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Supplementary Information for

An inhibitor of endothelial ETS transcription factors promotes physiologic and therapeutic vessel regression

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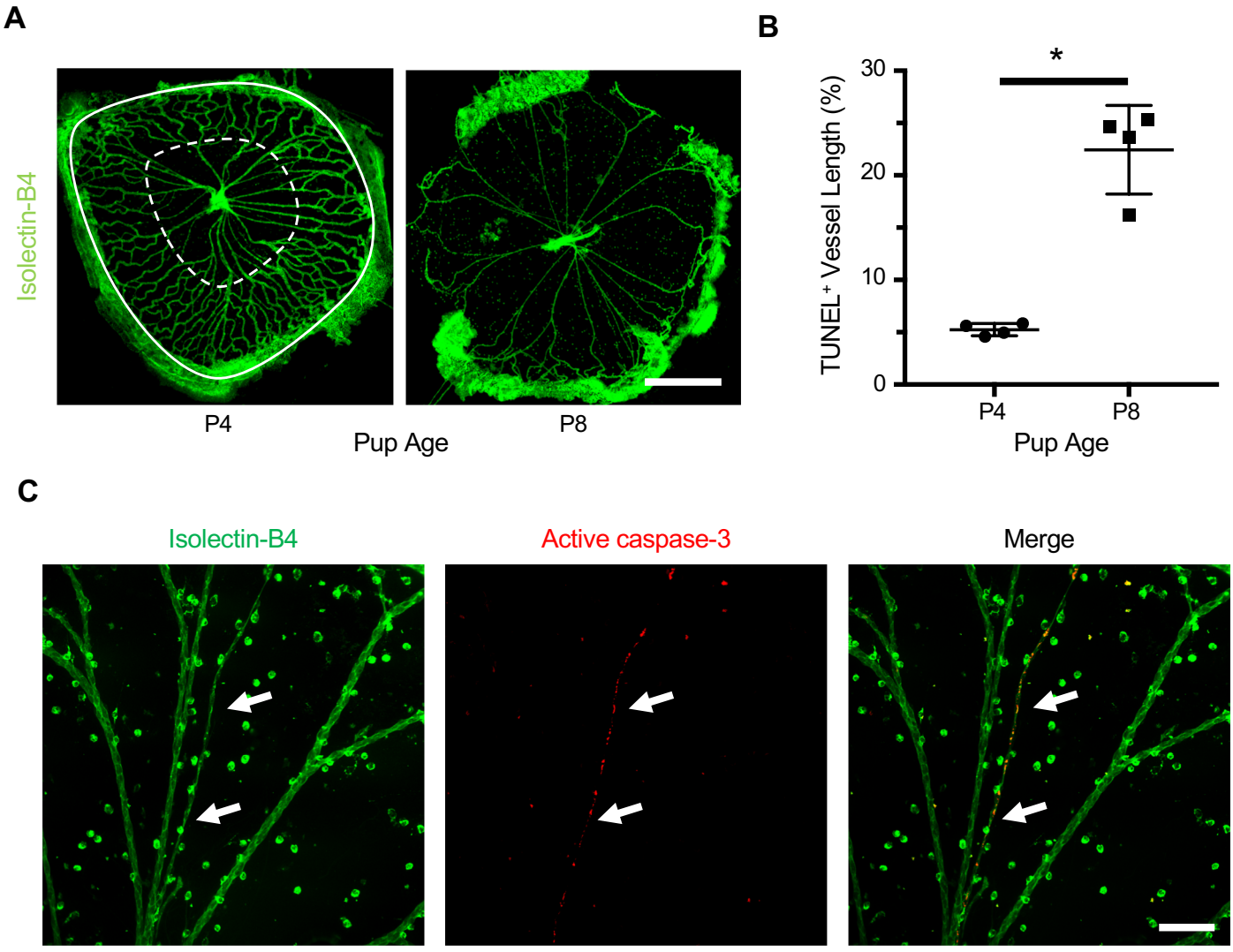

