FIG. 1

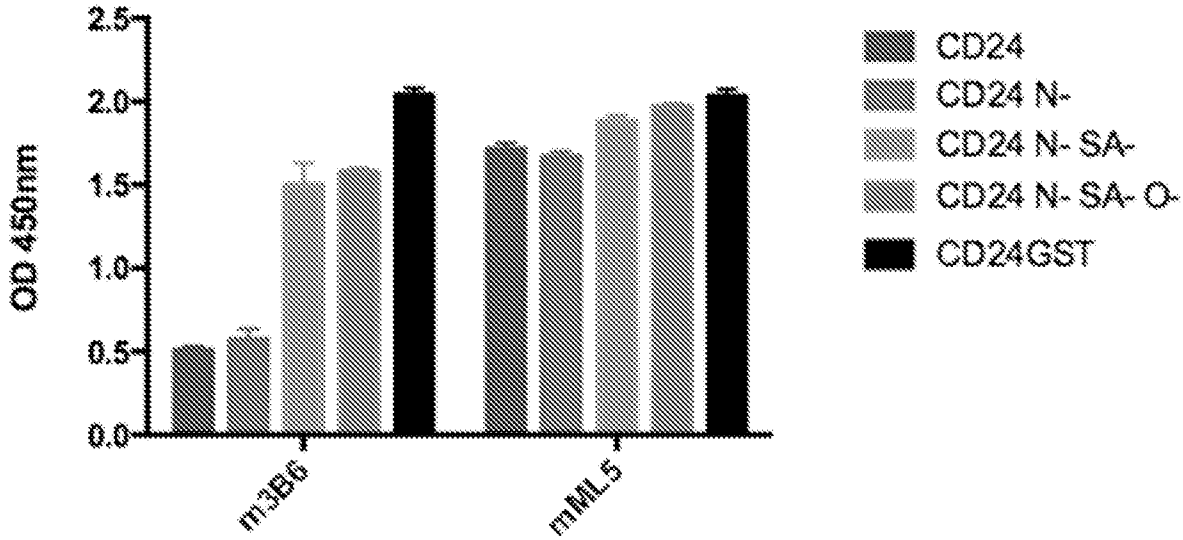

OD 450nm
2.5
2.0
1.5
1.0
0.5
0.0
m3B6
mML5
CD24
CD24 N-
CD24 N- SA-
CD24 N- SA- O-
CD24GST

FIG. 2A

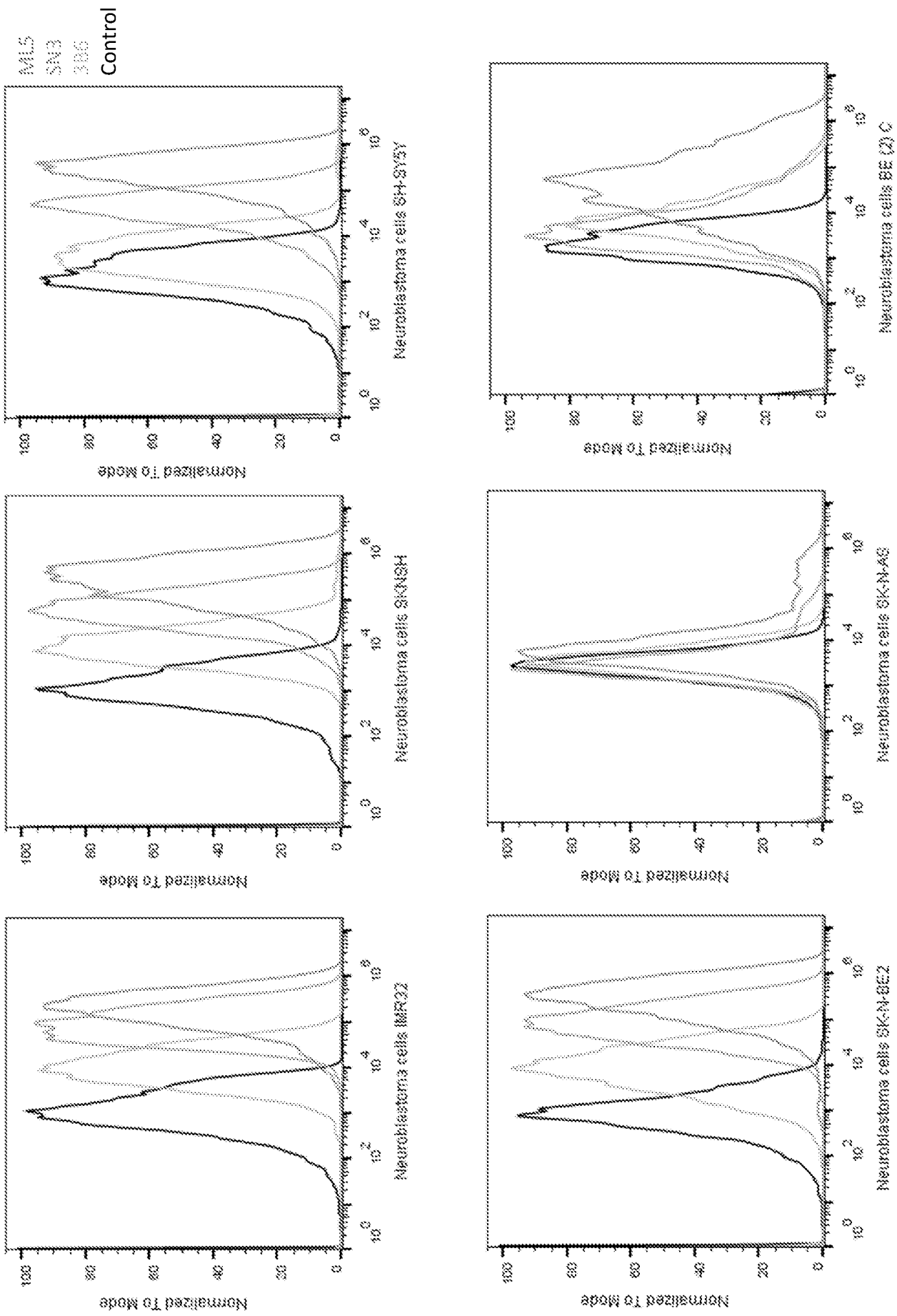

MLS
SN3
3B6
Control
Normalized To Mode
Neuroblastoma cells SH-SY5Y
Neuroblastoma cells SKNSH
Neuroblastoma cells IMR32
Neuroblastoma cells SK-N-AS
Neuroblastoma cells SK-N-BE2
Neuroblastoma cells BE (2) C

FIG. 2B

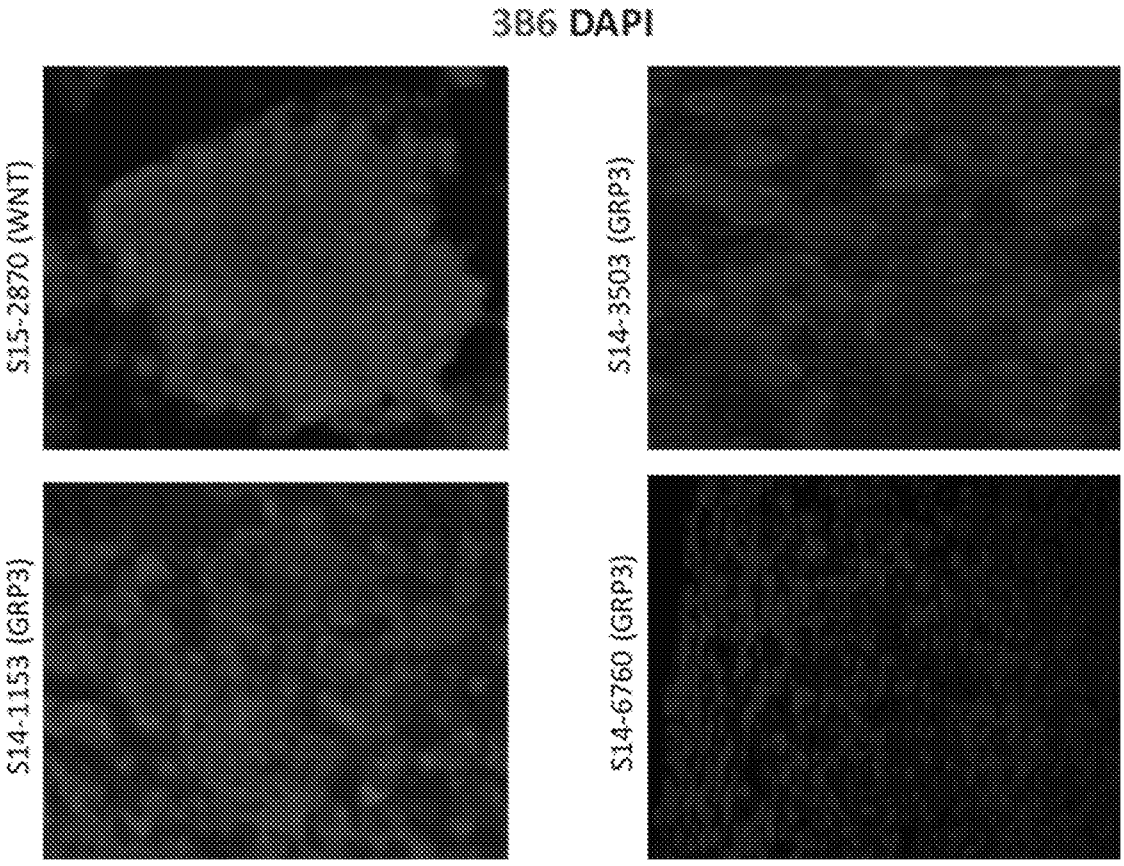
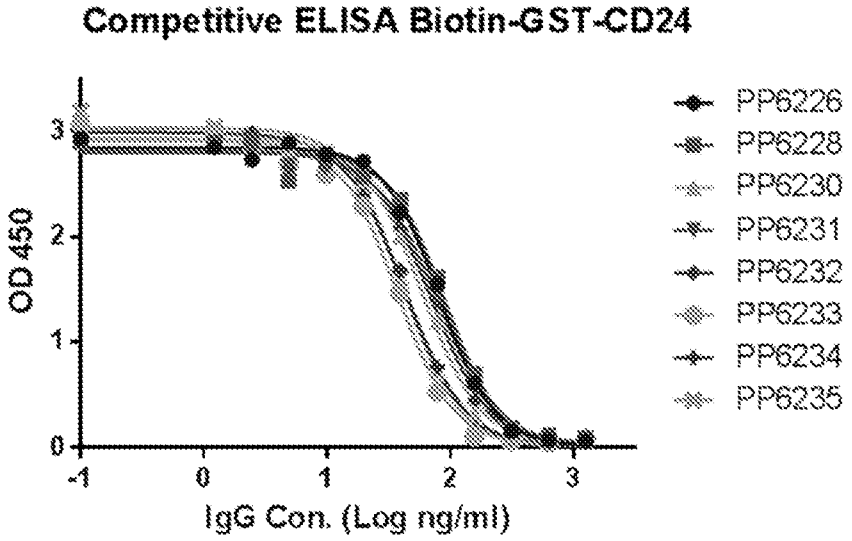
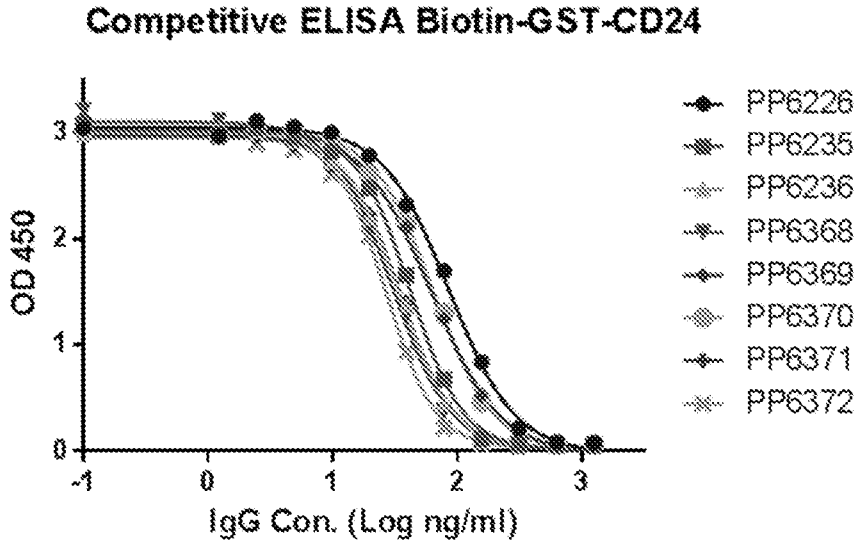

