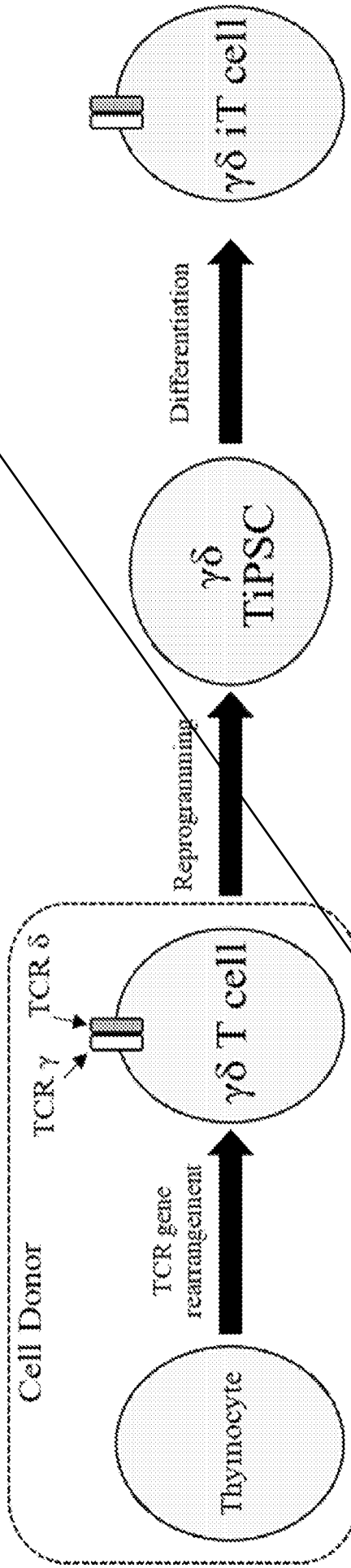

Cell Donor
Thymocyte
TCR gene rearrangement
γδ T cell
TCR γ
TCR δ
Reprogramming
γδ TiPSC
Differentiation
γδ iT cell

FIG. 1A

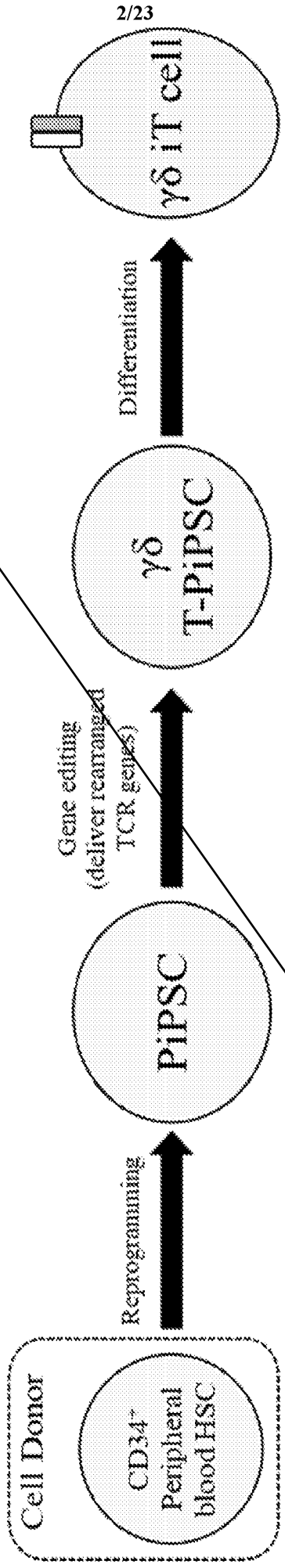

Cell Donor
CD34+ Peripheral blood HSC
Reprogramming
PiPSC
Gene editing (deliver rearranged TCR genes)
γδ T-PiPSC
Differentiation
γδ iT cell

FIG. 1B

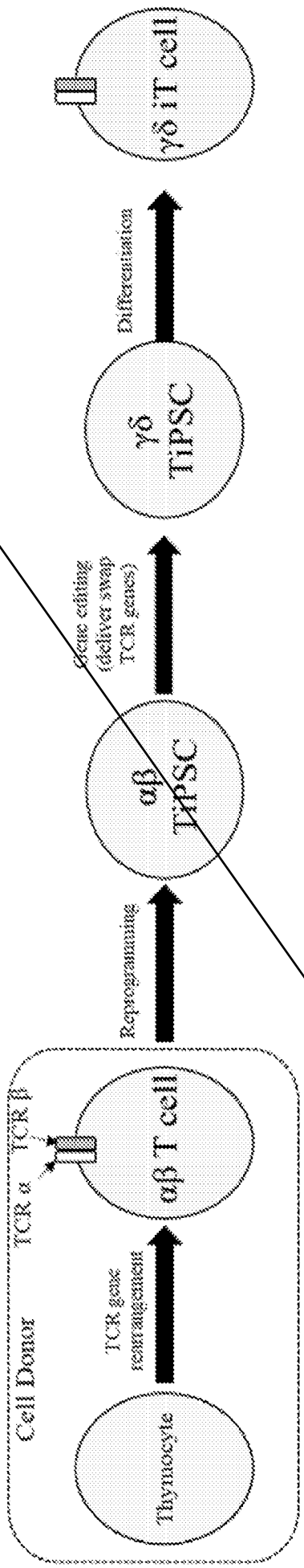

Cell Donor
Thymocyte
TCR gene rearrangement
αβ T cell
TCR α
TCR β
Reprogramming
αβ TiPSC
Gene editing (deliver swap TCR genes)
γδ TiPSC
Differentiation
γδ iT cell

FIG. 1C

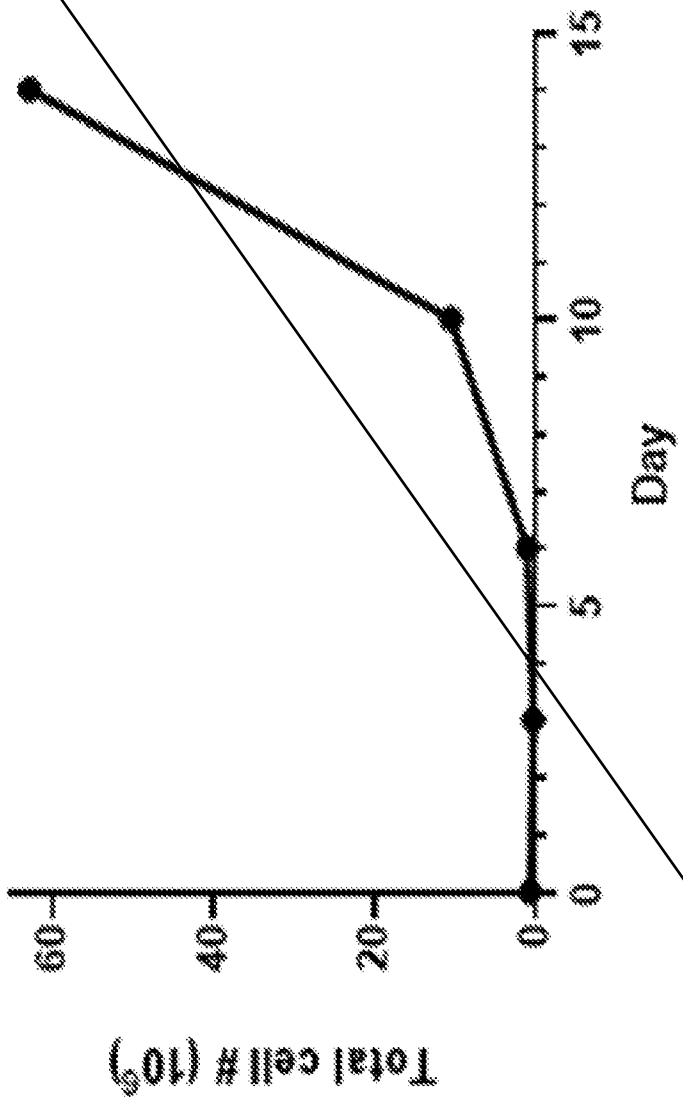


FIG. 2B

TiPSC ID	Vγ9	Vδ2	Vδ1	TCR Pair
gdTiPSC4.1	+	+	-	Vγ9/Vδ2
gdTiPSC4.2	-	-	-	Vγ?/Vδ?
gdTiPSC4.3	+	-	+	Vγ9/Vδ1
gdTiPSC4.4	+	+	-	Vγ9/Vδ2

FIG. 2C

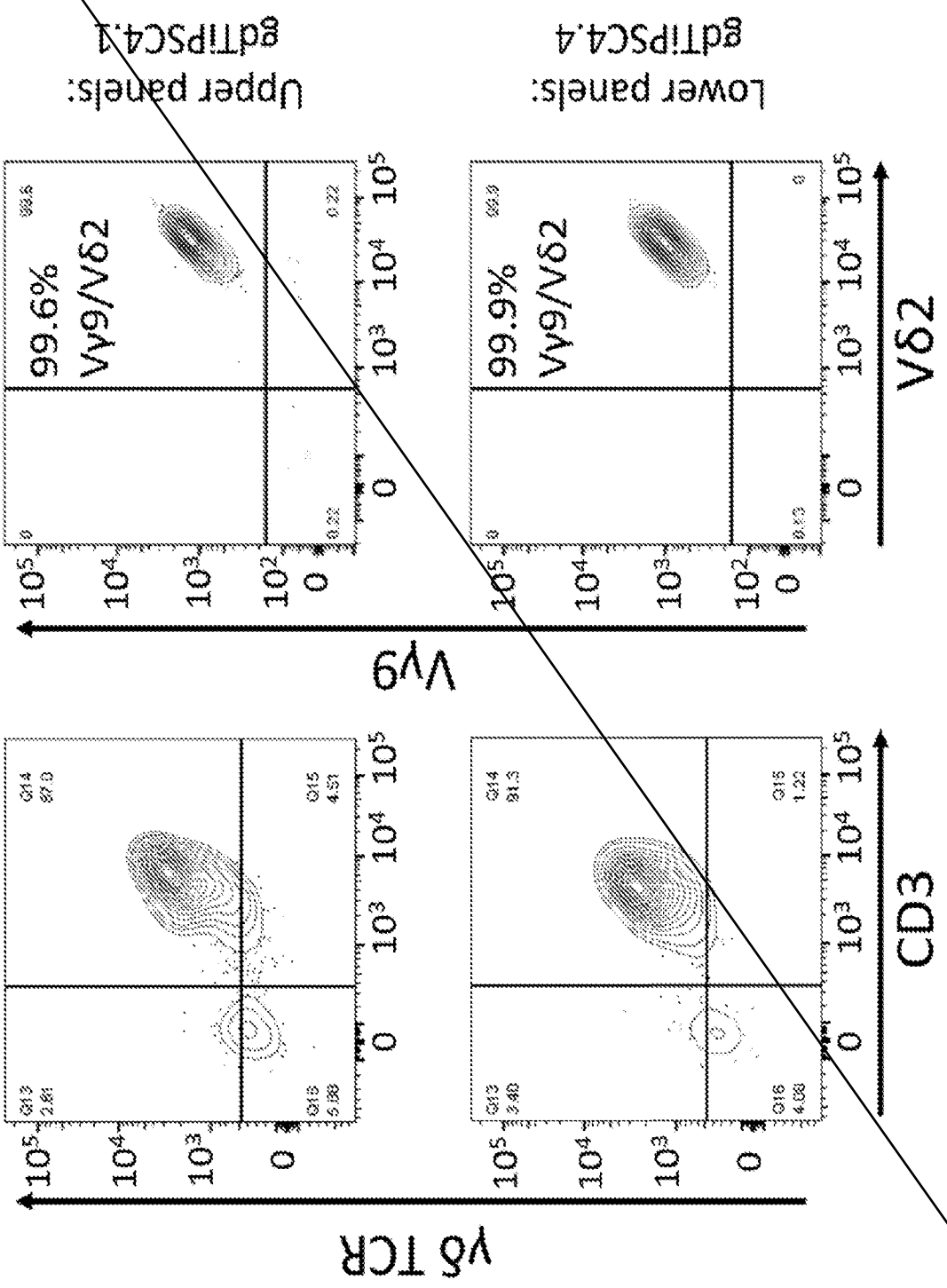

Upper panels:
γδTiPSC4.1
Lower panels:
γδTiPSC4.4
99.6%
Vγ9/Vδ2
99.9%
Vγ9/Vδ2
Vγ9
Vδ2
γδ TCR
CD3

FIG. 2D

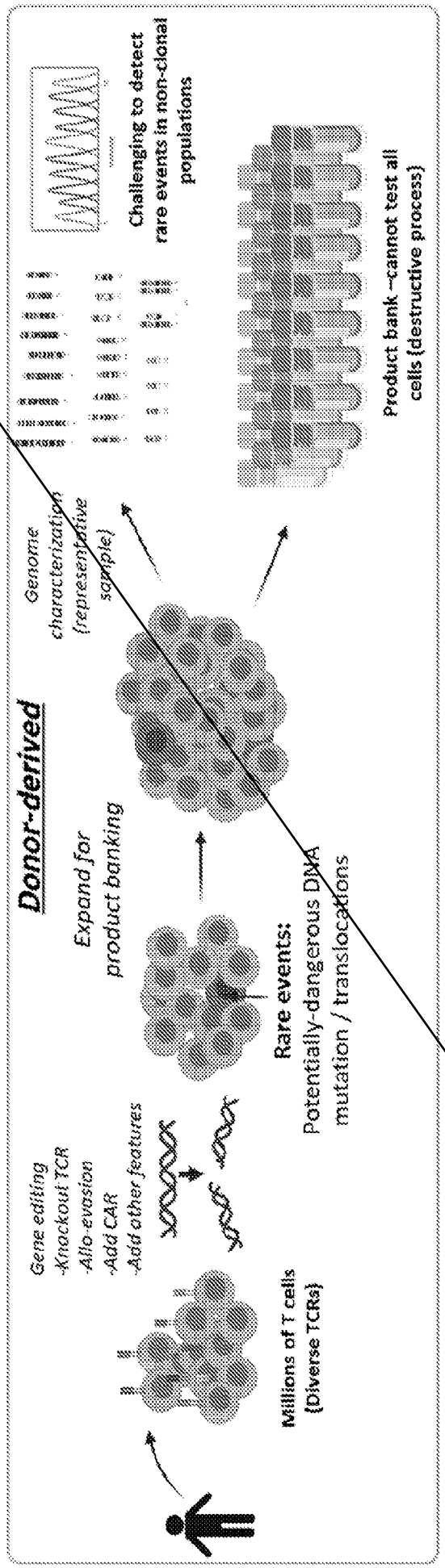

Donor-derived
Gene editing
-Knockout TCR
-Allo-evasion
-Add CAR
-Add other features
Millions of T cells
(Diverse TCRs)
Expand for
product banking
Rare events:
Potentially-dangerous DNA
mutation / translocations
Genome
characterization
(representative
sample)
Challenging to detect
rare events in non-clonal
populations
Product bank –cannot test all
cells (destructive process)

FIG. 4A

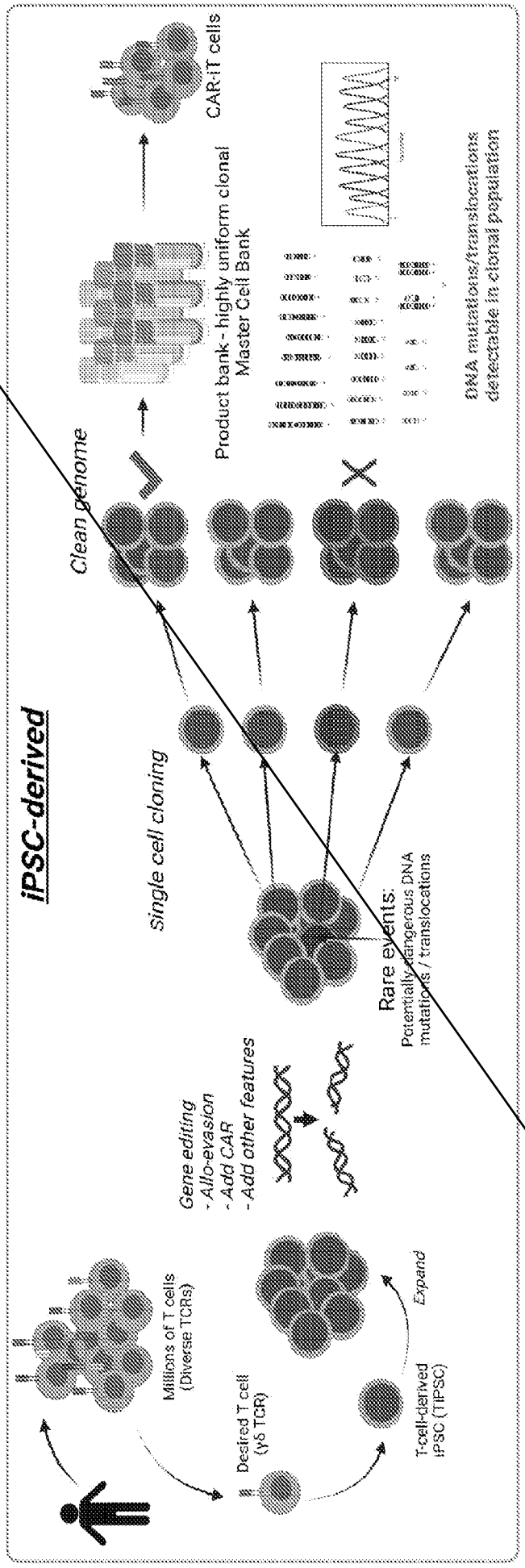

iPSC-derived
Millions of T cells (Diverse TCRs)
Desired T cell (γδ TCR)
T-cell-derived iPSC (TiPSC)
Expand
Gene editing
- Allo-evasion
- Add CAR
- Add other features
Single cell cloning
Rare events:
Potentially dangerous DNA mutations / translocations
Clean genome
Product bank - highly uniform clonal Master Cell Bank
DNA mutations/translocations detectable in clonal population
CAR-iT cells

FIG. 4B

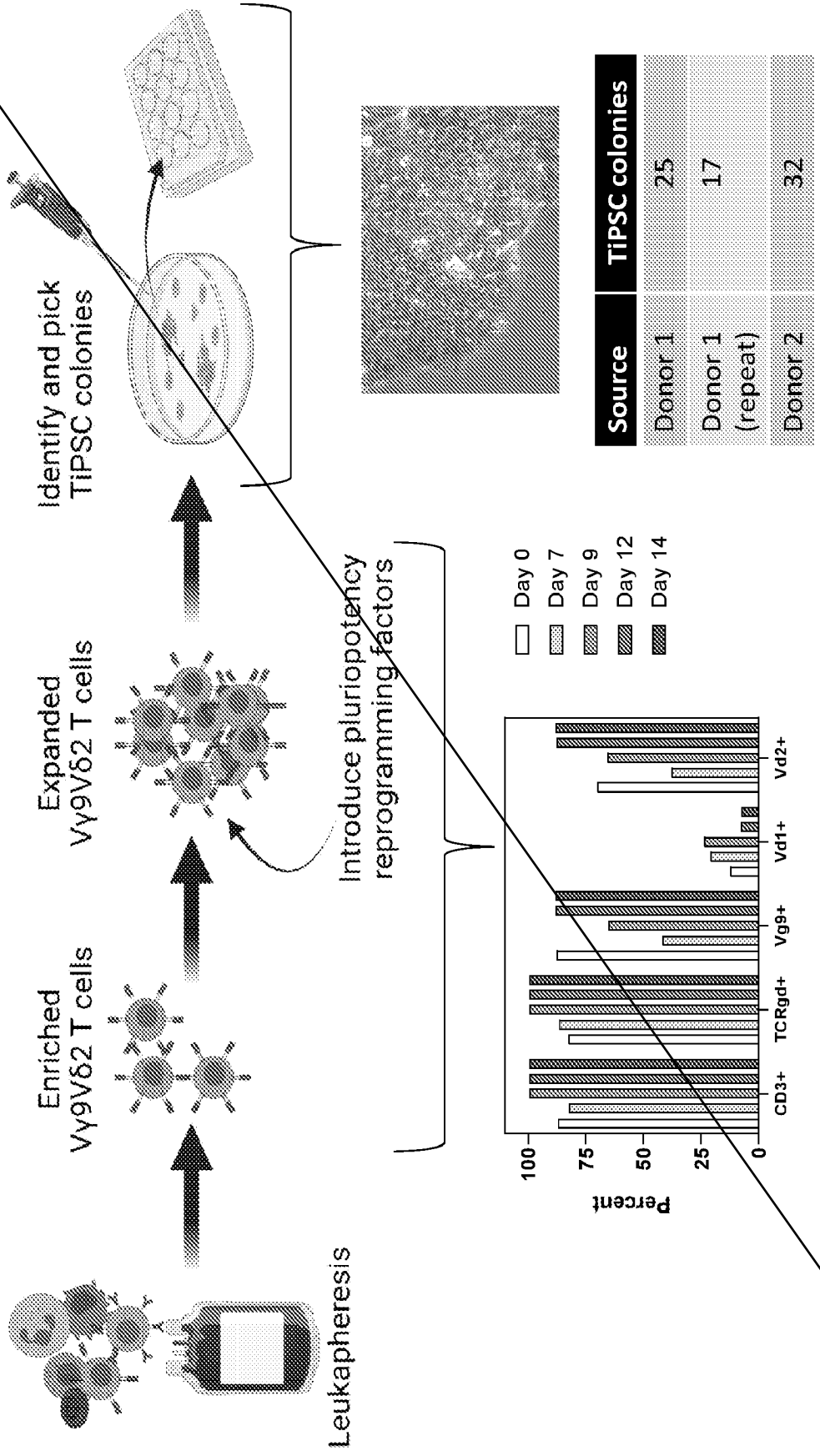

Leukapheresis
Enriched Vγ9Vδ2 T cells
Expanded Vγ9Vδ2 T cells
Introduce pluriopotency reprogramming factors
Identify and pick TiPSC colonies
Day 0
Day 7
Day 9
Day 12
Day 14
Percent
100
75
50
25
0
CD3+
TCRgd+
Vg9+
Vd1+
Vd2+
Source
TiPSC colonies
Donor 1
25
Donor 1 (repeat)
17
Donor 2
32

