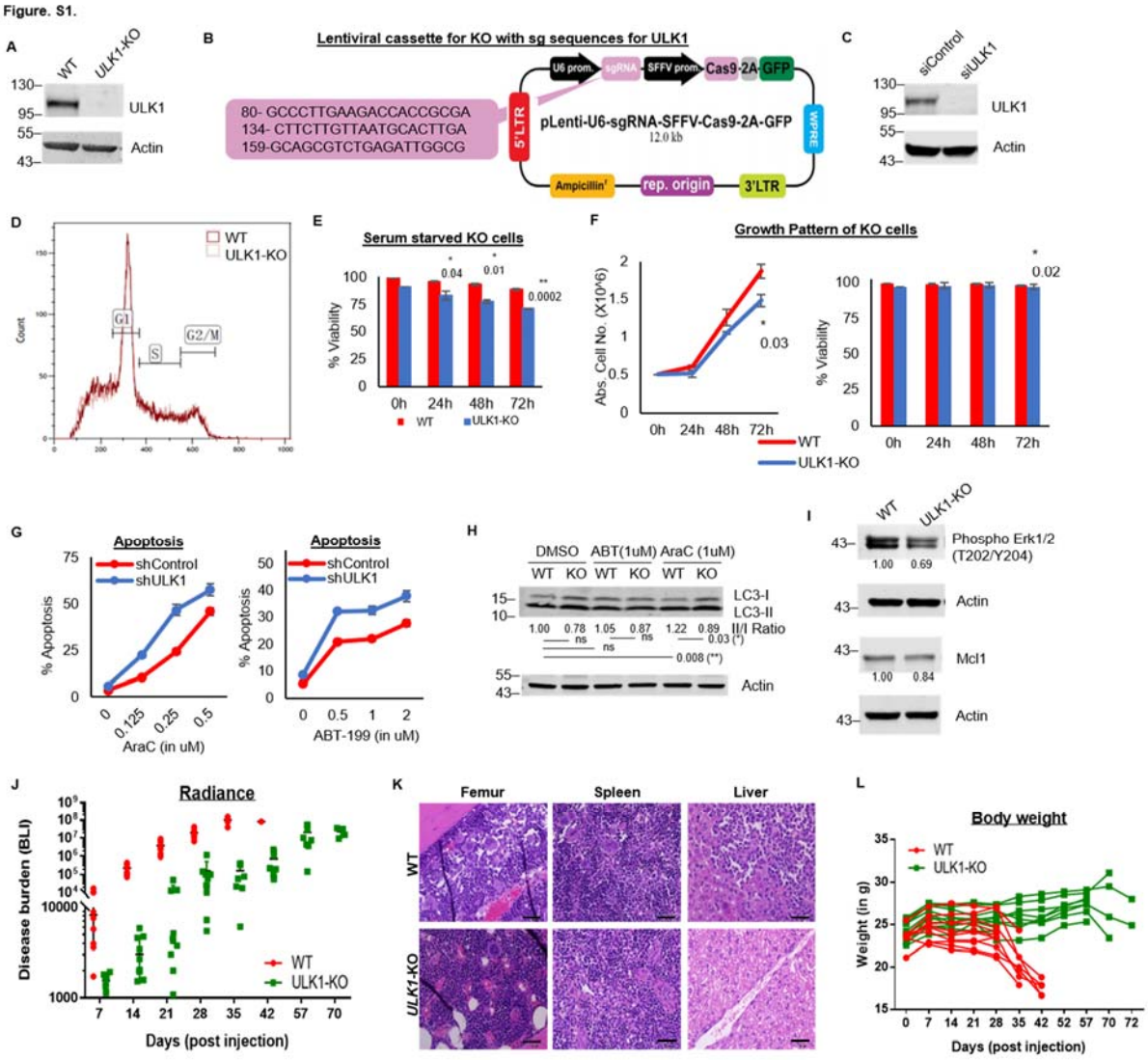
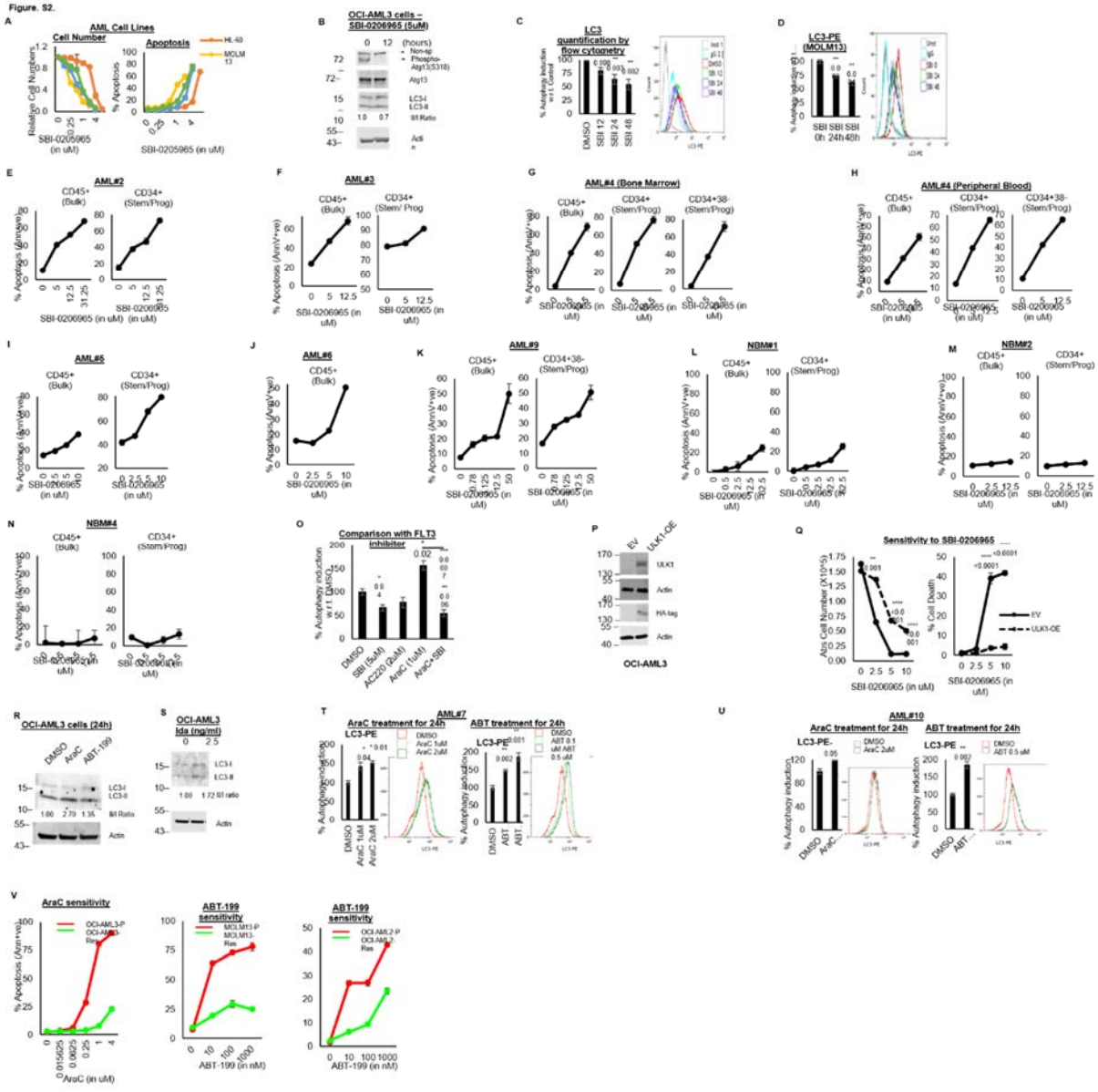
Supplementary Figures and Legends

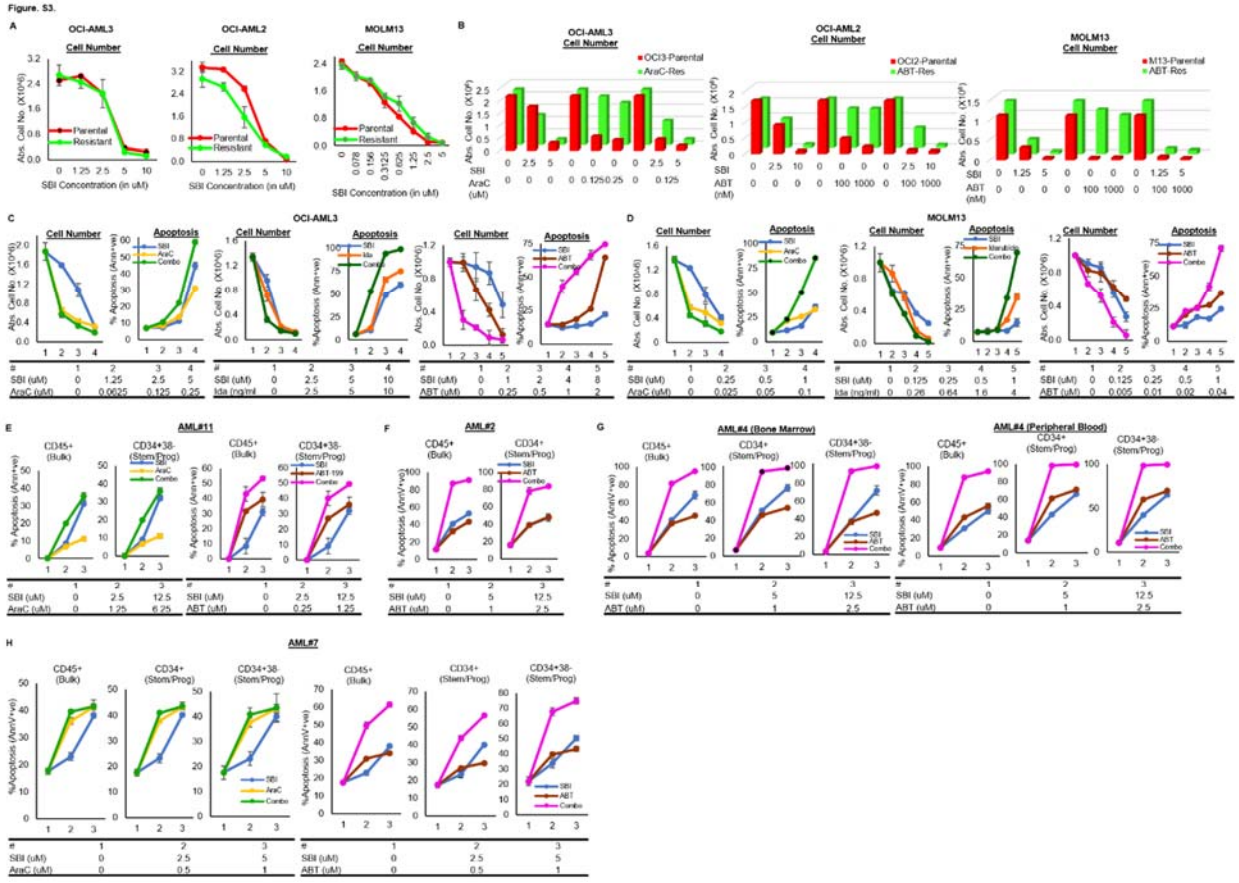


Supplementary Figure S1. Effect of genetic abrogation of ULK1 in vitro and in vivo. (A) Western blot analysis showing ULK1 expression in whole cell lysates of control (WT) and *ULK1*-KO cells generated in OCI-AML3. (B) Pictorial representation of the guide sequences and lentiviral cassette used for ULK1-KO. (C) Western blot analysis showing ULK1 expression in whole cell lysates of shControl and shULK1 cells generated in OCI-AML3. (D) Representative histograms from EdU incorporation assay showing a comparative analysis of cell cycle profile of control (WT) and ULK1-KO cells. (E) Representative bar graphs showing percent viability (from triplicates ± SD) measured