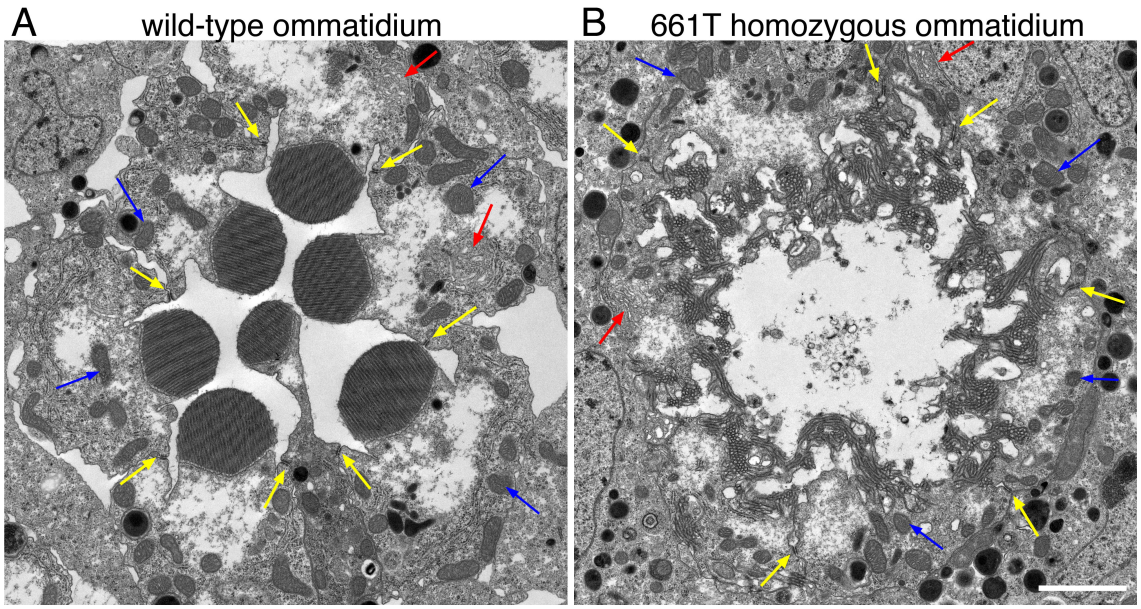

Fig. S1 Wild-type and 661T homozygous ommatidia at high resolution. Higher resolution of electron micrographs of the wild-type (A) and 661T homozygous ommatidia (B), shown in Fig. 1C and H. Yellow, red, and blue arrows indicate the adherens junctions, Golgi units, and mitochondria, respectively. Scale bar: 2 μm (A, B).

