Figure S1. Establishment of xenogeneic mouse model. (A) Persistence of human xenografts in NOD/SCID/γ_c^{null}, but not in SCID/bg mice. Mice were inoculated with 5 x 10^5 Raji tumor cells and six days later received adoptive transfer of 10^7 19z1+CD8+ T cells transduced with a 19z1-encoding vector. Mice were sacrificed 10 days post-T cell injection and tissues were enumerated for hCD3+ cells inside the PI- subset. Bone marrow was enumerated for hCD19+ cells inside the PI- subset; splenic hCD19+ populations represent non-specific staining as verified by experiments in which mice receiving no Raji cells were similarly enumerated for hCD19+ cells (data not shown). (B-F) Systemically injected Raji-GL tumor cells establish multifocal lesions in NOD/SCID/γ_c^{null} mice. Mice were inoculated intravenously with 5 x 10^5 Raji tumor cells. Three to four weeks later, mice developed hind limb paralysis and were euthanized. Photographs (H&E) are representative of three mice. (B) Femoral bone marrow with bone infarction. (C) Mesenteric lymph node. (D) Liver. (E) Cauda equina, nerve roots, vertebrae, and adjacent musculature. (F) Pituitary.

Figure S2. Raji tumor cells exhibit cytokine-independent growth in vitro and do not lose CD19 expression in vivo. (A) Exogenous IL-2, IL-7, IL-15, and IL-21 do not cause accumulation of Raji tumor cells. 10^5 Raji tumor cells were seeded with exogenous cytokine as indicated. Cells were fed at days 3.5, 7, and 10.5 at which time cytokine was replenished. Cells were split and replated on day seven. Viable cell number was assessed at days 3.5, 7, 10.5, and 14. Data are the average of two independent experiments. (B) End stage tumors from treated mice retain expression of the tumor antigen CD19. A mouse that failed treatment with 19z1-

ΔLNGFR-transduced T cells was euthanized 38 days post-tumor challenge and the liver mass analyzed by flow cytometry for eGFP-Firefly Luciferase fusion and CD19 expression. The bioluminescent image and dot plot are representative of three mice.

Figure S3. Expression of a PSMA-specific CAR (Pz1) by human primary T cells

Retroviral vectors for Pz1 expression were equivalent to those in Figure 1A except that Pz1 replaced 19z1. Dot plots indicate T cells six days post-AAPC-stimulation. Numbers at the bottom of the upper right quadrant represent the Pz1 MFI of the CD3+Pz1+ subset. CD4/CD8 dot plots have been gated on the CD3+Pz1+ subset.

Figure S4. Accumulation of transduced CD4+ and CD8+ T cells in response to tumor antigen exposure. T cells were transduced and exposed weekly to AAPCs. In parallel, control 19z1-ΔLNGFR T cells were cultured with titrating amounts of exogenously added IL-2, IL-7, IL-15, or IL-21. The number of viable 19z1+CD4+ and 19z1+CD8+ T cells were assessed at the indicated time points. Arrows denote AAPC-restimulation. The bottom right graph represents an overlay of the accumulation of cytokine-transduced CD4+ and CD8+ T cells. Data are average (+/- SEM) of three donors.

Figure S5. Formation of memory-phenotype CD4+ and CD8+ human T cells overexpressing IL-2, IL-7, IL-15, or IL-21. On day seven post-AAPC-stimulation, transduced T cells were assessed for expression of CD45RA and CD45RO. Untransduced cells were activated with PHA 14 days prior and maintained with 50 U/mL IL-2. Dot plots shown are from

