Supplemental Figures, Movies & Experimental Procedures:

Autophagy controls the kinetics and extent of mitochondrial apoptosis by regulating PUMA levels

Jacqueline Thorburn, Zdenek Andrysik, Leah Staskiewicz, Jacob Gump, Paola Maycotte, Andrew Oberst, Douglas R. Green, Joaquín M. Espinosa, Andrew Thorburn.

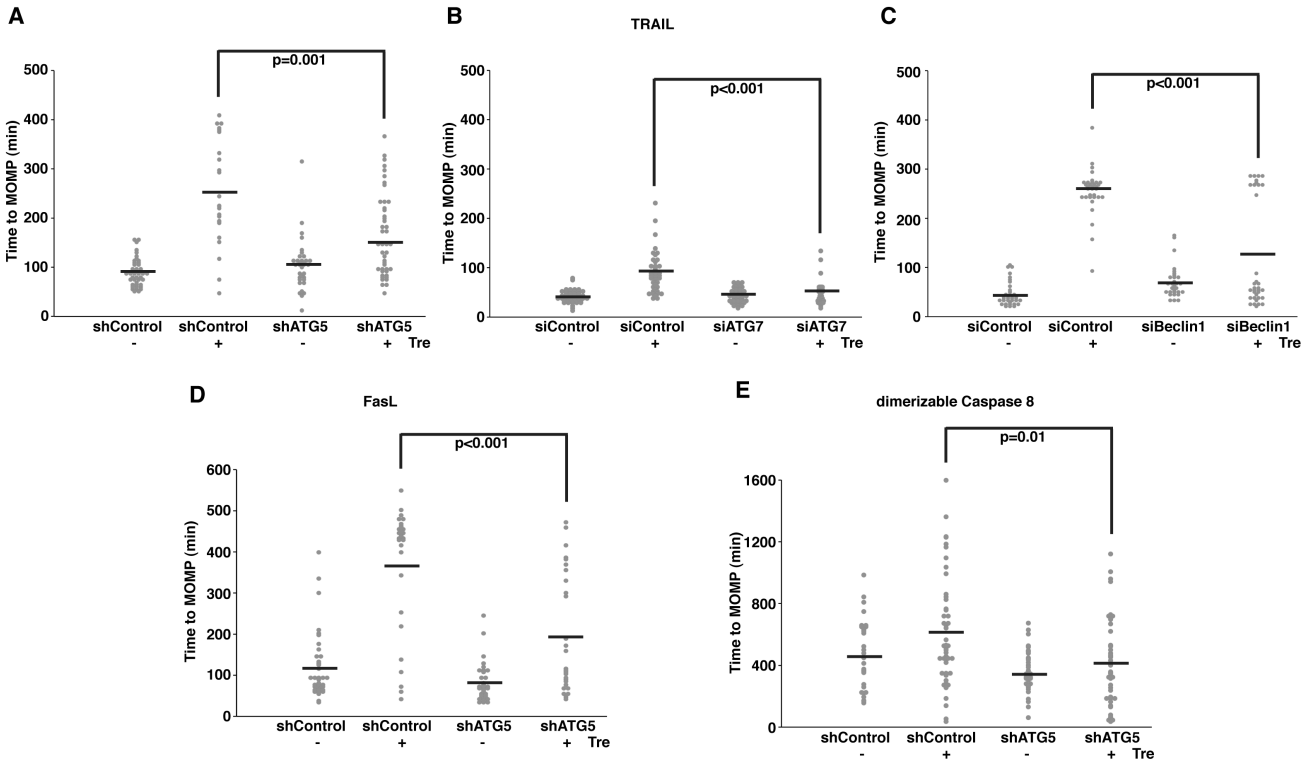

Figure S1, Autophagy regulates timing of MOMP. Related to Figure 1.

(A) Trehalose treatment delays MOMP after TRAIL treatment (25ng/ml) and this delay is blocked by autophagy inhibition using ATG5 shRNA. Cells were imaged individually after treatment with or without trehalose and shRNA as indicated.

(B) As in panel A using ATG7 siRNA.

(C) As in panel A using BECN1 siRNA.

(D) As in panel A using Fas Ligand treatment and ATG5 shRNA.

(E) As in panel A using artificial activation of caspase-8 by dimerization and ATG5 shRNA.

Bars indicate the mean time to MOMP, p values by Student's t- test.

