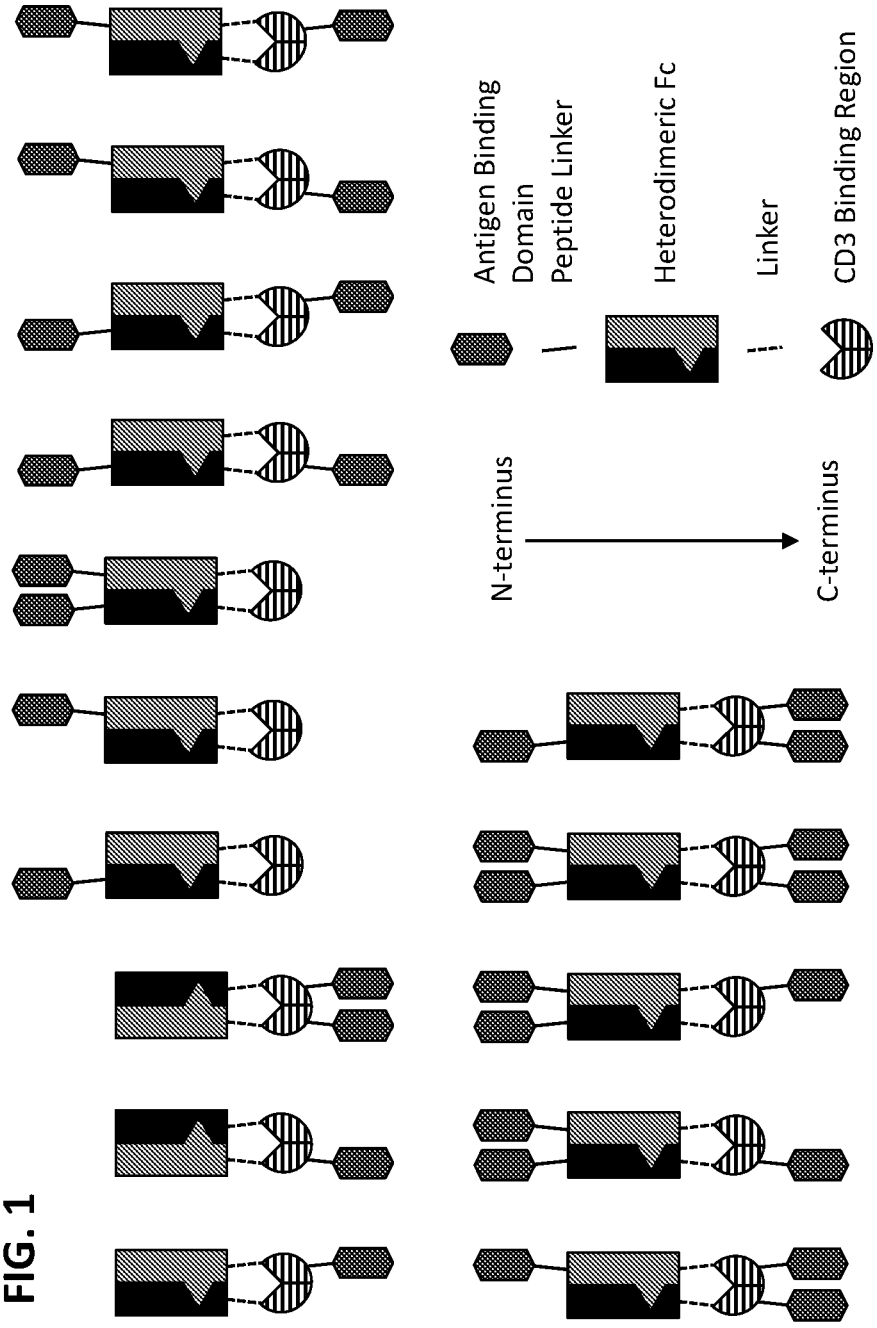

Antigen Binding Domain
Peptide Linker
Heterodimeric Fc
Linker
CD3 Binding Region
N-terminus
C-terminus

FIG. 1

FIG. 2A

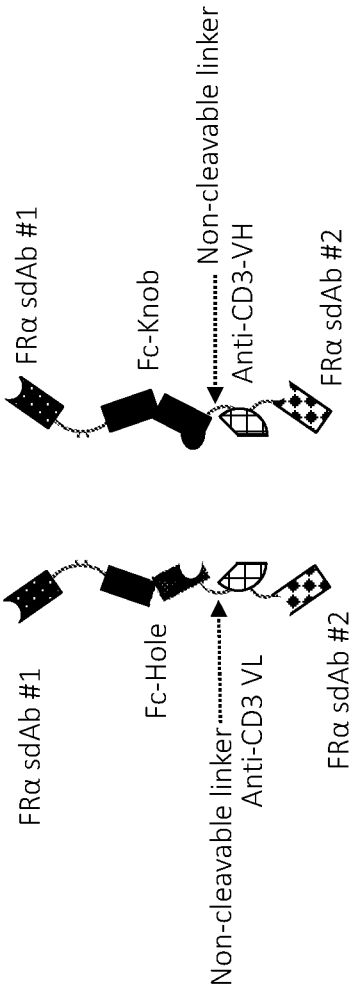


FIG. 2B

cx1356, cx1357, cx1358, cx1359, cx1360, cx681
FRα-targeted Constrained CD3: Non-cleavable linkers
Various length 3-18AA GlySer-based

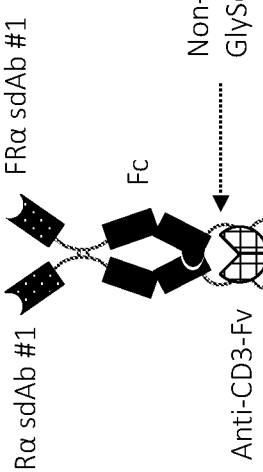


FIG. 3A

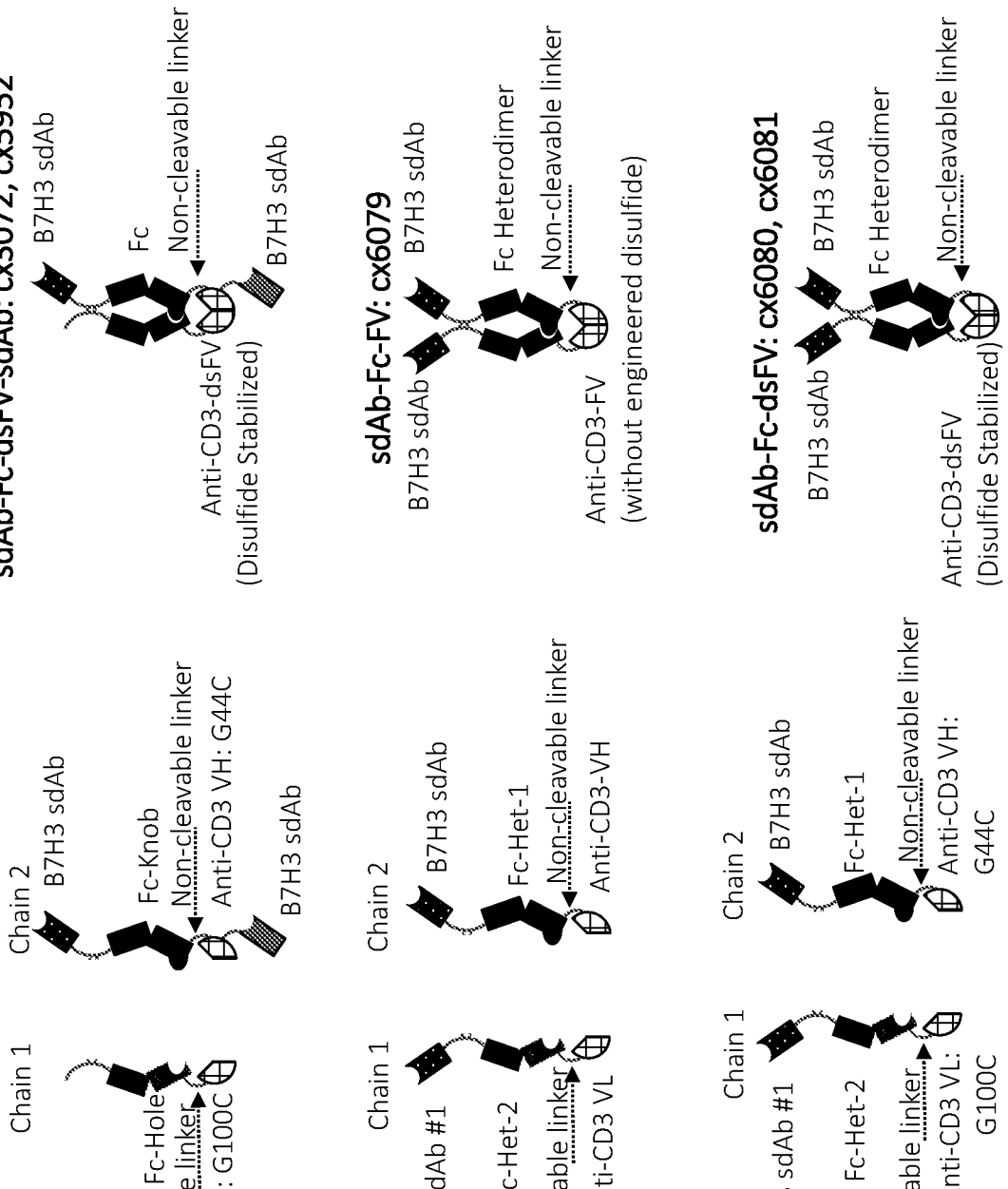

sdAb-Fc-dsFV-sdAb: cx3072, cx5952
Chain 1
Fc-Hole
Non-cleavable linker
Anti-CD3 VL: G100C
Chain 2
B7H3 sdAb
Fc-Knob
Non-cleavable linker
Anti-CD3 VH: G44C
B7H3 sdAb
B7H3 sdAb
Fc
Non-cleavable linker
Anti-CD3-dsFV
(Disulfide Stabilized)
sdAb-Fc-FV: cx6079
Chain 1
B7H3 sdAb #1
Fc-Het-2
Non-cleavable linker
Anti-CD3 VL
Chain 2
B7H3 sdAb
Fc-Het-1
Non-cleavable linker
Anti-CD3-VH
B7H3 sdAb
B7H3 sdAb
Fc Heterodimer
Non-cleavable linker
Anti-CD3-FV
(without engineered disulfide)
sdAb-Fc-dsFV: cx6080, cx6081
Chain 1
B7H3 sdAb #1
Fc-Het-2
Non-cleavable linker
Anti-CD3 VL:
G100C
Chain 2
B7H3 sdAb
Fc-Het-1
Non-cleavable linker
Anti-CD3 VH:
G44C
B7H3 sdAb
B7H3 sdAb
Fc Heterodimer
Non-cleavable linker
Anti-CD3-dsFV
(Disulfide Stabilized)

FIG. 3B

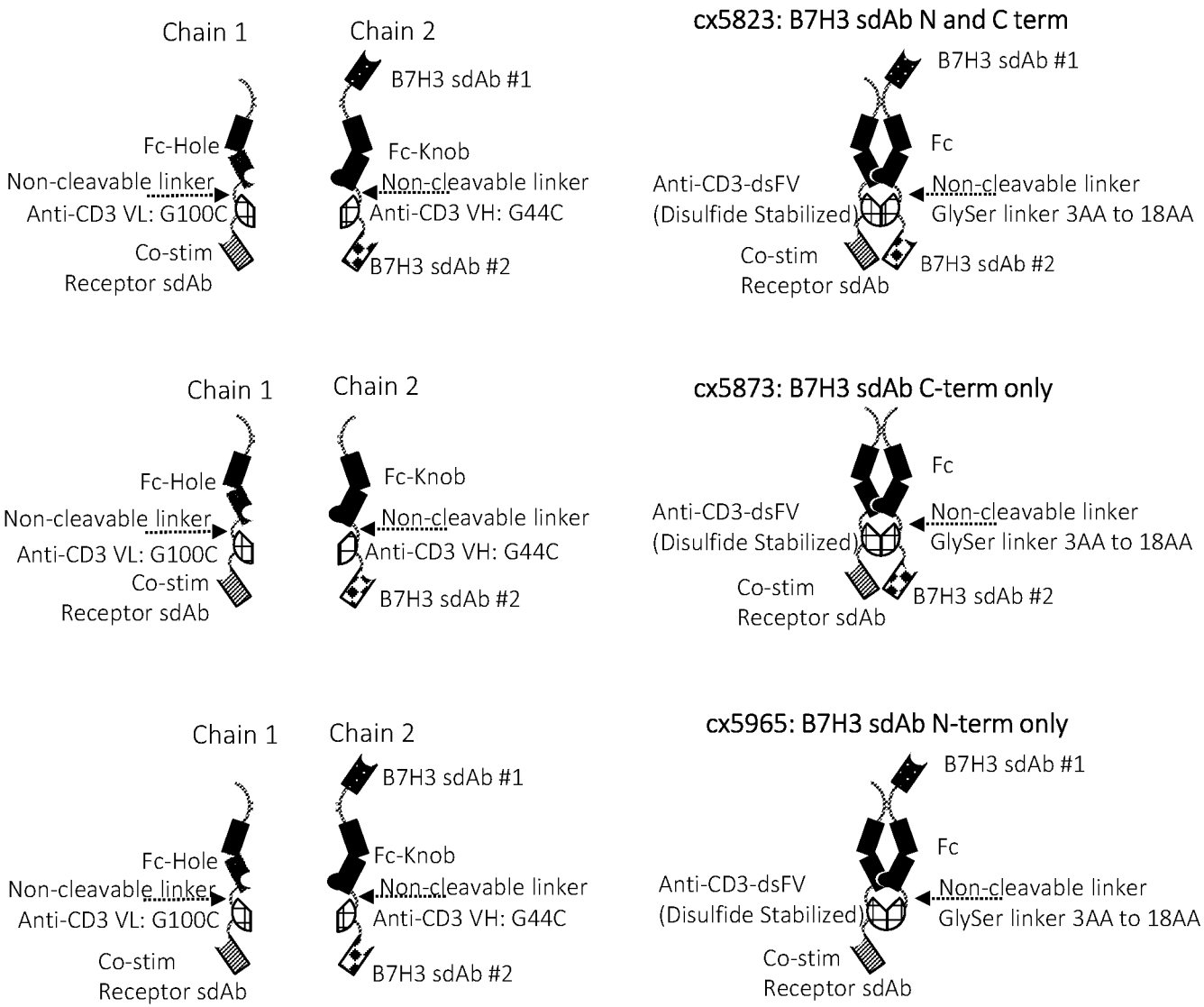

Chain 1
Chain 2
B7H3 sdAb #1
Fc-Hole
Fc-Knob
Non-cleavable linker
Anti-CD3 VL: G100C
Anti-CD3 VH: G44C
Co-stim
Receptor sdAb
B7H3 sdAb #2
cx5823: B7H3 sdAb N and C term
Fc
Anti-CD3-dsFV
(Disulfide Stabilized)
GlySer linker 3AA to 18AA
cx5873: B7H3 sdAb C-term only
cx5965: B7H3 sdAb N-term only

