FIG. 1

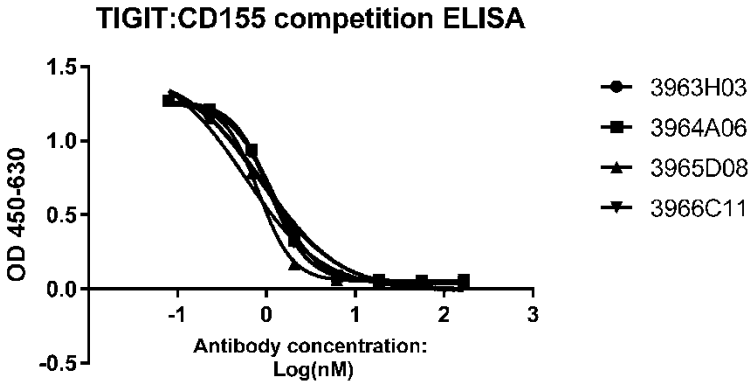

TIGIT:CD155 competition ELISA
OD 450-630
1.5
1.0
0.5
0.0
-0.5
-1
0
1
2
3
Antibody concentration:
Log(nM)
3963H03
3964A06
3965D08
3966C11

FIG. 2

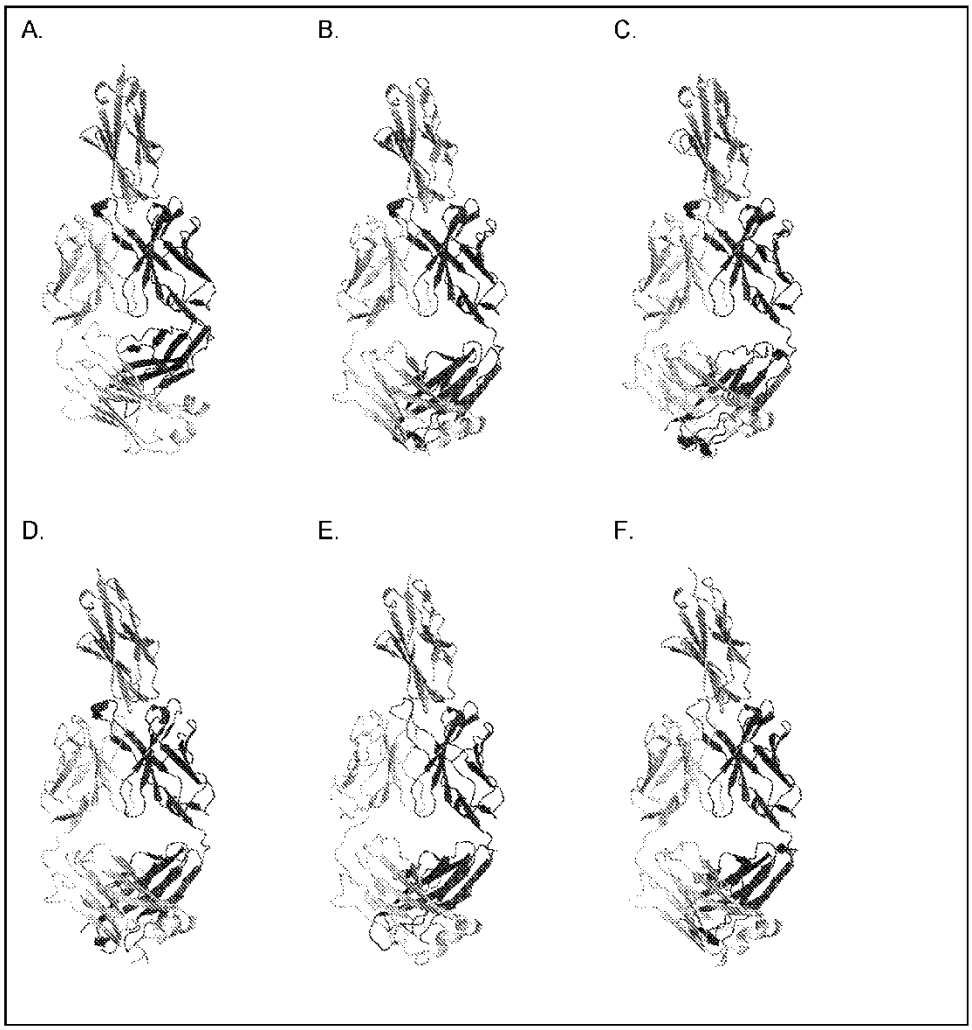


FIG. 3

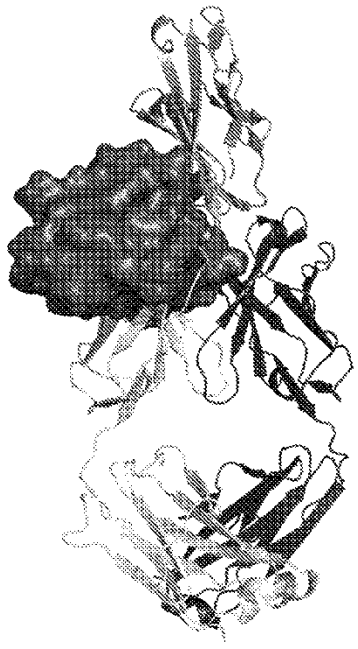


FIG. 4

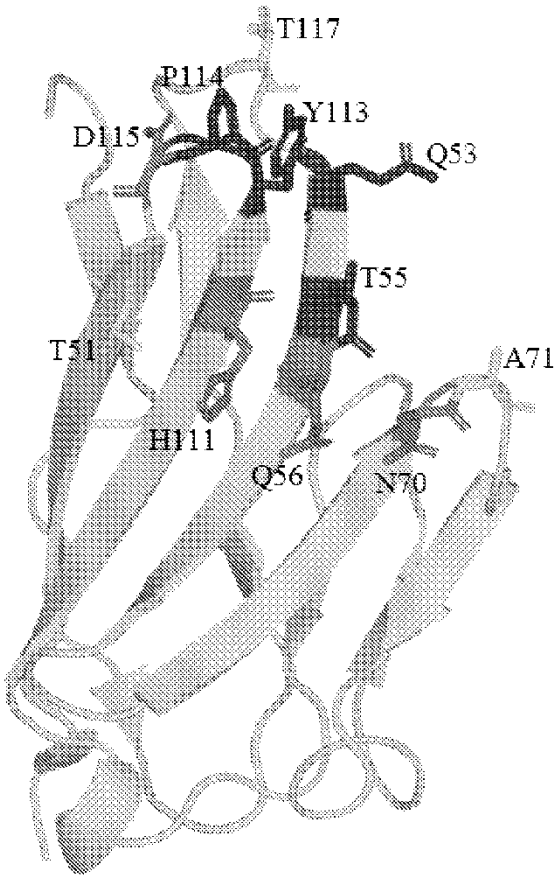


FIG. 5

Figure 5

KD(nM)	H03-12
huTIGIT ECD	2.1±0.1
huTIGIT mt(T51A)	1.8±0.4
huTIGIT mt(Q53A)	NB
huTIGIT mt(T55A)	NB
huTIGIT mt(Q56A)	33.9±0.9
huTIGIT mt(N70A)	31.5±6.0
huTIGIT mt(A71G)	7.0±0.4
huTIGIT mt(H111A)	30.9±0.2
huTIGIT mt(Y113A)	NB
huTIGIT mt(P114G)	NB
huTIGIT mt(D115A)	14.4±0.8
huTIGIT mt(T117A)	1.6±0.2

