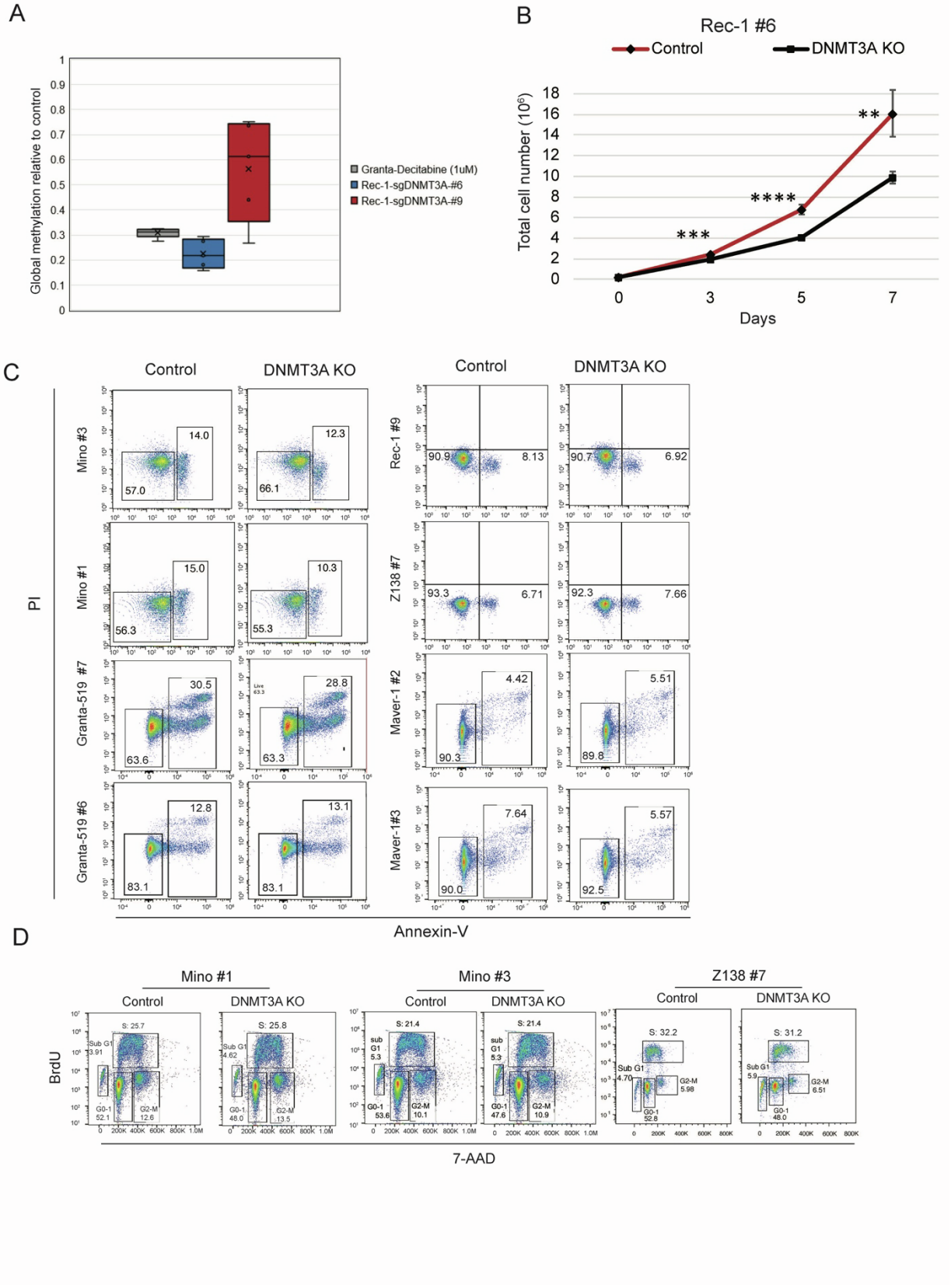
Cell Reports Medicine, Volume *5*

Supplemental information

Targeting DNMT3A-mediated oxidative phosphorylation to overcome ibrutinib resistance in mantle cell lymphoma

Nguyet-Minh Hoang, Yunxia Liu, Paul D. Bates, Alexa R. Heaton, Angelica F. Lopez, Peng Liu, Fen Zhu, Ruoyu Chen, Apoorv Kondapelli, Xiyu Zhang, Paul E. Selberg, Vu N. Ngo, Melissa C. Skala, Christian M. Capitini, and Lixin Rui

Supplemental Figure 1



Supplemental figure S1: DNMT3A knockout reduces DNA methylation and inhibits the growth of MCL cells but does not significantly affect cell cycle and apoptosis (related to Figure 1).

