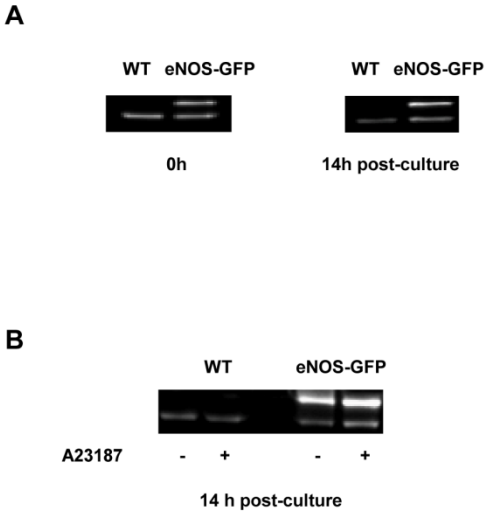

Figure S1: eNOS expression in retinal explants before and after Ca ionophore stimulation. **A** Freshly isolated retinas were incubated in the presence or absence of Ca ionophore and nitrite accumulation measured by Griess assay. **A** Representative western blot showing eNOS protein expression at 0h and 14 h post-ionophore stimulation. **B** Western blot showing eNOS expression after 14h with and without ionophore. There was no adverse effect on eNOS expression following culture.

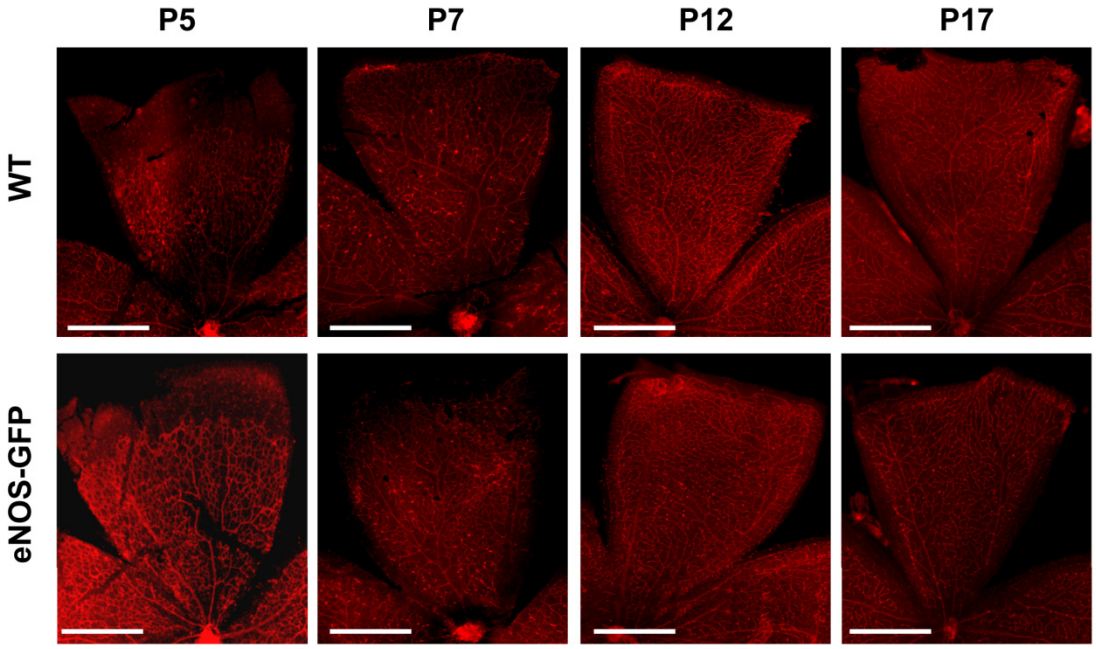

Figure S2: Development of the inner retinal vasculature of WT and eNOS-GFP animals at P5, P7, P12 and P17. Representative images from retinal flat mounts of WT and eNOS-GFP mice stained with lectin to show the vasculature. Retinal vascular development appears comparable throughout. Scale bars are 1 mm.

