Figure S1. Relative mRNA expression profile in erythroid progenitor cells from FL and AB samples as examined by reverse transcription-quantitative PCR. (A) The relative mRNA expression of two major fetal hemoglobin repressors, *BCL11A* and *LRF*. The relative mRNA expression of (B) four α-like globin genes, *HBZ*, *HBM*, *HBA* and *HBQ* and (C) four β-like globin genes, *HBE*, *HBG*, *HBD* and *HBB*. Gene expression levels were normalized to β-actin. Data are presented as the means ± SEM of relative fold-change of FL (n=3) relative to AB (n=3). *P<0.05, **P<0.005 and ****P<0.0001. FL, fetal liver; AB, adult peripheral blood; *BCL11A*, B-cell lymphoma/leukemia 11A; *LRF*, leukemia/lymphoma-related factor; *HBZ*, ζ-globin; *HBM*, μ-globin; *HBA*, α-globin; *HBQ*, θ-globin; *HBE*, ϵ-globin; *HBG*, γ-globin; *HBD*, δ-globin; *HBB*, β-globin.

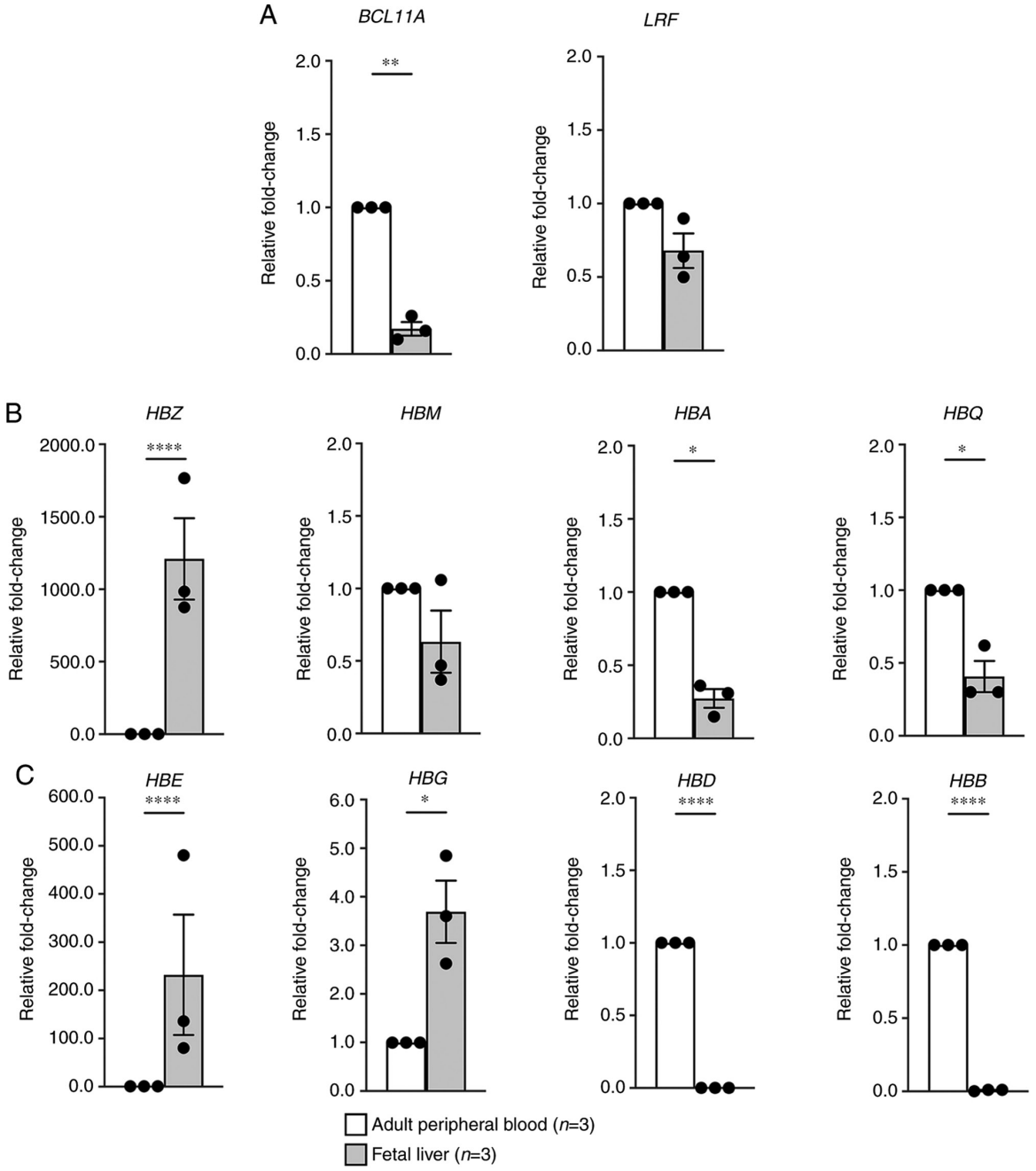

Figure S2. Time-course analysis of gene expression during erythroid differentiation. (A) Reverse transcription-quantitative PCR analysis for *KLF4*, *KLF1*, *BCL11A* and *LRF* mRNA expression levels upon time intervals of erythroid differentiation. Gene expression levels were normalized to β-actin. Data are presented as the means of relative fold-change of day 8 ± SEM of the healthy (n=3) and β ^{0}-thalassemia/HbE (n=3) groups. (B) Representative nuclear protein as evaluated by western blot analysis for KLF4, KLF1, BCL11A and LRF during erythroid differentiation of a healthy subject. Lamin A served as a loading control. KLF, Krüppel-like factor; HbE, hemoglobin E; BCL11A, B-cell lymphoma/leukemia 11A; LRF, leukemia/lymphoma-related factor; D, day.

