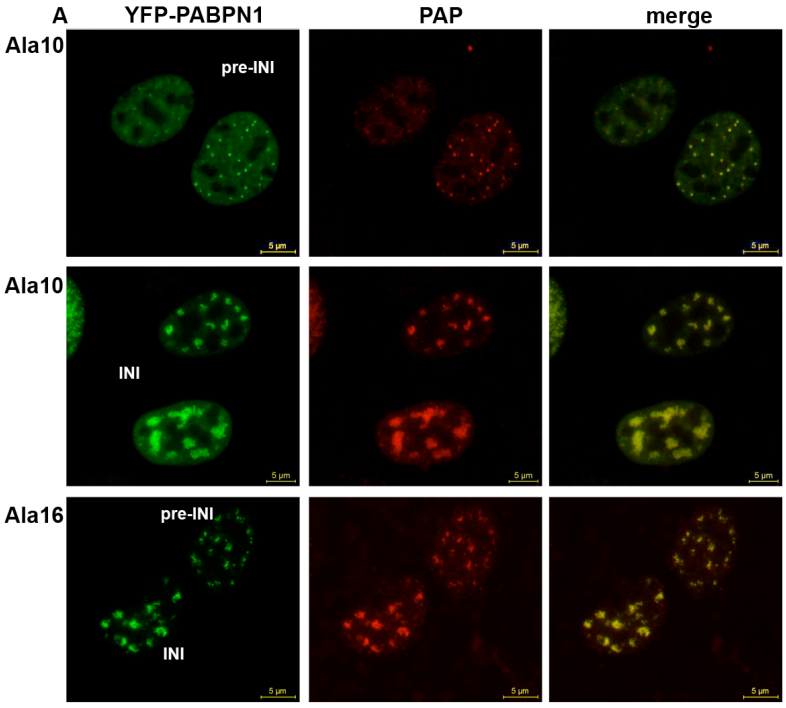
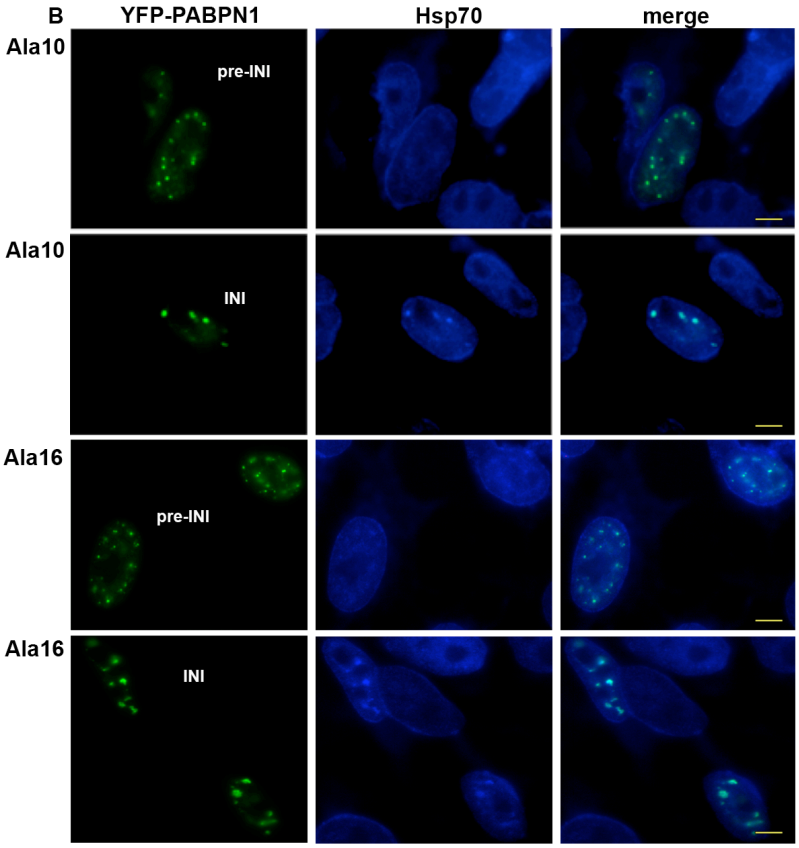
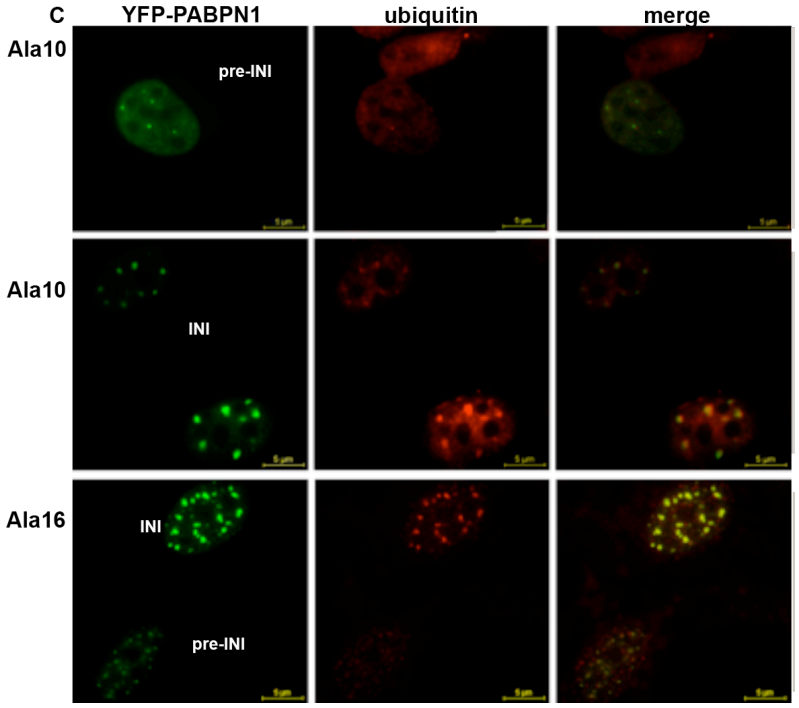
Raz et al.,
Supplementary material

