Figure 1

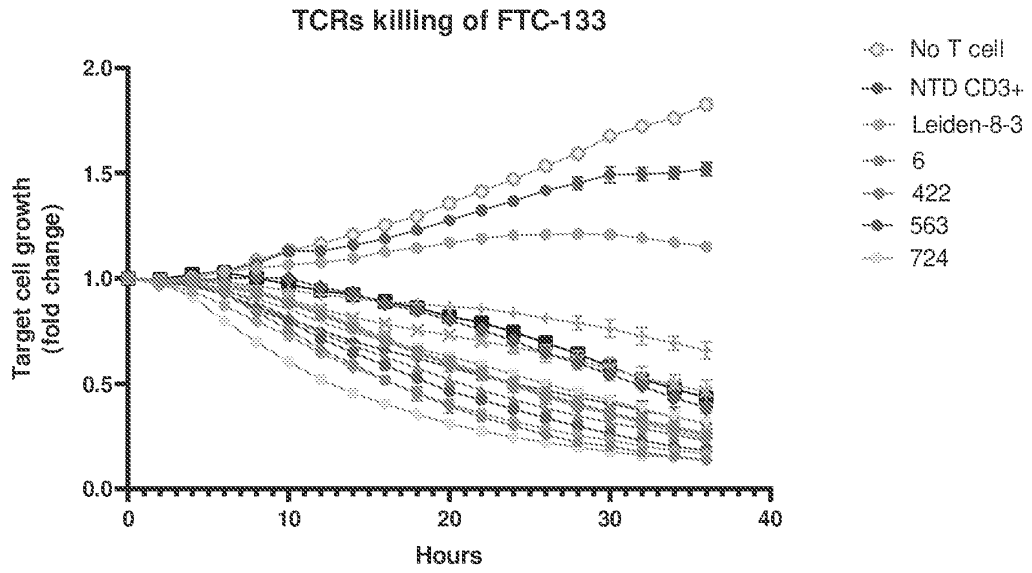


Figure 2

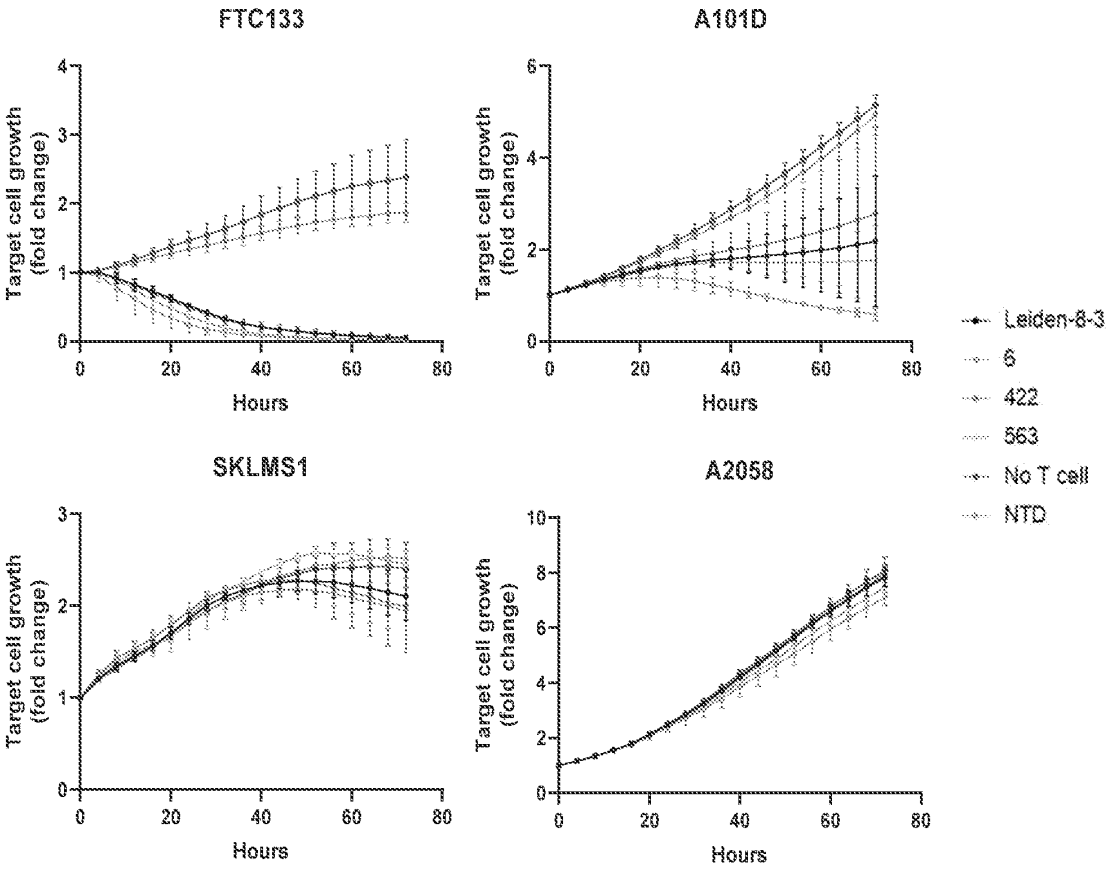


Figure 3A

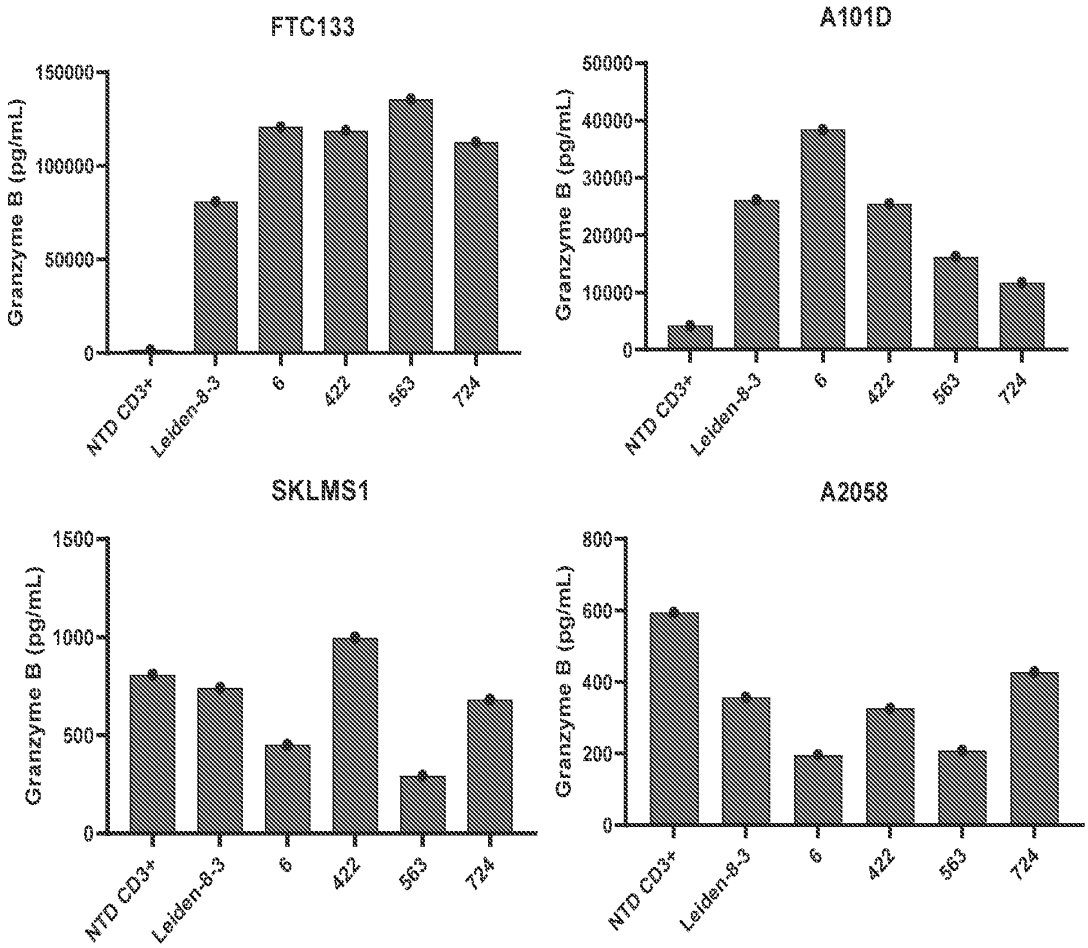

FTC133
A101D
SKLMS1
A2058
Granzyme B (pg/mL)
NTD CD3+
Leiden-8-3
6
422
563
724

Figure 3B

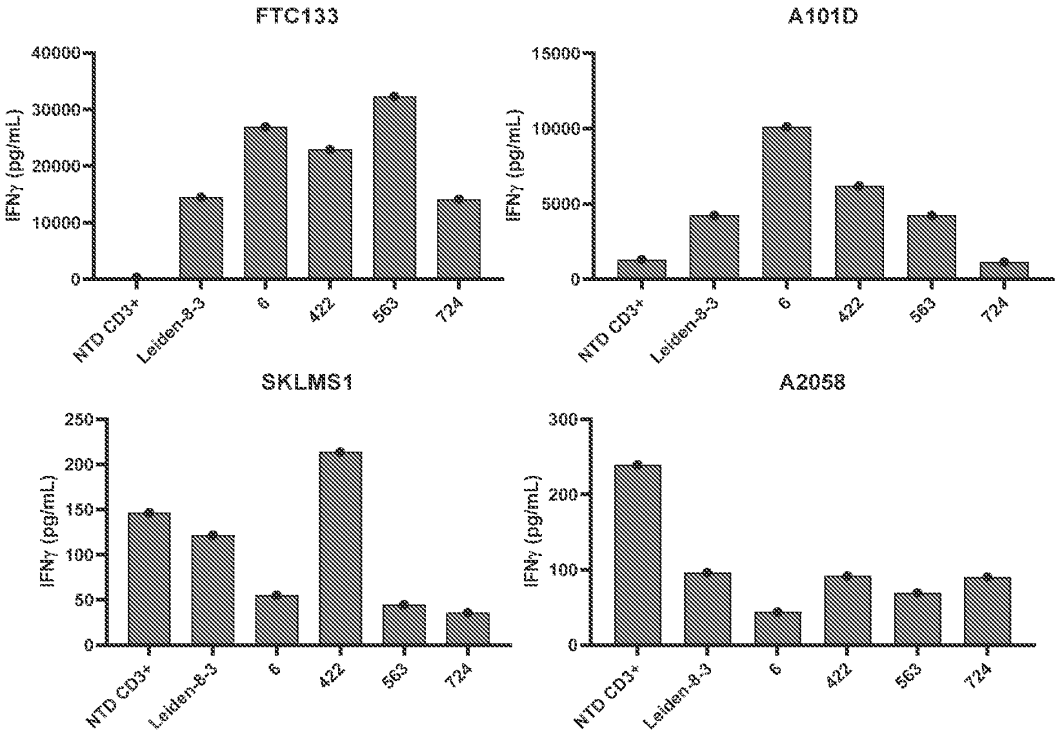

FTC133
A101D
SKLMS1
A2058
IFNγ (pg/mL)
NTD CD3+
Leiden-8-3
6
422
563
724

Figure 3C

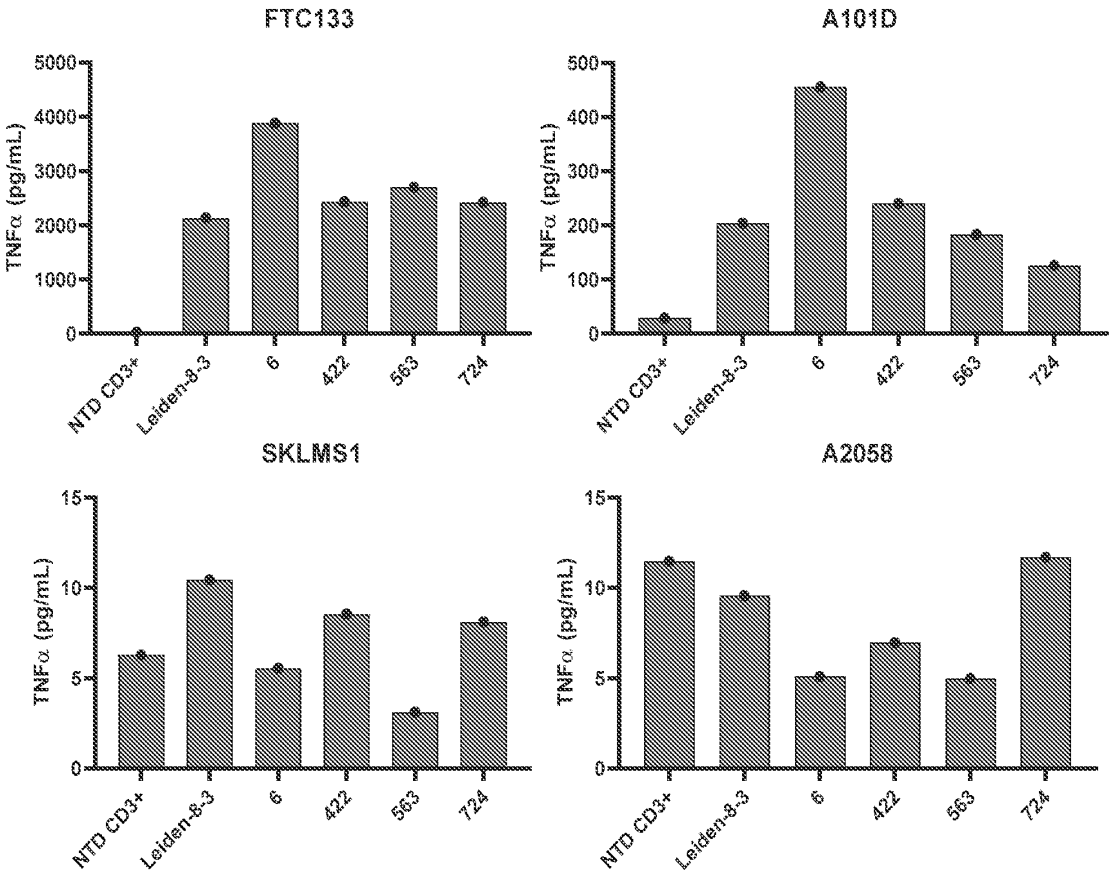
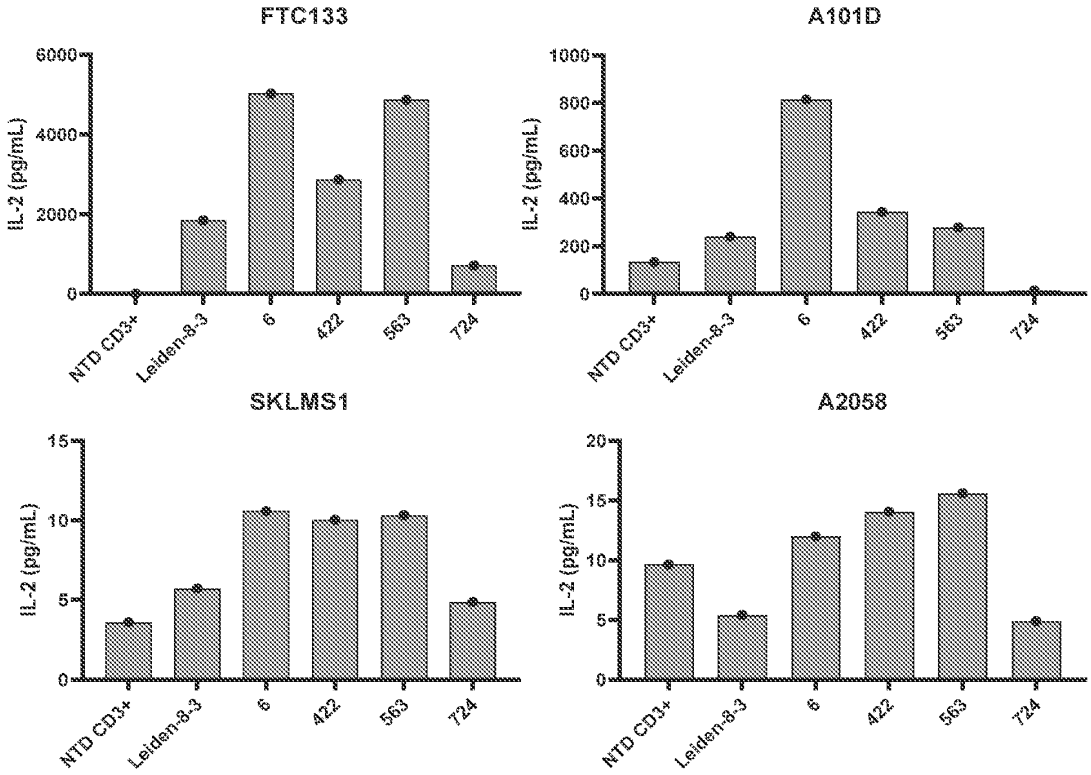

FTC133
A101D
