Supporting Information

Wei et al. 10.1073/pnas.1309276110

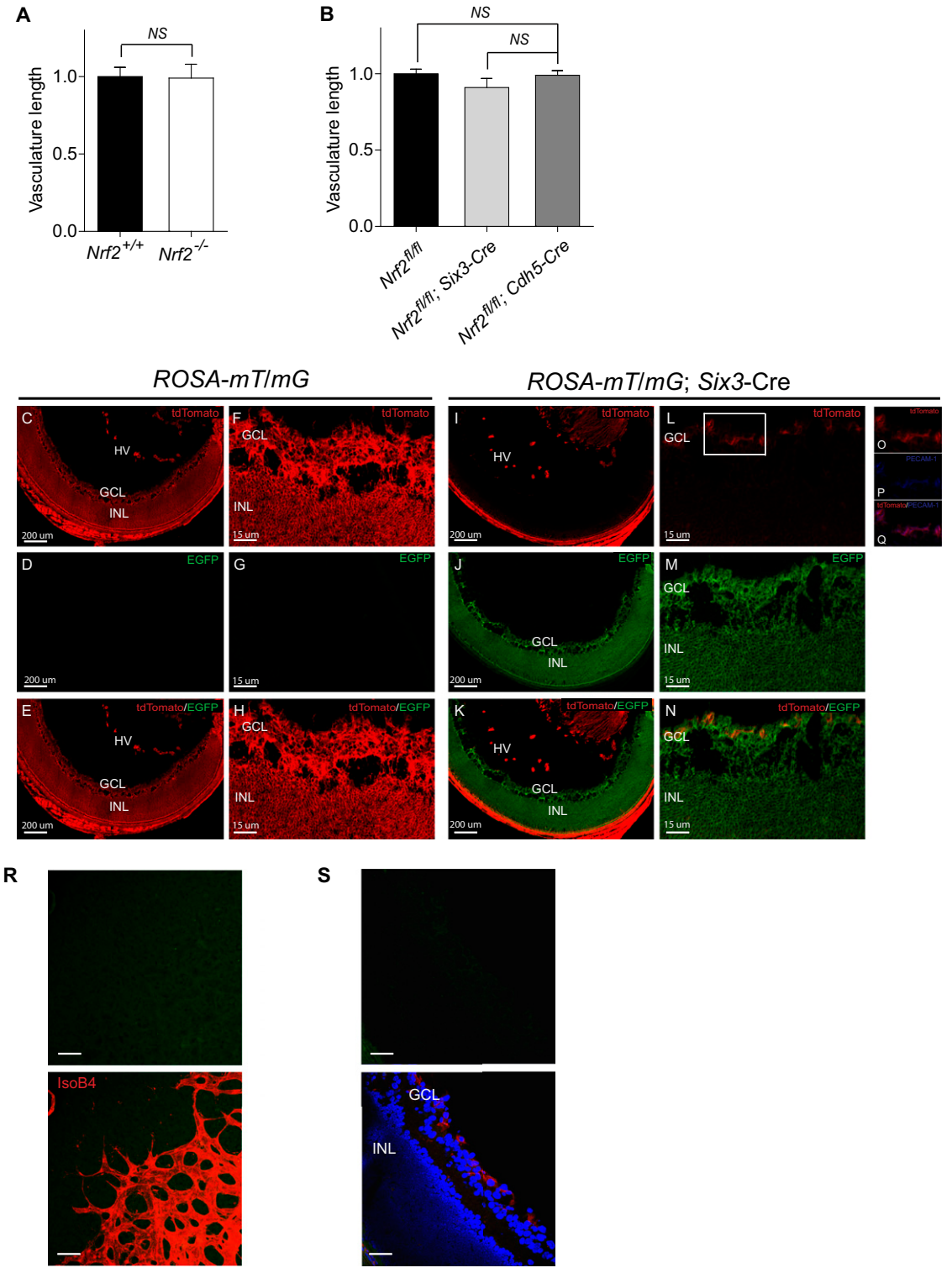

Fig. S1. (*A* and *B*) No effect on retinal vasculature length by genetic deletion of NF-E2–related factor 2 (Nrf2). Quantification of vasculature length in wild-type (WT) ($Nrf2^{+/+}$) and $Nrf2^{-/-}$ (*A*), control [$Nrf2^{flox/flox}$ ($Nrf2^{fl/fl}$)], $Nrf2^{fl/fl}$;*Six3-Cre*, and $Nrf2^{fl/fl}$;*Cdh5-Cre* (*B*) retinas at postnatal day (P) 5. (*C*–*Q*) Cre activity is

