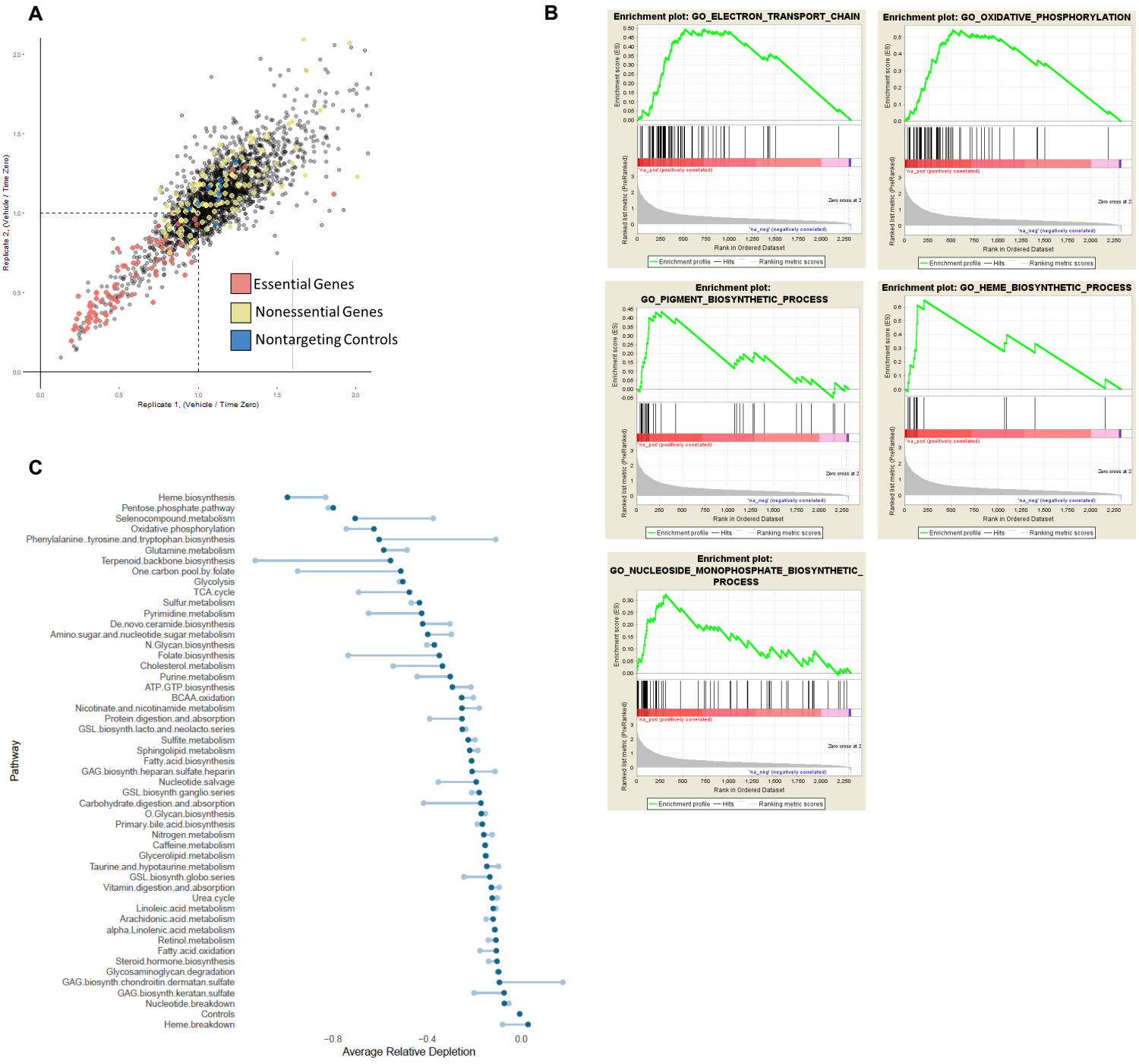

Figure S1: Characterization of screen reproducibility and pathway-level analysis, related to Figure 1.

(A) Replicate-to-replicate comparison of gene-level essentiality phenotypes in THP-1. Essential controls are shown in red, non-essential controls in yellow, and non-targeting controls in blue. **(B)** GSEA enrichment plots for five top-scoring pathways pulled out from pre-ranked GSEA analysis of a CRISPR-based metabolic screen performed in the presence versus absence of ABT-199. **(C)** Metabolic library genes were binned into metabolic pathways. Overall essentiality score for a pathway, shown in light blue, was calculated as the average essentiality TS of all genes binned in that pathway. Sensitizing score, shown in dark blue, was calculated as the average sensitizing TS of all genes binned.

