SUPPLEMENTARY FIGURE LEGENDS

Figure 1S. a. Hemoglobin (Hb) in wild-type (WT; *n*=6) and $Hbb^{th3/+}$mice (*n*=4) mice under either vehicle or bitopertin treatment at 3 or 10 mg/kg/d for 28 days, respectively. Data are presented as median and minimum/maximum; *p< 0.05 compared to WT mice; °p<0.05 compared to baseline values; 2-way ANOVA with Sidak's test for multiple comparisons. **b.** Red blood cell (RBC) and mean cell volume (MCV) in wild-type (WT; *n*=6) and $Hbb^{th3/+}$mice (*n*=4) mice under either vehicle or bitopertin treatment at 3 (*n*=4) or 10 (*n*=4) or 30 (*n*=6) mg/kg/d for 28 days, respectively. Data are presented as median and minimum/maximum; *p< 0.05 compared to WT mice; °p<0.05 compared to baseline values**;** 2-way ANOVA with Sidak's test for multiple comparisons. **c.** Circulating erythroblasts in $Hbb^{th3/+}$mice (*n*=4) mice under either vehicle or bitopertin treatment at 10 mg/kg for 28 days. Data are presented as median and minimum/maximum; °p<0.05 compared to baseline values, 2-way ANOVA with Sidak's test for multiple comparisons. **d.** Plasma erythropoietin (EPO) in $Hbb^{th3/+}$mice (*n*=6) mice under either vehicle or bitopertin treatment at 30 mg/kg/d for 28 days. Data are presented median and minimum/maximum; °p<0.05 compared to vehicle group; Mann-Whitney U test with multiple comparison corrections.

Figure 2S. a. Quantification of iron staining on Pearl's Prussian blue spleen from $Hbb^{th3/+}$mice (*n*=4) mice under either vehicle or bitopertin treatment at 30 mg/kg/d for 28 days. Data are presented as median and minimum/maximum; °p<0.05 compared to vehicle group; Mann-Whitney U test with multiple comparison corrections. **b.** Representative flow cytometric plots of the CD44-Ter119 gating strategy on the bone marrow (left panel) and spleen (right panel) from $Hbb^{th3/+}$ mice treated with either vehicle or bitopertin at the dosage of 30 mg/kg/d for 28 days. This strategy allows the identification of the following homogenous cell populations: population I corresponding to pro-erythroblasts; population II corresponding to basophilic erythroblasts; population III corresponding to polychromatic erythroblasts and population IV corresponding to orthochromatic erythroblasts (see also ref. #1). **c. Left panel.** Flow-cytometric analysis of the maturation pattern of erythroid precursors from the bone marrow (**left panel**) and spleen (**right panel**) of $Hbb^{th3/+}$ mice under either vehicle (black box) or bitopertin (gray box) treatment at 30 mg/kg/d

for 28 days, using the following surface markers: CD71 and TER119. Data are presented as median and minimum/maximum (*n*=5); °p<0.05 compared to vehicle; 2-way ANOVA with Sidak's test for multiple comparisons.

Figure 3S. a. Bar graph showing fold change in the amount of Annexin-V+ $CD44^{Low}TER119^{+}Fsc^{Low}$ cells and $CD44^{-}TER119^{+}Fsc^{Low}$ cells in spleen analyzed by flow cytometry from wild-type (WT) and $Hbb^{th3/+}$ mice with and without bitopertin treatment (10 mg/kg/d, 28 days). Data are presented as means ±SD (n=3); *p< 0.05 compared to WT; °p<0.05 compared to vehicle. **b.** Quantification of iron staining on Pearl's Prussian blue liver from wildtype (WT) and $Hbb^{th3/+}$ mice with and without bitopertin treatment (30 mg/kg/d, 28 days). Data are shown as median and minimum/maximum (*n*=4); **p*<0.05 compared to WT; °*p*<0.05 compared to vehicle; 2-way ANOVA with Sidak's test for multiple comparisons. **c. Upper panel.** Western-blot (Wb) analysis with specific antibodies anti-Phospho-Tyr705 STAT3 (P-STAT3), STAT3, Phospho-Thr202/Tyr204 -Erk1/2 (P-ERK1/2), total Erk1/2 of liver from wild-type (WT) and $Hbb^{th3/+}$ mice treated with either vehicle or bitopertin treatment (30 mg/kg/d, 28 days). GAPDH was used as protein loading control. One representative gel from four with similar results is presented. **Lower panel.** Densitometric analysis is shown in bar graph. Data are presented as median and minimum/maximum; °*p*<0.05 compared to vehicle**,** 2-way ANOVA with Sidak's test for multiple comparisons.

Figure 4S. a. Red blood Cells (RBC), hemoglobin (Hb) and circulating erythroblasts in wildtype (WT; *n*=3) and $Hbb^{th3/+}$ (*n*=4) mice under either vehicle or bitopertin treatment (10 mg/Kg/d, 28 days). Data are presented as median and minimum/maximum; *p< 0.05 compared to WT mice; °p<0.05 compared to baseline values; 2-way ANOVA with Sidak's test for multiple comparisons. **b.** Flow-cytometric analysis combining CD44-TER119 and cell size marker strategy ($CD44+/TER119+/Fsc^{high}$) on bone marrow and spleen from wildtype (WT) and $Hbb^{th3/+}$ mice with and without bitopertin treatment (10 mg/Kg/d, 28 days). Data are presented as median and minimum/maximum (n=4); *p< 0.05 compared to WT mice; °p<0.05 compared to vehicle; 2-way ANOVA with Sidak's test for multiple comparisons.

Figure 5S. a. RT-PCR expression of erythroferrone (*Erfe*; *Fam132b*) on sorted polychromatic erythroblasts (Pop III) and orthochromatic erythroblasts (Pop IV) from bone marrow of $Hbb^{th3/+}$ mice with and without bitopertin treatment (10 mg/kg/d, 28 days). Experiments were performed in triplicate. Data are shown as median and minimum/maximum; °p<0.05 compared to vehicle, ^p < 0.05 Pop IV *vs* Pop III; 2-way ANOVA with Sidak's test for multiple comparisons. **b**. RT-PCR expression of hepcidin (*Hamp)* on liver from wildtype (WT) and $Hbb^{th3/+}$ mice with and without bitopertin treatment (10 mg/kg/d, 28 days). Experiments were performed in triplicate. Data are presented as median and minimum/maximum; *p<0.05 compared to WT; °p<0.05 compared to vehicle; 2-way ANOVA with Sidak's test for multiple comparisons. **c.** RT-PCR expression of Id1 (*Hamp)* on liver from $Hbb^{th3/+}$ mice treated either with vehicle or bitopertin (10 mg/kg/d, 28 days). Experiments were performed in triplicate. Data are presented as median and minimum/maximum; °p<0.05 compared to vehicle; 2-way ANOVA with Sidak's test for multiple comparisons. **d. Upper panel.** Western-blot (Wb) analysis with specific antibodies anti- Phospho-Tyr705 STAT3 (P-STAT3) and anti- STAT3 of liver from $Hbb^{th3/+}$ mice treated with either vehicle or bitopertin (10 mg/kg/d, 28 days). GAPDH was used as protein loading control. One representative gel from four with similar results is presented. **Lower panel.** Densitometric analysis of immunoblots is shown in bar graph. Data are presented as median and minimum/maximum; °p<0.05 compared to vehicle; 2-way ANOVA with Sidak's test for multiple comparisons. **e. Left panel.** Western-blot (Wb) analysis with specific antibodies anti- Phospho-Thr202/Tyr204 -Erk1/2 (P-ERK1/2), Erk1/2 of liver from $Hbb^{th3/+}$ mice treated with either vehicle or bitopertin (10 mg/kg/d, 28 days). GAPDH was used as protein loading control. One representative gel from four with similar results is presented. Densitometric analysis of immunoblots is shown in the graph on the right. Data are presented as median and minimum/maximum; °p<0.05 compared to vehicle**;** 2-way ANOVA with Sidak's test for multiple comparisons. **f. Left panel.** Soluble fractions of liver from $Hbb^{th3/+}$ mice treated with either vehicle or bitopertin (10 mg/kg/d, 28 days). The samples were analyzed on 12% SDS-PAGE and subjected to oxyblot. The carbonylated proteins (1 mg) were detected by treating with DNPH and blotted with anti-DNP antibody. GAPDH was used as protein loading control. Quantification of band area was performed by densitometry and expressed as % of WT (**right panel**). The data are presented as median and minimum/maximum of at least four

experiments; °p<0.05 compared to vehicle**;** 2-way ANOVA with Sidak's test for multiple comparisons.

