

## SUPPLEMENTARY INFORMATION

### High-Efficiency Transduction of Primary Human Hematopoietic Stem/Progenitor Cells by AAV6 Vectors: Strategies for Overcoming Donor-Variation and Implications in Genome Editing

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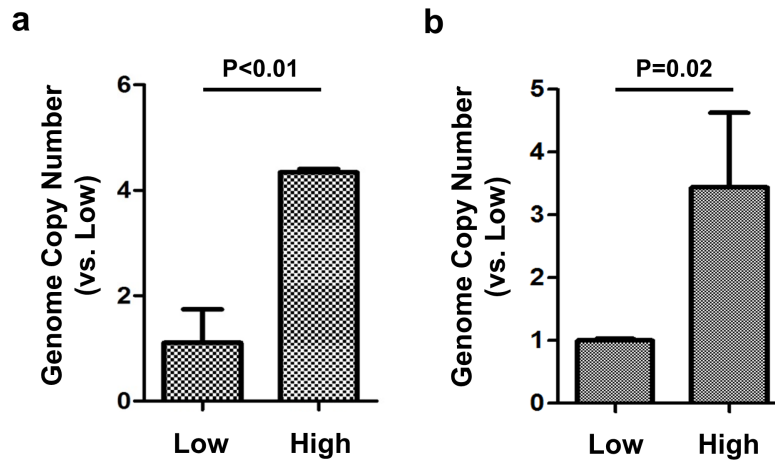
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41 **Supplementary Figures**



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**Supplementary Figure 1**

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45 **Supplementary Figure 1. AAV vector genome copy numbers.** Total DNAs were isolated from (a)  
46 K562 cells and (b) primary CD34<sup>+</sup> cells 2 hrs post-transduction with ssAAV6 vectors. The vector  
47 genome copy numbers/cell were determined by qPCR and were normalized to  $\beta$ -actin DNA  
48 copy number.

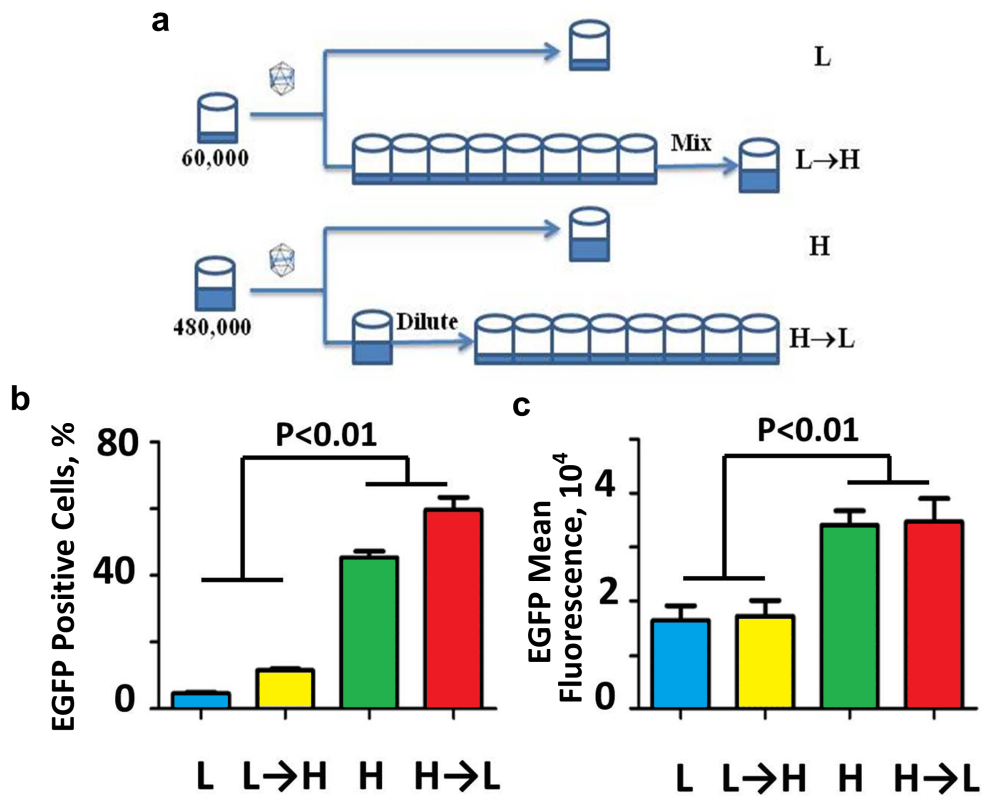
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### Supplementary Figure 2

