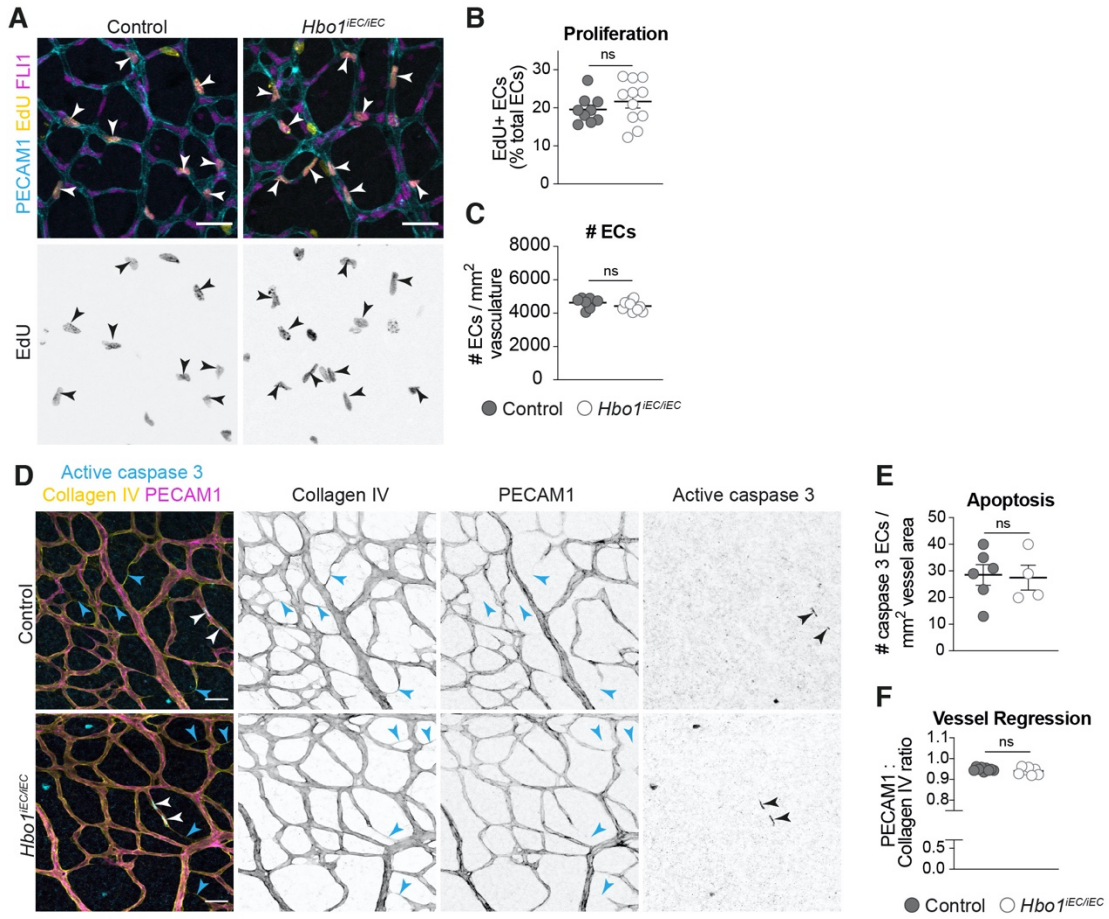

Fig. S1. Vessel patterning, EC Proliferation, EC apoptosis and vessel regression are normal in the absence of HBO1

(**A**) Proliferating ECs (indicated by arrowheads) in P6 control and *Hbo1*$^{iEC/iEC}$ retinas within the sprouting zone capillaries stained for PECAM1 (cyan), endothelial nuclear marker FLI1 (magenta) and EdU (yellow). Scale bar: 30 µm. Quantification of (**B**) proportion of proliferating ECs (control n = 9, *Hbo1*$^{iEC/iEC}$ n = 11, p = 0.35), (**C**) number of ECs per vessel area across whole retina (control n = 8, *Hbo1*$^{iEC/iEC}$ n = 11, p = 0.16). (**D**) P6 retinas from control and *Hbo1*$^{iEC/iEC}$ mice stained with collagen IV (yellow), PECAM1 (magenta) and active caspase 3 (cyan). Blue arrows indicate regressing vessels (PECAM1^{-} collagen IV^{+}) and white arrows indicate apoptotic ECs. Scale bar: 40 µm. Quantification of (**E**) apoptotic ECs per vessel area (control n = 6, *Hbo1*$^{iEC/iEC}$ n = 4, p = 0.87) and (**F**) vessel regression across retina (control n = 9, *Hbo1*$^{iEC/iEC}$ n = 7, p = 0.25) in P6 retinas. All statistical testing by Student's two-tailed t-test. All data are mean ± SEM. Each circle represents one individual animal.