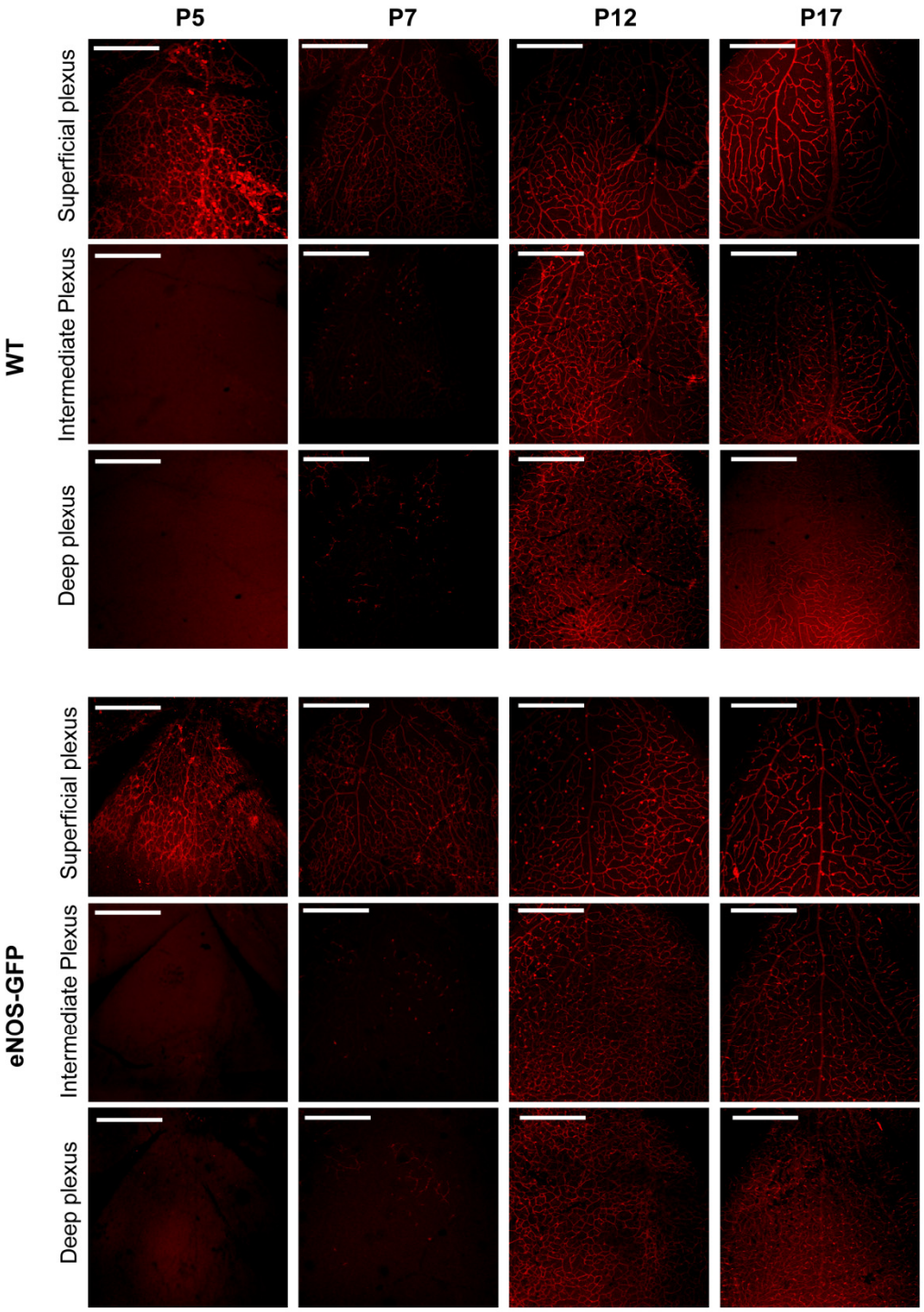

Figure S3: Visualisation of the deeper retinal layers. Representative images from retinal flat mounts of WT and eNOS-GFP mice stained with lectin and imaged using a confocal microscope to show the development of the superficial and deeper retinal plexi. As before, generation of these retinal beds appears similar between genotypes. Scale bars are 500 µm.

