

Supplementary Materials

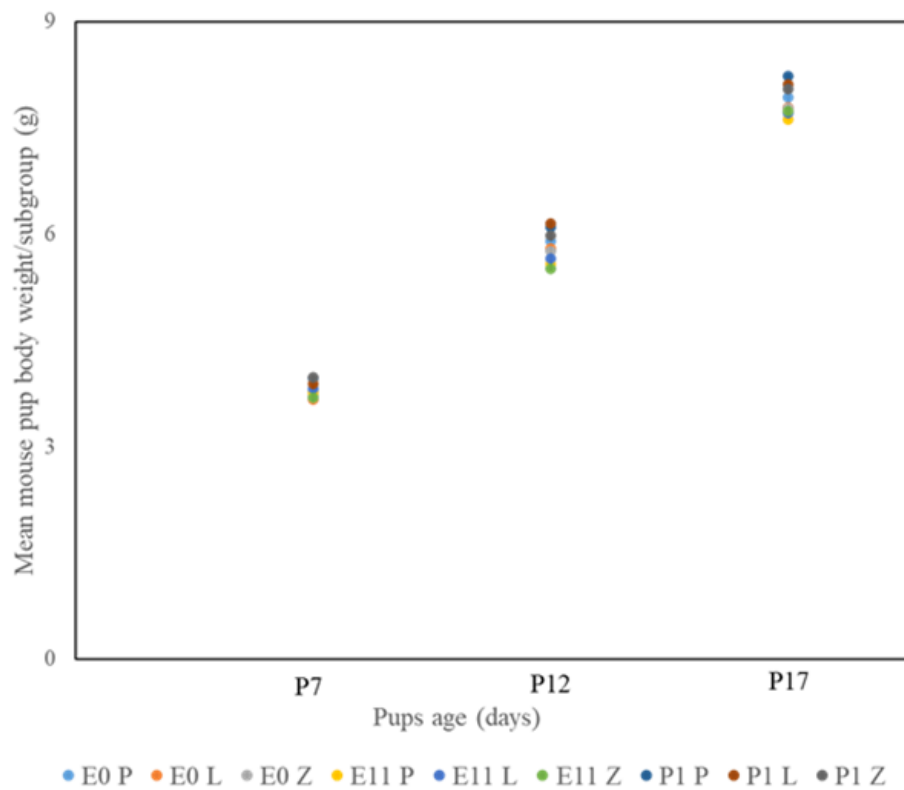
Online supporting material for "Prenatal Carotenoid Supplementation with Lutein or Zeaxanthin Ameliorates Oxygen-Induced Retinopathy (OIR) in *Bco2*^{-/-} Macular Pigment Mice".

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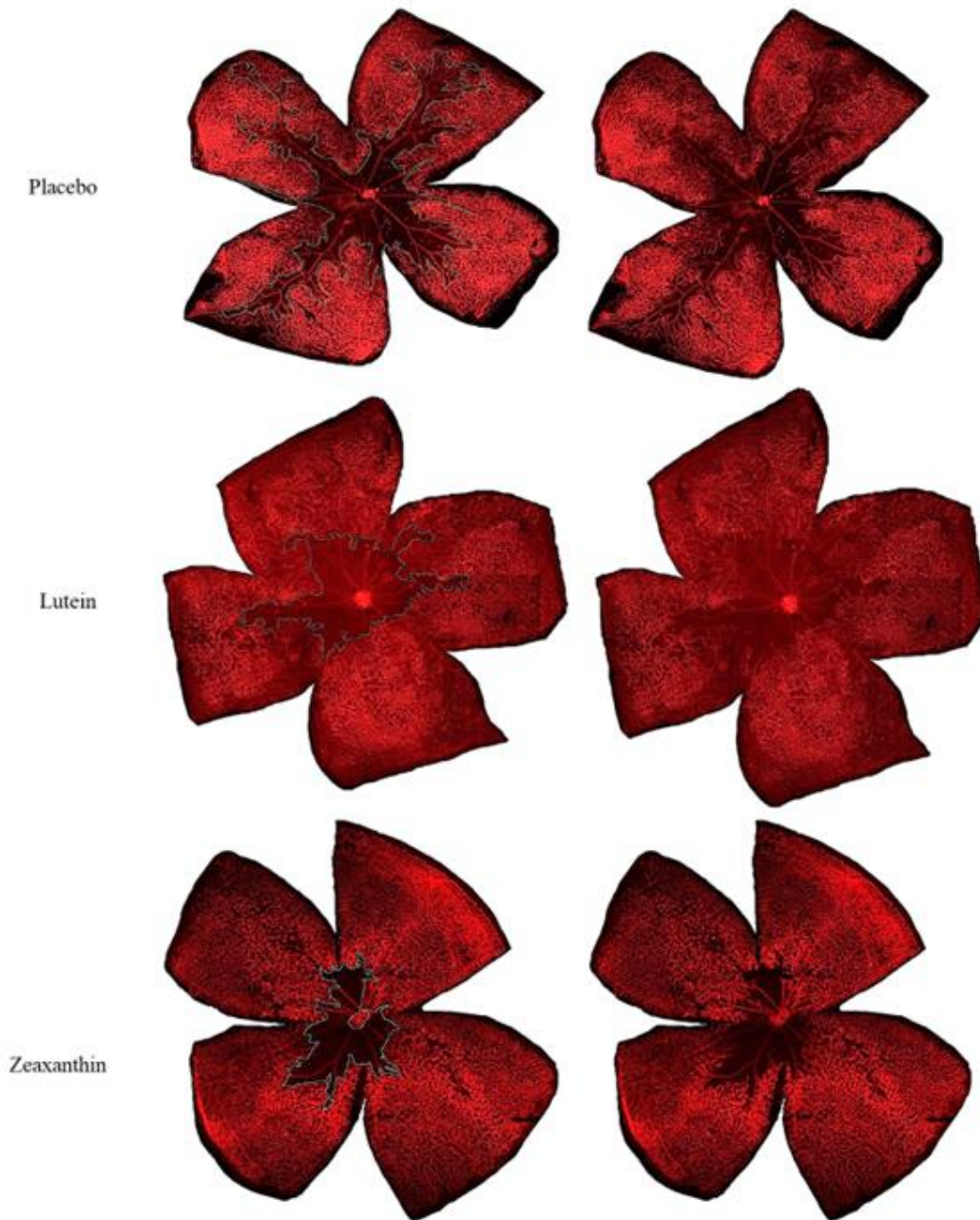
Mary Elizabeth Hartnett, and Paul S. Bernstein

Timeline	Pregnant <i>Bco2^{-/-}</i> mice (n = 2-4 mothers in each experimental group)		
	<p>Each group was subdivided into the L, Z, and placebo subgroups</p> <ul style="list-style-type: none"> • Pups from each experimental subgroup were pooled from 2-4 mothers. • Pups' body weights were assessed at P7, P12, and P17. 		
Prenatal L, Z, and placebo supplementation start at different pregnancy stages.	<p>Group I</p> <p>E0 – Embryonic day 0 (~ 1st trimester in humans)</p>	<p>Group II</p> <p>E11 – Embryonic day 11 (~ 2nd trimester in humans)</p>	<p>Group III</p> <p>P1 – Postnatal day 1 (~ preterm baby in humans)</p>
P7 – P12 Hyperoxia phase	<ul style="list-style-type: none"> • Nursing mothers and pups were exposed to 75% O₂ to initiate OIR • At the end of this phase, pups (n=4-6/subgroup) were sacrificed, and the retinas were analyzed for vaso-obliteration (VO). 		
P12 –17 Hypoxia phase	<ul style="list-style-type: none"> • Nursing mothers and the remaining pups were returned to 21% O₂. • At the end of this phase, the pups (n=3-5/subgroup) were sacrificed, and the retinas were analyzed for intravitreal neovascularization (INV). 		