(A) A summary of global methylation dot blots in two representative clones. The methylation level was quantitated using densitometry analysis by ImageJ. The methylation levels of DNMT3A KO (6 days post induction) or decitabine-treated (3 days post treatment) cells were normalized that of control cells. For DNMT3A KO condition, controls were uninduced sgDNMT3A clonal cells. For decitabine-treated cells, control were DMSO-treated cells. Decitabine-treated and DMSO-treated cells were included as controls every time this assay was performed. For each clonal cell line, the experiments were repeated as least twice. (**B)** DNMT3A KO reduced the growth of Rec-1 #6 clone in a time-dependent manner. Cells were counted every 2 days using trypan blue exclusion assay (Student's *t*-test, **p<0.01, ***p<0.001, ****p<0.0001, n=3). **(C, D)** Representative flow cytometry analyses of apoptosis and cell cycle in control and DNMT3A knockout (KO) MCL clonal cell lines 6 days after sgRNA induction.

Supplemental Figure 2

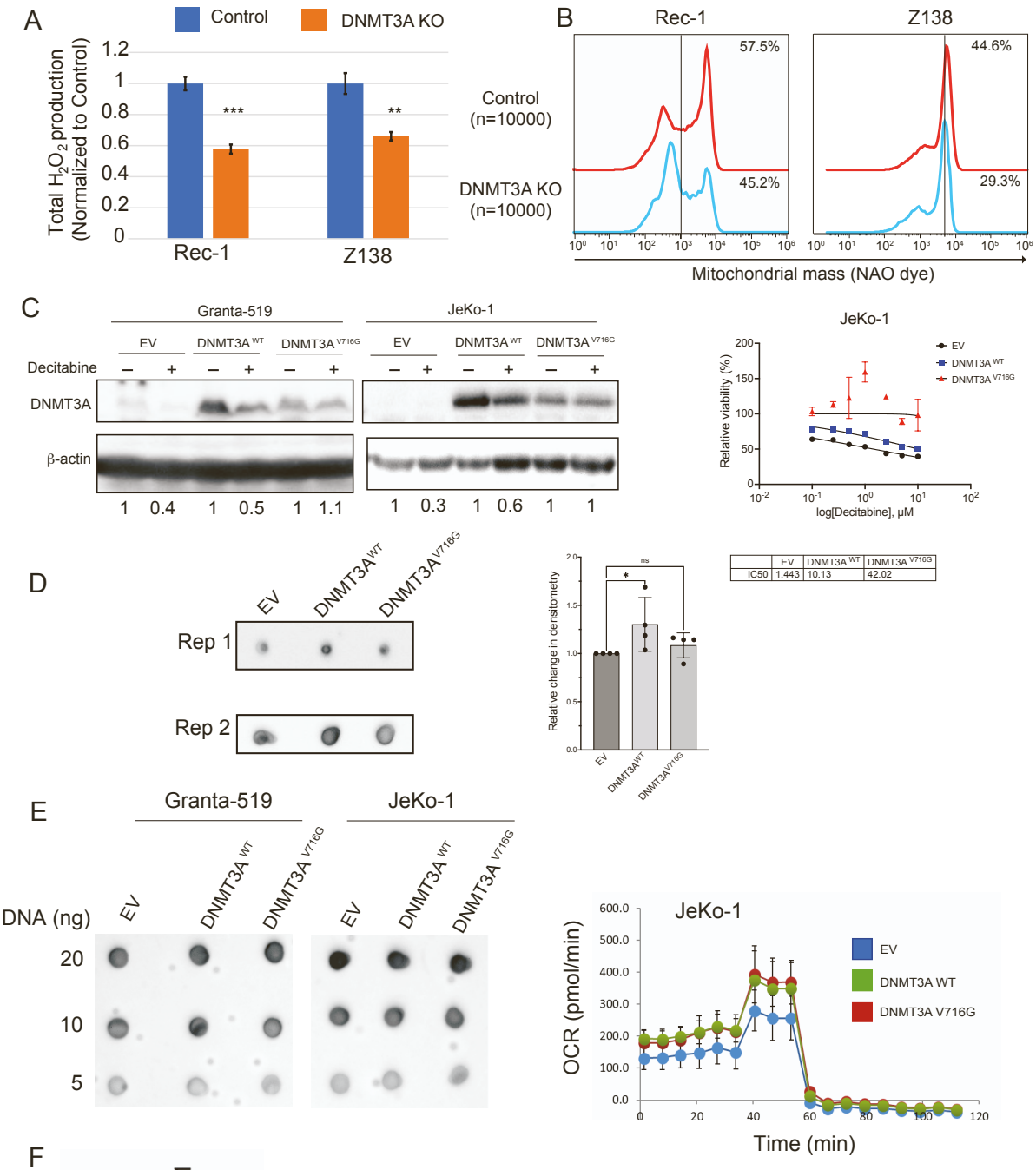


Figure S2: DNMT3A knockout reduces mitochondrial biogenesis in MCL cells independently of DNA methylation (related to Figure 2).

