
Steps:
1
2
3
6
8
10
Add DCs
Add DCs
Add DCs
DC prep 1
Tc stim 1
Tc stim 2
Tc stim 3
Readout

FIG. 1A

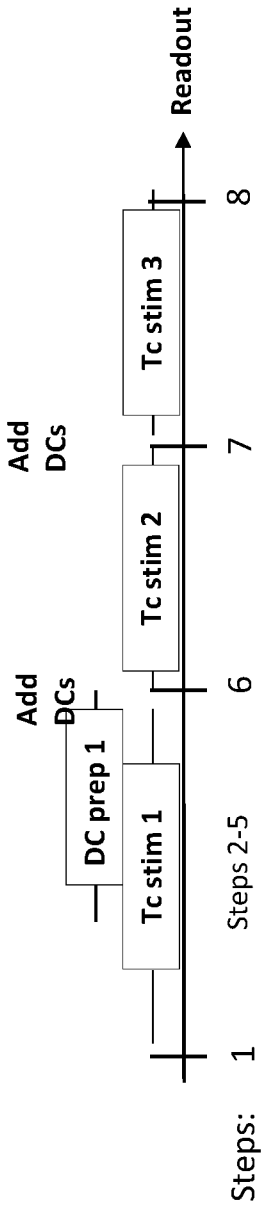

Steps:
1
Steps 2-5
6
7
8
Add DCs
Add DCs
DC prep 1
Tc stim 1
Tc stim 2
Tc stim 3
Readout

FIG. 1B

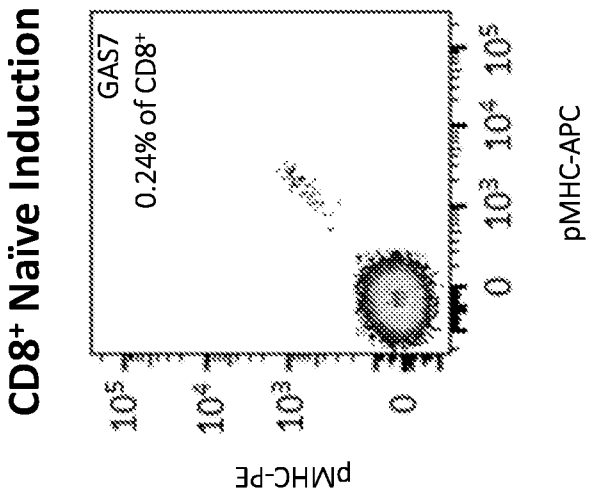

CD8+ Naïve Induction
GAS7
0.24% of CD8+
pMHC-PE
pMHC-APC
0
10^3
10^4
10^5

FIG. 3

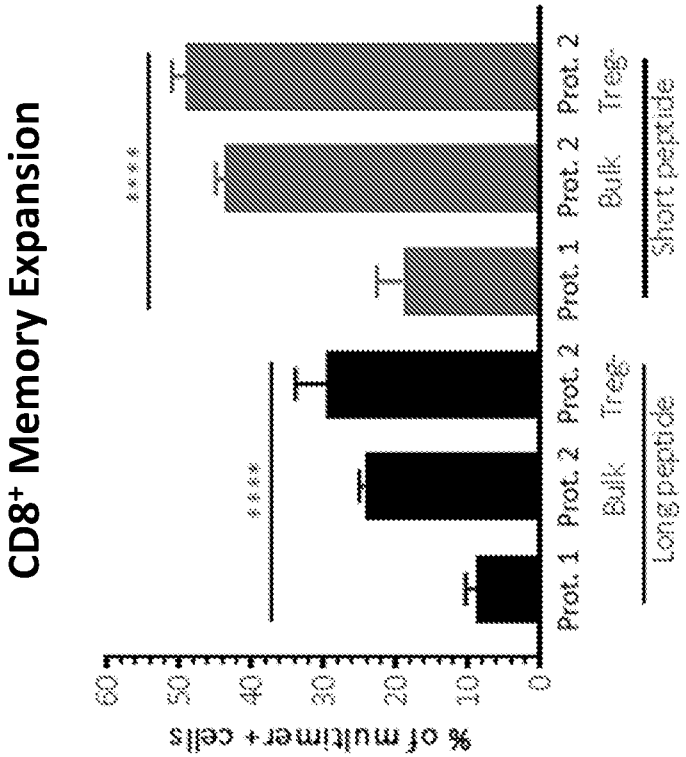

CD8+ Memory Expansion
% of multimer+ cells
0
10
20
30
40
50
60
****
****
Prot. 1
Prot. 2
Prot. 2
Prot. 1
Prot. 2
Prot. 2
Bulk
Treg
Bulk
Treg
Long peptide
Short peptide

FIG. 2

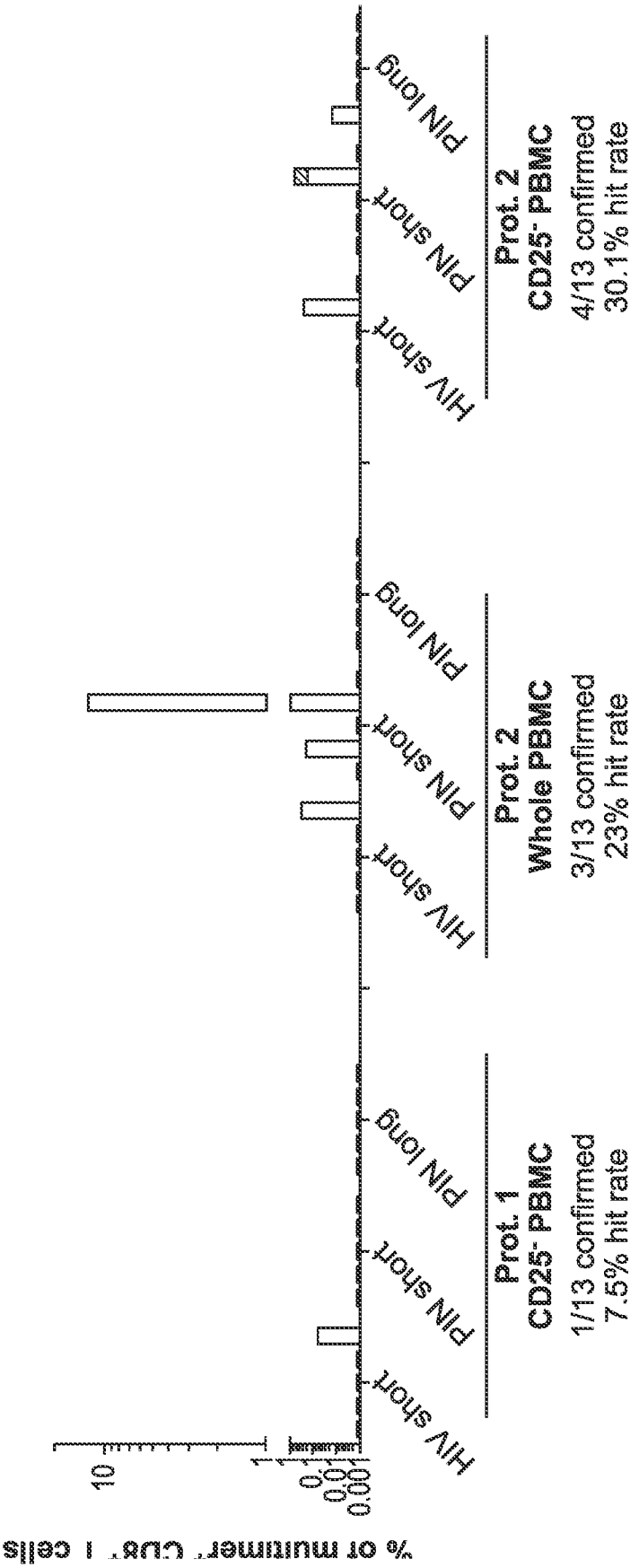

% of multimer+ CD8+ T cells
10
1
0.1
0.01
0.001
HIV short
PIN short
PIN long
HIV short
PIN short
PIN long
HIV short
PIN short
PIN long
Prot. 1
CD25- PBMC
1/13 confirmed
7.5% hit rate
Prot. 2
Whole PBMC
3/13 confirmed
23% hit rate
Prot. 2
CD25- PBMC
4/13 confirmed
30.1% hit rate

FIG. 4

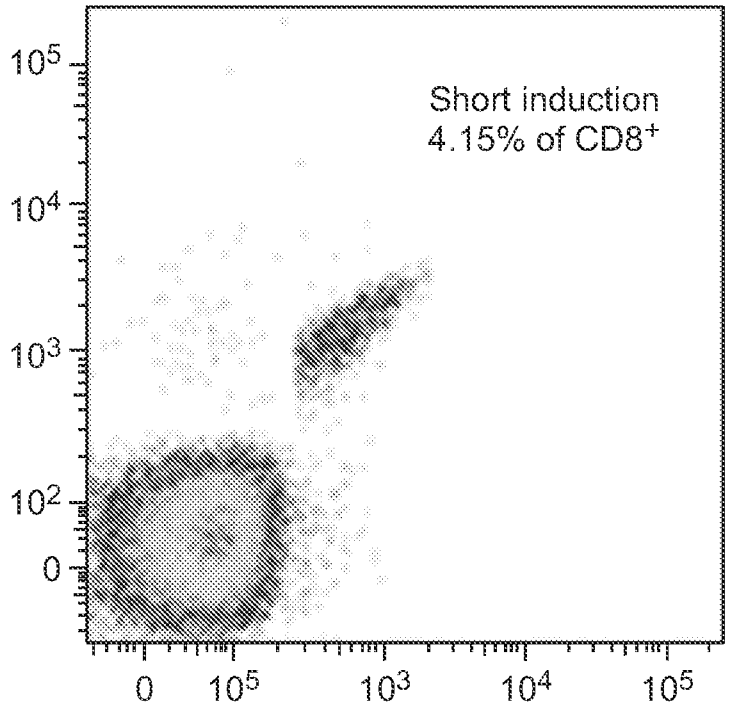

Short induction
4.15% of CD8+
10^5
10^4
10^3
10^2
0
0
10^5
10^3
10^4
10^5

FIG. 5A

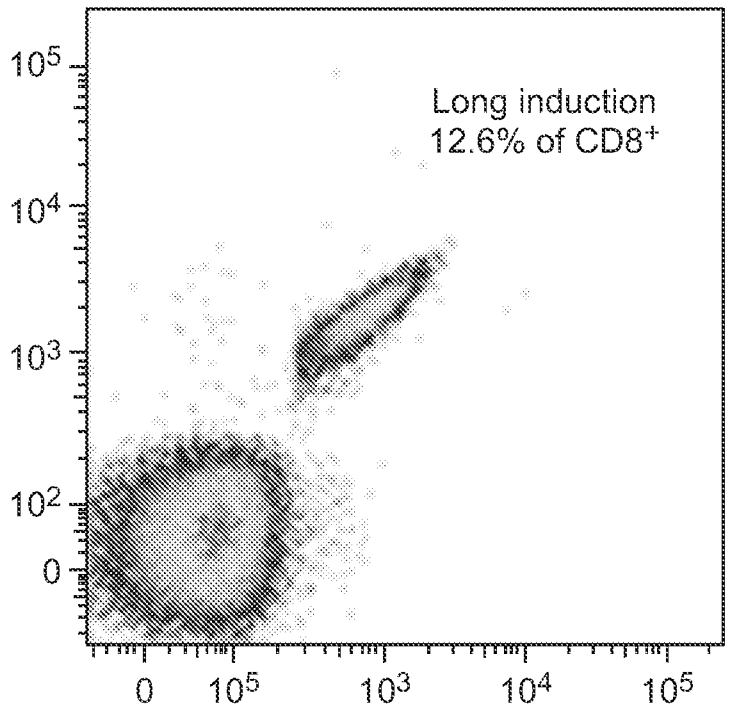

Long induction
12.6% of CD8+
10^5
10^4
10^3
10^2
0
0
10^5
10^3
10^4
10^5

FIG. 5B

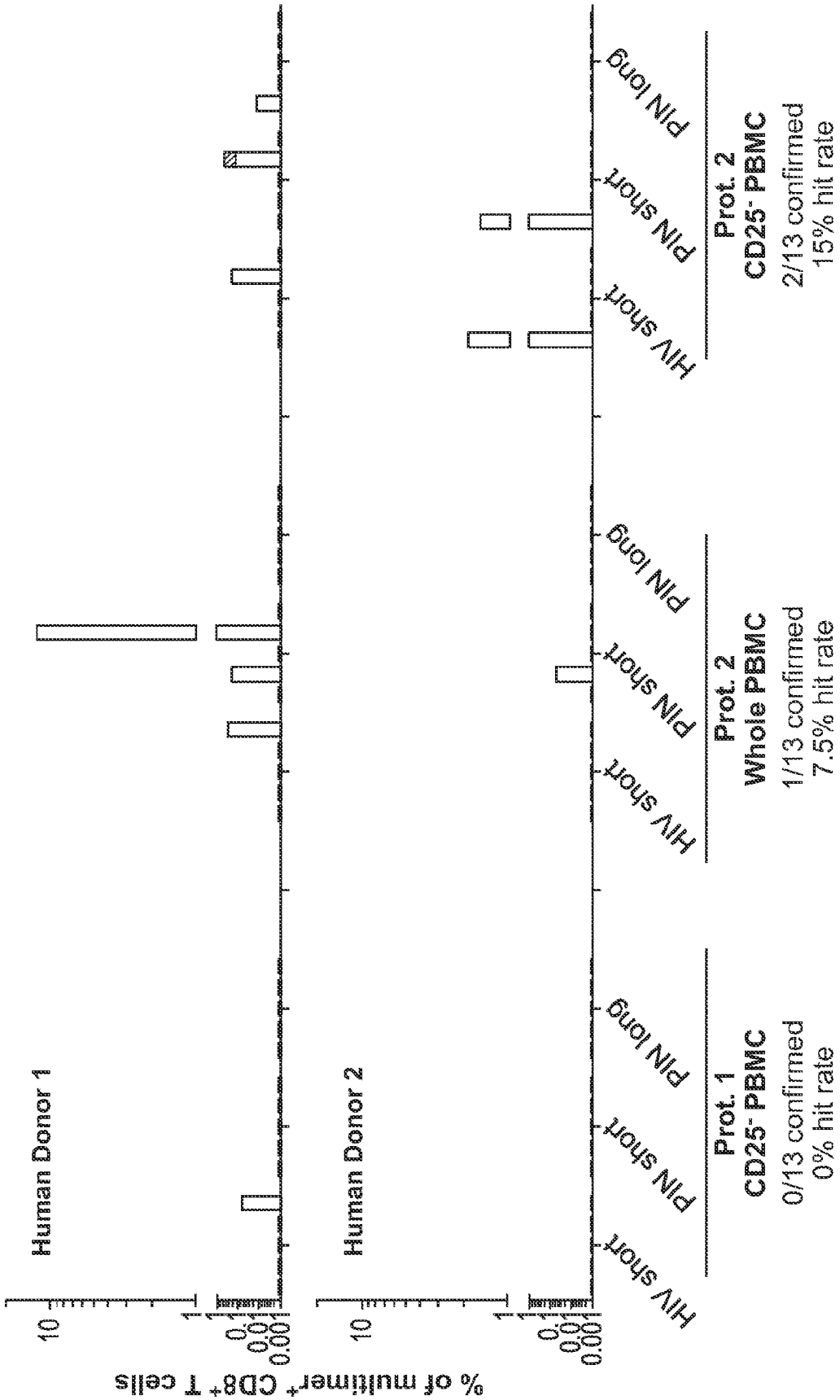

% of multimer+ CD8+ T cells
Human Donor 1
Human Donor 2
10
1
0.1
0.01
0.001
HIV short
PIN short
PIN long
Prot. 1
CD25- PBMC
0/13 confirmed
0% hit rate
Prot. 2
Whole PBMC
1/13 confirmed
7.5% hit rate
Prot. 2
CD25- PBMC
2/13 confirmed
15% hit rate

FIG. 6

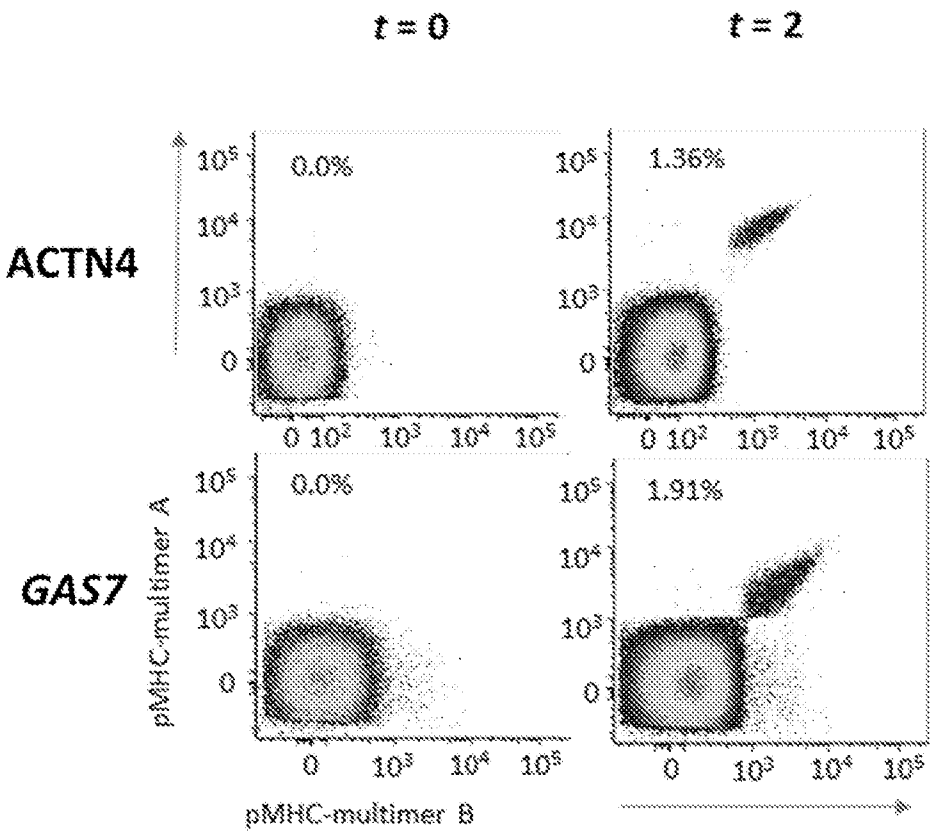



FIG. 7

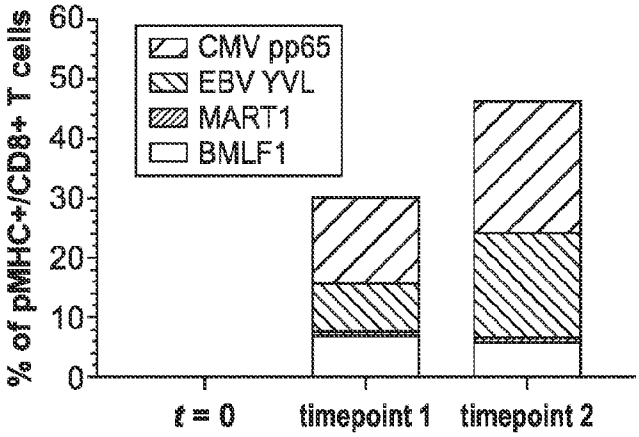

% of pMHC+/CD8+ T cells
60
50
40
30
20
10
0
CMV pp65
EBV YVL
MART1
BMLF1
t = 0
timepoint 1
timepoint 2

FIG. 8A

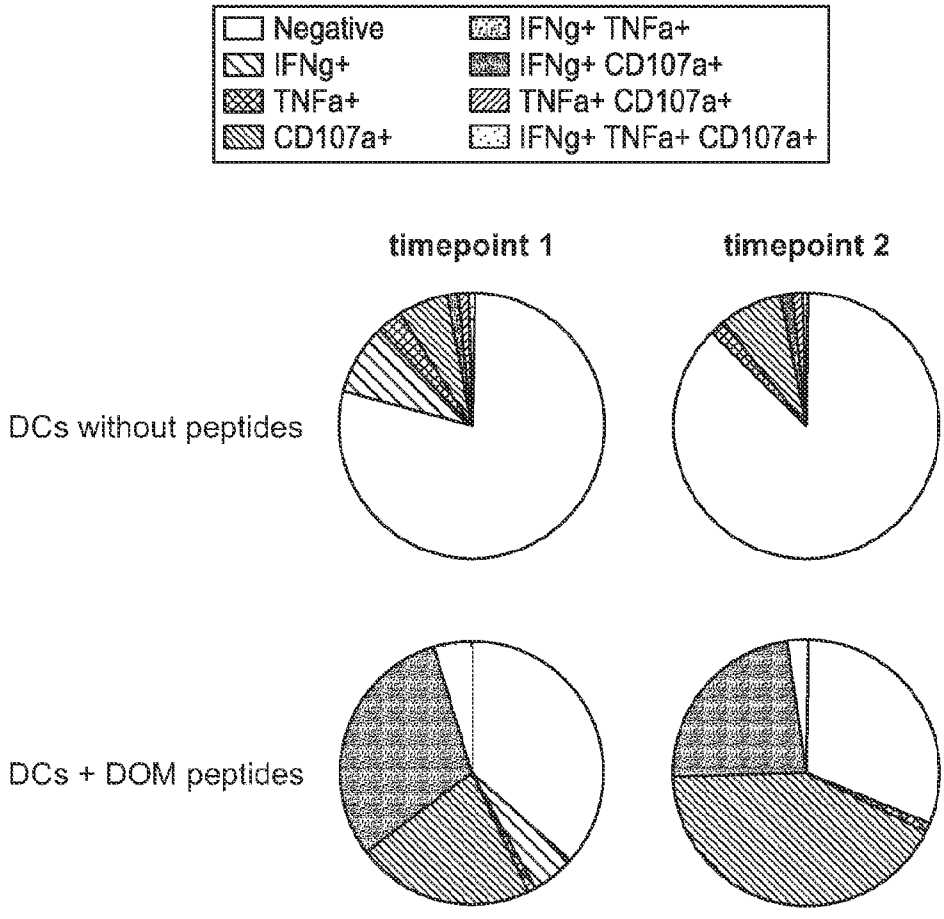

Negative
IFNg+
TNFa+
CD107a+
IFNg+ TNFa+
IFNg+ CD107a+
TNFa+ CD107a+
IFNg+ TNFa+ CD107a+
timepoint 1
timepoint 2
DCs without peptides
DCs + DOM peptides

