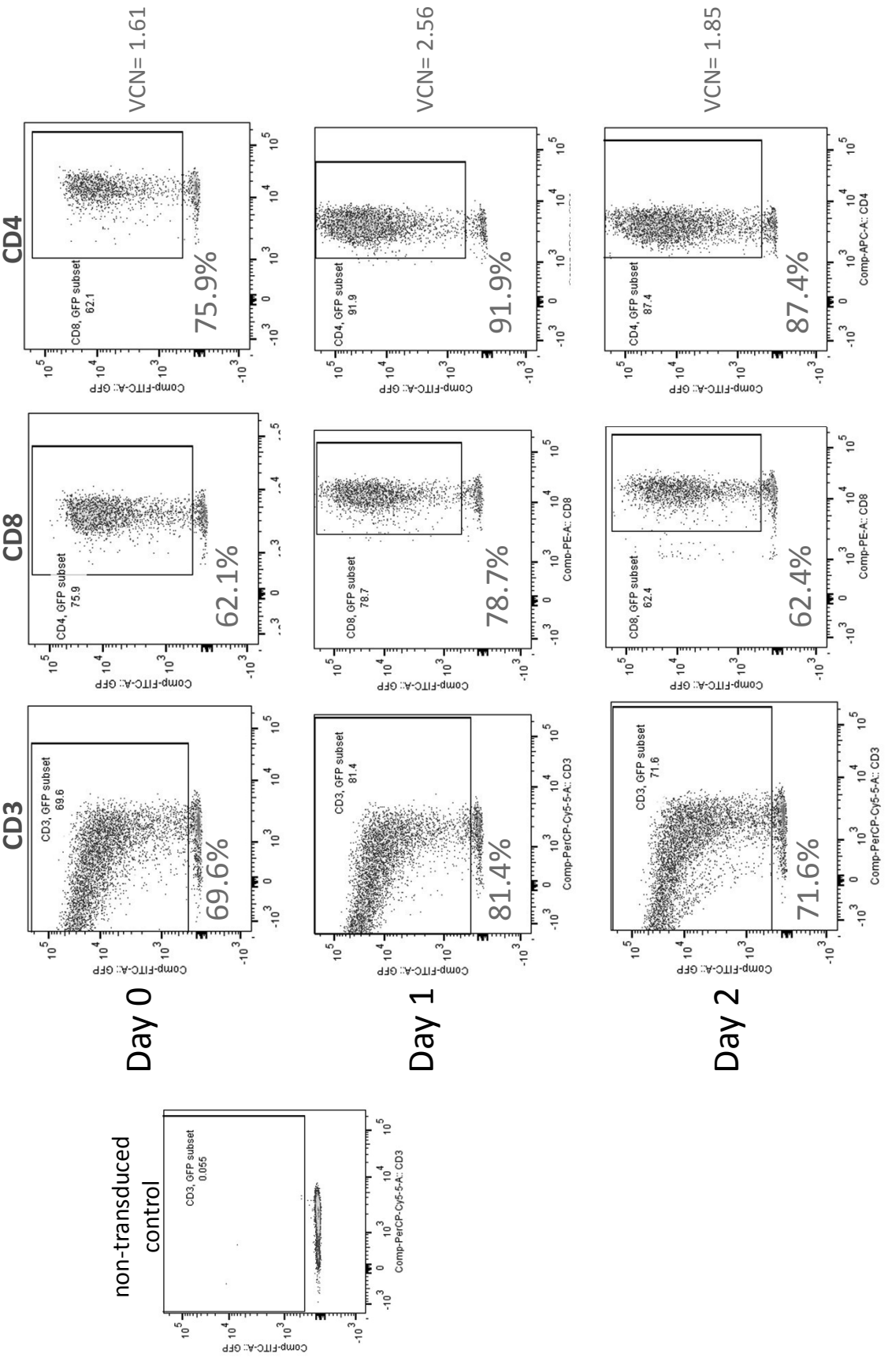

CD3
CD8
CD4
VCN= 1.61
VCN= 2.56
VCN= 1.85
Day 0
Day 1
Day 2
non-transduced control
CD3, GFP subset 69.6
69.6%
CD4, GFP subset 75.9
62.1%
CD8, GFP subset 62.1
75.9%
CD3, GFP subset 81.4
81.4%
CD8, GFP subset 78.7
78.7%
CD4, GFP subset 91.9
91.9%
CD3, GFP subset 71.6
71.6%
CD8, GFP subset 62.4
62.4%
CD4, GFP subset 87.4
87.4%
CD3, GFP subset 0.055
Comp-FITC-A:: GFP
Comp-PerCP-Cy5-5-A:: CD3
Comp-PE-A:: CD8
Comp-APC-A:: CD4

FIG. 1

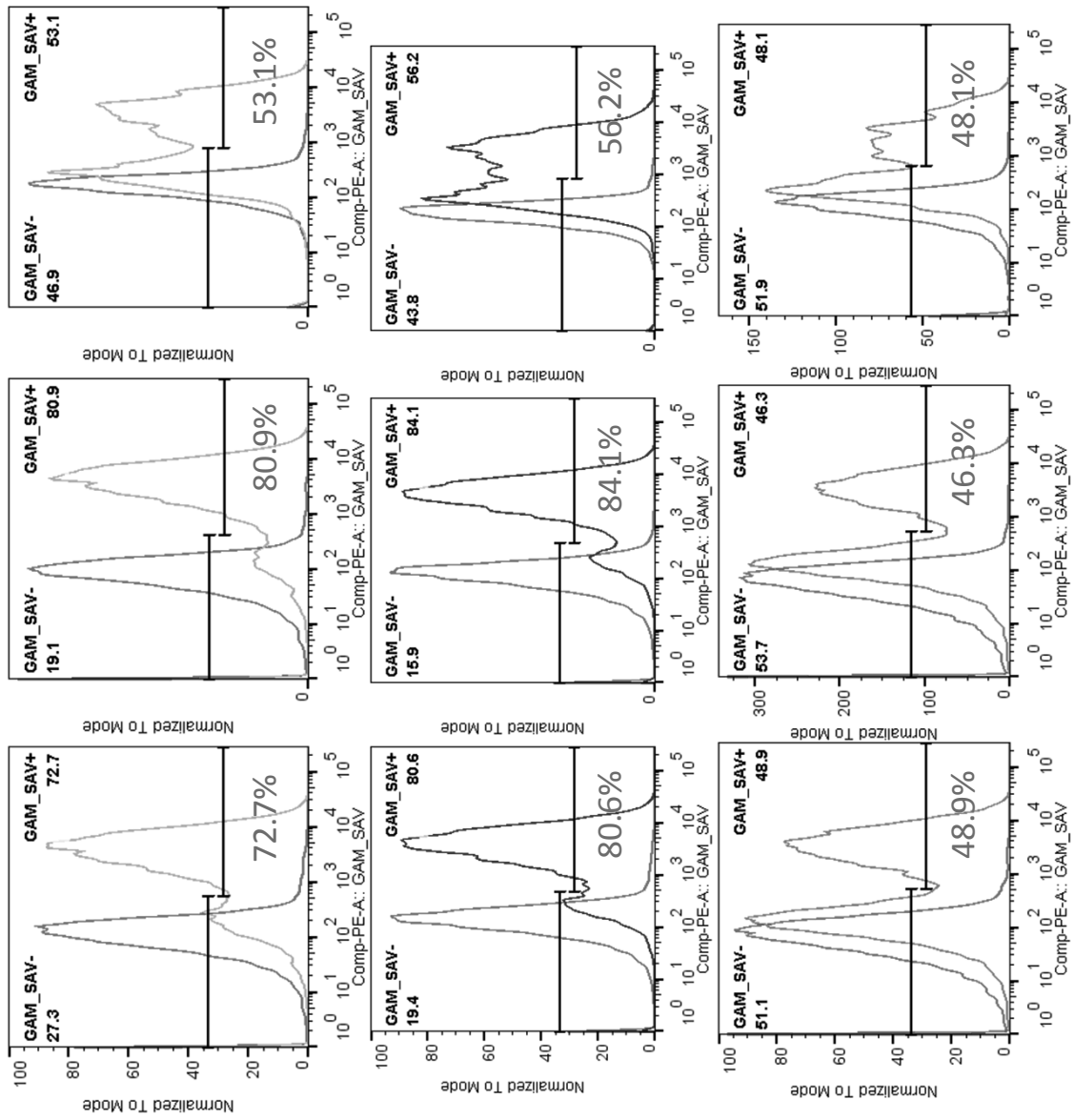

CD3
CD4
CD8
plate bound CD3/28
VCN=1.60
soluble CD3/28
VCN=1.95
beads CD3/28
VCN=1.32
GAM_SAV-
27.3
GAM_SAV+
72.7
72.7%
GAM_SAV-
19.1
GAM_SAV+
80.9
80.9%
GAM_SAV-
46.9
GAM_SAV+
53.1
53.1%
GAM_SAV-
19.4
GAM_SAV+
80.6
80.6%
GAM_SAV-
15.9
GAM_SAV+
84.1
84.1%
GAM_SAV-
43.8
GAM_SAV+
56.2
56.2%
GAM_SAV-
51.1
GAM_SAV+
48.9
48.9%
GAM_SAV-
53.7
GAM_SAV+
46.3
46.3%
GAM_SAV-
51.9
GAM_SAV+
48.1
48.1%
Normalized To Mode
Comp-PE-A:: GAM_SAV

