SUPPLEMENTARY FIGURE LEGENDS

Figure S1

Ambra1 degradation during apoptosis execution. (**a**) Total RNA was prepared from cells incubated with 2 μM staurosporine for 8 hrs and quantitative RT-PCR was performed using specific primers; the Ambra1 fold induction was normalized with ribosomal L34 mRNA levels (data represent average and SDs of three independent experiments ($P<0.05$); (**b-d**) A373, HeLa and Jurkat cells were treated or untreated with staurosporine for indicated time points and Ambra1, PARP fragment and Beclin1 protein levels were evaluated by western blotting (N = 3). (**e**) Mouse thymocytes were exposed 8 hrs to 100 ng/ml of anti-CD95 agonistic Ab (CD95) and Ambra1 and Beclin1 protein levels together with PARP fragment were evaluated by western blotting. Representative results from 1 of 7 mice are reported. Gapdh was used as loading control in all reported experiments (b-e).

Figure S2

Ambra1 down-regulation confers susceptibility to apoptotic cell death. (**a-b**) Ambra1 expression was down-regulated in 2F cells by RNA interference using specific oligonucleotides (siAmbra#1 and siAmbra#2; scrambled oligonucleotides was used as negative control, siCtrl). Cells were incubated with staurosporine (2 μM, 6 hrs) or etoposide (5 μg/ml, 24 hrs) and cell death was measured by FACS analysis of sub-G1 DNA content (a and b, respectively); (**c,d**) Colony formation assay of Ambra1 down-regulated cells treated with staurosporine. (**c**) Ambra1 expression was down-regulated in 2F cells by RNA interference using the siAmbra#2 oligonucleotides. Total RNA was prepared and quantitative RT-PCR was performed using specific primers. Ambra1 level was normalized to ribosomal L34 mRNA (**d**) Cell survival of staurosporine-treated 2F cells transfected as described in (c) was analysed by colony formation assay. Cells were

exposed for 6 hrs to staurosporine, plated at limiting dilution concentration and cultured for two more weeks. Cell colonies were stained with 5% Giemsa, as described in the Materials and Methods section, and counted. Data expressed as percentage of survival colony in staurosporine treated versus untreated condition, represent the average and SDs of three independent experiments ($P<0.05$). (**e-f**) Ambra1 and/or ATG7 expression were down-regulated in 2F cells by RNA interference using specific oligonucleotides (siAmbra#2 and siATG7; scrambled oligonucleotides was used as negative control, siCtrl). Total RNA was prepared and quantitative RT-PCR was performed using specific primers. Ambra1 or ATG7 fold induction was normalized with ribosomal L34 mRNA levels. Data represent average and SDs of three independent experiments ($P<0.05$). Cells were incubated with staurosporine (2 μM, 6 hrs) and cell death was measured by FACS analysis of sub-G1 DNA content (**f**).

Figure S3

Ambra1 protects cells from death. 2F cells were infected with a retroviral vector encoding β-Gal, Flag-Ambra1-WT or Flag-Ambra1-D482A mutant. Protein extracts were analysed by western blot using anti-Flag (Ambra1) or anti-Gapdh antibodies (**a**). Cells were exposed for 6 hrs to staurosporine, plated at limiting dilution concentration and cultured for two more weeks. Cell colonies were stained with Giemsa and counted. Data expressed as percentage of survival colony of staurosporine treated versus untreated condition, represent average and SDs, respectively, of three independent experiments ($P<0.05$) (**b**).
(**c**) Ambra1 WT/D482A subcellular localization was monitored by confocal analysis using anti-Flag with respect to the endoplasmic reticulum compartment which was visualized using an anti-ERp57 antibody. Images showing the merge of the two fluorescence signals are shown on the right panels. Representative images are reported. Scale bars, 8 μm.

Figure S4

Ambra1 degradation by caspases. Radioactively labelled recombinant Ambra1 was subjected to an in vitro caspase cleavage assay by using 300 (a), 100 (b) or 30 (c) ng of recombinant active caspase-3, -6, -7 or -8. Samples were subjected to SDS-PAGE and protein fragments visualized by autoradiography (N = 3).

Figure S5

Subcellular localization of Ambra1 C-terminal fragment. 2F cells were infected with a retroviral vector encoding the full length Flag-Ambra1 (Ambra1-WT) or the 482-1300 C-terminal fragment (Ambra1-CT). Ambra1 subcellular localization was monitored by confocal analysis by using anti-Flag antibody with respect to the endoplasmic reticulum compartment visualized by using an anti-ERp57 antibody (**a**) and the mitochondrial compartment visualized by using a respiratory chain complex V antibody (ATP synthase) (**b**). Images showing the merge of the two fluorescence signals are shown on the right panels. Representative images are reported. Scale bars, 8 μm.

Moreover, Protein extracts were analysed by western blot using anti-Flag (Ambra1) or anti-Gapdh antibodies (**c**). Cells were incubated with staurosporine (2 μM, 6 hrs) and cell death was measured by FACS analysis of sub-G1 DNA content (**d**). Data represent average and SDs of three independent experiments ($P<0.05$).