Schoborg et al., http://www.jcb.org/cgi/content/full/jcb.201304181/DC1

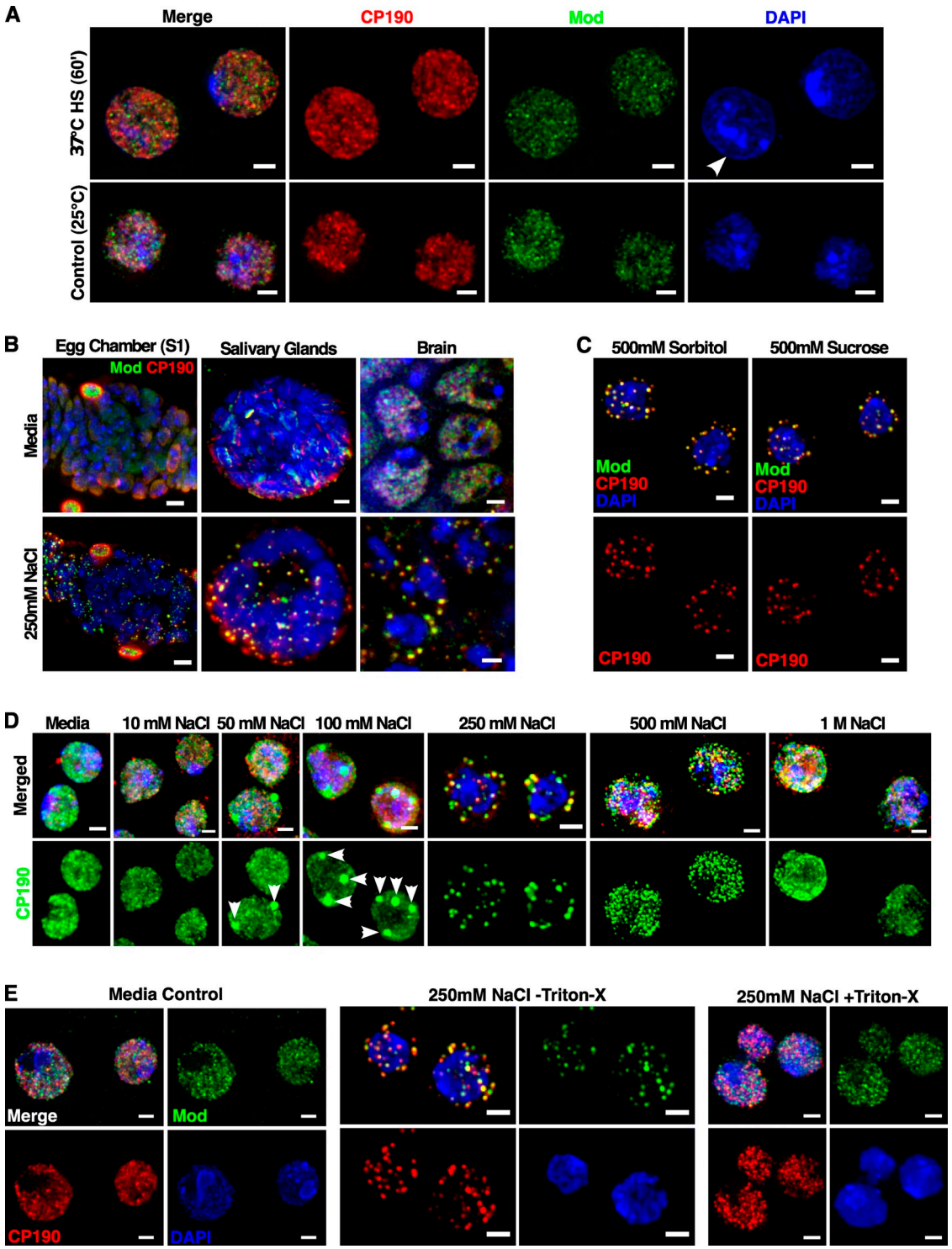


Figure S1. **Osmotic stress, but not heat shock, leads to insulator body formation.** (A) S2 cells heat shocked at 37°C for 60 min and untreated controls labeled with CP190 and Mod(mdg4). Arrowhead denotes chromatin rearrangement in HS nuclei. (B) Egg chambers, salivary glands (polytene nuclei), and brains treated with or without 250 mM NaCl and labeled with CP190 and Mod(mdg4). (C) S2 cells treated with 500 mM sorbitol or sucrose and labeled with CP190 and Mod(mdg4). (D) S2 cells treated with an increasing gradient of NaCl and labeled with CP190. Arrowheads mark CP190 bodies. (E) S2 cells treated with or without 0.2% Triton X-100 followed by addition of 250 mM NaCl and labeled with CP190 and Mod(mdg4). Bars: (A, B [brain], and C–E) 2 µm; (B, egg chamber) 4 µm; (B, salivary glands) 3 µm.

