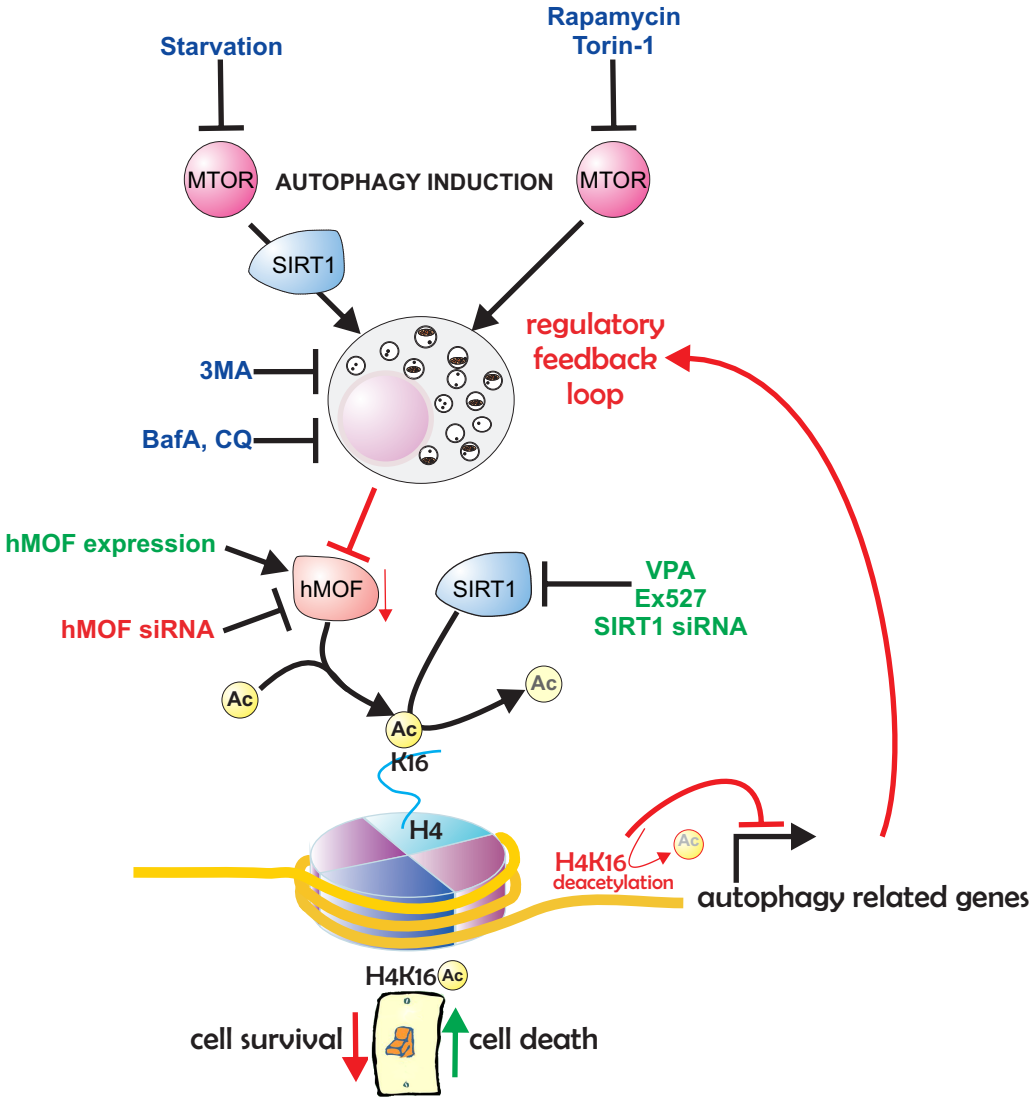

Figure S1 | Scheme illustrating the potential epigenetic regulatory feedback loop regulating the autophagic life and death decision via the hMOF-SIRT1 control of H4K16 acetylation/deacetylation.

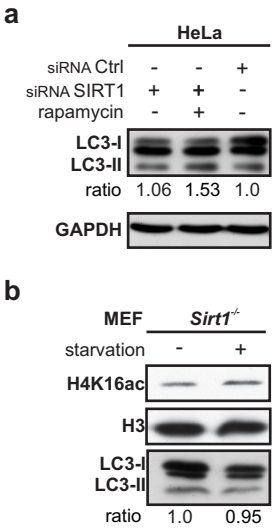

Figure S2 | SIRT1 is required for starvation-, but not rapamycin-, induced autophagy. (**a**) HeLa cells are still able to undergo autophagy, based on the increase in the LC3-II/LC3-I ratio) after rapamycin treatment when SIRT1 is knocked down with siRNA. (**b**) Starvation for 4 h did not reduce the H4K16ac level in *Sirt1* knockout MEF cells. Histone 3 is used as a loading control.