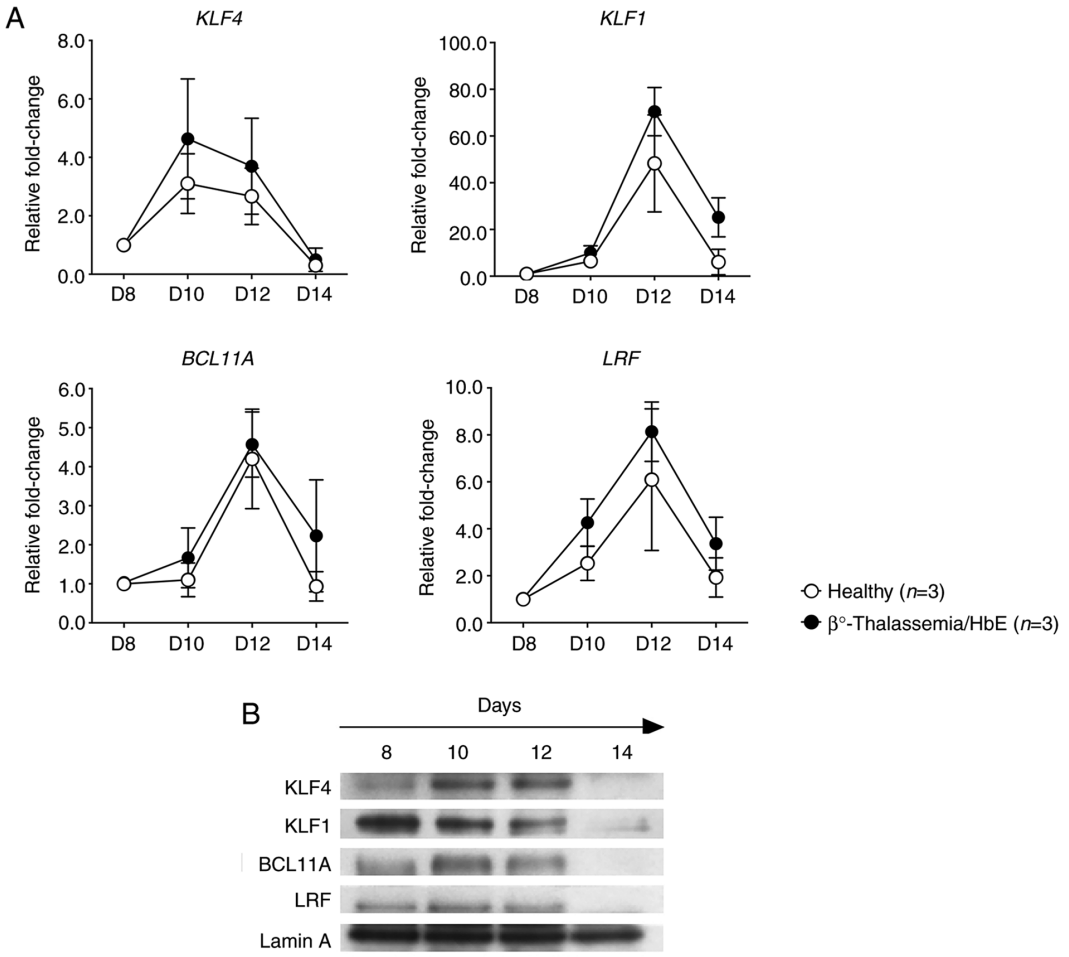

Figure S3. Hemoglobin composition after KLF4 knockdown. Representative high-performance liquid chromatography chromatograms from cultured cells on day 14. The bar graph represents the increase in the percentage of HbF (Δ%HbF) in KLF4-knockdown cells from the baseline level in shNTC-treated