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## Supplemental Information

# Cofilin-Mediated Actin Stress Response Is Maladaptive in Heat-Stressed Embryos

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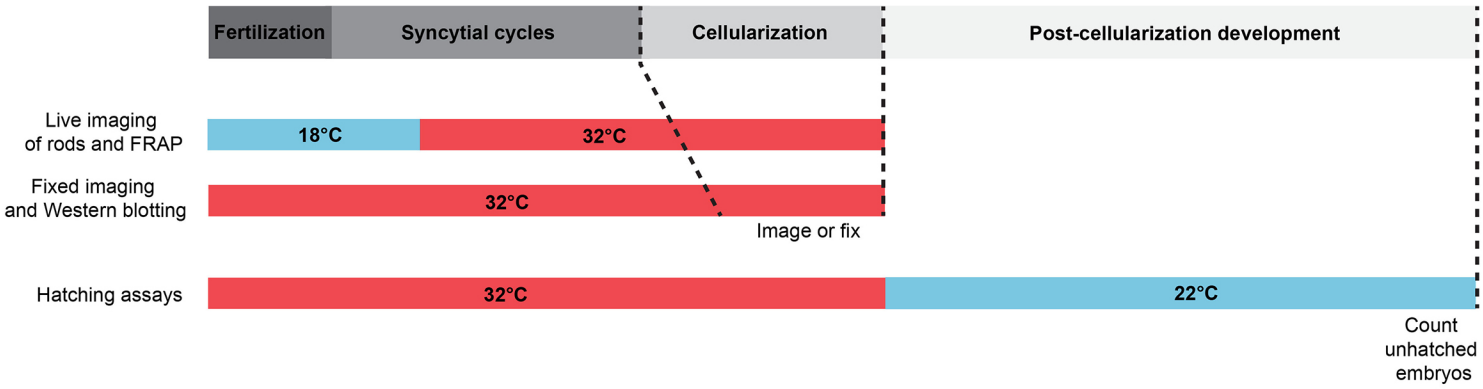


**Table S1. Timeline of heat stress experiments. Related to Figures 1-4.**
Schematic representation of the developmental timing of heat stress treatments for various experiments.

