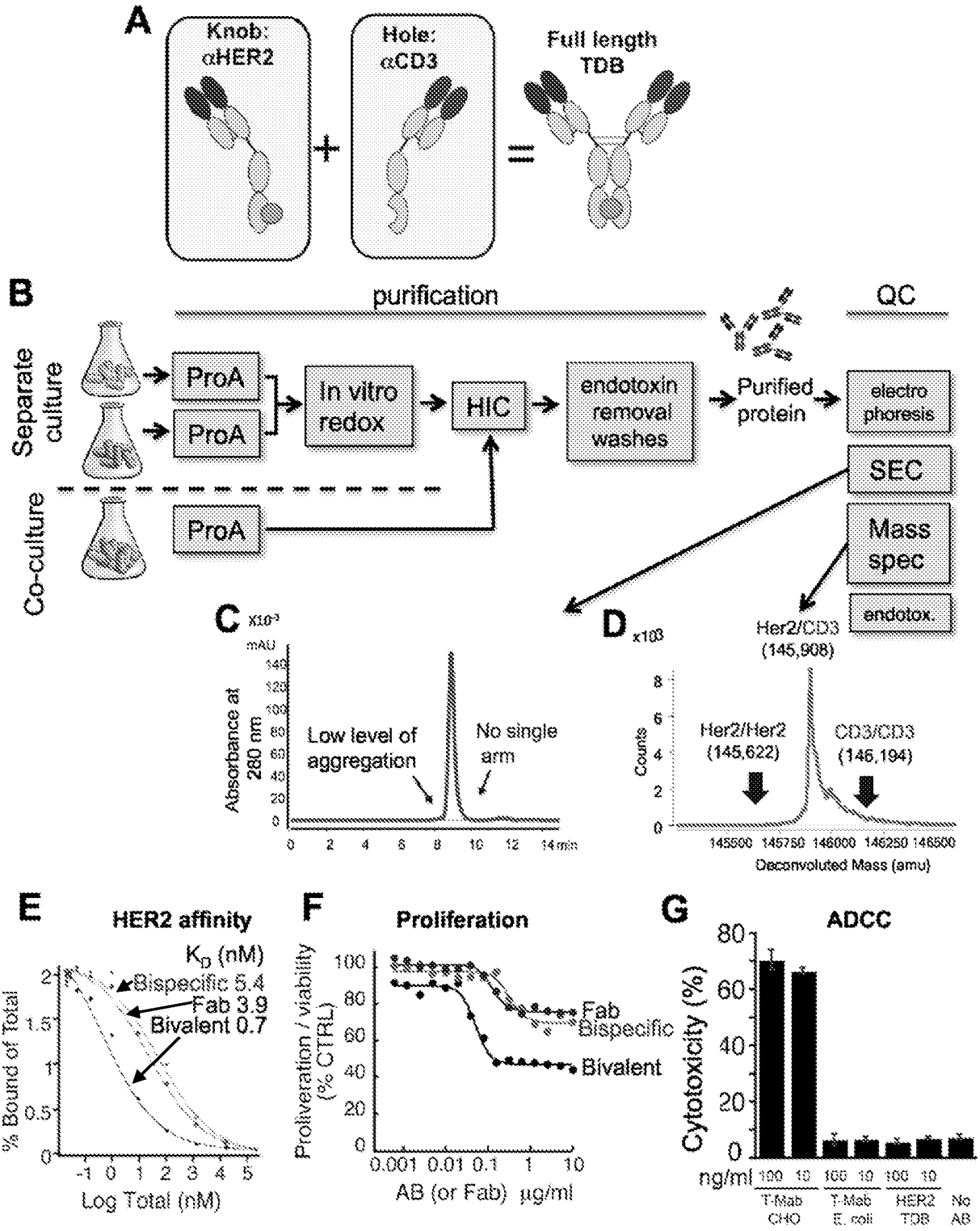

A
Knob: αHER2
Hole: αCD3
Full length TDB
B
purification
QC
Separate culture
Co-culture
ProA
ProA
ProA
In vitro redox
HIC
endotoxin removal washes
Purified protein
electro phoresis
SEC
Mass spec
endotox.
C
Absorbance at 280 nm
Low level of aggregation
No single arm
D
Her2/CD3 (145,908)
Her2/Her2 (145,622)
CD3/CD3 (146,194)
Counts
Deconvoluted Mass (amu)
E
HER2 affinity
K_D (nM)
Bispecific 5.4
Fab 3.9
Bivalent 0.7
% Bound of Total
Log Total (nM)
F
Proliferation
Proliferation / viability (% CTRL)
Fab
Bispecific
Bivalent
AB (or Fab) µg/ml
G
ADCC
Cytotoxicity (%)
ng/ml
T-Mab CHO
T-Mab E. coli
HER2 TDB
No AB

FIG. 1

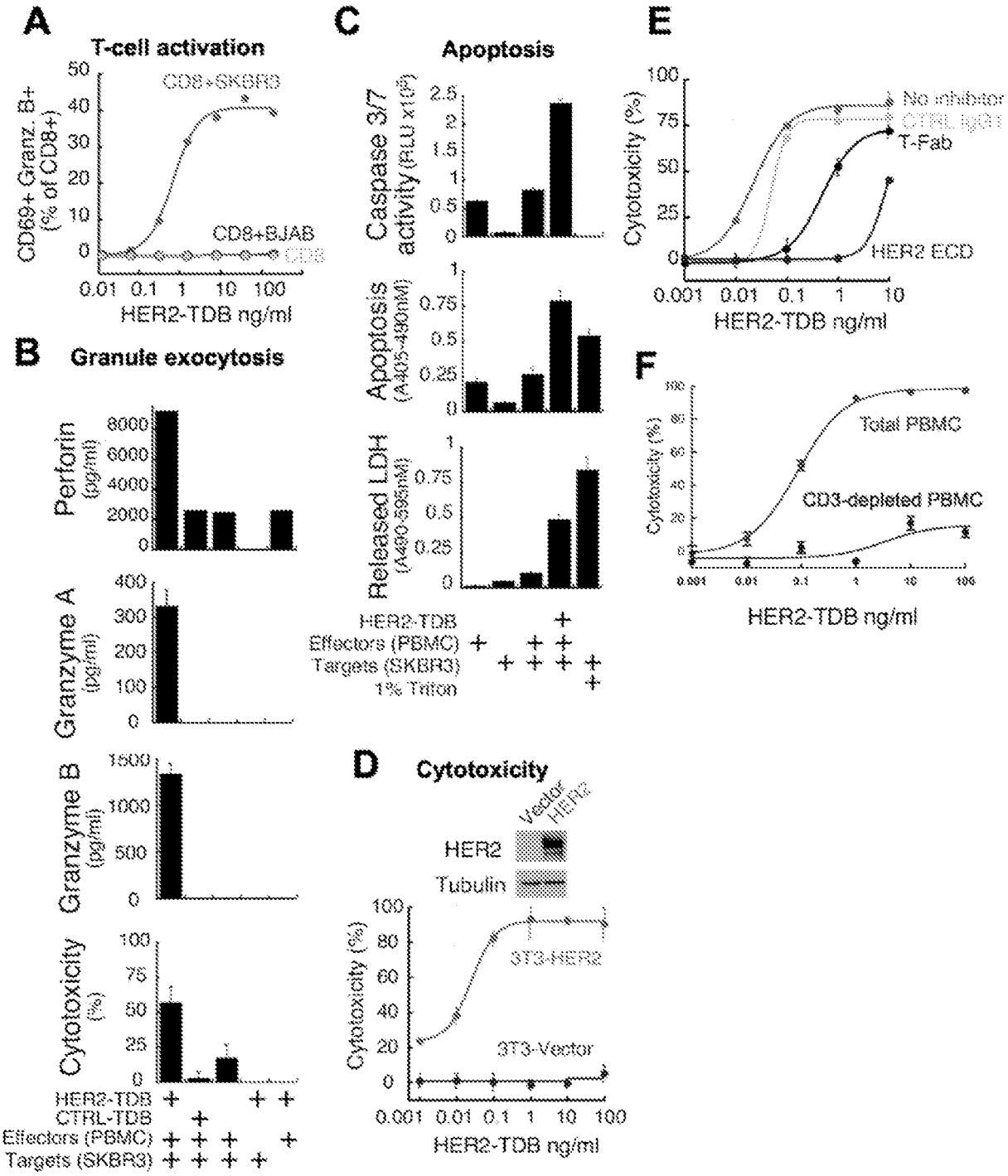

A
T-cell activation
CD69+ Granz. B+ (% of CD8+)
CD8+SKBR3
CD8+BJAB
HER2-TDB ng/ml
B
Granule exocytosis
Perforin (pg/ml)
Granzyme A (pg/ml)
Granzyme B (pg/ml)
Cytotoxicity (%)
HER2-TDB
CTRL-TDB
Effectors (PBMC)
Targets (SKBR3)
C
Apoptosis
Caspase 3/7 activity (RLU x10^3)
Apoptosis (A405-490nM)
Released LDH (A490-595nM)
HER2-TDB
Effectors (PBMC)
Targets (SKBR3)
1% Triton
D
Cytotoxicity
Vector
HER2
HER2
Tubulin
Cytotoxicity (%)
3T3-HER2
3T3-Vector
HER2-TDB ng/ml
E
Cytotoxicity (%)
No inhibitor
CTRL IgG1
T-Fab
HER2 ECD
HER2-TDB ng/ml
F
Cytotoxicity (%)
Total PBMC
CD3-depleted PBMC
HER2-TDB ng/ml