by Trypan Blue staining using a Vi-Cell counter in control (WT) and *ULK1*-KO cells cultured simultaneously under serum starved conditions. Cells were collected every 24 hours. (F) Representative line graphs showing absolute cell numbers (from triplicates ± SD; left) and representative bar graphs showing percent viability (from triplicates ± SD; right) measured by Trypan Blue staining using a Vi-Cell counter in control (WT) and *ULK1*-KO cells cultured simultaneously without replenishing media upto 72 hours. Cells were collected every 24

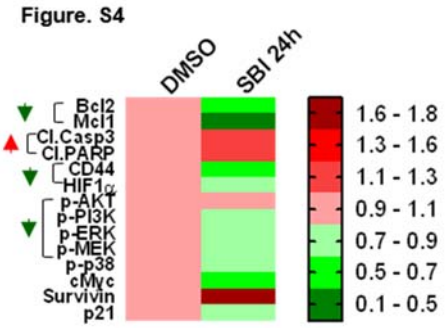
hours. (G) Representative line graphs showing percent apoptosis (from triplicates ± SD) from Annexin V/DAPI assay in shControl and shULK1 cells simultaneously treated with increasing doses as indicated of either AraC (left) or ABT-199 (right) for 72 hours. (H) Western blot analysis of whole cell lysates of control (WT) and *ULK1*-KO (KO) cells, treated with DMSO, AraC or ABT-199. (I) Western blot analysis of whole cell lysates of WT and *ULK1*-KO cells for phospho-Erk1/2 (T202/Y204) and Mcl1. Actin was used as the loading control and for densitometric measurements for all western blots. (J) Representative dot plot showing quantification of radiance from each individual mouse using the Living Image software (Caliper Life Sciences) based on BLI over time. (K) Representative images of H&E staining (40X magnification, 50 um scale bar) to analyze infiltration of leukemia in different organs as indicated in the control and *ULK1*-KO group. (L) Representative line graph showing body weights of individual mice during the course of the experiment.



