Supplementary Figure 1

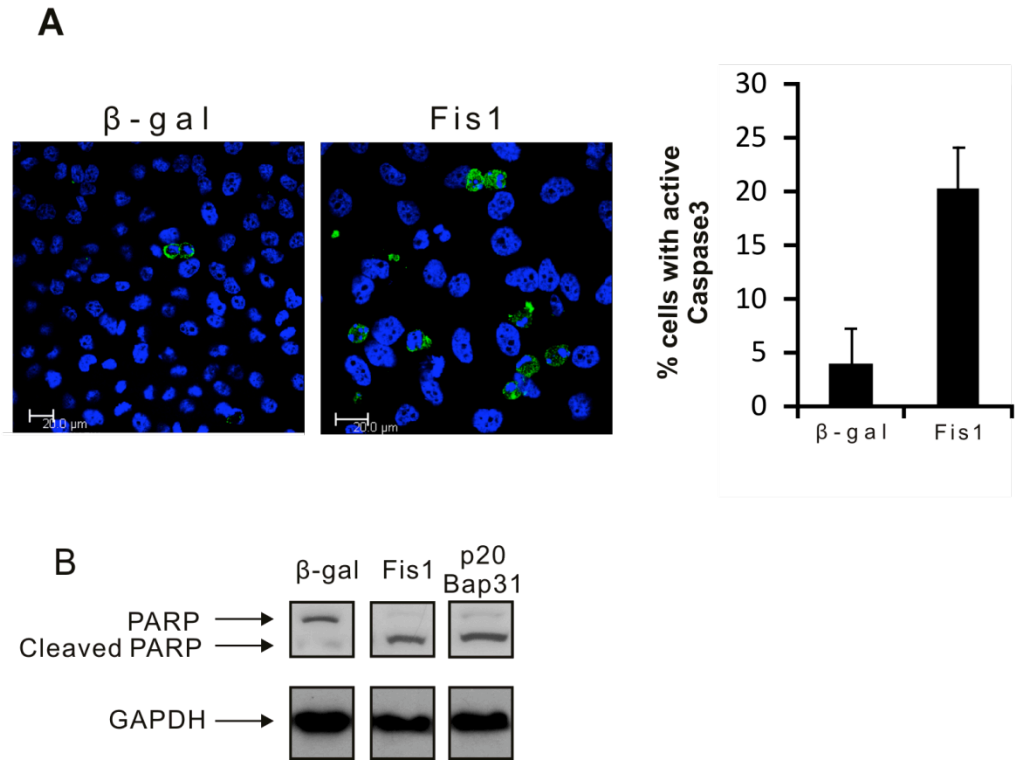

Fig. S1. Fis1 overexpression results in activation of procaspase-3 and cleavage of PARP. (**A**) HeLa cells were transfected either with a β-gal or Fis1 expression vector and the activation of procaspase-3 was determined after 48 hours post-transfection using an antibody specific for active-caspase-3 by immunofluorescence (left panel) and FACS (right panel). Scale bar indicate 20μm. (**B**) HeLa cells were transfected with the indicated genes and harvested 48 hours post-transfection. The cleavage of PARP was determined by immunoblotting. Note that all panels come from the same membrane with the same exposure time.

