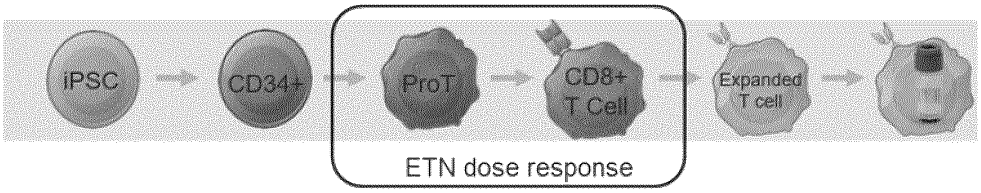
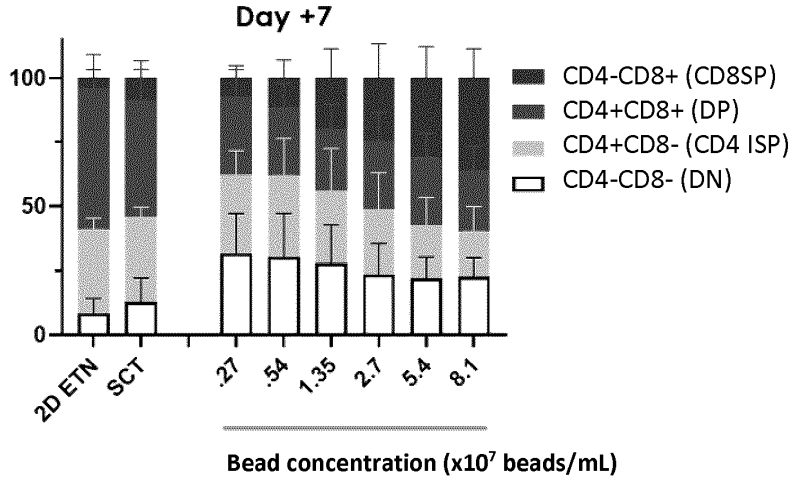
A


iPSC
CD34+
ProT
CD8+ T Cell
Expanded T cell
ETN dose response

B


Day +7
100
50
0
2D ETN
SCT
.27
.54
1.35
2.7
5.4
8.1
CD4-CD8+ (CD8SP)
CD4+CD8+ (DP)
CD4+CD8- (CD4 ISP)
CD4-CD8- (DN)
Bead concentration (x10⁷ beads/mL)

C

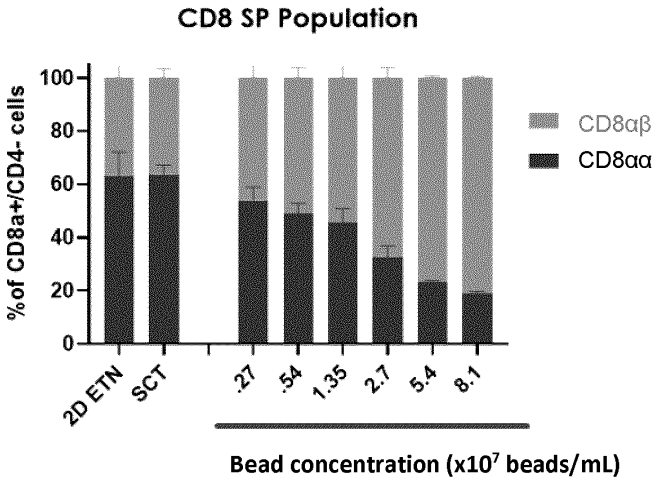
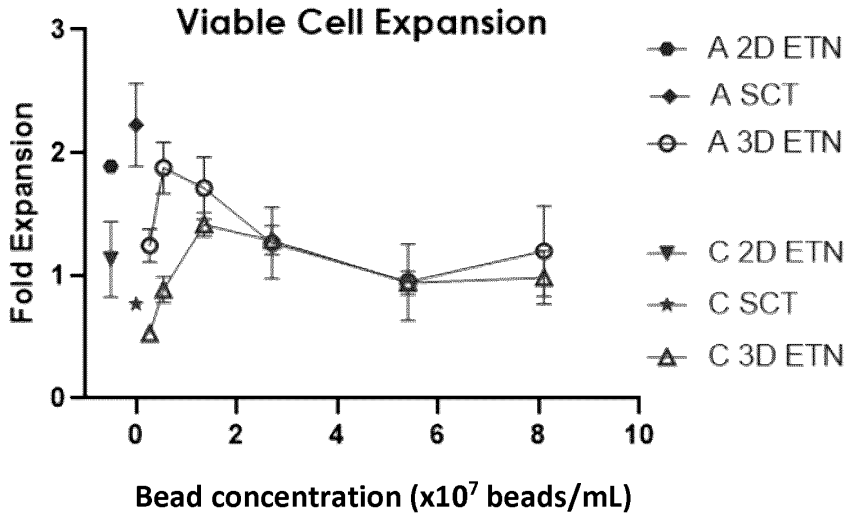

CD8 SP Population
% of CD8a+/CD4- cells
100
80
60
40
20
0
2D ETN
SCT
.27
.54
1.35
2.7
5.4
8.1
CD8αβ
CD8αα
Bead concentration (x10⁷ beads/mL)

FIGURE 1

A


Viable Cell Expansion
Fold Expansion
0
1
2
3
0
2
4
6
8
10
Bead concentration (x10^7 beads/mL)
A 2D ETN
A SCT
A 3D ETN
C 2D ETN
C SCT
C 3D ETN

B

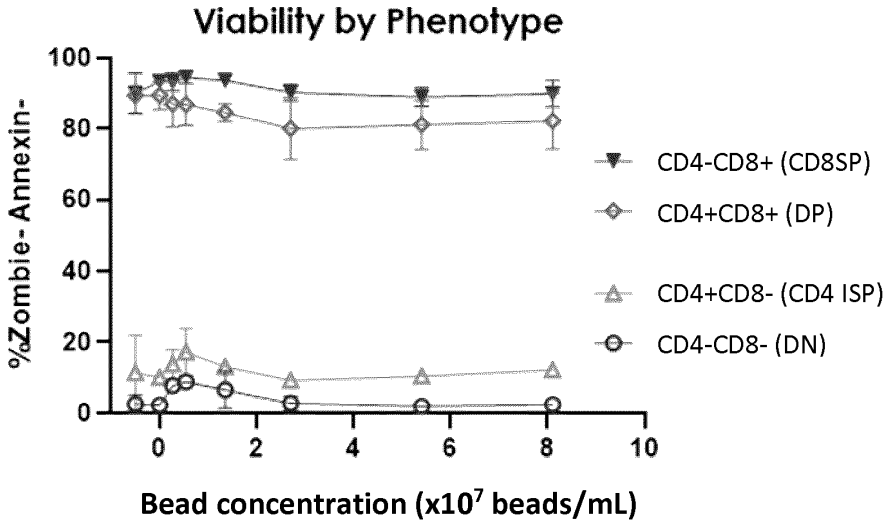
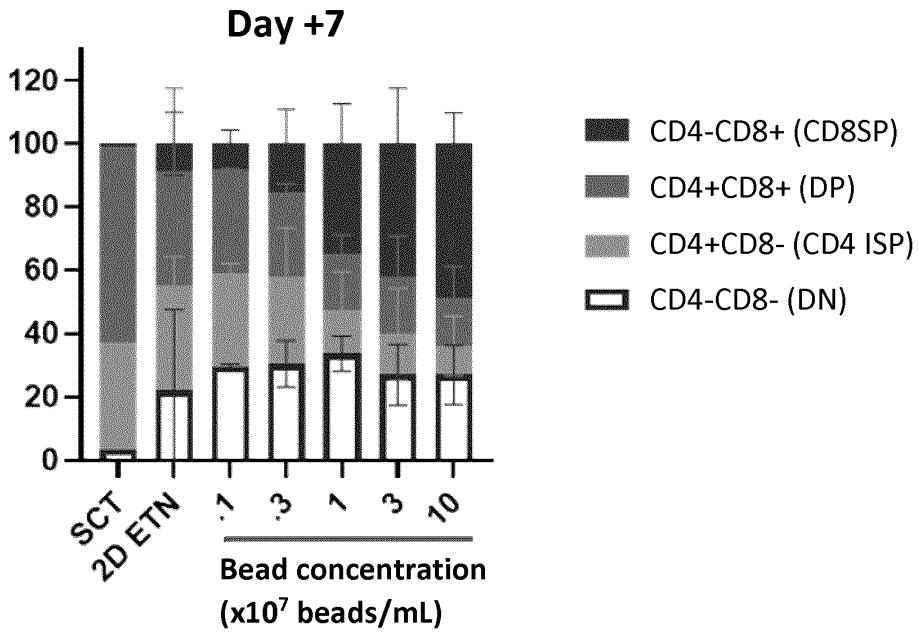

Viability by Phenotype
%Zombie- Annexin-
0
20
40
60
80
100
0
2
4
6
8
10
Bead concentration (x10^7 beads/mL)
CD4-CD8+ (CD8SP)
CD4+CD8+ (DP)
CD4+CD8- (CD4 ISP)
CD4-CD8- (DN)

FIGURE 2

A


Day +7
120
100
80
60
40
20
0
SCT
2D ETN
.1
.3
1
3
10
Bead concentration
(x10^7 beads/mL)
CD4-CD8+ (CD8SP)
CD4+CD8+ (DP)
CD4+CD8- (CD4 ISP)
CD4-CD8- (DN)

B

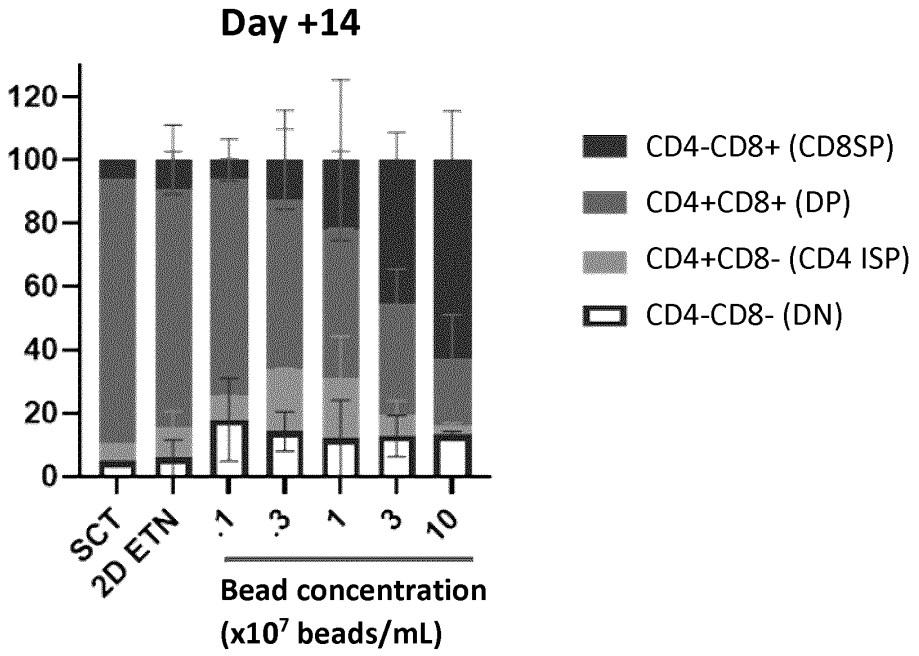

Day +14
120
100
80
60
40
20
0
SCT
2D ETN
.1
.3
1
3
10
Bead concentration
(x10^7 beads/mL)
CD4-CD8+ (CD8SP)
CD4+CD8+ (DP)
CD4+CD8- (CD4 ISP)
CD4-CD8- (DN)

FIGURE 3

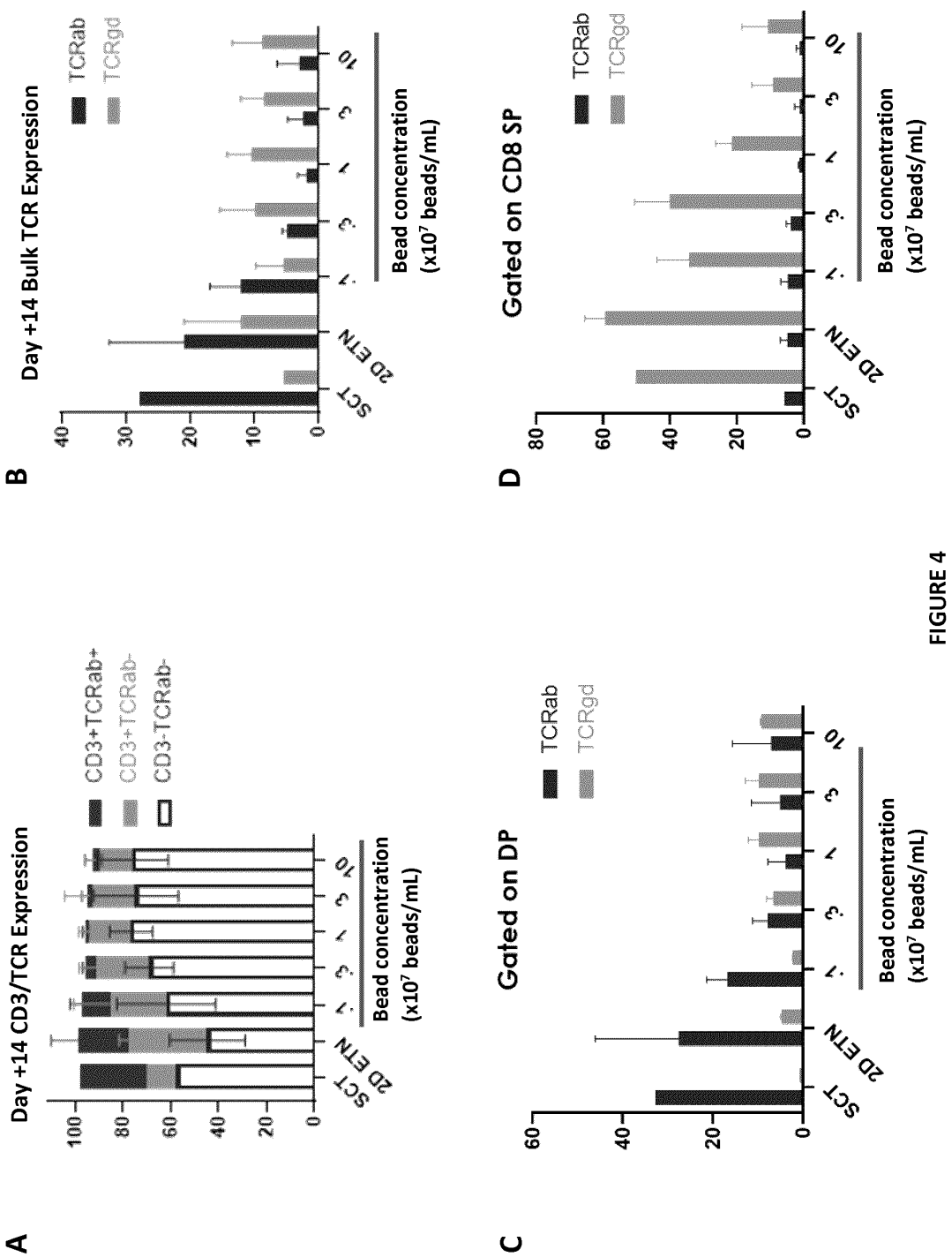

A
Day +14 CD3/TCR Expression
CD3+TCRAb+
CD3+TCRAb-
CD3-TCRAb-
100
80
60
40
20
0
SCT
2D ETN
1
3
1
3
1
3
10
Bead concentration
(x10⁷ beads/mL)
B
Day +14 Bulk TCR Expression
TCRAb
TCRgd
40
30
20
10
0
SCT
2D ETN
1
3
1
3
1
3
10
Bead concentration
(x10⁷ beads/mL)
C
Gated on DP
TCRAb
TCRgd
60
40
20
0
SCT
2D ETN
1
3
1
3
1
3
10
Bead concentration
(x10⁷ beads/mL)
D
Gated on CD8 SP
TCRAb
TCRgd
80
60
40
20
0
SCT
2D ETN
1
3
1
3
1
3
10
Bead concentration
(x10⁷ beads/mL)

FIGURE 4

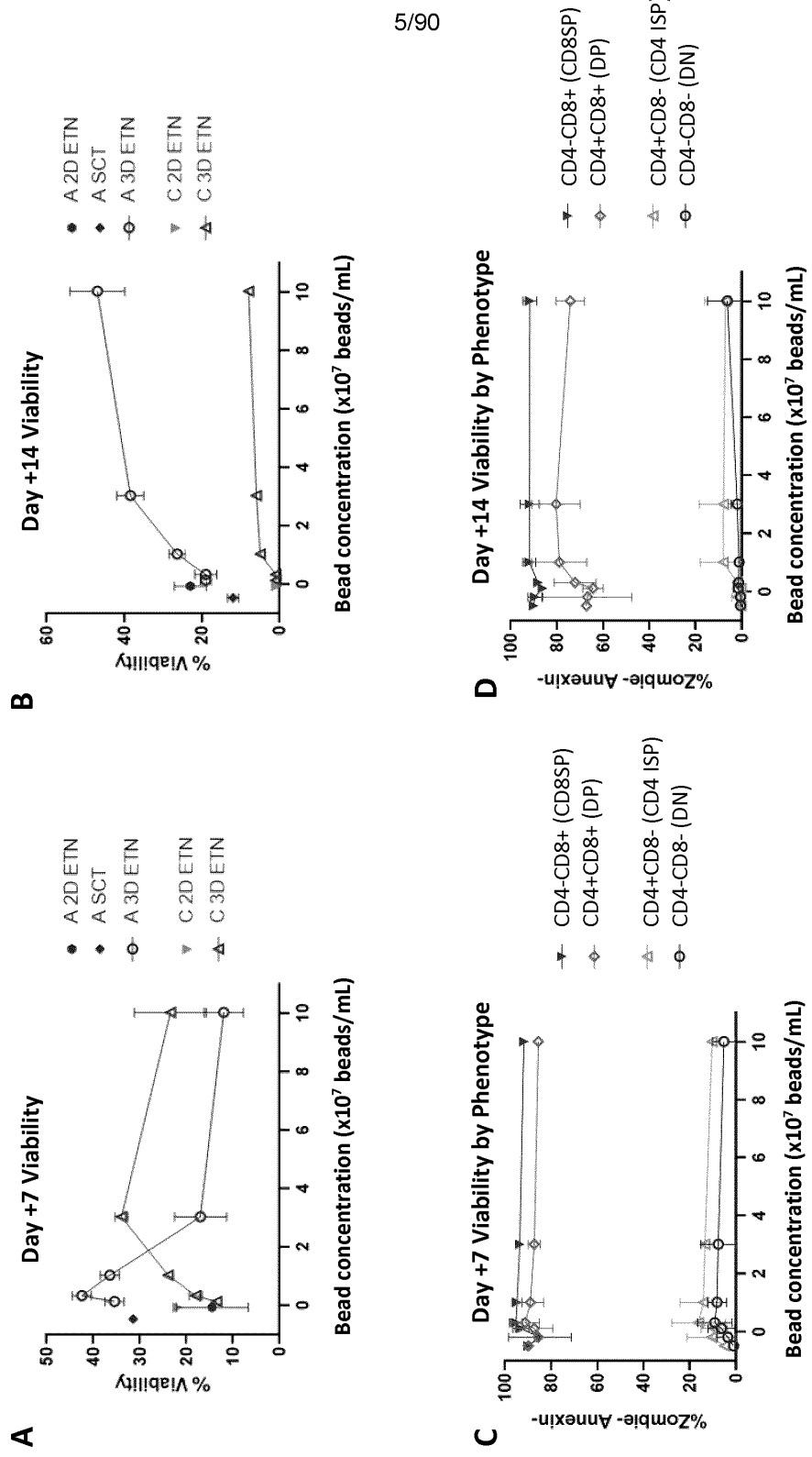

A
Day +7 Viability
% Viability
Bead concentration (x10^7 beads/mL)
A 2D ETN
A SCT
A 3D ETN
C 2D ETN
C 3D ETN
B
Day +14 Viability
C
Day +7 Viability by Phenotype
%Zombie- Annexin-
CD4-CD8+ (CD8SP)
CD4+CD8+ (DP)
CD4+CD8- (CD4 ISP)
CD4-CD8- (DN)
D
Day +14 Viability by Phenotype

FIGURE 5

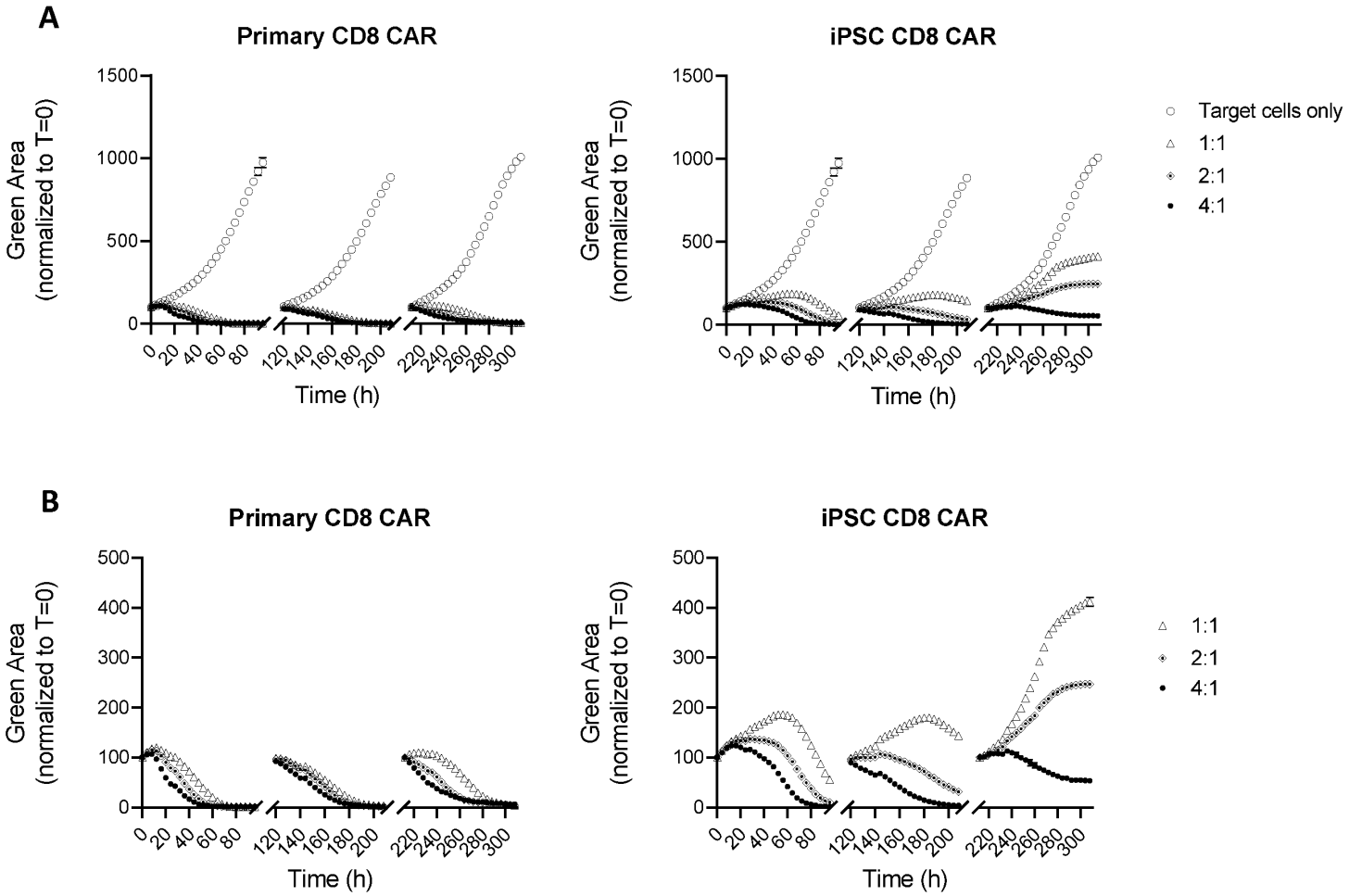

A
Primary CD8 CAR
iPSC CD8 CAR
Green Area (normalized to T=0)
Time (h)
Target cells only
1:1
2:1
4:1
B
Primary CD8 CAR
iPSC CD8 CAR
Green Area (normalized to T=0)
Time (h)
1:1
2:1
4:1

FIGURE 6

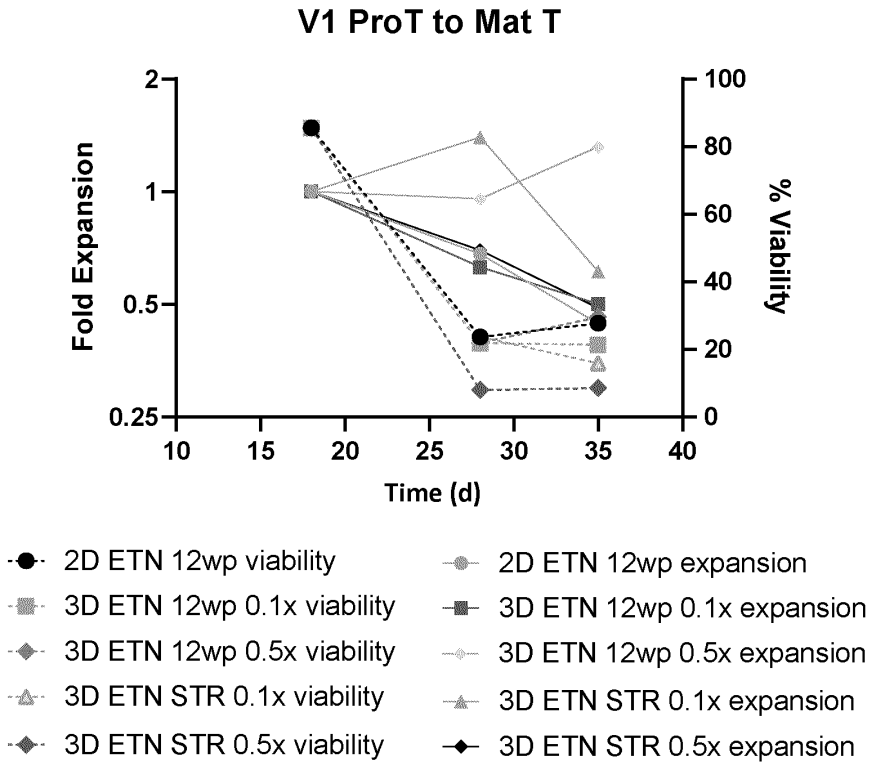
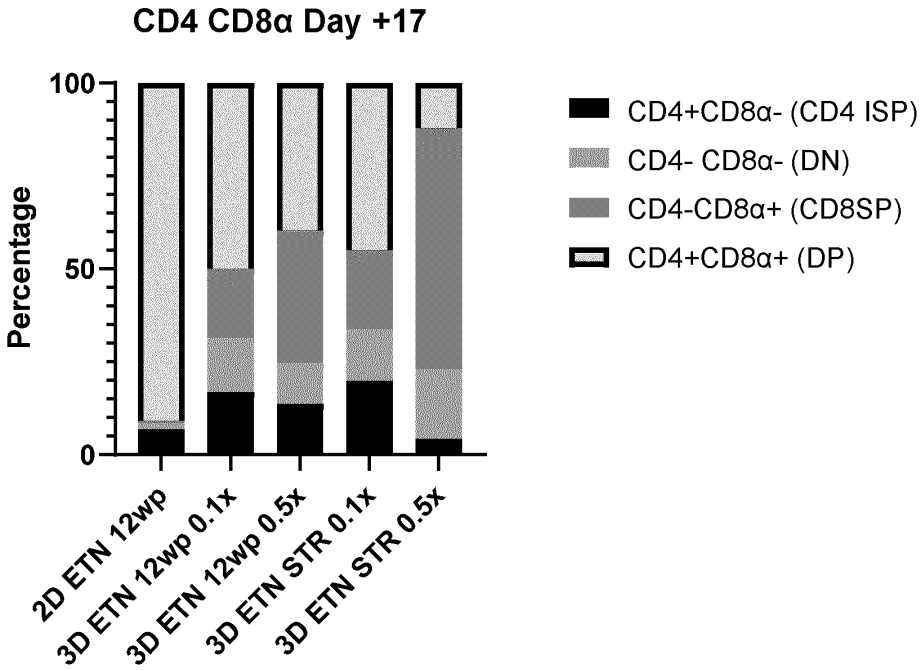

V1 ProT to Mat T
Fold Expansion
2
1
0.5
0.25
% Viability
100
80
60
40
20
0
10
15
20
25
30
35
40
Time (d)
2D ETN 12wp viability
3D ETN 12wp 0.1x viability
3D ETN 12wp 0.5x viability
3D ETN STR 0.1x viability
3D ETN STR 0.5x viability
2D ETN 12wp expansion
3D ETN 12wp 0.1x expansion
3D ETN 12wp 0.5x expansion
3D ETN STR 0.1x expansion
3D ETN STR 0.5x expansion

FIGURE 7

A


CD4 CD8α Day +17
Percentage
100
50
0
2D ETN 12wp
3D ETN 12wp 0.1x
3D ETN 12wp 0.5x
3D ETN STR 0.1x
3D ETN STR 0.5x
CD4+CD8α- (CD4 ISP)
CD4- CD8α- (DN)
CD4-CD8α+ (CD8SP)
CD4+CD8α+ (DP)

B

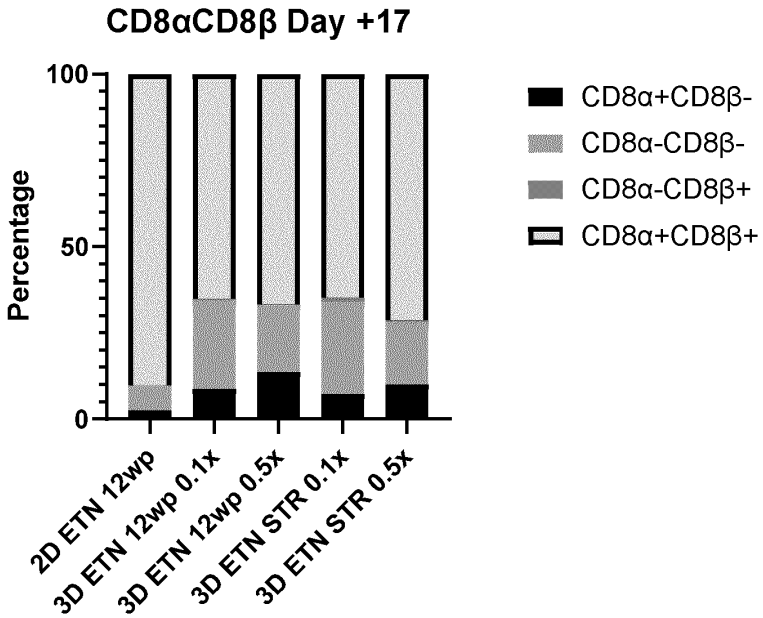

CD8αCD8β Day +17
Percentage
100
50
0
2D ETN 12wp
3D ETN 12wp 0.1x
3D ETN 12wp 0.5x
3D ETN STR 0.1x
3D ETN STR 0.5x
CD8α+CD8β-
CD8α-CD8β-
CD8α-CD8β+
CD8α+CD8β+

FIGURE 8

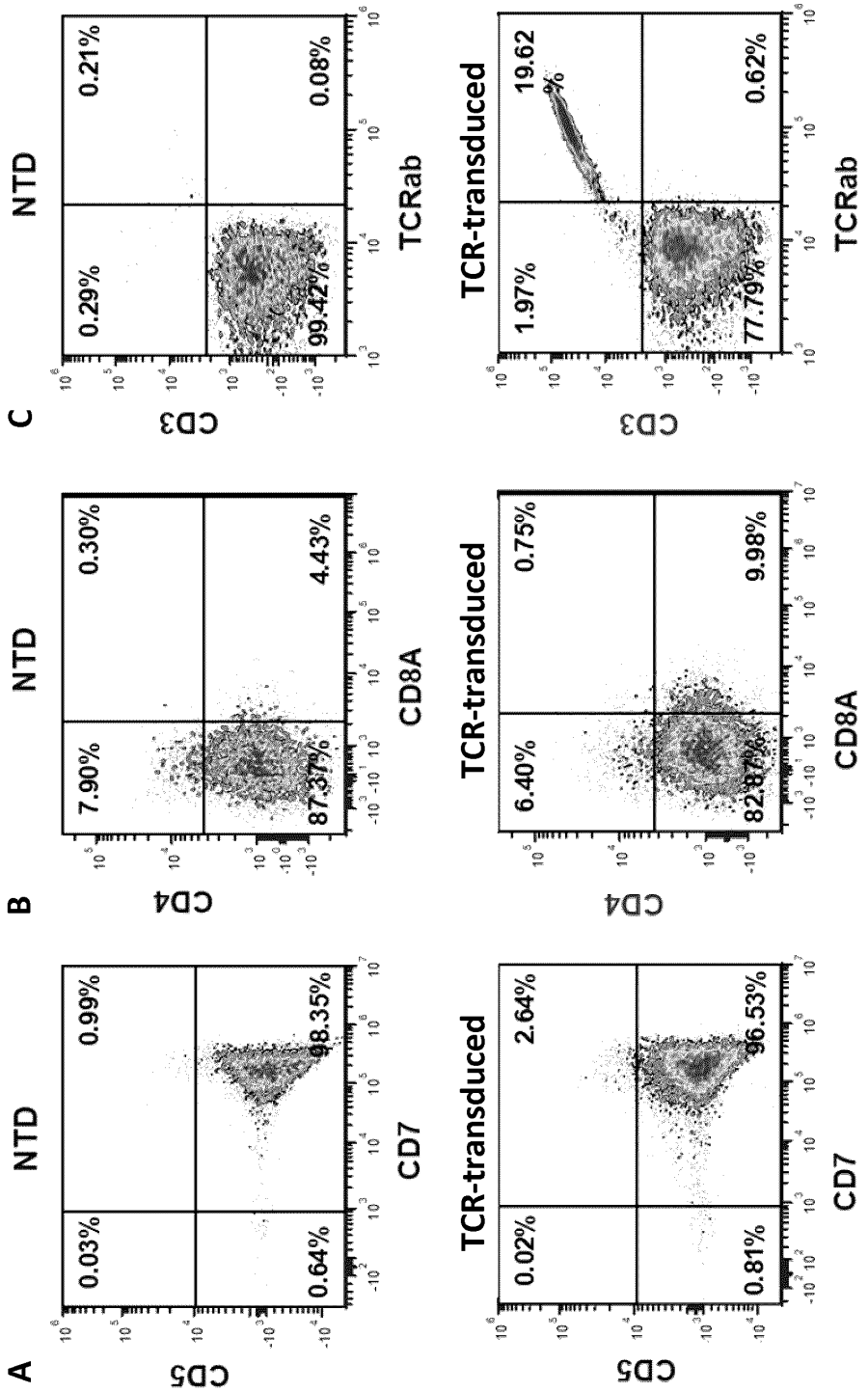

A
NTD
0.03%
0.99%
0.64%
98.35%
CD5
CD7
TCR-transduced
0.02%
2.64%
0.81%
96.53%
CD5
CD7
B
NTD
7.90%
0.30%
87.37%
4.43%
CD4
CD8A
TCR-transduced
6.40%
0.75%
82.87%
9.98%
CD4
CD8A
C
NTD
0.29%
0.21%
99.42%
0.08%
CD3
TCRab
TCR-transduced
1.97%
19.62%
77.79%
0.62%
CD3
TCRab

FIGURE 9

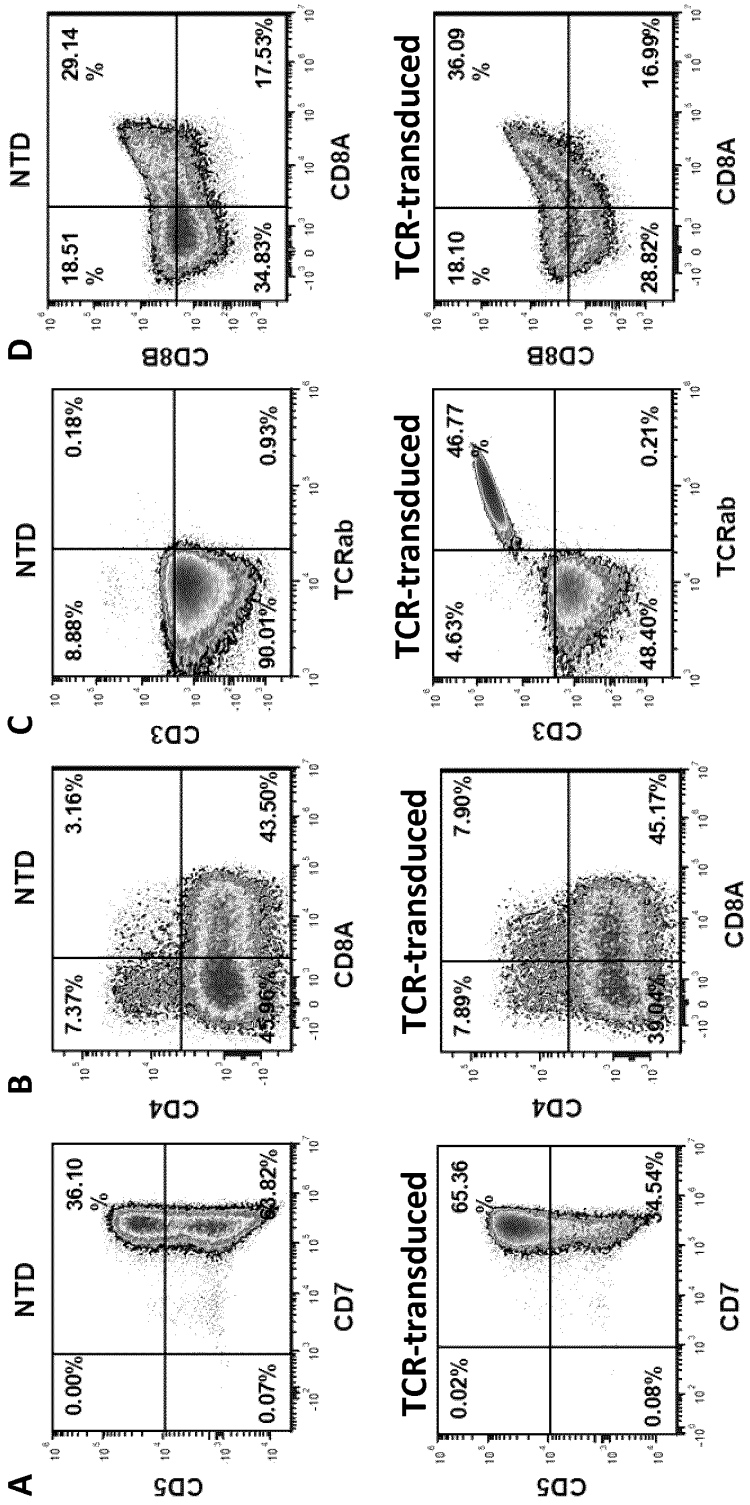

A
NTD
CD5
CD7
0.00%
0.07%
36.10 %
63.82%
TCR-transduced
CD5
CD7
0.02%
0.08%
65.36 %
34.54%
B
NTD
CD4
CD8A
7.37%
45.96%
3.16%
43.50%
TCR-transduced
CD4
CD8A
7.89%
39.04%
7.90%
45.17%
C
NTD
CD3
TCRab
8.88%
90.01%
0.18%
0.93%
TCR-transduced
CD3
TCRab
4.63%
48.40%
46.77 %
0.21%
D
NTD
CD8B
CD8A
18.51 %
34.83%
29.14 %
17.53%
TCR-transduced
CD8B
CD8A
18.10 %
28.82%
36.09 %
16.99%

