Supplemental Information

Supplemental Information Titles

Supplemental Figure 1. ***Ex vivo*** **erythroid differentiation of human CD34$^+$ progenitors is associated with a loss in oxidative phosphorylation.** Related to Figure 1.

Supplemental Figure 2. **αKG does not alter mitochondrial function during early stages of erythropoiesis and other TCA cycle metabolites do not inhibit enucleation.** Related to Figure 2.

Supplemental Figure 3. **Impact of vitamin C on αKG- and MitoPQ-induced oxidative stress in erythroid progenitors.** Related to Figures 3 and 4.

Supplemental Figure 4. **Ascorbic acid as well as other ROS scavengers rescue erythroid progenitors from oxidative stress**. Related to Figure 4.

Supplemental Figure 5. **The impact of Vitamin C on enucleation is independent of autophagy but is dependent on transferrin uptake.** Related to Figures 4 and 5.

Supplemental Figure 6. **Downregulation of IDH1 does not alter erythroid commitment or early erythroblast generation.** Related to Figure 6.

Supplemental Figure 7. **Vitamin C but no other ROS scavengers rescues enucleation and decreases mitochondrial stress in IDH1-downregulated progenitors.** Related to Figure 7.

Supplemental Information

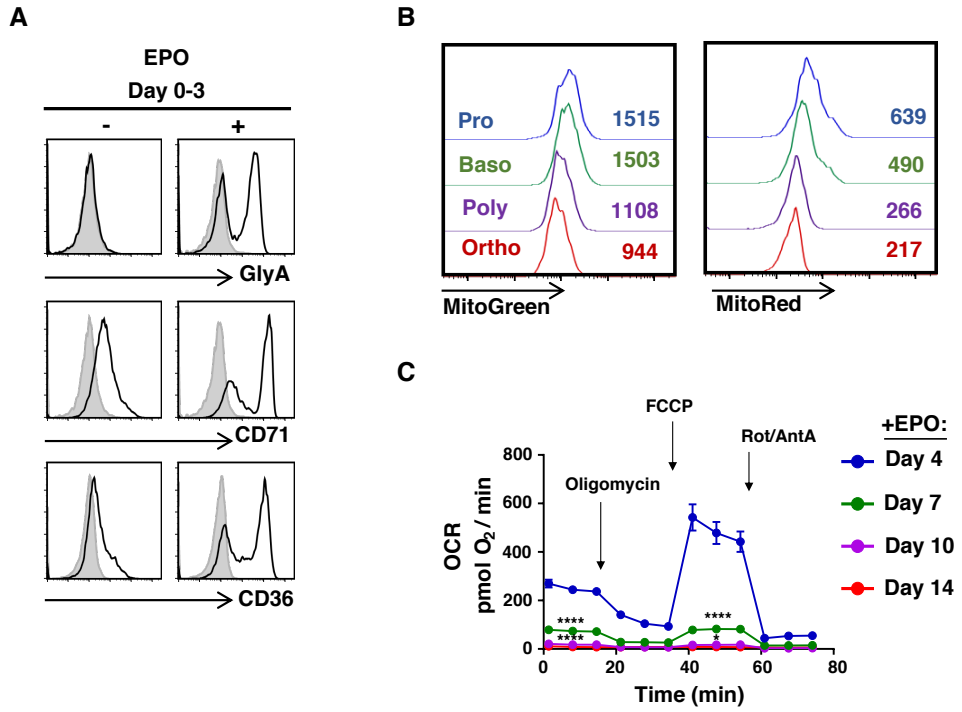

Figure S1. ***Ex vivo*** **erythroid differentiation of human CD34$^+$ progenitors is associated with a loss in oxidative phosphorylation. Related to Figure 1.** (**A**) The phenotype of CD34$^+$ progenitors differentiated in the absence (-) or presence (+) of recombinant erythropoietin (EPO). Representative histograms showing expression of GlyA, CD71, and CD36 are presented (data are representative of 1 of 10 independent experiments). (**B**) Representative histograms of mitochondrial biomass and polarization are shown for pro, basophilic, polychromatic, and orthochromatic erythroblasts, and mean levels are presented in Figure 1D. (**C**) CD34$^+$ progenitors were differentiated in the presence of recombinant erythropoietin (EPO) and OCR, a measure of oxidative phosphorylation, was monitored at the indicated days of erythroid differentiation. Basal oxygen consumption, maximal consumption following FCCP, and minimal levels following inhibition of the electron transport chain with rotenone/antimycin A (Rot/AntA) are presented as means ±SEM (n=6). ****p<0.0001

