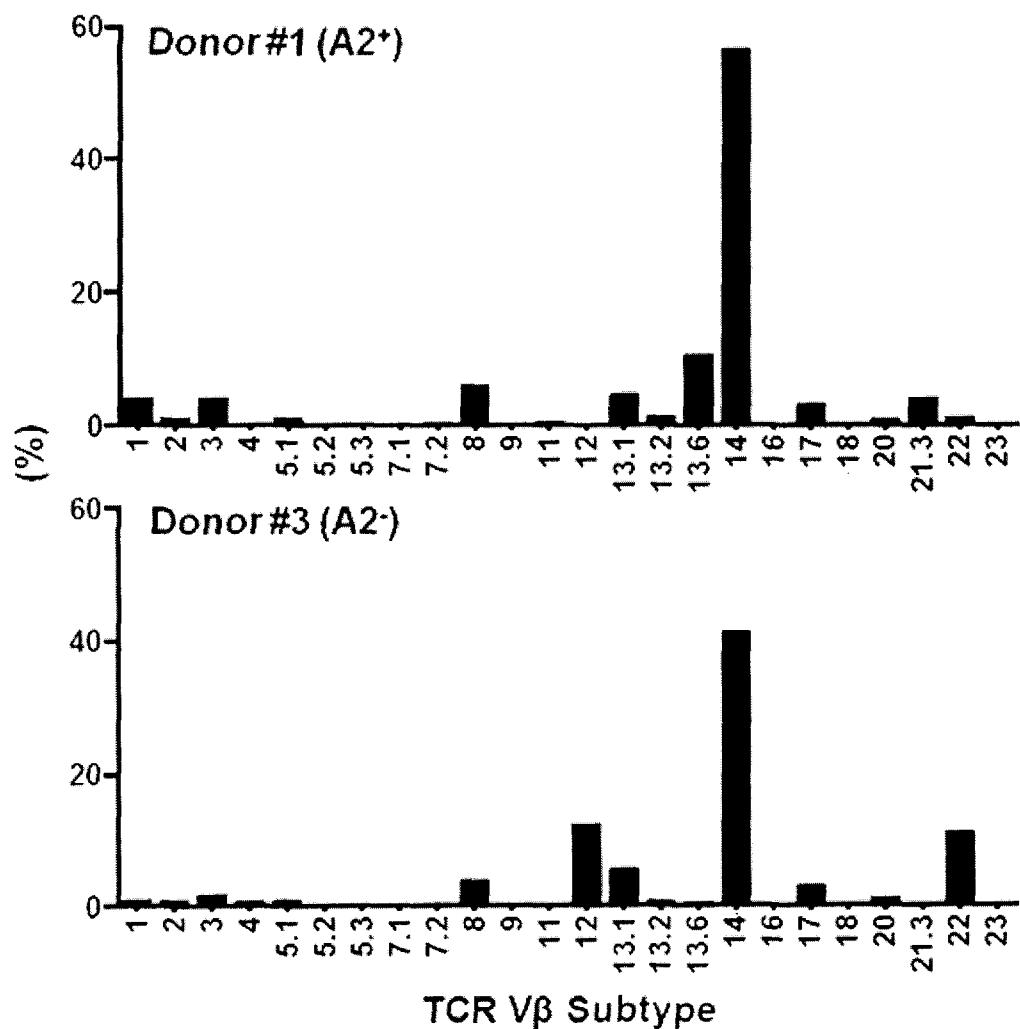
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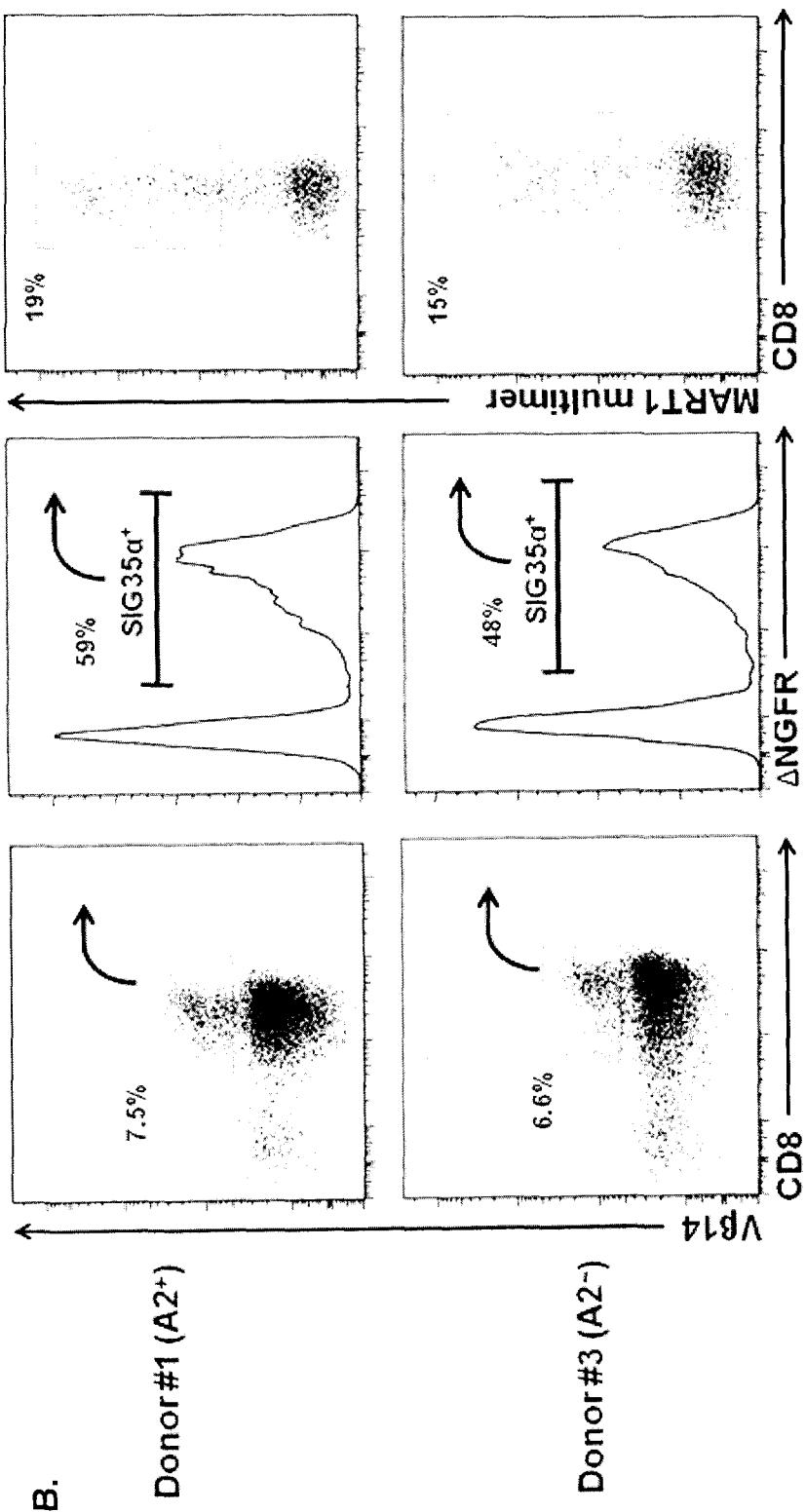
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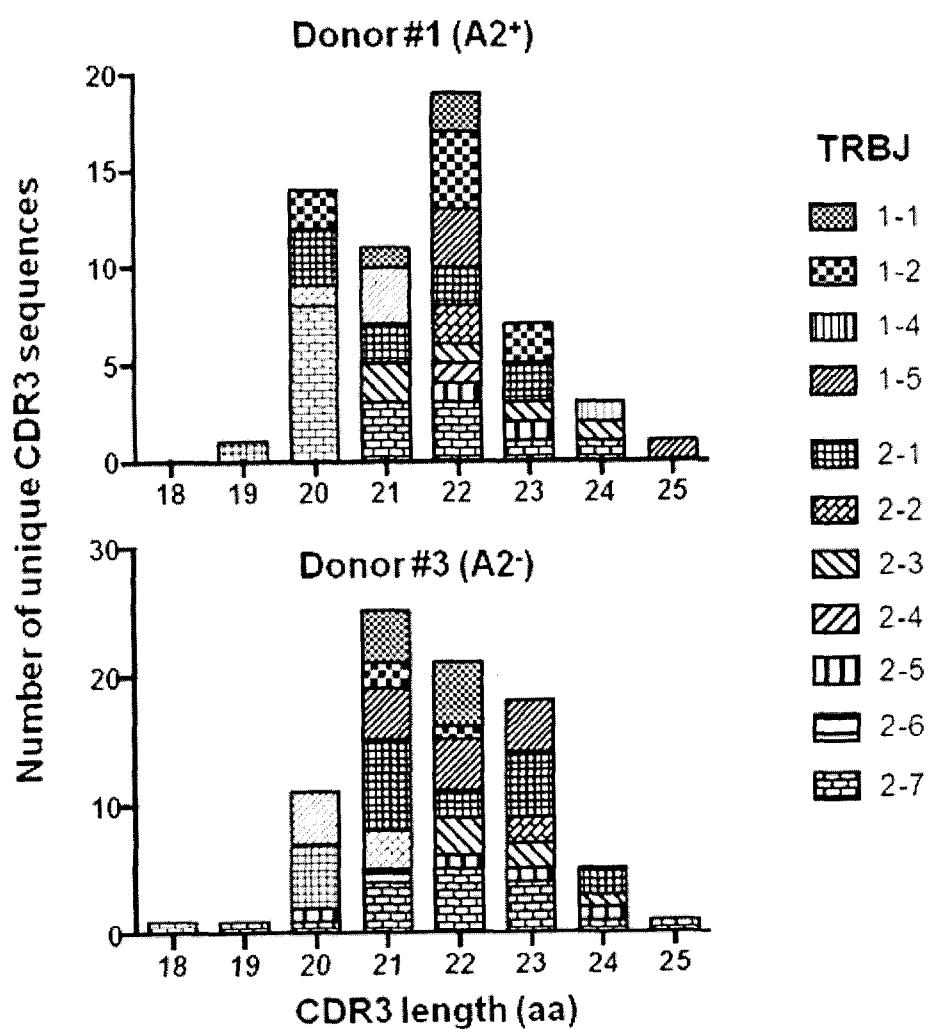
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Fig. 5
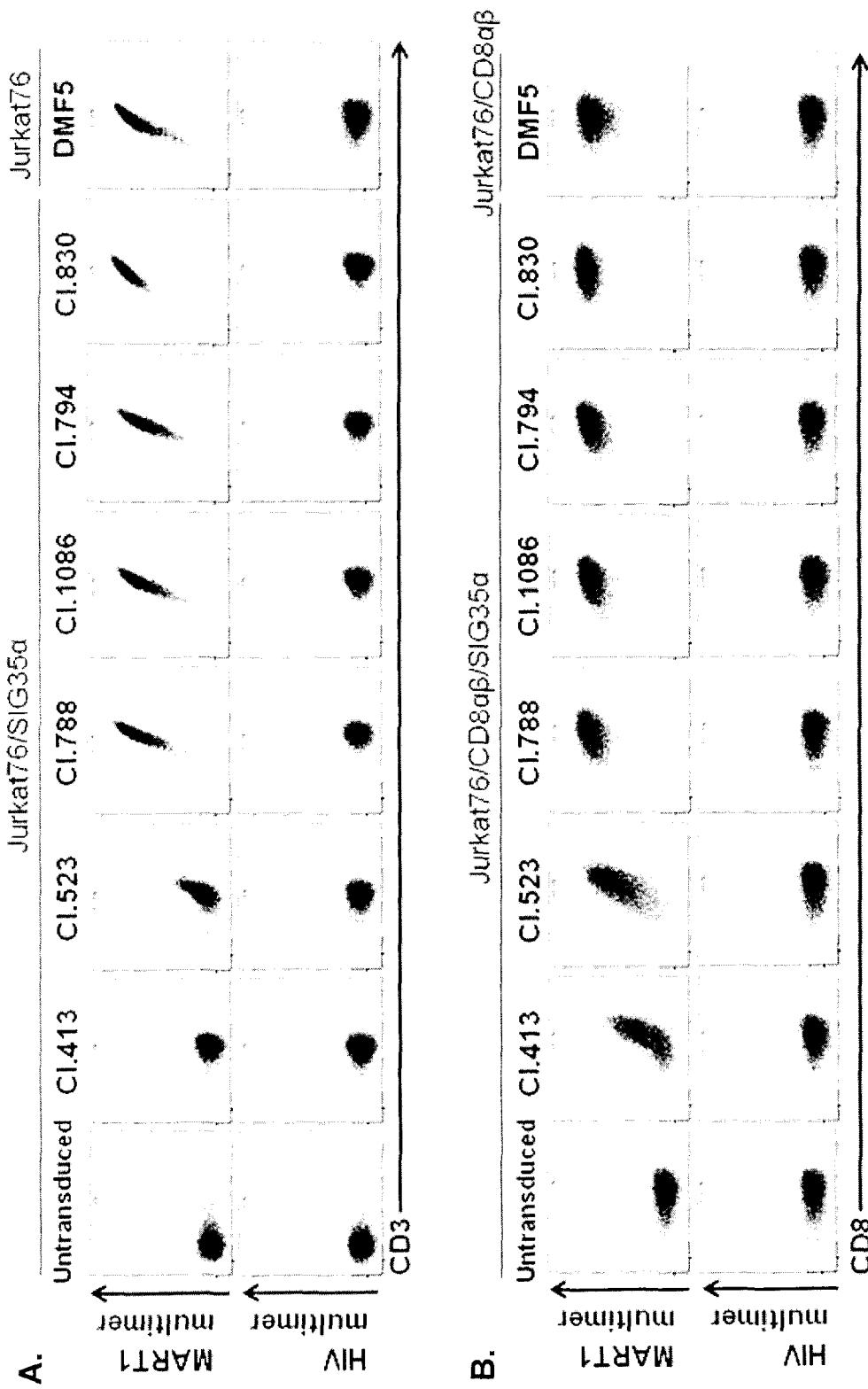