ΔΔG >3 >2 >0.7 **kcal/mol**

FIG. 6

ΔΔG (kcal/mol)	HD3-12
huTIGIT ECD	0.0
huTIGIT mt(T51A)	-0.1±0.2
huTIGIT mt(Q53A)	NB
huTIGIT mt(T55A)	NB
huTIGIT mt(Q56A)	1.7±0.0
huTIGIT mt(N70A)	1.6±0.1
huTIGIT mt(A71G)	0.7±0.0
huTIGIT mt(H111A)	1.6±0.0
huTIGIT mt(Y113A)	NB
huTIGIT mt(P114G)	NB
huTIGIT mt(D115A)	1.1±0.0
huTIGIT mt(T117A)	-0.2±0.1

ΔΔG >3 >2 >0.7 **kcal/mol**

FIG. 7

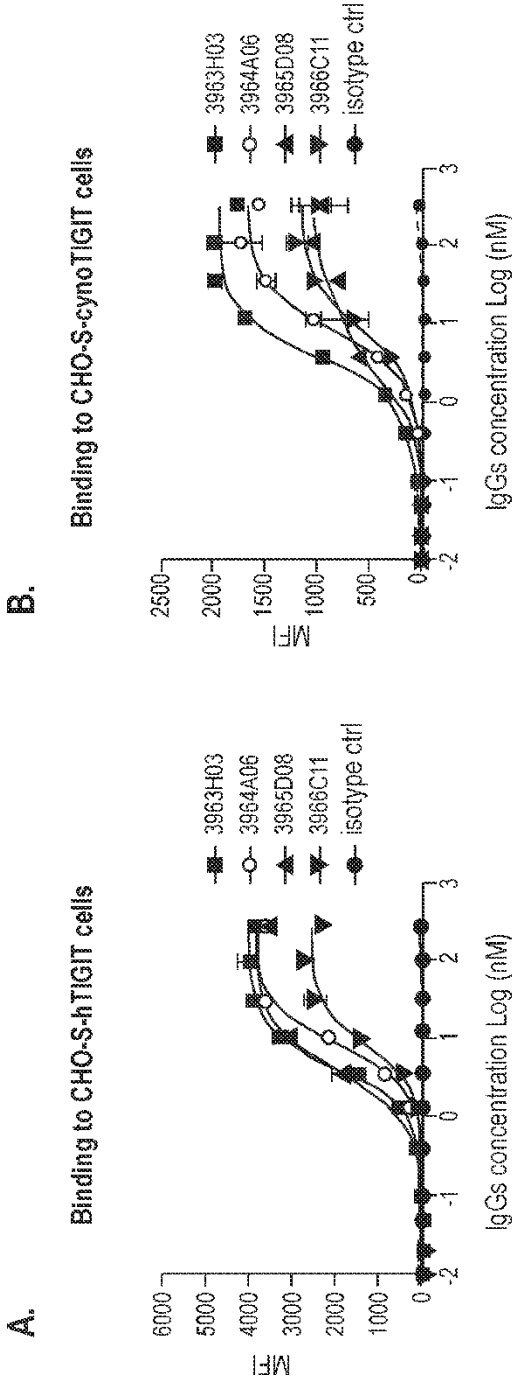

A.
Binding to CHO-S-hTIGIT cells
MFI
6000
5000
4000
3000
2000
1000
0
-2
-1
0
1
2
3
IgGs concentration Log (nM)
3963H03
3964A06
3965D08
3966C11
isotype ctrl
B.
Binding to CHO-S-cynoTIGIT cells
MFI
2500
2000
1500
1000
500
0
-2
-1
0
1
2
3
IgGs concentration Log (nM)
3963H03
3964A06
3965D08
3966C11
isotype ctrl

FIG. 8

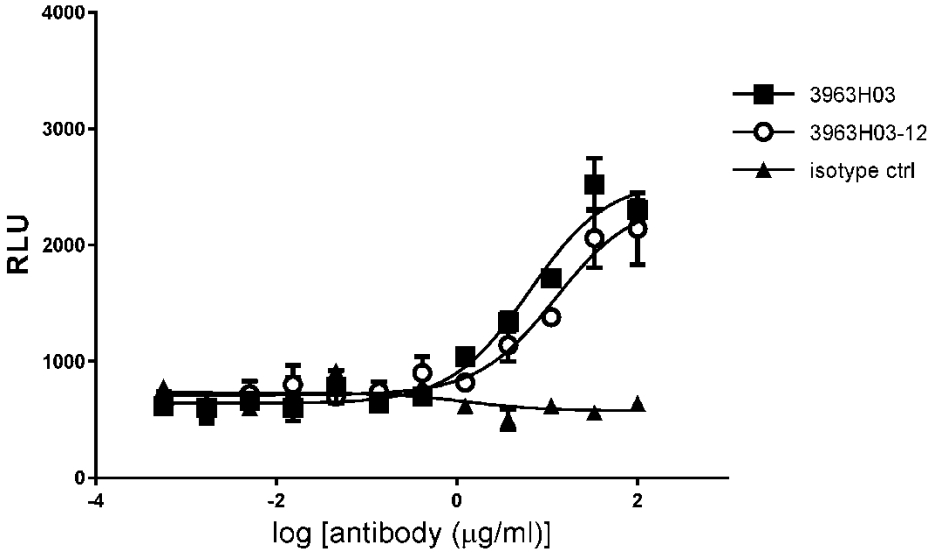

4000
3000
2000
1000
0
RLU
-4
-2
0
2
log [antibody (μg/ml)]
3963H03
3963H03-12
isotype ctrl

FIG. 9

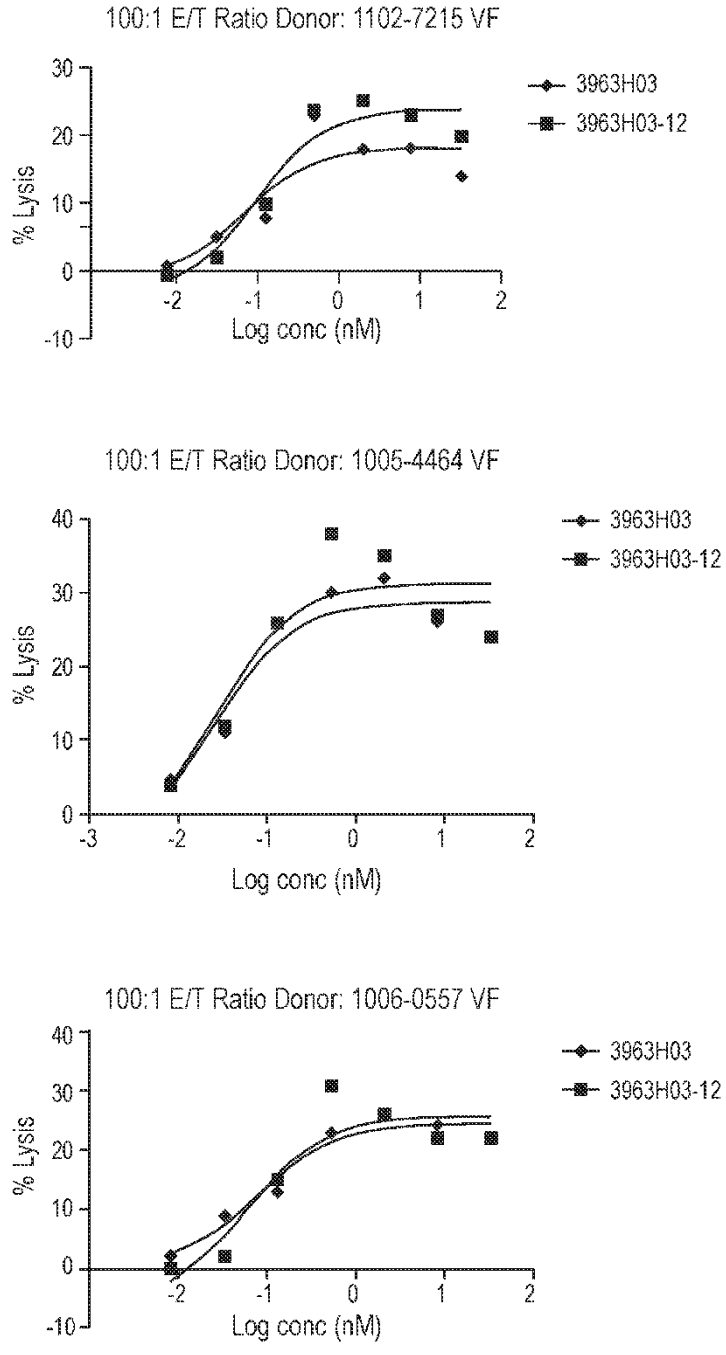

100:1 E/T Ratio Donor: 1102-7215 VF
% Lysis
Log conc (nM)
3963H03
3963H03-12
100:1 E/T Ratio Donor: 1005-4464 VF
% Lysis
Log conc (nM)
3963H03
3963H03-12
100:1 E/T Ratio Donor: 1006-0557 VF
% Lysis
Log conc (nM)
3963H03
3963H03-12

