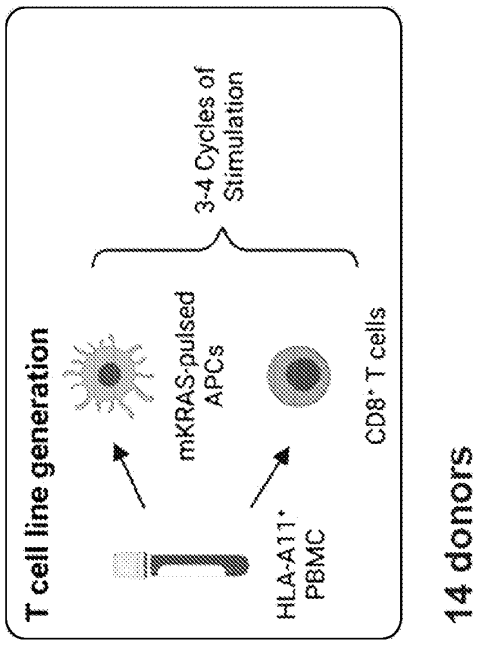

T cell line generation
HLA-A11+ PBMC
mKRAS-pulsed APCs
CD8+ T cells
3-4 Cycles of Stimulation
14 donors
No peptide
+mKRAS G12V peptide
TNFα
IFNγ
0.06
6
0.025
99.4
0.54
0.23
31.7
16.1
51.9

FIG. 1A

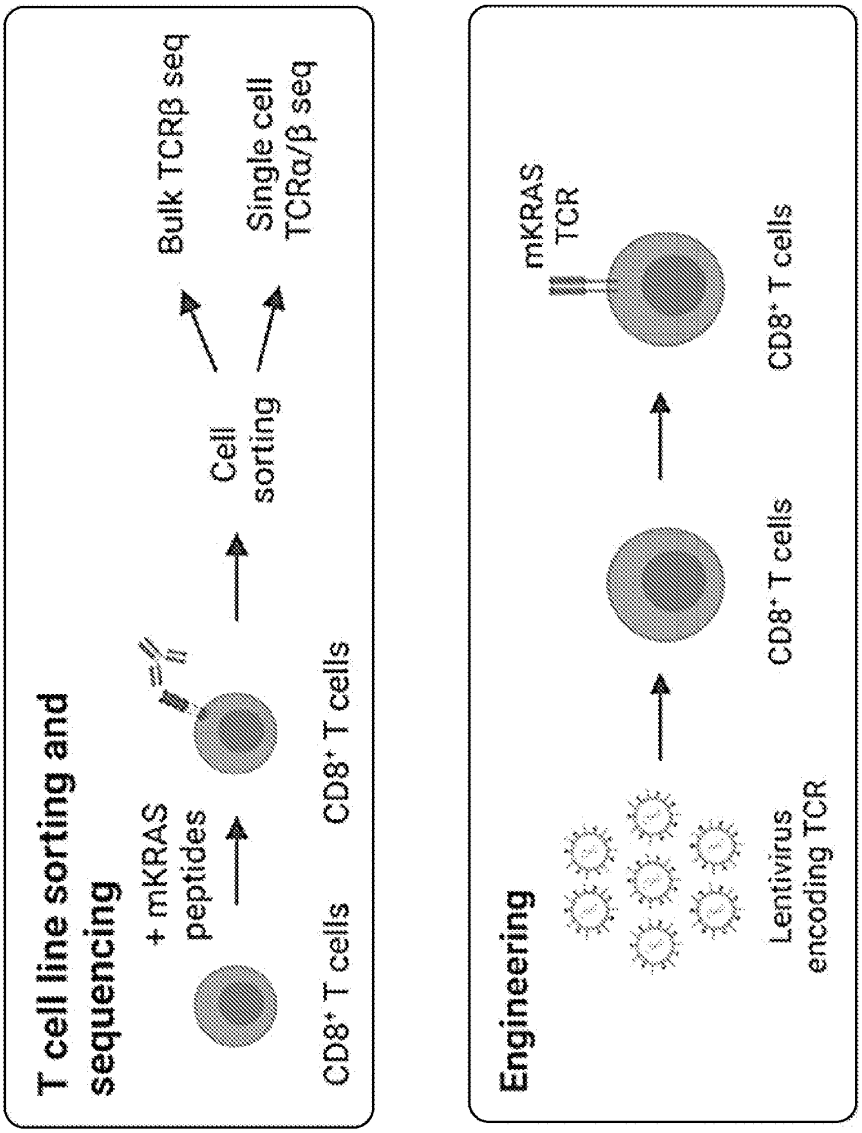

T cell line sorting and sequencing
+ mKRAS peptides
CD8+ T cells
CD8+ T cells
Cell sorting
Bulk TCRβ seq
Single cell TCRα/β seq
Engineering
Lentivirus encoding TCR
CD8+ T cells
CD8+ T cells
mKRAS TCR

FIG. 1B

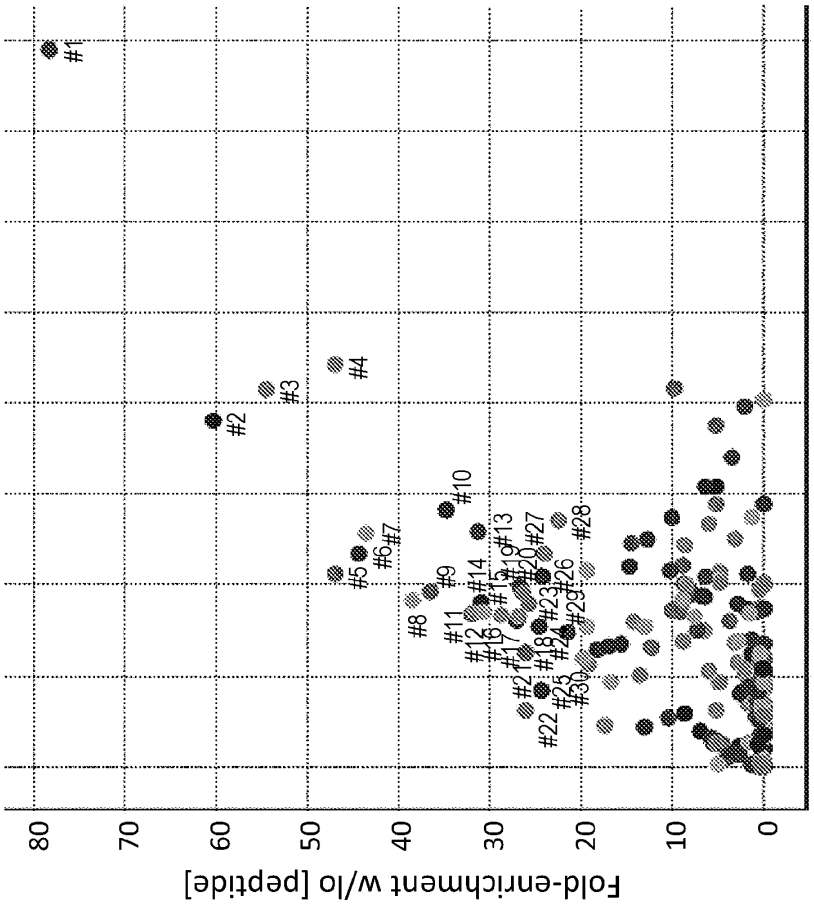

Fold-enrichment w/lo [peptide]
Fold-enrichment w/hi [peptide]
0
10
20
30
40
50
60
70
80
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
#13
#14
#15
#16
#17
#18
#19
#20
#21
#22
#23
#24
#25
#26
#27
#28
#29
#30

FIG. 1C

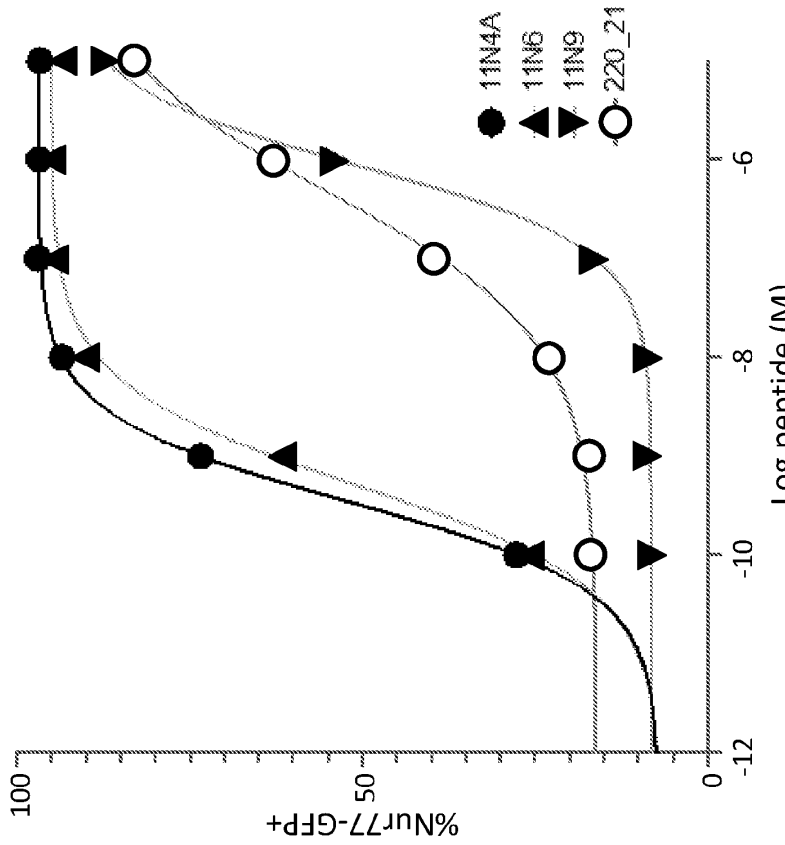


FIG. 1D

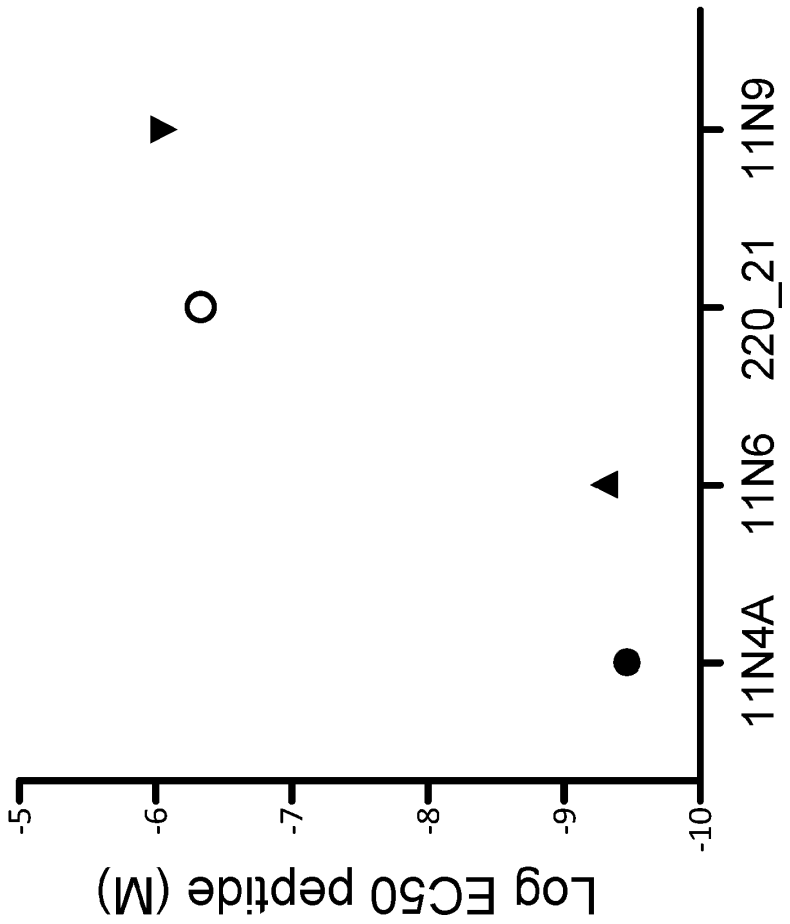


FIG. 1E

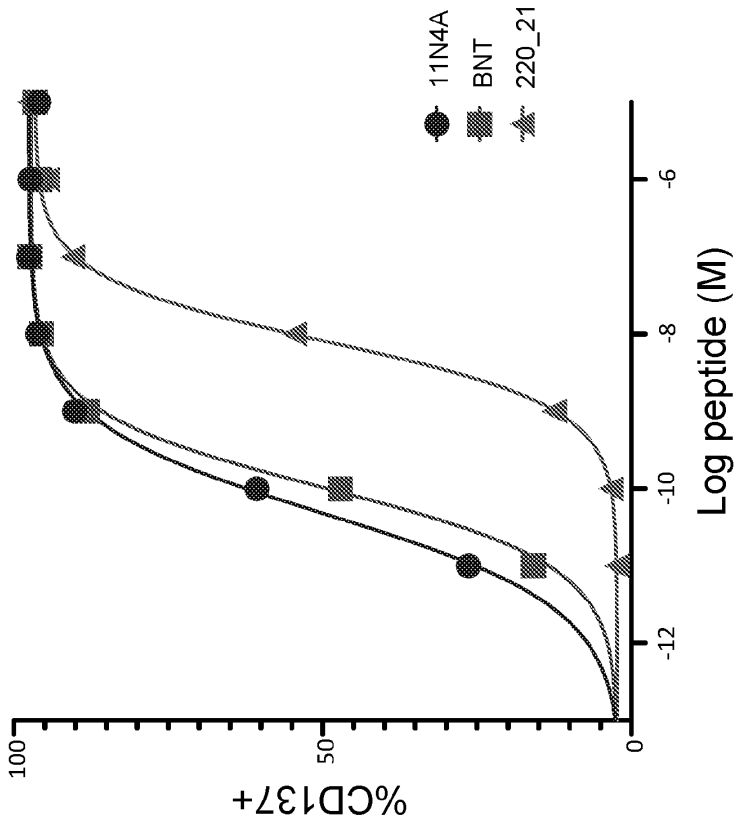


FIG. 2A

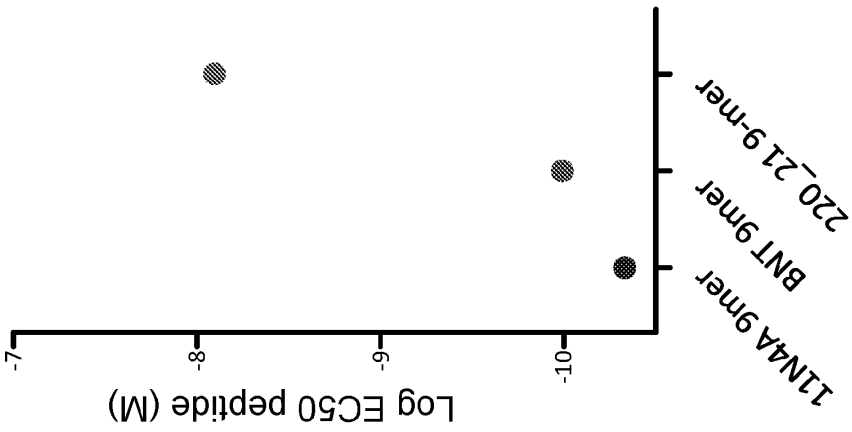


FIG. 2B

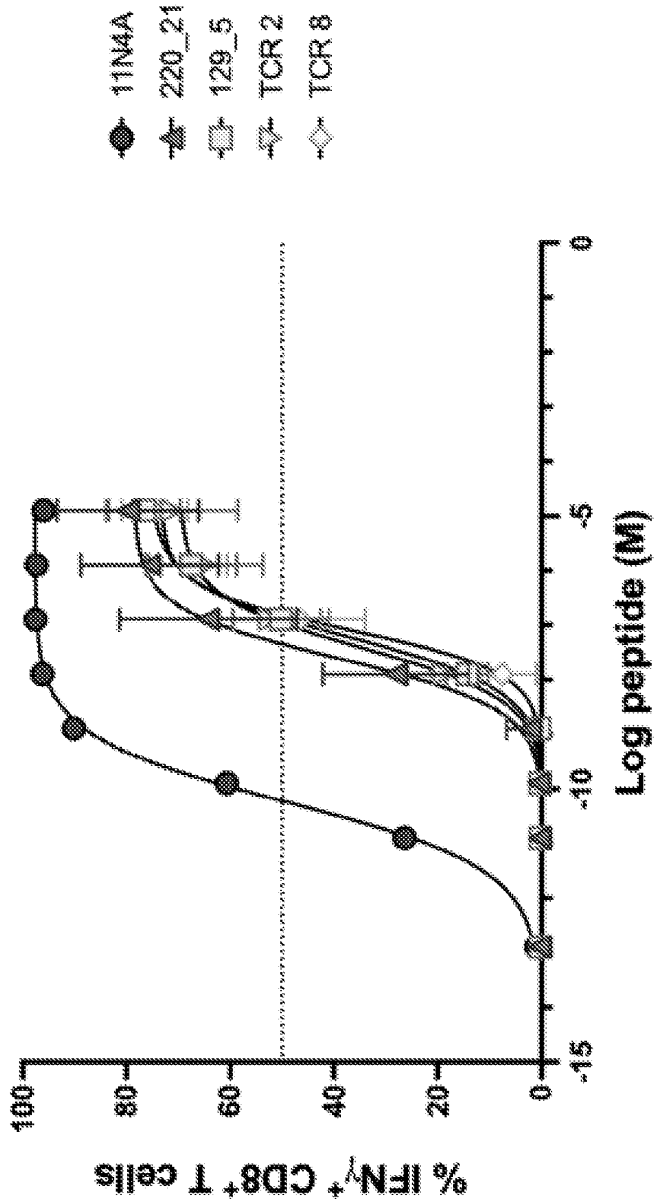

11N4A
220_21
129_5
TCR 2
TCR 8
% IFNγ+ CD8+ T cells
100
80
60
40
20
0
0
-5
-10
-15
Log peptide (M)

FIG. 2C

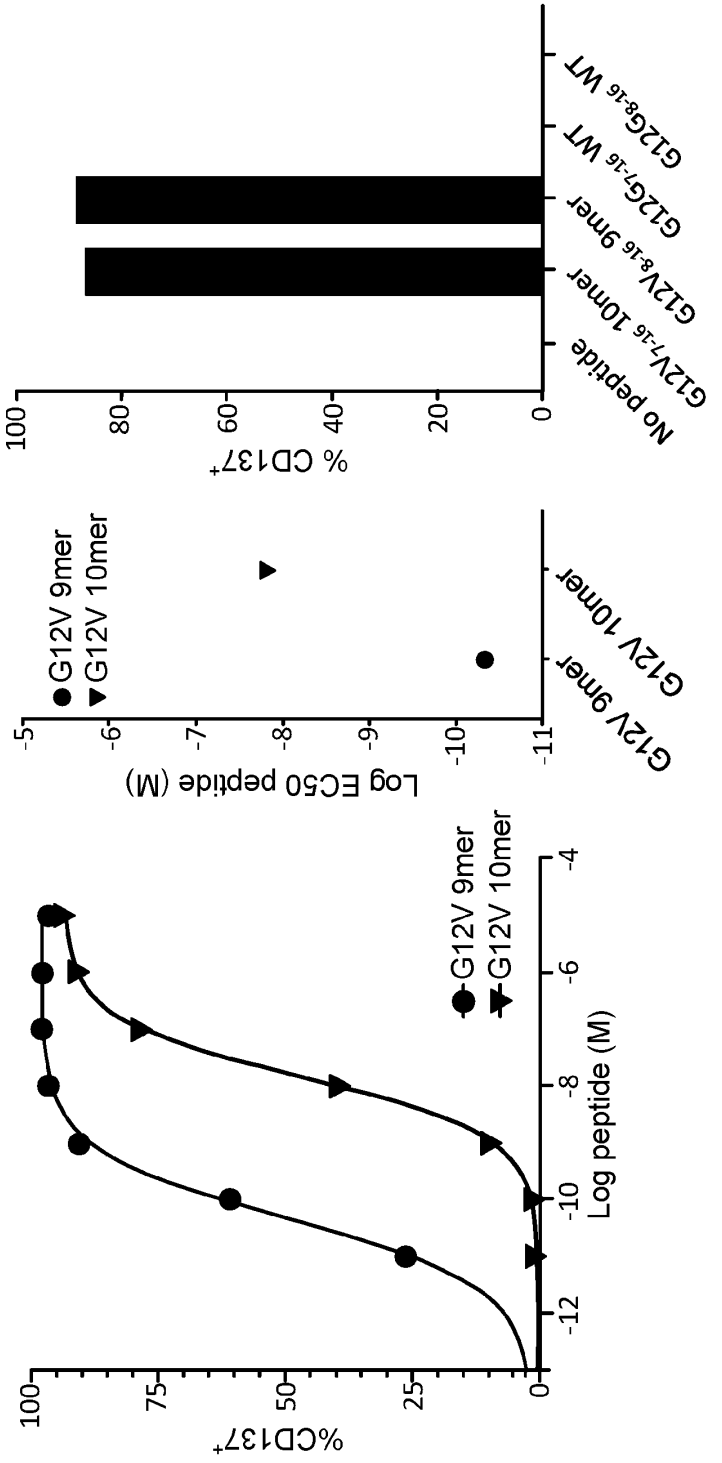


FIG. 2D

FIG. 2E

FIG. 2F

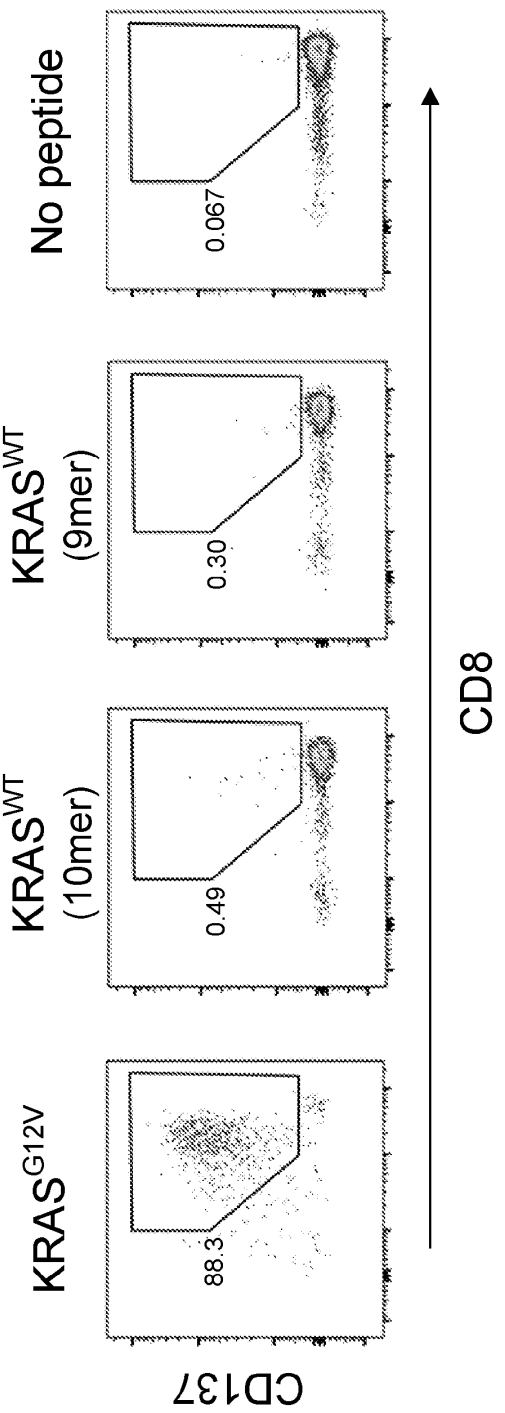

No peptide
0.067
KRAS^{WT} (9mer)
0.30
KRAS^{WT} (10mer)
0.49
KRAS^{G12V}
88.3
CD137
CD8

FIG. 2G

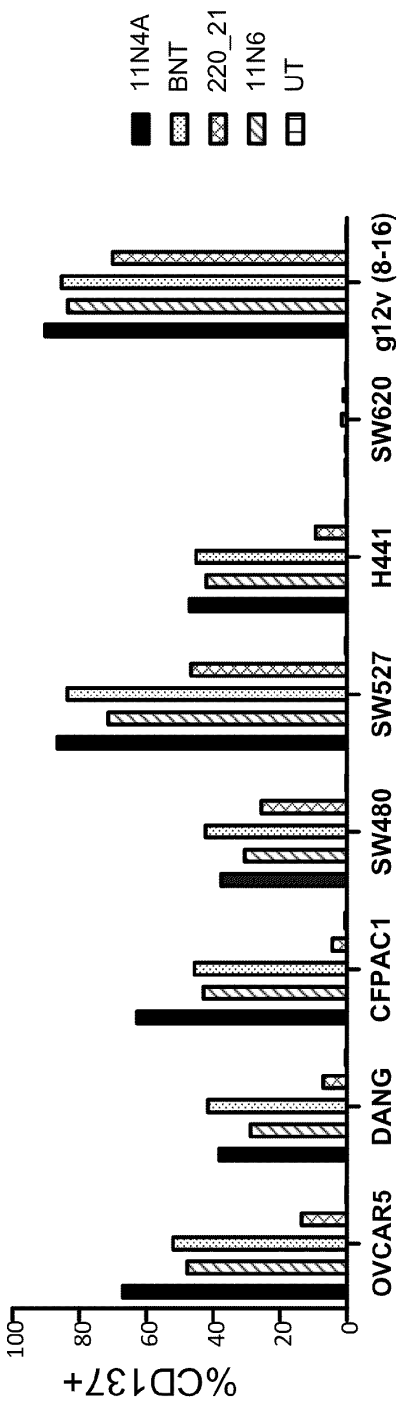

11N4A
BNT
220_21
11N6
UT
%CD137+
0
20
40
60
80
100
OVCAR5
DANG
CFPAC1
SW480
SW527
H441
SW620
g12v (8-16)