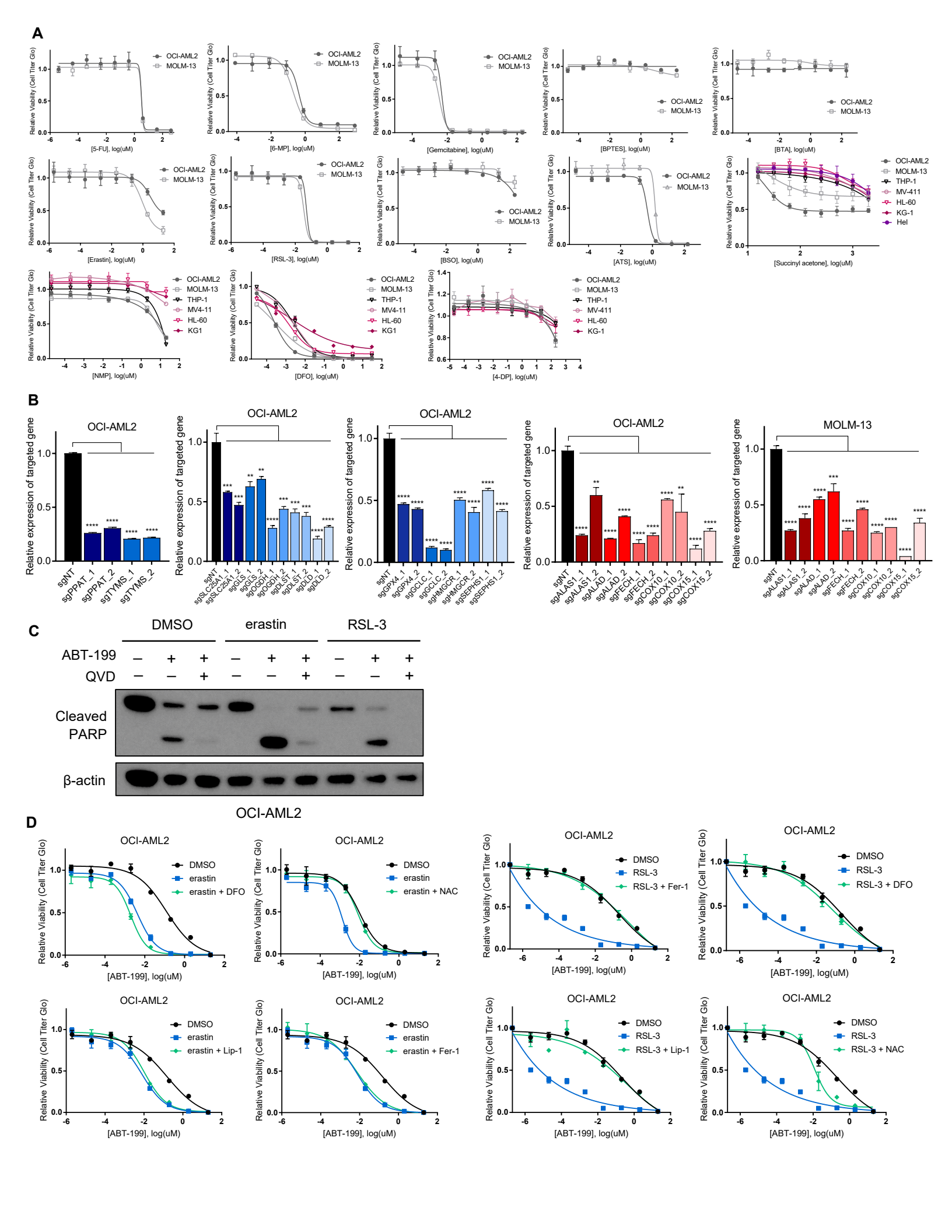
A
OCI-AML2
MOLM-13
Relative Viability (Cell Titer Glo)
[5-FU], log(uM)
[6-MP], log(uM)
[Gemcitabine], log(uM)
[BPTES], log(uM)
[BTA], log(uM)
[Erastin], log(uM)
[RSL-3], log(uM)
[BSO], log(uM)
[ATS], log(uM)
[Succinyl acetone], log(uM)
THP-1
MV-411
HL-60
KG-1
Hel
MV4-11
[NMP], log(uM)
[DFO], log(uM)
[4-DP], log(uM)
KG-1
B
OCI-AML2
MOLM-13
Relative expression of targeted gene
sgNT
sgPPAT_1
sgPPAT_2
sgTYMS_1
sgTYMS_2
sgSLC25A1_1
sgSLC25A1_2
sgGLS_1
sgGLS_2
sgOGDH_1
sgOGDH_2
sgDLST_1
sgDLST_2
sgDLD_1
sgDLD_2
sgGPX4_1
sgGPX4_2
sgGCLC_1
sgGCLC_2
sgHMGCR_1
sgHMGCR_2
sgSEPHS1_1
sgSEPHS1_2
sgALAS1_1
sgALAS1_2
sgALAD_1
sgALAD_2
sgFECH_1
sgFECH_2
sgCOX10_1
sgCOX10_2
sgCOX15_1
sgCOX15_2
C
DMSO
erastin
RSL-3
ABT-199
QVD
Cleaved PARP
β-actin
D
OCI-AML2
DMSO
erastin
erastin + DFO
erastin + NAC
erastin + Lip-1
erastin + Fer-1
RSL-3
RSL-3 + Fer-1
RSL-3 + DFO
RSL-3 + Lip-1
RSL-3 + NAC
[ABT-199], log(uM)