SKLMS1
A2058
TNFα (pg/mL)
5000
4000
3000
2000
1000
0
500
400
300
200
100
15
10
5
NTD CD3+
Leiden-8-3
6
422
563
724

Figure 3D


FTC133
A101D
SKLMS1
A2058
IL-2 (pg/mL)
NTD CD3+
Leiden-8-3
6
422
563
724

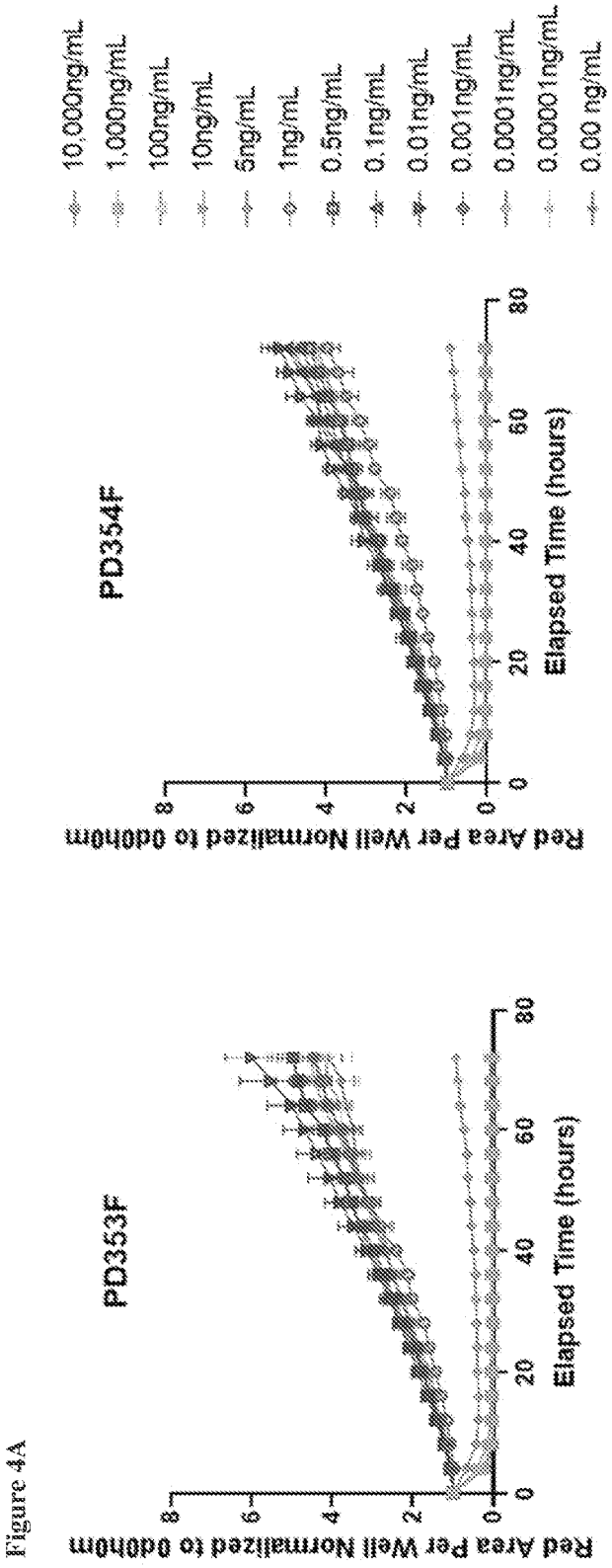

PD353F
PD354F
Red Area Per Well Normalized to 0 ohm
Elapsed Time (hours)
0
2
4
6
8
0
20
40
60
80
10,000ng/mL
1,000ng/mL
100ng/mL
10ng/mL
5ng/mL
1ng/mL
0.5ng/mL
0.1ng/mL
0.01ng/mL
0.001ng/mL
0.0001ng/mL
0.00001ng/mL
0.00 ng/mL