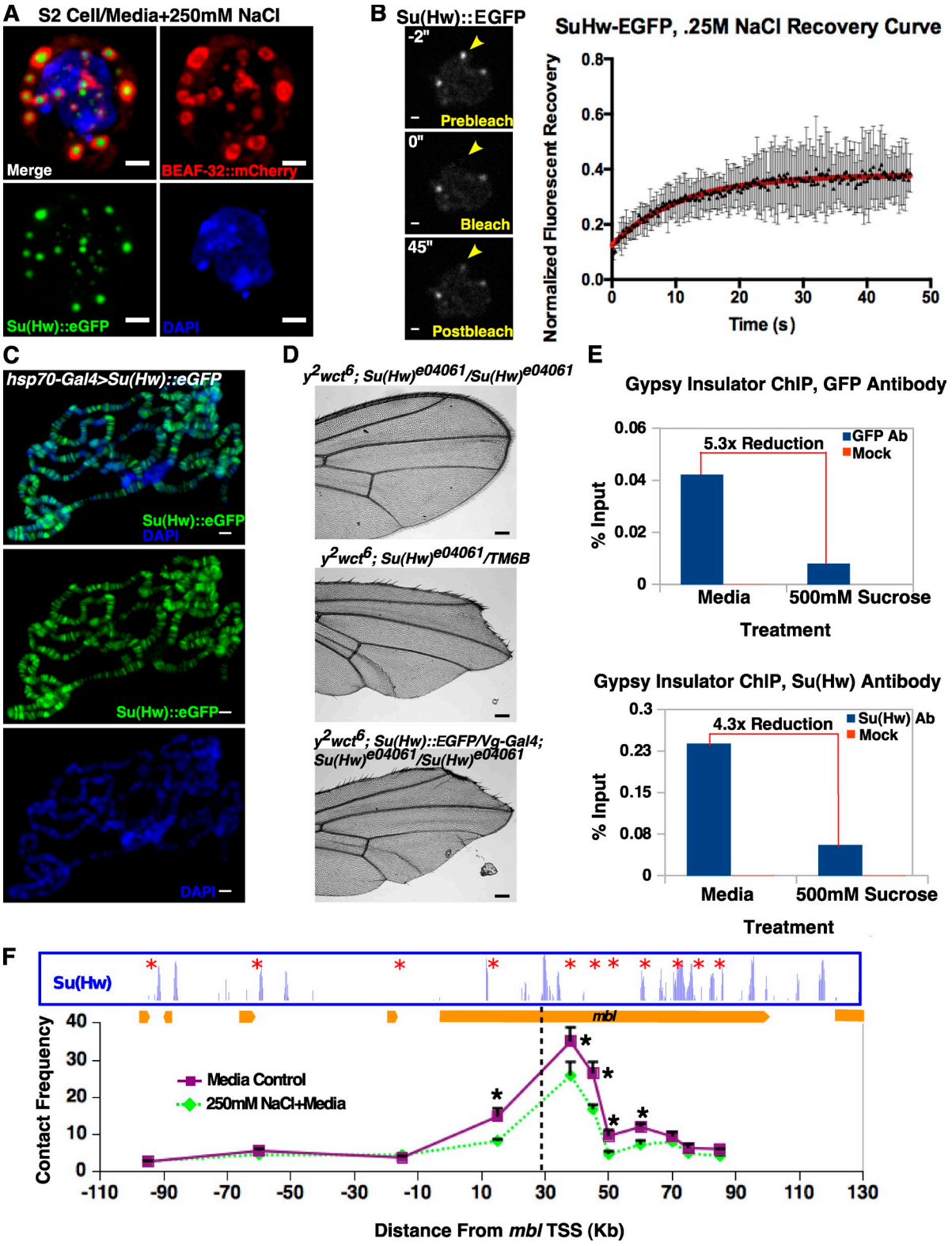


Figure S2. **The tagged Su(Hw)::EGFP accurately reproduces the behavior of the endogenous protein, and BEAF-32 donut formation is not an antibody artifact.** (A) S2 cell transfected with BEAF-32::mCherry and Su(Hw)::EGFP and stressed with 250 mM NaCl. (B) FRAP analysis of stationary bodies (error bars represent SEMs of three bodies from the same cell). Yellow arrowheads denote the location of one of the bodies used in the analysis. (C) Polytene chromosome spread from a Su(Hw)::EGFP-expressing third instar salivary gland. (D) Expression of this construct in a hypomorphic background of *su(Hw)* (*su(Hw)*^{e04061}) restores *gypsy* insulator function in *ct*^{6}. (E) ChIP using α-GFP from S2 cells expressing this construct treated with or without 500 mM sucrose shows a similar fold reduction at *gypsy* as the endogenous Su(Hw) protein. Ab, antibody. (F) 3C analysis of looping contacts throughout the *muscleblind* (*mbl*) locus in S2 cells after treatment with or without 250 mM NaCl for 5 min. The black dashed line marks the position of the 3C anchor primer with test primers noted by red asterisks. X axis denotes distance (in kilobases) from the *muscleblind* transcription start site (TSS). Blue peaks represent Su(Hw) binding sites. Statistically significant reductions are marked with black asterisks (Student's paired *t* test, P = 0.05; error bars represent SEMs). Bars: (A and B) 2 µm; (C) 3 µm; (D) 100 µm.