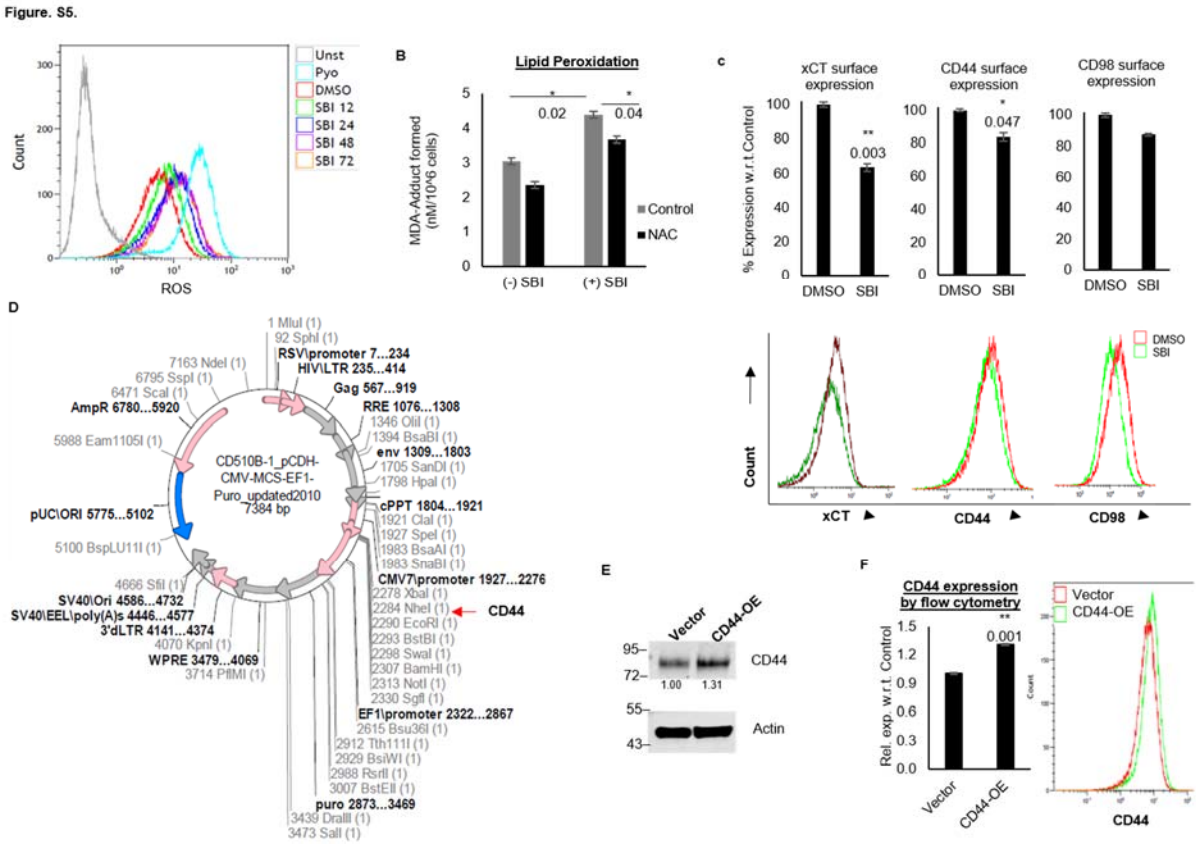
Supplementary Figure S2. ULK1 inhibitor SBI-0206965 has anti-leukemic activity and can overcome autophagic adaptation in drug-resistant cells. (A) Representative line graphs showing relative cell numbers (from triplicates ± SD; left) and percent apoptosis (from triplicates ± SD; right) from Annexin V/DAPI assay in 4 different AML cell lines (HL-60, MOLM13, MOLM14, and MV4;11) treated with increasing doses as indicated of SBI-0206965 for 72 hours. (B) Western blot analysis for Phospho-Atg13 (S318), Atg13 and LC3, in whole cell lysates of OCI-AML3 cells treated with 5uM of SBI-0206965 for indicated time points. (C-D) Representative bar graphs showing percent autophagy induction (calculated from triplicates ± SD, in LC3-PE flow cytometry) with respect to DMSO treated control cells as 100 (left) and representative histograms (right) in OCI-AML3 cells treated with 5uM SBI-0206965 (C), and MOLM13 cells treated with 1uM SBI-0206965 (D) for indicated time points. (E-N) Representative line graphs showing percent apoptosis (from triplicates ± SD) from Annexin V/DAPI assay in primary AML blasts (sample # 2, 3, 4, 5, 6 & 9) and normal bone marrow cells (NBM # 1, 2 & 4) treated with increasing doses as indicated of SBI-0206965 for 72 hours. Cell pellets were additionally stained with CD45, CD34 and CD38 surface markers to identify different subpopulations [bulk (CD45+ and stem/progenitor (CD34+ or CD34+CD38-)] by flow cytometry. (O) Representative bar graphs showing percent autophagy induction (calculated from triplicates ± SD, in LC3-PE flow cytometry) with respect to DMSO treated control cells as 100 in OCI-AML3 cells treated with indicated drugs for 24 hours. (P) Western blot analysis for ULK1 overexpression in OCI-AML3 cells using ULK1 and HA-tag antibodies. EV stands for empty vector and ULK1-OE stands for ULK1 overexpression. (Q). Representative line graphs showing absolute cell numbers (left) and percent apoptosis (right) from triplicates ± SD based on Annexin V/DAPI assay in EV and ULK1- OE cells treated with increasing doses as indicated of SBI-0206965 for 72 hours. (R) Western blot analysis of LC3 in whole cell lysates of OCI-AML3 cells treated with indicated drugs for 24 hours. (S) Western blot analysis of LC3 in whole cell lysates of OCI-AML3 cells treated with Idamycin for 24 hours. (T-U) Representative bar graphs showing percent autophagy induction (calculated from triplicates ± SD, in LC3-PE flow cytometry) with respect to DMSO treated control cells as 100 (left) and representative histograms (right). Primary AML blasts were treated with increasing doses as indicated of AraC (left panel) or ABT-199 (right panel) for 24 hours: sample # 7 (T) and sample # 10 (U). (V) Representative line graphs showing percent apoptosis (from triplicates ± SD) in Annexin V/DAPI assay performed on parental and drug-resistant cells treated with respective drugs against which the resistance was developed to verify the robustness of the system before further experiments were performed on these cells. Drug treatment was performed for 72 hours: AraC on AraC resistant OCI-AML3 cells (left); and ABT-199 on ABT-199 resistant OCI-AML2 cells (middle) and ABT-199 resistant MOLM13 cells (right). Actin was used as the loading control and for densitometric measurements in all western blots. The statistical significance of the experiments was calculated by standard Student's t-test and p-values are indicated in respective graphs.



