Yonamine et al., http://www.jcb.org/cgi/content/full/jcb.201004098/DC1

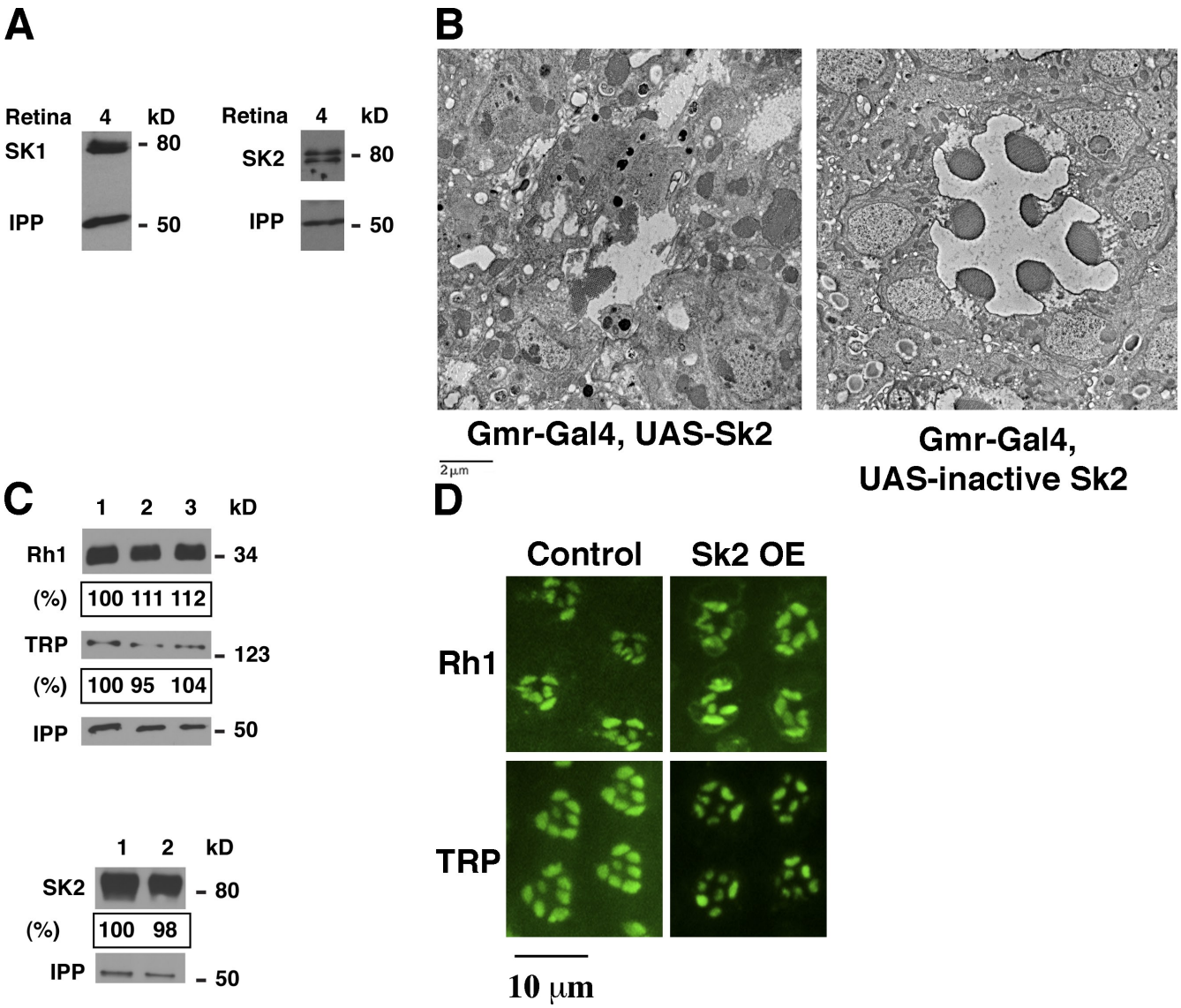

Figure S1. **Both sphingosine kinases are expressed in the retina, and overexpression of an inactive Sk2 does not result in photoreceptor degeneration.** (A) Both sphingosine kinase 1 and sphingosine kinase 2 are expressed in the retina. Retinal extracts prepared from w^{1118} flies were probed with affinity-purified antibodies to Sk1 and Sk2 proteins. 4 denotes the number of retinae. The blots were probed with anti-IPP as a loading control. The two endogenous bands detected for Sk1 and Sk2 likely represent posttranslationally modified forms. (B) Overexpression of an inactive form of Sk2 does not lead to photoreceptor degeneration. Photoreceptors expressing Sk2 or Sk2 ($GSGN^{312–315}DDDD$) in a C3 conserved catalytic domain were processed and observed by electron microscopy. (C) Rh1 and TRP levels are not reduced in flies expressing an Sk2-inactive form. Lane 1 shows w^{1118}, and lanes 2 and 3 show Rh1 and TRP in extracts from two Sk2-inactive transgenics. The percentages denote the amount of Rh1 and TRP in inactive Sk2 compared with w^{1118} after normalizing to the loading control IPP. The bottom shows a similar level of Sk2 overexpression in Sk2-active (lane 1) and Sk2-inactive (lane 2) transgenic flies. (D) Immunolocalization of Rh1 and TRP in control and Sk2-overexpressing photoreceptors. Retina from control and Sk2-overexpressing adult flies were stained for Rh1 and TRP. Control refers to *cn.bw* flies, and Sk2 overexpressors are in a *cn.bw* background to avoid red pigment that interferes with immunostaining. Sections show that Rh1 and TRP localize to the rhabdomeres of the photoreceptor cells in Sk2 overexpressors similar to control.

