

Supplemental Materials

Molecular Biology of the Cell

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL DATA

Figure S 1 (Related to Figure 6). Hsp70 is recruited to the centrosome during HS and protects it from damage.

- A. Heat shock induces Hsp70 recruitment to centrosomes (middle panel). Pericentrin (red); asterisk enlargement. Hsp70 depletion (shHsp70, right panel) inhibits its centrosome recruitment after HS compared with control (shNT, left panel).
- B. Hsp70 is a bona fide centrosome protein in stressed cells: pre-treatment with Noc does not prevent Hsp70 from its centrosomal localization in heat shocked cells (γ tub, green, Hsp70, red; asterisk, enlargement).
- C. Semi-quantitative intensity profiles of γ tub after HS show that the cells expressing EGFPHsp70wt have increased levels of γ tub when compared to GFP alone.
- D. Percent of the cells with positive γ tub after HS in RPE cells, transfected with GFP or EGFPHsp70 (n=3 experiments, n=500-600 cells/sample, data presented as mean \pm SD and analyzed by T test).

Figure S 2 (Related to Figure 6). Expression of centrosome-targeted (cHsp70) and centrosome-excluded (mHsp70) Hsp70 and verification of proper targeting.

- A. Maximum projection of confocal microscopy images showing that centrosome-targeted Hsp70 cHsp70 co-localizes with centrosome (centrosome marker, Cent2, red), white asterisk indicates enlarged cell.
- B. Maximum projection of confocal microscopy images showing that centrosome-excluded Hsp70 mHsp70 is expressed at the cell membrane, as expected for proteins with myristoylation signal.
- C. Immunoblot showing levels of expression for GFP, EGFPHsp70wt (Hsp70), GFPHsp70PACT (cHsp70), and myrEGFPHsp70 (mHsp70), endogenous Hsp/Hsc70, and GAPDH, loading control.
- D. Maximum projection showing that expressed centrosome-targeted PACT fusion protein cHsp70 does not displace endogenous pericentrin from centrosomes. TR, transfected cell, NTR, non-transfected cell (PCNT, green, Hsp70, red, bar, 5 μ m).). Insert, semi-quantitative intensity profile.
- E. To exclude recruitment of membrane-targeted Hsp70 to the centrosome after HS, cells were left untreated or subjected to HS, cytosolic fraction was washed by pre-permeabilization with non-ionic detergent for 45 sec, and cells were fixed with methanol. Cells expressing centrosome-targeted Hsp70 cHsp70 localized to the centrosome (γ tub, red, cHsp70 in green) in HS and non-HS cells. Centrosome-excluded mHsp70 and GFP alone did not localize to centrosomes in control or HS cells.

Figure S 3 (Related to Figure 6). Expression of centrosome-targeted (cHsp70) and centrosome-excluded (mHsp70) Hsp70 in rescue experiments and centrosome protection by cHsp70 in transiently transfected cells.

- A.** Maximum projections of confocal microscopy images showing expression of centrosome–targeted Hsp70 (cHsp70) and its co-localization with Cent2 and maximum projections of confocal microscopy images showing expression of centrosome –excluded Hsp70 (mHsp70) and disruption of Cent2 staining in heat shocked RPE cells (bar, 10 μ m).
- B.** Protective role for cHsp70, but not GFP, PACT or mHsp70, in transiently transfected cells after HS (percent of γ tubulin positive cells, (n=4 experiments, n=250-700 cells/sample, data presented as mean \pm SD and analyzed by one-way Anova combined with Tukey’s multiple comparison test).
- C.** Protective role for EGFPHsp70 (wtHsp70), EGFPHsp70PACT (cHsp70), but not GFP or myrEGFPHsp70 (mHsp70), in transiently transfected cells after HS (Centrin 2 signal, integrated intensity $\times 10^5$ arbitrary units; n=40-50 centrosomes/sample, data presented as mean \pm SEM and analyzed as in “B”).