Supplementary Figure S3. ULK1 inhibition reverses drug-resistance and synergizes with AML therapies. (A) Representative line graphs showing absolute cell numbers (from triplicates ± SD) in parental and drug-resistant cells treated with increasing doses as indicated of SBI-0206965 for 72 hours in Annexin V/DAPI assay. (B) Representative bar graphs comparing absolute cell numbers in parental versus drug-resistant cells. Data is based on Annexin V/DAPI assay after 72 hours of treatment with either SBI-0206965 alone or AML drugs alone or in combination (as indicated in the respective plots), in cell lines OCI-AML3 (left), OCI-AML2 (middle) and MOLM13 (right). (C-D) Representative line graphs showing absolute cell numbers (from triplicates ± SD; left) and percent apoptosis (from triplicates ± SD; right) from Annexin V/DAPI assay: OCI-AML3 (C), and MOLM13 (D) treated with either SBI-0206965 or AML drugs alone, and in combination (as indicated in the respective plots) for 72 hours. (E-H) Representative line graphs showing percent apoptosis (from triplicates ± SD) in an Annexin V/DAPI assay in primary AML blasts subjected to drug combinations (as indicated in the respective plots) for 72 hours: sample # 11 (E), sample # 2 (F), sample # 4 (G), and sample # 7 (H). Cell pellets were additionally stained with CD45, CD34 and CD38 surface markers to identify different subpopulations [bulk (CD45+) and stem/progenitor (CD34+ or CD34+CD38-)] by flow cytometry. The statistical significance of the experiments was calculated by standard Student's t-test and p-values are indicated in respective graphs.

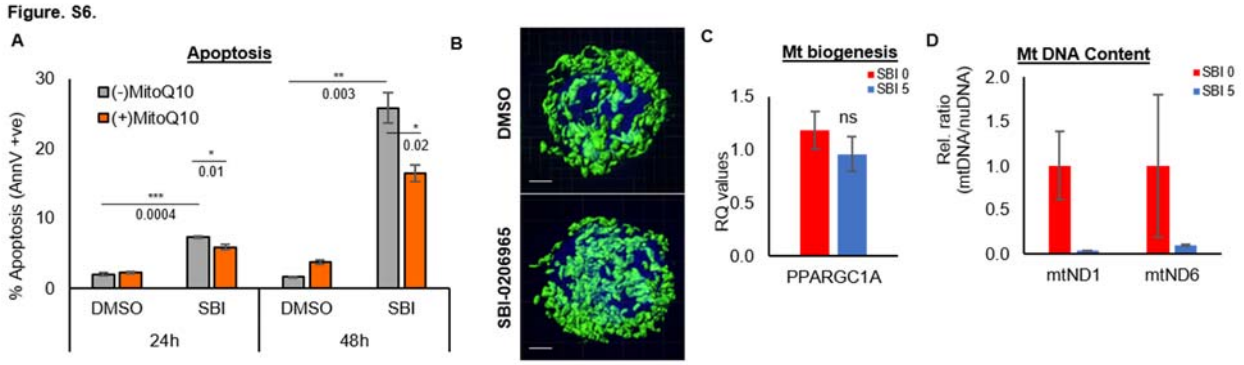


Supplementary Figure S4. ULK1 inhibition changes signaling in AML cells. Heat map representing CyTOF experiment (generated using GraphPad) to show the changes in protein expression of signaling molecules, based on different functional clusters in OCI-AML3 cells treated with 5uM of SBI-0206965 for 24 hours.

