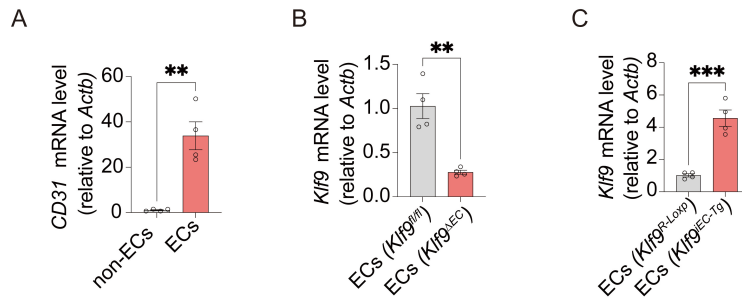
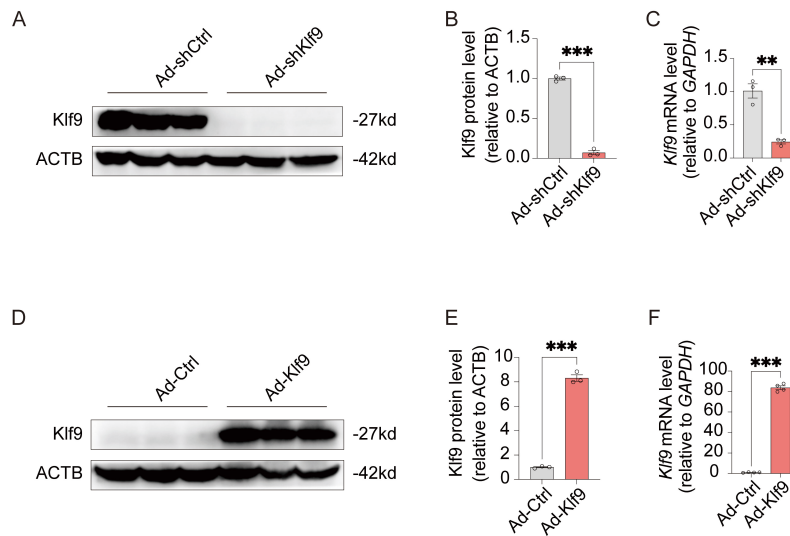


Supplementary figure 1



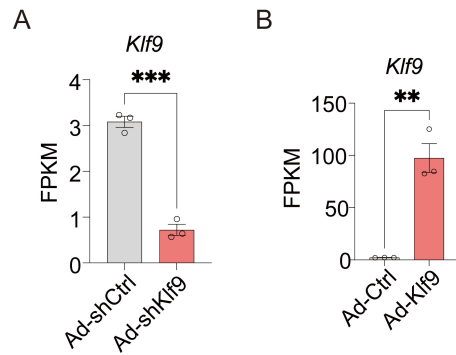
Supplementary Fig. 1 Establishment of endothelial-specific Klf9 deletion and overexpression in mice. (A) RT-qPCR confirmed the efficiency of magnetic bead sorting of CD31-positive cells from mouse retina (n = 4 per group). ** $p < 0.01$, mean \pm SEM, two-tailed unpaired Student's t test. (B) RT-qPCR confirmed the efficiency of endothelial-specific deletion of Klf9 from *Klf9* ^{Δ EC} mouse ECs compared with *Klf9*^{fl/fl} controls (n = 4 per group). ** $p < 0.01$, mean \pm SEM, two-tailed unpaired Student's t test. (C) RT-qPCR confirmed the efficiency of endothelial-specific overexpression of Klf9 from *Klf9*^{iEC-Tg} mouse ECs compared with *Klf9*^{R-Loxp} controls (n = 4 per group). *** $p < 0.001$, mean \pm SEM, two-tailed unpaired Student's t test.

