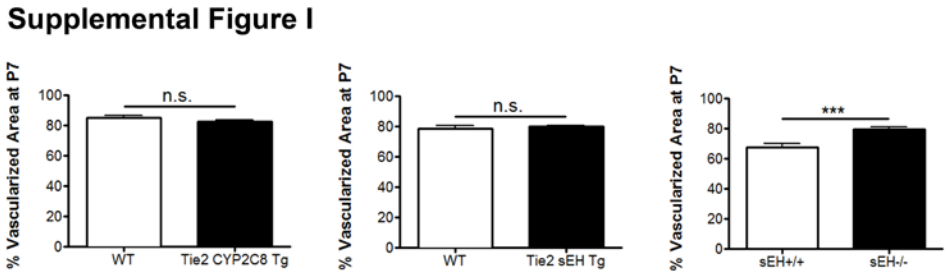
Zhuo et al., ATVB

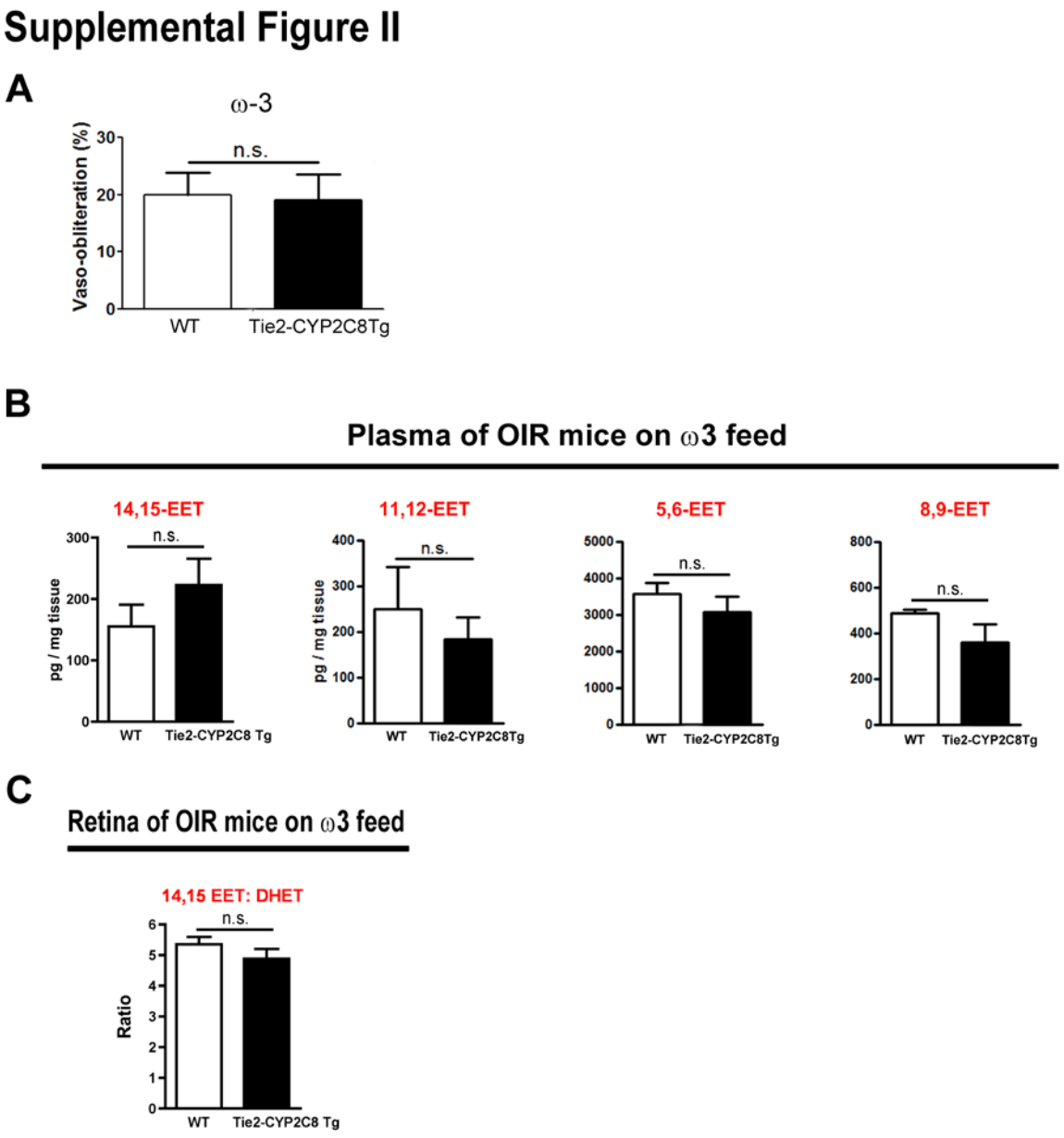
Cytochrome P450 2C8 ω3LCPUFA Metabolites Increase Mouse Retinal Pathologic Neovascularization