Supplementary Figure 2

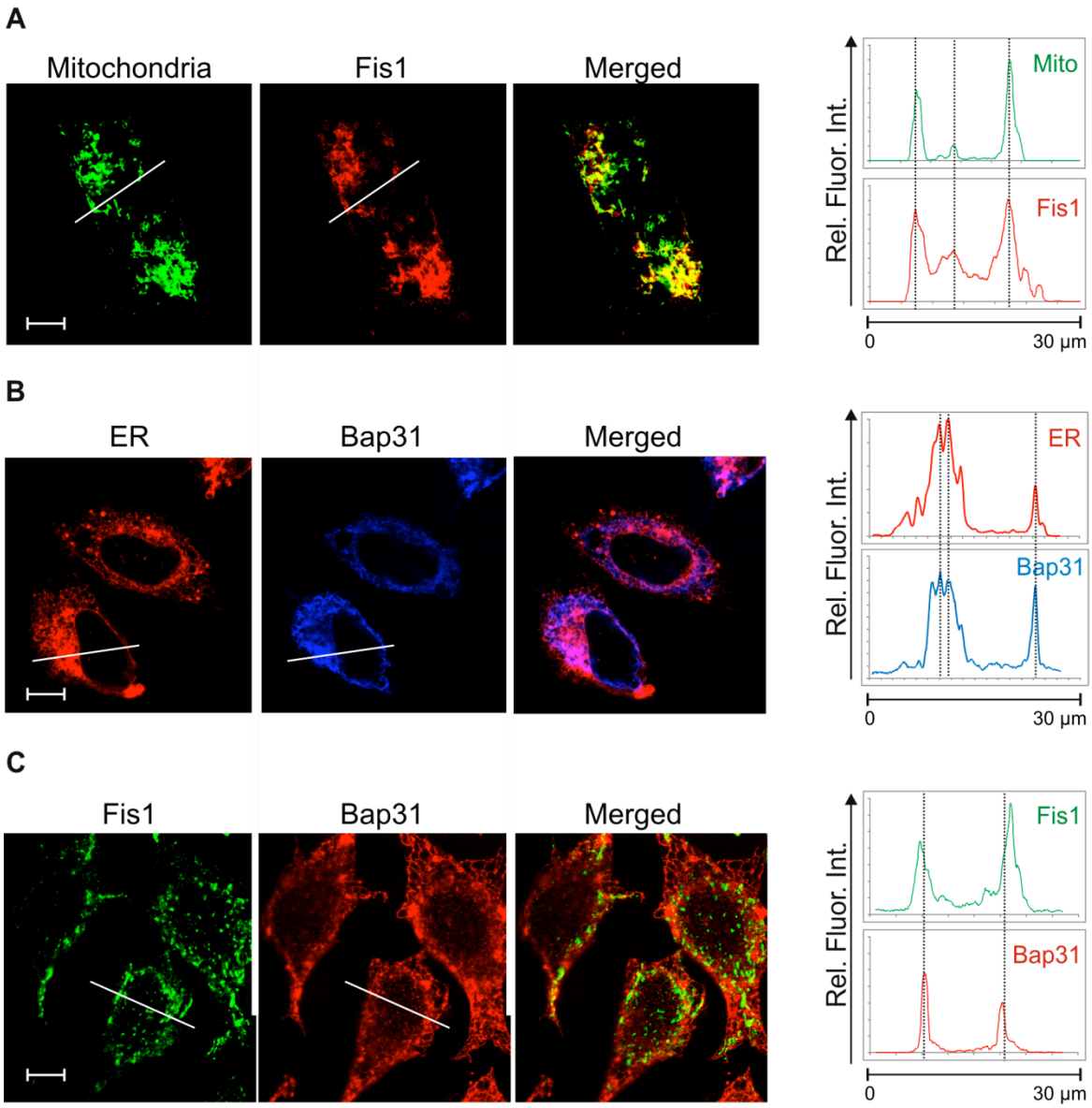

Fig. S2. Fis1 and Bap31 co-localize. HeLa cells were transfected with plasmids encoding Fis1-HA, Bap31-myc, DsRed-ER (specific for the endoplasmic reticulum) and YFP-mito (to stain mitochondria). The subcellular localisation of the proteins and the cellular organelles were detected using confocal microscopy. Distribution of the fluorescent signals of the mitochondrial YFP fluorescence (green) and Fis1 (red) (**A**), of the endoplasmic reticulum (red) and Bap31 (blue) (**B**), and of Fis1 (green) and the Bap31 (red) (**C**) are shown. Relative fluorescence intensity plots of representative sections (white lines) are shown in the right hand panels. Scale bars represent 10µm.