FIG. 8B

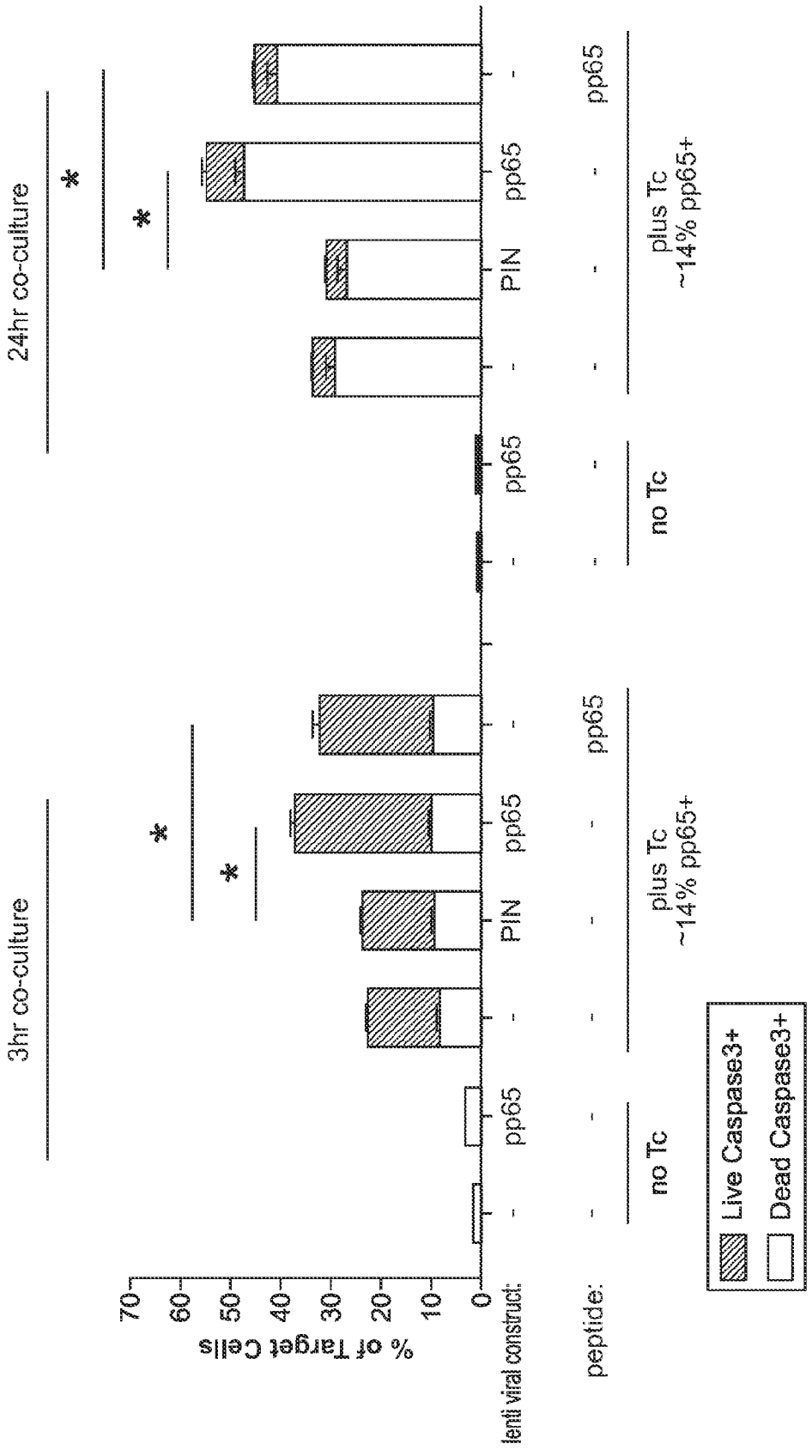

3hr co-culture
24hr co-culture
*
*
*
*
% of Target Cells
0
10
20
30
40
50
60
70
lenti viral construct:
-
pp65
-
PIN
pp65
-
pp65
-
PIN
pp65
-
peptide:
-
-
-
pp65
-
-
-
pp65
no Tc
plus Tc
~14% pp65+
no Tc
plus Tc
~14% pp65+
Live Caspase3+
Dead Caspase3+

FIG. 9

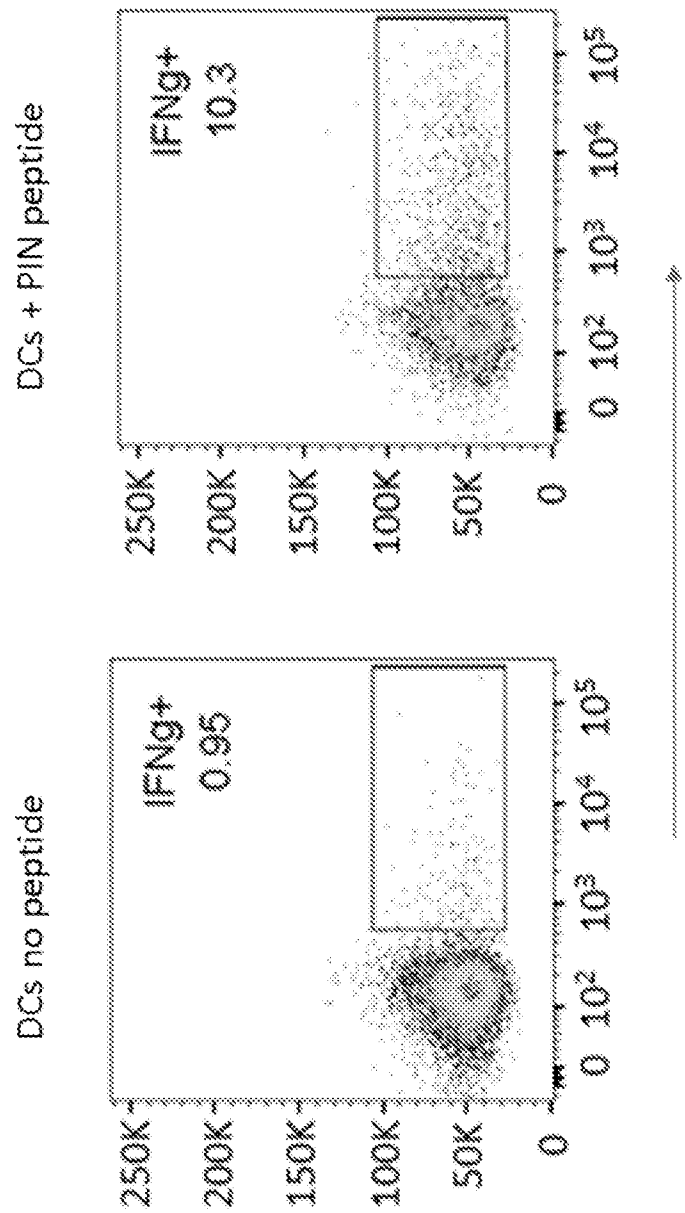

DCs no peptide
IFNg+
0.95
DCs + PIN peptide
IFNg+
10.3
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
IFN-gamma
(gated on CD4+ T cells)

FIG. 10

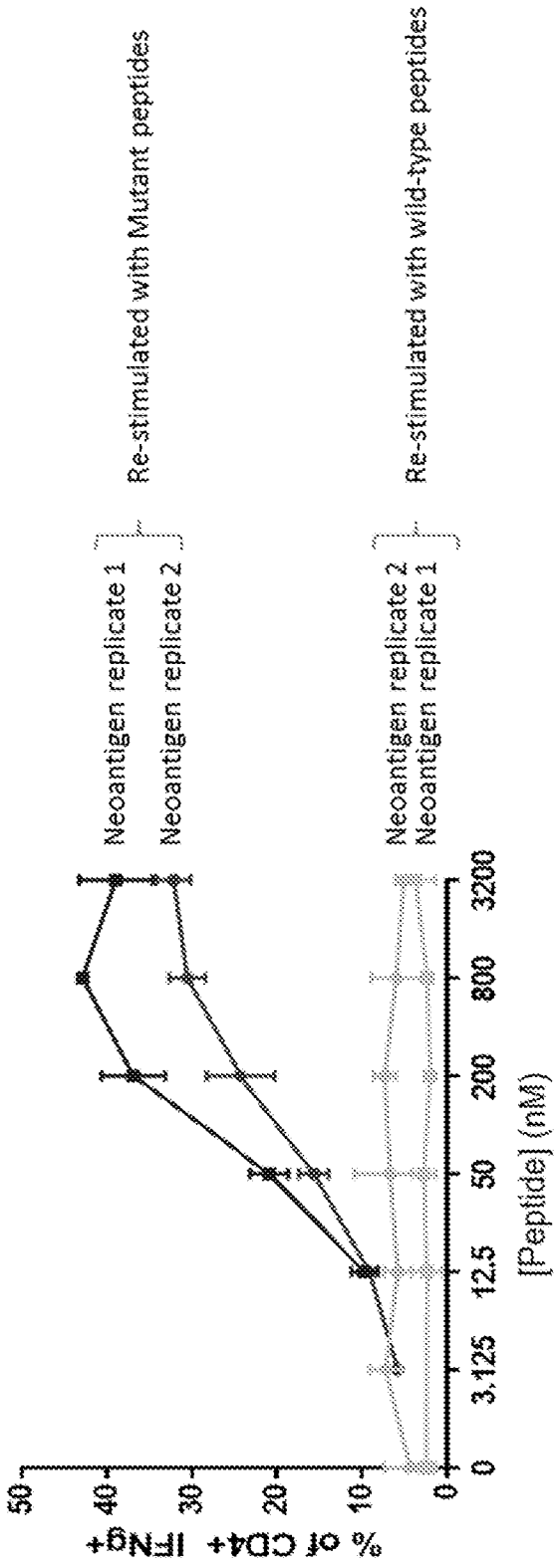

Re-stimulated with Mutant peptides
Re-stimulated with wild-type peptides
Neoantigen replicate 1
Neoantigen replicate 2
Neoantigen replicate 2
Neoantigen replicate 1
% of CD4+ IFNg+
50
40
30
20
10
0
0
3.125
12.5
50
200
800
3200
[Peptide] (nM)

FIG. 11

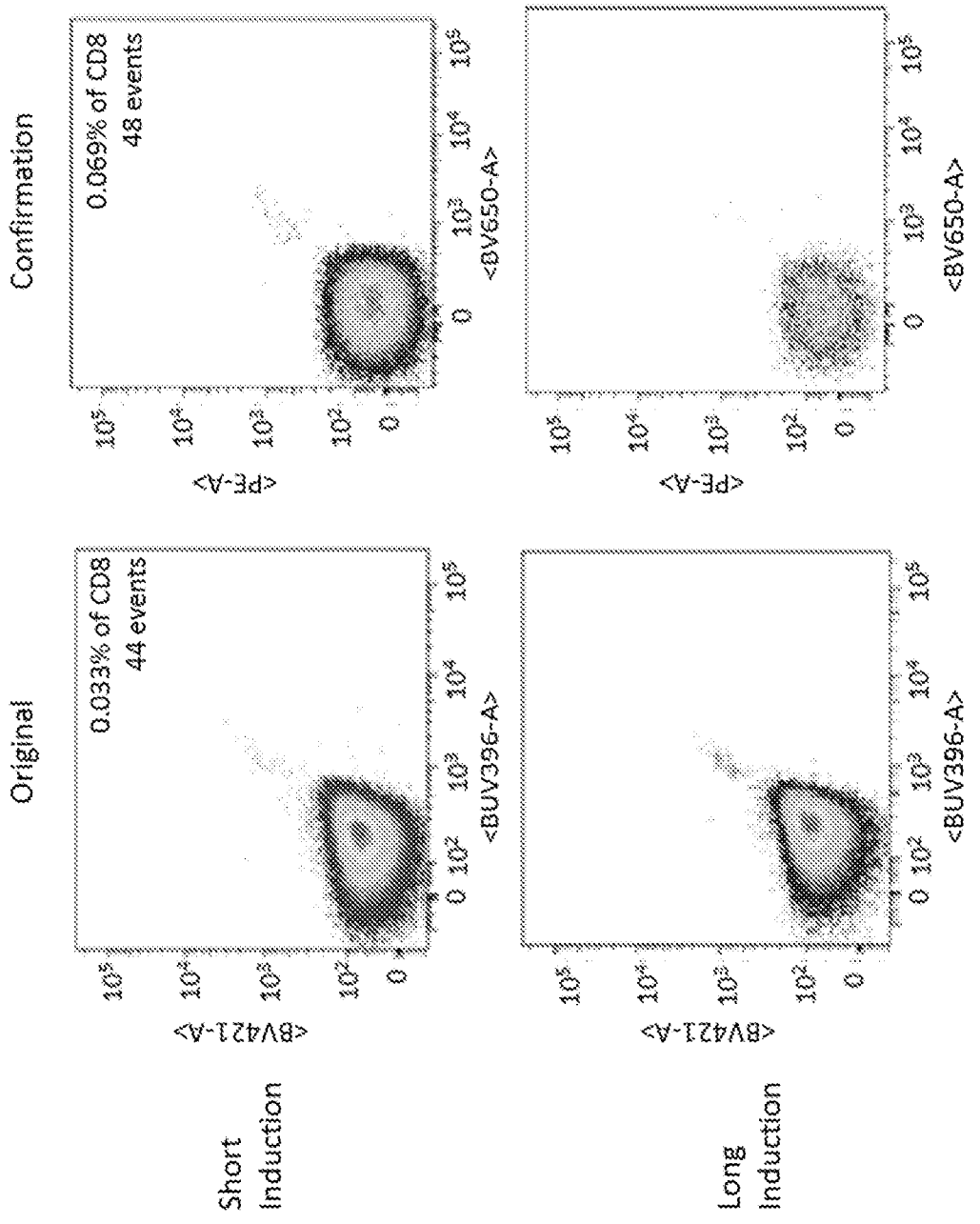

Original
Confirmation
Short Induction
Long Induction
0.033% of CD8
44 events
0.069% of CD8
48 events
<BV421-A>
<BUV396-A>
<PE-A>
<BV650-A>

FIG. 12

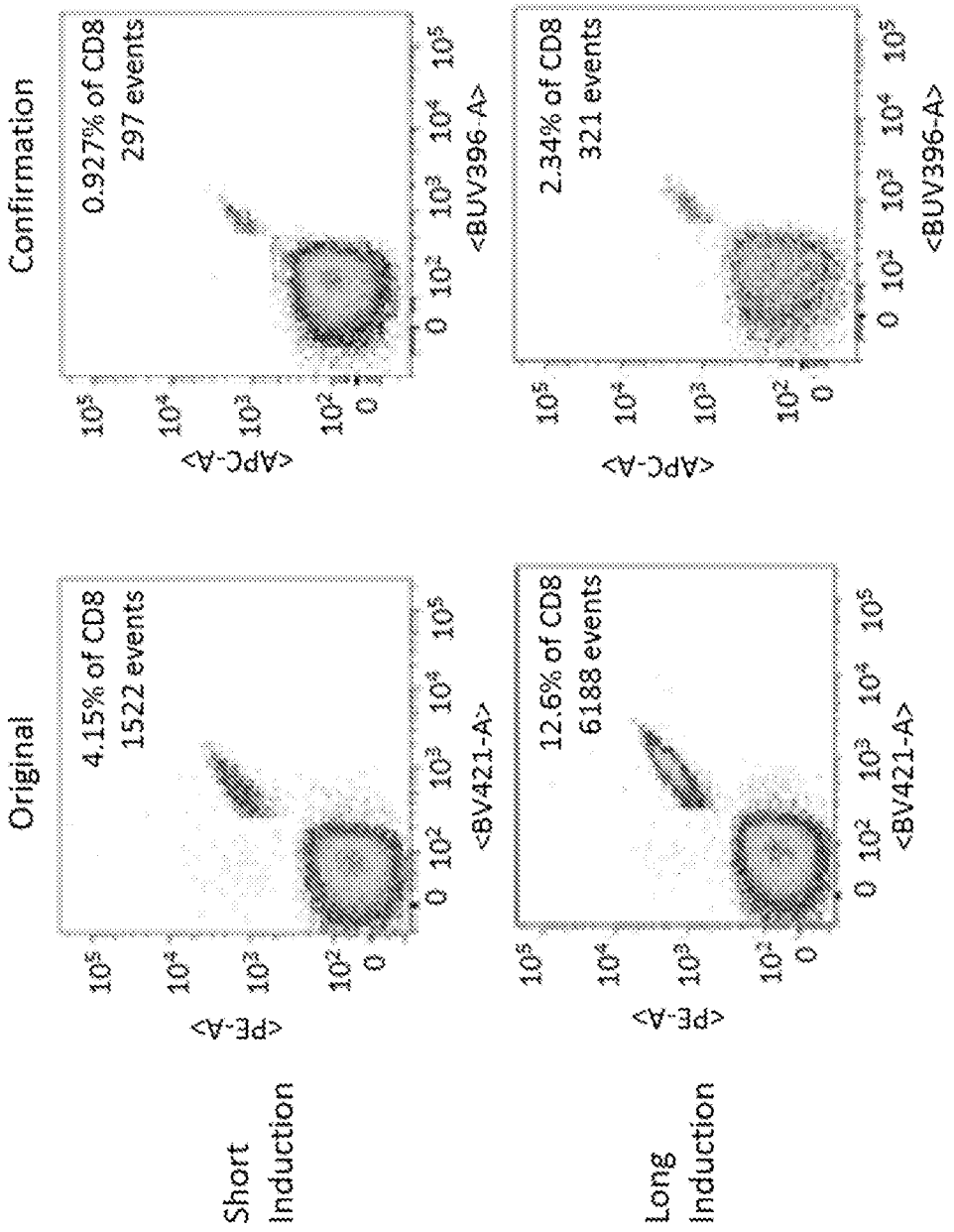

Original
Confirmation
Short Induction
Long Induction
4.15% of CD8
1522 events
0.927% of CD8
297 events
12.6% of CD8
6188 events
2.34% of CD8
321 events
<PE-A>
<APC-A>
<BV421-A>
<BUV396-A>
0
$10^2$
$10^3$
$10^4$
$10^5$

FIG. 13

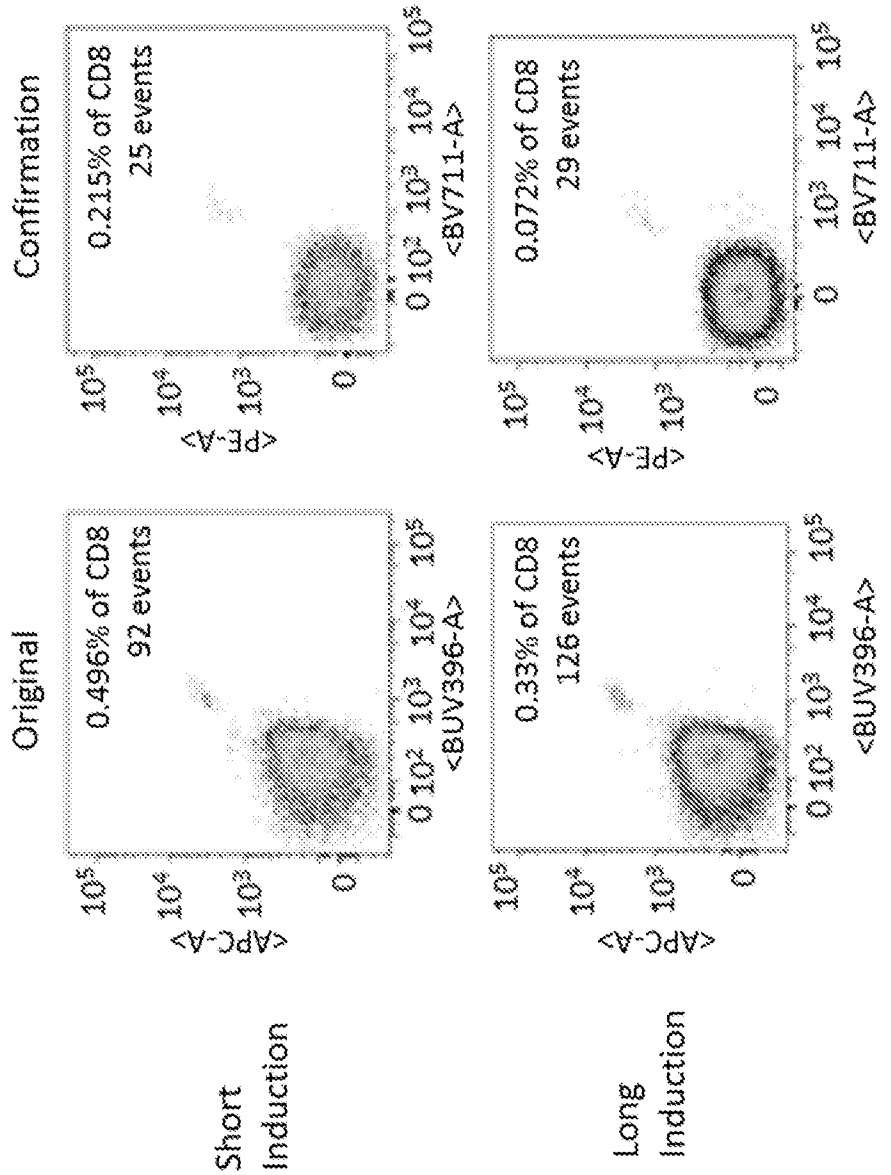

Original
Confirmation
Short Induction
Long Induction
0.496% of CD8
92 events
0.215% of CD8
25 events
0.33% of CD8
126 events
0.072% of CD8
29 events
<APC-A>
<PE-A>
<BUV396-A>
<BV711-A>

