Supplement data:

Figure 1: Negative controls of paraffin sections

Sagittal section of the cornea of a wildtype mouse stained with secondary antibodies used for Netrin-4 and Lyve-1 staining and AF488 conjugated isotype as negative controls. It shows a strong, unspecific staining of the corneal epithelium.
Figure 2: Netrin-4 and DAPI staining of paraffin section

Sagittal section of the cornea of a wildtype mouse 14 days after suture placement (A). Netrin-4 is visualized by Netrin-4 staining (red) and cell nuclei by DAPI staining (blue). Netrin-4 was expressed in the basement membrane of the corneal endothelium (Descemet membrane) (arrows, C) and epithelium (arrows, B) as well as in the basement membrane of corneal vessels.
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Figure 3: Netrin-4 staining of a corneal flatmount

Corneal flatmount of wildtype mouse 14 days after suture placement. Endothelium of vessels is visualized by CD31 staining (green) and Netrin-4 by Netrin-4 staining (red).
Figure 4: Differential regulation of VEGF-A, Netrin-4, Unc5H2 and Neogenin mRNA expression in HCET after stimulation with VEGF-A and Netrin-4

HCET were serum-starved with medium (Sasaki or KGM medium) for 12 hours. Quantitative PCR was performed to measure VEGF-A (A), Netrin-4 (B), Unc5H2 (C) and Neogenin (D) mRNA expression in HCET after stimulation for 2 hours with VEGF-A 1ng/ml and 50ng/ml and with Netrin-4 50ng/ml and 500ng/ml. VEGF-A, Netrin-4, Unc5H2 and Neogenin mRNA expression did not differ significantly in HCET between serum-free and VEGF-A or Netrin-4 stimulated cells.