FIGURE 10

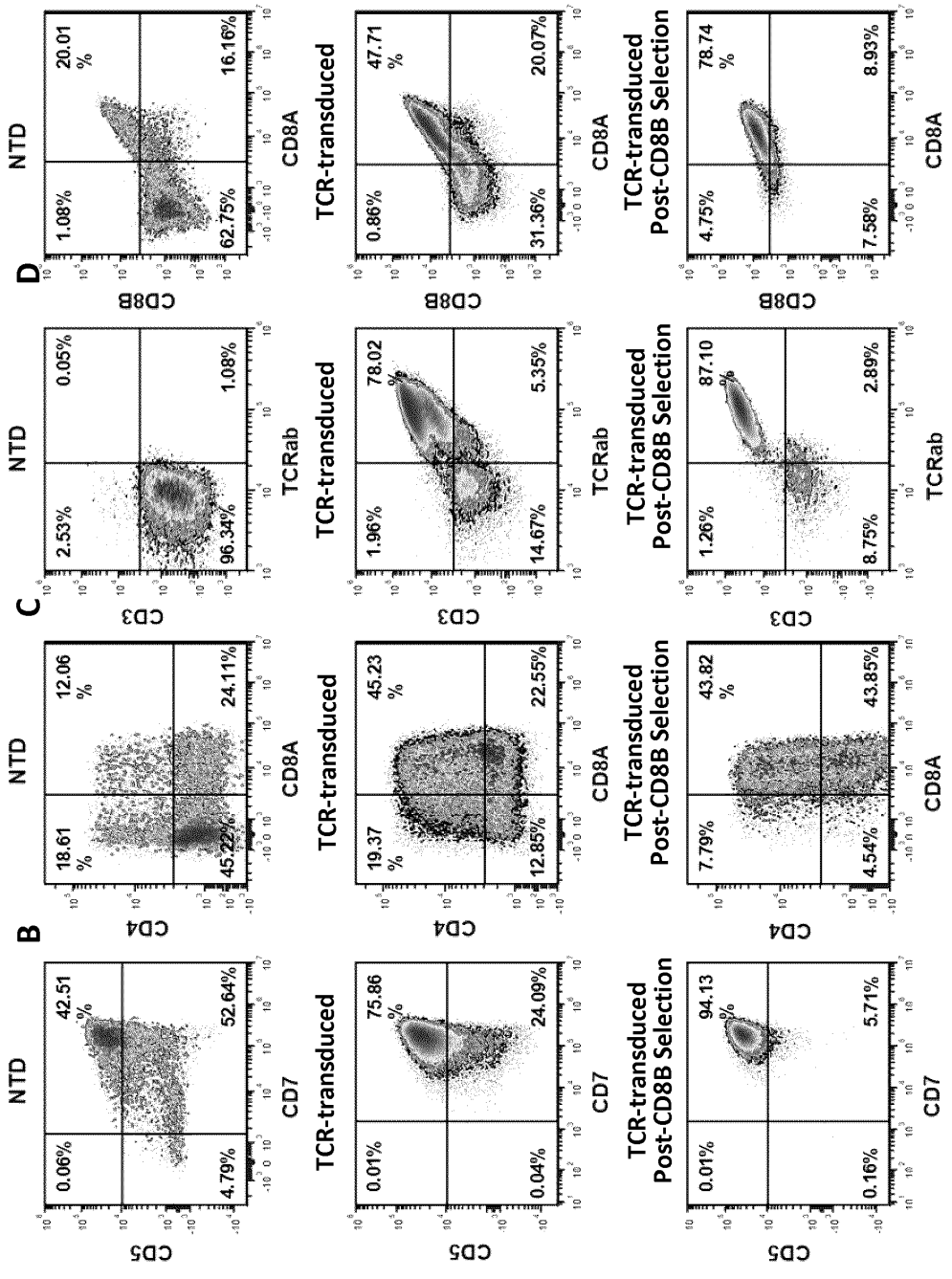

A
NTD
0.06%
42.51 %
4.79%
52.64%
CD5
CD7
TCR-transduced
0.01%
75.86 %
0.04%
24.09%
CD5
CD7
TCR-transduced Post-CD8B Selection
0.01%
94.13 %
0.16%
5.71%
CD5
CD7
B
NTD
18.61 %
45.22%
12.06 %
24.11%
CD4
CD8A
TCR-transduced
19.37 %
12.85%
45.23 %
22.55%
CD4
CD8A
TCR-transduced Post-CD8B Selection
7.79%
43.82 %
4.54%
43.85%
CD4
CD8A
C
NTD
2.53%
96.34%
0.05%
1.08%
CD3
TCRab
TCR-transduced
1.96%
14.67%
78.02 %
5.35%
CD3
TCRab
TCR-transduced Post-CD8B Selection
1.26%
8.75%
87.10 %
2.89%
CD3
TCRab
D
NTD
1.08%
62.75%
20.01 %
16.16%
CD8B
CD8A
TCR-transduced
0.86%
31.36%
47.71 %
20.07%
CD8B
CD8A
TCR-transduced Post-CD8B Selection
4.75%
7.58%
78.74 %
8.93%
CD8B
CD8A

FIGURE 11

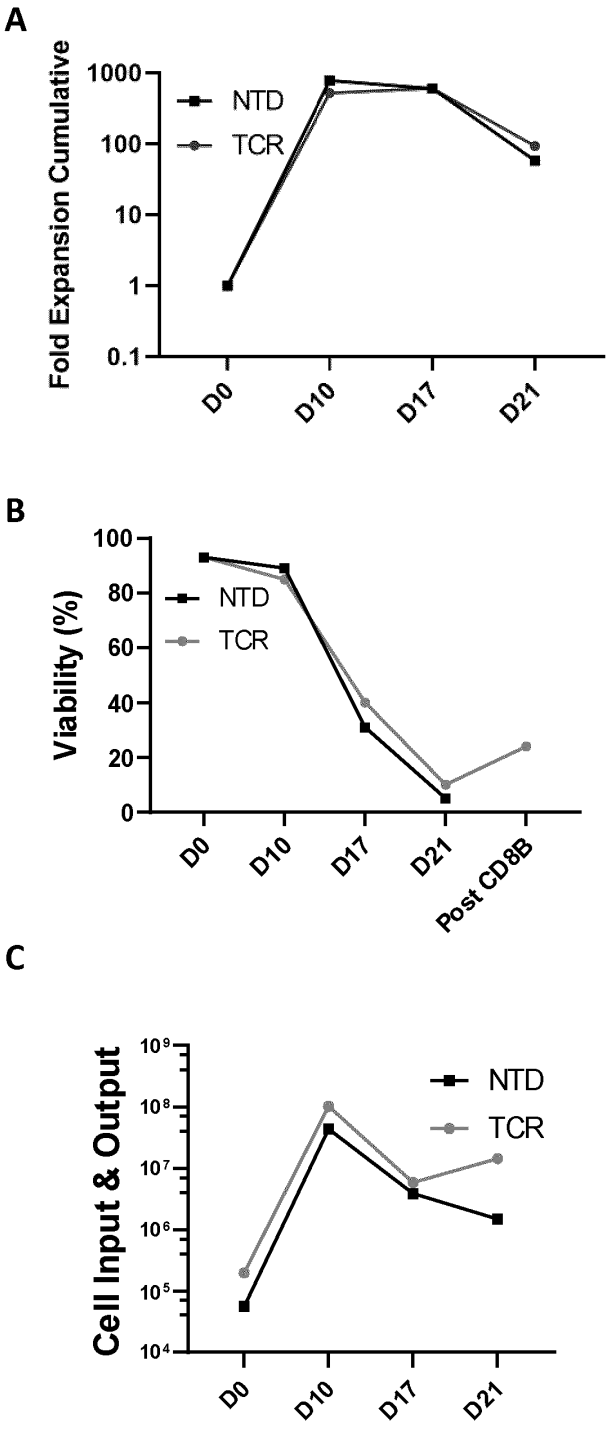
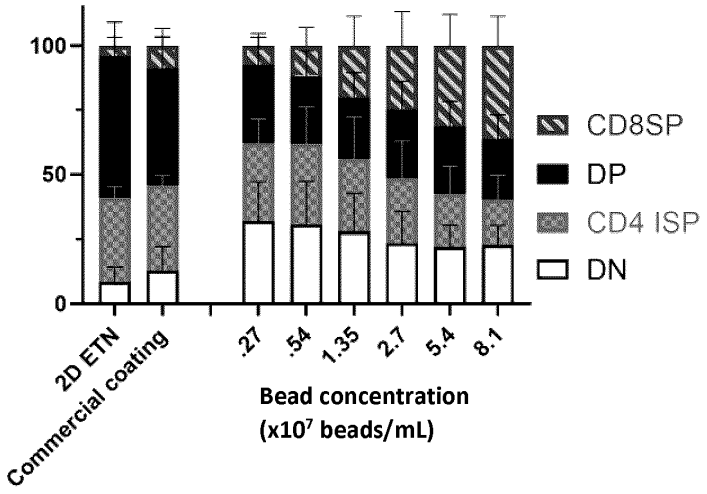

A
Fold Expansion Cumulative
1000
100
10
1
0.1
NTD
TCR
D0
D10
D17
D21
B
Viability (%)
100
80
60
40
20
0
NTD
TCR
D0
D10
D17
D21
Post CD8B
C
Cell Input & Output
$10^9$
$10^8$
$10^7$
$10^6$
$10^5$
$10^4$
NTD
TCR
D0
D10
D17
D21

FIGURE 12

FIGURE 13A



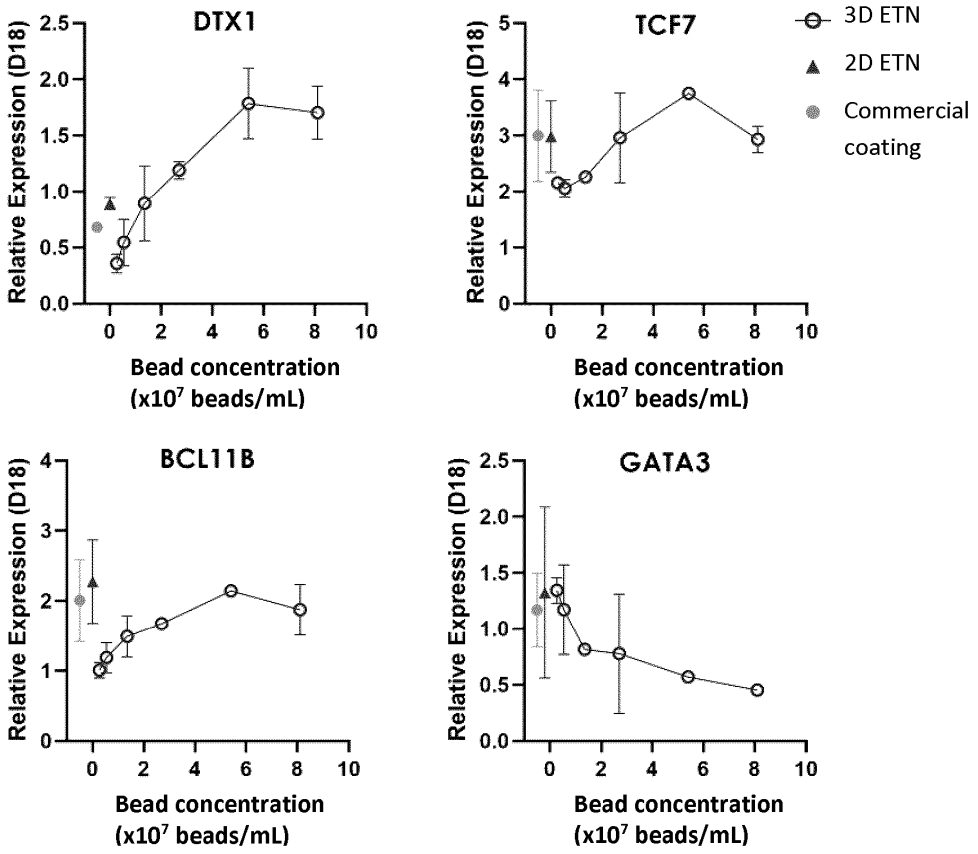




FIGURE 13B

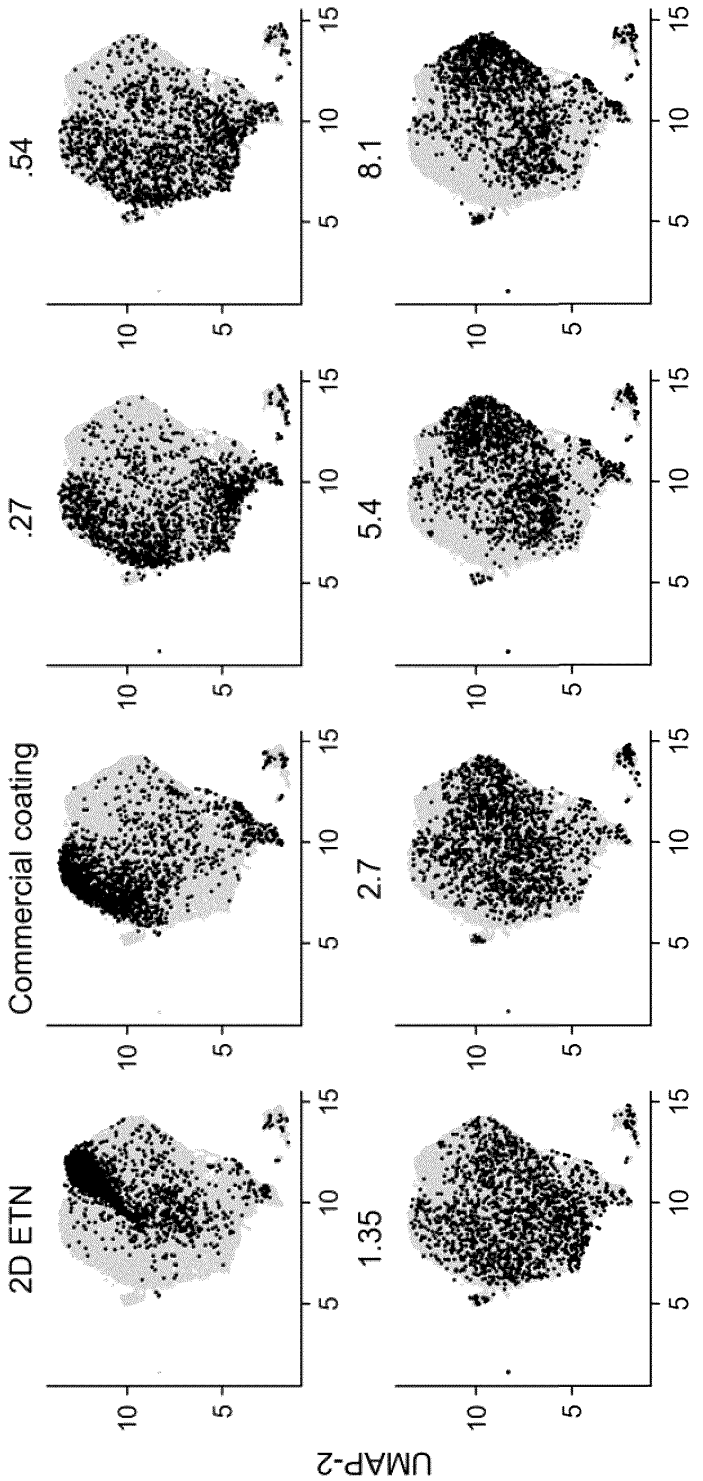

2D ETN
Commercial coating
.27
.54
1.35
2.7
5.4
8.1
UMAP-2
UMAP-1
5
10
15

FIGURE 13C

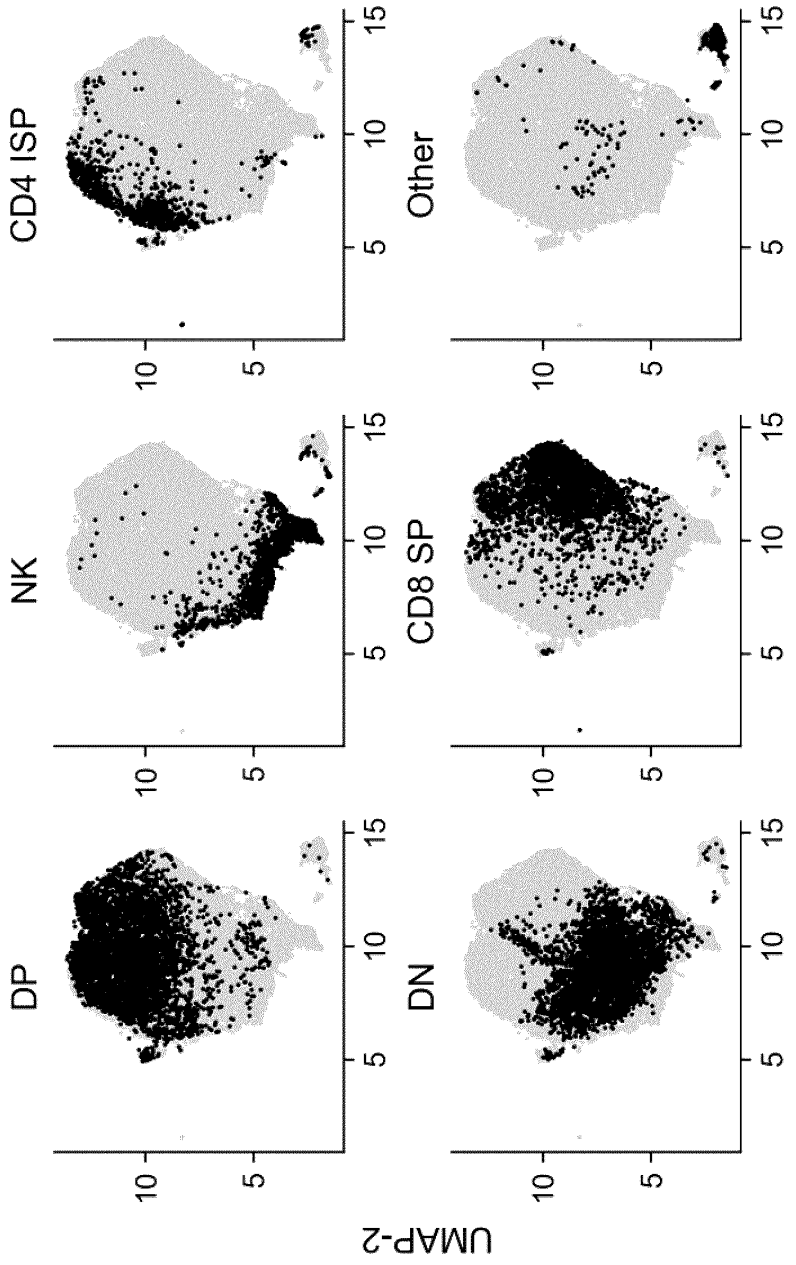
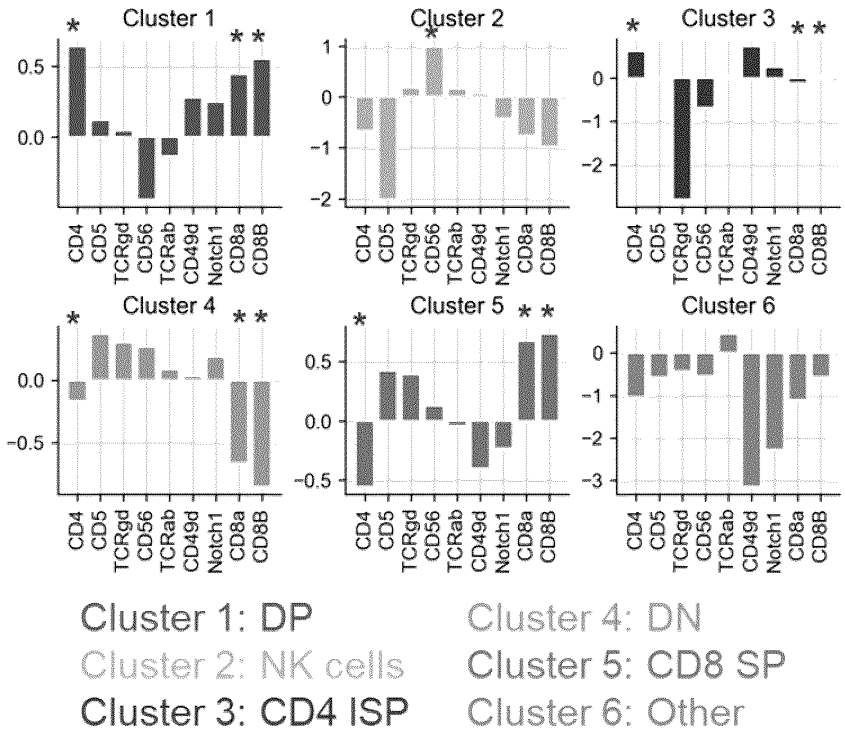



FIGURE 13D

**FIGURE 13E**



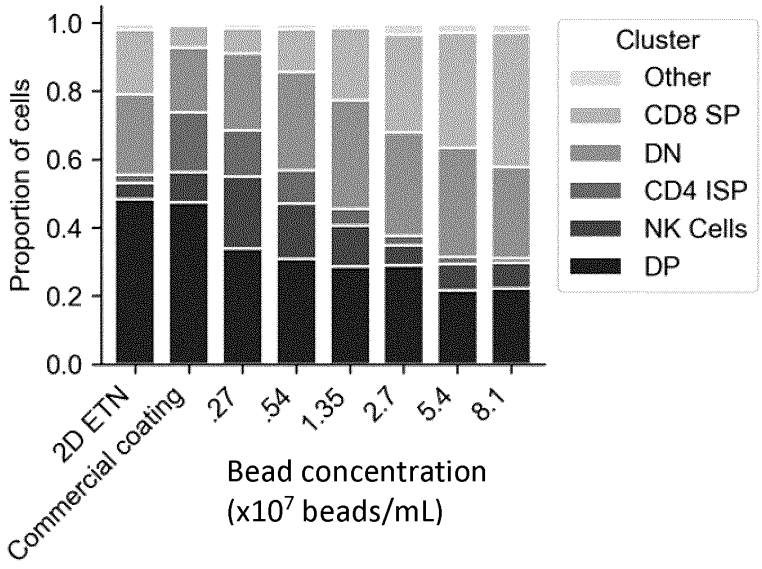




**FIGURE 13F**

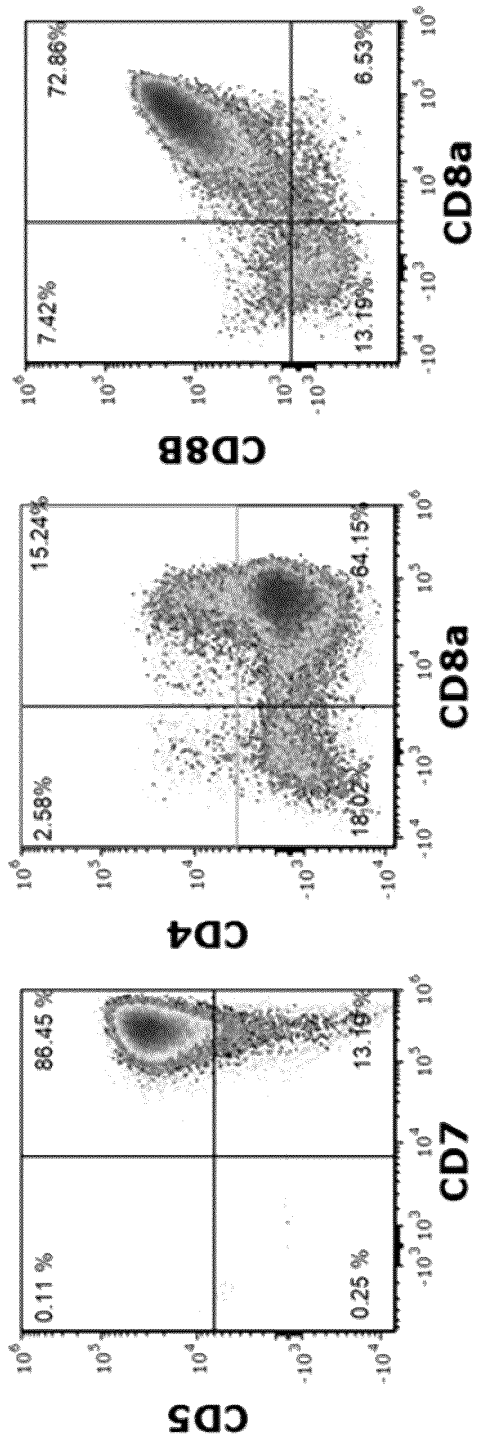

CD8B
CD8a
7.42%
72.86%
13.19%
6.53%
CD4
CD8a
2.58%
15.24%
18.02%
64.15%
CD5
CD7
0.11 %
86.45 %
0.25 %
13.19%

FIGURE 13G

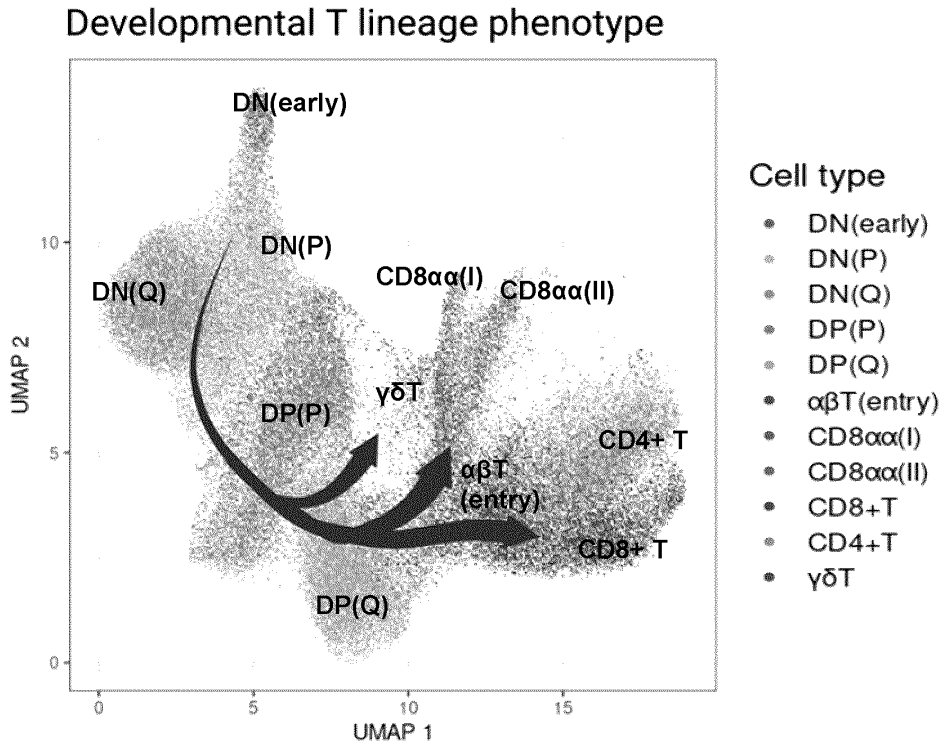

Developmental T lineage phenotype
DN(early)
DN(P)
DN(Q)
CD8αα(I)
CD8αα(II)
γδT
DP(P)
CD4+ T
αβT
(entry)
CD8+ T
DP(Q)
UMAP 2
UMAP 1
0
5
10
15
Cell type
DN(early)
DN(P)
DN(Q)
DP(P)
DP(Q)
αβT(entry)
CD8αα(I)
CD8αα(II)
CD8+T
CD4+T
γδT

FIGURE 14A

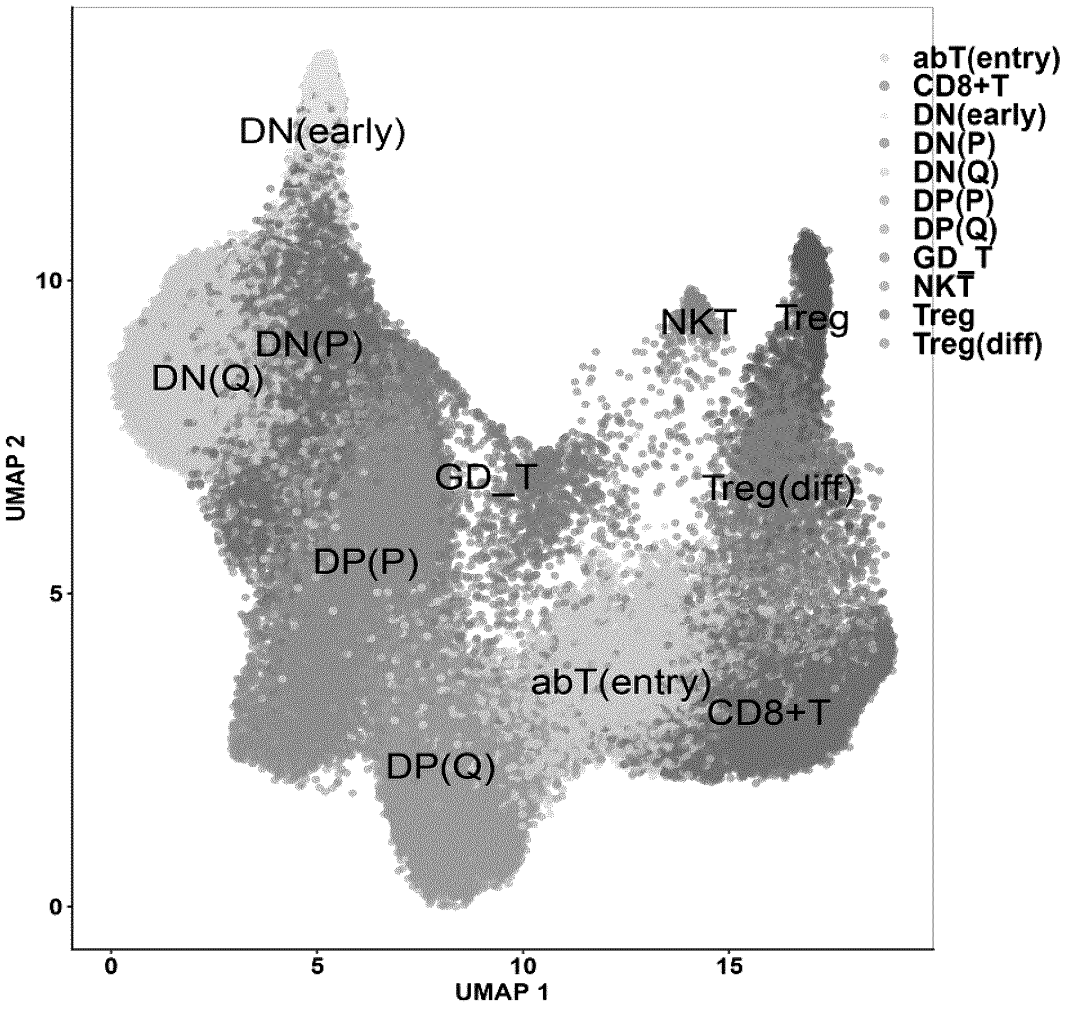

abT(entry)
CD8+T
DN(early)
DN(P)
DN(Q)
DP(P)
DP(Q)
GD_T
NKT
Treg
Treg(diff)
DN(early)
DN(P)
DN(Q)
NKT
Treg
GD_T
Treg(diff)
DP(P)
abT(entry)
CD8+T
DP(Q)
UMAP 2
UMAP 1
0
5
10
15

FIGURE 14B

FIGURE 15A Notch Signalling

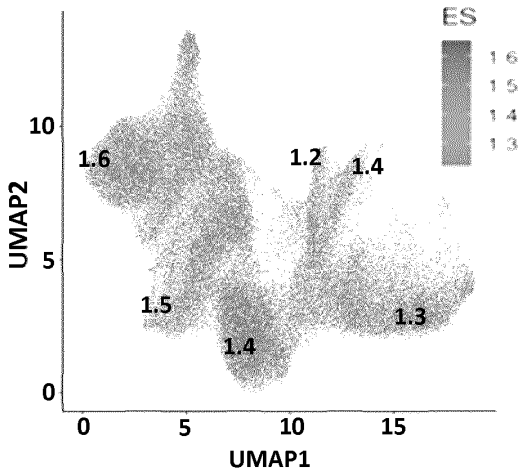
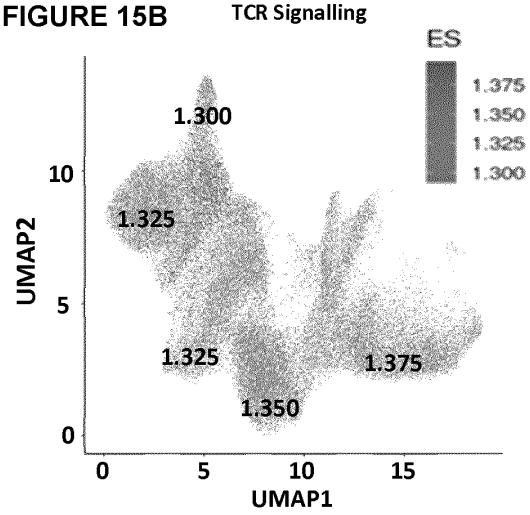



FIGURE 15B TCR Signalling



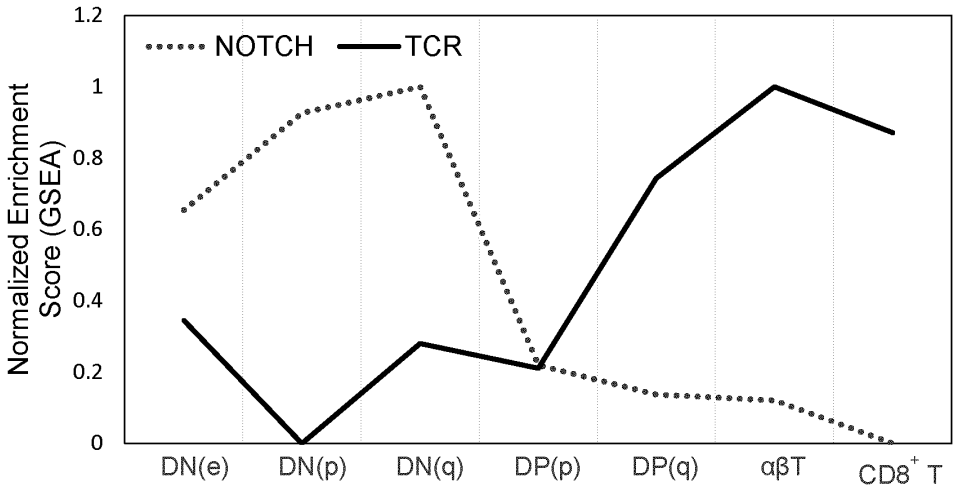




FIGURE 15C

FIGURE 15D

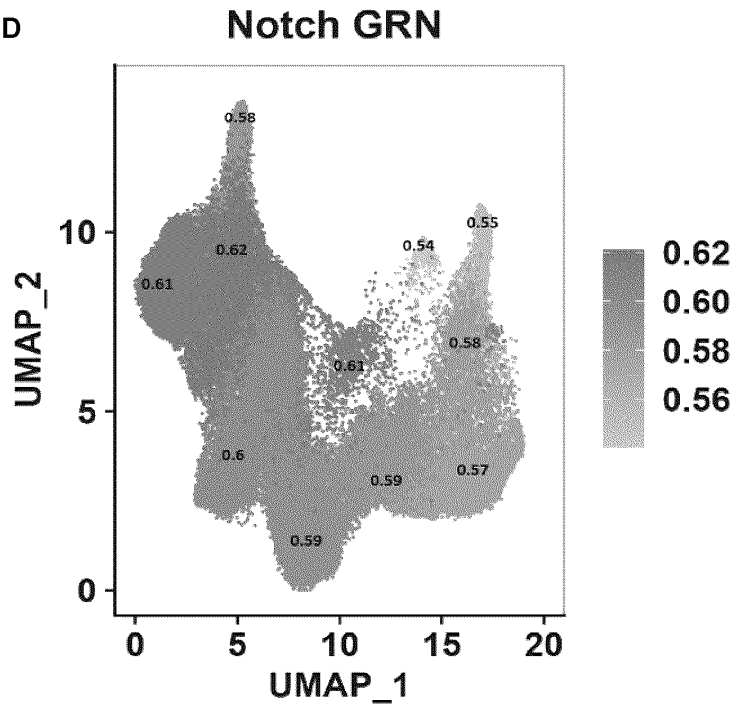
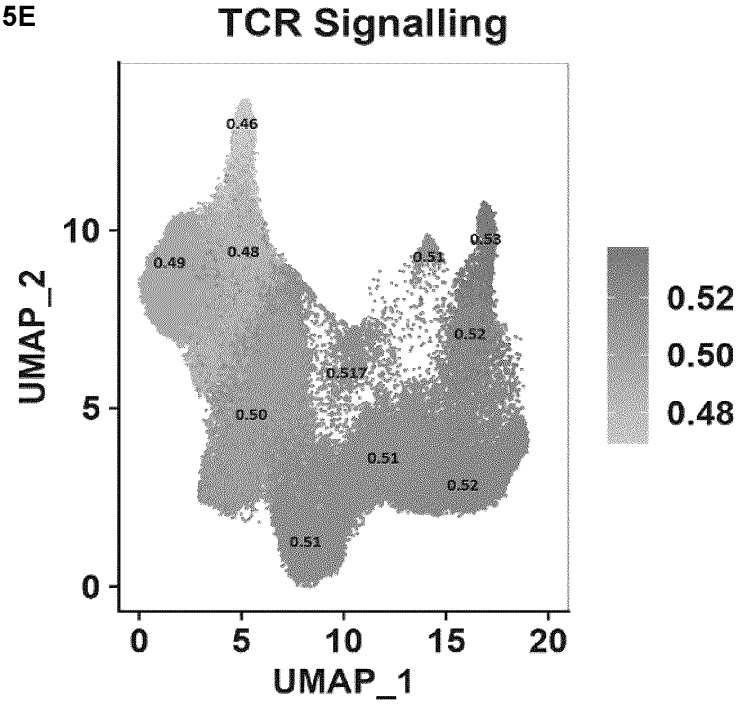



FIGURE 15E



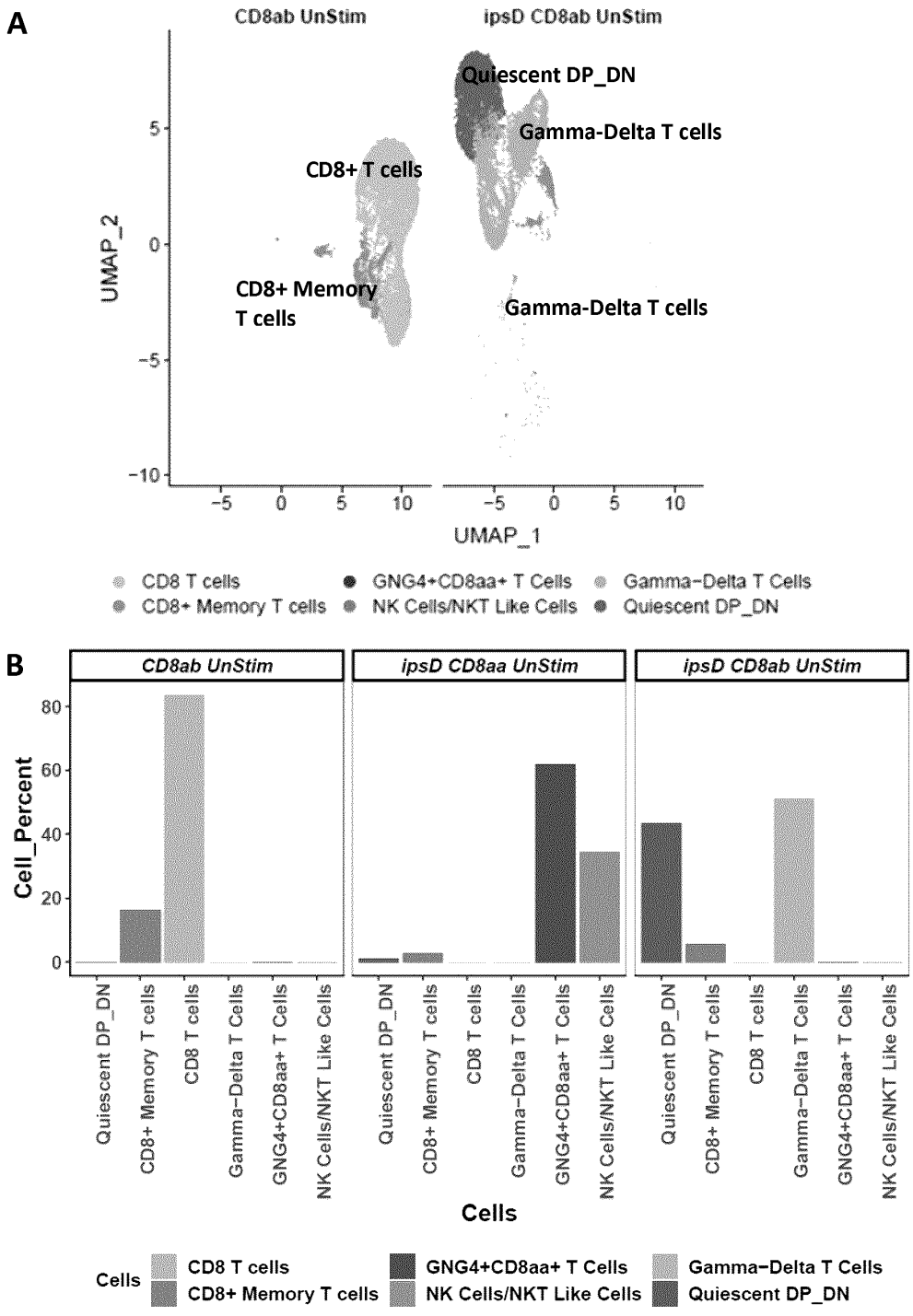

A
CD8ab UnStim
ipsD CD8ab UnStim
Quiescent DP_DN
Gamma-Delta T cells
CD8+ T cells
CD8+ Memory T cells
Gamma-Delta T cells
UMAP_2
UMAP_1
CD8 T cells
CD8+ Memory T cells
GNG4+CD8aa+ T Cells
NK Cells/NKT Like Cells
Gamma-Delta T Cells
Quiescent DP_DN
B
CD8ab UnStim
ipsD CD8aa UnStim
ipsD CD8ab UnStim
Cell_Percent
Quiescent DP_DN
CD8+ Memory T cells
CD8 T cells
Gamma-Delta T Cells
GNG4+CD8aa+ T Cells
NK Cells/NKT Like Cells
Cells
Cells
CD8 T cells
CD8+ Memory T cells
GNG4+CD8aa+ T Cells
NK Cells/NKT Like Cells
Gamma-Delta T Cells
Quiescent DP_DN

