

Legends for Supplemental figures Wang et. al.

Fig. S1. PARP cleavage and zVAD-fmk inhibition of PARP cleavage. (A) PARP cleavage after TNF α treatment. HeLa cells were treated with 50 ng/ml TNF α for 6 hours, harvested and western blotted with a PARP antibody. (B) PARP cleavage induced by STS was inhibited by broad based caspase inhibitor zVAD-fmk. Cells were treated for the indicated times in the presence of 50 μ M zVAD-fmk, where indicated, harvested and western blotted with a PARP antibody.

Fig. S2. Treatment of cells with latrunculin A disrupts cell cytoskeleton. HeLa cells were treated with 5 μ M latrunculin A (LA) for 1 hour, and the cells were observed under a phase microscope. Similarly to the STS-treated cells, LA-treated cells rounded up, indicating disruption of the cytoskeleton.

Fig. S3. Reduction of CAP1 level in CAP1-knockdown cells as detected by immunofluorescence. HeLa WT cells and CAP1 knockdown cells (S3-2) were labeled with mitotracker, cells were fixed and stained with anti-CAP1 antibody as in Fig. 2 and detected using an Alexa Fluor 350 goat anti-mouse IgG (H+L). The cells were observed for mitochondria staining and CAP1 level. CAP1 staining in S3-2 cells is significantly reduced.

Fig. S4. Translocation of Bak to mitochondria after STS treatment. NIH 3T3 cells were treated with STS for 3 hours to stimulate apoptosis. Cells were incubated with mitotracker, fixed, stained with Rabbit anti-Bak (Sigma) and Goat anti-rabbit Alexa Fluor 488. Cells were observed under confocal microscopy.

Fig. S5. Knockdown of cofilin did not prevent CAP1 translocation to mitochondria upon STS stimulation. HeLa cells grown on 100 mm plates were transfected with cofilin shRNA or a scrambled control using lipofectamine. 72 hours after transfection, the cells were treated with 1 μ M STS for 2 hours. The mitochondrial and cytosol fractions were collected and western blotted to determine CAP1 translocation to mitochondria. The cofilin knockdown efficiency was typically 50-75% in our experiments.

Fig. S6. Treatment with STS does not affect CAP1 association with actin. 293T cells were transfected with a plasmid expressing a 6xHis tagged CAP1. After 24 hours, the cells were treated with 1 μ M STS for 2 hours as indicated, harvested and the fusion protein was purified using nickel beads. After elution with a buffer containing imidazole, the samples were resolved on a SDS gel and stained using the Colloidal Blue Stain Kit (Invitrogen). The (His)CAP1 and actin bands are indicated with arrow and arrowheads, respectively.

Fig. S7. Reduction of cytochrome *c* release to cytosol in CAP1-knockdown cells detected by immunofluorescence. HeLa WT or CAP1 KD (S3-2) cells were incubated with mitotracker for 1 hour followed by treatment with 100 μ M etoposide for 24 hours. Cells were fixed, and stained with cytochrome *c* antibody and goat anti-rabbit Alexa Fluor 488. Cells were observed under fluorescence microscopy.