

SUPPLEMENTAL FIGURES

Supplemental Figure 1. Distribution of eIF4E in HeLa cells upon heat stress. Untreated (A) or heat stressed (B) HeLa cells were fixed, and stained for eIF4E and G3BP.

Supplemental Figure 2. SG formation upon heat shock is rapid. Wild-type and DKO MEFs where untreated (A) exposed to 44°C for 10 (A), 20 (B) or 30 (B) min, fixed, and stained for eIF4E and G3BP.

Supplemental Figure 3. Relocalization of eIF4E in DKO MEFs in the presence of 4E-BP1. WT MEFs are stably expressing GFP whereas DKO MEFs are stably expressing either GFP alone or GFP and 4EBP1. Cells are mock treated (A) or heat shocked at 42°C for 30min (B). They were then fixed and stained for eIF4E (red) or G3BP (blue). GFP (green) was used as an indicator of positively infected cells.

Supplemental Figure 4. SG formation is readily reversible. SG induction was performed on WT and DKO MEFs by exposing cells to 44°C/30 min, after which time, cells were transferred to 37°C for 1 (A), 2 (A), 3 (B), or 4(B) hours. Cells were then fixed and stained for eIF4E and G3BP.

Supplemental Figure 5. Cell viability following recovery from heat shock is not affected by the presence or absence of 4E-BP1/2. Following heat shock at 44°C/30 min, WT and DKO MEFs were allowed to recovery for 6 h at 37°C, after which time Annexin V-Propidium Iodide labelling was performed to determine the fraction of apoptotic cells.

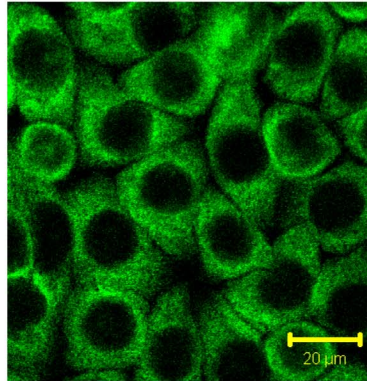
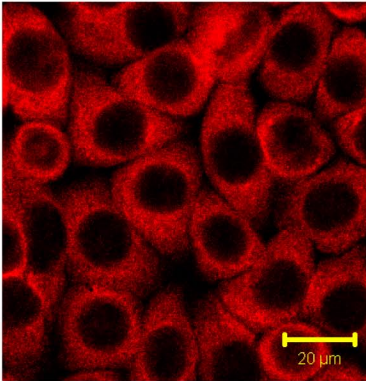
Supplemental Figure 6. Rapamycin alters the subcellular localization of eIF4E to SGs in wt, but not DKO, MEFs. WT (A) or DKO (B) MEF cells were exposed to vehicle (untreated) or to rapamycin (RAP: 20 nM for 1hr) or heat shock (HS: 44°C/30 min) or RAP treatment followed by a heat shock (RAP/HS). Cells were fixed and stained for eIF4E and G3BP.

Supplemental Figure 7. Consequences of HS and Rapamycin on 4E-BP1 phosphorylation. Western blot of 4E-BP1 from WT and DKO MEFs treated with Rapamycin (RAP: 20 nM for

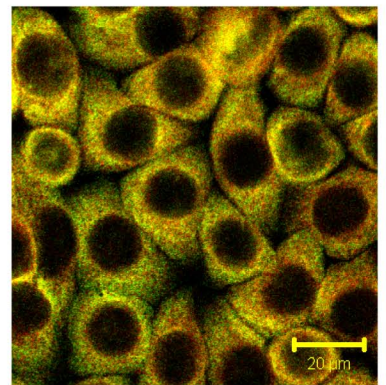
1hr) or heat shock (HS: 44°C/30 min) or RAP treatment followed by a heat shock (RAP/HS). β -Actin is used as a loading control.

HeLa
37°C
G3BP

eIF4E

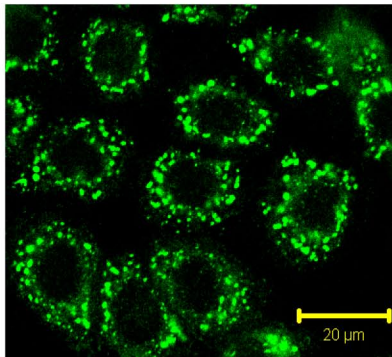
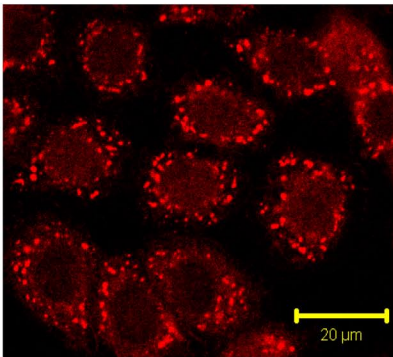


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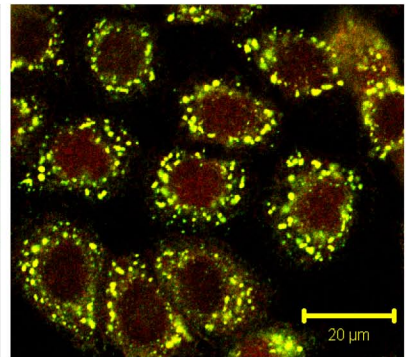


