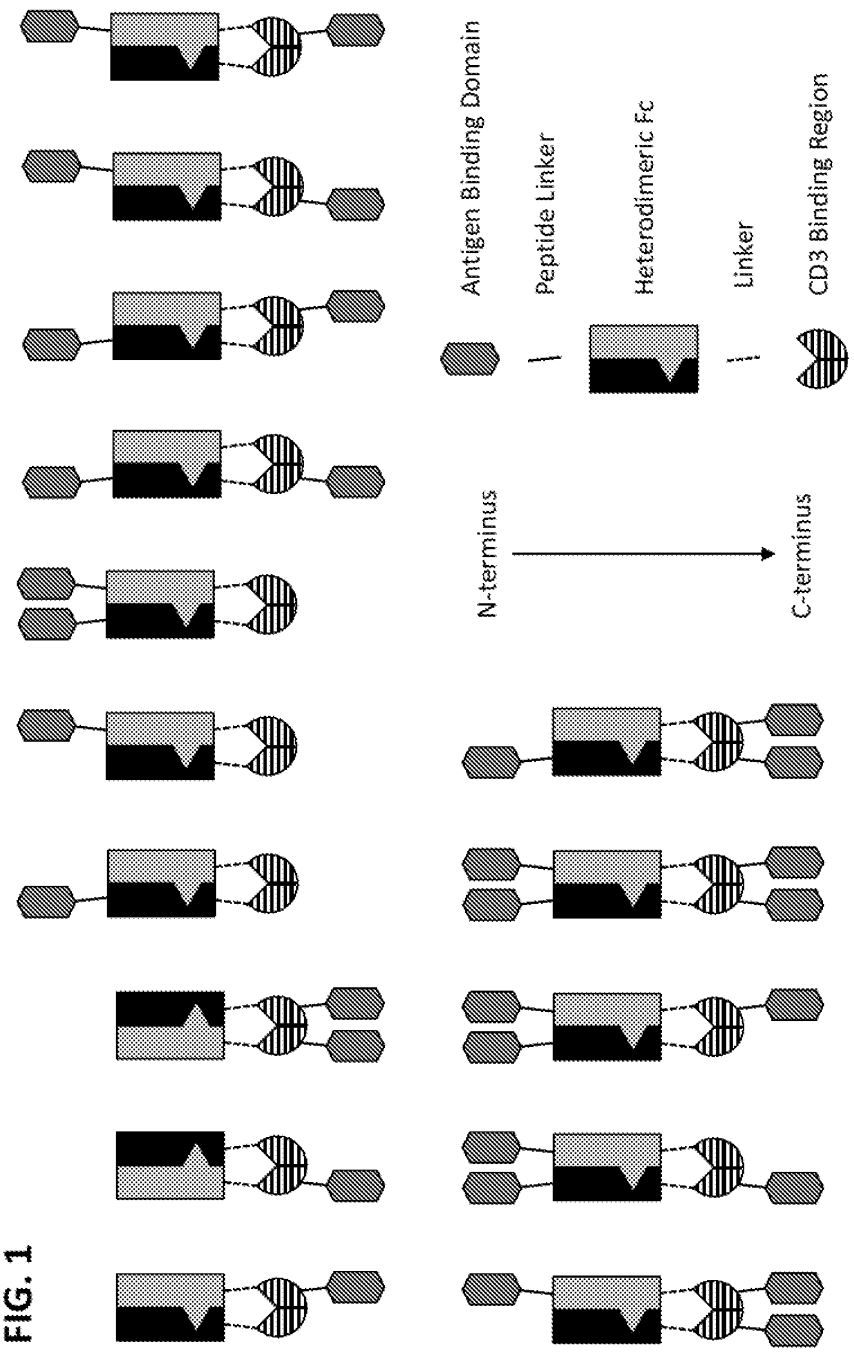

Antigen Binding Domain
Peptide Linker
Heterodimeric Fc
Linker
CD3 Binding Region
N-terminus
C-terminus

FIG. 1

FIG. 2

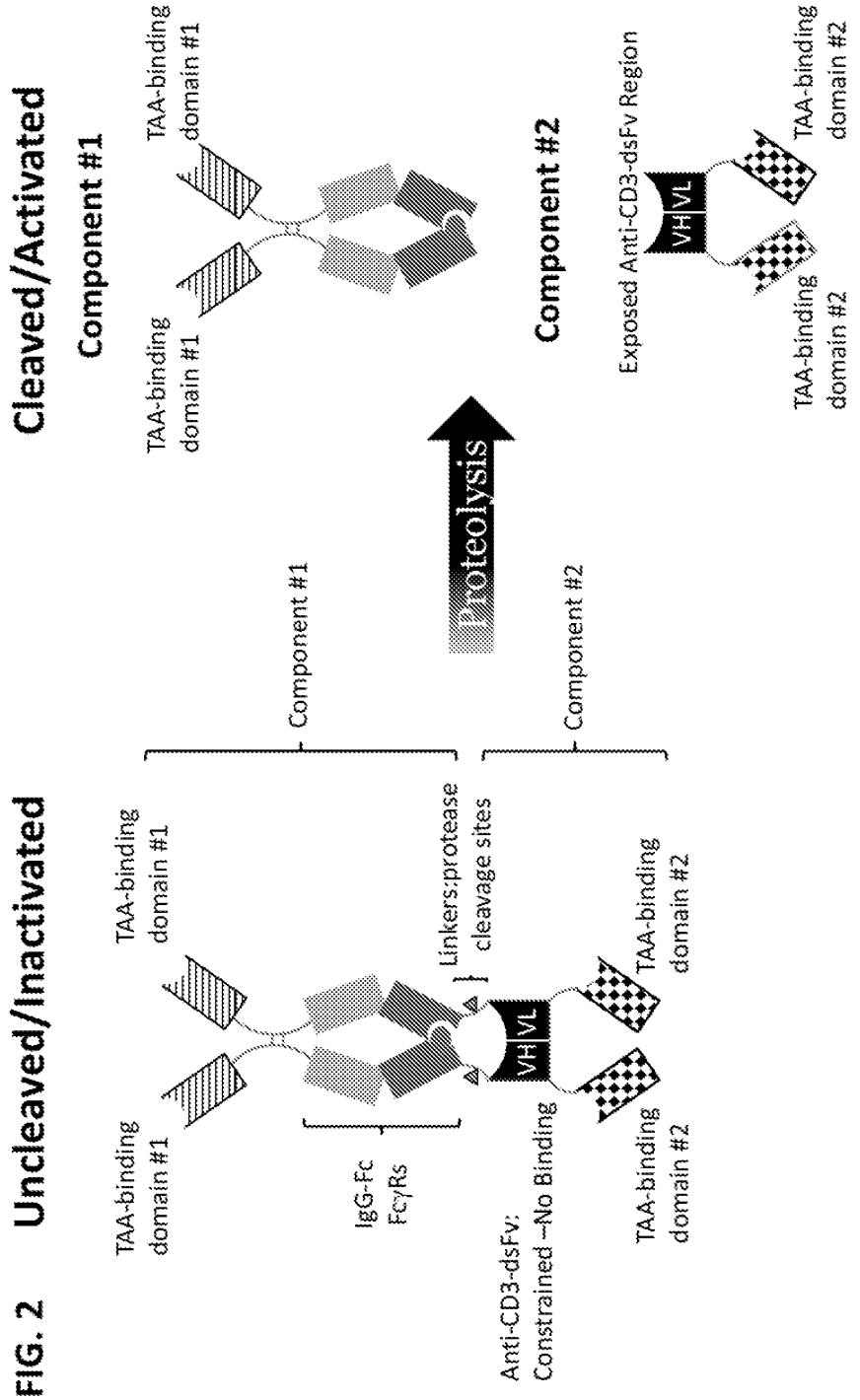

Uncleaved/Inactivated
TAA-binding domain #1
TAA-binding domain #1
IgG-Fc
FcγRs
Linkers:protease cleavage sites
VH
VL
Anti-CD3-dsFv:
Constrained –No Binding
TAA-binding domain #2
TAA-binding domain #2
Component #1
Component #2
Proteolysis
Cleaved/Activated
Component #1
TAA-binding domain #1
TAA-binding domain #1
Component #2
Exposed Anti-CD3-dsFv Region
VH
VL
TAA-binding domain #2
TAA-binding domain #2

FIG. 3

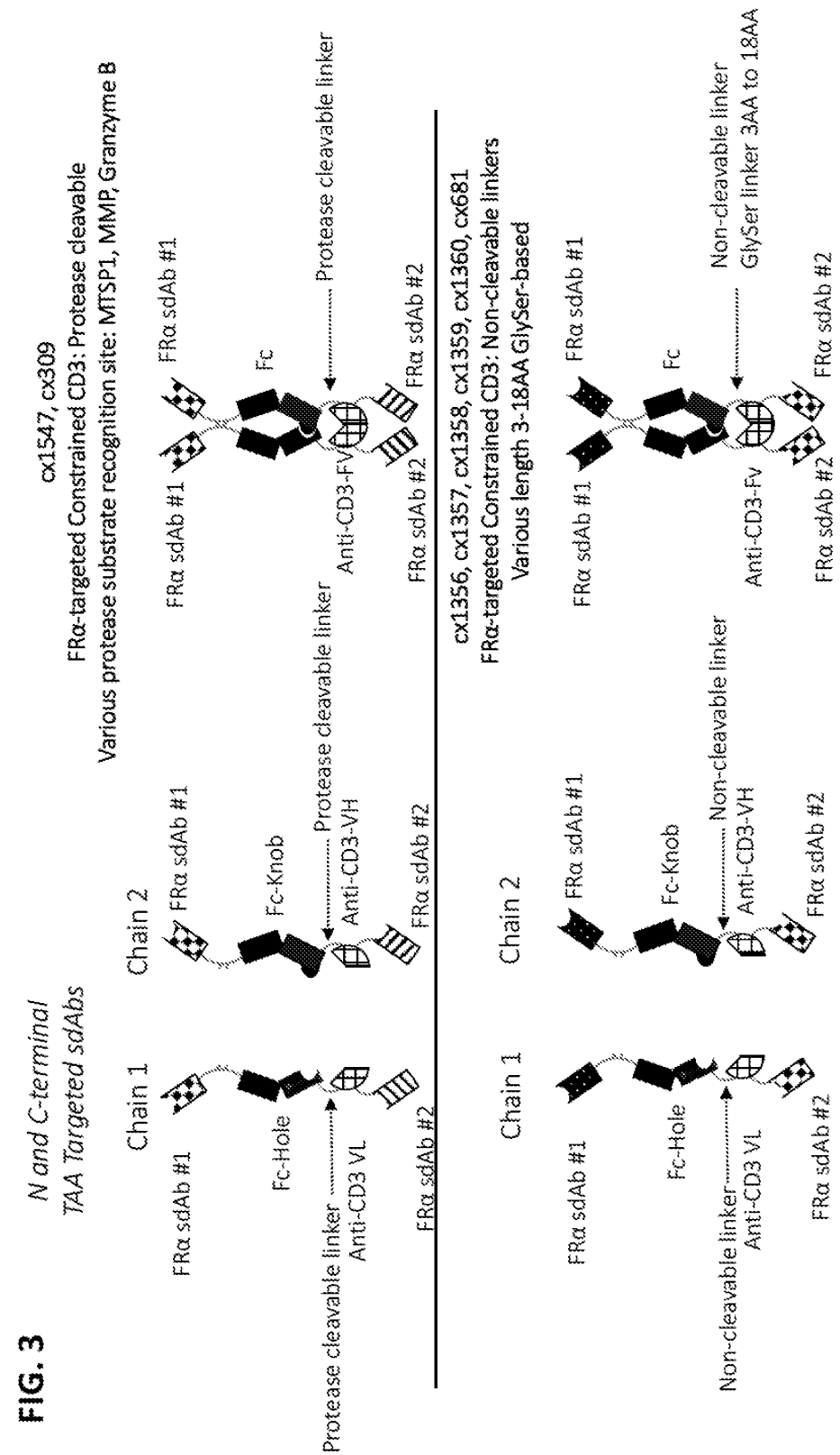

N and C-terminal
TAA Targeted sdAbs
cx1547, cx309
FRα-targeted Constrained CD3: Protease cleavable
Various protease substrate recognition site: MTSP1, MMP, Granzyme B
Chain 1
FRα sdAb #1
Fc-Hole
Protease cleavable linker
Anti-CD3 VL
FRα sdAb #2
Chain 2
FRα sdAb #1
Fc-Knob
Protease cleavable linker
Anti-CD3-VH
FRα sdAb #2
FRα sdAb #1
Fc
Protease cleavable linker
Anti-CD3-Fv
FRα sdAb #2
cx1356, cx1357, cx1358, cx1359, cx1360, cx681
FRα-targeted Constrained CD3: Non-cleavable linkers
Various length 3-18AA GlySer-based
Chain 1
FRα sdAb #1
Fc-Hole
Non-cleavable linker
Anti-CD3 VL
FRα sdAb #2
Chain 2
FRα sdAb #1
Fc-Knob
Non-cleavable linker
Anti-CD3-VH
FRα sdAb #2
FRα sdAb #1
Fc
Non-cleavable linker
GlySer linker 3AA to 18AA
Anti-CD3-Fv
FRα sdAb #2

FIG. 4A

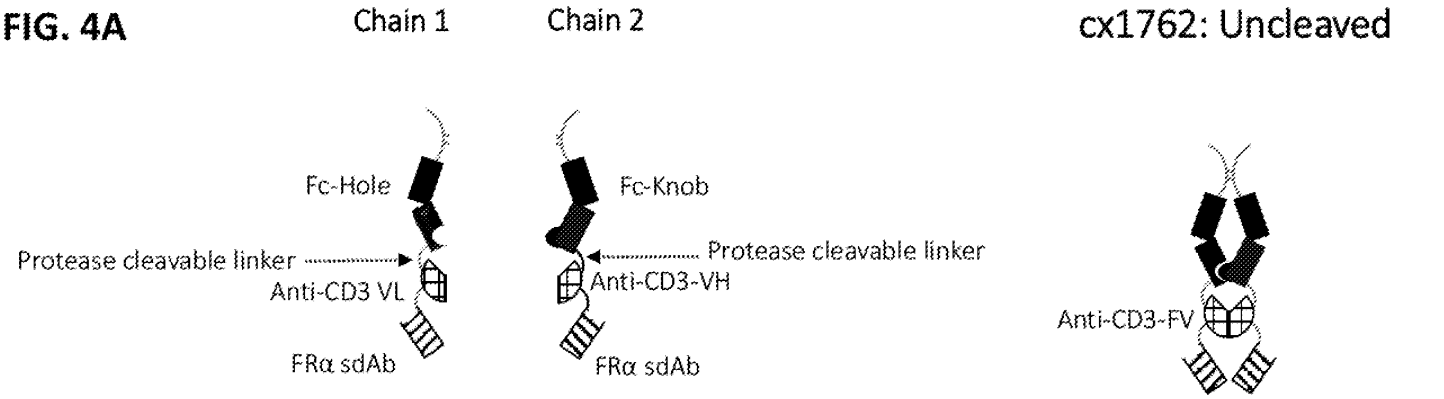


FIG. 4B

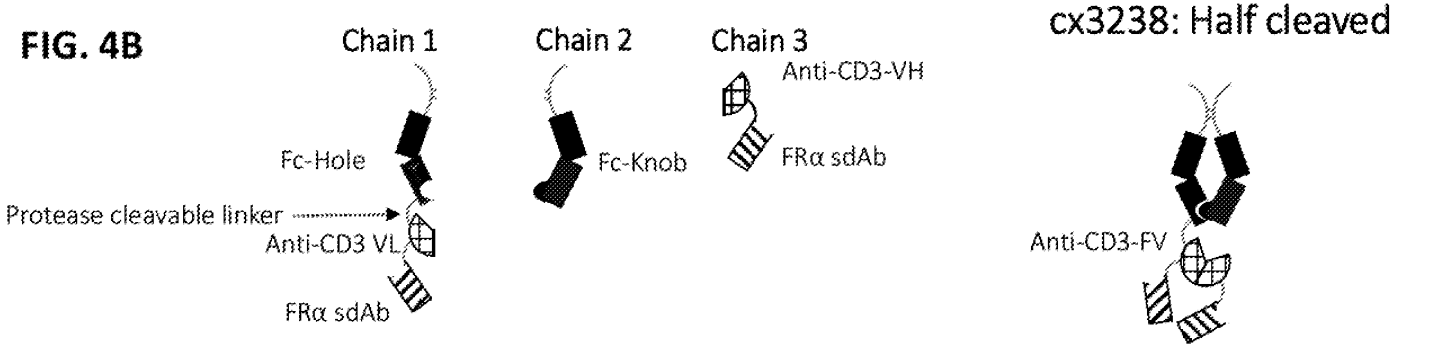


FIG. 4C

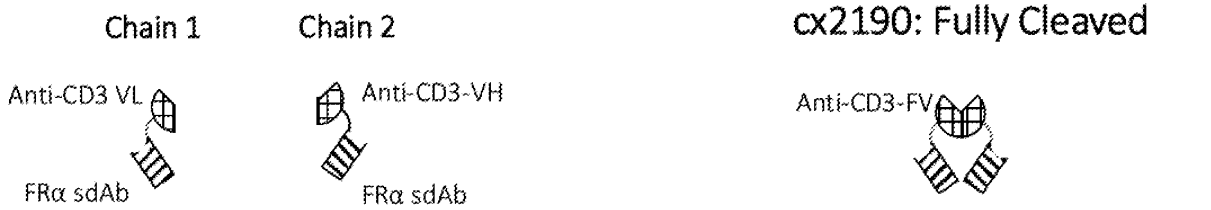


FIG. 5B

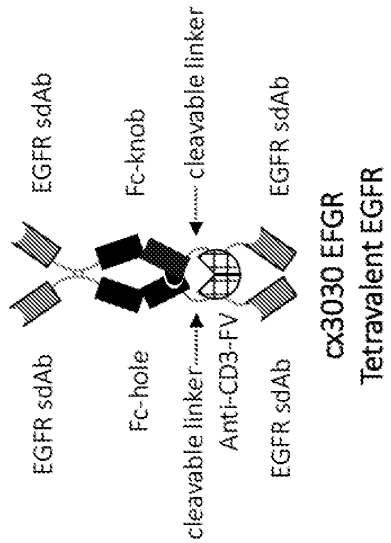

EGFR sdAb
Fc-knob
cleavable linker
EGFR sdAb
EGFR sdAb
Fc-hole
cleavable linker
Anti-CD3-FV
EGFR sdAb
cx3030 EFGR
Tetravalent EGFR

FIG. 5A

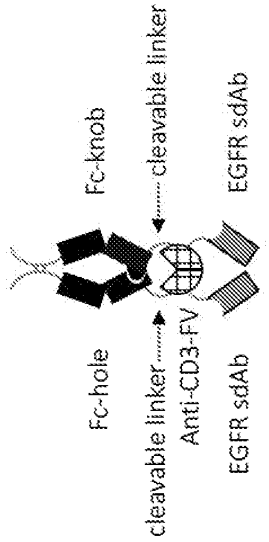
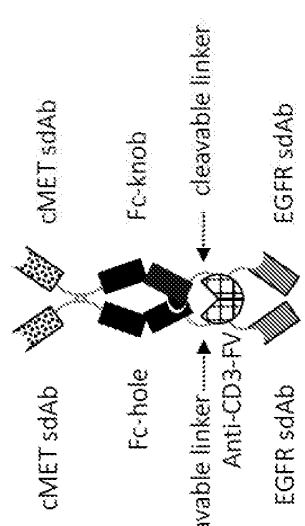
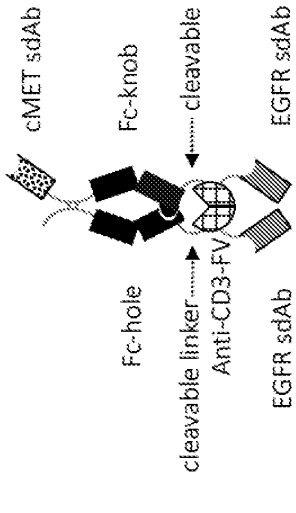

Fc-knob
cleavable linker
EGFR sdAb
Fc-hole
cleavable linker
Anti-CD3-FV
EGFR sdAb
cx2513 EFGR
Bivalent EGFR

FIG. 5C



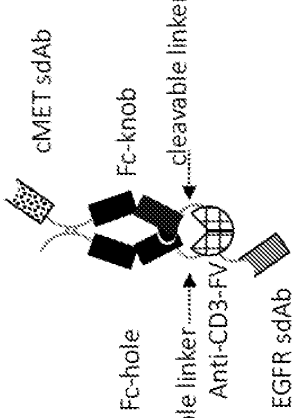
cx2973 cMet x EFGR
Bivalent cMet
Bivalent EGFR