Supplementary figure 2



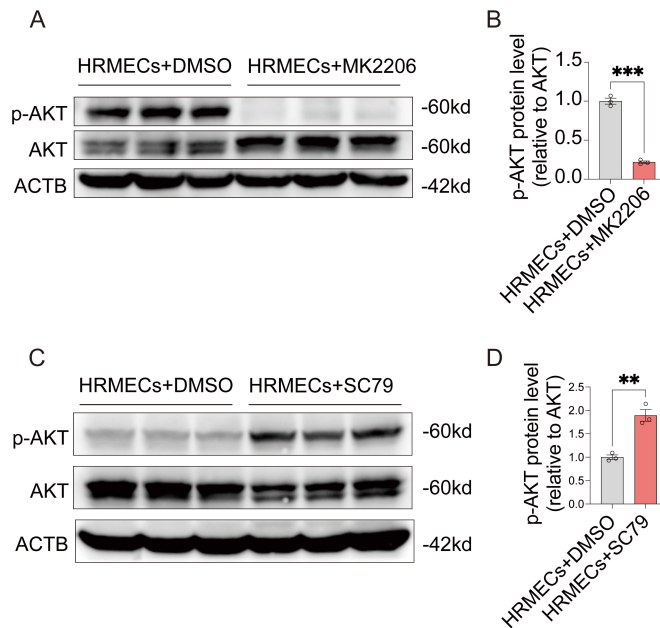
Supplementary Fig. 2 Establishment of Klf9 deletion and overexpression in HRMECs. (A, B) Quantification of Klf9 protein levels relative to GAPDH in the HRMECs transduced with Ad-shCtrl and Ad-shKlf9 (n = 3 biological replicates per group). *** $p < 0.001$, mean \pm SEM, two-tailed unpaired Student's t test. (C) RT-qPCR confirmed the efficiency of endothelial-specific deletion of Klf9 in HRMECs (n = 3 biological replicates per group). ** $p < 0.01$, mean \pm SEM, two-tailed unpaired Student's t test. (D, E) Quantification of Klf9 protein levels relative to GAPDH in the HRMECs transduced with Ad-Ctrl and Ad-Klf9 (n = 3 biological replicates per group). *** $p < 0.001$, mean \pm SEM, two-tailed unpaired Student's t test. (F) RT-qPCR confirmed the efficiency of endothelial-specific overexpression of Klf9 in HRMECs (n = 4 biological replicates per group). *** $p < 0.001$, mean \pm SEM, two-tailed unpaired Student's t test.

Supplementary figure 3



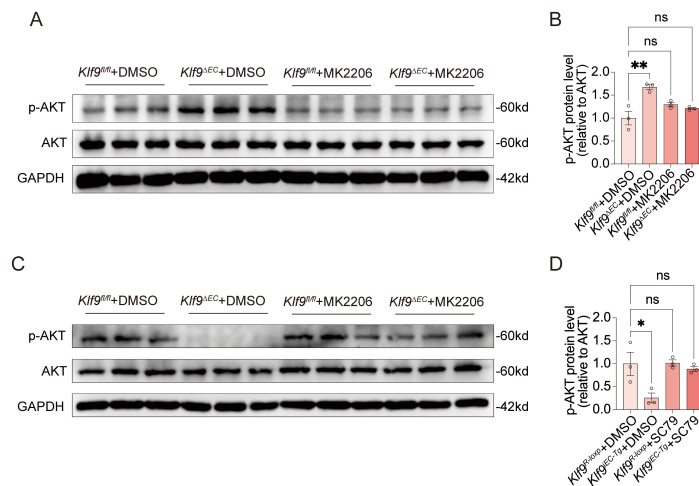
Supplementary Fig. 3 Validation of Klf9 knockdown and overexpression efficiency by RNA-seq. (A, B) FPKM values of Klf9 in HRMECs following knockdown or overexpression, as determined by RNA-seq (n = 3 biological replicates per group). ** $p < 0.01$, *** $p < 0.001$, mean \pm SEM, two-tailed unpaired Student's t test.

