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Supplemental Information

Safe and Effective Gene Therapy for Murine Wiskott-Aldrich Syndrome Using an Insulated Lentiviral Vector

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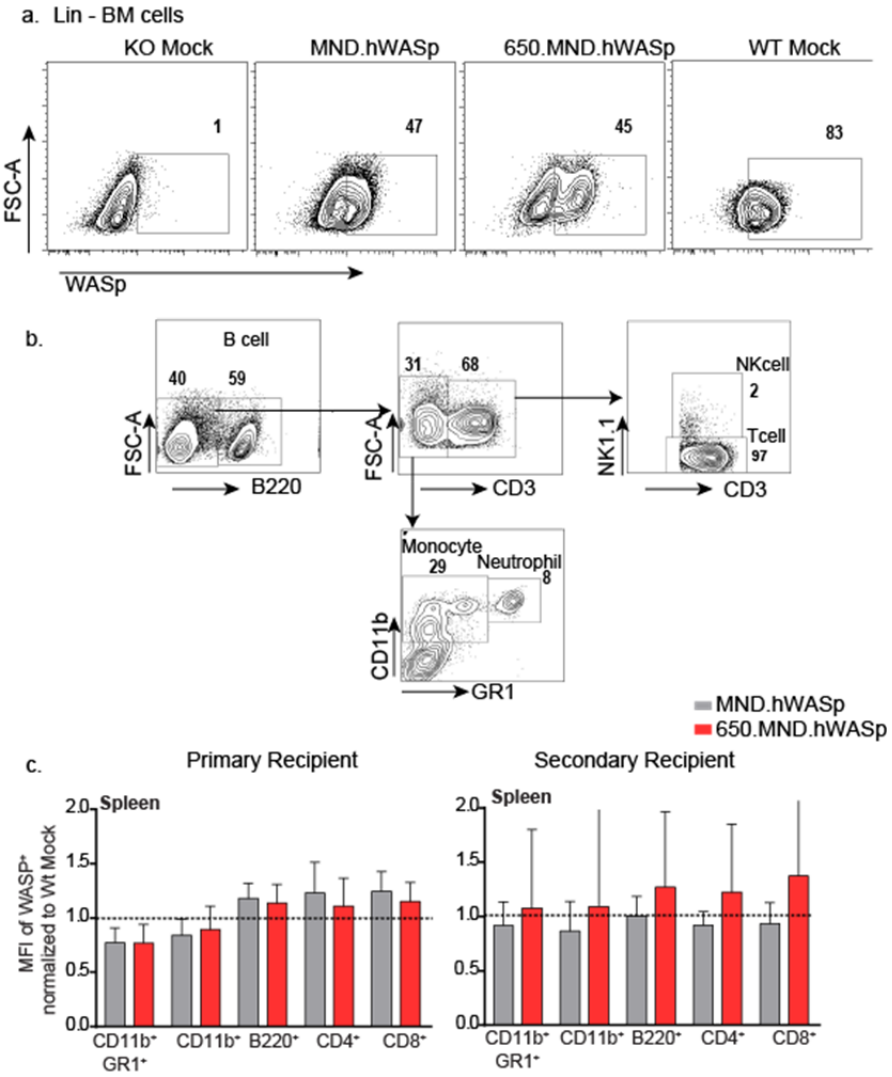

Figure S1. LV-delivered WASp expression is similar to that of endogenous *WAS* across multiple hematopoietic lineages. (**a)** Representative FACS plots of WASp expression after *in vitro* transduction of *Was*$^{-/-}$ mouse lin^{-} BM cells with either MND.hWASp or 650.MND.hWASp LV, or mock transduced *Was*$^{-/-}$ (KO) or WT lin^{-} BM cells. After transduction, cells were cultured for 7 days in StemSpan media supplemented with murine SCF and TPO cytokines. The percentage of WASp^{+} live cells is indicated. (**b)** Flow cytometry gating strategy for determining hematopoietic subsets. The same antibody panel was used for peripheral blood, BM and spleen. (**c)** Mean fluorescence intensity (MFI) of WASp^{+} cells in splenic hematopoietic cell subsets of primary and secondary recipients normalized to WT mock, shown as mean ± SD. For primary recipients, n = 3 (WT Mock), 5 (MND.hWASp), and 12 (650.MND.hWASp); for secondary recipients, n = 15 (WT Mock), 16 (MND.hWASp), and 50 (650.MND.hWASp).

