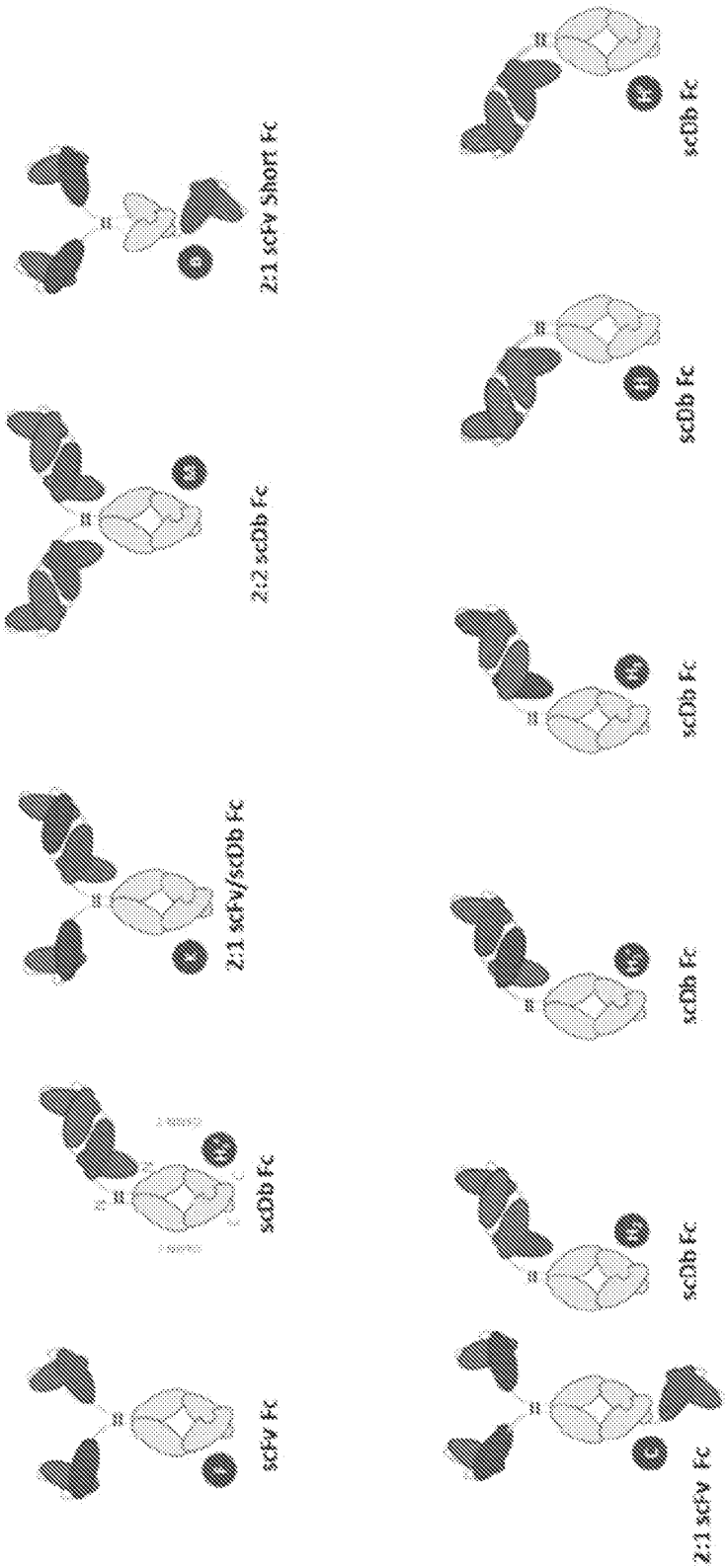

scFv Fc
scDb Fc
2:1 scFv/scDb Fc
2:2 scDb Fc
2:1 scFv Short Fc
2:1 scFv Fc
scDb Fc
scDb Fc
scDb Fc
scDb Fc

FIG. 1

Undesired KiH Related Impurities
Chain-1/Chain-2
Heterodimer
S-S stabilized bridge
H435R
Chain-1/Chain-1
Mis-Pair
Lower pI
Chain-2/Chain-2
Mis-Pair
H435R
H435R
2 Log decrease in
Protein A Binding
Higher pI

FIG. 2

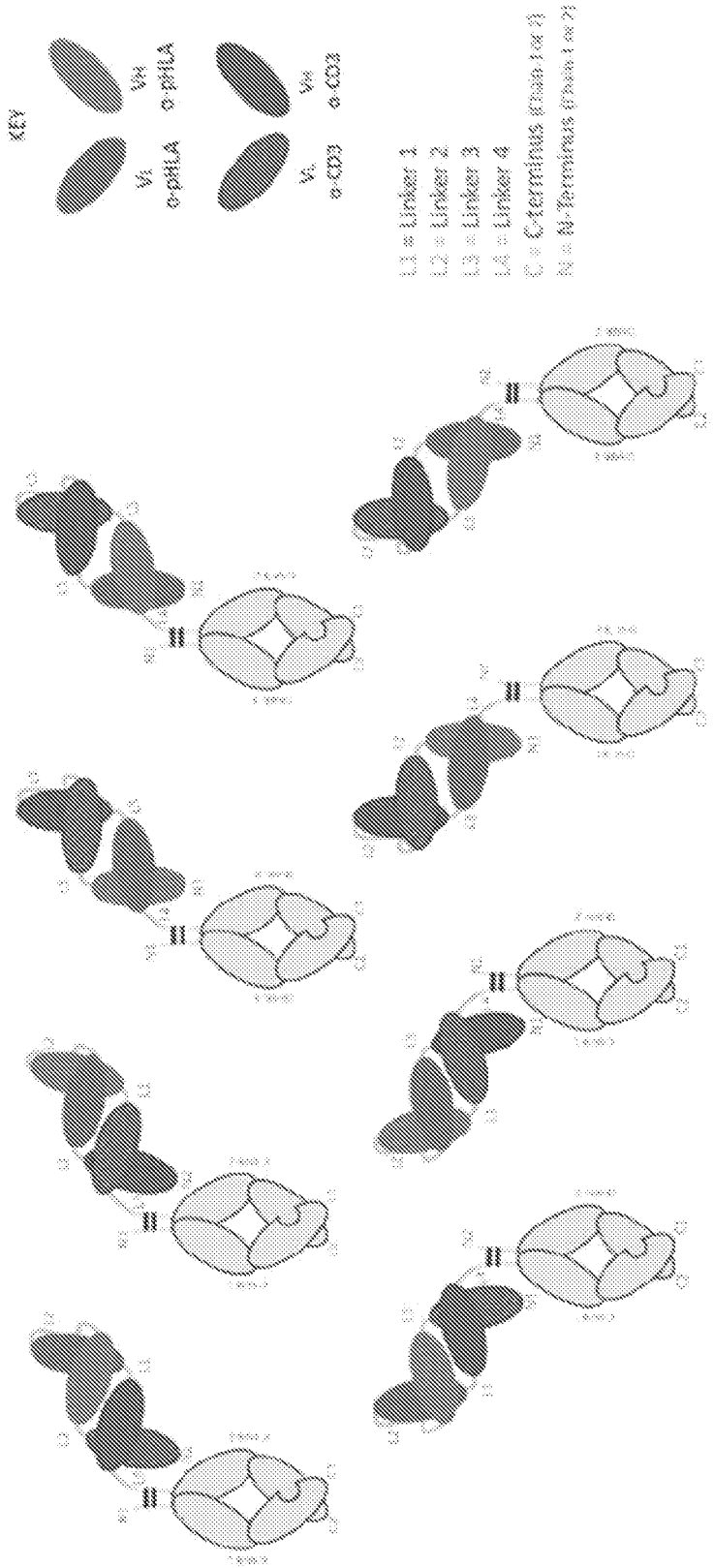

KEY
VH
α-pHLA
VL
α-pHLA
VH
α-CD3
VL
α-CD3
L1 = Linker 1
L2 = Linker 2
L3 = Linker 3
L4 = Linker 4
C = C-terminus (Exon 1 or 2)
N = N-Terminus (Exon 1 or 2)

FIG. 3

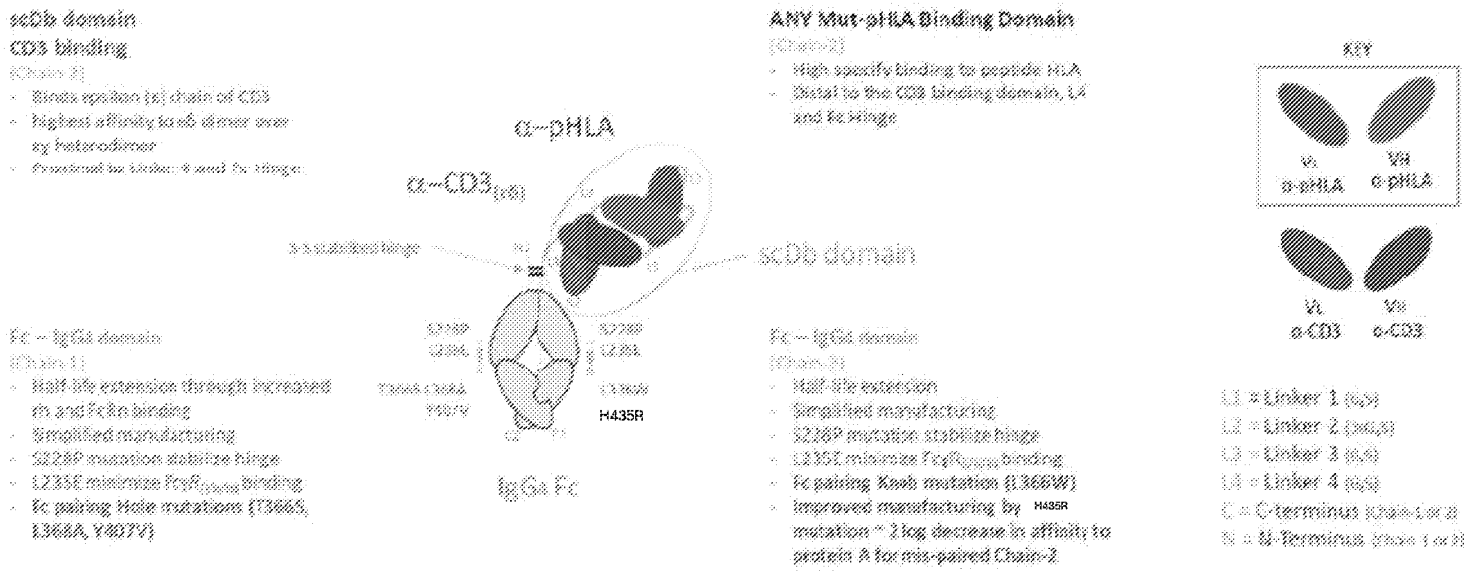


FIG. 4

This mutation changes the PI of the chain for effective separation of chain-1/chain-1 mis-pairs from heterodimeric drug and from chain-2/chain-2 mis-pairs.