(A) The byproduct of mitochondrial activity, mitochondrial ROS (mtROS), was approximated by hydrogen peroxide (H_2O_2) production. The H_2O_2 level of DNMT3A KO cells was normalized to control cells (Student's *t*-test, **p<0.01, ***p<0.001, n=3). **(B)** Control and DNMT3A KO clones (Rec-1 #9 and Z138 #7) were stained with 10-N-nonyl acridine orange (NAO) dye, a marker for mitochondrial mass. For each condition, approximately 10000 live cells were gated for measurement. **(C)** Left: JeKo-1 and Granta-519 were transduced with either $DNMT3A^{WT}$ or $DNMT3A^{V716G}$ or pCDH-GFP. After sorting, cells were treated with decitabine for 5 days at 40nM or DMSO. On the fifth day of treatment, cells were collected and processed for immunoblotting. Densitometry was performed using ImageJ. Right: 5,000 JeKo-1 cells transduced with either $DNMT3A^{WT}$ or $DNMT3A^{V716G}$ or pCDH-GFP were treated with decitabine at indicated concentrations in a 96-well plate; fresh drug was added every day. Cell viability was measured by CellTiter-Glo after 3 days of drug treatment. **(D)** HEK293T cells were transduced with either $DNMT3A^{WT}$ or $DNMT3A^{V716G}$ or pCDH-GFP. After sorting, gDNA of each condition was collected, quantified and globally methylation level was measured following methylation dot blot protocol **(E)** Left: The global methylation dot blot analysis showed unchanged methylation levels in Granta-519 and JeKo-1 cells transduced with $DNMT3A^{WT}$ or $DNMT3A^{V716G}$ compared to cells transduced with pCDH-GFP. Right: JeKo-1 cells transduced with $DNMT3A^{WT}$ or $DNMT3A^{V716G}$ showed an increase in the rate of oxygen consumption (OCR; pmol/min/cell).