FIG. 5D



cx2979 cMet x EFGR
Monovalent cMet
Bivalent EGFR

FIG. 5E



cx2977 cMet x EFGR
Monovalent cMet
Monovalent EGFR

FIG. 6A

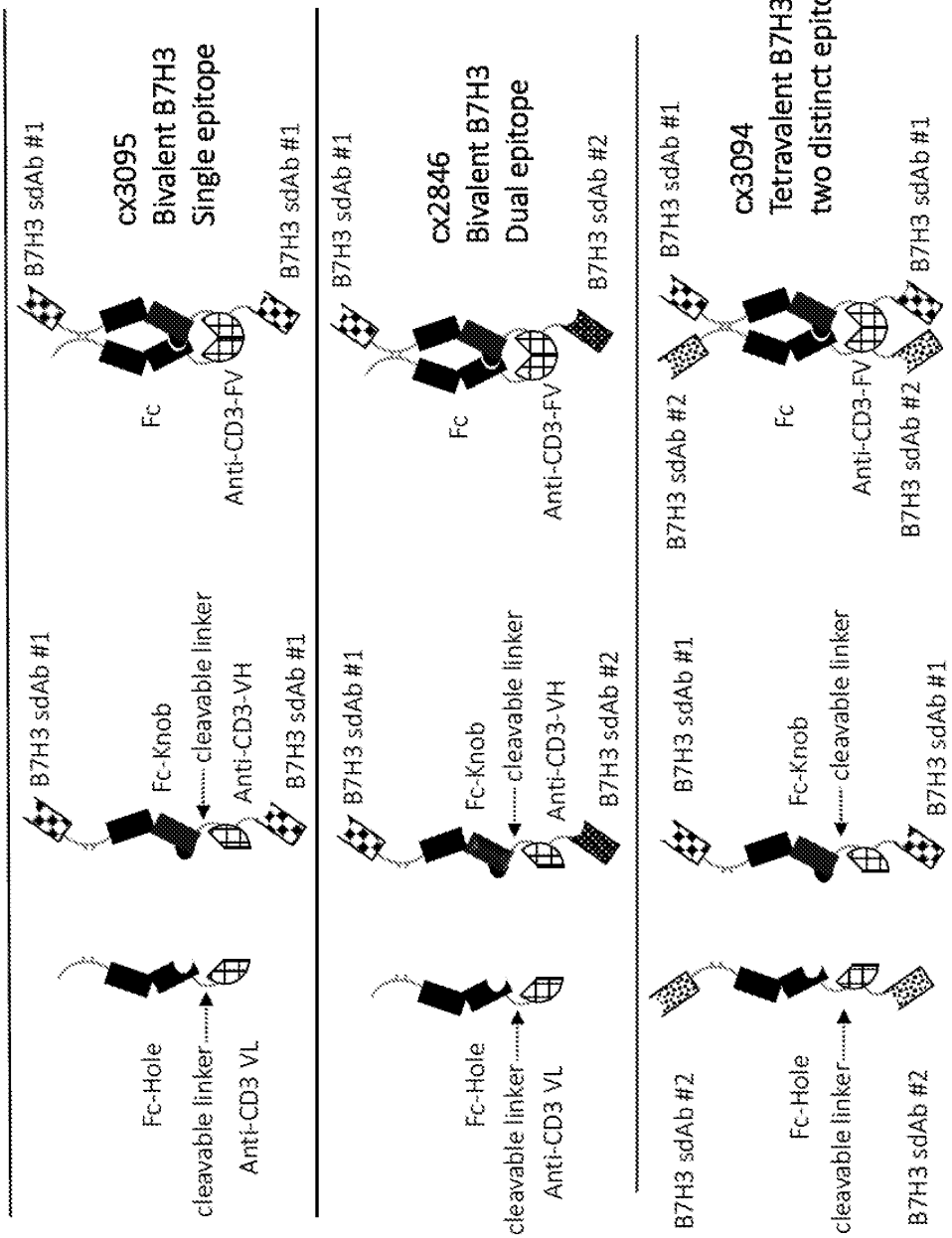

Chain 1
Chain 2
Fc-Hole
cleavable linker
Anti-CD3 VL
B7H3 sdAb #1
Fc-Knob
cleavable linker
Anti-CD3-VH
B7H3 sdAb #1
Fc
Anti-CD3-FV
B7H3 sdAb #1
cx3095
Bivalent B7H3
Single epitope
Fc-Hole
cleavable linker
Anti-CD3 VL
B7H3 sdAb #1
Fc-Knob
cleavable linker
Anti-CD3-VH
B7H3 sdAb #2
Fc
Anti-CD3-FV
B7H3 sdAb #2
cx2846
Bivalent B7H3
Dual epitope
B7H3 sdAb #2
B7H3 sdAb #1
Fc-Hole
cleavable linker
B7H3 sdAb #2
B7H3 sdAb #1
Fc-Knob
cleavable linker
B7H3 sdAb #1
Fc
Anti-CD3-FV
B7H3 sdAb #2
B7H3 sdAb #1
cx3094
Tetravalent B7H3,
two distinct epitopes

FIG. 6B

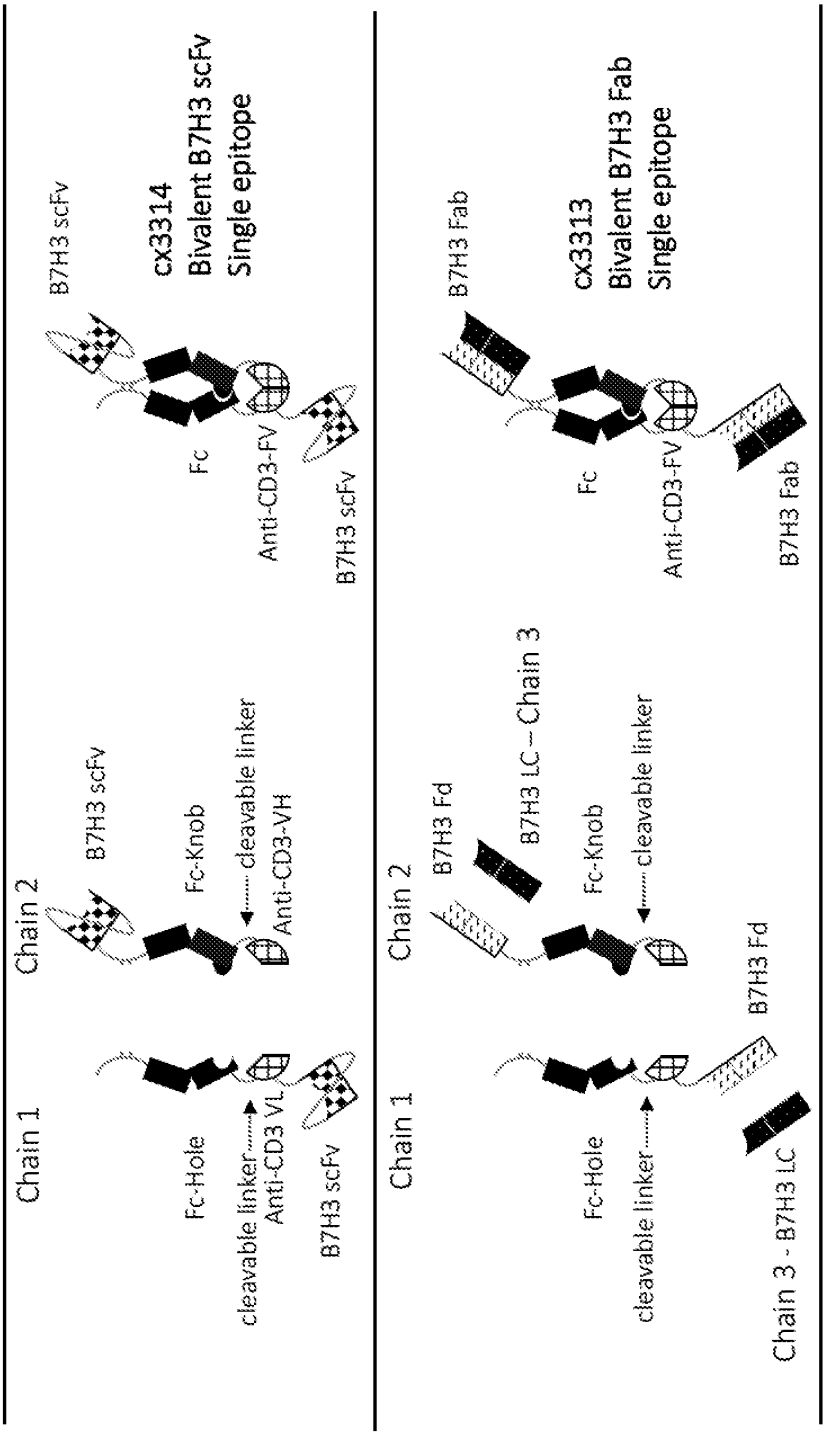
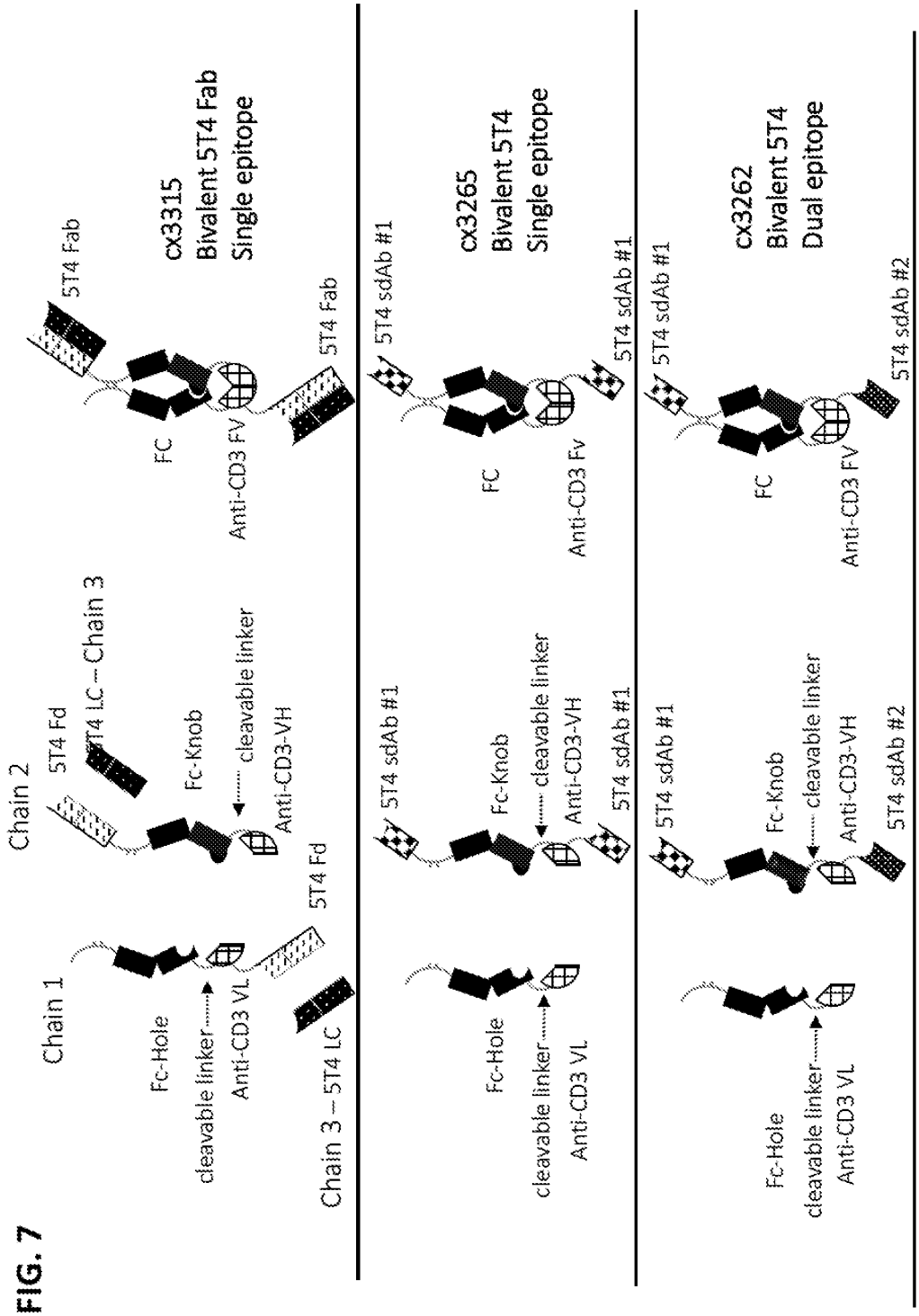

Chain 1
Chain 2
B7H3 scFv
Fc-Knob
cleavable linker
Anti-CD3-VH
Fc-Hole
cleavable linker
Anti-CD3 VL
B7H3 scFv
B7H3 scFv
Fc
Anti-CD3-FV
B7H3 scFv
cx3314
Bivalent B7H3 scFv
Single epitope
Chain 1
Chain 2
B7H3 Fd
B7H3 LC – Chain 3
Fc-Knob
cleavable linker
Fc-Hole
cleavable linker
B7H3 Fd
Chain 3 - B7H3 LC
B7H3 Fab
Fc
Anti-CD3-FV
B7H3 Fab
cx3313
Bivalent B7H3 Fab
Single epitope

FIG. 7


Chain 1
Fc-Hole
cleavable linker
Anti-CD3 VL
Chain 3 – 5T4 LC
Chain 2
5T4 Fd
5T4 LC – Chain 3
Fc-Knob
cleavable linker
Anti-CD3-VH
5T4 Fd
FC
Anti-CD3 FV
5T4 Fab
5T4 Fab
cx3315
Bivalent 5T4 Fab
Single epitope
Fc-Hole
cleavable linker
Anti-CD3 VL
5T4 sdAb #1
Fc-Knob
cleavable linker
Anti-CD3-VH
5T4 sdAb #1
FC
Anti-CD3 Fv
5T4 sdAb #1
cx3265
Bivalent 5T4
Single epitope
Fc-Hole
cleavable linker
Anti-CD3 VL
5T4 sdAb #1
Fc-Knob
cleavable linker
Anti-CD3-VH
5T4 sdAb #2
FC
Anti-CD3 FV
5T4 sdAb #2
cx3262
Bivalent 5T4
Dual epitope

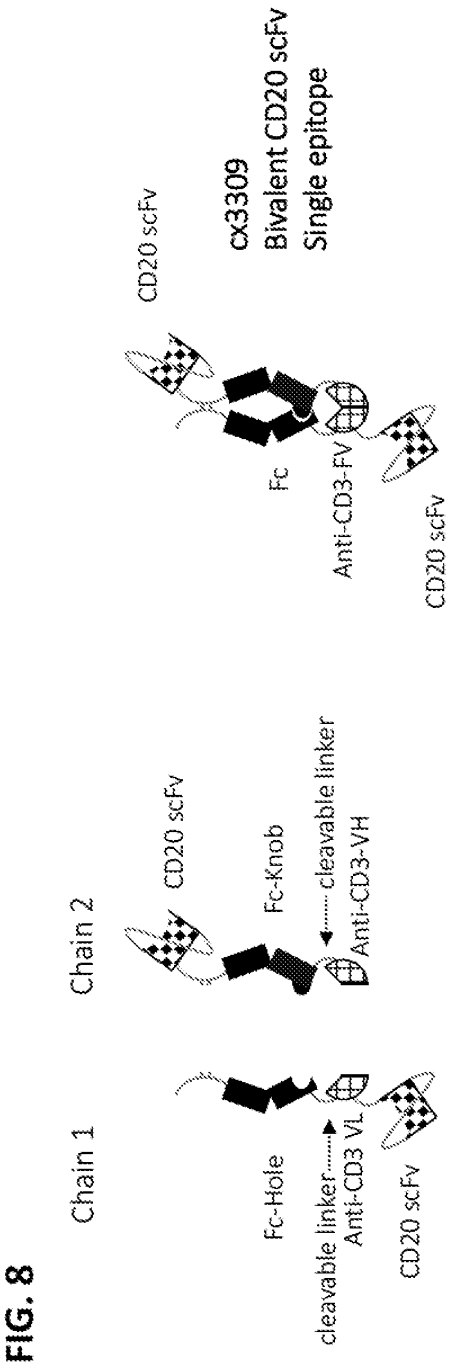
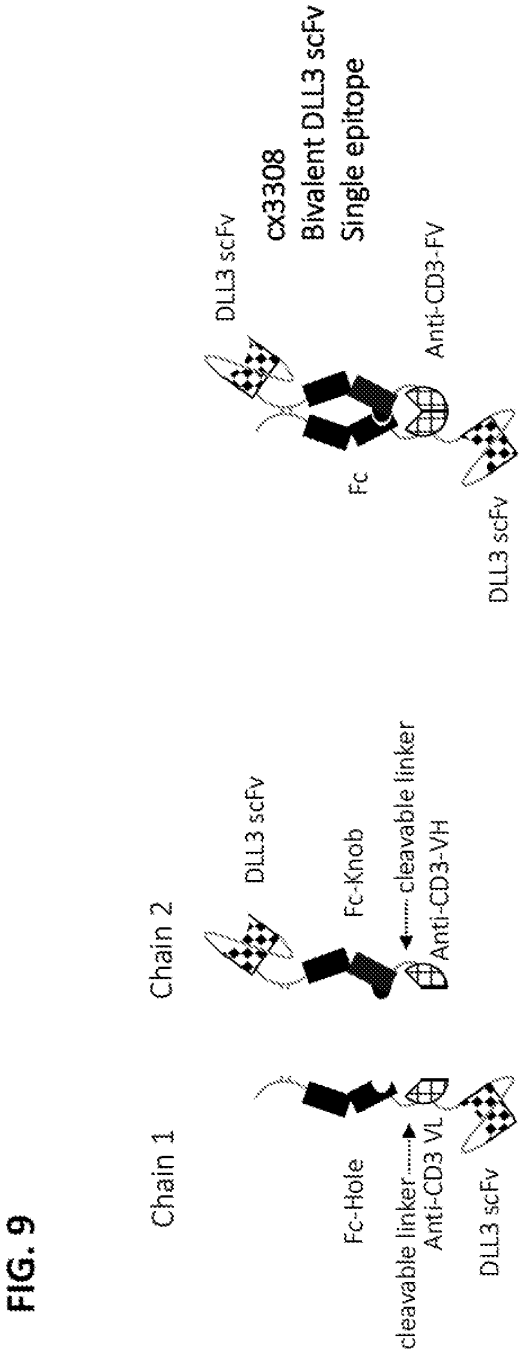

cx3309
Bivalent CD20 scFv
Single epitope
CD20 scFv
Fc
Anti-CD3-FV
CD20 scFv
Chain 2
CD20 scFv
Fc-Knob
cleavable linker
Anti-CD3-VH
Chain 1
Fc-Hole
cleavable linker
Anti-CD3 VL
CD20 scFv

FIG. 8

FIG. 9


cx3308
Bivalent DLL3 scFv
Single epitope
DLL3 scFv
Anti-CD3-FV
Fc
DLL3 scFv
Chain 2
DLL3 scFv
Fc-Knob
cleavable linker
Anti-CD3-VH
Chain 1
Fc-Hole
cleavable linker
Anti-CD3 VL
DLL3 scFv

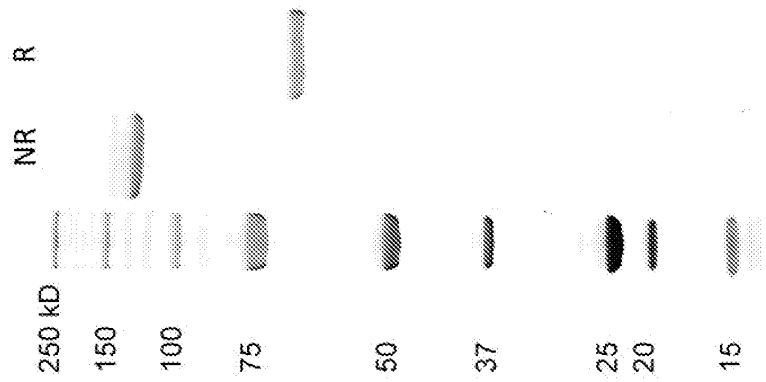
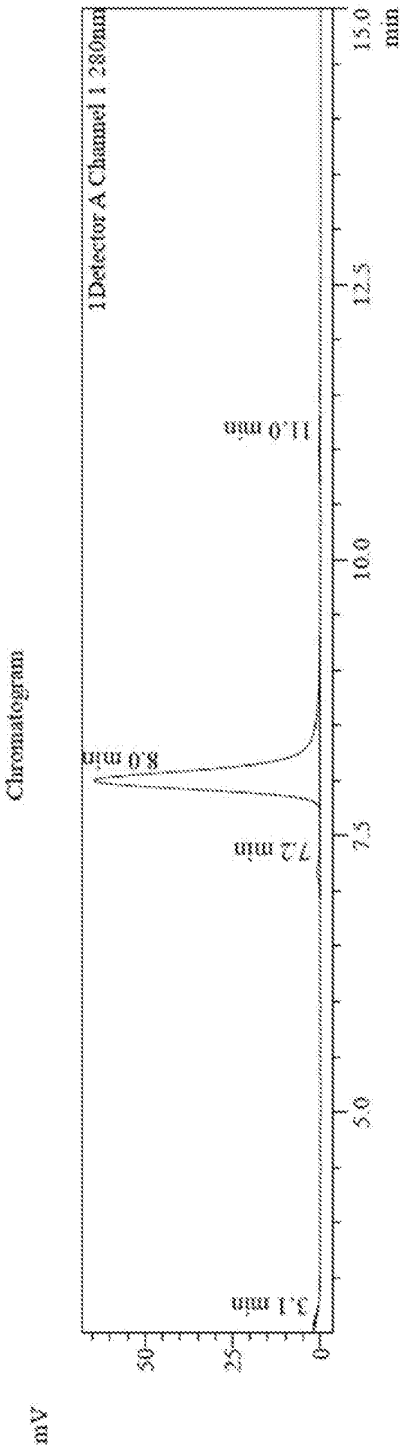


FIG. 10A

FIG. 10B



Peak Table

Detector A Channel 1 280nm

Ret. Time	Area	Area%
3.059 min	3065	0.346
7.174 min	9190	1.039
8.001 min	866802	97.960
10.993 min	5799	0.655
	884855	100.000

