Supplementary Note for

An erythroid-specific enhancer of *ATP2B4* mediates red blood cell hydration and malaria susceptibility

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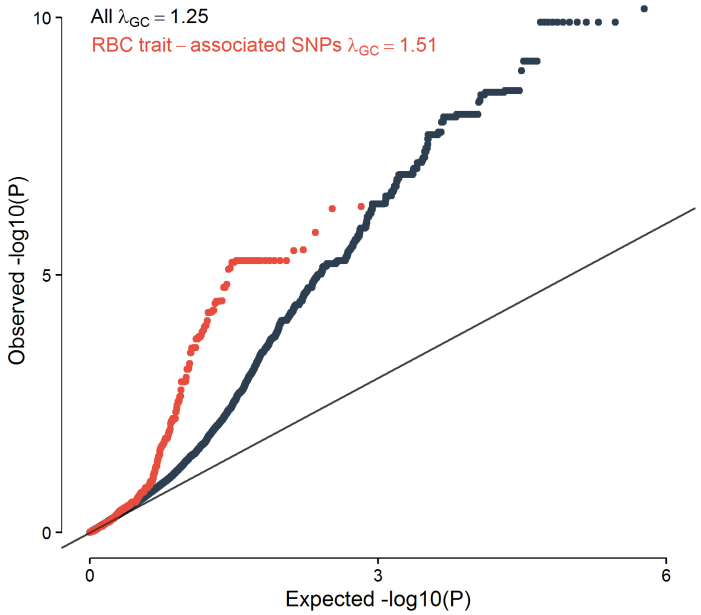

Figure S1.
Erythroblasts eQTL are enriched for RBC trait-associated SNPs. Quantile-quantile (QQ) plot of eQTL *P*-values for variants located within 100 kb of 479 AI genes in human erythroblasts (black, genomic control lambda (λ_{GC})=1.25). We find an enrichment of eQTL *P*-values for SNPs associated with RBC traits (red, λ_{GC}=1.51)(*7*). The number of different SNPs included when considering all tested variants and when subsetting on RBC trait-associated SNPs is 258,663 and 178 respectively.

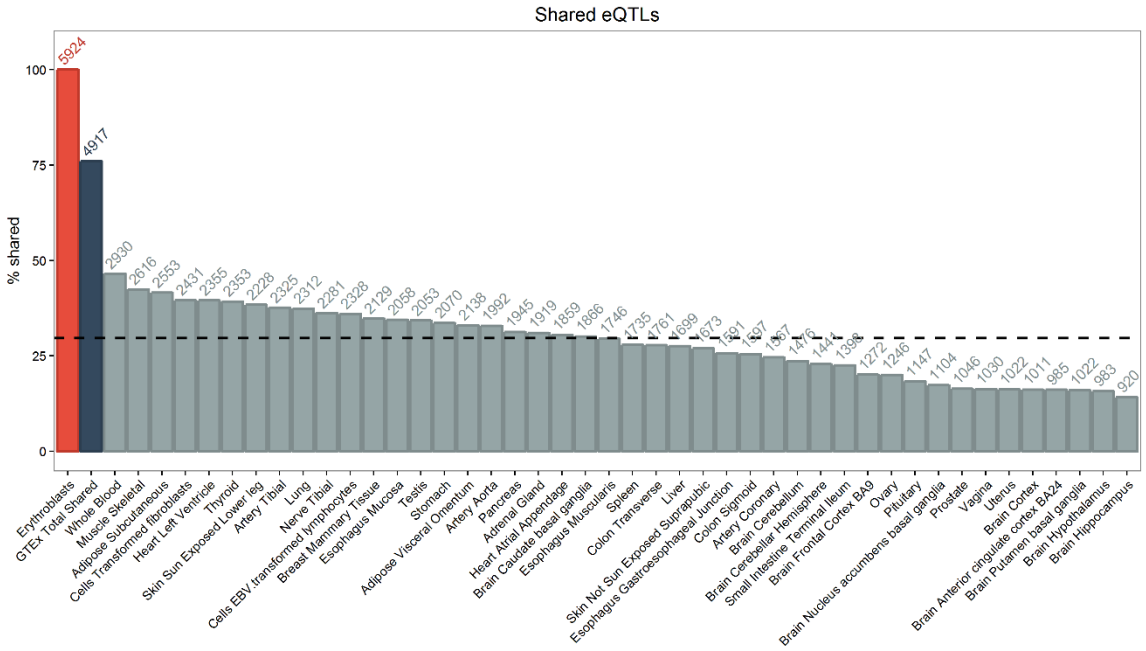

Figure S2.
Number of shared eQTLs between erythroblasts and GTEx samples. The dotted line represents the mean percentage of eQTLs shared between human erythroblasts and any of the GTEx tissues (mean=29.6%).

