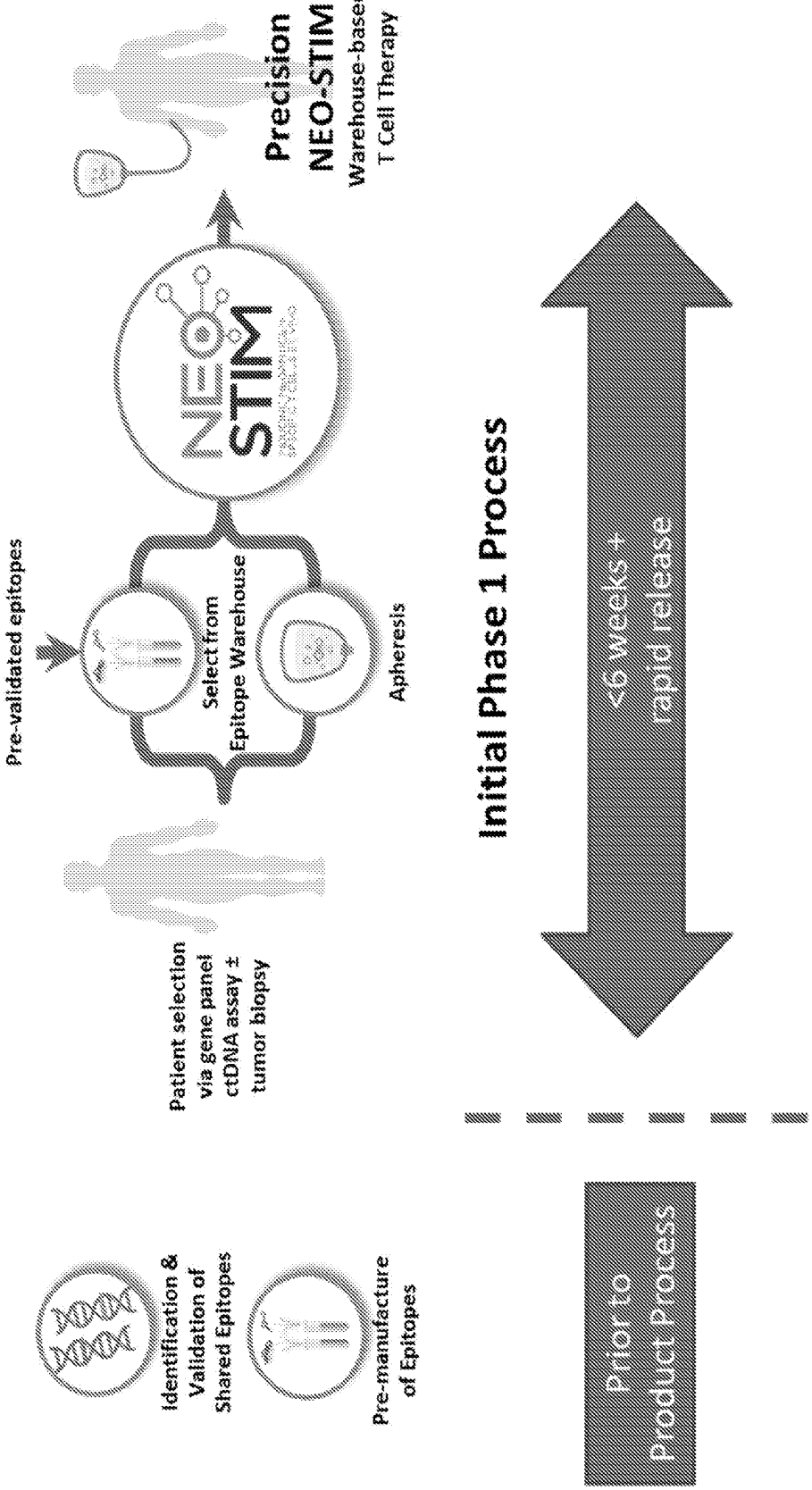

Prior to Product Process
Identification & Validation of Shared Epitopes
Pre-manufacture of Epitopes
Initial Phase 1 Process
Patient selection via gene panel ctDNA assay ± tumor biopsy
Pre-validated epitopes
Select from Epitope Warehouse
Apheresis
NEO STIM
Precision NEO-STIM
Warehouse-based T Cell Therapy
≤6 weeks + rapid release

FIG. 1A

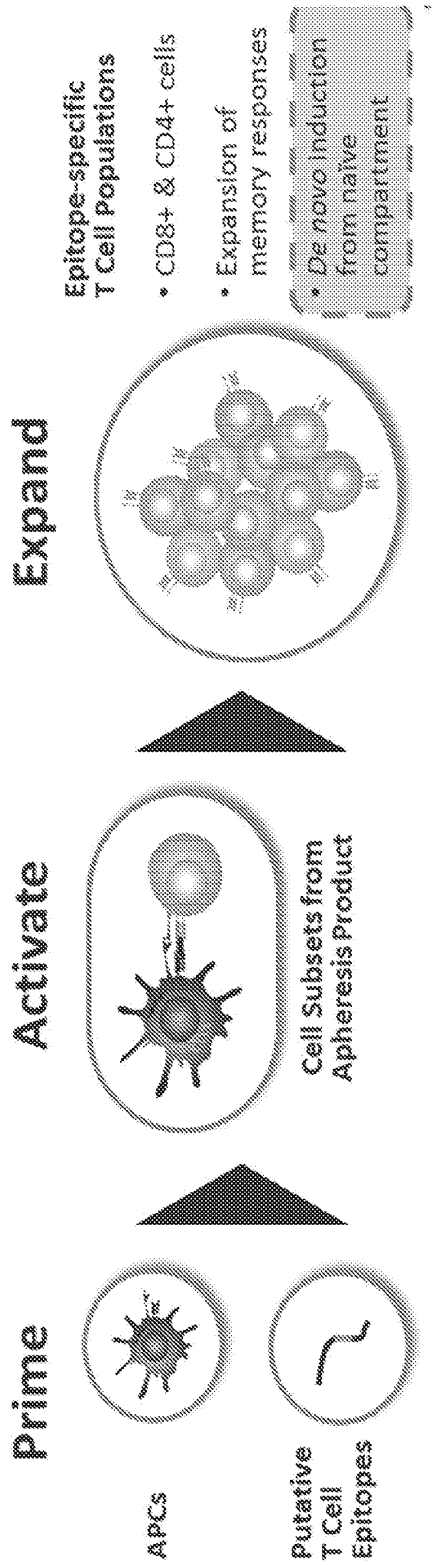

Prime
APCs
Putative T Cell Epitopes
Activate
Cell Subsets from Apheresis Product
Expand
Epitope-specific T Cell Populations
• CD8+ & CD4+ cells
• Expansion of memory responses
• De novo induction from naive compartment

FIG. 1B

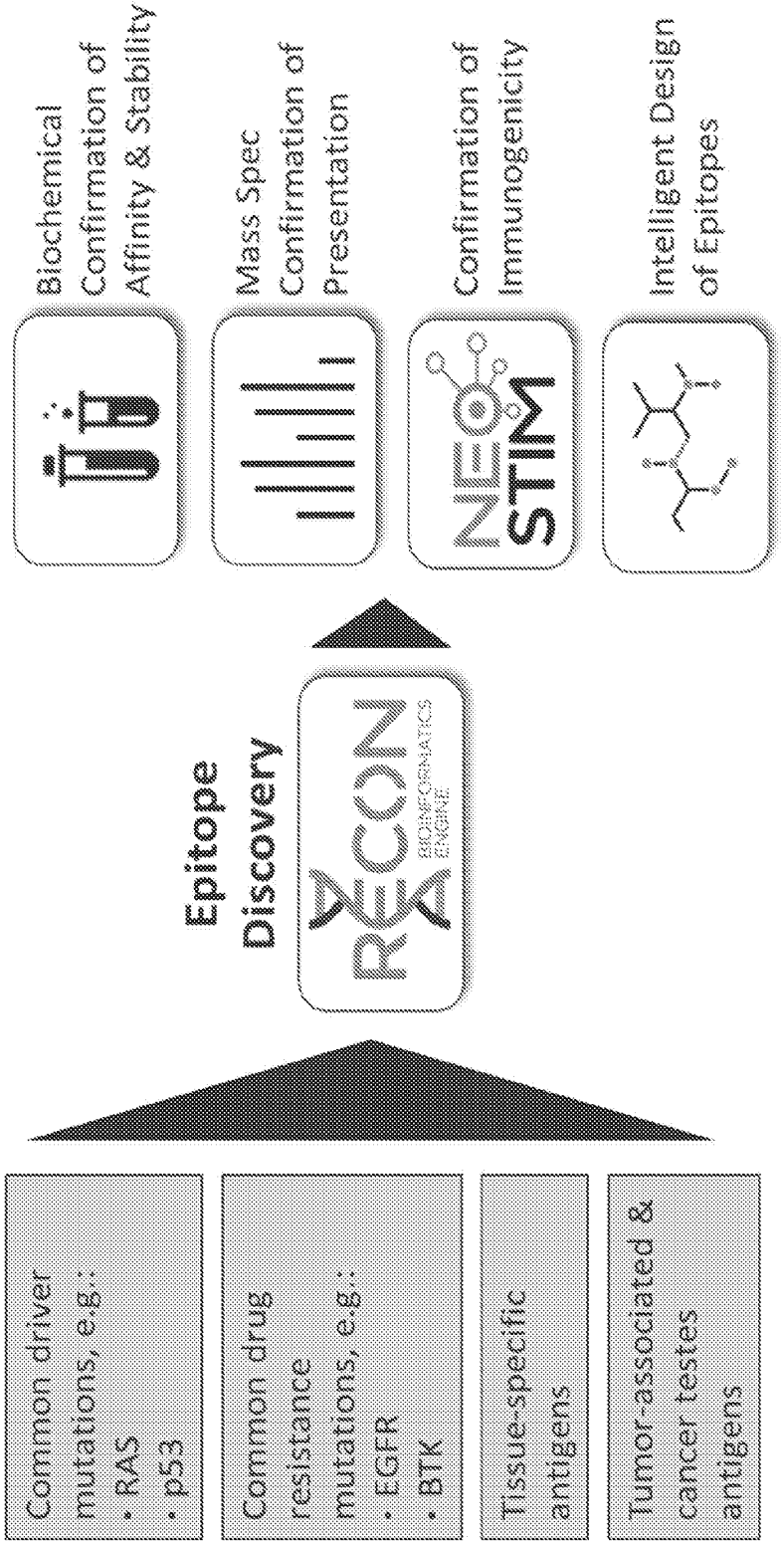

Common driver mutations, e.g.:
• RAS
• p53
Common drug resistance mutations, e.g.:
• EGFR
• BTK
Tissue-specific antigens
Tumor-associated & cancer testes antigens
Epitope Discovery
RECON
BIOINFORMATICS ENGINE
Biochemical Confirmation of Affinity & Stability
Mass Spec Confirmation of Presentation
Confirmation of Immunogenicity
NEO STIM
Intelligent Design of Epitopes

FIG. 2

Epitope	Allele	RECON % Rank	US Population Frequency	Validated as presented?
(v)vvgaDgvgk	A03:01	0.25, 1	21.8%	✓
	A11:01	0.17, 0.2	11.2%	✓
	A68:01	0.3	5.9%	✓
	A30:01	0.5	4.5%	
gaDgvgksa(l)	C05:01	0.12	13.6%	
	C03:03	0.5	8.8%	
	C08:02	0.04, 0.8	7.5%	✓
	B35:03	0.8	2.8%	
	C08:01	0.6, 0.9	2.1%	
Dgvgksal	B08:01	0.3	17.6%	
	B14:02	0.2	5.8%	
aDgvgksal	C07:01	1	26.2%	
	B37:01	0.6	2.2%	
gaDgvgks	C05:01	0.9	13.6%	

FIG. 3A

Epitope	Allele	RECON % Rank	US Population Frequency	Validated as presented?
(v)vvgaVgvgk	A03:01	0.12, 0.7	21.8%	✓
	A11:01	0.15, 0.15	11.2%	✓
	A68:01	0.25, 0.3	5.9%	✓
	A30:01	0.4	4.5%	
(g)aVgvgksal	B07:02	1	20.5%	
	C03:04	0.8, 0.8	14.7%	
	C03:03	0.7	8.8%	
	C08:02	1	7.5%	
	B35:03	1	2.8%	
	B48:01	0.7	1.1%	
	B46:01	0.7	0.8%	
	B81:01	0.5	0.6%	
klvvvgaVgv	A02:01	0.5	42.7%	
	A02:07	0.9	0.6%	
lvvvgaVgv	B68:02	0.4	3.6%	
Vgvgksal	B08:01	1	17.6%	

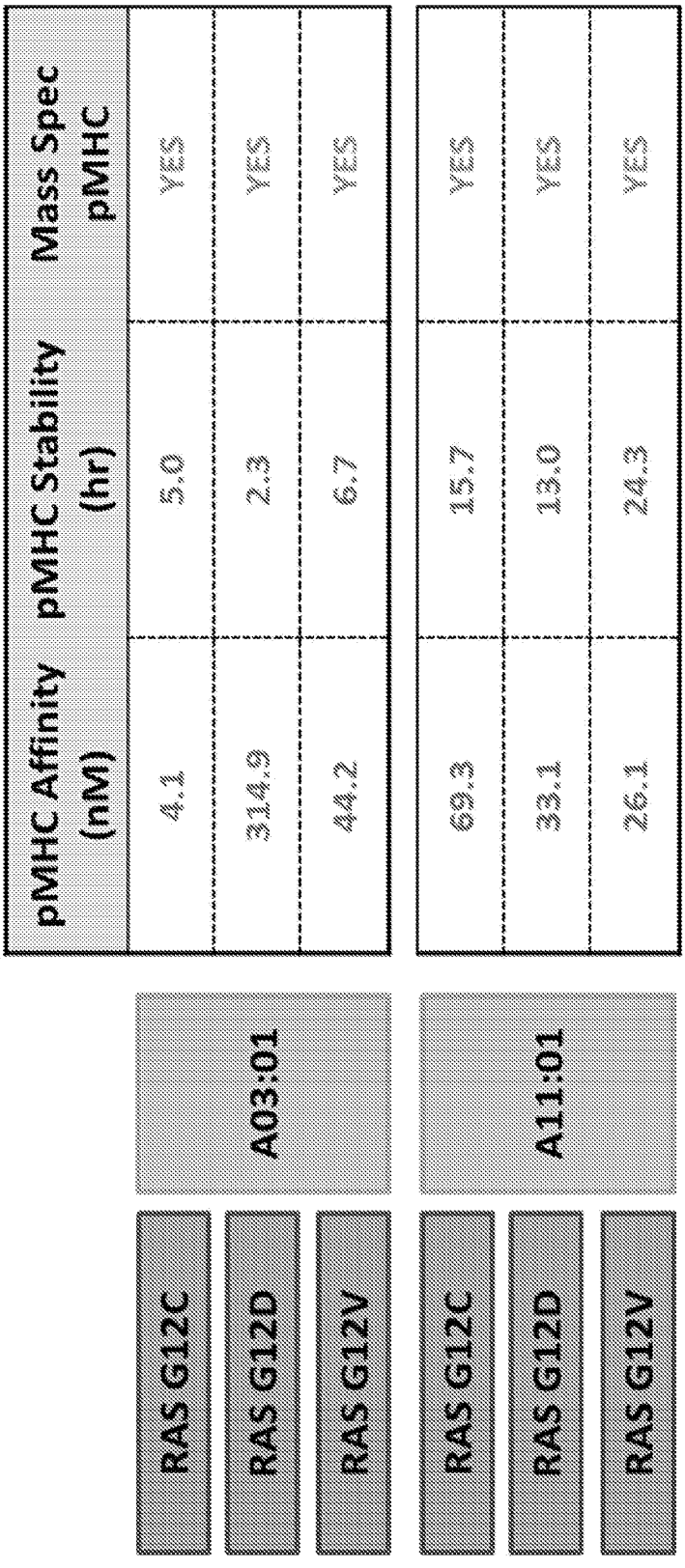
FIG. 3B

Epitope	Allele	RECON % Rank	US Population Frequency	Validated as presented?
(v)vvgaCgvgk	A03:01	0.25, 0.8	21.8%	✓
	A11:01	0.17, 0.3	11.2%	✓
	A68:01	0.4, 0.8	5.9%	✓
	A30:01	1	4.5%	

FIG. 3C

Epitope	Allele	RECON % Rank	US Population Frequency	Validated as presented?
(v)vvgaRgvgk	A03:01	0.13, 1	21.8%	✓
	A11:01	0.25, 0.4	11.2%	✓
	A68:01	0.4, 0.9	5.9%	✓
	A30:01	0.8	4.5%	✓
(a)Rgvgksal	B14:02	0.9	5.8%	
	B27:05	0.6	4.9%	
	B38:01	0.9	3.5%	
	B39:01	0.4	1.8%	
	C07:01	0.4, 0.7	26.2%	
	C07:02	0.3	24.7%	
gaRgvgksa	B55:01	0.6	2.7%	
	B46:01	0.5	0.8%	
eyklvvvgaR	A33:03	0.17	3.1%	

FIG. 3D



pMHC Affinity (nM)	pMHC Stability (hr)	Mass Spec pMHC
4.1	5.0	YES
314.9	2.3	YES
44.2	6.7	YES
69.3	15.7	YES
33.1	13.0	YES
26.1	24.3	YES