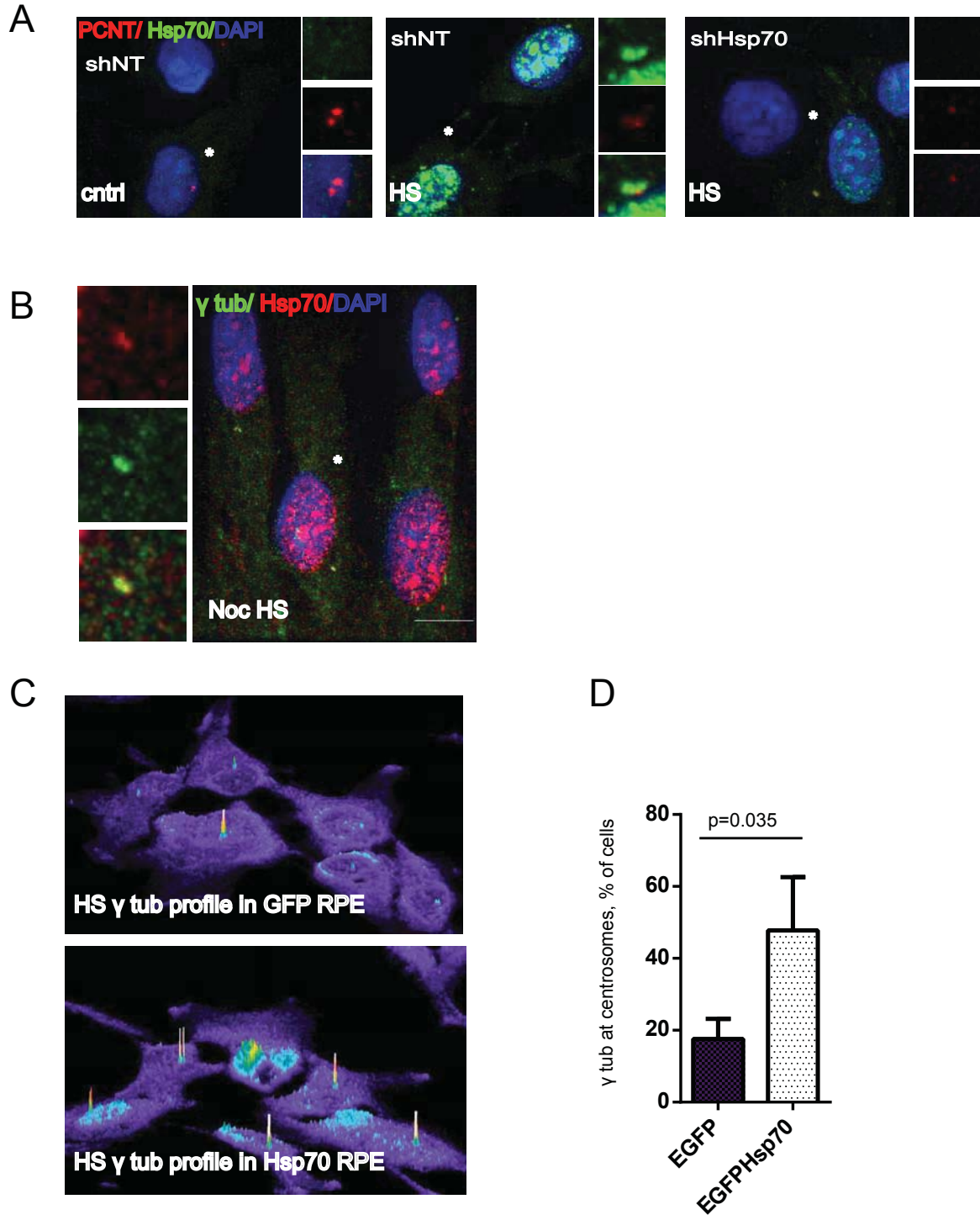


Figure S1. Related to Figure 6. Hsp70 is recruited to the centrosome during HS and protects it from damage.