386 DAPI
S15-2870 (WNT)
S14-3503 (GRP3)
S14-1153 (GRP3)
S14-6760 (GRP3)

FIG. 3



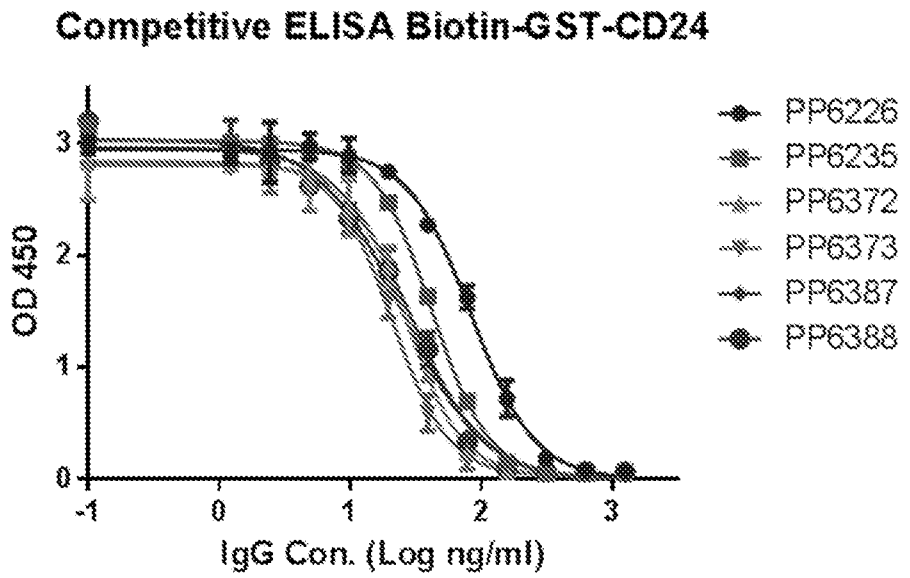
	PP6226	PP6228	PP6230	PP6231	PP6232	PP6233	PP6234	PP6235
IC50 (ng/ml)	81.38	88.82	58.94	76.77	71.25	37.24	42.61	41.09

FIG. 4



	PP6226	PP6235	PP6236	PP6368	PP6369	PP6370	PP6371	PP6372
IC50 (ng/ml)	85.64	41.87	33.63	29.85	61.12	64.23	34.52	26.89

FIG. 5



	PP6226	PP6235	PP6372	PP6373	PP6387	PP6388
IC50 (ng/ml)	83.39	41.48	26.83	20.68	28.43	24.86

FIG. 6

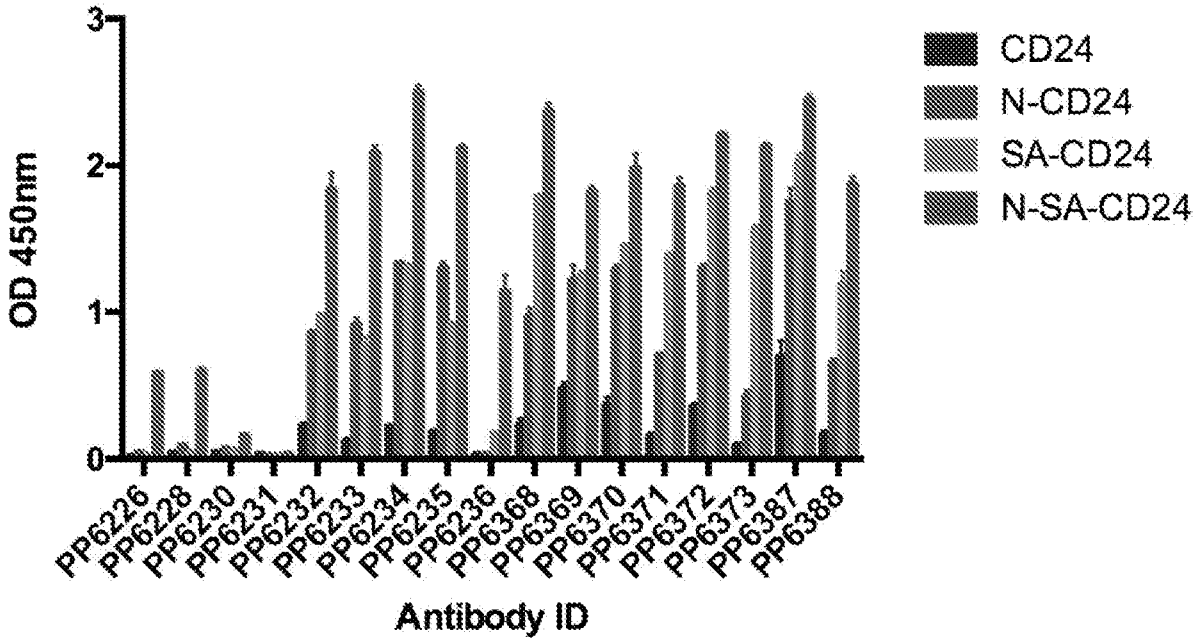

OD 450nm
3
2
1
0
PP6226
PP6228
PP6230
PP6231
PP6232
PP6233
PP6234
PP6235
PP6236
PP6368
PP6369
PP6370
PP6371
PP6372
PP6373
PP6387
PP6388
Antibody ID
CD24
N-CD24
SA-CD24
N-SA-CD24

FIG. 7

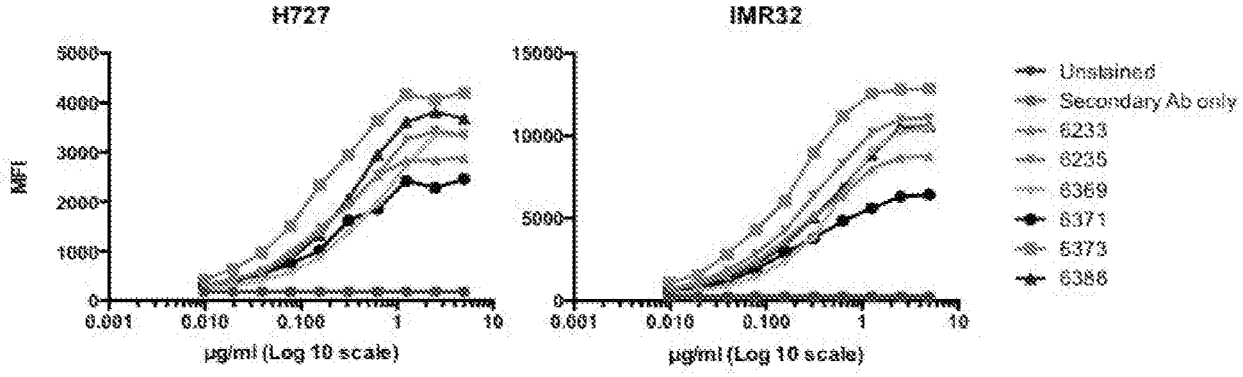

H727
IMR32
MFI
5000
4000
3000
2000
1000
0
15000
10000
5000
0.001
0.010
0.100
1
10
µg/ml (Log 10 scale)
Unstained
Secondary Ab only
6233
6235
6369
6371
6373
6386

FIG. 8

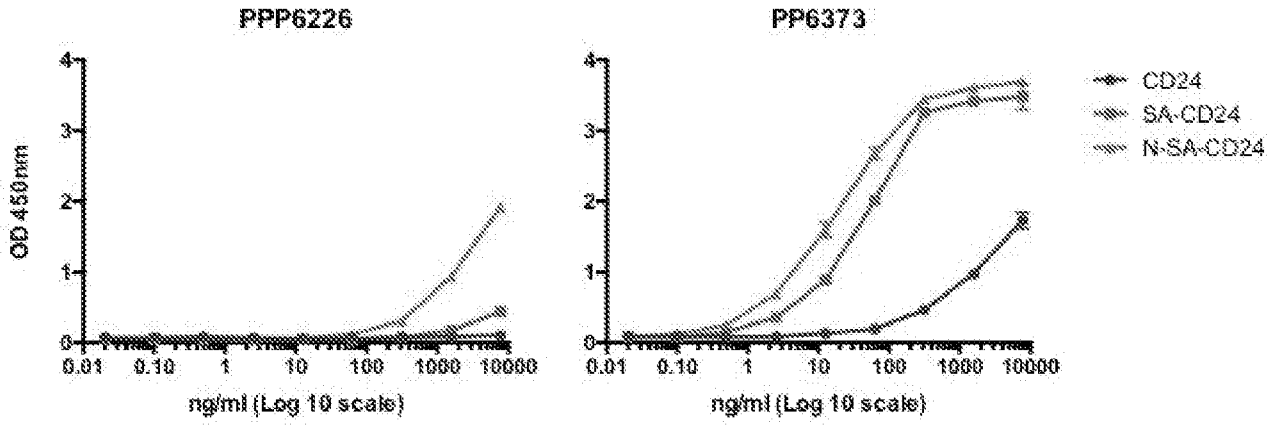

PPP6226
PP6373
OD 450nm
0
1
2
3
4
0.01
0.10
1
10
100
1000
10000
ng/ml (Log 10 scale)
CD24
SA-CD24
N-SA-CD24

