A. Supplemental methods

I. Human studies

Blood samples

Fresh peripheral blood samples were obtained from the three $ENT1_{null}$ individuals (Augustine-null patients, age range: 50-56) and healthy donors (age range: 18-70, as per Etablissement Français du Sang/EFS criteria) in accordance with the Declaration of Helsinki protocols and approved by the Ethical Committee of the National Institute of Blood Transfusion (INTS) (Paris, France). Blood samples from the EFS were from male and female Caucasian donors with a mean age of 50 years. $ENT1_{null}$ patients do not receive any chronic drug treatment. Of note, one of them (P1) is an occasional blood donor for the French national rare blood bank. Umbilical cord blood was obtained immediately following delivery of full-term infants after informed consent and approval by the "Committee for the Protection of Persons" (IRB).

Antibodies

Flow cytometric analysis of ENT1 surface expression was performed using the anti-AUG1 alloantibody from the alloimmunized Augustine-null proband (P3) previously described in the original paper[1]. Monoclonal antibodies used to characterize HSCs and erythropoiesis from human and mice are listed in the supplemental Table S3.

Ex vivo erythropoiesis: Purification and culture of $CD34^+$ cells

Peripheral blood

Peripheral blood mononuclear cells (PBMCs) were obtained from the blood samples of $ENT1_{null}$ patients (n=3) and healthy donors (n=20) using a Ficoll density gradient separation protocol (Pancoll 1.077 g/ml, PAN BIOTECH). $CD34^+$ cells were purified using anti-CD34 conjugated microbeads and manual cell separation columns as per the manufacturers' instructions (StemCell Technologies Inc; MACS Miltenyi Biotec). The purity of the selected

populations was evaluated by FACS-staining using an anti-CD34 mAb against an independent epitope, with the percentages of $CD34^+$ cells ranging from 85-90% (not shown). The cell culture procedure comprised of 2 phases as previously described by (Fibach et al., 1989; Reihani et al., 2016)[2,3]. Briefly, isolated peripheral blood $CD34^+$ cells were cultured for 7 days in Iscove's Modified Dulbecco's Medium IMDM Glutamax (Gibco) supplemented with 15% BIT 9500 (BSA, human insulin, human holo-transferin) (Stem Cell Technologies), 100 U/ml Penicillin Streptomycin (Gibco) and 2 mM L-Glutamine (Gibco), 100 ng/mL human recombinant (hr) interleukin-6 (IL-6) (Miltenyi Biotec), 10 ng/mL hr interleukin-3 (IL-3) (Miltenyi Biotec), and 50 ng/mL stem cell factor (SCF) (Miltenyi Biotec). On day 7, the cells were harvested and cultured for 12 days in the second phase medium (10 ng/mL hr IL-3, 100 ng/mL hr SCF, and 2 U/mL human recombinant Erythropoietin (EPO) at a density of 10^5 cells/ml. For cultures treated with adenosine, cells were incubated with 15 μM adenosine or dimethyl sulfoxide (DMSO) < 0.1 % (v/v) starting from day 0 of the differentiation phase.

Cord blood

$CD34^+$ cells were isolated from umbilical cord (UC) blood obtained immediately following delivery of full-term infants after informed consent and approval. $CD34^+$ cells were then isolated by positive selection (StemCell Technologies Inc; MACS Miltenyi Biotec) according to the manufacturers' recommendations. Progenitors were expanded in expansion media composed of IMDM Glutamax (ThermoFisher Scientific) supplemented with 15% BIT 9500 (BSA, human insulin, human holo-transferin) (Stem Cell Technologies), 5% fetal bovine serum (FBS), 25 ng/ml rhuSCF, 10 ng/ml rhuIL-3, and 10 ng/ml rhuIL-6 (Miltenyi Biotec). Erythropoiesis was induced by addition of 3 U/ml recombinant human erythropoietin (rEPO) using a three-phase culture system. Briefly, cells were cultured in 3% human serum, 2% human plasma, 15% BIT supplemented with 10 ng/mL stem cell factor, 1 ng/mL interleukin-3 (IL-3), and 3 IU/mL erythropoietin (EPO) (phase 1; Day 0 to day 7). In phase 2 (day 7 to day 10), IL-

3 was omitted from the culture medium. During phase 3 (lasting until day 17), SCF was removed from the culture medium. Cells were cultured at 37 °C in the presence of 5% CO_2 and maintained at a concentration of 10^5 cells/mL.

Virus production and transduction of $CD34^+$ progenitor cells

The lentiviral pLKO.1 plasmid harboring an shRNA directed against IDH1 (Clone ID: TRCN0000043645) was obtained from Sigma-Aldrich. Cells were transduced with either this shRNA or a plasmid where the 714bp sequence encoding for EGFP was inserted in place of the puromycin gene at the unique BamHI and KpnI restriction sites. Virions were generated by transient transfection of 293T cells with these vectors together with the Gag-Pol packaging construct 8.91 and a plasmid encoding the VSV-G envelope, pCMV-VSV-G, as described previously[4]. Viral supernatant was harvested 24 hours post-transfection and virions were concentrated by centrifugation overnight at 4°C at 4,000 rpm. Isolated virions were resuspended in RPMI with 1% BSA and stored at -80°C. Titers were determined by serial dilutions of vector preparations using Jurkat cells and are expressed as Jurkat transducing units (TU/ml). Three constructs were tested and the one inducing the highest levels of downregulation in Jurkat cells was used. For transduction of $CD34^+$ progenitors, cells were expanded for 3 days in Stem Span medium (Stem cell Technologies Inc) supplement with 5% fetal bovine serum (FBS), 25ng/ml rhuSCF (Amgen), 10 ng/ml rhuIL-3 and 10ng/ml rhuIL-6 (R&D) at 37°C. 5×10^5 cells were then exposed to viral supernatants containing 4.8×10^5 TU to 9.9×10^5 TU (representing a multiplicity of infection of 1-2). 72h after transduction, erythroid differentiation was initiated by the addition of rEPO (3U/ml). Transduction efficiency was monitored as the fraction of cells expressing GFP.

Quantitative Real Time PCR

Total RNA was isolated at the indicated time points using the RNeasy Mini kit (Qiagen). Quantitative PCR of cDNAs was performed using the Quantitect SYBR green PCR Master mix

(Roche) with 10 ng of cDNA and 0.5 µM primers in a final volume of 10µl. Primer sequences are as follows: ENT1: 5'- TGTCCTTGGTCACTGCTGAA-3' (forward)/ 5'-GGAGTTGAGGTAGGTGAATA-3' (reverse); and β-actin: 5'-GTCTTCCCCTCCATCGTG-3' (forward)/ 5'-TTCTCCATGTCGTCCCAG-3' (reverse). Amplification of cDNAs was performed using the LightCycler 480 (Roche). Cycling conditions comprised of a denaturation step for 5 min at 95°C, followed by 40 cycles of denaturation (95°C for 10 sec), annealing (63°C for 10 sec) and extension (72°C for 10 sec). After amplification, melting curve analysis was performed with denaturation at 95°C for 5 sec and continuous fluorescence measurement from 65°C to 97°C at 0.1°C/s increments. Each sample was amplified in triplicate. Relative expression was calculated by normalization to β-actin.

May- Grünwald Giemsa staining

1×10^5 cells were used for cytospin preparations on coated slides, using the Thermo Scientific Shandon 2 Cytospin. The slides were stained with May-Grunwald (Sigma MG500) solution for 5 minutes, with May- Grünwald diluted twice for 5 minutes, and subsequently stained with Giemsa solution (Sigma GS500) for 15 minutes. Following staining, the slides were rinsed twice in demineralized water for 4 minutes. The cells were imaged using a Nikon Eclipse Ti-S inverted microscope. The same protocol was used to stain blood smears. For blood smear preparation, a drop of blood was placed on one end of a glass slide and dispersed over the slide's length. MGG staining was performed in the INTS and Robert Debré hospital. For murine red cells, slides were stained using the Wright-Giemsa stain kit (WG16-500ml, Sigma-Aldrich).

