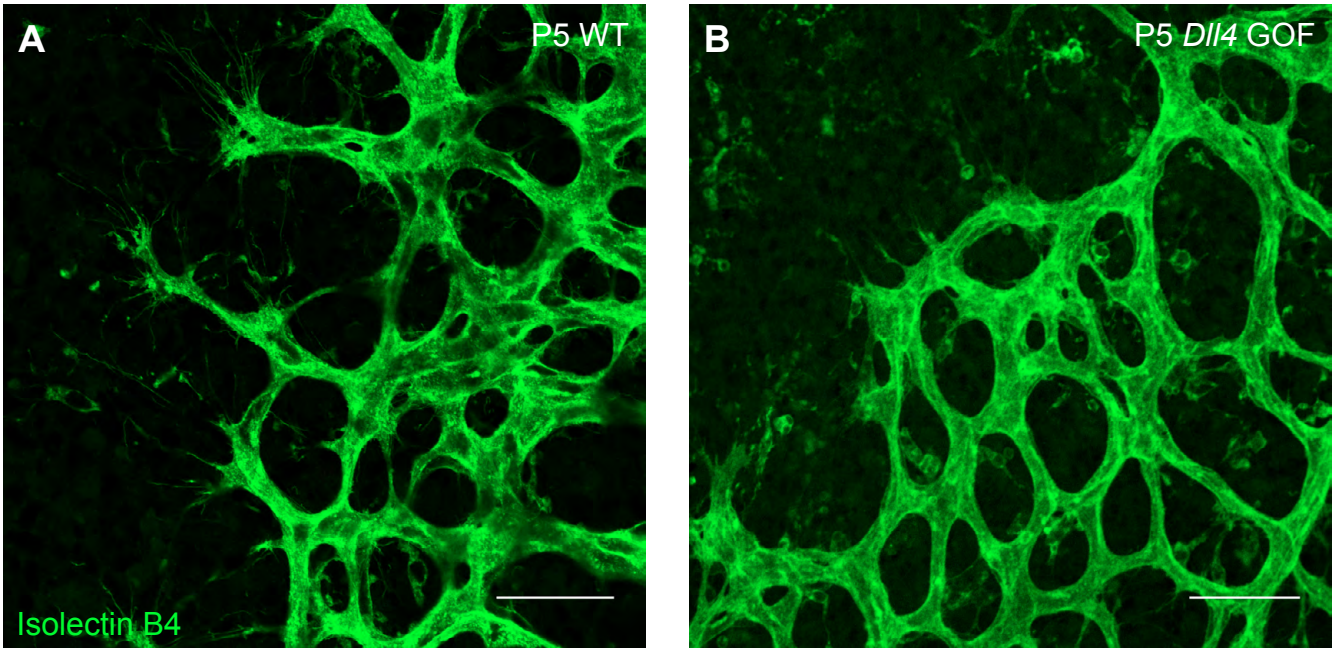


Fig. S1. *Dll4* overexpression decreases tip cell density in retina vasculature. (**A**,**B**) Confocal images of wild-type and *Dll4* gain-of-function retinas at postnatal day P5. Vascular plexus was stained for endothelial marker isolectin B4. *Dll4* overexpression significantly decreases the number of tip cells and filopodia extensions compared with control. Scale bar: 45 μm.