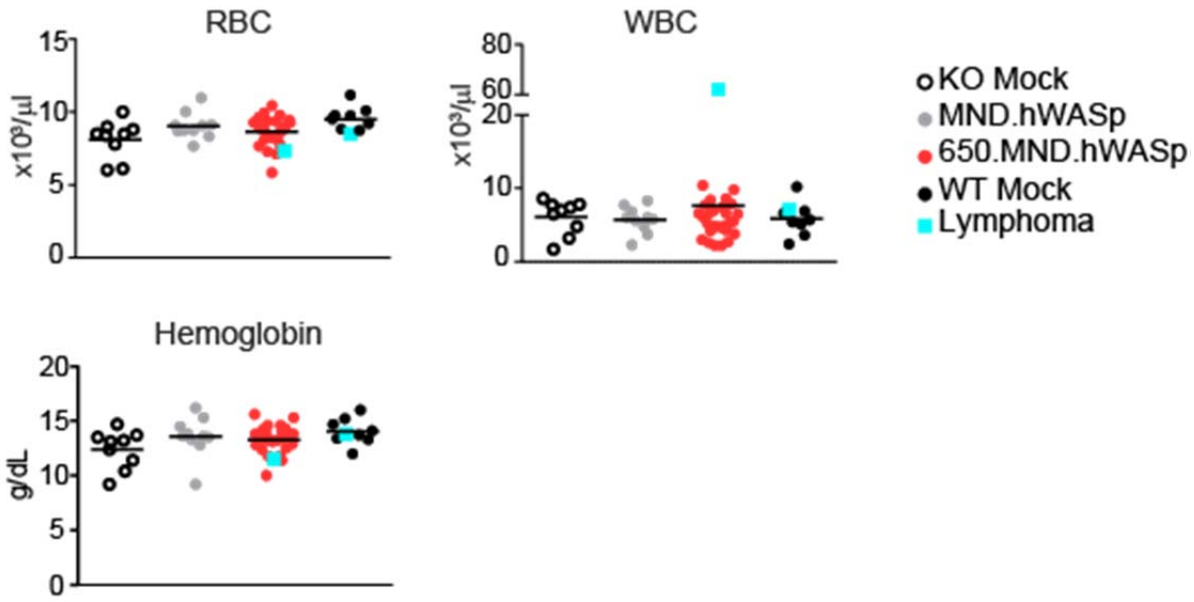

Figure S2. Peripheral blood cell counts for primary LV and control gene therapy recipients. Values from peripheral blood obtained 16 weeks post-transplant. Results are from 3 individual experiments; n =10 (KO Mock and MND.hWASp), 9 (WT Mock) and 29 (650.MND.hWASp). Mice with lymphomas are indicated in blue within their gene therapy cohort. RBC = Red blood cell; WBC = white blood cell.