Supplementary Figure 3

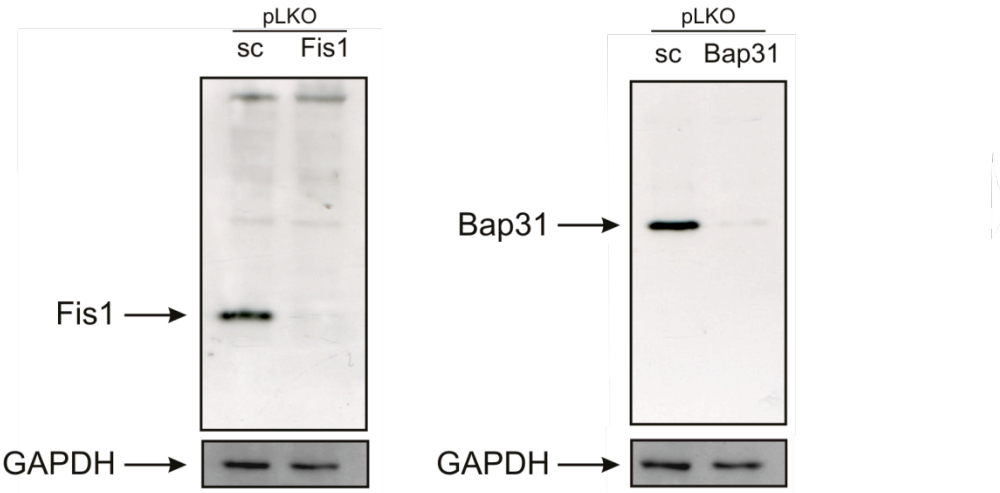

Fig. S3. HeLa cells were transduced with lentiviral shRNA particles to down-regulate Fis1 or Bap31. Total cell lysates were collected after six days of transduction (post 72 hours puromycin selection) and separated by SDS-PAGE. SC: scramble control cells.

Supplementary Figure 4

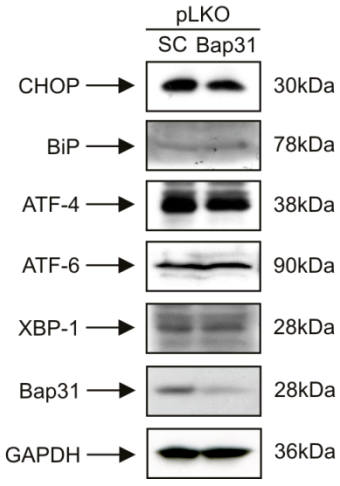

Fig. S4. Downregulation of Bap31 does not alter the expression level of ER stress markers. The whole cell lysates of HeLa cells stably downregulated for Bap31 (pLKO Bap31) or the control cell line (pLKO SC) were harvested, separated by SDS-PAGE and probed with the indicated antibodies.

