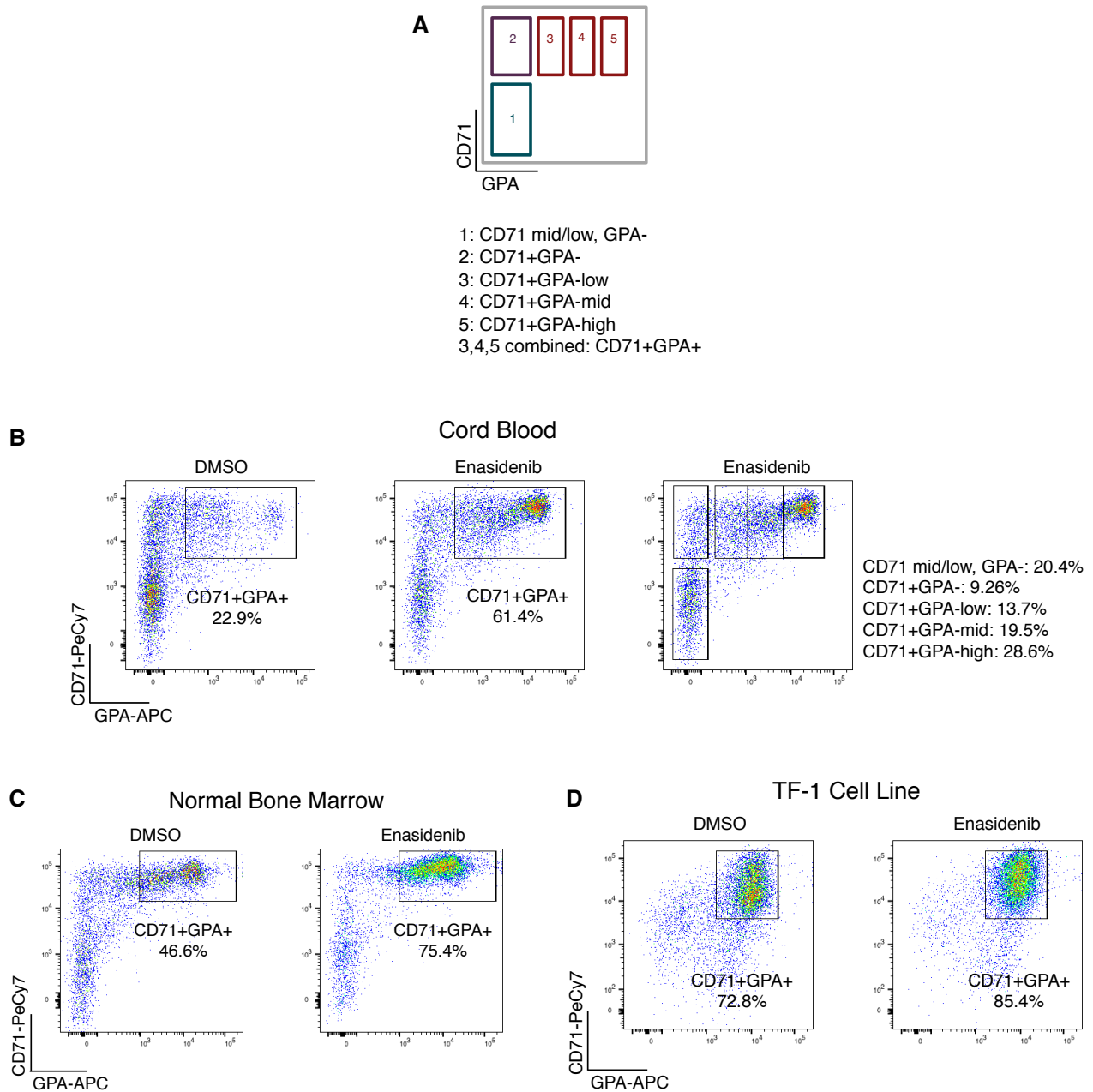
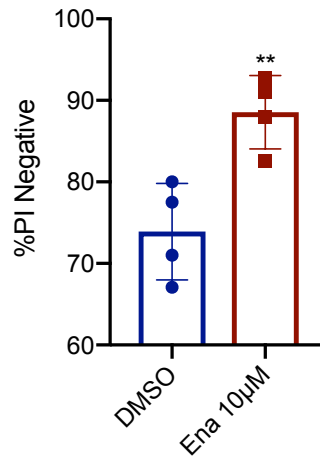


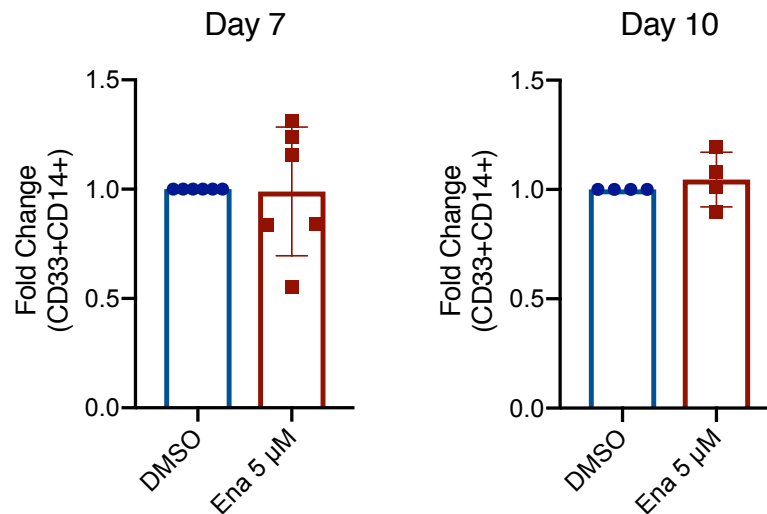
## Supplementary Materials:



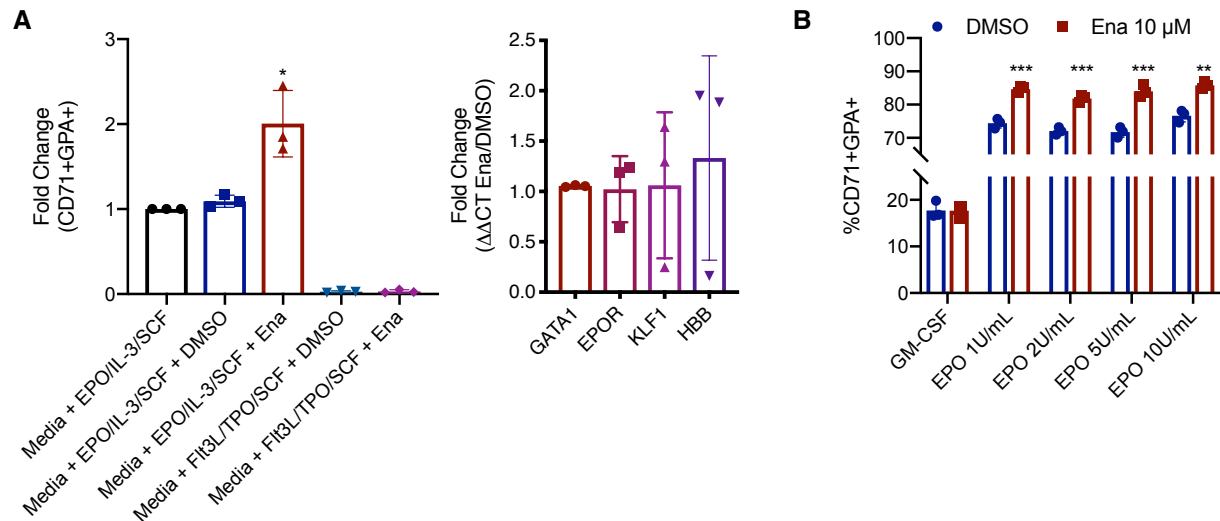
**Supplementary Figure 1. Gating Schema for CD71/GPA (A)** Gating schema for CD71 and GPA expression in CB-CD34<sup>+</sup>-derived cells (B) Representative flow plot of CB-CD34<sup>+</sup> cells at D8 of EDC with DMSO or enasidenib 10 $\mu$ M. (C) Representative flow plot of normal BM-CD34<sup>+</sup> cells at D8 of EDC with DMSO or enasidenib 10 $\mu$ M. (D) Representative flow plot of TF-1 erythroleukemic cells at D8 of EPO culture with DMSO or enasidenib 10 $\mu$ M.



