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Supplemental information

Development of an automated manufacturing process for large-scale production of autologous T cell therapies

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Supplemental Information

Table S1 Summary of Flow Cytometry Panels Used to Assess T Cell Phenotype At Different Timepoints

Panel	Timepoints	Marker	Fluorophore	Supplier
Cell Count/Viability	D1, D5, D7, D12	7AAD	N/A	BD Biosciences
Cellular Composition (Purity)	D0	CD45	VioBlue	Miltenyi Biotec
		CD4	VioGreen	Miltenyi Biotec
		CD3	FITC	Miltenyi Biotec
		CD56/CD16	PE	Miltenyi Biotec
		7AAD	N/A	BD Biosciences
		CD19	PE-Vio770	Miltenyi Biotec
		CD14	APC	Miltenyi Biotec
		CD8	APC-Vio770	Miltenyi Biotec
Exhaustion	D0, D7, D12	CD223 (LAG3)	VioBlue	Miltenyi Biotec
		CD4	VioGreen	Miltenyi Biotec
		CD3	FITC	Miltenyi Biotec
		7AAD	N/A	BD Biosciences
		CD279 (PD-1)	PE-Vio770	Miltenyi Biotec
		CD366 (TIM3)	APC	Miltenyi Biotec
		CD8	APC-Vio770	Miltenyi Biotec
Memory	D0, D7, D12	CCR7	VioBlue	Miltenyi Biotec
		CD4	VioGreen	Miltenyi Biotec
		CD3	FITC	Miltenyi Biotec
		7AAD	N/A	BD Biosciences
		CD45RA	PE-Vio770	Miltenyi Biotec
		CD95	APC	Miltenyi Biotec
		CD8	APC-Vio770	Miltenyi Biotec
Activation	D0, D1, D7, D12	CD4	VioBlue	Miltenyi Biotec
		CD8	VioGreen	Miltenyi Biotec
		CD25	PE	Miltenyi Biotec
		7AAD	N/A	BD Biosciences
		CD69	APC	Miltenyi Biotec
		CD3	APC-Vio770	Miltenyi Biotec
Transduction Efficiency	D7, D12	7AAD	N/A	BD Biosciences
		CD4	VioGreen	Miltenyi Biotec
		CD3	FITC	Miltenyi Biotec
		TCR peptide-specific dextramer	PE	Immudex
		CD8	APC-Vio770	Miltenyi Biotec

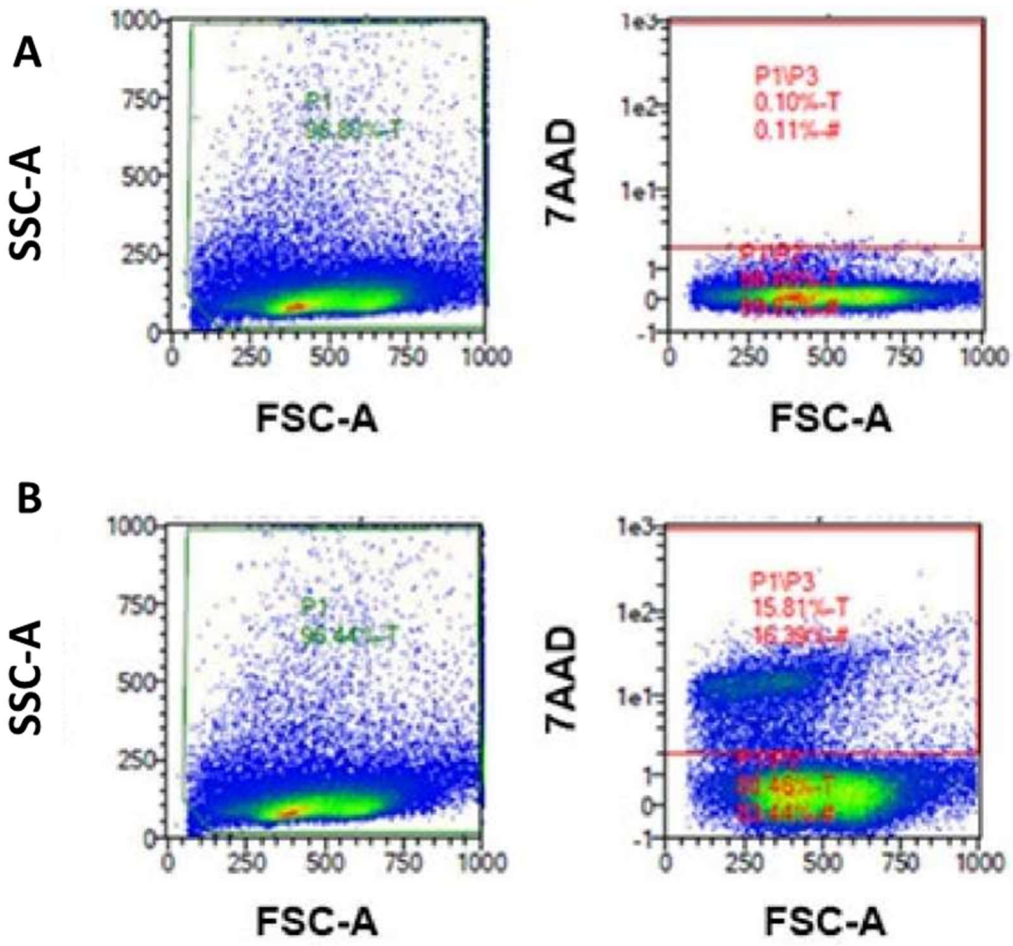

Figure S1 Gating strategy for Cell Count and Viability Panel. (A) Unstained control. (B) 7-AAD stained sample. The first gate (left) finds events the approximate size of T cells, the second gate (right) finds viable cells among these cells.