Figure S2: Validation of therapeutic / genetic perturbations, related to Figure 3.

(A) 72hr dose-response curves for drugs used to validate hits. Sub-lethal doses for combination treatment with ABT-199 were selected based on reported curves. **(B)** Relative mRNA expression of targeted metabolic genes measured by qRT-PCR. OCI-AML2 or MOLM-13 cells were transduced with CRISPR constructs targeting each of the stated genes. Reported values represent relative depletion of the targeted gene against nontargeting sgRNA. **(C)** Immunoblots of cleaved-PARP in OCI-AML2 cells treated for 24hr with ABT-199 30nM, QVD 20 μM, and erastin 4 μM or RSL-3 600nM. **(D)** 72hr ABT-199 dose-response curves for OCI-AML2 cells treated with background doses of erastin 4 μM or RSL-3 200nM, DFO 2.5 μM, NAC 1mM, Lip-1 1 μM, and Fer-1 1 μM.

** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$ by student's t-tests; n=3. Data are mean ± SD.

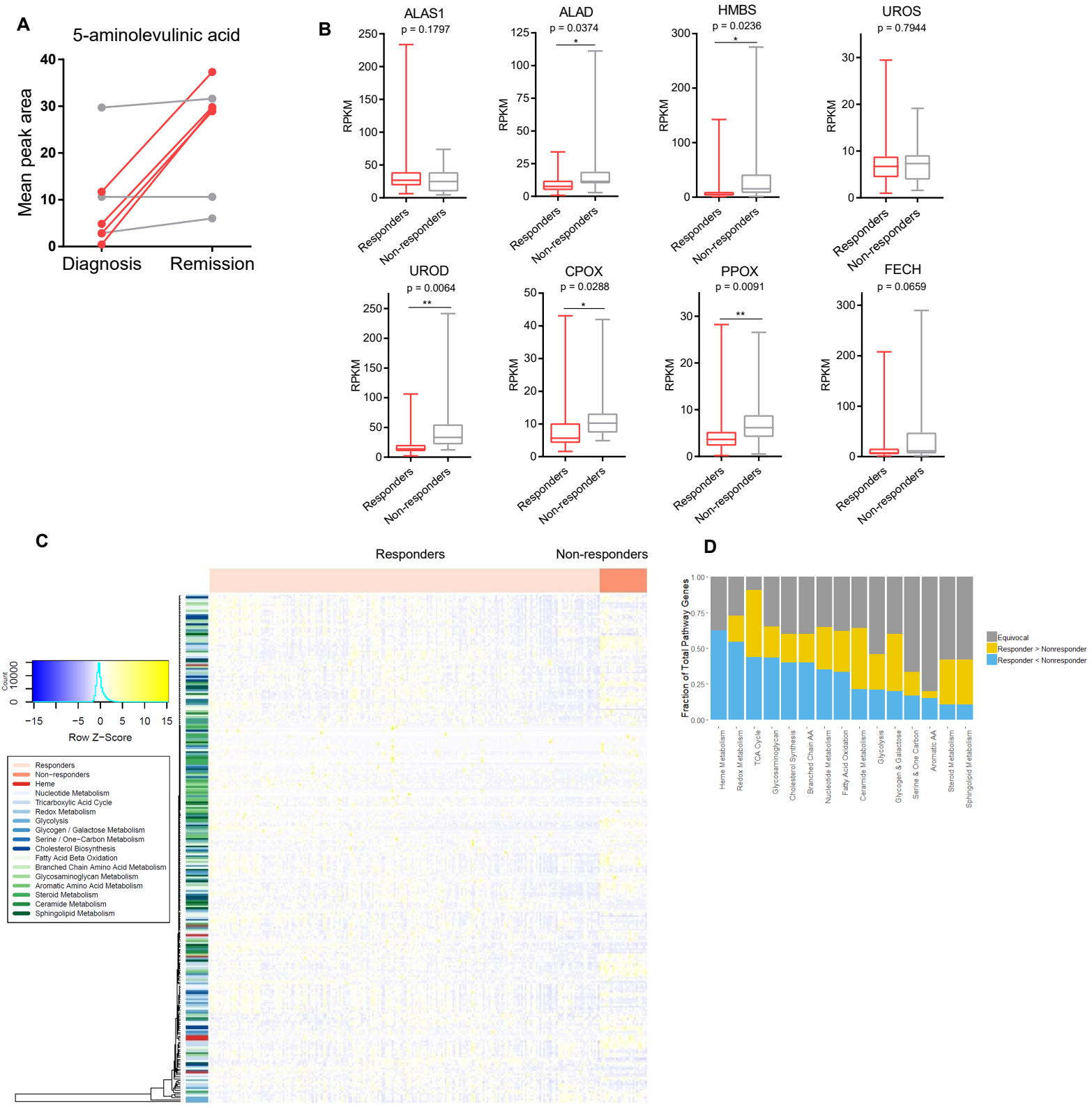

Figure S3: Matched patient samples exhibit restoration of heme pathway upon remission, related to Figure 4.

(A) Comparison of 5-aminolevulinic acid levels from pediatric AML patients taken at diagnosis vs. upon remission (n = 7). Data shown reflect matched 5-ALA levels for each individual. **(B)** Expression pattern of heme biosynthetic pathway genes in on-diagnosis samples from pediatric patients who achieved remission with induction therapy (responders) n = 213, or pediatric patients who failed to achieve remission within two rounds of induction therapy (non-responders) n = 25. *p ≤ 0.05, ** p ≤ 0.01 by student's t-tests with Welch's correction. Data are mean ± SD. **(C)** Heatmap of metabolic gene expression from responder samples and non-responder samples, with unsupervised clustering of rows. Data displayed as RPKM intensities, mean-centered across all samples. **(D)** Expression of each metabolic gene was individually compared between responder and non-responder groups and binned into one of three categories based on two-sided t-tests (p ≤ 0.05): no significant difference, significantly higher expression in responders, and significantly higher expression in non-responders. The proportion of pathway genes scoring in each category is displayed.

