Kunduri et al., http://www.jcb.org/cgi/content/full/jcb.201405020/DC1

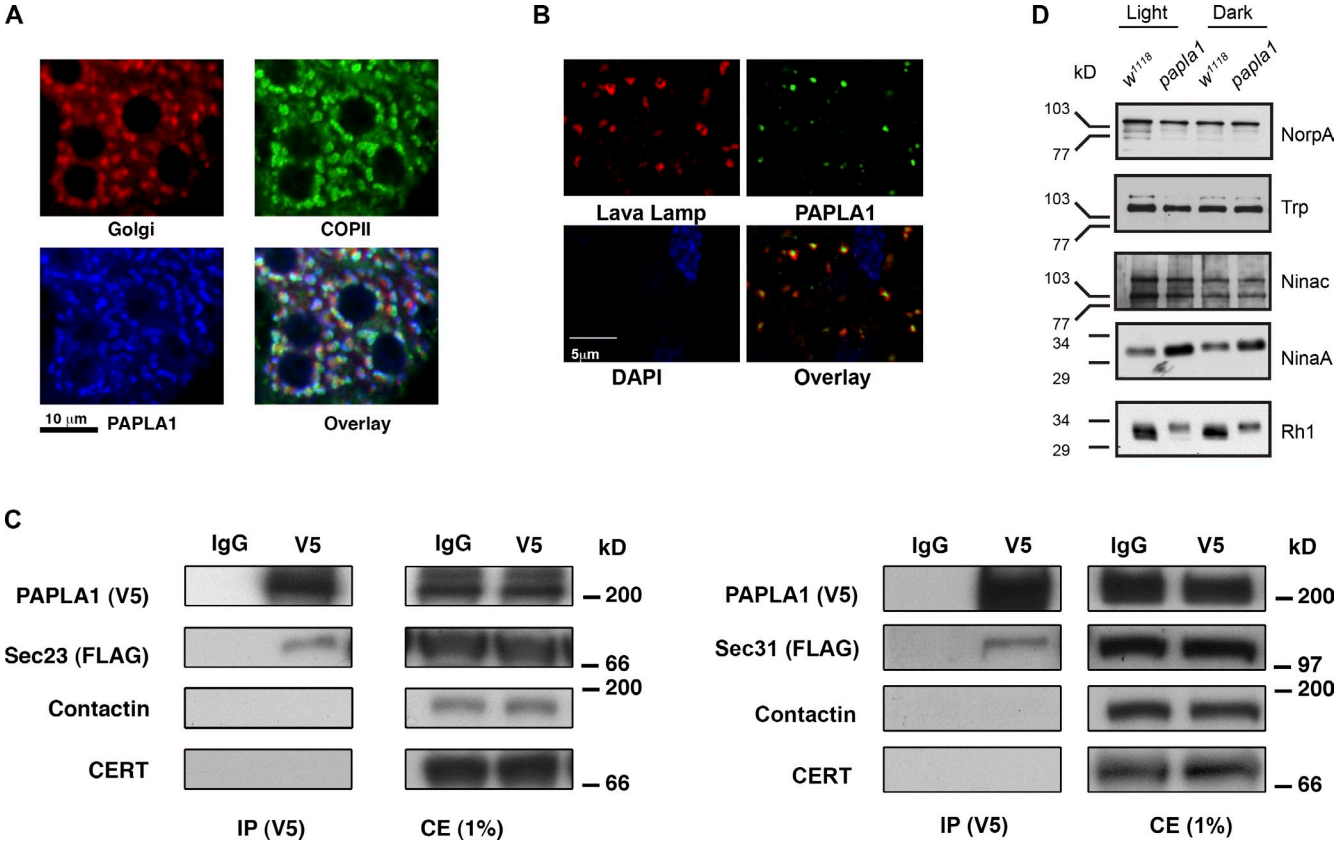

Figure S1. **Subcellular localization and interactions of PAPLA1.** (A) Immunolocalization of PAPLA1 and Sec23 in accessory glands of the male reproductive organs. PAPLA1 significantly overlaps with Sec23. (B) SR-SIM microscopic localization shows little overlap between PAPLA1 and Lava lamp, a peripheral Golgi protein. (C) PAPLA1 coimmunoprecipitates with Sec23 and Sec31. Stable S2 cells expressing V5 epitope–tagged PAPLA1 were stably transfected with either Flag-tagged Sec23 or the Flag-tagged Sec31 construct. After 24 h of induction of the proteins, PAPLA1 was immunoprecipitated using an antibody against V5. The precipitates and 1% of the flow through were then probed with anti-Flag antibody for Sec23 or Sec31. IgG was used for the control. In both instances, expressed Sec23 or Sec31 interacted specifically with PAPLA1 protein. The immunoprecipitates were probed for contactin and CERT as negative controls. (D) Western blot analysis of single fly head extracts from control and mutant flies. The left two series of blots were from 3-d-old flies that were exposed to a 12-h light/12-h dark cycle. The right two series of blots were from flies that were raised completely in the dark. The *papla1* mutant shows a decrease in the steady-state levels of Rh1 and is of a slightly higher molecular mass. In addition, the levels of the NinaA protein are increased in the mutant. CE, crude extract; IP, immunoprecipitation.

