Supplemental Figure 1. siRNA knock down of BIK in H1299 cells inhibits p53-induced release of Ca^{2+} from ER and fission of mitochondria. (A) H1299 cells were transfected with the indicated BIK siRNAs, infected with Ad p53 for 14 h, and analyzed for BIK and p53 expression by SDS-PAGE and immunoblotting using the indicated antibodies. (B) As in (A), except that Ca^{2+} levels in the ER were measured as described in Fig. 2A, and reported relative to the values obtained with control Ad vector, with the standard deviation indicated. (C) As in (A), except that mitochondrial fission was measured as described in Fig. 2D.

Supplemental Figure 2. Ad HA-BIK infected H1299 cells that exhibit release of cytochrome c to the cytoplasm also exhibit mitochondrial fragmentation. The analysis was conducted as described in Fig. 2B and D. Shown are representative images of the same field in which cells were stained with antibody against the mitochondrial marker TOM20 and against cytochrome c. Detection of diffuse (cytoplasmic) staining with anti-cytochrome c (*asterisk*) was associated with fragmented mitochondria.

Supplemental Figure 3. Noxa induces apoptosis only at late time points. (A) Induction of caspase activity after 48 hours infection with Ad Noxa. H1299 cells were infected with Ad Noxa or a control Ad vector (Ad Cre) for the indicated times after which cells were collected and analyzed for caspase-3-like activity using the fluorescent substrate DEVD-AMC. Shown are the results of 3 independent determinations ± S.D. (B) Absence of mitochondrial fragmentation by human Noxa at early time points. H1299 cells were infected with the indicated Ad vectors for 10 hours in presence of zVAD-fmk. Cells were fixed, stained with the mitochondrial marker TOM20 and the percentage of cells with mitochondrial fission was determined. Shown are the results of three independent

experiments ± S.D. (C) Human Noxa does not induce Ca^{2+} release from the ER. ER-Ca^{2+} content was determined as in Fig. 3 and the results were expressed as the difference in the 510 nm ratio before and after addition of TG. Shown are the results of at least 3 independent determinations ± S.D. BN = BIK + Noxa

Video1. Dynamic stretching and contraction of mitochondrial fragments in Ad HA-BIK-treated cells. H1299 cells were transfected with pOCT-YFP to visualize mitochondrial matrix and treated for 12 hours with Ad HA-BIK in the presence of zVAD-fmk. Frames were taken every two seconds. Images representing individual frames are shown in Fig. 3A.

Video2. CFP-DRP1(K38E) blocks BIK-induced dynamic stretching and contraction of mitochondria. H1299 cells were transfected with CFP-DRP1(K38E) as well as pOCT-YFP (to visualize mitochondrial matrix) and treated for 12 hours with Ad HA-BIK in presence of zVAD-fmk. Frames were taken every two seconds. Images representing individual frames are shown in Fig. 3B.

Video3.

DRP1-YFP remains associated with mitochondria undergoing morphological changes. COS-7 cells co-transfected with pOCT-CFP (red) and DRP1-YFP (green) were infected with Ad BIK for 8 hours in the presence of zVAD-fmk. Frames were taken every 4 seconds over 10 min.