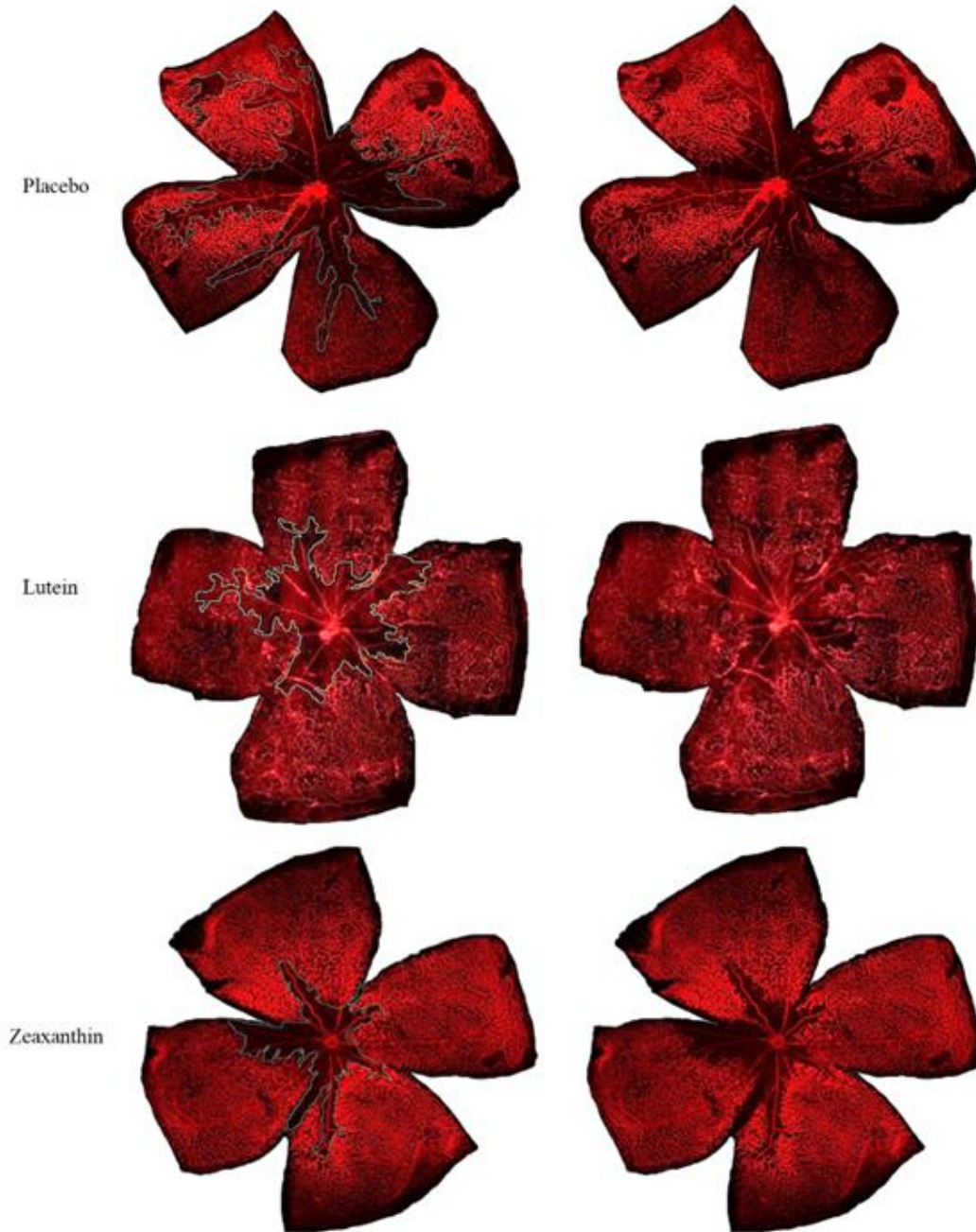
Supplementary Table A. Schematic representation of animal experimental design showing different treatment groups with their respective timelines and endpoints.