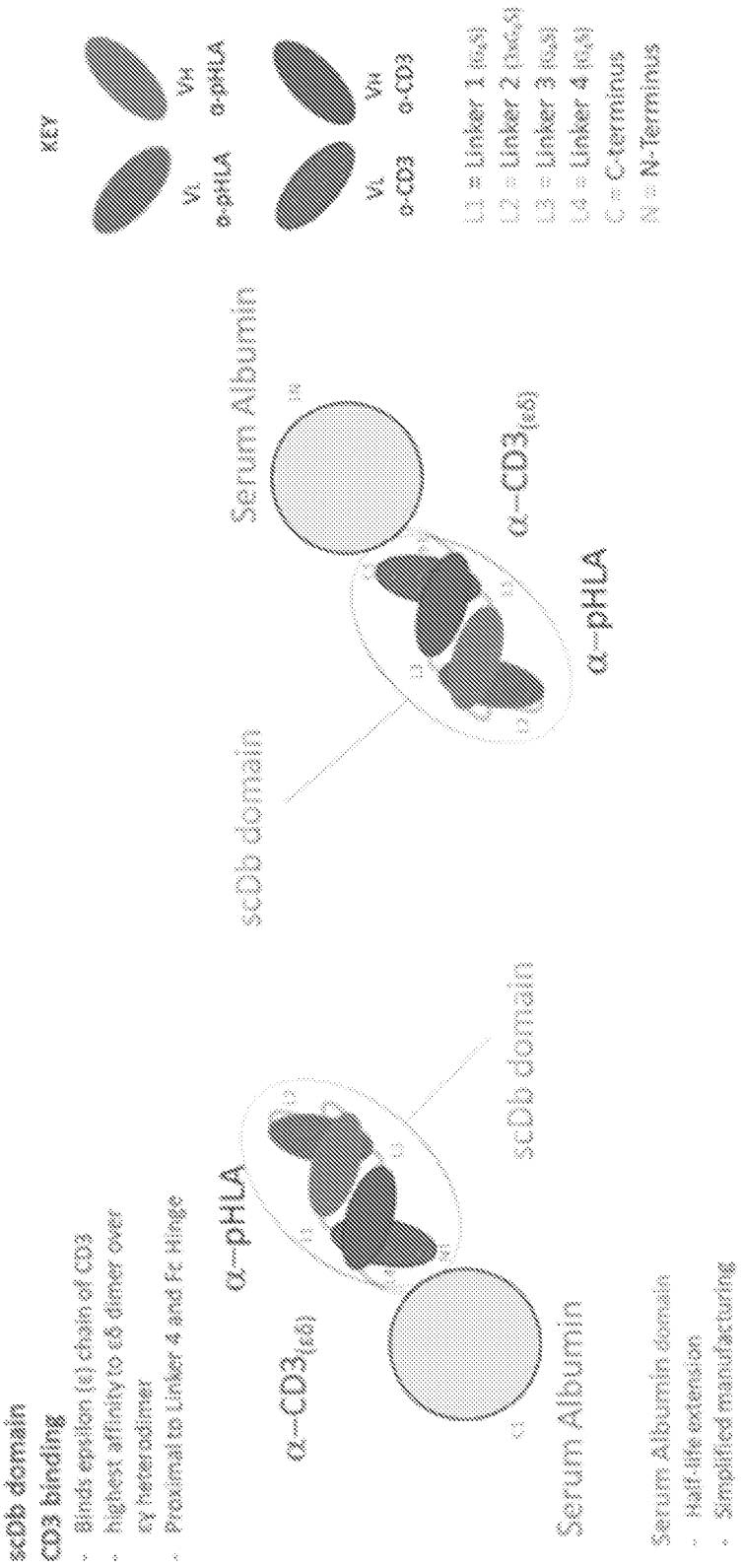

scDb domain
CD3 binding
Binds epsilon (ε) chain of CD3
highest affinity to εδ dimer over εγ heterodimer
Proximal to Linker 4 and Fc Hinge
α–pHLA
α–CD3(εδ)
Serum Albumin
scDb domain
Serum Albumin domain
Half-life extension
Simplified manufacturing
scDb domain
α–pHLA
α–CD3(εδ)
Serum Albumin
KEY
VL α-pHLA
VH α-pHLA
VL α-CD3
VH α-CD3
L1 = Linker 1
L2 = Linker 2
L3 = Linker 3
L4 = Linker 4
C = C-terminus
N = N-Terminus

FIG. 5

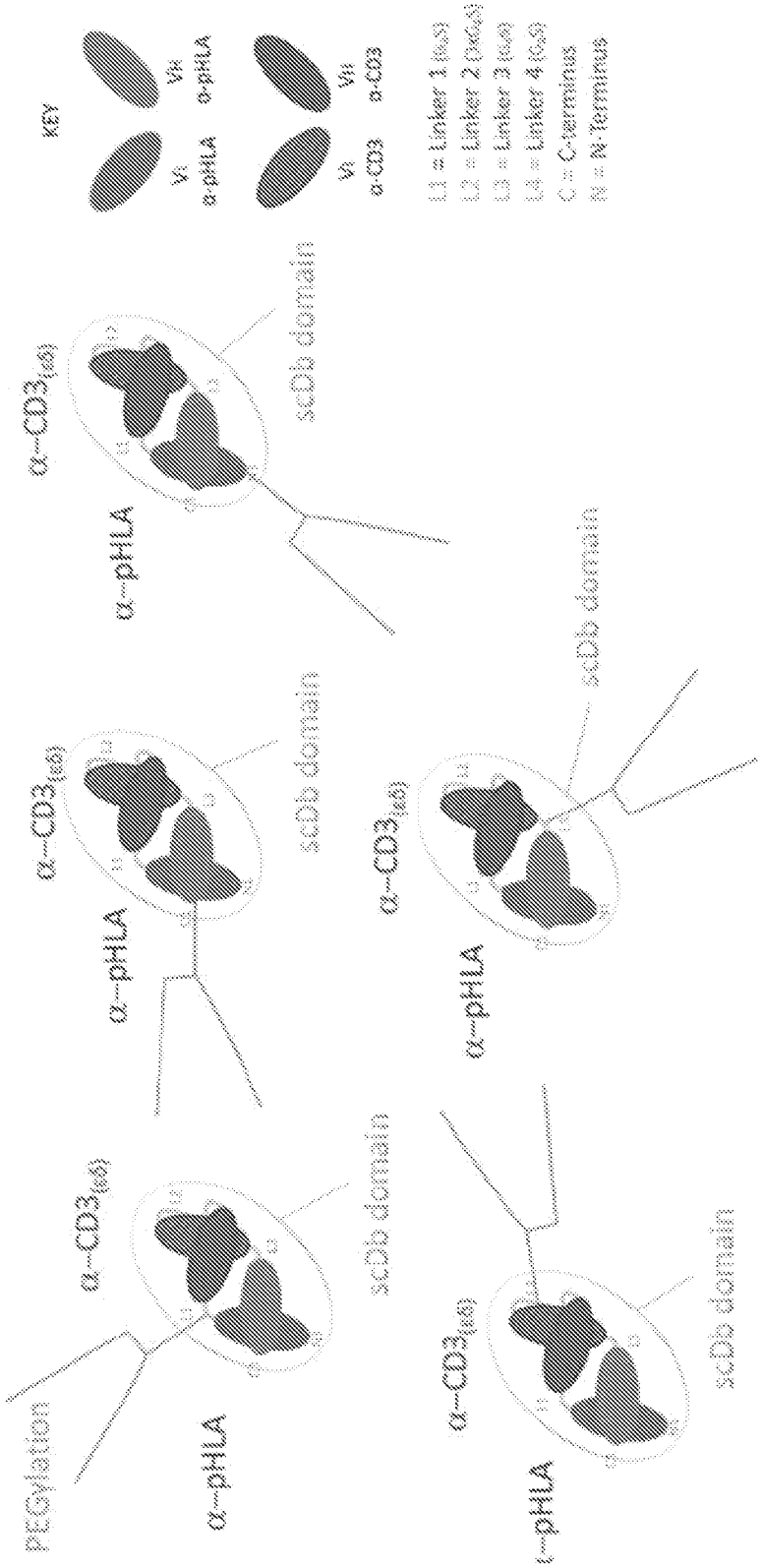

KEY
VH α-pHLA
VL α-pHLA
VH α-CD3
VL α-CD3
L1 = Linker 1 (G4S)
L2 = Linker 2 (GGGS)
L3 = Linker 3 (G4S)
L4 = Linker 4 (G4S)
C = C-terminus
N = N-Terminus
α–CD3(L6)
α–pHLA
scDb domain
PEGylation

FIG. 6

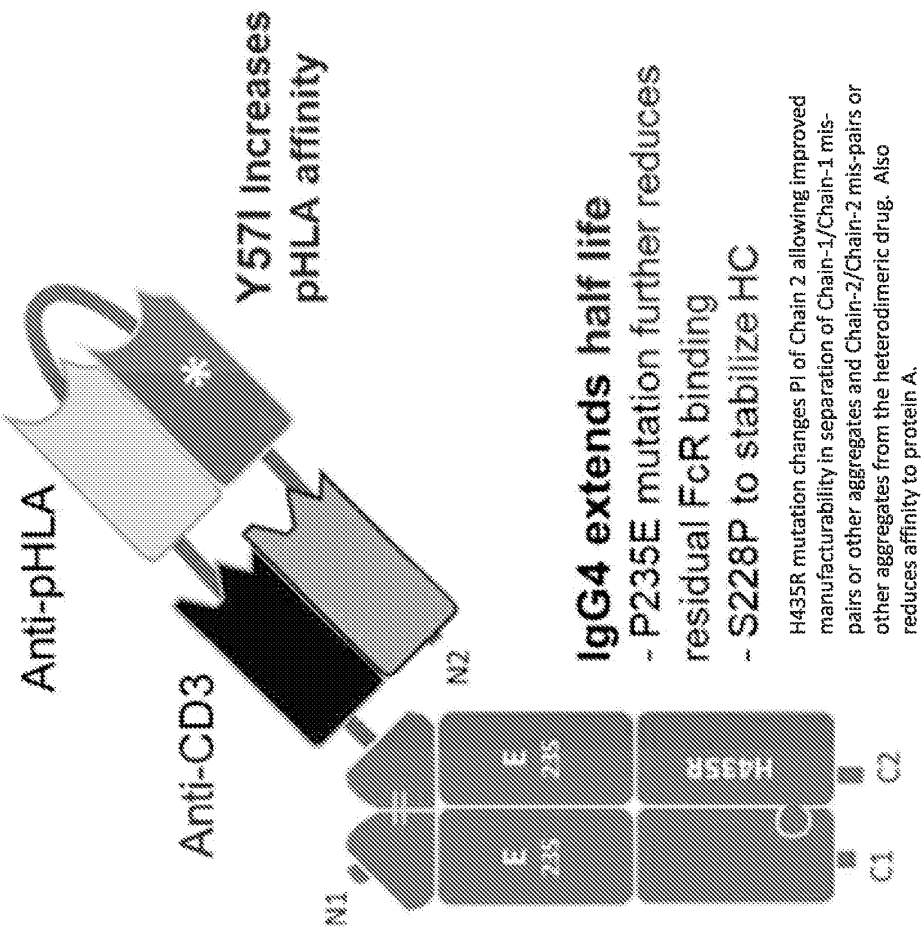

Anti-pHLA
Anti-CD3
Y57I Increases
pHLA affinity
N1
N2
C1
C2
IgG4 extends half life
- P235E mutation further reduces
residual FcR binding
- S228P to stabilize HC
H435R mutation changes PI of Chain 2 allowing improved manufacturability in separation of Chain-1/Chain-1 mis-pairs or other aggregates and Chain-2/Chain-2 mis-pairs or other aggregates from the heterodimeric drug. Also reduces affinity to protein A.

FIG. 7

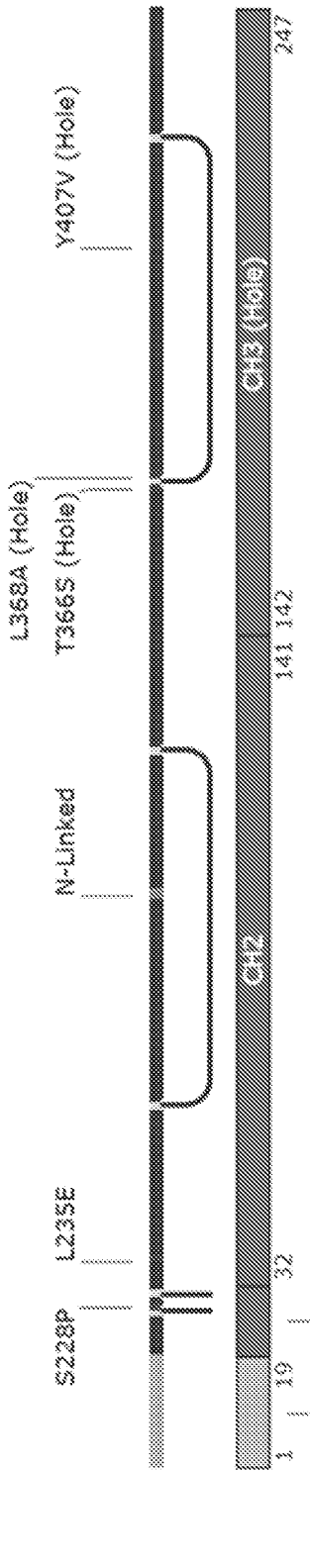

S228P
L235E
N-Linked
L368A (Hole)
T366S (Hole)
Y407V (Hole)
CH2
CH3 (Hole)
1
10
32
141
142
247
Leader Sequence
Hinge