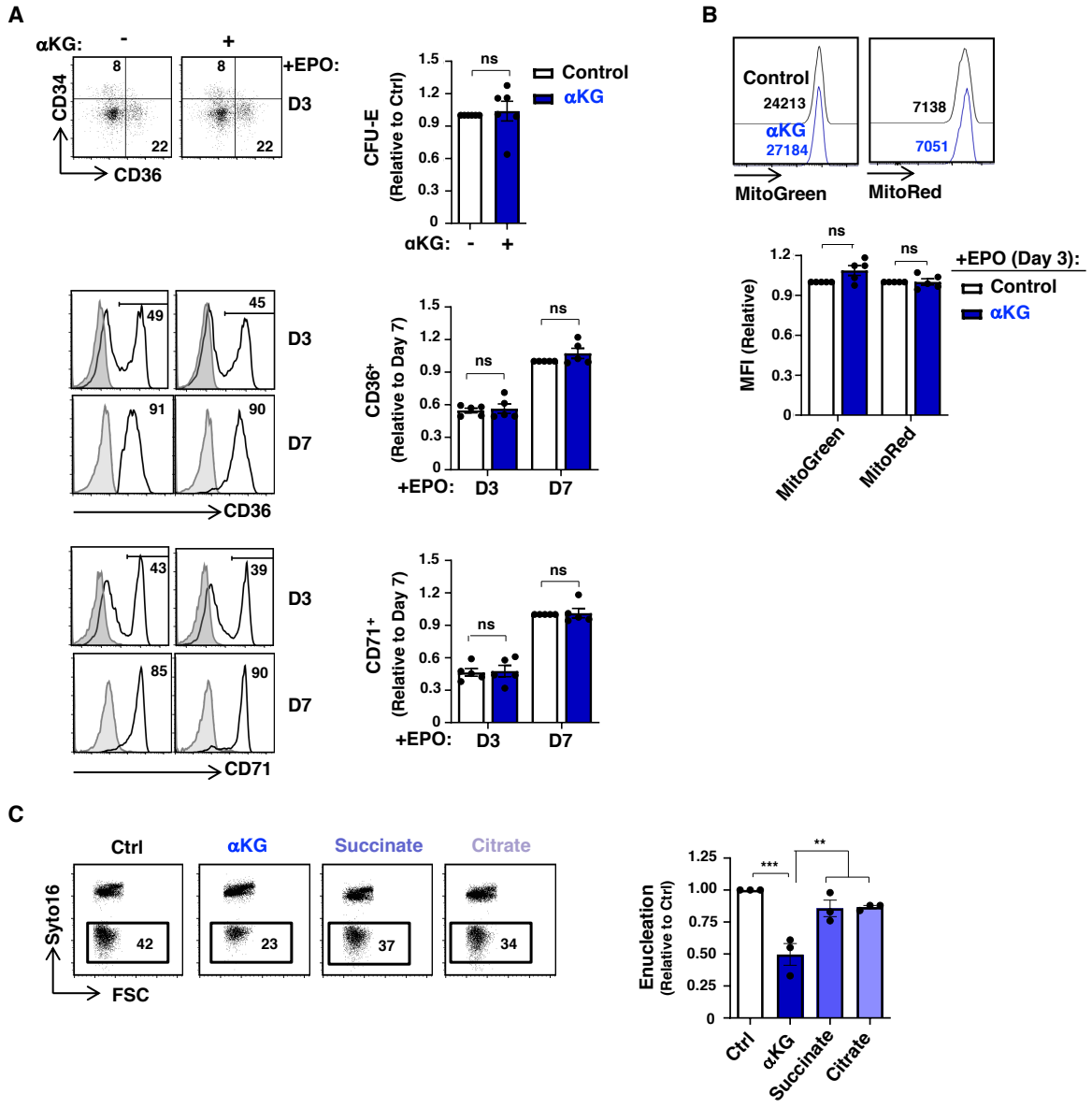

Figure S2. αKG does not alter mitochondrial function during early stages of erythropoiesis and other TCA cycle metabolites do not inhibit enucleation. Related to Figure 2. (**A**) The impact of αKG (3.5 mM) on early stages of erythropoiesis was evaluated at days 3 and 7 of EPO-induced differentiation. The induction of $CD36^{+}CD34^{-}$ CFU-E was monitored at day 3 and representative CD34/CD36 profiles as well as a quantification of the percentages of CFU-E relative to control are shown (top; n=6, non-significant, ns). Induction of CD36 (middle) and CD71 (bottom) during erythroid differentiation were monitored and representative histograms and quantifications (n=4-5) are presented (right). (**B**) Mitochondrial biomass and polarization were evaluated by flow cytometry and representative histograms as well as a quantification of MFI relative to control conditions are presented (n=5, bottom). (**C**) Differentiated erythroblasts were treated with αKG, succinate (5 mM) or citrate (8 mM) at day 7 and enucleation was evaluated at day 10 by Syto16 staining. Representative histograms (left) as well as means ±SEM (n=3 independent experiments) are shown. ** p<0.01, ***p<0.001, ns, non-significant

