Supplemental Material :

"Distinct visual pathways mediate *Drosophila* larval light avoidance and circadian clock entrainment"

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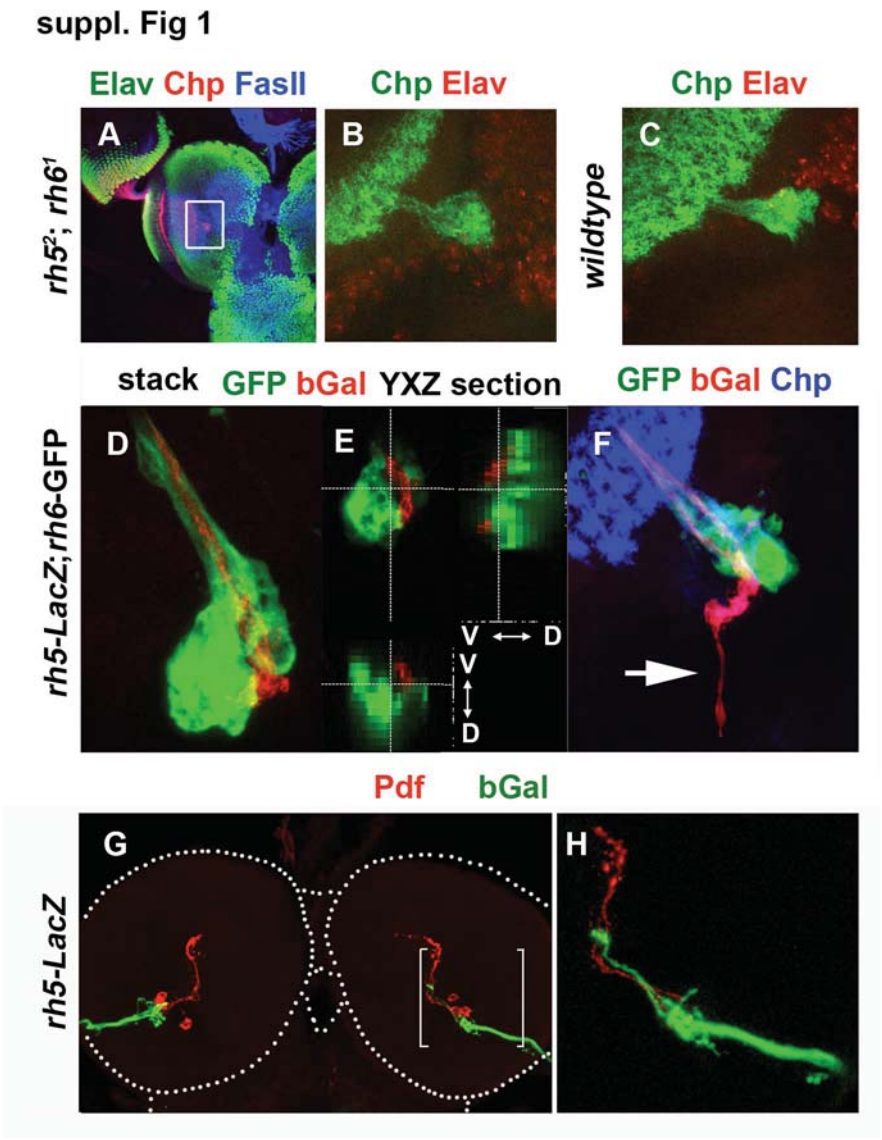
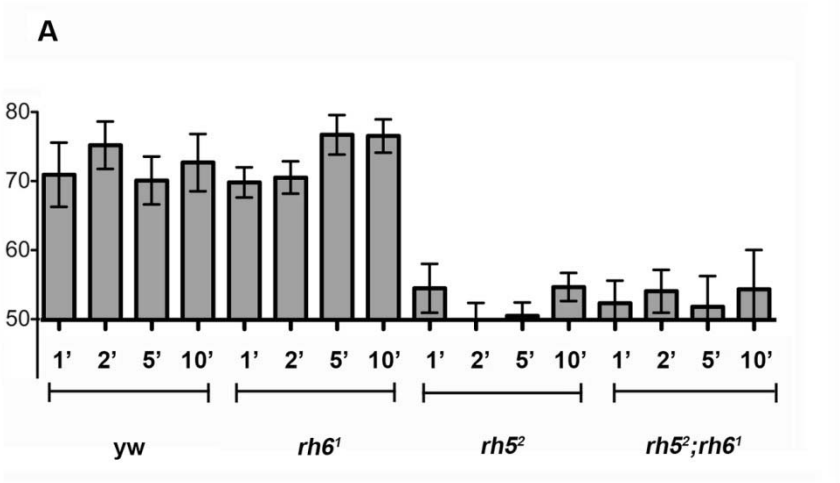
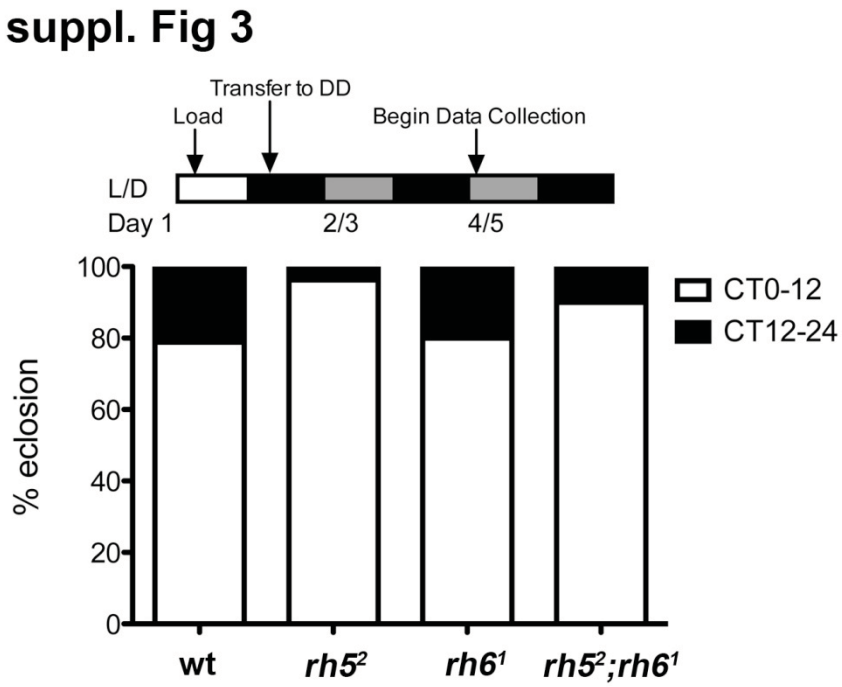