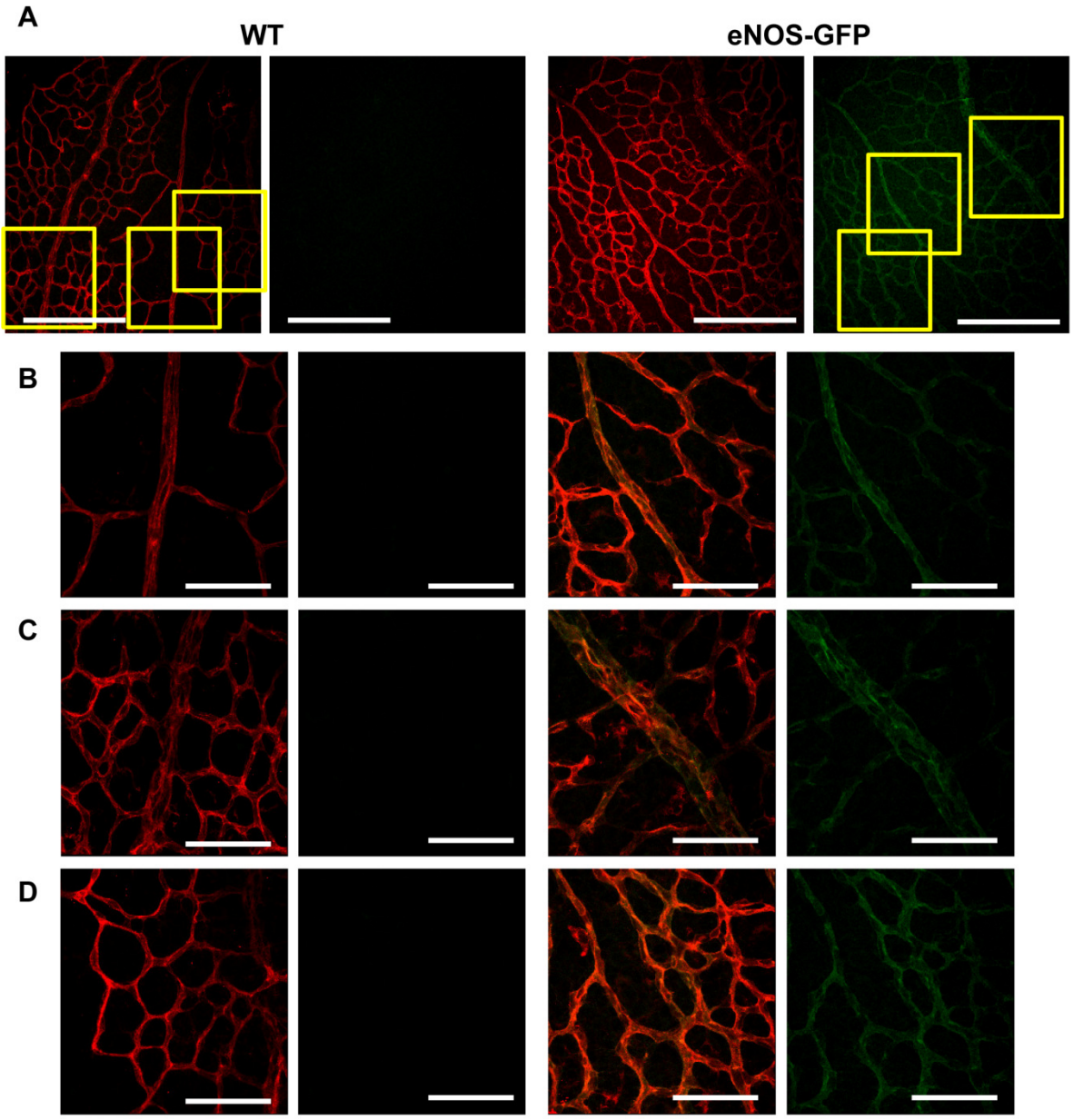

Figure S4: eNOS-GFP expression is clearly visible in the developing vasculature at P7. A Representative images of developing vessels in WT and eNOS-GFP retinal flat mounts stained with lectin. **B** High magnification images of those sections highlighted in the yellow box in with examples of arterioles (B), venules (C) and capillaries (D)A. Scale bars: A = 500 µm; B,C,D =100 µm