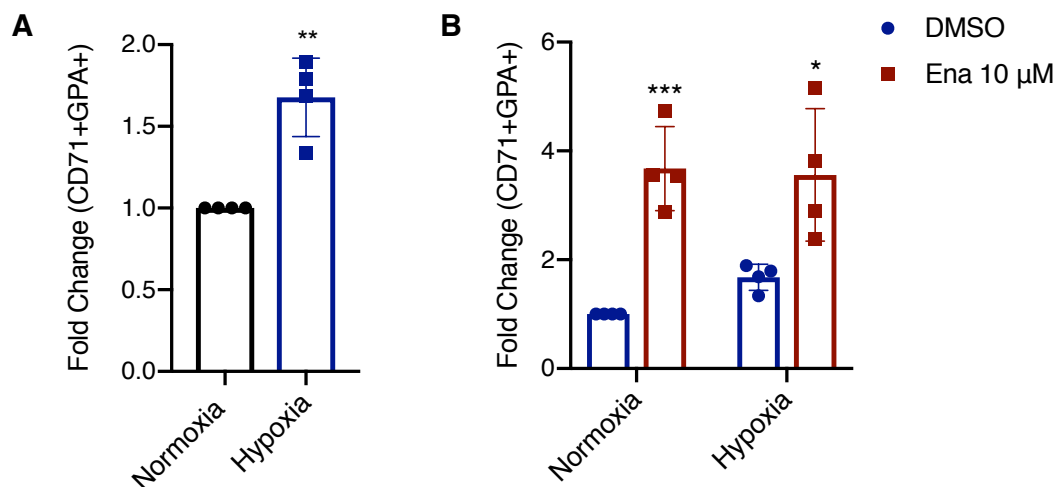
**Supplementary Figure 2. Enasidenib Does Not Decrease Viability** Proportion of PI-Negative (live) cells after D8 of EDC (n=4). Graph represents Mean  $\pm$  SD. Statistical significance was calculated using an unpaired two-tailed *t*-test.



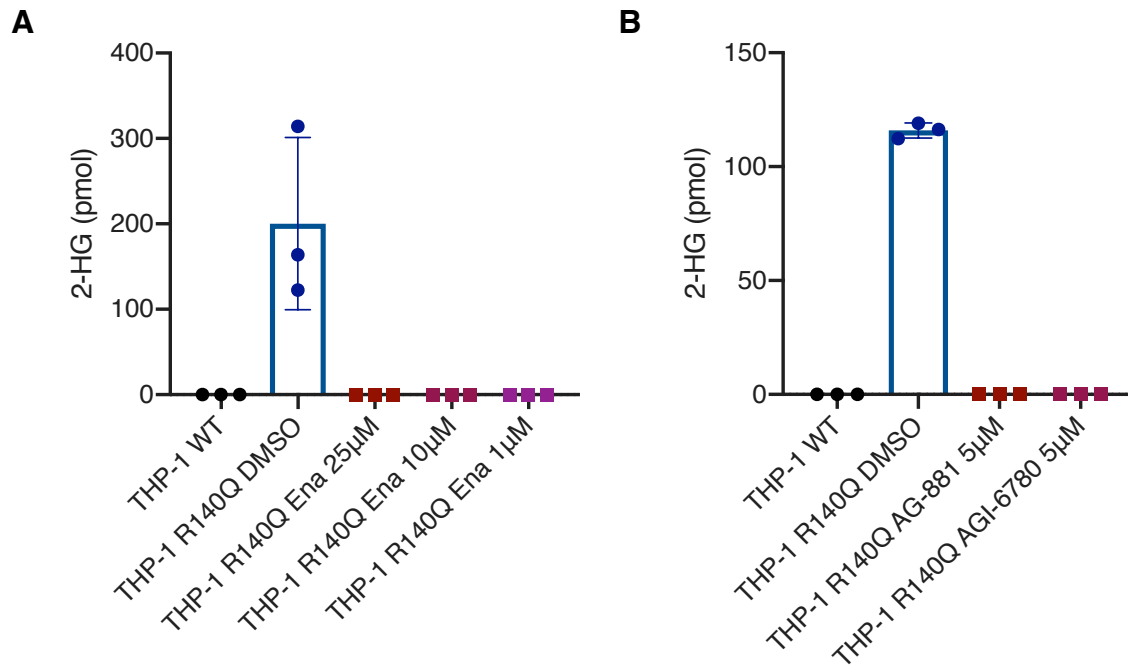
**Supplementary Figure 3. Enasidenib Does Not Increase Myeloid Differentiation** FC of %CD33+CD14+ cells after 7 (left) and 10 (right) days in myeloid differentiation culture (DMSO=1) (n=5 for Day 7, n=4 for Day 10). Graphs represent Mean  $\pm$  SD. Statistical significance was calculated using an unpaired two-tailed *t*-test.



