Science Advances
AAAS

Supplementary Materials for

# The generation of stable microvessels in ischemia is mediated by endothelial cell derived TRAIL

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**The PDF file includes:**

Figs. S1 to S8
Tables S1 to S8
Legend for data S1
References

**Other Supplementary Material for this manuscript includes the following:**

Data S1

# SUPPLEMENTARY FIGURES

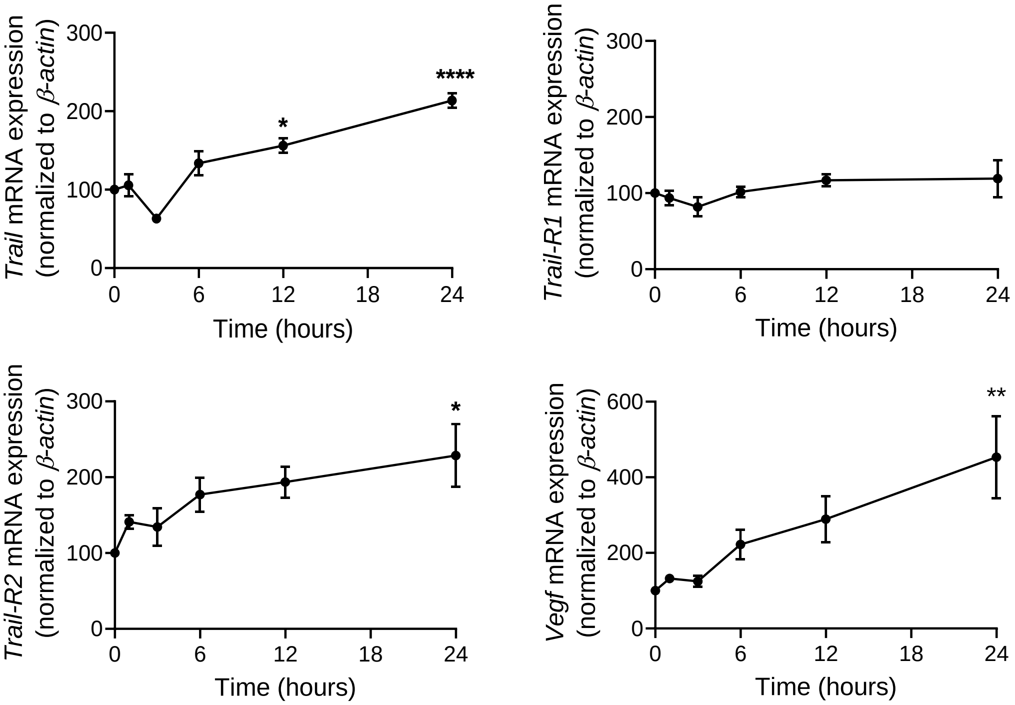
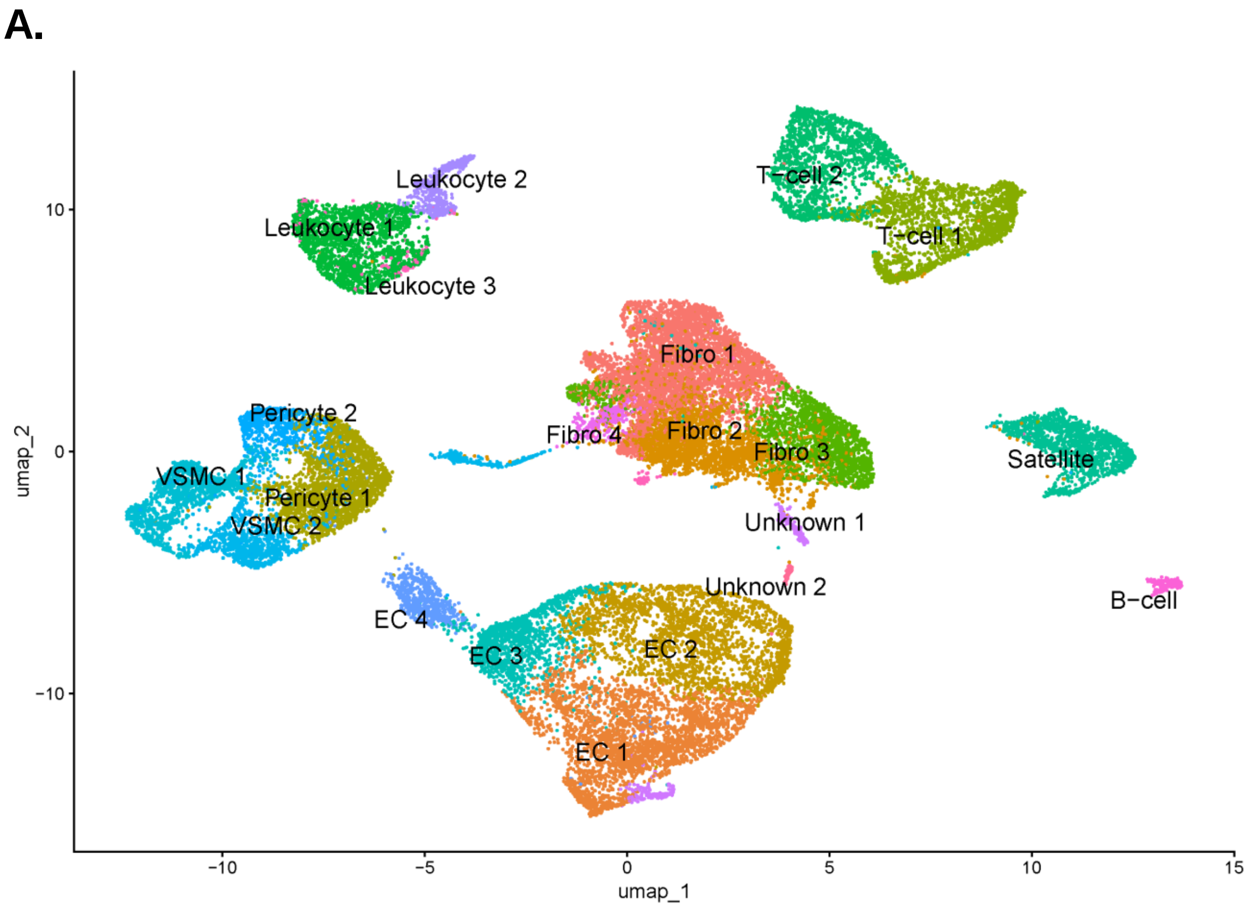
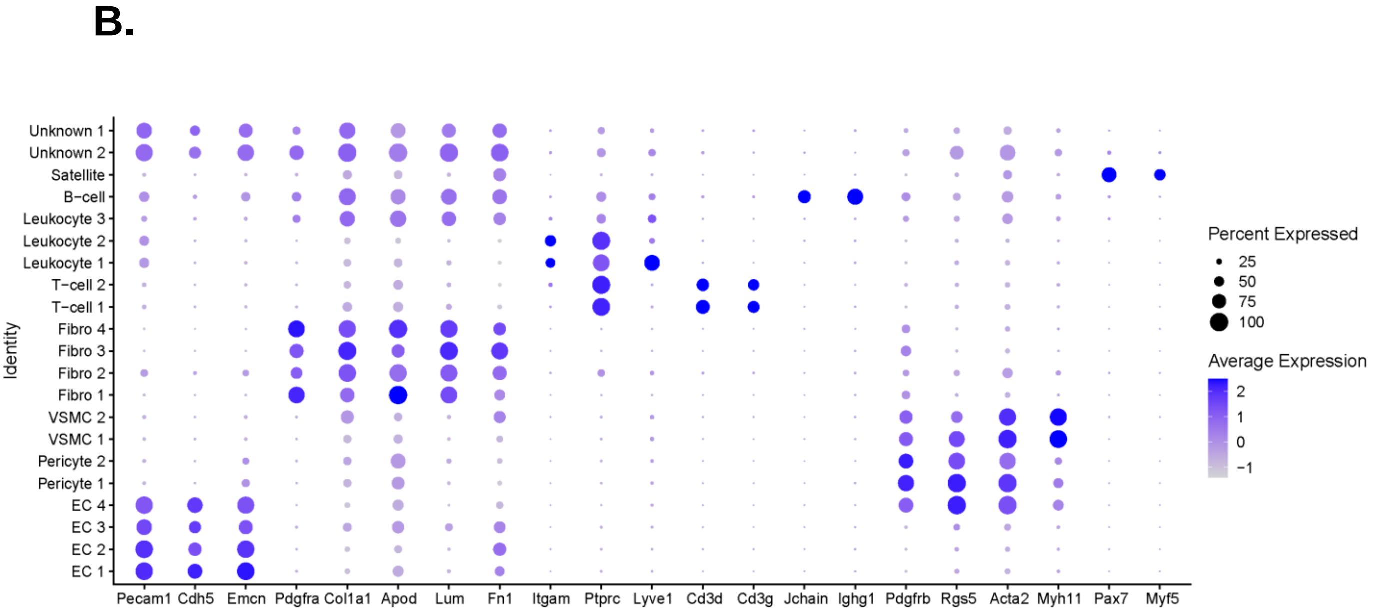


**Fig. S1.**

**Hypoxia increases *Trail* and *Trail-R2*, but not *Trail-R1* mRNA expression over 24 h**. Serum-arrested HMEC-1 were exposed to hypoxia (2% $O_2$) over 24 h. RNA harvested, and cDNA generated for qPCR. mRNA was normalized to *β-actin* (n=3/group). *Vegf* mRNA was assessed as a positive control. Results are mean±SEM; One-way ANOVA; **P*<0.05, ***P*<0.01 and *****P*<0.0001.