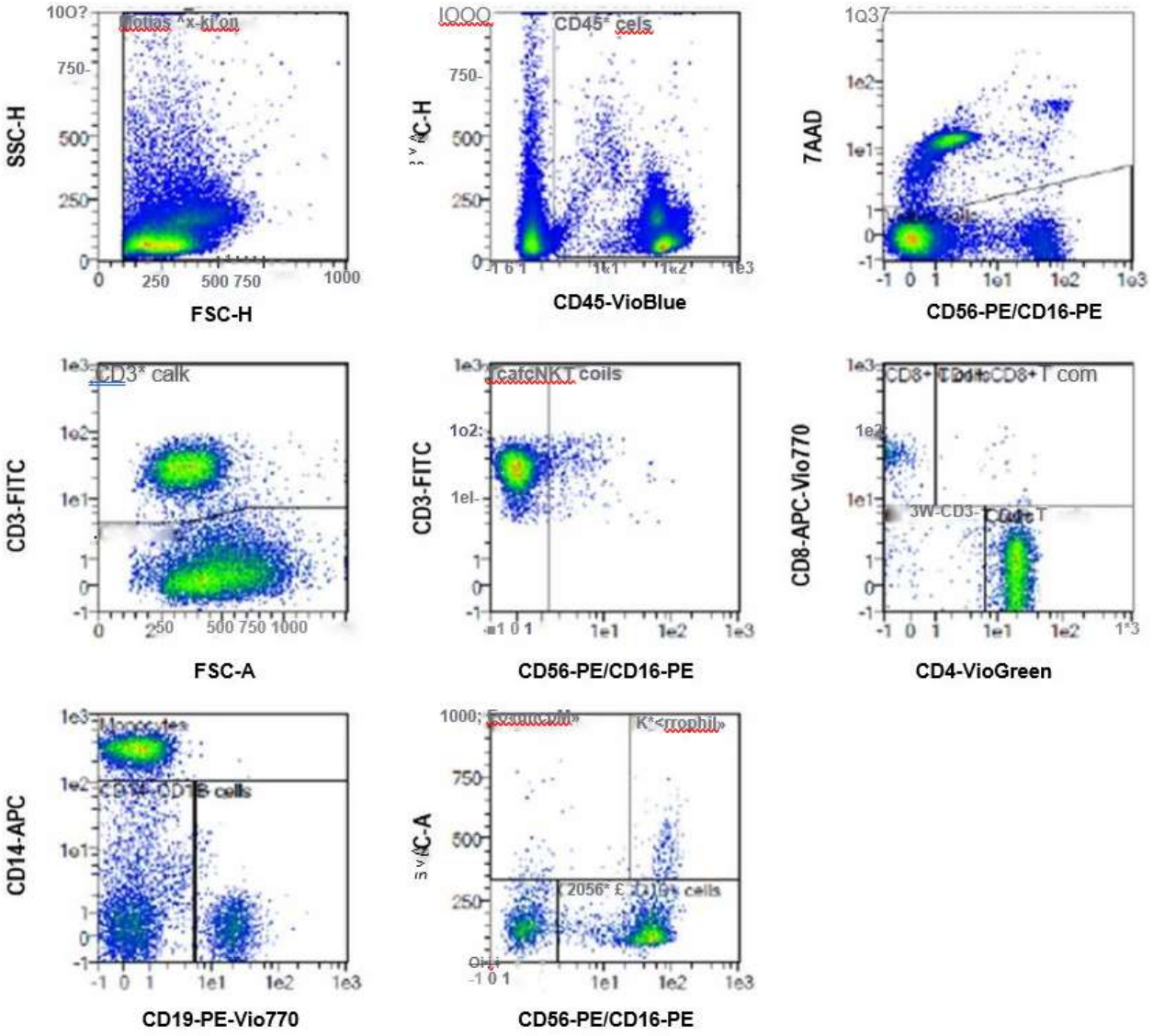

Figure S2 Gating Strategy for Cellular Composition Panel for Evaluation of T Cells and Other Immune Cell Types. The first gate (top left) excludes debris and red blood cells. The second gate (top centre) identifies CD45+ cells within the debris exclusion gate. The third plot identifies viable cells among CD45+ cells. The fourth plot (centre left) identifies CD3+ cells among viable cells. The fifth plot (centre) identifies T cells and NKT cells. The sixth plot (centre right) identifies CD4 and CD8 positive and negative populations within the T cell population. The seventh plot (bottom left) identifies monocytes and B cells within CD3+ cells. The eighth plot (bottom centre) identifies eosinophils and neutrophils among CD14-/CD19- cells.