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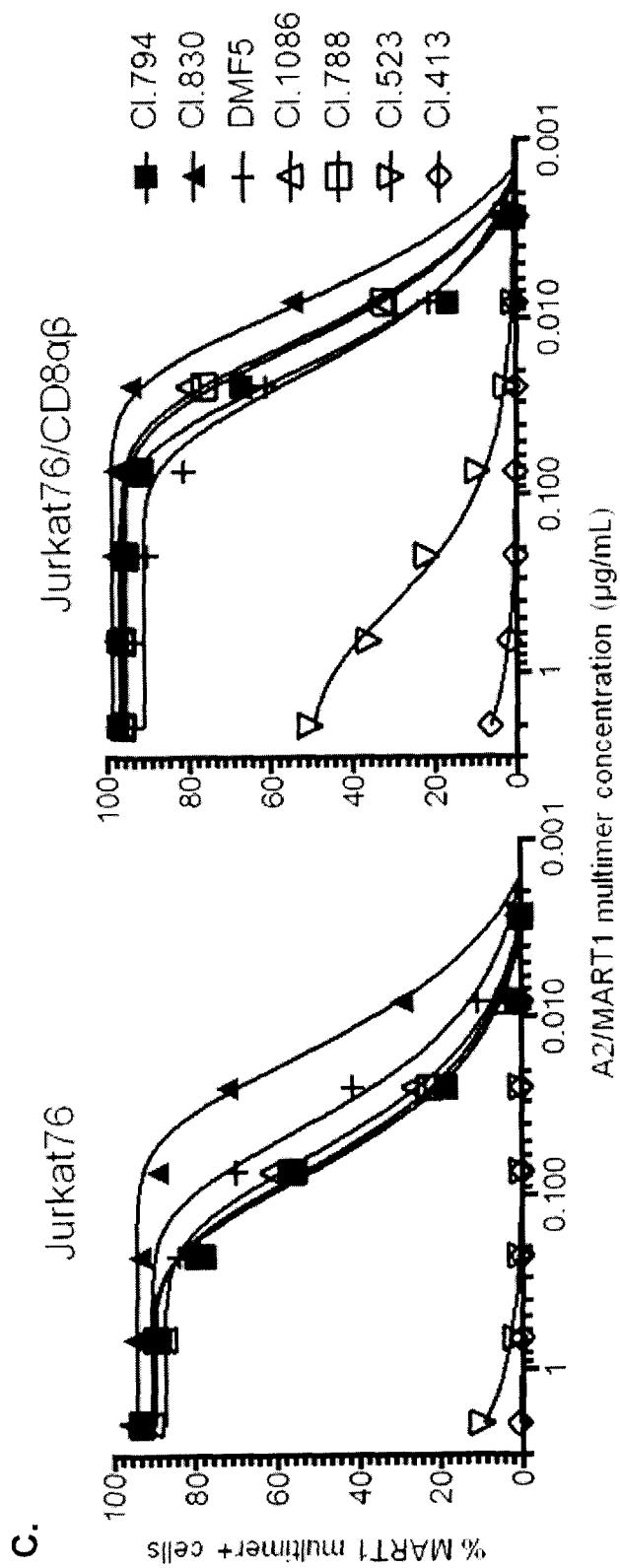
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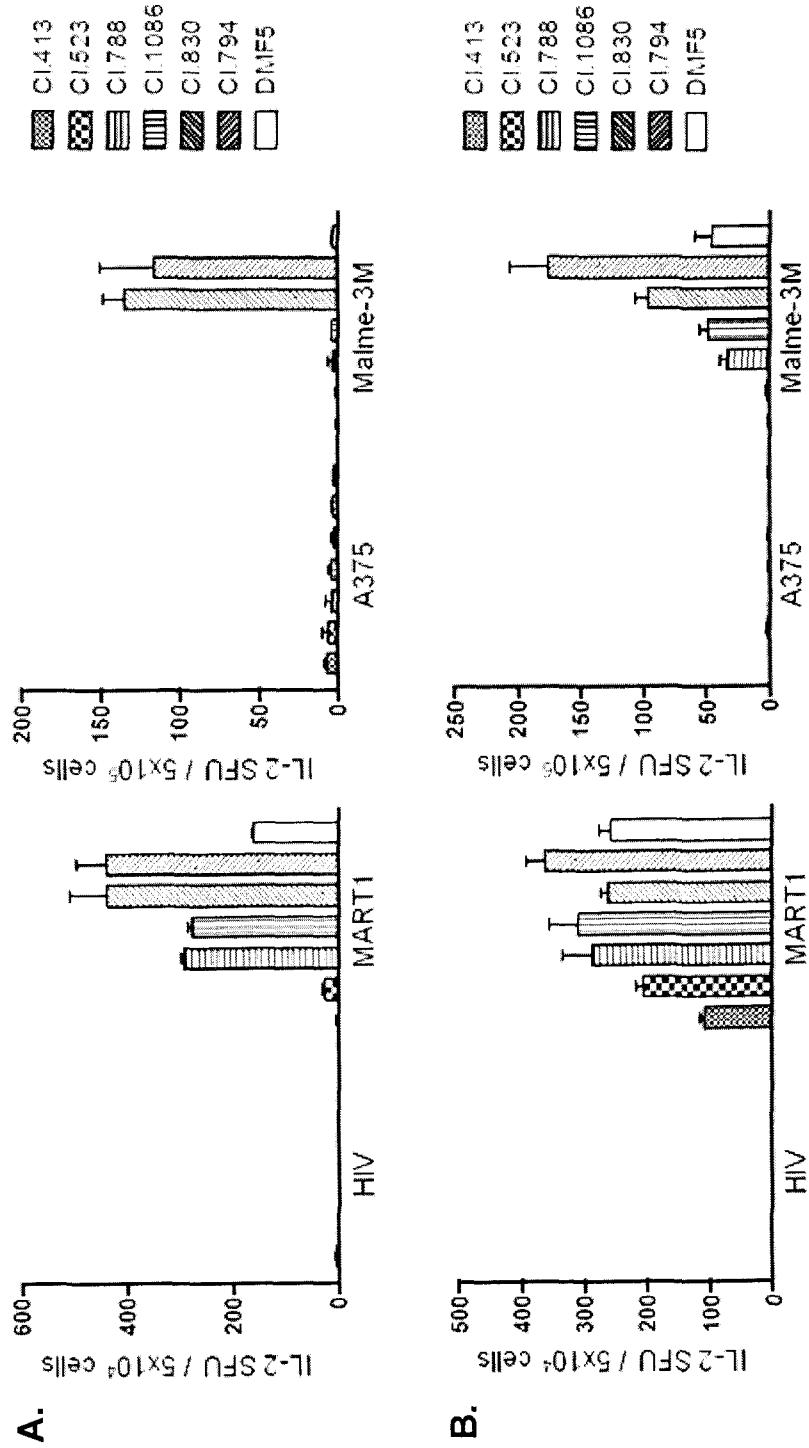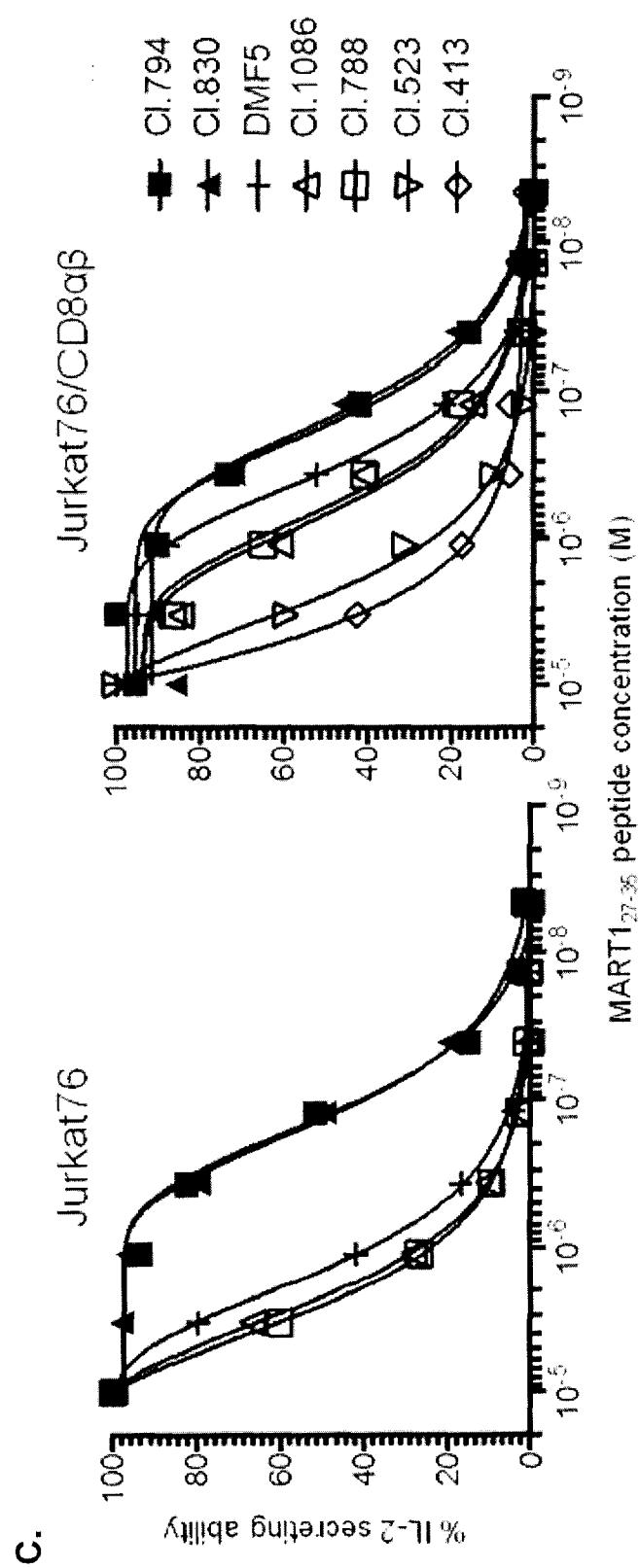
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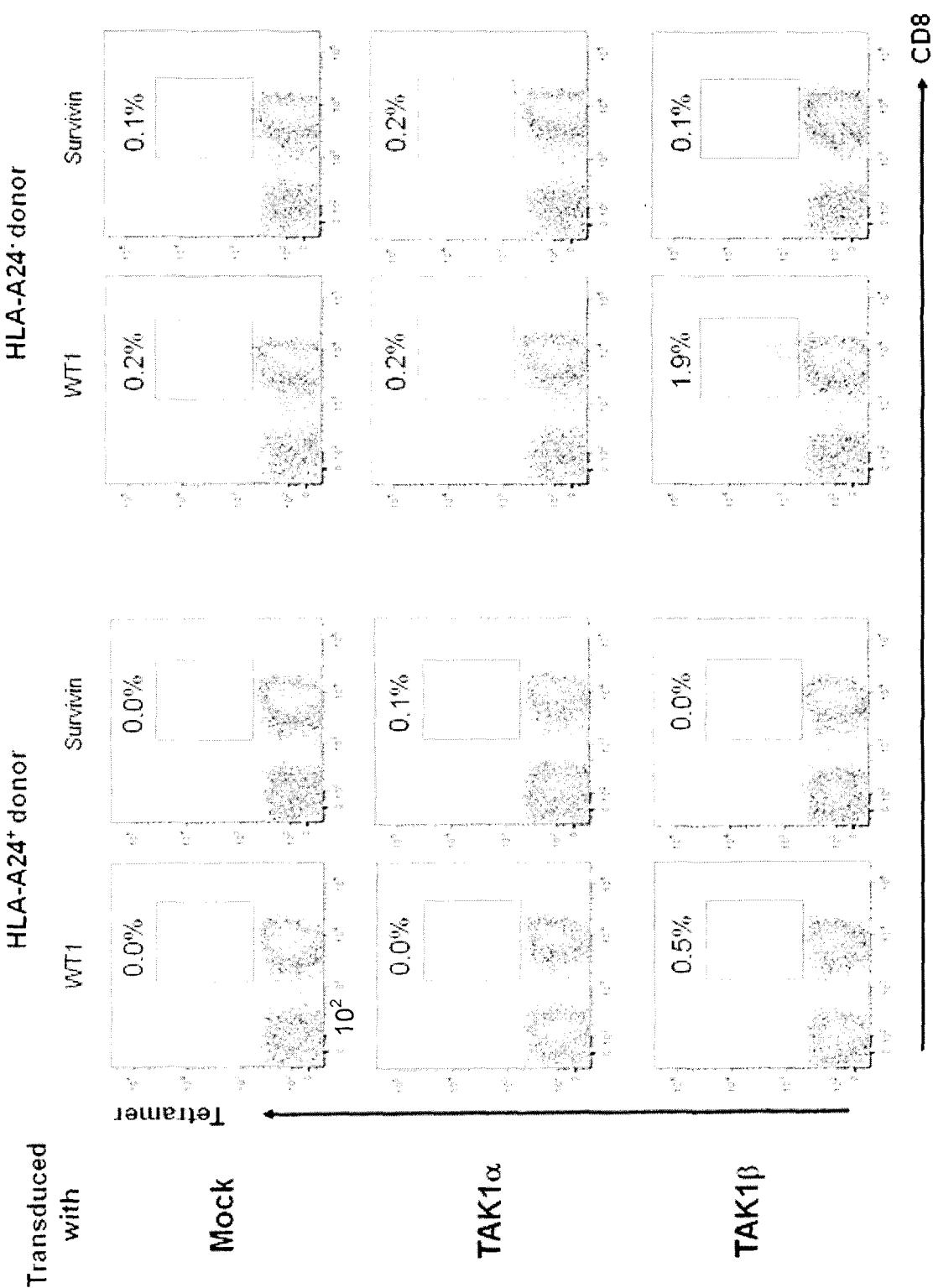
Fig. 7