FIG. 14

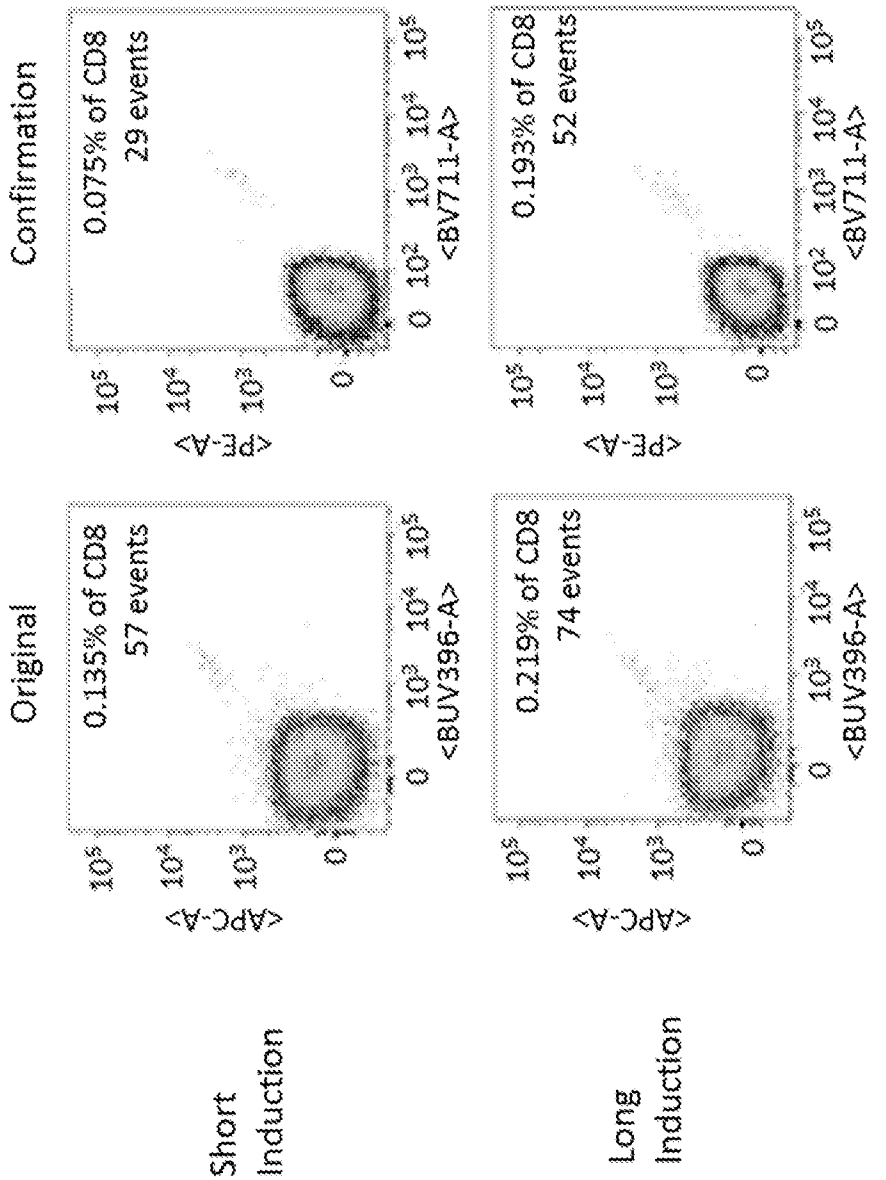

Original
Confirmation
Short Induction
Long Induction
0.135% of CD8
57 events
0.075% of CD8
29 events
0.219% of CD8
74 events
0.193% of CD8
52 events
<APC-A>
<PE-A>
<BUV396-A>
<BV711-A>
0
10³
10⁴
10⁵
10²

FIG. 15

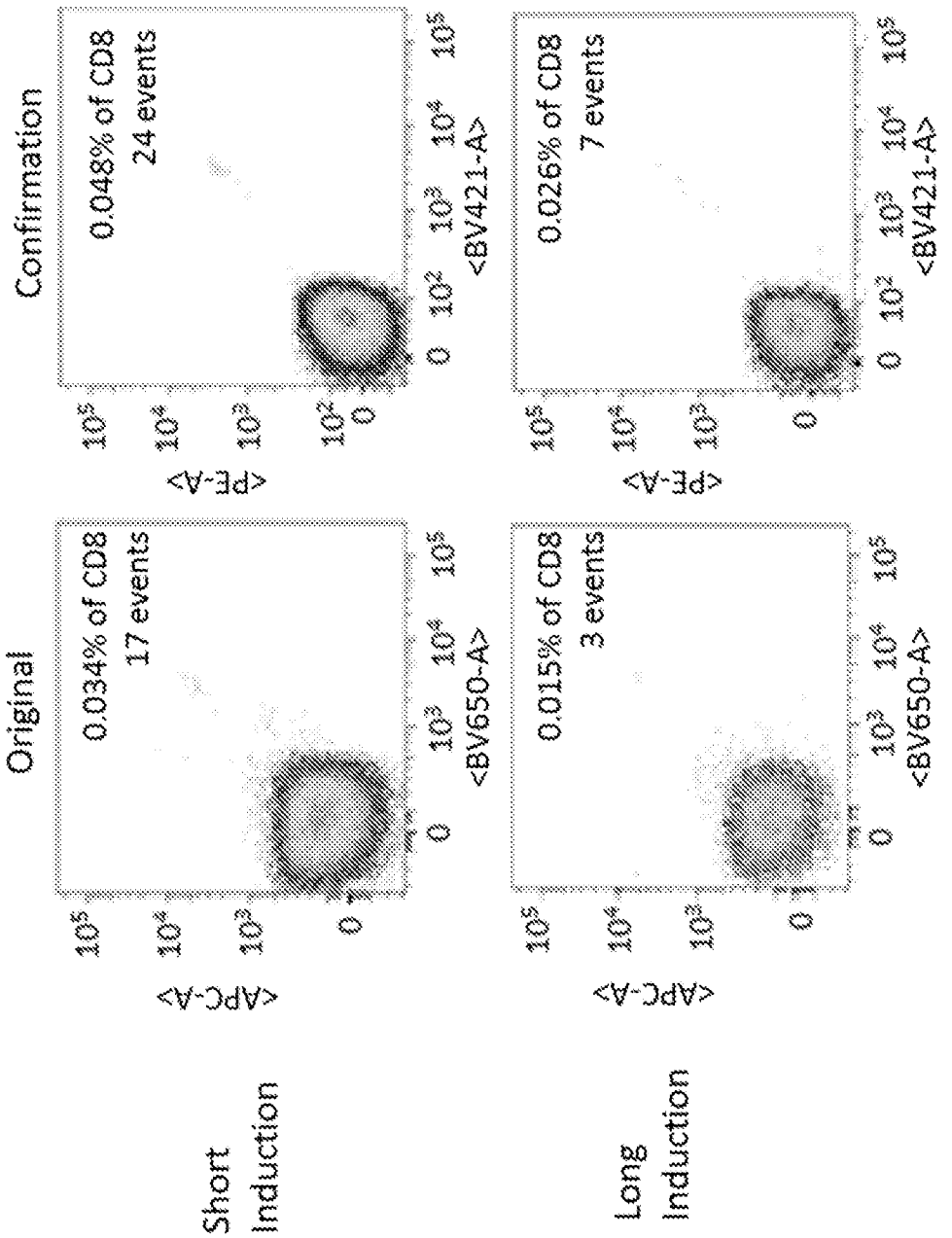

Original
Confirmation
Short Induction
Long Induction
0.034% of CD8
17 events
0.048% of CD8
24 events
0.015% of CD8
3 events
0.026% of CD8
7 events
<APC-A>
<PE-A>
<BV650-A>
<BV421-A>

FIG. 16

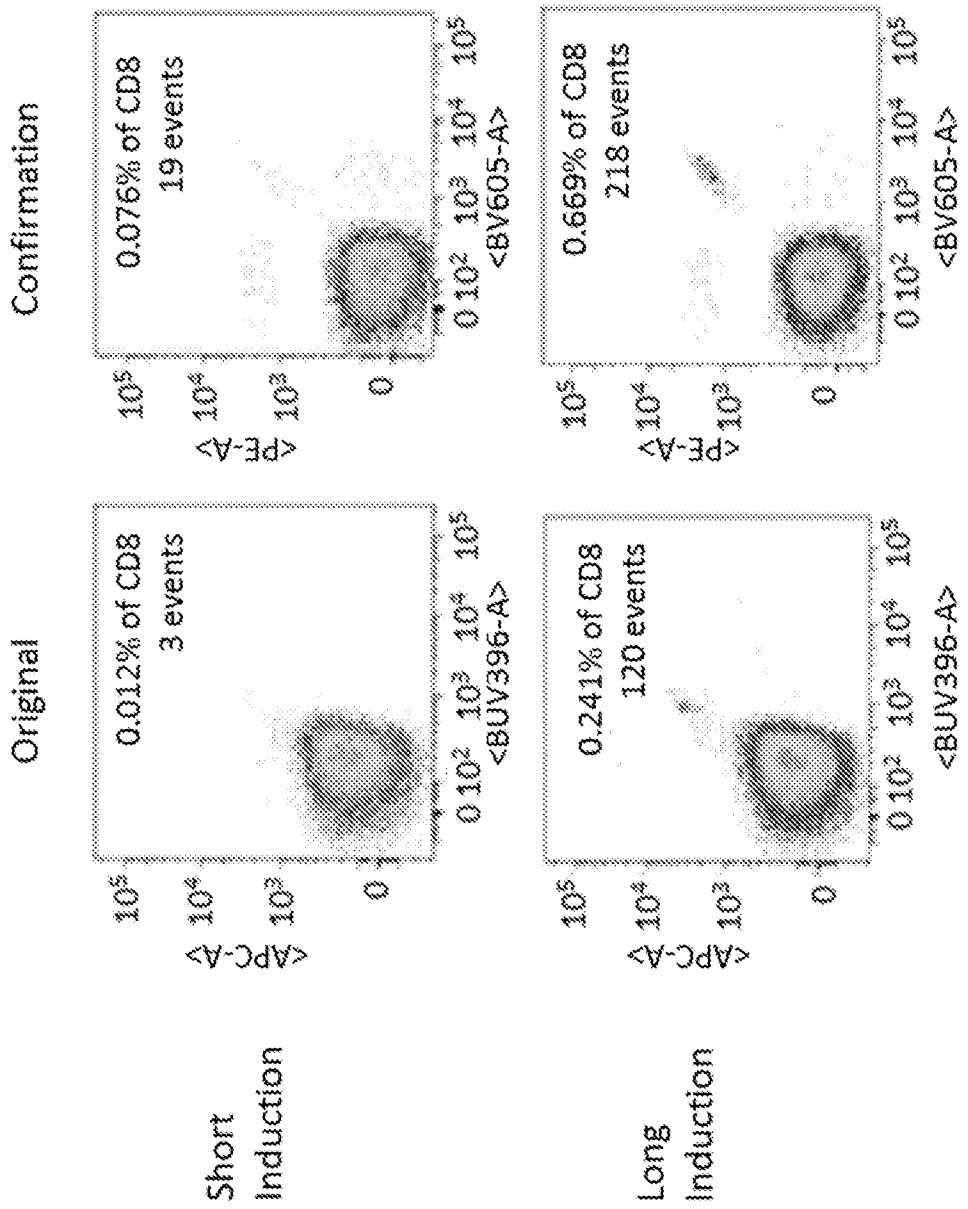

Original
Confirmation
Short Induction
Long Induction
0.012% of CD8
3 events
0.076% of CD8
19 events
0.241% of CD8
120 events
0.669% of CD8
218 events
<APC-A>
<PE-A>
<BUV396-A>
<BV605-A>
0
10²
10³
10⁴
10⁵

FIG. 17

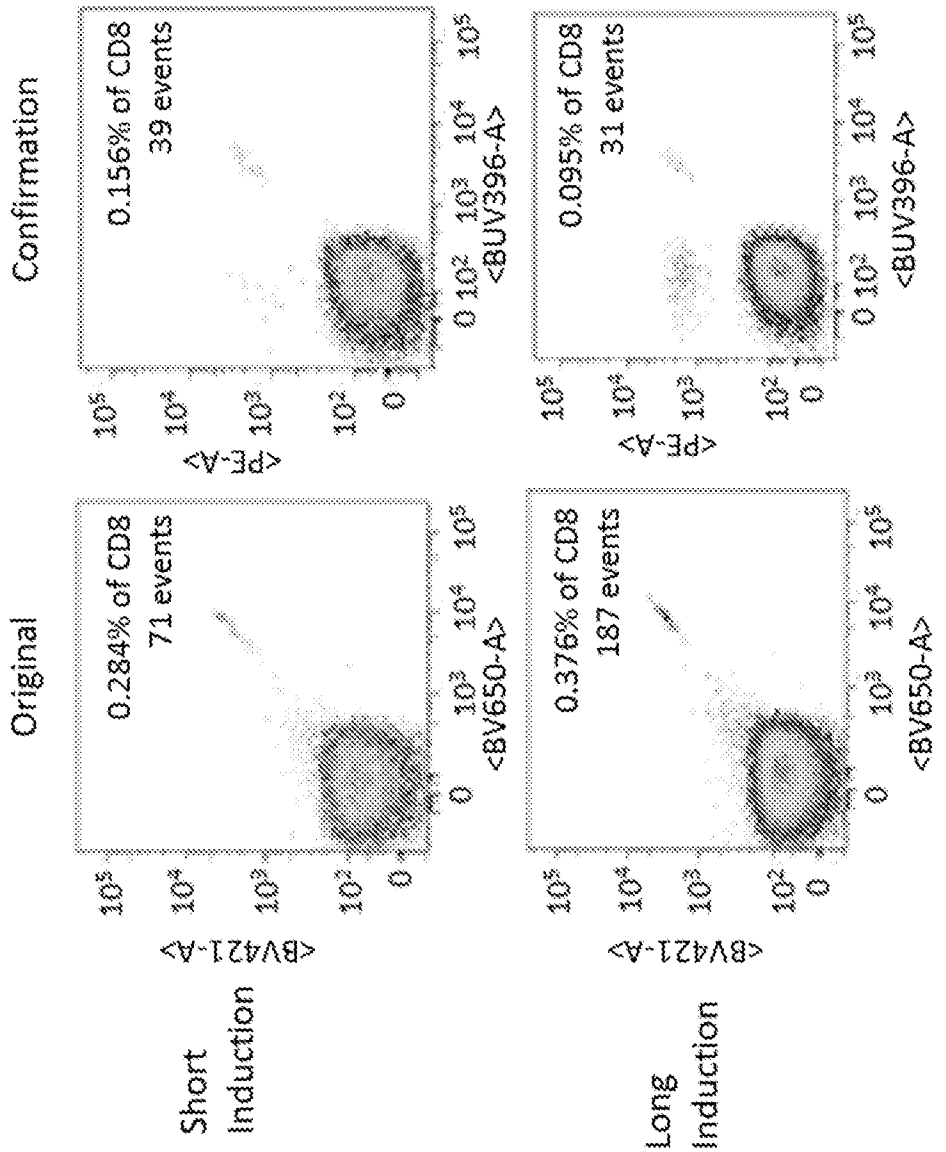

Original
Confirmation
Short Induction
Long Induction
0.284% of CD8
71 events
0.156% of CD8
39 events
0.376% of CD8
187 events
0.095% of CD8
31 events
<BV421-A>
<BV650-A>
<PE-A>
<BUV396-A>

FIG. 18

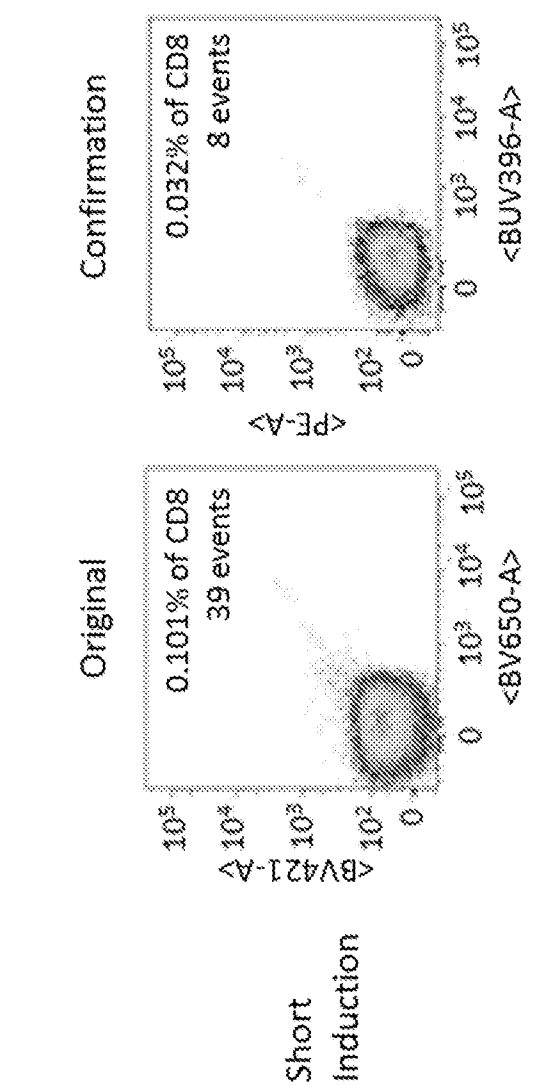



FIG. 19A

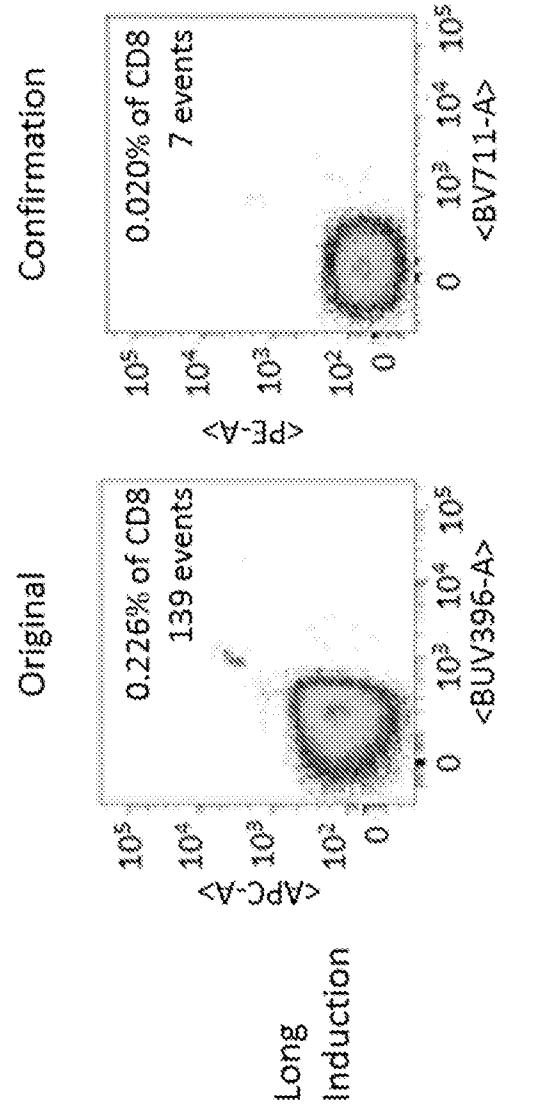



FIG. 19B

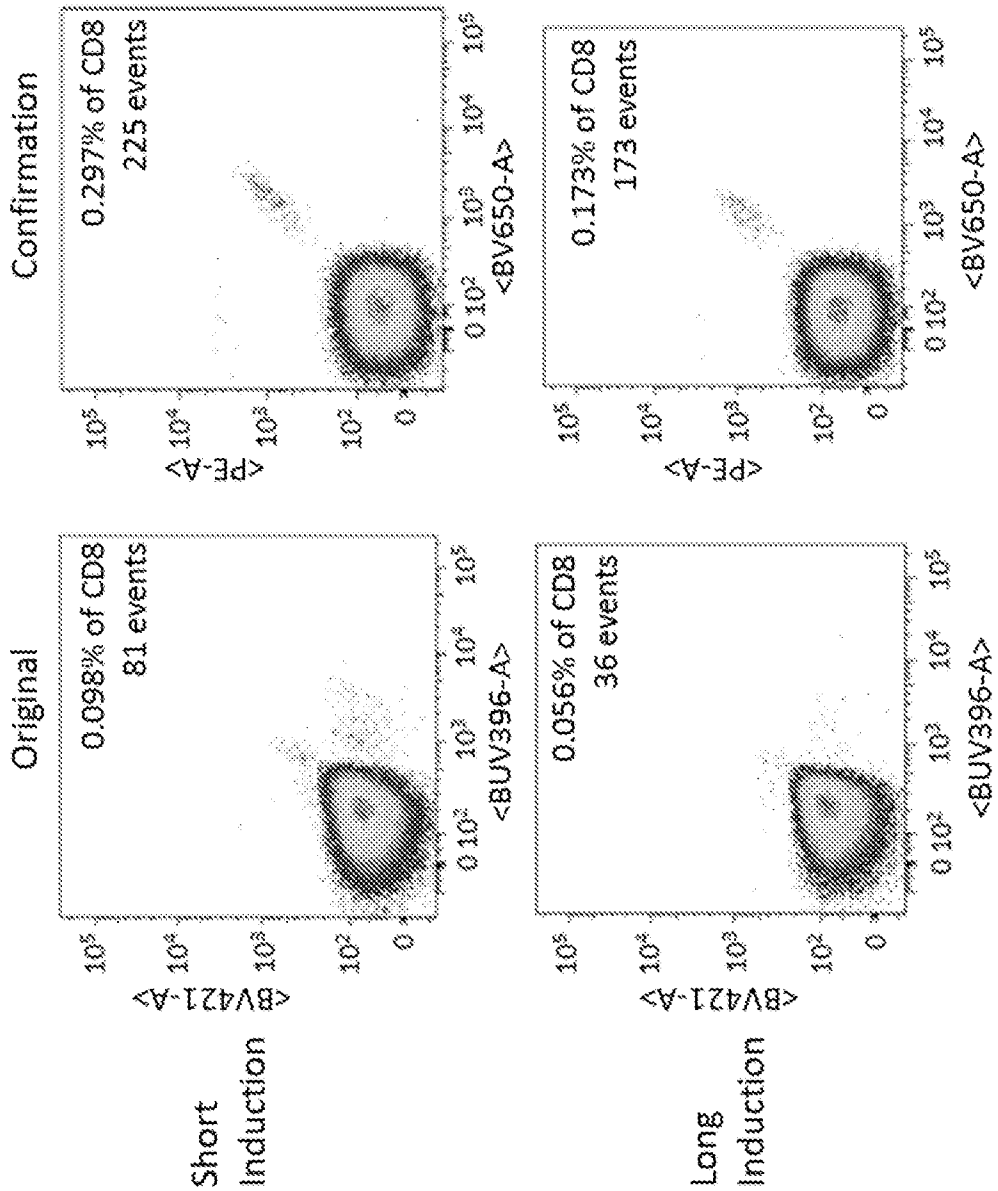

Original
Confirmation
Short Induction
Long Induction
0.098% of CD8
81 events
0.297% of CD8
225 events
0.056% of CD8
36 events
0.173% of CD8
173 events
<BV421-A>
<BUV396-A>
<PE-A>
<BV650-A>