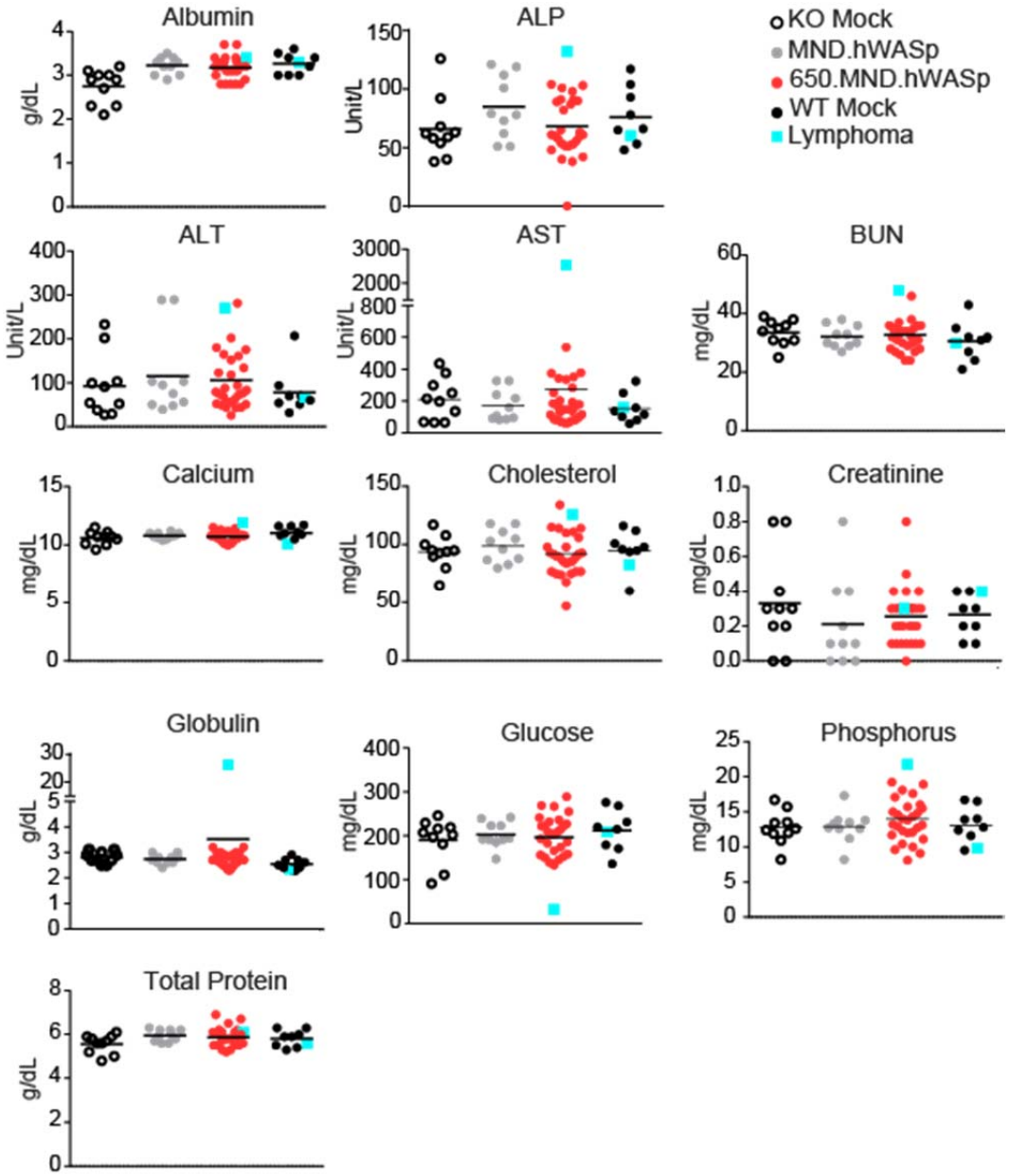

Figure S3: Serum chemistry for primary LV and control gene therapy recipients. Values from peripheral blood obtained 16 weeks post-transplant. Results compiled from 3 individual experiments; n =10 (KO Mock and MND.hWASp), 9 (WT Mock) and 29 (650.MND.hWASp). Mice with lymphomas are indicated in blue within their gene therapy cohort. ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen.

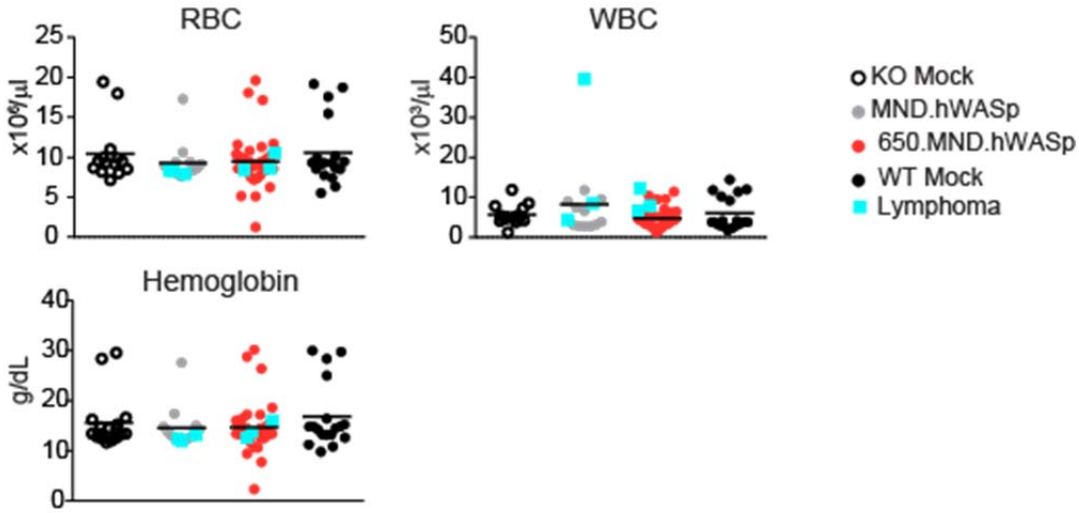

Figure S4. Peripheral blood cell counts for secondary LV and control gene therapy recipients. Values from peripheral blood obtained 16 weeks post-transplant. Results compiled from 3 individual experiments; n = 14 (KO and MND.hWASp), 18 (WT Mock) and 52 (650.MND.hWASp). Mice with lymphomas are indicated in blue within their gene therapy cohort. RBC = Red blood cell; WBC = white blood cell.