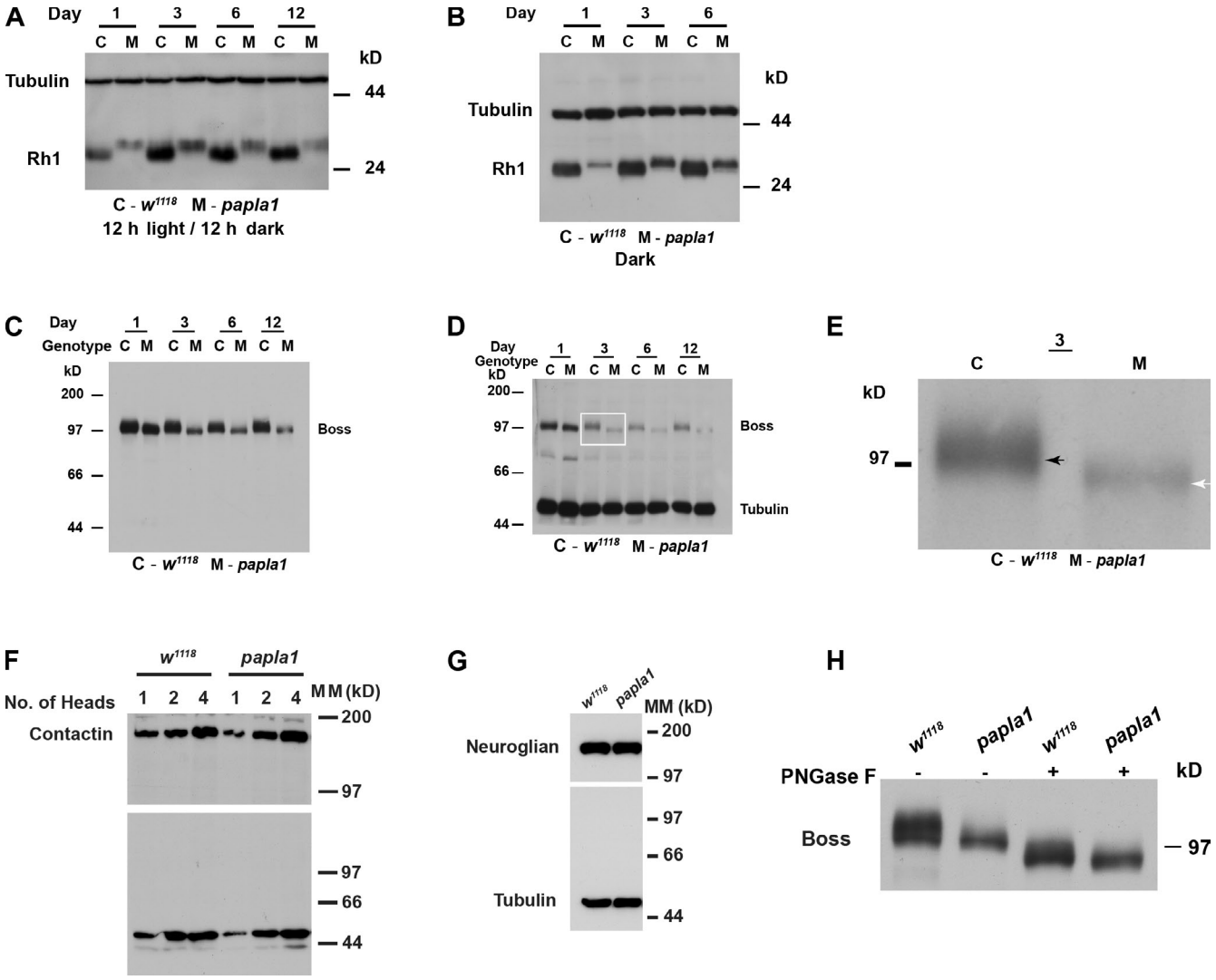

Figure S2. **Rh1 and Boss proteins are progressively lost in *papla1* mutants.** (A) Western analysis of head extracts from fly heads of 1- to 12-d-old flies. As can been seen, there is a progressive loss of Rh1 protein in the head extracts of *papla1* mutants exposed to a 12-h light and 12-h dark cycle. (B) A slight loss of Rh1 protein is also observed between day 3 and day 6 in flies kept completely in the dark, wherein activation of the phototransduction cascade is kept to a minimum. (C) Western blot analysis of the Boss protein in flies aged from day 1 to day 12 also shows a progressive decline in the steady-state levels of the protein in the *papla1* mutant. (D) The blot from C was probed with an antibody for tubulin. The previous probing for Boss is still visible, and we clearly see the small but consistent decrease in the molecular mass of the protein in *papla1* mutants. This difference is appreciated better in the enlarged section of the boxed area in E. The black and white arrows highlight mobility of Boss protein in the control and *papla1* mutant samples, respectively. (F) Proteins from one, two, and four head extracts from 3-d-old control and mutant flies were separated on a 6% SDS-PAGE gel and probed with an antibody against contactin. The blots were reprobed for tubulin as a loading control. (G) 