Fig. S1. *Visualization of hyaloid vessel regression.* **(A)** Hyaloid vessels from P4 and P8 mice were dissected and visualized by flat mount imaging with Isolectin-B4 (green). Regression was quantified (see Fig. 1A) by counting vessels crossing a 50% diameter of the hyaloid flat mount (dotted white line) Scale bar = 500 μm. **(B)** Quantification of TUNEL⁺ vessel length (n = 4) as a percent of total hyaloid vessel length in P4 and P8 wild type mice. **(C)** Flat mount hyaloid vessels dissected from a P6 wild type mouse were immunostained for Isolectin-B4 (green) and active caspase-3 (red). Active caspase-3 signal is confined to individual vascular branches that are constricted (indicated by white arrows) relative to adjacent vessel branches. Scale bar = 100 μm. *$P < 0.05$ (two-tailed Student's *t*-test). Error bars = S.D.

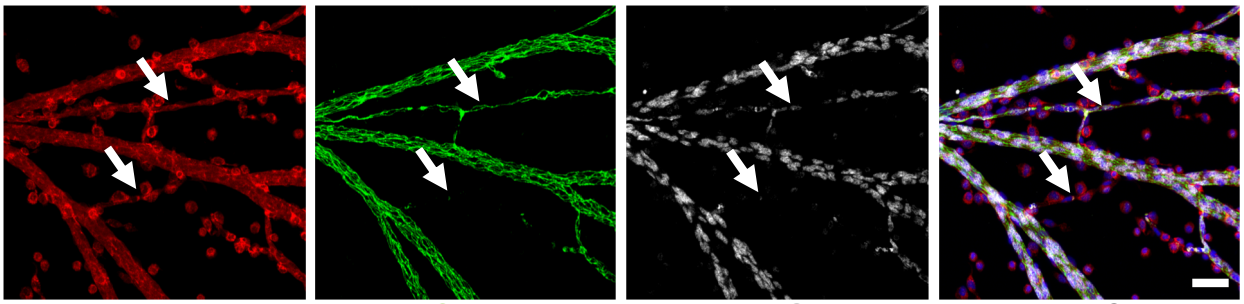

Fig. S2. *Downregulation of VE-Cadherin in constricted, regressing hyaloid vessels.* P6 hyaloid vessels were immunostained for Isolectin-B4 (red), ERG (white), and VE-Cadherin (green). Arrows indicate constricted hyaloid vessels with reduced expression of ERG and VE-Cadherin. Scale bar = 50 μm.