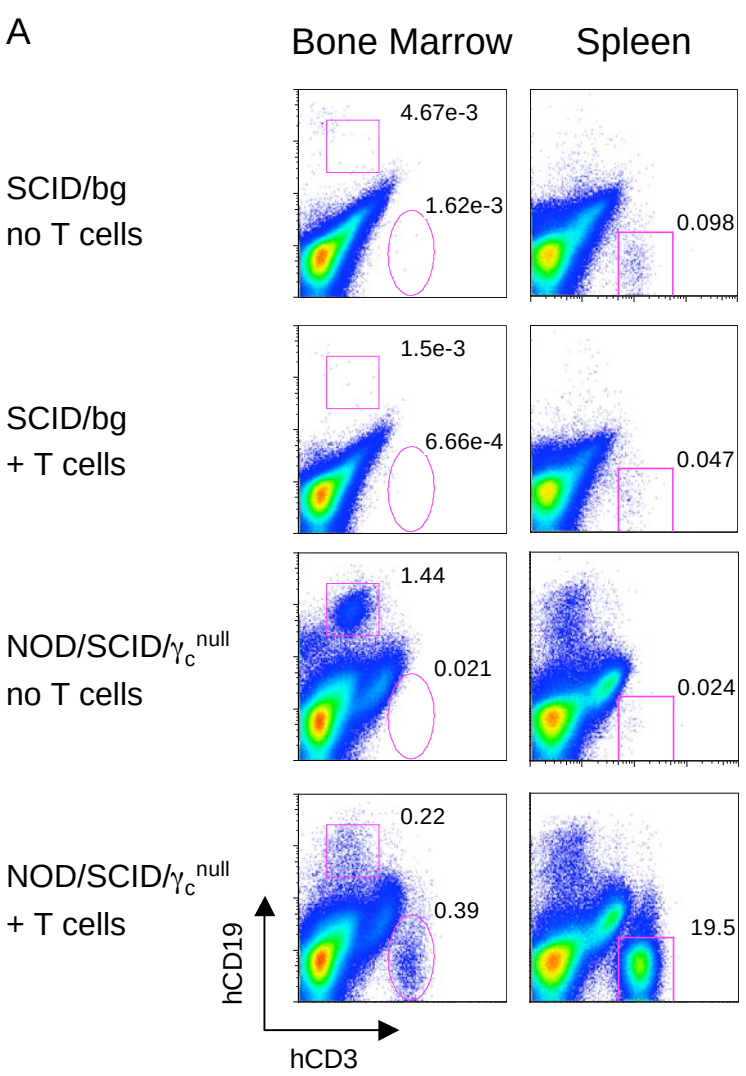
one representative donor of five. The bar graph indicates the average percent (+/- SEM) CD45RA-CD45RO+ of 19z1+CD4+ or 19z1+CD8+ T cells from five donors.

Figure S6. Overexpression of human primary T cells with IL-2, IL-7, IL-15, or IL-21 does not substantially or differentially accumulate regulatory-phenotype T cells

(A) Foxp3 protein expression of transduced CD4+ T cells. On day seven post-AAPC-stimulation, T cells were assessed for Foxp3 expression. Dot plots are representative of four donors and have been gated on the 19z1+CD4+ subset. The bar graph represents the average percentage (+/- SEM) of the foxp3+ after subtraction of the percentage of isotype+ within the 19z1+CD4+ subset. P = 0.7, 0.7, 0.9, and 0.06 for 19z1-IL2, 19z1-IL7, 19z1-IL15, and 19z1-IL21 versus 19z1-ΔLNGFR. (B) CD25 expression of transduced CD4+ T cells. On day seven post-AAPC-stimulation, 19z1+CD4+foxp3+ (thick line) and 19z1+CD4+foxp3- (thin line) T cells were assessed for CD25 expression. Histograms are representative of four donors.

Figure S7. Expression of CD62L by cytokine-transduced T cells in individual experiments. CD62L expression of CD8+ T cells seven days post-AAPC-stimulation. In all eight experiments, histograms have been gated on the 19z1+CD8+CD45RA-CD45RO+ subset.

Figure S1


A
Bone Marrow
Spleen
SCID/bg
no T cells
4.67e-3
1.62e-3
0.098
SCID/bg
+ T cells
1.5e-3
6.66e-4
0.047
NOD/SCID/γc null
no T cells
1.44
0.021
0.024
NOD/SCID/γc null
+ T cells
0.22
0.39
19.5
hCD19
hCD3