Figure 4A

Figure 4B

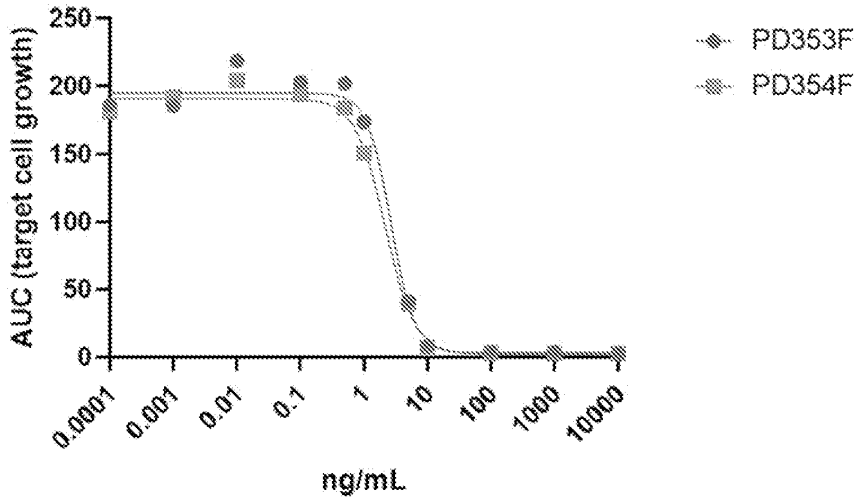


Figure 5A

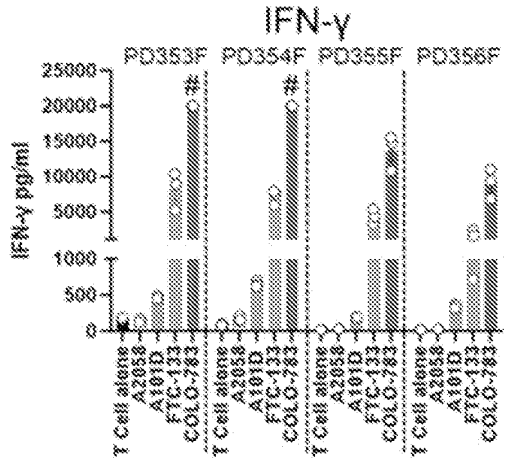


Figure 5B

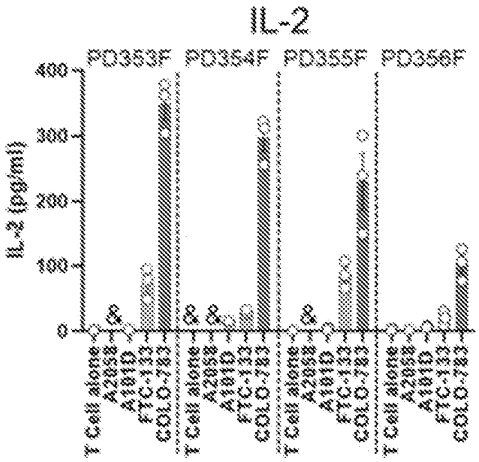


Figure 5C

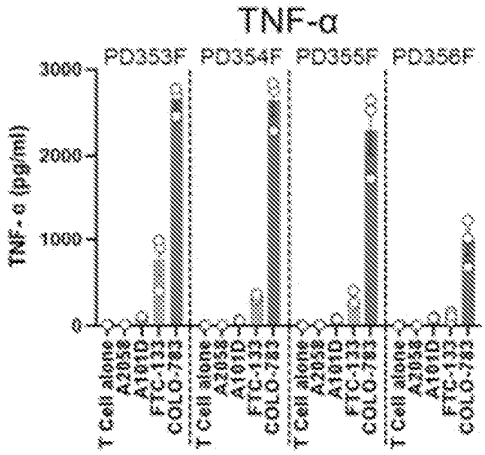


Figure 5D

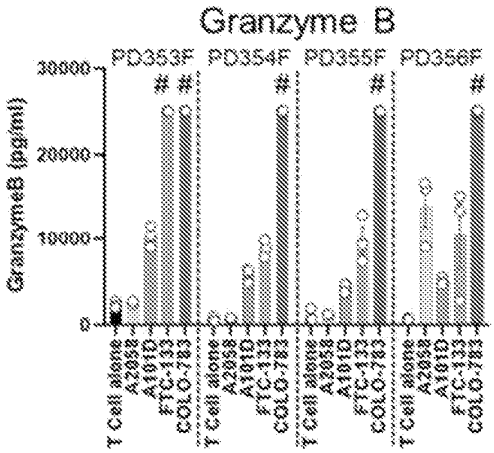


Figure 5E

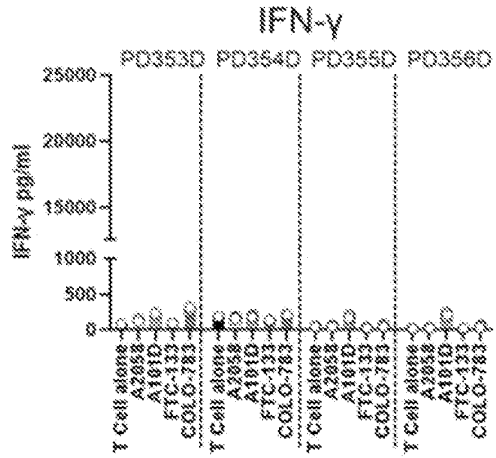


Figure 5F

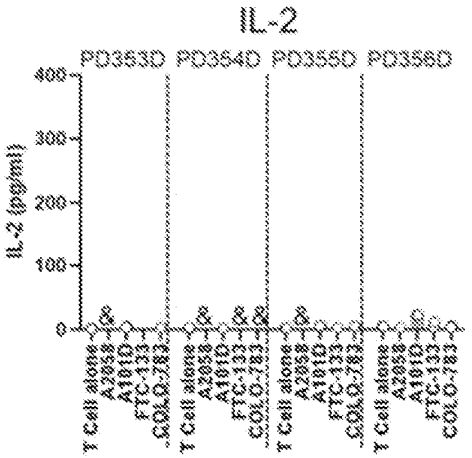


Figure 5G

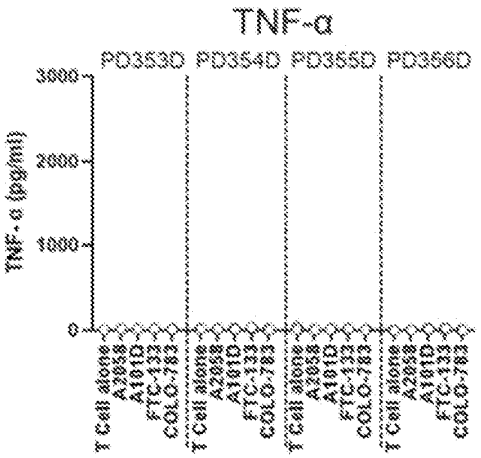
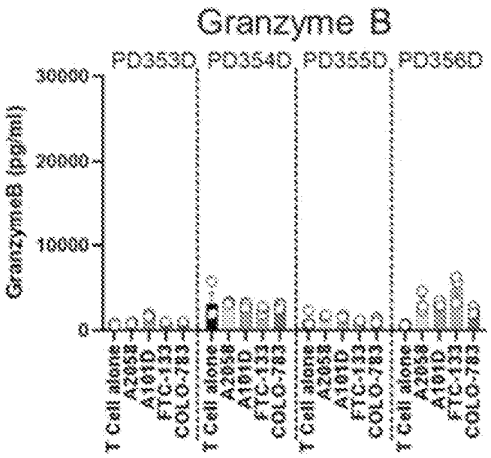
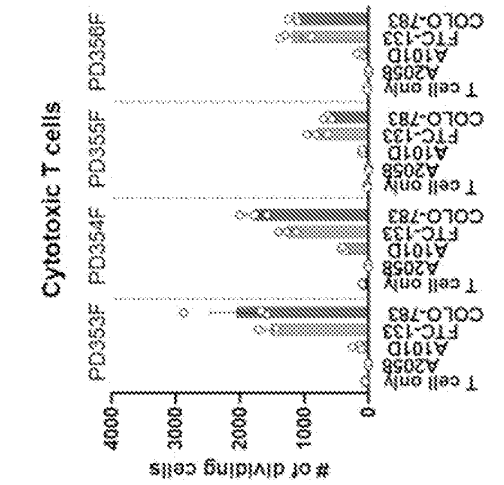
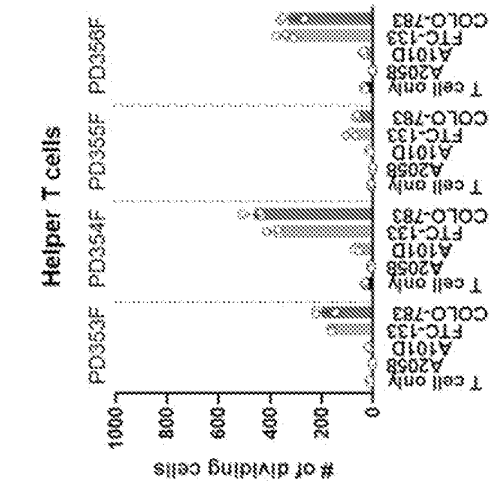


Figure 5H







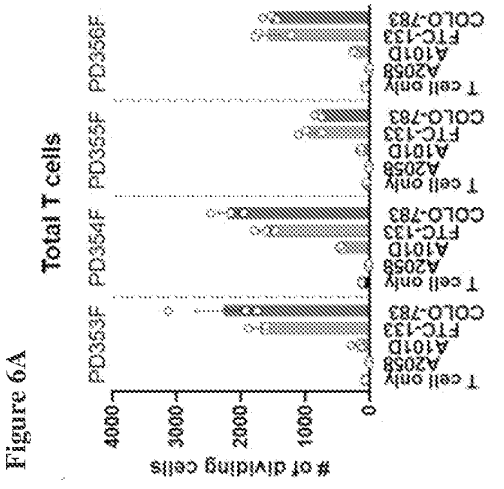
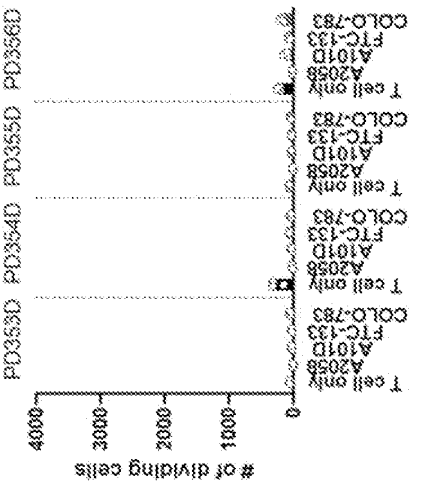
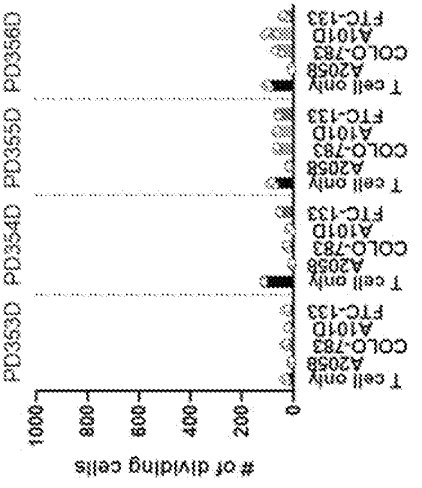


