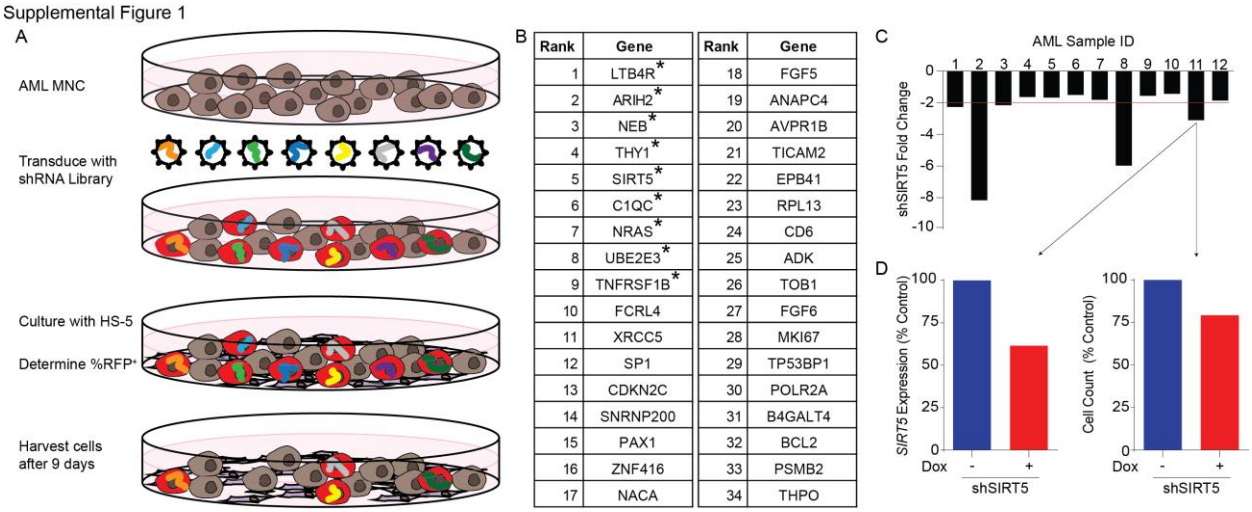
SUPPLEMENTAL FIGURES

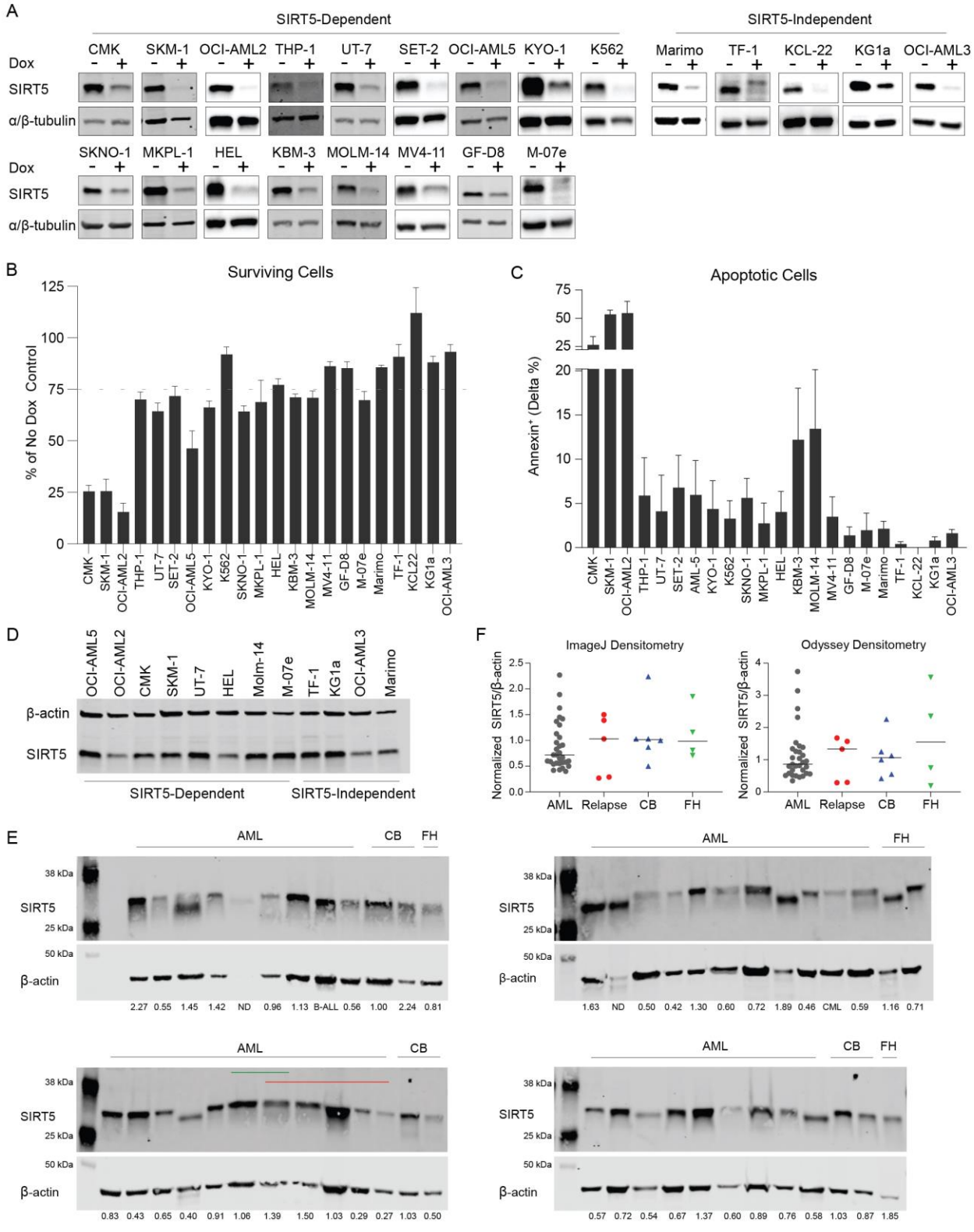


Rank	Gene	Rank	Gene
1	LTB4R*	18	FGF5
2	ARIH2*	19	ANAPC4
3	NEB*	20	AVPR1B
4	THY1*	21	TICAM2
5	SIRT5*	22	EPB41
6	C1QC*	23	RPL13
7	NRAS*	24	CD6
8	UBE2E3*	25	ADK
9	TNFRSF1B*	26	TOB1
10	FCRL4	27	FGF6
11	XRCC5	28	MKI67
12	SP1	29	TP53BP1
13	CDKN2C	30	POLR2A
14	SNRNP200	31	B4GALT4
15	PAX1	32	BCL2
16	ZNF416	33	PSMB2
17	NACA	34	THPO

Supplemental Figure 1.

