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Supplemental Information

HDAC inhibition prevents transgene expression downregulation and loss-of-function in T-cell-receptor-transduced T cells

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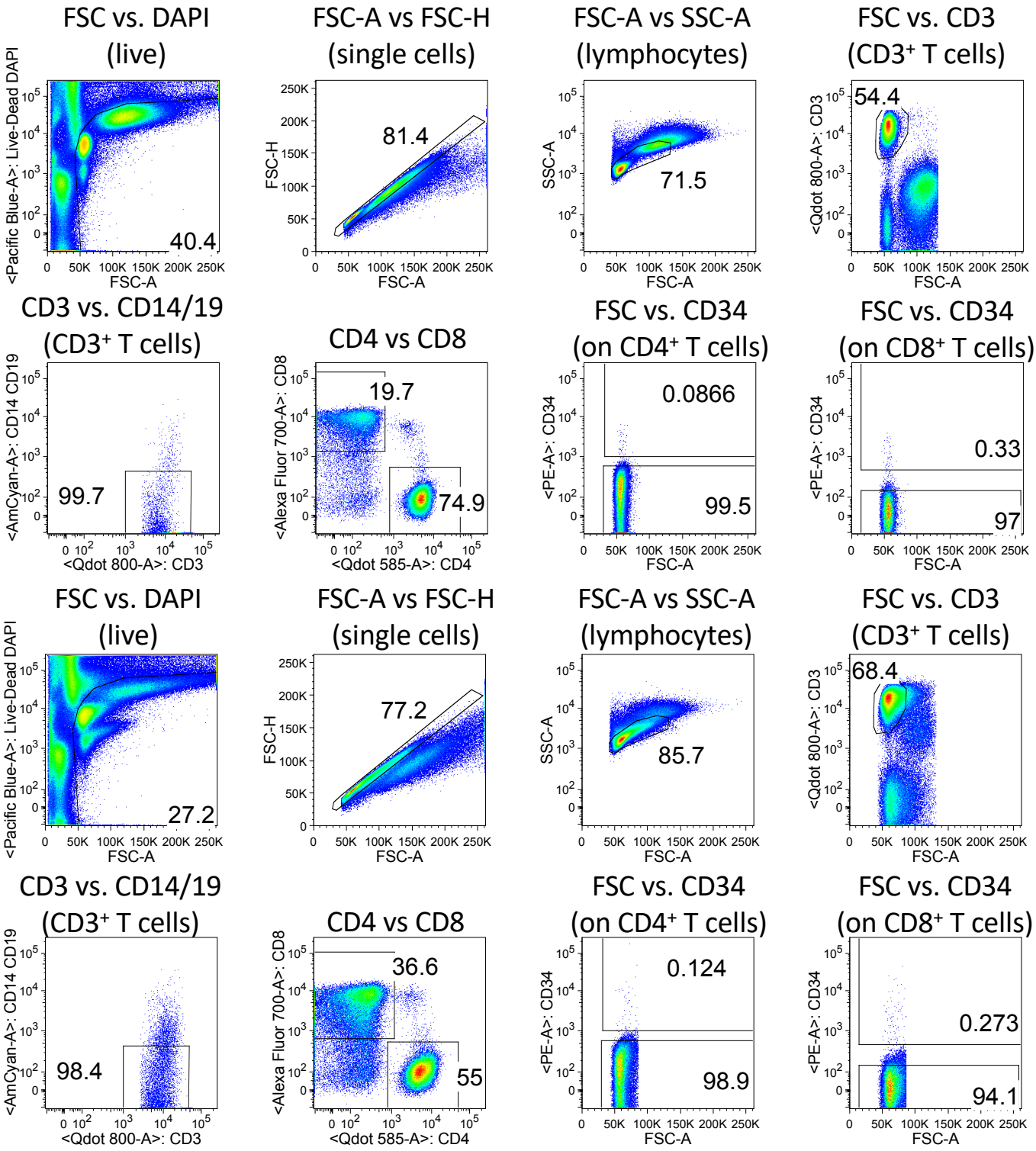
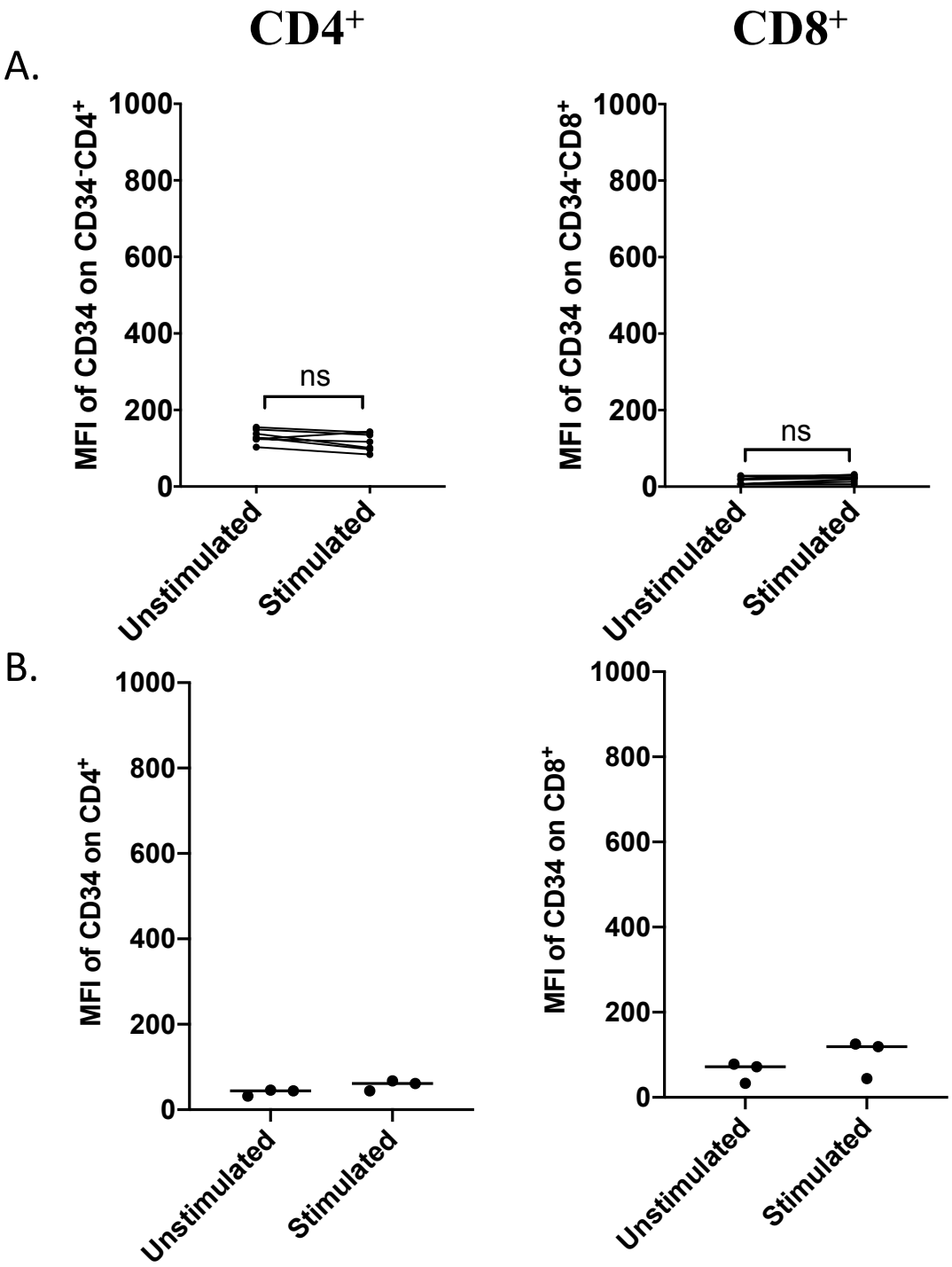
Supplemental Table S1: Patient and Transduced T cell Treatment Characteristics

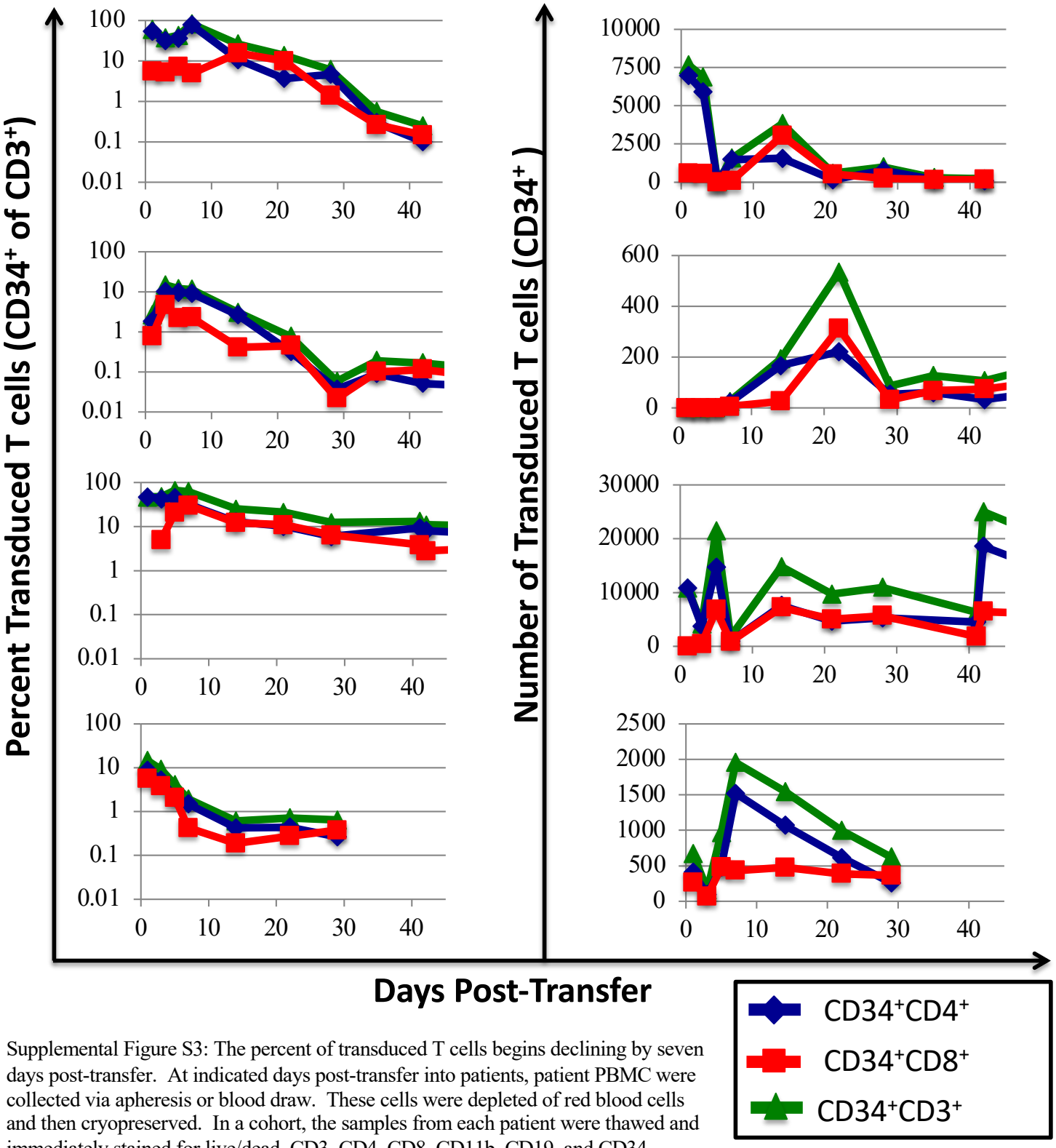
	Patient 4	Patient 5	Patient 6	Patient 7
Age at time of treatment	51	61	63	51
Sex	Female	Female	Male	Male
Stage of Melanoma at Diagnosis	T3aN1aM0, Stage IIIB	T2bN1aM0, Stage IIIB	T3bN2aMx, Stage IIIC	T1N0M0 Stage I
Prior therapies received	Nivolumab + Ipilimumab, Dabrafenib + trametinib, Pembrolizumab	Pembrolizumab, Ipilimumab, Nivolumab, Ipilimumab + Nivolumab	Ipilimumab, Nivolumab	Nivolumab, Ipilimumab
Stage of Melanoma at Treatment	Stage IV	Stage IV	Stage IV	Stage IV