FIG. 3C

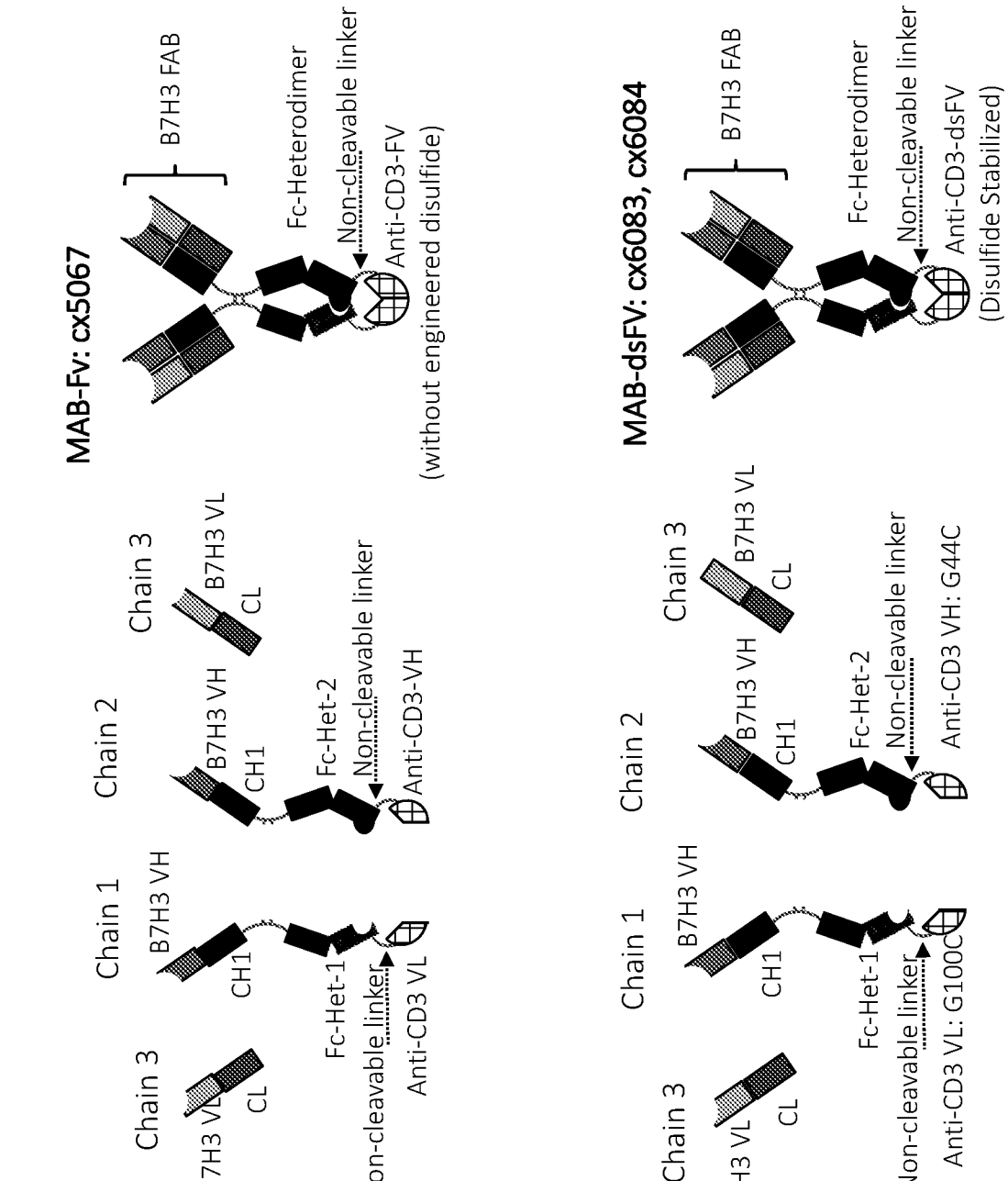

MAB-Fv: cx5067
B7H3 FAB
Fc-Heterodimer
Non-cleavable linker
Anti-CD3-FV
(without engineered disulfide)
Chain 1
B7H3 VH
CH1
Fc-Het-1
Non-cleavable linker
Anti-CD3 VL
Chain 2
B7H3 VH
CH1
Fc-Het-2
Non-cleavable linker
Anti-CD3-VH
Chain 3
B7H3 VL
CL
B7H3 VL
CL
MAB-dsFV: cx6083, cx6084
B7H3 FAB
Fc-Heterodimer
Non-cleavable linker
Anti-CD3-dsFV
(Disulfide Stabilized)
Chain 1
B7H3 VH
CH1
Fc-Het-1
Non-cleavable linker
Anti-CD3 VL: G100C
Chain 2
B7H3 VH
CH1
Fc-Het-2
Non-cleavable linker
Anti-CD3 VH: G44C
Chain 3
B7H3 VL
CL
B7H3 VL
CL

FIG. 4A

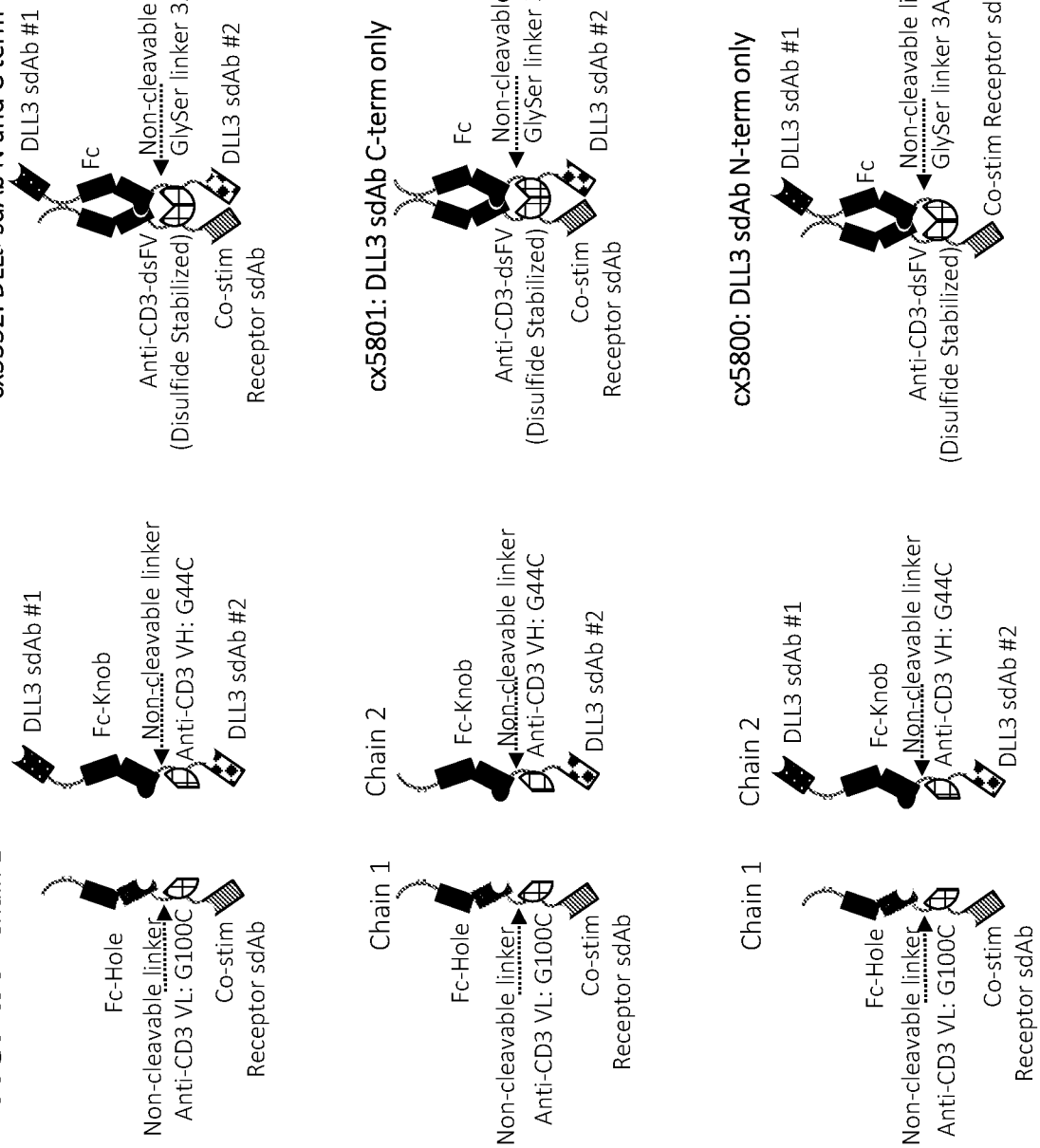

Chain 1
Fc-Hole
Non-cleavable linker
Anti-CD3 VL: G100C
Co-stim
Receptor sdAb
Chain 2
DLL3 sdAb #1
Fc-Knob
Non-cleavable linker
Anti-CD3 VH: G44C
DLL3 sdAb #2
cx5352: DLL3 sdAb N and C term
Fc
Non-cleavable linker
GlySer linker 3AA to 18AA
Anti-CD3-dsFV
(Disulfide Stabilized)
cx5801: DLL3 sdAb C-term only
cx5800: DLL3 sdAb N-term only
Co-stim Receptor sdAb

FIG. 4B

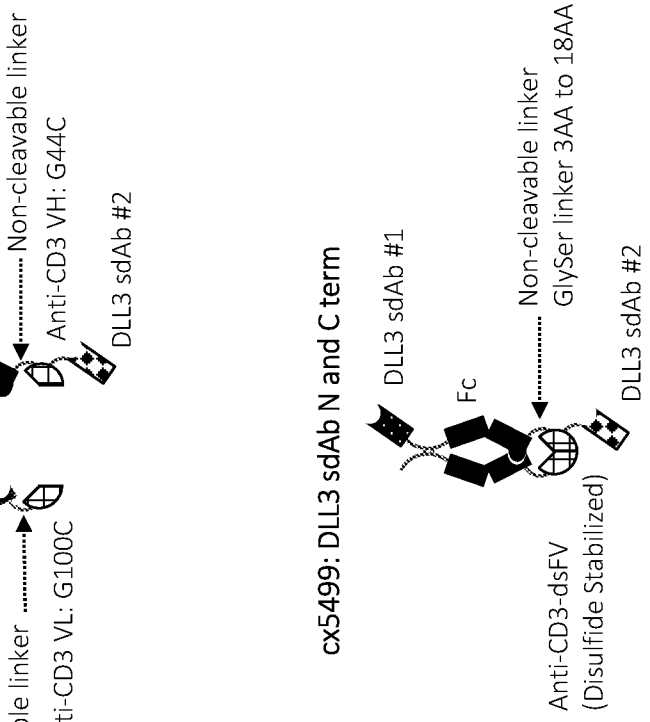

Chain 1
Chain 2
DLL3 sdAb #1
Fc-Knob
Non-cleavable linker
Anti-CD3 VH: G44C
DLL3 sdAb #2
Fc-Hole
Non-cleavable linker
Anti-CD3 VL: G100C
cx5499: DLL3 sdAb N and C term
DLL3 sdAb #1
Fc
Non-cleavable linker
GlySer linker 3AA to 18AA
DLL3 sdAb #2
Anti-CD3-dsFV
(Disulfide Stabilized)

FIG. 5A

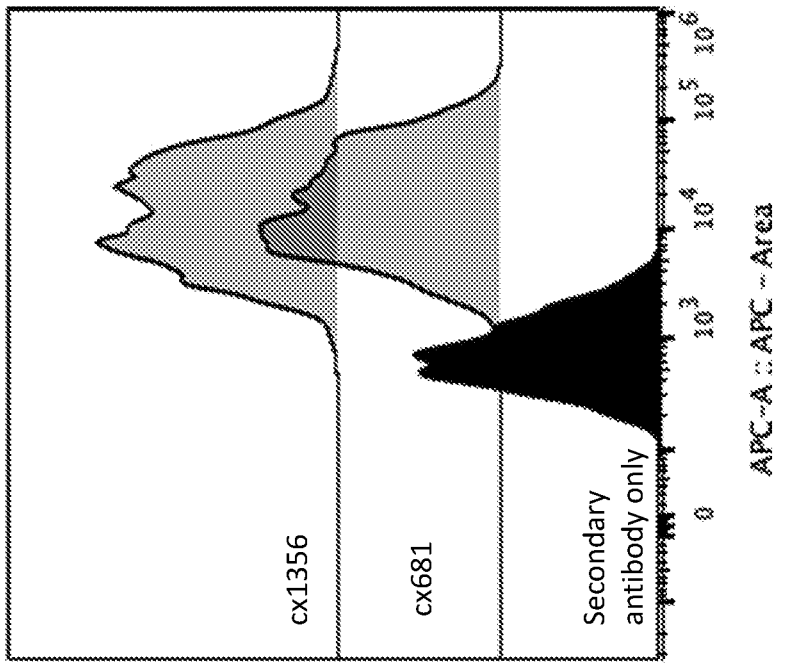
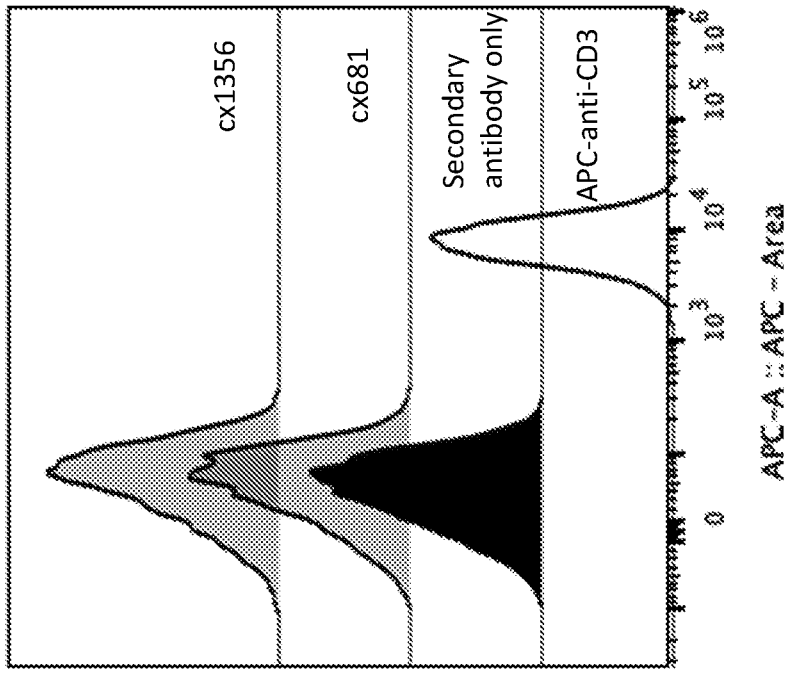