Figure 6S. a Flow-cytometric analysis combining CD44-TER119 and cell size marker strategy (CD44+/TER119+/Fsc^{high}) on bone marrow and spleen from Hbb$^{th3/+}$ mice treated with either bitopertin (30 mg/Kg/d) or bitopertin (30 mg/Kg/d) plus deferiprone (DFP). Data are presented as median and minimum/maximum (n=4); *p< 0.05 compared to bitopertin treated mice; °p<0.05 compared to baseline; 2-way ANOVA with Sidak's test for multiple comparisons. **b.** Representative flow cytometric profiles of the bone marrow (**upper panel**) and spleen (**lower panel**) from Hbb$^{th3/+}$ mice treated with either vehicle or bitopertin (30 mg/Kg/d) or bitopertin (30 mg/Kg/d) plus deferiprone (DFP), using the CD44-Ter119 and cell size marker strategy. Population I corresponding to pro-erythroblasts; population II corresponding to basophilic erythroblasts; population III corresponding to polychromatic erythroblasts and population IV corresponding to orthochromatic erythroblasts. **c.** ROS levels in red cells from Hbb$^{th3/+}$ mice treated with either bitopertin (30 mg/Kg/d) or bitopertin (30 mg/Kg/d) plus deferiprone (DFP) (n=4). Data are presented as median and minimum/maximum (n=6) *p< 0.05 compared to bitopertin treated mice; °p<0.05 compared to baseline, 2-way ANOVA with Sidak's test for multiple comparisons. **d**. TAU gel electrophoresis of red cell membrane from wild-type (vehicle) and Hbb$^{th3/+}$ mice under either vehicle or bitopertin (30 mg/Kg/d) or bitopertin (30 mg/Kg/d) plus deferiprone (DFP) (n=4) to evaluate the amount of α- globin and β-globins associated with red cell membrane. **Right panel.** Quantification of gel bands (OD) expressed as α- globin to β-globin ratio. Data are shown as median and minimum/maximum (n=6); *p< 0.05 compared to wild-type; °p< 0.05 compared to bitopertin treated mice; °p<0.05 compared to baseline; 2-way ANOVA with Sidak's test for multiple comparisons. **e.** Measurement of total and soluble hemoglobin (Hb) by Drabkin's method in hemolysates from Hbb$^{th3/+}$ mice under either vehicle or bitopertin (30 mg/Kg/d) or bitopertin (30 mg/Kg/d) plus deferiprone (DFP) (n=3). Data are shown as median and minimum/maximum (n=4); *p< 0.05 compared to bitopertin treated mice; °p<0.05 compared to baseline; 2-way ANOVA with Sidak's test for multiple comparisons. **f.** Plasma total bilirubin from Hbb$^{th3/+}$ mice under either vehicle or bitopertin (30 mg/Kg/d) or bitopertin (30 mg/Kg/d) plus deferiprone (DFP) (n=4). Data are presented as median and minimum/maximum, *p< 0.05 compared to

bitopertin treated mice; °p<0.05 compared to baseline; Mann-Whitney U test with multiple comparison corrections.

Figure 7S. a. Representative Western-blot (Wb) analysis of HRI phosphorylation in sorted population III, corresponding to polychromatic erythroblasts (Poly-Eb), and population IV, corresponding to orthochromatic erythroblasts (Ortho-EB) from wild-type (WT) mice treated with either vehicle or bitopertin treatment (10 mg/kg/d, 28 days). **b-c.** Densitometric analyses of the immunoblot bands similar to those shown are presented in Figure 4e, (DU: densitometric Unit). Data are shown as median and minimum/maximum (*n*=4); **p*<0.05 compared to WT; °*p*<0.05 compared to vehicle; 2-way ANOVA with Sidak's test for multiple comparisons.

Figure 8S. a. Western-blot (Wb) analysis of Glyt1 transporter in human erythroblasts from CD34+ derived cells of healthy control and β-thalassemic (β -Thal) subjects at 7 days (d) and 14 days (d) of culture, corresponding to early and late phase of erythropoiesis. **Lower panel.** Densitometric analyses of the immunoblot bands similar to those shown are presented at right (DU: densitometric Unit). Data are shown as median and minimum/maximum (*n*=4); § *p*<0.05 compared to 7 days of cell culture; 2-way ANOVA with Sidak's test for multiple comparisons. **b.** Dose response effect of bitopertin (1, 10, 100 nM) on cell proliferation of healthy erythroid precursors derived by in vitro liquid culture of $CD34^+$ cells isolated from peripheral blood of healthy donors (*n*=3). Arrows indicated when bitopertin was added to the culture medium. Data are presented as means± SD; * p<0.05 compared to vehicle treated cells; Wilcoxon-signed-rank test for multiple comparisons. **c.** Representative flow cytometric gating strategy of the human erythroid precursors at 14 days of cell culture. The use of the CD235a and CD71 markers allows to distinguish 4 different erythroid populations: erythroid cell forming units (CFU-E), Pro-erythroblasts (Pro-E), basophilic erythroblasts corresponding to intermediate erythroblasts (Int-E) and polychromatic and orthochromatic erythroblasts corresponding to the late erythroblasts (Late-E) (see also ref. #4 and 44).

Figure 9S. a. Effect of bitopertin (100 nM) on the amount of annexin-V + cells during healthy erythroid maturation from 4 separate individuals at 14 days of cell culture.

Data are shown as median and minimum/maximum (*n*=4); °p< 0.05 compared to vehicle treated cells. **b.** Effect of bitopertin (10 nM) on maturation pattern of healthy erythroid precursors from a single healthy subject. **Left panel.** Representative flow cytometric plot (left panel) and bar graphs of the differentiation pattern of healthy erythroid precursors at 14 days of culture with or without bitopertin (10 nM). The following surface markers were used: CD36, glycophorin-A and CD71. This allows the identification of the following homogenous cell populations: pro-erythroblasts (Pro-E), basophilic erythroblasts corresponding to intermediate erythroblasts (Int-E) and polychromatic and orthochromatic erythroblasts as late erythroblasts (Late- E) (see also ref. #4 and 44). **Right panel- Bar graph**. Data are expressed as percentages and shown as median and minimum/maximum (*n*=4); *p< 0.05 compared to vehicle treated cells; Wilcoxon-signed-rank test for multiple comparisons. **c.** Effect of bitopertin on the index of erythroid maturation of healthy cells from 4 separate individuals at 9 and 14 days of cell culture. *p< 0.05 compared to vehicle treated cells**;** Mann-Whitney U test with multiple comparison corrections. **d.** Effect of bitopertin (10 nM) on the amount of annexin-V + cells during β-thalassemic erythroid maturation from 4 separate individuals at 7 and 14 days of cell culture, *p< 0.05 compared to vehicle treated cells; Mann-Whitney U test with multiple comparison corrections.

SUPPLEMENTAL MATERIALS AND METHODS

Drugs and chemicals. NaCl, Na_3VO_4, bicine, TRIS, Tween 20, choline, $MgCl_2$, glycine, MOPS, hydrocortisone, benzamidine, β-mercaptoethanol, bromophenol blue, sodium dodecil sulphate (SDS), NaF, EDTA, May-Grunwald stain, Giemsa stain, erythropoietin (EPO), albumin from bovine serum (BSA), glycerol, Luminata Forte and Luminata Classico Hrp solutions, dithiotreithol (DTT) were obtained from Merck KGaA (Armstadt, Germany); protease inhibitor cocktail tablets were from Roche (Basel, Switzerland); Triton X-100, Prestained Protein Ladder and Temed were from GE Healthcare (Little Chalfont, UK); 40% Acrylamide/Bis Solution, 37.5:1 was from BIO-RAD (Hercules, CA, USA), Dulbecco's Phosphate Buffered Saline (DPBS) was from Lonza (Verviers, Belgium); Iscove's Modified Dulbecco's Medium (IMDM), α-MEM, Penicillin-Streptamicin, L-glutamine and Fetal Cow Serum (FCS) were from ThermoFisher (Waltham, MO, USA); Amphotericin was from Euroclone (Milan, Italy); MethoCult™ M3234 was from StemCell Techologies (Milan, Italy)..

Flow cytometric analysis of mouse erythroid precursors and molecular analysis of sorted erythroid cells. Flow cytometric analysis of erythroid precursors from bone marrow and spleen of mice from WT and $Hbb^{th3/+}$ mice was carried out as previously described using the CD44-TER-119 strategy. This strategy allows to identify 4 different erythroblast sub-populations: population I, II, III and IV corresponding to pro-erythroblasts, basophilic erythroblasts, polychromatic erythroblasts and orthochromatic erythroblasts, which were sorted from bone marrow of mice from WT and $Hbb^{th3/+}$ mice as previously reported. Flow cytometric analysis was carried out with the FACS CantoII™ flow cytometer (Becton Dickinson, San Jose, CA, USA). The biparametric scatter plots were analyzed with FlowJo software version 10 (Tree Star, Ashland, OR, USA). Whenever indicated we also used CD71-Ter119 gating strategy.

