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Supplemental Information

Lentiviral Vector-Based Dendritic Cell Vaccine Suppresses HIV Replication in Humanized Mice

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Supplementary Materials

Supplementary Methods

Viral RNA sequence analysis for escape mutants

Plasma and PBMC RNA prepared from HIV-1-infected BLT mice were reverse transcribed to generate cDNA from which amplicons spanning NL4-3 *gag* (bp 931-1111) in the region encoding SL9 were generated by nested PCR using the following primer pairs: outer primers: TGGGTGCGAGAGCGTCGGTAT and TCTATCCCATTCTGCAGCTTCCTC, inner primers: CCTGGCCTTTTAGAGACATCAG and GCTCTTCCTCTATCTTATCTAAGGC. Amplified DNA fragments were cloned using TOPO TA cloning (Invitrogen) and sequenced.

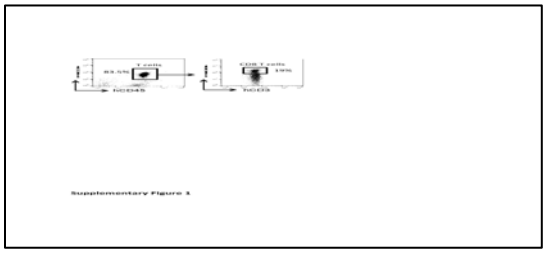

Figure S1. **BLT mice express high-levels of human T cells**. The gating strategy used to analyze human CD8 T cells from the peripheral blood of BLT humanized mice based on the expression of human CD45, CD3 and CD8 is shown.