FIG. 10

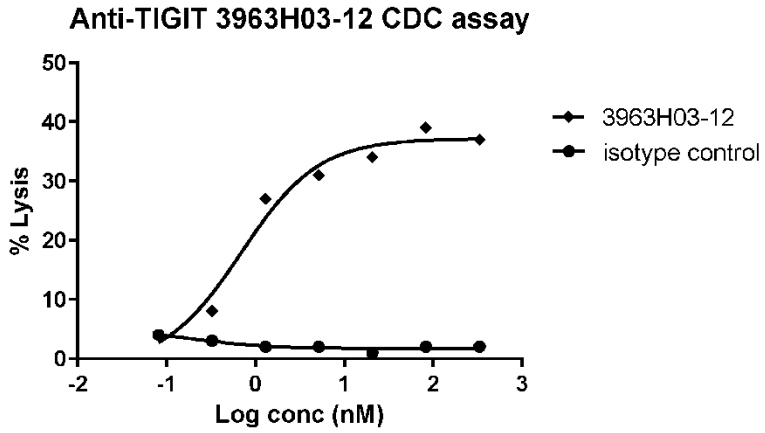

Anti-TIGIT 3963H03-12 CDC assay
% Lysis
50
40
30
20
10
0
-2
-1
0
1
2
3
Log conc (nM)
3963H03-12
isotype control

FIG. 11

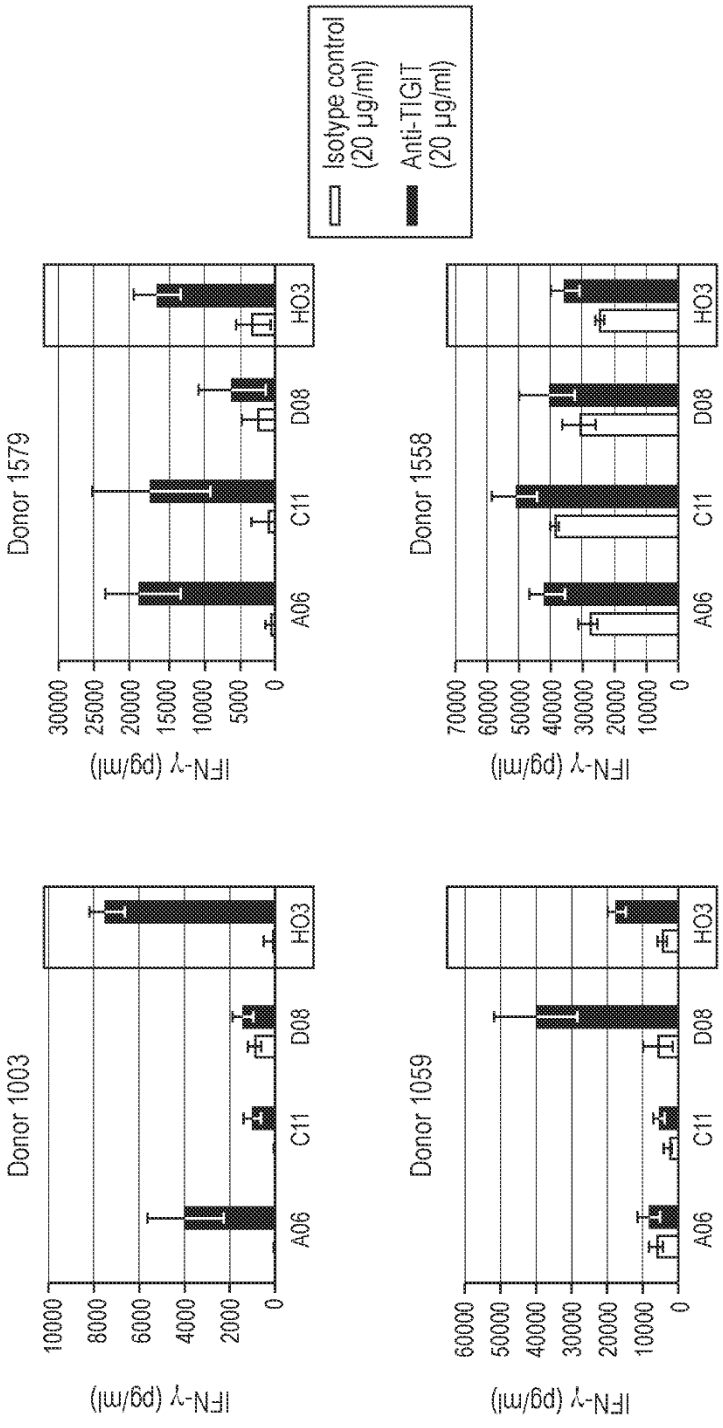

Isotype control (20 µg/ml)
Anti-TIGIT (20 µg/ml)
Donor 1579
Donor 1558
Donor 1003
Donor 1059
IFN-γ (pg/ml)
A06
C11
D08
HO3

FIG. 12

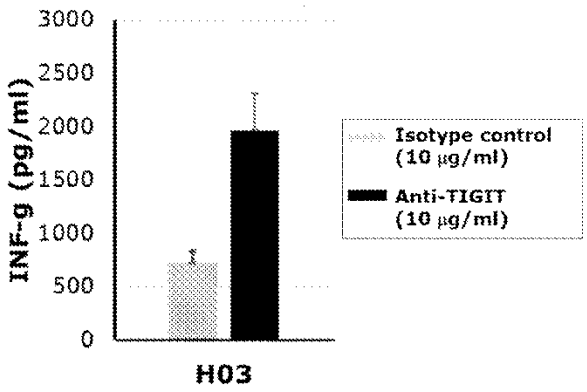
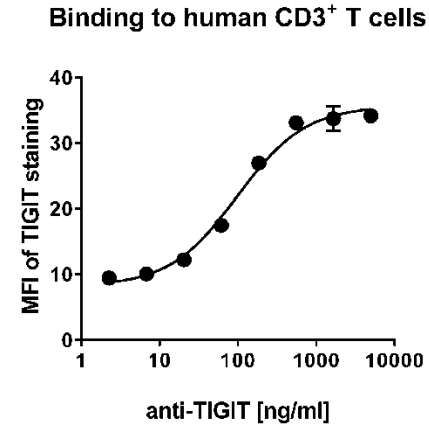

3000
2500
2000
1500
1000
500
0
INF-g (pg/ml)
H03
Isotype control
(10 μg/ml)
Anti-TIGIT
(10 μg/ml)

FIG. 13

A.


Binding to human CD3+ T cells
MFI of TIGIT staining
0
10
20
30
40
1
10
100
1000
10000
anti-TIGIT [ng/ml]

B.