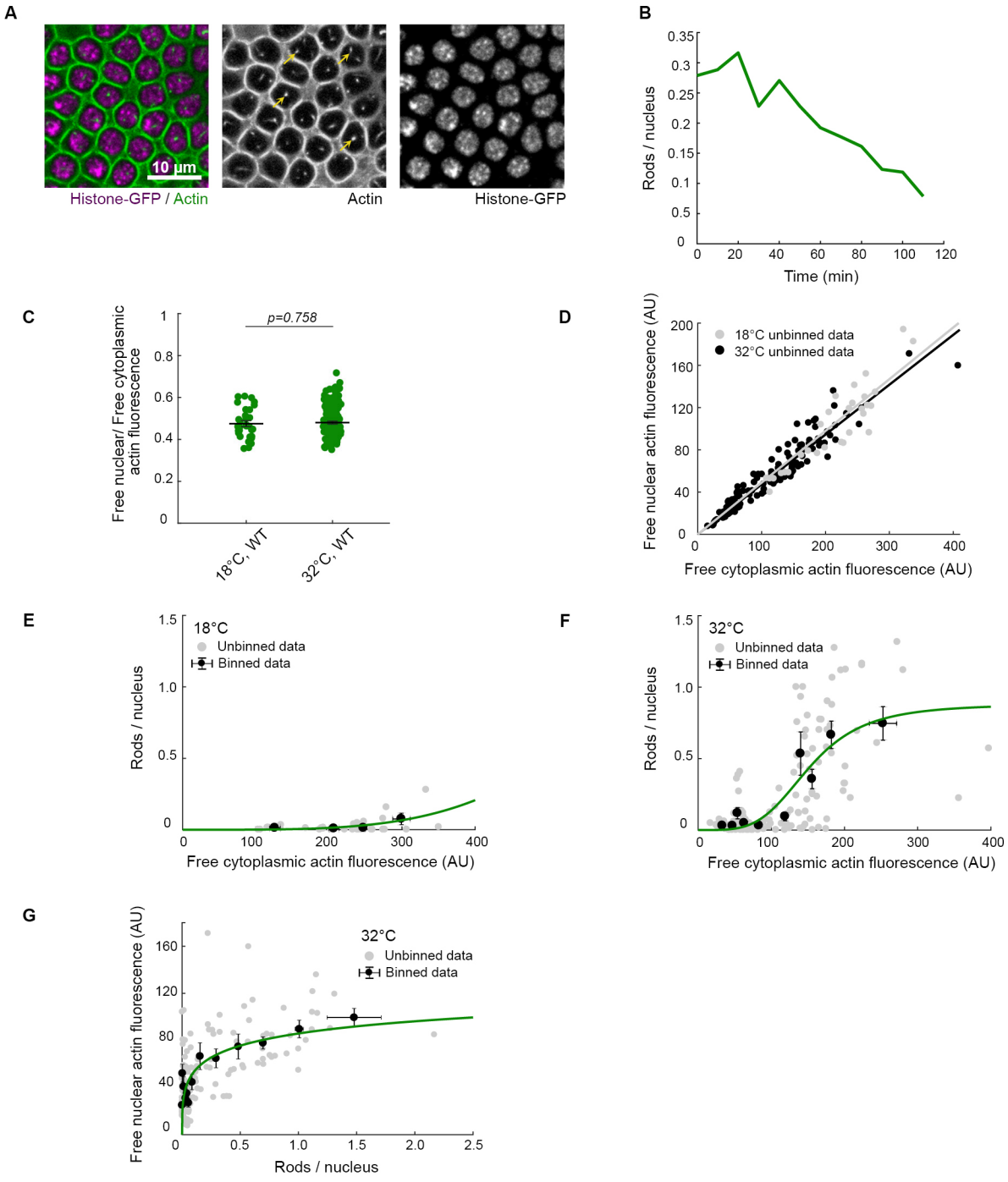


**Figure S1. Intra-nuclear actin rods assemble in heat stressed embryos. Related to Figure 2.**
(A) Surface views show G-actin$^{Red}$ (green) in furrow tips encircling nuclei (Histone-GFP, purple) in live wild-type (WT) embryos at 32°C. Rods (yellow arrows) assemble inside nuclei. (B) Rod abundance (rods / nucleus) in a single live embryo after downshift from heat stress at 32°C. (C) Ratio of free nuclear to free cytoplasmic actin fluorescence at indicated temperatures (n=147 embryos, with free actin fluorescence averaged from 3 nuclear or cytoplasmic regions per embryo). Each point represents one embryo. Horizontal lines are means ± SE. (D) Free nuclear versus free cytoplasmic actin fluorescence in live WT embryos at indicated temperatures (n=147 embryos, with free actin fluorescence intensity averaged from 3 nuclear or cytoplasmic regions per embryo). Each gray or black point represents one embryo at 18°C or 32°C, respectively. Gray or black lines are linear fits for corresponding data at 18°C or 32°, and fits yield slopes that are statistically indistinguishable. (E) and (F) Rod abundance (rods / nucleus) versus free cytoplasmic actin fluorescence in live WT embryos at indicated temperatures (n≥31 embryos, with rod abundance counted in ≥60 nuclei per embryo; free actin fluorescence averaged from 3 cytoplasmic regions per embryo). Related data shown in Figure 2C, 2D. (G) Free nuclear actin fluorescence versus rod abundance (rods / nucleus) in live WT embryos at 32°C (n=147 embryos, with rod abundance counted in ≥60 nuclei per embryo; free actin fluorescence averaged from 3 nuclei per embryo). Related data shown in Figure 2D.
*Each gray point represents one embryo, and black points are binned data (mean ± SE) in (E), (F), and (G).*
*Green line is binned data fitted to a Hill Function with Hill Coefficient=4 in (E) and (F), and Hill Coefficient=3.5 in (G).*
*Student's t-test used to calculate P value in (C).*