FIG. 2

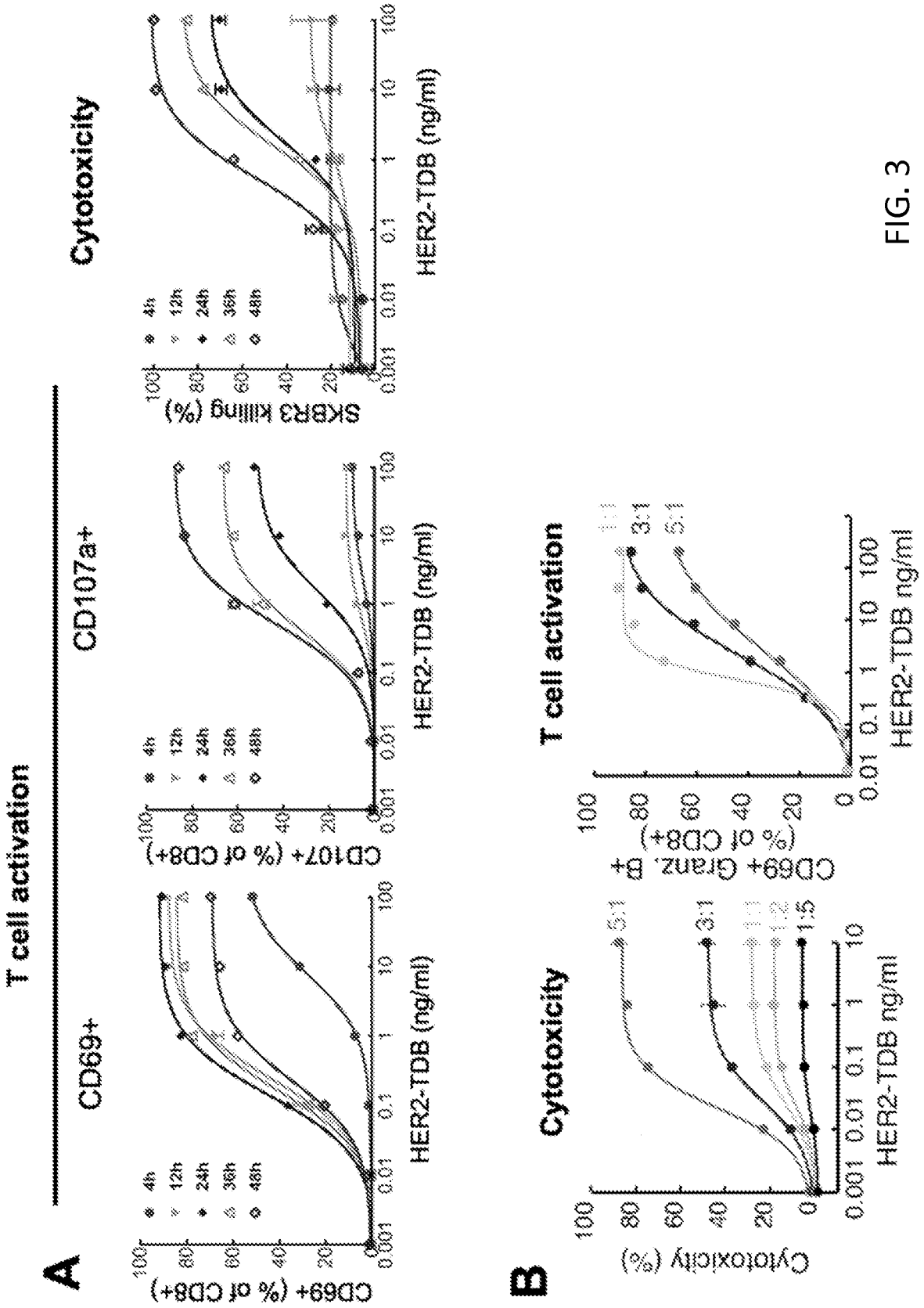

A
T cell activation
CD69+
CD107a+
Cytotoxicity
CD69+ (% of CD8+)
CD107+ (% of CD8+)
SKBR3 killing (%)
HER2-TDB (ng/ml)
4h
12h
24h
36h
48h
B
Cytotoxicity
T cell activation
Cytotoxicity (%)
CD69+ Granz. B+ (% of CD8+)
HER2-TDB ng/ml
6:1
3:1
1:1
1:2
1:5

FIG. 3

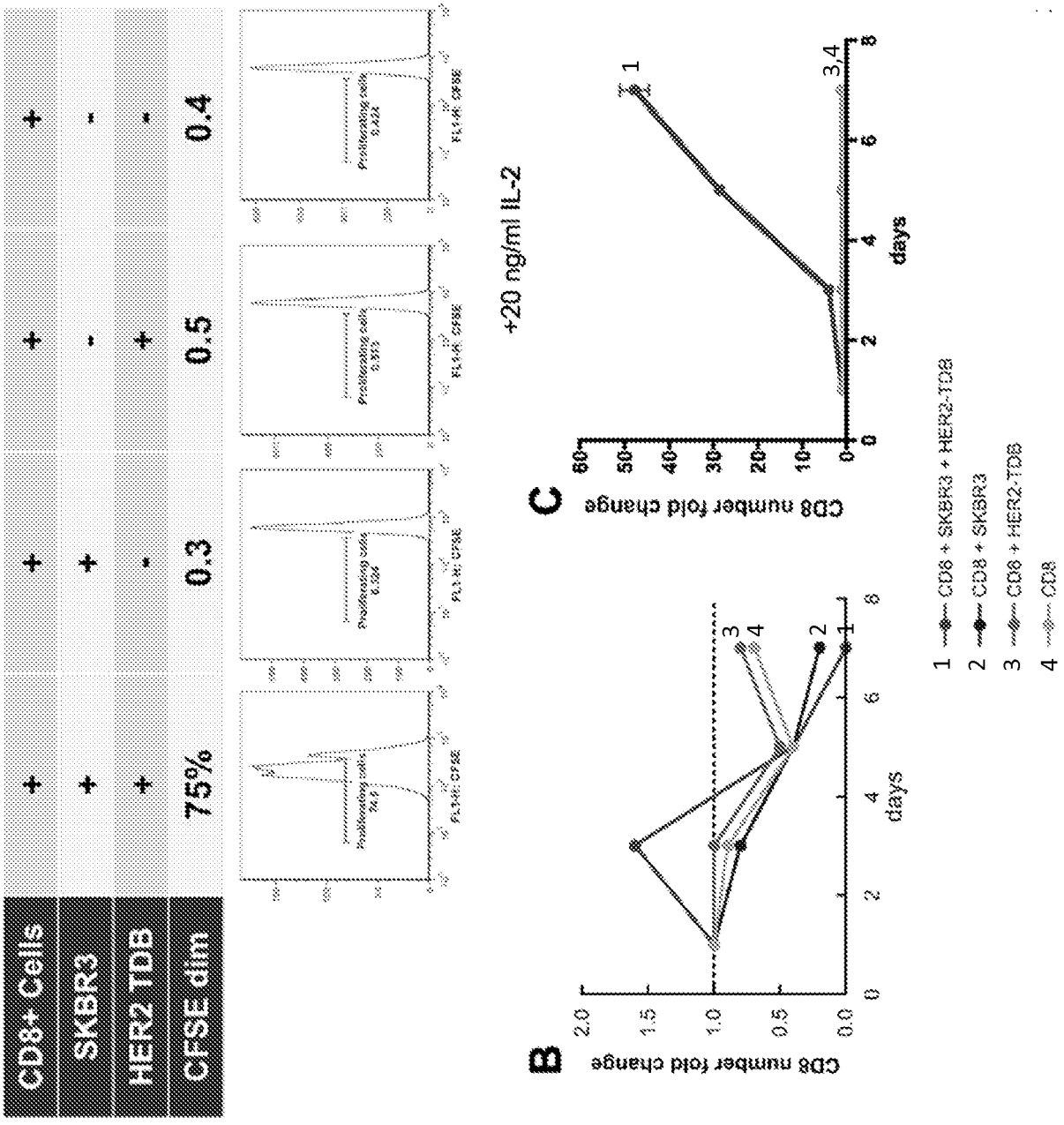

A
CD3+ Cells
SKBR3
HER2 TDB
CFSE dim
+
+
+
+
+
+
-
+
-
+
-
+
75%
0.3
0.5
0.4
B
CD8 number fold change
days
C
+20 ng/ml IL-2
CD8 number fold change
days
1 CD8 + SKBR3 + HER2-TDB
2 CD8 + SKBR3
3 CD8 + HER2-TDB
4 CD8