Methylcellulose assay

Methylcellulose assay was performed according to the manufacturer's instructions (Stemcell Technologies). Briefly, PBMCs were isolated from peripheral blood of $ENT1_{null}$ proband and healthy control, using a Ficoll density gradient centrifugation (Pancoll 1.077 g/ml, PAN BIOTECH). To analyze the colony-forming units derived from hematopoietic stem cells, 3×10^5

PBMCs were mixed with 3 mL MethoCult® media supplemented with recombinant cytokines without EPO (Stemcell Technologies, Inc., Vancouver, BC, Canada) or MethoCult® media supplemented with recombinant cytokines and 3 IU/ml rEPO (Stemcell Technologies, Inc., Vancouver, BC, Canada). The mixture was allowed to stand for 3 min to remove bubbles and a 1-ml mixture was dispensed by pipette for each 30-mm cell culture dish (triplicate assay). Cells were incubated for 3 weeks in a 5% CO_2 incubator at 37°C with humidifying water-containing dishes. After 3 weeks of culture, colonies were observed and counted under a Nikon Eclipse Ti-S inverted microscope, over a grid plate according to the shape and the size of the colonies.

Flow cytometry analysis

Monitoring the expression level of several surface markers using the corresponding monoclonal antibodies listed in Table S3 by flow cytometry was performed to characterize the erythroid differentiation of CD34+ progenitors. Briefly, 0.1×10^6 cells were suspended in 20µL PBS buffer supplemented with 2% bovine serum albumin (BSA) and stained with fluorochrome-conjugated antibodies for 30 minutes on ice. Cells were then washed three times with PBS 2% BSA before analysis. Each cocktail of antibodies contained a viability marker: 7-Aminoactinomycin D (7AAD) (BD Biosiciences) or Sytox Blue (Invitorgen). The Syto 16 green dye (Invitrogen) was used to identify the enucleated reticulocytes at the end of differentiation. Concerning the intracellular labelling of p-CREB, cells were fixed with 1% formaldehyde, 0.025%glutaraldehyde in PBS for 15 minutes. After two washes with PBS, RBCs were permeabilized in 0.1 M Octyl β-D-Glucopyranoside for 15 minutes, and saturated in PBS, 1% BSA, 2% goat serum for 30 minutes. Cells were then washed and the pellet was suspended in the saturation solution and stained with Alexa Fluor 647-mouse Anti-CREB (pS133) (1:20, BD Biosciences) for 30 minutes at 4 °C. At least 10 000 cells were acquired

using BD FACS Canto II flow cytometer coupled with the FACS Diva software (version 8.0.1), and analyzed by FlowJo software (V10).

RBC ghost preparation, SDS-PAGE and Western Blotting

RBC membranes (ghosts) were obtained by hypotonic lysis of freshly drawn RBCs in 5P8 buffer (5 mM Na2HPO4 pH 8.0 and 0.35 mM EDTA pH 8.0) supplemented with cocktails of protease and phosphatase inhibitors from Roche Diagnostics (Meylan, France), followed by four washes with the same buffer. Isolated ghost membranes were used for western blot and phosphoproteomic analysis. For western blot analysis ghost were solubilized in Laemmli buffer and proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membrane was then blocked and incubated with a polyclonal antibody directed against ENT1 (1:500; Applied Biological Materials, Richmond, BC, Canada) for 90 min at RT. Anti-ENT1 labeling was revealed with an anti-rabbit IgG(H+L) horseradish-peroxidaselinked goat antibody (1:5000; P.A.R.I. S Biotech) for 45 min at room temperature. The membrane was also blotted with an HRP coupled anti-actin antibody (Cell Signaling, 13E5, 1:5000), which was used as a loading control.

Hemorheology: Osmascan and Deformability Tests

100 to 200 µL of EDTA fresh whole blood samples were suspended in 5mL of polyvinylpyrrolidone isotonic buffer (PVP ISO, Mechatronics, Hoorn, The Netherlands) and were used for the analysis. The osmotic gradient curves reflect RBC deformability as a continuous function of suspending medium osmolality. Osmoscan test was evaluated at a fixed shear stress of 30Pa, under an osmotic gradient ranging from 50 to 500mOsm at 37°C. Analysis of RBC deformability (EI, elongation index) was determined at 37°C at 9 shear stresses ranging from 0.30 to 30 Pa by laser diffraction analysis (ektacytometry). Briefly, 25 µL of a 45% hematocrit whole blood sample were suspended in 5mL PVP ISO, then 1 mL of the mix was analyzed. The value of 3 Pa is often considered as the threshold between low/moderate shear

and high shear stress values. Both Osmoscan and deformability tests were performed using Laser-assisted Optical Rotation Cell Analyzer (LoRRca MaxSis, Mechatronics, Hoorn, The Netherlands) according to the manufacturer's instructions and as previously reported in detail[5,6].

Nucleotide metabolomics: Quantification of nucleotides

Intracellular pools of nucleotides were quantified based on previously described methods[7,8]. Briefly, cells were lysed in 20µL $HClO_4$ 1M, containing 4µM Adenosine-^{15}N5 5'-monophosphate, Adenosine-^{13}C10,^{15}N5 5'-triphosphate, Guanosine-^{13}C10,^{15}N5 5'-triphosphate, Cytidine-^{13}C9,^{15}N3 5'-triphosphate, 2′-Deoxyadenosine-^{13}C10,^{15}N5 5′-triphosphate and 2′-Deoxycytidine -^{13}C9,^{15}N3 5'-triphosphate, as internal standards. After 12,000g centrifugation for 5 min at 4 °C, supernatants were transferred to a 384-well plate and kept at 4 °C in an auto sampler before injection. Ten µL were injected onto a separation column (Acquity HSS T3 column, 1.8µm particle size, 2.1x100mm (Waters)) with a flow rate of 0.45 mL/min and analyzed with a tandem mass spectrometry system consisting of an Acquity Ultra Performance Liquid Chromatography (UPLC Waters) interfaced with a Xevo-TQ-S tandem quadrupole mass spectrometer (Waters). Mobile phase A was 5mM dibutylamine acetate in water and mobile phase B, 5mM dibutylamine acetate in acetonitrile. A programmed mobile phase-gradient was used during a 24min run: 0 min, 0% B; 20 min, 17% B ;20.1 min, 90% B; 22.1 min, 90% B; 22.3 min, 0% B; 24 min, 0% B. The content of the nucleotides AMP, ATP, dATP, cAMP, GTP, cGMP, CMP, CTP, dCTP and dCDP was quantified in the electrospray positive ion mode with multiple reaction monitoring (MRM). Transitions of m/z 348 > 136, 508 > 136, 492 > 136, 330 > 136, 524 > 152, 346 > 152, 324 > 112, 484 > 112, 468> 112, 388 > 112, were used for quantification of respectively AMP, ATP, dATP, cAMP, GTP, cGMP, CMP, CTP, dCTP and dCDP. Transitions of m/z 348 > 119, 508 > 410, 492 > 81, 330 > 97, 524 > 135, 346 > 135, 324 > 97, 484 > 97, 468 > 130, 388 > 81 were used for confirmation of

respectively AMP, ATP, dATP, cAMP, GTP, cGMP, CMP, CTP, dCTP and dCDP. Concentrations were determined by using calibration curves of the nucleotides. The linearity, exactitude and variability were determined for the technical validation of this assay. The linearity gave a correlation coefficient of the linear regression curves between 0.97 and 0.99 for each nucleotide.