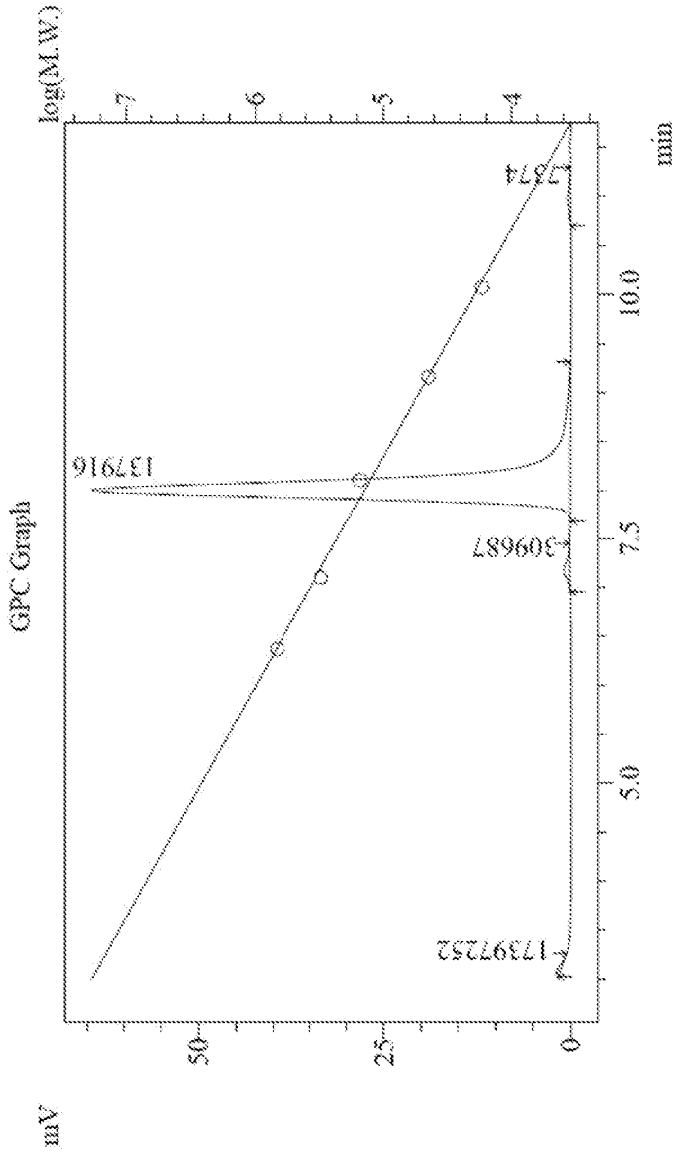

log(M.W.)
7
6
5
4
GPC Graph
mV
50
25
0
min
10.0
7.5
5.0
7374
1379716
309687
17397252

FIG. 10C

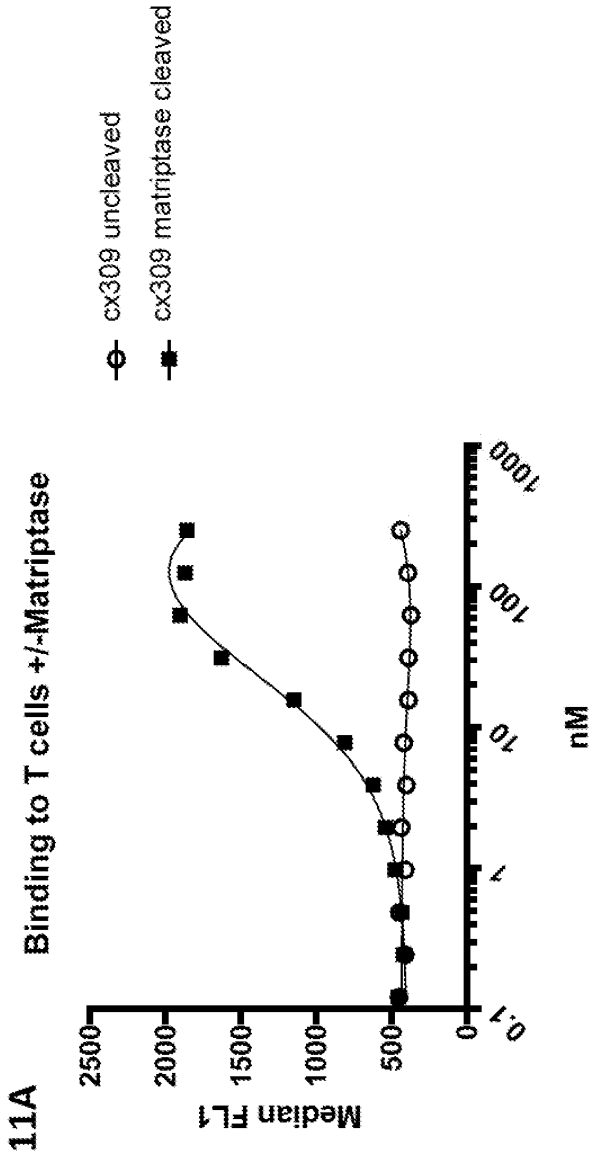

Binding to T cells +/-Matriptase
cx309 uncleaved
cx309 matriptase cleaved
Median FL1
2500
2000
1500
1000
500
0
0.1
1
10
100
1000
nM

FIG. 11A

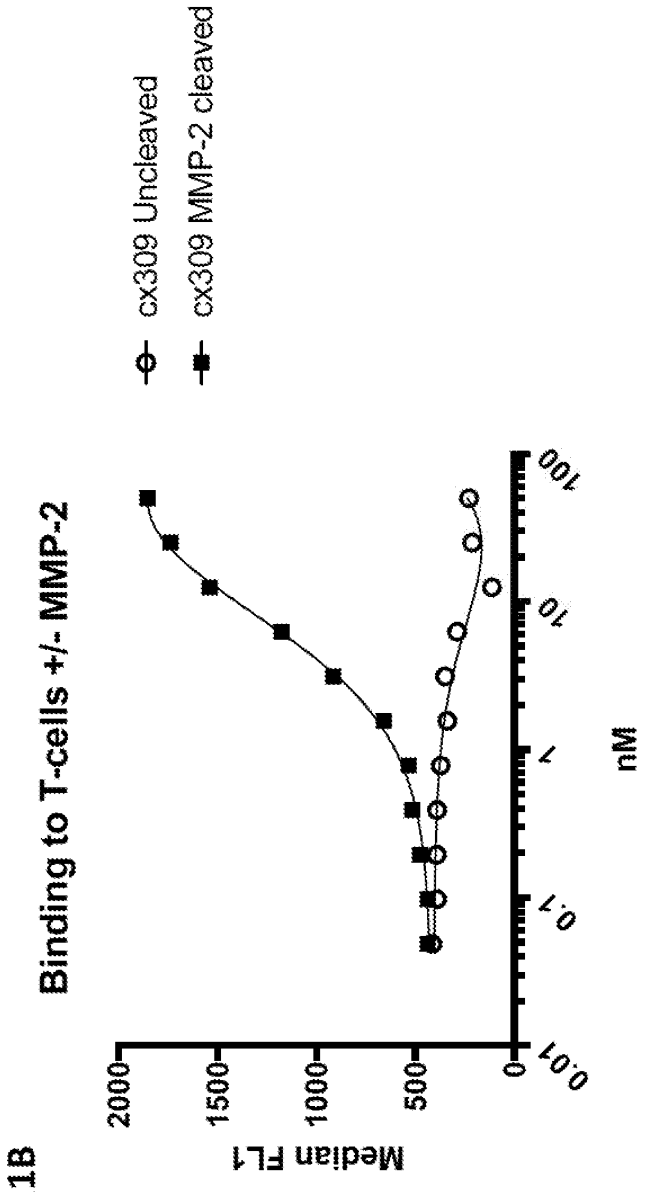

Binding to T-cells +/- MMP-2
cx309 Uncleaved
cx309 MMP-2 cleaved
Median FL1
2000
1500
1000
500
0
0.07
0.7
7
70
700
nM