Quantitative-Real time PCR on sorted erythroblasts. For quantitative real time (qRT)-PCR mRNA was isolated and reverse transcribed into high-purity cDNA using μMACS One-step cDNA Kit according to the manufacturer's instructions (Miltenyi

Biotec). We started from 400,000 sorted polychromatic and orthochromatic erythroblasts from bone marrow of the different mouse strains. 1/50th of the reactions were added to appropriate wells of the PCR plates. qRT-PCR was performed by SYBR Green PCR Master Mix (Applied Biosystems) by using Applied Biosystems Model 7900HT Sequence Detection System. Detailed primer sequences are available on request and Table 1S. All PCR reactions were performed in triplicate. Relative gene expression was calculated by using the $2^{-\Delta Ct}$ method, in which Ct indicates cycle threshold, the fractional cycle number where the fluorescent signal reaches the detection threshold. The ΔCt was computed by calculating the difference of the average Ct between the X-gene and the internal control GAPDH. The data are presented as mean ± the standard deviation (SD).

Liver molecular analysis. ***RNA isolation, cDNA preparation and quantitative qRT-PCR.*** Protocols used for RNA isolation, cDNA preparation, and quantitative qRT-PCR have been previously described.[1,5,6] Total RNA was extracted from mouse tissues using Trizol reagent (Life Technologies). Synthesis of cDNA from total RNA (2 μg) was performed using Super Script II First Strand kits (Life Technologies). Quantitative RT-PCR (qRT- PCR) was performed using the SYBR-green method as previously reported. Detailed primer sequences are available on request and on Table 1S. ***Liver Immuno-blot analysis.*** Frozen livers from WT and $Fyn^{-/-}$ mice were homogenized and lysed with iced lysis buffer (LB containing: 150mMNaCl, 25mM bicine, 0.1% SDS, 2% Triton X-100, 1mM EDTA, protease inhibitor cocktail tablets [Roche], 1mMNa_3VO_4 final concentration) followed by centrifugation for 30min at 4°C at 12,000 g. Proteins were quantified and analyzed by mono-dimensional SDS polyacrylamide gel electrophoresis. Gels were transferred to nitrocellulose membranes for immunoblot analysis with specific antibodies: anti-HO-1, STAT3 (C-20) and anti-NQO-1 (Santa Cruz Biotechnology, Texas, USA); Phospho-Thr202/Tyr204 p44/42 MAPK (P-Erk1/2), p44/42 MAPK (Erk1/2) were from Cell Signaling Technology (MA, USA); Phospho-Tyr705 STAT3 (P-STAT3, clone EP2147Y) was from AbCam (Cambridge, UK); anti-peroxiredoxin-2 (Prx2) (kindly provided by Prof. Ho Zoo Chae, School of Biological Science and Technology, Chonnam National University, Gwangju, Korea). Anti-GAPDH (clone D6, Santa Cruz Biotechnology, Texas, USA) was used as loading control. Secondary donkey anti-

rabbit IgG and anti-mouse IgG HRP conjugated were from GE Healthcare Life Sciences, secondary donkey anti goat HRP conjugated was from Santa Cruz Biotechnology (Texas, USA). Blots were developed by using the Luminata Forte Chemiluminescent HRP Substrate from Merck KGaA (Armstadt, Germany), and images were acquired by using Image Quant Las Mini 4000 Digital Imaging System (GE Healthcare Life Sciences).

Oxyblot was carried out as previously reported by Matte A *et al.*(Ref. 12).

Densitometric analyses were performed using the ImageQuant TL software (GE Healthcare Life Sciences).

***In vitro* erythropoiesis from CD34$^+$ cells from peripheral circulation of normal and β-thalassemia-intermedia subjects**

Cell culture, phenotypic and cell sorting strategy.

Light-density mononuclear cells were obtained by centrifugation on Lymphoprep (Nycomed Pharma, Oslo, Norway) density gradient, as previously described (11, 17). The CD34$^+$ cells were positively selected by anti-CD34-tagged magnetic beads (Mini-MACS columns; Miltenyi Biotech, Auburn, CA) according to the manufacturer's protocol. The recovery was more than 90% CD34$^+$ cells, as determined by flow cytometry. CD34$^+$ cells were grown at a density of 10^5 cells/mL in alpha-minimal essential medium (α-MEM; GIBCO, Grand Island, NY) supplemented with 100 U/mL penicillin-streptomycin, 2 mmol/L L-glutamine, 10^{-6} mol/L hydrocortisone, 10^{-3} g/L nucleotide, $25x10^{-3}$ mg/L gentamicin, 10^{-4} mol/L 2-mercaptoethanol, 1% deionized bovine serum albumin [BSA] (all from Sigma, St Louis, MO), 30% fetal bovine serum (FBS, GIBCO, Grand Island, NY), 1 μg/mL Cyclosporin A (Sigma, St Louis, MO). The following recombinant cytokines were added to the media: 3 U/mL recombinant human (rH) erythropoietin (rHuEPO, Janssen-Cilag, Milan, Italy), 20 ng/mL rH stem cell factor (SCF, PeproTech, London, UK), 10 ng/mL rH interleukin-3 (IL-3, PeproTech, London, UK). Cell samples were collected at day 7and 14 of culture for cell counting and determination of cell viability.

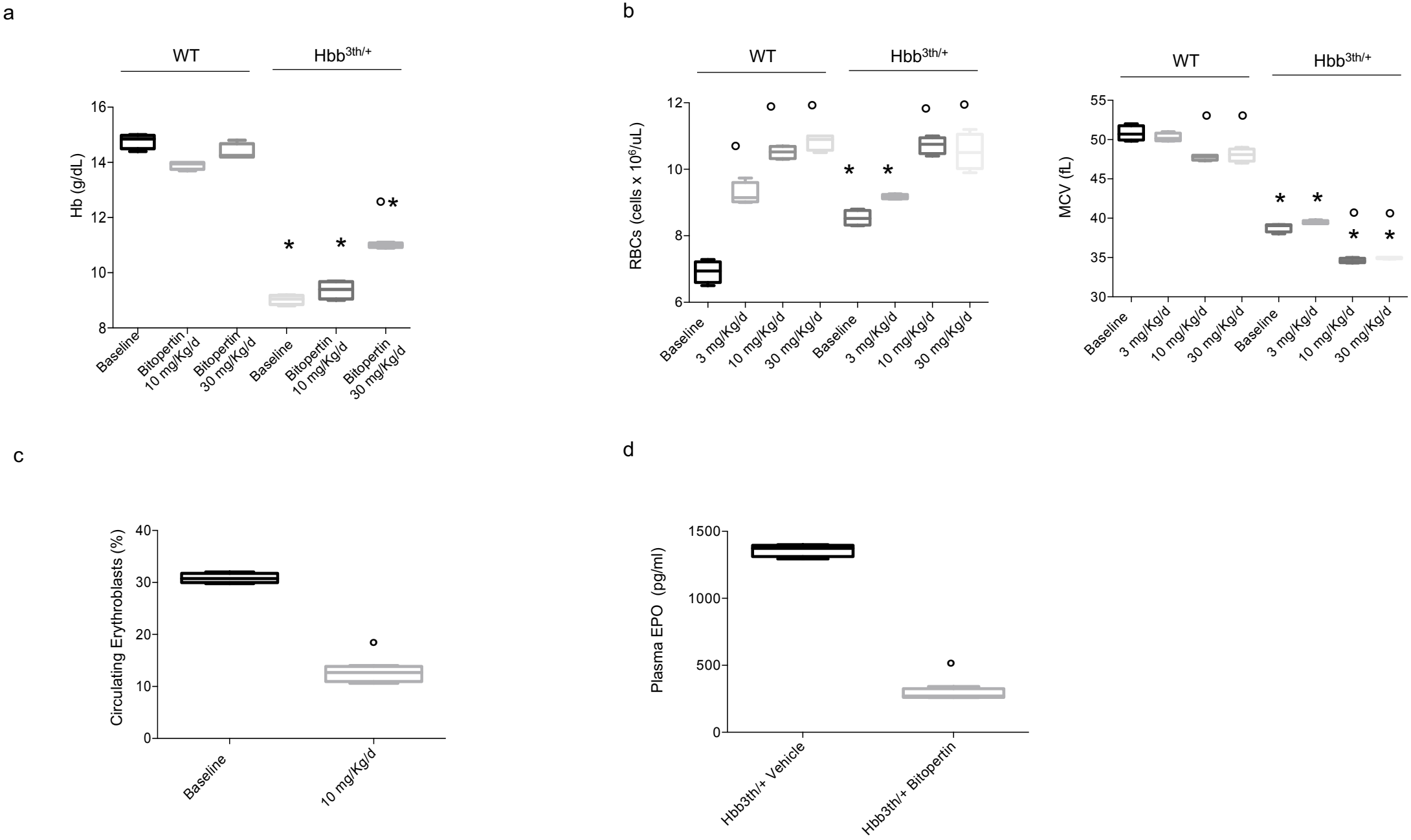
The erythroid cell antigen profile was analyzed using cytofluorimetric strategy with the following surface markers: CD71 (anti-CD71 allophycocyanin (APC)-conjugated; BD Biosciences, San Jose, CA, USA), glycophoryin A (GPA, phycoerythrin (PE)-conjugated anti- CD235a; BD Biosciences, San Jose, CA, USA) and CD36 (anti-

CD36 fluorescein isothiocyanate (FITC)-conjugated; BD Biosciences, San Jose, CA, USA) (22). All the analyses were performed with the flow cytometer FACS Canto II ™ (Becton Dickinson, San Jose, CA, USA). The biparametric scatter plots were analyzed with FlowJo software version 7.6.4 (Tree Star, Ashland, OR, USA). Unstained cells were used as a negative control. Cell apoptosis was determined on sorted CFU-E cells by double-staining the cells with FITC-conjugated Annexin-V and PI. The human Annexin-V-PE Apoptosis Detection Kit (Bender Medsystems, Vienna, Austria) was used, according to the manufacturer's instructions.