FIG. 9

Mapping 3B6 binding site through peptide inhibition

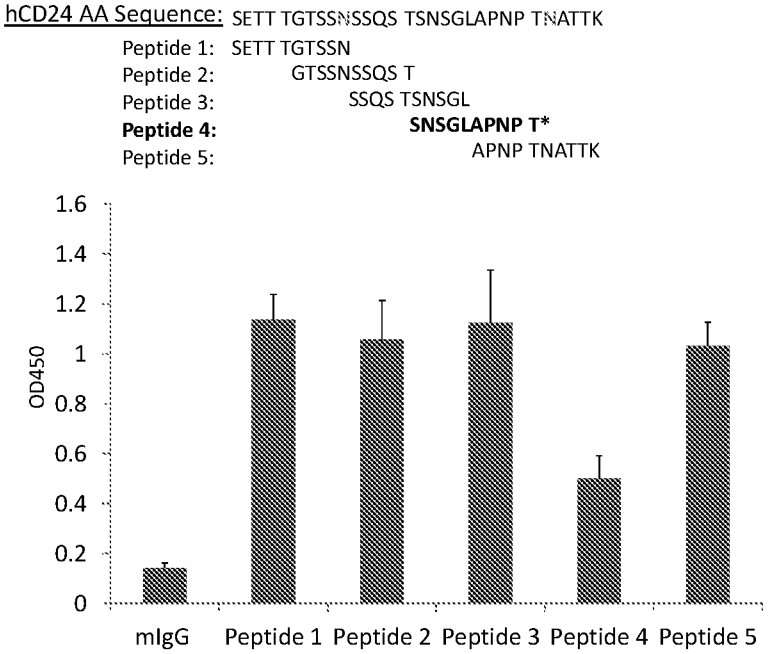
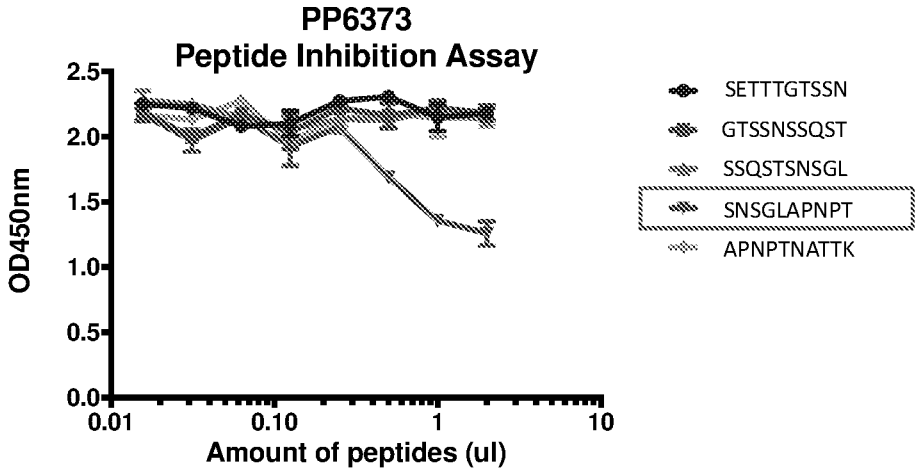


FIG. 10


PP6373
Peptide Inhibition Assay
OD450nm
2.5
2.0
1.5
1.0
0.5
0.0
0.01
0.10
1
10
Amount of peptides (ul)
SETTTGTSSN
GTSSNSSQST
SSQSTSNSGL
SNSGLAPNPT
APNPTNATTK

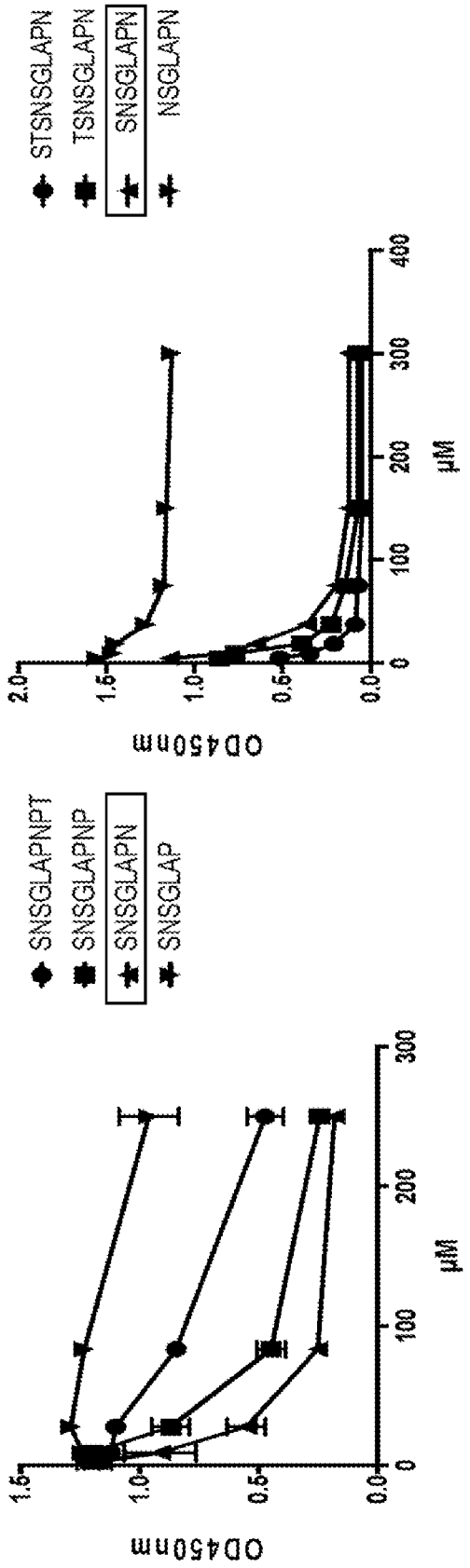

SNSGLAPNPT
SNSGLAPNP
SNSGLAPN
SNSGLAP
OD450nm
1.5
1.0
0.5
0.0
0
100
200
300
μM
STSNSGLAPN
TSNSGLAPN
SNSGLAPN
NSGLAPN
OD450nm
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
μM

FIG. 11

FIG. 12

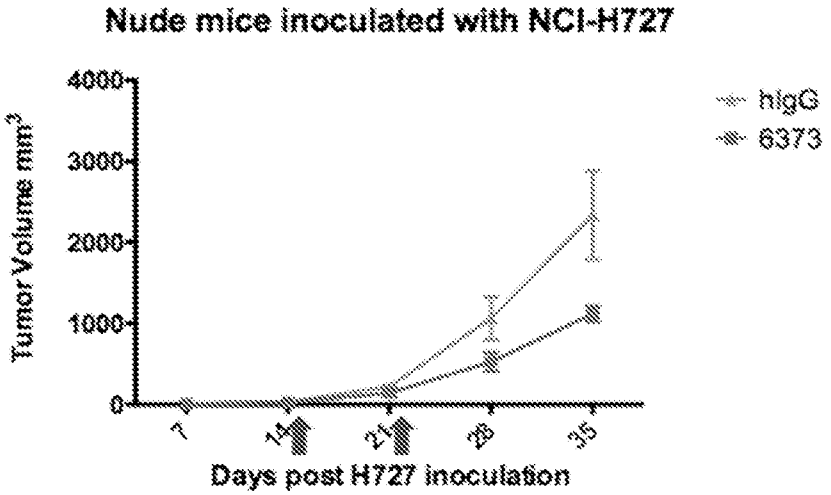

Nude mice inoculated with NCI-H727
Tumor Volume mm3
4000
3000
2000
1000
0
7
14
21
28
35
Days post H727 inoculation
hIgG
6373

FIG. 13

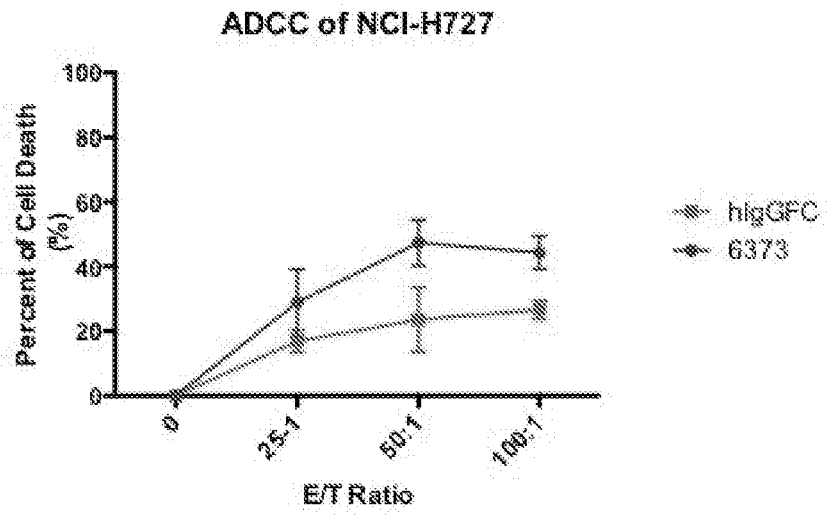

ADCC of NCI-H727
Percent of Cell Death (%)
100
80
60
40
20
0
0
25:1
50:1
100:1
E/T Ratio
hIgGFC
6373

FIG. 14

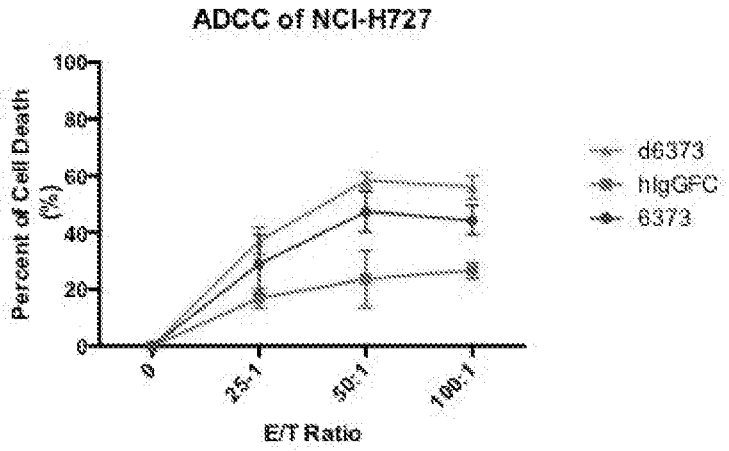
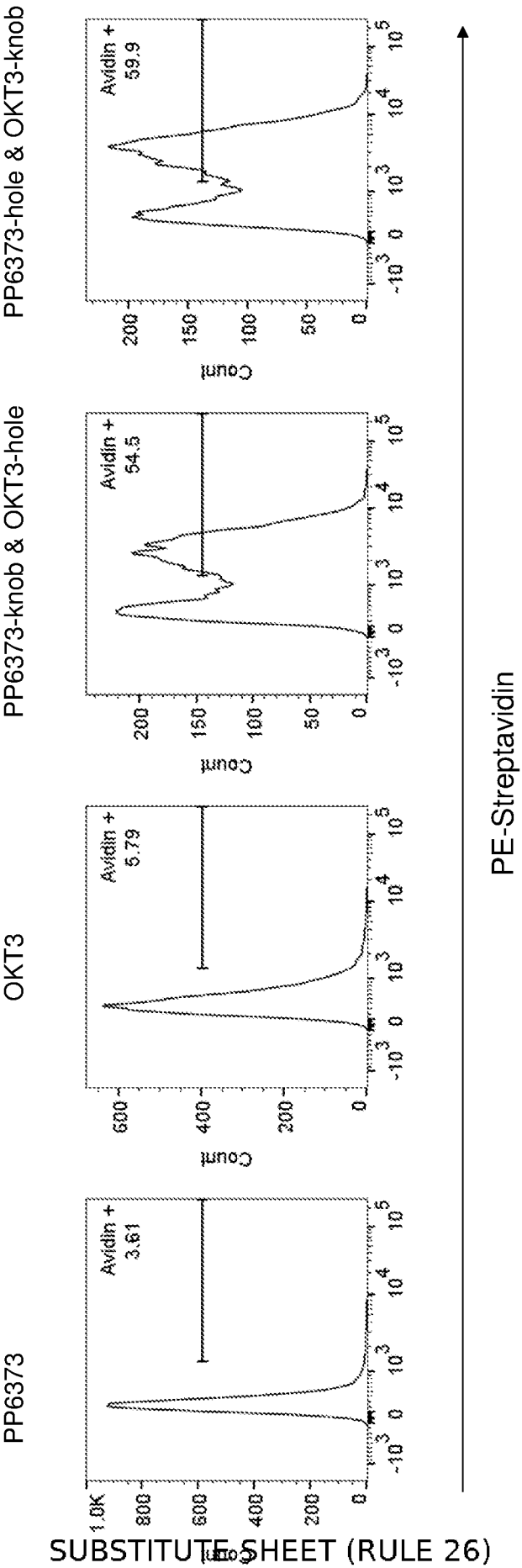