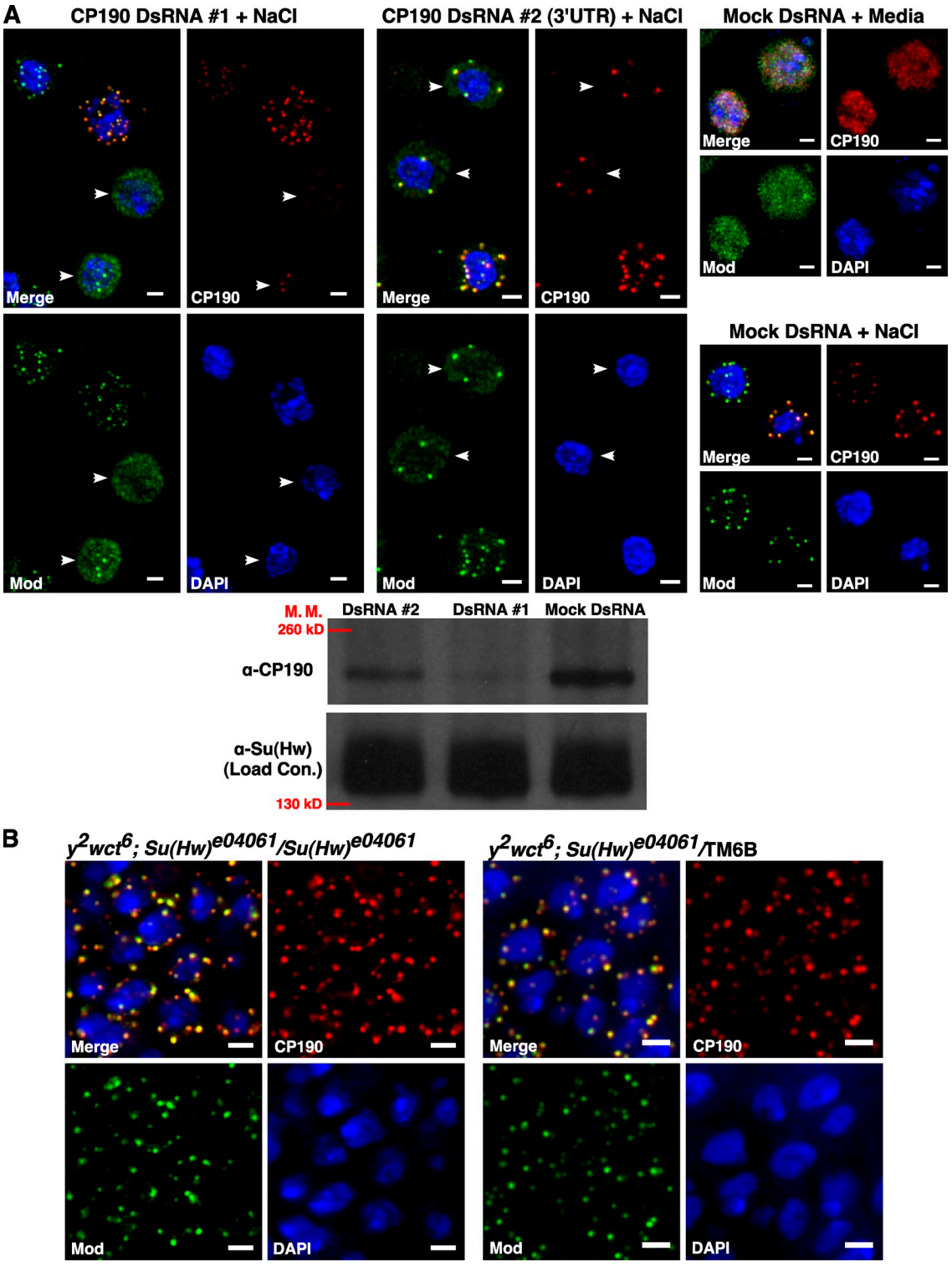


Figure S3. **DsRNA knockdown of CP190 disrupts Mod(mdg4) localization to insulator bodies in S2 cells.** (A) S2 cells soaked with two different CP190 DsRNA constructs (#1 and #2), treated with 250 mM NaCl, and labeled with CP190 and Mod(mdg4). Arrowheads mark nuclei with significantly reduced levels of CP190. Mock DsRNA controls treated with or without 250 mM NaCl are also shown. Western blot of lysates from knockdown lines compared with mock controls verifies CP190 reduction for both DsRNA constructs. Con., control; M.M., molecular mass. (B) Wing discs from $Su(Hw)^{e04061}$ homozygotes (left) and balanced controls (right) treated with 250 mM NaCl and labeled with CP190 and Mod(mdg4). Bars, 2 µm.

