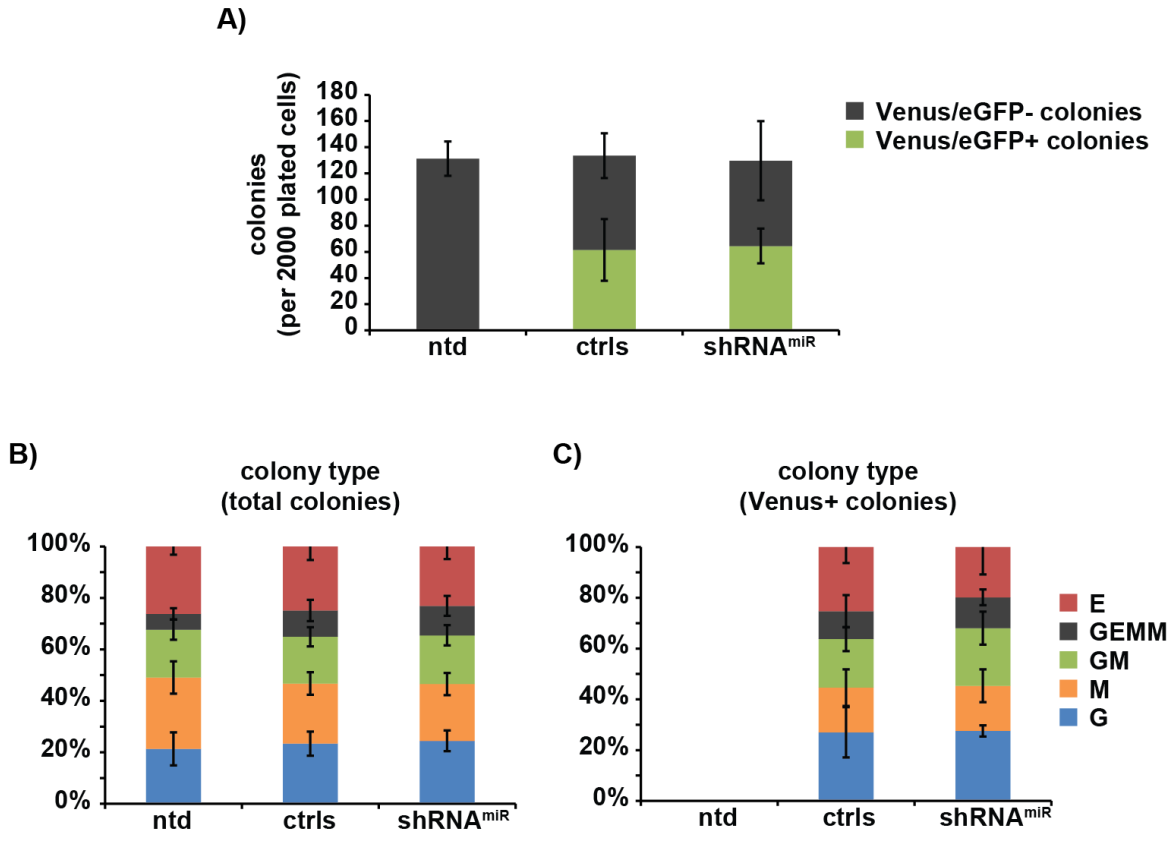
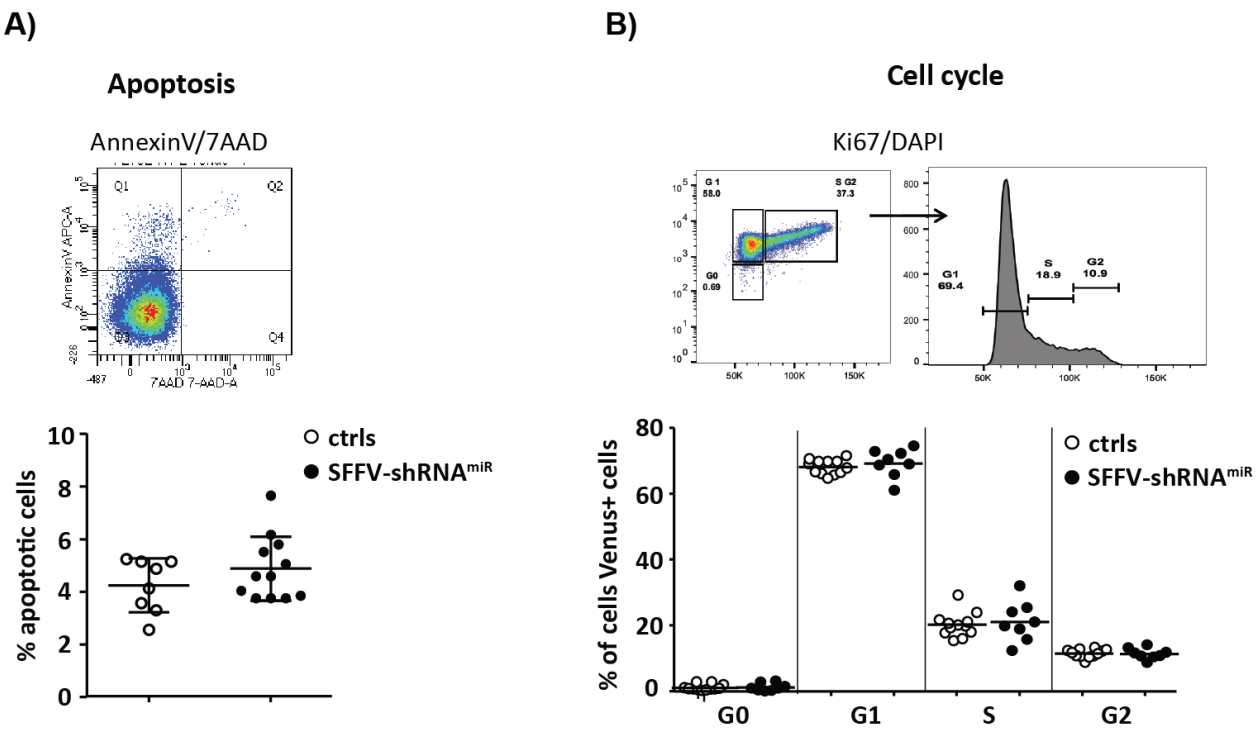
SUPPLEMENTARY MATERIALS