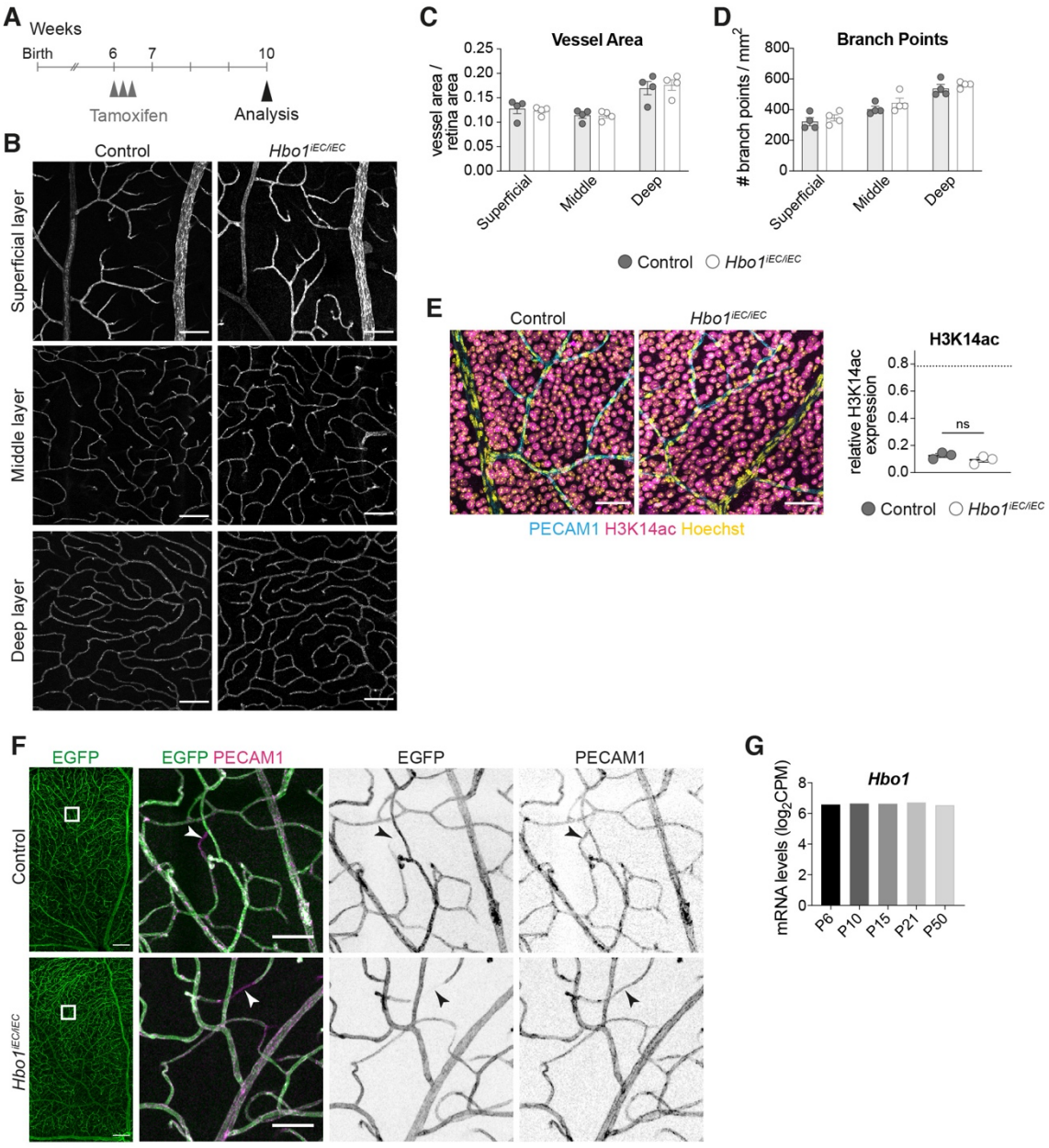

Fig. S2. Loss of HBO1 does not affect adult vasculature.