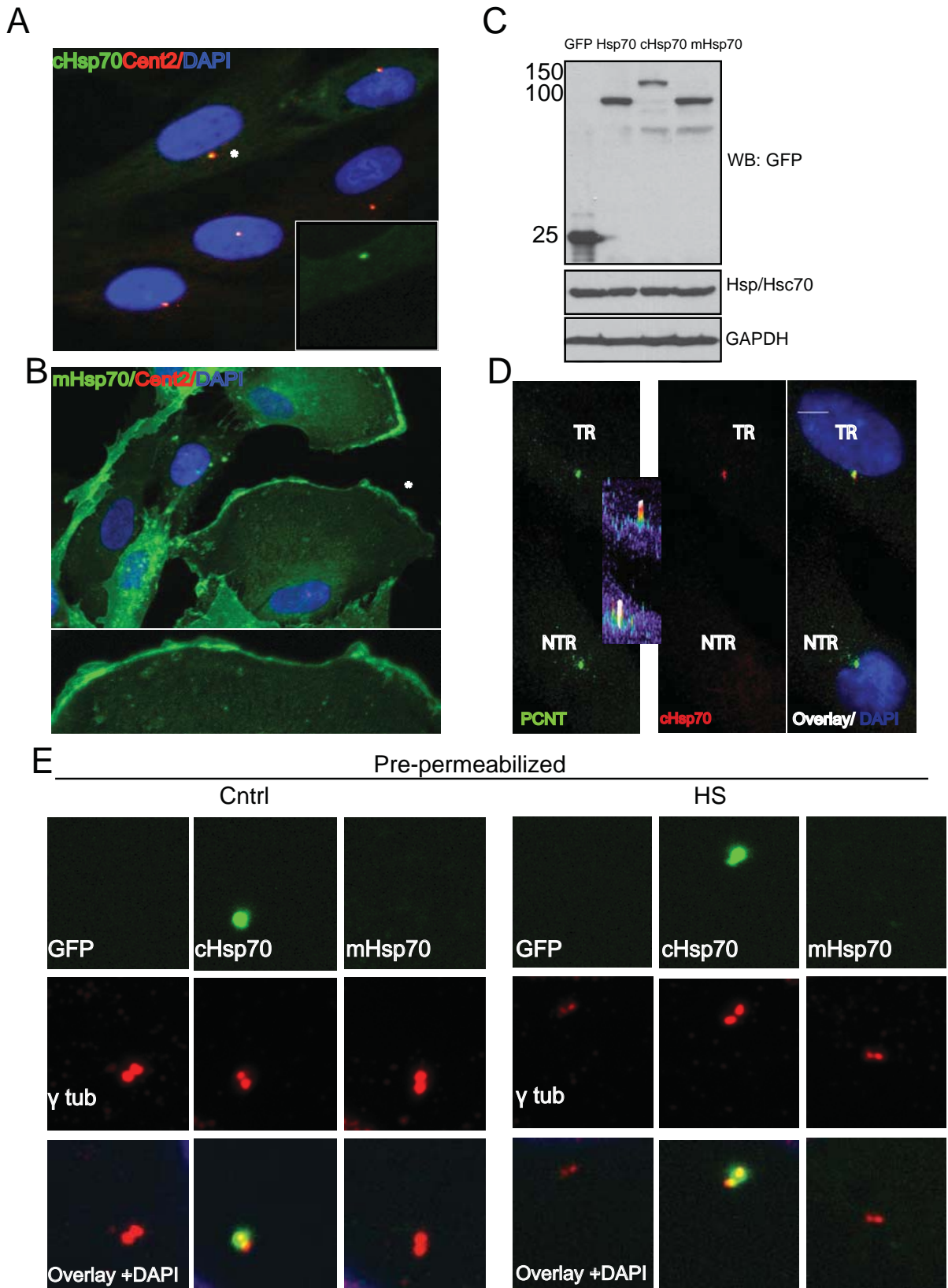
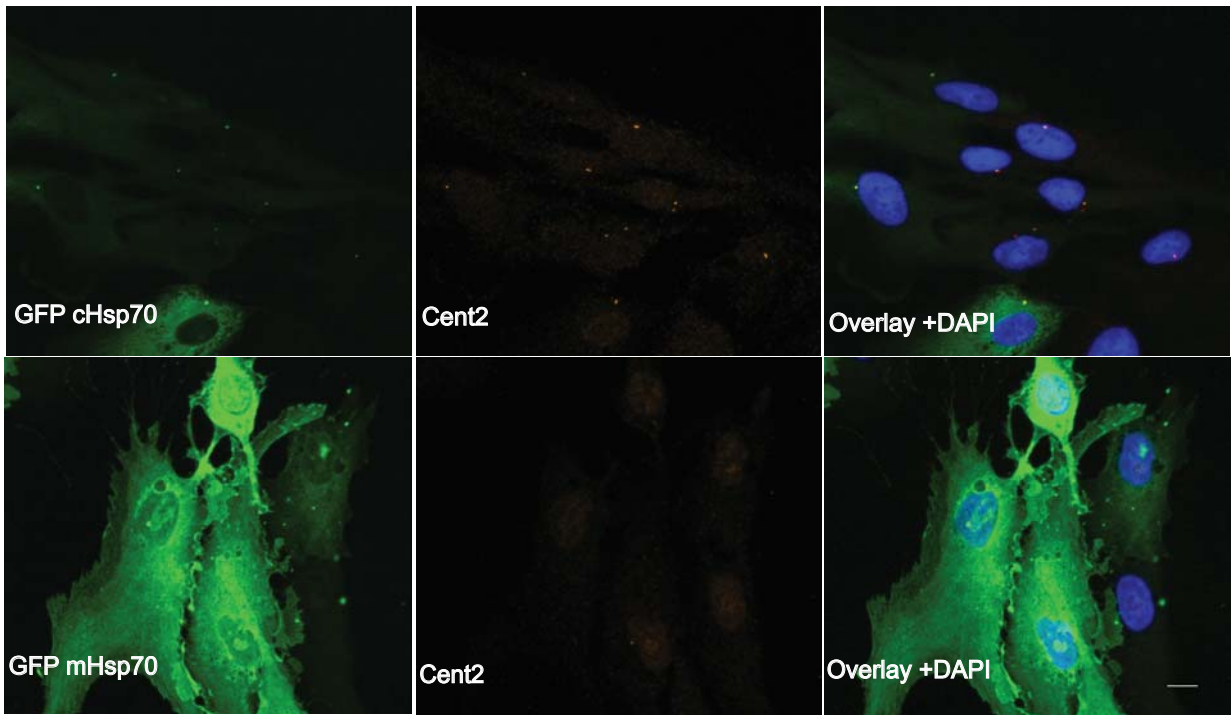