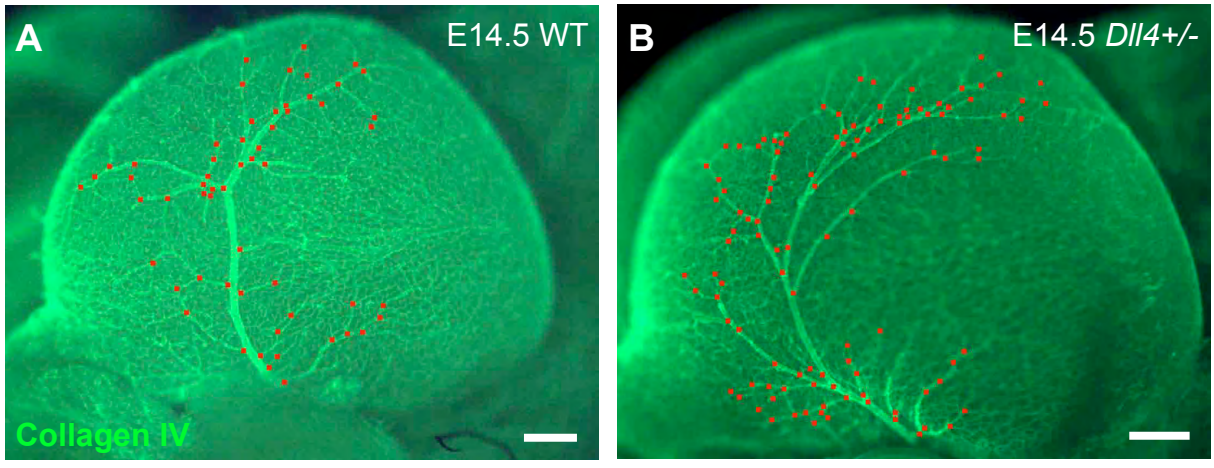


Fig. S2. Quantification of MCA branching in mouse embryos. (**A**,**B**) Epifluorescent images of wild-type and *Dll4*$^{+/-}$ brains stained for collagen IV at embryonic stage E14.5. Red dots highlight MCA branch points. Scale bar: 500 μm.