Supplemental Material

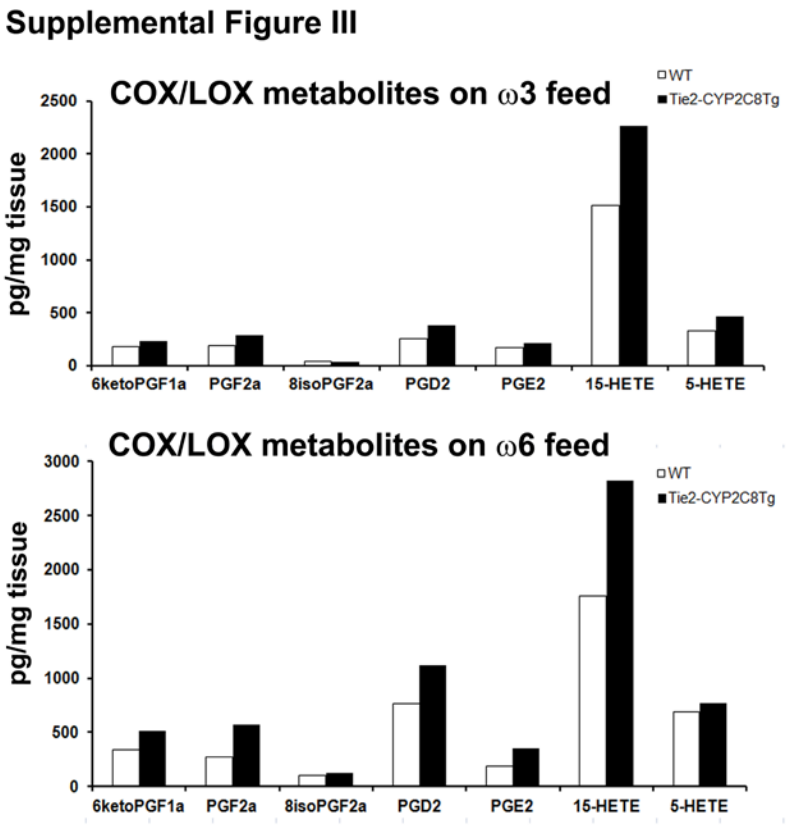
Supplemental Figure I: No difference in retinal vascular area coverage at P7 was seen in both *Tie2-CYP2C8-Tg* and *Tie2-sEH-Tg* compared with their wild-type (WT) littermate controls. Increased retinal vascular area at P7 was observed in *sEH*$^{-/-}$ mice compared with WT controls. Unpaired t test, n.s. not significant, ***p<0.001.