Plasma EPO measurement: the concentration of mouse erythropoietin in plasma was determined using the Mouse Erythropoietin Quantikine ELISA Kit (R&D Systems, Minneapolis, USA) following the manufacturer instructions.

Immunoblot analysis of sorted erythroid precursors. 1 x 10^6 cells at 7 and 14 days of normal and β-thalassemic cultures and 1.5 x 10^6 sorted erythroid precursors from WT and $Hbb^{th3/+}$ mice were solubilized as previously described.[3] Proteins were separated by monodimensional electrophoresis and transferred to membrane for immunoblot analysis with specific antibodies: anti- HRI, P-eIF2a, eIF2a, P-Ser2448-mTOR (P-mTOR, clone D9C2), mTOR(clone 7C10), Phospho- Thr202/Tyr204 p44/42 MAPK (P-Erk1/2), p44/42 MAPK (Erk1/2) were from Cell Signaling Technology (MA, USA); Rab5, P62/SQSTM1, Lamp-1 lysosome marker, Catalase, Phospho-Tyr705 STAT3 (P-STAT3, clone EP2147Y) were from AbCam (Cambridge, UK); anti-Trx-R1, STAT3 (C-20) were from Santa Cruz Biotechnology (Texas, USA); anti-Glyt1 (Origene Technology Inc, MD, USA); anti-peroxiredoxin-2 (Prx2) (kindly provided by Prof. Ho Zoo Chae, School of Biological Science and Technology, Chonnam National University, Gwangju, Korea). Anti-GAPDH (clone D6, Santa Cruz Biotechnology, Texas, USA) was used as loading control. When indicated, we carried out an immunoprecipitation assay as previously described. We carried out the densitometric analysis of the scanned images of unsaturated films (ImageJv 1.28 software).

Figure 1S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Hbb3th/+
RBCs (cells x 10^6/uL)
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10
8
6
Baseline
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WT
Hbb3th/+
MCV (fL)
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c
Circulating Erythroblasts (%)
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30
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1000
500
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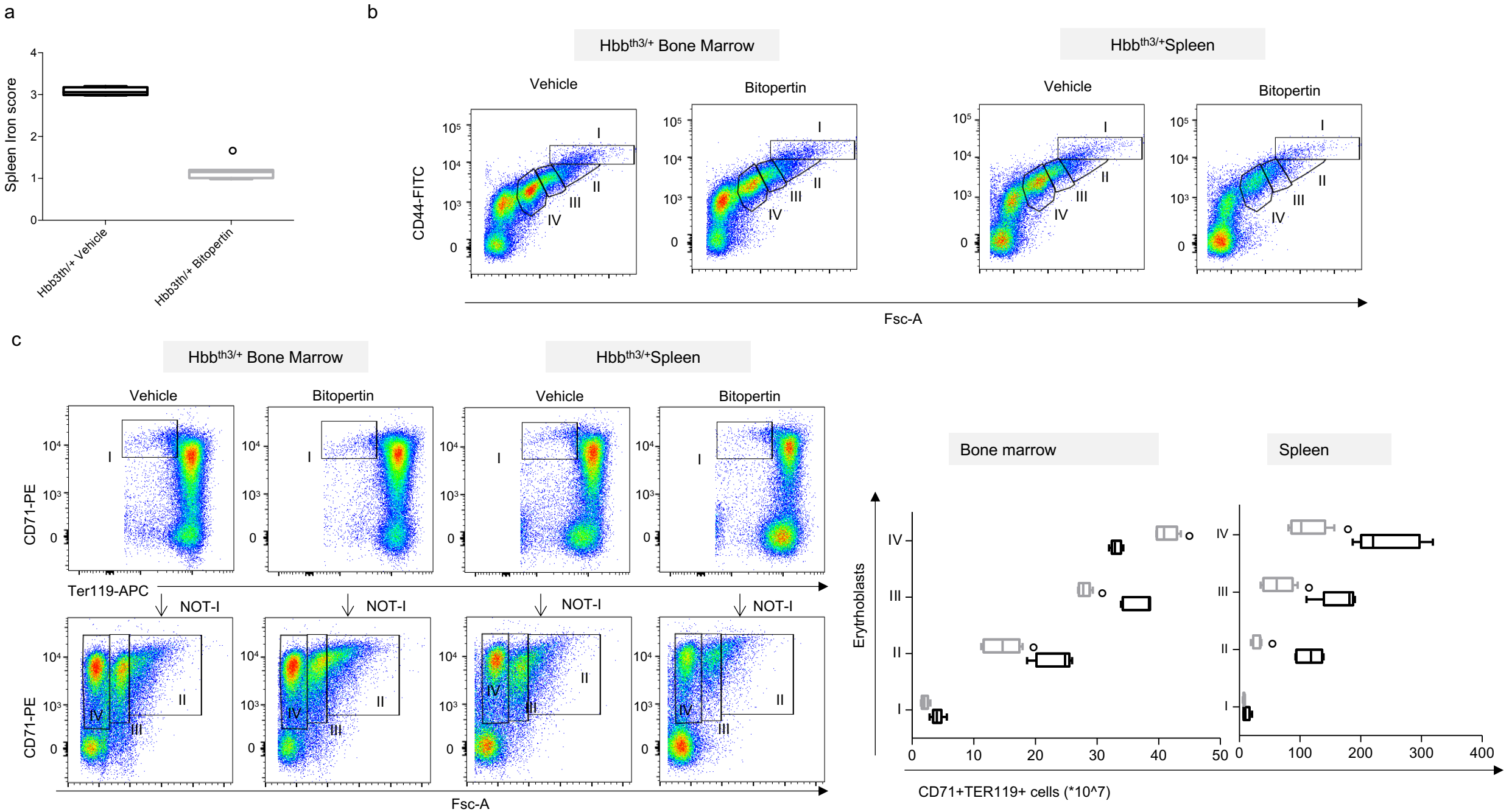
Hbb3th/+ Vehicle
Hbb3th/+ Bitopertin