FIG. 3A

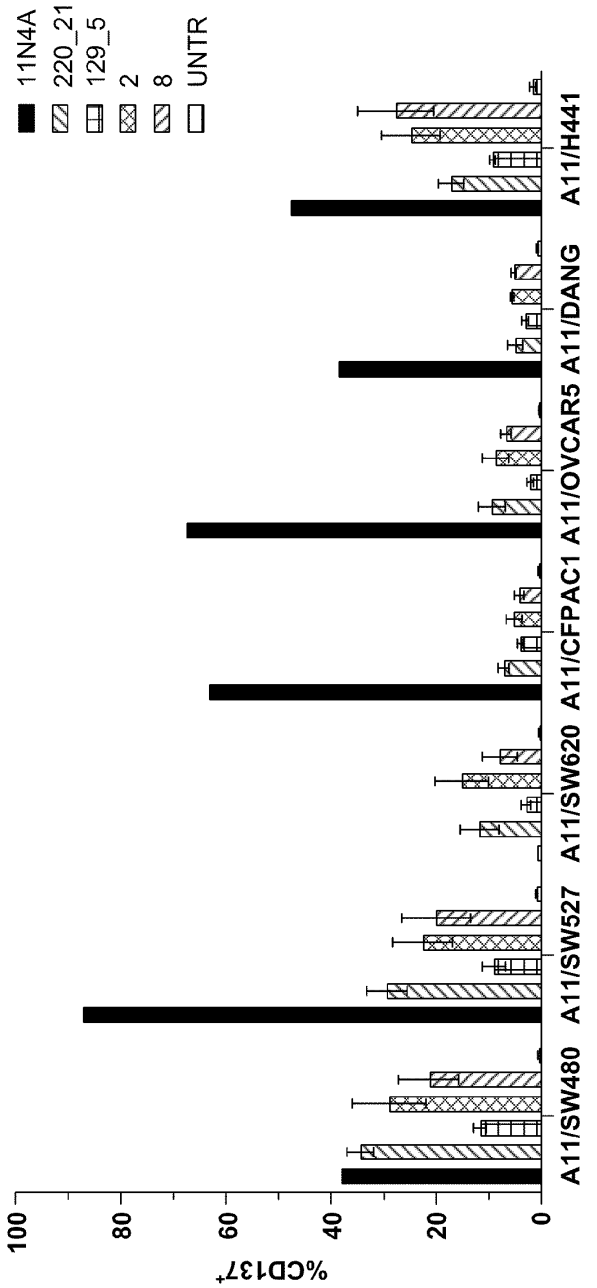

11N4A
220_21
129_5
2
8
UNTR
%CD137+
100
80
60
40
20
0
A11/SW480
A11/SW527
A11/SW620
A11/CFPAC1
A11/OVCAR5
A11/DANG
A11/H441
SW480 = Colon carcinoma
SW527 = Breast carcinoma
SW620 = Colon carcinoma
CFPAC1 = Pancreatic adenocarcinoma
OVCAR5= Ovarian serous adenocarcinoma
DAN-G = Pancreatic adenocarcinoma
NCI-H441 = Lung adenocarcinoma

FIG. 3B

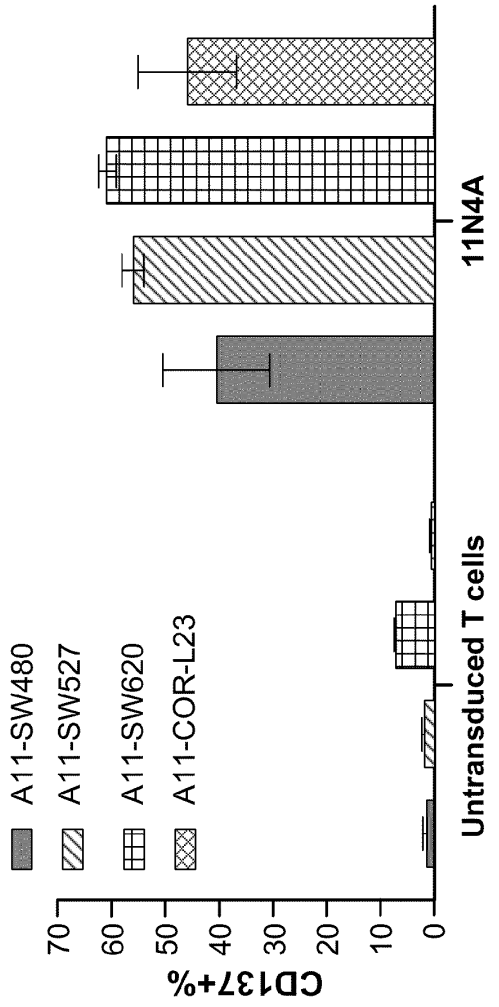

A11-SW480
A11-SW527
A11-SW620
A11-COR-L23
CD137+%
0
10
20
30
40
50
60
70
Untransduced T cells
11N4A

FIG. 3C

Cell lines used	Cancer derivation
CFPAC-1	Pancreatic
DAN-G	Pancreatic
SW620	Colon
SW480	Colon
COR-L23	Lung
NCIH441	Lung
SW527	Breast
PANC1 (KRAS G12v negative)	Pancreatic
HUCCT1 (KRAS G12V negative)	Cholangiocarcinoma

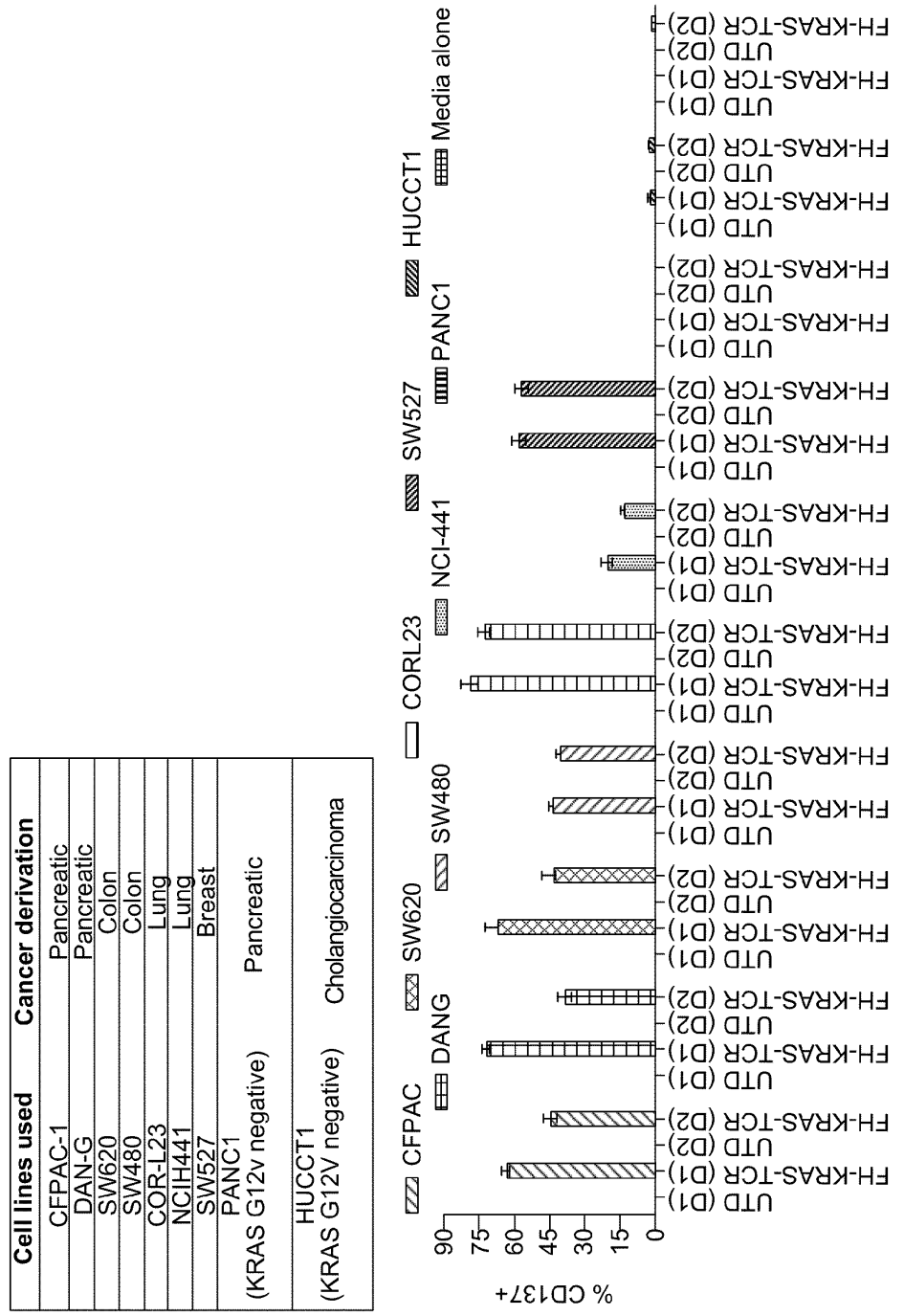


FIG. 3D

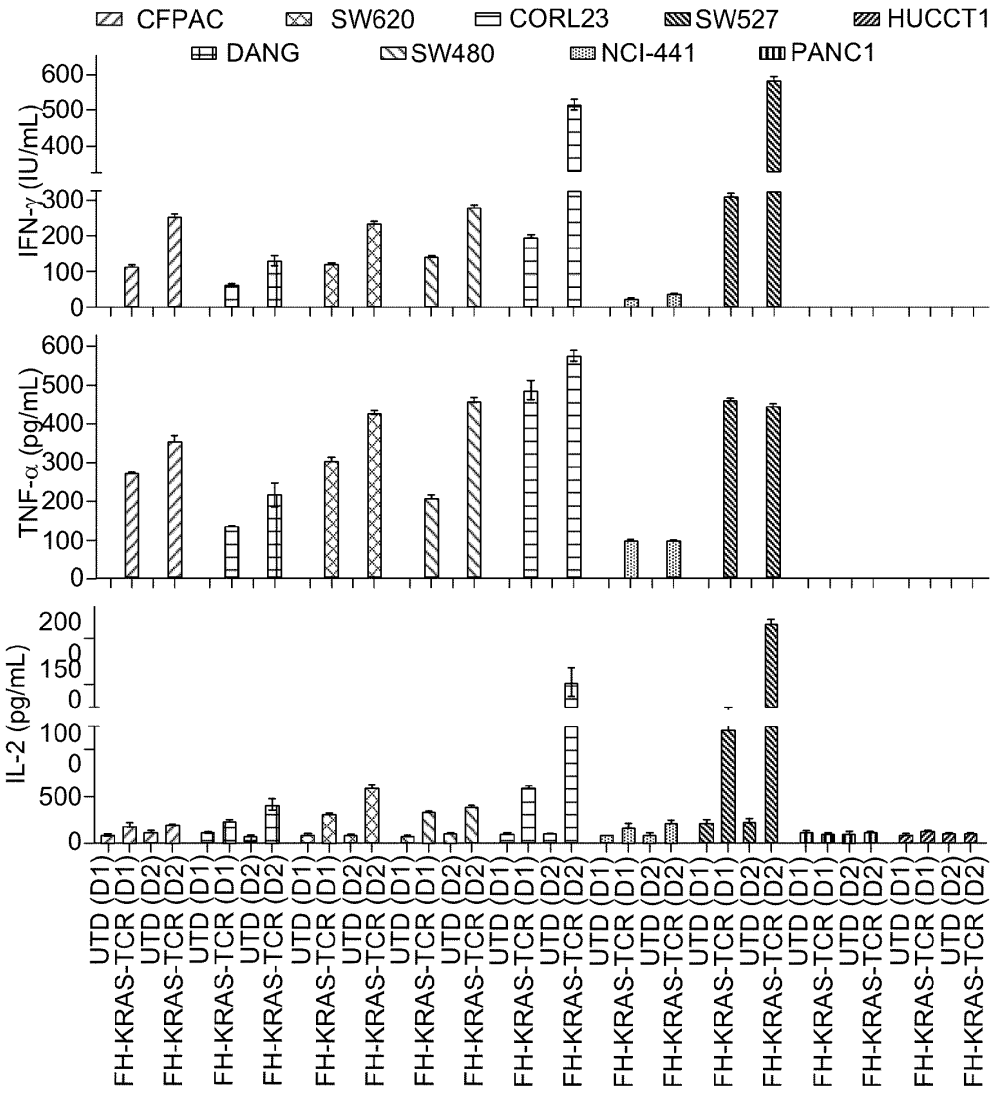

CFPAC
SW620
CORL23
SW527
HUCCT1
DANG
SW480
NCI-441
PANC1
IFN-γ (IU/mL)
TNF-α (pg/mL)
IL-2 (pg/mL)
UTD (D1)
FH-KRAS-TCR (D1)
UTD (D2)
FH-KRAS-TCR (D2)

FIG. 3E

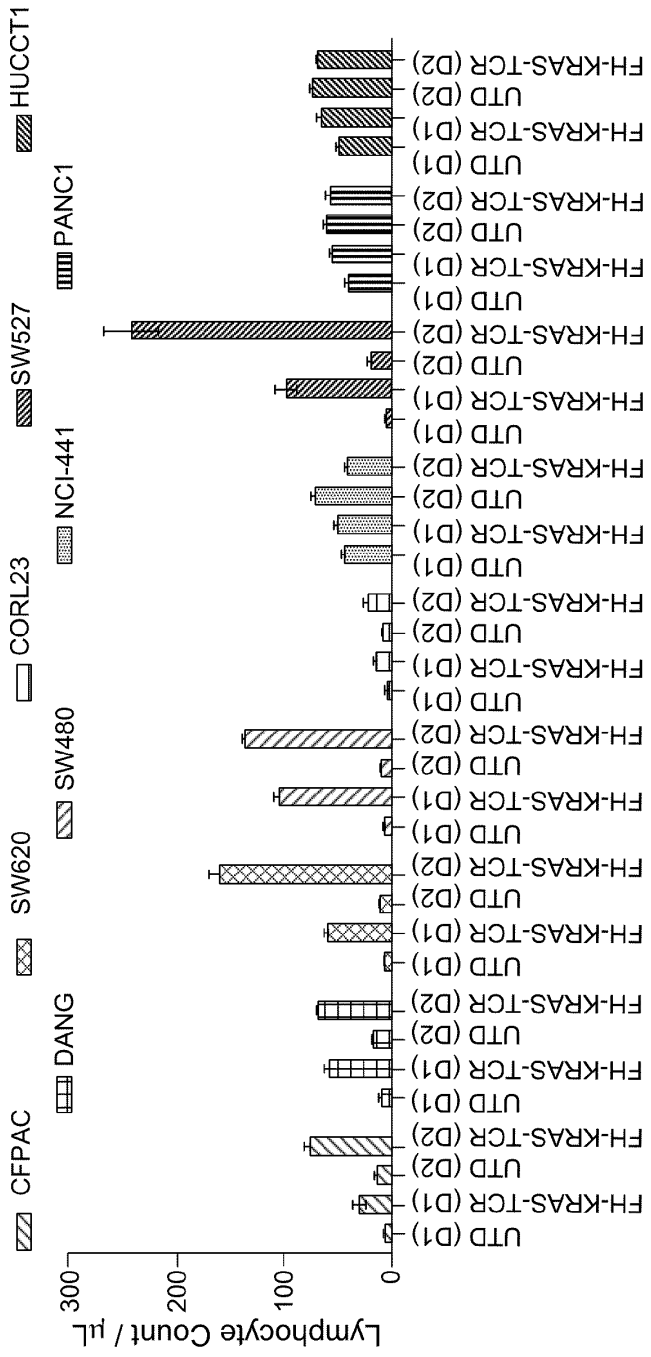
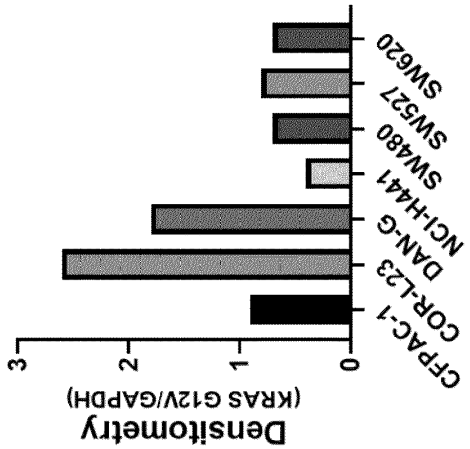

CFPAC
DANG
SW620
SW480
CORL23
NCI-441
SW527
PANC1
HUCCT1
Lymphocyte Count / μL
0
100
200
300
UTD (D1)
FH-KRAS-TCR (D1)
UTD (D2)
FH-KRAS-TCR (D2)
UTD (D1)
FH-KRAS-TCR (D1)
UTD (D2)
FH-KRAS-TCR (D2)
UTD (D1)
FH-KRAS-TCR (D1)
UTD (D2)
FH-KRAS-TCR (D2)
UTD (D1)
FH-KRAS-TCR (D1)
UTD (D2)
FH-KRAS-TCR (D2)
UTD (D1)
FH-KRAS-TCR (D1)
UTD (D2)
FH-KRAS-TCR (D2)
UTD (D1)
FH-KRAS-TCR (D1)
UTD (D2)
FH-KRAS-TCR (D2)
UTD (D1)
FH-KRAS-TCR (D1)
UTD (D2)
FH-KRAS-TCR (D2)

FIG. 3F


Densitometry
(KRAS G12V/GAPDH)
0
1
2
3
CFPAC-1
COR-L23
DAN-G
NCI-H441
SW480
SW527
SW620

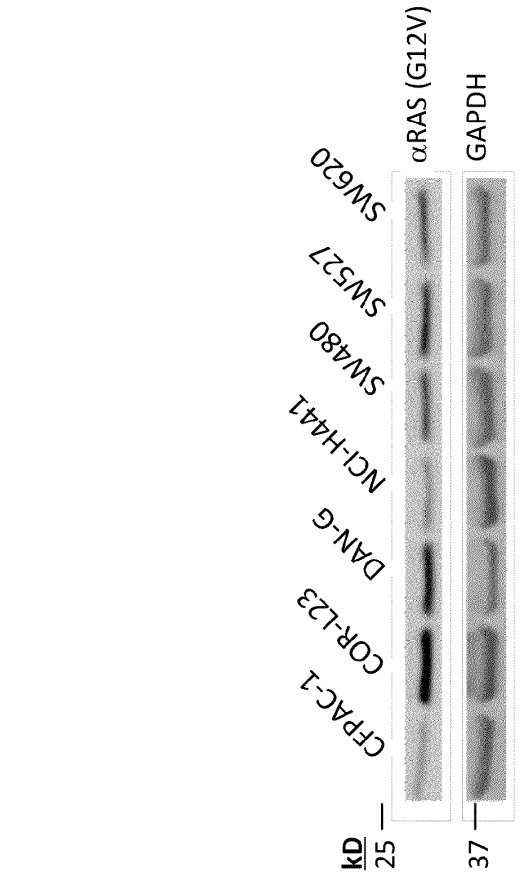

kD
25
37
αRAS (G12V)
GAPDH
CFPAC-1
COR-L23
DAN-G
NCI-H441
SW480
SW527
SW620

FIG. 3G

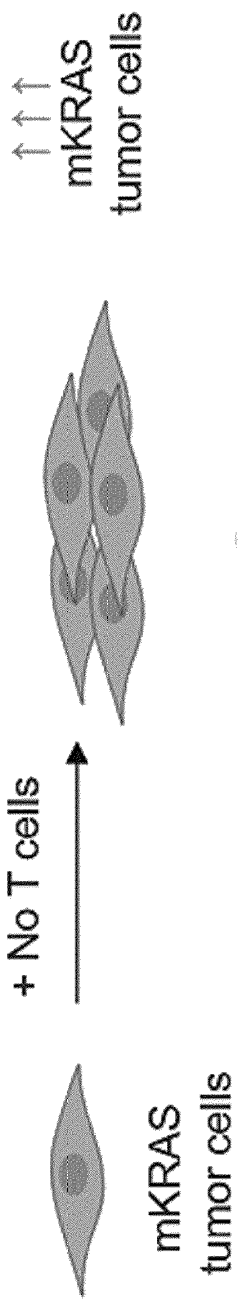


FIG. 4A

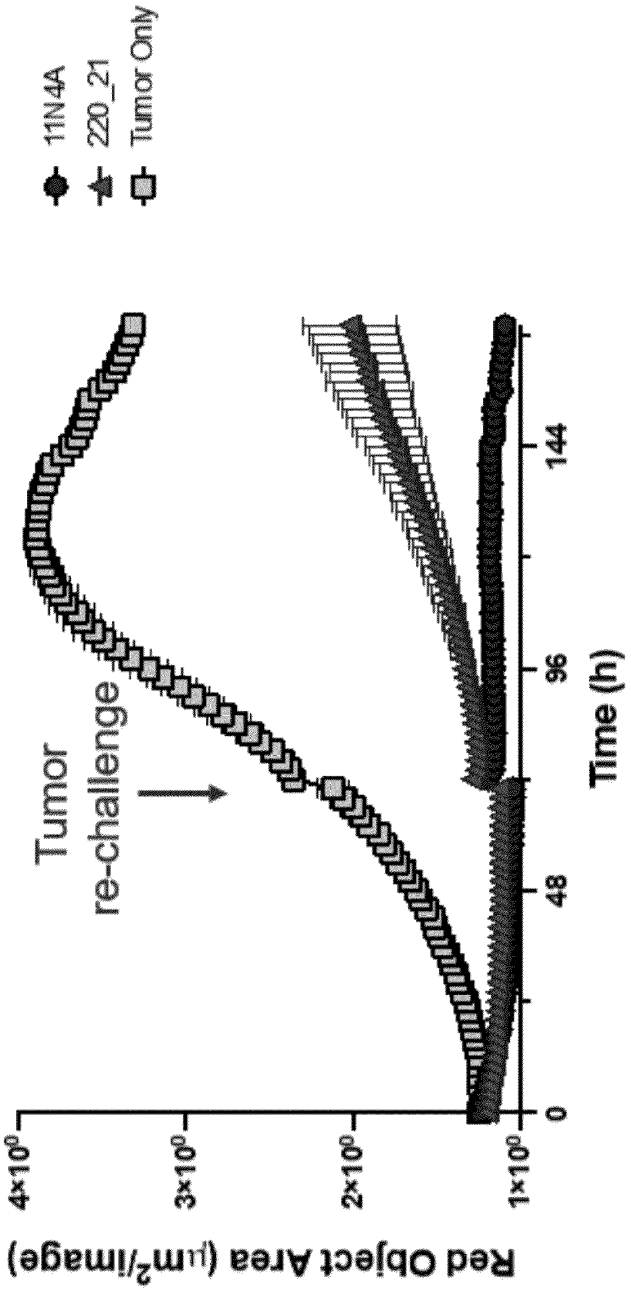

11N4A
220_21
Tumor Only
Tumor re-challenge
Red Object Area (μm²/image)
4×10⁰
3×10⁰
2×10⁰
1×10⁰
0
48
96
144
Time (h)

FIG. 4B

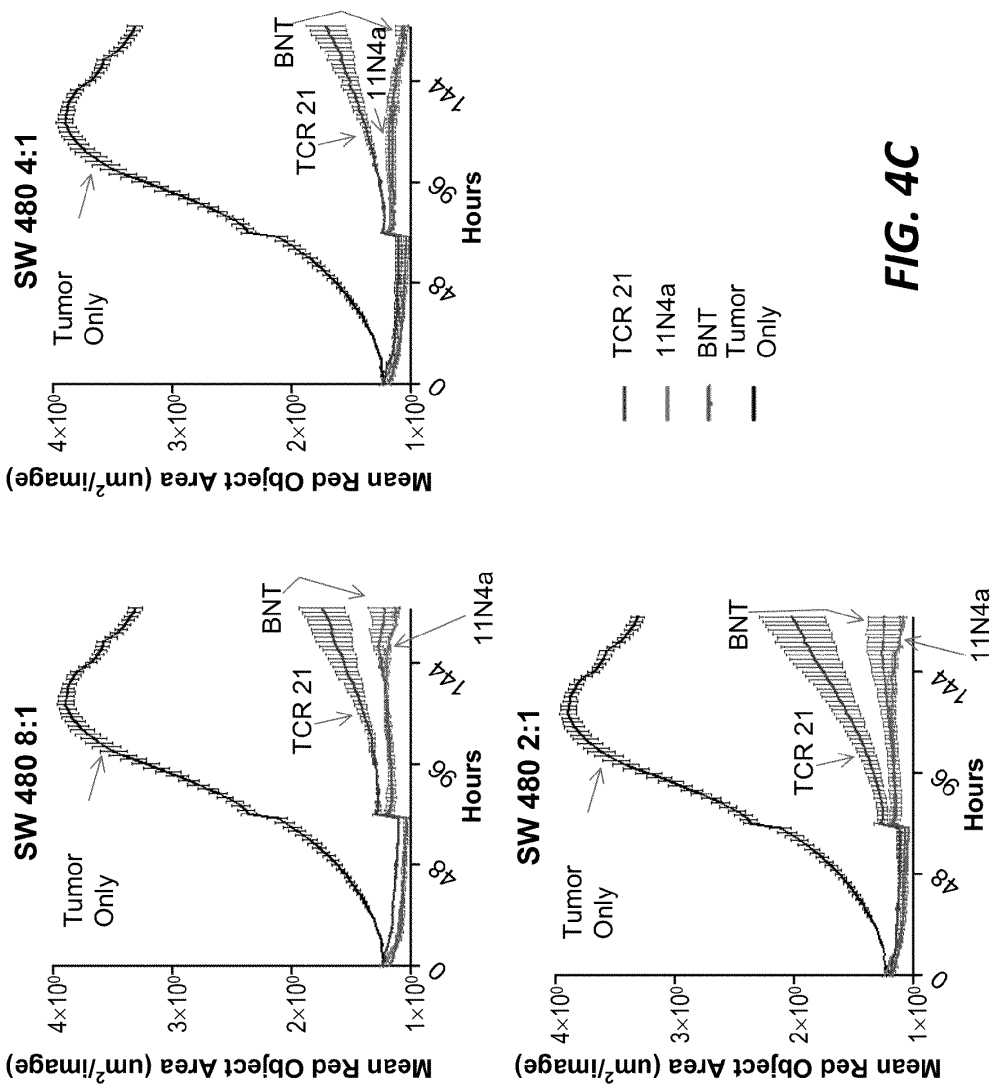

SW 480 4:1
SW 480 8:1
SW 480 2:1
Tumor Only
BNT
TCR 21
11N4a
Hours
0
48
96
144
Mean Red Object Area (um²/image)
1×10⁰
2×10⁰
3×10⁰
4×10⁰
TCR 21
11N4a
BNT
Tumor Only