Fig. 8
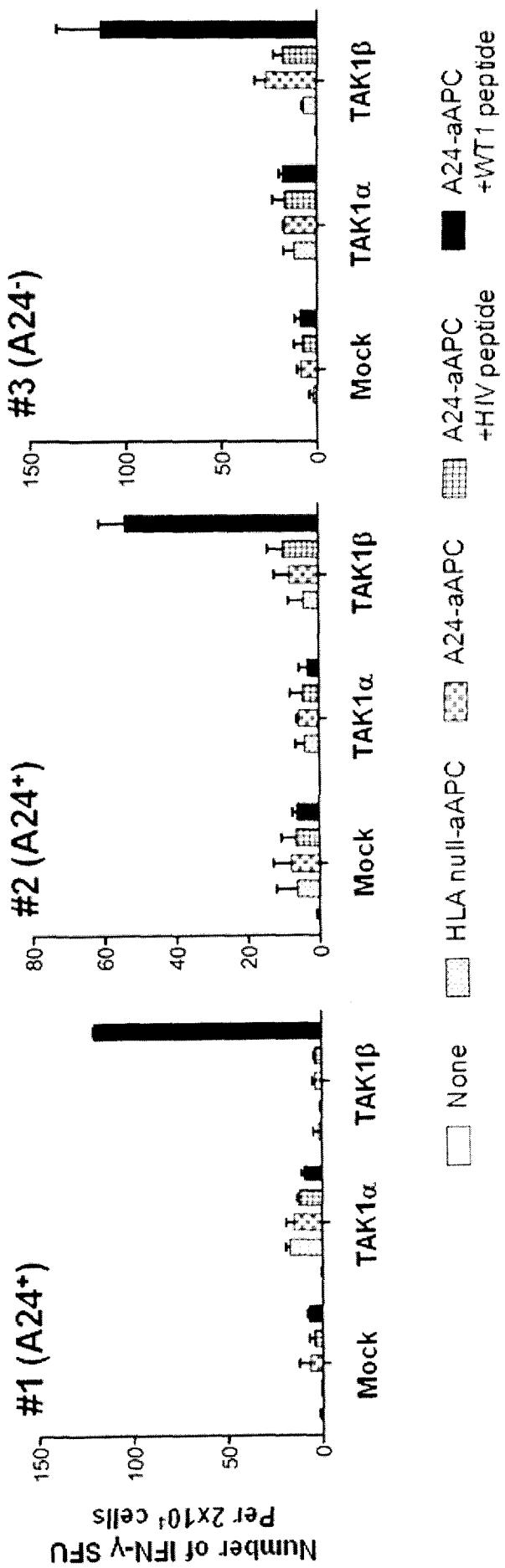
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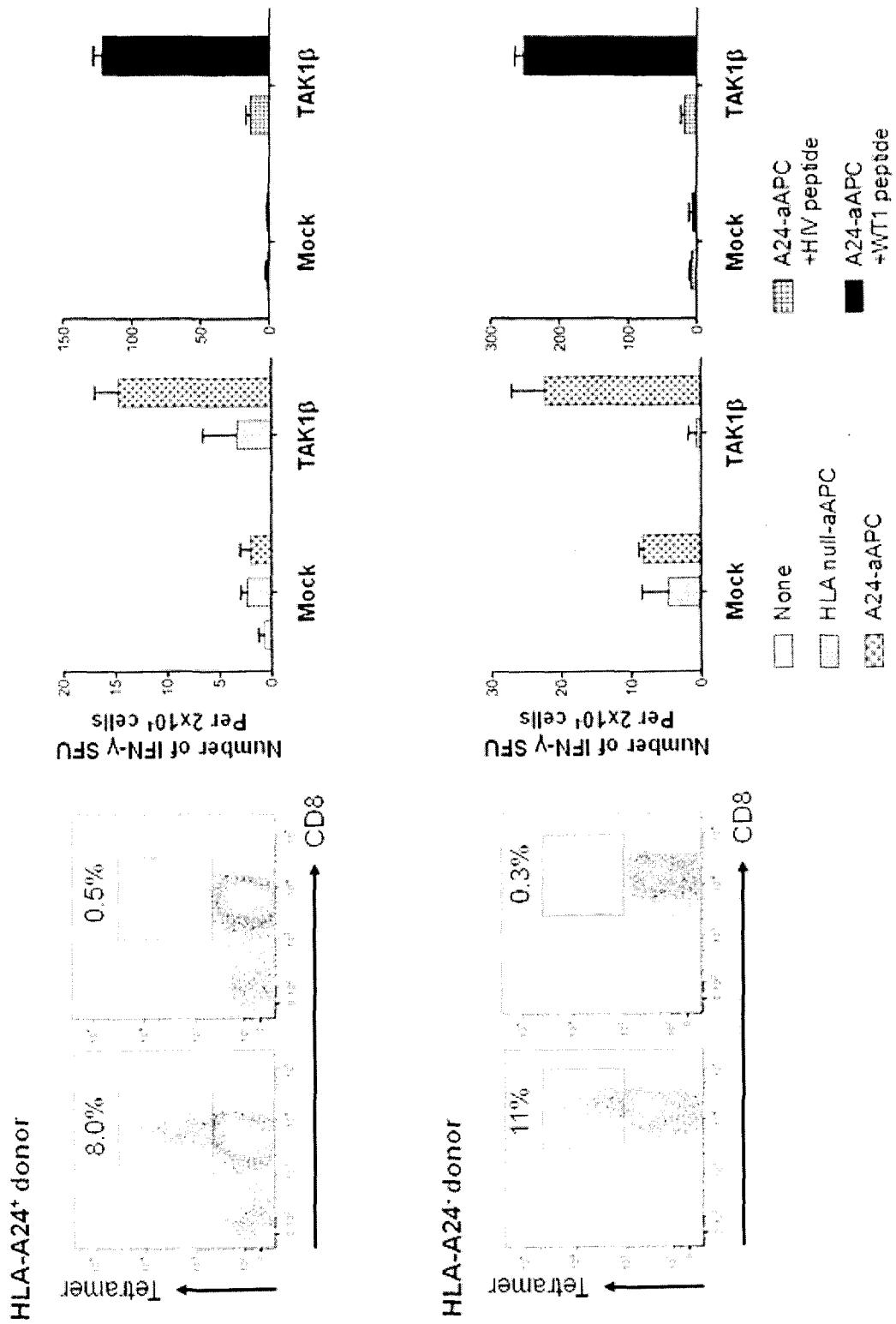
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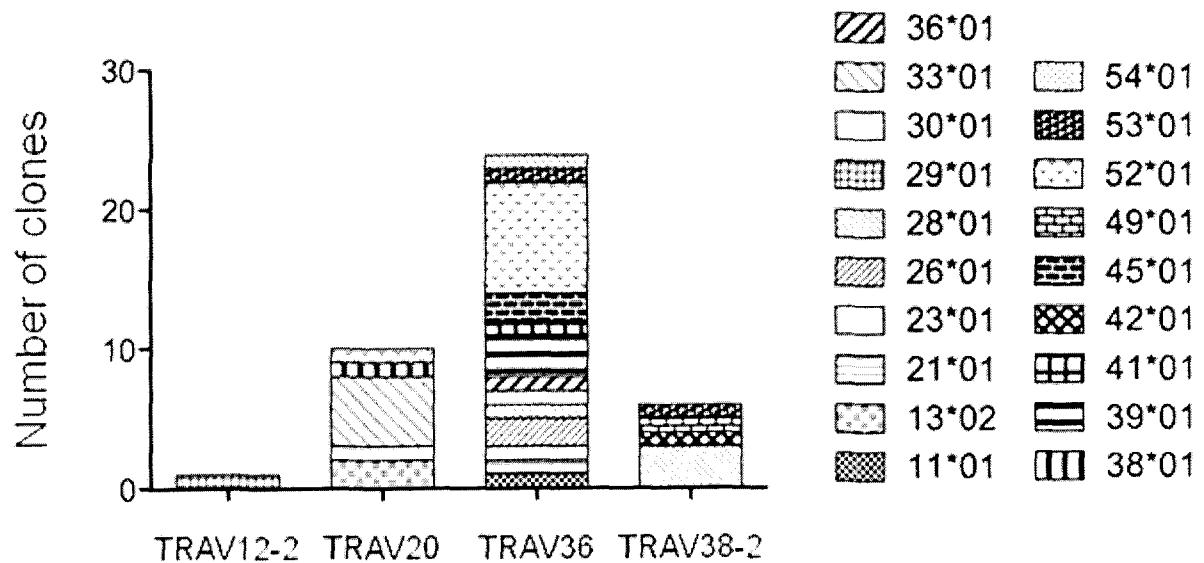
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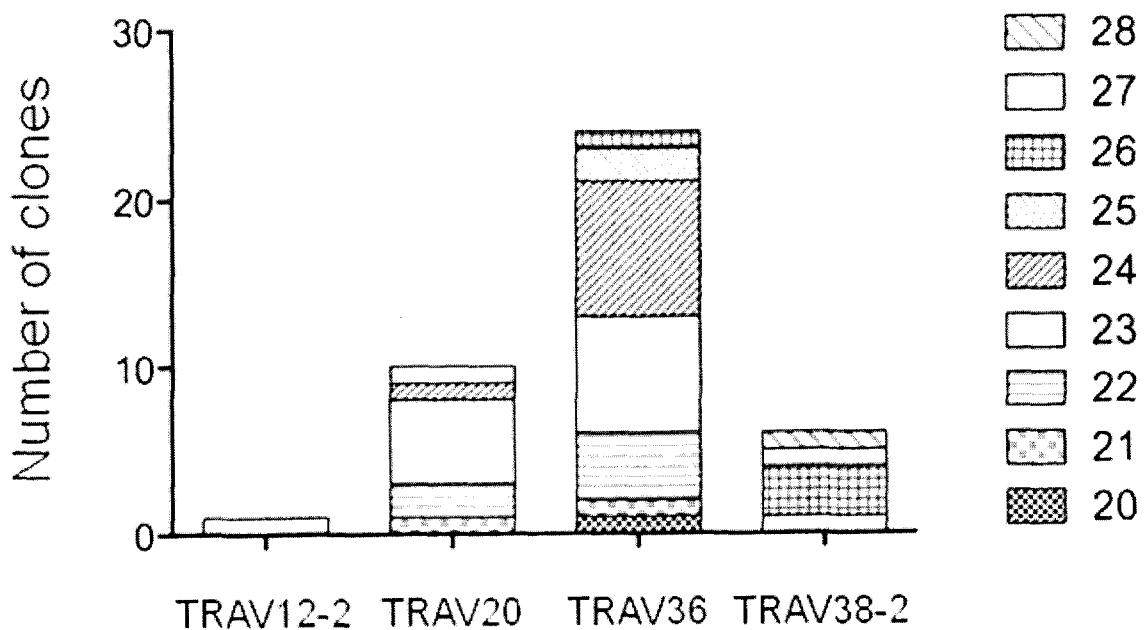
Fig.10