Supplemental Figure 1:
Neuropil formation in *rhodopsin* mutants and projections of larval PR-subtypes. **(A)** Projection of larval PRs is unaffected in *rh5*,*rh6* double mutants, neurons of the brain and optic lobe neuropil are labeled with anti Elav (green); anti-FasII (blue) labels PR axons and a subset of central brain axon fascicles; compare to Figure 1. B-F shows the LON in high-resolution, outlined section in A represents the area of display and orientation. **(B, C)** High magnification of LON labeled with anti-Chp (green) of *rh5*,*rh6* double mutants **(B)** and wild type **(C)** larvae (neurons shown with anti-Elav, red). **(D,E)** high magnification of projection domains of larval Rh6-PRs (shown with *rh6*-GFP, anti-GFP in green) and Rh5-PRs (shown with *rh5-LacZ*, anti-ßGal in red). **(D)** merge of whole stack shows the overall location of Rh5-termini in comparison to Rh6-termini, while XYZ cross section **(E)** shows the exact dimensions of and locations of terminal arbors. Rh5-PRs terminate in the medial-ventral part of the LON. **(F)** In several cases Rh5-PRs bypassed the LON projection into the central neuropile (Arrow). **(G)** Double labeling of *rh5-LacZ* larvae with anti-β-Gal (Green) and anti-PDF reveals Rh5-PRs project near PDF-neurons. **(H)** Single slice image reveals Rh5-PR projections both innervate and bypass the dendtrites of PDF-expressing LNs.