A.
Leukocyte 2
Leukocyte 1
Leukocyte 3
T−cell 2
T−cell 1
Fibro 1
Pericyte 2
Fibro 4
Fibro 2
Fibro 3
Satellite
VSMC 1
Pericyte 1
VSMC 2
Unknown 1
Unknown 2
B−cell
EC 4
EC 3
EC 2
EC 1
10
0
−10
umap_2
−10
−5
0
5
10
15
umap_1


B.
Unknown 1
Unknown 2
Satellite
B−cell
Leukocyte 3
Leukocyte 2
Leukocyte 1
T−cell 2
T−cell 1
Fibro 4
Fibro 3
Fibro 2
Fibro 1
VSMC 2
VSMC 1
Pericyte 2
Pericyte 1
EC 4
EC 3
EC 2
EC 1
Identity
Pecam1
Cdh5
Emcn
Pdgfra
Col1a1
Apod
Lum
Fn1
Itgam
Ptprc
Lyve1
Cd3d
Cd3g
Jchain
Ighg1
Pdgfrb
Rgs5
Acta2
Myh11
Pax7
Myf5
Percent Expressed
25
50
75
100
Average Expression
2
1
0
−1

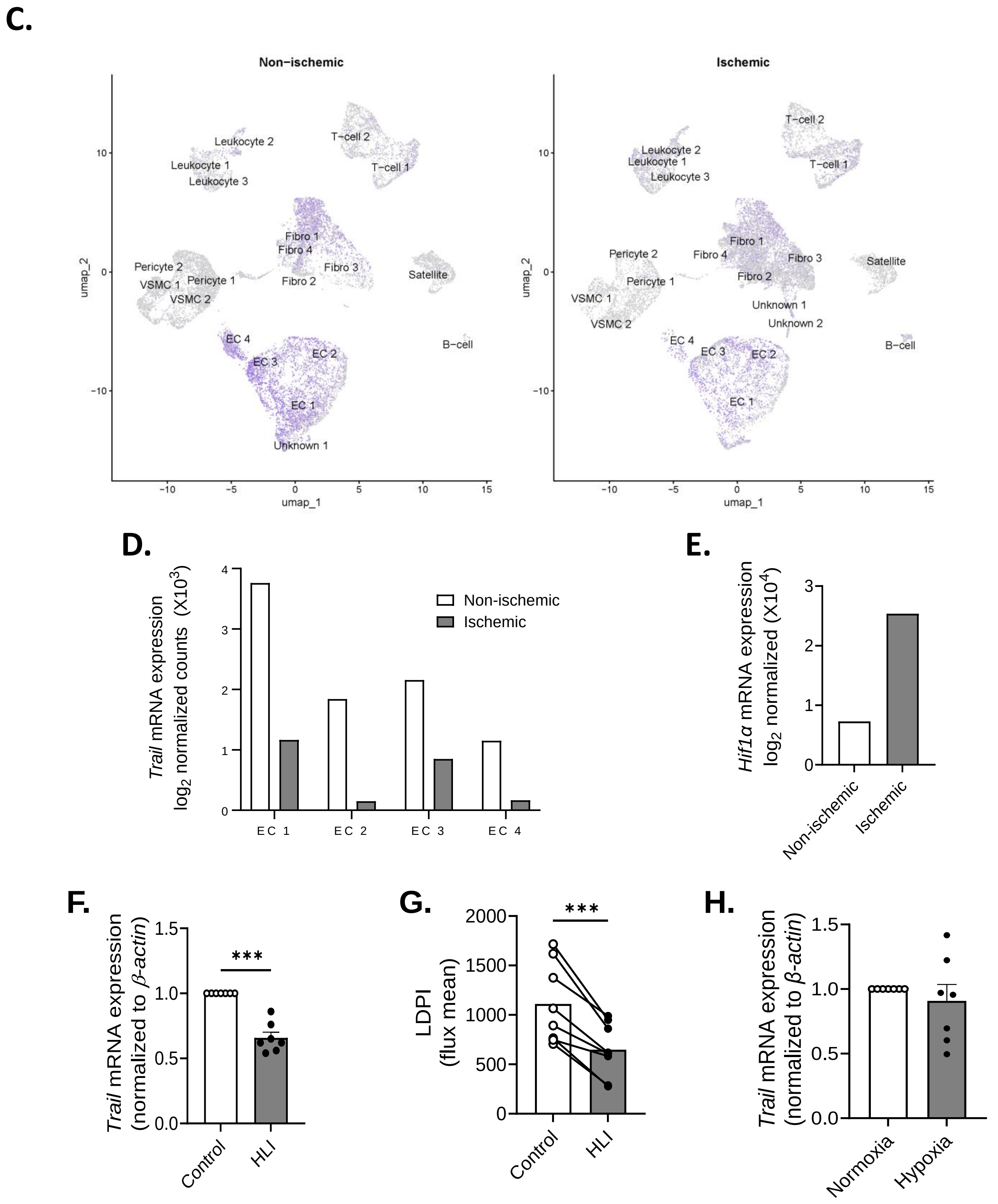


**Fig. S2.**

**ECs are a major source of TRAIL in human limb tissue.** (A) Principal component analysis followed by K-nearest neighbour clustering identified 4 EC clusters (EC 1-4). Additional cell clusters include: pericytes 1-2; vascular smooth muscle cell (VSMC 1-2); satellite cells (Satellite); leukocytes 1-3; T-cells 1-2; fibroblasts (Fibro 1-4); B-cells; (B) Dot plot showing differentially expressed genes for annotated cell populations. (C) *Trail* expression and distribution. (D) *Trail* mRNA expression in EC clusters 1 to 4, normalized to cell counts. (E) *Hif1α* mRNA expression in total ECs, normalized to cell counts. (F) *Trail* mRNA expression and (G) blood perfusion in C57Bl6 mouse limb tissues 14 d after hindlimb ischemia (HLI) (n=7). (H) *Trail* mRNA expression in HMEC-1 after 7 d of hypoxia (n=7/group). All mRNA was normalized to *β-actin*. Results are mean±SEM; Mann–Whitney *U*-test, Paired *t*-test; ****P*<00.1.

