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## Supplemental Information

# Efficient Non-viral Gene Delivery into Human Hematopoietic Stem Cells by Minicircle *Sleeping Beauty* Transposon Vectors

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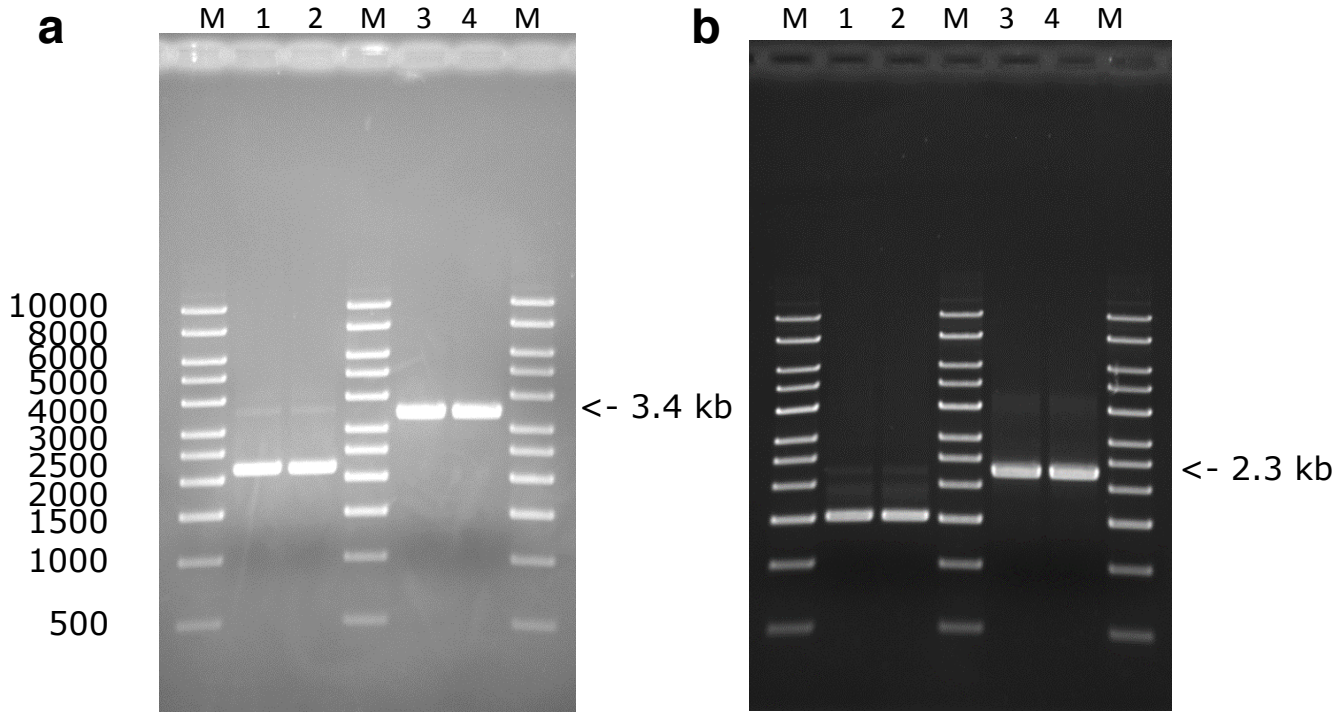
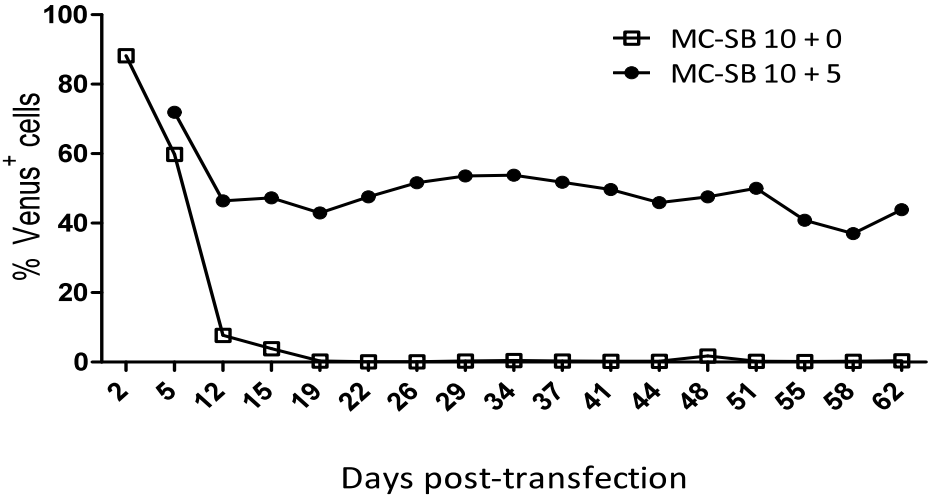
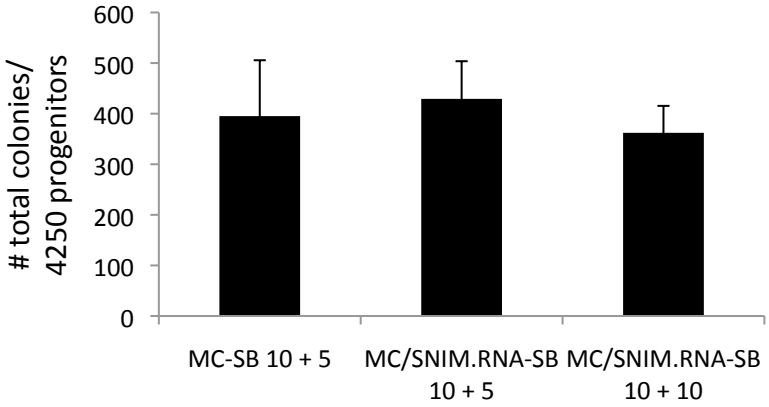