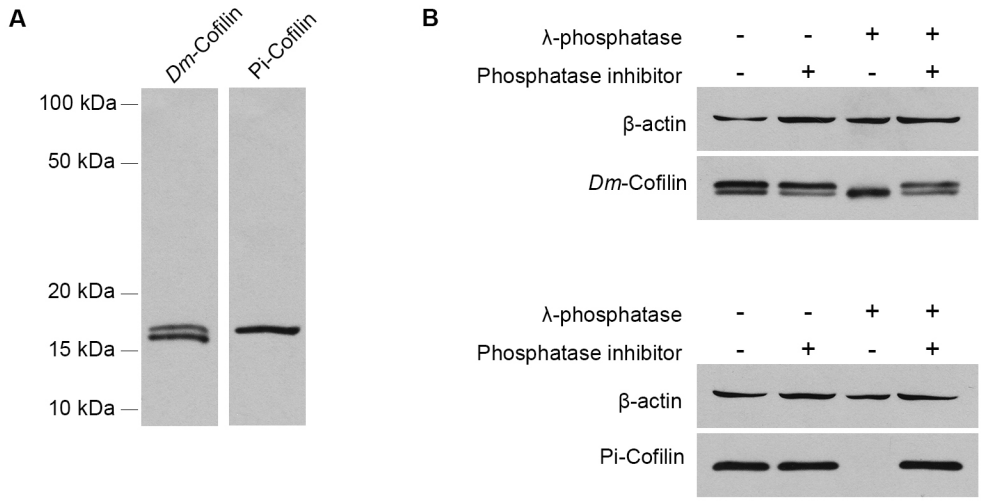


**Figure S2. Validation of Dm-Cofilin and Pi-Cofilin antibodies. Related to Figure 3.**
(A) Representative full lane Western blots for Dm-Cofilin and Pi-Cofilin antibodies from wild-type embryos at 25°C.
(B) Representative Western blots for Dm-Cofilin and Pi-Cofilin antibodies for lysates from wild-type embryos following indicated λ-phosphatase or Phosphatase inhibitor treatments. β-actin used as loading control.

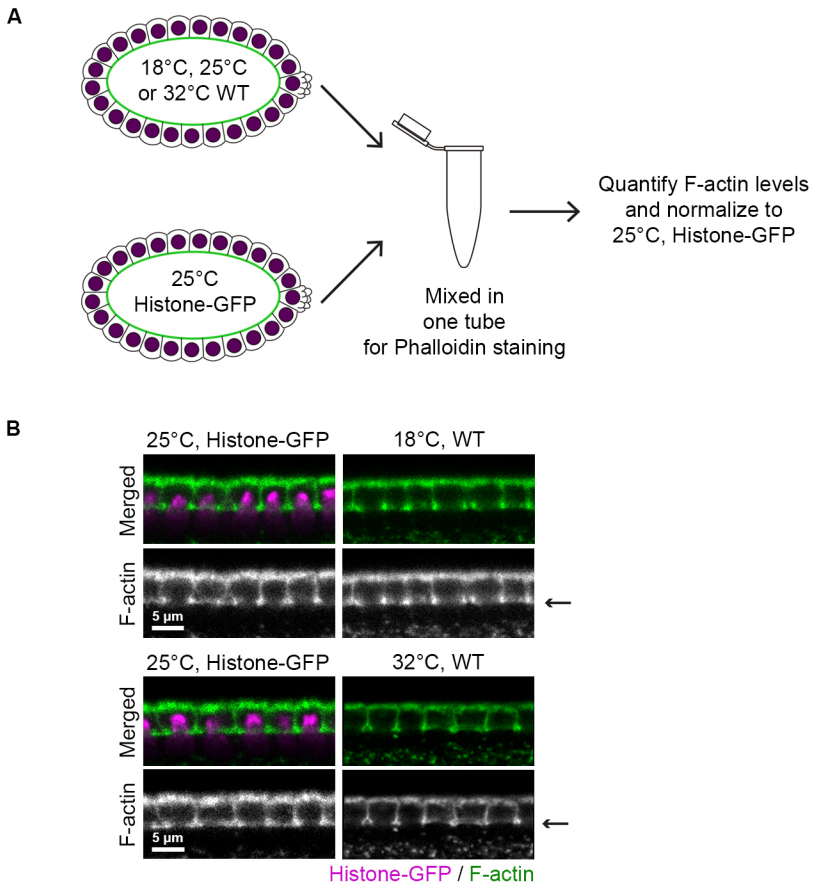


**Figure S3. Method used to quantify F-actin levels in embryos reared at different temperatures. Related to Figure 3.**
(A) Strategy used to quantify F-actin levels in embryos reared at different temperatures. To minimize experimental variability, embryos reared at each temperature were stained in the same tube as and normalized against internal control, Histone-GFP embryos reared at 25°C. (B) Cross sections show furrow tip F-actin (Phalloidin, green) and nuclei (Histone-GFP purple) in Histone-GFP and wild-type (WT) embryos at indicated temperatures. Arrows indicate furrow tip position where quantification was done.