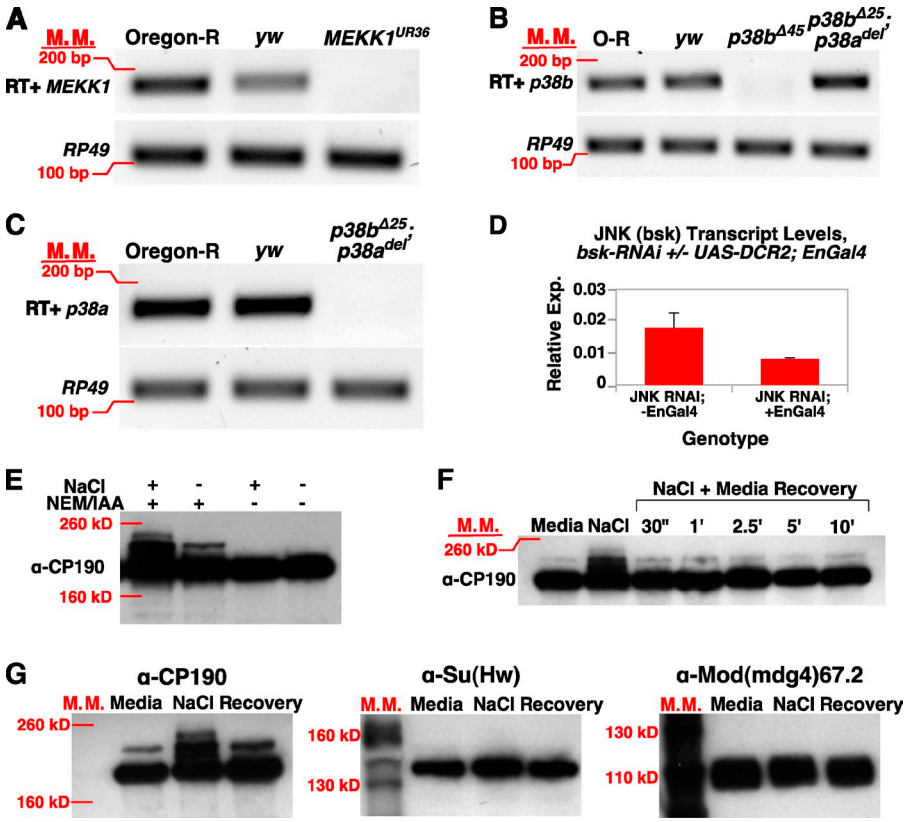


Figure S4. **Confirmation of mutant alleles for *dMEKK1* and *p38a/p38b*.** (A–C) RT-PCR from the indicated genotype confirms the absence of transcript in *dMEKK1*^{UR36} (A), *p38b*$^{\Delta 45}$ (B), and *p38a*^{del} (C) mutants (Rp49 indicates a loading control). O-R, Oregon-R. (D) qPCR analysis of *JNK* transcript levels in wing discs expressing *bsk-RNAi* under control of an *engrailed-Gal4; UAS-Dcr-2* driver, which is only active in the posterior compartment of the wing disc (error bars represent the SEMs of three biological replicates). Exp., expression. (E) Western Blot of CP190 from S2 cell lysates treated with or without 250 mM NaCl and in