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present in nonvascular tissue in *Six3-Cre* retina. (*C*–*H*) Retinal cryosections from *ROSA-mT/mG* mice at P5. Strong red fluorescence (tdTomato) was evident in the entire retina as well as hyaloid vessels (HV; *C*, *E*, *F*, and *H*), whereas EGFP was absent (*D*, *E*, *G*, and *H*). (*I*–*Q*) Retinal cryosections from *ROSA-mT/mG*;*Six3-Cre* mice at P5. The red fluorescence (tdTomato) was absent in the retina except in the vasculature (*I*, *K*, *L*, and *N*), and green fluorescence (i.e., EGFP) was evident instead in nonvascular retina (*J*, *K*, *M*, and *N*). Retinal vessels (*L* and *N*) and HV (*I* and *K*) expressed tdTomato. Staining with endothelial marker PECAM-1 verified the colocalization of tdTomato and PECAM-1 on retinal vessels, indicating the lack of cre activity in endothelial cells (ECs; *L*, *Inset*; *O*–*Q*). (*R* and *S*) Retinal flat-mount (*R*) and cryosection (*S*) stained with only secondary antibody and isolectin B4 using the Mouse on Mouse Fluorescein Kit described in *Materials and Methods*. No signal was detected with secondary antibody only. (Scale bar, 50 μm.) Data are presented as mean ± SEM ($n = 4$; NS, not significant).

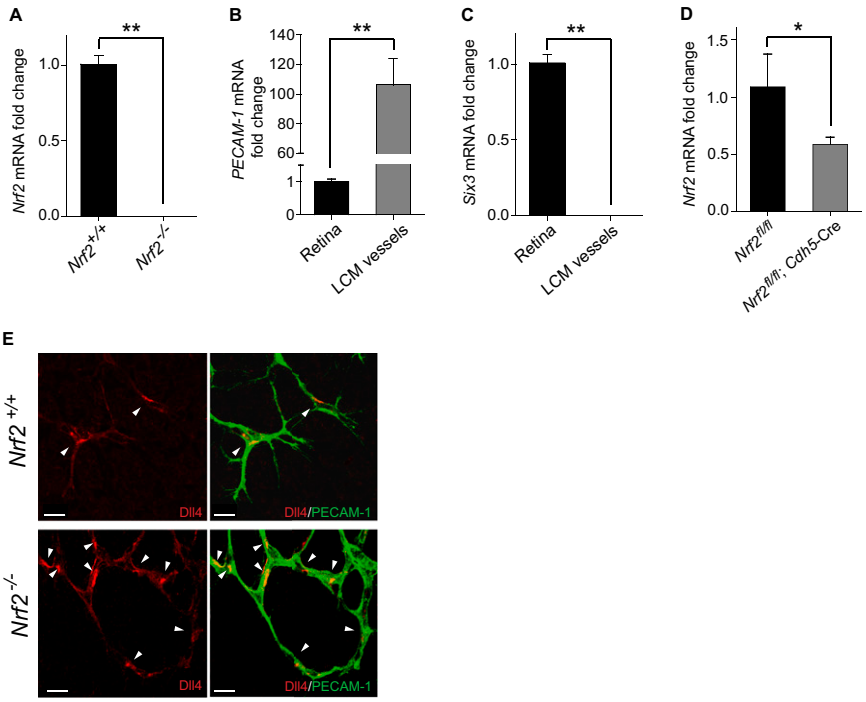

Fig. S2. (*A*) Quantitative RT-PCR analysis of WT *Nrf2* in $Nrf2^{+/+}$ and $Nrf2^{-/-}$ retina. (*B* and *C*) Quantitative RT-PCR analysis of *PECAM-1* (*B*) and *Six3* (*C*) in retina and microdissected retinal vessels from $Nrf2^{fl/fl}$;*Cdh5-Cre* mice. (*D*) Quantitative RT-PCR analysis of WT *Nrf2* in microdissected vessels from control ($Nrf2^{fl/fl}$) and $Nrf2^{fl/fl}$;*Cdh5-Cre* retinas at P5 ($n = 4$). (*E*) whole-mount immunofluorescence for Dll4 (red) and PECAM-1 (green) in P5 retinas. Increased Dll4 expression was observed in the angiogenic front (arrowheads) in $Nrf2^{-/-}$ retinas compared with control. (Scale bar, 25 μm.) Data are presented as mean ± SEM (*$P < 0.05$ and **$P < 0.01$).