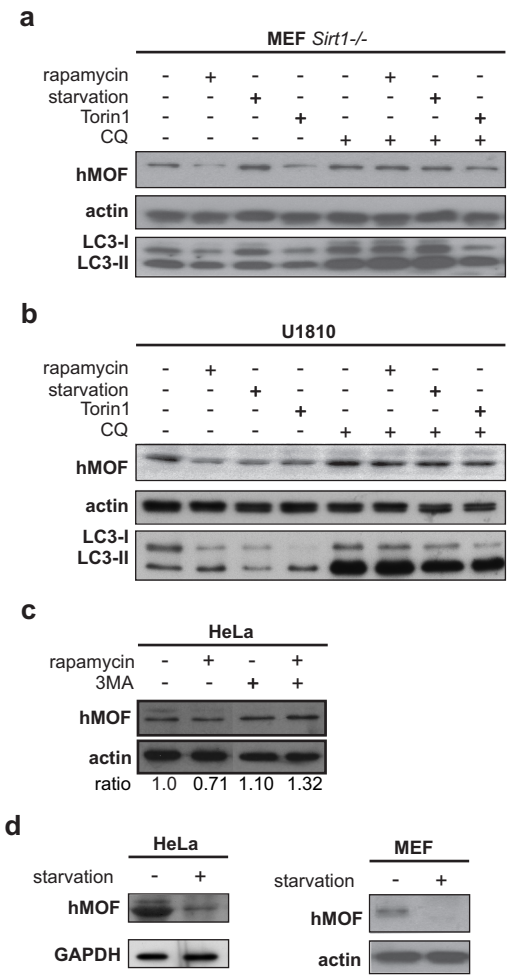

Figure S3 | hMOF levels are reduced upon autophagy induction. (**a**) hMOF levels are reduced in MEF *Sirt1*$^{-/-}$ cells upon autophagy induction using rapamycin (300 nM) or Torin1 (250 nM). When autophagy is induced in *Sirt1*$^{-/-}$ MEFs, LC3-I is not converted to LC3-II, and hMOF is not degraded, indicating that SIRT1 is required for starvation-induced autophagy. hMOF levels are decreased in the presence of rapamycin, but stabilized when autophagy is inhibited by chloroquine (CQ, 10 μM). (**b**) hMOF levels are reduced in U1810 cells when autophagy is induced by rapamycin, starvation or Torin1. Autophagy is monitored by an increase in the LC3-II to LC3-I ratio. When autophagy is inhibited by CQ, hMOF levels are stabilized. (**c**) hMOF levels are reduced in HeLa cells after rapamycin treatment. When autophagy is inhibited by 3-methyladenine (3MA, 5 mM) hMOF levels are stabilized. (**d**) hMOF levels are reduced in HeLa and MEF WT cells after starvation-induced autophagy.

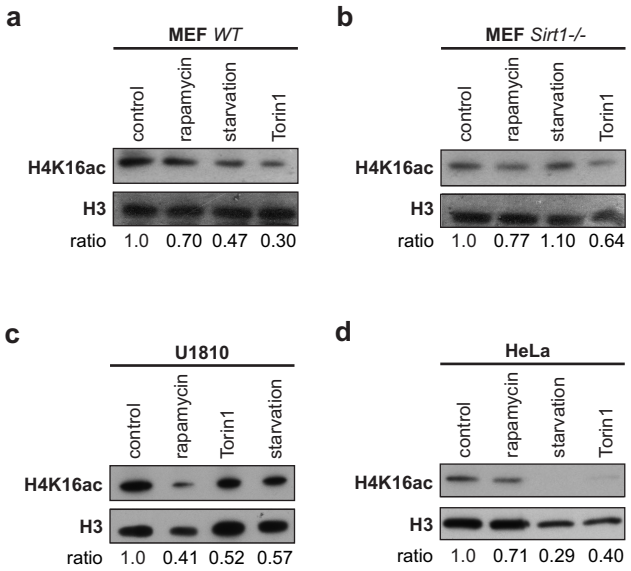

Figure S4 | H4K16ac levels are reduced after autophagy induction. (**a**) Autophagy induced by rapamycin, starvation or Torin1 reduced H4K16ac in MEF WT cells. (**b**) Autophagy induced by rapamycin or Torin1, but not starvation, reduced H4K16ac in *Sirt1*$^{-/-}$ MEF cells. Autophagy induced by rapamycin, starvation or Torin1 reduced H4K16ac in U1810 (**c**), or HeLa (**d**) cells. H3 is used as a loading control.

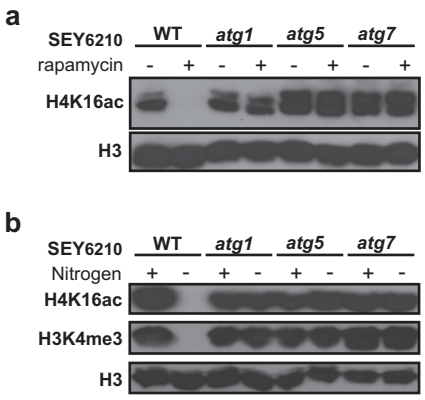

Figure S5 | Only the WT, but not the autophagy-deficient *atg1Δ*, *atg5Δ* and *atg7Δ* SEY6210 yeast cells, displayed a massive downregulation of H4K16ac and H3K4me3 upon (a) rapamycin treatment or (b) starvation.

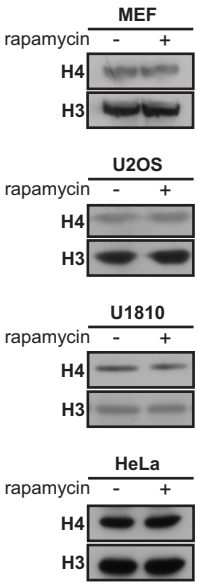

Figure S6 | Rapamycin treatment does not affect histone 4 (H4) levels. Rapamycin treatment for 48 h did not reduce the expression levels of histone H4 in MEF, U2OS, U1810 and HeLa cells. Histone H3 is used as a loading control.

