Supporting Information

Guma et al. 10.1073/pnas.0902659106

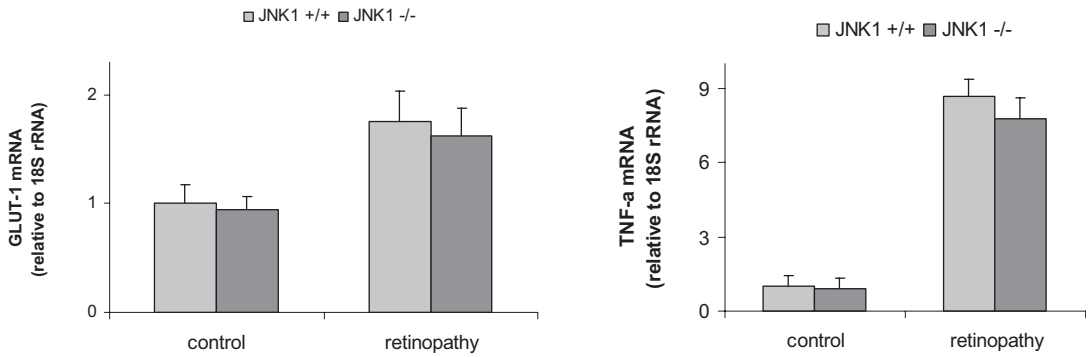

Fig. S1. JNK1 is not involved in regulation of hypoxia-inducible genes other than VEGF or inflammatory cytokines. RNA was extracted from retinas of normoxic or oxygen-induced retinopathy (OIR)-challenged mice at postnatal day 15 (P15), and mRNA expression was analyzed by quantitative (Q)-RT-PCR. Results are expressed as means ± SEM ($n = 4$ per genotype). GLUT-1 is a typical hypoxia-inducible gene, whereas TNF-α is an inflammatory cytokine.

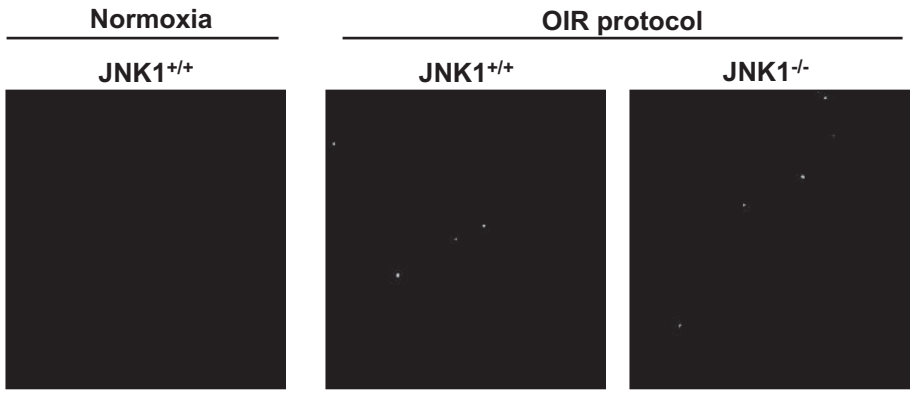

Fig. S2. JNK1 is not involved in retinal apoptosis. *Jnk1*$^{-/-}$ and *wild-type* (WT) retinas were examined by an in situ TUNEL assay at P14 of the OIR protocol for the presence of cells undergoing apoptosis.