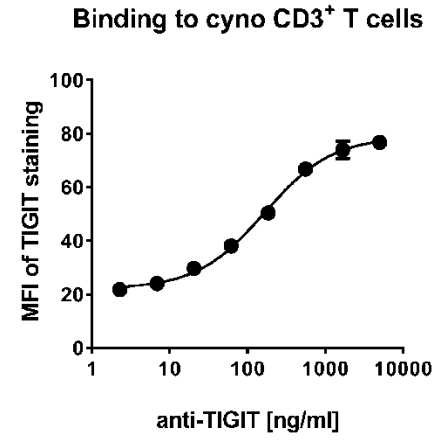
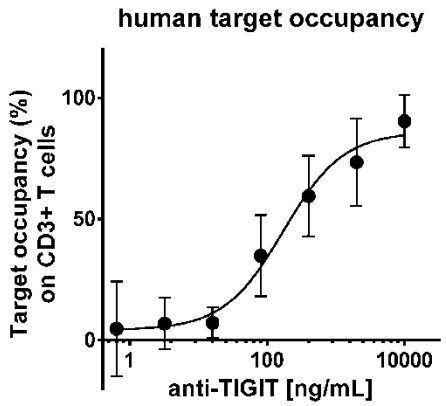

Binding to cyno CD3+ T cells
MFI of TIGIT staining
0
20
40
60
80
100
1
10
100
1000
10000
anti-TIGIT [ng/ml]

FIG. 14

A.


human target occupancy
Target occupancy (%) on CD3+ T cells
100
50
0
1
100
10000
anti-TIGIT [ng/mL]

B.

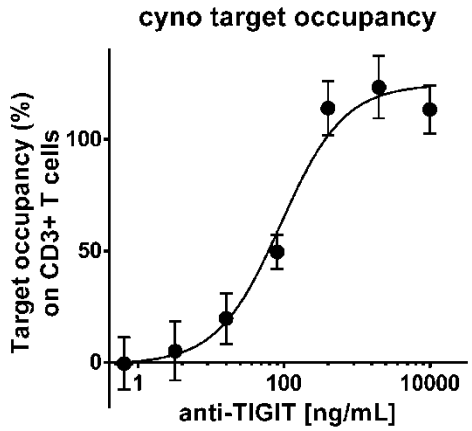
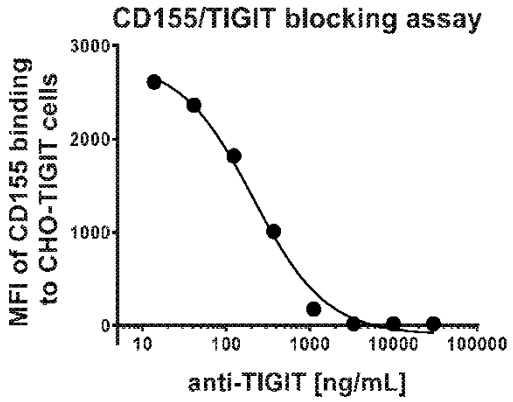

cyno target occupancy
Target occupancy (%) on CD3+ T cells
100
50
0
1
100
10000
anti-TIGIT [ng/mL]

FIG. 15

A.


CD155/TIGIT blocking assay
MFI of CD155 binding to CHO-TIGIT cells
3000
2000
1000
0
10
100
1000
10000
100000
anti-TIGIT [ng/mL]

B.

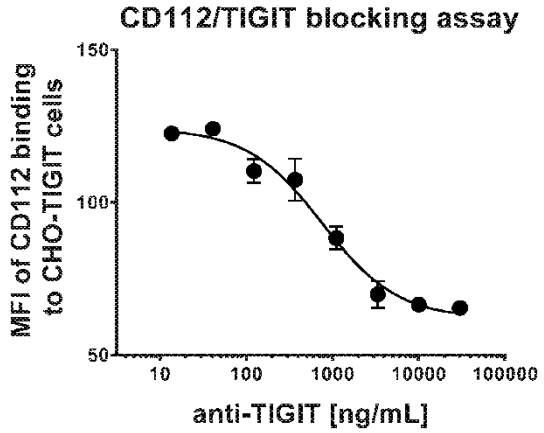
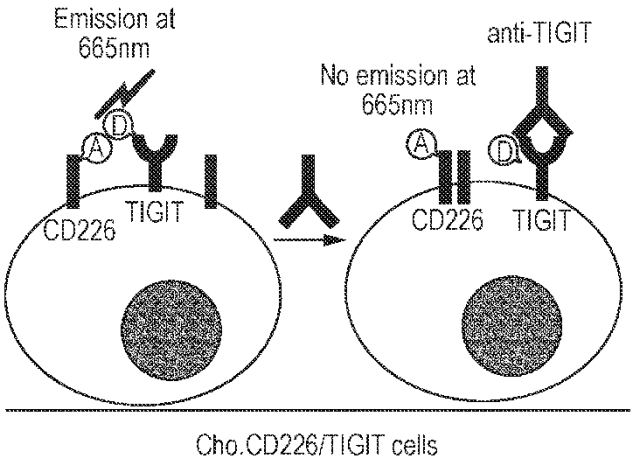

CD112/TIGIT blocking assay
MFI of CD112 binding to CHO-TIGIT cells
150
100
50
10
100
1000
10000
100000
anti-TIGIT [ng/mL]

FIG. 16

A


Emission at
665nm
anti-TIGIT
No emission at
665nm
A
D
A
D
CD226
TIGIT
CD226
TIGIT
Cho.CD226/TIGIT cells

B

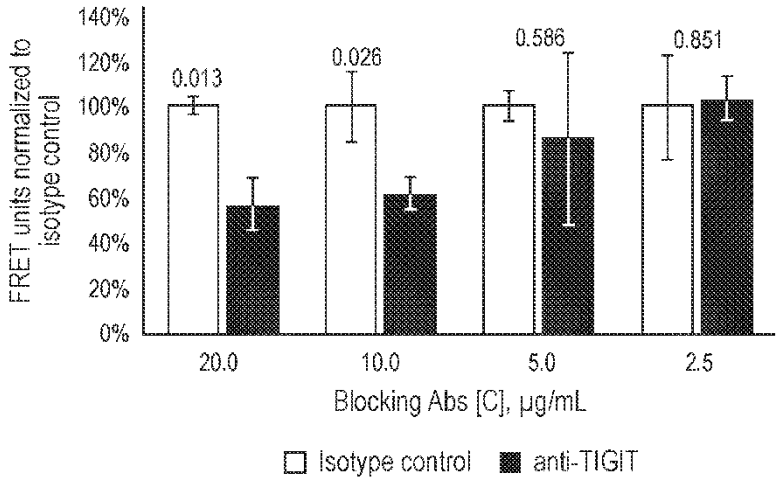

FRET units normalized to isotype control
140%
120%
100%
80%
60%
40%
20%
0%
0.013
0.026
0.586
0.851
20.0
10.0
5.0
2.5
Blocking Abs [C], µg/mL
Isotype control
anti-TIGIT