An shRNA library screen identifies SIRT5 dependence in primary AML cells. A. Schematic of the shRNA library screen. Cells were cultured on HS-5 stromal cells to mimic the BM microenvironment. **B.** Genes that met the following criteria: ≥2 shRNAs targeting the same mRNA with a fold-reduction ranking in the top 2% of all scores in ≥2/12 samples. Genes meeting the same criteria in ≥3/12 samples are indicated by an asterisk. **C.** Of the 12 AML samples analyzed, five showed at least a twofold reduction (red line) for an shRNA targeting *SIRT5*. **D.** The response to *SIRT5* knockdown was confirmed in AML sample 11, using sh*SIRT5*$^{2311/2312}$ ± doxycycline.

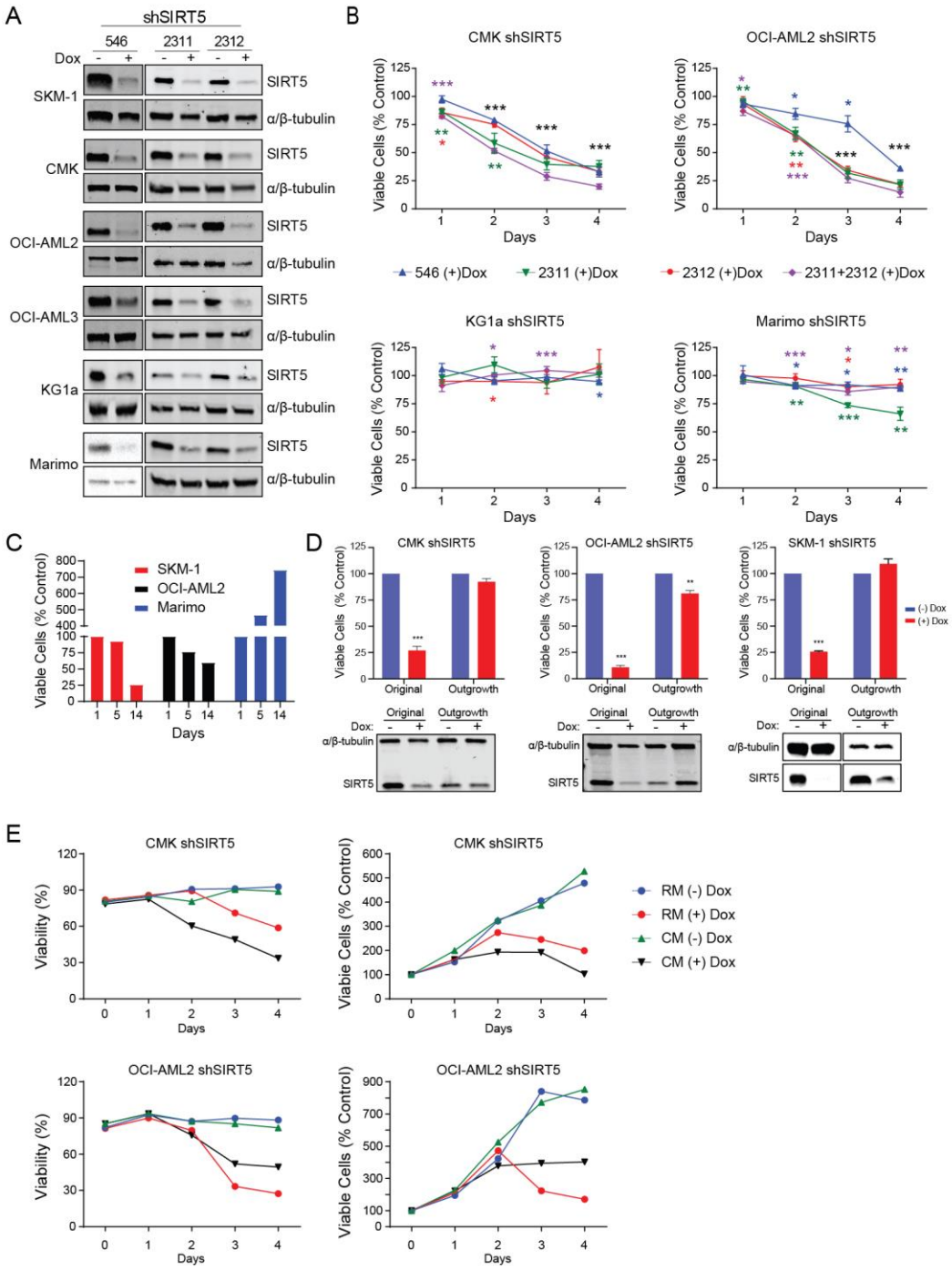
Supplemental Figure 2



Supplemental Figure 2.

SIRT5 dependence in AML cell lines and primary cells. (A-C) Twenty-two AML cell lines expressing dox-sh*SIRT5*$^{2311/2312}$ were cultured ± 100 ng/mL dox for 72 hours, followed by immunoblot analysis and functional studies. **A.** SIRT5 expression was assessed by immunoblot. **B.** Viable cells were quantified by MTS assay. **C.** Apoptosis was quantified by FACS for Annexin V. Note that data for CMK, SKM-1, OCI-AML2, Marimo, KG1a and OCI-AML3 cells are also shown in Fig. 1, and are included here for completeness. **(D-F)** SIRT5 expression was assessed by immunoblot. **D.** Exponentially growing representative AML cell lines. **E.** $CD34^+$ cells from AML patients. **F.** (left panel) Densitometry of immunoblots of primary AML cells using Image J. (right panel) Densitometry using Odyssey software.