ADCC of NCI-H727
Percent of Cell Death (%)
100
80
60
40
20
0
0
25:1
50:1
100:1
E/T Ratio
d6373
hIgGFC
6373

FIG. 15


PP6373
OKT3
PP6373-knob & OKT3-hole
PP6373-hole & OKT3-knob
Avidin + 3.61
Avidin + 5.79
Avidin + 54.5
Avidin + 59.9
Count
PE-Streptavidin

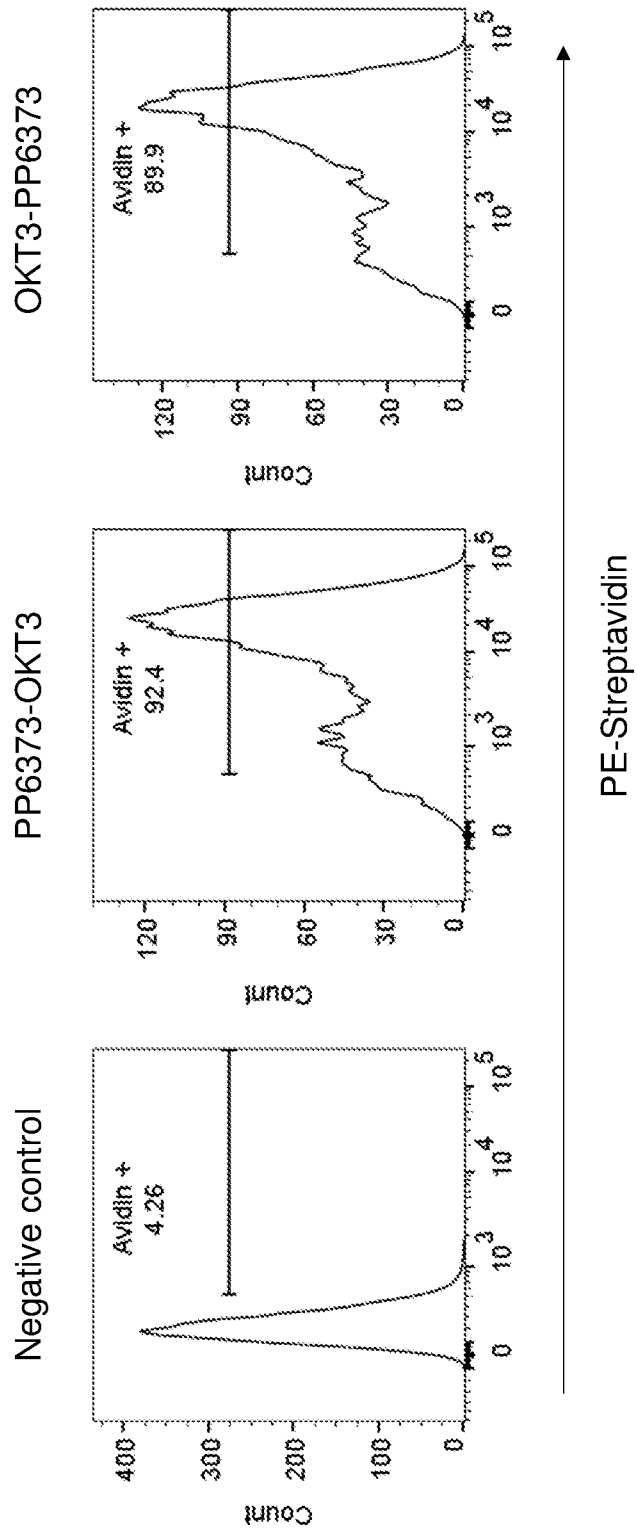

Negative control
PP6373-OKT3
OKT3-PP6373
Avidin +
4.26
Avidin +
92.4
Avidin +
89.9
Count
PE-Streptavidin

FIG. 16

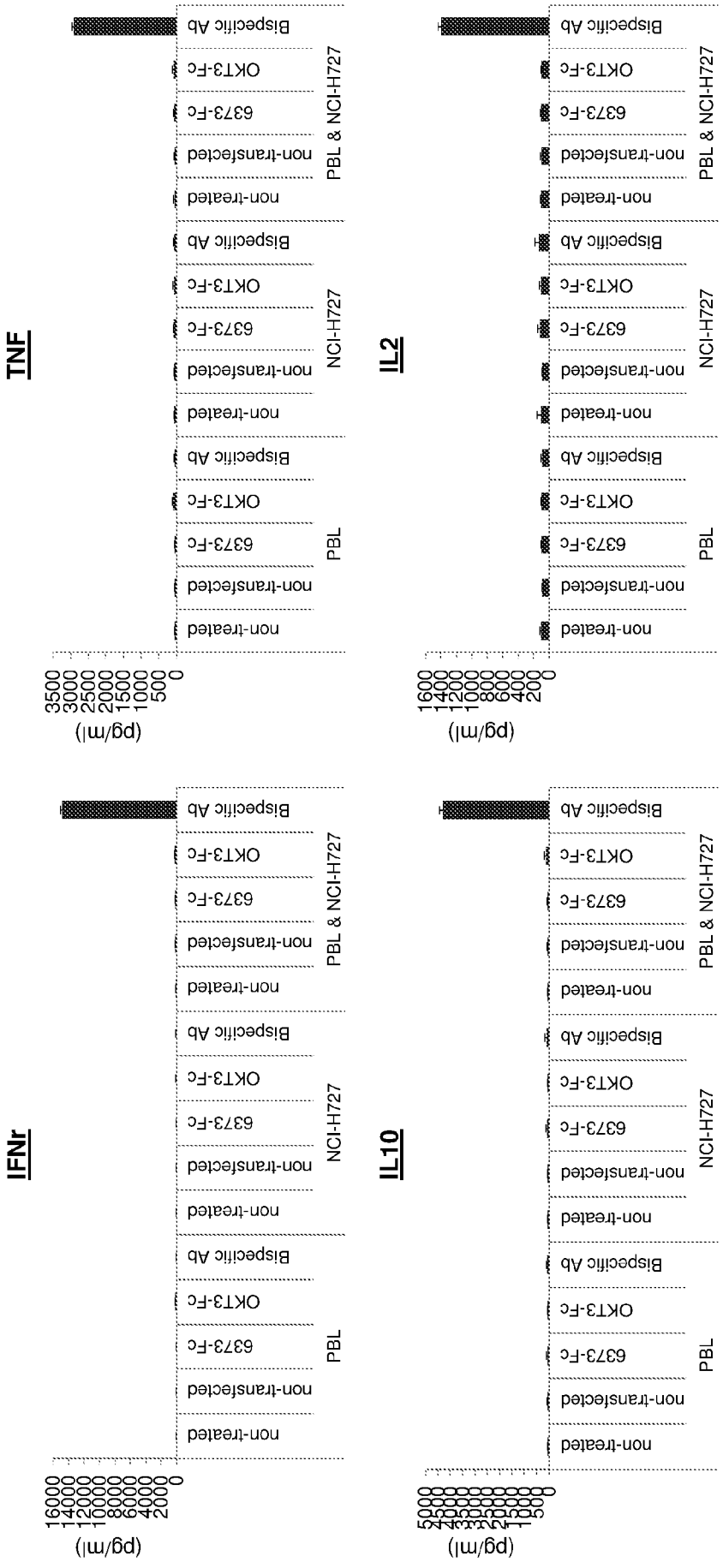

TNF
IL2
IFNr
IL10
(pg/ml)
non-treated
non-transfected
6373-Fc
OKT3-Fc
Bispecific Ab
PBL
PBL & NCI-H727
NCI-H727

FIG. 17

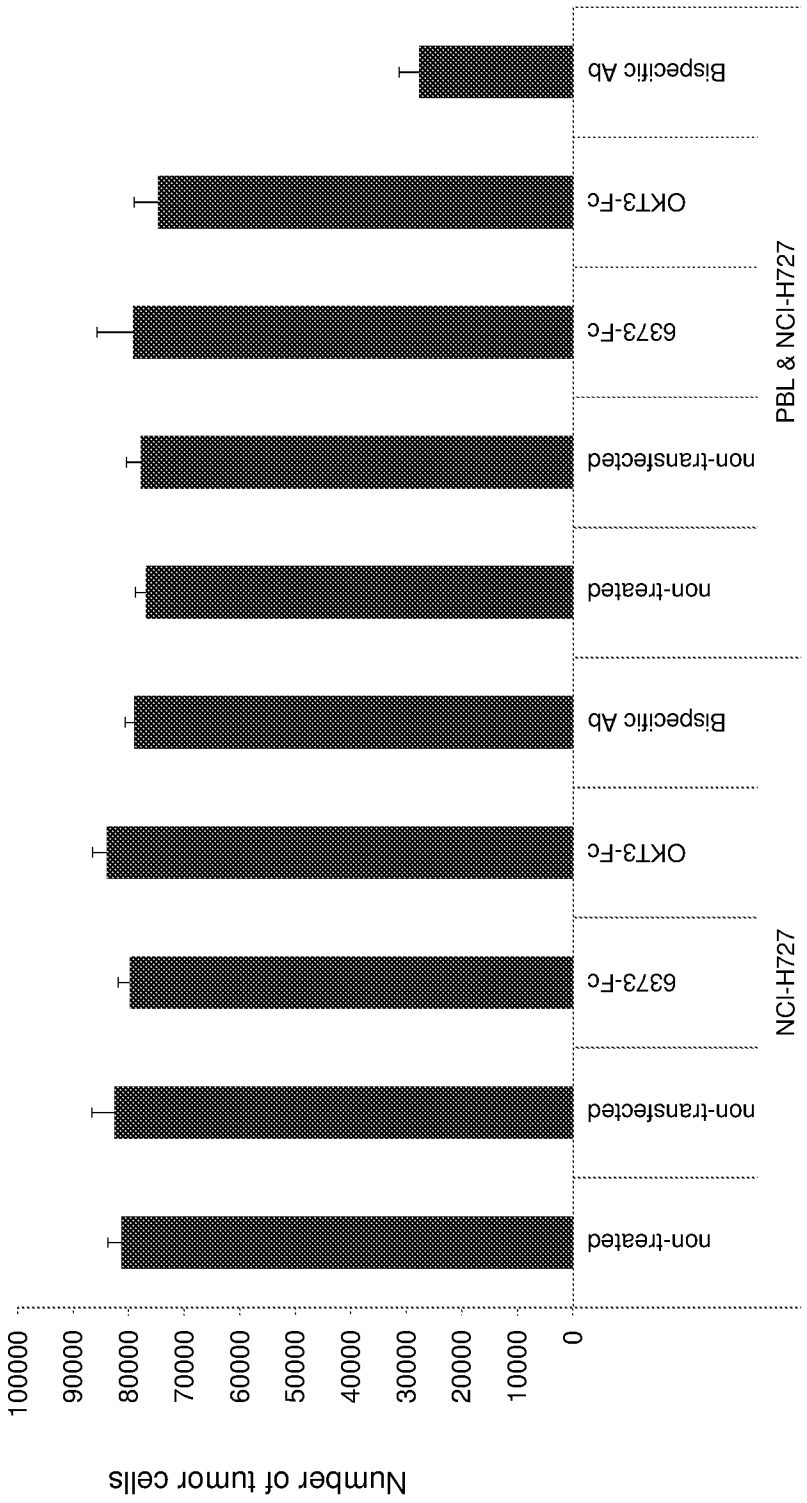
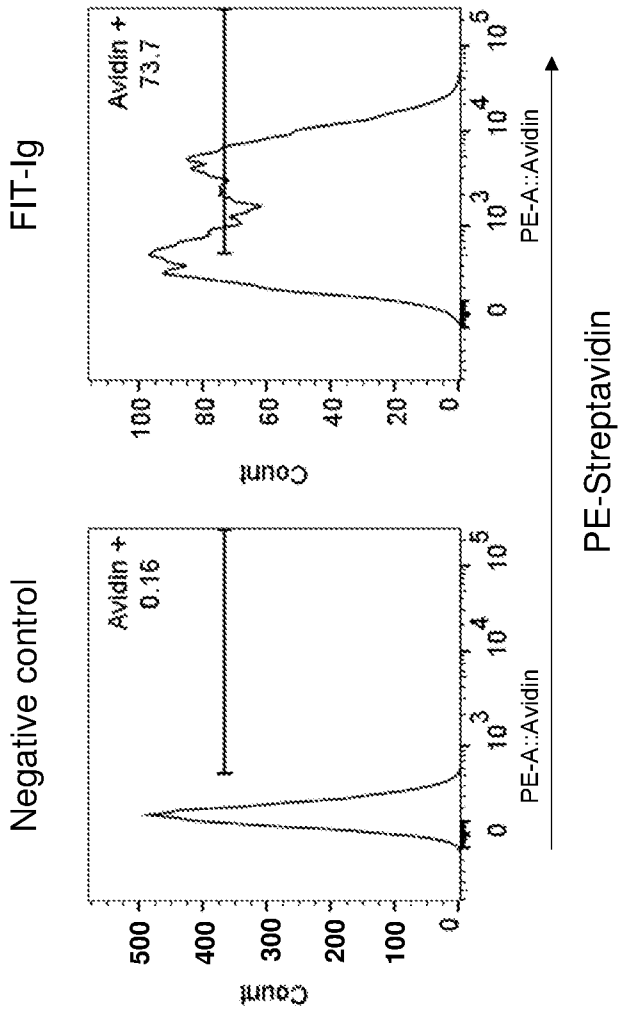