FIG. 4

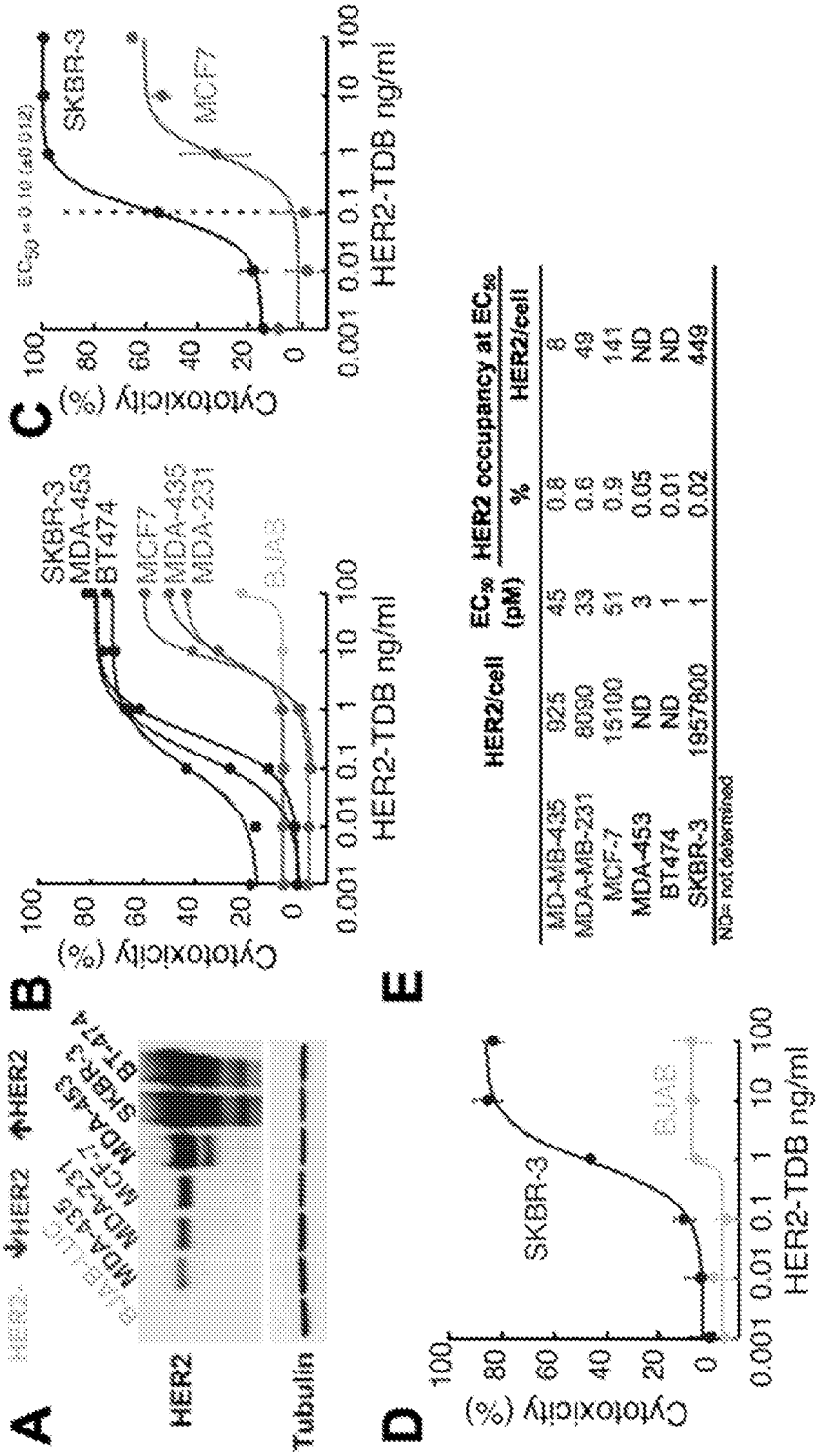

A
HER2-
HER2+
HER2
Tubulin
B
Cytotoxicity (%)
HER2-TDB ng/ml
SKBR-3
MDA-453
BT474
MCF7
MDA-435
MDA-231
BJAB
C
Cytotoxicity (%)
HER2-TDB ng/ml
SKBR-3
MCF7
D
Cytotoxicity (%)
HER2-TDB ng/ml
SKBR-3
BJAB
E
HER2/cell
EC50 (pM)
HER2 occupancy at EC50
% HER2/cell
MD-MB-435 925 45 0.8 8
MDA-MB-231 8030 33 0.6 48
MCF-7 16100 51 0.9 141
MDA-453 ND 3 0.05 ND
BT474 ND 1 0.01 ND
SKBR-3 1957800 1 0.02 449
ND= not determined

FIG. 5

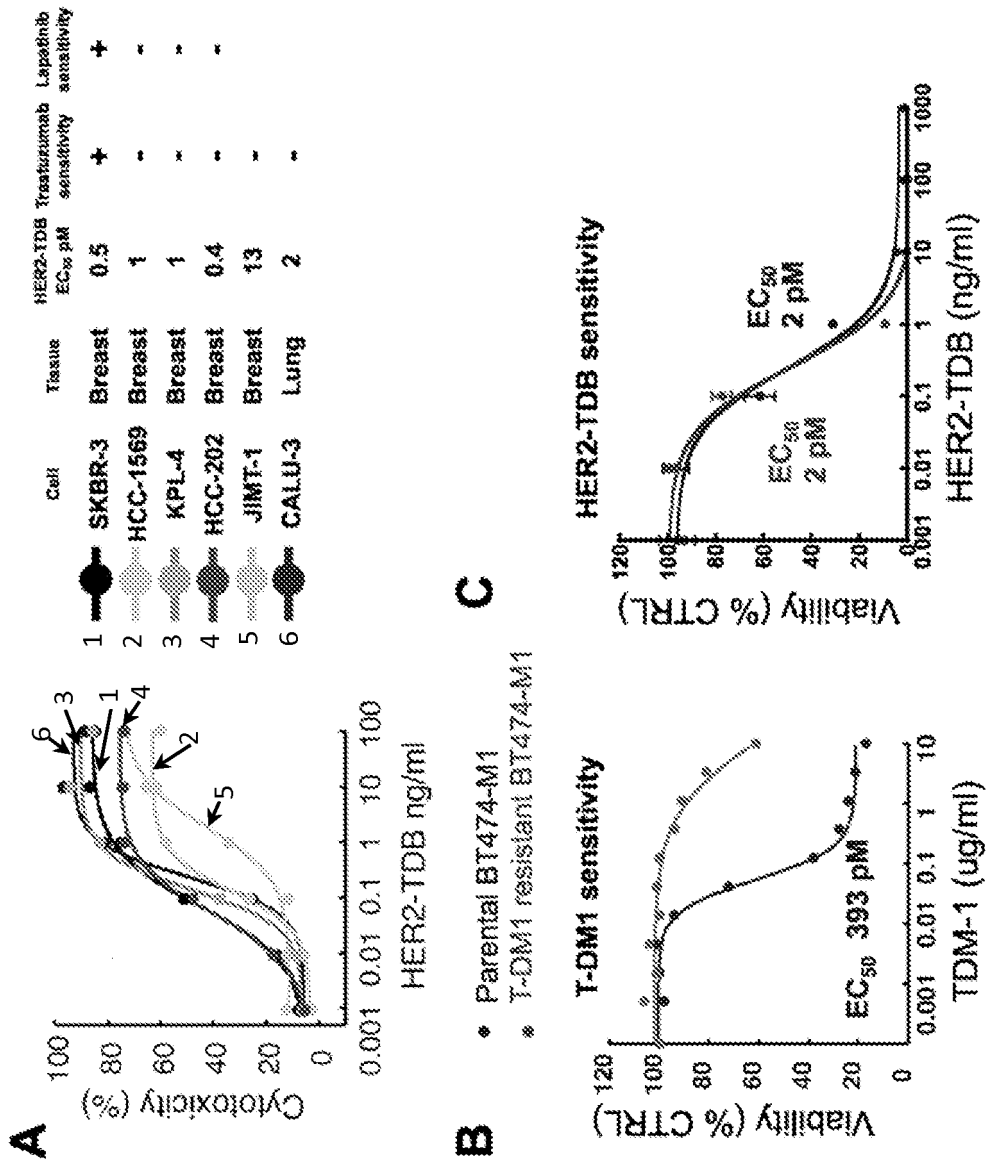

A
Cytotoxicity (%)
HER2-TDB ng/ml
Cell
Tissue
HER2-TDB EC50 pM
Trastuzumab sensitivity
Lapatinib sensitivity
1 SKBR-3 Breast 0.5 + +
2 HCC-1569 Breast 1 - -
3 KPL-4 Breast 1 - -
4 HCC-202 Breast 0.4 - -
5 JIMT-1 Breast 13 - -
6 CALU-3 Lung 2 - -
B
Parental BT474-M1
T-DM1 resistant BT474-M1
C
T-DM1 sensitivity
Viability (% CTRL)
EC50 393 pM
TDM-1 (ug/ml)
HER2-TDB sensitivity
EC50 2 pM
EC50 2 pM
HER2-TDB (ng/ml)