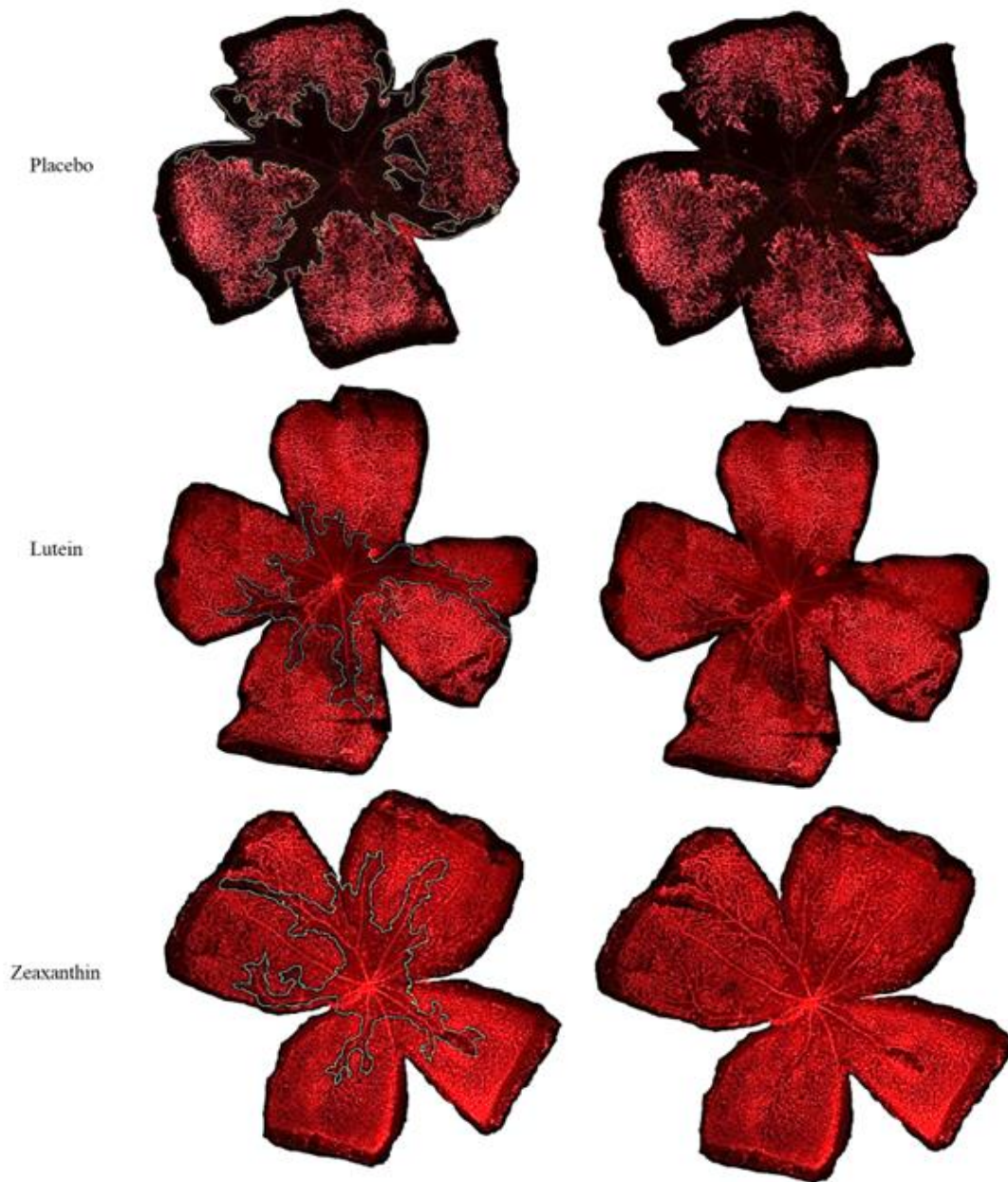
Supplementary Figure A. Mean body weight gain of *Bco2*^{-/-} mouse pups whose pregnant mothers were supplemented with either lutein (L), zeaxanthin (Z), or placebo (P) diets starting at E0, E11, or P1 and then subjected to our OIR protocol. Their body weights were measured at P7, P12, and P17.