Number of tumor cells
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
non-treated
non-transfected
6373-Fc
OKT3-Fc
Bispecific Ab
NCI-H727
non-treated
Bispecific Ab
OKT3-Fc
6373-Fc
non-transfected
non-treated
Bispecific Ab
PBL & NCI-H727

FIG. 18

FIG. 19


FIT-Ig
Negative control
Avidin +
0.16
Avidin +
73.7
Count
PE-A::Avidin
PE-Streptavidin

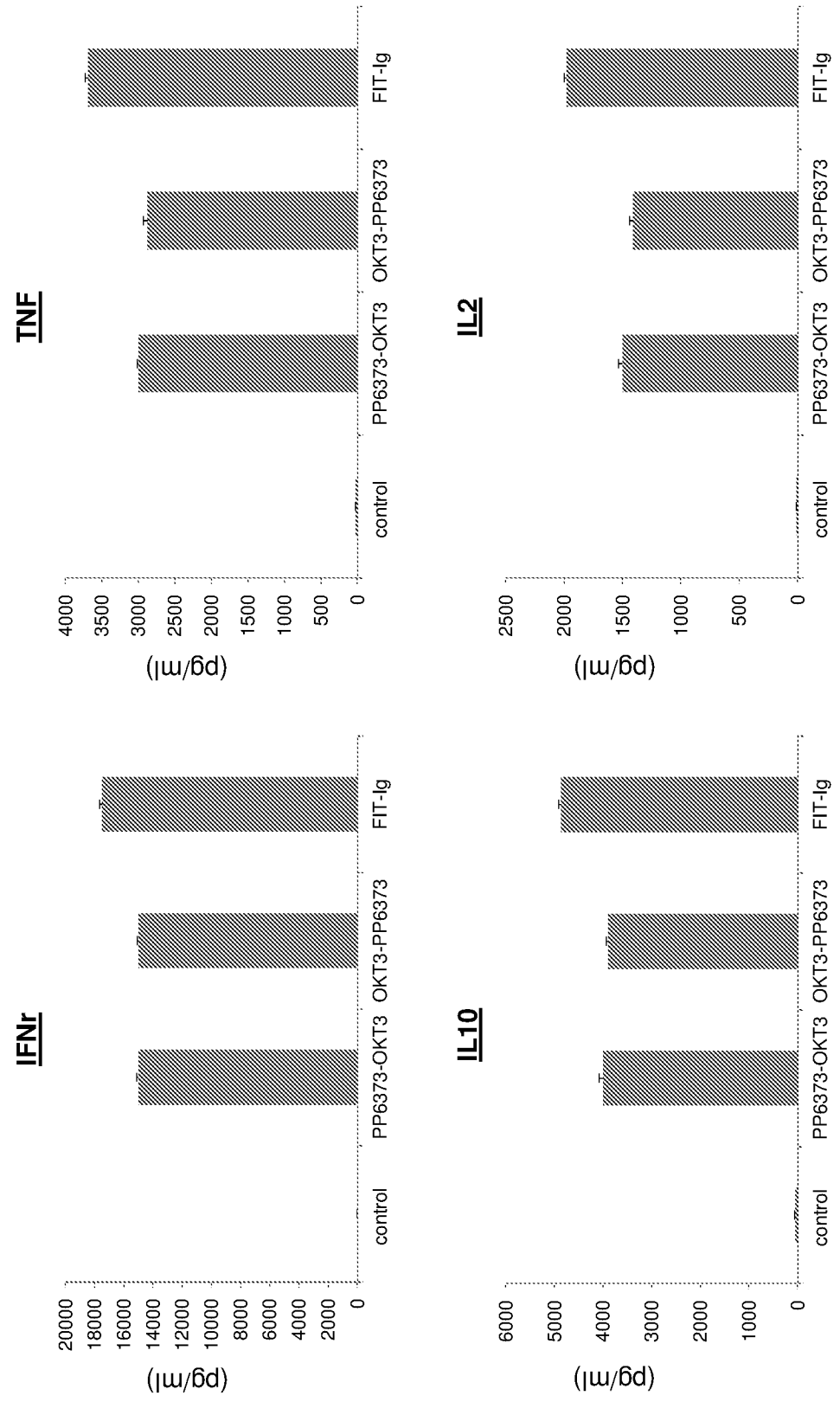

TNF
IL2
IFNr
IL10
(pg/ml)
control
PP6373-OKT3
OKT3-PP6373
FIT-Ig

FIG. 20

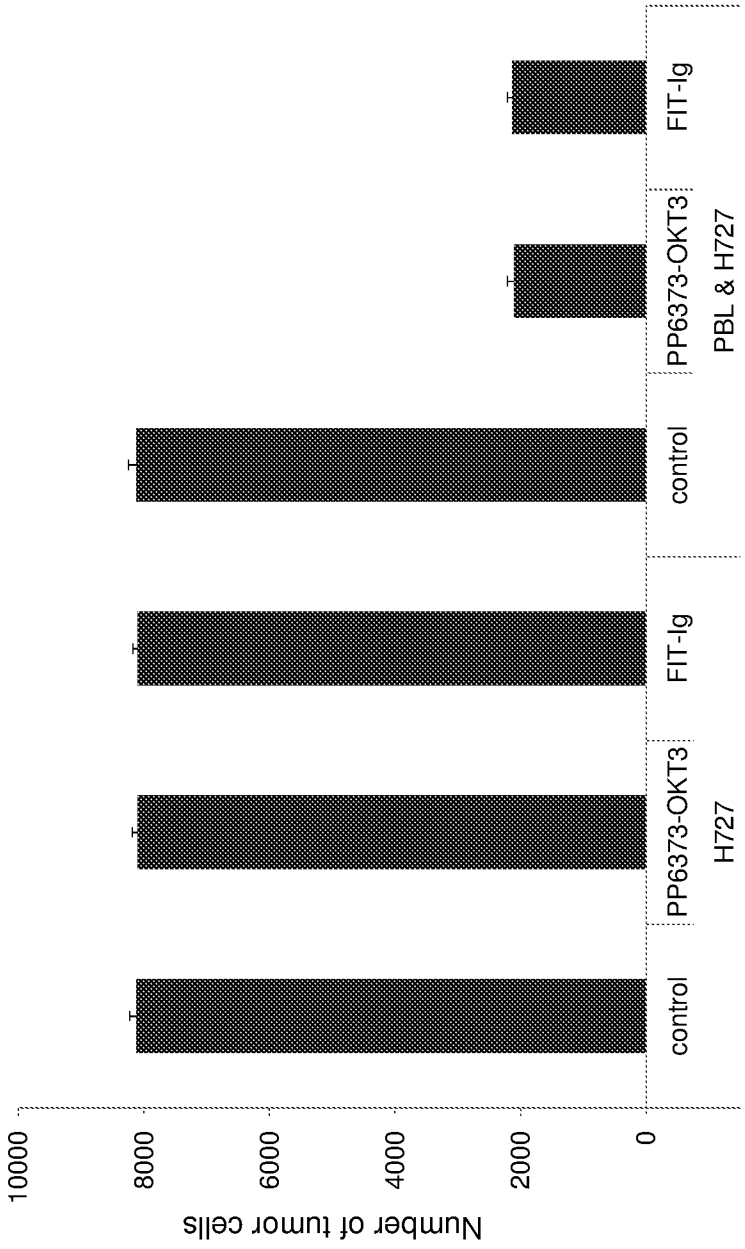

Number of tumor cells
10000
8000
6000
4000
2000
0
control
PP6373-OKT3
FIT-Ig
control
PP6373-OKT3
FIT-Ig
H727
PBL & H727