FIG. 6

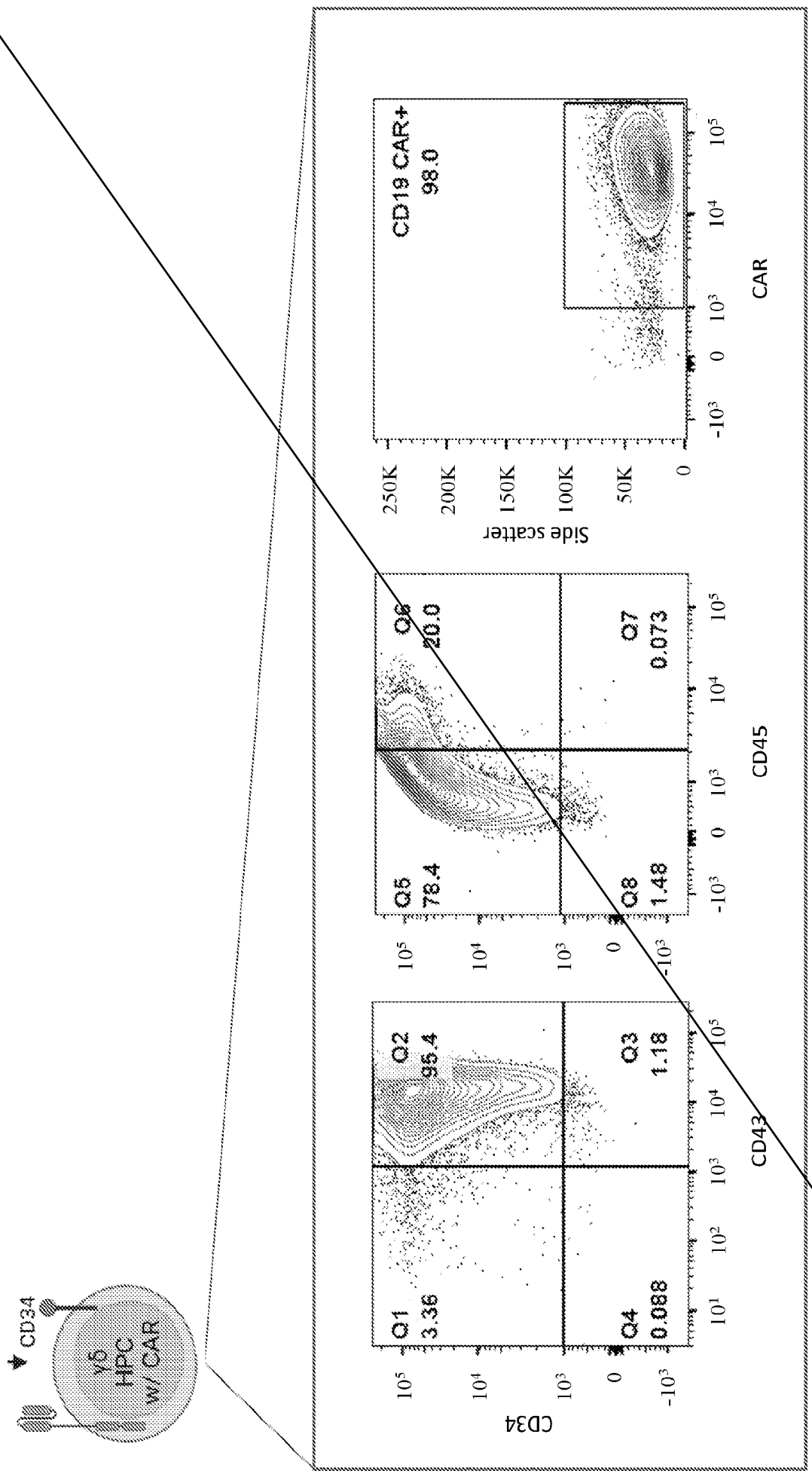

CD34
γδ HPC w/ CAR
CD34
CD43
Q1 3.36
Q2 95.4
Q3 1.18
Q4 0.088
CD45
Q5 78.4
Q6 20.0
Q7 0.073
Q8 1.48
Side scatter
CD19 CAR+ 98.0
CAR

FIG. 7B

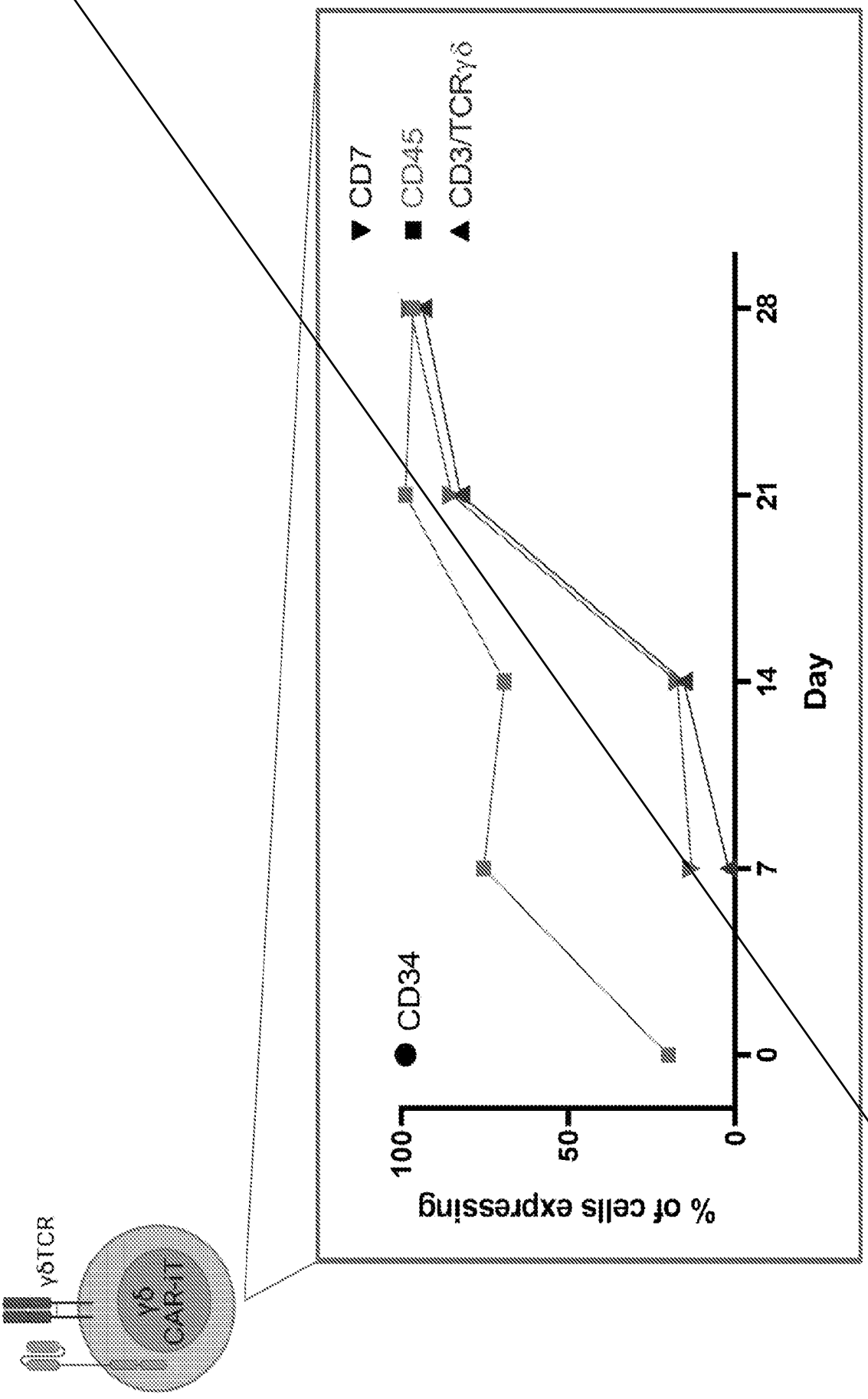

γδTCR
γδ CAR-T
% of cells expressing
100
50
0
CD34
CD7
CD45
CD3/TCRγδ
0
7
14
21
28
Day

FIG. 7C

Day 28 iT phenotypes of two TiPSC lines
TiPSC line A
TRGV2 / TRDV1
TiPSC line B
TRGV9 / TRDV2
CD4
CD8α
CD8
CD8α
Side scatter
CD19 CAR+
87.2
CD19 CAR+
98.2
CD19 CAR
(CD3z CD28)
Q13 0.59
Q14 0.051
Q15 18.5
Q16 80.8
Q13 0.99
Q14 2.09
Q15 55.2
Q16 41.7
Q21 4.29
Q22 1.89
Q23 15.3
Q24 78.5
Q21 8.13
Q22 29.3
Q23 25.4
Q24 37.2

FIG. 7D

FIG. 7D (continued)

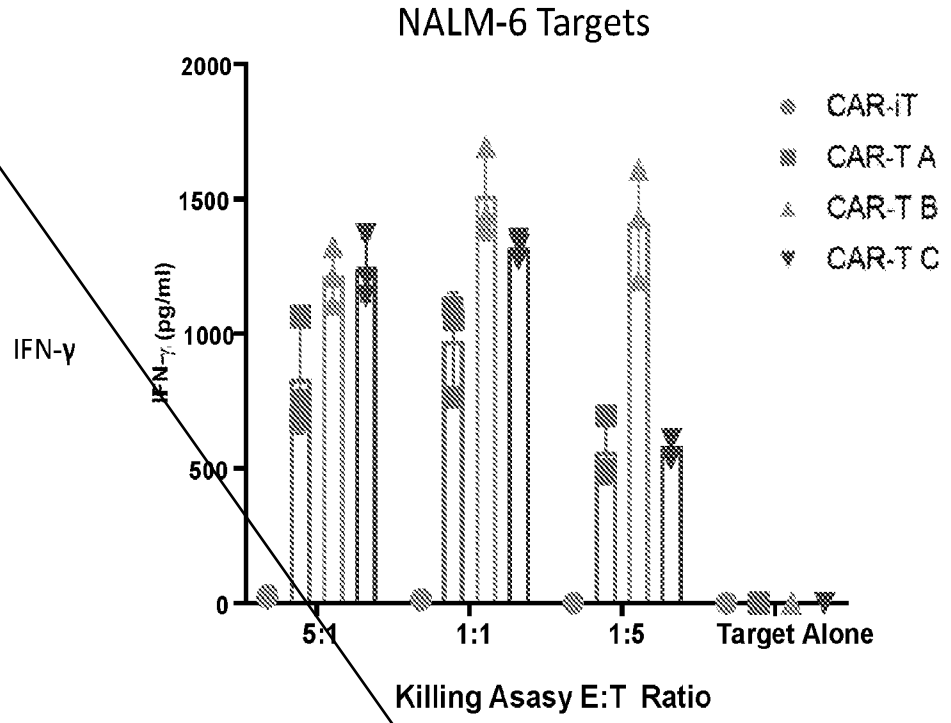

NALM-6 Targets
IFN-γ
IFN-γ (pg/ml)
2000
1500
1000
500
0
5:1
1:1
1:5
Target Alone
Killing Asasy E:T Ratio
CAR-iT
CAR-T A
CAR-T B
CAR-T C

FIG. 8E

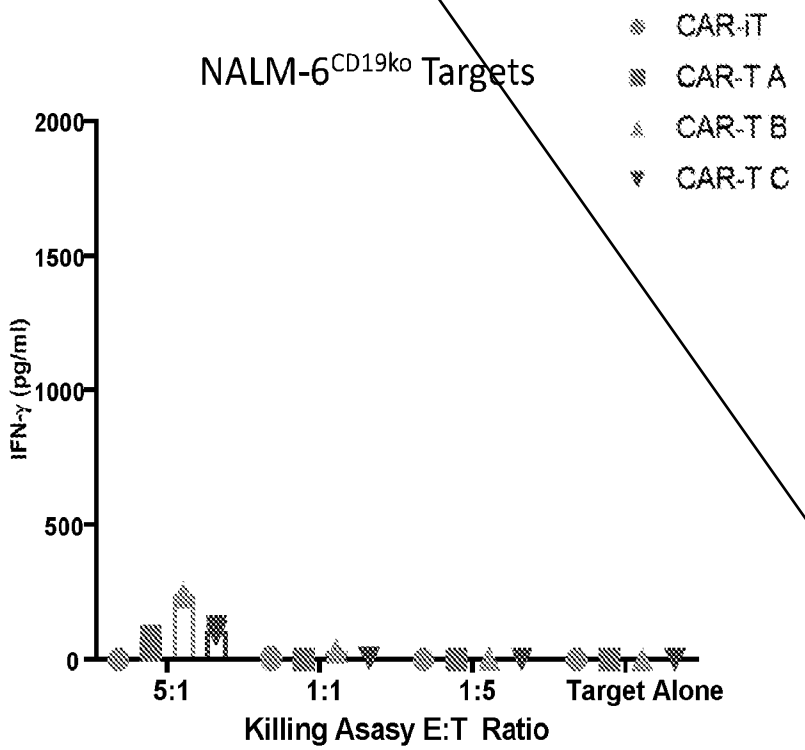

NALM-6CD19ko Targets
IFN-γ (pg/ml)
2000
1500
1000
500
0
5:1
1:1
1:5
Target Alone
Killing Asasy E:T Ratio
CAR-iT
CAR-T A
CAR-T B
CAR-T C

FIG. 8F

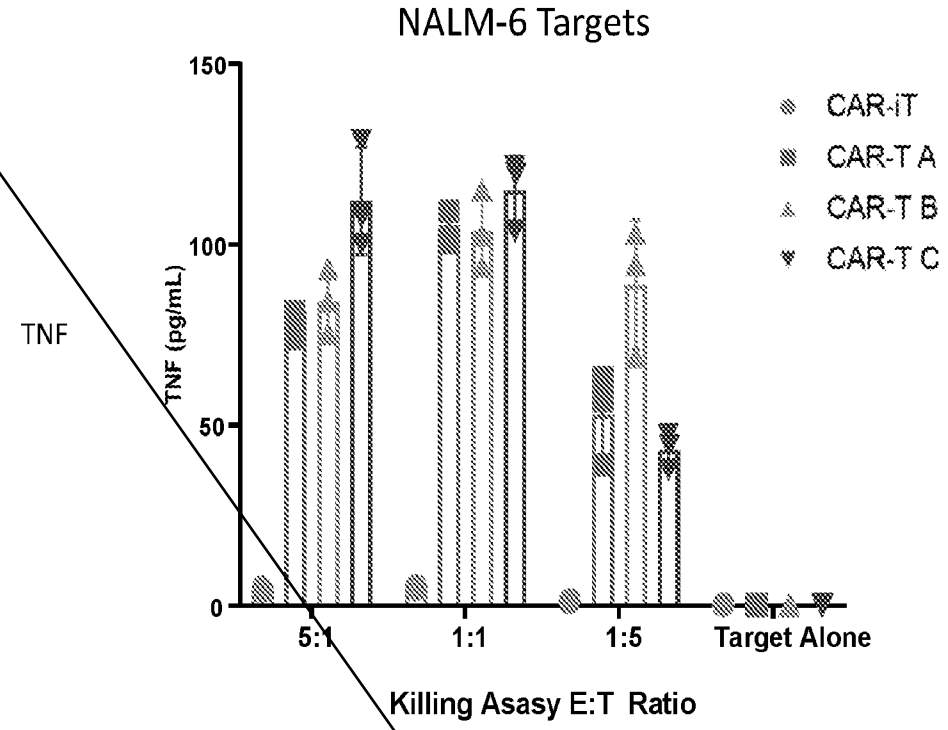

NALM-6 Targets
TNF
TNF (pg/mL)
150
100
50
0
CAR-iT
CAR-T A
CAR-T B
CAR-T C
5:1
1:1
1:5
Target Alone
Killing Asasy E:T Ratio

FIG. 8G

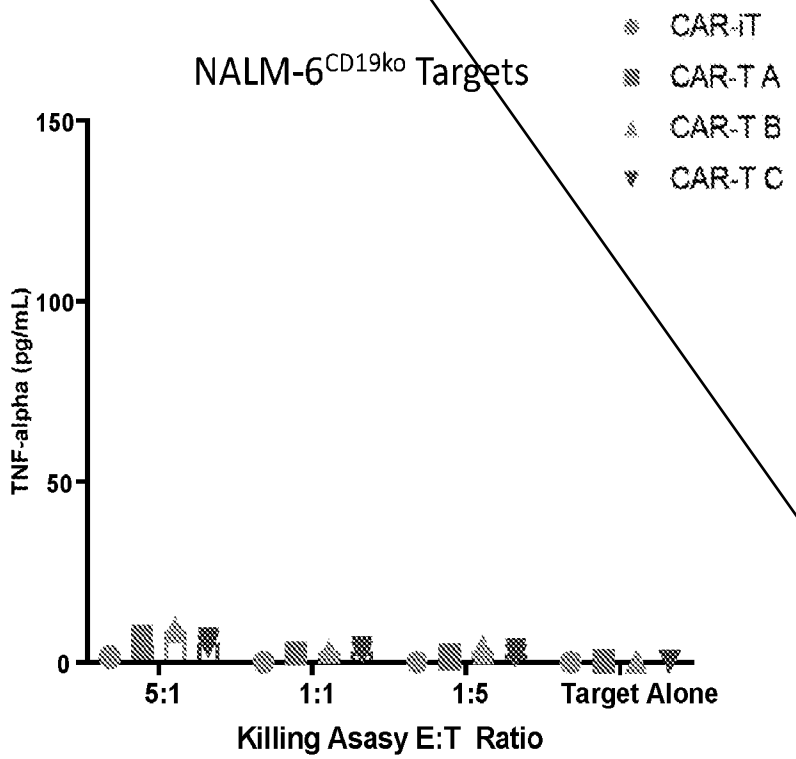

NALM-6CD19ko Targets
TNF-alpha (pg/mL)
150
100
50
0
CAR-iT
CAR-T A
CAR-T B
CAR-T C
5:1
1:1
1:5
Target Alone
Killing Asasy E:T Ratio

FIG. 8H

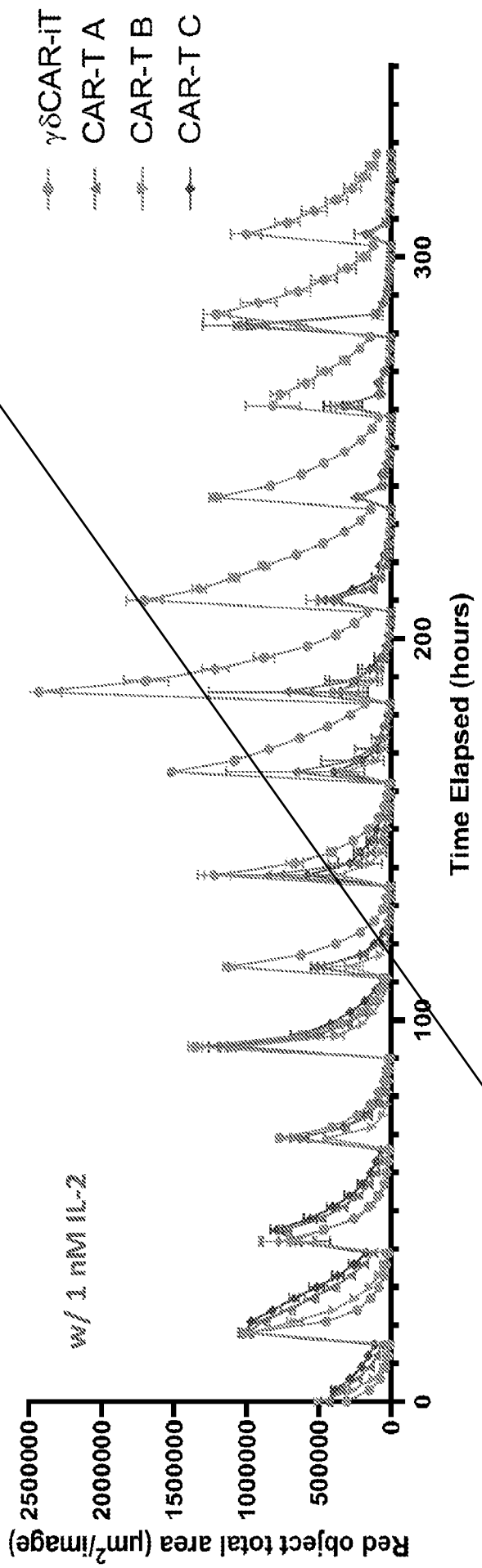

γδCAR-iT
CAR-T A
CAR-T B
CAR-T C
w/ 1 nM IL-2
Red object total area (μm²/image)
2500000
2000000
1500000
1000000
500000
0
0
100
200
300
Time Elapsed (hours)

FIG. 8K

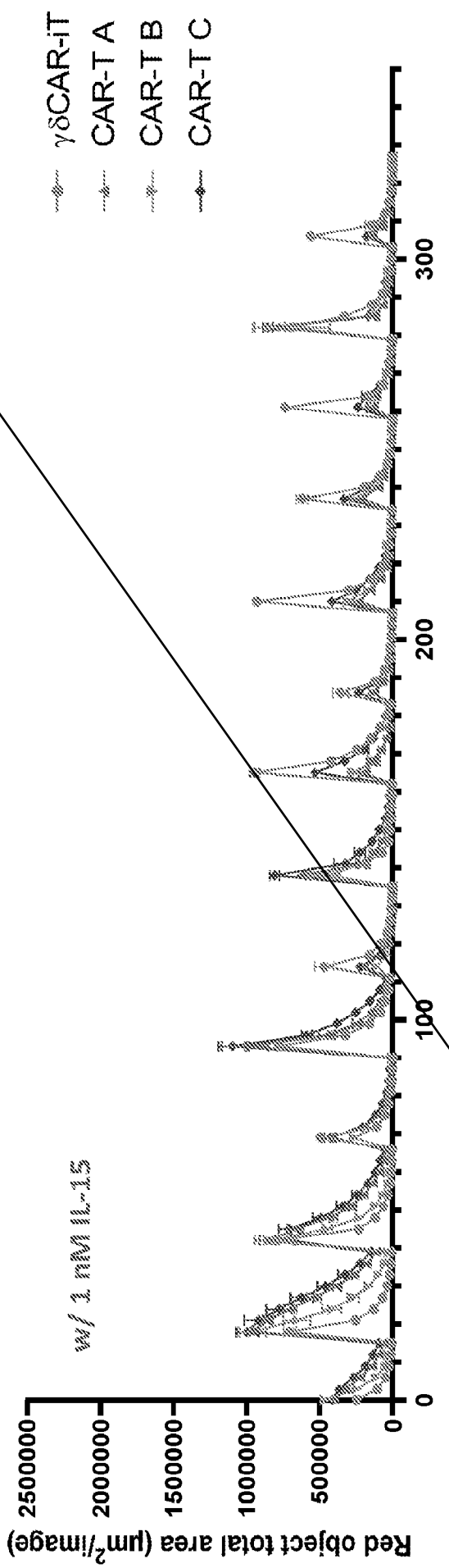

γδCAR-iT
CAR-T A
CAR-T B
CAR-T C
w/ 1 nM IL-15
Red object total area (μm²/image)
2500000
2000000
1500000
1000000
500000
0
0
100
200
300
Time Elapsed (hours)