FIG. 6

| | HER2-TDB | Trastuzumab |
|---|---|---|
| CL (mL/day/kg) | 10 ± 2 | 8 ± 1 |
| AUC (day*ug/mL) | 1328 ± 247 | 1553 ± 176 |
| $C_{max}$ (ug/mL) | 377 ± 31 | 314 ± 41 |
| $V_1$ (mL/kg) | 33 ± 3 | 40 ± 5 |
| $HL_{beta}$ (Day) | 7 ± 1 | 10 ± 1 |

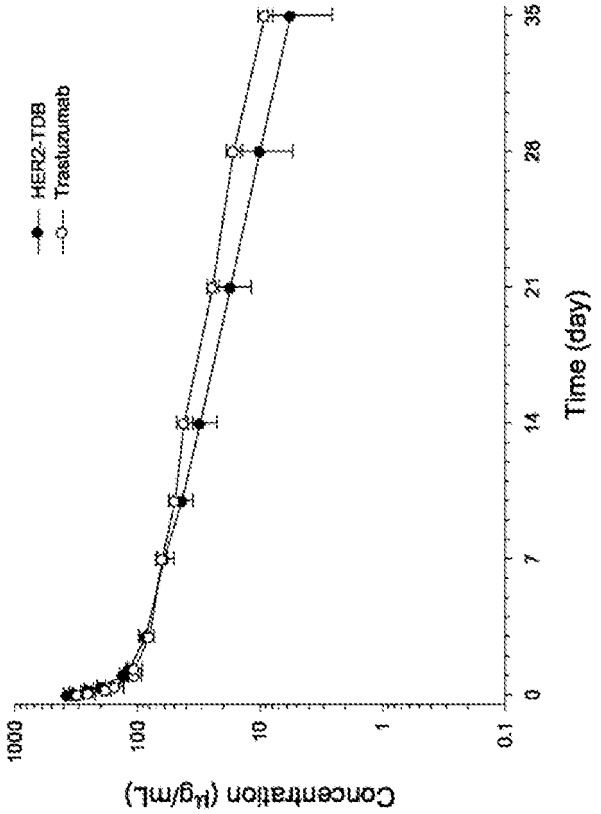



FIG. 7

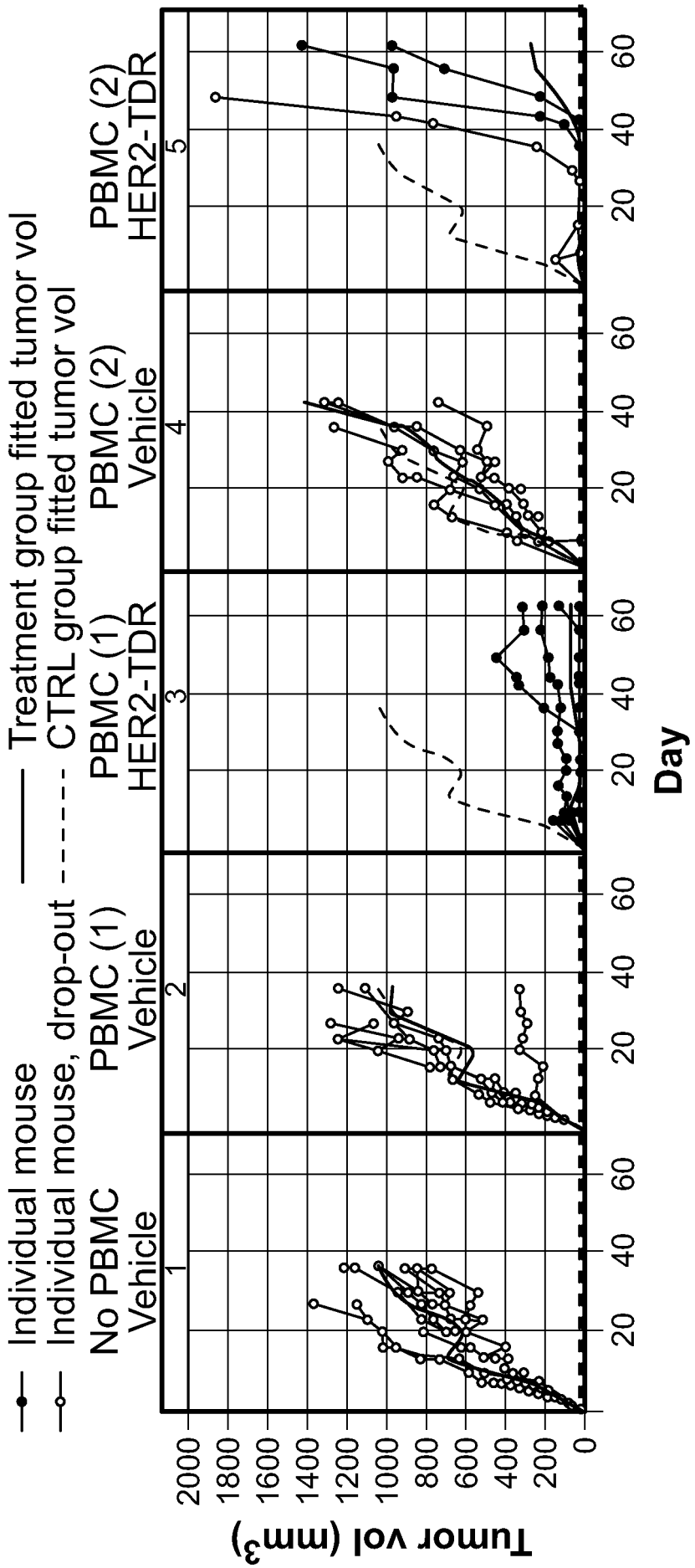

Individual mouse
Individual mouse, drop-out
Treatment group fitted tumor vol
CTRL group fitted tumor vol
No PBMC
Vehicle
PBMC (1)
Vehicle
PBMC (1)
HER2-TDR
PBMC (2)
Vehicle
PBMC (2)
HER2-TDR
Tumor vol (mm3)
Day
0
200
400
600
800
1000
1200
1400
1600
1800
2000
20
40
60
1
2
3
4
5