FIG. 17

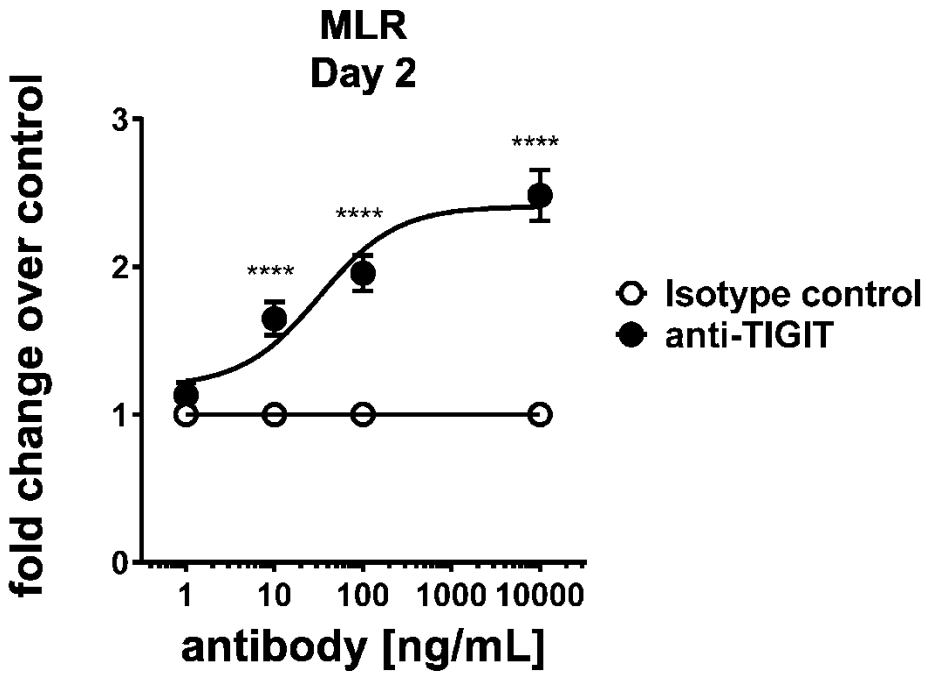

MLR
Day 2
fold change over control
3
2
1
0
****
****
****
1
10
100
1000
10000
antibody [ng/mL]
Isotype control
anti-TIGIT

FIG. 18

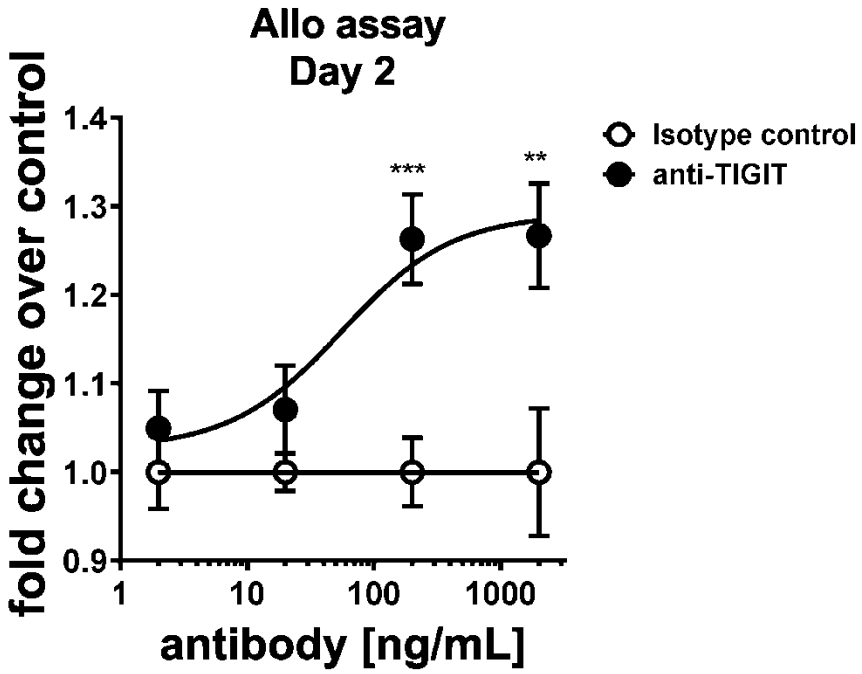

Allo assay
Day 2
fold change over control
1.4
1.3
1.2
1.1
1.0
0.9
1
10
100
1000
antibody [ng/mL]
***
**
Isotype control
anti-TIGIT

FIG. 19A

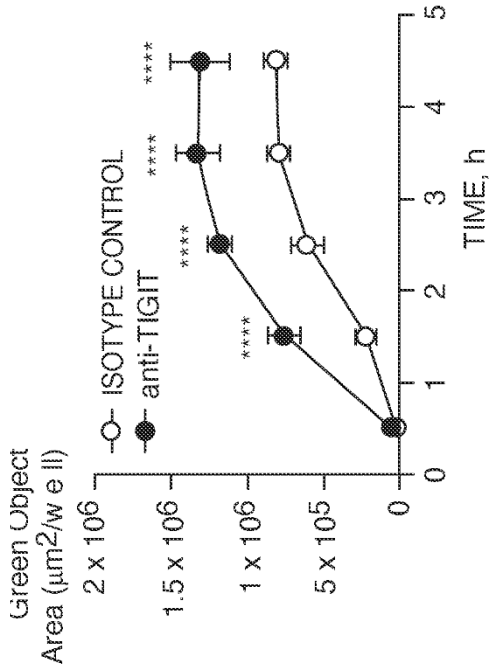


FIG. 19B

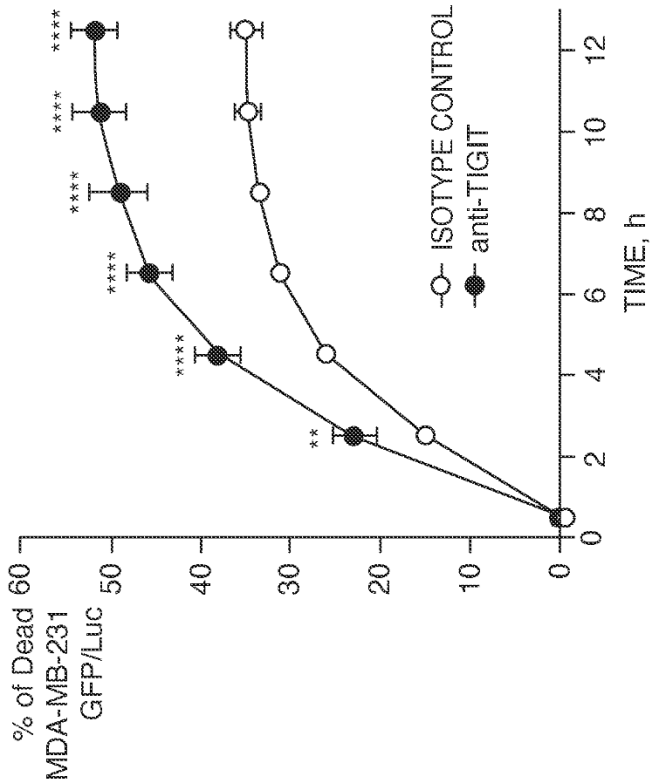


FIG. 20

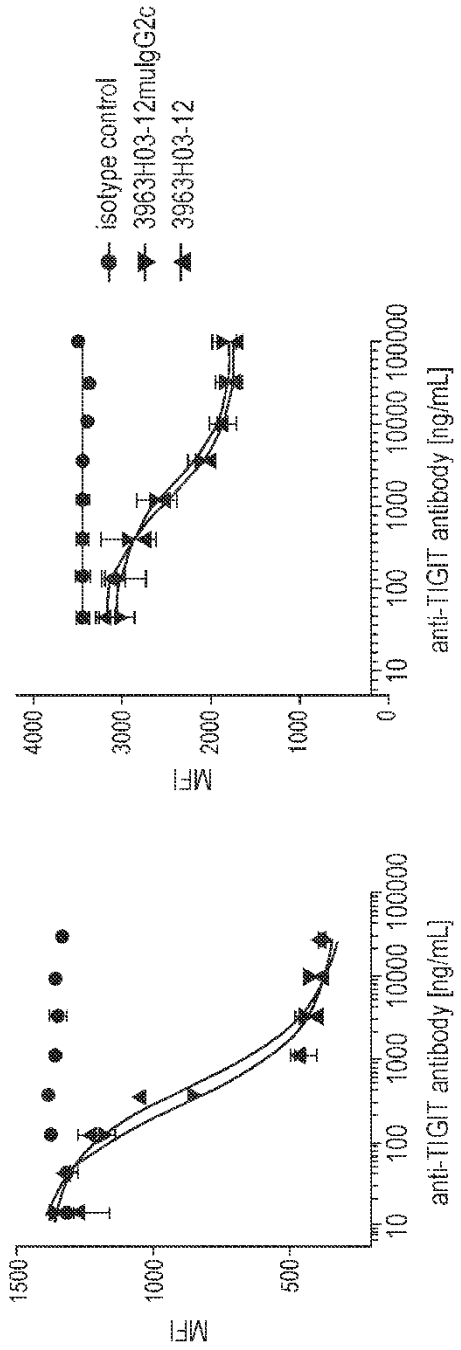

muCD155
muCD112
isotype control
3963H03-12mulgG2c
3963H03-12
MFI
anti-TIGIT antibody [ng/mL]
1500
1000
500
4000
3000
2000
1000
0
10
100
1000
10000
100000