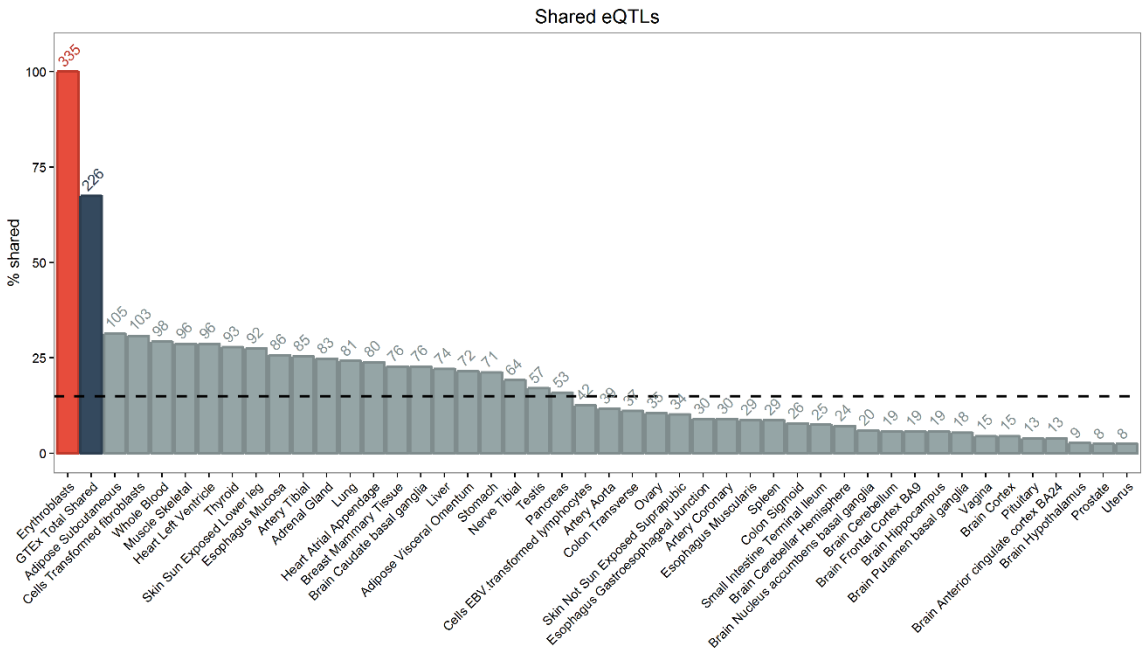

Figure S3.
Number of eQTLs in GATA1/TAL1 ChIP-seq peaks shared between erythroblasts and GTEx samples. The dotted line represents the mean percentage of shared eQTLs that map to GATA1/TAL1 ChIP-seq peaks between human erythroblasts and any of the GTEx tissues (mean=14.9%).

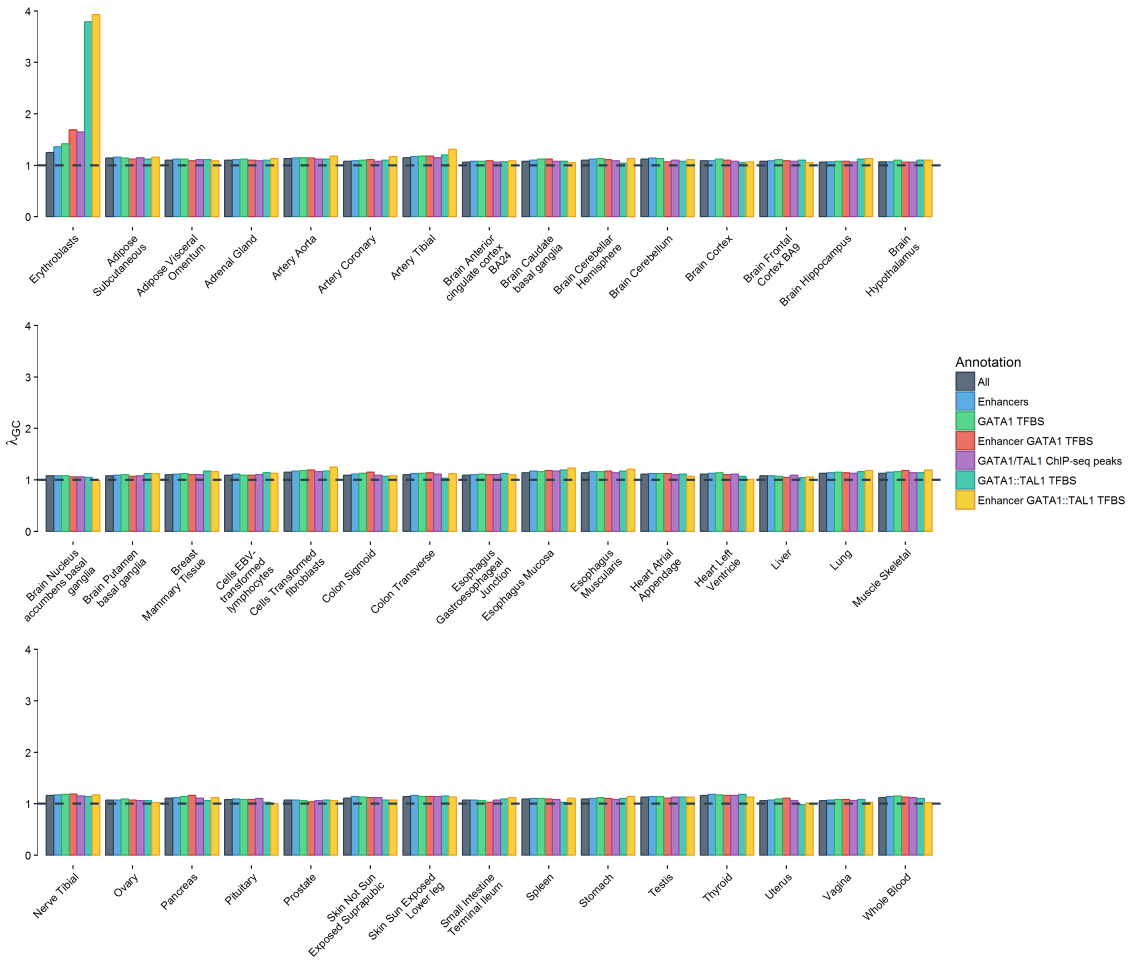

Figure S4.
Genomic control lambda (λ_{GC}) of eQTLs subsetted by erythroid regulatory regions. In this analysis, we compared inflation of eQTL signals observed in human erythroblasts (this study) with the inflation of the same SNPs but in different tissues available in the GTEx resource. We note a marked inflation of eQTL signals for erythroid annotations only in human erythroblasts. Dotted line is λ_{GC}=1.

