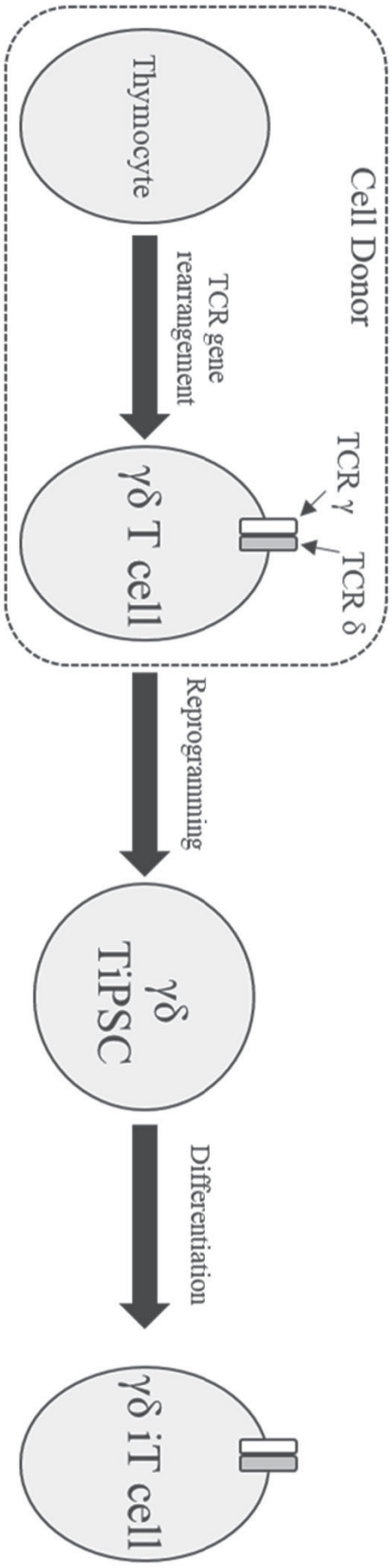

Cell Donor
Thymocyte
TCR gene rearrangement
γδ T cell
TCR γ
TCR δ
Reprogramming
γδ TiPSC
Differentiation
γδ iT cell

FIG. 1A

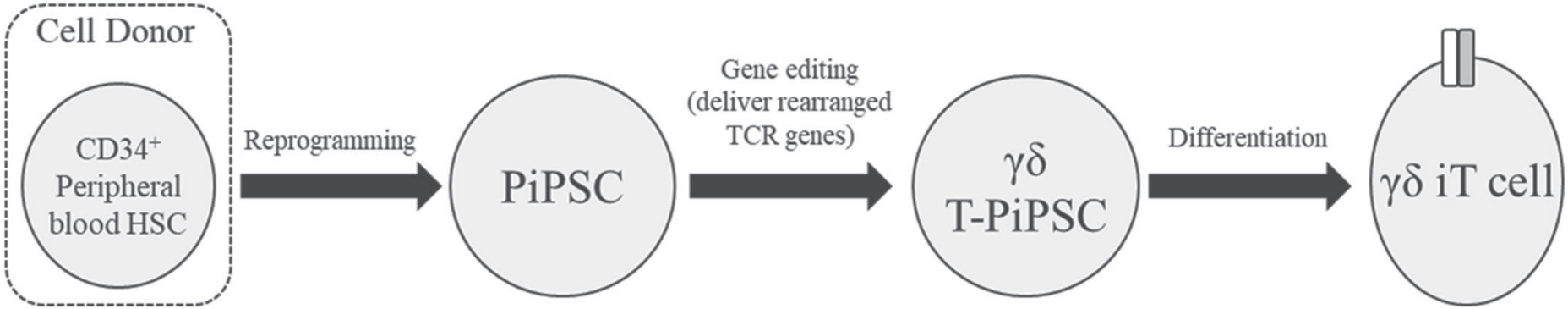

Cell Donor
CD34+ Peripheral blood HSC
Reprogramming
PiPSC
Gene editing (deliver rearranged TCR genes)
γδ T-PiPSC
Differentiation
γδ iT cell

FIG. 1B

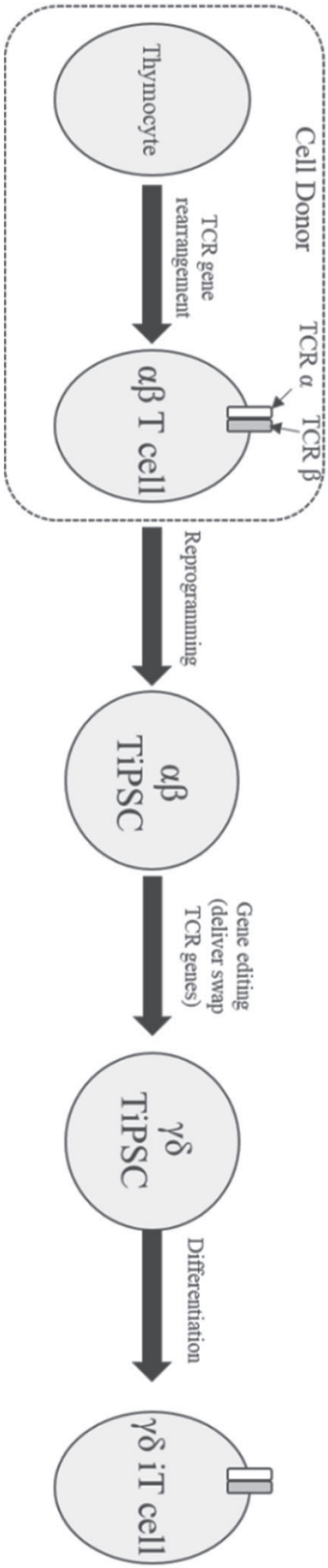

Cell Donor
Thymocyte
TCR gene rearrangement
αβ T cell
TCR α
TCR β
Reprogramming
αβ TiPSC
Gene editing (deliver swap TCR genes)
γδ TiPSC
Differentiation
γδ iT cell

FIG. 1C

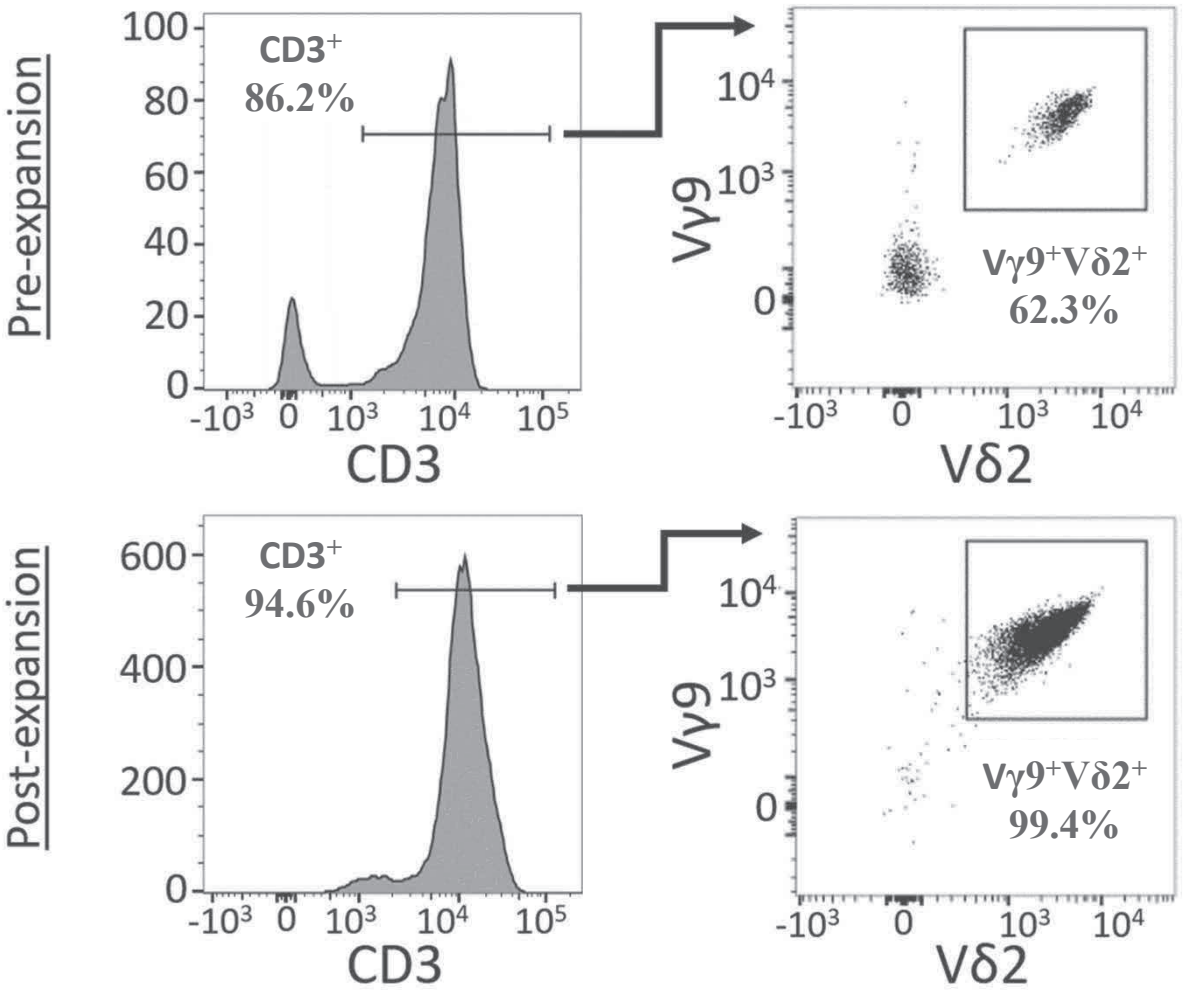

Pre-expansion
CD3+
86.2%
CD3
Vγ9
Vδ2
Vγ9+Vδ2+
62.3%
Post-expansion
CD3+
94.6%
CD3
Vγ9
Vδ2
Vγ9+Vδ2+
99.4%

FIG. 2A

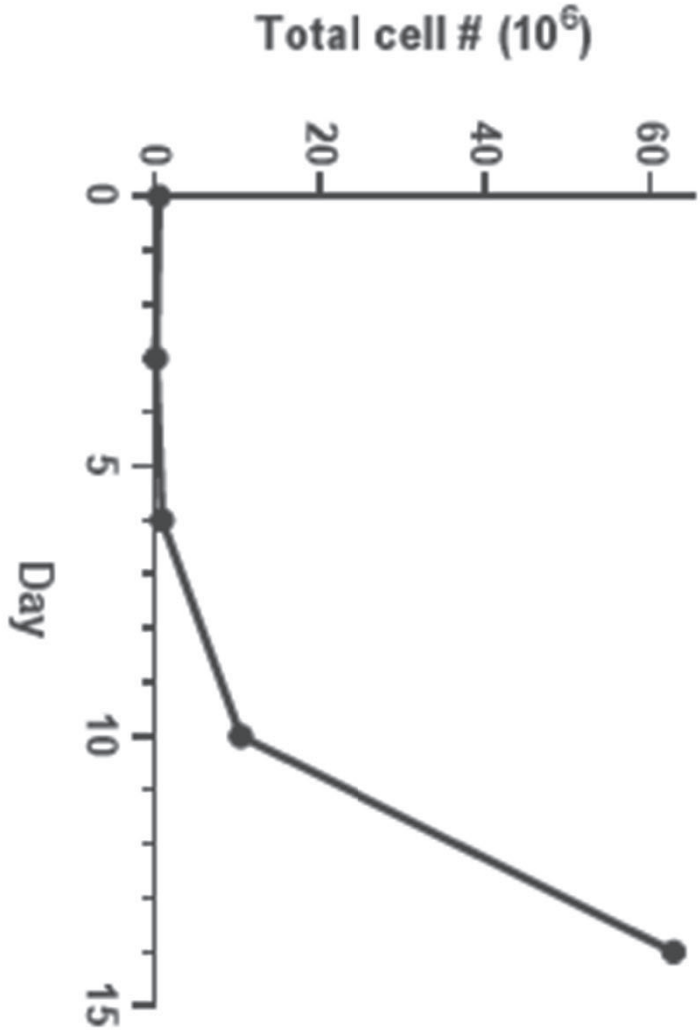


FIG. 2B

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

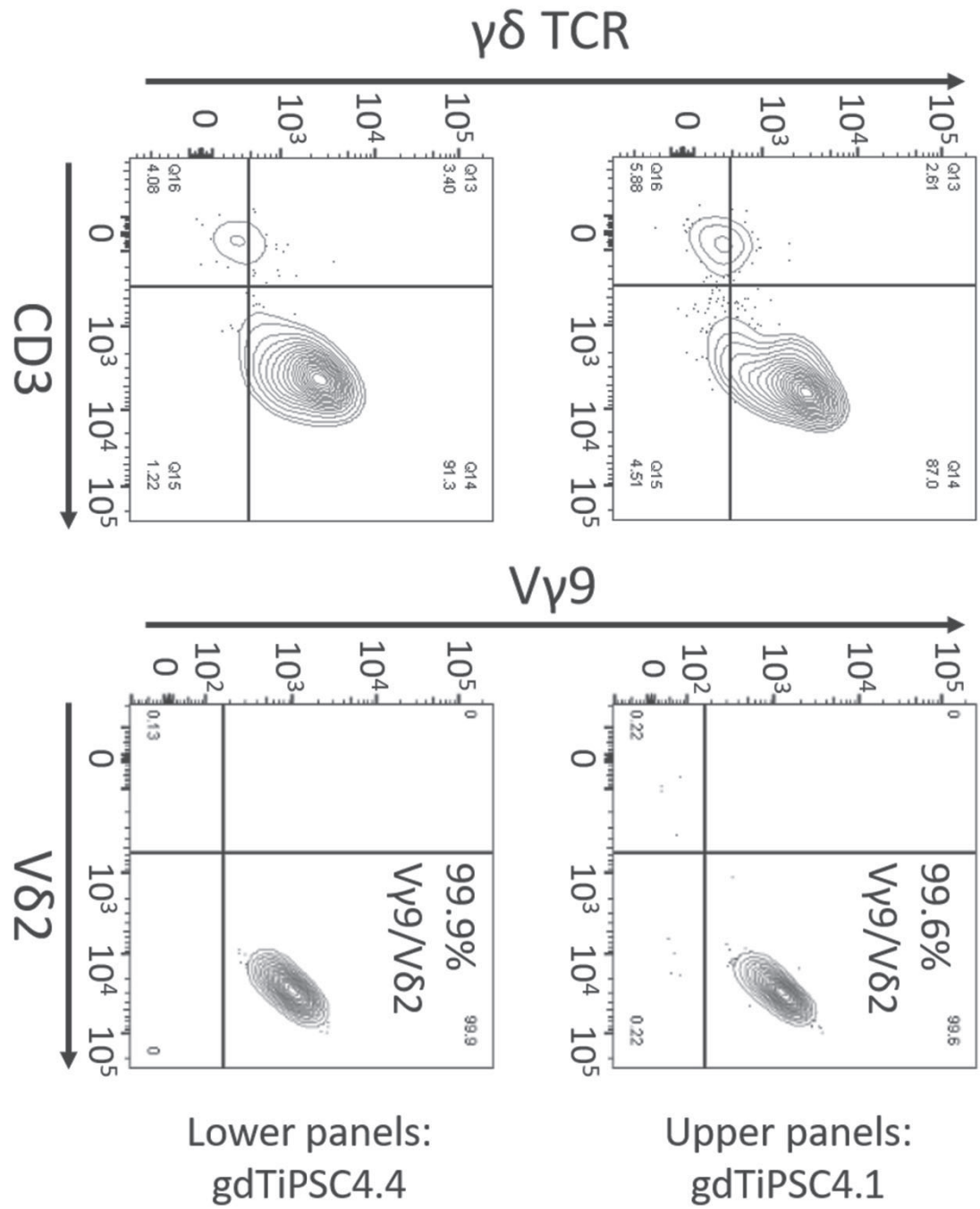

γδ TCR
CD3
Q13 2.61
Q16 5.88
Q14 87.0
Q15 4.51
Q13 3.40
Q16 4.08
Q14 91.3
Q15 1.22
Vγ9
Vδ2
0.22
99.6%
Vγ9/Vδ2
99.6
0.22
0.13
99.9%
Vγ9/Vδ2
99.9
0
Upper panels:
gdTiPSC4.1
Lower panels:
gdTiPSC4.4