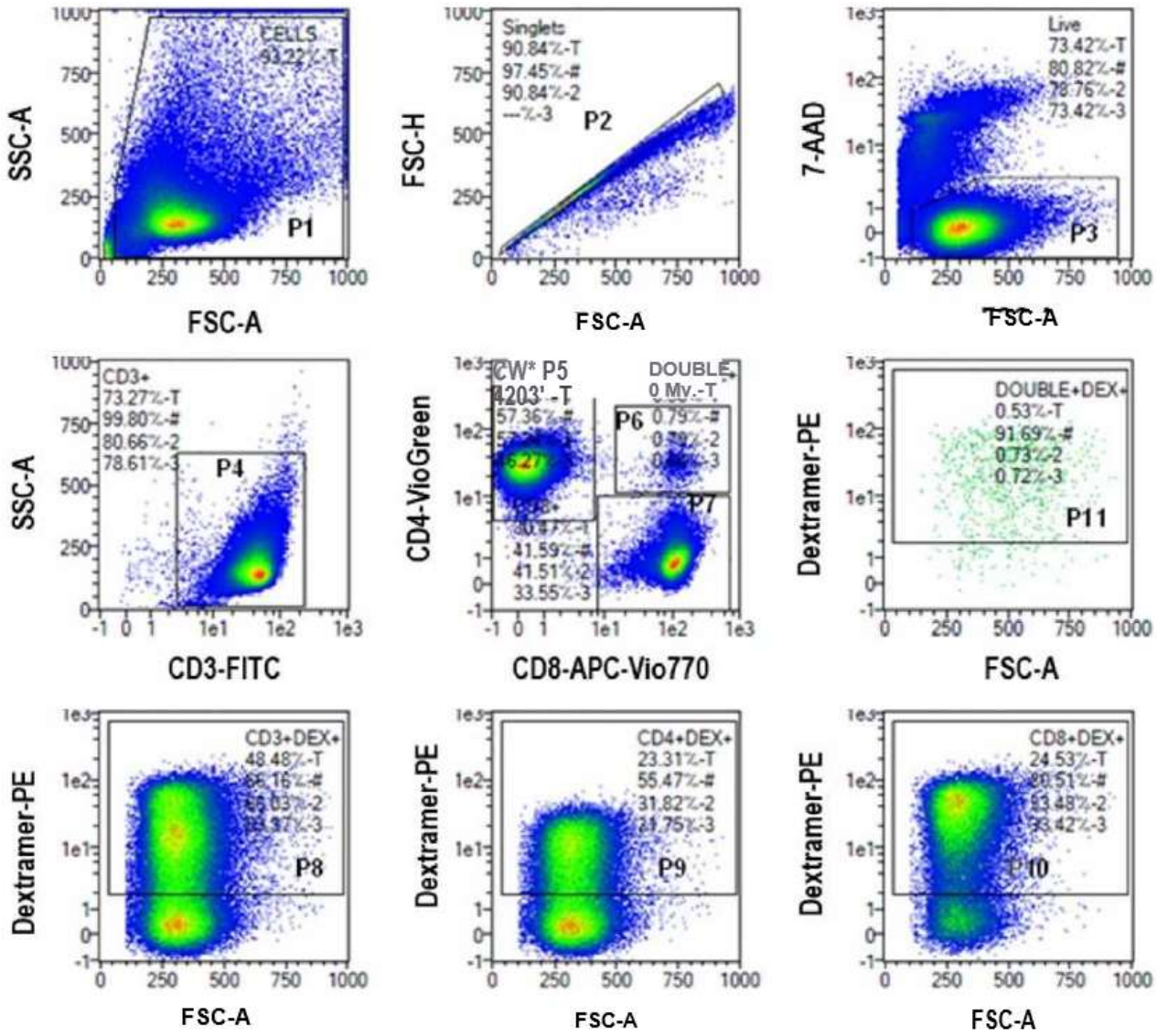

Figure S3 Gating strategy for Transduction Efficiency Panel. The first gate (top left) identifies cells. The second gate (top centre) identifies single cells. The third plot (top right) identifies viable cells. The fourth plot (centre left) identifies CD3+ cells. The fifth plot (centre) identifies CD4 and CD8 positive and negative populations within the T cell population. The sixth plot (centre right) identifies transduced cells within the CD4+/CD8+ population. The seventh plot (bottom left) identifies transduced cells within the CD3+ population. The eighth plot (bottom centre) identifies transduced cells within the CD4+ population. The ninth plot (bottom right) identifies transduced cells within the CD8+ population.