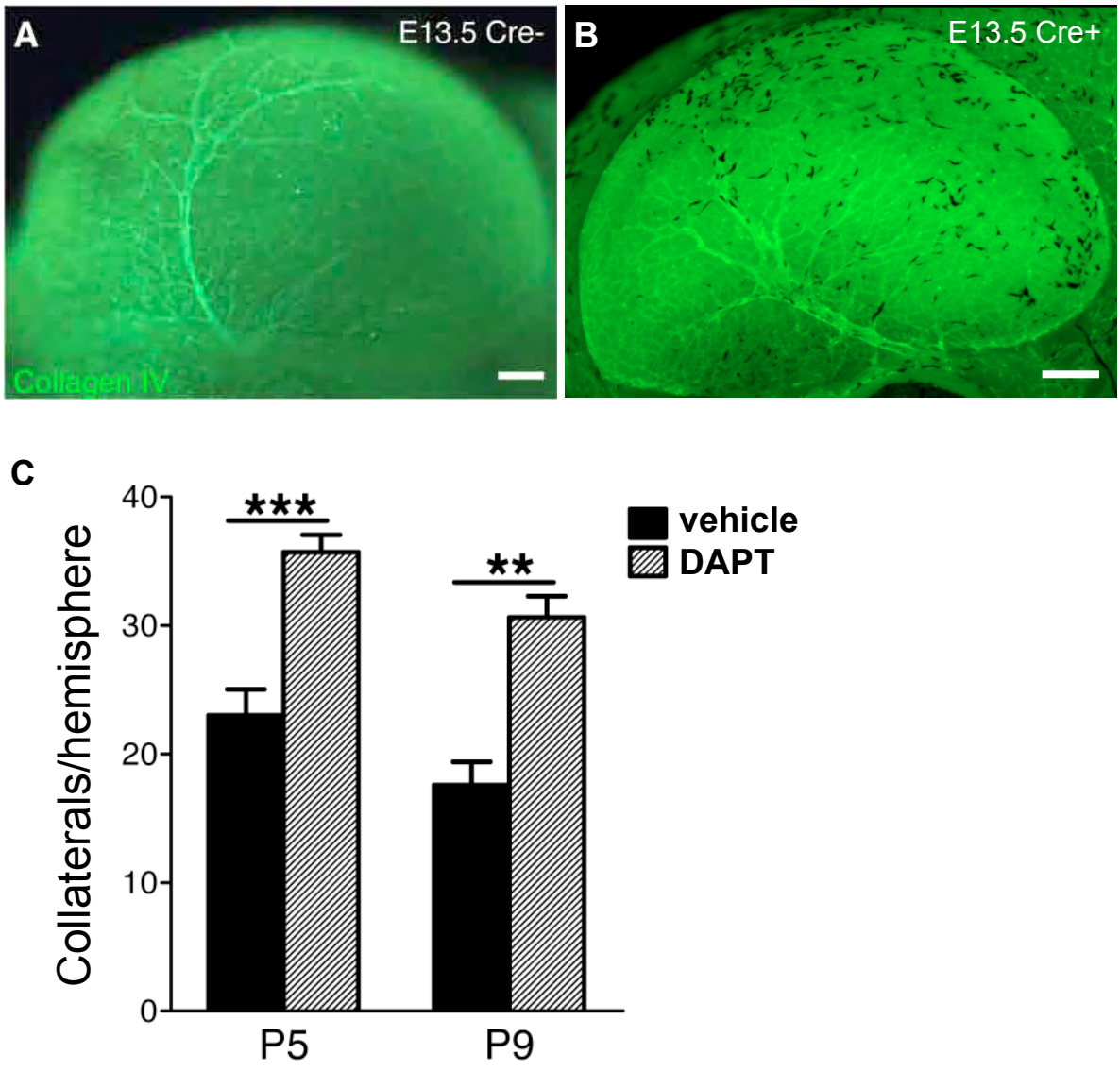


Fig. S3. Genetic and pharmacological inhibition of *Notch1* increases collateral numbers in the brain. (**A**,**B**) Inducible endothelium-specific inactivation of *Notch1* was obtained injecting tamoxifen into *Cdh5-CreERT2/R26R/Notch1*$^{floxed/floxed}$ mice at embryonic day E11.5. The E13.5 vascular plexus was stained for collagen IV and β-galactosidase (B, black). Even at low recombination frequence (B), MCA in *Notch1* loss-of-function brains shows hyperbranching, resembling *Dll4*$^{+/-}$ phenotype. Scale bar: 500 μm. (**C**) Bar graphs display collateral arteriolar density in P5 and P9 brains after treatment with Notch inhibitor DAPT (100 mg/kg body weight) or vehicle (corn oil) at day P2 and P4. Post-natal DAPT treatment increased pial collateral numbers. Data are shown as mean±s.e.m., n=5 mice per group. **P<0.01 versus vehicle; ***P<0.005 versus vehicle.