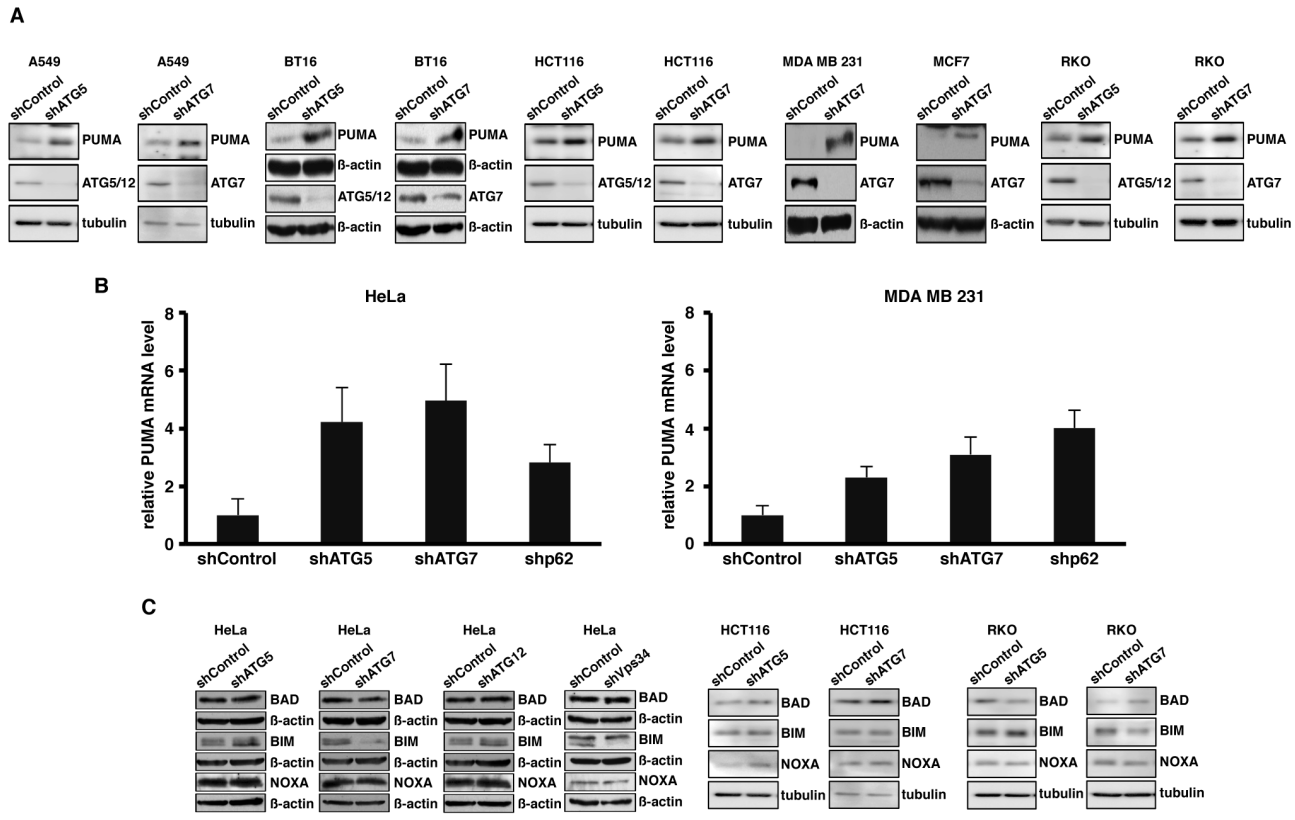

Figure S2. Autophagy selectively regulates levels of PUMA. Related to Figure 1.

(A) Autophagy regulator knockdown causes increase in PUMA levels in multiple cell lines.

(B) ATG5, ATG7 and p62/SQSTM1 knockdowns cause increase in PUMA mRNA levels. Q RT-PCR analysis of RNA isolated from knockdown cells demonstrates increased PUMA mRNA. Samples from three or four independent experiments were prepared and the average PUMA mRNA levels presented with error bars indicating standard deviation.

(C) ATG5 and ATG7 knockdown does not increase BAD, BIM or NOXA indicating selectivity of the effect on PUMA.