FIG. 2D

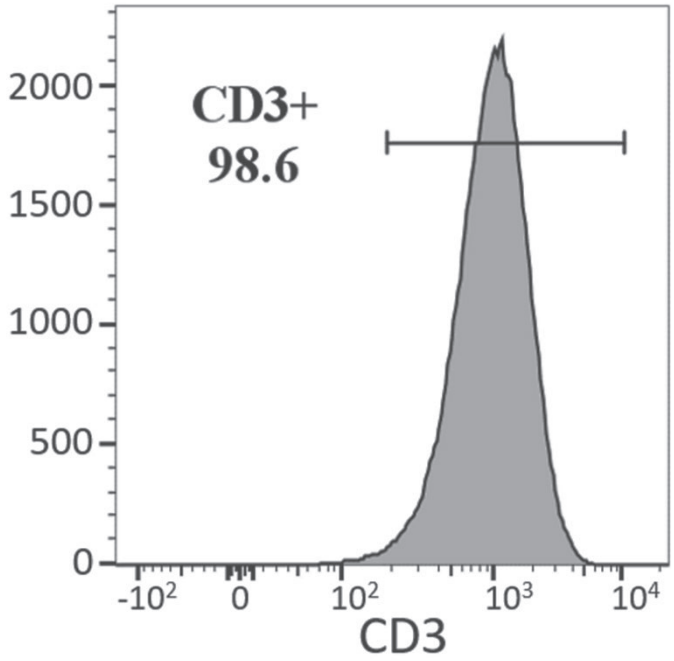

CD3+
98.6
2000
1500
1000
500
0
-10²
0
10²
10³
10⁴
CD3

FIG. 3A

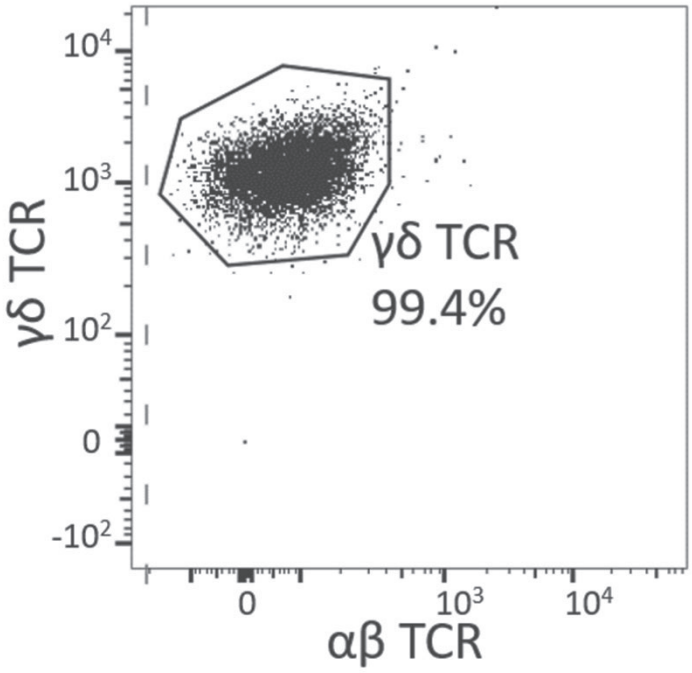

10⁴
10³
10²
0
-10²
γδ TCR
γδ TCR
99.4%
0
10³
10⁴
αβ TCR

FIG. 3B

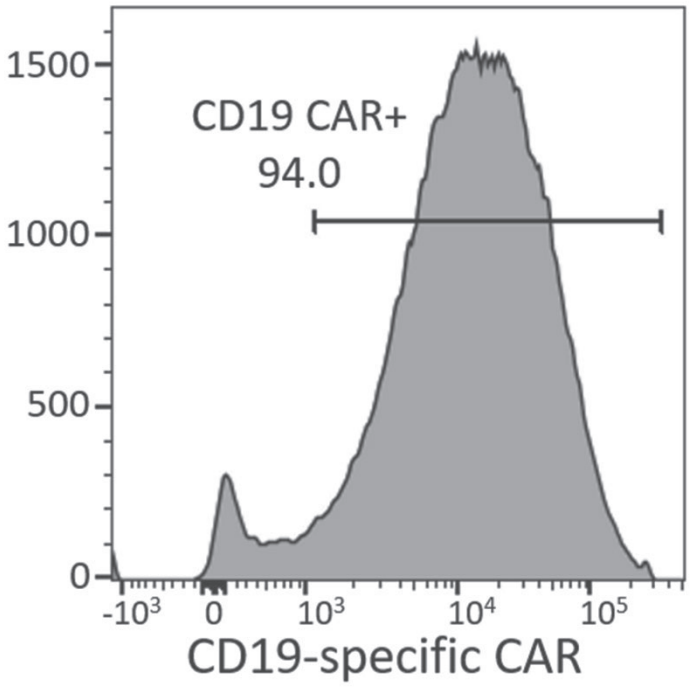

1500
1000
500
0
CD19 CAR+
94.0
-10³
0
10³
10⁴
10⁵
CD19-specific CAR

FIG. 3C

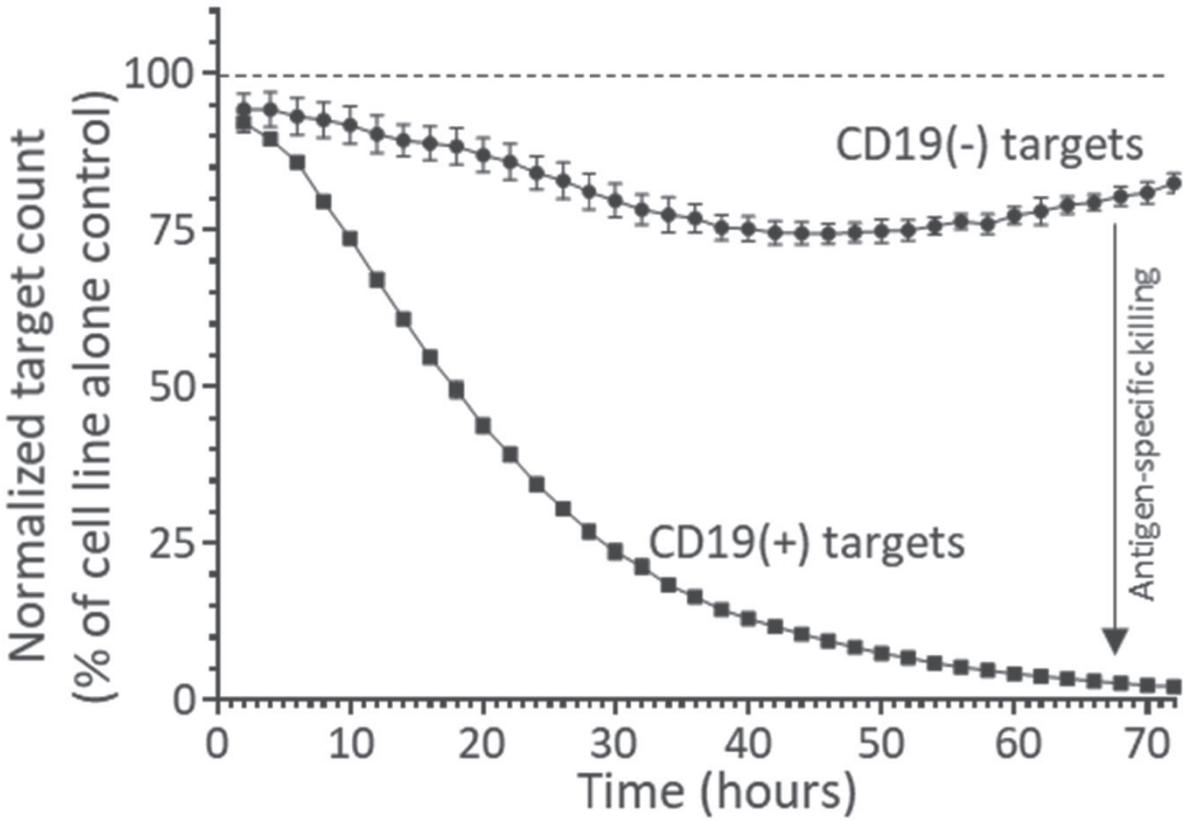

100
75
50
25
0
Normalized target count
(% of cell line alone control)
CD19(-) targets
CD19(+) targets
Antigen-specific killing
0
10
20
30
40
50
60
70
Time (hours)

FIG. 3D

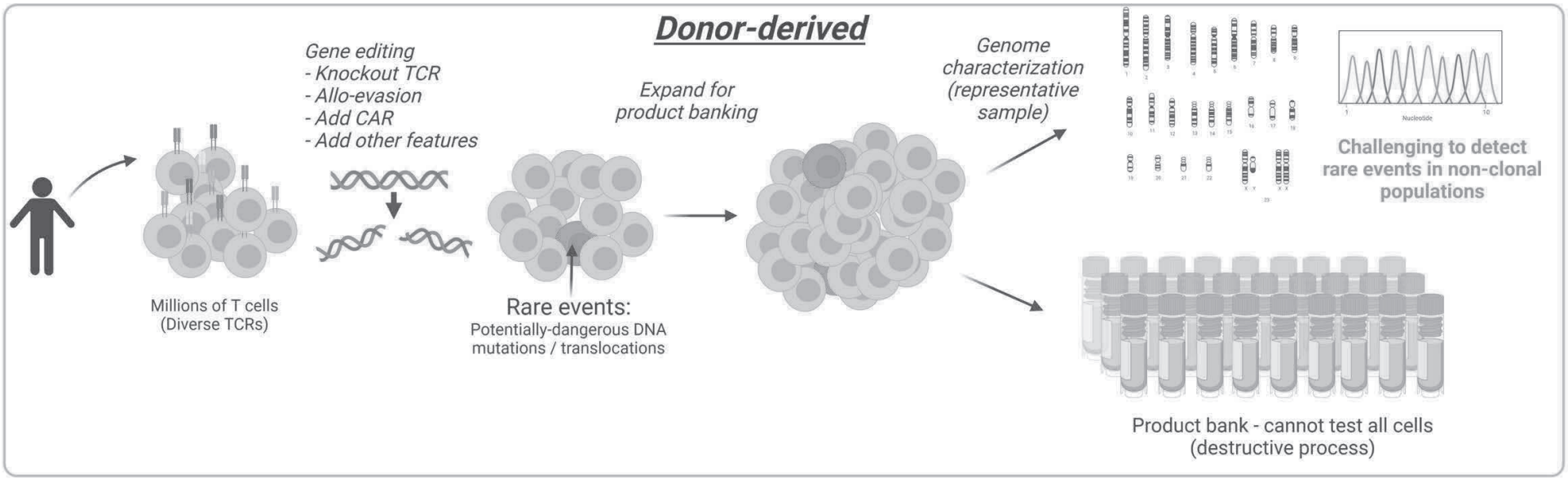

Donor-derived
Gene editing
- Knockout TCR
- Allo-evasion
- Add CAR
- Add other features
Expand for product banking
Genome characterization (representative sample)
Millions of T cells (Diverse TCRs)
Rare events:
Potentially-dangerous DNA mutations / translocations
Challenging to detect rare events in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

FIG. 4A

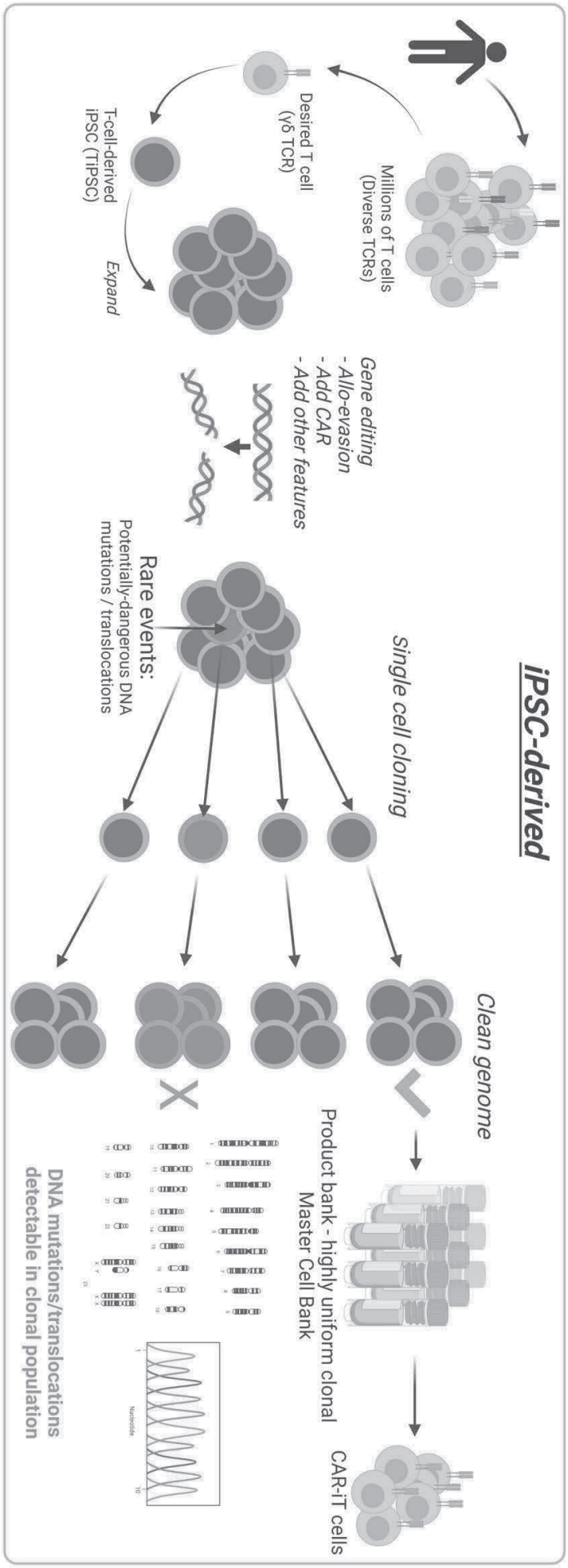

iPSC-derived
Millions of T cells
(Diverse TCRs)
Desired T cell
(γδ TCR)
T-cell-derived
iPSC (TiPSC)
Expand
Gene editing
- Allo-evasion
- Add CAR
- Add other features
Single cell cloning
Rare events:
Potentially-dangerous DNA
mutations / translocations
Clean genome
Product bank - highly uniform clonal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