Figure 6A





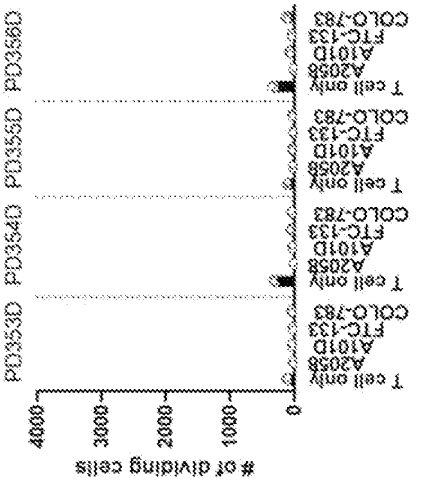


Figure 6B

Figure 7A

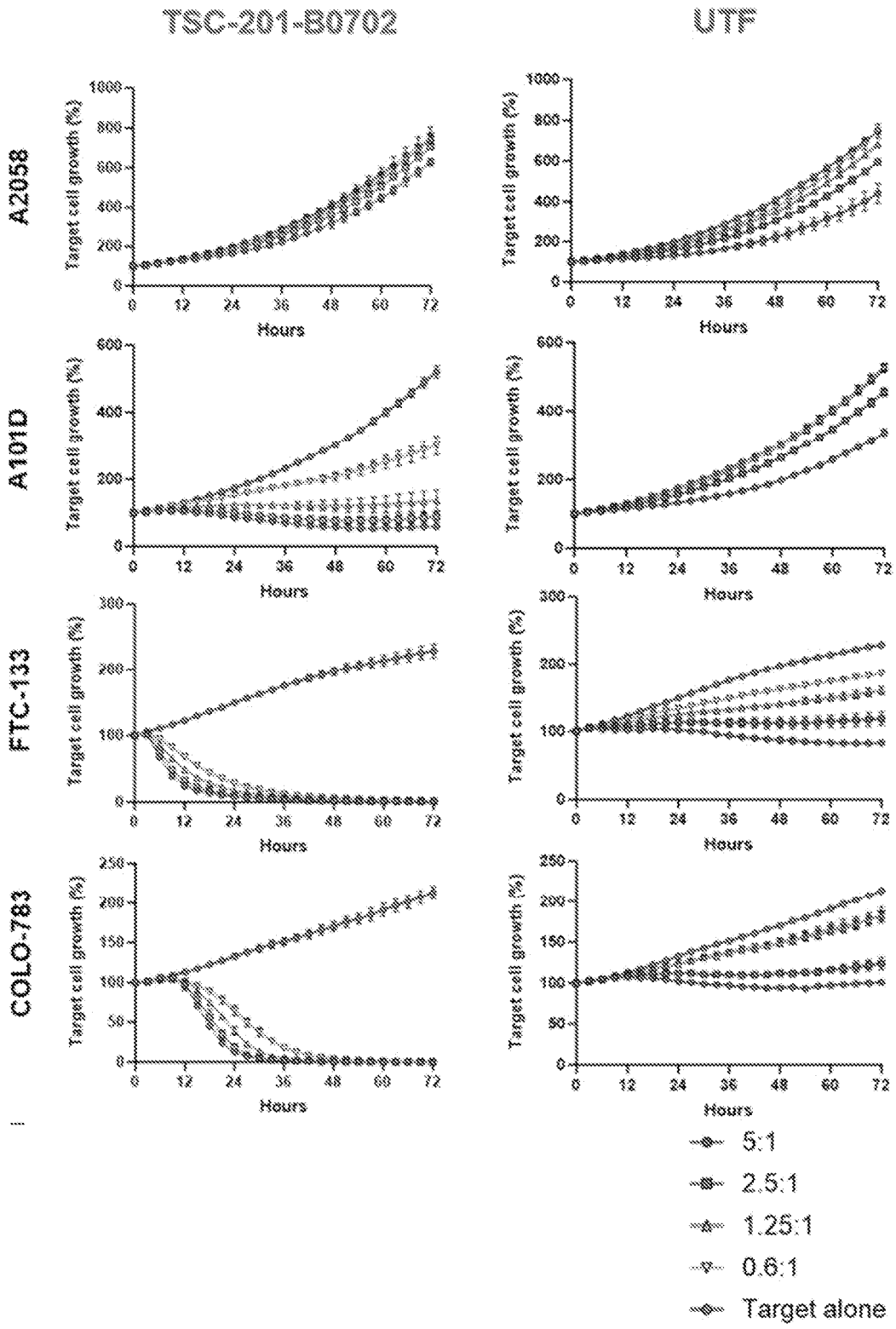

TSC-201-B0702
UTF
A2058
A101D
FTC-133
COLO-783
Target cell growth (%)
Hours
5:1
2.5:1
1.25:1
0.6:1
Target alone

Figure 7B

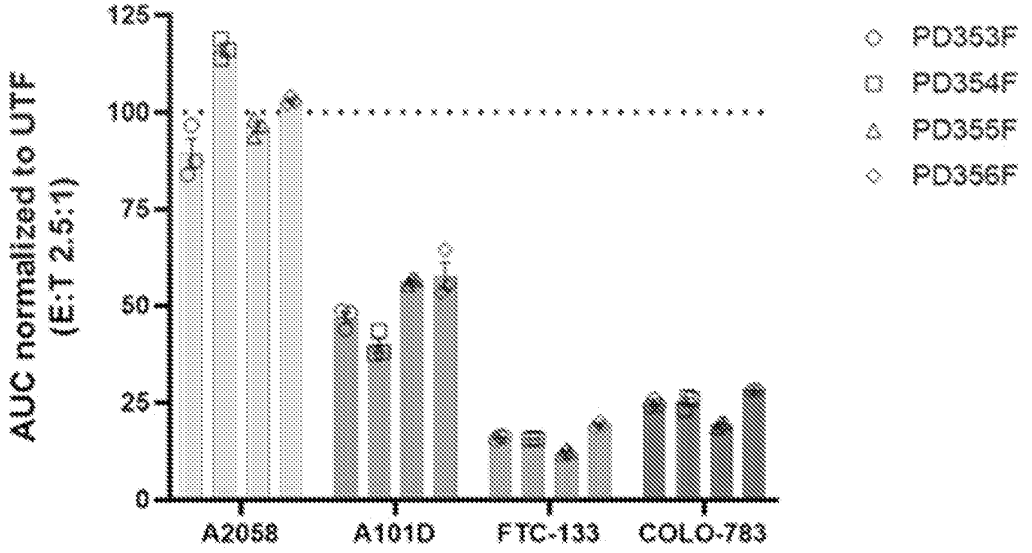
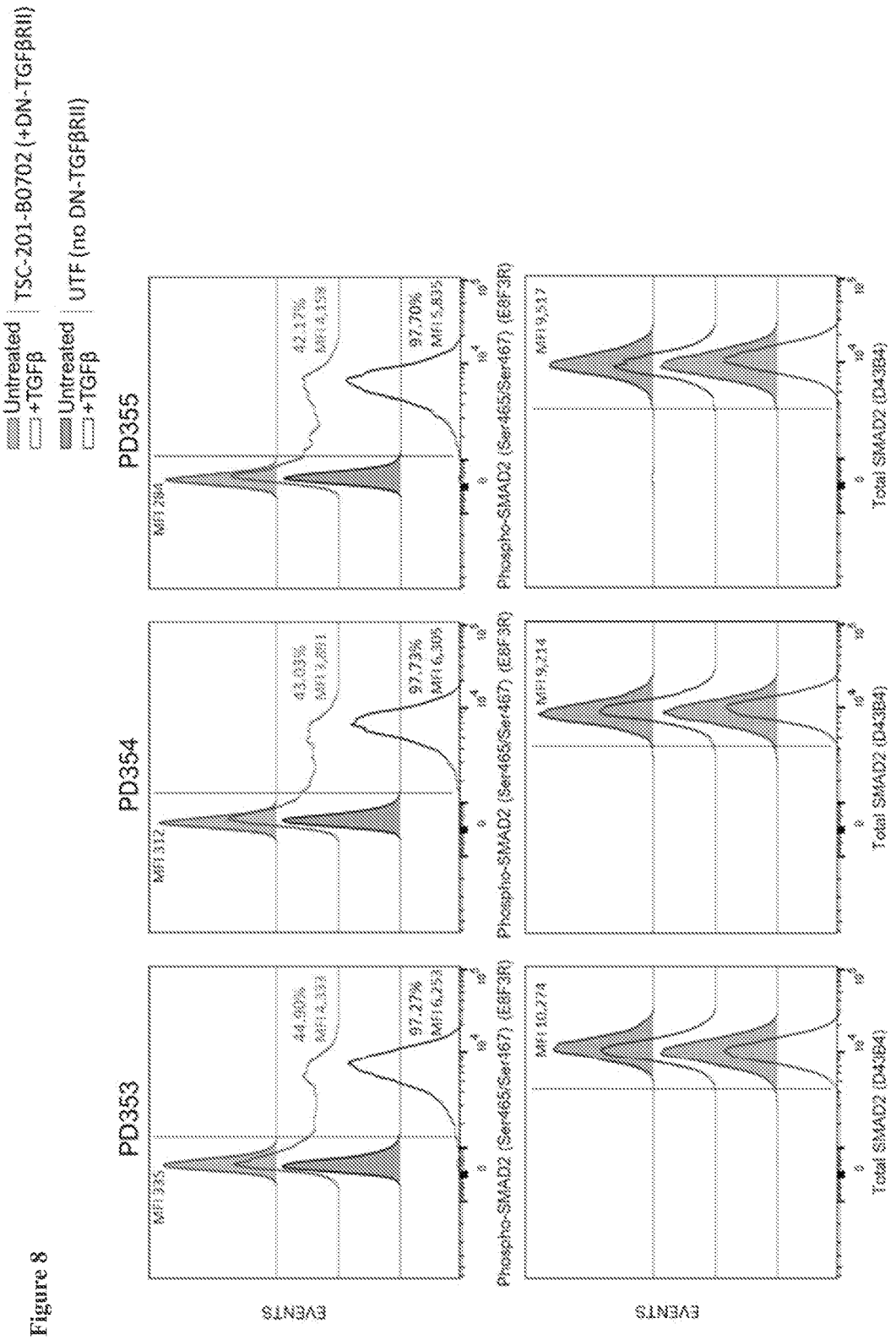

AUC normalized to UTF
(E:T 2.5:1)
125
100
75
50
25
0
A2058
A101D
FTC-133
COLO-783
PD353F
PD354F
PD355F
PD356F

Figure 8


Untreated
+TGFβ
TSC-201-B0702 (+DN-TGFβRII)
Untreated
+TGFβ
UTF (no DN-TGFβRII)
PD353
PD354
PD355
EVENTS
Phospho-SMAD2 (Ser465/Ser467) (E8F3R)
Total SMAD2 (D43B4)
MFI 325
MFI 4,332
44.80%
MFI 16,253
97.27%
MFI 317
MFI 1,891
43.03%
MFI 16,305
97.73%
MFI 384
MFI 4,159
42.17%
MFI 15,835
97.70%
MFI 10,274
MFI 9,214
MFI 9,537

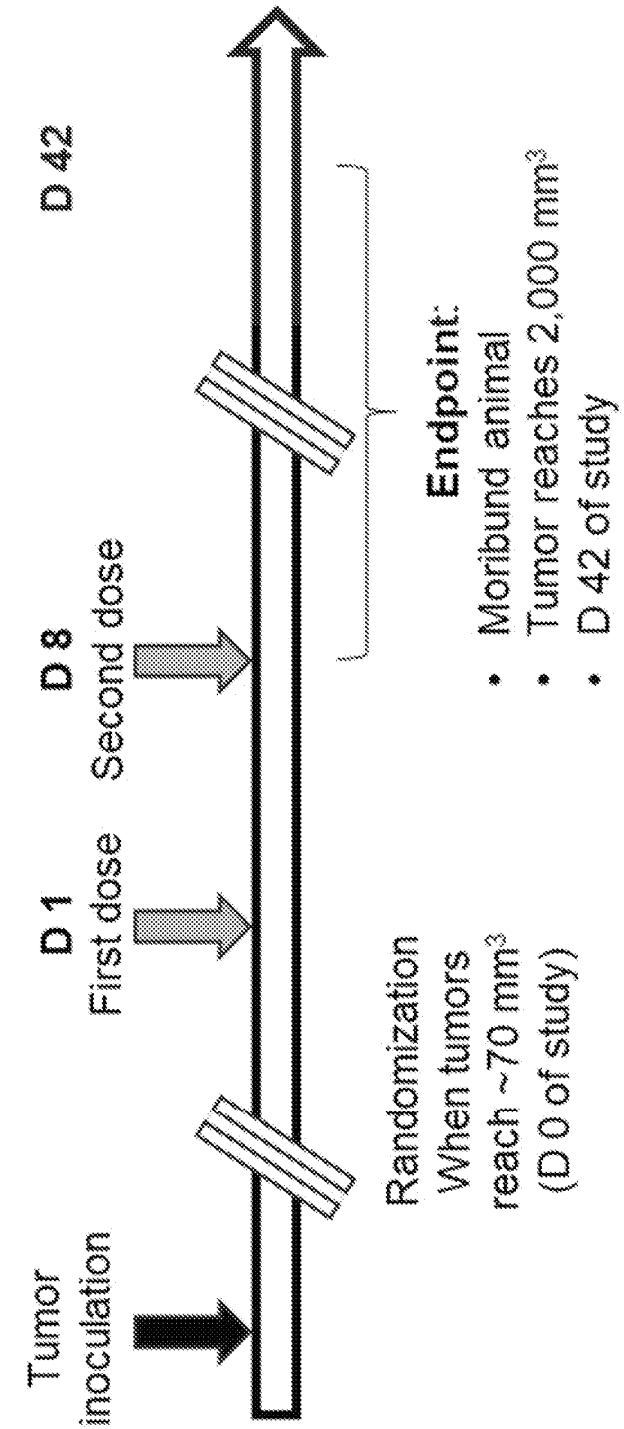

Tumor inoculation
D 1
First dose
D 8
Second dose
D 42
Randomization
When tumors reach ~70 mm3
(D 0 of study)
Endpoint:
• Moribund animal
• Tumor reaches 2,000 mm3
• D 42 of study

