

Figure S1. Efficiency of AAV-mediated UNC5B knockdown and overexpression in retinal endothelial cells. Control shRNA-AAV (shC) and UNC5B shRNA-AAV (shUNC5B), as well as control overexpression AAV (NC) and UNC5B overexpression AAV (oeUNC5B), all carrying an endothelial cell-specific promoter, were each delivered via a single retro-orbital vein injection. At ≥ 8 weeks after injection, retinal tissues were collected for analysis. (A) Immunofluorescence staining (scale bar, 100 μm) and (B) western blotting were used to verify the efficiency of UNC5B knockdown in retinal endothelial cells of the mice. (C) Immunofluorescence staining (scale bar, 100 μm) and (D) western blotting were used to verify the efficiency of UNC5B overexpression in retinal endothelial cells. The data are presented as the mean \pm SD. $n=5$ per group, except for western blotting experiments ($n=4$). Statistical comparisons of the fluorescence intensity between the shUNC5B/oeUNC5B and shC/NC groups were performed using two-tailed unpaired Student's *t*-tests. Statistical comparisons of the protein expression between the WT group and each of the other groups were performed using one-way ANOVA followed by Dunnett's multiple comparisons test. * $P < 0.05$ vs. shC/WT/NC. AAV, adeno-associated virus; DR, diabetic retinopathy; NC, negative control; oe, overexpression; shRNA, short hairpin RNA; shC, scramble control shRNA; shUNC5B, UNC5B shRNA; UNC5B, unc-5 netrin receptor B; WT, wild-type.

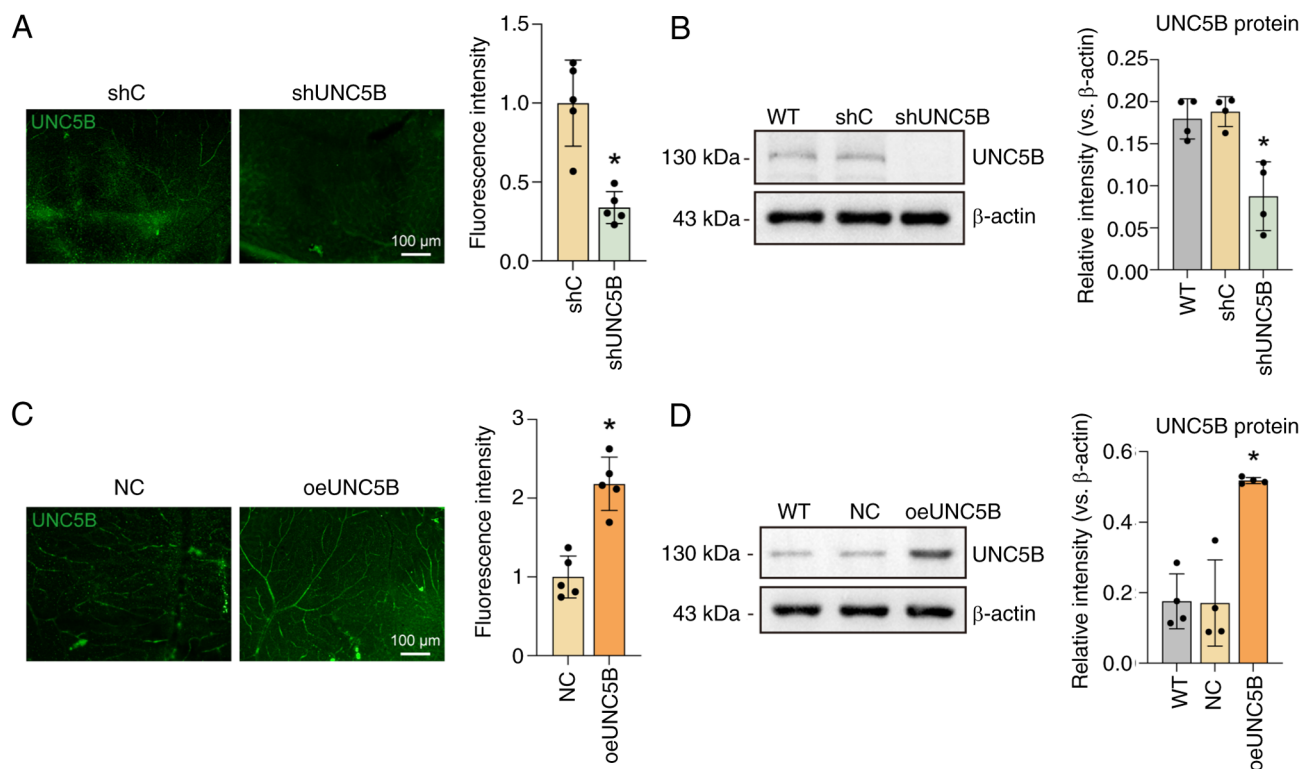


Figure S2. Transcriptomic analysis of differentially expressed genes following UNC5B knockdown in endothelial cells. Human retinal microvascular endothelial cells were transfected with lentiviral UNC5B shRNA, and stable UNC5B-silenced cell lines (shUNC5B) were constructed using puromycin selection. A control group was established by transfection with negative control shRNA (shC). A total of three samples from each group were used for transcriptome sequencing. (A) Volcano plot showing the distribution of differentially expressed genes between the shUNC5B and shC groups. The lollipop plots show the enrichment features of the two groups in terms of (B) cellular components and (C) molecular functions. P.adj, adjusted P-value; shRNA, short hairpin RNA; shC, scramble control shRNA; shUNC5B, UNC5B shRNA; UNC5B, unc-5 netrin receptor B.

