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Supplemental Information

CD46 Null Packaging Cell Line Improves Measles Lentiviral Vector Production and Gene Delivery to Hematopoietic Stem and Progenitor Cells

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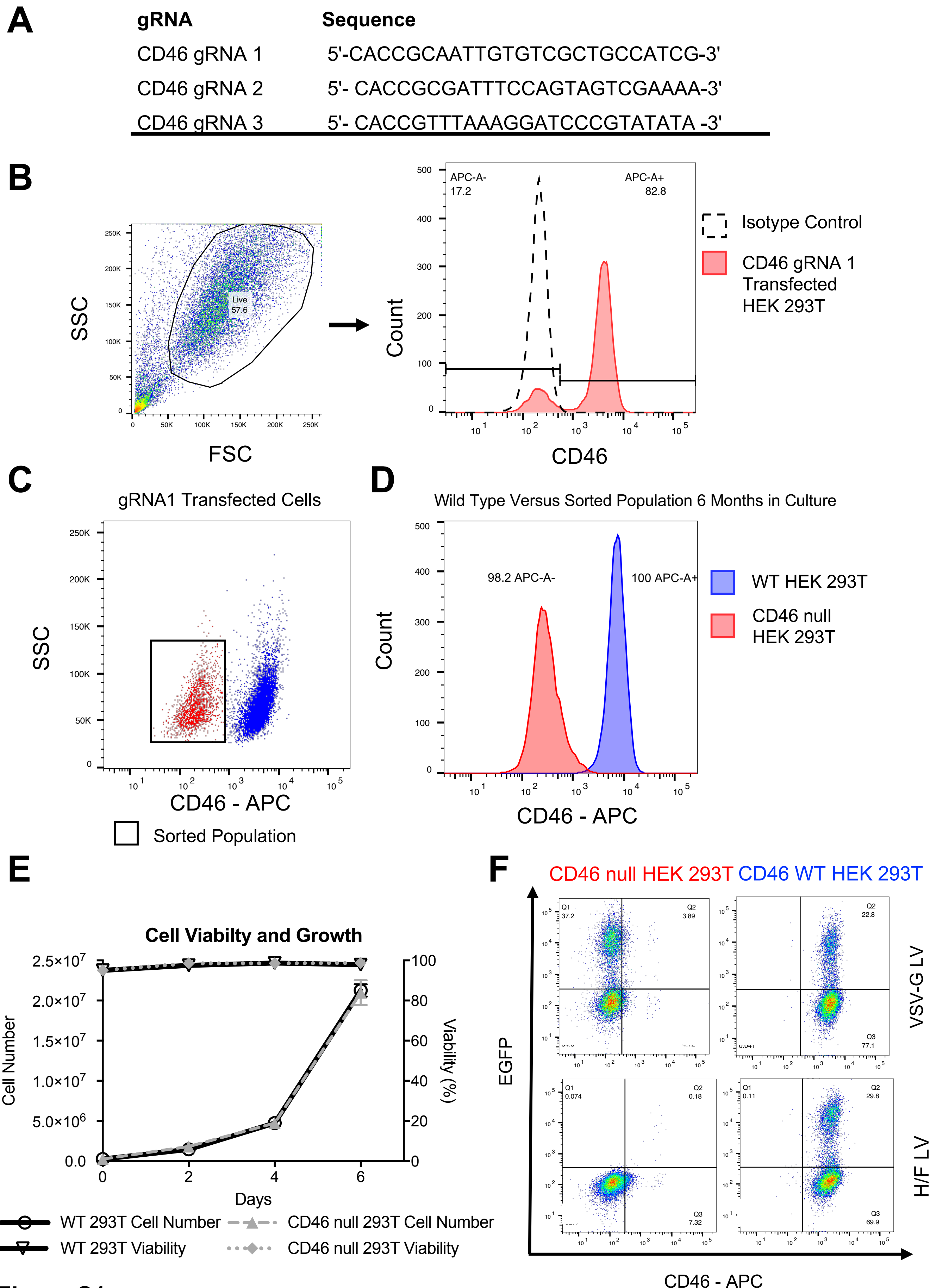
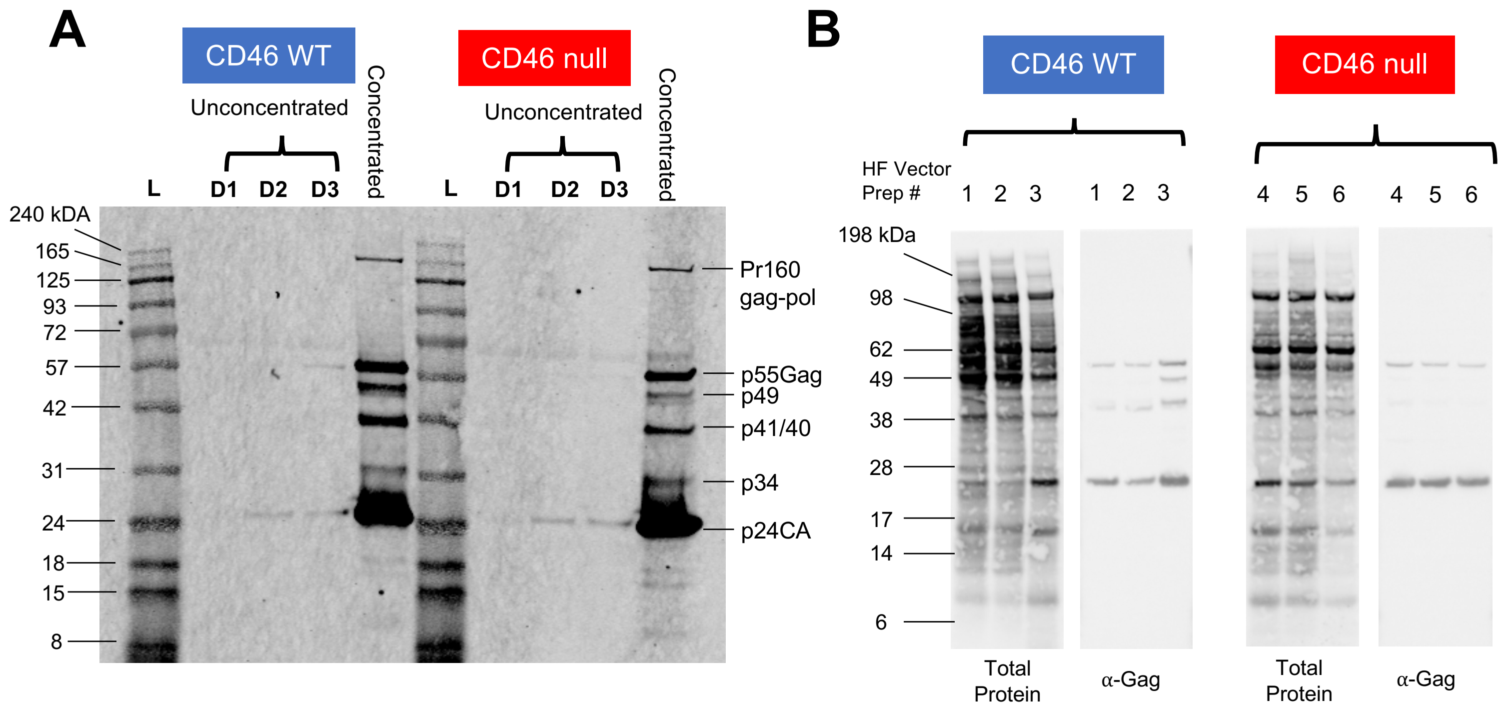