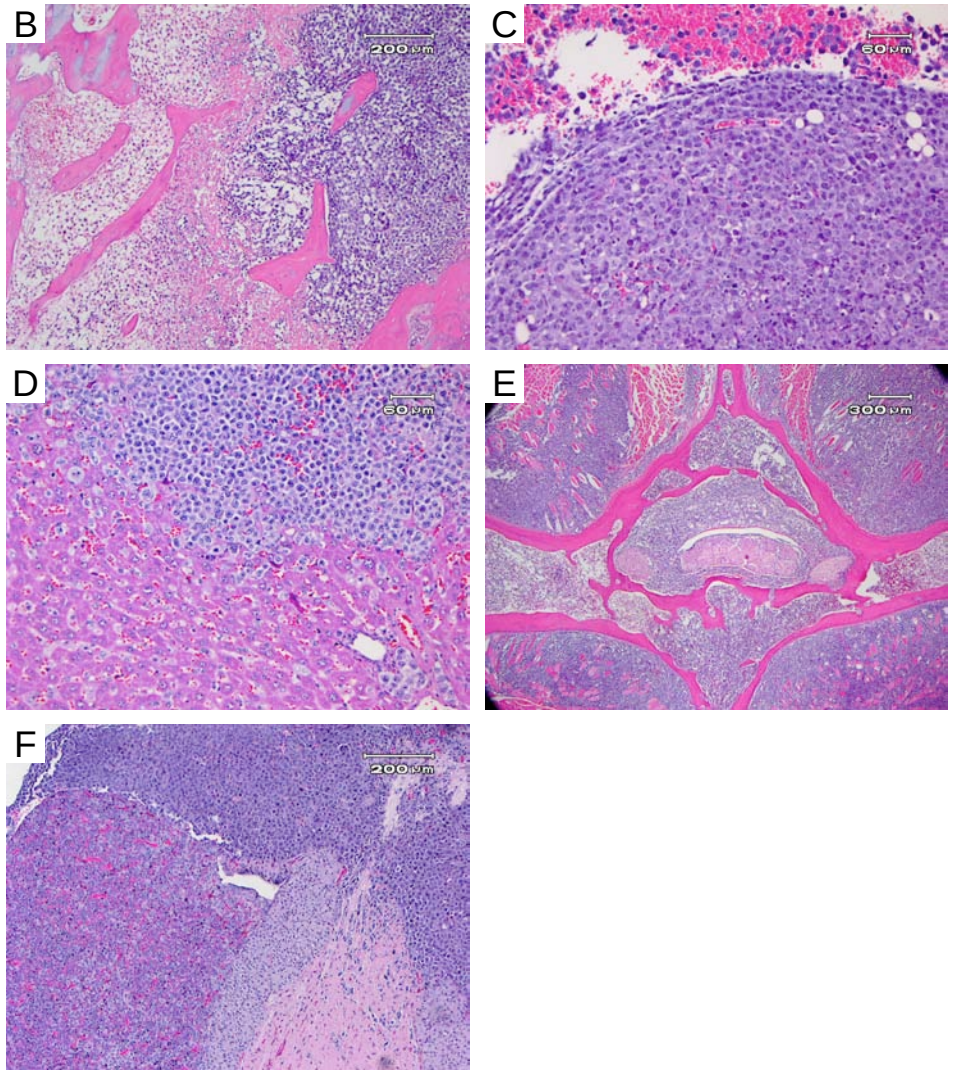
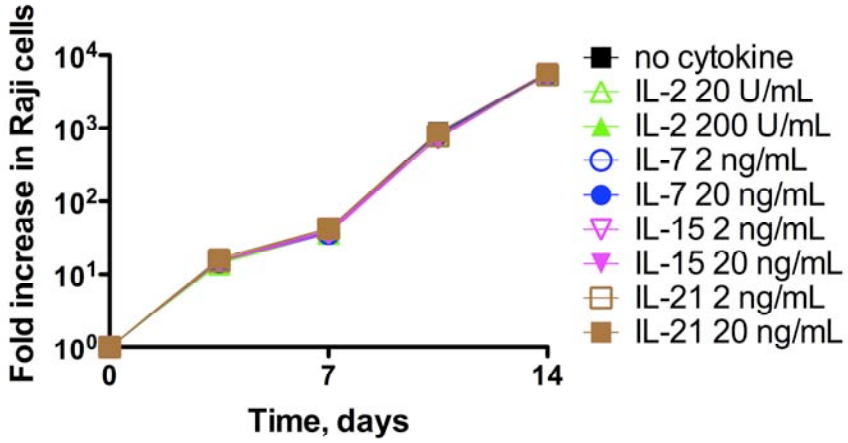

B
C
D
E
F

Figure S2

A


Fold increase in Raji cells
10^4
10^3
10^2
10^1
10^0
0
7
14
Time, days
no cytokine
IL-2 20 U/mL
IL-2 200 U/mL
IL-7 2 ng/mL
IL-7 20 ng/mL
IL-15 2 ng/mL
IL-15 20 ng/mL
IL-21 2 ng/mL
IL-21 20 ng/mL

