SUPPLEMENTAL MATERIAL

Sun et al., https://doi.org/10.1084/jem.20161645

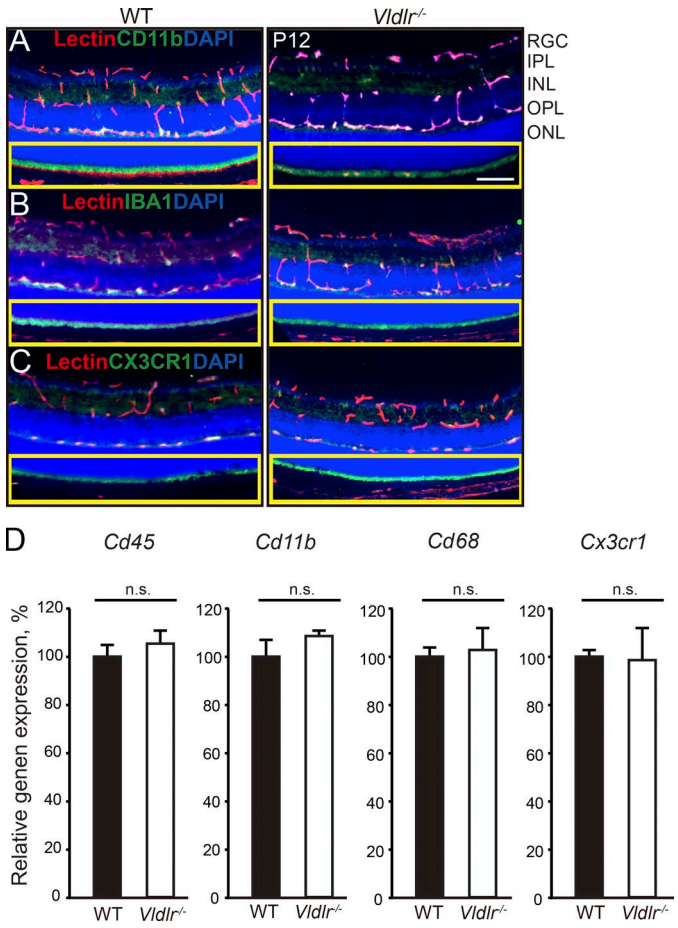

Figure S1. **There was no macrophage recruitment in P12 *Vldlr*$^{-/-}$ retinas.** (A–C) Macrophage markers (green) including CD11b, IBA1, and CX3CR1 costained with endothelial cell marker isolectin IB4 (red) and nuclear marker DAPI (blue). Macrophages were clustered around endothelial cells, and there was no macrophage recruitment into the deeper vascular layer and the subretinal space (yellow box) in both P12 WT and *Vldlr*$^{-/-}$ retinas. INL, inner nuclear layer; IPL, inner plexiform layer; OPL, outer plexiform layer; RGC, retinal ganglion cells. Bar,100 µm. (D) The mRNA levels of macrophage markers including *Cd45*, *Cd11b*, *Cd68*, and *Cx3cr1* were comparable between P12 WT and *Vldlr*$^{-/-}$ retinas. All data are representative of at least three independent experiments. Results are presented as mean ± SEM.