Supplemental Figure 3

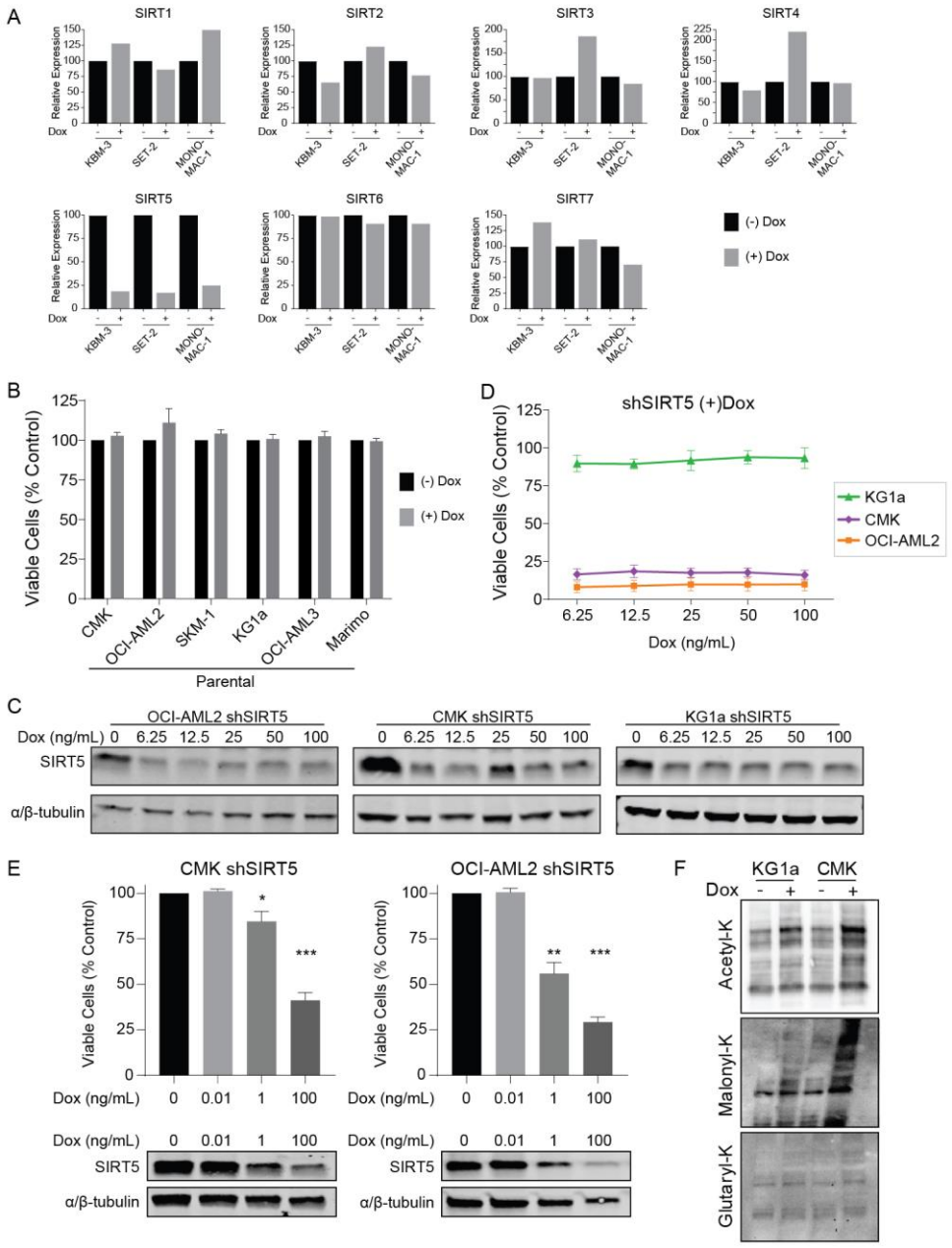


Supplemental Figure 3.

Experiments to validate SIRT5 dependence. (A,B) SKM-1, CMK and OCI-AML2 cells (SIRT5-dependent) and OCI-AML3, KG1a and Marimo cells (SIRT5-independent) were engineered to stably express dox-inducible shRNAs targeting three different SIRT5 sequences. Cells were cultured for 72 hours ± 100ng/mL dox. **A.** SIRT5 expression was assessed by immunoblot. **B.**

Viable cells were quantified by MTS assay at 1 – 4 days after adding dox. **C**. SKM-1 (SIRT5-dependent), OCI-AML2 (SIRT5-dependent) and Marimo cells (SIRT5-independent) were transduced with SIRT5 shRNA virus co-expressing a puromycin resistance gene and cultured for 2 weeks in the presence of puromycin. Viability was assessed by Viacount™ assay. **D.** CMK, OCI-AML2 and SKM-1 cells expressing dox-sh*SIRT5*$^{2311/2312}$ were cultured in 100 ng/mL dox, with regular medium changes irrespective of viability. After 4 – 6 weeks, cells started to recover and derivative lines were obtained that proliferated in the continuous presence of dox. Dox was removed from the resistant derivative lines for 96 hours, then re-added. (*upper panel*) Viable cells were quantified by Viacount™ assay and compared between parental and derivative cell lines at 72 hours after adding dox. (*lower panel*) SIRT5 expression was assessed by immunoblot. **E.** CMK cells expressing dox-sh*SIRT5*$^{2311/2312}$ were cultured ± dox for 96 hours in regular medium (RM) or HS-5 conditioned medium, as described in[48]. Viable cells were quantified by Viacount™ assay. (*left panel*) Viability. (*right panel*) Viable cell numbers compared to controls with multiple unpaired t tests.