Figure 9

Figure 10A

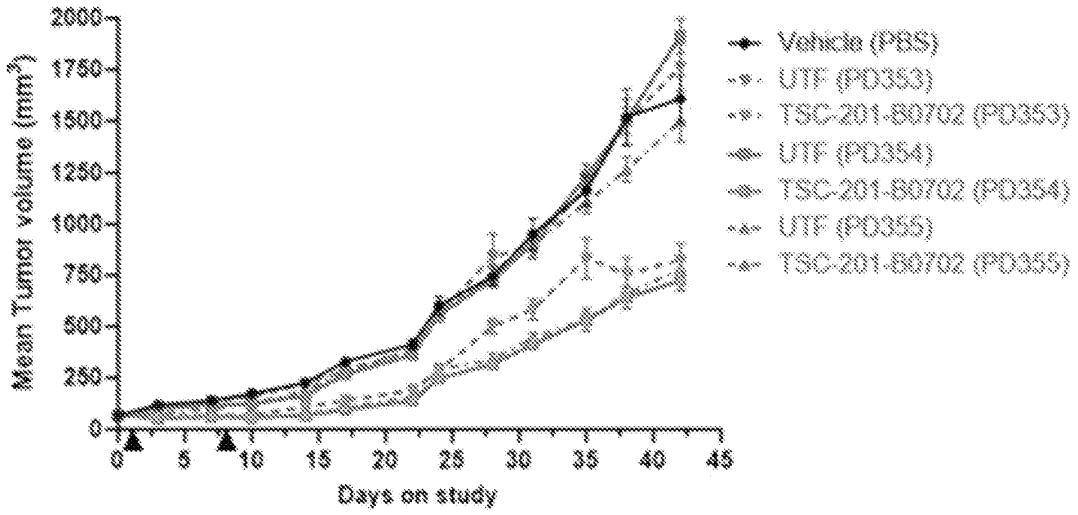


Figure 10B

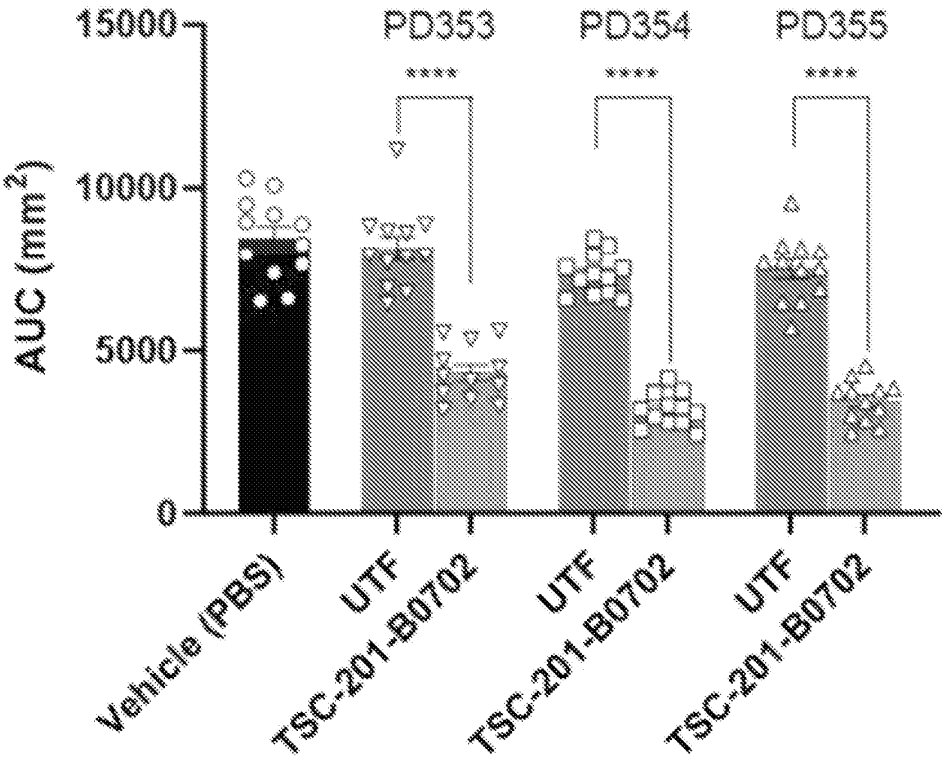


Figure 10C

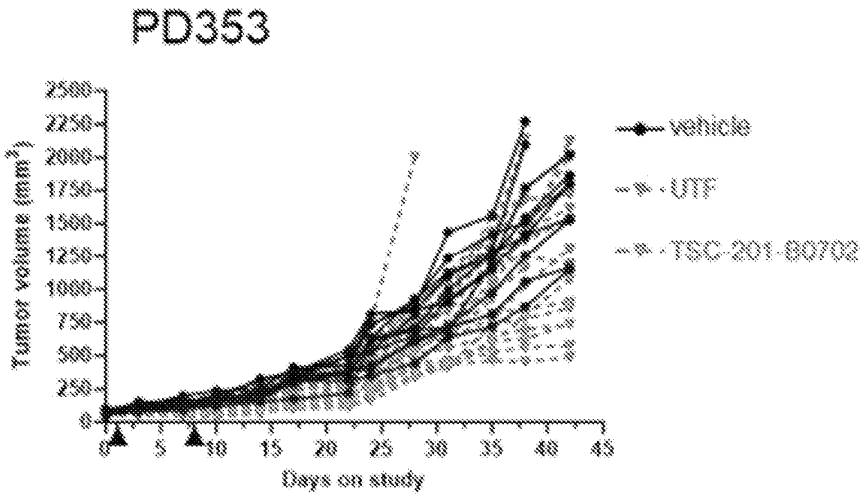


Figure 10D

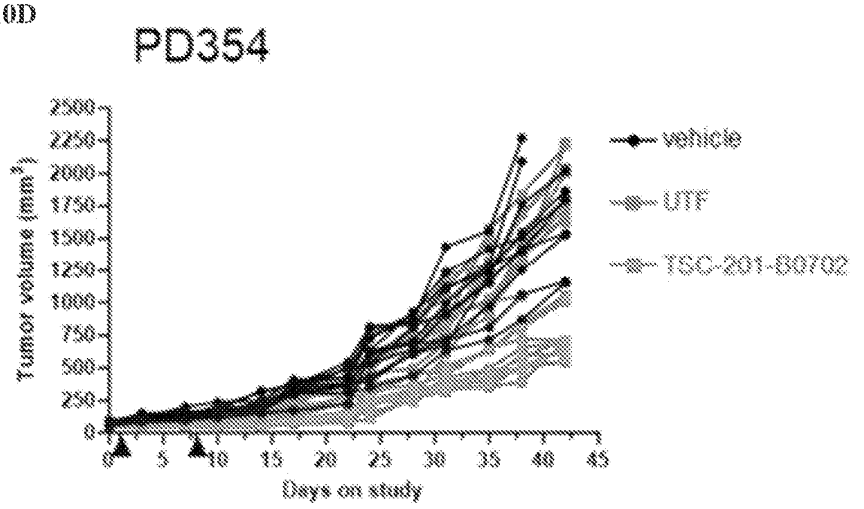
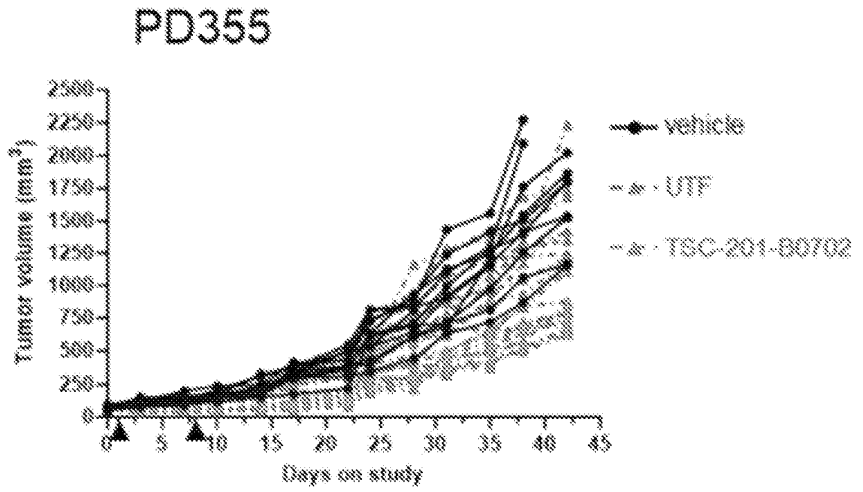