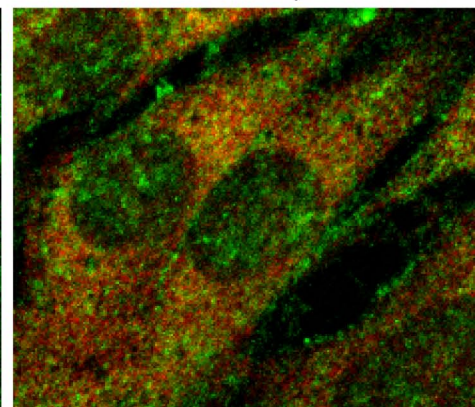
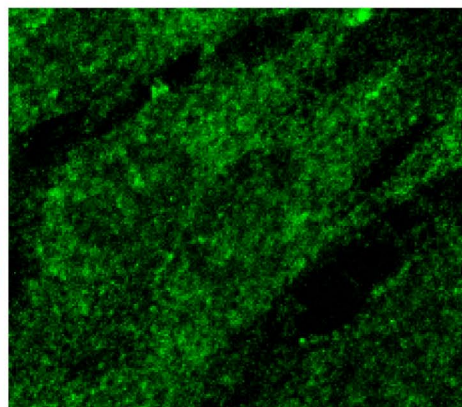
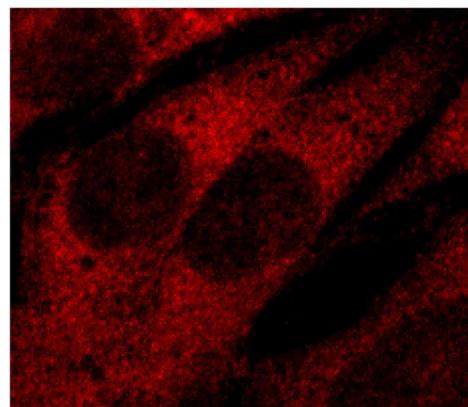
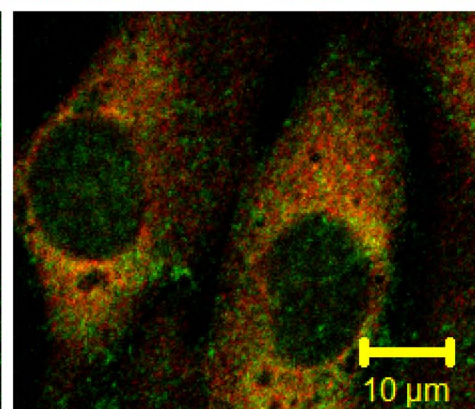
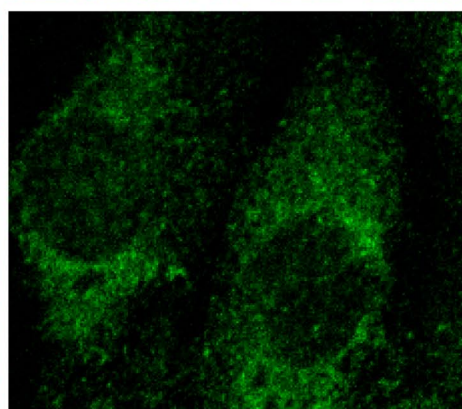
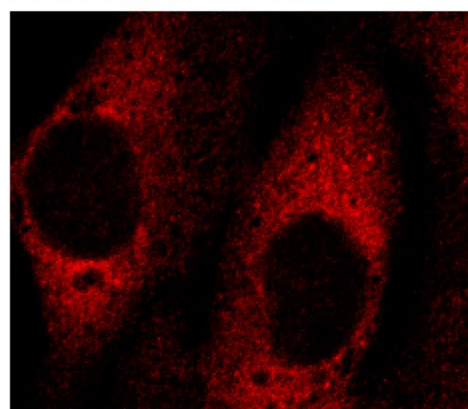
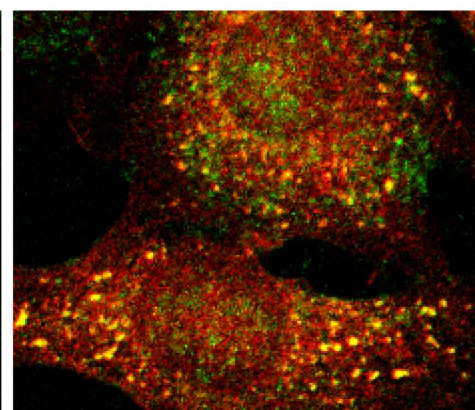
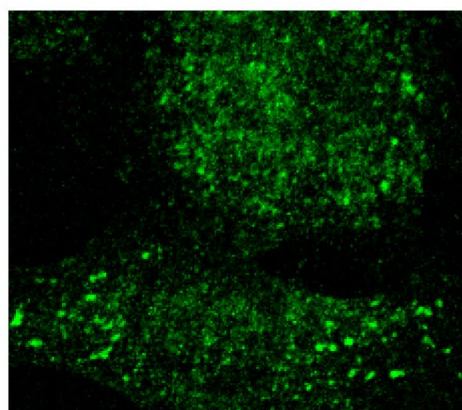
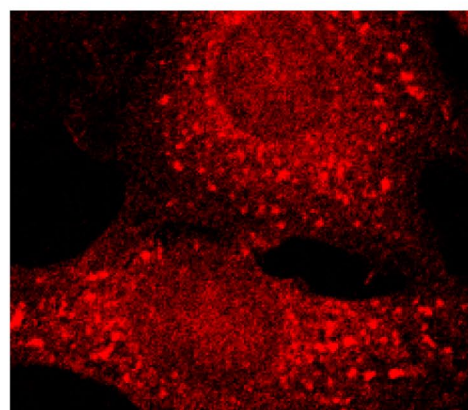
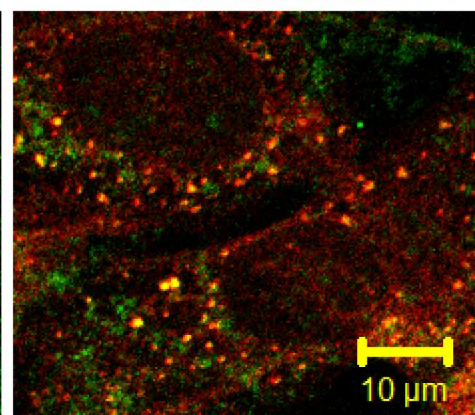
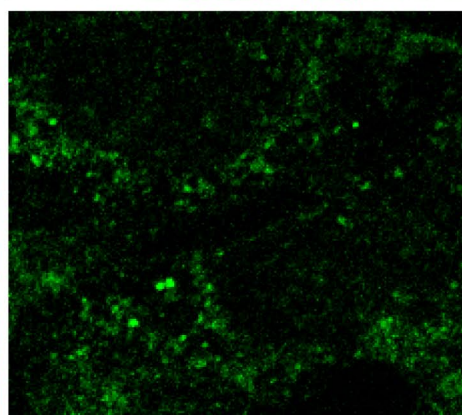
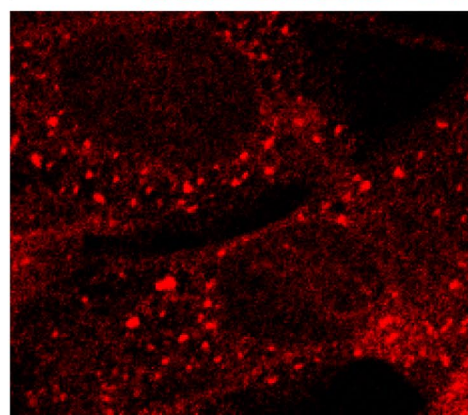
44°C
G3BP