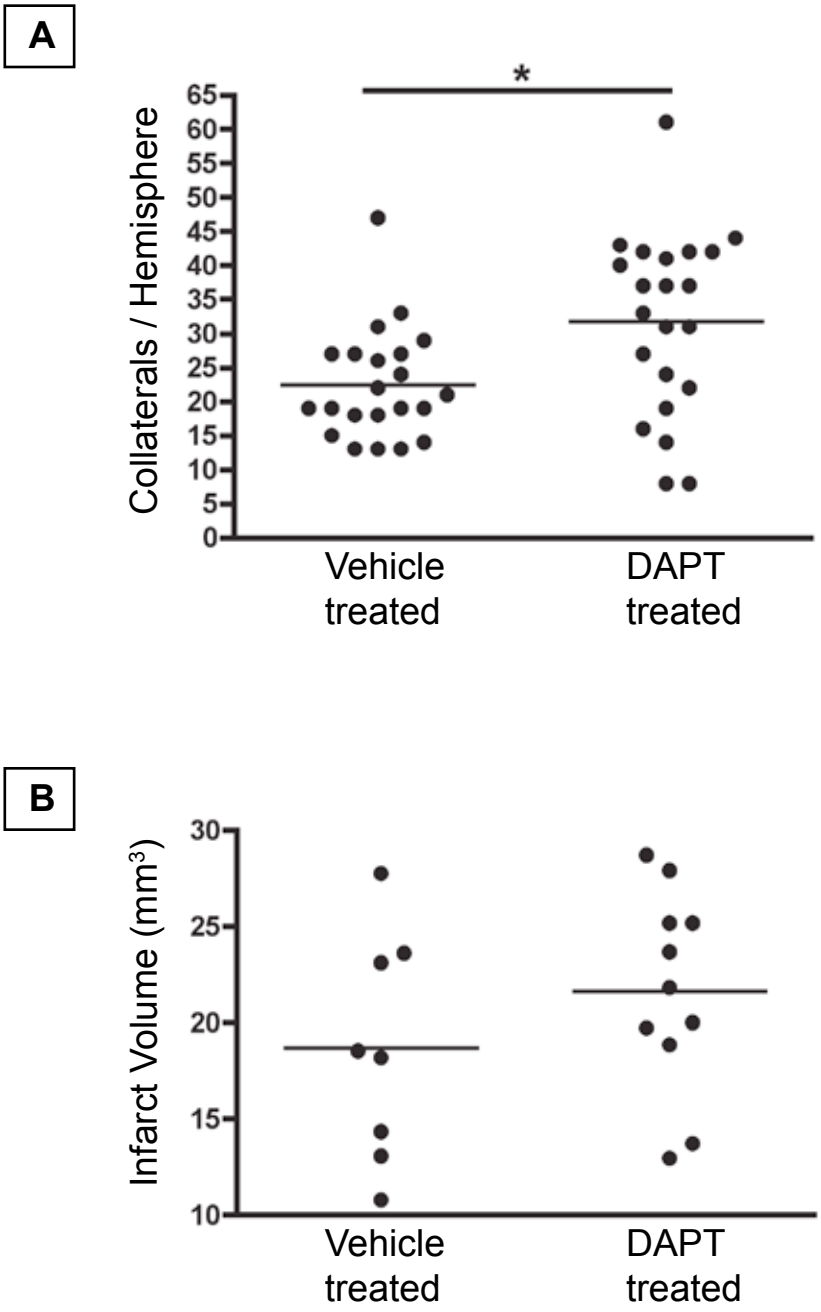


Fig. S4. Transient inhibition of notch signaling with DAPT augments collateral number without improving stroke volume upon MCA occlusion. (**A**) Pregnant mice were injected intraperitoneally at E10.5, E11.5 and E12.5 with vehicle (5% ethanol in corn oil) or DAPT at a final concentration of 100 mg/kg. Mice were sacrificed at 8 weeks of age and pial collaterals were counted after PECAM1 staining. There is a significant increase in collateral number in the DAPT-treated group. **P*<0.05. Mann-Whitney-U test. (**B**) Pregnant mice were injected as in A with vehicle or DAPT, and 8-week-old mice were subjected to MCA occlusion and infarct volume was measured. There were no significant differences in infarct volume between the treatment groups.