FIG. 8L

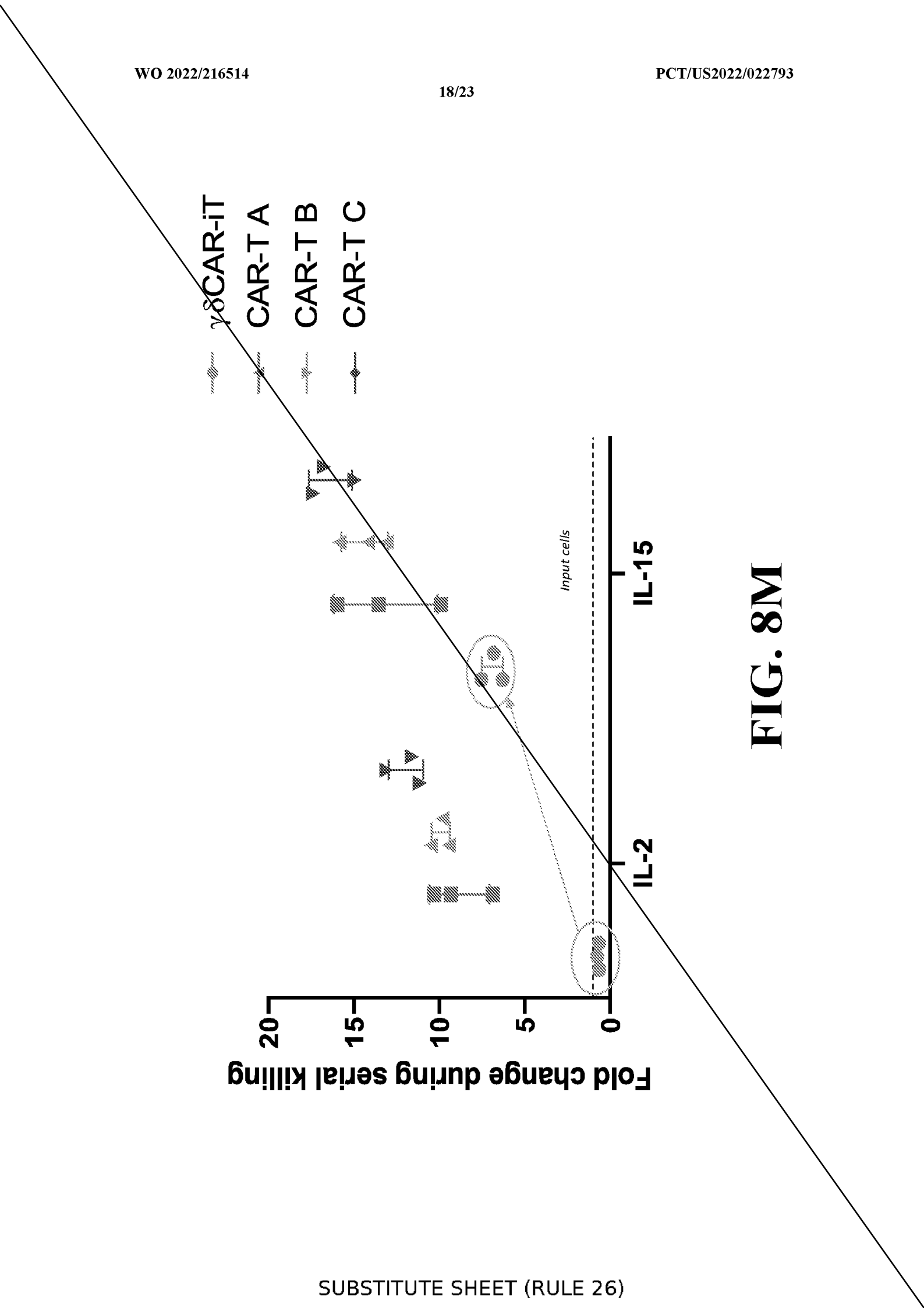

γδCAR-iT
CAR-T A
CAR-T B
CAR-T C
Fold change during serial killing
20
15
10
5
0
Input cells
IL-2
IL-15

FIG. 8M

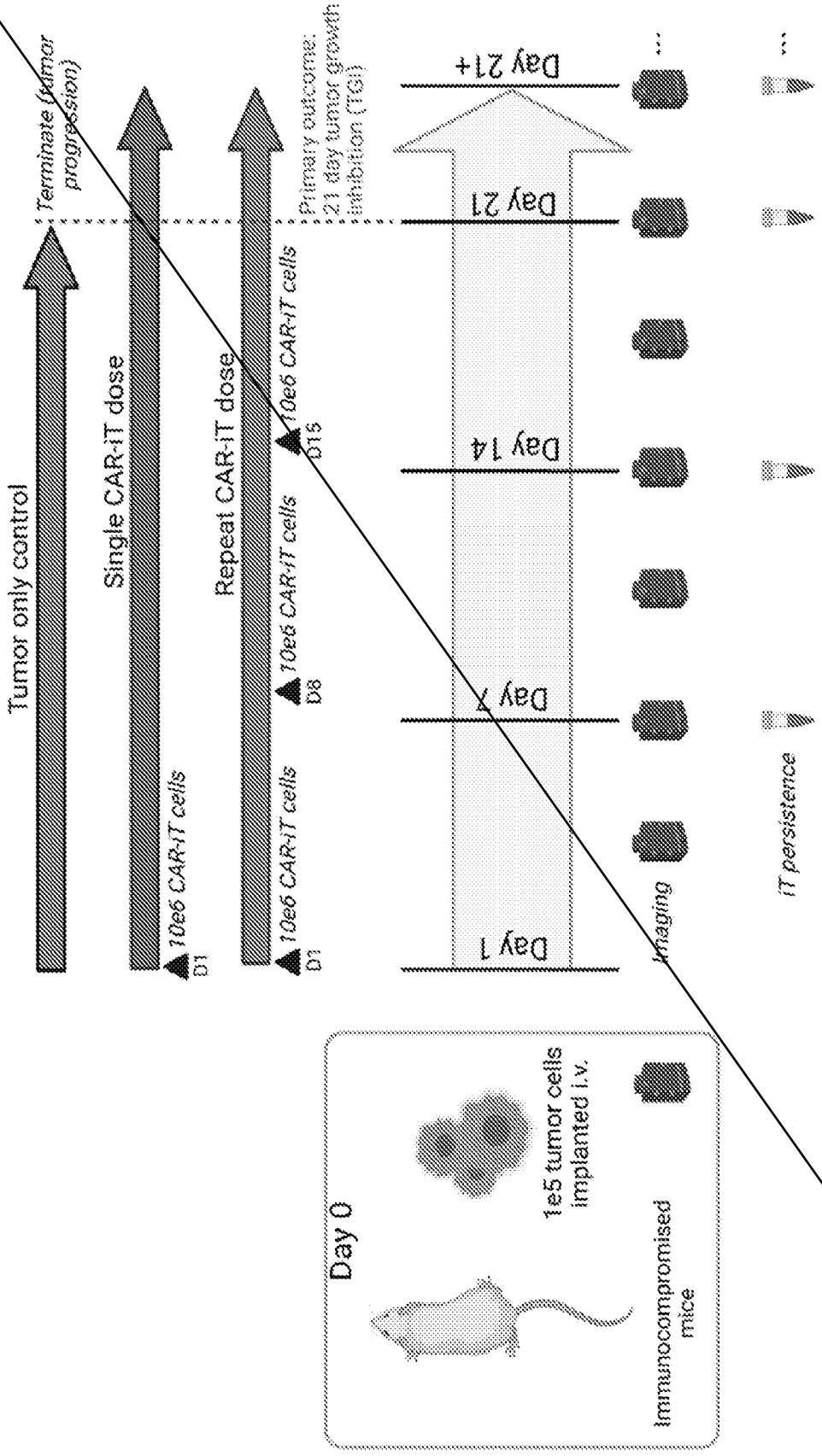

Tumor only control
Single CAR-iT dose
Repeat CAR-iT dose
Terminate (tumor progression)
Primary outcome: 21 day tumor growth inhibition (TGI)
10e6 CAR-iT cells
D1
10e6 CAR-iT cells
D8
10e6 CAR-iT cells
D15
Day 1
Day 7
Day 14
Day 21
Day 21+
Imaging
iT persistence
Day 0
1e5 tumor cells implanted i.v.
Immunocompromised mice

FIG. 9A

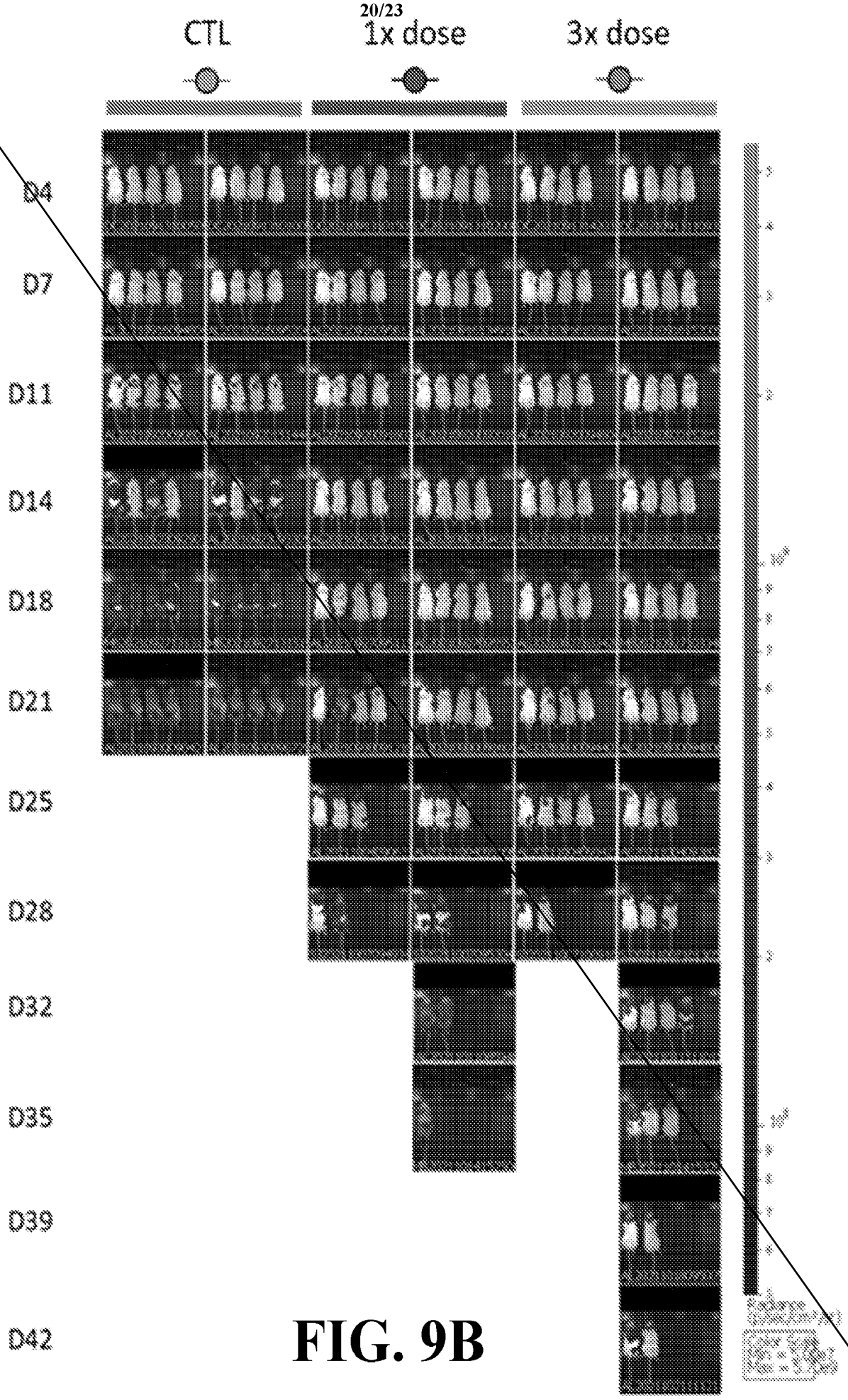


FIG. 9B

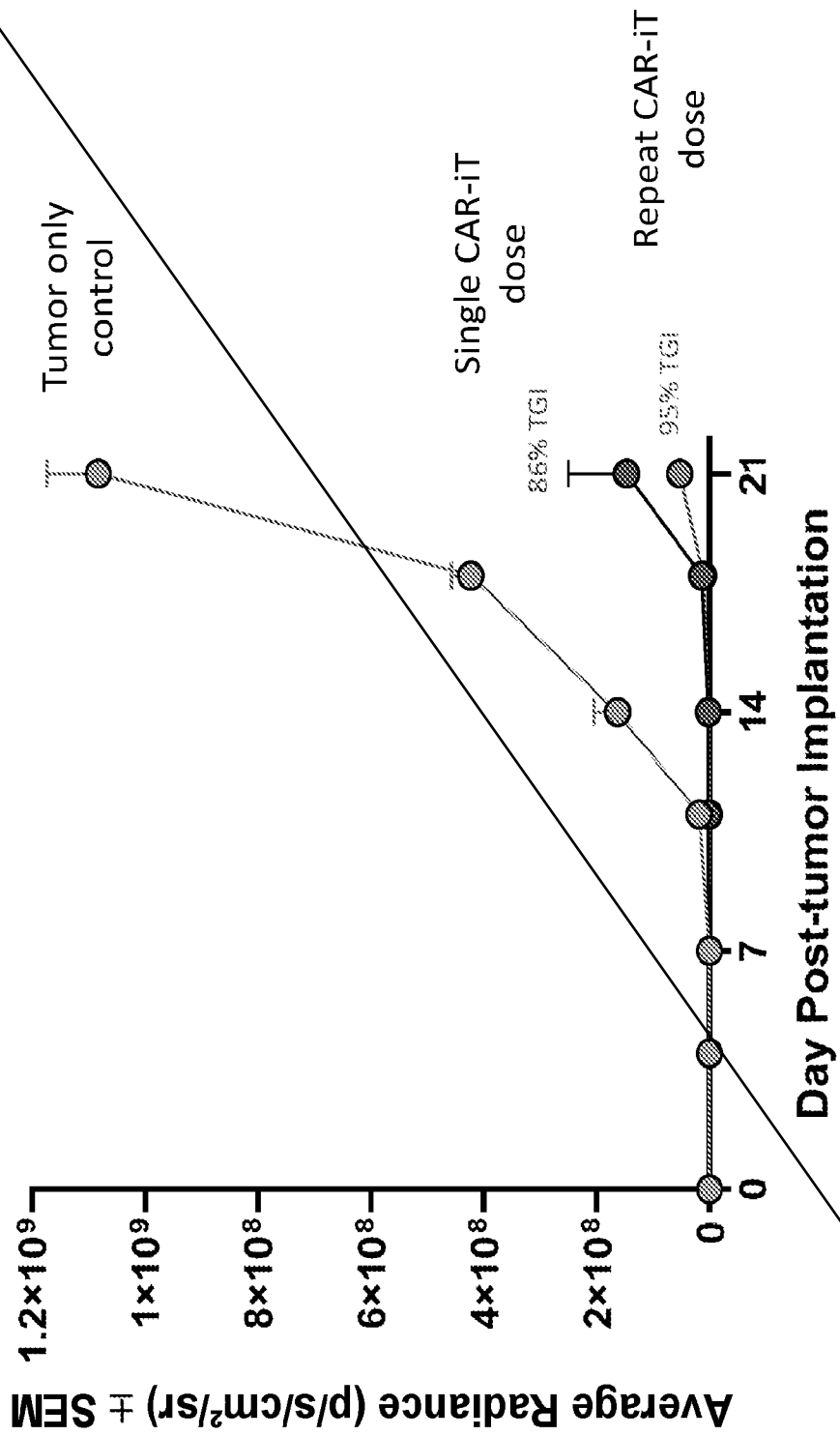

Average Radiance (p/s/cm²/sr) ± SEM
1.2×10⁹
1×10⁹
8×10⁸
6×10⁸
4×10⁸
2×10⁸
0
0
7
14
21
Day Post-tumor Implantation
Tumor only control
Single CAR-iT dose
Repeat CAR-iT dose
86% TGI
95% TGI

FIG. 9C

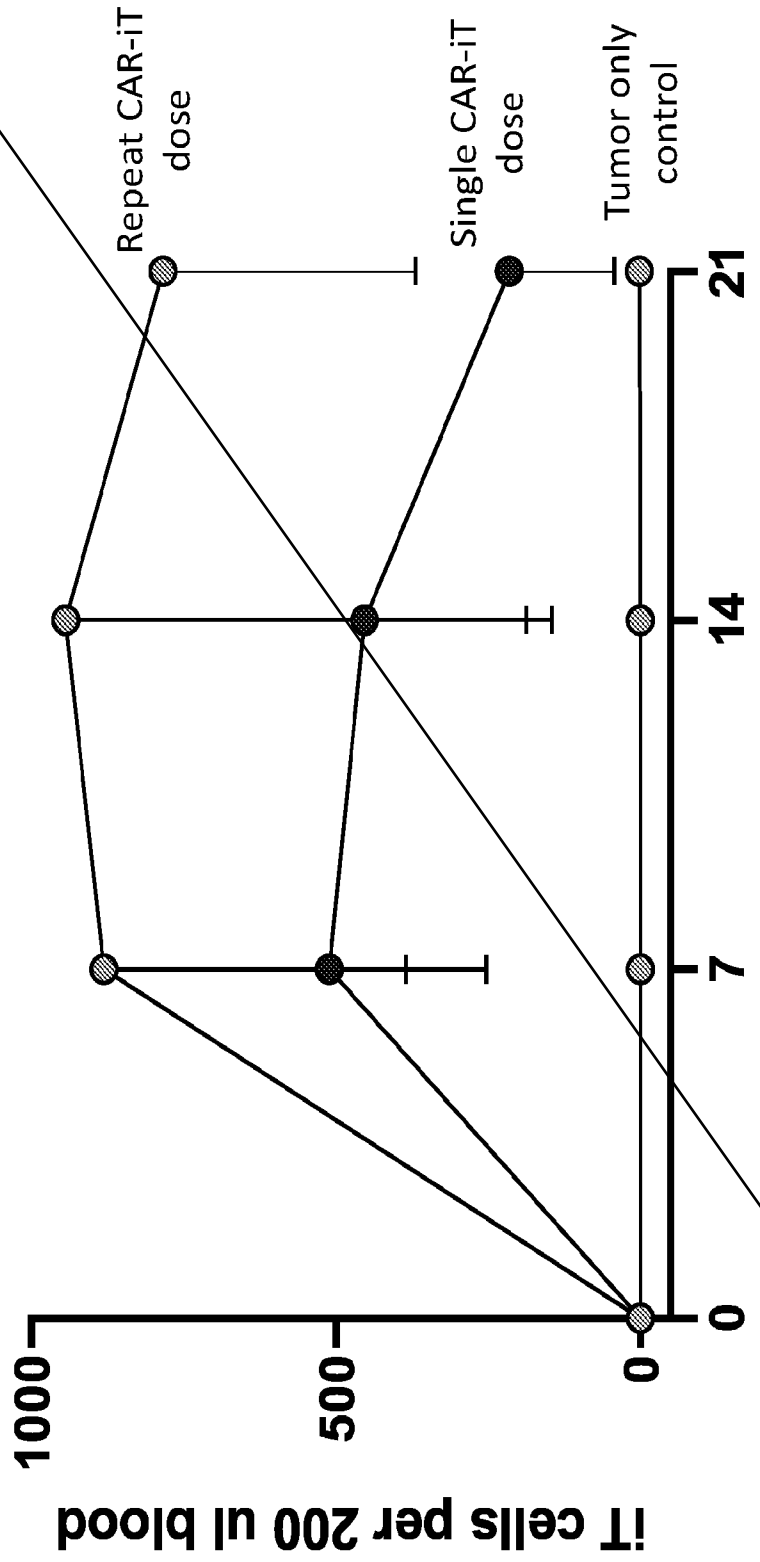

Repeat CAR-iT dose
Single CAR-iT dose
Tumor only control
1000
500
0
iT cells per 200 ul blood
0
7
14
21
Day

FIG. 9E

Dual color incucyte assay
One of:
Isotype (hIgG1)
Rituximab
Isotype
(low fucose hIgG1)
Obintuzumab
Vδ CAR-T
[FMC63]
Raji (CD19+ CD20+)
Nuclight Red
Raji (CD19ko CD20+)
Nuclight Green

FIG. 10

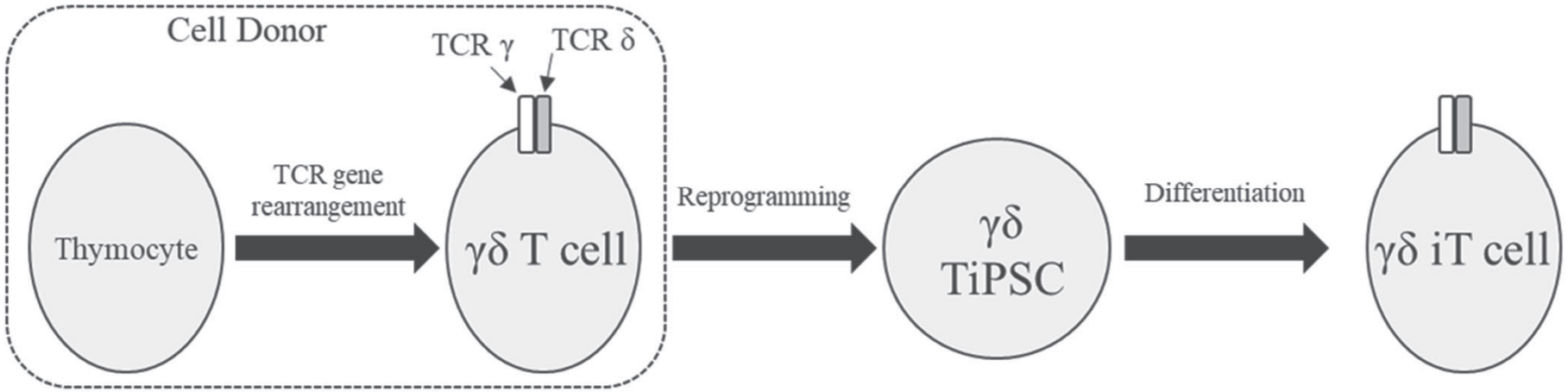

Cell Donor
TCR γ
TCR δ
Thymocyte
TCR gene rearrangement
γδ T cell
Reprogramming
γδ TiPSC
Differentiation
γδ iT cell

FIG. 1A

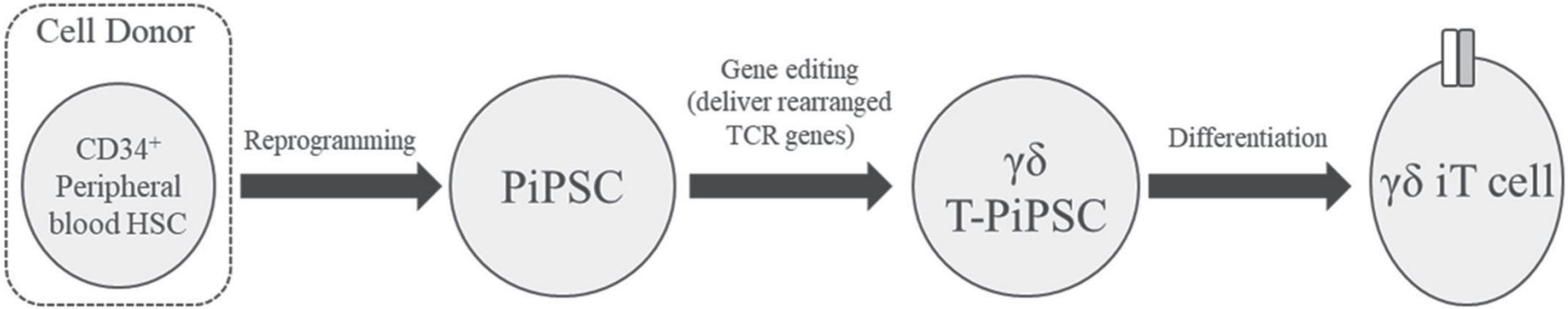

Cell Donor
CD34+ Peripheral blood HSC
Reprogramming
PiPSC
Gene editing (deliver rearranged TCR genes)
γδ T-PiPSC
Differentiation
γδ iT cell