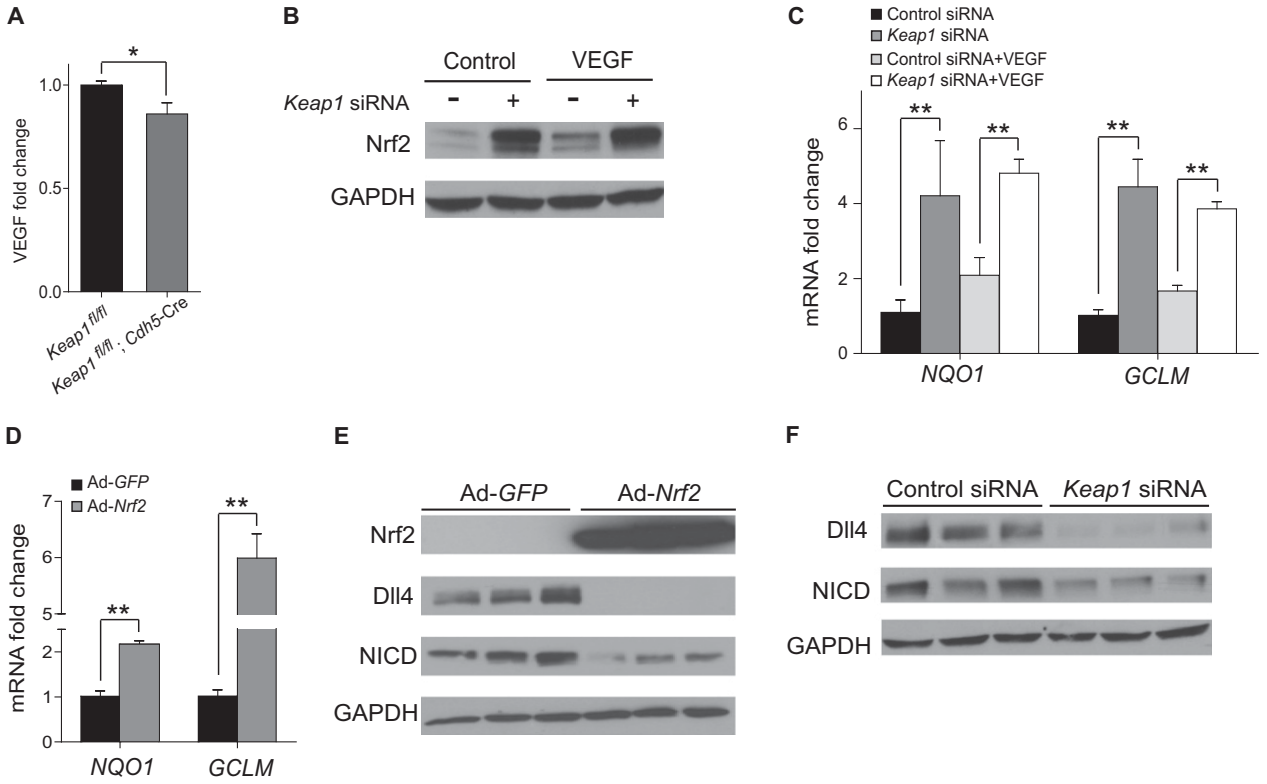

Fig. S3. (*A*) Retinal VEGF protein levels at P5 ($n = 5$). (*B*) Immunoblot analysis of Nrf2 in human retinal ECs (HRECs) treated with control siRNA or *Keap1* siRNA for 30 h. (*C*) Quantitative RT-PCR analysis of Nrf2 target genes (*NQO1* and *GCLM*) in HRECs treated with control siRNA or Keap1 siRNA for 30 h. (*D*) Quantitative RT-PCR analysis of Nrf2 target genes (*NQO1* and *GCLM*) in HRECs treated with control adenovirus (Ad-*GFP*) or adenovirus encoding Nrf2 (Ad-*Nrf2*) for 24 h. (*E*) Immunoblot analysis of Nrf2, Dll4 and cleaved Notch intracellular domain (NICD) in HRECs treated with Ad-*GFP* or Ad-*Nrf2* for 24 h. (*F*) Immunoblot analysis of Dll4 and NICD in HRECs treated with control siRNA or *Keap1* siRNA for 30 h. GAPDH was detected as a loading control. Data are presented as mean ± SEM (*$P < 0.05$ and **$P < 0.01$).