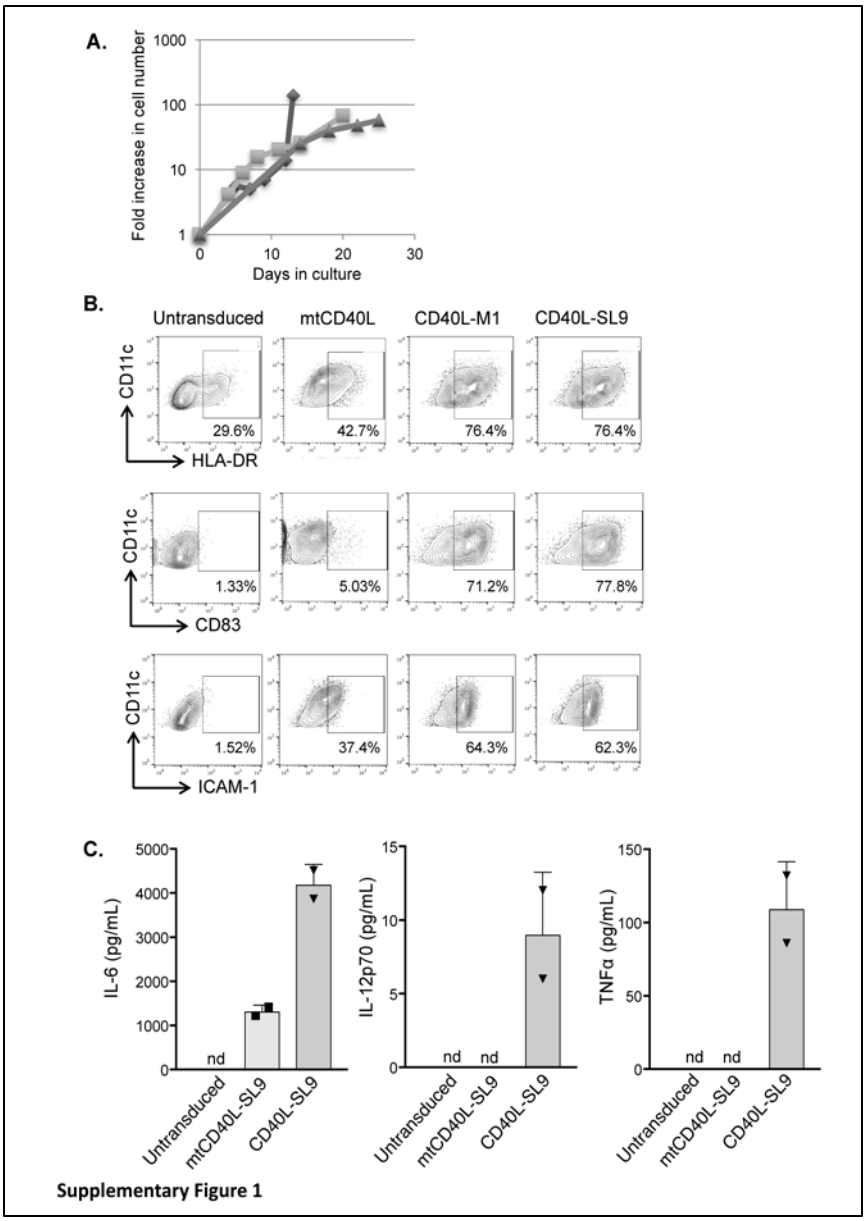

Figure S2. **HSCs treated with GM-CSF and SCF expand in log-scale to generate human DCs that mature in response to transduction with CD40L-expressing vectors**. (**A**) Fetal liver-derived HSCs were cultured for three weeks with GM-CSF and SCF to generate HSC-DCs. Shown are the number of cells in culture plotted as fold increase over time. (**B**) HSC-DCs were transduced with mtCD40L, CD40L-M1 or CD40L-SL9 vectors and after 72 h the percentage of HLA-DR+, CD83+ and ICAM-1+ cells was quantified by flow cytometry. Experiments were done in three donors. Representative blots from one donor are shown. (**C**) IL-6, IL-12p70, and TNFα in the transduced HSC-DC supernatant were quantified by cytokine bead array. Data represent mean ± SD from one donor analyzed in two independent experiments. nd = not detected.

Plasma RNA

Vaccination	Mice	Clones	WT SL9 epitopes (%)	Mutated SL9 epitopes (%)
No HSC-DCs	4	19	18/19 (95%)	SLYNTVA**A**L 1/19 (5%)
Untransduced HSC-DCs	3	13	8/13 (61%)	SLYNT**I**A**V**L 4/13 (31%) SLYNTVAT**P** 1/13 (8%)
CD40L-SL9 HSC-DCs	4	20	18/20 (90%)	**P**LYNTVATL 2/20 (10%)

PBMC cellular RNA

Vaccination	Mice	Clones	WT SL9 epitopes (%)	Mutated SL9 epitopes (%)
No HSC-DCs	5	22	20/22 (91%)	SLYNTVAT**F** 1/22 (4.5%) **T**LYNTVATL 1/22 (4.5%)
Untransduced HSC-DCs	2	9	8/9 (89%)	SLYNT**I**A**V**L 1/9 (11%)
CD40L-SL9 HSC-DCs	4	18	17/18 (94%)	SLYNT**I**A**V**L 1/18 (6%)

Supplementary Figure 3

Figure S3. **Virus rebound is not driven by SL9 escape mutations**. Plasma RNA and PBMC cellular RNA from unvaccinated and HSC-DC vaccinated humanized mice were reverse transcribed to generate cDNA from which amplicons spanning the HIV-1 Gag region encoding the SL9 epitope were generated by nested PCR and cloned by TOPA TA cloning. Up to five clones per mouse were sequenced and the number of WT and mutant SL9 epitopes and corresponding pie charts for plasma RNA (top) and cellular RNA (bottom) isolates are shown.