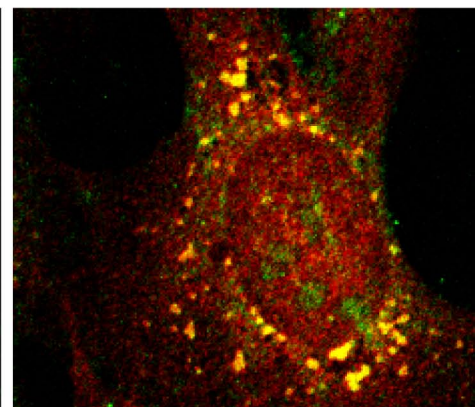
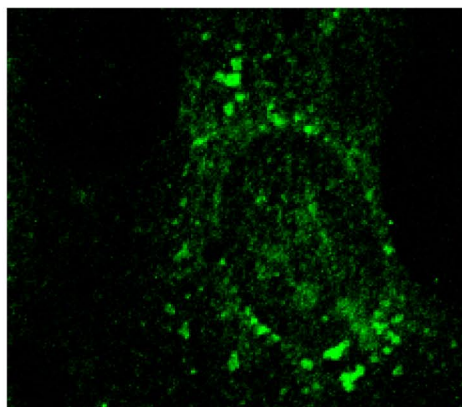
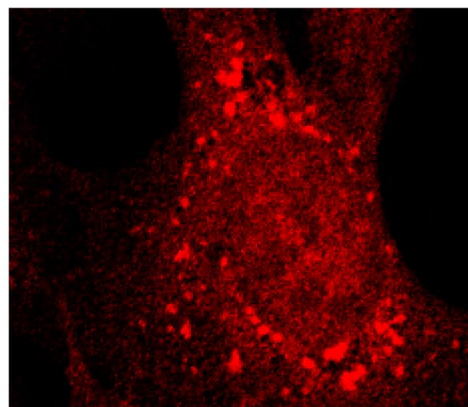
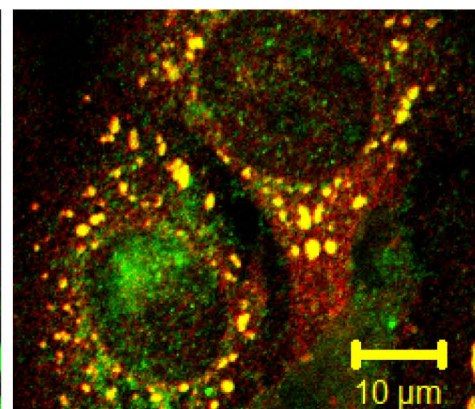
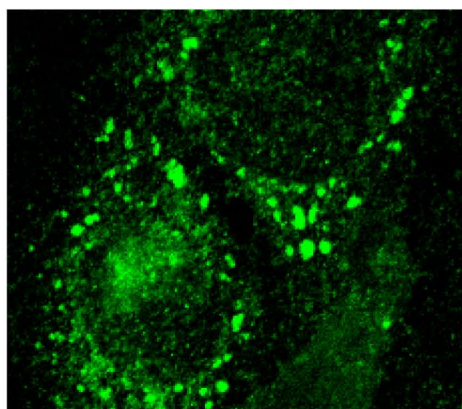
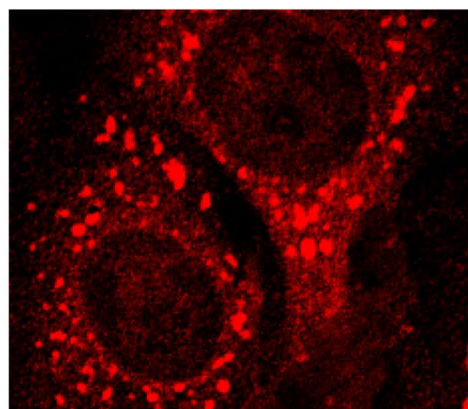
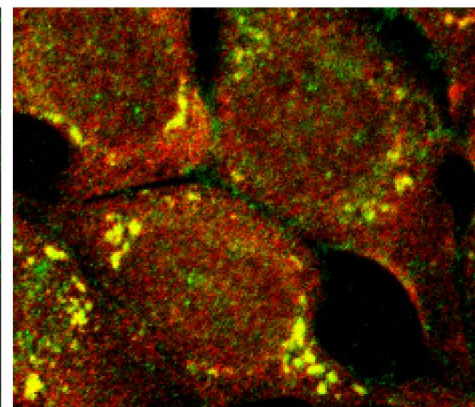
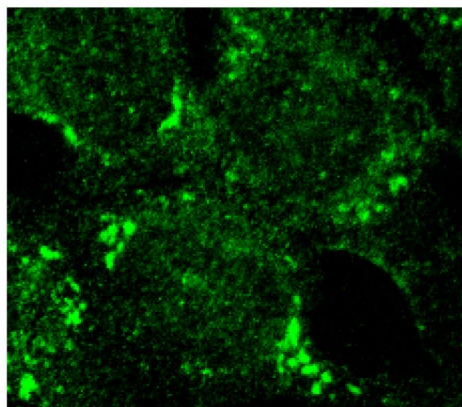
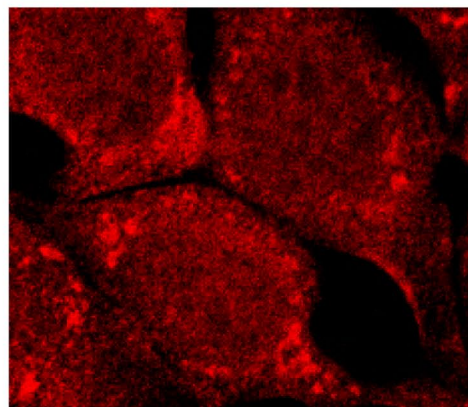
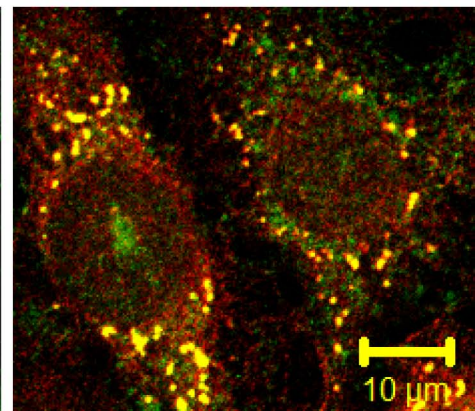
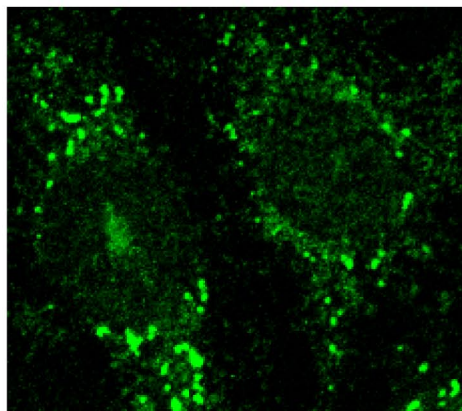
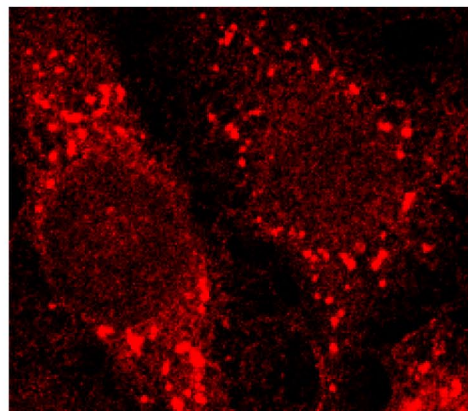
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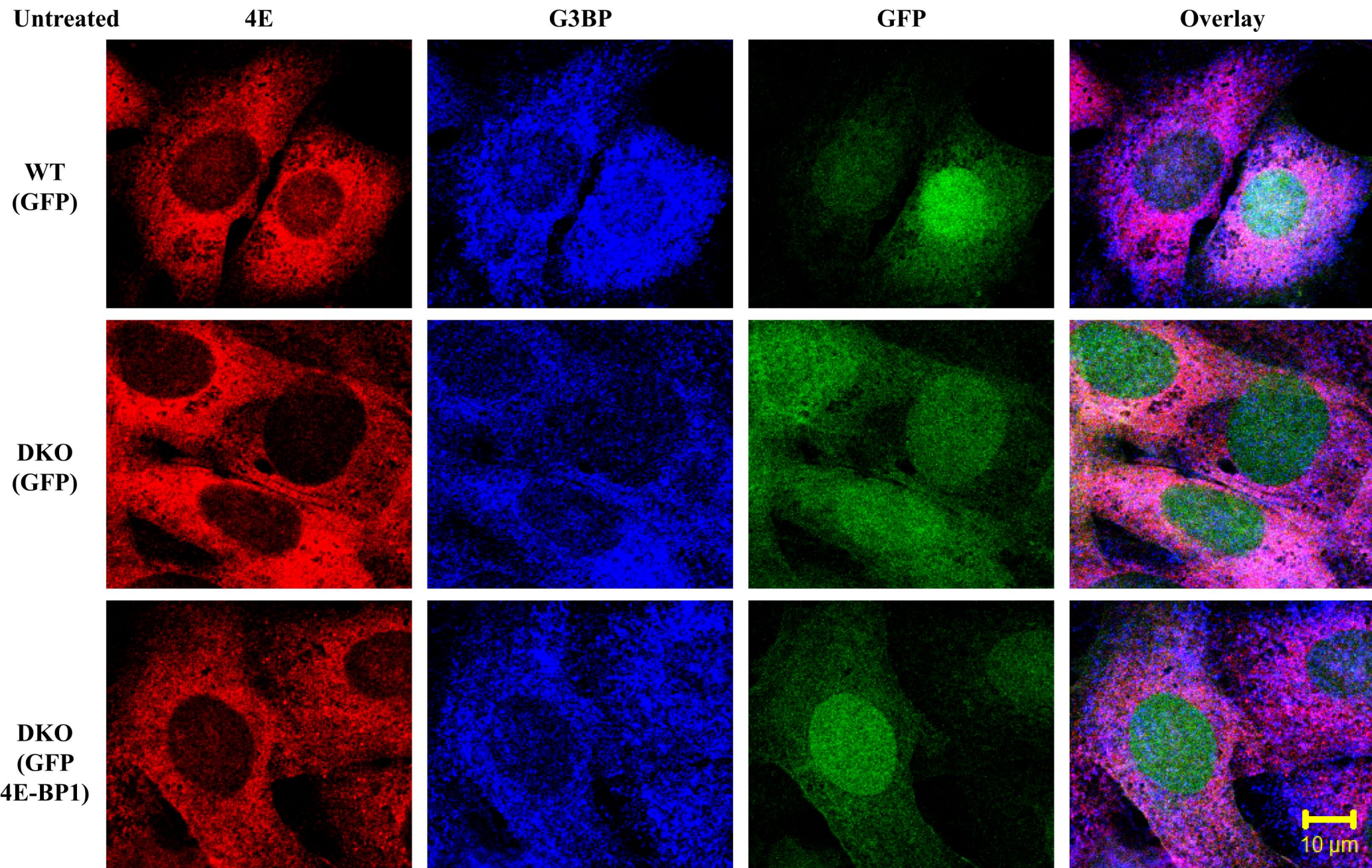