(**A**) Experimental overview for adult mice. Tamoxifen was administered for three consecutive days at six weeks of age and mice were analysed at ten weeks. (**B**) PECAM1 staining in superficial, middle and deep vessel layers of control and *Hbo1*$^{iEC/iEC}$ adults. Scale bar: 80 µm. Quantification of (**C**) vessel area per retina area for each vessel layer (control n = 4, *Hbo1*$^{iEC/iEC}$ n = 4) and (**D**) branch points (control n = 4, *Hbo1*$^{iEC/iEC}$ n = 4). (**E**) H3K14ac (magenta) staining and quantification in control (n = 9) and *Hbo1*$^{iEC/iEC}$ (n = 9) adult retinas. Co-stained for PECAM1 (cyan) and Hoechst 33342 (yellow). Scale bar: 50 µm. Dashed line indicates relative H3K14ac expression of P6 control mice, shown originally in Figure 1E. Student's two-tailed t-test, p = 0.22. (**F**) EGFP (green) and PECAM1 (magenta) in adult control and *Hbo1*$^{iEC/iEC}$ mice. White box in left-hand image enlarged in right-hand images. Scale bar: 200 µm (left)

and 50 µm (right). White/black arrows indicate EGFP$^-$ ECs. (**G**) Mean *Hbo1* mRNA expression (log_2 counts per million) in retinal ECs from P6 – P50 as analysed in data from Jeong *et al*[1]. Except in *G*, all data are mean ± SEM. Each circle represents one individual animal.

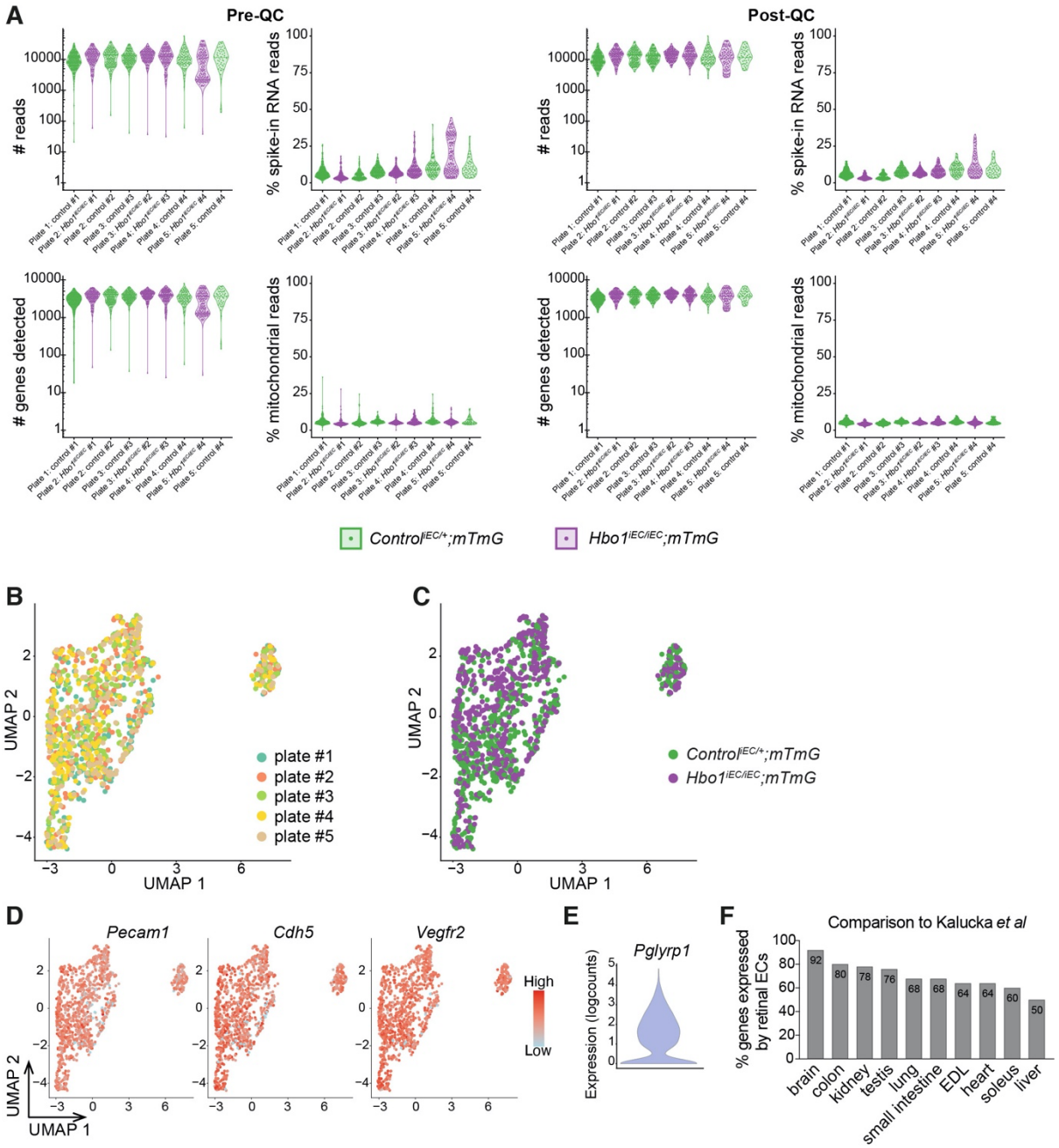

Fig. S3. Quality controls for scRNA-seq of retinal ECs.