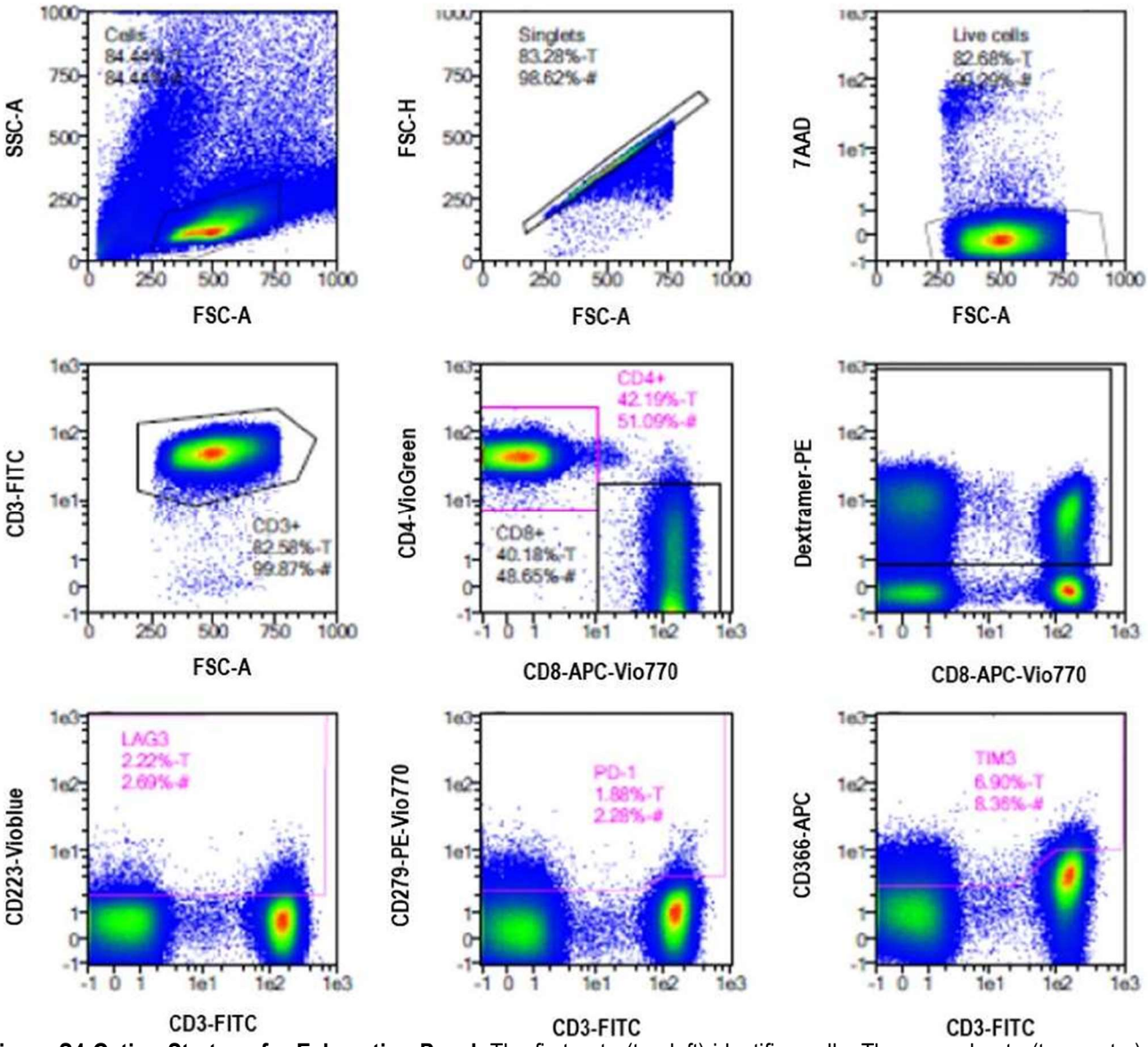

Figure S4 Gating Strategy for Exhaustion Panel. The first gate (top left) identifies cells. The second gate (top centre) identifies single cells. The third plot (top right) identifies viable cells. The fourth plot (centre left) identifies CD3+ cells. The fifth plot (centre) identifies CD4 and CD8 positive and negative populations within the T cell population. The sixth plot (centre right) identifies transduced cells within the CD4+/CD8+ population. The seventh plot (bottom left) identifies transduced cells within the CD3+ population. The eighth plot (bottom centre) identifies transduced cells within the CD4+ population. The ninth plot (bottom right) identifies transduced cells within the CD8+ population.

