Chartier et al., http://www.jcb.org/cgi/content/full/jcb.201203083/DC1

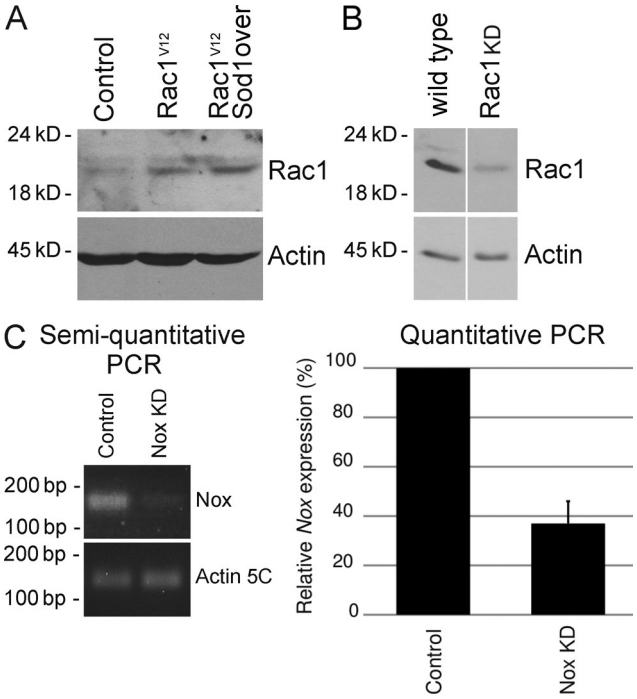

Figure S1. **Activation of Rac1 induces ROS production in *Drosophila*.** (A) Western blot showing that the expression of $Rac1^{V12}$ is not affected by concomitant overexpression of Sod1 in embryos. (B) Heads from control flies or from flies carrying Rac1 knockdown retinas were homogenized. Panels show a Western blot of Rac1 and Actin, which was used as loading control. Rac1 knockdown is efficient. (C) After RNA extraction and RT-PCR, a semiquantitative PCR was performed to assess knockdown of Nox. Actin 5C was used as control. The decrease in Nox expression was quantified by quantitative PCR (63% decrease; normalized to Actin 5C levels).

