The TRPC5 channel regulates angiogenesis to promote recovery from ischemic injury

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Supporting information

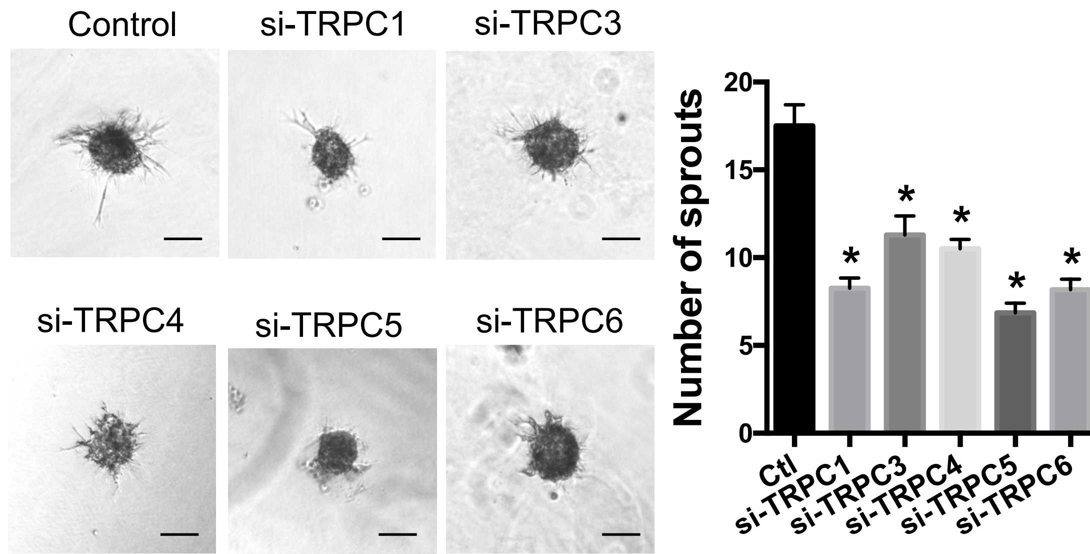

Figure S1. Involvement of TRPC channels in *in vitro* EC sprouting under hypoxia.

Left: representative images of spheroid sprouts in mouse primary mesenteric endothelial cells (MMECs) pretreated with control or TRPC siRNA under hypoxia (1% O_2). The values are the means ± SEM of $n = 6$ independent experiments. *$P < 0.05$ versus control (Ctl), one-way ANOVA and Dunnett's multiple comparison test. Scale bars: 100 μm.

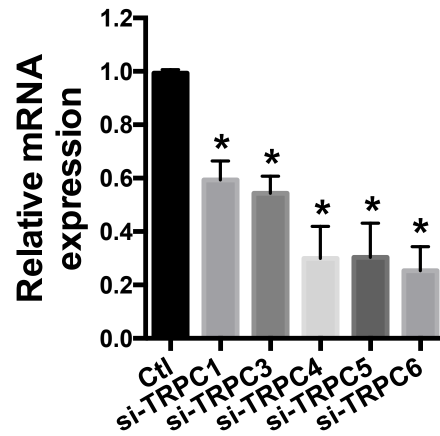

Figure S2. Effect of siRNA treatment on the mRNA expression of corresponding TRP channels.

Scrambled siRNA and targeted siRNAs were added to mouse primary mesenteric endothelial cells under hypoxia (1% O_2). After 48 hours, RNA was extracted, and quantitative real time-polymerase chain reaction was performed to determine the mRNA level of corresponding TRP channels. The values are the means ± SEM of $n = 3$ independent experiments. *$P < 0.05$ versus control (Ctl), one-way ANOVA and Dunnett's multiple comparison test.

