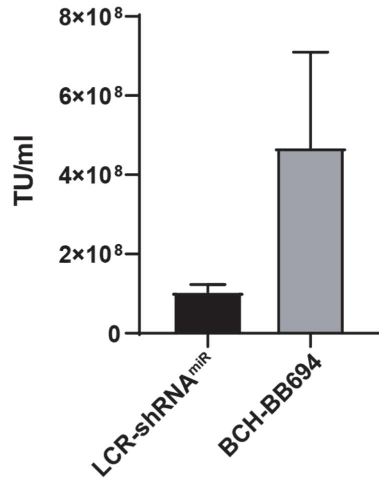
OMTM, Volume *17*

Supplemental Information

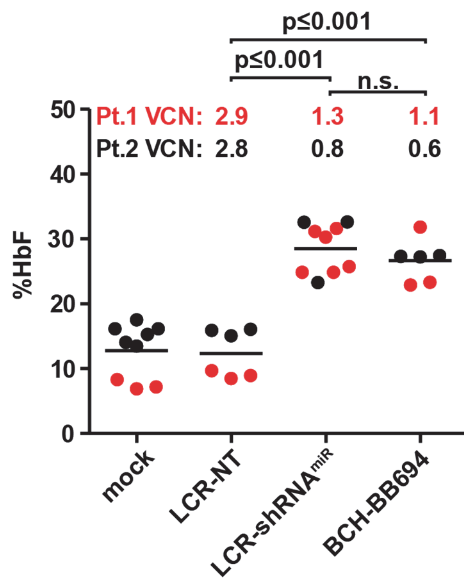
Preclinical Evaluation of a Novel Lentiviral Vector Driving Lineage-Specific BCL11A Knockdown for Sickle Cell Gene Therapy

Christian Brendel, Olivier Negre, Michael Rothe, Swaroopa Guda, Geoff Parsons, Chad Harris, Meaghan McGuinness, Daniela Abriss, Alla Tsytsykova, Denise Klatt, Martin Bentler, Danilo Pellin, Lauryn Christiansen, Axel Schambach, John Manis, Helene Trebeden-Negre, Melissa Bonner, Erica Esrick, Gabor Veres, Myriam Armant, and David A. Williams

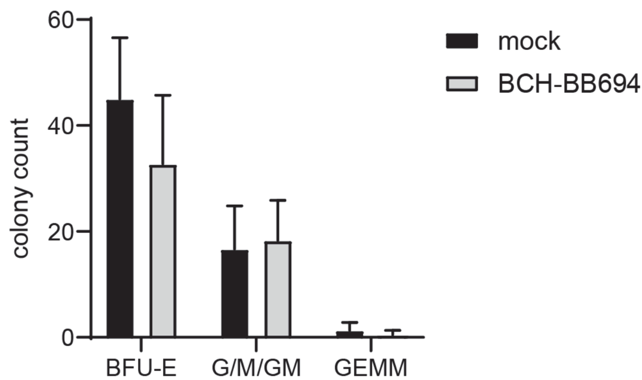
Supplementary Figures



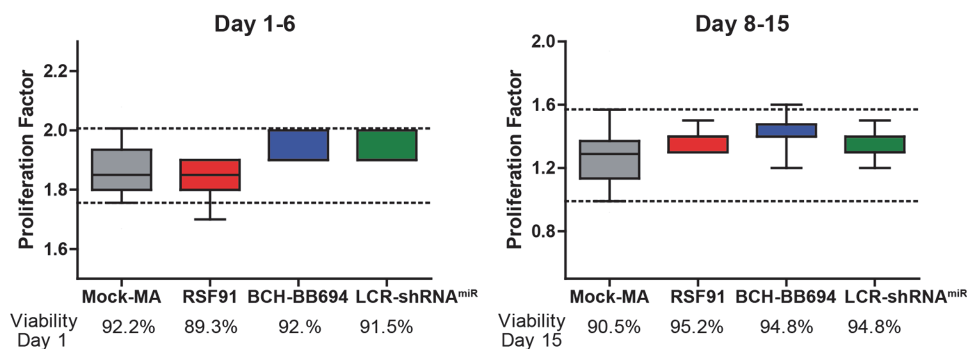
Supplementary Figure 1: Improvement of vector titers after changing the lentiviral vector backbone in independently performed large-scale production runs. Data represents the mean of 3 independent experiments. Error bars: SD.