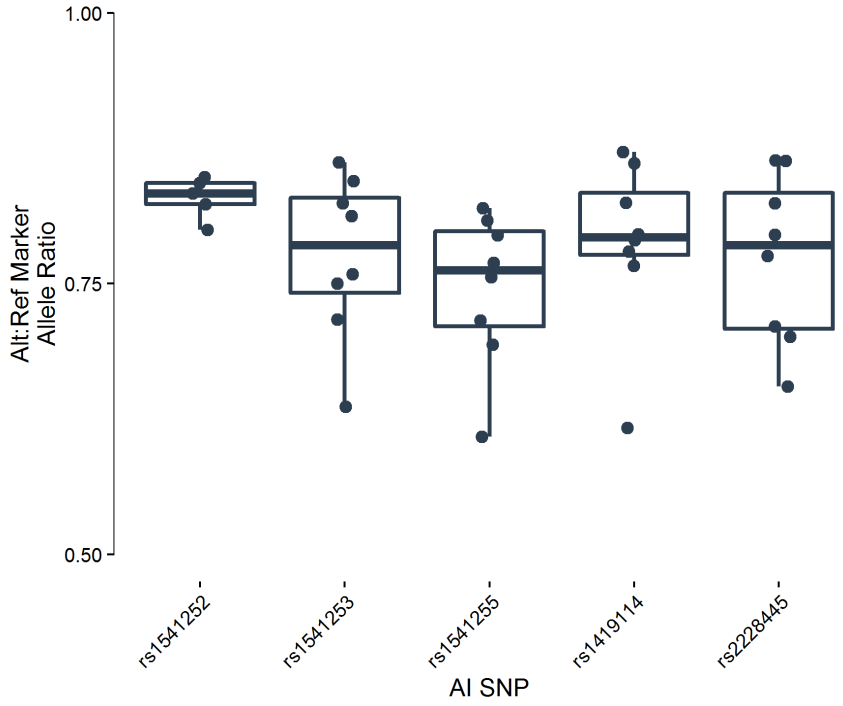

Figure S5.
ATP2B4 allelic imbalance in human erythroblasts. Ratio of reads of the alternate allele over the total number of reads overlapping *ATP2B4* exonic SNPs. In the absence of allelic imbalance and read mapping biases, the expected ratio is 0.5. All heterozygous samples at these different exonic SNPs are also heterozygotes for the top *ATP2B4* candidate eQTLs.

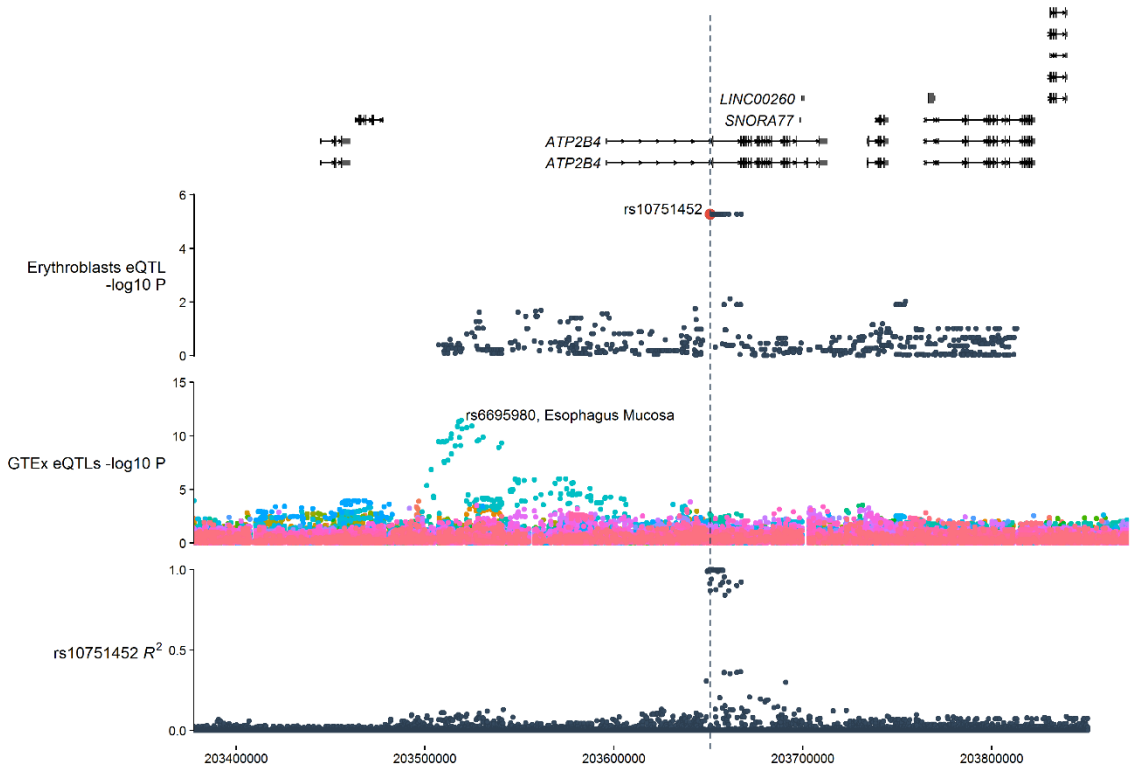

Figure S6.
Erythroblasts show an eQTL signal that is independent from *ATP2B4* eQTLs found in GTEx. The bottom track shows pairwise linkage disequilibrium (LD, r^2) between rs1075152 and surrounding SNPs. There is essentially no LD between the *ATP2B4* erythroblast eQTL (rs1075152) and eQTLs found in esophagus mucosa from the GTEx resource.