FIG. 4B

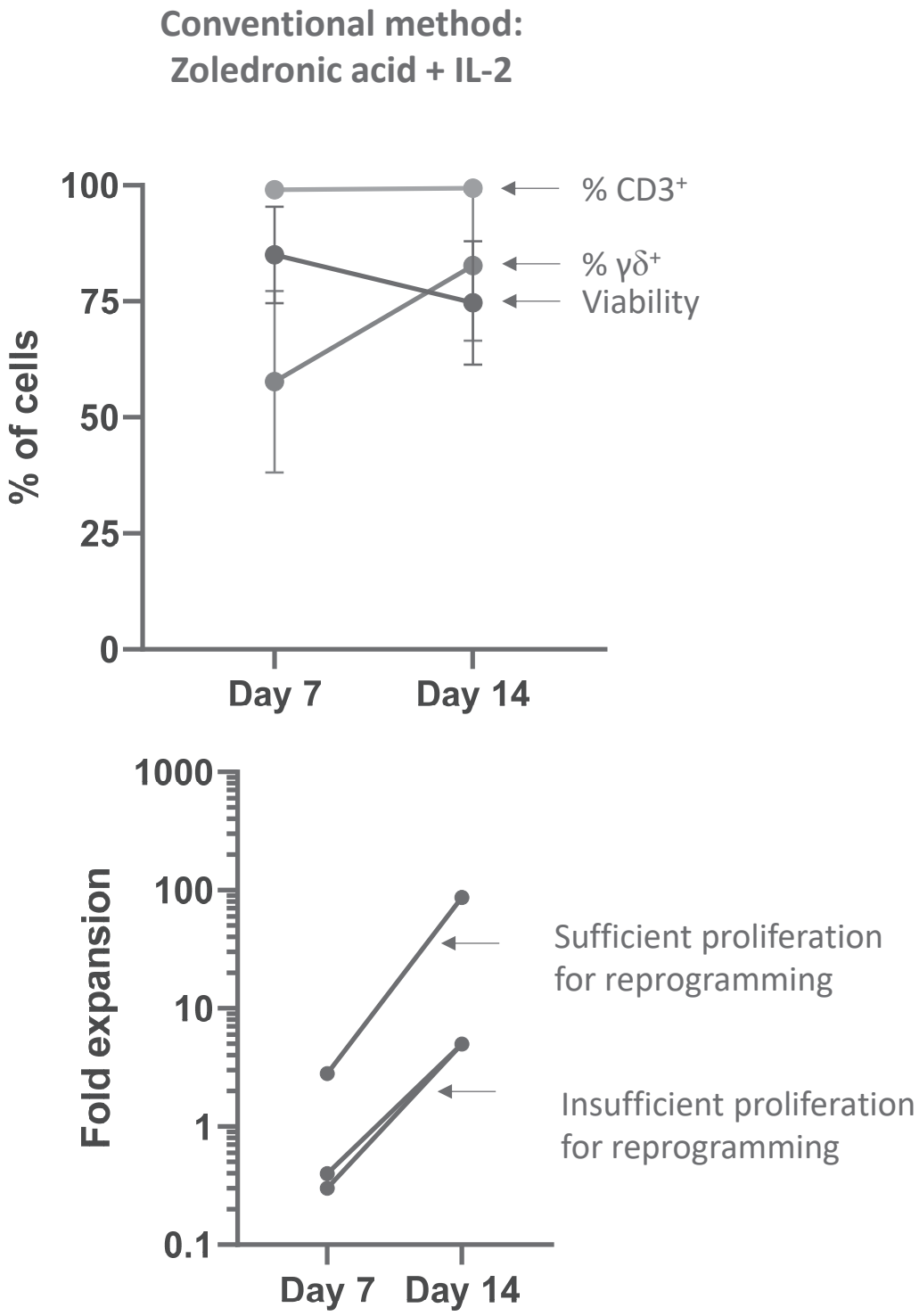

Conventional method:
Zoledronic acid + IL-2
100
75
50
25
0
% of cells
Day 7
Day 14
% CD3+
% γδ+
Viability
1000
100
10
1
0.1
Fold expansion
Day 7
Day 14
Sufficient proliferation
for reprogramming
Insufficient proliferation
for reprogramming

FIG. 5A

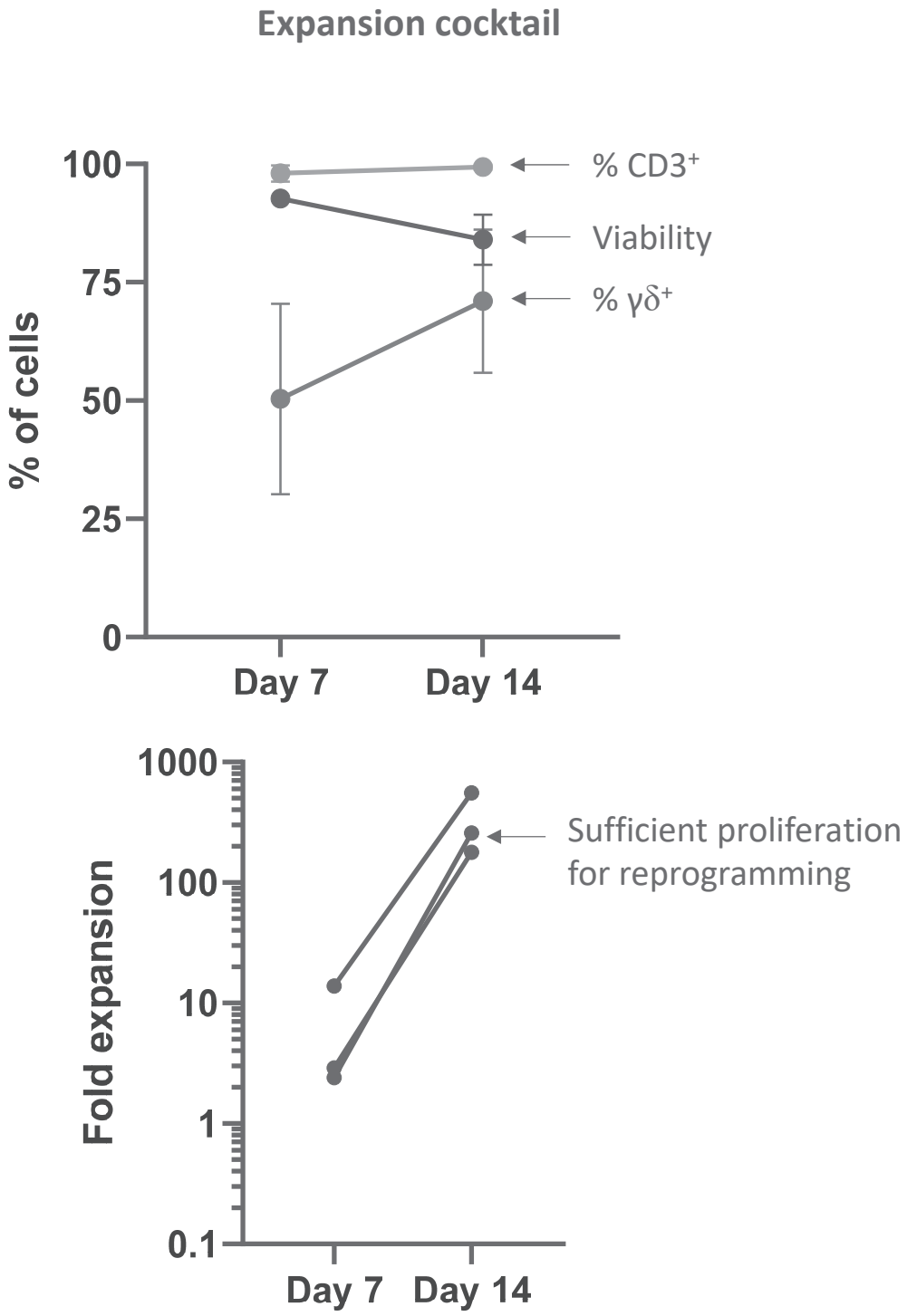
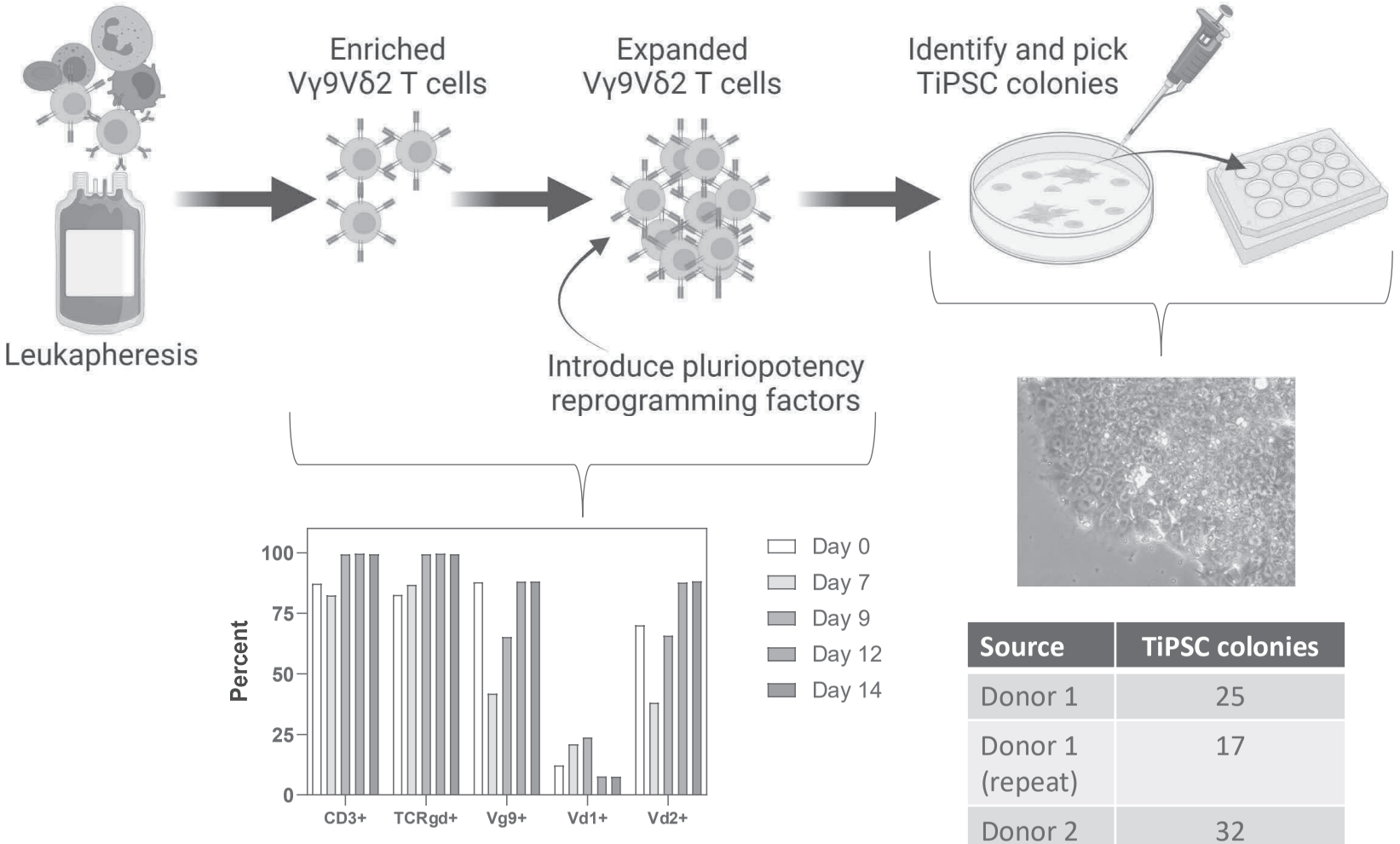

Expansion cocktail
100
75
50
25
0
% of cells
% CD3+
Viability
% γδ+
Day 7
Day 14
1000
100
10
1
0.1
Fold expansion
Sufficient proliferation
for reprogramming
Day 7
Day 14

FIG. 5B



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

FIG. 6

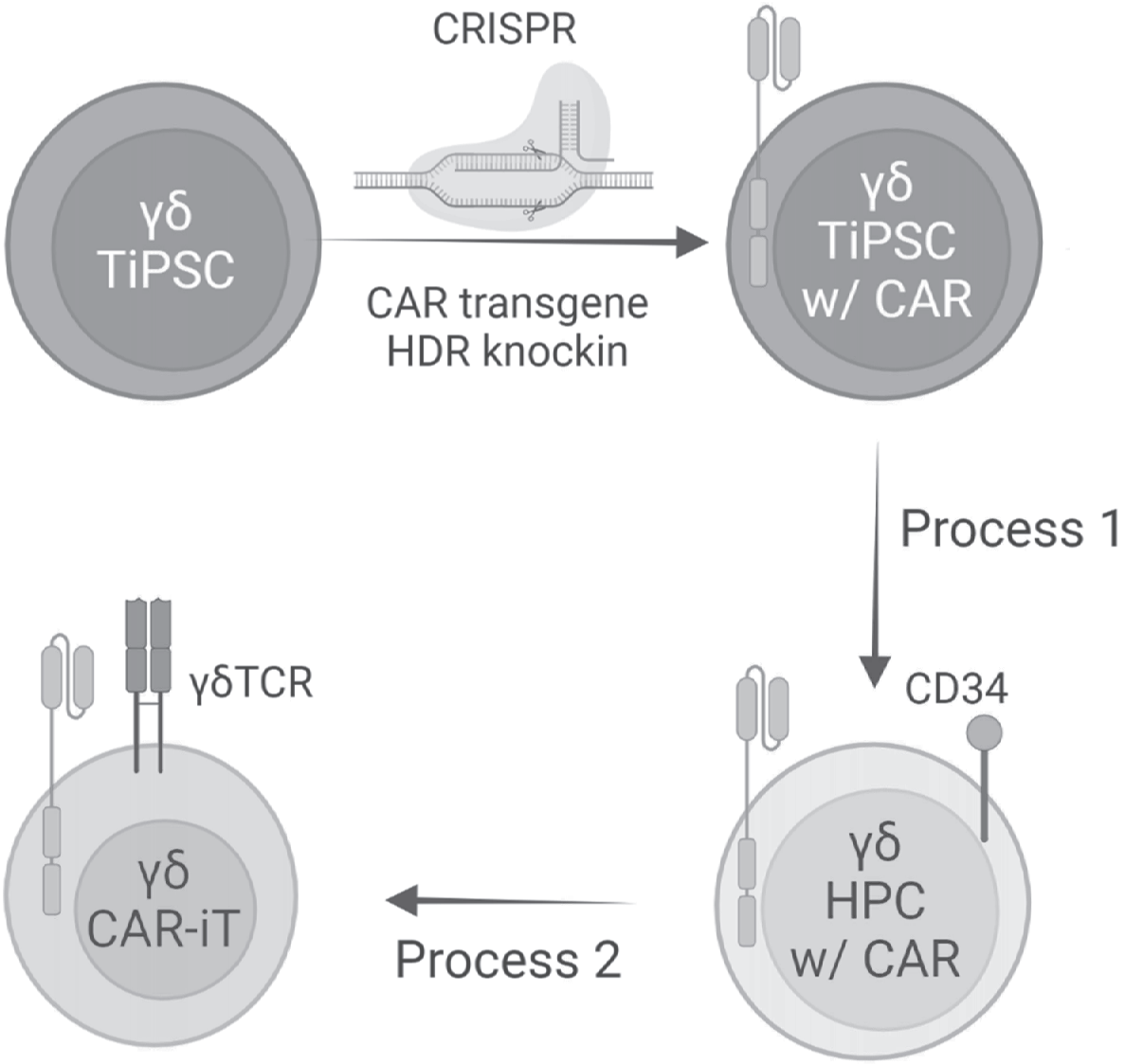

CRISPR
γδ
TiPSC
CAR transgene
HDR knockin
γδ
TiPSC
w/ CAR
Process 1
CD34
γδ
HPC
w/ CAR
Process 2
γδTCR
γδ
CAR-iT