FIG. 8A

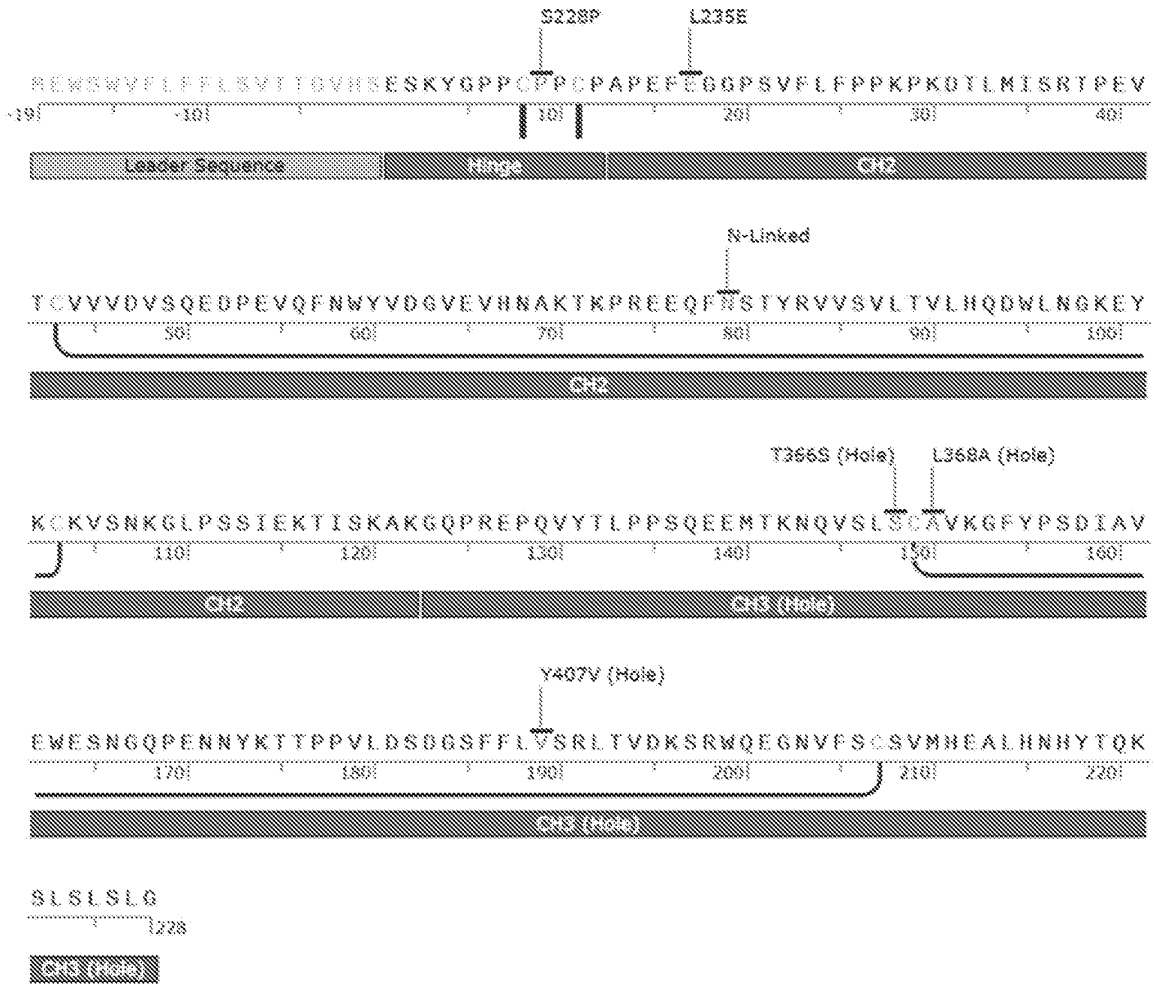

S228P
L235E
Leader Sequence
Hinge
CH2
N-Linked
CH2
T366S (Hole)
L368A (Hole)
CH2
CH3 (Hole)
Y407V (Hole)
CH3 (Hole)
CH3 (Hole)

FIG. 8B

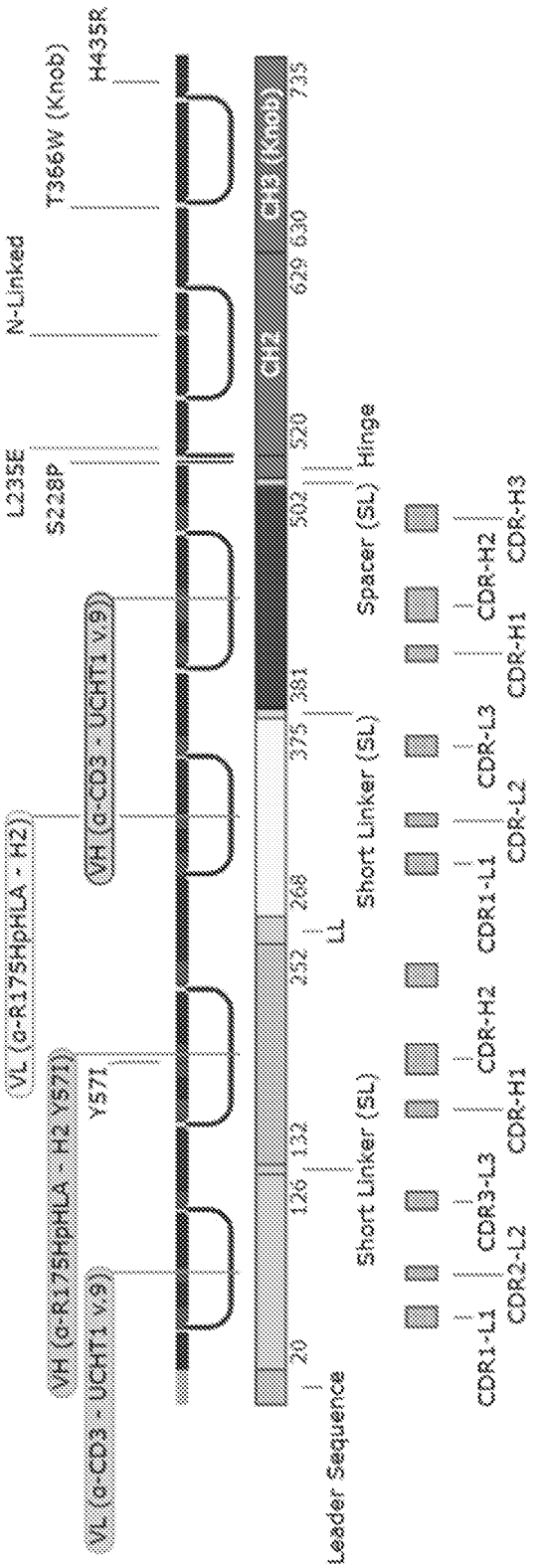

VL (α-R175HpHLA - H2)
VH (α-R175HpHLA - H2 Y571)
VL (α-CD3 - UCHT1 v9)
VH (α-CD3 - UCHT1 v9)
Y571
L235E
S228P
N-Linked
T366W (Knob)
H435R
CH2
CH3 (Knob)
Leader Sequence
Short Linker (SL)
Short Linker (SL)
LL
Spacer (SL)
Hinge
CDR1-L1
CDR2-L2
CDR3-L3
CDR-H2
CDR-H1
CDR1-L1
CDR-L2
CDR-L3
CDR-H1
CDR-H2
CDR-H3
20
126
132
252
268
375
381
503
520
629
630
735

FIG. 9A

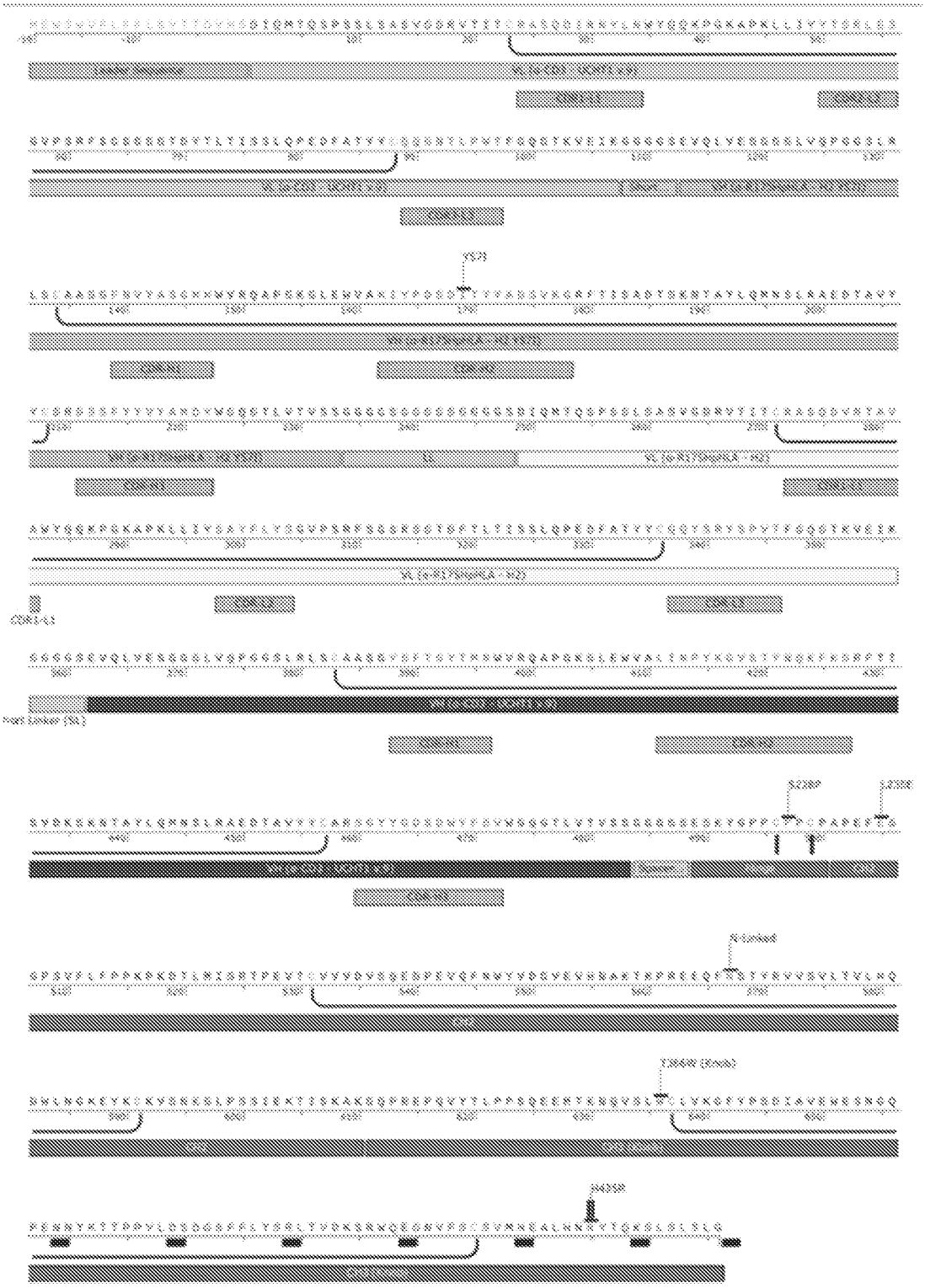


FIG. 9B

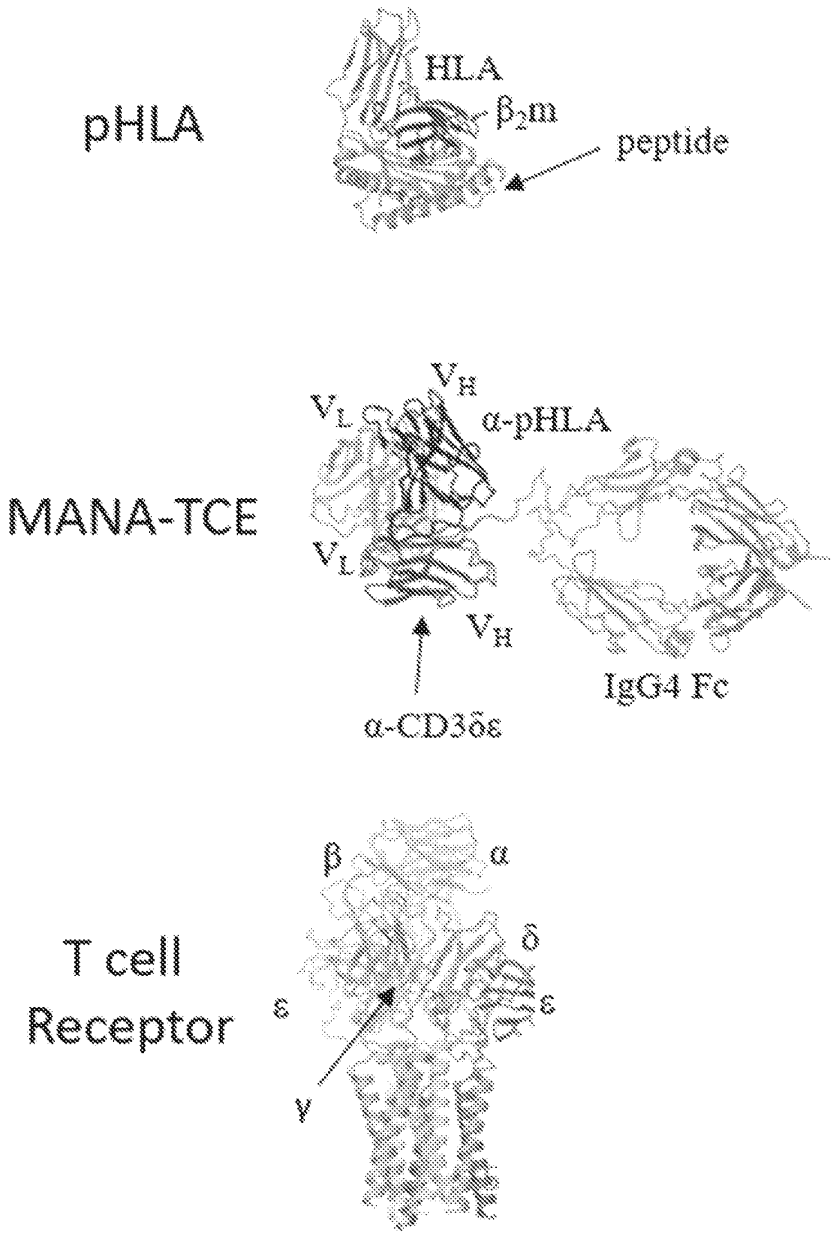

pHLA
HLA
β_2m
peptide
MANA-TCE
V_H
V_L
α-pHLA
V_L
V_H
IgG4 Fc
α-CD3δε
T cell
Receptor
β
α
δ
ε
ε
γ