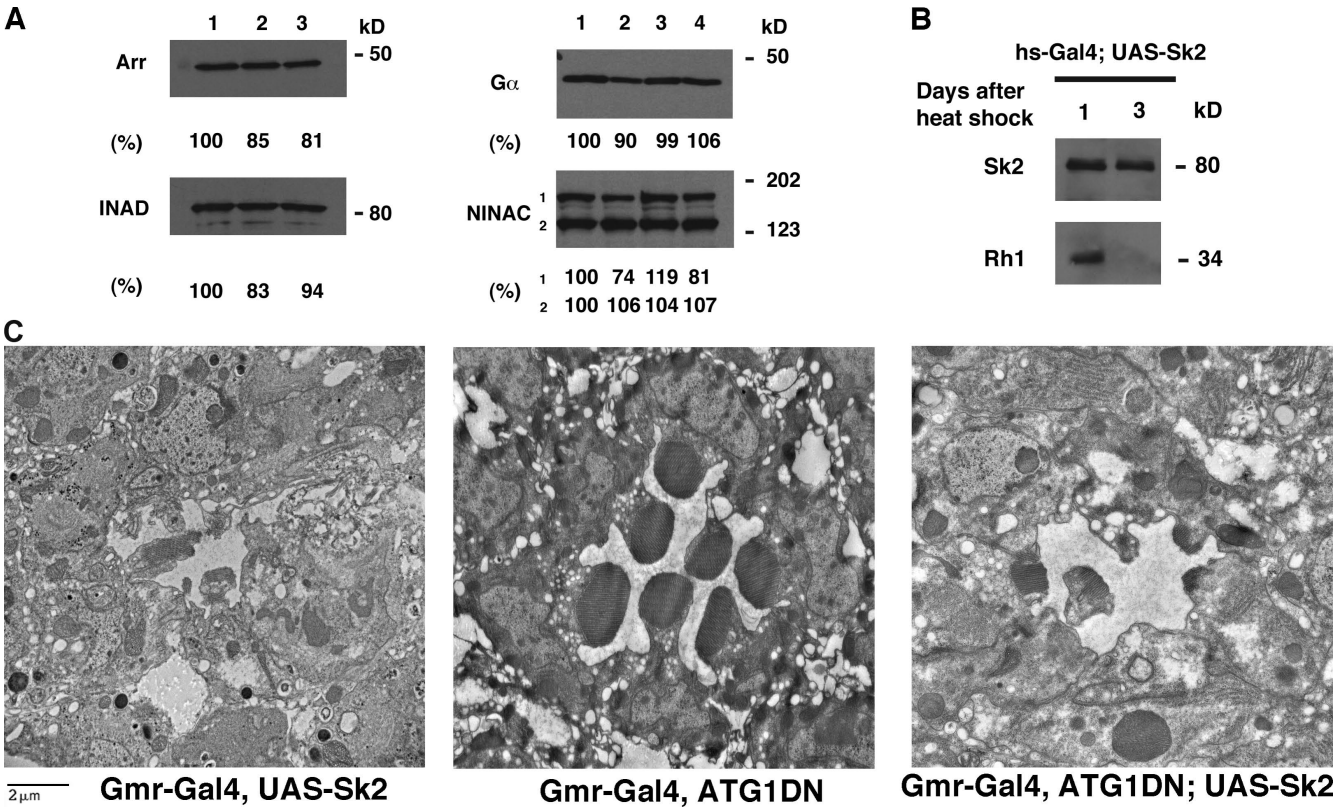

Figure S2. **Steady-state levels of some of the phototransduction components are not significantly affected in Sk2 overexpressors, and overexpression of a dominant-negative ATG1 does not suppress Sk2 degeneration.** (A) Analyses of steady-state levels of some phototransduction components in Sk2 overexpressors. Head extracts prepared from 7-d-old Sk2 overexpressors and different control flies were probed with antibodies to Arr2 (Arr), InaD, Gα, and NinaC. In the left blots, lane 1 represents w^{1118}, lane 2 represents UAS-Sk2, and lane 3 represents GMR-Gal4, UAS-Sk2. In the right blots, lane 1 represents w^{1118}, lane 2 represents GMR-Gal4, UAS-Sk2, lane 3 represents UAS-Sk2, and lane 4 represents GMR-Gal4. Percentages denote the amount of different proteins relative to control w^{1118}. (B) The Rh1 level decreases in Sk2 overexpressors driven by a heat shock promoter. Sk2 flies driven by hsp70-Gal4 were heat shocked at 37°C, and head extracts were subjected to Western analysis with an Rh1 antibody 1 and 3 d after induction. Despite the leaky expression of Sk2 under no heat shock condition, there was a significant induction (500–600%) of Sk2 expression upon heat shock. This is accompanied by a decrease