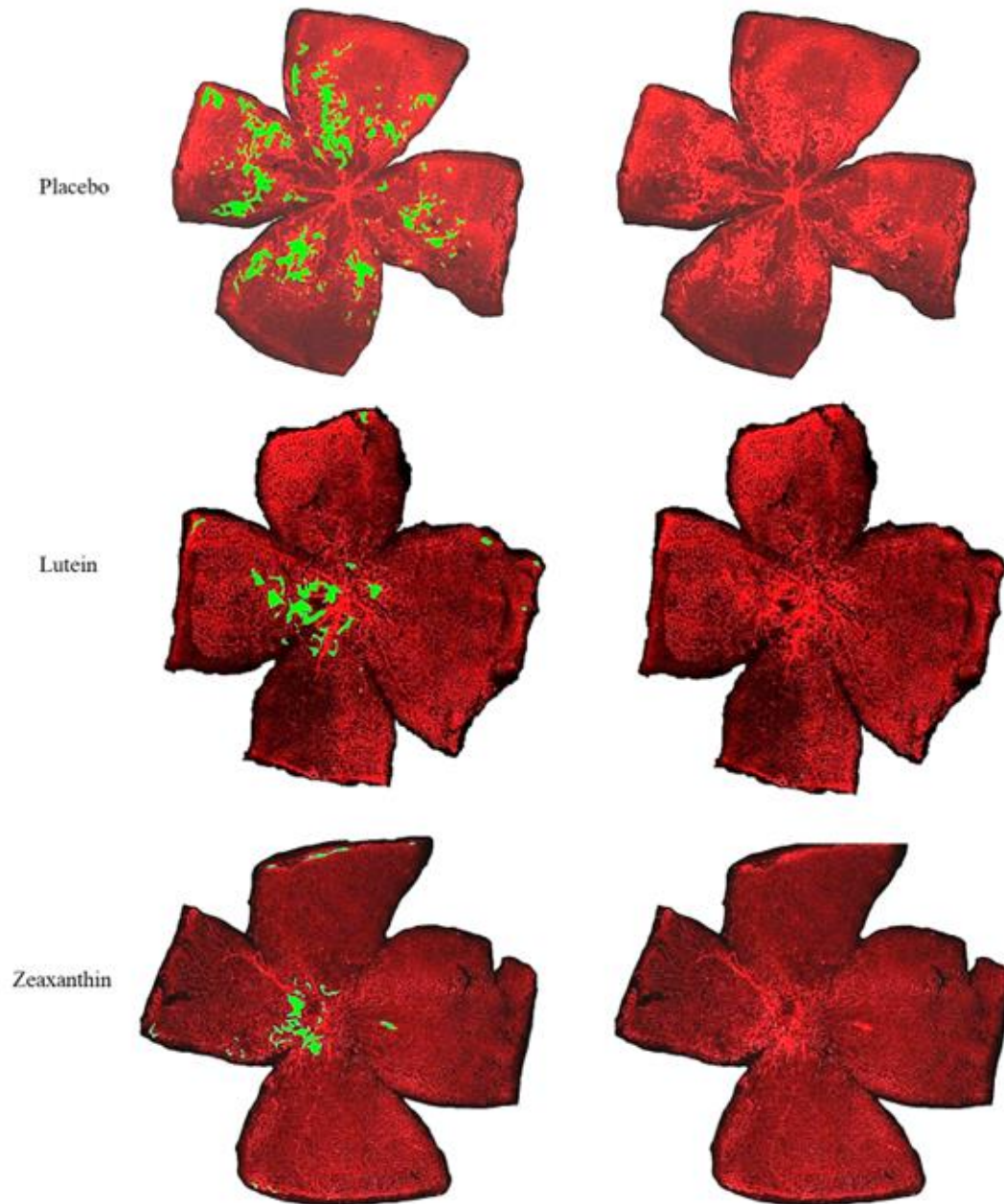
Supplementary Figure B. Representative lectin-stained flat mounts of retinas shown in **Figure 2**, marked (first column) and unmarked (second column) from *Bco2*^{-/-} mouse pups sacrificed at P12 showing vaso-oblivation (VO) after treatment under our OIR protocol. The blue marking shows the central avascular area. Their mothers started supplementation with L, Z, or placebo diets at E0.