FIG. 4C

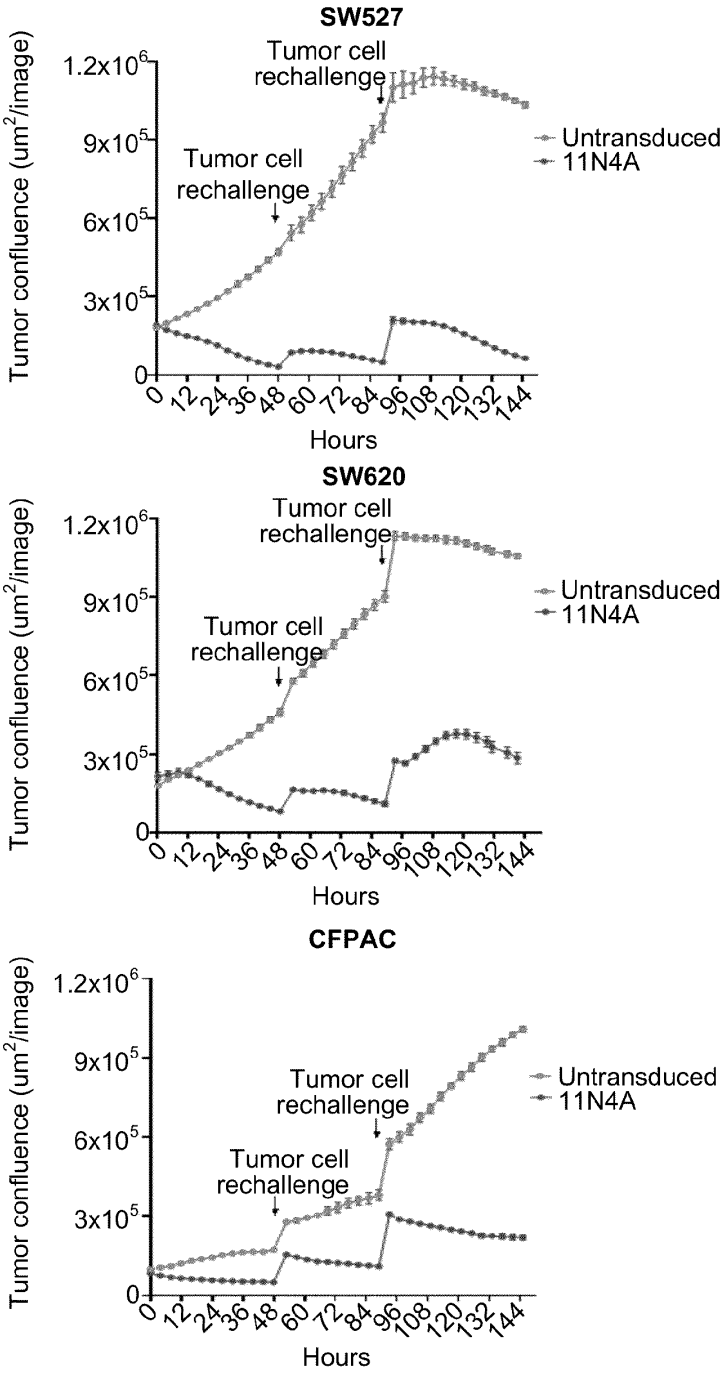

SW527
Tumor cell rechallenge
Tumor cell rechallenge
Untransduced
11N4A
Tumor confluence (um²/image)
1.2x10⁶
9x10⁵
6x10⁵
3x10⁵
0
0 12 24 36 48 60 72 84 96 108 120 132 144
Hours
SW620
Tumor cell rechallenge
Tumor cell rechallenge
Untransduced
11N4A
Tumor confluence (um²/image)
1.2x10⁶
9x10⁵
6x10⁵
3x10⁵
0
0 12 24 36 48 60 72 84 96 108 120 132 144
Hours
CFPAC
Tumor cell rechallenge
Tumor cell rechallenge
Untransduced
11N4A
Tumor confluence (um²/image)
1.2x10⁶
9x10⁵
6x10⁵
3x10⁵
0
0 12 24 36 48 60 72 84 96 108 120 132 144
Hours

FIG. 4D

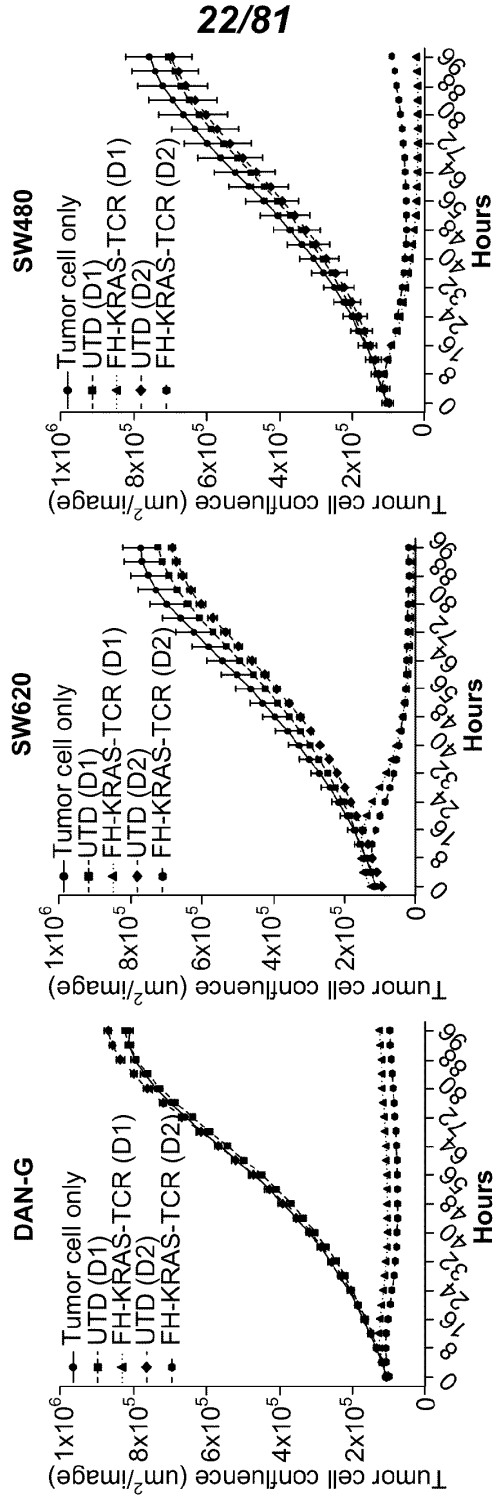

DAN-G
SW620
SW480
Tumor cell only
UTD (D1)
FH-KRAS-TCR (D1)
UTD (D2)
FH-KRAS-TCR (D2)
Tumor cell confluence (um²/image)
Hours
0
2x10⁵
4x10⁵
6x10⁵
8x10⁵
1x10⁶
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72
76
80
84
88
92
96

FIG. 4E

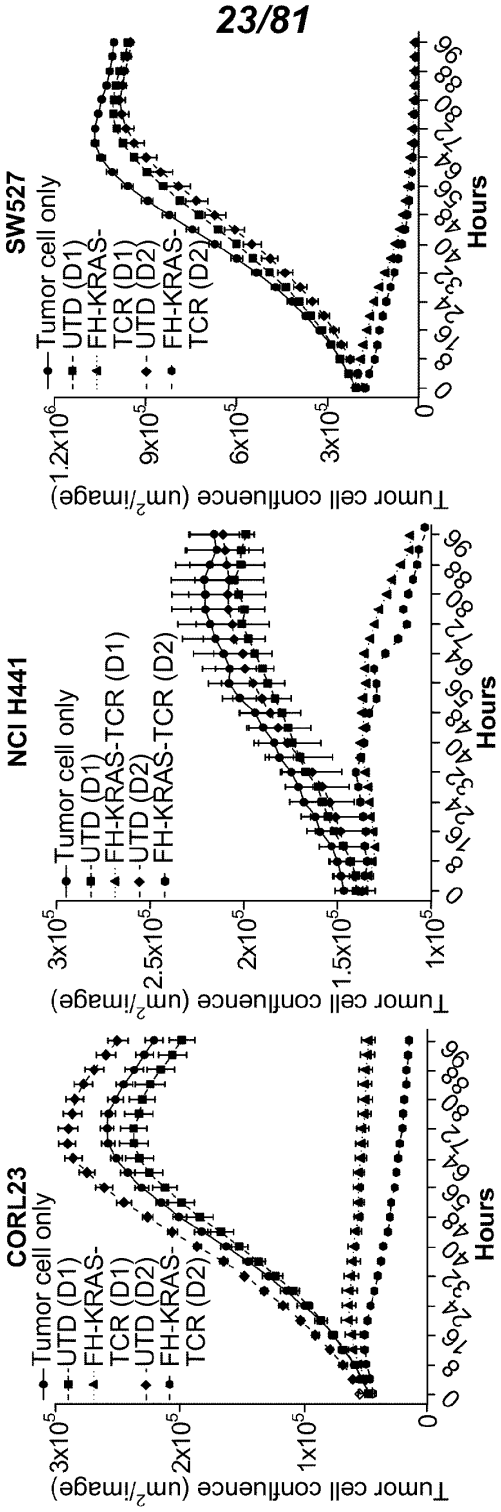

CORL23
NCI H441
SW527
Tumor cell only
UTD (D1)
FH-KRAS-TCR (D1)
UTD (D2)
FH-KRAS-TCR (D2)
Tumor cell confluence (um²/image)
Hours

FIG. 4F

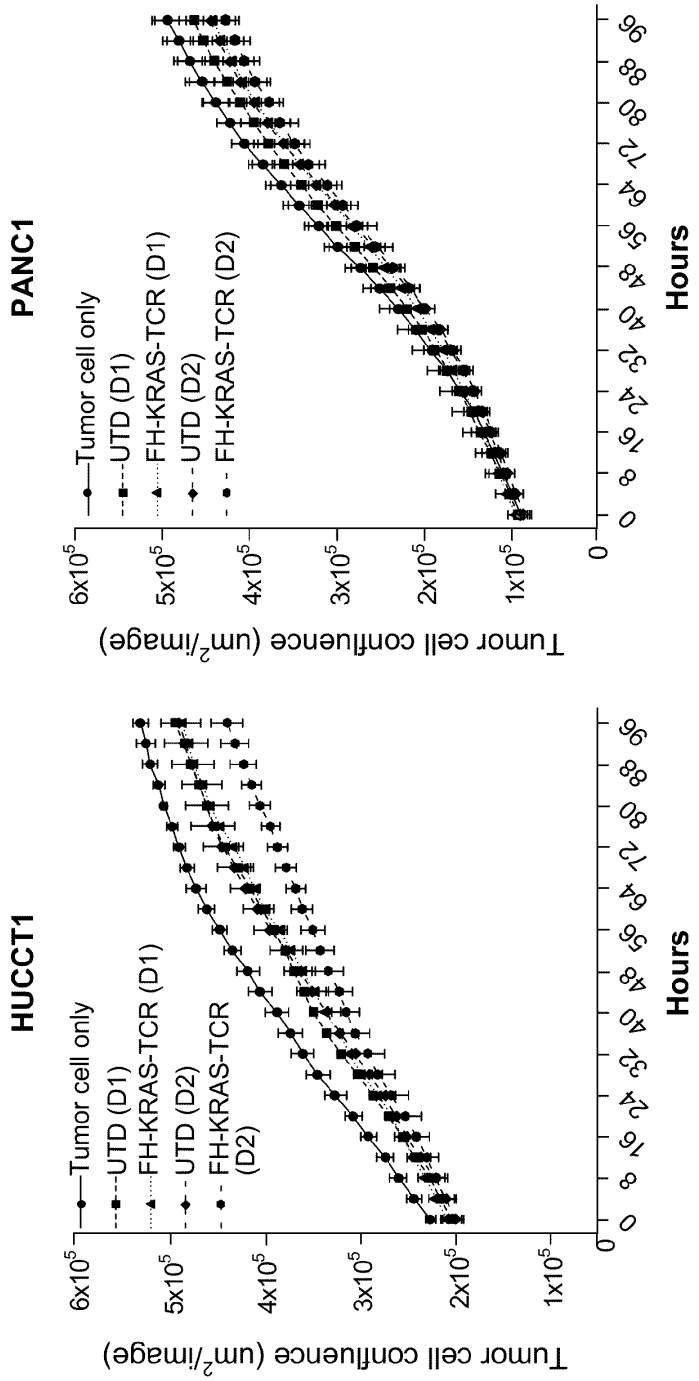

HUCCT1
PANC1
Tumor cell only
UTD (D1)
FH-KRAS-TCR (D1)
UTD (D2)
FH-KRAS-TCR (D2)
Tumor cell confluence (um²/image)
Hours
0
1x10⁵
2x10⁵
3x10⁵
4x10⁵
5x10⁵
6x10⁵
0
8
16
24
32
40
48
56
64
72
80
88
96

FIG. 4G

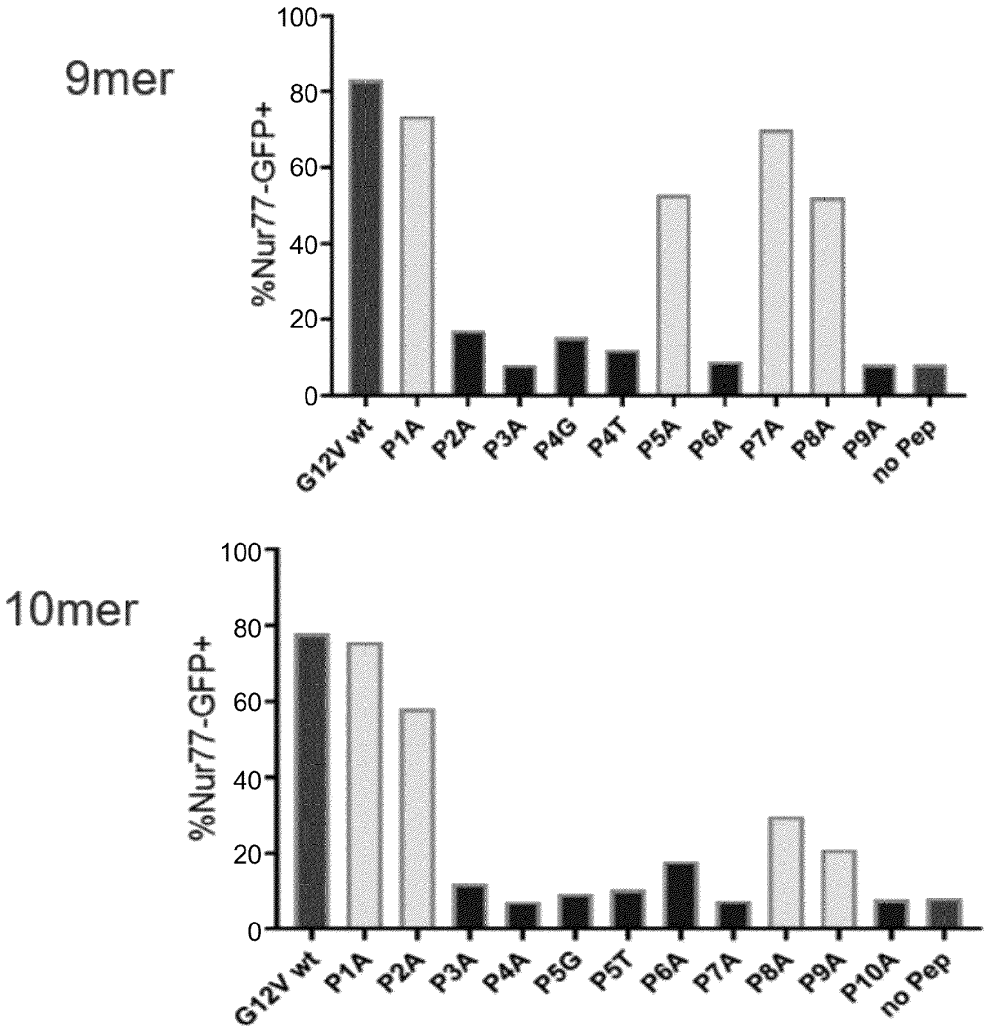

9mer
%Nur77-GFP+
100
80
60
40
20
0
G12V wt
P1A
P2A
P3A
P4G
P4T
P5A
P6A
P7A
P8A
P9A
no Pep
10mer
%Nur77-GFP+
100
80
60
40
20
0
G12V wt
P1A
P2A
P3A
P4A
P5G
P5T
P6A
P7A
P8A
P9A
P10A
no Pep

FIG. 5A

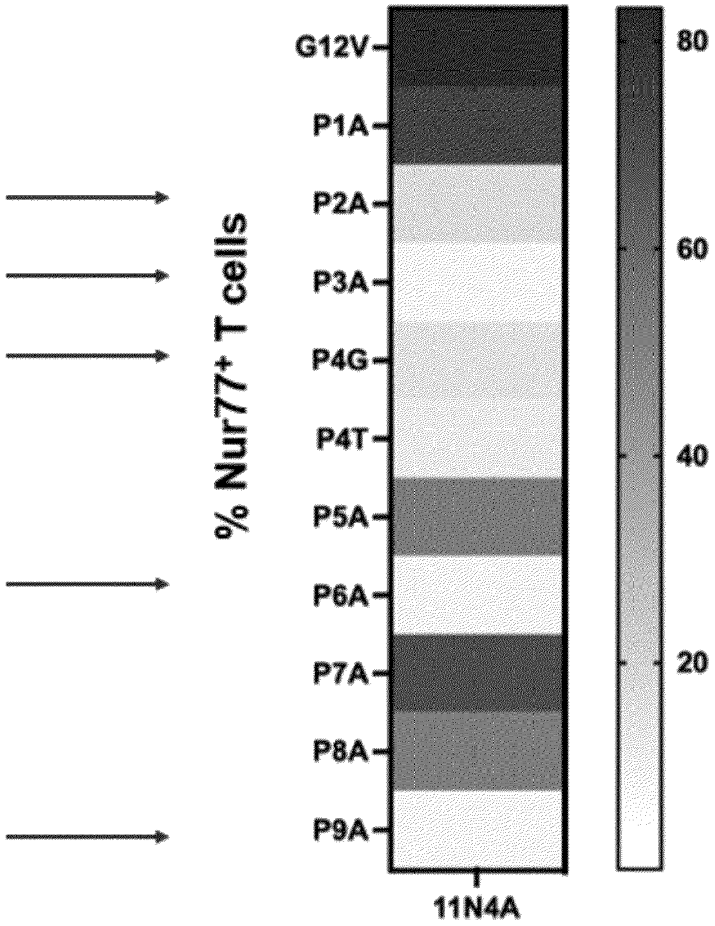

G12V
P1A
P2A
P3A
P4G
P4T
P5A
P6A
P7A
P8A
P9A
11N4A
% Nur77+ T cells
80
60
40
20

FIG. 5B

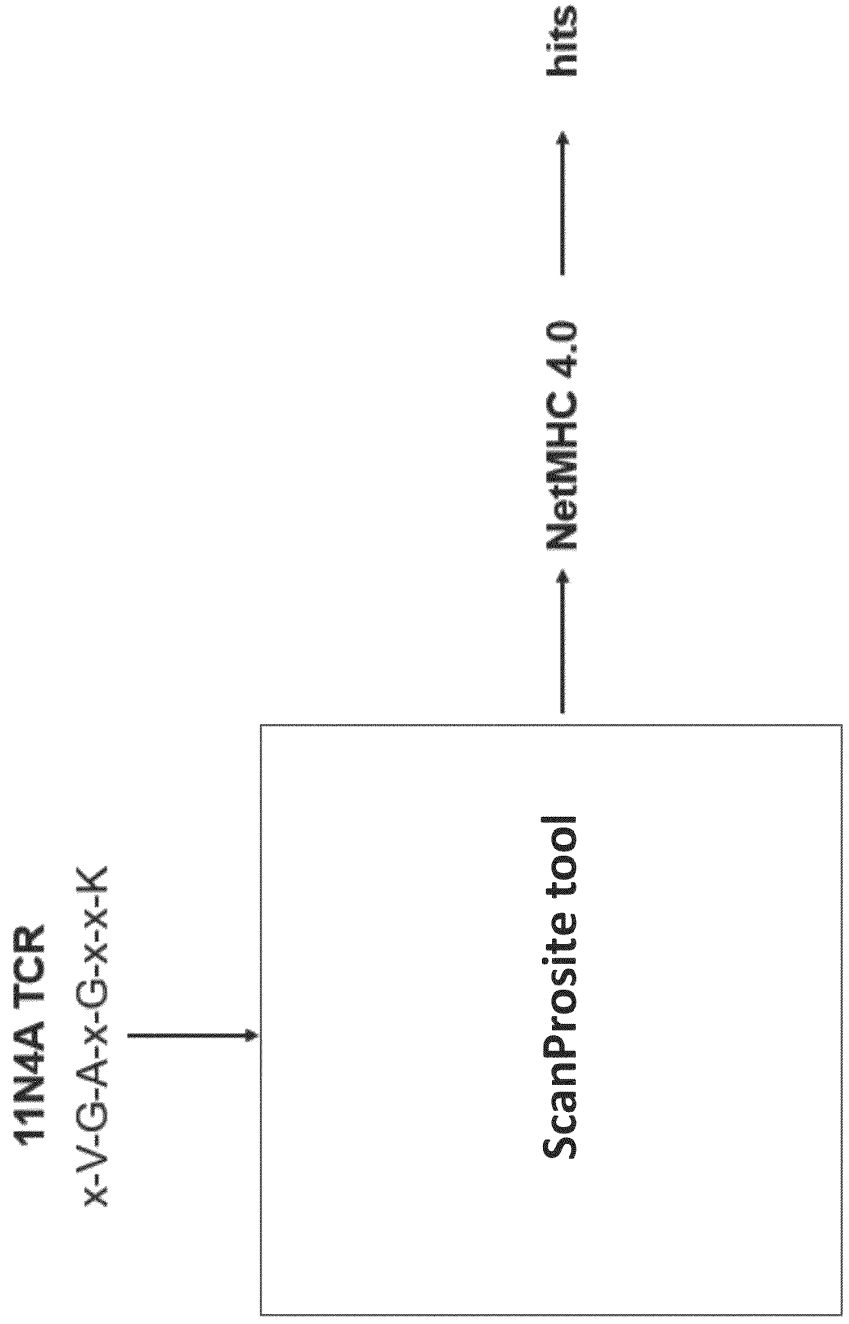


FIG. 5C

9mer Search String:	x-V-G-A-x-G-x-x-K	10 hits

Gene	peptide	start	end	score	rank
RAB7B	IVGAIGVGK	13	21	0.688497	0.15
ITA8	IVGAFGTGK	469	477	0.561758	0.24
ARRD4	AVGAEGRVK	10	18	0.328175	0.55
SMC5	IVGANGTGK	78	86	0.308533	0.59
MOGS	EVGAKGQLK	220	228	0.113675	1.3
GKN2	NVGAGGCAK	159	167	0.06972	1.7
CRNS1	VVGAGGVSK	398	406	0.74512	0.1
RASE	VVGASGVGK	46	54	0.690829	0.15
RSLBB	VVGASGVGK	38	46	0.690829	0.15
RASH	VVGAGGVGK	8	16	0.638055	0.18
RASK	VVGAGGVGK	8	16	0.638055	0.18
RASN	VVGAGGVGK	8	16	0.638055	0.18
MIRO2	VVGARGVGK	421	429	0.480429	0.33

FIG. 5D

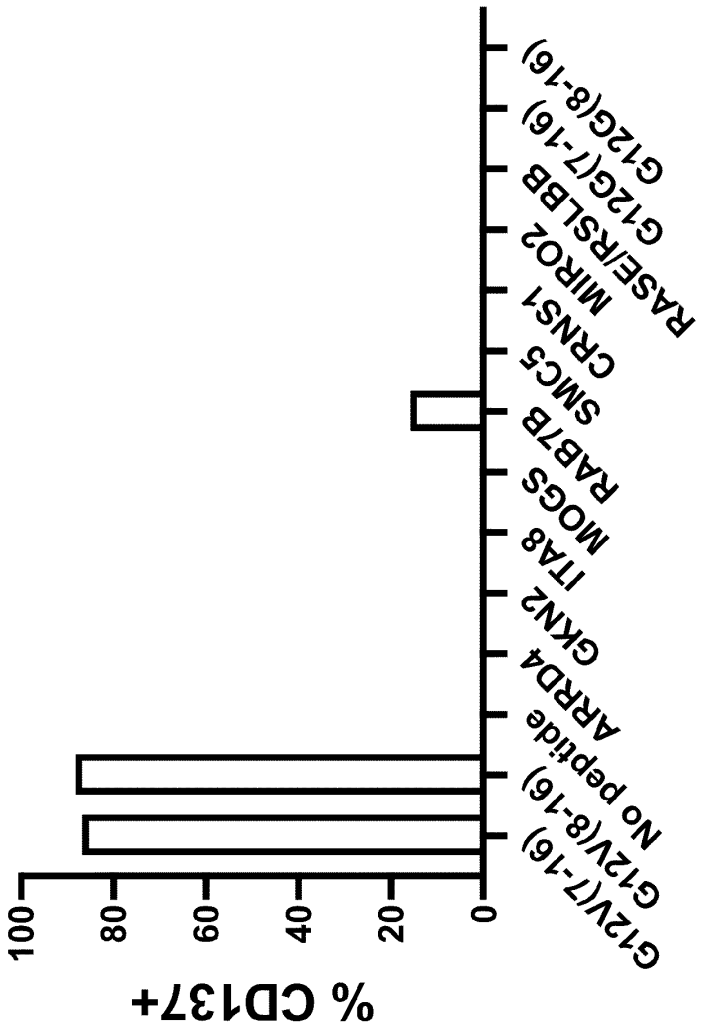

% CD137+
100
80
60
40
20
0
G12V(7-16)
G12V(8-16)
No peptide
ARRDA
GKN2
ITA8
MOGS
RAB7B
SMC5
CRNS1
MIRO2
RASE/RSLBB
G12G(7-16)
G12G(8-16)

FIG. 6A

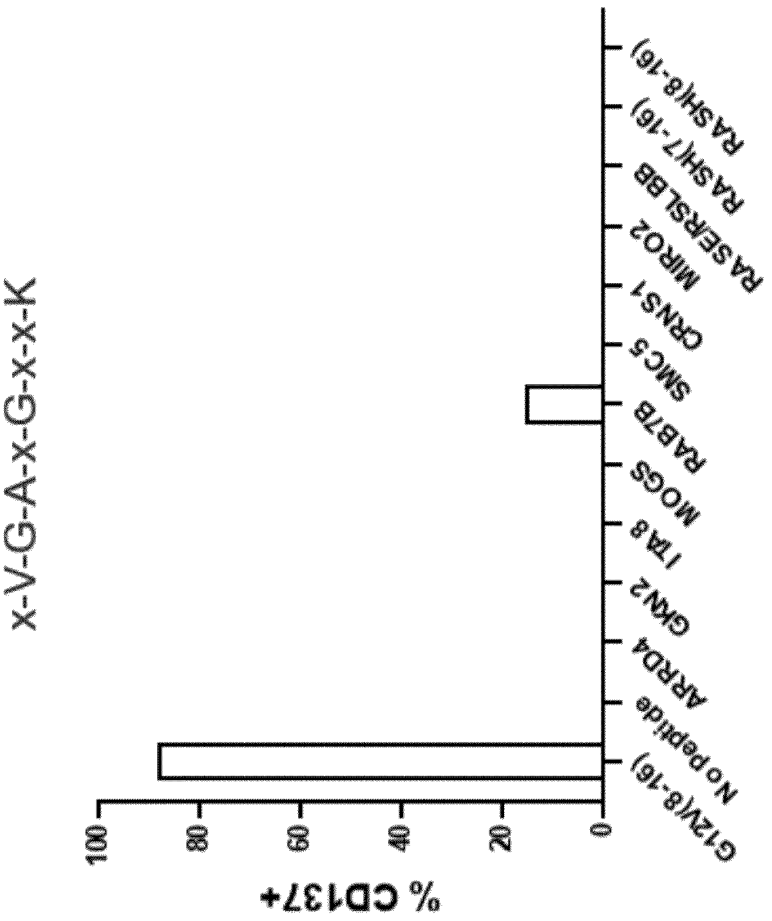

x-V-G-A-x-G-x-x-K
% CD137+
100
80
60
40
20
0
G12V(8-16)
No peptide
ARRDA
GKN2
ITA8
MOGS
RAB7B
SMC5
CRNS1
MIRO2
RASERSLBB
RASH(7-16)
RASH(8-16)