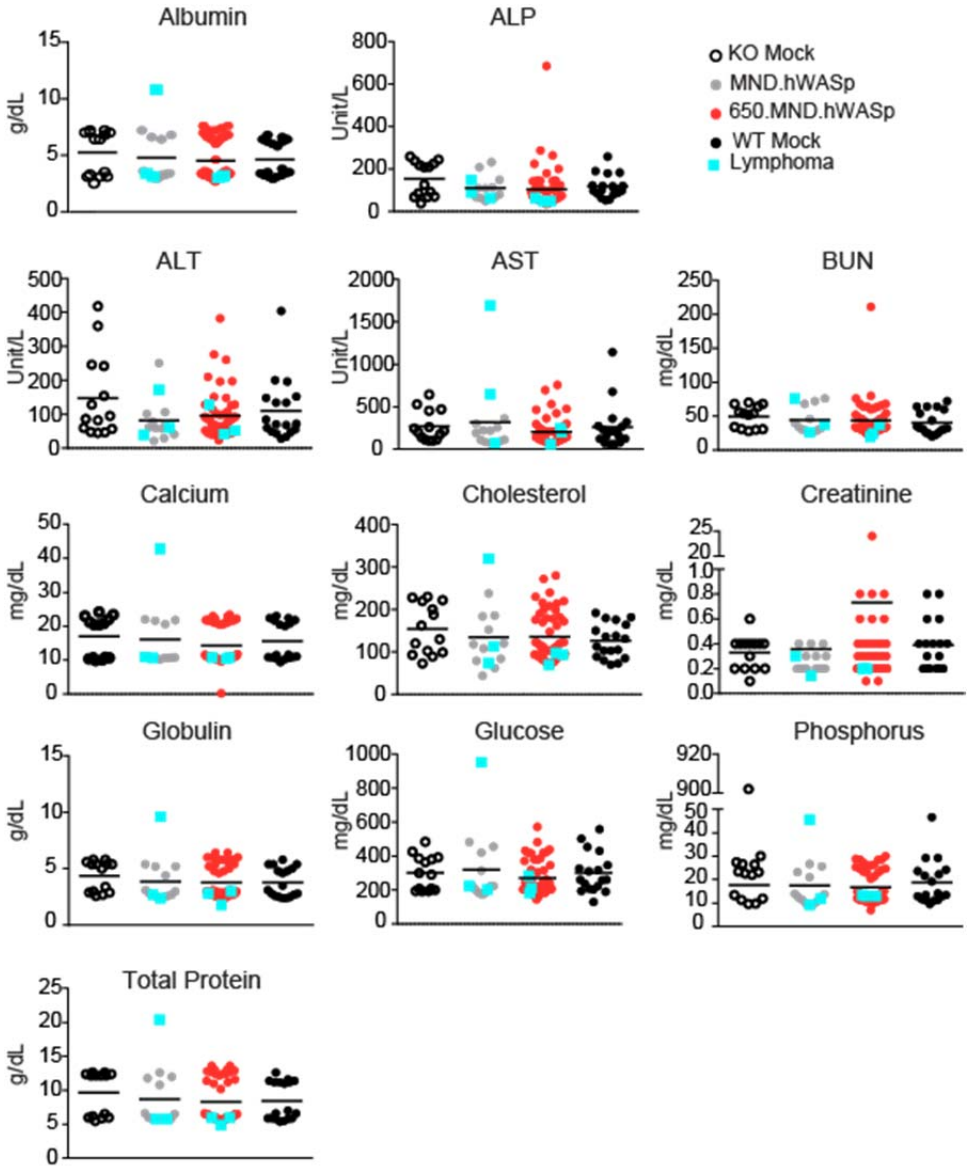

Figure S5. Serum chemistry for secondary LV and control gene therapy recipients. Values from peripheral blood obtained 16 weeks post-transplant. Results compiled from 3 individual experiments; n = 14 (KO and MND.hWASp), 18 (WT Mock) and 52 (650.MND.hWASp). Mice with lymphomas are indicated in blue within their gene therapy cohort. ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen.