FIG. 1B

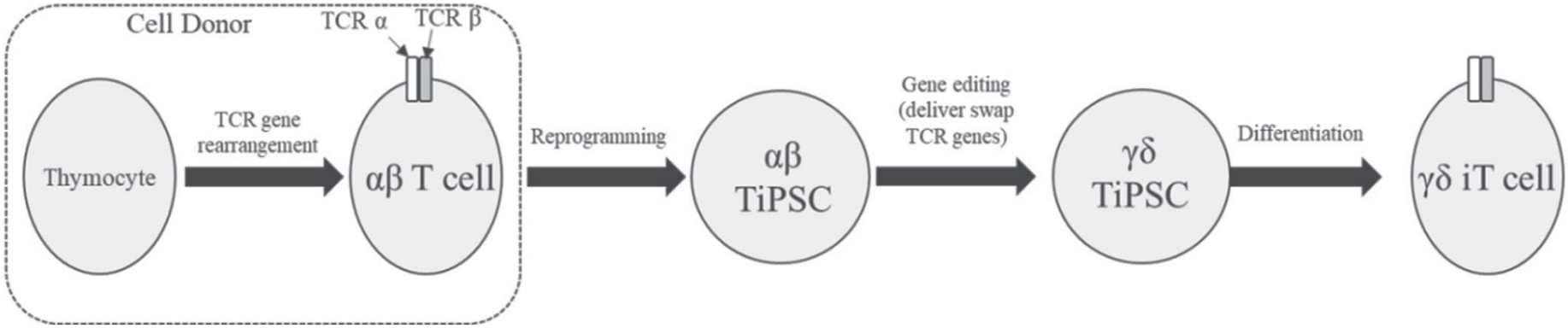

Cell Donor
TCR α
TCR β
Thymocyte
TCR gene rearrangement
αβ T cell
Reprogramming
αβ TiPSC
Gene editing (deliver swap TCR genes)
γδ TiPSC
Differentiation
γδ iT cell

FIG. 1C

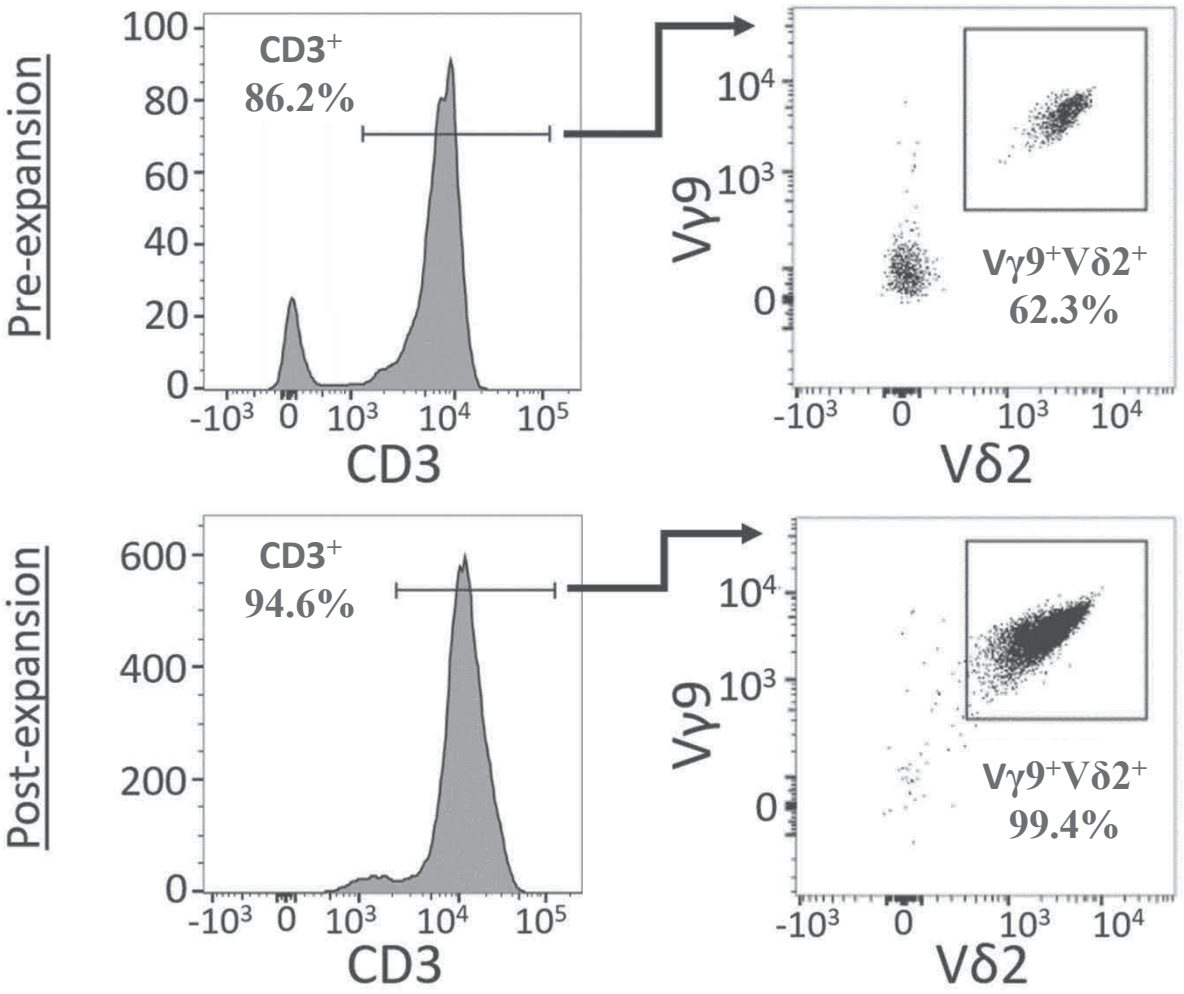

Pre-expansion
CD3+
86.2%
CD3
Vγ9
Vδ2
Vγ9+Vδ2+
62.3%
Post-expansion
CD3+
94.6%
CD3
Vγ9
Vδ2
Vγ9+Vδ2+
99.4%

FIG. 2A

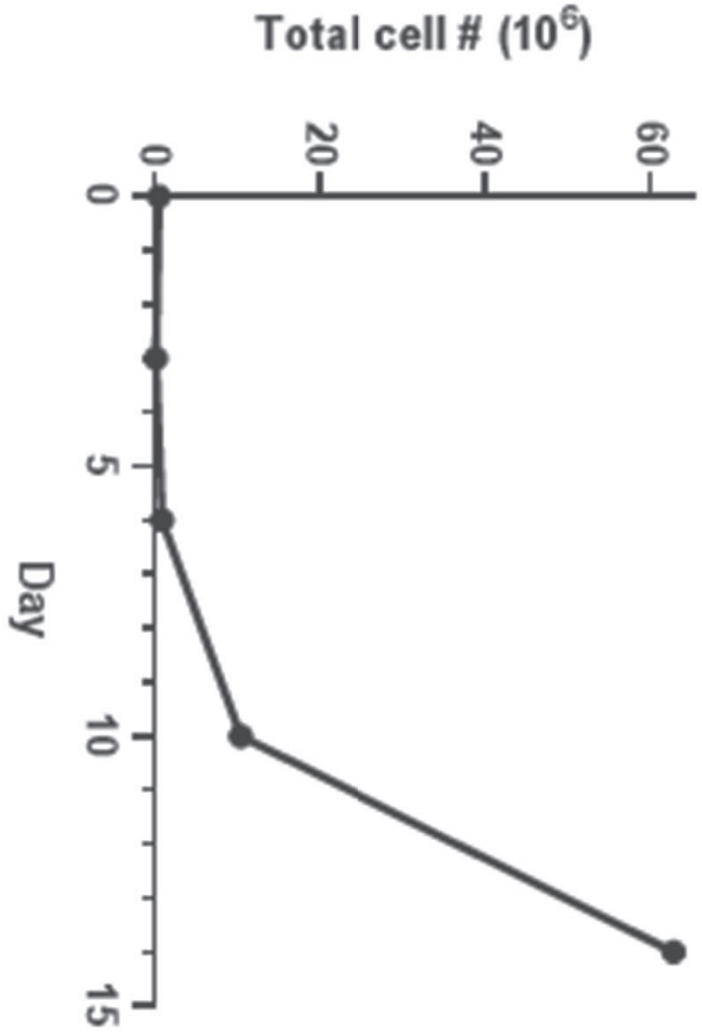


FIG. 2B

TiPSC ID	Vγ9	Vδ2	Vδ1	TCR Pair
gdTiPSC4.1	+	+	-	Vγ9/Vδ2
gdTiPSC4.2	-	-	-	Vγ?/Vδ?
gdTiPSC4.3	+	-	+	Vγ9/Vδ1
gdTiPSC4.4	+	+	-	Vγ9/Vδ2

FIG. 2C

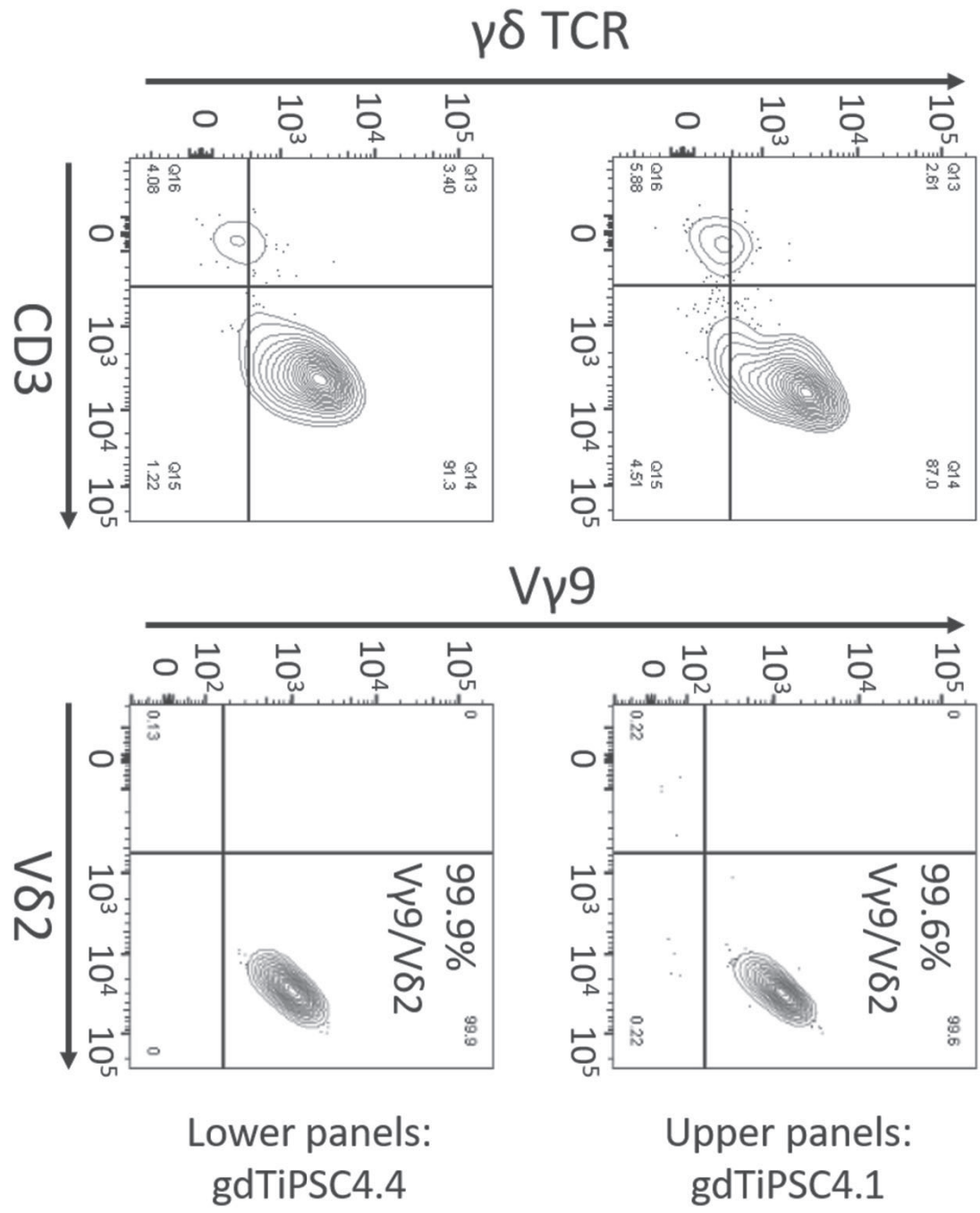

γδ TCR
CD3
Q13 2.61
Q16 5.88
Q14 87.0
Q15 4.51
Q13 3.40
Q16 4.08
Q14 91.3
Q15 1.22
Vγ9
Vδ2
0.22
99.6%
Vγ9/Vδ2
99.6
0.22
0.13
99.9%
Vγ9/Vδ2
99.9
0
Upper panels:
gdTiPSC4.1
Lower panels:
gdTiPSC4.4

FIG. 2D

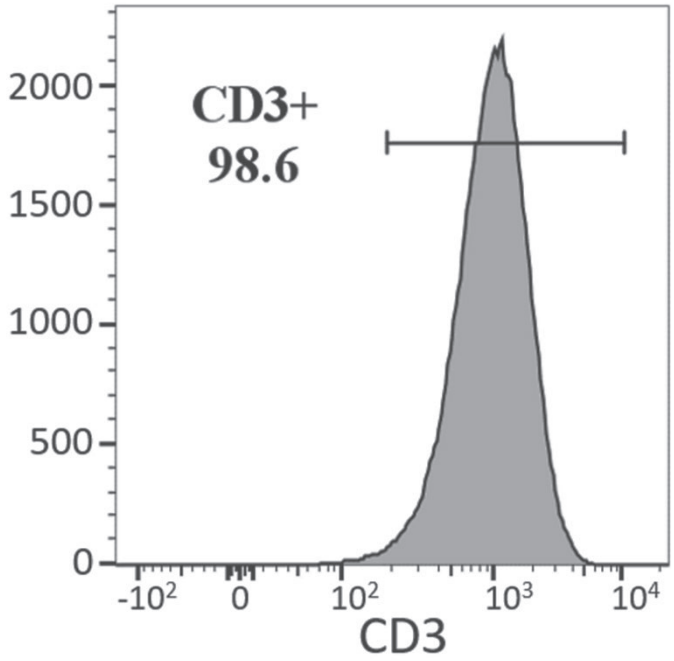

CD3+
98.6
2000
1500
1000
500
0
-10²
0
10²
10³
10⁴
CD3

FIG. 3A

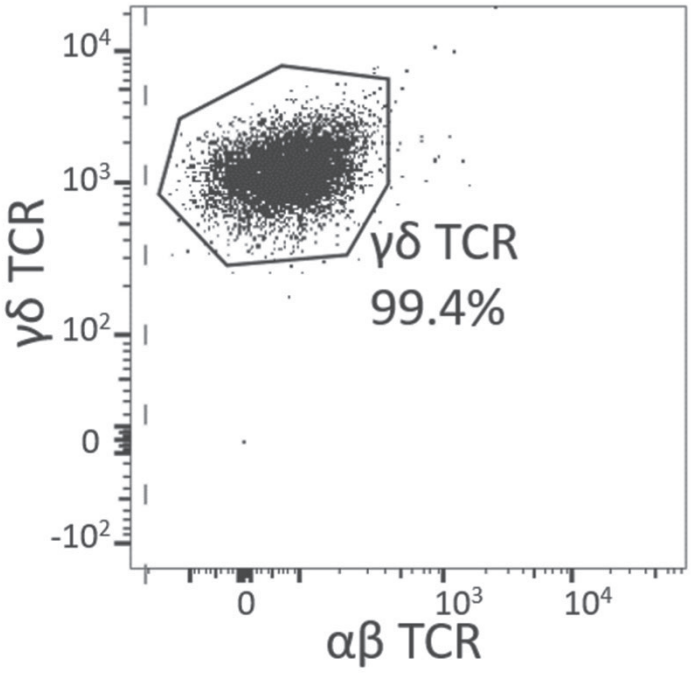

10⁴
10³
10²
0
-10²
γδ TCR
γδ TCR
99.4%
0
10³
10⁴
αβ TCR

FIG. 3B

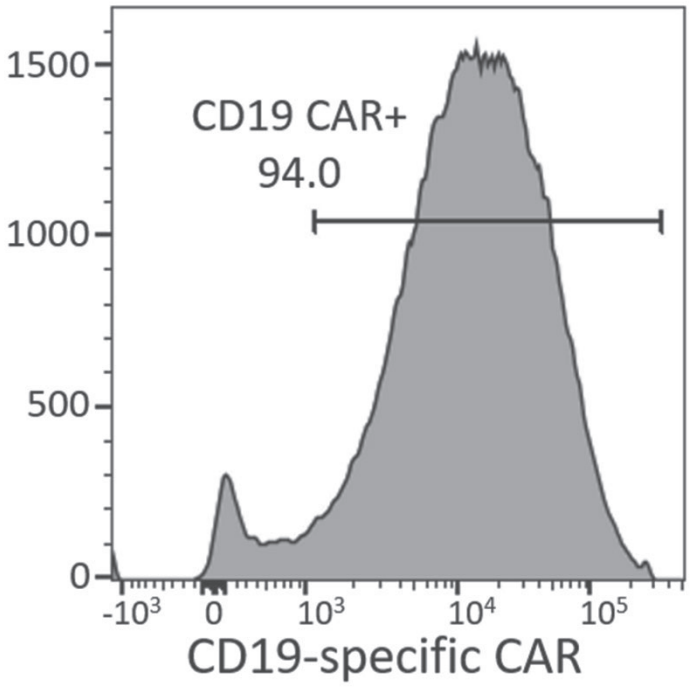

1500
1000
500
0
CD19 CAR+
94.0
-10³
0
10³
10⁴
10⁵
CD19-specific CAR

FIG. 3C

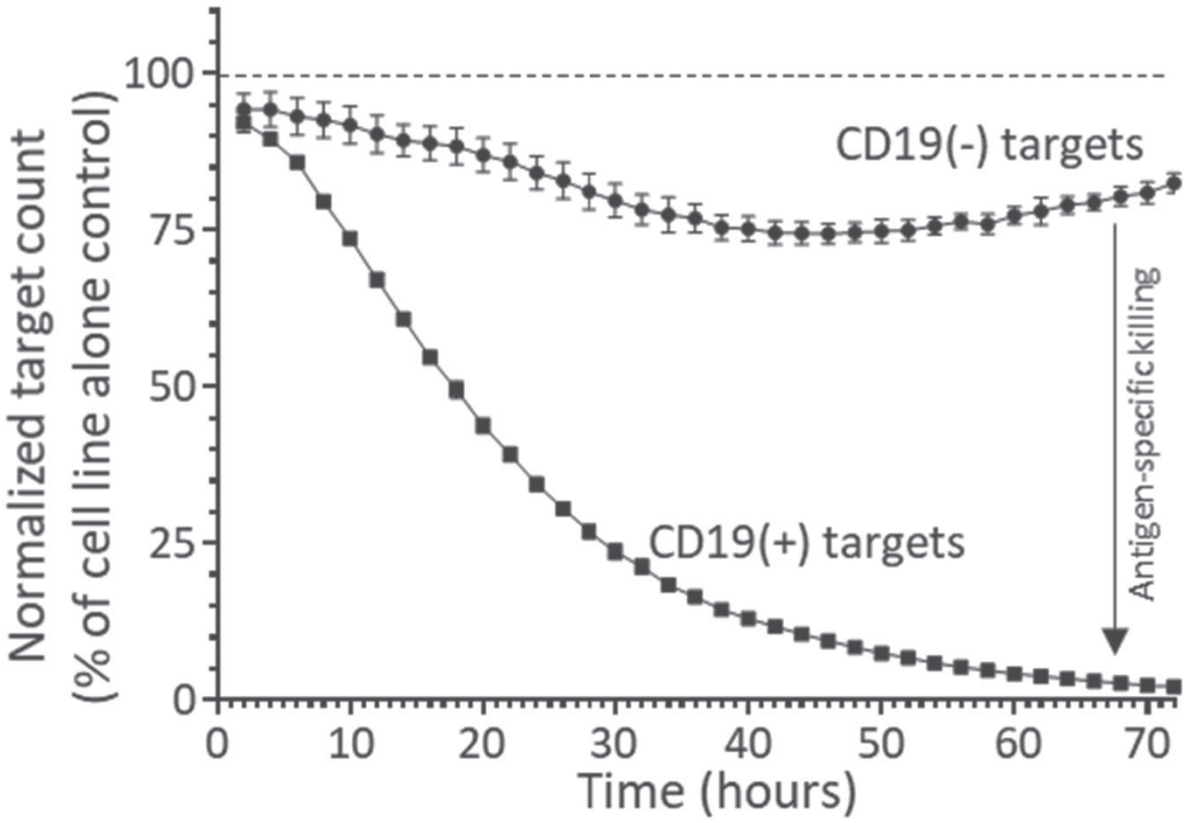

100
75
50
25
0
Normalized target count
(% of cell line alone control)
CD19(-) targets
CD19(+) targets
Antigen-specific killing
0
10
20
30
40
50
60
70
Time (hours)

FIG. 3D

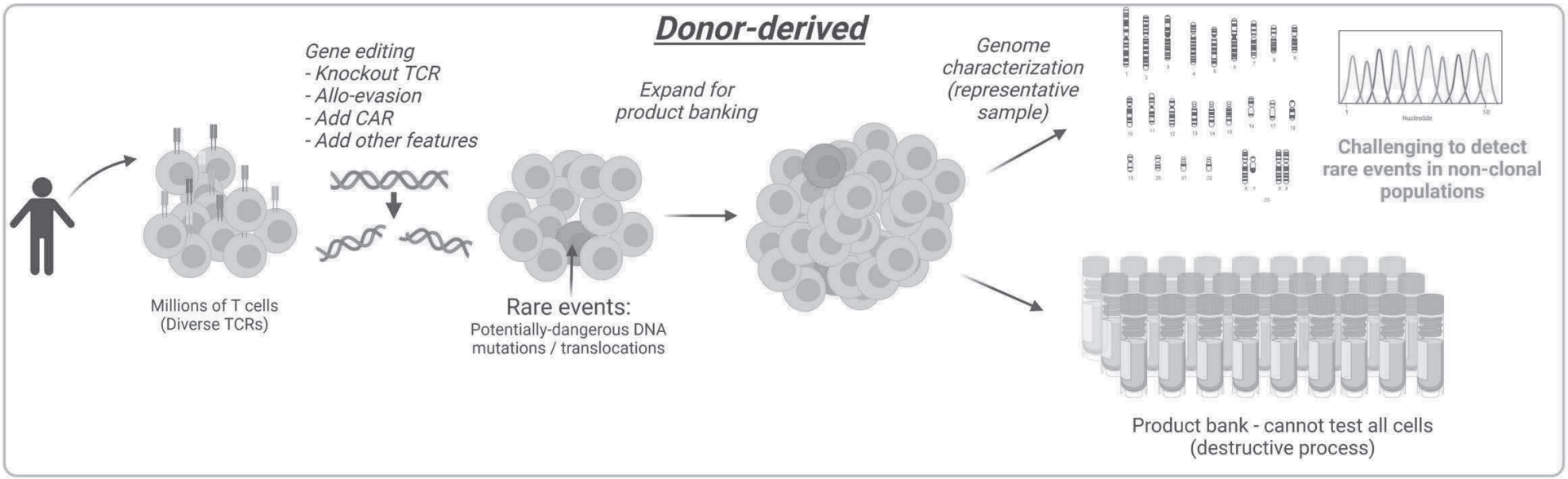

Donor-derived
Gene editing
- Knockout TCR
- Allo-evasion
- Add CAR
- Add other features
Expand for product banking
Genome characterization (representative sample)
Challenging to detect rare events in non-clonal populations
Millions of T cells (Diverse TCRs)
Rare events:
Potentially-dangerous DNA mutations / translocations
Product bank - cannot test all cells (destructive process)