Phosphoproteomics

Sample preparation and mass spectrometry

100 µg of ghost proteins from $ENT1_{null}$ (n=3) and control (n=5) samples, obtained as described above, were solubilized in 2% SDS pH 7.5 and heated at 95°C for 5 minutes. Samples were subsequently reduced with 0.1 M DTT final concentration at 60°C for 1 hour. MS sample preparation was performed using a FASP method (filter aided sample preparation) according to Lipecka et al[9]. Briefly Proteins were transferred to Microcon filter units (30 kDa cut-off) and were washed twice with 200 µl buffer UA (0.05 M ammonium bicarbonate, 8 M urea) and concentrated by centrifugation at 14,000 rpm for 15 minutes. The proteins were alkylated with 100 µl of IAA buffer (0.05 M iodoacetamide,) at room temperature in the dark for 20 minutes and were centrifuged at 14,000 rpm for 10 minutes. The proteins were then washed twice by adding 100 µl of UA buffer before centrifugation at 14,000 rpm for 10 minutes. Proteins were washed twice by 100 µl of 0.05 M ammonium bicarbonate and centrifugation at 14,000 rpm for 10 minutes. Filters units were transferred to new collection tubes and samples were incubated with 40 µl of 0.05 M ammonium bicarbonate containing 2 µg of tryspin in a thermomixer at 37°C for 18 hours. Tubes were centrifuged at 14,000 rpm for 10 minutes, 40 µl of 0.05 M ammonium bicarbonate were added and tubes were centrifuged again. Peptides were finally recovered in the collection tubes.

Phosphopeptide enrichment by titanium dioxide (TiO2)

Phosphopeptide enrichment was carried out using a Titansphere TiO2 Spin tips (3 mg/200 µL, Titansphere PHOS-TiO, GL Sciences Inc, Japan) on the digested proteins for each biological replicate. Briefly, the TiO_2 Spin tips were conditioned with 20µL of solution A (80% acetonitrile, 0,4% TFA), centrifuged at 3,000 x g for 2min and equilibrated with 20µL of solution B (75% acetonitrile, 0,3% TFA, 25% lactic acid) followed by centrifugation at 3,000 x g for 2 min. Peptides were dissolved in 20µL of solution A, mixed with 100µL of solution B and centrifuged at 1,000 x g for 10min. Sample was applied back to the TiO_2 Spin tips two more times in order to increase the adsorption of the phosphopeptides to the TiO_2. Spin tips were washed with, sequentially, 20µL of solution B and two times with 20µL of solution A. Phosphopeptides were eluted by the sequential addition of 50µL of 5% NH4OH and 50µL of 5% pyrrolidine. Centrifugation was carried out at 1,000 x g for 5 min.

Phosphopeptides were purified using GC Spin tips (GL-Tip, Titansphere, GL Sciences Inc, Japan). Briefly, the GC Spin tips were conditioned with 20µL of solution A, centrifuged at 3,000 x g for 2min and equilibrated with 20µL of solution C (0,1% TFA in HPLC-grade water) followed by centrifugation at 3,000 x g for 2min. Eluted phosphopeptides from the TiO_2 Spin tips were added to the GC Spin tips and centrifuged at 1,000 x g for 5min. GC Spin tips were washed with 20µL of solution C. Phosphopeptides were eluted with 70µL of solution A (1,000 x g for 5min) and vacuum dried.

nanoLC-MS/MS protein identification and quantification

Peptides for total proteome analysis were resuspended in 0.1% TFA in HPLC-grade water, 10% acetonitrile and 500ng of each sample was injected in a nanoRSLC-Q Exactive PLUS (RSLC Ultimate 3000, Thermo Scientific, MA, USA). Phosphopeptides were resuspended in 42µL of 0.1% TFA in HPLC-grade water and 5µL of each sample was injected in the mass spectrometer. Samples were loaded onto a µ-precolumn (Acclaim PepMap 100 C18, cartridge, 300 µm i.d.×5 mm, 5 µm, Thermo Scientific, MA, USA), and were separated on a 50 cm reversed-phase liquid

chromatographic column (0.075 mm ID, Acclaim PepMap 100, C18, 2 µm, Thermo Scientific, MA, USA). Chromatography solvents were (A) 0.1% formic acid in water, and (B) 80% acetonitrile, 0.08% formic acid. Samples were eluted from the column with the following gradient 5% to 40% B (120 min), 40% to 80% (6 minutes). At 127 min, the gradient returned to 5% to re-equilibrate the column for 20 minutes before the next injection. One blank was run between biological replicates to prevent sample carryover. Samples eluting from the column were analyzed by data dependent MS/MS, using top-10 acquisition method. Peptides and phosphopeptides were fragmented using higher-energy collisional dissociation (HCD). Briefly, the instrument settings were as follows: resolution was set to 70,000 for MS scans and 17,500 for the data dependent MS/MS scans in order to increase speed. The MS AGC target was set to 3.10^6 counts with maximum injection time set to 60ms, while MS/MS AGC target was set to 1.10^5 with maximum injection time set to 120ms. The MS scan range was from 400 to 2000 m/z. Dynamic exclusion was set to a duration of 30 sec.

The MS files were processed with the MaxQuant software version 1.5.8.3 and searched with Andromeda search engine against the database of human from Swiss-Prot (downloaded 15/04/2019, 20415 entries). MS files from TiO2 enriched peptides (phosphoproteome) were searched using a mass deviation of 4.5 ppm and 20 ppm for parent mass and fragment ions. Strict specificity for trypsin/P cleavage was required, allowing up to two missed cleavage sites. Carbamidomethylation (Cys) was set as fixed modification, whereas oxidation (Met) and N-term acetylation and phosphorylation on S, T and Y were set as variable modifications. The false discovery rates (FDRs) at the protein and peptide level were set to 1%. Scores were calculated in MaxQuant as described previously[10,11]. Match between runs was allowed. The reverse hits were removed from MaxQuant output. Phosphopeptides were quantified according to the MaxQuant label-free algorithm using intensities. Statistical and bioinformatic analysis, including heatmaps, volvano plots and clustering, were performed with Perseus software

(version 1.6.2.3) freely available at www.perseus-framework.org[12]. For statistical comparison we set two groups Control (C) and Patient (P). Each sample was run in technical triplicates as well. We analysed the Phospho(STY).txt file. Phosphopeptides intensities were transformed in log 2, site table was expanded to analyze all phosphosites separately. We filtered the data to keep only proteins with at least 3 valid values in at least one group. Data were imputed to fill missing using a Gaussian distribution of random numbers with a standard deviation of 30% relative to the standard deviation of the measured values and 3 standard deviation downshift of the mean to simulate the distribution of low signal values. We performed a t-test, FDR<0.05 (250 randomizations), S0=0.5.

Whole-exome sequencing and data analysis

Genomic DNA was extracted from leukocytes of $ENT1_{null}$ patients using the MagnaPure system (Roche). Exome capture was performed with the Sure Select Human All Exon Kit (Agilent Technologies). Agilent Sure Select Human All Exon (58 Mb, V6) libraries were prepared from 3 µg of genomic DNA sheared with an ultrasonicator (Covaris) as recommended by the manufacturer. Barcoded exome libraries were pooled and sequenced with a HiSeq2500 system (Illumina), generating paired-end reads. After demultiplexing, sequences were mapped on the human genome reference (NCBI build 37, hg19 version) with Burrows-Wheeler Aligner (BWA). The mean depth of coverage obtained for the three patients' exome libraries was >120X with > =95% and >=94% of the targeted exonic bases covered at least 15 and 30 independent sequencing reads. Variant calling was carried out with the Genome Analysis Toolkit (GATK), SAMtools, and Picard tools. Single-nucleotide variants were called with GATK Unified Genotyper, whereas indel calls were made with the GATK IndelGenotyper_v2. All the variants were annotated and filtered with PolyWeb, an in-house annotation software program.

Sanger sequencing of *ABCC4*

Exon 5 of *ABCC4* was amplified from the genomic DNA of $ENT1_{null}$ patients and the corresponding PCR product was purified with SureClean Plus (Bioline) and sequenced by ABI BigDye terminator chemistry (GATC Biotech). Detailed PCR conditions and primer sequences are available upon request.