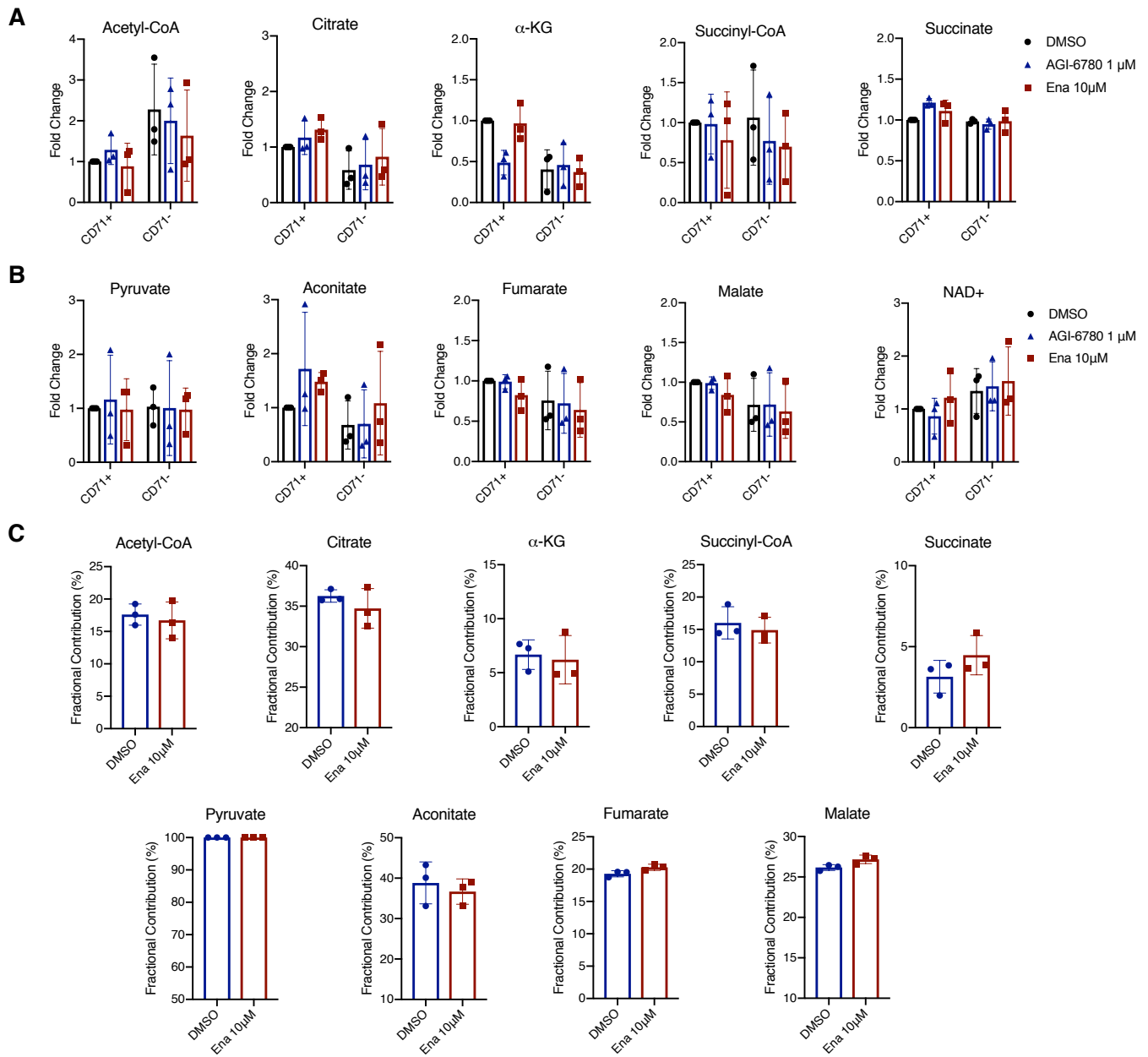
**Supplementary Figure 4. Enasidenib is Dependent on EPO Signaling to Drive Erythroid Differentiation** (A) (Left) FC of %CD71+GPA+ in EDM (EPO, IL-3, SCF) or Stem Cell Retention media (Flt3L, TPO, SCF). (EDM only=1) (n=3). (Right) qPCR detection of relative RNA expression of erythroid signature genes in enasidenib-treated CB-CD34+-derived cells at D8 in Stem Cell Retention Media (Flt3L, TPO, SCF) (DMSO=1) (n=3). (B) Proportion of CD71+GPA+ TF-1 erythroleukemic cells at D8 of culture with GM-CSF or different EPO concentrations (n=3). Graphs represent Mean  $\pm$  SD. Statistical significance was calculated using an unpaired two-tailed *t*-test.



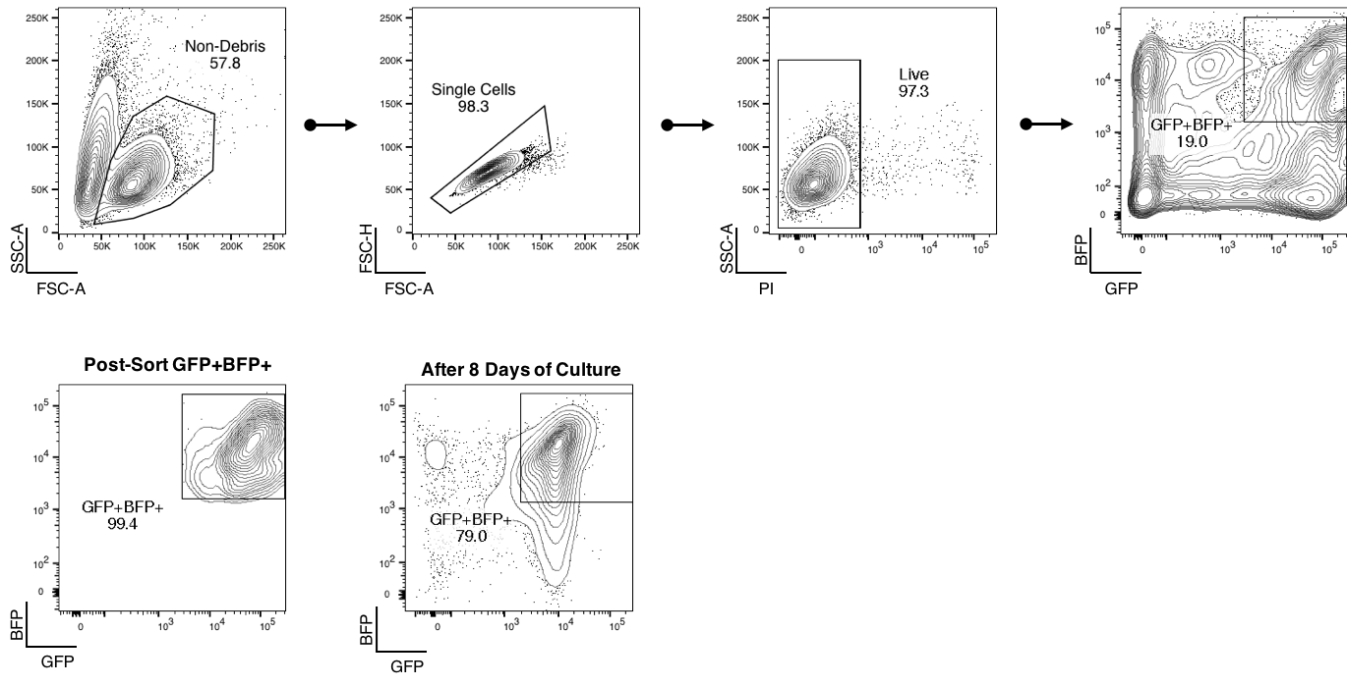
**Supplementary Figure 5. Enasidenib Drives Erythroid Differentiation in Hypoxic Conditions** (A) FC of %CD71+GPA+ in EDC at D8 in normoxic or hypoxic culture conditions (Normoxia=1) (n=4). (B) FC of %CD71+GPA+ in EDC at D8 in normoxic or hypoxic culture conditions with DMSO or enasidenib (DMSO with normoxia=1) (n=4). Graphs represent Mean  $\pm$  SD. Statistical significance was calculated using an unpaired two-tailed *t*-test, with all comparisons made to DMSO in respective culture conditions.



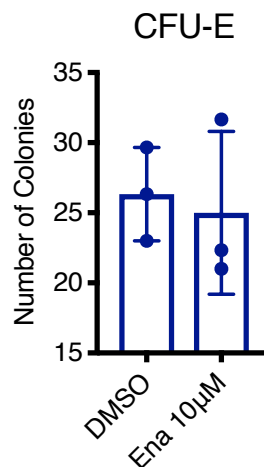
**Supplementary Figure 6. Enasidenib, AG-881, and AGI-6780 Suppress D-2-HG Production by mutant IDH2 D-2-HG in the parental THP-1 cell line and an inducible IDH2-mutant R140Q THP-1 cell line, after treatment with (A) enasidenib (n=3) or (B) AGI-881 and AGI-6780 (n=3).** Graphs represent Mean  $\pm$  SD. Statistical significance was calculated using an unpaired two-tailed *t*-test.



