SUPPLEMENTARY APPENDIX

Induction of adult levels of β-globin in human erythroid cells that intrinsically express embryonic or fetal globin by transduction with KLF1 and BCL11A-XL

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Supplementary Table 1. PCR and qPCR primer sequences.

BCL11A-XL	5' AGATCCCTTCCTTAGCTTCG	5' TCAACACTCGATCACTGTGC
BCL11A-L(1)	5' GACGATGGCACTGTTAATGG	5' GGGTGTGTGAAGAACAAGTG
BCL11A-L(2)	5'-GACGATGGCACTGTTAATGG	5' AATGGGGGTGTGTGAAGAAC
BCL11A-S(1)	5' CATGACCTCCTCACCTGTGG	5' GGTGTGTGAAGAACCCGCGG
BCL11A-S(2)	5' CATGACCTCCTCACCTGTGG	5' ATGGGGGTGTGTGAAGAACC
KLF1	5' GCCCTCCATCAGCACACT	5' GATCCTCCGAACCCAAAAG
β-globin	5' CTTTAGTGATGGCCTGGCTC	5' GGCAGAATCCAGATGCTCAA
β-globin (qPCR)	5' GCAAGGTGAACGTGGATGAA	5'TCACCTTAGGGTTGCCCATAACT
PABPC1 (qPCR)	5'AGCTGTTCCCAACCCTGTAATC	5' GGATAGTATGCAGCACGGTTCTG

Supplementary Figure Legends

Supplementary Figure 1. Expression of BCL11A variants in K562 and adult erythroid cells.

Transcripts for the BCL11A variants BCL11A-XL, BCL11A-L and BCL11A-S in K562 cells and erythroblasts differentiated from peripheral blood progenitors at day 9 of culture as a positive control, analysed by PCR. Two primer sets were used for BCL11A-S and BCL11A-L. Sequences of primers used are shown in Suppl Table 1.

Supplementary Figure 2. Co-transfection of K562 cells with KLF1 and different variants of BCL11A.

K562 cells were co-transfected with 5 μg of pBp HA- KLF1 and 5 μg of pCDNA3-3Flag-BCL11A-XL (XL), pCDNA3-3Flag-BCL11A-L (L) or pCDNA3-3Flag-BCL11A-S (S). Cells were collected at 17 hours post transfection. Total protein from co-transfected and K562 control (Ctrl) cells was resolved on an 8% (for BCL11A-L and –XL) and 12% (for BCL11A-S) SDS-PAGE gel and western blotted. Membranes were probed with BCL11A, KLF1 and β-globin antisera. Western blots were stripped and re-probed with an antibody to Tubulin as a loading control.

Supplementary Figure 3. Co-transduction and co-transfection of K562 cells with KLF1 and BCL11A-XL increase levels of β-globin.

K562 cells were transduced with pXLG3-eGFP-BCL11A-XL, pXLG3-KLF1 or with both constructs (A), or transfected with 5 μg of pCDNA3-3Flag-BCL11A-XL (B-XL), 5 μg of pBp HA-KLF1 or co-transfected with 5 μg of each plasmid (B). Western blots of whole cell lysates were probed with an antibody to β-globin. Blots were stripped and an antibody to tubulin was used as a protein loading control.

Supplementary Figure 4. Erythroid phenotype of HiDEP-1 cells.
(A) Undifferentiated (undif) and HiDEP-1 cells differentiated for 4, 8 and 12 days in erythroid culture medium stained with May-Grunwald Giemsa reagent and analyzed by light microscopy. Arrows indicate developmental cell types: white, proerythroblast; blue, basophilic erythroblast; red, polychromatic erythroblast; black, orthochromatic erythroblast (B) Western blot (5 μg whole cell lysate) of erythroid cells differentiated from adult (PB) and cord blood (CB) progenitors for up to 20 days in erythroid culture media, and undifferentiated (undif) and HiDEP-1 cells differentiated for up to 12 days in erythroid culture medium incubated with antibodies to Glycophorin A (GPA) and Band 3. (C) confocal images of HiDEP-1 cells at day 5 in erythroid culture incubated with antibodies to Glycophorin A (GPA), Band 3, Glycophorin C (GPC), Rh antigen, Rh associated glycoprotein (RhAg) and transferrin receptor (CD71). Nuclei were stained with DAPI (blue). Arrows indicate enucleated cells.