Supplemental Figure 4

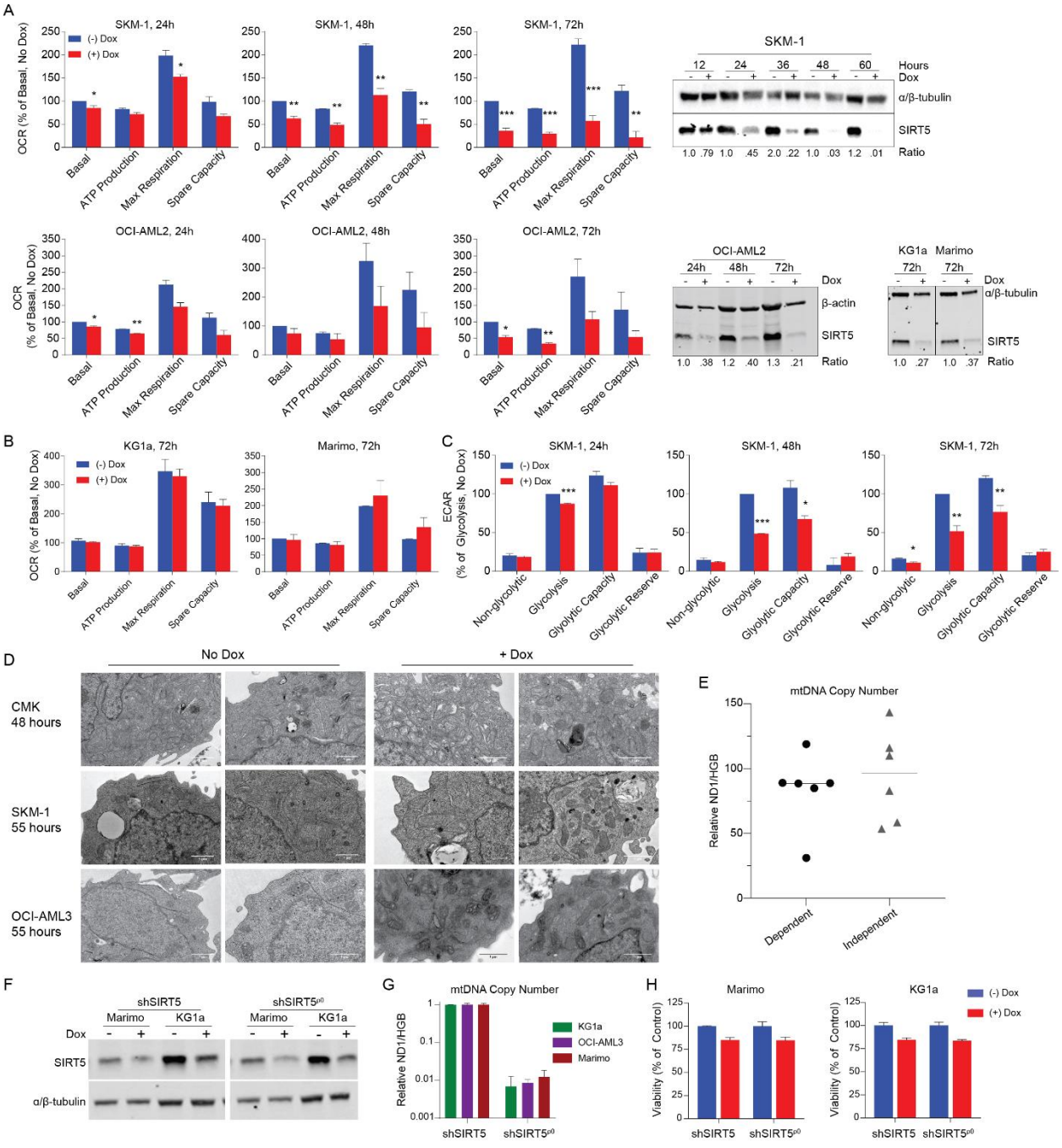


Supplemental Figure 4.

sh*SIRT5*^{2311} and sh*SIRT5*^{2312} target specifically *SIRT5* and doxycycline is non-toxic to AML cell lines. A. KBM-3 and SET2 cells expressing dox-sh*SIRT5*$^{2311/2312}$ were cultured in 100 ng/mL dox for 72 hours. *SIRT1*, *SIRT2*, *SIRT3*, *SIRT4*, *SIRT5, SIRT6* and *SIRT7* mRNA expression was measured by qRT-PCR. **B.** SIRT5-dependent and SIRT5-indpendent cell lines were cultured ± 100 ng/mL dox and viable cells quantified after 96 hours. **(C-D)** OCI-AML2, CMK and KG1a cells

expressing dox-sh*SIRT5*$^{2311/2312}$ were cultured in graded concentration of dox, starting from 6.25 ng/mL, for 96 hours. **C.** SIRT5 expression was analyzed by immunoblot at 96 hours. **D.** Viable cells were quantified by MTS assay at 96 hours. **E.** CMK and OCI-AML2 cells expressing dox-sh*SIRT5*$^{2311/2312}$ were cultured with dox over a 4-log dose concentration range for 96 hours. (*upper panel*) Viable cells were quantified by MTS assay and compared with an unpaired t test. (*lower panel*) SIRT5 expression was assessed by immunoblot. **F**. KG1a and CMK cells were incubated with dox and lysates analyzed with antibodies against acetyl-lysine, malonyl-lysine, and glutaryl-lysine.