FIG. 11B

FIG. 12A

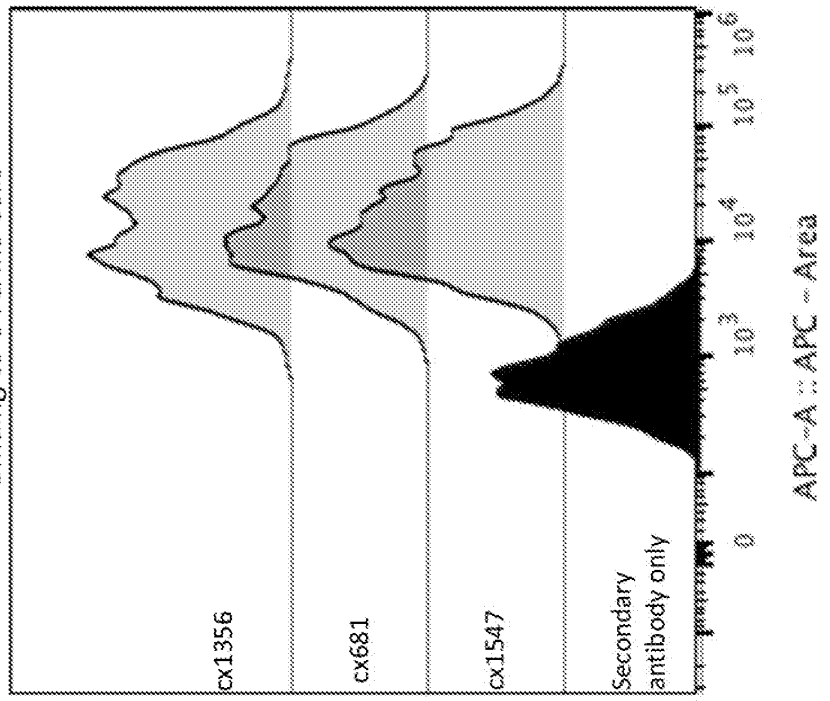


FIG. 12B

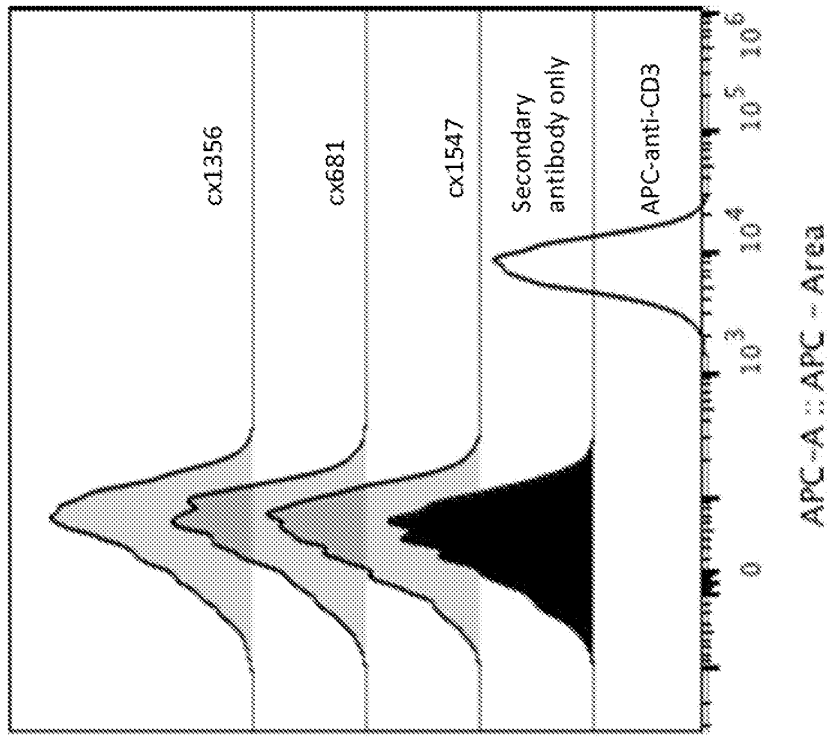


FIG. 12C

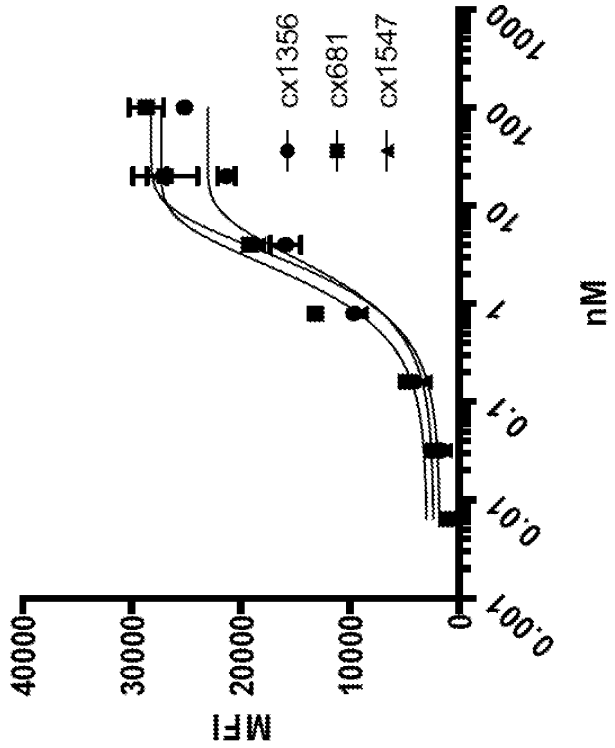


FIG. 12D

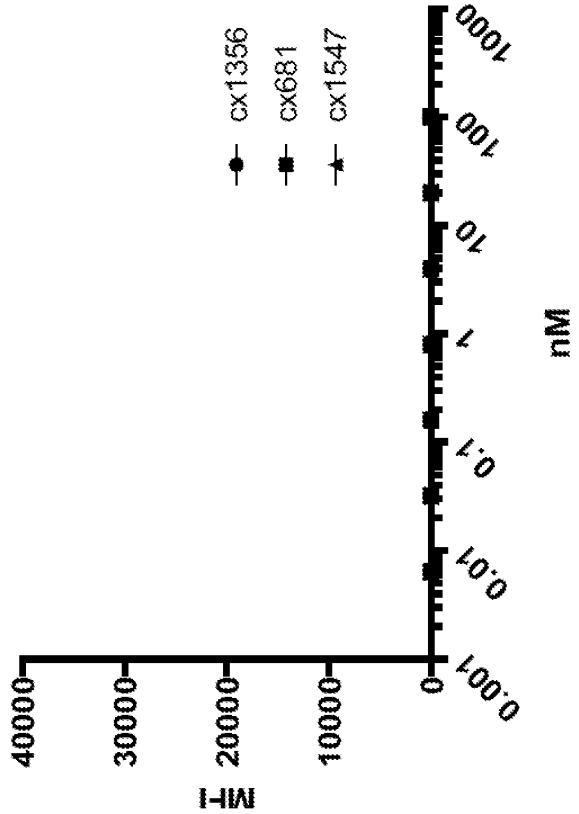


FIG. 13A

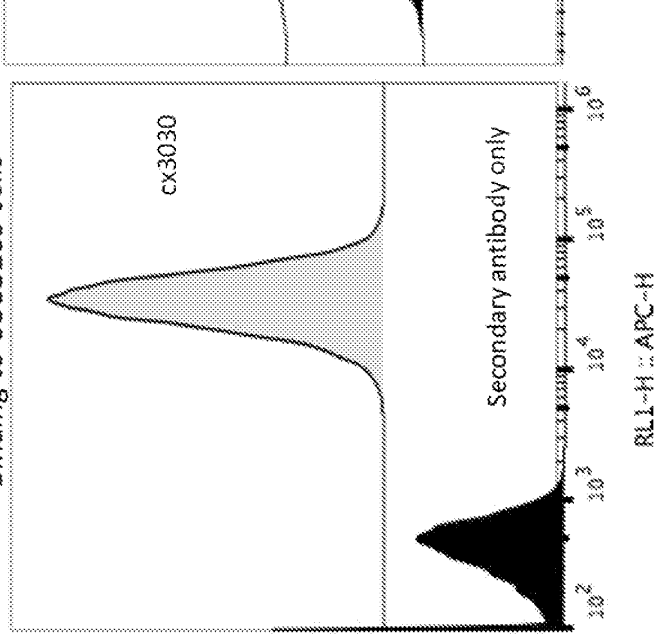


FIG. 13B

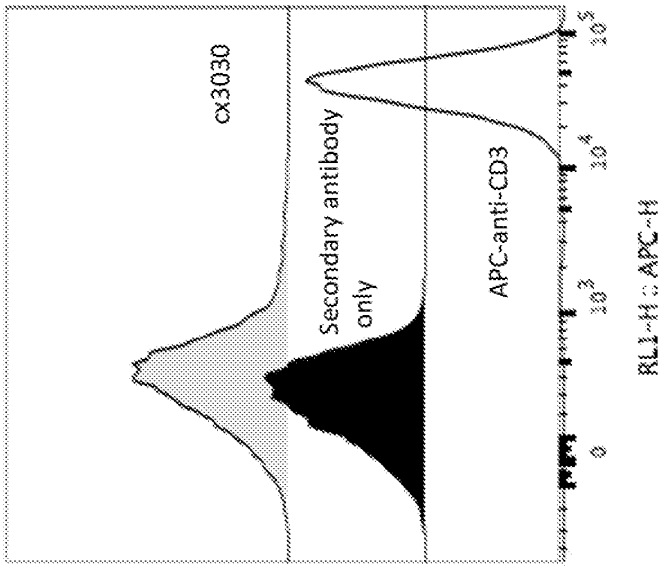


FIG. 14A

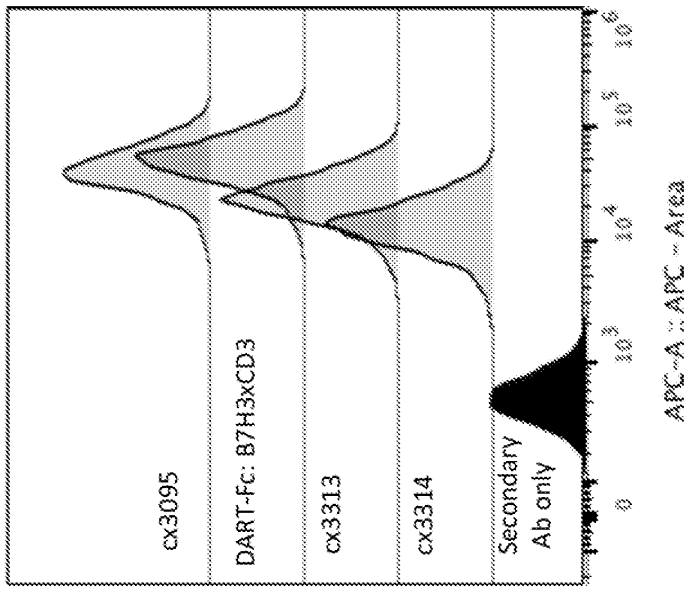


FIG. 14B

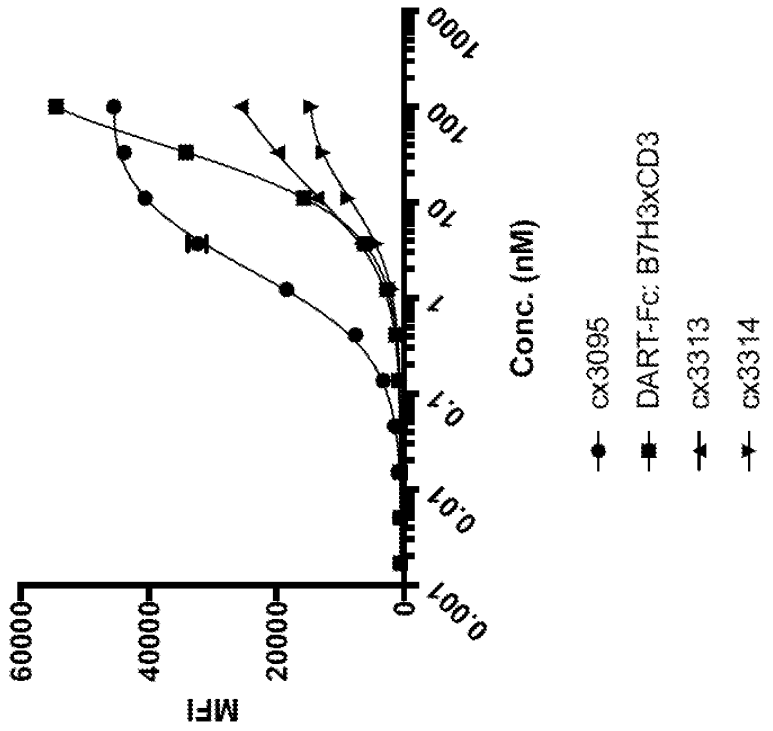


FIG. 14C

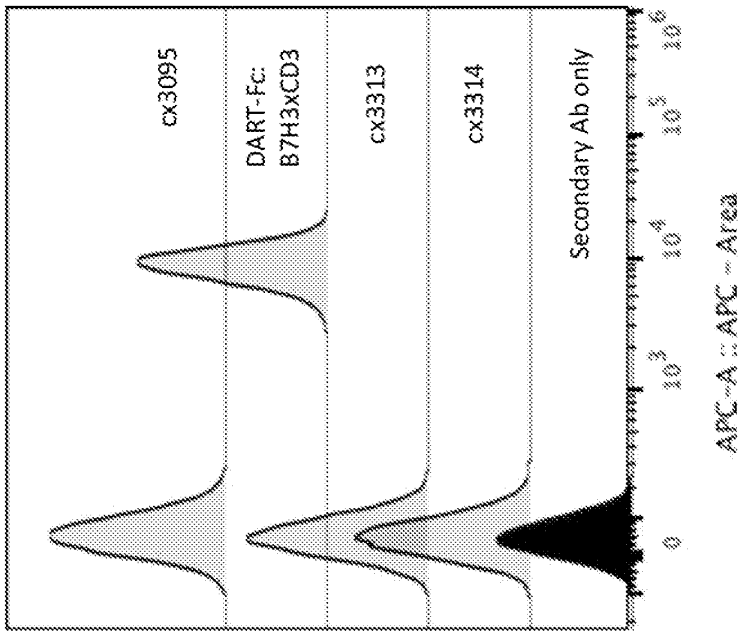


FIG. 14D

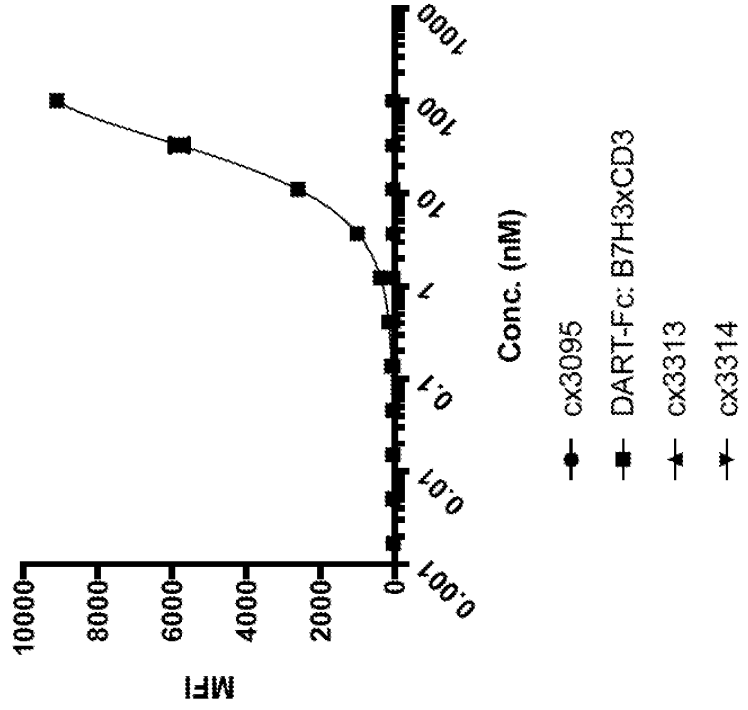


FIG. 15A

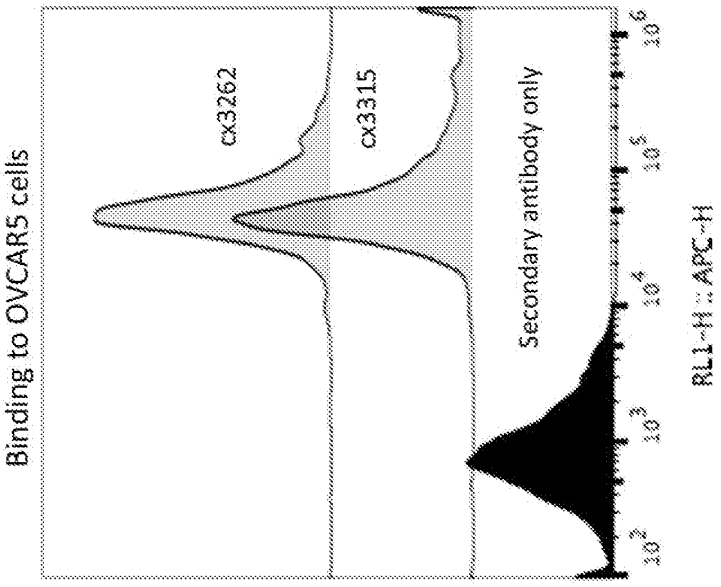


FIG. 15B

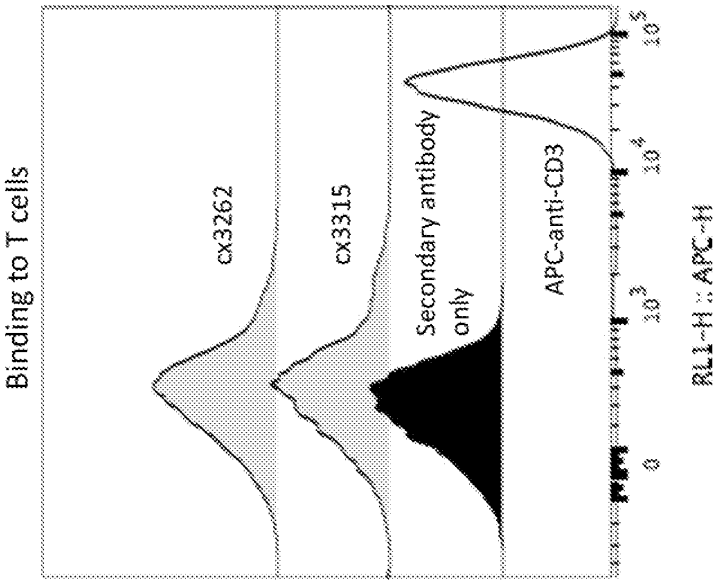


FIG. 16A

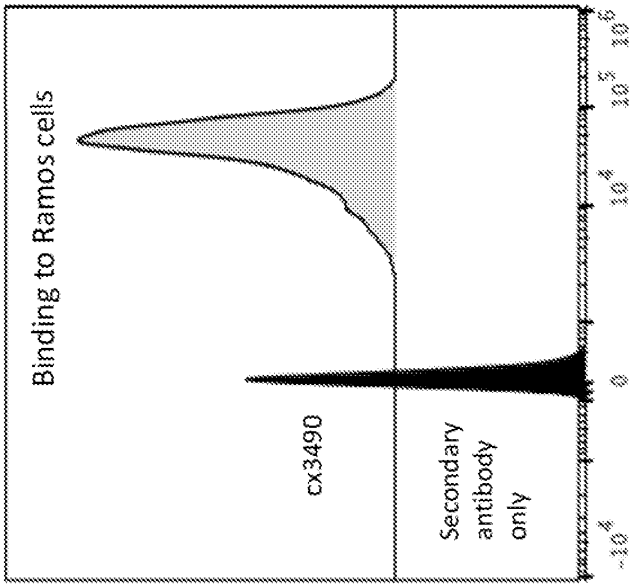


FIG. 16B

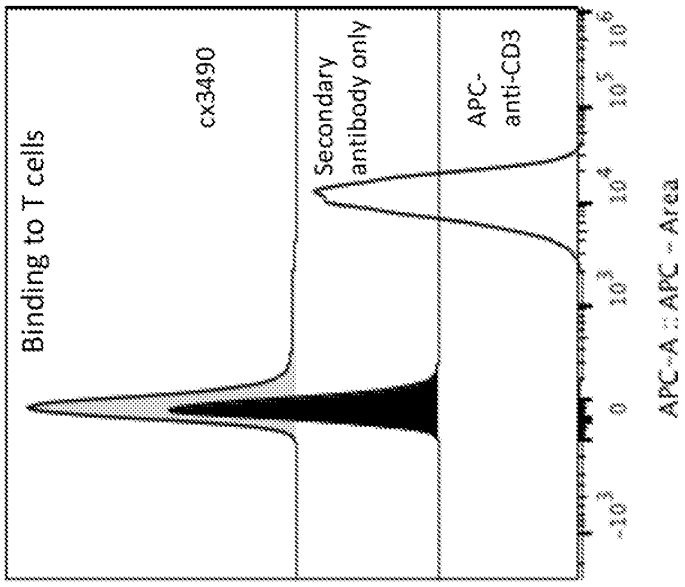


FIG. 16C

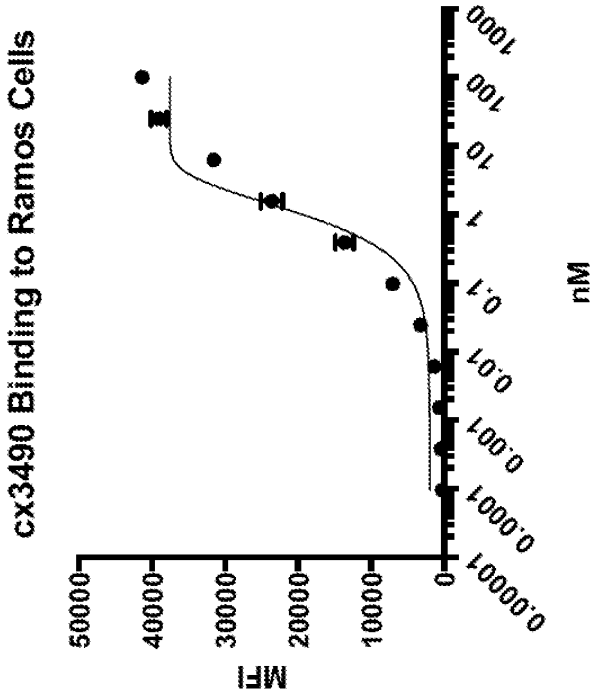


FIG. 16D

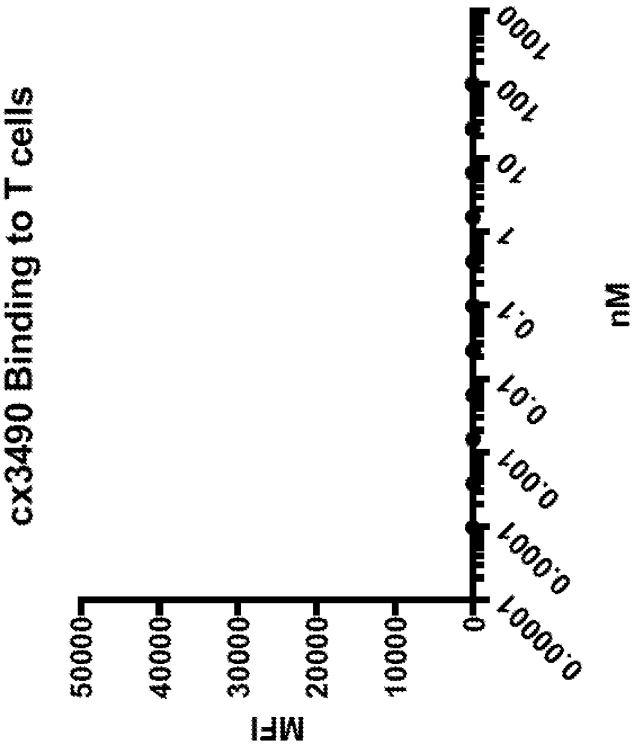


FIG. 17

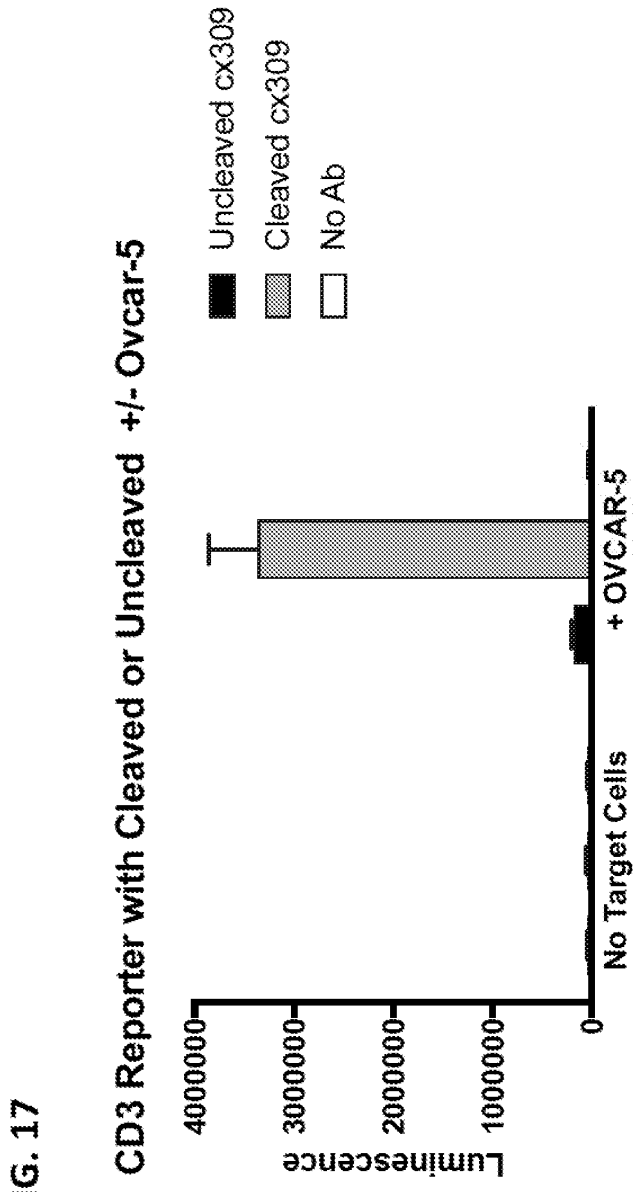

CD3 Reporter with Cleaved or Uncleaved +/- Ovcar-5
Uncleaved cx309
Cleaved cx309
No Ab
Luminescence
4000000
3000000
2000000
1000000
0
No Target Cells
+ OVCAR-5

FIG. 18

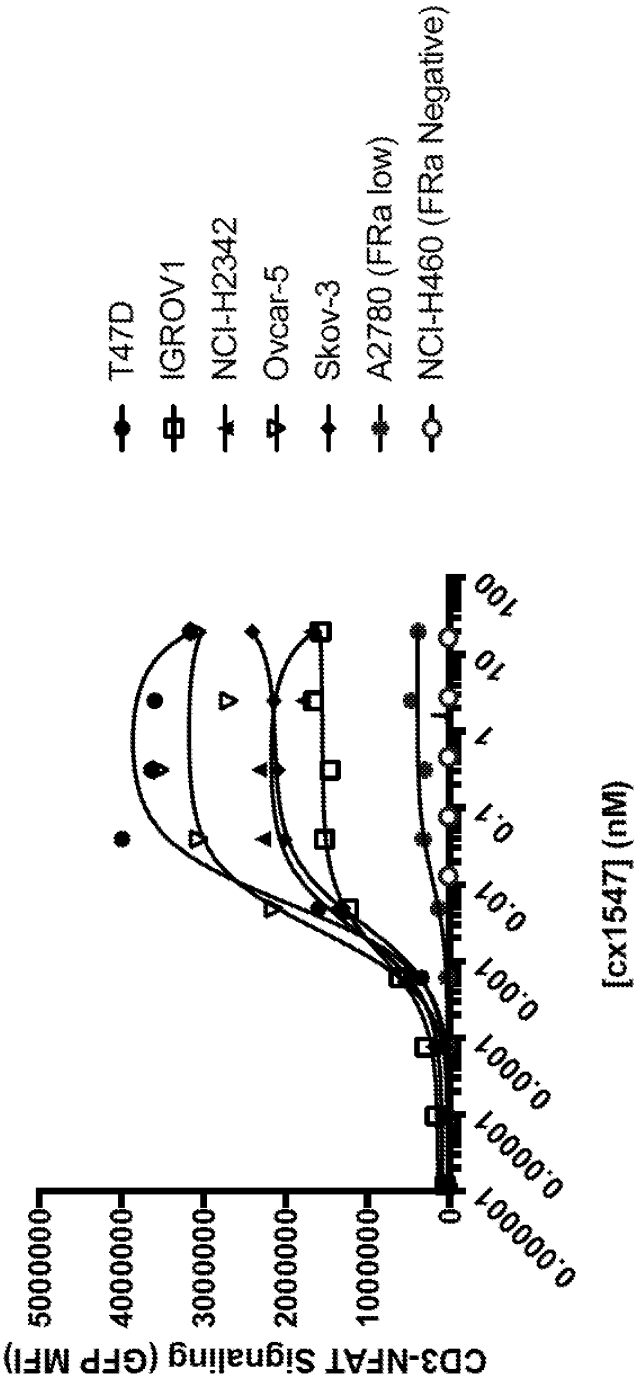

Jurkat CD3-NFAT Reporter: Various Target Cell Lines
CD3-NFAT Signaling (GFP MFI)
5000000
4000000
3000000
2000000
1000000
0
T47D
IGROV1
NCI-H2342
Ovcar-5
Skov-3
A2780 (FRa low)
NCI-H460 (FRa Negative)
100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
[cx1547] (nM)

FIG. 19A

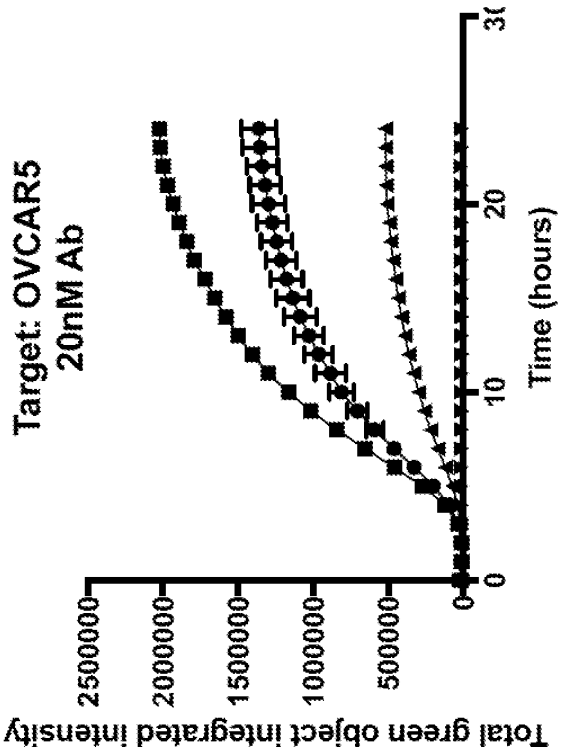


FIG. 19B

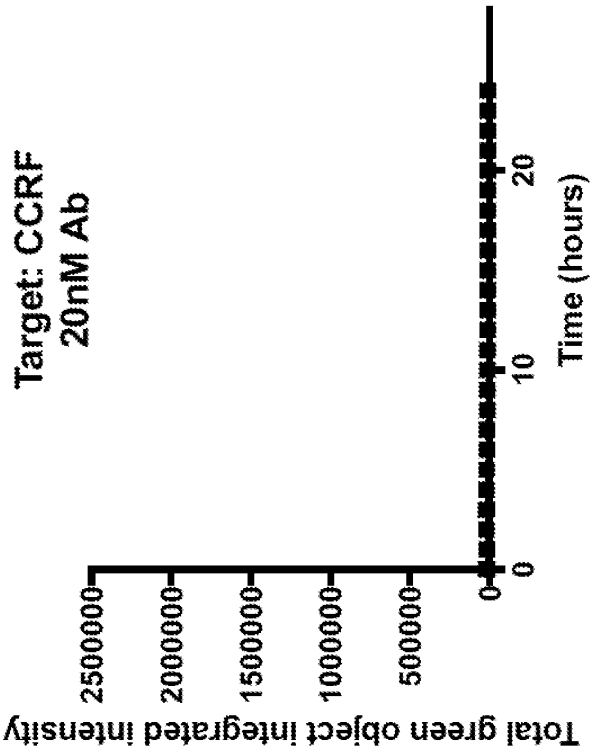


FIG. 19C

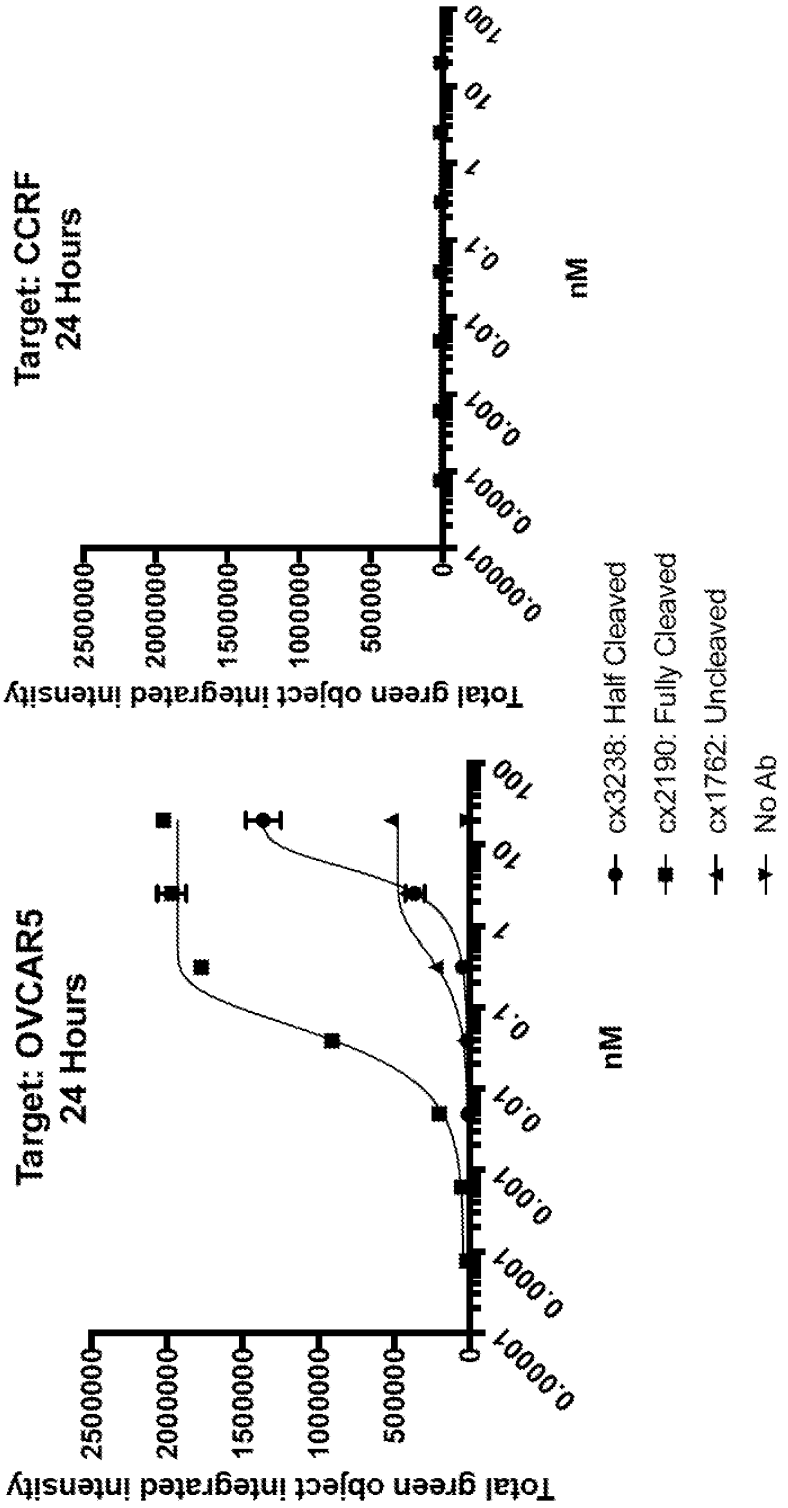


FIG. 19D

FIG. 20A

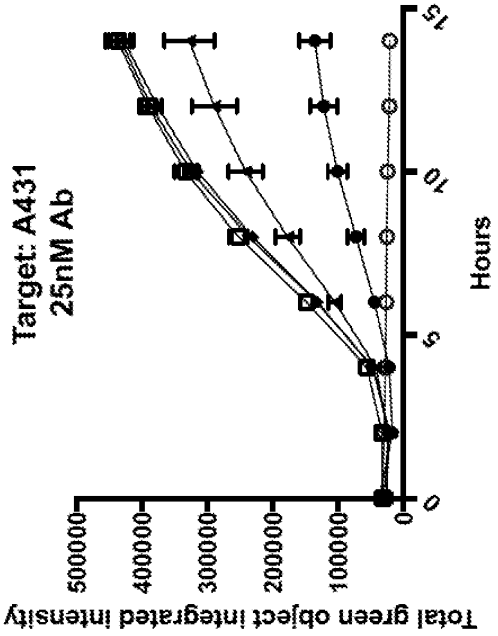
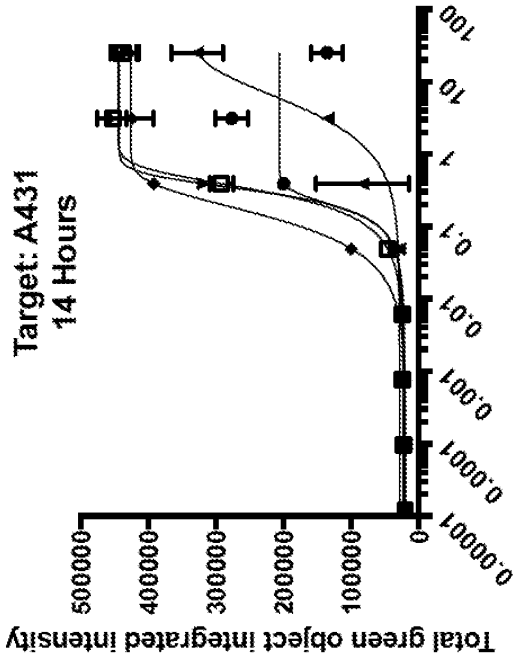