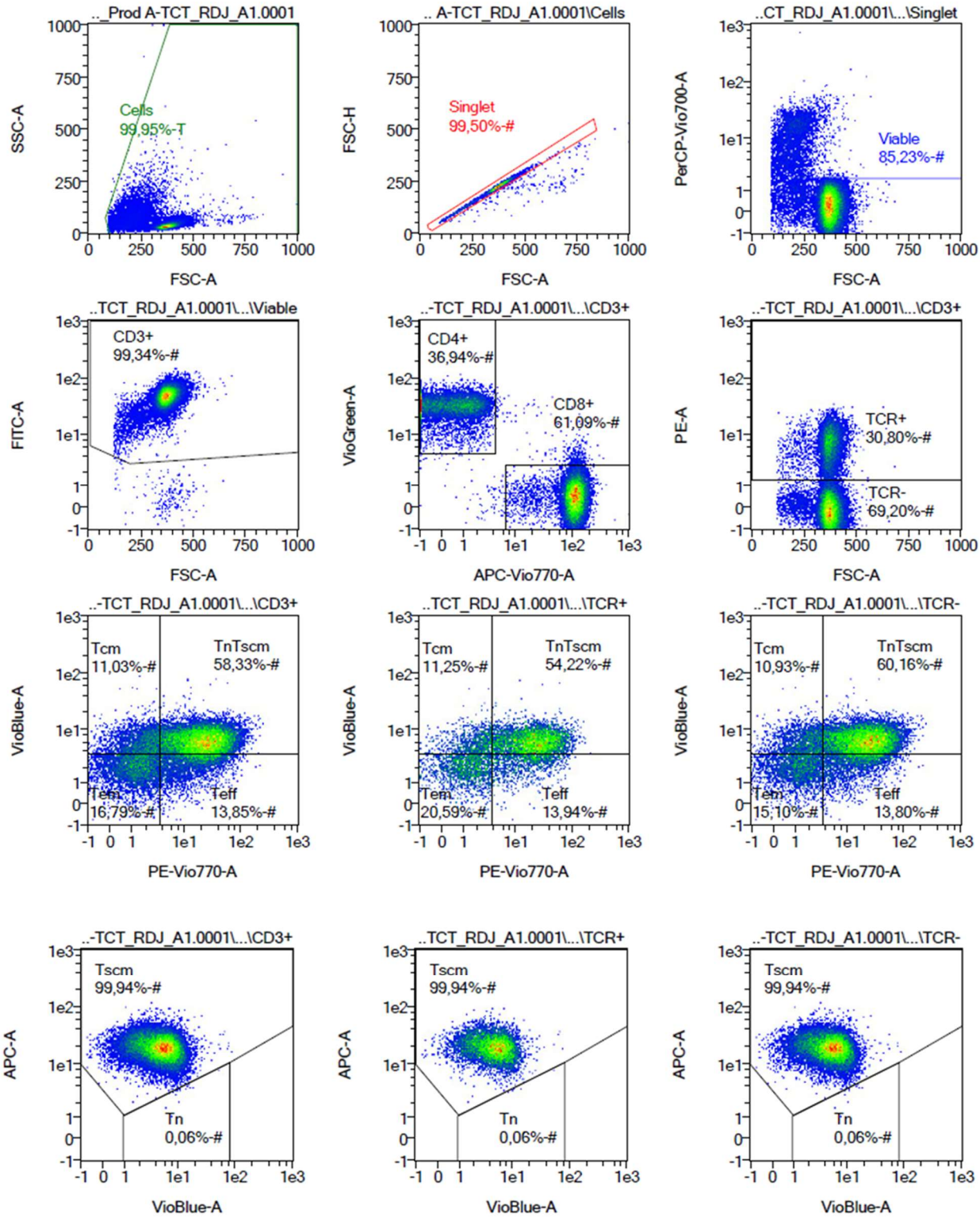

Figure S5 Gating Strategy for Differentiation Panel for Evaluation of T Cell Memory Subsets. The first gate (top left) identifies cells. The second gate (top centre) identifies single cells. The third plot (top right) identifies viable cells. The fourth gate (centre left) identifies CD3+ cells. The fifth plot (centre) identifies CD4 and CD8 positive and negative populations within the T cell population. The sixth plot (centre right) identifies transduced (TCR+) and non-transduced (TCR-) cells within the CD3+ population. The seventh, eighth and ninth plots (second row from bottom) identifies TCM, TEM and TEFF sub-populations within the CD3+, TCR+ and TCR- populations respectively. The tenth, eleventh, and twelfth plots identify TSCM and TN sub-populations within the CD3+, TCR+ and TCR- populations, respectively.

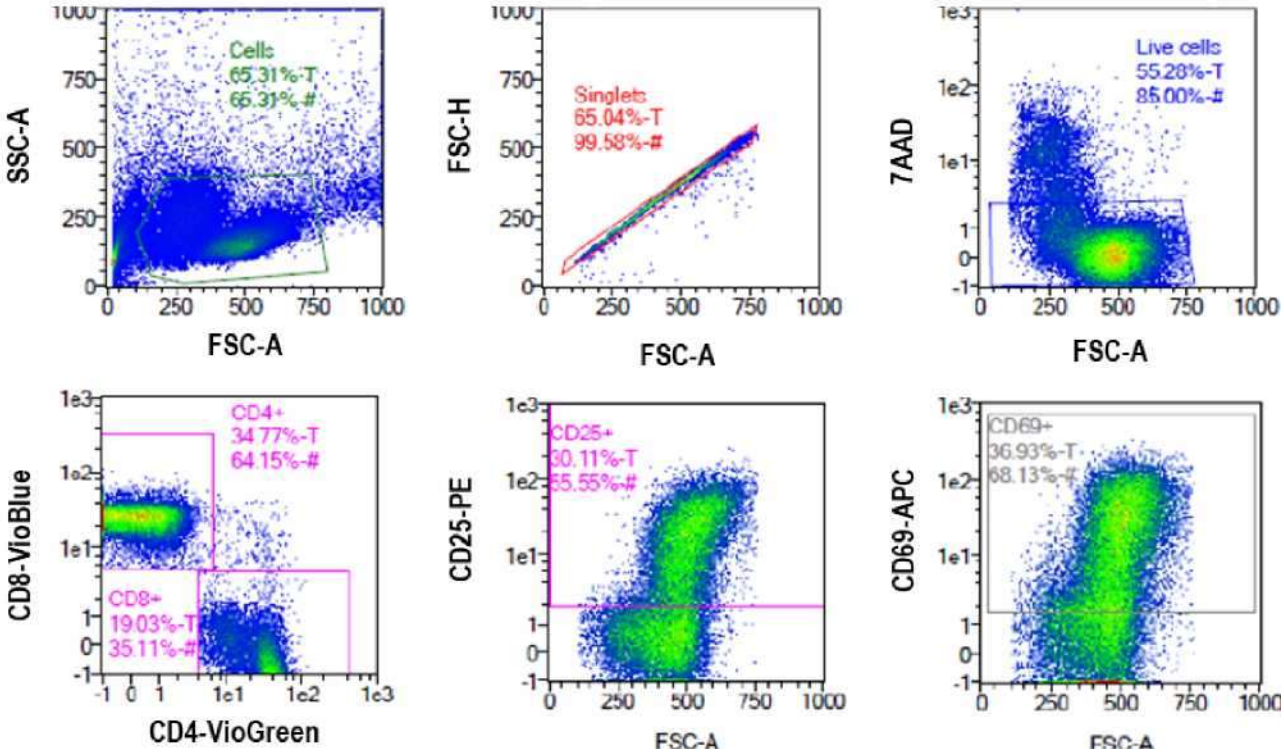

Figure S6 Gating Strategy for Activation Panel. The first gate (top left) identifies cells. The second gate (top centre) identifies single cells. The third plot (top right) identifies viable cells. The fourth plot (bottom left) identifies CD4+ and CD8+ cells. The fifth plot (bottom centre) identifies CD25+ cells. The sixth plot (bottom right) identifies CD69+ cells.