FIG. 20

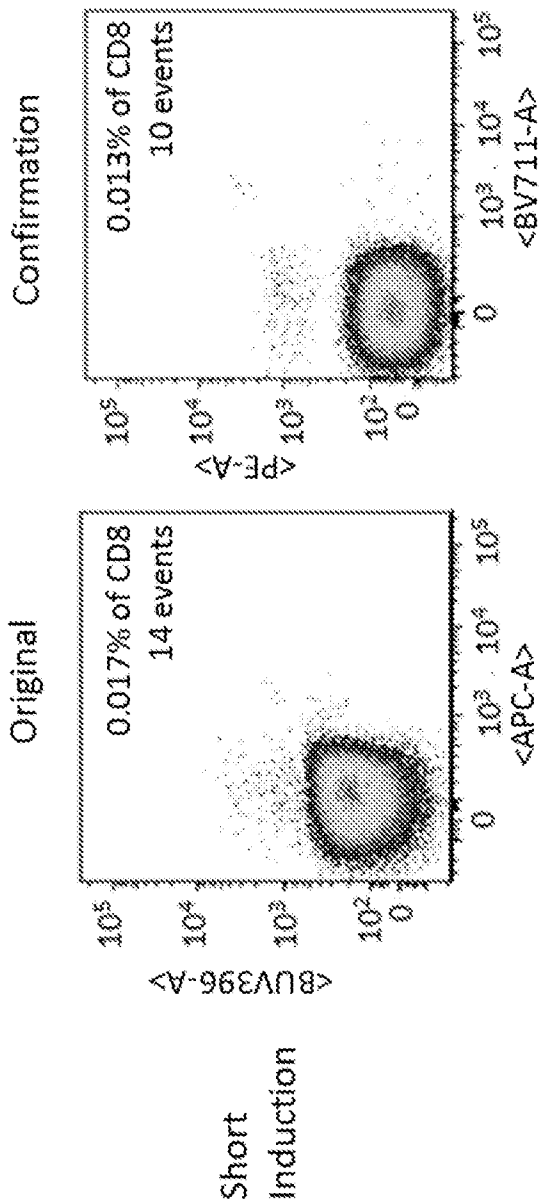

Short Induction
Original
Confirmation
0.017% of CD8
14 events
0.013% of CD8
10 events
<BUV396-A>
<APC-A>
<PE-A>
<BV711-A>

FIG. 21

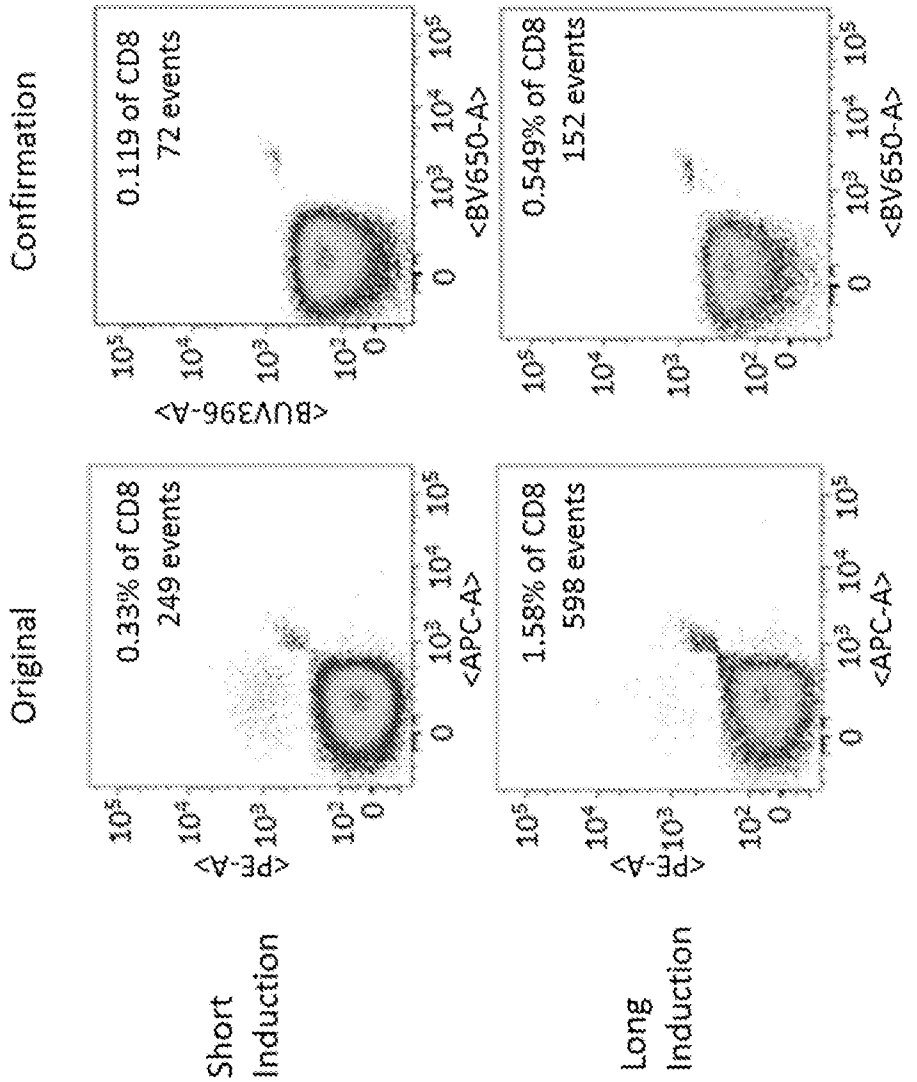

Original
Confirmation
Short Induction
Long Induction
0.33% of CD8
249 events
0.119 of CD8
72 events
1.58% of CD8
598 events
0.549% of CD8
152 events
<APC-A>
<BV650-A>
<BUV396-A>

FIG. 22

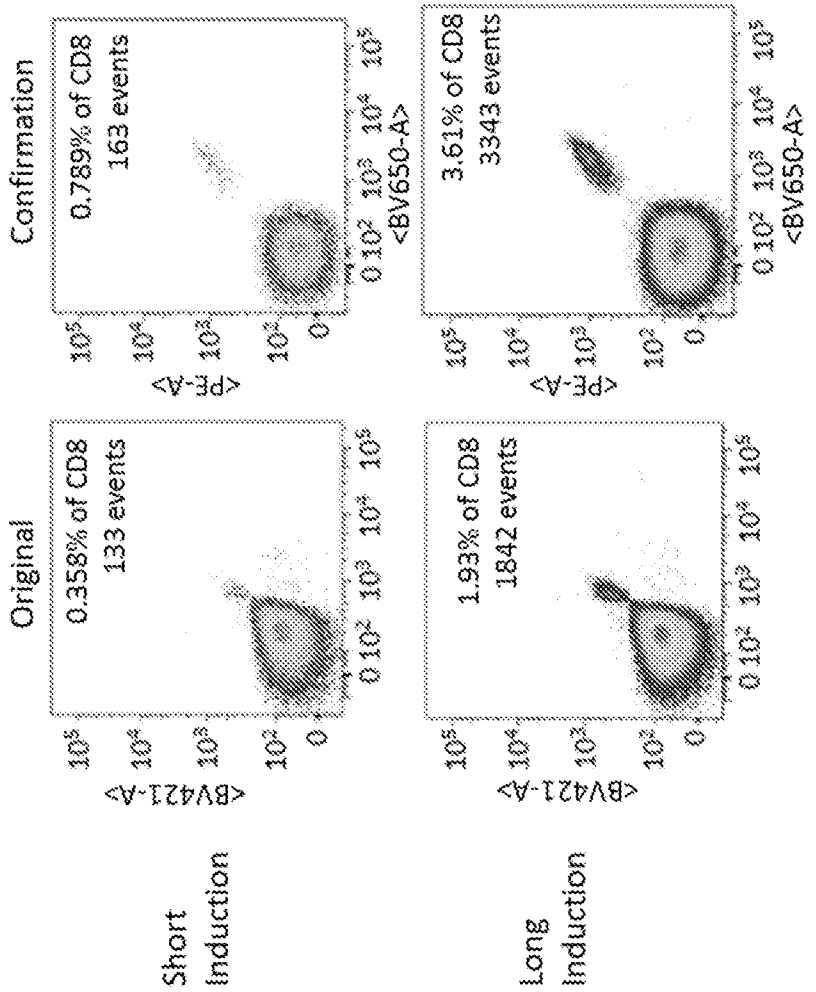

Original
Confirmation
Short Induction
Long Induction
0.358% of CD8
133 events
0.789% of CD8
163 events
1.93% of CD8
1842 events
3.61% of CD8
3343 events
<BV421-A>
<PE-A>
<BV650-A>

FIG. 23

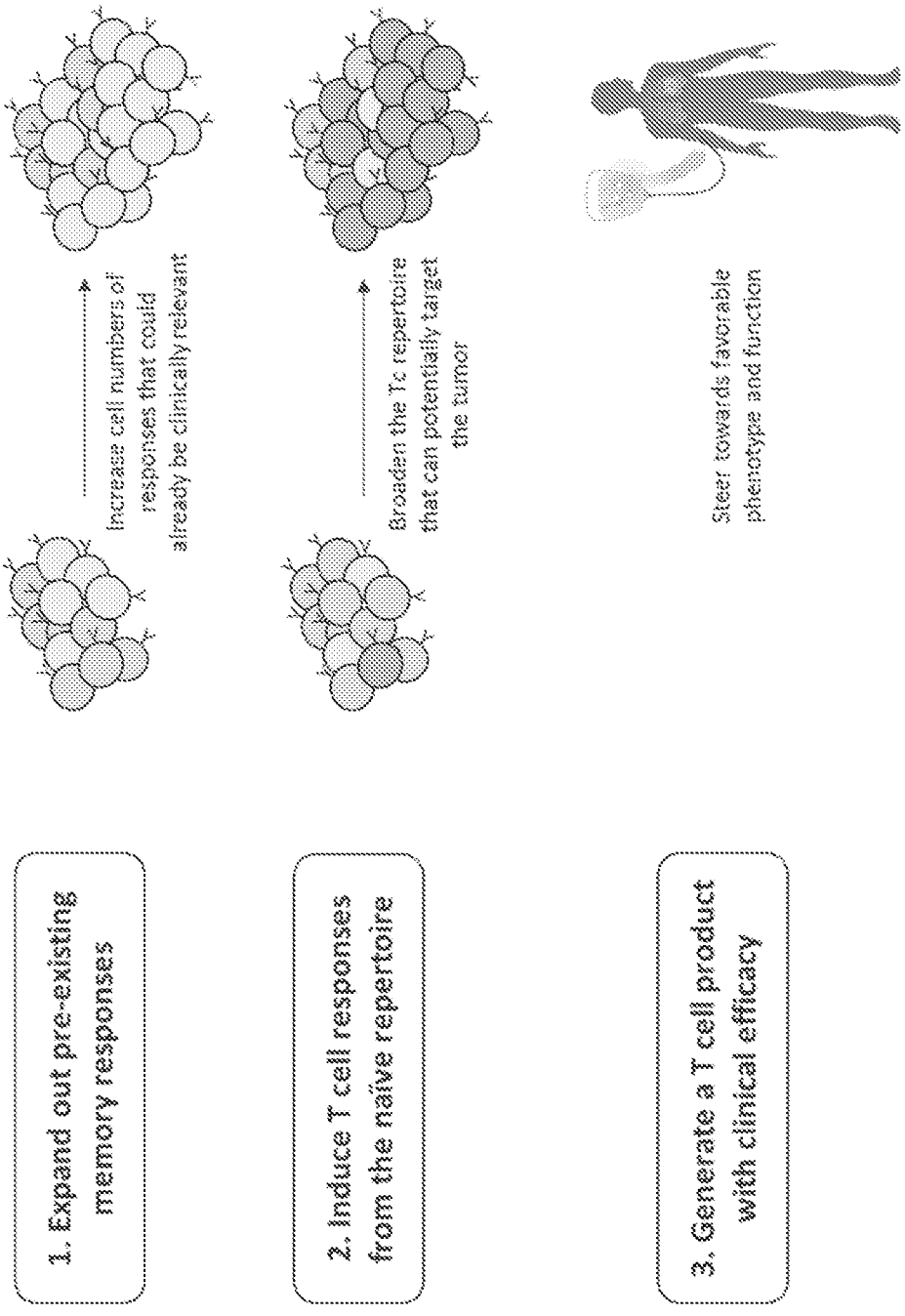

1. Expand out pre-existing memory responses
Increase cell numbers of responses that could already be clinically relevant
2. Induce T cell responses from the naïve repertoire
Broaden the Tc repertoire that can potentially target the tumor
3. Generate a T cell product with clinical efficacy
Steer towards favorable phenotype and function

FIG. 24

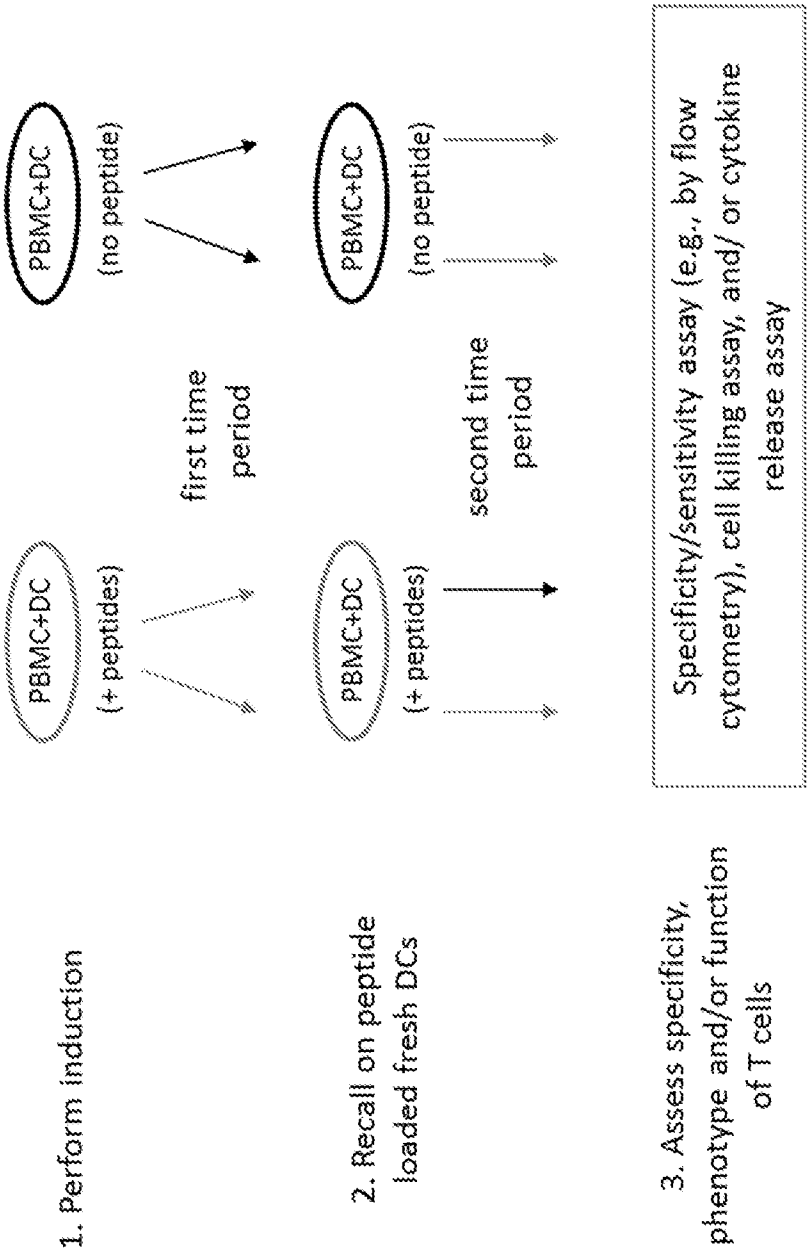

1. Perform induction
PBMC+DC
(+ peptides)
first time period
PBMC+DC
(no peptide)
2. Recall on peptide loaded fresh DCs
PBMC+DC
(+ peptides)
second time period
PBMC+DC
(no peptide)
3. Assess specificity, phenotype and/or function of T cells
Specificity/sensitivity assay (e.g., by flow cytometry), cell killing assay, and/ or cytokine release assay

FIG. 25

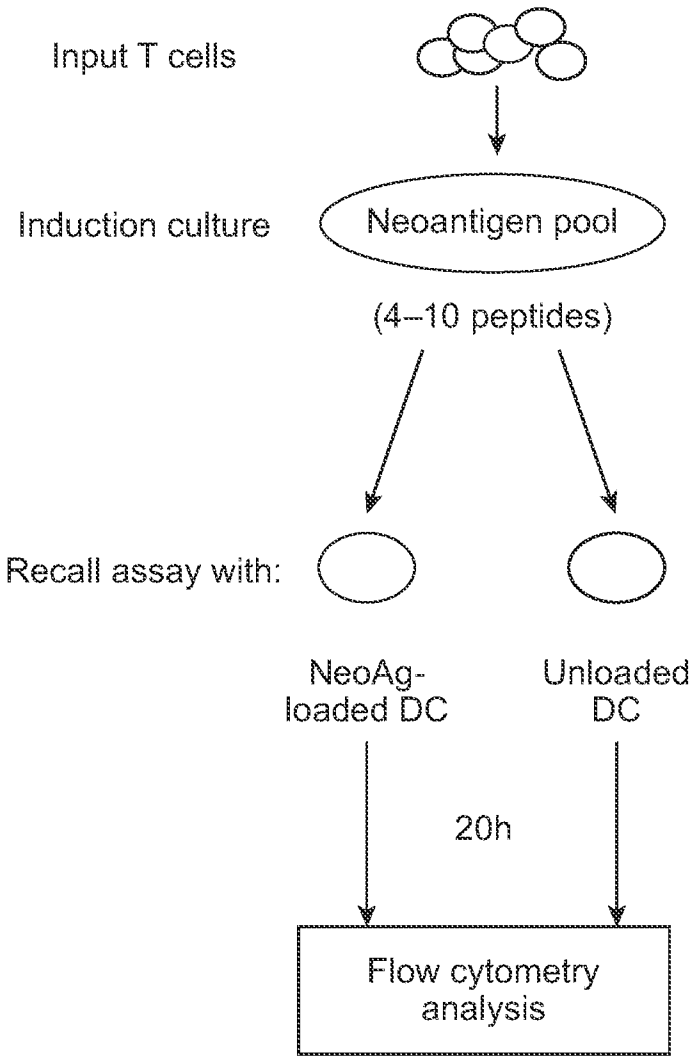

Input T cells
Induction culture
Neoantigen pool
(4–10 peptides)
Recall assay with:
NeoAg-loaded DC
Unloaded DC
20h
Flow cytometry analysis

FIG. 26

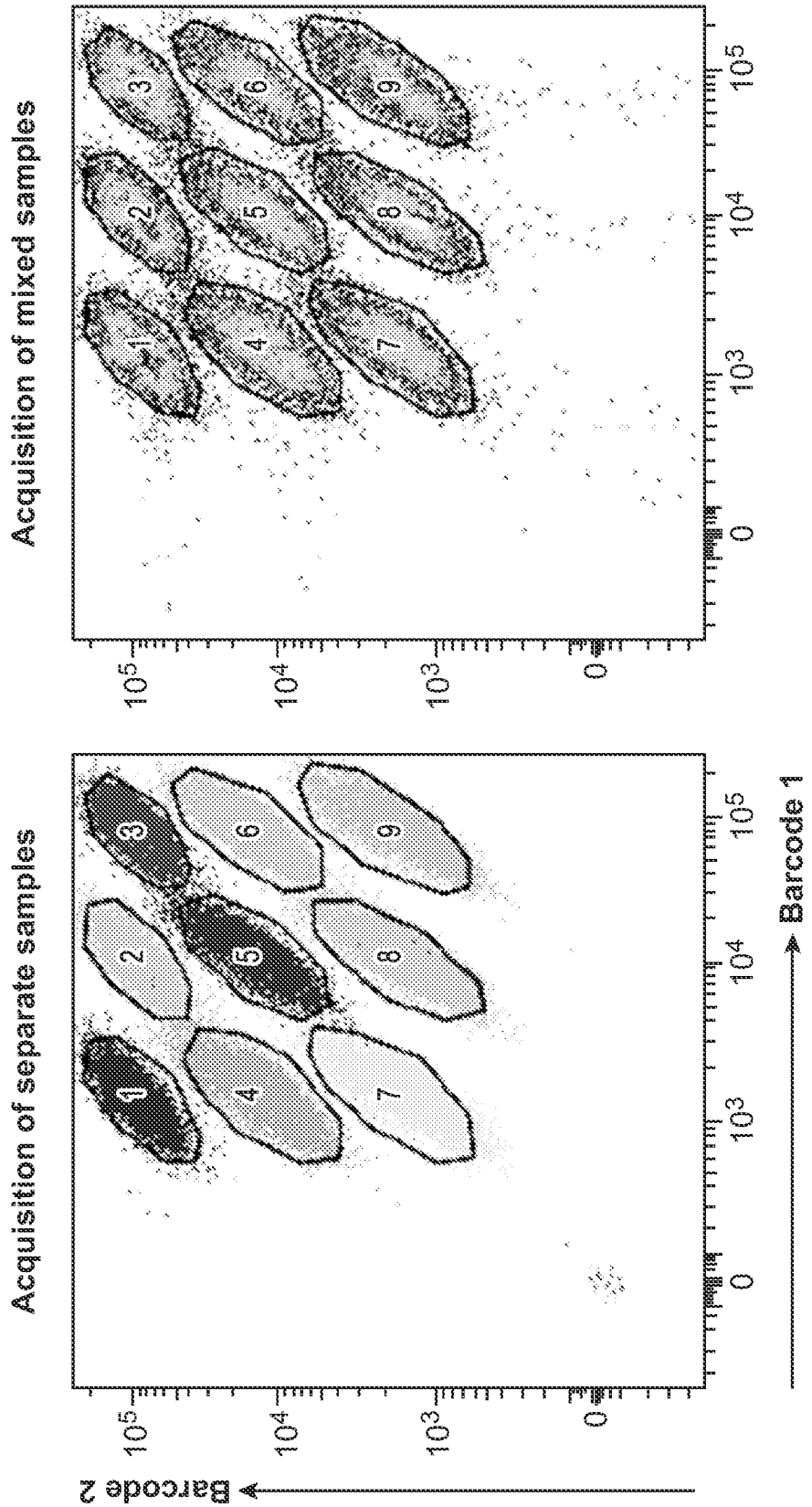

Acquisition of separate samples
Acquisition of mixed samples
Barcode 2
Barcode 1
0
$10^3$
$10^4$
$10^5$
1
2
3
4
5
6
7
8
9