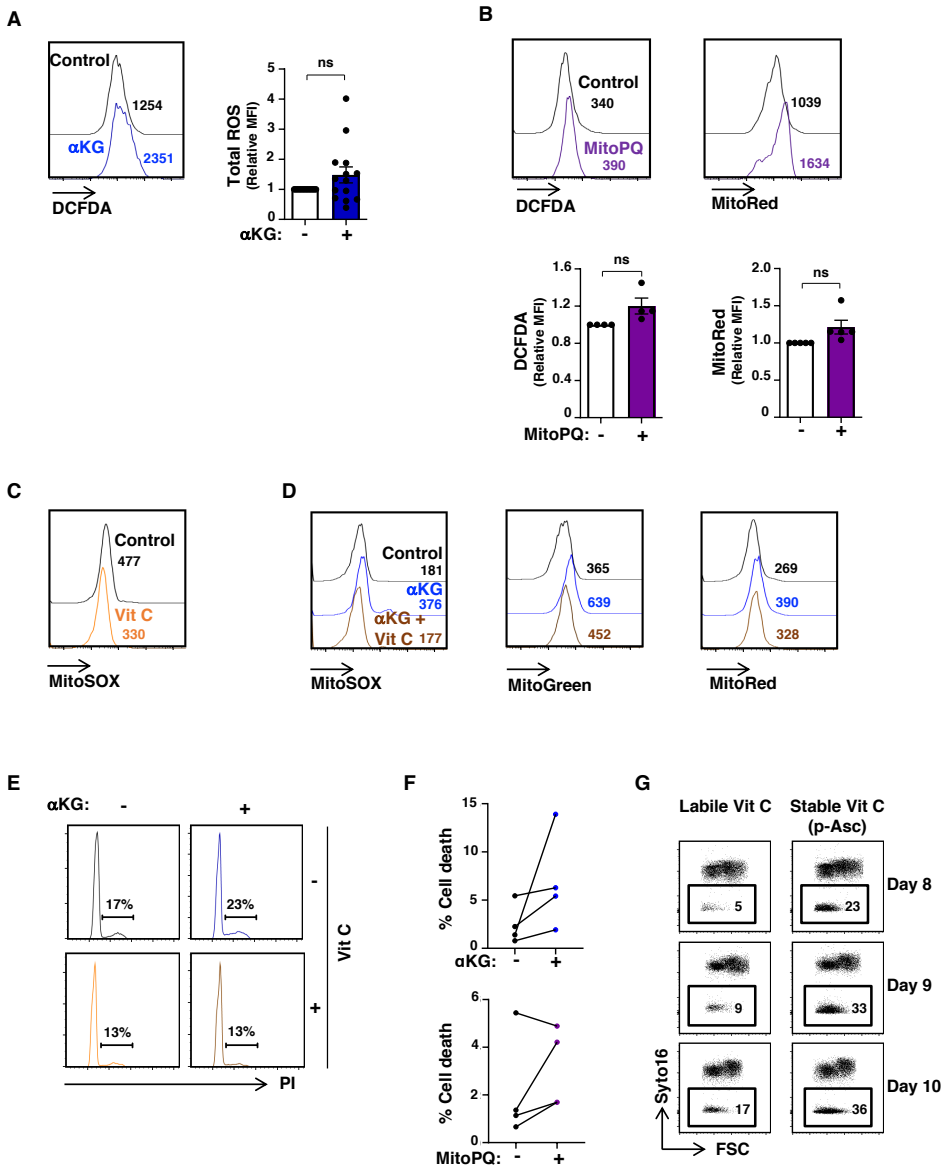

Figure S3. Impact of vitamin C on αKG- and MitoPQ-induced oxidative stress in erythroid progenitors. Related to Figures 3 and 4. (**A**) Total intracellular ROS levels were evaluated on erythroblasts differentiated from day 7-10 in the absence (control) or presence of αKG, as a function of CM-H2DCFDA staining. Representative histograms (left) and quantifications in 12 independent experiments (right) are shown. (**B**) Total ROS levels and mitochondrial polarization were evaluated in the absence or presence of MitoPQ and representative histograms (top) and MFI quantifications (bottom) are presented (n=4). (**C**) The impact of L-ascorbic acid 2-phosphate (100µM) was evaluated on the generation of mitochondrial superoxide between days 7 and 10 after EPO induction and representative histograms are shown with quantification provided in panel 4E. (**D**) The ability of Vit C to alter αKG-mediated increases in mitochondrial superoxide, biomass and membrane potential was evaluated by flow cytometry and representative histograms are shown. (**E**) Representative histograms of PI staining show the percentages of cells in S-G2/M of the cell cycle with quantifications provided in panel 4F. (**F**) Cell death was evaluated in the absence or presence of αKG (3.5mM, top) and MitoPQ (50µM, bottom) and data from 4 independent experiments are presented. (**G**) $CD34^+$ HSPCs were differentiated to the erythroid lineage by treatment with rEPO for 7 days. At day 7, erythroid cells were cultured in the presence of L-ascorbate and ascorbate 2-phosphate (100µM), presenting labile and stable vitamin C, respectively (Hata and Senoo, 1989). Enucleation was evaluated 24, 48 and 72h later; at days 8, 9, and 10 of differentiation, respectively. Representative dot plots of Syto16 staining are shown and the percentages of enucleated cells are indicated.