FIG. 7A

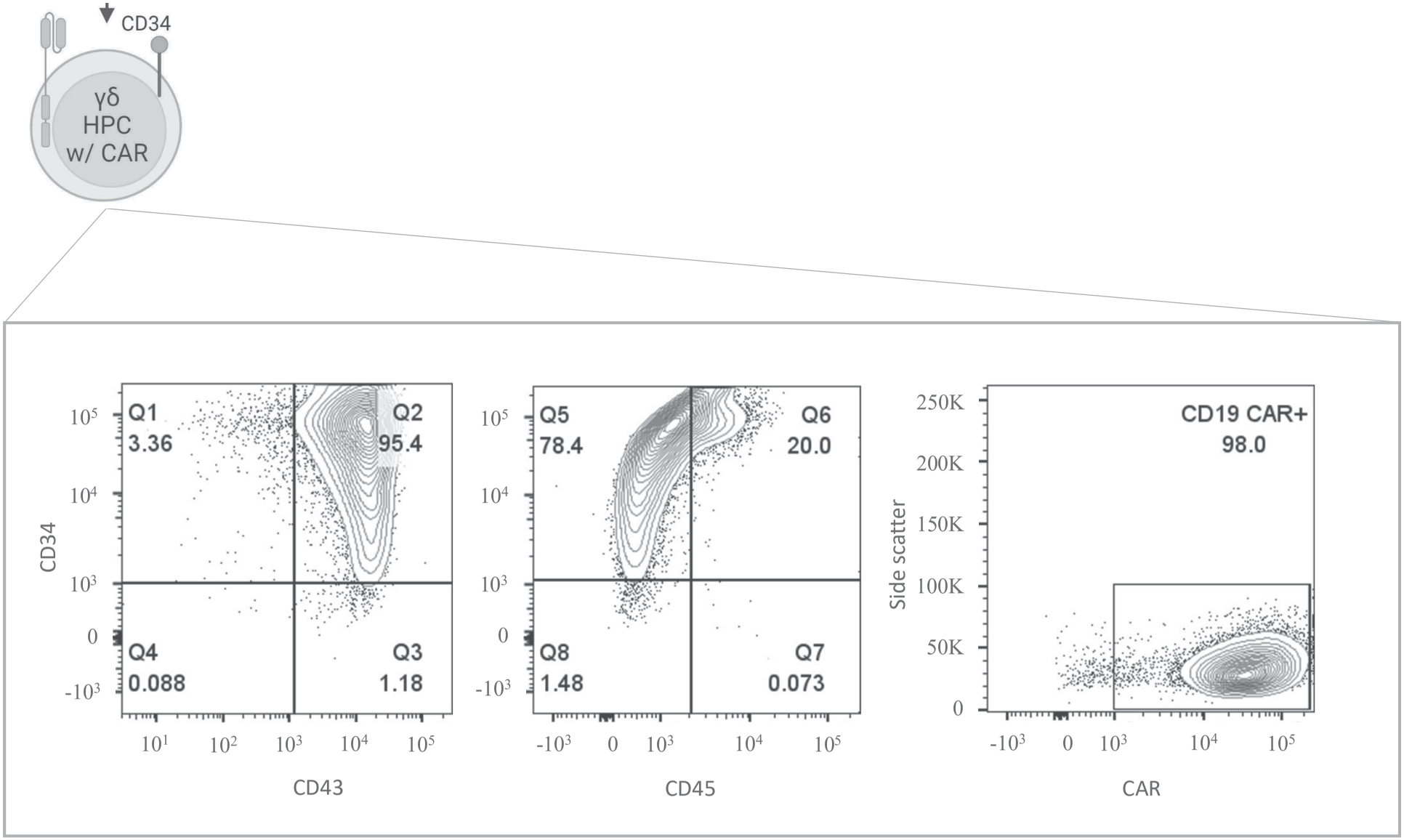

CD34
γδ
HPC
w/ CAR
Q1
3.36
Q2
95.4
Q4
0.088
Q3
1.18
CD34
CD43
Q5
78.4
Q6
20.0
Q8
1.48
Q7
0.073
CD45
CD19 CAR+
98.0
Side scatter
CAR

FIG. 7B

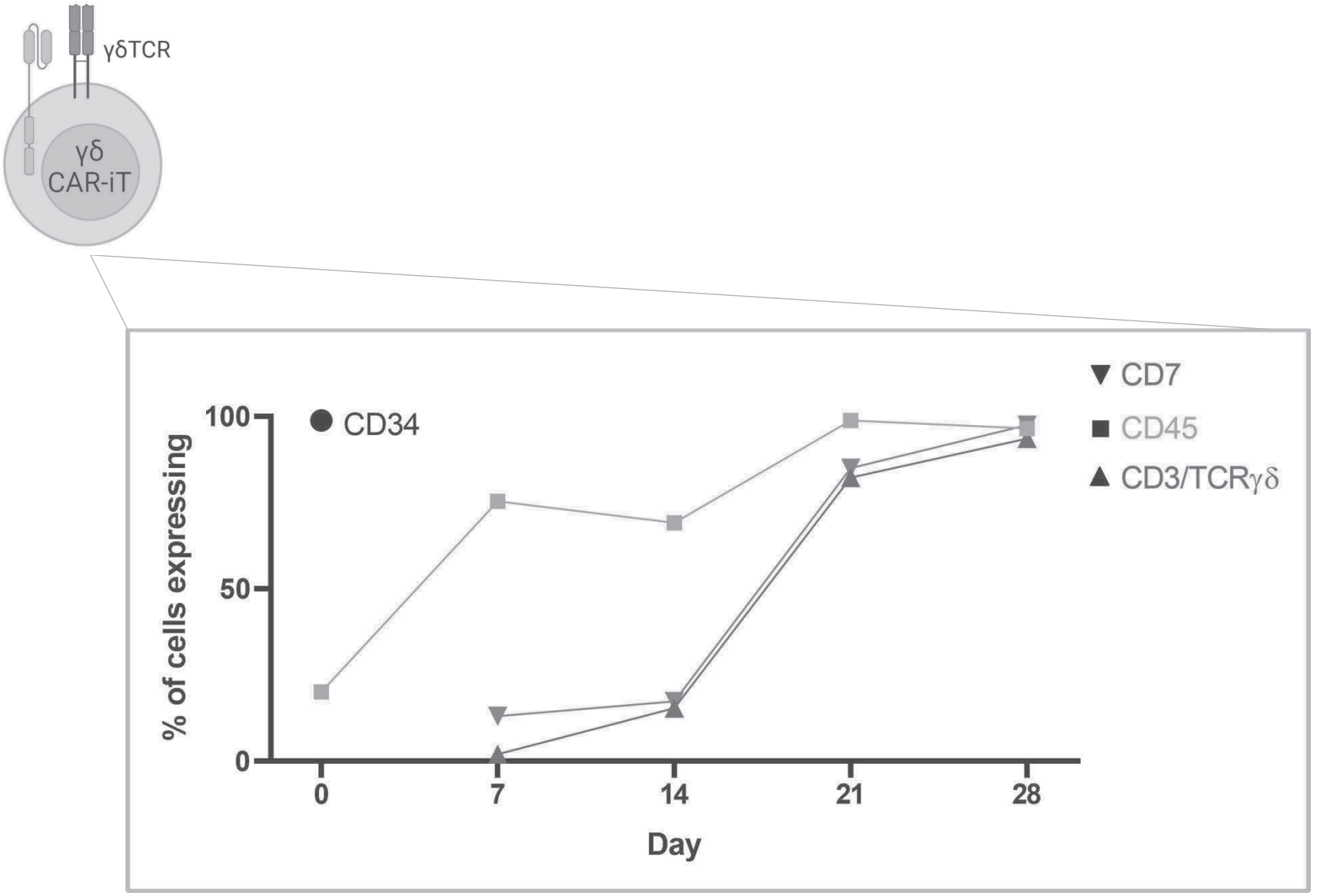

γδTCR
γδ
CAR-iT
CD34
CD7
CD45
CD3/TCRγδ
% of cells expressing
100
50
0
0
7
14
21
28
Day

FIG. 7C

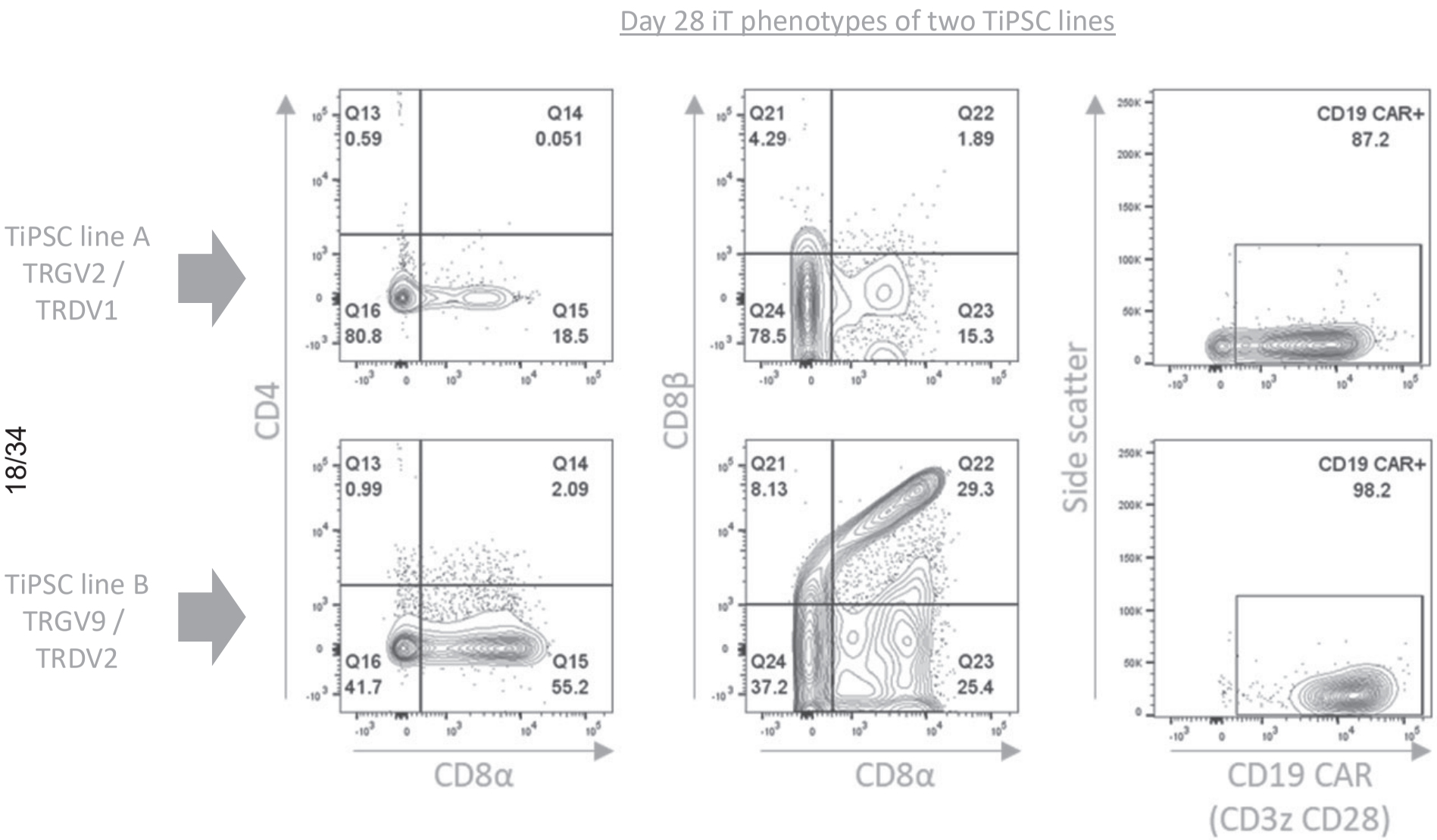

Day 28 iT phenotypes of two TiPSC lines
TiPSC line A
TRGV2 /
TRDV1
TiPSC line B
TRGV9 /
TRDV2
CD4
CD8α
Q13
0.59
Q14
0.051
Q16
80.8
Q15
18.5
Q13
0.99
Q14
2.09
Q16
41.7
Q15
55.2
CD8β
CD8α
Q21
4.29
Q22
1.89
Q24
78.5
Q23
15.3
Q21
8.13
Q22
29.3
Q24
37.2
Q23
25.4
Side scatter
CD19 CAR
(CD3z CD28)
CD19 CAR+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