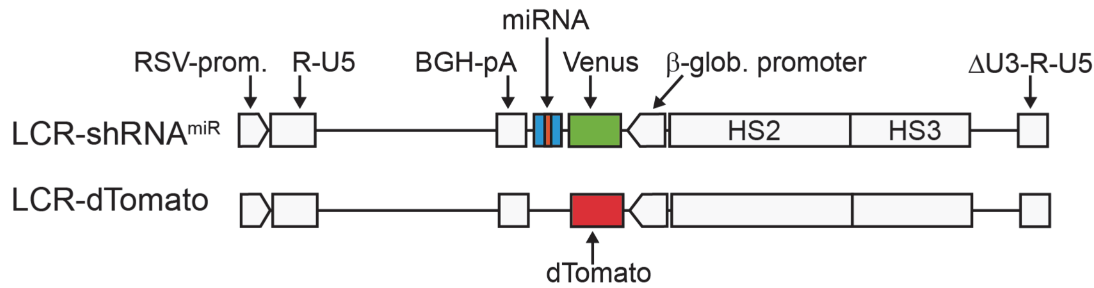
Supplementary Figure 2: Comparison of HbF induction in healthy donor CD34^{+} derived erythroid cells[44] between the original LCR-shRNA^{miR} and the new modified BCH-BB694 vector. CD34^{+} cells from two different healthy donors were left untransduced or transduced with a non-targeting control vector (LCR-NT), LCR-shRNA^{miR} or BCH-BB694 followed by erythroid *in vitro* differentiation. HbF induction was determined by HPLC of the bulk population, including many untransduced cells. At similar VCN the levels of HbF induction were comparable for LCR-shRNA^{miR} and BCH-BB694 vectors.



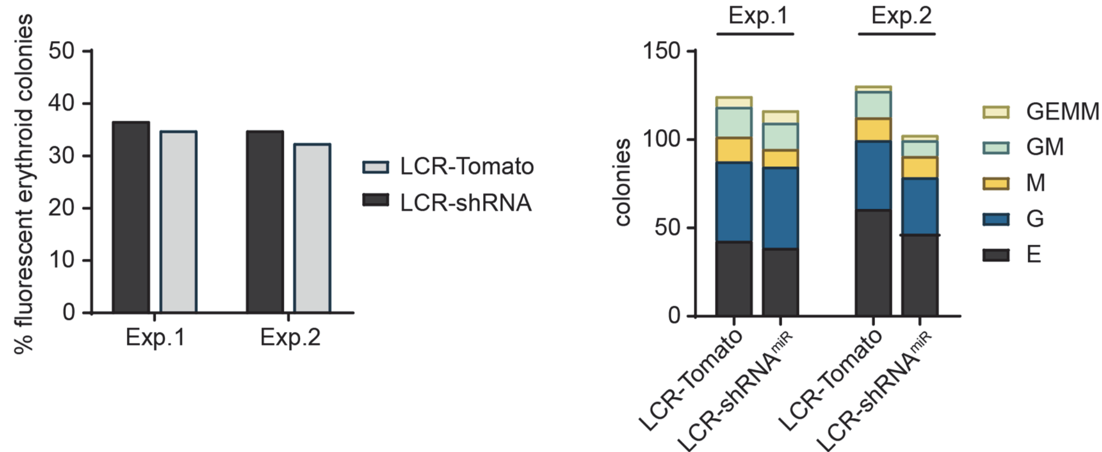
Supplementary Figure 3: Absolute colony numbers of transduced $CD34^+$ cells transduced with BCH-BB694. Mobilized peripheral blood $CD34^+$ cells from two healthy and two SCD donors were transduced with BCH-BB694 and seeded onto cytokine supplemented semisolid media. Untransduced cells served as control. The frequency of different colony types was scored after two weeks. BFU-E: burst forming unit of erythroid cells; G/M/GM: granulocyte or macrophage or mixed colony, GEMM: granulocyte, erythroid, macrophage, megakaryocyte mixed colony. Error bar: SD. Differences between groups are statistically not significant.