FIG. 20B



EGFR/cMET Positive

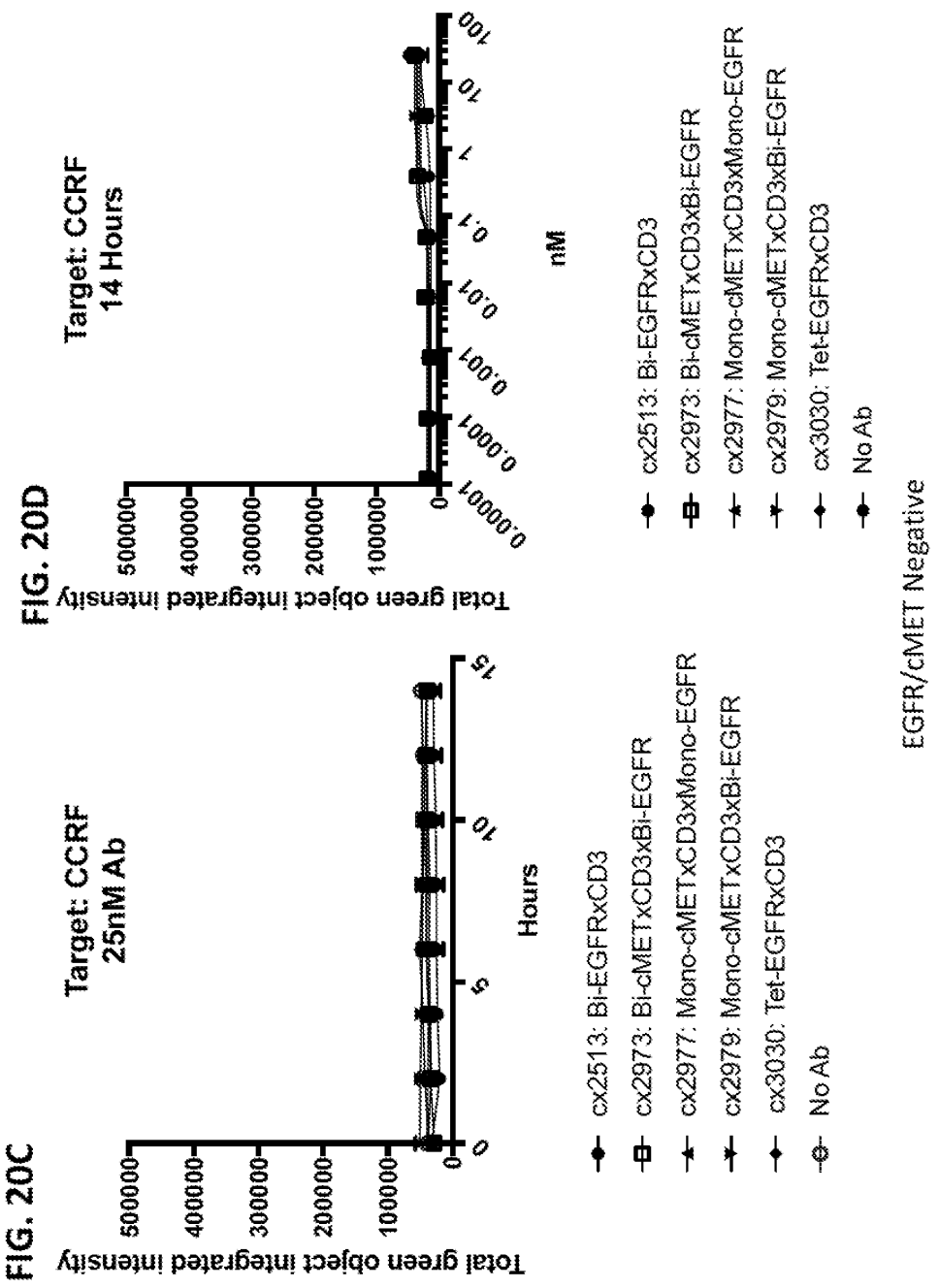


FIG. 20C
Target: CCRF
25nM Ab
Total green object integrated intensity
500000
400000
300000
200000
100000
0
0
5
10
15
Hours
FIG. 20D
Target: CCRF
14 Hours
Total green object integrated intensity
500000
400000
300000
200000
100000
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
nM
cx2513: Bi-EGFRxCD3
cx2973: Bi-cMETxCD3xBi-EGFR
cx2977: Mono-cMETxCD3xMono-EGFR
cx2979: Mono-cMETxCD3xBi-EGFR
cx3030: Tet-EGFRxCD3
No Ab
EGFR/cMET Negative

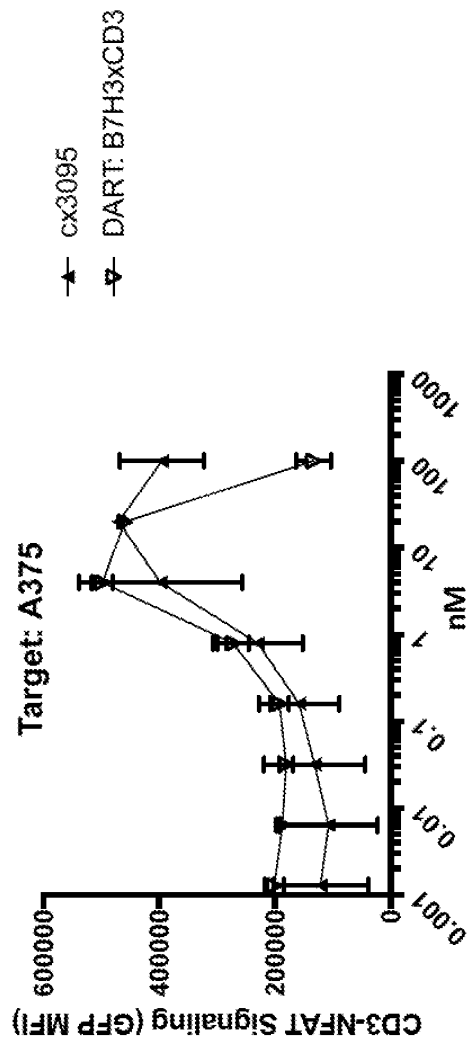


FIG. 21A

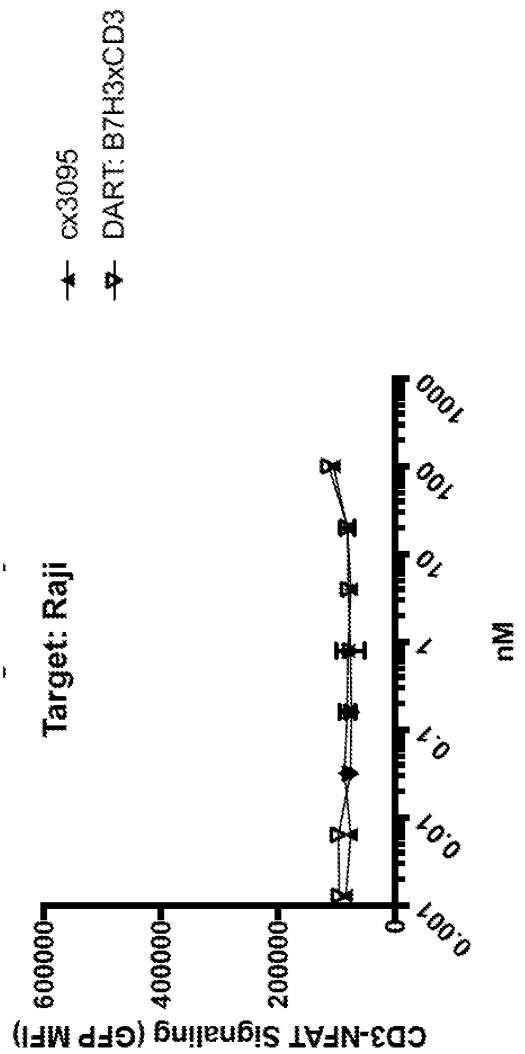


FIG. 21B

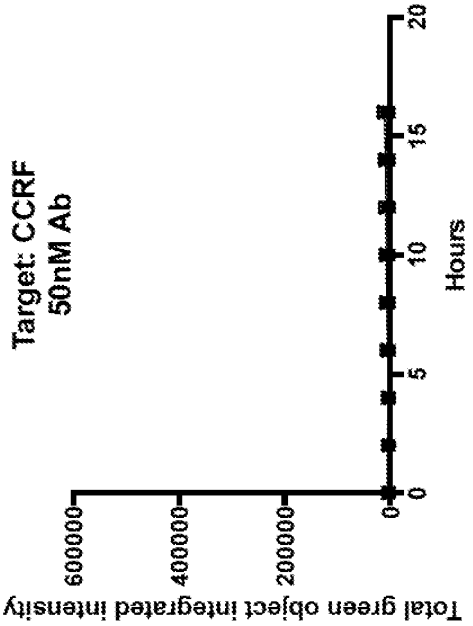


FIG. 22B

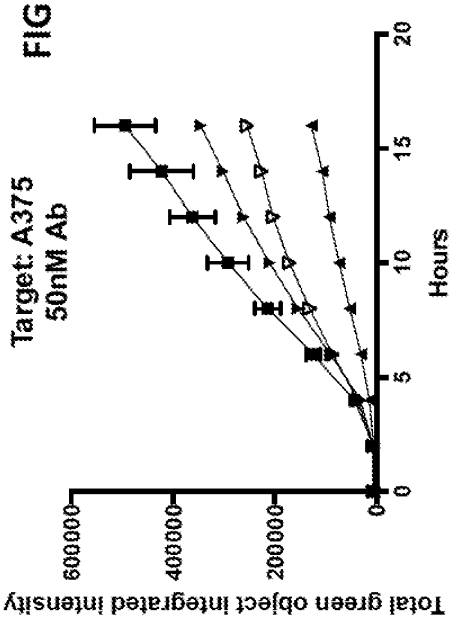


FIG. 22A

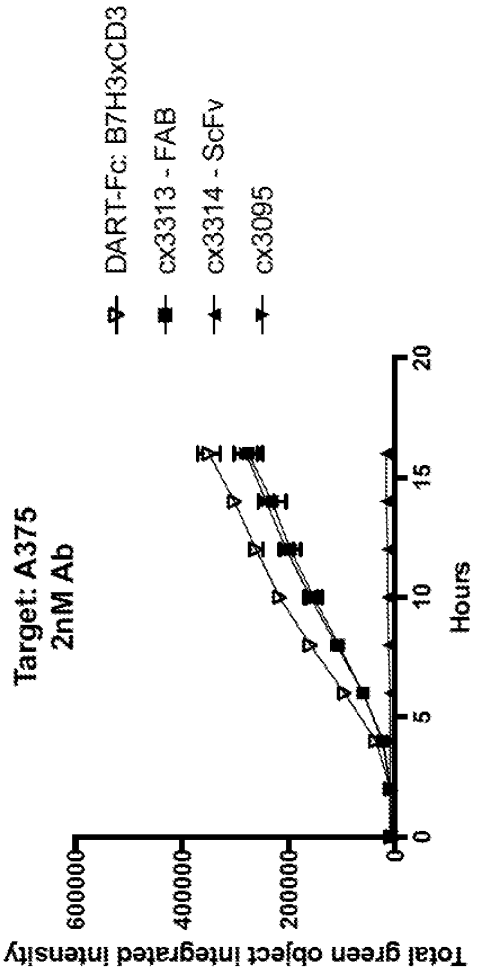


FIG. 22C

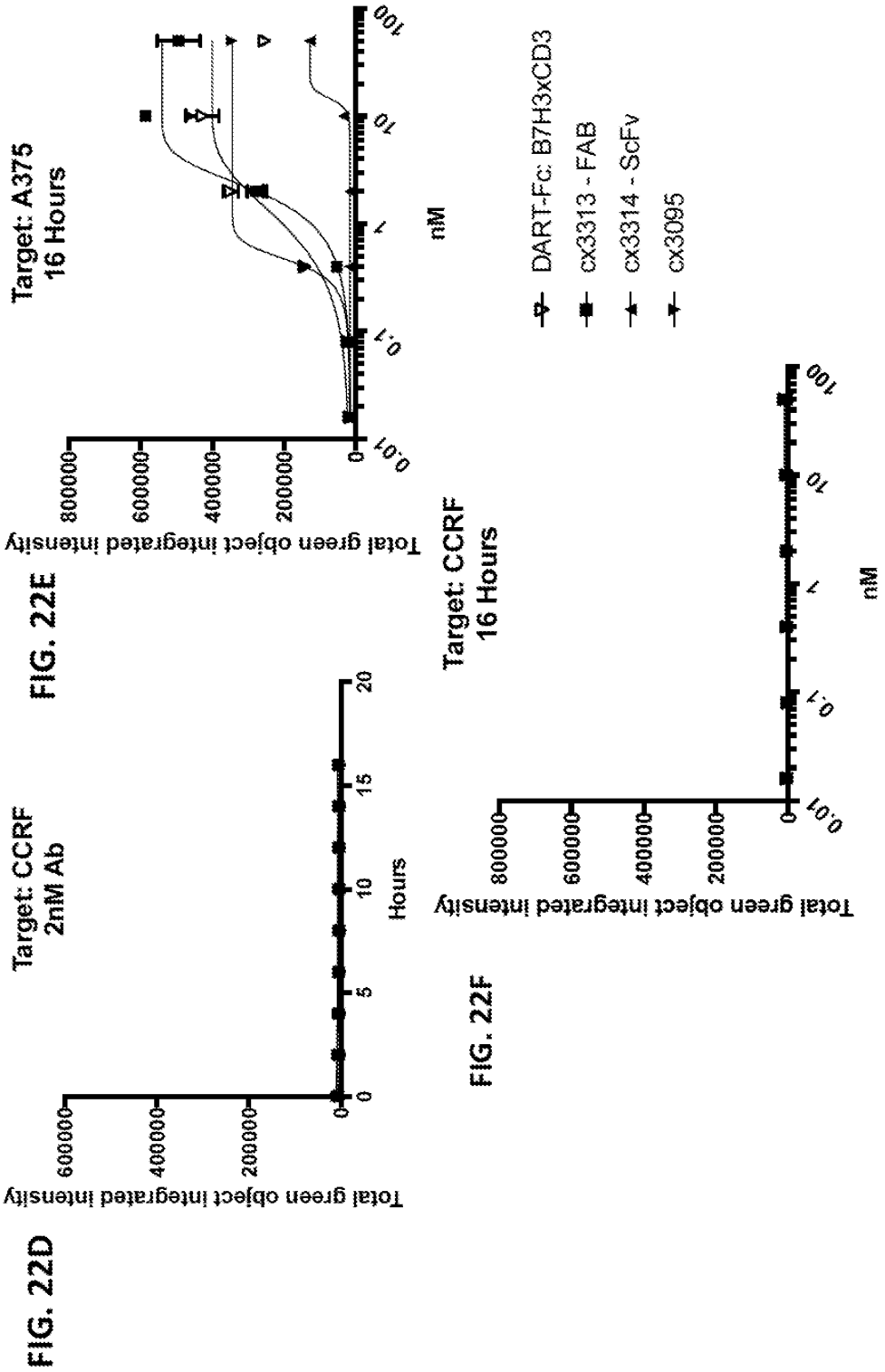


FIG. 22E

FIG. 22F

FIG. 22D

FIG. 23A

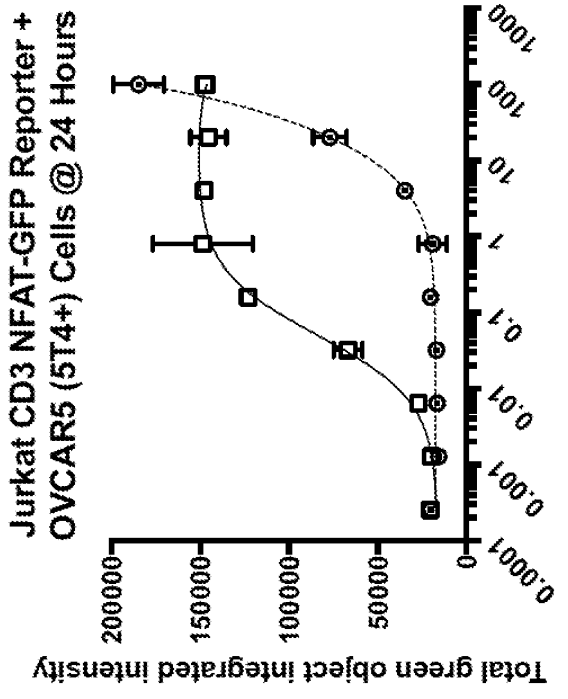


FIG. 23B

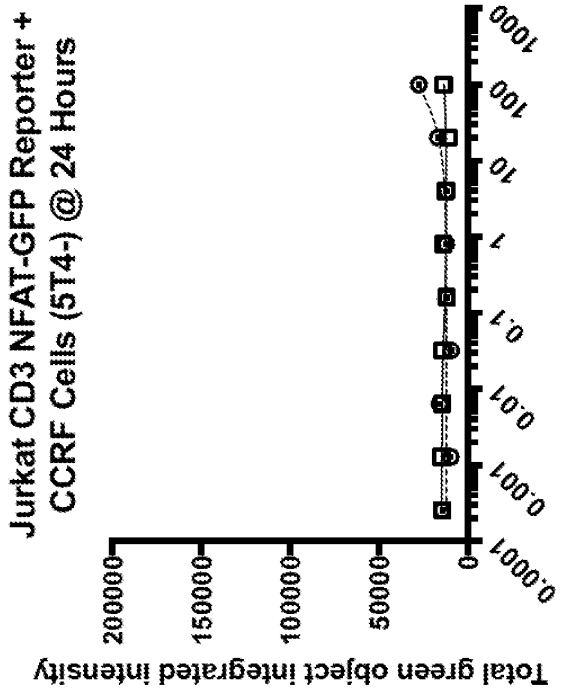
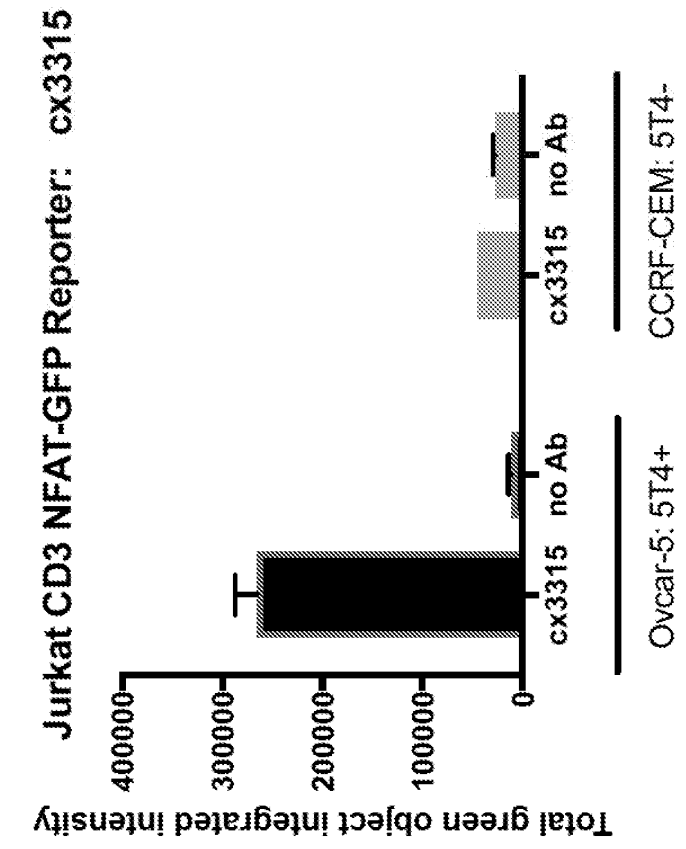


FIG. 24


Jurkat CD3 NFAT-GFP Reporter: cx3315
Total green object integrated intensity
400000
300000
200000
100000
0
cx3315
no Ab
cx3315
no Ab
Ovcar-5: 5T4+
CCRF-CEM: 5T4-

