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Supplemental Information

An Optimized Lentiviral Vector Efficiently Corrects the Human Sickle Cell Disease Phenotype

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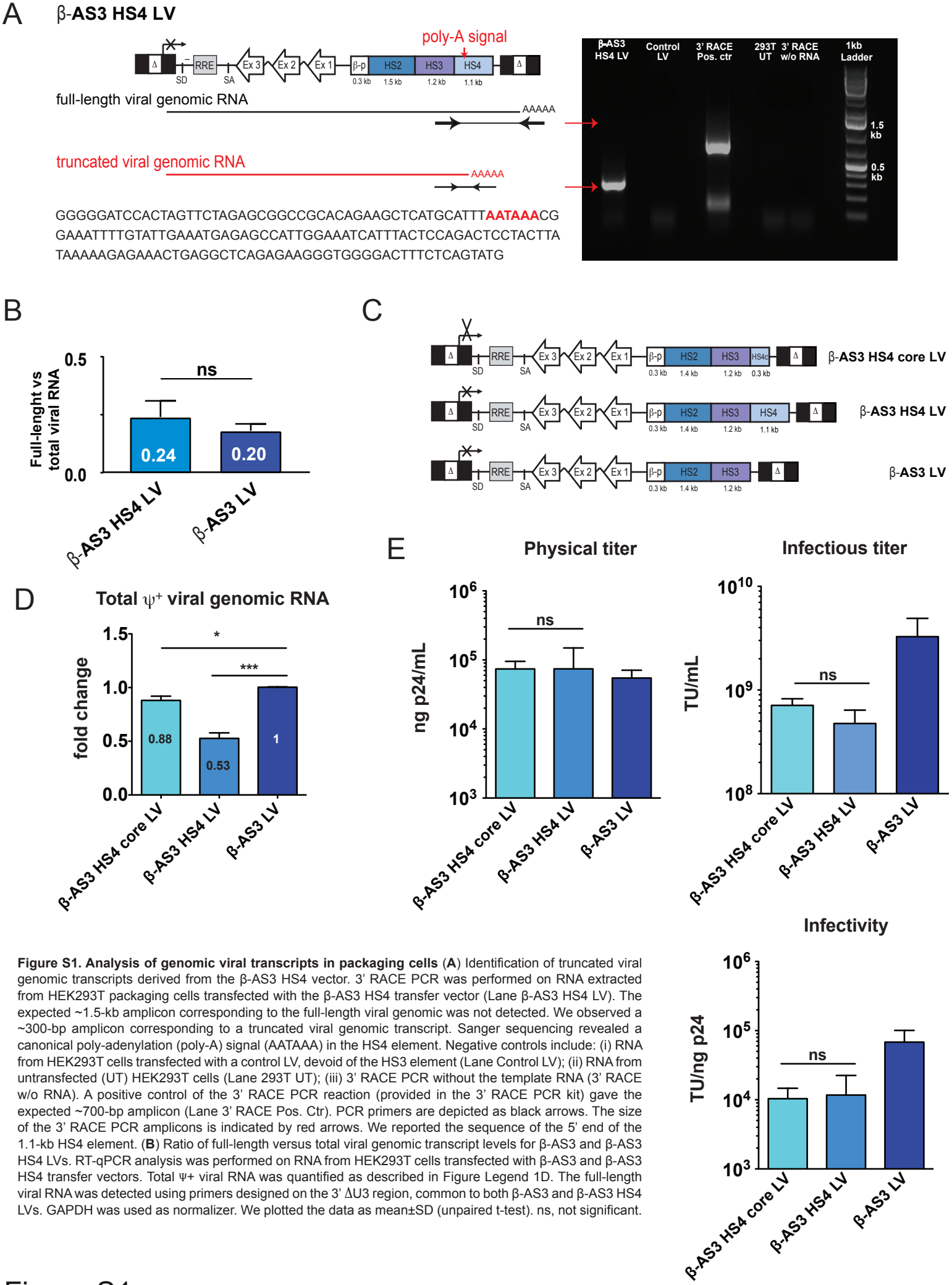