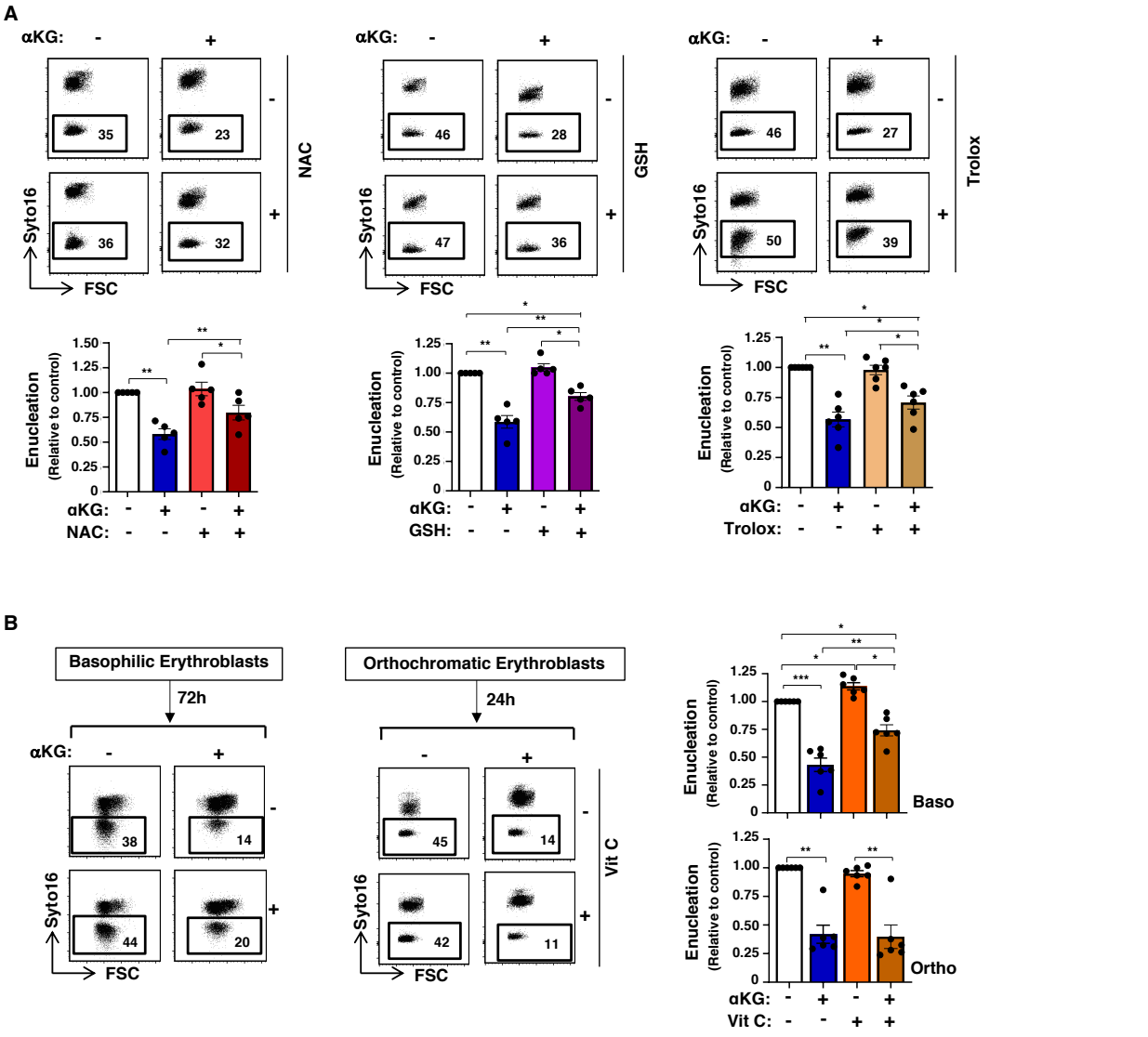

Figure S4. Ascorbic acid as well as other ROS scavengers rescue erythroid progenitors from oxidative stress. Related to Figure 4. (**A**) The impact of ROS scavengers on the αKG-mediated decrease in enucleation was evaluated for NAC (100μM, left), GSH (100μM, middle) and Trolox (100μM, right). Representative plots (top) and means ± SEM of 5-6 independent experiments (bottom) are presented. (**B**) Early basophilic (left) and late orthochromatic (right) subsets were FACS-sorted and then differentiated for 72h or 24h, respectively, in the absence (-) or presence (+) of αKG and/or Vit C as indicated. Enucleation was evaluated by Syto16 staining and representative plots are presented (left). Enucleation in experimental groups was calculated relative to control conditions and mean levels are presented (right, n=6). ns, not significant; *p<0.05, ** p<0.01, ***p<0.001