FIG. 7D

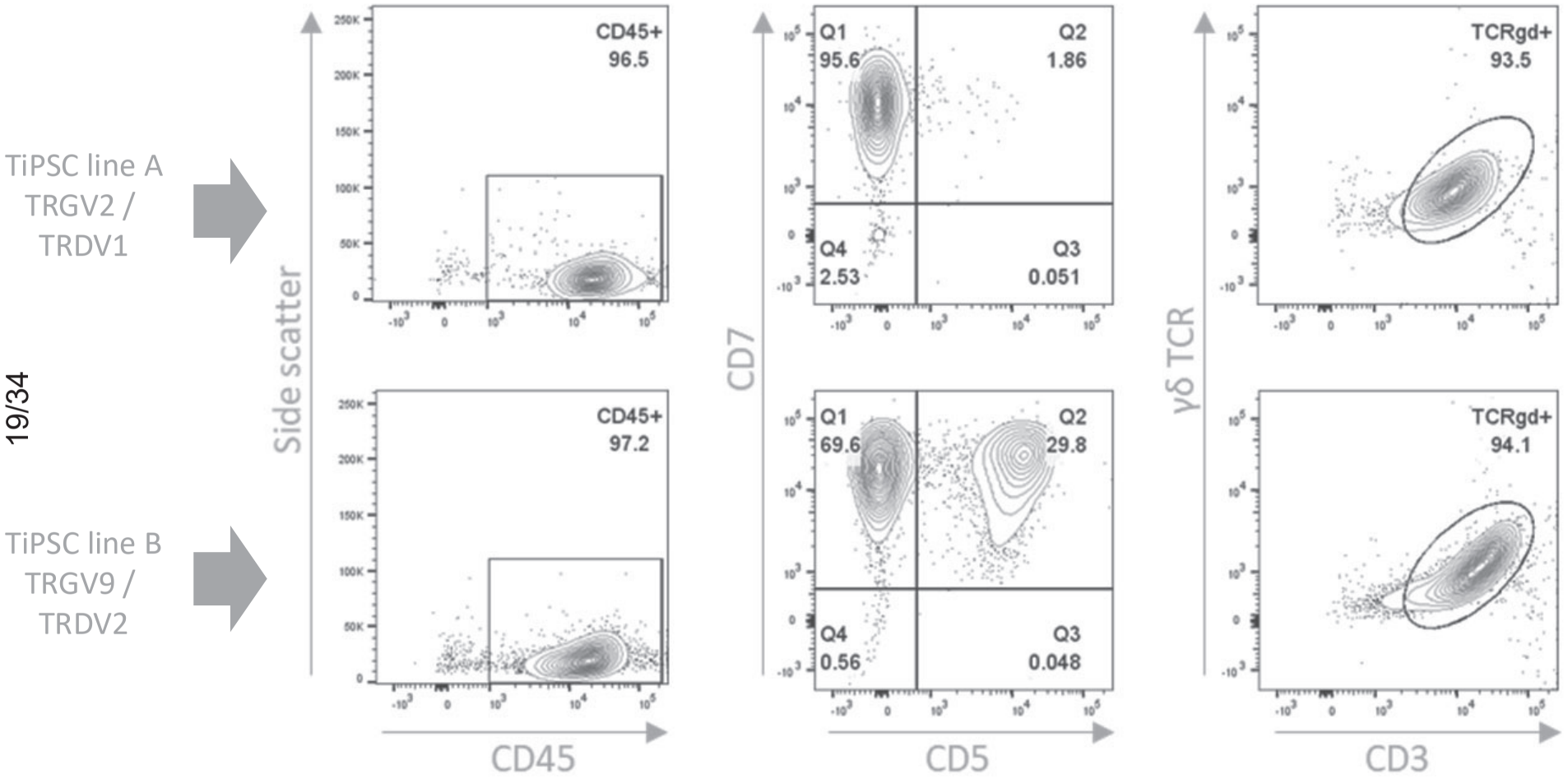


FIG. 7D (continued)

Nalm-6 Cells
(CD19^{Hi})

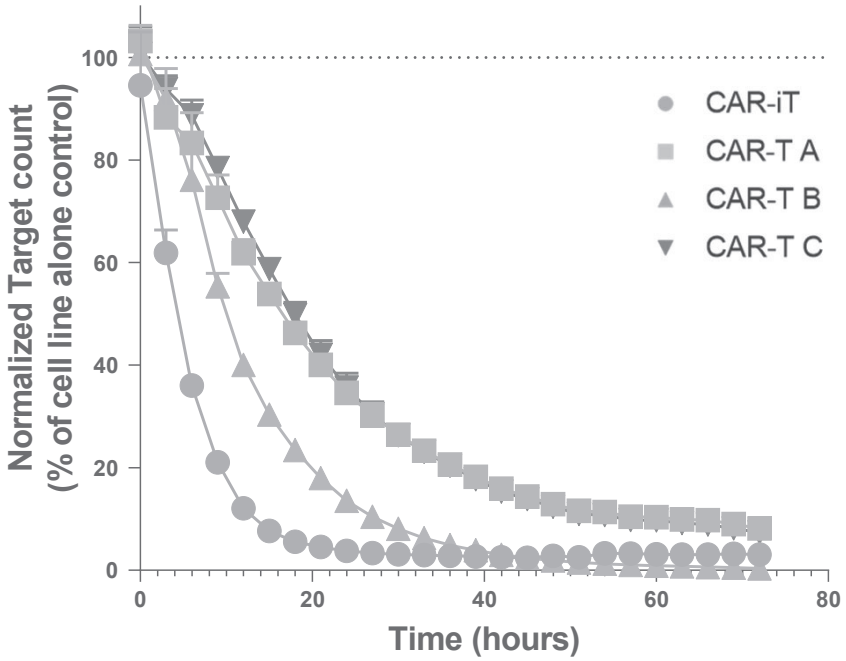


FIG. 8A

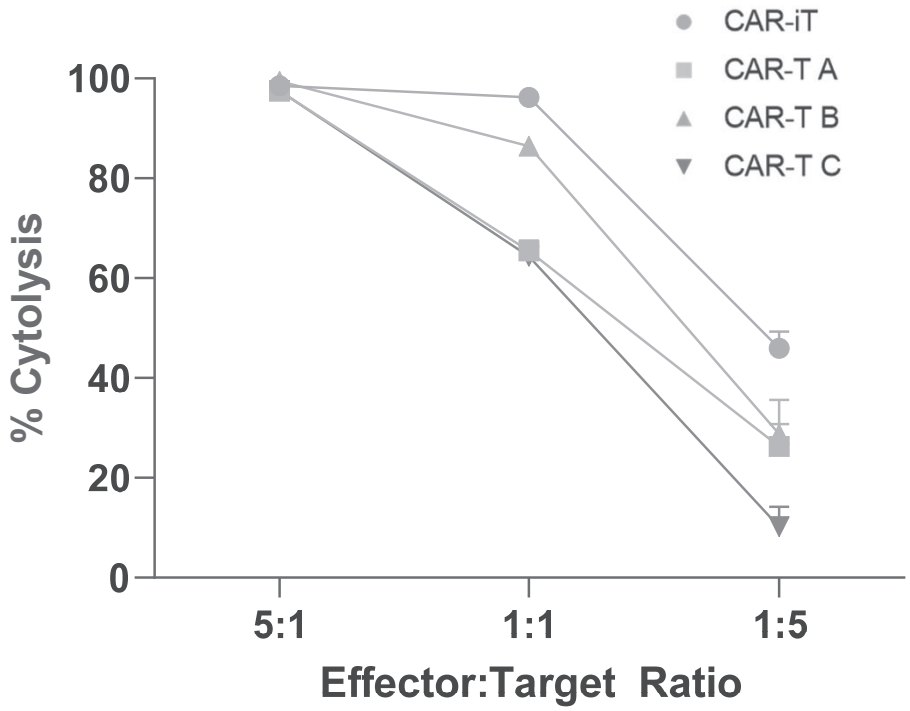


FIG. 8B

Reh Cells

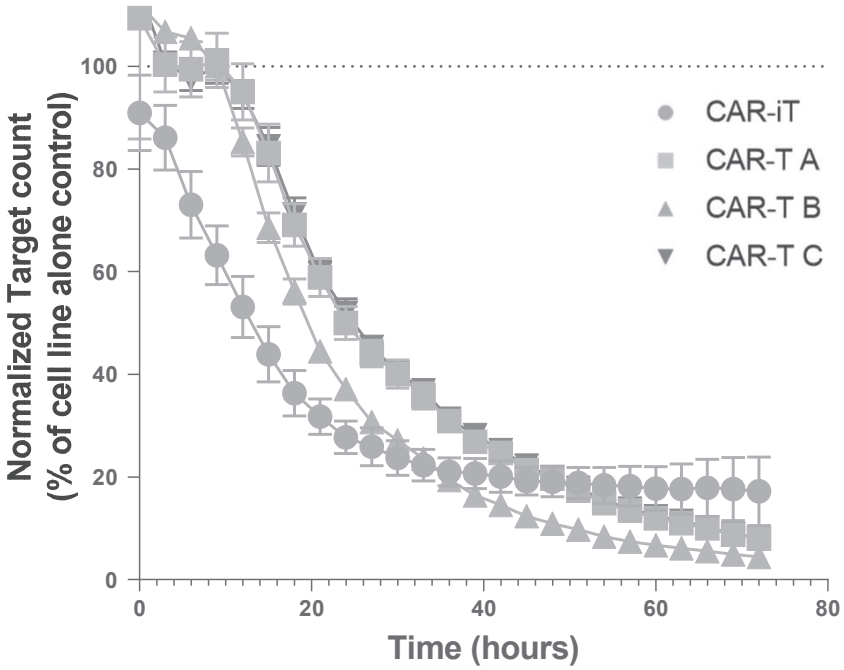


FIG. 8C

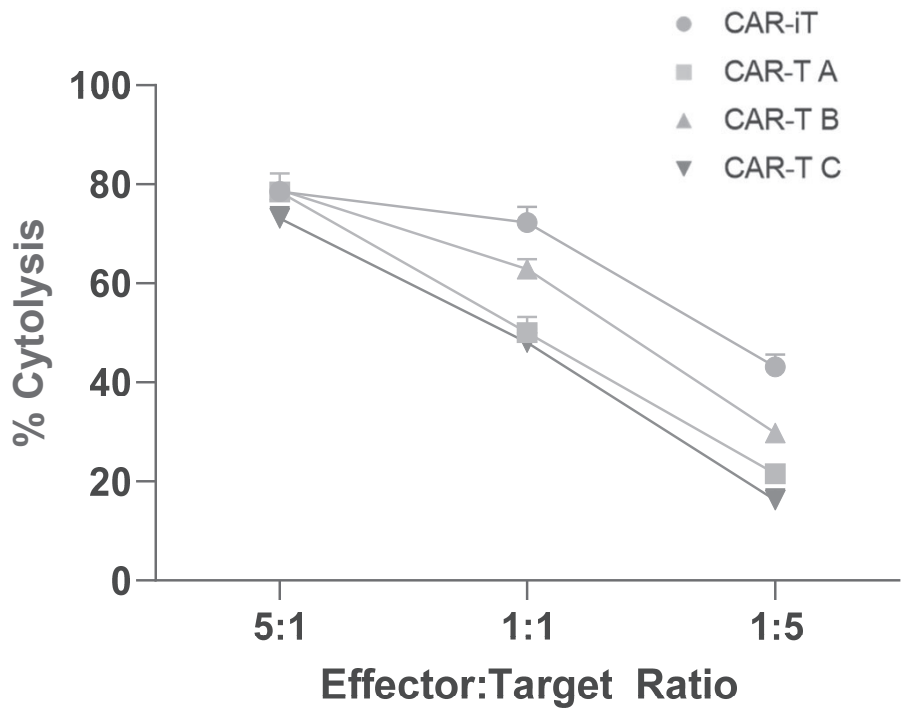


FIG. 8D

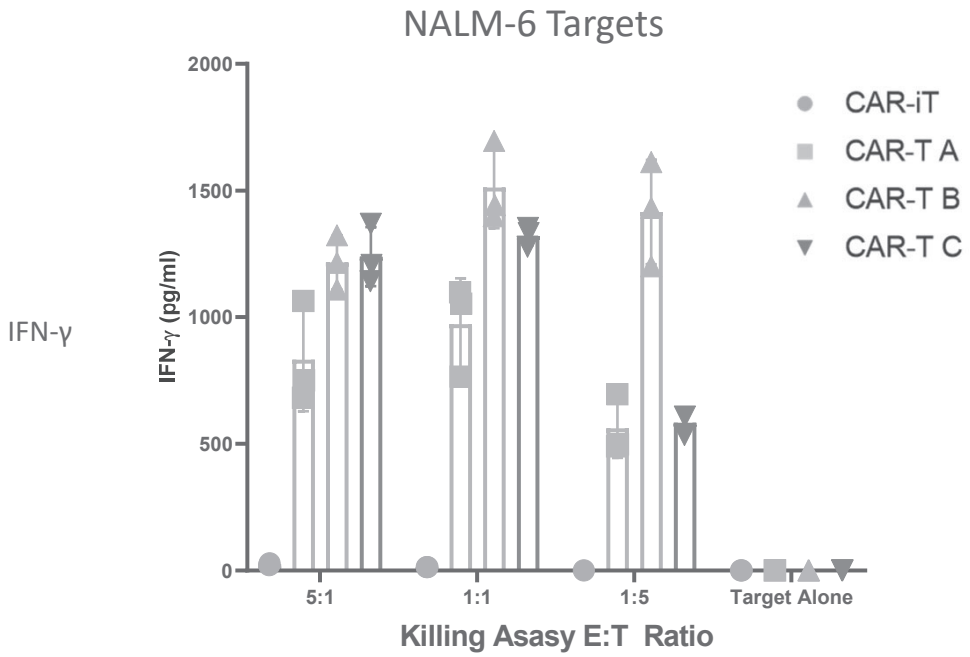

NALM-6 Targets
IFN-γ
IFN-γ (pg/ml)
2000
1500
1000
500
0
CAR-iT
CAR-T A
CAR-T B
CAR-T C
5:1
1:1
1:5
Target Alone
Killing Asasy E:T Ratio

FIG. 8E

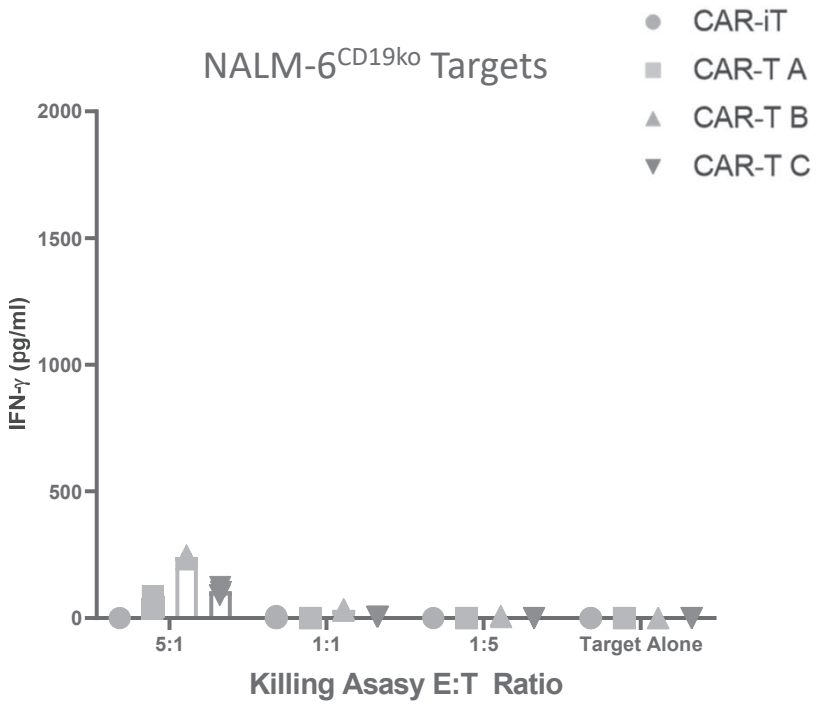

NALM-6CD19ko Targets
IFN-γ (pg/ml)
2000
1500
1000
500
0
CAR-iT
CAR-T A
CAR-T B
CAR-T C
5:1
1:1
1:5
Target Alone
Killing Asasy E:T Ratio