FIGURE 16

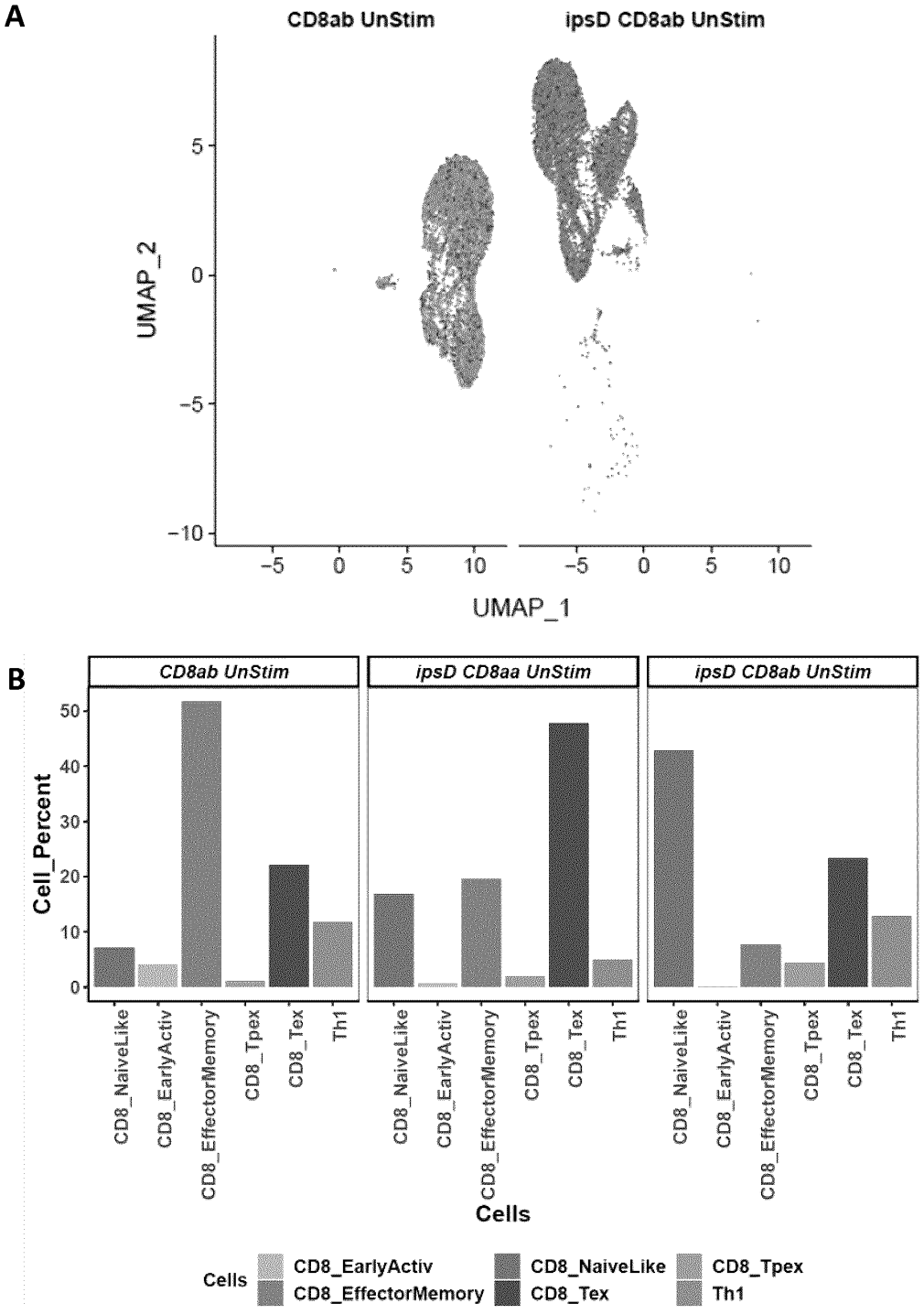

A
CD8ab UnStim
ipsD CD8ab UnStim
UMAP_2
UMAP_1
B
CD8ab UnStim
ipsD CD8aa UnStim
ipsD CD8ab UnStim
Cell_Percent
CD8_NaiveLike
CD8_EarlyActiv
CD8_EffectorMemory
CD8_Tpex
CD8_Tex
Th1
Cells
Cells
CD8_EarlyActiv
CD8_EffectorMemory
CD8_NaiveLike
CD8_Tex
CD8_Tpex
Th1

FIGURE 17

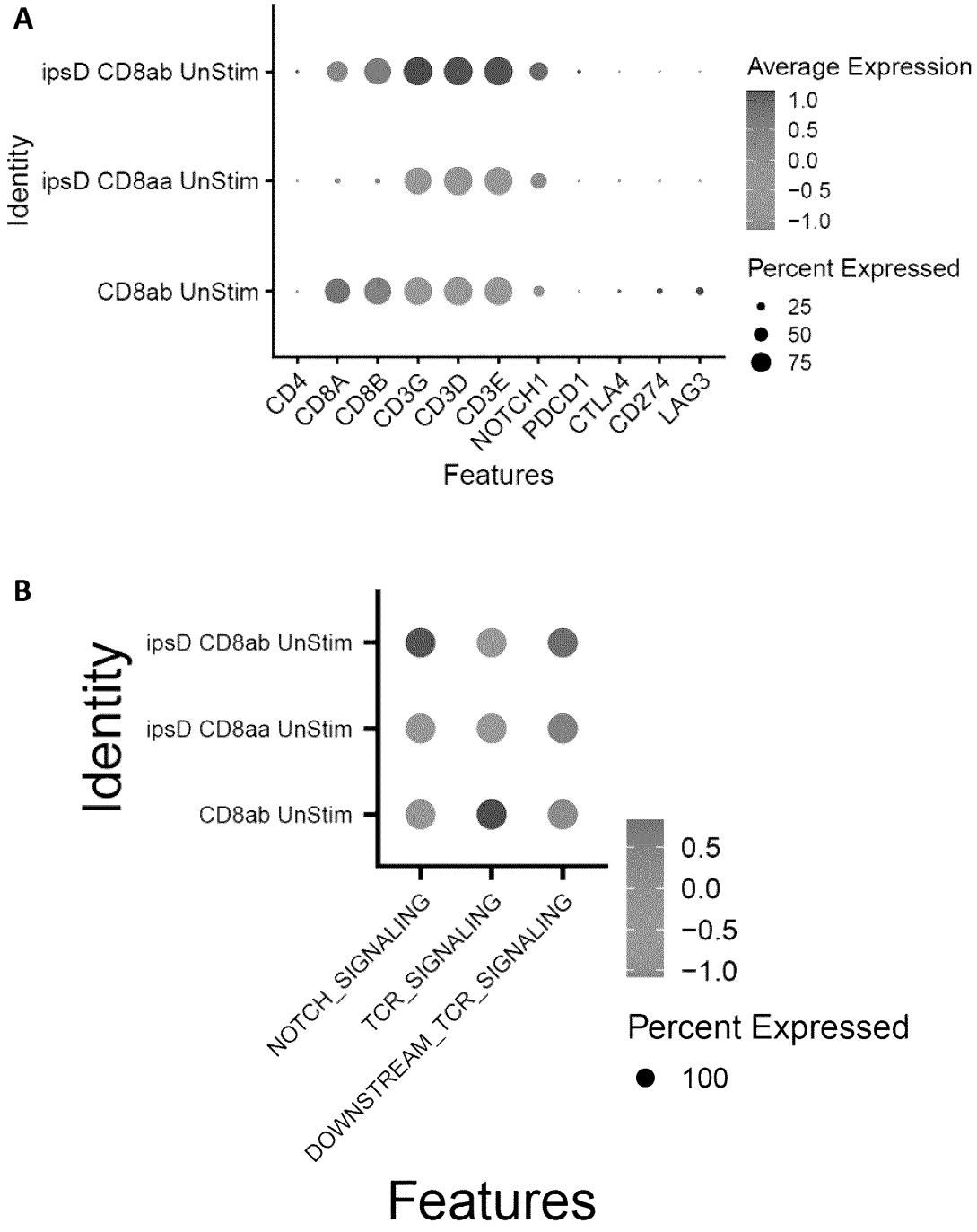

A
ipsD CD8ab UnStim
ipsD CD8aa UnStim
CD8ab UnStim
Identity
CD4
CD8A
CD8B
CD3G
CD3D
CD3E
NOTCH1
PDCD1
CTLA4
CD274
LAG3
Features
Average Expression
1.0
0.5
0.0
−0.5
−1.0
Percent Expressed
25
50
75
B
ipsD CD8ab UnStim
ipsD CD8aa UnStim
CD8ab UnStim
Identity
NOTCH_SIGNALING
TCR_SIGNALING
DOWNSTREAM_TCR_SIGNALING
0.5
0.0
−0.5
−1.0
Percent Expressed
100
Features

FIGURE 18

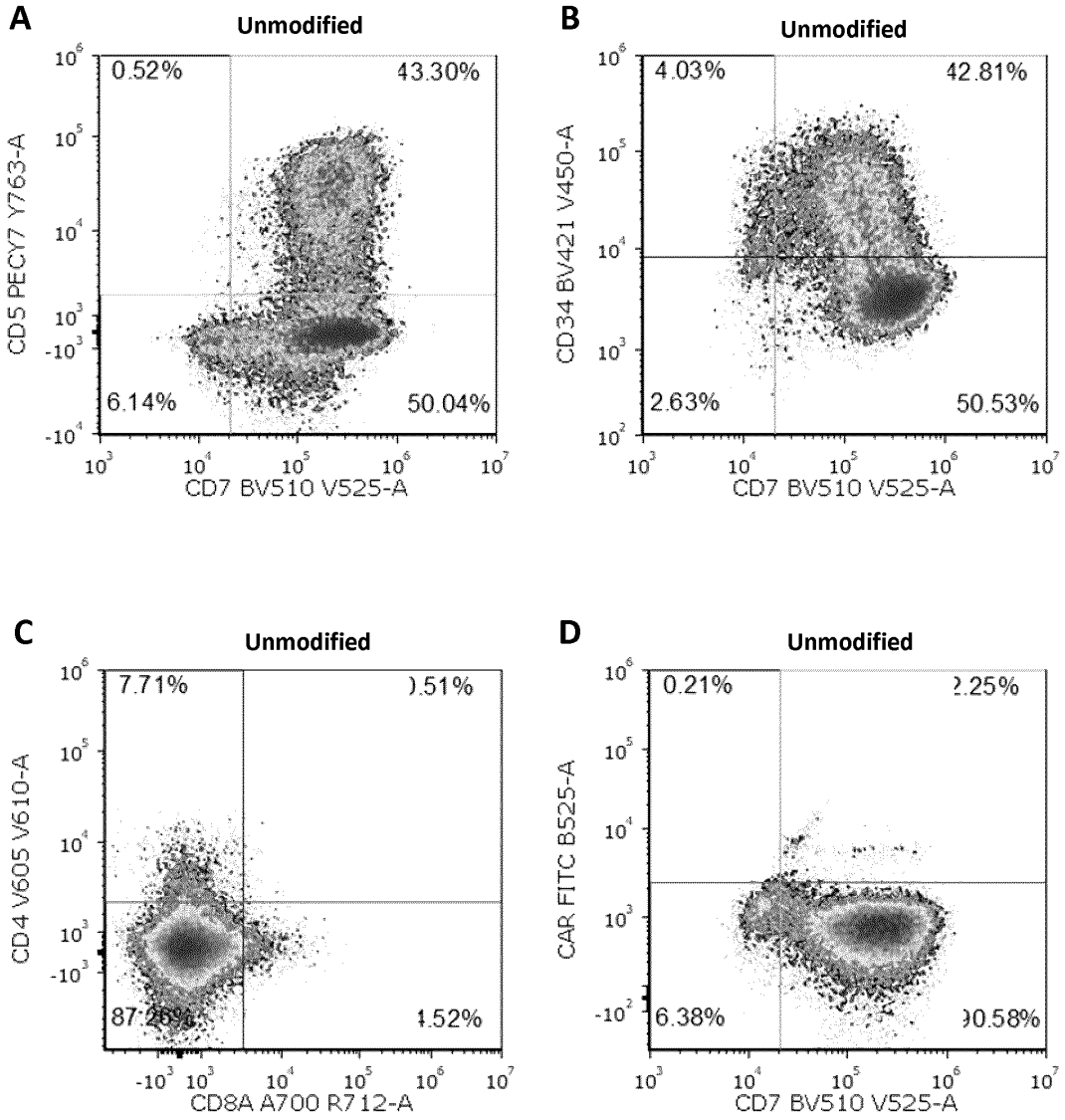

A
Unmodified
0.52%
43.30%
6.14%
50.04%
CD5 PECY7 Y763-A
CD7 BV510 V525-A
B
Unmodified
4.03%
42.81%
2.63%
50.53%
CD34 BV421 V450-A
CD7 BV510 V525-A
C
Unmodified
7.71%
1.52%
CD4 V605 V610-A
CD8A A700 R712-A
D
Unmodified
0.21%
6.38%
CAR FITC B525-A
CD7 BV510 V525-A

FIGURE 19

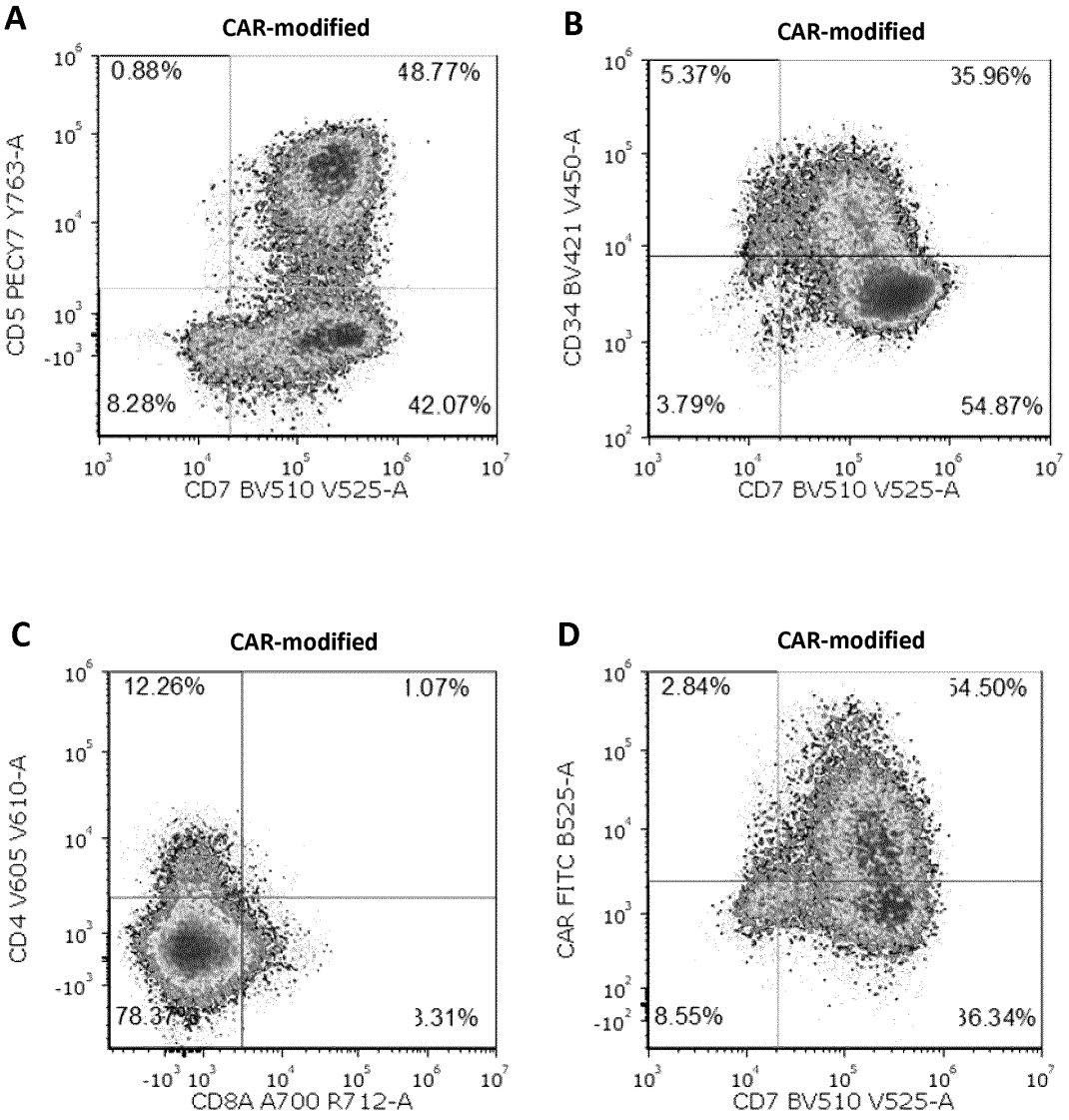

A
CAR-modified
0.88%
48.77%
8.28%
42.07%
CD5 PECY7 Y763-A
CD7 BV510 V525-A
B
CAR-modified
5.37%
35.96%
3.79%
54.87%
CD34 BV421 V450-A
CD7 BV510 V525-A
C
CAR-modified
12.26%
1.07%
78.37%
3.31%
CD4 V605 V610-A
CD8A A700 R712-A
D
CAR-modified
2.84%
54.50%
8.55%
36.34%
CAR FITC B525-A
CD7 BV510 V525-A

FIGURE 20

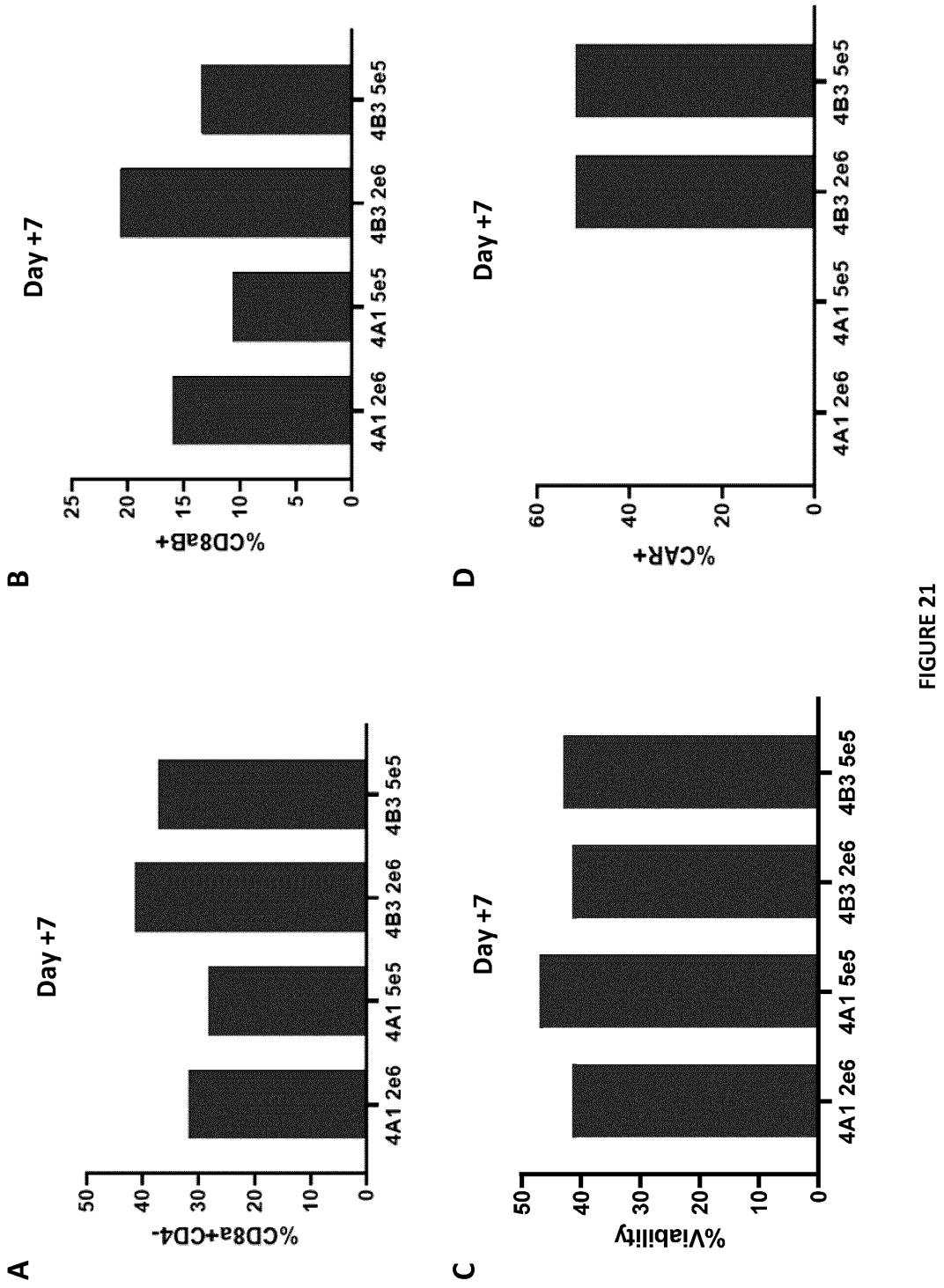

A
Day +7
%CD8a+CD4-
0
10
20
30
40
50
4A1 2e6
4A1 5e5
4B3 2e6
4B3 5e5
B
Day +7
%CD8aB+
0
5
10
15
20
25
4A1 2e6
4A1 5e5
4B3 2e6
4B3 5e5
C
Day +7
%Viability
0
10
20
30
40
50
4A1 2e6
4A1 5e5
4B3 2e6
4B3 5e5
D
Day +7
%CAR+
0
20
40
60
4A1 2e6
4A1 5e5
4B3 2e6
4B3 5e5

FIGURE 21

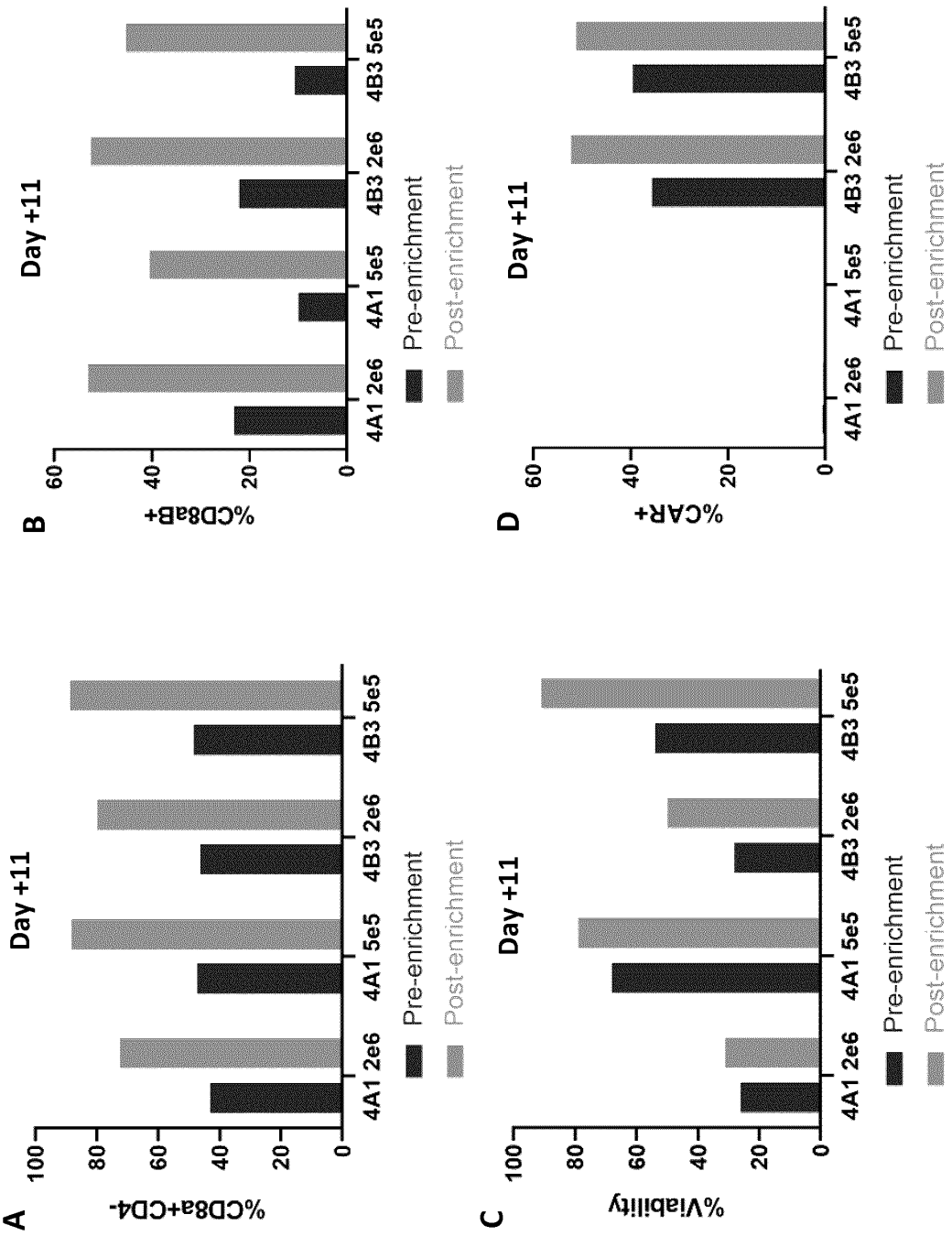

A
Day +11
%CD8a+CD4-
100
80
60
40
20
0
4A1 2e6
4A1 5e5
4B3 2e6
4B3 5e5
Pre-enrichment
Post-enrichment
B
Day +11
%CD8aB+
60
40
20
0
4A1 2e6
4A1 5e5
4B3 2e6
4B3 5e5
Pre-enrichment
Post-enrichment
C
Day +11
%Viability
100
80
60
40
20
0
4A1 2e6
4A1 5e5
4B3 2e6
4B3 5e5
Pre-enrichment
Post-enrichment
D
Day +11
%CAR+
60
40
20
0
4A1 2e6
4A1 5e5
4B3 2e6
4B3 5e5
Pre-enrichment
Post-enrichment

FIGURE 22

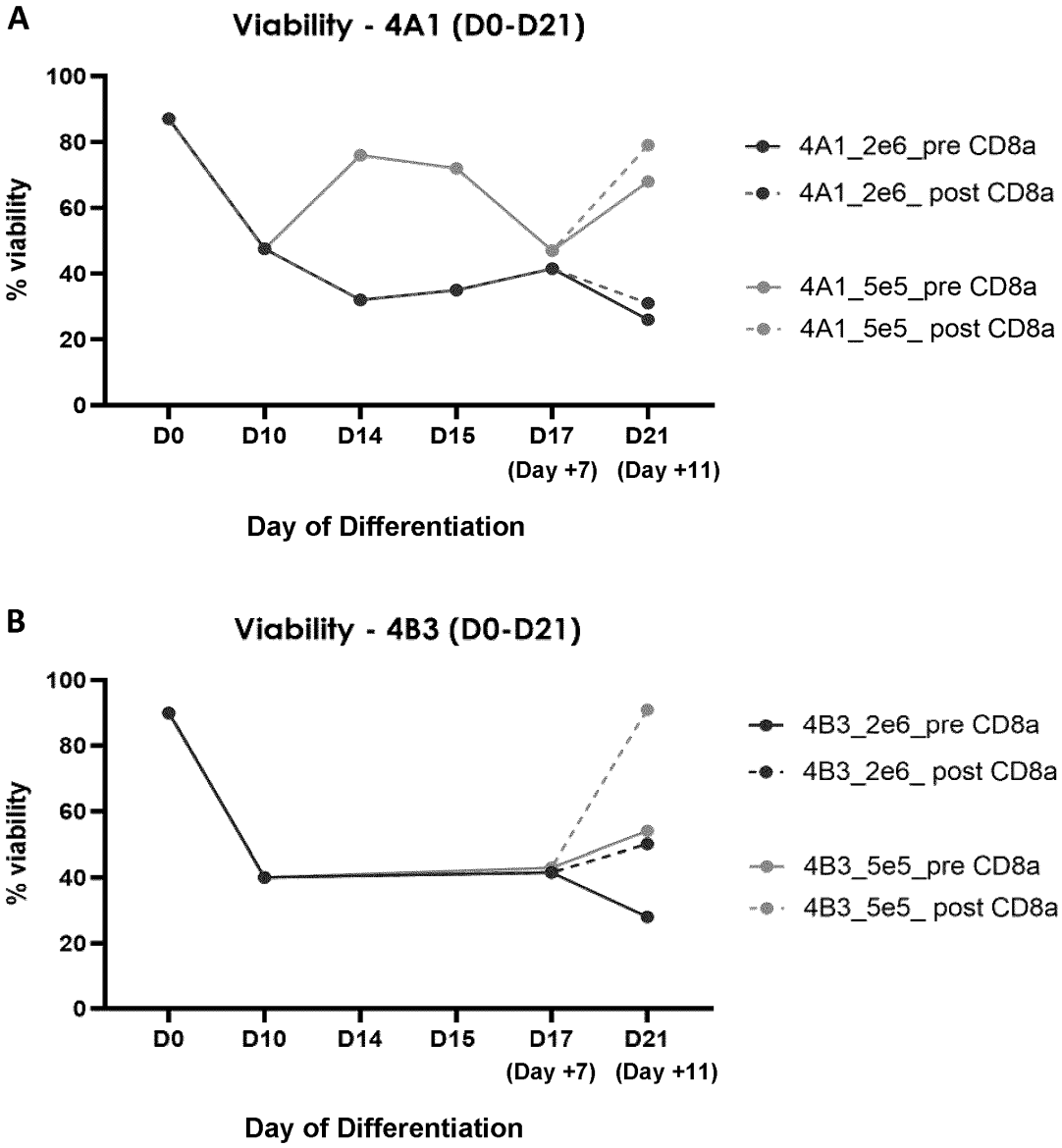

A
Viability - 4A1 (D0-D21)
% viability
100
80
60
40
20
0
D0
D10
D14
D15
D17
(Day +7)
D21
(Day +11)
Day of Differentiation
4A1_2e6_pre CD8a
4A1_2e6_ post CD8a
4A1_5e5_pre CD8a
4A1_5e5_ post CD8a
B
Viability - 4B3 (D0-D21)
% viability
100
80
60
40
20
0
D0
D10
D14
D15
D17
(Day +7)
D21
(Day +11)
Day of Differentiation
4B3_2e6_pre CD8a
4B3_2e6_ post CD8a
4B3_5e5_pre CD8a
4B3_5e5_ post CD8a

FIGURE 23

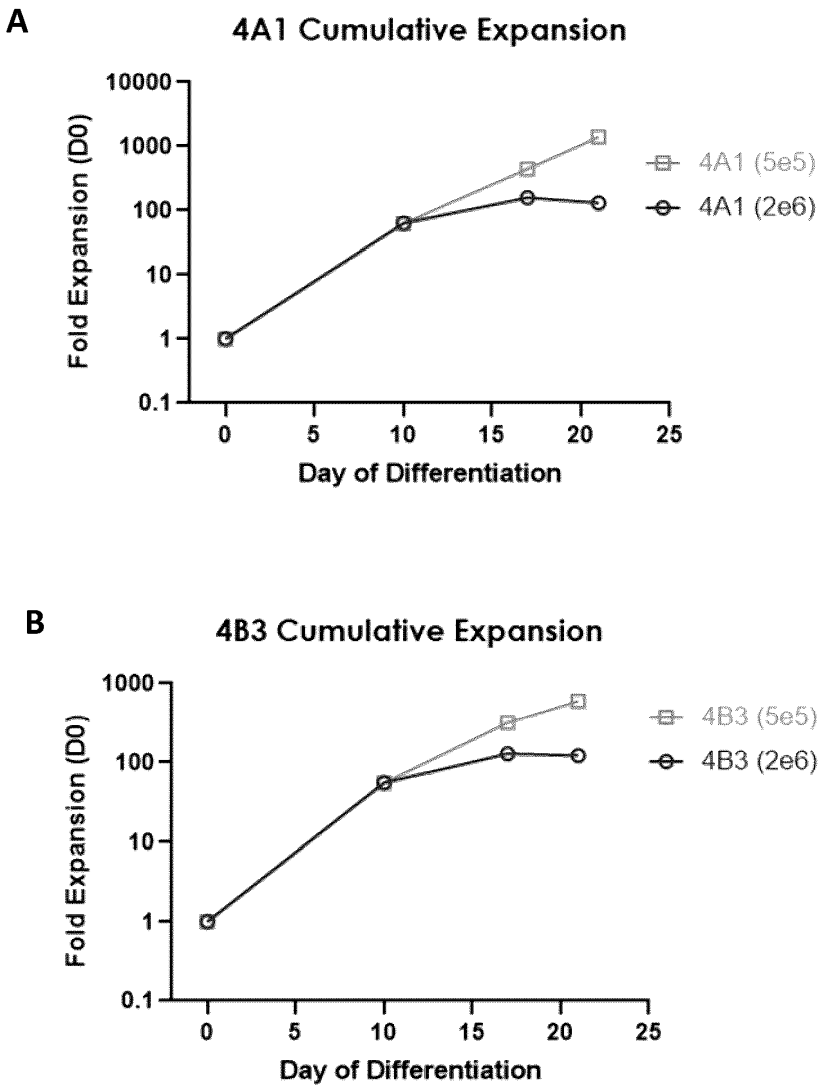

A
4A1 Cumulative Expansion
Fold Expansion (D0)
10000
1000
100
10
1
0.1
0
5
10
15
20
25
Day of Differentiation
4A1 (5e5)
4A1 (2e6)
B
4B3 Cumulative Expansion
Fold Expansion (D0)
1000
100
10
1
0.1
0
5
10
15
20
25
Day of Differentiation
4B3 (5e5)
4B3 (2e6)

FIGURE 24

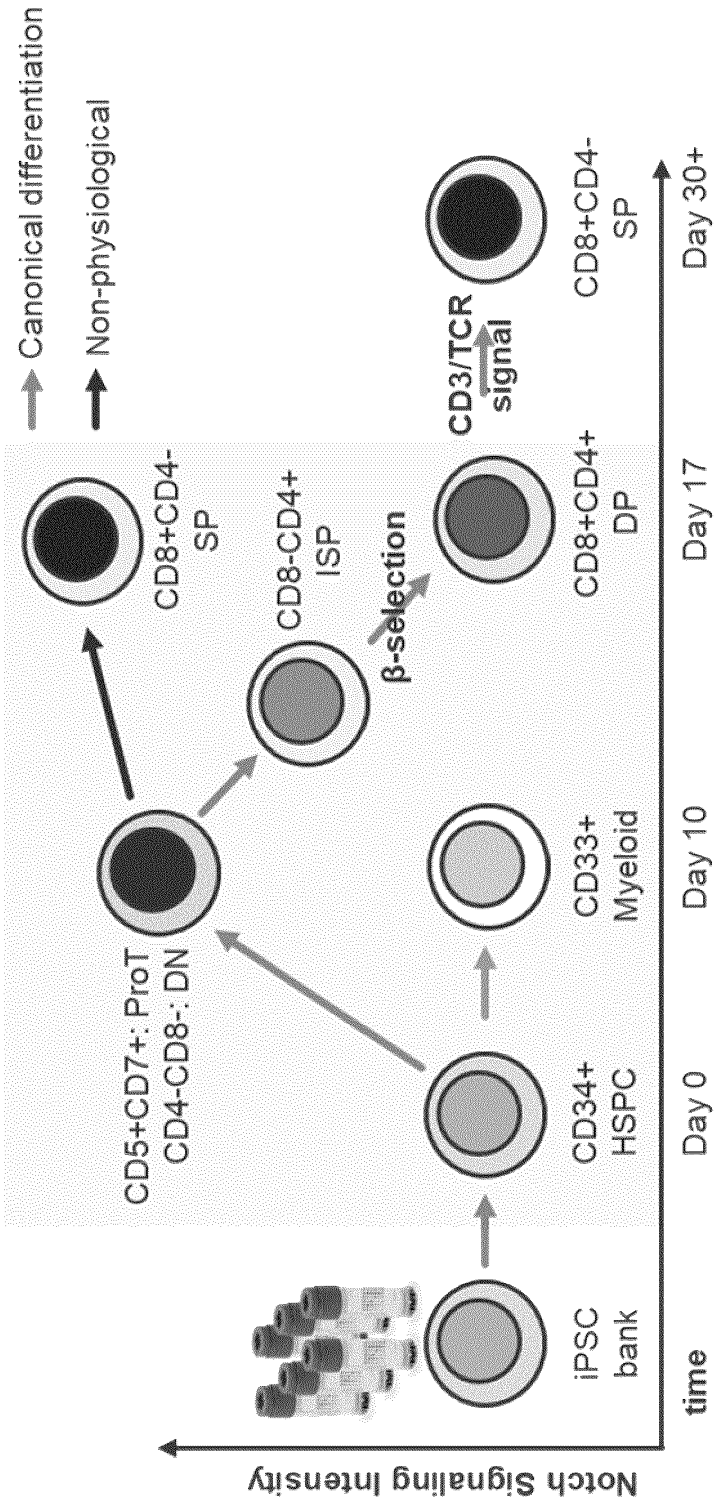

Canonical differentiation
Non-physiological
CD5+CD7+: ProT
CD4-CD8-: DN
CD8+CD4-
SP
CD8-CD4+
ISP
β-selection
CD8+CD4+
DP
CD3/TCR
signal
CD8+CD4-
SP
iPSC
bank
CD34+
HSPC
CD33+
Myeloid
time
Day 0
Day 10
Day 17
Day 30+
Notch Signaling Intensity

FIGURE 25

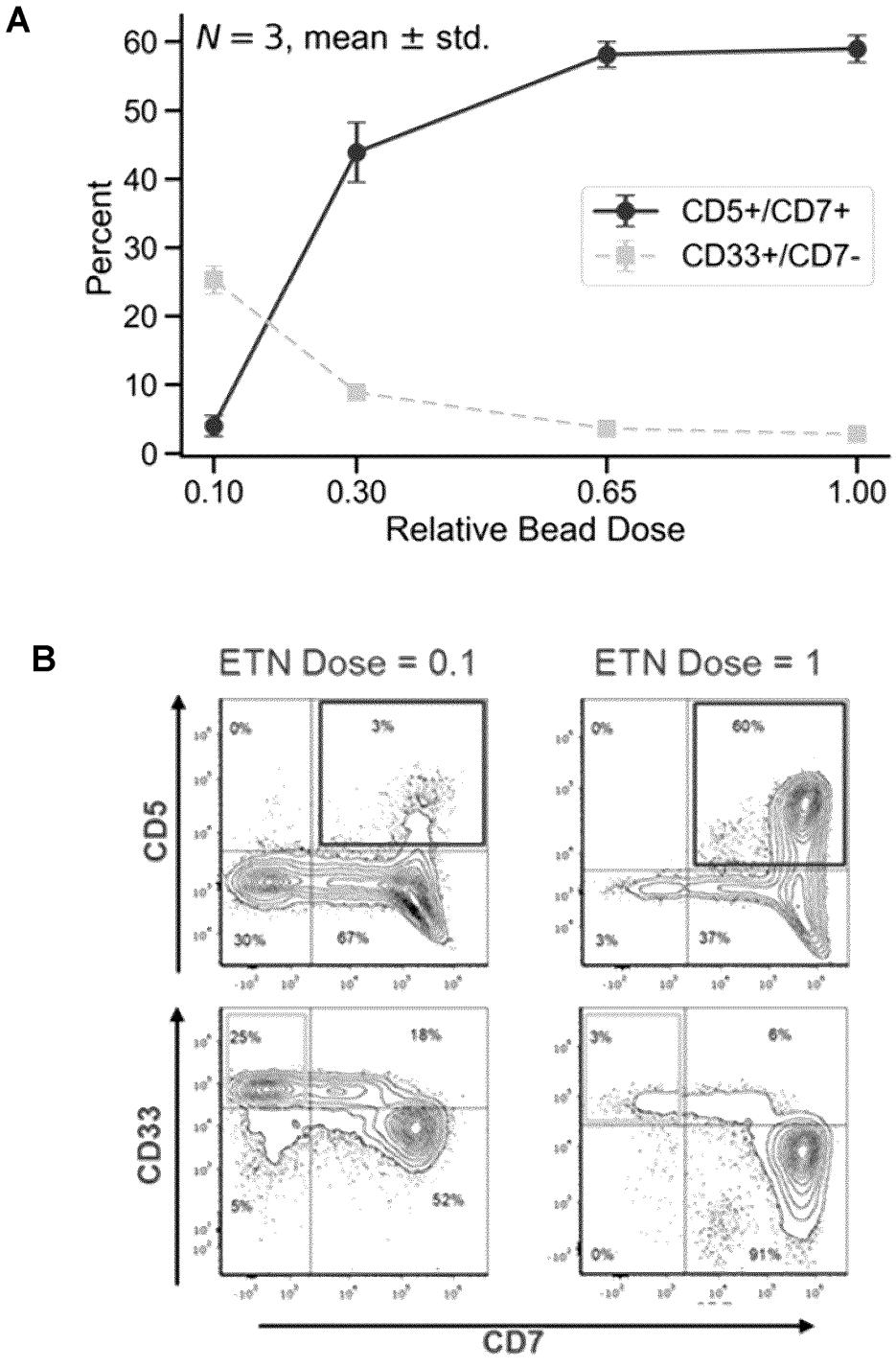

A
N = 3, mean ± std.
Percent
60
50
40
30
20
10
0
0.10
0.30
0.65
1.00
Relative Bead Dose
CD5+/CD7+
CD33+/CD7-
B
ETN Dose = 0.1
ETN Dose = 1
CD5
CD33
CD7
0%
3%
30%
67%
0%
60%
3%
37%
25%
18%
5%
52%
3%
6%
0%
91%