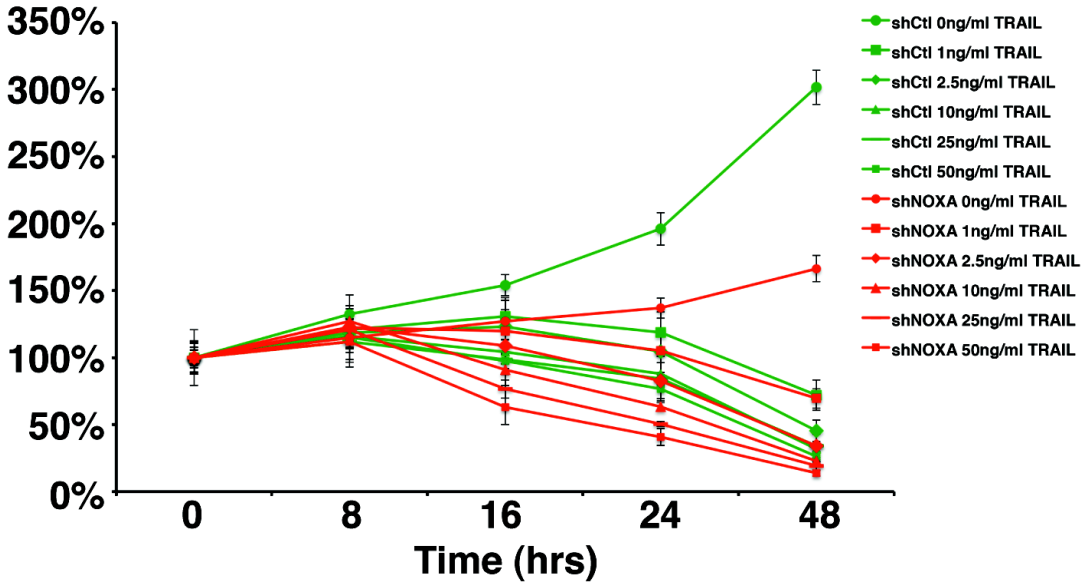

Figure S3. NOXA does not regulate sensitivity to TRAIL-induced apoptosis. Related to Figure 4.

Quantitation of cell viability by continuous monitoring of mixed populations of control (Green) or shRNA knockdown (Red) cells. HeLa cells were marked with nuclear GFP or mCherry and cell viability after treatment with different doses of TRAIL was determined by continual monitoring using the IncuCyte system. shRNA knockdown of NOXA has no significant effect to protect or potentiate TRAIL induced death.

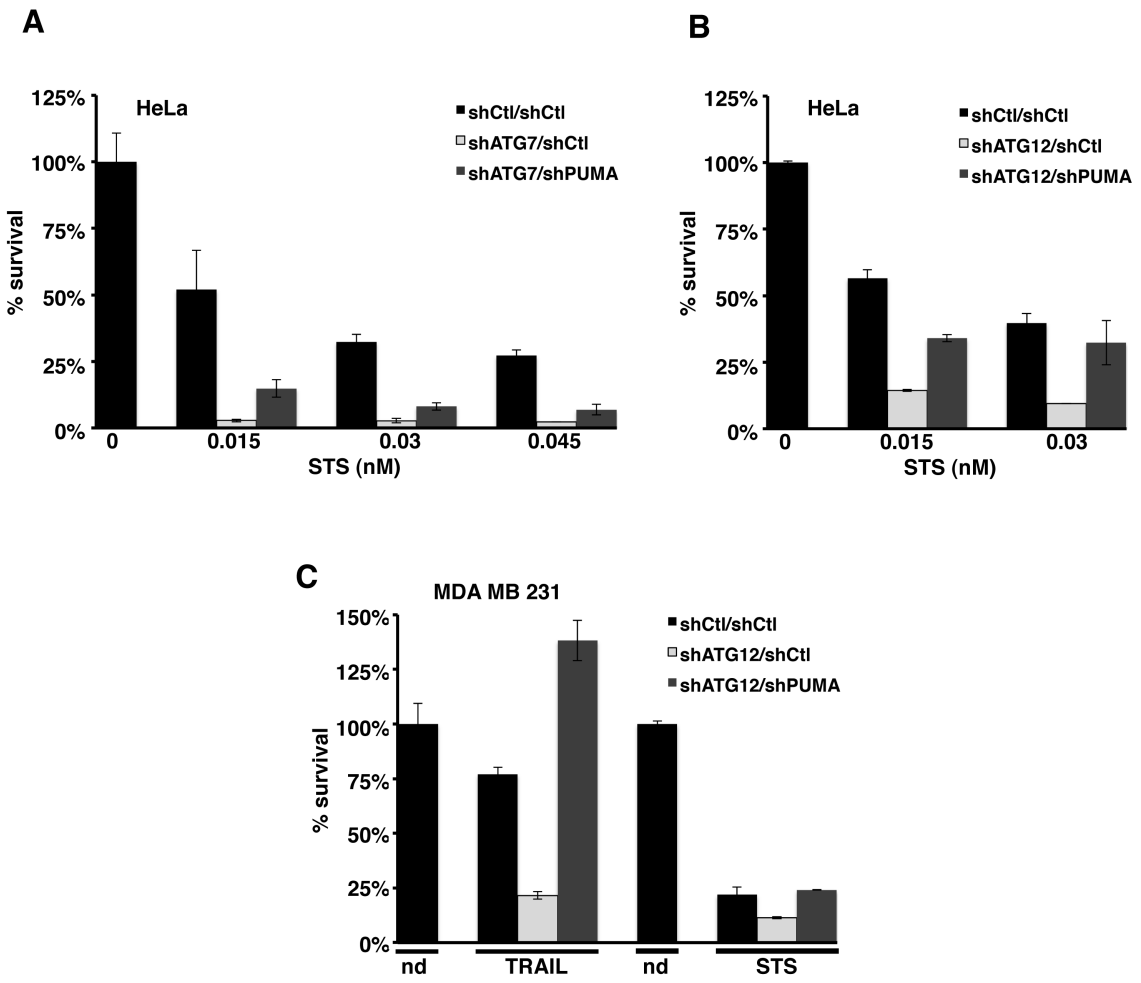

Figure S4. Autophagy potentiates Staurosporine-induced death via PUMA. Related to Figure 4.