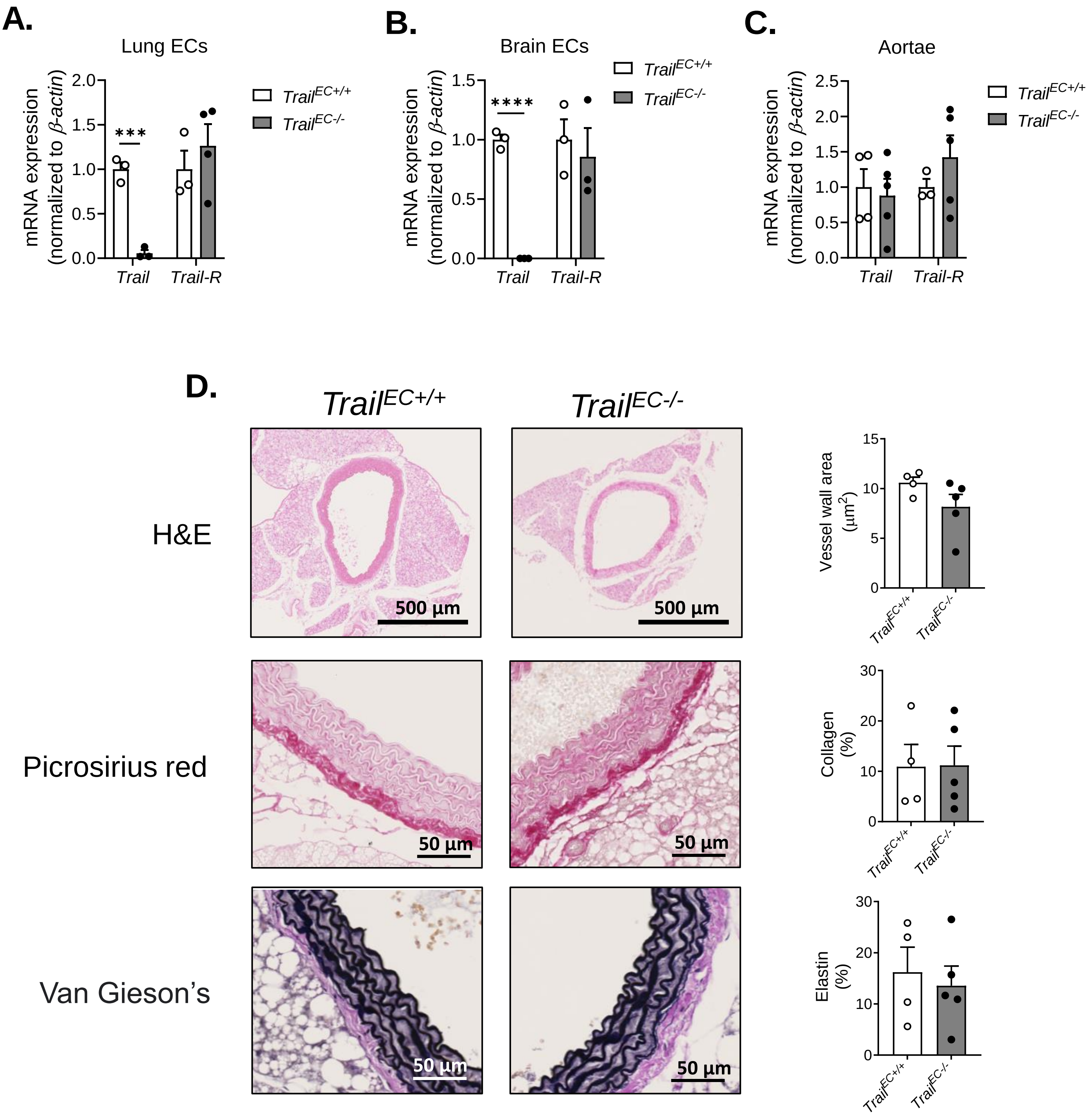


**Fig. S3.**

**TRAIL deletion is specific to ECs, with no effect on large vessel structure in *Trail*$^{EC-/-}$ mice.** *Trail*, but not *Trail-R* mRNA expression is reduced in ECs isolated from (A) lung and (B) brain from *Trail*$^{EC-/-}$ mice (n=3/group). (C) *Trail* or *Trail-R* mRNA in aortae of *Trail*$^{EC-/-}$ and *Trail*$^{EC+/+}$ mice. mRNA normalized to *β-actin* (n=3-5/group). (D) Paraffin-embedded cross section of aortae from *Trail*$^{EC-/-}$ and *Trail*$^{EC+/+}$ mice stained for hematoxylin and eosin (H&E), picrosirius red (collagen) and Van Gieson's (elastin); scale bars as indicated (n=4-5/genotype). Results are mean±SEM. Mann–Whitney *U*-test; ****P*<0.001 and *****P*<0.0001.

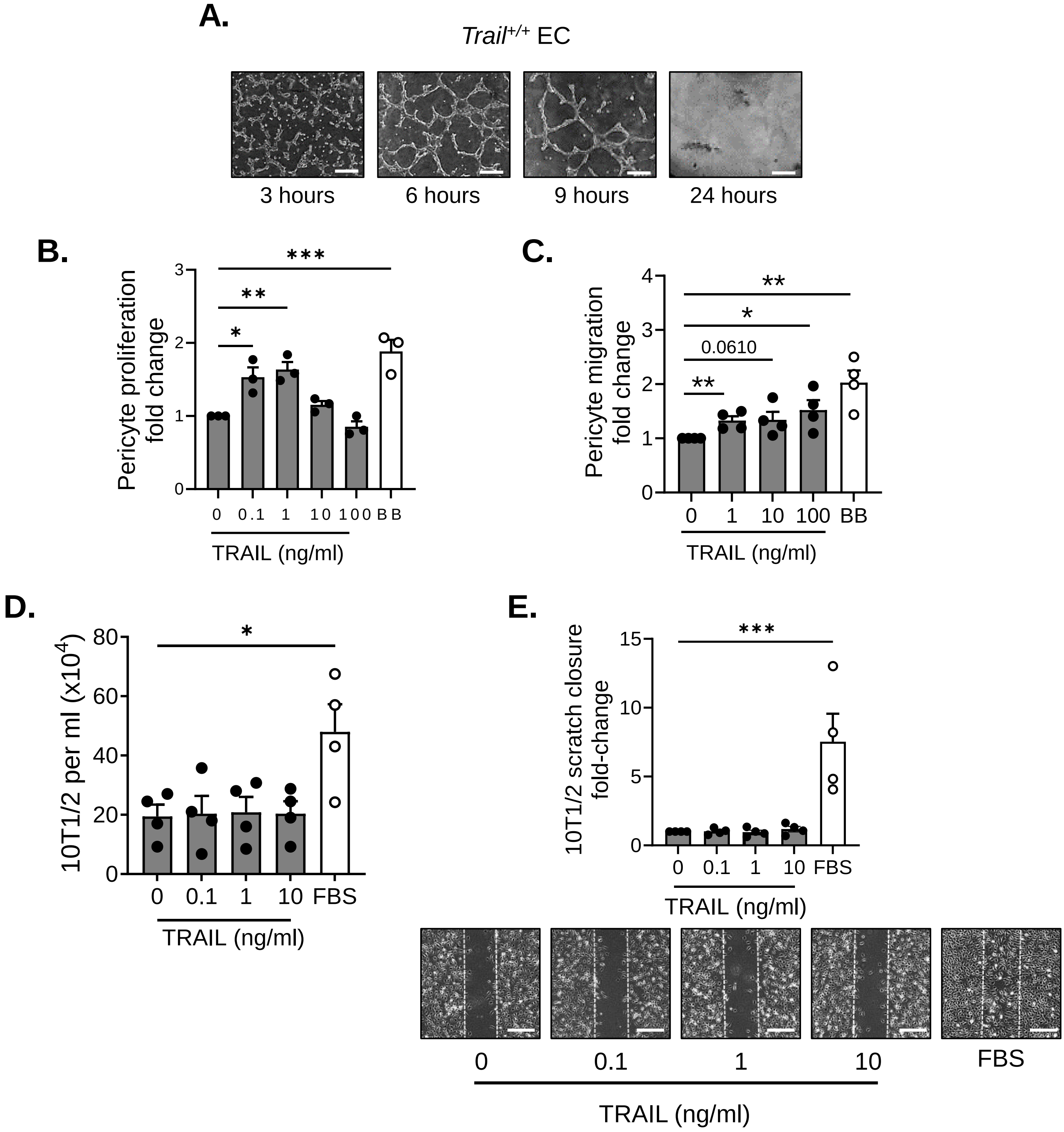


**Fig. S4.**

**Tubulogenesis and pericyte migration.** (A) Representative image showing *Trail*$^{+/+}$ ECs formed tubules by 6-9 h but dissociated by 24 h; scale bar 250 µm. (B) Pericyte proliferation at 24 h (n=3/group) and (C) pericyte Transwell migration to recombinant TRAIL at 8 h (n=4/group). PDGF-BB (BB; 20 ng/ml) was used as positive control. ECs and pericytes were isolated from mouse brain. (D) 10T1/2 cell proliferation at 24 h. (E) 10T1/2 migration via scratch assay at 24 h; *top*, quantification; *bottom*, representative images. Both are unaltered with recombinant human TRAIL at 24 h. 10% FBS was used as a positive control. Dotted lines indicate denuded zone; scale bar 200 µm (n=4/group). Results are mean±SEM; one-way ANOVA or Students *t*-test; *$P<0.05$, **$P<0.01$ and ***$P<0.001$.