FIG. 10A

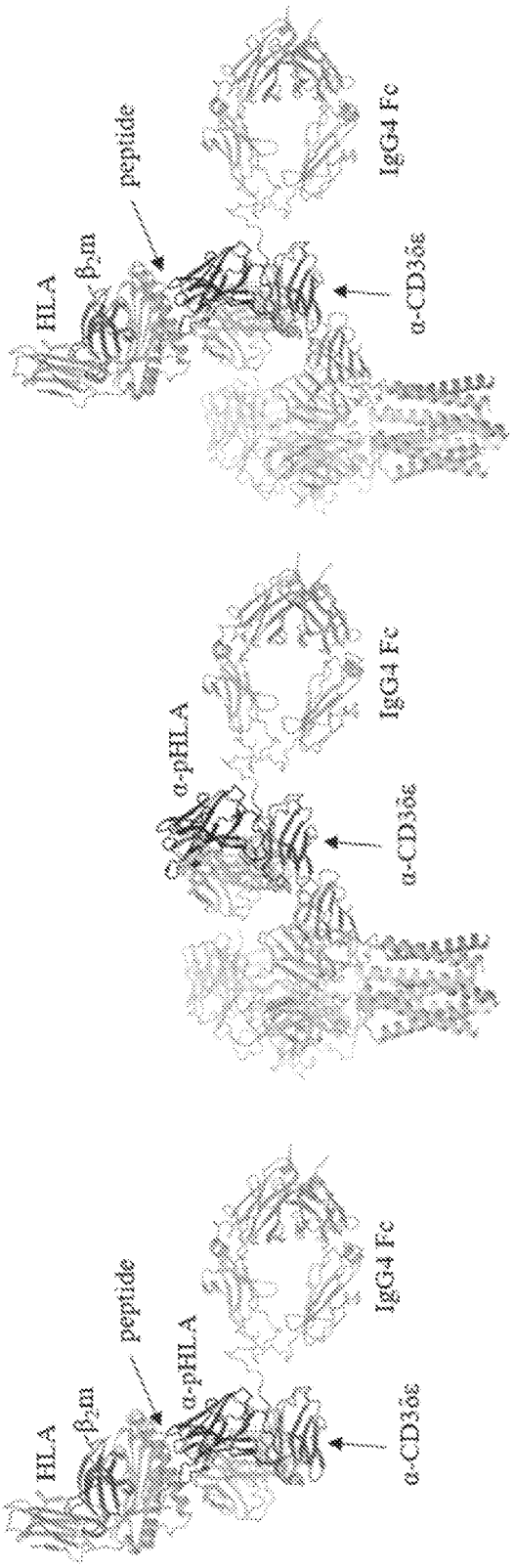

pHLA:MANA-TCE Dimer
HLA
β-m
peptide
α-pHLA
α-CD3ε
IgG4 Fc
TCR:MANA-TCE Dimer
α-pHLA
α-CD3ε
IgG4 Fc
pHLA:MANA-TCE:TCR Trimer
HLA
β-m
peptide
α-CD3ε
IgG4 Fc

FIG. 10B

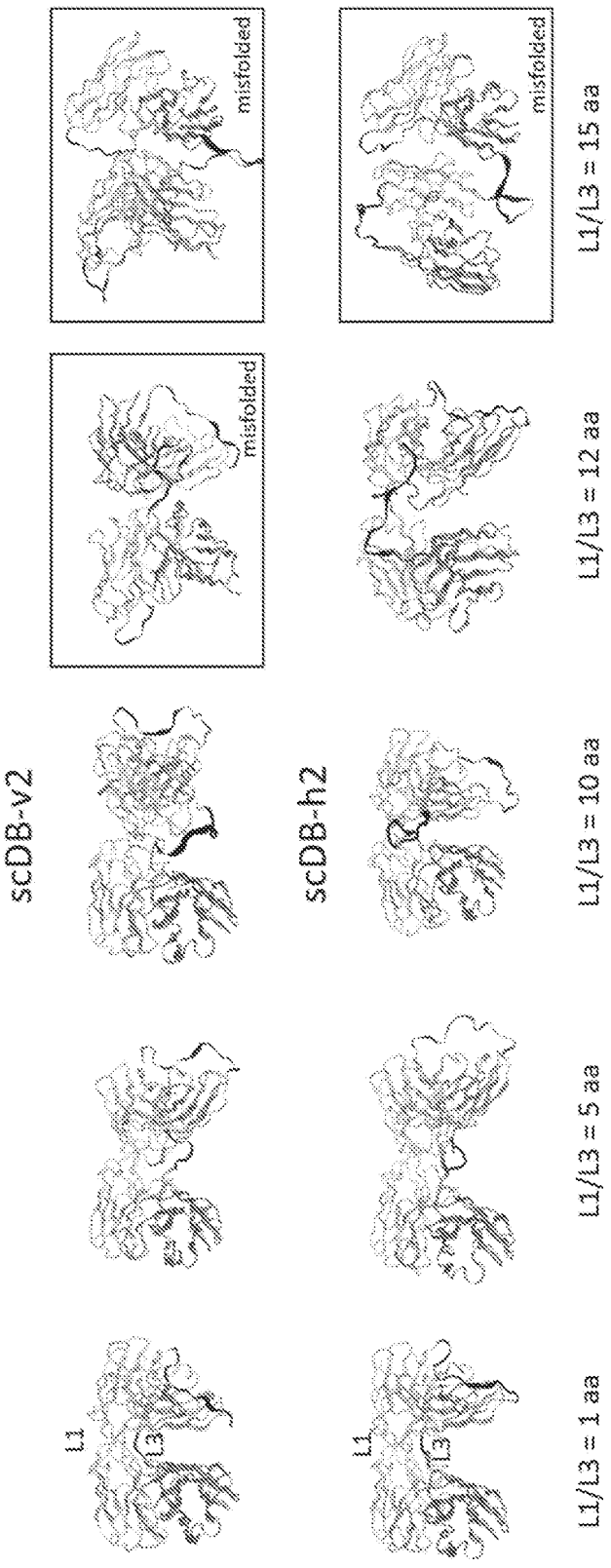

scDB-v2
scDB-h2
misfolded
misfolded
L1
L3
L1/L3 = 1 aa
L1/L3 = 5 aa
L1/L3 = 10 aa
L1/L3 = 12 aa
L1/L3 = 15 aa

FIG. IIA

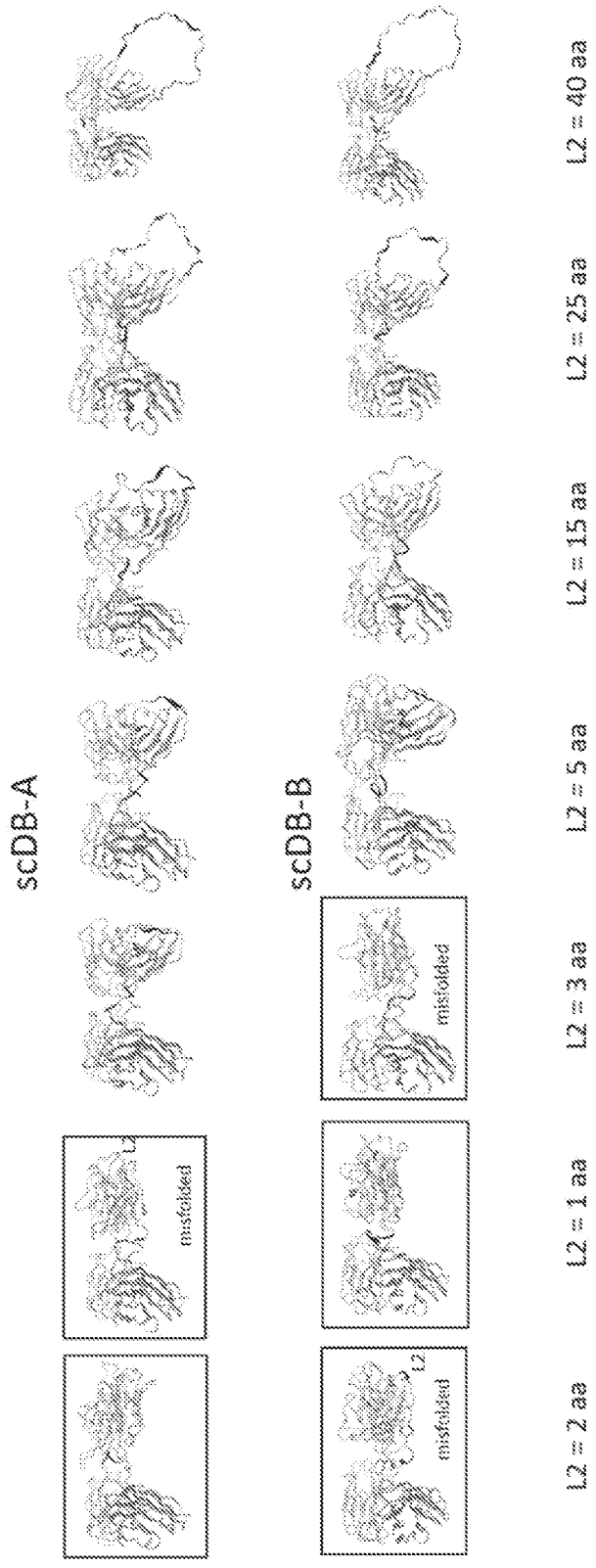

scDB-A
misfolded
scDB-B
misfolded
misfolded
L2 = 2 aa
L2 = 1 aa
L2 = 3 aa
L2 = 5 aa
L2 = 15 aa
L2 = 25 aa
L2 = 40 aa

FIG. 11B

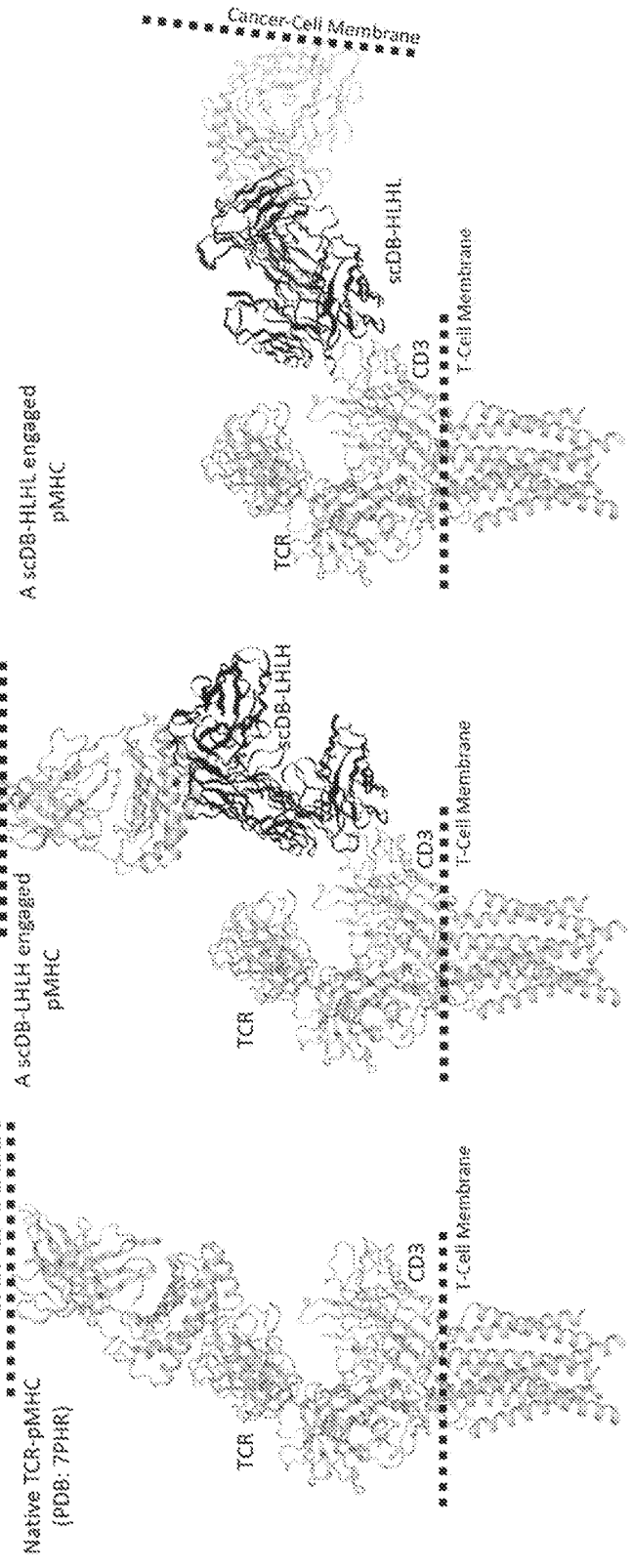

Native TCR-pMHC
(PDB: 7PHR)
Cancer-Cell Membrane
TCR
CD3
T-Cell Membrane
A scDB-LHLH engaged
pMHC
scDB-LHLH
A scDB-HLHL engaged
pMHC
scDB-HLHL