Figure 2S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CD44-FITC
Fsc-A
c
Hbbth3/+ Bone Marrow
Hbbth3/+Spleen
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Bone marrow
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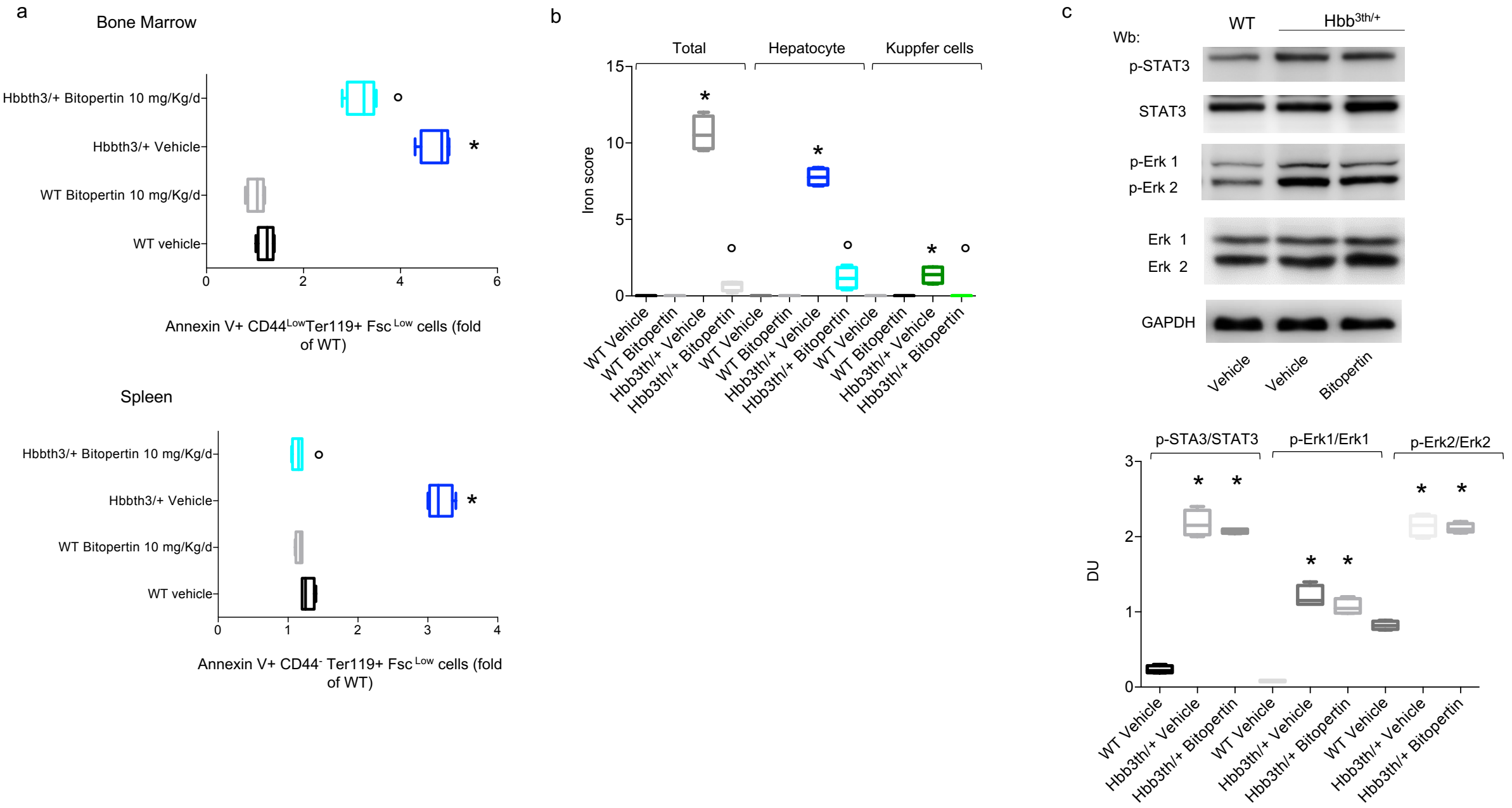
Erythroblasts
CD71+TER119+ cells (*10^7)

Figure 3S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Hbbth3/+ Vehicle
WT Bitopertin 10 mg/Kg/d
WT vehicle
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4
6
Annexin V+ CD44LowTer119+ FscLow cells (fold of WT)
Spleen
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Hbbth3/+ Vehicle
WT Bitopertin 10 mg/Kg/d
WT vehicle
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1
2
3
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Annexin V+ CD44- Ter119+ FscLow cells (fold of WT)
b
Total
Hepatocyte
Kuppfer cells
Iron score
15
10
5
0
WT Vehicle
WT Bitopertin
Hbb3th/+ Vehicle
Hbb3th/+ Bitopertin
c
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Wb:
p-STAT3
STAT3
p-Erk 1
p-Erk 2
Erk 1
Erk 2
GAPDH
Vehicle
Vehicle
Bitopertin
p-STA3/STAT3
p-Erk1/Erk1
p-Erk2/Erk2
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2
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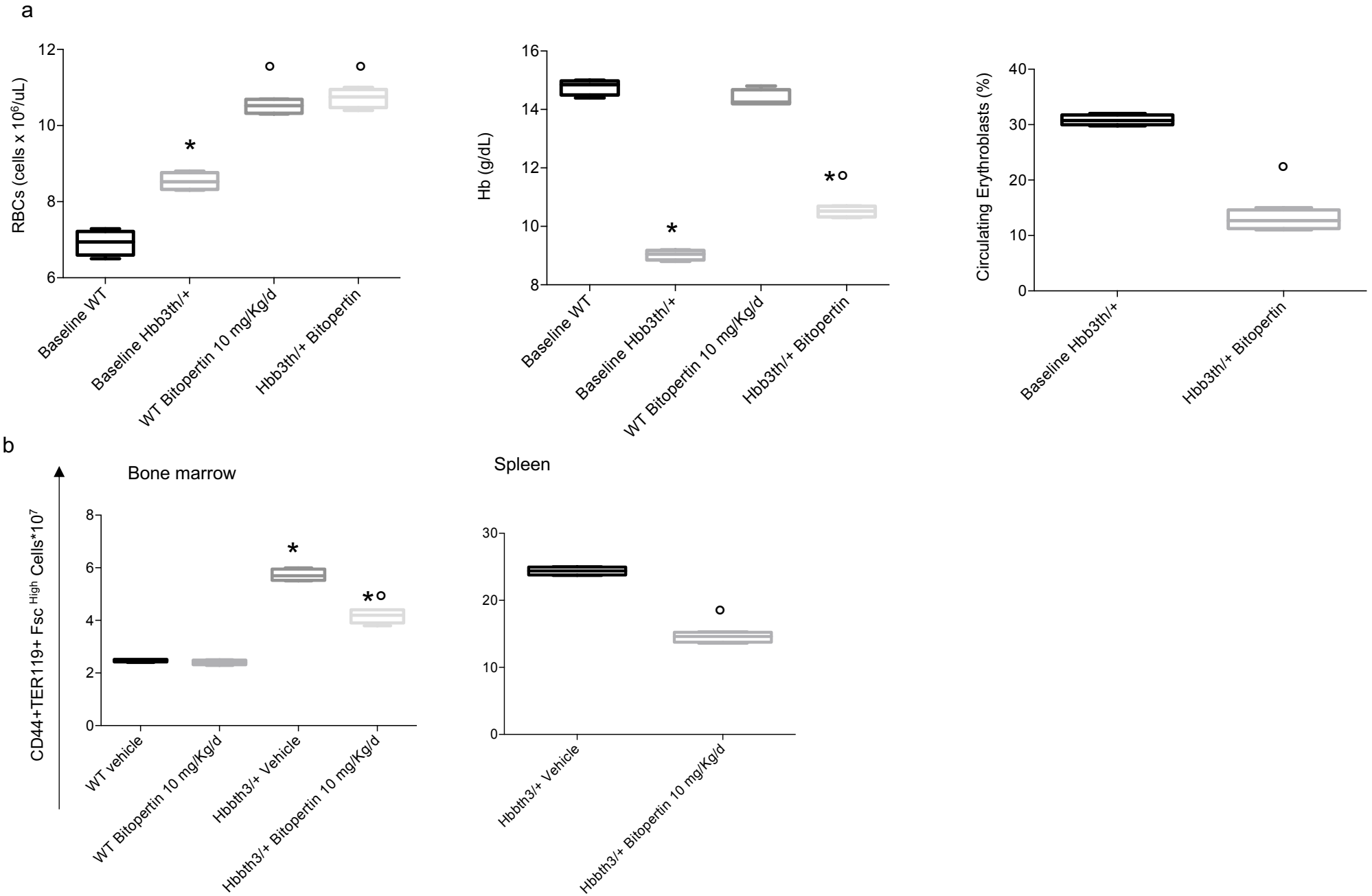
WT Vehicle
Hbb3th/+ Vehicle
Hbb3th/+ Bitopertin

Figure 4S


a
RBCs (cells x 10^6/uL)
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WT Bitopertin 10 mg/Kg/d
Hbb3th/+ Bitopertin
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16
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12
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Baseline WT
Baseline Hbb3th/+
WT Bitopertin 10 mg/Kg/d
Hbb3th/+ Bitopertin
Circulating Erythroblasts (%)
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20
10
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Baseline Hbb3th/+
Hbb3th/+ Bitopertin
b
Bone marrow
CD44+TER119+ Fsc High Cells*10^7
8
6
4
2
0
*
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WT vehicle
WT Bitopertin 10 mg/Kg/d
Hbbth3/+ Vehicle
Hbbth3/+ Bitopertin 10 mg/Kg/d
Spleen
30
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Hbbth3/+ Vehicle
Hbbth3/+ Bitopertin 10 mg/Kg/d