Figure S1



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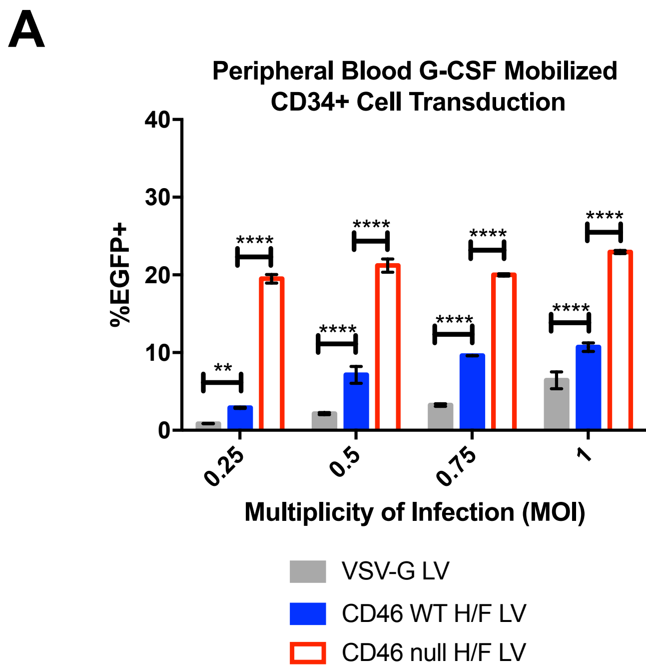
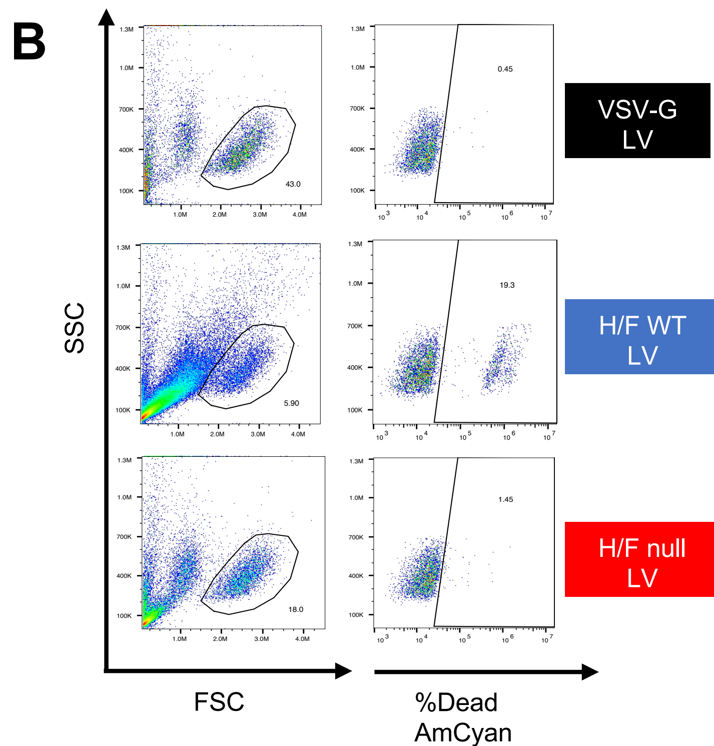
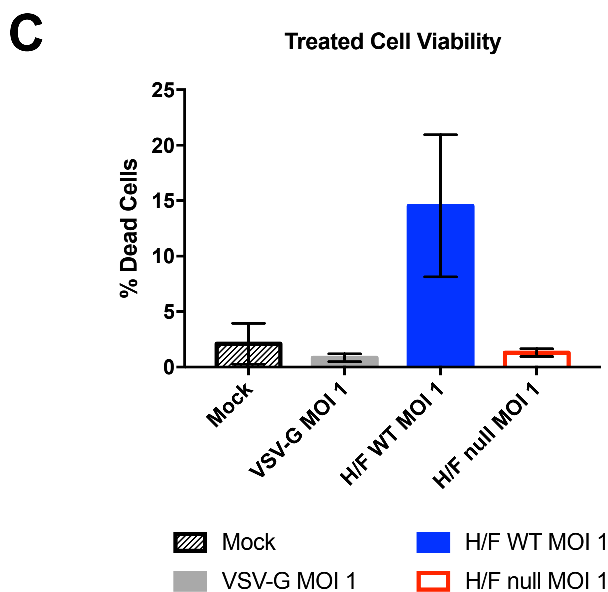
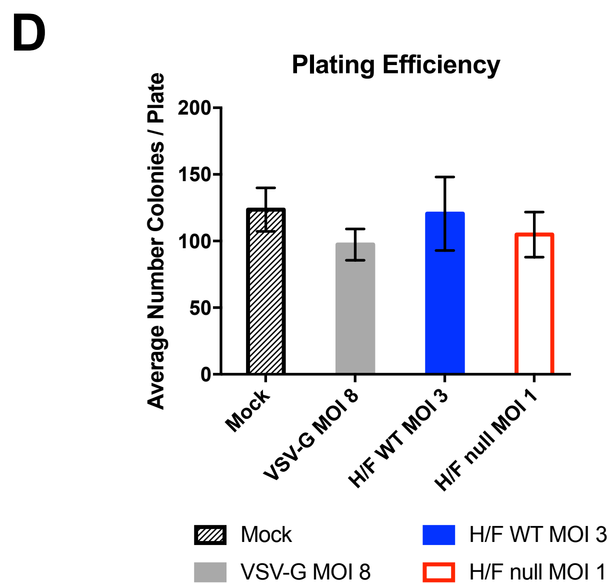
Producer Cell Type	HF LV Prep #	% p24 CA of Total Gag	%p24 CA of Total Protein
WT 293T	1	71.92	3.18
	2	63.72	2.65
	3	56.61	3.52
	Mean	64.09	3.12
CD46 null 293T	4	83.4	7.56
	5	86.78	5.56
	6	88.64	6.07
	Mean	86.27	6.40