3-d-old control and mutant fly head extracts were run on a 6% gel, probed for neuroglian, and reprobed for tubulin as a loading control. (H) Western blot analysis of Boss molecular masses before and after treatment with PNGase F in control w^{1118} and *papla1* mutants. The small difference in the molecular masses of the Boss protein from the control and the mutant dissipates upon treatment with PNGase F, and both now migrate as a lower molecular mass protein. C, control; M, mutant; MM, molecular mass.

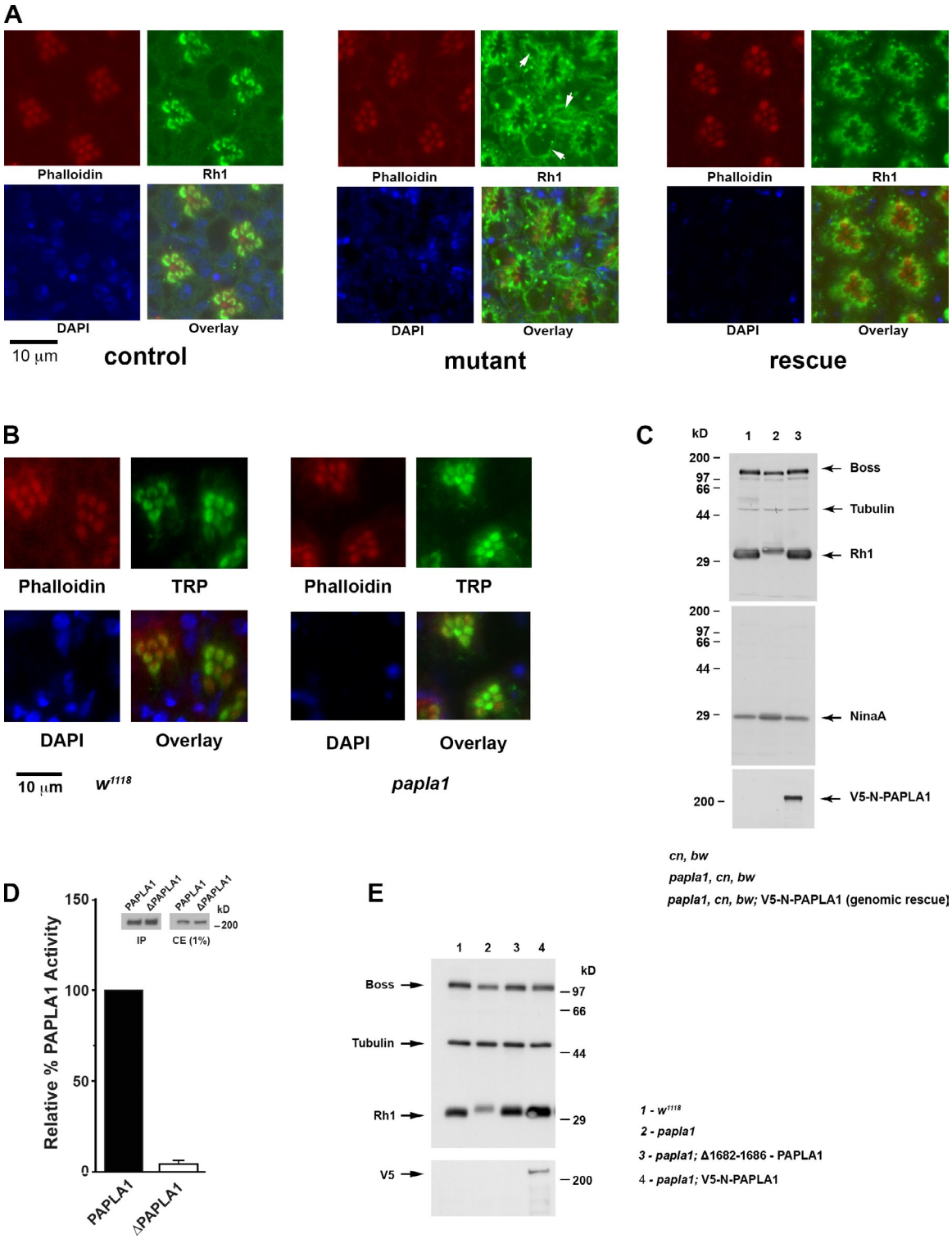

Figure S3. **Immunolocalization experiments and rescue of *papla1* mutant.** (A) Whole-mount immunostaining of retina from control w^{1118}, mutant *papla1*, and mutant *papla1* with V5-N-PAPLA1 genomic rescue. The rhabdomeres were stained with Alexa Fluor 568 phalloidin, Rh1 was stained with a monoclonal antibody, and nuclei was stained with DAPI. (top) Rh1 is almost wholly localized to the rhabdomere in the control photoreceptor cells in whole-mount immunostaining of ommatidia. (middle) However, in the mutant, although we do see staining at the base of the rhabdomeres, we also see ectopic staining of Rh1 in photoreceptor cells. V5-N-PAPLA1 restores the staining of the mutant Rh1 to wild type–like distribution. The aberrantly localized Rh1s are highlighted by the white arrows. (B) Whole-mount immunostaining of retina