FIG. 4A

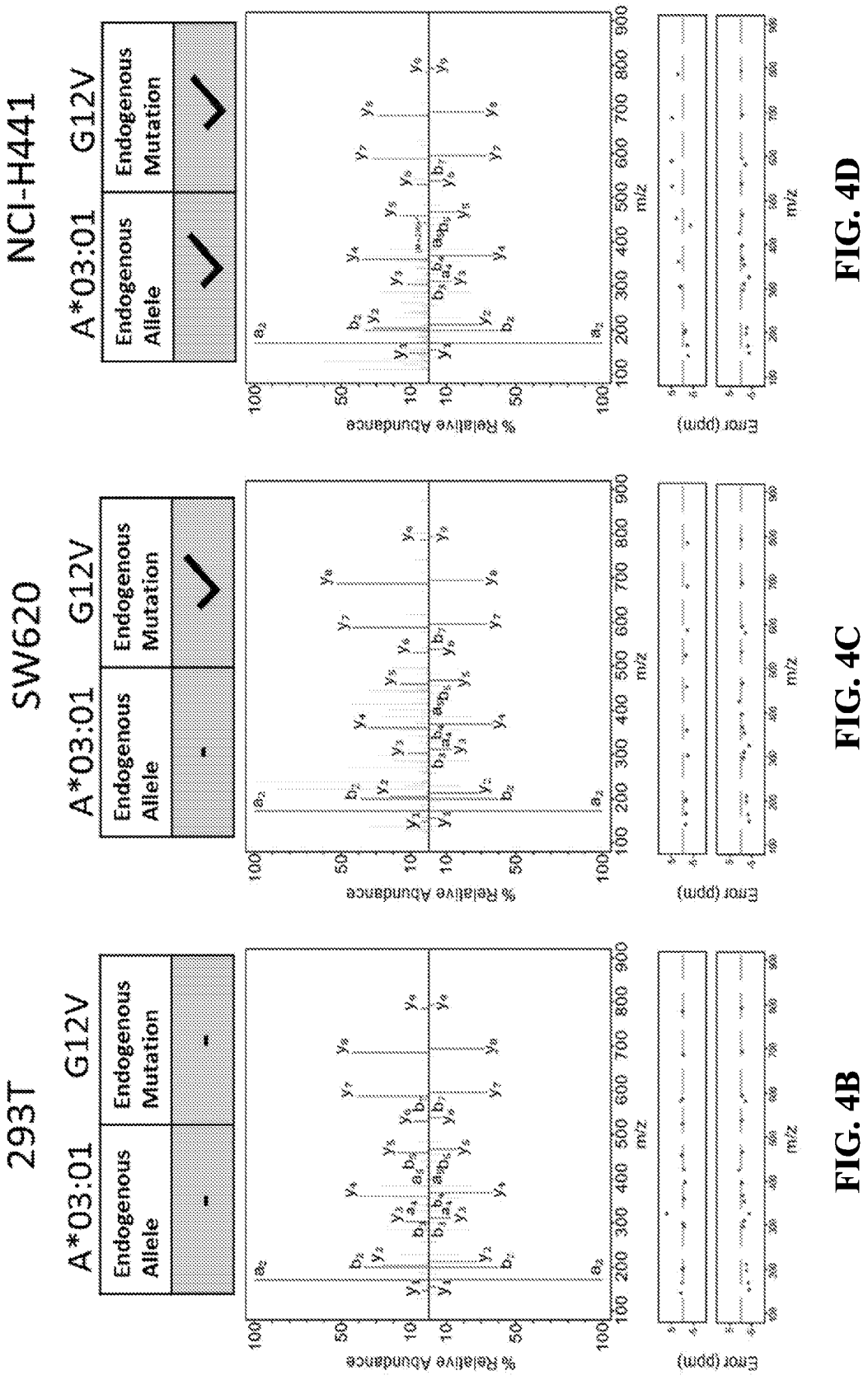


FIG. 4B FIG. 4C FIG. 4D

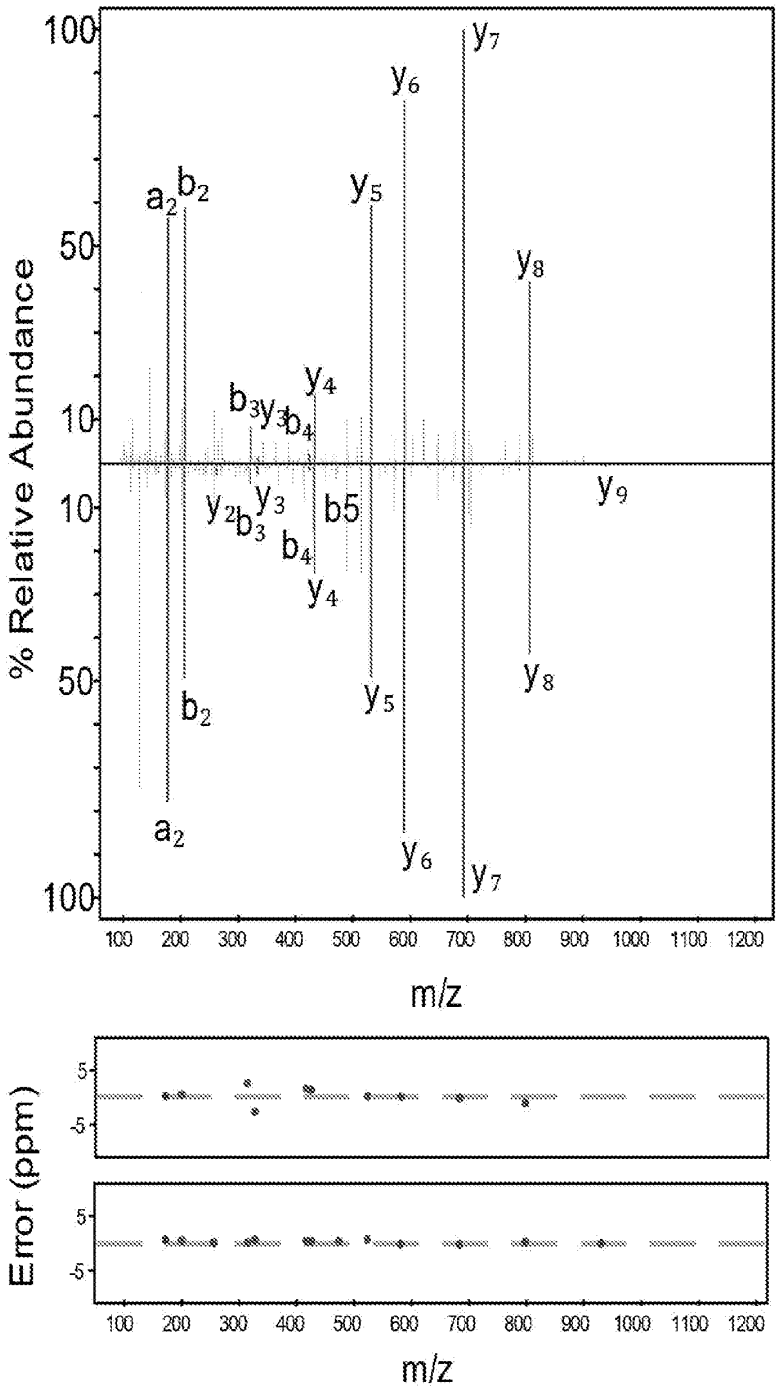

100
50
10
10
50
100
% Relative Abundance
y7
y6
y5
y8
y4
a2
b2
b3
y3
b4
y2
b5
y9
100 200 300 400 500 600 700 800 900 1000 1100 1200
m/z
5
-5
5
-5
Error (ppm)
100 200 300 400 500 600 700 800 900 1000 1100 1200
m/z

FIG. 4E

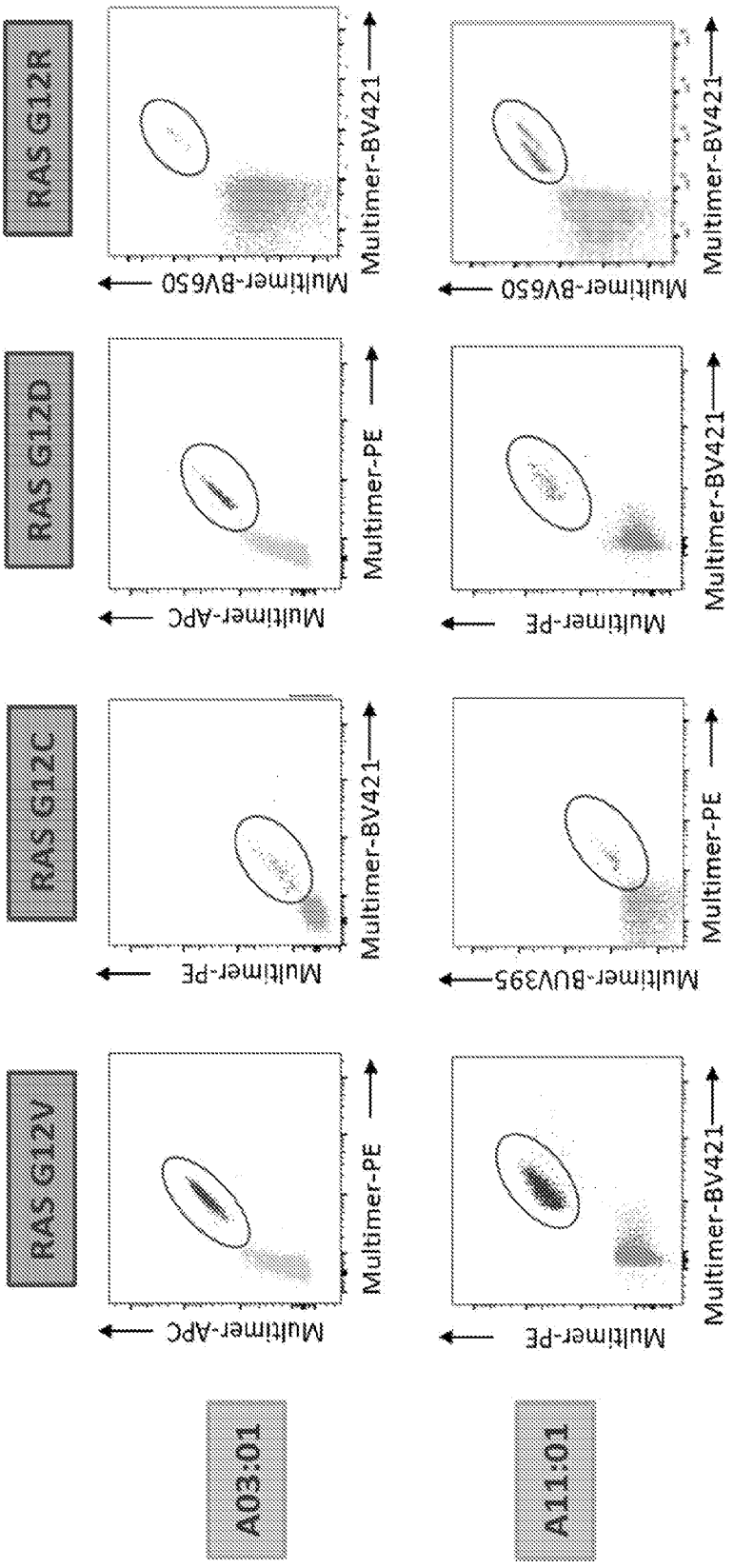

A03:01
A11:01
RAS G12V
RAS G12C
RAS G12D
RAS G12R
Multimer-APC
Multimer-PE
Multimer-BV421
Multimer-BV650
Multimer-BUV395

FIG. 5

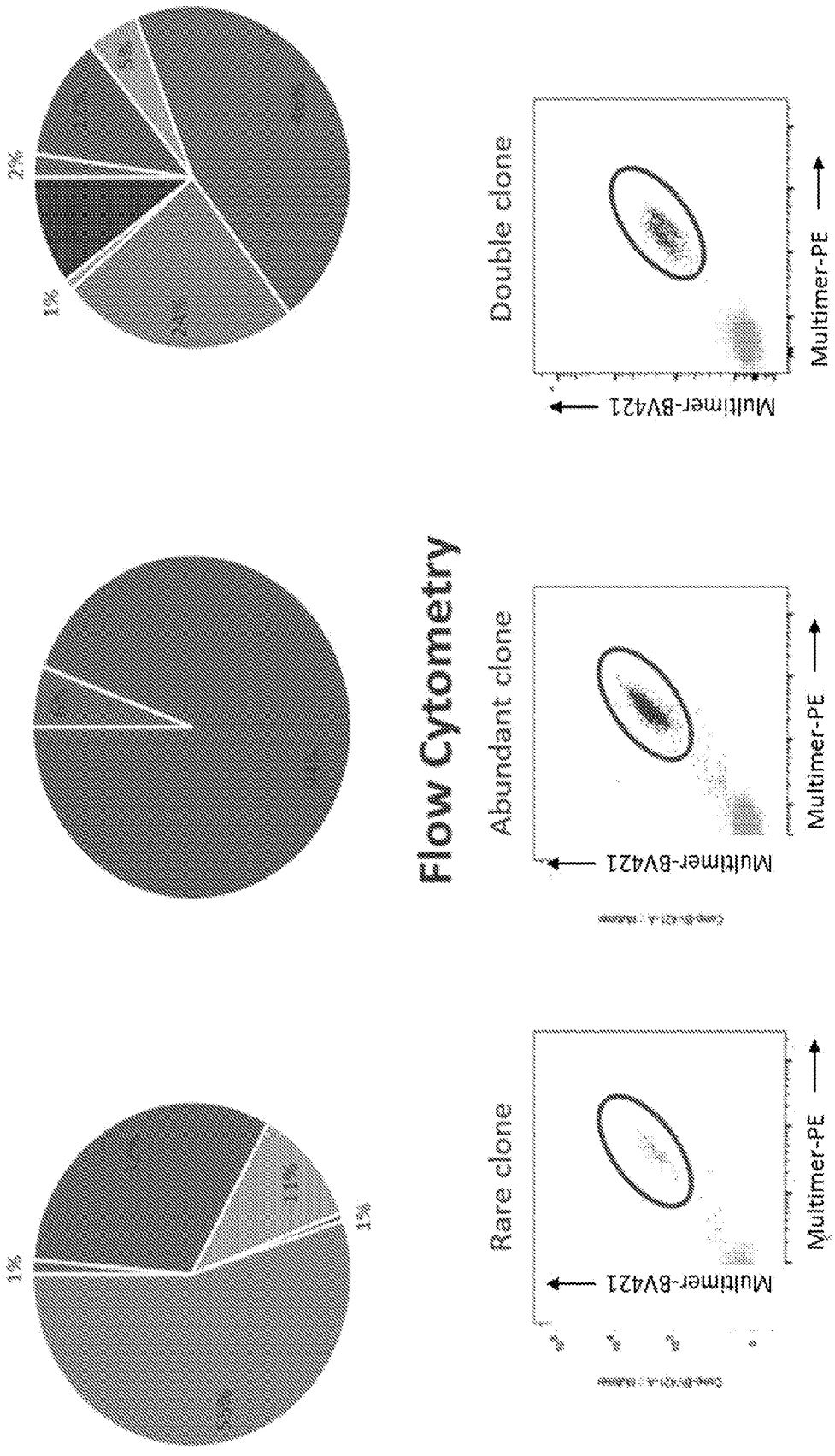

Flow Cytometry
Double clone
Abundant clone
Rare clone
Multimer-BV421
Multimer-PE
2%
1%
1%
1%

FIG. 6

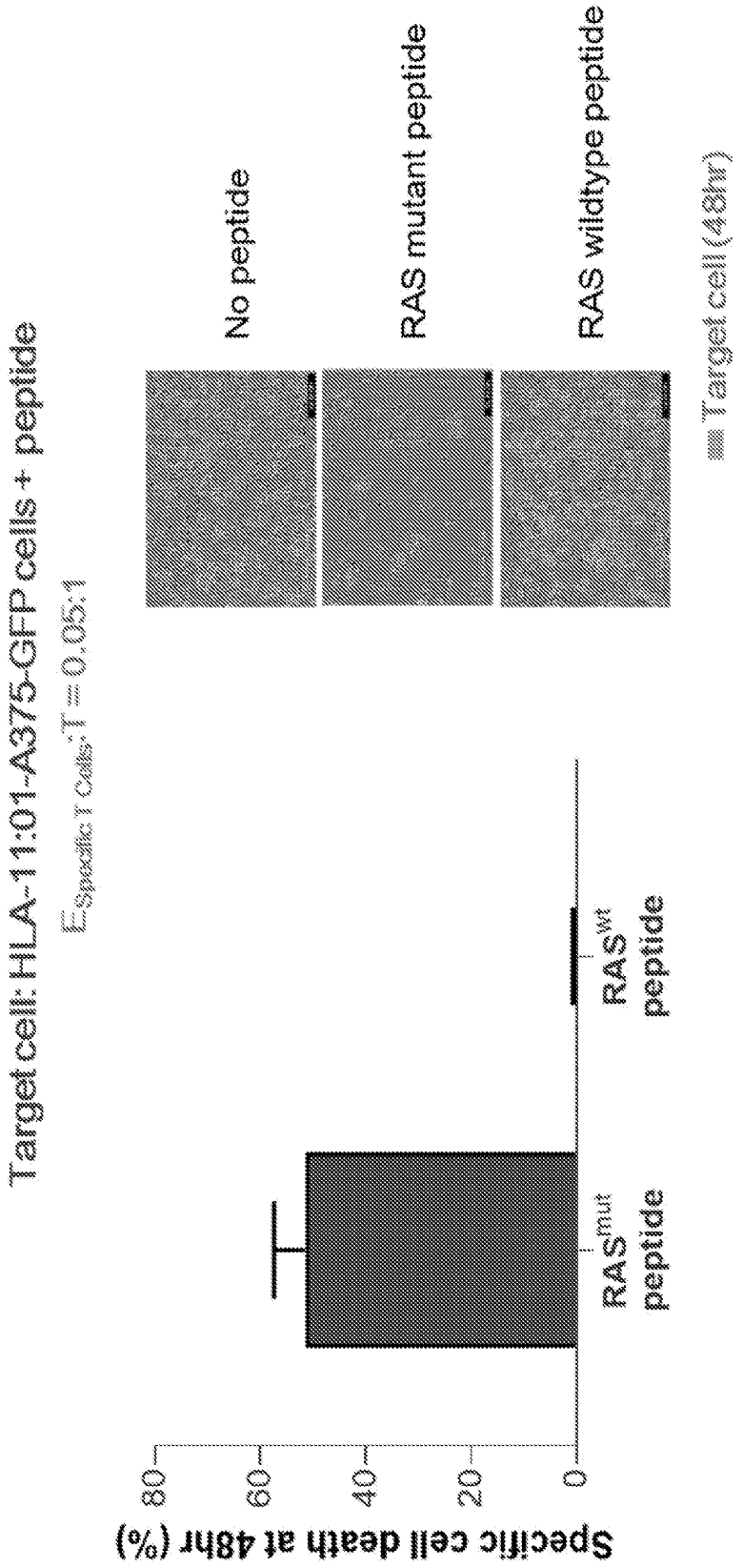

Target cell: HLA-11:01-A375-GFP cells + peptide
$E_{Specific\ T\ Cells}$:T = 0.05:1
No peptide
RAS mutant peptide
RAS wildtype peptide
Target cell (48hr)
Specific cell death at 48hr (%)
0
20
40
60
80
RAS^mut peptide
RAS^wt peptide

FIG. 7

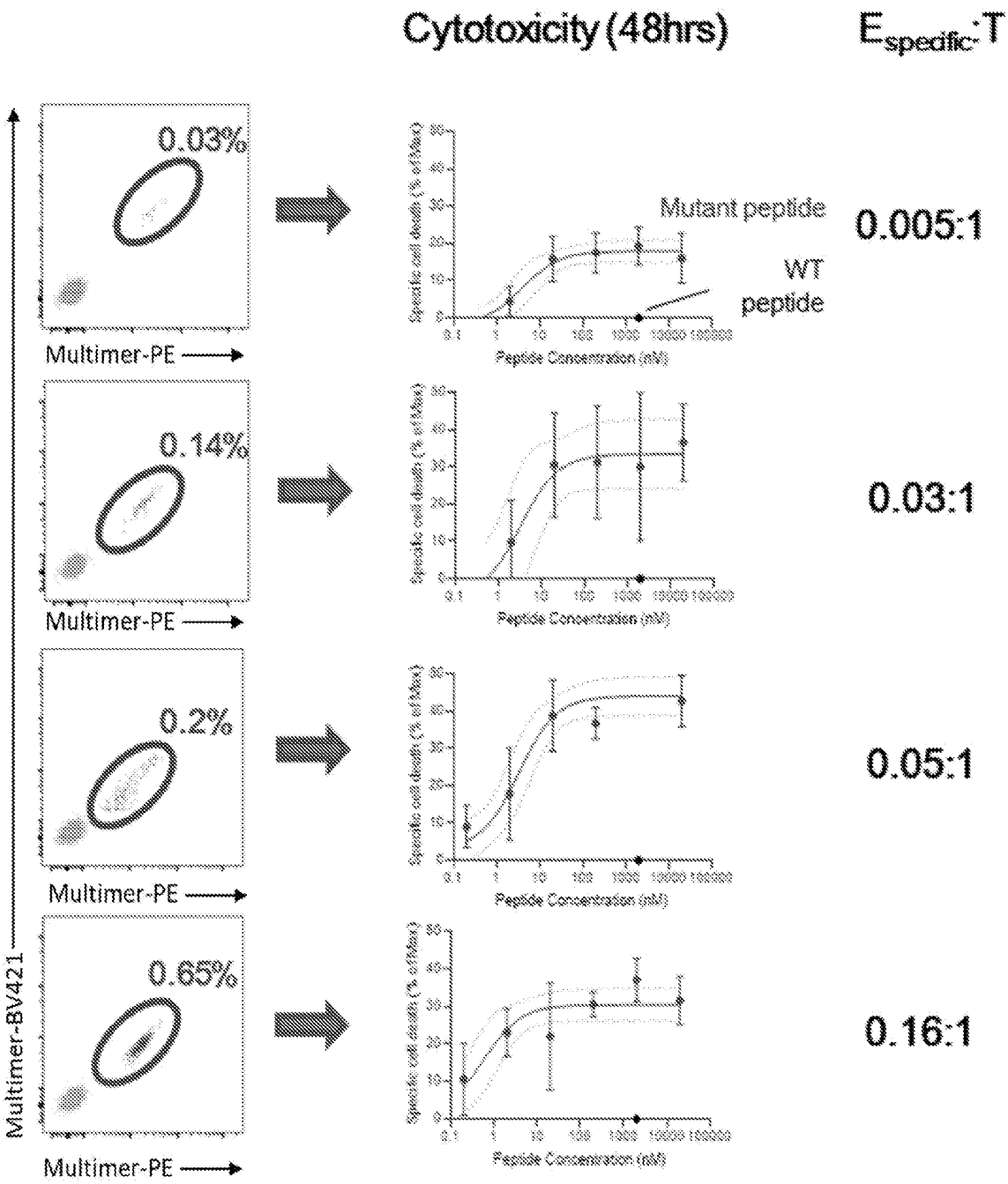

Cytotoxicity (48hrs)
$E_{specific}$:T
Multimer-BV421
Multimer-PE
0.03%
0.14%
0.2%
0.65%
Mutant peptide
WT peptide
Peptide Concentration (nM)
0.005:1
0.03:1
0.05:1
0.16:1