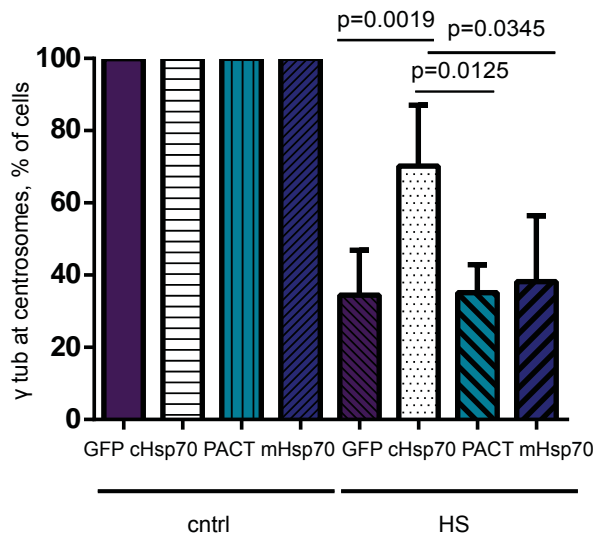
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A

HS



B



C

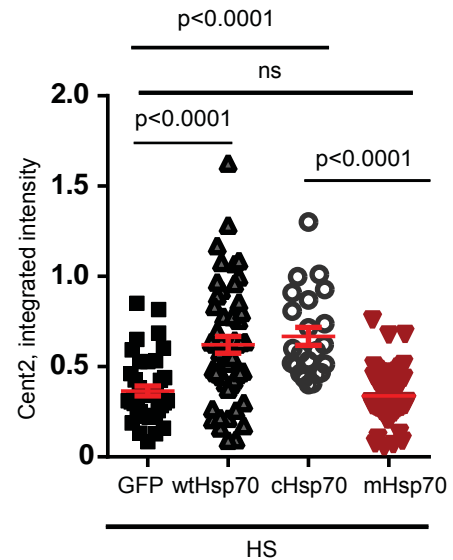


Figure S3. Related to Figure 6. Expression of centrosome-targeted (cHsp70) and centrosome-excluded (mHsp70) in rescue experiments and centrosome protection by cHsp70 in transiently transfected cells