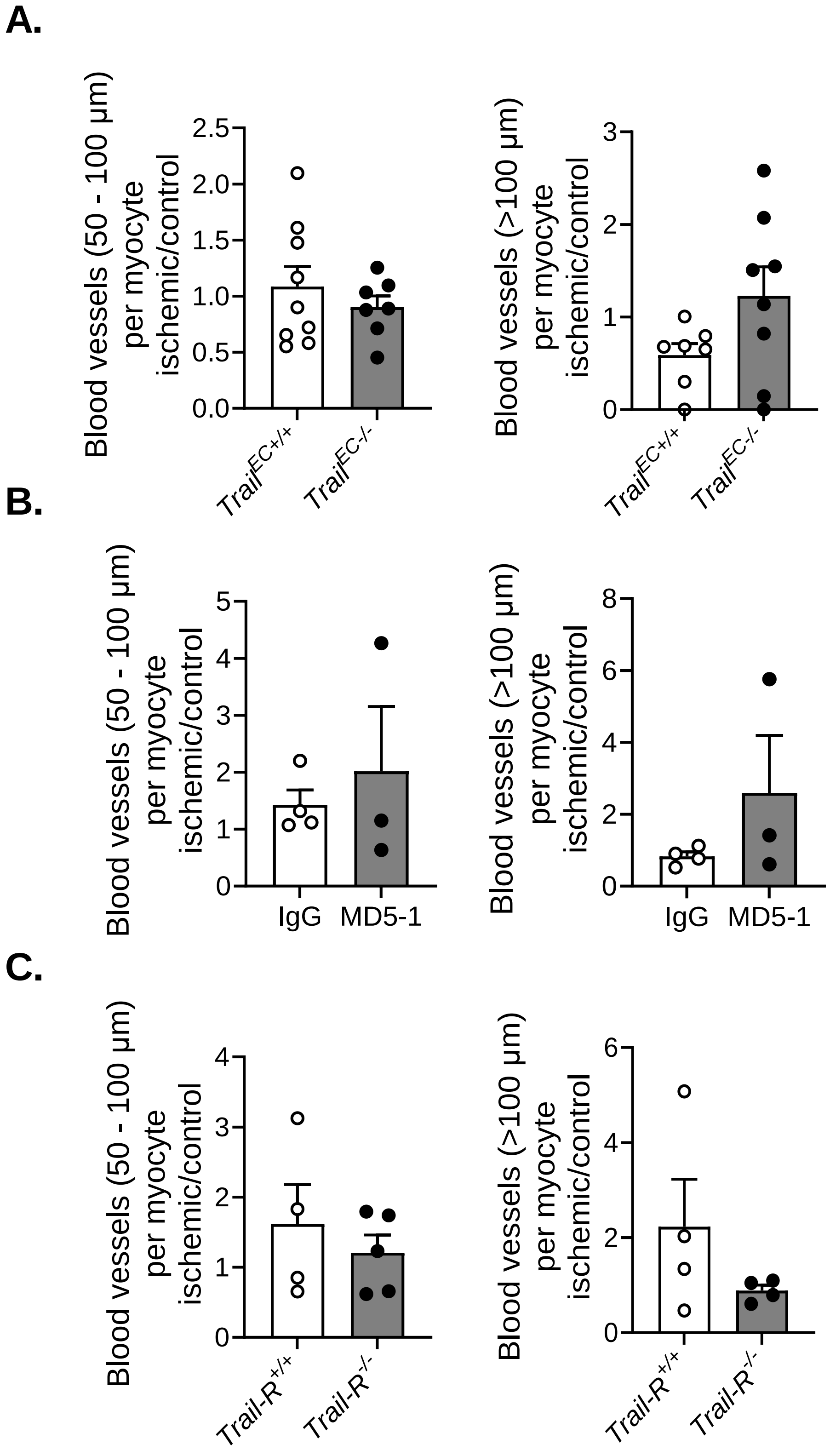
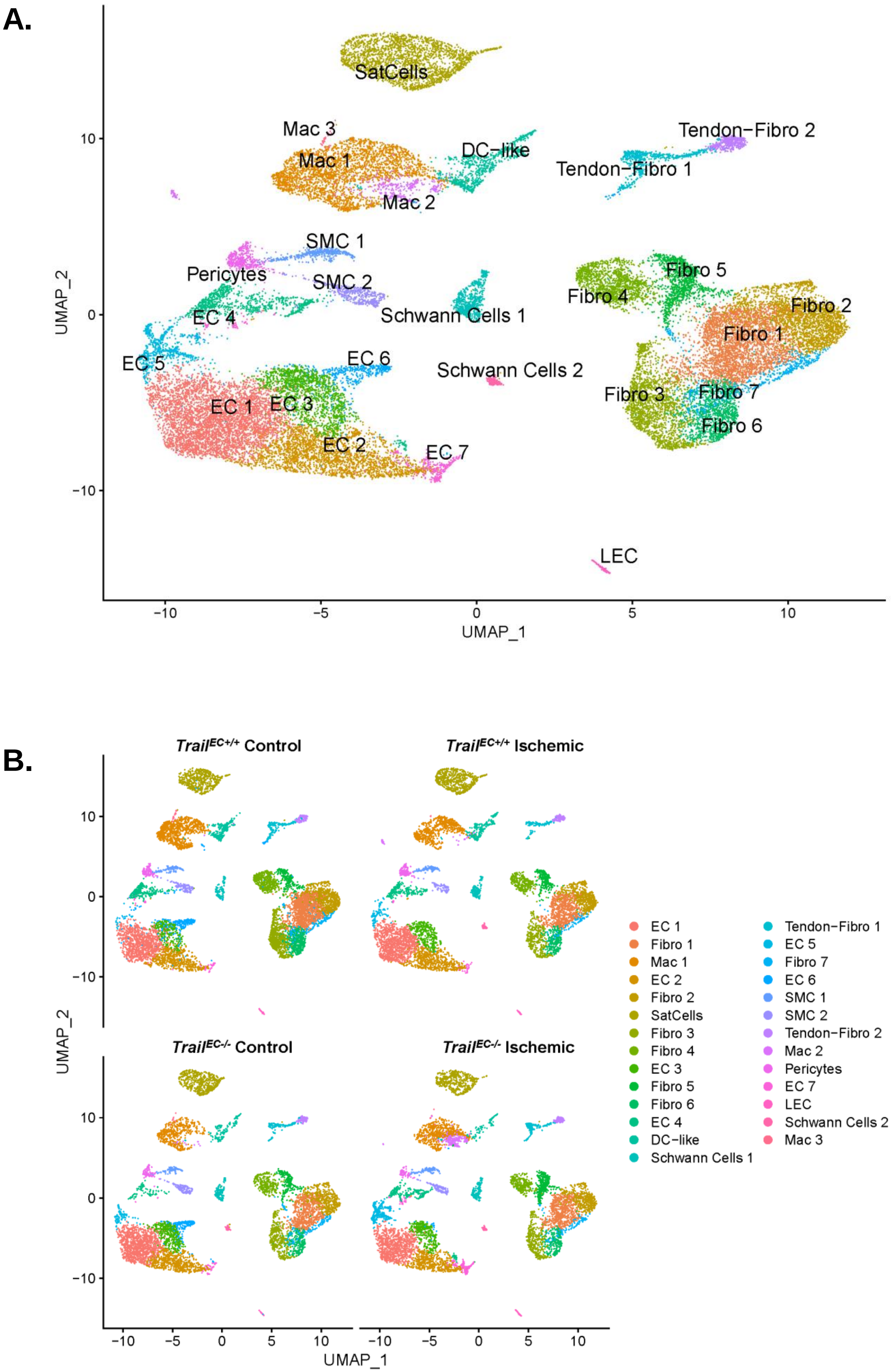
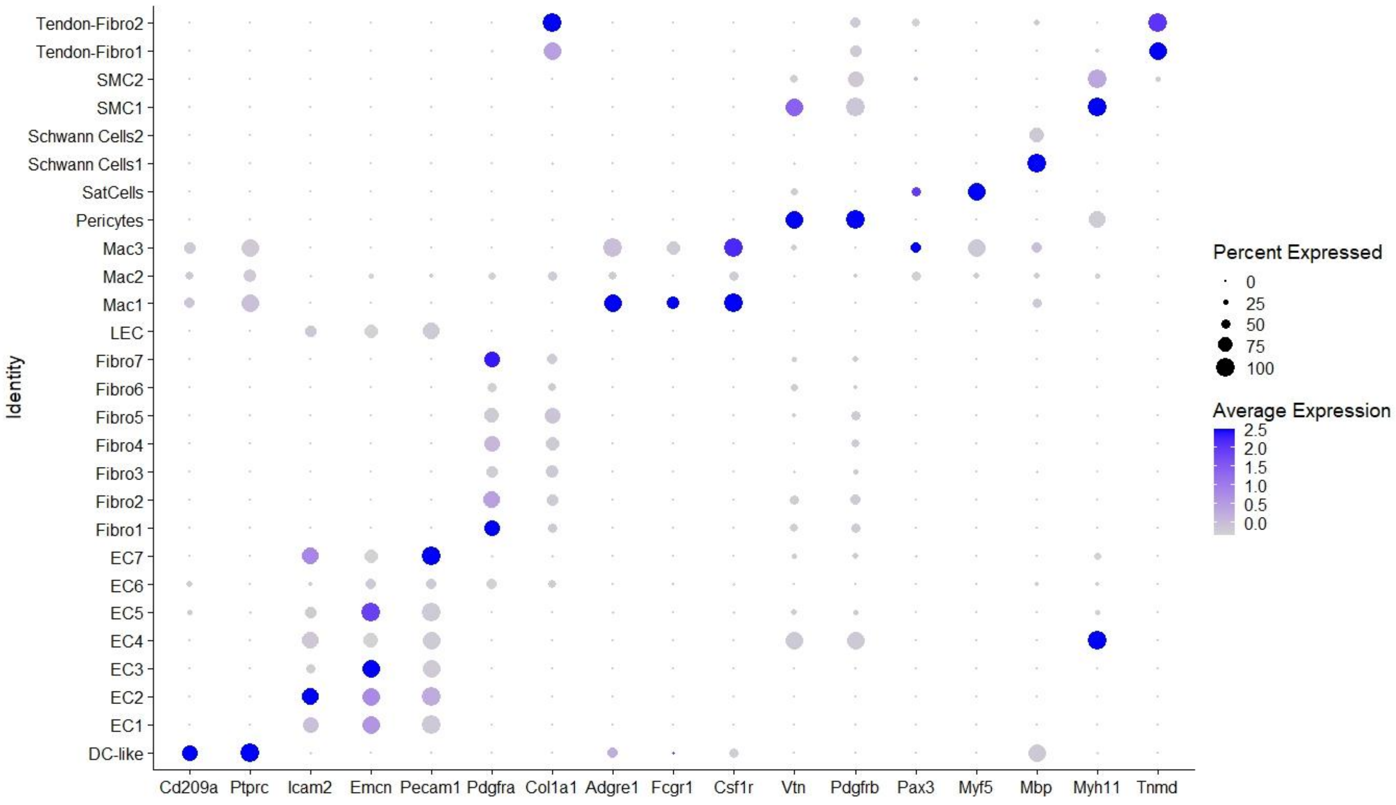