FIG. 8F

TNF

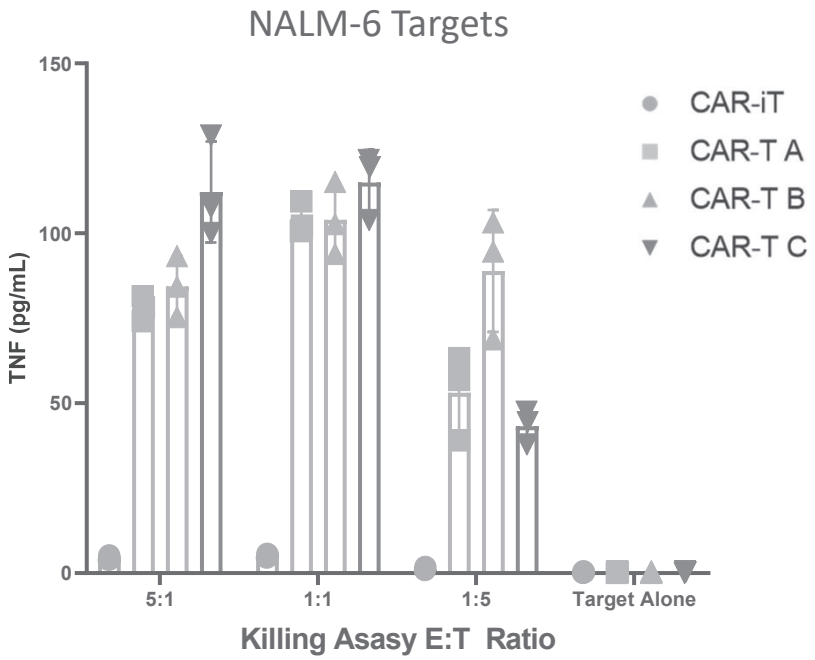


FIG. 8G

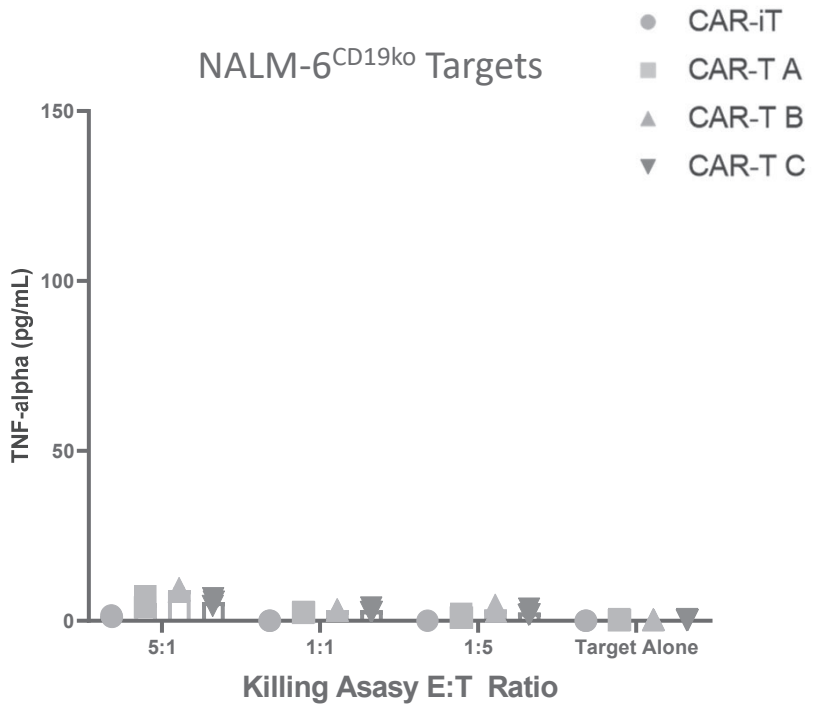


FIG. 8H

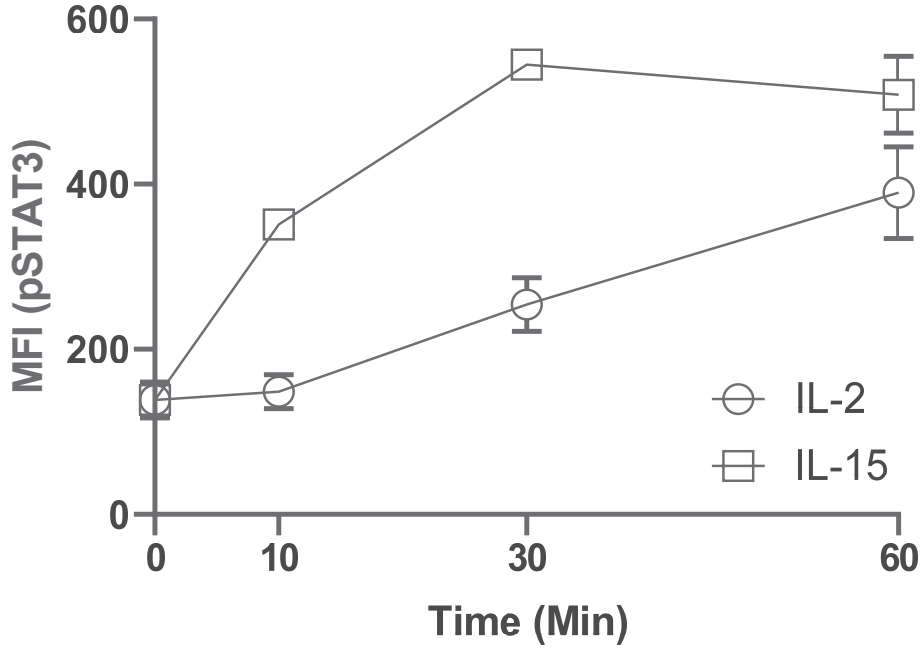

600
400
200
0
MFI (pSTAT3)
0
10
30
60
Time (Min)
IL-2
IL-15

FIG. 8I

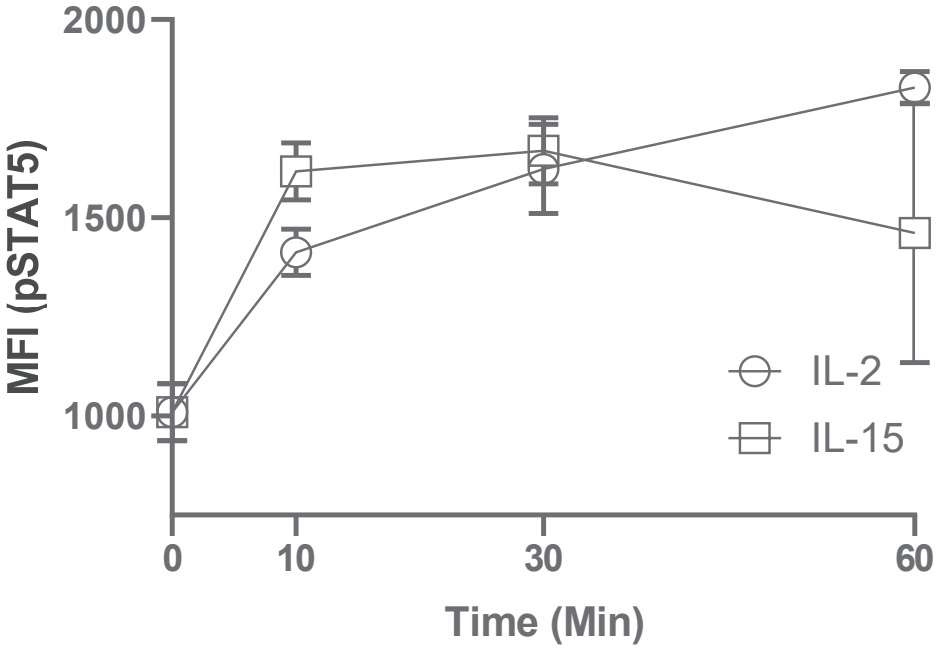

2000
1500
1000
MFI (pSTAT5)
0
10
30
60
Time (Min)
IL-2
IL-15

FIG. 8J

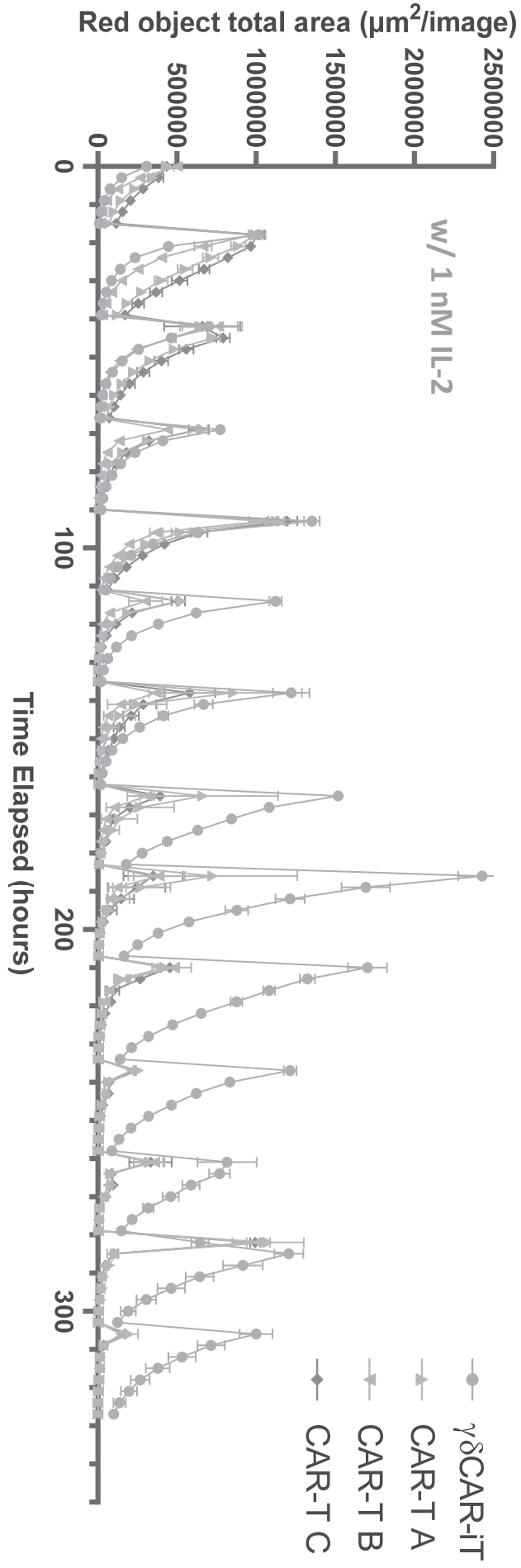

Red object total area (μm²/image)
0
500000
1000000
1500000
2000000
2500000
w/ 1 nM IL-2
0
100
200
300
Time Elapsed (hours)
γδCAR-iT
CAR-T A
CAR-T B
CAR-T C

FIG. 8K

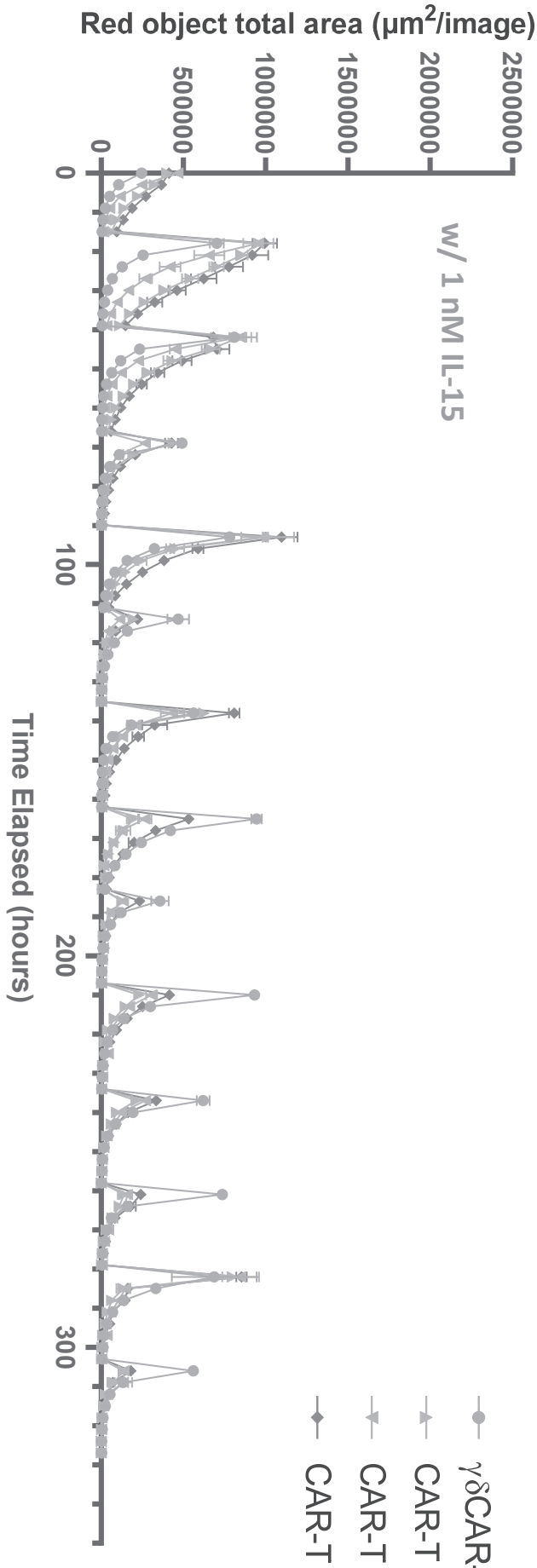

Red object total area (μm²/image)
0
500000
1000000
1500000
2000000
2500000
w/ 1 nM IL-15
0
100
200
300
Time Elapsed (hours)
γδCAR-iT
CAR-T A
CAR-T B
CAR-T C

FIG. 8L

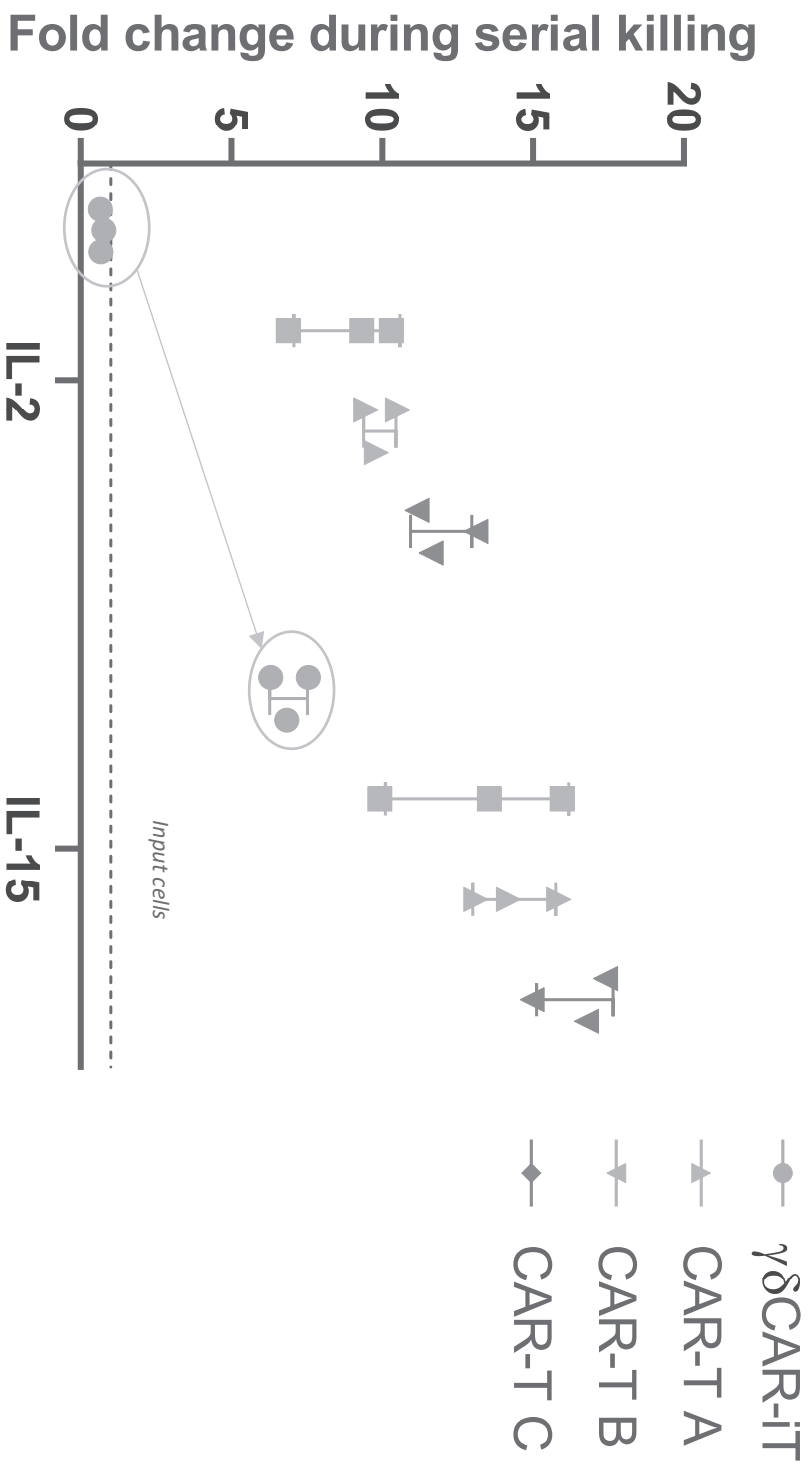

Fold change during serial killing
0
5
10
15
20
IL-2
IL-15
Input cells
γδCAR-iT
CAR-T A
CAR-T B
CAR-T C