B

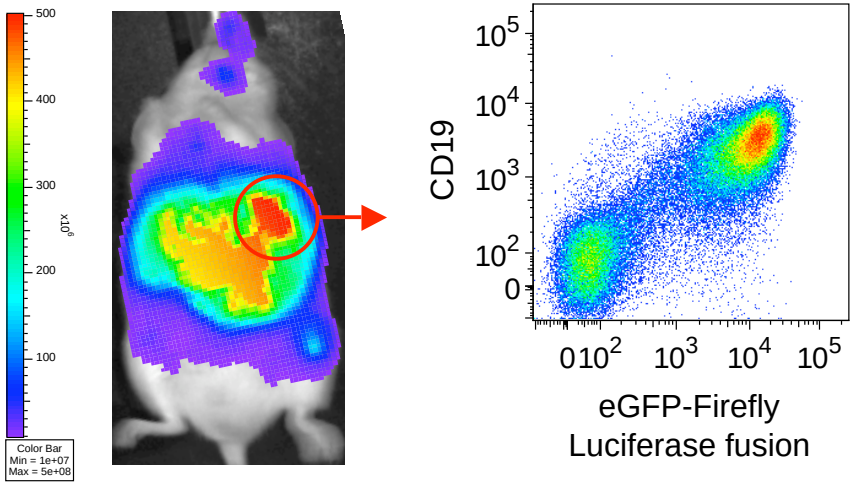

500
400
300
x10⁶
200
100
Color Bar
Min = 1e+07
Max = 5e+08
CD19
10^5
10^4
10^3
10^2
0
0
10^2
10^3
10^4
10^5
eGFP-Firefly
Luciferase fusion

Figure S3

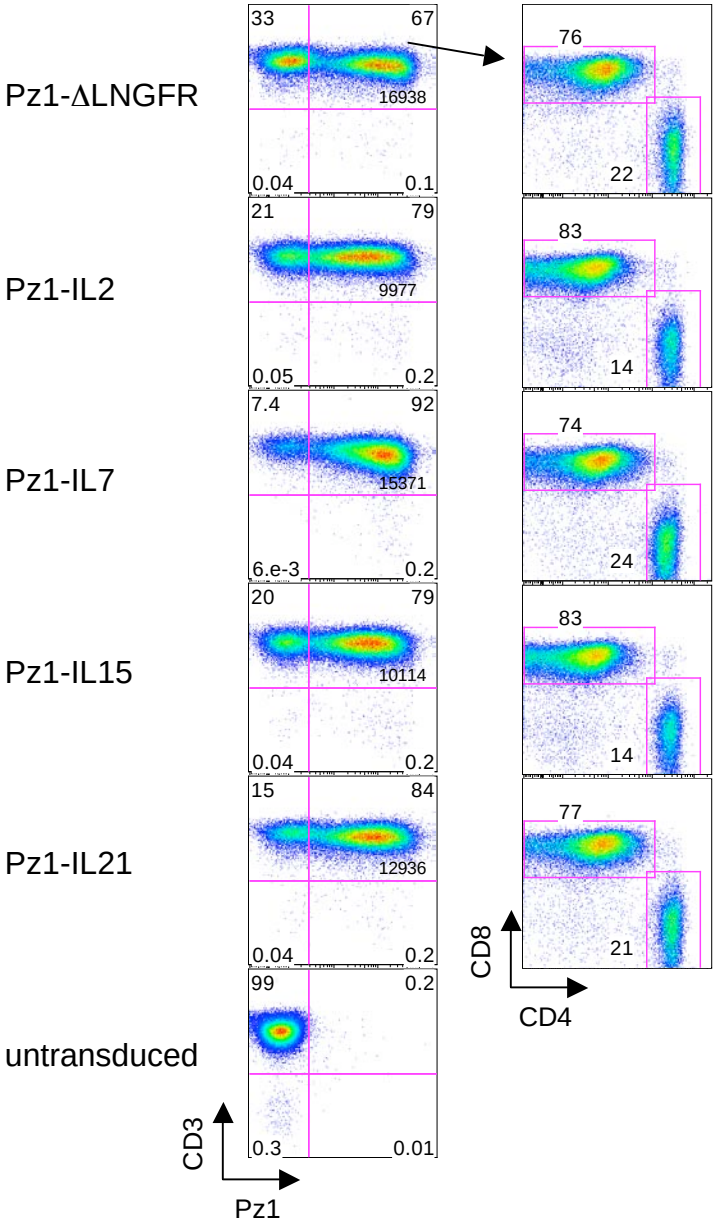

Pz1-ΔLNGFR
Pz1-IL2
Pz1-IL7
Pz1-IL15
Pz1-IL21
untransduced
33
67
16938
0.04
0.1
76
22
21
79
9977
0.05
0.2
83
14
7.4
92
15371
6.e-3
0.2
74
24
20
79
10114
0.04
0.2
83
14
15
84
12936
0.04
0.2
77
21
99
0.2
0.3
0.01
CD8
CD4
CD3
Pz1