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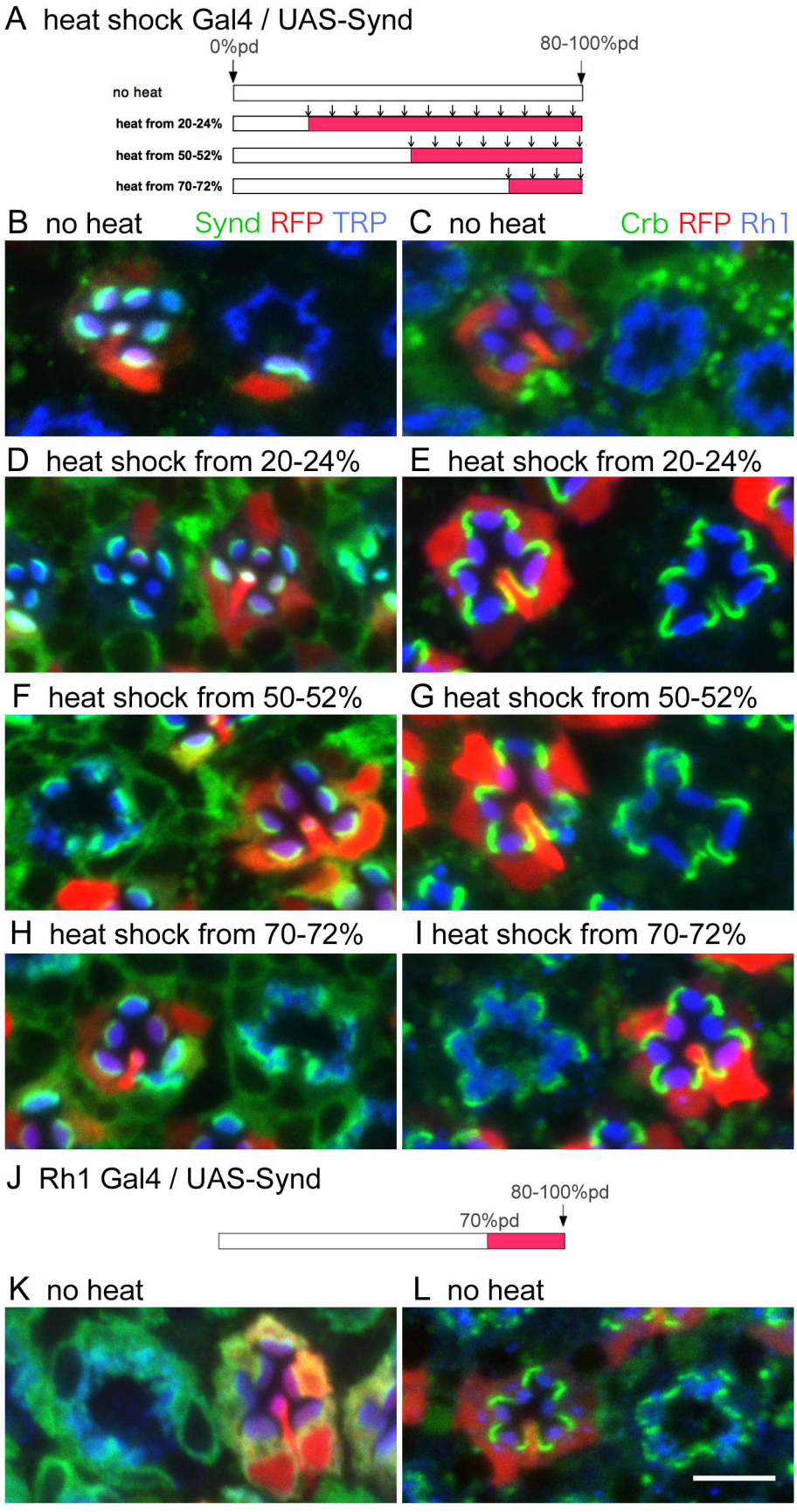

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Fig. S2 Synd expression rescues the 661T mutant phenotype. Immunostaining of the late pupal 661T mutant mosaic eyes expressing Synd full length protein driven by a heat shock-Gal4 driver (B–I) or an Rh1-Gal4 driver (K, L). RFP (red) indicates wild-type cells. (A) Schematic representation of the heat shock procedure (arrow) and Synd expression (pink bar) driven by the heat shock-Gal4 driver. (J) Schematic representation of Synd expression (pink bar) driven by the Rh1-Gal4 driver. (B, D, F, H, K) Green represents Synd and blue represents TRP. (C, E, G, I, L) Green represents Crb and blue represents Rh1. 8, 6, 5, 5, 4, 4, 3, 4, 5 and 3 independent eyes were observed in B, C, D, E, F, G, H, I, K and L, respectively. Scale bar: 5 μm (B–I, K, L).