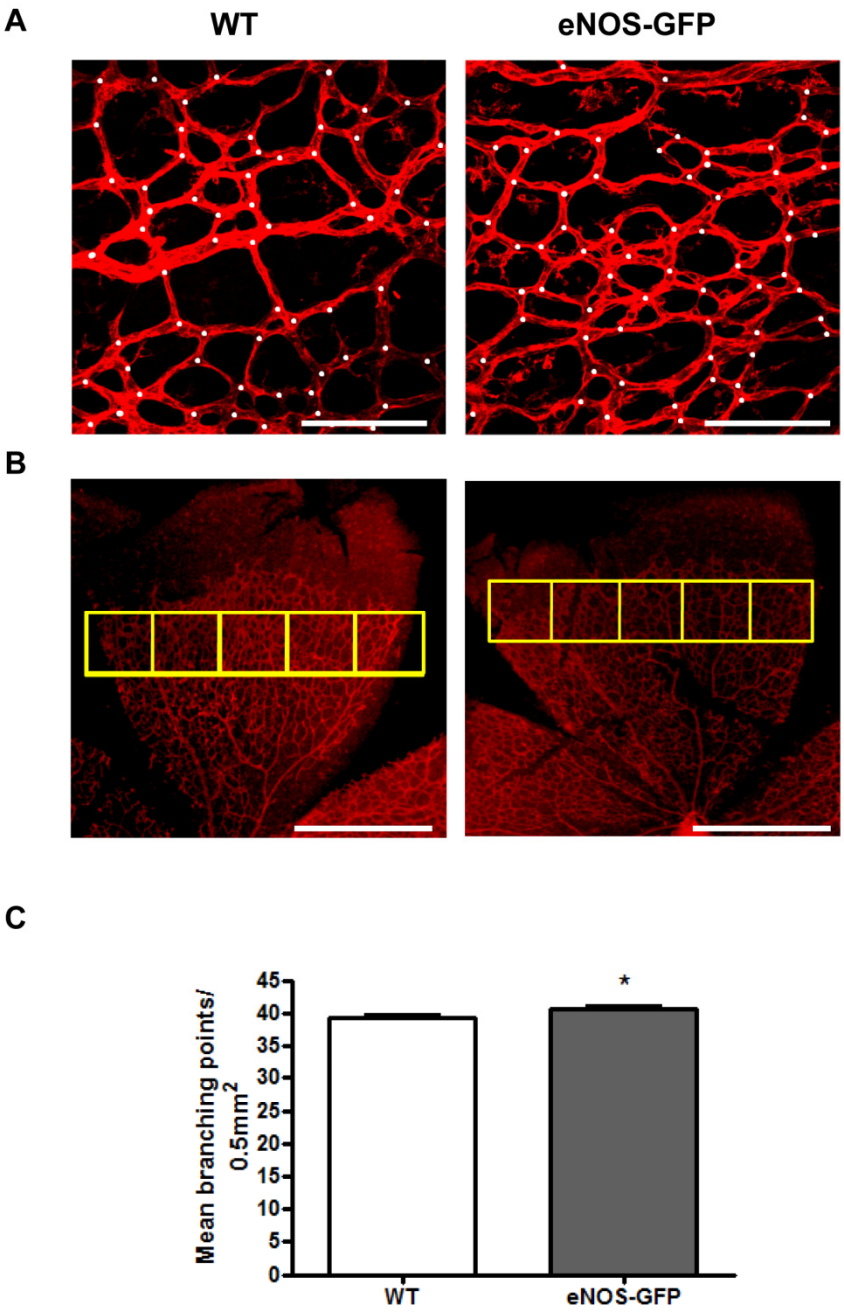

Figure S5: Vascular branching in WT and eNOS-GFP animals at P5. Retinal flat mounts were stained with lectin prior to imaging**. A** Representative images of branching in WT and eNOS-GFP flat mounts, branching points are marked with white dots. **B** Representative images of branching in the peripheral retina. n=5-8 per group; * $P < 0.05$ Scale bars = 100 µm (A), 1mm (B). **C** Quantification of retinal branching.