(**A**) Violin plots of total number of reads, total number of genes detected, proportion of reads mapped to spike-in RNA and proportion of reads mapped to mitochondrial genes before and after cells that failed to achieve quality control (QC) cut-offs were removed from the analysis. See Table S6 for QC cut-offs. (**B**) UMAP plot colour coded for batch/plate analysed and (**C**) genotype (*Control*$^{iEC/+}$*;mTmG* n = 4, *Hbo1*$^{iEC/iEC}$*;mTmG* mice n = 4). (**D**) UMAP plots colour-coded for expression of indicated endothelial genes. Colour scale: red = high expression, blue = low expression. (**E**) Violin plot of *Pglyrp1* expression across all ECs sequenced and analysed. (**F**) Proportion (value shown on bar plot) of the top 50 tissue specific capillary markers from

Kalucka *et al*[2] that are expressed by retinal ECs. EDL: extensor digitorum longus skeletal muscle. All figure panels from scRNA-seq data and samples include ECs from *Control*$^{iEC/+}$*;mTmG* n = 4, *Hbo1*$^{iEC/iEC}$*;mTmG* n = 4. Data were analysed as described in the Material and Methods section.

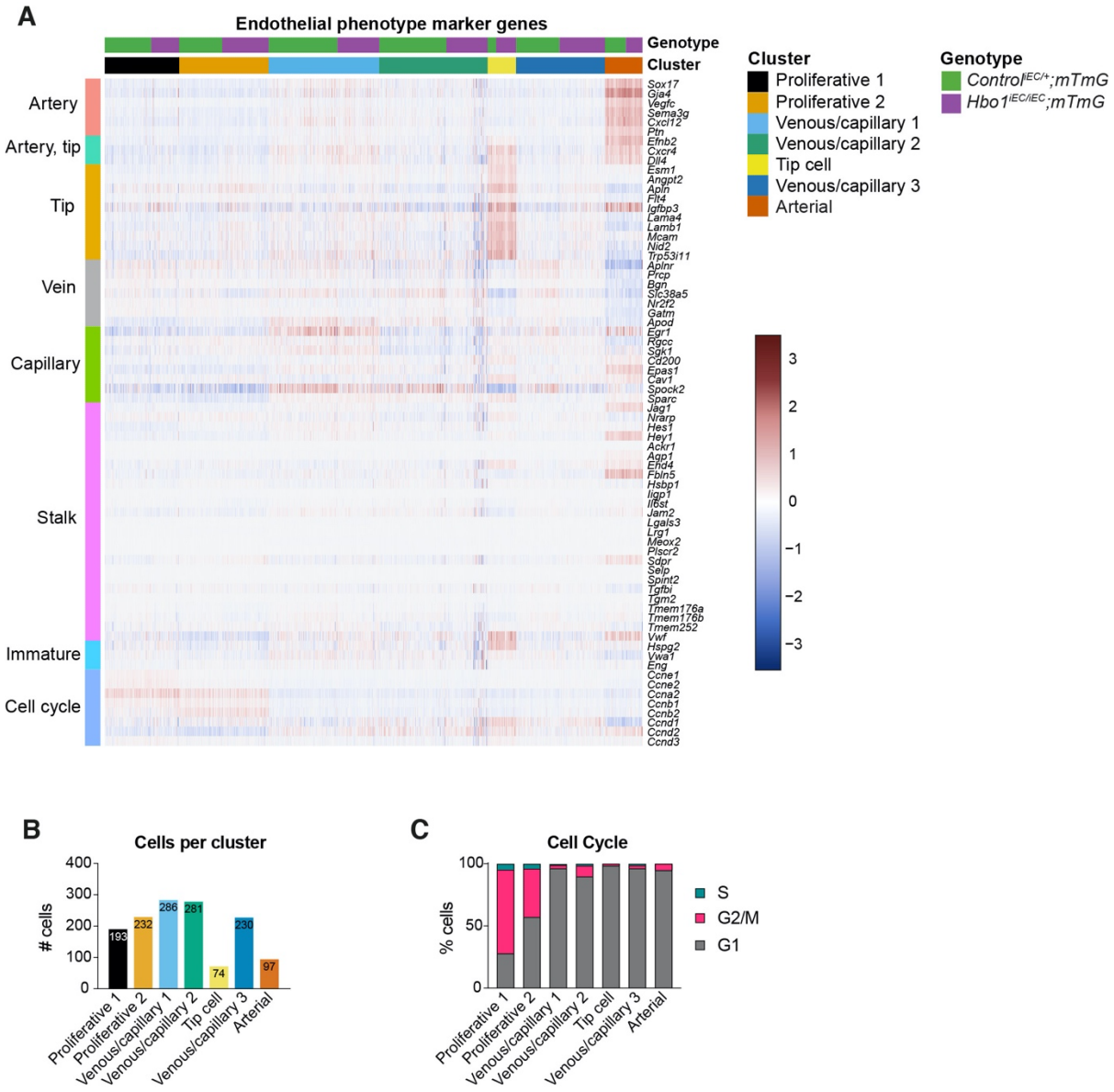

Fig. S4. Intratissue heterogeneity of retinal ECs.

(**A**) Heatmap displaying batch corrected logcounts (row-normalised) for EC subtype marker genes from Kalucka *et al*[2], Goveia *et al*[3] and Zhao *et al*[4]. Colour scale: red = high expression, blue = low expression. (**B**) Total number of cells within each cluster (includes both *Control*$^{iEC/+}$*;mTmG* and *Hbo1*$^{iEC/iEC}$*;mTmG* mice). Number of cells indicated on bar plot. (**C**) Proportion of cells in each cluster in each stage of the cell cycle as determined by cyclone classifier analysis. All figure panels from scRNA-seq data and samples include ECs from *Control*$^{iEC/+}$*;mTmG* n = 4, *Hbo1*$^{iEC/iEC}$*;mTmG* n = 4. Data were analysed as described in the Material and Methods section.