Fig. 11
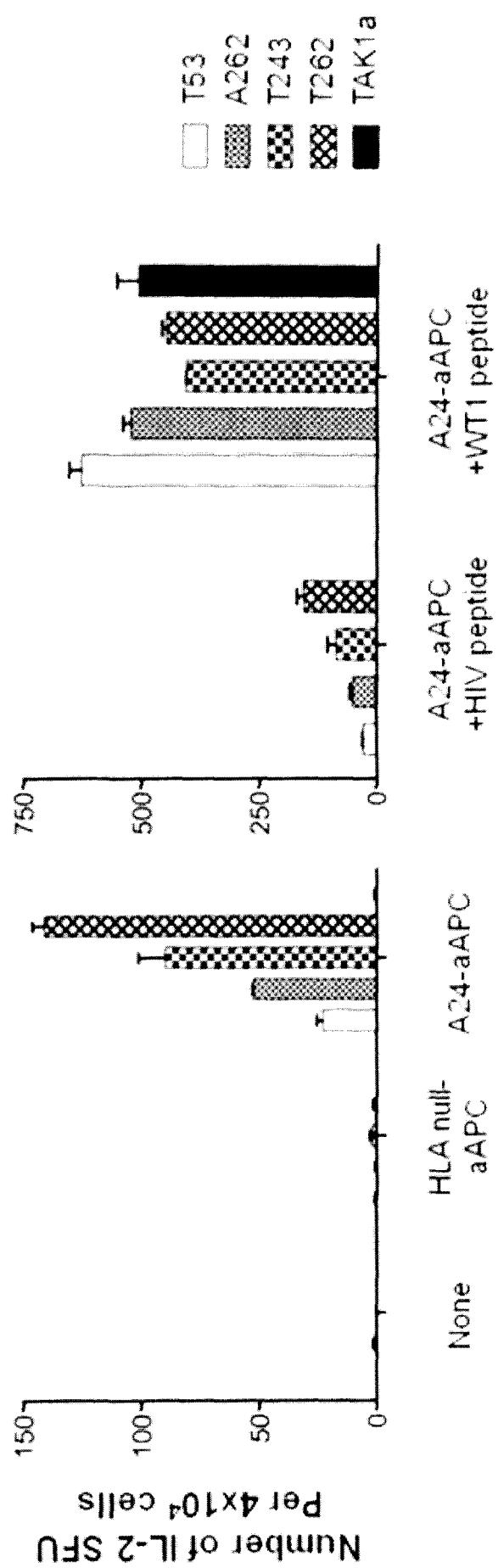
Fig. 12

SEQ ID NO:1

TCRα chain, Clone SIG35α nucleotide sequence

ATGATGCGGCCCATCGTCGTGCTGGTGCTGCTGTTCGCCACAAGCGCCCTGGCCCAGAAAGAGGTGGAACAGAACAGCGGCCCCTCTGAGCGTGCCCG
AGGGCGCCATTGCCAGCCTGAACTGCACCTACAGCGACCGGGGCTCCCAGAGCTTCTTCTGGTACAGACAGTACAGCGGCAAGAGCCCCGAGCT
GATCATGTTCATCTACAGCAACGGCGACAAAGAGGACGGCCGGTTCACCGCCCAGCTGAACAAGGCCAGCCAGTACGTGTCCCTGCTGATCCGG
                    CDR1
GACAGCCAGCCCAGCGACAGCGCCACCTACCTGTGTGCCGTGTCCATCGGCTTCGGCAACGTGCTGCACTGCGGCAGCGGCACCCAGGTCATCG
    CDR2   CDR3
TGCTGCCCAACATCCAGAACCCCGACCCGGCCGTGTACCAGCTGCGGGGACAGCAGCAGAAGAGCGTGTGCCTGTTCACCGACTTTGA
CAGCCAGACCAACGTGTCCCAGAGCAAGGACAGCGATAAGACAGTGCTGGACATGCGGAGCATGGACTTCAAGAGCAAC
AGCGCCGTGGCCTGGTCCAACAAGAGCGACTTCGCCTGCGCCAACGCCTTCAACAACAGCATCATCCCCGAGGACACATTCTTTCCCAAGCCCCG
AGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACCGACACCAACCTGAACTTCCAGAACCTGAGCGTGATCGGCTTCAGAATCCT
GCTGCTGAAGGTGGCCGGCTTCAACCTGCTGATGACCCTGCGCGGCCTGTGGTCCTCGTGA