FIG. 4A

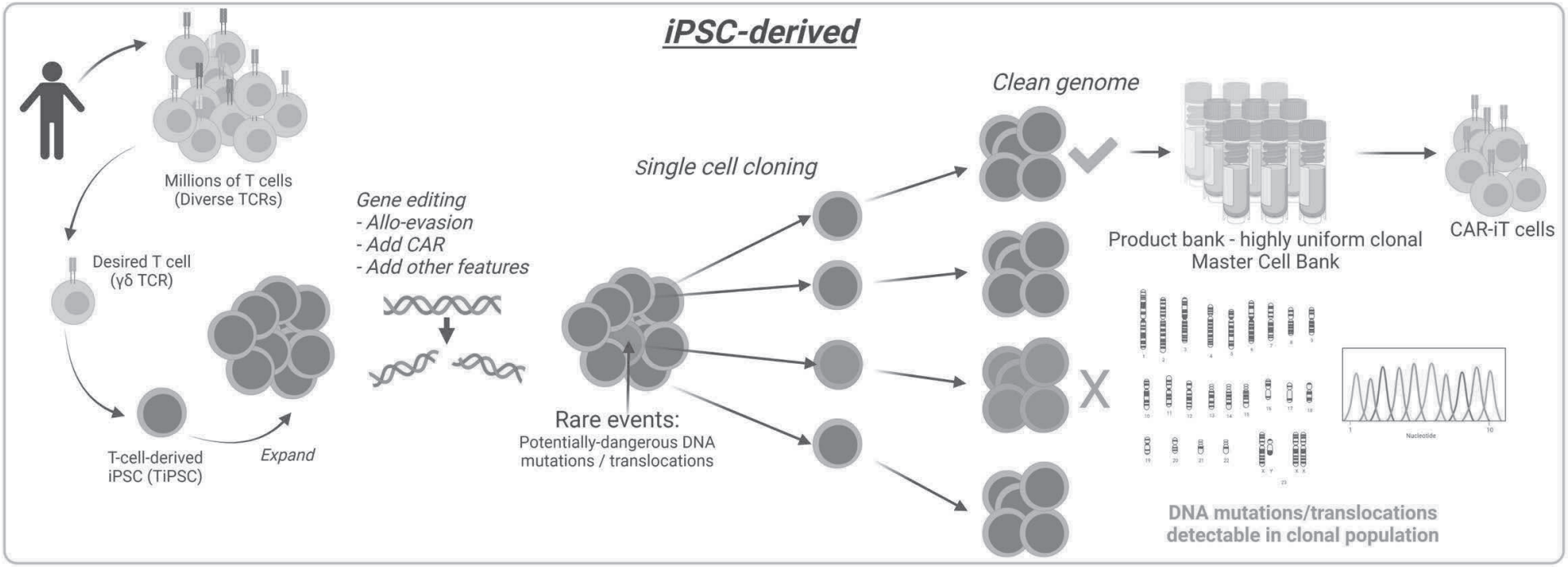

iPSC-derived
Millions of T cells
(Diverse TCRs)
Desired T cell
(γδ TCR)
T-cell-derived
iPSC (TiPSC)
Expand
Gene editing
- Allo-evasion
- Add CAR
- Add other features
single cell cloning
Rare events:
Potentially-dangerous DNA
mutations / translocations
Clean genome
Product bank - highly uniform clonal
Master Cell Bank
CAR-iT cells
DNA mutations/translocations
detectable in clonal population

FIG. 4B

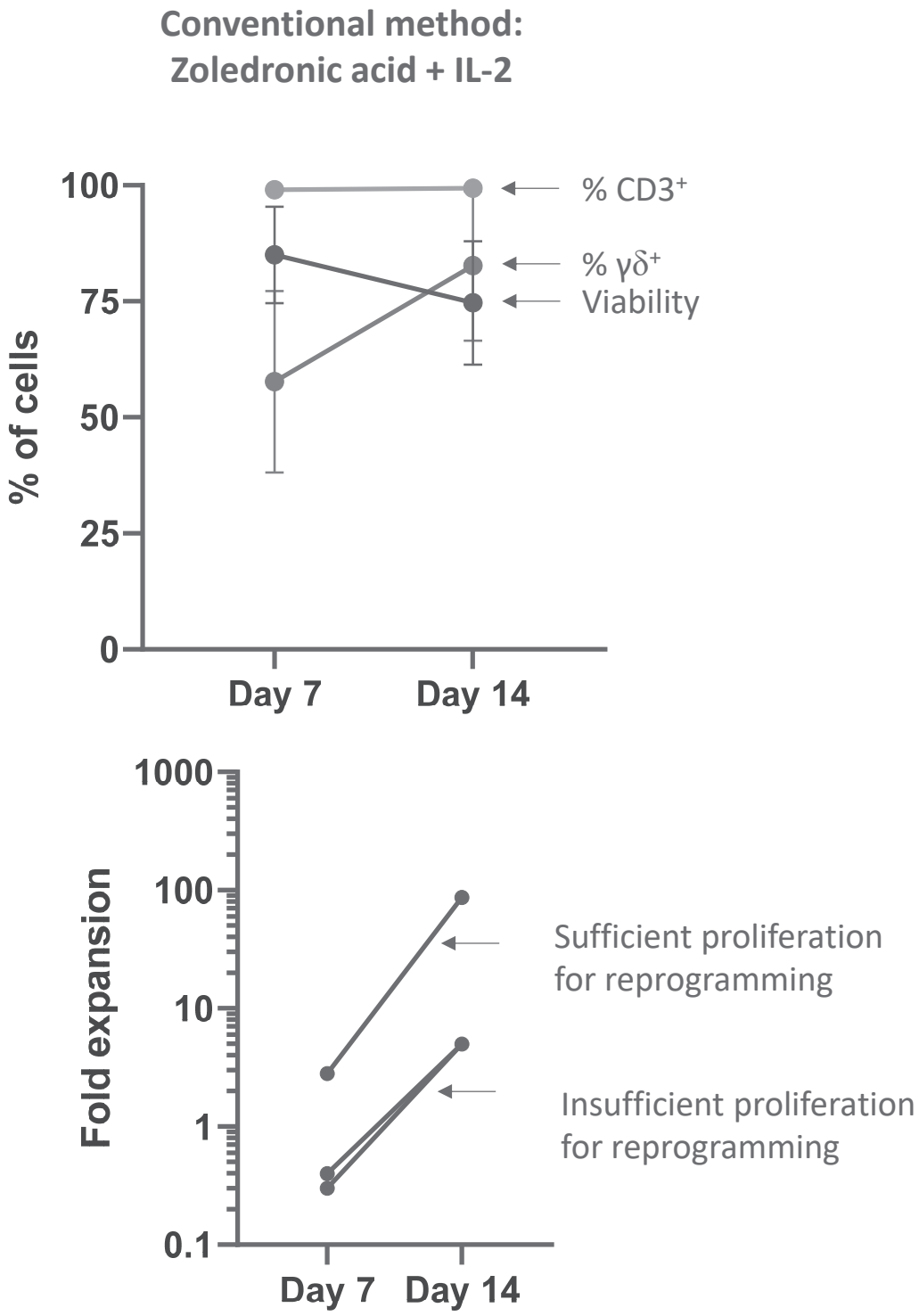

Conventional method:
Zoledronic acid + IL-2
100
75
50
25
0
% of cells
Day 7
Day 14
% CD3+
% γδ+
Viability
1000
100
10
1
0.1
Fold expansion
Day 7
Day 14
Sufficient proliferation
for reprogramming
Insufficient proliferation
for reprogramming

FIG. 5A

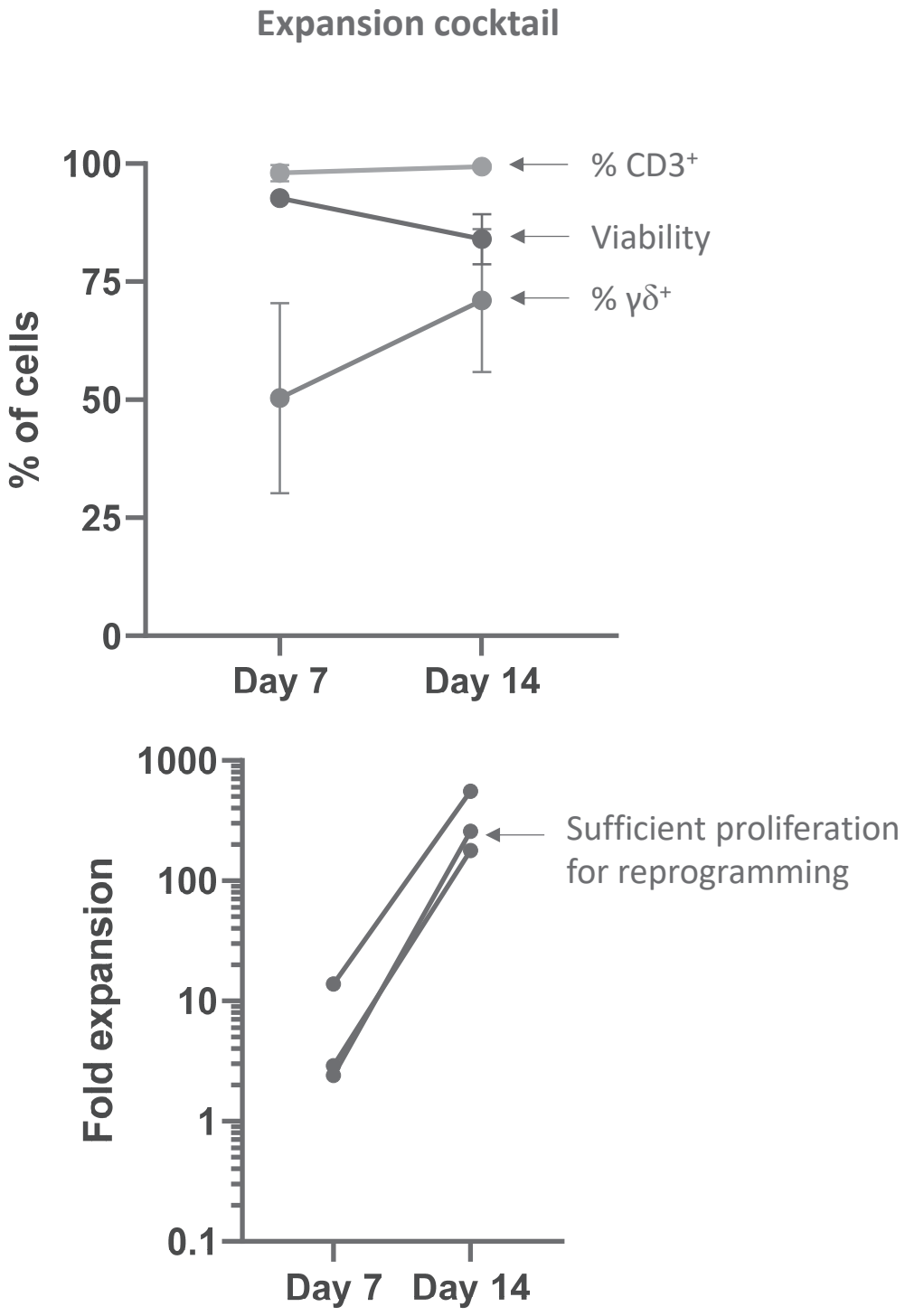
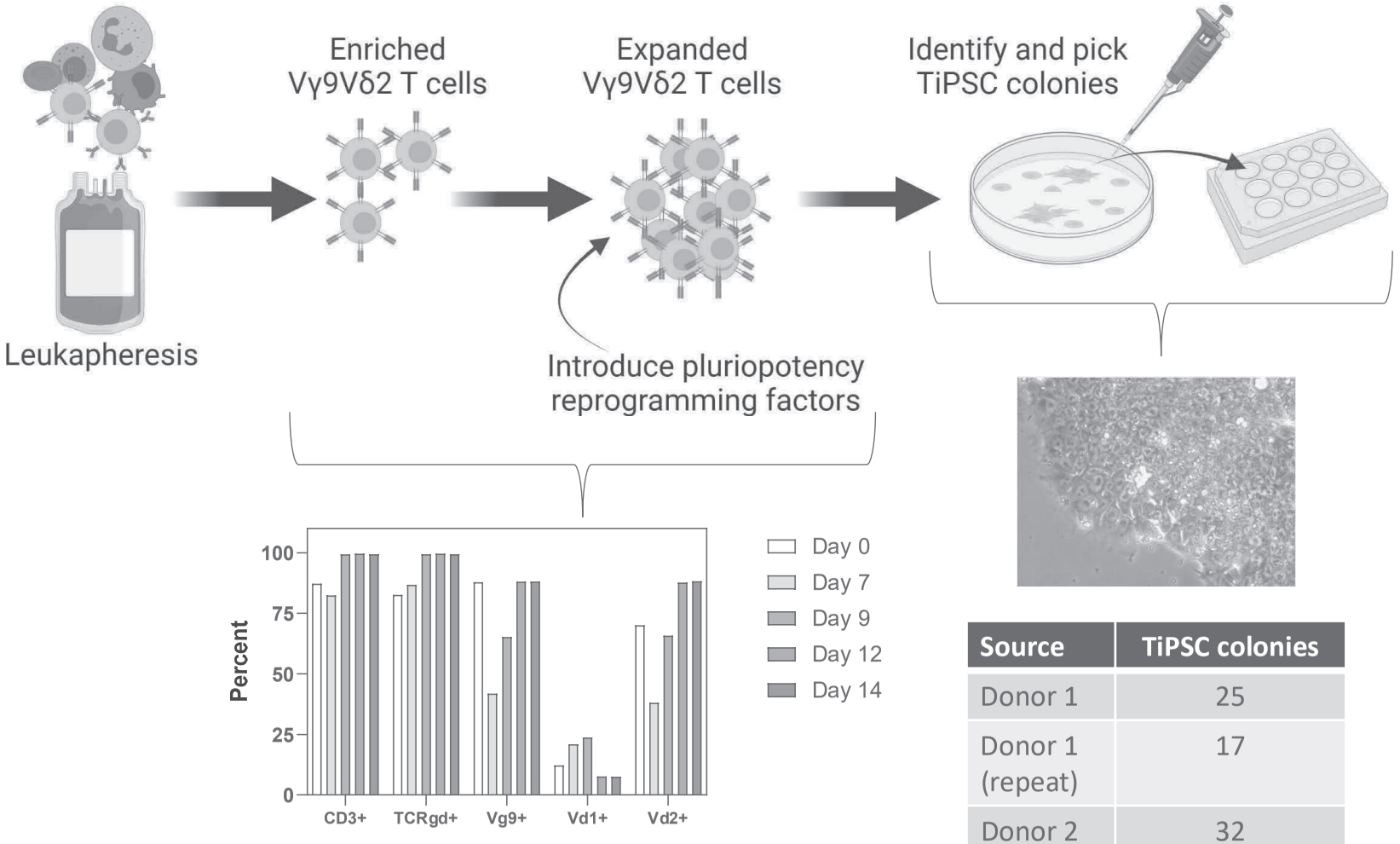

Expansion cocktail
100
75
50
25
0
% of cells
Day 7
Day 14
% CD3+
Viability
% γδ+
1000
100
10
1
0.1
Fold expansion
Day 7
Day 14
Sufficient proliferation
for reprogramming

FIG. 5B



Source	TiPSC colonies
Donor 1	25
Donor 1 (repeat)	17
Donor 2	32

FIG. 6

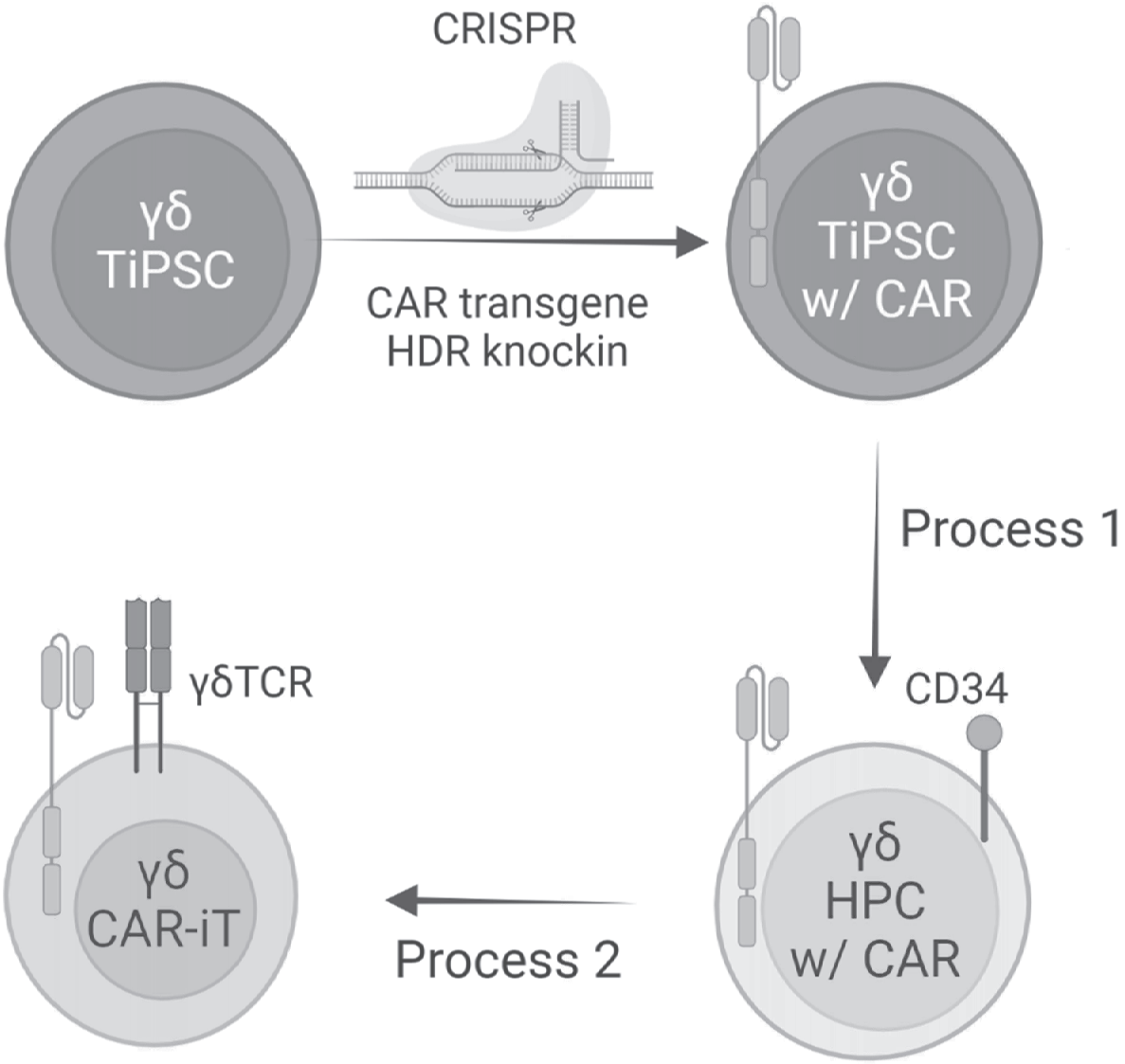

CRISPR
γδ
TiPSC
CAR transgene
HDR knockin
γδ
TiPSC
w/ CAR
Process 1
CD34
γδ
HPC
w/ CAR
Process 2
γδTCR
γδ
CAR-iT

FIG. 7A

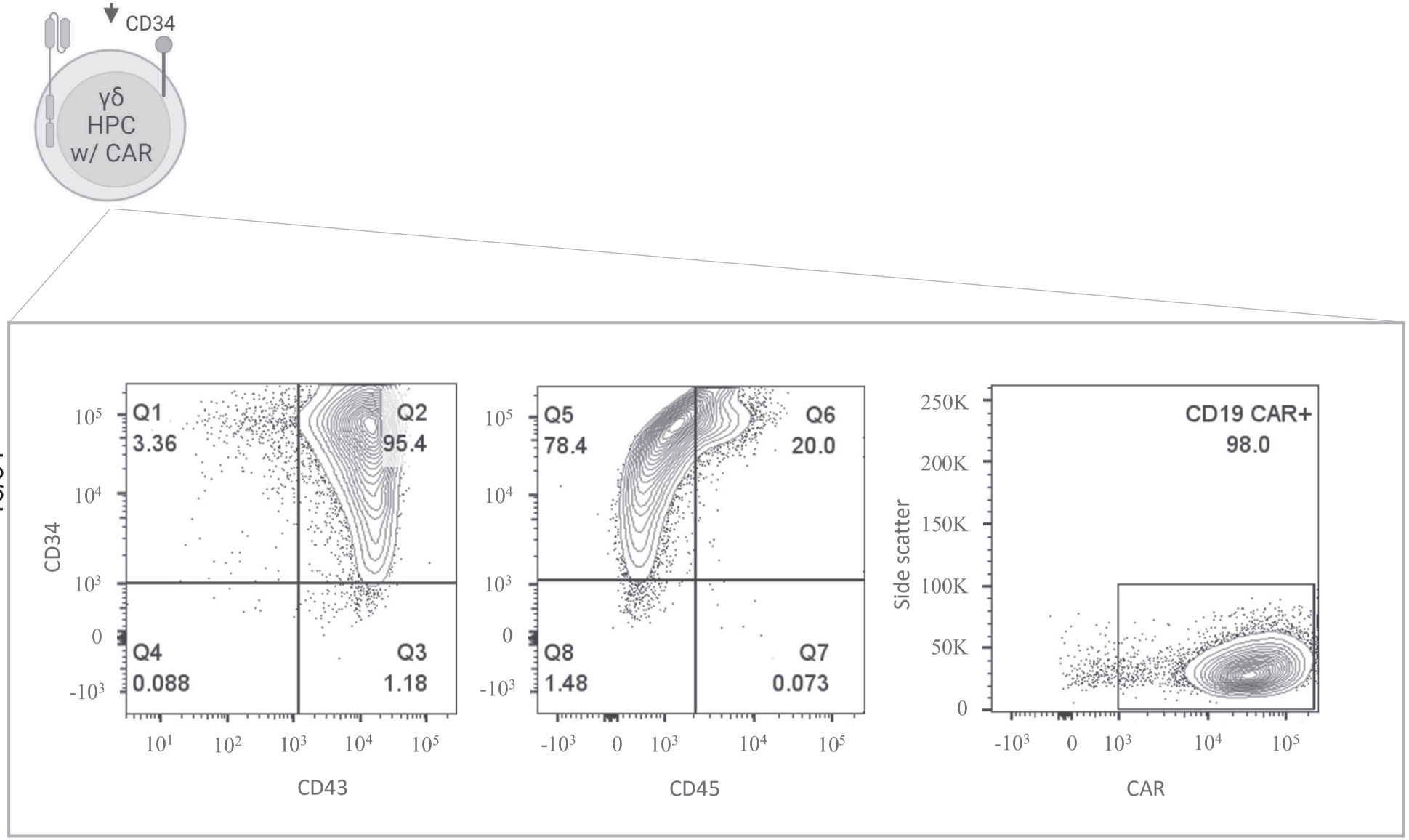

CD34
γδ
HPC
w/ CAR
Q1
3.36
Q2
95.4
Q4
0.088
Q3
1.18
CD34
CD43
Q5
78.4
Q6
20.0
Q8
1.48
Q7
0.073
CD45
CD19 CAR+
98.0
Side scatter
CAR