FIG. 8

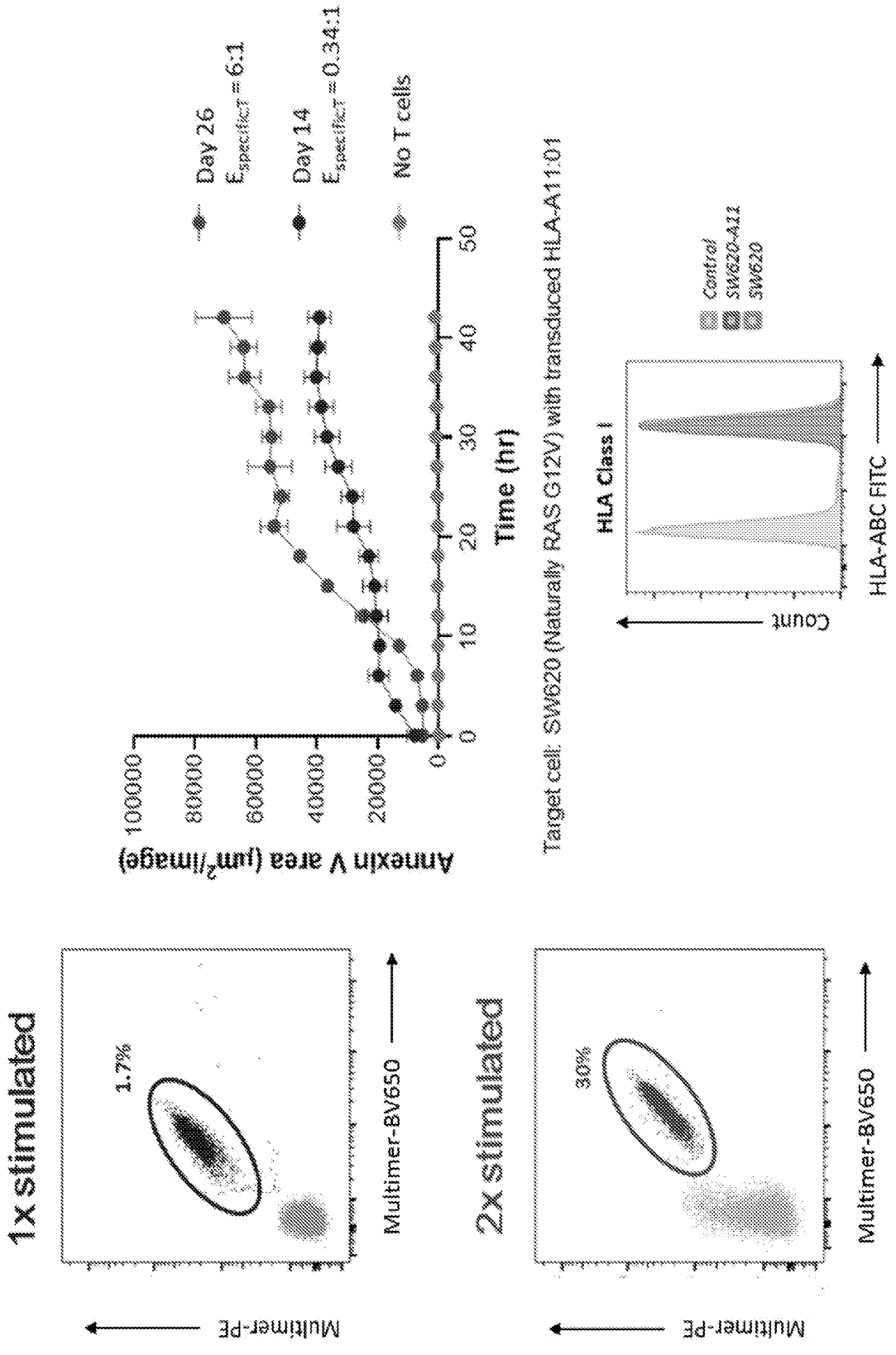

1x stimulated
1.7%
Multimer-PE
Multimer-BV650
2x stimulated
30%
Multimer-PE
Multimer-BV650
Annexin V area (μm²/Image)
100000
80000
60000
40000
20000
0
0
10
20
30
40
50
Time (hr)
Day 26
E specificT = 6:1
Day 14
E specificT = 0.34:1
No T cells
Target cell: SW620 (Naturally RAS G12V) with transduced HLA-A11:01
HLA Class I
Count
HLA-ABC FITC
Control
SW620-A11
SW620

FIG. 9

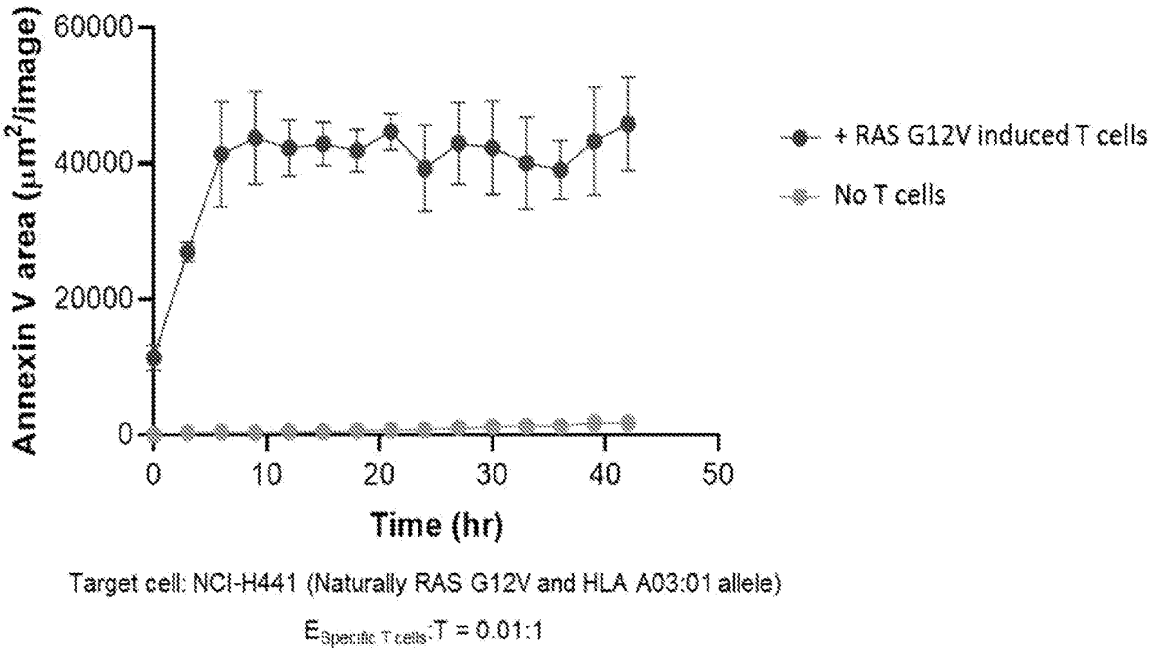


FIG. 10

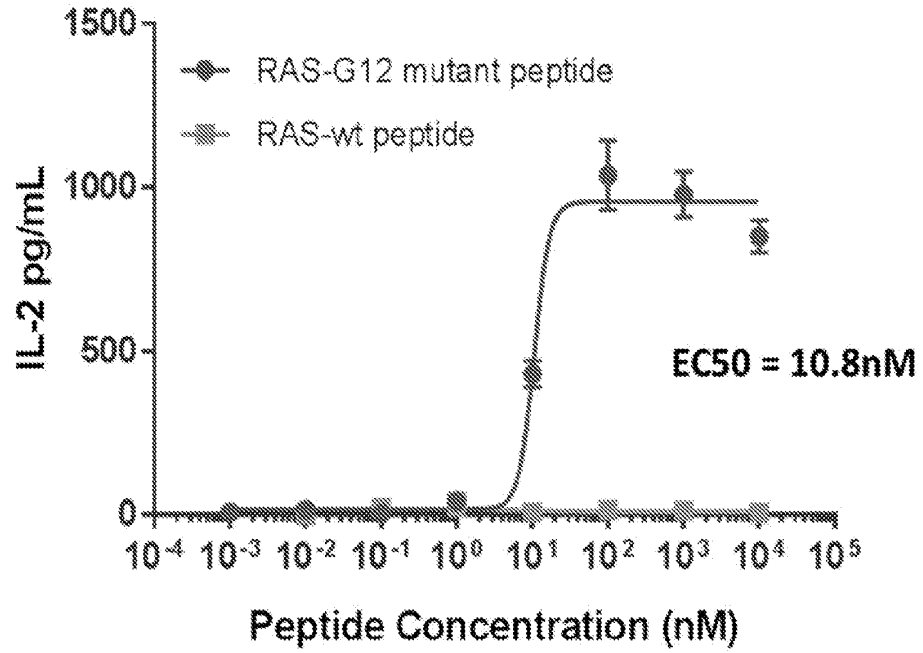


FIG. 11A

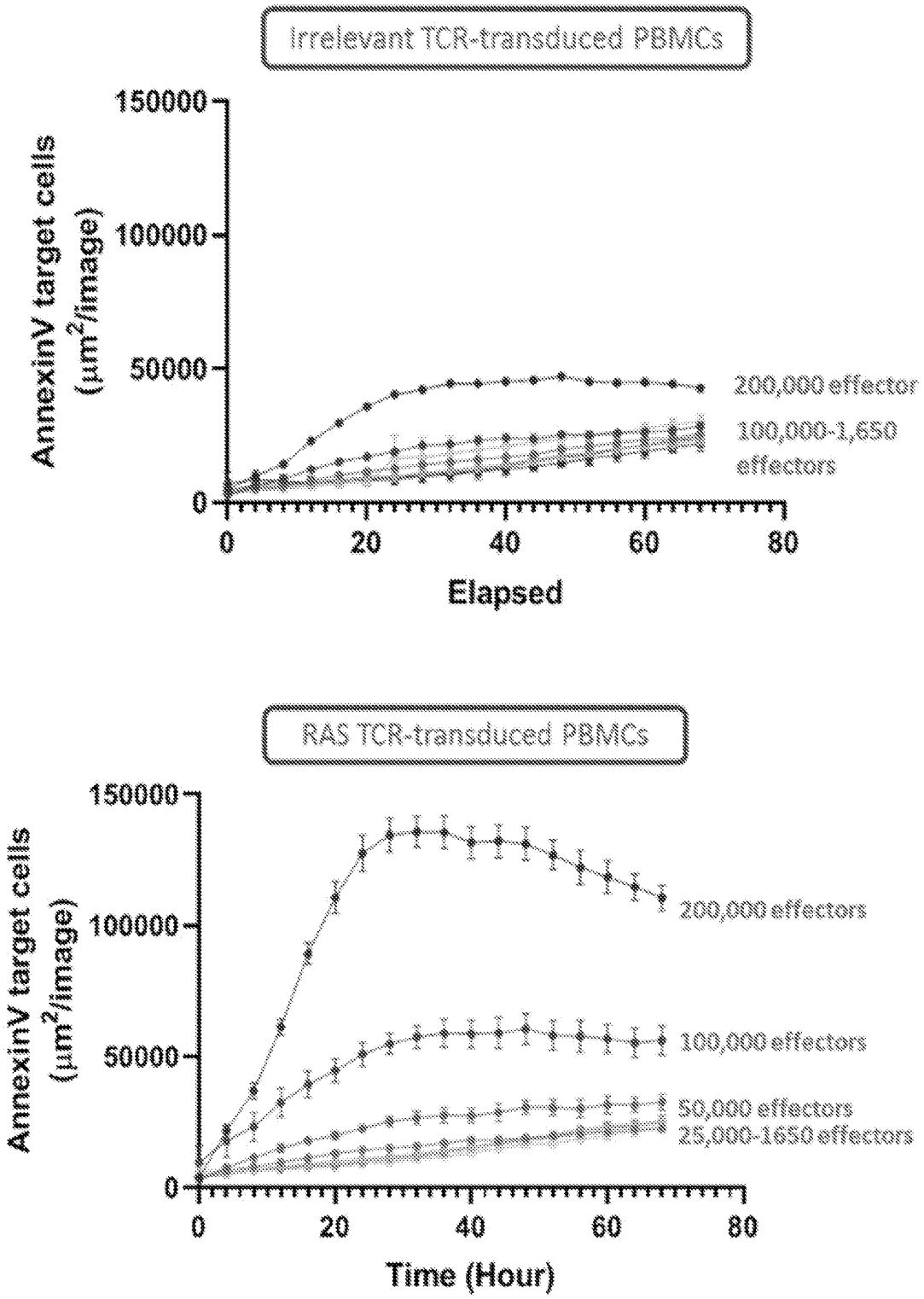

Irrelevant TCR-transduced PBMCs
AnnexinV target cells
(μm²/image)
150000
100000
50000
0
0
20
40
60
80
Elapsed
200,000 effector
100,000-1,650
effectors
RAS TCR-transduced PBMCs
AnnexinV target cells
(μm²/image)
150000
100000
50000
0
0
20
40
60
80
Time (Hour)
200,000 effectors
100,000 effectors
50,000 effectors
25,000-1650 effectors

FIG. 11B

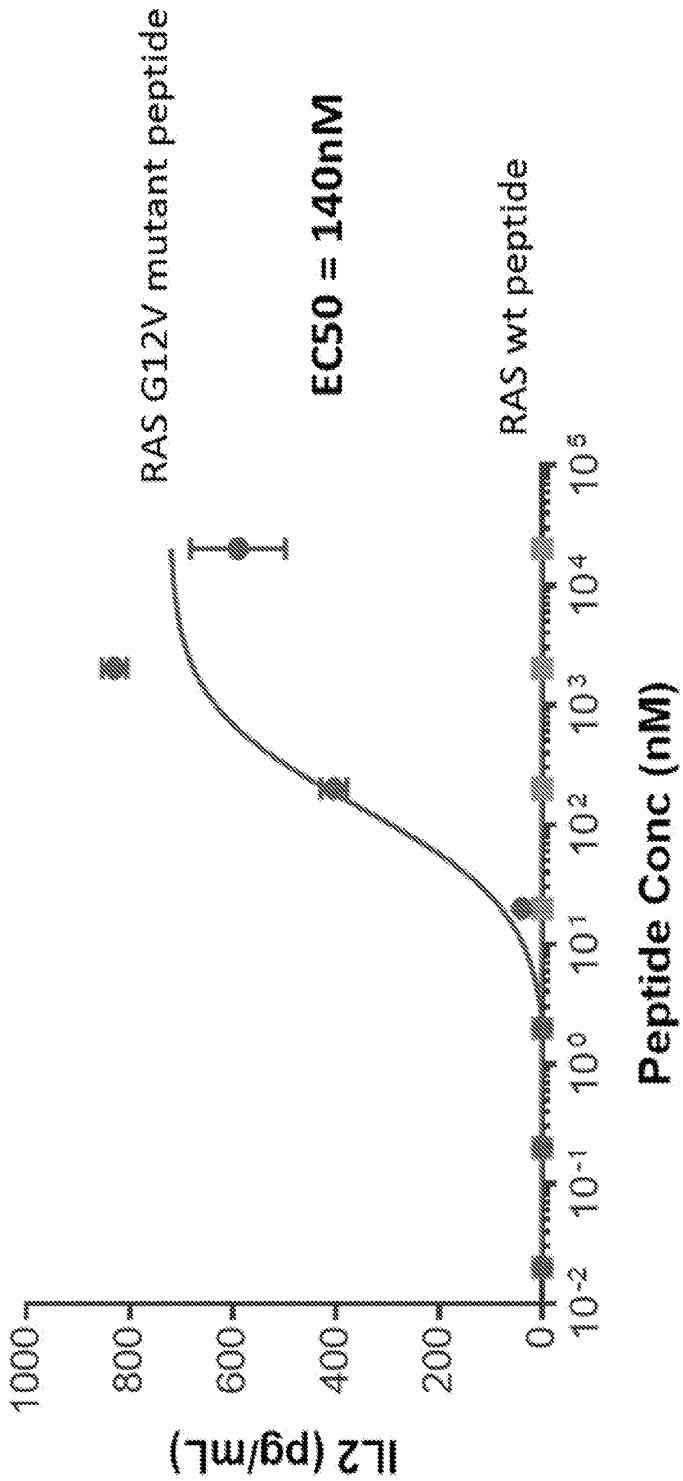

RAS G12V mutant peptide
EC50 = 140nM
RAS wt peptide
IL2 (pg/mL)
1000
800
600
400
200
0
10-2
10-1
10°
10¹
10²
10³
10⁴
10⁵
Peptide Conc (nM)

FIG. 11C

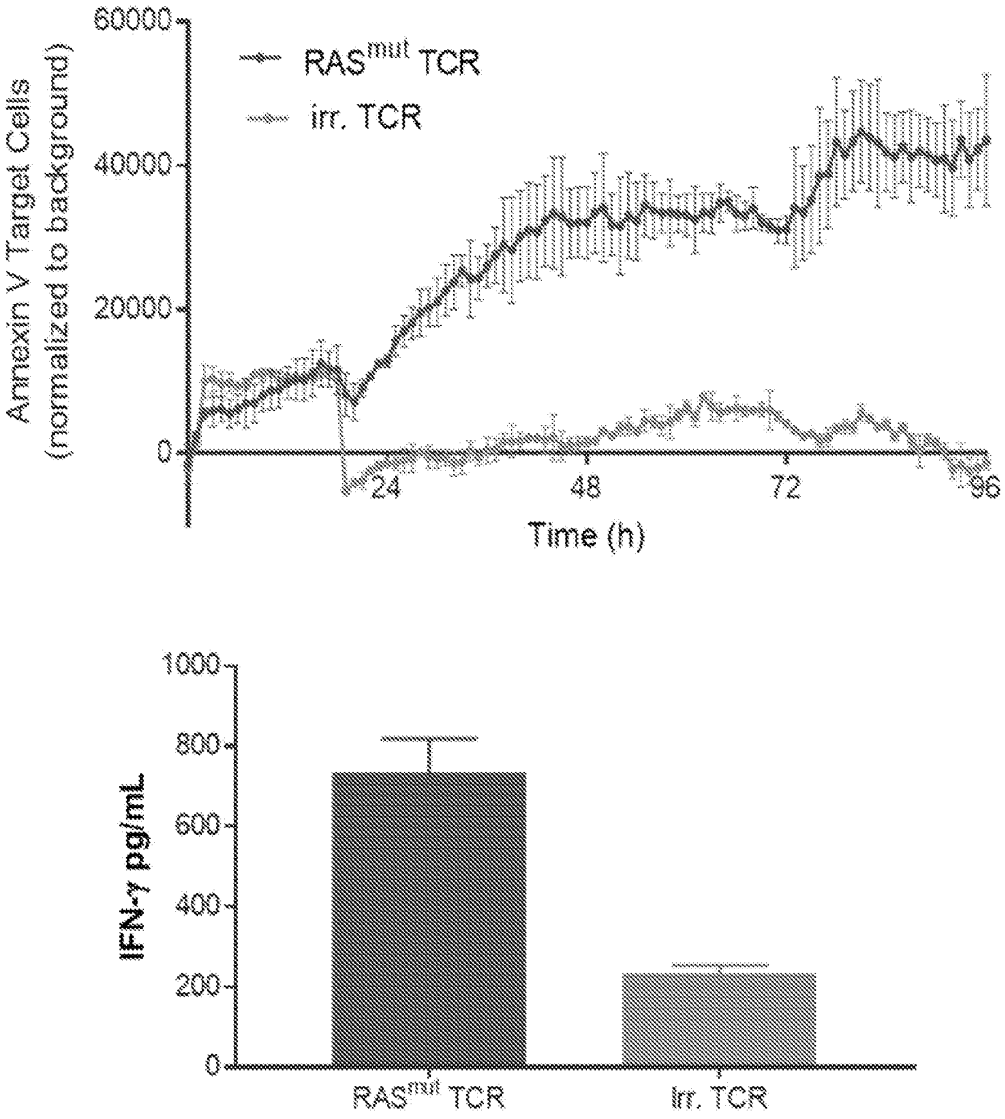

Annexin V Target Cells
(normalized to background)
60000
40000
20000
0
RAS^mut TCR
irr. TCR
24
48
72
96
Time (h)
IFN-γ pg/mL
1000
800
600
400
200
0
RAS^mut TCR
Irr. TCR