Supplement 1: Co-localization of PAP, HSP70 and ubiquitin with pre-INI and inclusions.



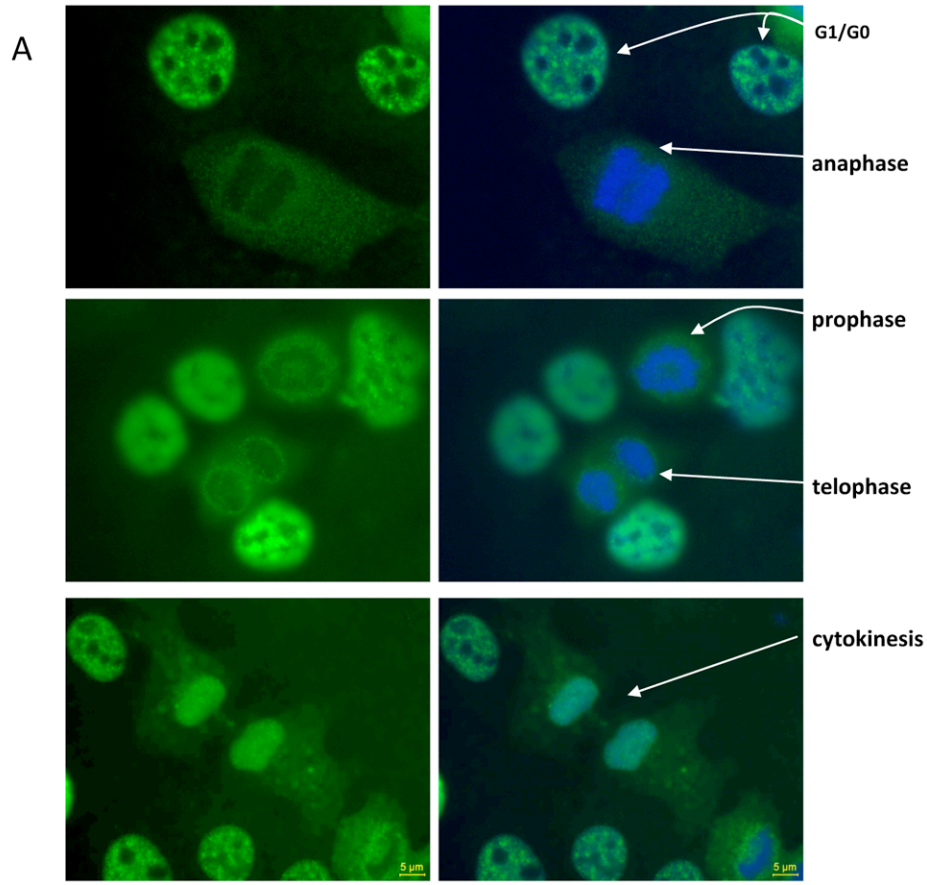




U2OS cells were transfected with YFP-Ala10-PABPN1 or YFP-Ala16-PABPN1 and 30 hours post transfection were fixed. Immuno-labeling was carried our with anti PAP (A);

anti HSP-70 (B) or anti-Ubiquitin (C) antibodies, which were visualized with Alexa 594-conjugated secondary antibody (A;C) or Deac-conjugated (B). Scale bars equal 5 μm. Pre-I are labeled with PAP while inclusions co-localizes with HSP70, PAP and Ubiquitin. Pre-I of PABPN1-Ala16 but not of PABPN1-Ala10 are also labeled with ubiquitin.

Supplement 2: Changes in PABPN1 sub-cellular distribution during mitosis



Changes in PABPN1 subcellular localization during cell division in GFP-Ala10-PABPN1 transfected U2OS cells. Cells were fixed 24 hours post transfection. GFP-PABPN1 is shown in green and DNA was counterstained with DAPI (blue). Selected steps in cell cycle are indicated.

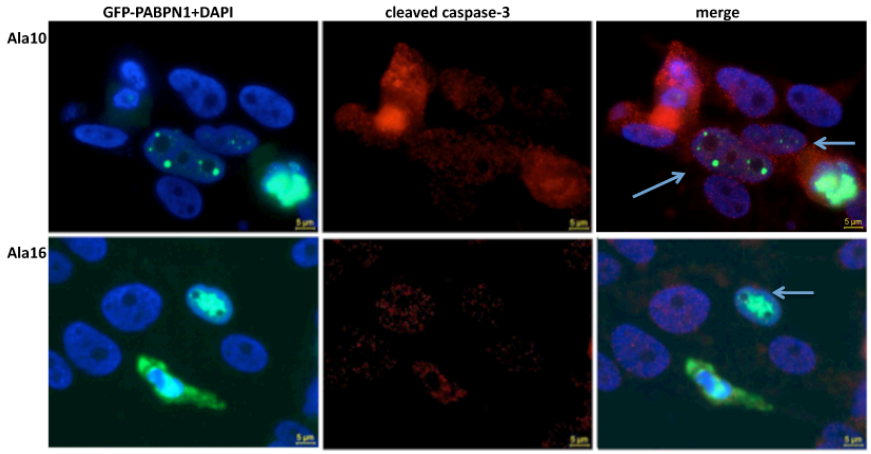
B. Change in PABPN1 aggregation during mitosis