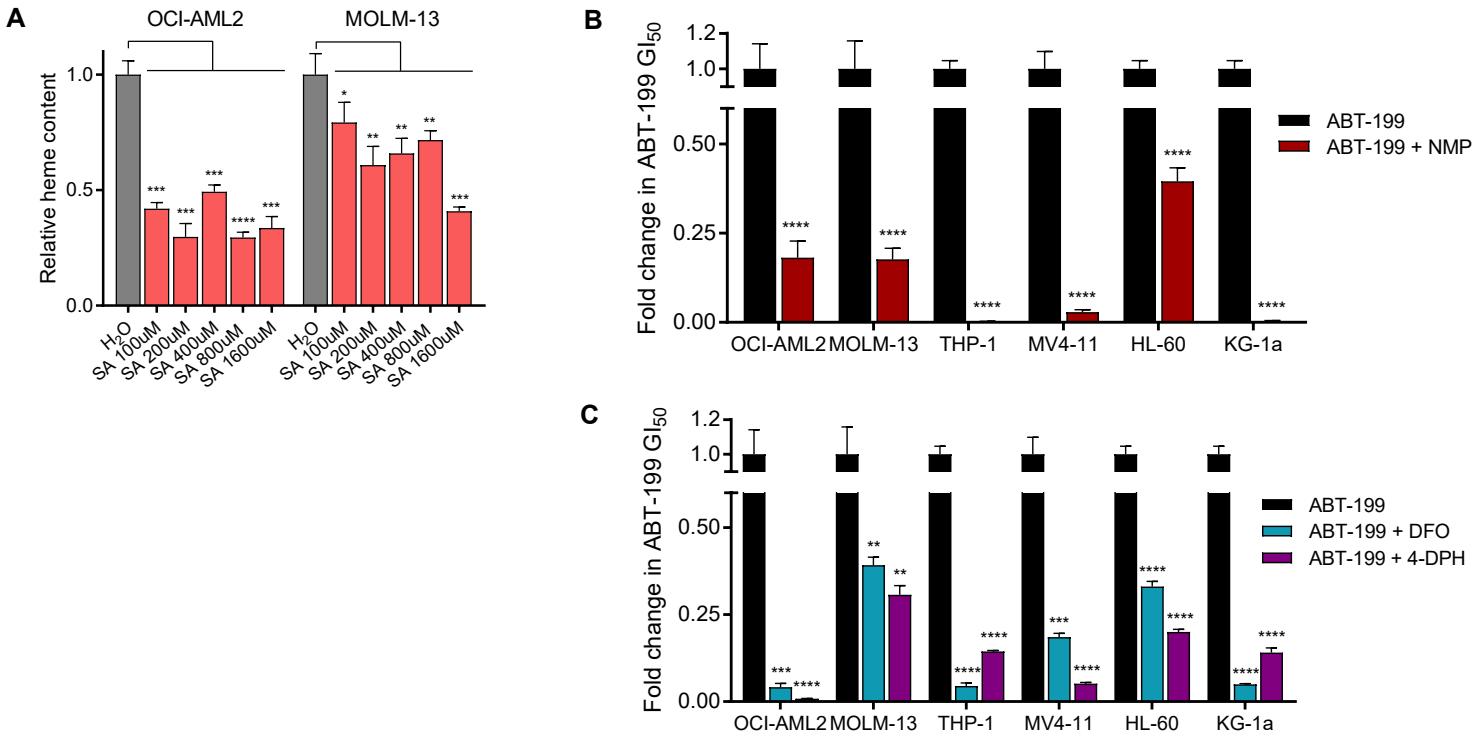

Figure S4: Effects of heme manipulation on heme levels and ABT-199 sensitivity, related to Figure 5.

(A) Heme assay assessing relative heme content. OCI-AML2 and MOLM-13 cells were treated for 48h with increasing doses of SA. **(B)** Fold change in 72hr GI_{50} values derived from ABT-199 dose-response curves, in the presence and absence of NMP. OCI-AML2 cells were pre-treated with NMP for 48h before plating (OCI-AML2: 10μM, MOLM-13: 12μM, THP-1: 6μM, MV-411: 10μM, HL-60: 4μM, KG-1: 10μM). Absolute GI_{50} values were normalized to viability in NMP alone to permit observation of supra-additive effects. **(C)** Fold change in ABT-199 72hr GI_{50} values derived from ABT-199 dose-response curves, in the presence and absence of DFO or 4-DPH. OCI-AML2 cells were pre-treated with DFO (OCI-AML2: 4μM, MOLM-13: 2.5μM, THP-1: 4μM, MV-411: 4μM, HL-60: 4μM, KG-1: 4μM) or 4-DPH (OCI-AML2: 3mM, MOLM-13: 3mM, THP-1: 6mM, MV4-11: 6mM, HL-60: 6mM, KG-1: 6mM) for 48h before plating. Absolute GI_{50} values were normalized to viability in each background drug alone.

*p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001 by student's t-tests; n=3, data are mean ± SD.