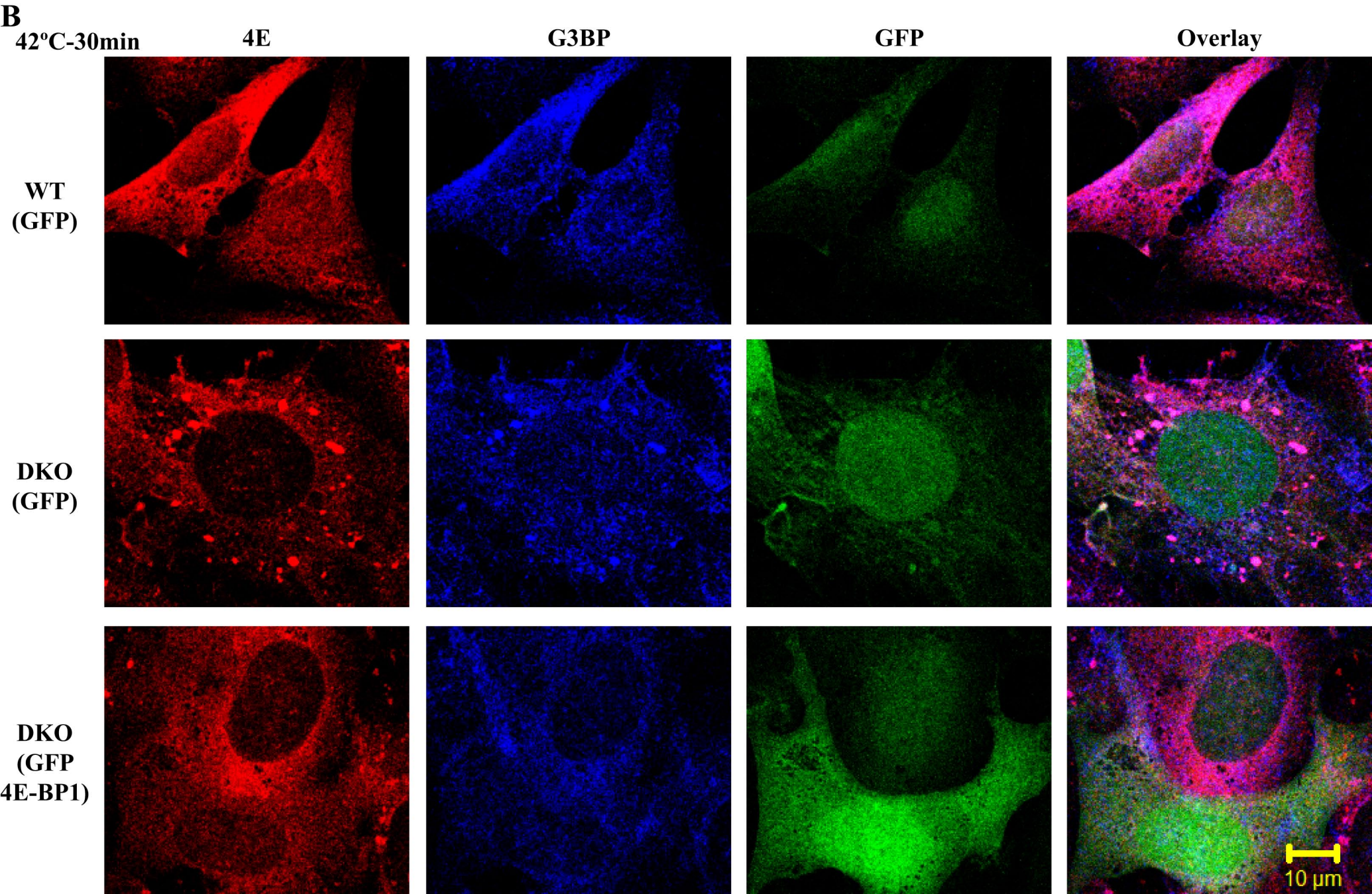
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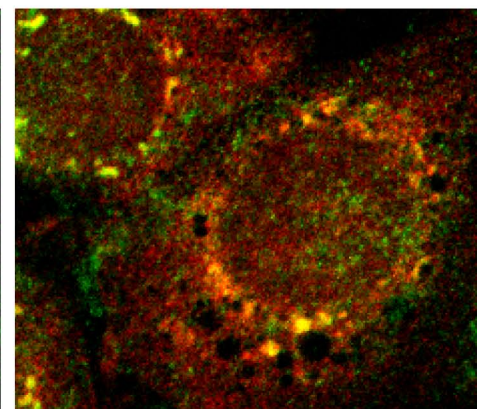
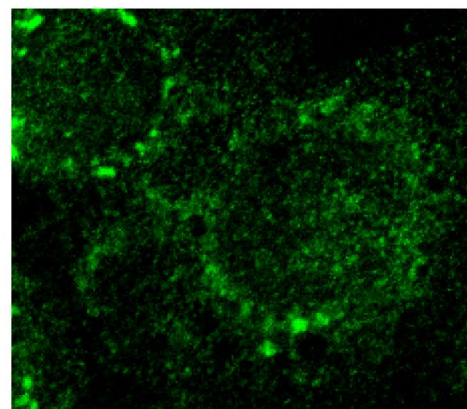
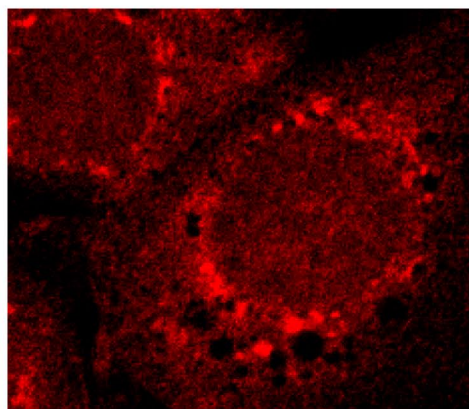
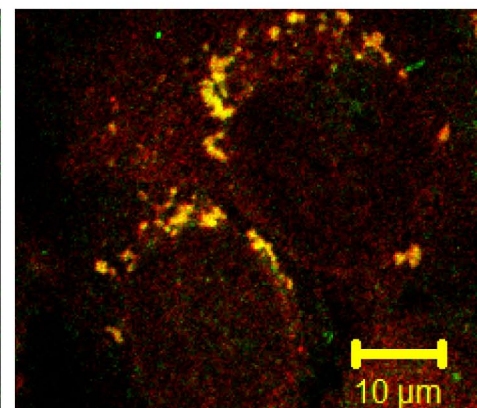
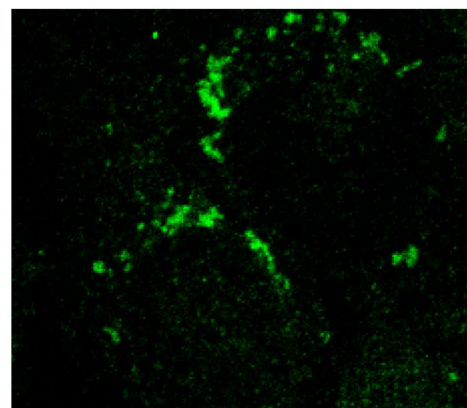