either the presence or absence of the SUMO isopeptidase inhibitors *N*-ethylmaleimide (NEM) and indole-3-acetate (IAA). (F) Western Blot of CP190 from S2 cell lysates treated with 250 mM NaCl and allowed to recover in isotonic media for the indicated period of time before lysing in the presence of *N*-ethylmaleimide and indole-3-acetate. (G) Western blot of CP190, Su(Hw), and Mod(mdg4) from S2 cells under conditions of media, osmostress, or recovery in fresh media (*N*-ethylmaleimide and indole-3-acetate included in lysis buffer). Molecular mass (M.M.) markers are indicated.

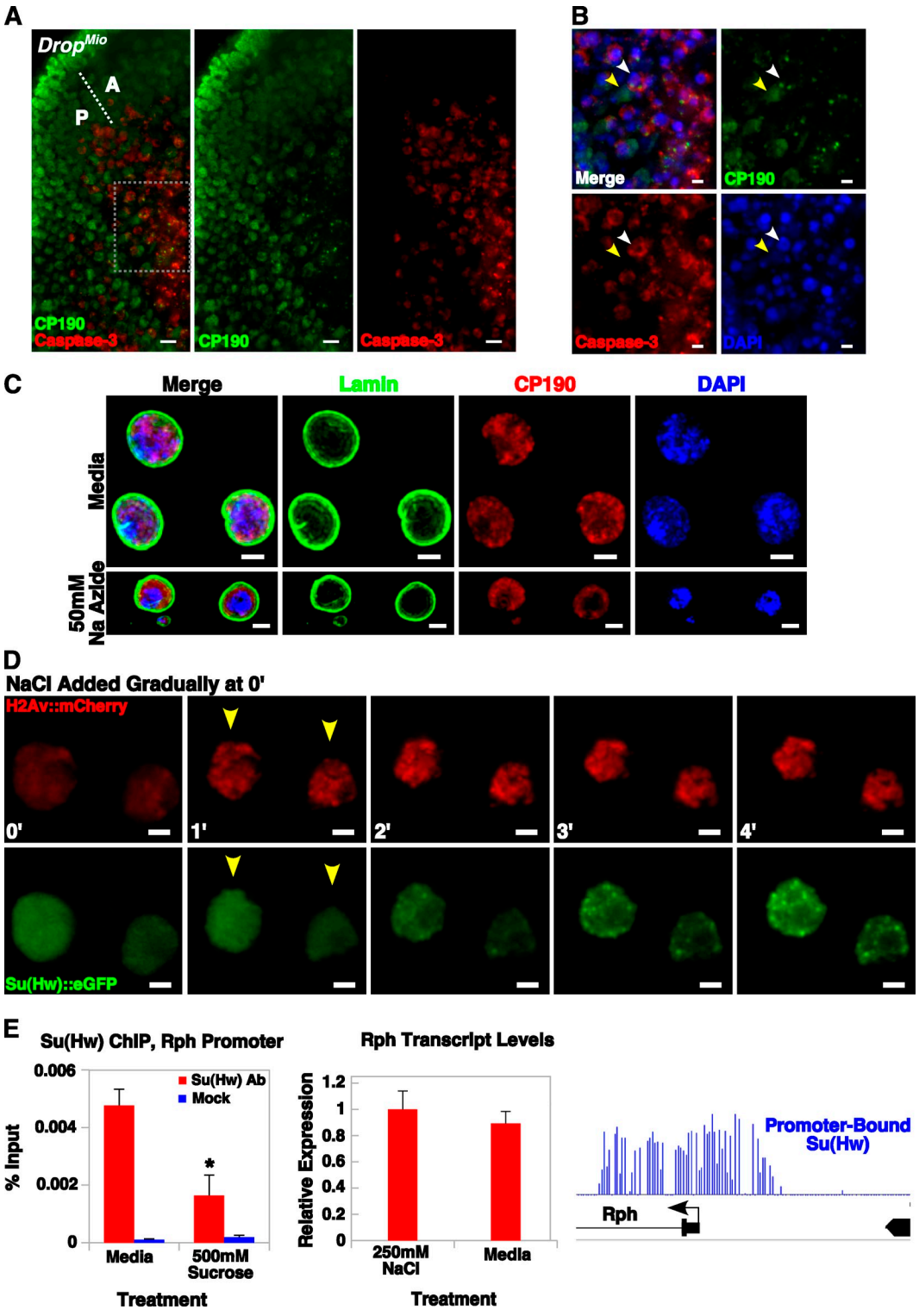
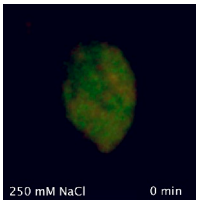
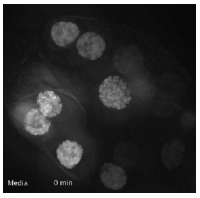