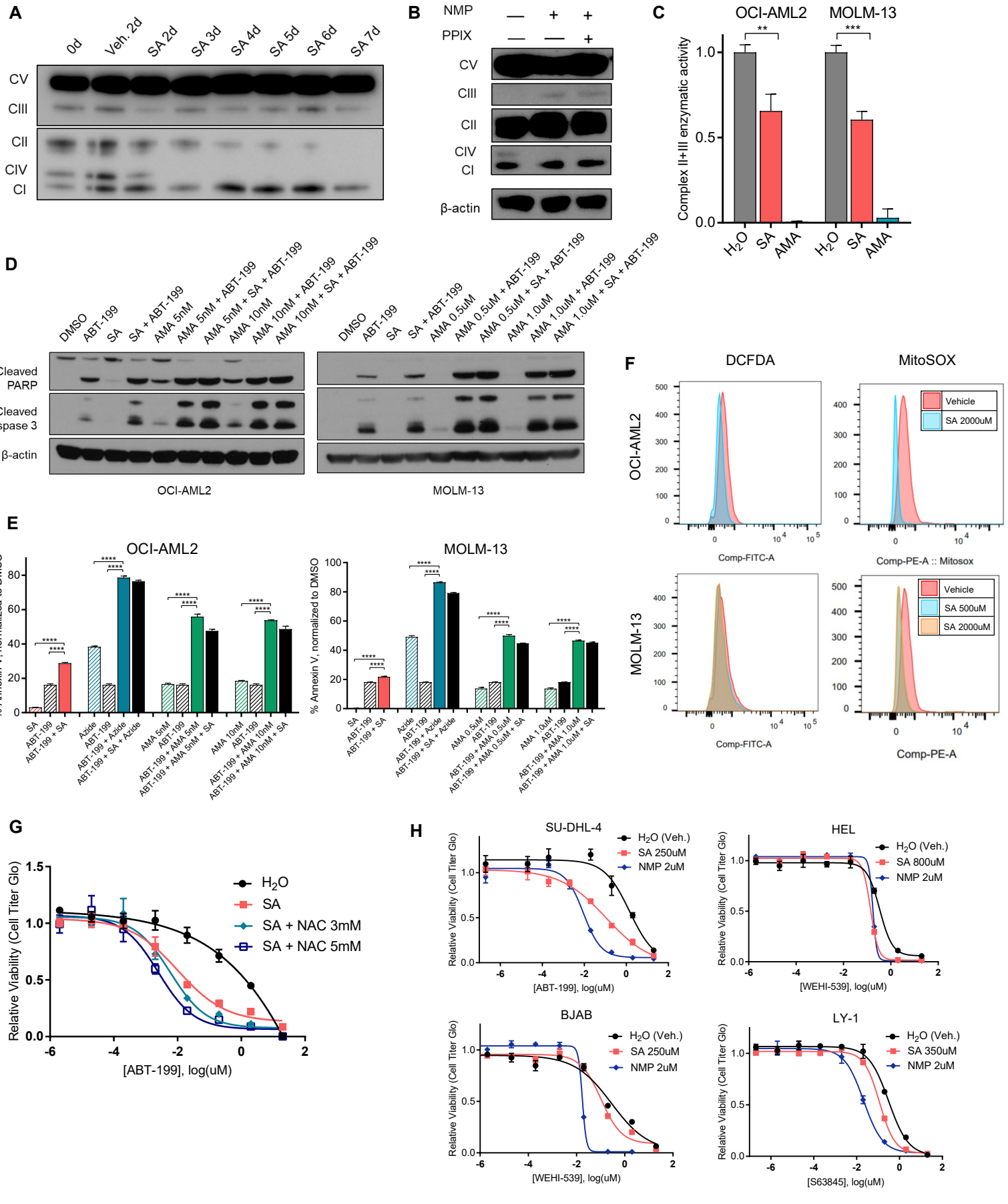
A
0d
Veh. 2d
SA 2d
SA 3d
SA 4d
SA 5d
SA 6d
SA 7d
CV
CIII
CII
CIV
CI
B
NMP
PPIX
CV
CIII
CII
CIV
CI
β-actin
C
OCI-AML2
MOLM-13
Complex II+III enzymatic activity
H2O
SA
AMA
D
DMSO
ABT-199
SA
SA + ABT-199
AMA 5nM
AMA 5nM + ABT-199
AMA 5nM + SA + ABT-199
AMA 10nM
AMA 10nM + ABT-199
AMA 10nM + SA + ABT-199
AMA 0.5uM
AMA 0.5uM + ABT-199
AMA 0.5uM + SA + ABT-199
AMA 1.0uM
AMA 1.0uM + ABT-199
AMA 1.0uM + SA + ABT-199
Cleaved PARP
Cleaved caspase 3
β-actin
OCI-AML2
MOLM-13
E
OCI-AML2
MOLM-13
% Annexin V, normalized to DMSO
F
DCFDA
MitoSOX
OCI-AML2
MOLM-13
Vehicle
SA 2000uM
SA 500uM
Comp-FITC-A
Comp-PE-A :: Mitosox
Comp-PE-A
G
Relative Viability (Cell Titer Glo)
H2O
SA
SA + NAC 3mM
SA + NAC 5mM
[ABT-199], log(uM)
H
SU-DHL-4
HEL
BJAB
LY-1
H2O (Veh.)
SA 250uM
SA 800uM
SA 350uM
NMP 2uM
[ABT-199], log(uM)
[WEHI-539], log(uM)
[S63845], log(uM)

Figure S5: Effects of heme manipulation on the ETC and apoptosis, related to Figure 6.

(A) Immunoblot of subunits of ETC complexes I – V in OCI-AML2 cells treated with SA 250μM for between 2 and 7 days. Complex IV is durably suppressed over 2-day to 7-day SA treatment. Complex II is suppressed around Day 4. **(B)** Immunoblot of subunits of ETC complexes I – V in OCI-AML2 cells treated with NMP 5μM and NMP + PPIX 2.5μM for 48h. **(C)** Enzymatic activity of complexes II + III