Supplementary Figure 5. Expression of eGFP-BCL11A-XL in nuclei of HiDEP-1 cells.
Confocal images of HiDEP-1 cells and HiDEP-1 cells transduced with pXLG3-eGFP-BCL11A-XL (green). Nuclei with stained with DAPI (blue).

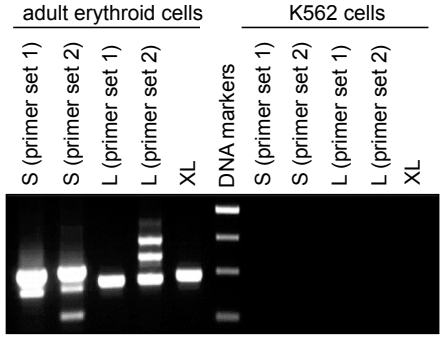
Supplementary Figure 6. Erythroid phenotype of differentiated HiDEP-1 cells before and after transduction with eGFP-BCL11A-XL. HiDEP-1 cells and HiDEP-1 cells transduced with eGFP-BCL11A-XL differentiated in tertiary erythroid culture medium for 12 days were (A) stained with May-Grunwald Giemsa reagent and analyzed by light microscopy, and (B) incubated with antibody to GPA (red). Nuclei were stained with DAPI (blue).

Supplementary Figure 7. Co-transduction of erythroid cells differentiated *in vitro* from cord blood progenitors with KLF1 and BCL11A-XL increase level of β-globin.

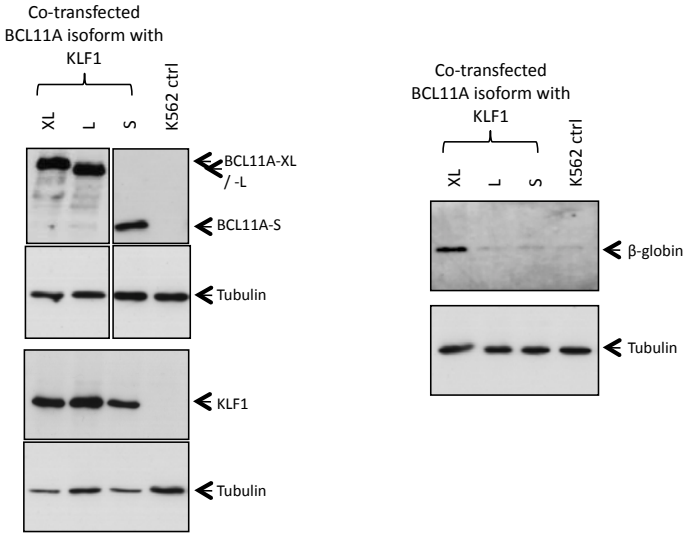
Western blot (A) of erythroid cell differentiated from cord blood progenitors transduced with CSII-EF-BCL11A-IRES-Puro or CSII-EF-BCL11A-IRES-Puro and pXLG3-KLF1 probed with antibody to β-globin. An antibody to β-actin was used as a protein loading control (B) levels of β-globin normalised to respective β-actin control and calibrated to level in non-transduced control cells.

Supplementary Figure 8. Level of Mi2β in erythroid cells differentiated *in vitro* from adult and cord blood progenitors and HiDEP-1 cells, and in K562 cells.
Western blot of whole cell lysates (30 μg) of erythroid cells derived from adult and CB progenitors, HiDEP-1 and K562 cells probed with antibody to Mi2β. Blot was stripped and re-probed with antibody to β-actin as a protein loading control.