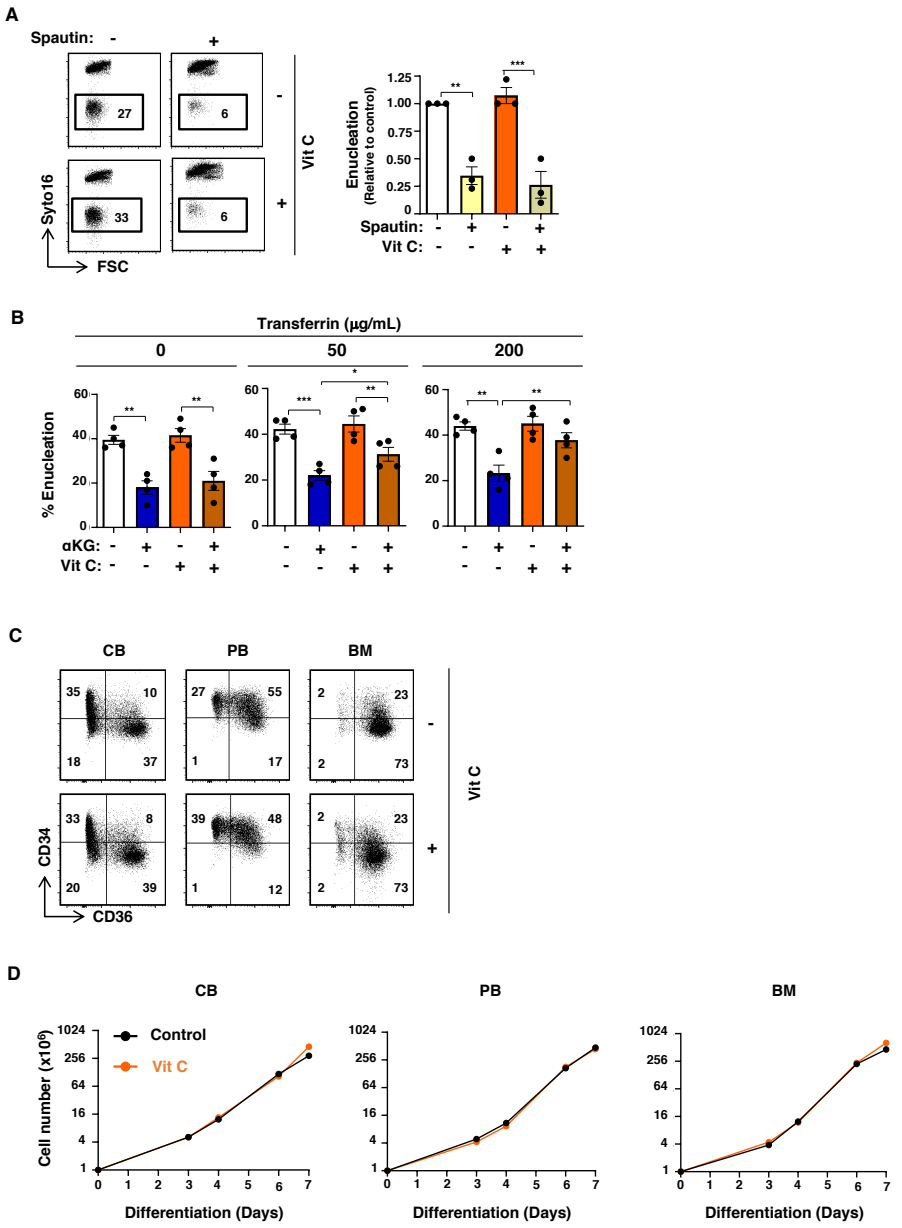

Figure S5. The impact of Vitamin C on enucleation is independent of autophagy but is dependent on transferrin uptake. Related to Figures 4 and 5. (**A**) The impact of Vit C on erythroid enucleation under conditions of a Spautin (10 µM)-mediated inhibition of autophagy was evaluated at day 10 of erythroid differentiation. Representative plots (left) and means ± SEM of 3 independent experiments (right) are shown. (**B**) Erythroblasts were differentiated in the absence or presence of transferrin (50 or 200 µg/ml), αKG (3.5mM) and Vit C (100µM) between days 7 and 10 of differentiation and enucleation was monitored by Syto16 staining. Means ± SEM of 4 independent experiments are presented. (**C**) CD34 HSPCs isolated from CB, PB, and BM were differentiated with EPO (+) in the absence (-) or presence of vitamin C (Vit C) for 4 days. The differentiation of CFU-E ($CD34^{-}CD36^{+}$) was monitored as a function of CD34/CD36 staining and representative plots are shown. (**D**) Growth of EPO-differentiated CB-, PB- and BM-derived progenitors was evaluated in the absence (-) or presence of vitamin C. Fold-increase at days 3, 4, 6 and 7 differentiation is shown for one representative experiment (n=2). *p<0.05, ** p<0.01, ***p<0.001