in cells treated with SA (OCI-AML2: 250μM, MOLM-13: 750μM) and antimycin A (AMA) 5μM for 48hr. Enzymatic activity normalized to vehicle treatment. **(D)** Immunoblots of cleaved-PARP and cleaved-caspase 3 in cells treated with SA for 48hr (OCI-AML2: 100μM, MOLM-13: 750μM), AMA for 48hr (OCI-AML2: 5nM and 10nM, MOLM-13: 0.5μM and 1.0μM), ABT-199 for 8h (OCI-AML2: 50nM, MOLM-13: 30nM), or the combinations. **(E)** Percentage of drug-induced apoptosis (annexin V+) in cells treated with SA for 48hr (OCI-AML2: 100μM, MOLM-13: 750μM), azide (OCI-AML2: 200μM, MOLM13: 2mM) for 48hr, AMA (OCI-AML2: 5nM and 10nM, MOLM-13: 0.5μM and 1.0μM) for 48hr, ABT-199 (OCI-AML2: 50nM, MOLM-13: 30nM) for 8h, or in shown combinations. **(F)** Cytosolic and mitochondrial ROS production assessed by flow cytometry using H_2DCFDA and MitoSOX, with SA treatment for 24h in OCI-AML2 and MOLM-13. **(G)** 72hr ABT-199 dose-response curves for OCI-AML2 cells treated with background dose of SA 300μM and rescued with NAC 3mM and 5mM. **(H)** ABT-199 dose response curves for SU-DHL-4 cells (BCL-2-dependent), WEHI-539 dose response curves for HEL cells and BJAB cells (BCL-xL-dependent), and S63845 dose response curves for OCI-LY-1 cells (MCL-1-dependent), in indicated background doses of SA and NMP, demonstrating sensitization to BH3 mimetics in non-BCL2-dependent, non-AML models.

** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$ by student's t-tests; n=3, data are mean ± SD.

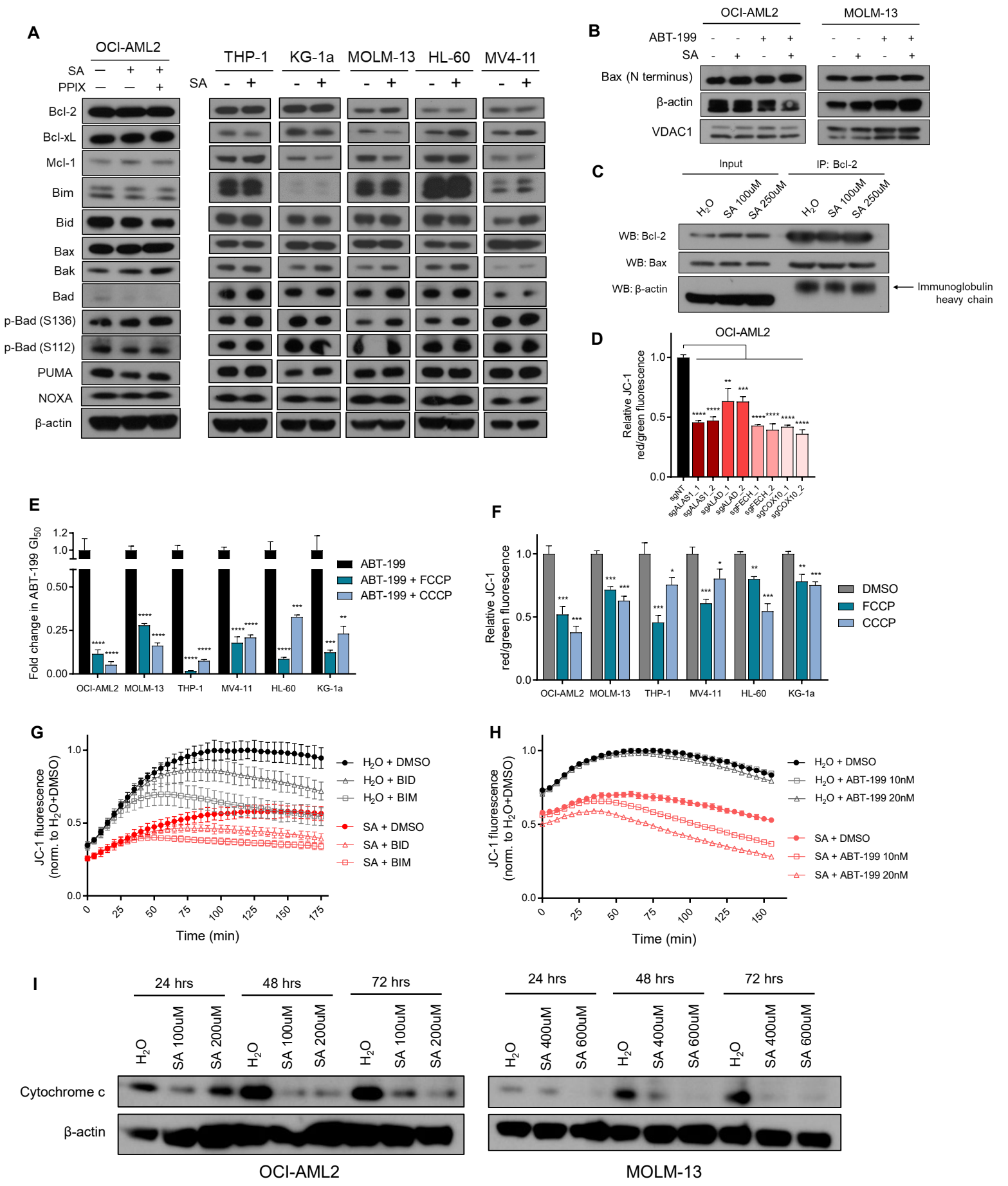
A
OCI-AML2
SA
PPIX
THP-1
KG-1a
MOLM-13
HL-60
MV4-11
Bcl-2
Bcl-xL
Mcl-1
Bim
Bid
Bax
Bak
Bad
p-Bad (S136)
p-Bad (S112)
PUMA
NOXA
β-actin
B
OCI-AML2
MOLM-13
ABT-199
SA
Bax (N terminus)
β-actin
VDAC1
C
Input
IP: Bcl-2
H2O
SA 100uM
SA 250uM
WB: Bcl-2
WB: Bax
WB: β-actin
Immunoglobulin heavy chain
D
OCI-AML2
Relative JC-1 red/green fluorescence
sgNT
sgALAS1_1
sgALAS1_2
sgALAD_1
sgALAD_2
sgFECH_1
sgFECH_2
sgCOX10_1
sgCOX10_2
E
Fold change in ABT-199 GI50
ABT-199
ABT-199 + FCCP
ABT-199 + CCCP
OCI-AML2
MOLM-13
THP-1
MV4-11
HL-60
KG-1a
F
Relative JC-1 red/green fluorescence
DMSO
FCCP
CCCP
G
JC-1 fluorescence (norm. to H2O+DMSO)
Time (min)
H2O + DMSO
H2O + BID
H2O + BIM
SA + DMSO
SA + BID
SA + BIM
H
JC-1 fluorescence (norm. to H2O+DMSO)
Time (min)
H2O + DMSO
H2O + ABT-199 10nM
H2O + ABT-199 20nM
SA + DMSO
SA + ABT-199 10nM
SA + ABT-199 20nM
I
24 hrs
48 hrs
72 hrs
H2O
SA 100uM
SA 200uM
SA 400uM
SA 600uM
Cytochrome c
β-actin
OCI-AML2
MOLM-13