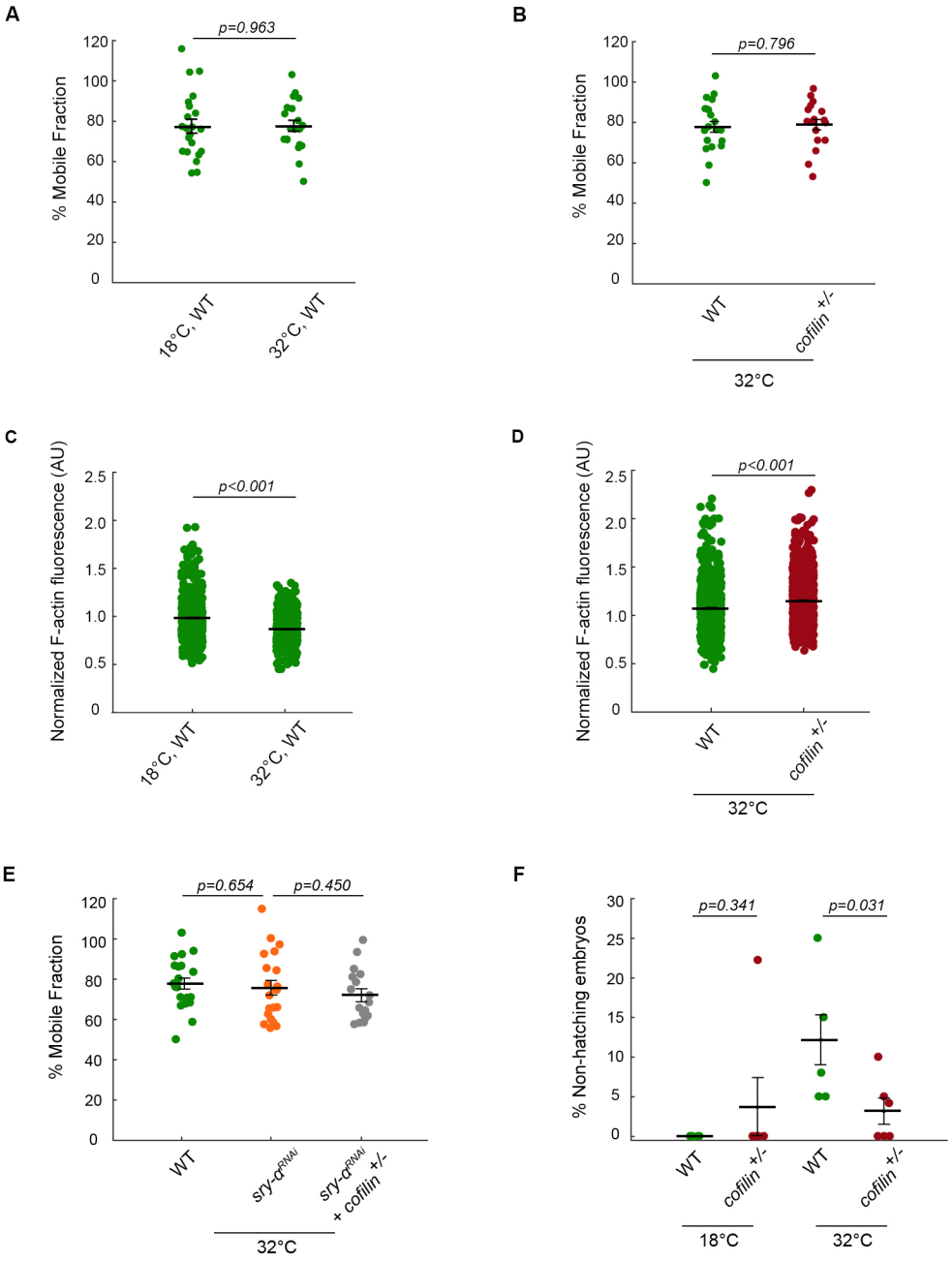


**Figure S4. Percent mobile fraction from all FRAP experiments and scatter plots for F-actin quantifications and hatching assays. Related to Figures 3 and 4.**

(A) and (B) Percent mobile fraction for furrow tip F-actin in wild-type (WT) or *cofilin*$^{+/-}$ embryos at indicated temperatures (n≥20 embryos per temperature, with 1-3 furrows analyzed per embryo). (C) F-actin levels in furrow tips in WT embryos at indicated temperatures (n≥29 embryos per temperature, with 15 furrows analyzed per embryo). (D) F-actin levels in furrow tips in WT and *cofilin*$^{+/-}$ embryos at 32°C (n≥48 embryos per condition, with 15 furrows analyzed per embryo). (E) Percent mobile fraction for furrow tip F-actin in indicated genotypes at 32°C (n≥20 embryos per temperature, with 1-3 furrows analyzed per embryo). (F) Larval hatching rates for indicated conditions (n≥5 independent experiments, with ≥9 embryos per experiment).

*Each point represents one embryo, and horizontal lines are means ± SE for (A), (B), (E) and (F).*

*Method shown in Figure S3 for (C) and (D).*

*Each point represents an individual pairwise comparison between all WT embryos and all Histone-GFP embryos, and horizontal lines are means ± SE for (C) and (D).*

*Student's t-test used to calculate P values in (A)-(F).*