Fig. S1: Cells of the persistent primary vitreous mass consist of vascular and pigmented cells. (A-F) Serial sections of a P7 ephrin-A5<sup>−/−</sup> eye is shown in bright-field (A) and immunostained for markers indicative of endothelial cells (CD-31 and Collagen-IV, B and C), smooth muscle cells (αSMA) (D), pericytes (PDGFR-β) (E), and melanocytes (TRP-1) (F). Scale bars = 100 μm.
Fig. S2: Macrophage expression observed in the retrolental mass in ephrin-A5⁻/⁻ animals. (A and B) Retrolental tissue of the P7 ephrin-A5⁻/⁻ eyes stained for macrophage markers. F4/80+ (A) and ED-1+ (B) cells are observed throughout the mass. Scale bars = 100 μm. (D and E) The primary vitreous shows expression of F4/80 in both wild-type (D) and ephrin-A5⁻/⁻ (E) tissues at embryonic stages. Scale bars = 100 μm.

Fig. S3: The primary vitreous is made of cells consisting of both the neural crest and mesoderm. Quantification of the percentage of cells that are AP2β+ and CD31+ within the primary vitreous at E11 shows no significant differences between the wild-type and ephrin-A5⁻/⁻ animals (p > 0.05, n=4 per group).
Fig. S4: Apoptotic cells present during development of primary vitreous in wild-type and ephrin-A5⁻/⁻ animals. (A and B) Cells labeled for cleaved caspase-3 present in wild-type and ephrin-A5⁻/⁻ primary vitreous cells at E14 (A) and E16 (B). Cleaved caspase-3 is observed in both wild-type and ephrin-A5⁻/⁻ tissues. Scale bar = 100 μm. (C) Quantification of the percentage of cleaved caspase-3 positive cells in the wild-type and ephrin-A5⁻/⁻ primary vitreous. The percentage of cells positive for cleaved caspase-3 was not found to be significantly different between wild-type and ephrin-A5⁻/⁻ animals at either E14 or E16 (P>0.05, n=6 per group).