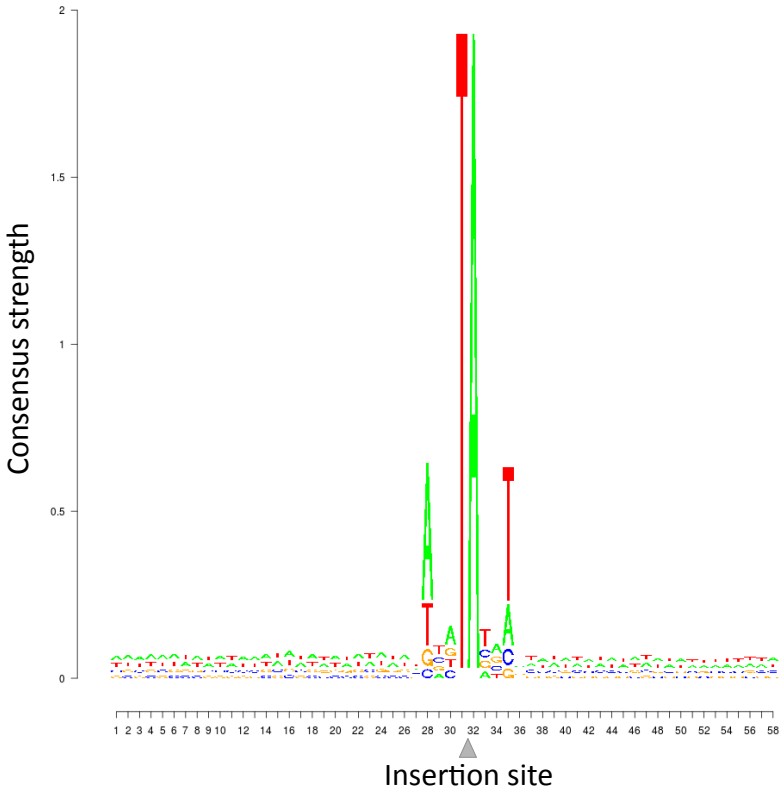

**Supplementary Figure S1. Minicircle *Sleeping Beauty* transposon vectors. a**) Agarose gel electrophoresis of the minicircle MC.T2-CAGGS-Venus (MC1390). Lanes M: 1-kb DNA size marker (PlasmidFactory Item no. MSM-865-50), lanes 1,2: undigested minicircle DNA almost monomeric supercoiled form, lanes 3,4: minicircle DNA linearized with *Pme*I (NEB) to determine the size (appr. 3.4 kb). **b**) Agarose gel electrophoresis of the minicircle MC.SB100X (MC1420). Lanes M: 1-kb DNA size marker (PlasmidFactory Item no. MSM-865-50), lanes 1,2: undigested minicircle DNA almost monomeric supercoiled form, lanes 3,4: minicircle DNA linearized with *Pac*I (NEB) to determine the size (appr. 2.3 kb).