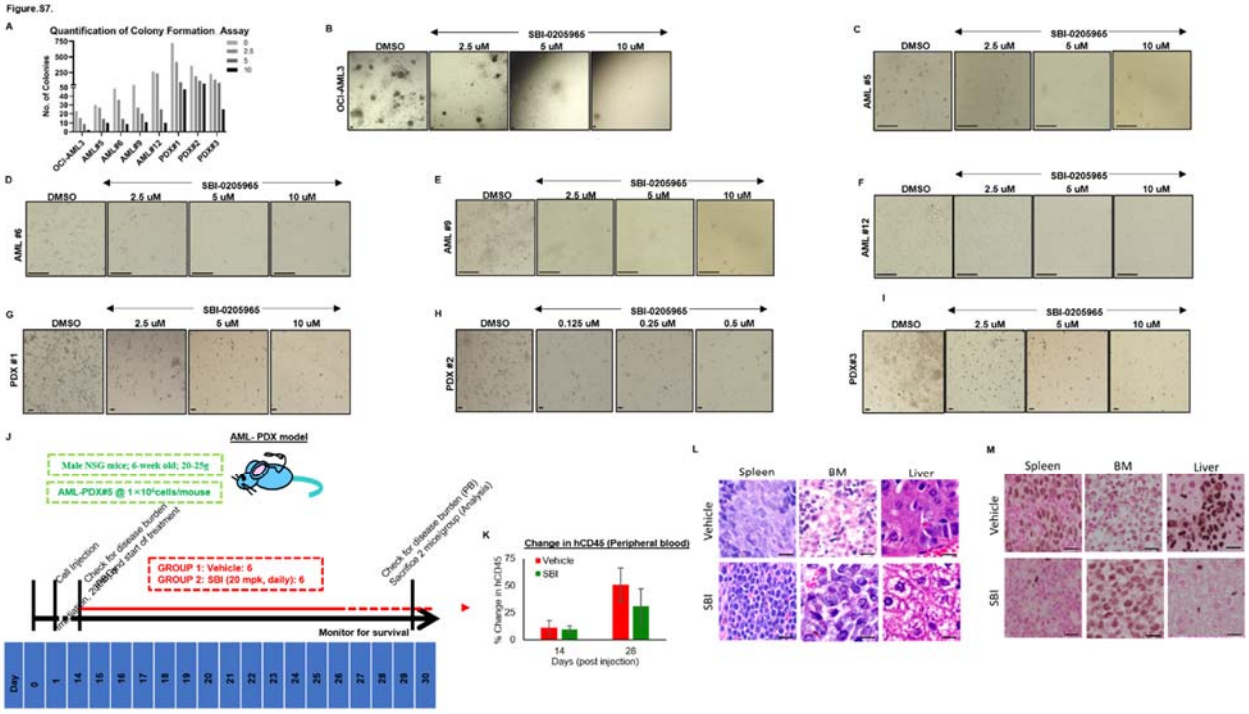


Supplementary Figure S5. ULK1 inhibition hampers ROS. (A) Representative histograms of the ROS estimation shown in Figure 6A (in OCI-AML3). (B) Representative bar graphs showing the amount MDA-adduct formed in nM per 10^6 cells (from triplicates ± SD) to quantify lipid peroxidation (colorimetrically) in OCI-AML3 cells treated with or without SBI-0206965 in the presence or absence of NAC for 24 hours. (C) Representative bar graphs (mean intensity from triplicates ± SD) (upper panel) and representative histograms (lower panel) from the flow cytometric experiment to determine surface expression of indicated molecules in OCI-AML3 cells treated with DMSO or SBI-0206965 for 24 hours. (D) Pictorial representation of the lentiviral

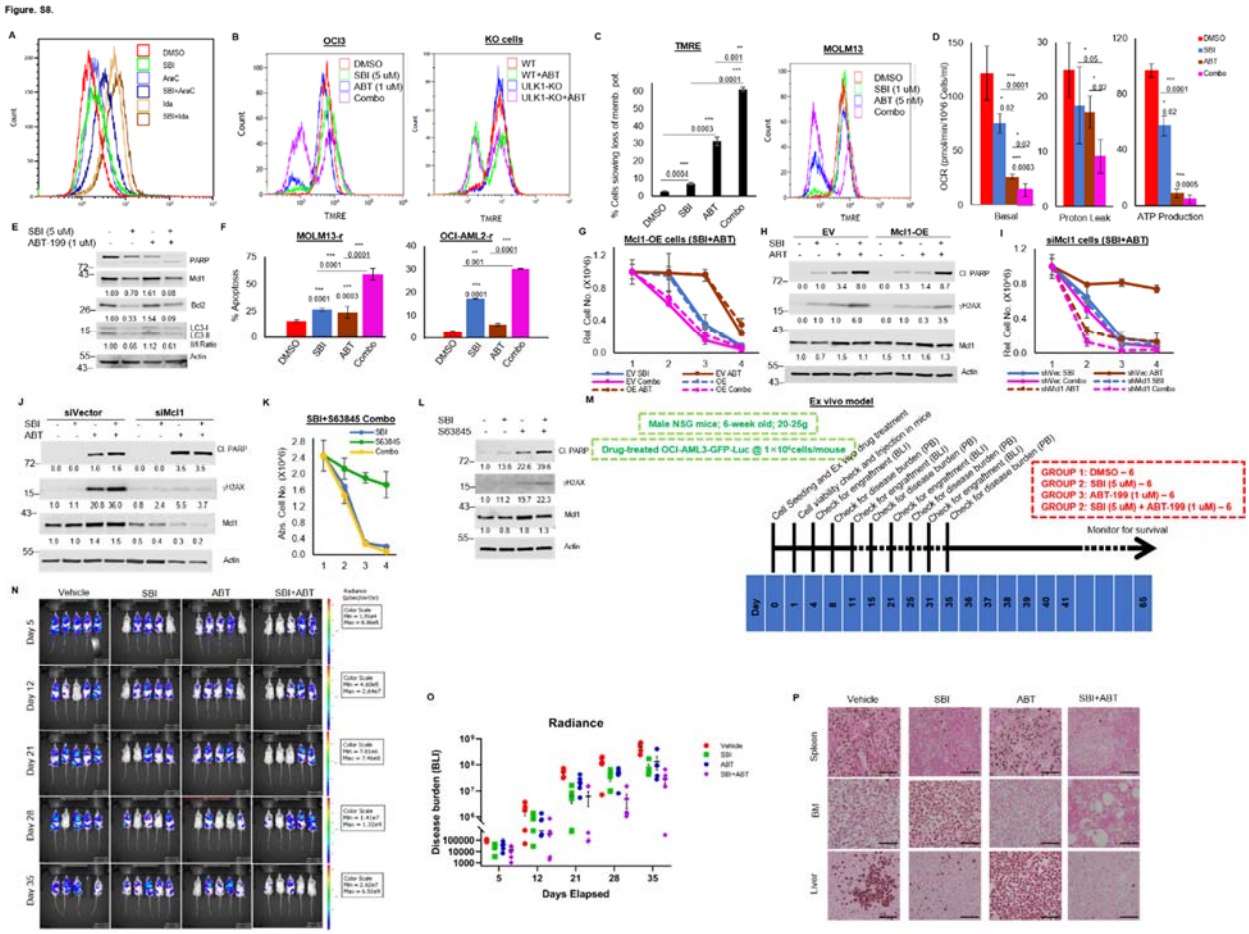
vector used for CD44 overexpression. (E) Western blot analysis showing transient overexpression of CD44 in whole cell lysates of OCI-AML3 cells. Actin was used as the loading control. (F) Representative bar graph (mean intensity from triplicates ± SD) (left) and representative histograms (right) from the flow cytometric experiment to determine surface expression of CD44 in vector and CD44 overexpressing cells. The statistical significance of the experiments was calculated by standard Student's t-test and p-values are indicated in respective graphs.'