FIG. 8A

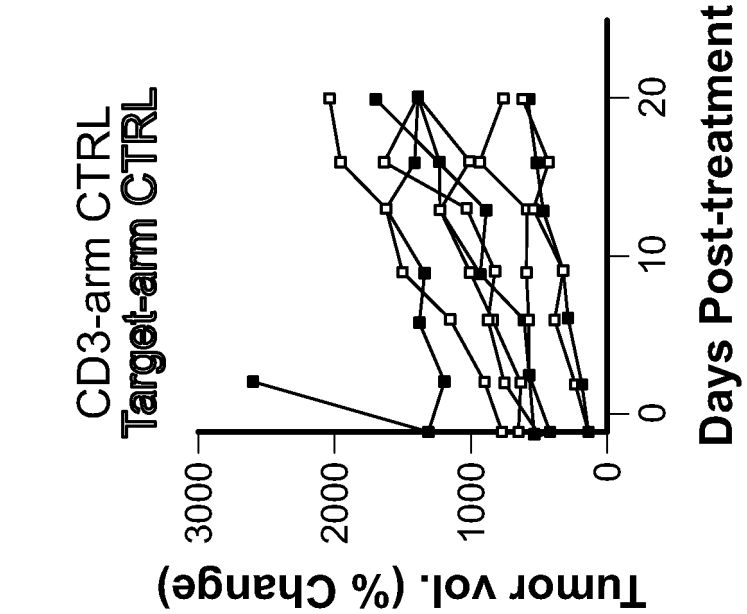



FIG. 8E

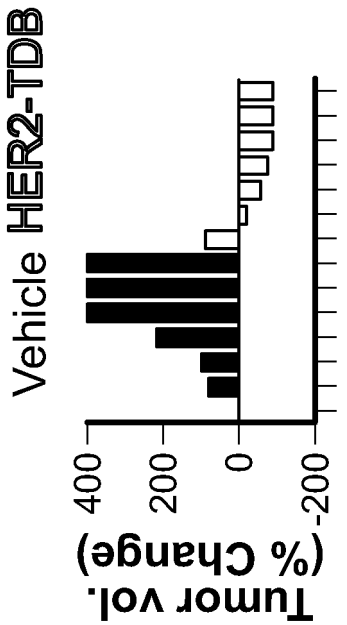



FIG. 8C

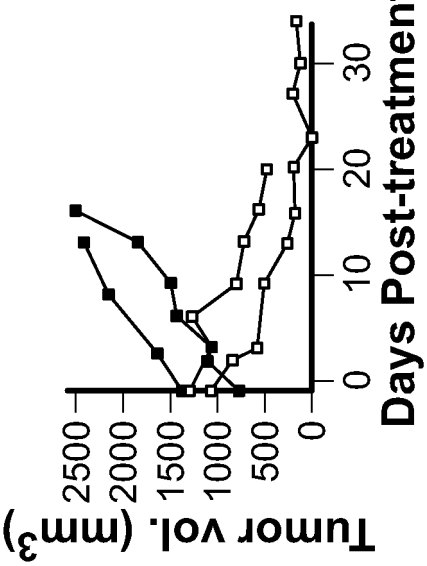



FIG. 8D

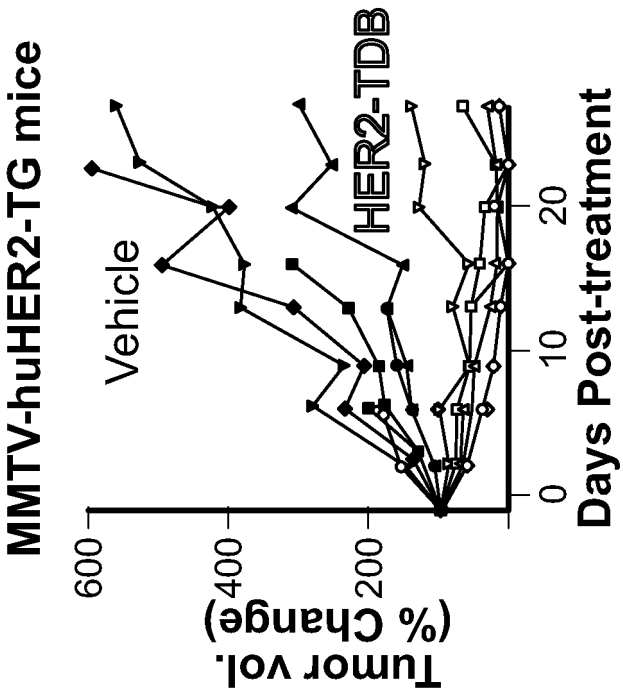



FIG. 8B

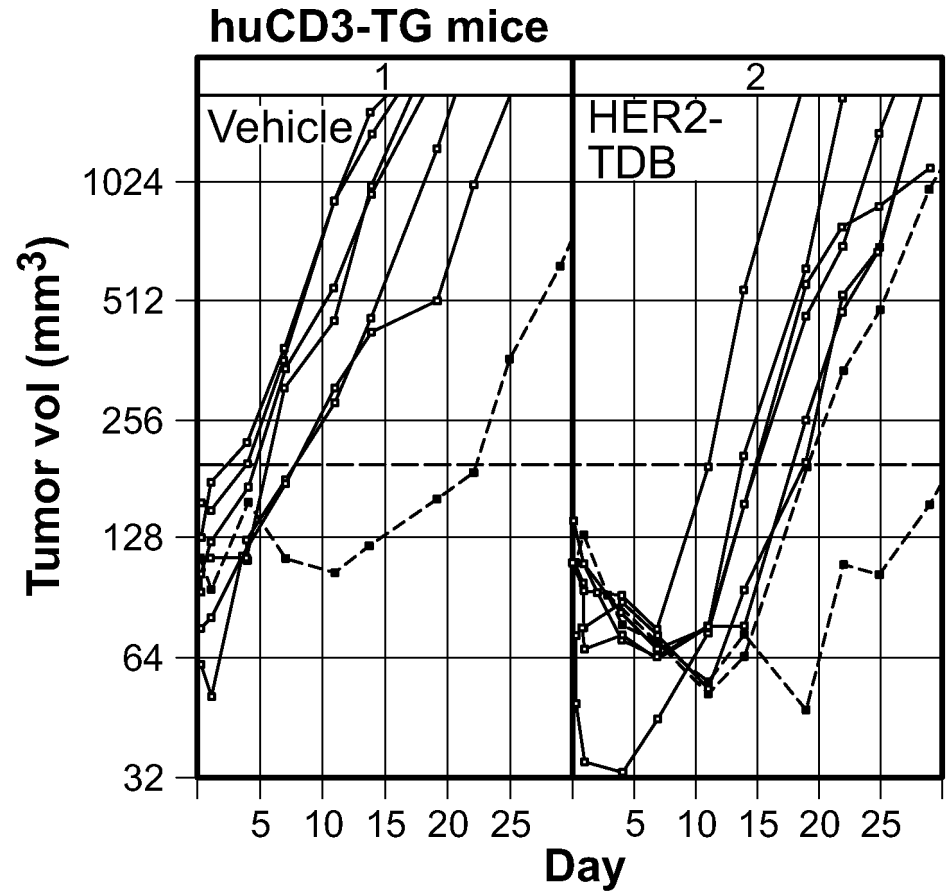

huCD3-TG mice
1
Vehicle
2
HER2-TDB
1024
512
256
128
64
32
Tumor vol (mm3)
5 10 15 20 25
5 10 15 20 25
Day

FIG. 8F

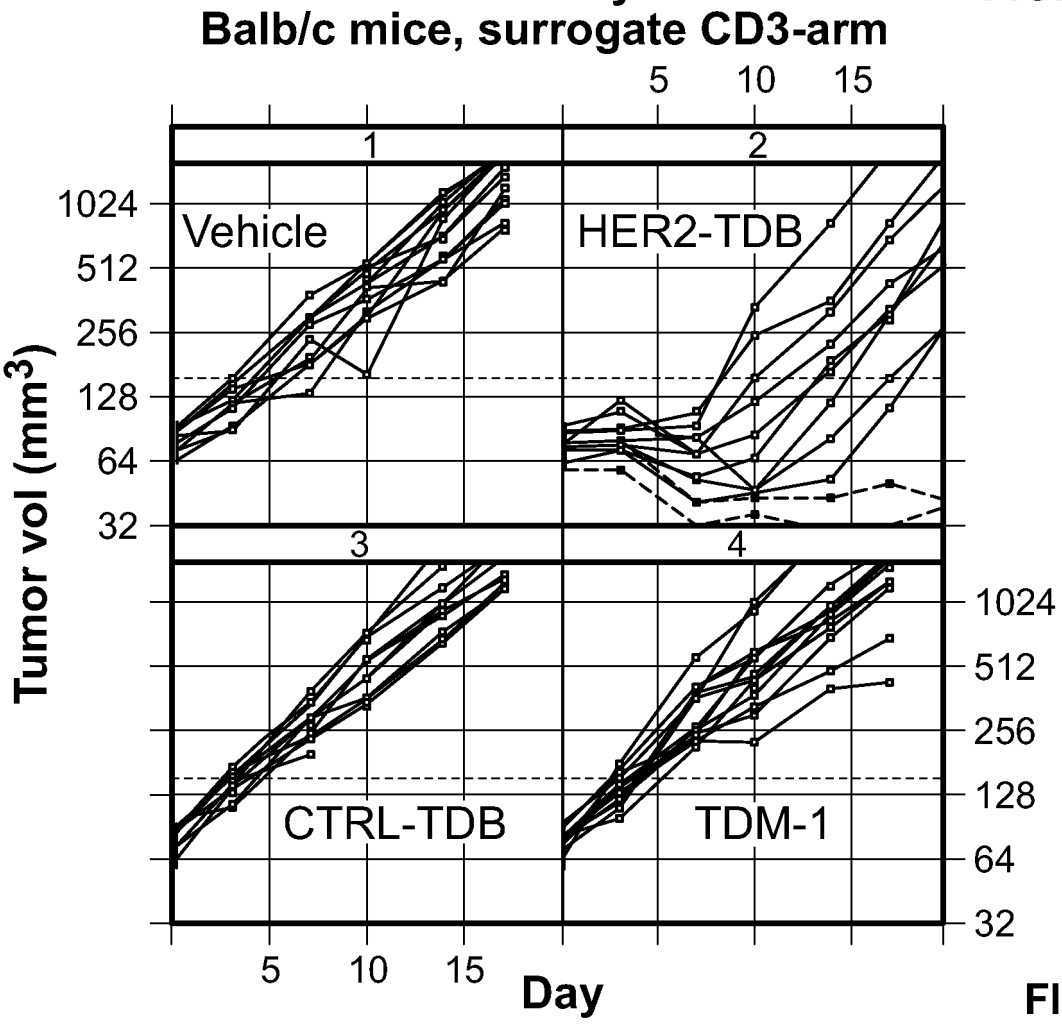

Balb/c mice, surrogate CD3-arm
5 10 15
1
Vehicle
2
HER2-TDB
3
CTRL-TDB
4
TDM-1
1024
512
256
128
64
32
Tumor vol (mm3)
5 10 15
Day