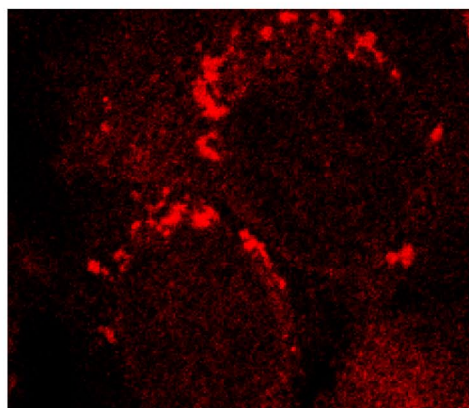
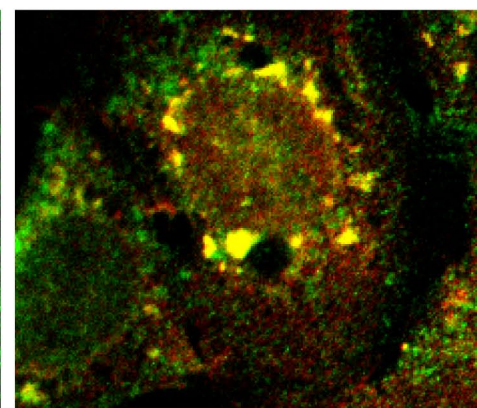
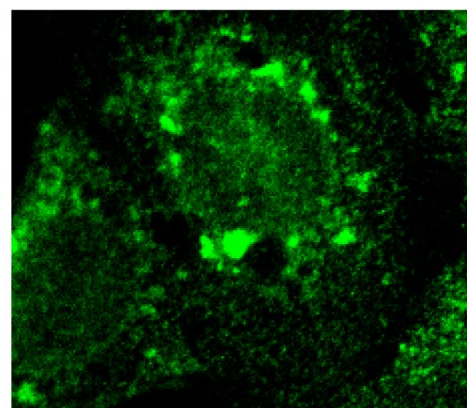
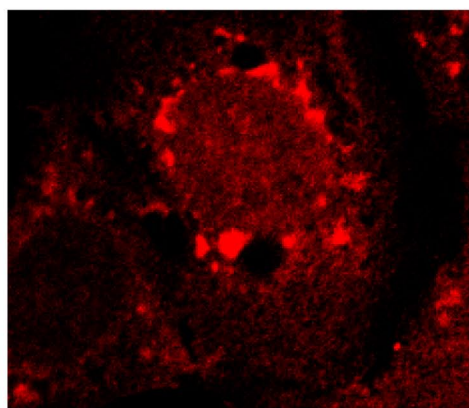
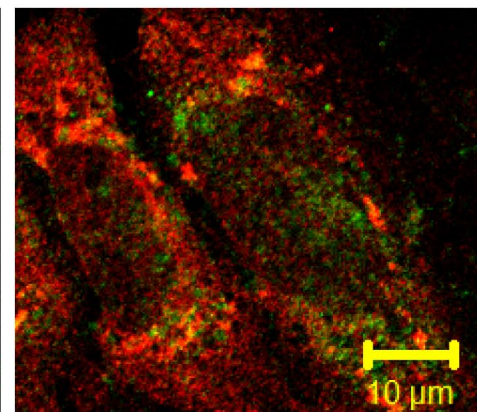
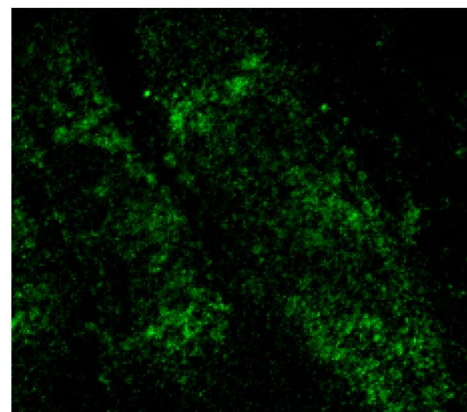
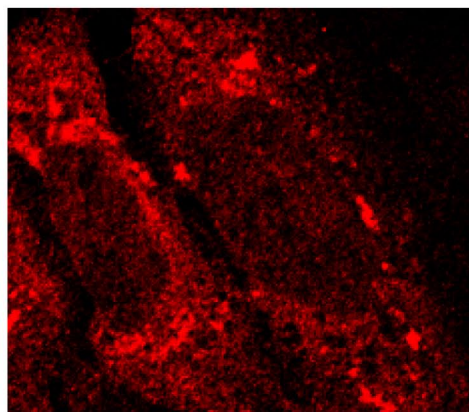