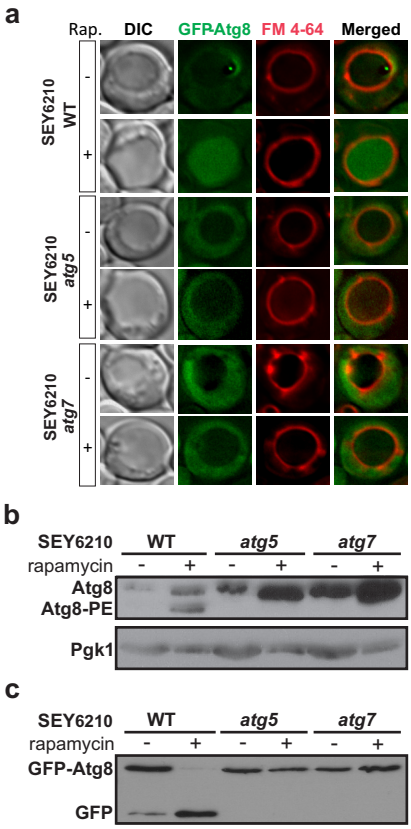

Figure S7 | Autophagy can only be induced in WT, but not *atg5?* and *atg7?* mutant yeast cells. (**a**) WT, and *atg5Δ* and *atg7Δ* mutant SEY6210 cells were transformed with a plasmid expressing GFP-Atg8 and stained with FM 4-64, which marks the vacuole membrane; autophagy is monitored by the appearance of GFP fluorescence within the vacuole lumen. (**b**) Rapamycin only induces the formation of Atg8-phosphatidylethanolamine (Atg8-PE) when Atg5 and Atg7 are present. Phosphoglycerate kinase 1 (Pgk1) is used as a loading control. (**c**) Additionally, rapamycin-induced cleavage of GFP-Atg8 only occurs in WT, but not in *atg5?* and *atg7*? SEY6210 cells. Upon autophagy-dependent cleavage, the GFP-Atg8 band disappears and a free GFP band is observed.