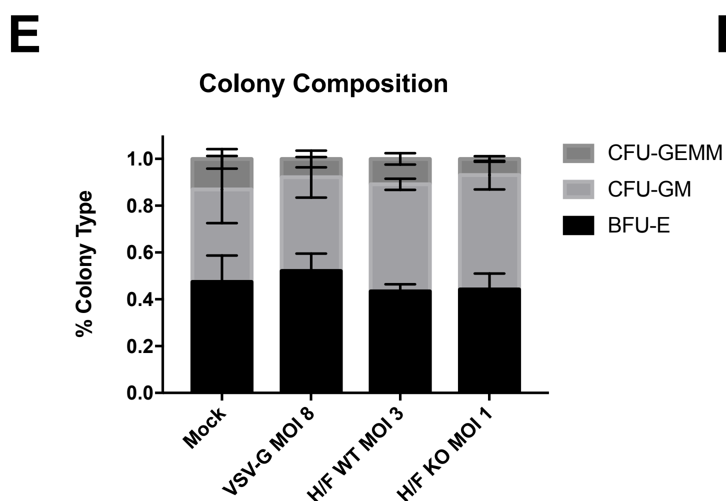
Figure S2











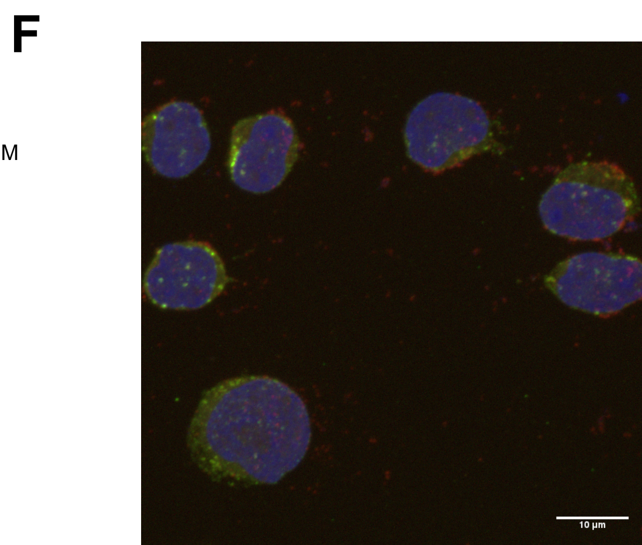


Figure S3

Figure S1. CRISPR/Cas9 knockout of CD46 on HEK 293T cells renders them resistant to H/F LV but not VSV-G LV transduction but does not alter cellular growth. (**A**) Sequences of tested guide RNAs targeting protospacer adjacent motifs in the first exon of the human CD46 gene. (**B**) HEK 293T cells transiently transfected with Cas9 + gRNA1, before evaluation by flow cytometric analysis for CD46 expression. (**C**) Fluorescence-assisted cell sorting scheme for puromycin selected Cas9/gRNA1 transfected HEK 293T cells, gating to exclude CD46 expressing cells (blue) and isolate CD46 null cells (red). (**D**) Wild-type (blue) and CD46 null sorted cells (red) maintained distinct CD46 expression profiles after 6 months of continued culture. (**E**) Wild-type and CD46 null HEK 293T cells demonstrate identical viability and proliferation profiles. (**F**) Representative FACS plots indicating transduction of CD46 null and wild-type HEK 293T cells by VSV-G LV (MOI 10) and H/F LV (MOI 1). Both cell types showed efficient transduction by VSV-G LV, while CD46 null HEK 293Ts were not transduced by H/F LV.

Figure S2. H/F LV generated in CD46 null cells have increased purity and processed Gag content. (**A**) Representative SDS-PAGE gel of H/F LV containing supernatant from wild-type and CD46 knockout HEK 293T cell collected 24 (D1), 48 (D2) and 72 hours (D3) post-transfection. Collected supernatant was also concentrated by ultracentrifugation over 20% sucrose, before matching for total protein and visualization by anti-Gag western blot. (**B**) Three vector preps each of wild-type and CD46 null cell generated H/F LV were evaluated by SDS-PAGE, quantifying total protein and anti-Gag western blot. (**C**) Quantification of mature p24 CA protein as a percent of total protein and total Gag signal in vector preps generated in wild-type or CD46 null producer cells.