FIG. 27A

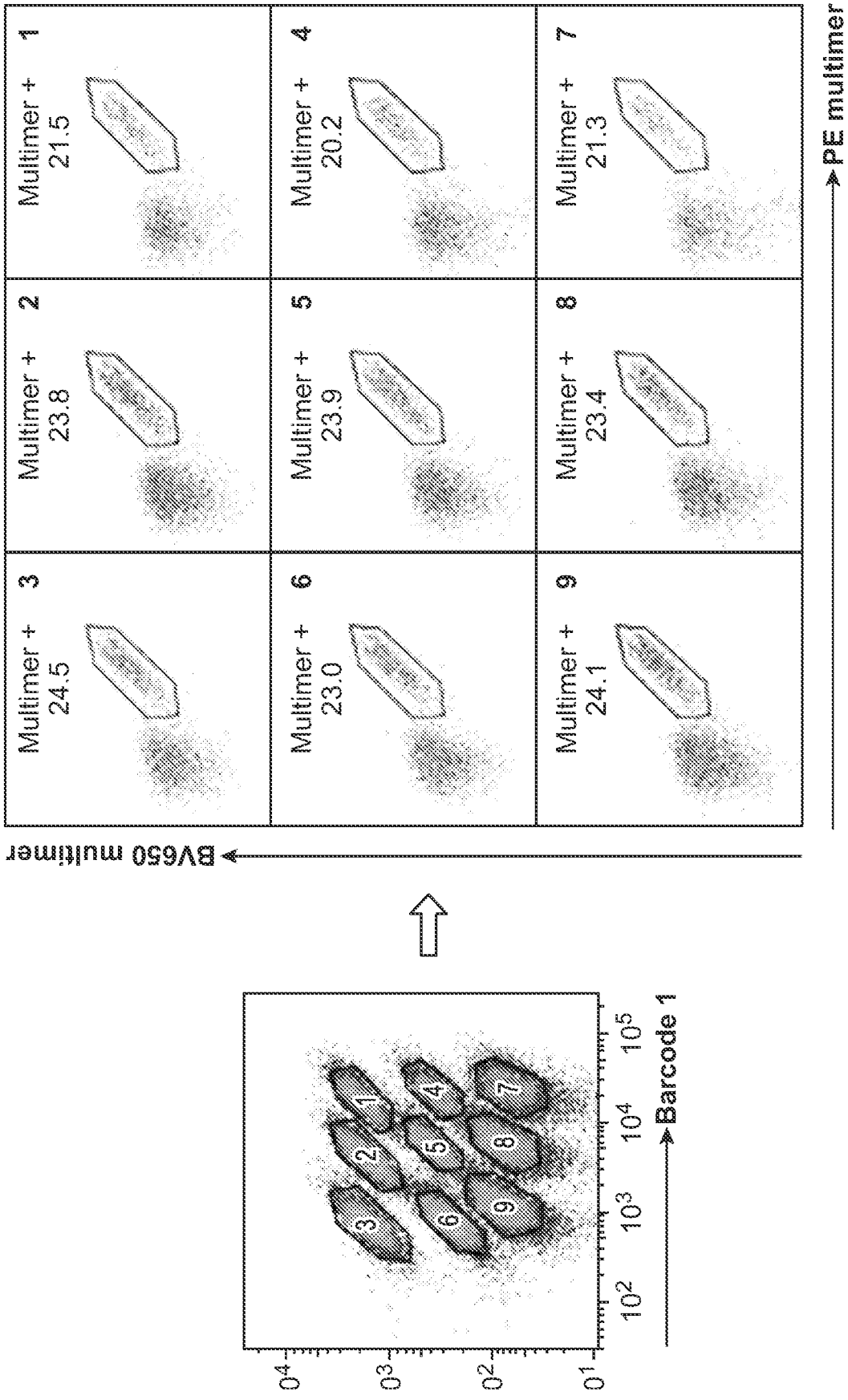

Multimer + 1
21.5
Multimer + 2
23.8
Multimer + 3
24.5
Multimer + 4
20.2
Multimer + 5
23.9
Multimer + 6
23.0
Multimer + 7
21.3
Multimer + 8
23.4
Multimer + 9
24.1
BV650 multimer
PE multimer
Barcode 1
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$

FIG. 27B

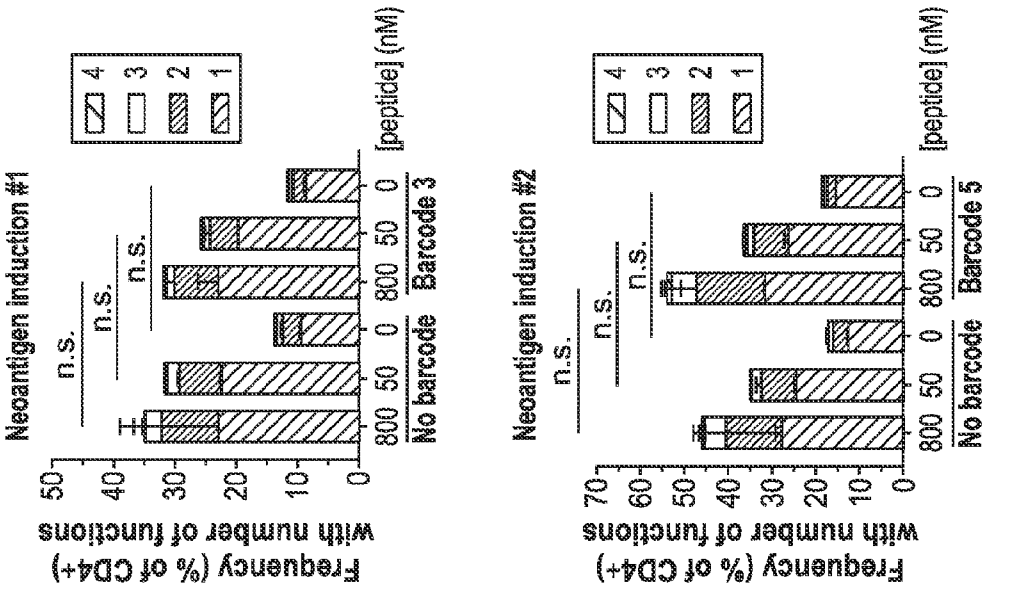



FIG. 28B

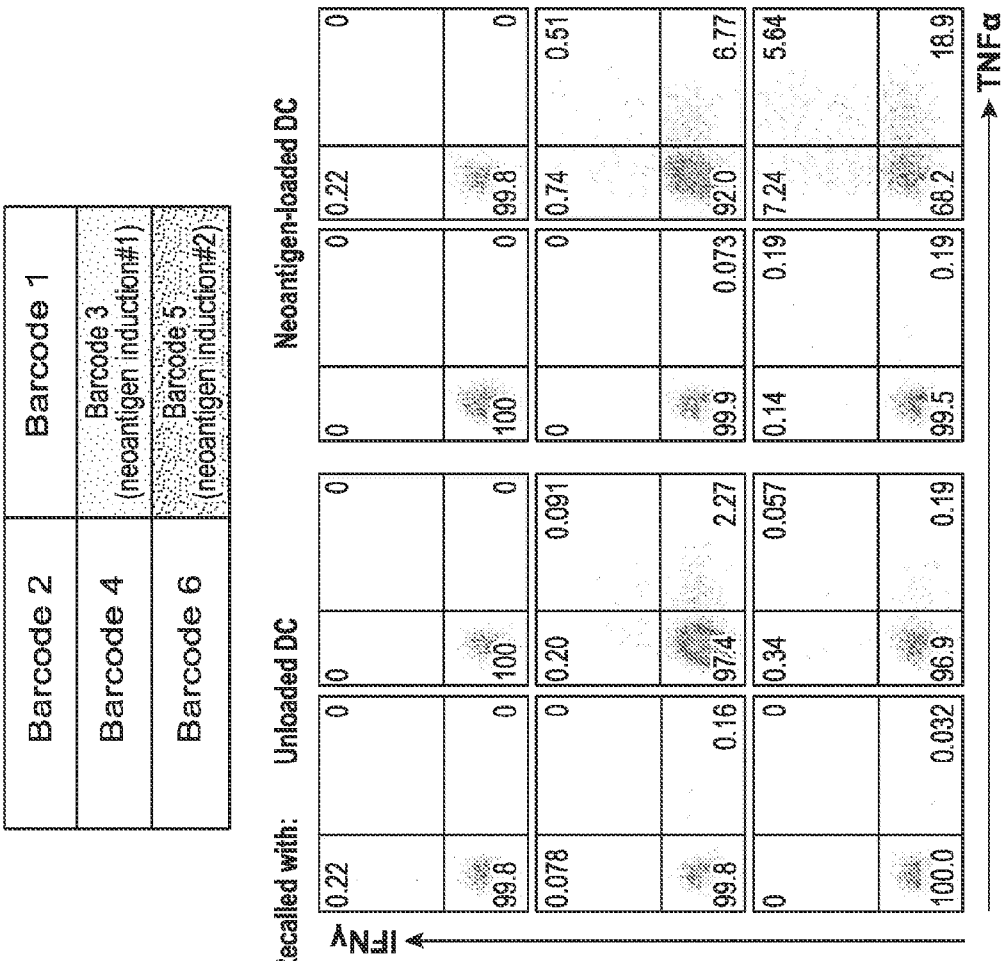



FIG. 28A

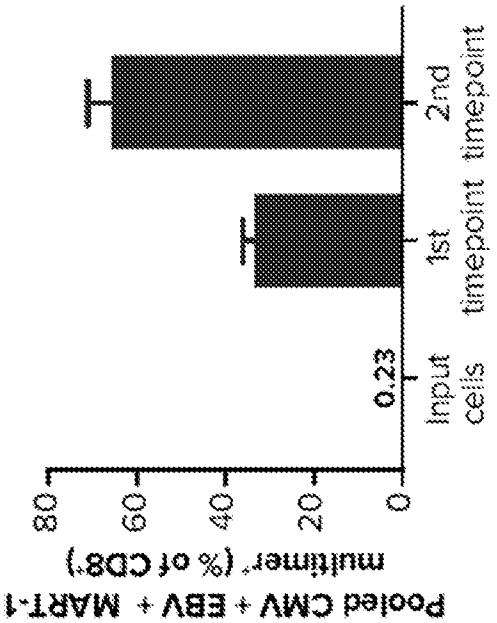



FIG. 29A

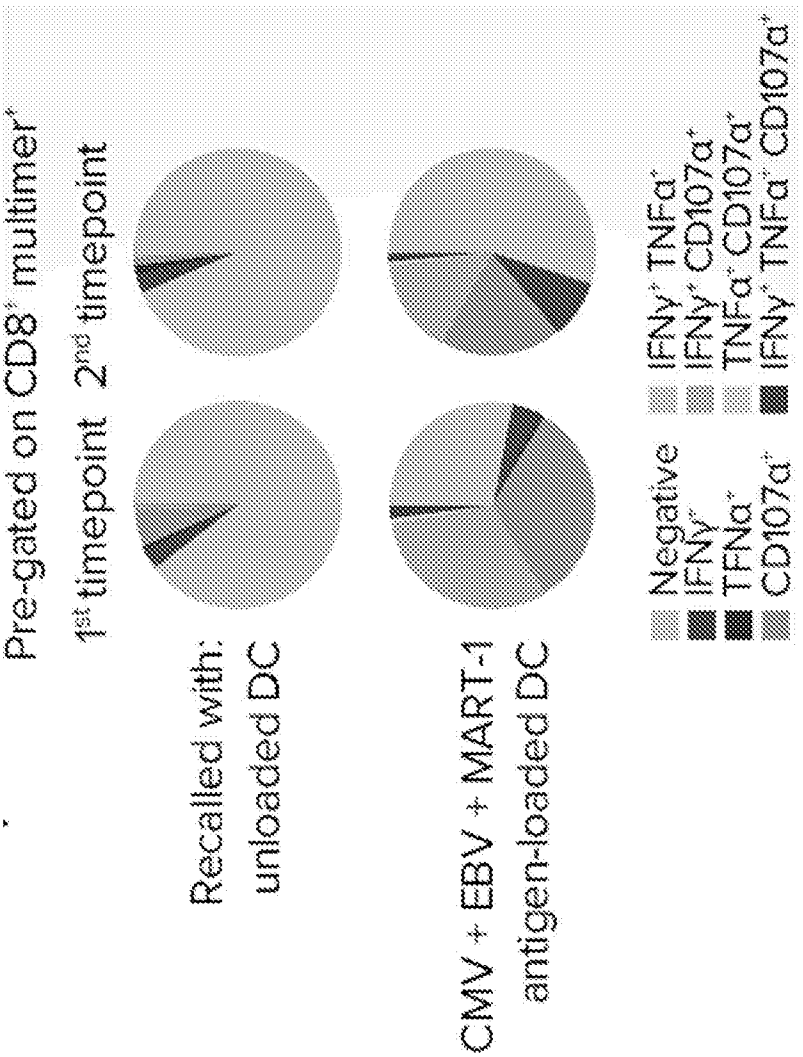



FIG. 29B

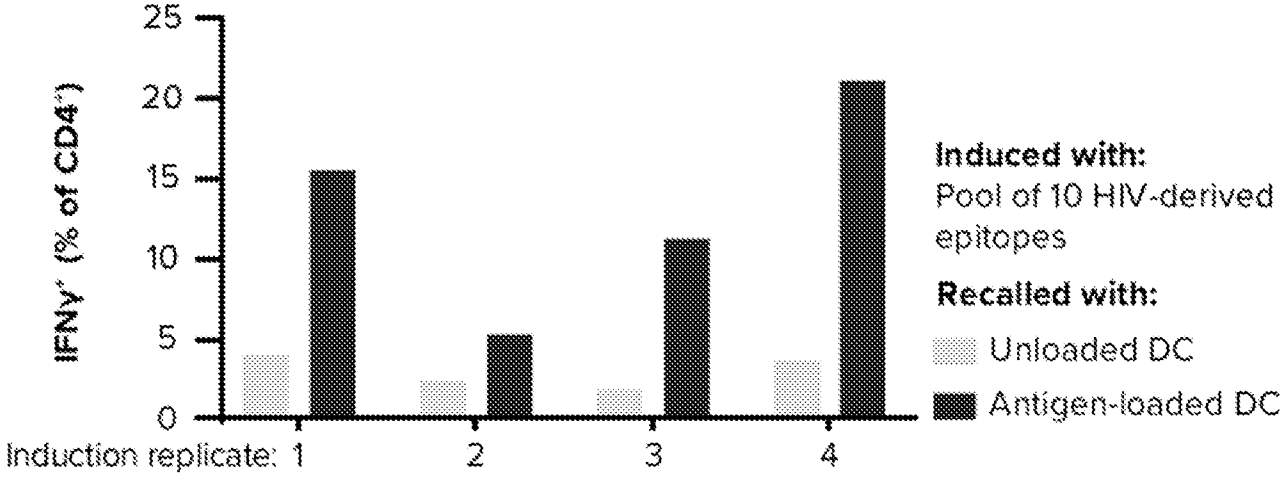



FIG. 30A

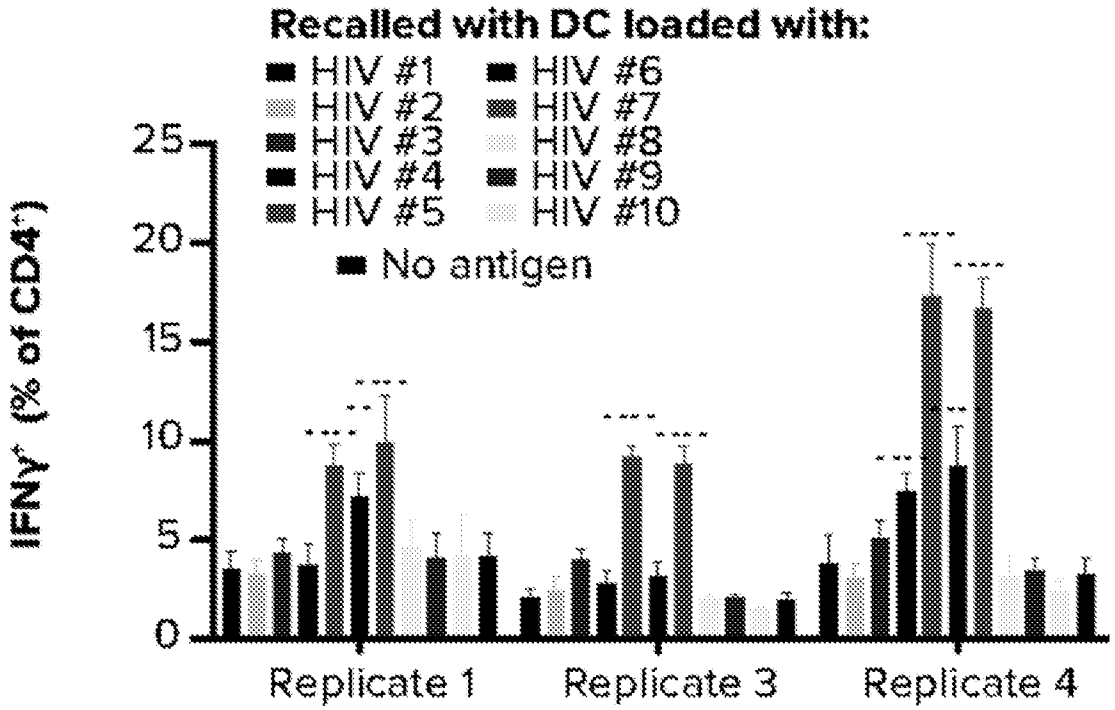



FIG. 30B

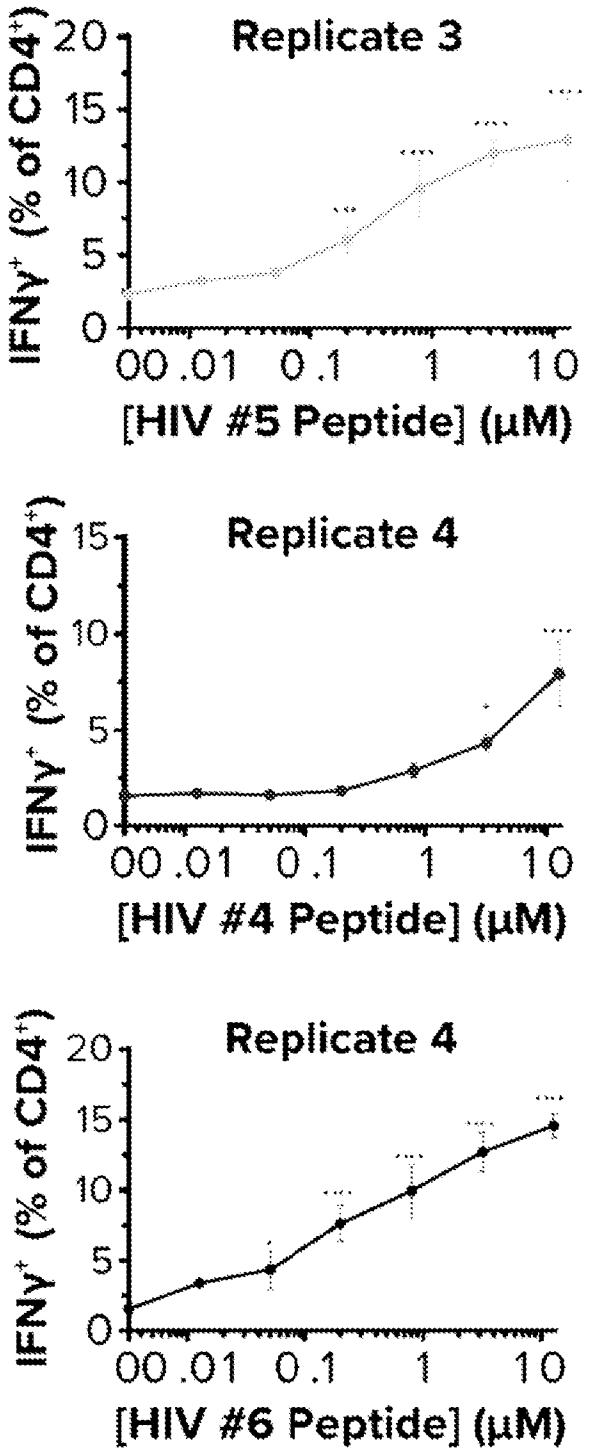

Replicate 3
IFNγ+ (% of CD4+)
20
15
10
5
0
00 .01 0.1 1 10
[HIV #5 Peptide] (μM)
Replicate 4
IFNγ+ (% of CD4+)
15
10
5
0
00 .01 0.1 1 10
[HIV #4 Peptide] (μM)
Replicate 4
IFNγ+ (% of CD4+)
20
15
10
5
0
00 .01 0.1 1 10
[HIV #6 Peptide] (μM)