FIG. 21

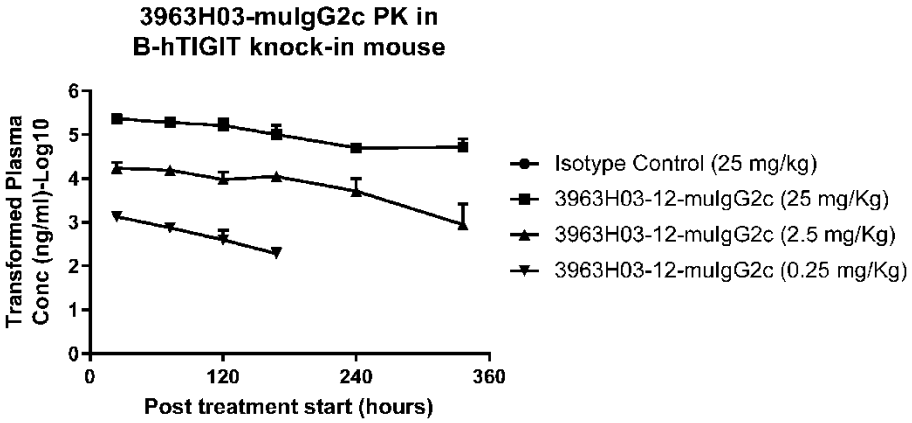

3963H03-mulgG2c PK in
B-hTIGIT knock-in mouse
Transformed Plasma
Conc (ng/ml)-Log10
0
1
2
3
4
5
6
0
120
240
360
Post treatment start (hours)
Isotype Control (25 mg/kg)
3963H03-12-mulgG2c (25 mg/Kg)
3963H03-12-mulgG2c (2.5 mg/Kg)
3963H03-12-mulgG2c (0.25 mg/Kg)

FIG. 22

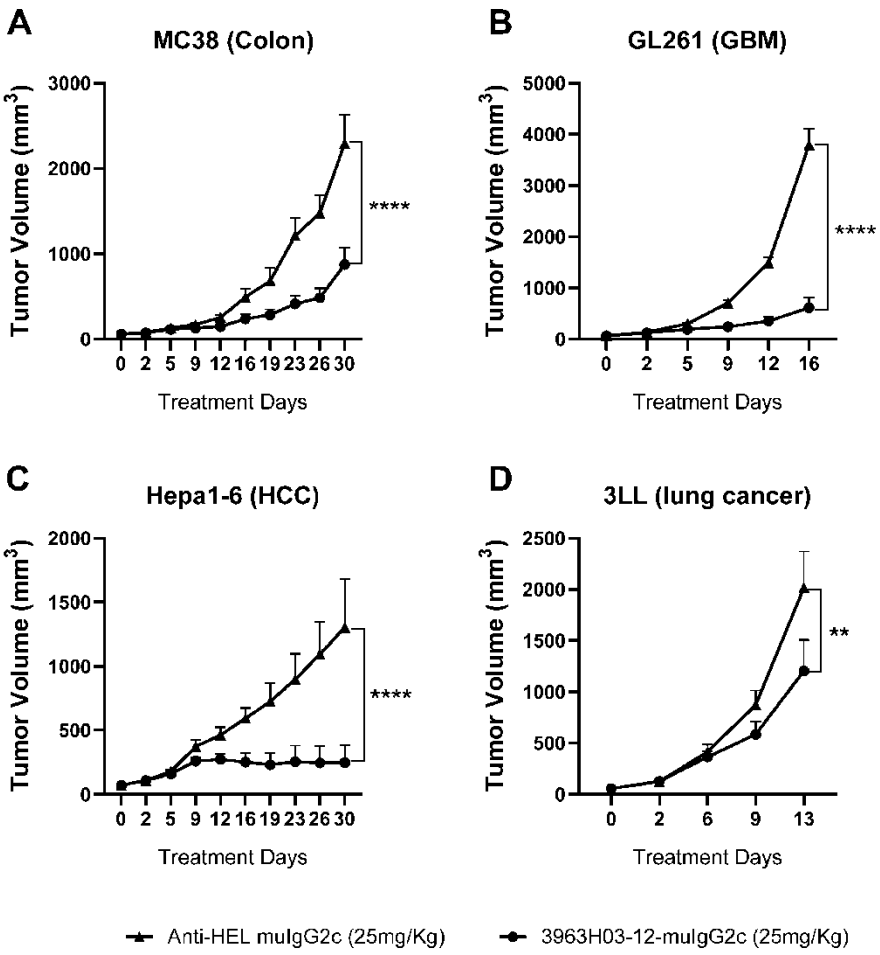

A
MC38 (Colon)
Tumor Volume (mm3)
3000
2000
1000
0
0 2 5 9 12 16 19 23 26 30
Treatment Days
****
B
GL261 (GBM)
Tumor Volume (mm3)
5000
4000
3000
2000
1000
0
0 2 5 9 12 16
Treatment Days
****
C
Hepa1-6 (HCC)
Tumor Volume (mm3)
2000
1500
1000
500
0
0 2 5 9 12 16 19 23 26 30
Treatment Days
****
D
3LL (lung cancer)
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0 2 6 9 13
Treatment Days
**
Anti-HEL mulgG2c (25mg/Kg)
3963H03-12-mulgG2c (25mg/Kg)