Supplementary Figure 1. Transduction of mouse lineage negative HSPCs with SFFV-shRNA^{miR} is not associated with alteration of apoptosis or cell cycle in vitro

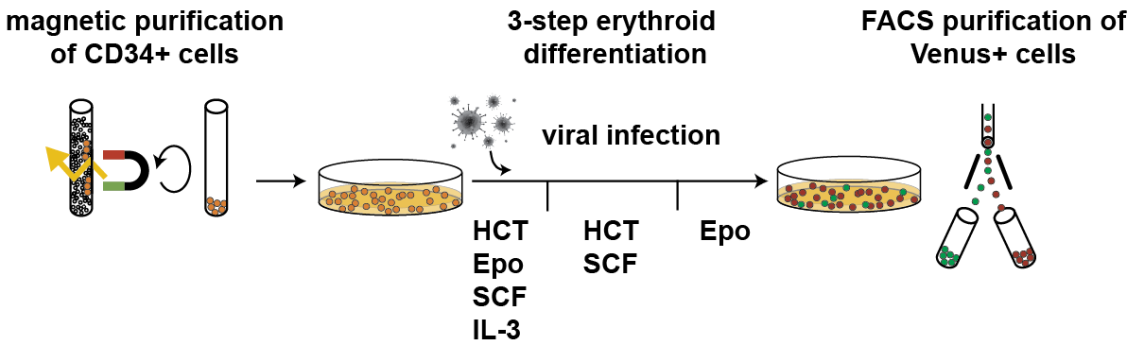
A) Mouse lineage negative bone marrow cells were transduced with control vectors (SFFV-eGFP and SFFV-shRNA^{miR}NT) or BCL11A knock down vectors. Three days later colony assays were performed to assess myelo-erythroid colony forming potential. The total number of transduced (Venus+, green) and untransduced colonies is shown (grey). B) Colony phenotyping of all colonies was performed to assess if lineage skewing occurs. C) Similarly, the fraction of Venus positive colonies was scored to determine the effect of the vectors relative to control groups.