Binding to OVCAR5 cells
cx1356
cx681
Secondary antibody only
0
10^3
10^4
10^5
10^6
APC-A :: APC – Area

FIG. 5B


Binding to T cells
cx1356
cx681
Secondary antibody only
APC-anti-CD3
0
10^3
10^4
10^5
10^6
APC-A :: APC – Area

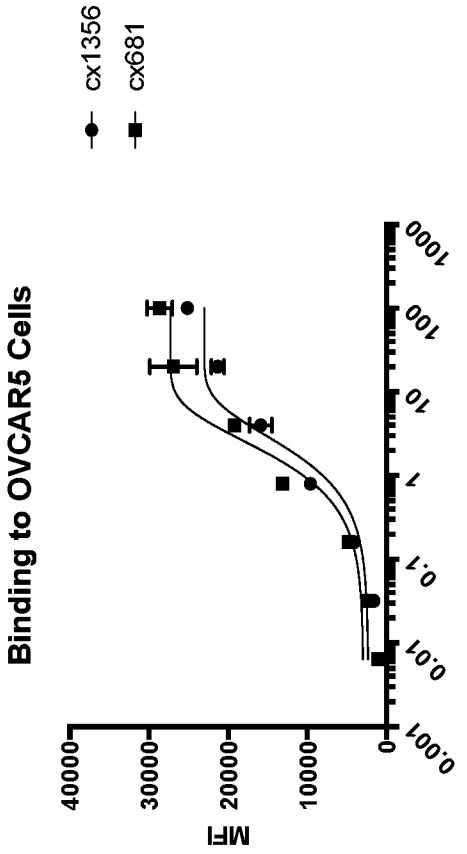

Binding to OVCAR5 Cells
cx1356
cx681
MFI
40000
30000
20000
10000
0
0.001
0.01
0.1
1
10
100
1000
nM

FIG. 5C

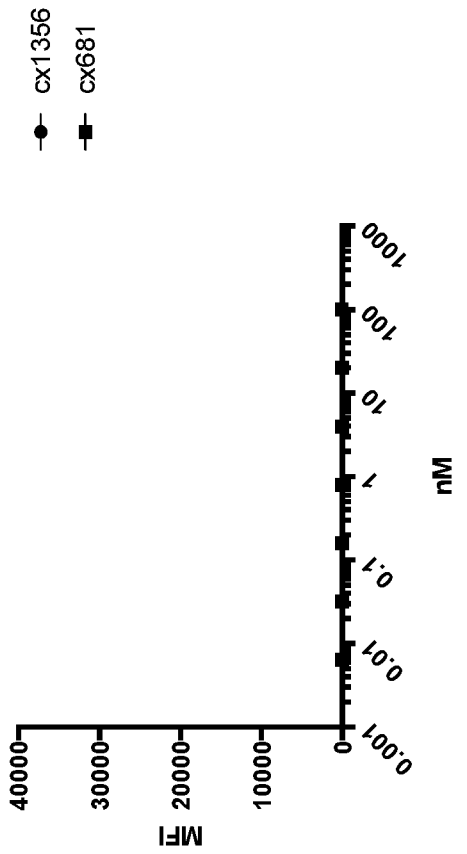

Binding to T cells
cx1356
cx681
MFI
40000
30000
20000
10000
0
0.001
0.01
0.1
1
10
100
1000
nM

FIG. 5D

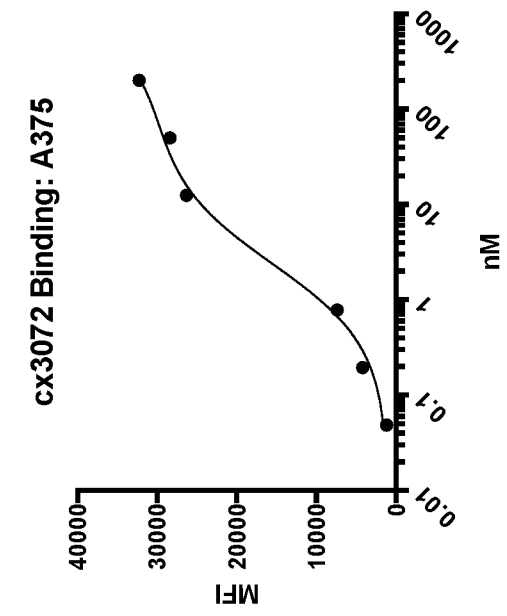

cx3072 Binding: A375
MFI
40000
30000
20000
10000
0
0.01
0.1
1
10
100
1000
nM

FIG. 6A

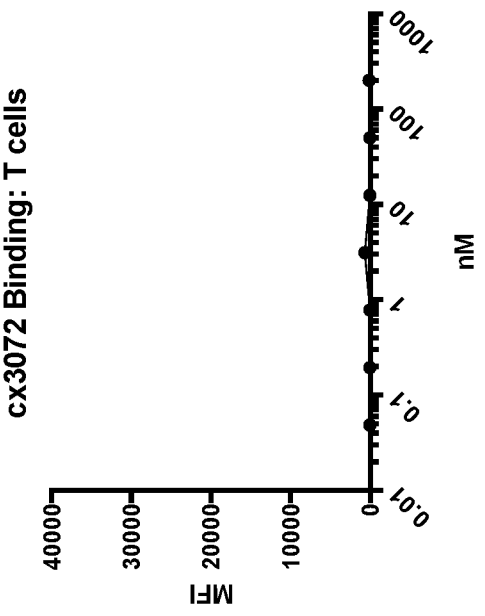

cx3072 Binding: T cells
MFI
40000
30000
20000
10000
0
0.01
0.1
1
10
100
1000
nM

FIG. 6B

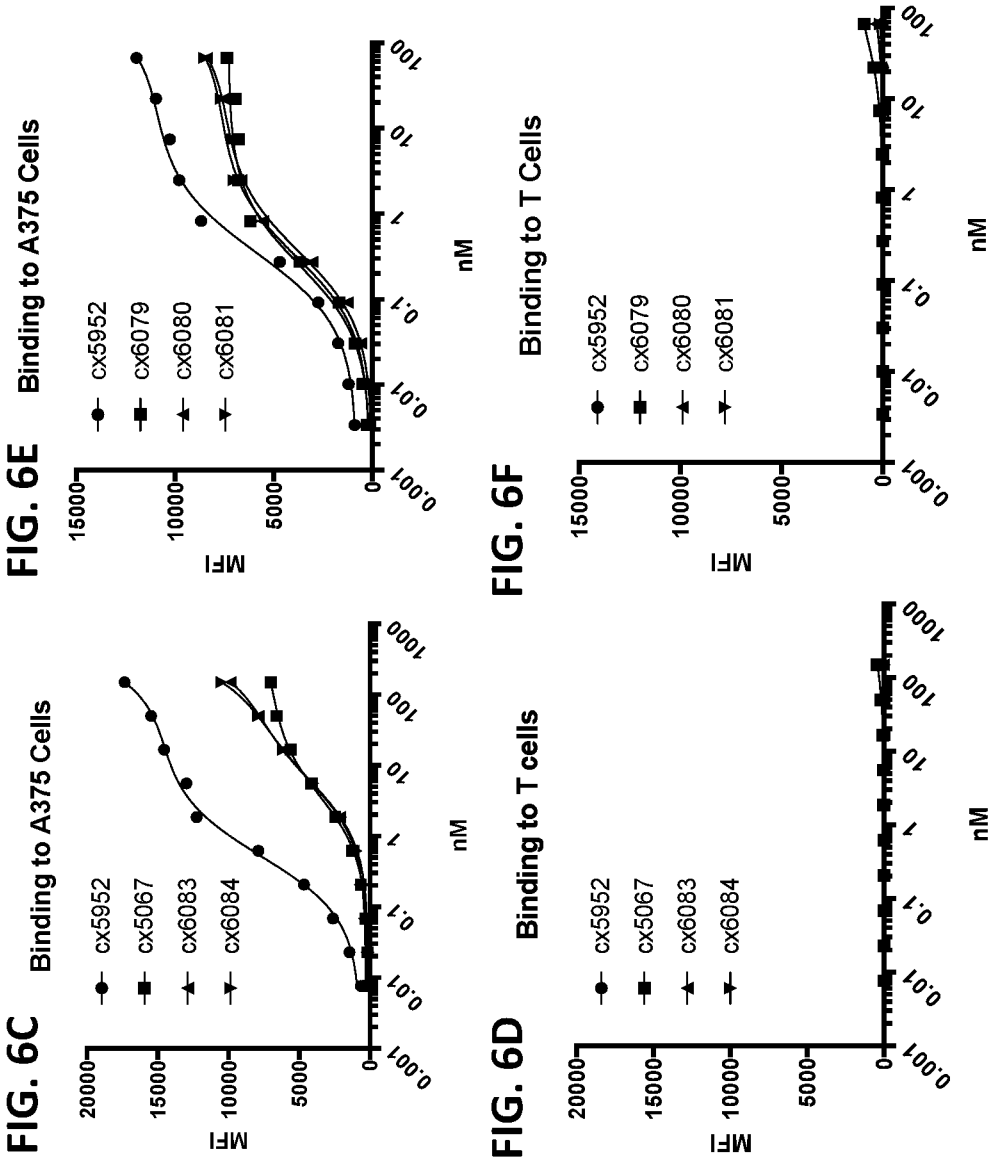


FIG. 6E
Binding to A375 Cells
cx5952
cx6079
cx6080
cx6081
MFI
nM
FIG. 6F
Binding to T Cells
cx5952
cx6079
cx6080
cx6081
MFI
nM
FIG. 6C
Binding to A375 Cells
cx5952
cx5067
cx6083
cx6084
MFI
nM
FIG. 6D
Binding to T cells
cx5952
cx5067
cx6083
cx6084
MFI
nM

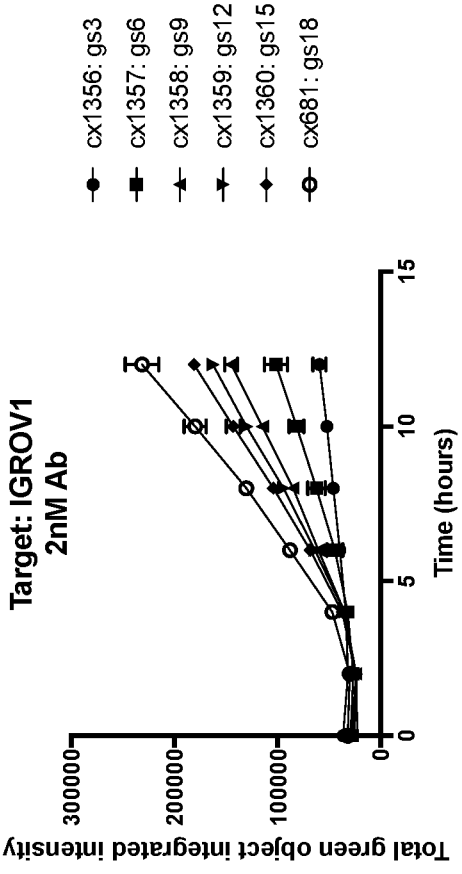

Target: IGROV1
2nM Ab
cx1356: gs3
cx1357: gs6
cx1358: gs9
cx1359: gs12
cx1360: gs15
cx681: gs18
Total green object integrated intensity
300000
200000
100000
0
0
5
10
15
Time (hours)

FIG. 7A

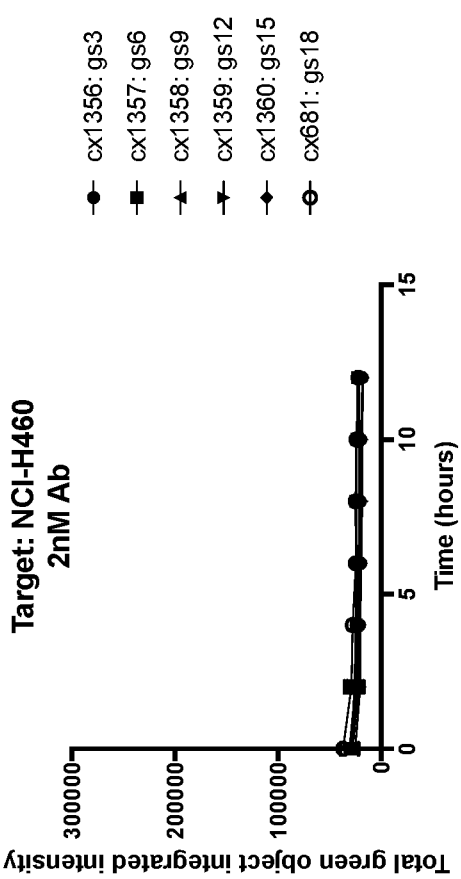

Target: NCI-H460
2nM Ab
cx1356: gs3
cx1357: gs6
cx1358: gs9
cx1359: gs12
cx1360: gs15
cx681: gs18
Total green object integrated intensity
300000
200000
100000
0
0
5
10
15
Time (hours)

FIG. 7B

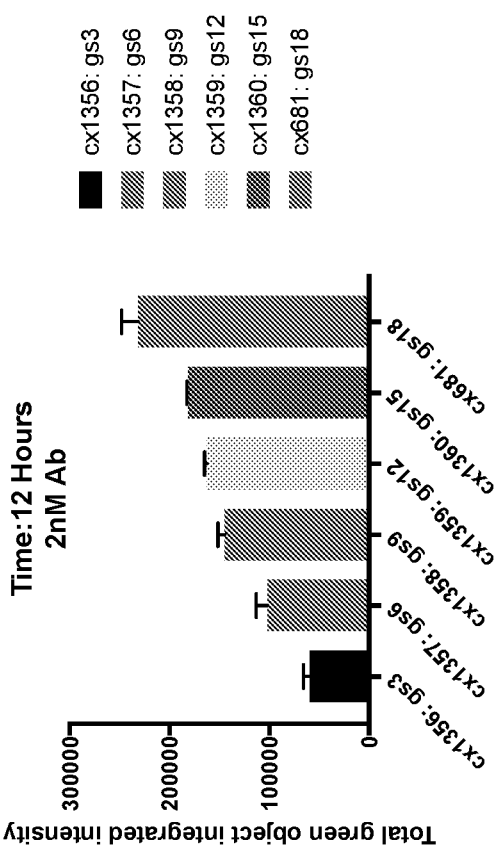

cx1356: gs3
cx1357: gs6
cx1358: gs9
cx1359: gs12
cx1360: gs15
cx681: gs18
Time:12 Hours
2nM Ab
Total green object integrated intensity
300000
200000
100000
0
cx1356: gs3
cx1357: gs6
cx1358: gs9
cx1359: gs12
cx1360: gs15
cx681: gs18