FIG. 23

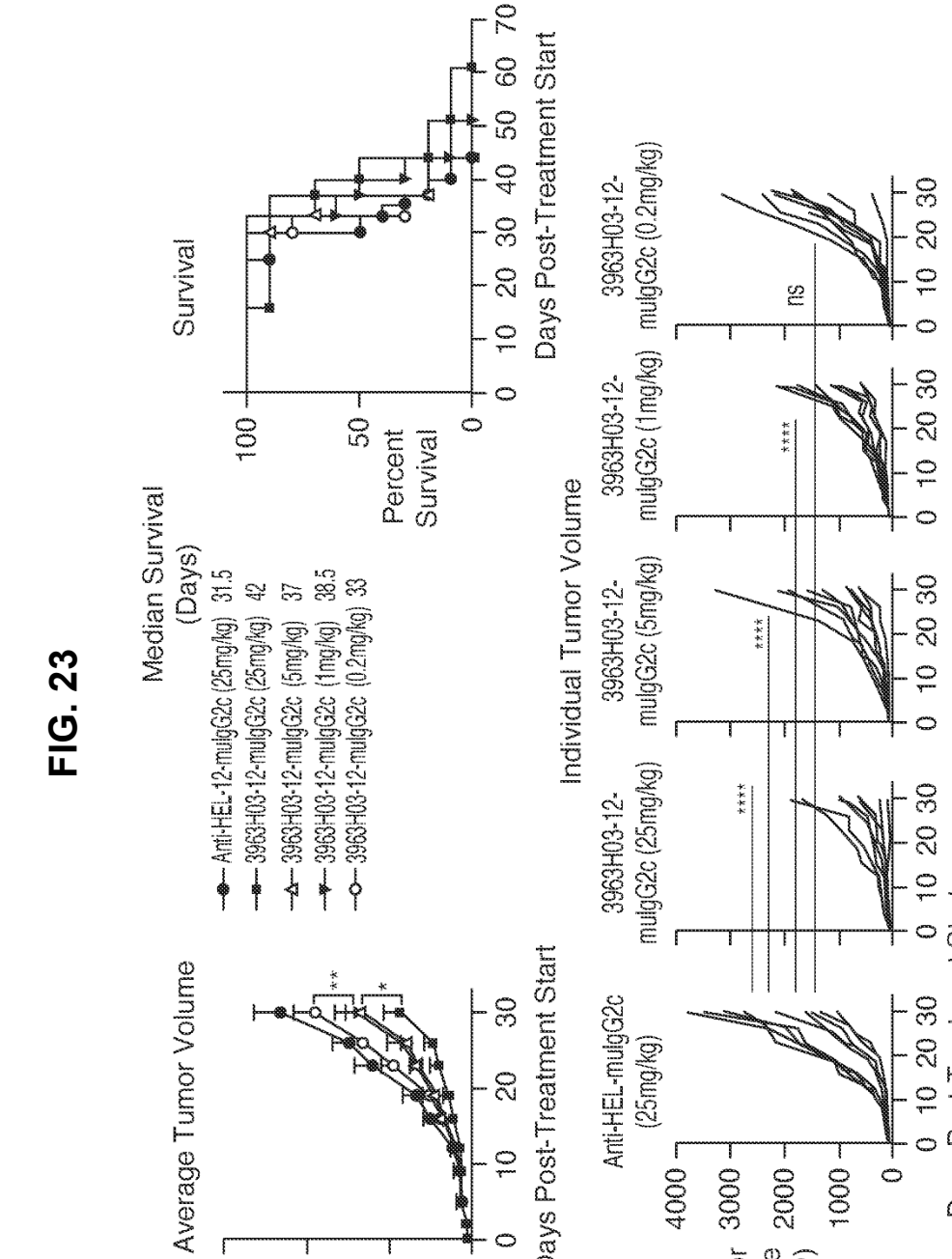

Median Survival (Days)
Anti-HEL-12-mulgG2c (25mg/kg) 31.5
3963H03-12-mulgG2c (25mg/kg) 42
3963H03-12-mulgG2c (5mg/kg) 37
3963H03-12-mulgG2c (1mg/kg) 38.5
3963H03-12-mulgG2c (0.2mg/kg) 33
Survival
Percent Survival
Days Post-Treatment Start
Average Tumor Volume
Tumor Volume (mm3)
Days Post-Treatment Start
Individual Tumor Volume
Anti-HEL-mulgG2c (25mg/kg)
3963H03-12-mulgG2c (25mg/kg)
3963H03-12-mulgG2c (5mg/kg)
3963H03-12-mulgG2c (1mg/kg)
3963H03-12-mulgG2c (0.2mg/kg)
Tumor Volume (mm3)
Days Post-Treatment Start
****
ns

FIG. 24

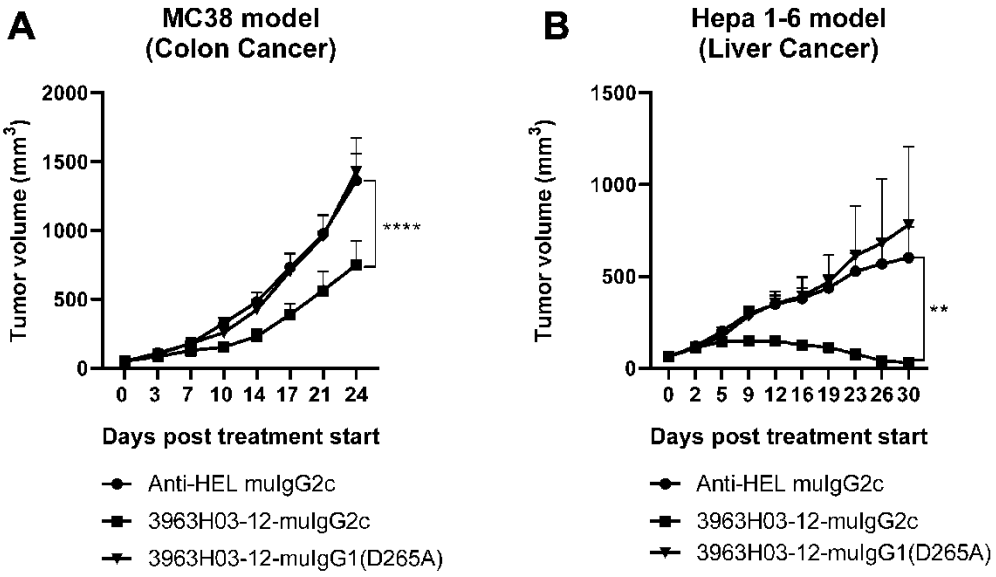

A
MC38 model
(Colon Cancer)
Tumor volume (mm3)
2000
1500
1000
500
0
0 3 7 10 14 17 21 24
****
Days post treatment start
Anti-HEL mulgG2c
3963H03-12-mulgG2c
3963H03-12-mulgG1(D265A)
B
Hepa 1-6 model
(Liver Cancer)
Tumor volume (mm3)
1500
1000
500
0
0 2 5 9 12 16 19 23 26 30
**
Days post treatment start
Anti-HEL mulgG2c
3963H03-12-mulgG2c
3963H03-12-mulgG1(D265A)