Figure S4

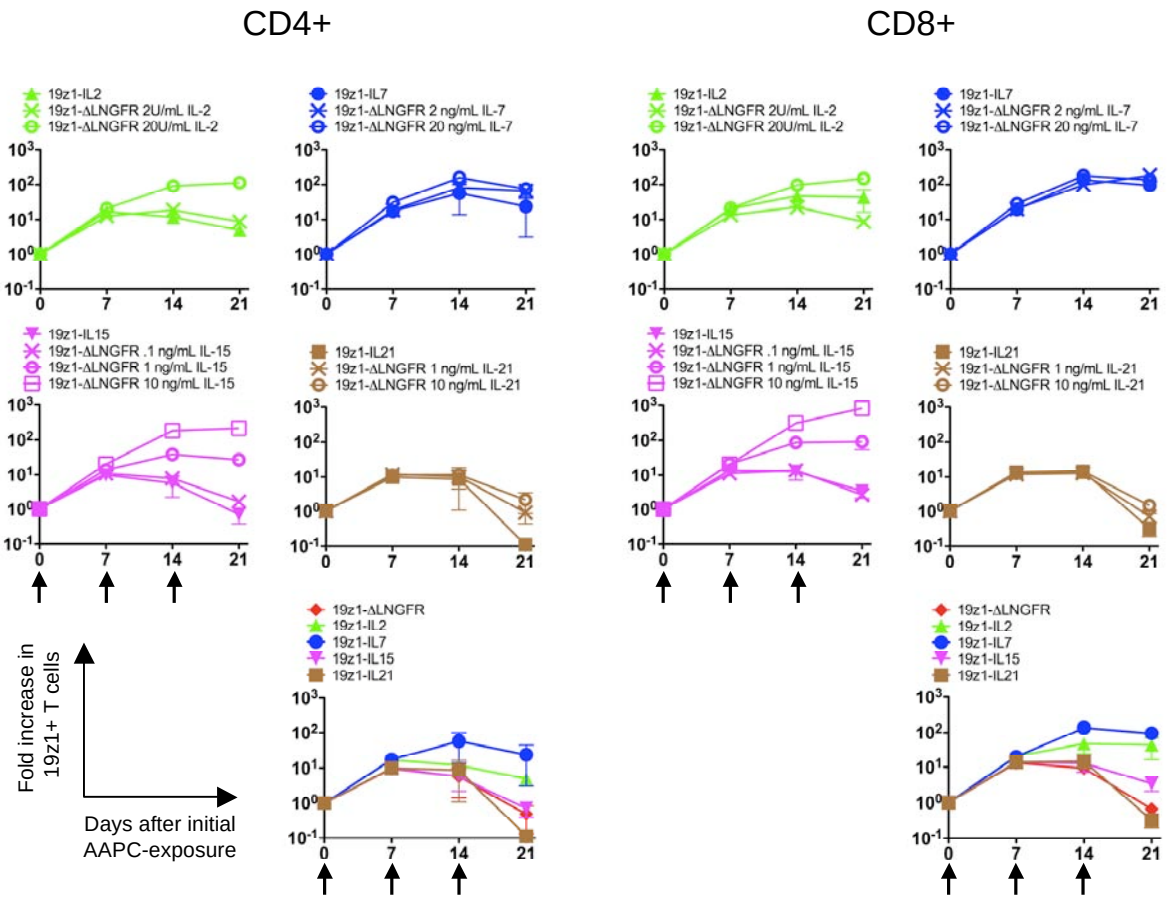
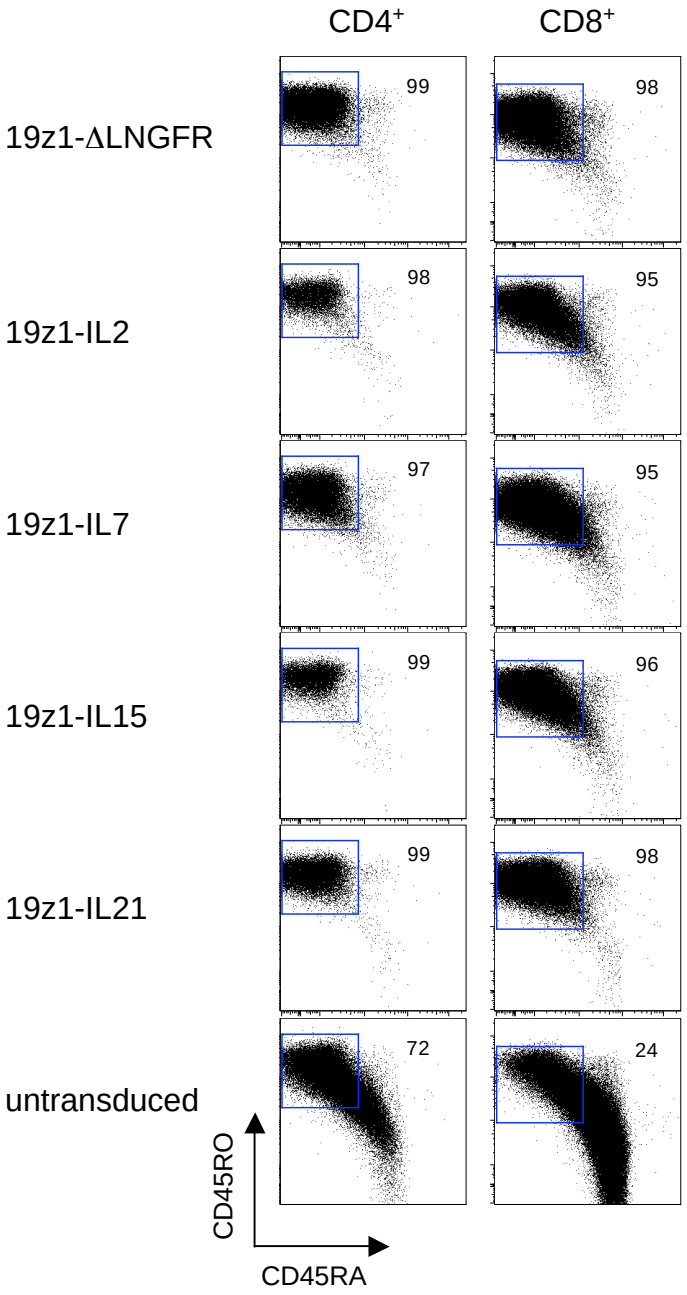