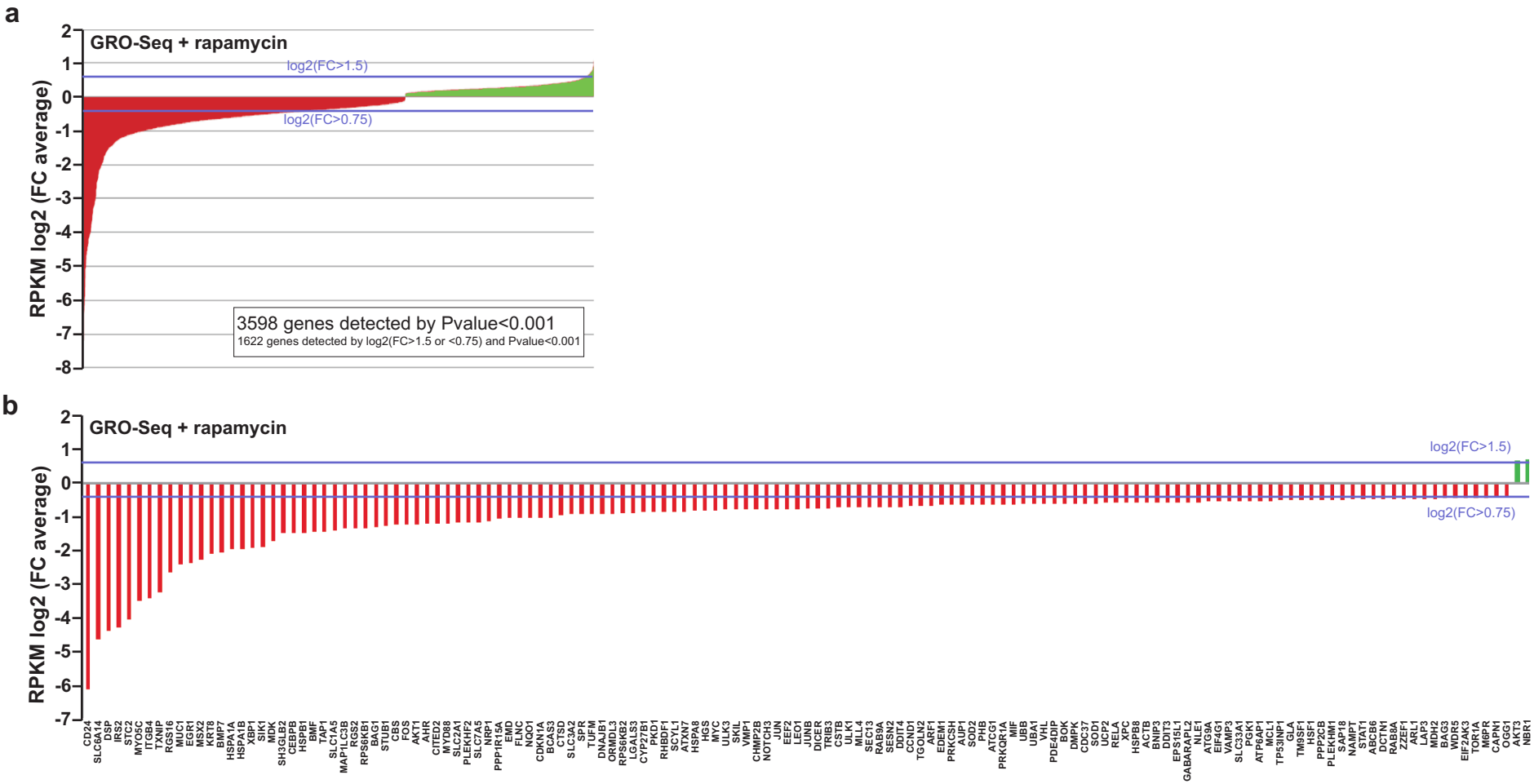

Figure S8 | Rapamycin treatment is associated with transcriptional regulation of autophagy-related genes. De novo detection of transcripts using GRO-Seq analysis was performed in 8 h rapamycin-treated U1810 cells and compared to untreated U1810 cells. (**a**) An illustration of the RPKM log2 fold-change values for 3598 rapamycin-regulated genes detected by Pvalue>0.001 is depicted. (**b**) An illustration of the RPKM (reads per kilobase and million mappable reads) log2 fold-change values for 141 rapamycin-regulated autophagy-related genes is depicted.

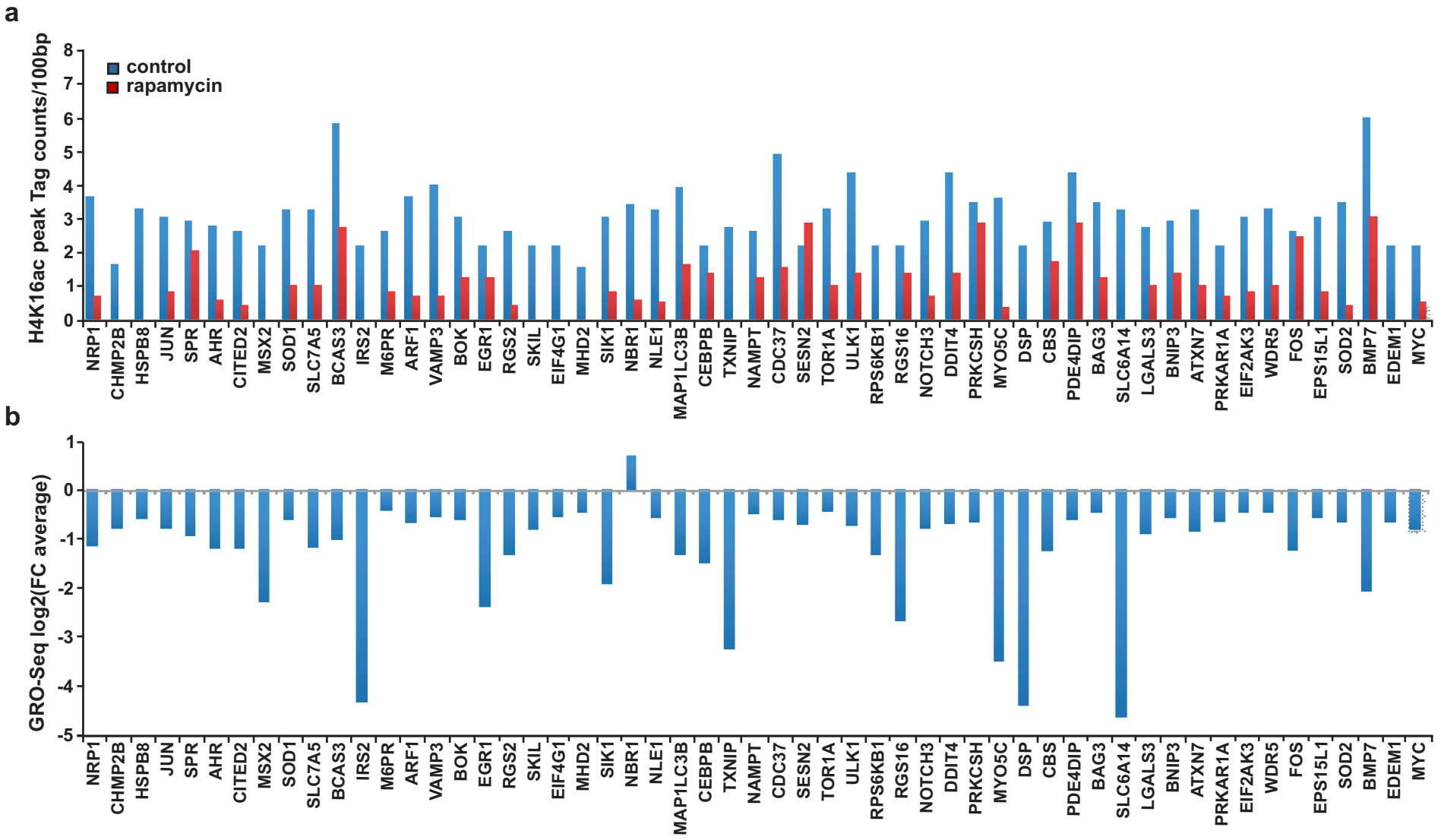

Figure S9 | H4K16 deacetylation in autophagy-annotated genes. (**a**) H4K16 deacetylation presented as tag counts per 100 bp in 55 regions defined as peaks in the no treatment sample and where the peaks have been annotated to an autophagy-related gene. (**b**) 55 rapamycin-regulated autophagy-related genes sorted by H4K16ac peak distance to the transcription start site.