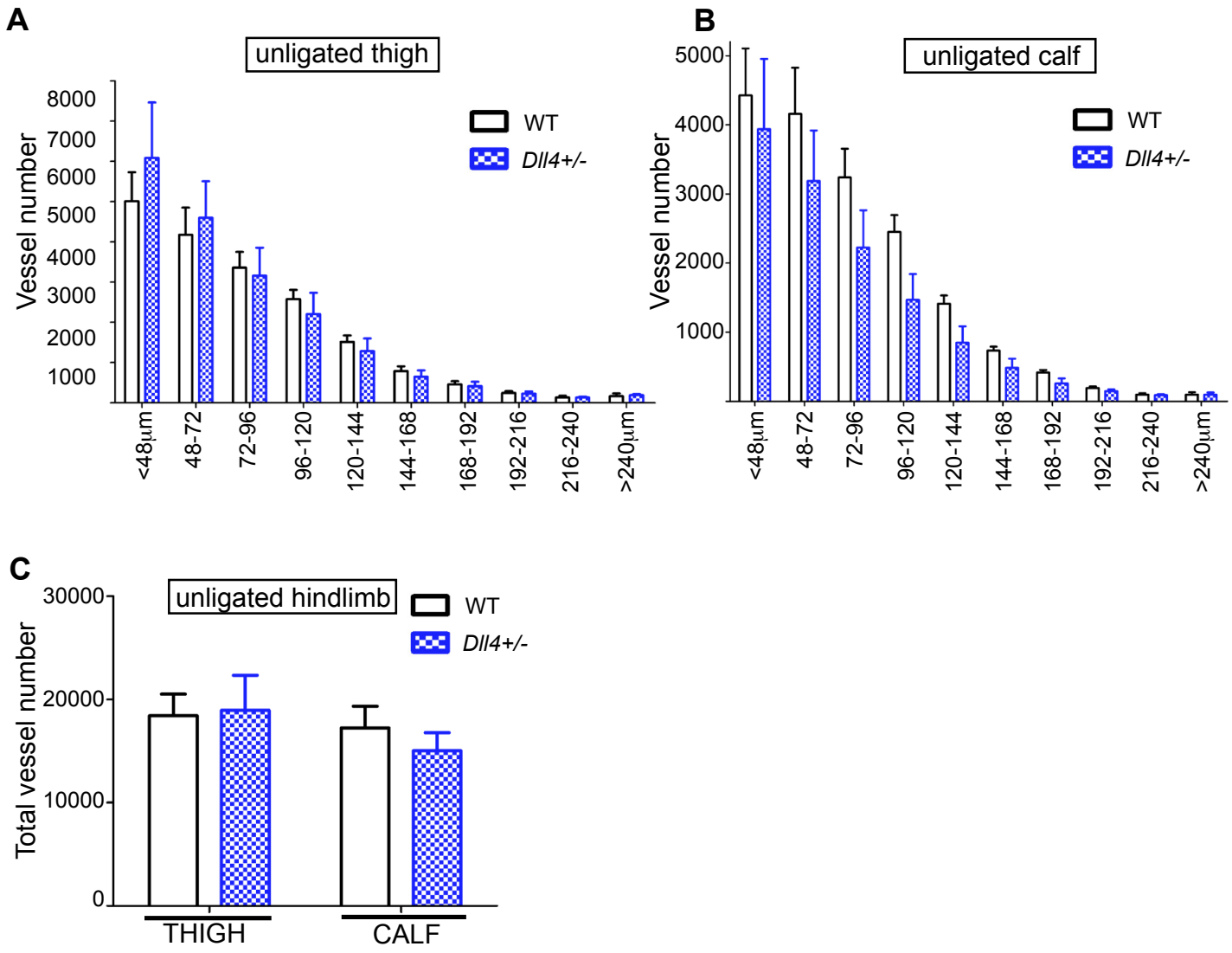


Fig. S5. Micro-CT analysis of unligated control hindlimb vasculature. (**A**,**B**) Quantitative analysis of micro-CT images in thigh and calf of unligated left hindlimb in wild type (n=4) and *Dll4*$^{+/-}$ (n=3). Shown are total numbers of vascular structures per indicated diameter class in consecutive z-axis slices. (**C**) Total arteriolar number in unligated left thigh and calf of wild-type and *Dll4*$^{+/-}$ mice based on micro-CT imaging. Vessel numbers are comparable in *Dll4*$^{+/-}$ and wild type. The data are shown as mean±s.e.m.

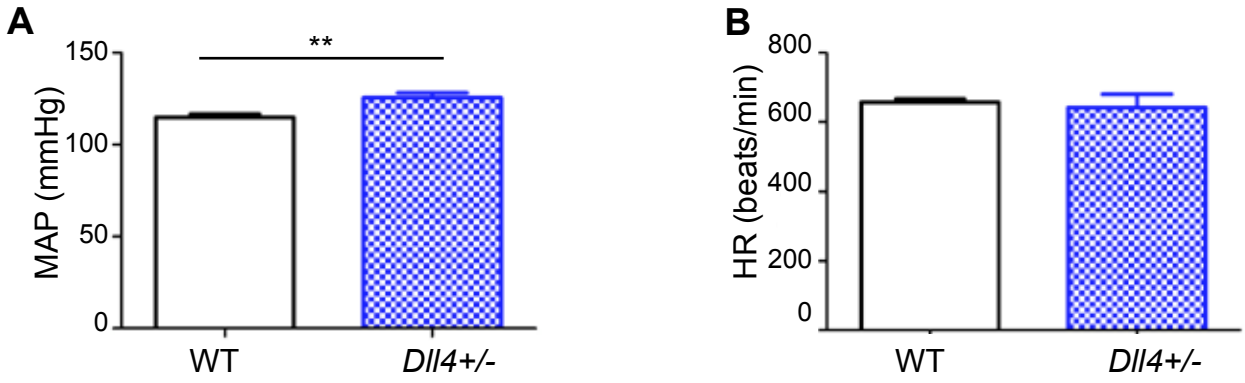
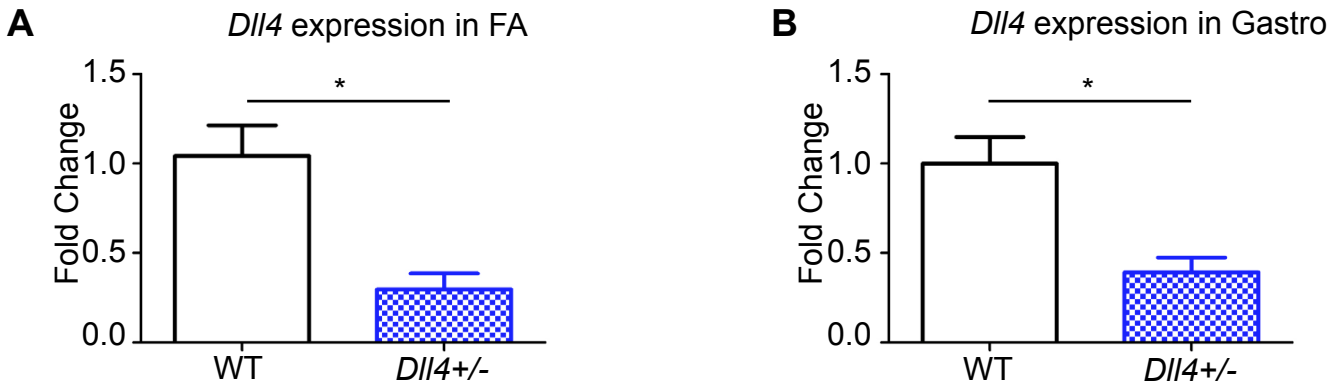


Fig. S6. Arterial blood pressure and heart rate. Mean arterial pressure (MAP in mmHg) (**A**) and heart rate (HR in beats per minute) (**B**) in wild-type and *Dll4*$^{+/-}$ mice. The data are shown as mean±s.e.m; wild type, n=5; *Dll4*$^{+/-}$, n=6; $^{**}P<0.01$.