FIG. 21

FIG. 22

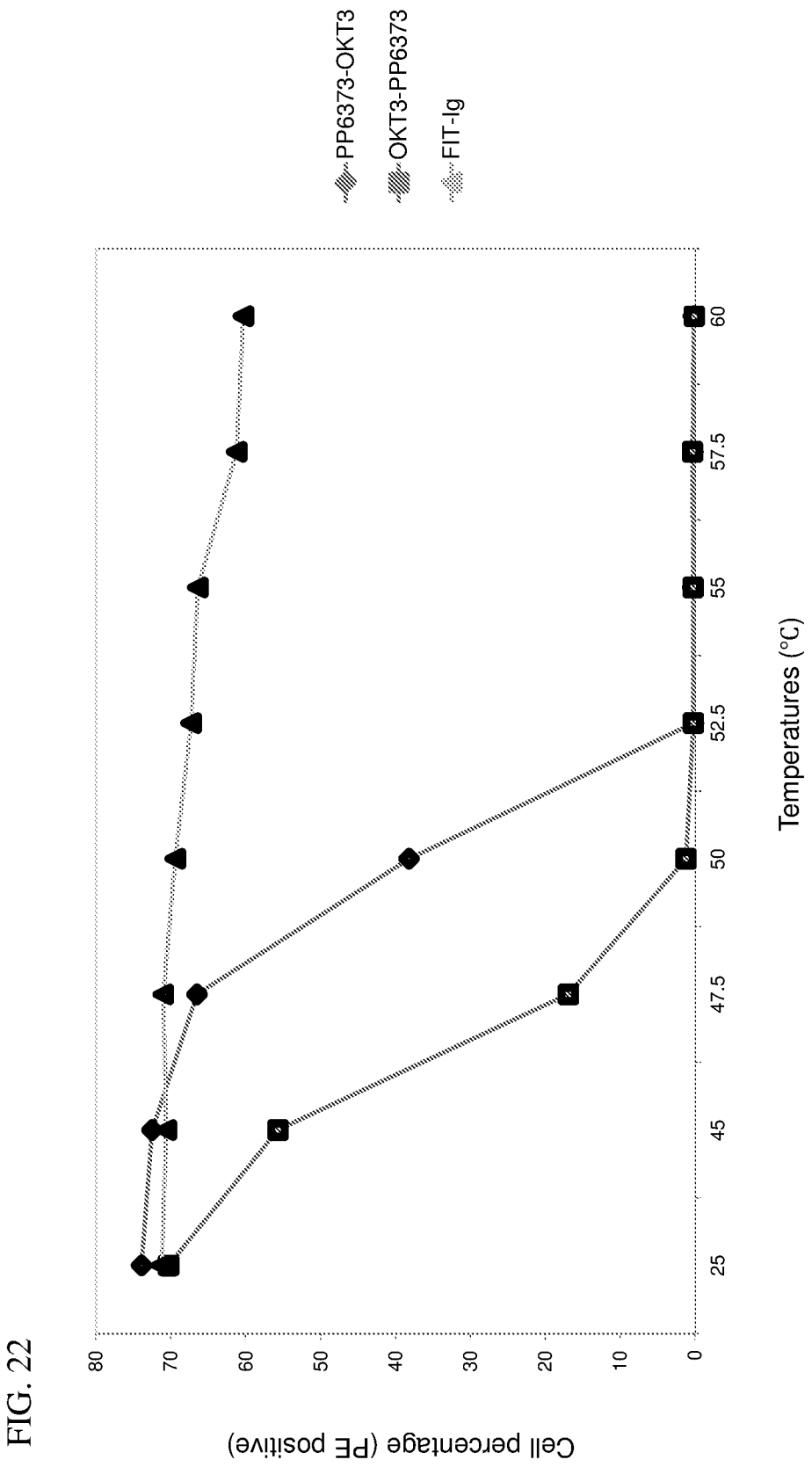

PP6373-OKT3
OKT3-PP6373
FIT-Ig
Cell percentage (PE positive)
0
10
20
30
40
50
60
70
80
25
45
47.5
50
52.5
55
57.5
60
Temperatures (°C)

FIG. 23

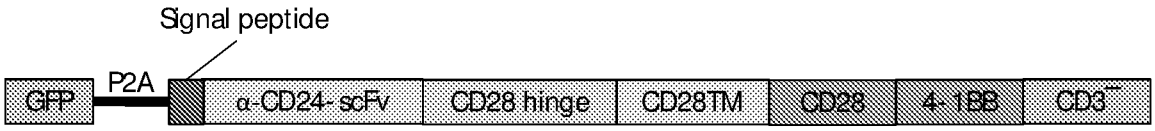

Signal peptide
GFP
P2A
α-CD24- scFv
CD28 hinge
CD28TM
CD28
4-1BB
CD3ζ

FIG. 24

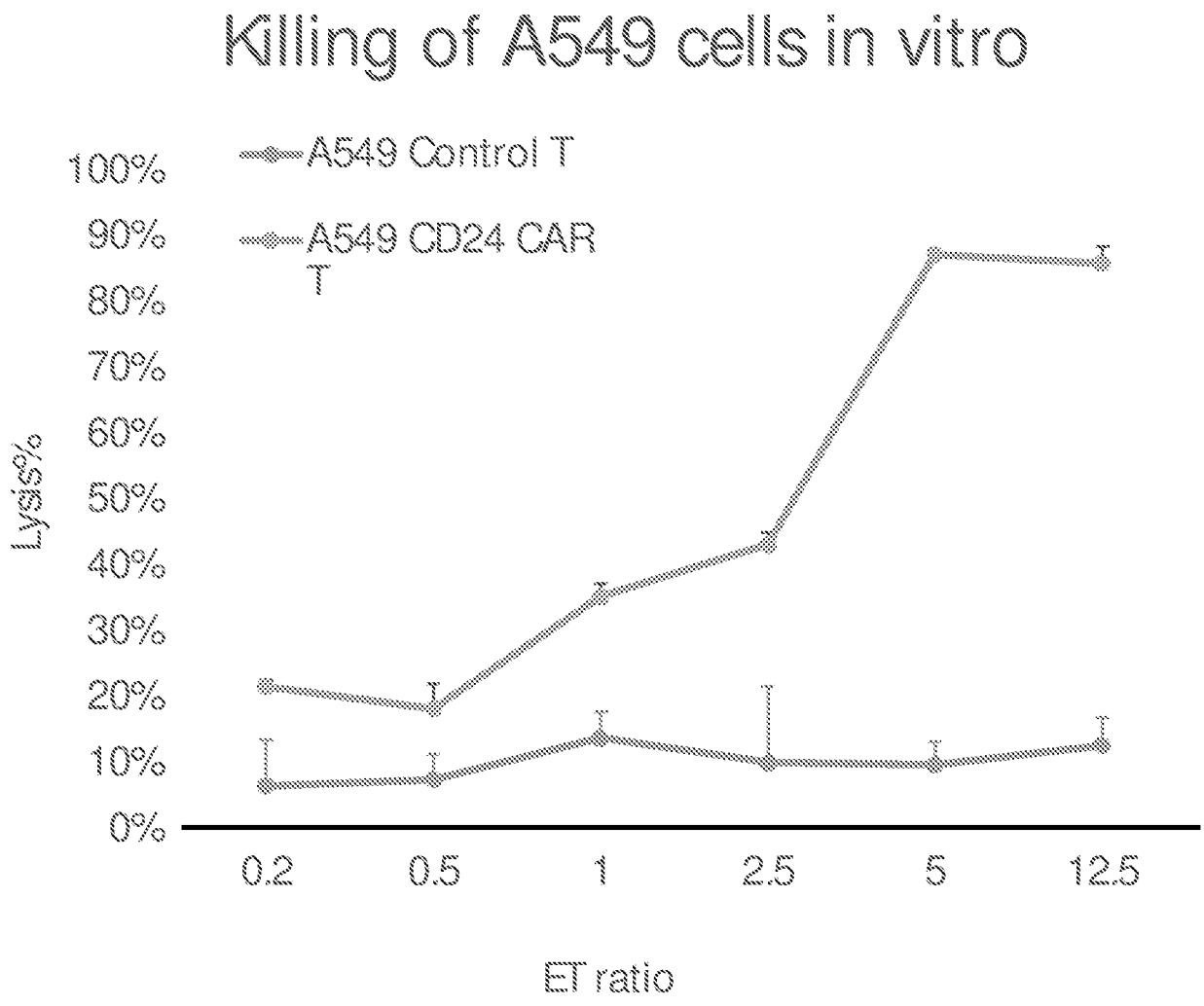

Killing of A549 cells in vitro
A549 Control T
A549 CD24 CAR T
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Lysis%
0.2
0.5
1
2.5
5
12.5
ET ratio

FIG. 25

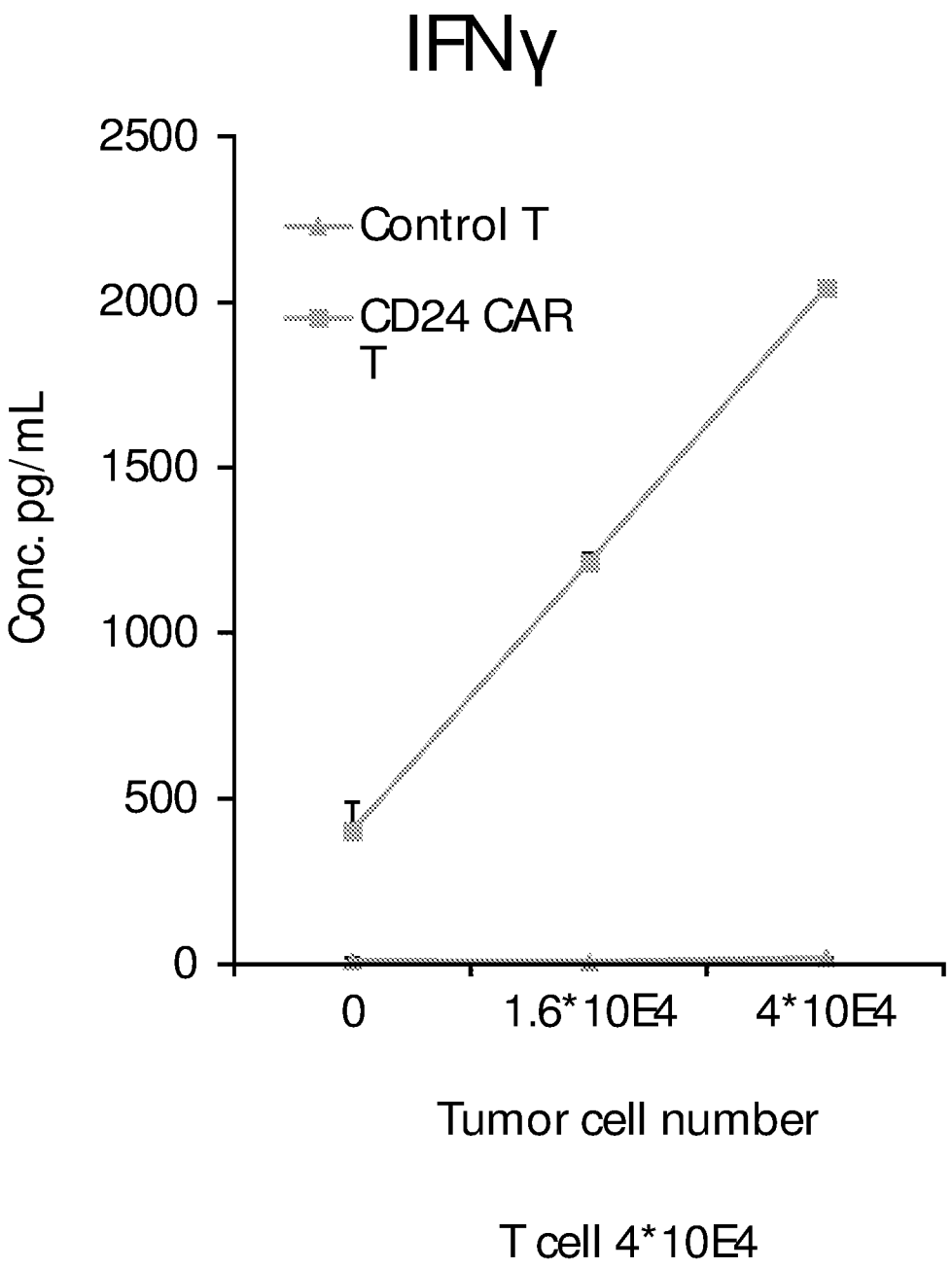

IFNγ
2500
2000
1500
1000
500
0
Conc. pg/mL
Control T
CD24 CAR T
0
1.6*10E4
4*10E4
Tumor cell number
T cell 4*10E4