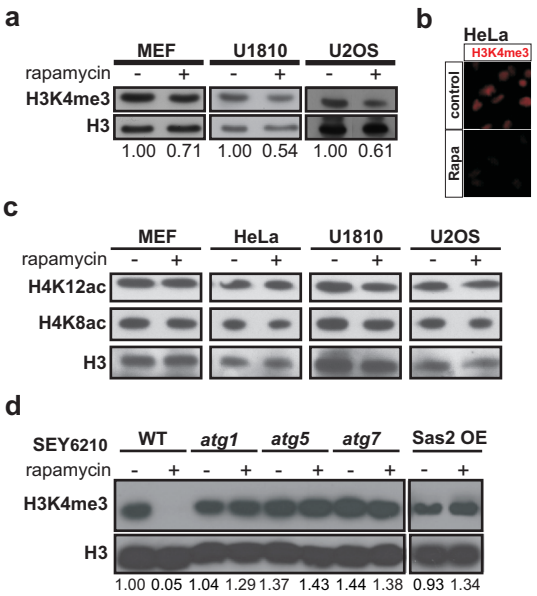

Figure S10 | The H4K16ac-associated histone posttranslational modification H3K4me3 is downregulated upon rapamycin induction of autophagy. Genome-wide investigations provide the compelling evidence that the H4K16ac and H3K4me3 histone marks reside within single nucleosomal units in human cells[21,22]. The coexistence of H4K16ac with H3K4me3 marks is consistent with the identification of multiple molecular interactions between the enzymes that are responsible for installing these marks[21,23,31]. Rapamycin-induced autophagy led to downregulation of H3K4me3 at 48 h as seen by immunobloting of (**a**) histone extracts of MEF, U1810 and U20S cells or (**b**) confocal imaging in HeLa cells. (**c**) H4K16ac-independent histone marks (H4K12ac and H4K8ac) were not downregulated after rapamycin treatment in MEF, HeLa, U1810 and U2OS cells, showing the specificity of the H4K16ac downregulation during autophagy and specific correlation of H3K4me3 and H4K16ac levels. (**d**) The joint downregulation of the H4K16ac and the H3K4me3 histone modifications was also observed upon rapamycin treatment in WT yeast, but mot *atg1?*, *atg5?* and *atg7?* autophagy-deficient yeast or Sas2-overexpressing yeast.

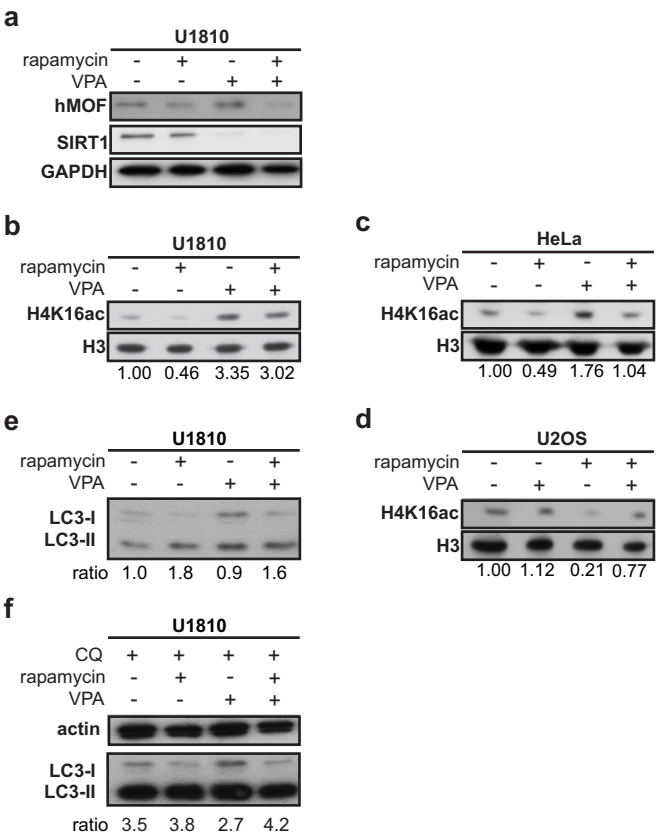

Figure S11 | Rapamycin-induced hMOF downregulation promotes deacetylation of H4K16. (**a**) Rapamycin treatment (48 h) promoted the downregulation of the histone acetyltransferase hMOF expression level, but left SIRT1 protein expression unaffected in U1810 cells. VPA (1 mM) treatment reduced SIRT1 expression. GAPDH is used as a standard for equal loading of protein. VPA treatment counteracted rapamycin-induced H4K16ac downregulation in (**b**) U1810 cells (**c**) HeLa cells and (**d**) U2OS cells. Histone 3 (H3) is used as a standard for equal loading of protein. VPA treatment decreased the LC3 ratio (**e**). Cotreatment with chloroquine (CQ), showed that the decrease in LC3 ratio was a result of an increase in autophagic flux (**f**).