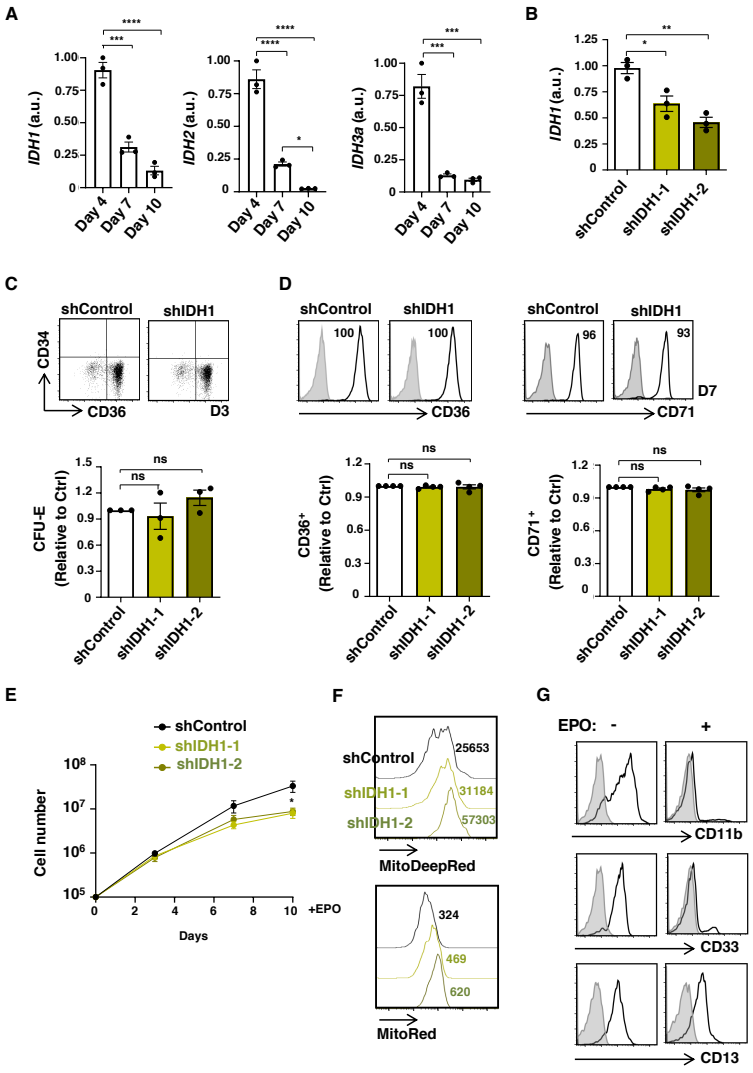

Figure S6. Downregulation of IDH1 does not alter erythroid commitment or early erythroblast generation. Related to Figure 6. (**A**) mRNA levels of IDH1, IDH2 and IDH3 were evaluated by qRT-PCR and means ± SEM relative to β-actin are shown (technical triplicates, representative of 1 of 2 independent experiments). (**B**) The efficacy of the two shIDH1 vectors was evaluated by monitoring IDH1 mRNA levels by qRT-PCR. Mean levels ±SEM relative to β-actin following transduction with shIDH1-1 and shIDH1-2 are presented. (**C**) Generation of CFU-E, defined as $CD34^{-}CD36^{+}$, were evaluated in shControl- and shIDH1-transduced progenitors at day 3 of differentiation. Representative dot plots (top) and quantification of the relative induction of CFU-E (bottom) are shown (n=3). (**D**) Differentiation of erythroblasts was evaluated by induction of the CD36 and CD71 markers and representative histograms (top) and quantifications (bottom) are shown (n=4). (**E**) The expansion of control and IDH1-downregulated progenitors was evaluated at the indicated day and means ± SEM of 5 independent experiments are shown. (**F**) Representative histograms of MitoDeepRed (top) and MitoRed (bottom) staining in shControl- and shIDH1-transduced progenitors at day 10 of differentiation. (**G**) The phenotype of $CD34^{+}$ progenitors expanded in the absence (-) or presence (+) of EPO is shown at day 7 of differentiation as a function of CD11b, CD33 and CD13 staining. Solid line and shaded histograms represent specific and non-specific staining, respectively. ns, non-significant; * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