FIG. 8G

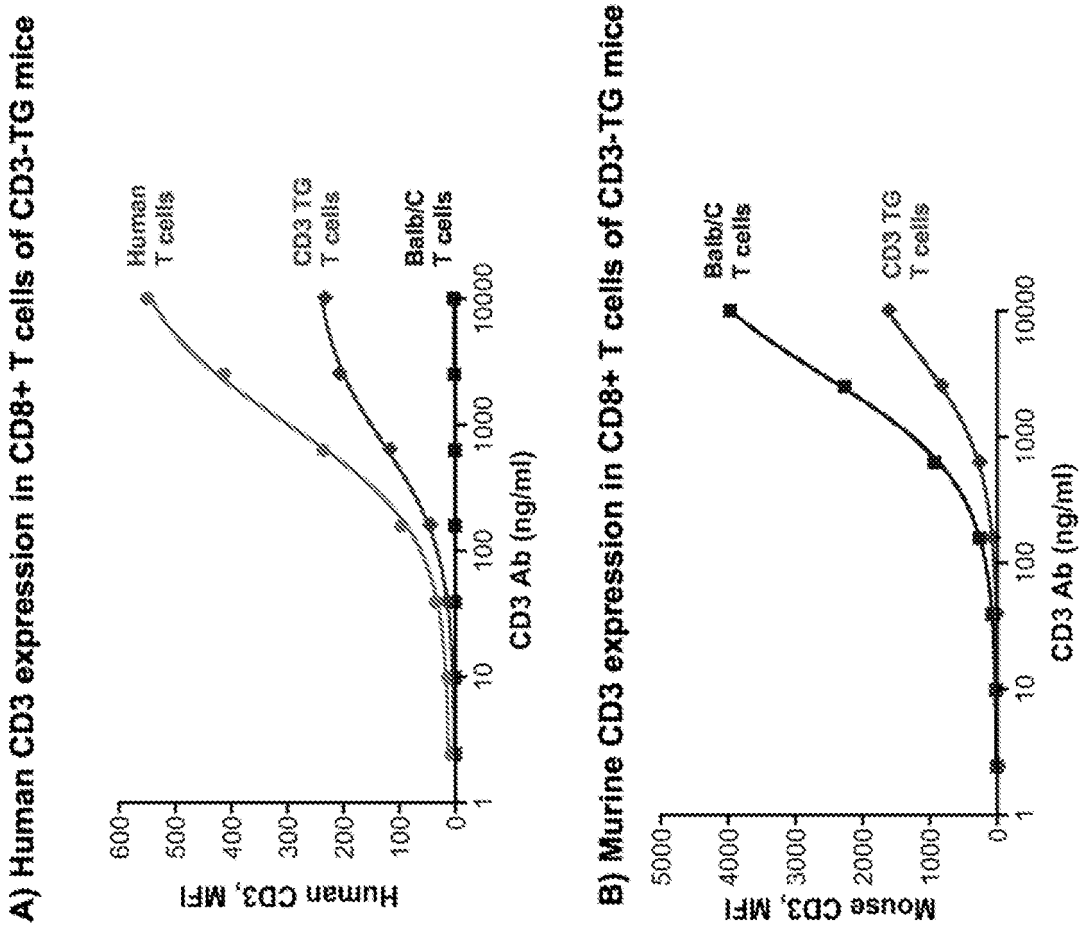

A) Human CD3 expression in CD8+ T cells of CD3-TG mice
Human CD3, MFI
600
500
400
300
200
100
0
1
10
100
1000
10000
CD3 Ab (ng/ml)
Human T cells
CD3 TG T cells
Balb/C T cells
B) Murine CD3 expression in CD8+ T cells of CD3-TG mice
Mouse CD3, MFI
5000
4000
3000
2000
1000
0
1
10
100
1000
10000
CD3 Ab (ng/ml)
Balb/C T cells
CD3 TG T cells

FIG. 9

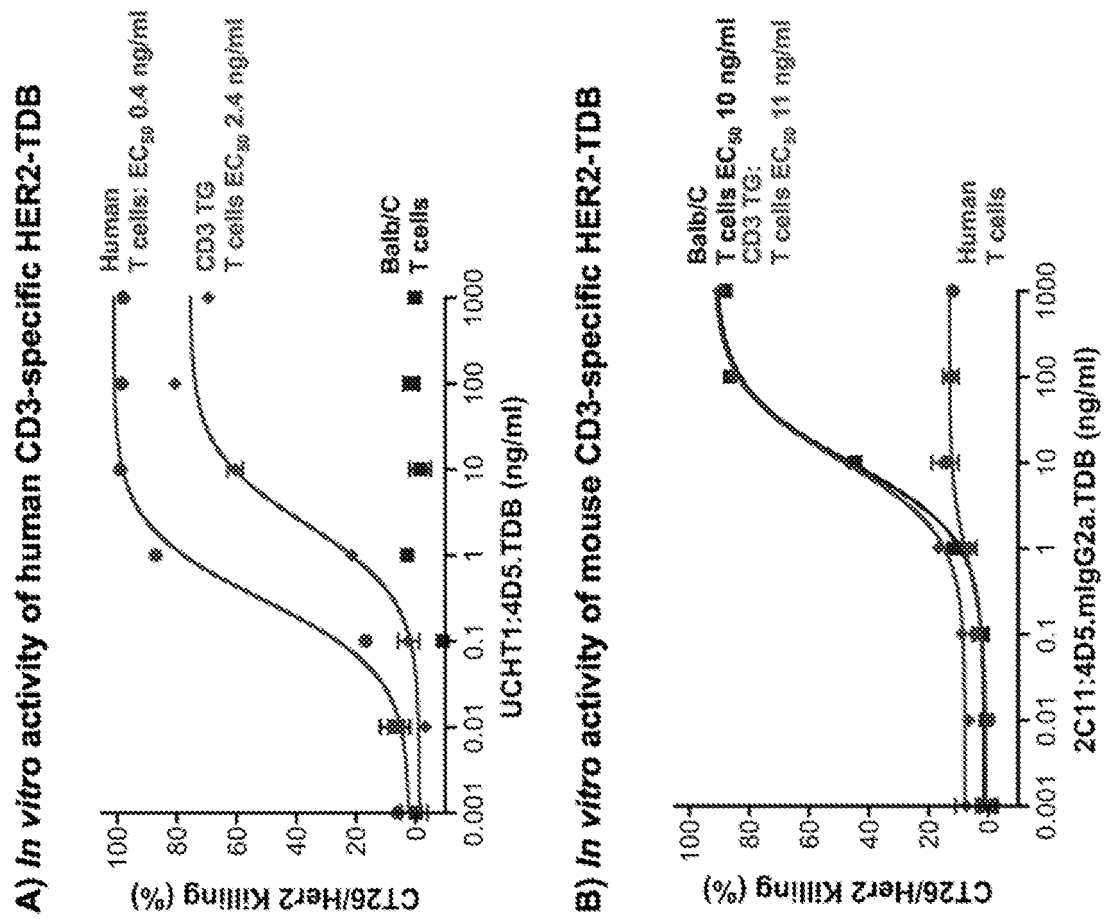

A) In vitro activity of human CD3-specific HER2-TDB
CT26/Her2 Killing (%)
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
1000
UCHT1;4D5.TDB (ng/ml)
Human
T cells: EC50 0.4 ng/ml
CD3 TG
T cells EC50 2.4 ng/ml
Balb/C
T cells
B) In vitro activity of mouse CD3-specific HER2-TDB
CT26/Her2 Killing (%)
Balb/C
T cells EC50 10 ng/ml
CD3 TG:
T cells EC50 11 ng/ml
Human
T cells
2C11;4D5.mIgG2a.TDB (ng/ml)

FIG. 10

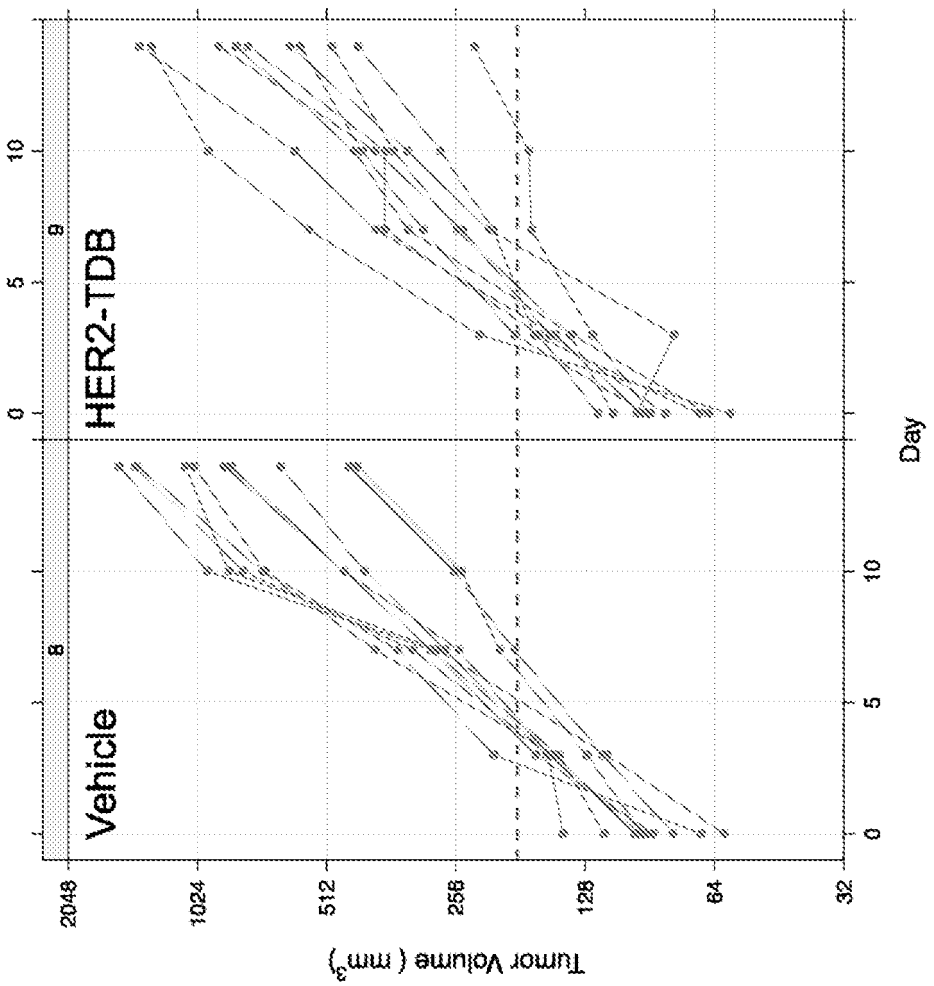

Vehicle
HER2-TDB
8
8
0
5
10
0
5
10
2048
1024
512
256
128
64
32
Tumor Volume ( mm3)
Day