Tissue Involvement	Ovaries, brain, subcutaneous LNs, adrenals, stomach	In transit mets in lower extremities, lung, pelvic LNs	Lung, liver, axillary LNs, spleen, bones	Rectal, liver, bones, lungs
Time (at treatment) since initial diagnosis (months)	50	26	39	16
Largest Decrease in Tumor Size (cm)	R. Adrenal: 6.9 → 6.0 Retroperitoneal: 2.5 x 2.8 → 2.1 x 1.5	No Response	L. axilla: 2.2 x 2.3 x 3.5 → 1.9 x 1.5 x 3.3	No Response
Observed effects on tumors (pre-treatment to four weeks post-treatment)	Mixed	None	Stable Disease	None
Response to therapy (RECIST)	Progressive Disease	Progressive Disease	Progressive Disease	Progressive Disease
Percent $CD34^+$ $CD3^+$ pre-sort	30.7	31.3	9.7	15.1
Percent $V\beta12^+$ $CD3^+$ pre-sort	7.61	19.4	3.9	7.4
Percent $CD34^+$ $CD3^+$ post-sort	99.9	98.5	99.4	93.6
Percent $V\beta12^+$ $CD3^+$ post-sort	33.7	67.4	34.8	44.2
Percent $CD34^+CD4^+$ post-sort	64.0	74.3	62.4	60.2
Percent $CD34^+CD8^+$ post-sort	36.7	25.1	36.5	36.3
Percent $V\beta12^+CD4^+$ post-sort	25.1	50.7	34.5	35.1
Percent $V\beta12^+CD8^+$ post-sort	8.6	18.5	17.7	16.4
Copy number post-sort	2.8	2.8	1.8	2.1