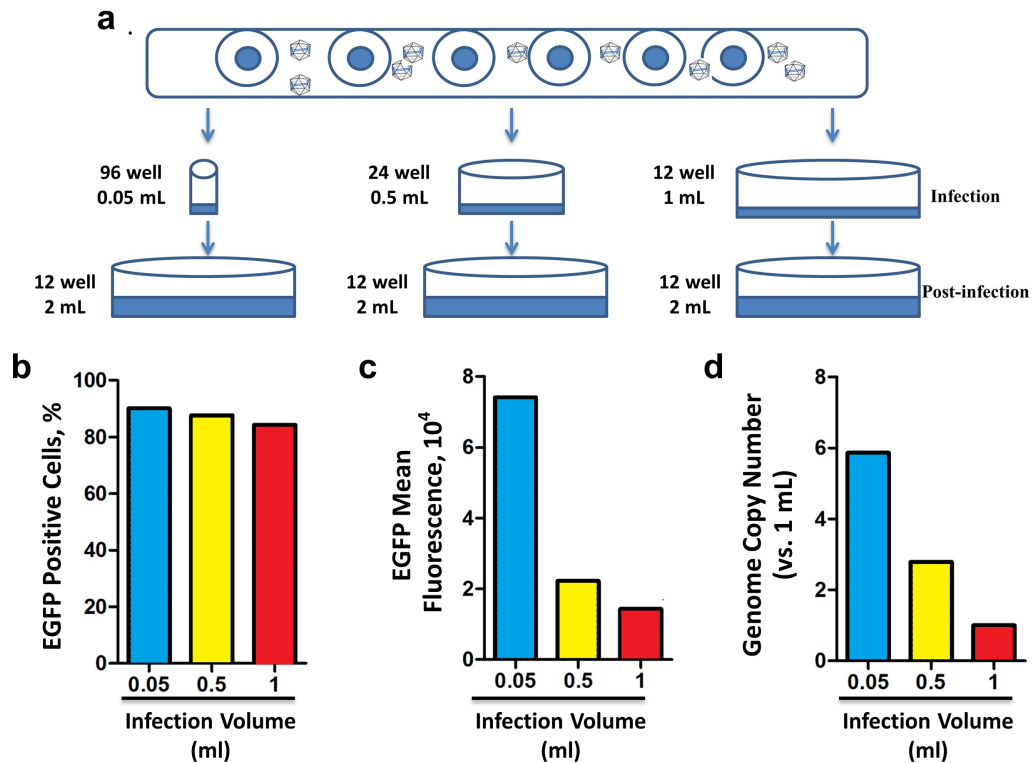
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57 **Supplementary Figure 2. Effect of initial cell-cell contact.** (a) Schematics of the experiment  
 58 design. K562 cells (60,000 cells/50  $\mu$ l) and 8 replicate cultures were transduced at low-density  
 59 (L) with TM-sCAAV6-CBAp-EGFP vectors at an MOI of 3,000 vgs/cell for 2 hrs and subsequently  
 60 pooled together and centrifuged to reach high-density (L→H), and conversely, cells (480,000  
 61 cells/50  $\mu$ l) were transduced at high-density (H), and 2 hrs post-transduction, were diluted to  
 62 low-density (H→L). (b) Transgene expression in each cell population was analyzed by  
 63 fluorescence microscopy 48 hrs post-transduction. (c) Mean fluorescence intensity of transgene  
 64 expression.