Supplemental Figure 3

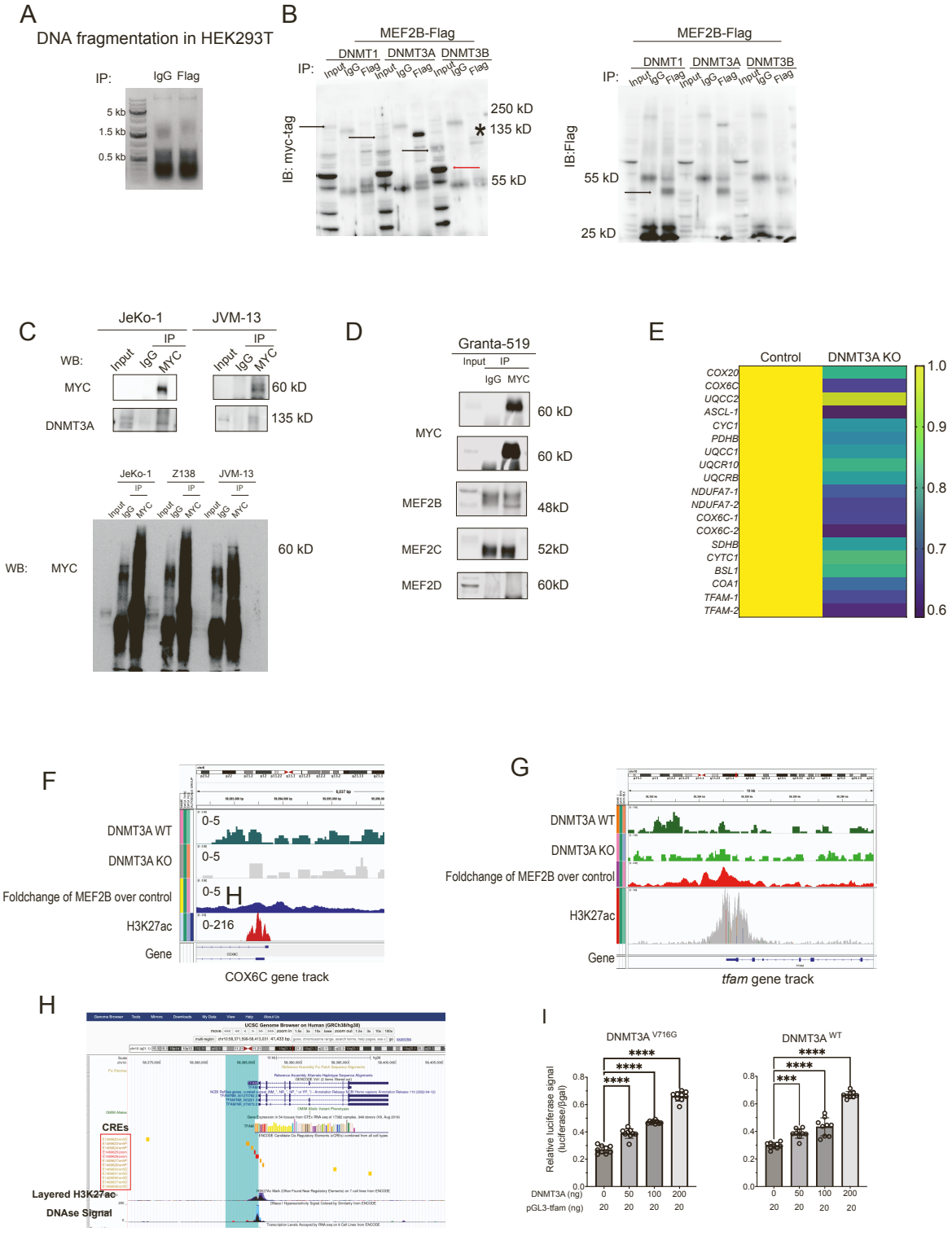


Figure S3: DNMT3A, MEF2B and MYC form a transcriptional activating complex to regulate genes involved in mitochondrial biogenesis in MCL (related to Figure 3). **(A)** Representative images of DNA fragmentation by DNAse (6 μg/mL) during the immunoprecipitation (IP) experiments. After overnight immunoprecipitation, eluates were collected and incubated with proteinase K for 2 hours and then analyzed on an agarose gel. **(B)** Co-IP experiments using anti-flag (M2 clone) antibody to pulldown flag-tagged MEF2B in HEK293T cells which was co-transfected with flag-tagged MEF2B and myc-tagged DNMT3A or DNMT3B or DNMT1. Blots were incubated with anti-flag and anti-myc tag antibody (clone 9E10). Black arrows indicate myc-tagged DNMTs (left) and flag-tagged MEF2B (right). Red arrow indicates possible endogenous MYC of HEK293T in the input (left). Star indicates an unspecific band. (**C)** MYC antibody that only recognizes full-length MYC protein (clone Y69) was used to pulldown endogenous MYC in JeKo-1 and JVM-13 cell lines and then blotted for DNMT3A. **(D)** Pulldown of endogenous MYC in Granta-519 cell line then blotted for MEF2B, MEF2C, and MEF2D. **(E)**The relative mRNA expression of genes involved in mitochondrial biogenesis normalized to PCNA housekeeping gene in Z138 #7 control and DNMT3A KO cells. **(F,G)** Gene tracks of *COX6A* and *tfam* from ChIP-seq data of DNMT3A (WT vs. KO), MEF2B and H3K27ac. **(H)** The presence of cis-regulatory elements (CREs) in the +3.5kb upstream region of *tfam* to which DNMT3A and MEF2B bind and that is marked by H3K27ac. **(I)** $DNMT3A^{WT}$ or $DNMT3A^{V716G}$ alone can induce the transcription of *tfam* in a dose-dependent manner. Student's *t*-test was used in **(D)** and **(H)**, **p<0.01, ***p<0.001, ****p<0.0001, n=3.