Supplementary Figure 5

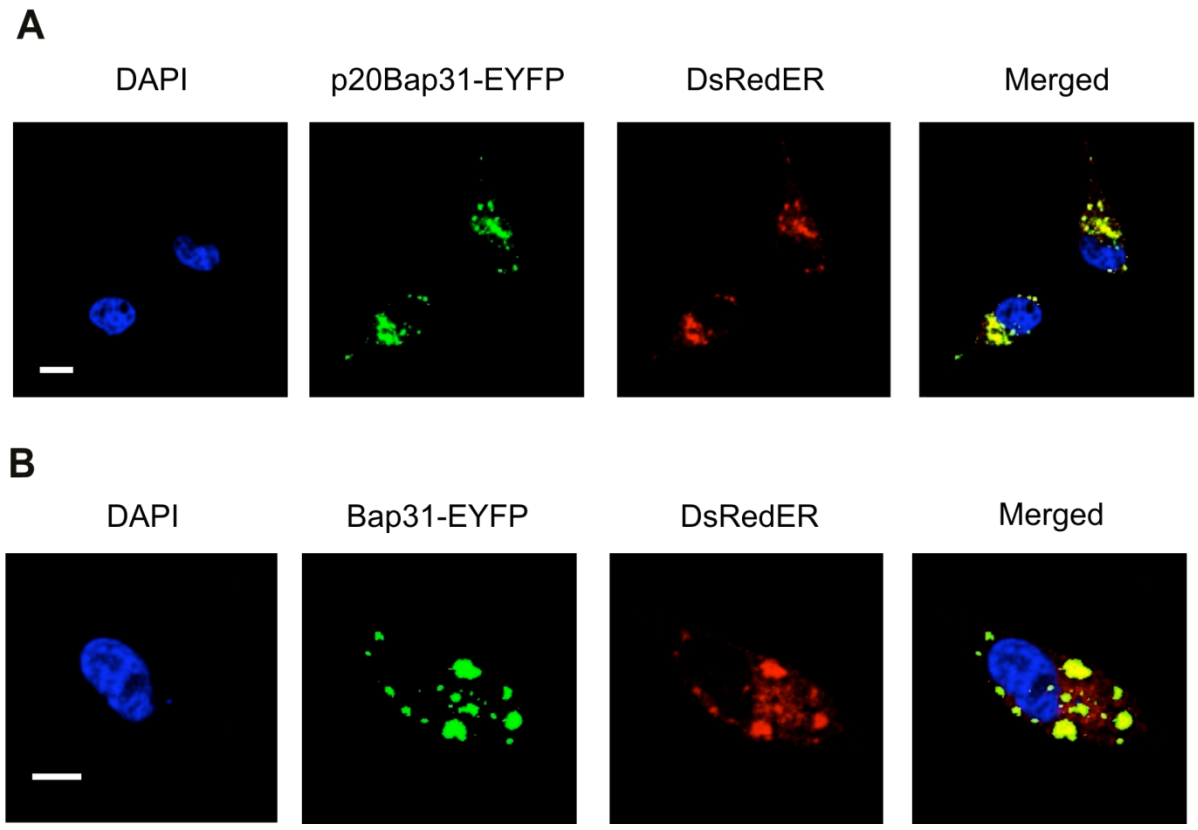

Fig. S5. p20Bap31 and Bap31 localize to the ER. HeLa cells were transfected with p20Bap31-EYFP and DsRed-ER (**A**) or Bap31-EYFP and DsRed-ER (**B**). Localization of the proteins was determined 24 hours post-transfection by direct fluorescence. All scale bars indicate 10μm.

Supplementary Figure 6

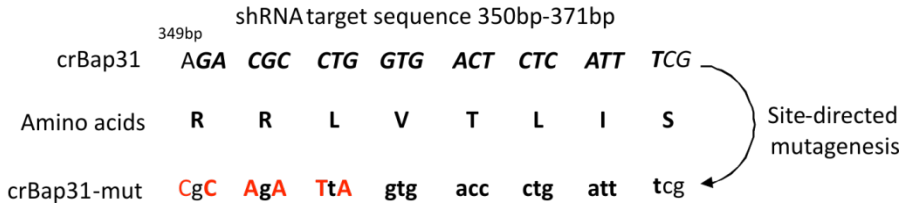

Fig. S6. Schematic diagram to explain the site-directed mutagenesis of crBap31. A site-directed mutagenesis was performed on the mammalian expression vector coding for a caspase cleavage-resistant Bap31 (crBap31), which harbours two mutations at D164A and D238A, to introduce six silent mutations (indicated as red) so that the lentiviral shRNAs expressed in pLKO Bap31 cells (indicated as bold letters) is unable to target this construct.