Avi files of time laps imaging of GFP-Ala10-PABPN1 or GFP-Ala16-PABPN1 expressing cells 30 hours post transfection. Images were taken in 30 minutes intervals for 18 hours. Scale bar is indicated. Film shows disassemble of PABPN1 aggregates during cell division.

Supplement 3: pre-I reversion.

Videos of time laps imaging of foci in GFP-Ala10-PABPN1 or GFP-Ala16-PABPN1 expressing cells 30 hours post transfection. Images were taken in 30 minutes intervals for 18 hours. Scale bar is indicated. Film shows reversion in GFP-Ala10-PABPN1 but not in GFP-Ala16-PABPN1 expressing cells.

Supplement 4: Cleaved-Caspase-3 expression in PABPN1 expressing cells



In U2OS cells that showed high overexpression of either Ala10-PABPN1 or Ala16-PABPN1 activation of apoatosis was observed. These cells were excluded from our studies. U2OS cells were transfected with YFP-Ala10-PABPN1 construct, and cells were fixed 40 hours post transfection. Immuno-labeling was carried out with the anti Cleaved-caspase-3 antibodies (R&D systems) visualized with Alexa 594-conjugated secondary antibody.
The cleaved caspase-3 is found in cells with high overexpression of PABPN1, but not in cells expressing PABPN1 2-4 fold over the endogene level (indicated by arrows).