FIG. 7C

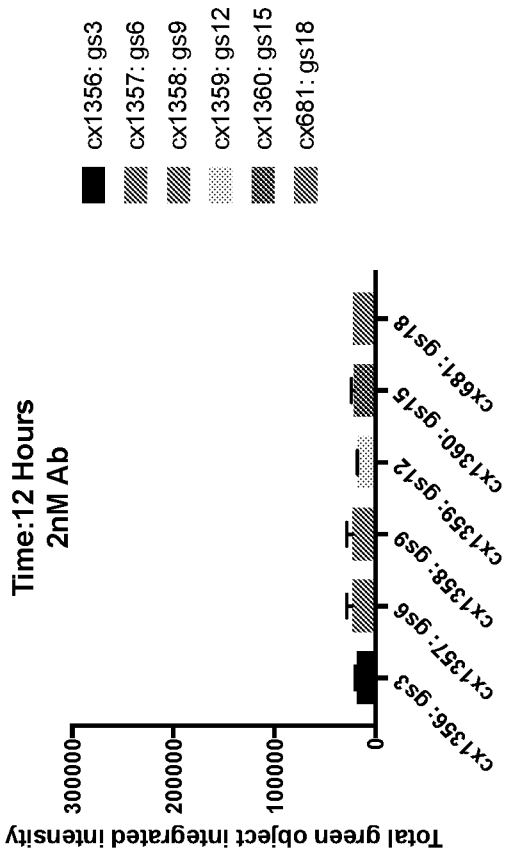

cx1356: gs3
cx1357: gs6
cx1358: gs9
cx1359: gs12
cx1360: gs15
cx681: gs18
Time:12 Hours
2nM Ab
Total green object integrated intensity
300000
200000
100000
0
cx1356: gs3
cx1357: gs6
cx1358: gs9
cx1359: gs12
cx1360: gs15
cx681: gs18

FIG. 7D

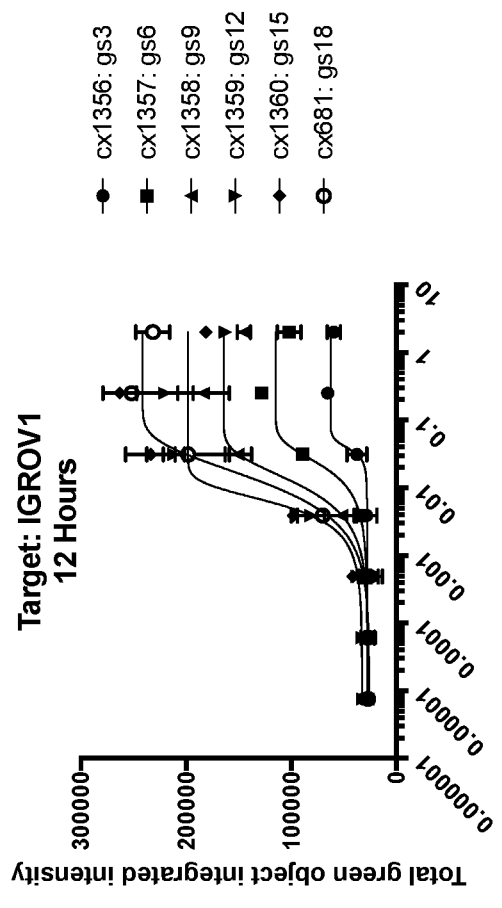

Target: IGROV1
12 Hours
Total green object integrated intensity
300000
200000
100000
0
cx1356: gs3
cx1357: gs6
cx1358: gs9
cx1359: gs12
cx1360: gs15
cx681: gs18
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
nM

FIG. 7E

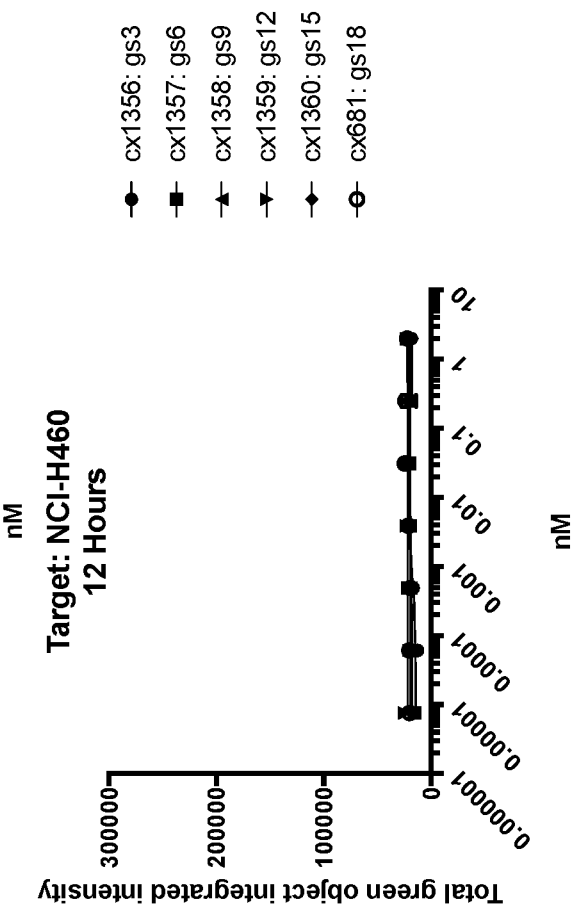

Target: NCI-H460
12 Hours
Total green object integrated intensity
300000
200000
100000
0
cx1356: gs3
cx1357: gs6
cx1358: gs9
cx1359: gs12
cx1360: gs15
cx681: gs18
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
nM

FIG. 7F

FIG. 8A

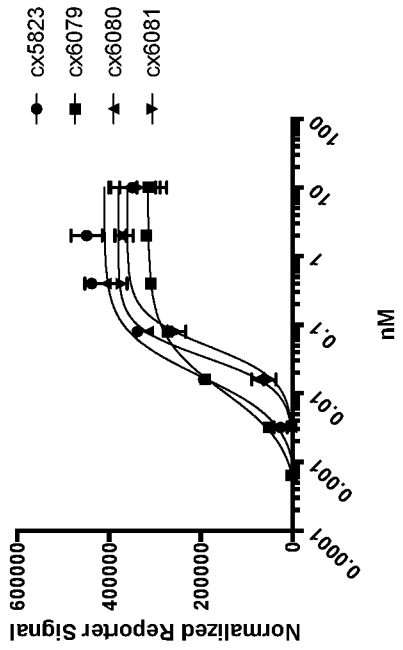


FIG. 8B

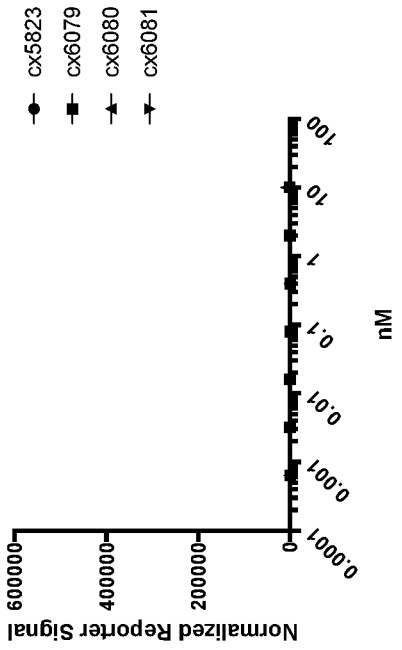


FIG. 8C

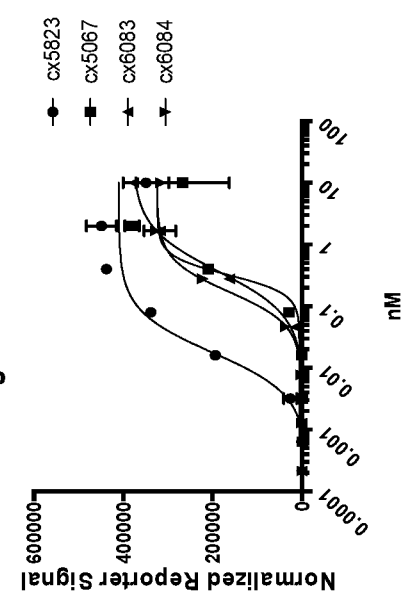
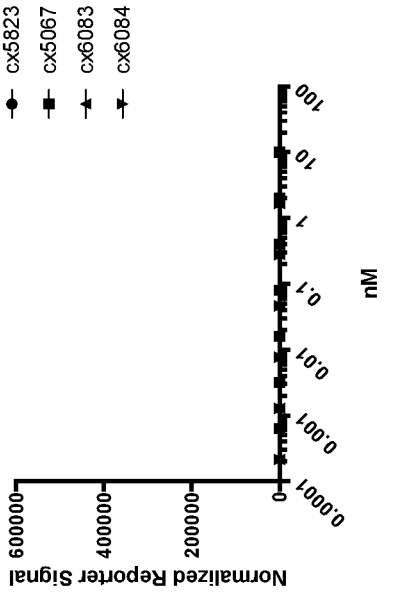


FIG. 8D



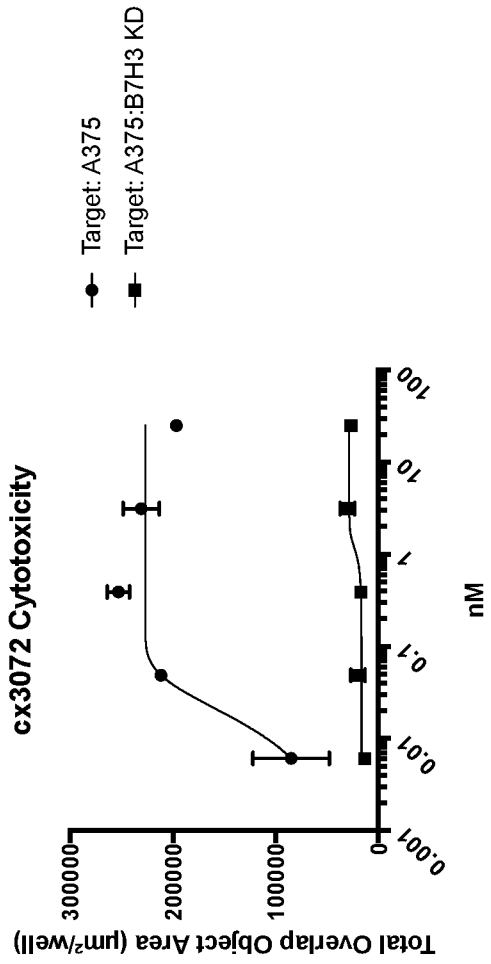

cx3072 Cytotoxicity
Target: A375
Target: A375:B7H3 KD
Total Overlap Object Area (µm²/well)
300000
200000
100000
0
0.001
0.01
0.1
1
10
100
nM

FIG. 9A

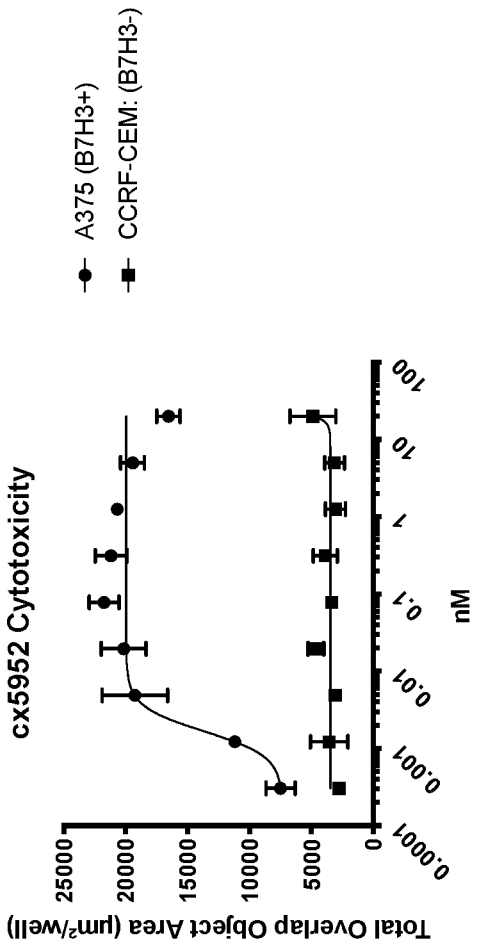

cx5952 Cytotoxicity
A375 (B7H3+)
CCRF-CEM: (B7H3-)
Total Overlap Object Area (µm²/well)
25000
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
100
nM