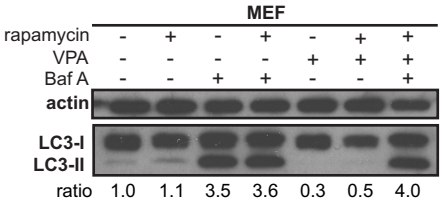

Figure S12 | VPA causes an increase in autophagic flux. VPA and VPA+rapamycin treatment resulted in a major decrease of the LC3-II band, resulting in a reduction of the LC3-II to LC3-I ratio. However, when degradation of LC3-II was blocked by the late inhibitor of autophagy, bafilomycin A1 (BafA), LC3-II was increased by VPA co-treatment. This proves an increase in turnover of LC3 and autophagic flux after VPA treatment.

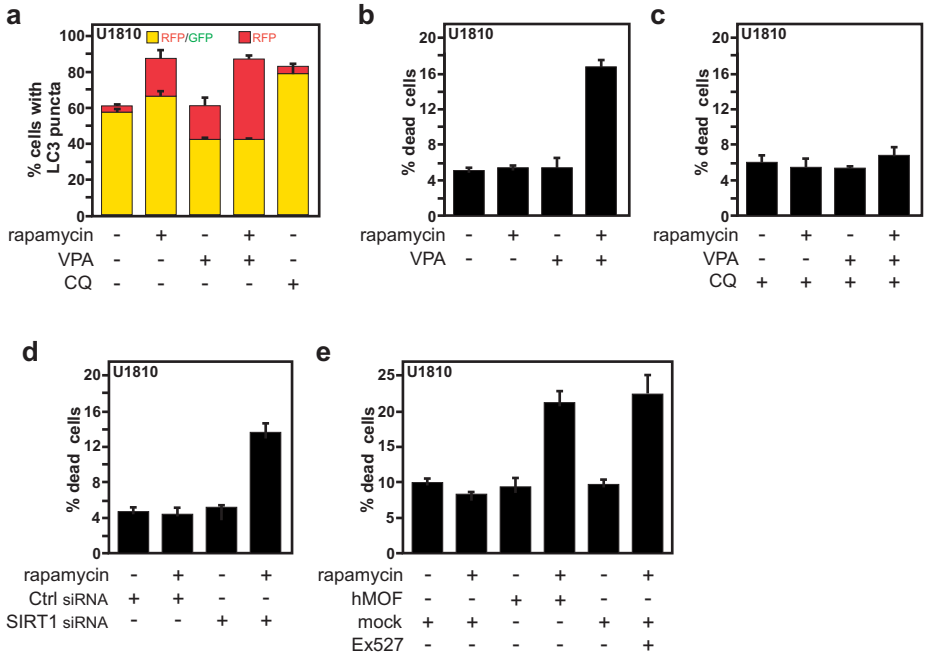

Figure S13 | Inhibition of H4K16ac downregulation upon autophagy induction results in cell death in U1810 cells. (**a**) VPA increases the autophagic flux in rapamycin-treated cells. LC3 puncta were counted in cells transfected with the tandem reporter construct, mRFP-GFP-LC3. This probe allows distinguishing between autophagosomes (GFP+/RFP+ yellow puncta) and autolysosomes (GFP-/RFP+ red puncta). Rapamycin treatment induced an increase in the percentage of cells with LC3 puncta, whereas VPA increased autophagic flux as represented by the quenching of GFP. Chloroquine (CQ) is used as a control for a block in autophagic flux. (**b**) Neither rapamycin (300 nM) nor VPA (1 mM) alone promoted cell death of U1810 cells. However, co-treatment with VPA and rapamycin led to increased cell death. (**c**) Co-treatment with (CQ, 10 μM) abrogated VPA+rapamycin-induced cell death. (**d-e**) Increasing H4K16ac levels by either inhibition of SIRT1 by siRNA or the chemical inhibitor Ex527 or overexpression of hMOF, promoted cell death upon rapamycin treatment.