FIG. 2

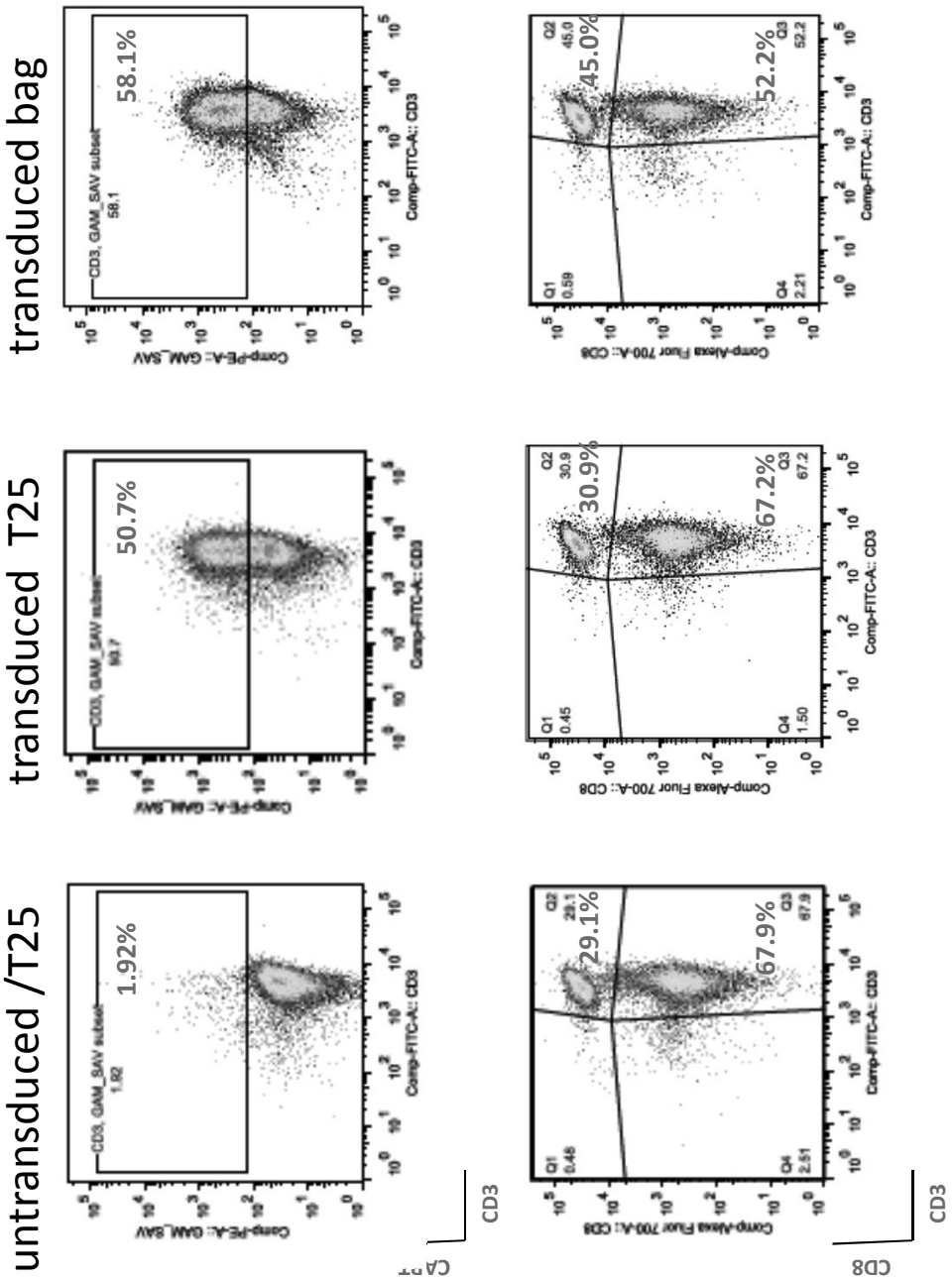

untransduced /T25
transduced T25
transduced bag
1.92%
50.7%
58.1%
29.1%
30.9%
45.0%
67.9%
67.2%
52.2%
CD3
CD8

FIG. 3

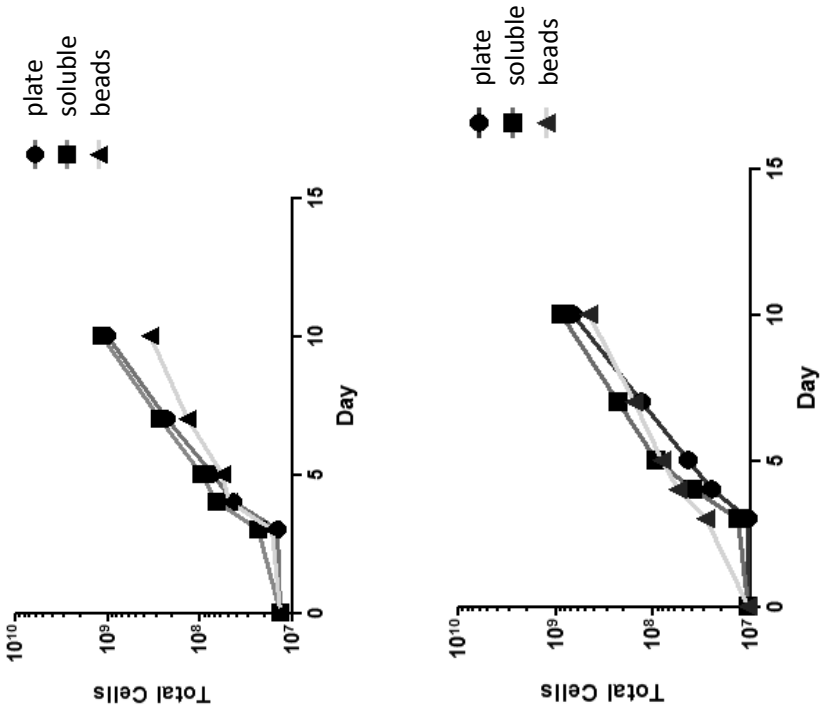
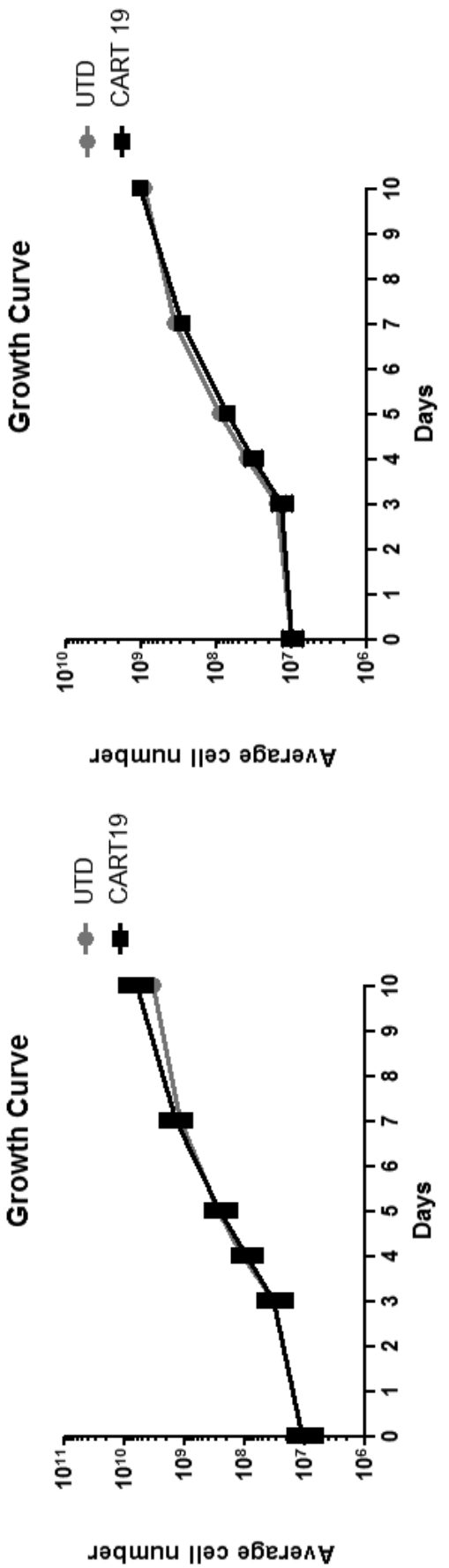

plate
soluble
beads
Total Cells
Day
10^{10}
10^{9}
10^{8}
10^{7}
0
5
10
15
plate
soluble
beads
Total Cells
Day
10^{10}
10^{9}
10^{8}
10^{7}
0
5
10
15

FIG. 4



KP36253- 43.8 % lymphocytes in PBMC
KP36307- 51% lymphocytes in PBMC
KP36649- 22.3% lymphocytes in PBMC

KP36943- 69 % lymphocytes in PBMC
KP37050- 61% lymphocytes in PBMC
KP36649- 22.3% lymphocytes in PBMC