Figure S1. Analysis of genomic viral transcripts in packaging cells (**A**) Identification of truncated viral genomic transcripts derived from the β-AS3 HS4 vector. 3' RACE PCR was performed on RNA extracted from HEK293T packaging cells transfected with the β-AS3 HS4 transfer vector (Lane β-AS3 HS4 LV). The expected ~1.5-kb amplicon corresponding to the full-length viral genomic was not detected. We observed a ~300-bp amplicon corresponding to a truncated viral genomic transcript. Sanger sequencing revealed a canonical poly-adenylation (poly-A) signal (AATAAA) in the HS4 element. Negative controls include: (i) RNA from HEK293T cells transfected with a control LV, devoid of the HS3 element (Lane Control LV); (ii) RNA from untransfected (UT) HEK293T cells (Lane 293T UT); (iii) 3' RACE PCR without the template RNA (3' RACE w/o RNA). A positive control of the 3' RACE PCR reaction (provided in the 3' RACE PCR kit) gave the expected ~700-bp amplicon (Lane 3' RACE Pos. Ctr). PCR primers are depicted as black arrows. The size of the 3' RACE PCR amplicons is indicated by red arrows. We reported the sequence of the 5' end of the 1.1-kb HS4 element. (**B**) Ratio of full-length versus total viral genomic transcript levels for β-AS3 and β-AS3 HS4 LVs. RT-qPCR analysis was performed on RNA from HEK293T cells transfected with β-AS3 and β-AS3 HS4 transfer vectors. Total Ψ+ viral RNA was quantified as described in Figure Legend 1D. The full-length viral RNA was detected using primers designed on the 3' ΔU3 region, common to both β-AS3 and β-AS3 HS4 LVs. GAPDH was used as normalizer. We plotted the data as mean±SD (unpaired t-test). ns, not significant.