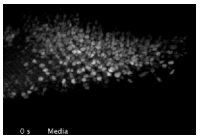
Figure S5. **Insulator bodies are also evident in tissues undergoing cell death, and body formation is not dependent on chromatin condensation nor does it lead to alterations in gene expression.** (A) Insulator bodies are also evident in tissues undergoing cell death, marked by cleaved caspase-3 in eye discs derived from *Drop*^{Mio} larvae (A/P marks the anterior–posterior orientation of the tissue). (B) A close up the gray boxed region from A shows diffuse staining for CP190, lack of cleaved caspase-3, and decondensed DAPI in healthy cells (yellow arrowheads) and formation of CP190 bodies in cells marked with caspase and condensed DAPI (white arrowheads). (C) S2 cells treated with or without low concentrations of Na azide to induce chromatin compaction independently of osmostress and labeled with lamin and CP190. (D) Time-lapse imaging of a S2 cell transfected with H2Av::mCherry to mark chromatin and Su(Hw)::EGFP stressed with 250 mM NaCl at time 0 with frames taken at 1-min intervals. First evidence of chromatin compaction is indicated by yellow arrowheads. (E) ChIP of Su(Hw) from the promoter of *Rabphilin* (*Rph*) after osmostress and the expression levels of *Rabphilin*. Asterisk marks statistically significant reductions of Su(Hw), but expression levels are not significantly altered (Student's paired *t* test, P = 0.05; error bars represent SEMs of three biological replicates). Bars: (A) 5 µm; (B–D) 2 µm.



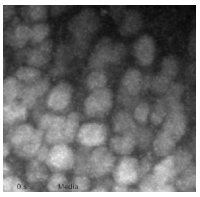
Video 1. **Insulator bodies nucleate from smaller speckles during osmostress and can undergo rounds of fusion to produce larger structures.** S2 cells transfected with Su(Hw)::EGFP (green) and BEAF-32::mCherry (red) were gradually exposed to 250 mM NaCl/SFX media starting at 0 min and imaged using time-lapse epifluorescence microscopy (DM6000 B). Z stacks for each frame were acquired every 2 min for 36 min.



Video 2. **Insulator body formation is readily reversible.** A third instar larvae salivary gland expressing Su(Hw)::EGFP (gray) was subjected to 250 mM NaCl/SFX media at 10 min and allowed to recover in isotonic SFX media at 23 min and imaged using time-lapse epifluorescence microscopy (DM6000 B). Z stacks for each frame were acquired every minute for 33 min.



Video 3. **Multiple rounds of rapid insulator body assembly and disassembly are observable in diploid tissues subjected to two rounds of 250 mM NaCl/SFX stress and isotonic media recovery, respectively.** Eye disc from a third instar *GMR-Gal4>UAS-Su(Hw)::EGFP* larvae was imaged using time-lapse epifluorescence microscopy (DM6000 B). Z stacks for each frame were acquired every 100 s for 9,300 s.



Video 4. **Close up of eye disc from Video 3 showing the initial 250 mM NaCl/SFX stress treatment on a subset of nuclei.** Cropped from 63× magnification from Video 3 using ImageJ.