FIG. 25

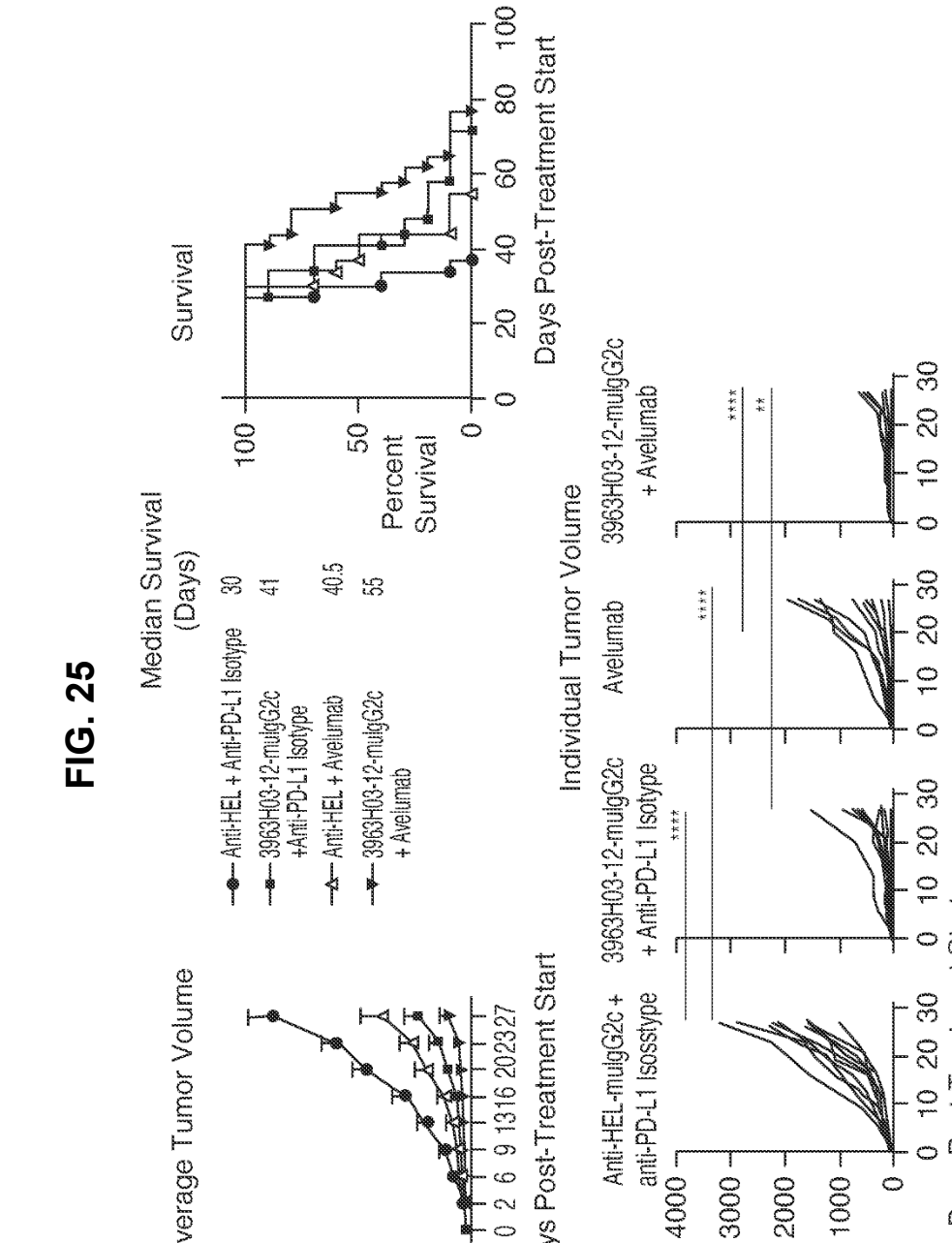

Average Tumor Volume
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0 2 6 9 13 16 20 23 27
Days Post-Treatment Start
Median Survival (Days)
Anti-HEL + Anti-PD-L1 Isotype 30
3963H03-12-mulgG2c +Anti-PD-L1 Isotype 41
Anti-HEL + Avelumab 40.5
3963H03-12-mulgG2c + Avelumab 55
Survival
Percent Survival
100
50
0
0 20 40 60 80 100
Days Post-Treatment Start
Individual Tumor Volume
Anti-HEL-mulgG2c + anti-PD-L1 Isosstype
3963H03-12-mulgG2c + Anti-PD-L1 Isotype
Avelumab
3963H03-12-mulgG2c + Avelumab
Tumor Volume (mm3)
4000
3000
2000
1000
0
****
****
**
0 10 20 30
Days Post-Treatment Start

FIG. 26

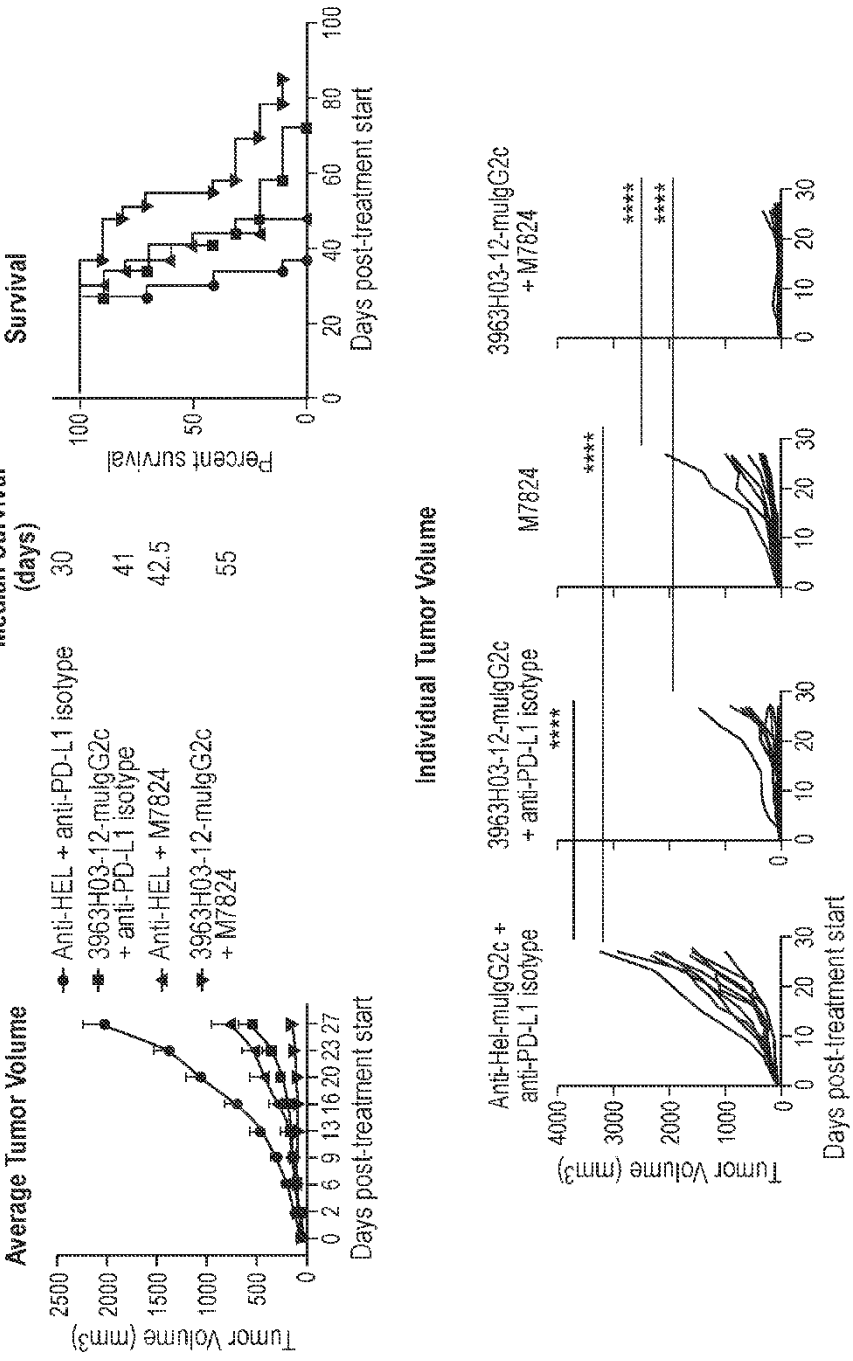
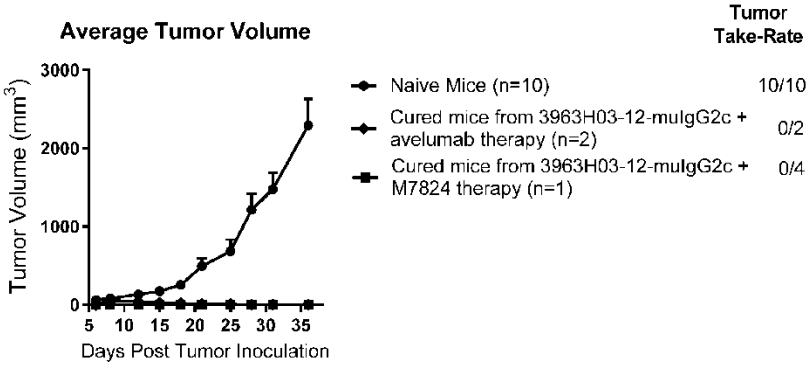

Average Tumor Volume
Tumor Volume (mm3)
Days post-treatment start
Anti-HEL + anti-PD-L1 isotype
3963H03-12-mulgG2c + anti-PD-L1 isotype
Anti-HEL + M7824
3963H03-12-mulgG2c + M7824
Median Survival (days)
30
41
42.5
55
Survival
Percent survival
Days post-treatment start
Individual Tumor Volume
Anti-Hel-mulgG2c + anti-PD-L1 isotype
3963H03-12-mulgG2c + anti-PD-L1 isotype
M7824
3963H03-12-mulgG2c + M7824
****
Tumor Volume (mm3)
Days post-treatment start

FIG. 27


Average Tumor Volume
Tumor Volume (mm3)
3000
2000
1000
0
5
10
15
20
25
30
35
Days Post Tumor Inoculation
Tumor Take-Rate
Naive Mice (n=10)
10/10
Cured mice from 3963H03-12-muIgG2c + avelumab therapy (n=2)
0/2
Cured mice from 3963H03-12-muIgG2c + M7824 therapy (n=1)
0/4