Figure 10E



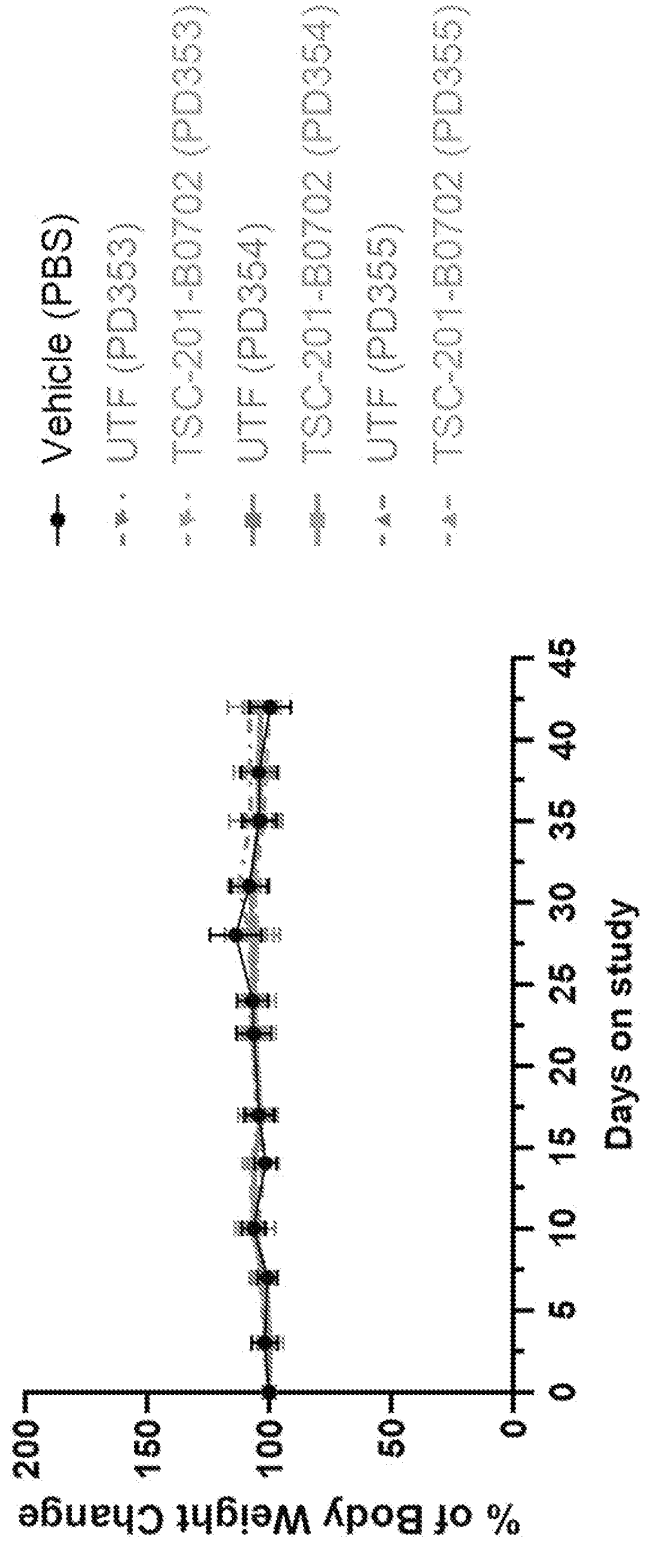

Vehicle (PBS)
UTF (PD353)
TSC-201-B0702 (PD353)
UTF (PD354)
TSC-201-B0702 (PD354)
UTF (PD355)
TSC-201-B0702 (PD355)
% of Body Weight Change
200
150
100
50
0
0
5
10
15
20
25
30
35
40
45
Days on study

Figure 11

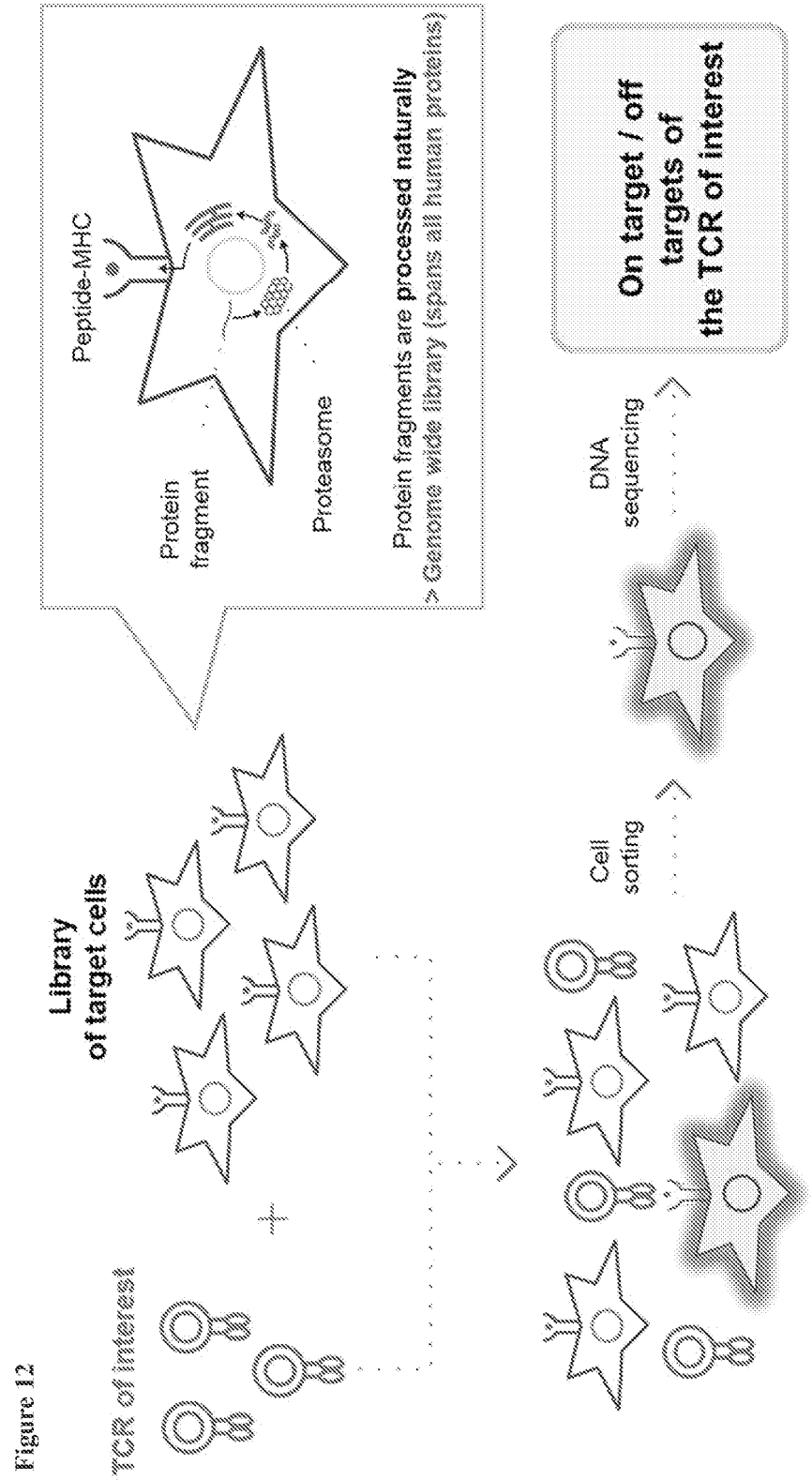

Peptide-MHC
Protein fragment
Proteasome
Protein fragments are processed naturally
> Genome wide library (spans all human proteins)
Library of target cells
TCR of interest
Cell sorting
DNA sequencing
On target / off targets of the TCR of interest

Figure 12

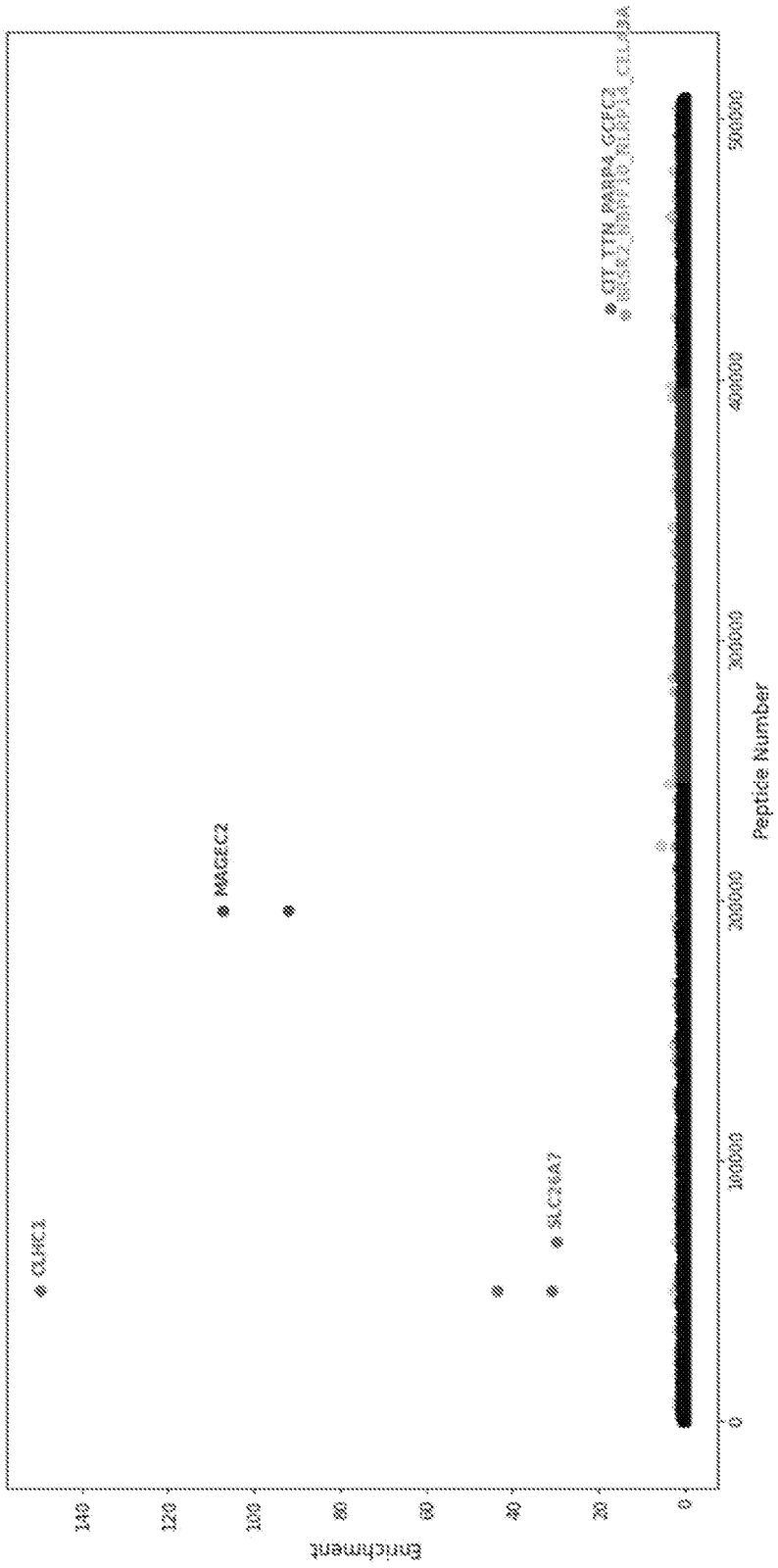

Enrichment
Peptide Number
0
20
40
60
80
100
120
140
0
1000000
2000000
3000000
4000000
5000000
CLEC3A
MAGEC2
SLC39A7
GTL1TN_PARP4_GCEC2_CELLDEA
SELL2_MAPK10_SSPN_CELLDEA