FIG. 11

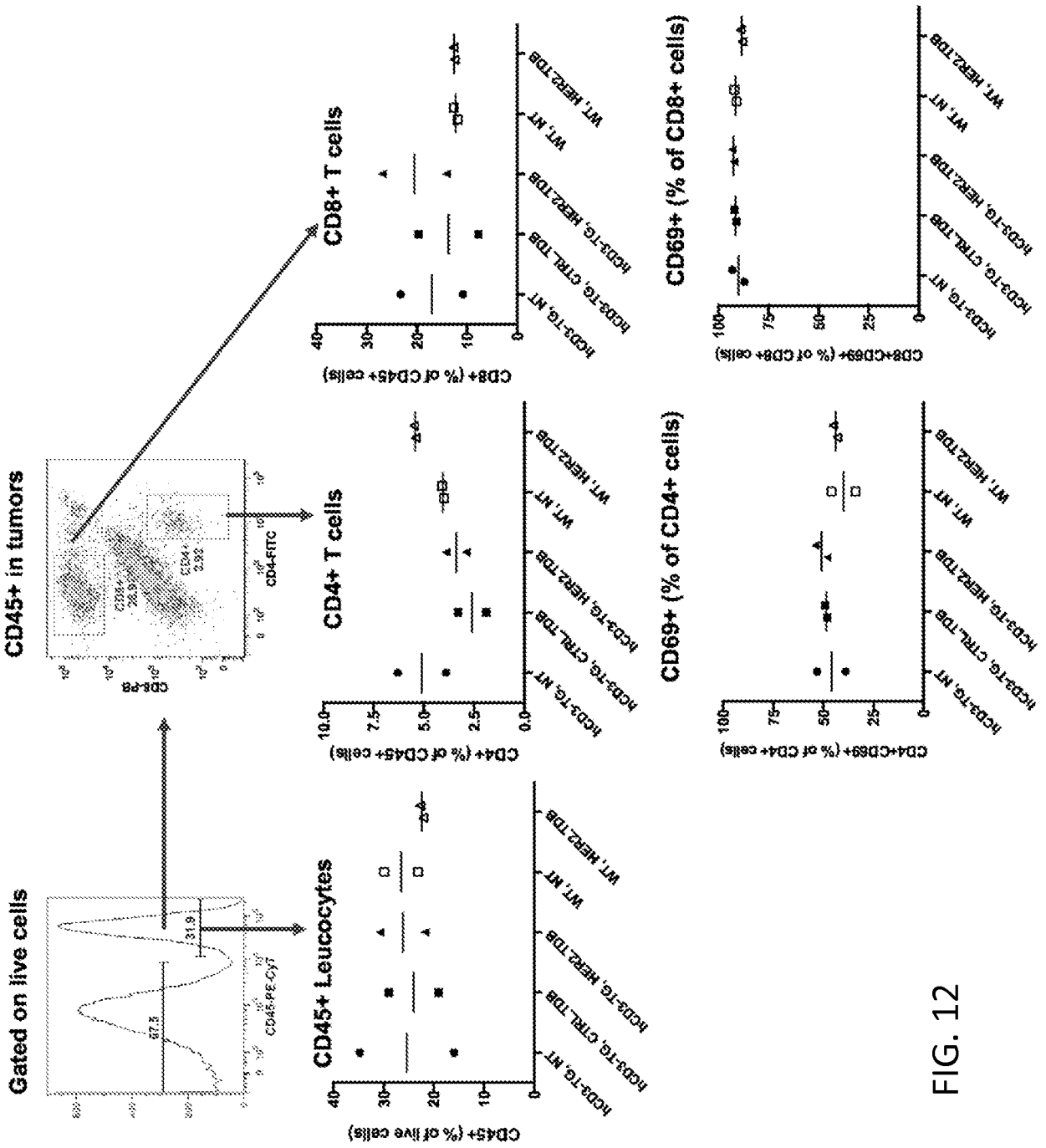

Gated on live cells
CD45+ in tumors
CD45+ Leucocytes
CD4+ T cells
CD8+ T cells
CD69+ (% of CD4+ cells)
CD69+ (% of CD8+ cells)
hCD3-TG, NT
hCD3-TG, CTRL_TDB
hCD3-TG, HER2_TDB
WT, NT
WT, HER2_TDB

FIG. 12

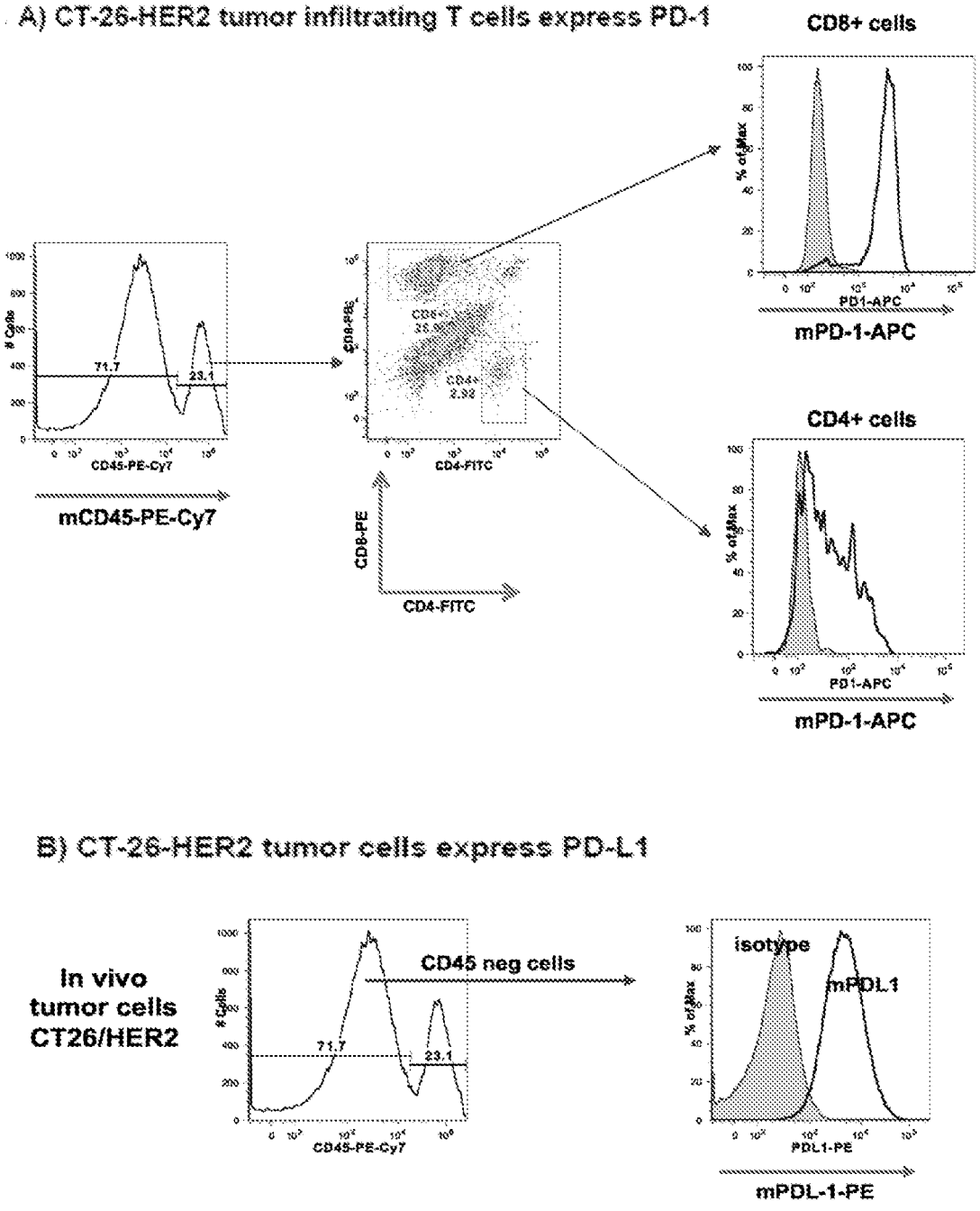

A) CT-26-HER2 tumor infiltrating T cells express PD-1
CD8+ cells
mPD-1-APC
CD4+ cells
mPD-1-APC
mCD45-PE-Cy7
CD8-PE
CD4-FITC
71.7
23.1
CD45-PE-Cy7
CD4-FITC
PD1-APC
% of Max
# Cells
B) CT-26-HER2 tumor cells express PD-L1
In vivo tumor cells CT26/HER2
CD45 neg cells
isotype
mPDL1
PDL1-PE
mPDL-1-PE