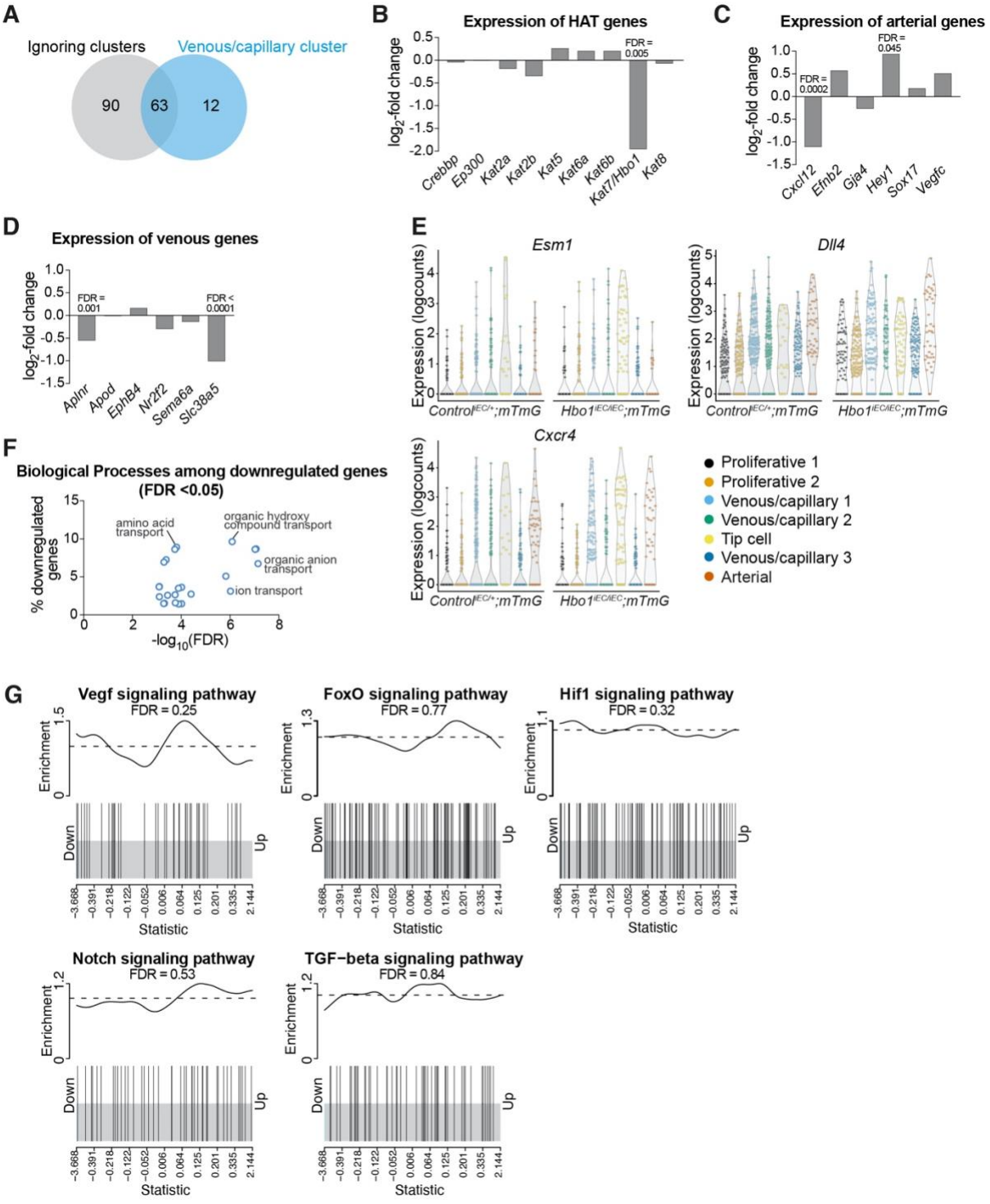

Fig. S5. Deletion of *Hbo1* causes changes in retinal EC gene expression.

(**A**) Overlap between differentially expressed genes ignoring clusters and in the venous/capillary clusters. mRNA levels of genes (**B**) encoding histone acetyltransferases, (**C**) expressed by arterial ECs and (**D**) expressed by venous ECs in *Hbo1*$^{iEC/iEC}$*;mTmG* retinal ECs relative to *Control*$^{iEC/+}$*;mTmG*. (**E**) Violin plots of tip cell gene marker expression across all clusters for *Control*$^{iEC/+}$*;mTmG* and *Hbo1*$^{iEC/iEC}$*;mTmG* mice. Each circle indicates the expression (in logcounts) for a single cell. (**F**) Scatterplot of the top 30 significantly

downregulated biological processes GO terms in *Hbo1*$^{iEC/iEC}$*;mTmG* ECs compared to *Control*$^{iEC/+}$*;mTmG*. Each dot represents the significance of one biological process against the proportion of genes associated with that term that are downregulated in *Hbo1*$^{iEC/iEC}$*;mTmG* ECs compared to *Control*$^{iEC/+}$*;mTmG*. This figure excludes GO terms that have a total of fewer than 25 genes per annotation. For the full list of terms see Supp Table 3. (**G**) Barcode plots showing enrichment of indicated pathways in *Hbo1*$^{iEC/iEC}$ retinal ECs compared to control. The vertical lines ('barcode') represent all pathway genes expressed in the ECs. At the top, the horizontal dotted line represents what is considered neutral or no enrichment and worm represents the enrichment of pathway genes in *Hbo1*$^{iEC/iEC}$ ECs. FDR was calculated by rotation gene set tests, testing whether the gene set is differentially expressed in either direction. All figure panels from scRNA-seq data and samples include ECs from *Control*$^{iEC/+}$*;mTmG* n = 4, *Hbo1*$^{iEC/iEC}$*;mTmG* n = 4. Data were analysed as described in the Material and Methods section.