FIG. 30C

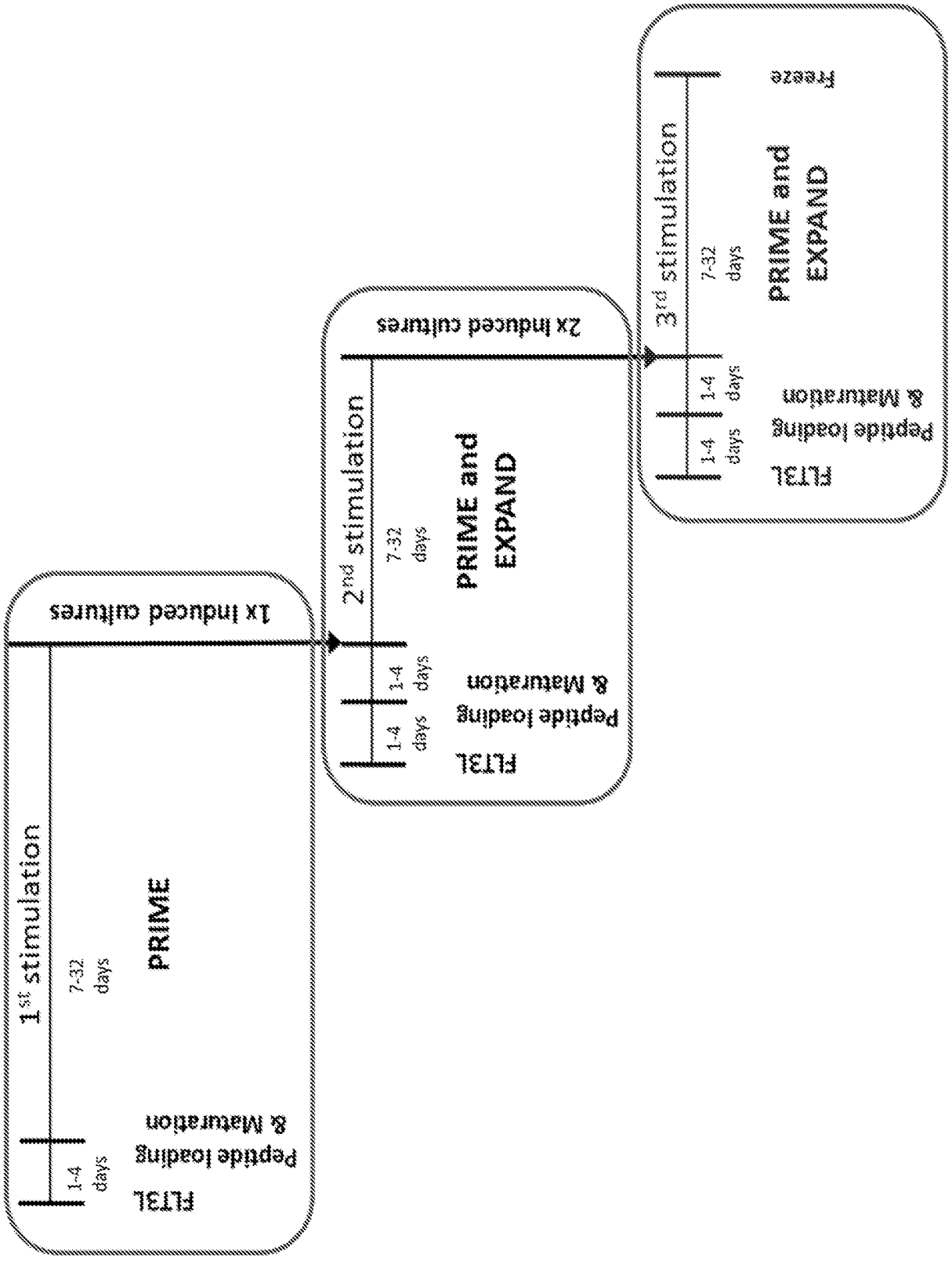

1x induced cultures
1st stimulation
7-32 days
PRIME
FLT3L
Peptide loading & Maturation
1-4 days
2x induced cultures
2nd stimulation
7-32 days
PRIME and EXPAND
FLT3L
Peptide loading & Maturation
1-4 days
3rd stimulation
7-32 days
PRIME and EXPAND
FLT3L
Peptide loading & Maturation
1-4 days
Freeze

FIG. 31

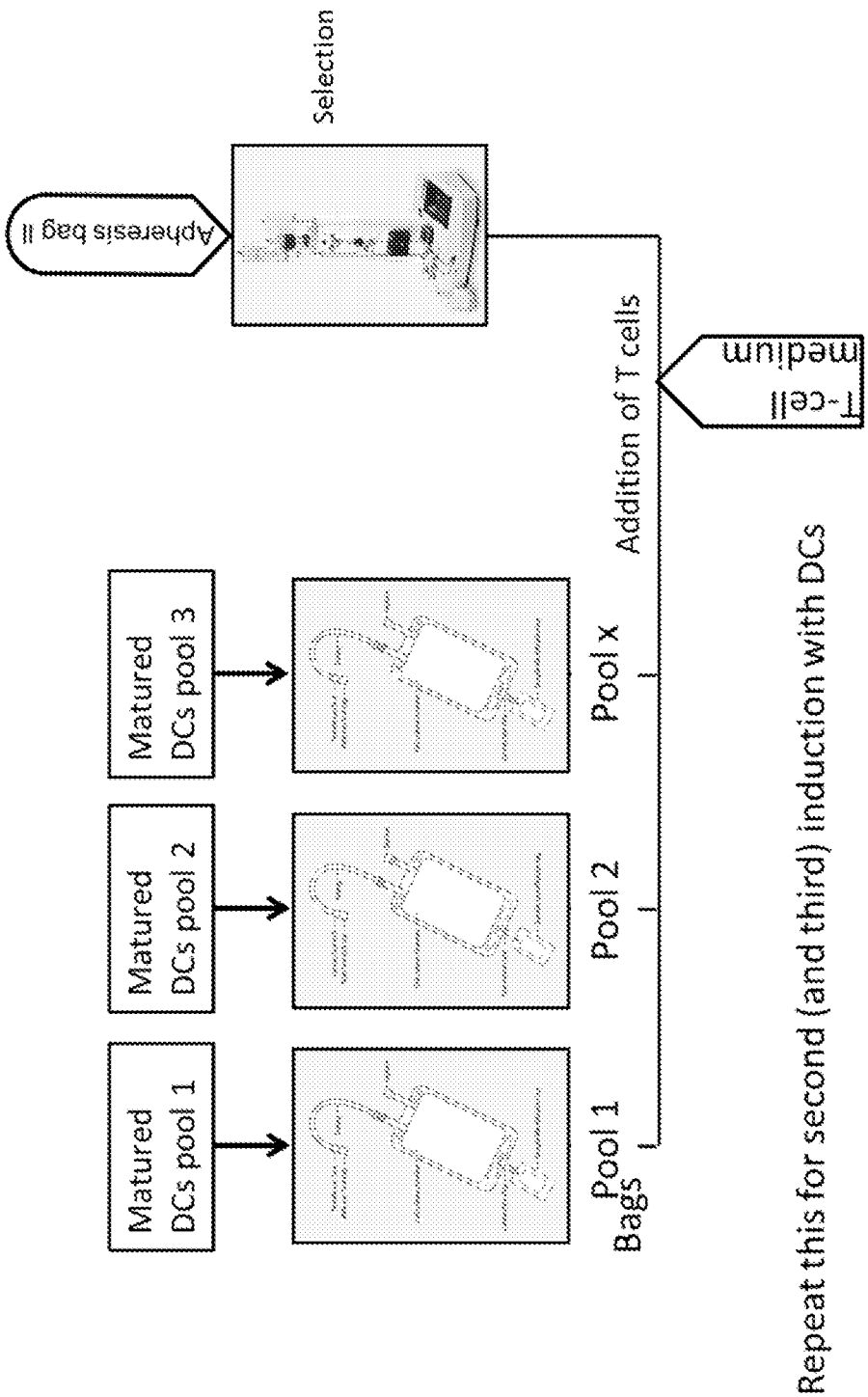

Aphaeresis bag
Selection
T-cell medium
Addition of T cells
Matured DCs pool 1
Matured DCs pool 2
Matured DCs pool 3
Pool 1
Pool 2
Pool x
Bags
Repeat this for second (and third) induction with DCs

FIG. 32

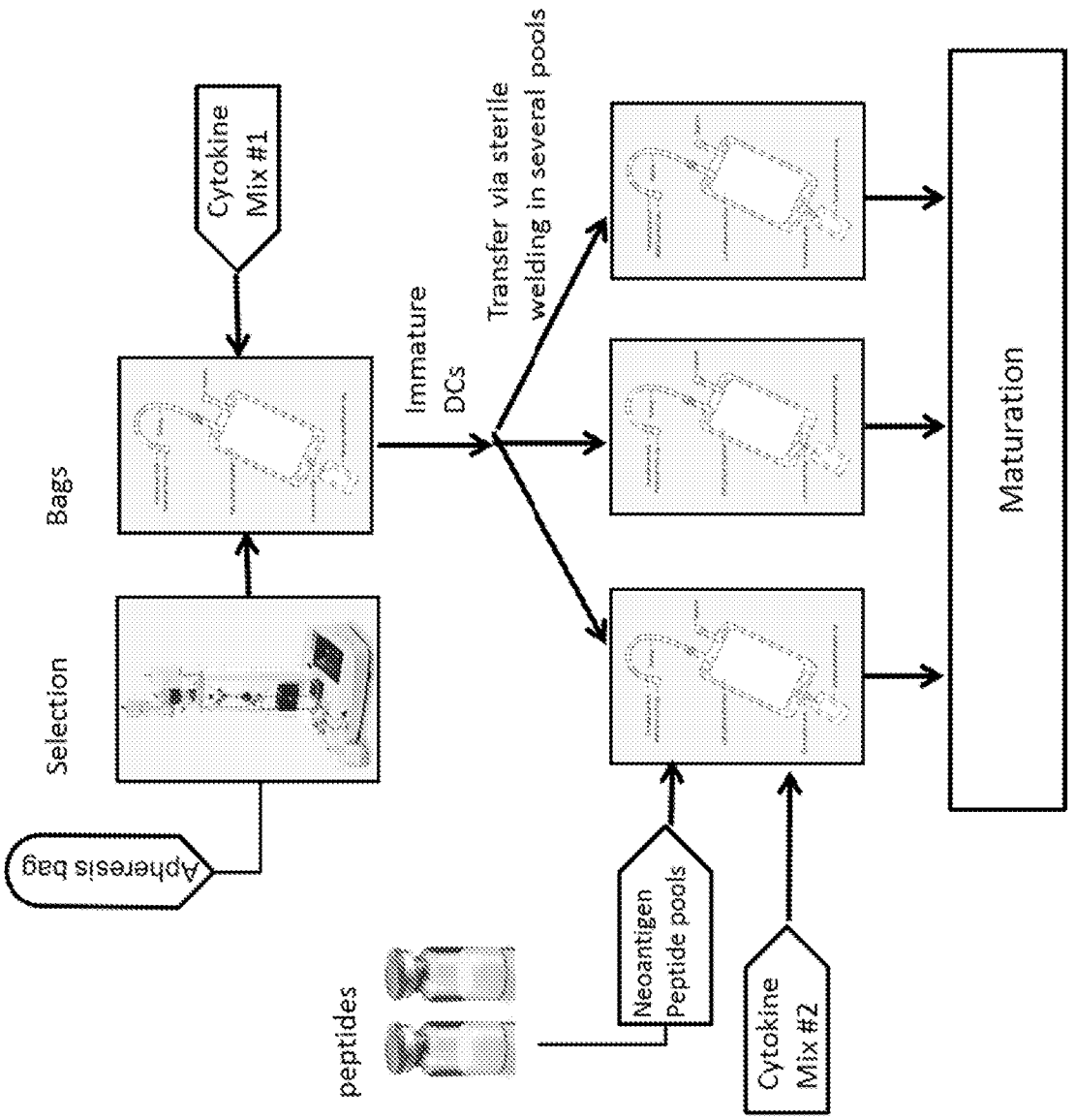

Apheresis bag
Selection
Bags
Cytokine Mix #1
Immature DCs
Transfer via sterile welding in several pools
peptides
Neoantigen Peptide pools
Cytokine Mix #2
Maturation

FIG. 33

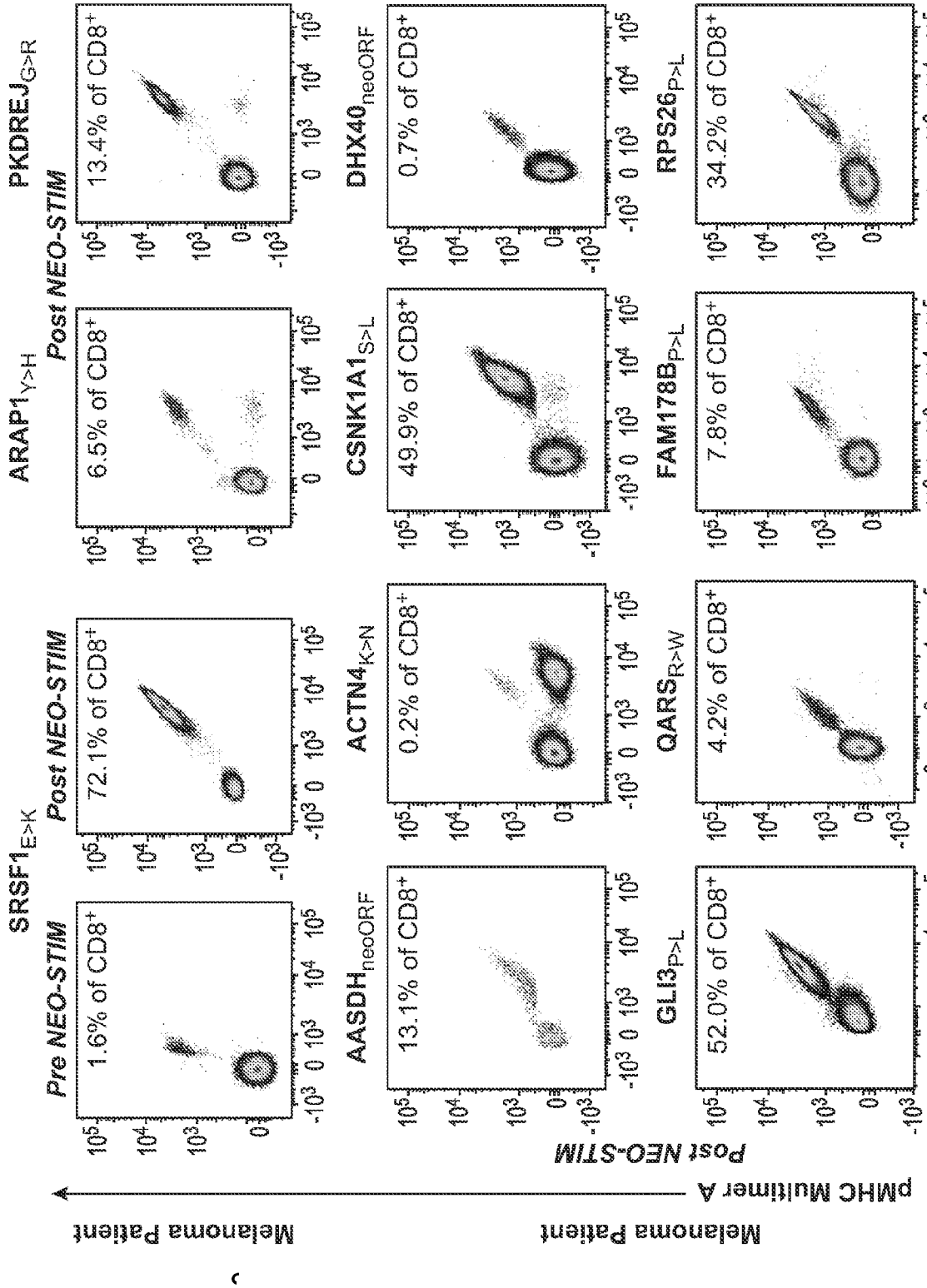

SRSF1E>K
ARAP1Y>H
PKDREJG>R
Pre NEO-STIM
Post NEO-STIM
Post NEO-STIM
1.6% of CD8+
72.1% of CD8+
6.5% of CD8+
13.4% of CD8+
AASDHneoORF
ACTN4K>N
CSNK1A1S>L
DHX40neoORF
13.1% of CD8+
0.2% of CD8+
49.9% of CD8+
0.7% of CD8+
GLI3P>L
QARSR>W
FAM178BP>L
RPS26P>L
52.0% of CD8+
4.2% of CD8+
7.8% of CD8+
34.2% of CD8+
Melanoma Patient
Melanoma Patient
pMHC Multimer A
Post NEO-STIM

FIG. 34

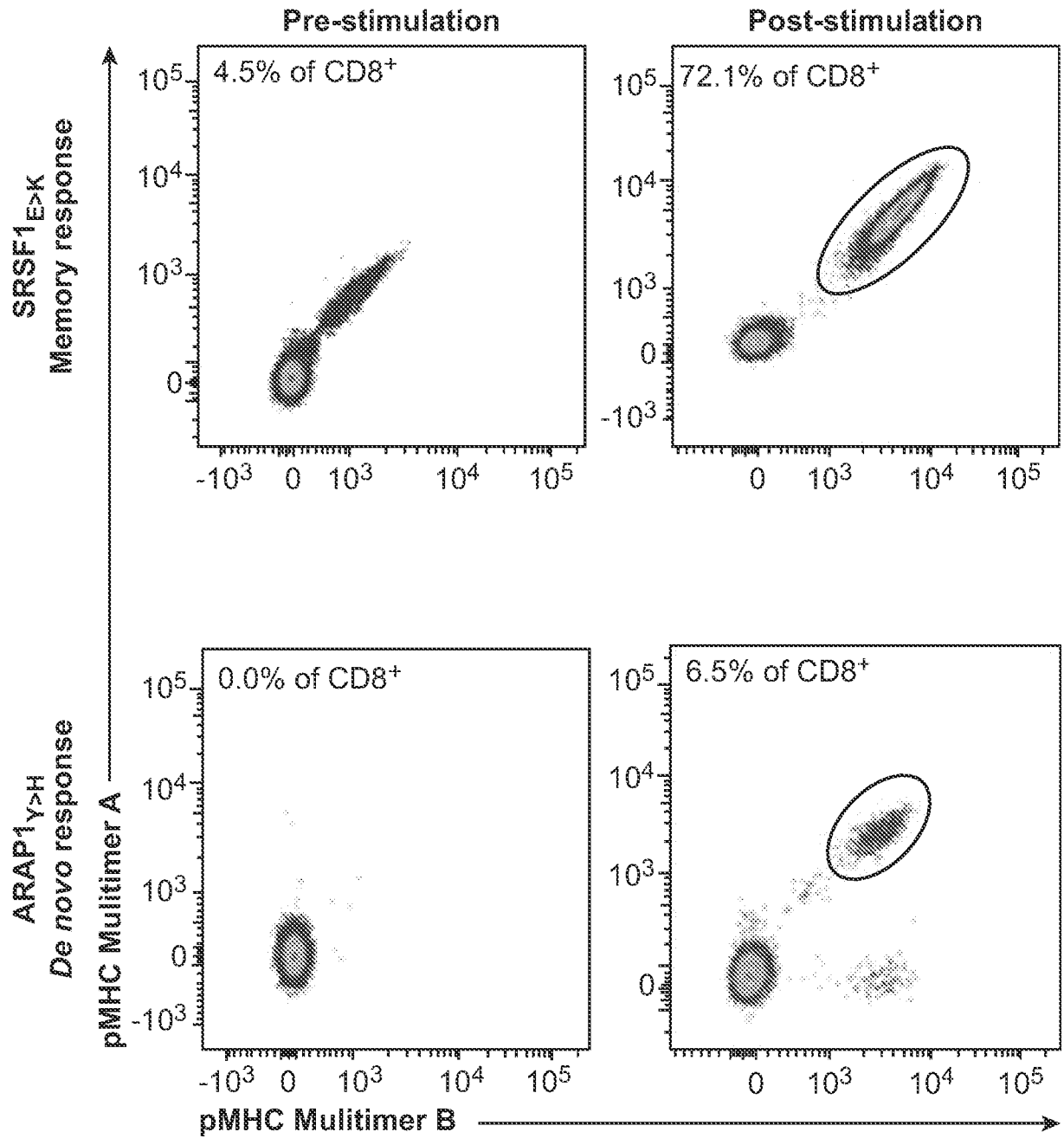

Pre-stimulation
Post-stimulation
4.5% of CD8+
72.1% of CD8+
0.0% of CD8+
6.5% of CD8+
SRSF1E>K
Memory response
ARAP1Y>H
De novo response
pMHC Mulitimer A
pMHC Mulitimer B
10^5
10^4
10^3
0
-10^3

FIG. 35

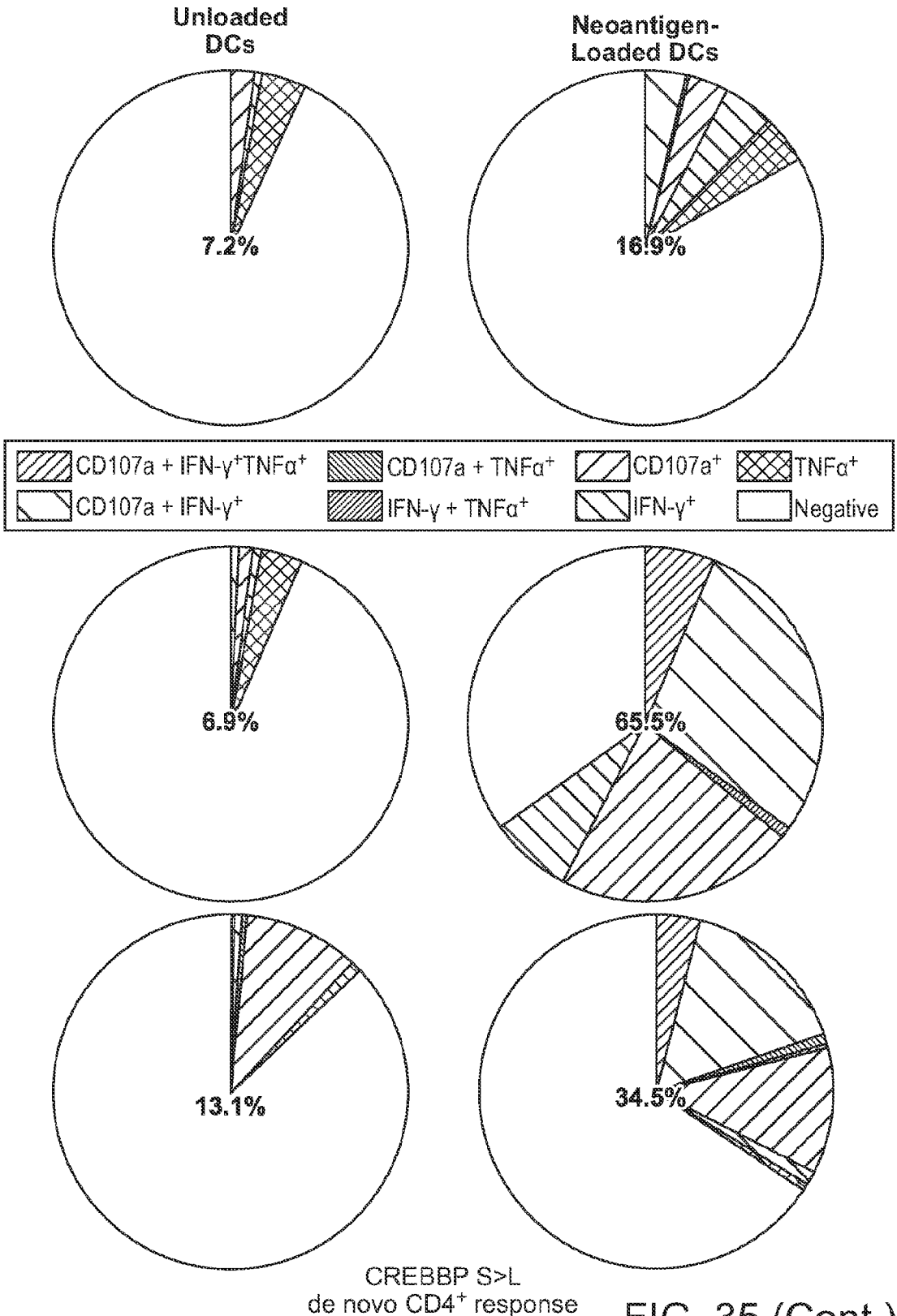

Unloaded DCs
Neoantigen-Loaded DCs
7.2%
16.9%
CD107a + IFN-γ+TNFα+
CD107a + TNFα+
CD107a+
TNFα+
CD107a + IFN-γ+
IFN-γ + TNFα+
IFN-γ+
Negative
6.9%
65.5%
13.1%
34.5%
CREBBP S>L
de novo CD4+ response

FIG. 35 (Cont.)