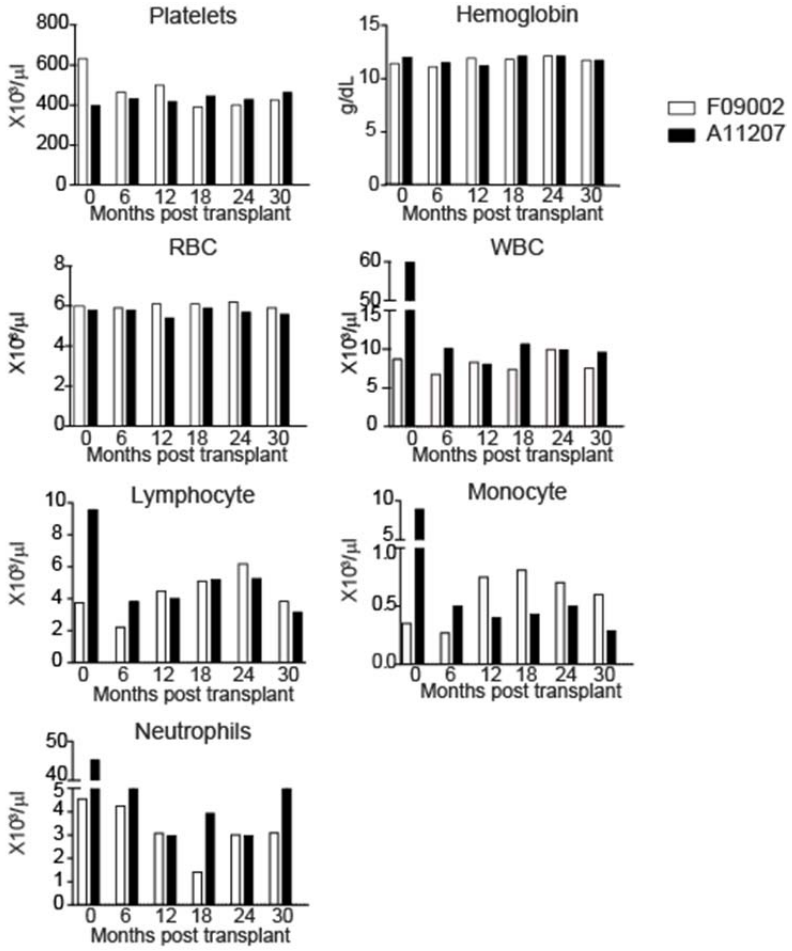

Figure S6. Peripheral blood cell counts for non-human primates F09002 and A11207. Time course of cell counts on the indicated month post-transplant with gene therapy treated cells. RBC = Red blood cell; WBC = white blood cell.

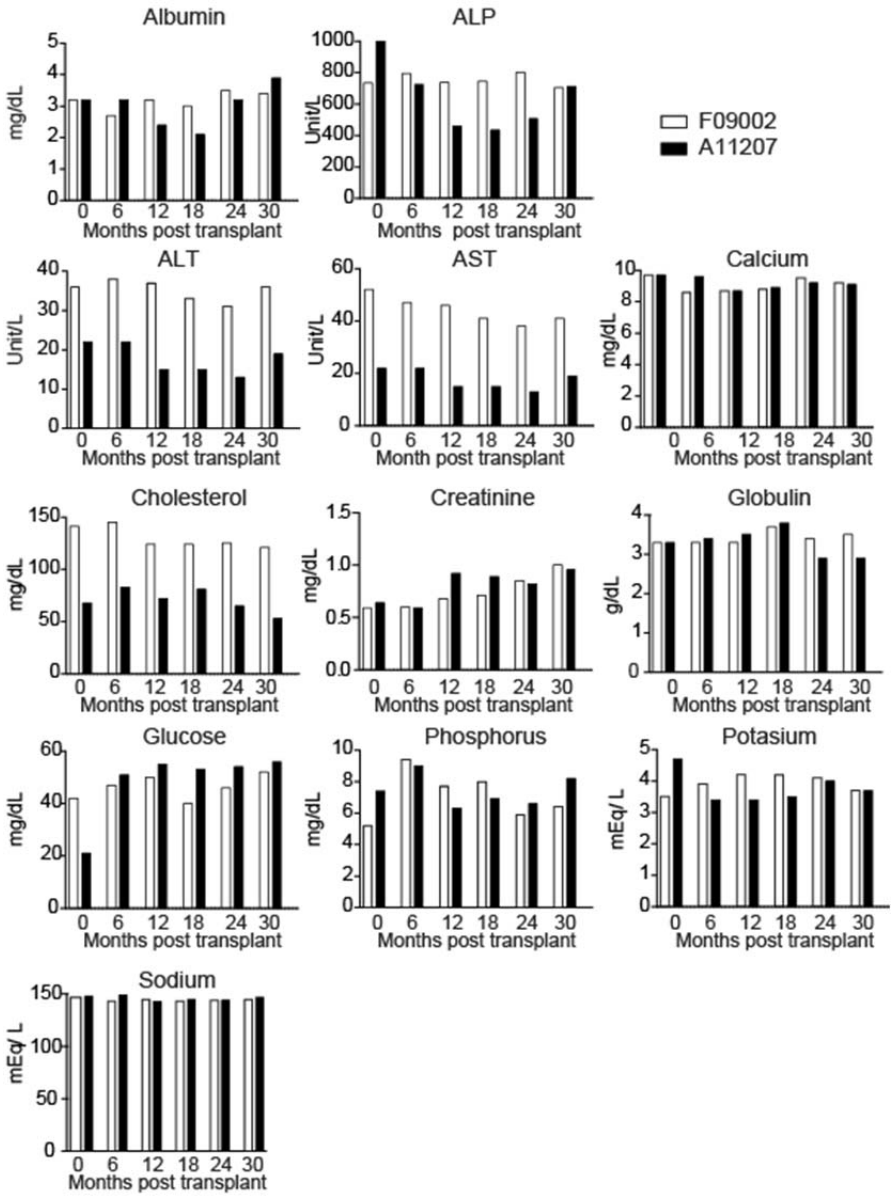

Figure S7. Serum chemistry for non-human primates F09002 and A11207. Time course of serum chemistry on the indicated month post-transplant with gene therapy treated cells. ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen.