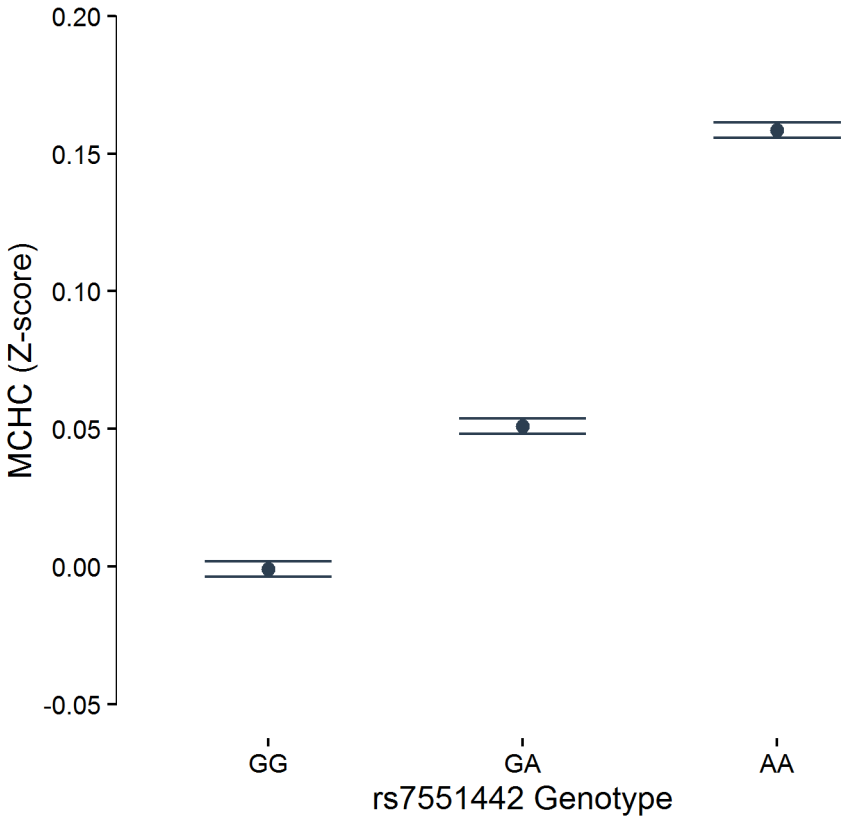

Figure S7.
Association of rs7551442 with mean corpuscular hemoglobin concentration (MCHC) in the UK Biobank. Dots represent the mean normalized MCHC by genotype, while the error-bars represent the standard errors.