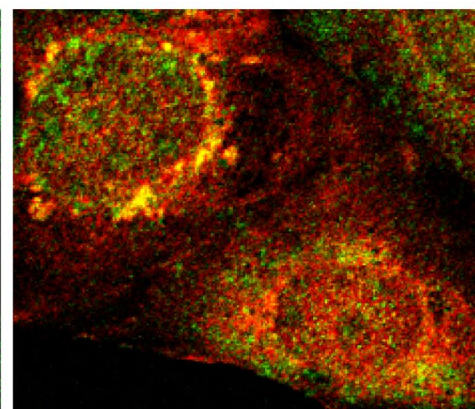
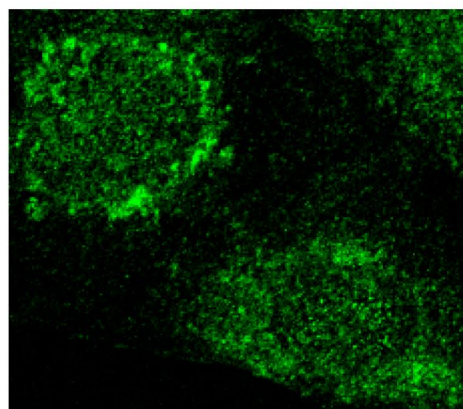
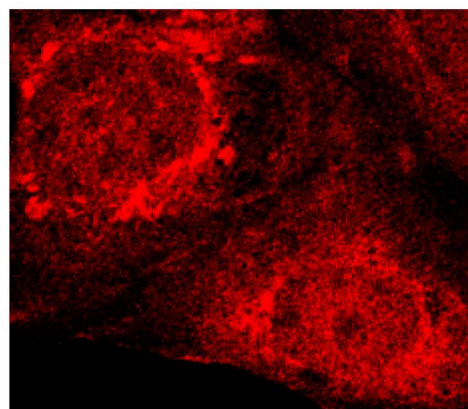
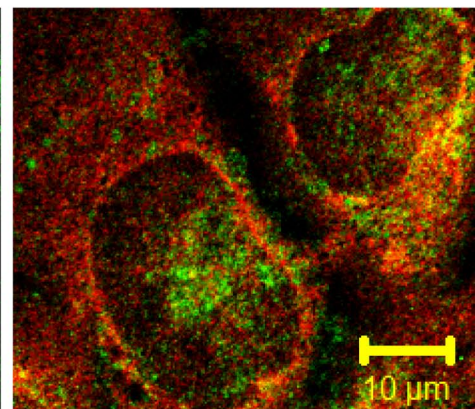
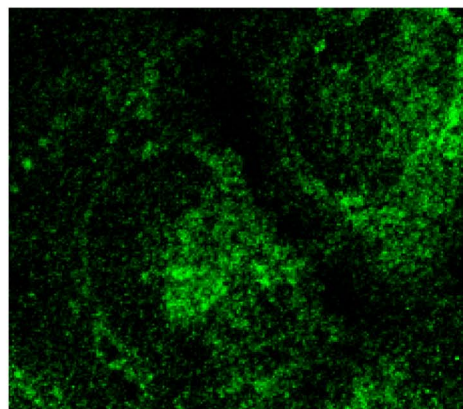
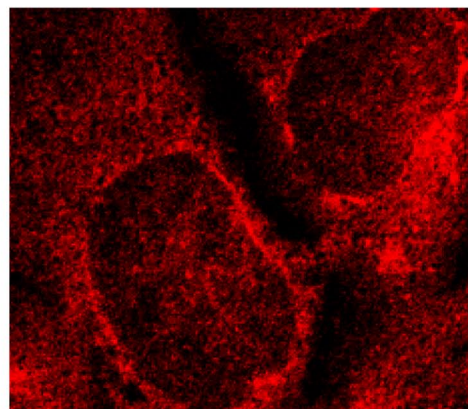
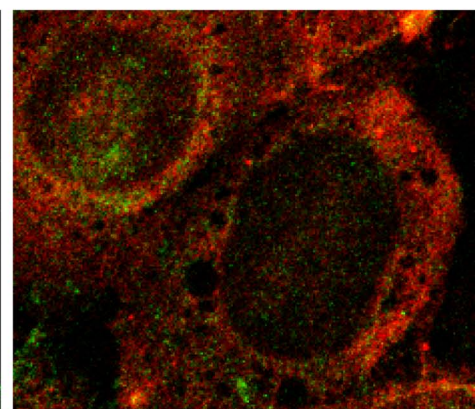
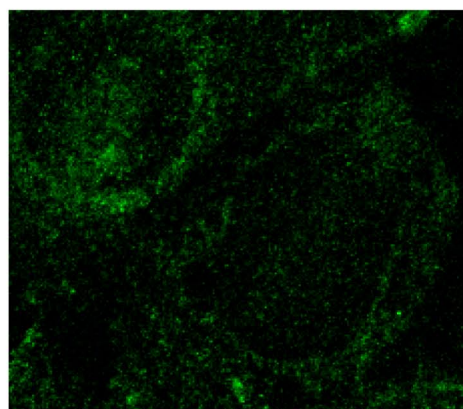
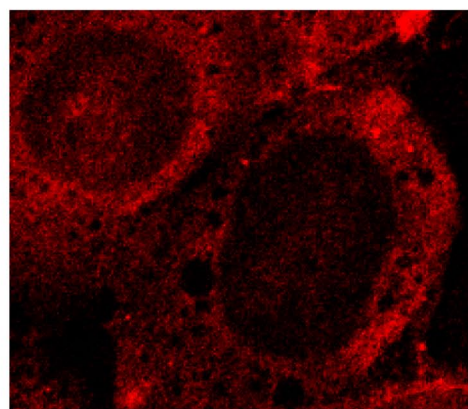
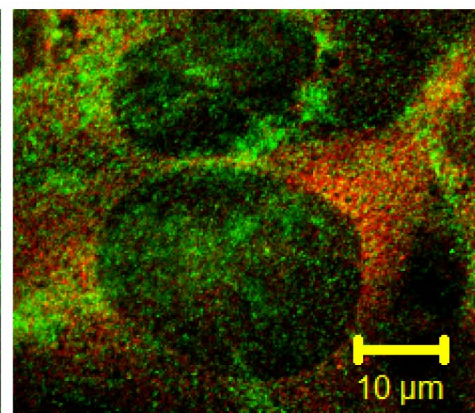
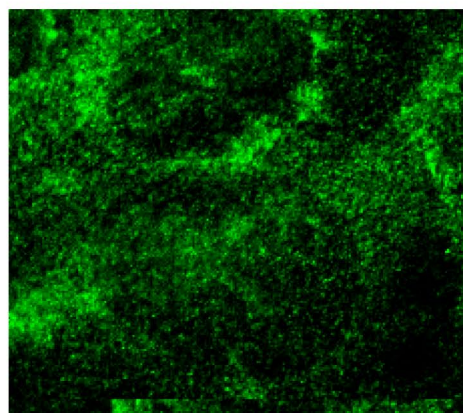
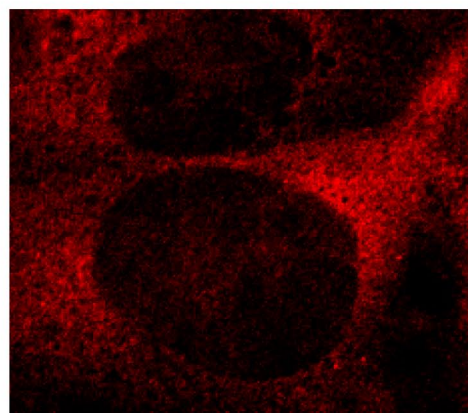
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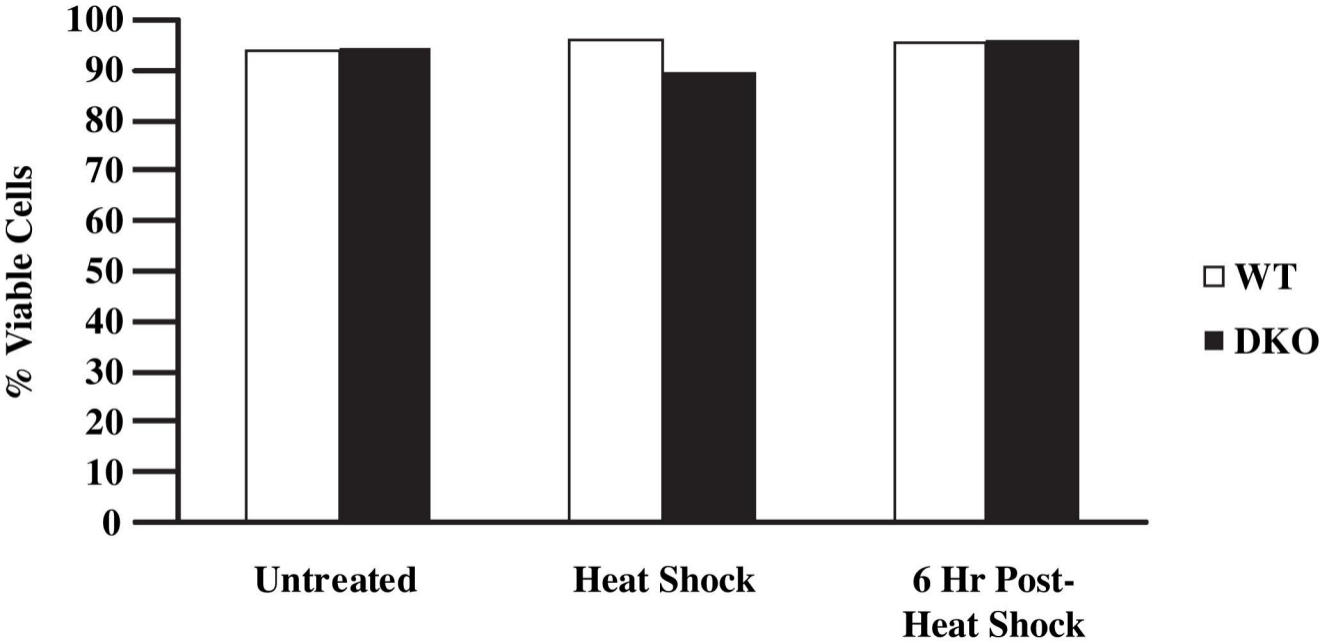
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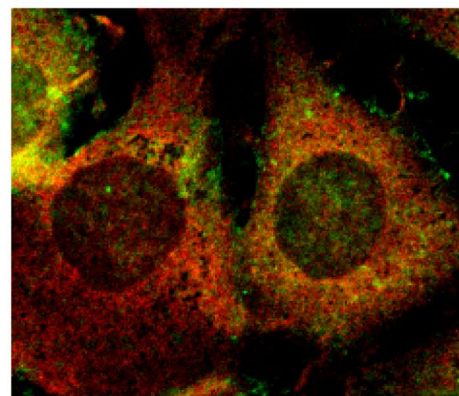