FIG. 5

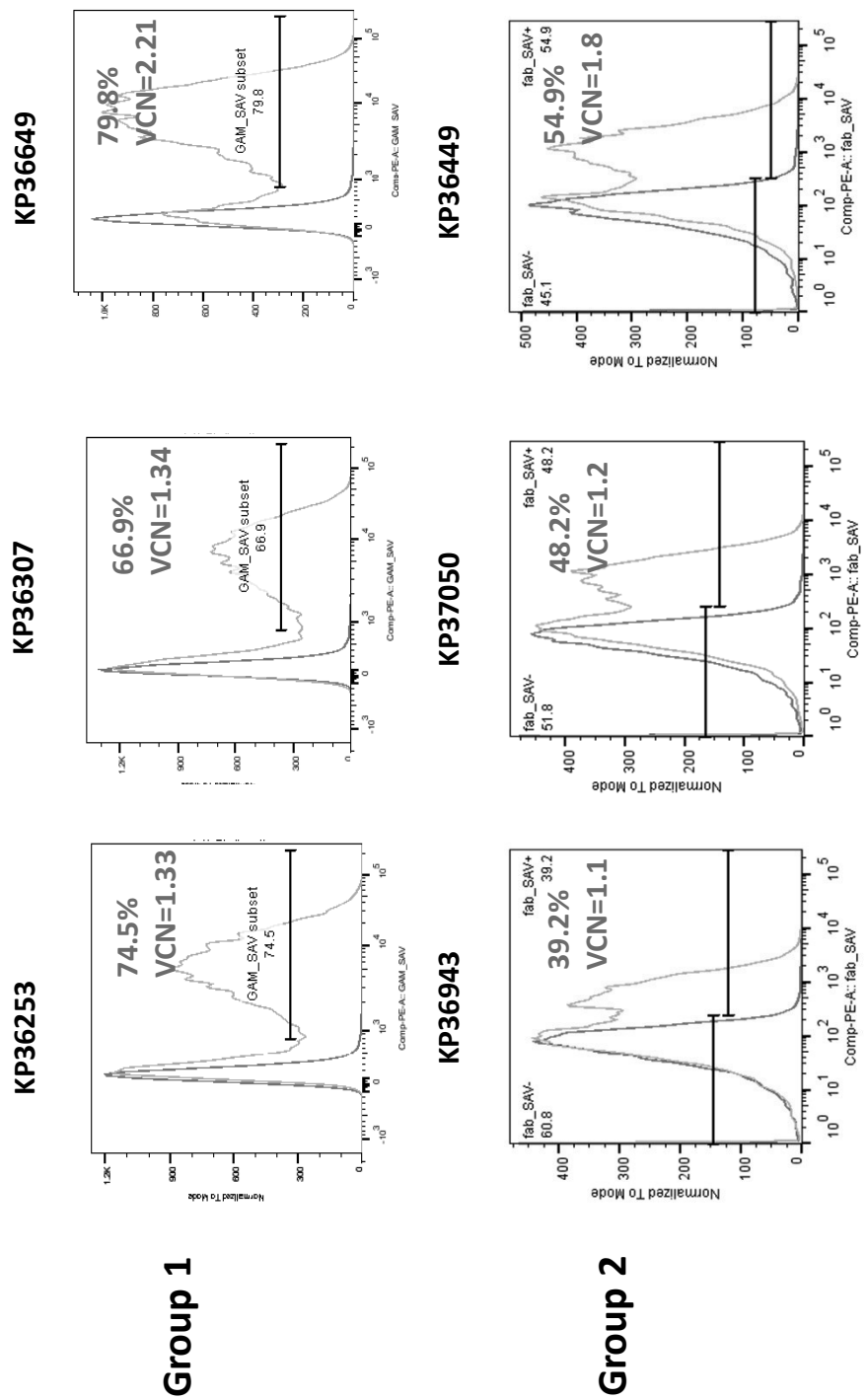

KP36649
KP36307
KP36253
KP36449
KP37050
KP36943
79.8%
VCN=2.21
GAM_SAV subset
79.8
66.9%
VCN=1.34
GAM_SAV subset
66.9
74.5%
VCN=1.33
GAM_SAV subset
74.5
54.9%
VCN=1.8
fab_SAV+
54.9
fab_SAV-
45.1
48.2%
VCN=1.2
fab_SAV+
48.2
fab_SAV-
51.8
39.2%
VCN=1.1
fab_SAV+
39.2
fab_SAV-
60.8
Normalized To Mode
Comp-PE-A::fab_SAV
Comp-PE-A::GAM_SAV
Group 1
Group 2

FIG. 6

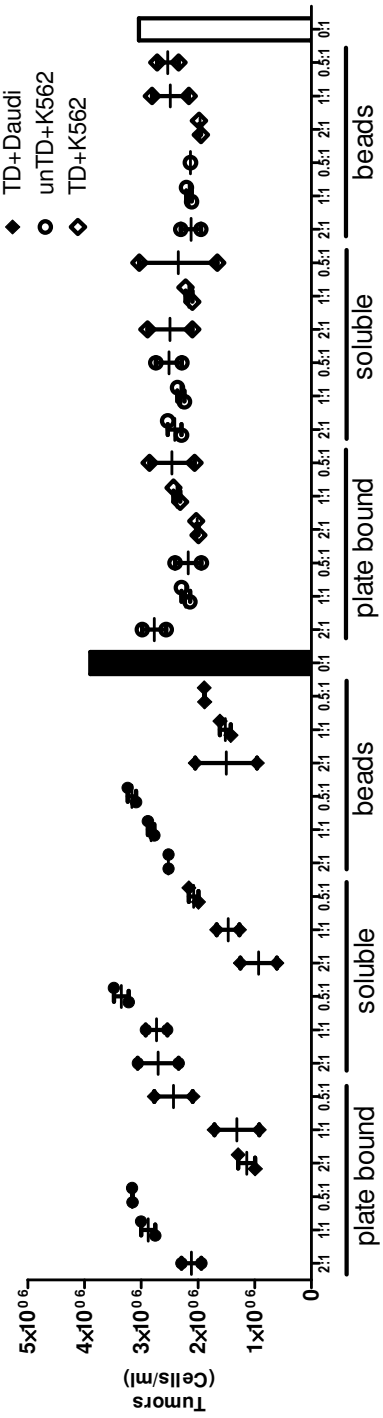

Antigen-specific Tumor Clearance
unTD+Daudi
TD+Daudi
unTD+K562
TD+K562
Tumors (Cells/ml)
5x10^6
4x10^6
3x10^6
2x10^6
1x10^6
0
plate bound
soluble
beads
plate bound
soluble
beads
2:1 1:1 0.5:1 2:1 1:1 0.5:1 2:1 1:1 0.5:1 0:1 2:1 1:1 0.5:1 2:1 1:1 0.5:1 2:1 1:1 0.5:1 0:1

FIG. 7

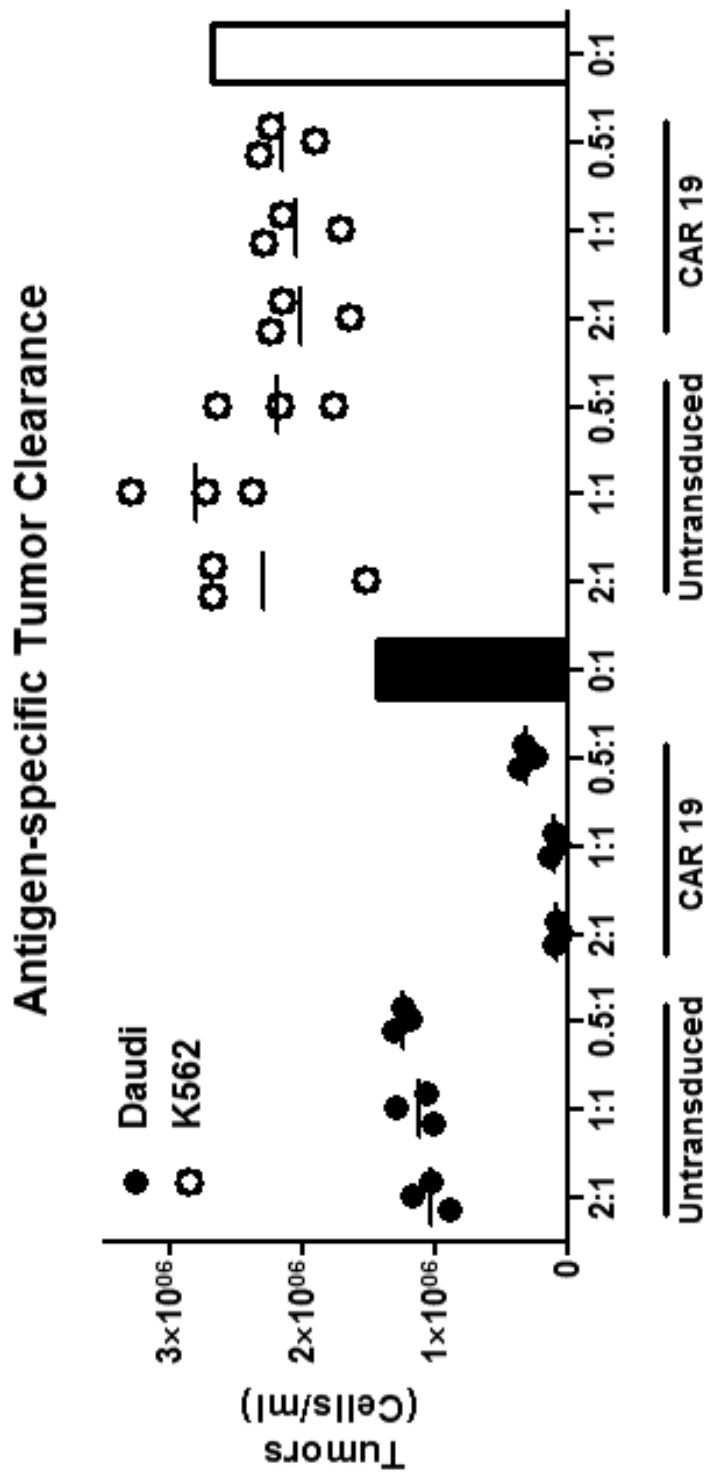

Antigen-specific Tumor Clearance
Daudi
K562
Tumors (Cells/ml)
3×10^06
2×10^06
1×10^06
0
2:1
1:1
0.5:1
0:1
2:1
1:1
0.5:1
0:1
Untransduced
CAR 19
Untransduced
CAR 19
T cell:Tumor ratio