**Fig. S5.**

**Large vessel numbers are unaltered in ischemic limbs.** Large vessel numbers 50-100 and >100 µm are unaltered in ischemic gastrocnemius muscle from: (A) *Trail*$^{EC+/+}$ vs. *Trail*$^{EC-/-}$ mice 28 d post-HLI (n=7-9/genotype); (B) *Trail*$^{EC-/-}$ treated with MD5-1 or IgG 7 d post-HLI (n=3-4/treatment); and (C) *Trail-R*$^{+/+}$ vs. *Trail-R*$^{-/-}$ mice 28 d post-HLI (n=4-5/genotype). Results are mean±SEM. Students *t*-test.

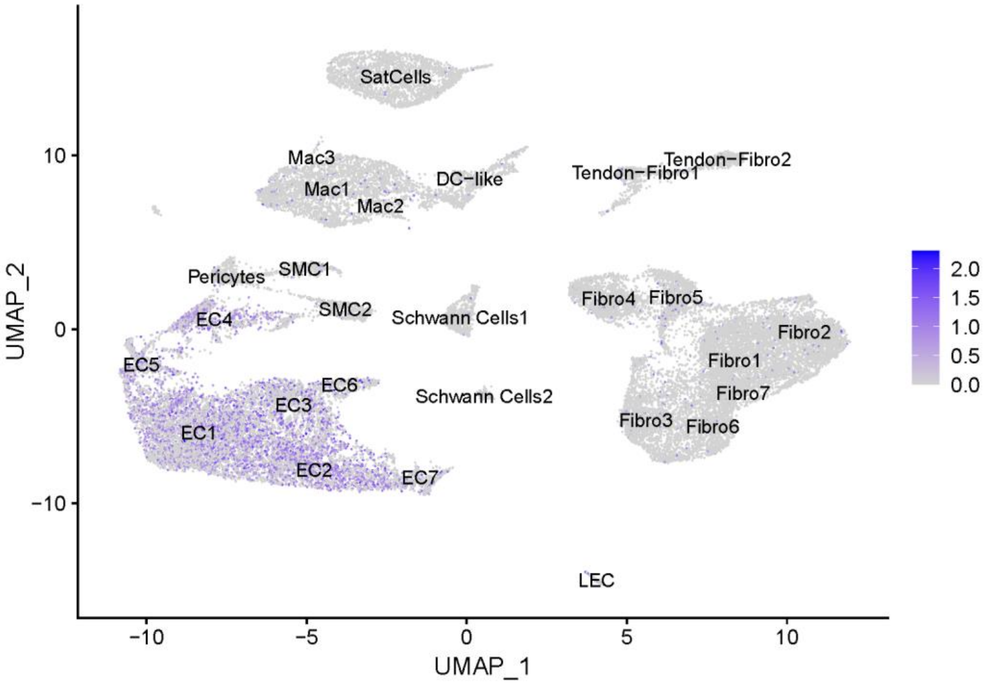

A.
SatCells
Mac 3
Mac 1
DC−like
Mac 2
Tendon−Fibro 2
Tendon−Fibro 1
SMC 1
Pericytes
SMC 2
Schwann Cells 1
EC 4
EC 5
EC 6
Schwann Cells 2
EC 1
EC 3
EC 2
EC 7
Fibro 5
Fibro 4
Fibro 2
Fibro 1
Fibro 3
Fibro 7
Fibro 6
LEC
UMAP_2
UMAP_1
10
0
−10
−10
−5
0
5
10
B.
Trail^EC+/+ Control
Trail^EC+/+ Ischemic
Trail^EC-/- Control
Trail^EC-/- Ischemic
UMAP_2
UMAP_1
−10
−5
0
5
10
EC 1
Fibro 1
Mac 1
EC 2
Fibro 2
SatCells
Fibro 3
Fibro 4
EC 3
Fibro 5
Fibro 6
EC 4
DC−like
Schwann Cells 1
Tendon−Fibro 1
EC 5
Fibro 7
EC 6
SMC 1
SMC 2
Tendon−Fibro 2
Mac 2
Pericytes
EC 7
LEC
Schwann Cells 2
Mac 3

