

Figure S1:

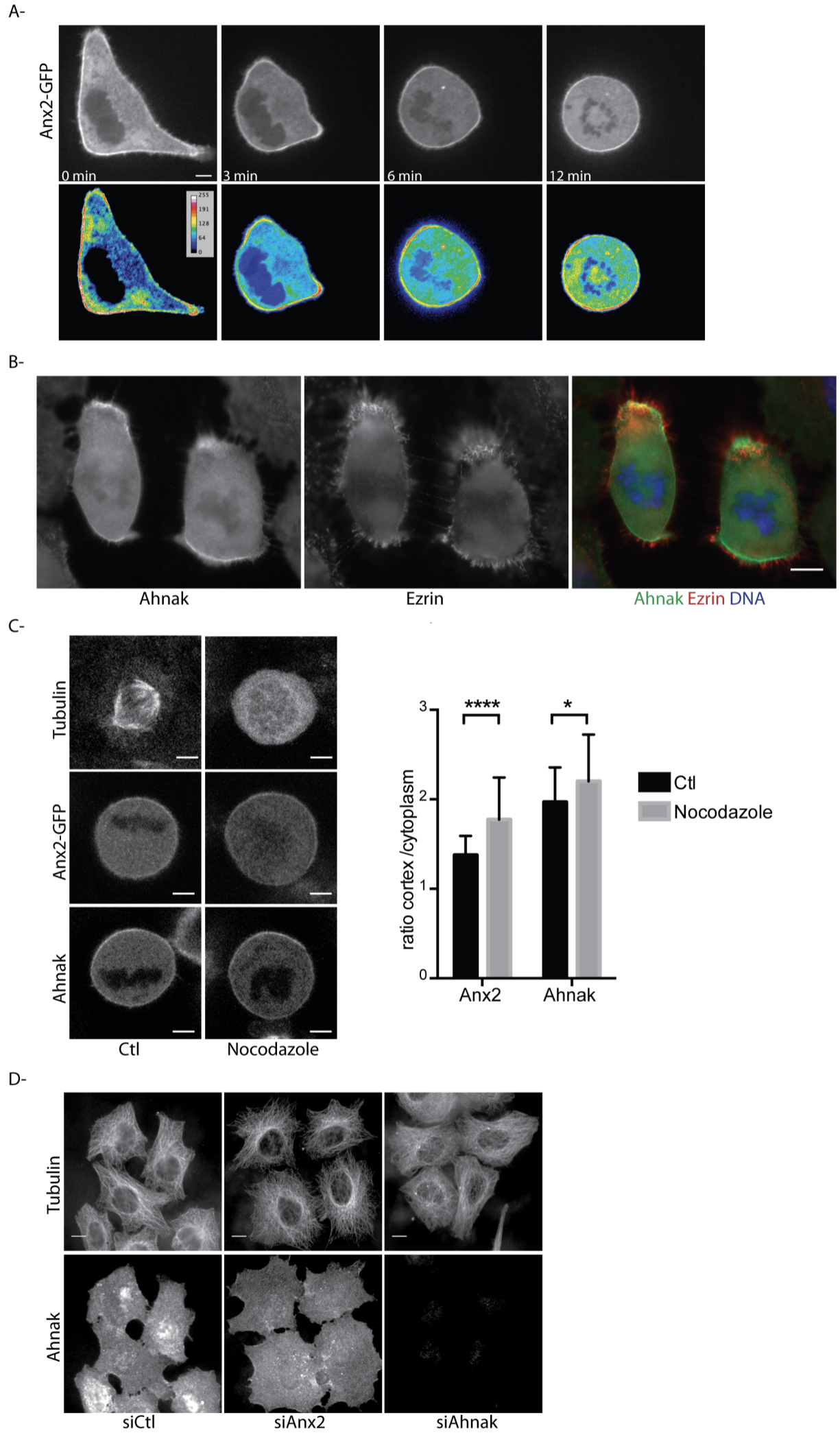


Fig. S1. Adhesion controls cortical distribution of Anx2 and Ahnak. **A-** Live time-lapse images of Anx2-GFP HeLa cells plated on L-shaped micropatterns imaged through early mitotic progression. Grey level (top) and 16 color ramp (bottom) images are shown. Scale bars: 5 μm . **B-** Staining of endogenous ahnak and ezrin protein in rounding prometaphase cells. Scale bars: 10 μm . **C-(left panel)** Confocal images of tubulin, Ahnak and Anx2-GFP (anti-GFP) metaphase cells in presence or absence of 10 μM nocodazole for 1hr. **(right panel)** Quantification of Anx2-GFP and Ahnak cortical intensity (cortex/cytoplasm) in control and nocodazole treated cells. $n>30$; ****, $P < 0.0001$; *, $P = 0,0473$ (Anx2, Student t-test; Ahnak, Mann-Whitney