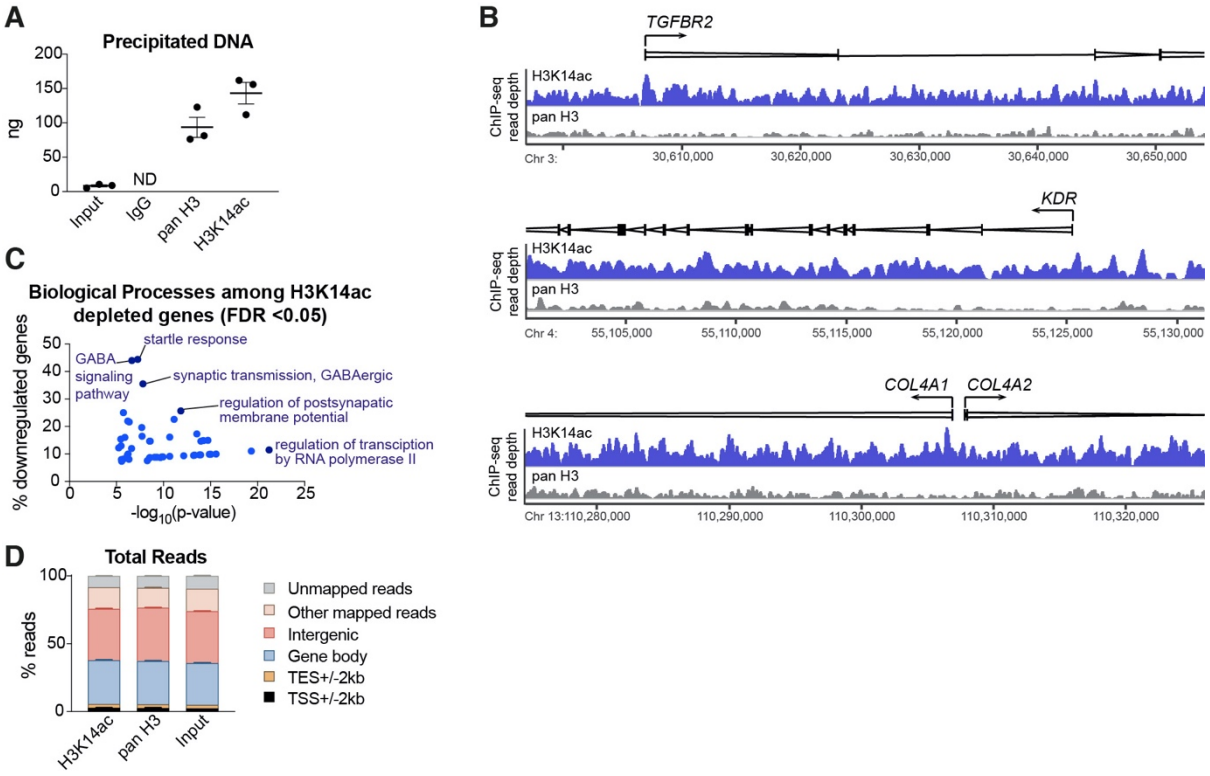

Fig. S6. H3K14ac is widely distributed in the endothelial cell genome.

(**A**) Total DNA precipitated by ChIP, ND = not detected. Data are mean ± SEM. Each circle represents one sample (n = 3). (**B**) Read depth plot of H3K14ac (blue) and pan H3 (grey) as assessed by ChIP-seq in HUVECs at the transcription start sites (TSS) of *TGFBR2, KDR, COL4A1* and *COL4A2* loci. H3K14ac samples were sequenced with twice the depth as pan H3 samples as described in methods. (**C**) Scatterplot of the top 100 biological processes GO terms at genes depleted for H3K14ac (n = 3) compared to pan H3 (n = 2). Each dot represents the significance of one biological process against the percentage of genes associated with that process that are depleted for H3K14ac. For the full list of GO terms see Supp Table 5. (**D**) Proportion of total reads that were mapped to the human genome and those that correspond to intergenic regions (defined as ≥10 kb away from any gene), gene bodies, TSS ± 2 kb and transcription end sites (TES) ± 2 kb. H3K14ac n = 3, pan H3 n = 2, input n = 2. Data are mean ± SEM. Data were analysed as described in the Material and Methods section.