FIG. 8

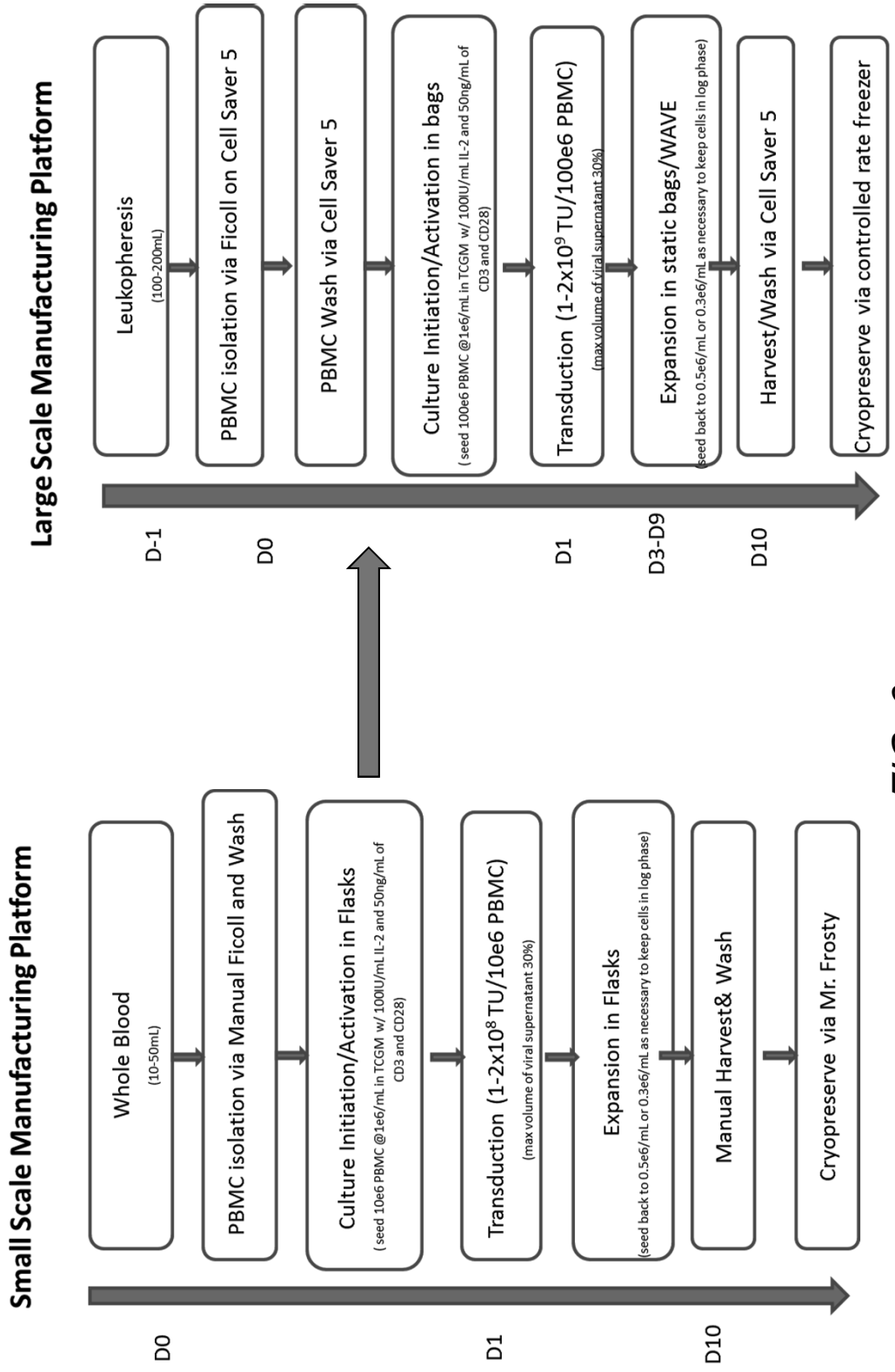

Small Scale Manufacturing Platform
Whole Blood
(10-50mL)
PBMC isolation via Manual Ficoll and Wash
Culture Initiation/Activation in Flasks
(seed 10e6 PBMC @1e6/mL in TCGM w/ 100IU/mL IL-2 and 50ng/mL of CD3 and CD28)
Transduction (1-2x10^8 TU/10e6 PBMC)
(max volume of viral supernatant 30%)
Expansion in Flasks
(seed back to 0.5e6/mL or 0.3e6/mL as necessary to keep cells in log phase)
Manual Harvest& Wash
Cryopreserve via Mr. Frosty
D0
D1
D10
Large Scale Manufacturing Platform
Leukopheresis
(100-200mL)
PBMC isolation via Ficoll on Cell Saver 5
PBMC Wash via Cell Saver 5
Culture Initiation/Activation in bags
(seed 100e6 PBMC @1e6/mL in TCGM w/ 100IU/mL IL-2 and 50ng/mL of CD3 and CD28)
Transduction (1-2x10^9 TU/100e6 PBMC)
(max volume of viral supernatant 30%)
Expansion in static bags/WAVE
(seed back to 0.5e6/mL or 0.3e6/mL as necessary to keep cells in log phase)
Harvest/Wash via Cell Saver 5
Cryopreserve via controlled rate freezer
D-1
D0
D1
D3-D9
D10