Supplementary Figure 1



Supplementary Figure 2



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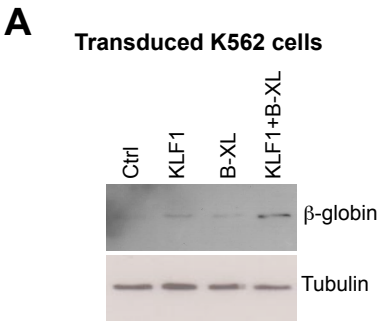
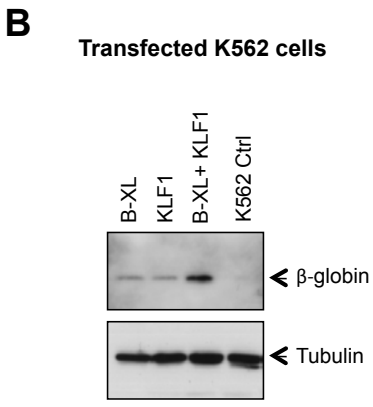
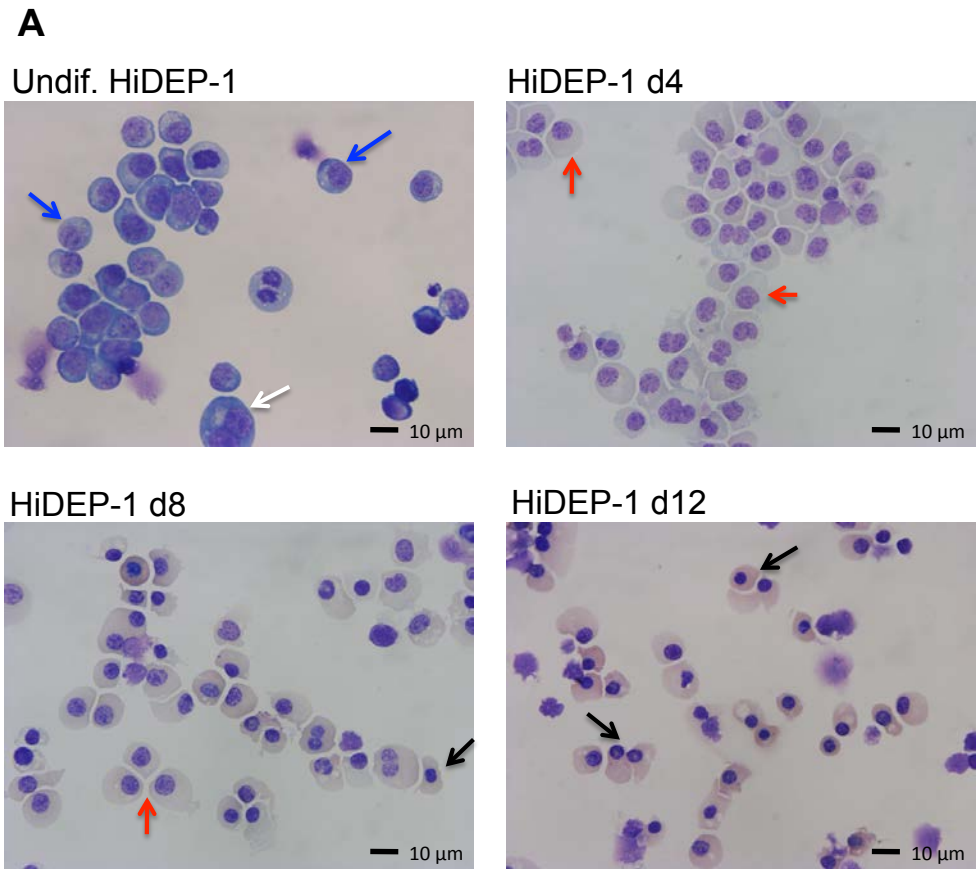
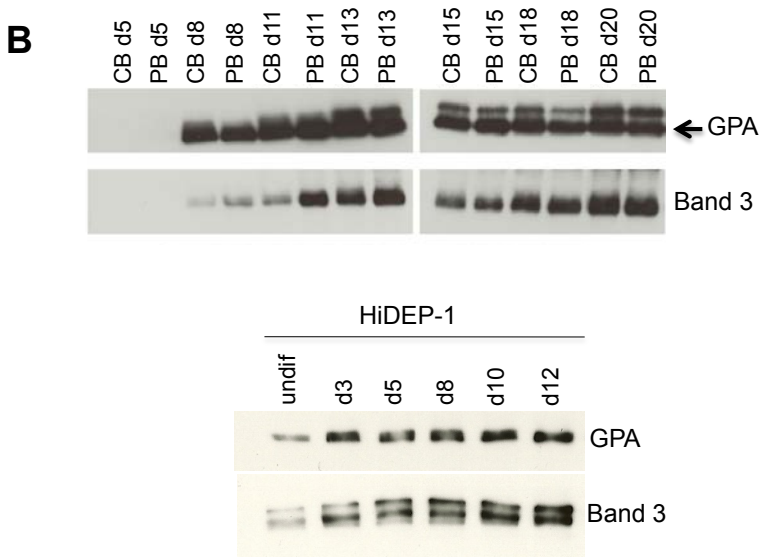
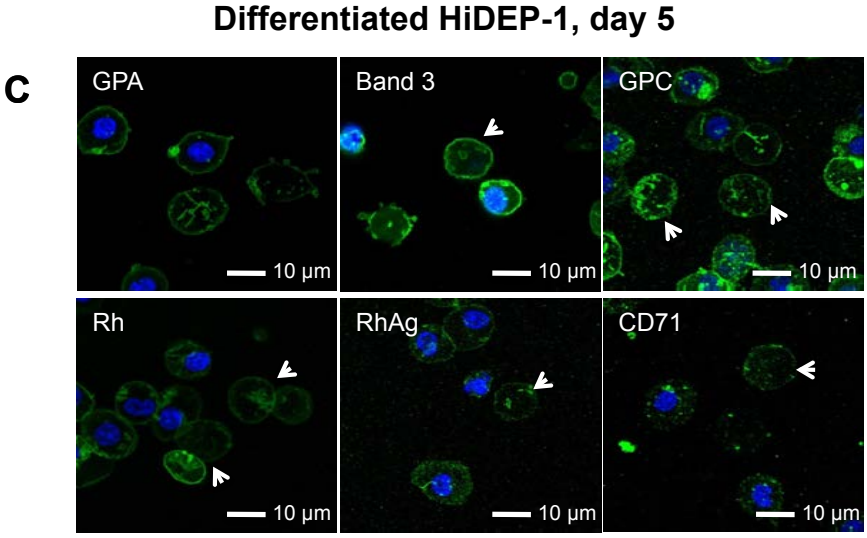