Supplemental Figure 4

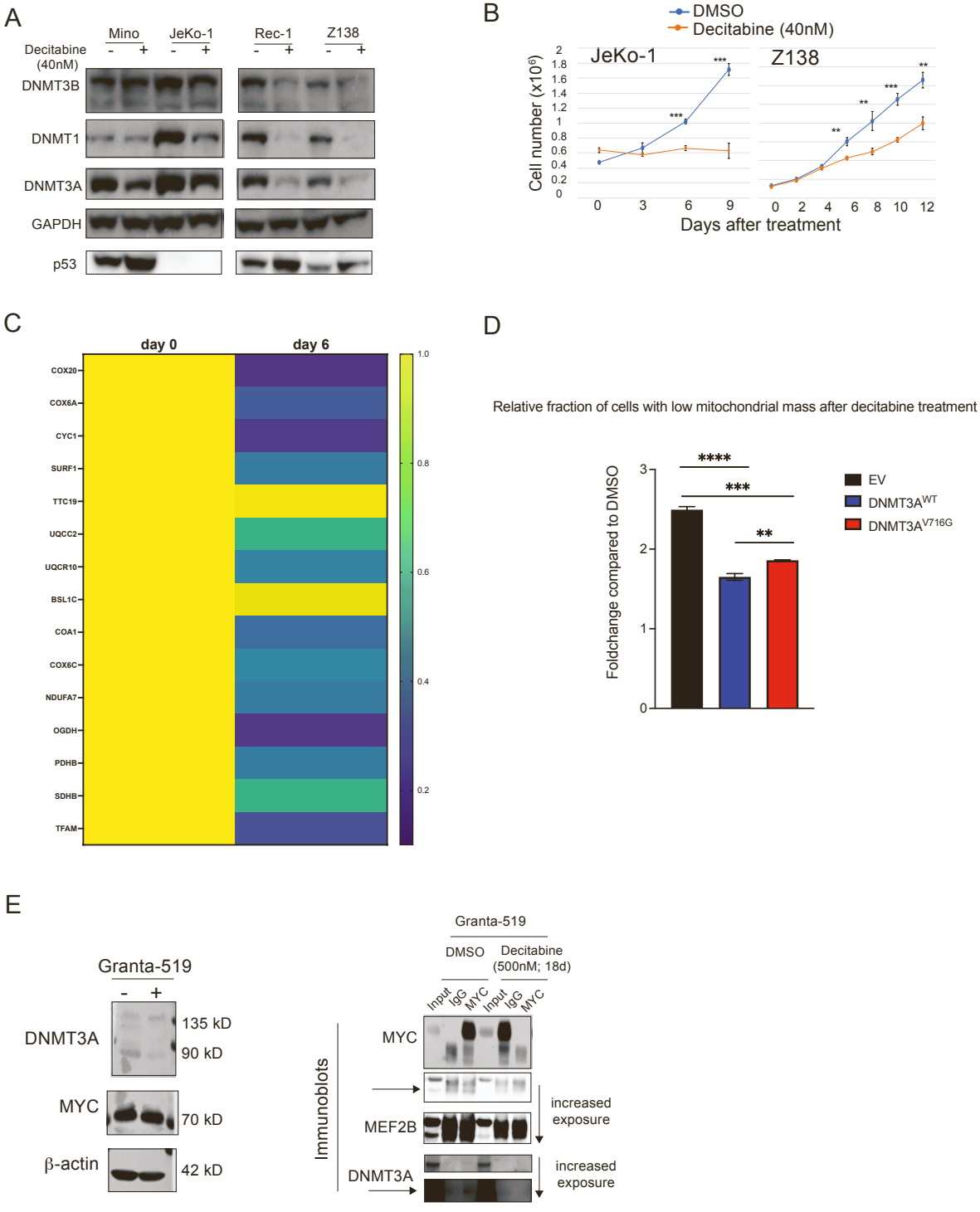
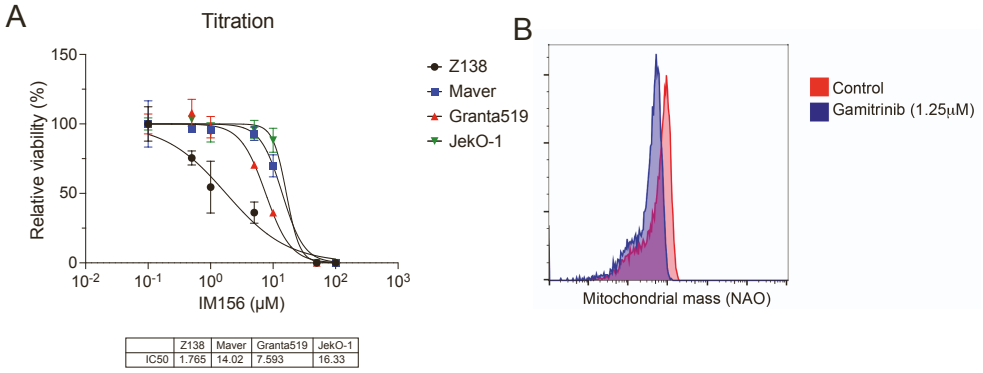


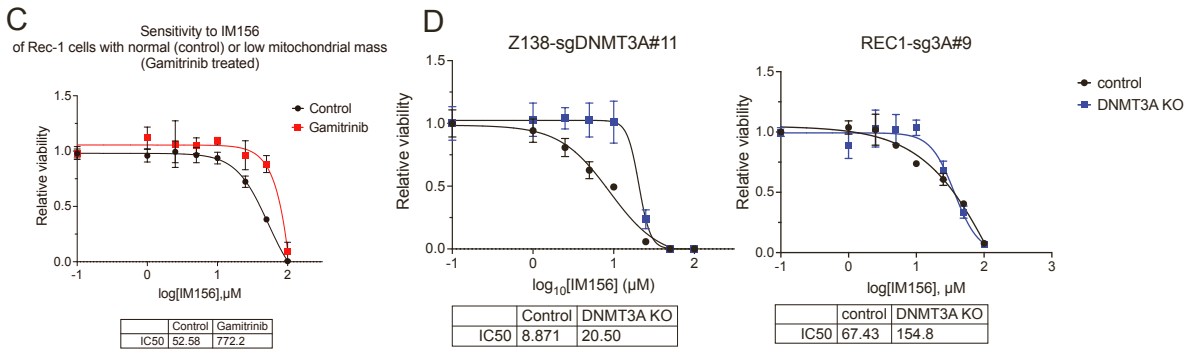
Figure S4: Decitabine reduces tumor growth partly by inhibiting mitochondrial activity through DNMT3A-MEF2B-MYC complex in a DNA-methylation-independent manner (related to Figure 4).

(A) A low dose treatment of decitabine (40nM; 6 days) induced the degradation of DNMTs. Immunoblots of DNMTs and p53 and GAPDH loading control are shown. **(B)** Decitabine reduced the *in vitro* growth of MCL cell lines over time. **(C)** The relative mRNA expression of genes involved in mitochondrial biogenesis normalized to PCNA housekeeping gene in Z138 treated with DMSO or decitabine after 6 days. **(D)** JeKo-1 cells transduced with $DNMT3A^{WT}$ or $DNMT3A^{V716G}$ or pCDH-GFP were treated with decitabine (100nM) for four days. Cells were stained with mitoDeep Red and DAPI, analyzed on a flow cytometer. **(E)** Decitabine treatment led to dissociation of DNMT3A/MEF2B/MYC complex. Granta-519 was treated with decitabine (500nM) for 18 days. Cells were collected for immunoblotting and for immunoprecipitation of endogenous MYC. Student's *t*-test was used in **(B)** and **(D)**, **p<0.01, ***p<0.001, ****p<0.0001, n=3. All experiment were performed independently twice, each with 3 technical replicates.