Figure S3. Improved transduction of peripheral blood mobilized CD34+ cells, reduced cytotoxicity and no difference in UCB CD34$^+$ colony forming potential or type after transduction with CD46 null H/F LV. (**A**) G-CSF mobilized peripheral blood-derived CD34$^+$ cells were transduced with either H/F LV derived from WT or CD46 null HEK 293T cells or VSV-G LV. Vector application was matched for MOI determined by serial dilution of vector on HEK 293T cells. Data presented as bar graphs (mean ± s.d.). Statistical analysis using 2-way ANOVA with Tukey's multiple comparisons test; ns, not significant *$p<0.0332$, **$p<0.0021$, ***$p<0.0002$, ****$p<0.0001$. (**B**) UCB–derived CD34$^+$ HSPCs were treated with VSV-G LV, H/F LV derived from WT or CD46 null HEK 293T cells matched for MOI and evaluated for cell viability by flow cytometry 24 hours later. (**C**) Quantification of cell viability from flow cytometry analysis described above (n=2 donors, mean ± s.d.). (**D**) Methocult colony-forming unit assay for UCB-derived CD34$^+$ cells transduced with VSV-G LV, wild-type HEK 293T produced H/F LV and CD46 null HEK 293T produced H/F LV show no difference in plating efficiency or (**E**) colony distribution for any vector treatment system, evaluated 14 days after plating (n=3). CFU-GEMM, colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte; CFU-GM, colony forming unit-granulocyte, monocyte; BFU-E, erythroid burst-forming units. Data presented as bar graphs (mean ± s.d.). (**F**) H/F LV generated in CD46 null HEK 293Ts show no contaminating cellular debris when applied to UCB-derived CD34$^+$ HSPCs as observed by confocal microscopy. Images taken of cells fixed 15 minutes post-vector addition, stained for EEA1+ endosomes (green), vector p24+ protein (red) and Hoescht stained for cell nuclei (blue).