Triton extractability and western blot analysis

Membrane proteins from whole ghost lysates (G) or Triton X-100 soluble (S) and insoluble pellet fractions (P) were separated on SDS-PAGE, transferred to nitrocellulose membrane and immunoblotted with the indicated primary antibodies. These antibodies were described previously[13]. Following washing, the nitrocellulose membrane was incubated with the appropriate peroxidase-conjugated secondary antibody (anti-rabbit or anti-mouse, IgG) (Biosys, Compiegne, France). Immunoblots were visualized using the ECL chemiluminescent system (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Statistics

Statistical analyses were performed using GraphPad Prism 7. Statistical tests corresponding to each experiment are indicated in the figure legends. Briefly, comparisons between values were performed using Student's t test, unless otherwise indicated. All comparisons between multiple groups were performed using one-way or two-way analysis of variance (ANOVA). For all statistical analyses, $p < 0.05$ was considered statistically significant. The results are expressed as the means ± SEM, unless otherwise mentioned.

II. Mice and MK-571 preclinical studies

All protocols involving animal studies were reviewed and approved by the Institutional Animal Welfare Committee of the University of Texas Health Science Center at Houston. *Ent1*$^{-/-}$ mice were C57BL/6 background[14]. Mice were purchased from Jackson Lab. Eight to twelve-week-old and sex-matched (including both male and female) C57BL/6 and *Ent1*$^{-/-}$ mice were used for experiments. MK-571 was purchased from sigma Andrich (Cat#: M7571). Eight to twelve-

week-old and sex-matched *Ent1*$^{-/-}$ and WT mice were treated with vehicle or MK-571 at a dosage of 10mg/kg every two days for one week by retro-orbital plexus injection[15]. Mice were sacrificed at the end of the experiments. One mL whole blood was collected with EDTA as an anti-coagulant for complete blood count (CBC) analysis in the University of Texas Health Science Center at Houston. Bone marrow and spleen were isolated, and flow cytometry analysis was performed as below. For bone marrow single-cell suspension preparations, mouse tibia and femur were flushed with HBSS/0.5% BSA/2mM EDTA and the resulting cell suspension was gently passed through a 40mm nylon membrane.

Flow cytometry

Whole bone marrow cells were isolated and stained on ice with various antibody cocktails to identify each progenitor compartment as previously described before[16]. All antibodies were obtained from Biolegend and used at a concentration of 1:100 unless otherwise indicated. For hematopoietic stem cell or progenitor cell analysis, whole bone marrow was treated with red blood cell lysis buffer (Gbico, A1049201) and then further stained with Pacific Blue-conjugated lineage markers (Mac-1, Gr-1, CD4, CD8, B220, and Ter119), IL7Ra-Percp-cy5.5, Sca-1-APC, c-Kit-PEcy7, CD16/32-APC/CY7 and CD34-FITC (RAM34, at 1:50; BD Biosciences) for 1 hour or overnight on ice. Cells were then spun down, resuspended and analysis was accomplished on alive cells with an BD LSR-II (BD Biosciences). To evaluate the differences between *Ent1*$^{-/-}$ and WT mice, data were analyzed for statistical significance by using GraphPad Prism 8, and expressed as the mean ± SD. Differences between the means of multiple groups were compared by two-way ANOVA, followed by a Turkey's multiple comparisons test. P value < 0.05 was considered significant.

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B. Supplemental figures

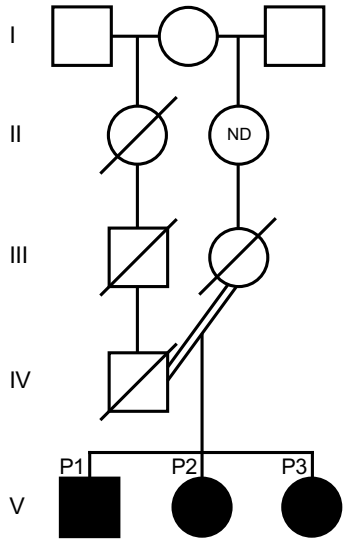

Figure S1. Pedigree of the family of the three ENT1null individuals. Black-filled symbols represent individuals with the Augustine-null blood type, therefore ENT1null. The diagonal lines on symbols represents individuals with a heterozygous mutation in the SLC29A1 gene. ND, no data.

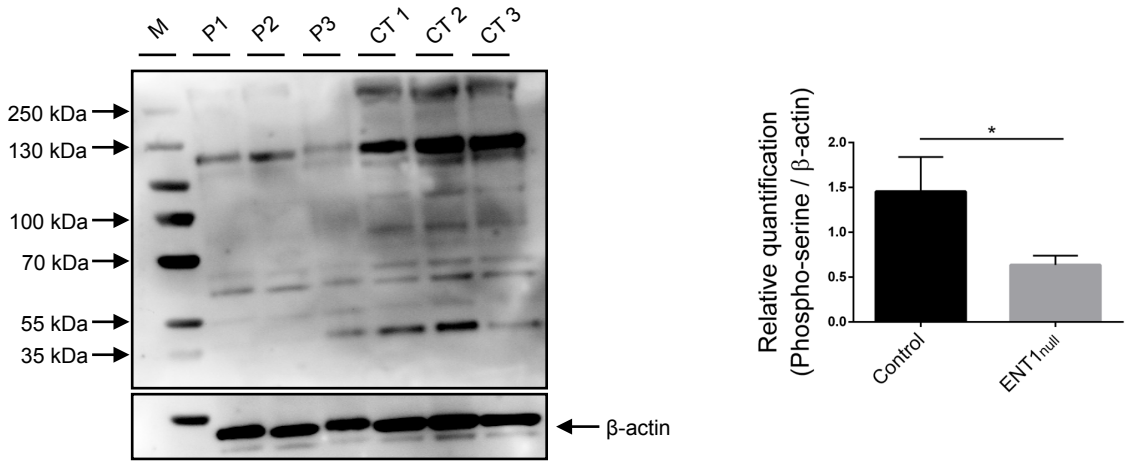

Figure S2. Decreased serine phosphorylation in RBC from ENT1null patients. Western blot of global serine phosphorylation and the corresponding β-actin expression in RBC membranes from the 3 patients (P1, P2, P3) and 3 controls (CT1, CT2, CT3). M corresponds to the protein molecular weight markers (left). Serine phosphorylation was quantified relative to the expression of β-actin and means ± SD are presented (right). *$p<0.05$.