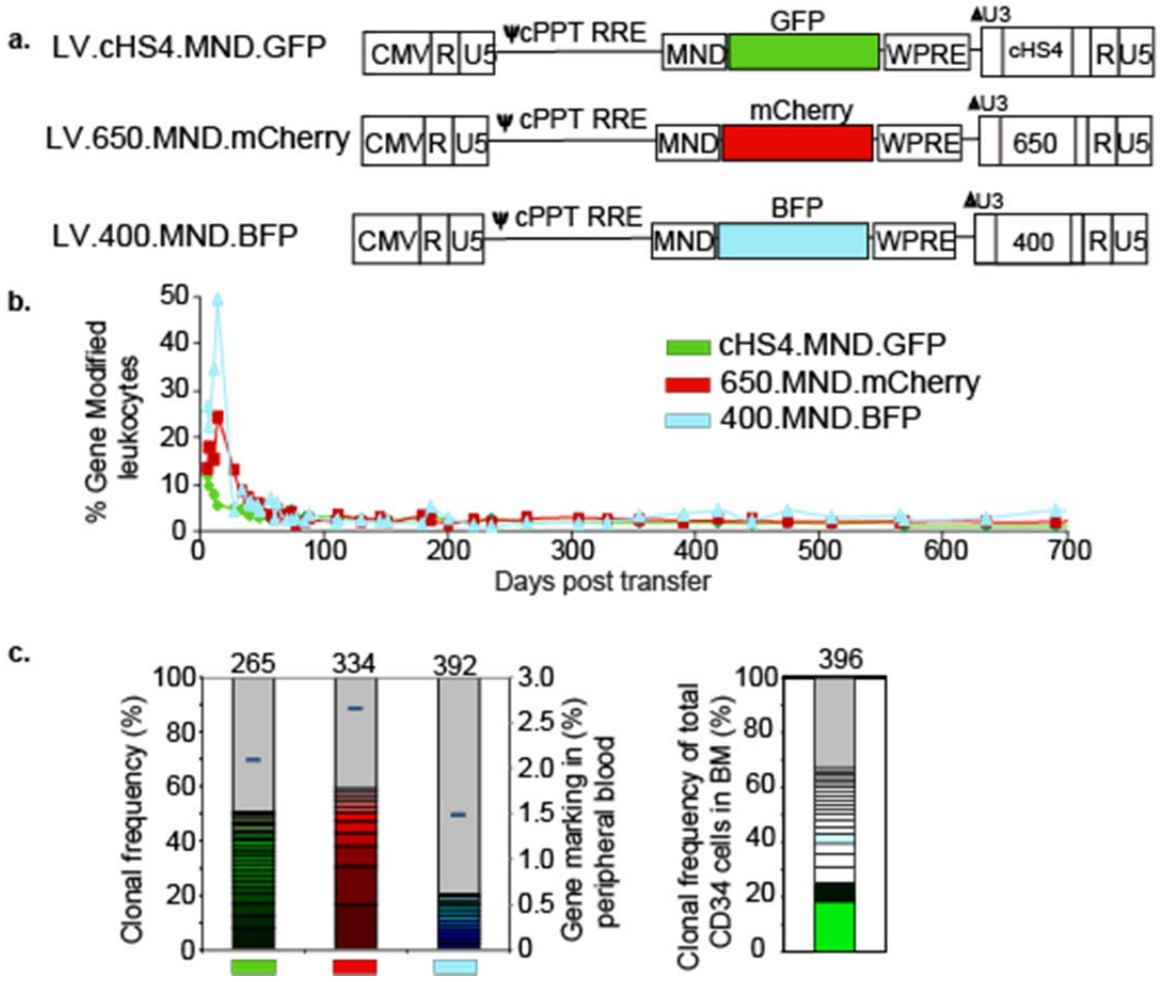

Figure S8. Direct comparison of expression and polyconality of insulated and non-insulated MND promoters in a non-human primate gene therapy model. (a) Schematic diagram of the SIN-LV vectors used for this study. All LV vectors were based on the SIN LV pCL backbone. *Macaca nemestrina* #A11207 was transplanted with bone marrow transduced with three different SIN-LV MND-fluorochrome expression cassettes, each insulated using a distinct fragment of the 1.2 kb cHS4 chromatin insulating element. The GFP expression cassette was insulated by the full-length insulator, mCherry was insulated by the 650 bp cHS4 fragments, and BFP was insulated by a 400 bp fragment as described (Arumugon et at, Wielgosz et al). **(b)** Plot shows % lentivirus transduced leukocytes in peripheral blood vs. time post-transfer. Fluorophores are from the LV in (**a**) and were detected using flow cytometry. **(c)** Clonal diversity of sorted fluorochrome^{+} peripheral blood (*left*) and BM CD34^{+} (*right*) leukocytes in animal A11207 221 days (peripheral blood) or 223 days (BM CD34+ cells) after transplant**.** The thickness of the colored bars indicates the frequency of a particular clone; clones in the gray shaded area are too infrequent to distinguish width. The total number of clones identified in each sample is listed at the top of the corresponding bar. The percentage of fluorochrome^{+} cells within peripheral blood (*right y-axis*) is indicated by a dark blue bar.