Supplemental Figure 5



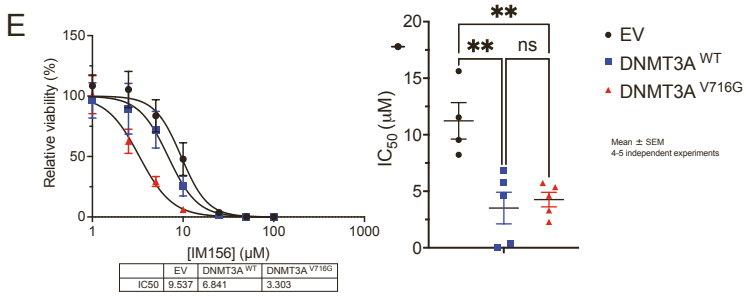
	Z138	Maver	Granta519	JekO-1
IC50	1.765	14.02	7.593	16.33



	Control	Gamitrinib
IC50	52.58	772.2

	Control	DNMT3A KO
IC50	8.871	20.50

	control	DNMT3A KO
IC50	67.43	154.8



	EV	DNMT3A WT	DNMT3A V716G
IC50	9.537	6.841	3.303

F

Cell line	ZIP	Loewe	HSA	Bliss
Mino	20.56	23.7	31.28	20.47
JVM-13	15.94	15.8	23.25	16.06
Granta-519	13.01	13.23	24.56	13.01
JeKo-1	3.55	5.61	8.4	3.1
Z138	8.9	2.65	13.62	9.23
Maver	11.61	15.75	18.98	11.84

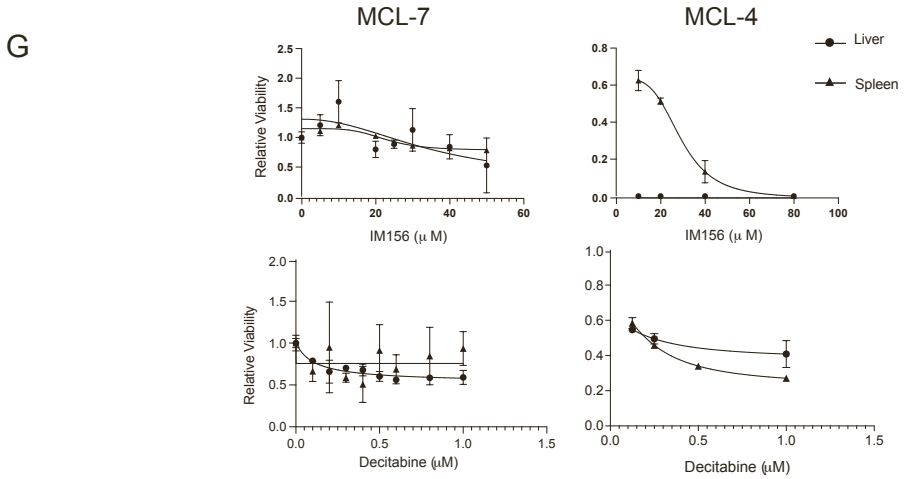


Figure S5: Decitabine synergizes with a novel complex I inhibitor to inhibit the growth of cell lines and primary MCL cells (related to Figure 5).
(A) Titration of IM156 in MCL cell lines. 5000 cells were seeded in a 96-well plate and treated with indicated concentrations of IM156 for 3 days. Cell viability was measured using CellTiter-Glo. Relative viability was calculated as the ratio of luminescence unit of decitabine-treated to DMSO-treated cells. **(B)** Representative image of mitochondrial mass of gamitrinib-treated and solvent-treated MCL cells measured by NAO flow cytometric assay. **(C)** The sensitivity of gamitrinib-treated and solvent-treated MCL cells to IM156. **(D)** The sensitivity of control and DNMT3A KO MCL cell lines to IM156. **(E) Left:** The sensitivity of JeKo-1 cells transduced with $DNMT3A^{WT}$ or $DNMT3A^{V716G}$ or pCDH-GFP to IM156. **Right:** The summary of the IC_{50} of JeKo-1 cells transduced with indicated constructs. Each data point represents an independent experiment. **(F)** A summary of synergy scores of decitabine and IM156 in MCL cell lines from different models using SynergyFinder plus. A score above 0 indicated a synergistic effect of the two drugs**. (G)** The titrations of decitabine and IM156 as single agent in MCL primary cells (MCL-7 and MCL-4). Cells were seeded in a 96-well plate (10,000 cells per well in triplicates) and treated with DMSO, decitabine or IM156 for 3 days. On the third day, cells were stained with DAPI and analyzed on a flow cytometer. Live cells in each concentration were then normalized to live cells in DMSO treated wells.