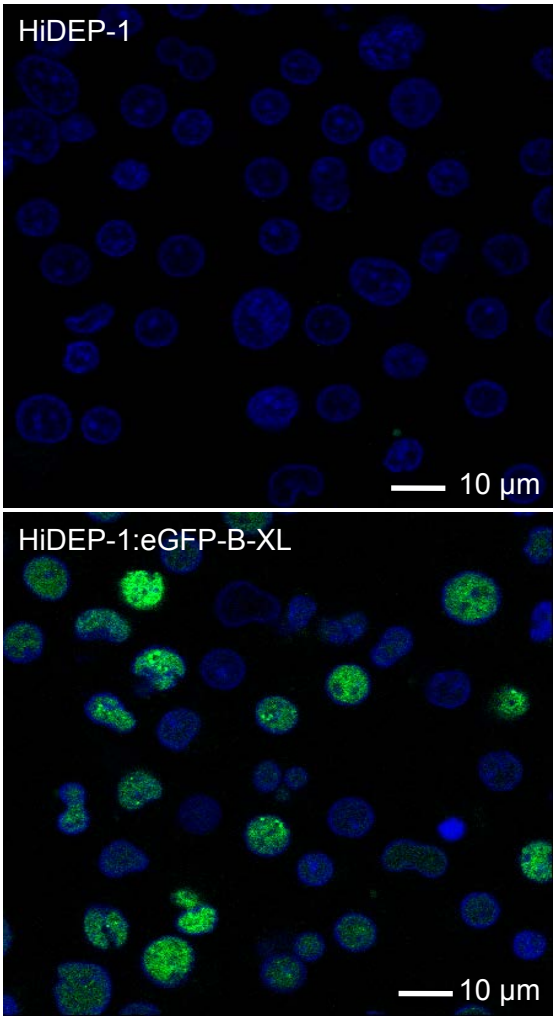
Supplementary Figure 4



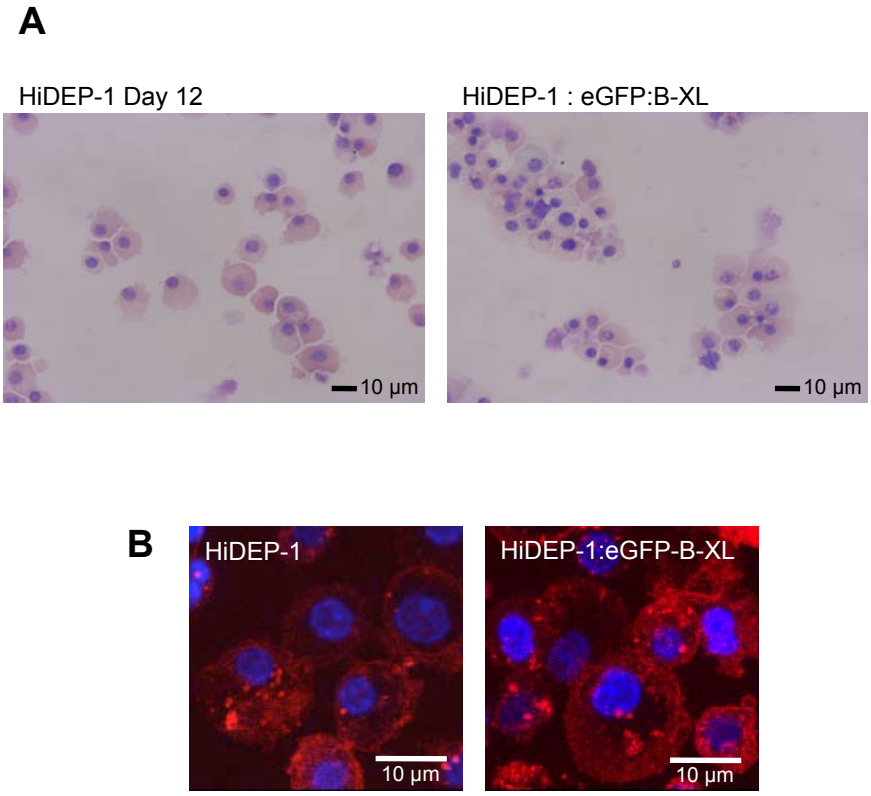

B
CB d5
PB d5
CB d8
PB d8
CB d11
PB d11
CB d13
PB d13
CB d15
PB d15
CB d18
PB d18
CB d20
PB d20
GPA
Band 3
HiDEP-1
undif
d3
d5
d8
d10
d12
GPA
Band 3


Differentiated HiDEP-1, day 5
C
GPA
Band 3
GPC
Rh
RhAg
CD71
10 µm

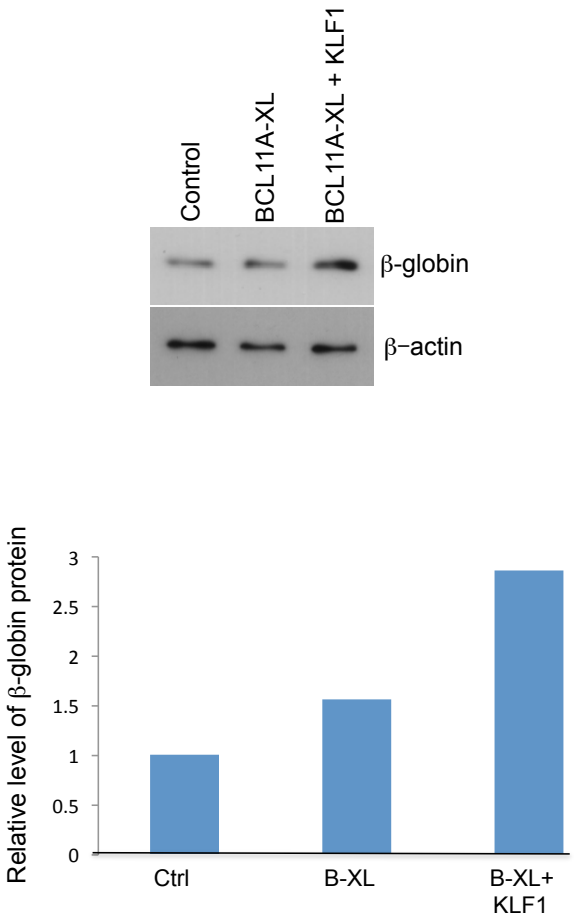
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8