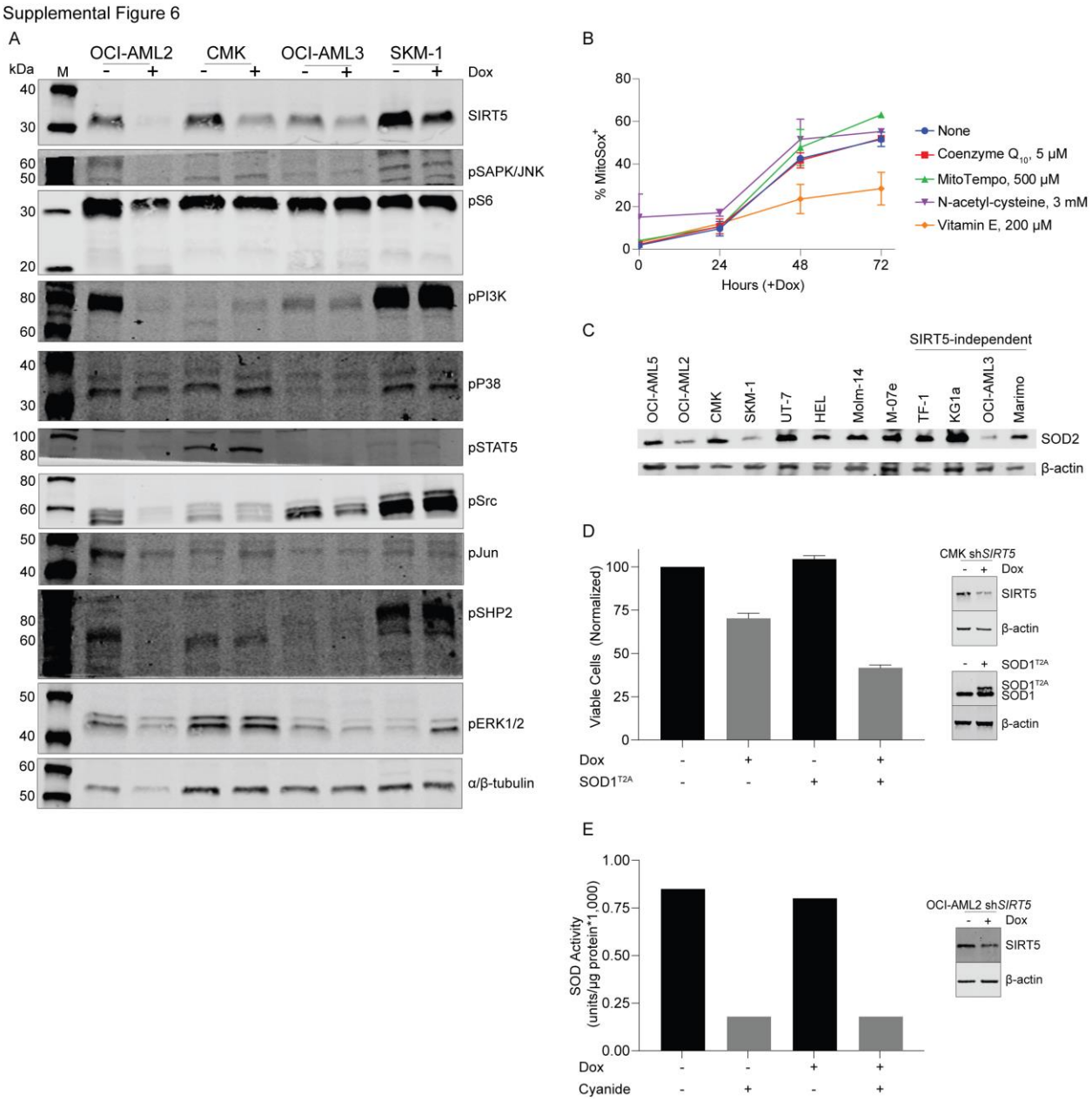
Supplemental Figure 5



Supplemental Figure 5.

***SIRT5* knockdown reduces oxygen consumption and extracellular acidification in SIRT5-dependent, but not SIRT5-independent cell lines. A.** SKM-1 and OCI-AML2 cells (SIRT5-dependent) expressing dox-sh*SIRT5*$^{2311/2312}$ were cultured ± 100 ng/mL dox. Oxygen consumption rate (OCR) was measured on an Agilent Seahorse XFe96 Analyzer at 12-hour intervals (only

results from 24, 48 and 72 hours are shown, comparisons by multiple unpaired t tests). Mitochondrial respiration was assessed under basal conditions, with sequential addition of 1 µM oligomycin (ATP synthase inhibitor), 1 µM carbonilcyanide p-trifluoromethoxy phenylhydrazone (FCCP, mitochondrial oxidative phosphorylation uncoupler), and 0.5 µM rotenone and antimycin A (electron transport chain complex I and III inhibitors). **B.** OCR was measured in KG1A and Marimo cells (SIRT5-independent). Comparisons were performed with multiple unpaired t tests. **C.** Extracellular acidification rate (ECAR) as a proxy for glycolysis was measured upon SIRT5 knockdown in SKM-1 cells under basal conditions, with sequential addition of 10 mM glucose, 1 µM oligomycin, and 50 mM 2-deoxy-glucose (2-DG, hexokinase inhibitor). **D.** Mitochondrial morphology was assessed by transmission electron microscopy in CMK, SKM-1 and OCI-AML3 cells expressing dox-sh*SIRT5*$^{2311/2312}$ cultured for 48-55 hours ± dox, Representative images are shown. **E.** Mitochondrial DNA copy number in SIRT5-dependent (CMK, SKM1, OCI-AML2, K562, MV411, Molm14) and SIRT5-independent (KG1a, OCI-AML3, Marimo, KCL22, KBM5, TF1) AML cells lines was assessed by quantitative PCR for the mitochondrial gene NADH:ubiquinone oxidoreductase core subunit 1 (ND1) normalized for the nuclear gene hemoglobin**. (F-H).** KG1a, OCI-AML3 and Marimo cells (SIRT5-independent) and CMK, SKM-1 and OCI-AML2 cells (SIRT5-dependent) were cultured in ethidium bromide to deplete mitochondrial DNA. Derivative lines (ρ_0) were established from SIRT5-independent lines only, while SIRT5-dependent lines failed to grow out. **F.** Mitochondrial DNA copy numbers in parental lines and ρ_0 derivatives were cultured ± dox and SIRT5 expression was assessed by immunoblot. **G.** Marimo and KG1a cells and their ρ_0 derivatives. **H.** Viability was measured by MTS assay after 96 hours. Data represent the mean ± SEM from three independent experiments. *p<.05; **p<.01; ***p<.001



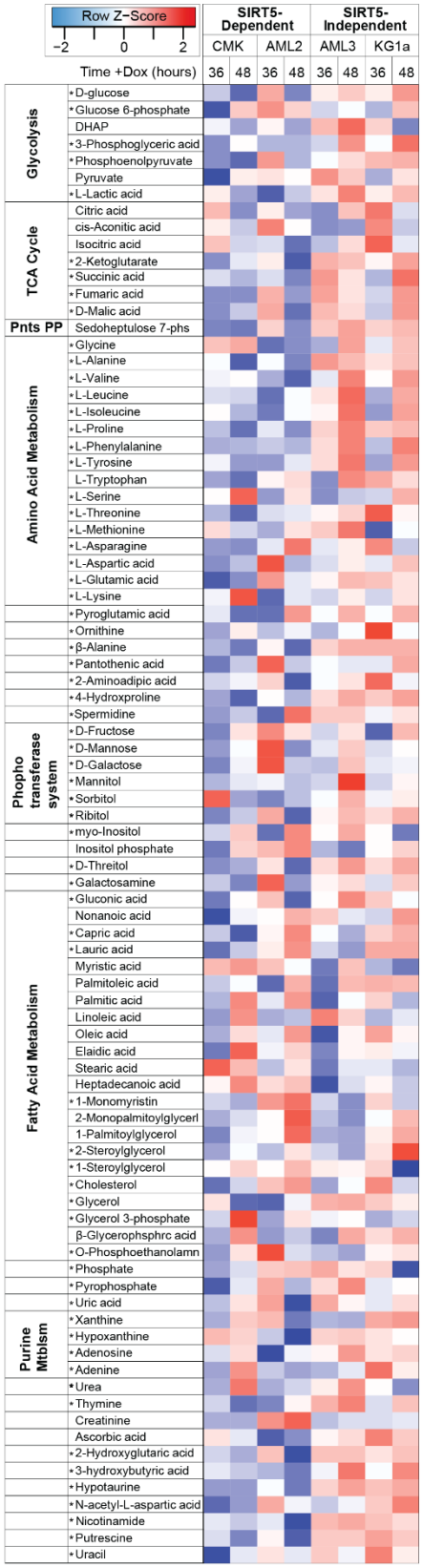
Supplemental Figure 6.