Figure S1

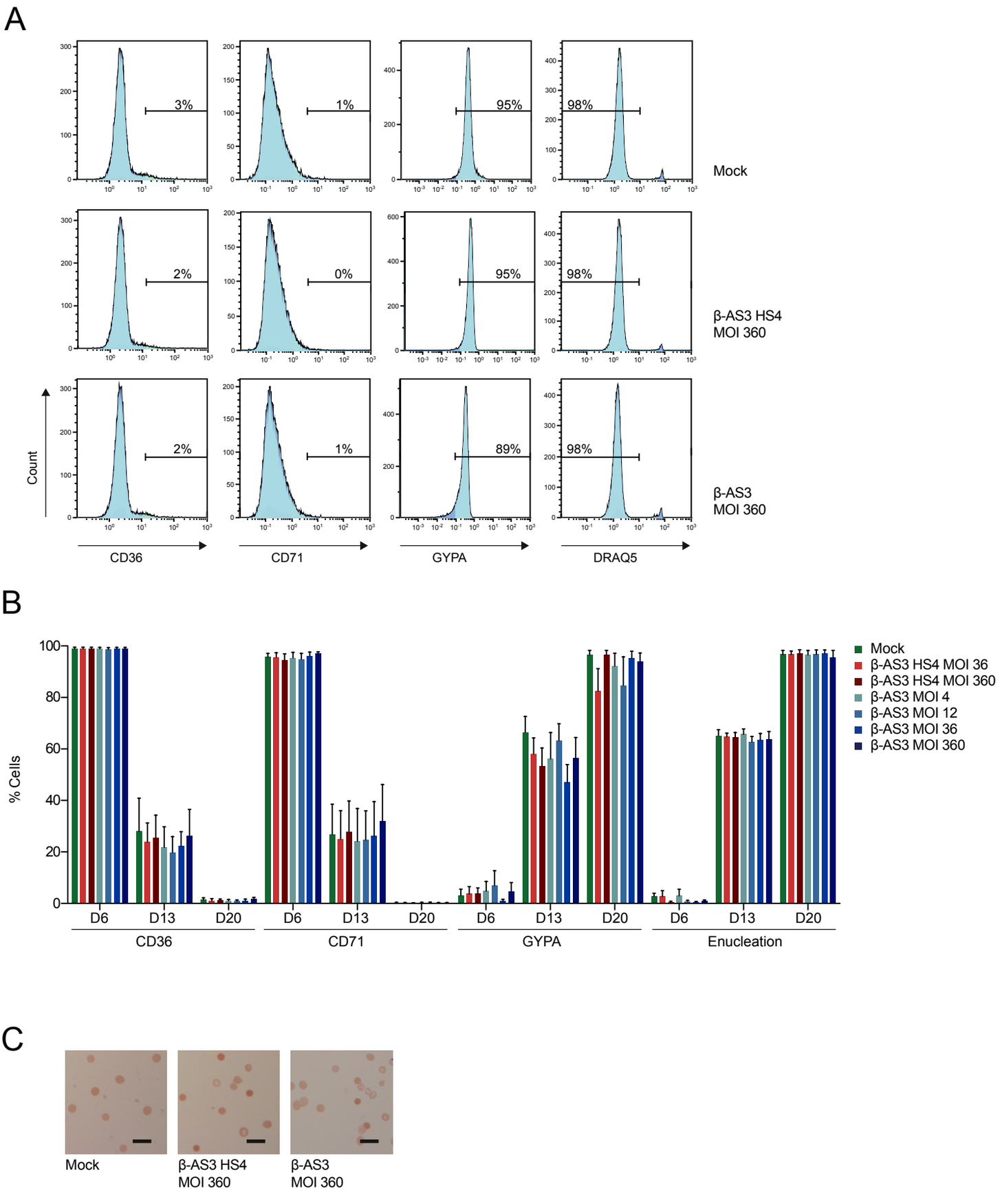


Figure S2. Erythroid differentiation of LV-transduced SCD HSPCs. (**A**) Representative FACS histograms showing the frequency of cells expressing the early erythroid markers CD36 and CD71 and the late erythroid marker Glycophorin A (GYPA), and the proportion of DRAQ5- enucleated cells. RBCs derived from mock-, β-AS3 HS4- and β-AS3- transduced SCD HSPCs were analyzed at day 20 of erythroid differentiation. (**B**) Time-course FACS analysis of CD36, CD71 and GYPA expression and enucleation in samples derived from mock-, β-AS3 HS4- and β-AS3- transduced samples. Cells were analyzed at day 6 (D6), day 13 (D13) and day 20 (D20) of erythroid differentiation. Values shown are mean±SEM of 3 experiments (n=2 donors). (**C**) Representative photomicrographs of RBCs obtained at day 20 of erythroid differentiation and stained with May-Grünwald Giemsa. Scale bars, 20 µm.

Figure S2

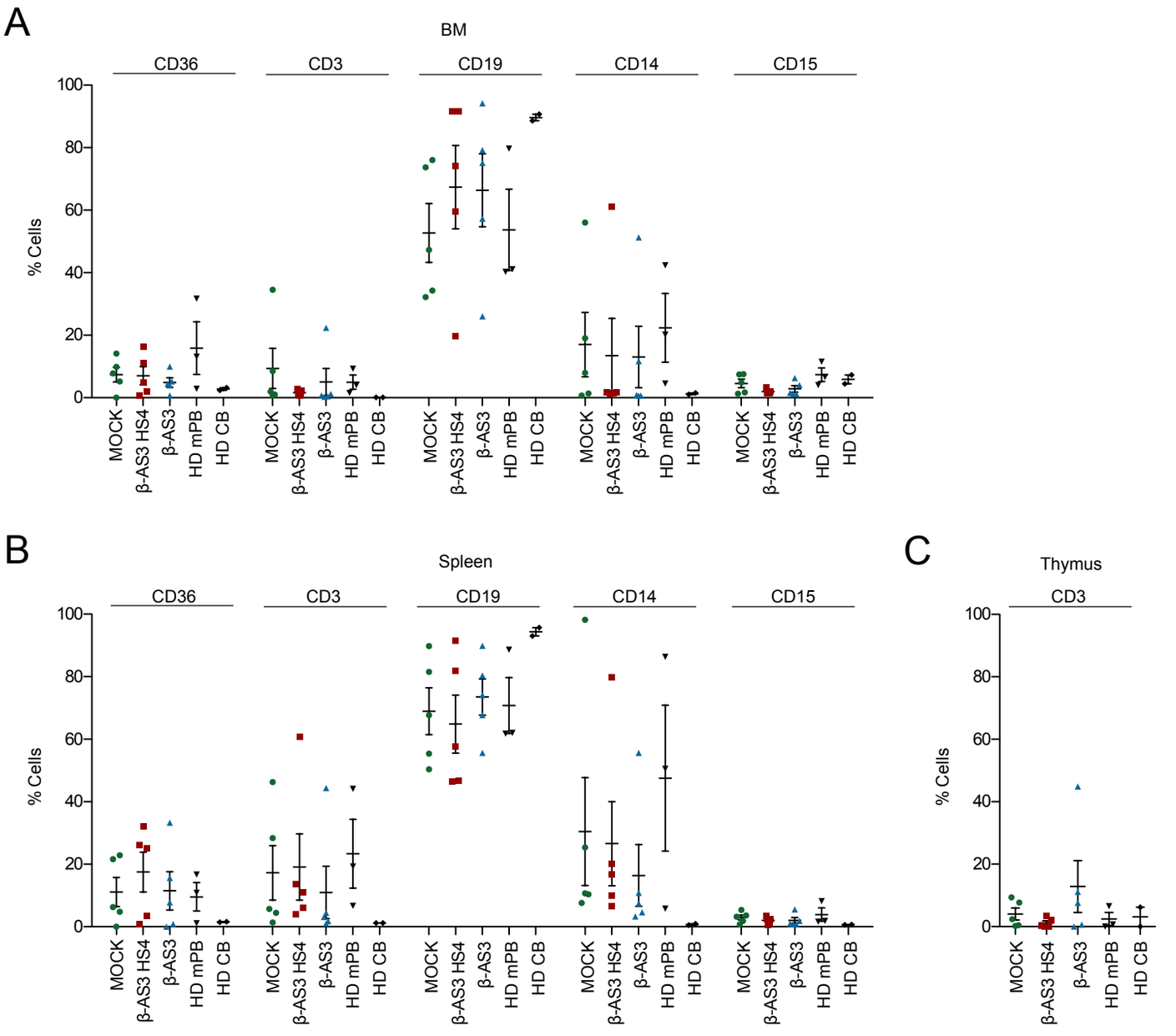
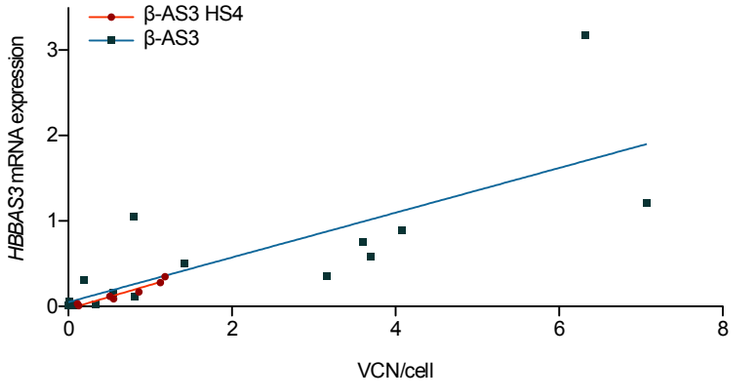