Supplemental Figure 6-1

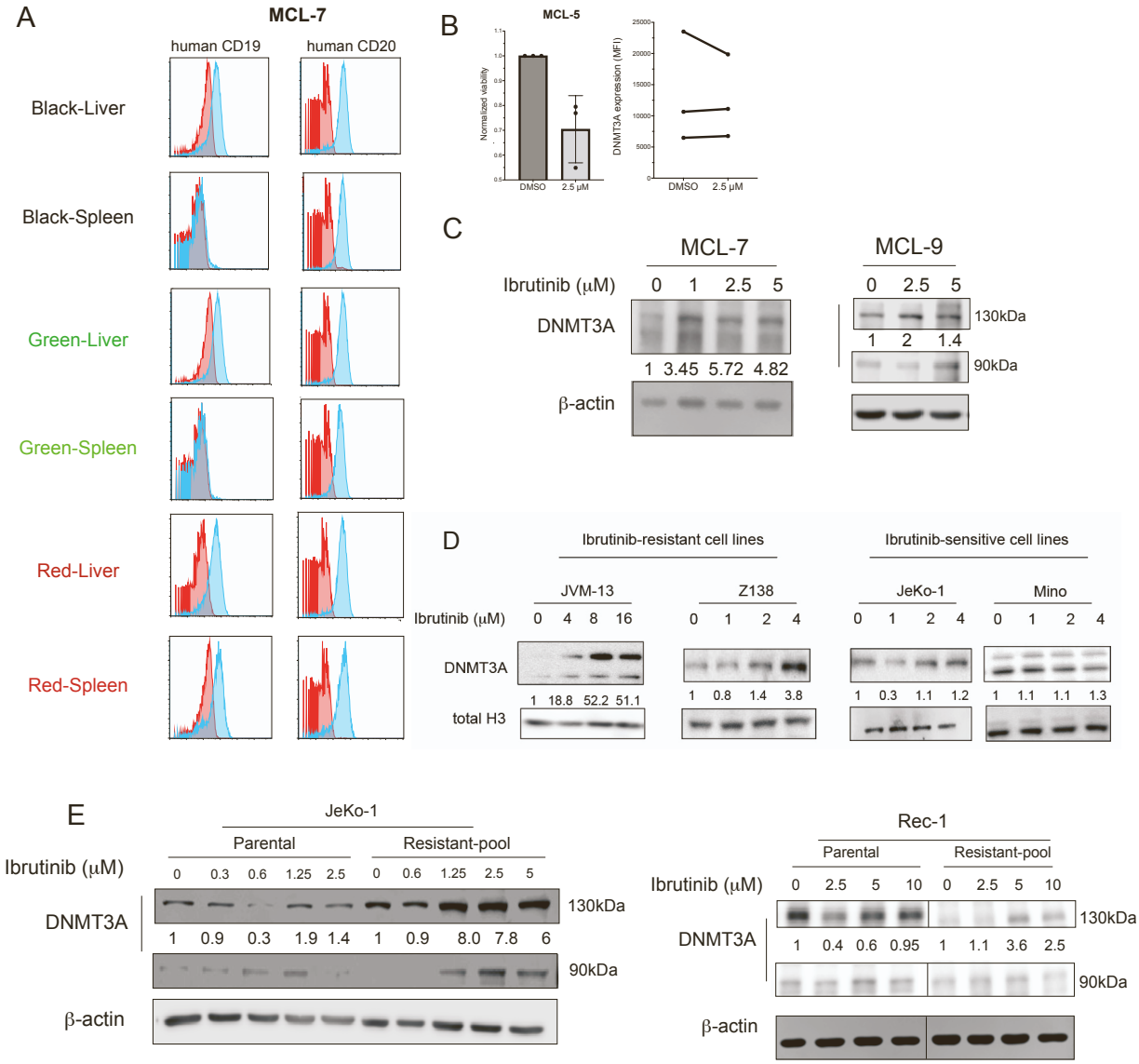


Figure S6-1: Human CD19 and CD20 expression in the xenografted primary MCL cells and the sensitivity and DNMT3A expression upon ibrutinib treatment (related to Figure 6).

(A) An example of the heterogeneity of the surface expression of human CD19 and CD20 from MCL-7 sample expanded in different mice and between different organs within a given mouse from the first *in vivo* expansion. (**B)** MCL-5 exhibited sensitivity to ibrutinib (top) and the expression of DNMT3A in these samples remains unchanged or slightly reduced (bottom). **(C)** Immunoblotting of DNMT3A in 2 primary MCL samples from *in vivo* passage 2 **(D)** Immunoblotting of DNMT3A in 2 ibrutinib-resistant cell lines (JVM-13 and Z138) and 2 ibrutinib-sensitive cell lines (JeKo-1 and Rec-1). The quantification is based on the signal of the longest isoform (130kDa) relative to internal control(s) and then normalized to the DMSO control sample. **(E)** DNMT3A expression was increased after decitabine treatment in acquired ibrutinib-resistant JeKo-1 and Rec-1 cell lines but not in their respective parental cell lines. All experiments were collected on day 3 of ibrutinib treatment unless otherwise indicated.