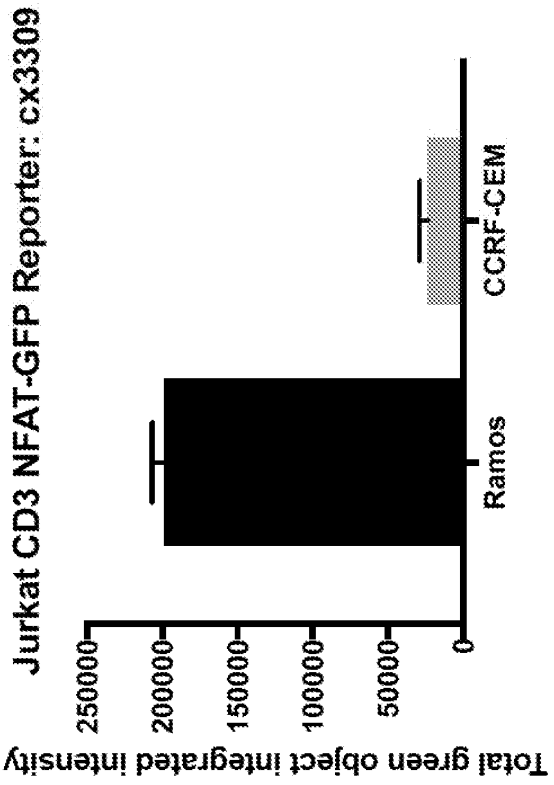

Jurkat CD3 NFAT-GFP Reporter: cx3309
Total green object integrated intensity
250000
200000
150000
100000
50000
0
Ramos
CCRF-CEM

FIG. 25

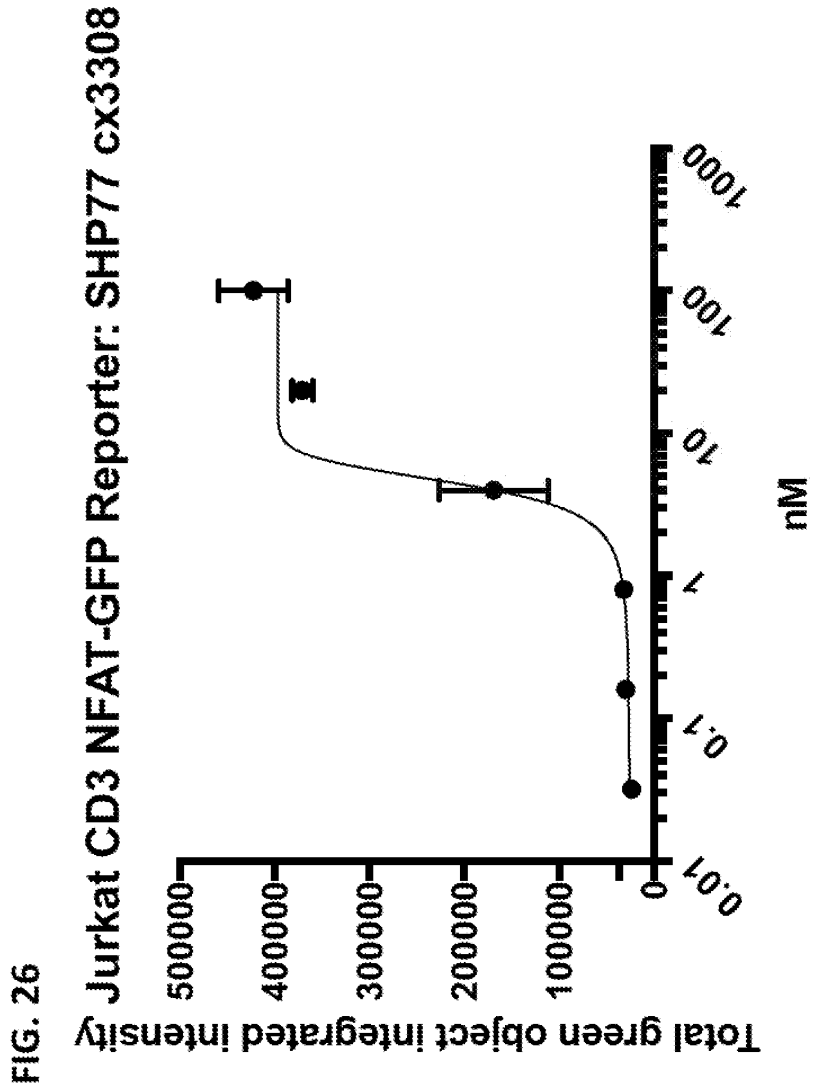

Jurkat CD3 NFAT-GFP Reporter: SHP77 cx3308
Total green object integrated intensity
500000
400000
300000
200000
100000
0
0.07
0.7
7
10
100
1000
nM

FIG. 26

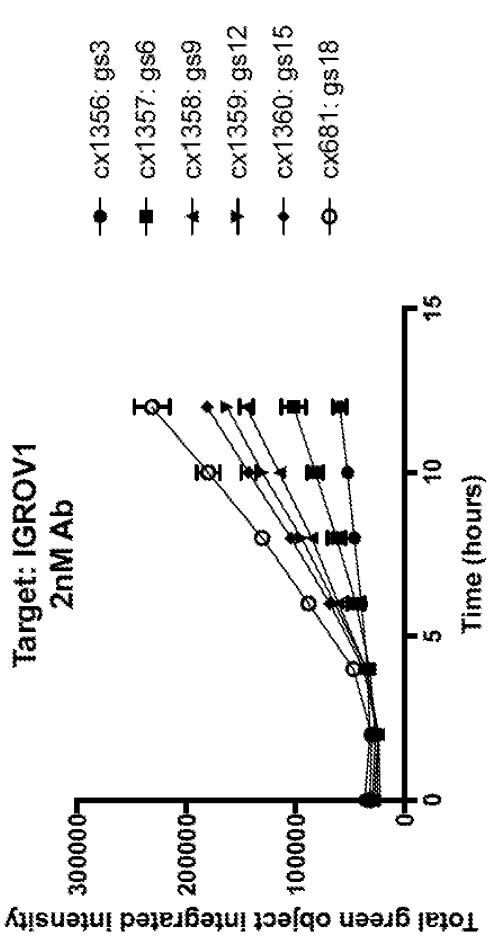


FIG. 27A

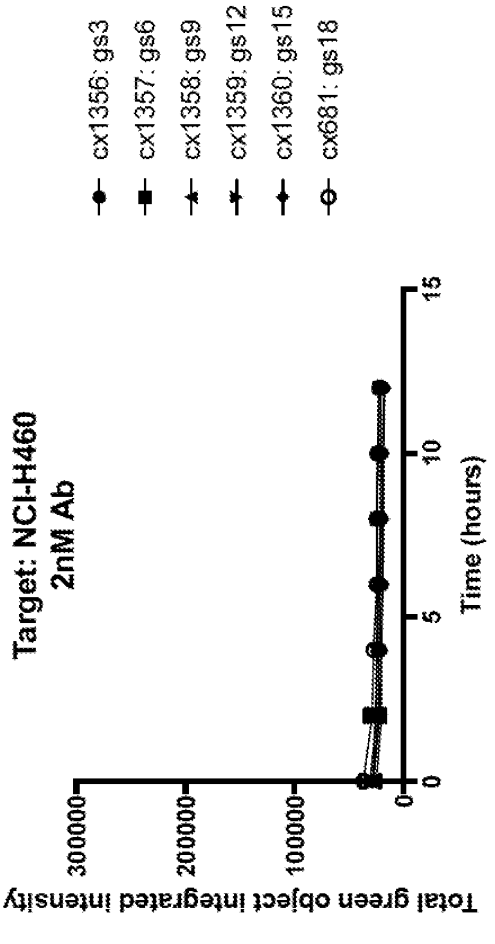


FIG. 27B

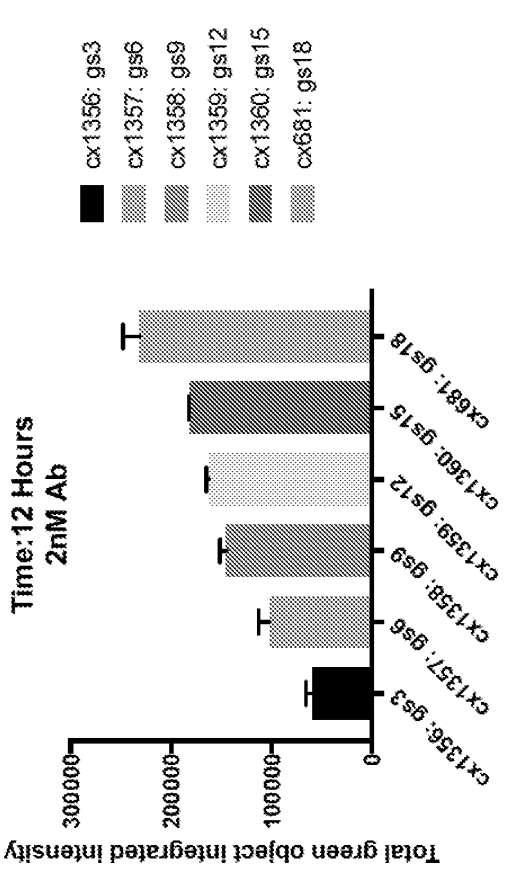


FIG. 27C

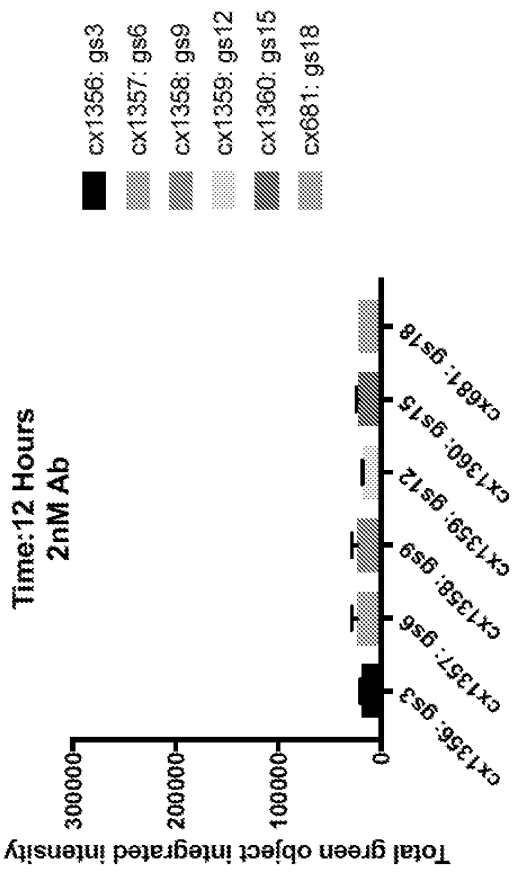


FIG. 27D

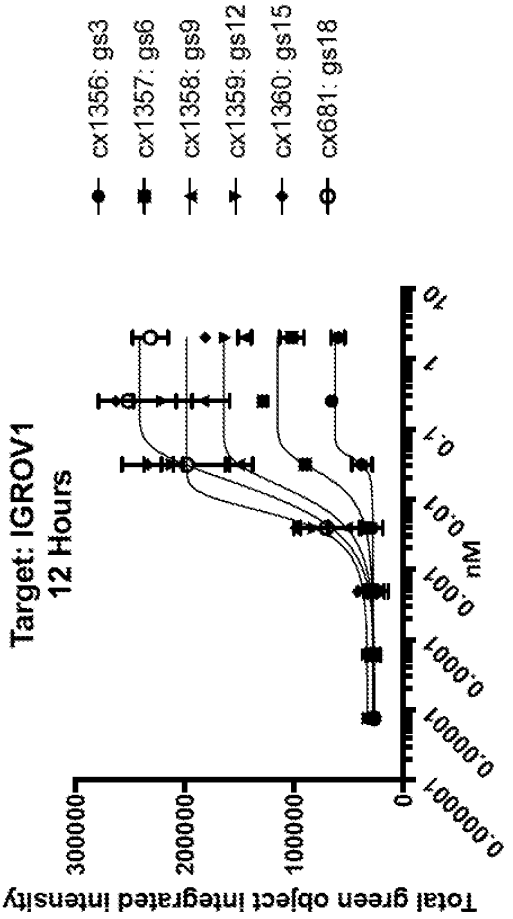


FIG. 27E

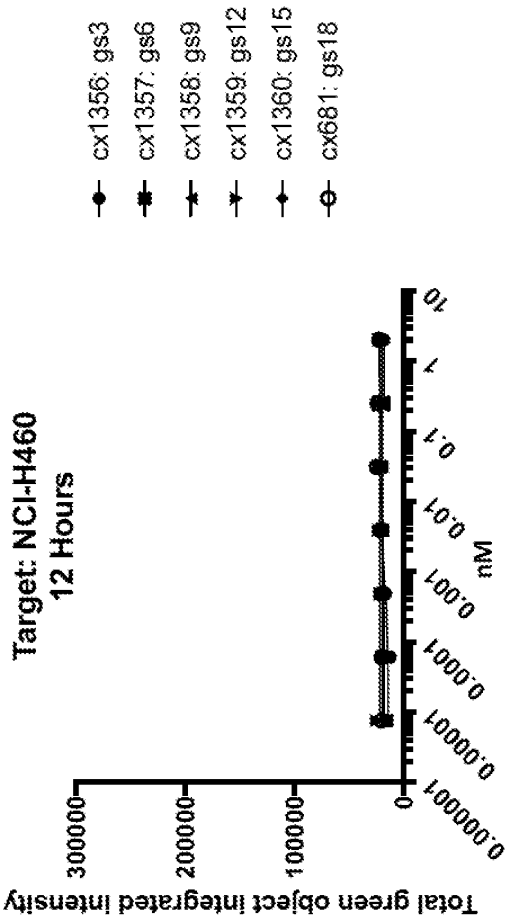


FIG. 27F

FIG. 28A

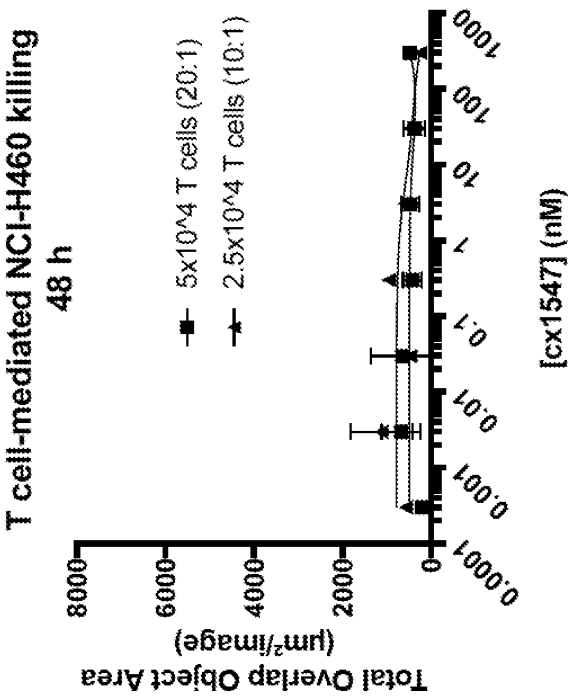
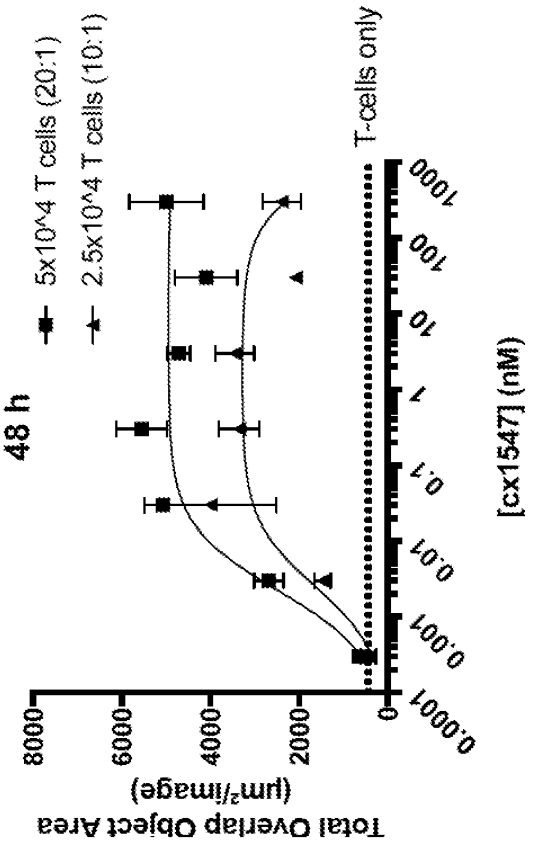


FIG. 28B



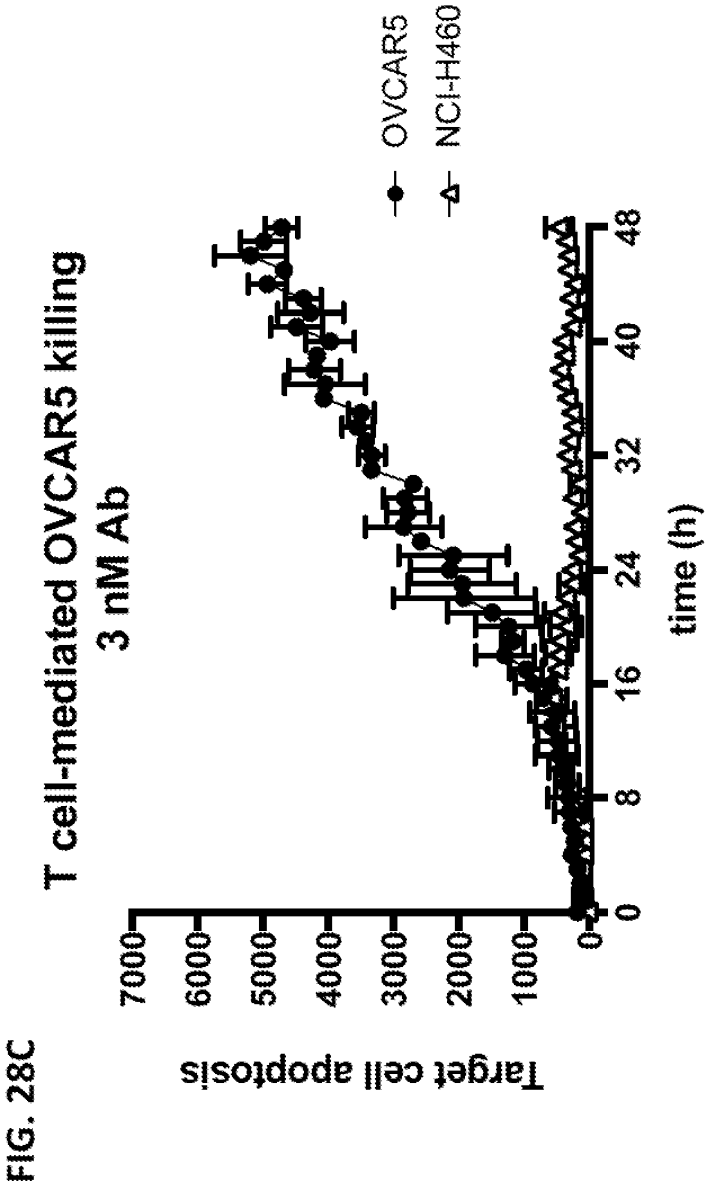

T cell-mediated OVCAR5 killing
3 nM Ab
OVCAR5
NCI-H460
Target cell apoptosis
7000
6000
5000
4000
3000
2000
1000
0
0
8
16
24
32
40
48
time (h)