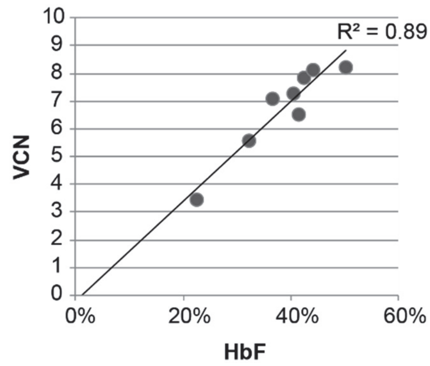
Supplementary Figure 4: Proliferation and viability of mouse lineage negative bone marrow cells in liquid culture. Cells were left untransduced (mock), transduced with the mutagenic positive control vector RSF91 or the BCH-BB694 or LCR-$shRNA^{miR}$ vectors. The cells were cultured for 15 days and viability and proliferation were assessed at early and late timepoints.



Supplementary Figure 5: Schematic overview over the vectors (in plasmid configuration) used in competitive repopulation experiments. The LCR-dTomato vector is derived from LCR-shRNA^{miR}; Venus has been replaced with dTomato and the miRNA hairpin has been removed.



Supplementary Figure 6: Characterization of the cell product used for competitive mouse repopulation experiments from two independent experiments. Left: Fraction of gene modified erythroid colonies in methylcellulose as assessed by fluorescent marker expression. Cells were transduced with the empty LCR-dTomato vector or the BCL11A targeting vector LCR-shRNA^{miR}. Right: absolute number of different colony types.



Supplementary Figure 7: Correlation of HbF induction and VCN in erythroid cells derived from individual NSG mice treated with the BCH-BB694 gene therapy vector.

Group (pre-transplantation VCN)	PB	total BM	hCD45 enriched BM*	hCD34 from BM*	total spleen cells	total thymic cells
BCH-BB694 (10.13)	8.24	7.24	6.92	8.21	7.43	4.76
	7.37	5.62	7.02	5.58	6.54	3.19
	7.43	6.45	6.26	7.28	6.60	5.24
	7.04	6.70	7.38	6.52	6.74	7.14
	9.35	6.25	6.46	3.44	6.54	3.37
	6.95	7.84	8.26	7.83	7.02	3.00
	7.82	7.02	7.00	8.15	6.14	7.32
	7.86	7.23	7.81	7.08	6.54	7.98
SFFV-GFP (4.85)	4.18	3.49	3.25	2.56	3.29	3.74
	4.71	3.56	3.32	2.88	3.28	3.06
	3.20	3.05	2.86	2.53	3.28	2.38
	4.00	3.46	3.42	2.99	3.41	3.37
	3.54	3.19	3.54	2.46	3.63	4.24

Supplementary Table 1: Vector copy number in peripheral blood (PB), bone marrow (BM), spleen and thymus isolated from NSG animals 8 weeks post transplantation. qPCR primers and probes are specific for human cells only. *CD34 and hCD45 cells were isolated by MACS (Miltenyi, Germany) from the bone marrow of transplanted animals. CD34 cells were cultured *in vitro* for cell expansion under erythroid differentiation conditions as described in materials and methods.