FIG. 7B

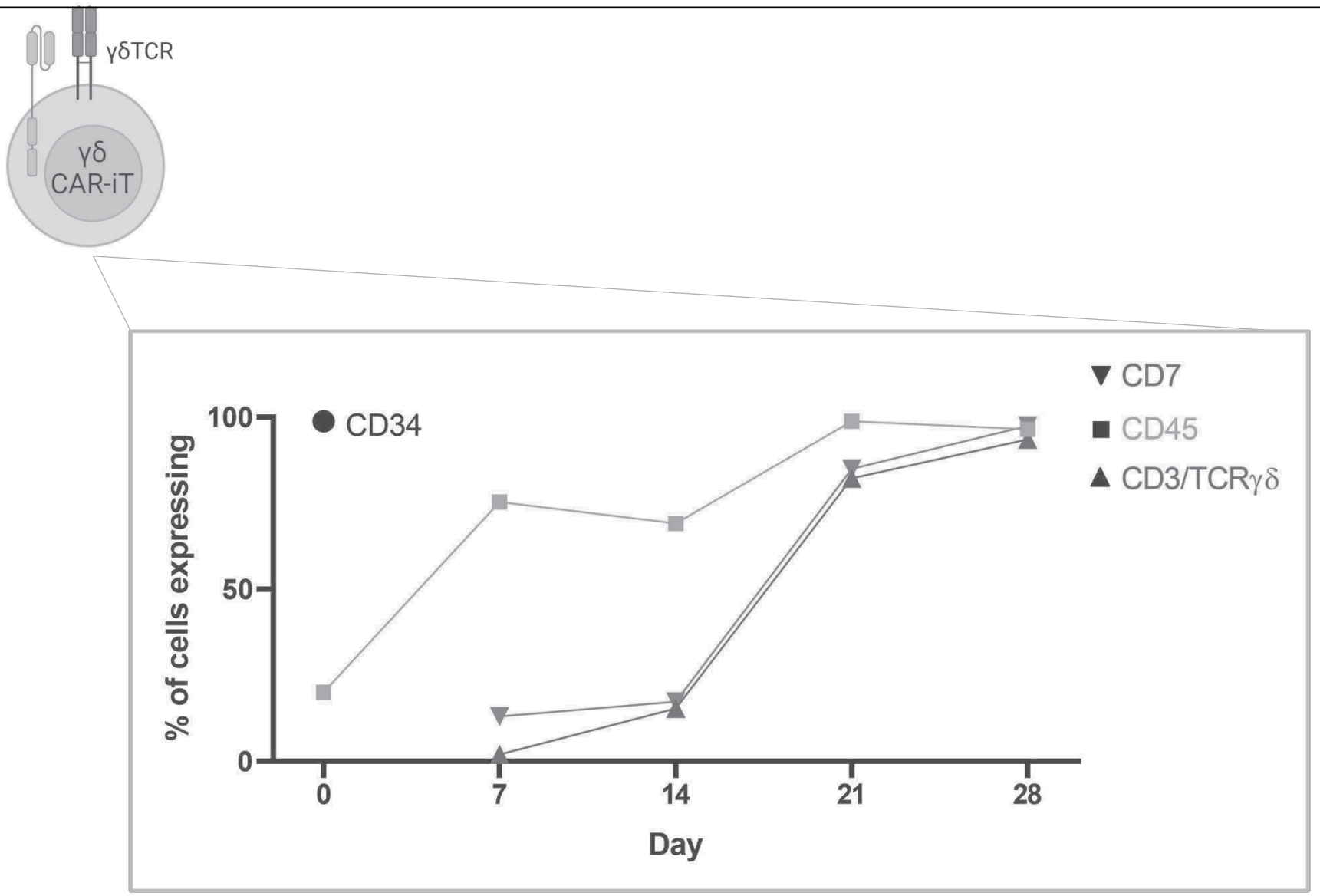

γδTCR
γδ
CAR-iT
CD34
CD7
CD45
CD3/TCRγδ
% of cells expressing
100
50
0
0
7
14
21
28
Day

FIG. 7C

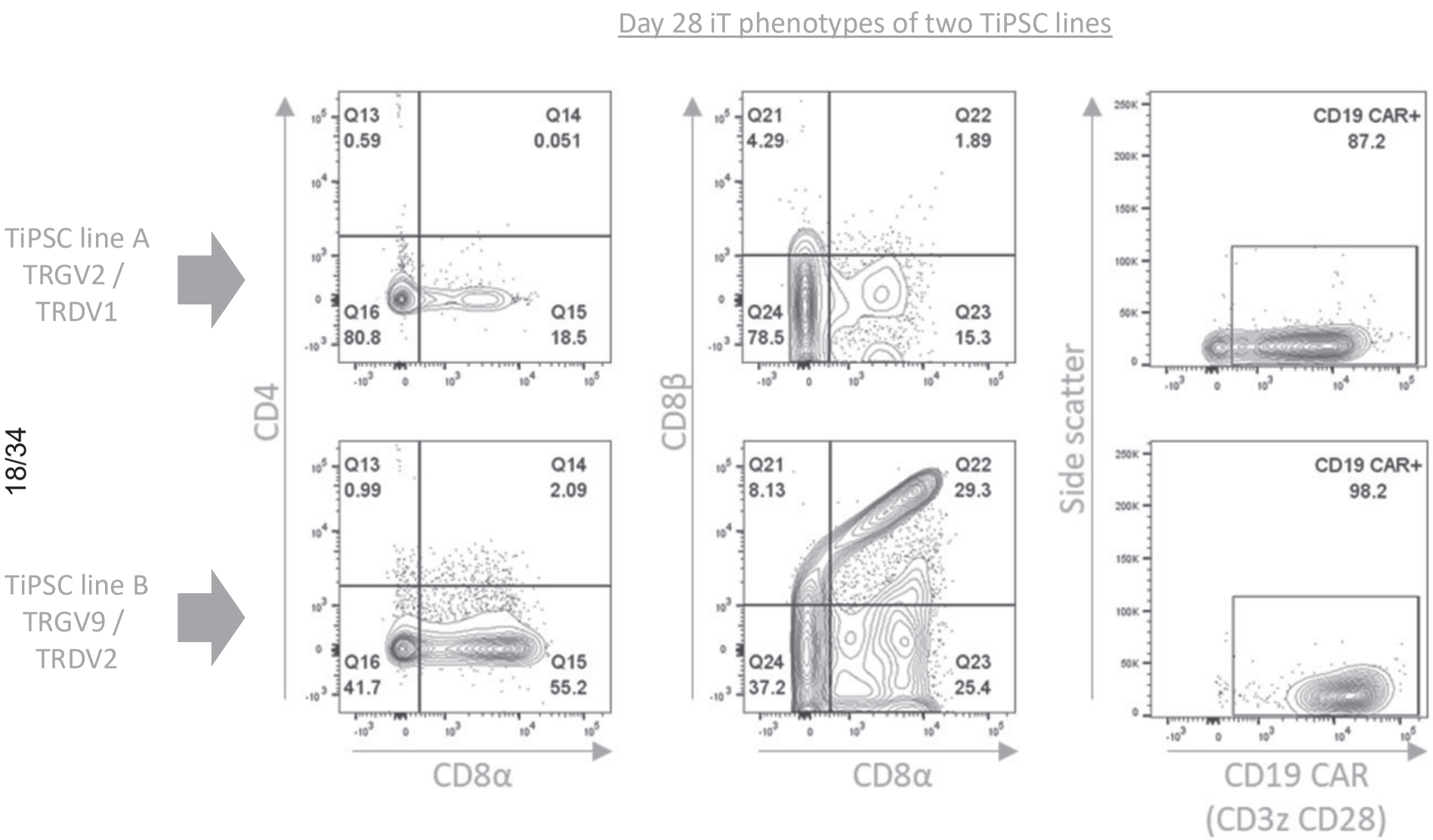

Day 28 iT phenotypes of two TiPSC lines
TiPSC line A
TRGV2 /
TRDV1
TiPSC line B
TRGV9 /
TRDV2
CD4
CD8α
Q13
0.59
Q14
0.051
Q16
80.8
Q15
18.5
Q13
0.99
Q14
2.09
Q16
41.7
Q15
55.2
CD8β
CD8α
Q21
4.29
Q22
1.89
Q24
78.5
Q23
15.3
Q21
8.13
Q22
29.3
Q24
37.2
Q23
25.4
Side scatter
CD19 CAR
(CD3z CD28)
CD19 CAR+
87.2
CD19 CAR+
98.2

FIG. 7D

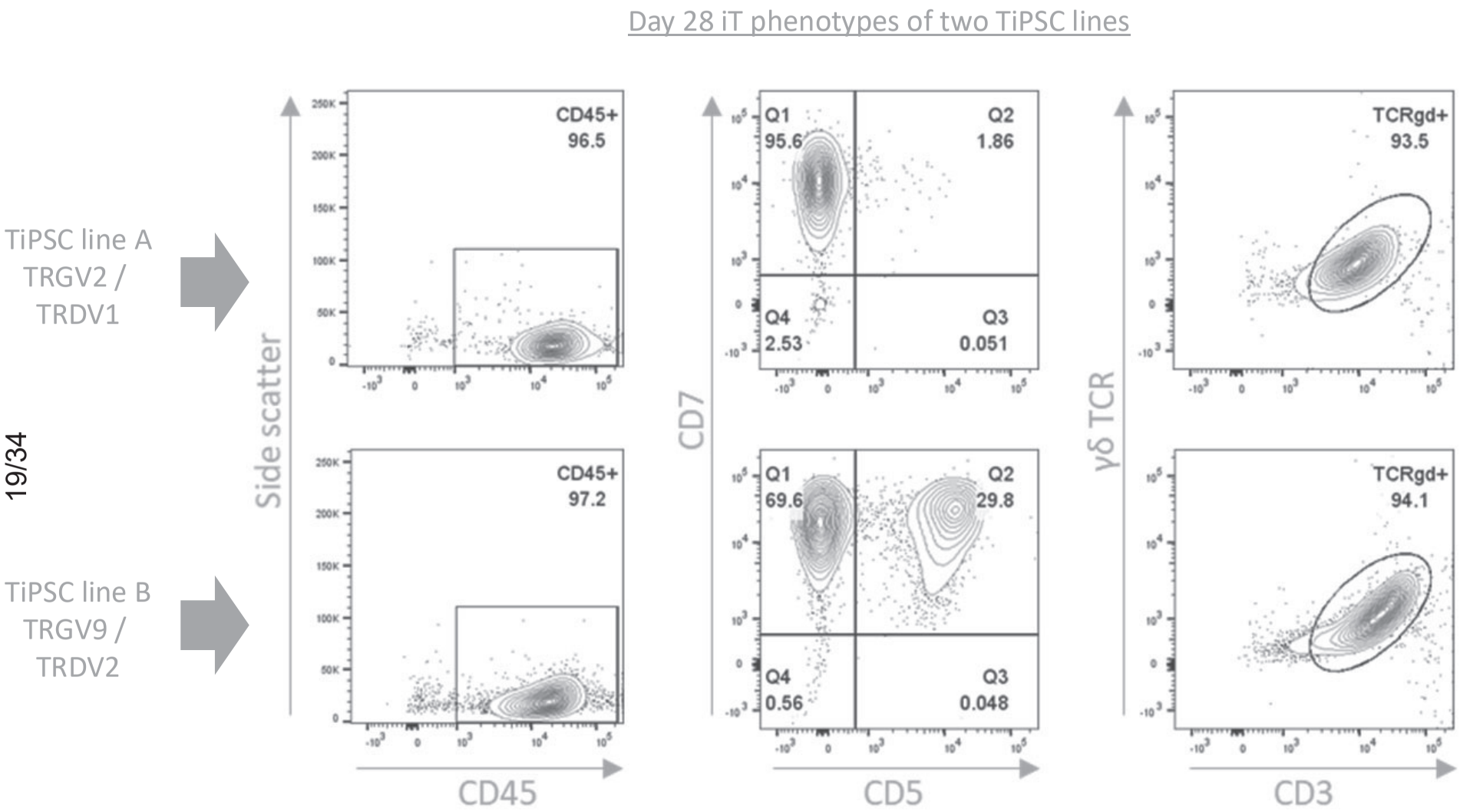


FIG. 7D (continued)

Nalm-6 Cells
(CD19^{Hi})

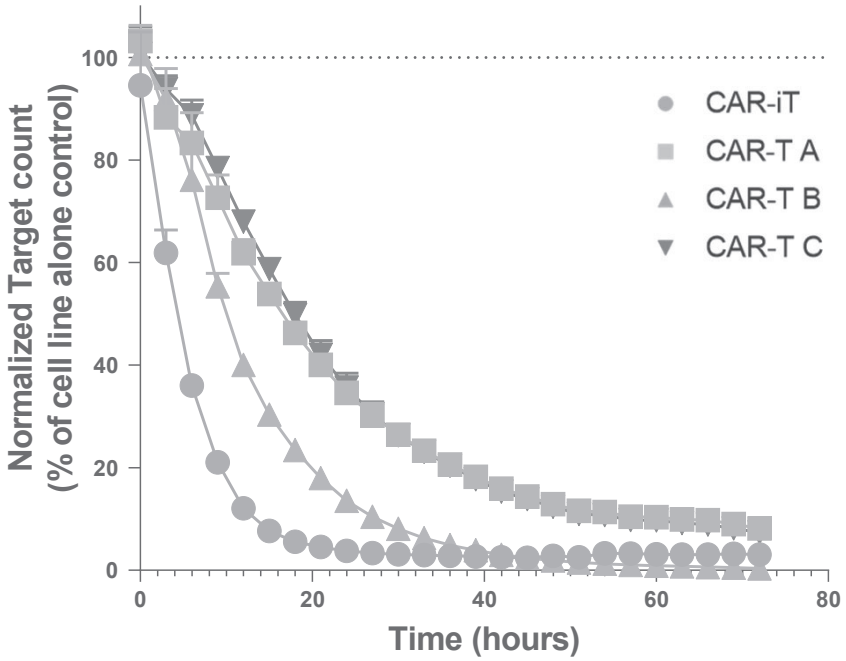


FIG. 8A

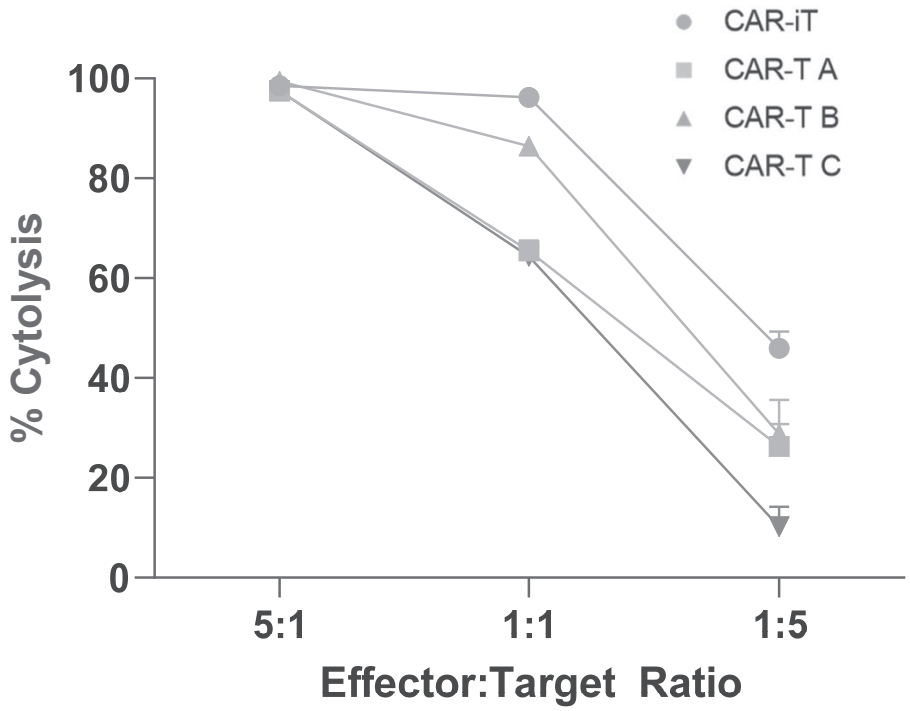


FIG. 8B

Reh Cells

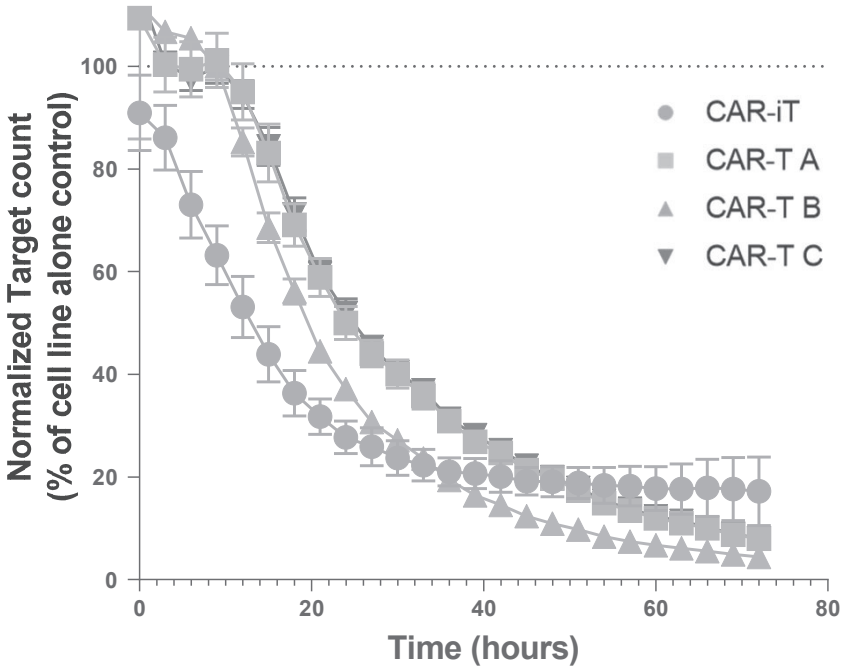


FIG. 8C

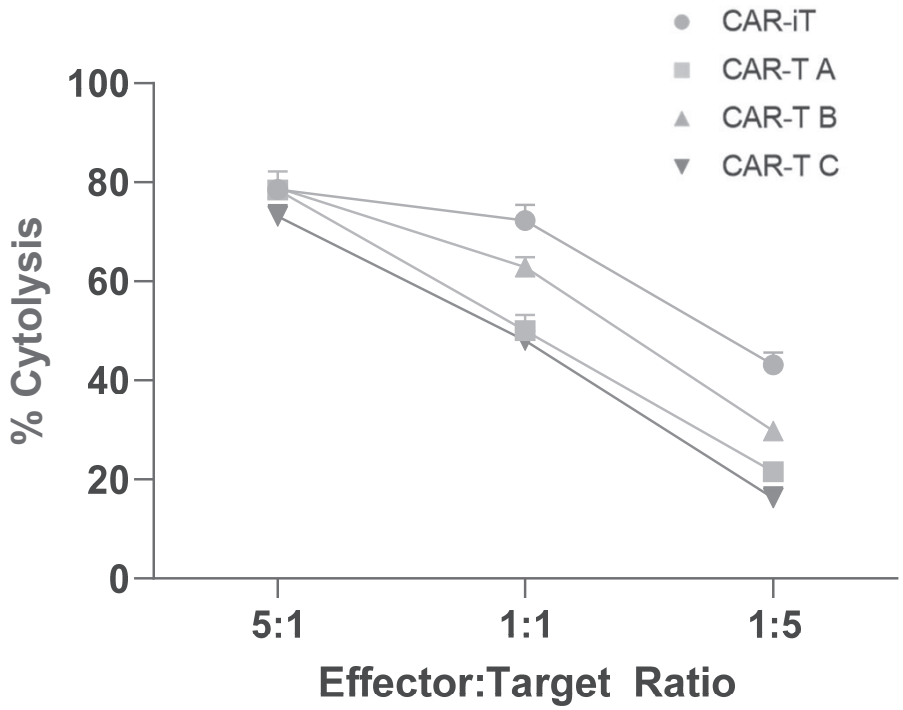


FIG. 8D

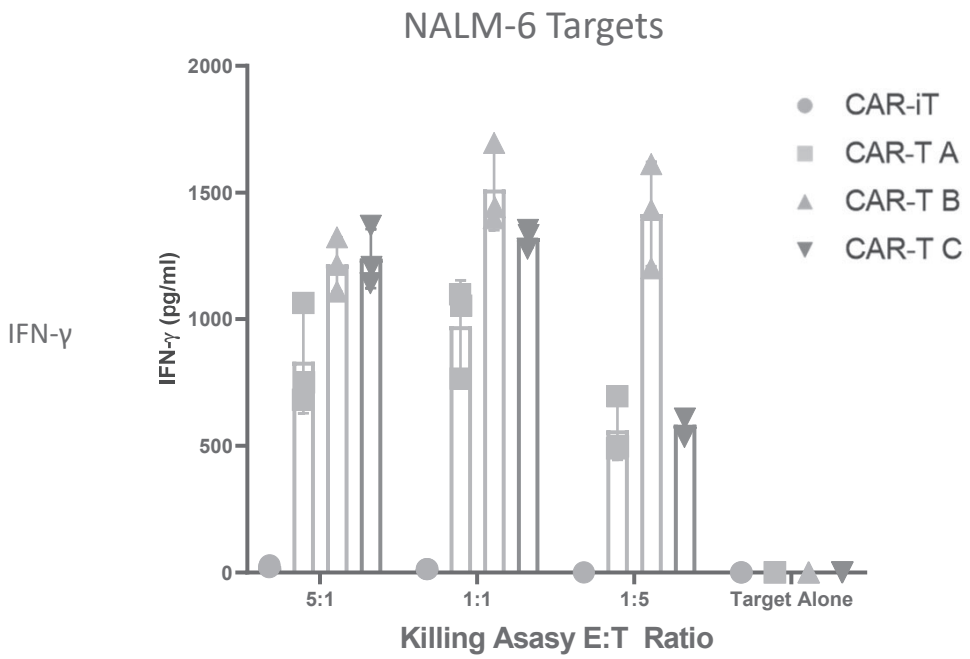

NALM-6 Targets
IFN-γ
2000
1500
1000
500
0
IFN-γ (pg/ml)
CAR-iT
CAR-T A
CAR-T B
CAR-T C
5:1
1:1
1:5
Target Alone
Killing Asasy E:T Ratio

FIG. 8E

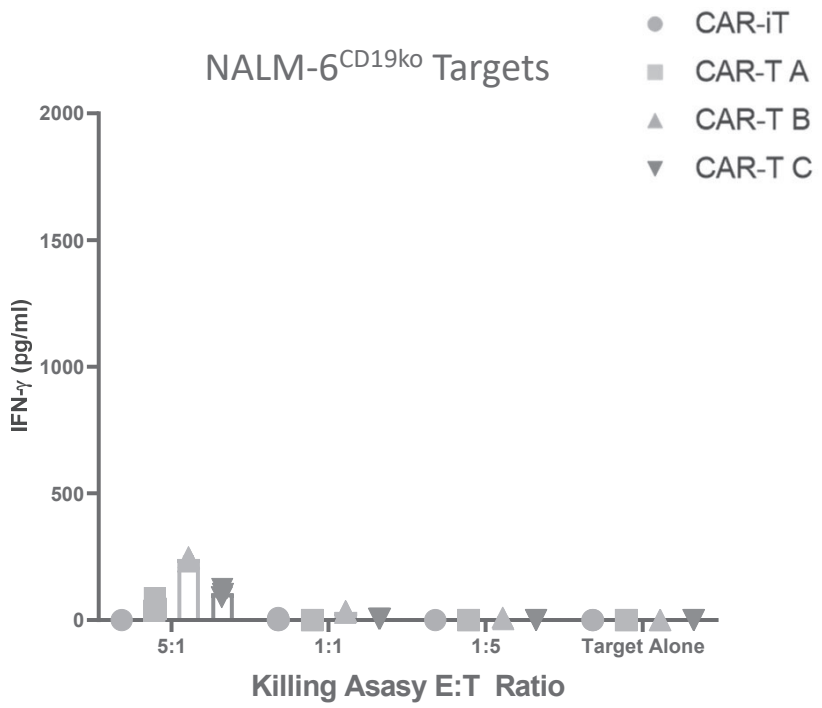

NALM-6CD19ko Targets
2000
1500
1000
500
0
IFN-γ (pg/ml)
CAR-iT
CAR-T A
CAR-T B
CAR-T C
5:1
1:1
1:5
Target Alone
Killing Asasy E:T Ratio