FIGURE 26

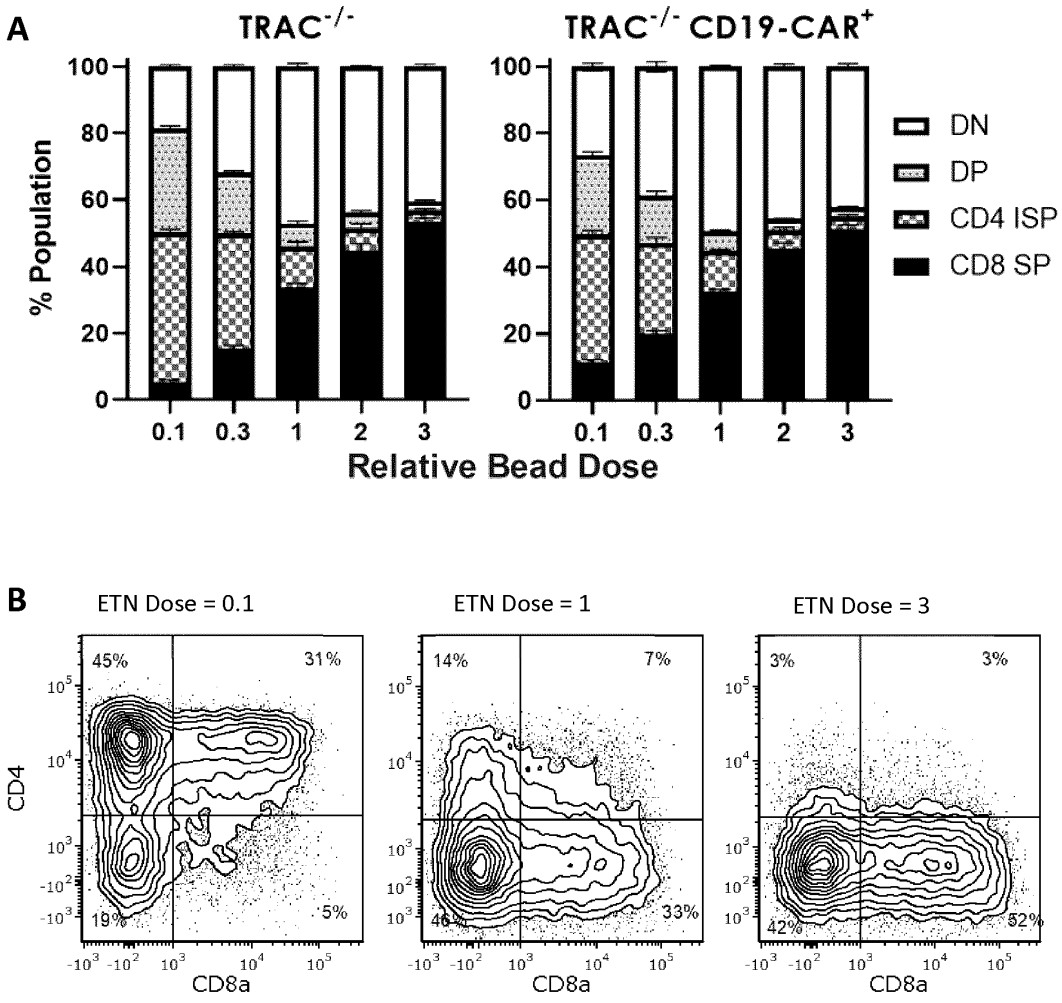

A
TRAC-/-
TRAC-/- CD19-CAR+
% Population
100
80
60
40
20
0
0.1
0.3
1
2
3
Relative Bead Dose
DN
DP
CD4 ISP
CD8 SP
B
ETN Dose = 0.1
ETN Dose = 1
ETN Dose = 3
CD4
CD8a
45%
31%
19%
5%
14%
7%
46%
33%
3%
3%
42%
52%

FIGURE 27

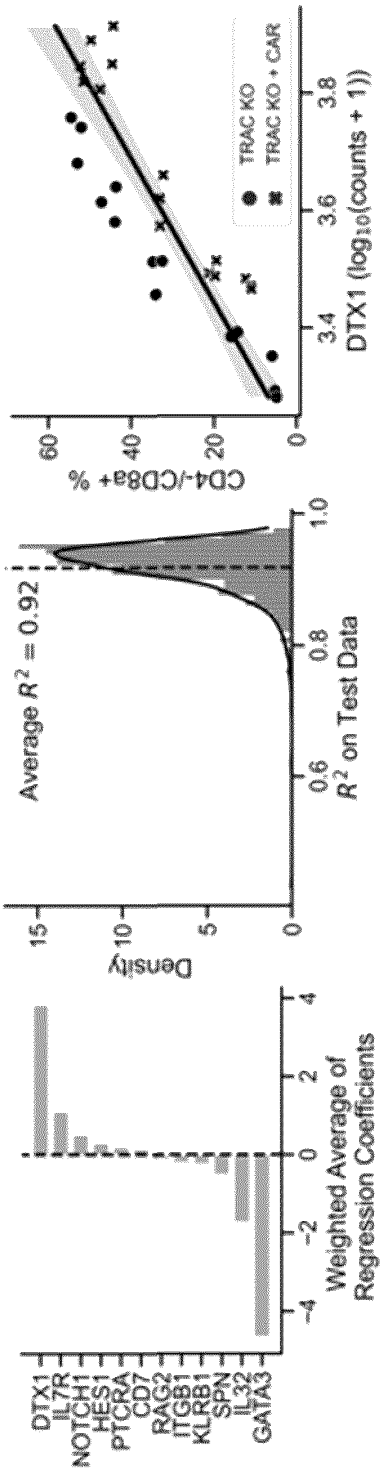



FIGURE 28A




FIGURE 28B




FIGURE 28C

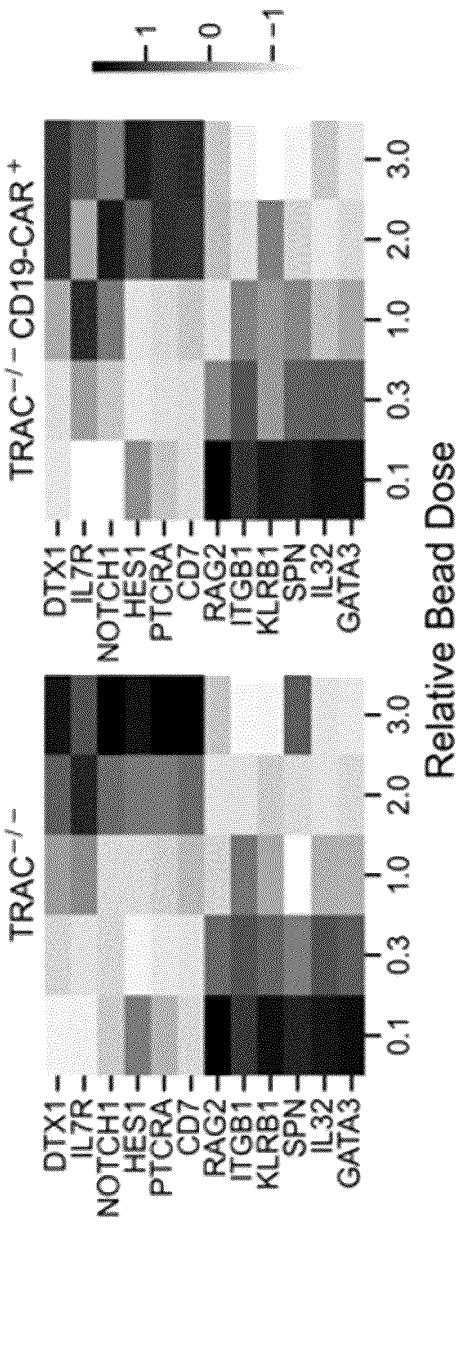



FIGURE 28D

FIGURE 28E

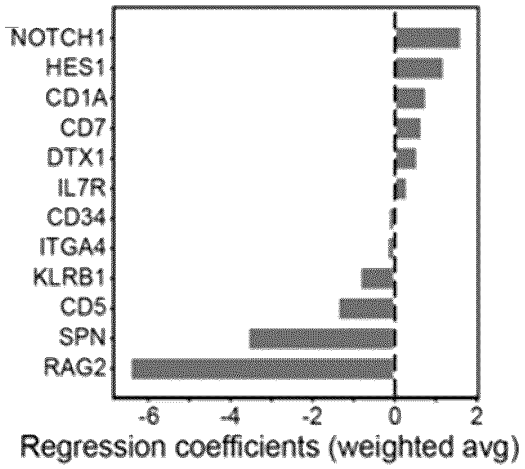
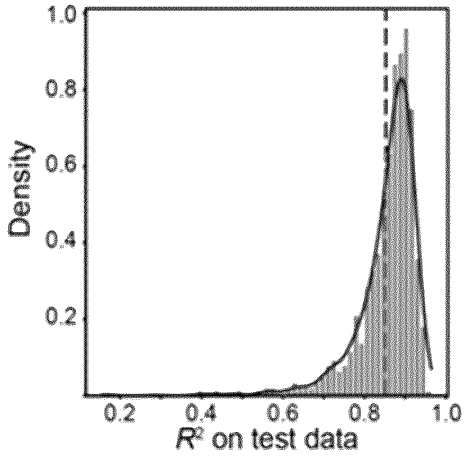



FIGURE 28F



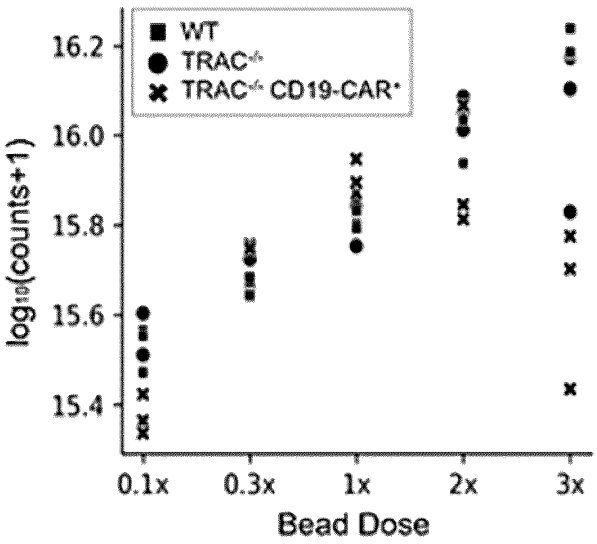




FIGURE 28G

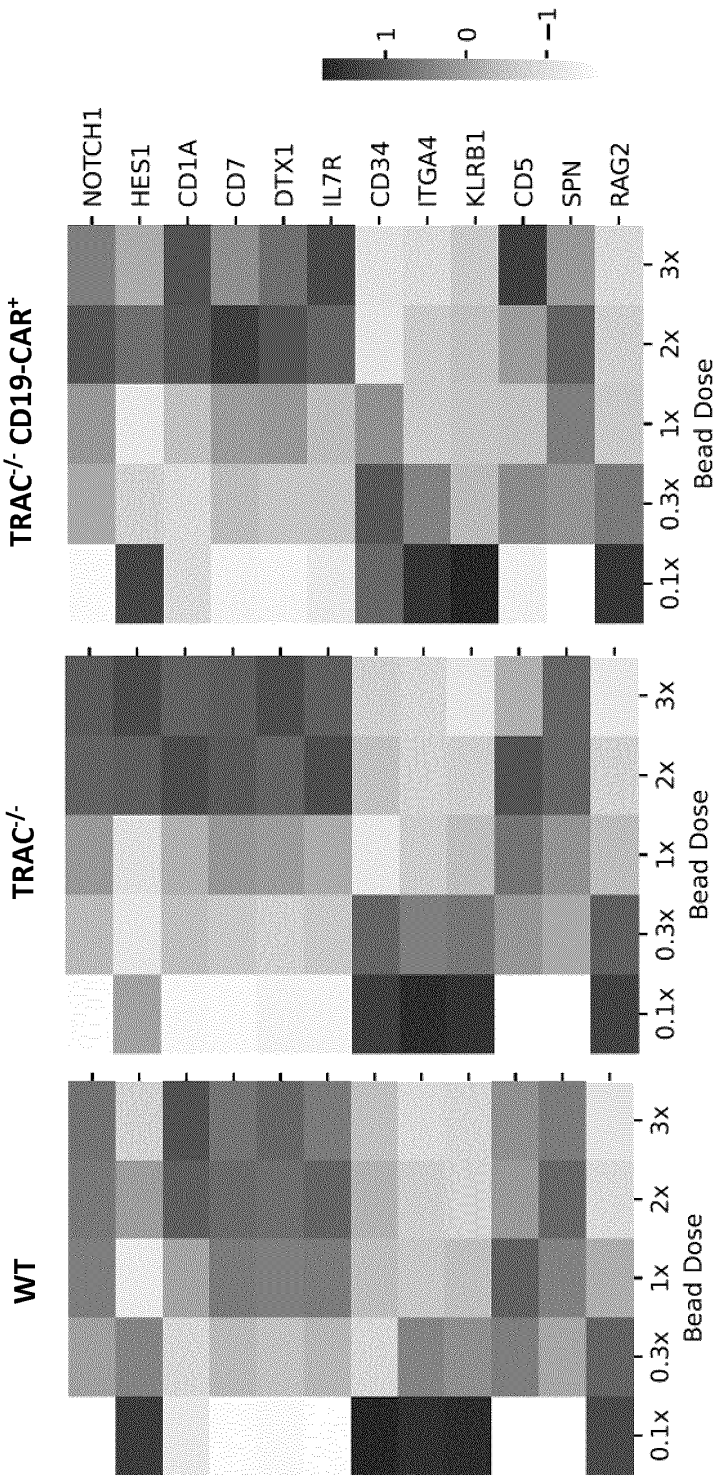
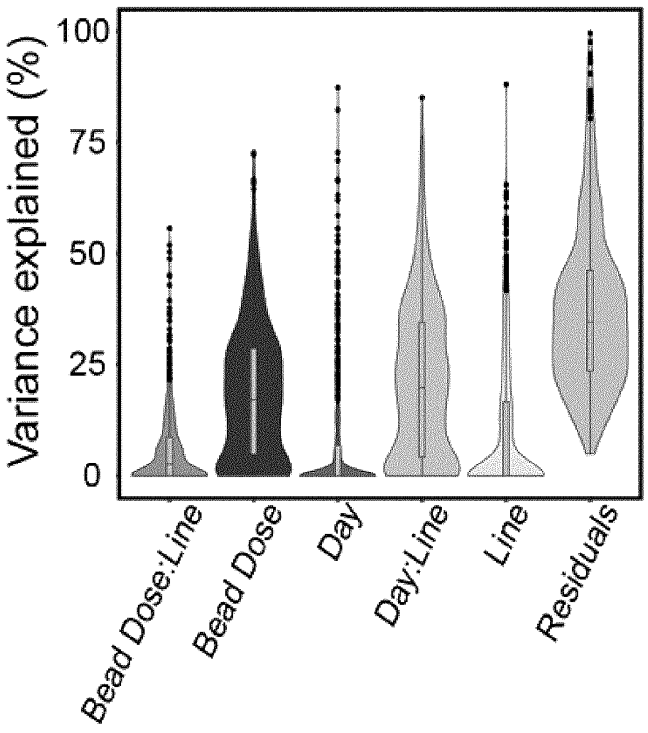

1
0
−1
NOTCH1
HES1
CD1A
CD7
DTX1
IL7R
CD34
ITGA4
KLRB1
CD5
SPN
RAG2
TRAC-/- CD19-CAR+
TRAC-/-
WT
0.1x
0.3x
1x
2x
3x
Bead Dose

FIGURE 28H

FIGURE 28I



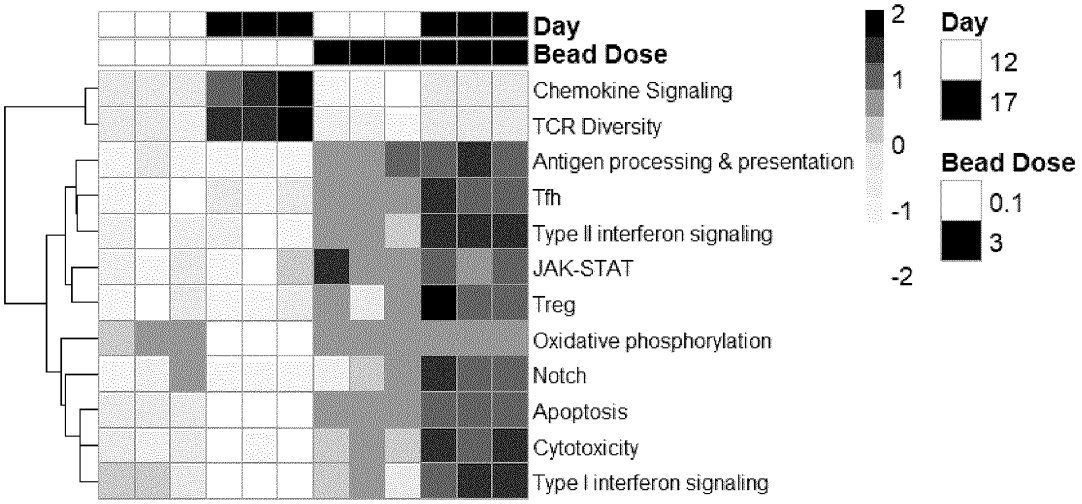
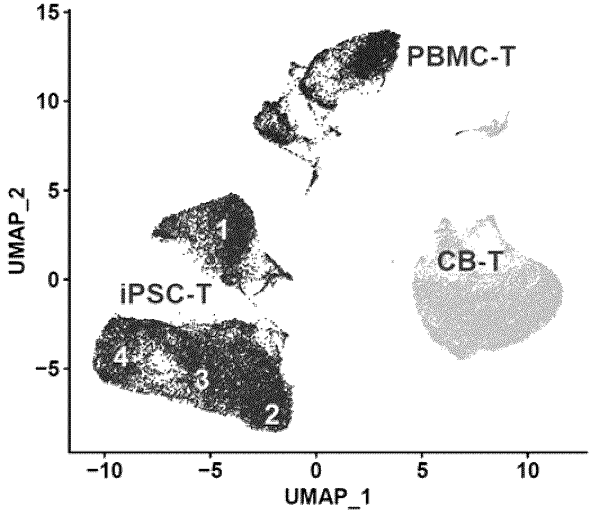




FIGURE 28J

FIGURE 29A



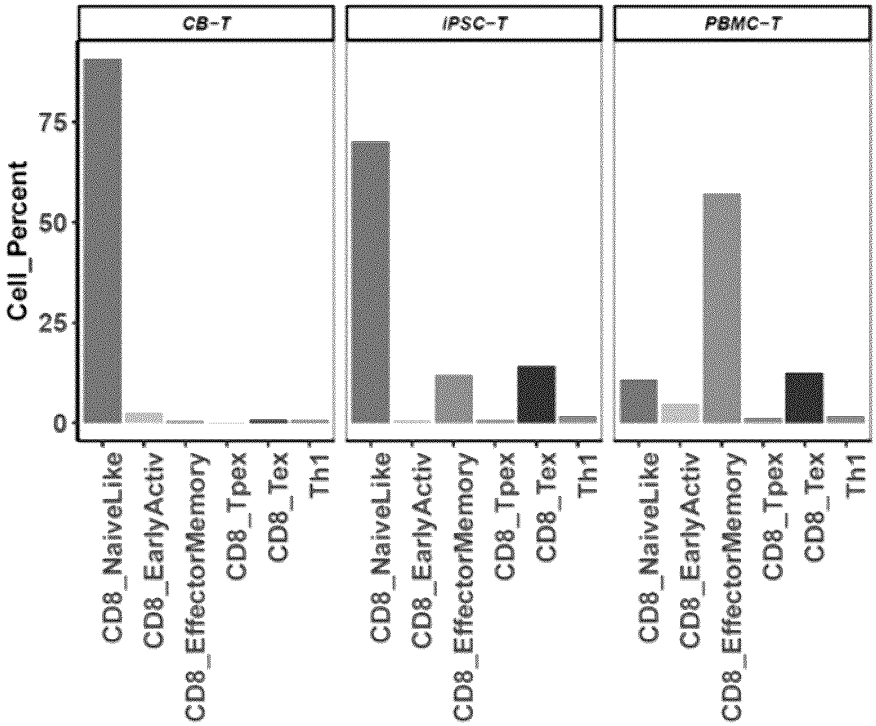
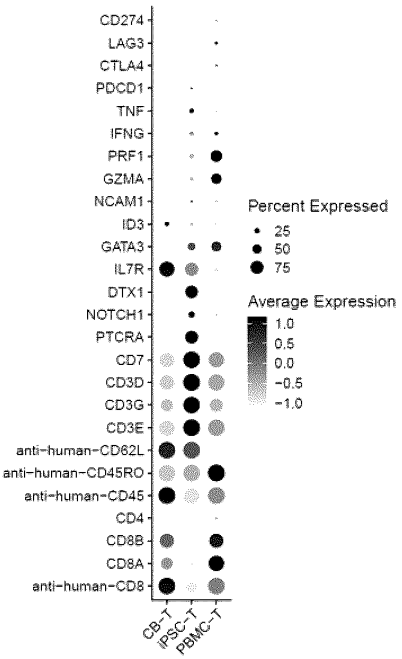




FIGURE 29B

FIGURE 29C



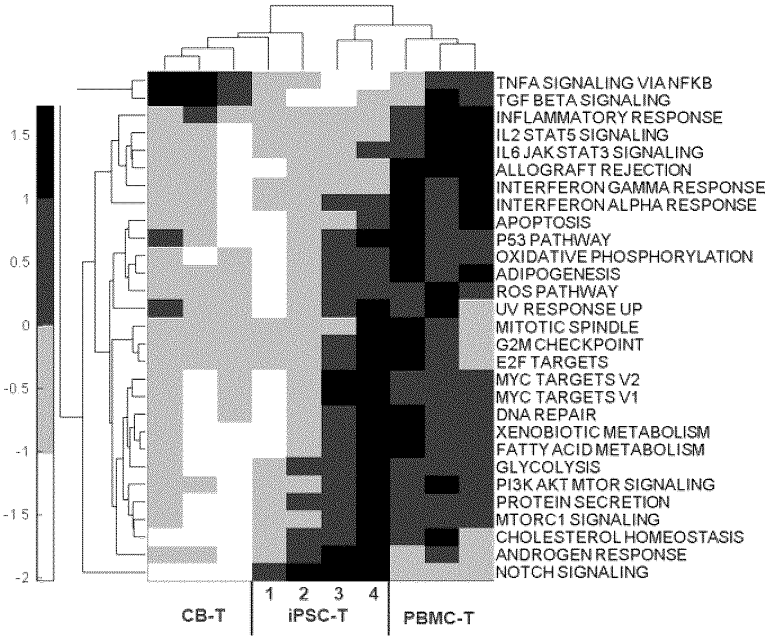




FIGURE 29D

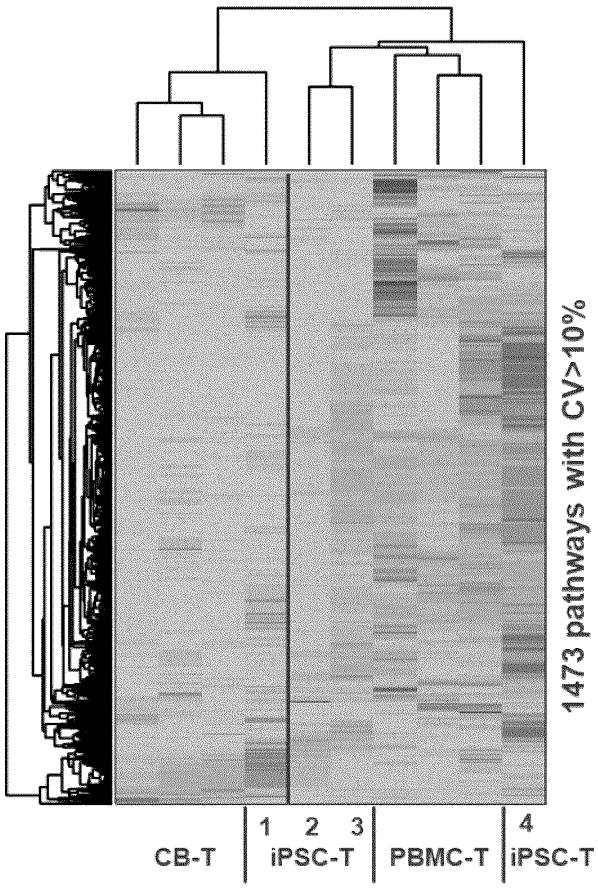



FIGURE 29E

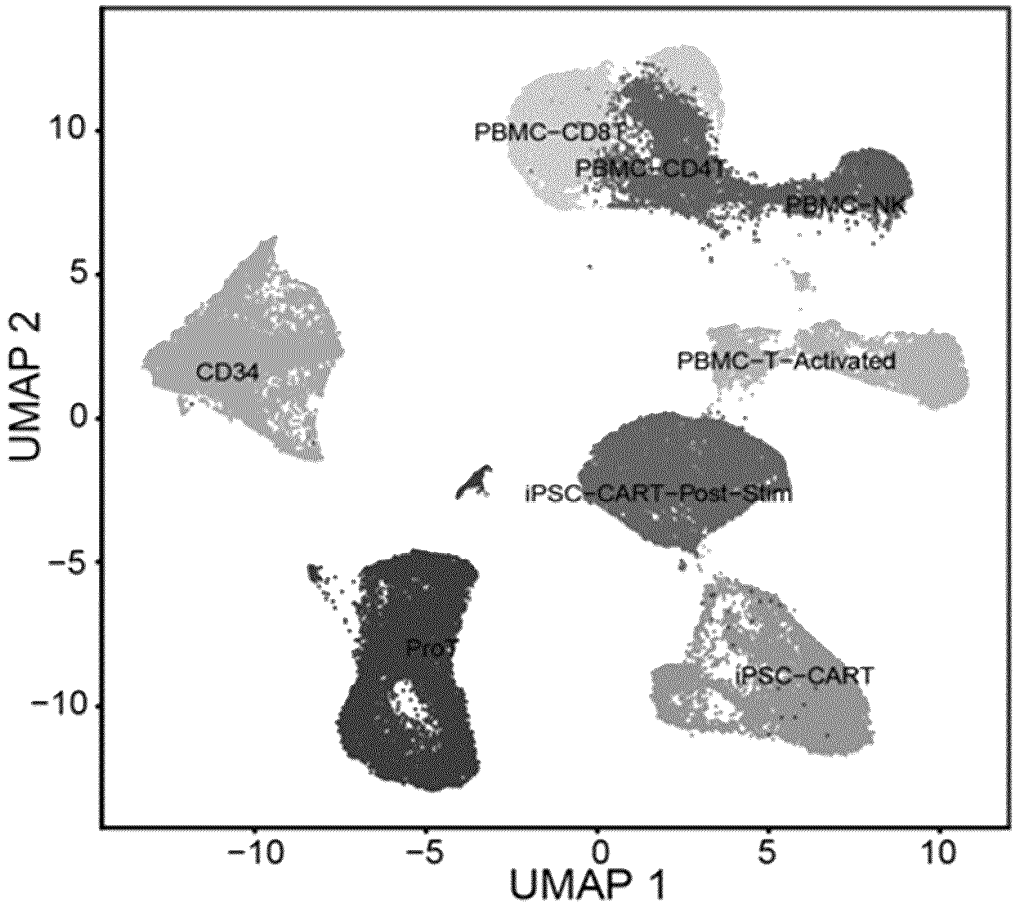

PBMC-CD8T
PBMC-CD4T
PBMC-NK
CD34
PBMC-T-Activated
iPSC-CART-Post-Stim
ProT
iPSC-CART
UMAP 2
UMAP 1
10
5
0
−5
−10
−10
−5
0
5
10

FIGURE 29F

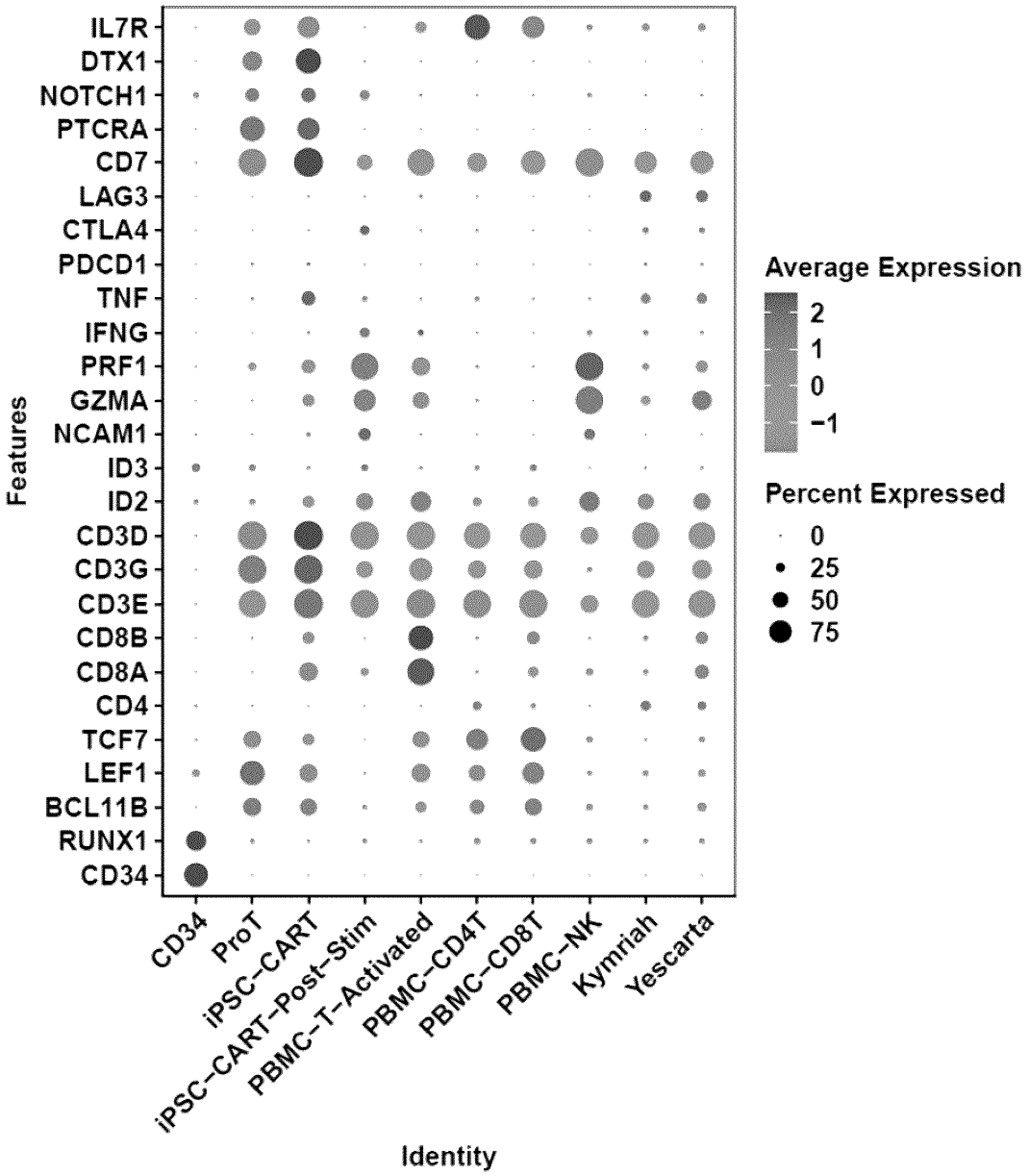

IL7R
DTX1
NOTCH1
PTCRA
CD7
LAG3
CTLA4
PDCD1
TNF
IFNG
PRF1
GZMA
NCAM1
ID3
ID2
CD3D
CD3G
CD3E
CD8B
CD8A
CD4
TCF7
LEF1
BCL11B
RUNX1
CD34
Features
CD34
ProT
iPSC-CART
iPSC-CART-Post-Stim
PBMC-T-Activated
PBMC-CD4T
PBMC-CD8T
PBMC-NK
Kymriah
Yescarta
Identity
Average Expression
2
1
0
−1
Percent Expressed
0
25
50
75

FIGURE 29G

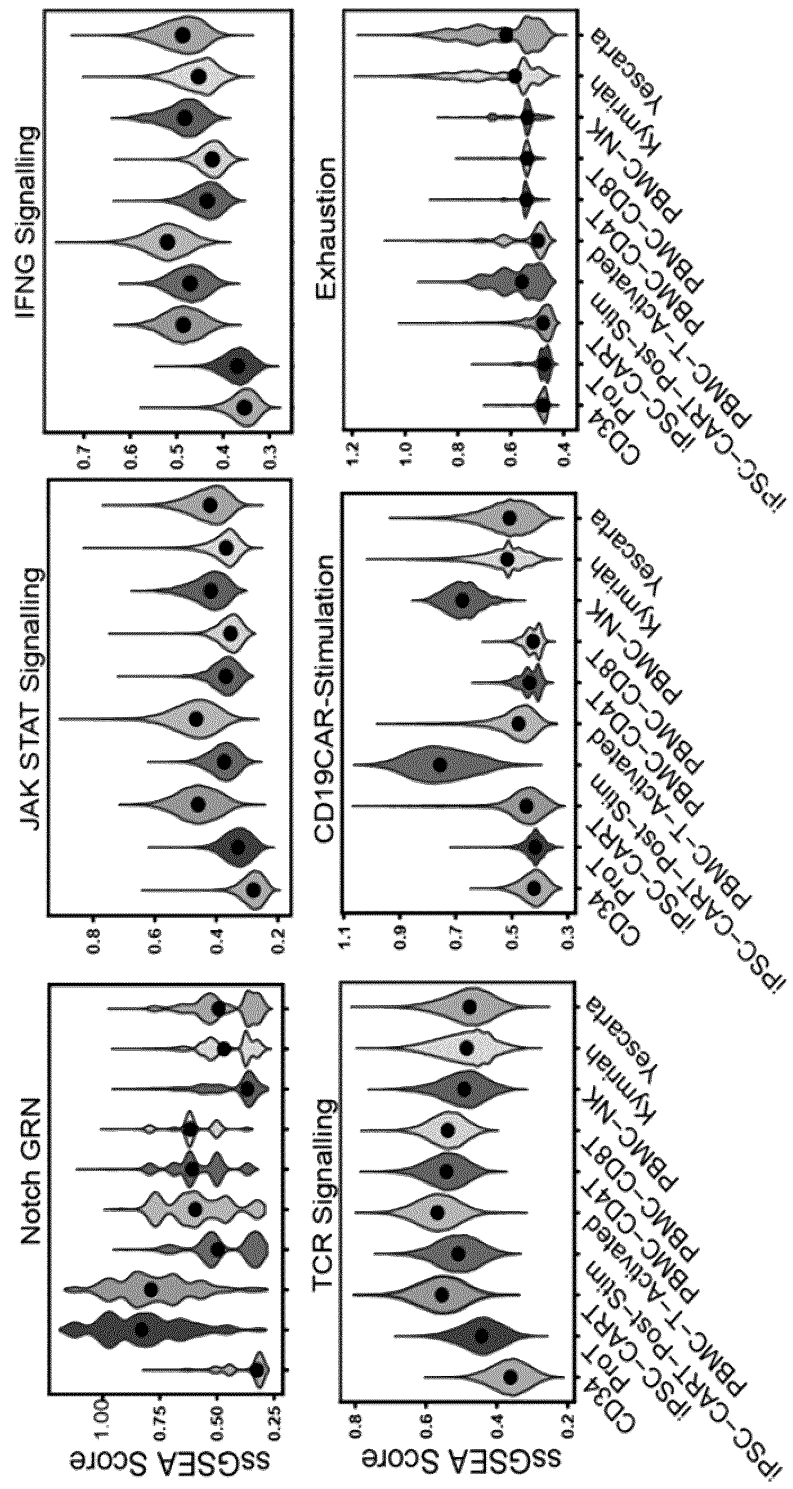

Notch GRN
JAK STAT Signalling
IFNG Signalling
TCR Signalling
CD19CAR-Stimulation
Exhaustion
ssGSEA Score
CD34
ProT
iPSC-CART
iPSC-CART-Post-Stim
PBMC-T-Activated
PBMC-CD4T
PBMC-CD8T
PBMC-NK
Kymriah
Yescarta

FIGURE 29H

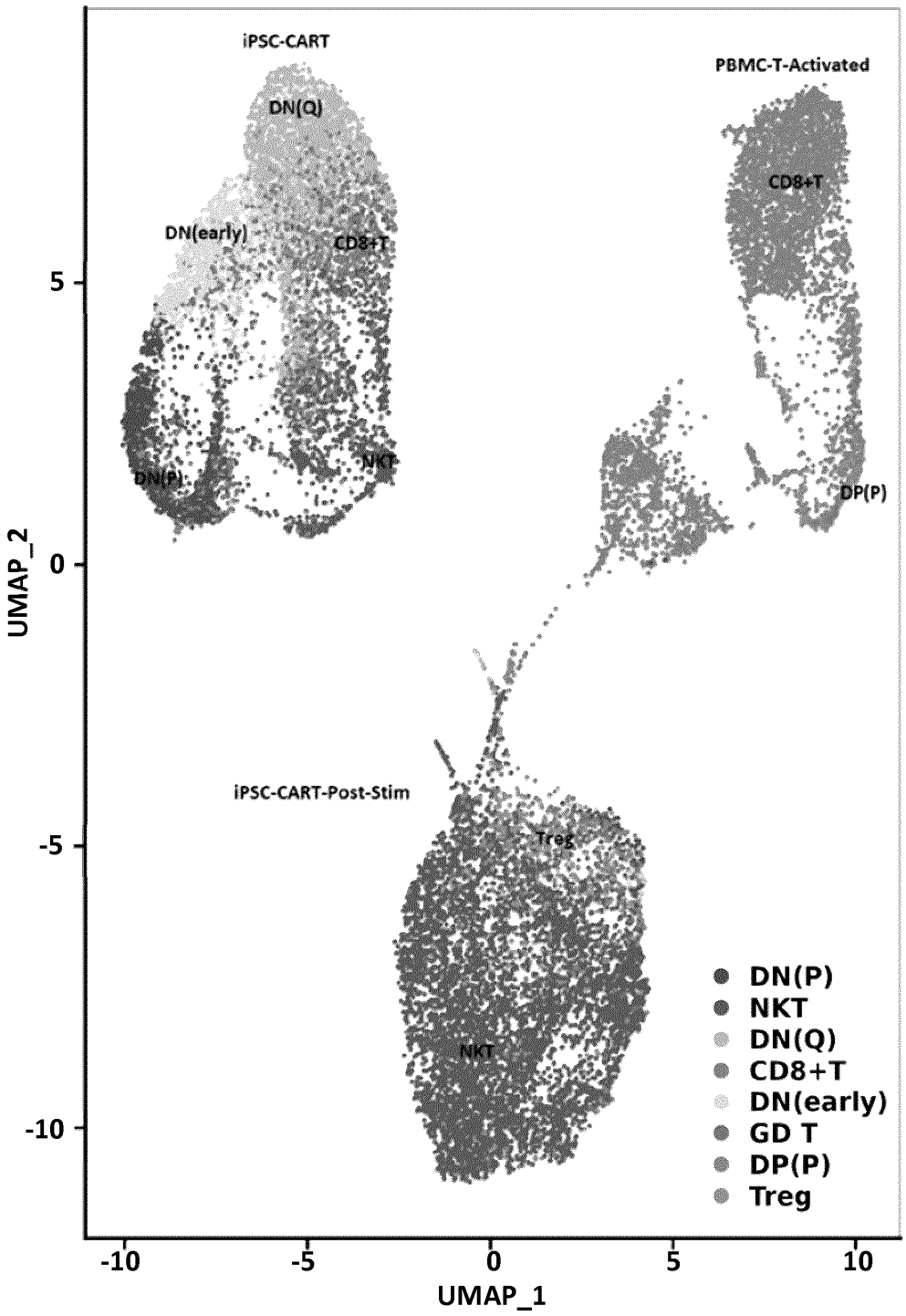
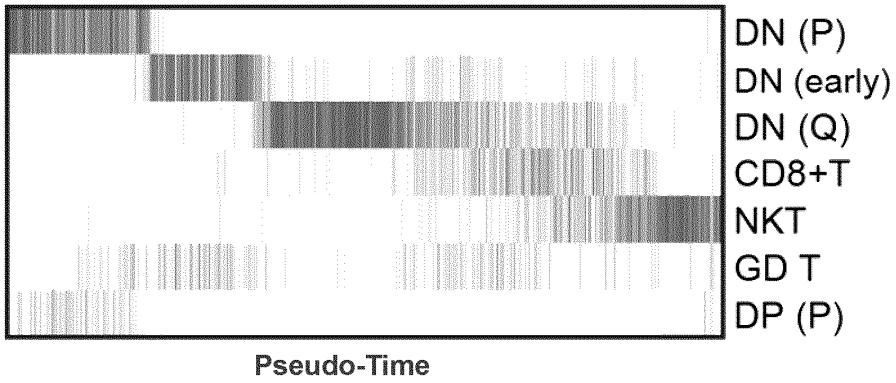

iPSC-CART
DN(Q)
DN(early)
CD8+T
DN(P)
NKT
PBMC-T-Activated
CD8+T
DP(P)
iPSC-CART-Post-Stim
Treg
NKT
DN(P)
NKT
DN(Q)
CD8+T
DN(early)
GD T
DP(P)
Treg
UMAP_2
5
0
-5
-10
-10
-5
0
5
10
UMAP_1