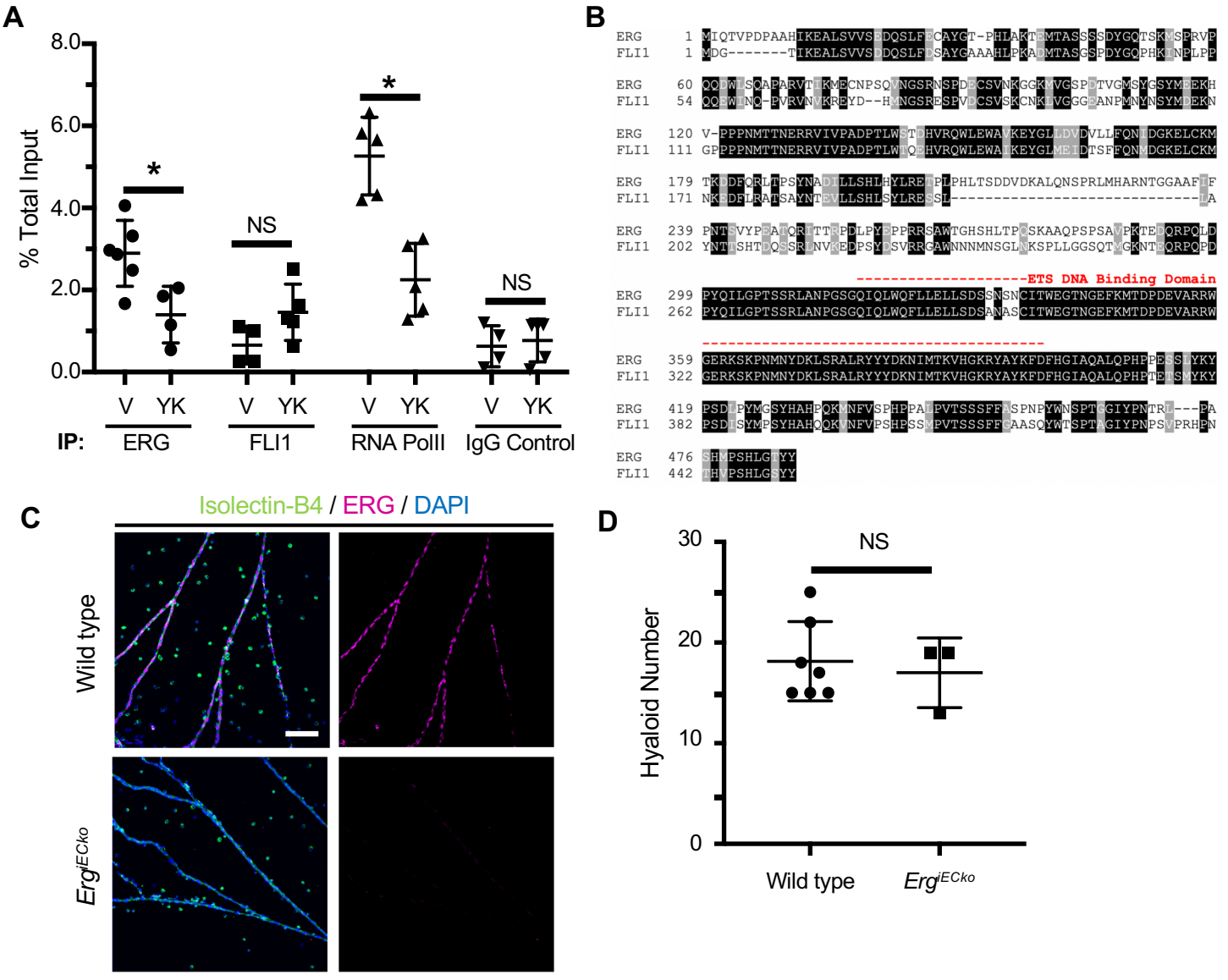

Fig. S3. *Functional compensation between ERG and FLI1*. **(A)** Chromatin immunoprecipitation (ChIP) was performed using antibodies against ERG, FLI1, RNA Polymerase II (RNA PolII), or rabbit IgG (control) on HUVECs cultured with 10μM YK-4-279 (YK) or vehicle (V) for 6h. Protein binding was quantified by qPCR at a known ERG binding site in the *Cdh5* promoter and was graphed as a percentage of the total input signal. **(B)** Alignment of murine E-26 transformation specific (ETS) related gene (ERG) and FLI1 protein sequences with identical (black highlight) and similar (grey highlight) residues indicated. The highly conserved ETS DNA binding domain is indicated in red. **(C)** Flat mount hyaloid vessels from P7 littermate wild type and Erg^{iECko} mice were immunostained for Isolectin-B4 (green), ERG (magenta), and DAPI (blue). Tamoxifen was orally administered at P3, P4, and P5 to induce genetic deletion of *Erg* in ECs of the Erg^{iECko} mice. Scale bar = 100μM. **(D)** Quantification of regression in P7 hyaloids from Erg^{iECko} (n = 3) mice and wild type (n = 4) littermates imaged as in (C). $^*P < 0.05$ (2-way ANOVA for A; two-tailed Student's *t*-test for D), NS = not significant. Error bars = S.D.