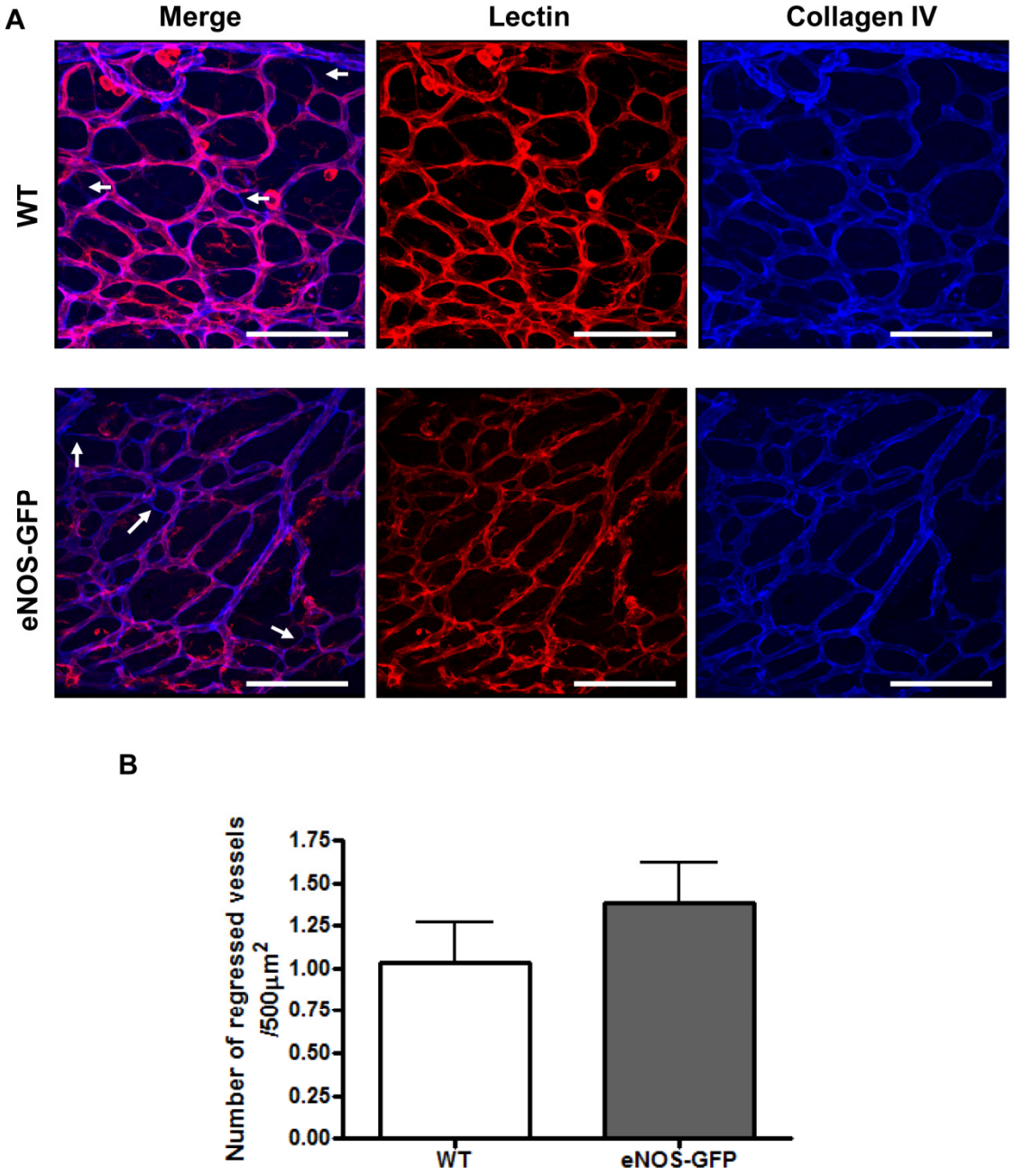

Figure S6: Vessel regression in developing P5 retinal vasculature. Flat mounted retinas from WT and eNOS-GFP mice were stained with lectin and collagen IV to investigate vessel remodelling. **A** Remodelled vessels were those that are collagen IV positive and lectin negative (arrows). **B** Quantification of collagen IV positive, lectin negative vessel fragments (n=5-8 per group from $*P < 0.05$). There was no significant difference in the number of regressed vessel in WT and eNOS-GFP retinas. Scale bars are 100µm.