FIG. 11C

ka (1/Ms)	kd (1/s)	KD (nM)	Chi²	U-Value
2.889x10³	0.007369	25.5	0.0224	1

ka (1/Ms)	kd (1/s)	KD (nM)	Chi²	U-Value
3.329x10³	0.0002121	0.6	0.238	1

FIG. 12

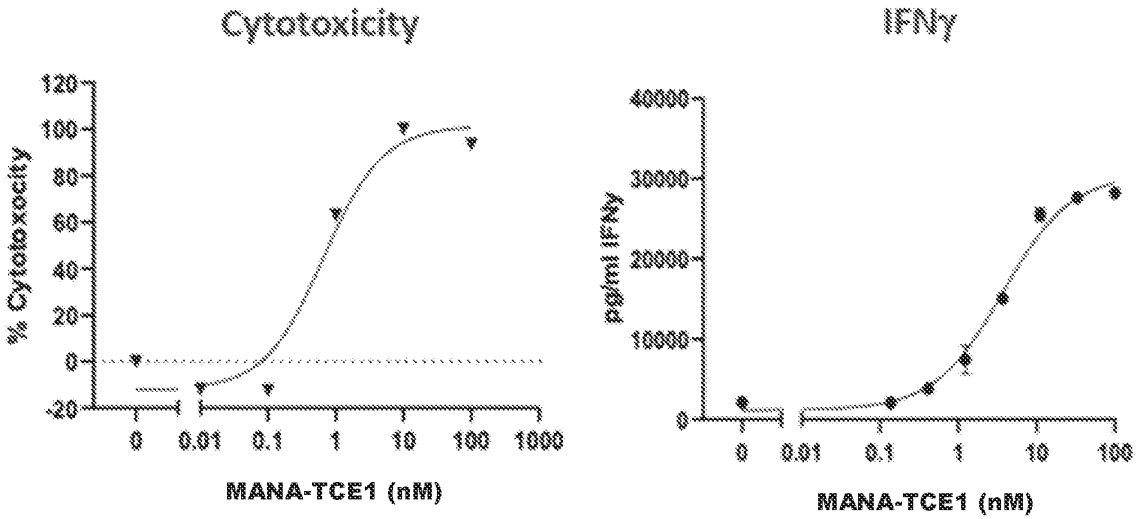


FIG. 13A

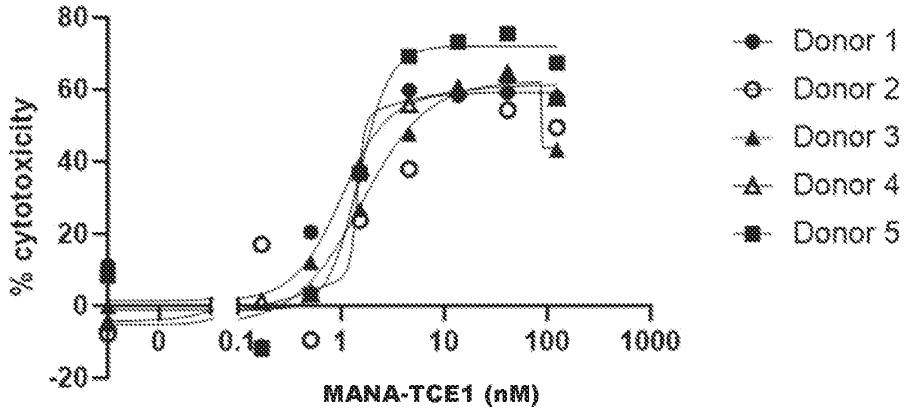


FIG. 13B

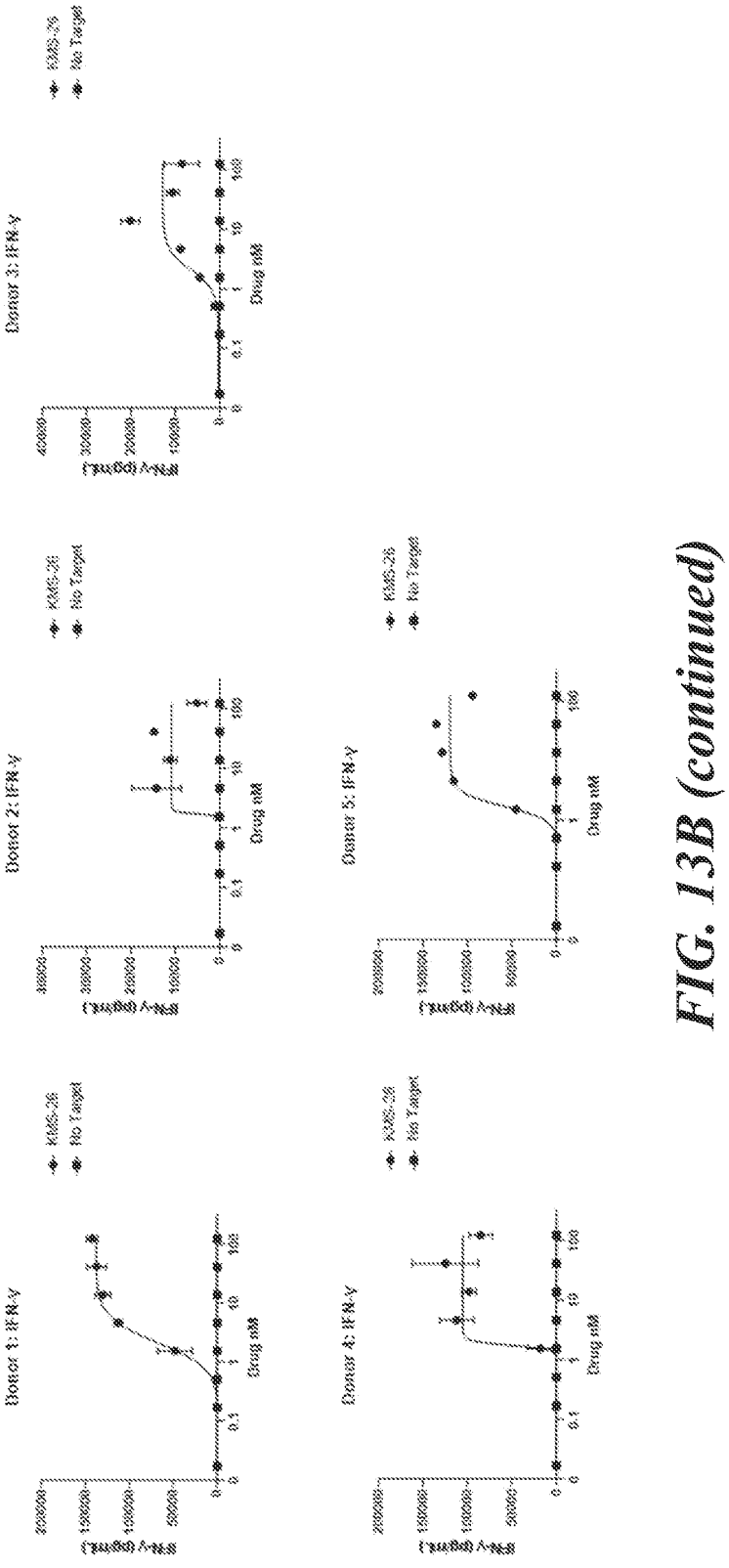
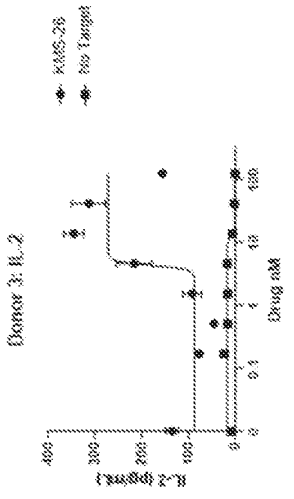
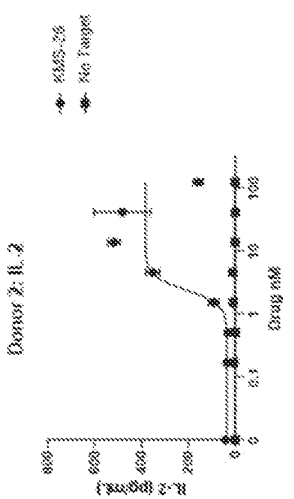
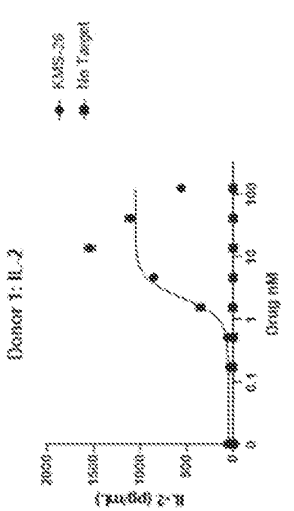
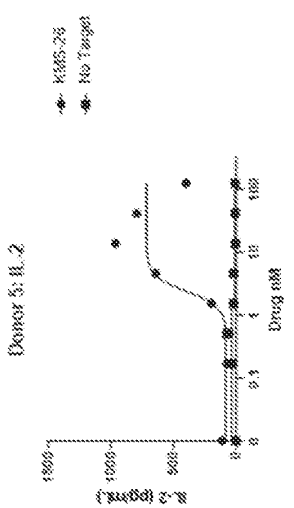


FIG. 13B (continued)









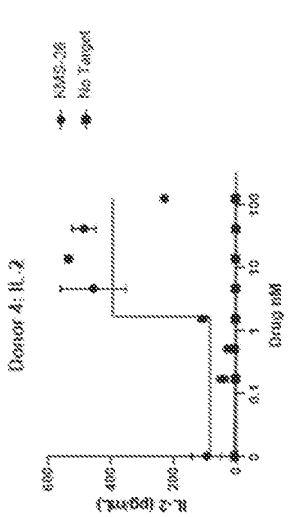


FIG. 13B (continued)

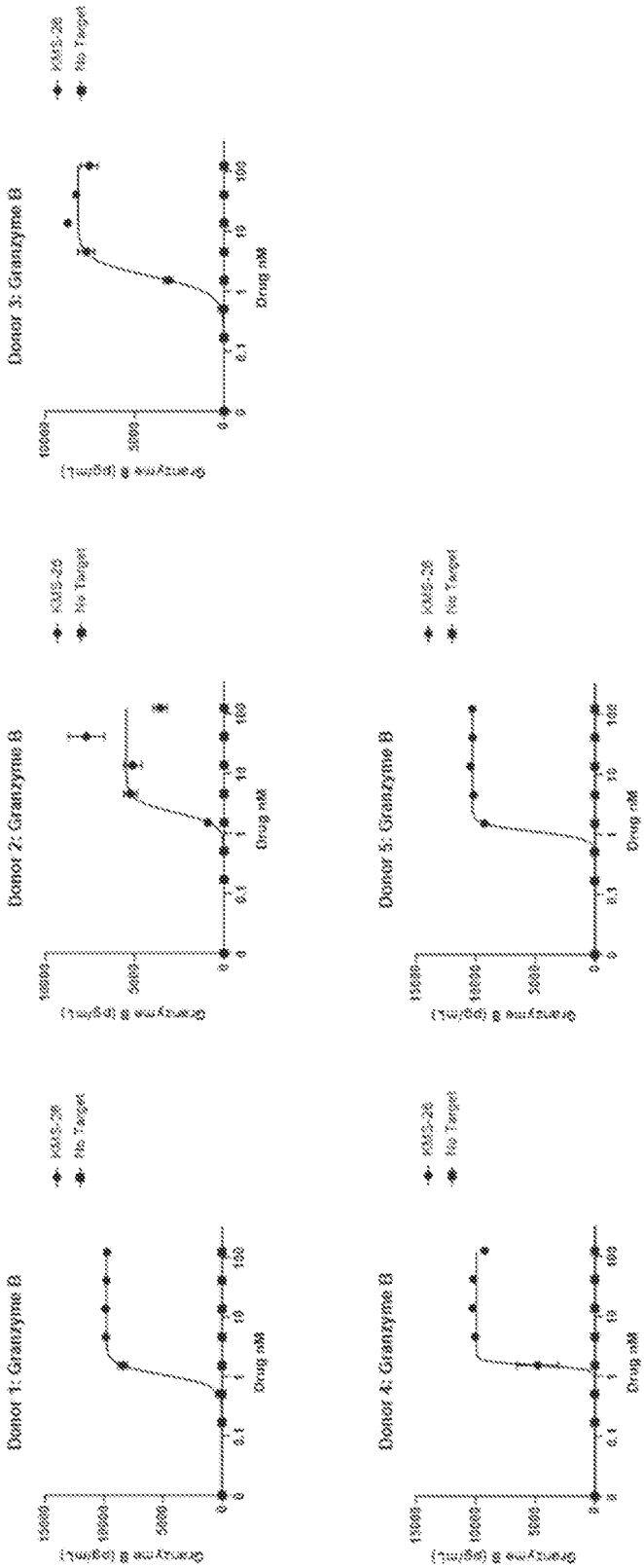


FIG. 13B (continued)