SEQ ID NO:2

TCRα chain, Clone SIG35α amino acid sequence

MMRPIVLVLLFATSALAQKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPELIMFIYSNGDKEDGRFTAQLNKASQYVSLLIR
                                              CDR1   CDR2
DSQPSDSATYLCAVSIGFGNVLHCGSGTQVIVLPNIQNPDPAVYQIRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSN
          CDR3
SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLIVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS

Fig. 13

SEQ ID NO:3

TCRβ chain, Clone 794 nucleotide sequence

ATGGGCCCCCCAGCTCCTTGGCTATGTGGTCCTTTGCCTTTCTAGGAGCAGGCCCCCTGGAAGCCCAAGTGACCCAGAACCCAAGATACCTCATC
ACAGTGACTGGAAAAGAAGTTAACAGTGACTTGGTTCTCAGAATATGAACCATGAGTATATGAGTTGGTATCGTGGTATCGACAAGACCCAGGGCTGGGCTTA
AGGCAGATCTACTATTCAATGAATGTTGAGGTGACTGATAAAGGGAGATGTTCCTGAAGGGTACAAAGTCTCTCGAAAAGAGAAGAGAGAATTTC
CCCCTGATCCTGGAGTCGCCCAGCCCCAACCAGAGACCTCTCTGTACTTTCTGTGCCAGCAGTCTACTCGGGGACTATGGCTACACCTTCGGTTCG
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AGATCGTCAGCGCGCGAGCCTGGGGTAGAGCCAGACTGTGGCTTTACCTCGGTGTCCTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGA
GATCCTGCTAGGGAAGGCCACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAGAAAAGGATTTCTGA

SEQ ID NO:4

TCRβ chain, Clone 794 amino acid sequence

MGPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSRKEKRNF

PLILESPSPNQTSIYFCASSLLGDYGYTFGSGTRLTVVEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSG

VSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSATILY

EILLGKATLYAVLVSALVLMAMVKRKDF

Fig. 14

SEQ ID NO:5

TCRβ chain, Clone 830 nucleotide sequence

ATGGGCCCCAGCTCCTTGGCTATGTGGTCCTTTGCCTTGTTCTAGGAGCAGGCCCCCTGGAAGCCCAAGTGACCCAGAACCCAAGATACCTCATCA
CAGTGACTGGAAAGAAGTTAACAGTTGACTTGTTTCTCAGAATATGAACCATGAGTATATGTCCTGGTATCGACAAGACCCAGGGCTGGGCTTAAG
                                                                        CDR1
GCAGATCTACTATTCAATGAATGTTGAGGTGACTGATAAGGGAGAGTGTTCCTGAAAAGAGAGAAGAGAGGAATTTCCCC
                CDR2
CTGATCCTGGAGTCGCCCCAACCAGACCCTCTGTACTTCTGTGCCAGCAGTTTAGGGGGTGCCTACGAGCAGTACTTCGGGCCGGGCA
                                                                      CDR3
CCAGGCTCACGGGTCACACAGGAGGACCTGAAAAACGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAA
GGCCACACTGGTGTGTGCCTGGCCACAGGGCTTCTACCCCGACCACGTGGAGCTGGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGC
ACAGACCCGCAGCCCTCAAGGAGCAGCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTGTCTCGGCCAAACCTGTCACCAGAT
ACCCCCGCAACCACTTCCGCTCCGTCGTCAAGTCCAGTTCTACGGGCTTCACCTCCGAGTCTTACCCAGCAATGGTCAAGTGGTCCTGTCTGCTGATGAGATC
CGTCAGCGCCGAGGCCTGGGGTAGAGCAGCAGACTGTGCGTGGGTCAATGGCAATGGTCAAGTGGTCAAGAGAAAGGATTCCAGAGGCTAG
TTGCTAGGGAAGGCCACCTTGTATGCCGTGCTGGTCAGTGCCCTGCAGTGCTGCTGGCAATGGTCAAGAGAAAGGATTCCAGAGGCTAG

SEQ ID NO:6

TCRβ chain, Clone 830 amino acid sequence

MGPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLITVTCSQMMNHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSRKEKRNFP
                                             CDR1   CDR2
LILESPSPNQTSLYFCASSLGGAYEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVS
                   CDR3
TDPQPLKEQPALNDSRYCLSSRLRVSATFWQMPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAMGRADCGFTSESYQQGVLSATILYEI
LLGKATLYAVLVSALVLMAMVKRKDSRG

Fig. 15

SEQ ID NO:7

TCRα chain, Clone T53 nucleotide sequence

ATGATGAAGTGTCCACAGGCTTTACTAGCTATCTTTTGGCTTCTACTGAGCTGGGTGAGCAGTGAAGACAAGGTGGTACAAAGCCCTCTATCT

CTGGTTGTCCACGAGGGAGACACTGTAACTCTCAATTGCAGTTATGAAGTGACTAACTTTCGAAGCCTACTATGGTACAAGCAGGAAAAGAAA

        CDR1

GCTCCCACATTTCTATTTATGCTAACTTCAAGTGGAATTGAAAAGAAGTCAGGAAGACTAAGTAGCATATTAGATAAGAAAGAACTTTTCAGC

  CDR2

ATCCTGAACATCACAGCCACCCAGACCGGAGACTCGGCCGGCCGTCTACCCTCTGTGCTGTGATAACTGGTGGTACTAGCTATGGAAAGCTGACATTT

           CDR3

GGACAAGGGACCATCTTGACTGTCCATCCAAATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCT

GTCTGCCTATTCACCGATTTTGATTCTCAAAACAACAAATGTGTCAAAAGTCACAGACAAAAACTGTGCTAGACATG

AGGTCTATGGACTTCAAGAGCAACAGCTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACAGCATTATTCCA

GAAGACACTTCTTCCCAGCCAGAAAGTTCCTGTGATGTCGAGAAAGCTTTGAAAACAGATACGAACCTAAACTTTCAAAAAC

CTGTCAGTGATTGGGGTTCCGAATCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCCAGCTGA

SEQ ID NO:8

TCRα chain, Clone T53 amino acid sequence

MMKCPQALLAIFWLLLSWVSSEDKVVQSPLSLVVHEGDTVTLNCSYEVTNFRSLLWYKQEKKAPTFLFMLTSSGIEKKSGRLSSILDKKELFS

                       CDR1   CDR2

ILNITATQTGDSAVYLCAVTGGTSYGKLTFGQGTILTVHPNIQMPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLD

          CDR3

RSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS

Fig. 16

SEQ ID NO:9

TCRα chain, Clone A262 nucleotide sequence

ATGATGAAGTGTCCACAGGCTTTACTAGCTATCTTTTGGCTTTCTACTGAGCTGGGTGAGCAGTGAGCAGTGAAGACAAGGTGGTACAAAGCCCTCTATCT
CTGGTTGTCCACGAGGGAGACACTGTAACTCTCAATTGCAGTTATGAAGTGACTAACTTTCGAAGCTACTATGGTACAAGCAGGAAAAGAAA

CDR1

GCTCCCACATTTCTATTTATGCTAACTTCAAGTGGAATTGAAAAGAAGTCAGGAAGACTAAGTAGCATATTAGATAAAGAAAGAACTTTTCAGC

CDR2

ATCCTGAACATCACAGCCACCCAGACCGGAGACTCAGCCACTTATCTCTGTGCTGTGCAGAATGCTGGTGGTACTAGCTATGGAAAGCTGACA

CDR3

TTTTGGACAAGGGACCATCTTGACTGTCCATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAG
TCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTCACAGACAAAACTGTGCTAGAC
ATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGTGATGTCAAATCTGACTTTGCATGTCAAACGCCTTCAACAACAGCATTATT
CCAGAAGAAGACACCTTCTTCCCCAGCCCAGAAGTTCCTGTGATGTCAGCTGGTCAGGAAAAAGCTTTGAAAACAGCTTTGAAAACAGCTTTGAAAACTTTCAA
AACCTGTCAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGGTTTAATCTGCTCATGACGCTGGCTGTGGTGTCCAGCTGA

SEQ ID NO:10

TCRα chain, Clone A262 amino acid sequence

MMKCPQALLAIFWLLLSWVSSEDKVVQSPLSLVVHEGDTVTLNCSYEVTNFRSLLWYKQEKKAPTFLFMLTSSGIEKKSGRLSSILDKKELFS

                                                CDR1   CDR2

ILNITATQTGDSATYLCAVQNAGGTSYGKLTFGQGTILTVHPNIQNPDRAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLD

    CDR3

MRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS

Fig. 17

SEQ ID NO:11

TCRα chain, Clone T243 nucleotide sequence

ATGATGAAGTGTCCACAGGCTTTACTAGCTATCTTTTGGCTTCTACTGAGCTGGGTGAGCAGTGAAGACAAGGTGGTACAAAGCCCTCTATCT
CTGGTTGTCCACGAGGGGAGAGACACTGTAACTCTCAATTGCAGTTATGAAGTGACTAACTTTCGAAGCCTACTATGGTACAAGCAGGAAAAGAAA

<u>CDR1</u>

GCTCCCACATTTCTATTTATGCTAACTTCAAGTGGAATTGAAAAAGAAGTCAGGAGACTAAGTAGCATATTAGATAAGAAAGAACTTTTCAGC

<u>CDR2</u>

ATCCTGAACATCACAGCCACCCAGACCGGGGACTCGGCCGTCACAACCCTGACCCTCTGTGCTGTGCTTACCCAAACTGGGGCAAACAACCTCTTCTTTGGG

<u>CDR3</u>

ACTGGAACGAGACTCACCGTTATTCCCTATATCCAGAACCCTGACCCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTC
TGCCTATTCACCGATTTTGATTCTCAAAACAAATGTGTCTGGAGCAACAAATCTGACTTTGCAGTGTCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAA
TCTATGGACTTCAAGAGCAACAGTGCCTGTGGCCTGTGTGATGTGTCAAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAAACTTTCAAAACCTG
GACACCTTCTTCCCCCAGCCCAGAAATCCTCCCTCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCAGCTGA