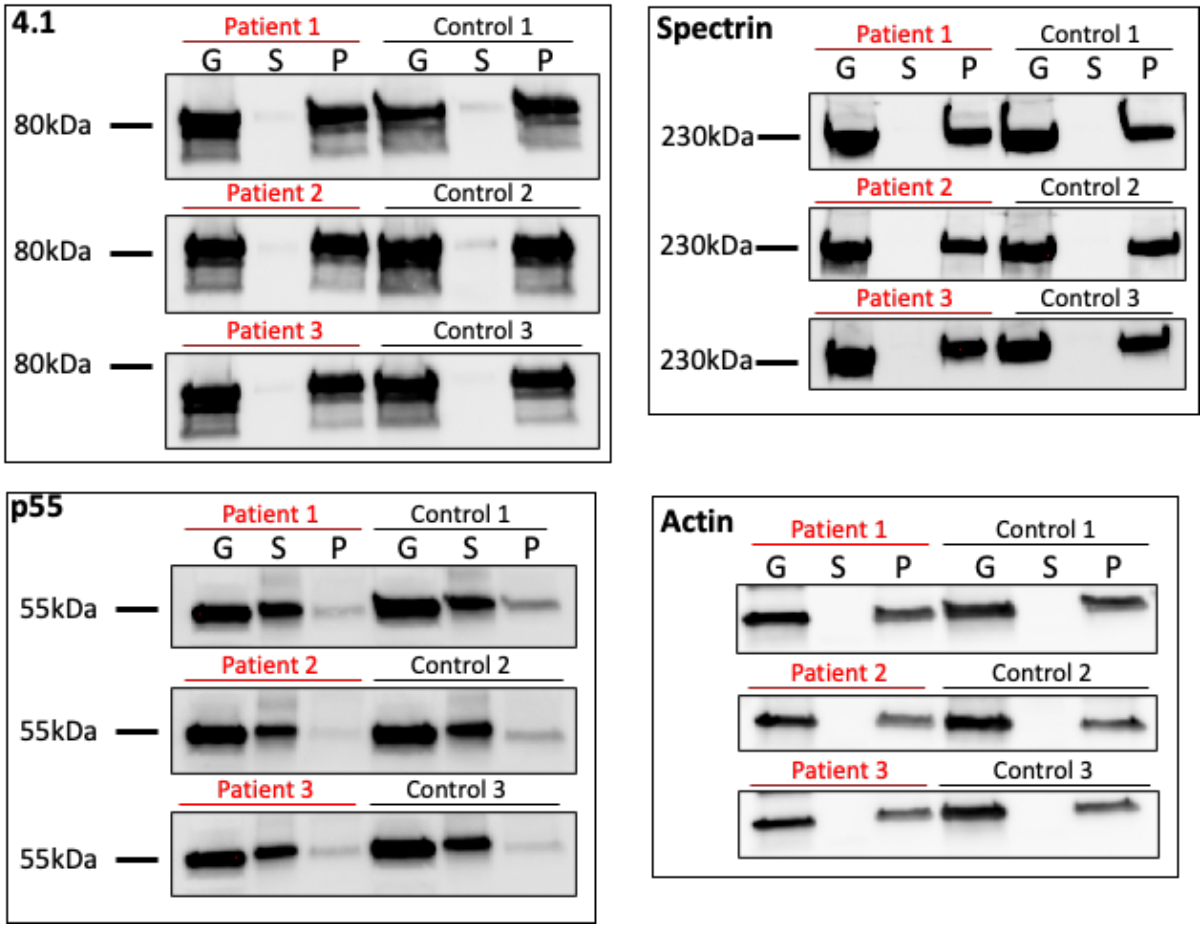

Figure S3. Triton X-100 extractability of skeletal proteins is not altered in ENT1null RBC. Erythrocyte ghosts (G) from control and ENT1$_{null}$ RBCs (Patient 1, 2, and 3) were extracted with 1% Triton X-100 and the soluble (S) and pellet (P) fractions were analyzed. The relative levels of 4.1, spectrin, p55 and actin in the G, S, and P fractions were evaluated by immunoblotting with the corresponding antibody as indicated.

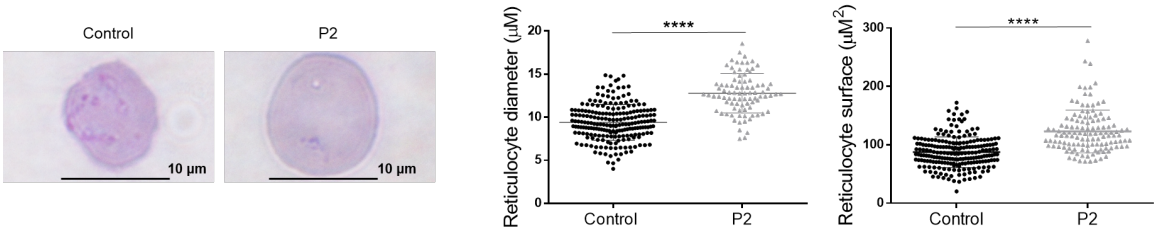

Figure S4. Reticulocyte size and surface area are significantly larger in ENT1null patients than healthy controls. CD34^{+} cells from peripheral blood of control or the P2 ENT1$_{null}$ patient were differentiated *in vitro*. At day 12 of EPO-induced erythroid differentiation, cytospins were stained with MGG to identify reticulocytes. Representative cells (left) as well as cell diameter and (middle) and surface area (right) were measured using ImageJ software (n=213 cells for control and n=88 cells for P2). Data are shown as mean ± SD; ****p<0.0001.

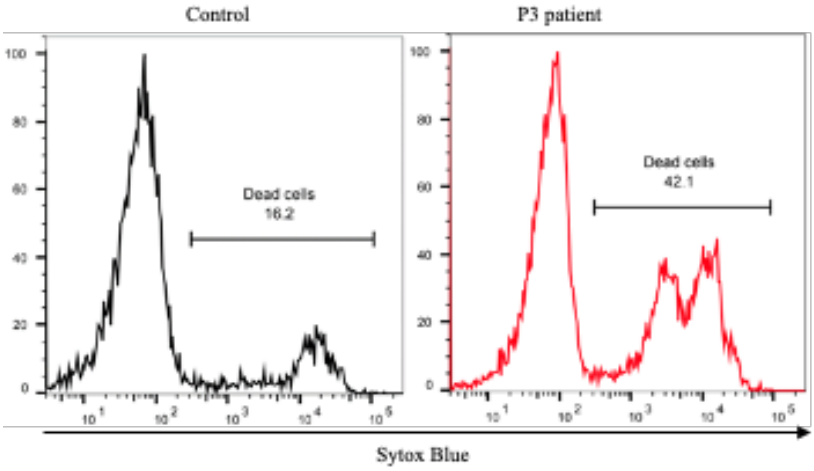

Figure S5. ENT1 deficiency in P3 increased cell death in *ex vivo* erythropoiesis. Dead cells were determined by Sytox blue staining at day 5 of differentiation.

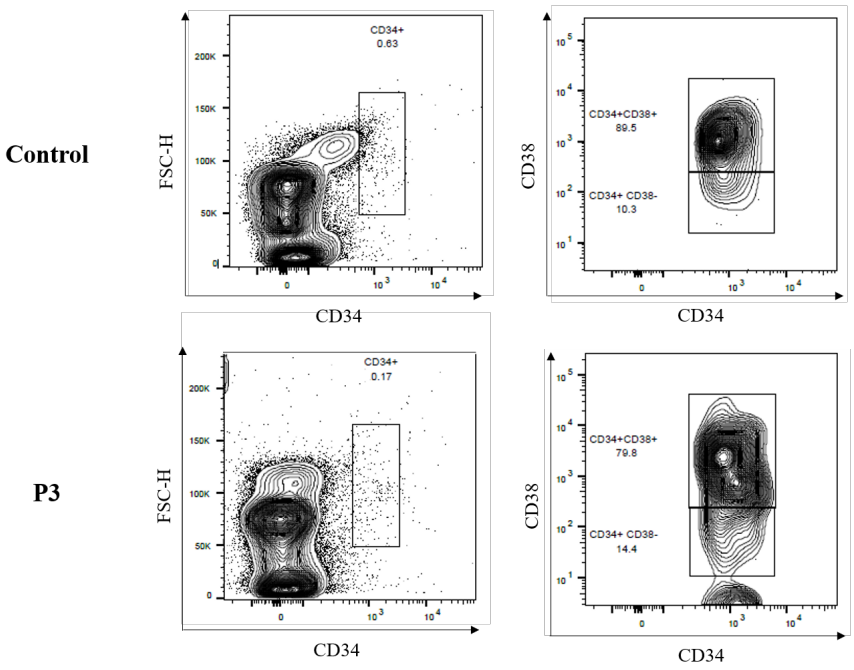

Figure S6. The percentage of $CD34^{+}CD38^{+}$ and $CD34^{+}CD38^{-}$ progenitors in PBMCs from P3 patient and control. PBMC were isolated from peripheral blood of control and the P3 $ENT1_{null}$ patient following by CD34 and CD38 labelling. CD38 expression was analyzed within the CD34 positive cells. Representative percentages are indicated.