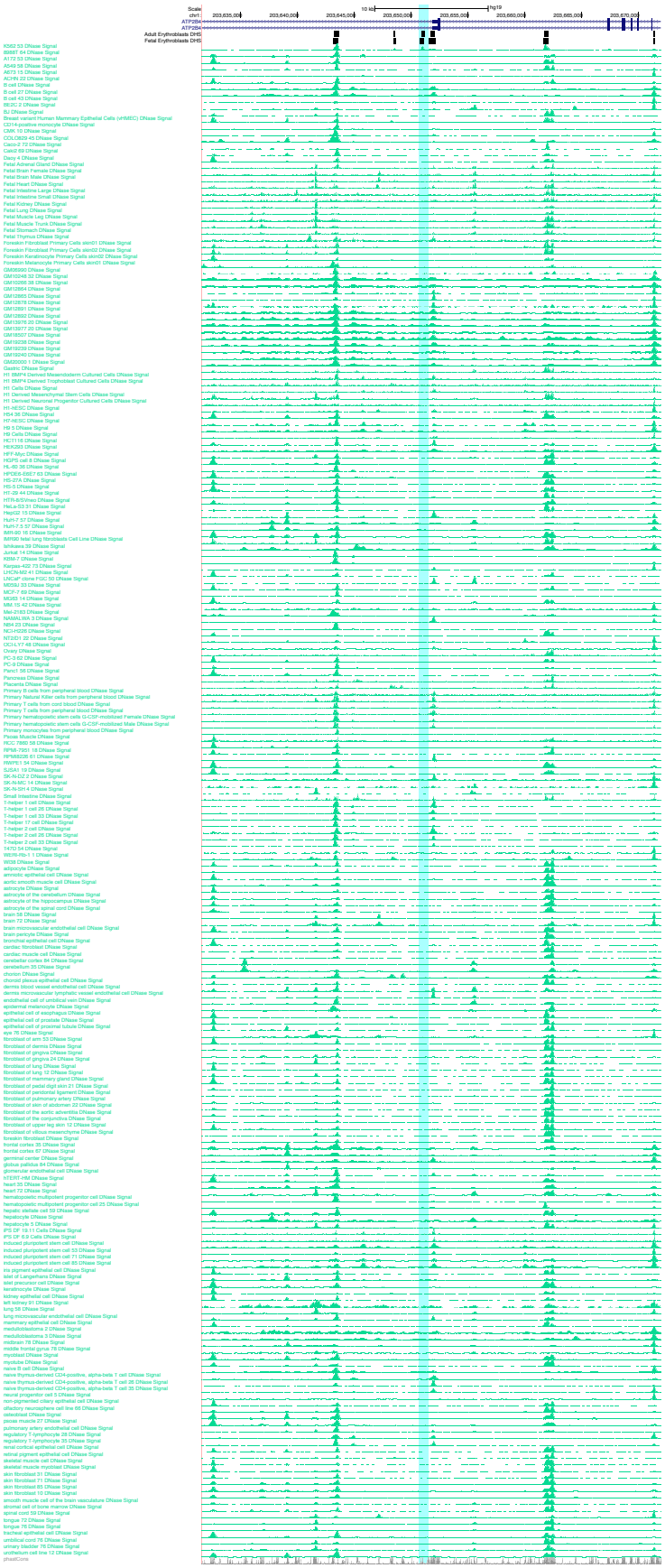
Scale
chr1
203,635,000
203,640,000
203,645,000
10 kb
hg19
203,650,000
203,655,000
203,660,000
203,665,000
203,670,000
ATP2B4
Adult Erythroblasts DHS
Fetal Erythroblasts DHS
K562 53 DNase Signal
BM8R7 64 DNase Signal
A172 53 DNase Signal
A549 58 DNase Signal
A673 15 DNase Signal
AG16 22 DNase Signal
B cell DNase Signal
B cell 27 DNase Signal
B cell 43 DNase Signal
BE2C 2 DNase Signal
BJ DNase Signal
Breast variant Human Mammary Epithelial Cells (vHMEC) DNase Signal
CD14-positive monocyte DNase Signal
CMK 10 DNase Signal
COLO829 45 DNase Signal
Caco-2 72 DNase Signal
Caki2 69 DNase Signal
Daoy 4 DNase Signal
Fetal Adrenal Gland DNase Signal
Fetal Brain Female DNase Signal
Fetal Brain Male DNase Signal
Fetal Heart DNase Signal
Fetal Intestine Large DNase Signal
Fetal Intestine Small DNase Signal
Fetal Kidney DNase Signal
Fetal Lung DNase Signal
Fetal Muscle Leg DNase Signal
Fetal Muscle Trunk DNase Signal
Fetal Stomach DNase Signal
Fetal Thymus DNase Signal
Foreskin Fibroblast Primary Cells skin01 DNase Signal
Foreskin Fibroblast Primary Cells skin02 DNase Signal
Foreskin Keratinocyte Primary Cells skin02 DNase Signal
Foreskin Melanocyte Primary Cells skin01 DNase Signal
GM06990 DNase Signal
GM10248 32 DNase Signal
GM10266 38 DNase Signal
GM12864 DNase Signal
GM12865 DNase Signal
GM12878 DNase Signal
GM12891 DNase Signal
GM12892 DNase Signal
GM13976 20 DNase Signal
GM13977 20 DNase Signal
GM18507 DNase Signal
GM19238 DNase Signal
GM19239 DNase Signal
GM19240 DNase Signal
GM20000 1 DNase Signal
Gastric DNase Signal
H1 BMP4 Derived Mesendoderm Cultured Cells DNase Signal
H1 BMP4 Derived Trophoblast Cultured Cells DNase Signal
H1 Cells DNase Signal
H1 Derived Mesenchymal Stem Cells DNase Signal
H1 Derived Neuronal Progenitor Cultured Cells DNase Signal
H1-hESC DNase Signal
H54 36 DNase Signal
H7-hESC DNase Signal
H9 5 DNase Signal
H9 Cells DNase Signal
HCT116 DNase Signal
HEK293 DNase Signal
HFF-Myc DNase Signal
HGPS cell 8 DNase Signal
HL-60 36 DNase Signal
HPDE6-E6E7 63 DNase Signal
HS-27A DNase Signal
HS-5 DNase Signal
HT-29 44 DNase Signal
HTR-8/SVneo DNase Signal
HeLa-S3 31 DNase Signal
Hepg2 15 DNase Signal
Huh7 57 DNase Signal
Huh7.5 57 DNase Signal
IMR-90 16 DNase Signal
IMR90 fetal lung fibroblasts Cell Line DNase Signal
Ishikawa 39 DNase Signal
Jurkat 14 DNase Signal
KIRK-7 DNase Signal
Kasgas-422 73 DNase Signal
LHCN-M2 41 DNase Signal
LNCaP clone FGC 59 DNase Signal
MDSU 33 DNase Signal
MCF-7 69 DNase Signal
Mz65 14 DNase Signal
MM 1S 42 DNase Signal
Mel-2183 DNase Signal
NAMALWA 3 DNase Signal
NB4 23 DNase Signal
NCI-H226 DNase Signal
NT2/D1 22 DNase Signal
OCI-LY7 48 DNase Signal
Ovary DNase Signal
PC-3 62 DNase Signal
PC-9 DNase Signal
Panc1 56 DNase Signal
Pancreas DNase Signal
Placenta DNase Signal
Primary B cells from peripheral blood DNase Signal
Primary Natural Killer cells from peripheral blood DNase Signal
Primary T cells from cord blood DNase Signal
Primary T cells from peripheral blood DNase Signal
Primary hematopoietic stem cells G-CSF-mobilized Female DNase Signal
Primary hematopoietic stem cells G-CSF-mobilized Male DNase Signal
Primary monocytes from peripheral blood DNase Signal
Psoas Muscle DNase Signal
RCC 7860 58 DNase Signal
RPMI-7951 18 DNase Signal
RPMI8226 61 DNase Signal
RWPE1 54 DNase Signal
SJSA1 19 DNase Signal
SK-N-DZ 2 DNase Signal
SK-N-MC 14 DNase Signal
SK-N-SH 4 DNase Signal
Small Intestine DNase Signal
T-helper 1 cell DNase Signal
T-helper 1 cell 26 DNase Signal
T-helper 1 cell 33 DNase Signal
T-helper 17 cell DNase Signal
T-helper 2 cell DNase Signal
T-helper 2 cell 26 DNase Signal
T-helper 2 cell 33 DNase Signal
T47D 54 DNase Signal
WERI-Rb-1 1 DNase Signal
WI38 DNase Signal
adipocyte DNase Signal
amniotic epithelial cell DNase Signal
aortic smooth muscle cell DNase Signal