from control w^{1118} and mutant *papla1* with Trp antibody. In both instances, Trp is localized to the rhabdomere, indicating that its transport is not compromised in the *papla1* mutant. (C) V5-N-PAPLA1 rescues the biochemical defect observed in the *papla1* mutant. 3-d-old control w^{1118}, *papla1* mutant, and V5-N-PAPLA1 rescued fly head extracts were run on an 8% gel and probed for Rh1, tubulin, and Boss, successively. They were also subsequently probed for the NinaA protein. For the V5 detection, head extracts were run from the respective genotypes on a 6% gel and probed with a monoclonal antibody against V5. (D) The C-terminally V5 epitope–tagged wild type and the active site deletion mutant (Δ1,682–1,686) were expressed in the S2 cells and immunoprecipitated using V5 antibody (inset). PAPLA1 is the wild-type protein, ΔPAPLA1 is the deletion mutant, and CE is the crude extract. The immunoprecipitated proteins were analyzed for PAPLA1 activity. The active site mutant is inactive with only background activity. The error bar indicates standard error of mean. IP, immunoprecipitation. (E) The active site mutant restores the level and glycosylation pattern of *papla1* mutant G proteins to that of the wild type. 1-d-old fly head extracts were used in the set of experiments shown in the figure. The data were similar from 3-d-old flies also (not depicted).