Figure S3. Human hematopoietic cell reconstitution in NSG mice transplanted with BM SCD cells. Frequency of human erythroid (CD36), T (CD3) and B (CD19) lymphoid and myeloid (CD14 and CD15) cells in BM (**A**) and spleen (**B**) of mice transplanted with mock-, β-AS3 HS4- or β-AS3-transduced CD34+ cells. (C) Percentage of CD3+ T lymphoid cells in the thymus of transplanted mice. Mice transplanted with healthy donor peripheral blood G-CSF-mobilized (HD mPB) or cord blood (HD CB) HSPCs were used as controls. Each data point represents an individual mouse.

Figure S3

A



B

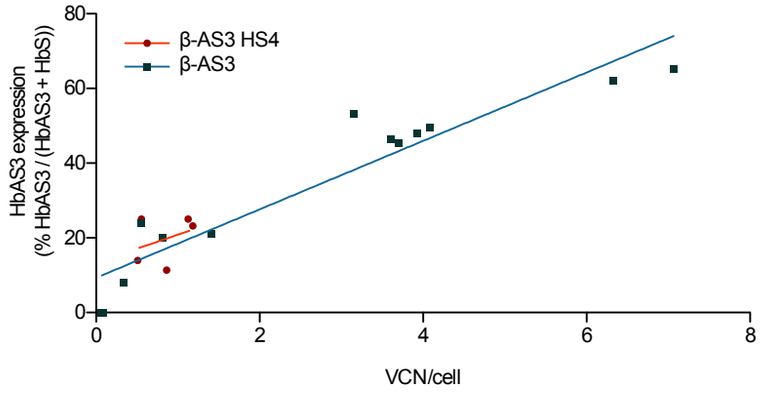


Figure S4. Transgene expression in BFU-E derived from β-AS3 HS4- and β-AS3- transduced SCD BM HSPCs. (**A**) Correlation between the levels of HBBAS3 mRNA expression. The slopes of the linear regression lines for samples transduced with β-AS3 HS4 (n=7) or β-AS3 (n=16) LVs were not significantly different (P=0.9672; n=2 donors). Equations that define the best fit lines were y= 0.28x – 0.03 (R2=0.9339 and P=0.0004) for β-AS3 HS4 samples and y= 0.26x + 0.05 (R2=0.5913 and P=0.0005) for β-AS3 samples. (**B**) HbAS3 expression and the average VCN/cell in pools of BFU-E. The slopes of the linear regression lines for samples transduced with β-AS3 HS4 (n=5) or β-AS3 (n=13) LVs were not significantly different (P=0.8963; n=2 donors). Equations that define the best fit lines were y= 7.51x + 13.35 (R2=0.1282 and P=0.5540) for β-AS3 HS4 samples and y= 9.16x + 9.32 (R2=0.8902 and P<0.0001) for β-AS3 samples.