Canonical signaling pathways upon *SIRT5* knockdown, response to antioxidants and SOD2 expression. A. OCI-AML2, CMK, and SKM-1 cells (SIRT5-dependent) and OCI-AML3 cells (SIRT5-independent) expressing dox-sh*SIRT5*$^{2311/2312}$ were cultured with 100 ng/mL dox for 48 hours. The activation status of canonical signaling pathways was analyzed by immunoblot as indicated. **B.** CMK cells were cultured with various antioxidants ± dox, and mitochondrial superoxide measured by FACS. **C.** SOD2 expression was measured by immunoblot in AML cell

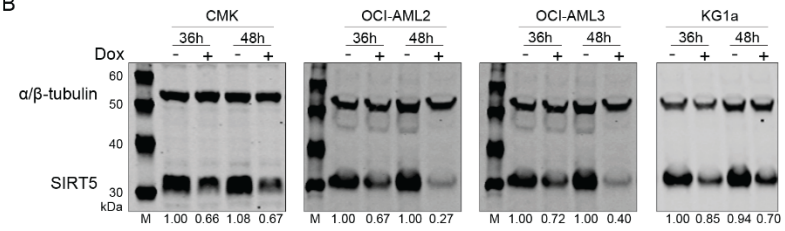
lines exhibiting various degrees of SIRT5 dependence. **D**. Viable cells were quantified with CellTiter-Glo following overexpression of SOD1 in CMK cells expressing dox-sh*SIRT5*$^{2311/2312}$. **E**. SOD activity was measured in OCI-AML2 cells at 36 hours after the addition of dox with a commercial kit that generates superoxide and measures its presence. The addition of cyanide eliminates activity from SOD1 and SOD3, leaving SOD2 as the only active superoxide dismutase in the lysate.

Supplemental Figure 7

A



B



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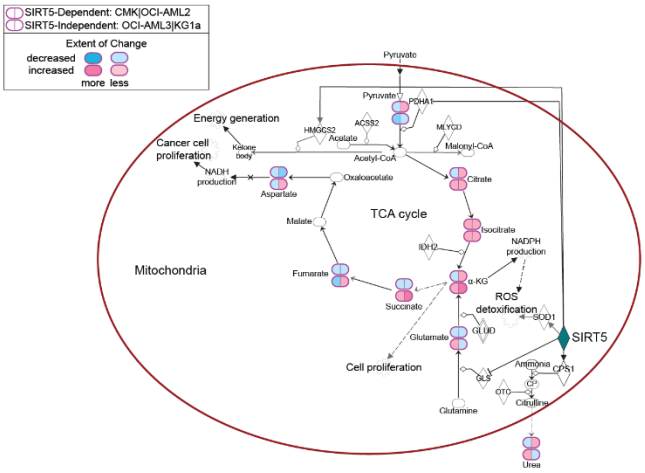


D

Upstream Regulator	Molecular Function	SIRT5-Dependent				SIRT5-Independent			
		CMK		OCI-AML2		OCI-AML3		KG1a	
		36h	48h	36h	48h	36h	48h	36h	48h
sirolimus	mTOR inhibitor	2.5					3.0		2.6
afatinib	EGFR inhibitor	3.0					2.2		2.2
glutamine	metabolite		2.6						
GLS	glutaminase		2.2						
IDH1	dehydrogenase	2.2	2.2						
BCR-ABL1	tyrosine kinase	2.2	2.3						
ERBB2	tyrosine kinase								
STK11	serine/threonine kinase								
α-ketoglutarate	metabolite		2.0						
elaidic acid	unsaturated fatty acid		2.0	2.0					2.0

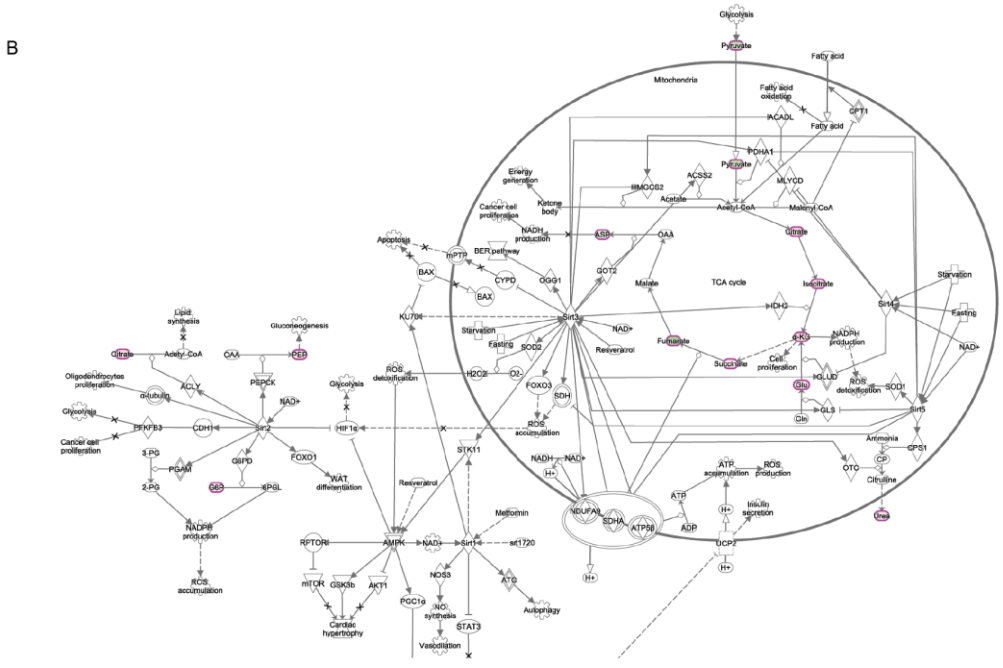
Supplemental Figure 7.