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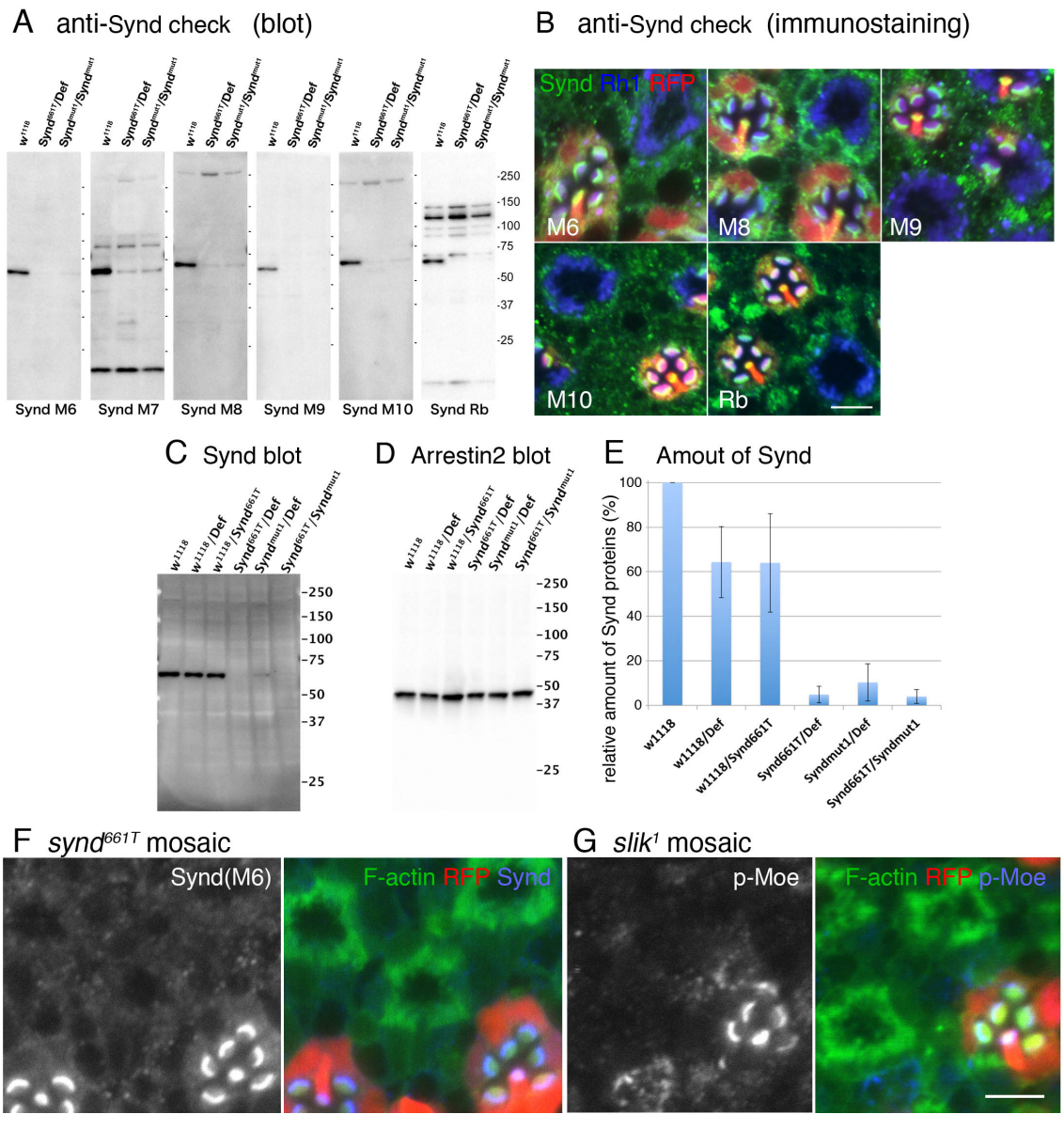

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Fig. S3 Verification of anti-Synd and anti-Moe antisera, and characterization of the Synd^{661T} allele. (A) Immunoblotting of the fly head extracts of w^{1118} (wild-type), Synd^{661T}/Def, and Synd^{mut1} homozygous flies using mouse anti-Synd (M6-10) and rabbit anti-Synd (Rb) antibodies. (B) Immunostaining of the Synd^{661T} mutant mosaic eyes using mouse anti-Synd (M6, 8-10) and Rb anti-Synd antibodies (green). The mosaic eyes were also stained with Rh1 to visualize the rhabdomeres (blue). RFP (red) indicates wild-type cells. Scale bar: 5 μm. 1, 2, 2, 5 and 3 independent eyes were observed in M6, M8, M9, M10 and Rb, respectively. (C, D) Immunoblotting of the fly head extracts of w^{1118} (wild-type), w^{1118}/Def, w^{1118}/ Synd^{661T}, Synd^{661T}/Def, Synd^{mut1}/Def, and Synd^{661T}/Synd^{mut1} flies using mouse anti-Synd M6 (C) or anti-Arrestin2 antibodies (D). (E) Quantification of Synd normalized by Arrestin2 in the fly head extracts of w^{1118} (wild-type), w^{1118}/Def, w^{1118}/ Synd^{661T}, Synd^{661T}/Def, Synd^{mut1}/Def, and Synd^{661T}/Synd^{mut1} flies. Data are expressed as the means ± s.d. (n = 3). (F, G) Immunostaining of *Synd^{661T}* (F) and *Slik^{1}* (G) mutant mosaic eyes by high concentration of mouse anti-Synd (1/40) (F) or anti-pMoe antibodies (1/12) (blue) (G). Secondary antibodies are also used at high concentration (1/100). Phalloidin staining is shown in green. RFP (red) indicates wild-type cells. 9 and 7 independent eyes were observed in F and G, respectively. Scale bar: 5 μm.