astrocyte DNase Signal
astrocyte of the cerebellum DNase Signal
astrocyte of the hippocampus DNase Signal
astrocyte of the spinal cord DNase Signal
brain 58 DNase Signal
brain 72 DNase Signal
brain microvascular endothelial cell DNase Signal
brain pericyte DNase Signal
bronchial epithelial cell DNase Signal
cardiac fibroblast DNase Signal
cardiac muscle cell DNase Signal
cerebellar cortex 84 DNase Signal
cerebellum 35 DNase Signal
chorion DNase Signal
choroid plexus epithelial cell DNase Signal
dermis blood vessel endothelial cell DNase Signal
dermis microvascular lymphatic vessel endothelial cell DNase Signal
endothelial cell of umbilical vein DNase Signal
epidermal melanocyte DNase Signal
epithelial cell of esophagus DNase Signal
epithelial cell of prostate DNase Signal
epithelial cell of proximal tubule DNase Signal
eye 76 DNase Signal
fibroblast of arm 53 DNase Signal
fibroblast of dermis DNase Signal
fibroblast of gingiva DNase Signal
fibroblast of gingiva 24 DNase Signal
fibroblast of lung DNase Signal
fibroblast of lung 12 DNase Signal
fibroblast of mammary gland DNase Signal
fibroblast of pedal digit skin 21 DNase Signal
fibroblast of periorbital ligament DNase Signal
fibroblast of pulmonary artery DNase Signal
fibroblast of skin of abdomen 22 DNase Signal
fibroblast of the aortic adventitia DNase Signal
fibroblast of the conjunctiva DNase Signal
fibroblast of upper leg skin 12 DNase Signal
fibroblast of villous mesenchyme DNase Signal
foreskin fibroblast DNase Signal
frontal cortex 35 DNase Signal
frontal cortex 67 DNase Signal
germinal center DNase Signal
globus pallidus 64 DNase Signal
glomerular endothelial cell DNase Signal
hTERT-RM DNase Signal
heart 35 DNase Signal
heart 72 DNase Signal
hematopoietic multipotent progenitor cell DNase Signal
hematopoietic multipotent progenitor cell 25 DNase Signal
hepatic stellate cell 59 DNase Signal
hepatocyte DNase Signal
hepatocyte 5 DNase Signal
iPS DF 19.11 Cells DNase Signal
iPS DF 6.9 Cells DNase Signal
induced pluripotent stem cell DNase Signal
induced pluripotent stem cell 53 DNase Signal
induced pluripotent stem cell 71 DNase Signal
induced pluripotent stem cell 85 DNase Signal
iris pigment epithelial cell DNase Signal
islet of Langerhans DNase Signal
islet precursor cell DNase Signal
keratinocyte DNase Signal
kidney epithelial cell DNase Signal
left kidney 91 DNase Signal
lung 58 DNase Signal
lung microvascular endothelial cell DNase Signal
mammary epithelial cell DNase Signal
medulloblastoma 2 DNase Signal
medulloblastoma 3 DNase Signal
midbrain 78 DNase Signal
middle frontal gyrus 78 DNase Signal
myotube DNase Signal
myotube DNase Signal
naive B cell DNase Signal
naive thymus-derived CD4-positive, alpha-beta T cell DNase Signal
naive thymus-derived CD4-positive, alpha-beta T cell 26 DNase Signal
naive thymus-derived CD4-positive, alpha-beta T cell 35 DNase Signal
neural progenitor cell 5 DNase Signal
non-pigmented ciliary epithelial cell DNase Signal
olfactory neurosphere cell line 66 DNase Signal
osteoblast DNase Signal
psoas muscle 27 DNase Signal
pulmonary artery endothelial cell DNase Signal
regulatory T-lymphocyte 28 DNase Signal
regulatory T-lymphocyte 35 DNase Signal
renal cortical epithelial cell DNase Signal
retinal pigment epithelial cell DNase Signal
skeletal muscle cell DNase Signal
skeletal muscle myoblast DNase Signal
skin fibroblast 31 DNase Signal
skin fibroblast 71 DNase Signal
skin fibroblast 85 DNase Signal
skin fibroblast 92 DNase Signal
smooth muscle cell of the brain vasculature DNase Signal
stromal cell of bone marrow DNase Signal
spinal cord 59 DNase Signal
tongue 72 DNase Signal
tongue 76 DNase Signal
tracheal epithelial cell DNase Signal
umbilical cord 76 DNase Signal
urinary bladder 76 DNase Signal
urothelium cell line 12 DNase Signal
phastCons