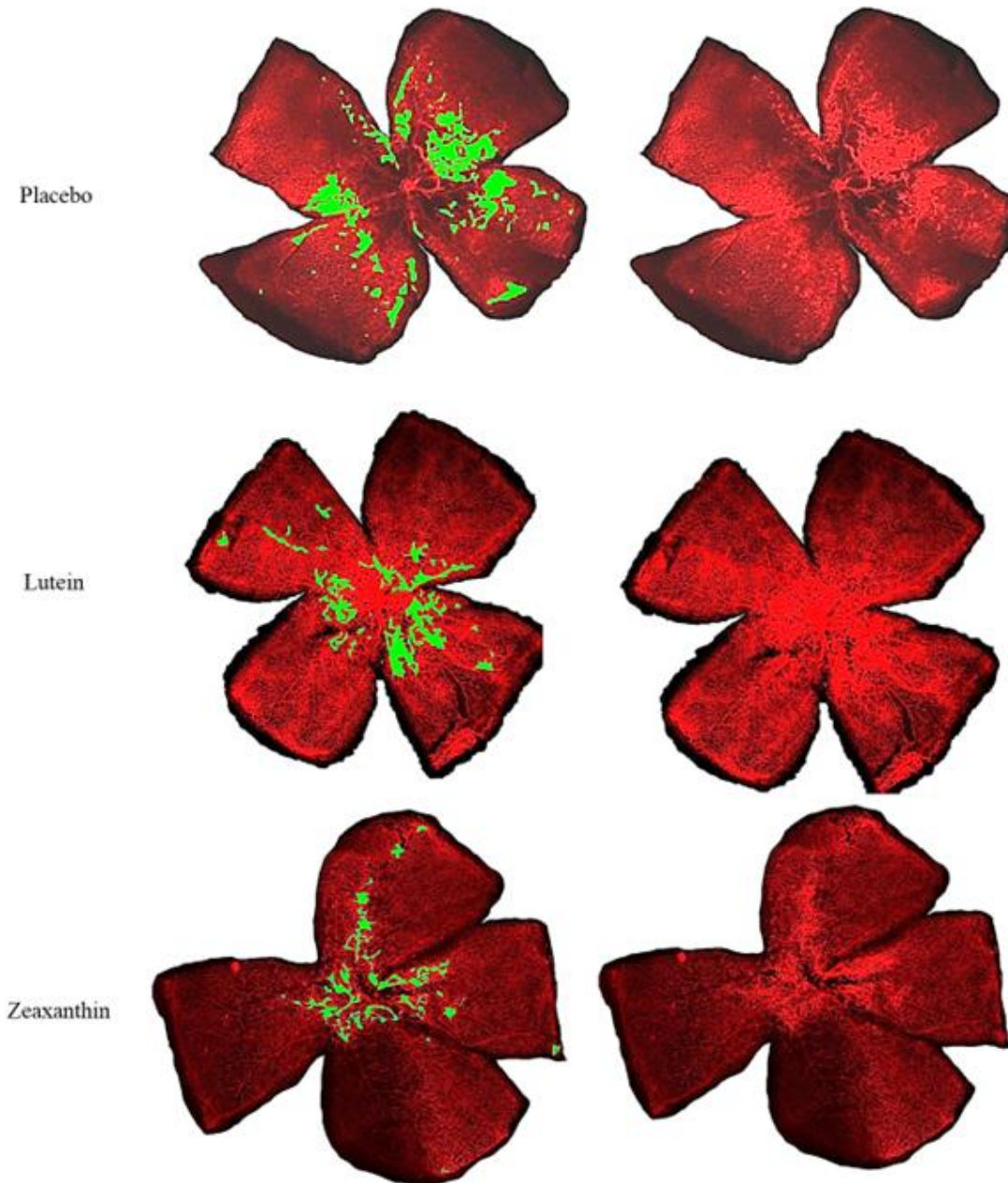
Supplementary Figure C. Representative lectin-stained flat mounts of retinas shown in **Figure 3**, marked (first column) and unmarked (second column) from *Bco2*^{-/-} mouse pups sacrificed at P12 showing VO after treatment under our OIR protocol. The blue marking shows the central avascular area. Their mothers started supplementation with L, Z, or placebo diets at E11.