**C.**



**D.**

*Trail* expression



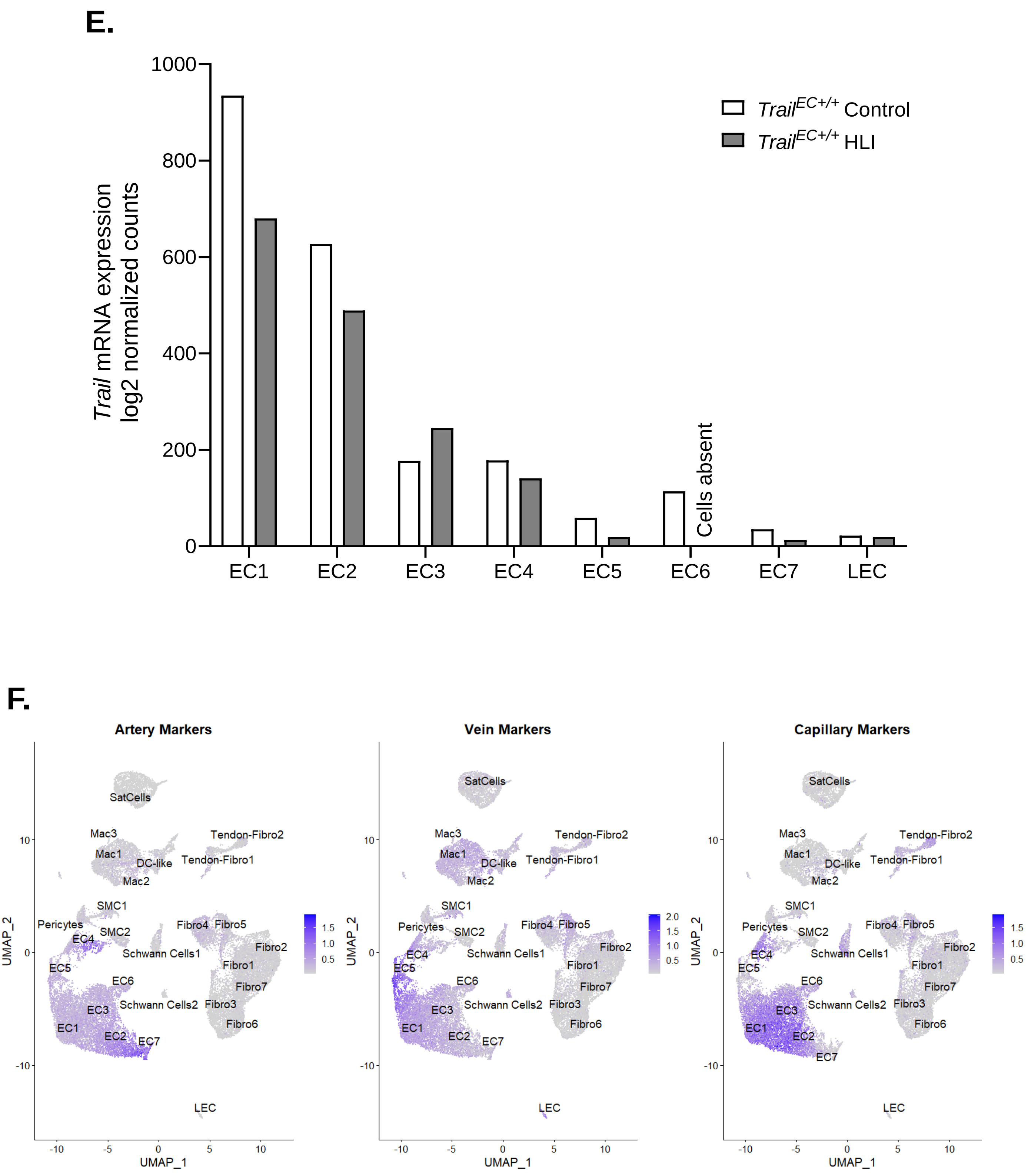
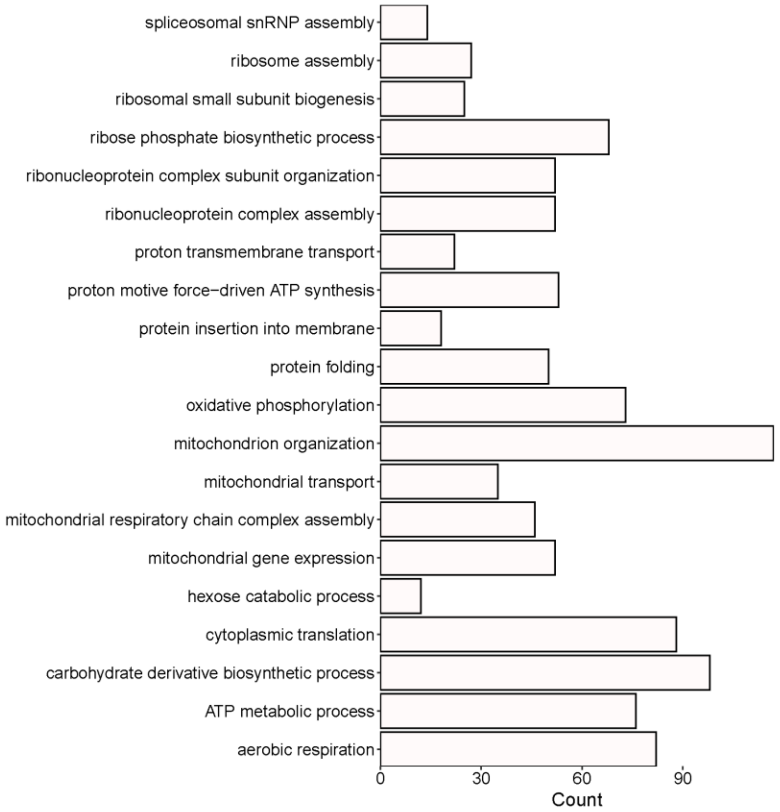
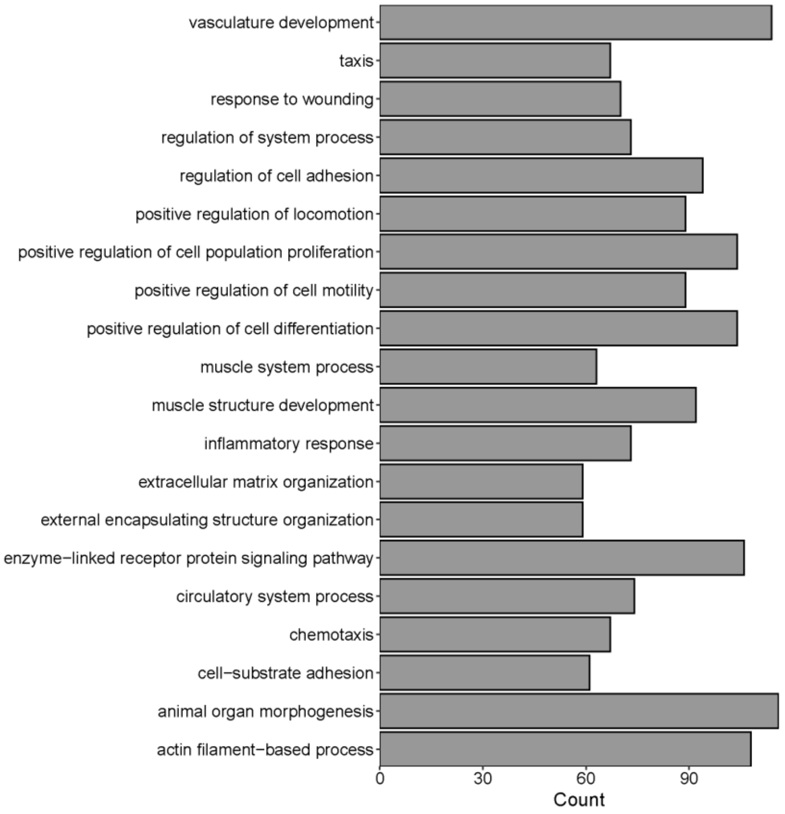


**Fig. S6.**
**scRNA-seq of gastrocnemius muscle.** (A) Principal component analysis followed by K-nearest neighbour clustering identified 8 EC (EC1-7, LEC) and 1 Pericyte cluster. Additional cell clusters included: smooth muscle cells (SMC1-2); satellite cells (SatCell); dendritic cell-like (DC-like); macrophages (Mac1-3); fibroblasts (Fibro1-7); Schwann cells 1-2; tendon fibroblasts (Tendon-Fibro1-2). (B) Cell clusters in each group. (C) Dot plot showing differentially expressed genes for annotated cell populations. *Trail* expression in (D) all clusters or in (E) EC subtypes. (F) Clusters marking arteries, veins and capillaries.