FIG. 9

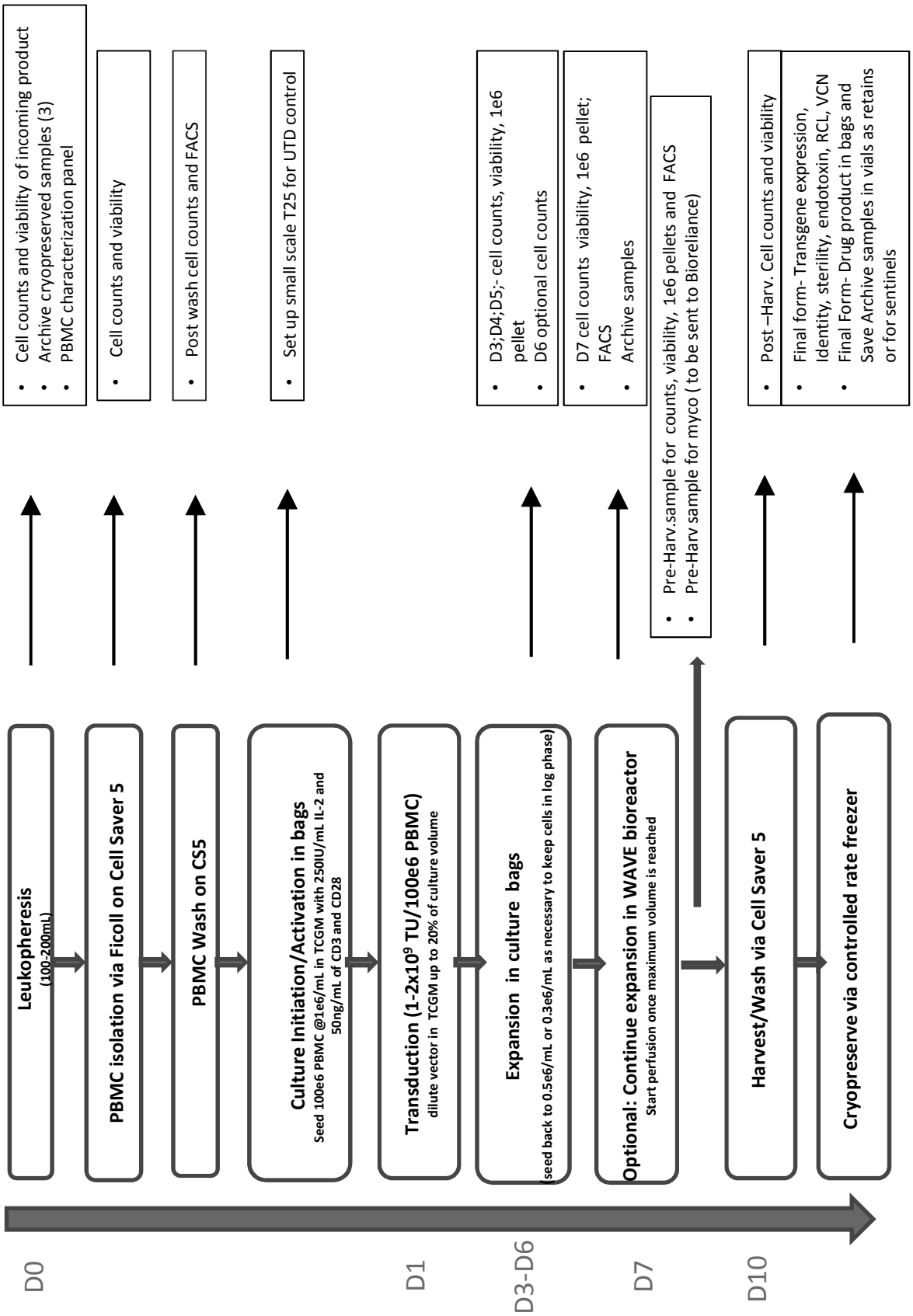

D0
D1
D3-D6
D7
D10
Leukopheresis
(100-200mL)
PBMC isolation via Ficoll on Cell Saver 5
PBMC Wash on CS5
Culture Initiation/Activation in bags
Seed 100e6 PBMC @1e6/mL in TCGM with 250IU/mL IL-2 and 50ng/mL of CD3 and CD28
Transduction (1-2x10^9 TU/100e6 PBMC)
dilute vector in TCGM up to 20% of culture volume
Expansion in culture bags
seed back to 0.5e6/mL or 0.3e6/mL as necessary to keep cells in log phase)
Optional: Continue expansion in WAVE bioreactor
Start perfusion once maximum volume is reached
Harvest/Wash via Cell Saver 5
Cryopreserve via controlled rate freezer
• Cell counts and viability of incoming product
• Archive cryopreserved samples (3)
• PBMC characterization panel
• Cell counts and viability
• Post wash cell counts and FACS
• Set up small scale T25 for UTD control
• D3;D4;D5;- cell counts, viability, 1e6 pellet
• D6 optional cell counts
• D7 cell counts viability, 1e6 pellet; FACS
• Archive samples
• Pre-Harv.sample for counts, viability, 1e6 pellets and FACS
• Pre-Harv sample for myco (to be sent to Bioreliance)
• Post –Harv. Cell counts and viability
• Final form- Transgene expression, Identity, sterility, endotoxin, RCL, VCN
• Final Form- Drug product in bags and Save Archive samples in vials as retains or for sentinels

FIG. 10

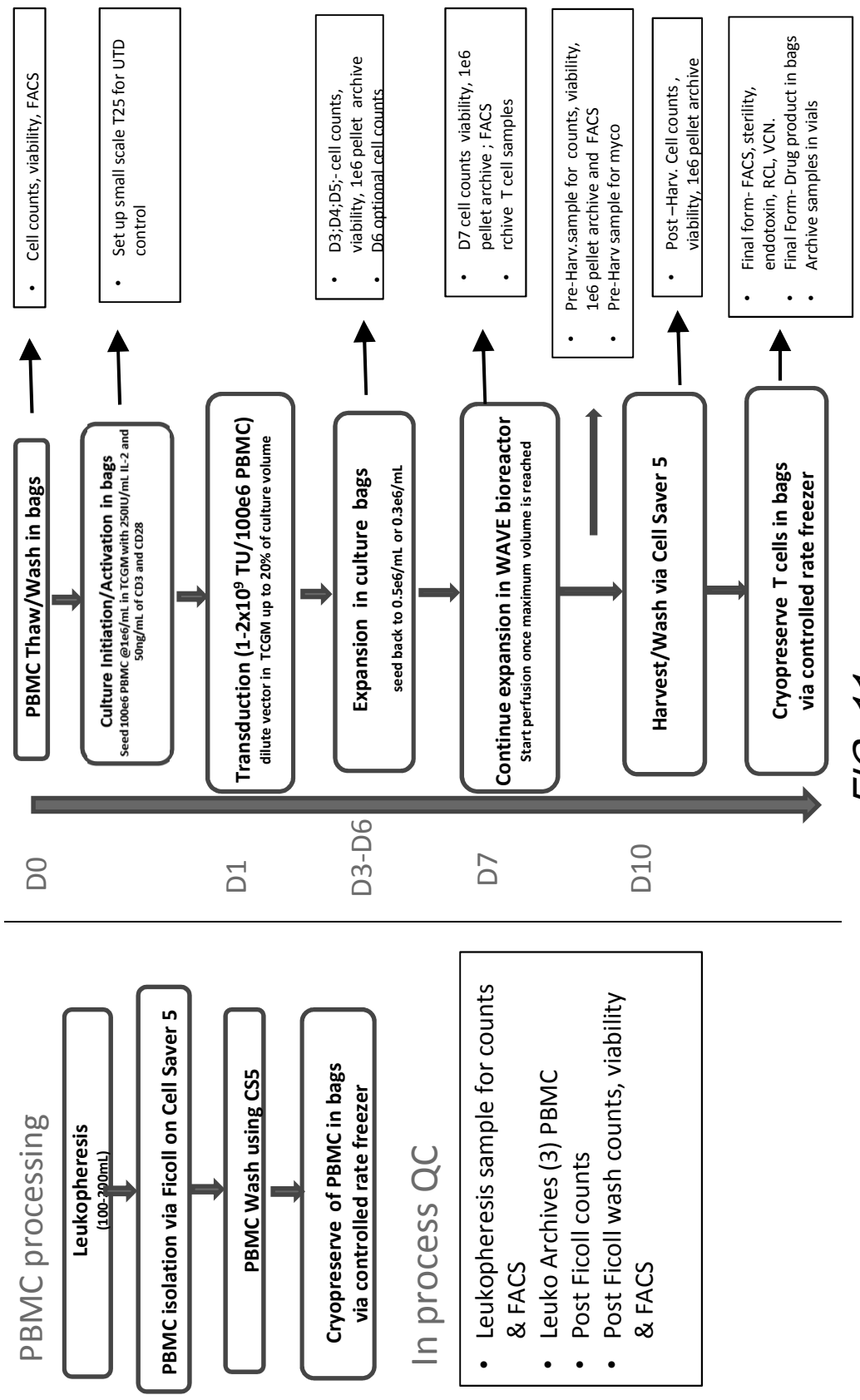

PBMC processing
Leukopheresis
(100-200mL)
PBMC isolation via Ficoll on Cell Saver 5
PBMC Wash using CS5
Cryopreserve of PBMC in bags via controlled rate freezer
In process QC
Leukopheresis sample for counts & FACS
Leuko Archives (3) PBMC
Post Ficoll counts
Post Ficoll wash counts, viability & FACS
D0
D1
D3-D6
D7
D10
PBMC Thaw/Wash in bags
Cell counts, viability, FACS
Culture Initiation/Activation in bags
Seed 100e6 PBMC @1e6/mL in TCGM with 250IU/mL IL-2 and 50ng/mL of CD3 and CD28
Set up small scale T25 for UTD control
Transduction (1-2x10^9 TU/100e6 PBMC)
dilute vector in TCGM up to 20% of culture volume
Expansion in culture bags
seed back to 0.5e6/mL or 0.3e6/mL
D3;D4;D5;- cell counts, viability, 1e6 pellet archive
D6 optional cell counts
Continue expansion in WAVE bioreactor
Start perfusion once maximum volume is reached
D7 cell counts viability, 1e6 pellet archive ; FACS
rchive T cell samples
Pre-Harv.sample for counts, viability, 1e6 pellet archive and FACS
Pre-Harv sample for myco
Harvest/Wash via Cell Saver 5
Post –Harv. Cell counts , viability, 1e6 pellet archive
Cryopreserve T cells in bags via controlled rate freezer
Final form- FACS, sterility, endotoxin, RCL, VCN.
Final Form- Drug product in bags
Archive samples in vials