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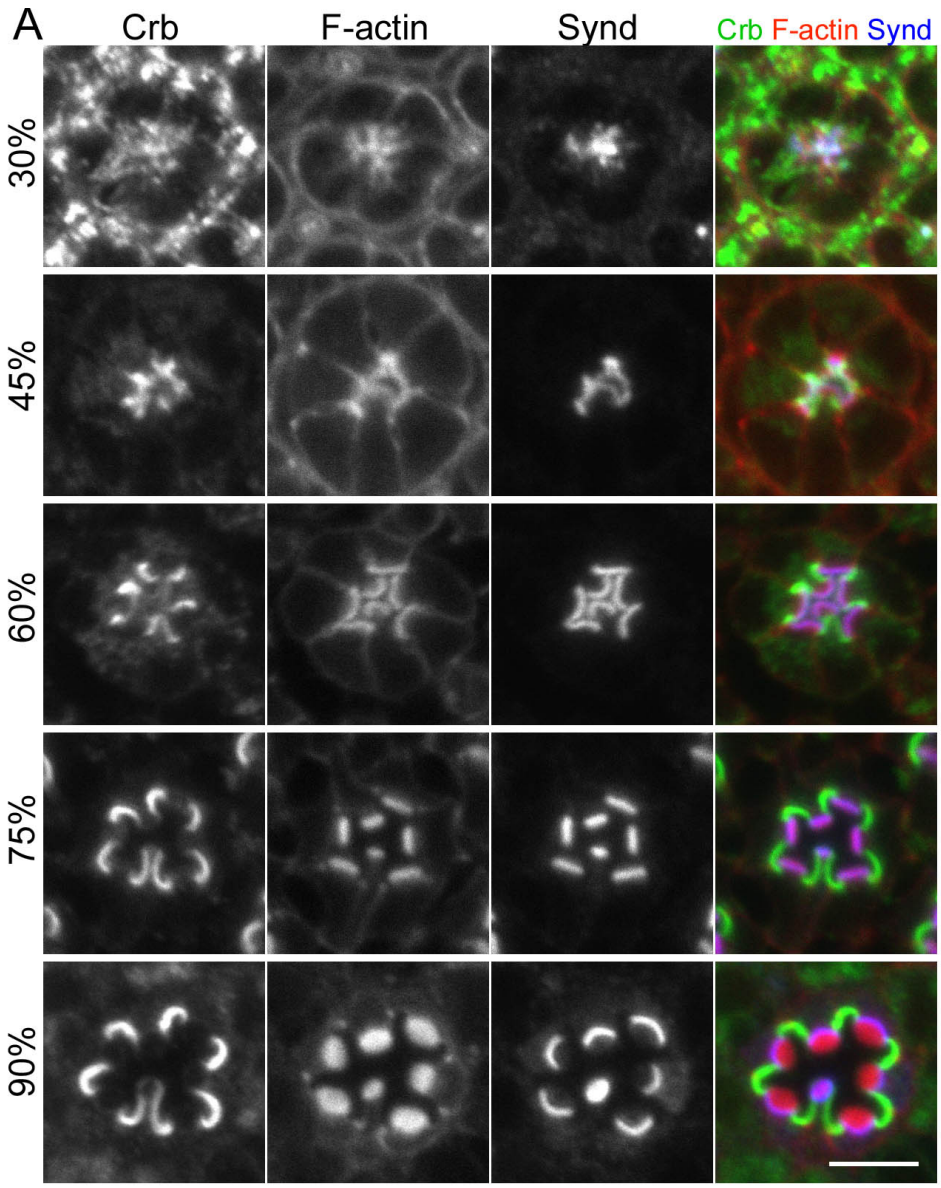