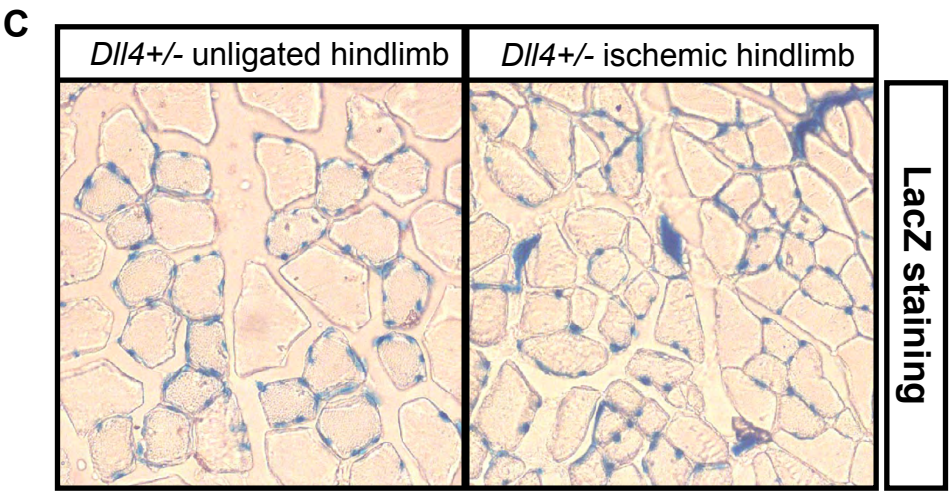


Fig. S7. *Dll4* expression in the mouse hindlimb. (**A**,**B**) Taqman analysis of *Dll4* expression in the femoral artery (FA) (A) and gastrocnemius muscle (Gastro) (B) of wild-type (n=8) and *Dll4*$^{+/-}$ (n=8) mice. The data are shown as mean±s.e.m.; $^{*}P<0.05$. (**C**) β-galactosidase staining (*lacZ*) staining on hindlimb cryosections in *Dll4*$^{+/-}$ mice. There is strong staining in vessels.

Table S1. Real-time PCR primers and probes

Gene	Forward primer	Reverse primer	Probe
Hif1a	TCAGAGGAAGCGAAAAATGGA	AGTCACCTGGTTGCTGCAATAA	FAM-5′-TTCCAATTCCTGCTGCTTGAAAAAGGG-3′-TAMRA
Hif1b	GCATGGGCTCACGAAGGT	AACAGGGTCCACGGAGCTAGT	FAM-5′-TTCATCTGCCGCATGAGGTGTGG-3′-TAMRA
Vegfa	GCAGGCTGCTGTAACGATGAA	TTTGATCCGCATGATCTGCAT	FAM-5′-CCCTGGAGTGCGTGCCCA-3′-TAMRA
Kdr	ACTGCAGTGATTGCCATGTTCT	CCTTCATTGGCCCGCTTAA	FAM-5′-TGGCTCCTTCTTGTCATTGTCCTACGGA-3′-TAMRA
Notch1	CATCCGTGGCTCCATTGTCTA	GACGCAAGAGCACCTAGGAA	FAM-5′-TGGAGATCGACAACCGGCAATGT-3′-TAMRA
Dll4	CGAATGCCCCCCCAACT	GTTCGGCTTGGACCTCTGTTC	FAM-5′-CGGCTCTAACTGTGAGAAGAAAGTAGACAGG-3′-TAMRA
Efnb2	TCCAGAGCTAGAAGCTGGTACAAATG	ATGCCCCGCGCTGTTG	FAM-5′-ATCGGTGCTAGAACCTGGATTTGG-3′-TAMRA
Nrp1	ACAGCAAGCGCAAGGCTAA	AGGTGAAAACGTCCGAAGCT	FAM-5′-TCGTTCGAAGGCAACAACAACTATG-3′-TAMRA