FIG. 6B

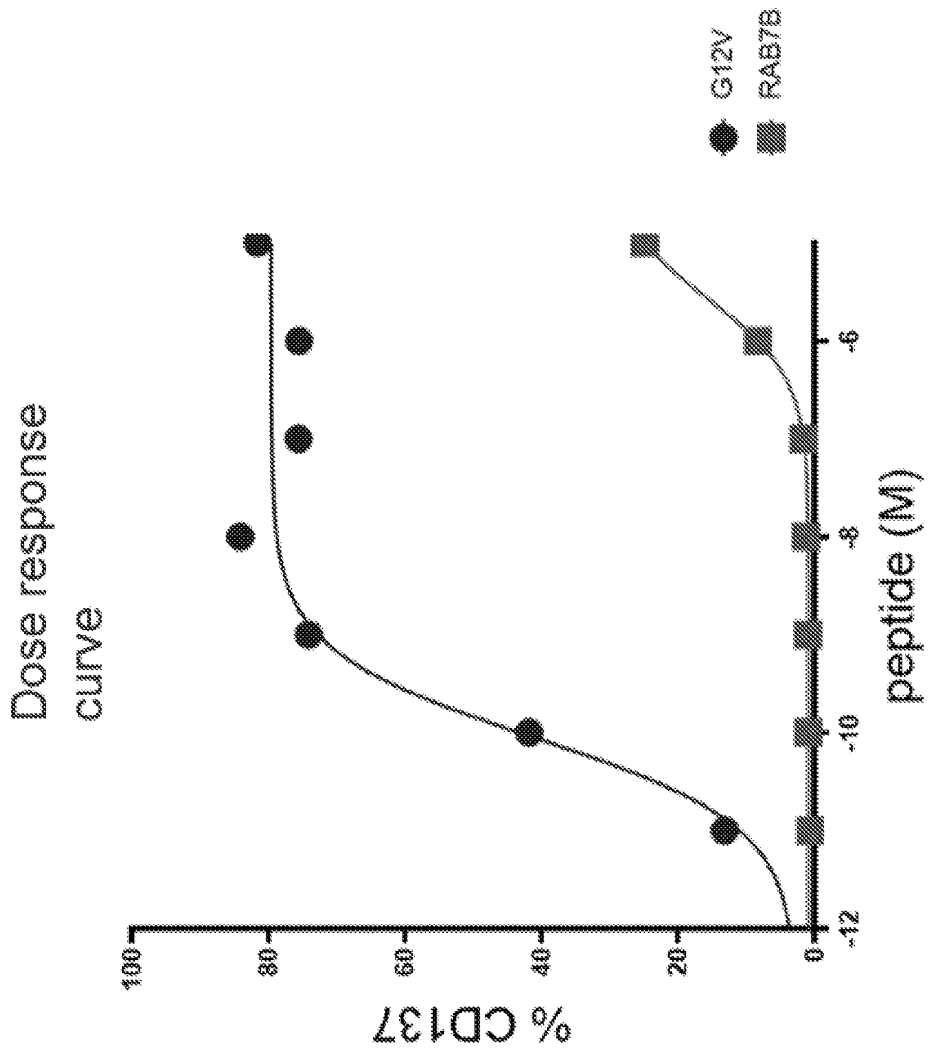

Dose response curve
% CD137
100
80
60
40
20
0
-12
-10
-8
-6
peptide (M)
G12V
RAB7B

FIG. 6C

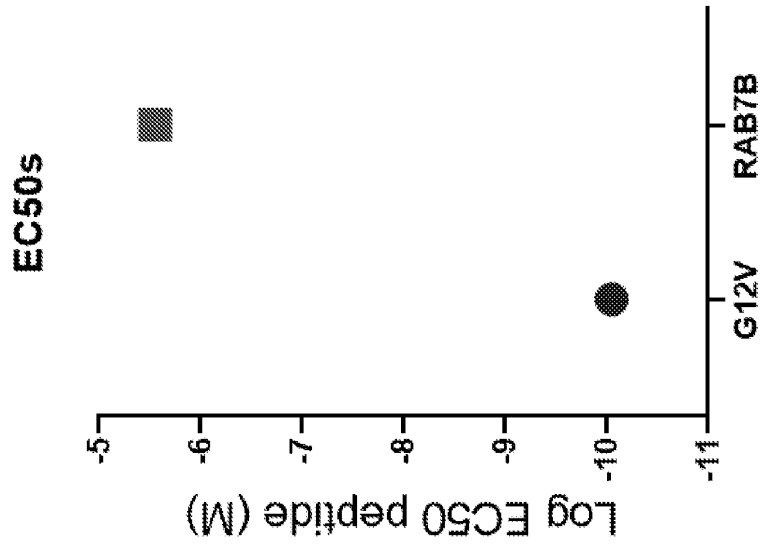
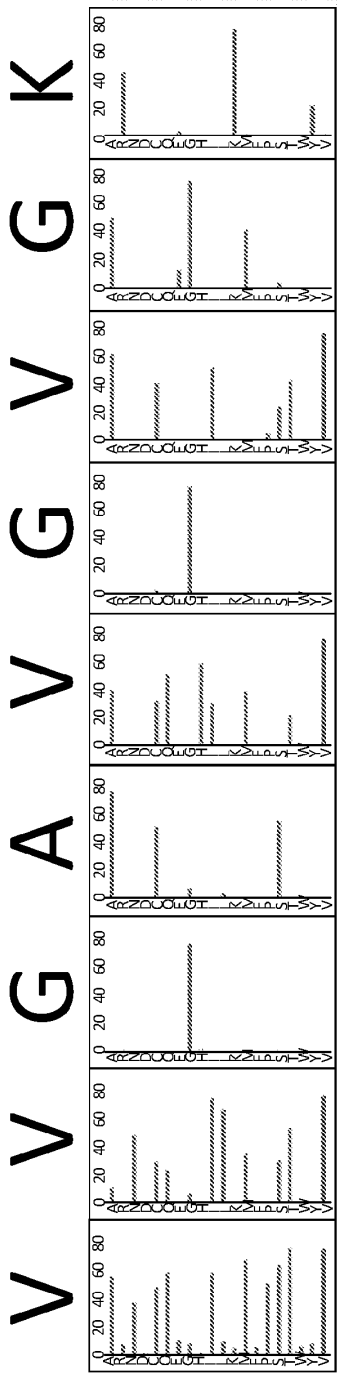


FIG. 6D



Prosite search string: [ANCQIMPSTV]-[NCQILMSTV]-G-[ACS]-[ACQHIMTV]-G-[ACISTV]-[AGM]-[RKY]

FIG. 6E

FIG. 6E, cont'd

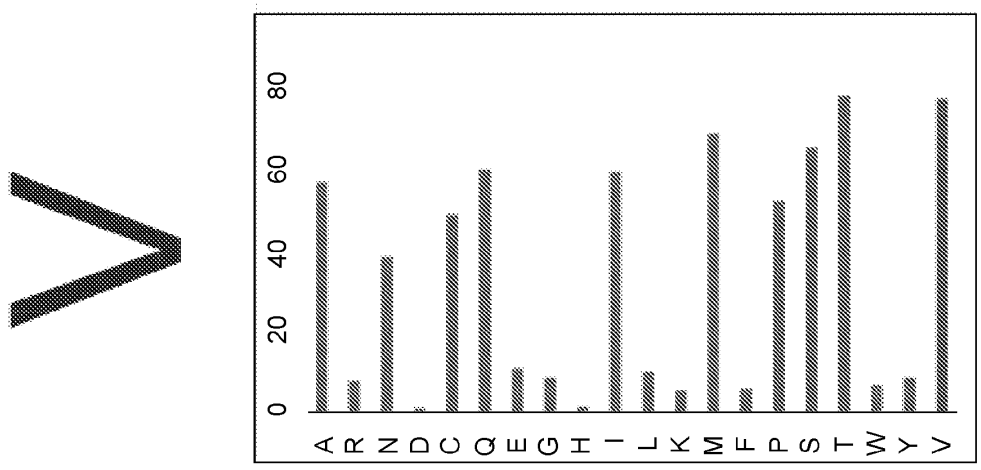


FIG. 6E, cont'd

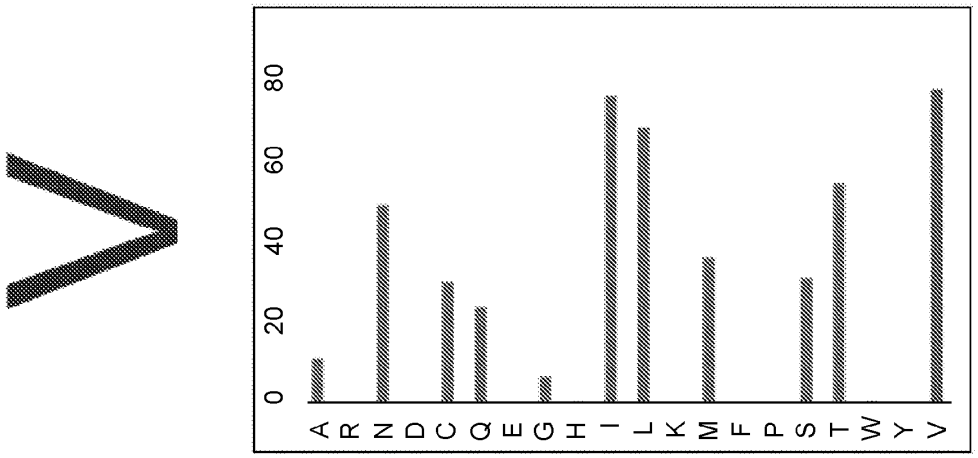


FIG. 6E, cont'd

G

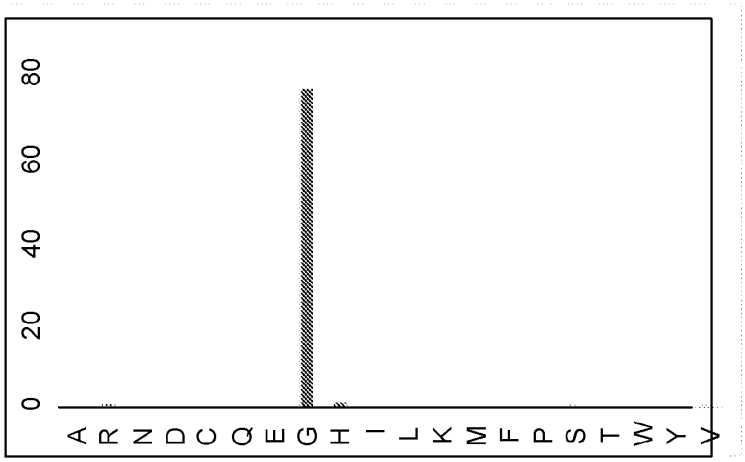


FIG. 6E, cont'd

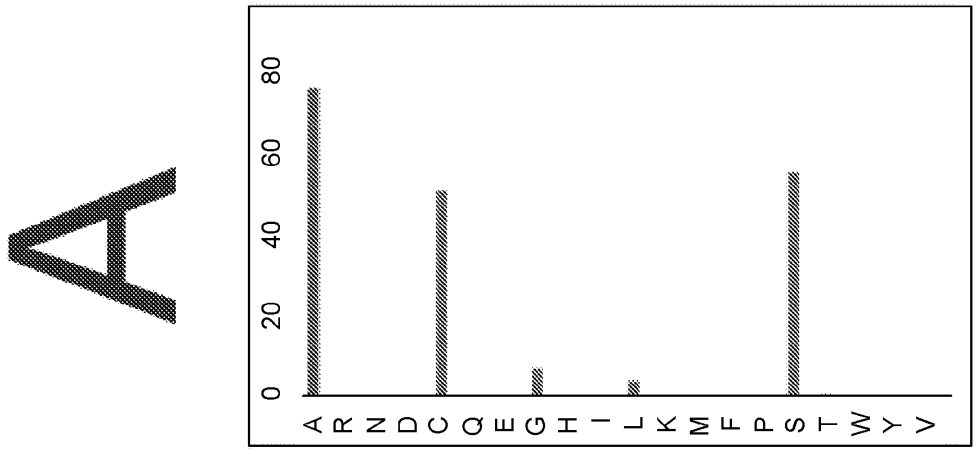


FIG. 6E, cont'd

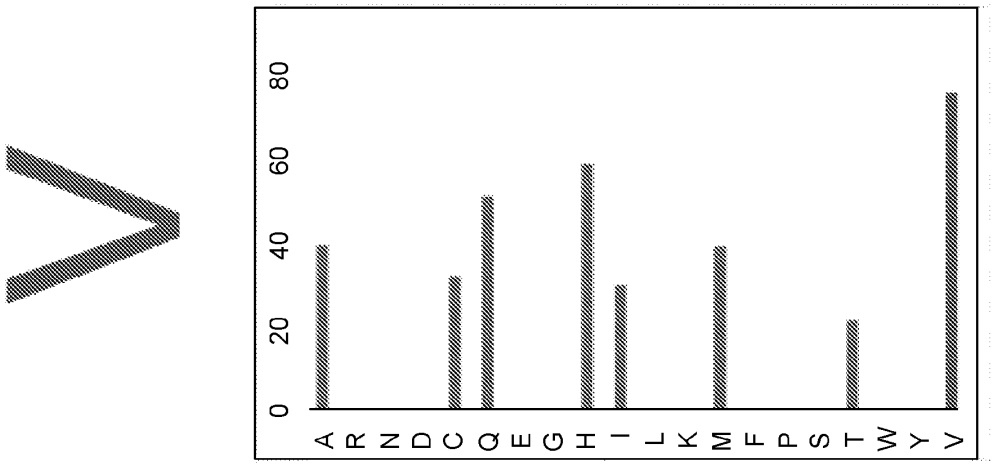


FIG. 6E, cont'd

G

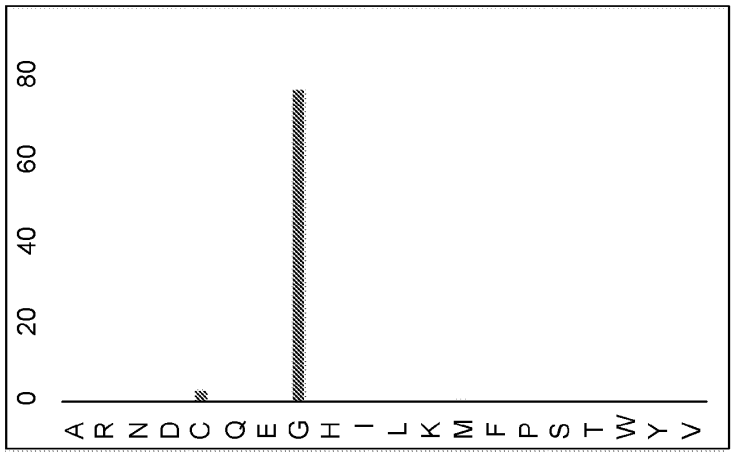


FIG. 6E, cont'd

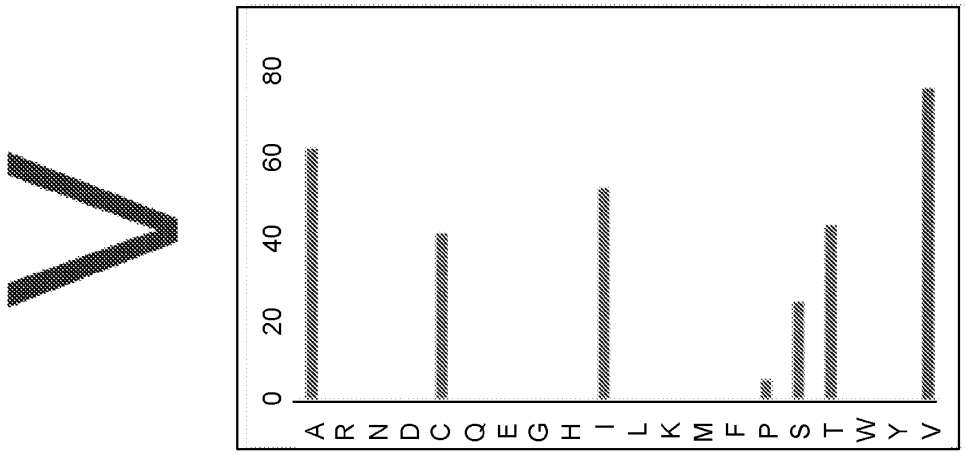


FIG. 6E, cont'd

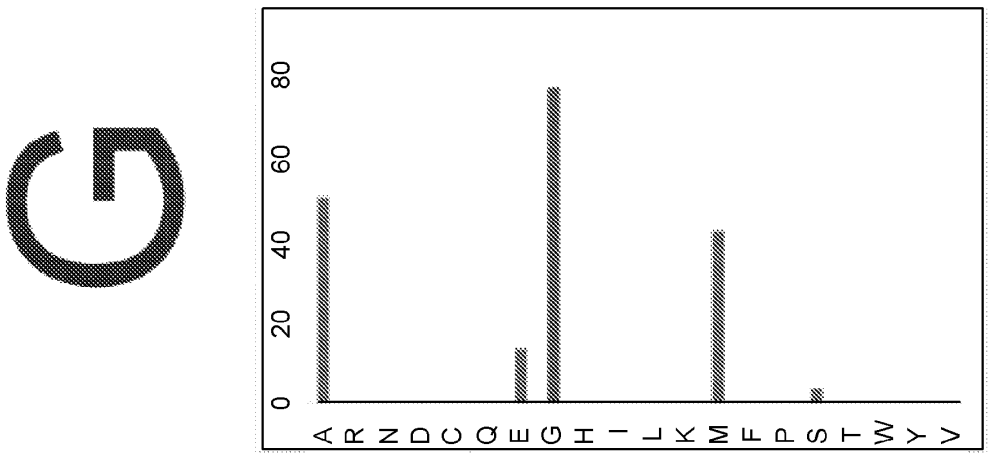
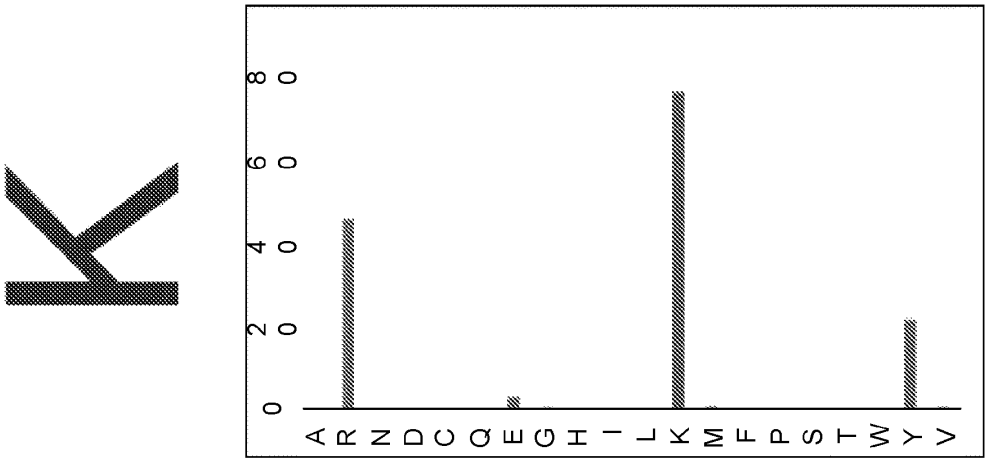


FIG. 6E, cont'd



Gene	start	end	peptide	score	%rank
KRAS G12V	8	16	VVGAVGVGK	0.755168	0.1
HTR5B	1570	1578	ASGAVGSAK	0.701693	0.14
RAB7B	13	21	IVGAIGVGK	0.688497	0.15
MED1	1383	1391	NVGSTGVAK	0.425281	0.39
RAB4B	13	21	VIGSAGTGK	0.401558	0.43
NAL12	215	223	MQGAAGIGK	0.248652	0.72
MOD5	28	36	ILGATGTGK	0.116715	1.3
DYH2	2082	2090	IVGCTGSGK	0.069626	1.7
MRP5	593	601	ICGSVGSGK	0.0073	4.7
AFDDT	155	163	QMGAAGSGR	0.00474	5.5
ANKR9	240	248	PVGAAGSAR	0.002816	6.7
EP300	192	200	MNGSIGAGR	0.002772	6.7
SCGR4	80	88	TCGSCGCGY	0.000111	21

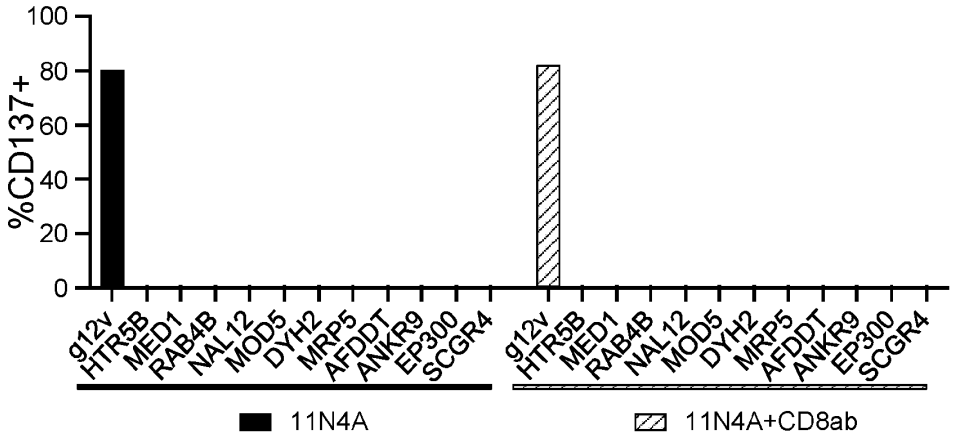


FIG. 6F

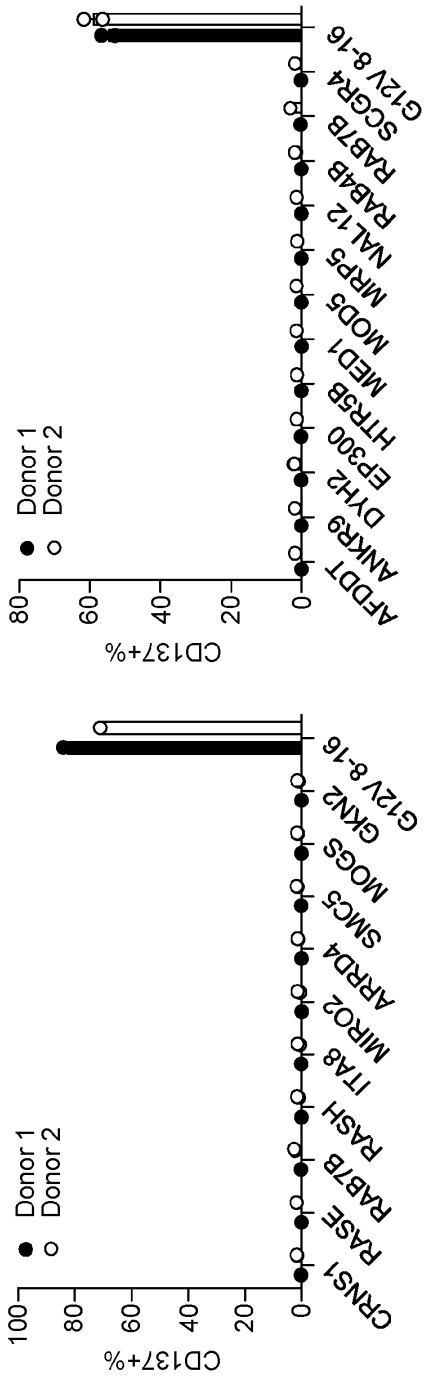

CD137+%
0
20
40
60
80
100
Donor 1
Donor 2
CRNS1
RASE
RAB7B
RASH
ITA8
MIR02
ARRD4
SMC5
MOGS
GKN2
G12V 8-16
CD137+%
0
20
40
60
80
Donor 1
Donor 2
AFDDT
ANKR9
DYH2
EP300
HTR5B
MED1
MOD5
MRP5
NAL12
RAB4B
RAB7B
SCGR4
G12V 8-16

FIG. 6G

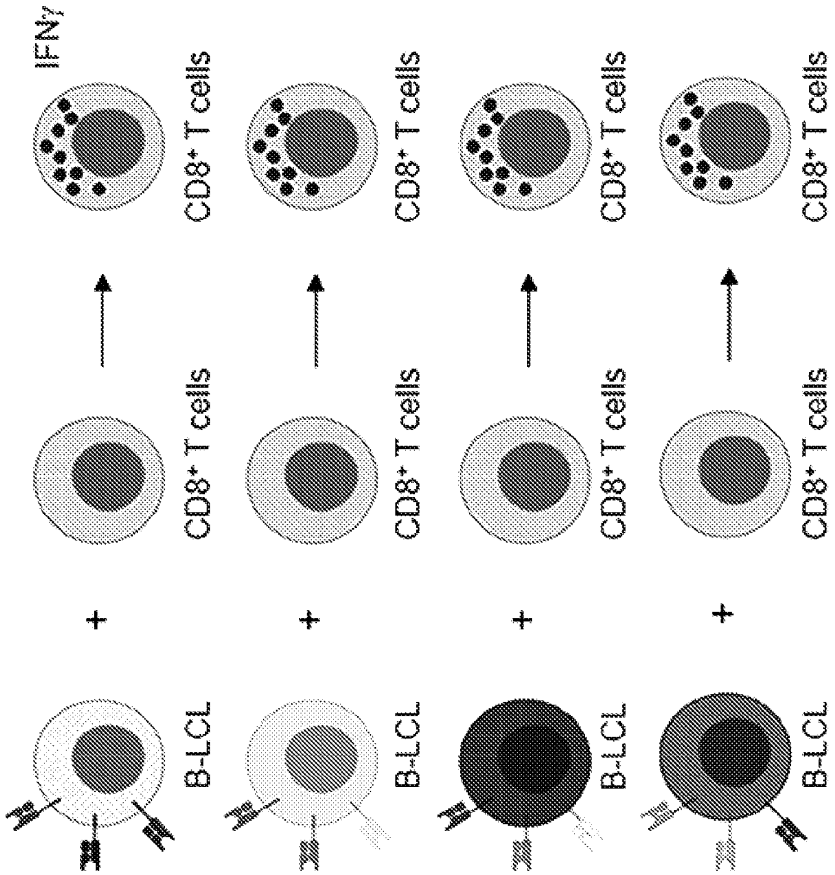

IFNγ
CD8+ T cells
CD8+ T cells
B-LCL
+
CD8+ T cells
CD8+ T cells
B-LCL
+
CD8+ T cells
CD8+ T cells
B-LCL
+
CD8+ T cells
CD8+ T cells
B-LCL
+