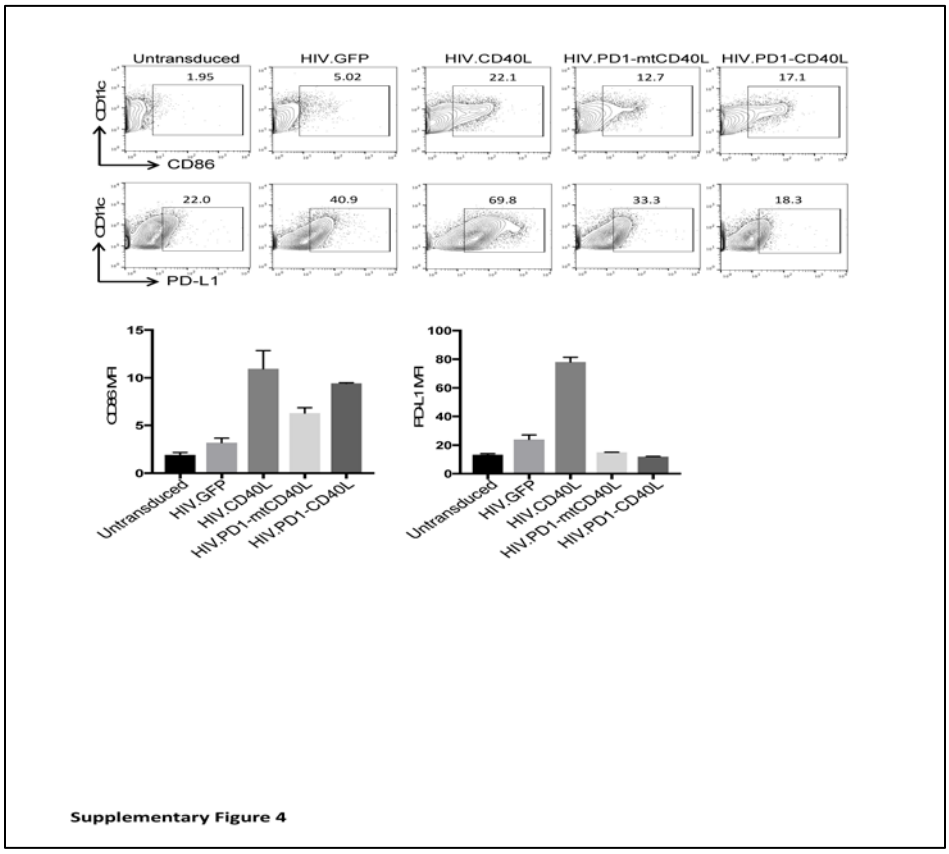

Figure S4. **Co-expression of CD40L and PD-1 microbody enhances HSC-DC maturation while masking PD-L1**. HSC-DCs were untransduced or transduced with HIV.GFP, HIV.CD40L, HIV.PD1-mtCD40L or HIV.PD1-CD40L vectors and 72 h later the frequency of CD86+ or PD-L1+ CD11c+ cells was measured by flow cytometry. Representative blots are shown (top). Pooled data showing the CD86 and PD-L1 MFIs of CD11c+ cells derived from two different aliquots of donor CD34+ HSCs that were separately differentiated to HSC-DCs and transduced with the lentiviral vectors are shown (bottom). Data represent mean ± SEM.

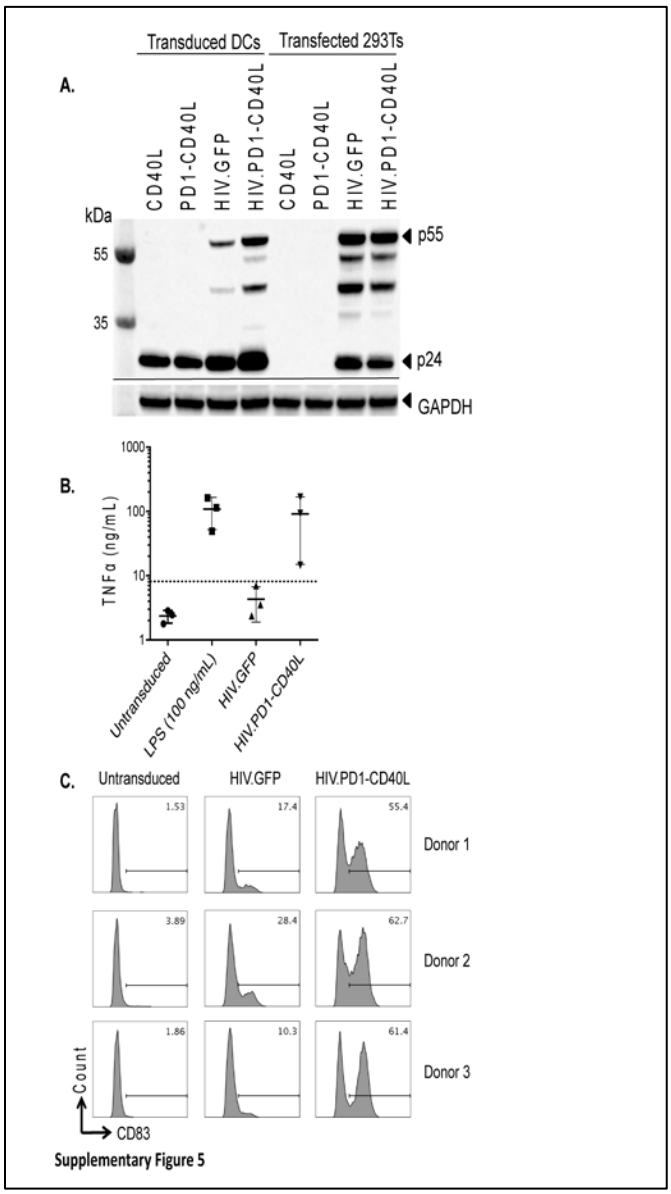

Figure S5. **HIV.PD1-CD40L-transduced MDDCs express HIV proteins and mature and become activated**. (**A**) Donor MDDCs were transduced with CD40L, PD1-CD40L, HIV.GFP or HIV.PD1-CD40L vectors (left) or transfected with the corresponding plasmids (right). At 72 h post-transduction or post-transfection, Gag protein and GAPDH in the cell lysates was detected by western blot. (**B**) MDDCs were transduced with HIV.GFP or HIV.PD1-CD40L vectors. Untransduced and LPS-treated MDDCs served as controls. After 72 h, supernatant TNFα was quantified by cytokine bead array and flow cytometry. Pooled results from three donors are shown. Data represent mean ± SD. The dotted line indicates the limit of detection. (**C**) MDDC expression of the maturation marker CD83 is shown for each donor.