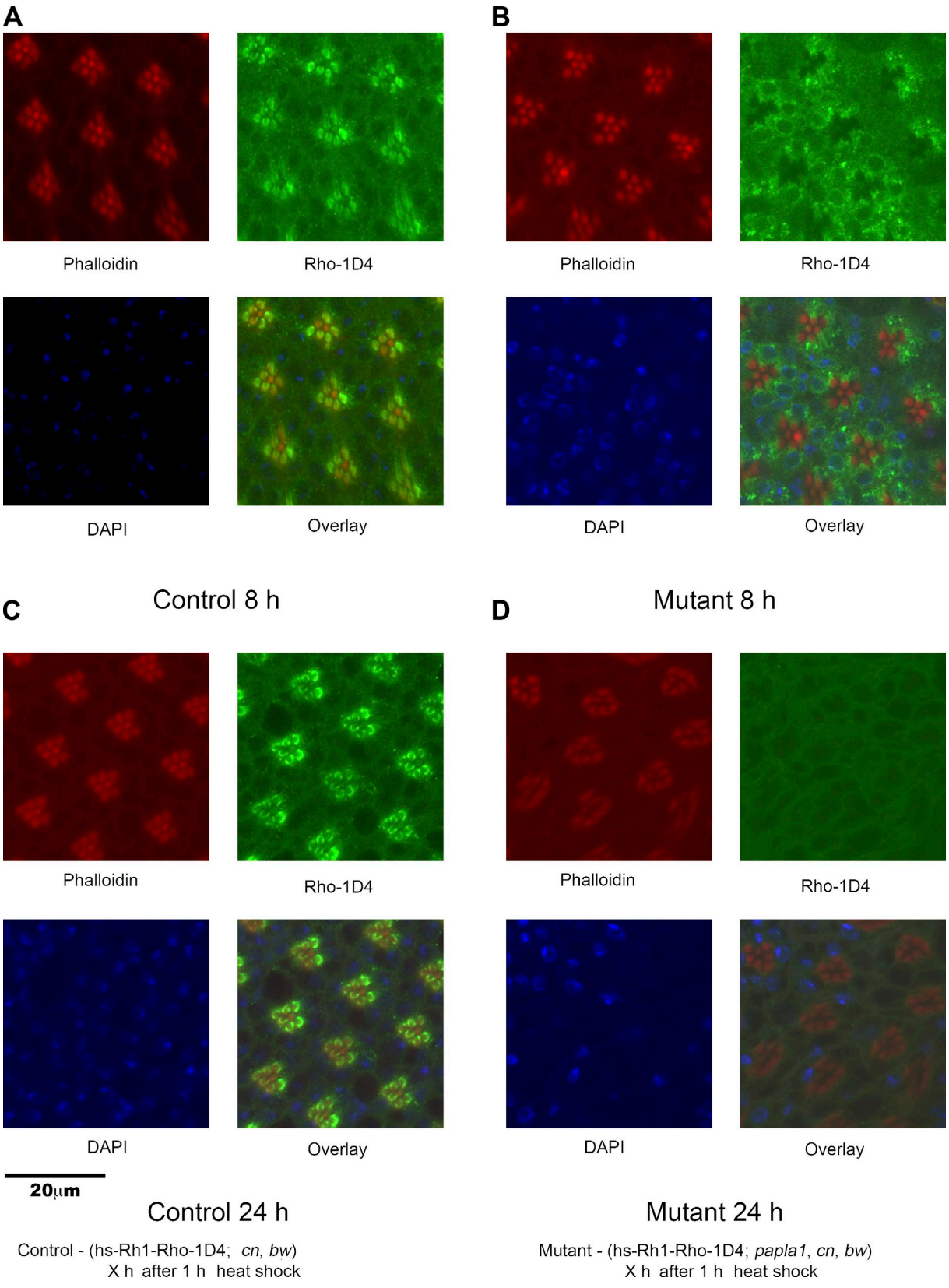

Figure S4. **hs-Rh1-1D4 experiments in control and *papla1* mutant photoreceptor cells.** (A–D) hs-Rh1-1D4 was induced in control (A and C) and *papla1* (B and D) photoreceptors with a 1-h hs at 37°C, and the retinae were dissected after 8 h (A and B) or 24 h (C and D). By 8 h, Rh1 is beginning to be concentrated in the wild-type retina. In the mutant, although some crescent-shaped staining is visible at the base of the retina, much of the Rh1 is distributed around the nucleus and other ectopic regions of the cell. At 24 h after hs, the Rh1 staining is predominantly in the rhabdomeres in the control, whereas in the mutant, the rhabdomeric staining of the Rh1 is minimal, and much of the signal seen is in the other ectopic regions.