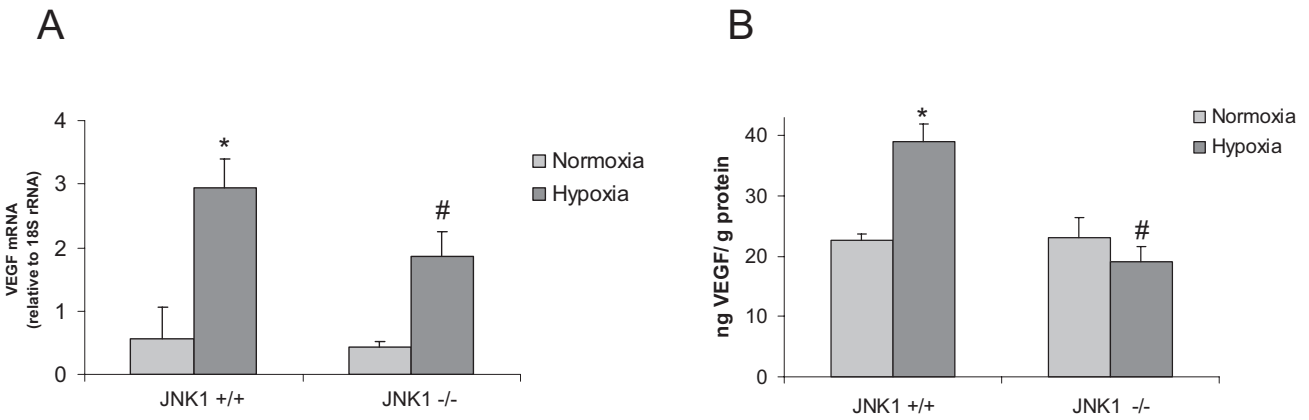

Fig. S3. JNK1 regulates hypoxia-induced VEGF expression in murine embryonic fibroblasts (MEFs). *Jnk1*$^{+/+}$ or *Jnk1*$^{-/-}$ MEFs were exposed to normoxia (Po_2 = 21%) or hypoxia (Po_2 = 0.5%) for 4 h. (*A*) RNA was extracted, and VEGF mRNA expression was quantified by Q-RT-PCR. Results are expressed as means ± SEM. *, $P < 0.05$ vs. *Jnk1*$^{+/+}$ MEFs under normoxia; #, $P < 0.05$ vs. *Jnk1*$^{+/+}$ MEFs under hypoxia. (*B*) VEGF protein expression was quantified by ELISA. Results are expressed as means ± SEM. *, $P < 0.05$ vs. *Jnk1*$^{+/+}$ MEFs under normoxia; #, $P < 0.05$ vs. *Jnk1*$^{+/+}$ MEFs under hypoxia.

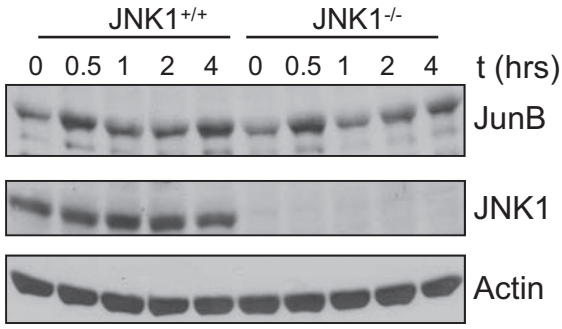

Fig. S4. JNK1 does not control JunB expression. *Jnk1*$^{+/+}$ and *Jnk1*$^{-/-}$ BMDM were cultured under normoxia (Po_2 = 21%) or hypoxia (Po_2 = 0.5%) for different times. Cell lysates were obtained, and protein expression was analyzed by immunoblotting.