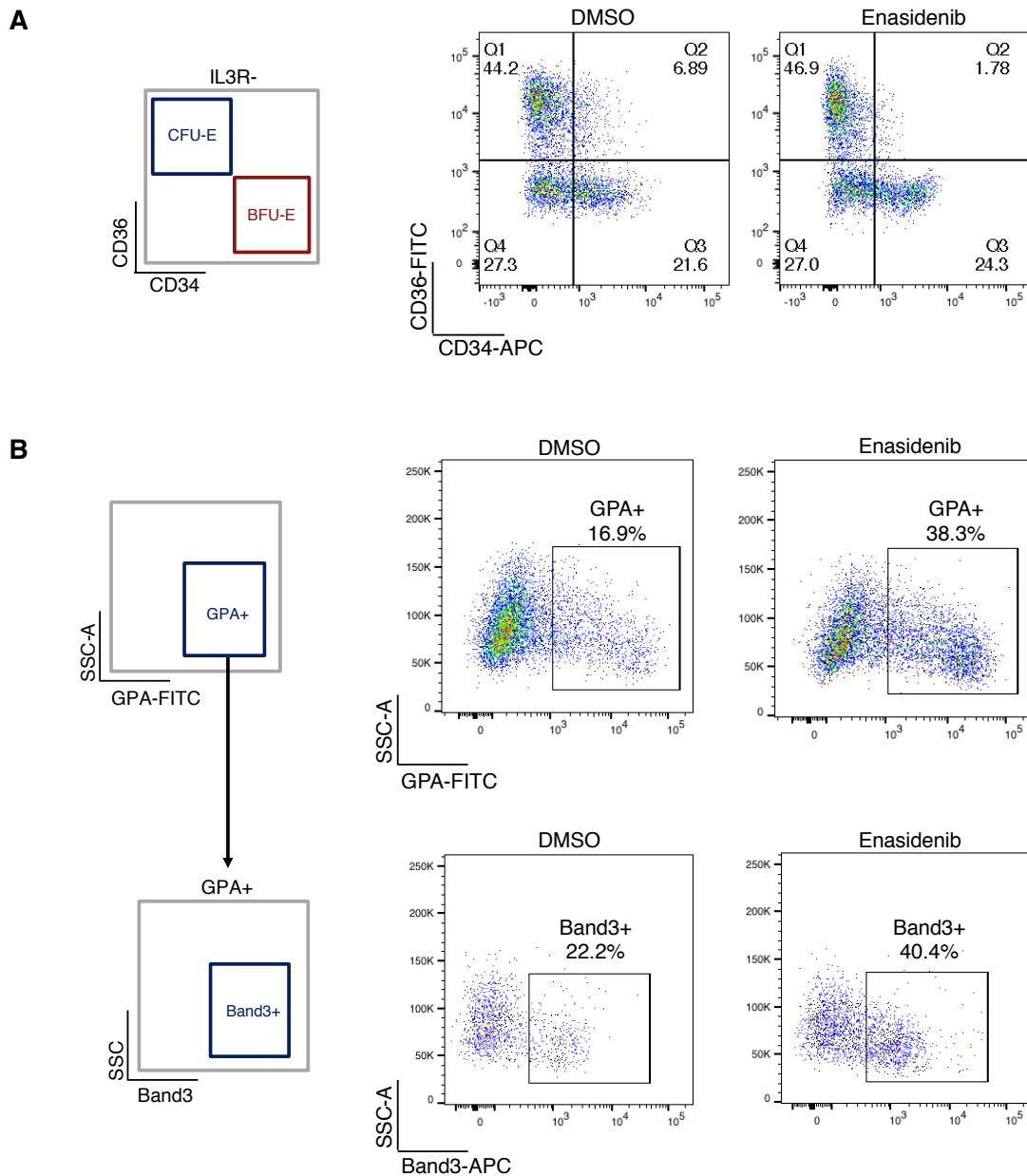
**Supplementary Figure 7. Enasidenib Does Not Alter Levels or Flux of TCA Cycle Metabolites** (A) and (B) FC of LC-MS measurement of TCA cycle metabolites after 16 hours of treatment with DMSO, AGI-6780, or enasidenib in sorted CD71+ or CD71- CB-CD34+ derived cells (DMSO=1) (n=3). (C) Fractional contributions showing the percentage of carbons in each metabolite derived from the labeled tracer nutrient in sorted CD71+ CB-CD34+ derived cells after 16 hours of treatment with DMSO or enasidenib (n=3). Graphs represent Mean  $\pm$  SD. Statistical significance was calculated using an unpaired two-tailed *t*-test.



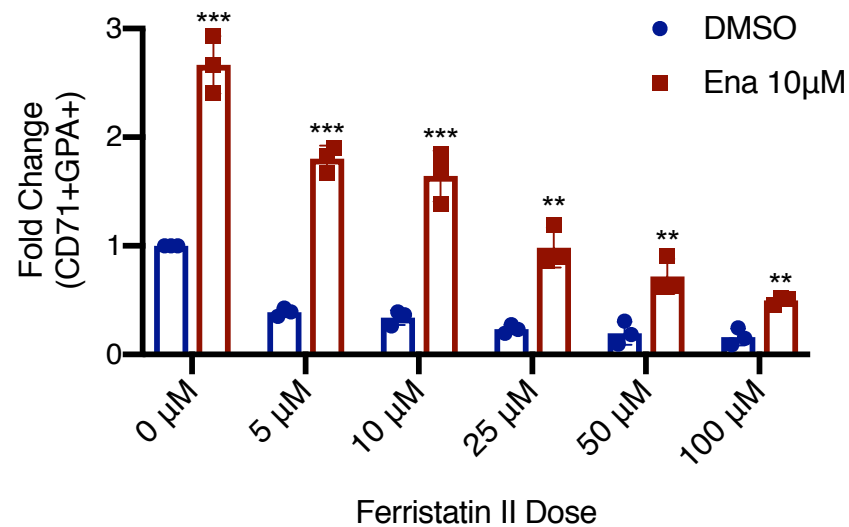
**Supplementary Figure 8. Schema for IDH2-KO Sorting** (Top panel) Schema depicting the sorting strategy for IDH2-KO cells edited by CRISPR-Cas9. GFP+BFP+ cells were plated into EDC after sorting. (Bottom left) Representative plot of the post-sort purity check of GFP+BFP+ cells (Bottom right). Representative plot of GFP+BFP+ cells in culture after 8 days. Cells were gated on the GFP+BFP+ population before further analysis of erythroid differentiation.



**Supplementary Figure 9. Enasidenib Does Not Alter CFU-E Colony Formation** Methylcellulose colony forming assay of CB-CD34+ cells indicating the number of CFU-E colonies observed with DMSO or enasidenib treatment after 7 days (n=3).