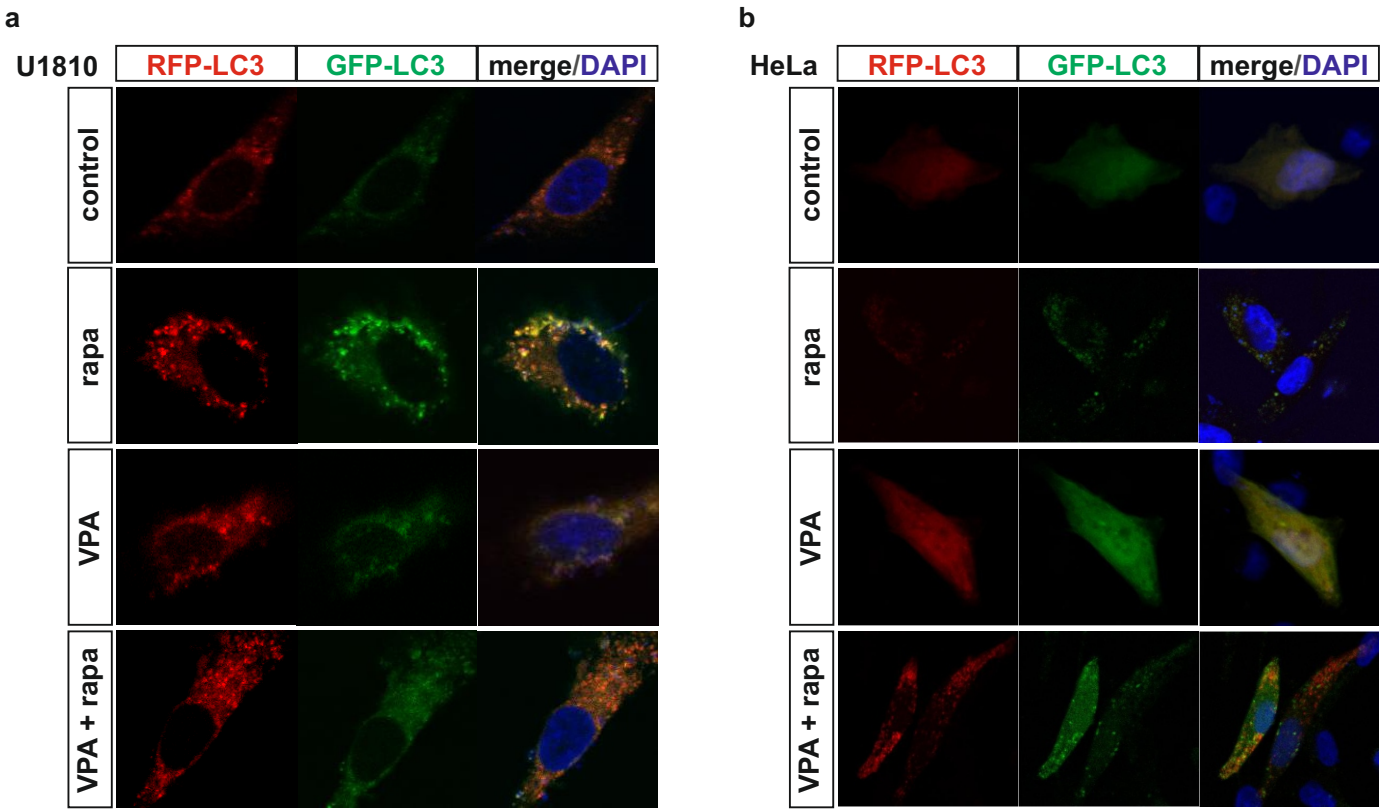

Figure S14 | Effect of VPA and rapamycin combined treatment on the autophagic flux. Analysis of mRFP-GFP-LC3-expressing U1810 (**a**) and HeLa (**b**) cells shows accumulation of autophagosomes (GFP+/RFP+ yellow puncta) in rapamycin-treated cells, and autolysosomes (GFP-/RFP+ red puncta) upon cotreatment with VPA.

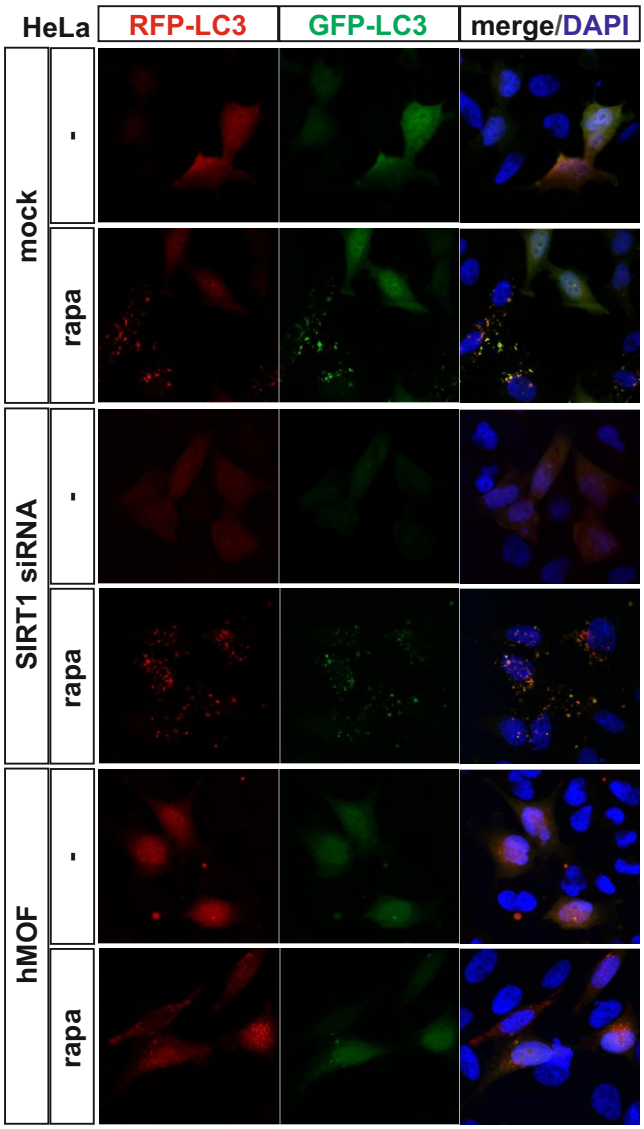

Figure S15 | SIRT1 knockdown or hMOF overexpression increases the autophagic flux in rapamycin-treated cells. Analysis of mRFP-GFP-LC3-expressing U1810 cells showed accumulation of autophagosomes (GFP+/RFP+ yellow puncta) and autolysosomes (GFP-/RFP+ red puncta) upon SIRT1 silencing or hMOF overexpression in rapamycin-treated cells.