U test). Scale bars: 5 μm . **D-** Immunofluorescent images illustrating the ability of control, Anx2 siRNA and Ahnak siRNA treated cells to adhere and spread onto fibronectin coated coverslips. Cells were stained for α -tubulin and Ahnak. Scale bars: 10 μm . Graphs show means \pm SD from three independent experiments.

Figure S2:

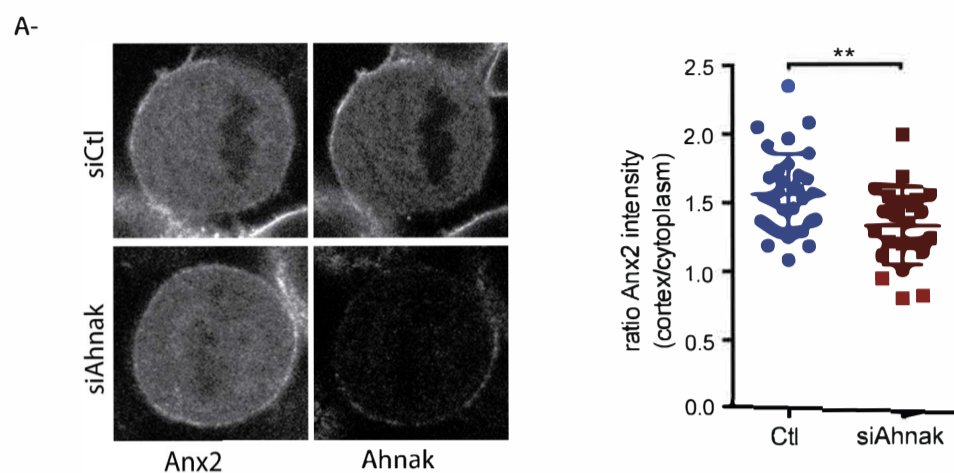


Fig. S2. Influence of Ahnak on cortical Anx2 localization. A-(left panel) Confocal images of Anx2 and Ahnak staining in metaphase HeLa control cells or cells treated with Ahnak siRNA. (right panel) Quantification of Anx2 cortical intensity (cortex/cytoplasm) in control and Ahnak siRNA treated cells. $n > 30$; $P = 0,0042$ (Mann-Whitney U test). Graphs show means \pm SD from three independent experiments.

Figure S3:

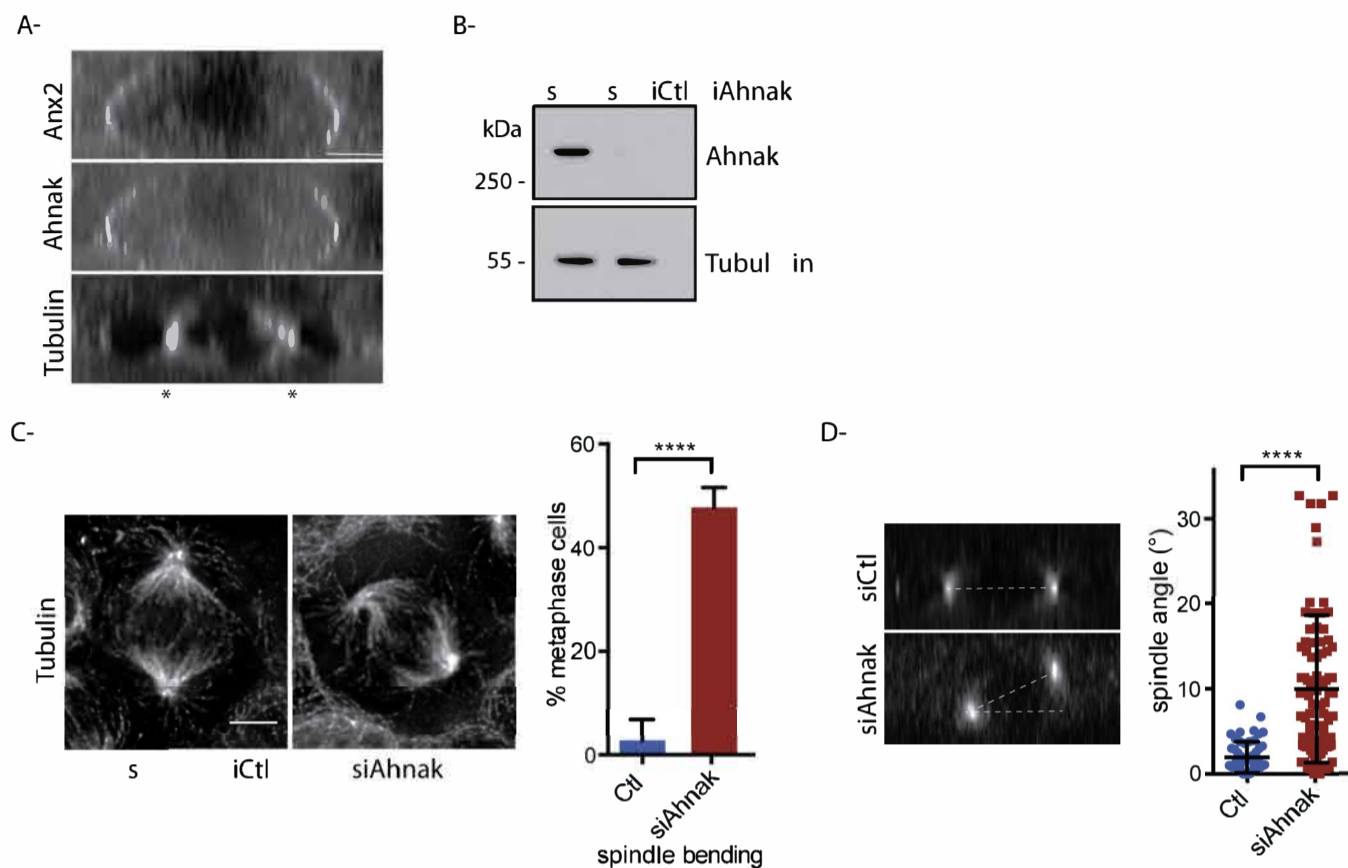
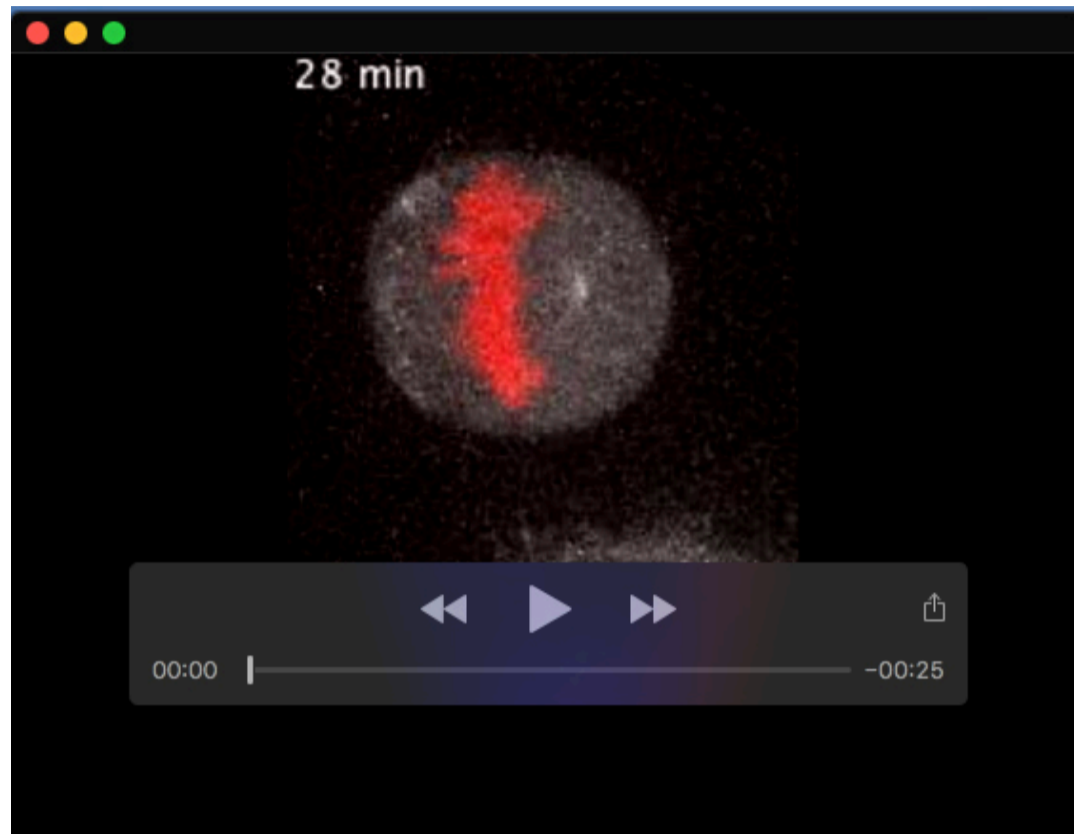