Figure 5S

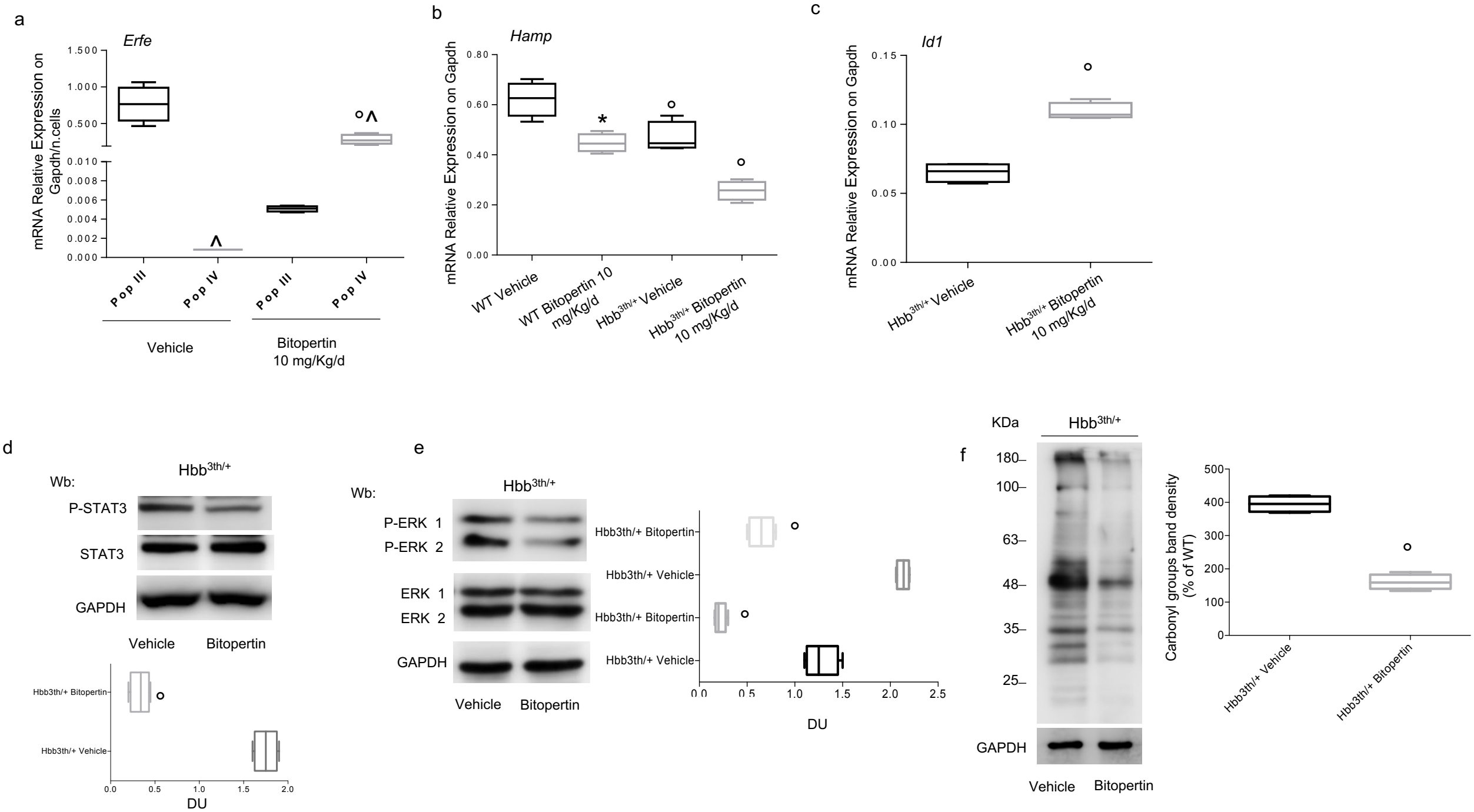
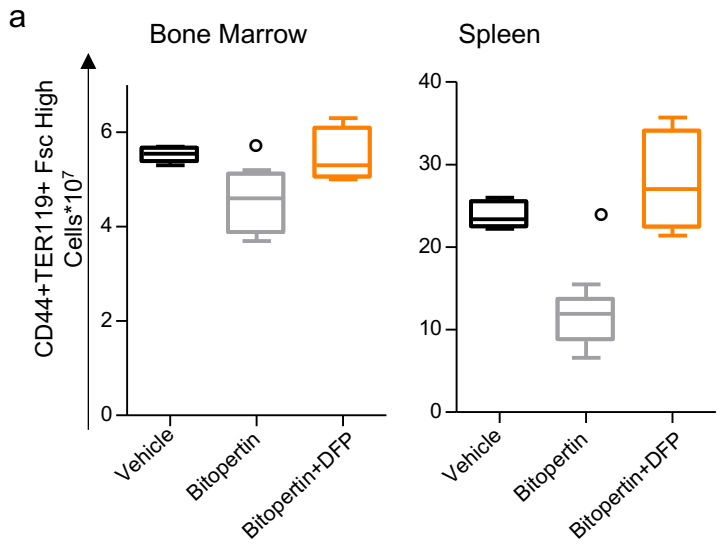
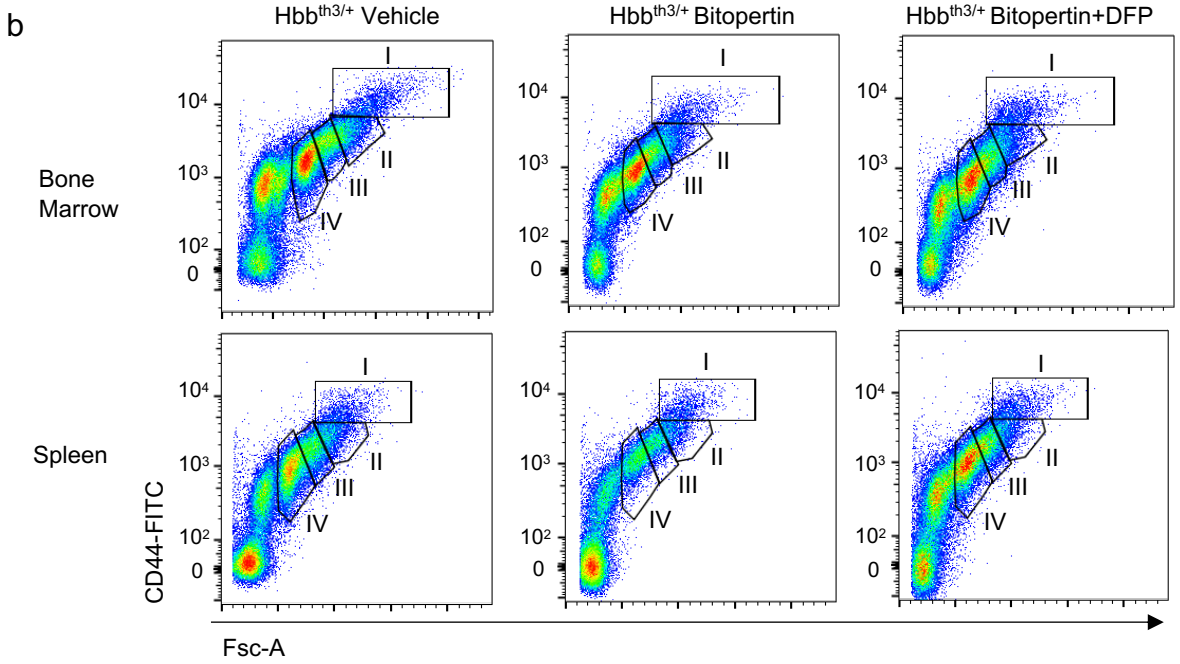
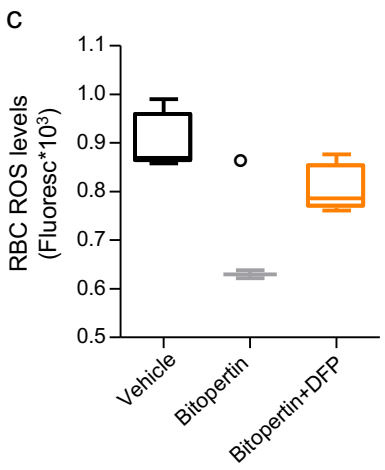
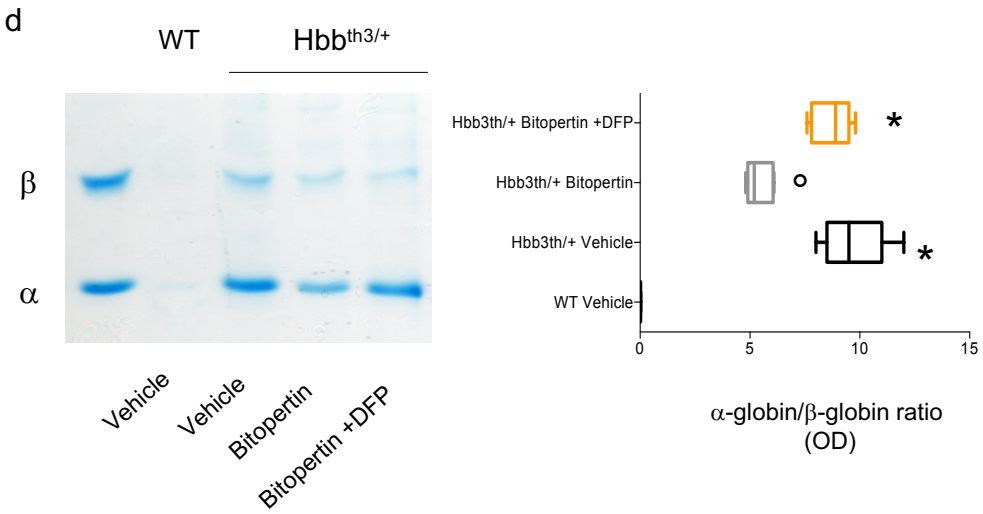