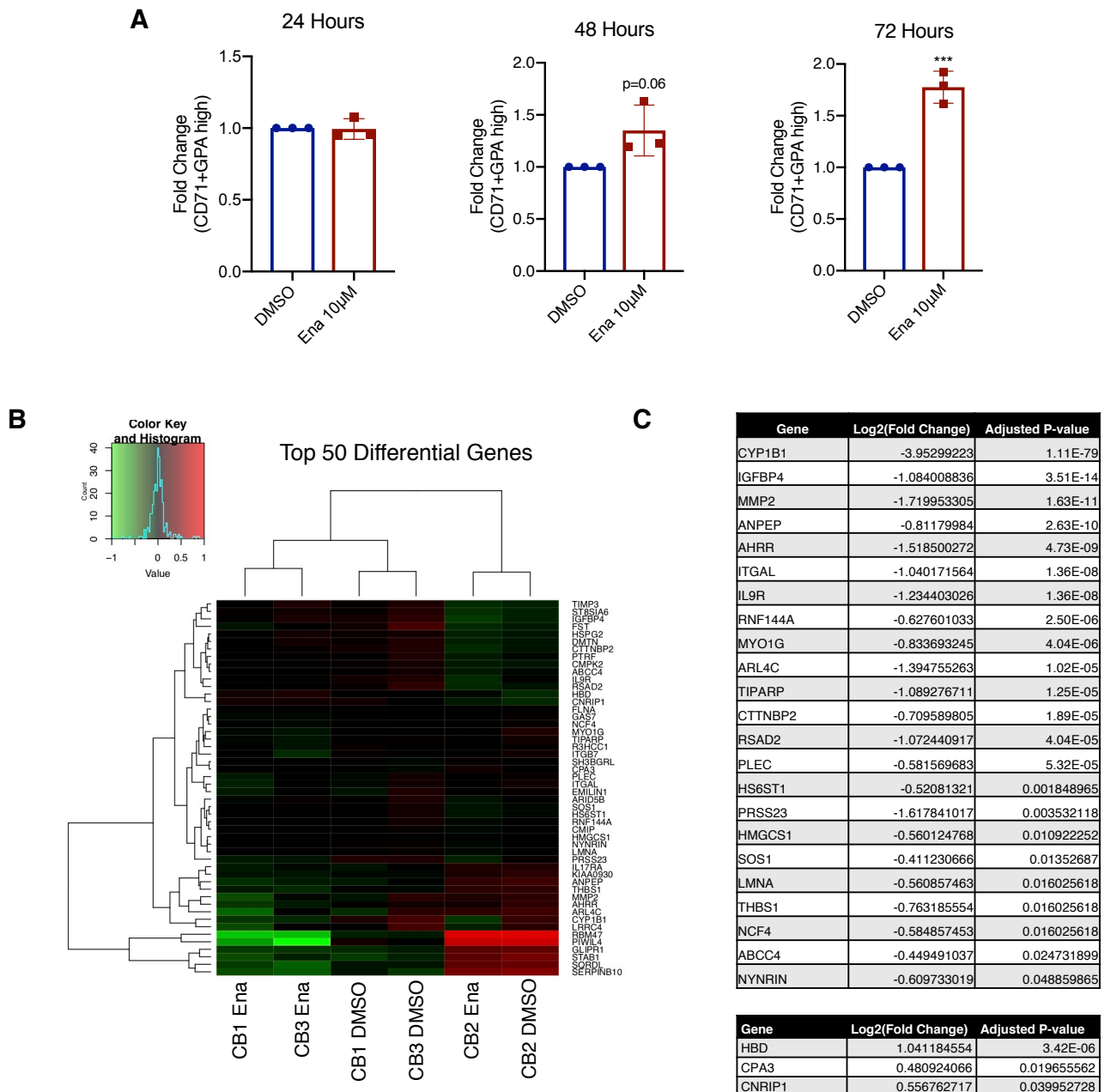


**Supplementary Figure 10. Gating Schema for Erythroid Progenitors** (A) Gating schema and representative flow plots for BFU-E and CFU-E. Cells are first gated on IL3R- and then on CD34 and CD36 as depicted for DMSO and enasidenib 10 $\mu$ M treated cells. (B) Gating schema and representative flow plots for GPA+ and Band3+. Cells are first gated on GPA+ as shown in the top panel and then gated on Band3+ for DMSO and enasidenib 10 $\mu$ M treated cells.

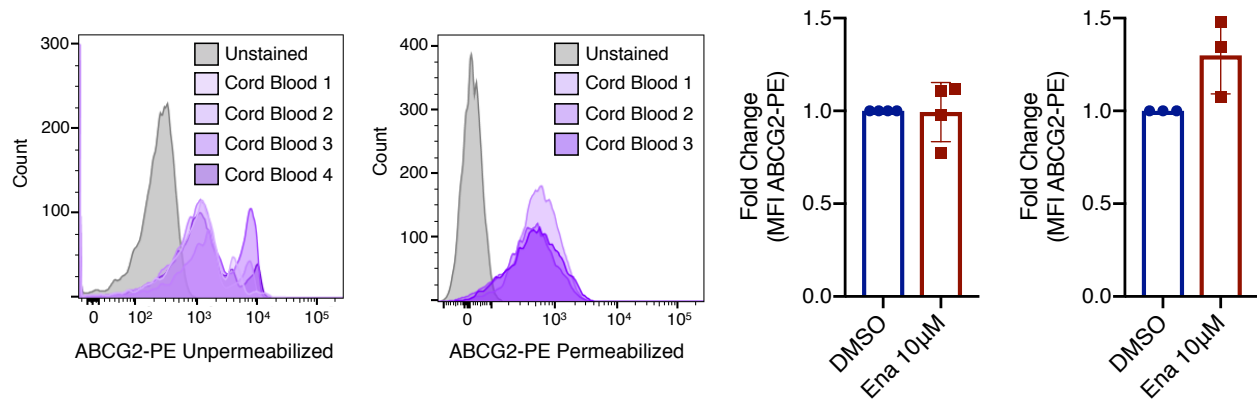


**Supplementary Figure 11. Enasidenib-Mediated Erythroid Differentiation is Reduced by Inhibition of CD71 FC of %CD71+GPA+ cells 8 days in EDC with indicated doses of ferristatin II, a small molecule that promotes degradation of CD71 (DMSO with 0 µM ferristatin II = 1) (n=3).** Graphs represent Mean  $\pm$  SD. Statistical significance was calculated using an unpaired two-tailed *t*-test and all comparisons were made to the respective DMSO condition.