FIG. 11D

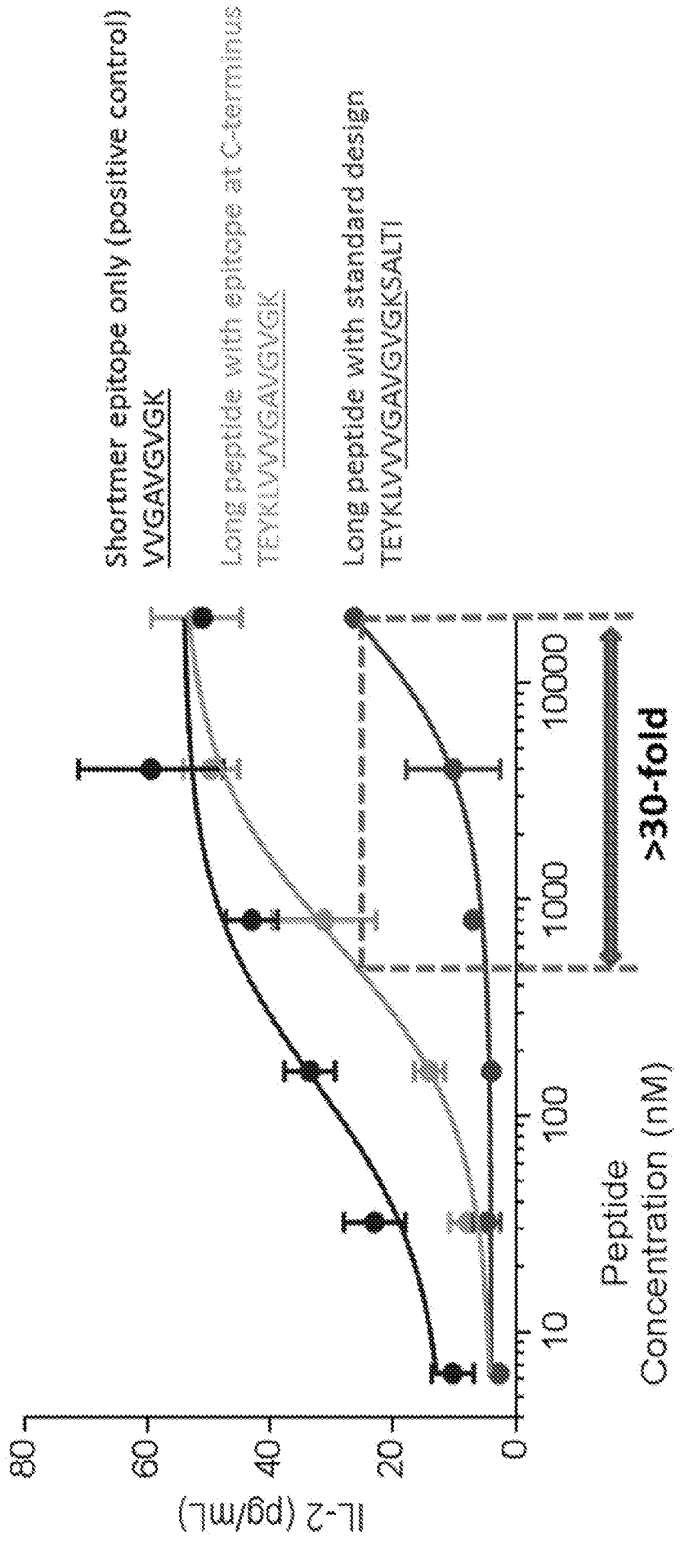

Shortmer epitope only (positive control)
VVGAVGVGK
Long peptide with epitope at C-terminus
TEYKLVVVGAVGVGK
Long peptide with standard design
TEYKLVVVGAVGVGKSALTI
IL-2 (pg/mL)
0
20
40
60
80
10
100
1000
10000
Peptide Concentration (nM)
>30-fold

FIG. 12A

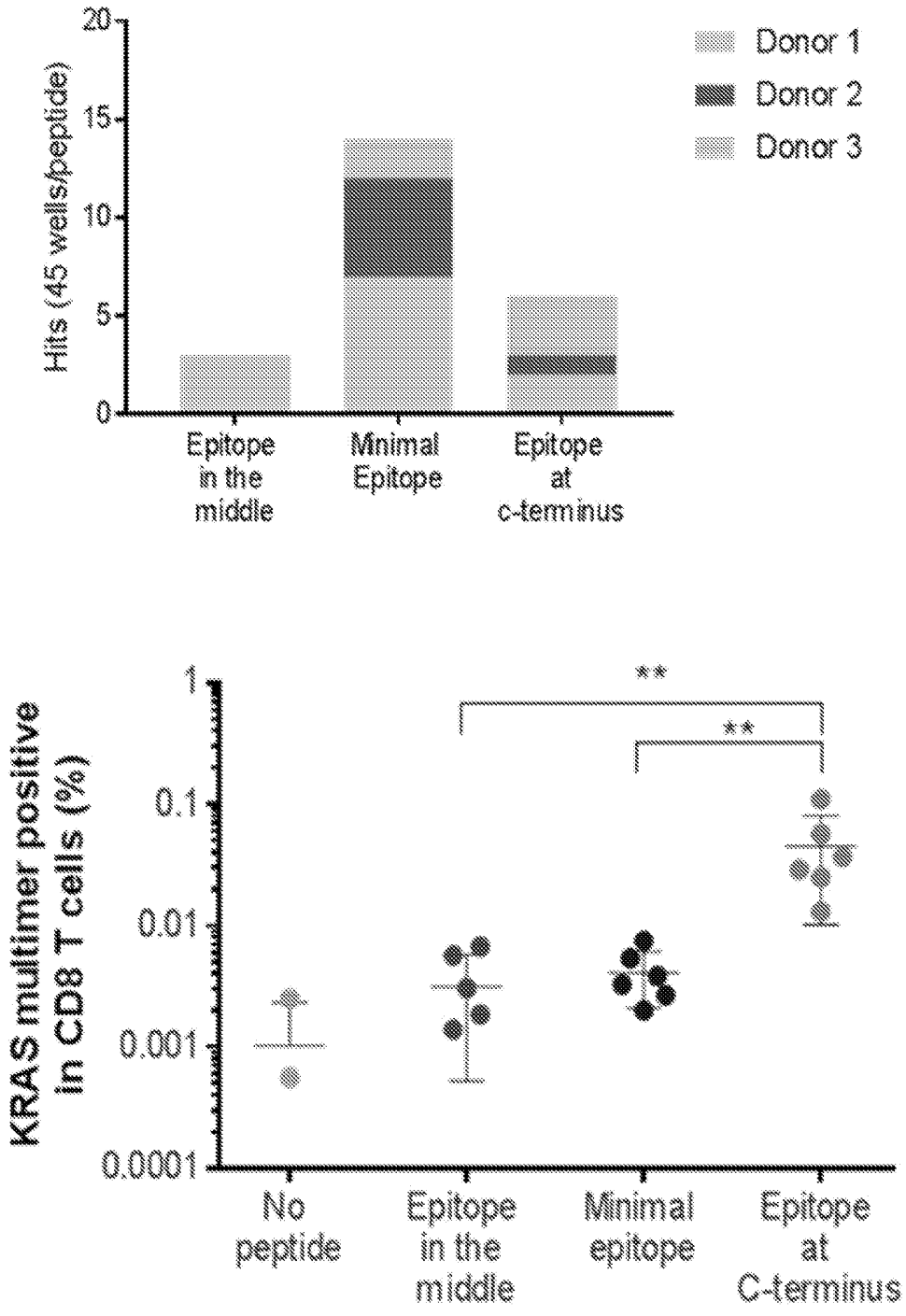

Hits (45 wells/peptide)
20
15
10
5
0
Donor 1
Donor 2
Donor 3
Epitope in the middle
Mnimal Epitope
Epitope at c-terminus
KRAS multimer positive in CD8 T cells (%)
1
0.1
0.01
0.001
0.0001
**
**
No peptide
Epitope in the middle
Minimal epitope
Epitope at C-terminus

FIG. 12B

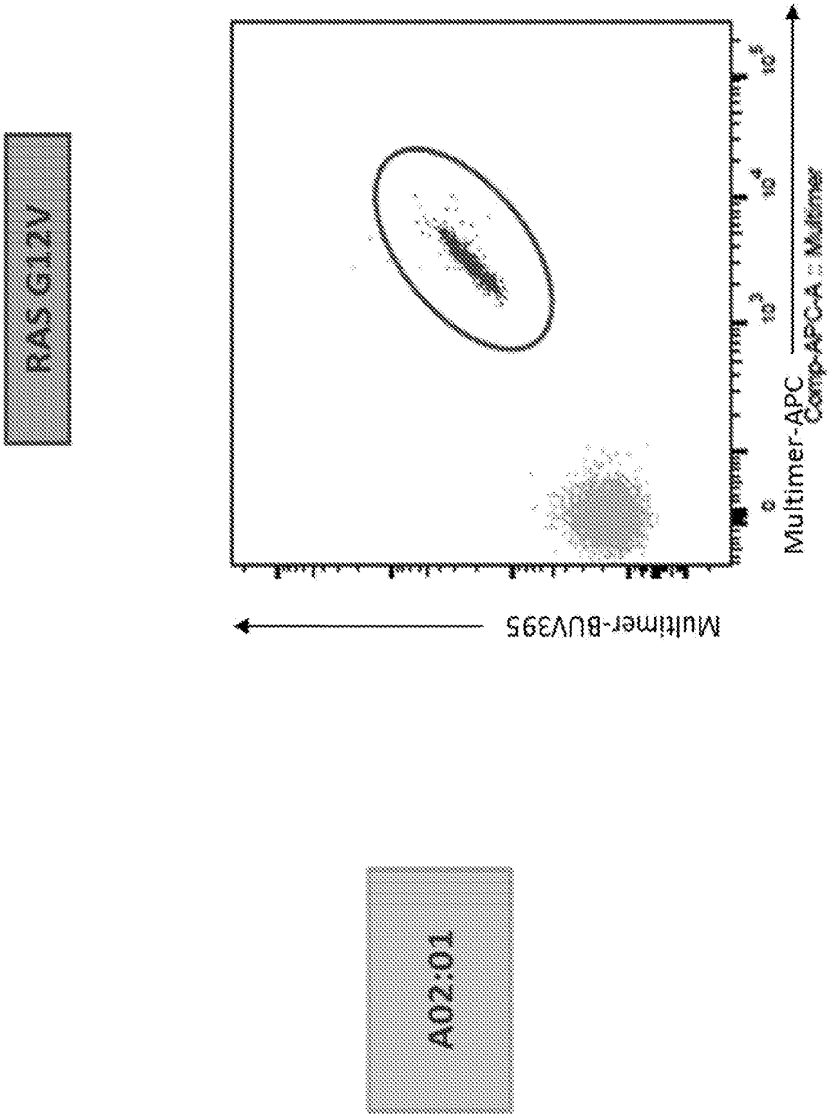

RAS G12V
A02:01
Multimer-BUV395
Multimer-APC
Comp-APC-A :: Multimer
0
10^3
10^4
10^5

FIG. 13

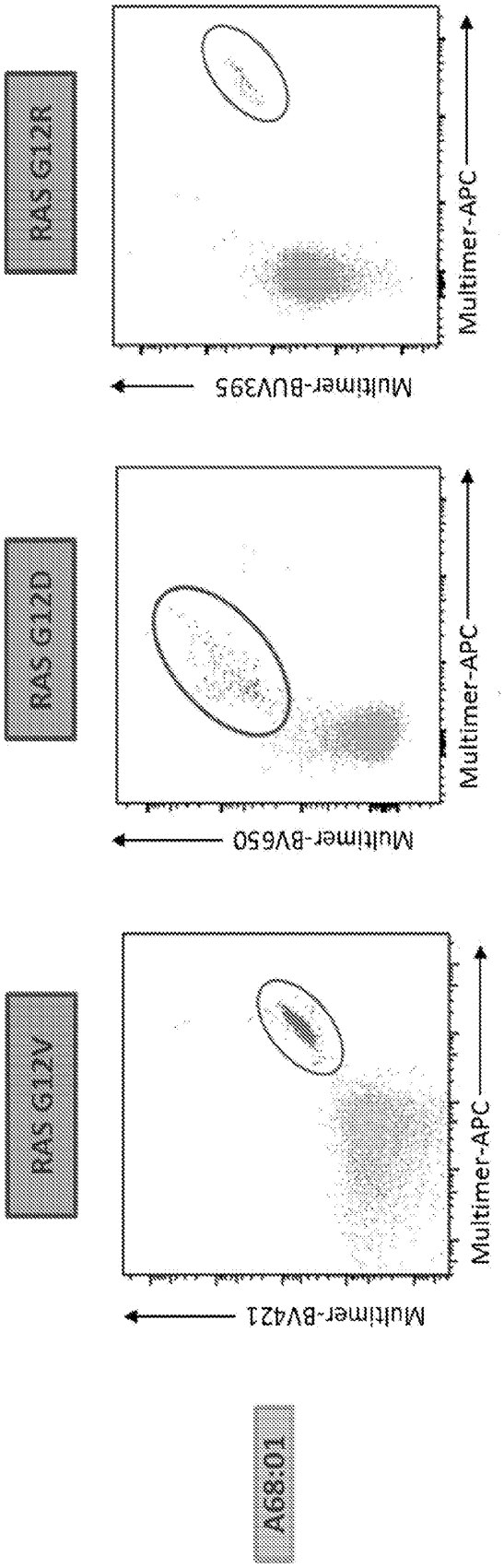

A68:01
RAS G12V
RAS G12D
RAS G12R
Multimer-BV421
Multimer-BV650
Multimer-BUV395
Multimer-APC
Multimer-APC
Multimer-APC