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### Supplementary Figure 3

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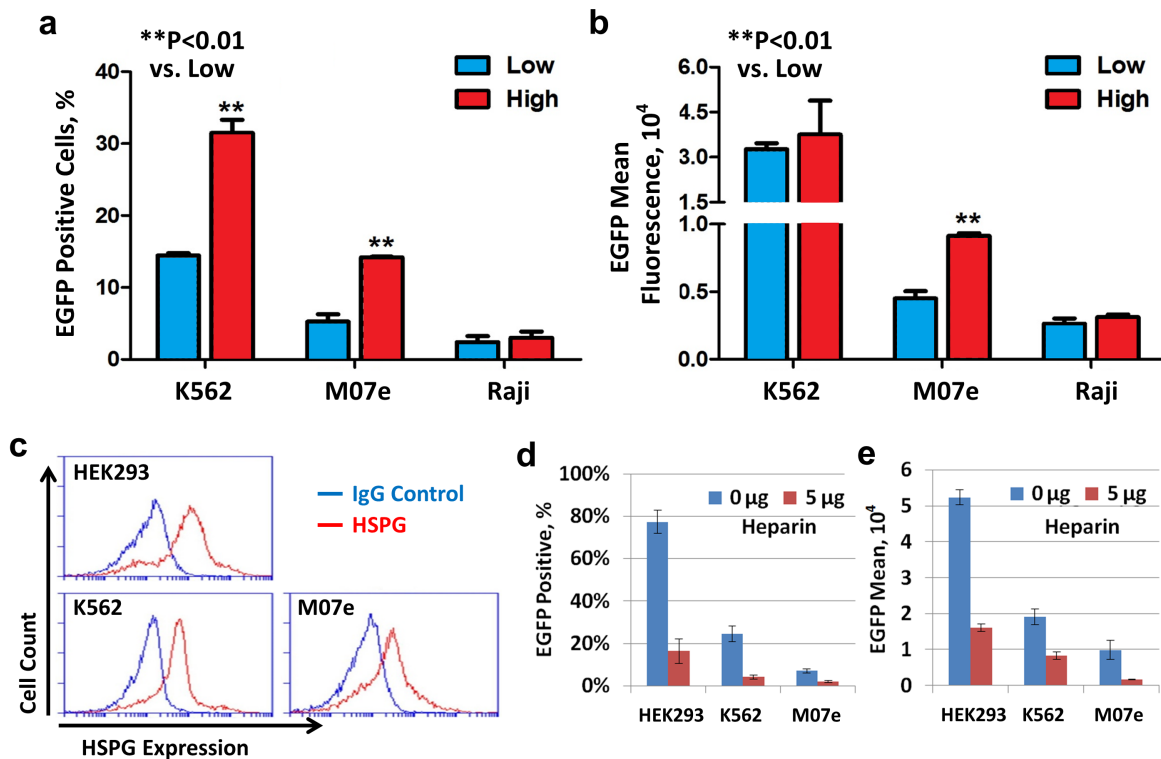
71 **Supplementary Figure 3. Effect of culture volume.** (a) Schematics of the experiment design. A  
 72 fixed number of K562 cells were transduced with viral vectors in various indicated volumes for 2  
 73 hrs and subsequently diluted to 2.0 ml. (b) Transgene expression in each cell population was  
 74 analyzed by flow cytometry 48 hrs post-transduction. (c) Mean fluorescence intensity of  
 75 transgene expression. (d) Vector genome copy numbers/cell were determined by qPCR and  
 76 were normalized to  $\beta$ -actin DNA copy number as described above.

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#### Supplementary Figure 4

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84 **Supplementary Figure 4. Transduction efficiency of AAV2 vectors in various human**  
 85 **hematopoietic cell lines at low and high cell densities. (a)** Human K562, M07e, and Raji cells  
 86 were transduced with scAAV2-CBAp-EGFP vectors at an MOI of 5,000 vgs/cell at either low  
 87 ( $1 \times 10^6$  cells/ml) or high ( $1 \times 10^7$  cells/ml) cell density. Transgene expression was analyzed by flow  
 88 cytometry 48 hrs post-transduction. **(b)** Mean fluorescence intensity of transgene expression in  
 89 each cell type. **(c)** FACS analyses of the level of expression of membrane heparan sulfate  
 90 proteoglycan in various human cell types. **(d)** Transduction efficiency of AAV2 vectors in human  
 91 cell lines in the absence or the presence of heparin. Each cell type was transduced with TM-  
 92 scAAV6-CBAp-EGFP vector at an MOI of 5,000 vgs/cell at high ( $1 \times 10^7$  cells/ml) cell density, and  
 93 transgene expression was analyzed by flow cytometry 48 hrs post-transduction. **(e)** Mean  
 94 fluorescence intensity of transgene expression in each cell type

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96 **Supplementary Table 1: Transduction efficiency of WT and mutant AAV2 and AAV6 serotype**  
 97 **vectors in primary human HSPCs from various donors.**

	WT- AAV2	WT- AAV6	TM- AAV6-	WT- AAV2	QM- AAV2-	WT- AAV6	TM- AAV6-
	Low Cell Density			High Cell Density			
<b>Donor #1</b>	ND	1.3% 5,090	ND	ND	ND	19.4% 9,080	ND
<b>Donor #2</b>	1.4%, 4,184	9.8% 6,904	ND	7.2% 5,810	31.1% 15,672	27.0% 9,994	45.2% 25,373
<b>Donor #3</b>	ND	ND	28.6%, 8,246	ND	ND	ND	58.5% 23,846
<b>Donor #4</b>	4.4%, 3,720	9.5%, 6,512	ND	11.6%, 6,686	19.6%, 13,001	23.9%, 15,434	ND

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 99 Primary human bone marrow-derived CD34<sup>+</sup> cells from various donors were transduced in  
 100 triplicate with the WT-AAV2, WT-AAV6, Y444+500+730F+T491T-AAV2 quadruple-mutant (QM),  
 101 or Y705+731F+T492V-AAV6 triple-mutant (TM) scAAV-EGFP vectors at an MOI of 3,000 vgs/cell.  
 102 Transgene expression was analyzed by flow cytometry 48 hrs post-transduction. Data are  
 103 presented as % EGFP positive cells, and EGFP mean fluorescence intensities.

104 ND = Not done.

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