A Jurkat cells were transfected with mtYFP and pre-treated with BAPTA-AM before inducing AICD. Representative reconstructions of confocal z-stacks of the mtYFP fluorescence 24 h after AICD induction are shown. Scale bar, 5 μm.

B Morphometric analysis of mitochondrial shape of cells treated as in (A) is shown. Data represent mean ± SE of three independent experiments (n = 30 cells per condition in each experiment).

C AICD and BAPTA pre-treatment were performed as previously described. Viability was determined cytofluorimetrically as Annexin-V/PI double-negative cells. Data represent mean ± SE of three independent experiments.

D Jurkat cells were co-transfected with mtYFP or mtRFP, the dominant-negative form of calmodulin (DChA1S12Q) or the mutant form of DRP1 (DRP15637D). Representative reconstructions of confocal z-stacks of the mtYFP or mtRFP fluorescence 24 h after AICD induction are shown.

E Morphometric analysis of mitochondrial shape of cells transfected as in (D) is shown. Data represent mean ± SE of three independent experiments (n = 30 cells per condition in each experiment).

F Jurkat cells were transfected as indicated and 32 h after AICD induction viability was determined cytofluorimetrically. Data represent mean ± SE of three independent experiments.

Data information: P-values: *P < 0.05; **P < 0.01; ***P < 0.001.
Figure EV2. Parkin knock-down-dependent detrimental effect on AICD depends on increased depolarization of mitochondria and OPA1 long to short forms’ conversion.

A Jurkat cells were transfected with scramble, AMPK or Parkin siRNA 24 h before AICD induction. Viability was determined cytofluorimetrically as Annexin-V/PI double-negative cells. Data represent mean ± SE of three independent experiments.

B Mitochondrial depolarization upon AICD induction was analysed as indicated in cells transfected with scramble and Parkin siRNA. Data represent mean ± SE of three independent experiments.

C Jurkat cells were transfected as in (B); 24 h after transfection, protein samples (20 µg) were separated by SDS–PAGE and immunoblotted with the indicated antibodies. WBs are representative of at least four independent experiments.

D, E Jurkat cells were transfected as in (B). OPA1 total level and the ratio between short and long OPA1 isoforms are shown. Data represent mean ± SE of four independent experiments.
Figure EV3. AMPK inhibition leads to inactivation of its downstream autophagic effectors ULK1 and ATG13.

A–H Protein samples (20 μg) from Jurkat cells treated as indicated were separated by SDS–PAGE and immunoblotted with the indicated antibodies. WBs are representative of at least four independent experiments. Levels of proteins and phosphorylation status of AMPK (P-T172) (A, B), ULK1 (P-S555) (C, D), ATG13 (P-S318) (E, F) and mTOR (P-S2448) (G, H) were quantified and analysed as shown. Signals referring to both phosphorylated and total proteins have been normalized to actin before being compared, to normalize for loading errors during the WBs. Data represent mean ± SE of four independent experiments.
Figure EV4. Inhibition of PKA results in reduced AMPK$^{485/491}$ phosphorylation and increased LC3-I/LC3-II conversion.

A–C Protein samples (20 μg) from Jurkat cells overexpressing empty vector or AMPK$^{S173A}$ were subjected to AICD. At the indicated time points, protein samples were prepared and immunoblotted with the indicated antibodies. (A) LC3-I/LC3-II conversion during AICD is shown. (B–C) Levels of proteins and phosphorylation status of ULK1 (P S555) were quantified and analysed as shown. Data represent mean ± SE of three independent experiments.

D, E Levels of mitochondrial markers and quantitative analysis of the ratio between the protein levels of mitochondrial markers and actin during AICD are shown. Data represent mean ± SE of four independent experiments.

F, G Jurkat cells were subjected to AICD and, where indicated, 10 μM H89 was added. At selected times, protein samples (20 μg) were separated by SDS–PAGE and immunoblotted with the indicated antibodies. Phosphorylation status of AMPK$^{485/491}$ and LC3-I/LC3-II conversion during AICD in the presence of 10 μM H89 is shown. WBs are representative of at least four independent experiments.

H Protein samples (20 μg) from Jurkat cells treated as indicated were separated by SDS–PAGE and immunoblotted with the indicated antibodies. WBs are representative of at least three independent experiments.

I Quantitative analysis of the ratio between the protein levels of mitochondrial markers and actin during AICD is shown. Data represent mean ± SE of three independent experiments.

J Mitochondrial depolarization upon AICD induction was analysed in cells treated as indicated. Data represent mean ± SE of three independent experiments.
Figure EV4.
Figure EV5. Rapamycin induces autophagy in activated hPB T and Jurkat cells and reduces the number of cells with depolarized mitochondria.

A Activated hPB T cells were pre-treated with 100 nM rapamycin before protein samples were prepared. Protein samples (20 μg) from cells treated as described were separated by SDS–PAGE and immunoblotted with the indicated antibodies.

B Jurkat cells were treated with 100 nM rapamycin during AICD. Protein samples (20 μg) from cells treated as described were separated by SDS–PAGE and immunoblotted with the indicated antibodies.

C Jurkat cells were co-transfected with mtRFP and the mutant form of DRP1 (DRP1S637A) and treated as indicated. Representative reconstructions of confocal z-stacks of the mtRFP fluorescence 24 h after AICD induction are shown.

D, E AICD induction and rapamycin treatment were carried out as previously described, except that at the indicated times cells were incubated with 100 nM MitoTracker Green (D) or 2 nM TMRM (E). MitoTracker Green and TMRM fluorescence changes were analysed cytofluorimetrically. Mean fluorescent intensity of MitoTracker Green (D), and images of TMRM-stained (E) Jurkat cells are shown. Data represent mean ± SE of three independent experiments.