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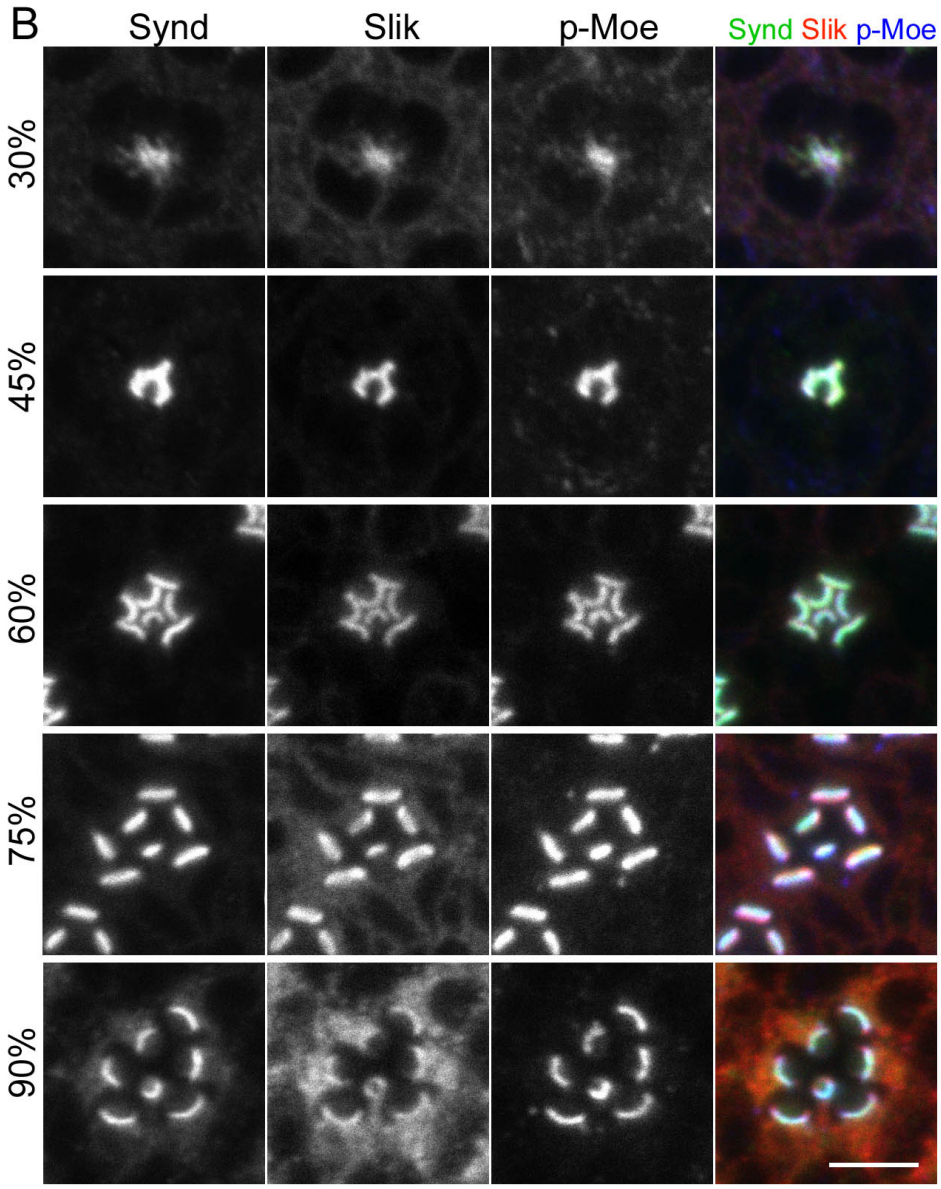

Fig. S4 Synd, Crb, p-Moe, and Slik localization in ommatidia during development. (A) Immunostaining of the w^{1118} (wild-type) retinas from the pupae at 30%, 45%, 60%, 75%, and 90% pd by anti-Crb (green), phalloidin (red), and anti-Synd antibodies (blue). 4, 4, 3, 4 and 4 independent eyes were observed in 30%, 45%, 60%, 75% and 90%, respectively. (B) Immunostaining of w^{1118} (wild-type) retinas from the pupae at 30%, 45%, 60%, 75%, and 90% pd by anti-Synd (green), Slik (red), and anti-p-Moe antibodies (blue). 7, 10, 4, 4 and 3 independent eyes were observed in 30%, 45%, 60%, 75% and 90%, respectively. Scale bar: 5 μm (A, B).