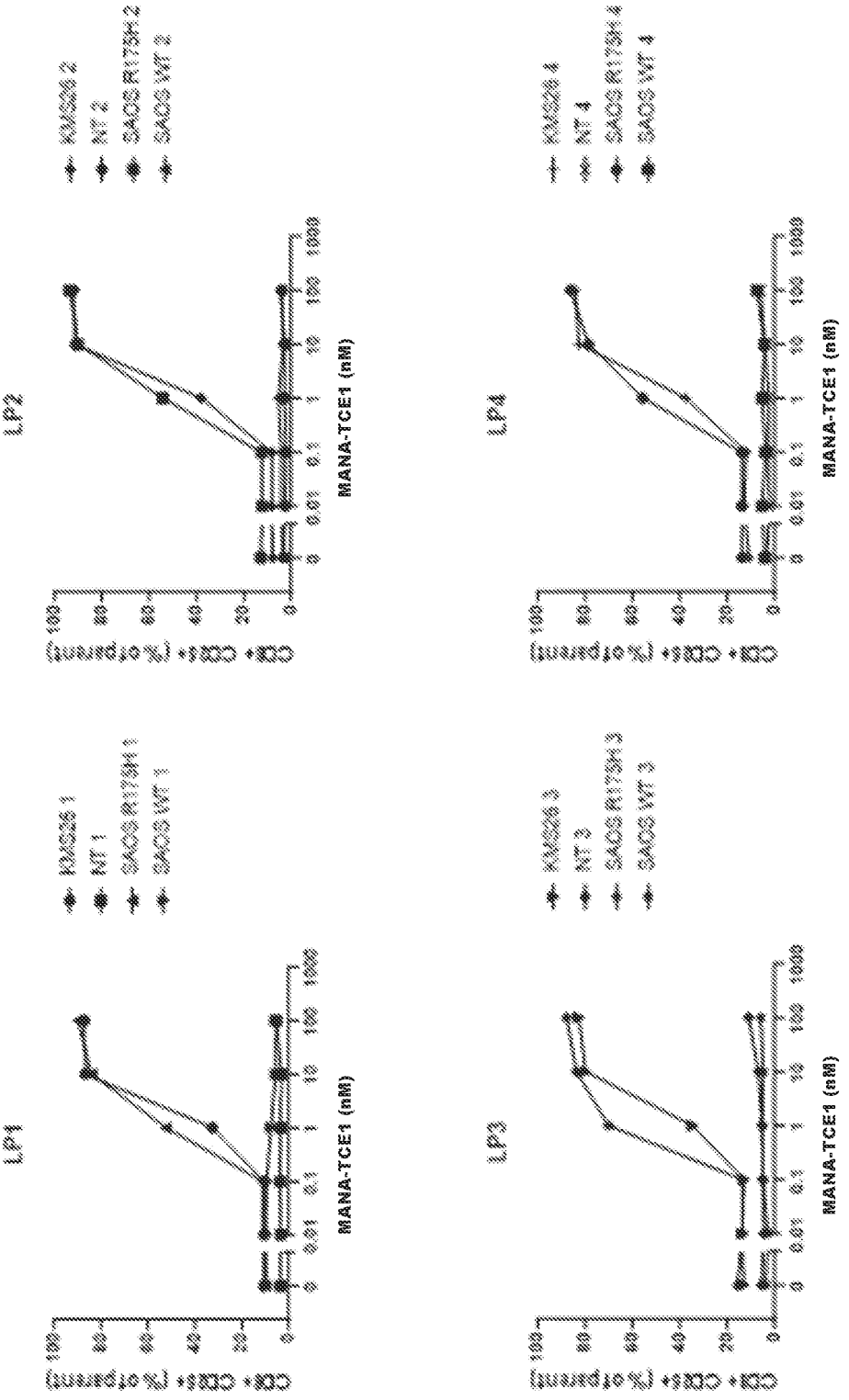

LP1
LP2
LP3
LP4
KMS26 1
NT 1
SAOS R175H 1
SAOS WT 1
KMS26 2
NT 2
SAOS R175H 2
SAOS WT 2
KMS26 3
NT 3
SAOS R175H 3
SAOS WT 3
KMS26 4
NT 4
SAOS R175H 4
SAOS WT 4
CD8+ CD3+ (% of parent)
MANA-TCE1 (nM)
0
0.01
0.1
1
10
100
1000
0
20
40
60
80
100

FIG. 14

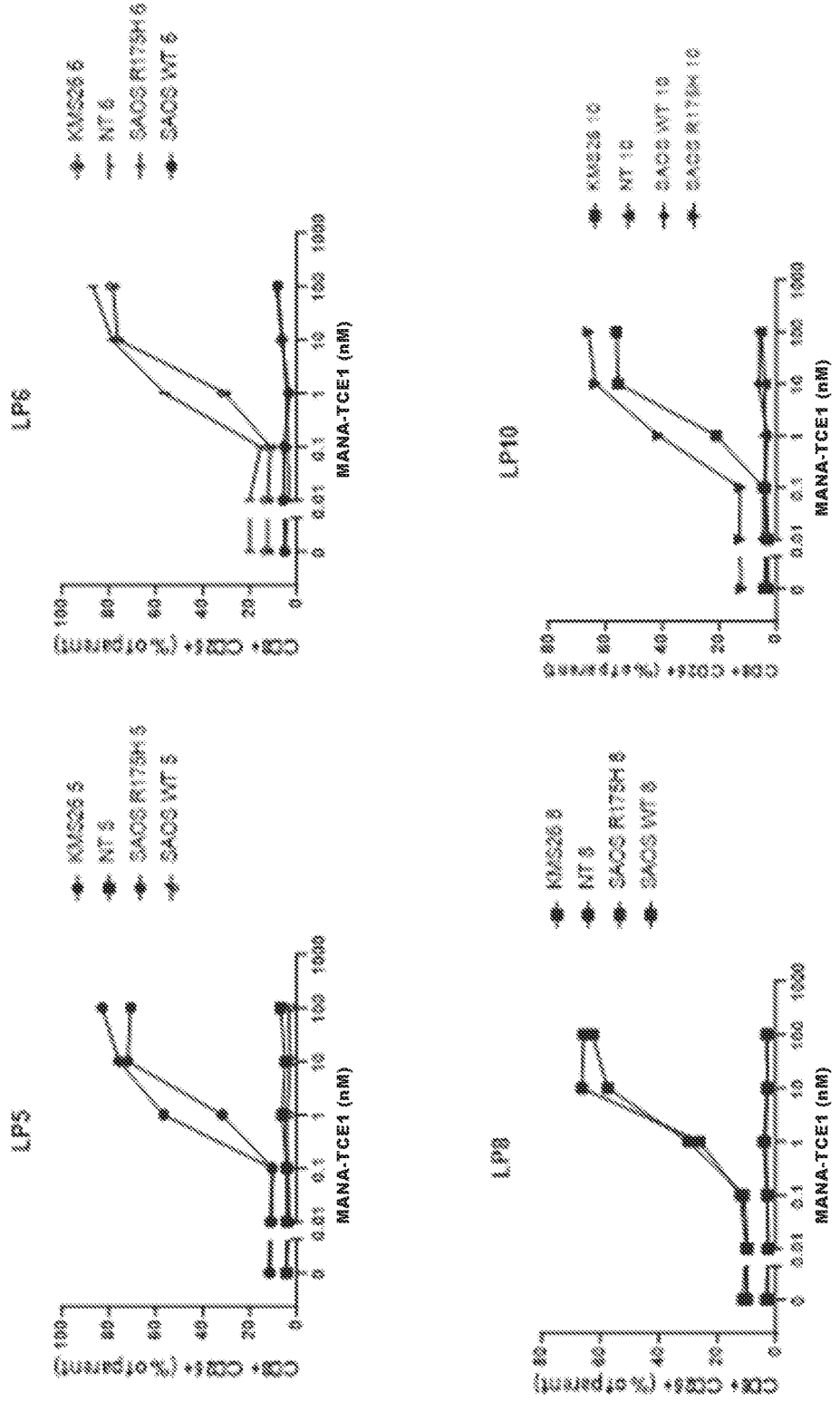


FIG. 14 (continued)

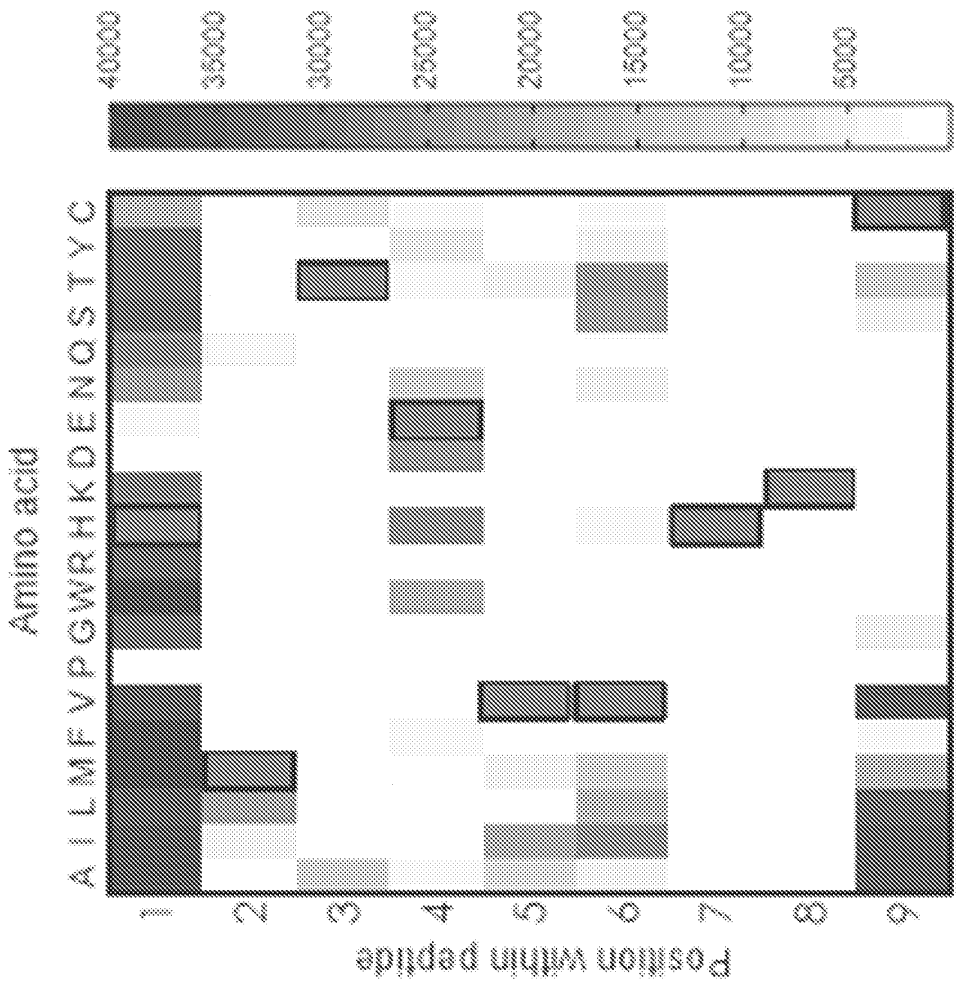

40000
35000
30000
25000
20000
15000
10000
5000
Amino acid
A I L M F V P G W R H K D E N Q S T Y C
1
2
3
4
5
6
7
8
9
Position within peptide