FIG. 9B

FIG. 10A

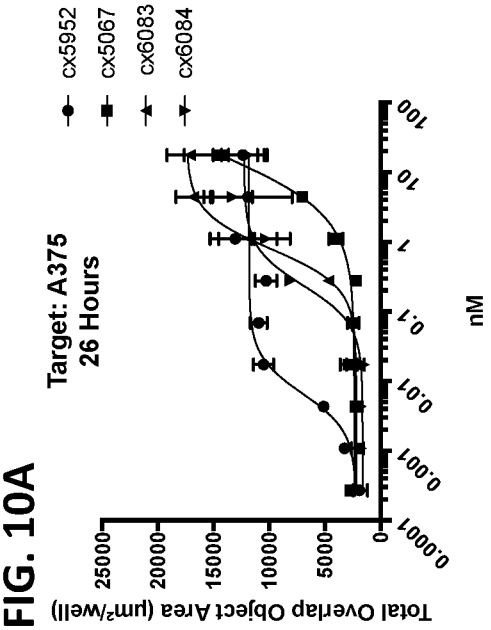


FIG. 10B

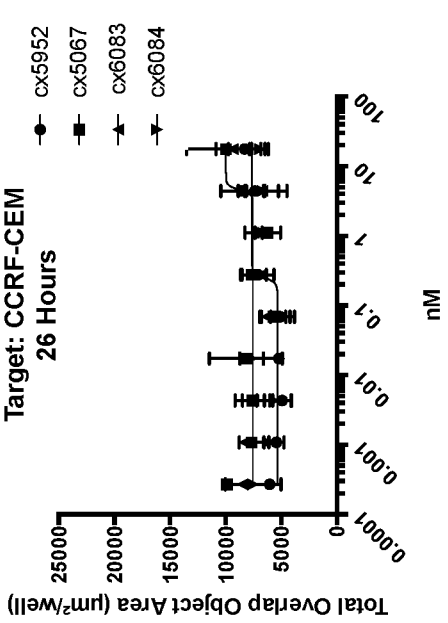


FIG. 10C

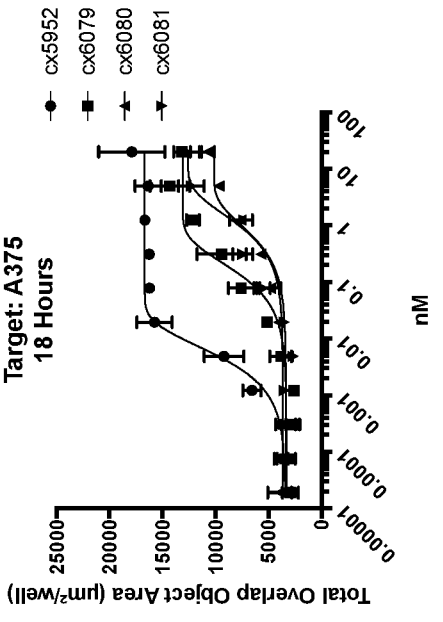
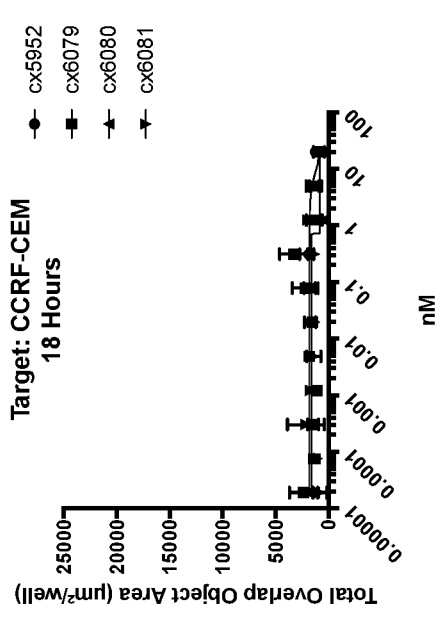


FIG. 10D



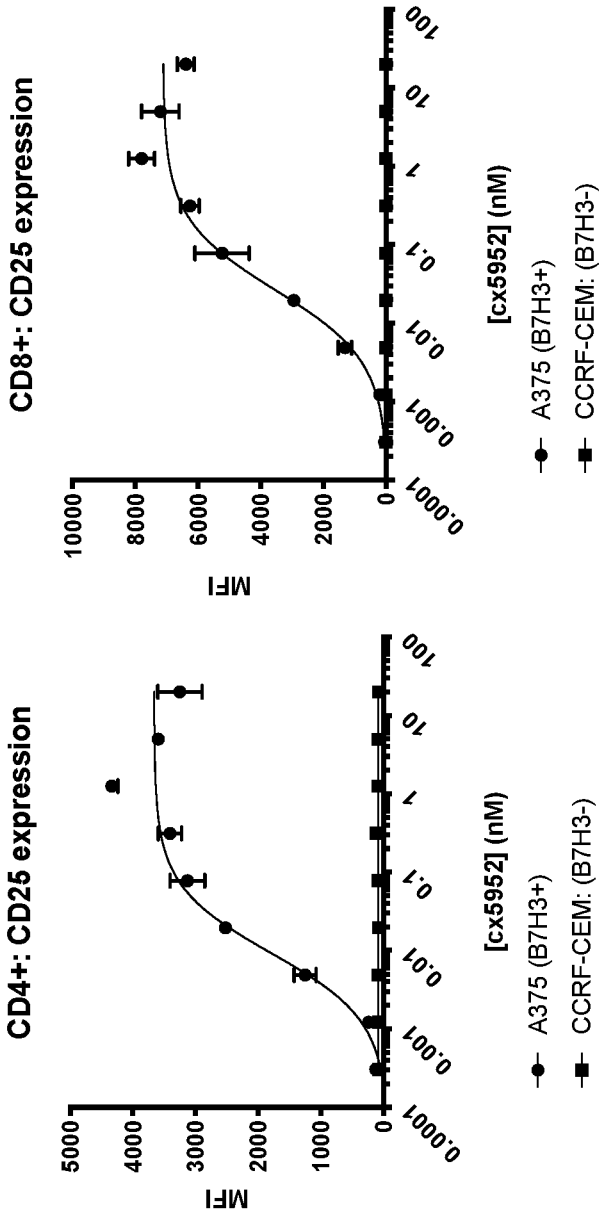

CD8+: CD25 expression
MFI
10000
8000
6000
4000
2000
0
0.0001
0.001
0.01
0.1
1
10
100
[cx5952] (nM)
A375 (B7H3+)
CCRF-CEM: (B7H3-)
CD4+: CD25 expression
MFI
5000
4000
3000
2000
1000
0
0.0001
0.001
0.01
0.1
1
10
100
[cx5952] (nM)
A375 (B7H3+)
CCRF-CEM: (B7H3-)

FIG. 11A

FIG. 11B

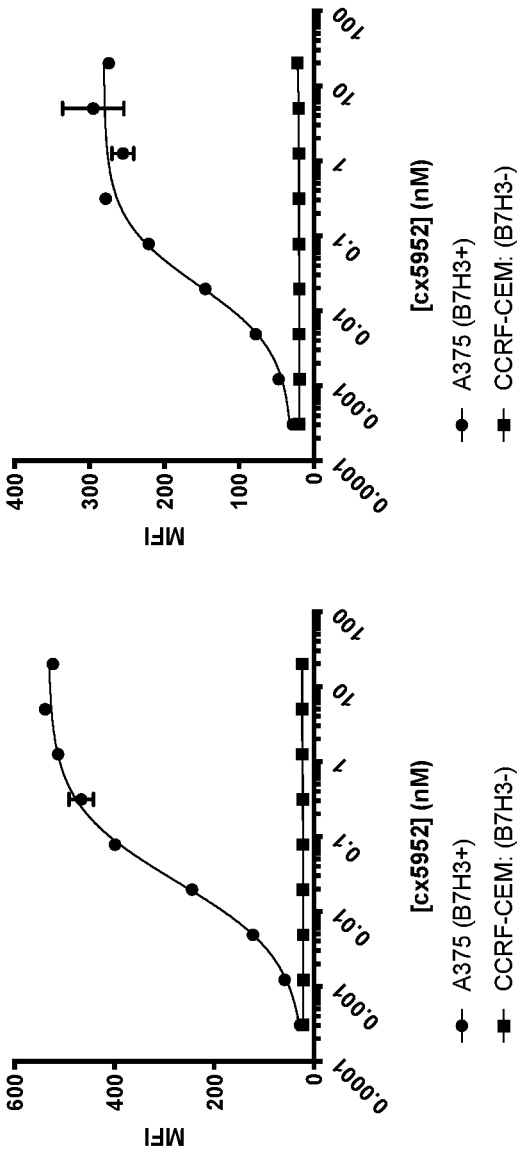
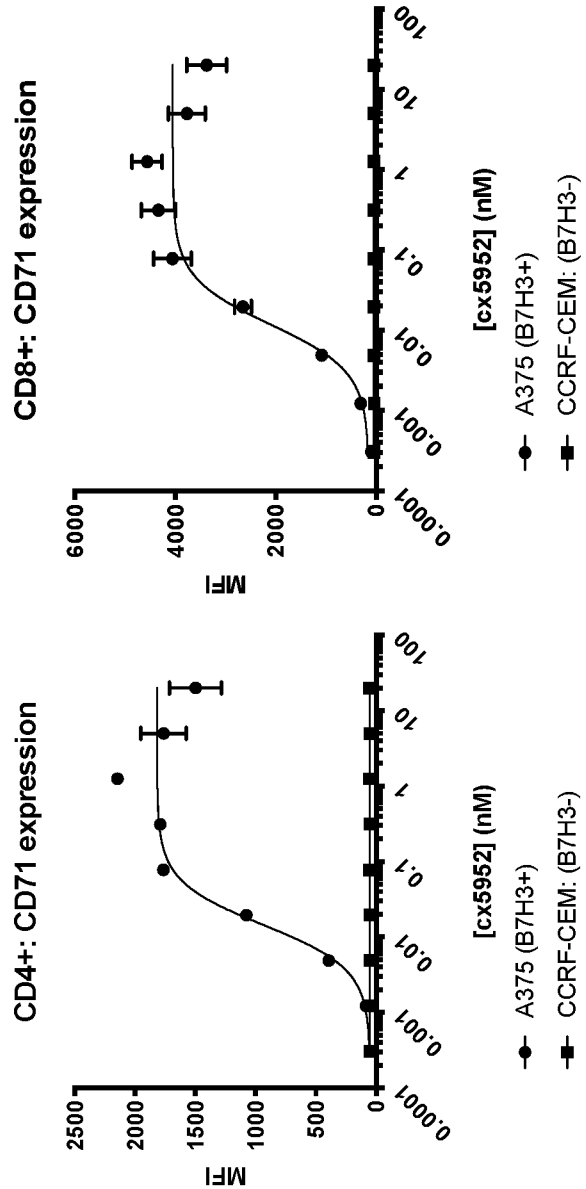

CD4+: CD69 expression
MFI
600
400
200
0
0.0001
0.001
0.01
0.1
1
10
100
[cx5952] (nM)
A375 (B7H3+)
CCRF-CEM: (B7H3-)
CD8+: CD69 expression
MFI
400
300
200
100
0
0.0001
0.001
0.01
0.1
1
10
100
[cx5952] (nM)
A375 (B7H3+)
CCRF-CEM: (B7H3-)

FIG. 11C


CD4+: CD71 expression
MFI
2500
2000
1500
1000
500
0
0.0001
0.001
0.01
0.1
1
10
100
[cx5952] (nM)
A375 (B7H3+)
CCRF-CEM: (B7H3-)
CD8+: CD71 expression
MFI
6000
4000
2000
0
0.0001
0.001
0.01
0.1
1
10
100
[cx5952] (nM)
A375 (B7H3+)
CCRF-CEM: (B7H3-)

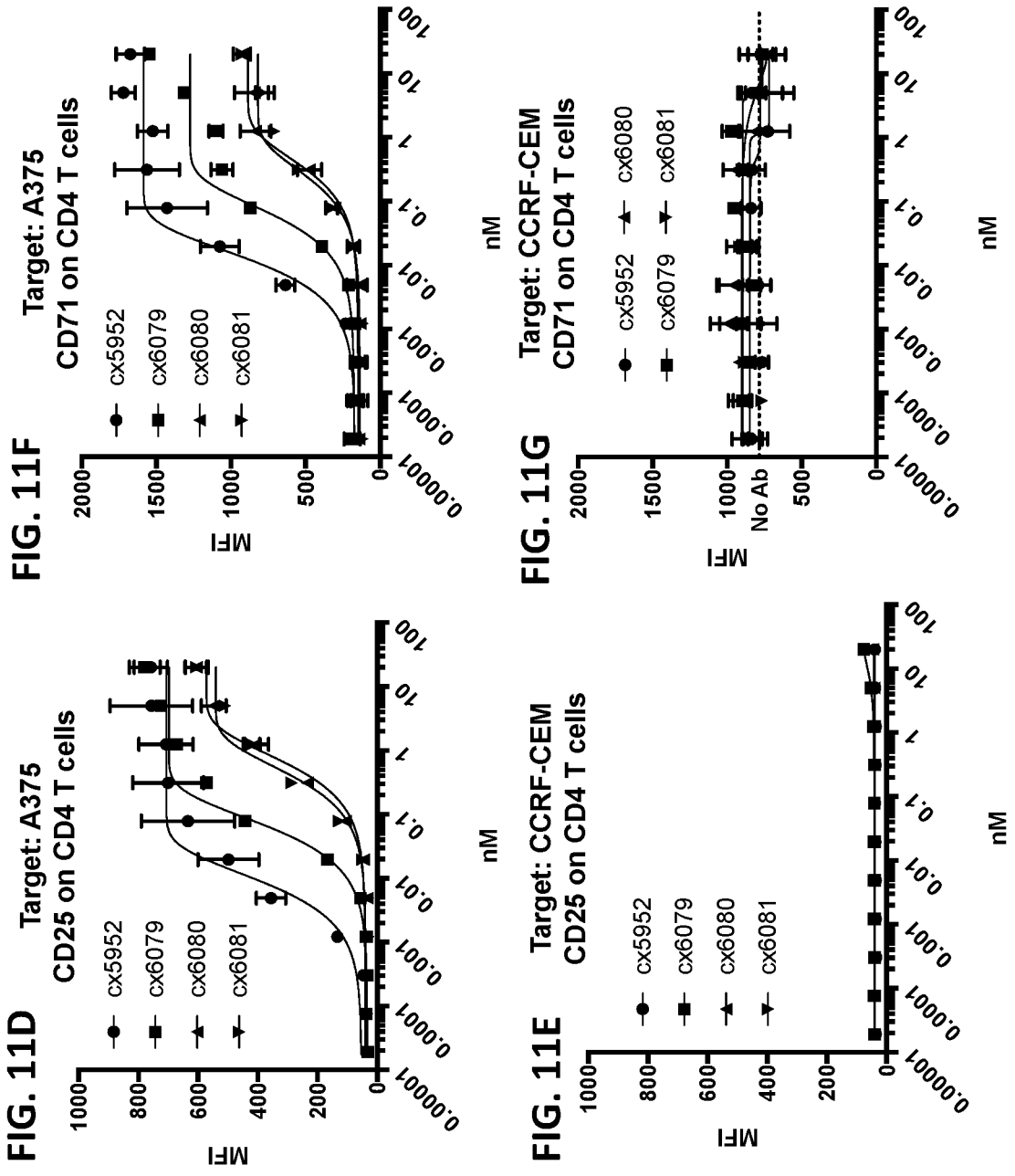


FIG. 11D
Target: A375
CD25 on CD4 T cells
cx5952
cx6079
cx6080
cx6081
MFI
nM
FIG. 11F
Target: A375
CD71 on CD4 T cells
FIG. 11E
Target: CCRF-CEM
CD25 on CD4 T cells
FIG. 11G
Target: CCRF-CEM
CD71 on CD4 T cells
No Ab