FIG. 7A

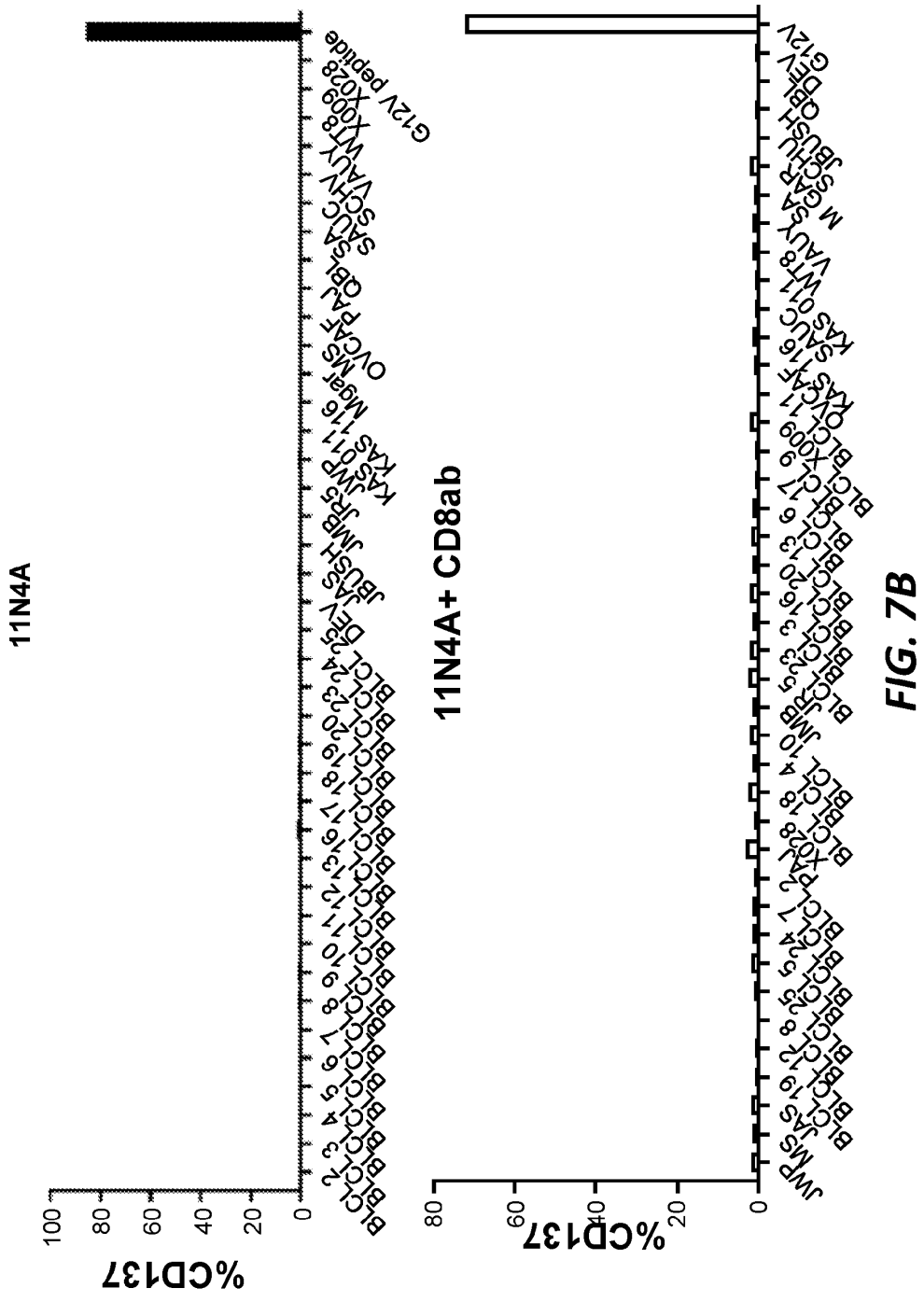

11N4A
%CD137
100
80
60
40
20
0
G12V peptide
11N4A+ CD8ab
%CD137
80
60
40
20
0

FIG. 7B

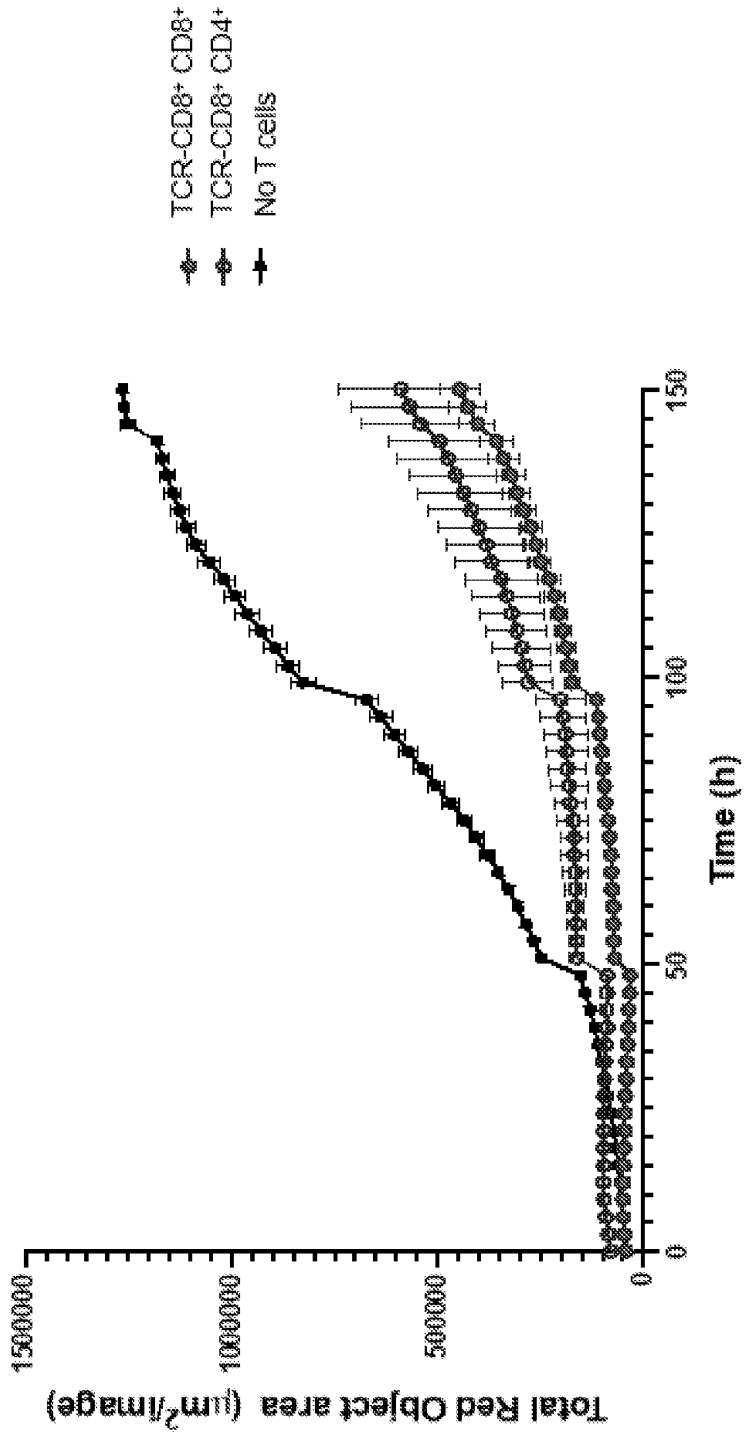

TCR-CD8+ CD8+
TCR-CD8+ CD4+
No T cells
Total Red Object area (μm²/Image)
1500000
1000000
500000
0
0
50
100
150
Time (h)

FIG. 8

11N4A TCR alpha – *original nucleotide sequence* (SEQ ID NO.:5)

atggccatgctcctgggggcatcagtgctgattctgtggcttcagccagactgggtaaacagtcaacagaagaatgatgaccagcaagtt
aagcaaaattcaccatccctgagcgtccaggaaggaagaatttctattctgaactgtgactatact**aacagcatgtttgattat**ttcctatg
gtacaaaaaataccctgctgaaggtcctacattcctgatatct**ataagttccattaaggataaa**aatgaagatggaagattcactgtcttc
ttaaacaaaagtgccaagcacctctctctgcacattgtgccctcccagcctggagactctgcagtgtacttc**tgtgcagcaagtggggtttc**
aggaaacacacctcttgtctttggaaagggcacaagactttctgtgattgcaaatatccagaaccctgaccctgccgtgtaccagctgag
agactctaaatccagtgacaagtctgtctgcctattcaccgattttgattctcaaacaaatgtgtcacaaagtaaggattctgatgtgtata
tcacagacaaaactgtgctagacatgaggtctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgactttgcatgtg
caaacgccttcaacaacagcattattccagaagacaccttcttccccagcccagaaagttcctgtgatgtcaagctggtcgagaaaagct
ttgaaacagatacgaacctaaactttcaaaacctgtcagtgattgggttccgaatcctcctcctgaaagtggccgggtttaatctgctcatg
acgctgcggctgtggtccagctga

11N4A TCR beta – *original nucleotide sequence* (SEQ ID NO.:6)

atgggctccaggctgctctgttgggtgctgctttgtctcctgggagcaggcccagtaaaggctggagtcactcaaactccaagatatctga
tcaaaacgagaggacagcaagtgacactgagctgctcccctatc**tctgggcataggagt**gtatcctggtaccaacagaccccaggacag
ggccttcagttcctctttgaa**tacttcagtgagacacag**agaaacaaaggaaacttccctggtcgattctcagggcgccagttctctaact
ctcgctctgagatgaatgtgagcaccttggagctgggggactcggccctttatctt**tgcgccagcagcgtcgggactgtggagcagtactt**
cgggccgggcaccaggctcacggtcacagaggacctgaaaaacgtgttcccacccgaggtcgctgtgtttgagccatcagaagcagag
atctcccacacccaaaaggccacactggtgtgcctggccacaggcttctaccccgaccacgtggagctgagctggtgggtgaatgggaa
ggaggtgcacagtggggtcagcacagacccgcagcccctcaaggagcagcccgccctcaatgactccagatactgcctgagcagccgc
ctgagggtctcggccaccttctggcagaacccccgcaaccacttccgctgtcaagtccagttctacgggctctcggagaatgacgagtgg
acccaggatagggccaaacctgtcacccagatcgtcagcgccgaggcctggggtagagcagactgtggcttcacctccgagtcttacca
gcaaggggtcctgtctgccaccatcctctatgagatcttgctagggaaggccaccttgtatgccgtgctggtcagtgccctcgtgctgatgg
ccatggtcaagagaaaggattccagaggctag

Key:
CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
Signal peptide
Cysteine modified **C**

FIG. 9A

11N4A TCRbeta-P2A-alpha *Codon-optimization A* (SEQ ID NO.:7)

ATGGGCTCTAGACTGTTGTTGTGGGTTCTGCTGTGTCTGCTTGGAGCTGGACCTGTGAAAGCTGGAGTT
ACCCAGACACCCAGATATCTGATCAAGACCAGAGGACAGCAGGTGACACTGAGCTGTAGCCCTATTTCT
GGCCACAGGAGCGTTAGCTGGTATCAGCAAACACCCGGGCAGGGACTACAATTTCTATTCGAGTACTTC
AGCGAGACCCAGCGGAATAAGGGCAATTTTCCTGGCAGAATTTAGCGGCAGGTTCAGCAACAGCAG
AAGCGAGATGAACGTGAGCACCCTGGAATTAGGCGATTCTGCTCTGTACCTGTGCGCTCTTCTGTGGG
AACAGTGGAGCAGTACTTTGGCCCCGGCACGAGACTGACAGTGACAGAGGACCTGAAGAACGTGTTCCC
CCCCAGAGGTGGCCGTGTTCGAGCCTAGCGAGGCCGAGATCAGCCACACCCAGAAAGCCACCCTCGTG
TGCCTGGCCACCGGCTTTTACCCCGACCACGTGGAACTGTCTTGGTGGGTCAACGGCAAAGAGGTGCAC
AGCGGCGTCTGCACCGACCCCCAGCCCCTGAAAGAGCAGCCCGCCCTGAACGACAGCCGGTACTGTCTG
AGCAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCCGGAACCACTTCAGATGCCAGGTGCAG
TTCTACGGCCTGAGCGAGAACGACGAGTGGACCCAGGACCGGGCCAAGCCCGTGACCCAGATCGTGTCC
TGCTGAGGCCTGGGGCAGAGCCGATTGCGGCTTCACCAGCGAGAGCTACCAGCAGGGCGTGCTGAGCG
CCACCATCCTGTACGAGATCCTGCTGGGCAAGGCCACCCTGTACGCCGTGCTGGTGTCCGCCCTGGTGCT
GATGGCCATGGTCAAGCGGAAGGACAGCCGGGGCGGTTCCGGAGCCACGAACTTCTCTCTGTTAAAGC
AAGCAGGAGACGTGGAAGAAAACCCCGGTCCCATGGCCATGTTACTAGGAGCGAGCGGTGCTGATTCTG
TGGTTACAGCCTGATTGGGTGAACTCTCAGCAGAAGAACGACGATCAGCAGGTGAAGCAGAATAGCCC
CTCTCTGTCTGTGCAGGAGGGCAGAATCTCTATCCTGAATTGCGACTACACCAACAGCATGTTCGACTAT
TTTCTGTGGTACAAAAAATACCCCGCCGAGGGCCCTACATTCCTGATCAGCATCAGCTCTATCAAGGACA
AGAACGAGGATGGCAGATTTACCGTGTTCCTGAACAAGAGCGCCAAGCACCTGAGCCTGCACATTGTGC
CTTCTCAACCTGGCGATTCTGCTGTGTACTTTTGTGCTGCCTCTGGAGTGAGCGGCAATACACCTCTAGT
GTTCGGGAAGGGCACAAGACTGTCTGTTATTGCAAACATTCAAAACCCCGACCCTGCTGTGTACCAGCT
GCGGGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTCACCGACTTCGACAGCCAGACCAACGTGT
CCCAGAGCAAGGACAGCGACGTGTACATCACCGATAAGTGCGTGCTGGACATGCGGAGCATGGACTTC
AAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGACTTCGCCTGCGCCAACGCCTTCAACAACAGC
ATTATCCCCGAGGACACATTCTTCCCAAGCCCCGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAG
CTTCGAGACAGACACCAACCTGAACTTCCAGAACCTCAGCGTGATCGGCTTCCGGATCCTGCTGCTGAA
GGTGGCCGGCTTCAACCTGCTGATGACCCTGCGGCTGTGGTCCAGCTGA

Key:
CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
GSG linker/P2A
Signal peptide

FIG. 9B

11N4A TCRbeta-P2A-alpha *Codon-optimization B* (SEQ ID NO.:8)

ATGGGATCTAGATTGCTTTGTTGGGTGCTGCTGTGCCTGCTCGGAGCCGGACCTGTGAAAGCTGGCGTT
ACCCAGACACCTAGATACCTGATCAAGACCAGAGGCCAGCAAGTGACCCTGAGCTGCTCTCCTATC**AGC**
GGCCACAGAAGCGTGTCCTGGTATCAGCAGACACCTGGACAGGGCCTGCAGTTCCTGTTCGAG**TACTTC**
AGCGAGACACAGCGGAACAAGGGCAACTTCCCCGGCAGATTTTCCGGCAGACAGTTCAGCAACAGCCG
CAGCGAGATGAACGTGTCCACACTGGAACTGGGCGACAGCGCCCTGTATCTG**TGTGCCTCTTCTGTGGG**
CACCGTGGAACAGTACTTTGGCCCTGGCACCAGACTGACCGTGACCGAGGATCTGAAGAACGTGTTCCC
ACCTGAGGTGGCCGTGTTCGAGCCTTCTGAGGCCGAGATCAGCCACACACAGAAAGCCACACTCGTGTG
TCTGGCCACCGGCTTCTATCCCGATCACGTGGAACTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACAG
CGGCGTCTGTACCGATCCTCAGCCTCTGAAAGAGCAGCCCGCTCTGAACGACAGCAGATACTGCCTGAG
CAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCAGAAACCACTTCAGATGCCAGGTGCAGTT
CTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGATAGAGCCAAGCCTGTGACACAGATCGTGTCTG
CCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGAGCTACCAGCAGGGCGTGCTGTCTGCC
ACAATCCTGTACGAGATCCTGCTGGGCAAAGCCACTCTGTACGCCGTGCTGGTTTCTGCCCTGGTGCTGA
TGGCCATGGTCAAGCGGAAGGATTCTAGAGGC**GGATCCGGAGCCACCAACTTCAGCCTGCTTAAACAG**
GCCGGCGACGTGGAAGAGAACCCTGGACCT ATGGCTATGCTGCTGGGAGCCTCTGTGCTGATCCTGTG
GCTGCAACCCGATTGGGTCAACAGCCAGCAGAAGAACGACGACCAGCAAGTCAAGCAGAACAGCCCCA
GCCTGAGCGTGCAAGAGGGCAGAATCAGCATCCTGAACTGCGACTACACC**AACTCTATGTTCGACTAC**T
TTCTGTGGTACAAGAAGTACCCCGCCGAGGGACCCACCTTCCTGATCAGC**ATCAGCAGCATCAAGGACA**
AGAACGAGGACGGCCGGTTCACCGTGTTTCTGAACAAGAGCGCCAAGCACCTGAGCCTGCACATCGTG
CCTTCTCAGCCTGGCGATAGCGCCGTGTACTTT**TGTGCTGCCAGCGGCGTGTCAGGCAACACCCCTCTGG**
TTTTTGGCAAGGGCACACGCCTGTCCGTGATCGCCAACATTCAGAACCCTGATCCTGCCGTGTACCAGCT
GAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTCACCGACTTCGACAGCCAGACCAACGTGT
CCCAGAGCAAGGACAGCGACGTGTACATCACCGATAAGTGCGTGCTGGACATGCGGAGCATGGACTTC
AAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGTCCGATTTCGCCTGCGCCAACGCCTTCAACAACAGC
ATTATCCCCGAGGACACATTCTTCCCAAGTCCTGAGTCCAGCTGCGACGTGAAGCTGGTGGAAAAGAGC
TTCGAGACAGACACCAACCTGAACTTCCAGAATCTGAGCGTGATCGGCTTCAGAATCCTGCTGCTGAAG
GTGGCCGGATTCAACCTGCTGATGACCCTCAGACTGTGGTCCAGCTGA

Key:
CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
GSG linker/P2A
Signal peptide

FIG. 9C

CD8alpha-T2A-CD8beta-P2A-11N4A TCRbeta-P2A-alpha *Codon-optimization A* (SEQ ID NO.:9)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCCGCTAGACCCAGCCAGT
TCAGAGTGTCCCCTCTGGACAGAACCTGGAACCTGGGCGAGACAGTGGAACTGAAGTGCCAGGTGCTG
CTGAGCAATCCTACCAGCGGCTGCAGCTGGCTGTTTCAGCCTAGAGGTGCTGCCGCCTCTCCTACCTTTC
TGCTGTACCTGAGCCAGAACAAGCCCAAGGCCGCCGAAGGACTGGACACCCAGAGATTCAGCGGCAAG
AGACTGGGCGACACCTTCGTGCTGACCCTGAGCGACTTCAGAAGAGAGAACGAGGGCTACTACTTCTGC
AGCGCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCGTGTTTCTGCCCGCCAAGCCTACAA
CAACCCCTGCTCCTAGACCTCCTACACCAGCTCCTACAATCGCCAGCCAGCCTCTGTCTCTGAGGCCAGA
AGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAGAGGACTGGATTTCGCCTGCGACATCTACAT
CTGGGCCCCTCTGGCTGGAACATGTGGCGTGCTGCTGCTGTCCCTGGTCATCACCCTGTACTGCAACCAC
CGGAACAGGCGGAGAGTGTGCAAGTGCCCTAGACCTGTGGTCAAGAGCGGCGACAAGCCTAGCCTGA
GCGCCAGATATGTT**GGCAGCGGAGAAGGCAGAGGCTCCCTGCTTACATGCGGCGACGTGGAAGAGAA**
CCCCGGACCTATGAGGCCTAGACTGTGGCTGCTTCTGGCTGCCCAGCTGACAGTGCTGCACGGCAATTC
TGTCCTGCAGCAGACCCCTGCCTACATCAAGGTGCAGACCAACAAGATGGTCATGCTGAGCTGCGAGGC
CAAGATCAGCCTGTCCAACATGCGGATCTACTGGCTGCGGCAGAGACAGGCCCCTAGCTCTGATAGCCA
CCACGAGTTTCTGGCCCTGTGGGATTCTGCCAAGGGCACCATTCACGGCGAGGAAGTGGAACAAGAGA
AGATCGCCGTGTTCCGGGACGCCAGCAGATTCATCCTGAACCTGACCAGCGTGAAGCCCGAGGACAGC
GGCATCTATTTCTGCATGATCGTGGGCAGCCCCGAGCTGACATTTGGCAAGGGAACACAGCTGAGCGTG
GTGGACTTCCTGCCTACTACAGCCCAGCCTACCAAGAAGTCTACCCTGAAGAAACGCGTGTGCAGACTG
CCCAGGCCTGAGACACAAAAGGGCCCTCTGTGCAGCCCTATCACACTGGGATTGCTGGTGGCTGGCGTT
CTGGTCCTGCTGGTGTCTCTGGGAGTTGCCATCCACCTGTGCTGTAGAAGAAGGCGGGCCAGACTGCGG
TTCATGAAGCAGTTCTACAAA**GGCAGCGGCGCCACCAACTTCAGCCTGCTGAAACAAGCCGGCGACGTC**
GAGGAAAATCCTGGACCT **ATGGGCTCTAGACTGTTGTGTTGGGTTCTGCTGTGTCTGCTTGGAGCTGGA**
CCTGTGAAAGCTGGAGTTACCCAGACACCCAGATATCTGATCAAGACCAGAGGACAGCAGGTGACACT
GAGCTGTAGCCCTATT**TCTGGCCACAGGAGC**GTTAGCTGGTATCAGCAAACACCCGGGCAGGGACTACA
ATTTCTATTCGAG**TACTTCAGCGAGACCCAG**CGGAATAAGGGCAATTTTCCTGGCAGATTTAGCGGCAG
GCAGTTCAGCAACAGCAGAAGCGAGATGAACGTGAGCACCCTGGAATTAGGCGATTCTGCTCTGTACCT
GTGTGCCTCTTCTGTGGGAACAGTGGAGCAGTACTTTGGCCCCGGCACGAGACTGACAGTGACAGAGG
ACCTGAAGAACGTGTTCCCCCCAGAGGTGGCCGTGTTCGAGCCTAGCGAGGCCGAGATCAGCCACACCC
AGAAAGCCACCCTCGTGTGCCTGGCCACCGGCTTTTACCCCGACCACGTGGAACTGTCTTGGTGGGTCA
ACGGCAAAGAGGTGCACAGCGGCGTCTGCACCGACCCCCAGCCCCTGAAAGAGCAGCCCGCCCTGAAC

Key:
CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
GSG linker/P2A
GSG linker/T2A
Signal peptide

FIG. 9D

CD8alpha-T2A-CD8beta-P2A-11N4A TCRbeta-P2A-alpha *Codon-optimization A*
(SEQ ID NO.:9, cont'd)

GACAGCCGGTACTGTCTGAGCAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCCGGAACCAC
TTCAGATGCCAGGTGCAGTTCTACGGCCTGAGCGAGAACGACGAGTGGACCCAGGACCGGGCCAAGCC
CGTGACCCAGATCGTGTCTGCTGAGGCCTGGGGCAGAGCCGATTGCGGCTTCACCAGCGAGAGCTACC
AGCAGGGCGTGCTGAGCGCCACCATCCTGTACGAGATCCTGCTGGGCAAGGCCACCCTGTACGCCGTGC
TGGTGTCCGCCCTGGTGCTGATGGCCATGGTCAAGCGGAAGGACAGCCGGGGC**GGTTCCGGAGCCACG**
AACTTCTCTCTGTTAAAGCAAGCAGGAGACGTGGAAGAAAACCCCGGTCCCATGGCCATGTTACTAGGA
GCGAGCGTGCTGATTCTGTGGTTACAGCCTGATTGGGTGAACTCTCAGCAGAAGAACGACGATCAGCA
GGTGAAGCAGAATAGCCCCTCTCTGTCTGTGCAGGAGGGCAGAATCTCTATCCTGAATTGCGACTACAC
C**AACAGCATGTTCGACTAT**TTTCTGTGGTACAAAAAATACCCCGCCGAGGGCCCTACATTCCTGATCAGC
ATCAGCTCTATCAAGGACAAGAACGAGGATGGCAGATTTACCGTGTTCCTGAACAAGAGCGCCAAGCAC
CTGAGCCTGCACATTGTGCCTTCTCAACCTGGCGATTCTGCTGTGTACTTT**TGTGCTGCCTCTGGAGTGA**
GCGGCAATACACCTCTAGTGTTCGGGAAGGGCACAAGACTGTCTGTTATTGCAAACATTCAAAACCCCG
ACCCTGCTGTGTACCAGCTGCGGGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTCACCGACTTCG
ACAGCCAGACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGATAAGTGCGTGCTGGAC
ATGCGGAGCATGGACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGACTTCGCCTGCGC
CAACGCCTTCAACAACAGCATTATCCCCGAGGACACATTCTTCCCAAGCCCCGAGAGCAGCTGCGACGT
GAAGCTGGTGGAAAAGAGCTTCGAGACAGACACCAACCTGAACTTCCAGAACCTCAGCGTGATCGGCTT
CCGGATCCTGCTGCTGAAGGTGGCCGGCTTCAACCTGCTGATGACCCTGCGGCTGTGGTCCAGCTGA

Key:
CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
GSG linker/P2A
Signal peptide

FIG. 9E

CD8alpha-T2A-CD8beta-P2A-11N4A TCRbeta-P2A-alpha *Codon-optimization B* (SEQ ID NO.:10)