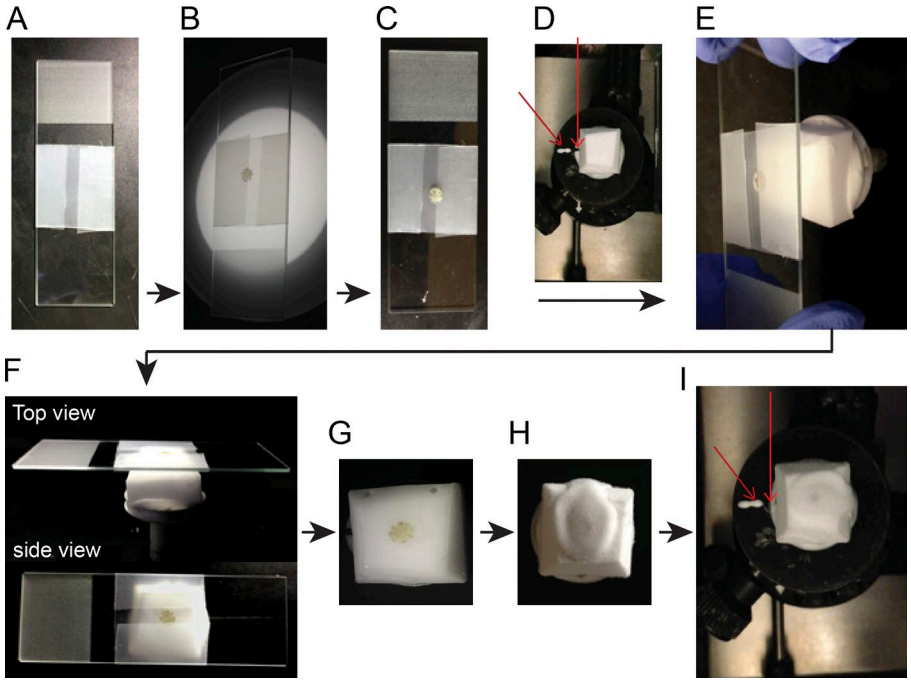

Figure S2. **Diagram of retinal layer sectioning.** (A) Wrap a microscope slide with parafilm to make a smooth flat surface of parafilm on one side. (B and C) Dissect a retina, make eight evenly spaced radial cuts, and place the dissected retina on the flat surface of the parafilm. Place a drop of OCT medium on top of the retina. (D) Freeze a block of OCT medium, and place the block firmly on the cryostat. Trim the OCT block, and fix the cryostat settings until a flat surface is achieved. Mark any edge of the OCT medium with a marker (red arrow) and corresponding direction on the cryostat with OCT medium (red arrow). (E–G) Transfer the OCT block onto a bench top, flip the slide upside down, and gently touch the flat surface of the parafilm-wrapped slide to the flat surface of the trimmed OCT block for 1–2 min. Make sure the retina has successfully transferred onto the OCT block. (H) Apply enough OCT medium to fully cover the retina and freeze the block until the retina is no longer visible. (I) Place the OCT block with the retina back on the cryostat in the correct orientation, as marked previously (red arrows), to ensure a flat cut of each retinal section. Use the same cryostat settings, and cut the OCT block at 20 µm/section. Collect each section into a separate RNAnase-free tube for ∼12 sections.