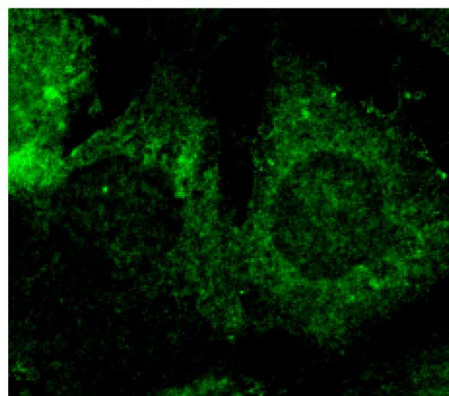
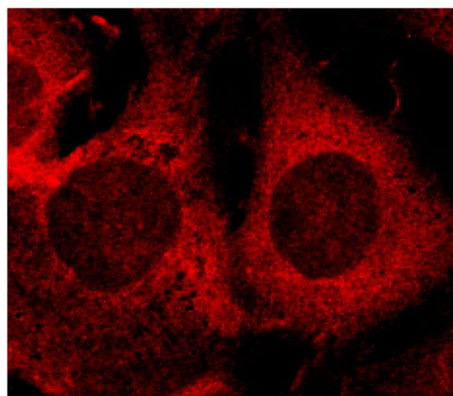
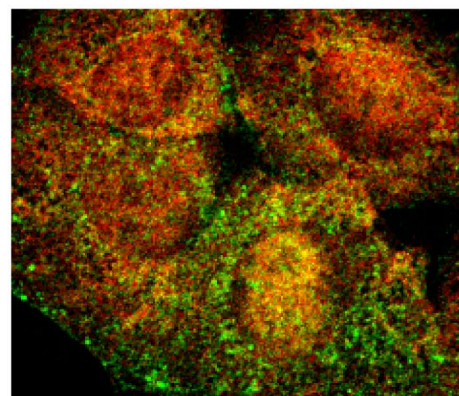
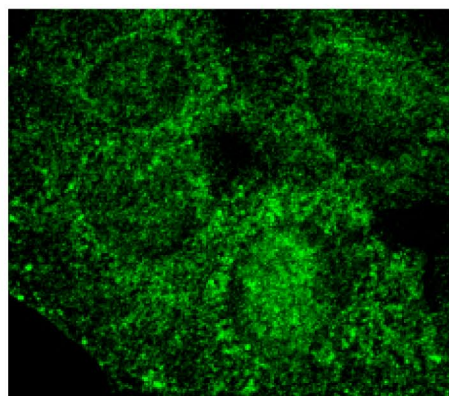
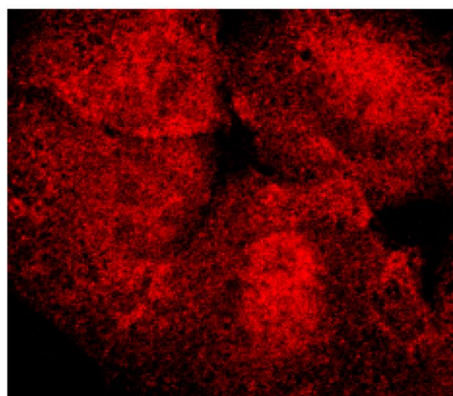
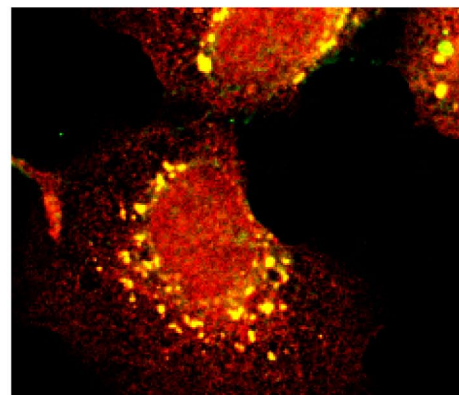
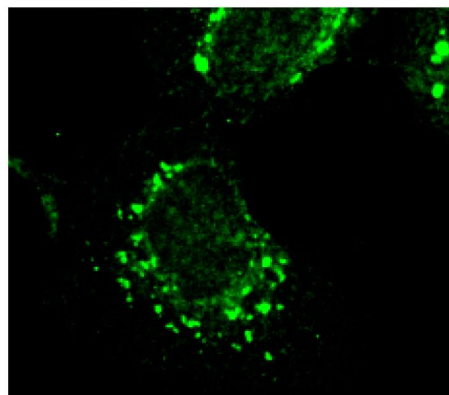
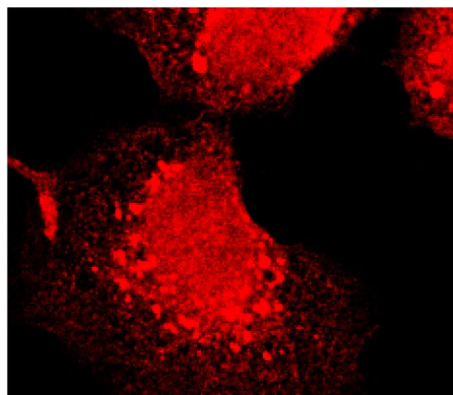
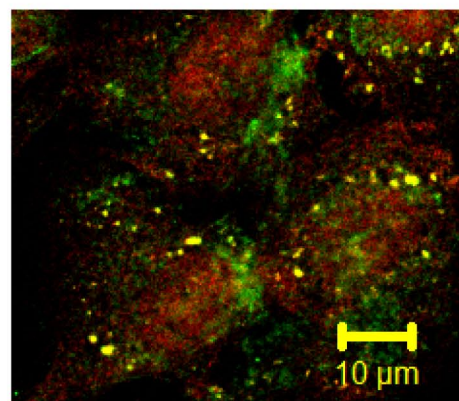
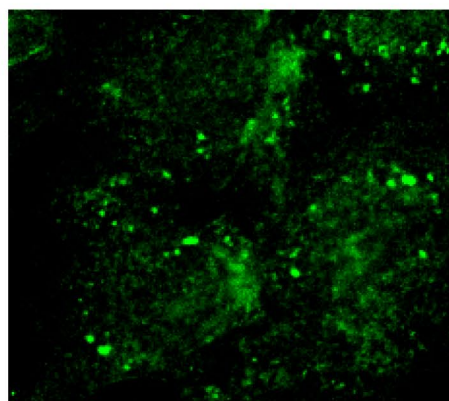
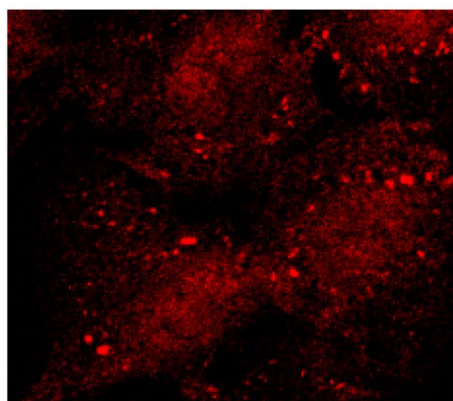
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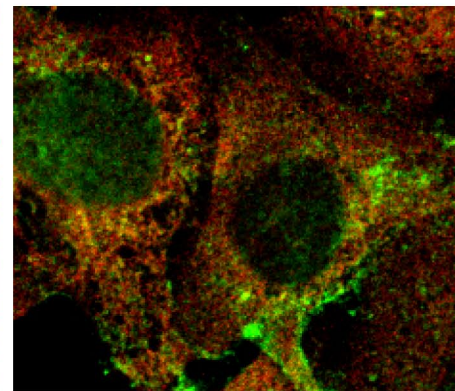
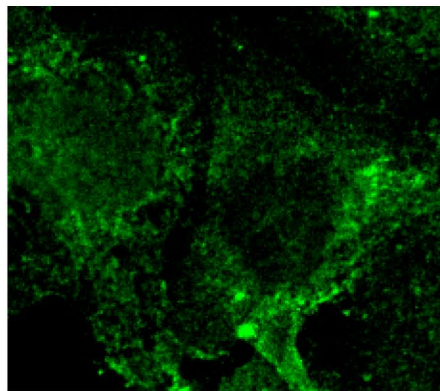
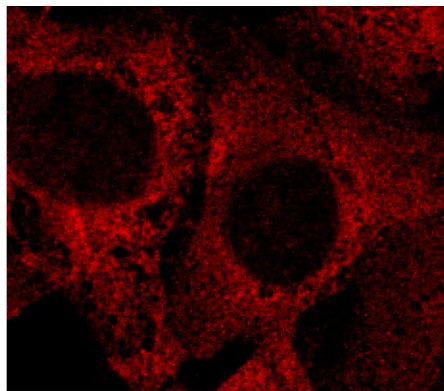
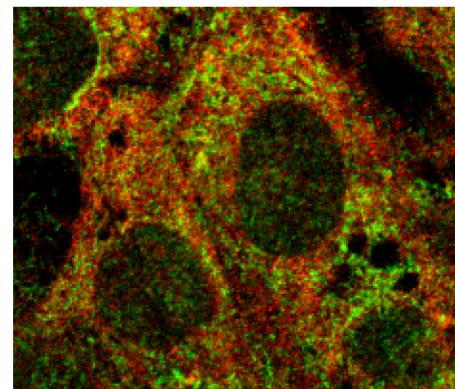
