

Description of Additional Supplementary Files

File Name: Supplementary Movie 1

Description: *Live fluorescence imaging of HeLa cells stably expressing Apaf1- GFP, treated with ABT-737.* Movie showing Apaf1-GFP (green) foci formation followed by cell shrinkage and death. Mitochondria were stained with MitoTracker DeepRed (magenta). Image acquisition time (h:min) after ABT-737 treatment is indicated at the top left. Imaging frame rate: 15 minutes. Scale bar: 10 μ m

File Name: Supplementary Movie 2

Description: *Live fluorescence imaging of a HeLa cell stably expressing Apaf1- GFP, treated with ABT-737.* Movie showing Apaf1-GFP (green) foci formation and disassembly in an apoptotic cell. Mitochondria were stained with MitoTracker DeepRed (magenta). Image acquisition time (h:min) after ABT-737 treatment is indicated at the top left. Imaging frame rate: 15 minutes. Scale bar: 10 μ m.

File Name: Supplementary Movie 3

Description: *Live fluorescence imaging of a HeLa cell stably expressing Apaf1- GFP, treated with ABT-737 and QVD.* Movie showing Apaf1-GFP (green) foci formation and disassembly in apoptotic cells. Mitochondria were stained with MitoTracker DeepRed (magenta). Image acquisition time (h:min) after ABT-737 and QVD treatment is indicated at the top left. Imaging frame rate: 15 minutes. Scale bar: 10 μ m.

File Name: Supplementary Movie 4

Description: *Live fluorescence imaging of a HeLa cell stably expressing Apaf1- GFP, treated with ABT-737 and QVD.* Movie showing two consecutive events of Apaf1-GFP (green) foci formation in an apoptotic cell. Mitochondria were stained with MitoTracker DeepRed (magenta). Image acquisition time (h:min) after ABT-737 treatment is indicated at the top left. Imaging frame rate: 15 minutes. Scale bar: 10 μ m.

File Name: Supplementary Movie 5

Description: *Electron tomogram of Apaf1-GFP in resin-embedded cell.* Movie through virtual slices of the electron tomogram shown in Figure 3B, obtained by CLEM on resinembedded HeLa cells expressing Apaf1-GFP, treated with ABT-737 and QVD. Scale bar: 200 nm.

File Name: Supplementary Movie 6

Description: *Electron tomogram of Apaf1-GFP in resin-embedded cell.* Movie through virtual slices of the electron tomogram shown in Figure 3D, obtained by CLEM on resinembedded HeLa cells expressing Apaf1-GFP, treated with ABT-737 and QVD. Scale bar: 200 nm.

File Name: Supplementary Movie 7

Description: *Live fluorescence imaging of a HeLa cell transiently expressing Apaf1-SNAP, treated with ABT-737 and QVD.* Movie showing Apaf1-SNAP647 (magenta) foci formation and disassembly in apoptotic cells. Mitochondria were stained with MitoSpy green (green). Image acquisition time (h:min) after ABT-737 and QVD treatment is indicated at the top left. Imaging frame rate: 15 minutes. Scale bar: 10 μ m.

File Name: Supplementary Movie 8

Description: *Cryo-electron tomogram of Apaf1-GFP in vitrified, cryo-FIB-milled cell.* Movie through virtual slices of the cryo-electron tomogram shown in Figure 3G, obtained by preFIB milling cryo-CLEM of HeLa cells expressing Apaf1-GFP, treated with ABT-737 and QVD. Scale bar: 100 nm.

File Name: Supplementary Movie 9

Description: *Cryo-electron tomogram of Apaf1-SNAP647 in vitrified, cryo-FIBmilled cell.* Movie through virtual slices of the cryo-electron tomogram shown in Figure 3L, obtained by pre- and post-FIB milling cryo-CLEM of HeLa cells expressing Apaf1-SNAP-tag labelled with SNAP-Cell 647-SiR, treated with ABT-737 and QVD. Scale bar: 100 nm.

File Name: Supplementary Movie 10

Description: *Live fluorescence imaging of a HeLa cells stably expressing Apaf1- GFP, microinjected with cyt c.* Movie showing Apaf1-GFP (green) foci formation and dynamics upon microinjection of cyt c and rhodamine dextran (magenta). Imaging time (h:min) after the start of the microinjection session is indicated at the top left. Imaging frame rate: 10 minutes. Scale bar: 10 μ m.

File Name: Supplementary Movie 11

Description: *Live fluorescence imaging of a HeLa cells expressing Apaf1-DWD40- GFP.* Movie showing Apaf1-DWD40-GFP (green) foci formation and disassembly upon expression in cells. Imaging time (h:min) after the transfection is indicated at the top left. Imaging frame rate: 15 minutes. Scale bar: 10 μ m.