a
Erfe
mRNA Relative Expression on Gapdh/n.cells
1.500
1.000
0.500
0.010
0.008
0.006
0.004
0.002
0.000
Pop III
Pop IV
Pop III
Pop IV
Vehicle
Bitopertin 10 mg/Kg/d
b
Hamp
mRNA Relative Expression on Gapdh
0.80
0.60
0.40
0.20
0.00
WT Vehicle
WT Bitopertin 10 mg/Kg/d
Hbb3th/+ Vehicle
Hbb3th/+ Bitopertin 10 mg/Kg/d
c
Id1
mRNA Relative Expression on Gapdh
0.15
0.10
0.05
0.00
Hbb3th/+ Vehicle
Hbb3th/+ Bitopertin 10 mg/Kg/d
d
Hbb3th/+
Wb:
P-STAT3
STAT3
GAPDH
Vehicle
Bitopertin
Hbb3th/+ Bitopertin
Hbb3th/+ Vehicle
0.0
0.5
1.0
1.5
2.0
DU
e
Hbb3th/+
Wb:
P-ERK 1
P-ERK 2
ERK 1
ERK 2
GAPDH
Vehicle
Bitopertin
Hbb3th/+ Bitopertin
Hbb3th/+ Vehicle
Hbb3th/+ Bitopertin
Hbb3th/+ Vehicle
0.0
0.5
1.0
1.5
2.0
2.5
DU
f
KDa
Hbb3th/+
180
100
63
48
35
25
GAPDH
Vehicle
Bitopertin
Carbonyl groups band density (% of WT)
500
400
300
200
100
0
Hbb3th/+ Vehicle
Hbb3th/+ Bitopertin

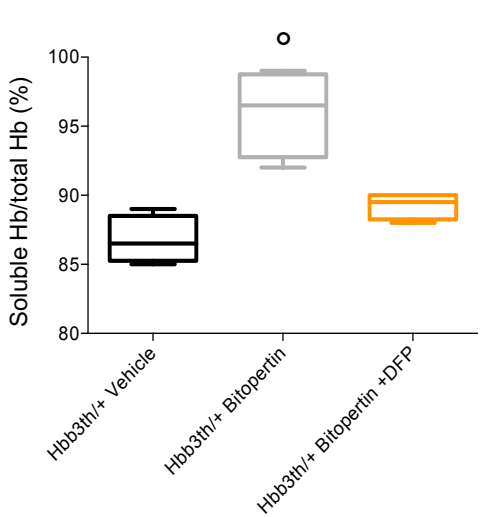
Figure 6S


a
Bone Marrow
Spleen
CD44+TER119+ Fsc High Cells*10^7
Vehicle
Bitopertin
Bitopertin+DFP


b
Hbb^th3/+ Vehicle
Hbb^th3/+ Bitopertin
Hbb^th3/+ Bitopertin+DFP
Bone Marrow
Spleen
CD44-FITC
Fsc-A
I
II
III
IV


c
RBC ROS levels (Fluoresc*10^3)
Vehicle
Bitopertin
Bitopertin+DFP


d
WT
Hbb^th3/+
β
α
Vehicle
Vehicle
Bitopertin
Bitopertin +DFP
Hbb3th/+ Bitopertin +DFP
Hbb3th/+ Bitopertin
Hbb3th/+ Vehicle
WT Vehicle
α-globin/β-globin ratio (OD)


e
Soluble Hb/total Hb (%)
Hbb3th/+ Vehicle
Hbb3th/+ Bitopertin
Hbb3th/+ Bitopertin +DFP

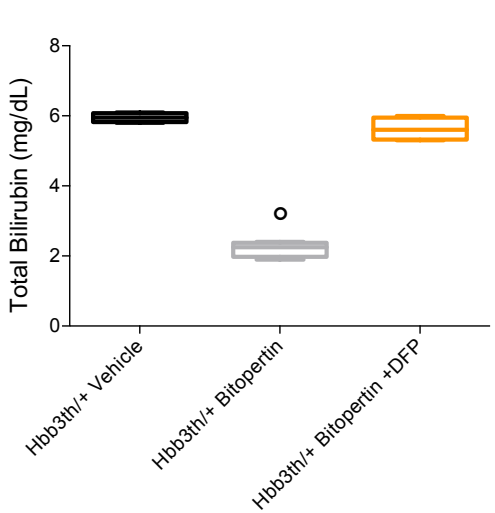

f
Total Bilirubin (mg/dL)
Hbb3th/+ Vehicle
Hbb3th/+ Bitopertin
Hbb3th/+ Bitopertin +DFP

Figure 7S

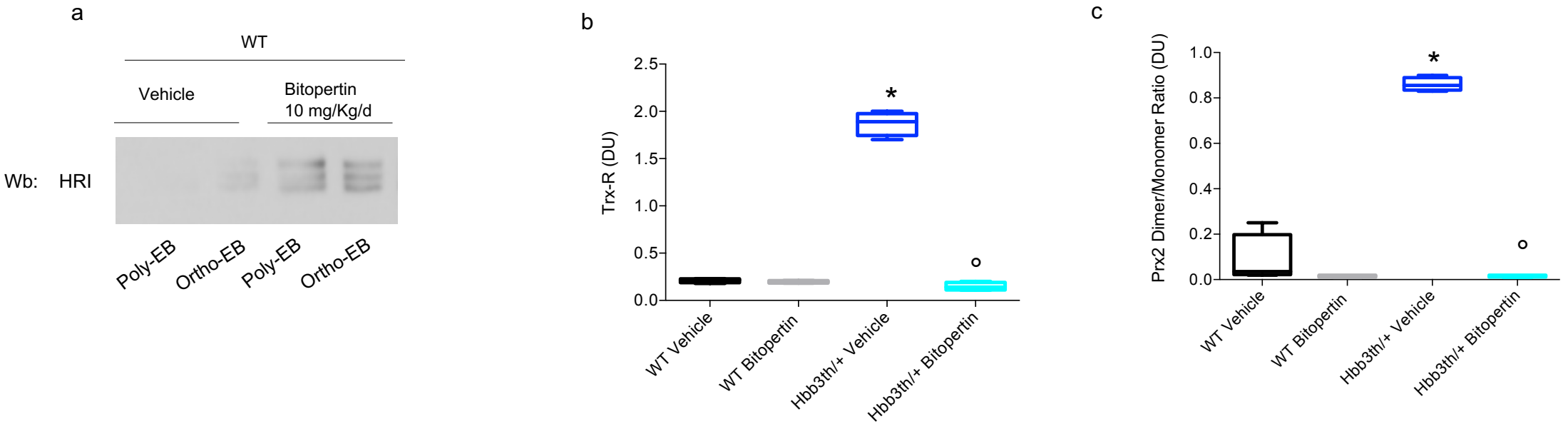

a
WT
Vehicle
Bitopertin
10 mg/Kg/d
Wb: HRI
Poly-EB
Ortho-EB
Poly-EB
Ortho-EB
b
Trx-R (DU)
2.5
2.0
1.5
1.0
0.5
0.0
*
°
WT Vehicle
WT Bitopertin
Hbb3th/+ Vehicle
Hbb3th/+ Bitopertin
c
Prx2 Dimer/Monomer Ratio (DU)
1.0
0.8
0.6
0.4
0.2
0.0
*
°
WT Vehicle
WT Bitopertin
Hbb3th/+ Vehicle
Hbb3th/+ Bitopertin