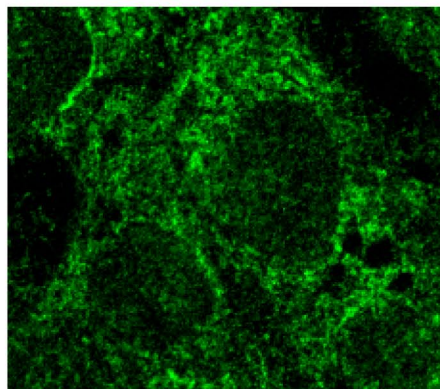
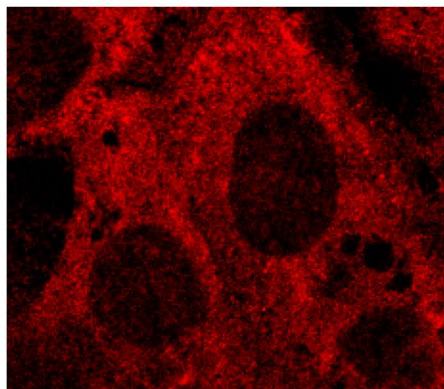
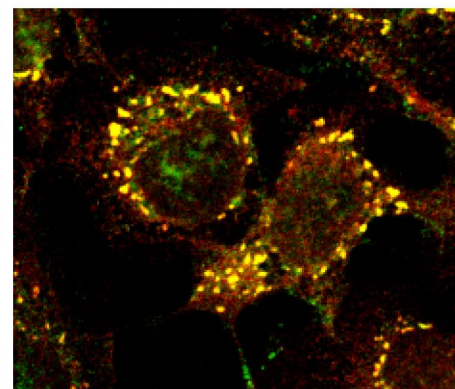
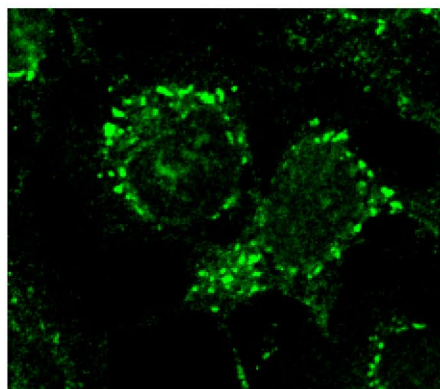
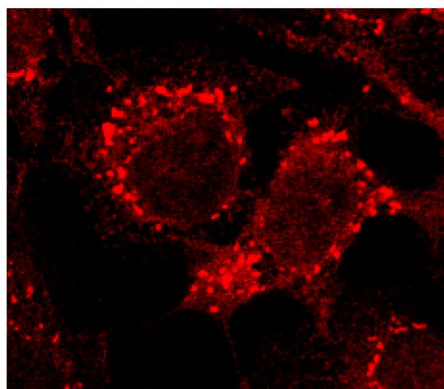
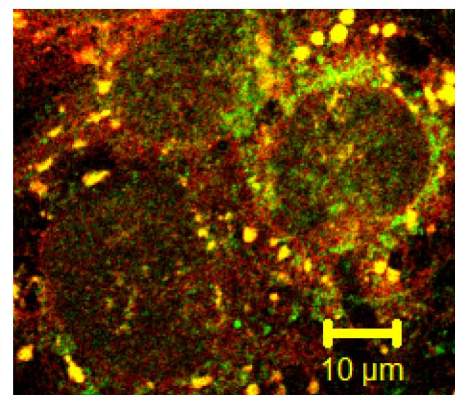
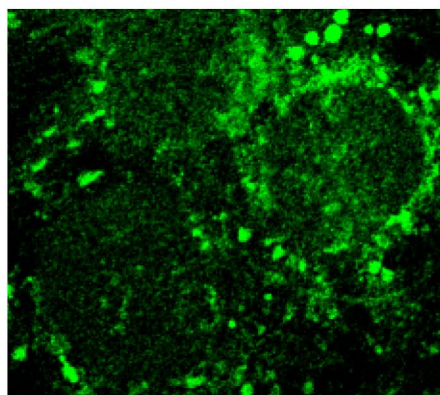
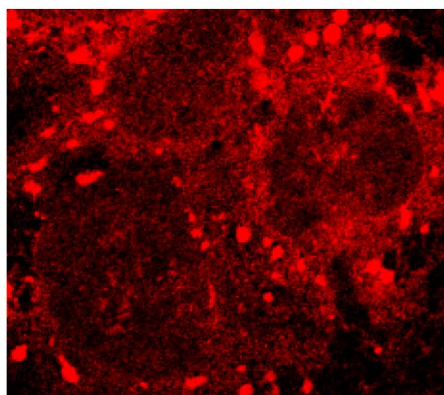


A**44°C-30min
Rescue 1h****4E****G3BP****Overlay****WT****DKO****44°C-30min
Rescue 2h****WT****DKO**

B**44°C-30min
Rescue 3h****4E****G3BP****Overlay****WT****DKO****44°C-30min
Rescue 4h****WT****DKO**



A**4E****G3BP****Overlay****Untreated****RAP****HS****HS+RAP****10 μ m**

B**4E****G3BP****Overlay****Untreated****RAP****HS****HS+RAP****10 μm**