FIGURE 29I

FIGURE 29J



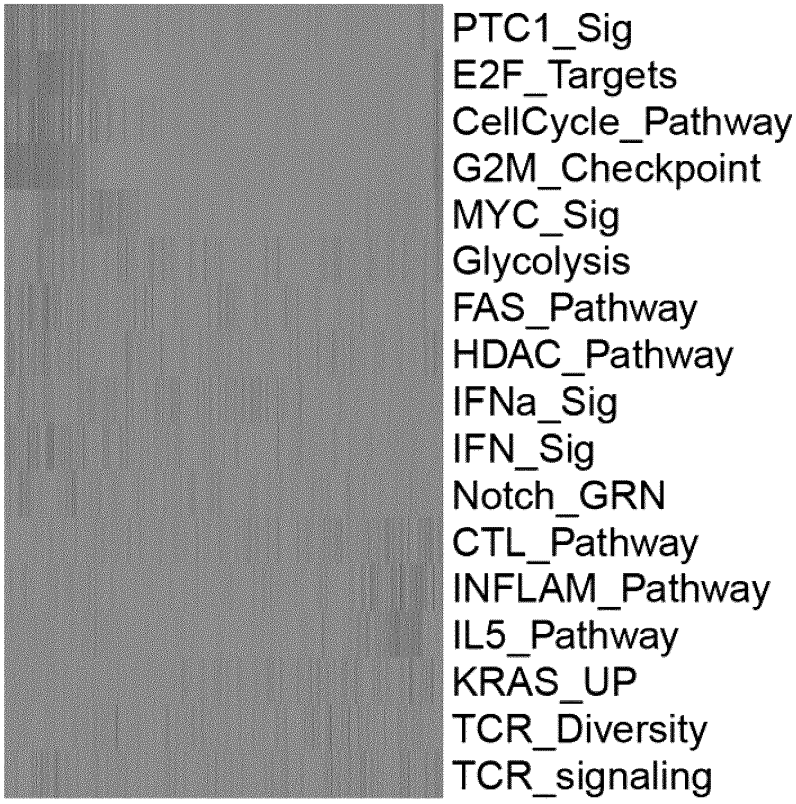




FIGURE 29K

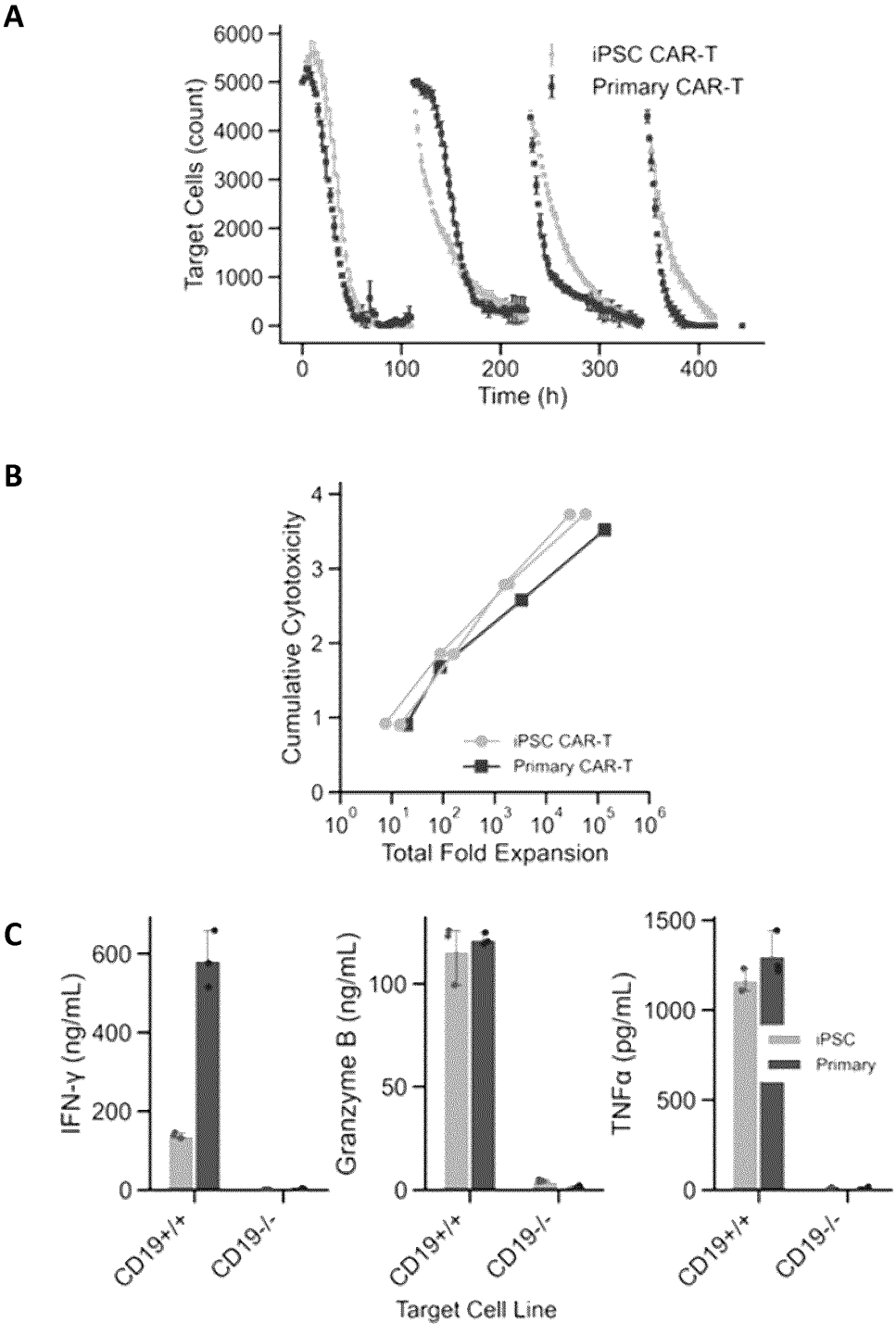
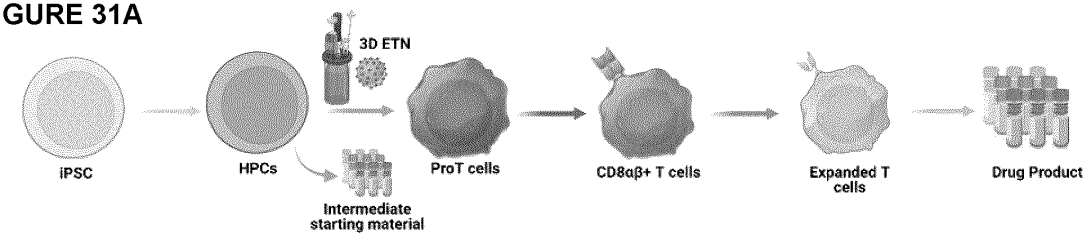

A
Target Cells (count)
Time (h)
iPSC CAR-T
Primary CAR-T
B
Cumulative Cytotoxicity
Total Fold Expansion
iPSC CAR-T
Primary CAR-T
C
IFN-γ (ng/mL)
Granzyme B (ng/mL)
TNFα (pg/mL)
CD19+/+
CD19-/-
Target Cell Line
iPSC
Primary

FIGURE 30

FIGURE 31A



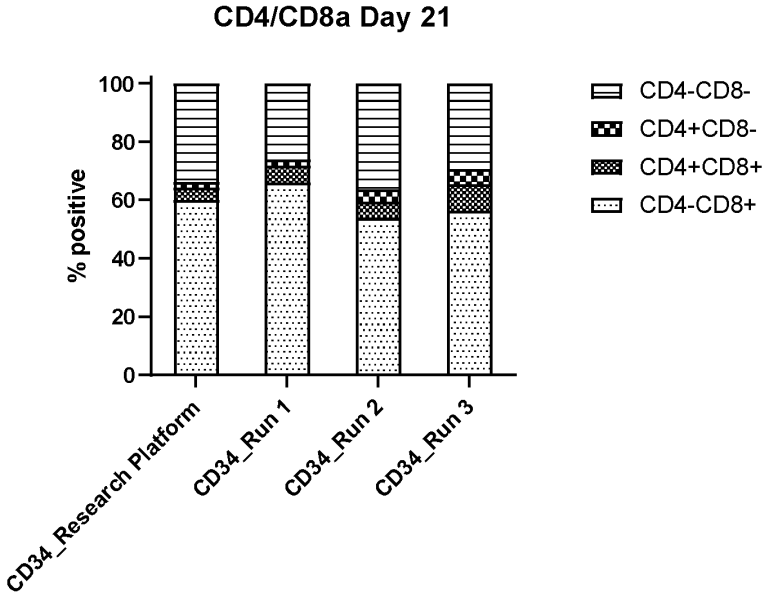




FIGURE 31B

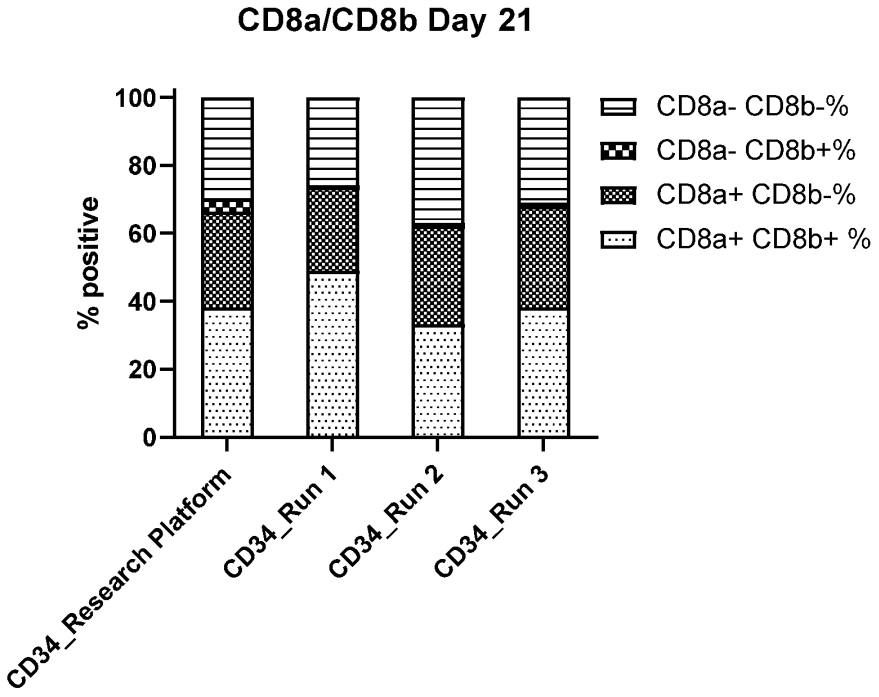
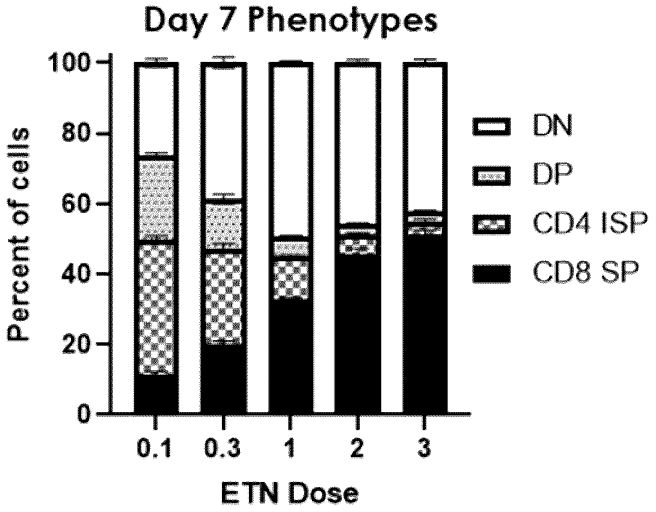

CD8a/CD8b Day 21
% positive
100
80
60
40
20
0
CD8a- CD8b-%
CD8a- CD8b+%
CD8a+ CD8b-%
CD8a+ CD8b+ %
CD34_Research Platform
CD34_Run 1
CD34_Run 2
CD34_Run 3

FIGURE 31C

A


Day 7 Phenotypes
Percent of cells
100
80
60
40
20
0
0.1
0.3
1
2
3
ETN Dose
DN
DP
CD4 ISP
CD8 SP

B

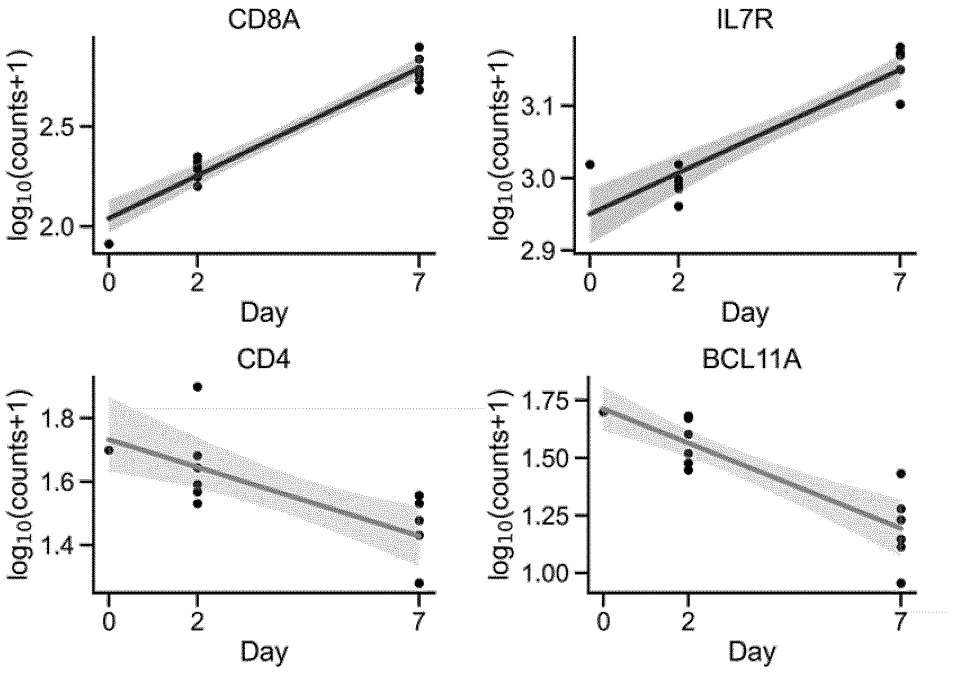

CD8A
IL7R
CD4
BCL11A
log10(counts+1)
Day
0
2
7
2.0
2.5
2.9
3.0
3.1
1.4
1.6
1.8
1.00
1.25
1.50
1.75

FIGURE 32

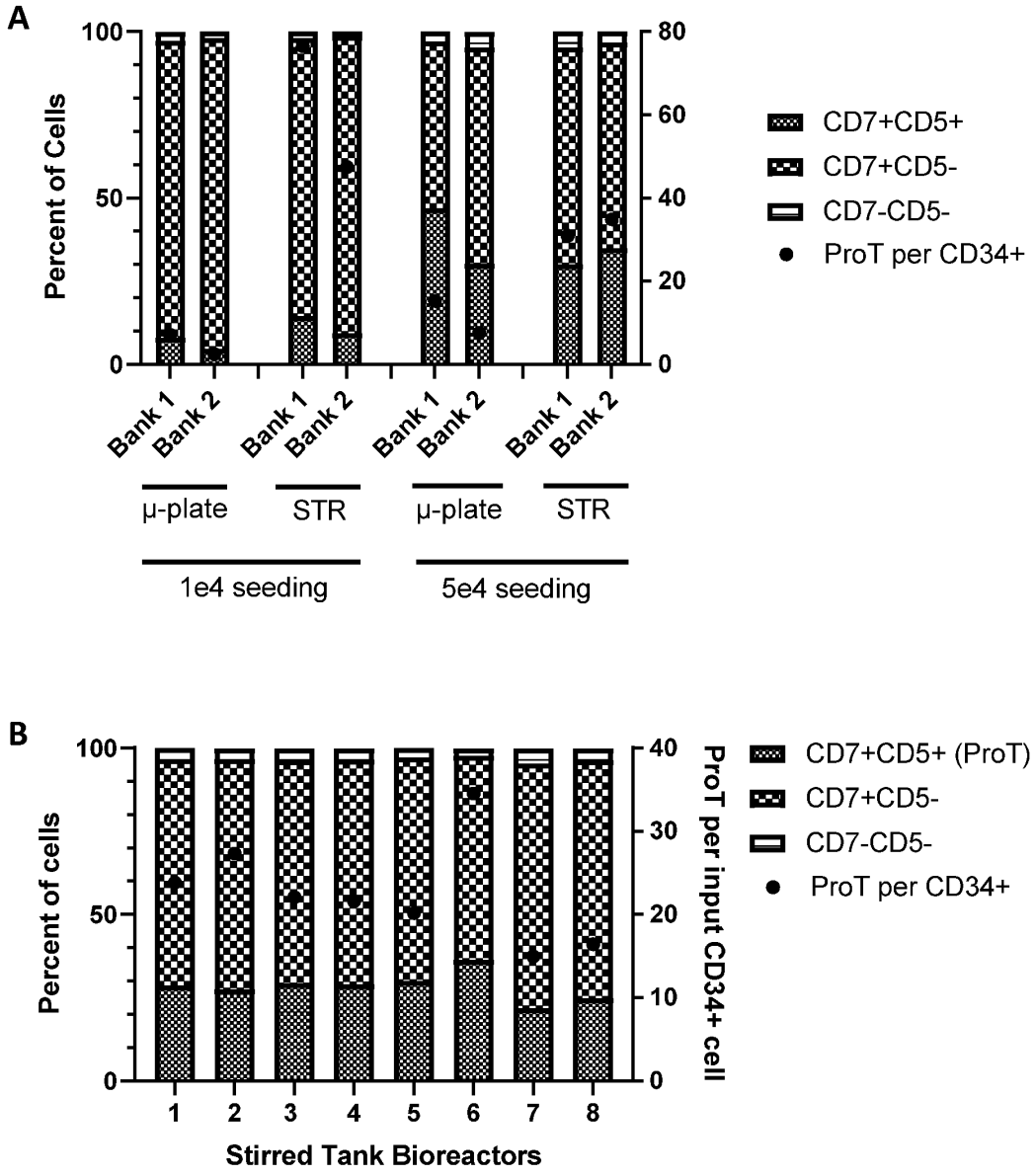

A
Percent of Cells
100
50
0
80
60
40
20
0
Bank 1
Bank 2
Bank 1
Bank 2
Bank 1
Bank 2
Bank 1
Bank 2
µ-plate
STR
µ-plate
STR
1e4 seeding
5e4 seeding
CD7+CD5+
CD7+CD5-
CD7-CD5-
ProT per CD34+
B
Percent of cells
100
50
0
40
30
20
10
0
ProT per input CD34+ cell
1
2
3
4
5
6
7
8
Stirred Tank Bioreactors
CD7+CD5+ (ProT)
CD7+CD5-
CD7-CD5-
ProT per CD34+

FIGURE 33

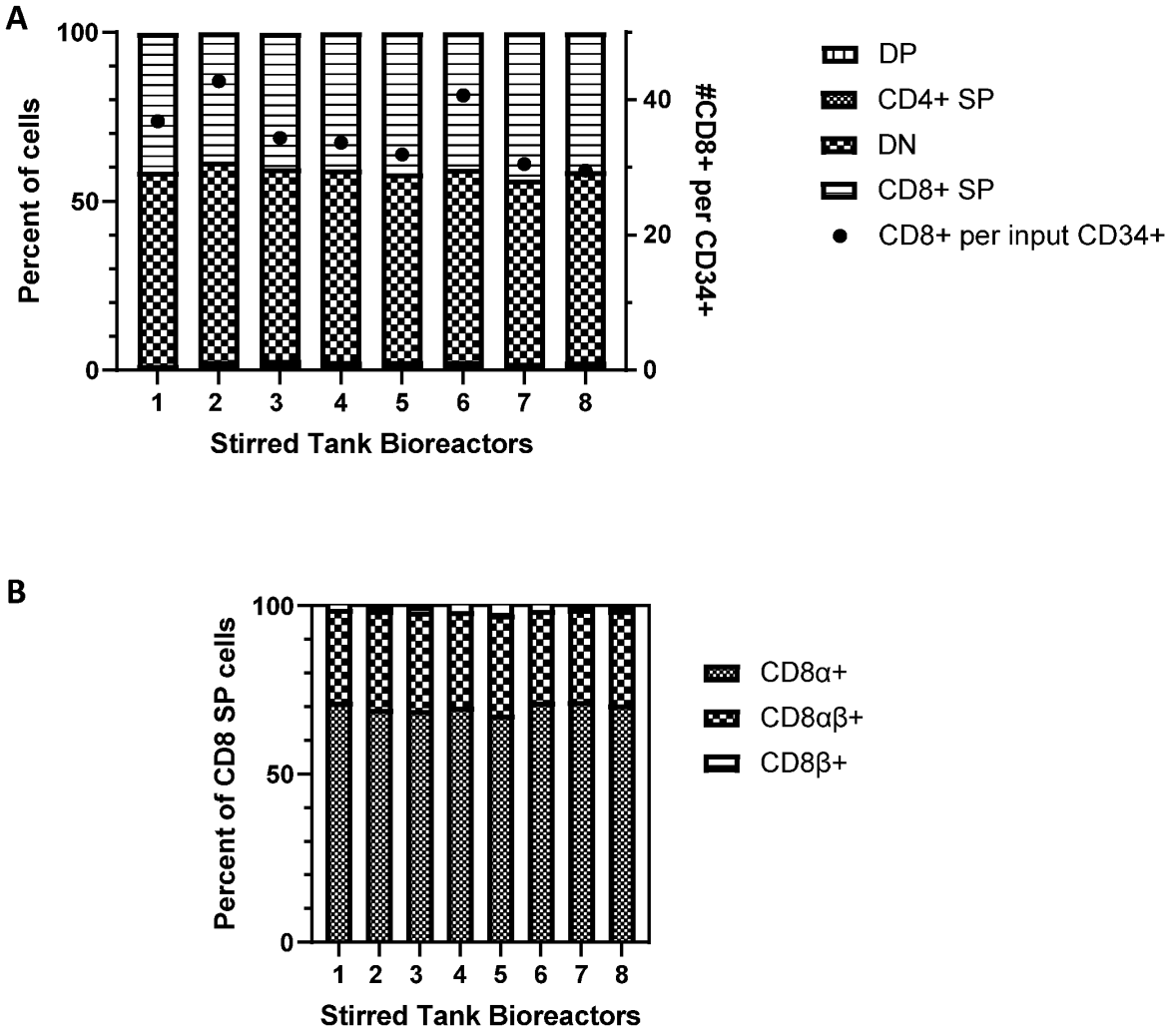

A
100
50
0
Percent of cells
40
20
0
#CD8+ per CD34+
1 2 3 4 5 6 7 8
Stirred Tank Bioreactors
DP
CD4+ SP
DN
CD8+ SP
CD8+ per input CD34+
B
100
50
0
Percent of CD8 SP cells
1 2 3 4 5 6 7 8
Stirred Tank Bioreactors
CD8α+
CD8αβ+
CD8β+

FIGURE 34

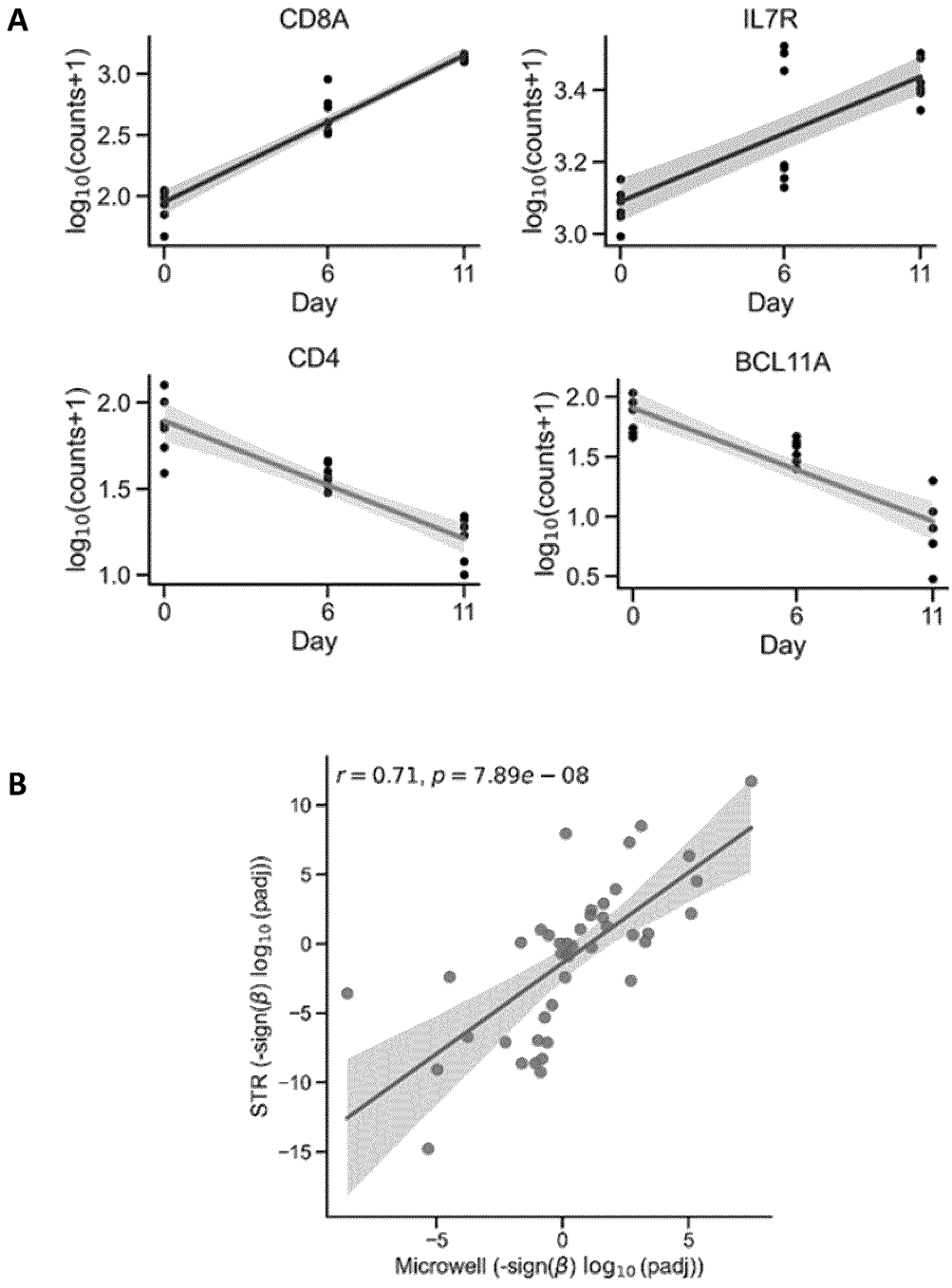
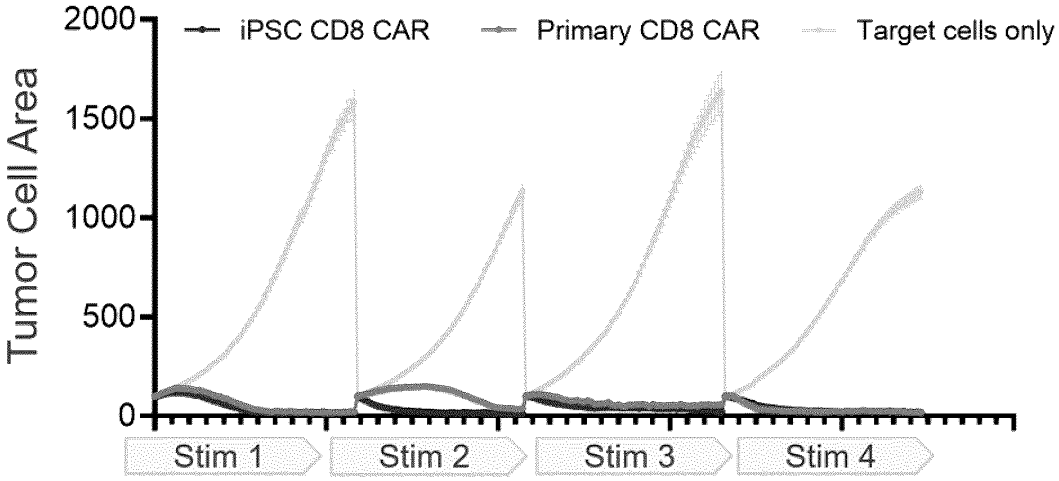

A
CD8A
IL7R
CD4
BCL11A
log10(counts+1)
Day
B
r = 0.71, p = 7.89e − 08
STR (-sign(β) log10 (padj))
Microwell (-sign(β) log10 (padj))

FIGURE 35

**FIGURE 36A**



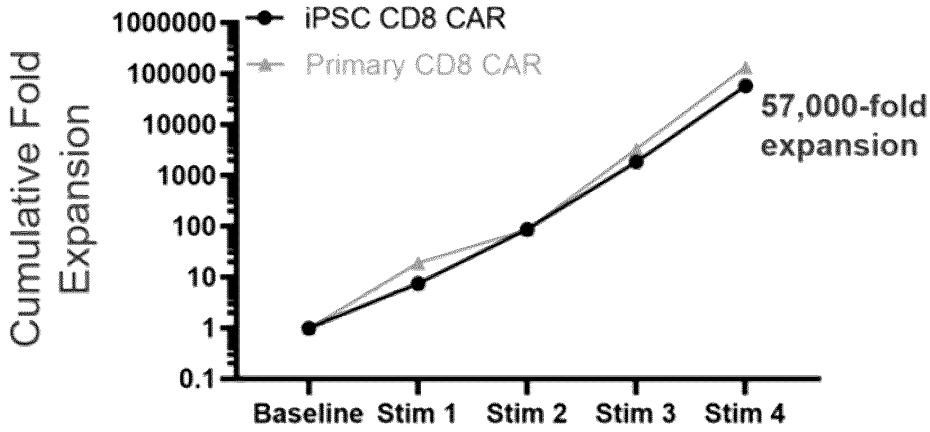




**FIGURE 36B**

FIGURE 36C

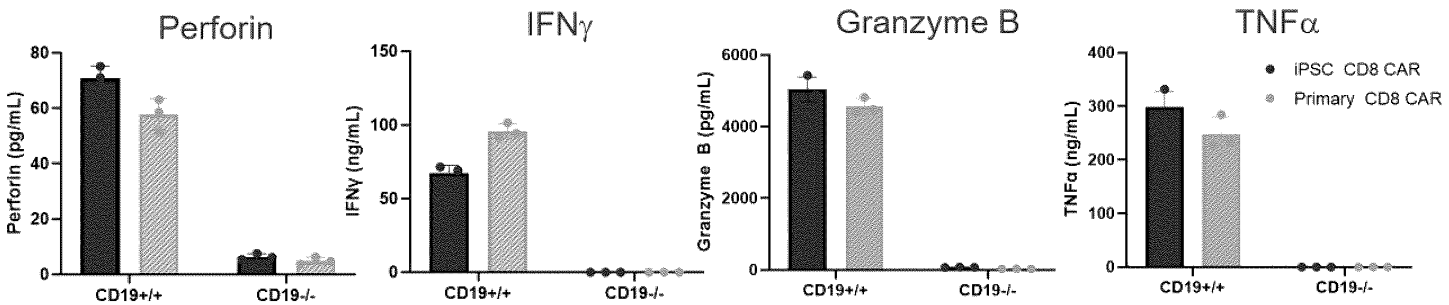



FIGURE 36D

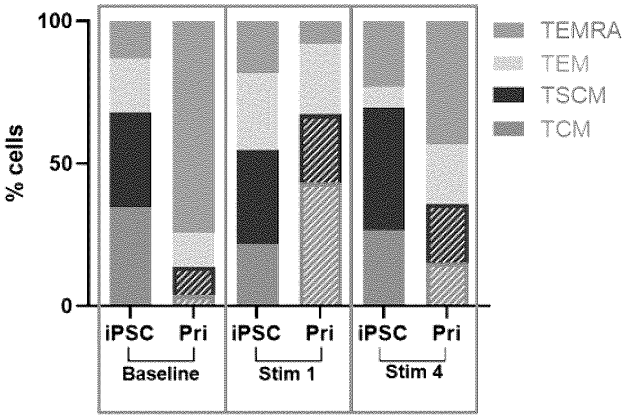
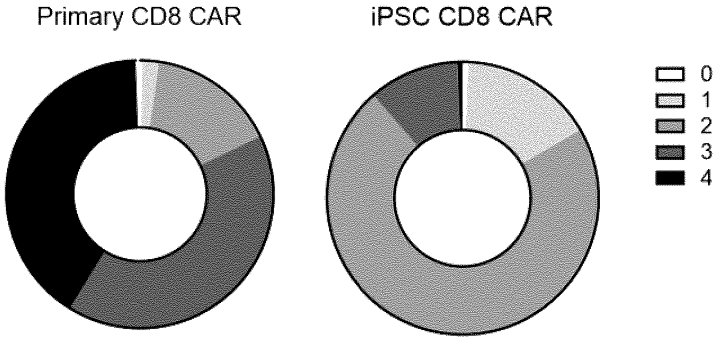



FIGURE 36E



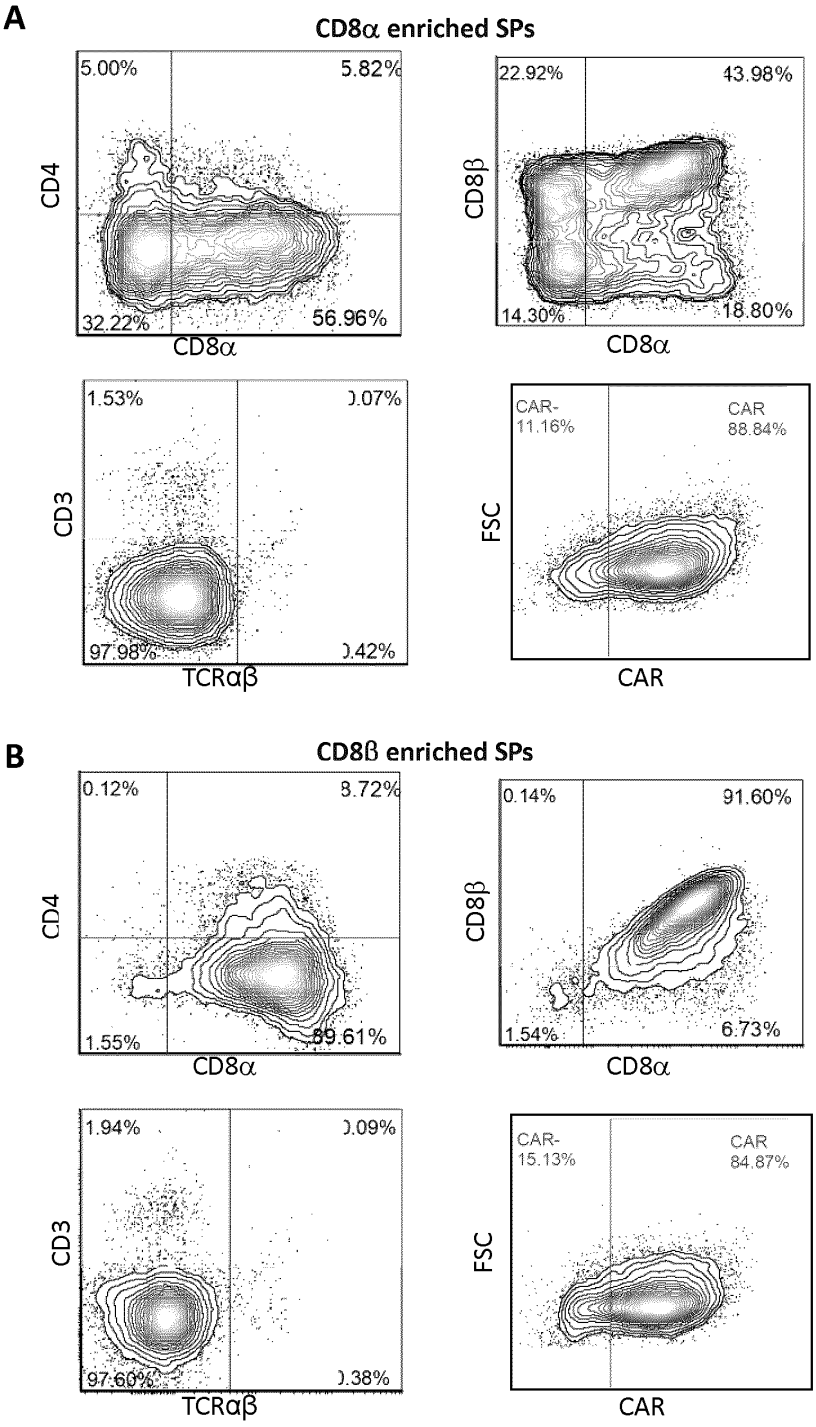

A
CD8α enriched SPs
5.00%
5.82%
32.22%
56.96%
CD4
CD8α
22.92%
43.98%
14.30%
18.80%
CD8β
CD8α
1.53%
97.98%
CD3
TCRαβ
CAR-
11.16%
CAR
88.84%
FSC
CAR
B
CD8β enriched SPs
0.12%
1.55%
89.61%
CD4
CD8α
0.14%
91.60%
1.54%
6.73%
CD8β
CD8α
1.94%
97.60%
CD3
TCRαβ
CAR-
15.13%
CAR
84.87%
FSC
CAR

FIGURE 37

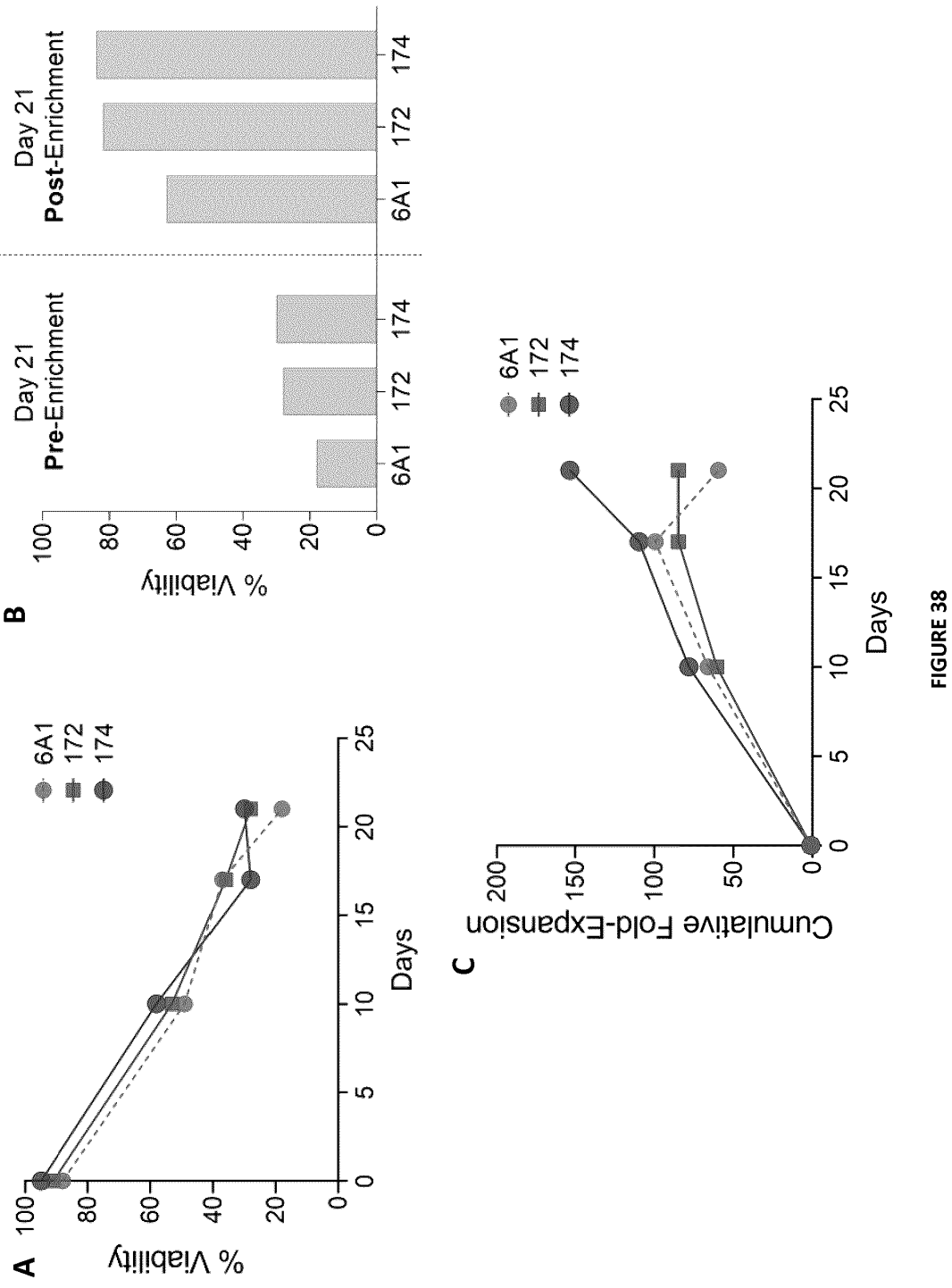

A
% Viability
Days
6A1
172
174
B
% Viability
Day 21
Pre-Enrichment
Day 21
Post-Enrichment
6A1
172
174
C
Cumulative Fold-Expansion
Days
6A1
172
174