FIG. 14

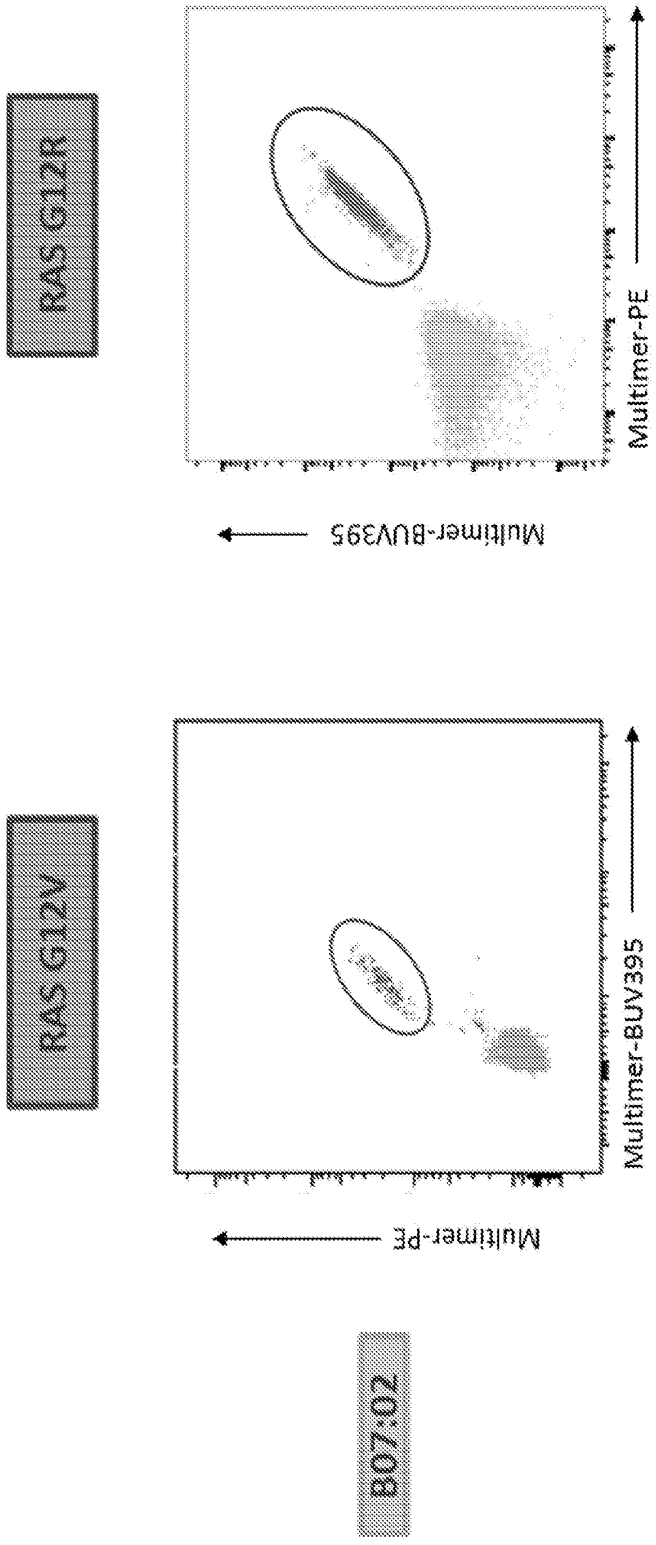

B07:02
RAS G12V
Multimer-PE
Multimer-BUV395
RAS G12R
Multimer-BUV395
Multimer-PE

FIG. 15

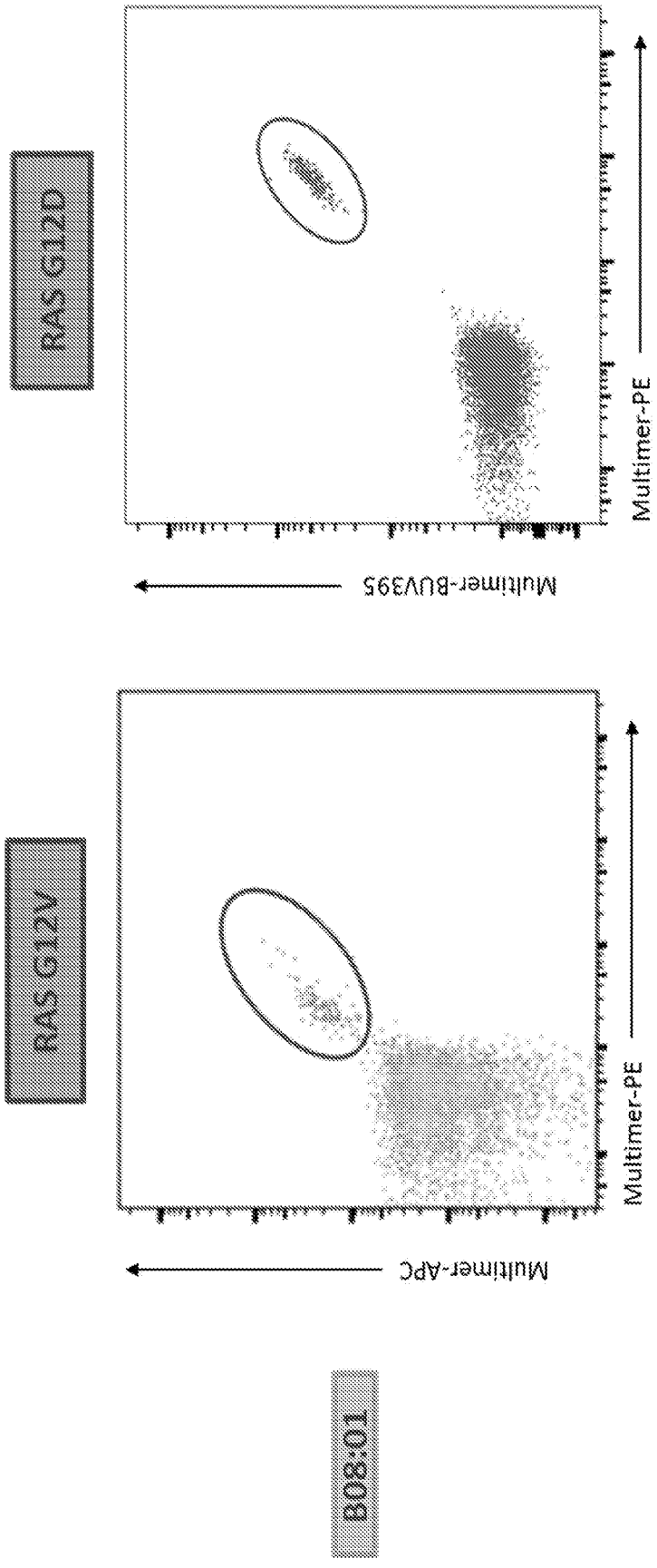

B08:01
RAS G12V
RAS G12D
Multimer-APC
Multimer-PE
Multimer-BUV395
Multimer-PE

FIG. 16

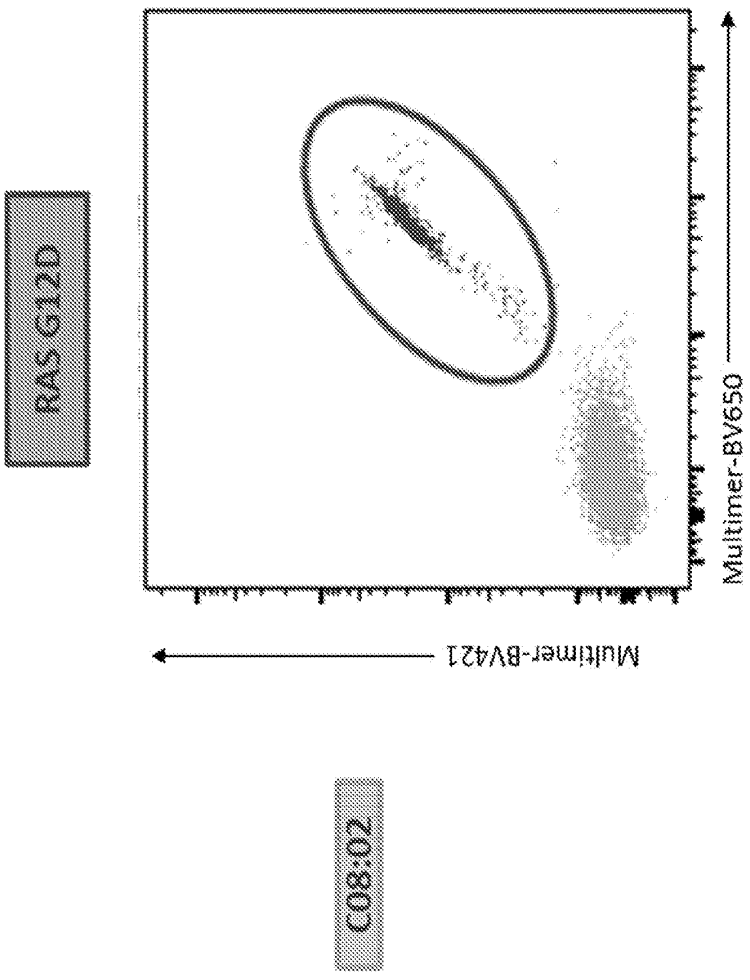


FIG. 17

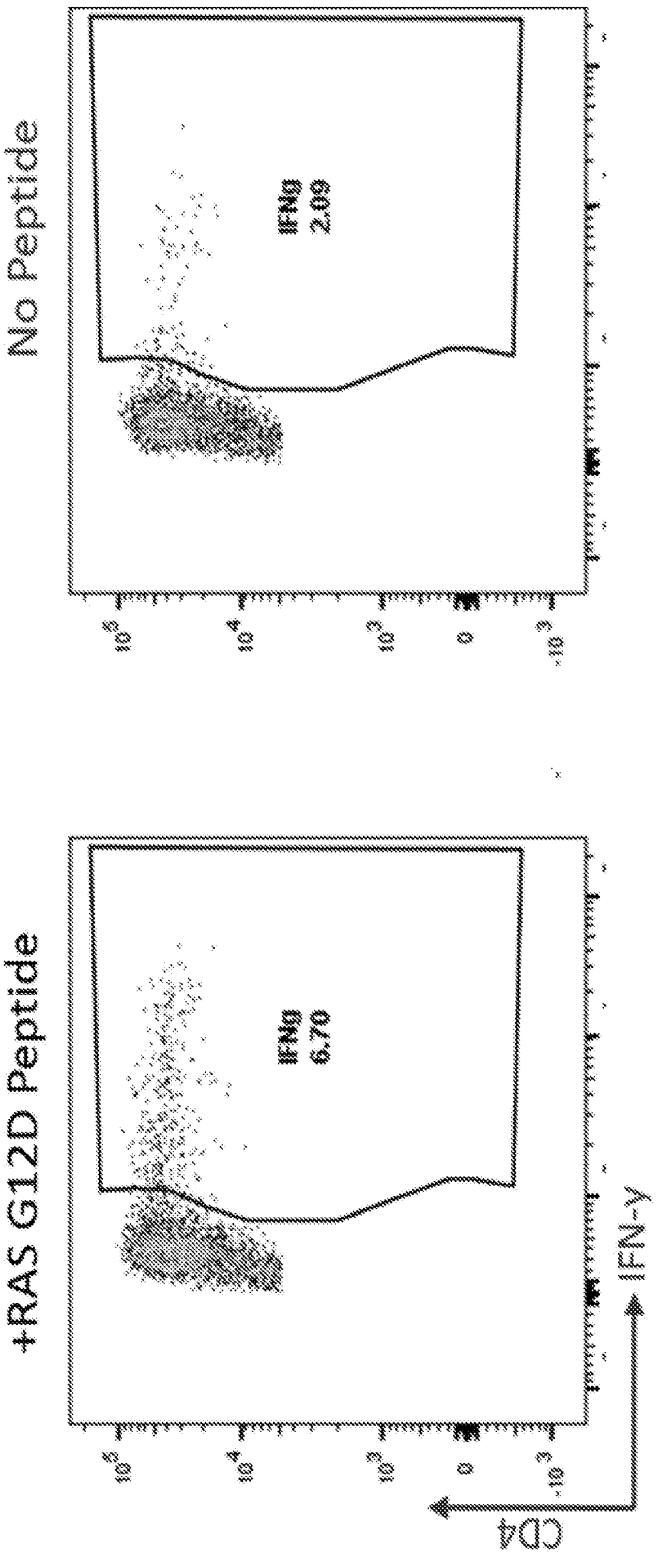


FIG. 18

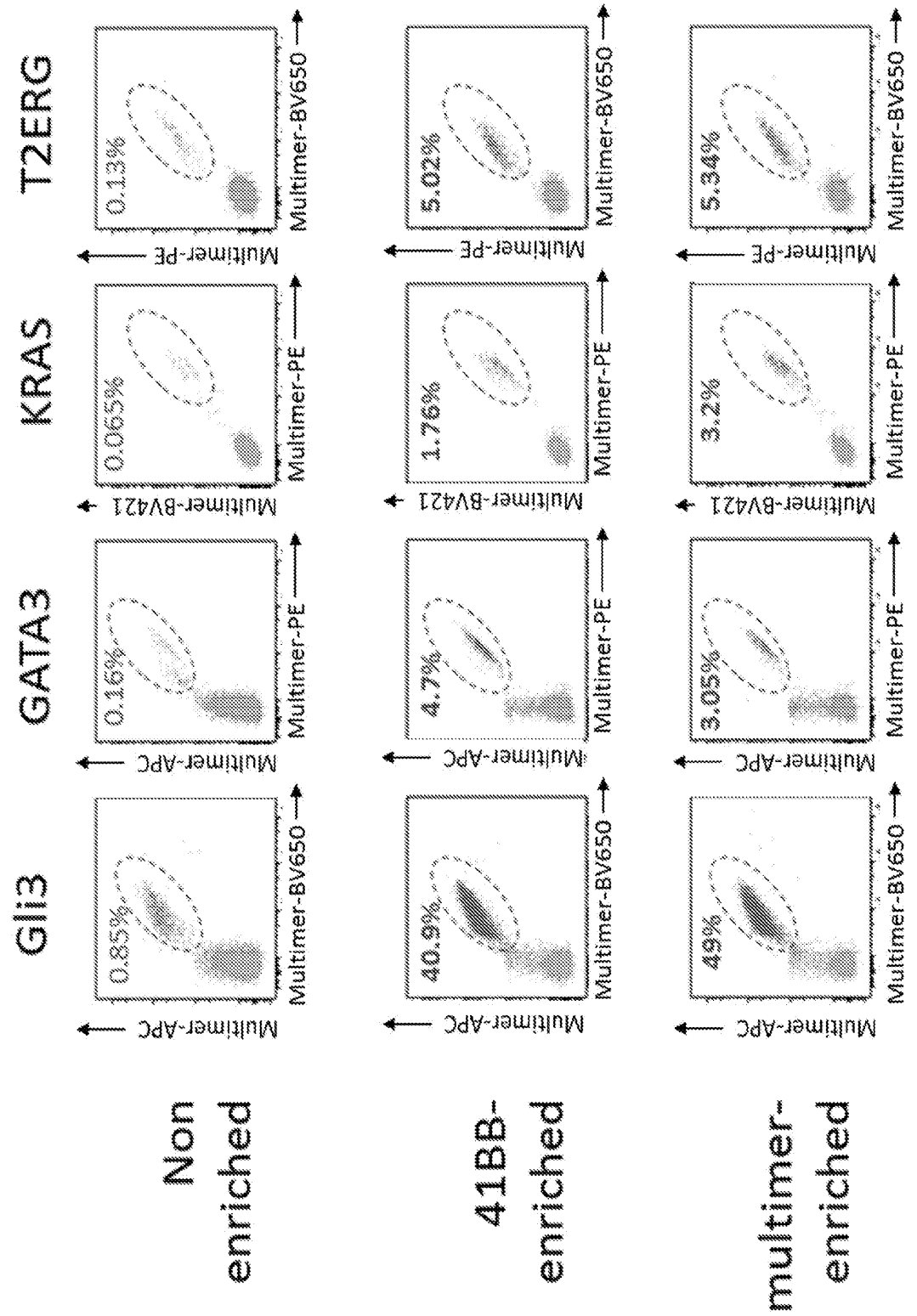

Gli3
GATA3
KRAS
T2ERG
Non enriched
41BB-enriched
multimer-enriched
0.85%
0.16%
0.065%
0.13%
40.9%
4.7%
1.76%
5.02%
49%
3.05%
3.2%
5.34%
Multimer-APC
Multimer-BV650
Multimer-BV421
Multimer-PE

FIG. 19A

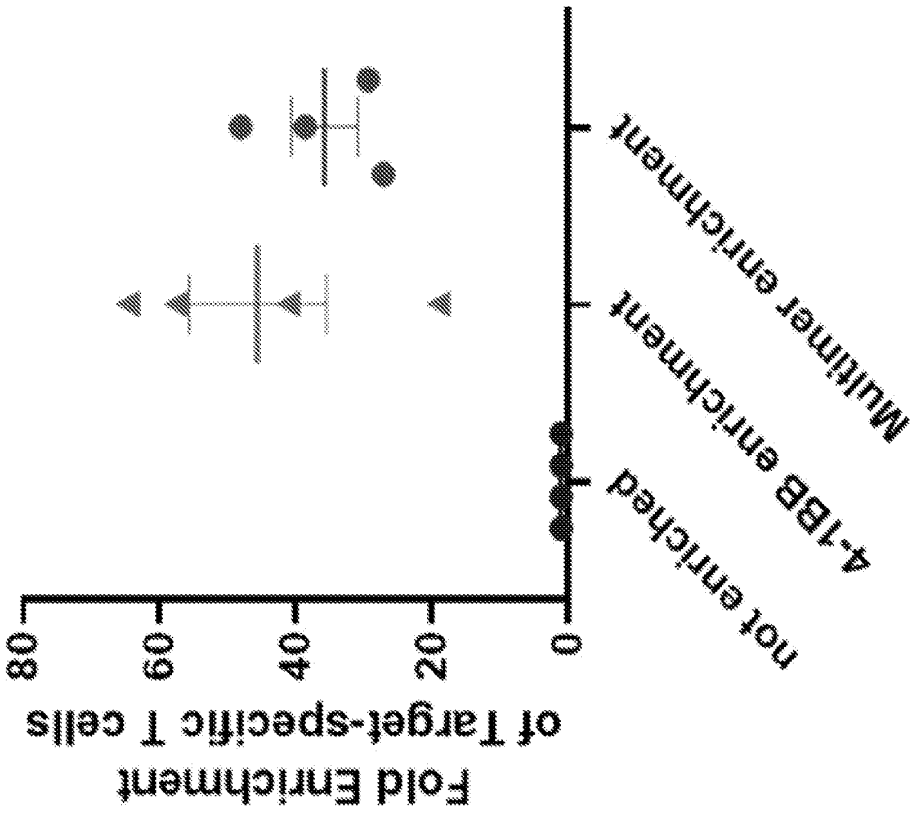

Fold Enrichment
of Target-specific T cells
0
20
40
60
80
not enriched
4-1BB enrichment
Multimer enrichment

FIG. 19B

Allele	Neoepitope	Observed MHC Affinity (nM)	Observed MHC Stability (hr)	Detected by MS?
A*02:01	MLTGPPARV	11	5.8	✔
	SMLTGPPARV	14	21.7	✔
	TLQRSSLWCL	118	0.5	-
	YMFLKAESKI	141	0.6	-
A*03:01	KIMFATLQR	3	5.6	✔
	YMFLKAESK	80	0.3	-
A*11:01	KIMFATLQR	23	8.9	✔
B*07:02	FATLQRSSL	14	0.7	-
	KPKRDGYMF	28	8.6	✔
	KPKRDGYMFL	98	3.3	✔
	GPPARVPAV	221	1.6	-
	MFATLQRSSL	267	0	-
B*08:01	ESKIMFATL	18	1.3	✔
	FLKAESKIM	22	1.2	-
	FATLQRSSL	27	0	-
	YMFLKAESKI	32	0.4	-
	IMKPKRDGYM	33	0.4	-
	MFATLQRSSL	53	0	-
	FLKAESKIMF	82	0	-
	LHFCRSSIM	119	0	-

FIG. 20

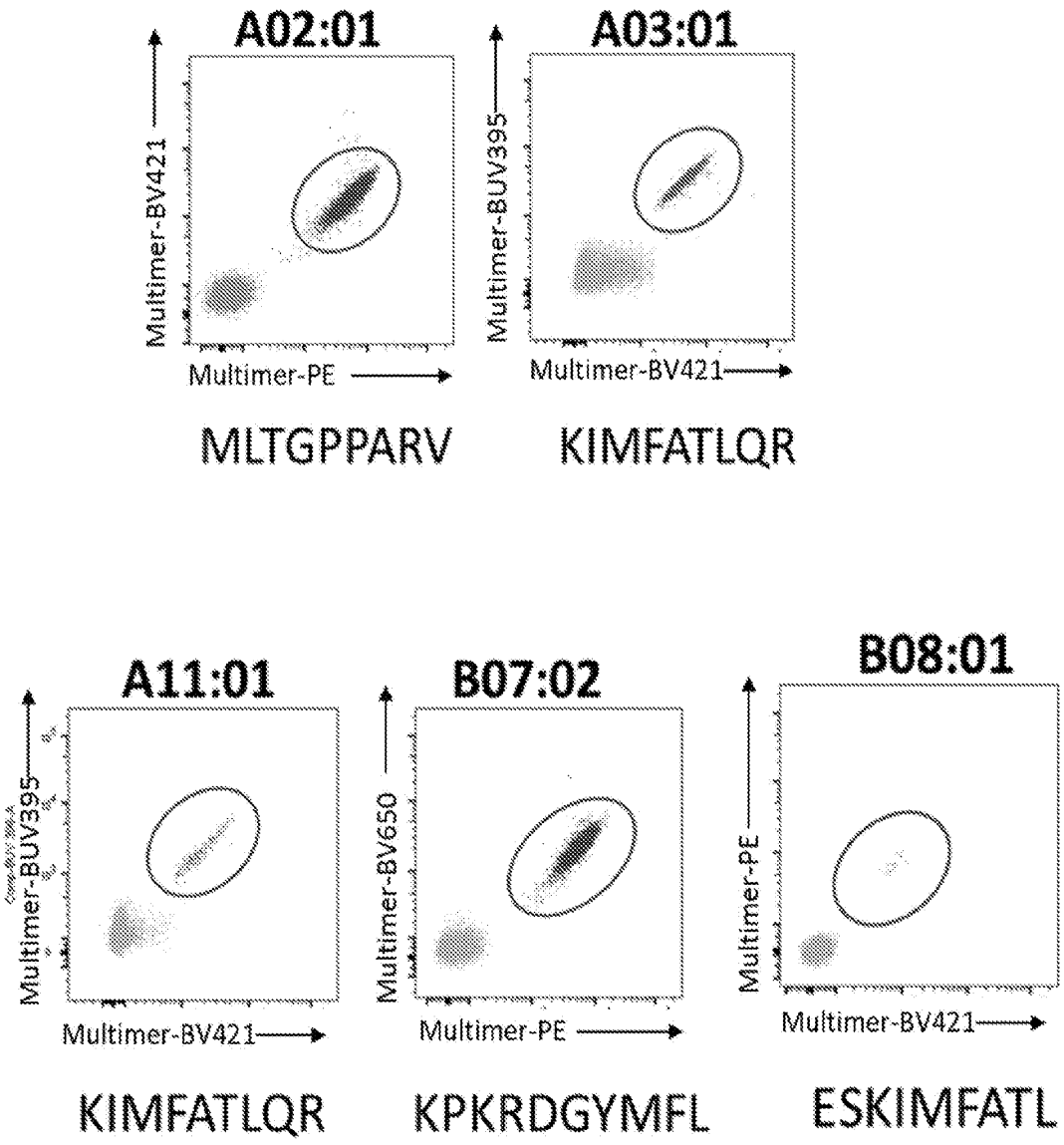

A02:01
Multimer-BV421
Multimer-PE
MLTGPPARV
A03:01
Multimer-BUV395
Multimer-BV421
KIMFATLQR
A11:01
Multimer-BUV395
Multimer-BV421
KIMFATLQR
B07:02
Multimer-BV650
Multimer-PE
KPKRDGYMFL
B08:01
Multimer-PE
Multimer-BV421
ESKIMFATL

FIG. 21

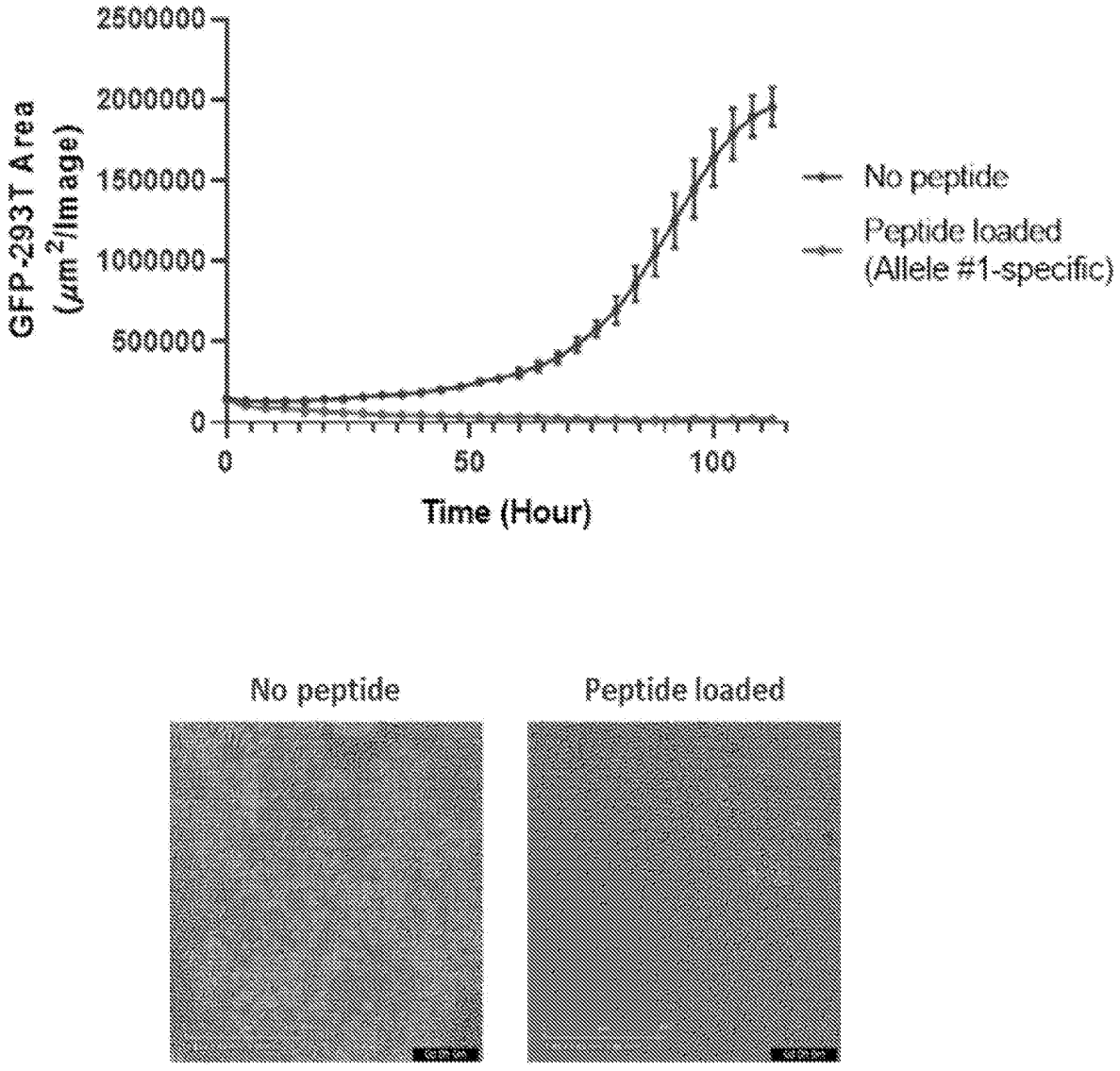

2500000
2000000
1500000
1000000
500000
0
GFP-293T Area
(μm²/Image)
0
50
100
Time (Hour)
No peptide
Peptide loaded
(Allele #1-specific)
No peptide
Peptide loaded
Target cell: 293T-GFP cells +/- 2uM peptide
E specific T cells:T = 0.7:1