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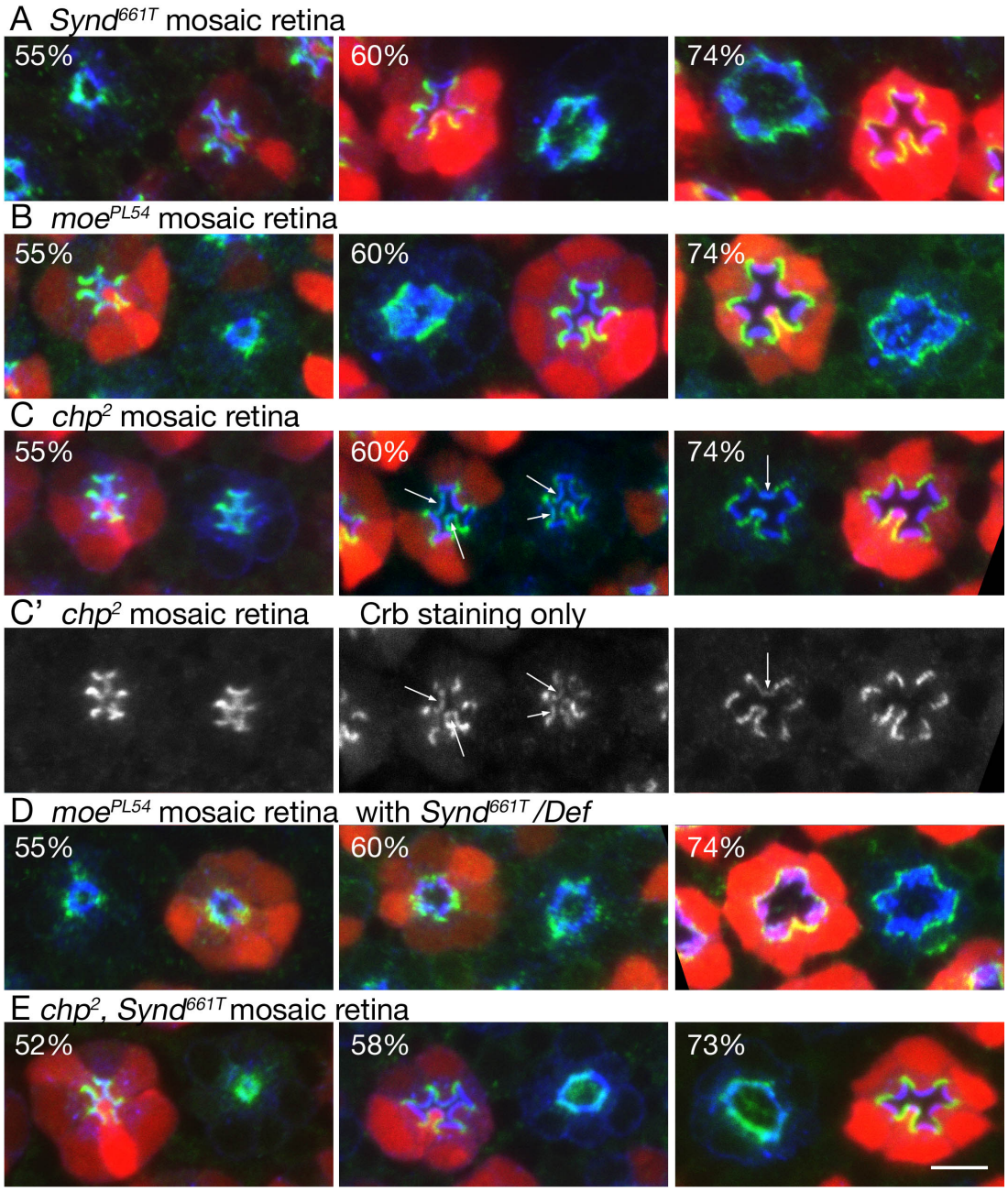