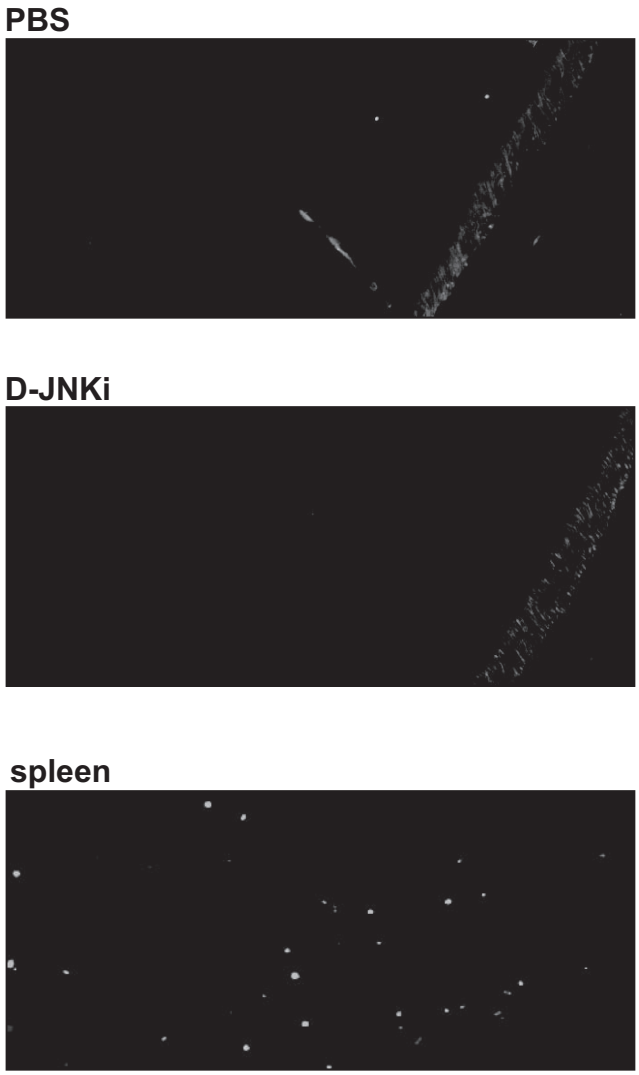

Fig. S5. The D-JNKi peptide does not induce retinal apoptosis. Retinas from PBS or D-JNKi-treated eyes (injected at P18 and assessed at P19) do not show increased apoptosis. Spleen tissue served as a control. Apoptotic cells were visualized by an in situ TUNEL assay.

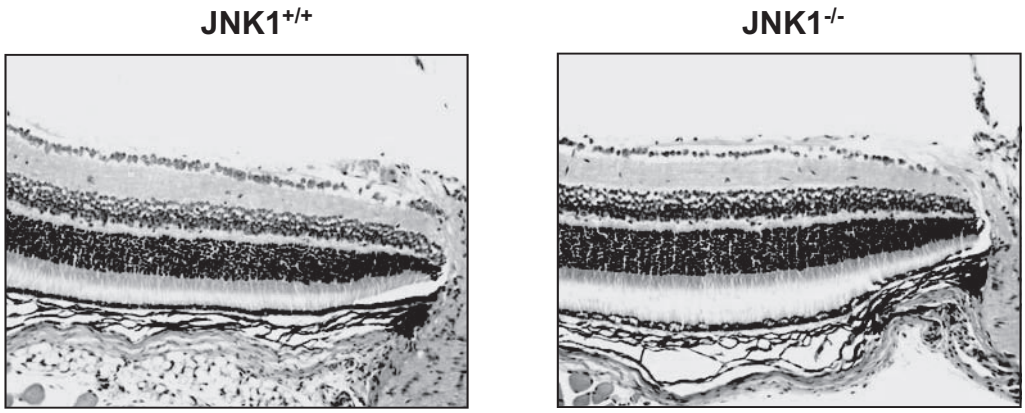

Fig. S6. *Jnk1*$^{-/-}$ mice do not show any retinal morphological abnormalities. Retinas from *Jnk1*$^{-/-}$ mice show normal histology in retinal cross-sections stained with hematoxylin and eosin (P18) under normoxic conditions.

Table S1. Evaluation of retinal parameters as indicators of acute treatment toxicity

Parameters	JNK1$^{+/+}$	JNK1$^{-/-}$	PBS-treated	D-JNKi-treated
Ganglion cells*†	30.3 ± 4.1	31.6 ± 2.8	31.2 ± 5.8	32.9 ± 5.8
Width whole retina†	2.9 ± 0.41	2.7 ± 0.32	2.7 ± 0.32	2.9 ± 0.33
Width inner nuclear layer†	0.53 ± 0.1	0.47 ± 0.11	0.49 ± 0.1	0.49 ± 0.1
Width outer nuclear layer†	0.69 ± 0.13	0.7 ± 0.12	0.69 ± 0.13	0.67 ± 0.11

*Number of ganglion cells.
†Values are means ± SD ($n > 10$).