FIG. 13

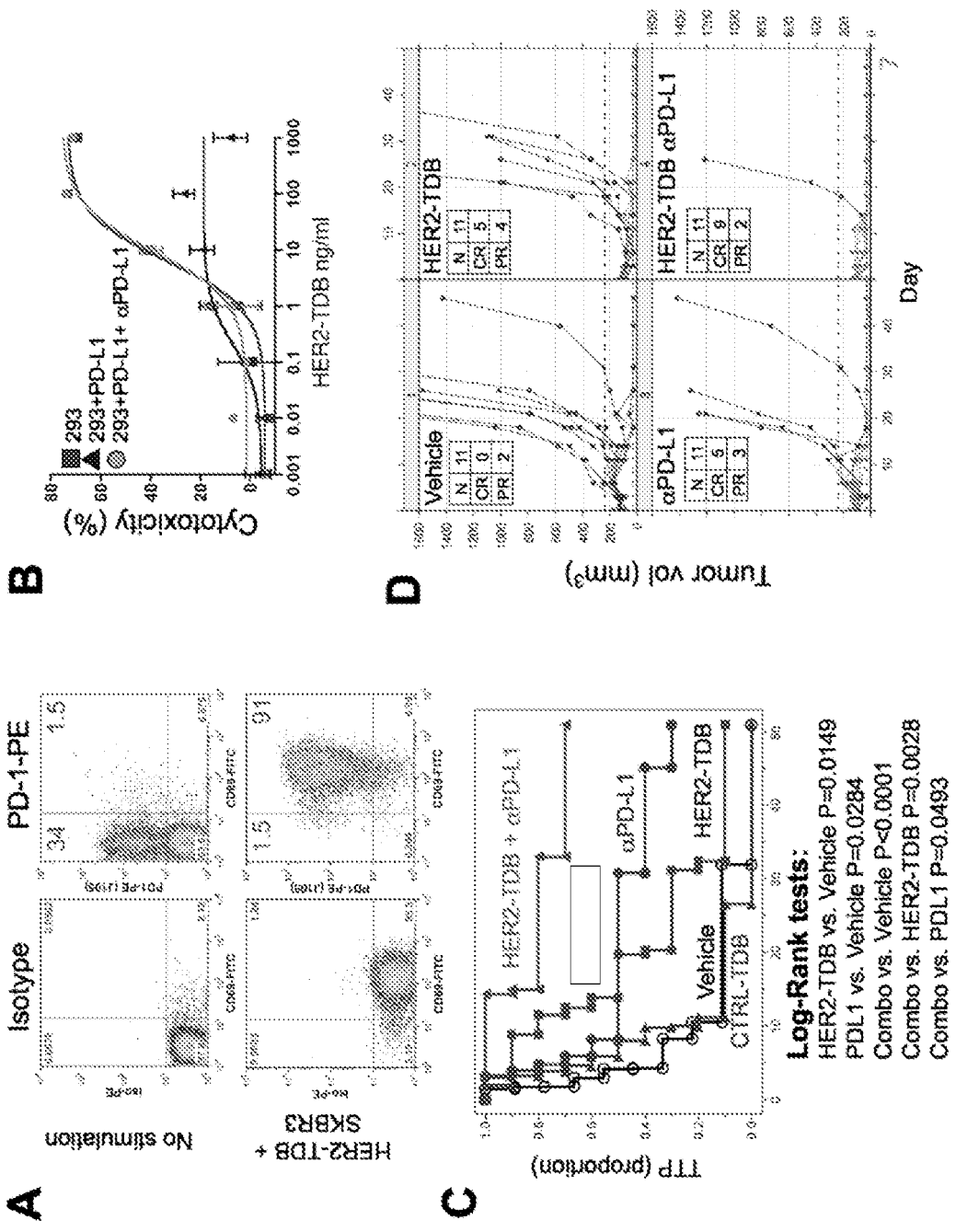

A
Isotype
PD-1-PE
No stimulation
HER2-TDB + SKBR3
B
Cytotoxicity (%)
293
293+PD-L1
293+PD-L1+ αPD-L1
HER2-TDB ng/ml
C
TTP (proportion)
HER2-TDB + αPD-L1
αPD-L1
HER2-TDB
Vehicle
CTRL-TDB
Log-Rank tests:
HER2-TDB vs. Vehicle P=0.0149
PDL1 vs. Vehicle P=0.0284
Combo vs. Vehicle P<0.0001
Combo vs. HER2-TDB P=0.0028
Combo vs. PDL1 P=0.0493
D
Tumor vol (mm³)
Vehicle
HER2-TDB
αPD-L1
HER2-TDB αPD-L1
Day

FIG. 14

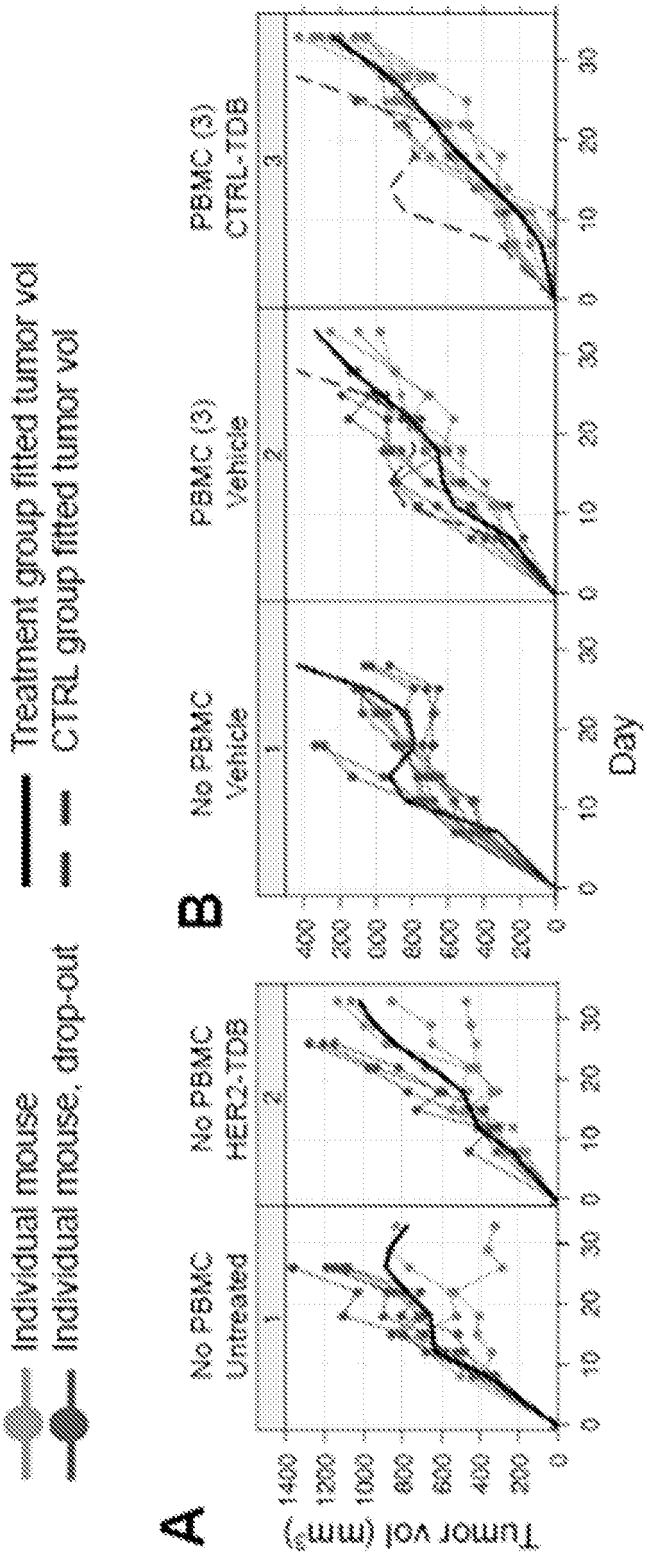

Individual mouse
Individual mouse, drop-out
Treatment group fitted tumor vol
CTRL group fitted tumor vol
A
No PBMC
Untreated
No PBMC
HER2-TDB
B
No PBMC
Vehicle
PBMC (3)
Vehicle
PBMC (3)
CTRL-TDB
Tumor vol (mm³)
Day

FIG. 15