Figure S6: Capacity of heme depletion to sensitize ABT-199 is driven by baseline mitochondrial membrane depolarization, not by induction of ROS or BCL-2-specific interaction, related to Figure 6.

(A) Immunoblots of anti-apoptotic proteins Bcl-2, Bcl-xL, Mcl-1 and pro-apoptotic proteins Bim, Bid, Bax, Bak, Bad, phospho-Bad sites (S112 and S136), PUMA, and NOXA in cells treated with SA for 48hr (OCI-AML2: SA 250μM and PPIX 5μM, THP1: 2mM, KG-1α: 1.5mM, MOLM13: SA 750μM, HL-60: 2mM, MV-411: 1mM). **(B)** Immunoblots of Bax (N terminus epitope) in cells treated with SA for 48hr (OCI-AML2: 100μM, MOLM-13: 750μM) and ABT-199 (OCI-AML2: 50nM, MOLM-13: 30nM) for 8h. Cells were collected and fractionated. Shown are the membrane fractions. (C) Immunoblots examining Bax bound to Bcl-2, after immunoprecipitation for Bcl-2. OCI-AML2 cells were treated with SA 100μM and 250μM for 48hr. **(D)** JC-1 fluorescence in OCI-AML2 cells transduced with two independent sgRNAs targeting ALAS1, ALAD, FECH, and COX10, relative to a non-targeting sgRNA. **(E)** Fold change in 72hr GI_{50} values derived from ABT-199 dose-response curves treated in the presence and absence of FCCP (all lines: 2μM) and CCCP (OCI-AML2: 4μM, MOLM-13: 5μM, THP-1: 3μM, MV-411: 3μM, KG-1α: 6μM, HL-60: 3μM). Absolute GI_{50} values were normalized to viability in FCCP or CCCP alone. **(F)** JC-1 fluorescence in OCI-AML2 cells treated with FCCP (all lines: 2μM) and CCCP (OCI-AML2: 4μM, MOLM-13: 5μM, THP-1: 3μM, MV-411: 3μM, KG-1α: 6μM, HL-60: 3μM) for 72hr. **(G)** BH3 profiling. OCI-AML2 cells treated with SA 100μM for 48hr, then JC-1 fluorescence was read out over 3hr after addition of Bim 100μM and Bid 100μM. Shown are fluorescence units relative to the maximum fluorescence in control condition (H_2O + DMSO). **(H)** Membrane potential timecourse, with JC-1 fluorescence read out over 2.5hr after addition of ABT-199. Shown are fluorescence units relative to the maximum fluorescence in control condition (H_2O + DMSO). **(I)** Immunoblots of cytochrome c and beta-actin in OCI-AML2 and MOLM-13 cells treated with SA for 24h, 48h, and 72h.

** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$ by student's t-tests; n=3, data are mean ± SD.