Figure 8S

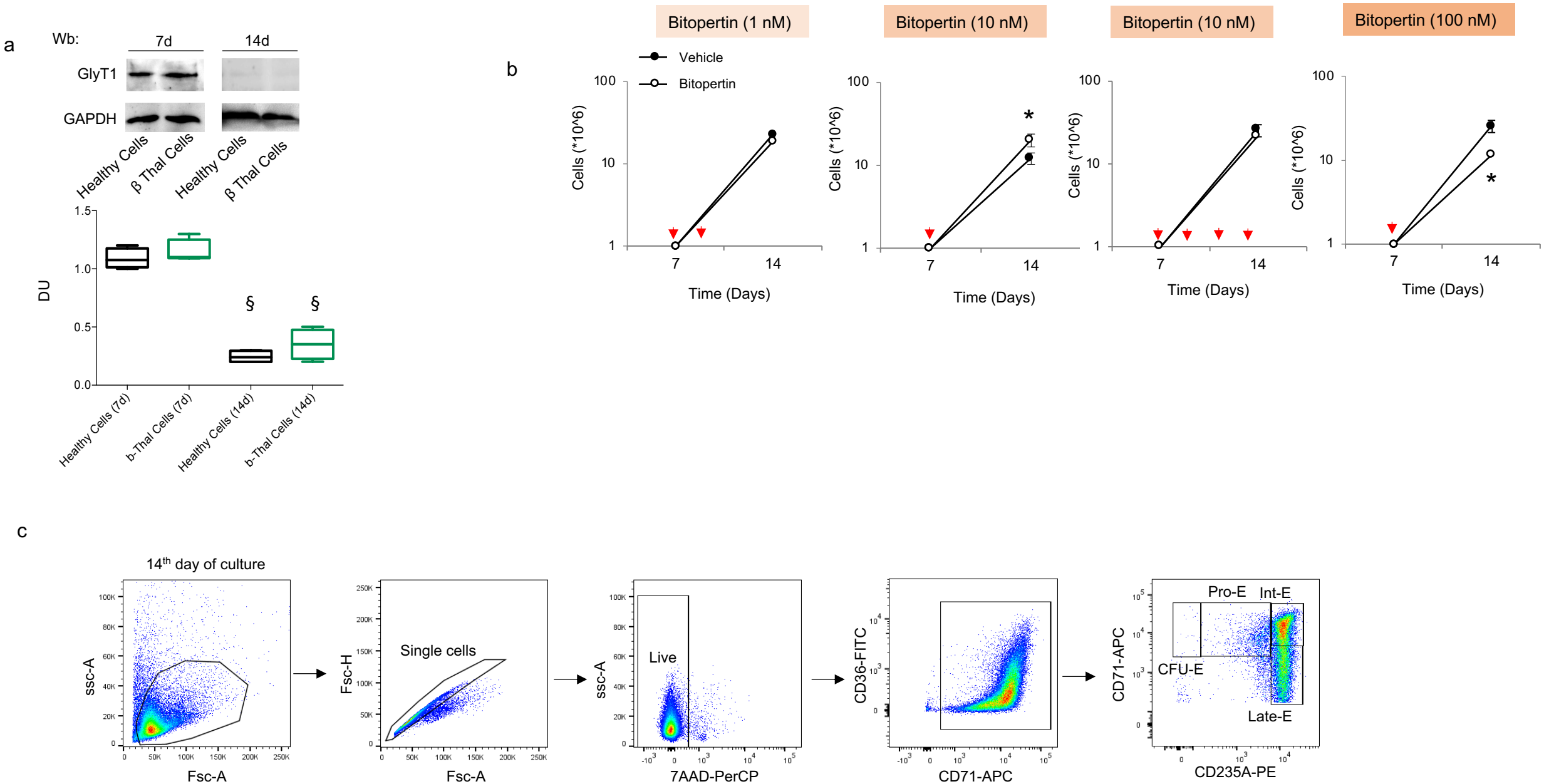

a
Wb:
7d
14d
GlyT1
GAPDH
Healthy Cells
β Thal Cells
Healthy Cells
β Thal Cells
DU
1.5
1.0
0.5
0.0
§
§
Healthy Cells (7d)
b-Thal Cells (7d)
Healthy Cells (14d)
b-Thal Cells (14d)
b
Bitopertin (1 nM)
Bitopertin (10 nM)
Bitopertin (10 nM)
Bitopertin (100 nM)
Vehicle
Bitopertin
Cells (*10^6)
100
10
1
7
14
Time (Days)
*
*
c
14th day of culture
ssc-A
Fsc-A
Fsc-H
Single cells
Live
7AAD-PerCP
CD36-FITC
CD71-APC
CD71-APC
CD235A-PE
Pro-E
Int-E
CFU-E
Late-E

Figure 9S

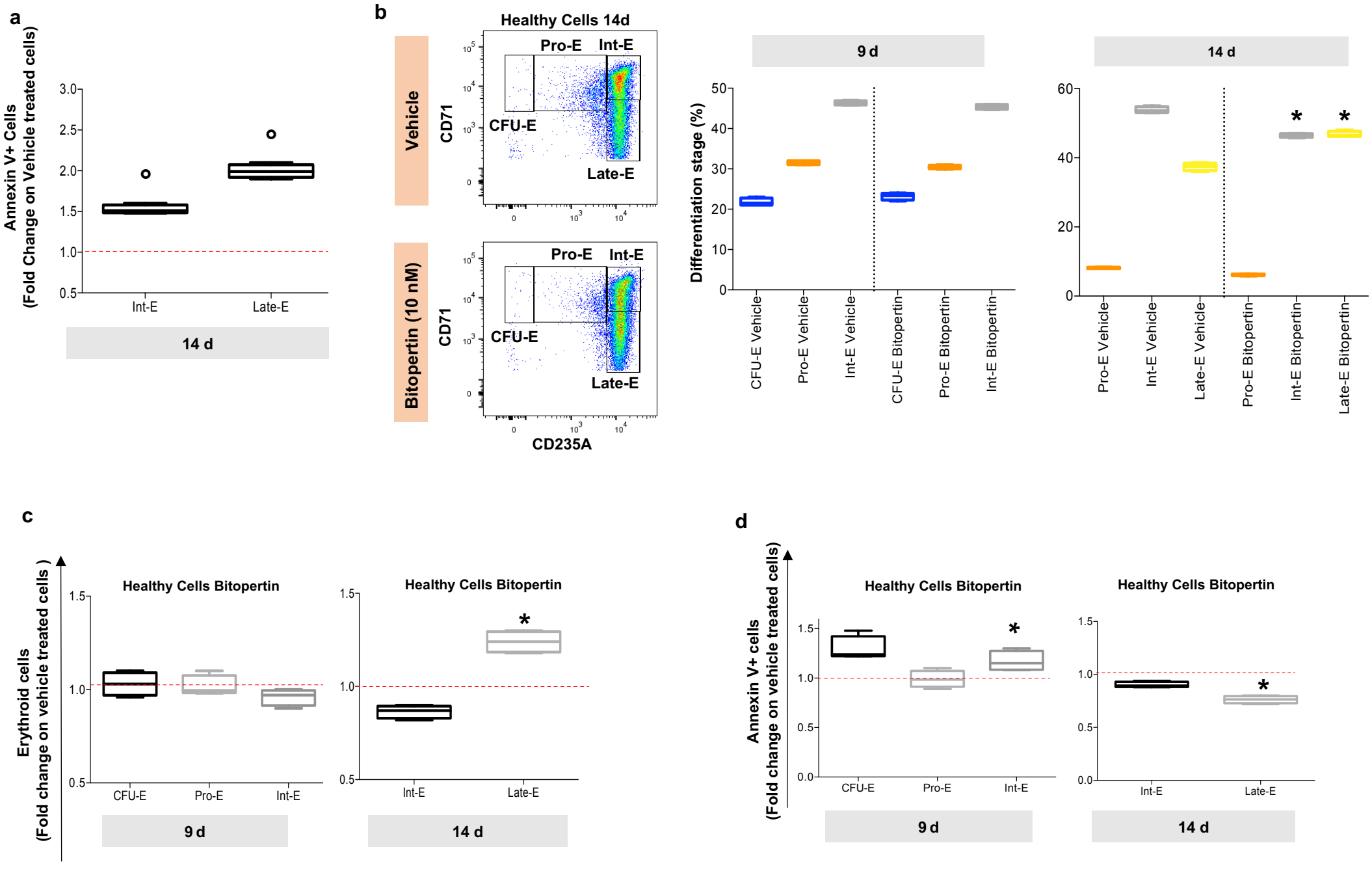

a
Annexin V+ Cells
(Fold Change on Vehicle treated cells)
3.0
2.5
2.0
1.5
1.0
0.5
Int-E
Late-E
14 d
b
Healthy Cells 14d
Vehicle
Bitopertin (10 nM)
CD71
CD235A
Pro-E
Int-E
CFU-E
Late-E
9 d
14 d
Differentiation stage (%)
CFU-E Vehicle
Pro-E Vehicle
Int-E Vehicle
CFU-E Bitopertin
Pro-E Bitopertin
Int-E Bitopertin
Late-E Vehicle
Late-E Bitopertin
c
Erythroid cells
(Fold change on vehicle treated cells)
Healthy Cells Bitopertin
CFU-E
Pro-E
Int-E
Late-E
9 d
14 d
d
Annexin V+ cells
(Fold change on vehicle treated cells)
Healthy Cells Bitopertin

TABLE 1S: LIST OF GENES PRIMERS ANALYSED BY QUANTITATIVE RT-PCR

Gene	Forward Primer Sequence (5'→ 3')	Reverse Primer Sequence (5'→ 3')
Hamp	GCCTGAGCAGCACCACCTAT	TTCTTCCCCGTGCAAAGGCT
Cish	GGATCTGCTGTGCATAGCCAA	GCCTCGCTGGCTGTAATAGAAC
Id1	CACTGAGGGACCAGATGGACTC	GGTGGCTGCGGTAGTGTCTT
Erfe	ATGGGGCTGGAGAACAGC	TGGCATTGTCCAAGAAGACA
Nqo1	TGCTCGTAGCAGGATTTGCC	CCAGTGGTGATAGAAAGCAAGGT
Gapdh	CCACATCGCTCAGACACCAT	AGTTAAAAGCAGCCCTGGTGAC
GAPDH	CCACATCGCTCAGAC	AGTTAAAAGCAGCCC