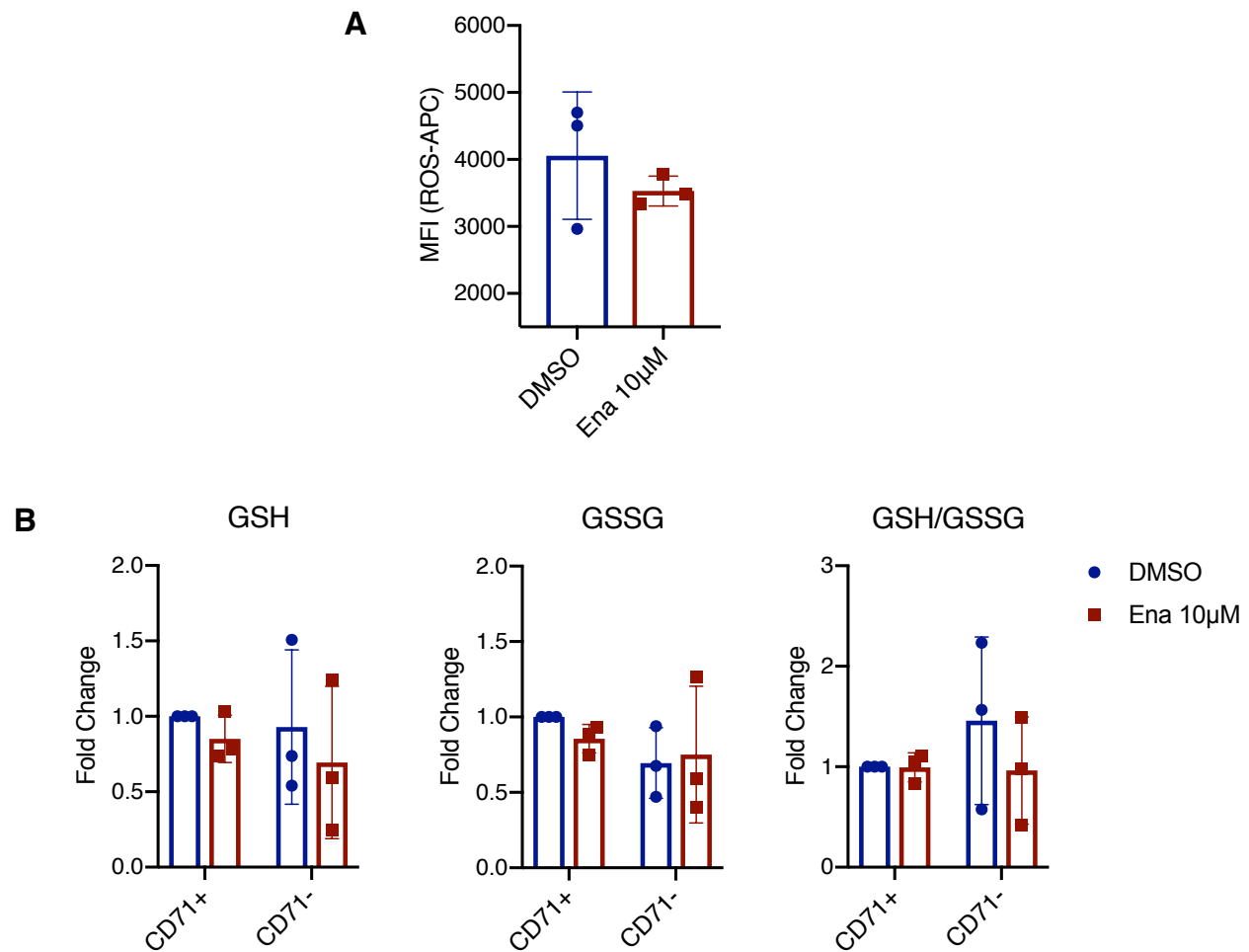




**Supplementary Figure 12. RNA-Seq Reveals Limited Transcriptional Changes at Early Timepoints of Enasidenib Treatment (A)** FC of %CD71+GPA-high cells in CD71+ sorted erythroid progenitors after 24, 48, and 72 hours of culture with DMSO or enasidenib (n=3). Graphs represent Mean  $\pm$  SD. Statistical significance was calculated using an unpaired two-tailed *t*-test. **(B)** Heatmap depicting the top 50 differentially expressed genes between DMSO and enasidenib conditions at 24 hours (n=3). **(C)** (Top) Log<sub>2</sub>(Fold Change) of all significantly downregulated genes in enasidenib-treated cells (adjusted *p*-value <0.05) (Bottom) Log<sub>2</sub>(Fold Change) of all significantly upregulated genes in enasidenib-treated cells (adjusted *p*-value <0.05).



**Supplementary Figure 13. Enasidenib Does Not Change Expression Levels of ABCG2**  
(Leftmost) Extracellular ABCG2 expression on unfixed, unpermeabilized CB-CD34<sup>+</sup>-derived cells at D4 of EDC (n=4) compared to unstained cells. (Left middle) Total ABCG2 expression on fixed and permeabilized CB-CD34<sup>+</sup>-derived cells at D4 of EDC (n=3) compared to unstained cells. (Right middle) FC of ABCG2 expression, measured by mean fluorescence intensity (MFI), in unfixed cells (DMSO=1) (n=4). (Rightmost) FC of ABCG2 expression, measured by MFI, in fixed, permeabilized cells (DMSO=1) (n=3).



**Supplementary Figure 14. Enasidenib Does Not Increase ROS or Oxidative Stress in Erythroid Progenitors** (A) Reactive oxygen species (ROS) measurements in CB-CD34+ cells after D8 of EDC (n=3). (B) FC of LC-MS measurements of GSH and GSSG after 16 hours of treatment with enasidenib in sorted CD71+ and CD71- CB-derived cells (DMSO=1) (n=3). Graphs represent Mean  $\pm$  SD. Statistical significance was calculated using an unpaired two-tailed *t*-test.

## Supplemental Methods

### *Statistics and Figure Generation*

Prism8 (GraphPad) was used to perform statistics. Error bars represent SD. An unpaired (two-tailed) t-test was used to define statistical significance (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 and \*\*\*\*P < 0.0001). One-way ANOVA tests were performed for experiments with more than two conditions. Experiments were performed with at least 3 biological replicates, with technical triplicates or duplicates per biological samples unless otherwise noted. Figure 4J was created with Biorender.com.

### *Differentiation Assays*

For erythroid differentiation assays, CD34<sup>+</sup> cells derived from umbilical cord blood or normal bone marrow were plated in StemSpan SFEM II (STEMCELL Technologies) supplemented with 1X StemSpan Erythroid Expansion Supplement (STEMCELL Technologies) and 1X Penicillin-Streptomycin (P/S, Gibco) (termed “Erythroid Differentiation Media”). Cells were plated at an initial density of 50,000 cells/mL and additional media was added on day 4 of culture. TF-1 cells were obtained from ATCC and cultured in RPMI 1640 + Glutamax (Gibco) with 10% fetal bovine serum (FBS, Omega Scientific Australian Source), 1X P/S with 2ng/mL of GM-CSF (Peprotech). In order to induce erythroid differentiation, cells were washed 4X with PBS to remove GM-CSF and cultured in RPMI 10% FBS 1X P/S with 1U/mL, 2U/mL, 5U/mL, or 10U/mL of EPO (Peprotech). For myeloid differentiation assays, CB-CD34<sup>+</sup> cells were plated in Myelocult H5100 (STEMCELL Technologies) with added SCF, Flt-3L, IL-3, IL-6, GM-CSF, G-CSF (all 20 ng/mL, Peprotech), 0.5 µg/ml Hydrocortisone, and 1X P/S. Unless otherwise noted, cells were cultured in 37°C and 5% CO<sub>2</sub>. Cells in hypoxic conditions were cultured in a hypoxic tri-gas chamber (37°C, 5% CO<sub>2</sub>, 2% O<sub>2</sub>, 5% N<sub>2</sub>).

### *Primary Human Samples*

Cord blood was collected with written informed consent from the mother before delivery of full-term pregnancies at the Lucile Packard Children's Hospital or purchased from the New York Blood Center (NYBC). Samples were processed using a Ficoll-Paque PLUS (GE Healthcare) density gradient media to isolate mononuclear cells, followed by ACK lysis (ACK Lysing Buffer, Thermo Fisher Scientific) to remove remaining red blood cells. CD34 enrichment was performed by magnetic cell separation using MACS CD34 Microbead kit (Miltenyi Biotec).

### *Drug Assays*

Enasidenib and AG-120 were purchased from Chemietek. AG-881 was purchased from SelleckChem; AGI-6780, Ko143, and Ferristatin II were purchased from Sigma-Aldrich. All drugs except ferristatin II were resuspended in DMSO and added at the indicated concentrations at the beginning of the culture period. Ferristatin II was resuspended in water. Fresh drug was added with media replenishment at day 4 of culture.

For drug-washout experiments, cells were plated in Erythroid Differentiation Media with DMSO or enasidenib 10  $\mu$ M at 50,000 cells/mL and were washed 4X with PBS at the indicated timepoints. Cells were then re-plated in fresh Erythroid Differentiation Media with no DMSO or enasidenib and erythroid differentiation was read out by %CD71+GPA+ expression at day 8 of culture.

### *Flow Cytometry/Fluorescence-Activated Cell Sorting*

Cells were analyzed using a FACSCanto II (BD), FACS Aria II (BD), or CytoFLEX (Beckman Coulter) and sorted using the FACS Aria II (BD). Cells were washed with FACS Buffer (PBS 2% FBS 2mM EDTA) and stained for 30 minutes on ice in a 50  $\mu$ L total volume. Cells were then washed in FACS buffer before analysis or sorting. To enumerate number of CD71/GPA cells, CountBright Absolute Counting Beads (ThermoFisher Scientific) were utilized according to

manufacturer's protocol. For intracellular ABCG2 staining, 200,000 cells were fixed and permeabilized using the BD Cytofix/Cytoperm kit prior to staining. For PPIX fluorescence measurement, cells were washed twice with FACS buffer and fluorescence was measured using a 405 laser with 635LP 670/30BP filter (BV650 channel) on a FACSAria II (BD). For sorting of CD71/GPA populations, cord-blood derived cells were cultured in Erythroid Differentiation Media for 6 days and stained with CD71 and GPA. Cells were sorted into StemSpan SFEM II and purity of >95% was verified using post-sort analysis. Cells were subsequently plated in Erythroid Differentiation Media + enasidenib or DMSO. The following antibodies were utilized for flow cytometry: Anti-human CD71 (eBioscience, PE-Cy7, Clone OKT9, 1:50), Anti-human GPA/CD235a (Biolegend, Clone HIR2, APC, 1:25), Anti-human CD338/ABCG2 (Invitrogen, Clone 5D3, PE, 1:25), Anti-human CD33 (BD, Clone WM53, PE, 1:50), Anti-human CD14 (BD, Clone M $\phi$ P9, APC-Cy7, 1:25), Anti-human CD123 (eBioscience, Clone 6H6, PE, 1:200), Anti-human CD36 (BD Biosciences, Clone CB38, FITC, 1:200), Anti-human CD34 (BD Biosciences, APC, 1:200), Anti-human CD235a (BD Biosciences, Clone HIR2, FITC, 1:200), Anti-human CD49d (Miltenyi Biotec, Clone MZ18-24A9, PE, 1:200), Anti-human Band3 (Noncommercial from Narla Lab, New York Blood Center) (1). Propidium iodide was utilized as a live/dead stain. Data was analyzed using FlowJo Software (BD). Cells were first gated on live and singlet cells before further analysis.