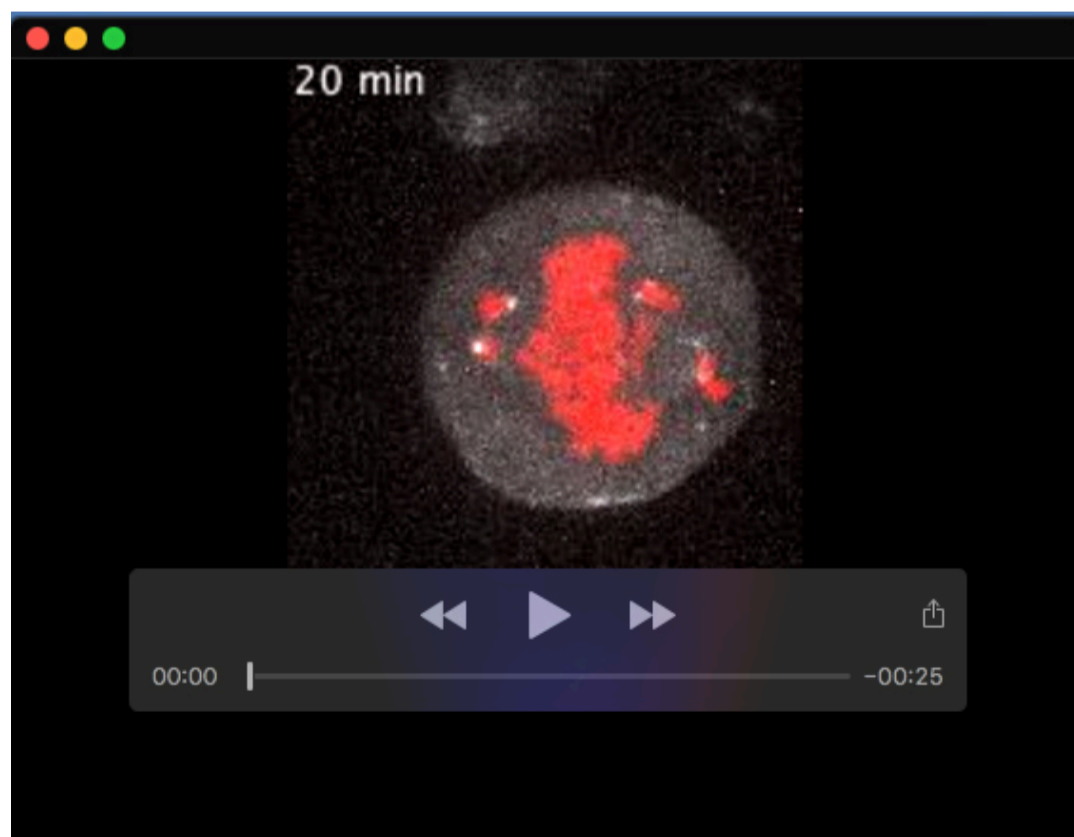