FIG. 11

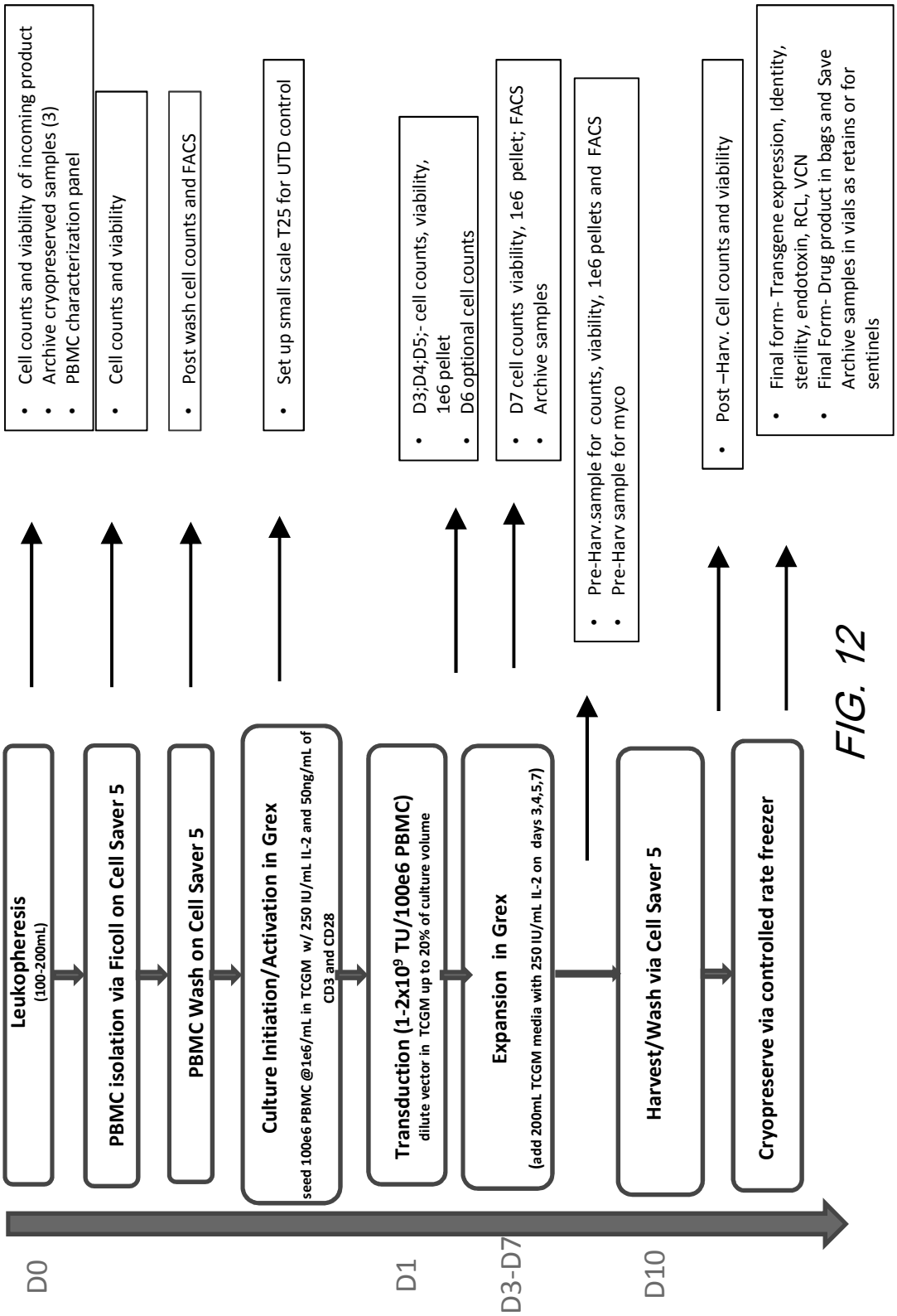

Leukopheresis
(100-200mL)
PBMC isolation via Ficoll on Cell Saver 5
PBMC Wash on Cell Saver 5
Culture Initiation/Activation in Grex
seed 100e6 PBMC @1e6/mL in TCGM w/ 250 IU/mL IL-2 and 50ng/mL of CD3 and CD28
Transduction (1-2x10⁹ TU/100e6 PBMC)
dilute vector in TCGM up to 20% of culture volume
Expansion in Grex
(add 200mL TCGM media with 250 IU/mL IL-2 on days 3,4,5,7)
Harvest/Wash via Cell Saver 5
Cryopreserve via controlled rate freezer
D0
D1
D3-D7
D10
Cell counts and viability of incoming product
Archive cryopreserved samples (3)
PBMC characterization panel
Cell counts and viability
Post wash cell counts and FACS
Set up small scale T25 for UTD control
D3;D4;D5;- cell counts, viability, 1e6 pellet
D6 optional cell counts
D7 cell counts viability, 1e6 pellet; FACS
Archive samples
Pre-Harv.sample for counts, viability, 1e6 pellets and FACS
Pre-Harv sample for myco
Post –Harv. Cell counts and viability
Final form- Transgene expression, Identity, sterility, endotoxin, RCL, VCN
Final Form- Drug product in bags and Save Archive samples in vials as retains or for sentinels