FIG. 26

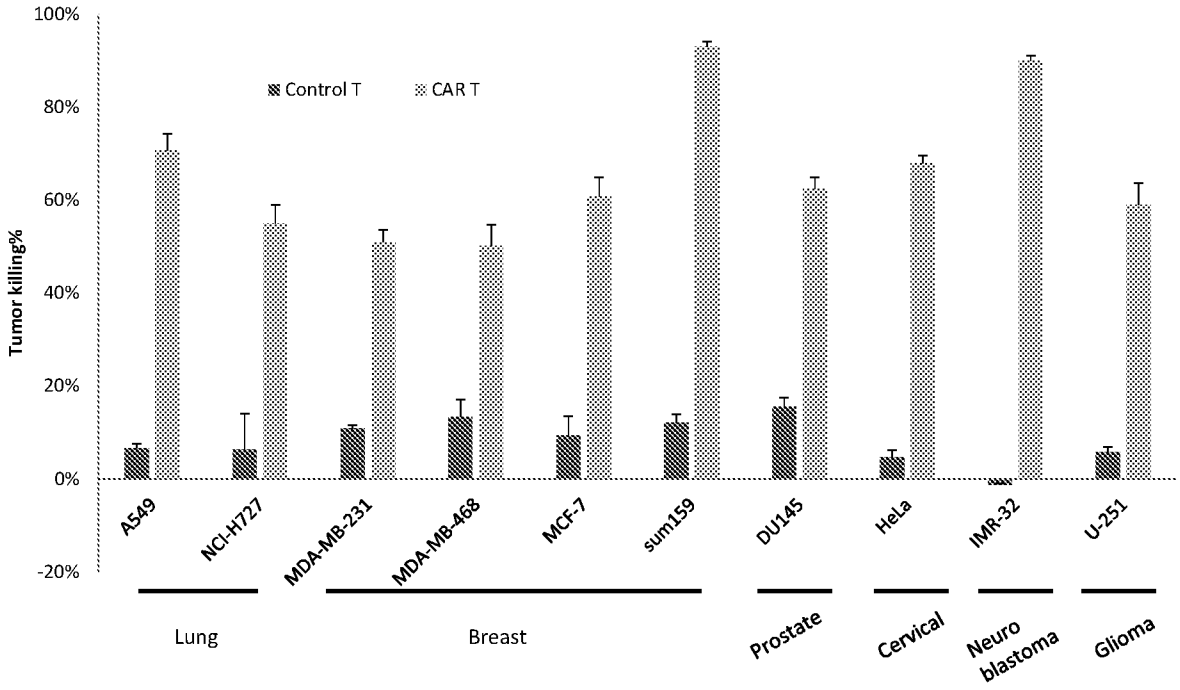

100%
80%
60%
40%
20%
0%
-20%
Tumor killing%
Control T
CAR T
A549
NCI-H727
MDA-MB-231
MDA-MB-468
MCF-7
sum159
DU145
HeLa
IMR-32
U-251
Lung
Breast
Prostate
Cervical
Neuro blastoma
Glioma

FIG. 27

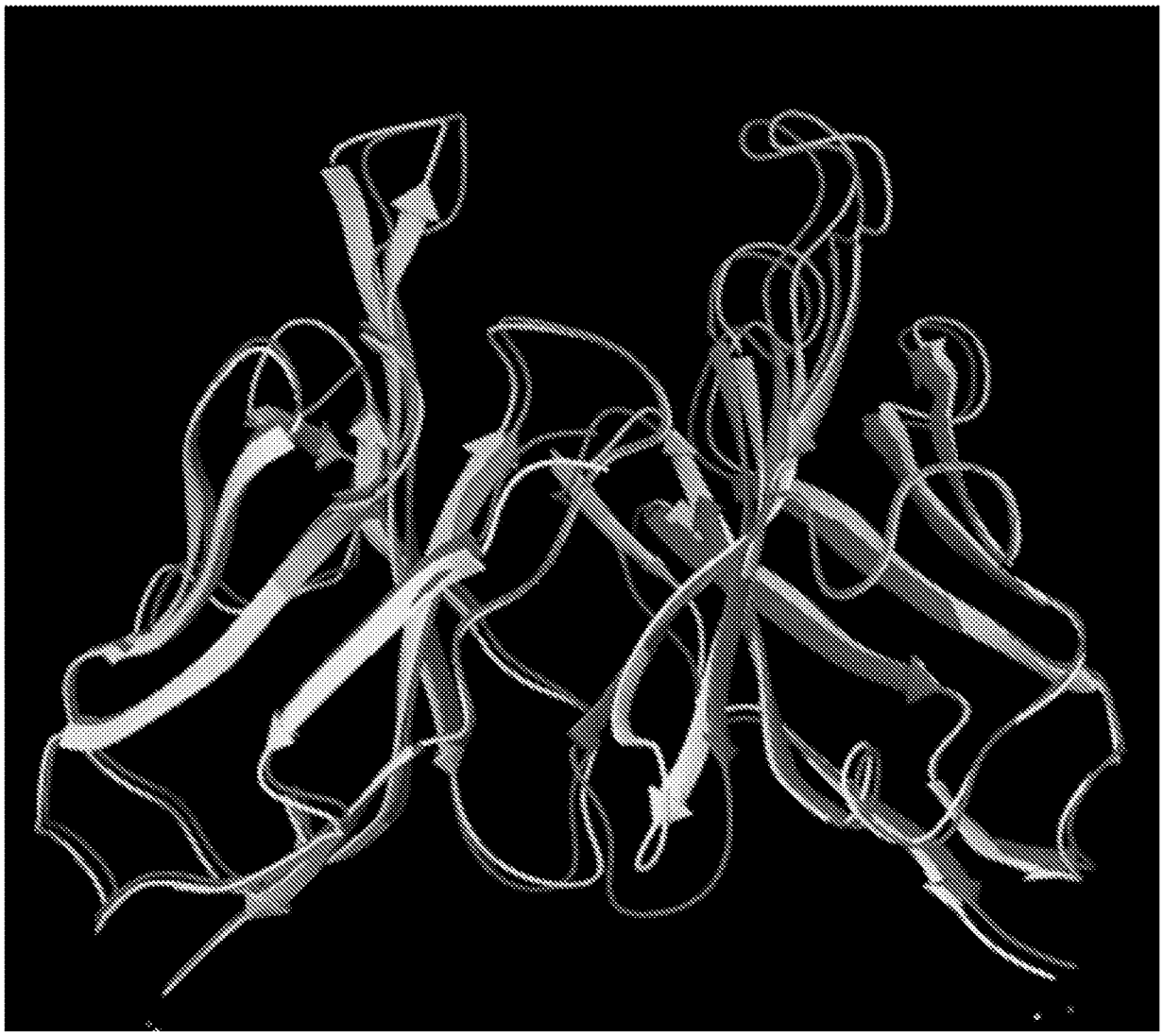


FIG. 28

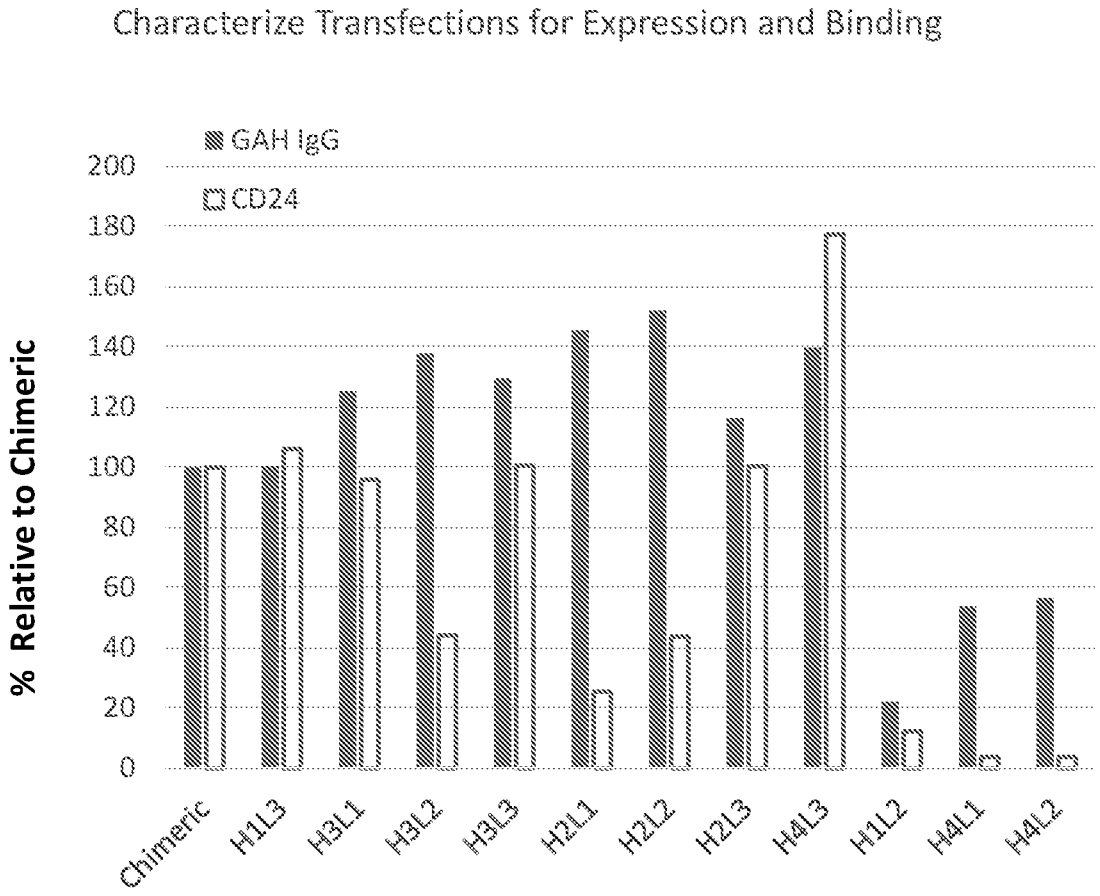

Characterize Transfections for Expression and Binding
GAH IgG
CD24
% Relative to Chimeric
200
180
160
140
120
100
80
60
40
20
0
Chimeric
H1L3
H3L1
H3L2
H3L3
H2L1
H2L2
H2L3
H4L3
H1L2
H4L1
H4L2

FIG. 29

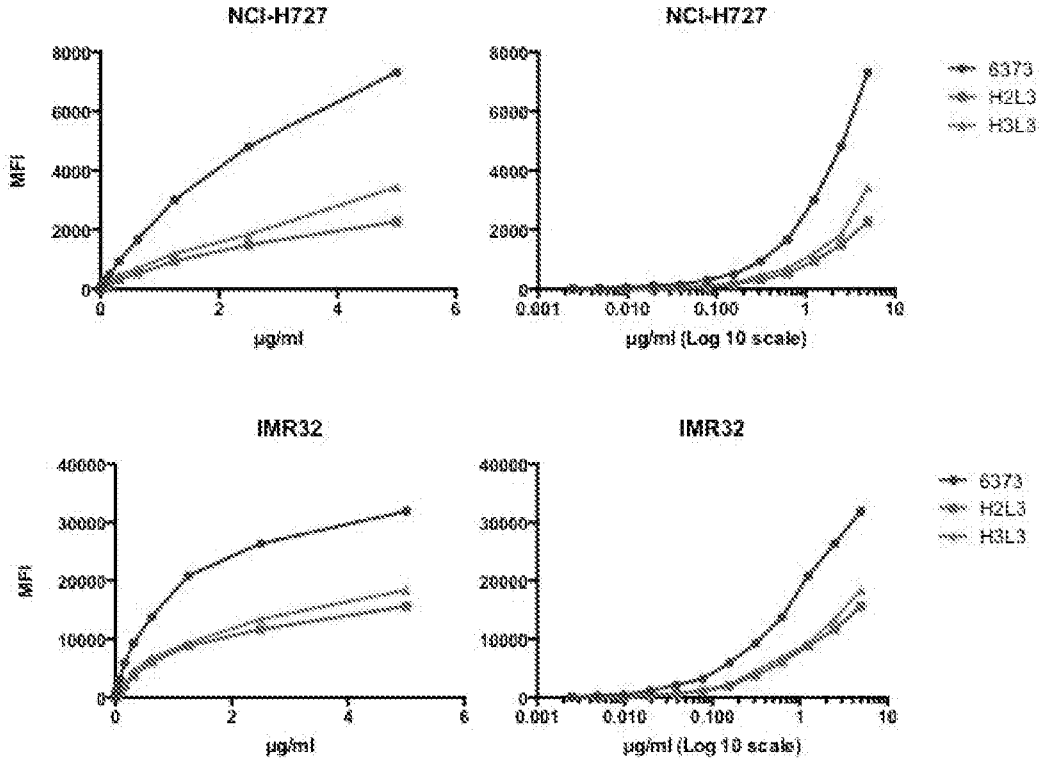

NCI-H727
NCI-H727
MFI
8000
6000
4000
2000
0
0
2
4
6
µg/ml
0.001
0.010
0.100
1
10
µg/ml (Log 10 scale)
6373
H2L3
H3L3
IMR32
IMR32
40000
30000
20000
10000
6373
H2L3
H3L3

