Supplemental Information

Stem cell-derived CAR T cells show greater

persistence, trafficking, and viral control

compared to *ex vivo* transduced CAR T cells

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Supplemental Material

Figure S1:

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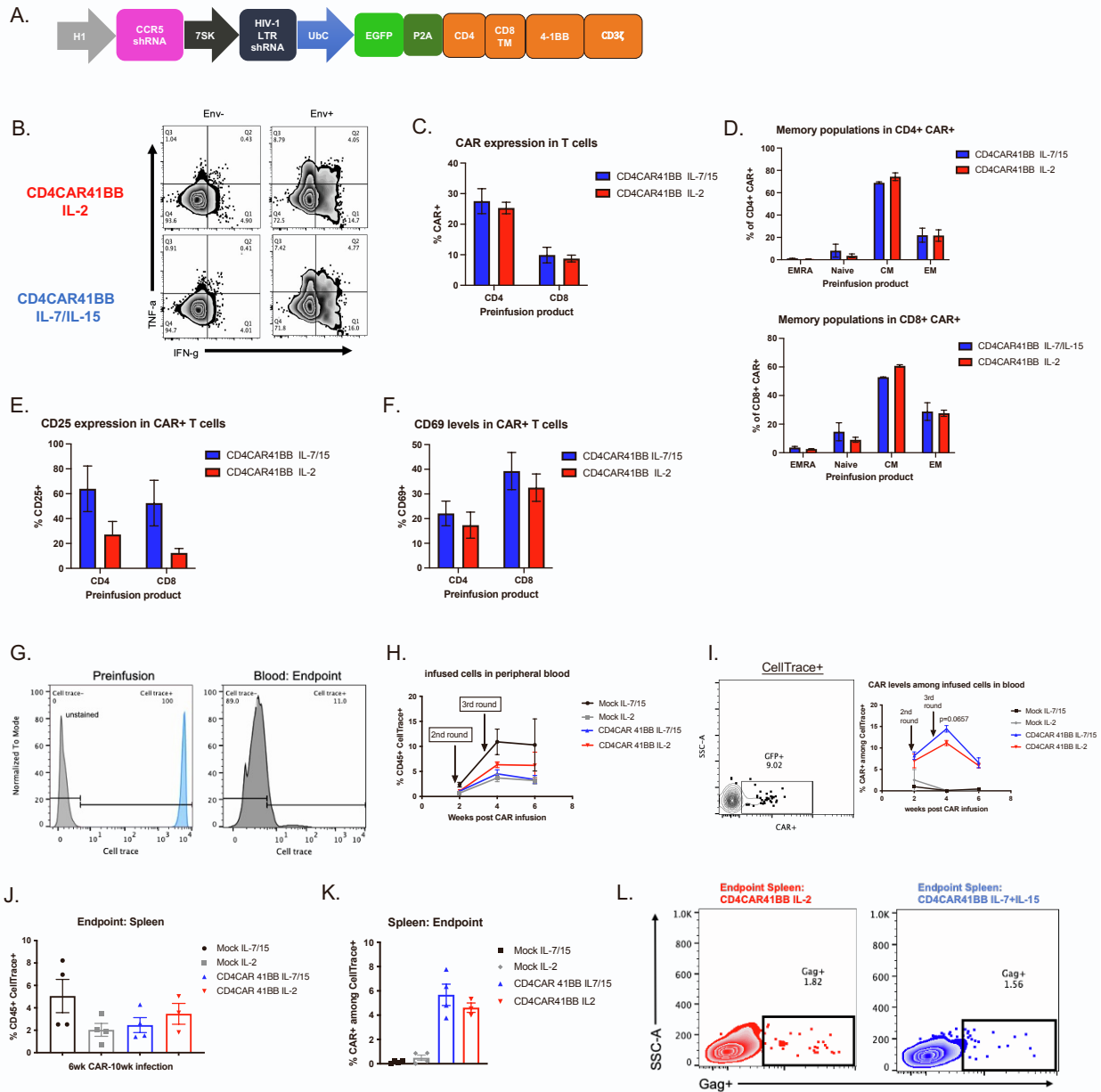**H.**