Fig. S5 Stalk-rhabdomere segregation in *Synd*^{661T}, *Moe*^{PL54}, and *chp*^{2} single and double mutant ommatidia during development. (A–D) Immunostaining of the mosaic retinas with indicated genotypes from the pupae at 55%, 60%, and 74% pd by anti-Crb (green) and anti-TRP antibodies (blue). RFP (red) indicates the wild-type (A–C) or *Synd*^{661T} single mutant photoreceptors (D). C' shows anti-TRP staining of *chp*^{2} mosaic retina (C). (E) Immunostaining of *chp*^{2} and *Synd*^{661T} double mutant mosaic retinas from the pupae at 52%, 58%, and 73% pd by anti-Crb (green) and anti-TRP antibodies (blue). RFP (red) indicates the wild-type. Scale bar: 5 μm (A–E).

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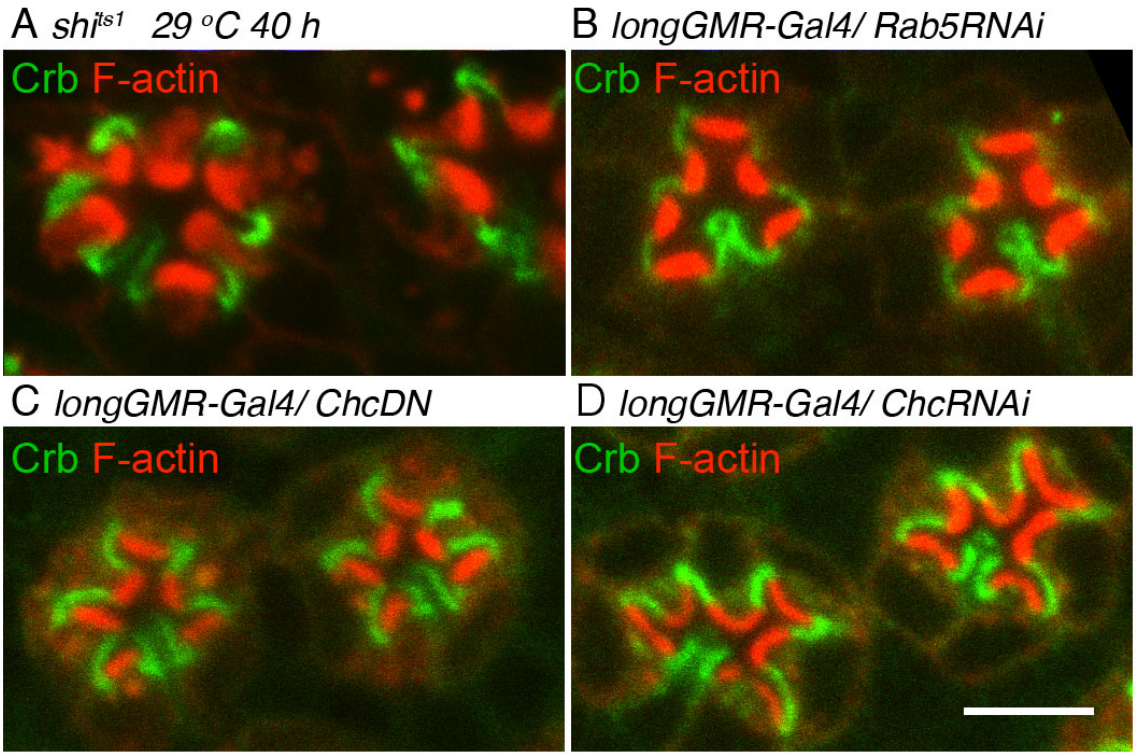

Fig. S6 Endocytosis is not required for stalk-rhabdomere segregation. (A-D) (A) Immunostaining of retinas from *shi*^{ts1} homozygous flies by anti-Crb antibody (green) and F-actin (red). *shi*^{ts1} homozygous flies are maintained at 20 °C, and unstaged pupae were randomly corrected and incubated at 29°C for 40 h, and pupae with gray-wings were fixed, which are supposed to be 80-100% pd. (B-D) Immunostaining of retinas expressing Rab5RNAi (B), ChcDN (C) or Chc RNAi (D) by longGMR-Gal4 by using anti-Crb antibody (green) and F-actin (red). 2, 3, 4 and 5 independent eyes were observed in G, H, I and J, respectively. Scale bar: 5 μm (A, B).

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