Figure S8.
ENCODE and Roadmap Epigenomic Consortia DNAse I hypersensitive sites (DHSs) signals at the *ATP2B4* locus.

Table S1 (separate file).
Genes with significant allelic imbalance in human erythroblasts. For each sample, we report exonic SNPs displaying significant allelic imbalance along with their overlapping genes. REF: Reference allele of the SNP. ALT: Alternative allele. REF_count: Number of RNA-sequencing reads with the reference allele. ALT_count: Number of reads containing the alternative allele. Total_count: Total number of overlapping reads. Ratio: Ratio of reads with the reference allele over the total number of reads. P: Binomial test P-value (see Methods for details).

Table S2 (separate file).
Significant eQTLs around genes with allelic imbalance in human erythroblasts. eQTL SNPs correspond to candidate regulatory SNPs, whereas AI SNPs correspond to the exonic SNP which we used to test for allelic imbalance. N hets: Number of samples that are heterozygote for the eQTL SNP and for the AI SNP. These samples should display AI if the eQTL SNP is a real regulatory variant. N homs: Number of samples that are homozygote for the eQTL SNP, but heterozygote for the AI SNP. These samples should not display AI. Mean hets: Mean AI ratio of the previously defined heterozygotes. Mean homs: Mean AI ratio of the previously defined homozygotes. AI P: one-sided t-test P-value between homozygotes and heterozygotes. LM Beta: Beta statistic of the linear regression model between the eQTL SNP and gene expression. LM P: P-value of the linear regression model. Combined P: Combined P-values of the linear regression model and AI ratio t-test (see Methods for details). The combined P-value corresponds to the linear regression P-value when it was not possible to test for the difference in AI ratios due to a limited number of samples. q-value: False discovery rate-adjusted P-value. Associated Blood Index corresponds to SNP associated with red blood cell trait from Astle et *al*., *Cell*, 2016. RDW: Red cell distribution width; MCH: Mean corpuscular hemoglobin; MCV: Mean corpuscular volume; IRF: Immature fraction of reticulocytes; MCHC: Mean corpuscular hemoglobin concentration; HGB: Hemoglobin; HLSR#: High light scatter reticulocyte count; HLSR%: High light scatter reticulocyte percentage of red cells; RBC#: Red blood cell count.

Table S3 (separate file).
Genes with erythroblast-specific eQTLs. This table includes genes without any shared eQTL in GTEx. See also Figure 1D.

Table S4.
Top gene ontology terms and mouse phenotypes significantly enriched among erythroblast eGenes.

ID	Name	*P*-value	FDR B&H	Genes from Input	Genes in Annotation
GO: Molecular Function					
GO:0046977	TAP binding	2.18E-05	1.92E-02	3	7
GO:0016404	15-hydroxyprostaglandin dehydrogenase (NAD+) activity	7.52E-05	2.80E-02	2	2
GO:0005344	oxygen transporter activity	2.17E-04	2.80E-02	3	14
GO:0002060	purine nucleobase binding	2.22E-04	2.80E-02	2	3
GO:0016822	hydrolase activity, acting on acid carbon-carbon bonds	2.22E-04	2.80E-02	2	3
GO:0004351	glutamate decarboxylase activity	2.22E-04	2.80E-02	2	3
GO:0016823	hydrolase activity, acting on acid carbon-carbon bonds, in ketonic substances	2.22E-04	2.80E-02	2	3
GO: Biological Processes					
GO:0002486	antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway, TAP-independent	6.59E-07	2.42E-03	3	3
GO:0006779	porphyrin-containing compound biosynthetic process	4.78E-06	4.93E-03	5	29
GO:0019885	antigen processing and presentation of endogenous peptide antigen via MHC class I	5.24E-06	4.93E-03	4	14
GO:0033014	tetrapyrrole biosynthetic process	7.94E-06	4.93E-03	5	32
GO:0006335	DNA replication-dependent nucleosome assembly	9.29E-06	4.93E-03	5	33
GO:0034723	DNA replication-dependent nucleosome organization	9.29E-06	4.93E-03	5	33
GO:0002483	antigen processing and presentation of endogenous peptide antigen	9.39E-06	4.93E-03	4	16
GO:0019883	antigen processing and presentation of endogenous antigen	1.96E-05	9.00E-03	4	19
GO:0001916	positive regulation of T cell mediated cytotoxicity	3.62E-05	1.29E-02	4	22
GO:0048534	hematopoietic or lymphoid organ development	4.19E-05	1.29E-02	21	905
GO:0071103	DNA conformation change	4.27E-05	1.29E-02	11	285
GO:0006778	porphyrin-containing compound metabolic process	4.90E-05	1.29E-02	5	46
GO:1901566	organonitrogen compound biosynthetic process	5.27E-05	1.29E-02	28	1445
GO:0002484	antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway	5.28E-05	1.29E-02	3	9
GO:0002480	antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-independent	5.28E-05	1.29E-02	3	9
GO:0030097	hematopoiesis	6.08E-05	1.40E-02	20	858
Mouse Phenotypes					
MP:0008956	decreased cellular hemoglobin content	8.88E-07	1.22E-03	4	8
MP:0011177	abnormal erythroblast number	1.48E-06	1.22E-03	6	34
MP:0011188	increased erythrocyte protoporphyrin level	1.58E-06	1.22E-03	4	9

MP:0008954	abnormal cellular hemoglobin content	2.62E-06	1.51E-03	4	10
MP:0011176	abnormal erythroblast morphology	4.02E-06	1.85E-03	6	40
MP:0000611	jaundice	4.93E-06	1.90E-03	5	24
MP:0010375	increased kidney iron level	6.07E-06	2.00E-03	4	12
MP:0002642	anisocytosis	8.16E-06	2.23E-03	6	45
MP:0008742	abnormal kidney iron level	8.70E-06	2.23E-03	4	13
MP:0002812	spherocytosis	1.63E-05	3.14E-03	4	15
MP:0004147	increased porphyrin level	1.63E-05	3.14E-03	4	15
MP:0011989	abnormal porphyrin level	1.63E-05	3.14E-03	4	15
MP:0003657	abnormal erythrocyte osmotic lysis	2.18E-05	3.87E-03	5	32
MP:0010163	hemolysis	2.96E-05	4.88E-03	5	34
MP:0010067	increased red blood cell distribution width	4.14E-05	5.73E-03	7	87

Table S5.
Comparison of the association of the rs7551442 A-allele with red blood cell (RBC) traits in the first release of the UK Biobank and effect of the *Atp2b4* targeted deletion on RBC phenotypes in mice. In the UK Biobank, effect sizes for the A-allele at rs7551442 (betas and standard errors (SE)) are in standard deviation units. In the mouse, effect sizes for the deletion allele are in the following units: hemoglobin (HGB, g/dL), hematocrit (HCT, ratio), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), RBC count (RBC, 10^9/L), reticulocyte count (RETIC, %), RBC distribution width (RDW, %), mean corpuscular volume (MCV, fL).