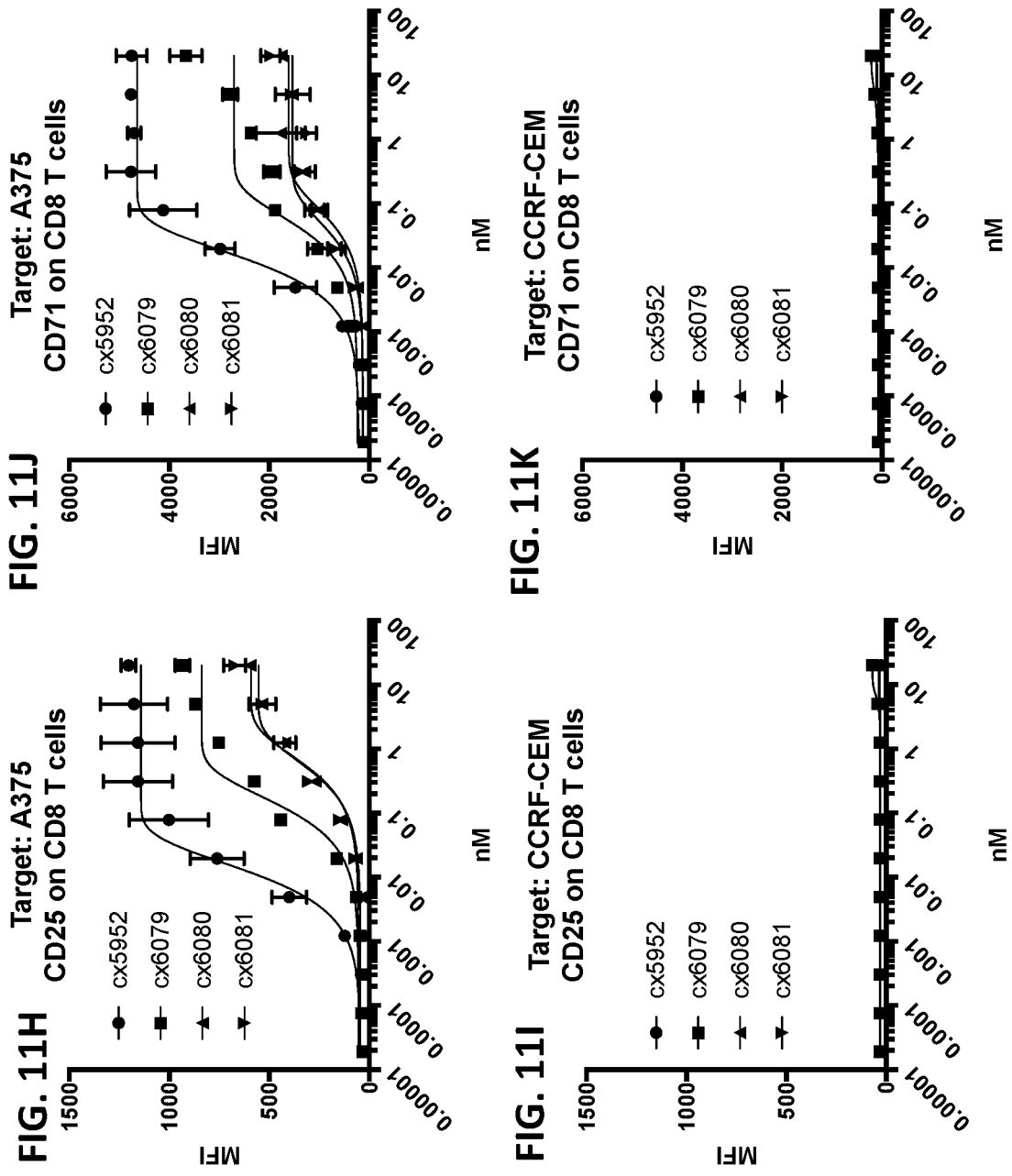


FIG. 11H
Target: A375
CD25 on CD8 T cells
cx5952
cx6079
cx6080
cx6081
MFI
1500
1000
500
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
nM
FIG. 11I
Target: CCRF-CEM
CD25 on CD8 T cells
FIG. 11J
Target: A375
CD71 on CD8 T cells
6000
4000
2000
FIG. 11K
Target: CCRF-CEM
CD71 on CD8 T cells

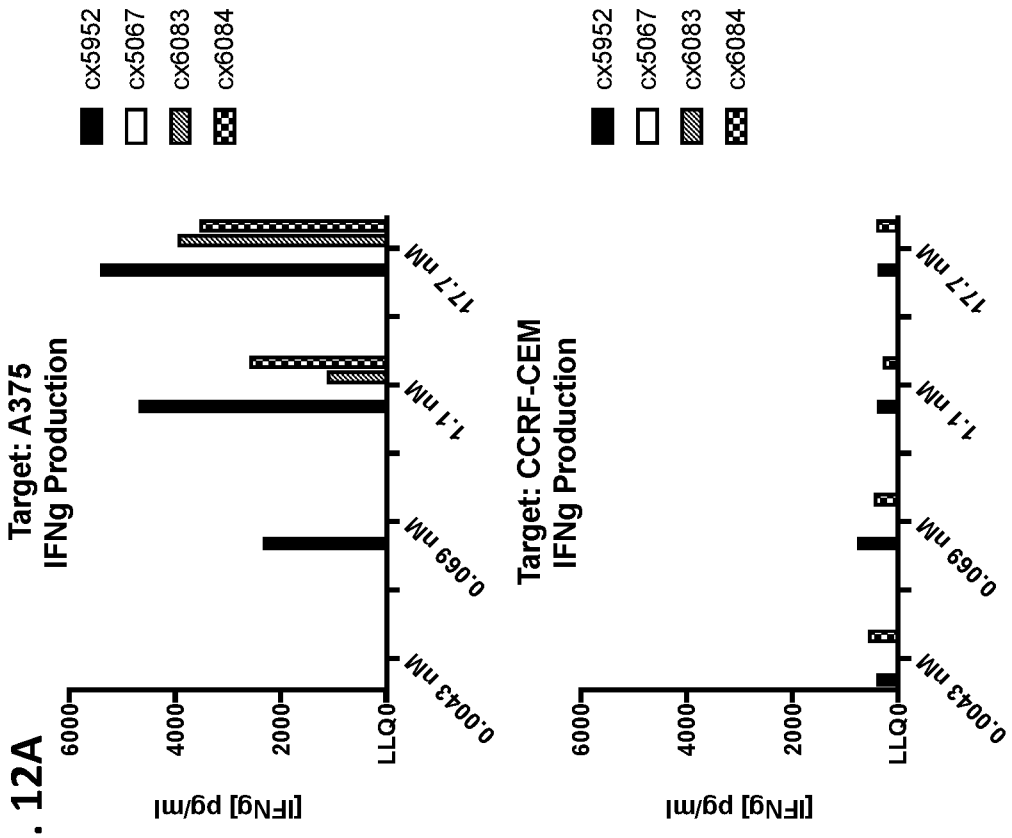

Target: A375
IFNg Production
cx5952
cx5067
cx6083
cx6084
[IFNg] pg/ml
6000
4000
2000
LLQ0
0.0043 nM
0.069 nM
1.1 nM
17.7 nM
Target: CCRF-CEM
IFNg Production
cx5952
cx5067
cx6083
cx6084
[IFNg] pg/ml
6000
4000
2000
LLQ0
0.0043 nM
0.069 nM
1.1 nM
17.7 nM

FIG. 12A

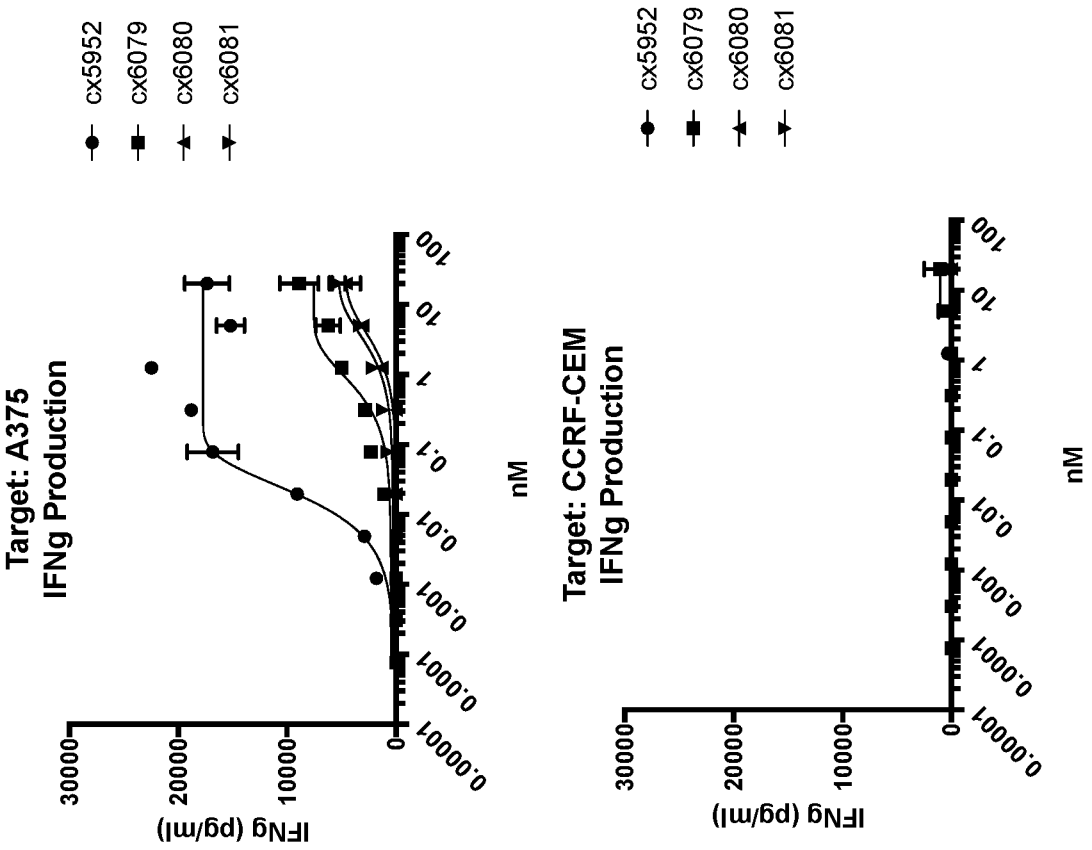

Target: A375
IFNg Production
cx5952
cx6079
cx6080
cx6081
IFNg (pg/ml)
30000
20000
10000
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
nM
Target: CCRF-CEM
IFNg Production
cx5952
cx6079
cx6080
cx6081
IFNg (pg/ml)
30000
20000
10000
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
nM