Pre-Rep Cell number (Cells undergoing REP)	1.7×10^8 (8×10^6)	2.98×10^8 (8×10^6)	7.2×10^7 (8×10^6)	5.75×10^7 (8×10^6)
Day 4 cell number	2.78×10^8	2.68×10^8	1.66×10^8	1.88×10^8
Day 6 cell number	5.98×10^8	6.5×10^8	4.1×10^8	4.39×10^8
Doubling time (days/division)	1.81	1.56	1.54	1.63
REP day 5 cell number	3.7×10^8	8.45×10^8	9.0×10^8	1.1×10^9
REP day 7 cell number	9.81×10^8	4.58×10^9	1.8×10^9	2.1×10^9
REP day 11 cell number (day 10 for patients 6 and 7)	5.1×10^9	4.38×10^9	5.4×10^9	3.90×10^9
REP Doubling time (days/division) (day 5 to 7)	1.422	0.820	2.0	2.144
Final $\%CD34^+$ $CD3^+$	98.2	95.6	86.3	95.2
Final $\%V\beta12^+$ $CD3^+$	21.2	23.6	26.9	21.3
Final $\%CD4^+CD34^+$	61.0	42.9	48.0	31.7
Final $\%CD8^+CD34^+$	36.8	54.2	38.2	63.5
REP d5 IFN-γ: T2+Tyro (T2 alone)	12732 (982)	7611 (12)	15533 (79)	9379 (201)
REP d5 IFN-γ: 624-Mel (624-28 A2- Mel)	10023 (508)	3743 (14)	11941 (91)	13551 (186)
REP d8 IFN-γ: T2+Tyro (T2 alone) [pg/mL]	5614 (12)	3882 (70)	58234 (61)	16088 (67)
REP d8 IFNγ: 624-Mel (624-28 A2-neg) [pg/mL]	2739 (0)	1828 (14)	20592 (48)	23493 (0)