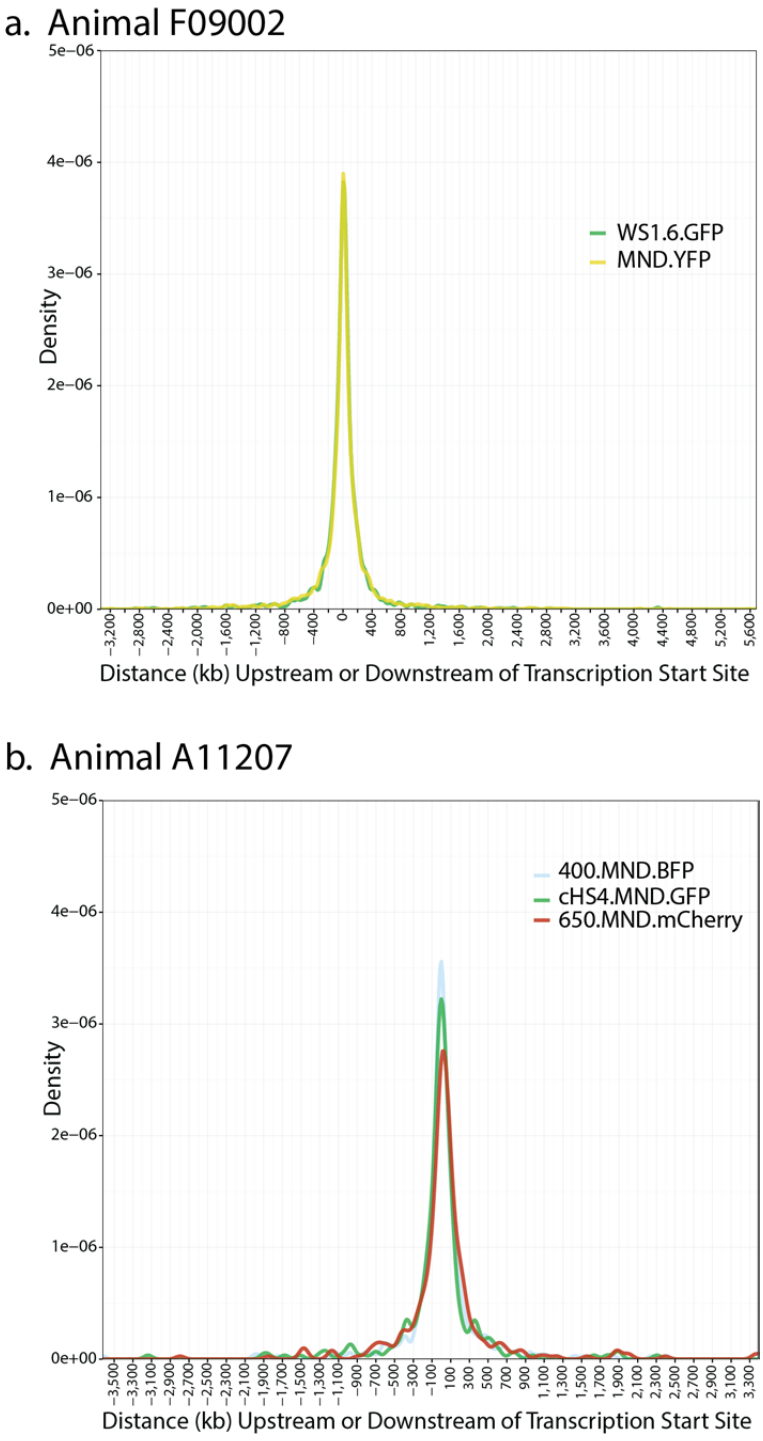

Figure S9. Integration Site Distances to Transcription Start Sites. Integration Site Distances to Transcription Start Sites. The probability density function was calculated using a Kernel Density Estimation (*geom_density* function of the *ggplot2 R* package) of distance between integration site and transcription start site for each fluorescent protein (colored) in subject (**a**) F09002 and (**b**) A11207. The area under the curve sums to 1. The probability of observing an integration site in any given window is given as the area under the curve between those two points on the x axis. Upstream and downstream are relative to the direction of transcription.