FIG. 12B

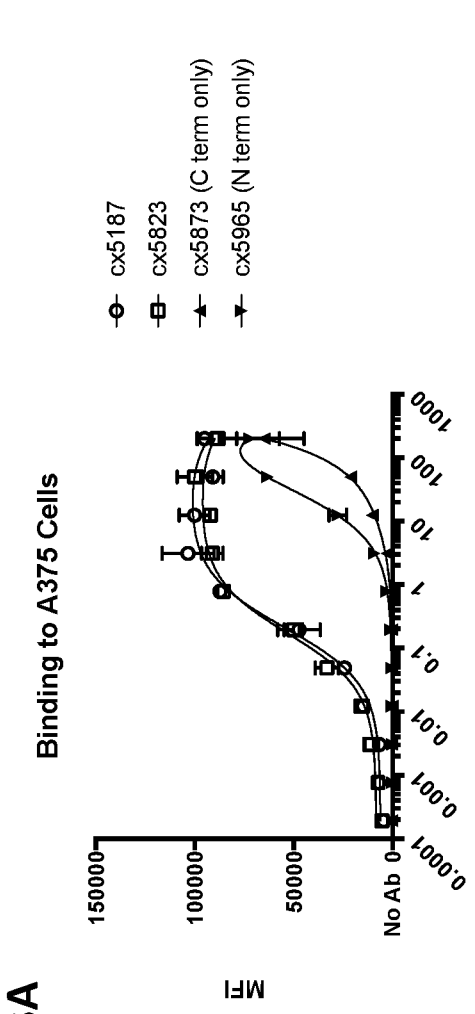


FIG. 13A

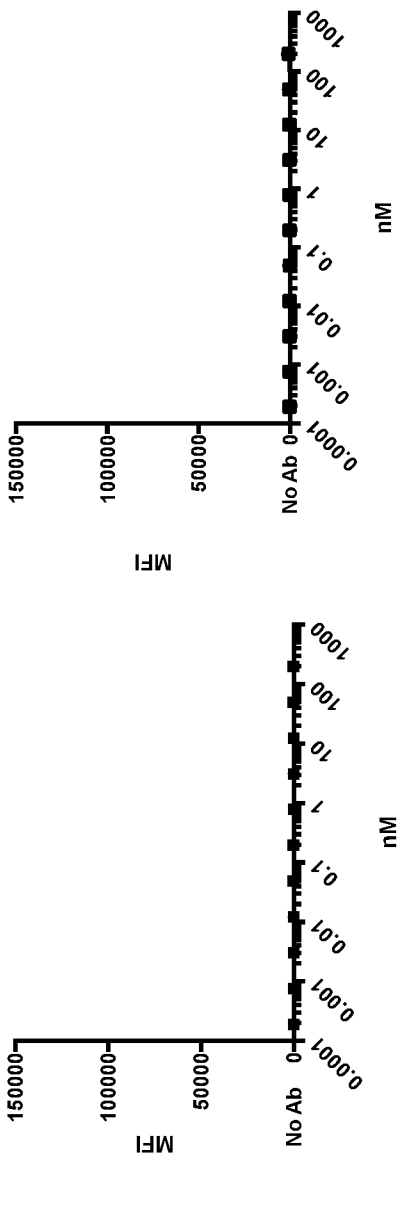


FIG. 13B

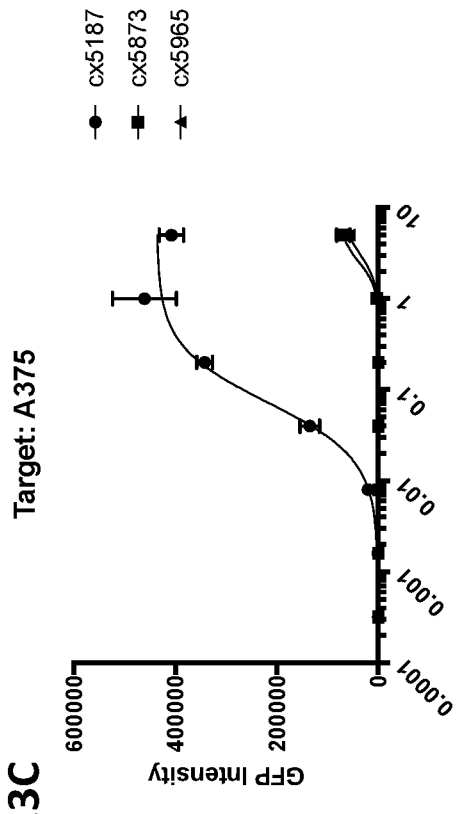


FIG. 13C

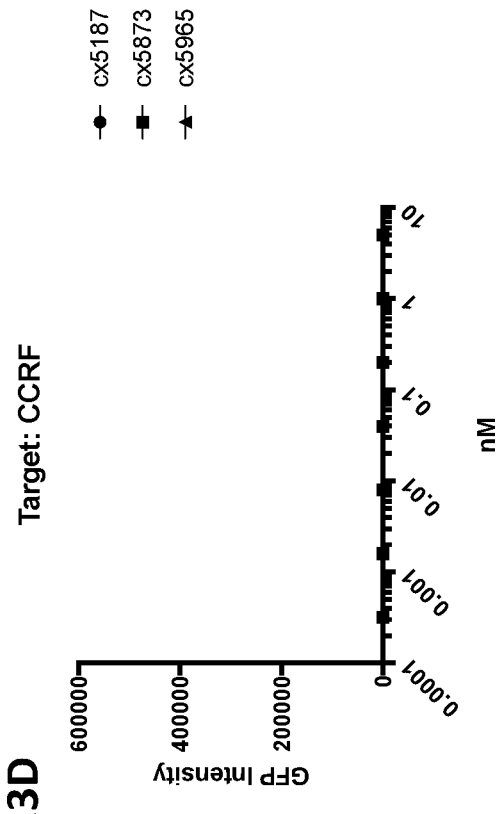


FIG. 13D

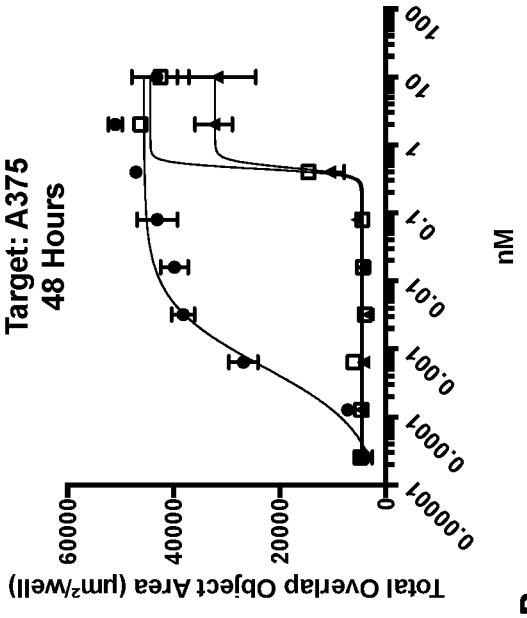


FIG. 14A

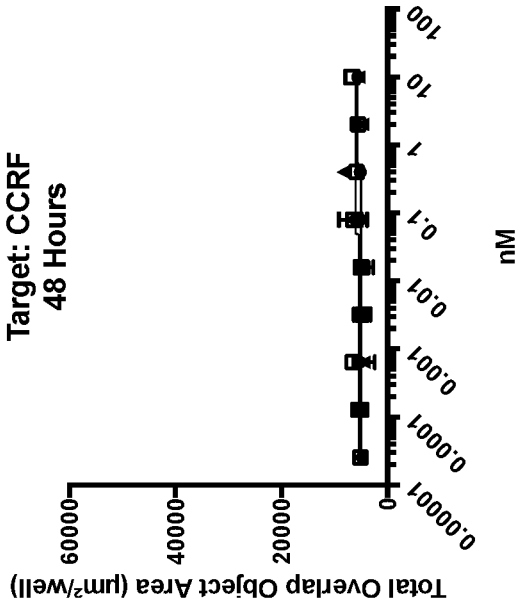


FIG. 14B

FIG. 15A

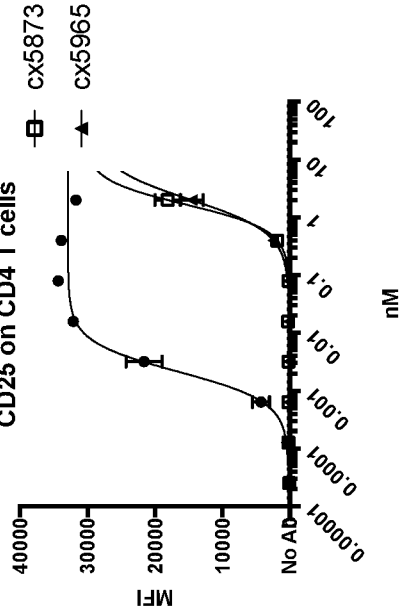


FIG. 15B

FIG. 15C

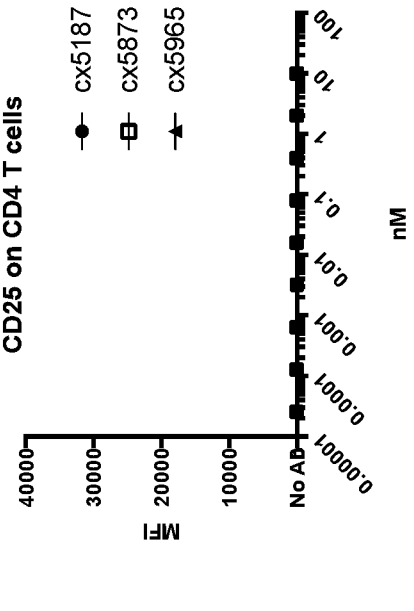


FIG. 15D

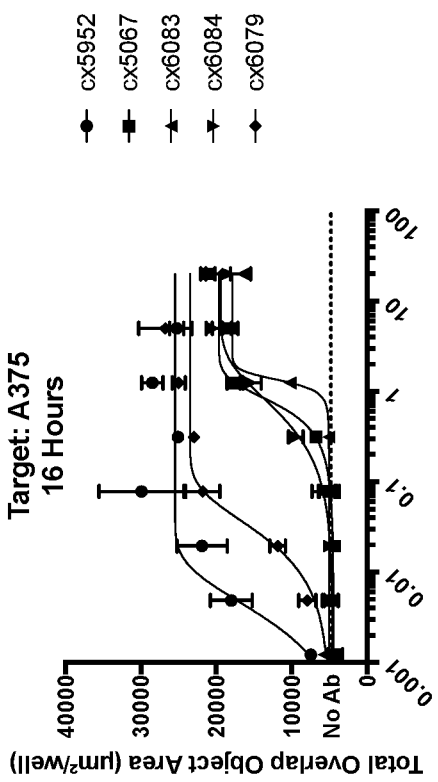


FIG. 16A

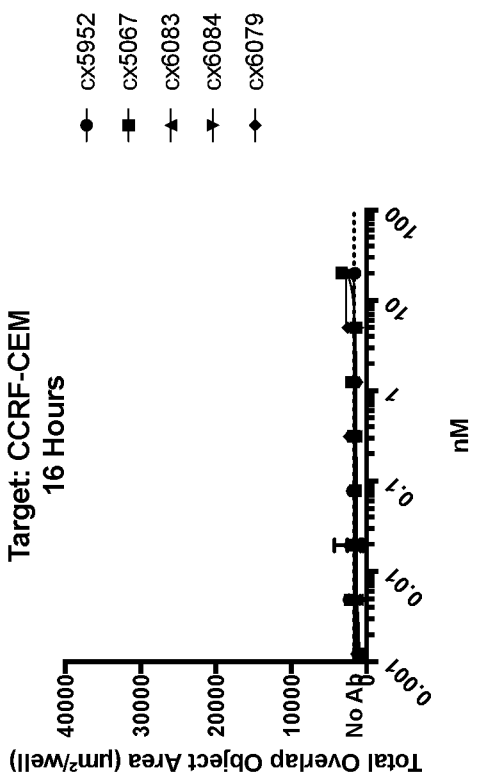


FIG. 16B

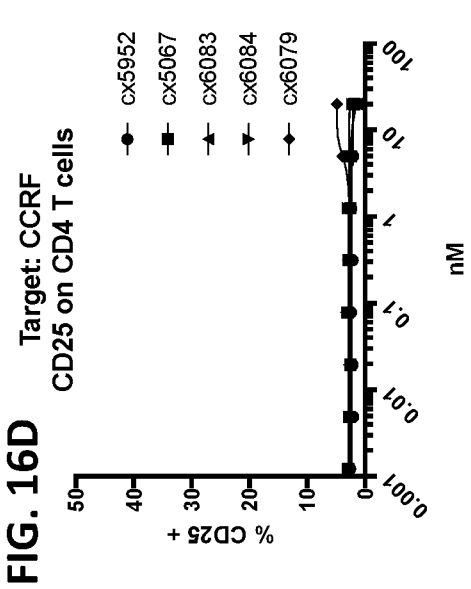


FIG. 16D

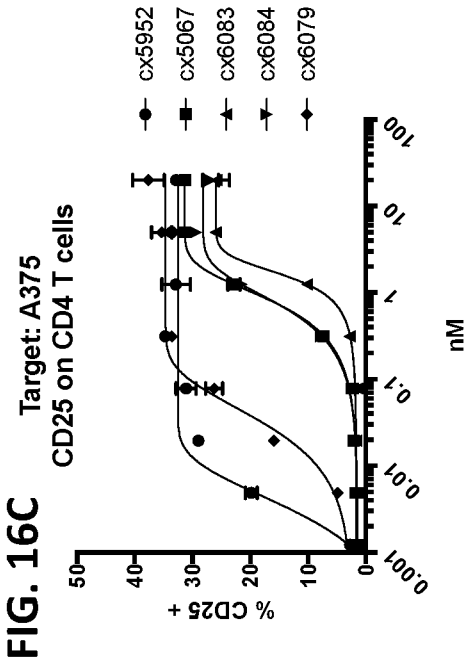


FIG. 16C

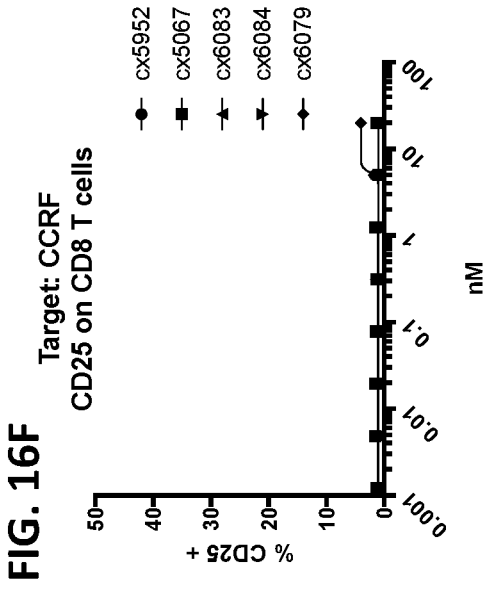


FIG. 16F

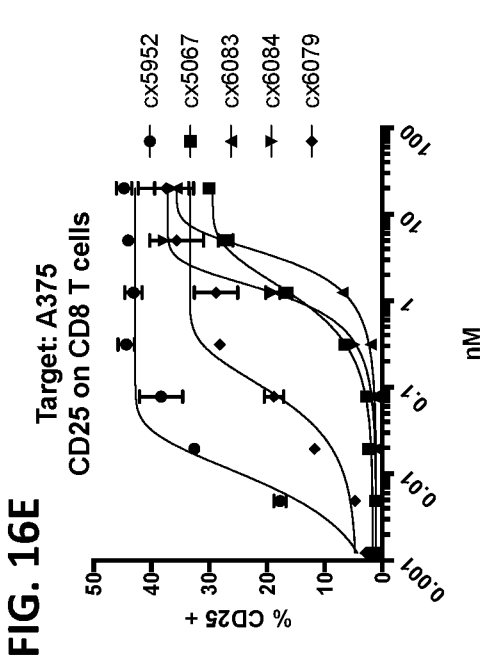


FIG. 16E

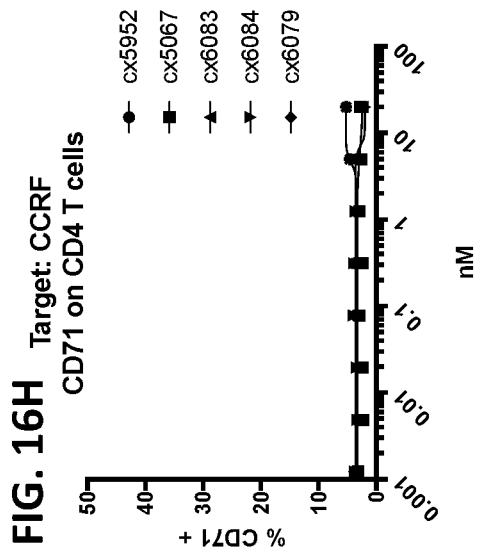


FIG. 16H

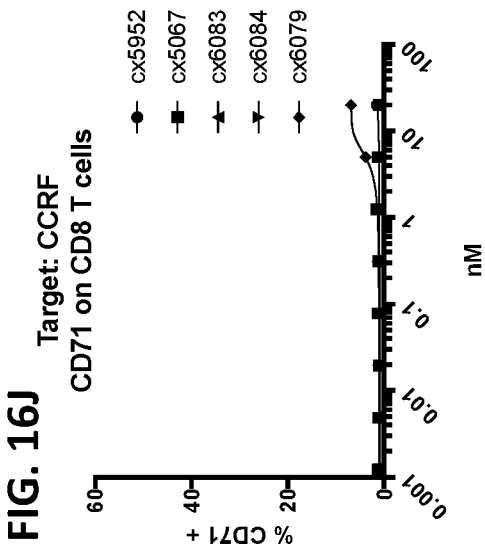


FIG. 16J

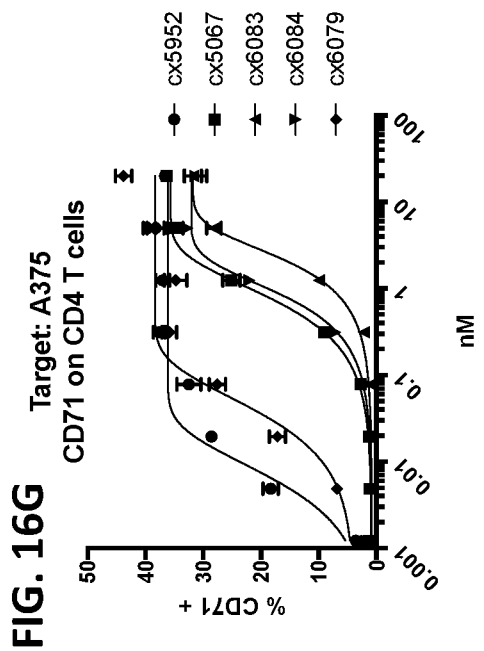