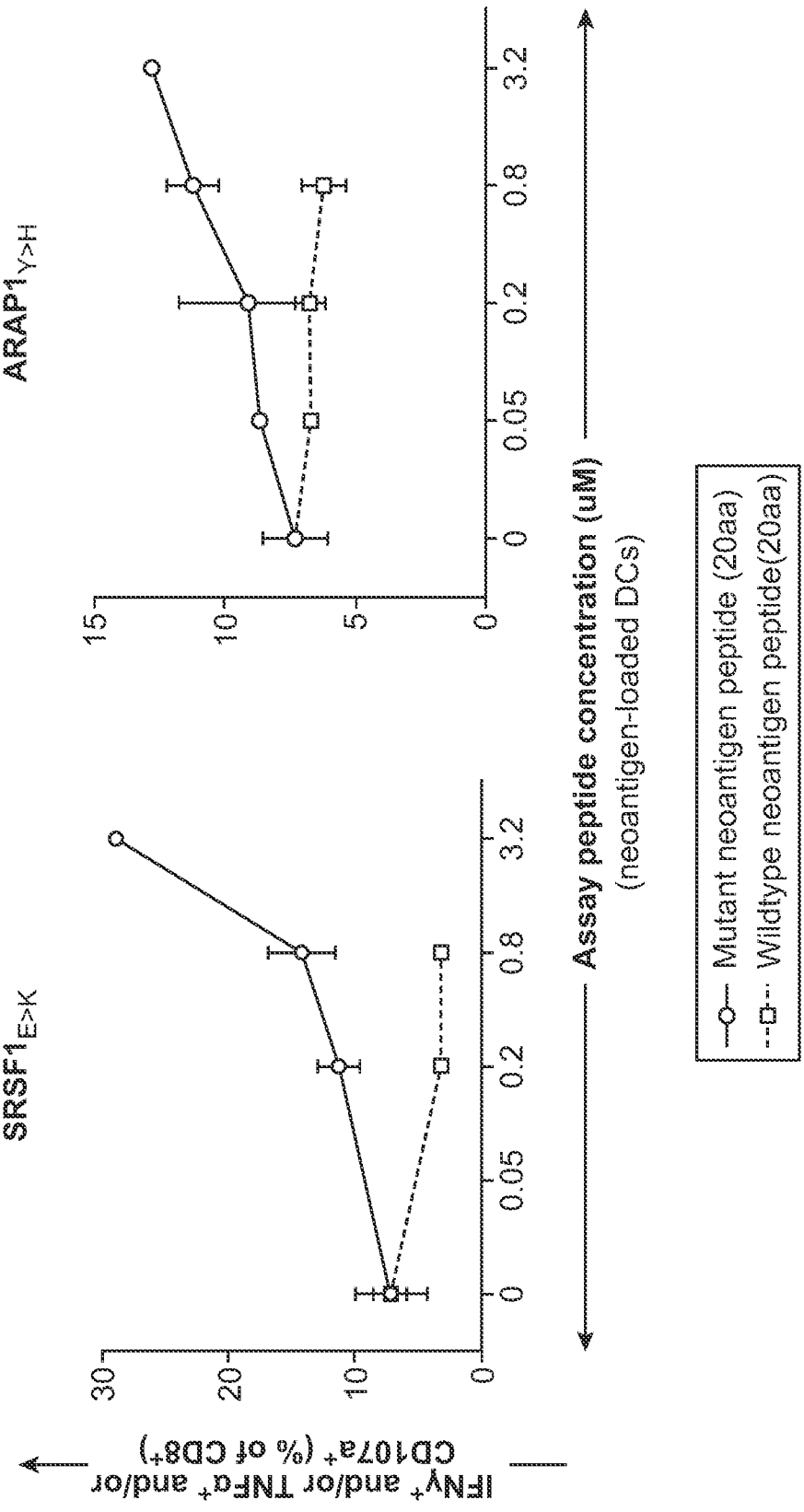

SRSF1E>K
ARAP1Y>H
IFNγ+ and/or TNFα+ and/or CD107a+ (% of CD8+)
Assay peptide concentration (uM)
(neoantigen-loaded DCs)
Mutant neoantigen peptide (20aa)
Wildtype neoantigen peptide(20aa)
0
10
20
30
5
15
0.05
0.2
0.8
3.2

FIG. 36

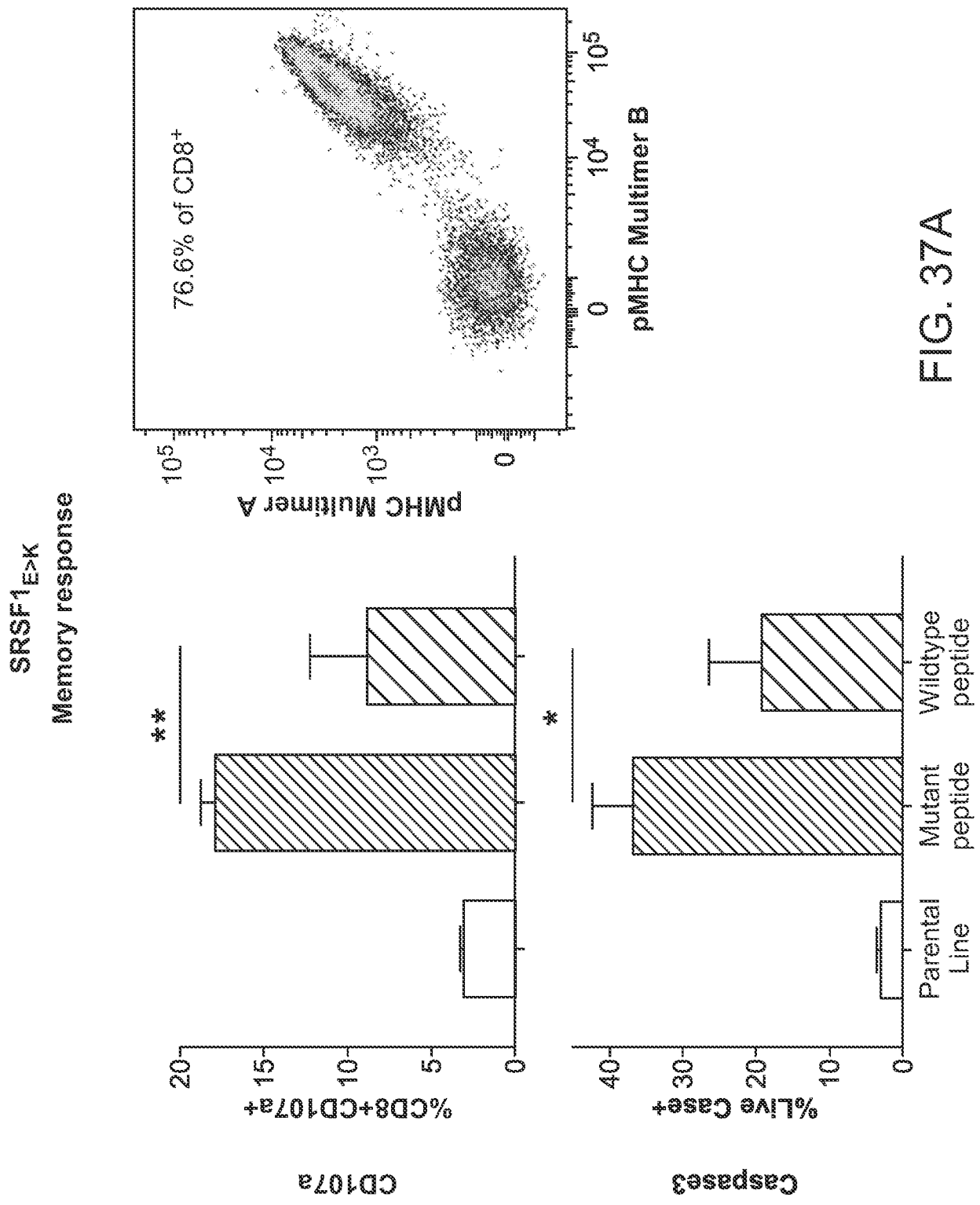

SRSF1E>K
Memory response
76.6% of CD8+
pMHC Multimer A
pMHC Multimer B
CD107a
%CD8+CD107a+
Caspase3
%Live Case+
Parental Line
Mutant peptide
Wildtype peptide

FIG. 37A

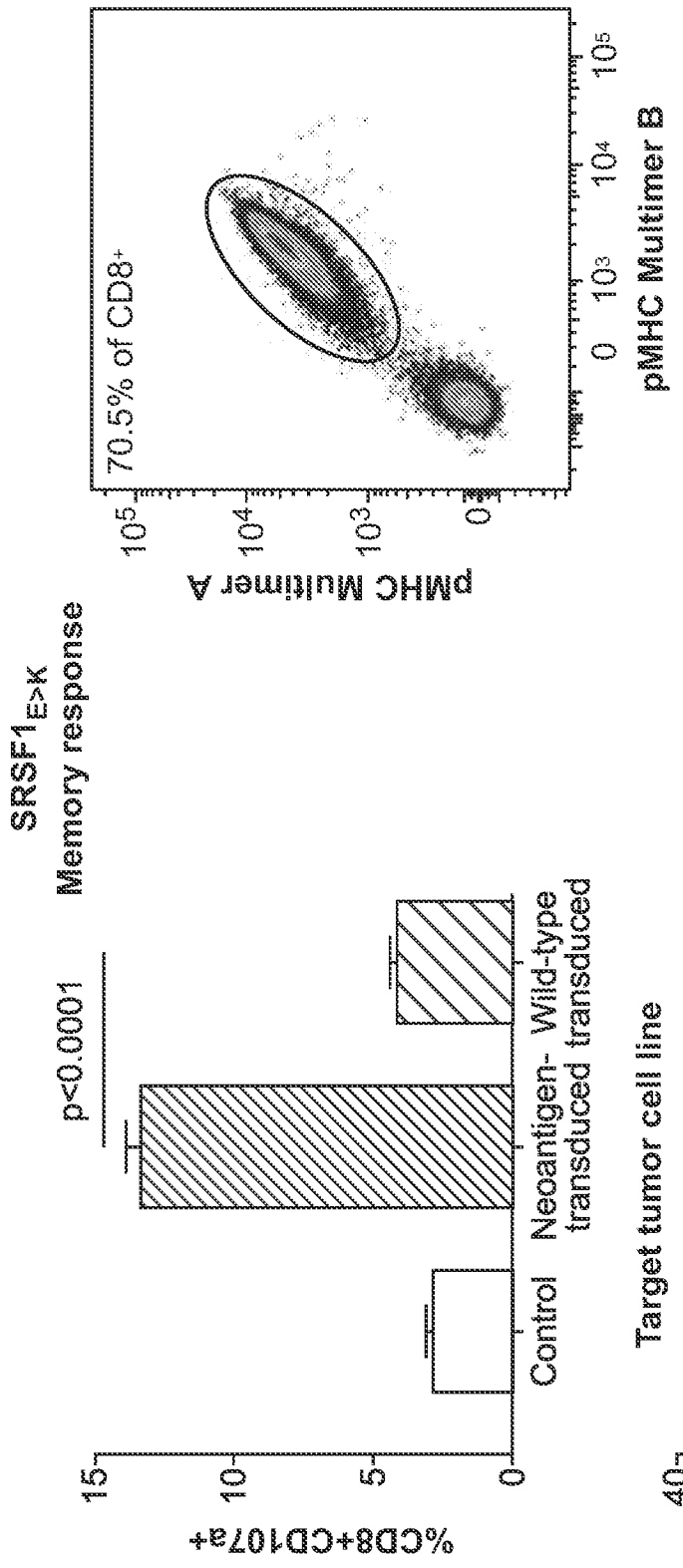

SRSF1E>K
Memory response
p<0.0001
%CD8+CD107a+
Control
Neoantigen-transduced
Wild-type transduced
Target tumor cell line
70.5% of CD8+
pMHC Multimer A
pMHC Multimer B
p<0.0001
%Live+Cas3+
Control
Neoantigen-transduced
Wild-type transduced
Target tumor cell line

FIG. 37B

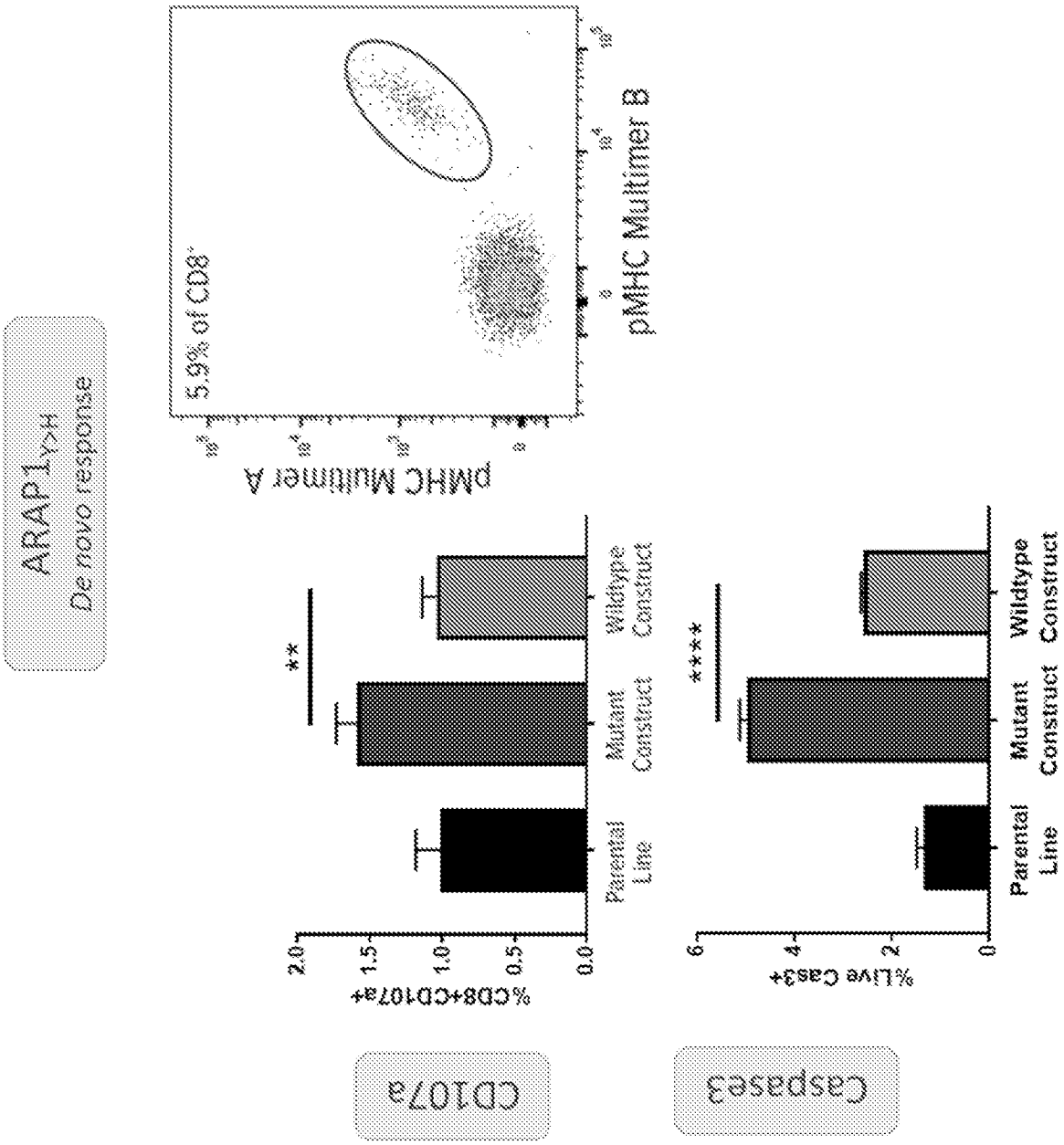

ARAP1Y>H
De novo response
5.9% of CD8+
pMHC Multimer A
pMHC Multimer B
CD107a
%CD8+CD107a+
Parental Line
Mutant Construct
Wildtype Construct
**
Caspase3
%Live Cas3+
****

FIG. 37C

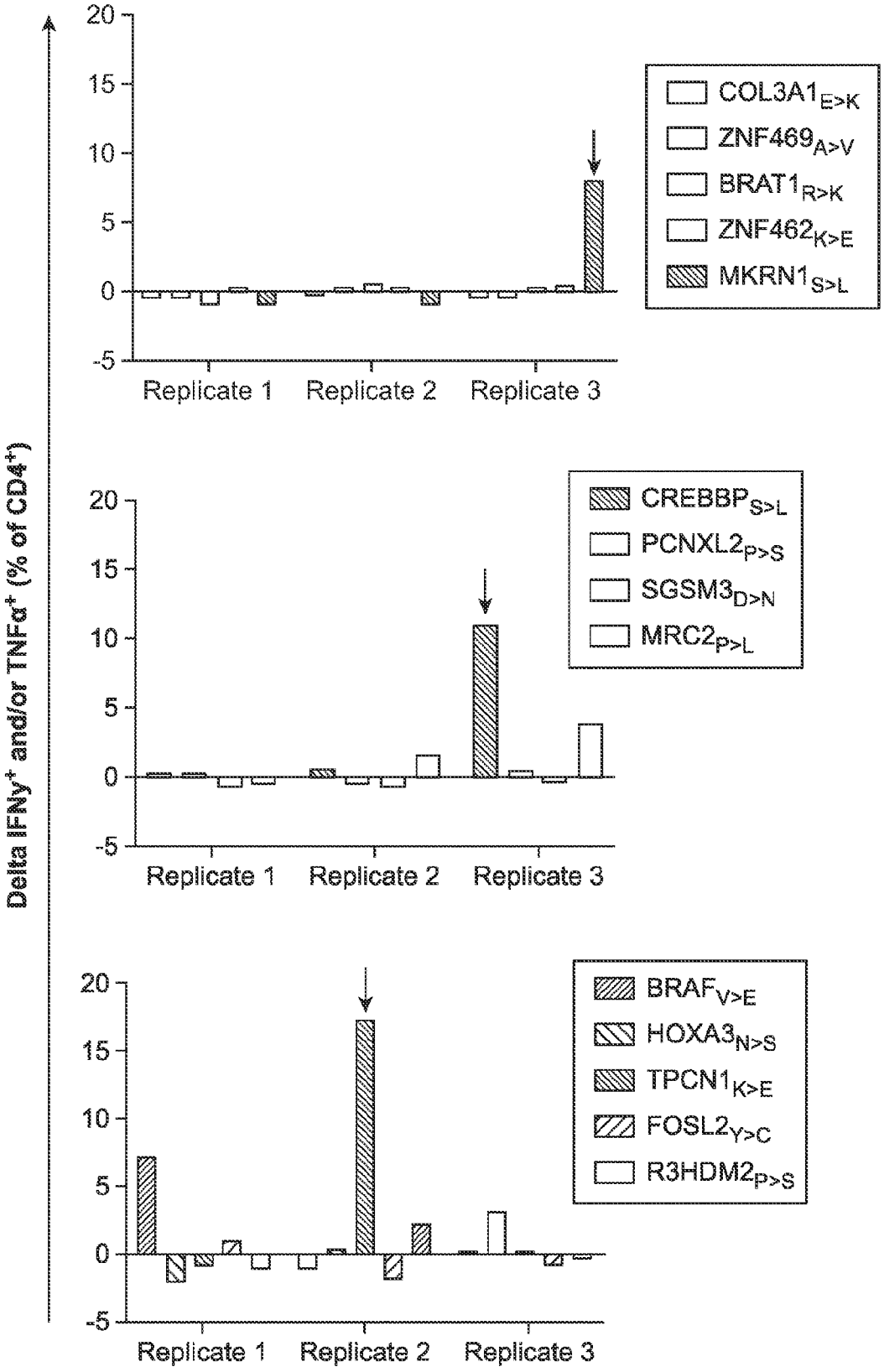

Delta IFNγ+ and/or TNFα+ (% of CD4+)
20
15
10
5
0
-5
Replicate 1
Replicate 2
Replicate 3
COL3A1E>K
ZNF469A>V
BRAT1R>K
ZNF462K>E
MKRN1S>L
CREBBPS>L
PCNXL2P>S
SGSM3D>N
MRC2P>L
BRAFV>E
HOXA3N>S
TPCN1K>E
FOSL2Y>C
R3HDM2P>S