Figure 13



Figure 14

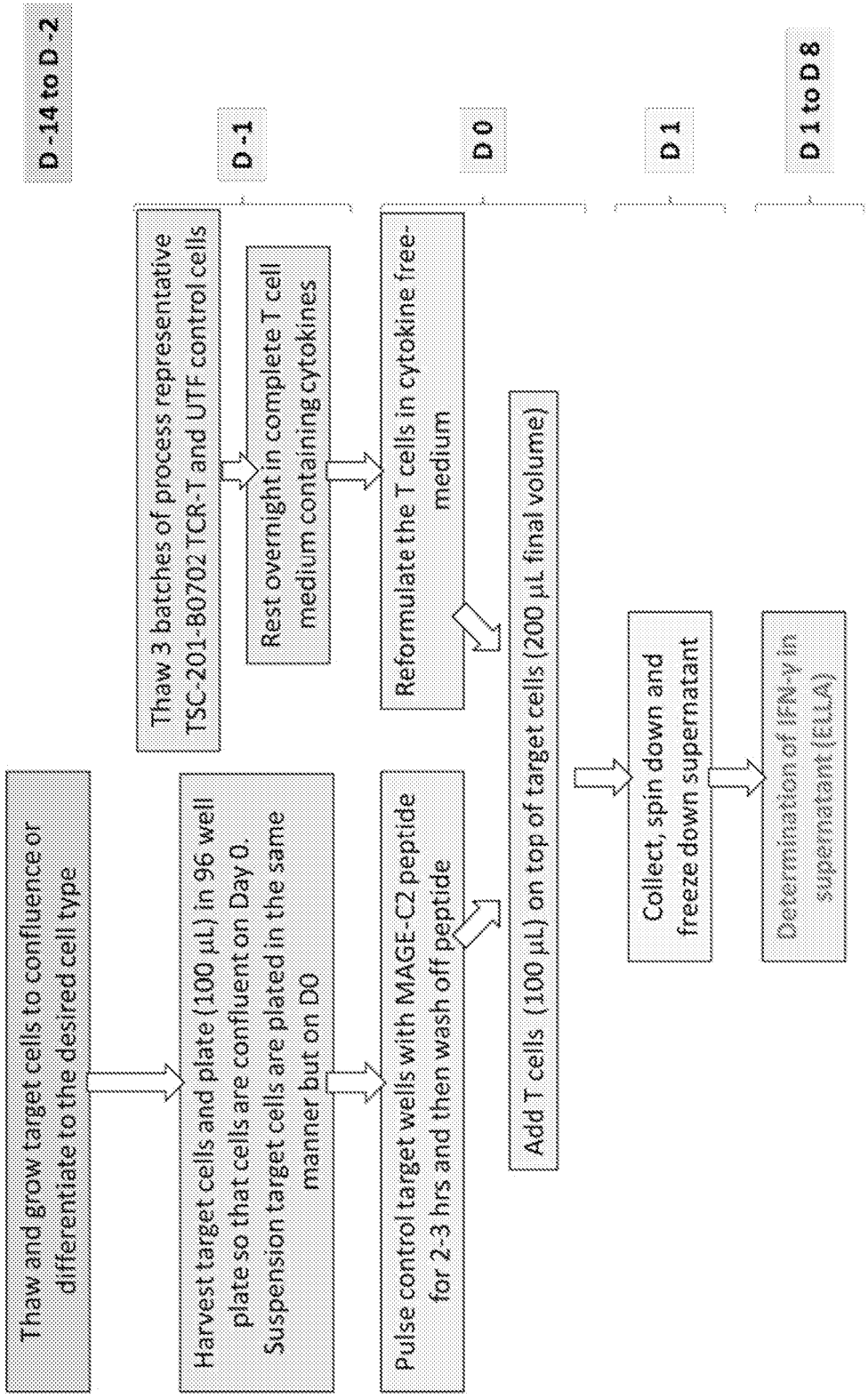

D -14 to D -2
Thaw and grow target cells to confluence or differentiate to the desired cell type
D -1
Thaw 3 batches of process representative TSC-201-B0702 TCR-T and UTF control cells
Rest overnight in complete T cell medium containing cytokines
Harvest target cells and plate (100 µL) in 96 well plate so that cells are confluent on Day 0. Suspension target cells are plated in the same manner but on D0
D 0
Reformulate the T cells in cytokine free-medium
Pulse control target wells with MAGE-C2 peptide for 2-3 hrs and then wash off peptide
Add T cells (100 µL) on top of target cells (200 µL final volume)
D 1
Collect, spin down and freeze down supernatant
D 1 to D 8
Determination of IFN-γ in supernatant (ELLA)

Figure 15

Figure 16

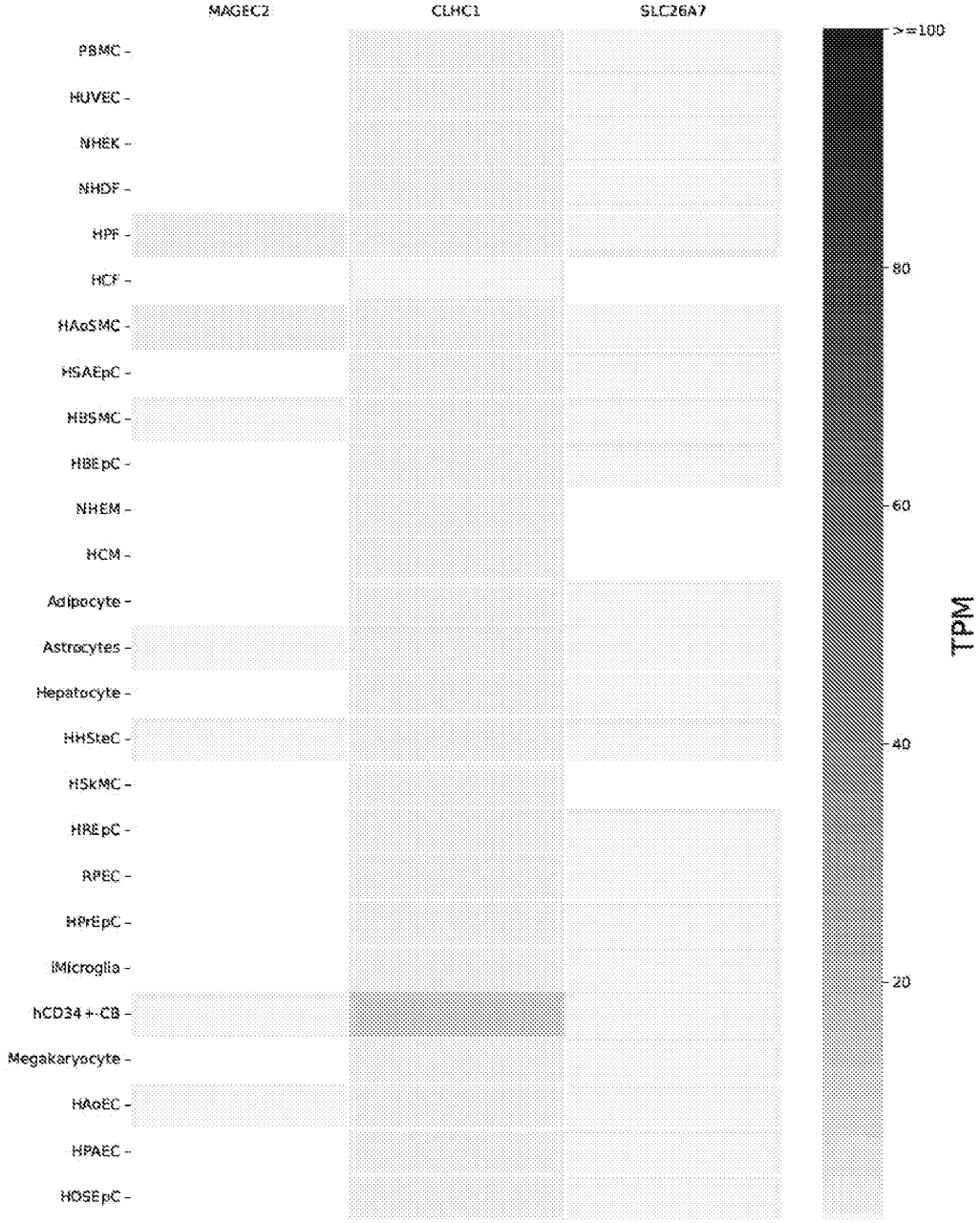

MAGEC2
CLHC1
SLC26A7
PBMC
HUVEC
NHEK
NHDF
HPF
HCF
HAoSMC
HSAEpC
HBSMC
HBEpC
NHEM
HCM
Adipocyte
Astrocytes
Hepatocyte
HHSteC
HSkMC
HREpC
RPEC
HPrEpC
iMicroglia
hCD34+-CB
Megakaryocyte
HAoEC
HPAEC
HOSEpC
>=100
80
60
40
20
TPM

Figure 17A

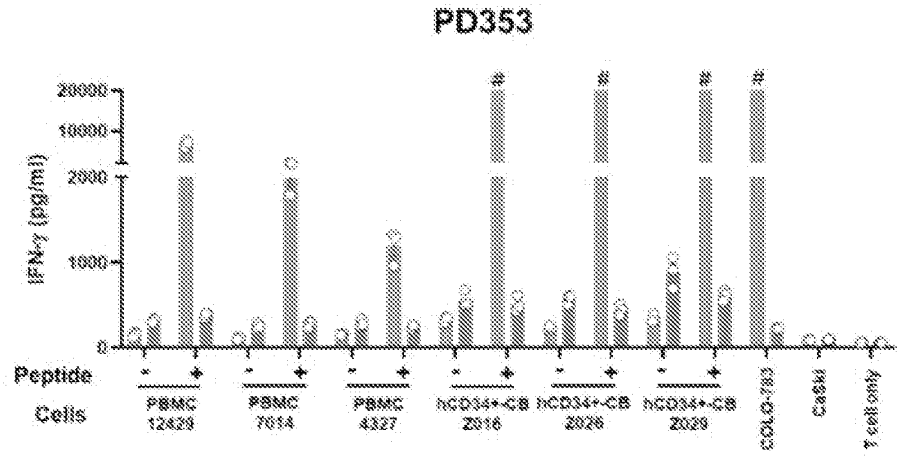


Figure 17B

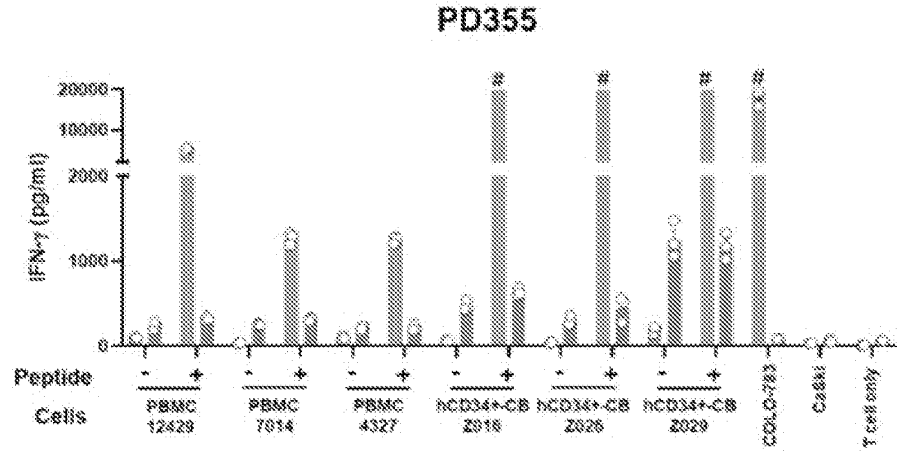
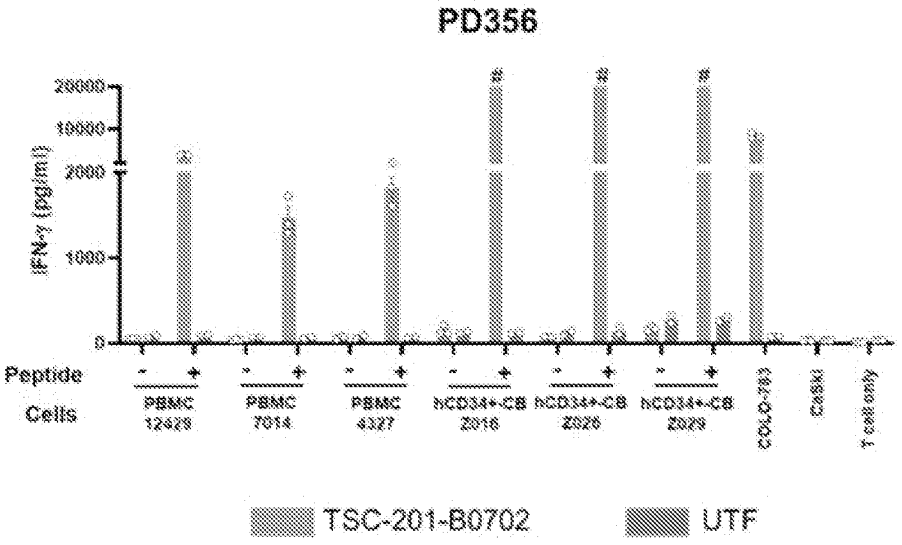