Supplementary figure 4



Supplementary Fig. 4 Establishment of Akt signaling activation and inhibition in HRMECs. (A, B) Quantification of p-AKT protein levels relative to AKT in HRMECs treated with DMSO and MK2206 (n = 3 biological replicates per group). *** $p < 0.001$, mean \pm SEM, two-tailed unpaired Student's t test. (C, D) Quantification of p-AKT protein levels relative to AKT in HRMECs treated with DMSO and SC79 (n = 3 biological replicates per group). *** $p < 0.001$, mean \pm SEM, two-tailed unpaired Student's t test.

Supplementary figure 5



Supplementary Fig. 5 Modulation of Akt signaling by *Klf9* deficiency or overexpression following MK2206 or SC79 treatment. (A, B) Quantification of p-AKT protein levels relative to AKT in *Klf9^{fl/fl}*+DMSO, *Klf9^{ΔEC}*+DMSO, *Klf9^{fl/fl}*+MK2206 and *Klf9^{ΔEC}*+MK2206 (n = 3 per group). ***p* < 0.01, mean ± SEM, one-way ANOVA with Tukey's multiple comparisons test. (C, D) Quantification of p-AKT protein levels relative to AKT in *Klf9^{R-loxp}*+DMSO, *Klf9^{iEC-Tg}*+DMSO, *Klf9^{R-loxp}*+SC79 and *Klf9^{iEC-Tg}*+SC79 (n = 3 per group). ***p* < 0.01, mean ± SEM, one-way ANOVA with Tukey's multiple comparisons test.

Supplementary Table 1. Primers for RT-qPCR and ChIP-qPCR.

Primers for RT-qPCR	Forward	Reverse
Mouse <i>Klf9</i>	CGAGCGGCTGCGACTACCTG	GGGCTGTGGGAAGGACTCGAC
Mouse <i>Actb</i>	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Mouse <i>Gapdh</i>	AGGTCGGTGTGAACGGATTG	TGTAGACCATGTAGTTGAGGTCA
Mouse <i>CD31</i>	AGCCTAGTGTGGAAGCCAAC	AGCCTCCGTTCTCTTGGTG
Human <i>Klf9</i>	GCCGCCTACATGGACTTCG	GGATGGGTCGGTACTTGTTCA
Human <i>GAPDH</i>	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
Human <i>AKT1</i>	AGCGACGTGGCTATTGTGAAG	GCCATCATTCTTGAGGAGGAAGT
Human <i>PTK2</i>	GCTTACCTTGACCCCAACTTG	ACGTTCCATAACCAGTACCCAG
Human <i>RAC1</i>	ATGTCCGTGCAAAGTGGTATC	CTCGGATCGCTTCGCAAACA
Human <i>VCL</i>	CTCGTCCGGGTTGGAAAAGAG	AGTAAGGGTCTGACTGAAGCAT
Human <i>MYL9</i>	TCTTCGCAATGTTGACCAGT	GTTGAAAGCCTCCTTAAACTCCT
Human <i>RAPGEF1</i>	CTGAGGTGTCCGTAAAGATTCC	AGGGTAGCCCTCTGGTAGAAA
/	/	/
Primers for ChIP-qPCR	Forward	Reverse
Human <i>AKT1</i>	ATTCGTCCCTGACCTGTCTC	GGGCTGGGCCGCTAAC
Human <i>PTK2</i>	GACCCCAAGGATGTCCGC	CTCGCGTAATTTGTCCTGTAGTG
Human <i>RAC1</i>	CTCGTGACCTCAGGTGATCC	CGCTCCCGGAGTTTCTCTG
Human <i>VCL</i>	GGGCAATCATACCTAGTTCCA	GCCCTCCTCAAAGACATTCTG
Human <i>MYL9</i>	CCCAGAGCGAACCCCCAC	CCTTATTTGGCCTCGACCCAC
Human <i>RAPGEF1</i>	CAGAGTCACCAGCTCCAGAC	ACGTCCTGGGGTGGAAAATC