SEQ ID NO:12

TCRα chain, Clone T243 amino acid sequence

MMKCPQALLAIFWMLLLSWVSSEDKVVQSPLSLVVHEGDTVTLNCSYEVTNFRSLLWYKQEKKAPTFLFMLTSSGIEKKSGRLSSILDKKELFS

<u>CDR1</u>   <u>CDR2</u>

ILNITATQTGDSAVYLCAVITQTGANNLFFGTGTRLTVIPYIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMR

<u>CDR3</u>

SMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS

Fig. 18

SEQ ID NO:13

TCRα chain, Clone T262 nucleotide sequence

```
ATGGAGAAAAATGTTGGAGTGTGCATTCATAGTCTTGTGGCTTCAGCTTGGCTGGTTGAGTGGAGAAGACCAGGTGACGCAGAGTCCCGAGGCC
CTGAGACTCCAGGAGGGAGAGAGAGTCAGTCTCAACTGCAGTAGCAGTGTTCTGGTATAGGCAAGATCCTGGG
                                                                            CDR1
AAAGGCCCTGAATTCCTCTTCACCCTGTATTCAGCTGGGAAGAAAAGGAGAAGAAAGGCTAAAAGCCACATTAACAAAGAAGGAAAGCTTT
                              CDR2
CTGCACATCACAGCCCCTAAACCTGAAGACTCAGCCACTTATCTCTGTGCAGGCCTTAAGGAATAATGCTGGCAACAACCGTAAGCTG
                                                          CDR3
ATTTGGGGATTGGGAACAAGCCTGGCAGTAAATCCGAATATCCAGAACCCCTGACCCTGCCGTGTACCAGCTGAGAGAGACTCTAAATCCAGTGAC
AAGTCTGTCTGCCTATTCCACCGATTTTGATTCTCAAAACAAATGTGTCTGGAGCAACAAATCTGACTTTGCATGTGCAAAAGTTCTGATGTGTATATCACAGACAACAAAACTGTGCTA
GACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGTGGTCCTGTGATGTGTCAAAAGTTCCTGTGATGTGTCGAGAAAAAGTTTGAAAACAGATACGAACCTAAACTTT
ATTCCAGAAGACACCTTCTTCCCCCAGCCCAGAAATCCTCCTGAAAGTTTAATCTGCTCATGACGCTGCGGGCTGTGGTCCCAGCTGA
CAAAACCTGTCAGTGTCAGTGATTGGGTTCCGAATCCTCCTGAAAGTTTAATCTGCTCATGACGCTGCGGGCTGTGGTCCCAGCTGA
```

SEQ ID NO:14

TCRα chain, Clone T262 amino acid sequence

```
MEKMLECAFIVLMLQLGWLSGEDQVTQSPEALRLQEGESSLNCSYTVSGLRGLFWYRQDPGKGPEFLFTLYSAGEEKEKERLKATLTKKESF
                                            CDR1                              CDR2
LHITAPKPEDSATYLCAVQALRNNAGNNRKLIWGLGTSLAVNPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVL
                  CDR3
DMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLKVAGFNLLMTLRIWSS
```

Fig. 19

SEQ ID NO:15

TCRβ chain, Clone TAK1 nucleotide sequence

```
ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCGGCGTGACCCAGACCCCTAGATACCTGATCAAGACCAGAGGC
CAGCAGGTCACACTGGAGCTGCAGCCCCTATCAGCGGCCACAGAAGCGGTGTCCTGGTATCAGCAGGCAGACCCCAGGGCCTGCAGTTCCTGTTC
                                  CDR1
GAGTACTTCAGCGAGACACAGCGGGAACAAGGGCAACTTCCCCGGCAGATTCAGCGGCGCAGACAGTTCAGCAACAGCCGCGAGATGAACGTG
  CDR2
TCCACCCTGGAACTGGGCGACAGCGCCCTGTACCTGTGTGCCTTCTCTCTGGGCTGGGGGAAAACCTACAACGAGCAGTTCTTCGGCCCTGGC
                                                CDR3
ACCAGACTGACCGTGCTGGAAGATCTGAAGAACGTGTTCCCCCCAGAGGTGGCCGTGTTCGAGCCTTCTGAGGCCGAGATCAGCCACACCCAG
AAAGCCACCCTCGTGTGTCTGGCCACCGGCTTCTACCCCGACCACGTGGAACTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTC
AGCACCGACCCCGAGCCTCAGCCCTCTGAAAGAGCAGCCCGCCCTGAACGCCGGTCAGTCTCTGAGCGACGAGAGCGACCCAAGCCTGTGACC
CAGAACCCCCGGAACCCACTTCAGATGCCCAGGTGCAGTTCTACGGCCAGTTGCGGGCTTTACCAGCGAGAGCTACCAGCAGGCGTGCTGACCC
CAGATCGTGTGTGCTGGGCAAGCCCACCCTGTATGCCGTGCTGGTGTCAGCCCTGGTGCTGATGGCTATGGTCAAGCGGAAGGACAGCCGGCTGA
```

SEQ ID NO:16

TCRβ chain, Clone TAK1 amino acid sequence

```
MMRPIVIVLLFATSALAGVTQTPRYLIKTRGQQVTLSCSPISGHRSVSWYQQTPGQGLQFLFEYFSETQRMKGNFPGRFSGRQFSNSRSEMNV
                                    CDR1                                       CDR2
STLELGDSALYLCASSLGWRETYNEQFFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGV
                CDR3
STDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILY
EILLGKATLYAVLVSALVLMAMVKRKDSRG
```

Endogenous
TCRs with
thymic selection
experience

Exogenous TCRα

Newly-paired
TCRs without
thymic selection
experience

Peripheral T cells

Fig.20

TCRα or TCRβ without stop codon	Furin recognition sequence	SGSG linker	F2A	ΔNGFR

Fig.21

Antitumor TCR αβ Chains vs. α or β Hemi-Chain Gene Therapy

	αβ chains	α or β hemi-chain
No. of genes transduced	Two	One
TCR clonality	Monoclonal	Highly polyclonal
TCR affinity	Fixed (high)	Highly broad (Ultra high ~ low)
GVHD risk	1	≪1/2