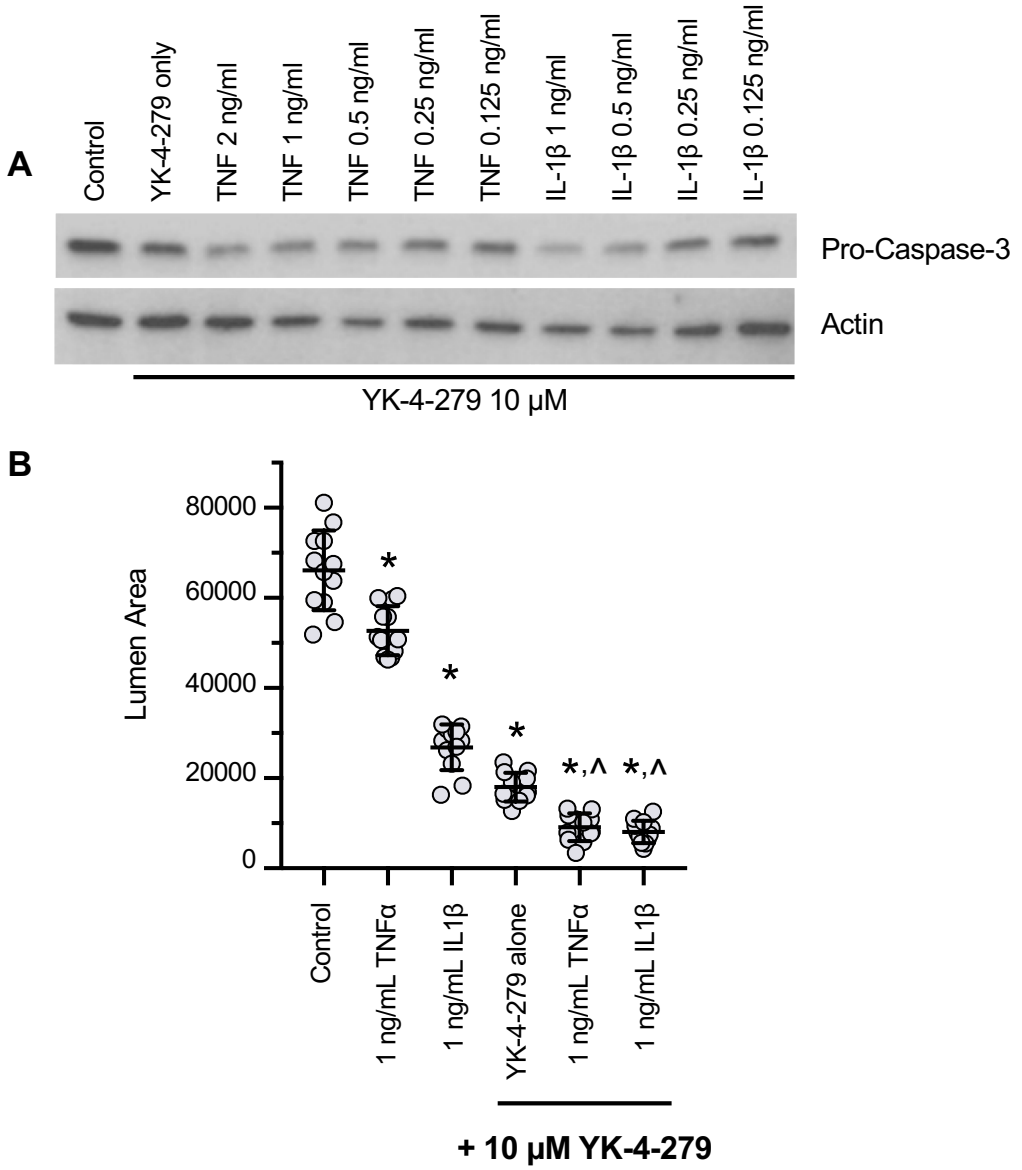

Fig. S4. *YK-4-279 enhances capillary regression induced by the inflammatory cytokines TNFα and IL1β*. **(A)** Western blot of 3D HUVEC cultures treated with the indicated YK-4-279, TNFα, and IL1β concentrations for 24 hr and probed for pro-caspase-3 and actin. Reduced pro-caspase-3 signal following YK-4-279 treatment indicates elevated EC apoptosis, which is further increased by co-treatment with low concentrations of both TNFα and IL1β. **(B)** Quantification of 3D HUVEC lumen area (n = 12) after treatment with the indicated concentrations of YK-4-279, TNFα, and IL1β for 24 hr. Co-incubation of TNFα or IL1β with YK-4-279 further increases the extent of vascular regression in vitro. *P < 0.05 (versus Control, unpaired Student's t-test); ^P < 0.05 (versus YK-4-279 alone, unpaired Student's t-test). Error bars = S.D.