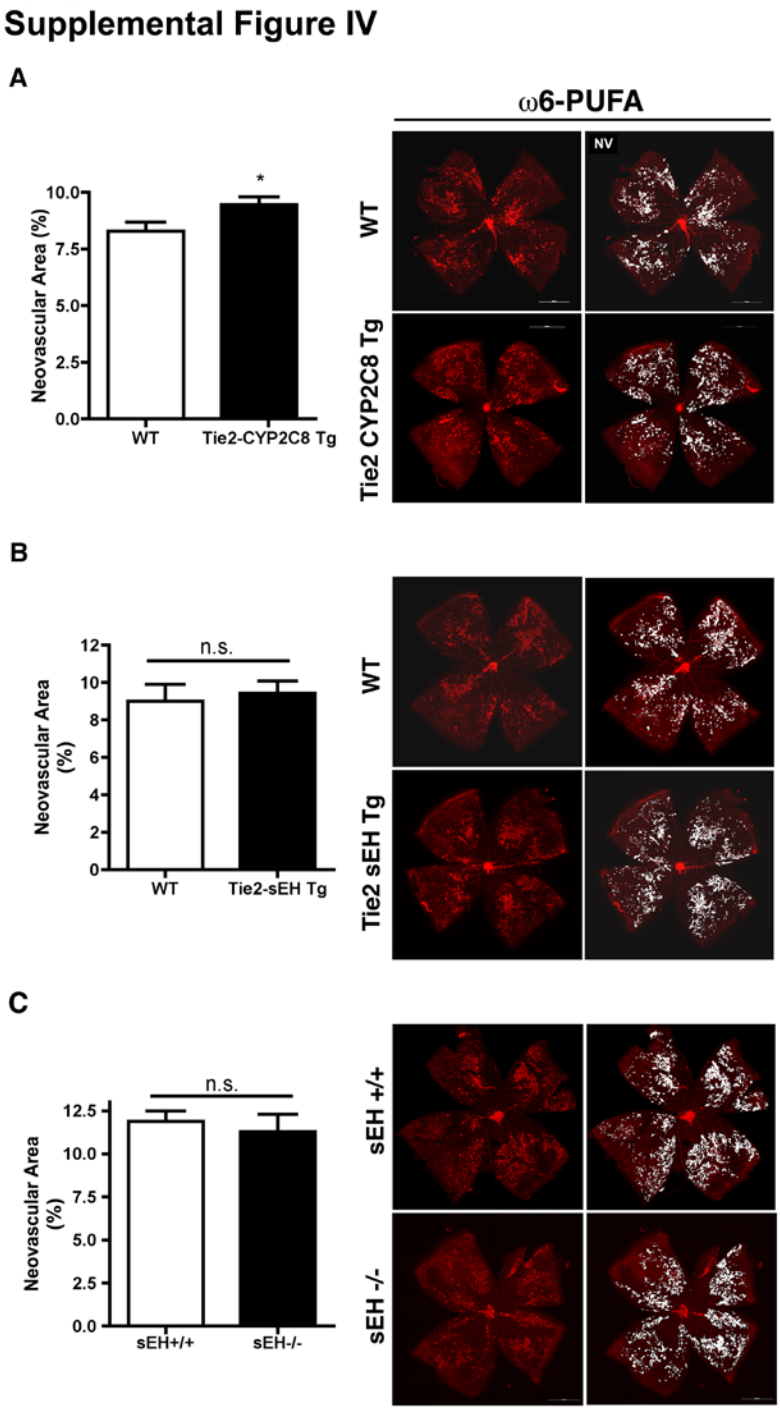
Supplemental Figure II: (A) No change in retinal vaso-obliteration was observed between WT and *Tie2-CYP2C8-Tg* mice with ω3LCPUFA feed. (B) No significant change was shown in plasma 14,15-EET, 11,12-EET, 5,6-EET and 8,9-EET between WT and *Tie2-CYP2C8-Tg* mice with ω3LCPUFA feed. (C) The retinal 14,15EET:DHET ratio in *Tie2-CYP2C8-Tg* was comparable with that in WT mice.



Supplemental Figure III: No global suppression of plasma COX metabolites 6-keto-PGF1α, PGF2α, 8-iso-PGF2α, PGD2, PGE2 levels and LOX metabolites 15-HETE and 5-HETE levels in WT and Tie-2 CYP2C8Tg mice with ω3 or ω6 LCPUFA feeds.



Supplemental Figure IV: With ω6LCPUFA feed, *Tie2-CYP2C8-Tg* induces OIR-neovascularization versus WT; no difference was seen with *Tie2-sEH-Tg* or *sEH*$^{-/-}$. (A-C) Percentage of neovascularization of *Tie2-CYP2C8-Tg* (A), *Tie2-sEH-Tg* (B), *sEH*$^{-/-}$ (C) mice versus WT littermate controls (n=8-35/group) Scale bar: 500μm. Unpaired t test, n.s. not significant, *p<0.05.



Supplemental Figure V: In OIR versus normoxia at P14 (on normal chow), the retinal 14,15-EET:14,15-DHET is increased. Plasma 14,15-EET and retinal 14,15-EET:14,15-DHET ratio are increased with *Tie2-CYP2C8-Tg* versus WT With 14,15-EET treatment, aortic ring sprouting was similar in *Tie2-sEH-Tg*, *sEH*$^{-/-}$ and WT mice. (A) The ratio of corresponding 14,15EET epoxides to diols by LC/MS/MS oxylipid analysis. n=5/group. Two-way ANOVA with Bonferroni post-test, *p<0.05, **p<0.01. (B) With ω6PUFA feed, plasma 14,15EET levels in *Tie2-CYP2C8-Tg* and *Tie2-sEH-Tg* mice versus WT littermates. (C) The retinal 14,15EET:DHET ratio in *Tie2-CYP2C8-Tg* and *Tie2-sEH-Tg* mice versus WT controls (n=4-6/group). (D) Vascular sprouting from *Tie2-sEH-Tg* and *sEH*$^{-/-}$ aortas treated with 14,15EET (n=4-8/group). Unpaired t test, *p<0.05, n.s.: not significant.