#### *Quantitative RT-PCR*

RNA was isolated using the MiniPrep Plus Kit (Qiagen) and reverse transcribed using the High Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific). TaqMan Universal PCR Master Mix (ThermoFisher Scientific) was used to prepare the reaction mixture, and RNA levels were recorded on the QuantStudio 7 Flex Real-Time PCR Instrument (Applied Biosystems). TaqMan Primer-

Probes were purchased from ThermoFisher Scientific: *GAPDH* (Hs02786624\_g1), *KLF1* (Hs00610592\_m1), *GATA1* (Hs01085823\_m1), *EPOR* (Hs00959427\_m1), *GATA2* (Hs00231119\_m1), *SPI1* (Hs02786711\_m1), *HBG1/HBG2* (Hs00361131\_g1), *HBA2/HBA1* (Hs00361191\_g1), *HBD* (Hs00426283\_m1), *HBB* (Hs00747223\_g1).

#### *Western Blot*

Cells were lysed with standard RIPA buffer. Cell extracts were fractionated with SDS-polyacrylamide gel electrophoresis (NuPAGE 4-12% Bis-Tris Gel, Invitrogen) and transferred onto 0.45-micron nitrocellulose membranes (Amersham Protan, GE Healthcare Life Sciences). Primary antibodies utilized were IDH2 Clone D8E3B (rabbit, Cell Signaling) and Vinculin Clone V284 (mouse, Biorad). Primary antibodies were detected with horseradish peroxidase-conjugated secondary antibodies (anti-rabbit and anti-mouse, Cell Signaling). Antibodies were detected using Clarity Western ECL Substrate (Biorad).

#### *Microscopy*

Cord-blood derived cells treated with enasidenib or DMSO in Erythroid Differentiation Media for 8 days were resuspended at  $1 \times 10^6$  cells/mL in 0.1% bovine serum albumin (BSA) in PBS and cytopun onto slides. For Wright-Giemsa staining, slides were fixed in 100% methanol and subsequently stained with May-Grünwald solution (Sigma), washed with DI water, and stained with Giemsa solution (Sigma) before washing and mounting with a glass cover slip. For benzidine staining, cells were stained in 1% 3,3-dimethoxybenzidine (benzidine, Sigma) in methanol and subsequently transferred into 2.5% hydrogen peroxide/EtOH solution and washed with tap water. Slides were then stained with 1x Hematoxylin 7211 (Richard-Allan Scientific, ThermoFisher Scientific) before washing with water and mounting. Slides were imaged using the confocal microscope LSM710 (Zeiss). For PPIX microscopy, cells were fixed in 4% paraformaldehyde

solution and 200,000 cells were cytopun onto slides, Slides were mounted and imaged using the confocal microscope LSM710 (Zeiss) using the HeNe 633nm laser for excitation. Images were processed using Fiji (ImageJ).

#### *Hemoglobin Colorimetric Assay*

$2 \times 10^6$  CB-derived cells were collected per condition after 14 days of culture in erythroid expansion media as described above. Hemoglobin was measured using the Hemoglobin Assay Kit (Sigma-Aldrich) per manufacturer's instructions. Absorbance was measured at 400nm using SpectraMax M3 Microplate Reader.

#### *D-2-HG Assays*

THP-1 or cord blood samples were plated at 250,000 cells/mL with addition of enasidenib, AGI-6780, or AG-881 in culture conditions described above. The THP-1 IDH2-R140Q cell line is a doxycycline-inducible system previously established in our lab (2). Doxycycline was added to THP-1 IDH2-R140Q cells at 1  $\mu\text{g}/\text{mL}$  to induce IDH2-R140Q expression at the beginning of the culture period. After three days, cell pellets ( $1 \times 10^6$  cells) or supernatant was collected for D-2-HG measurement. For cell pellet samples, cells underwent three freeze thaw cycles and were lysed using CellLytic M (Sigma). Cell culture supernatant or cell lysate was incubated overnight at 37°C with Proteinase K (400  $\mu\text{g}/\text{mL}$ ) and deproteinized using deproteinization kit (Biovision). D-2-HG was subsequently measured using the D-2-HG Assay Kit (Sigma) following the manufacturer's instructions. For addition of 2-HG to culture, we added the membrane-permeant precursor of 2-HG, (2R)-octyl-alpha-2HG (Cayman Chemicals) dissolved in DMSO, to culture at 50 or 200  $\mu\text{M}$ .

#### *Colony Assays*

5000 CB-CD34<sup>+</sup> were added to 4 mL of MethoCult H4434 (STEMCELL Technologies) with DMSO or 10  $\mu\text{M}$  enasidenib and plated in triplicate in a SmartDish (STEMCELL Technologies).



Plates were incubated at 37°C and 5% CO<sub>2</sub>. The number of colonies in each sample was scored at 14 days to determine the numbers of BFU-Es, CFU-GEMM/M/G/GM and at 7 days to determine the number of CFU-E colonies.

### *Metabolomics*

CB-CD34<sup>+</sup> cells were plated in Erythroid Differentiation Media for 6 days. 750,000 CD71<sup>+</sup> and CD71<sup>-</sup> cells were sorted and plated in RPMI 1640 (+) L-glutamine (-) D-glucose (Gibco) with 10% dialyzed FBS (OneShot FBS, ThermoFisher Scientific), 1X P/S, and U13C glucose (Cambridge Isotope Laboratories) at 2g/L with enasidenib, AGI-6780, or DMSO. Cells were collected after 16 hours of culture, washed 2x in PBS, and pellets were flash frozen and stored in -80°C until metabolite analysis. Metabolomics was performed with UCLA Metabolomics Core. Cell pellets were resuspended in 1 ml cold 80% methanol (-80C) and kept on dry ice for 15 min with occasional vigorous vortexing. After centrifugation for 10 min at 16,000g at 4C, supernatants were transferred to a glass vial. The remaining pellet was re-extracted with 100µl 80% methanol, and after clearing the supernatant was combined with the first extraction volume. Extracts were dried in a Genevac evaporator at 30°C and stored at -80°C.

Dried metabolites were resuspended in 50% ACN and 5µl loaded onto a Luna 3µm NH<sub>2</sub> 100A (150 × 2.0 mm) column (Phenomenex). The chromatographic separation was performed on an UltiMate 3000 RSLC (ThermoFisher Scientific) with mobile phases A (5 mM NH<sub>4</sub>AcO pH 9.9) and B (ACN) and a flow rate of 200µl/min. The gradient from 15% A to 95% A over 18 min was followed by 9 min isocratic flow at 95% A and reequilibration to 15% A. Metabolite detection was achieved with a Thermo Scientific Q Exactive mass spectrometer run with polarity switching (+3.0 kV/- 2.25 kV) in full scan mode with an m/z range of 65-975. Metabolite peaks were identified with TraceFinder 4.1 (ThermoFisher Scientific) using accurate mass measurements (< 3 ppm) and

retention time and quantified by integrating area under the curve. Relative amounts of metabolites were calculated by summing up all isotopologues of a given metabolite and normalized to cell number. Fractional contribution values, showing the percentage of carbons of each metabolite derived from the labeled tracer nutrient, were calculated using the mass isotopologue distribution values for each isotopologue of the metabolite.

