Supplementary Data

Hsp72 and Nek6 cooperate to cluster amplified centrosomes in cancer cells

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SUPPLEMENTARY MATERIAL

Supplementary Figure S1. Protein expression of Hsp72, Hsc70, Nek6 and Nek7 in different cell types following different treatments

A. Western blots of Hsp72, Hsc70 and GAPDH in MDA-MB-231 cells that were mock-depleted or depleted with siRNAs against GAPDH or human Hsp72 (oligo 6 and oligo 7). **B**. Western blots of Hsp72, Hsc70 and GAPDH in NIE-115 cells that were mock-depleted or depleted with siRNAs against GAPDH or mouse Hsp72 (oligo 5 and oligo 7). **C**. Western blots of Nek6, Nek7 and GAPDH in MDA-MB-231 cells that were mock-depleted or depleted with siRNAs as indicated. **D**. Western blots of Nek6, Nek7 and GAPDH in MDA-MB-231 cells that were mock-depleted or depleted with siRNAs against GAPDH, Nek6 or Nek7. **E**. Western blots of Hsp72, Hsc70, Nek6 and GAPDH in PBL and ALL cells. **F.** Western blots of Nek6, Hsp72 and GAPDH in MDA-MB-231 cells that were either untreated (control) or incubated for 4 hours with Aurora-A (AurA) or Plk1 inhibitors. M. wts (kDa) are shown on the left of each panel.

Supplementary Figure S2. Hsp70 inhibition does not block clustering of acentrosomal spindle poles.

A. Time-course for microtubule regrowth assay used in Fig. 4E-G. **B, C.** NIE-115 (B) and HeLa (C) cells were incubated with nocodazole according to the protocol shown in Supp. Fig. S2A before being transferred to nocodazole-free media with or without 10 μM Hsp70i. Cells were fixed at the times indicated and stained with α-tubulin (top panels; red in merge). Merge panels also include centrin-2 (green) and DNA (blue). Scale bars, 5 μm**.**

Supplementary Figure S3. Hsp70 inhibition does not perturb mitotic progression in RPE1 cells

A. HeLa and RPE1 cells were either untreated (control) or treated with Hsp70i as indicated for 4 h before being fixed and stained for phospho-histone H3 (pHH3, red) and DNA (blue). Magnified views of mitotic cells are shown. Scale bar, 50 μm. **B.** RPE1 cells were incubated with SiR-tubulin

for 7 h prior to time-lapse confocal imaging with images captured every 7 mins. Stills are shown of SiR-tubulin alone or merged with brightfield (BF) at the times indicated from control or Hsp70i-treated cells. Scale bar, 20 μm. **C.** Time-lapse imaging was used to follow RPE1 cells in the absence (control) or presence of Hsp70i. Each bar is representative of a single cell with the time spent in each stage of mitosis indicated (see Fig. 2B). **D.** Western blots for the proteins indicated were used to assess relative expression in MDA-MB-231, HeLa, RPE1 and HBL-100 cells. M. wts (kDa) are indicated. **E.** The protein expression of Hsp72 and Nek6 was determined relative to GAPDH for each cell line from D; n=2.