Untargeted metabolomic profiling identifies disrupted glutamine pathway upon SIRT5 knockdown in SIRT5-dependent cells. SIRT5-dependent (CMK, OCI-AML2 and SIRT5-independent (OCI-AML3, KG1a) AML cell lines expressing dox-shSIRT5$^{2311/2312}$ were grown in 100 ng/mL dox for 36 and 48 hours and subjected to untargeted metabolomics analysis. **A.** Heatmap of metabolites (log2 fold-changes). Data was sum normalized, log transformed and auto-scaled, without clustering or ranking. Metabolites with significant change ($p<.05$) in at least one comparison are indicated by an asterisk. **B.** SIRT5 expression was analyzed by immunoblot. **C.** IPA-predicted sirtuin metabolic (enzyme-related) network at 48h after adding dox. Solid lines denote direct and dashed lines indirect relationships. The IPA output diagram was simplified to represent only changes linked to SIRT5 activity (extended output in Fig. S8B). **D.** IPA was used to identify potential upstream regulators whose activation/inhibition is consistent with the observed changes in metabolites. The overall activation/inhibition status of the regulator was determined from the level of consistency in the observed metabolite changes, statistically represented by z-score. Potential regulators are shown if they were identified at least twice across the eight conditions (four cell lines and two time points) with a z-score ≥|2|.

Supplemental Figure 8

A

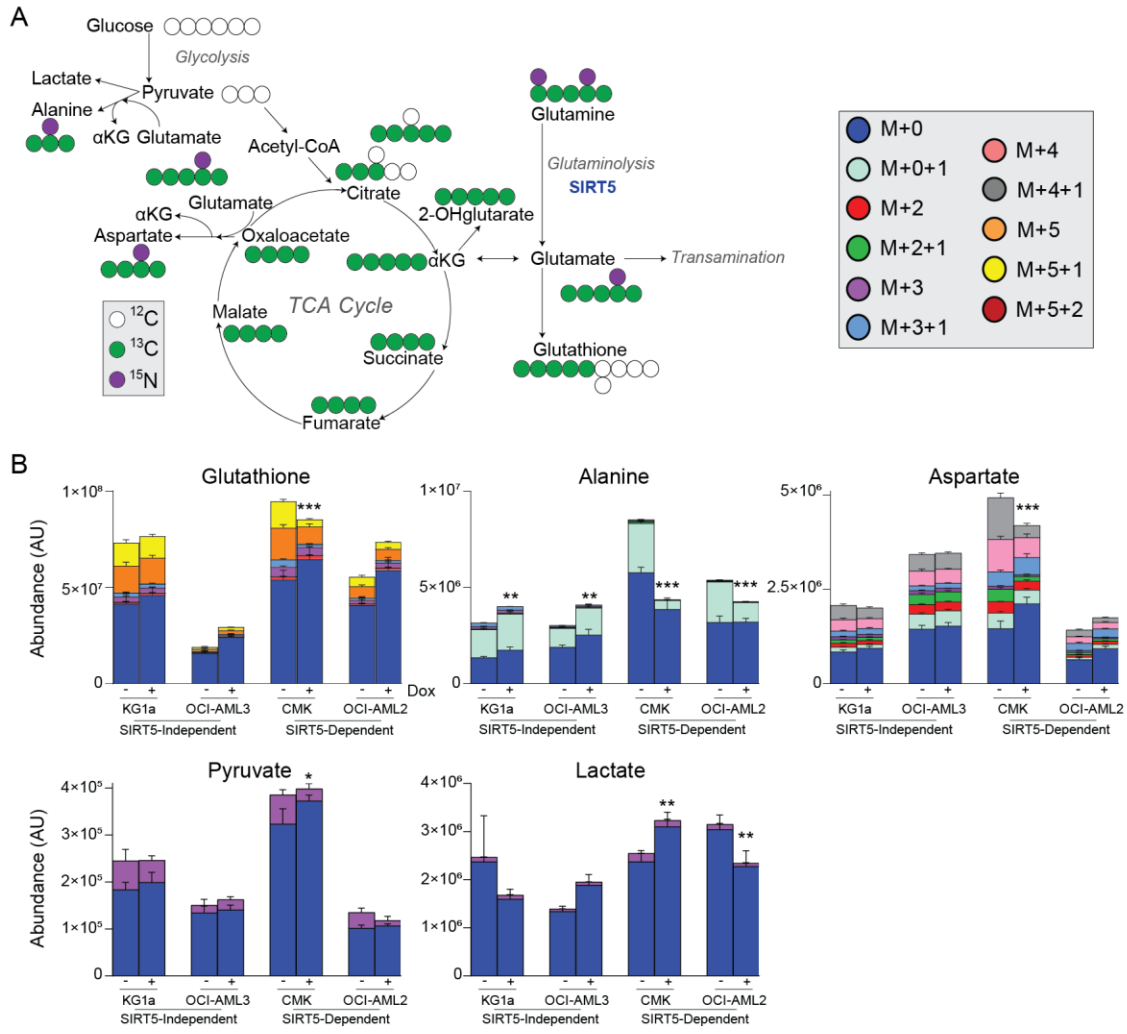
B



Supplemental Figure 8.