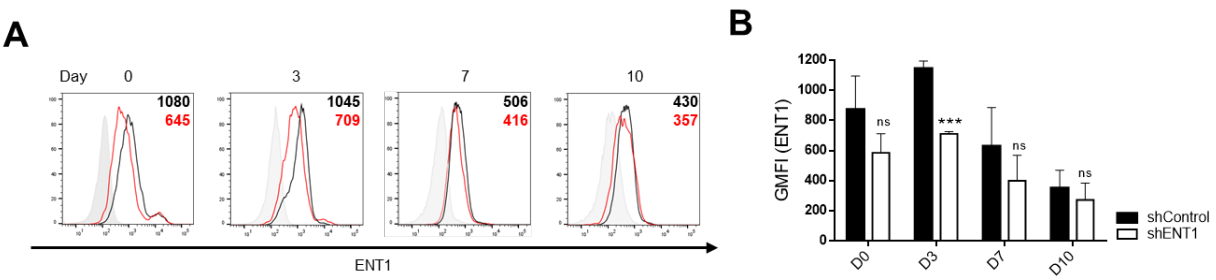

Figure S7. Transduction of CD34$^+$ progenitors with shENT1 lentiviral vector results in an early downregulation of surface ENT1 levels. Adult CD34$^+$ cells from cord blood of healthy donors were expanded for 7 days then differentiated using a three-phase culture system (containing rEPO), recapitulating human erythropoiesis up to the enucleated reticulocyte. Cells were transduced either by ENT1 shRNA or a scramble shRNA at day 4 of the expansion phase. **(A)** Flow cytometric analysis of ENT1 at day 0, 3, 7 and 10 of differentiation, in progenitors transduced with shENT1 (red line) or shControl (black line) vectors. The geometric mean of fluorescence intensity (GMFI) is shown in the plot for shENT1 (red), shControl (black) and non-specific IgG staining (grey shaded). **(B)** Quantification of ENT1 expression at day 0, 3, 7 and 10 of erythroid differentiation was monitored as a function of GMFI and mean levels ± SEM in shENT1- (open bars) and shControl- (black bars) transduced cells are presented. ns, non-significant, ***p<0.001.

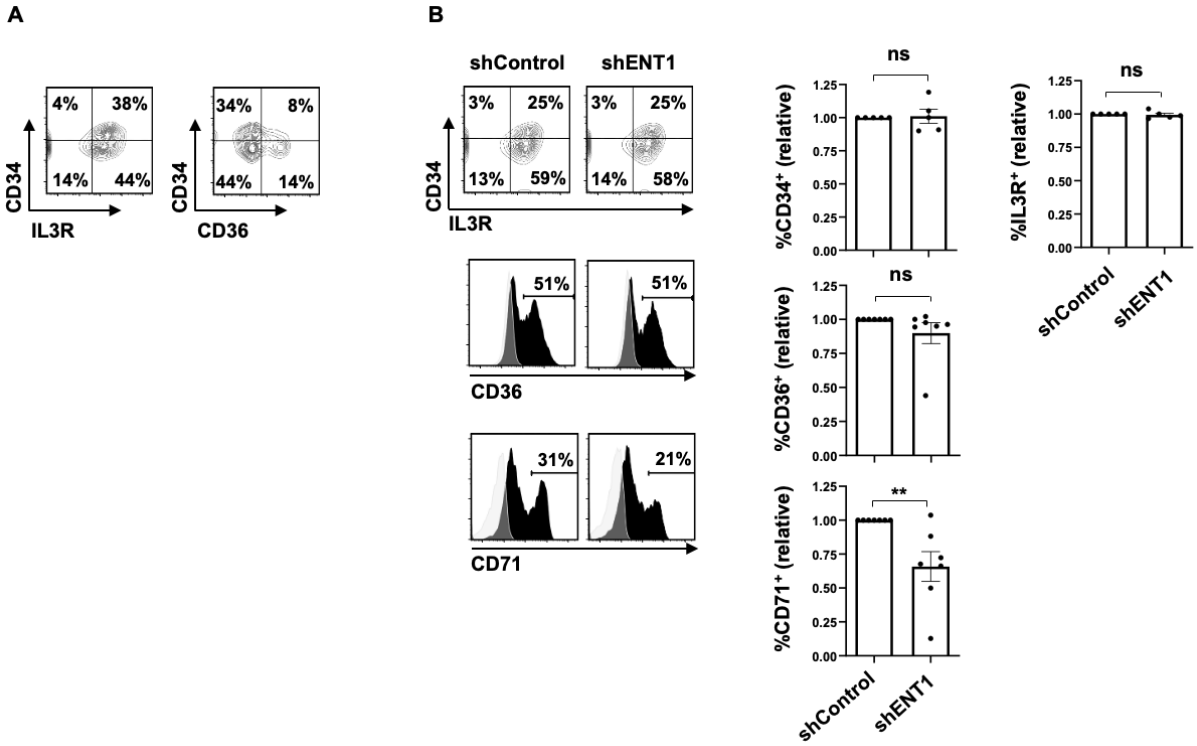

Figure S8. Phenotype of progenitors upon shRNAmediated downregulation of ENT1. (A) Representative profiles of CD34/IL-3R and CD34/CD36 staining at day 4 of expansion of CD34+ progenitors in the presence of recombinant SCF, IL-3 and IL-6. Mean levels of C34+ cells c day 4 was 67±8.6% (n=6). **(B)** Progenitors (shown in A) were transduced with shControl and shENT1 vectors at day 4 of expansion and the phenotypes of the cells were evaluated at day 7, immediately prior to induction of erythroid differentiation by rEPO. Representative dot plots showing CD34/IL-3R profiles, as well as histograms of CD36 and CD71 staining (black)

are presented (left). Isotype staining is shown in grey and percentages of positive-stained cells are indicated. Quantification of expression in shControl- and shENT1-transduced cells are presented and levels were normalized to shControl cells (arbitrarily set at "1"; n=7).

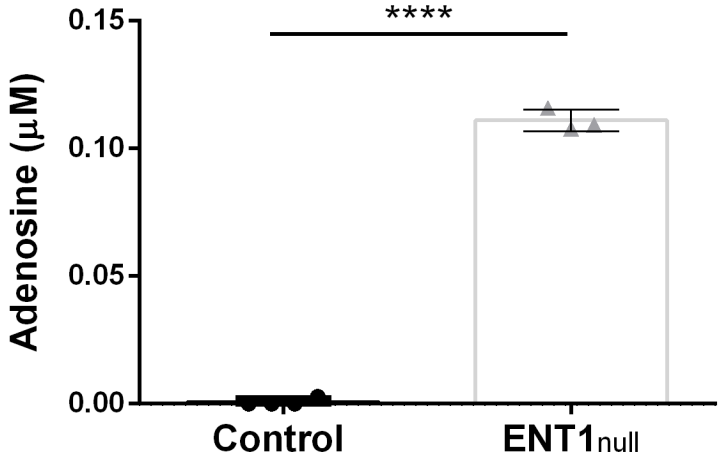

Figure S9. Plasma adenosine levels are significantly elevated in ENT1null patients. Plasma adenosine levels in healthy controls and $ENT1_{null}$ patients were measured by a liquid chromatography–mass spectrometry approach (n=4 controls and n=3 patients). ****p< 0.0001.