	UK Biobank			***Atp2b4*$^{-/-}$ mice**		
Trait	**N**	**Beta (SE)**	***P***	**N**	**Beta (SE)**	***P***
HBG	128,311	0.034 (0.0066)	$2.1\text{x}10^{-7}$	26	0.0557 (0.098)	0.58
HCT	128,311	0.011 (0.0066)	0.098	26	-0.009 (0.003)	$2.6\text{x}10^{-3}$
MCH	128,253	0.012 (0.0065)	0.062	26	-0.095 (0.106)	0.38
MCHC	128,224	0.058 (0.0065)	$2.6\text{x}10^{-19}$	26	0.893 (0.267)	$2.7\text{x}10^{-3}$
RBC	128,312	0.021 (0.0065)	$1.4\text{x}10^{-3}$	26	0.099 (0.056)	0.088
RETIC	128,348	0.022 (0.0066)	0.022	26	-0.295 (0.130)	0.033
RDW	128,231	-0.064 (0.0065)	$1.2\text{x}10^{-22}$	26	0.409 (0.173)	0.026
MCV	128,311	-0.019 (0.0065)	$2.8\text{x}10^{-3}$	26	-1.420 (0.214)	$7.1\text{x}10^{-7}$

Table S6.
Single guide RNA design.

Single-guide RNA Name	Single-guide RNA sequence	Genomic cleavage position (hg19)
ATP2B4 sgL1	GGACTACGTAACCGGGGCTG	chr1:203650624
ATP2B4 sgR1	TAAATGGTTACGACCTCTGA	chr1:203651551
ATP2B4 sgL2	TTAGCACAGCACCAGAACAT	chr1:203650637
ATP2B4 sgR2	CACAGGTCCCTCAAATAGAC	chr1:203651526
ATP2B4 sg1	CTCCCTTGCTAACCTTGCTG	chr1:203650938
ATP2B4 sg2	TCTCAATTCAGATAGAGGAT	chr1:203650951
ATP2B4 sg3	ATACCTCTCAATTCAGATAG	chr1:203650956
ATP2B4 sg4	TATCCTCTATCTGAATTGAG	chr1:203650964
ATP2B4 sg5	CATTAAAGTTTCTCTGGAGT	chr1:203650988
ATP2B4 sg6	AGCATTAAAGTTTCTCTGGAG	chr1:203650989
ATP2B4 sg7	CCTGAGCATTAAAGTTTCTC	chr1:203650994
ATP2B4 sg8	ACCTCTGAGGAGGGAAGATA	chr1:203651026
ATP2B4 sg9	GGACAGAAGGACCTCTGAGG	chr1:203651036
Non-targeting sg1	CACGGAGGCTAAGCGTCGCAA	
Non-targeting sg2	GCGCTTCCGCGGCCCGTTCAA	
Non-targeting sg3	GATCGTTTCCGCTTAACGGCG	
Non-targeting sg4	GTAGGCGCGCCGCTCTCTAC	
Non-targeting sg5	GCCATATCGGGGCGAGACATG	
Non-targeting sg6	GTACTAACGCCGCTCCTACAG	
Non-targeting sg7	GTGAGGATCATGTCGAGCGCC	
Non-targeting sg8	GGGCCCGCATAGGATATCGC	
Non-targeting sg9	GTAGACAACCGCGGAGAATGC	
Non-targeting sg10	GACGGGCGGCTATCGCTGACT	

Table S7.
qPCR design.

Forward Primer	Reverse Primer	Description
ATP2B4_enhancer_out_F AGAGGATTGTCAGGAATCGGC	*ATP2B4_enhancer_out_R* TGACTCAAGAGAGGCCCGTT	Deletion amplicon detection
ATP2B4_enhancer_in_F CCCTAGTTAGCATGCGTGAGA	*ATP2B4_enhancer_in_R* CTTTGGCAGTTGGTGACGCA	Non-deletion (internal) amplicon detection
ATP2B4_ enhancer_out_F AGAGGATTGTCAGGAATCGGC	*ATP2B4_enhancer_in_R2* CAGCATTTCTCCCCTCAGAA	Non-inversion amplicon detection
ATP2B4_enhancer_out_F AGAGGATTGTCAGGAATCGGC	*ATP2B4_enhancer_in_F2* CTAACGGGCTTTGGAGGATTT	Inversion amplicon detection
ATP2B4_enh_core_F TTCTGAGGGGAGAAATGCTG	*ATP2B4_ enh_core_R* AAGAGGCTTTTGGGGAGAAG	Deletion/non-deletion amplicon detection
ATP2B4_enh_core_F2 CCCTAGTTAGCATGCGTGAGA	*ATP2B4_enh_core_R2* AAATCCTCCAAAGCCCGTTAG	Amplicon Sanger sequencing
ATP2B4_RT_F1 AAAGACCCCATGTTGCTCTC	*ATP2B4_RT_R1* CCCCTTCGTCATCCTCATTG	RT-qPCR