(A) Cell viability quantified by the IncuCyte at different doses of Staurosporine was determined in matched control, ATG7 or ATG7/PUMA knockdown cells. Autophagy inhibition by ATG7 knockdown increases Staurosporine-induced death and this is partly rescued by simultaneous knockdown of PUMA.

(B) As in panel A except using ATG12 knockdown to inhibit autophagy.

(C) MDA MB 231 cells display sensitization to both TRAIL and Staurosporine-induced cell death by autophagy inhibition with ATG12 knockdown. This potentiation is inhibited for TRAIL-induced and Staurosporine-induced death by PUMA knockdown.

Supplemental Movies:

Movie S1. Inefficient MOMP followed by cellular recovery and division in PUMA knockdown cells treated with TRAIL. The movie starts with the fluorescence image one frame before MOMP, which is seen as dissolution of the punctate mitochondrial staining. Note that after slow contraction the cell recovers normal morphology and divides. Isolated frames from this movie are shown in Figure 3B

Movie S1. Inefficient MOMP followed by cellular recovery and division in PUMA knockdown cells treated with TRAIL. Another example of MOMP, followed by slow contraction and cellular recovery and division in TRAIL-treated PUMA knockdown cells. Isolated frames form this movie are shown in Figure 3C.

Supplemental Experimental Procedures.

siRNA and shRNA experiments.

SMARTpool:ON TARGET PLUS human ATG5, ATG7, ATG12 and Beclin1 siRNAs were obtained from Thermoscientific (USA) and transiently transfected using T*rans*-IT-LT1. pLKO.1 shRNA lentivirus constructs were obtained from the University of Colorado Cancer Center Functional Genomics Shared Resource: pLKO.1 human shATG5: Target sequence: CCTTTCATTCAGAAGCTGTTT; ID TRCN0000151474. pLKO.1 human shATG7: Target sequence: GCCTGCTGAGGAGCTCTCCAT; ID TRCN0000007584. pLKO.1 human shATG12: Target sequence: CCAAGGACTCATTGACTTCAT; ID TRCN0000007392 and target sequence: TGGAACTCTCTATGAGTGTTT; ID TRCN0000007394. pLKO.1 human shBBC3 (PUMA): Target sequence: CGTGAAGAGCAAATGAGCCAA; ID TRCN0000033609 and target sequence: GTACAATCTCATCATGGGACT; ID TRCN0000033610. pKLO.1 human BECN1: Target sequence: CCCGTGGAATGGAATGAGATT; ID TRCN0000033549**.** pLKO.1 human shBID: Target sequence: CCTCCAAAGCTGTTCTGACAA; ID TRCN0000062708. pLKO.1 human shPIK3C3 (VPS34): Target sequence: GAGATGTACTTGAACGTAATG; ID TRCN0000196840. pLKO.1 human shPmaip1 (NOXA): Target sequence: CTCAGGAGATTTGGAGACAAA; ID TRCN0000338863. pKLO.1 human shSQSTM1 (p62): Target sequence: CGAGGAATTGACAATGGCCAT; ID TRCN0000007234. Lentiviral particles were produced by co-transfecting HEK293FT cells (Clontech, Mountain View, CA, USA) with the structural plasmids Rev, RRE and VSVG along with pKLO.1 shRNA lentivirus, pLJMGFP-3xNLS-puro and pLJMmCherry-3xNLS-puro constructs using T*rans*IT-LT1.

Q RT-PCR.

Total RNA was extracted from cells using an RNeasy kit (Qiagen). The RNA concentration was analyzed by spectral absorption method and 0.5-1.0 µg of the total RNA was used to prepare template cDNA (RT^2 First Strand Synthesis kit, Qiagen). cDNA was subjected to PCR reaction with SYBR Green PCR Master Mix (Applied Biosystems, Life Technologies). Primers for PUMA detection were Forward TCAACGCACAGTACGAGCG, Reverse AAGGGCAGGAGTCCCATGAT, 18s RNA (used for normalization) primer sequences were Forward GCCGCTAGAGGTGAAATTCTTG and Reverse CTTTCGCTCTGGTCCGTCTT.