FIG. 8M

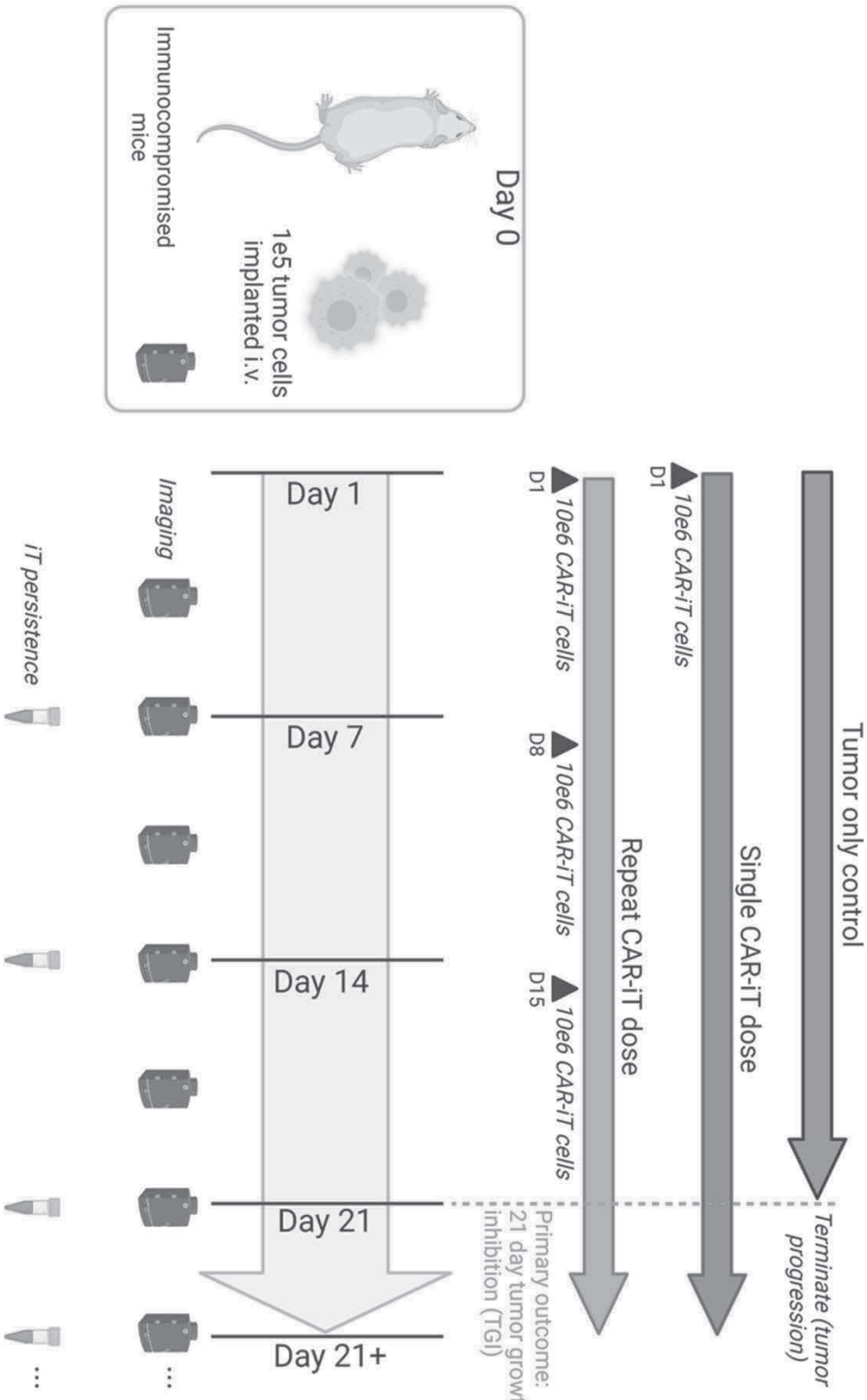

Day 0
Immunocompromised mice
1e5 tumor cells implanted i.v.
Tumor only control
Terminate (tumor progression)
Single CAR-iT dose
D1
10e6 CAR-iT cells
Repeat CAR-iT dose
D1
10e6 CAR-iT cells
D8
10e6 CAR-iT cells
D15
10e6 CAR-iT cells
Primary outcome: 21 day tumor growth inhibition (TGI)
Day 1
Day 7
Day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

FIG. 9A

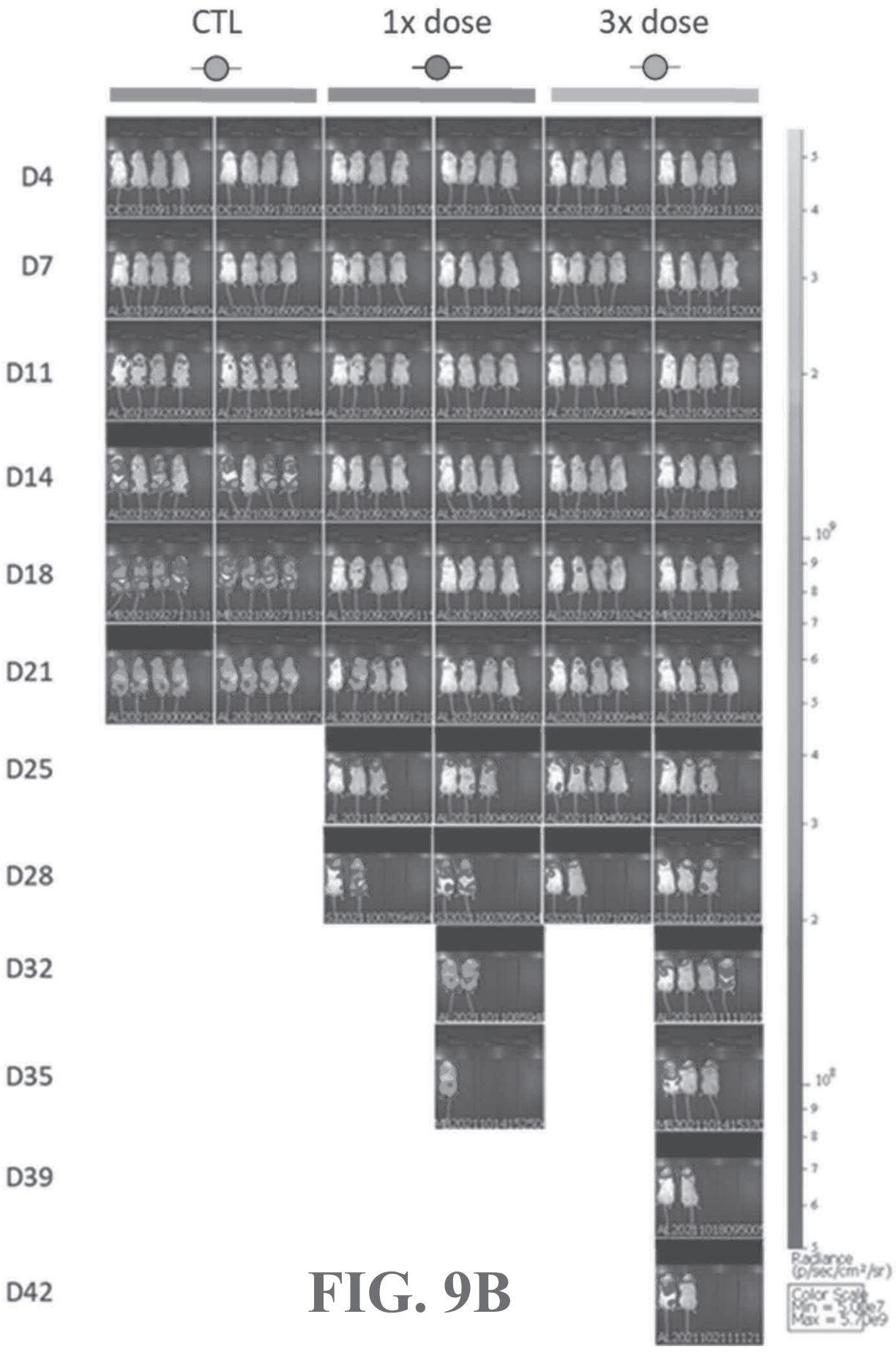

CTL
1x dose
3x dose
D4
D7
D11
D14
D18
D21
D25
D28
D32
D35
D39
D42
Radiance (p/sec/cm²/sr)

FIG. 9B

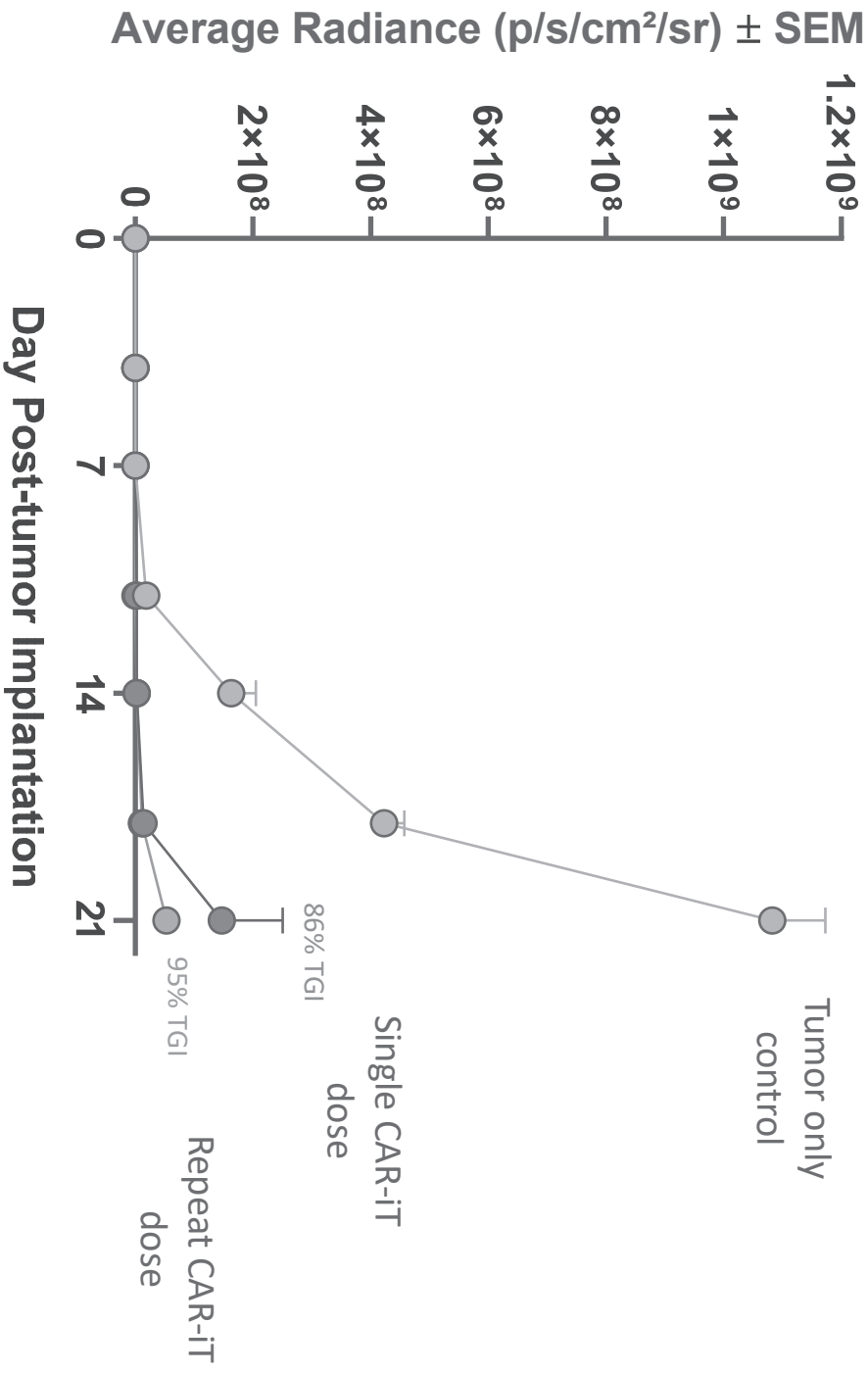

Average Radiance (p/s/cm²/sr) ± SEM
1.2×10⁹
1×10⁹
8×10⁸
6×10⁸
4×10⁸
2×10⁸
0
0
7
14
21
Day Post-tumor Implantation
Tumor only control
Single CAR-iT dose
Repeat CAR-iT dose
86% TGI
95% TGI