cells. Data are presented as means ± SEM in the healthy (n=3) and β^0-thalassemia/HbE (n=3) groups. KLF, Krüppel-like factor; HbF, fetal hemoglobin; KLF4sh, shRNAs targeting KLF4; shRNA, short hairpin RNA; shNTC, non-targeting control.

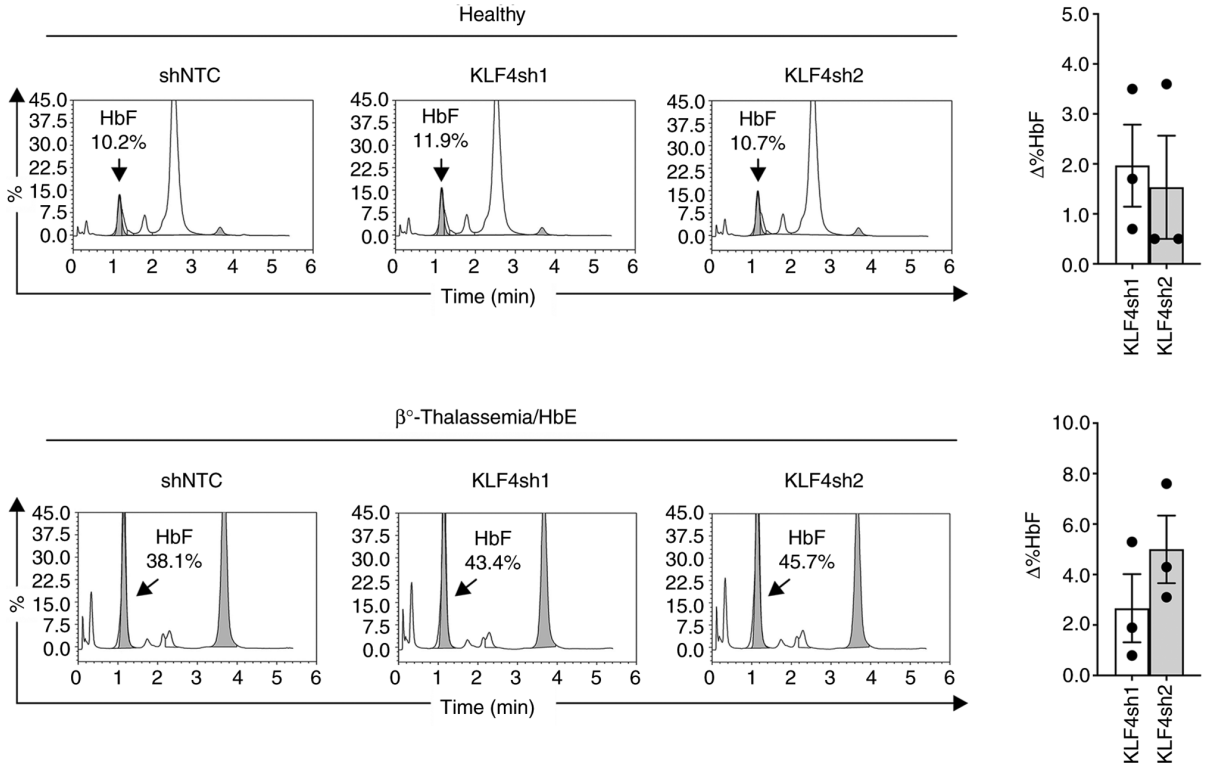

Table SI. Hematological data of subjects from the present study.