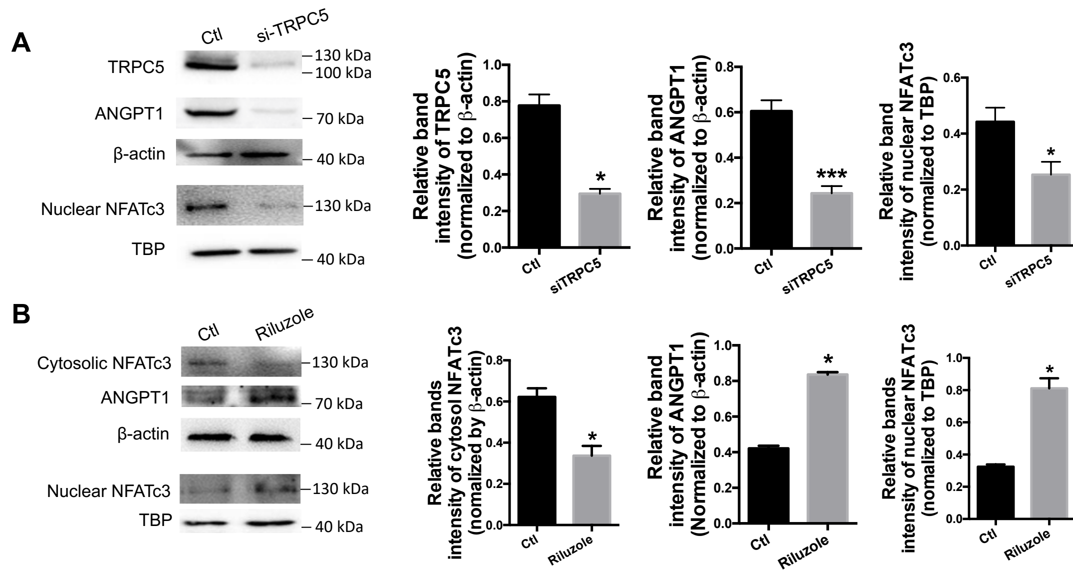

Figure S3. TRPC5 influences NFATc3 and ANGPT1 expression.

(A) Mouse primary mesenteric endothelial cells (MMECs) were treated with TRPC5-siRNA or scrambled-siRNA. Nuclear protein extracts were used for NFATc3 analysis. Whole-cell lysates were used for TRPC5 and ANGPT1 analyses. For TRPC5 analysis, *$P < 0.05$ versus control (Ctl), unpaired Mann Whitney test. For NFATc3 and ANGPT1 analysis, *$P < 0.05$, ***$P < 0.001$ versus Ctl. Student's unpaired two-tailed t test. (**B)** Primary MMECs were treated with or without riluzole (50 μM) for 12 hours. Cytoplasmic and nuclear extracts for NFATc3 detection and whole cell lysates for ANGPT1 detection were analyzed by western blot. *$P < 0.05$ versus Ctl, unpaired Mann-Whitney test. (A and B) TBP (TATA-binding protein) is the loading control in the nuclear, and β-actin protein is the control in cytoplasmic or whole cell proteins.

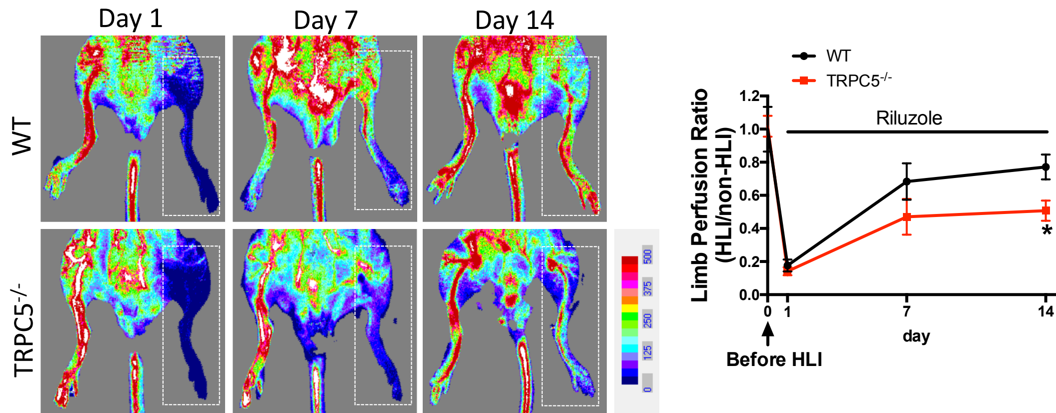

Figure S4. The restoration of hind-limb blood flow after ischemia surgery in TRPC5 wildtype and TRPC5$^{-/-}$ mice under riluzole treatment.

Hind-limb ischemia was surgically induced in WT and TRPC5$^{-/-}$ mice. Mice were treated with riluzole (10mg/kg/day) after the surgery. The blood flow was determined by laser Doppler imager at days 1, 7 and 14 after hind-limb ischemia. Left: representative images of hind-limb blood flow. Ischemic limbs are highlighted by white dashed box. Right: Comparison of limb perfusion ratios between WT and TRPC5$^{-/-}$ mice measured on days 1, 7, and 14 after HLI. The final blood flow values are expressed as the ratios of ischemic to non-ischemic hind limb perfusion. $n = 4$. $*P < 0.05$ versus control at day14, Student's unpaired two-tailed t test.

