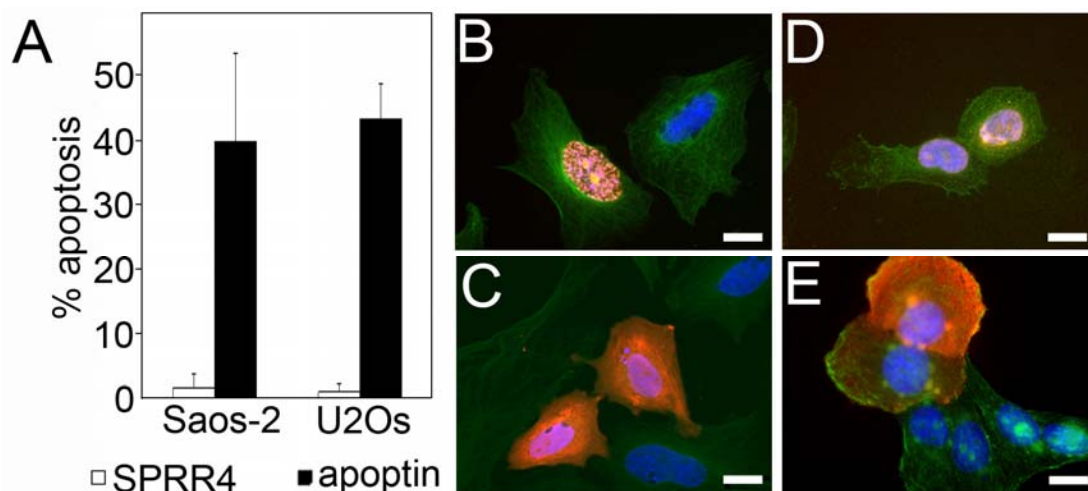


## Supplementary Information:

# Mitotic catastrophe triggered in human cancer cells by the viral protein apoptin

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**Supplementary Figure S1.** Apoptin but not SPRR4 induces apoptosis in osteosarcoma cell lines. Saos-2 and U2Os cells were transfected with plasmid encoding flag-apoptin or flag-SPRR4 (control) and were fixed 72 h or 48 h post transfection (Saos-2 and U2Os respectively). The percentage of apoptosis (represented as means  $\pm$  standard deviation) induced by apoptin and SPRR4 in Saos-2 and U2Os cells was scored by aberrant DNA staining<sup>30</sup>. SPRR4 did not induce apoptosis in these cells compared to apoptin (A). Flag-tagged apoptin or flag-tagged SPRR4 were stained with a flag-specific antibody conjugated to rhodamine (red), endogenous tubulin with a FITC-labeled tubulin-specific antibody (green) and DNA with Hoechst (blue). Apoptin localizes to the nucleus of interphase cells (panels B, D) and SPRR4 to both nucleus and cytoplasm (panels C, E). Saos-2 cells (B, C); U2Os cells (C, E). Scale bar: 20 $\mu$ m.

### **Original movie files corresponding to the film-strips of figure 3:**

**Movie A:** U2Os cells were transiently transfected with plasmids encoding GFP-tubulin (green) and counterstained with Hoechst 33342 (blue). Live cell imaging was performed 40h after transfection on an Olympus IX81 time-lapse microscope equipped with an MT10 lamp (Olympus) and adequate excitation and emission filters (Olympus). Images were captured every 2 min and analyzed with the Cell M software version 3.1 (Olympus). Several U2Os cells transfected solely with GFP-tubulin and dividing normally (1 h to complete mitosis) are shown (= control experiment).

[http://cellobservatory.leidenuniv.nl/videos/CCDis/Lanz\\_2013\\_CCDIs\\_movie\\_A.avi](http://cellobservatory.leidenuniv.nl/videos/CCDis/Lanz_2013_CCDIs_movie_A.avi)

**Movie B:** U2Os cells were transiently transfected with plasmids encoding GFP-tubulin (green) and mCherry-apoptin (red) and counterstained with Hoechst 33342 (blue). Live cell imaging was performed 40h after transfection on an Olympus IX81 time-lapse microscope equipped with an MT10 lamp (Olympus) and adequate excitation and emission filters (Olympus). Images were captured every 2 min and movies were analyzed with the Cell M software version 3.1 (Olympus). Apoptin-positive cells show abnormal spindle formation, complete mitosis but undergo apoptosis during telophase after progression through mitosis has been seriously delayed.

[http://cellobservatory.leidenuniv.nl/videos/CCDis/Lanz\\_2013\\_CCDIs\\_movie\\_B.avi](http://cellobservatory.leidenuniv.nl/videos/CCDis/Lanz_2013_CCDIs_movie_B.avi)

**Movie C:** U2Os cells were transiently transfected with plasmids encoding GFP-tubulin (green) and mCherry-apoptin (red) and counterstained with Hoechst 33342 (blue). Live cell imaging was performed 40h after transfection on an Olympus IX81 time-lapse microscope equipped with an MT10 lamp (Olympus) and adequate excitation and emission filters (Olympus). Images were captured every 2 min and movies were analyzed with the Cell M software version 3.1 (Olympus). An apoptin-positive U2Os cell with apparently normal spindle formation is arrested during metaphase and eventually undergoes apoptosis during the same phase. Untransfected cells complete mitosis with normal kinetics in the same culture.

[http://cellobservatory.leidenuniv.nl/videos/CCDis/Lanz\\_2013\\_CCDIs\\_movie\\_C.avi](http://cellobservatory.leidenuniv.nl/videos/CCDis/Lanz_2013_CCDIs_movie_C.avi)

**Movie D:** Saos2 cells were transiently transfected with plasmids encoding GFP-tubulin (green) and mCherry-apoptin (red) and counterstained with Hoechst 33342 (blue). Live cell imaging was performed 65h after transfection on an Olympus IX81 time-lapse microscope equipped with an MT10 lamp (Olympus) and adequate excitation and emission filters (Olympus). Images were captured every 2 min and movies were analyzed with the Cell M software version 3.1 (Olympus). An apoptin-positive cell undergoes seemingly normal but prolonged mitosis and apoptotic cell death occurs after cytokinesis in the two daughter cells.

[http://cellobservatory.leidenuniv.nl/videos/CCDis/Lanz\\_2013\\_CCDIs\\_movie\\_D.avi](http://cellobservatory.leidenuniv.nl/videos/CCDis/Lanz_2013_CCDIs_movie_D.avi)