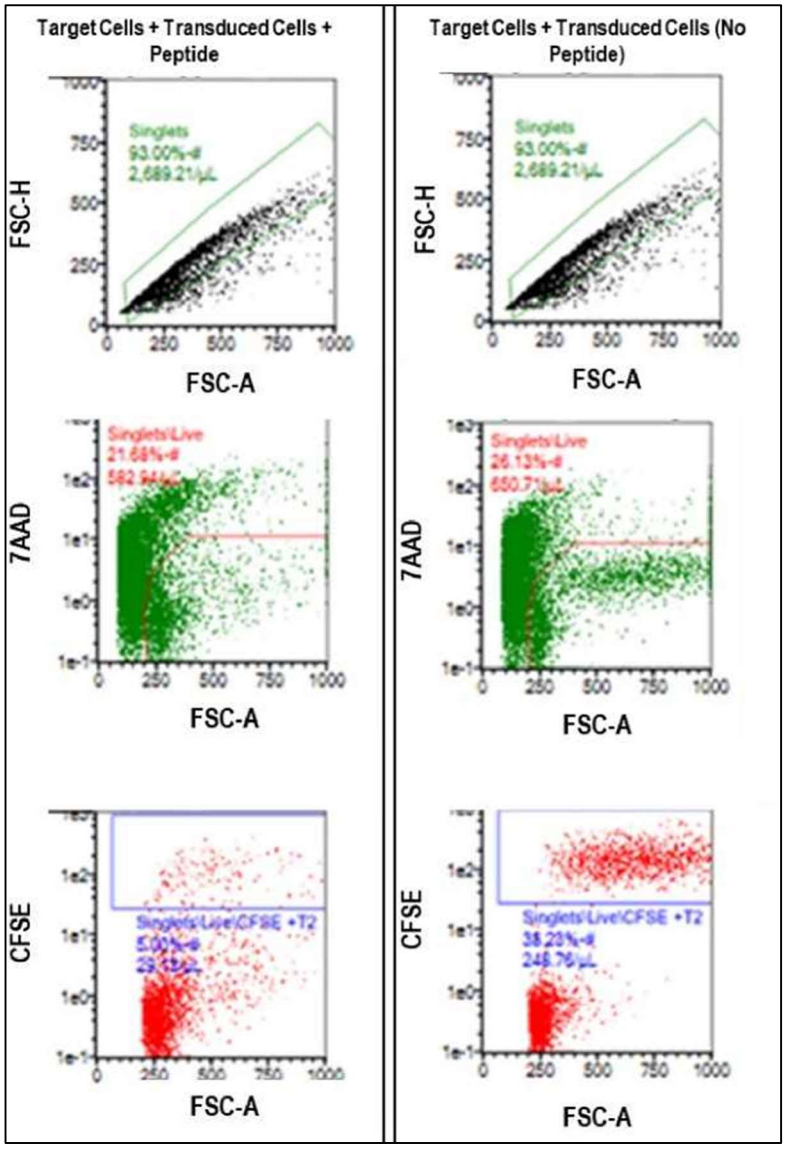

Figure S7 Gating strategy for cytotoxicity assay. Left panel: target cells + transduced cells + peptide. Right panel: No-peptide control. Top gate identifies singlets. Centre gate identifies viable cells within singlets. Bottom gates identify CFSE-labelled target cells within viable cells.