**A.** Upregulated genes; ischemic vs. non-ischemic limbs of *Trail*$^{EC+/+}$



**B.** Upregulated genes; ischemic vs. non-ischemic limbs of *Trail*$^{EC-/-}$



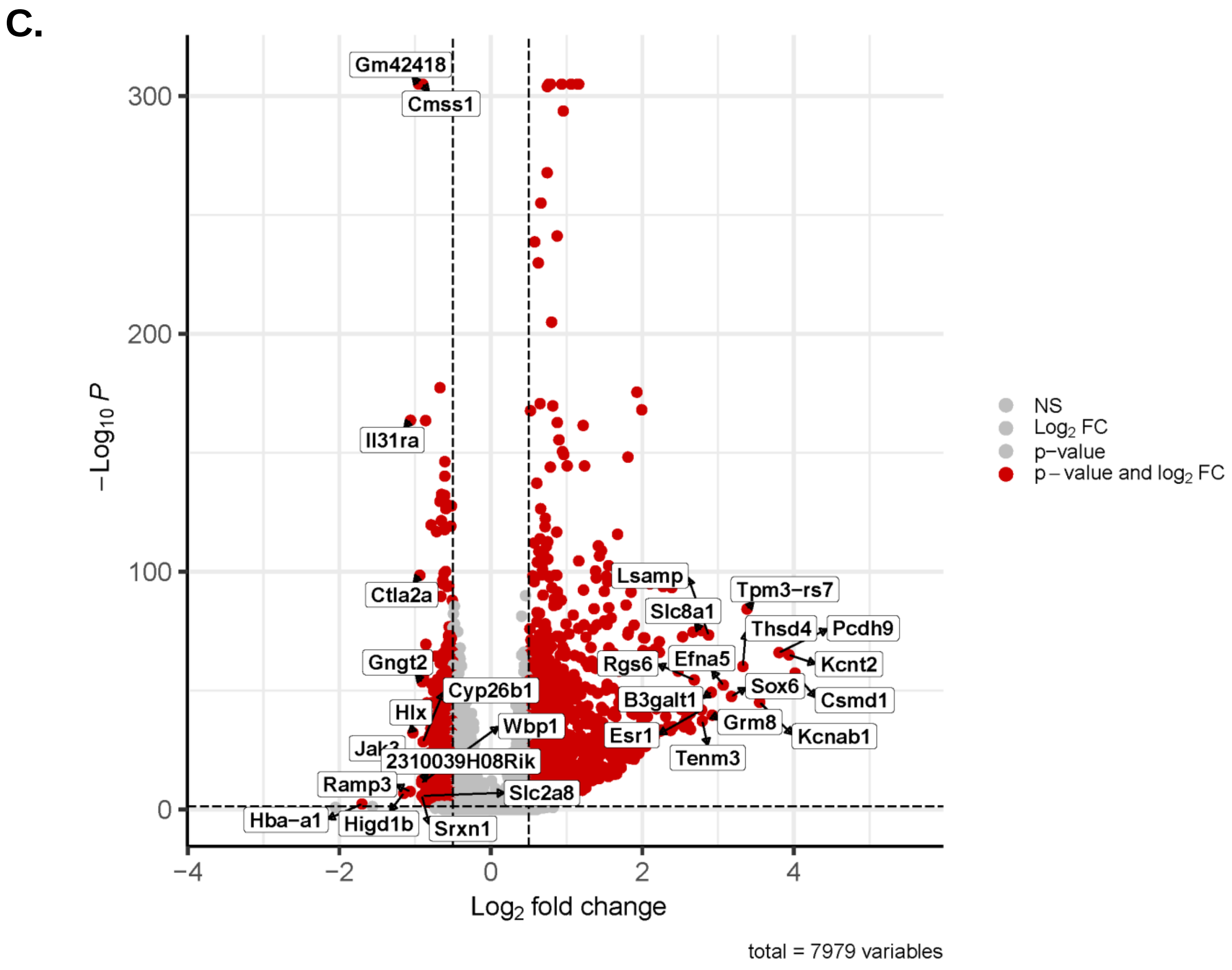
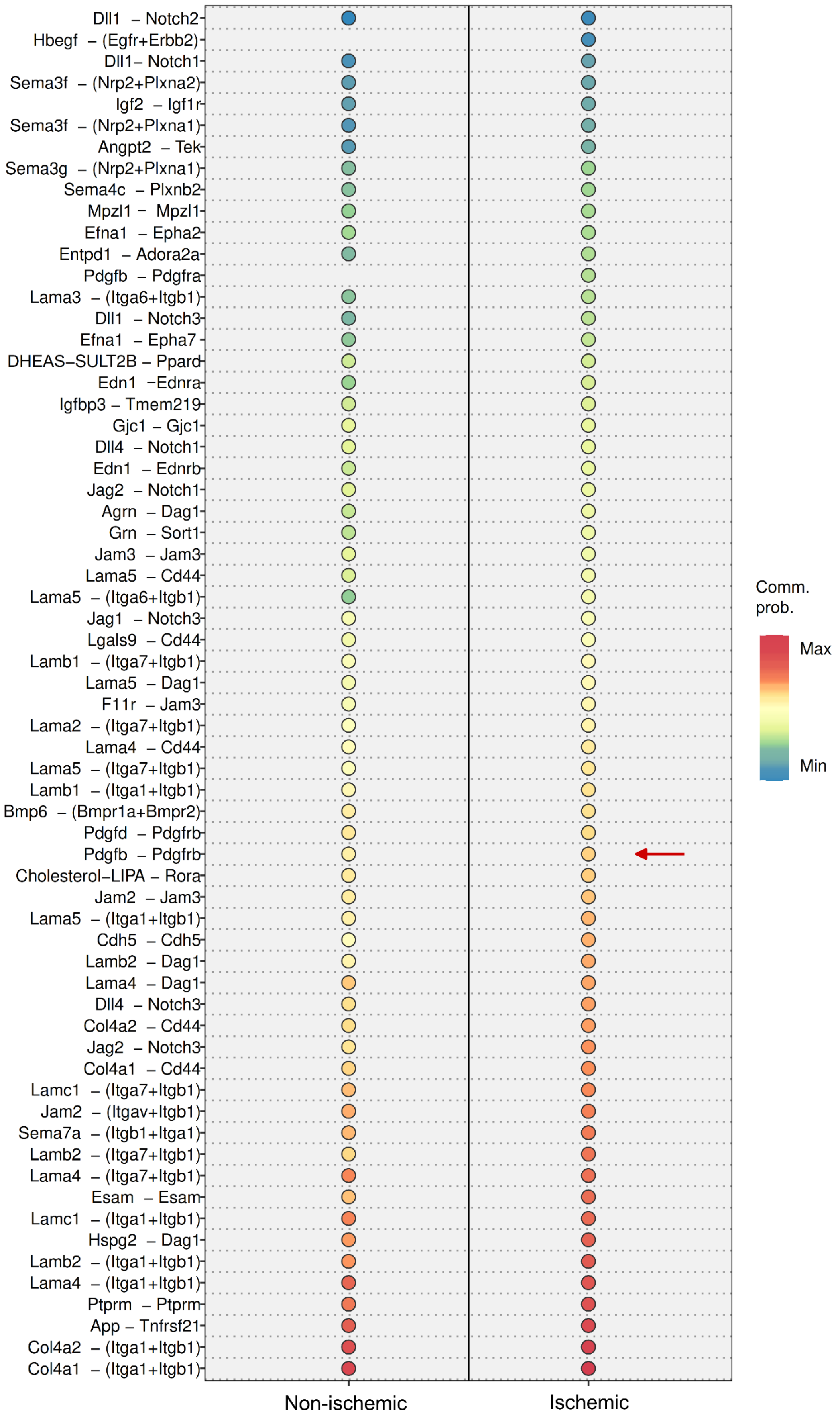


**Fig. S7.**

**Differentially expressed genes in EC microvessel populations are altered in limbs of *Trail*$^{EC-/-}$ mice.** (A) *Trail*$^{EC+/+}$ and (B) *Trail*$^{EC-/-}$ EC1-EC7 clusters in ischemic tissues showing altered biological processes. (C) Volcano plot showing top up-regulated and down-regulated differentially expressed genes from *Trail*$^{EC-/-}$ vs *Trail*$^{EC+/+}$ ischemic tissues.