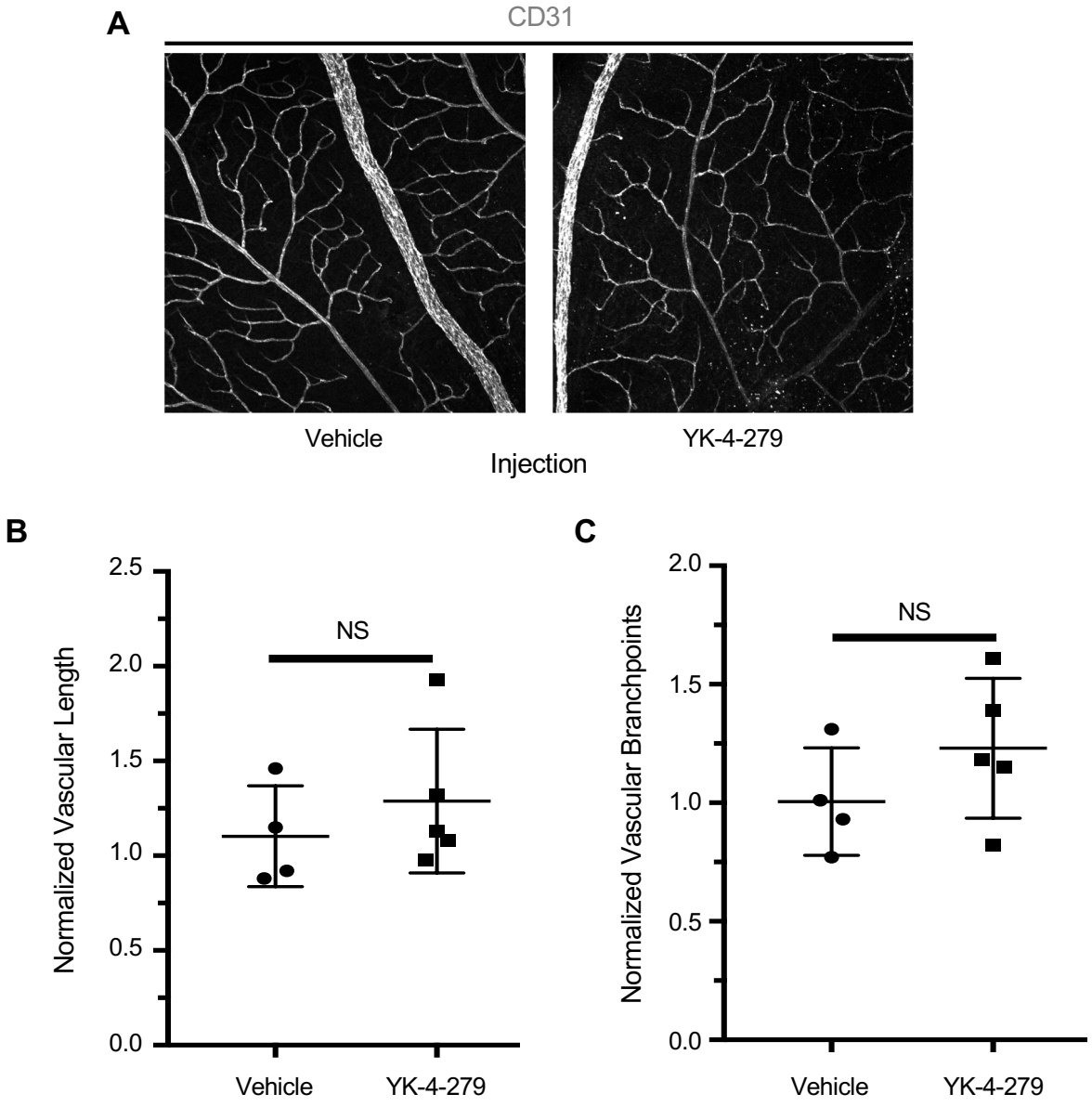

Fig. S5. *YK-4-279 does not affect wild type healthy retinal vessels*. **(A)** Flat mounts of retinal vasculature from adult wild type mice were immunostained for CD31 (white) 48 hr after intravitreal injection of YK-4-279 or a vehicle control. Vascular length **(B**; *n* = 4**)** and branch points **(C**; *n* = *4***)** from retinas treated as in (A) were quantified using AutoTube (1) and normalized to retinal area per image. NS = not significant (two-tailed Student's *t*-test). Error bars = S.D.

SI Reference

1. Montoya-Zegarra, J.A., et al., AutoTube: a novel software for the automated morphometric analysis of vascular networks in tissues. *Angiogenesis*. 22, 223-236 (2019).