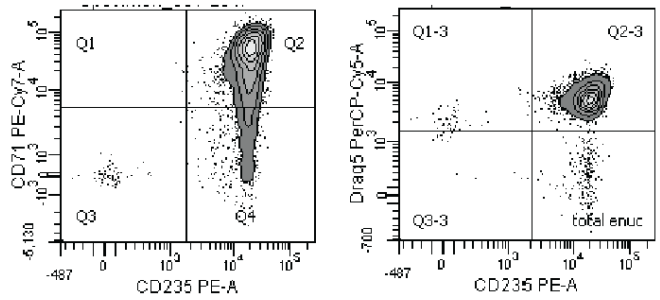
Supplementary Figure 2. Transduction of hCD34+ cells with SFFV-shRNA^{miR} is not associated with alteration of apoptosis or cell cycle in vitro

A) Following a 24h prestimulation human CD34+ cells were transduced with control vector (SFFV-eGFP) or BCL11A knock down vector (SFFV-shRNA^{miR}). Three days later the fraction of apoptotic cells was analyzed by AnnexinV/7AAD staining. B) The cell cycle status was assessed on the same day using Ki-67 / DAPI staining. Each dot represents an independent replicate. No statistical significant differences between treatment groups were observed.

A)

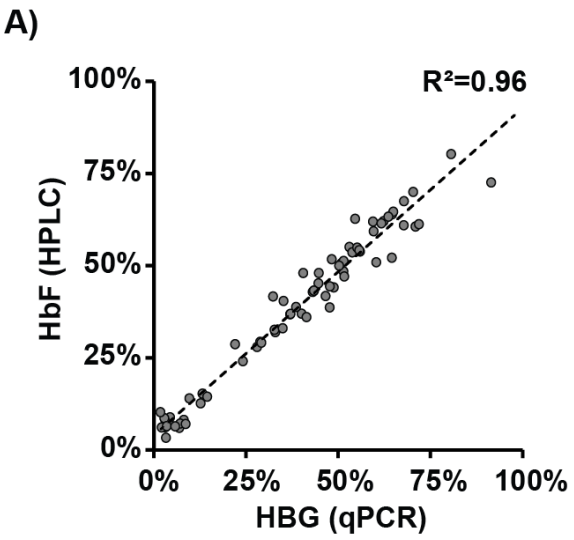


B)



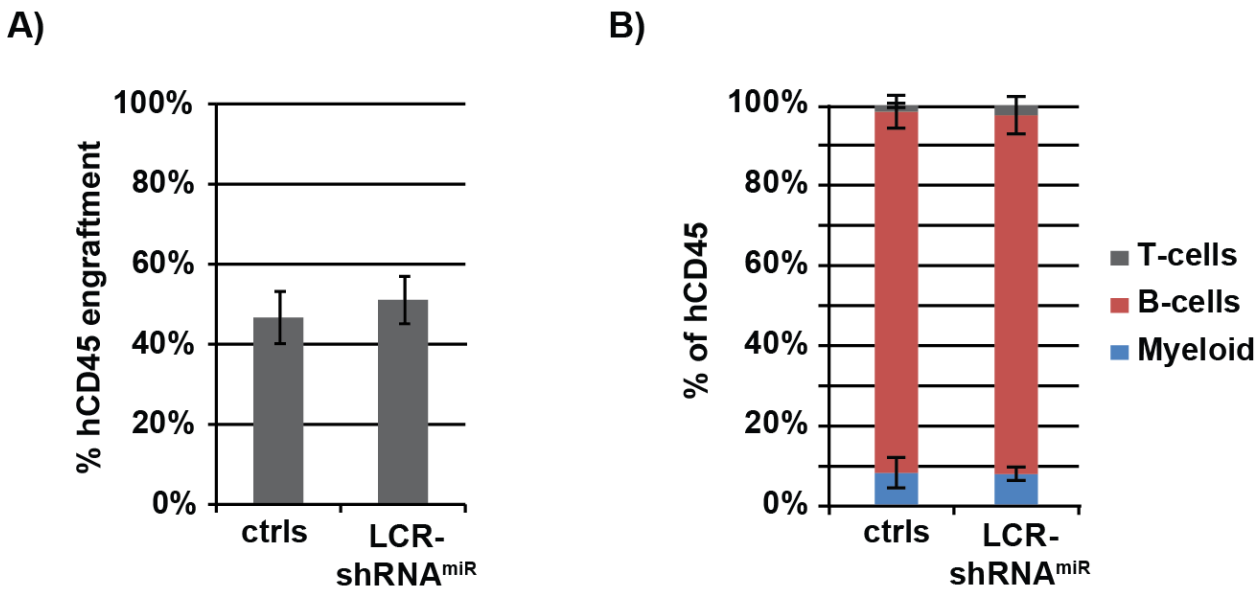
Supplementary Figure 3. Procedure of erythroid in vitro differentiation of CD34+ cells, cell phenotype and vector expression kinetics