Group	Experimentally Induced Background Lesions	Primary Mice Total/Affected	Secondary Mice Total/Affected
650 MND hWAS-M	Rad-Pneumonitis	20/19	44/37
	Rad-Glomerulopathy	20/20	44/44
	Rad-Testicular Atrophy	20/19	44/44
	Collection trauma	20/18	44/40
650 MND hWAS-F	Rad-Pneumonitis	10/9	9/8
	Rad-Glomerulopathy	10/9	9/8
	Rad-Ovarian Atrophy	10/10	9/8
	Collection trauma	10/9	9/7
KO Mock-M	Rad-Pneumonitis	7/7	3/3
	Rad-Glomerulopathy	7/7	3/3
	Rad-Testicular Atrophy	7/7	3/3
	Collection trauma	7/7	3/3
KO Mock-F	Rad-Pneumonitis	3/2	11/8
	Rad-Glomerulopathy	3/3	11/11
	Rad-Ovarian Atrophy	3/1	11/11
	Collection trauma	3/3	11/10
WT Mock-M	Rad-Pneumonitis	5/4	6/6
	Rad-Glomerulopathy	5/5	6/6
	Rad-Testicular Atrophy	5/5	6/6
	Collection trauma	5/4	6/6
WT Mock-F	Rad-Pneumonitis	4/3	9/9
	Rad-Glomerulopathy	4/3	9/9
	Rad-Ovarian Atrophy	4/4	9/9
	Collection trauma	4/2	9/7

Table S1. Background histopathology lesions identified in the murine gene therapy controls. Rad = lesions found in all cohorts receiving total body irradiation.

Days Post Transplant	Chromosome	Locus of Integration (bp)	Nearest Oncogene TSS (kb)	Nearest TSS Oncogene Name	IS Genomic Feature
96	5	146057509-146057541	69.4	FBXW7	Intronic (FBXW7)
	11	62386621-62386439	33.0	WIF1	Intronic (WIF1)
221	1	60908469-60908512	1210.8	JUN	Intergenic
	4	37524395-37524458	142.1	HMGA1	Intergenic
	7	144707316-144707370	124.2	GTF2A1	Intronic (TSHR)
	8	95057086-95057034	128.0	NBN	Intergenic
	11	63162615-63162678	38.0	HMGA2	Intronic (HMGA2)
	12	60750713-60750671	68.4	SF3B1	Intergenic
	13	132794273-132794169	110.7	ERCC3	Intergenic
	13	47318184-47318119	171.7	FBXO11	Intergenic
	13	61658259-61658311	51.5	XPO1	Intergenic
	15	89202976-89203017	1652.1	GNAQ	Intergenic
	20	4182934-4182988	202.3	CREBBP	Intergenic
	X	154605195-154605319	29.8	MTCP1NB	Intronic (MTCP1)
392	5	146057509-146057541	69.4	FBXW7	Intronic (FBXW7)
	7	129555065-129554970	25.7	GPHN	Intronic (GPHN)
	11	48069006-48069149	3.4	ATF1	Intronic (ATF1)
	14	114860698-114860772	194.0	ZBTB16	Intergenic
	16	32431104-32431030	99.4	CDK12	Intergenic
	16	62004778-62004811	58.0	DDX5	Intergenic
	20	65374479-65374403	62.8	CBFB	Intronic (CBFB)

Table S2. Lentivirus insertion sites associated with known oncogenes for animal A11207. Genes proximal to the integration sites identified at different time points (96, 221 and 392 days after transplant) are shown. Yellow highlight shows clones with identical integration sites. TSS= Transcription start site, IS= integration site.

Mouse antigen:	Fluorophore	Clone	Supplier
B220	QDot 655	RA3-6B2	Life Technologies
CD11b	FITC	M1/70	eBioscience
GR-1	eFluor450	RB6-8C5	eBioscience
NK1.1	PE	PK163	Biolegend
CD3	APC-Cy7	17A2	eBioscience
CD45.2	Pe-Cy7	104	Southern BioTech
CD45.1	APC	A20	eBioscience
CD62L	APC	MEL-14	eBioscience
CD25	FITC	PC61	Biolegend
CD44	APC-Cy7	IM7	Biolegend
CD8	PE	53- 6.7	eBioscience
CD4	PerCp-Cy5.5	GK1.5	Biolegend
CD45.1	Pacific Blue	A20	eBioscience
CD23	APC	B3B4	Life Technologies
CD21	PE	7G6	BD Bioscience
CD45.1	FITC	A20	eBioscience
CD24	Pacific Blue	M1/69	Biolegend
Sca-1	PerCp-Cy5.5	D7	eBioscience
c-Kit	PE	2B8	eBioscience
CD41	FITC	MWReg30	BD Bioscience
Mouse hematopoietic lineage cocktail	eFluor450	17A2, RA3-6B2, M1/70, TER-119, RB6-8C5	eBioscience
WASp			Hans Ochs
F(ab\')2 anti-rabbit IgG	Alexa568	polyclonal	Life Technologies

Table S3. List of antibodies used for flow cytometry.