FIG. 15

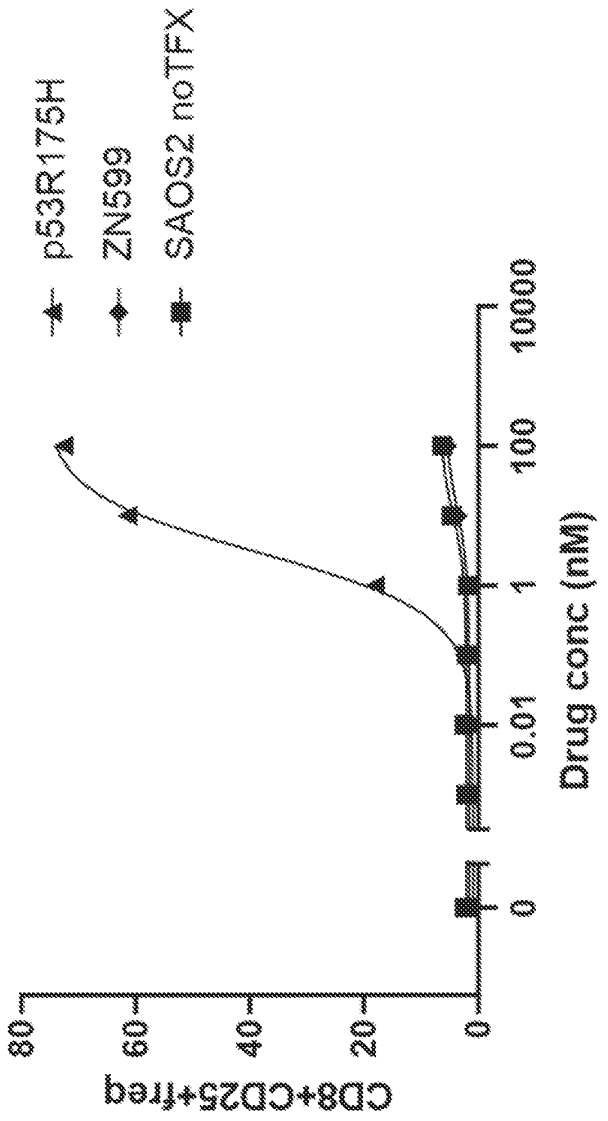

p53R175H
ZN599
SAOS2 noTFX
CD8+CD25+freq
0
20
40
60
80
0
0.01
1
100
10000
Drug conc (nM)

FIG. 16

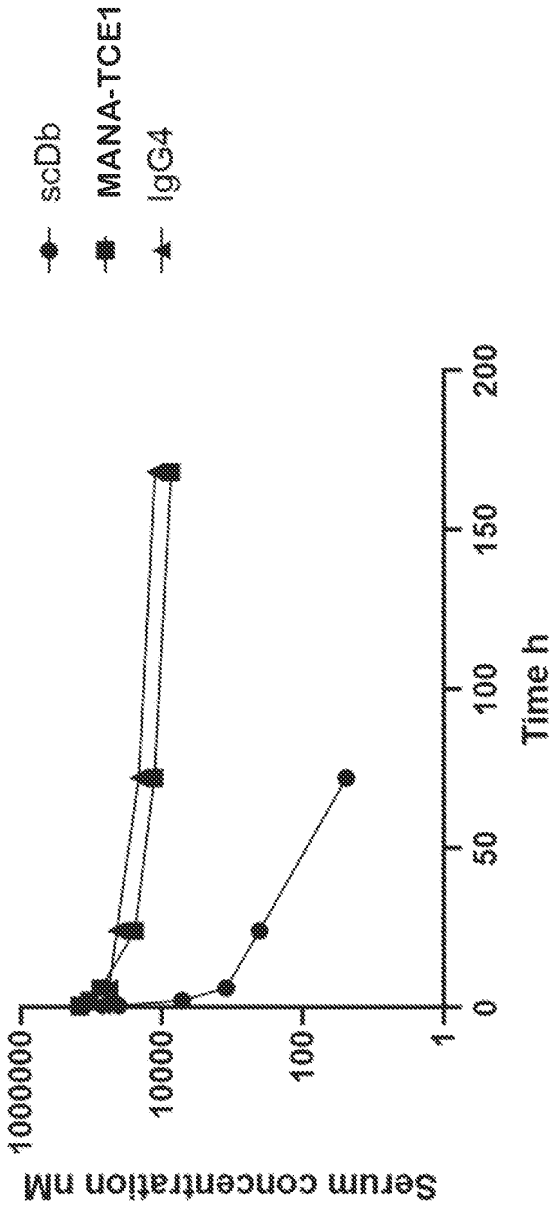

scDb
MANA-TCE1
IgG4
Serum concentration nM
1000000
10000
100
1
0
50
100
150
200
Time h

FIG. 17

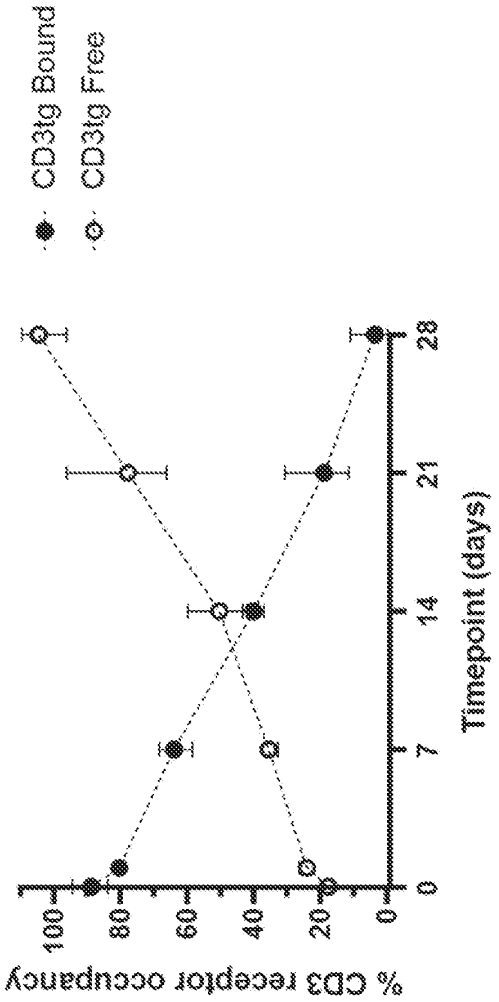

CD3tg Bound
CD3tg Free
% CD3 receptor occupancy
100
80
60
40
20
0
0
7
14
21
28
Timepoint (days)

FIG. 19

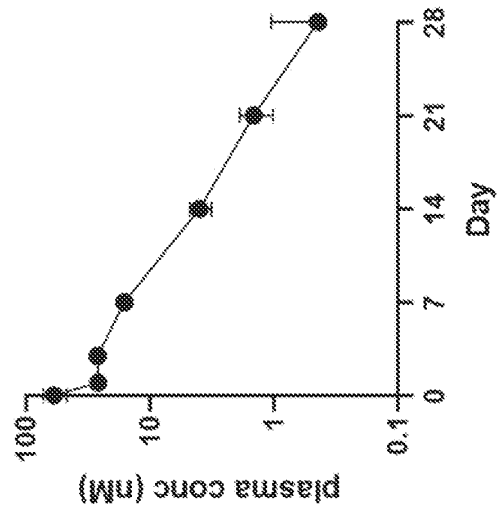

plasma conc (nM)
100
10
1
0.1
0
7
14
21
28
Day

FIG. 18

FIG. 20

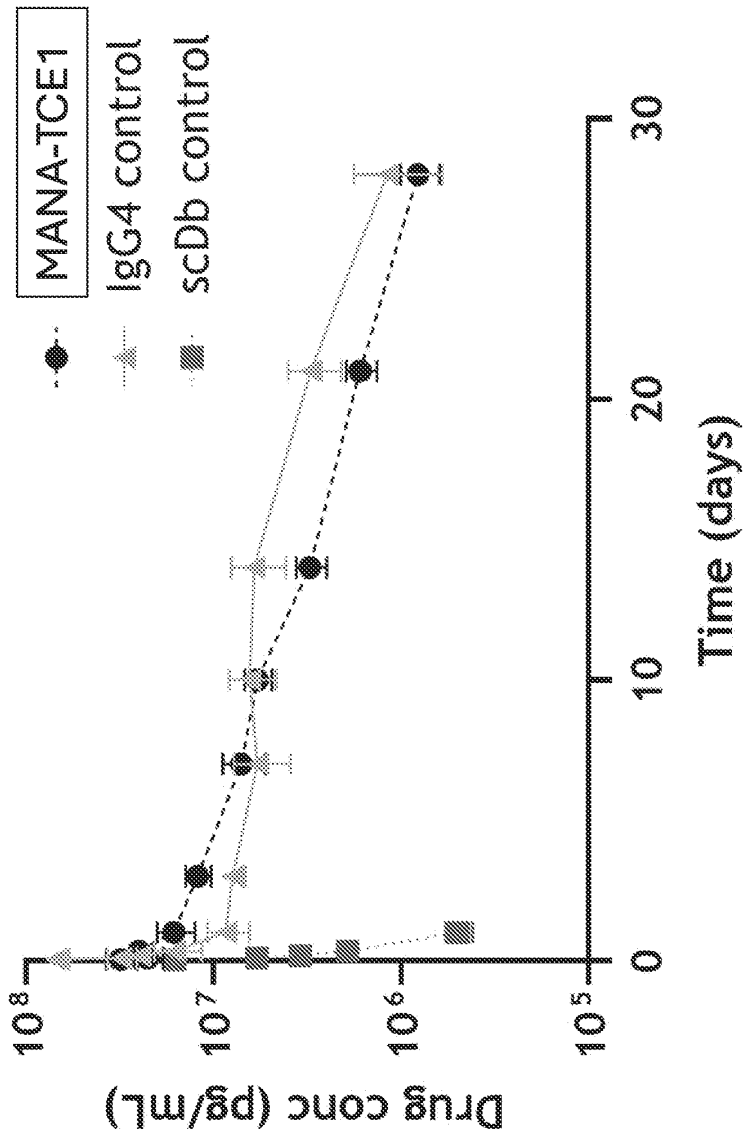

MANA-TCE1
IgG4 control
scDb control
Drug conc (pg/mL)
10^8
10^7
10^6
10^5
0
10
20
30
Time (days)