Fig.22
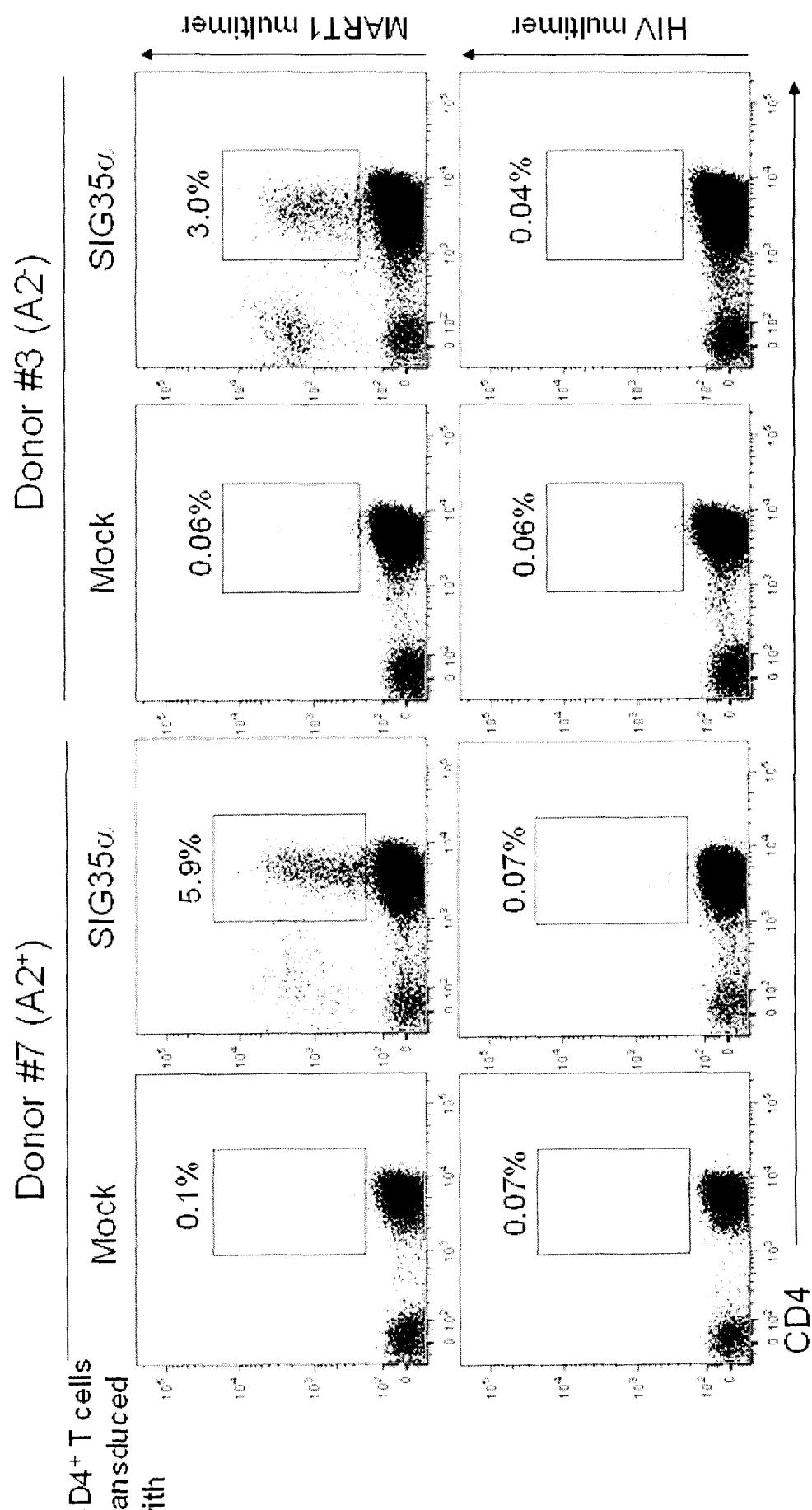
Fig. 23

Fig. 24
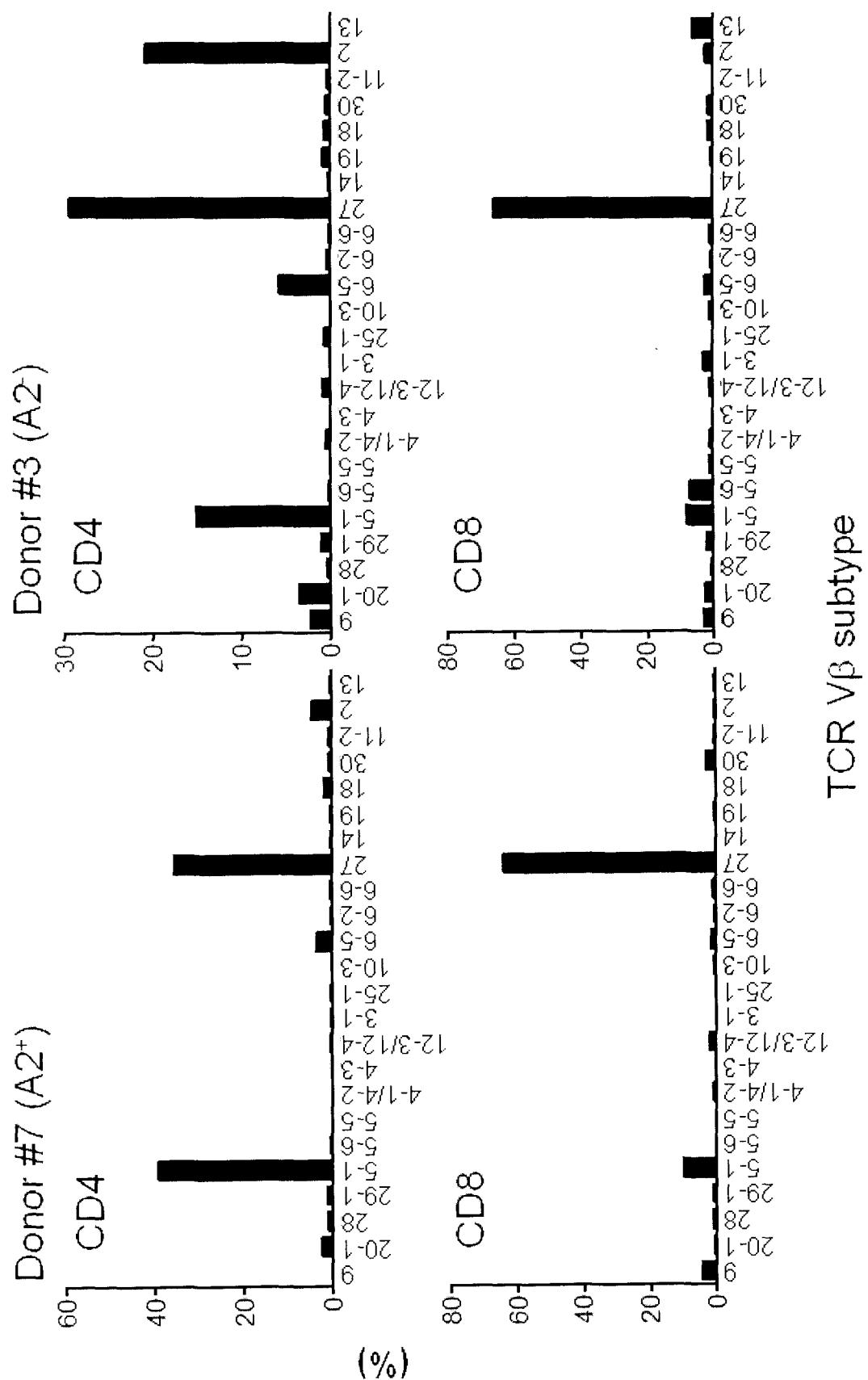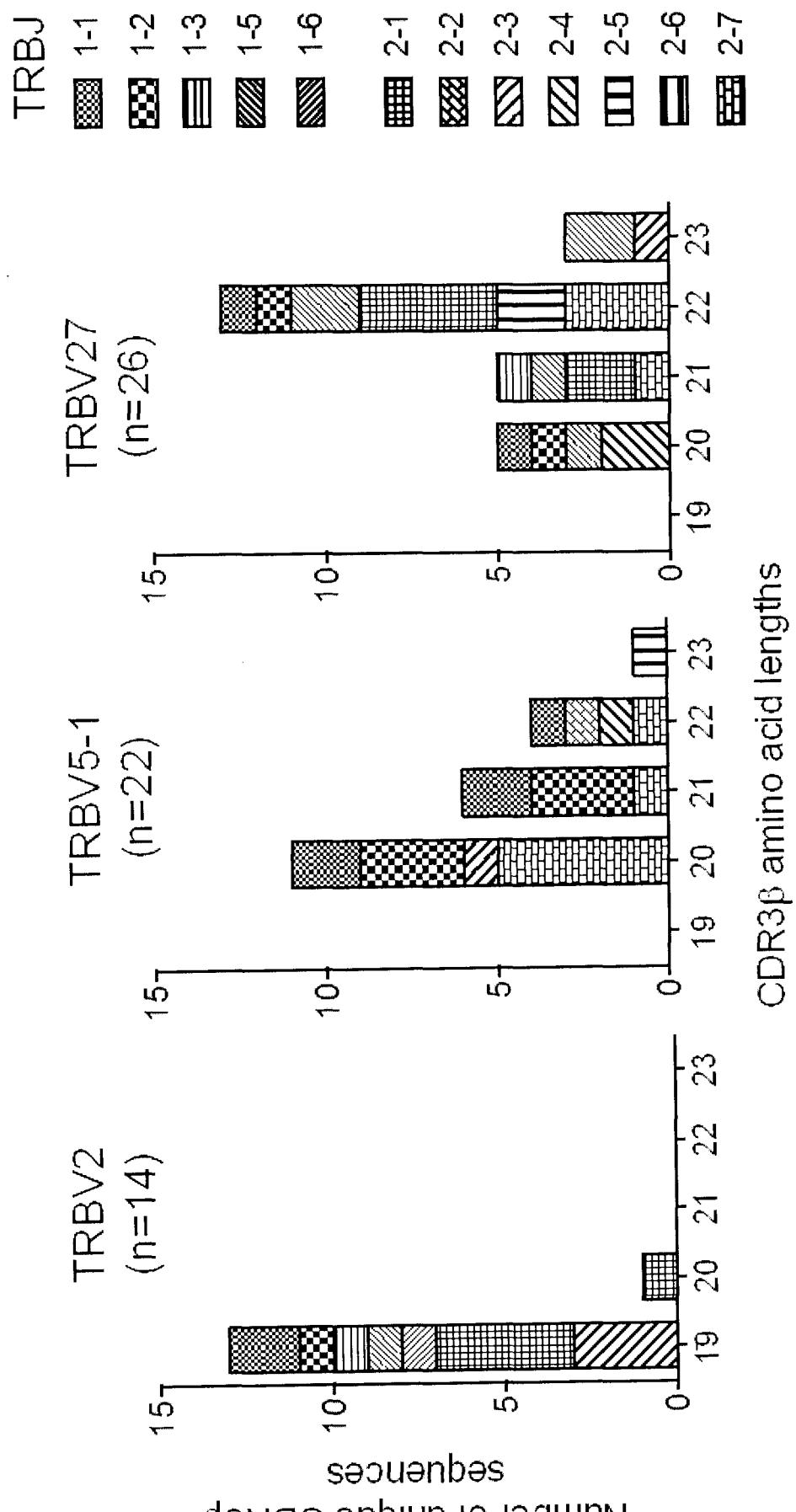
Fig. 25
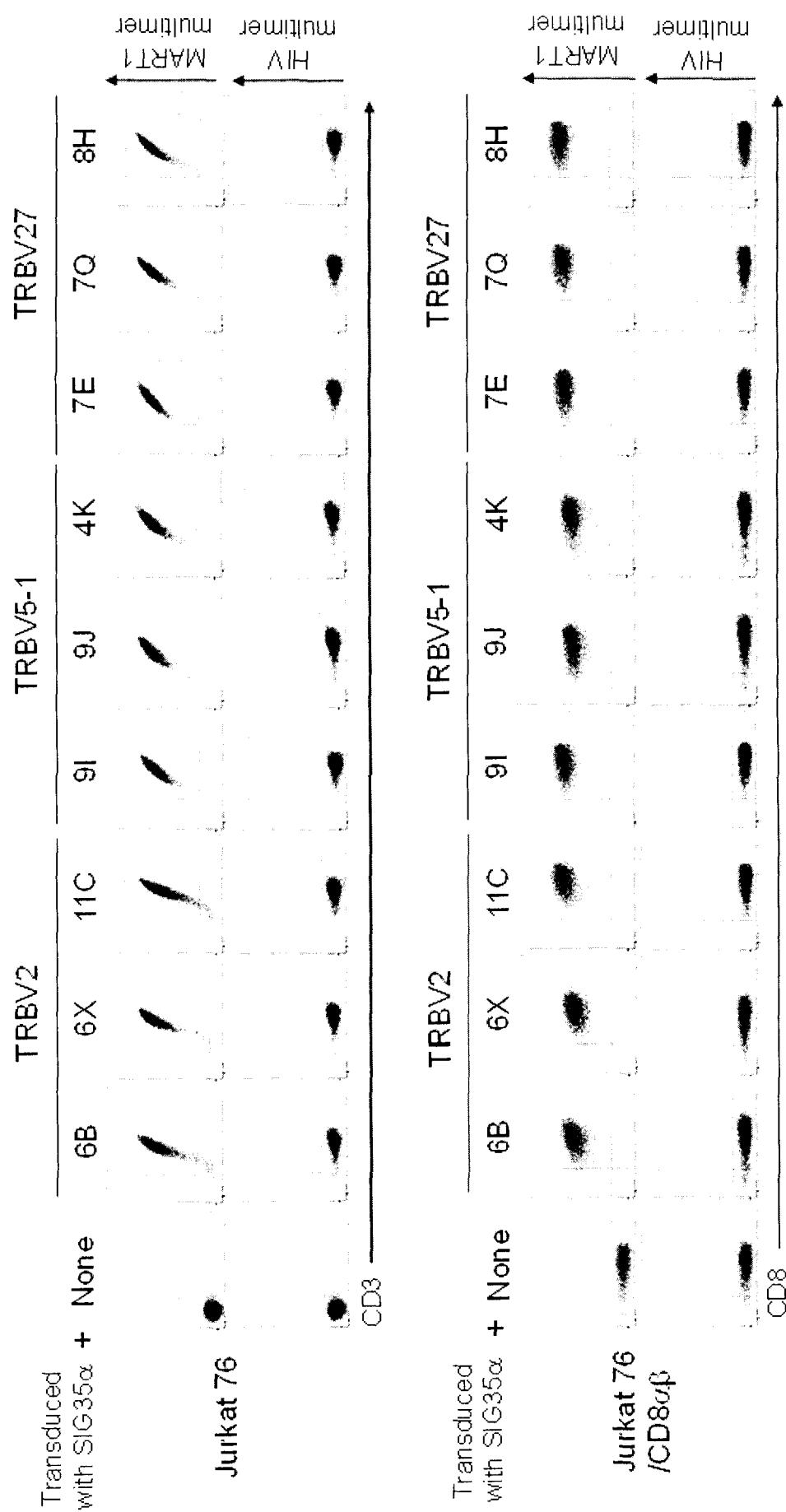
Fig. 26
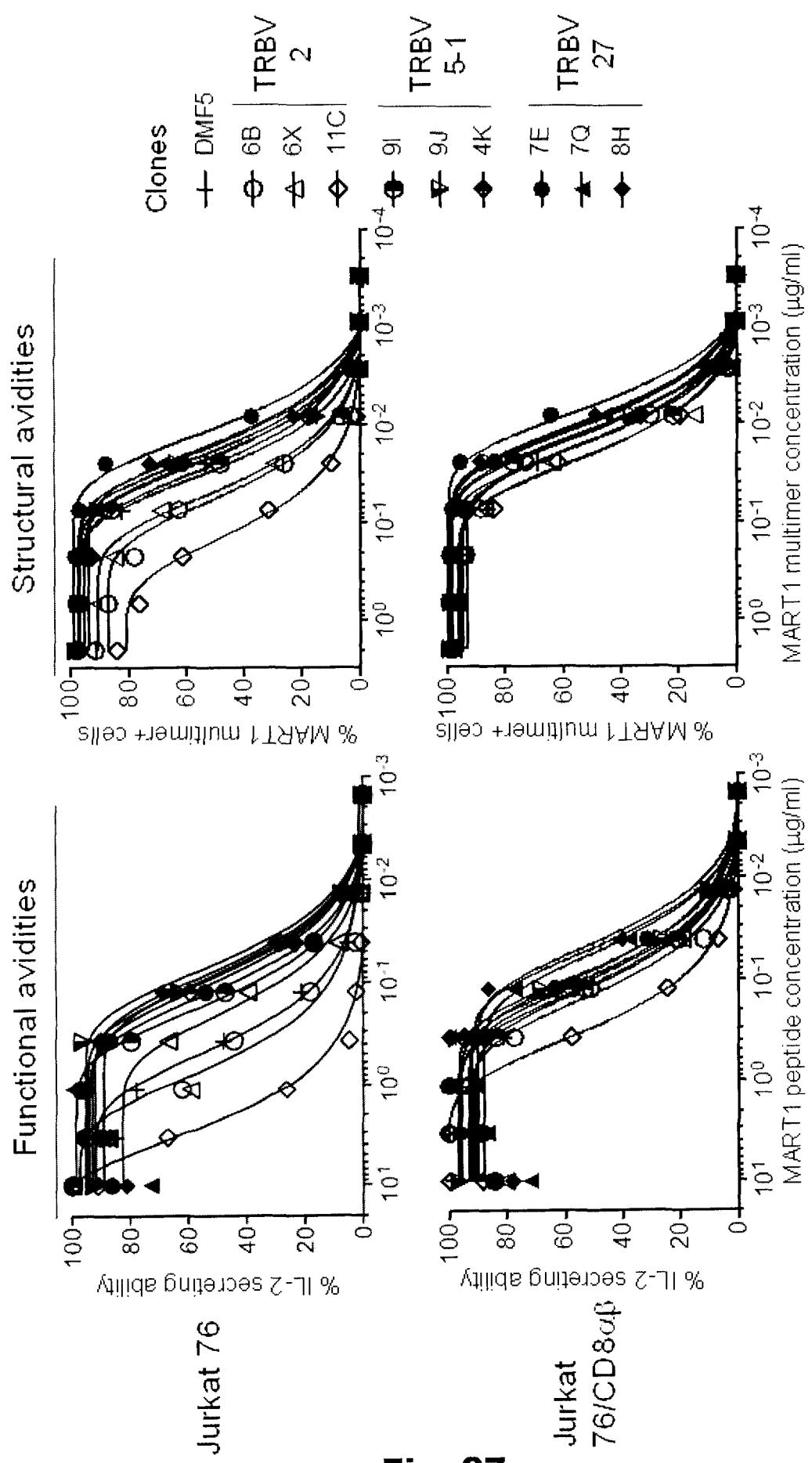
Fig. 27