Patient no.	Gender	Age (years)	*HBB* alleles	WBC ($x10^3/\mu l$)	RBC ($x10^6/\mu l$)	Hb (g/dl)[a]	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dl)	NRBC/100 WBC	HbF (%)	HbA2/E (%)[b]	HbA (%)
N1	Male	28	WT:WT	4.77	4.70	13.8	41	88.1	29.4	33.3	0	0.1	2.2	97.7
N2	Male	26	WT:WT	5.14	5.21	15.9	47	90.2	30.5	34.2	0	1.7	3.2	95.1
N3	Female	25	WT:WT	5.69	4.68	12.1	38	78.4	25.9	33.0	0	0.2	2.7	97.1
BE1	Female	43	Codon41/42(-TTCT): Codon26(G>A)	8.74	3.54	8.3	24	67.8	23.4	34.6	8	43.5	53.1	3.4
BE2	Male	22	Codon41/42(-TTCT): Codon26(G>A)	8.25	4.34	7.7	25	57.6	17.7	30.8	ND	18.0	76.4	5.6
BE3	Male	36	Codon41/42(-TTCT): Codon26(G>A)	6.00	2.45	5.5	17	69.4	22.4	32.4	6	39.5	58.3	2.2

[a]Hemoglobin levels and hemoglobin typing determined by HPLC were performed from peripheral blood at steady state. [b]HbA2 and HbE coexisted as a single peak and could not be separated by HPLC. N1-3, healthy adults; BE1-3, β^0-thalassemia/HbE patients. HPLC, high-performance liquid chromatography; Hb, hemoglobin; WT, wild-type; WBC, white blood cell; RBC, red blood cell; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; NRBC, nucleated red blood cell; ND, not determined.

Table SII. Primers used for reverse transcription-quantitative PCR in the present study.

Primer	Sequence (5' to 3')	
	Forward	Reverse
KLF1	CACACAGGGGAGAAGCCATA	GAAAAAGCACGTGGGCAGAG
KLF2	CTGAGTGAACCCATCCTGCC	CGGCCAGCGCTCCTG
KLF3	CCTGTCTCAGTGTCATACCCA	CTTGTTCACCGTGAGGTCCA
KLF4	TGCCCCGAATAACCGCTG	GGTCTCTCTCCGAGGTAGGG
KLF5	TGCGATTACCCTGGTTGCAC	CCTGTGTGCTTCCGGTAGTG
KLF6	AGTACTGGCAACAGACCTGC	CTCGGGCTGATGAGAGTGTC
KLF7	GCAGCAGACATGCCTTGAAT	AAGCGTGGAGGAAACAGTCC
KLF8	GGTCACTGCTTCTGTTCGGA	TCAACTGGTTCCACTTGGGG
KLF9	GGAAACACGCCTCCGAAAAG	GCCGTTCACCTGTATGCACT
KLF10	CAGCATTTTGTTTGACTCCACCT	CTTGTTGCCTGAGCTTTGGG
KLF11	CCATTCTTTATCGACTCTGTGCAT	ACAGGTGTCCTTGTCGATGG
KLF12	TTTCCTGAGAACTGCAGAGAGC	GTCACATTGATCCTGAACAGAAG
KLF13	GCTGCGAGAAAGTTTACGGG	AAGGGCCTCTCACCTGTGT
KLF14	TCTGGTGTCTCACCCATCCA	CCAAAGGCCTCTCACCATCC
KLF15	GCCAAGTTCAGCCGCCAC	CCAACCAGCCTATCACCCAG
KLF16	GACGCACACAGGGGAACG	GCACAGAGGGCAGGAGAAG
KLF17	CCGTGGTGGCTGGTTC	AGTTCTCGTTATCCTGGGCA
KLF18	CAAGCACACCGGGGAGAA	TTGTGGACCTTTGCATGCTG
BCL11A	GGGAATTCTCGCCCGAG	GGGAAGTTCATCTGGCACT
LRF	CTTCACCAGGCAGGACAA	GGTTCTTCAGGTCGTAGTTGTG
HBZ	TGAGAGGACCATCATTGTGTCC	AAGTGCGGGAAGTAGGTCTT
HBM	CTCAGGCTCTTCACGGTGTA	ATTAGCAGCGGAAAGTTGGC
HBA	TGGACCCGGTCAACTTCAAG	TCACAGAAGCCAGGAACTTGTC
HBQ	GAAGAAGCTGGGCAGCAA	GTGGGCTCTGACTTGTGAGG
HBE	GAATGTGGAAGAGGCTGGAG	GGCTTGAGGTTGTCCATGTT
HBG	TCACAGAGGAGGACAAGGCTA	GCTTTATGGCATCTCCCAAG
HBD	CAAAGTGAACGTGGATGCAG	CTGAGAAAAAGTGCCCTTGAG
HBB	GAAGGCTCATGGCAAGAAAG	CACTGGTGGGGTGAATTCTT
ACTB	GGCACCACACCTTCTACAATG	GGTCTCAAACATGATCTGGGTC