FIG. 9C

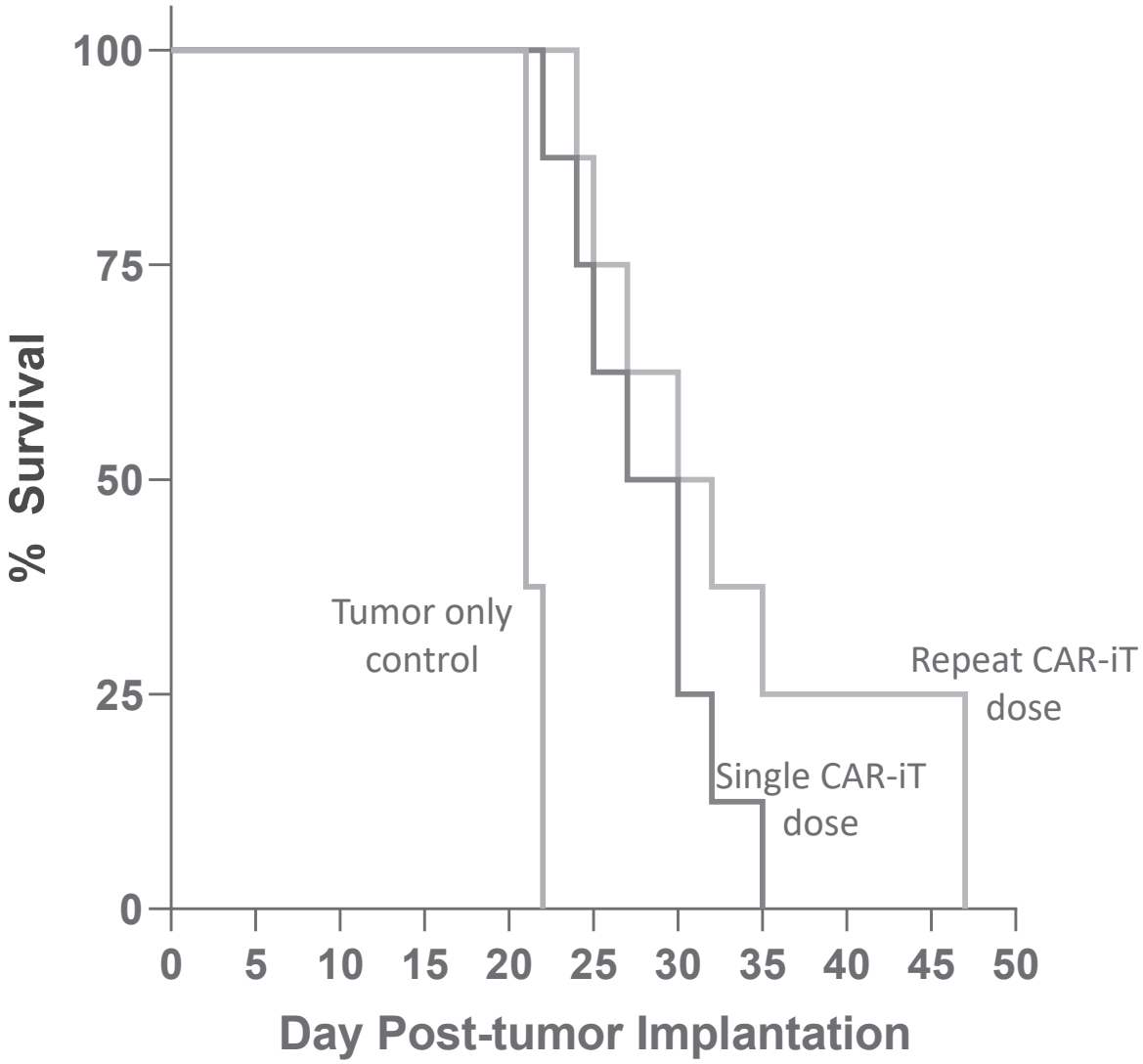

100
75
50
25
0
% Survival
Tumor only control
Repeat CAR-iT dose
Single CAR-iT dose
0 5 10 15 20 25 30 35 40 45 50
Day Post-tumor Implantation

FIG. 9D

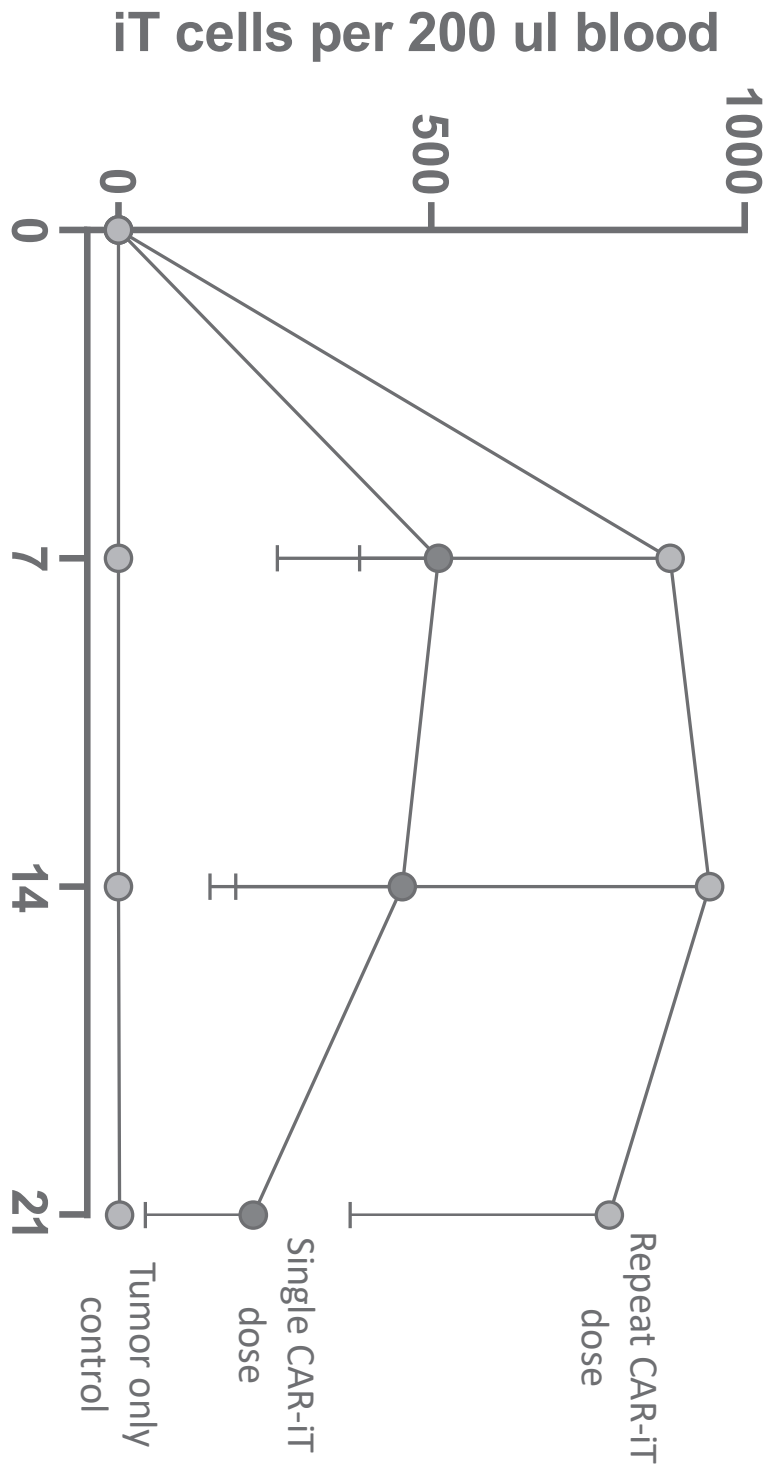

iT cells per 200 ul blood
1000
500
0
0
7
14
21
Day
Repeat CAR-iT dose
Single CAR-iT dose
Tumor only control

FIG. 9E

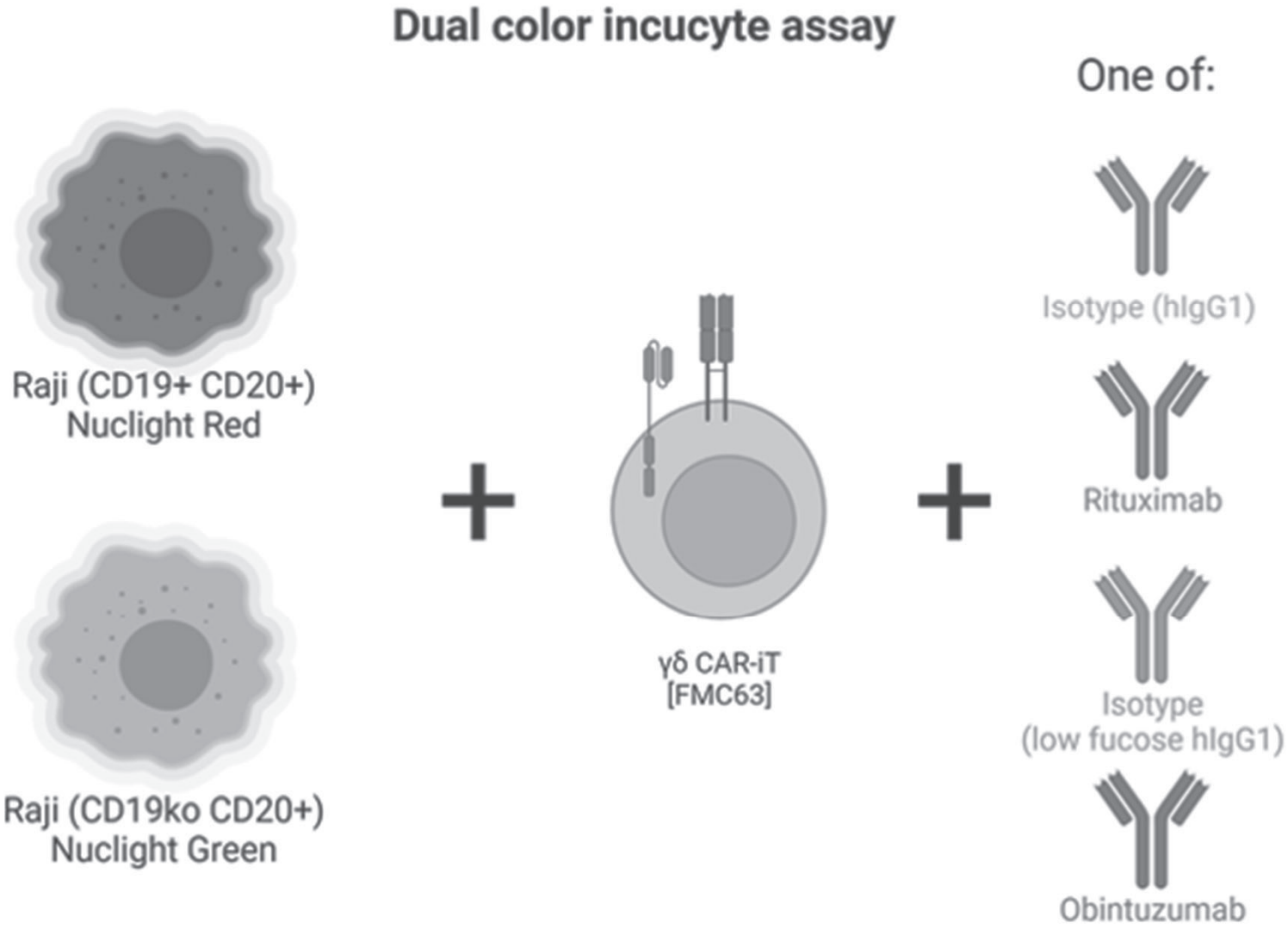

Dual color incucyte assay
One of:
Raji (CD19+ CD20+)
Nuclight Red
Raji (CD19ko CD20+)
Nuclight Green
γδ CAR-iT
[FMC63]
Isotype (hIgG1)
Rituximab
Isotype
(low fucose hIgG1)
Obintuzumab

FIG. 10

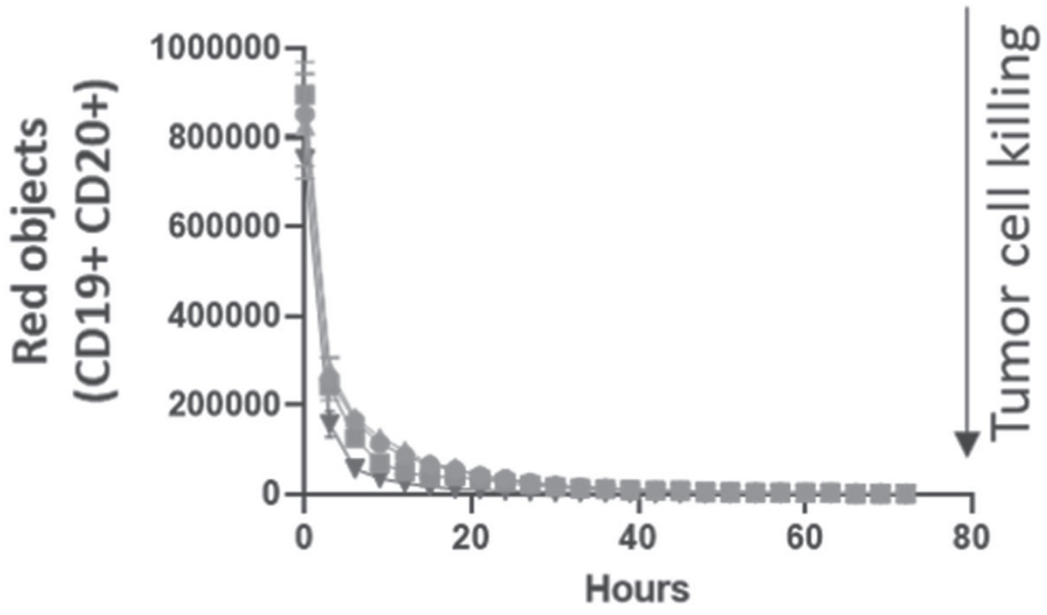


FIG. 11A

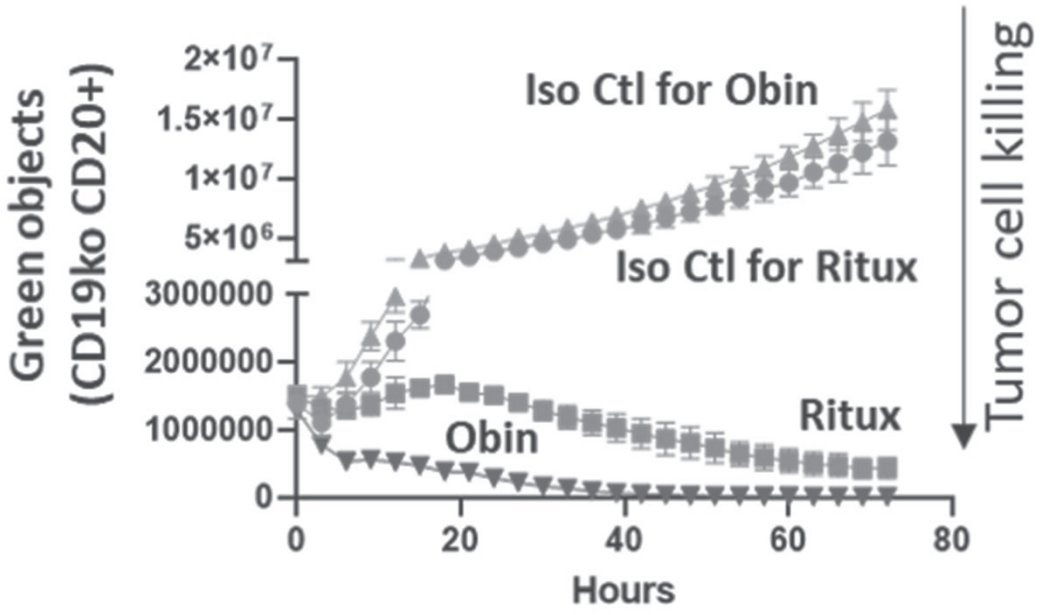


FIG. 11B