FIG. 38A

SU

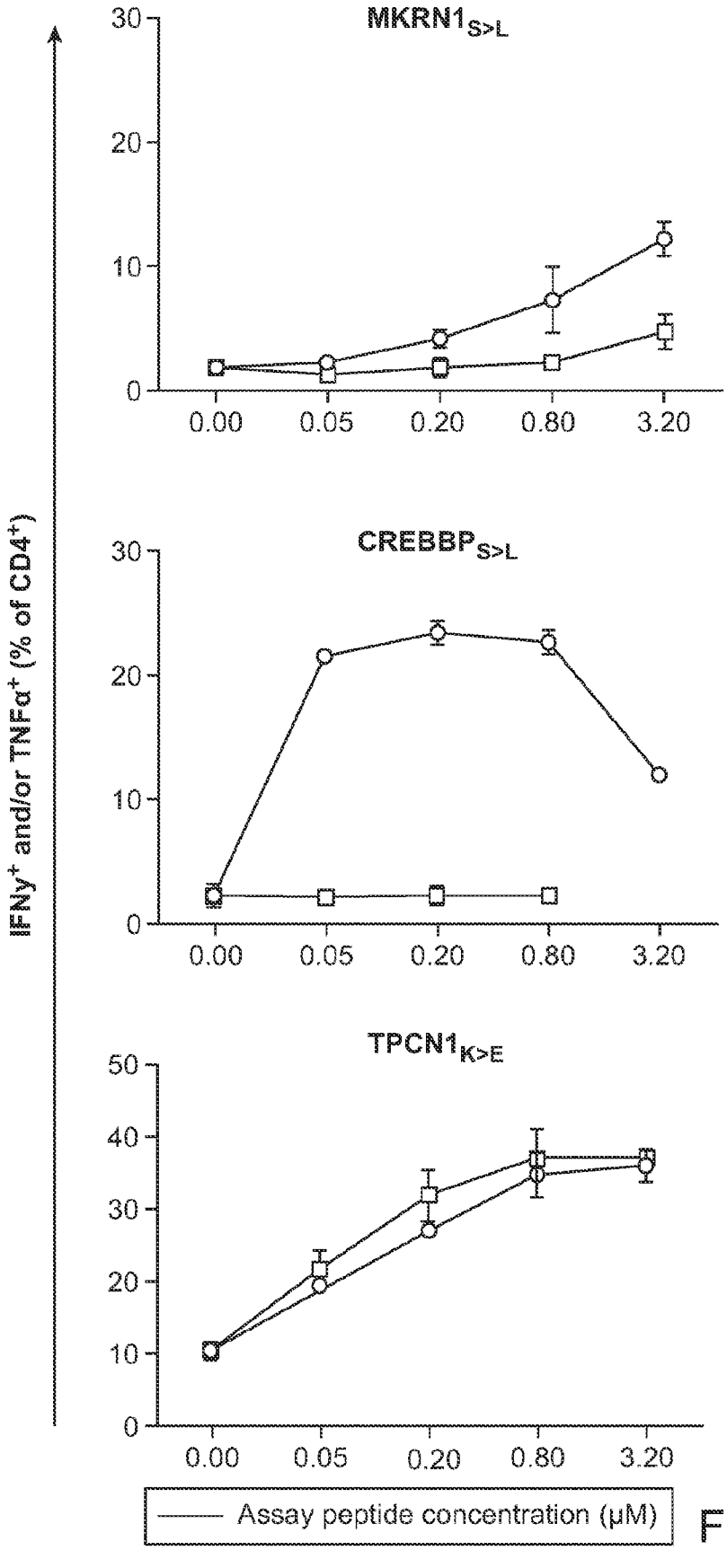

MKRN1S>L
CREBBPS>L
TPCN1K>E
IFNγ+ and/or TNFα+ (% of CD4+)
30
20
10
0
50
40
0.00
0.05
0.20
0.80
3.20
Assay peptide concentration (μM)

FIG. 38B

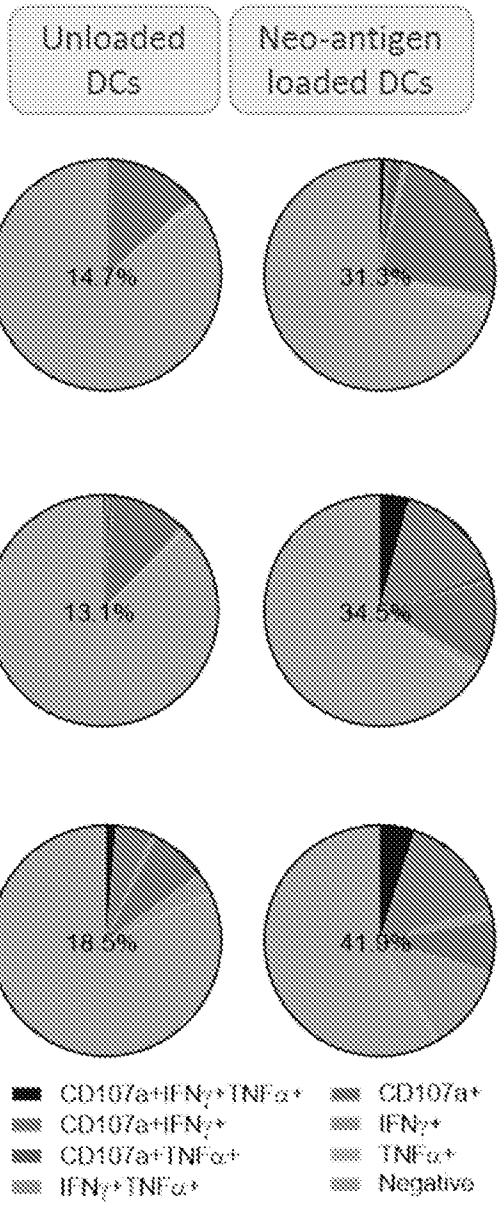

Unloaded DCs
Neo-antigen loaded DCs
14.7%
31.3%
13.1%
34.5%
18.5%
41.3%
CD107a+IFNγ+TNFα+
CD107a+IFNγ+
CD107a+TNFα+
IFNγ+TNFα+
CD107a+
IFNγ+
TNFα+
Negative

FIG. 38C

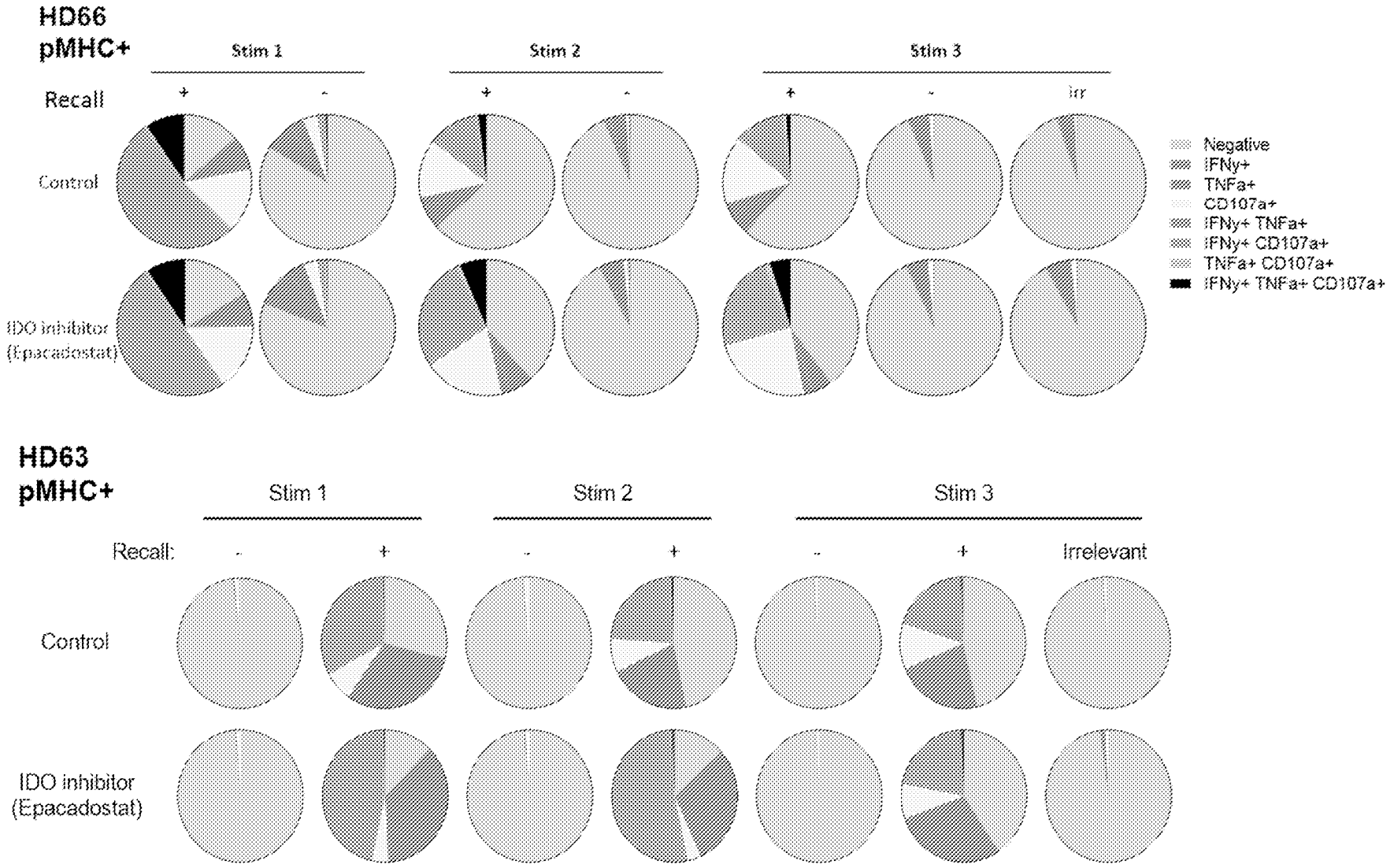

HD66
pMHC+
Stim 1
Stim 2
Stim 3
Recall
+
-
Irr
Control
IDO inhibitor
(Epacadostat)
Negative
IFNγ+
TNFa+
CD107a+
IFNγ+ TNFa+
IFNγ+ CD107a+
TNFa+ CD107a+
IFNγ+ TNFa+ CD107a+
HD63
pMHC+
Stim 1
Stim 2
Stim 3
Recall:
-
+
Irrelevant
Control
IDO inhibitor
(Epacadostat)

FIG. 39

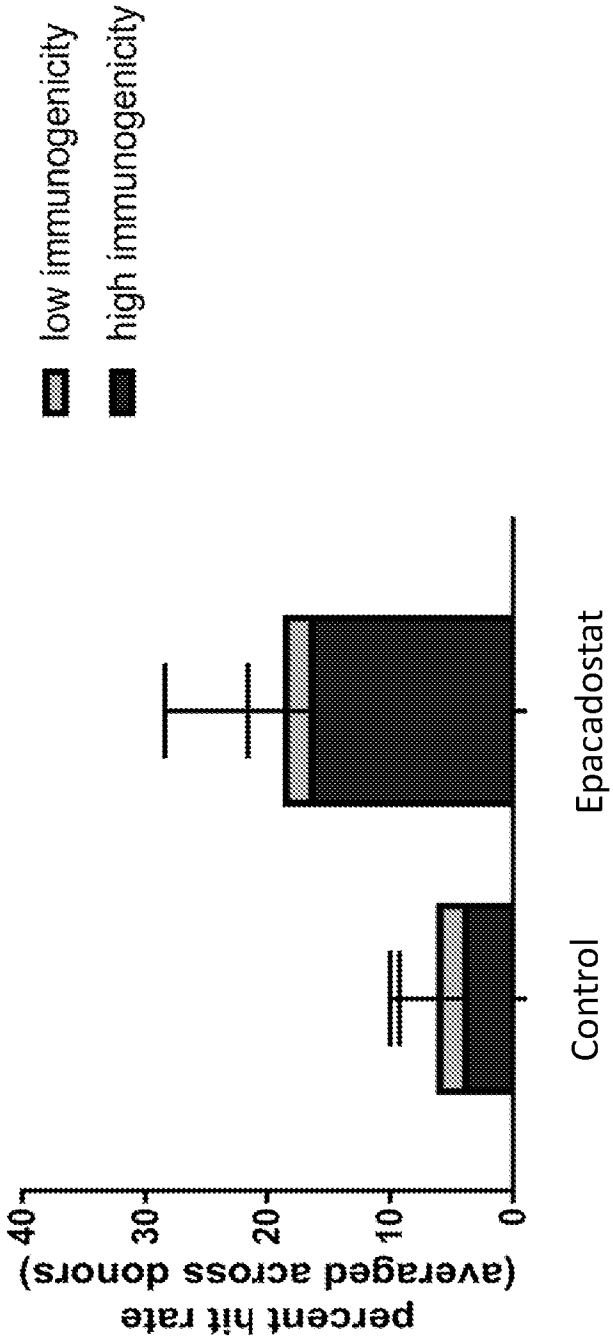

low immunogenicity
high immunogenicity
percent hit rate
(averaged across donors)
40
30
20
10
0
Control
Epacadostat

FIG. 40

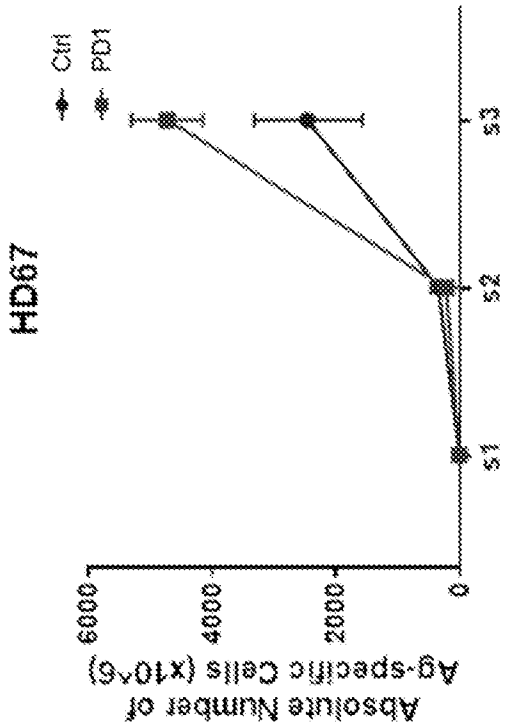



FIG. 41B

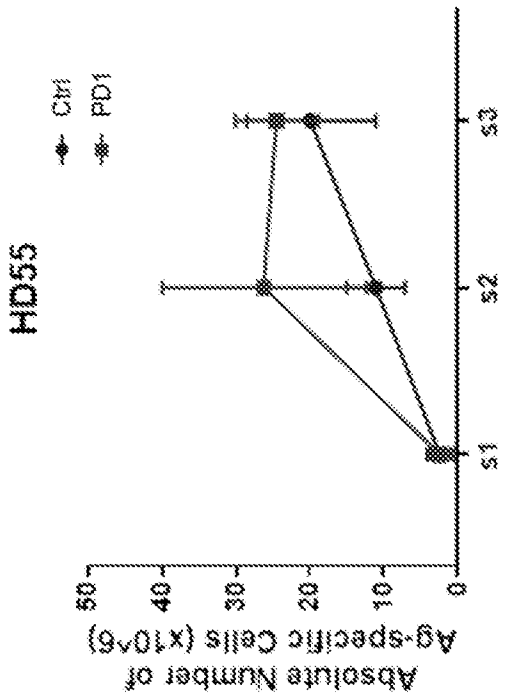



FIG. 41A

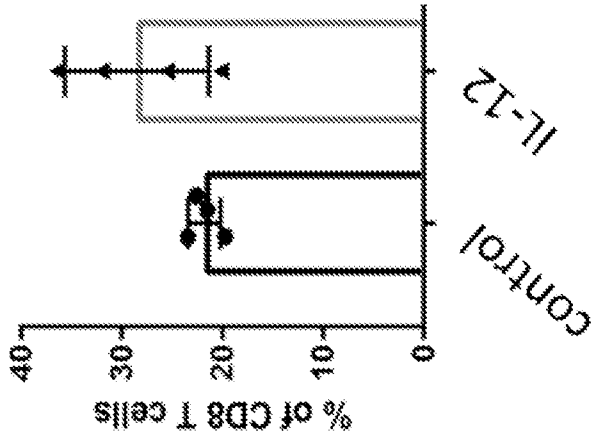



FIG. 42B

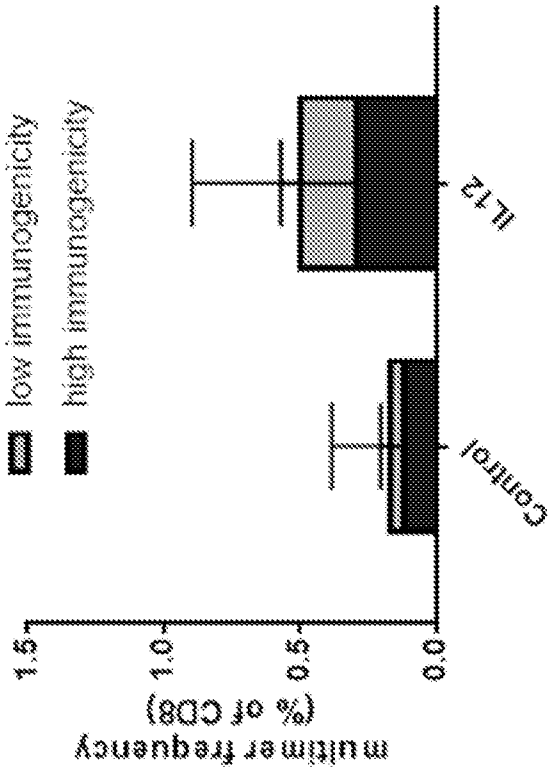



FIG. 42A

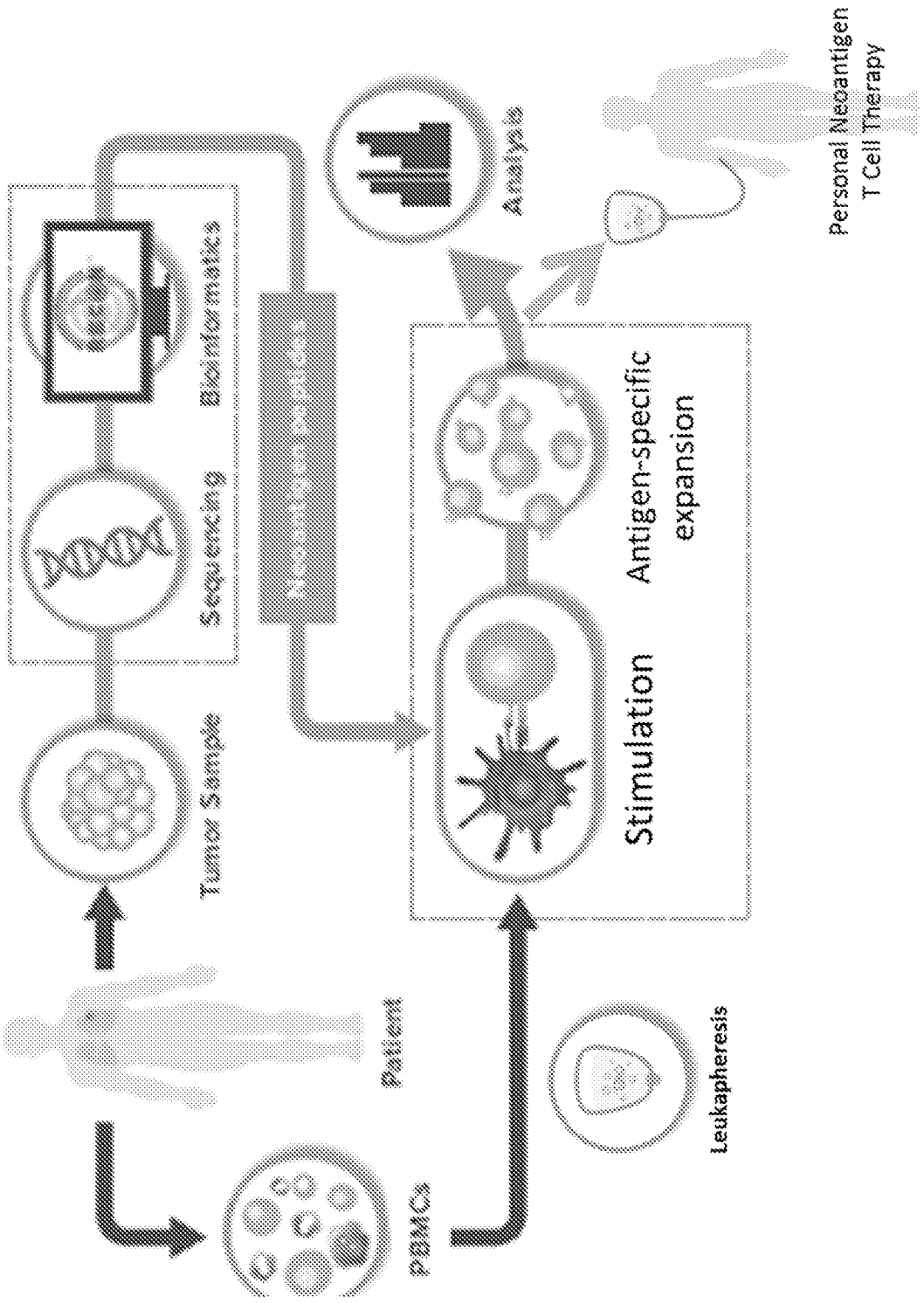

Tumor Sample
Sequencing
Bioinformatics
Patient
PBMCs
Leukapheresis
Stimulation
Antigen-specific expansion
Personal Neoantigen T Cell Therapy

FIG. 43