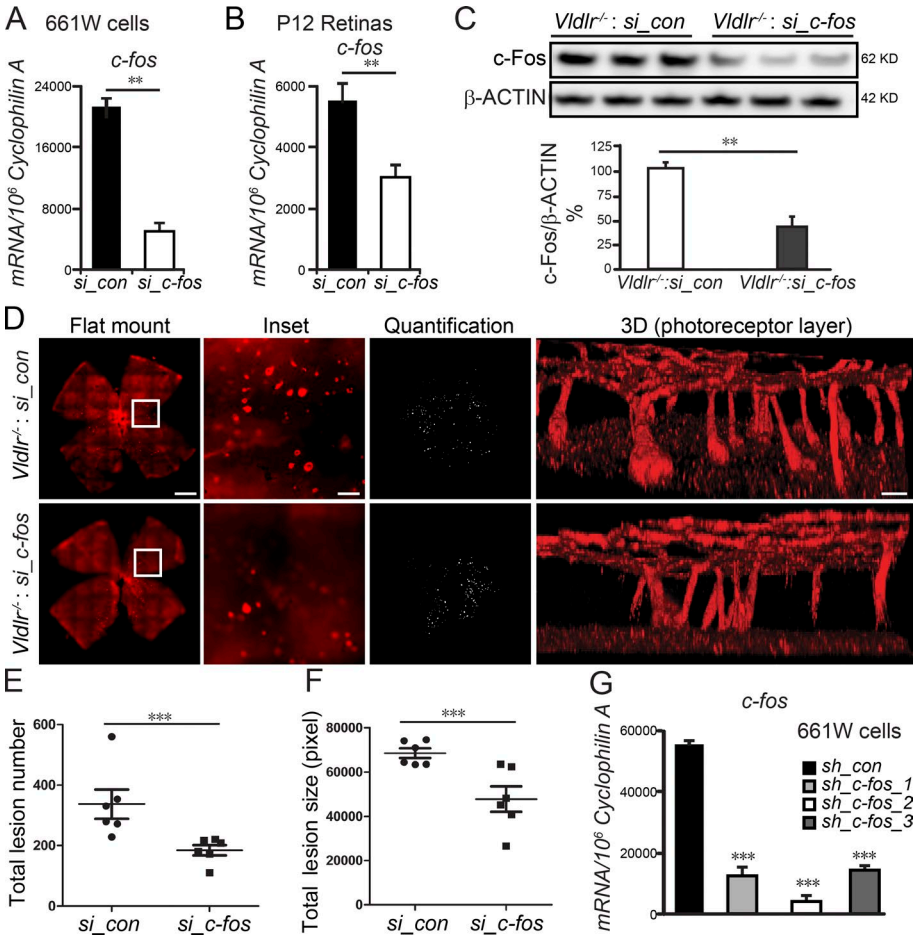

Figure S3. **Knocking down *c-fos* reduced neovascularization in *Vldlr*$^{-/-}$ retinas.** (A) The knockdown efficiency of siRNA targeting *c-fos* was validated in photoreceptor 661W cells. (B and C) *c-fos* mRNA and protein levels were reduced in *si_c-fos*–treated WT retinas compared with *si_control*–treated retinas. (B) $n = 6$. (C) $n = 3$. (D–F) Knocking down *c-fos* with *si_c-fos* reduced neovascularization. (D) Representative flat-mount images of *si_control*– or *si_c-fos*–treated- *Vldlr*$^{-/-}$ retinas stained with isolectin IB4 for endothelial cells. White dots show newly formed vessel tips on the RPE for quantification. 3D reconstruction of representative confocal images and enlarged flat-mount images (inset) show reduced neovascularization in photoreceptor layers. Bars: (whole-mount images) 1,000 µm; (Inset) 250 µm; (3D) 100 µm. (E and F) Quantification of total lesion number and total lesion size (pixels) for *si_control*– or *si_c-fos*–treated *Vldlr*$^{-/-}$ retinas. Both lesion number and lesion size were reduced significantly by *si_c-fos* treatment. $n = 6–8$. (G) The knockdown efficiency of three AAV2-hRK-shRNA targeting *c-fos* was validated in photoreceptor 661W cells. $n = 6$. *si_con*, *si_control*. All data are representative of at least three independent experiments. **, $P < 0.01$; ***, $P < 0.001$. Results are presented as mean ± SEM.

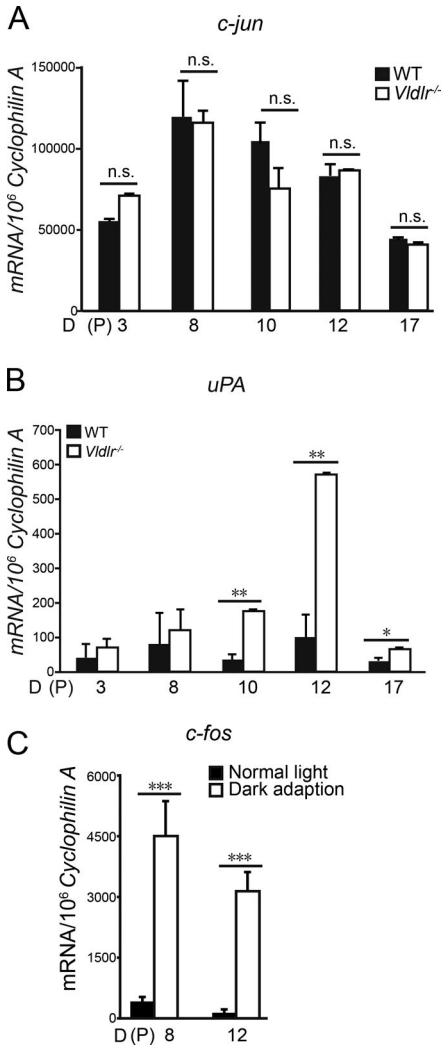

Figure S4. **The mRNA expression of c-*Jun* and *uPA* in WT and *Vldlr*$^{-/-}$ mice and the mRNA expression of c-fos in dark-adapted *Vldlr*$^{-/-}$ retinas.** (A) *c-Jun* expression was not changed during retinal development in *Vldlr*$^{-/-}$ retinas compared with littermate WT controls. $n = 6$. (B) *uPA* mRNA expression was increased during retinal development in *Vldlr*$^{-/-}$ retinas compared with littermate WT controls. (C) *c-fos* expression was dramatically increased in dark-adapted *Vldlr*$^{-/-}$ retinas compared with normal light *Vldlr*$^{-/-}$ controls. $n = 6$. All data are representative of at least three independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Results are presented as mean ± SEM.

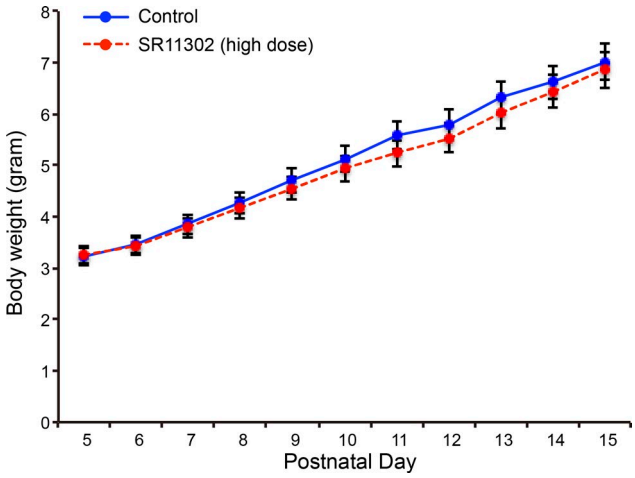

Figure S5. **Body weight of mice treated with a high dose of SR11302 or control from P5 to P15.** The body weights between high dose of the SR11302-treated group and control-treated group were comparable. $n = 6$. All data are representative of at least three independent experiments. Results are presented as mean ± SEM.

Table S1 is included as an Excel file and shows upstream regulator prediction of the microarray data.