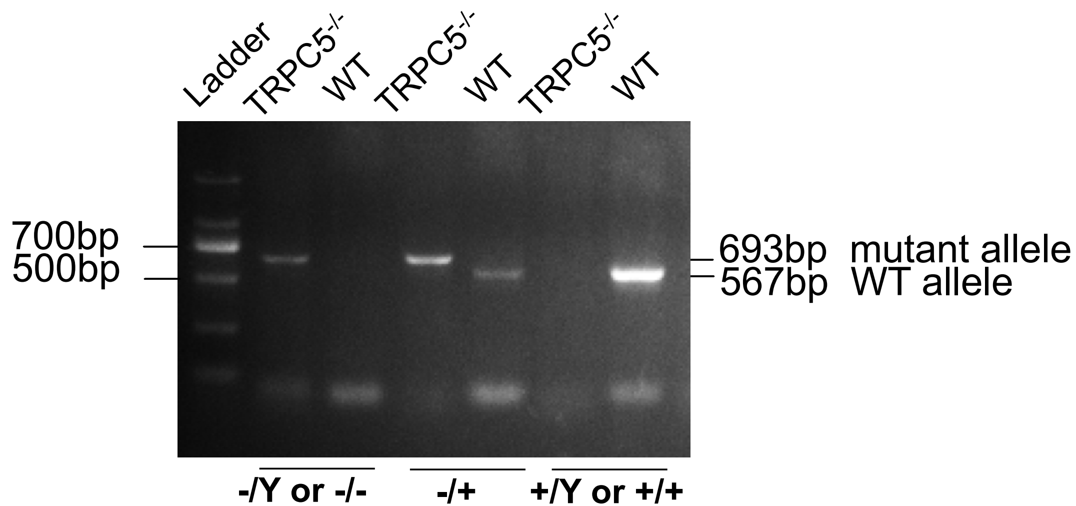

Figure S5. Genotyping of TRPC5 wildtype and knockout mice.

Representative PCR genotyping gel image of TRPC5$^{-/-}$, wildtype (WT), and heterozygous mice. Mouse tail genomic DNA was used to determine the genotypes. The sequences of the primers for TRPC5$^{-/-}$ mouse genotyping were as follows: forward primer 1 (F1)-5'GTAAGTGATACTAGGTATGGGGTATGGAGG, reverse primer 1 (R1)-5'GTCGACACACGTATAAGGCATACTCTTG 3'. The sequences of the primers for TRPC5 WT mouse genotyping were as follows: F1-5'GTAAGTGATACTAGGTATGGGGTATGGAGG 3', reverse primer 2 (R2)-5'CTAACCATTCTTCTCACCTCTCTCTCCTC 3'. The F1/R1 primer combination generated an amplicon of 693 bp, and the F1/R2 primer combination generated one amplicon of 567 bp. The PCR conditions were as follows: 1) 95 °C for 2 min; 35 cycles of 2) 95 °C for 30 sec; 3) 58 °C for 45 sec; 4) 72 °C for 1 min; and a final step of, 5) 72 °C for 10 min.

Table S1 TRPC gene-specific primers for RT-PCR

Target gene	Primer sequence (5'-3')
TRPC1	Forward: AGCCTCTTGACAAACGAGGA
	Reverse: TCTTACAGGTGGGCTTACGG
TRPC3	Forward: GCCTTCATGTTCGGTGCTC
	Reverse: GCGTTCTGGCCCATGTAGT
TRPC4	Forward: GGCGGACTCCAGGATTACATC
	Reverse: CCATGATTCCCGTGGGTTCAG
TRPC5	Forward: GGGCTGAGACTGAGCTGTC
	Reverse: TTGCGGATGGCGTAGAGTAAT
TRPC6	Forward: AGCCAGGACTATTTGCTGATGG
	Reverse: AACCTTCTTCCCTTCTCACGA
GAPDH	Forward: CAAGAAGGTGGTGAAGCAGG
	Reverse: TCAAAGGTGGAGGAGTGGGT

TRPC, transient receptor potential canonical; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.