FIG. 30

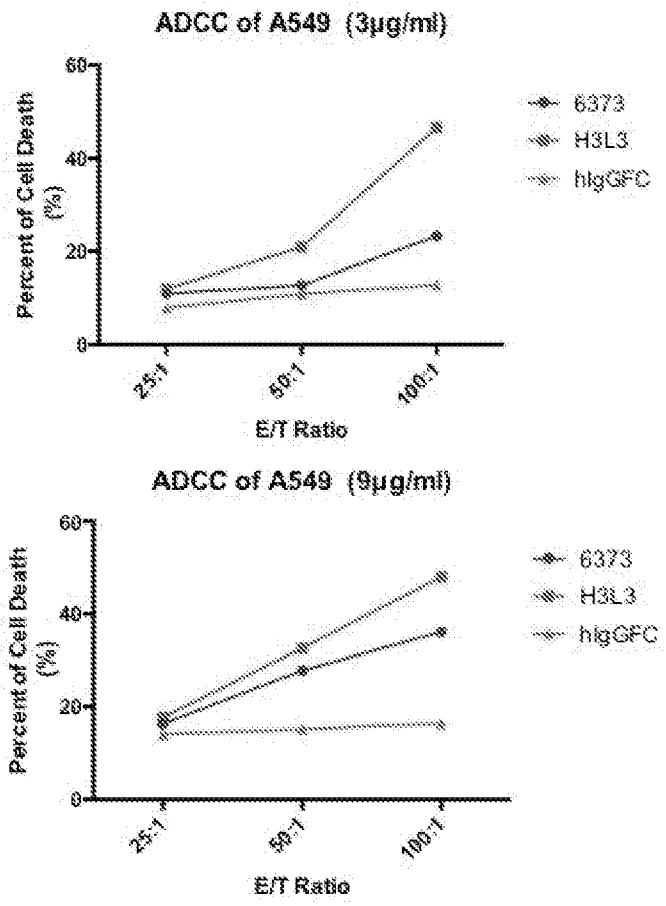

ADCC of A549 (3µg/ml)
Percent of Cell Death (%)
60
40
20
0
25:1
50:1
100:1
E/T Ratio
6373
H3L3
hIgGFC
ADCC of A549 (9µg/ml)
Percent of Cell Death (%)
60
40
20
0
25:1
50:1
100:1
E/T Ratio
6373
H3L3
hIgGFC

FIG. 31

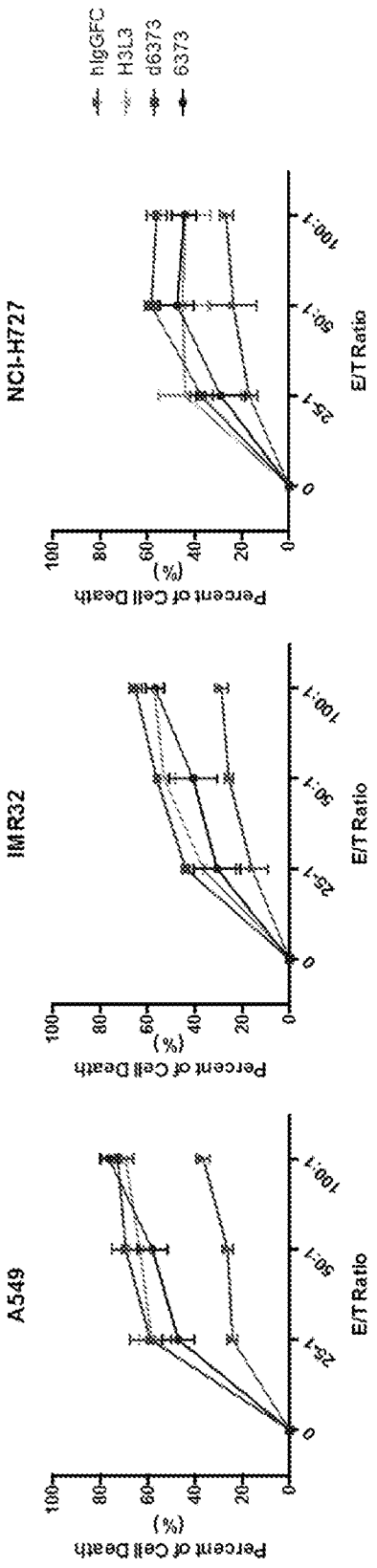
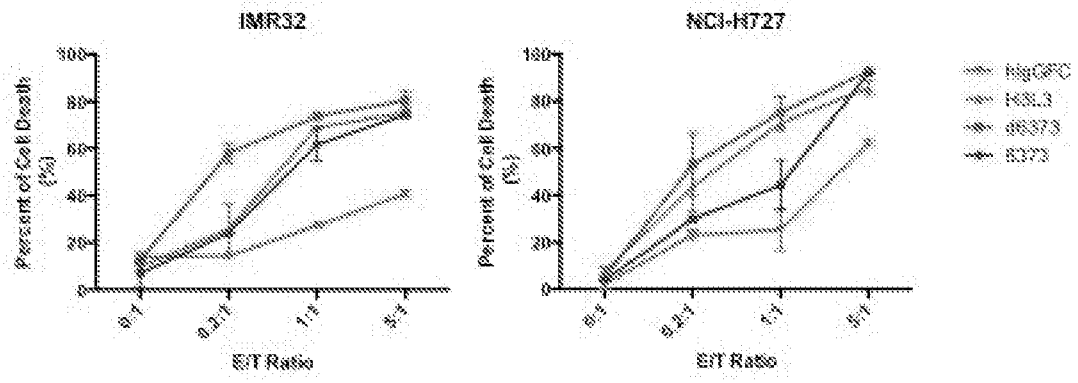

hIgGFC
H3L3
d6373
6373
NCI-H727
IMR32
A549
Percent of Cell Death
(%)
0
20
40
60
80
100
E/T Ratio
0
25:1
50:1
100:1

FIG. 32


IMR32
NCI-H727
Percent of Cell Death (%)
E/T Ratio
0:1
0.2:1
1:1
5:1
0
20
40
60
80
100
HigGFC
H3L3
d6373
8373

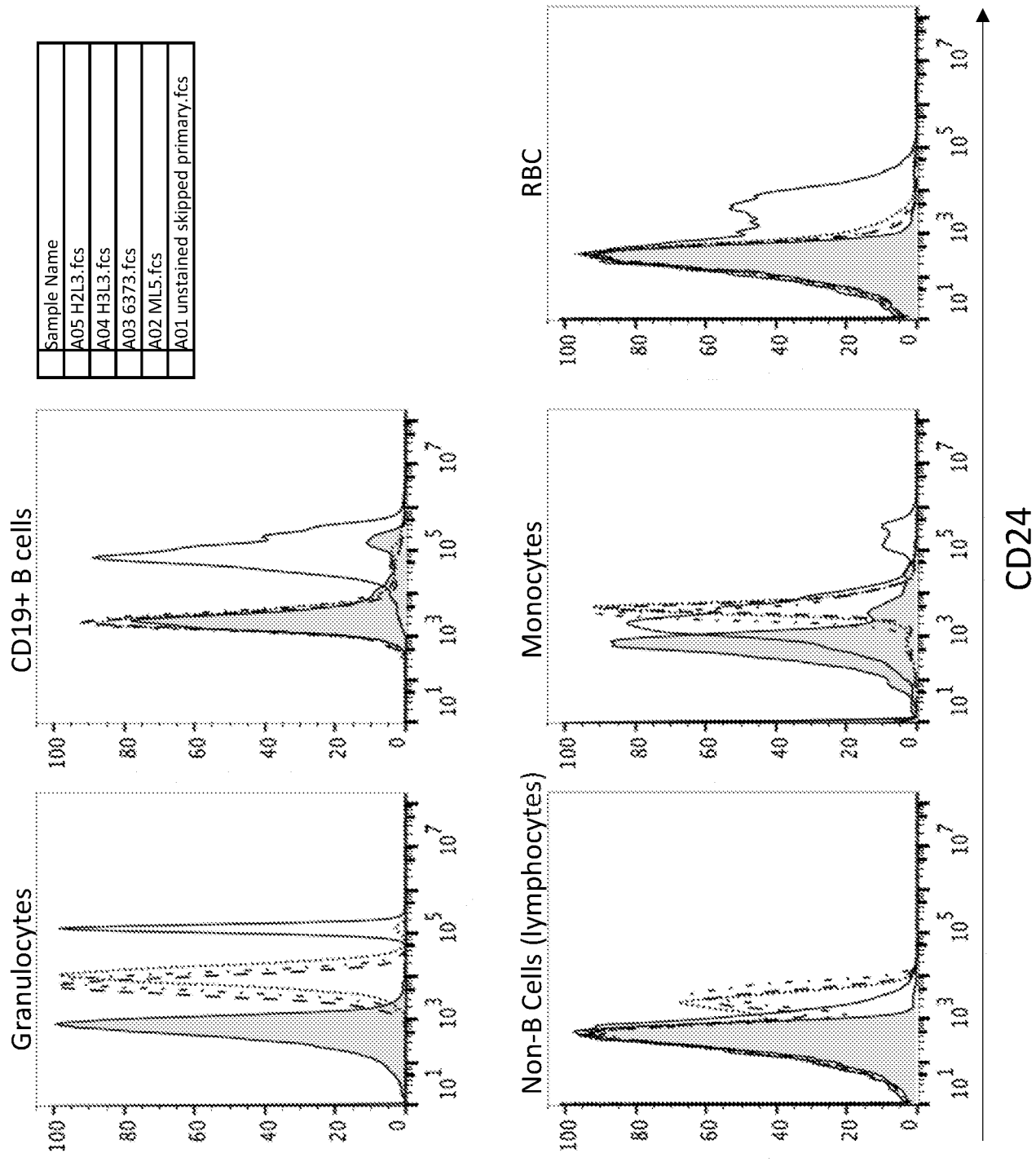

Sample Name
A05 H2L3.fcs
A04 H3L3.fcs
A03 6373.fcs
A02 ML5.fcs
A01 unstained skipped primary.fcs
Granulocytes
CD19+ B cells
Non-B Cells (lymphocytes)
Monocytes
RBC
CD24

FIG. 33