FIG. 22

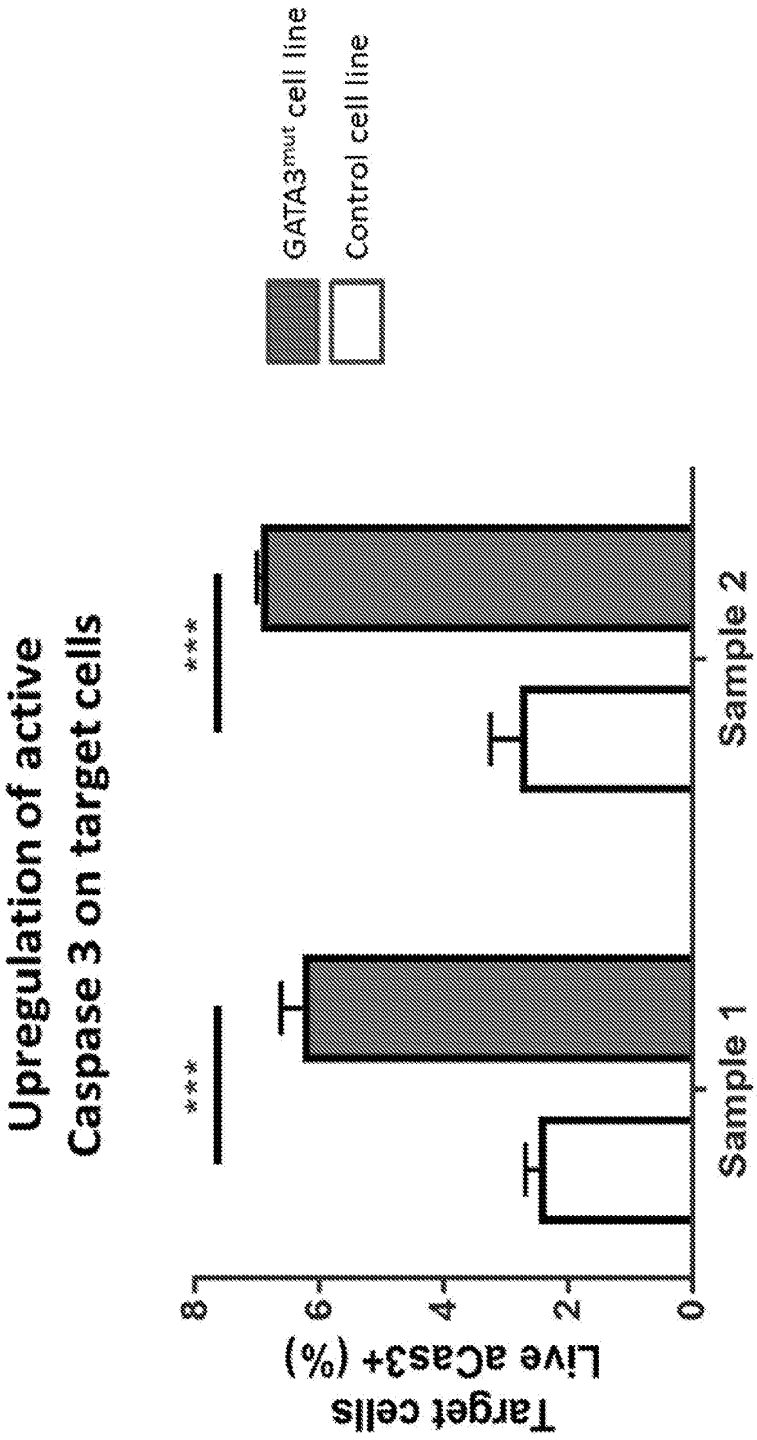

Upregulation of active
Caspase 3 on target cells
GATA3mut cell line
Control cell line
***
***
Target cells
Live aCas3+ (%)
0
2
4
6
8
Sample 1
Sample 2

FIG. 23

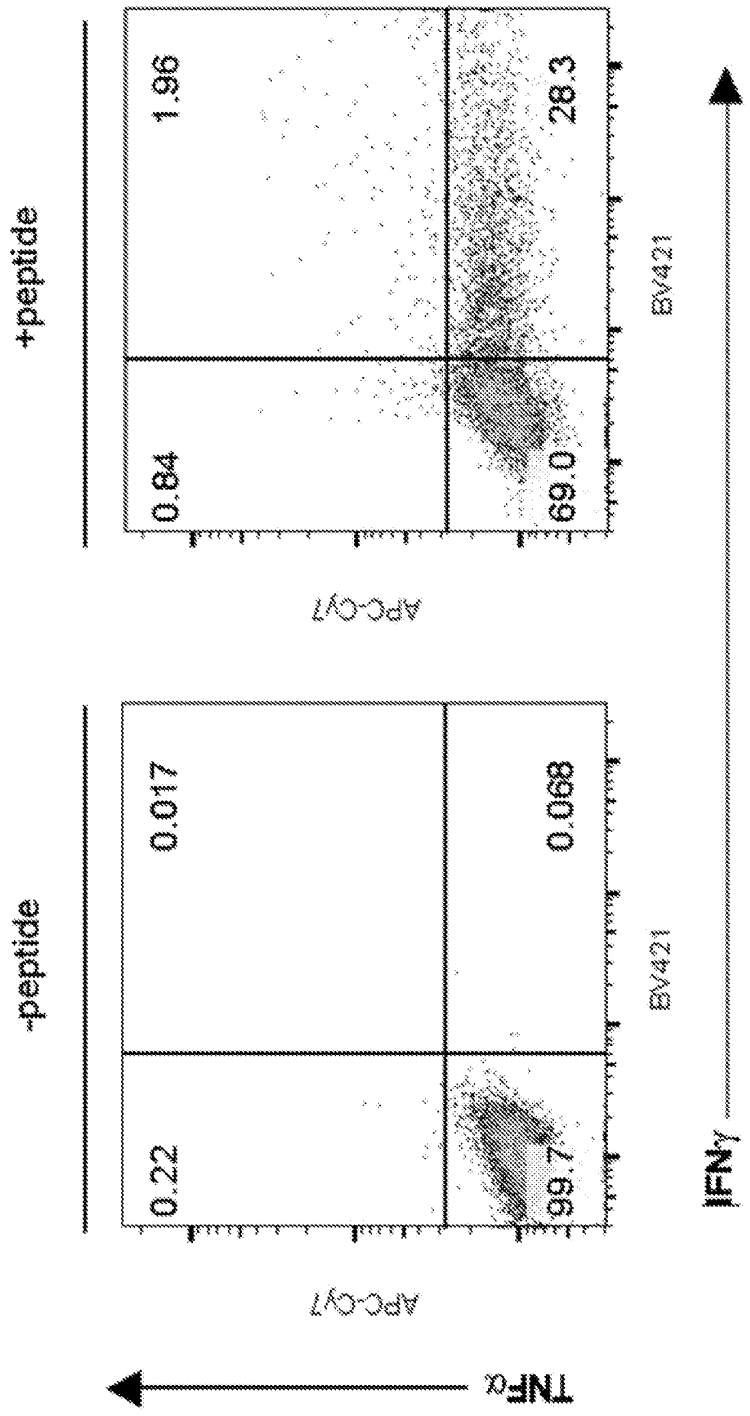

+peptide
1.96
28.3
0.84
69.0
APC-Cy7
BV421
+peptide
0.017
0.068
0.22
99.7
APC-Cy7
BV421
TNFα
IFNγ

FIG. 24

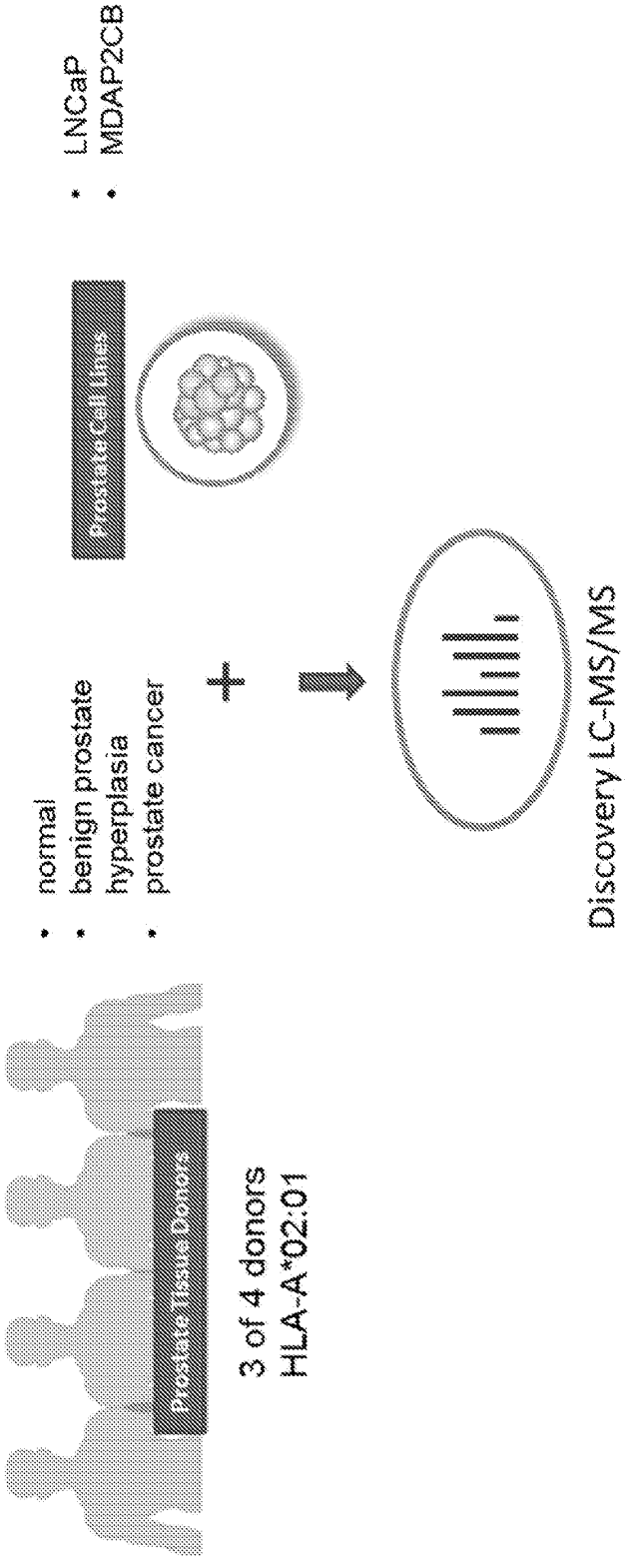

Prostate Cell Lines
LNCaP
MDAP2CB
normal
benign prostate hyperplasia
prostate cancer
+
Discovery LC-MS/MS
Prostate Tissue Donors
3 of 4 donors
HLA-A*02:01

FIG. 25A

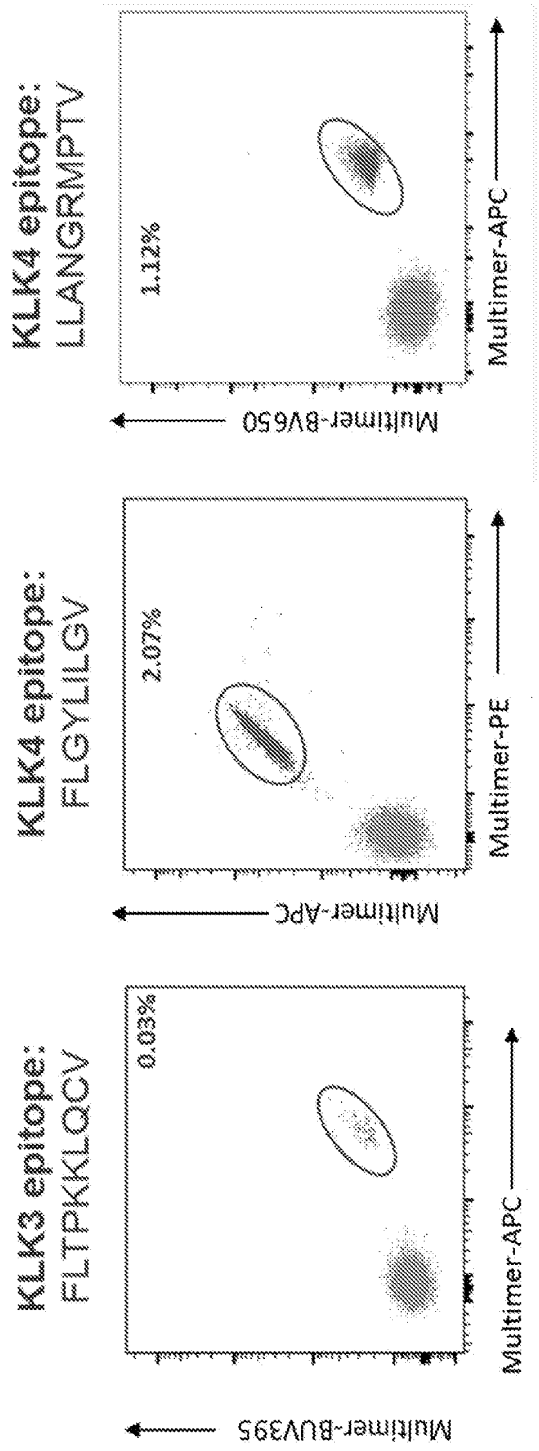

NEO-STIM Validation (A02:01)
KLK3 epitope: FLTPKKLQCV
0.03%
Multimer-BUV395
Multimer-APC
KLK4 epitope: FLGYLILGV
2.07%
Multimer-APC
Multimer-PE
KLK4 epitope: LLANGRMPTV
1.12%
Multimer-BV650
Multimer-APC

FIG. 25B

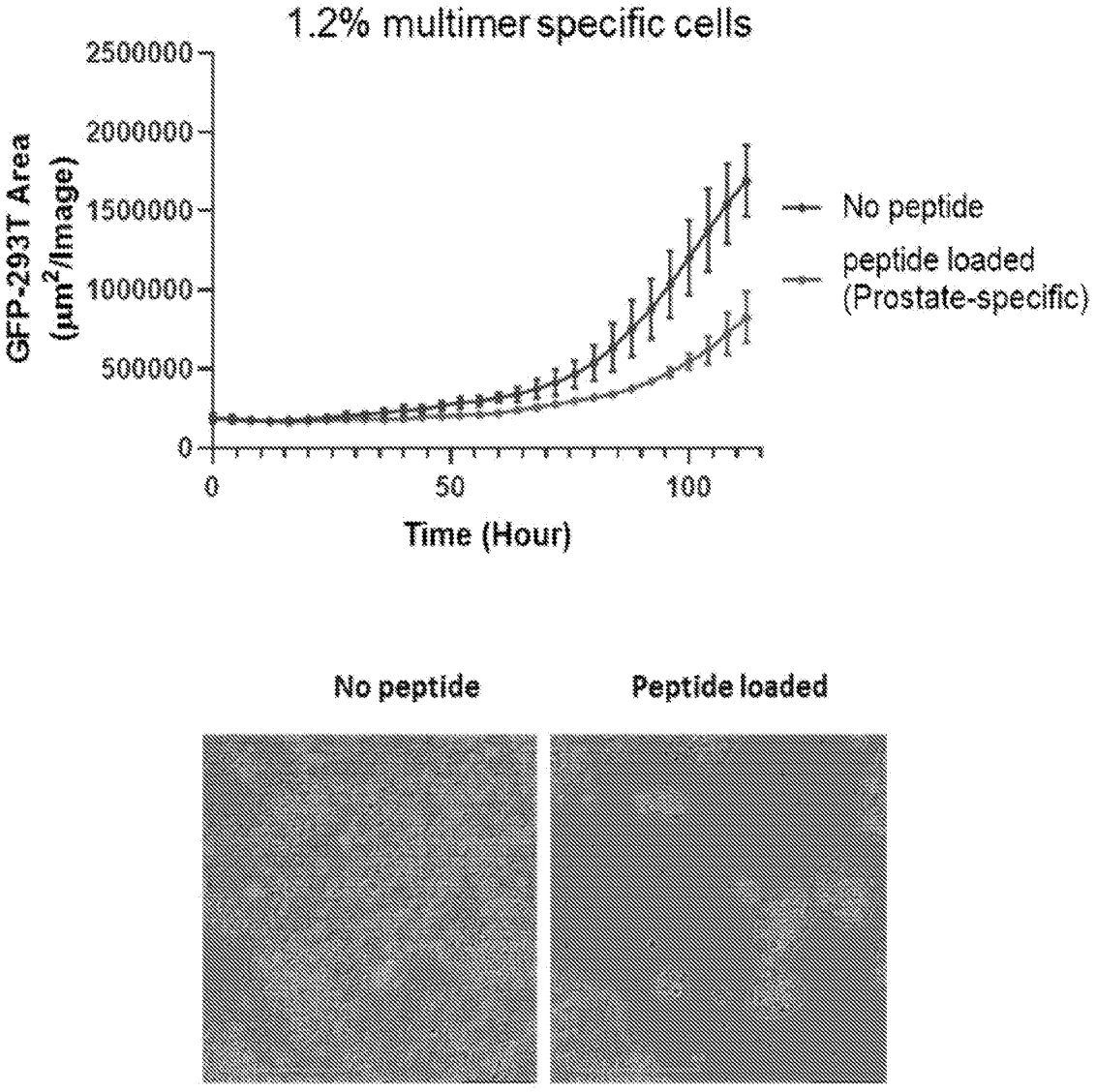

1.2% multimer specific cells
GFP-293T Area (μm²/Image)
2500000
2000000
1500000
1000000
500000
0
0
50
100
Time (Hour)
No peptide
peptide loaded (Prostate-specific)
No peptide
Peptide loaded
Target cell: 293T-GFP cells +/- 2uM peptide
Effector specific T cells:Target = 0.01:1