Ingenuity pathway analysis. SIRT5-dependent (CMK, OCI-AML2) and SIRT5-independent (OCI-AML3, KG1a) AML cell lines expressing dox-shSIRT5$^{2311/2312}$ were cultured in 100 ng/mL dox for 36 and 48 hours then subjected to untargeted metabolomics analysis. **A.** Enriched pathways identified by Ingenuity pathway analysis (IPA) based on metabolite changes associated with SIRT5 knockdown at 36h and 48h in SIRT5-dependent (CMK, OCI-AML2) and SIRT5-independent (OCI-AML3, KG1a) AML cell lines. Green bars denote the percentage of downregulated and red bars the percentage of upregulated metabolites in shSIRT5 cells cultured with dox relative to cells cultured without dox. Total numbers of metabolites present within a pathway are shown on the right. The orange line denotes the likelihood [-log (p-value)] that the specific pathway was affected. Threshold was set at $p<0.00005$ (i.e., 4.30 log-scale). Pathways are shown according to their –log (p-value) as output by IPA. **B.** IPA-predicted sirtuin metabolic network in OCI-AML3 48h after adding dox. Solid lines denote direct and dashed lines indirect relationships. The IPA output diagram was simplified and represents only mitochondrial and cytoplasmic relationships.

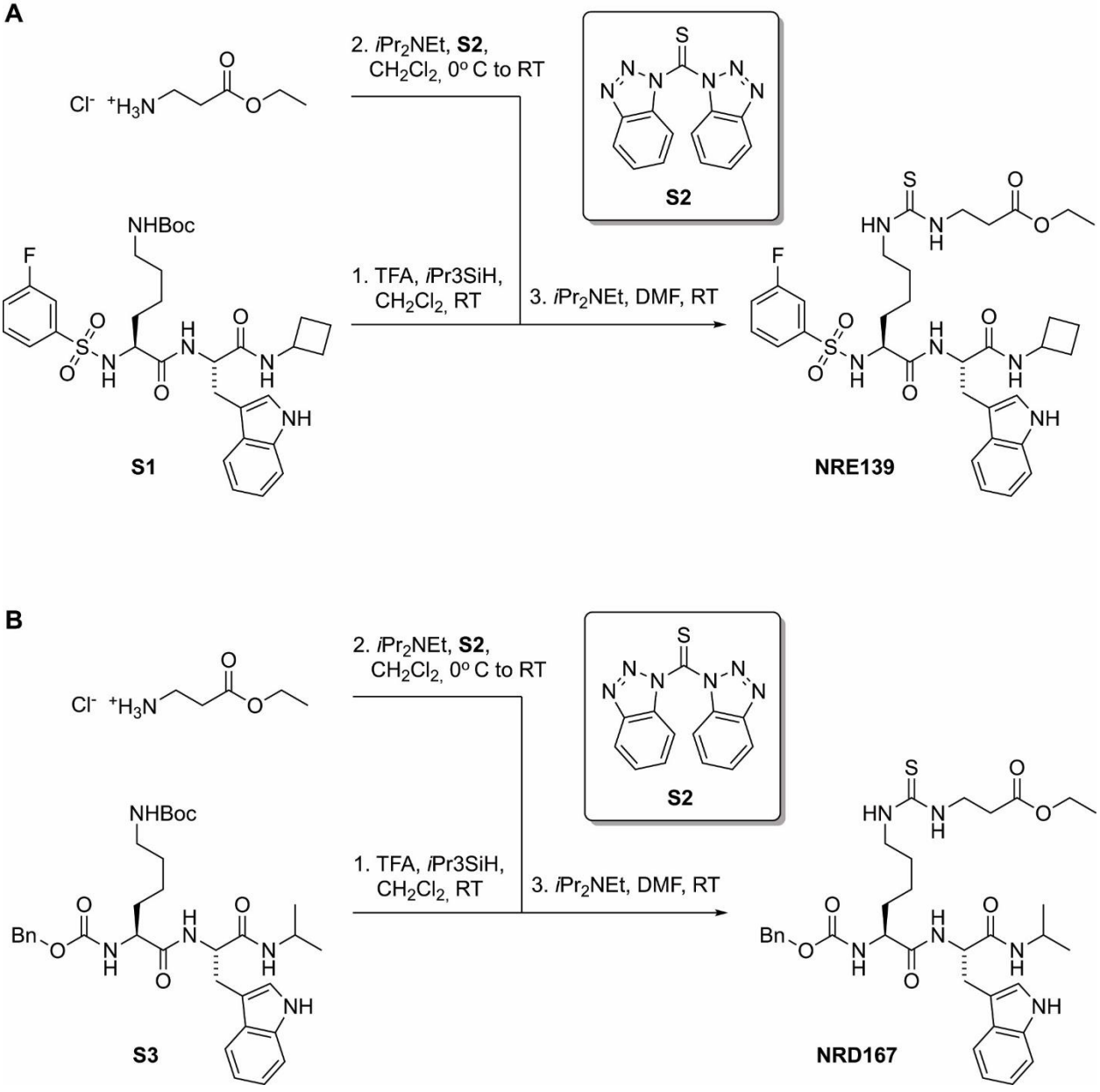
Supplemental Figure 9



Supplemental Figure 9.

[$^{13}C_5{}^{15}N_2$]-L-glutamine flux on SIRT5-dependent and –independent AML cell lines. A. Overview of predicted isotopologues for metabolites downstream to [$^{13}C_5$,$^{15}N_2$]-glutamine in tracing experiments. **B.** KG1a, OCI-AML3 cells (SIRT5-independent), and CMK and OCI-AML2 cells (SIRT5-dependent) expressing dox-sh*SIRT5*$^{2311/2312}$ were cultured in 100 ng/mL dox then moved to [$^{13}C_5{}^{15}N_2$]-L-glutamine-containing medium for 4 hours (36 hours total in dox). Data are from the same experiments as those in Fig.6, with additional metabolites are shown. Comparisons were performed with a two-way ANOVA with Tukey's correction for multiple comparisons.

Supplemental Figure 10.



Supplemental Figure 10.

Synthesis scheme for SIRT5 inhibitors. A. Structures of known intermediates (S1 and S2) and synthesis of compound NRE139. **B**. Synthesis steps for NRD167. Complete details of the synthesis steps are published elsewhere: Rajabi N, Auth M, Troelsen KR, Pannek M, Bhatt DP, Fontenas M*, et al.* Mechanism-Based Inhibitors of the Human Sirtuin 5 Deacylase: Structure-

Activity Relationship, Biostructural, and Kinetic Insight. Angew Chem Int Ed Engl **2017**;56(47):14836-41 doi 10.1002/anie.201709050.