Supplementary Figure 7

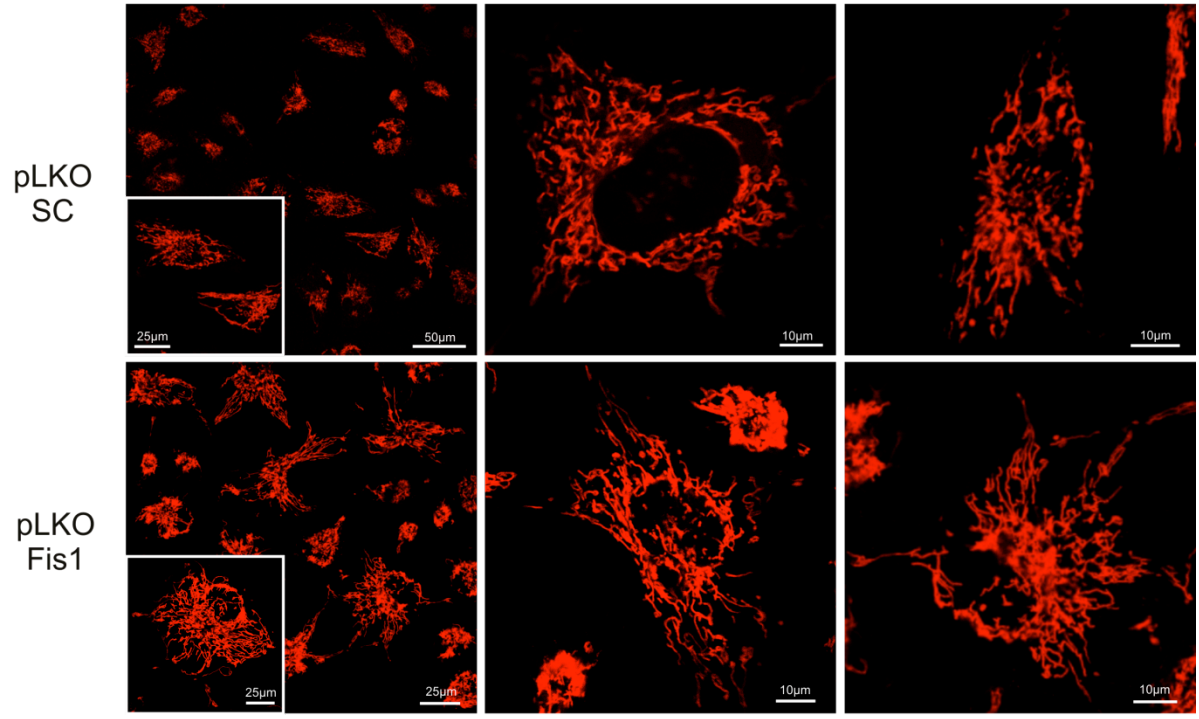

Fig. S7. Effect of Fis1 downregulation on the mitochondrial network. Endogenous Fis1 was stably downregulated with lentiviral shRNA particles. Mitochondria from control cell line (pLKO SC) and Fis1 knock-down cells (pLKO Fis1) were analysed with confocal microscopy using the mitochondrial dye TMRE. One representative field (left panels) and two representative cells (right panels) are shown for each cell line.

Supplemental Experimental Procedures

Immunofluorescence

HeLa cells were transfected and grown for 24 hs, fixed in paraformaldehyde, and blocked with BSA in TBS with 0.02% saponine (TBSS). Cells were then incubated with the primary antibody. Mouse monoclonal anti-HA antibody (Sigma) and rabbit polyclonal anti-myc antibody (Santa Cruz Biotechnology) were used at a dilution of 1:100 in TBSS. Cells were rinsed and incubated with the secondary anti-mouse Alexa Fluor 633 antibody or the anti-rabbit Alexa Fluor 405 antibody (Molecular Probes, Invitrogen) at a dilution of 1:500 in TBSS. Cells were washed and incubated with DAPI (2ug/ml, Invitrogen). p20Bap31-EYFP, Bap31-EYFP, YFP-mito and DsRed-ER were observed by direct fluorescence. Expression vectors for DsRed-ER and YFP-mito were from Dr Remy Sadoul (Inserm U836, Grenoble, France). Tetramethylrhodamine ethyl ester (TMRE, Invitrogen) was used to stain mitochondria. Cells were observed by Leica TCS SP5 confocal laser-scanning microscope. HCX PL APO lambda blue 63x/1.4 oil immersion lens was used for the acquisition. Data were acquired with LAS AF software and analysed with LAS AF lite software.

Active caspase-3 was determined using the rabbit polyclonal anti-cleaved caspase-3 Asp175 at a dilution of 1:200 (Cell Signaling). Anti-rabbit Alexa Fluo 488 was used at a dilution of 1:500 (Molecular Probes, Invitrogen) for the analysis by both confocal microscopy and flow cytometry.

Immunoblotting. HeLa cells were transfected as indicated, lysed in RIPA buffer and the total cell lysates subjected to separation by SDS-PAGE. The immunoblotting procedure was followed as described in materials and methods. Antibodies used were: rabbit anti-PARP (cell signalling); goat α-BiP ,mouse anti-GAPDH, and goat α-XBP-1 (Santa Cruz Biotechnology); mouse α-CHOP and goat α-ATF-4 (Abcam); rabbit Bap31 (Proteintech); rabbit α-ATF-6 (Imagenex); rabbit HRP (Sigma); and mouse HRP (Invitrogen). Rabbit Fis1 antibody was produced in our laboratory.