FIGURE 38

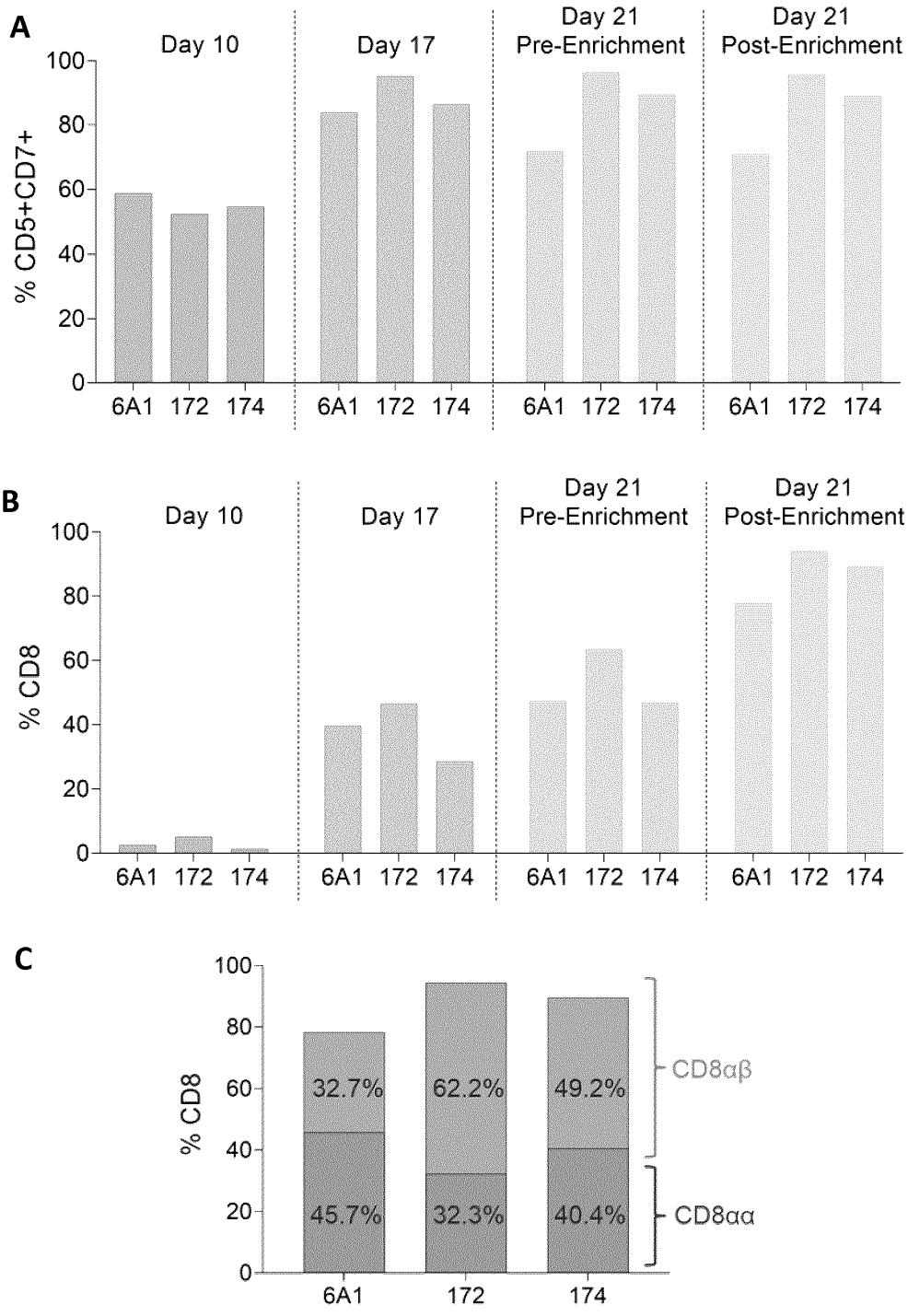

A
Day 10
Day 17
Day 21
Pre-Enrichment
Day 21
Post-Enrichment
% CD5+CD7+
100
80
60
40
20
0
6A1 172 174
B
% CD8
C
32.7%
45.7%
62.2%
32.3%
49.2%
40.4%
CD8αβ
CD8αα

FIGURE 39

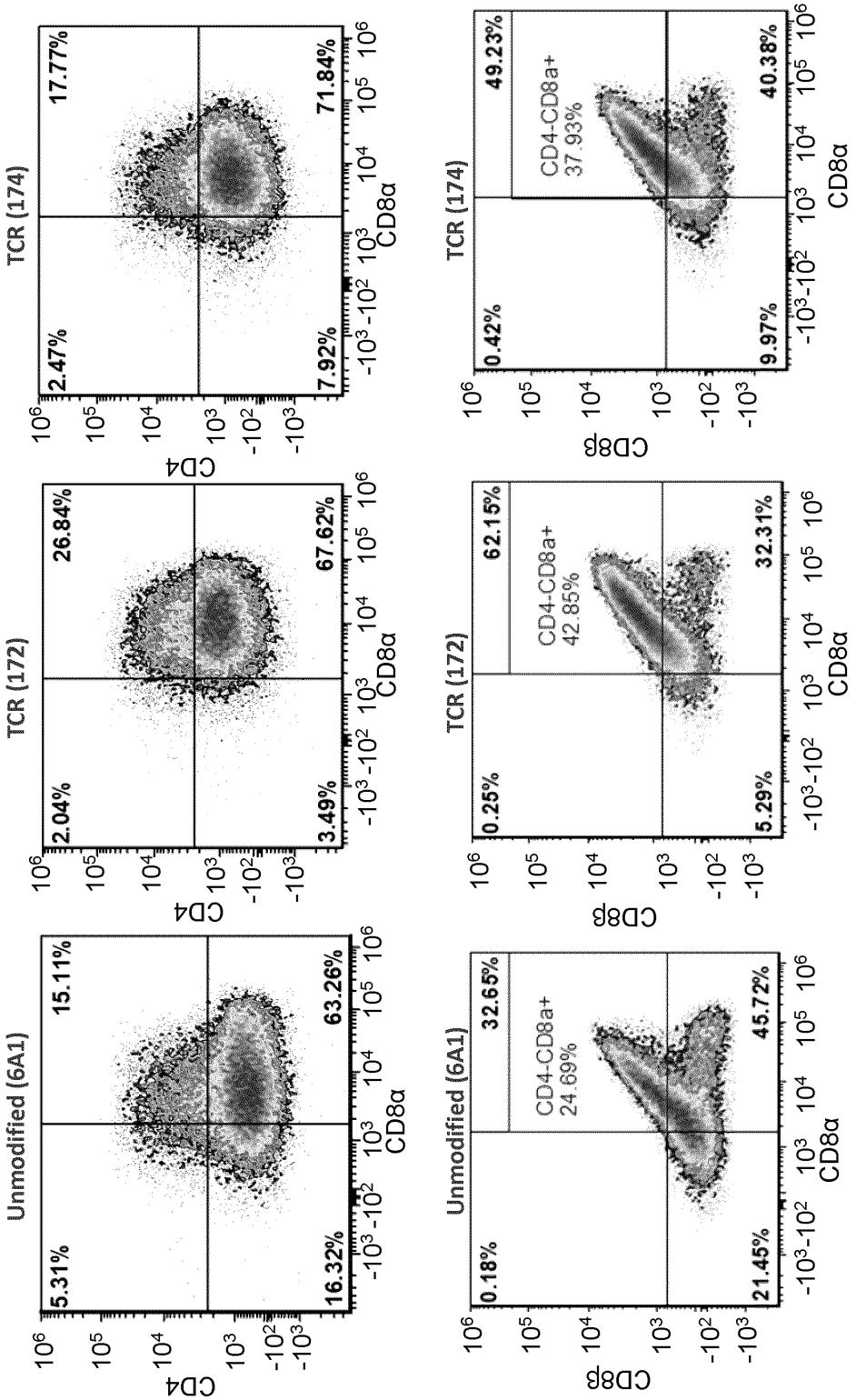

Unmodified (6A1)
TCR (172)
TCR (174)
CD4
CD8α
CD8β
5.31%
15.11%
16.32%
63.26%
2.04%
26.84%
3.49%
67.62%
2.47%
17.77%
7.92%
71.84%
0.18%
32.65%
21.45%
45.72%
CD4-CD8a+
24.69%
0.25%
62.15%
5.29%
32.31%
CD4-CD8a+
42.85%
0.42%
49.23%
9.97%
40.38%
CD4-CD8a+
37.93%

FIGURE 40

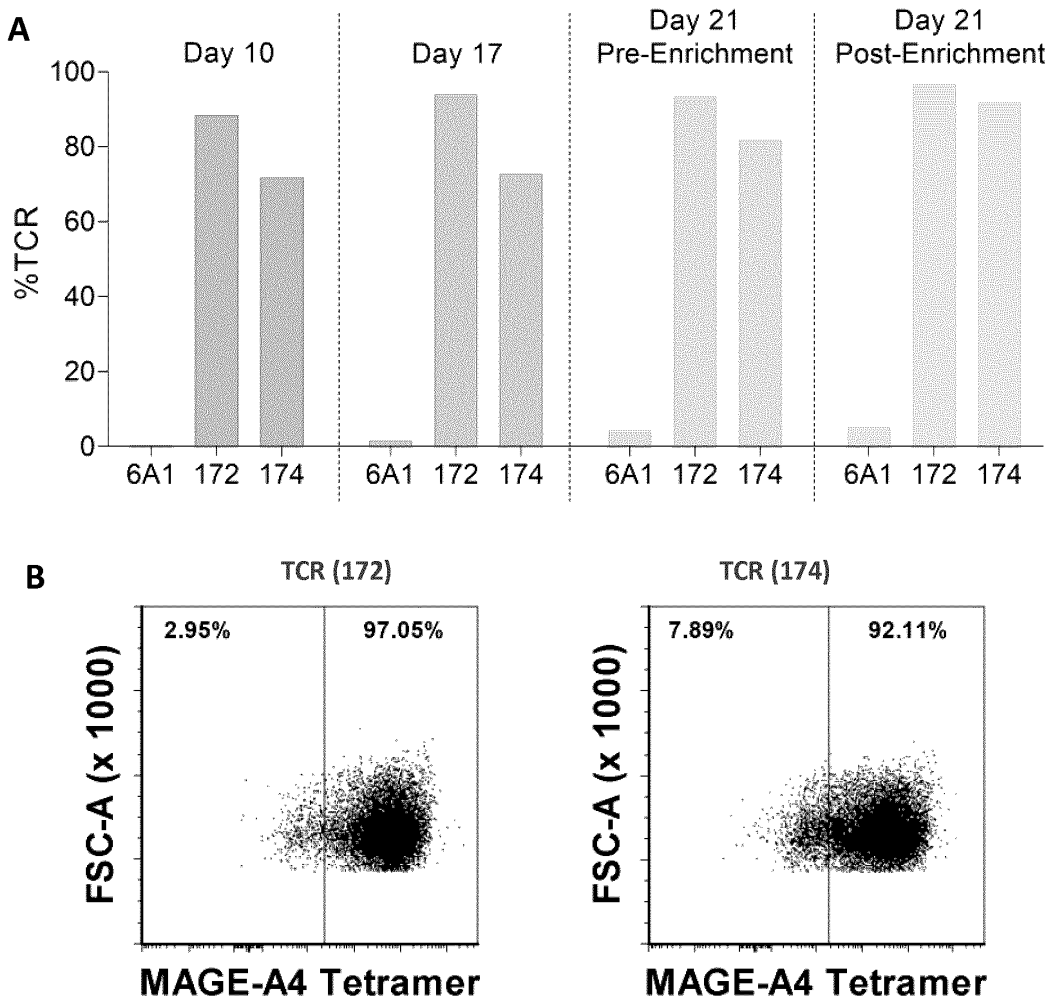

A
Day 10
Day 17
Day 21
Pre-Enrichment
Day 21
Post-Enrichment
%TCR
100
80
60
40
20
0
6A1 172 174
6A1 172 174
6A1 172 174
6A1 172 174
B
TCR (172)
TCR (174)
2.95%
97.05%
7.89%
92.11%
FSC-A (x 1000)
FSC-A (x 1000)
MAGE-A4 Tetramer
MAGE-A4 Tetramer

FIGURE 41

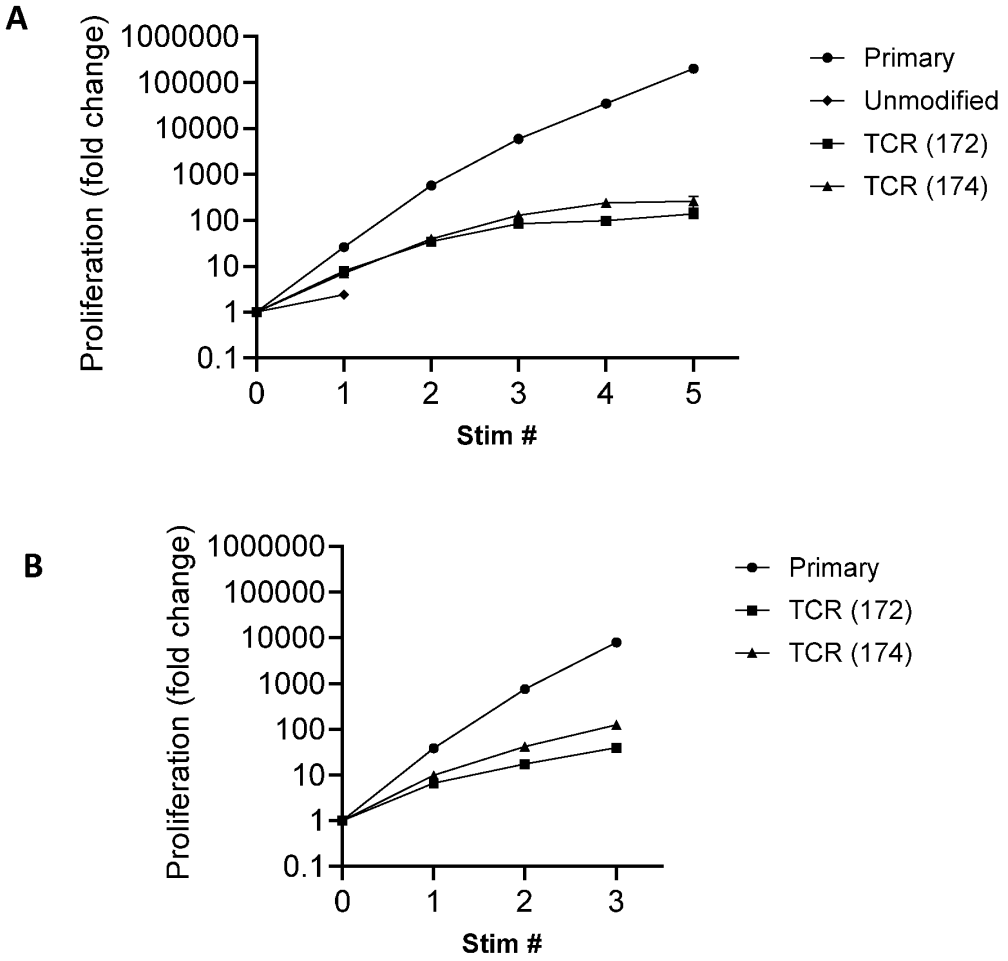

A
Proliferation (fold change)
1000000
100000
10000
1000
100
10
1
0.1
0
1
2
3
4
5
Stim #
Primary
Unmodified
TCR (172)
TCR (174)
B
Proliferation (fold change)
1000000
100000
10000
1000
100
10
1
0.1
0
1
2
3
Stim #
Primary
TCR (172)
TCR (174)

FIGURE 42

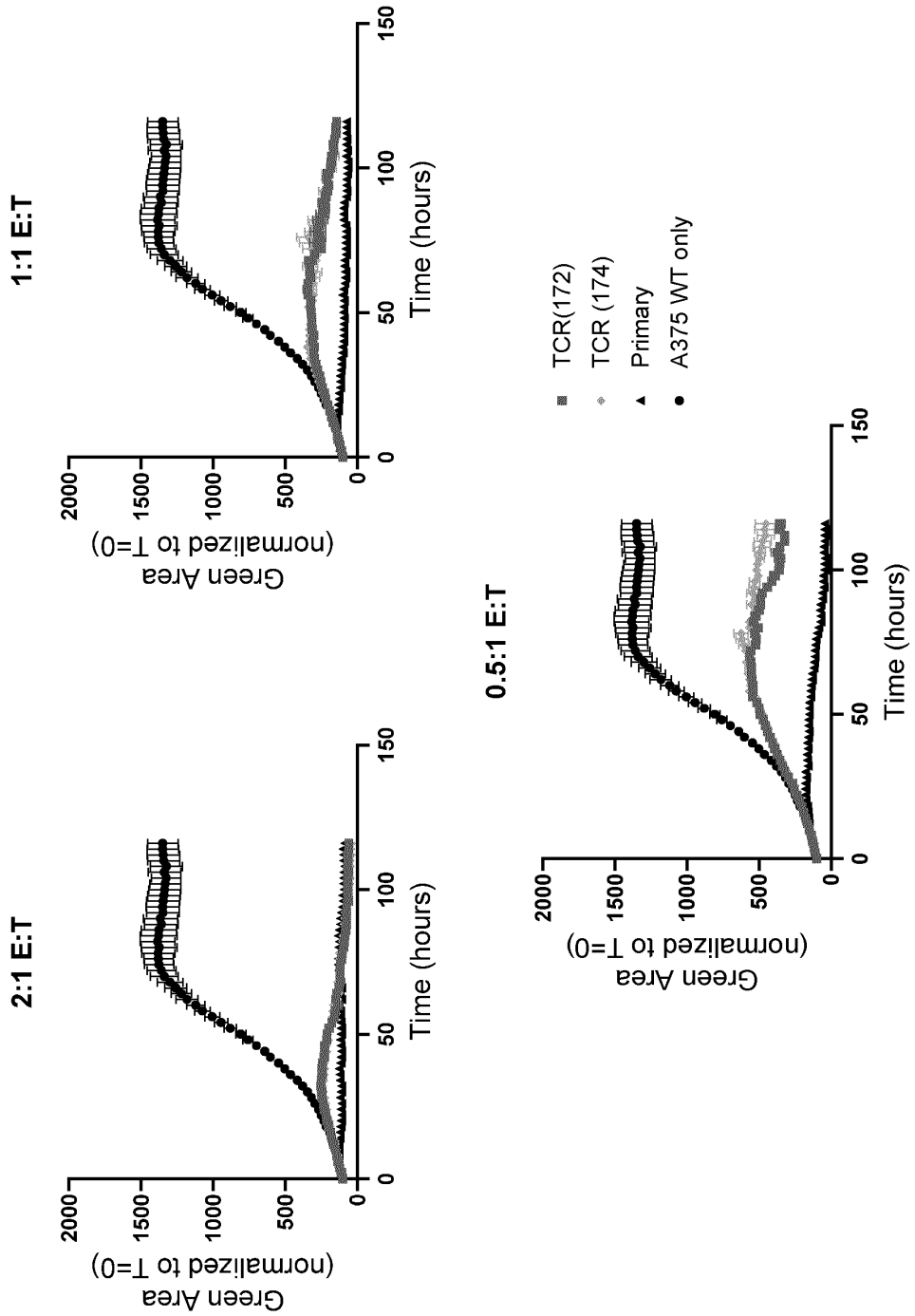

1:1 E:T
2:1 E:T
0.5:1 E:T
Green Area (normalized to T=0)
Time (hours)
0
500
1000
1500
2000
50
100
150
TCR(172)
TCR (174)
Primary
A375 WT only

FIGURE 43

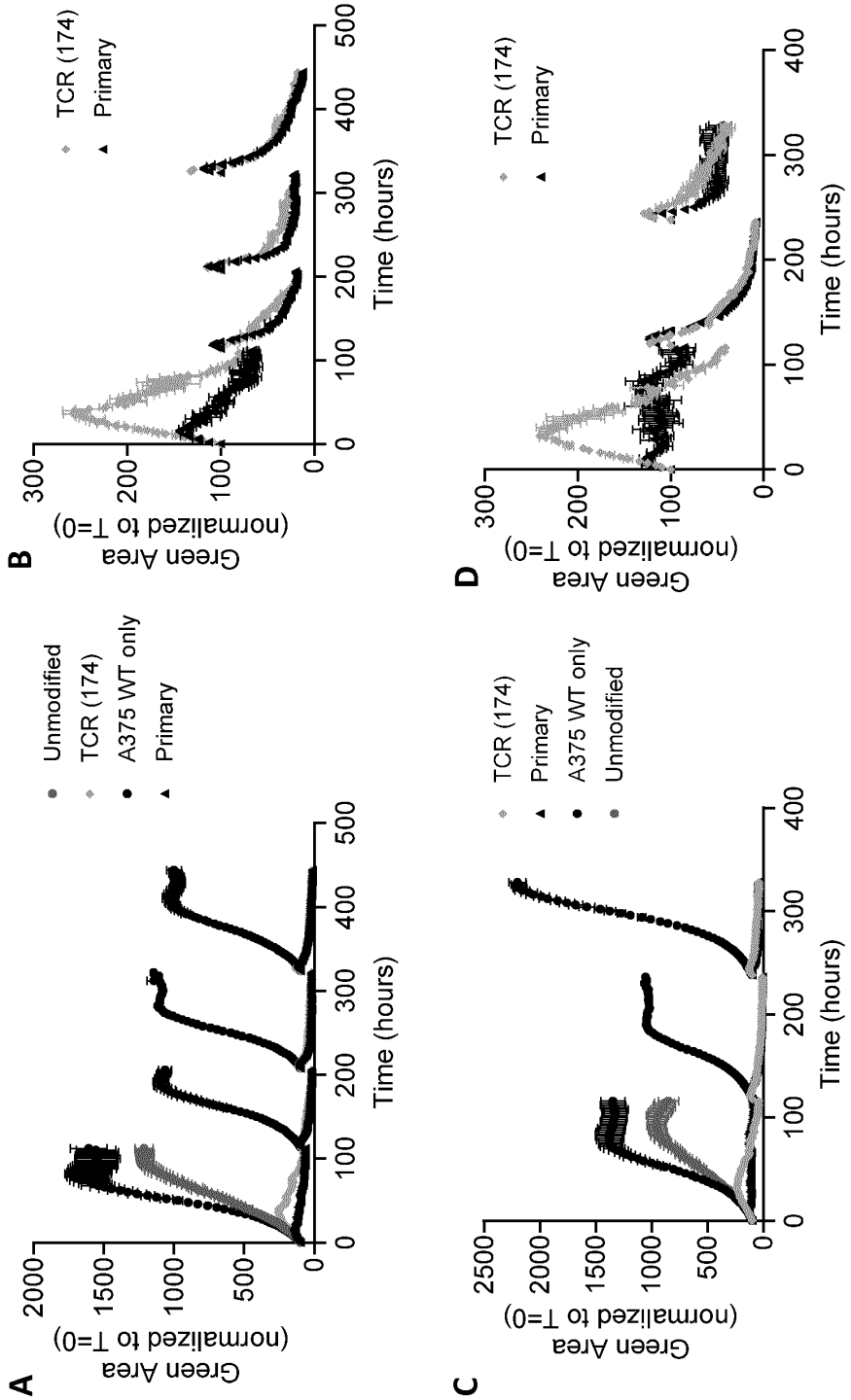

A
Green Area (normalized to T=0)
Time (hours)
Unmodified
TCR (174)
A375 WT only
Primary
B
Green Area (normalized to T=0)
Time (hours)
TCR (174)
Primary
C
Green Area (normalized to T=0)
Time (hours)
TCR (174)
Primary
A375 WT only
Unmodified
D
Green Area (normalized to T=0)
Time (hours)
TCR (174)
Primary

FIGURE 44

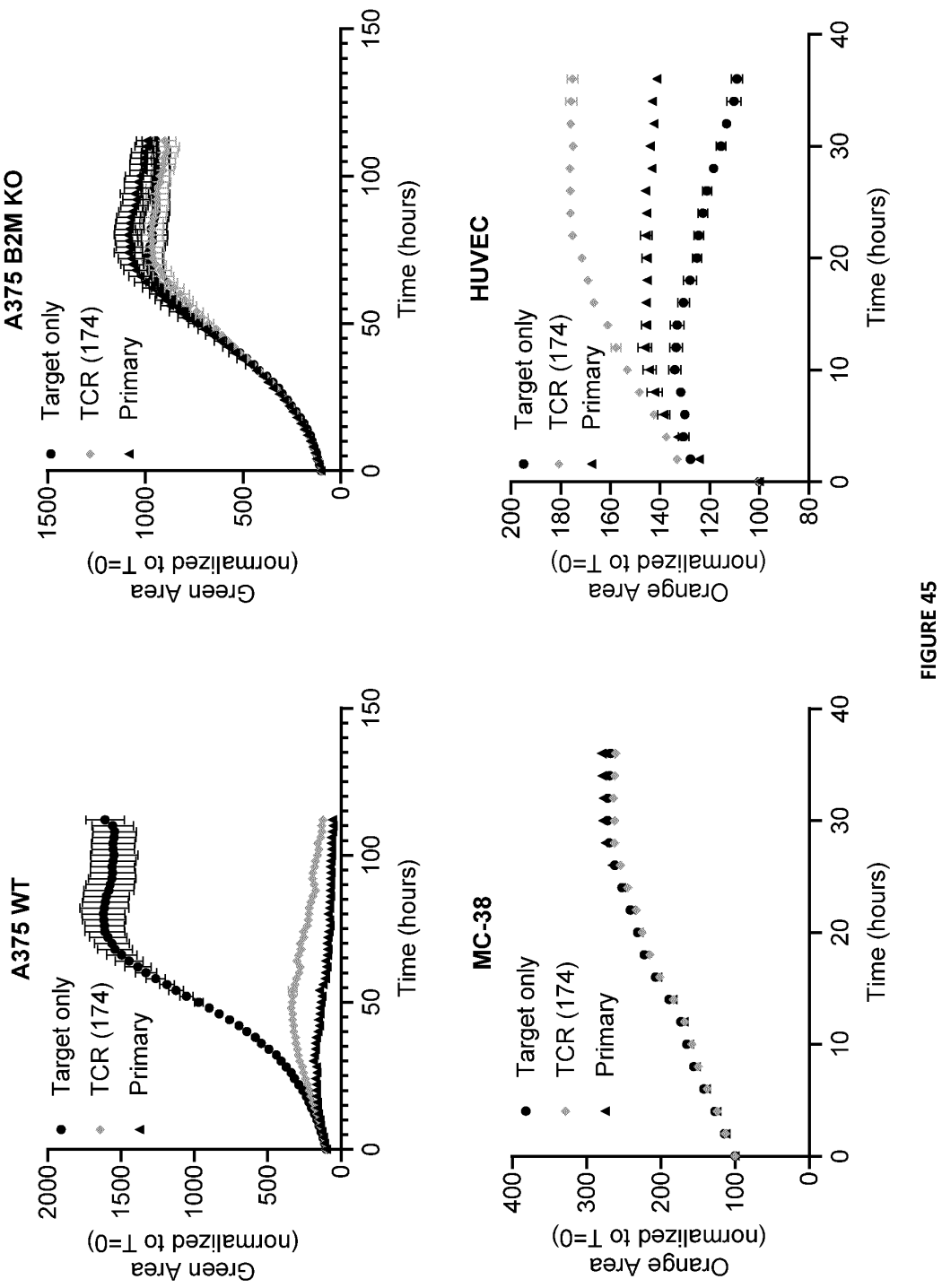

A375 WT
A375 B2M KO
HUVEC
MC-38
Target only
TCR (174)
Primary
Green Area (normalized to T=0)
Orange Area (normalized to T=0)
Time (hours)

FIGURE 45

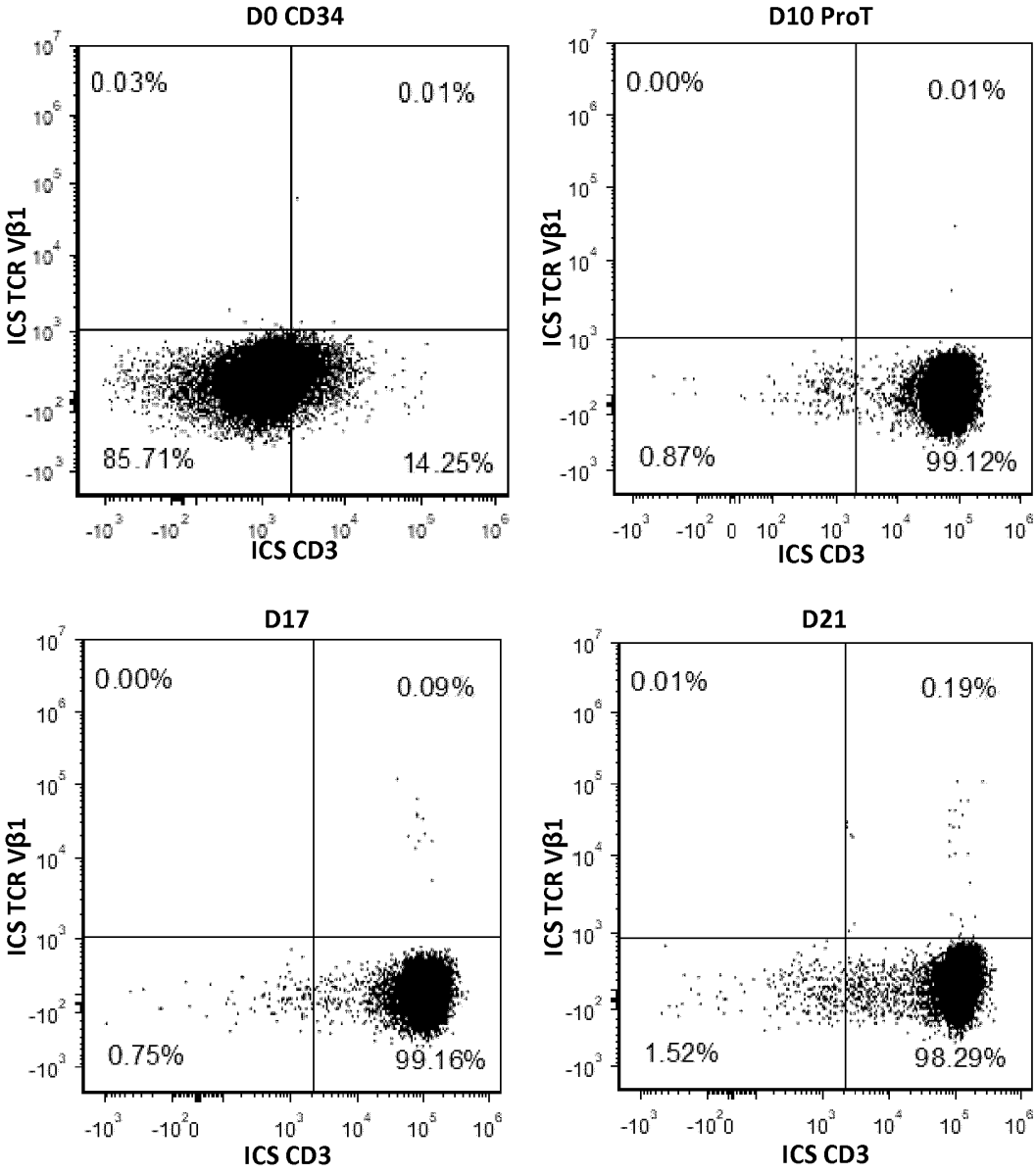
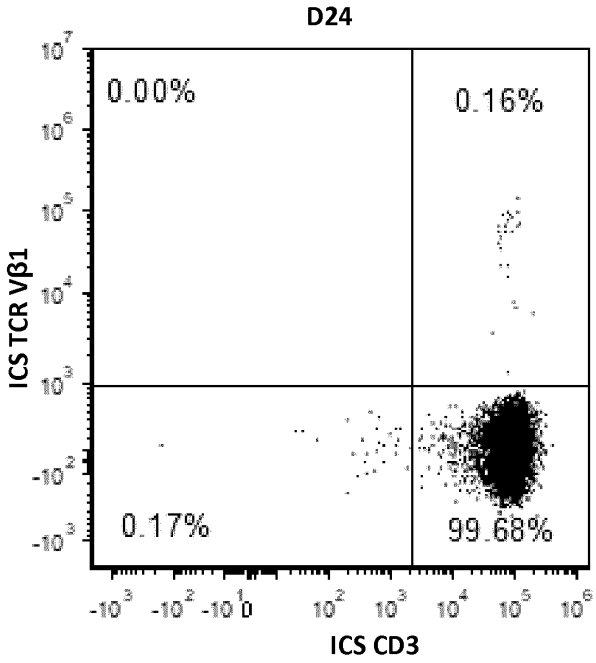

D0 CD34
0.03%
0.01%
85.71%
14.25%
D10 ProT
0.00%
0.01%
0.87%
99.12%
D17
0.00%
0.09%
0.75%
99.16%
D21
0.01%
0.19%
1.52%
98.29%
ICS TCR Vβ1
ICS CD3

FIGURE 46A

FIGURE 46B



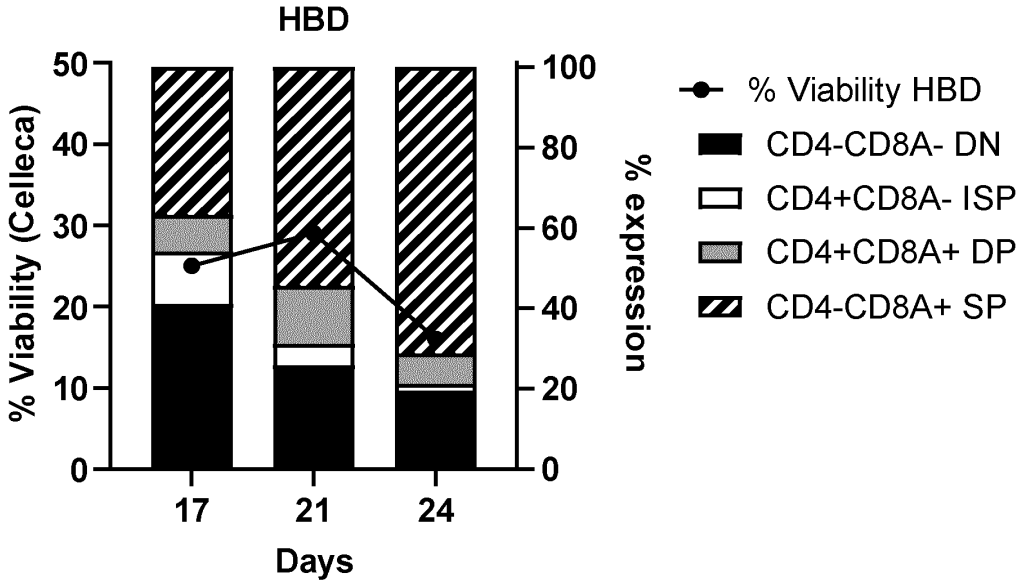




FIGURE 46C

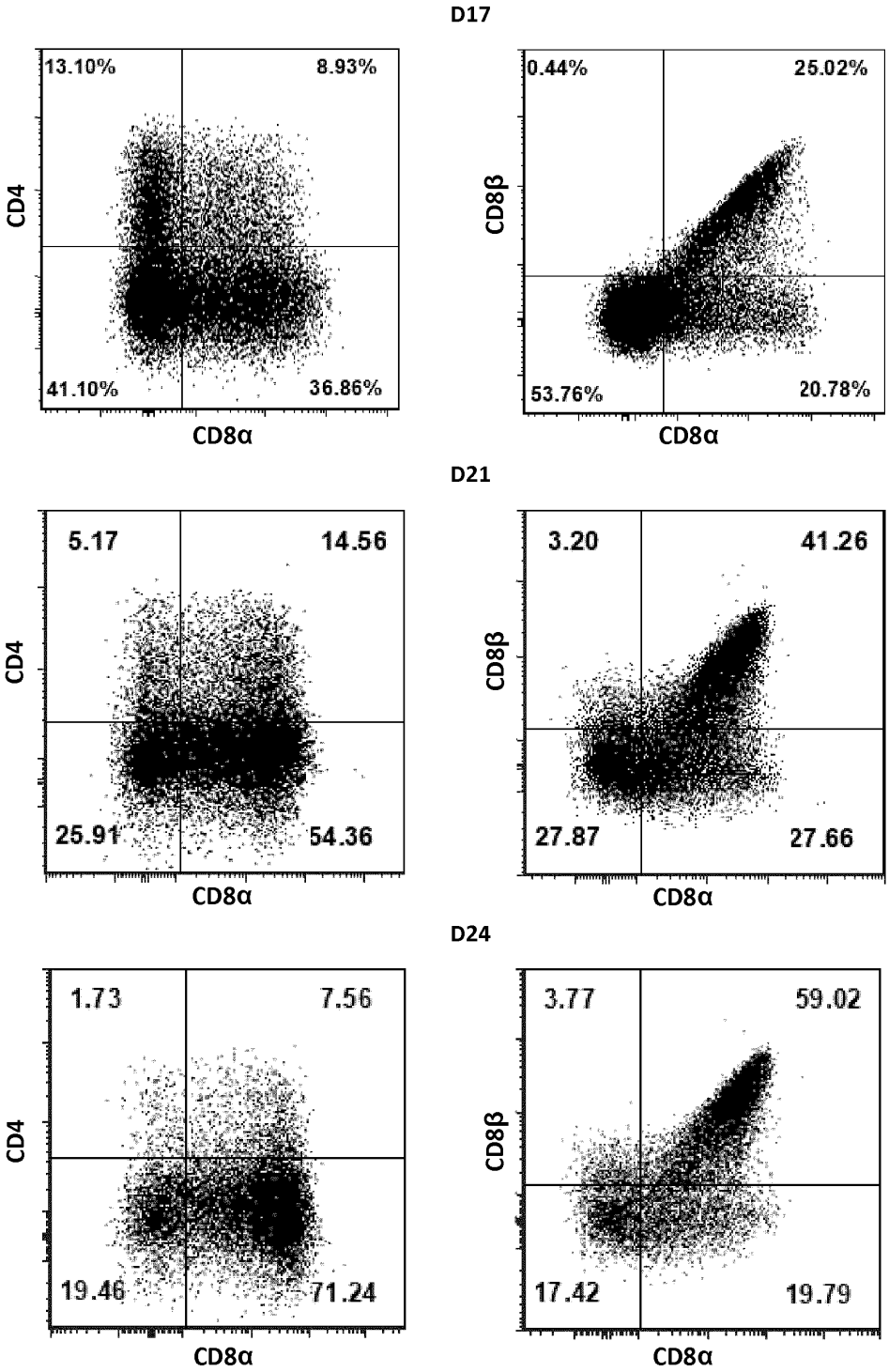
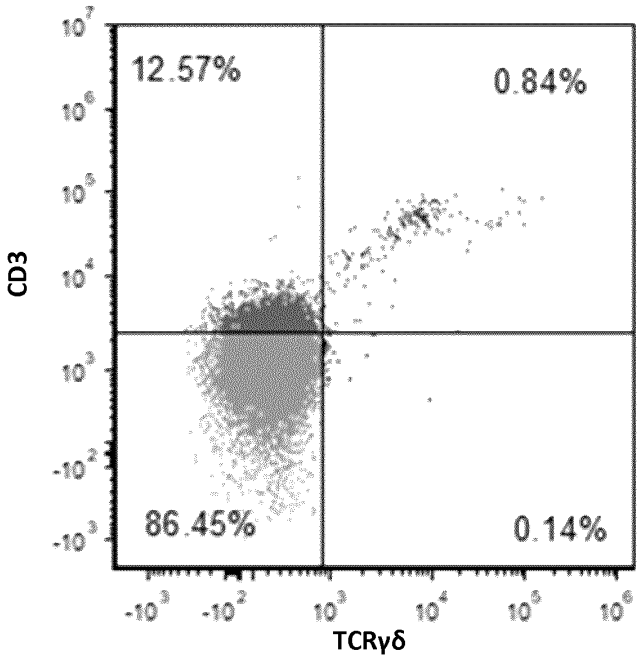

D17
13.10%
8.93%
41.10%
36.86%
CD4
CD8α
0.44%
25.02%
53.76%
20.78%
CD8β
CD8α
D21
5.17
14.56
25.91
54.36
CD4
CD8α
3.20
41.26
27.87
27.66
CD8β
CD8α
D24
1.73
7.56
19.46
71.24
CD4
CD8α
3.77
59.02
17.42
19.79
CD8β
CD8α