Fig. 28

SEQ ID NO:93

TCRβ chain, Clone 8H nucleotide sequence

ATGGGCCCCCAGCTCCTCTTGGCTATGTGGTCCTTTGCCTTCCTAGGAGCAGGCCCCCTGGAAGCCCAAGTGACCCAGAACCCAAGATACCTCATC
ACAGTGACTGGAAAGAAGTTAACAGTGACTTGTTCTCAGAATATGAATCATGAGTATATGTCCTGGTATCGACAAGACCCAGGGCTGGGCTTA

<u>CDR1</u>

AGGCAGATCTACTATTCAATGAATGTTGAGGTGACTGATAAGGGAGATGTTCCTGAAGGGTACAAAGTCTCTCGAAAAGAGAAGAGGAATTTC

<u>CDR2</u>

CCCCTGATCCTGGAGTCGCCCAACCAGACCTCTCTGTACTTCTGTGCCAGCAGTCCCCTGGGGCCATGGAGCAGTACTTCGGGGCCG

<u>CDR3 (novel)</u>

GGCACCCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACC
CAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTACCCCGACCACGTTGGAGCTGAGCTGGGTGGTGAGCTGGAAGGAGGTGCACAGTGGG
GTCAGCAGAGACCCGCAGCCCCGCAACCACTTCCGCTGTCCAGTTCTCAAGTCCTGGCCTGTGTGGGCTCAGGGTAGGGCCTGAGAGTGGGTC
TGGCAGAACCCCGCAACCACTTCCGCTGTCCAGTTCTCAAGTCCTGGCCTGTGTGGGCTCAGGGTAGGGCCAAACCCGTC
ACCCAGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTCTTACCCGAGTCTTACCAGCAAGGGGTCCTGTCTGCCGCCATCCTC
TATGAGATCTTGCTAGGGAAGGCCACCTTGTATGCCGTGCTGGTCAGTGCCCTCGTGTTATGCCGTGATGGCCTGATGGCCTGAGAGAAAAGGATTCCAGAGGC
TAG

SEQ ID NO:94

TCRβ chain, Clone 8H amino acid sequence

MGPQLLGYVVLCLLGAGPIEAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSRKEKRNF
               <u>CDR1</u>   <u>CDR2</u>

PLILESPSPNQTSLYFCASSPLGAMEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSMWVNGKEVHSG
             <u>CDR3 (novel)</u>

VSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATIL
YEILLGKATLYAVLVSALVLMAMVKRKDSRGZ

Fig. 29

SEQ ID NO:95
TCRβ chain, Clone 7Q nucleotide sequence

ATGGGCCCCAGCTCCTTGGCTATGTGGTCCTTTGCCTTCTAGGAGCAGGCCCCCCTGGAAGCCCAAGTGACCGAGAACCCAAGATACCTCATC
ACAGTGACTGGAAAGAAGTTAACAGTGACTTGTTCTTCAGAATATGAACCATGAGAATGACTGATATGTCCTGGTATCGACAAGACCCAGGGCTTGGGCTTA
AGGCAGATCTACTATTCAATGAATGTTGAGGTGACTGATAAGGGAGATGTTCCTGAAGGGTACAAAAGTCTTCTCGAAAAAGAGAGAAGAGAATTTC
                                                        CDR1
                                CDR2
CCCCTGATCCTGGAGTCGCCGCCCAGCCCCAACCAGACCCTCTGCTGTCTGTGCCAGCAGTCCCTACACTGATGAACACTGAAGCTTTCTTTGGA
                                                                    CDR3 (novel)
CAAGGCACCAGACTCACAGTTGTAGAGGACCTGAACAAGGTGTTCCCACCCGAGGTCGCTGTGTTGAGCCATCAGAAGCAGAGATCTCCCAC
ACCCAAAAAGGCCACACACTGGTGTGCCTGGCCACACGTGGAGCTGGAGTGAGCGCCTGAGCAGATACTGCCTGACAATGAGAATGACGAGTGGAACCCAGGAGTCTCGGCACCC
GGGGTCAGGACACGGACCCCGCAGCCCCTCAAGGAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGACAATGAGAATGACGAGTGGAACCCAGGAGTCTCGGCACCC
TTCTGGGCAGAAACCCCCGCCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGCTTTCTACCTCGGCTTTCTTACCTCGGCTTTCTTACCTCGGCTTTCTTGCTGGCTAGAGAGCAGGCCCTGGGGGTAGAGCAGGCCCTGGGGGTAGAGCAGGCCCTGGGGGTAGAGCAGCCCTGTCCTGCTCCTGTCCTGCTCCTGTCCTGCTCCTGGTCCTGCTCCTGTCCTG
GTCACCCAGATCGTCAGCGCCGAAGCGCCTGAGCGACCAAGGGGGTCCTGTCTGCTGCCACCATC
CTCTATGAGAGATCCTGCTAGGGGAAGGGCTAGGGGCCACCCTGTGTGCTGATGGCCATGGTCAAGAGAAAGGGATTTCTGA

SEQ ID NO:96
TCRβ chain, Clone 7Q amino acid sequence

MGPQLLGYVVLCLLGAGPLEAAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSRK
                                                                      CDR1
                                              CDR2
EKRNFPLILESPSPNQTSLYFCASSPYMMNTEAFFGQGTRLITVVEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSW
                      CDR3 (novel)
WVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF
TSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF

Fig. 30

SEQ ID NO:97
TCRβ chain, Clone 9J nucleotide sequence

ATGGGGCTCCAGGCTGCTCTGTTGGGTGCTGCTTTGTCTCCTGGGAGCAGGCCCAGTAAAGGCTGGAGTCACTCAAACTCCAAGATATCTGATC
AAAACGAGAGGACAGCAAGTGACACTGAGCTGCTCCCCTATCTCTGGGCATAGGAGGTGTATCCTGGTACCAACAGACCCCAGGACAGGGCCTT
CDR1
CAGTTCCTCTTTGAATACTTCAGTGAGACACAGAGAAACAAAGGAAACTTCCCTGGTCGATTCTCAGGGCGCCAGTTCTCTAACTCTCGCTCT
CDR2
GAGATGAATGTGAGCACCTTGGAGCTGGGGGACTCGGCCCTTTATCTTTGCCGCCAGCAGCTGGACAGGGGATGGCTACACCTTCGGTTCGGGG
CDR3 (novel)
ACCAGGTTAACCGTTGTAGAGGACCTGAACAAGGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCAGCCATCAGAAGCAGAGATCTCCCACACCAA
AAGGCCACACTGGGTGTGCCTGGCCACAGGTCTTCTTCCCTGACCACGTGGAGCTGGAGTGGAATGGGAAGGAGGTGCACAGTGGGGGTC
AGCACGGACCCGCAGCCCCTCAAGAGCAGCCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCGCCTGAGGGGTCTCGGCCACCTTCTGG
CAGAACCCCGCAACCACTTCCGCTGTCAAGTCCGCTGTGGGGTAGGCAGCAGTGTGGCTTTACCTCGGTGTCCTGTGTTGATGGCCTGCTGCCACC
CAGATCGTCGTCAGCGCGCCGAGGCCTGGGGTAGGCAGCCACCCCTGTGTGCTGTGTATGCTGTGCTGTCAGCGCGCCCTTGTGTTGATGGCCTATCTTAT
GAGATCCTGCTGCTAGGGGAAGGCCACCCCTGTGTGCTGTGTATGCTGTGCTGTCAGCGCGCCCTTGTGTTGATGGCCATGGTCAAGAGAGAAAGGATTTCTGA

SEQ ID NO:98
TCRβ chain, Clone 9J amino acid sequence

MGSRLLCWVILCLIGAGPVKAGVTQTPRYLIIKTRGQQVTLSCSPISGHRSVSWYQQTPGQGLQFLFEYFSETQRNKGNFPGRFSGRQFSNSRS
CDR1   CDR2
EMNVSTLELGDSALYLCASSWTGDGYTFGSGTRLTVVEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGV
CDR3 (novel)
STDPQPIKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSATILY
EILLGKATIYAVLVSAIVLMAMVKRKDFZ

Donor#1 (A2+)

Donor#3 (A2-)

(%)

TCR Vβ Subtype