FIG. 28C

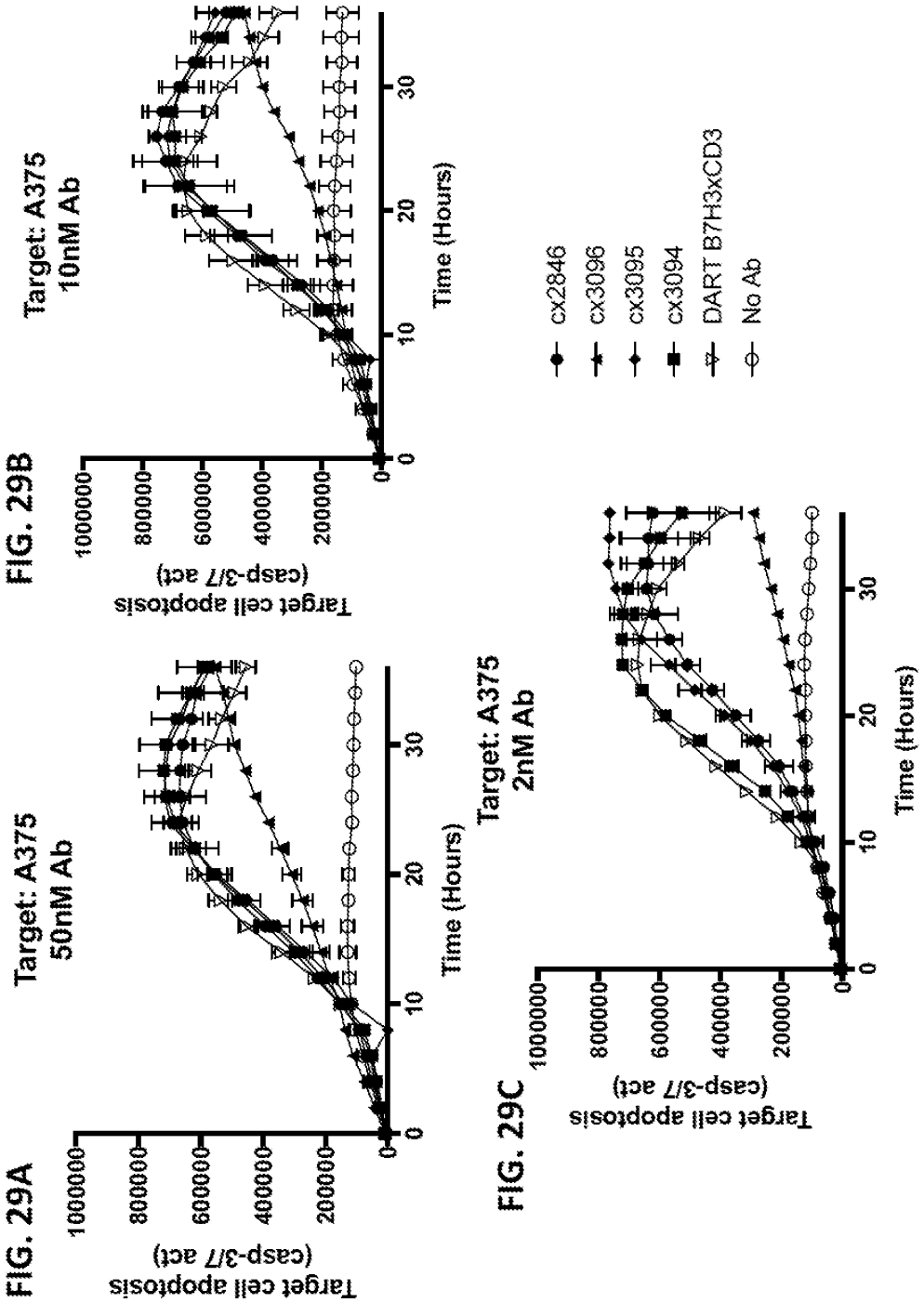


FIG. 29A

FIG. 29B

FIG. 29C

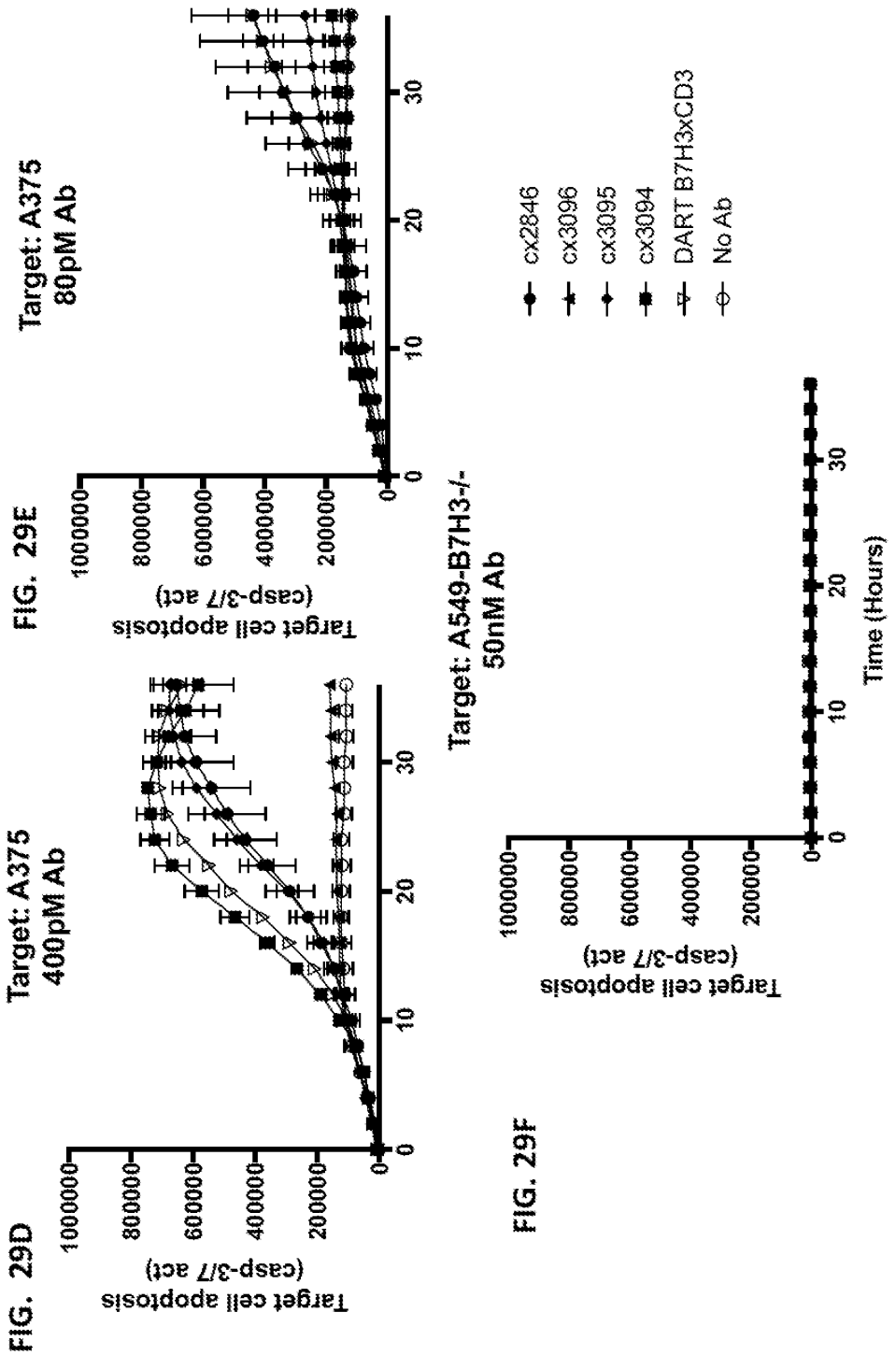


FIG. 29D

FIG. 29E

FIG. 29F

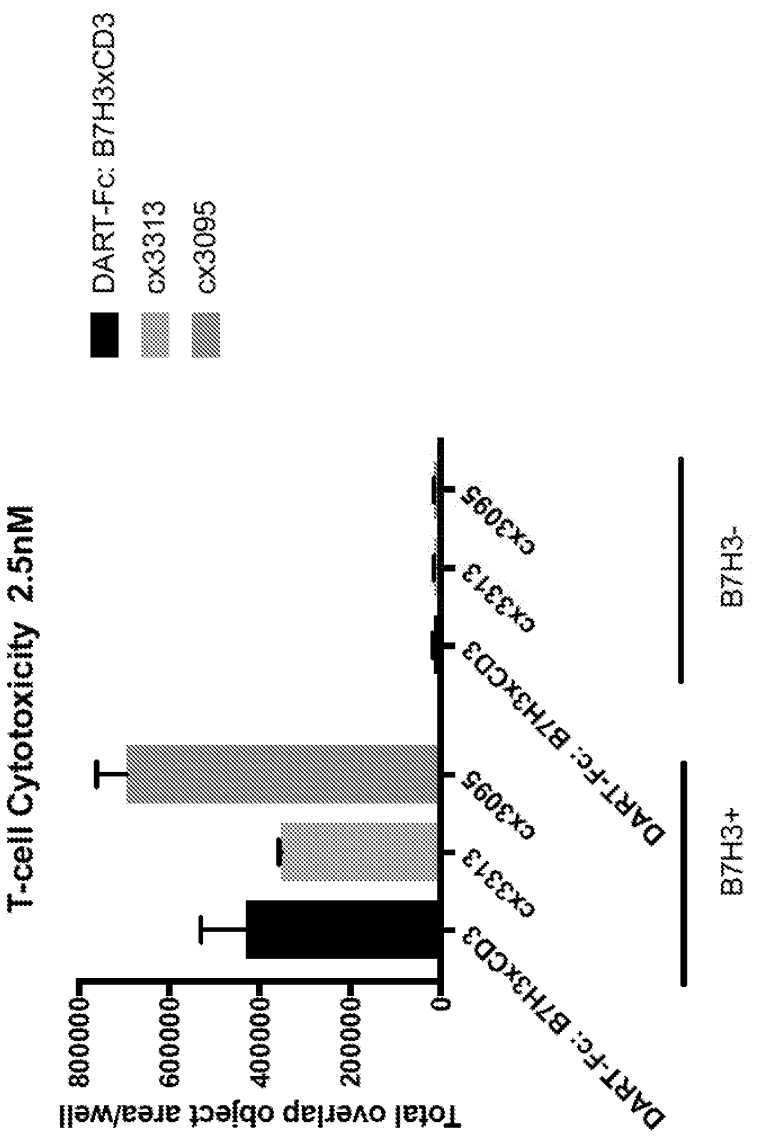

DART-Fc: B7H3xCD3
cx3313
cx3095
T-cell Cytotoxicity 2.5nM
Total overlap object area/well
800000
600000
400000
200000
0
DART-Fc: B7H3xCD3
cx3313
cx3095
DART-Fc: B7H3xCD3
cx3313
cx3095
B7H3+
B7H3-

FIG. 30

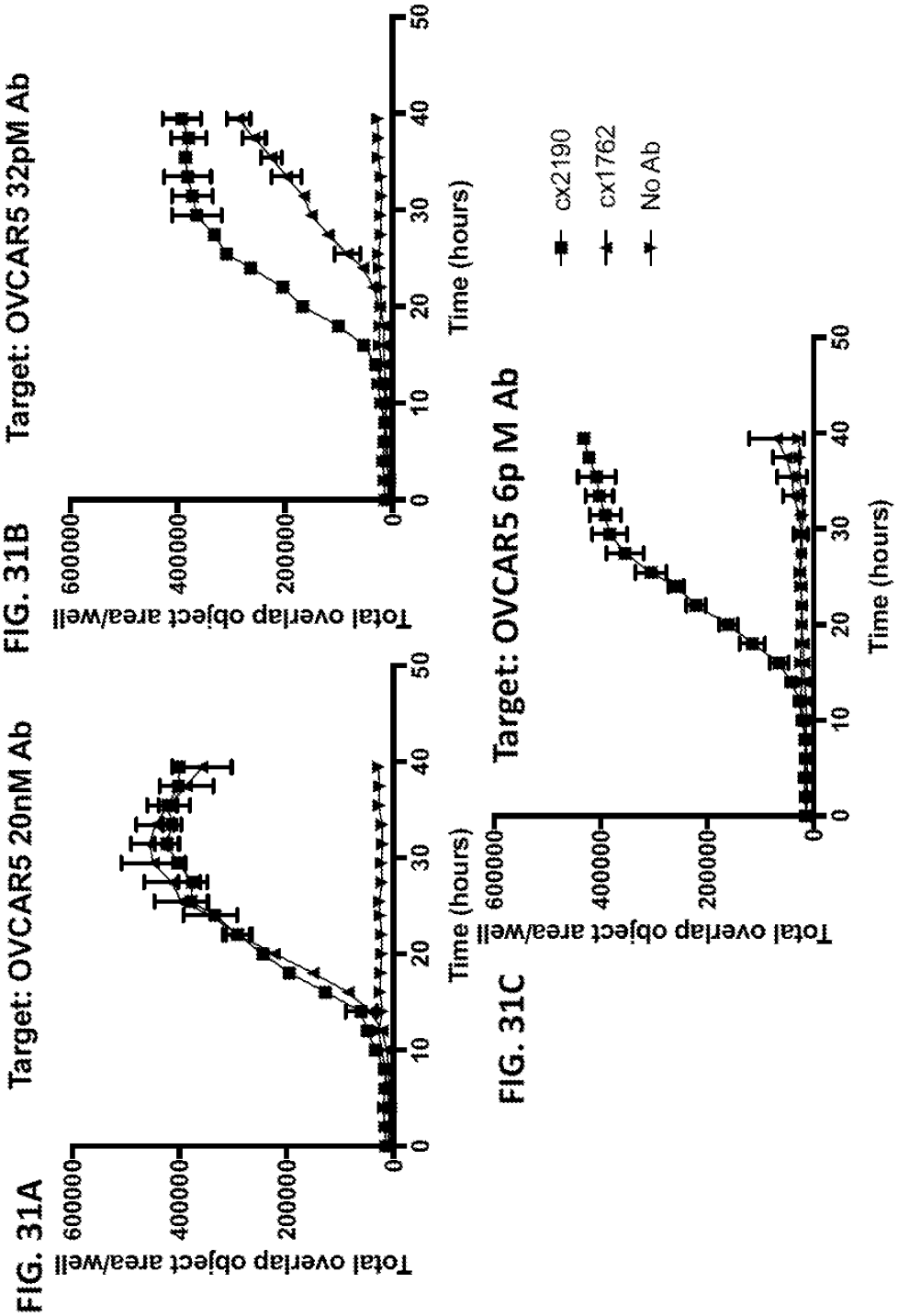


FIG. 31A
Target: OVCAR5 20nM Ab
Total overlap object area/well
600000
400000
200000
0
0
10
20
30
40
50
Time (hours)
FIG. 31B
Target: OVCAR5 32pM Ab
Total overlap object area/well
600000
400000
200000
0
0
10
20
30
40
50
Time (hours)
FIG. 31C
Target: OVCAR5 6p M Ab
cx2190
cx1762
No Ab
Total overlap object area/well
600000
400000
200000
0
0
10
20
30
40
50
Time (hours)

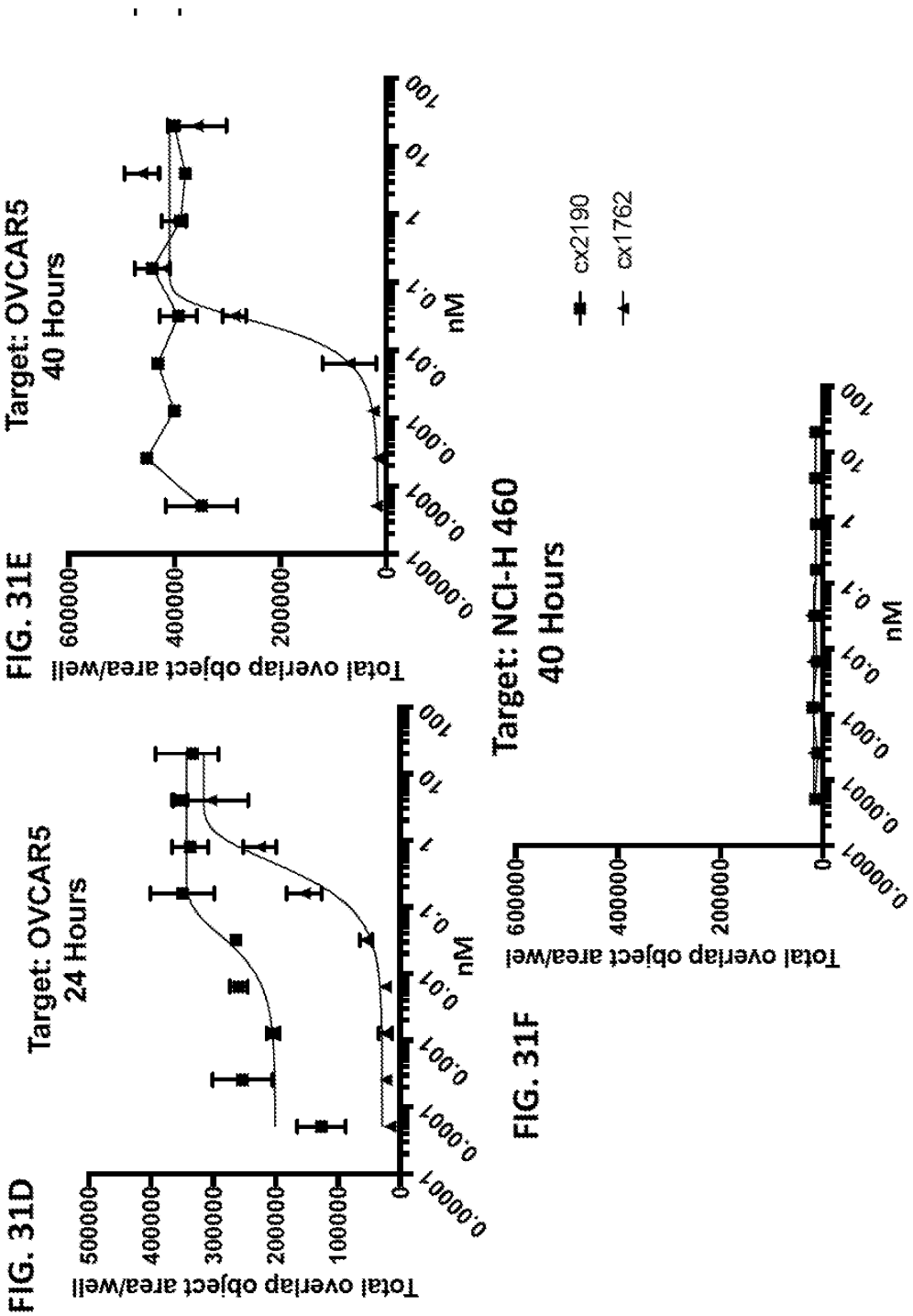


FIG. 31D
Target: OVCAR5
24 Hours
Total overlap object area/well
500000
400000
300000
200000
100000
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
nM
FIG. 31E
Target: OVCAR5
40 Hours
Total overlap object area/well
600000
400000
200000
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
nM
FIG. 31F
Target: NCI-H 460
40 Hours
cx2190
cx1762
Total overlap object area/wel
600000
400000
200000
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
nM

FIG. 32

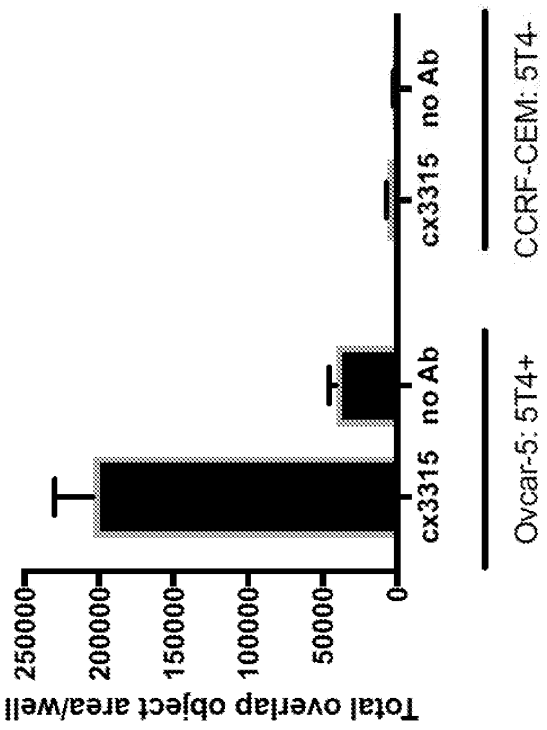

T-cell Cytotoxicity
Total overlap object area/well
250000
200000
150000
100000
50000
0
cx3315
no Ab
cx3315
no Ab
Ovcar-5: 5T4+
CCRF-CEM: 5T4-

FIG. 33

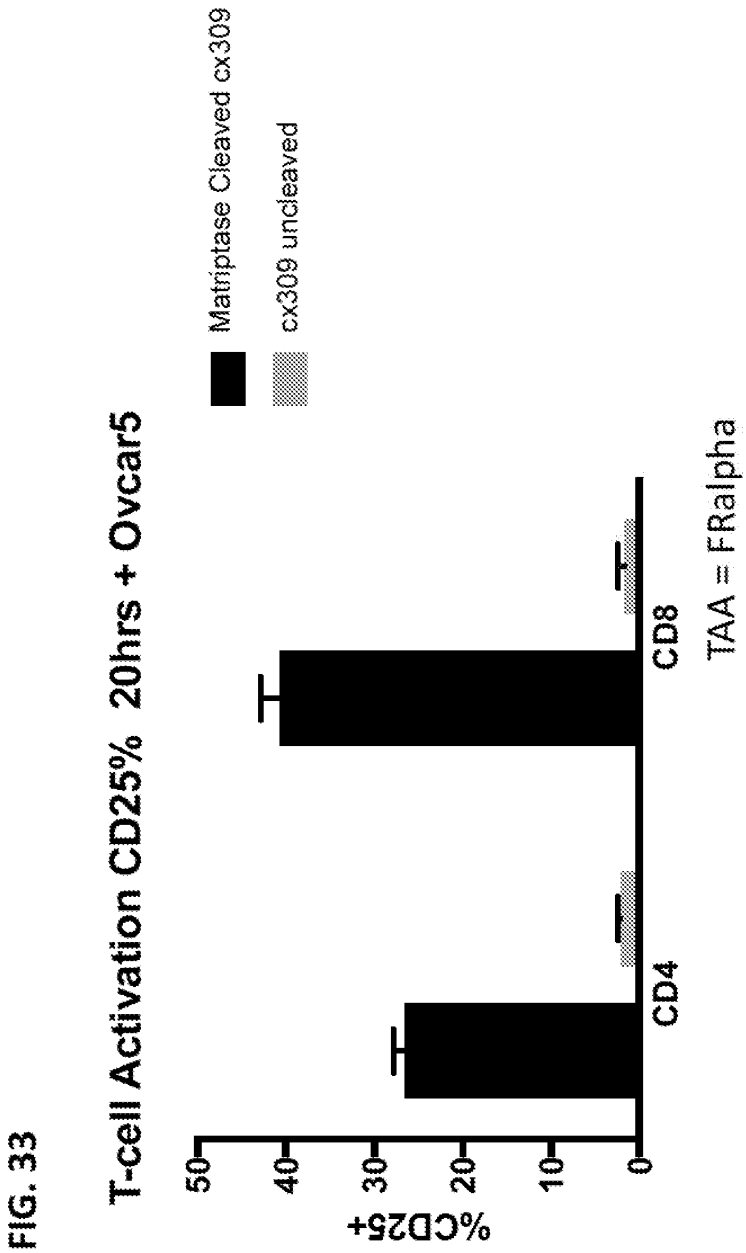

T-cell Activation CD25% 20hrs + Ovcar5
Matriptase Cleaved cx309
cx309 uncleaved
%CD25+
50
40
30
20
10
0
CD4
CD8
TAA = FRalpha