FIG. 12

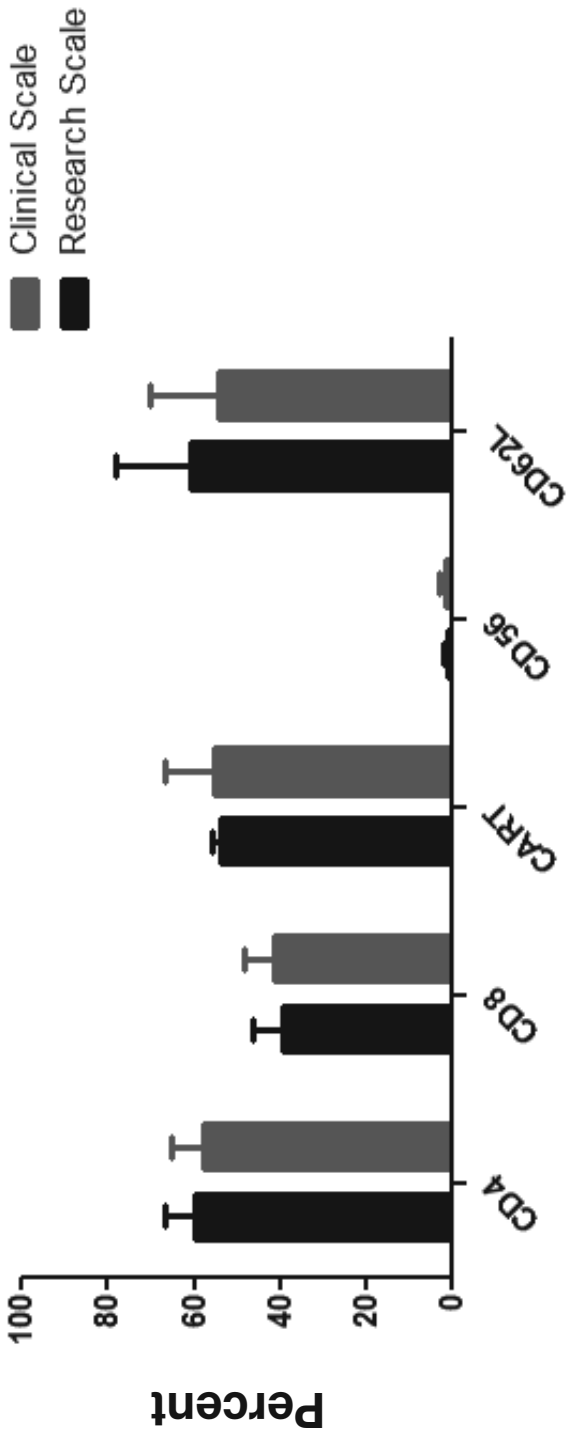

Clinical Scale
Research Scale
Percent
100
80
60
40
20
0
CD4
CD8
CART
CD56
CD62L

FIG. 13

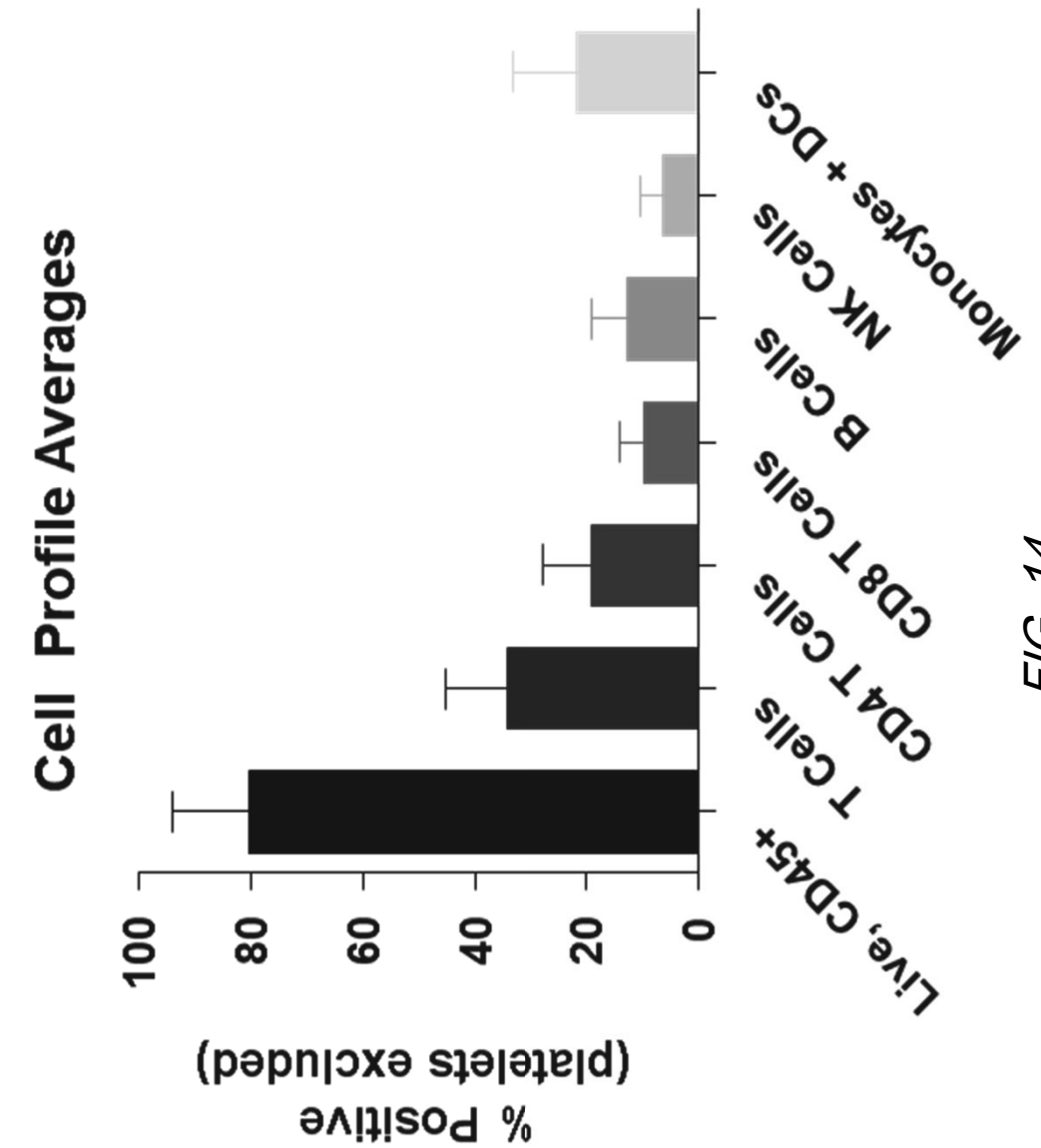

Cell Profile Averages
% Positive
(platelets excluded)
100
80
60
40
20
0
Live, CD45+
T cells
CD4 T cells
CD8 T cells
B cells
NK cells
Monocytes + DCs

FIG. 14

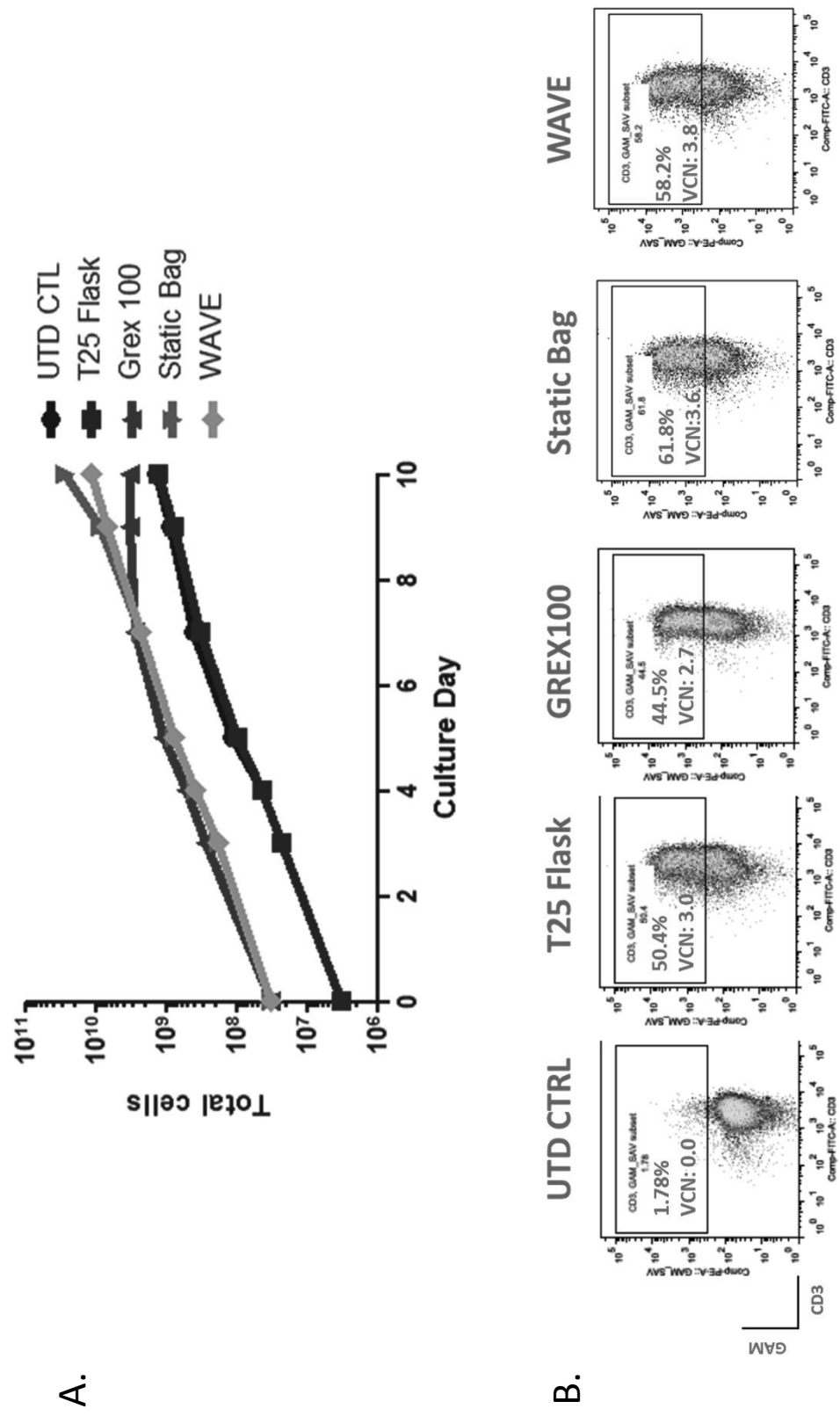

A.
UTD CTL
T25 Flask
Grex 100
Static Bag
WAVE
Total cells
Culture Day
B.
UTD CTRL
T25 Flask
GREX100
Static Bag
WAVE
GAM
CD3
1.78%
VCN: 0.0
50.4%
VCN: 3.0
44.5%
VCN: 2.7
61.8%
VCN:3.6
58.2%
VCN: 3.8

FIG. 15

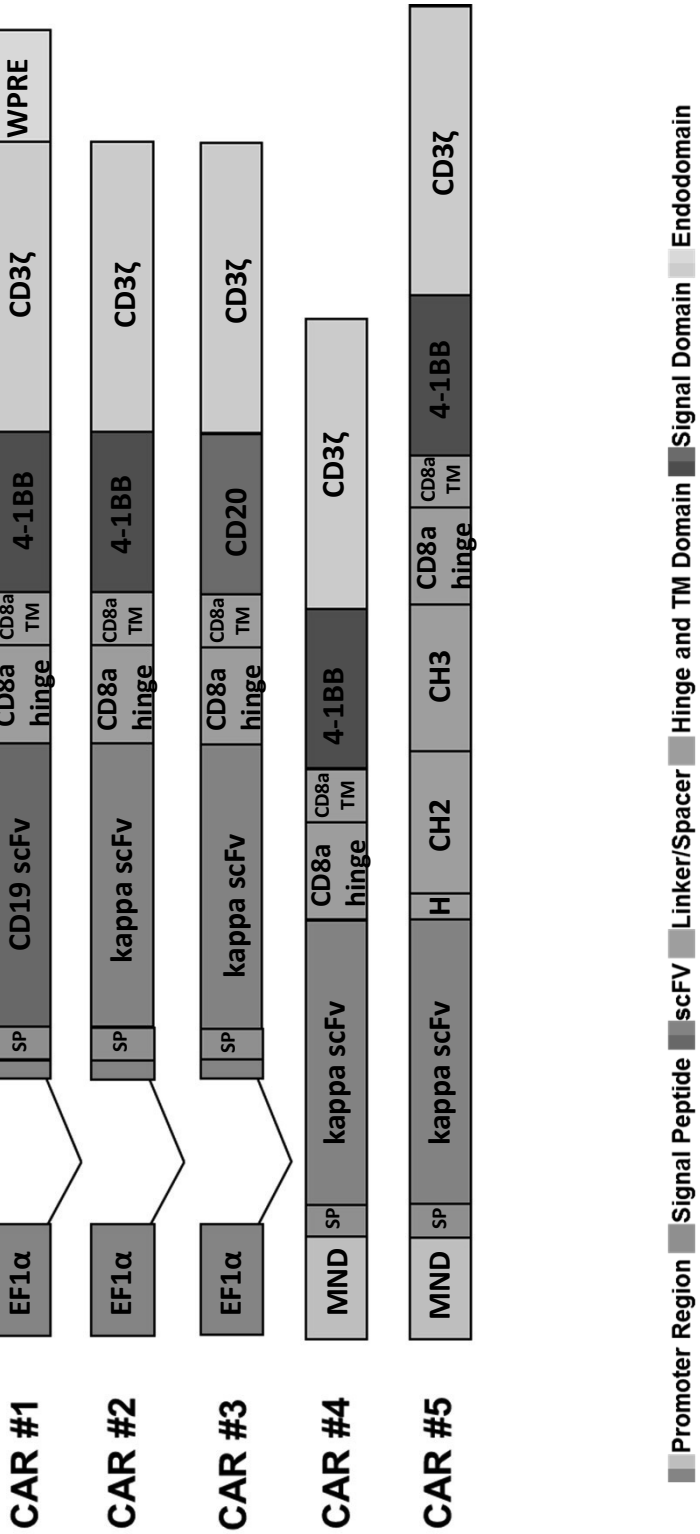

CAR #1
EF1α
SP
CD19 scFv
CD8a hinge
CD8a TM
4-1BB
CD3ζ
WPRE
CAR #2
EF1α
SP
kappa scFv
CD8a hinge
CD8a TM
4-1BB
CD3ζ
CAR #3
EF1α
SP
kappa scFv
CD8a hinge
CD8a TM
CD20
CD3ζ
CAR #4
MND
SP
kappa scFv
CD8a hinge
CD8a TM
4-1BB
CD3ζ
CAR #5
MND
SP
kappa scFv
H
CH2
CH3
CD8a hinge
CD8a TM
4-1BB
CD3ζ
Promoter Region
Signal Peptide
scFV
Linker/Spacer
Hinge and TM Domain
Signal Domain
Endodomain