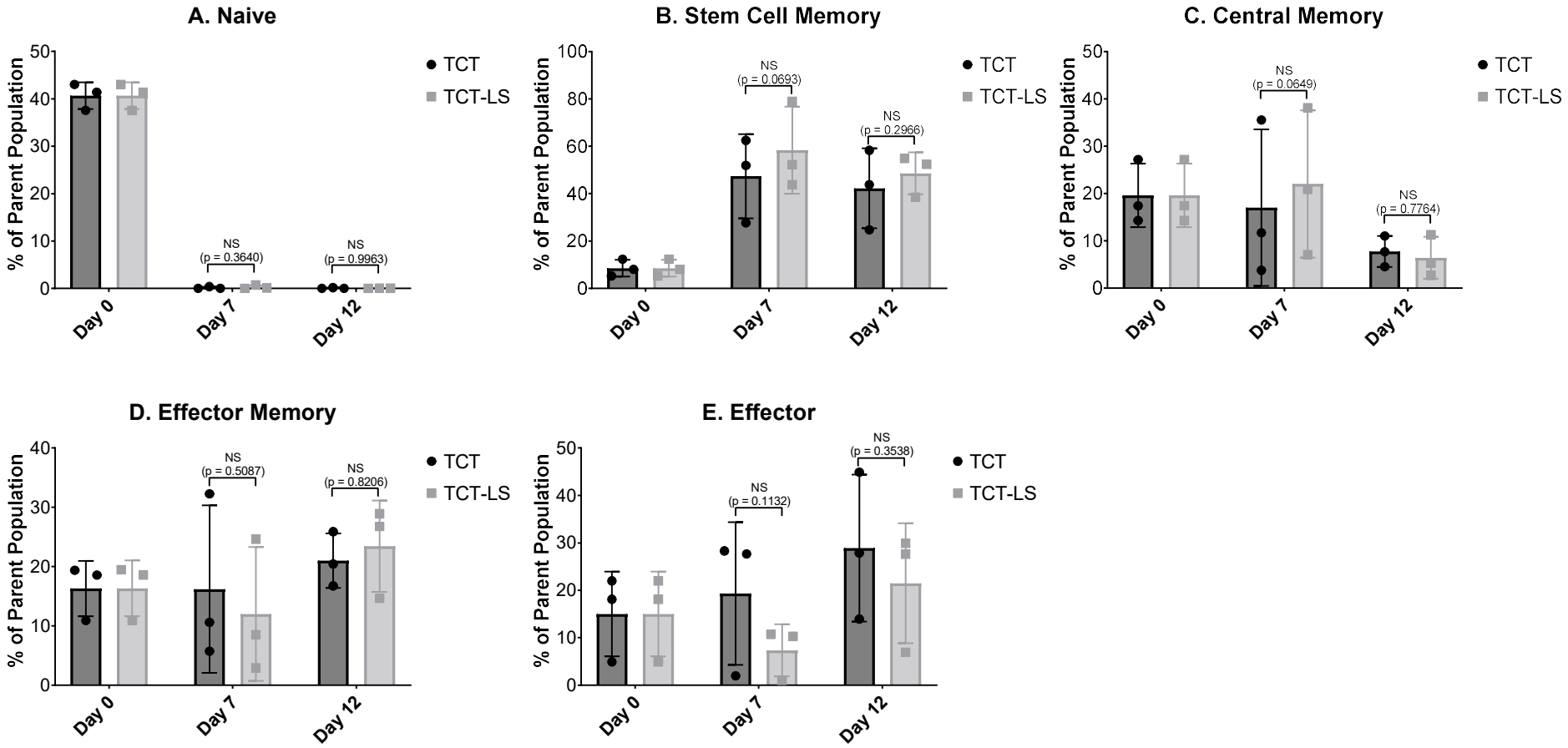

Figure S8 Memory phenotype in TCT and TCT-LS Processes at days 0, 7 and 12 from T cells manufactured using either the TCT or TCT-LS process using cells from matched healthy donors (n = 3). A: Naive T cells are present at day 0 but not at days 7 and 12; B: Stem cell memory T cells increase on days 7 and 12 compared to day 0; C: Central memory T cells decrease at day 12 compared to days 0 and 7; D: Effector memory T cells vary between time points; E: Effector T cells increase at day 12 compared to day 0. Two-way ANOVA with multiple comparisons shows no significant difference between TCT and TCT-LS processes at any time point (day 0 time point shows same data for both processes as the arms were divided after this time point). Graphs show individual data points with mean and standard deviation