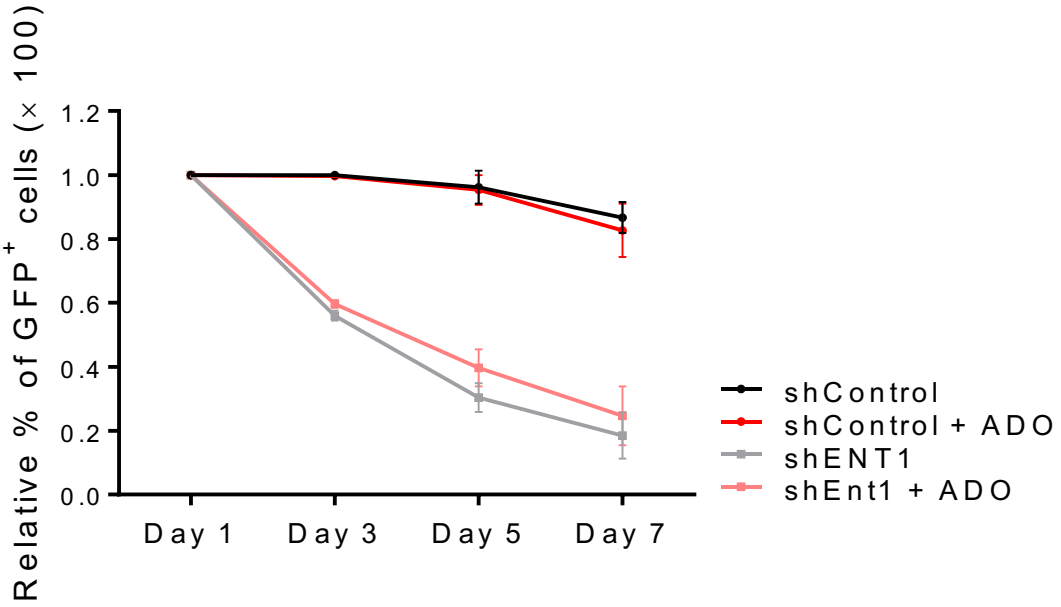

Figure S10. Negative selection of erythroid progenitors with downregulated ENT1. The evolution of shControl- and shENT1-transduced progenitors was monitored as a function of the presence of the GFP transgene, following differentiation for 1-7 days in the presence (+ADO) or absence of adenosine. The percentages of GFP^+ cells relative to day 1 are shown. Means ± SEM from three independent experiments are presented. GFP^+ cells were significantly decreased in shENT1 and shENT1+ ADO conditions compared to shControl and shControl+ ADO (Test two-way ANOVA, p<0.001).

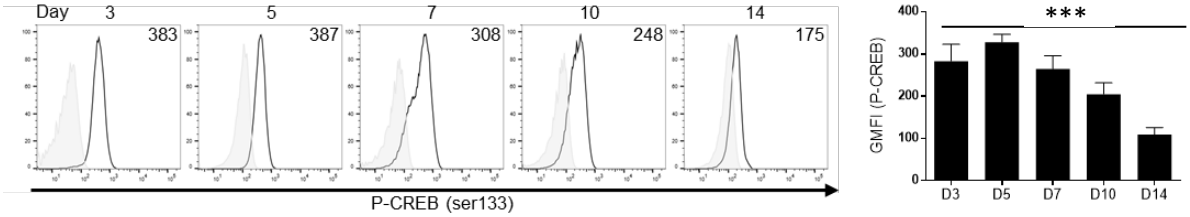

Figure S11. CREB phosphorylation decreases during erythroid differentiation. The phosphorylation state of CREB was monitored by intracellular staining with an anti-phospho-CREB antibody and representative histograms are shown at days 3, 5, 7, 10 and 14 of erythroid differentiation. GMFIs are indicated on each histogram (left). Quantification of GMFI from 10 different experiments was monitored and means ± SEM are presented (right). ***p= 0.0005.

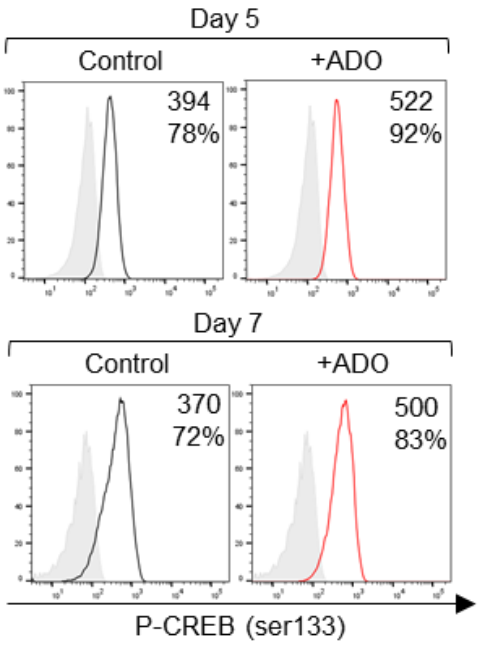

Figure S12. CREB phosphorylation is increased by extracellular adenosine. The phosphorylation of CREB was evaluated at days 5 and 7 of erythroid differentiation in the presence (+ADO) or absence of adenosine. Representative histograms showing phosphorylated CREB in control (black lines) and adenosine-treated cells (red lines) are presented. The percentages of positively stained cells relative to the negative control (grey histograms) as well as the GMFIs are indicated.

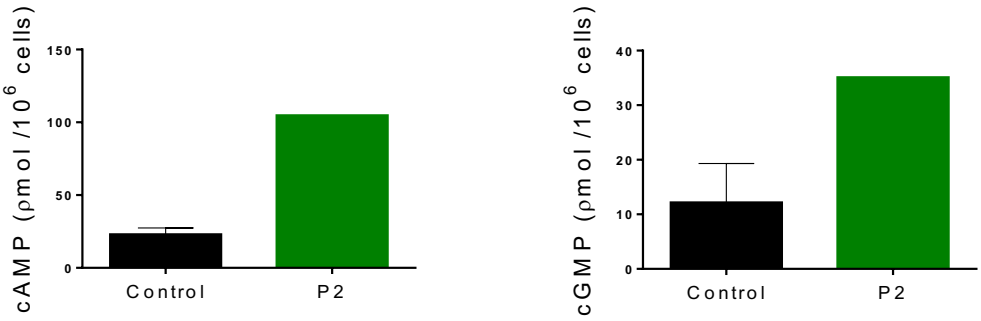

Figure S13. Increased cAMP and cGMP levels in erythroblasts from an ENT1$_{null}$ patient (P2) relative to control erythroblasts. The intracellular concentrations of cAMP (left) and cGMP (right) in immature erythroblasts at day 2 of differentiation were measured by a liquid chromatography–mass spectrometry approach. Quantifications for control (n=3, black bars) and P2 (n=1, green bars) were evaluated and means ± SEM are presented.

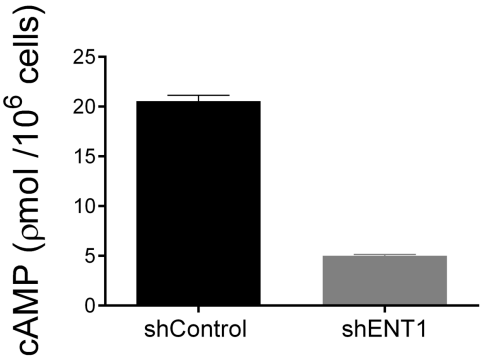

Figure S14. Decreased cAMP levels following downregulation of ENT1 in CD34^{+} progenitors. Intracellular concentrations of cAMP were evaluated at day 3 of EPO-induced erythroid differentiation of shControl- and shENT1-transduced progenitors. cAMP was measured by liquid chromatography–mass spectrometry and mean levels ± SEM from 2 independent experiments are shown.