FIG. 16G

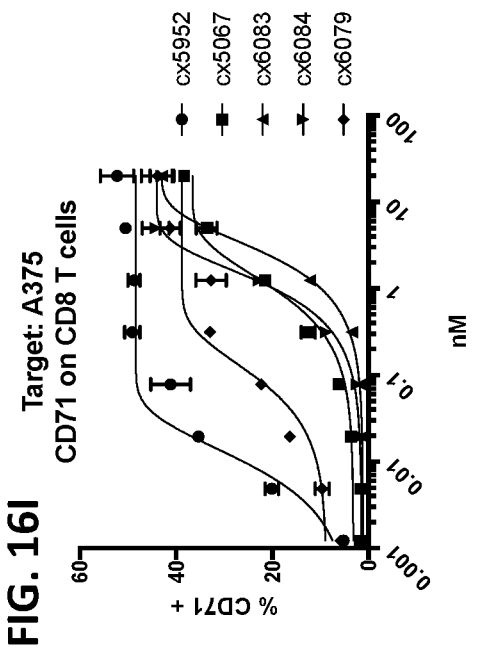


FIG. 16I

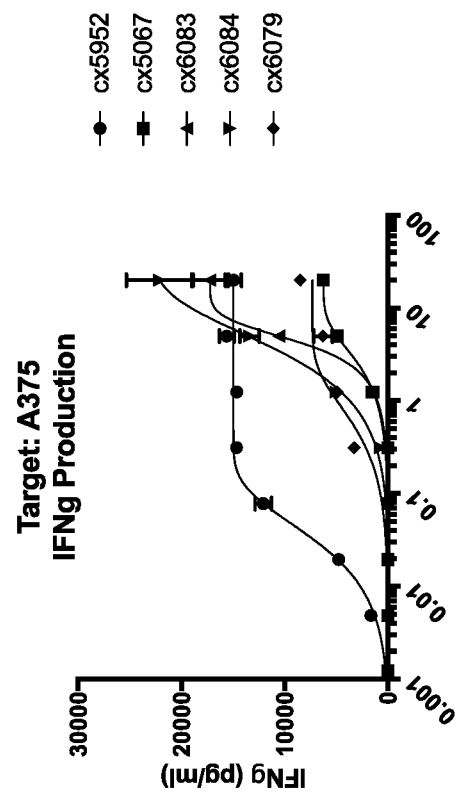


FIG. 16K

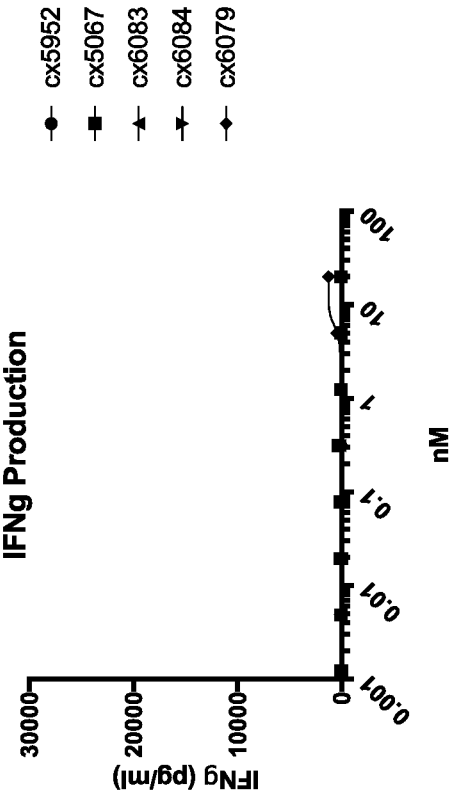


FIG. 16L

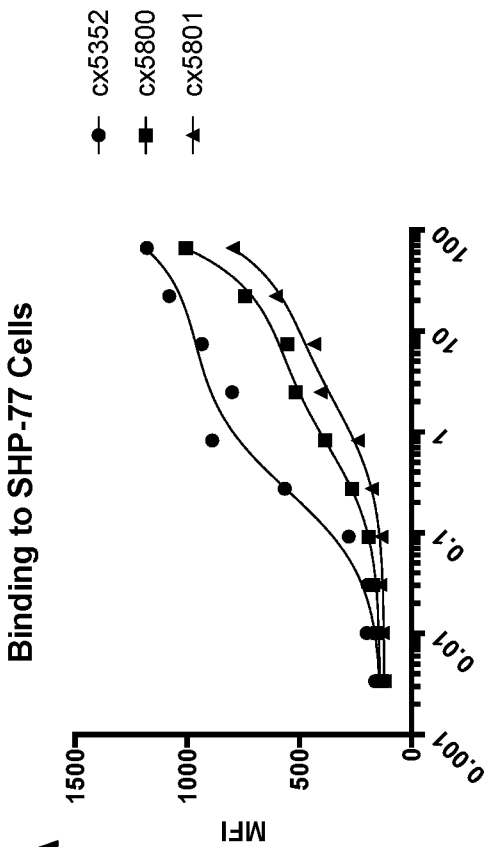


FIG. 17A

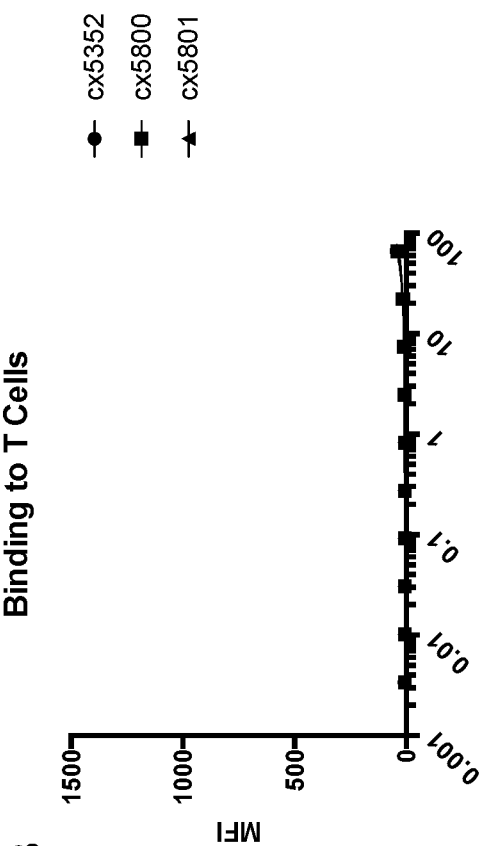


FIG. 17B

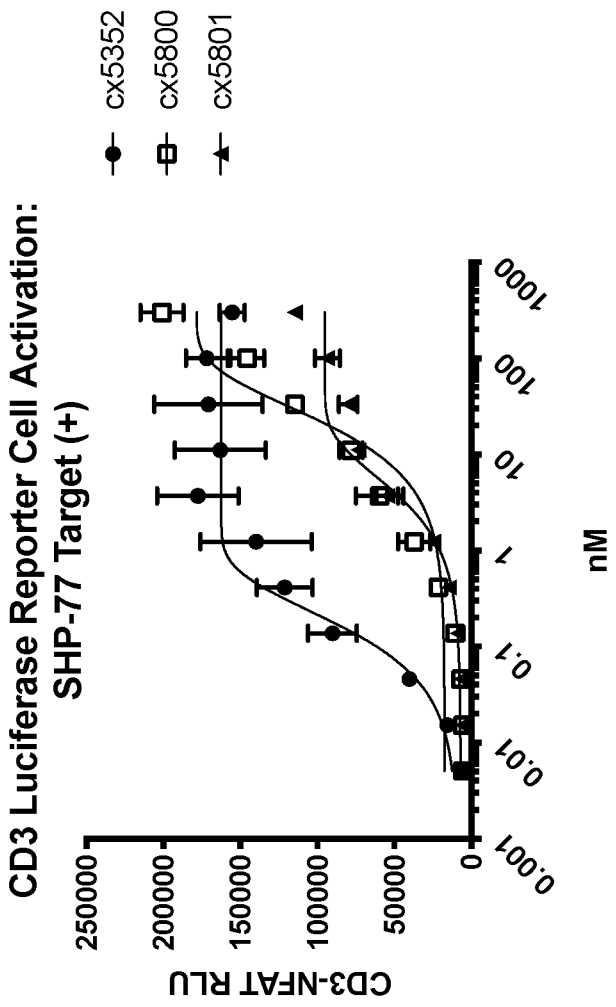

CD3 Luciferase Reporter Cell Activation:
SHP-77 Target (+)
cx5352
cx5800
cx5801
CD3-NFAT RLU
250000
200000
150000
100000
50000
0
0.001
0.01
0.1
1
10
100
1000
nM

FIG. 17C

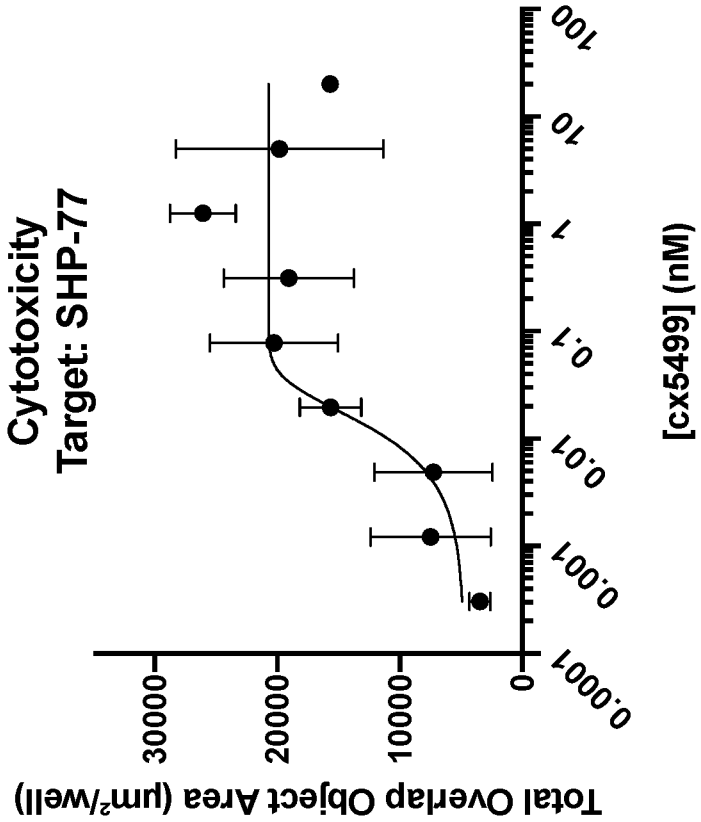

Cytotoxicity
Target: SHP-77
Total Overlap Object Area (μm²/well)
30000
20000
10000
0
0.0001
0.001
0.01
0.1
1
10
100
[cx5499] (nM)

FIG. 18A

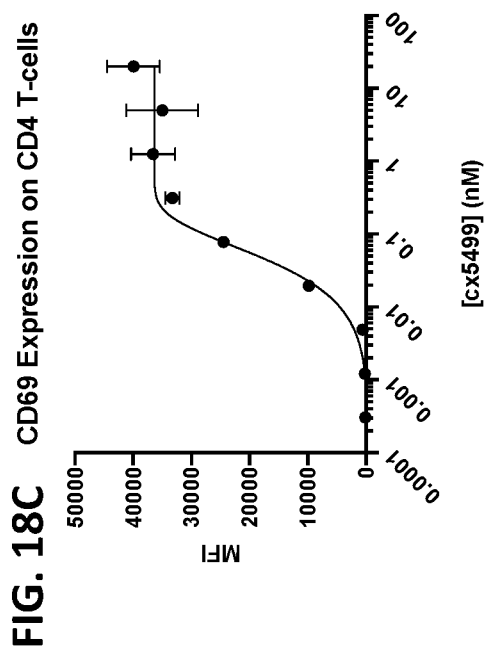


FIG. 18C

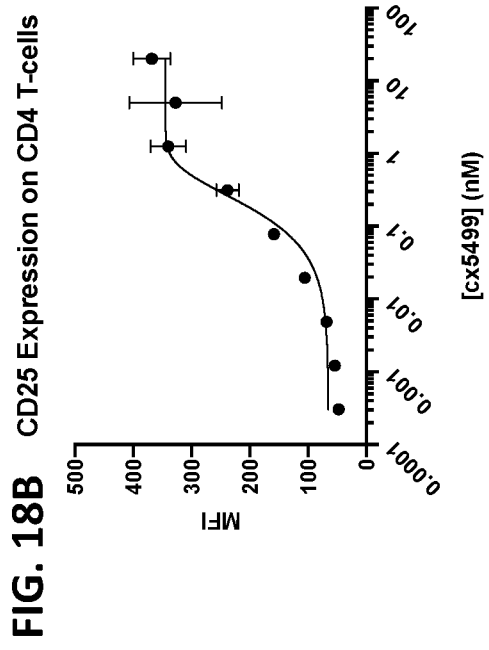


FIG. 18B

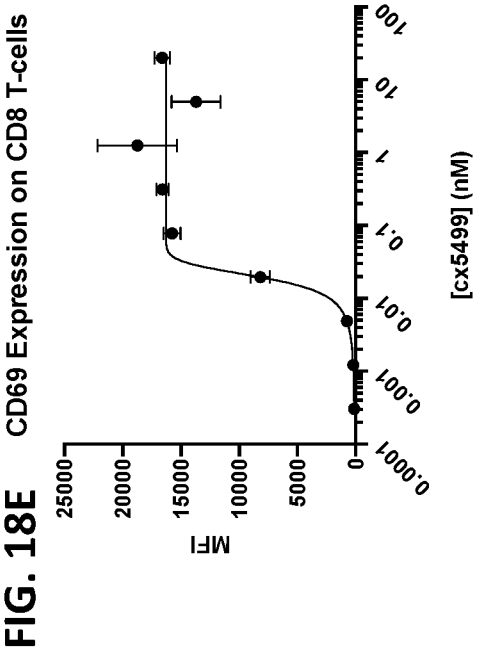


FIG. 18E

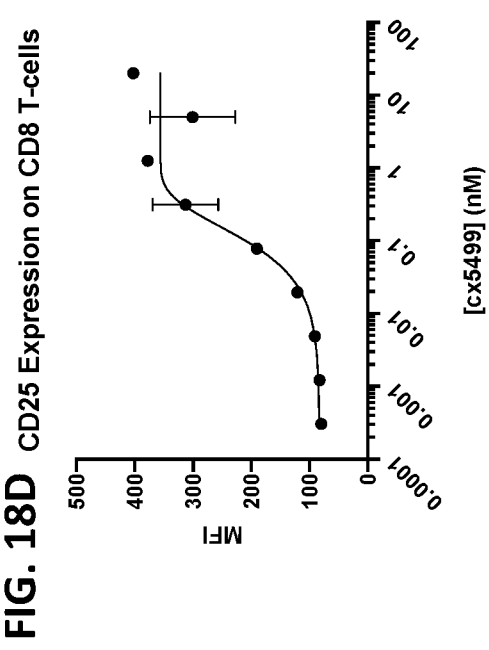


FIG. 18D