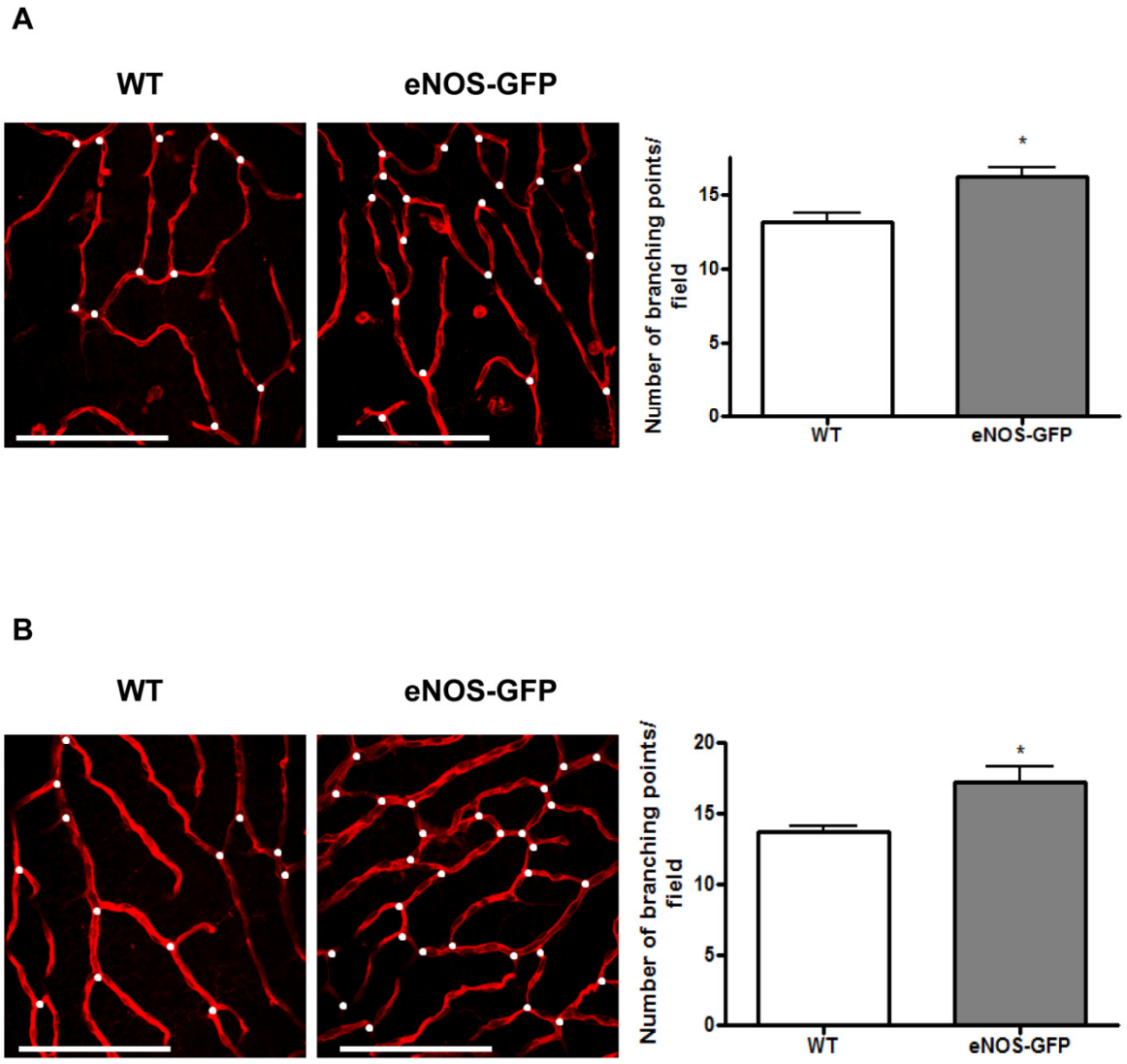

Figure S7: Comparison of vascular branching in the central and peripheral retinal zones of WT and eNOS-GFP animals at P17. Retinal flat mounts were stained with lectin prior to imaging**. A** Representative images of branching in WT and eNOS-GFP of the central retina in flat mounts, branching points are marked with white dots. **B** Representative images of branching in the peripheral retina (n=3 per group from $*P < 0.05$). Scale bars = 100 µm.