Supplementary Figure S6. ULK1 inhibition accumulates dysfunctional mitochondria. (A) Representative bar graphs (mean percentage from triplicates ± SD) from Annexin V/DAPI assay to estimate the percentage of apoptosis in OCI-AML3 cells treated with or without SBI-0206965, in the presence or absence of mitochondria specific antioxidant MitoQ10, for 24 or 48 hours. (B) Representative 3D-reconstructed images to quantify mitochondrial volume in DMSO and SBI-0206965 treated cells using Imaris software "Surfaces" (scale bar 2 um). (C) Representative bar graphs (mean RQ value from triplicates ± SD) from qRT-PCR assay performed for *PPARGC1A* on RNA isolated from OCI-AML3 cells treated with 5uM of SBI-0206965 for 24 hours. RQ values were generated from Ct values ($2^{-\Delta\Delta Ct}$). (D) Representative bar graphs (mean of relative ratio from triplicates ± SD) from qRT-PCR assay performed simultaneously for mtND1 or mtND6 with nuclear 18S rRNA on genomic DNA isolated from OCI-AML3 cells treated with 5uM of SBI-0206965 for 24 hours. Relative ratio of expression was generated based on Ct values ($\Delta Ct_{Target}/\Delta Ct_{18S}$). The statistical significance of the experiments was calculated by standard Student's t-test and p-values are indicated in respective graphs.