FIG. 8F

TNF

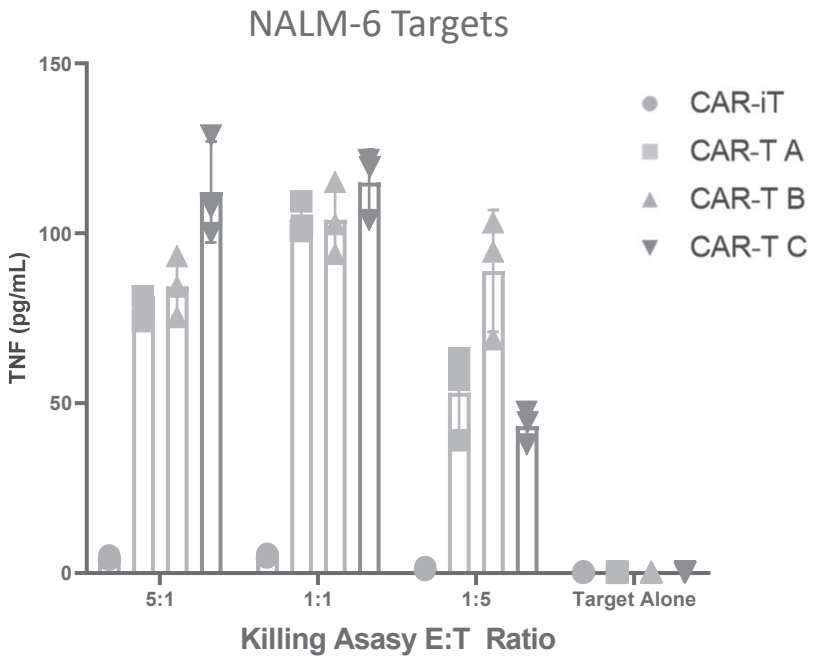


FIG. 8G

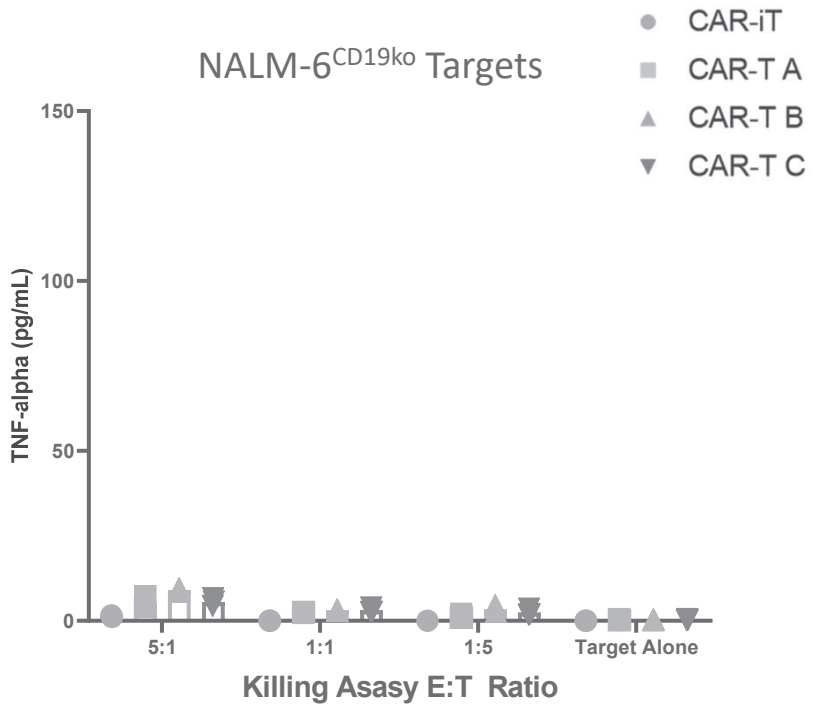


FIG. 8H

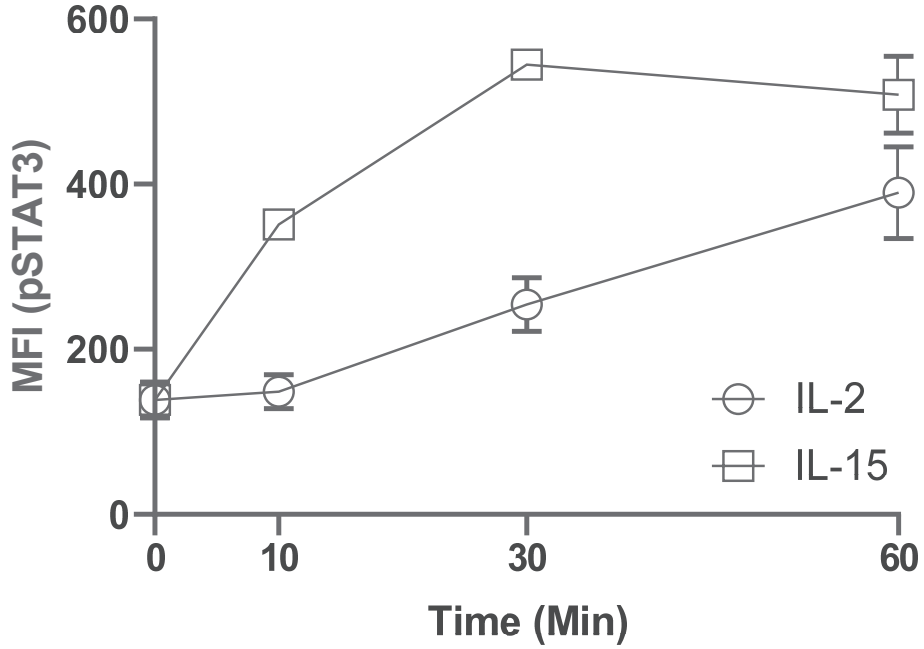

600
400
200
0
MFI (pSTAT3)
0
10
30
60
Time (Min)
IL-2
IL-15

FIG. 8I

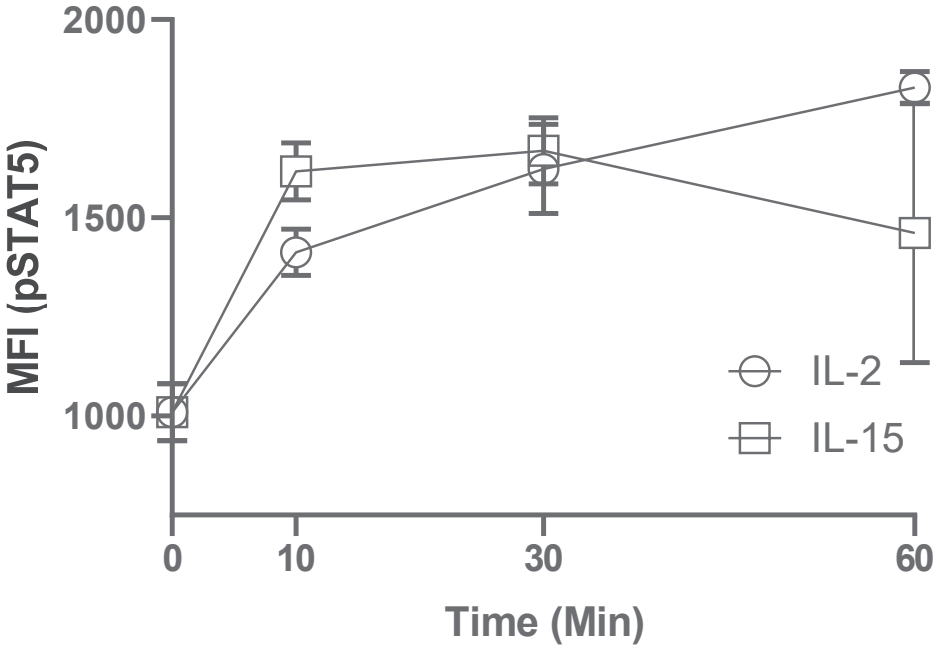

2000
1500
1000
MFI (pSTAT5)
0
10
30
60
Time (Min)
IL-2
IL-15

FIG. 8J

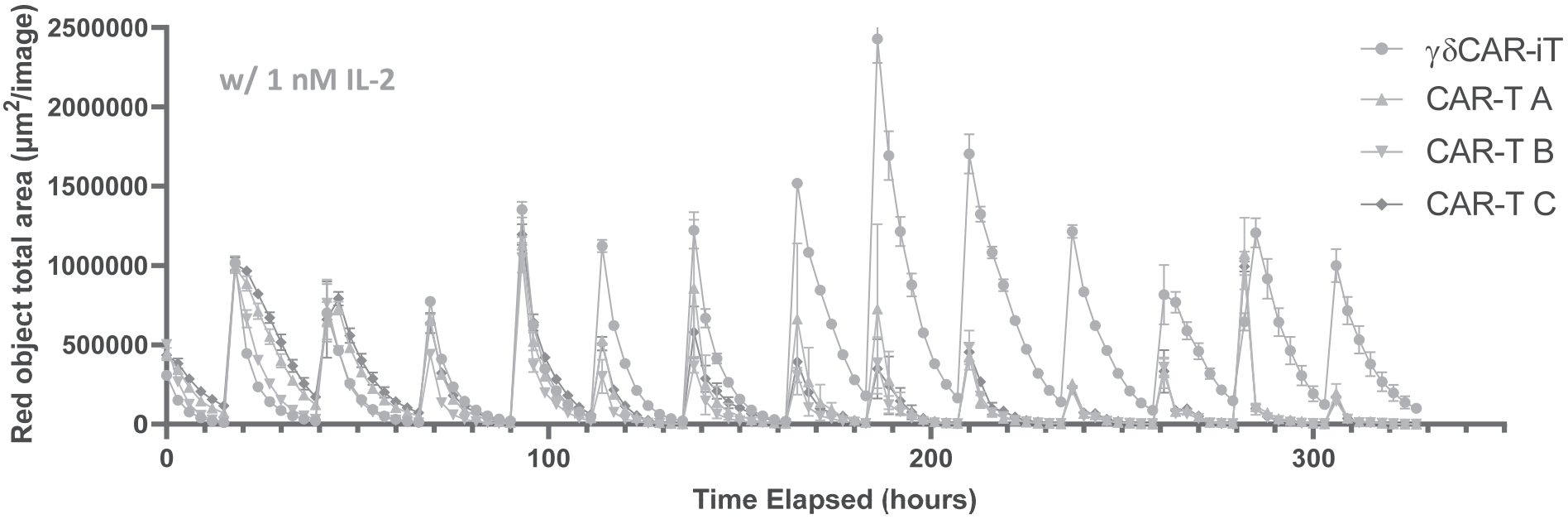

w/ 1 nM IL-2
Red object total area (μm²/image)
2500000
2000000
1500000
1000000
500000
0
0
100
200
300
Time Elapsed (hours)
γδCAR-iT
CAR-T A
CAR-T B
CAR-T C

FIG. 8K

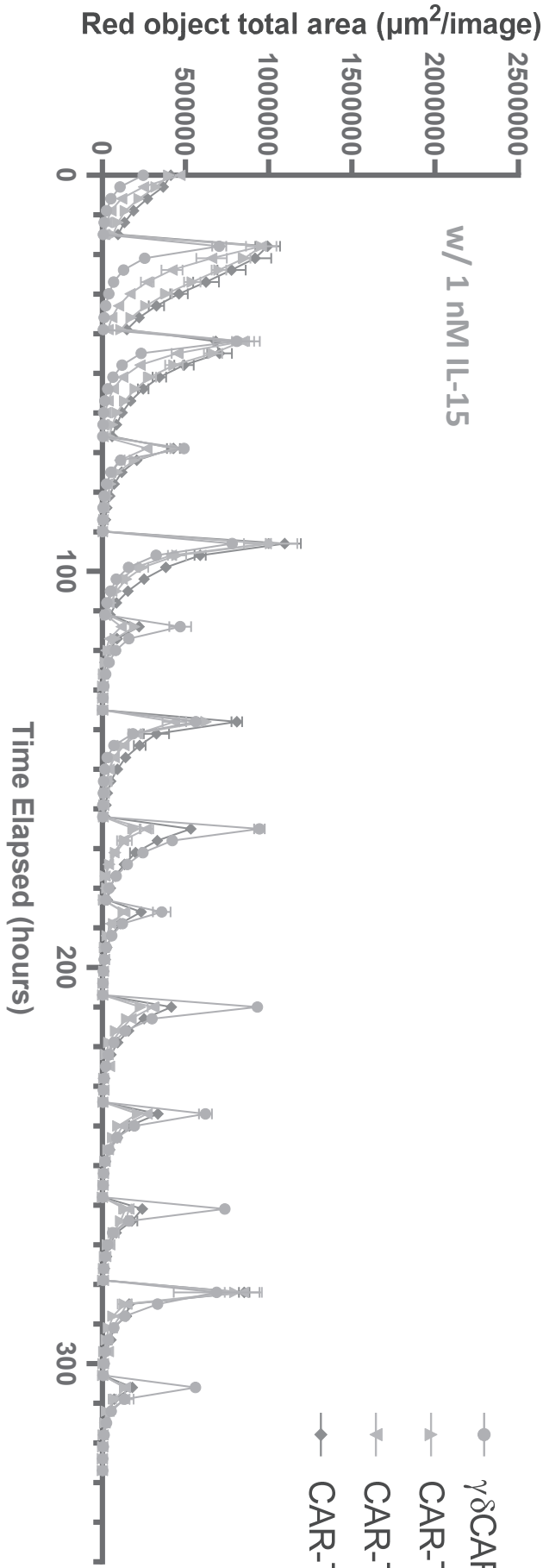

Red object total area (μm²/image)
0
500000
1000000
1500000
2000000
2500000
w/ 1 nM IL-15
0
100
200
300
Time Elapsed (hours)
γδCAR-iT
CAR-T A
CAR-T B
CAR-T C

FIG. 8L

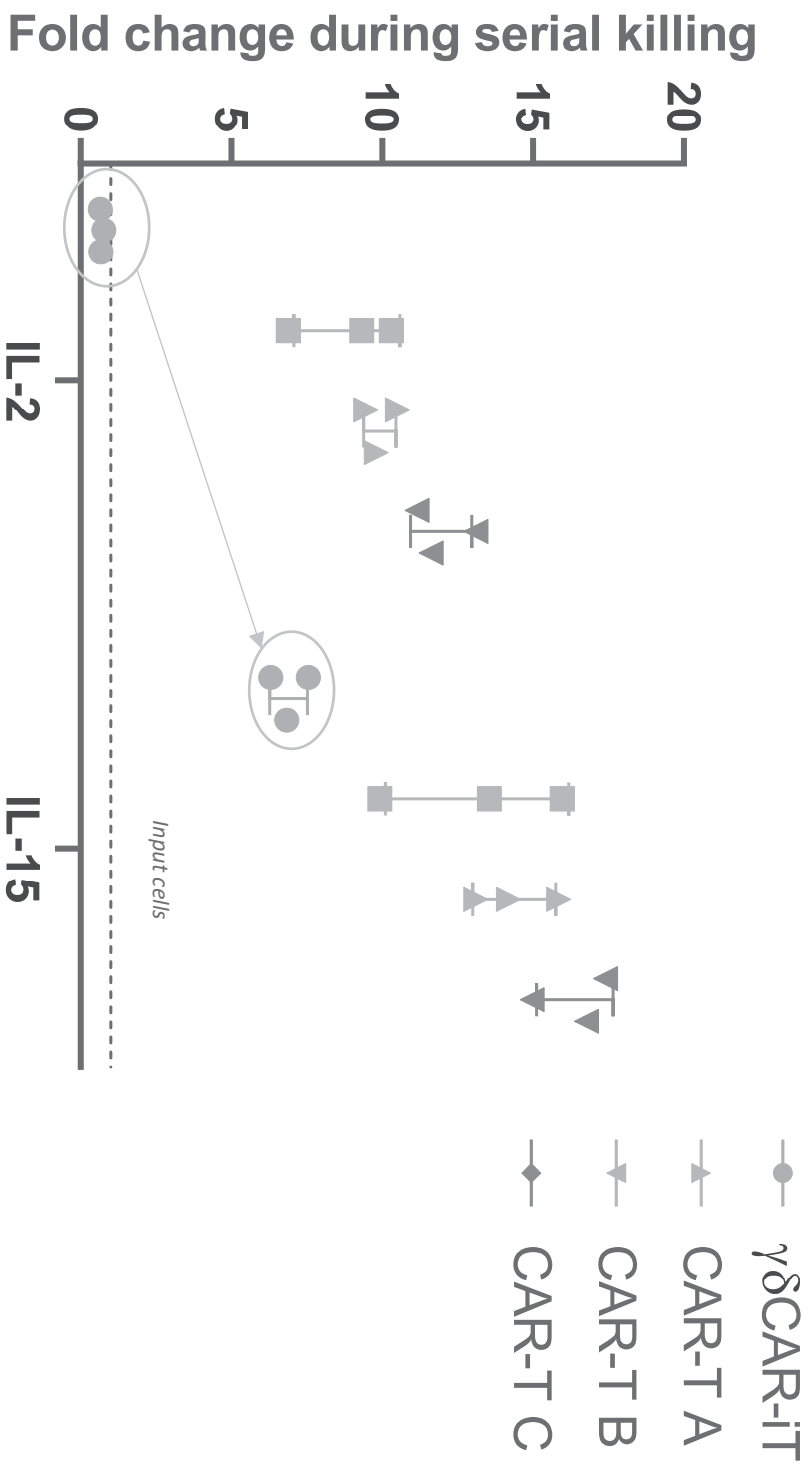

Fold change during serial killing
0
5
10
15
20
IL-2
IL-15
Input cells
γδCAR-iT
CAR-T A
CAR-T B
CAR-T C

FIG. 8M

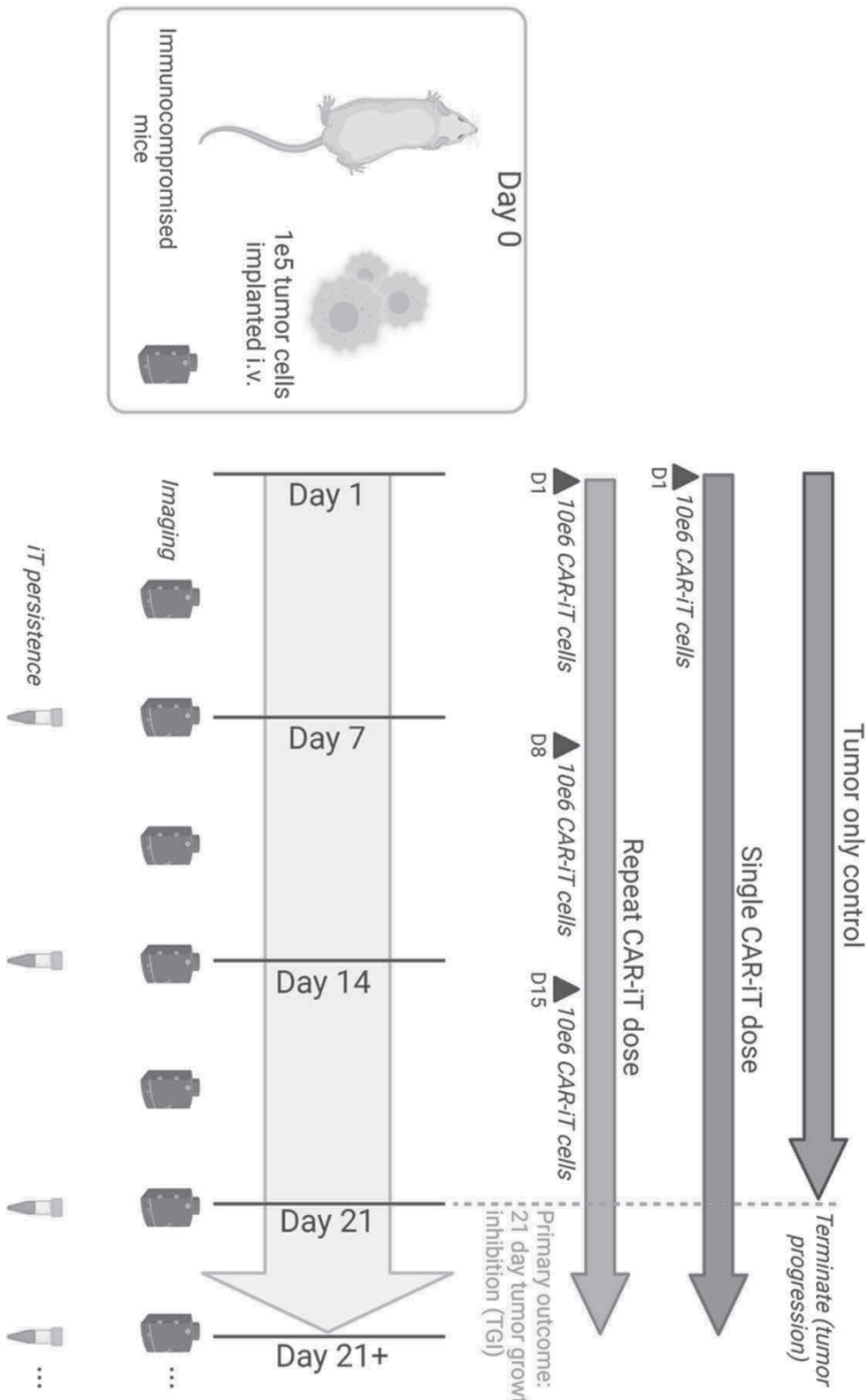

Day 0
Immunocompromised mice
1e5 tumor cells implanted i.v.
Imaging
iT persistence
Day 1
Day 7
Day 14
Day 21
Day 21+
Tumor only control
Terminate (tumor progression)
Single CAR-iT dose
10e6 CAR-iT cells
D1
Repeat CAR-iT dose
10e6 CAR-iT cells
D1
10e6 CAR-iT cells
D8
10e6 CAR-iT cells
D15
Primary outcome:
21 day tumor growth inhibition (TGI)