ATGGCATTGCCTGTTACAGCTCTGCTGCTGCCCCTGGCTCTGCTTCTGCATGCTGCTAGACCCAGCCAGTT
CAGAGTGTCCCCTCTGGACAGAACCTGGAACCTGGGCGAGACAGTGGAACTGAAGTGCCAGGTGCTGC
TGAGCAATCCTACCAGCGGCTGCAGCTGGCTGTTTCAGCCTAGAGGTGCTGCCGCCTCTCCTACCTTTCT
GCTGTACCTGAGCCAGAACAAGCCCAAGGCCGCCGAAGGACTGGACACCCAGAGATTCAGCGGCAAGA
GACTGGGCGACACCTTCGTGCTGACCCTGAGCGACTTCAGAAGAGAGAACGAGGGCTACTACTTCTGCA
GCGCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCGTGTTTCTGCCCGCCAAGCCTACAAC
AACCCCTGCTCCTAGACCTCCTACACCAGCTCCTACAATCGCCAGCCAGCCTCTGTCTCTGAGGCCAGAA
GCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAGAGGACTGGATTTCGCCTGCGACATCTACATC
TGGGCCCCTCTGGCTGGAACATGTGGCGTGCTGCTGCTGTCTCTGGTCATCACCCTGTACTGCAACCACC
GGAACAGGCGGAGAGTGTGCAAGTGCCCTAGACCTGTGGTCAAGAGCGGCGACAAGCCTAGCCTGAG
CGCCAGATATGTT**GGCAGCGGAGAAGGCAGAGGCAGCCTGCTTACATGCGGCGACGTGGAAGAGAAC**
CCCGGACCTATGAGGCCTAGACTGTGGCTGCTTCTGGCTGCCCAGCTGACAGTGCTGCACGGCAATTCT
GTCCTGCAGCAGACCCCTGCCTACATCAAGGTGCAGACCAACAAGATGGTCATGCTGAGCTGCGAGGCC
AAGATCAGCCTGTCCAACATGCGGATCTACTGGCTGCGGCAGAGACAGGCCCCTAGCAGCGATTCTCAC
CACGAGTTTCTGGCCCTGTGGGATAGCGCCAAGGGAACCATTCACGGCGAGGAAGTGGAACAAGAGAA
GATCGCCGTGTTCCGGGACGCCAGCAGATTCATCCTGAACCTGACCAGCGTGAAGCCCGAGGACAGCG
GCATCTATTTCTGCATGATCGTGGGCAGCCCCGAGCTGACATTTGGCAAGGGAACACAGCTGAGCGTGG
TGGACTTCCTGCCTACTACAGCCCAGCCTACCAAGAAGTCTACCCTGAAGAAACGCGTGTGCAGACTGC
CCAGGCCTGAGACACAAAAGGGCCCTCTGTGCAGCCCTATCACACTGGGATTGCTGGTGGCTGGCGTTC
TGGTCCTGCTGGTTTCTCTGGGAGTTGCCATCCACCTGTGCTGCAGACGCAGAAGGGCCAGACTGCGGT
TCATGAAGCAGTTCTACAAA**GGCAGCGGCGCCACCAACTTCAGCCTGCTGAAACAAGCCGGCGACGTCG**
AAGAAAATCCTGGACCAATGGGCAGCAGACTGCTGTGCTGGGTTCTGCTGTGTCTGCTTGGAGCCGGAC
CTGTGAAAGCTGGCGTGACCCAGACACCTAGATACCTGATCAAGACCAGAGGCCAGCAAGTGACACTG
AGCTGTAGCCCCATC**AGCGGCCACAGAAGC**GTGTCCTGGTATCAGCAGACTCCTGGACAGGGCCTGCA

Key:
CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
GSG linker/P2A
GSG linker/T2A
Signal peptide

FIG. 9F

CD8alpha-T2A-CD8beta-P2A-11N4A TCRbeta-P2A-alpha *Codon-optimization B*
(SEQ ID NO.:10, cont'd)

GTTCCTGTTCGAG**TACTTCTCCGAGACACAG**AGGAACAAGGGCAACTTCCCCGGCAGATTCTCCGGCAG
ACAGTTCAGCAACTCCCGCAGCGAGATGAACGTGTCCACACTGGAACTGGGAGATAGCGCCCTGTACCT
G**TGTGCCTCTTCTGTGGGAACCGTGGAACAGTACTTC**GGCCCTGGCACAAGACTGACCGTGACCGAGGA
CCTGAAGAACGTGTTCCCACCTGAGGTGGCCGTGTTCGAGCCTTCTGAGGCCGAGATCTCTCACACCCA
GAAAGCCACACTCGTGTGTCTGGCCACCGGCTTCTATCCCGATCACGTGGAACTGTCTTGGTGGGTCAAC
GGCAAAGAGGTGCACAGCGGCGTCTGTACCGATCCTCAGCCACTGAAAGAGCAGCCCGCTCTGAACGA
CAGCAGATACTGCCTGTCCTCCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCAGAAACCACTTC
AGGTGTCAGGTGCAGTTTTACGGCCTGAGCGAGAACGACGAGTGGACCCAGGATAGAGCCAAGCCTGT
GACACAGATCGTGTCTGCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGAGCTACCAGC
AGGGCGTTCTGTCTGCCACCATCCTGTACGAGATCCTGCTGGGCAAAGCCACTCTGTACGCCGTGTTGGT
GTCTGCCCTGGTGCTGATGGCCATGGTCAAGCGGAAGGATTCTAGAGGC**GGATCCGGAGCCACAAATT**
TCTCACTGCTGAAGCAGGCCGGGGATGTTGAGGAAAACCCAGGACCTATGGCTATGCTGCTGGGAGCC
TCTGTGCTGATCCTGTGGCTGCAACCCGATTGGGTCAACAGCCAGCAGAAGAACGACGACCAGCAAGTC
AAGCAGAACAGCCCCAGCCTGAGCGTGCAAGAGGGCAGAATCAGCATCCTGAACTGCGACTACACC**AA**
CTCTATGTTCGACTACTTTCTGTGGTACAAGAAGTACCCCGCCGAGGGACCCACCTTCCTGATCAGC**ATC**
AGCAGCATCAAGGACAAGAACGAGGACGGCCGGTTCACCGTGTTTCTGAACAAGAGCGCCAAGCACCT
GAGCCTGCACATCGTGCCTTCTCAGCCTGGCGATAGCGCCGTGTACTTT**TGTGCTGCCAGCGGCGTGTCA**
GGCAACACCCCTCTGGTTTTTGGCAAGGGCACACGCCTGTCCGTGATCGCCAACATTCAGAACCCTGATC
CTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTCACCGACTTCGAC
AGCCAGACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGATAAGTGCGTGCTGGACAT
GCGGAGCATGGACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGTCCGATTTCGCCTGCGCCAA
CGCCTTCAACAACAGCATTATCCCCGAGGACACATTCTTCCCAAGTCCTGAGTCCAGCTGCGACGTGAAG
CTGGTGGAAAAGAGCTTCGAGACAGACACCAACCTGAACTTCCAGAATCTGAGCGTGATCGGCTTCAGA
ATCCTGCTGCTGAAGGTGGCCGGATTCAACCTGCTGATGACCCTCAGACTGTGGTCCAGCTGA

Key:
CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
GSG linker/P2A
Signal peptide

FIG. 9G

11N4A TCR alpha – *original protein* (SEQ ID NO.:11)
MAMLLGASVLILWLQPDWVNSQQKNDDQQVKQNSPSLSVQEGRISILNCDYTNSMFDYFLWYKKYPAEG
PTFLISISSIKDKNEDGRFTVFLNKSAKHLSLHIVPSQPGDSAVYFCAASGVSGNTPLVFGKGTRLSVIANIQNP
DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANA
FNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS*

11N4A TCR beta – *original protein* (SEQ ID NO.:12)
MGSRLLCWVLLCLLGAGPVKAGVTQTPRYLIKTRGQQVTLSCSPISGHRSVSWYQQTPGQGLQFLFEYFSET
QRNKGNFPGRFSGRQFSNSRSEMNVSTLELGDSALYLCASSVGTVEQYFGPGTRLTVTEDLKNVFPPEVAVF
EPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATF
WQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKAT
LYAVLVSALVLMAMVKRKDSRG*

Key:
CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
Signal peptide

FIG. 9H

11N4A TCRbeta-P2A-alpha – *Protein* (SEQ ID NO.:31)

MGSRLLCWVLLCLLGAGPVKAGVTQTPRYLIKTRGQQVTLSCSPISGHRSVSWYQQTPGQGLQFLFEYFSET
QRNKGNFPGRFSGRQFSNSRSEMNVSTLELGDSALYLCASSVGTVEQYFGPGTRLTVTEDLKNVFPPEVAVF
EPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVCTDPQPLKEQPALNDSRYCLSSRLRVSATF
WQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKAT
LYAVLVSALVLMAMVKRKDSRGGSGATNFSLLKQAGDVEENPGPMAMLLGASVLILWLQPDWVNSQQKN
DDQQVKQNSPSLSVQEGRISILNCDYTNSMFDYFLWYKKYPAEGPTFLISISSIKDKNEDGRFTVFLNKSAKHL
SLHIVPSQPGDSAVYFCAASGVSGNTPLVFGKGTRLSVIANIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTN
VSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFET
DTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS*

Key:
CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
GSG linker/P2A
Signal peptide
Cysteine modified C

FIG. 9I

CD8alpha-T2A-CD8beta-P2A-11N4A TCRbeta-P2A-alpha – *Protein* (SEQ ID NO.:32)

MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQPRGAAASPTFLLYLS
QNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYYFCSALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTP
APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVV
KSGDKPSLSARYVGSGEGRGSLLTCGDVEENPGPMRPRLWLLLAAQLTVLHGNSVLQQTPAYIKVQTNKMV
MLSCEAKISLSNMRIYWLRQRQAPSSDSHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASRFILNLTSVKPEDS
GIYFCMIVGSPELTFGKGTQLSVVDFLPTTAQPTKKSTLKKRVCRLPRPETQKGPLCSPITLGLLVAGVLVLVSL
GVAIHLCCRRRRARLRFMKQFYKGSGATNFSLLKQAGDVEENPGPMGSRLLCWVLLCLLGAGPVKAGVTQT
PRYLIKTRGQQVTLSCSPISGHRSVSWYQQTPGQGLQFLFEYFSETQRNKGNFPGRFSGRQFSNSRSEMNVS
TLELGDSALYLCASSVGTVEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVEL
SWWVNGKEVHSGVCTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQD
RAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRGGSGAT.
NFSLLKQAGDVEENPGPMAMLLGASVLILWLQPDWVNSQQKNDDQQVKQNSPSLSVQEGRISILNCDYT N
SMFDYFLWYKKYPAEGPTFLISISSIKDKNEDGRFTVFLNKSAKHLSLHIVPSQPGDSAVYFCAASGVSGNTPLV
FGKGTRLSVIANIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRSMDFKSNS
AVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTL
RLWSS*

Key:

CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
GSG linker/P2A
GSG linker/T2A
Signal peptide
Cysteine modified C

FIG. 9J

11N6 TCR alpha – *original nucleotide sequence* (SEQ ID NO.:33)

atggaaactctcctgggagtgtctttggtgattctatggcttcaactggctagggtgaacagtcaacagggagaagaggatcctcaggcc
ttgagcatccaggagggtgaaaatgccaccatgaactgcagttacaaa**actagtataaacaat**ttacagtggtatagacaaaattcagg
tagaggccttgtccacctaattttaa**tacgttcaaatgaaagagag**aaacacagtggaagattaagagtcacgcttgacacttccaagaa
aagcagttccttgttgatcacggcttcccgggcagcagacactgcttcttacttc**tgtgctacggaccctatgaacaccaatgcaggcaaa**
tcaacctttgggggatgggactacgctcactgtgaagccaaatatccagaaccctgaccctgccgtgtaccagctgagagactctaaatcc
agtgacaagtctgtctgcctattcaccgattttgattctcaaacaaatgtgtcacaaagtaaggattctgatgtgtatatcacagacaaaa
ctgtgctagacatgaggtctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgactttgcatgtgcaaacgccttca
acaacagcattattccagaagacaccttcttccccagcccagaaagttcctgtgatgtcaagctggtcgagaaaagctttgaaacagata
cgaacctaaactttcaaaacctgtcagtgattgggttccgaatcctcctcctgaaagtggccgggtttaatctgctcatgacgctgcggctg
tggtccagctga

11N6 TCR beta – *original nucleotide sequence* (SEQ ID NO.:34)

atgggcaccaggctcctctgctgggcggccctctgtctcctgggagcagaactcacagaagctggagttgcccagtctcccagatataag
attatagagaaaaggcagagtgtggctttttggtgcaatcctata**tctggccatgctacc**ctttactggtaccagcagatcctgggacagg
gcccaaagcttctgattcag**tttcagaataacggtgta**gtggatgattcacagttgcctaaggatcgattttctgcagagaggctcaaagg
agtagactccactctcaagatccaacctgcaaagcttgaggactcggccgtgtatctc**tgtgccagcagcccctacgggggagcgtctc**
ctacgagcagtacttcgggccgggcaccaggctcacggtcacagaggacctgaaaaacgtgttcccacccgaggtcgctgtgtttgagc
catcagaagcagagatctcccacacccaaaaggccacactggtgtgcctggccacaggcttctaccccgaccacgtggagctgagctgg
tgggtgaatgggaaggaggtgcacagtggggtcagcacagacccgcagcccctcaaggagcagcccgccctcaatgactccagatact
gcctgagcagccgcctgagggtctcggccaccttctggcagaacccccgcaaccacttccgctgtcaagtccagttctacgggctctcgga
gaatgacgagtggacccaggatagggccaaacctgtcacccagatcgtcagcgccgaggcctggggtagagcagactgtggcttcacct
ccgagtcttaccagcaaggggtcctgtctgccaccatcctctatgagatcttgctagggaaggccaccttgtatgccgtgctggtcagtgcc
ctcgtgctgatggccatggtcaagagaaaggattccagaggctag

Key:
CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
Signal peptide

FIG. 10A

11N6 TCRbeta-P2A-alpha *Codon-optimized* (SEQ ID NO.:35)

ATGGGCACAAGACTTCTCTGTTGGGCTGCACTGTGCTTGGAGCTGACAGAAGCTGGAGTT
GCCCAATCTCCTAGGTACAAGATCATCGAGAAGCGGCAGTCTGTGGCCTTTTGGTGCAATCCCATTAGC
GGACATGCCACCCTGTACTGGTATCAGCAAATTCTGGGACAGGGCCCTAAACTGCTGATCCAGTTCCAG
AATAACGGCGTGGTGGACGATTCTCAACTGCCTAAGGACCGGTTTTCTGCCGAGAGACTGAAAGGCGTT
GATAGCACCCTGAAGATCCAACCTGCCAAACTGGAGGATTCTGCCGTGTACCTGTGTGCTAGCAGCCCTT
ATGGAGGATCTGTGTCTTATGAGCAGTACTTCGGACCTGGCACCAGACTGACCGTGACTGAAGACCTGA
AGAACGTGTTCCCCCCAGAGGTGGCCGTGTTCGAGCCTAGCGAGGCCGAGATCAGCCACACCCAGAAA
GCCACCCTCGTGTGCCTGGCCACCGGCTTTTACCCCGACCACGTGGAACTGTCTTGGTGGGTCAACGGC
AAAGAGGTGCACAGCGGCGTCTGCACCGACCCCCAGCCCCTGAAAGAGCAGCCCGCCCTGAACGACAG
CCGGTACTGTCTGAGCAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCCGGAACCACTTCAG
ATGCCAGGTGCAGTTCTACGGCCTGAGCGAGAACGACGAGTGGACCCAGGACCGGGCCAAGCCCGTGA
CCCAGATCGTGTCTGCTGAGGCCTGGGGCAGAGCCGATTGCGGCTTCACCAGCGAGAGCTACCAGCAG
GGCGTGCTGAGCGCCACCATCCTGTACGAGATCCTGCTGGGCAAGGCCACCCTGTACGCCGTGCTGGTG
TCCGCCCTGGTGCTGATGGCCATGGTCAAGCGGAAGGACAGCCGGGGCGGTTCCGGAGCCACGAACTT
CTCTCTGTTAAAGCAAGCAGGAGACGTGGAAGAAAACCCCGGTCCCATGGAGACACTGCTTGGCGTATC
ACTGGTGATTCTGTGGCTGCAACTGGCTAGAGTGAACTCTCAGCAGGGAAGAAGAGGATCCTCAAGCTCT
GAGCATTCAGGAAGGCGAAAACGCAACCATGAATTGCTCATACAAGACCAGCATCAACACCTGCAGTG
GTACCGGCAGAATAGCGGAAGAGGACTGGTTCACCTGATTTTAATCAGGTCTAATGAAAGGGAGAAGC
ACAGCGGCAGACTGAGAGTTTACCCTGGACACATCCAAGAAATCTTCTCTCTGCTGATTACAGCCTCTAG
AGCCGCCGATACAGCCAGCTACTTTTGTGCCACAGATCCCATGAACACCAATGCCGGAAAGAGCACATT
CGGCGATGGCACAACCCTGACAGTTAAGCCCAATATCCAGAATCCCGATCCTGCCGTGTACCAGCTGCG
GGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTCACCGACTTCGACAGCCAGACCAACGTGTCCC
AGAGCAAGGACAGCGACGTGTACATCACCGATAAGTGCGTGCTGGACATGCGGAGCATGGACTTCAAG
AGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGACTTCGCCTGCGCCAACGCCTTCAACAACAGCATT
ATCCCCGAGGACACATTCTTCCCAAGCCCCGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTC
GAGACAGACACCAACCTGAACTTCCAGAACCTCAGCGTGATCGGCTTCCGGATCCTGCTGCTGAAGGTG
GCCGGCTTCAACCTGCTGATGACCCTGCGGCTGTGGTCCAGCTGA

Key:

CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
GSG linker/P2A
Signal peptide

FIG. 10B

CD8alpha-T2A-CD8beta-P2A-11N6 TCRbeta-P2A-alpha *Codon-optimized* (SEQ ID NO.:36)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCCGCTAGACCCAGCCAGT
TCAGAGTGTCCCCTCTGGACAGAACCTGGAACCTGGGTGAGACAGTGGAACTGAAGTGCCAGGTGCTG
CTGAGCAATCCTACCAGCGGCTGCAGCTGGCTGTTTCAGCCTAGAGGTGCTGCCGCCTCTCCTACCTTTC
TGCTGTACCTGAGCCAGAACAAGCCCAAGGCCGCCGAAGGACTGGACACCCAGAGATTCAGCGGCAAG
AGACTGGGCGACACCTTCGTGCTGACCCTGAGCGACTTCAGAAGAGAGAACGAGGGCTACTACTTCTGC
AGCGCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCGTGTTTCTGCCCGCCAAGCCTACAA
CAACCCCTGCTCCTAGACCTCCTACACCAACCTGCCAGCCCAGCCTCTGTCTCTGAGGCCAGA
AGCTTGTAGACCTGCTGGCTGGCGGAGCCGTGCATACAAGAGGACTGGATTTCGCCTGCGACATCTACAT
CTGGGCCCCTCTGGCTGGAACATGTGGCGTGCTGCTGTCCCTGGTCATCACCCTGTACTGCAACCAC
CGGAACAGGCGGAGAGTGTGCAAGTGCCCTAGACCTGTGGTCAAGAGCGGCGACAAGCCTAGCCTGA
GCGCCAGATATGTT GGCAGCGGAGAAGGCAGAGGCTCCCTGCTTACATGCGGCGACGTGGAAGAGAA
CCCCGGACCT ATGAGGCCTAGACTGTGGCTGCTTCTGGCTGCCCAGCTGACAGTGCTGCACGGCAATTC
TGTCCTGCAGCAGACCCCTGCCTACATCAAGGTGCAGACCAACAAGATGGTCATGCTGAGCTGCGAGGC
CAAGATCAGCCTGTCCAACATGCGGATCTACTGGCTGCGGCAGAGACAGGCCCCTAGCTCTGATAGCCA
CCACGAGTTTCTGGCCCTGTGGGATTCTGCCAAGGGCACCATTCACGGCGAGGAAGTGGAACAAGAGA
AGATCGCCGTGTTCCGGGACGCCAGCAGATTCATCCTGAACCTGACCAGCGTGAAGCCCGAGGACAGC
GGCATCTATTTCTGCATGATCGTGGGCAGCCCCGAGCTGACATTTGGCAAGGGAACACAGCTGAGCGTG
GTGGACTTCCTGCCTACTACAGCCCAGCCTACCAAGAAGTCTACCCTGAAGAAACGGCGTGTGCAGACTG

Key:
GSG linker/T2A

FIG. 10C

CD8alpha-T2A-CD8beta-P2A-11N6 TCRbeta-P2A-alpha *Codon-optimized* (SEQ ID NO.:36, cont'd)

CCCAGGCCTGAGACACAAAAGGCCCTCTGTGCAGCCCTATCACACTGGGATTGCTGGTGGCTGGCGTT

CTGGTCCTGCTGGTGTCTCTGGGAGTTGCCATCCACCTGTGCTGTAGAAGAAGGCGGGGCCAGACTGCGG

TTCATGAAGCAGTTCTACAAA GGCAGCGGCGCCACCAACTTCAGCCTGCTGAAACAAGCCGGCGACGTC

GAGGAAAATCCTGGACCT ATGGGCACAAGACTTCTCTGTTGGGCTGCACTGTGCTTGCTTGGAGCTGAG

CTGACAGAAGCTGGAGTTGCCAATCTCCTAGGTACAAGATCATCGAGAAGCGGCAGTCTGTGGCCTTT

TGGTGCAATCCCATT AGCGGACATGCCACCCTGTACTGGTATCAGCAAATTCTGGGACAGGGCCCTAAA

CTGCTGATCCAGTTCCAGAATAACGGCGTGGTGGACGATTCTCAACTGCCTAAGGACCGGTTTTCTGCCG

AGAGACTGAAAGGCGTTGATAGCACCCTGAAGATCCAACCTGCCAAACTGGAGGATTCTGCCGTGTACC

TGTGTGCTAGCAGCCCTTATGGAGGATCTGTGTCTTATGAGCAGTACTTCGGACCTGGCACCAGACTGA

CCGTGACTGAAGACCTGAACAAGGTGTTCCCCCCAGAGGTGGCCGTGTTCGAGCCTAGCGAGGCCGAG

ATCAGCCACACCCAGAAAGCCACCCTCGTGTGCCTGGCCACCGGCTTTTACCCCGACCACGTGGAACTGT

CTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCTGCACCGACCCCCAGCCCCTGAAAGAGCAG

CCCGCCCTGAACGACAGCCGGTACTGTCTGAGCAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAAC

CCCCGGAACCACTTCAGATGCCAGGTGCAGTTCTACGGCCTGAGCGAGAACGACGAGTGGACCCAGGA

CCGGGCCAAGCCCGTGACCCAGATCGTGTCTGCTGAGGCCTGGGGCAGAGCCGATTGCGGCTTCACCA

GCGAGAGCTACCAGCAGGGCGTGCTGAGCGCCACCATCCTGTACGAGATCCTGCTGGGCAAGGCCACC

Key:

CDR1 (alpha and beta)

CDR2 (alpha and beta)

CDR3 (alpha and beta)

GSG linker/P2A

Signal peptide

FIG. 10D

CD8alpha-T2A-CD8beta-P2A-11N6 TCRbeta-P2A-alpha *Codon-optimized* (SEQ ID NO.:36, cont'd)

CTGTACGCCGTGCTGGTGTCCGCCCTGGTGCTGATGGCCATGGTCAAGCGGAAGGACAGCCGGGGC GG

TTCCGGAGCCACCAACTTCAGCCTGCTTAAACAGGCCGGCGACGTGGAAGAGAACCCTGGACCT ATGGA

GACACTGCTTGGCGTATCACTGGTGATTCTGGCTGCAACTGGCTAGAGTGAACTCTCAGCAGGGAGA

AGAGGATCCCTCAAGCTCTGAGCATTCAGGAAGGCGAAAACGCAACCATGAATTGCTCATACAAG ACCAG

CATCAACAACCTGCAGTGGTACCGGCAGAATAGCGGAAGAGGACTGGTTCACCTGATTTTA ATCAGGTC

TAATGAAAGGGAGAAGCACAGCGGCAGACTGAGAGTTACCCTGGACACATCCAAGAAATCTTCTTCTCT

GCTGATTACAGCCTCTAGAGCCGCCGATACAGCCACTACTTT TGTGCCACAGATCCCATGAACACCAAT

GCCGGAAAGAGCACATTC GGCGATGGCACAACCCTGACAGTTAAGCCCAATATCCAGAATCCCGATCCCT

GCCGTGTACCAGCTGCGGGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTCACCGACTTCGACAG

CCAGACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGATAAGTGCGTGCTGGACATGC

GGAGCATGGACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGACTTCGCCTGCGCCAAC

GCCTTCAACAACAGCATTATCCCCGAGGACACATTCTTCCCAAGCCCCGAGAGCAGCTGCGACGTGAAG

CTGGTGGAAAAGAGCTTCGAGACAGACACCAACCTGAACTTCCAGAACCTCAGCGTGATCGGCTTCCGG

ATCCTGCTGCTGAAGGTGGCCGGCTTCAACCTGCTGATGACCCTGCGGCTGTGGTCCAGCTGA

Key:

CDR1 (alpha and beta)

CDR2 (alpha and beta)

CDR3 (alpha and beta)

GSG linker/P2A

Signal peptide

FIG. 10E

11N6 TCR alpha – *original protein* (SEQ ID NO.:37)

METLLGVSLVILWLQLARVNSQQGEEDPQALSIQEGENATMNCSYKTSINNLQWYRQNSGRGLVHLIL**IRSN**
EREKHSGRLRVTLDTSKKSSSLLITASRAADTASYF**CATDPMNTNAGKSTF**GDGTTLTVKPNIQNPDPAVYQL
RDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPE
DTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS*

11N6 TCR beta – *original protein* (SEQ ID NO.:47)

MGTRLLCWAALCLLGAELTEAGVAQSPRYKIIEKRQSVAFWCNPI**SGHAT**LYWYQQILGQGPKLLIQ**FQNNG**
VVDDSQLPKDRFSAERLKGVDSTLKIQPAKLEDSAVYL**CASSPYGGSVSYEQYF**GPGTRLTVTEDLKNVFPPEV
AVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVS
ATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLG
KATLYAVLVSALVLMAMVKRKDSRG*

Key:
CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
Signal peptide

FIG. 10F

11N6 TCRbeta-P2A-alpha – *Protein* (SEQ ID NO.:57)

MGTRLLCWAALCLLGAELTEAGVAQSPRYKIIEKRQSVAFWCNPISGHATLYWYQQILGQGPKLLIQ**FQNNG**
VVDDSQLPKDRFSAERLKGVDSTLKIQPAKLEDSAVYL**CASSPYGGSVSYEQYF**GPGTRLTVTEDLKNVFPPEV
AVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGV**C**TDPQPLKEQPALNDSRYCLSSRLRVS
ATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLG
KATLYAVLVSALVLMAMVKRKDSRG**GSGATNFSLLKQAGDVEENPGP**METLLGVSLVILWLQLARVNS**QQG**
EEDPQALSIQEGENATMNCSYKTSINNLQWYRQNSGRGLVHLILIRSNEREKHSGRLRVTLDTSKKSSSLLITA
SRAADTASYFC**ATDPMNTNAGKSTF**GDGTTLTVKPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQS
KDSDVYITDK**C**VLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNL
NFQNLSVIGFRILLLKVAGFNLLMTLRLWSS*

Key:
CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
GSG linker/P2A
Signal peptide
Cysteine modified **C**

FIG. 10G

CD8alpha-T2A-CD8beta-P2A-11N6 TCRbeta-P2A-alpha – *Protein* (SEQ ID NO.:58)

MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQPRGAAASPTFLLYLS
QNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYYFCSALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTP
APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVV
KSGDKPSLSARYV**GSGEGRGSLLTCGDVEENPGPMRPRLWLLLAAQLTVLHGNSV**LQQTPAYIKVQTNKMV
MLSCEAKISLSNMRIYWLRQRQAPSSDSIHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASRFILNLTSVKPEDS
GIYFCMIVGSPELTFGKGTQLSVVDFLPTTAQPTKKSTLKKRVCRLPRPETQKGPLCSPITLGLLVAGVLVLLVSL
GVAIHLCCRRRRARLRFMKQFYK**GSGATNFSLLKQAGDVEENPGPMGTRLLCWAALCLLGAELTEA**GVAQS
PRYKIIEKRQSVAFWCNPI**SGHATL**YWYQQILGQGPKLLIQ**FQNNGV**VDDSQLPKDRFSAERLKGVDSTLKIQ
PAKLEDSAVYL**CASSPYGGSVSYEQY**FGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYP
DHVELSWWVNGKEVHSGVCTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDE
WTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRGG.
SGATNFSLLKQAGDVEENPGPMETLLGVSLVILWLQLARVNSQQGEEDPQALSIQEGENATMNCSYK**TSIN**
NLQWYRQNSGRGLVHLIL**IRSNEREK**HSGRLRVTLDTSKKSSSLLITASRAADTASYF**CATDPMNTNAGKSTF**
GDGTTLTVKPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRSMDFKSNSA
VAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLR
LWSS*

Key:

CDR1 (alpha and beta)
CDR2 (alpha and beta)
CDR3 (alpha and beta)
GSG linker/P2A
GSG linker/T2A
Signal peptide
Cysteine modified C

FIG. 10H

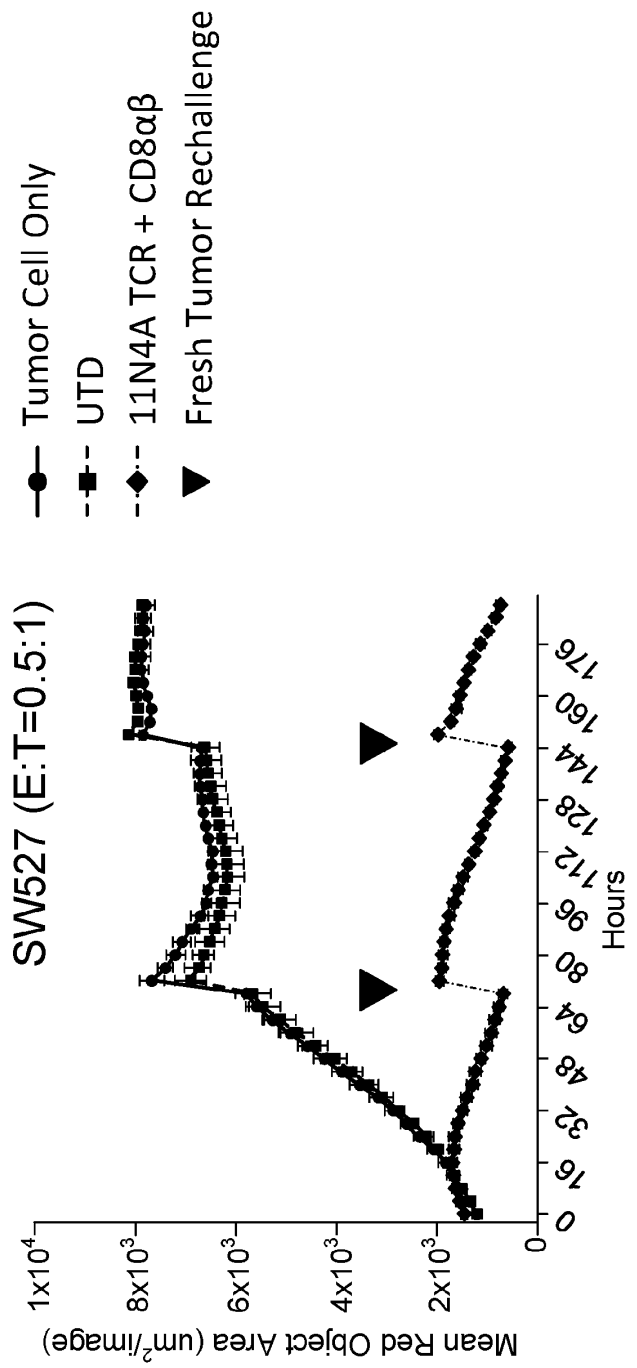

SW527 (E:T=0.5:1)
Tumor Cell Only
UTD
11N4A TCR + CD8αβ
Fresh Tumor Rechallenge
Mean Red Object Area (um²/image)
1x10⁴
8x10³
6x10³
4x10³
2x10³
0
0
16
32
48
64
80
96
112
128
144
160
176
Hours

FIG. 11A

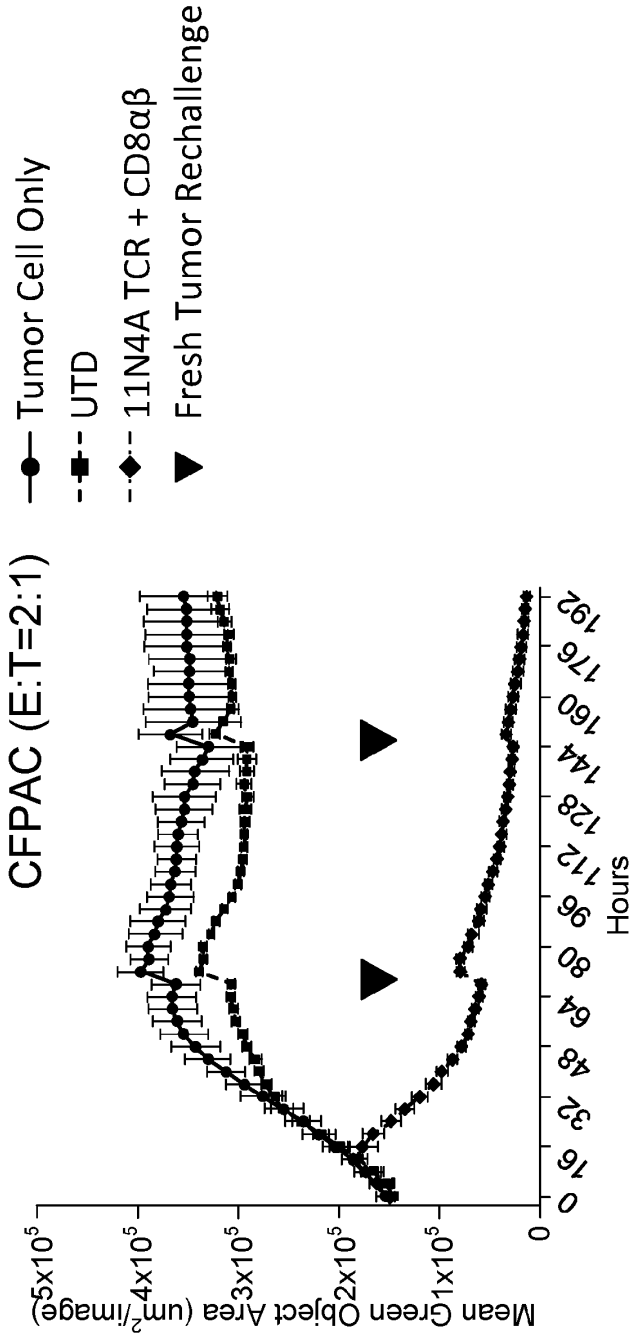

CFPAC (E:T=2:1)
Tumor Cell Only
UTD
11N4A TCR + CD8αβ
Fresh Tumor Rechallenge
Mean Green Object Area (um²/image)
5x10⁵
4x10⁵
3x10⁵
2x10⁵
1x10⁵
0
0
16
32
48
64
80
96
112
128
144
160
176
192
Hours

FIG. 11B

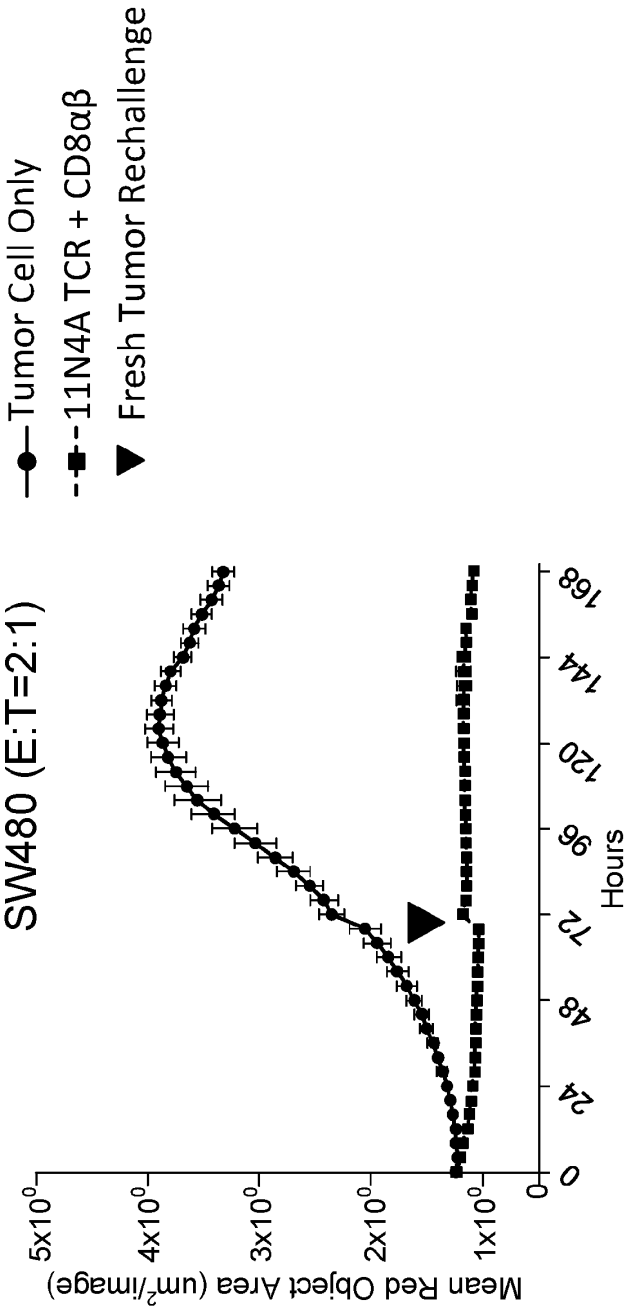

SW480 (E:T=2:1)
Tumor Cell Only
11N4A TCR + CD8αβ
Fresh Tumor Rechallenge
Mean Red Object Area (um²/image)
5x10⁰
4x10⁰
3x10⁰
2x10⁰
1x10⁰
0
0
24
48
72
96
120
144
168
Hours

FIG. 11C

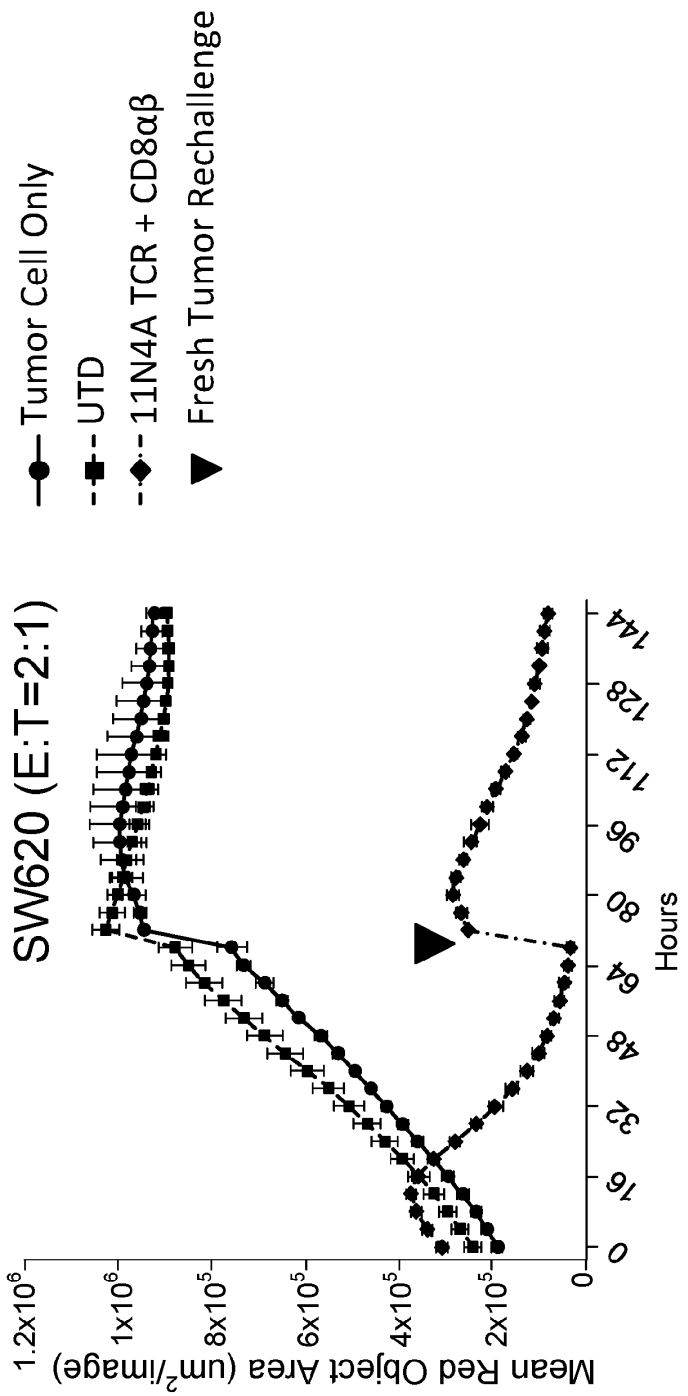

SW620 (E:T=2:1)
Tumor Cell Only
UTD
11N4A TCR + CD8αβ
Fresh Tumor Rechallenge
Mean Red Object Area (um²/Image)
1.2x10⁶
1x10⁶
8x10⁵
6x10⁵
4x10⁵
2x10⁵
0
0
16
32
48
64
80
96
112
128
144
Hours

FIG. 11D

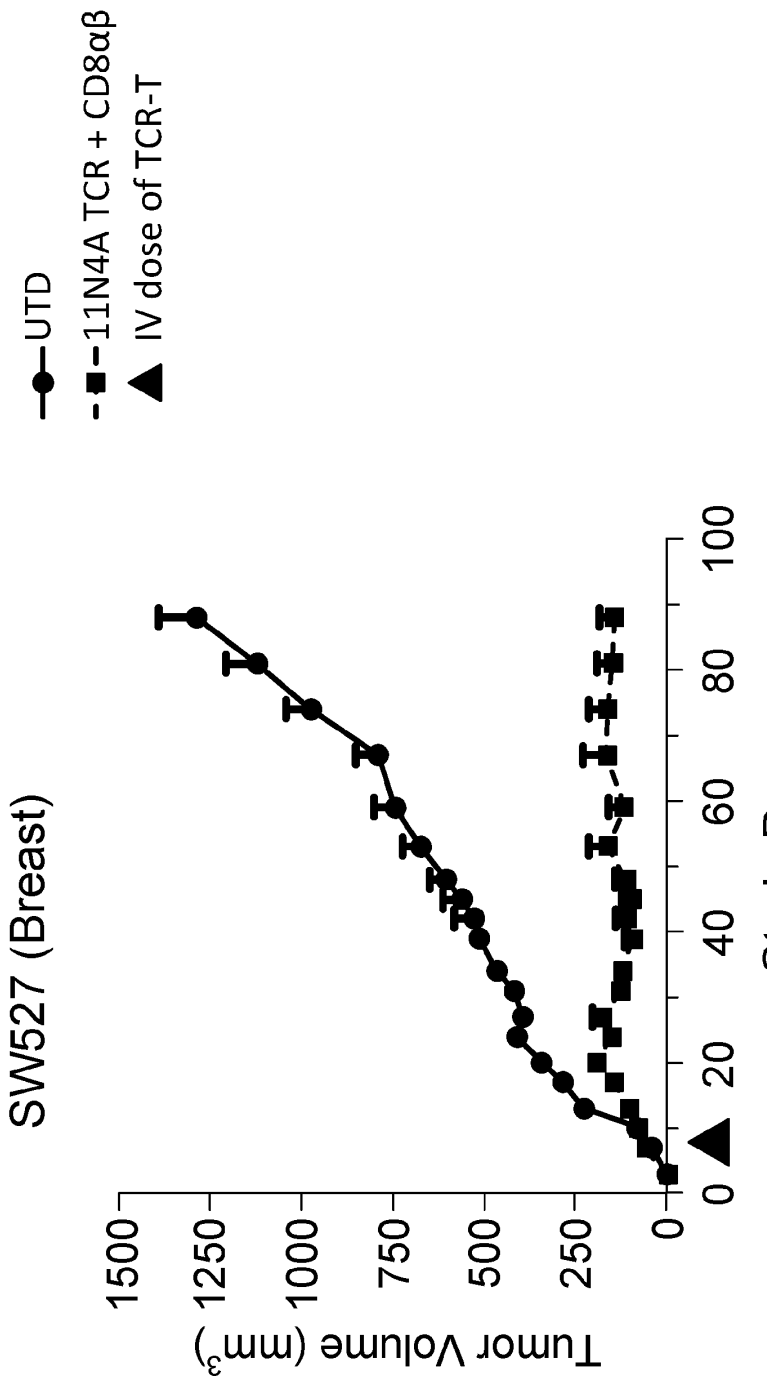

UTD
11N4A TCR + CD8αβ
IV dose of TCR-T
SW527 (Breast)
Tumor Volume (mm³)
1500
1250
1000
750
500
250
0
0
20
40
60
80
100
Study Day

FIG. 12A

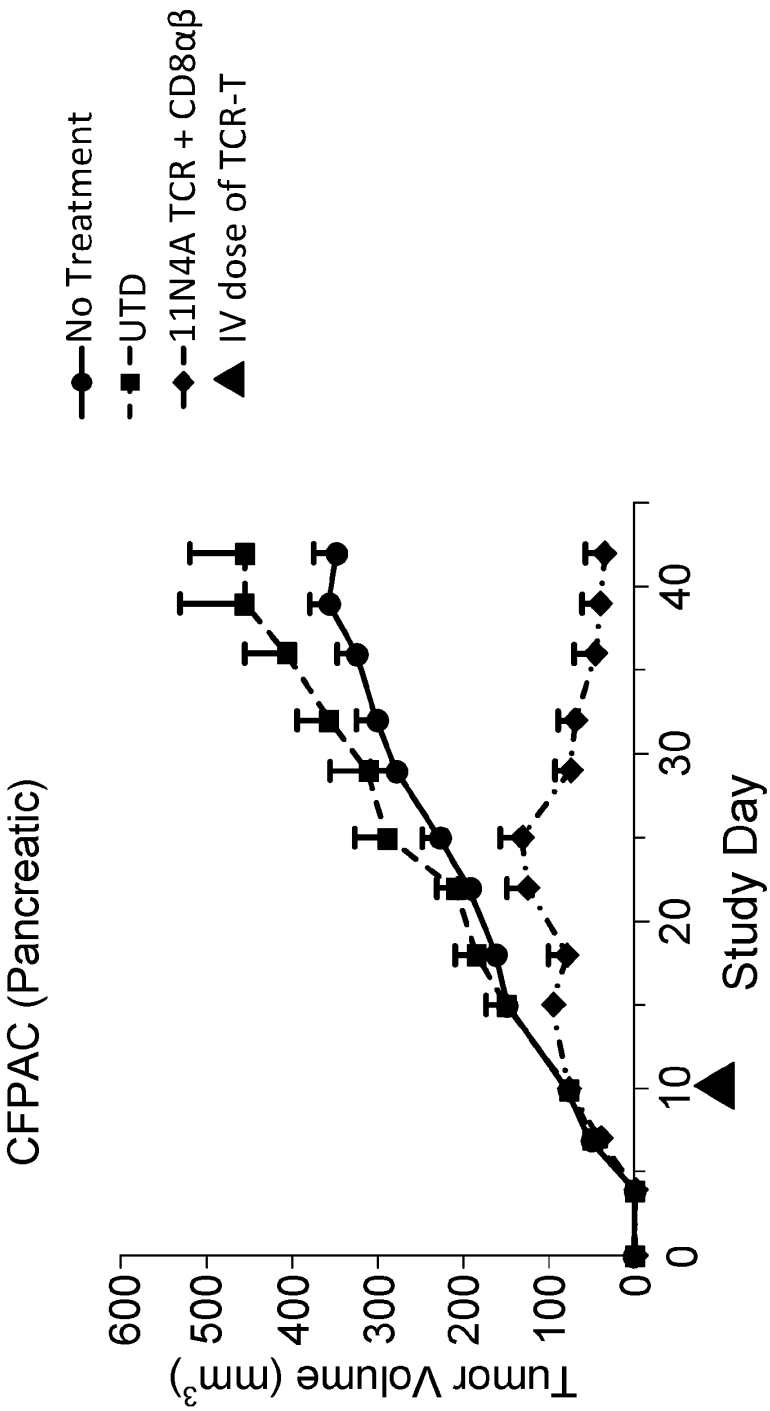

No Treatment
UTD
11N4A TCR + CD8αβ
IV dose of TCR-T
CFPAC (Pancreatic)
Tumor Volume (mm³)
0
100
200
300
400
500
600
0
10
20
30
40
Study Day

FIG. 12B

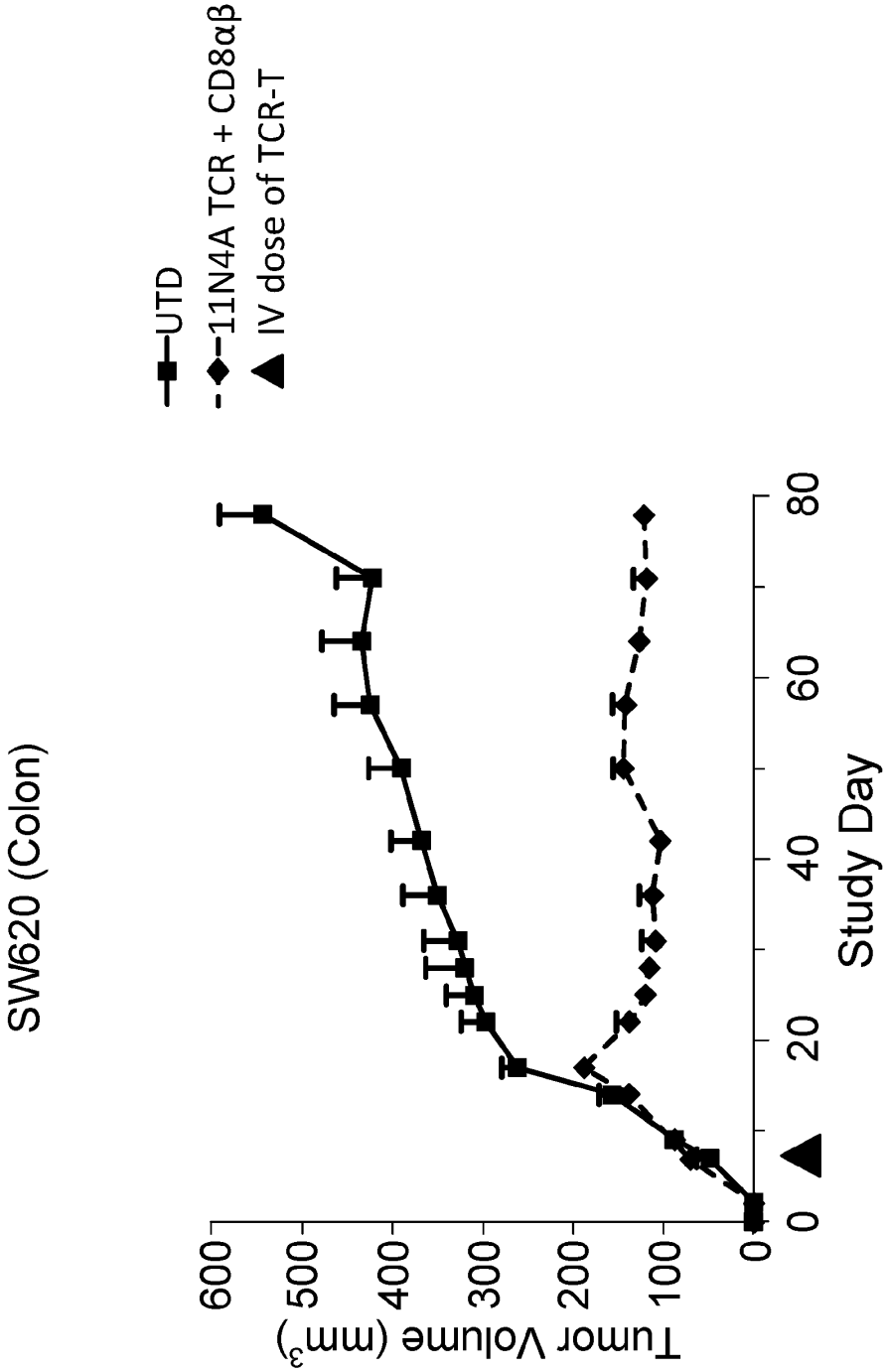

SW620 (Colon)
UTD
11N4A TCR + CD8αβ
IV dose of TCR-T
Tumor Volume (mm³)
600
500
400
300
200
100
0
0
20
40
60
80
Study Day