Supplemental Figure 6-2

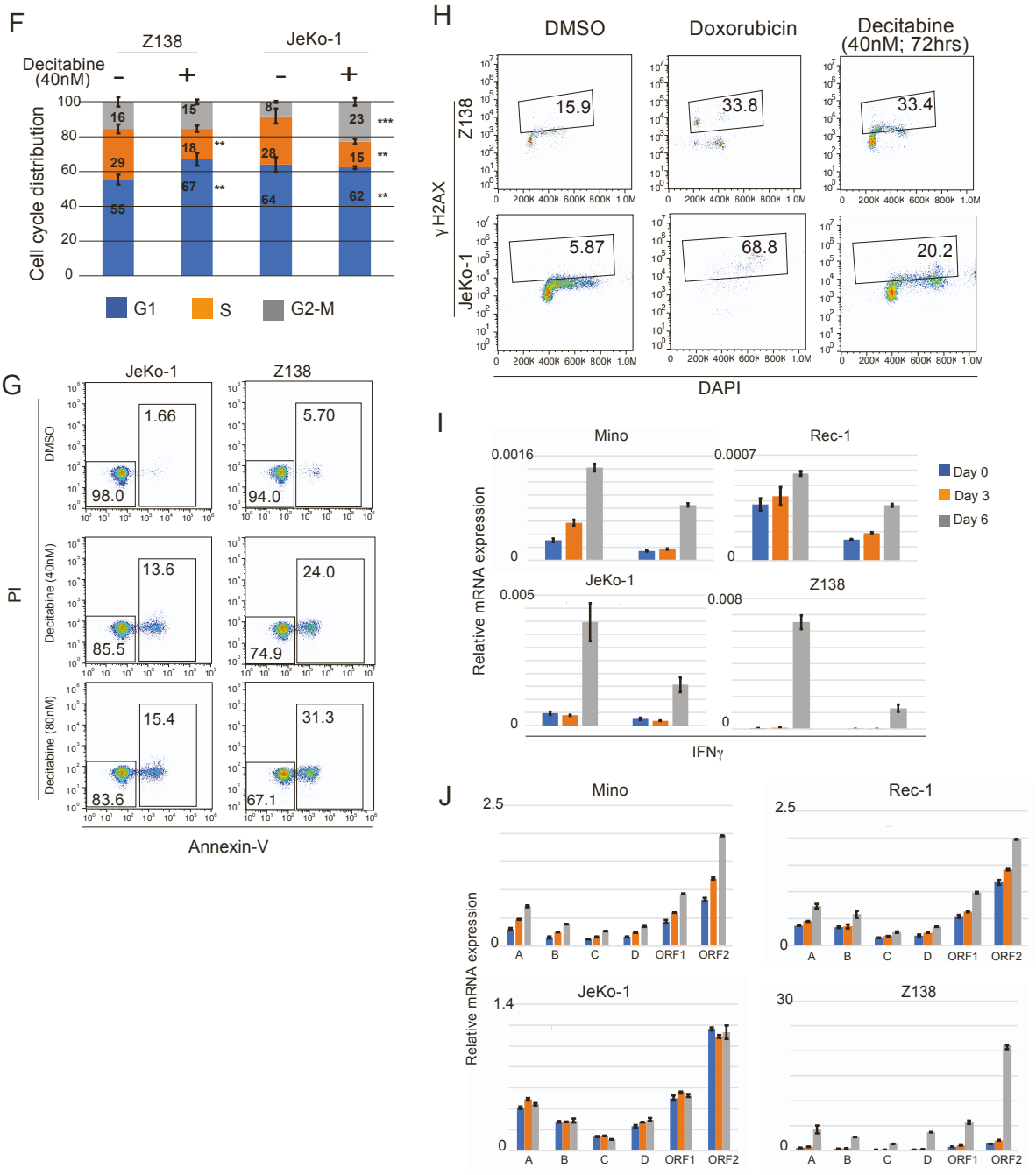


Figure S6-2: Decitabine reduces the growth of MCL cells and causes DNA damage, cell cycle arrest and apoptosis, likely due to the increase in the activation of interferon gamma through the reactivation of LINE-1, an endogenous retrotransposable element (related to Discussion)

(F) Decitabine induced cell cycle arrest in MCL cells. **(G)** Decitabine increased apoptotic cells. **(H)** Decitabine triggered DNA damage as measured by γH2AX. Doxorubicin treated cells (1μM; 24 hours) were included as a control for gating γH2AX positive cells. **(I)** Decitabine increased the expression of IFNγ in MCL cell lines. **(J)** The expression of L1 retrotransposable elements was increased after decitabine treatment in at least 3 MCL cell lines, except for JeKo-1 cells. All experiments were measured on day 6 of decitabine treatment at 40 nM unless otherwise indicated. The relative mRNA expression of genes of interest was normalized to *gapdh* housekeeping gene.