KLF, Krüppel-like factor; BCL11A, B-cell lymphoma/leukemia 11A; LRF, leukemia/lymphoma-related factor; HBZ, ζ-globin; HBM, μ-globin; HBA, α-globin; HBQ, θ-globin; HBE, ϵ-globin; HBG, γ-globin; HBD, δ-globin; HBB, β-globin; ACTB, β-actin.

Table SIII. Antibodies used for western blot analysis in the present study.

Antibody	Cat. no. (manufacturer)	Dilution
Rabbit polyclonal antibody to human KLF4	Ab106629 (Abcam)	1:200
Rabbit polyclonal antibody to human KLF1 (H-210)	Sc-14034 (Santa Cruz Biotechnology, Inc.)	1:5,000
Mouse monoclonal antibody to human BCL11A [14B5]	Ab19487 (Abcam)	1:2,000
Rabbit polyclonal antibody to human LRF	13097 (Cell Signaling Technology, Inc.)	1:5,000
Rabbit antibody to human lamin A	L1293 (EMD Millipore)	1:5,000
Mouse monoclonal antibody to human β-actin (HRP) [AC-15]	Ab49900 (Abcam)	1:50,000
Goat polyclonal antibody to rabbit IgG-H&L (HRP)	Ab97051 (Abcam)	1:10,000
Goat polyclonal antibody to mouse IgG-H&L (HRP)	Ab136815 (Abcam)	1:10,000

KLF, Krüppel-like factor; BCL11A, B-cell lymphoma/leukemia 11A; LRF, leukemia/lymphoma-related factor; IgG, immunoglobulin.

Table SIV. KLF gene expression in hematopoietic progenitor cells from FL (n=3) and AB (n=3) analyzed from previous microarray data (18).

Transcript cluster ID	Chromosome	Genomic position	Gene symbol	Description	Fold-change (FL vs. AB)	P-value
16869359	chr19	12995236-12998017	KLF1	Krüppel-like factor 1 (erythroid)	1.25	0.06327
16859314	chr19	16435651-16438345	KLF2	Krüppel-like factor 2 (lung)	0.90	0.27010
16966008	chr4	38665790-38703134	KLF3	Krüppel-like factor 3 (basic)	0.98	0.81659
17096827	chr9	110247133-110252763	KLF4	Krüppel-like factor 4 (gut)	0.79	0.06495
16775364	chr13	73629114-73651676	KLF5	Krüppel-like factor 5 (intestinal)	1.03	0.32501
16711280	chr10	3818188-3827473	KLF6	Krüppel-like factor 6; NULL	0.94	0.61026
16907623	chr2	207938861-208031991	KLF7	Krüppel-like factor 7 (ubiquitous); NULL	1.09	0.19002
16907641	chr2	207985608-207986768	KLF7-IT1	Null; KLF7 intronic transcript 1 (non-protein coding)	0.84	0.01423
17104159	chrX	56258822-56314686	KLF8	Krüppel-like factor 8	0.97	0.60585
17094761	chr9	72999503-73029573	KLF9	Krüppel-like factor 9	1.91	0.00628
17079910	chr8	103661005-103668130	KLF10	Krüppel-like factor 10	1.09	0.15792
16877007	chr2	10182976-10194963	KLF11	Krüppel-like factor 11	0.96	0.59992
16779766	chr13	74260149-74708394	KLF12	Krüppel-like factor 12	0.86	0.23042
16798872	chr15	31619058-31727868	KLF13; LOC100499221	Krüppel-like factor 13; uncharacterized LOC100499221	0.94	0.50395
17062938	chr7	130417396-130418888	KLF14	Krüppel-like factor 14	1.07	0.94710
16958638	chr3	126061478-126076285	KLF15	Krüppel-like factor 15	0.88	0.12081
16866815	chr19	1852398-1863564	KLF16	Krüppel-like factor 16	0.94	0.23679
16663851	chr1	44509600-44570647	KLF17	Krüppel-like factor 17	0.91	0.54223
16663863	chr1	44584522-44600812	KLF17	Krüppel-like factor 17	1.08	0.71197

FL, fetal liver; AB, adult peripheral blood.