FIG. 25C

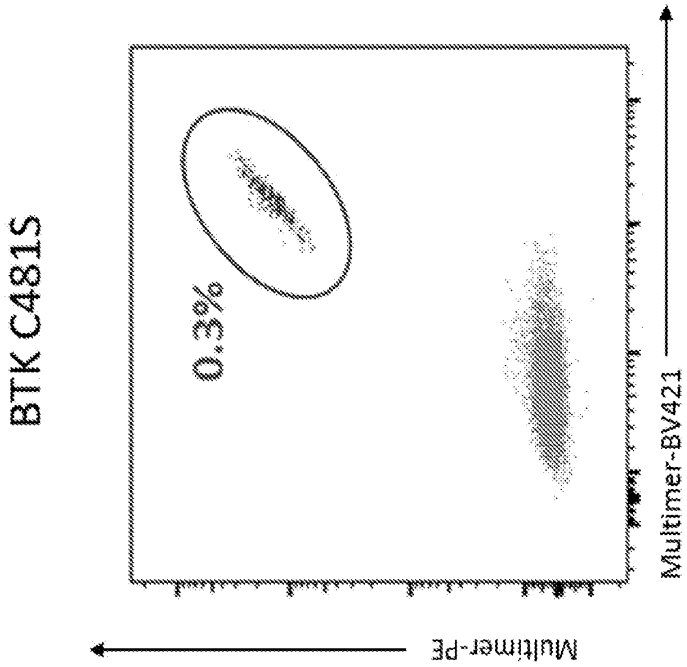


FIG. 26

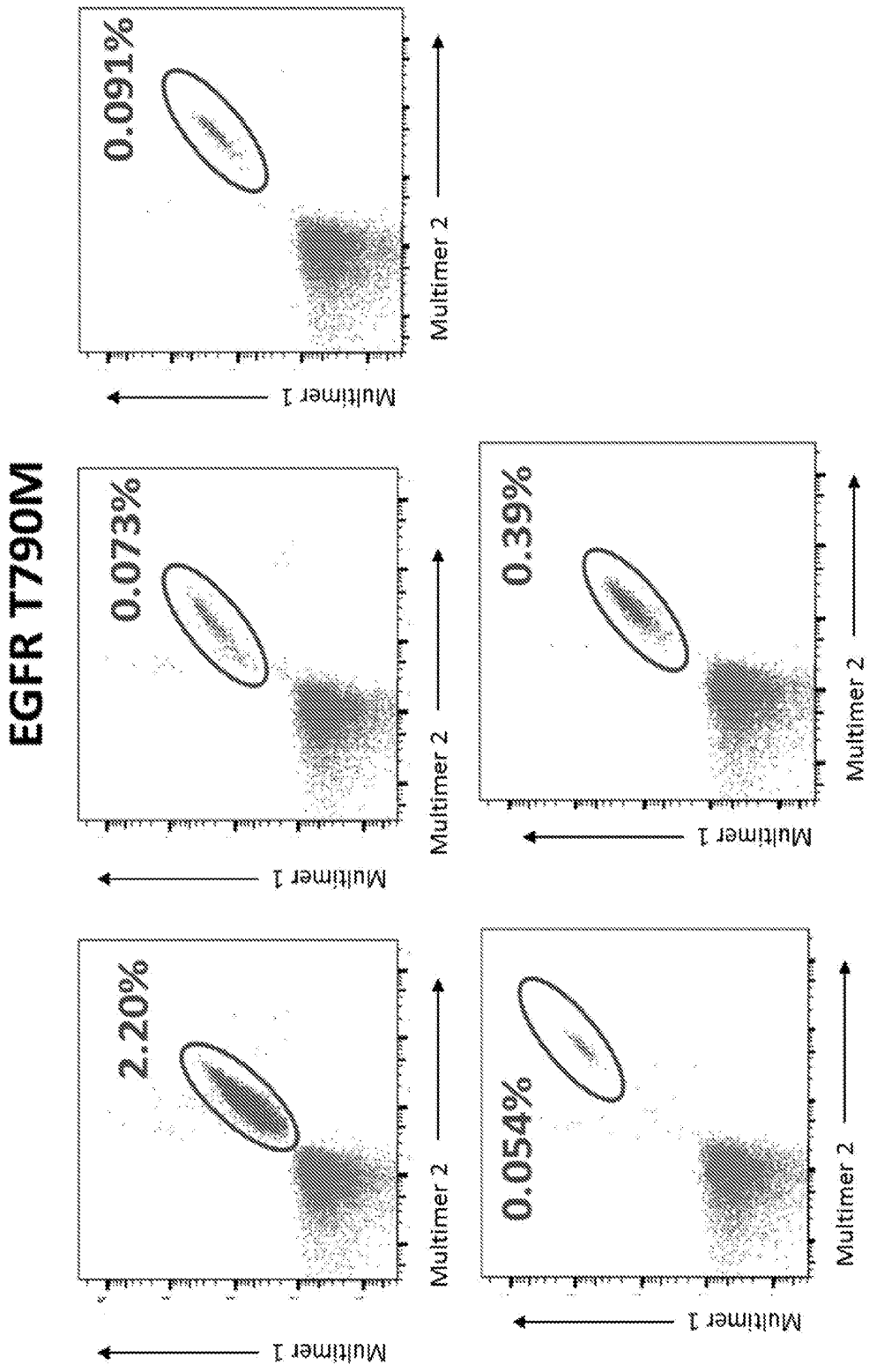


FIG. 27

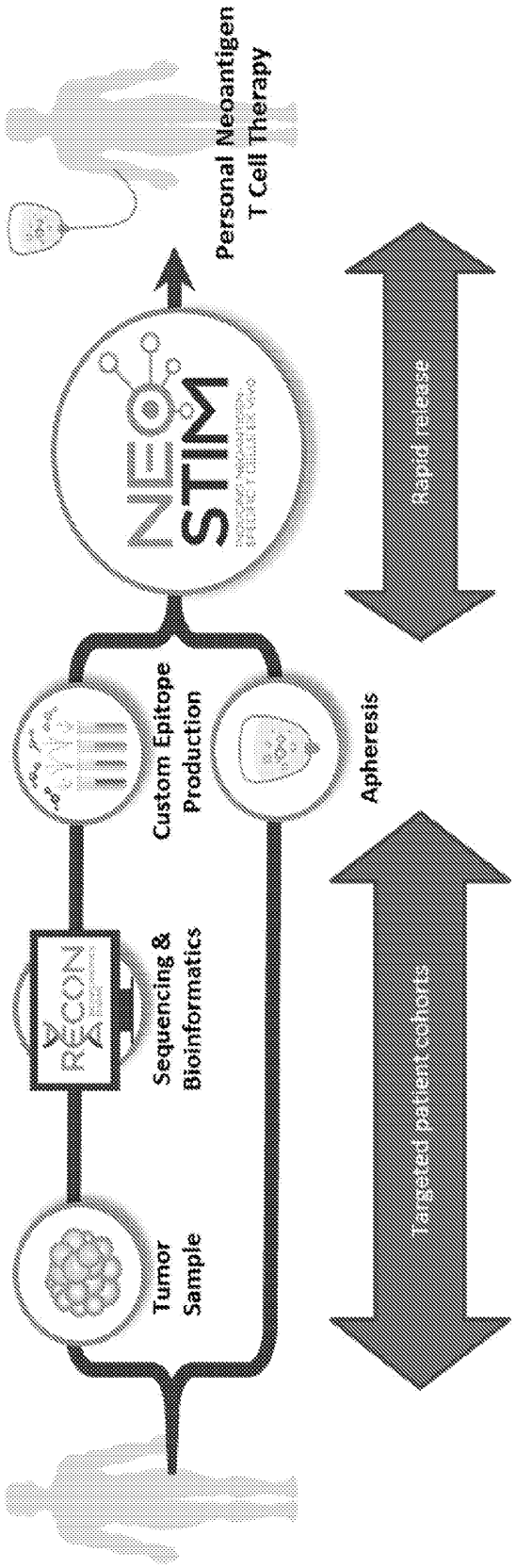


FIG. 28A

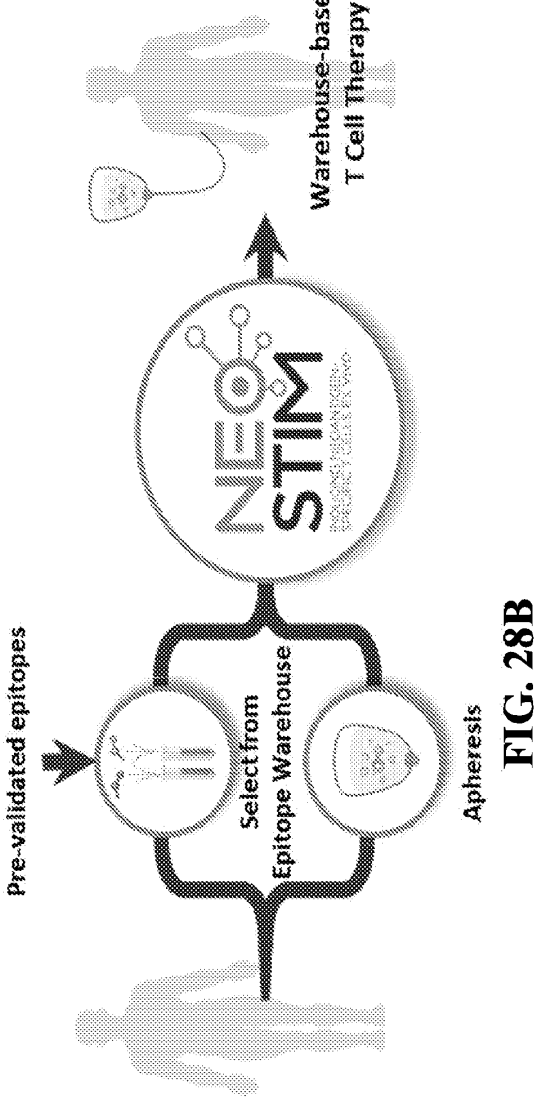


FIG. 28B

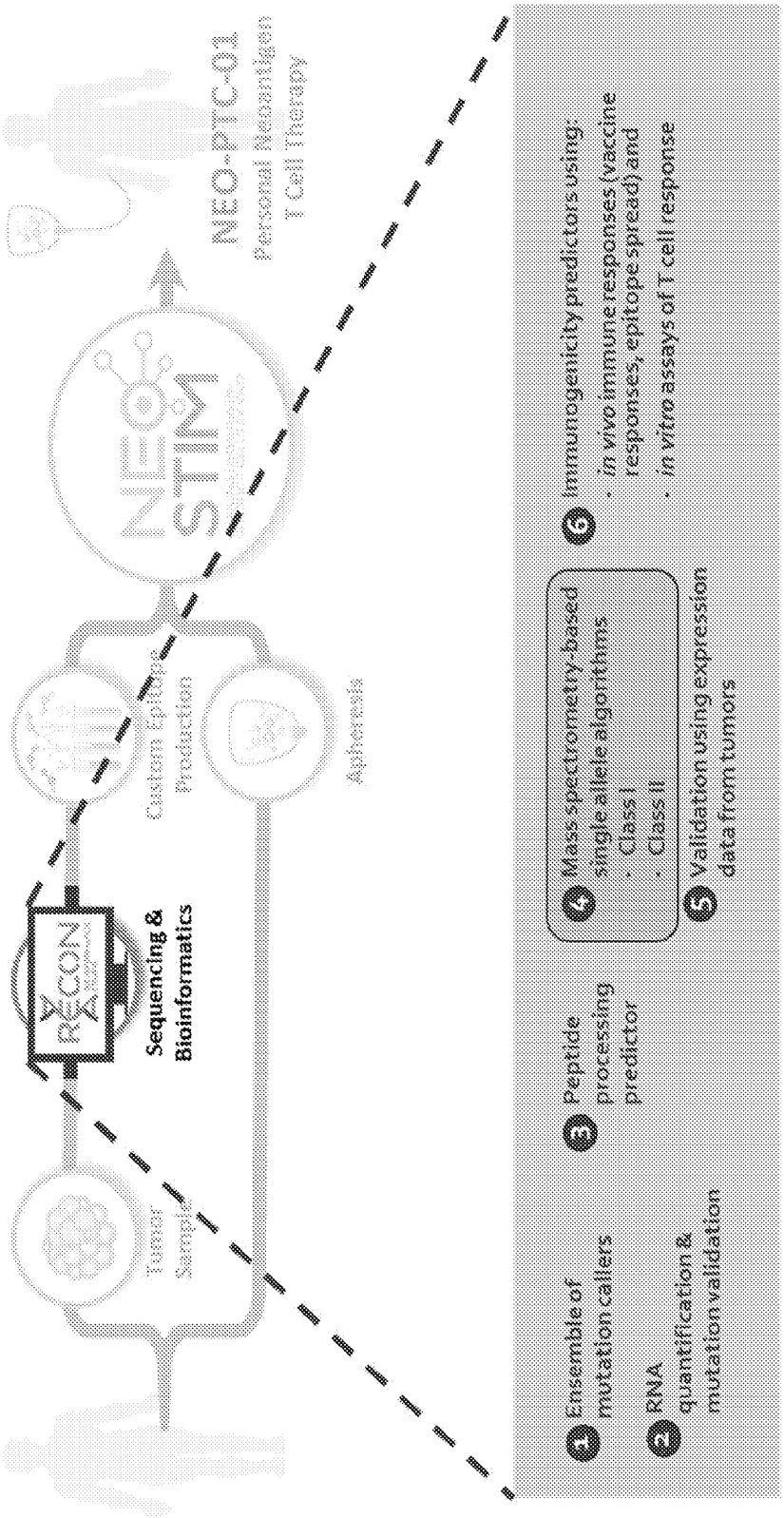

NEO-PTC-01
Personal Neoantigen T Cell Therapy
Sequencing & Bioinformatics
Custom Epitope Production
Apheresis
Tumor Sample
1 Ensemble of mutation callers
2 RNA quantification & mutation validation
3 Peptide processing predictor
4 Mass spectrometry-based single allele algorithms
· Class I
· Class II
5 Validation using expression data from tumors
6 Immunogenicity predictors using
· in vivo immune responses (vaccine responses, epitope spread) and
· in vitro assays of T cell response

FIG. 29

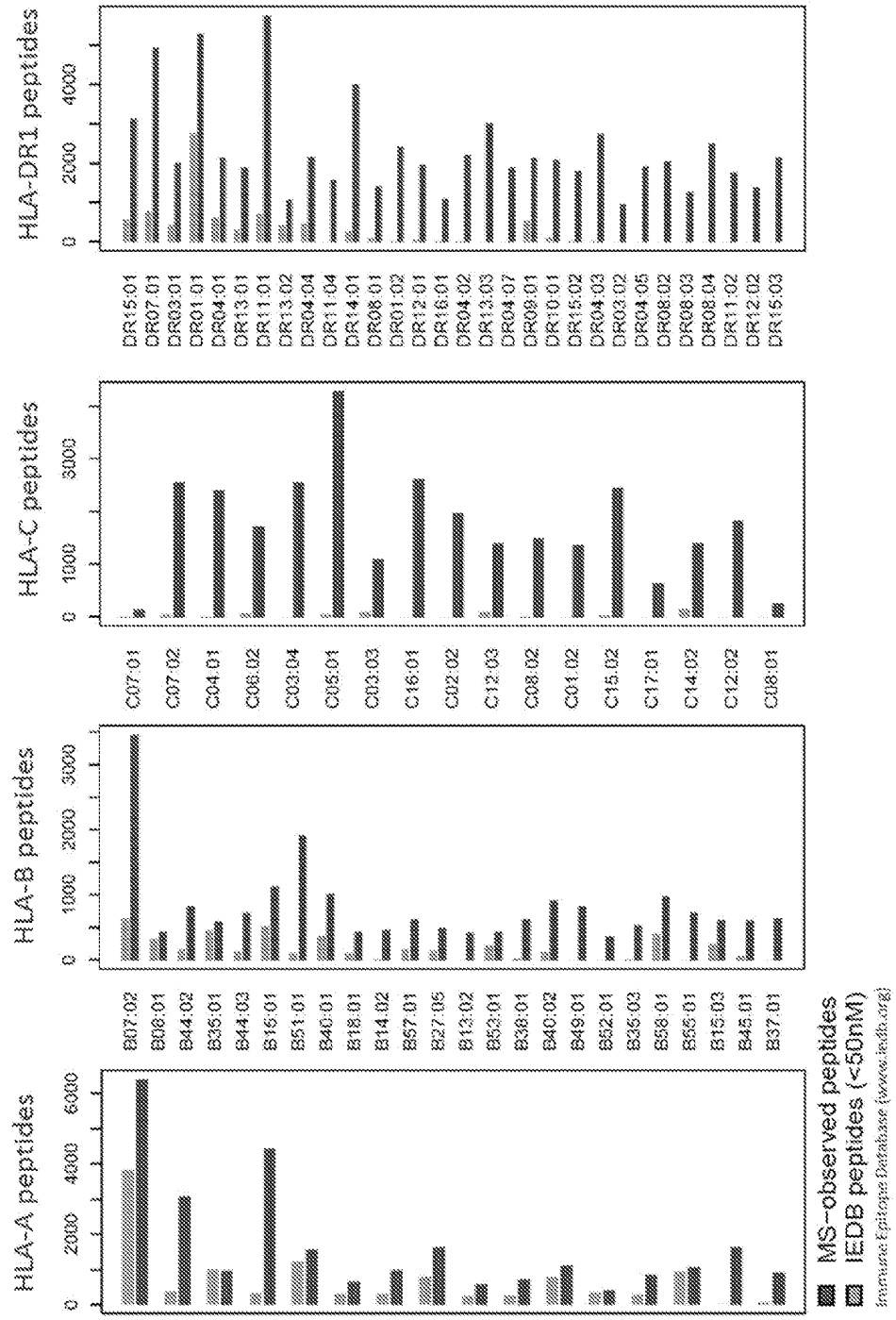
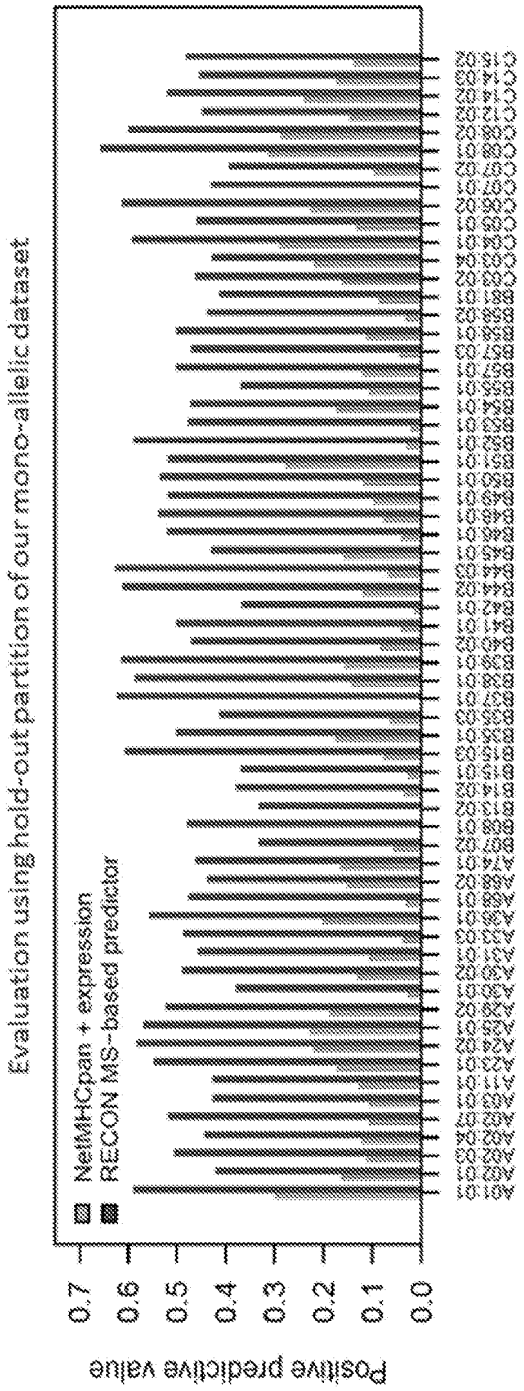

HLA-A peptides
HLA-B peptides
HLA-C peptides
HLA-DR1 peptides
Highest frequency alleles
Lower frequency alleles
A02:01
A01:01
A03:01
A24:02
A11:01
A29:02
A23:01
A68:01
A26:01
A32:01
A31:01
A30:01
A30:02
A68:02
A33:03
A25:01
B07:02
B08:01
B44:02
B35:01
B44:03
B15:01
B51:01
B40:01
B18:01
B14:02
B57:01
B27:05
B13:02
B53:01
B38:01
B40:02
B49:01
B52:01
B35:03
B58:01
B55:01
B15:03
B45:01
B37:01
C07:01
C07:02
C04:01
C06:02
C03:04
C05:01
C03:03
C16:01
C02:02
C12:03
C08:02
C01:02
C15:02
C17:01
C14:02
C12:02
C08:01
DR15:01
DR07:01
DR03:01
DR01:01
DR04:01
DR13:01
DR11:01
DR13:02
DR04:04
DR11:04
DR14:01
DR08:01
DR01:02
DR12:01
DR16:01
DR04:02
DR13:03
DR04:07
DR09:01
DR10:01
DR15:02
DR04:03
DR03:02
DR04:05
DR08:02
DR08:03
DR08:04
DR11:02
DR12:02
DR15:03
MS−observed peptides
IEDB peptides (<50nM)
Immune Epitope Database (www.iedb.org)

FIG. 30


Validation of T cell epitope prediction model on in vitro data set
Evaluation using hold-out partition of our mono-allelic dataset
NetMHCpan + expression
RECON MS-based predictor
Positive predictive value
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7

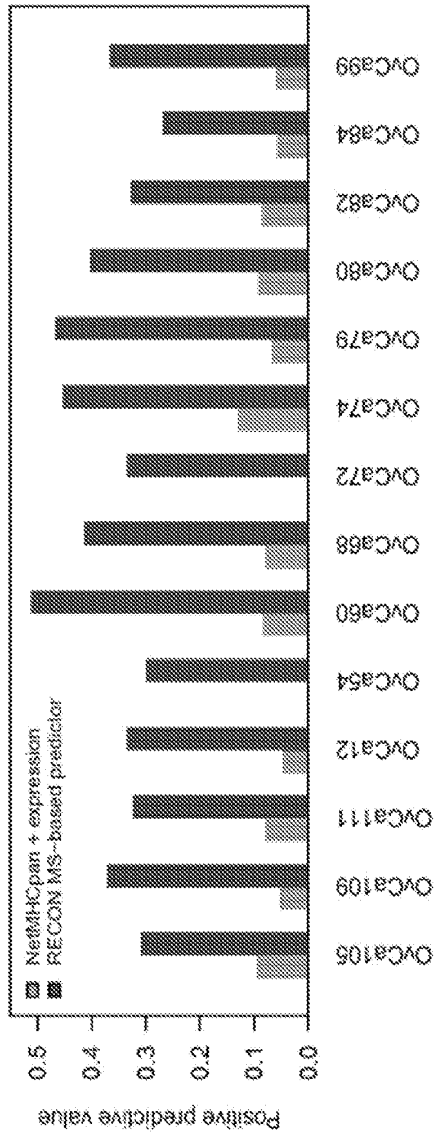

Validation of T cell epitope prediction model on in vivo tumor biopsy data set
Evaluation using ovarian tumors profiled by MS (Schuster, PNAS, 2017)
NetMHCpan + expression
RECON MS-based predictor
Positive predictive value
0.0
0.1
0.2
0.3
0.4
0.5
OvCa105
OvCa109
OvCa111
OvCa12
OvCa54
OvCa60
OvCa68
OvCa72
OvCa74
OvCa79
OvCa80
OvCa82
OvCa84
OvCa99

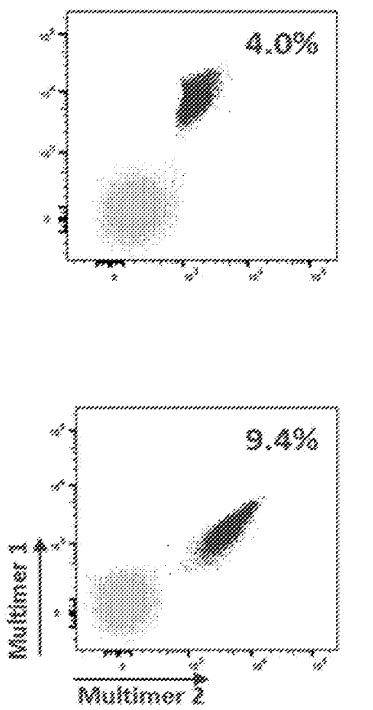
FIG. 31

A02:01 neoepitopes (melanoma samples)
High ranking RECON epitopes (top 0.3%)

Target gene	Mutation class	T cell Immunogenicity (NEO-STIM inductions)
C5orf42	missense	Confirmed
GLI3	missense	Confirmed
USP34	missense	Confirmed
SHANK2	missense	Confirmed
FAR1	missense	Confirmed
FLI1	missense	-
EPAS1	missense	Confirmed
GTPBP1	missense	Confirmed
IAH1	frameshift	Confirmed
CHD8	frameshift	-
ATP6AP2	missense	Confirmed
GLI3	missense	Confirmed
SERPINE2	missense	-