FIG. 12C

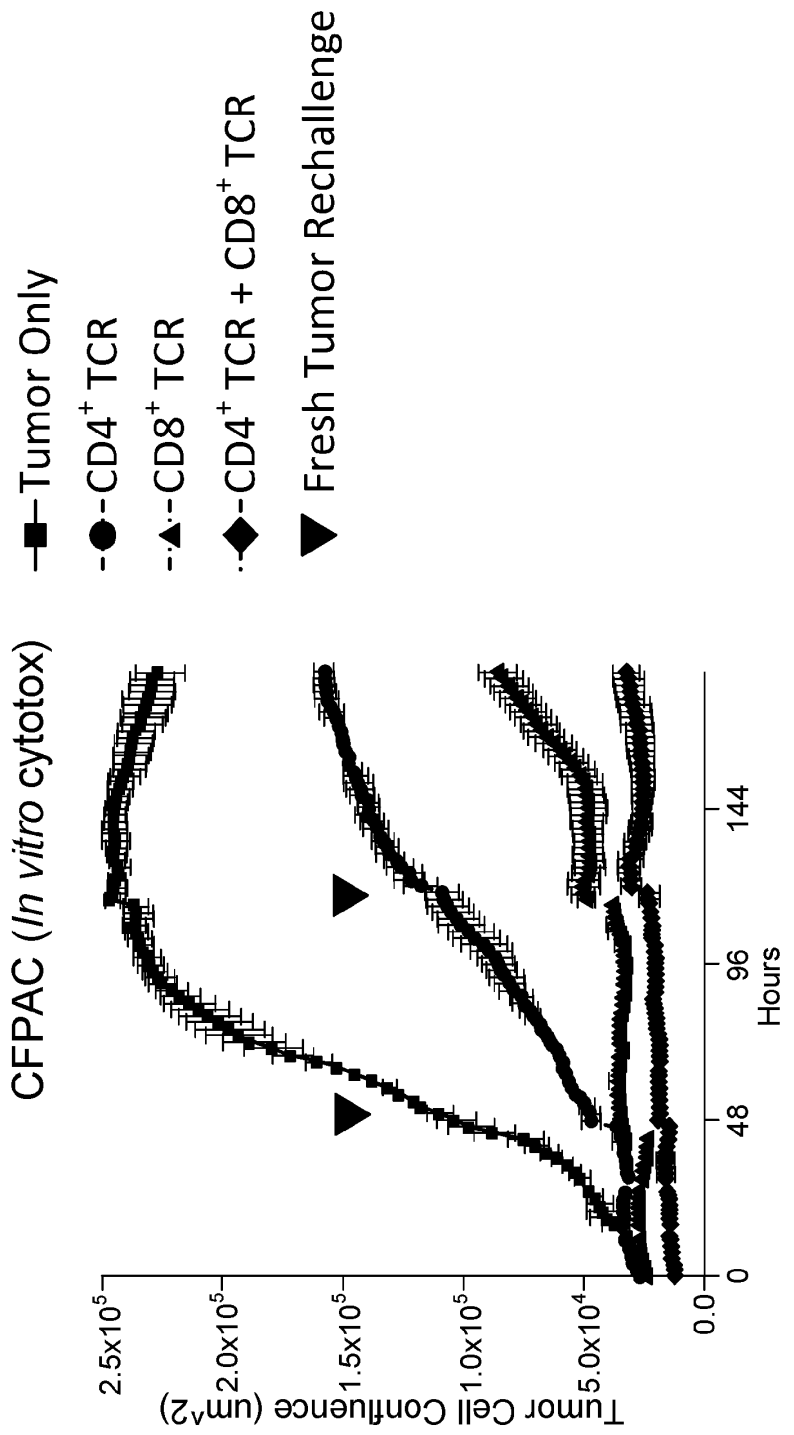

CFPAC (*In vitro* cytotox)
Tumor Only
CD4+ TCR
CD8+ TCR
CD4+ TCR + CD8+ TCR
Fresh Tumor Rechallenge
Tumor Cell Confluence (um^2)
2.5x10^5
2.0x10^5
1.5x10^5
1.0x10^5
5.0x10^4
0.0
0
48
96
144
Hours

FIG. 13A

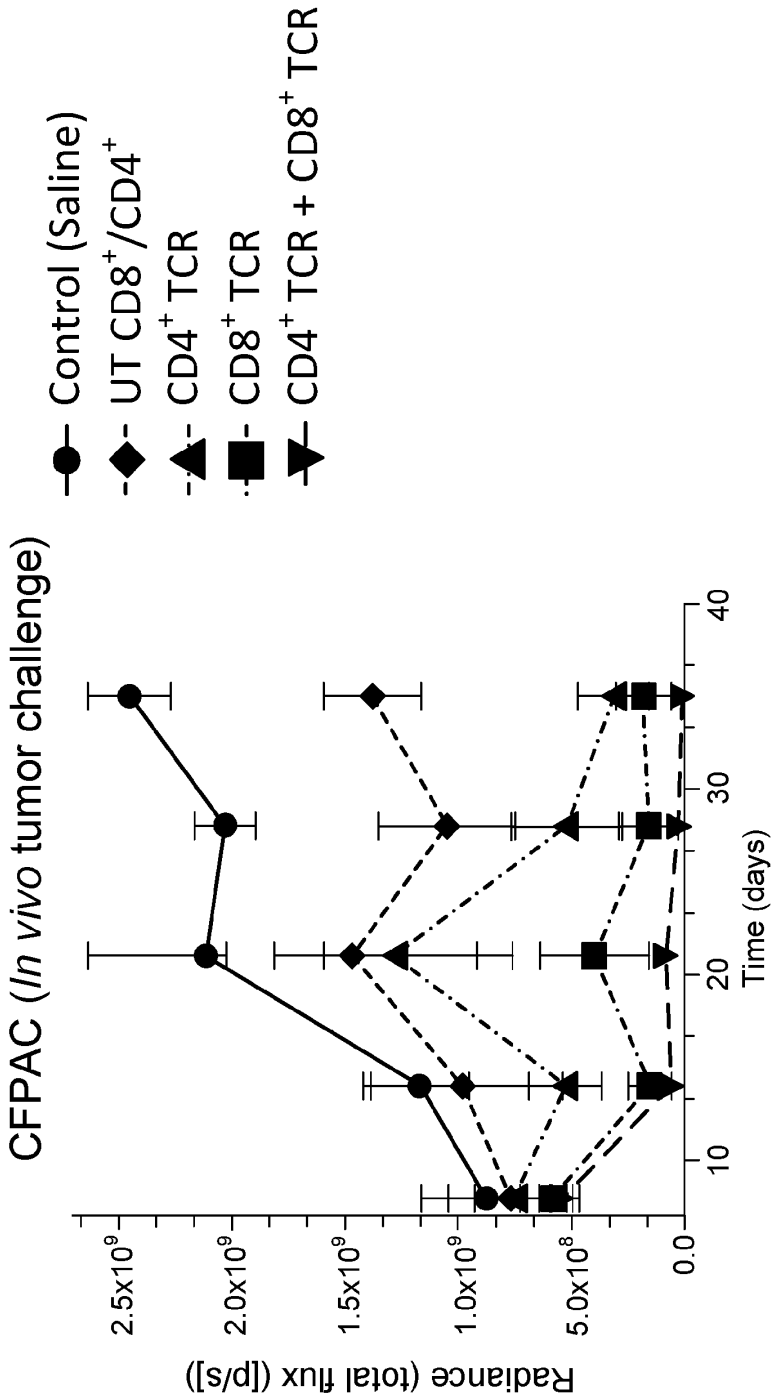

CFPAC (*In vivo* tumor challenge)
Control (Saline)
UT CD8+/CD4+
CD4+ TCR
CD8+ TCR
CD4+ TCR + CD8+ TCR
Radiance (total flux ([p/s])
2.5x10^9
2.0x10^9
1.5x10^9
1.0x10^9
5.0x10^8
0.0
10
20
30
40
Time (days)

FIG. 13B

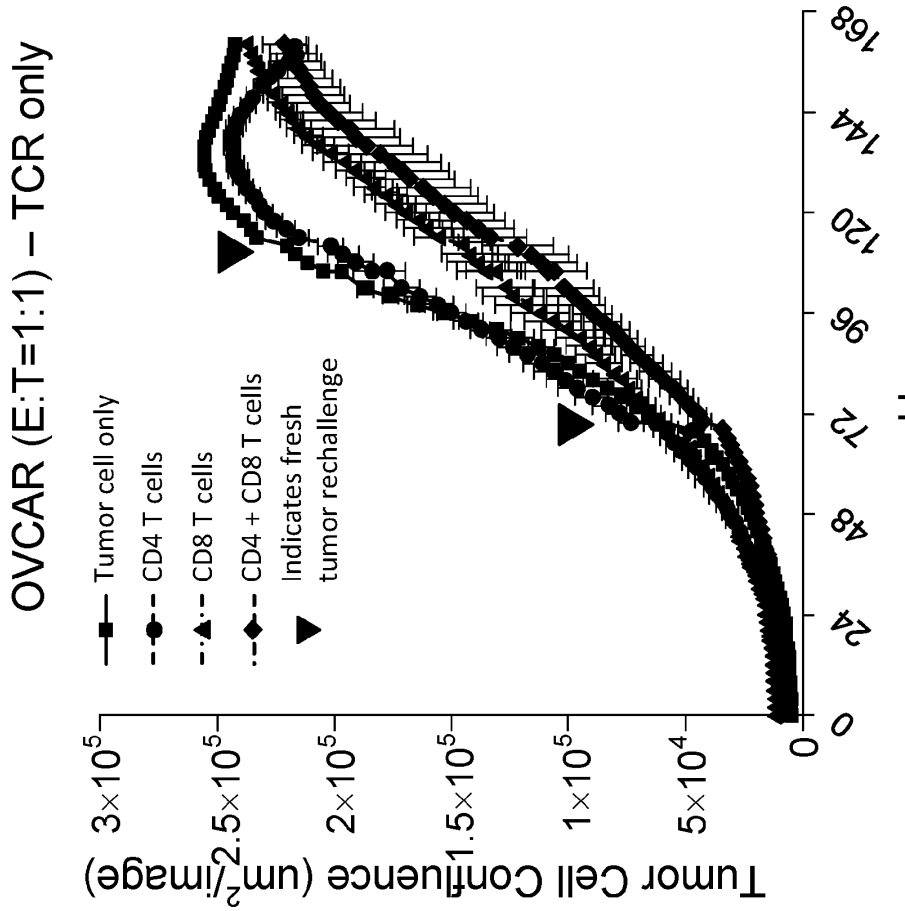

OVCAR (E:T=1:1) – TCR only
Tumor cell only
CD4 T cells
CD8 T cells
CD4 + CD8 T cells
Indicates fresh tumor rechallenge
Tumor Cell Confluence (um²/image)
3×10⁵
2.5×10⁵
2×10⁵
1.5×10⁵
1×10⁵
5×10⁴
0
0
24
48
72
96
120
144
168
Hours

FIG. 13C

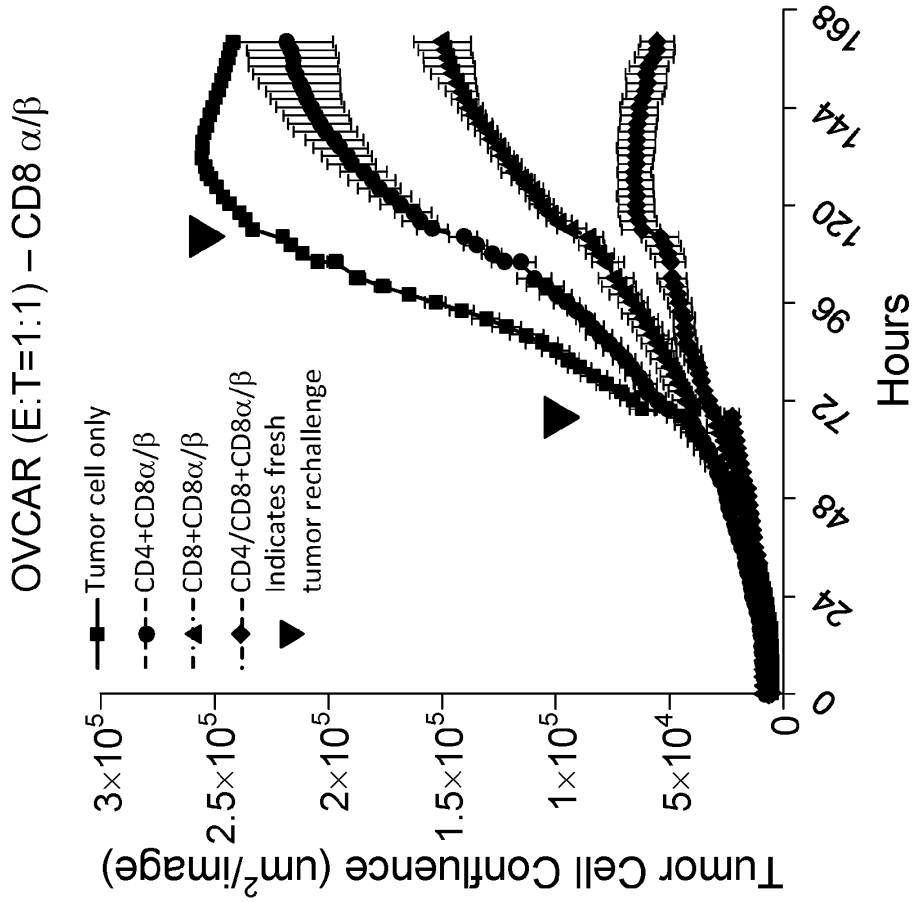

OVCAR (E:T=1:1) – CD8 α/β
Tumor cell only
CD4+CD8α/β
CD8+CD8α/β
CD4/CD8+CD8α/β
Indicates fresh tumor rechallenge
Tumor Cell Confluence (um²/image)
3×10⁵
2.5×10⁵
2×10⁵
1.5×10⁵
1×10⁵
5×10⁴
0
0
24
48
72
96
120
144
168
Hours

FIG. 13D

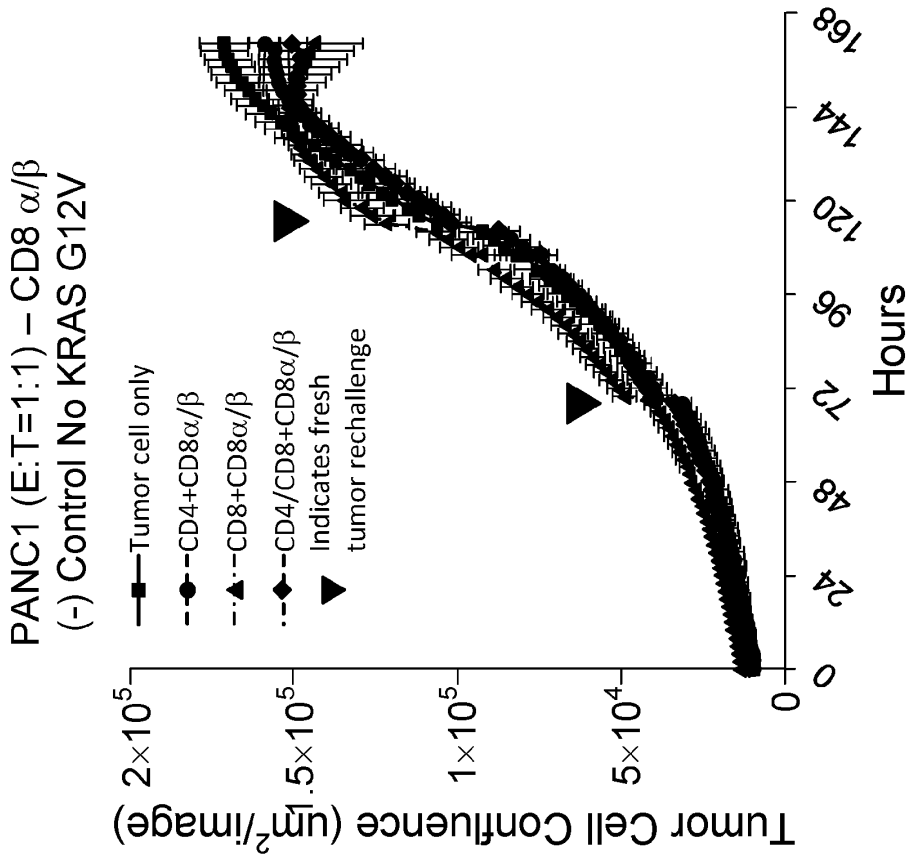

PANC1 (E:T=1:1) – CD8 α/β
(-) Control No KRAS G12V
Tumor cell only
CD4+CD8α/β
CD8+CD8α/β
CD4/CD8+CD8α/β
Indicates fresh tumor rechallenge
Tumor Cell Confluence (μm²/image)
2×10⁵
1.5×10⁵
1×10⁵
5×10⁴
0
0
24
48
72
96
120
144
168
Hours

FIG. 13E

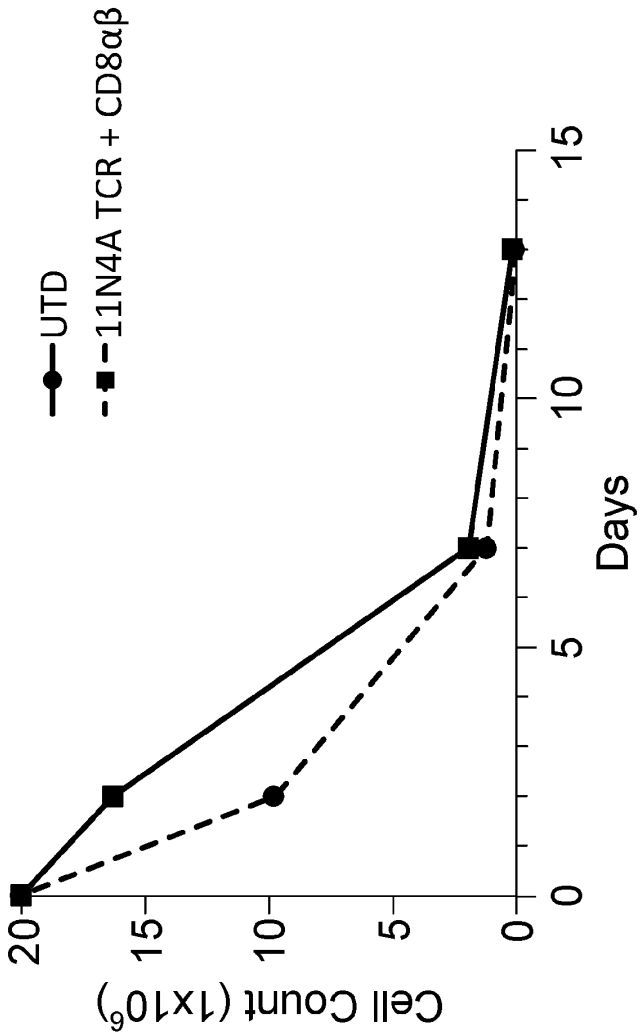


FIG. 14

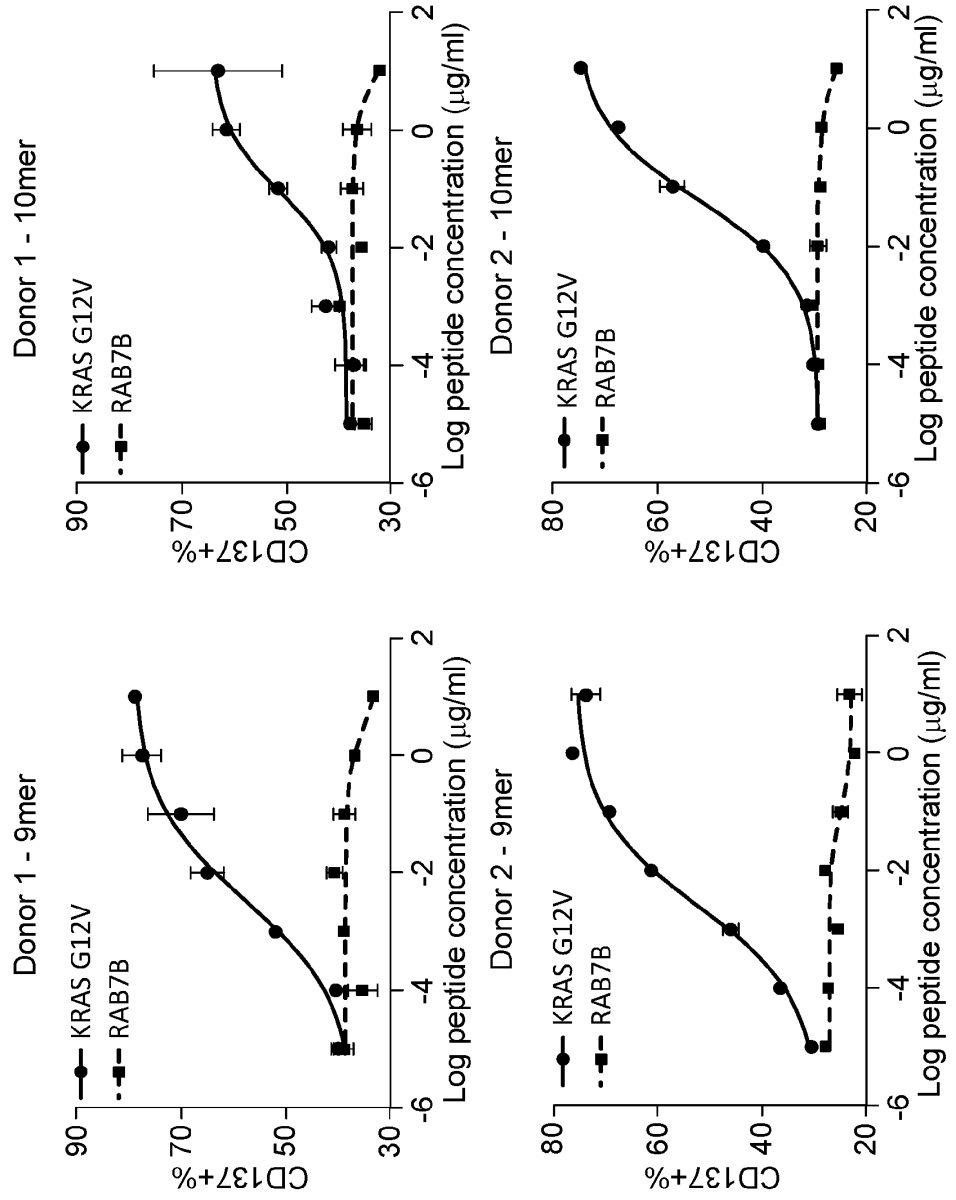

Donor 1 - 10mer
Donor 2 - 10mer
Donor 1 - 9mer
Donor 2 - 9mer
KRAS G12V
RAB7B
CD137+%
Log peptide concentration (μg/ml)

FIG. 15

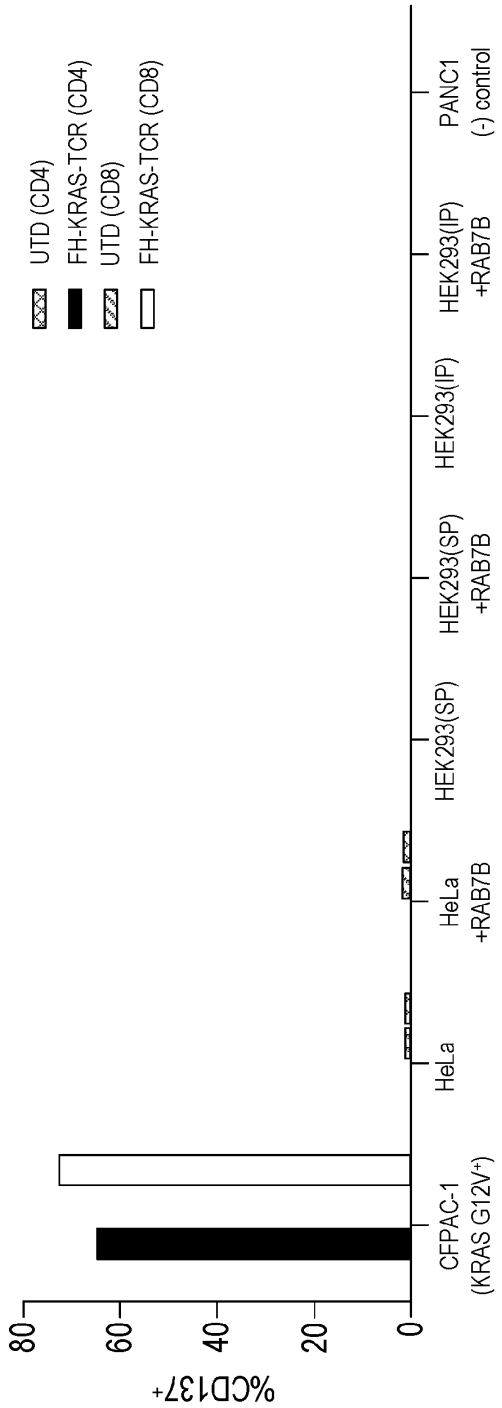

UTD (CD4)
FH-KRAS-TCR (CD4)
UTD (CD8)
FH-KRAS-TCR (CD8)
%CD137+
0
20
40
60
80
CFPAC-1 (KRAS G12V+)
HeLa
HeLa +RAB7B
HEK293(SP)
HEK293(SP) +RAB7B
HEK293(IP)
HEK293(IP) +RAB7B
PANC1 (-) control

FIG. 16

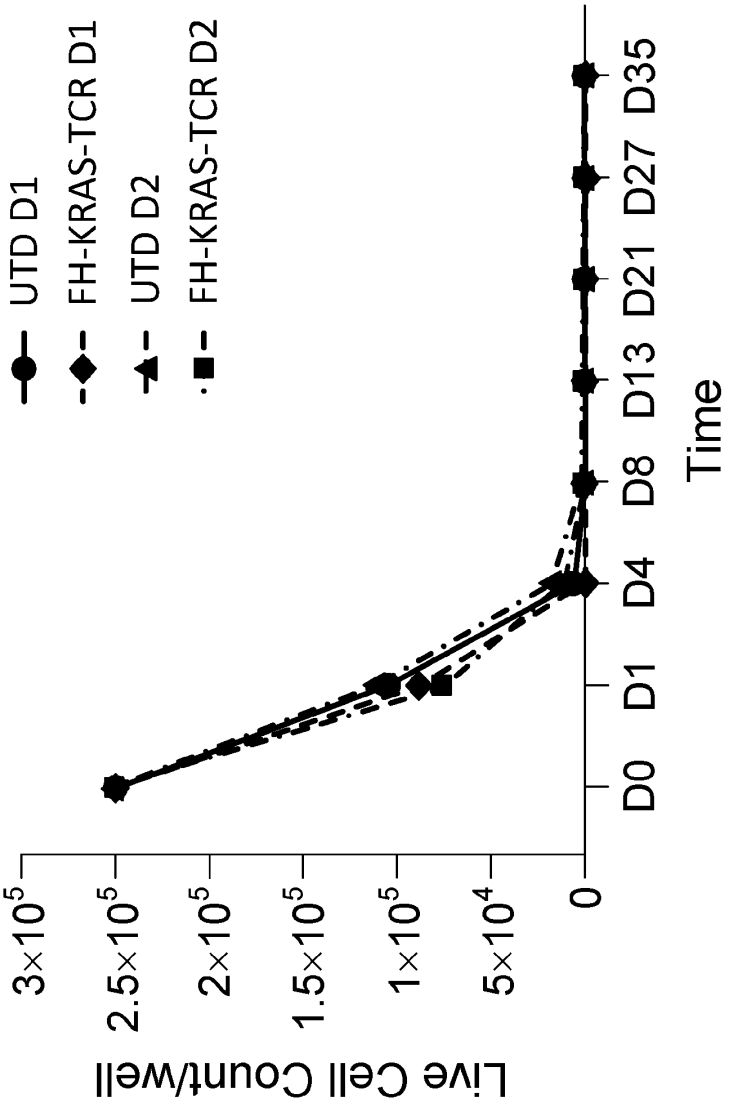

UTD D1
FH-KRAS-TCR D1
UTD D2
FH-KRAS-TCR D2
Live Cell Count/well
3×10^5
2.5×10^5
2×10^5
1.5×10^5
1×10^5
5×10^4
0
D0
D1
D4
D8
D13
D21
D27
D35
Time

FIG. 17