FIGURE 47

D21




D24

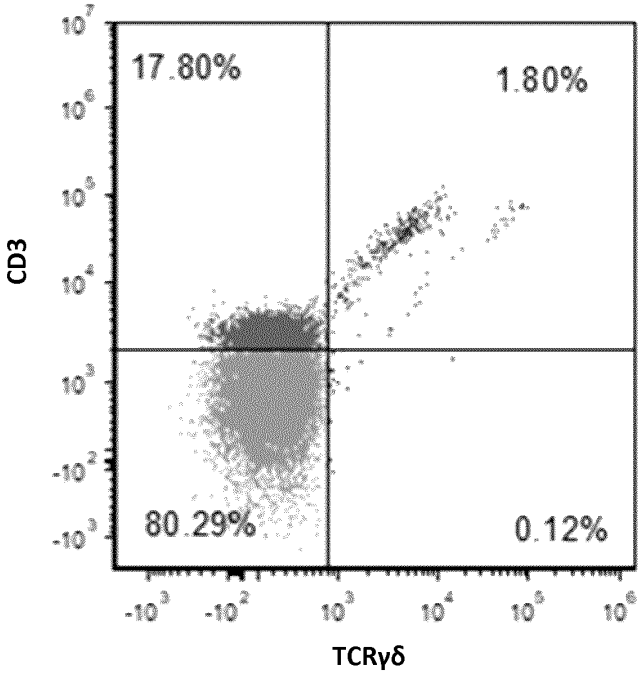



FIGURE 48

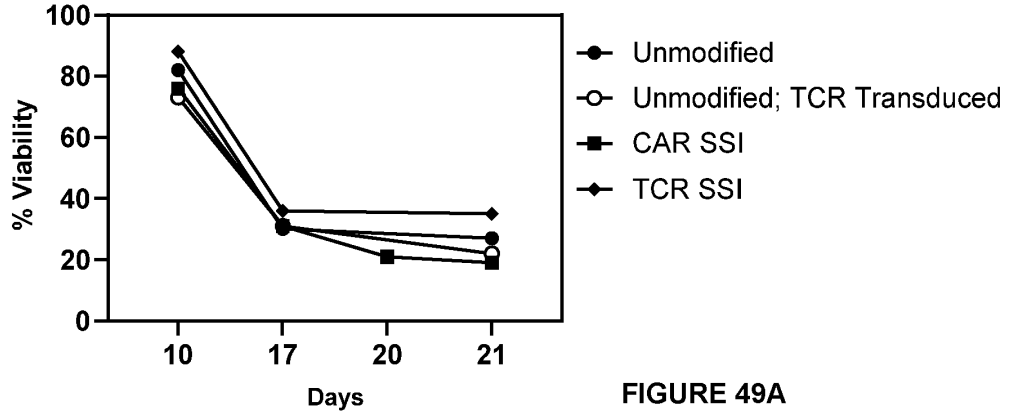



FIGURE 49A

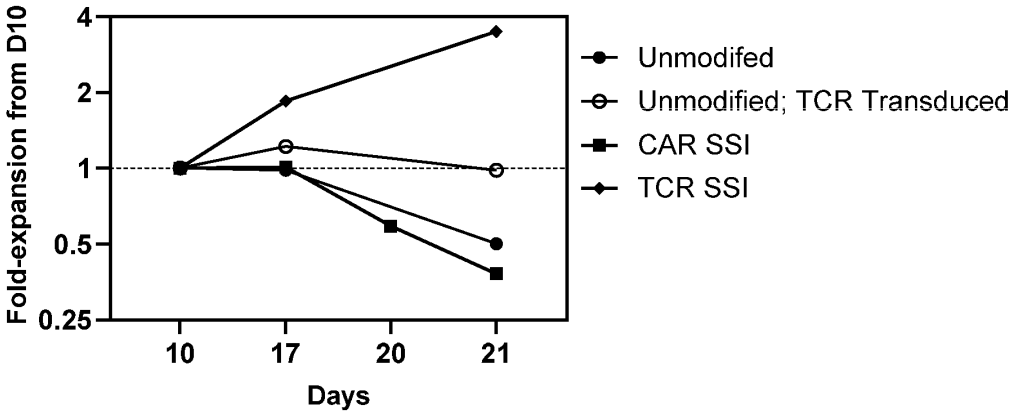



FIGURE 49B

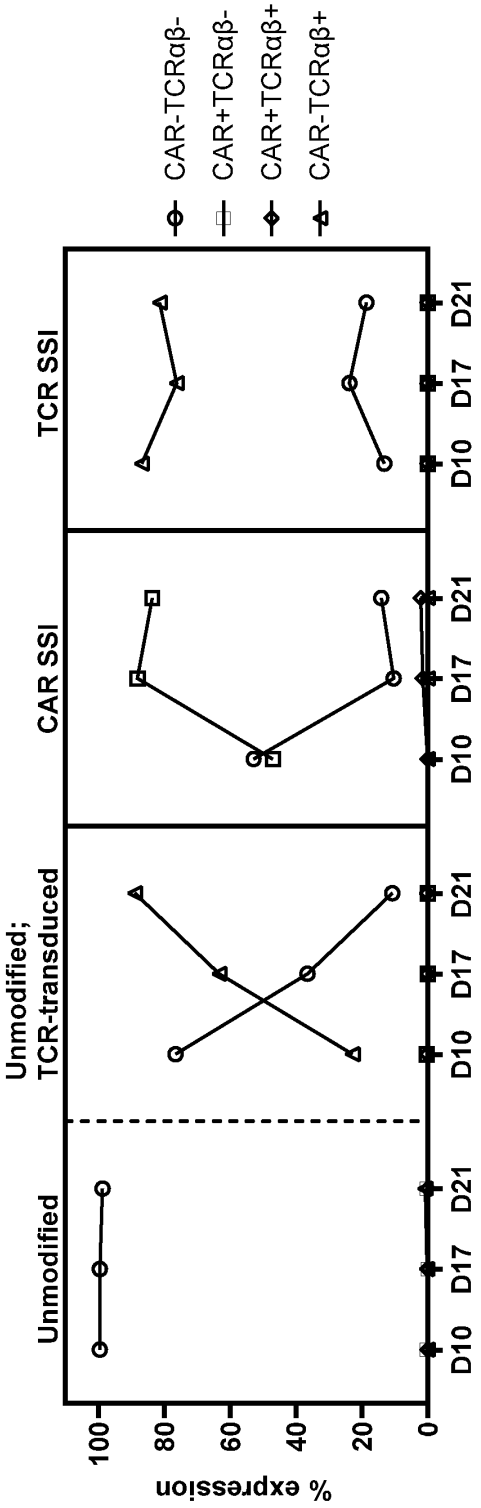

CAR-TCRαβ-
CAR+TCRαβ-
CAR+TCRαβ+
CAR-TCRαβ+
% expression
100
80
60
40
20
0
Unmodified
Unmodified; TCR-transduced
CAR SSI
TCR SSI
D10
D17
D21

FIGURE 49C

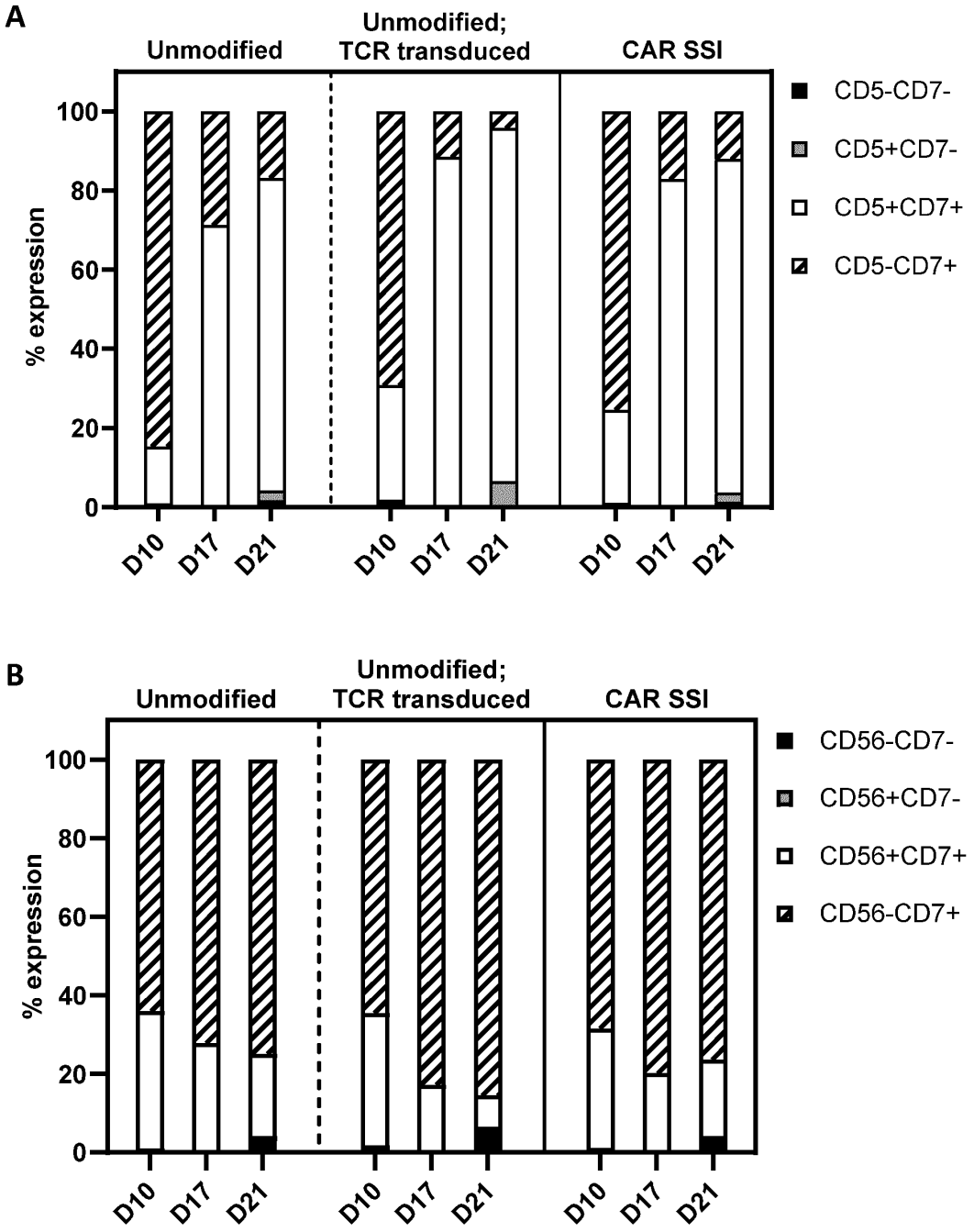

A
Unmodified
Unmodified;
TCR transduced
CAR SSI
% expression
100
80
60
40
20
0
D10
D17
D21
CD5-CD7-
CD5+CD7-
CD5+CD7+
CD5-CD7+
B
Unmodified
Unmodified;
TCR transduced
CAR SSI
% expression
CD56-CD7-
CD56+CD7-
CD56+CD7+
CD56-CD7+

FIGURE 50

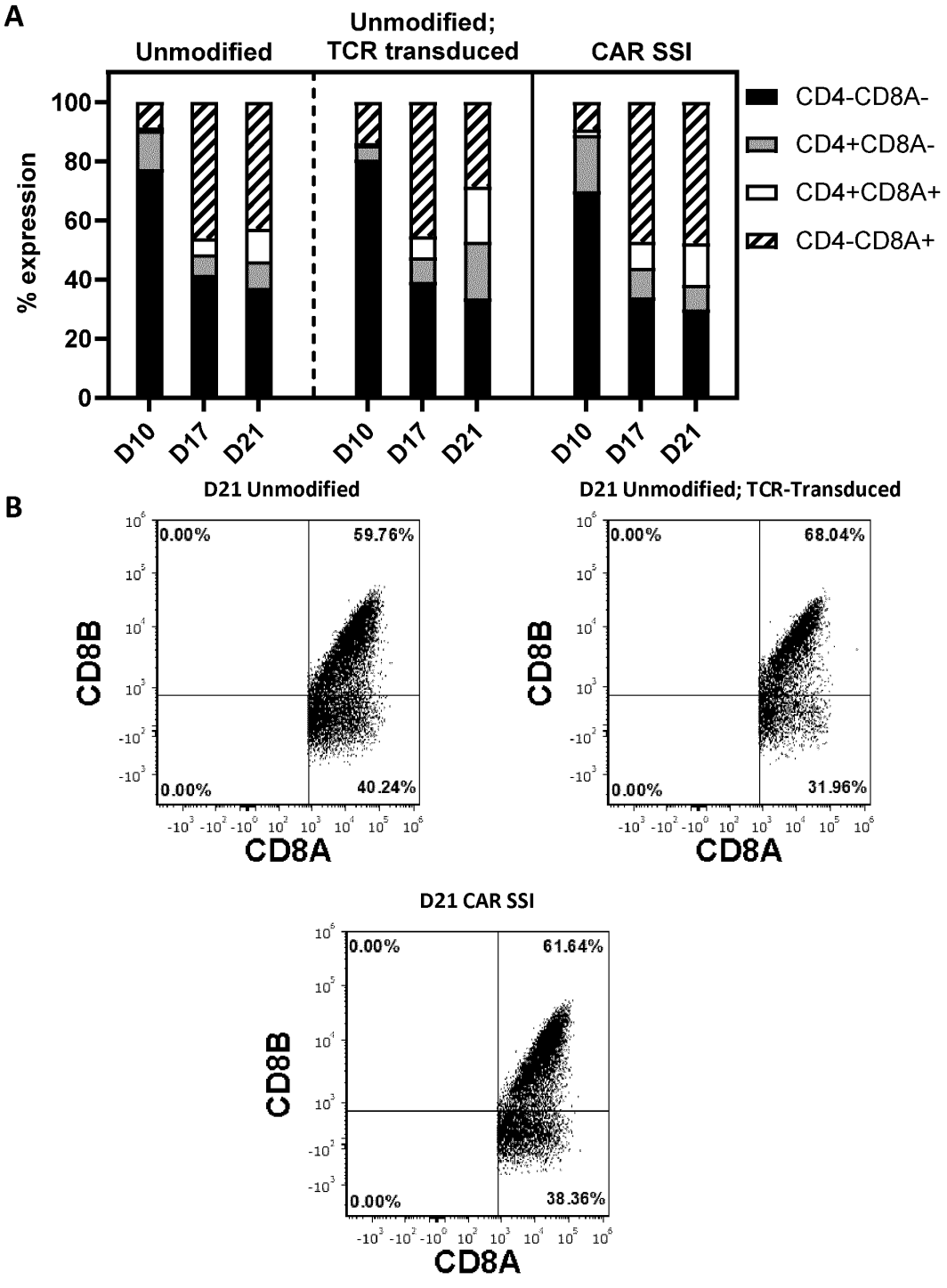

A
Unmodified
Unmodified;
TCR transduced
CAR SSI
% expression
100
80
60
40
20
0
D10
D17
D21
CD4-CD8A-
CD4+CD8A-
CD4+CD8A+
CD4-CD8A+
B
D21 Unmodified
0.00%
59.76%
0.00%
40.24%
CD8B
CD8A
D21 Unmodified; TCR-Transduced
0.00%
68.04%
0.00%
31.96%
CD8B
CD8A
D21 CAR SSI
0.00%
61.64%
0.00%
38.36%
CD8B
CD8A

FIGURE 51

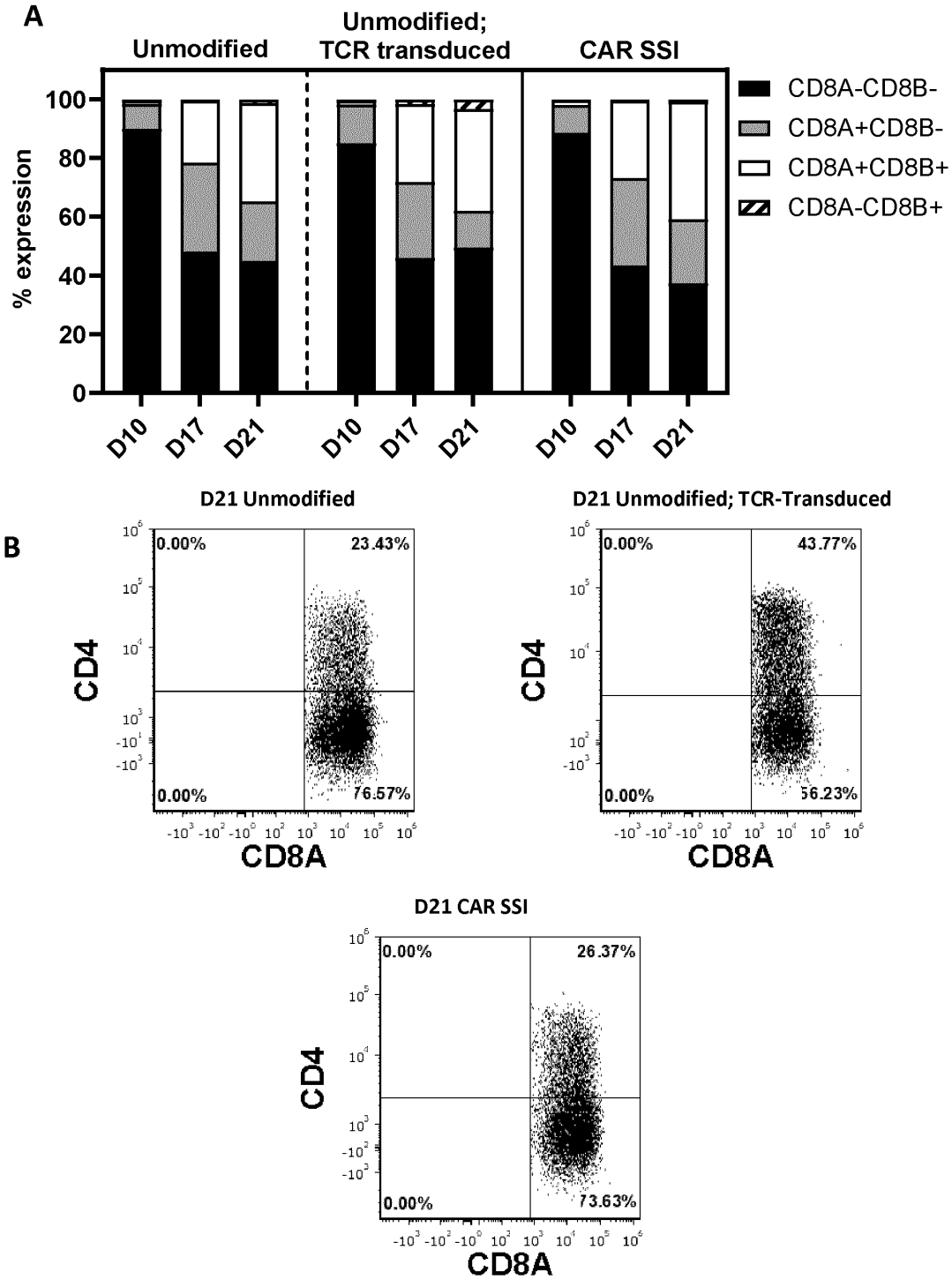

A
Unmodified
Unmodified; TCR transduced
CAR SSI
% expression
100
80
60
40
20
0
D10
D17
D21
CD8A-CD8B-
CD8A+CD8B-
CD8A+CD8B+
CD8A-CD8B+
B
D21 Unmodified
0.00%
23.43%
0.00%
76.57%
CD4
CD8A
D21 Unmodified; TCR-Transduced
0.00%
43.77%
0.00%
56.23%
CD4
CD8A
D21 CAR SSI
0.00%
26.37%
0.00%
73.63%
CD4
CD8A

FIGURE 52

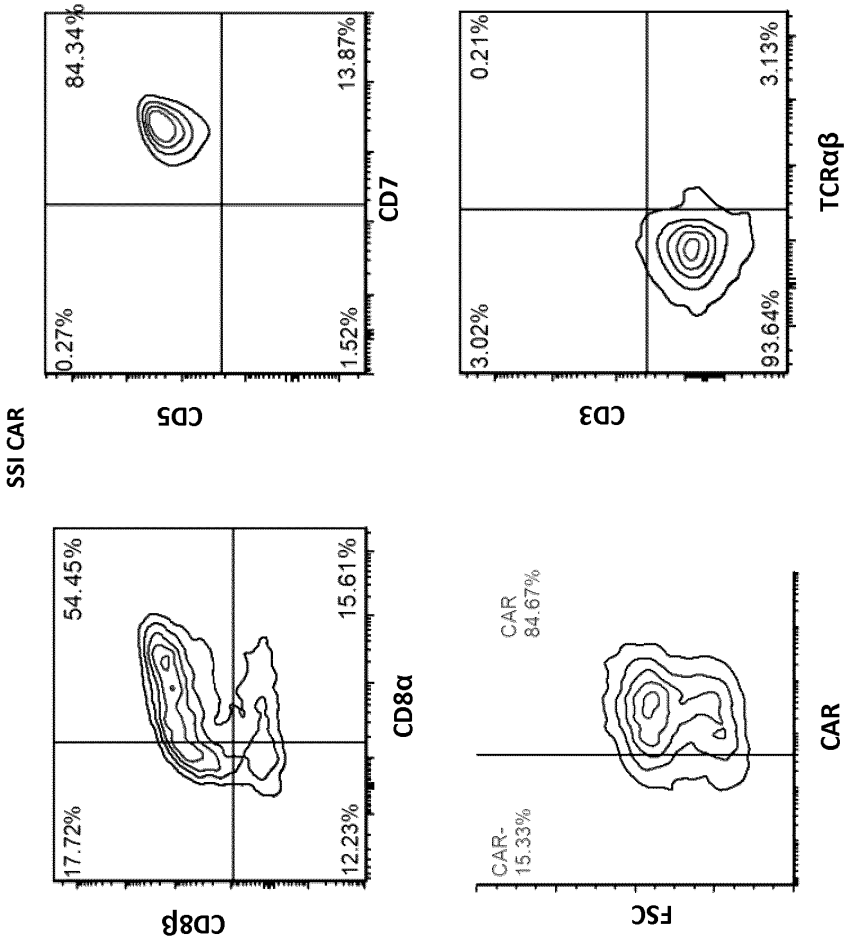

SSI CAR
CD5
84.34%
13.87%
0.27%
1.52%
CD7
CD3
0.21%
3.13%
3.02%
93.64%
TCRαβ
CD8β
54.45%
15.61%
17.72%
12.23%
CD8α
FSC
CAR
84.67%
CAR-
15.33%
CAR

FIGURE 53

A
SSI CAR
Fold Expansion
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
Stim #
B
CD8α+CD8β+
CD8α+CD8β-
CD8α-CD8β-
CD8α-CD8β+
SSI CAR
% Live Singlets
100
80
60
40
20
0
Baseline
Post Stim 1
Post Stim 3
C
SSI CAR (CD19 +/+)
SSI CAR (CD19 -/-)
Raji Target only (CD19 +/+)
Raji Target only (CD19 -/-)
GFP Area (normalized %)
4000
3000
2000
1000
0
0
100
200
300
Time (hours)

FIGURE 54

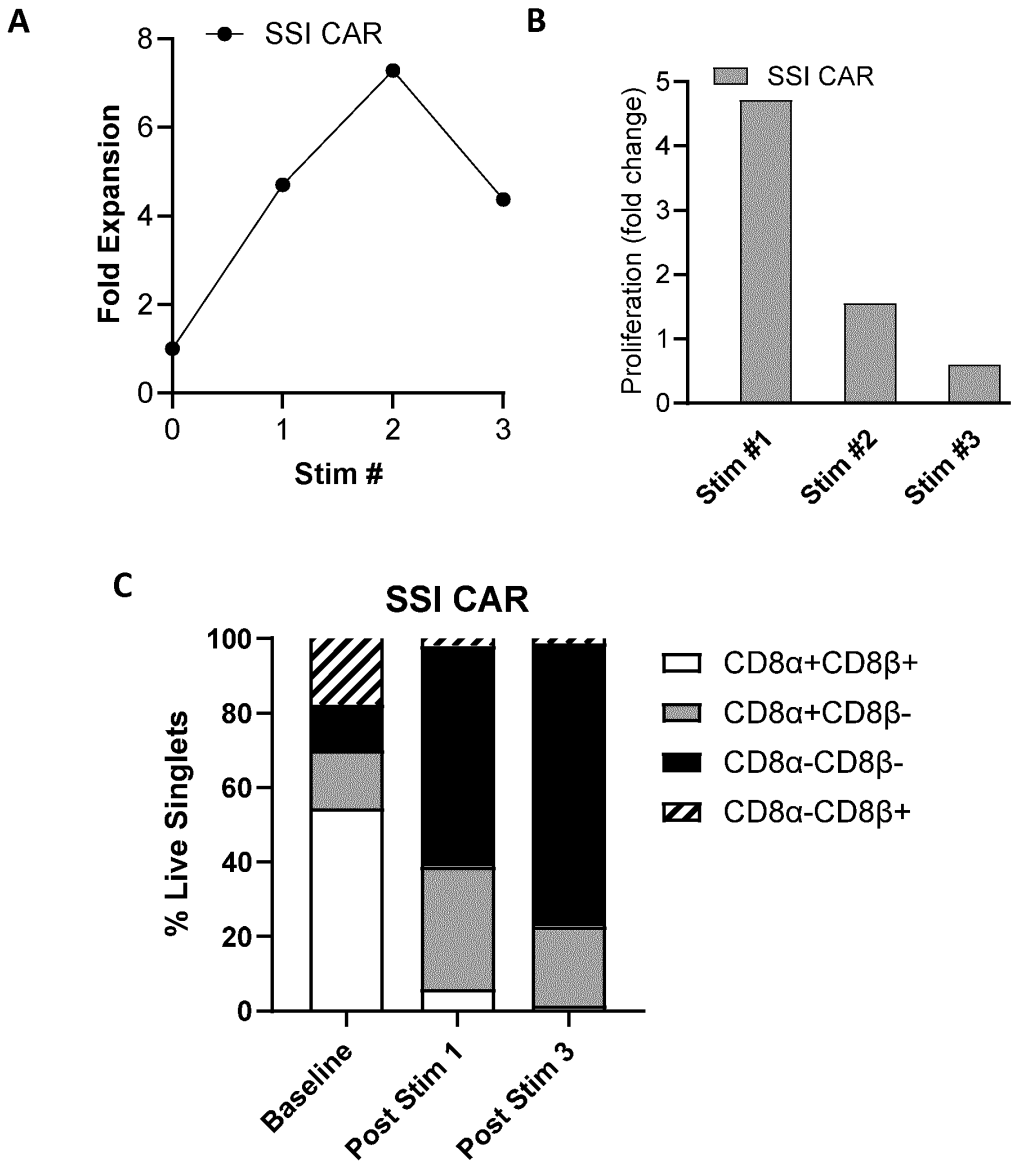

A
SSI CAR
Fold Expansion
Stim #
B
SSI CAR
Proliferation (fold change)
Stim #1
Stim #2
Stim #3
C
SSI CAR
% Live Singlets
CD8α+CD8β+
CD8α+CD8β-
CD8α-CD8β-
CD8α-CD8β+
Baseline
Post Stim 1
Post Stim 3

FIGURE 55

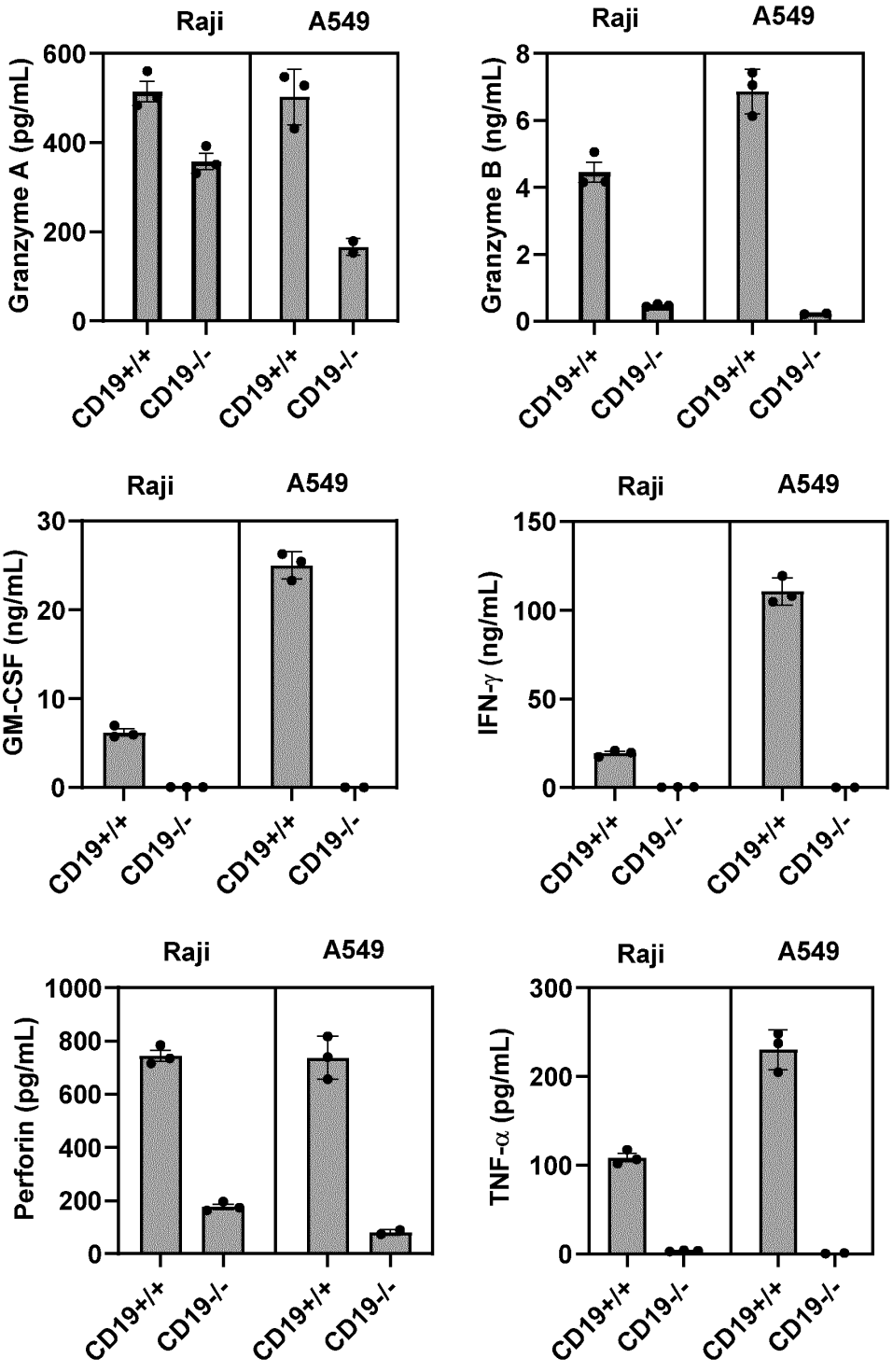
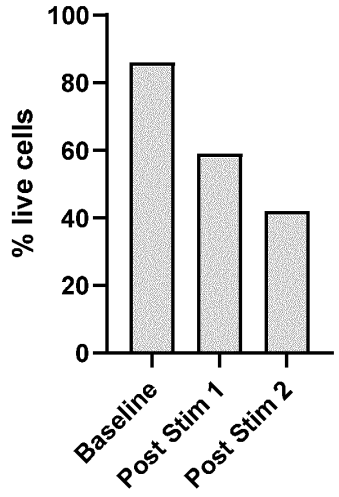

Raji
A549
Granzyme A (pg/mL)
600
400
200
0
CD19+/+
CD19-/-
CD19+/+
CD19-/-
Raji
A549
Granzyme B (ng/mL)
8
6
4
2
0
CD19+/+
CD19-/-
CD19+/+
CD19-/-
Raji
A549
GM-CSF (ng/mL)
30
20
10
0
CD19+/+
CD19-/-
CD19+/+
CD19-/-
Raji
A549
IFN-γ (ng/mL)
150
100
50
0
CD19+/+
CD19-/-
CD19+/+
CD19-/-
Raji
A549
Perforin (pg/mL)
1000
800
600
400
200
0
CD19+/+
CD19-/-
CD19+/+
CD19-/-
Raji
A549
TNF-α (pg/mL)
300
200
100
0
CD19+/+
CD19-/-
CD19+/+
CD19-/-

FIGURE 56

Raji 4:1 E:T

A




A549-CD19 2:1 E:T

B

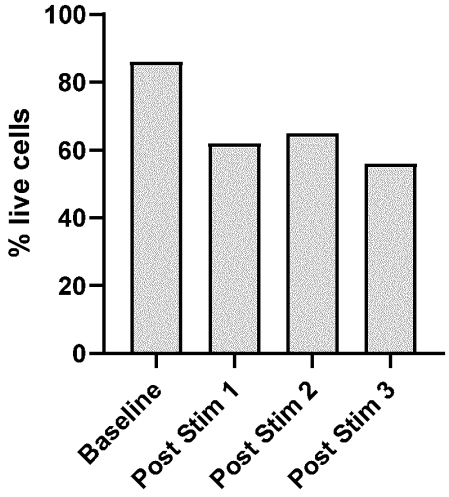



FIGURE 57

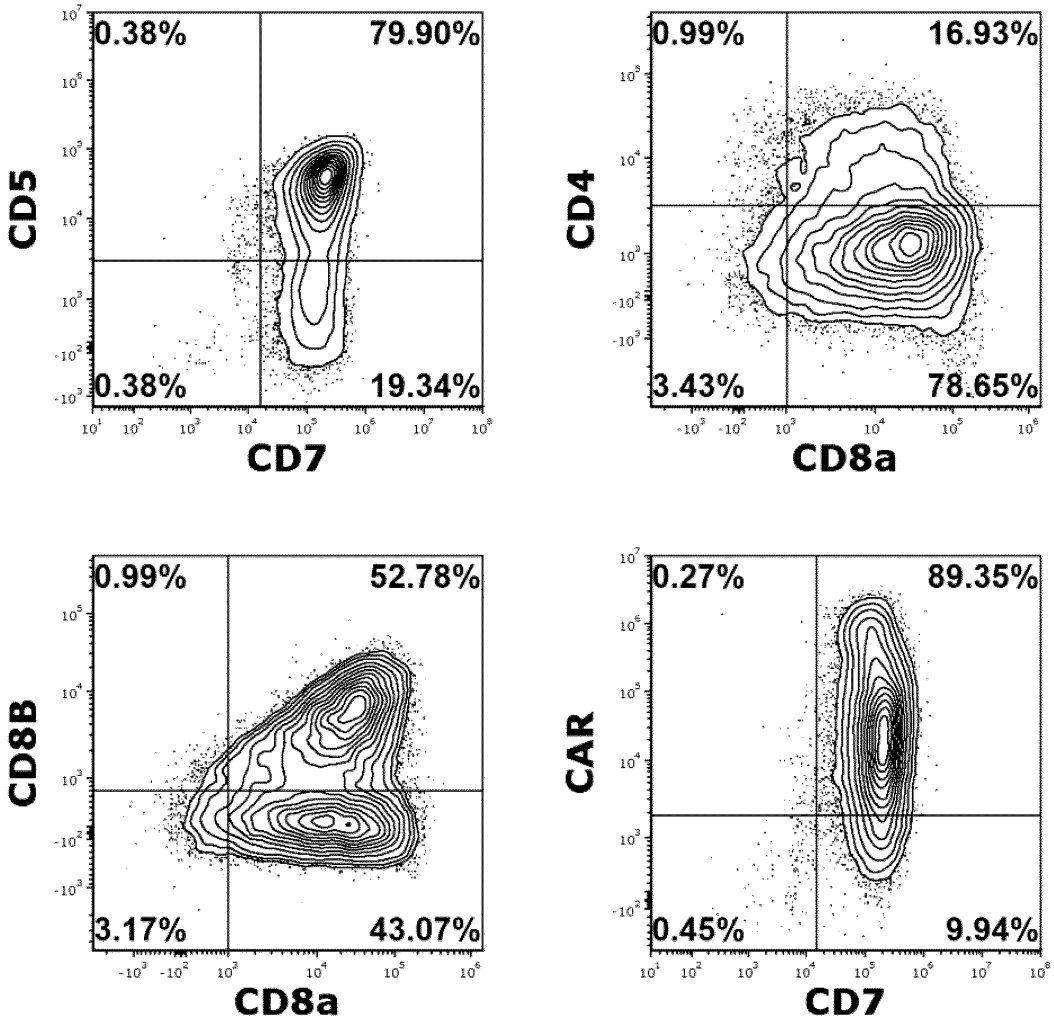

0.38%
79.90%
0.38%
19.34%
CD5
CD7
0.99%
16.93%
3.43%
78.65%
CD4
CD8a
0.99%
52.78%
3.17%
43.07%
CD8B
CD8a
0.27%
89.35%
0.45%
9.94%
CAR
CD7

FIGURE 58

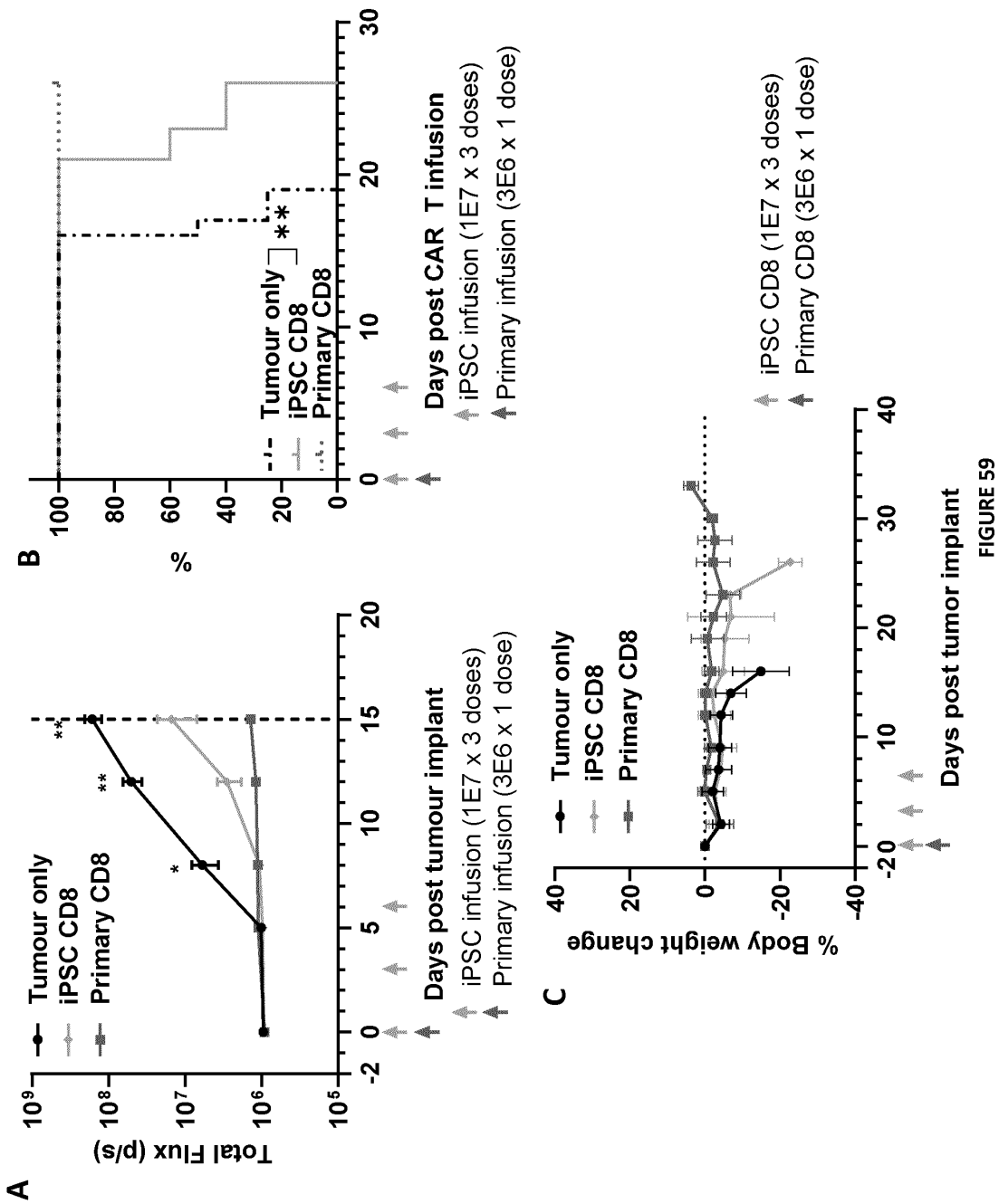

A
Total Flux (p/s)
Tumour only
iPSC CD8
Primary CD8
Days post tumour implant
iPSC infusion (1E7 x 3 doses)
Primary infusion (3E6 x 1 dose)
B
%
Tumour only
iPSC CD8
Primary CD8
Days post CAR T infusion
iPSC infusion (1E7 x 3 doses)
Primary infusion (3E6 x 1 dose)
C
% Body weight change
Tumour only
iPSC CD8
Primary CD8
iPSC CD8 (1E7 x 3 doses)
Primary CD8 (3E6 x 1 dose)
Days post tumor implant