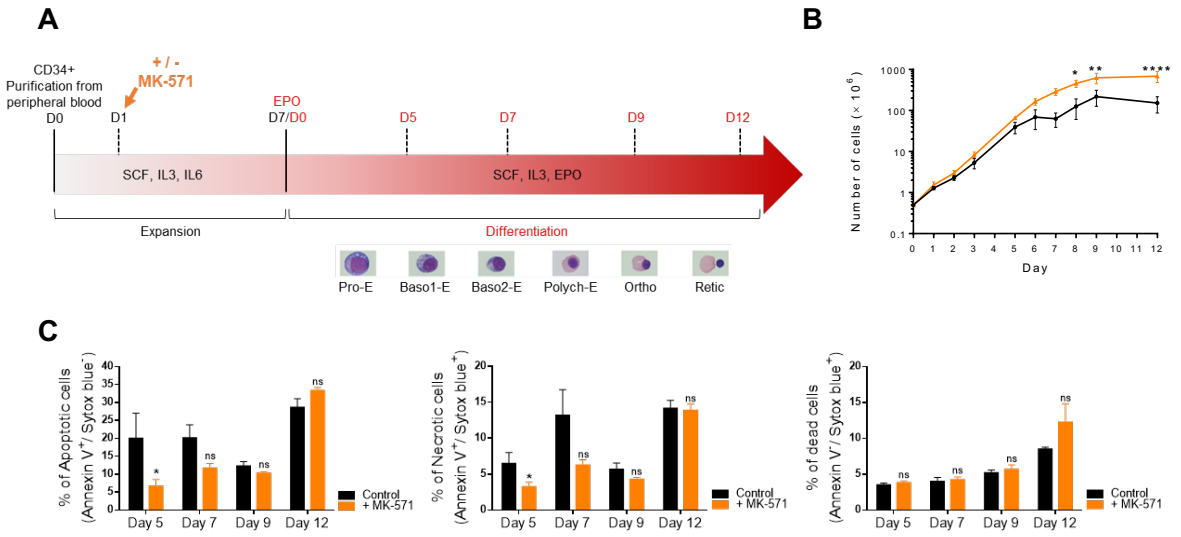

Figure S15. Inhibition of ABCC4 during *in vitro* erythropoiesis of healthy CD34$^+$ cells augments cell expansion without altering cell death. (A) Schematic representation of the schema used to evaluate the role of an MK-571-mediated inhibition on *ex vivo* human erythropoiesis. Adult CD34$^+$ cells isolated from peripheral blood of healthy donors and treated with or without MK-571 (100 µM) starting at day 1. Following a 7-day expansion, progenitors were differentiated with rEPO in a liquid culture system. **(B)** Growth curves of progenitors differentiated in the presence or absence of MK-571 (orange and black lines, respectively) are shown for the indicated day of erythroid differentiation (n=3 independent experiments). *p<0.05, **p<0.01, ****p<0.0001 (unpaired t-test). **(C)** Apoptotic, necrotic and dead cells in control and MK-571-treated cultures were monitored at the indicated days by flow cytometry using Sytox blue and Annexin V and mean levels ± SEM are presented. ns, non-significant, *p<0.05.

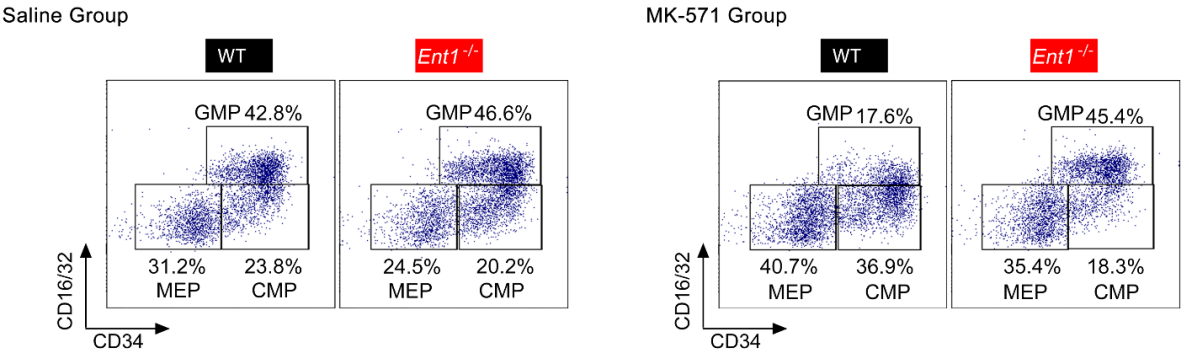

Figure S16. Inhibition of ABCC4 increases the percentages of megakaryocyte erythroid progenitors (MEPs) in BM of WT and *Ent1*$^{-/-}$ mice. Representative dot plots of Lin$^-$cKit$^+$ progenitors from BM of WT and *Ent1*$^{-/-}$ mice under control conditions (saline, left) or following a 6 day treatment with MK-571 (10mg/kg, right). CMP, MEP and GMP were evaluated as a function of their CD34/CD16/32 staining profiles and the percentages of cells in each subset are indicated.

C. Supplemental Tables

Table S1. Hematological parameters of ENT1$_{null}$ patients

	Patient 1	Patient 2	Patient 3	Normal values
RBCs (10^{12}/L)	4.31	3.91	4.22	3.80 - 5.40
Hb (g/dL)	15.8	13.3	14.4	12.5 - 15.5
HCT (%)	46	40	41	37 - 47
MCV (fL)	107#	104#	108#	82 - 97
MCH (pg)	37#	35#	34#	27 - 32
MCHC (g/dL)	35	34	35	32 - 36
RET (%)	1.5	1.1	2.1	0.5 – 2.5
WBCs (10^9/L)	8.42	5.63	8.77	4.00 - 10.00
PLT (10^9/L)	202	233	346	150 - 400
MPV (fL)	11	8	10	7 - 11

RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RET, reticulocytes; WBC, white blood cell; PLT, platelet; MPV, mean platelet volume. #Abnormal values

Table S2. Hematological parameters of wild-type and *Ent1*$^{-/-}$ mice

	C57 Bl/6 (Male, n=5)	*Ent1*$^{-/-}$ (Male, n=5)	*C57 Bl/6* (Female, n=4)	*Ent1*$^{-/-}$ (Female, n=4)
RBC ($1x10^6$cells μl^{-1})	9.12 ± 0.16	7.64 ± 0.48 ***	8.99 ± 0.22	7.22 ± 0.77 **
Hb (gdl^{-1})	13.70 ± 0.43	12.8 ± 0.57 *	13.25 ± 0.73	12.62 ± 0.69
HCT (%)	44.78 ± 1.16	41.38 ± 2.15 *	44.6 ± 1.68	40.2 ± 1.98 *
MCV (fl)	49.12 ± 2.08	54.18 ± 1.33 **	49.27 ± 1.47	57.17 ± 3.38 **
MCH (pg)	15.44 ± 0.15	17.27 ± 0.12**	15.05 ± 0.44	16.95 ± 0.31**
WBC ($1x10^3$cells μl^{-1})	6.00 ± 1.47	5.50 ± 2.95	5.56 ± 3.44	2.39 ± 1.34

*p <0.05, **p <0.01, ***p <0.001 for parameters in *Ent1*$^{-/-}$ mice versus WT C57BL/6 mice of the same gender.

Table S3. Antibodies used for flow cytometry

Antibody	Fluorescence	Clone	Dilution	Brand	Reference
		Human			
CD235a (GPA)	BV 421	GA-R2 (HIR2)	1/20	BD Pharmingen	562938
CD34	PE	563	1/20	BD Pharmingen	550761
CD36	APC	CB38 (NL07)	1/20	BD Pharmingen	550956
CD71	PE	M-A712	1/20	BD Pharmingen	555537
CD49d (α4β1 integrin)	APC	MZ18-24A9	1/20	Miltenyi Biotec	130-093-281
CD233 (Band3)	PE	BRIC6	1/100	IBGRL	9439PE
CD38	BV421	HIT2	1/20	BD Pharmingen	562445
CD11a	PE	TS2/4	1/20	Biolegend	350605
SYTOX blue	Pacific Blue		1/1000	Invitrogen	S34857
Syto 16 green	FITC		1/1000	Invitrogen	S7578
CD123 (IL-3R)	FITC	7G3	1/20	BD Pharmingen	558663
CD33	BV421	WM53	1/20	BD Pharmingen	565949
CREB (pS133)	Alexa Fluor 647	J151-21	1/20	BD Pharmingen	558435
PKA [RIIβ] (pS114)	Alexa Fluor 488	47/PKA	1/20	BD Pharmingen	560204
		Mouse			
Mac-1	Pacific Blue	M1/70	1/100	Biolegend	101217
Gr-1	Pacific Blue	RB6-8C5	1/100	Biolegend	108417
CD3	Pacific Blue	17A2	1/100	Biolegend	100210
B220	Pacific Blue	RA3-6B2	1/100	Biolegend	103225
Ter119	Pacific Blue	Ter-119	1/100	Biolegend	116232
IL7Ra	Percp-cy5.5	A7R34	1/100	Biolegend	135022
Sca-1	APC	D7	1/100	Biolegend	108112
c-Kit	PEcy7	2B8	1/100	Biolegend	105814
CD16/32	APCcy7	93	1/50	Biolegend	101328
CD34	FITC	RAM34	1/50	BD Pharmingen	553733