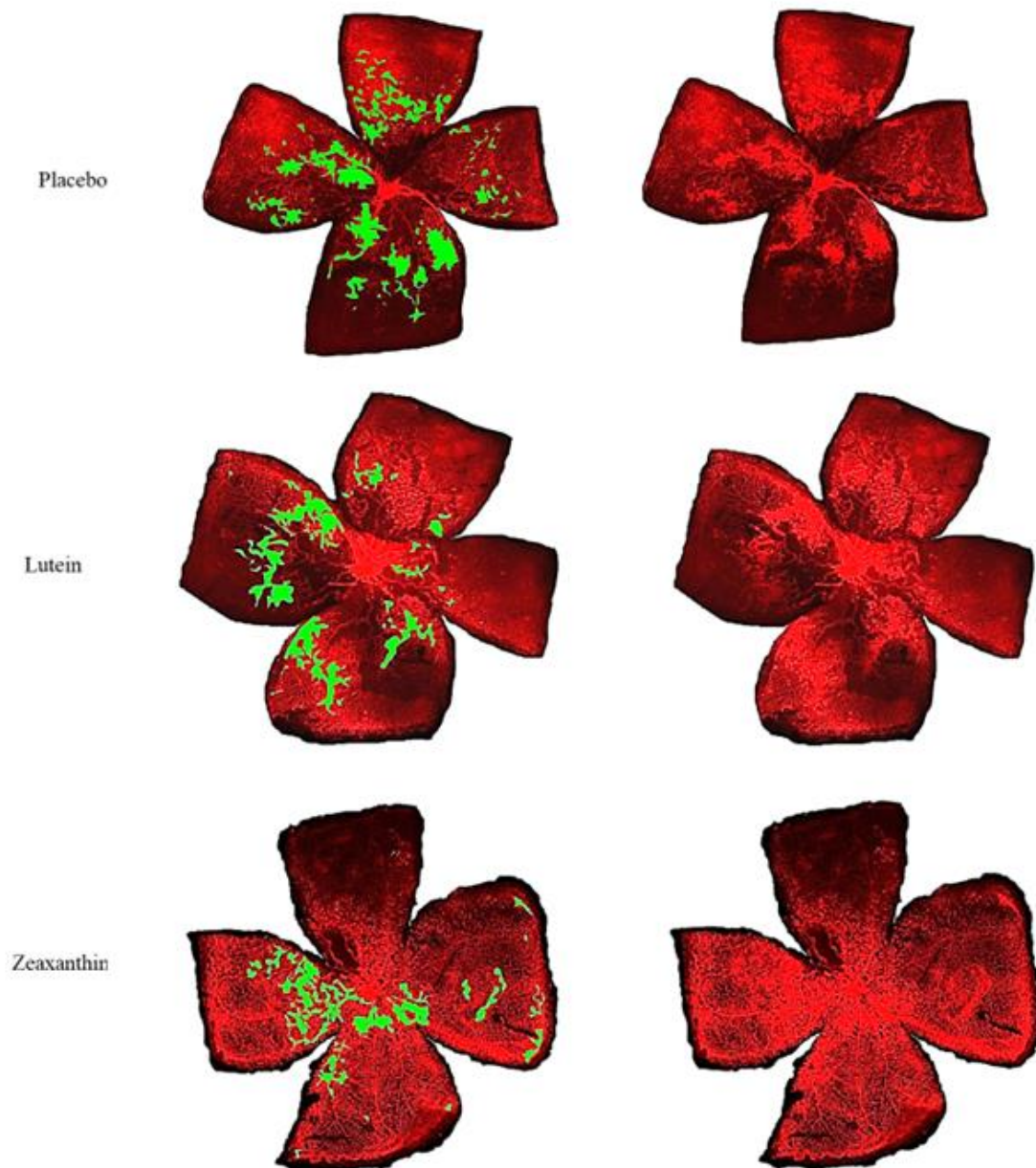
Supplementary Figure D. Representative lectin-stained flat mounts of retinas shown in **Figure 4**, marked (first column) and unmarked (second column) from *Bco2*^{-/-} mouse pups sacrificed at P12 showing VO after treatment under our OIR protocol. The blue marking shows the central avascular area. Their mothers started supplementation with L, Z, or placebo diets at P1.



Supplementary Figure E. Representative lectin-stained flat mounts of retinas shown in **Figure 6**, marked (first column) and unmarked (second column) from *Bco2*^{-/-} mouse pups sacrificed at P17 showing intravitreal neovascularization (INV) under our OIR protocol. The green marking shows the INV area. Their mothers started supplementation with L, Z, or placebo diets at E0.



Supplementary Figure F. Representative lectin-stained flat mounts of retinas shown in **Figure 7**, marked (first column) and unmarked (second column) from *Bco2*^{-/-} mouse pups sacrificed at P17 showing INV after treatment under our OIR protocol. The green marking shows the INV area. Their mothers started supplementation with L, Z, or placebo diets at E11.



Supplementary Figure G. Representative lectin-stained flat mounts of retinas shown in **Figure 8**, marked (first column) and unmarked (second column) from *Bco2*^{-/-} mouse pups sacrificed at P17 showing INV after treatment under our OIR protocol. The green marking shows the INV area. Their mothers started supplementation with L, Z, or placebo diets at P1.