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Figure S1: In vitro function and in vivo tracking of CD4CAR41BB T cells cultured in IL-2 or IL-7/IL-15. A) Schematic of lentiviral vector expressing CD4CAR41BB and protective shRNAs against CCR5 and HIV LTR. B) FACS plot showing upregulation of human cytokines in CD4CAR41BB T cells cultured in IL-2 or IL-7/IL-15 after co-incubation with stimulated ACH2 cells (Env+) or unstimulated ACH2 cells (Env-). T cells derived from healthy donors. C) CD4CAR41BB expression in CD4 and CD8 T cells (derived from uninfected BLT mice) cultured in IL-2 or IL-7/IL-15. D) Frequency of memory populations in CD4+ CAR+ T cells (top) and CD8+ CAR+ T cells (bottom) from (C) before infusion. E) Frequency of CD25+ in CAR+ CD4 and CD8 T cells cultured in IL-2 or IL-7/IL-15 from (C). F) Frequency of CD69+ in CAR+ CD4 and CD8 T cells cultured in IL-2 or IL-7/L-15 from (C). G) Representative histogram plots showing CellTrace+ among CD45+ population before infusion (left) and after infusion at endpoint of study in peripheral blood. H) Percentage of human CD45+ CellTrace+ cells in peripheral blood of mock or CAR treated humanized mice. I) Representative FACS plot showing CAR+ (GFP+) gating in CellTrace+ population (left) and summary of percentage of CAR+ (GFP+) cells among CellTrace+ population in peripheral blood (right). J) Percentage of human CD45+ CellTrace+ cells in spleen. K) Percentage of CAR+ (GFP) cells among CellTrace+ in spleen. L) FACS plots (3-4 CAR mice combined) showing Gag expression in CAR+ (GFP+) cells cultured in IL-2 or IL-7/IL-15 in spleen. Error bars represent +/- SEM with n=3-4.
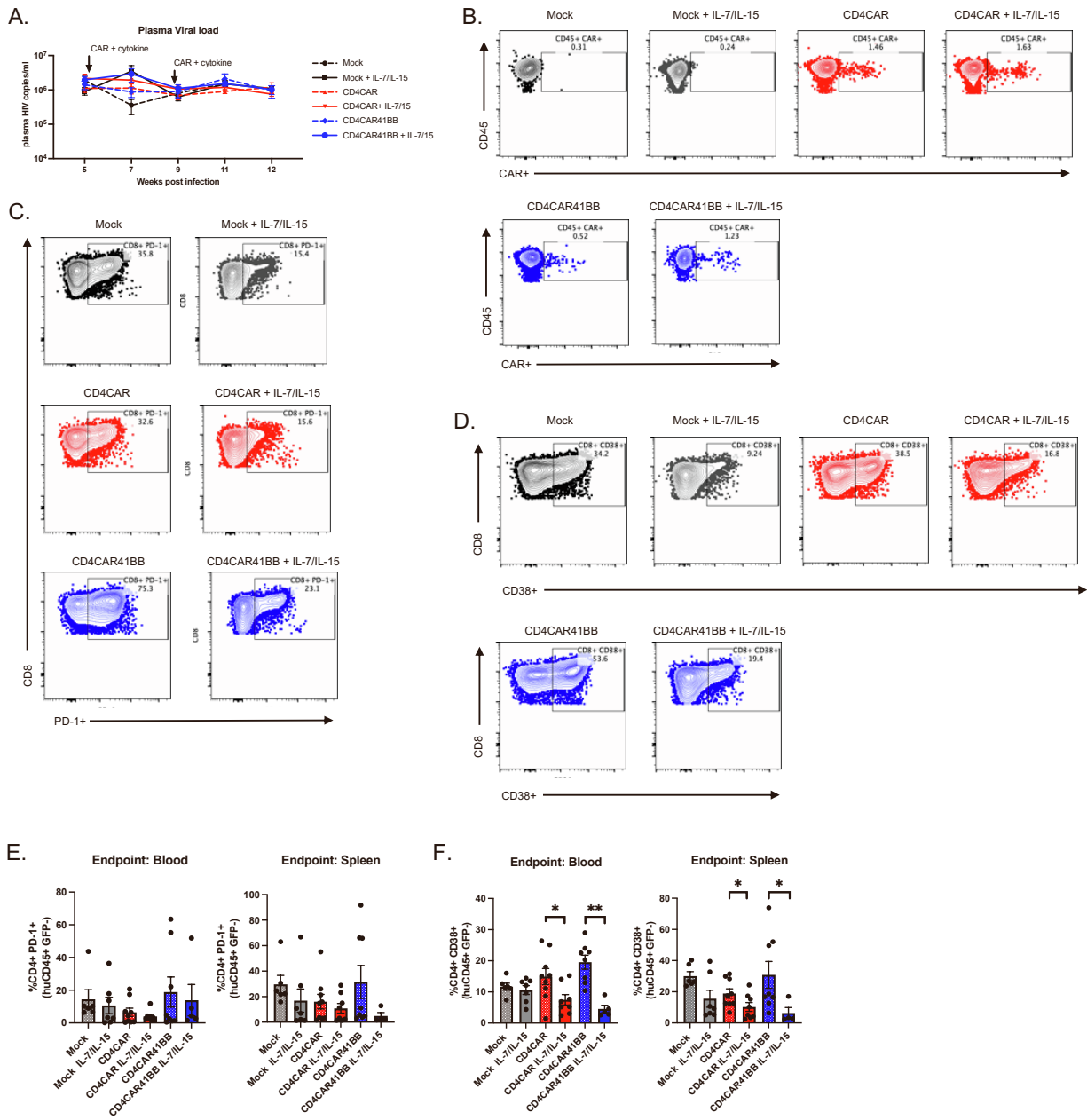
Figure S2:

Figure S2: IL-7 and IL-15 administration reduces expression of exhaustion and activation markers in CAR negative T cells. A) Plasma HIV RNA copies measured by RT-PCR. Arrows indicate CAR T cell and cytokine treatment. B) Representative FACS plots showing percentage of CAR+ T cells among total human CD45 detected in peripheral blood 2 weeks post infusion. C) Representative FACS plots showing PD-1 expression in CD8+ CAR negative T cells in mice that received mock or CAR-T with or without cytokine treatments. D) Representative FACS plots showing CD38 expression in CD8+ CAR negative T cells in mice that received mock or CAR-T with or without cytokine treatments. E) Percentage of CD4+ CAR negative T cells expressing PD-1 in blood (left) and spleen (right) at endpoint of study. F) Percentage of CD4+ CAR negative T cells expressing CD38 in blood (left) and spleen (right) at the endpoint of study. Error bars indicate SEM. **p<0.01; *p<0.05; Mann-Whitney test. n=4-6 per group with two independent experiments.
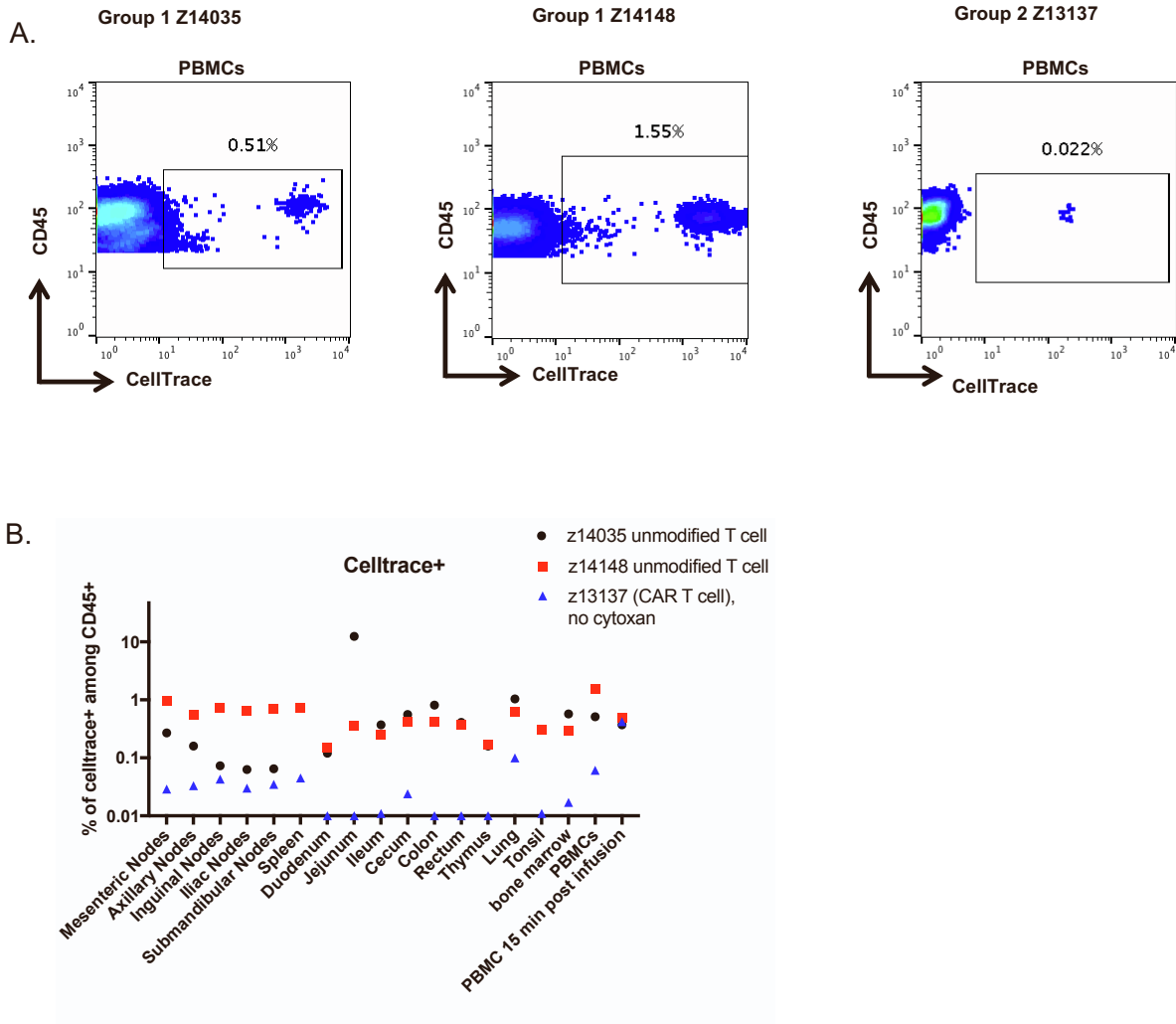
Figure S3:

Figure S3: Cyclophosphamide preconditioning enhances autologous adoptive T cell engraftment after infusion in SHIV infected NHP animals. To track T cell trafficking, group 1 animals were infused with cell trace labeled T cells and group 2 animal was infused with cell trace labeled CAR-T cells 3 days before necropsy. A) Representative flow plot showing detection of cell trace+ cells in PBMCs of group 1 and group 2 animals. B) % of cell trace positive cells in lymphoid tissues.
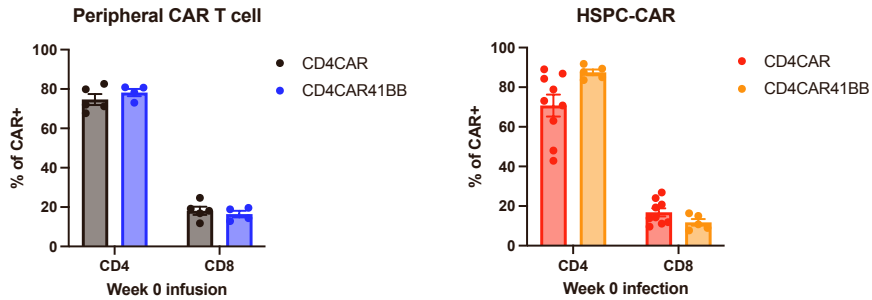
Figure S4:

Figure S4: Adoptive CAR T cell treated mice and stem cell CAR mice have similar engraftment levels of CD4 and CD8 CAR+ T cell. Percentage of CD4 and CD8 CAR+ (GFP+) T cells in peripheral blood at week 0 of CAR T infusion (left) or week 0 of infection in CAR-stem cell mice (right). Error bars indicate SEM. n=4-5 per group with 1-2 independent experiments.

Table S1. HSPC transplantation conditioning and cell doses.

Animal ID	1. HSPC Transplantation						2. SHIV infection	3. T Cell Adoptive Transfer #1				3. T Cell Adoptive Transfer #2				4. Necropsy
	Conditioning	Mobilization	Cells/kg	Infusion CFU Lenti Gene Marking (%)	Lenti+ CFU (Count)	Actin+ CFU (Count)	Years Post-Transplant	Infusion Product	Total Cell Dose (cells/kg)	CAR+ Cell Dose (cells/kg)	CellTrace Labeled?	Infusion Product	Total Cell Dose (cells/kg)	CAR+ Cell Dose (cells/kg)	CellTrace Labeled?	Notes
Z14160	TBI	G-CSF/SCF-Primed Marrow	1.24E+07	43.53	37	85	-	-	-	-	-	-	-	-	-	Acute kidney injury following HSPC transplantation
Z14279	TBI	G-CSF/SCF-Primed Marrow	1.23E+07	85.71	6	7	1.32	-	-	-	-	-	-	-	-	Hemolytic-Uremic Syndrome (HUS)
Z14123	TBI	G-CSF/SCF-Primed Marrow	2.30E+07	6.90	6	87	1.22	CD4CAR T-Cell	6.11E+07	1.21E+06	No	-	-	-	-	Hemolytic-Uremic Syndrome (HUS)
Z14035	TBI	G-CSF/SCF-Primed Marrow	1.82E+07	69.32	61	88	1.34	CD4CAR T-Cell	4.84E+07	2.62E+06	No	Control T-Cell	2.03E+07	-	Yes	Disseminated, moderate to extensive lymphoid reactivity
Z14148	TBI	G-CSF/SCF-Primed Marrow	1.67E+07	72.29	60	83	1.38	CD4CAR T-Cell	4.99E+07	1.48E+06	No	Control T-Cell	6.75E+07	-	Yes	Disseminated, moderate to extensive lymphoid reactivity
Z13137	TBI	G-CSF/SCF-Primed Marrow	1.60E+07	44.44	36	81	1.53	CD4CAR T-Cell	3.29E+07	2.42E+06	Yes	CD4CAR T-Cell	1.22E+08	1.18E+07	Yes	N/A