Transduced Treatment Cell Number (total cell number)	5.79×10^8 (5.9×10^8)	8.42×10^8 (8.85×10^8)	5.9×10^8 (6.95×10^8)	1.76×10^9 (1.87×10^9)



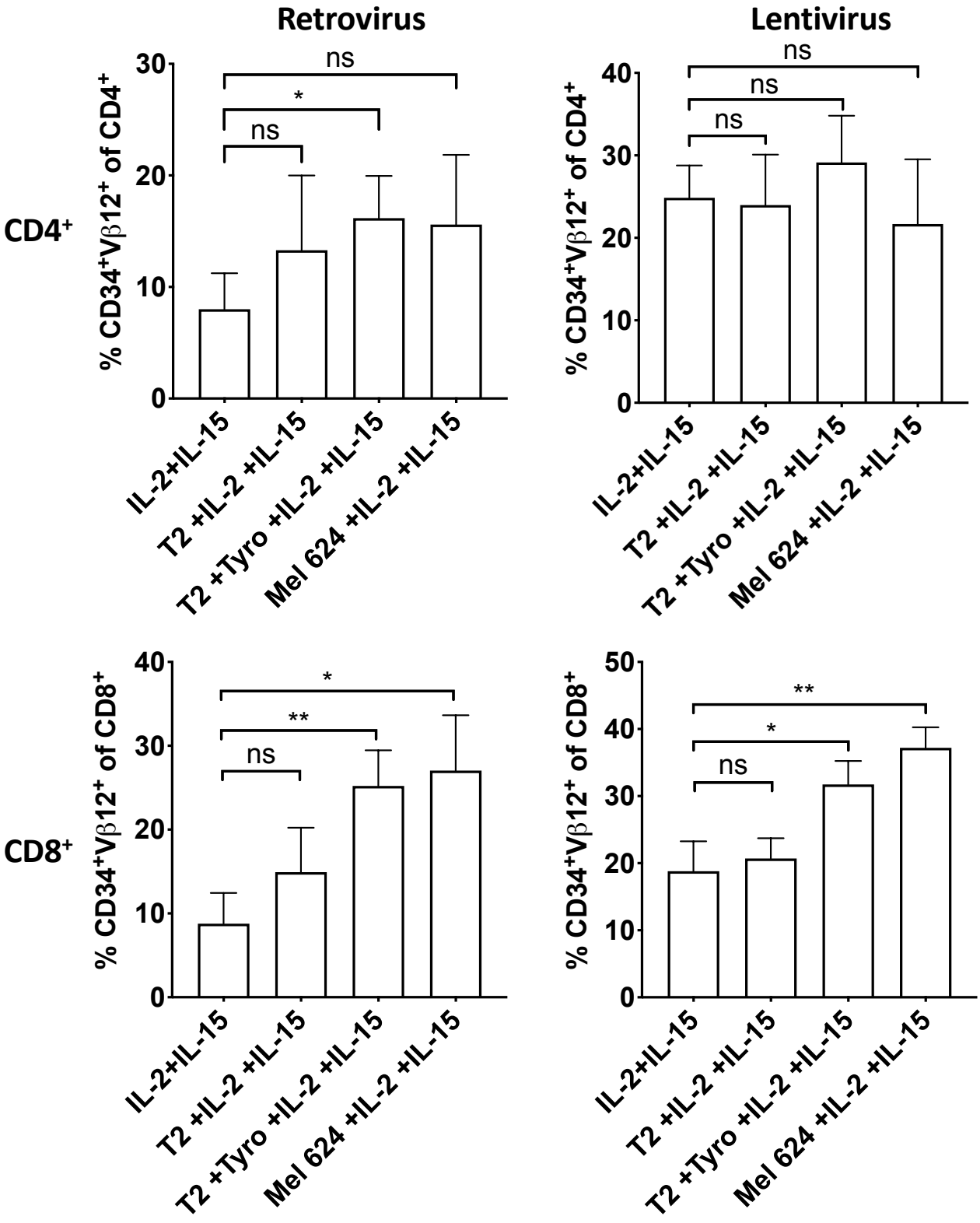
Supplemental Figure S1: Gating strategy for identifying CD34+ T cells



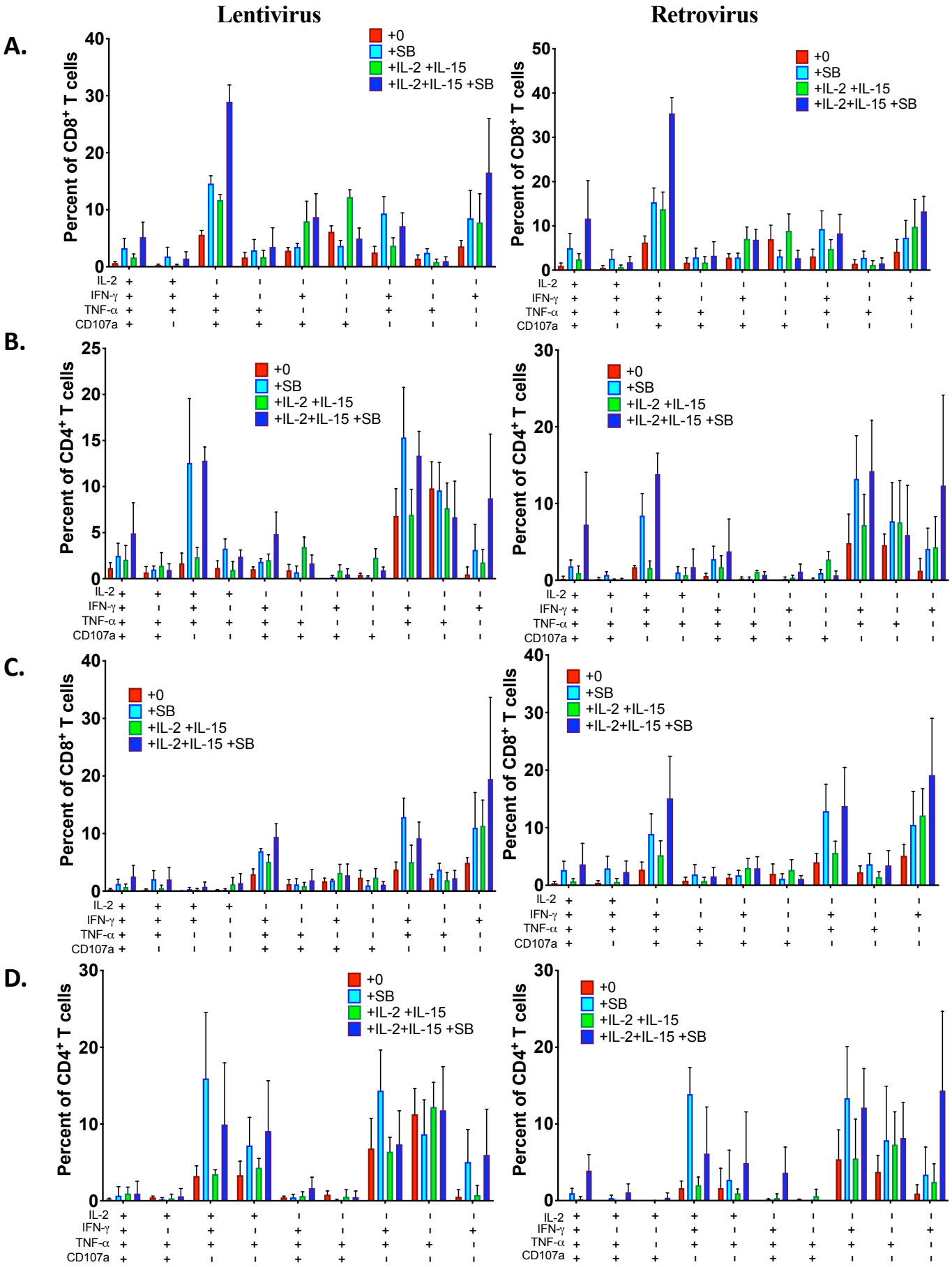
Supplemental Figure S2: Non-transduced T cells have low levels of CD34 with and without stimulation. A. At 28 days post-transfer into patients, patient PBMC were collected via apheresis or blood draw. These cells were depleted of red blood cells and then cryopreserved. In a cohort, the cells were thawed and either left untreated (Unstimulated) or rested in complete media with IL-2 (300IU/mL) and IL-15 (100ng/mL) for 2 days, then stimulated with anti-CD3/anti-CD28 beads (1:1 bead:cell ratio) (Stimulated). Cells were stained for CD3, CD4, CD8, and CD34, and gated as shown in Supplemental Figure 2. The calculated MFI values for CD34 on $CD34^{-}CD4^{+}$ and $CD34^{-}CD8^{+}$ T cells