Figure 17C



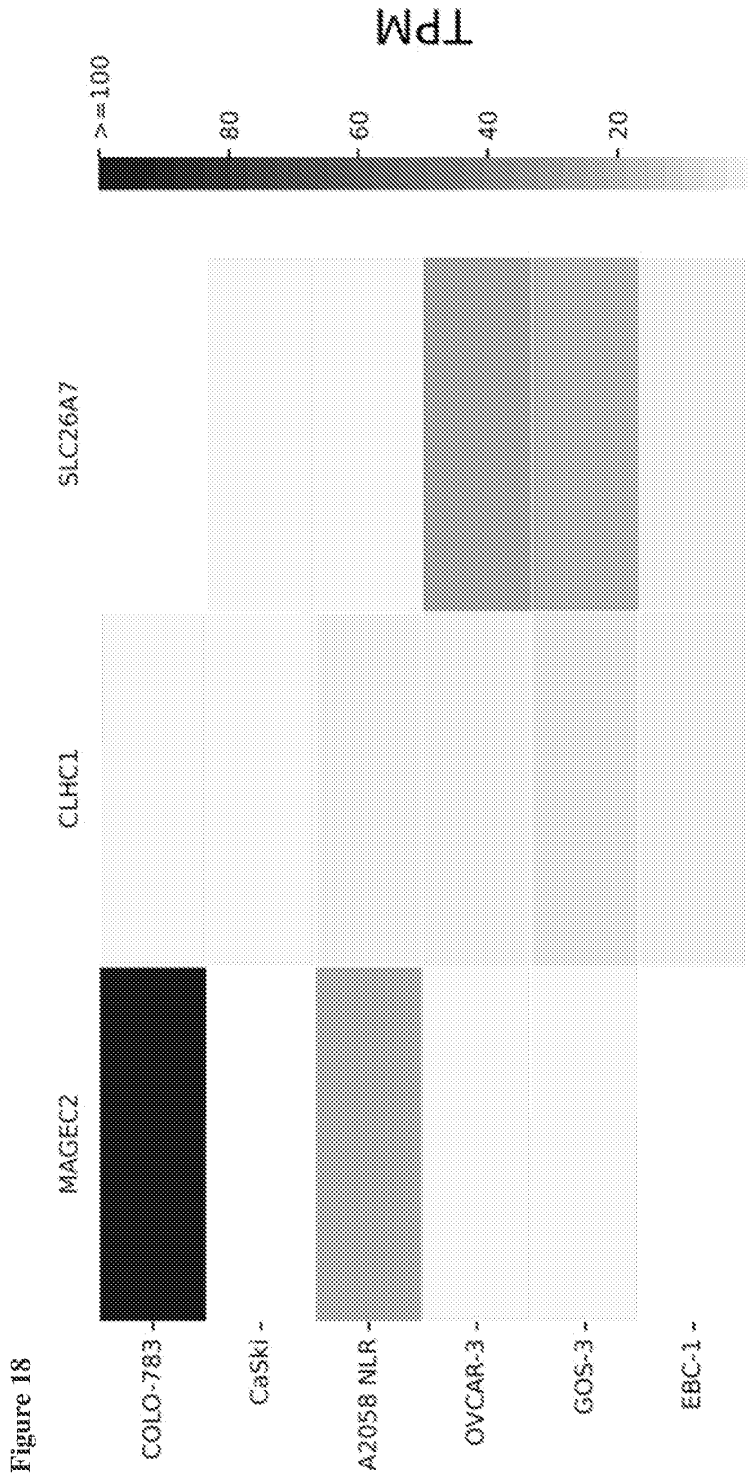

TPM
>=100
80
60
40
20
MAGEC2
CLHC1
SLC26A7
COLO-783
CaSki
A2058 NLR
OVCAR-3
GOS-3
EBC-1

Figure 18

Figure 19A

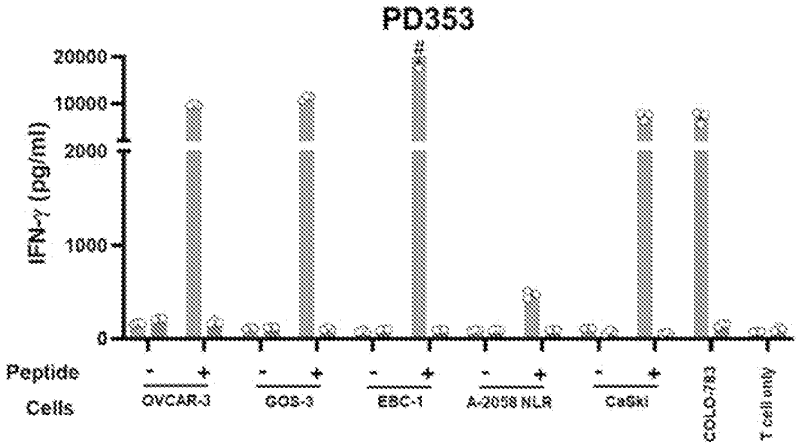


Figure 19B

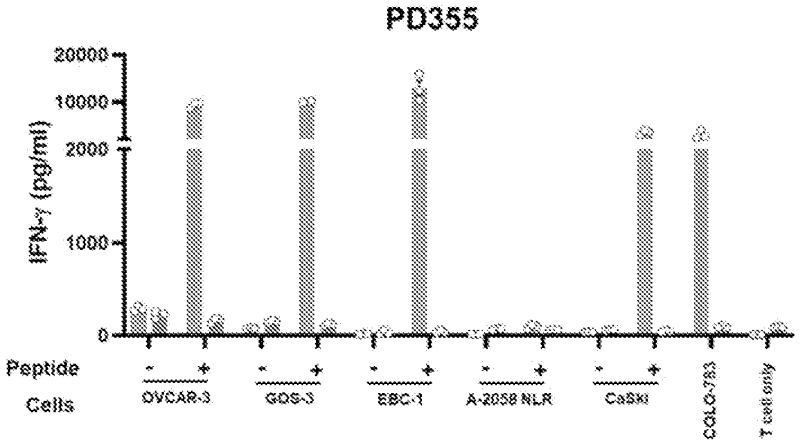
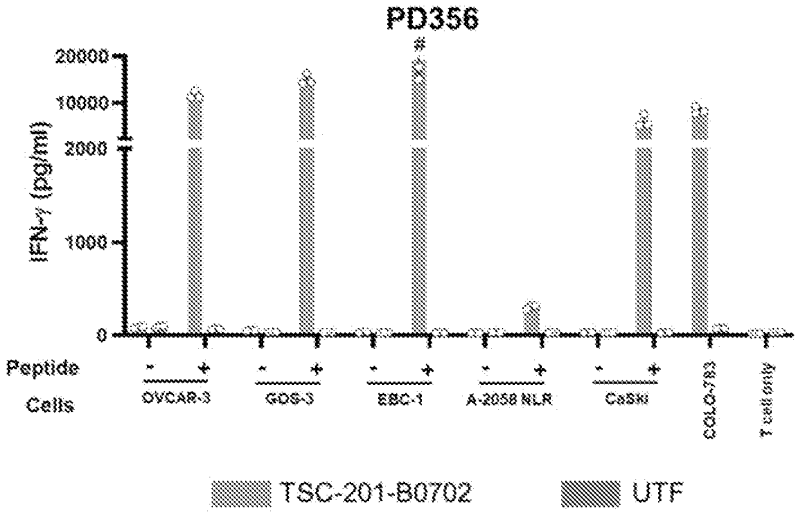


Figure 19C




MAGE-C2 Relative to GAPDH
0.3
0.2
0.1
0.0
Adrenal Gland
Bone marrow
Brain
Bronchus
Cervix
Colon
Descending part of duodenum
Epididymis
Esophagus
Heart
Heart, ventricle (left)
Heart, ventricle (right)
Intestine (small)
Kidney
Larynx
Liver
Lung
Lymph node
Lymphocytes
Mammary gland
Muscle
Nasal muscosa
Optic nerve
Ovary
Oviduct
Pancreas
Penis
Pericardium
Pituitary
Placenta
Prostate
Retina
Salivary gland
Skin
Spinal cord
Spleen
Stomach
Testis
Thymus
Thyroid
Tongue
Tonsil
Trachea
Ureter
Urinary bladder
Uterus
Uvula
Vagina

Figure 20

Figure 21A


pNVVD187_TSC-201-B07_TCR-6_MSCV-TCR6-CD8-EF1a-dnTGFbRII-DHFRdm
8028 bp
8000
1000
2000
3000
4000
5000
6000
7000
R6K origin
RNA-OUT
TK
SV40 poly(A) signal
DHFRdm
P2A
Furin cleavage site
QBend/10 epitope
CD8 α chain
CD8 β chain
TCR β chain
TCR α chain
BGH poly(A) signal

Figure 21B


pNVVD188_TCR-422_MSCV-TCR- 422-CD8-EF1α-dnTGFbRII-DHFR
8058 bp
8000
1000
2000
3000
4000
5000
6000
7000
f1 ori
R6K origin
RNA-OUT
MSCV promoter
TCR β chain
Furin cleavage site
P2A
TCR α chain
QBend/10 epitope
Furin cleavage site
P2A
CD8 α chain
Furin cleavage site
P2A
EF1α promoter
dnTGFbRII
SV40 poly(A) signal
DHFR
SV40 poly(A) signal
P2A

Figure 21C


pNVVD189_TCR-724_MSCV-TCR- 724-CD8-EF1α-dnTGFbRII-DHFR
8061 bp
9000
1000
2000
3000
4000
5000
6000
7000
8000
R6K origin
RNA-OUT
MSCV promoter
Furin cleavage site
P2A
QBend/10 epitope
CD8 α chain
SV40 poly(A) signal
EF1α promoter
TCR β chain
TCR α chain
DHFR

Figure 21D


pNVVD190_TCR-563_MSCV-TCR- 563-CD8-FF1a-dnTGFbRII-DHFR
8052 bp
8000
1
F1 ori
R6K origin
RNA-OUT
TR
MSCV promoter
1000
TCR β chain
2000
Furin cleavage site
P2A
TCR α chain
3000
CBend/10 epitope
Furin cleavage site
P2A
3830
CD8 α chain
4000
P2A
CD8 β chain
5000
hGH poly(A) signal
6000
F1 ori
6500
EF1α promoter
7000
P2A
DHFR
SV40 poly(A) signal
F1 ori

Figure 21E


pNVVD186_TCR-Leiden-8-3_MSCV-TCR-Leiden-8-3-CD8-EF1a-dnTGFbRII-DHFR
9061 bp
8000
1000
2000
3000
4000
5000
6000
7000
9000
AmpR
SV40 poly(A) signal
R6K origin
RNA-OUT
TK
MSCV promoter
TCR β chain
Furin cleavage site
P2A
TCR α chain
QBend/10 epitope
Furin cleavage site
P2A
P2A
CD8 α chain
EF1α promoter
dnTGFbRII
P2A
DHFR