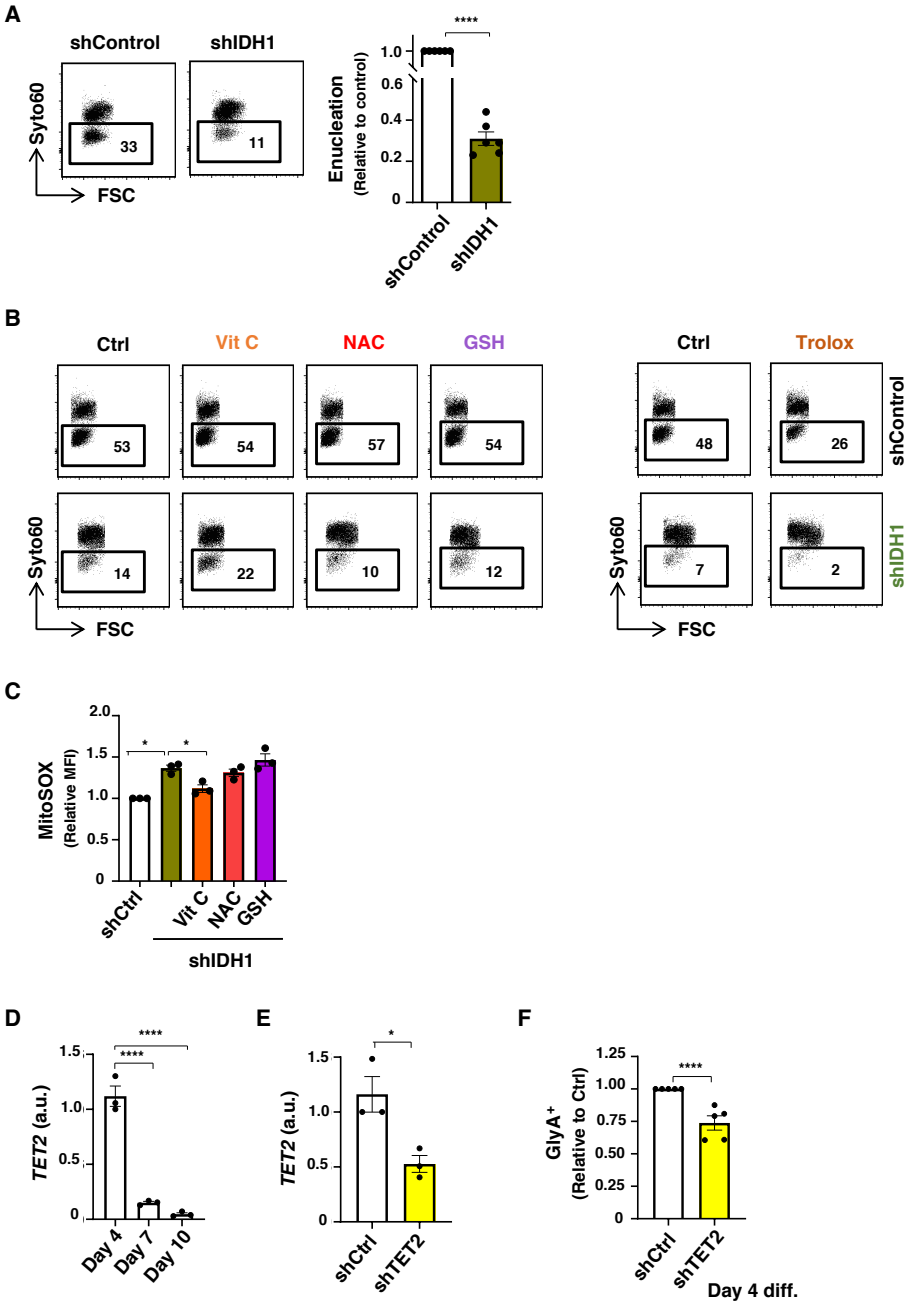

Figure S7. Vitamin C but no other ROS scavengers rescues enucleation and decreases mitochondrial stress in IDH1-downregulated progenitors. Related to Figure 7. (**A**) IDH1 was downregulated in erythroid progenitors (day 4) by transduction with shIDH1-encoding lentiviral particles and enucleation was monitored at day 10 of differentiation. Representative plots (left) and means ± SEM (right) are presented (n=6, 1-way ANOVA with Tukey's post-hoc test). (**B**) The impact of ROS scavengers (Vit C, NAC, GSH and Trolox; 100 µM) on the shIDH1-mediated decrease in enucleation was evaluated at day 10 of differentiation. Representative plots are presented (n=2). (**C**) The impact of Vit C, NAC, GSH and Trolox (with DMSO as a control) was evaluated on the shIDH1-mediated increase in MitoSOX at day 10. Means ± SEM of 3 independent experiments are shown. (**D**) TET2 mRNA levels were evaluated at days 4, 7 and 10 of differentiation and means ± SEM of 3 independent experiments are shown. (**E**) The shTET2-mediated downregulation of TET2 was evaluated by qRT-PCR and means ± SEM relative to β-actin are shown. (**F**) The impact of shTET2 on erythroid development was evaluated at day 4 of differentiation as a function of GlyA staining. The relative levels of GlyA in 5 independent experiments were normalized to control progenitors. ns, not significant; *p<0.05, ****p<0.0001