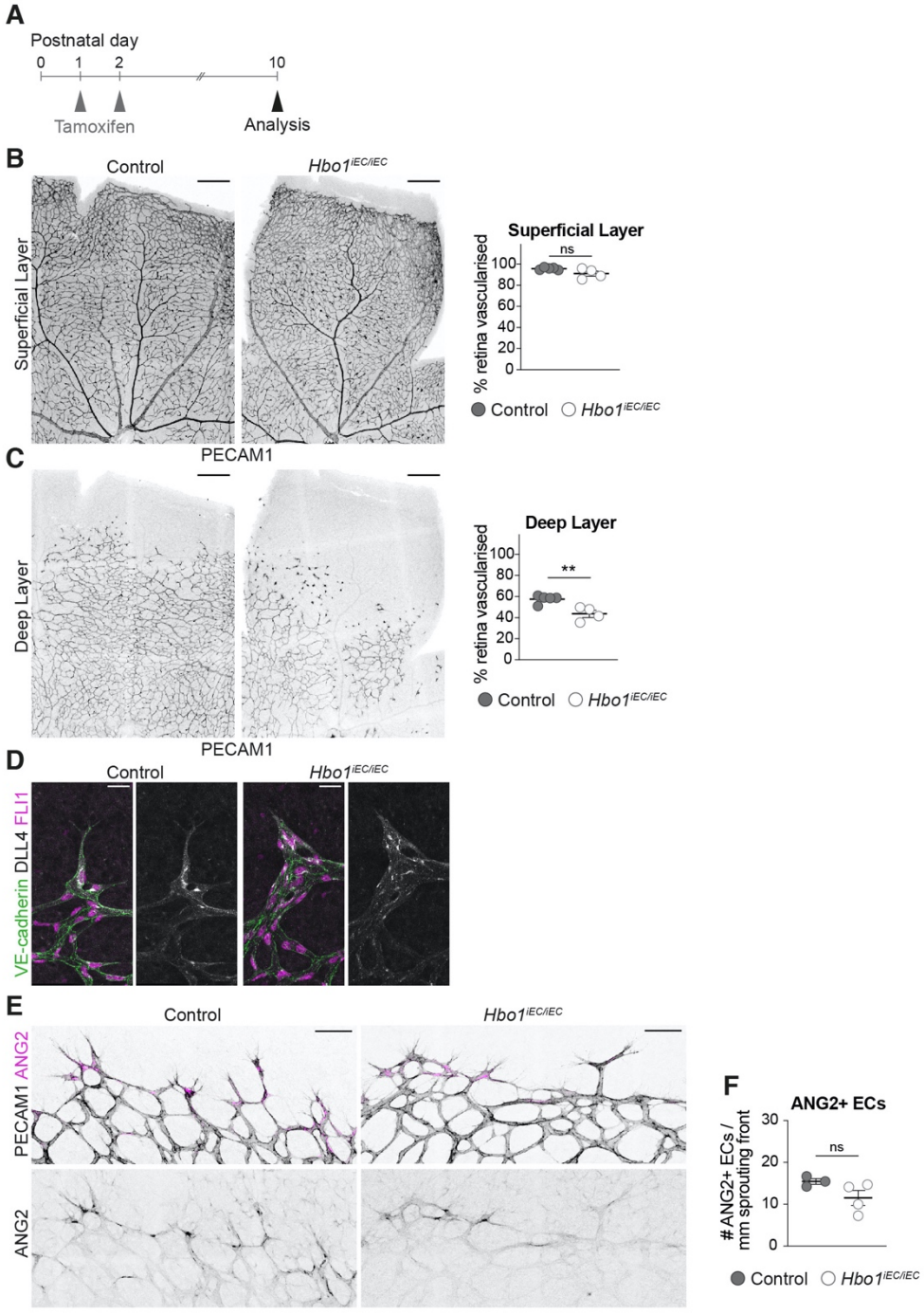

Fig. S7. Loss of HBO1 impairs sprouting into deeper retinal vessel layers.

(**A**) Experimental overview for mice analysed at P10. Tamoxifen was administered at P1 and P2 to induce cre recombination. (**B**) PECAM1 staining and quantification of proportion of the superficial retina vascularised in P10 retinas (control n = 5, *Hbo1*$^{iEC/iEC}$ n = 4, p = 0.067). (**C**) PECAM1 staining and quantification of proportion of the deep retina vascularised in P10 retinas (control n = 5, *Hbo1*$^{iEC/iEC}$ n = 4, p = 0.005). (**D**) Representative example of DLL4 expression (grey) in tip cells of control and *Hbo1*$^{iEC/iEC}$ retinas. Retinas also stained with VE-cadherin (green) and FLI1 (magenta). Scale bar: 30 µm. (**E**) Vessels in the sprouting front of

P6 control and *Hbo1*$^{iEC/iEC}$ retinas stained for PECAM1 (greyscale) and tip-cell marker ANG2 (magenta). Scale bar: 80 µm. (**F**) Quantification of the number of ANG2+ ECs per length of sprouting front (control n = 3, *Hbo1*$^{iEC/iEC}$ retinas n = 4, p = 0.13). All statistical testing by Student's two-tailed t-test. All data are mean ± SEM. Each circle represents one individual animal.

Table S1. Retinal scRNA-seq cluster marker genes

Click here to download Table S1

Table S2. Differentially expressed genes in Hbo1 deleted ECs by scRNA-seq Supplementary