A) Procedure overview for erythroid differentiation of human CD34+ cells. B) Differentiation status of erythroid cells after 18 days in culture using CD71 and GpA and Draq5 to assess enucleation.



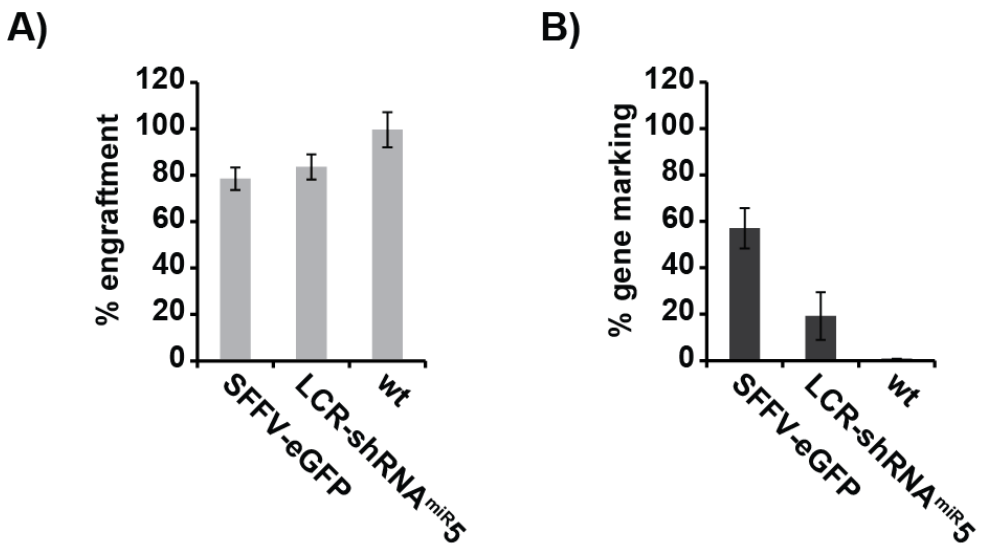
Supplementary Figure 4. Correlation between γ-globin expression, HbF levels and the degree of BCL11A knock down

A) Correlation between qPCR based detection of HBG transcripts and HbF as measured by HPLC.



Supplementary Figure 5. Engraftment and lineage distribution of human cells in NSG-mice

The engraftment of human CD45 positive cells (of total CD45) and their lineage identity was assessed in the bone marrow of NSG-mice 14 to 16 weeks post transplantation. A) Human chimerism in the bone marrow of transplanted animals. The control group consists of animals which received untransduced cells, SFFV-eGFP or LCR-shRNA^{miR}NT transduced cells. The treatment group received cells transduced with BCL11A targeting vectors LCR-shRNA^{miR}3, 5 or 8 (error bars: SEM). B) Lineage distribution of human CD45 positive bone marrow cells (error bars: SD). Statistical analysis revelaled no significant differences between treatment groups for figure A) or B).



Supplementary Figure 6. Engraftment and gene marking of mice transplanted with Berkeley-SCD cells

The engraftment and gene marking was assessed 10 weeks post transplantation in animals which received Berkeley-SCD cells which were transduced with SFFV-eGFP or LCR-shRNA^{miR} vectors or untransduced wt-cells, respectively. A) Chimerism in CD45+ peripheral blood myeloid cells. B) Fraction of Venus/eGFP+ cells in whole blood.