Hemin, PPIX, and ZnPPIX measurements were performed at the Iron and Heme Core facility at the University of Utah using the following protocol. Six CB-CD34<sup>+</sup> samples cultured in enasidenib or DMSO in Erythroid Differentiation Media for 8 days and combined into two groups (CB1/2/3 and CB4/5/6) to reach  $130 \times 10^6$  cells per condition for metabolite analysis. Samples were subsequently washed with PBS, pelleted, and flash frozen. The cells were suspended in two pellet volumes of 50mM potassium phosphate pH7.4 and homogenized by sonication. The resulting homogenate was assayed for BCA protein content, and then adjusted to about 10mg/mL in the same homogenization buffer (50mM KPi pH7.4). Extraction solvent (EA) was prepared by mixing four volumes of ethyl acetate to one volume of glacial acetic acid. While being vortexed vigorously, 200 $\mu$ L EA was slowly added to 50 $\mu$ L adjusted sample and the shaken for another 60 seconds. The mixture was centrifuged at 16,000xg for 0.5 minutes and the supernatant was collected, which was around 90% of the total volume. About 10 $\mu$ L of the supernatant solution above was injected into a Waters Acquity UPLC system which included a binary solvent manager, sample manager, photodiode array detector (PDA), fluorescence detector (FLR), column heater and an Acquity UPLC BEH C18, 1.7  $\mu$ M, 2.1 x 100 mm column. The detector settings were as follows: PDA to measure hemin absorbance at 398nm and the FLR to measure fluorescence of protoporphyrin IX (PPIX) at 404 nm excitation and 630 nm emission and of Zn protoporphyrin IX (ZnPPIX) at 406 nm excitation and 586 nm emission. The sample chamber was kept dark and at

ambient temperature. Solvent A was 0.2% aqueous formic acid while Solvent B was 0.2% formic acid in methanol. The flow rate was kept at 0.40 mL per minute and the column maintained at 60°C for the total run time of 7 min. The following successive linear gradient settings for run time in minutes versus Solvent A were as follows: 0.0, 80%; 2.5, 1%; 4.5, 1%; 5.0, 80%. For standards, extract solutions of known concentrations of authentic hemin, PPIX, and ZnPPIX dissolved in 1% aqueous trimethylamine.

### *CRISPR-Cas9 Editing*

To generate IDH2 knockout in human hematopoietic progenitors, we used CRISPR/Cas9 and AAV6, following the methodology previously published (3). In short, single guide RNAs (sgRNAs) were designed to target IDH2 at exon 3, which is expressed in all known IDH2 isoforms (genomic sgRNA target sequences with PAM in bold: GGTCACGGTTTGGGAGCCCG**AGG**). sgRNAs were chemically modified with three terminal nucleotides at both the 5' and 3' ends containing 2' O-Methyl 3' phosphorothioate (Synthego) and precomplexed with purified Cas9 protein (IDT) at a molar ratio of 1:2.5 (Cas9:sgRNA) at 25°C for 10 min immediately prior to use. CB-CD34<sup>+</sup> cells were expanded in StemSpan SFEM II (STEMCELL Technologies) with SCF, Flt3-L, IL-6 (all 20ng/mL, Peprotech), TPO (100ng/mL, Peprotech) and UM-171 (35nM) for 48 hours in a hypoxic tri-gas chamber (37°C, 5% CO<sub>2</sub>, 2% O<sub>2</sub>, 5% N<sub>2</sub>) and then electroporated using the Lonza Nucleofector 4D (program DZ-100) in P3 buffer and 150µg/ml Cas9 protein. AAV vector plasmids were cloned in the pAAV-MCS plasmid (Agilent Technologies) containing ITRs from AAV serotype 2 (AAV2). Vectors contained 400bp homology arms flanking the CRISPR/Cas9 cut site and a reporter gene (either turboGFP or mtagBFP2) driven by a SFFV promoter followed by and BGH polyA. AAV6 particles were produced in 293FT cells transfected using standard PEI transfection with ITR-containing plasmid and pDGM6 containing the AAV6

cap genes, AAV2 rep genes and adenovirus five helper genes, harvested after 72h, purified using the AAVpro Purification Kit (Takara Bio Inc.), according to the manufacturer's instructions and then stored at -80°C until further use. As a negative control, we targeted the AAVS1 locus (genomic target sequence: GGGGCCACTAGGGACAGGATTGG), which acts as a safe harbor. Cells were cultured for an additional three days in hypoxic conditions, after which the BFP+GFP+ double positive population was sorted for both the AAVS1 and IDH2-KO cells using a FACSAriaII (BD) with a post-sort analysis to verify purity of >95%. Cells were plated immediately after sorting for further molecular characterization or in erythroid differentiation assays. Proper integration of the AAV donor DNA into the genomic IDH2 locus was confirmed using PCR, with a reverse primer within the AAV construct (within SFFV) and a forward primer within the genome (in IDH2).

#### *RNA-seq*

RNA was isolated using the RNeasy Mini Kit (Qiagen). Samples were sequenced and analyzed in with Girihlet. RNA with RIN 9 was reverse transcribed to cDNA. cDNA libraries were prepared using 500ng of total RNA using the TruSeq RNA Sample Preparation Kit v2 (Illumina). 2.3. mRNA-seq and data analysis cDNA libraries were sequenced on the Illumina Nextseq platform to obtain 80-bp single-end reads. The reads were trimmed, compressed and mapped to the human genome as previously described (4). The raw read count matrix was normalized to account for variability between different cord blood samples and differential expression was determined using DESeq2 (5).

#### *ABCG2/BCRP Inhibition Assay*

Assay was carried out with Eurofins Discovery (Assay BCRP-CHO, Hoechst 33342 substrate). Recombinant CHO-K1 cells with human BCRP were seeded in a 96-well culture plate at 30,000

cells/well and were used on days 2 or 3 post-seeding. On the day of assay, enasidenib was prepared in assay buffer (HBSS-HEPES, pH 7.4), added to the cell plate at 1  $\mu$ M, 10  $\mu$ M, and 25  $\mu$ M, and pre-incubated at 37 °C for 15 min. Subsequently, Hoechst 33342 was added to the plate followed by 20-min incubation at 37 °C. The plate was then washed with cold assay buffer followed by fluorescence reading. The percent of control activity is calculated by comparing the signal in the presence of the enasidenib to DMSO (control). The percent inhibition is then calculated by subtracting the percent control activity from 100. Ko143 was used as the reference inhibitor in the assay.

#### *ROS Measurements*

ROS was measured using the CellROX Deep Red Flow Cytometry Assay Kit (Molecular Probes) as per manufacturer's instructions after eight days of culture in erythroid differentiation media.

#### **Supplementary Materials References:**

1. Hu J, et al. Isolation and functional characterization of human erythroblasts at distinct stages: implications for understanding of normal and disordered erythropoiesis in vivo. *Blood* 2013;121(16):3246–3253.
2. Chan SM, et al. Isocitrate dehydrogenase 1 and 2 mutations induce BCL-2 dependence in acute myeloid leukemia. *Nat. Med.* 2015;21(2):178–184.
3. Bak RO, et al. Multiplexed genetic engineering of human hematopoietic stem and progenitor cells using CRISPR/Cas9 and AAV6. *eLife* 2017;6:e27873.
4. Howarth DL, et al. Activating Transcription Factor 6 Is Necessary and Sufficient for Alcoholic Fatty Liver Disease in Zebrafish. *PLoS Genet.* 2014;10(5):e1004335.

5. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15(12):550.