Figure S4

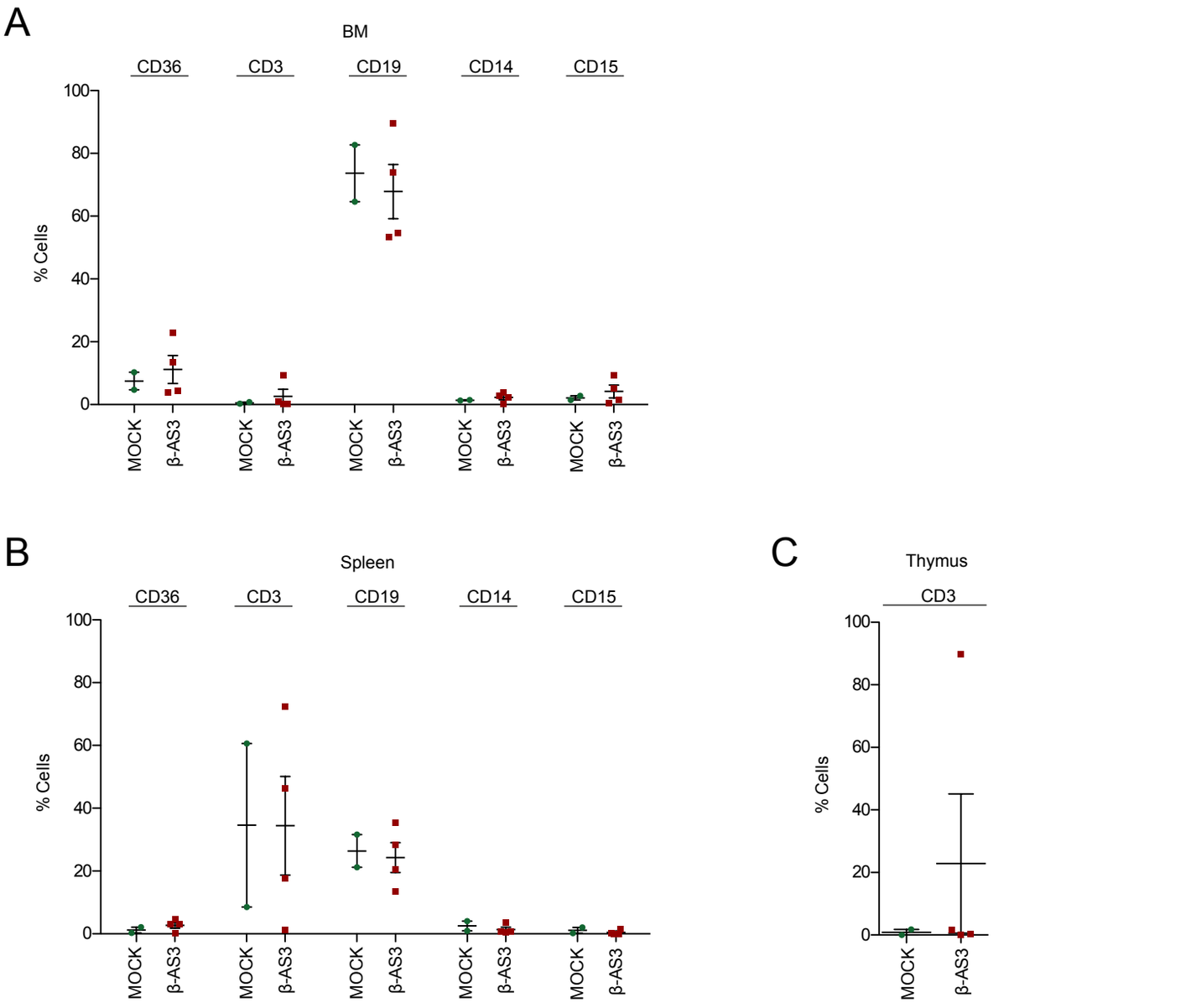


Figure S5. Human hematopoietic cell reconstitution in NSG mice transplanted with Plerixafor-mobilized SCD cells. Percentage of erythroid, T and B lymphoid and myeloid markers in BM (**A**) and spleen (**B**) of mice transplanted with mock- or β-AS3-transduced HSPCs. (**C**) Frequency of T lymphoid cells in the thymus of transplanted mice. Each data point represents an individual mouse.

Figure S5