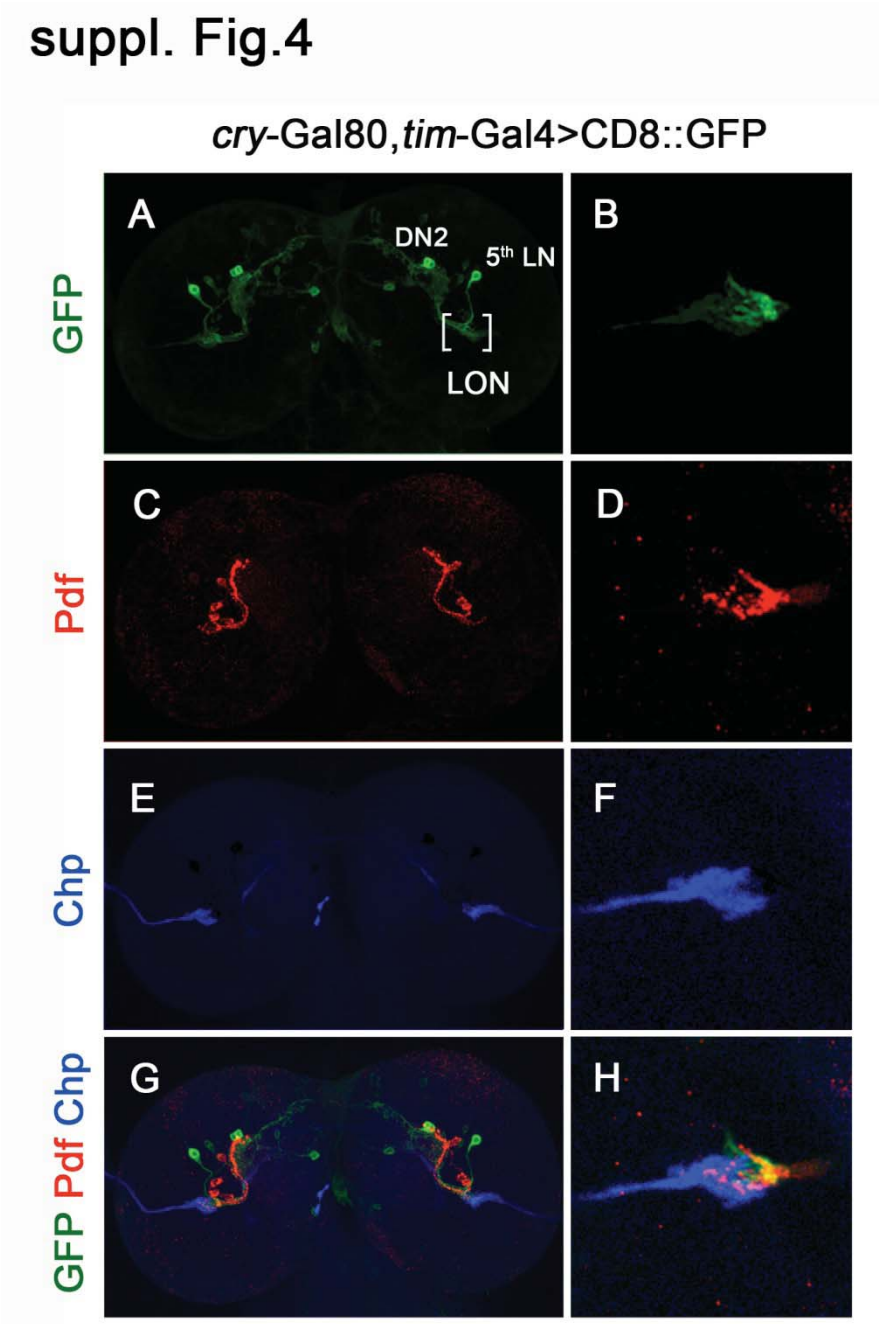
suppl. Fig 2



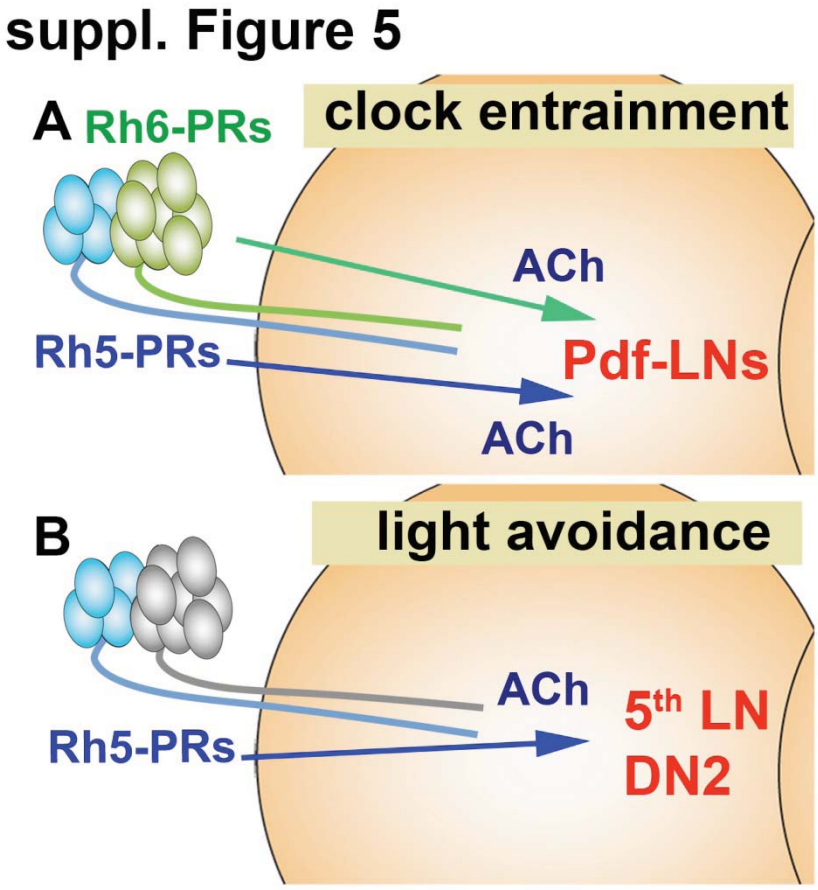
Supplemental Figure 2.
Time-course confirms involvement of *rh*5, but not *rh*6 in light avoidance. To deterimine the time-course of light avoidance we developed a novel lightbox assay. The protocol remains consistent with the light avoidance assay from Mazzoni et al (2005) except larvae half the Petri-dish is illuminated from the bottom. The percentage of larvae on dark was quantified at 1, 2, 5 and 10 minutes. At all time-points tested *rh6* larvae did not differ from *yw* controls, while *rh5* and *rh5*, *rh6* double mutants had reduced light avoidance.



Supplemental Figure 3.
Rh5 and Rh6 are dispensable for larval entrainment of eclosion rhythms.
Larvae mutant for either PR PR-subtype by itself, or both-PRs combined display robust preference for subjective daytime-eclosion. Presented are the cumulative percentage of eclosions from CT0-CT12 (open bars) and CT12-24 (black bars).



Supplemental Figure 4.
Triple labeling of *tim*-Gal4, *cry*-Gal80 confirms that PDF negative LNs are innervated by BO.
tim-Gal4, *cry*-Gal80 flies harboring the UAS-*CD8::GFP* transgenes were dissected and and stained with anti-GFP **(A,B)** anti-PDF **(C,D)** and Anti-Chp **(E, F)**. **(A,C,E,G)** represent whole-brain confocal stacks. **(B,D,F,H)** represent 1μm single-slice image of a single brain hemisphere. Overlay **(G, H)** reveals that both anti-PDF and anti-GFP co-localize with anti-Chp, suggesting both PDF and non-PDF expressing LNs are innervated by BO.



Supplemental Figure 5.
Schematic representation Rh5- and Rh6 PR function.
(A) Either PR-subtype by itself is sufficient for light-induced TIM degradation in LNs. **(B)** Rh5-PRs and DN2s are required for light avoidance. Both Rh5 and Rh6 signal through ACh.