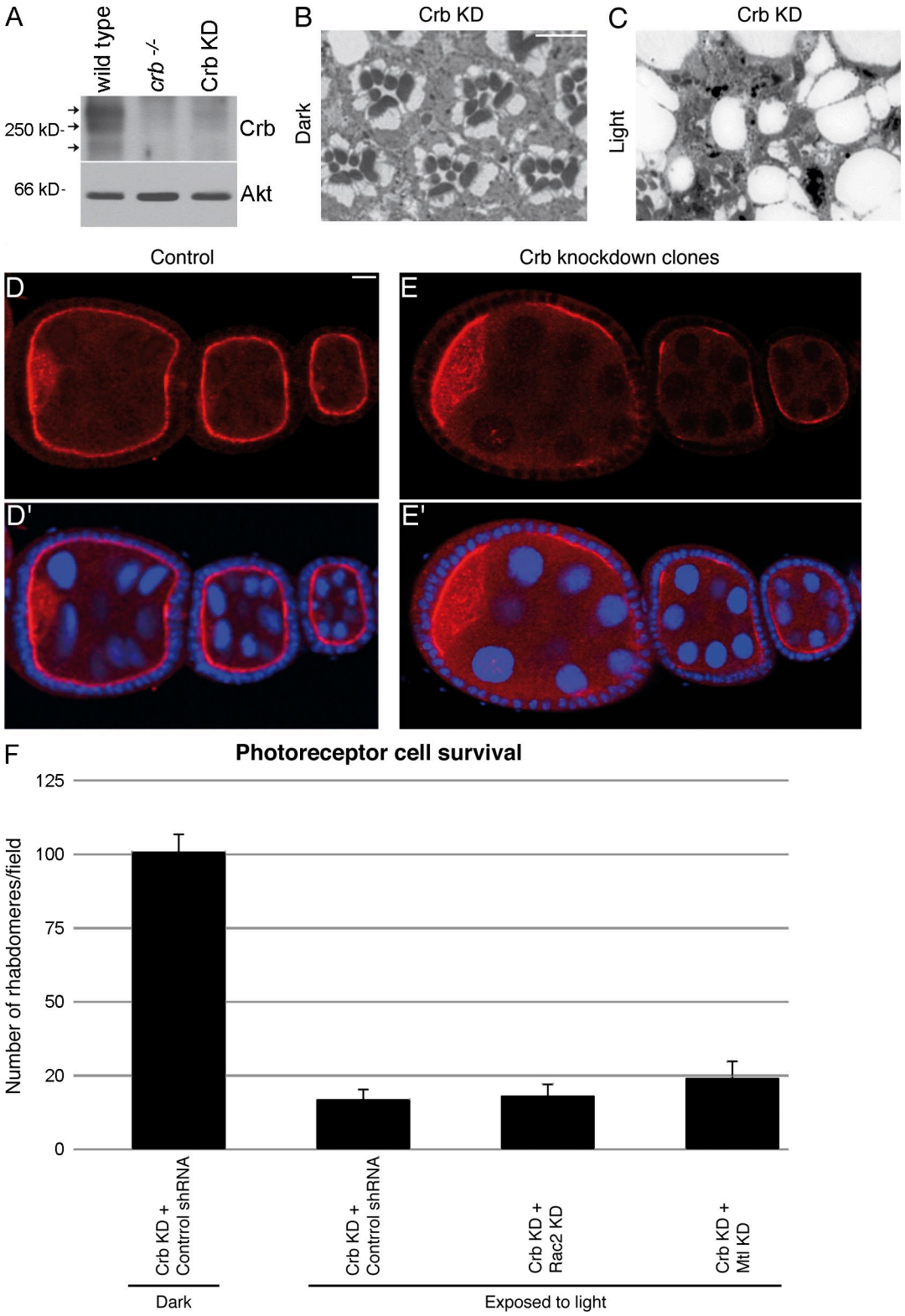

Figure S2. **Crb knockdown in the retina mimics *crb* mutations.** (A) Comparison of Crb levels in wild-type, *crb* mutant, and Crb knockdown retinas. Arrows point to *crb* gene products. The shRNA used strongly suppressed Crb expression. A Western blot showing Akt levels demonstrates that reduction of Crb expression is not caused by a general down-regulation of protein amount. (B) Panel shows a cross section of a retina knocked-down for Crb maintained in the dark. Crb knockdown and *crb* mutant retinas show similar phenotypes, including widened rhabdomeres (Fig. 4 B; Johnson et al., 2002; Pellikka et al., 2002). (C) Cross section of a Crb knockdown retina exposed to constant illumination. As observed for *crb* knockout retinas (Fig. 4 C; Johnson et al., 2002), retinas in which Crb abundance is reduced by knockdown show light dependent degeneration. (D) Crb staining in control follicles. (E) shRNA directed against *crb* under the control of a UAS promoter were expressed clonally in the follicular epithelium using *enGAL4*. Gaps in Crb staining show that the knockdown is efficient. (F) Heads from flies maintained under constant illumination (exposed to light) or kept in total darkness (dark) were fixed, sectioned, and stained for light microscopy analysis. These flies were knocked down for Crb and expressed a control shRNA (directed against luciferase), knocked down for Crb and Rac2, or knocked down for Crb and Mtl. Rhabdomeres were counted on retinal cross sections to quantify the number of surviving photoreceptor cells after 7 d of constant illumination. Reduction of Rac2 or Mtl did not significantly increase the number of surviving photoreceptor cells in Crb knockdown retinas. This suggests that Rac1 plays a specific role in photoreceptor cell death associated with the loss of Crb. However, we cannot rule out the idea that Rac2 and Mtl also play a role, as we used a knockdown approach and, consequently, a residual amount of these proteins could be sufficient to promote cell death of Crb knockdown cells. In addition, it is possible that Rac1 is redundant with Rac2 and/or Mtl. Error bars represent the 95% confidence level. Bars: (B and C) 5 µm; (D and E) 20 µm.

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