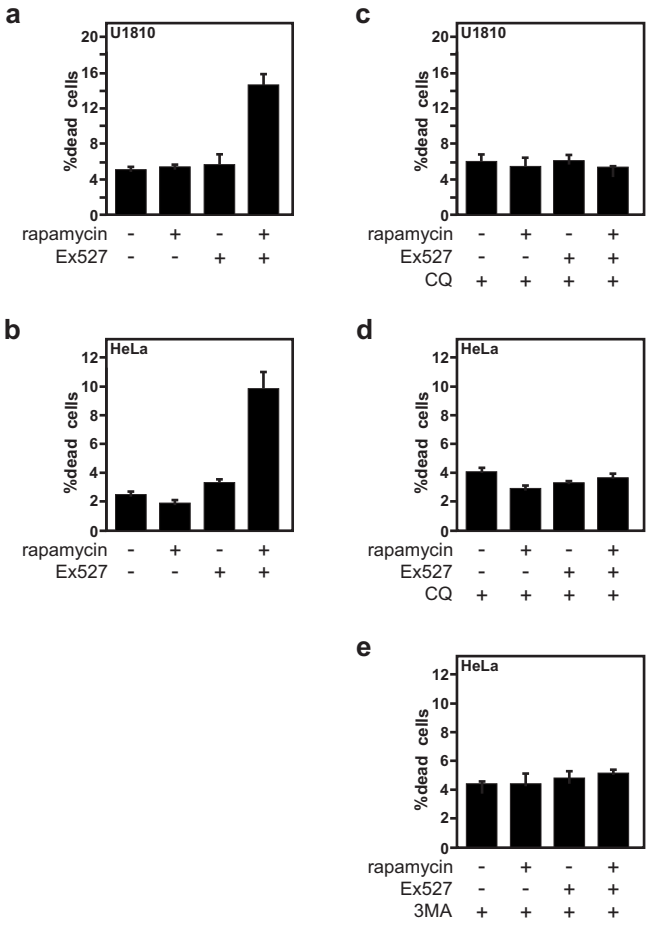

Figure S16 | Inhibition of SIRT1 by the specific inhibitor Ex527 upon autophagy induction results in cell death. (**a-b**) Neither rapamycin (300 nM) nor Ex527 (10 nM) alone promoted cell death of U1810 (**a**) or HeLa (**b**) cells. However, co-treatment with Ex527 and rapamycin led to increased cell death in all human cell types tested. (**c-d**) Co-treatment with chloroquine (CQ, 10 μM), an autophagy inhibitor, abrogated Ex527+rapamycin-induced cell death in the tested cell lines.(**e**) Co-treatment with the early inhibitor of autophagy 3-methyladenine (3MA, 5 mM) also abrogated Ex527+rapamycin-induced cell death.

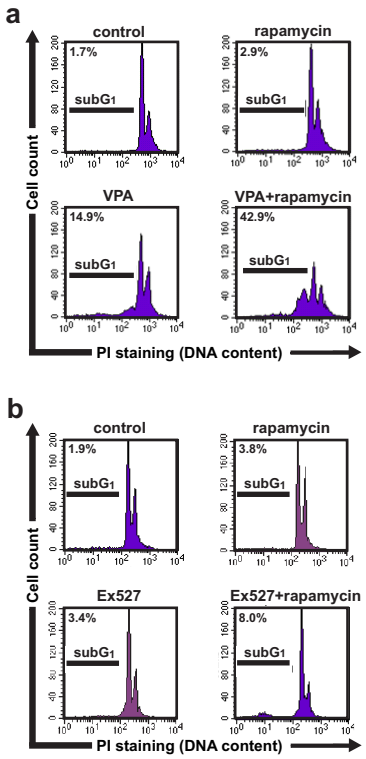

Figure S17 | Inhibition of H4K16ac downregulation upon autophagy induction results in cell death. (**a-b**) Neither rapamycin (300 nM) nor VPA (1 mM) or Ex527 (10 μM) treatment alone caused a significant appearance of a hypodiploid sub-G_1 peak in HeLa cells in HeLa cells, as monitored by FACS analysis upon propidium iodide (PI) staining. However, co-treatment with VPA and rapamycin (**a**) or with Ex527 (10 μM) and rapamycin (**b**) promoted the emergence of a sub-G_1 peak. Percentages of cells in sub-G_1 are displayed. Results are representative for at least 3 independent experiments.

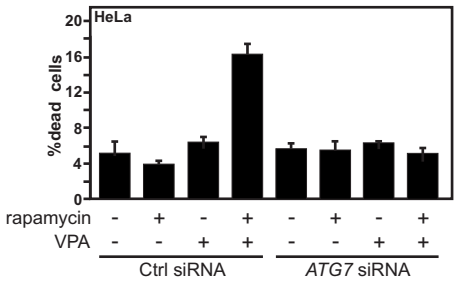

Figure S18 | ATG7 silencing inhibits rapamycin+VPA induced cell death. Neither rapamycin (300 nM) nor VPA (1 mM) caused cell death in scramble siRNA or ATG7 siRNA (25 nM) transfected HeLa cells. However, combined treatment with rapamycin+VPA induced cell death in scramble siRNA transfected cells, but not in ATG7 siRNA transfected cells.