FIG. 21

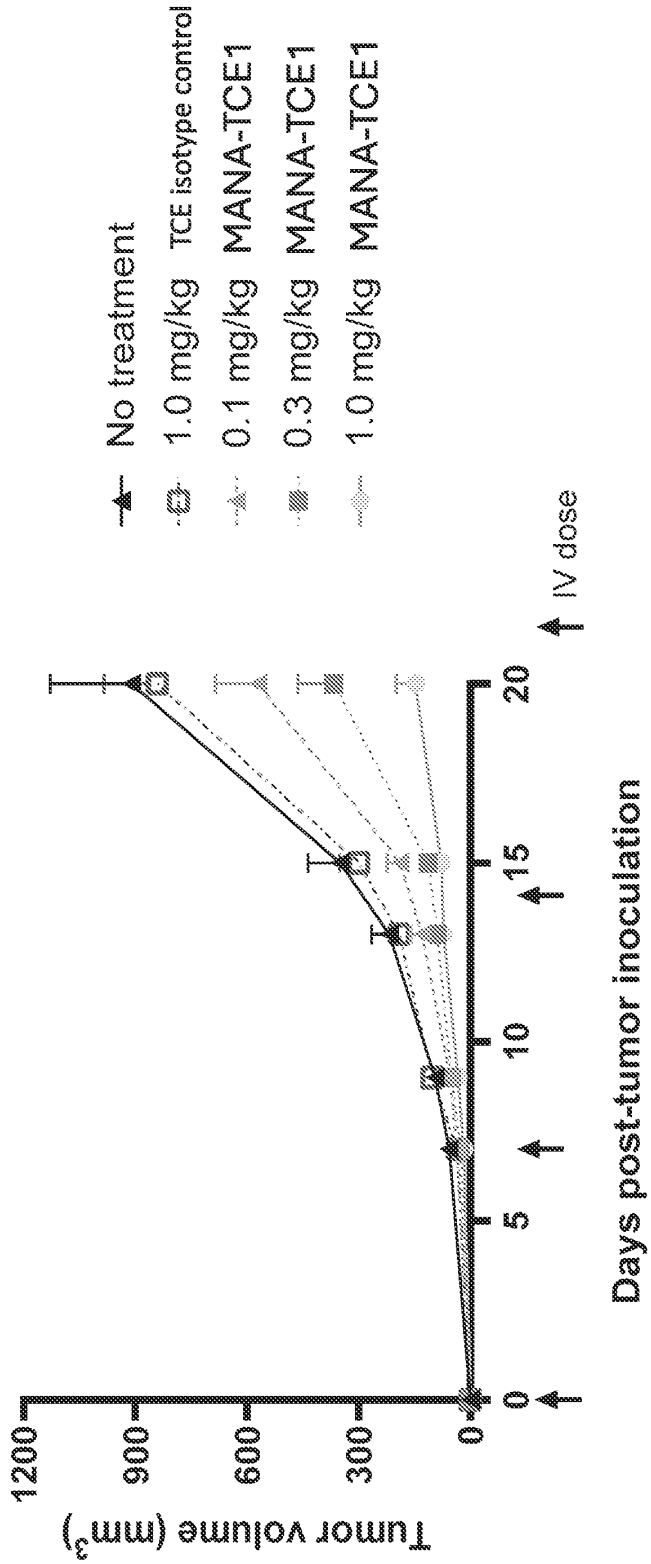
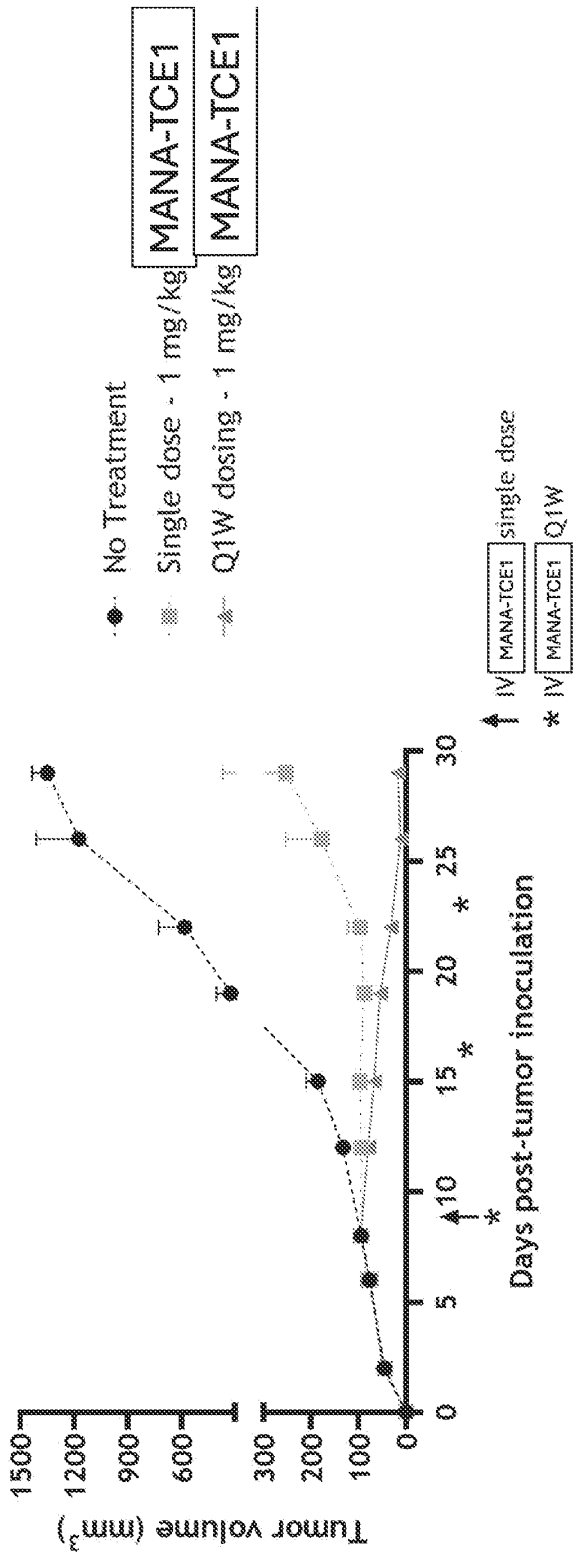
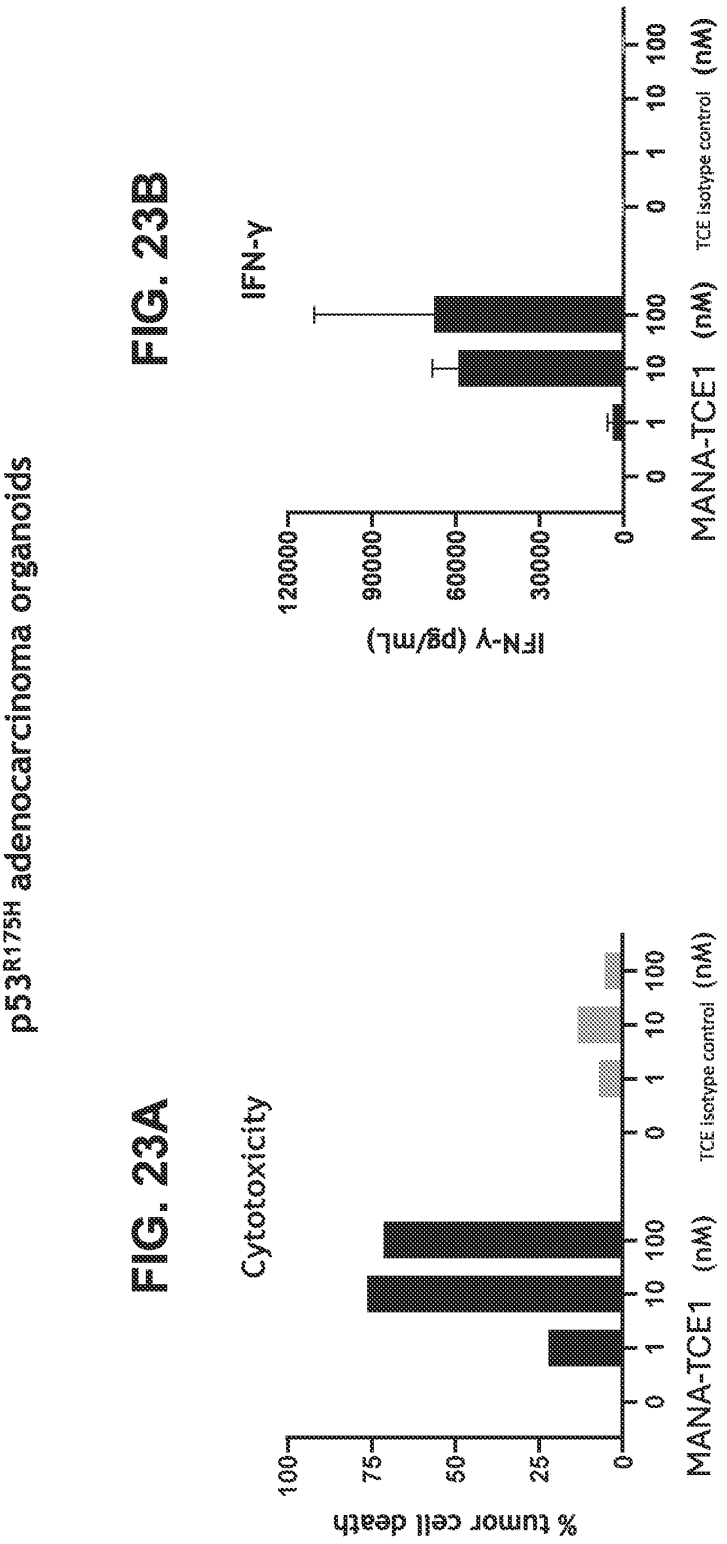

No treatment
1.0 mg/kg TCE isotype control
0.1 mg/kg MANA-TCE1
0.3 mg/kg MANA-TCE1
1.0 mg/kg MANA-TCE1
IV dose
Tumor volume (mm3)
1200
900
600
300
0
0
5
10
15
20
Days post-tumor inoculation

FIG. 22


No Treatment
Single dose - 1 mg/kg MANA-TCE1
Q1W dosing - 1 mg/kg MANA-TCE1
Tumor volume (mm³)
1500
1200
900
600
300
200
100
0
0
5
10
15
20
25
30
Days post-tumor inoculation
IV MANA-TCE1 single dose
IV MANA-TCE1 Q1W


p53R175H adenocarcinoma organoids
FIG. 23A
Cytotoxicity
% tumor cell death
100
75
50
25
0
0 1 10 100 0 1 10 100
MANA-TCE1 (nM) TCE isotype control (nM)
FIG. 23B
IFN-γ
IFN-γ (pg/mL)
120000
90000
60000
30000
0
0 1 10 100 0 1 10 100
MANA-TCE1 (nM) TCE isotype control (nM)

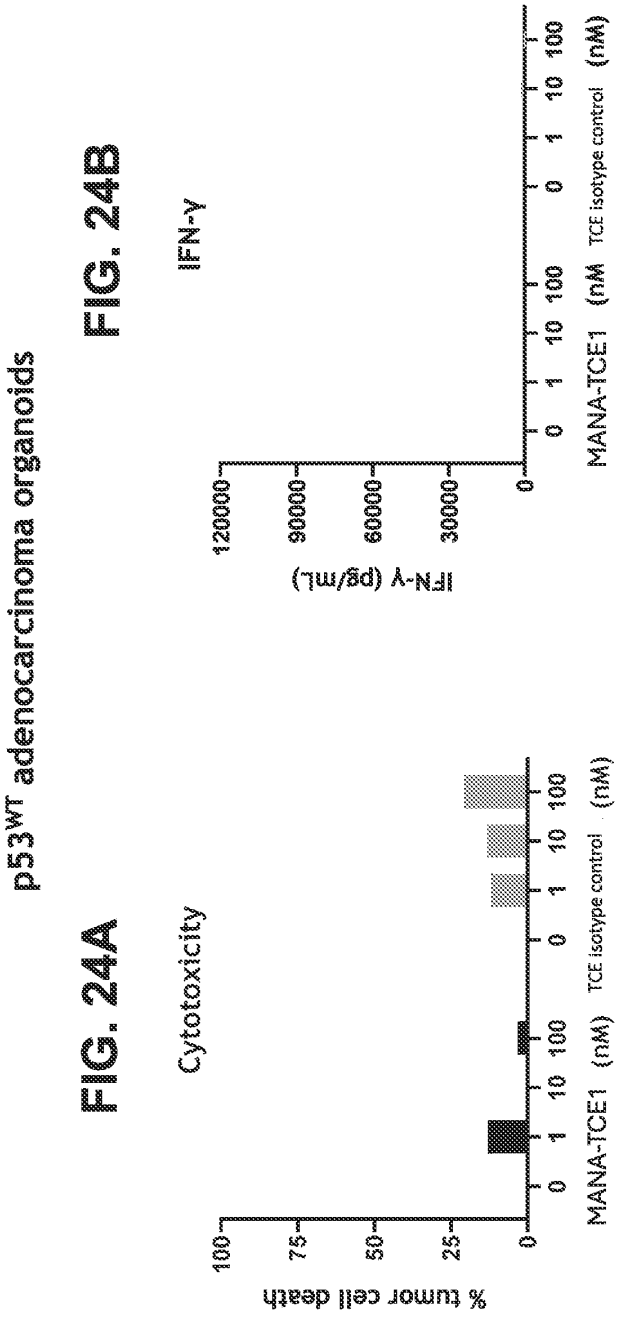
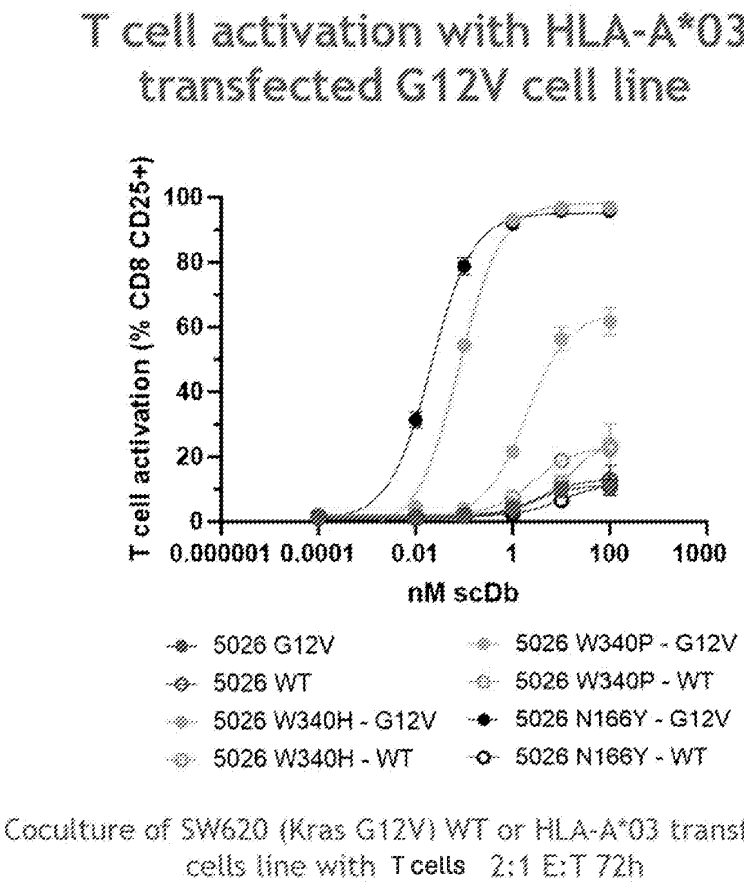


FIG. 24A

FIG. 24B

FIG. 25


T cell activation with HLA-A*03 transfected G12V cell line
T cell activation (% CD8 CD25+)
100
80
60
40
20
0
0.000001 0.0001 0.01 1 100 1000
nM scDb
5026 G12V
5026 WT
5026 W340H - G12V
5026 W340H - WT
5026 W340P - G12V
5026 W340P - WT
5026 N166Y - G12V
5026 N166Y - WT
Coculture of SW620 (Kras G12V) WT or HLA-A*03 transfected cells line with T cells 2:1 E:T 72h