CD4+
CD8+
19z1-IL2
19z1-ΔLNGFR 2U/mL IL-2
19z1-ΔLNGFR 20U/mL IL-2
19z1-IL7
19z1-ΔLNGFR 2 ng/mL IL-7
19z1-ΔLNGFR 20 ng/mL IL-7
19z1-IL15
19z1-ΔLNGFR .1 ng/mL IL-15
19z1-ΔLNGFR 1 ng/mL IL-15
19z1-ΔLNGFR 10 ng/mL IL-15
19z1-IL21
19z1-ΔLNGFR 1 ng/mL IL-21
19z1-ΔLNGFR 10 ng/mL IL-21
19z1-ΔLNGFR
Fold increase in 19z1+ T cells
Days after initial AAPC-exposure

Figure S5


CD4+
CD8+
19z1-ΔLNGFR
19z1-IL2
19z1-IL7
19z1-IL15
19z1-IL21
untransduced
99
98
98
95
97
95
99
96
99
98
72
24
CD45RO
CD45RA

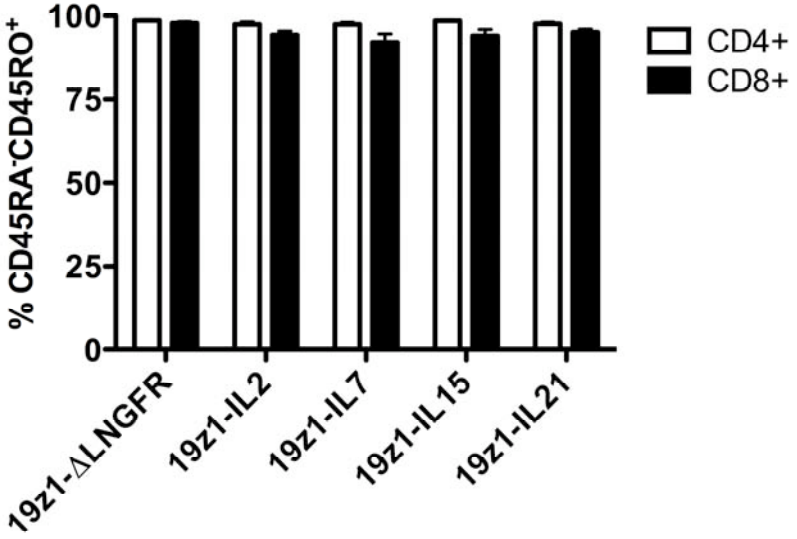

% CD45RA-CD45RO+
100
75
50
25
0
CD4+
CD8+
19z1-ΔLNGFR
19z1-IL2
19z1-IL7
19z1-IL15
19z1-IL21

Figure S6

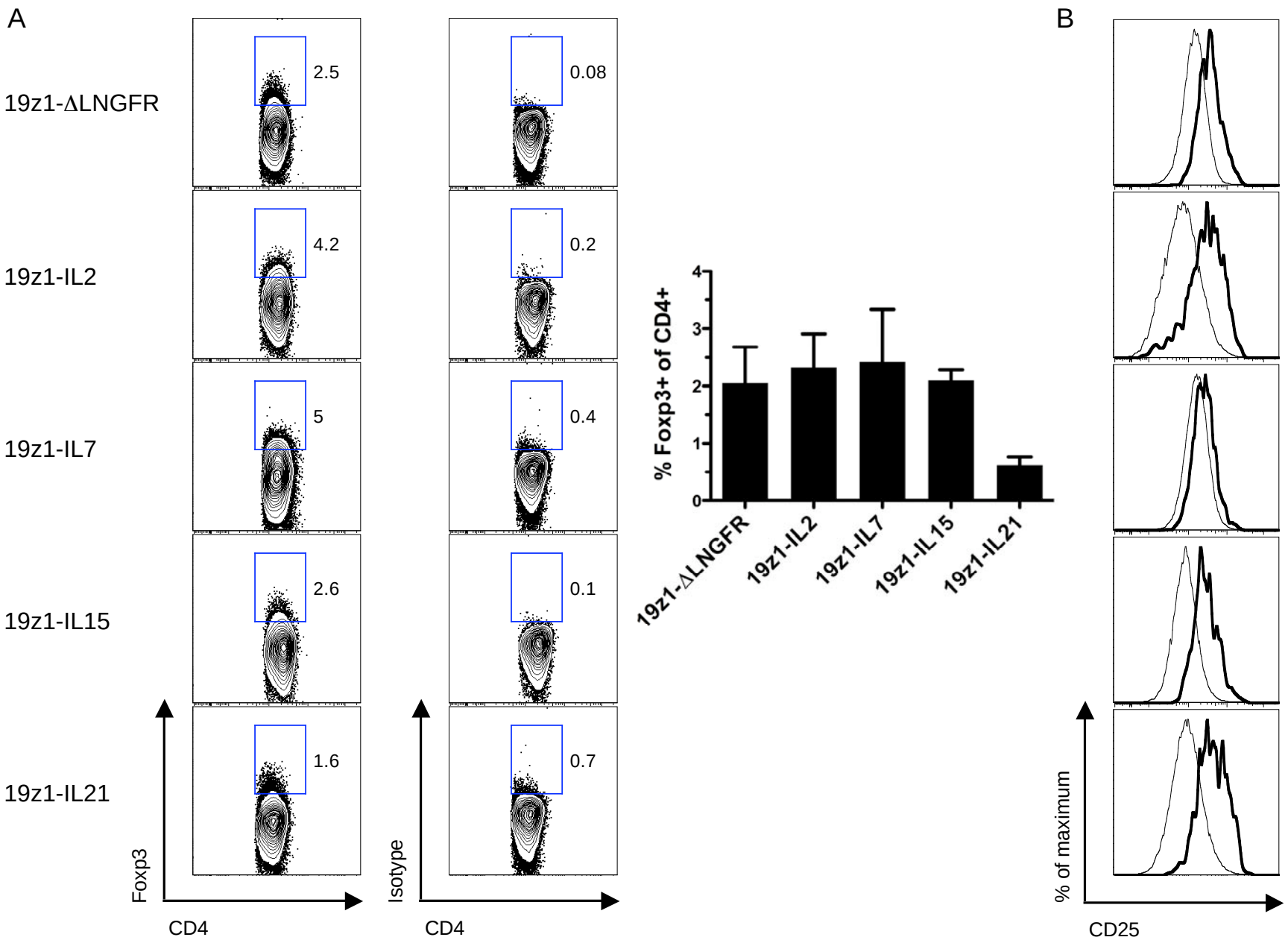
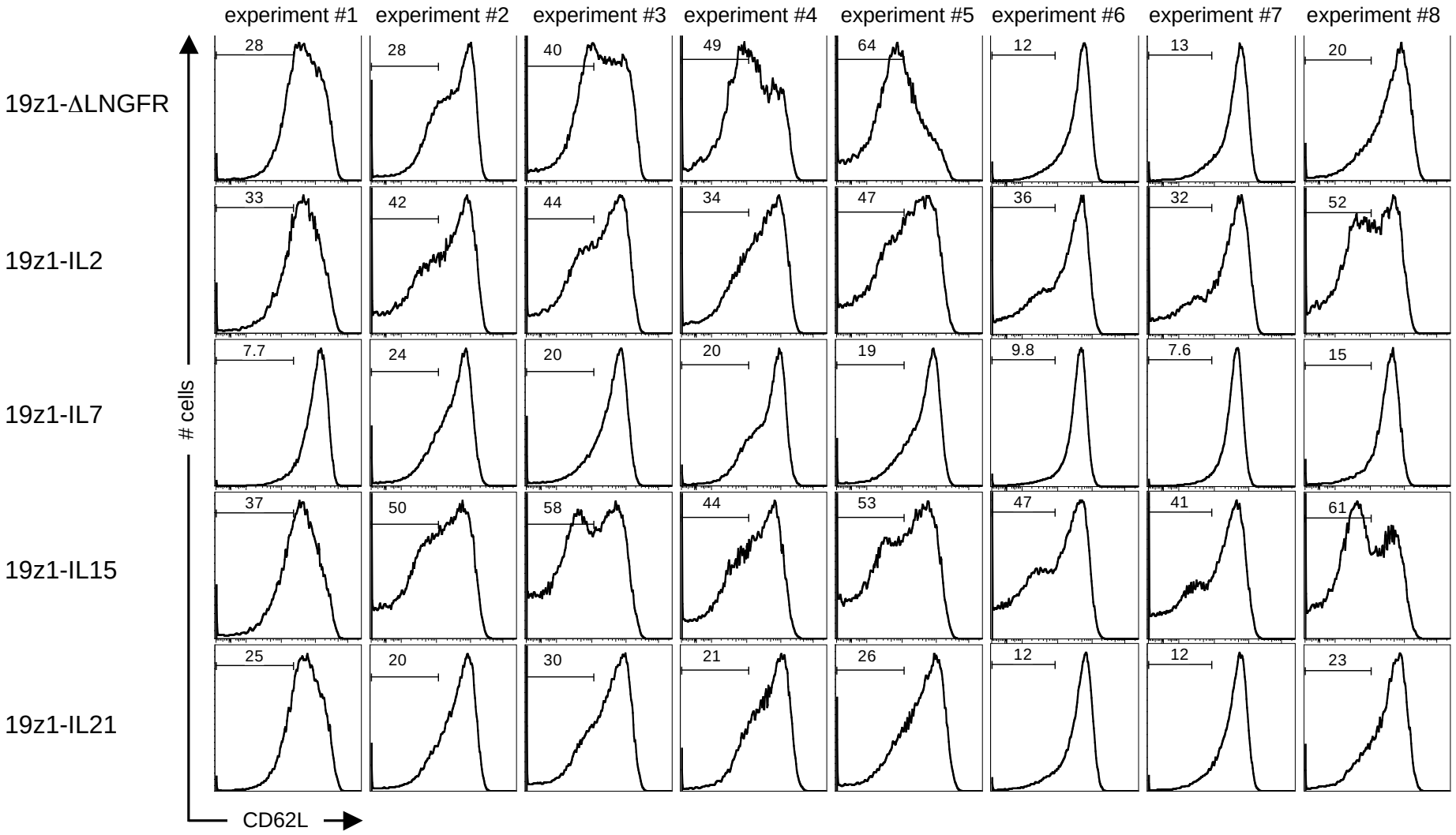

A
19z1-ΔLNGFR
19z1-IL2
19z1-IL7
19z1-IL15
19z1-IL21
2.5
0.08
4.2
0.2
5
0.4
2.6
0.1
1.6
0.7
Foxp3
CD4
Isotype
CD4
% Foxp3+ of CD4+
0
1
2
3
4
19z1-ΔLNGFR
19z1-IL2
19z1-IL7
19z1-IL15
19z1-IL21
B
% of maximum
CD25

Figure S7


experiment #1
experiment #2
experiment #3
experiment #4
experiment #5
experiment #6
experiment #7
experiment #8
19z1-ΔLNGFR
19z1-IL2
19z1-IL7
19z1-IL15
19z1-IL21
cells
CD62L
28
28
40
49
64
12
13
20
33
42
44
34
47
36
32
52
7.7
24
20
20
19
9.8
7.6
15
37
50
58
44
53
47
41
61
25
20
30
21
26
12
12
23