FIG. 16

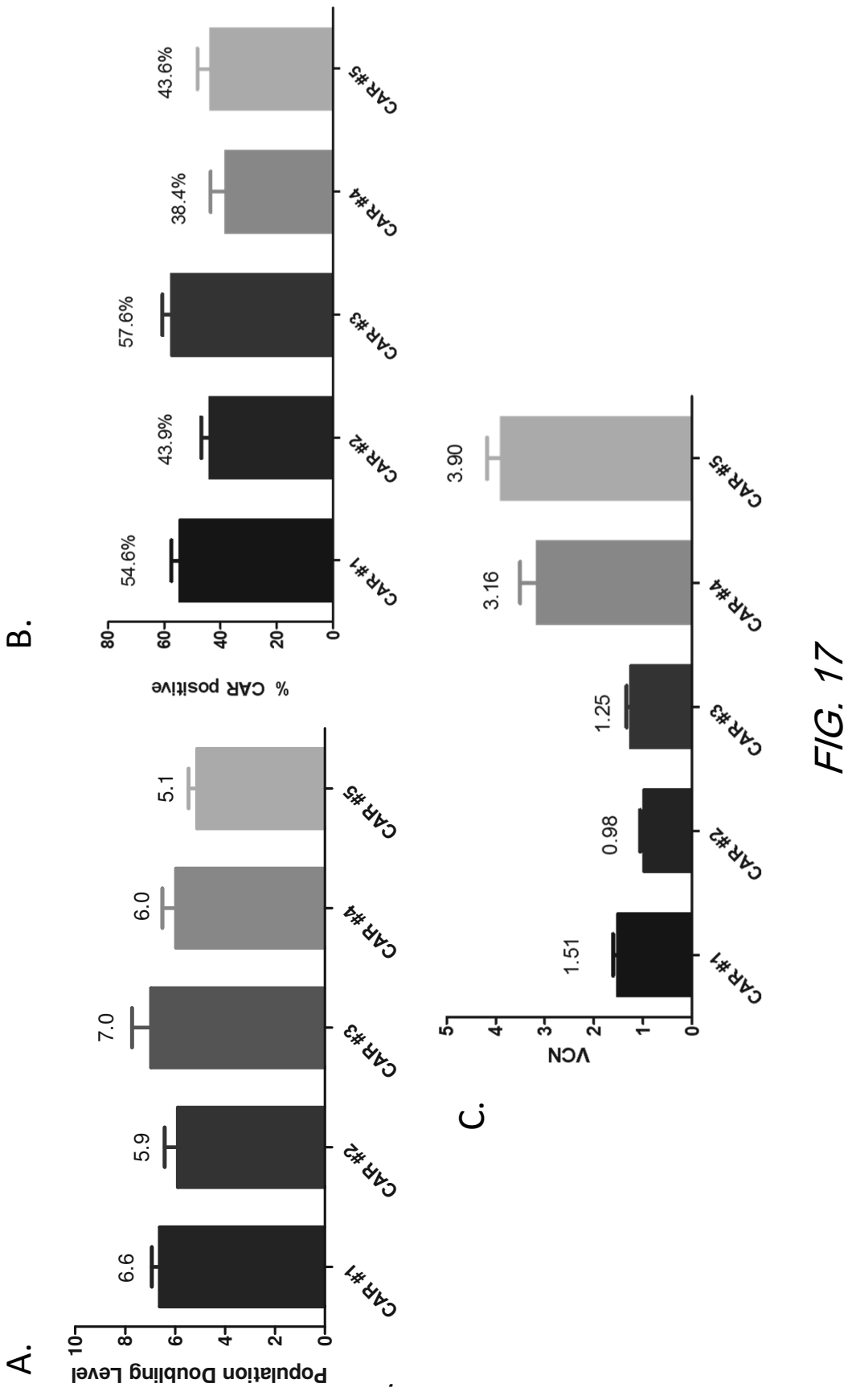

A.
Population Doubling Level
10
8
6
4
2
0
6.6
5.9
7.0
6.0
5.1
CAR #1
CAR #2
CAR #3
CAR #4
CAR #5
B.
% CAR positive
80
60
40
20
0
54.6%
43.9%
57.6%
38.4%
43.6%
CAR #1
CAR #2
CAR #3
CAR #4
CAR #5
C.
VCN
5
4
3
2
1
0
1.51
0.98
1.25
3.16
3.90
CAR #1
CAR #2
CAR #3
CAR #4
CAR #5

FIG. 17

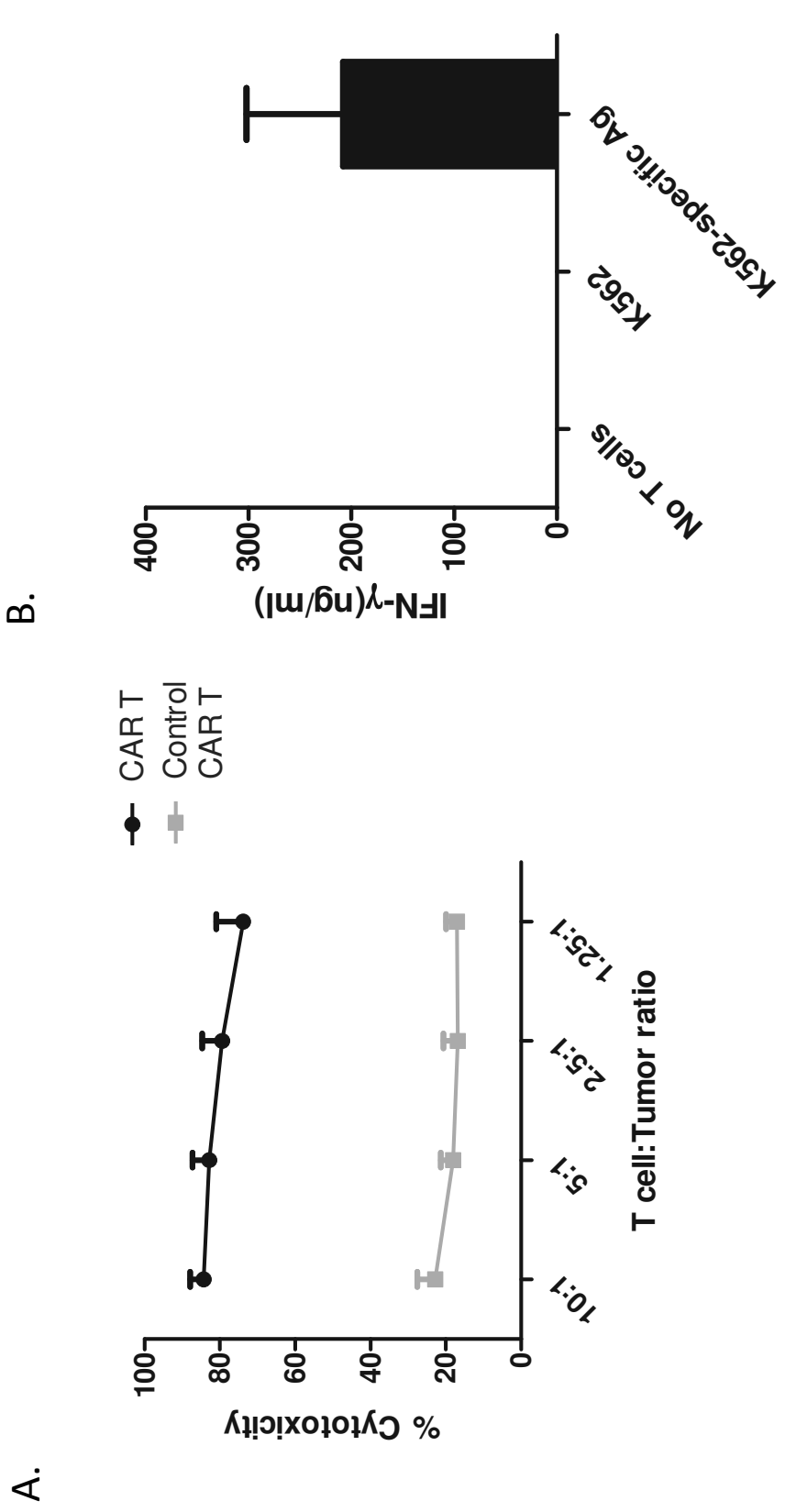

A.
% Cytotoxicity
100
80
60
40
20
0
10:1
5:1
2.5:1
1.25:1
T cell:Tumor ratio
CAR T
Control CAR T
B.
IFN-γ(ng/ml)
400
300
200
100
0
No T cells
K562
K562-specific Ag

FIG. 18