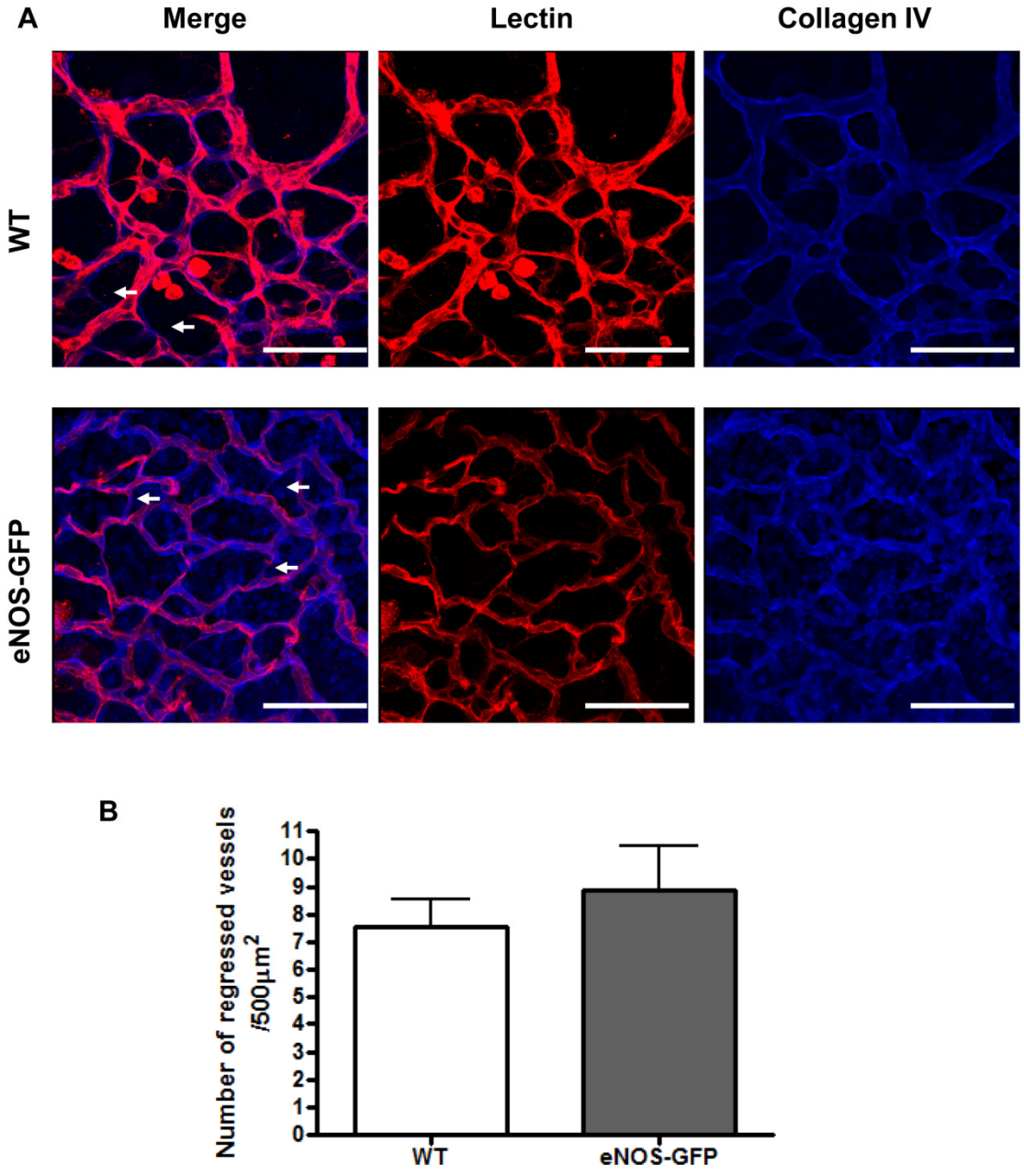

Figure S8: Vessel regression in developing P17 retinal vasculature. Flat mounted retinas from WT and eNOS-GFP mice were stained with lectin and collagen IV to investigate vessel remodelling. Remodelled vessels were those that are collagen IV positive and lectin negative (arrows). **B** Quantification of collagen IV positive, lectin negative vessel fragments (n=8 per group from $*P < 0.05$). Scale bars are 100µm.