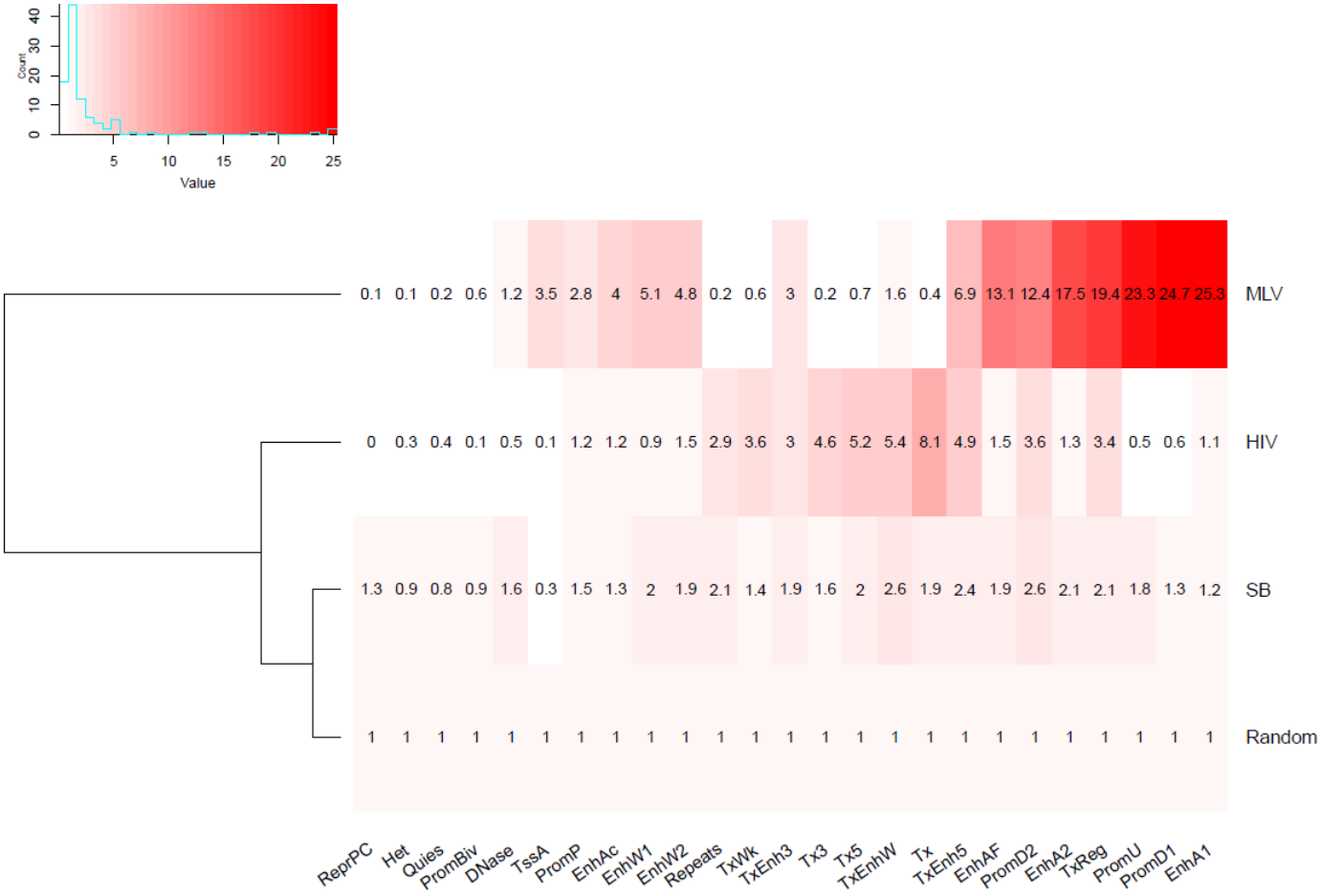

**Supplementary Figure S2. Long-term persistence of transgene expression in CD34⁺ cells following *Sleeping Beauty* transposon-mediated stable gene delivery *in vitro*.** Percentages of Venus-expressing cells after nucleofection of CD34⁺ cells with (MC-SB 10+5) or without (MC-SB 10+0) SB transposase.




**Supplementary Figure S3. Comparative numbers of colonies generated after nucleofection of CD34$^+$ cells determined by CFU assays.** Numbers of total CFUs after seeding of 4,000-4,500 purified CD34$^+$ cells following nucleofection with MC DNA only and with MC DNA/SNIM.RNA are shown. Data are expressed as means ± SEM; n = 3 per group.