10/13 (77%) of RECON predicted epitopes validated by NEOSTIM

Example NEO-STIM T cell inductions to confirm immunogenicity



Dual multimer stain for neoantigen-specific T cells

FIG. 32

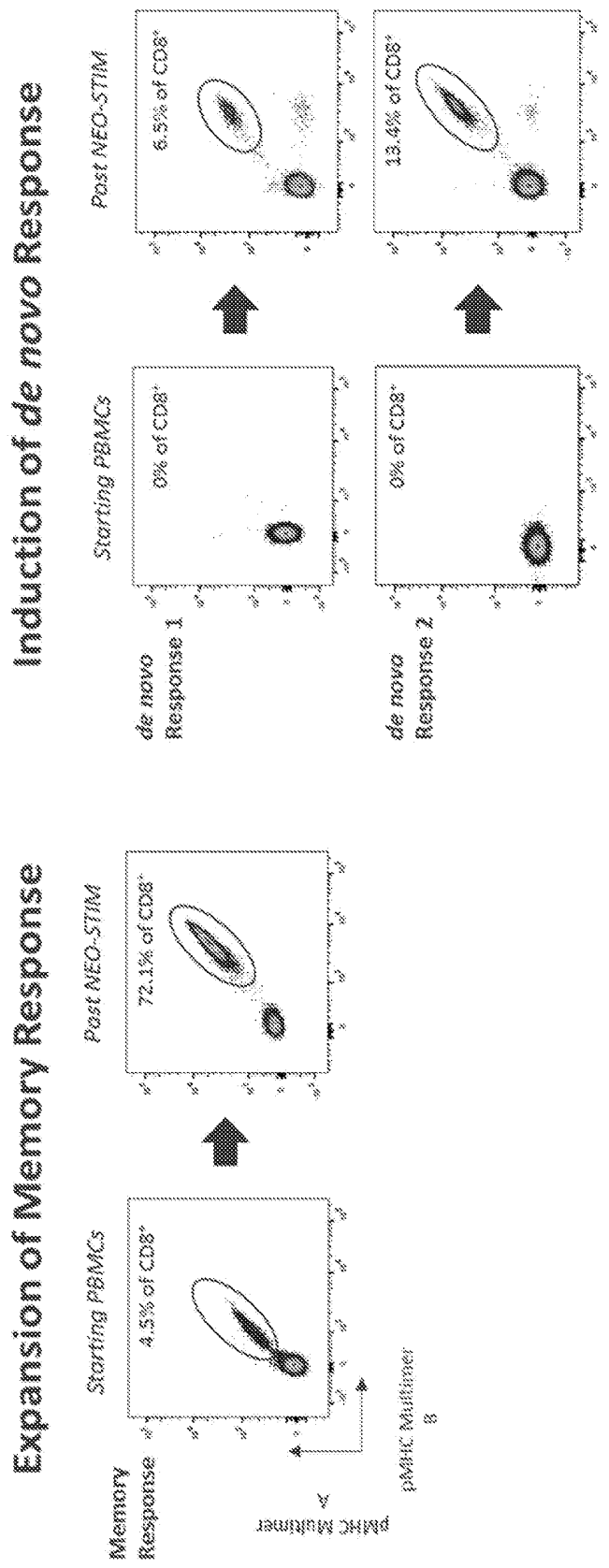

Induction of de novo Response
Starting PBMCs
Post NEO-STIM
de novo Response 1
0% of CD8+
6.5% of CD8+
de novo Response 2
0% of CD8+
13.4% of CD8+
Expansion of Memory Response
Starting PBMCs
Post NEO-STIM
Memory Response
4.5% of CD8+
72.1% of CD8+
pMHC Multimer A
pMHC Multimer B

FIG. 33

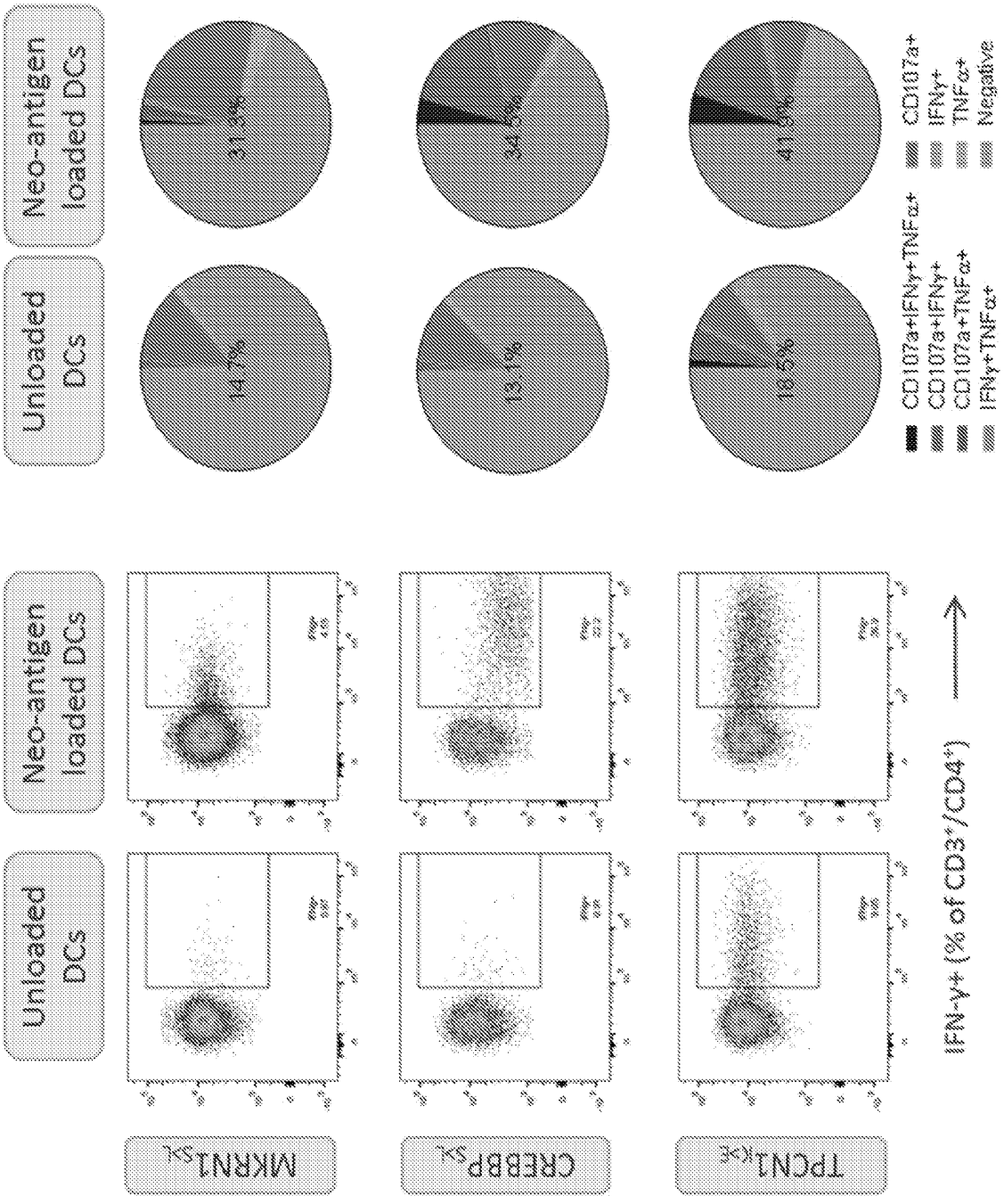

Unloaded DCs
Neo-antigen loaded DCs
MKRN1SxL
CREBBPSxL
TPCN1KxE
IFN-γ+ (% of CD3+/CD4+)
14.7%
31.3%
13.1%
34.5%
18.5%
41.3%
CD107a+IFNγ+TNFα+
CD107a+IFNγ+
CD107a+TNFα+
IFNγ+TNFα+
CD107a+
IFNγ+
TNFα+
Negative

FIG. 34

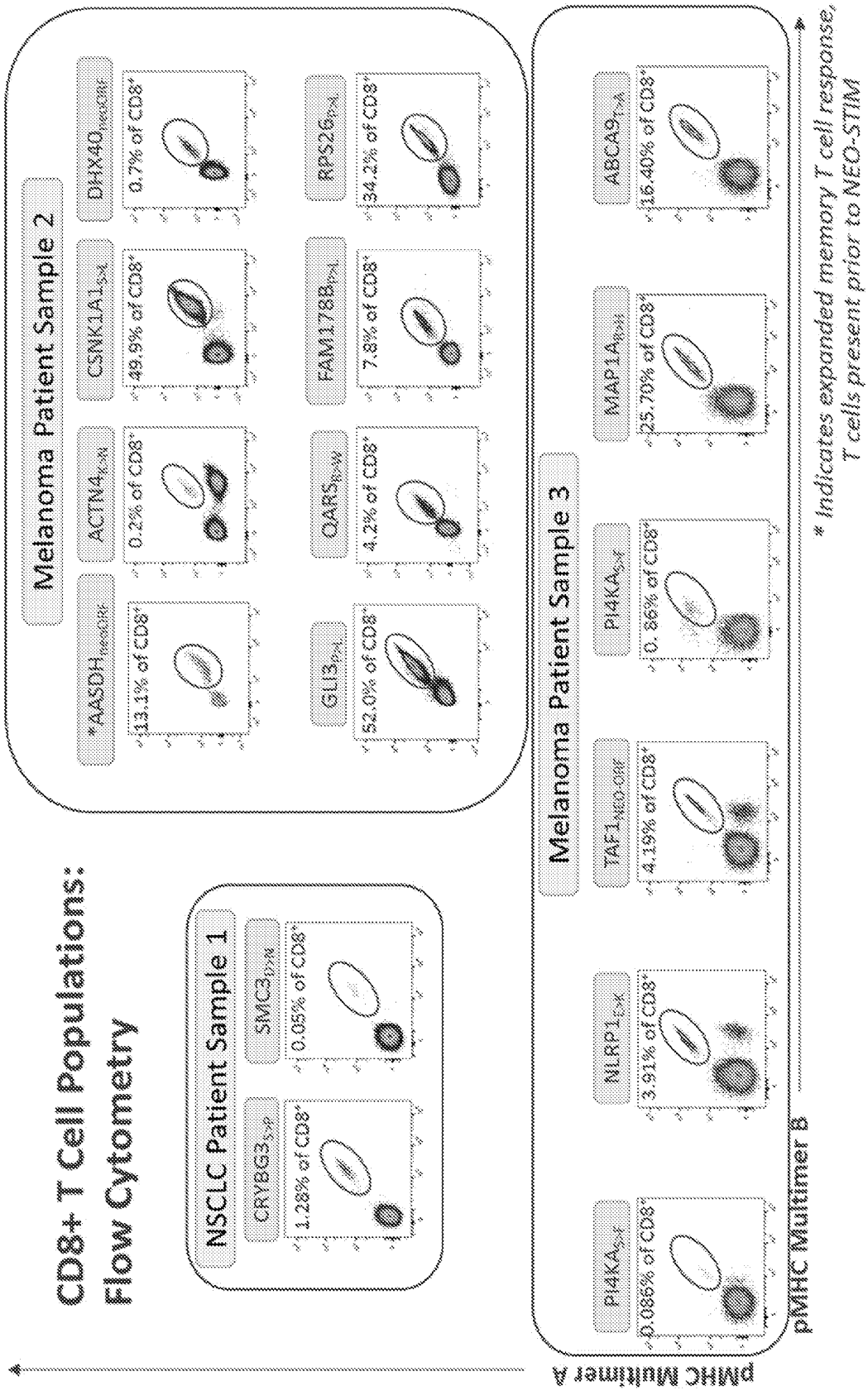

CD8+ T Cell Populations: Flow Cytometry
NSCLC Patient Sample 1
CRYBG3 E>K
1.28% of CD8+
SMC3 E>K
0.05% of CD8+
Melanoma Patient Sample 2
*AASDH TRIM33 R>K
13.1% of CD8+
ACTN4 R>K
0.2% of CD8+
CSNK1A1 S>A
49.9% of CD8+
DHX40 TRIM28 R>K
0.7% of CD8+
GLI3 R>K
52.0% of CD8+
OARS R>K
4.2% of CD8+
FAM178B R>K
7.8% of CD8+
RPS26 R>K
34.2% of CD8+
Melanoma Patient Sample 3
PI4KA S>F
0.086% of CD8+
NLRP1 E>K
3.91% of CD8+
TAF1 NEO-ORF
4.19% of CD8+
PI4KA S>F
0. 86% of CD8+
MAP1A R>H
25.70% of CD8+
ABCA9 R>A
16.40% of CD8+
pMHC Multimer A
pMHC Multimer B
* Indicates expanded memory T cell response, T cells present prior to NEO-STIM

FIG. 35

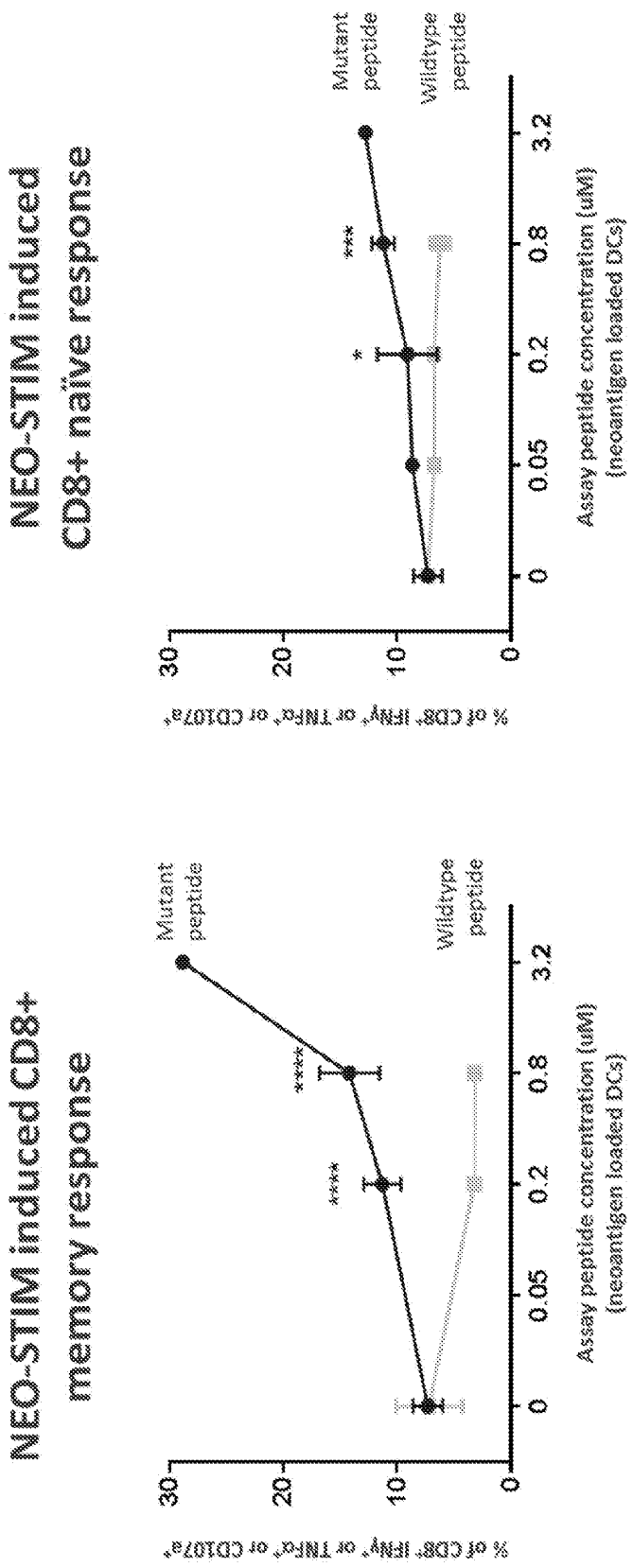

NEO-STIM induced CD8+ naïve response
NEO-STIM induced CD8+ memory response
% of CD8+ IFNγ+ or TNFα+ or CD107a+
Assay peptide concentration (uM) (neoantigen loaded DCs)
Mutant peptide
Wildtype peptide
0
10
20
30
0
0.05
0.2
0.8
3.2
*
***
****
****

FIG. 36

NEO-STIM induced T cells demonstrate cytokine release (IFNγ and TNFα) and degranulation (CD107a) upon activation
3 markers: IFNγ + TNFα + CD107a
2 markers observed
1 marker observed
No activation markers
Control
Neoantigen-activated
CD8+ memory response
7.2%
16.9%
CD8+ de novo response
6.9%
65.5%
CD4+ de novo response
13.1%
34.5%

FIG. 37

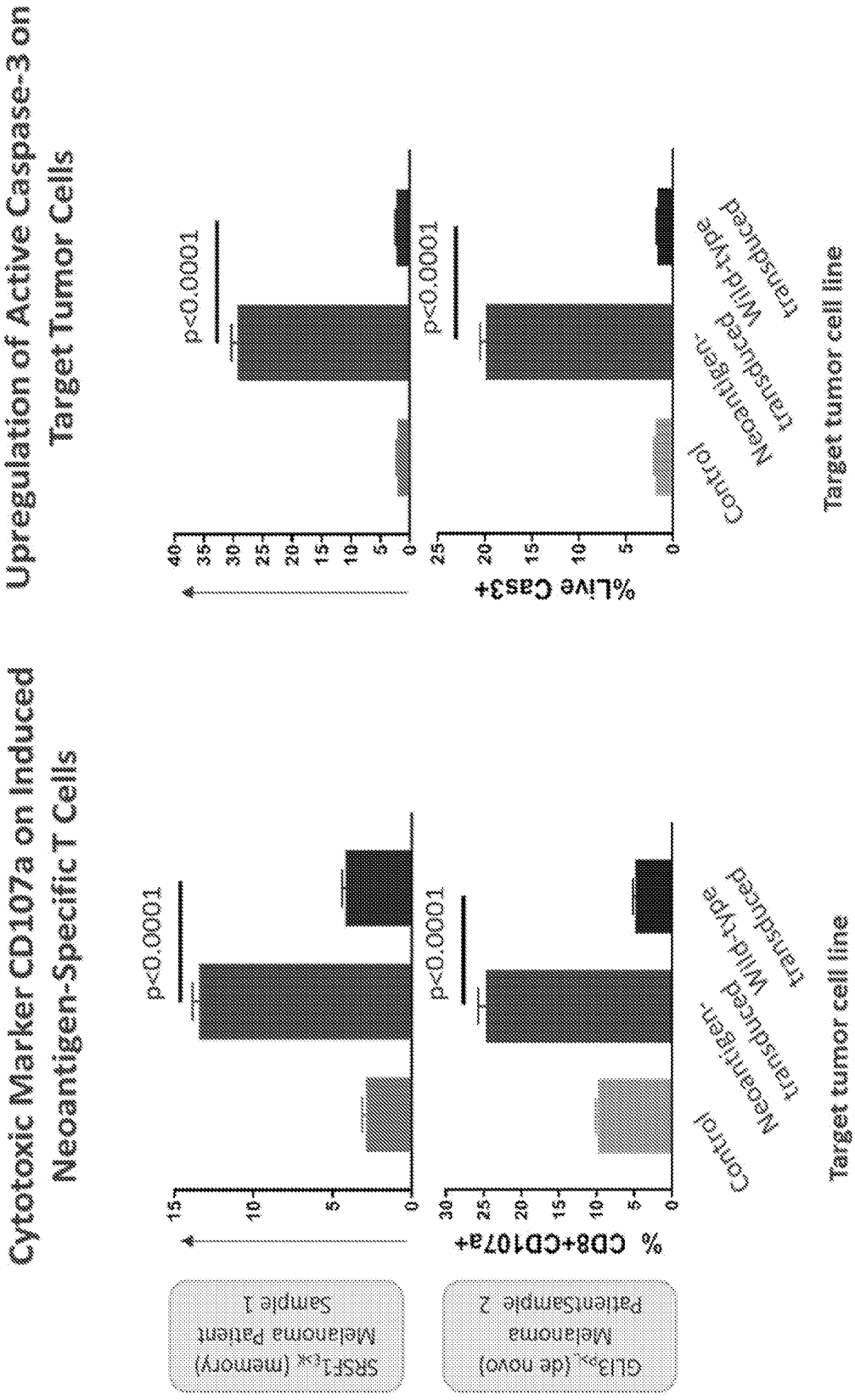

Upregulation of Active Caspase-3 on Target Tumor Cells
%Live Cas3+
p<0.0001
p<0.0001
Control
Neoantigen-transduced
Wild-type transduced
Target tumor cell line
Cytotoxic Marker CD107a on Induced Neoantigen-Specific T Cells
% CD8+CD107a+
p<0.0001
p<0.0001
GL13$_{K}$ (de novo) Melanoma PatientSample 2
SRSF1$_{K}$ (memory) Melanoma Patient Sample 1
Control
Neoantigen-transduced
Wild-type transduced
Target tumor cell line

FIG. 38