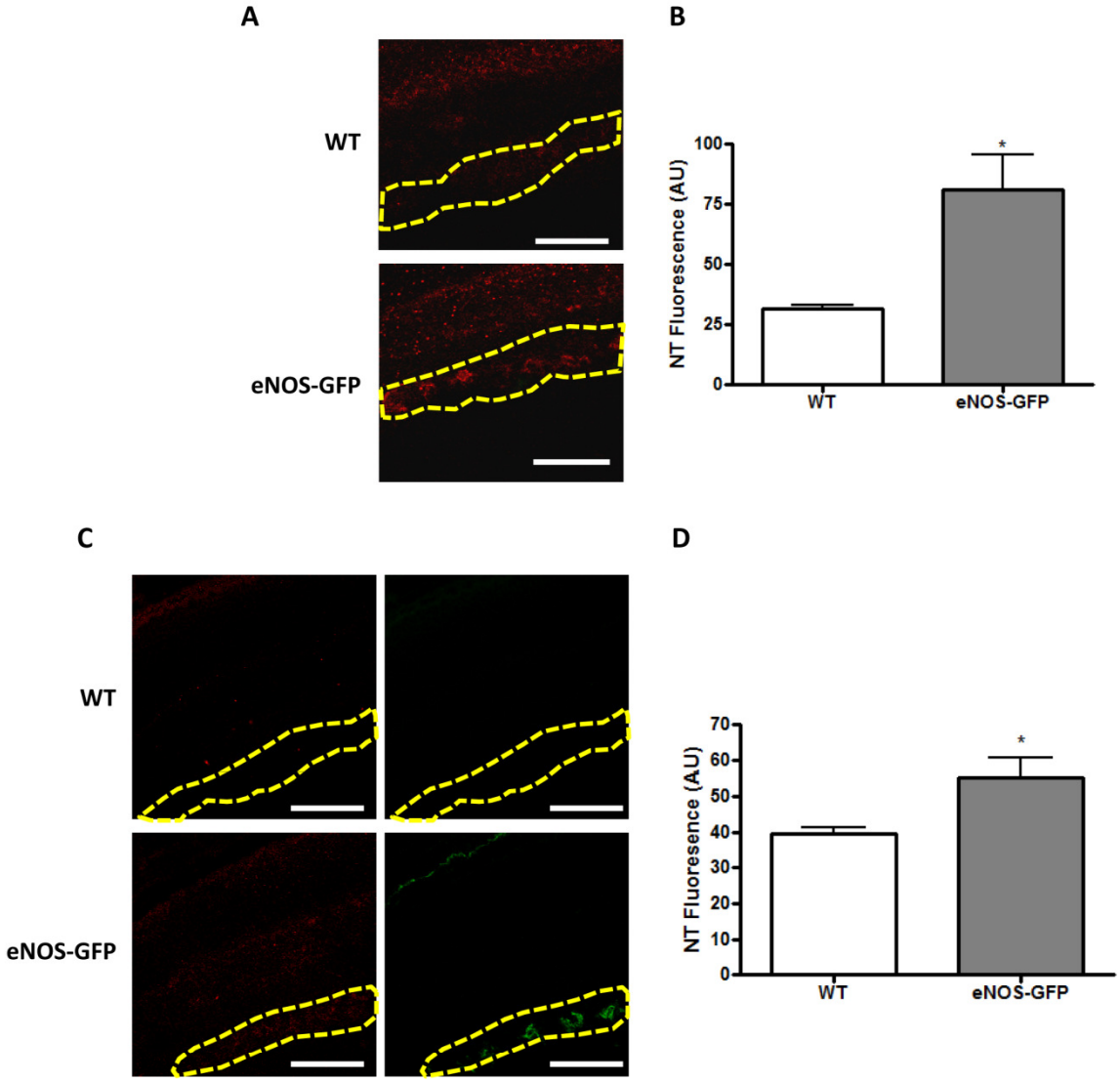

Figure S9: Quantification of nitrotyrosine (NT) immunolabelling in the ganglion cell layer (GCL) of retinal sections at P12 post-OIR. **A** Representative image of NT (red) staining intensity in the GCL demarcated with the yellow outline in the avascular area. **B** Quantification of NT staining shown in A. **C** Representative image of NT staining intensity in the GCL of the peripheral vasculature. **D** Quantification of NT staining shown in C,*P<0.05, n=3 from 3 independent OIR experiments. Scale bars are 50µm. NT is significantly increased in GCL of retinal sections from eNOS-GFP mice compared to WT in both avascular (A &B) and vascular (C &D) regions.

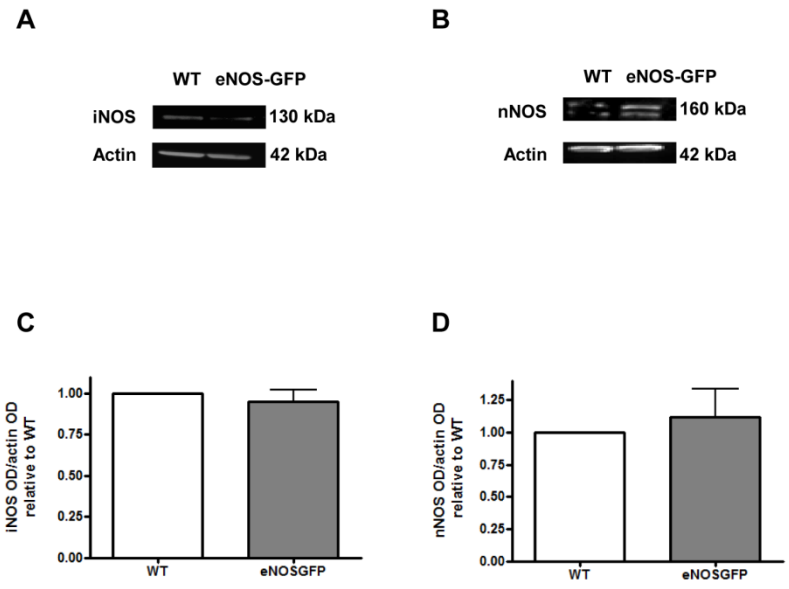

Figure S10: iNOS and nNOS protein levels in P17 OIR eNOS-GFP retina. Four retinas were pooled for each sample and protein was extracted for western blotting. **A** Representative western blot image for iNOS protein. **B** Representative western blot image for nNOS protein. **C** There is no significant difference in iNOS protein level, n=3 groups of 4 pooled retinas. **D** There is no significant difference in nNOS protein level, n=3 groups of 4 pooled retinas.