are shown. B. Cells from the aphereses of three healthy donors were thawed and either left untreated (Unstimulated) or cultured with IL-2 and IL-15 for two days then stimulated with anti-CD3/anti-CD28 beads (1:1 bead:cell ratio) (Stimulated). The calculated MFI values for CD34 on $CD4^{+}$ (left) or $CD8^{+}$ (right) T cells are shown. P values were calculated by a ratio paired t test and summarized with ns indicating not significant ($p > 0.05$).



Supplemental Figure S3: The percent of transduced T cells begins declining by seven days post-transfer. At indicated days post-transfer into patients, patient PBMC were collected via apheresis or blood draw. These cells were depleted of red blood cells and then cryopreserved. In a cohort, the samples from each patient were thawed and immediately stained for live/dead, CD3, CD4, CD8, CD11b, CD19, and CD34 expression. Stained samples were analyzed by flow cytometry for the percent of $CD34^+$ of total, $CD4^+$, and $CD8^+$ T cells. The number of $CD34^+$ T cells per milliliter of blood was estimated by multiplying the percent $CD34^+CD3^+$ (or $CD34^+CD4^+CD3^+$ or $CD34^+CD8^+CD3^+$) of viable cells by the total number of viable white blood cells per milliliter of blood drawn.



Supplemental Figure S4: The presence of antigen appears to maintain higher transgene expression in the presence of cytokines. Healthy donor T cells from three independent donors were activated, transduced with lentivirus (pLVX-1383I, left) or retrovirus (SAMEN-1383I, RV, right), then sorted using magnetic beads (Miltenyi) for $CD34^+$ cells, and rapidly expanded. Retroviral and lentiviral transduced T cells were cultured with IL-2 and IL-15 in the presence or absence of T2 cells, T2 cells pulsed with tyrosinase peptide (T2/tyrosinase) and MEL 624 cells. Tumor cells were added at a ratio of 1:4 tumor:T cell. Percentages of $CD34^+$ $V\beta12^+$ cells within $CD4^+$ T cells (top) and $CD8^+$ T cells (bottom) are shown. Error bars represent standard deviation. Significance is shown by Student's t-test with p-values summarized as *** < 0.001, ** <0.01, * ≤ 0.05, and ns > 0.05.



Supplemental Figure S5: CD4+ and CD8+ T cell responses to antigen in the presence or absence of cytokines and sodium butyrate. T cells were transduced, sorted, expanded, stimulated and stained as described in Figure 5. Shown are the percentages of lentiviral and retroviral transduced cells making each combination of cytokines specified. A. CD8+ T cells cocultured with 624 MEL cells. B. CD4+ T cells cocultured with 624 MEL cells. C. CD8+ T cells cocultured with T2 cells pulsed with tyrosinase peptide. D. CD4+ T cells cocultured with T2 cells pulsed with tyrosinase peptide. Error bars represent SD.

SAMEN-1383I

pLVX-1383I

Supplemental Figure S6: Maps of the retroviral and lentiviral constructs used to transduce T cells. LTR are long terminal repeat sequences. The MMLV-CMV LTR is a hybrid LTR containing both essential MMLV LTR components and a CMV promoter sequence. CD34t is the truncated non-signaling human CD34 sequence.