FIGURE 59

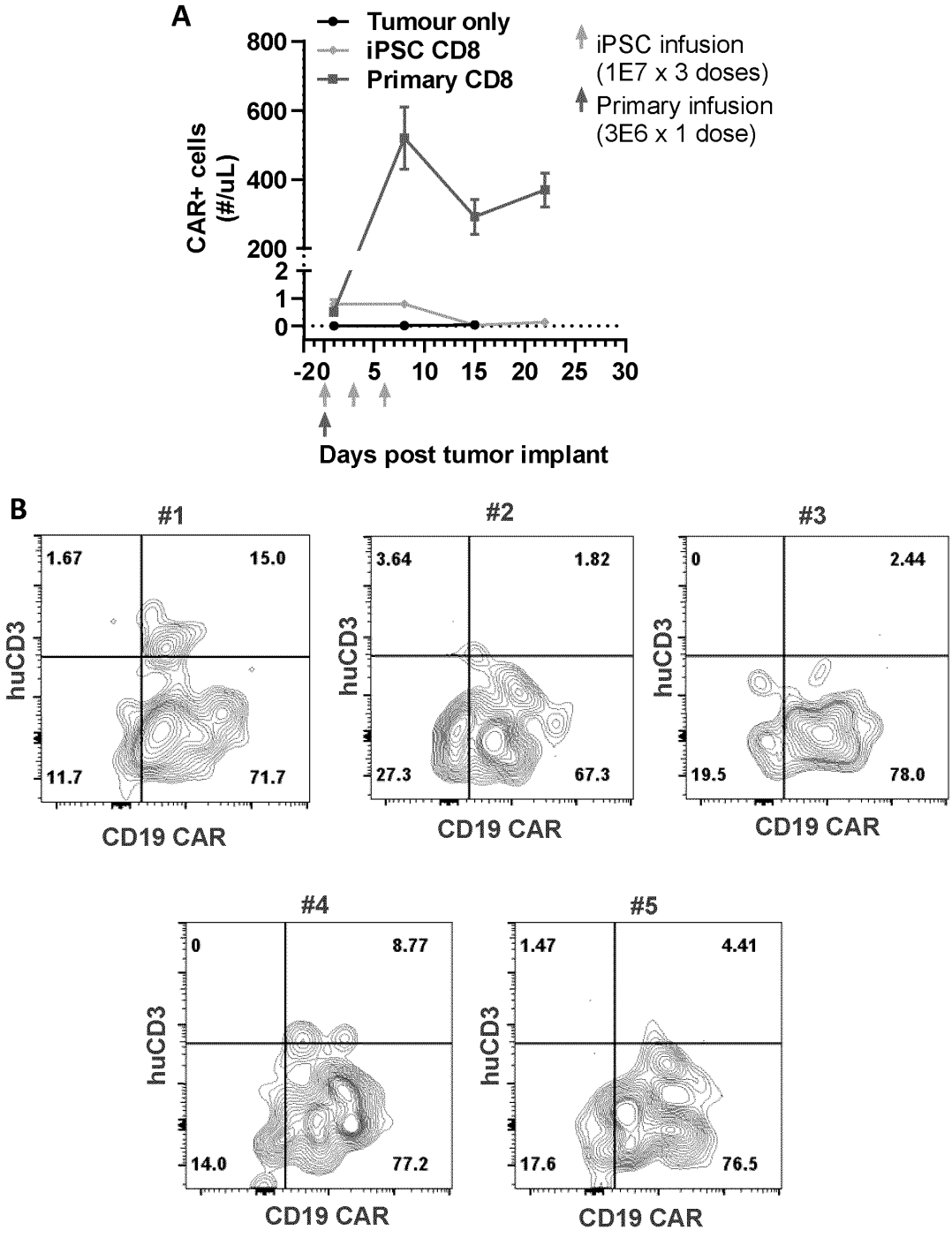

A
Tumour only
iPSC CD8
Primary CD8
iPSC infusion
(1E7 x 3 doses)
Primary infusion
(3E6 x 1 dose)
CAR+ cells
(#/uL)
800
600
400
200
2
1
0
-20
5
10
15
20
25
30
Days post tumor implant
B
#1
1.67
15.0
11.7
71.7
#2
3.64
1.82
27.3
67.3
#3
0
2.44
19.5
78.0
#4
0
8.77
14.0
77.2
#5
1.47
4.41
17.6
76.5
huCD3
CD19 CAR

FIGURE 60

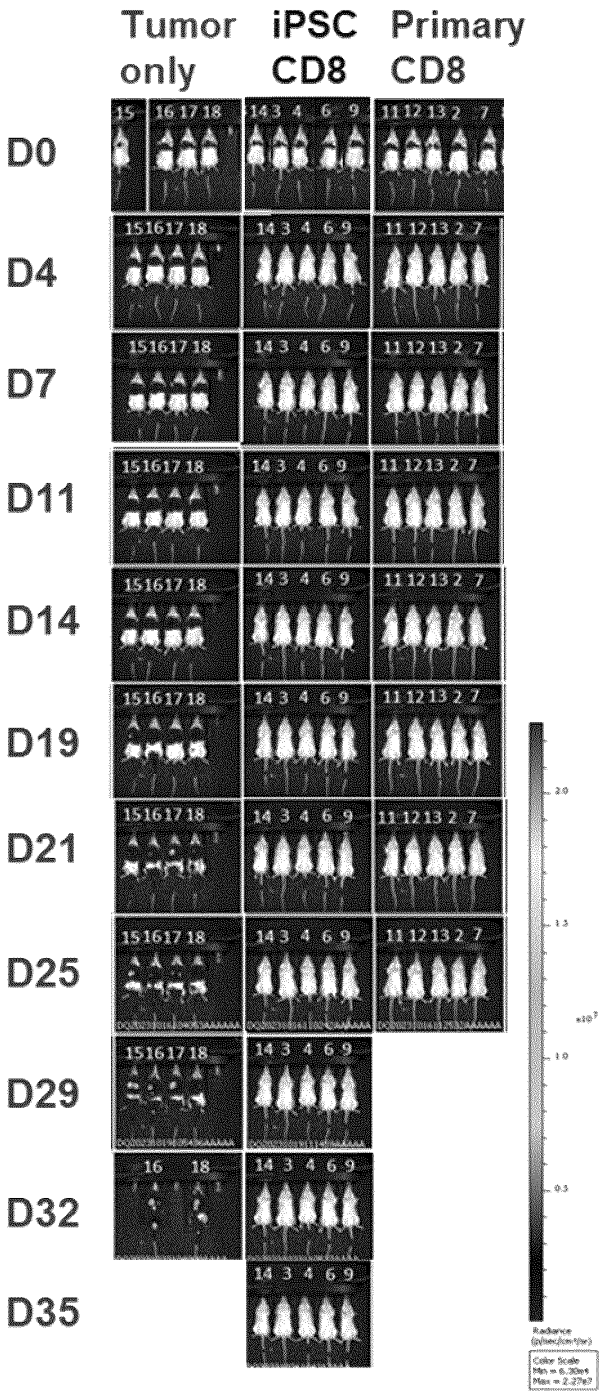

Tumor only
iPSC CD8
Primary CD8
D0
D4
D7
D11
D14
D19
D21
D25
D29
D32
D35

FIGURE 61A

FIGURE 61B

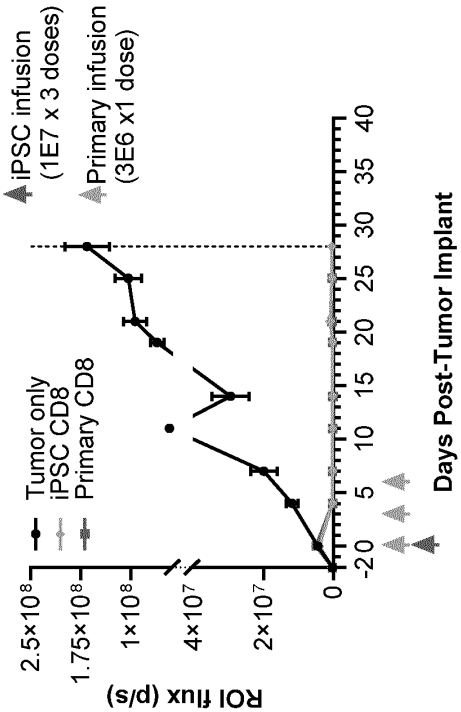



FIGURE 61C

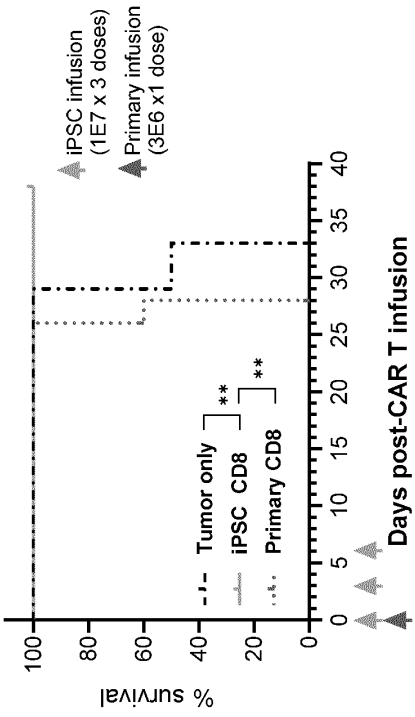



FIGURE 61D

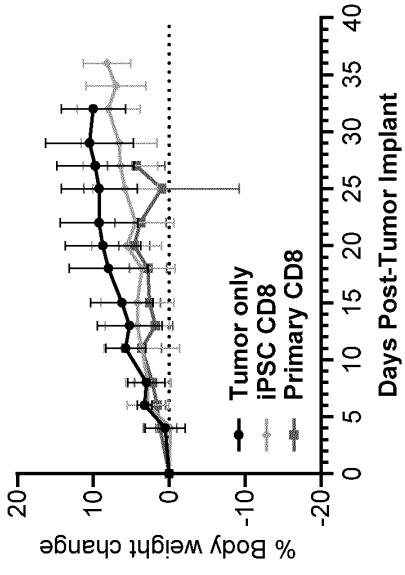
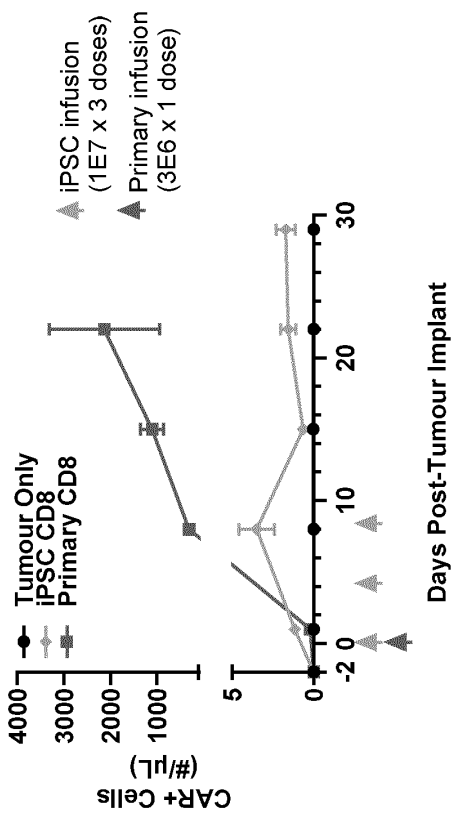
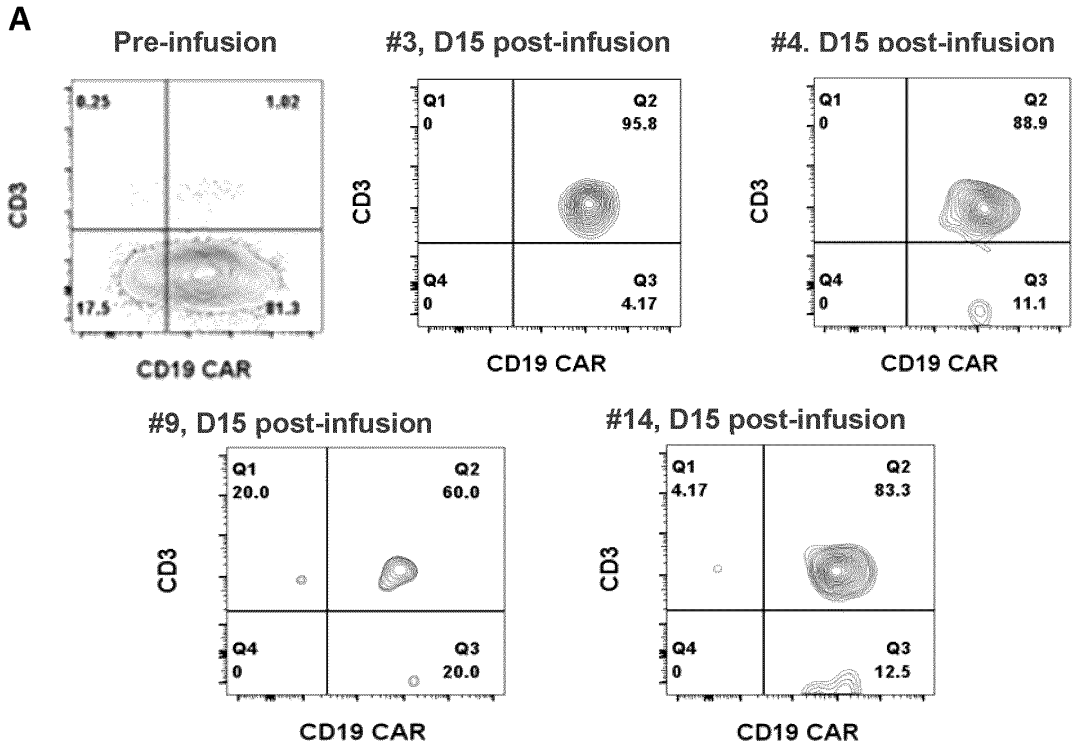



FIGURE 61E




A
Pre-infusion
#3, D15 post-infusion
#4. D15 post-infusion
#9, D15 post-infusion
#14, D15 post-infusion
CD3
CD19 CAR
Q1
Q2
Q3
Q4
0.25
1.02
17.5
81.3
95.8
4.17
88.9
11.1
20.0
60.0
20.0
4.17
83.3
12.5

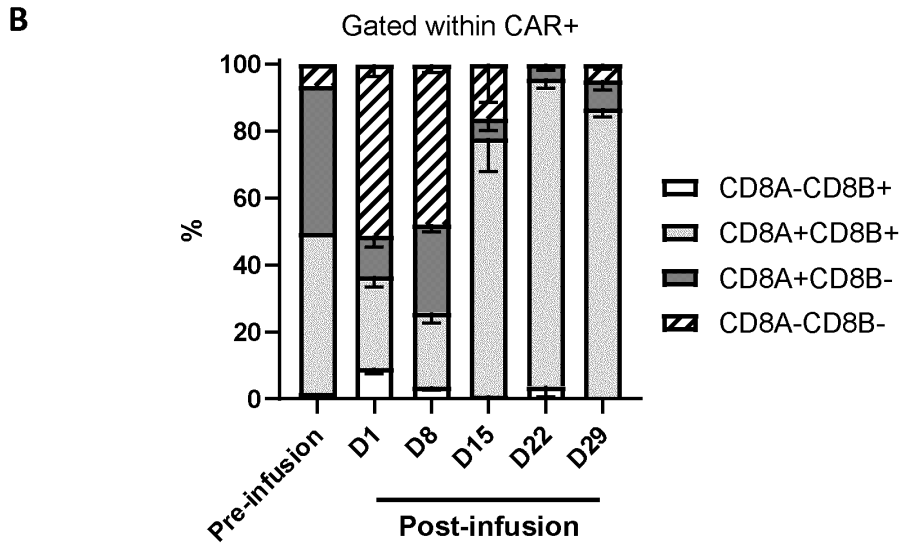

B
Gated within CAR+
%
100
80
60
40
20
0
Pre-infusion
D1
D8
D15
D22
D29
Post-infusion
CD8A-CD8B+
CD8A+CD8B+
CD8A+CD8B-
CD8A-CD8B-

FIGURE 62

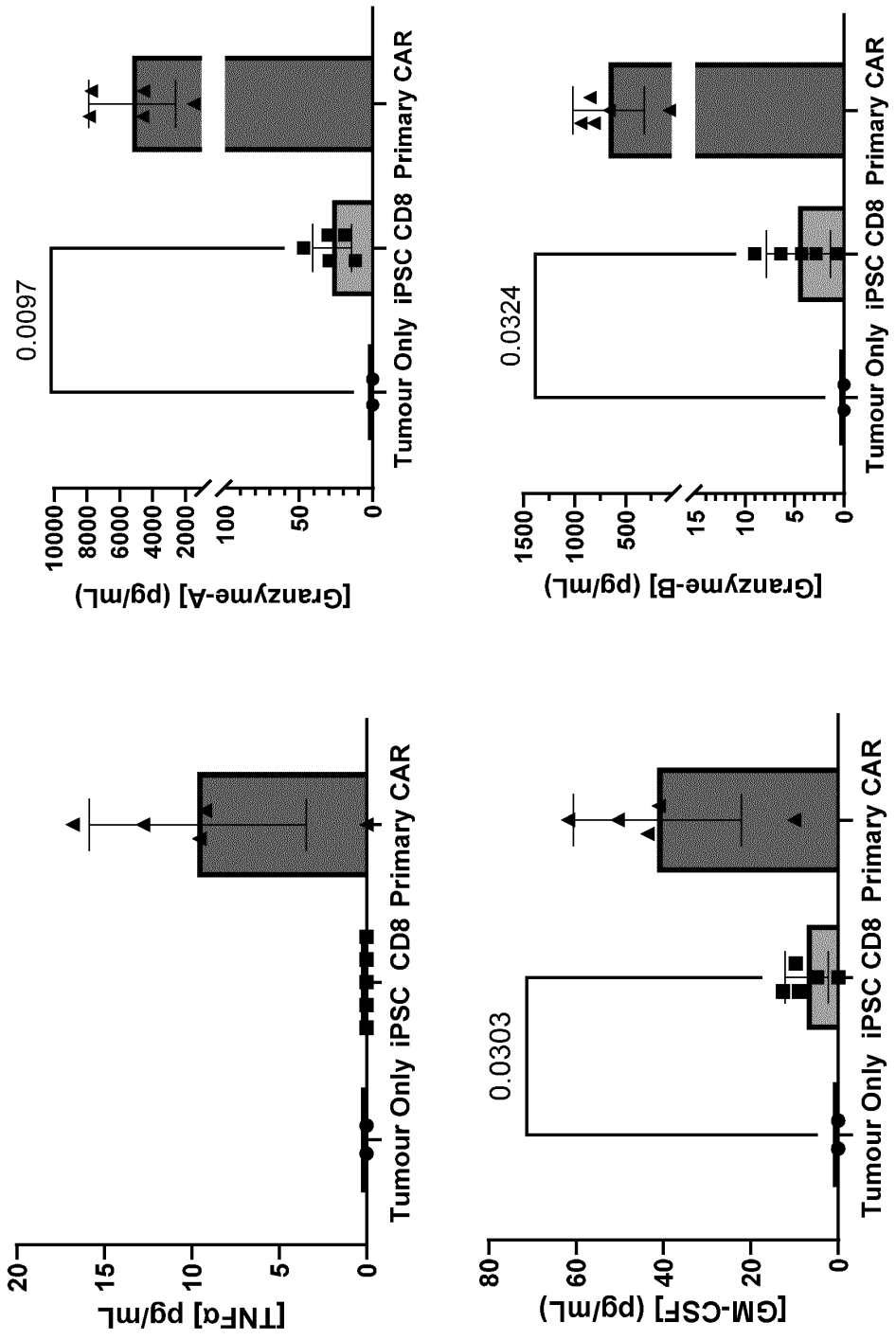
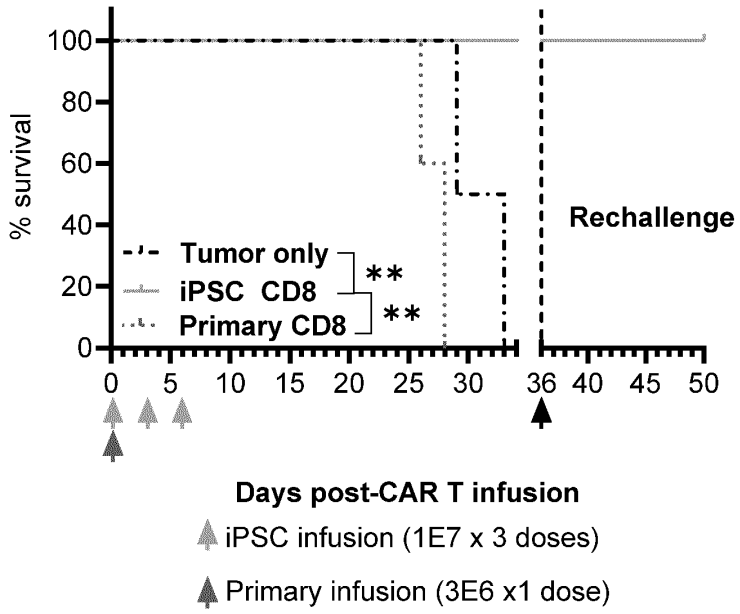

[Granzyme-A] (pg/mL)
10000
8000
6000
4000
2000
100
50
0
0.0097
Tumour Only
iPSC CD8
Primary CAR
[Granzyme-B] (pg/mL)
1500
1000
500
15
10
5
0
0.0324
Tumour Only
iPSC CD8
Primary CAR
[TNFα] pg/mL
20
15
10
5
0
Tumour Only
iPSC CD8
Primary CAR
[GM-CSF] (pg/mL)
80
60
40
20
0
0.0303
Tumour Only
iPSC CD8
Primary CAR

FIGURE 63

A


100
80
60
40
20
0
% survival
Tumor only
iPSC CD8
Primary CD8
**
**
Rechallenge
0 5 10 15 20 25 30 36 40 45 50
Days post-CAR T infusion
iPSC infusion (1E7 x 3 doses)
Primary infusion (3E6 x1 dose)

B

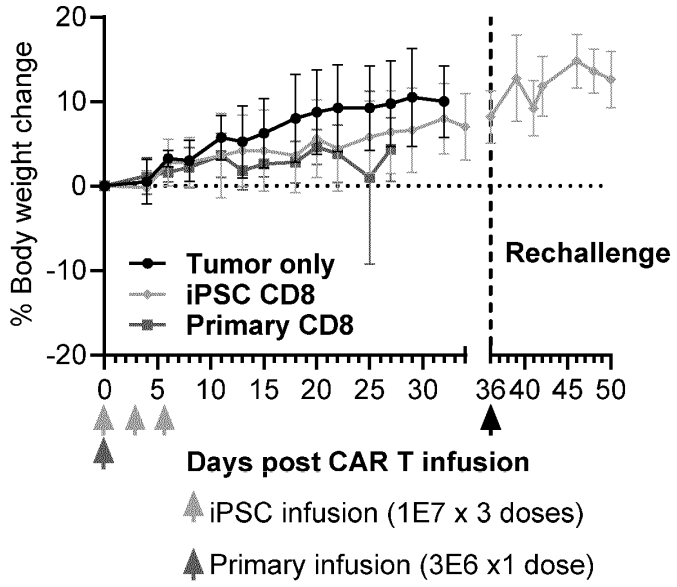

20
10
0
-10
-20
% Body weight change
Tumor only
iPSC CD8
Primary CD8
Rechallenge
0 5 10 15 20 25 30 36 40 45 50
Days post CAR T infusion
iPSC infusion (1E7 x 3 doses)
Primary infusion (3E6 x1 dose)

FIGURE 64

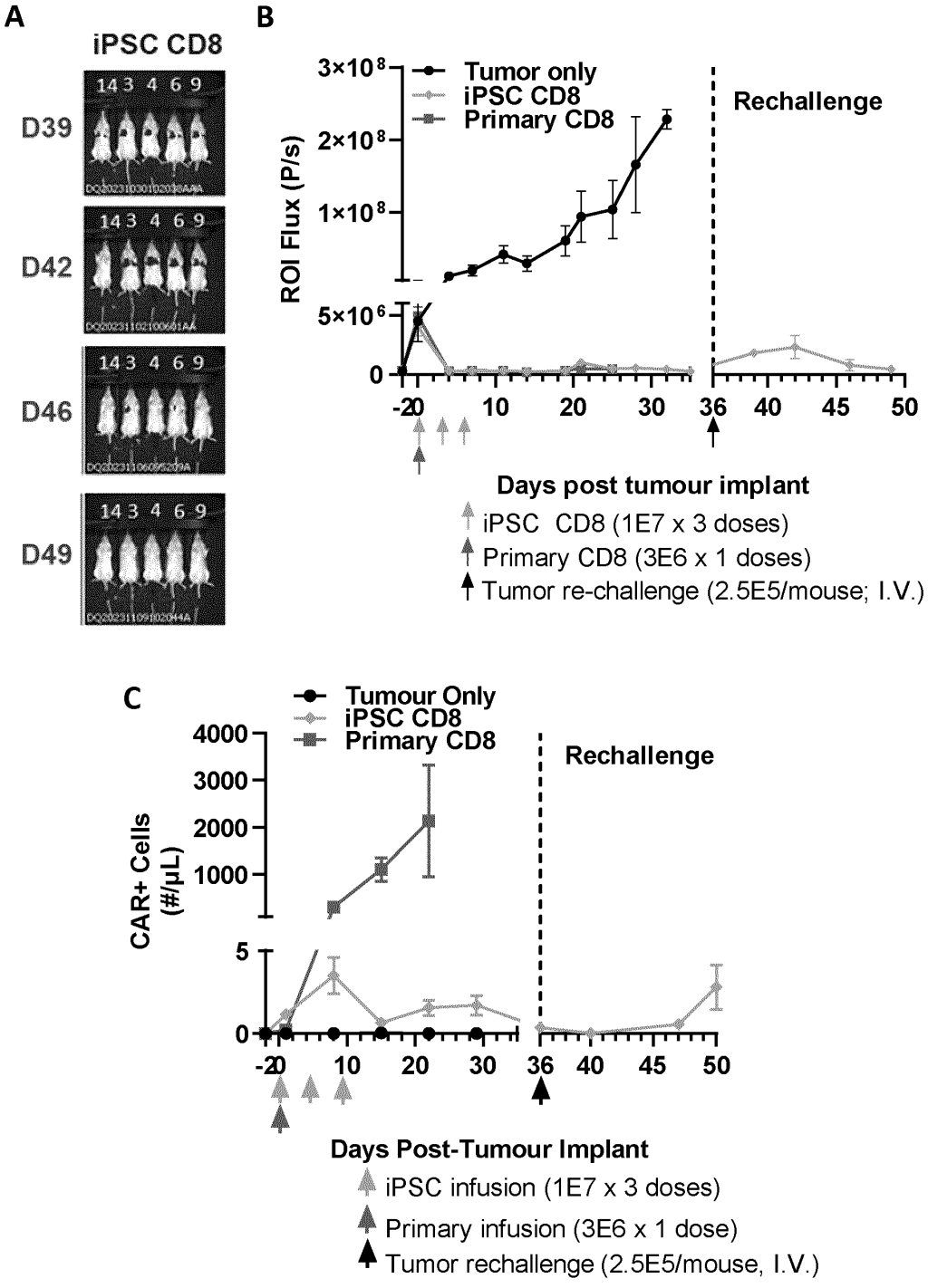

A
iPSC CD8
D39
D42
D46
D49
B
Tumor only
iPSC CD8
Primary CD8
Rechallenge
ROI Flux (P/s)
Days post tumour implant
iPSC CD8 (1E7 x 3 doses)
Primary CD8 (3E6 x 1 doses)
Tumor re-challenge (2.5E5/mouse; I.V.)
C
Tumour Only
iPSC CD8
Primary CD8
Rechallenge
CAR+ Cells (#/µL)
Days Post-Tumour Implant
iPSC infusion (1E7 x 3 doses)
Primary infusion (3E6 x 1 dose)
Tumor rechallenge (2.5E5/mouse, I.V.)

FIGURE 65

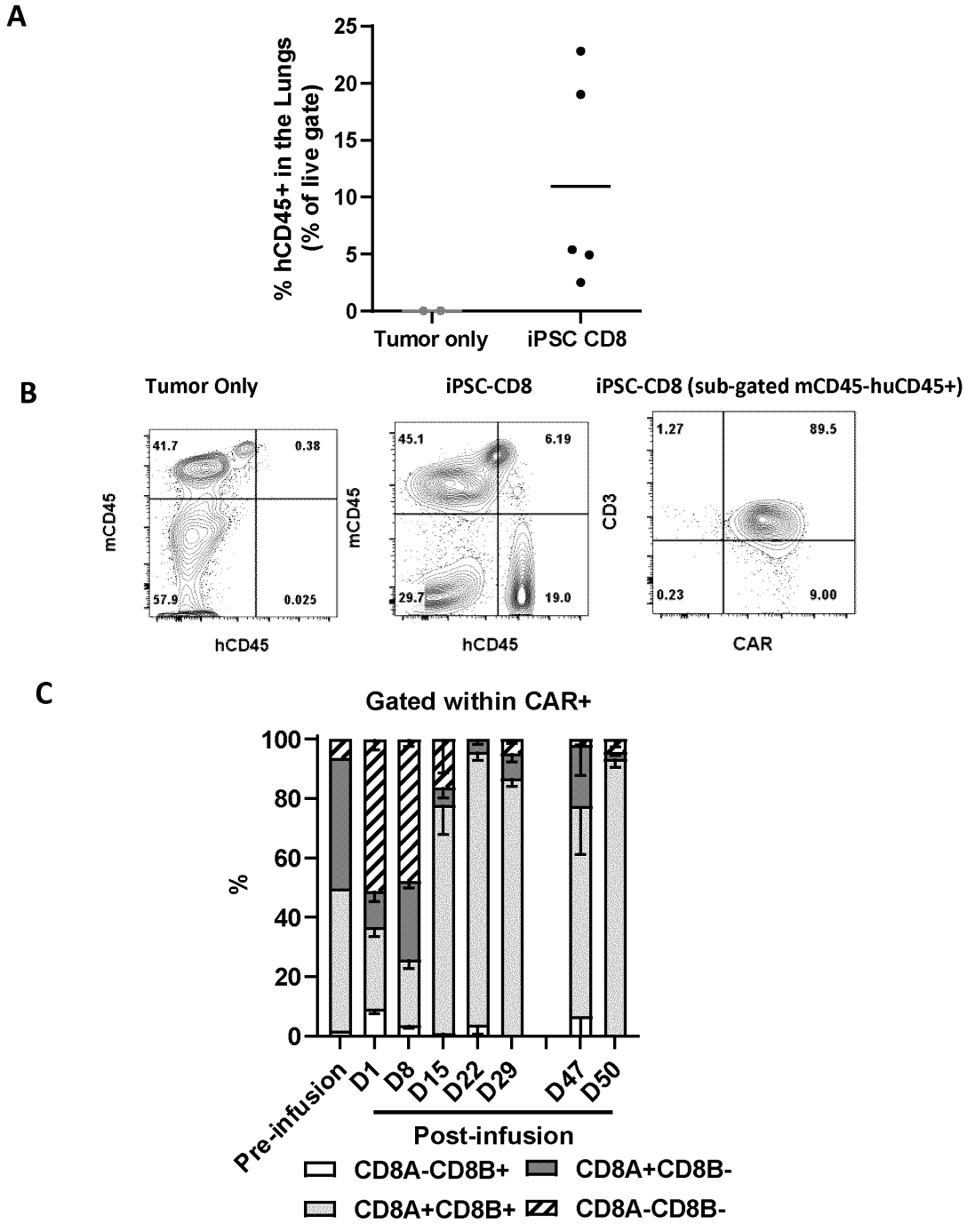

A
% hCD45+ in the Lungs (% of live gate)
25
20
15
10
5
0
Tumor only
iPSC CD8
B
Tumor Only
iPSC-CD8
iPSC-CD8 (sub-gated mCD45-huCD45+)
mCD45
hCD45
41.7
0.38
57.9
0.025
45.1
6.19
29.7
19.0
CD3
CAR
1.27
89.5
0.23
9.00
C
Gated within CAR+
%
100
80
60
40
20
0
Pre-infusion
D1
D8
D15
D22
D29
D47
D50
Post-infusion
CD8A-CD8B+
CD8A+CD8B-
CD8A+CD8B+
CD8A-CD8B-

FIGURE 66

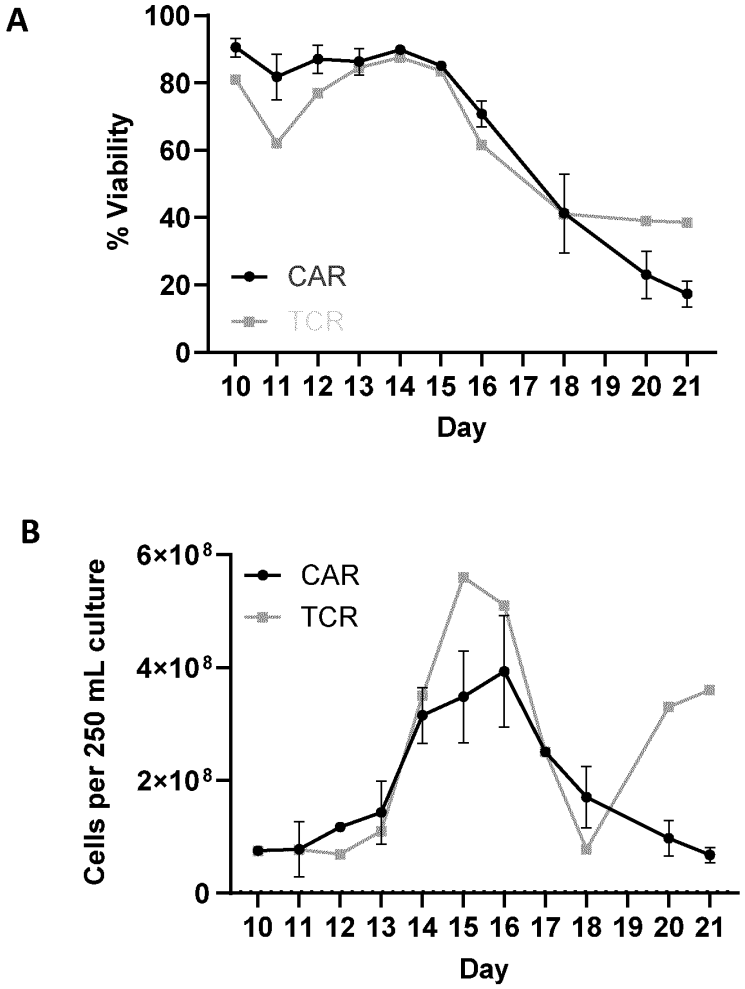

A
100
80
60
40
20
0
% Viability
CAR
TCR
10 11 12 13 14 15 16 17 18 19 20 21
Day
B
6×10⁸
4×10⁸
2×10⁸
0
Cells per 250 mL culture
CAR
TCR
10 11 12 13 14 15 16 17 18 19 20 21
Day

FIGURE 67

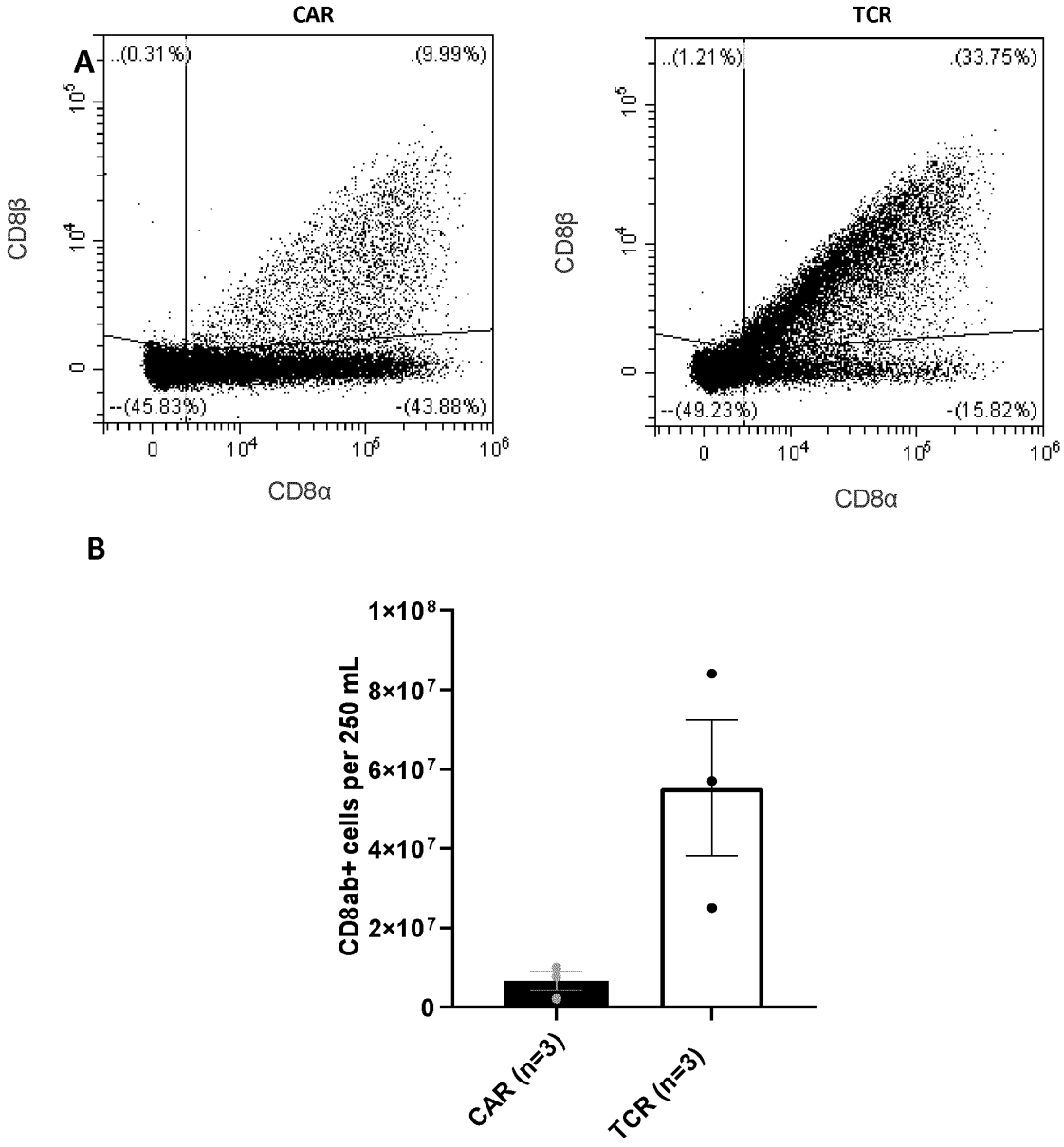

A
CAR
TCR
..(0.31%)
.(9.99%)
--(45.83%)
-(43.88%)
..(1.21%)
.(33.75%)
--(49.23%)
-(15.82%)
CD8β
CD8α
0
10^4
10^5
10^6
B
CD8ab+ cells per 250 mL
1×10^8
8×10^7
6×10^7
4×10^7
2×10^7
0
CAR (n=3)
TCR (n=3)

FIGURE 68

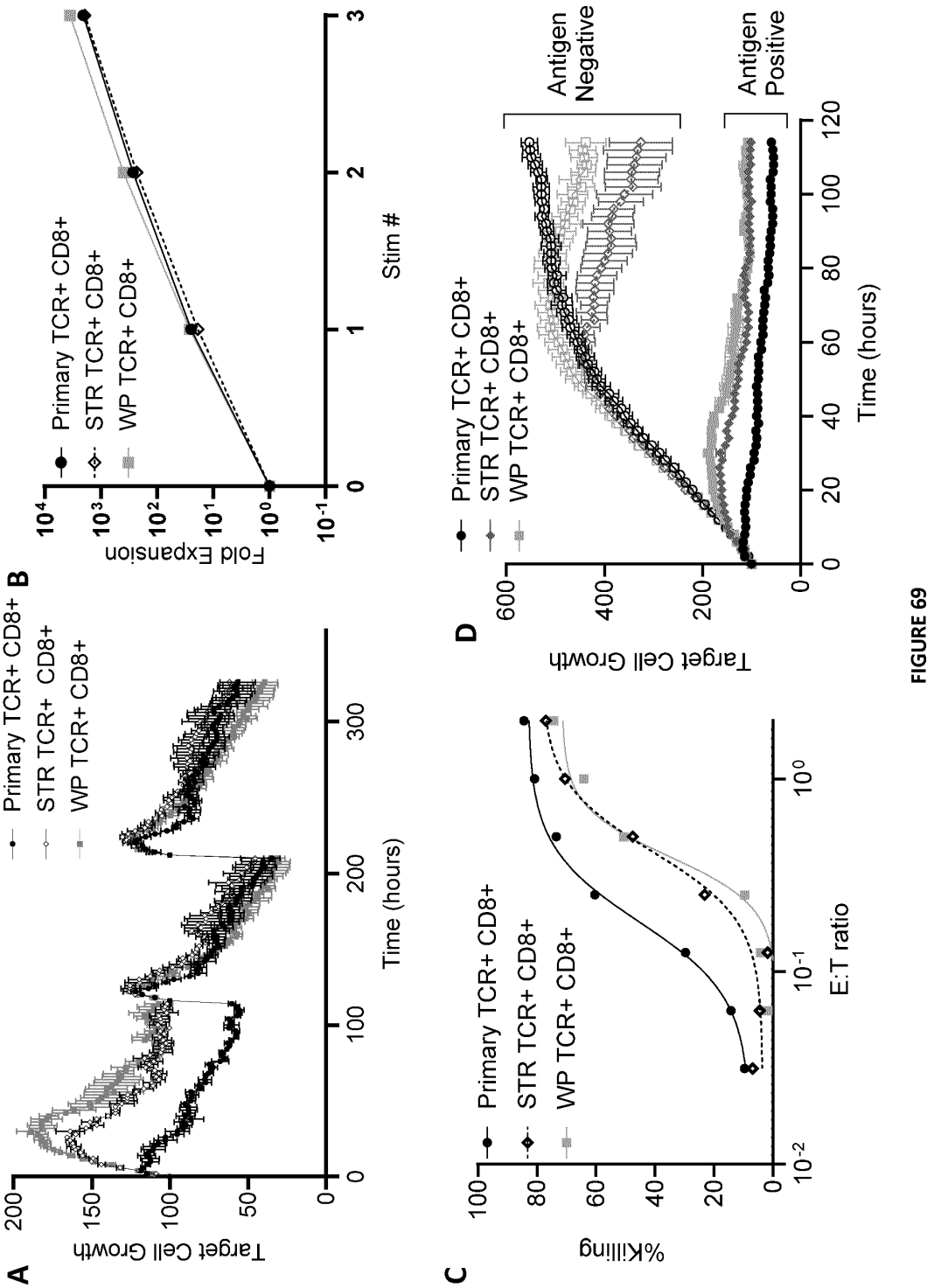

A
Target Cell Growth
Time (hours)
Primary TCR+ CD8+
STR TCR+ CD8+
WP TCR+ CD8+
B
Fold Expansion
Stim #
C
%Killing
E:T ratio
D
Target Cell Growth
Time (hours)
Antigen Negative
Antigen Positive

FIGURE 69