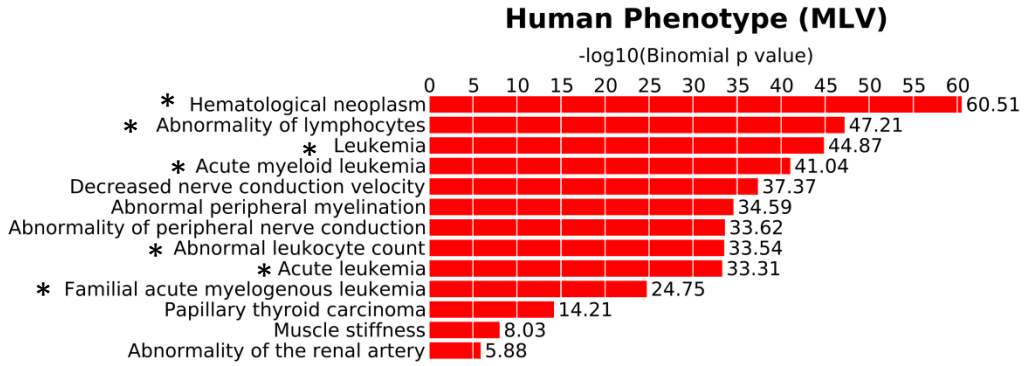


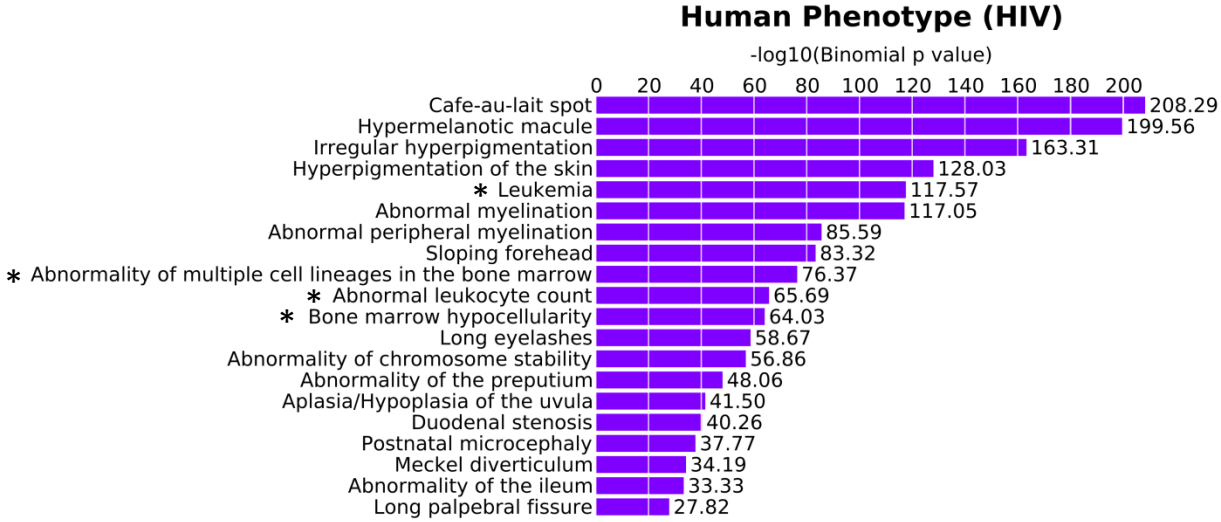

**Supplementary Figure S4. Sequence logo of *Sleeping Beauty* insertion sites in CD34$^{+}$ cells.** The figure was generated on the base frequency matrix of genomic sequences immediately flanking the SB insertion sites in HCSs using the *seqLogo* package in *R*.

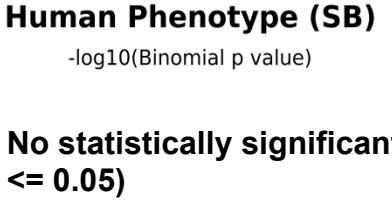



**Supplementary Figure S5. Distribution of *Sleeping Beauty* transposon, MLV-derived gammaretroviral and HIV-1-derived lentiviral integrants in a 25-state chromatin segmentation dataset of CD34⁺ cells.** Color intensities depict the degree of deviation from the expected random distribution. The cladogram was drawn on the basis of row means. The abbreviations stand for the following genomic states:

| | |
|---|---|
| TssA | Active TSS |
| PromU | Promoter Upstream TSS |
| PromD1 | Promoter Downstream TSS 1 |
| PromD2 | Promoter Downstream TSS 2 |
| Tx5 | Transcribed - 5' preferential |
| Tx | Strong transcription |
| Tx3 | Transcribed - 3' preferential |
| TxWk | Weak transcription |
| TxReg | Transcribed & regulatory (Prom/Enh) |
| TxEnh5 | Transcribed 5' preferential and Enh |
| TxEnh3 | Transcribed 3' preferential and Enh |
| TxEnhW | Transcribed and Weak Enhancer |
| EnhA1 | Active Enhancer 1 |
| EnhA2 | Active Enhancer 2 |
| EnhAF | Active Enhancer Flank |
| EnhW1 | Weak Enhancer 1 |
| EnhW2 | Weak Enhancer 2 |
| EnhAc | Primary H3K27ac possible Enhancer |
| DNase | Primary DNase |
| ZNF/Rpts | ZNF genes & repeats |
| Het | Heterochromatin |
| PromP | Poised Promoter |
| PromBiv | Bivalent Promoter |
| ReprPC | Repressed Polycomb |
| Quies | Quiescent/Low |

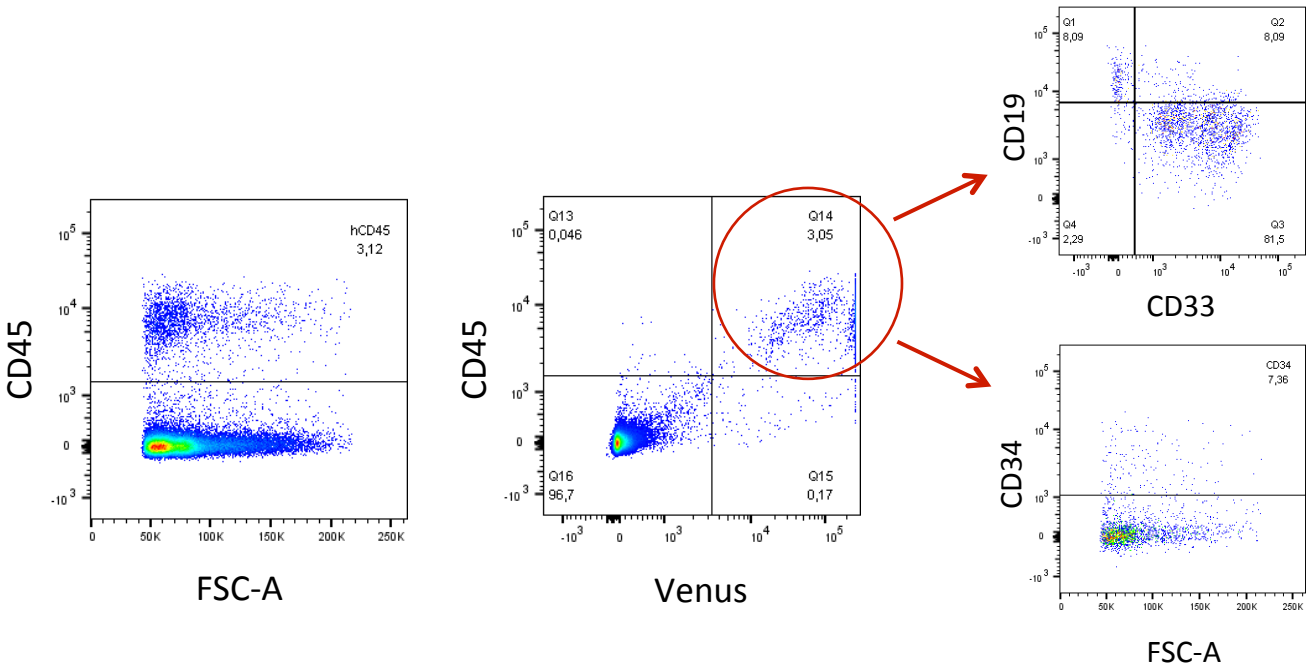



**Supplementary Figure S6. Viral gene vector insertions have a tendency to associate with genes also linked to human disease**. Shown are the statistically most significant human phenotype ontology categories in association with vector insertions within expressed genes of human HSCs. The GREAT algorithm[1] was used to calculate association based on two measures of enrichment: a binomial test over genomic regions and a hypergeometric test over genes. Any target region was considered statistically significant if the FDR Q-values for both statistical tests were <= 0.05. Asterisks mark phenotypes related to hematopoietic functions.

1. McLean CY, Bristor D, Hiller M, et al. GREAT improves functional interpretation of cis-regulatory regions. *Nature biotechnology.* 2010;28(5):495-501.




**Supplementary Figure S7:** Representative analyses showing the expression of the Venus marker gene in human myeloid (CD33), lymphoid (CD19) and CD34$^{+}$ cells engrafting NSG mice. Data correspond to one mouse transplanted with cells nucleofected with the MC/SNIM.RNA-SB constructs.