FIG. 34A

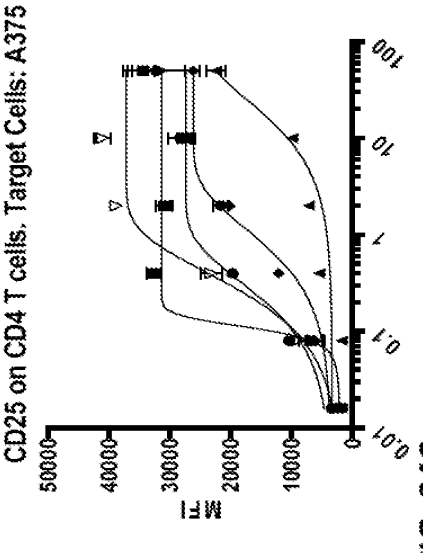


FIG. 34B

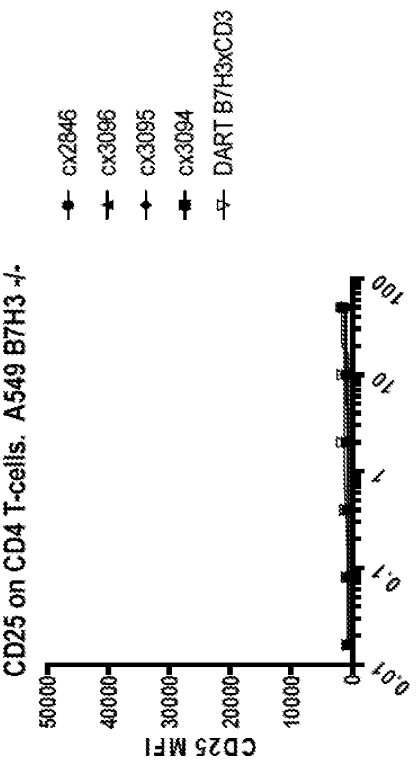


FIG. 34C

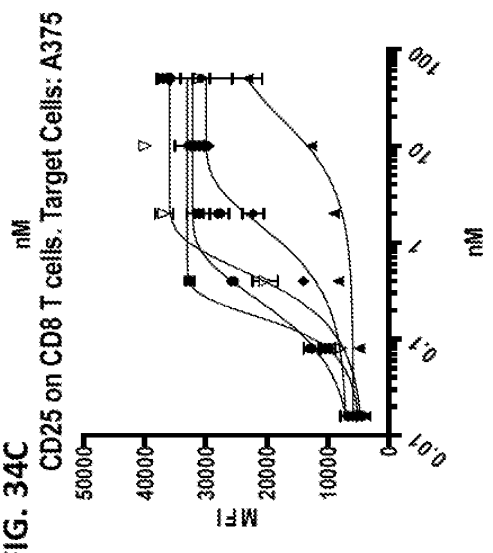
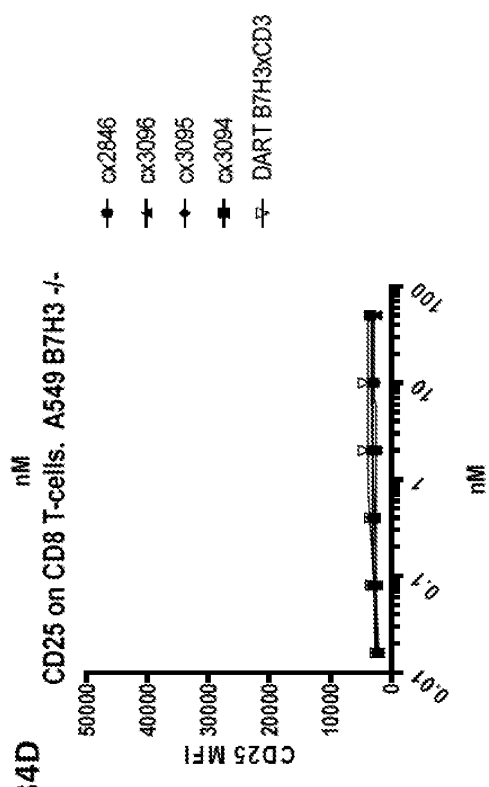


FIG. 34D



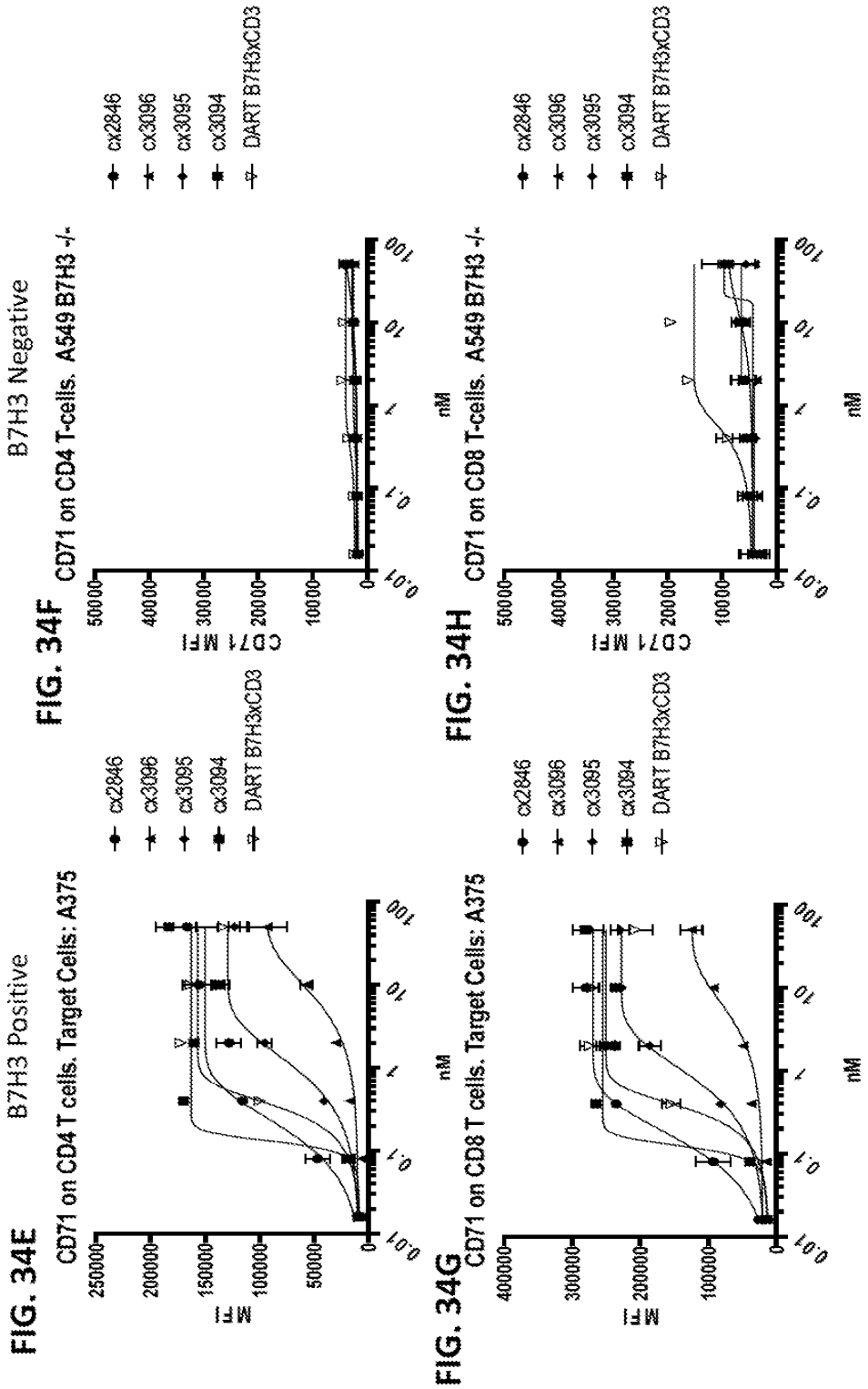


FIG. 34E

FIG. 34F

FIG. 34G

FIG. 34H

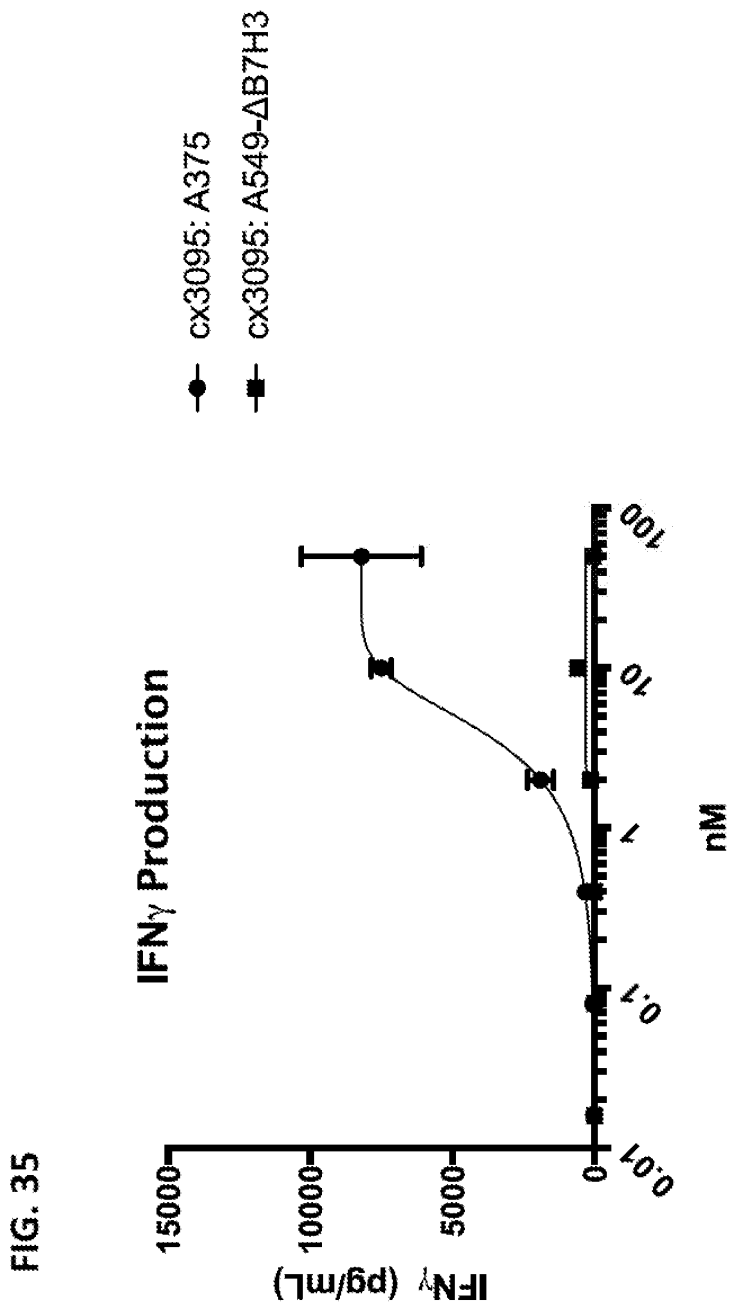

cx3095: A375
cx3095: A549-ΔB7H3
IFNγ Production
IFNγ (pg/mL)
15000
10000
5000
0
0.07
0.7
7
10
100
nM

FIG. 35

FIG. 36A

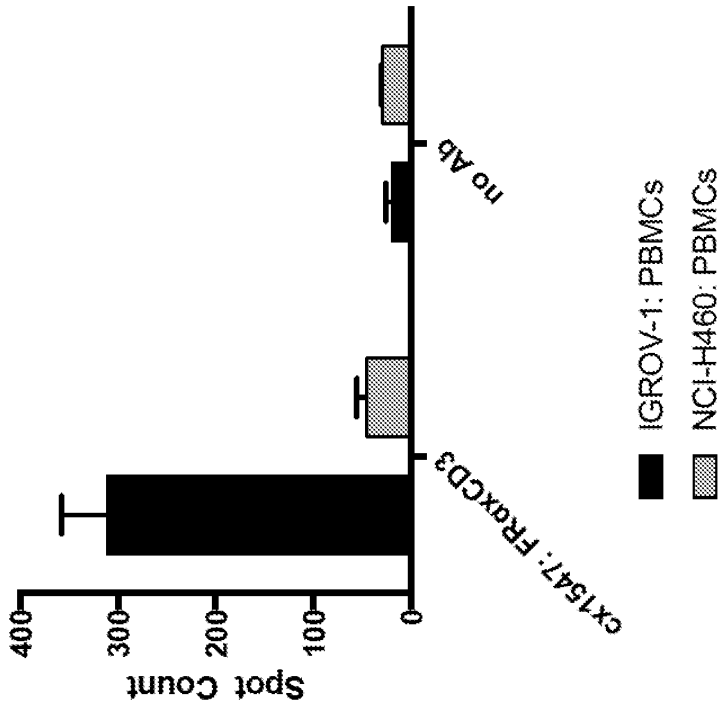
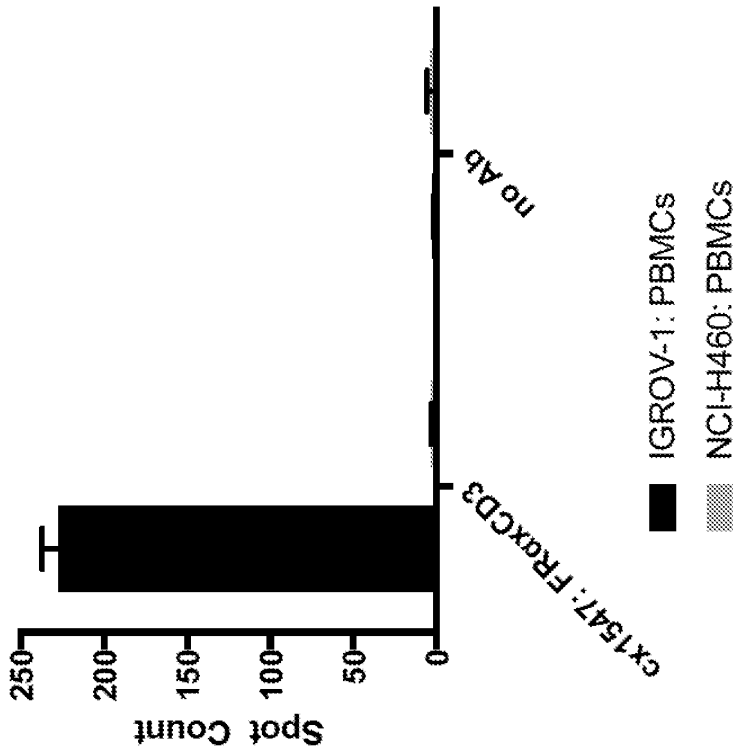


FIG. 36B



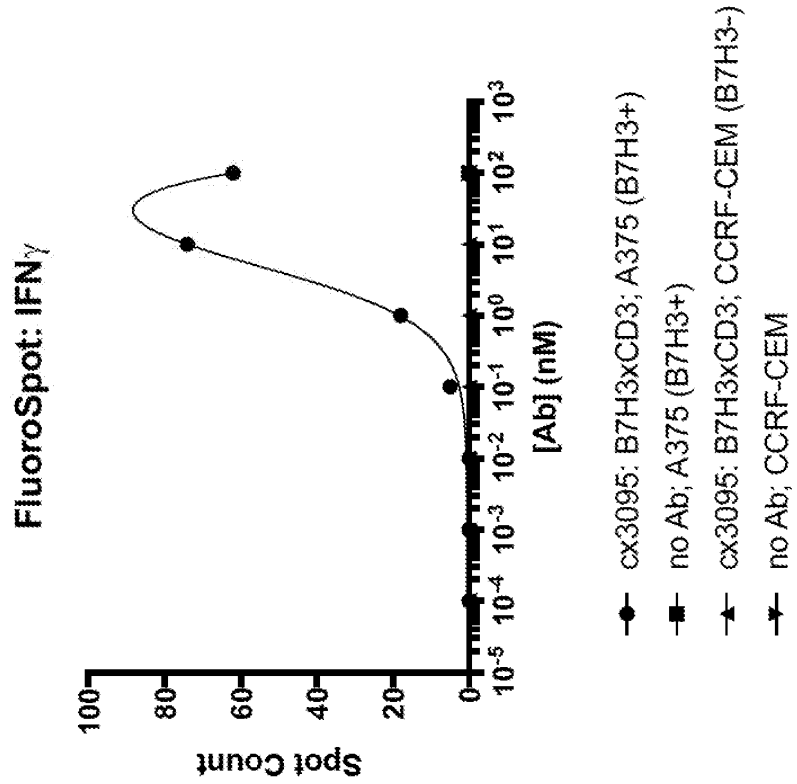

FluoroSpot: IFNγ
Spot Count
100
80
60
40
20
0
10-5 10-4 10-3 10-2 10-1 100 101 102 103
[Ab] (nM)
cx3095: B7H3xCD3; A375 (B7H3+)
no Ab; A375 (B7H3+)
cx3095: B7H3xCD3; CCRF-CEM (B7H3-)
no Ab; CCRF-CEM

FIG. 37

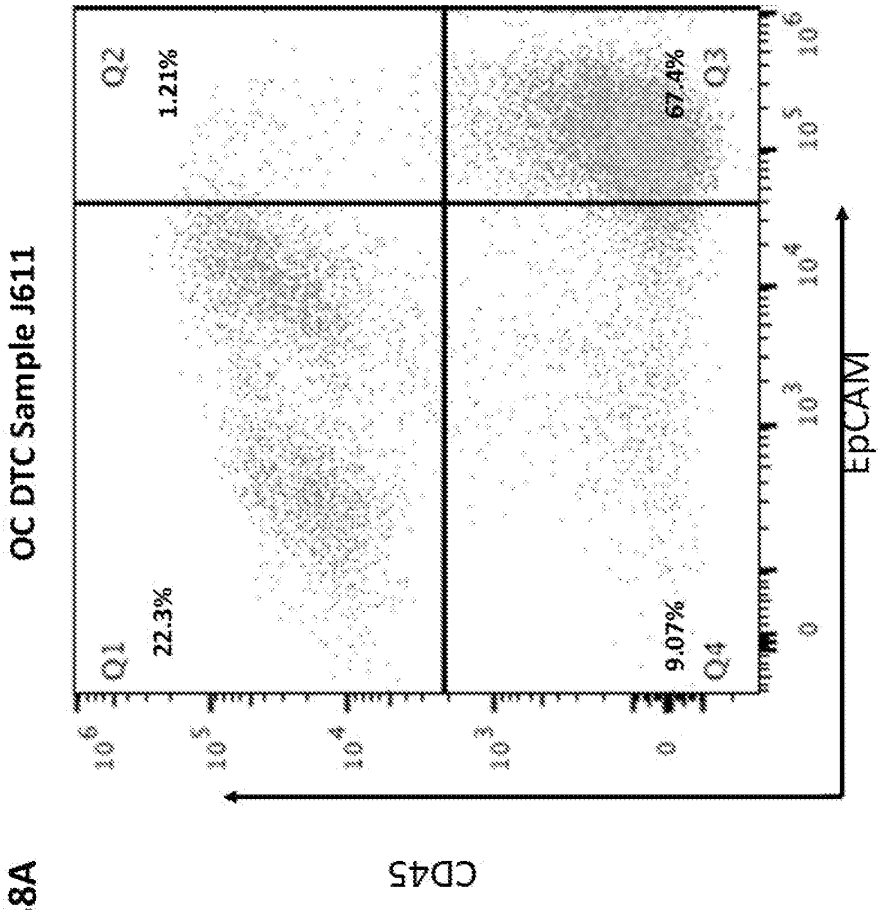

OC DTC Sample J611
Q1
22.3%
Q2
1.21%
Q3
67.4%
Q4
9.07%
CD45
EpCAM
0
10^3
10^4
10^5
10^6

FIG. 38A

FIG. 38B

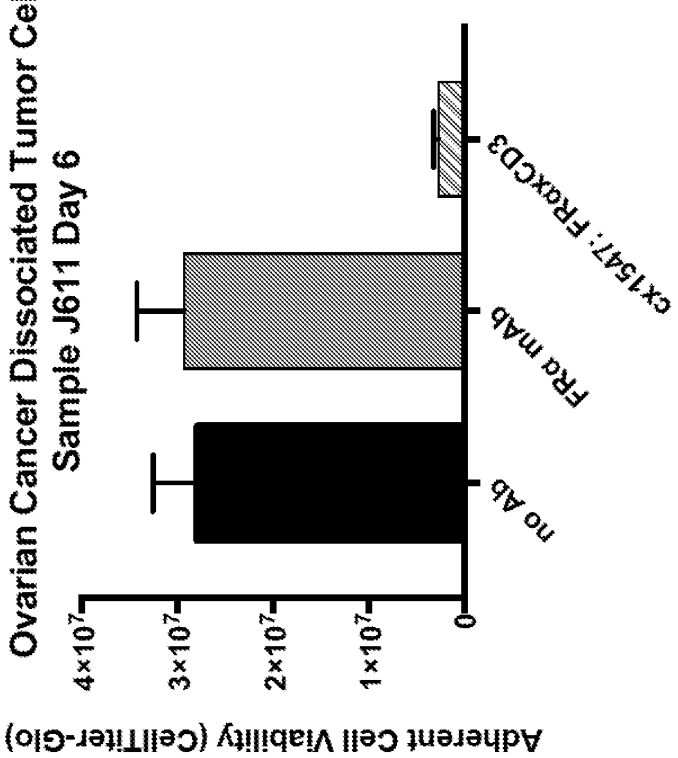


FIG. 38C

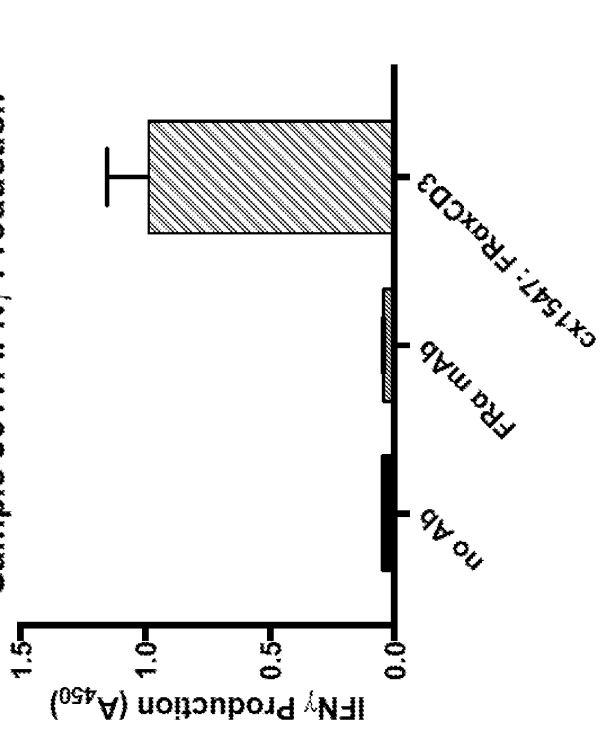


FIG. 38D

No Treatment
FRα mAb
Cx1547: FRα x CD3
Tumor cell viability (adherent cells remaining) was assessed via CellTiter Glo reagent after washing away lymphocytes (suspension cells)