FIG. 9A

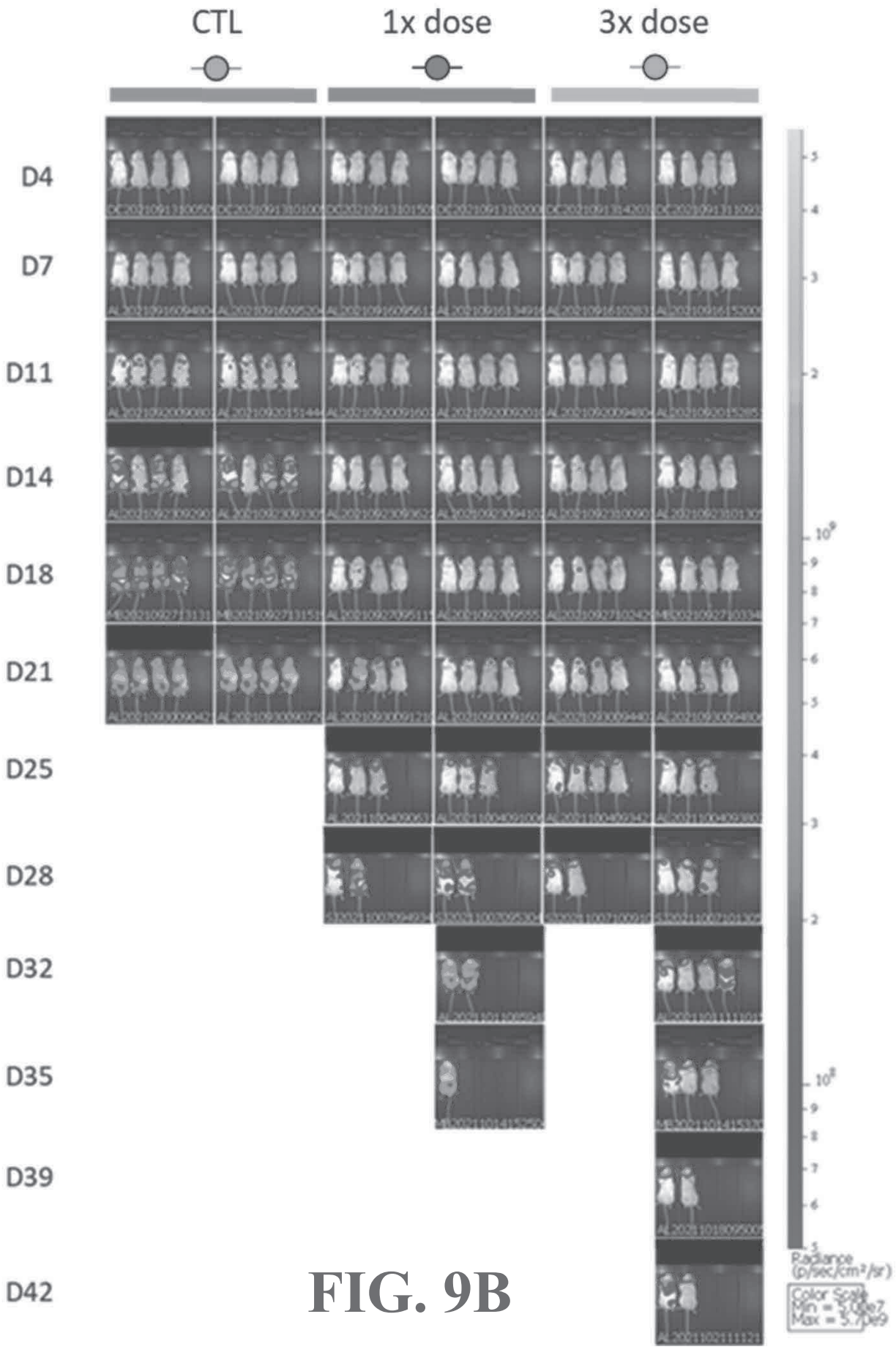

CTL
1x dose
3x dose
D4
D7
D11
D14
D18
D21
D25
D28
D32
D35
D39
D42
Radiance (p/sec/cm²/sr)

FIG. 9B

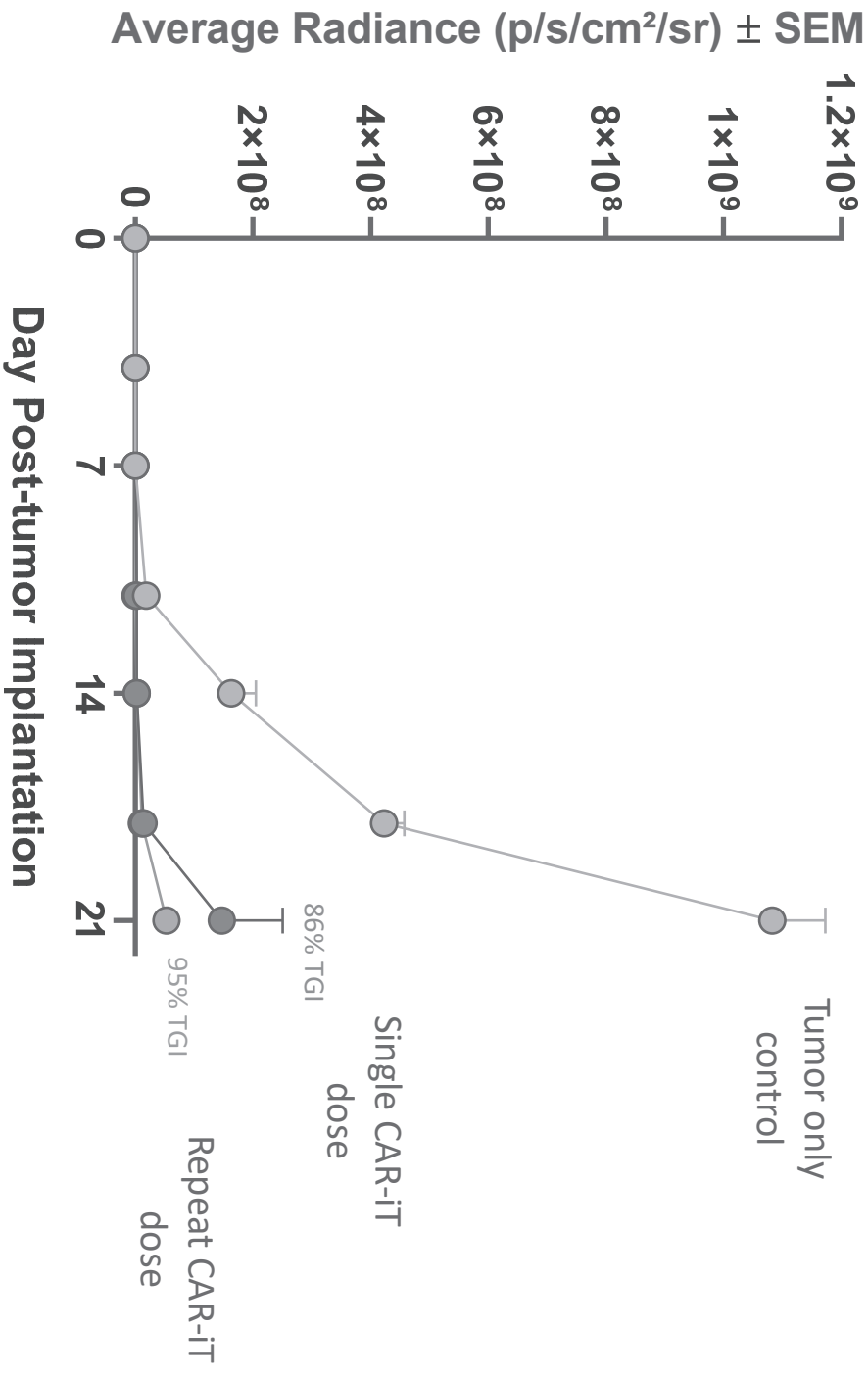

Average Radiance (p/s/cm²/sr) ± SEM
1.2×10⁹
1×10⁹
8×10⁸
6×10⁸
4×10⁸
2×10⁸
0
0
7
14
21
Day Post-tumor Implantation
Tumor only control
Single CAR-iT dose
Repeat CAR-iT dose
86% TGI
95% TGI

FIG. 9C

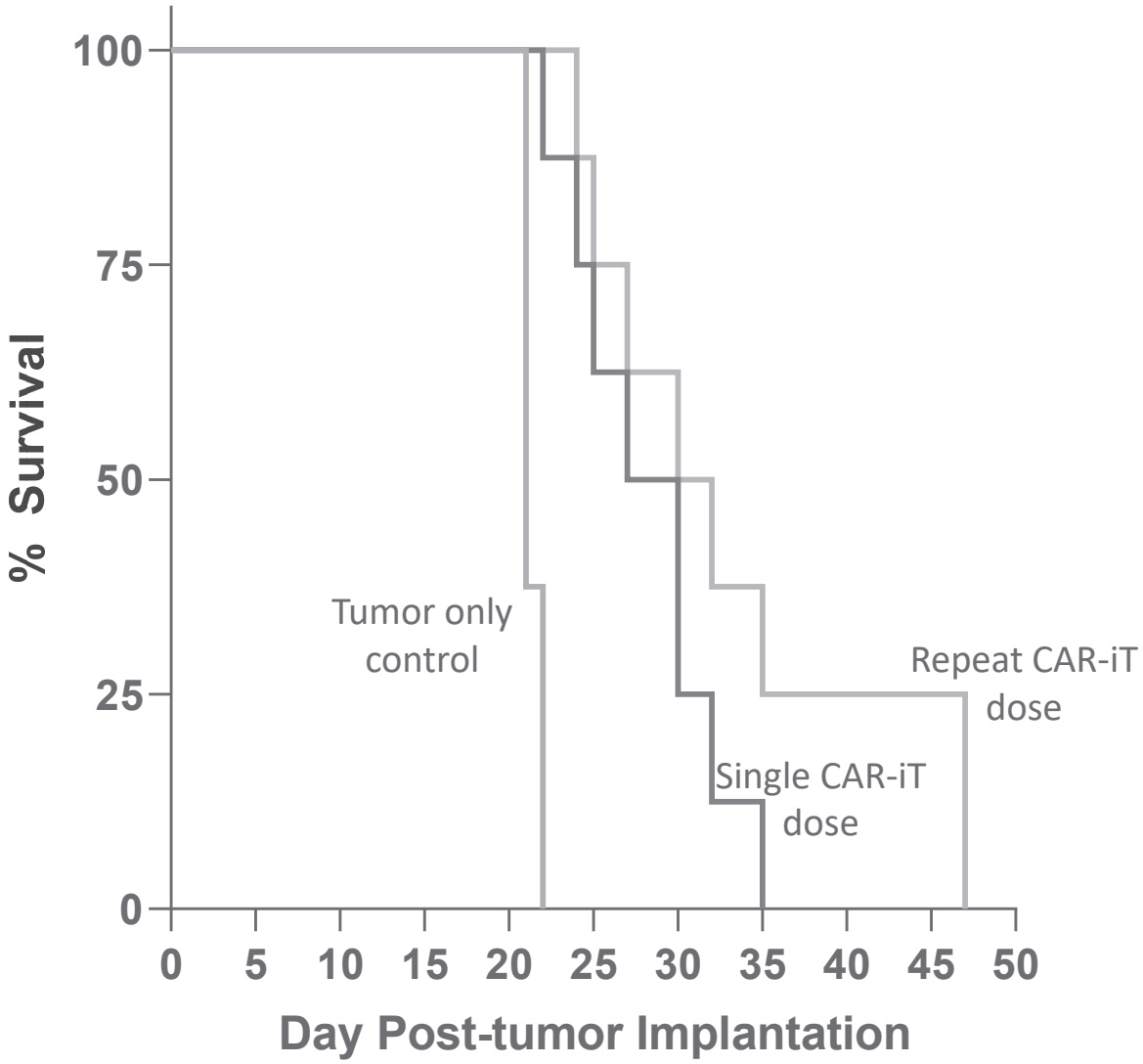

100
75
50
25
0
% Survival
Tumor only control
Repeat CAR-iT dose
Single CAR-iT dose
0 5 10 15 20 25 30 35 40 45 50
Day Post-tumor Implantation

FIG. 9D

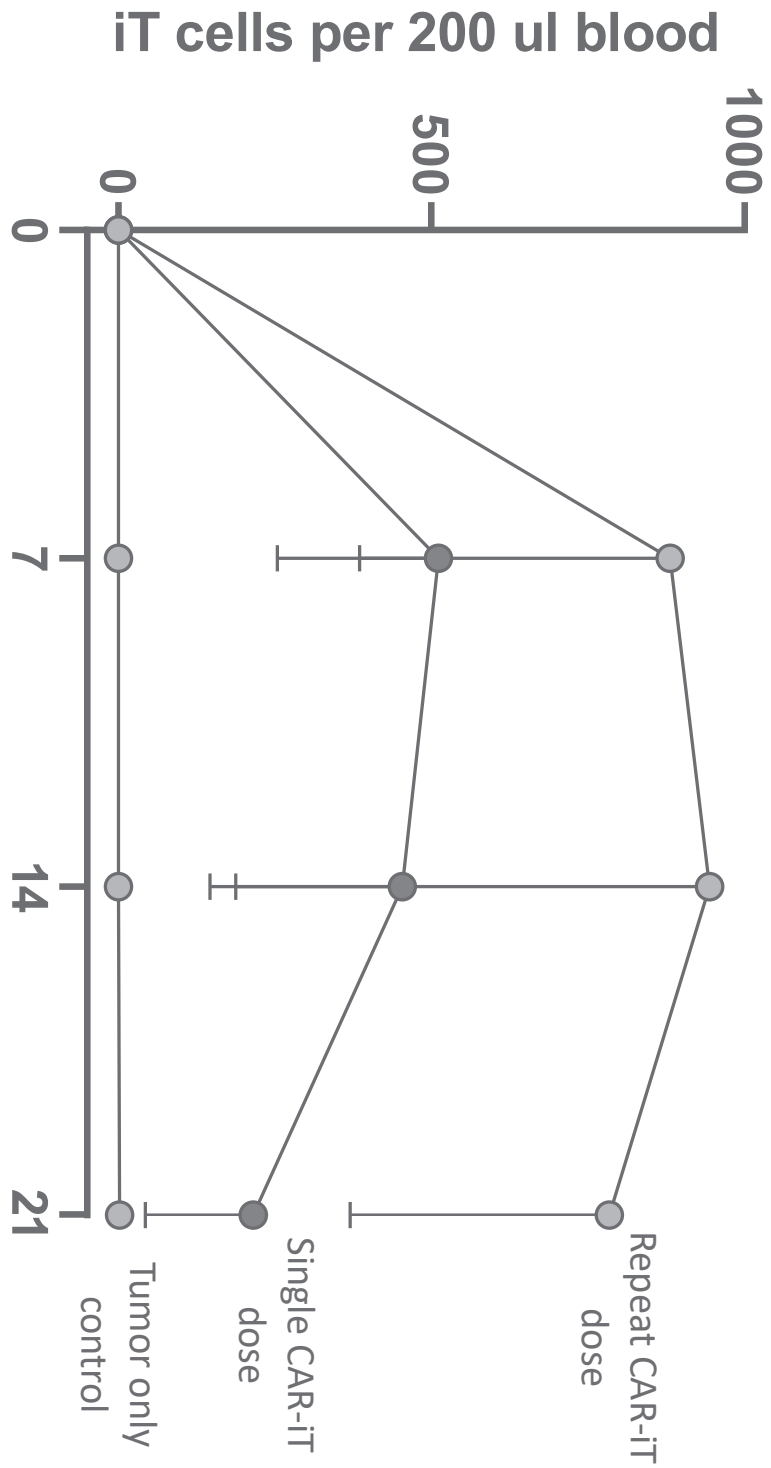

iT cells per 200 ul blood
1000
500
0
0
7
14
21
Day
Repeat CAR-iT dose
Single CAR-iT dose
Tumor only control

FIG. 9E

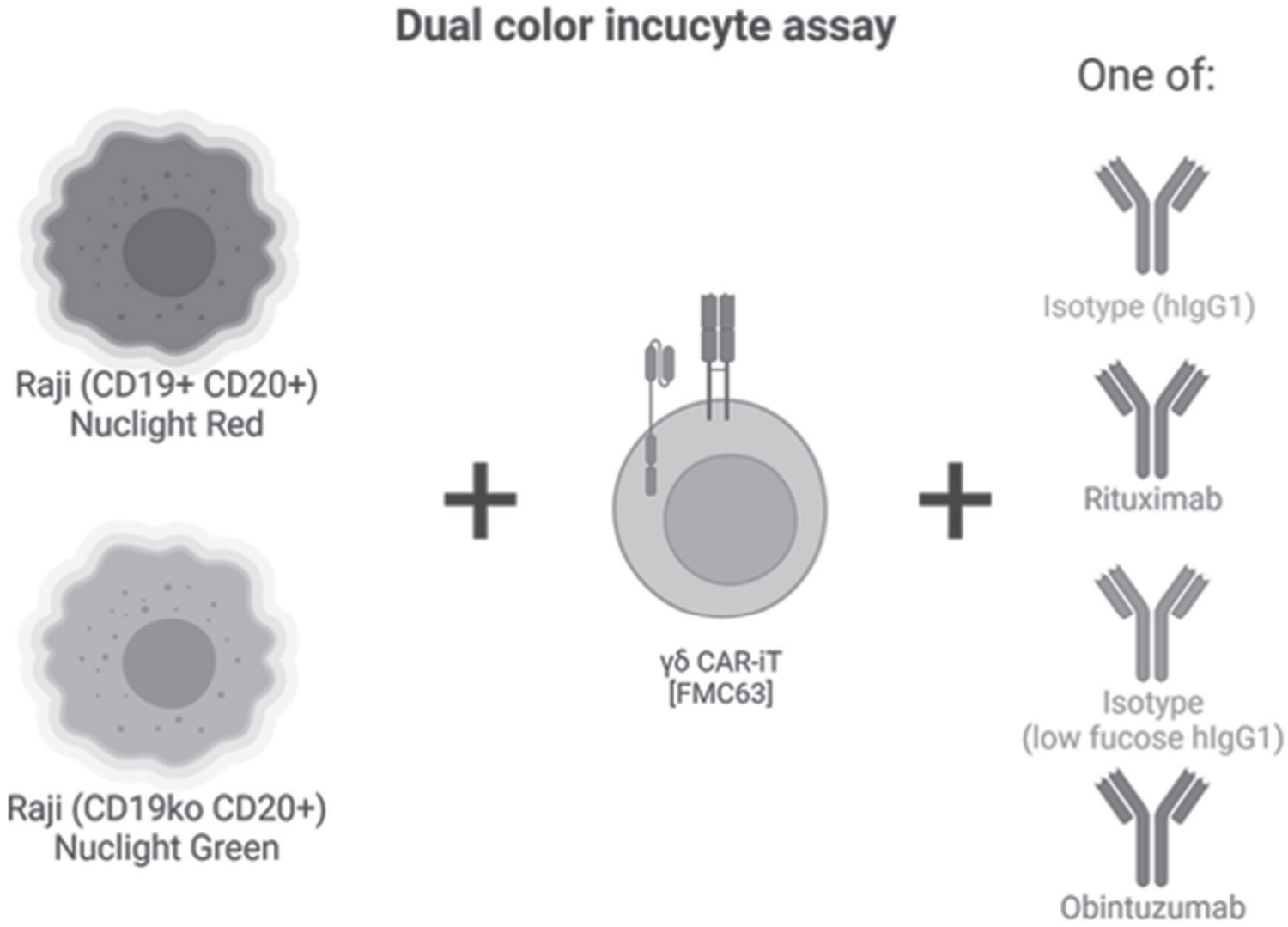

Dual color incucyte assay
One of:
Raji (CD19+ CD20+)
Nuclight Red
Raji (CD19ko CD20+)
Nuclight Green
γδ CAR-iT
[FMC63]
Isotype (hIgG1)
Rituximab
Isotype
(low fucose hIgG1)
Obintuzumab

FIG. 10

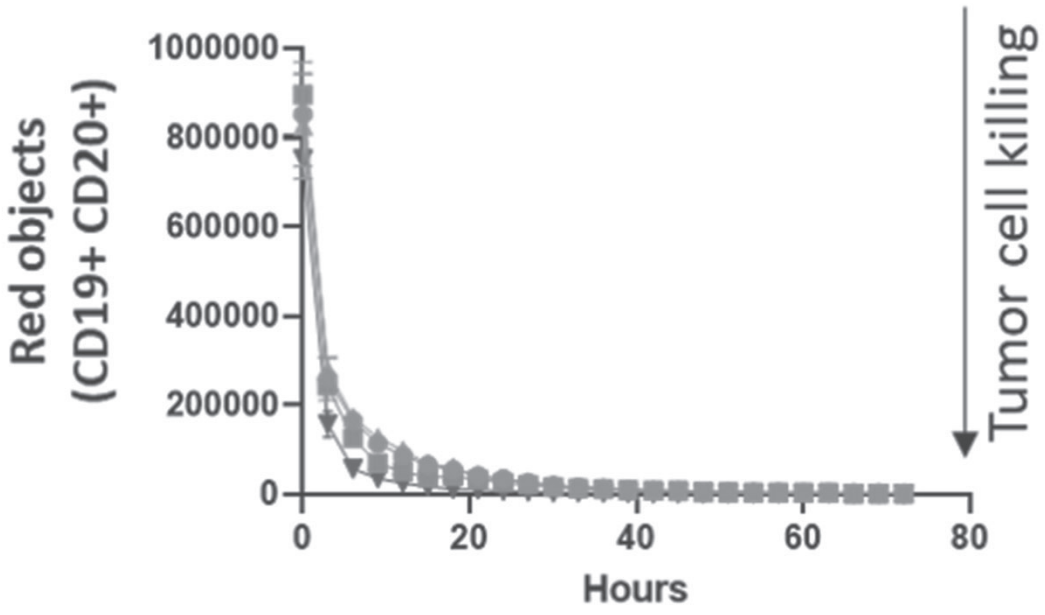


FIG. 11A

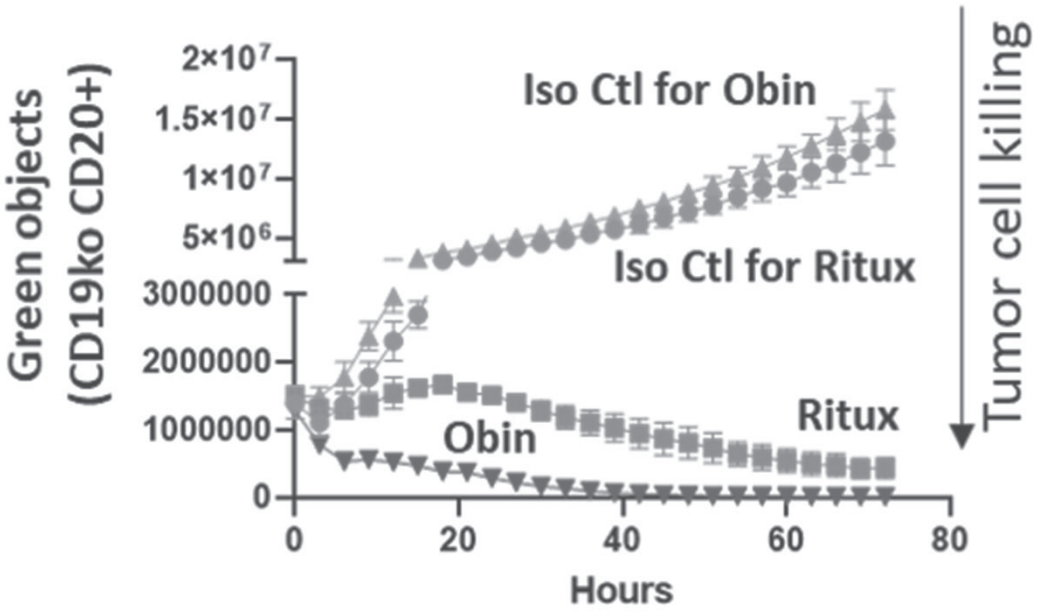


FIG. 11B