Supplementary Figure S7. ULK1 inhibition impairs colony forming ability and delays leukemia progression. (A) Representative bar graph showing the number of colonies formed in different primary AML blasts in the presence of increasing doses of SBI-0206965. (B) Representative images of colony formation assay performed in OCI-AML3 cells treated with increasing doses of SBI-0206965 as indicated for 24 hours (4X magnification, 1 mm scale bar). (C-F) Representative images of colony formation assay performed in primary AML blasts treated with increasing doses of SBI-0206965 as indicated for 24 hours (20X magnification, 200 um scale bar): AML sample # 5, 6, 9 and 12. (G-I) Representative images of colony formation assay performed in primary AML blasts treated with increasing doses of SBI-0206965 as indicated for 24 hours (20X magnification, 500 um scale bar): PDX sample # 1, 2 and 3. (J) Schema showing experimental details of the AML-PDX model: 6 weeks old male NOD scid-IL2Rγ^{null} (NSG) mice were irradiated (200mGy) 1 day prior to injection. Previously characterized AML-PDX cells were injected on day 0 and engraftment was checked after 2 weeks by analysis of circulating human CD45 in peripheral blood. Mice were randomly grouped and treated daily with vehicle and SBI-0206965 (6 mice in each group). Disease burden was estimated in peripheral blood by quantifying human CD45 positive cells by flow cytometry, in tissue specimens by H&E and human Ku80 immunohistochemical staining, and the survival was monitored over time. (K) Representative bar graph showing the percentage change in circulating human CD45+ve cells by flow cytometry from peripheral blood, to measure the disease burden over the course of the experiment. (L-M) Representative images at 400X magnification (10 um scale bar) to analyze infiltration of leukemic cells in different organs as indicated in the vehicle control and SBI-0206965 treated mice by H&E staining (L) and immunohistochemistry of human Ku80 (M).



Supplementary Figure S8. In vitro and in vivo synergy of ULK1 inhibition with AML therapies. (A) Representative histograms of the ROS estimation shown in Figure 8A. (B) Representative histograms of TMRE assay performed in OCI-AML3 cells treated with single agents SBI-0206965 or ABT-199, and in combination for 24 hours (left), and in WT and *ULK1*-KO cells in the presence or absence of ABT-199 (right). (C) Representative bar graphs (mean percentage from triplicates ± SD) (left), with representative histograms (right) of TMRE assay to quantify loss of membrane potential in MOLM13 cells treated with single agents SBI-0206965 or ABT-199, and in combination for 24 hours. (D) Representative bar graphs (from triplicates ± SD) from the Seahorse Mito Stress test shown in Figure 8E with quantification of basal respiration (left), proton leak (middle) or ATP production (right). (E) Western blot analysis for PARP, Mcl1, Bcl2 and LC3 in whole cell lysates of OCI-AML3 cells treated with single agents SBI-0206965 or ABT-199, and in combination for 24 hours. (F) Representative bar graphs (mean percentage from triplicates ± SD) from Annexin V/DAPI assay to estimate the percentage of apoptosis in ABT-199 resistant cells treated with single agents SBI-0206965 or ABT-199, and in combination for 72 hours. (G) Representative line graphs showing relative cell numbers (from triplicates ± SD) from flow cytometry experiment in vector control and Mcl1-OE cells treated with single agents SBI-0206965 or ABT-199, and in combination for 72 hours. (H) Western blot analysis for apoptosis markers and Mcl1 in whole cell lysates from vector control and Mcl1-OE cells treated with single agents SBI-0206965 or ABT-199, and in combination for 24 hours. (I) Representative line graphs

showing relative cell numbers (from triplicates ± SD) from flow cytometry experiment in vector control and shMcl1 cells treated with single agents SBI-0206965 or ABT-199, and in combination for 72 hours. (J) Western blot analysis for apoptosis markers and Mcl1 in whole cell lysates from vector control and shMcl1 cells treated with single agents SBI-0206965 or ABT-199, and in combination for 24 hours. (K) Representative line graphs showing relative cell numbers (from triplicates ± SD) from flow cytometry experiment in OCI-AML3 cells treated with single agents SBI-0206965 or Mcl1 inhibitor (S63845), and in combination for 72 hours. (L) Western blot analysis for apoptosis markers and Mcl1 in whole cell lysates from OCI-AML3 cells treated with single agents SBI-0206965 or Mcl1 inhibitor (S63845), and in combination for 24 hours. (M) Schema showing experimental details of the ex vivo model: Luciferized OCI-AML3 cells were treated with vehicle and either SBI-0206965 or ABT-199 alone, and in combination, for 24 hours. Cell viability was measured thereafter, and equal number of viable cells were injected into 6 weeks old male NSG mice. Engraftment was checked after 3 days by BLI. Disease burden was estimated in whole body by BLI, in peripheral blood by quantifying human CD45 positive cells by flow cytometry, in tissue specimens by human Ku80 staining, and the survival was monitored over time. (N) BLI of ex vivo experiment showing vehicle, SBI-0206965, ABT-199 and combination groups monitored over time for disease progression. (O) Representative dot plot showing quantification of radiance from each individual mouse using the Living Image software (Caliper Life Sciences) based on BLI over time in the ex vivo model. (P) Representative images of immunohistochemistry of human Ku80 (100X magnification, 40 um scale bar) to analyze infiltration of leukemia in different organs as indicated, in the ex vivo model. Actin was used as the loading control in all western blots. The statistical significance of the experiments was calculated by standard Student's t-test and p-values are indicated in respective graphs.