in Rh1 (50%) by day 1 after heat shock when compared with no heat shock control, and it further decreased by day 3. (C) Expression of $ATG1^{K38Q}$ does not suppress photoreceptor degeneration in Sk2 overexpressors. The left image shows degenerating photoreceptors of Sk2 overexpressors, the middle image shows that overexpression of $ATG1^{K38Q}$ does not significantly affect photoreceptor structure, and the right image shows Sk2 photoreceptors expressing $ATG1^{K38Q}$. About 4% of rhabdomeres are intact in Sk2 overexpressors, 95% are intact in $ATG1^{K38Q}$ overexpressors, and 16% are intact in Sk2 and $ATG1^{K38Q}$ overexpressors.

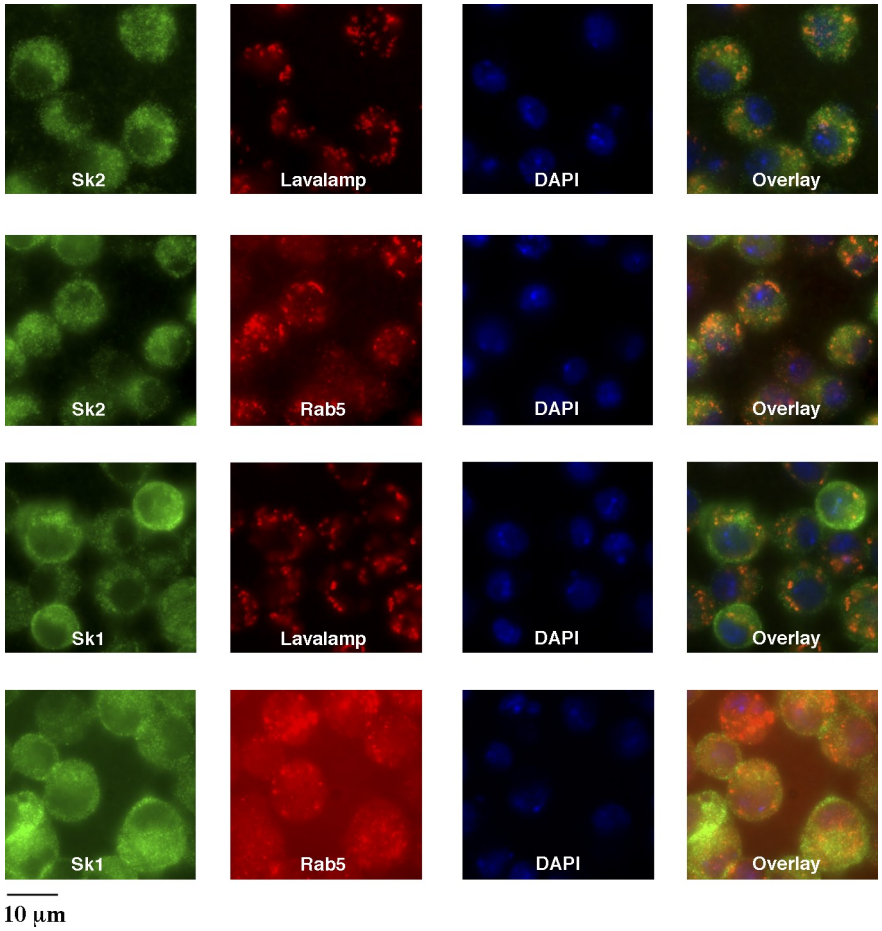

Figure S3. **Immunofluorescence analyses of Sk1 and Sk2 proteins in Schneider cells.** The top row shows uninduced stable cells expressing V5-tagged Sk2 stained with V5 antibody and an antibody to Lavalamp, a Golgi marker. The second row shows staining with Rab5, an early endosomal marker. Sk2 staining does not overlap with Lavalamp or Rab5. The third row shows uninduced stable cells expressing V5-tagged Sk1 stained with antibodies to V5 and Lavalamp, and the last row shows cells stained with antibodies to V5 and Rab5. Sk1 does not show colocalization with Lavalamp or Rab5.