Click here to download Table S2

Table S3. Gene Ontology terms (biological processes) upregulated and downregulated in Hbo1 deleted ECs by scRNA-seq

Click here to download Table S3

Table S4. Genes enriched or depleted for H3K14ac

Click here to download Table S4

Table S5. Gene Ontology terms (biological processes) for H3K14ac enriched and depleted gene bodies by ChIP-seq

Click here to download Table S5

Table S6. Retinal EC scRNA-seq quality control metrics

Click here to download Table S6

Supplementary Data References

1 Jeong, H. W. *et al.* Transcriptional regulation of endothelial cell behavior during sprouting angiogenesis. *Nat Commun* **8**, 726, doi:10.1038/s41467-017-00738-7 (2017).

2 Kalucka, J. *et al.* Single-Cell Transcriptome Atlas of Murine Endothelial Cells. *Cell* **180**, 764-779 e720, doi:10.1016/j.cell.2020.01.015 (2020).

3 Goveia, J. *et al.* An Integrated Gene Expression Landscape Profiling Approach to Identify Lung Tumor Endothelial Cell Heterogeneity and Angiogenic Candidates. *Cancer Cell* **37**, 21-36 e13, doi:10.1016/j.ccell.2019.12.001 (2020).

4 Zhao, Q. *et al.* Single-Cell Transcriptome Analyses Reveal Endothelial Cell Heterogeneity in Tumors and Changes following Antiangiogenic Treatment. *Cancer Res* **78**, 2370-2382, doi:10.1158/0008-5472.CAN-17-2728 (2018).