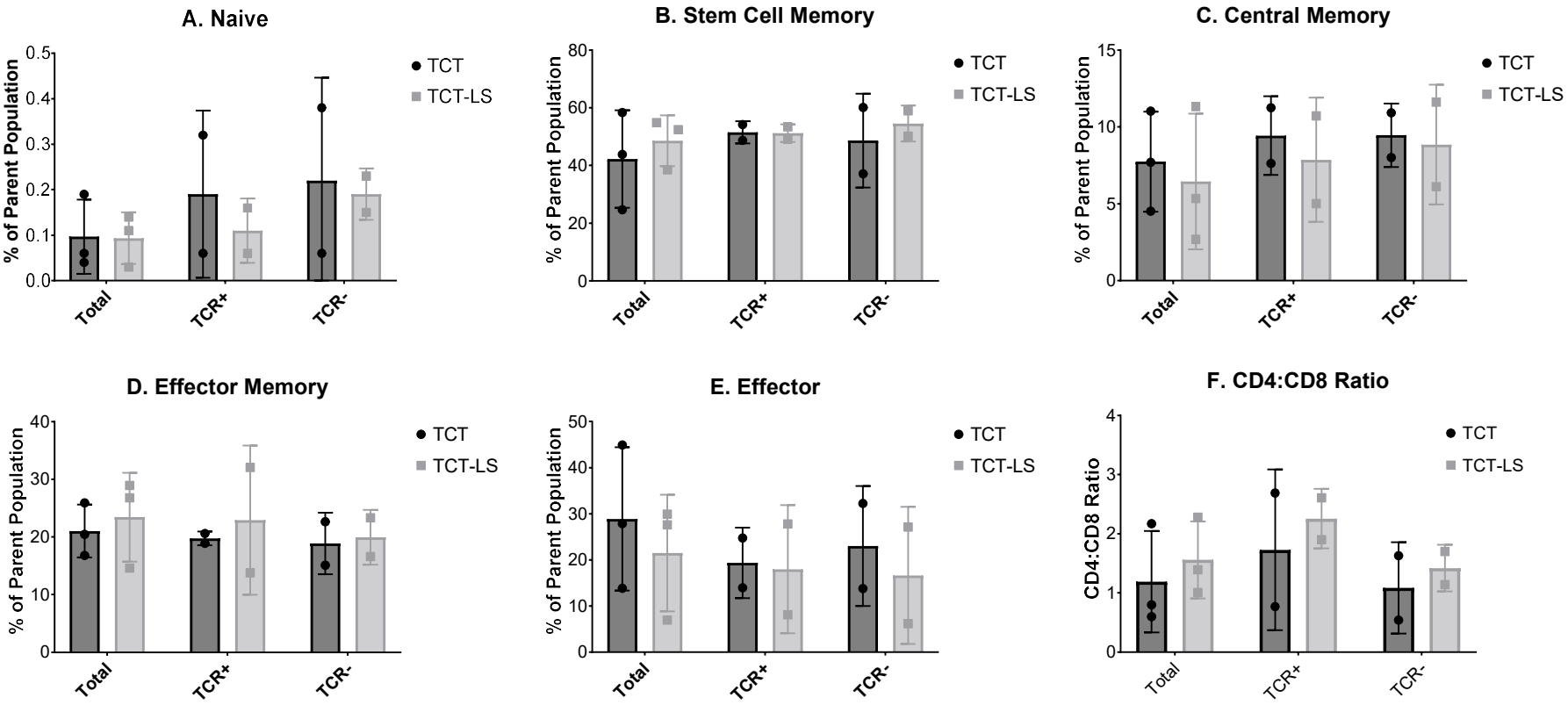

Figure S9 Memory phenotype and CD4:CD8 ratio in TCT and TCT-LS Processes in total, transduced (TCR+) and untransduced (TCR-) populations at day 12 from T cells manufactured using either the TCT or TCT-LS process using cells from matched healthy donors (n = 3). A: Naive T cells; B: Stem cell memory T cells; C: Central memory T cells; D: Effector memory T cells; E: Effector T cells; F: CD4:CD8 ratio. Two-way ANOVA with multiple comparisons shows no significant difference between TCT and TCT-LS processes for any population. Graphs show individual data points with mean and standard deviation.

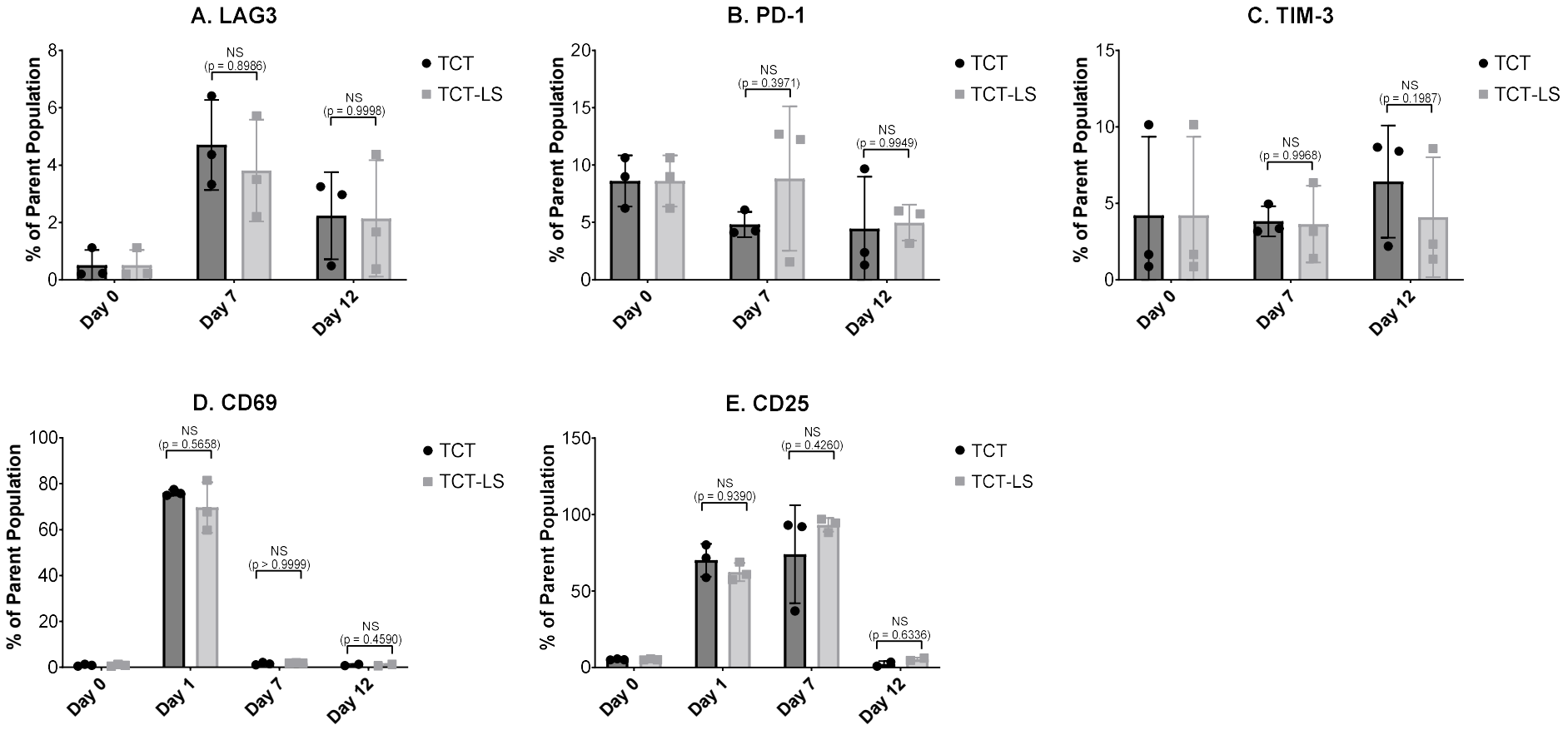

Figure S10 Activation and Exhaustion Markers in TCT and TCT-LS Processes at days 0, 7 and 12 from T cells manufactured using either the TCT or TCT-LS process using cells from matched healthy donors (n = 3). A: Expression of exhaustion marker LAG-3. B: Expression of exhaustion marker PD-1. C: Expression of exhaustion marker TIM-3. D: CD69 peaks at day 1. E: CD25 expression peaks at day 7 but is also upregulated at days 1 and 12. Two-way ANOVA with multiple comparisons shows no significant difference between TCT and TCT-LS processes at any time point. Graphs show individual data points with mean and standard deviation.