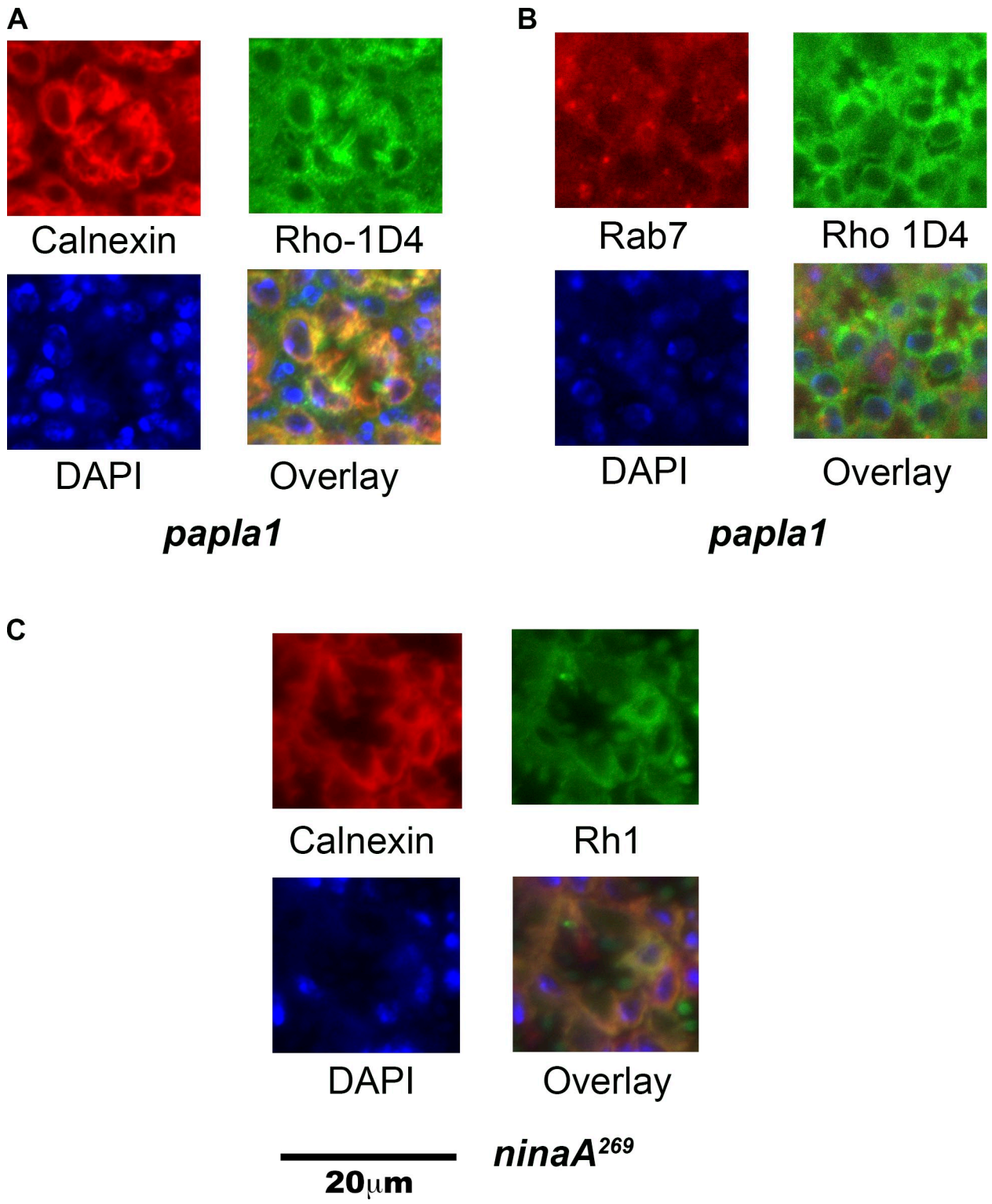

Figure S5. **Aberrant localization of Rh1 in hs-Rh1-1D4 *papla1* mutants 10 h after hs.** (A and B) Rh1 colocalizes with calnexin in papla1 mutants (A), whereas Rab7 shows very little overlap in the staining pattern (B). Rab 7 appeared in punctate structures but generally showed a diffuse staining that showed no specific pattern of overlap with the Rh1. (C) Rh1 immunolocalization of Rh1 in ninaA^{269} mutants shows that much of it colocalizes with calnexin, an ER-resident protein.