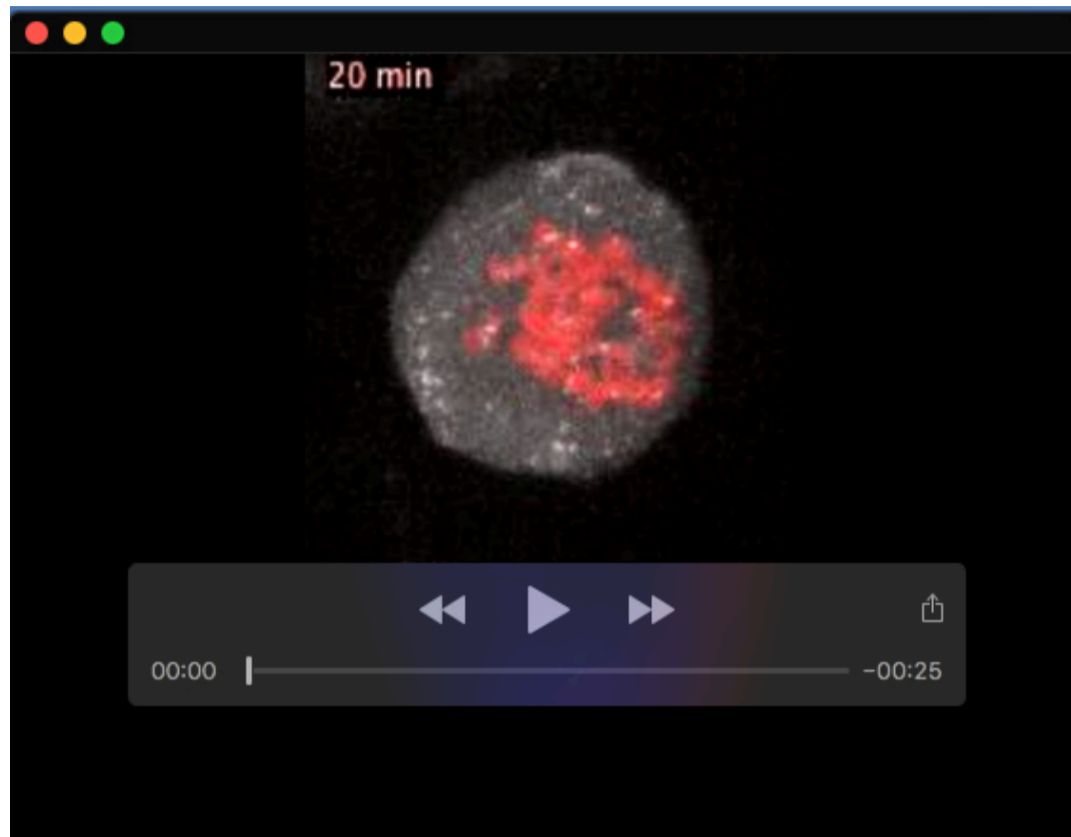
Fig. S3. Anx2 and Ahnak are required for proper spindle positioning in MDCK cells. **A-** x-z projection images of Anx2, Ahnak and tubulin staining in metaphase MDCK cells (stars indicates spindle pole). **B-** Western blot of lysates from control and Ahnak siRNA treated MDCK cells. **C-** (left panel) Deconvoluted images of tubulin staining of mitotic spindles in control and Ahnak siRNA treated metaphase cells. (right panel) Quantification of the percentage of control ($n = 57$) and Ahnak siRNA ($n = 84$) treated mitotic cells displaying spindle bending as illustrated in the left panel. ****, $P < 0.0001$ (Mann-Whitney U test). **D-** (left panel) x-z projection images of centrosome staining in control and Ahnak siRNA treated metaphase cells. (right panel) Quantification of metaphase spindle angles relative to the adhesion plane in control ($n = 55$) and Ahnak siRNA treated cells ($n = 85$) each from three independent experiments. ****, $P < 0.0001$ (Mann-Whitney U test). Graphs show means \pm SD from three independent experiments.



Movie 1. Dynein in control cells. HeLa DynHC-GFP cells expressing H2B-mChery were synchronized at the G2/M transition, released and imaged from prophase to anaphase onset. Dynein is in grey and DNA in red. Images were acquired every 4 min for 40 min. Max projection of Z stack presented. Movie 5 frame per seconds



Movie 2. Dynein in cells invalidated for Ahnak. Experimental conditions identical to movie 1



Movie 3. Dynein in cells invalidated for Anx2. Experimental conditions identical to movie 1