**A.**


Dll1 – Notch2
Hbegf – (Egfr+Erbb2)
Dll1– Notch1
Sema3f – (Nrp2+Plxna2)
Igf2 – Igf1r
Sema3f – (Nrp2+Plxna1)
Angpt2 – Tek
Sema3g – (Nrp2+Plxna1)
Sema4c – Plxnb2
Mpzl1 – Mpzl1
Efna1 – Epha2
Entpd1 – Adora2a
Pdgfb – Pdgfra
Lama3 – (Itga6+Itgb1)
Dll1 – Notch3
Efna1 – Epha7
DHEAS–SULT2B – Ppard
Edn1 –Ednra
Igfbp3 – Tmem219
Gjc1 – Gjc1
Dll4 – Notch1
Edn1 – Ednrb
Jag2 – Notch1
Agrn – Dag1
Grn – Sort1
Jam3 – Jam3
Lama5 – Cd44
Lama5 – (Itga6+Itgb1)
Jag1 – Notch3
Lgals9 – Cd44
Lamb1 – (Itga7+Itgb1)
Lama5 – Dag1
F11r – Jam3
Lama2 – (Itga7+Itgb1)
Lama4 – Cd44
Lama5 – (Itga7+Itgb1)
Lamb1 – (Itga1+Itgb1)
Bmp6 – (Bmpr1a+Bmpr2)
Pdgfd – Pdgfrb
Pdgfb – Pdgfrb
Cholesterol–LIPA – Rora
Jam2 – Jam3
Lama5 – (Itga1+Itgb1)
Cdh5 – Cdh5
Lamb2 – Dag1
Lama4 – Dag1
Dll4 – Notch3
Col4a2 – Cd44
Jag2 – Notch3
Col4a1 – Cd44
Lamc1 – (Itga7+Itgb1)
Jam2 – (Itgav+Itgb1)
Sema7a – (Itgb1+Itga1)
Lamb2 – (Itga7+Itgb1)
Lama4 – (Itga7+Itgb1)
Esam – Esam
Lamc1 – (Itga1+Itgb1)
Hspg2 – Dag1
Lamb2 – (Itga1+Itgb1)
Lama4 – (Itga1+Itgb1)
Ptprm – Ptprm
App – Tnfrsf21
Col4a2 – (Itga1+Itgb1)
Col4a1 – (Itga1+Itgb1)
Non-ischemic
Ischemic
Comm.
prob.
Max
Min

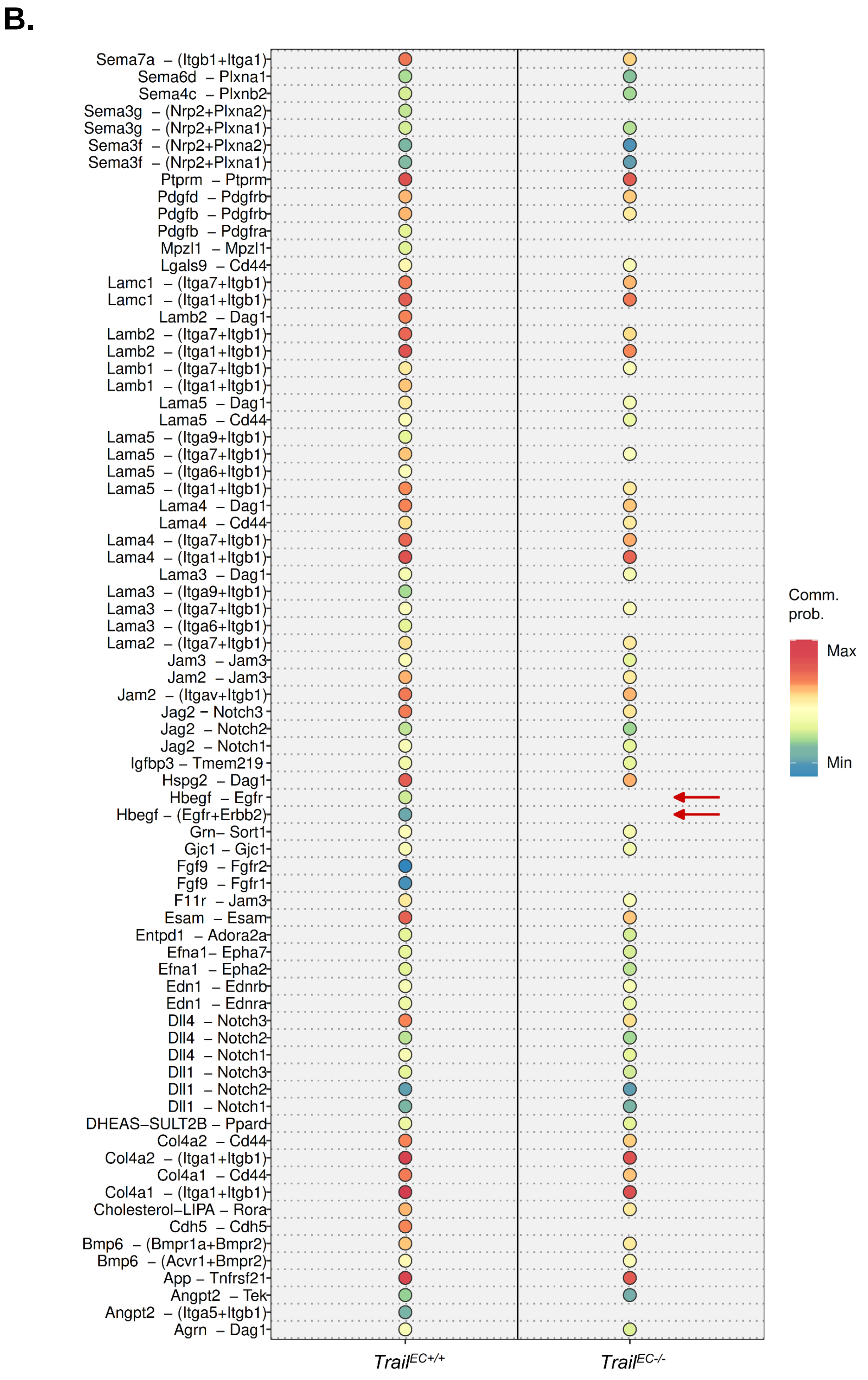


**Fig. S8.**

**TRAIL-dependent EC-pericyte interactions.** (A) CellChat analysis showing EC-pericyte interactions, control vs ischemic limb of *Trail*$^{EC+/+}$ mice. Red arrow depicting PDGFB-PDGFRβ interactions. (B) CellChat analysis showing EC-pericyte interactions of ischemic limbs between *Trail*$^{EC+/+}$ and *Trail*$^{EC-/-}$ mice. Red arrows depicting loss of HBEGF-EGFR and HBEGF-EGFR/ERBB2 interactions in ischemic limbs of *Trail*$^{EC-/-}$ mice.

# SUPPLEMENTARY TABLES

**Table S1.**

**Human demographics.**

| **Characteristic** | **PAD Patients**<br>**n=11** | **Healthy Controls**<br>**n=12** |
|---|---|---|
| Age (y) Mean (SD) | 66.1 (10.0) | 38.9 (12.1) |
| Female (%) | 4 (36) | 8 (67) |
| Body Mass Index Mean (SD) | 30.0 (7.4) | ND |
| Hypertension (%) | 7 (63.6) | N/A |
| Diabetic (%) | 9 (81.8) | N/A |
| Hypercholesterolemia (%) | 8 (72.7) | N/A |
| Smoker (%) | 9 (81.8) | ND |
| Myocardial Infarction (%) | 7 (63.6) | N/A |

ND, not determined; N/A, not applicable.

**Table S2.**

**Plasma chemistries of adult *Trail*$^{EC+/+}$ and *Trail*$^{EC-/-}$ mice**

| | *Trail*$^{EC+/+}$ | *Trail*$^{EC-/-}$ | *P* value | *n per group* |
|---|---|---|---|---|
| **Glucose mmol/L** | 10.29 ± 0.42 | 10.12 ± 0.44 | 0.787 | 6-7 |
| **Insulin mg/L** | 0.3± 0.07 | 0.5 ± 0.22 | 0.105 | 6-8 |
| **Cholesterol mg/dL** | 115.6 ± 13.22 | 107.8 ± 23.18 | 0.310 | 6-8 |
| **TRAIL pg/mL** | 10782 ± 1963 | 4649 ± 338 | 0.002 | 6 |

**Table S3.**

**Markers used to identify artery, vein, and capillaries from EC populations.**

| Vessel Type | Marker |
|---|---|
| Artery | *Acvrl1* |
| | *Bmx* |
| | *Crip1* |
| | *Efnb2* |
| | *Fbln2* |
| | *Gja4* |
| | *Gja5* |
| | *Gkn3* |
| | *Hey1* |
| | *Hey2* |
| | *Mecom* |
| | *Nrp1* |
| | *Sat1* |
| | *Sema3g* |
| | *Sox17* |
| Vein | *Apoe* |
| | *Bgn* |
| | *Ctla2a* |
| | *Emcn* |
| | *Ephb4* |
| | *Il6st* |
| | *Nr2f2* |
| | *Nrp2* |
| | *Ptgs1* |
| | *Selp* |
| | *Tmsb10* |
| | *Tmsb4x* |
| | *Vwf* |
| Capillary | *Car4* |
| | *Kdr* |
| | *Mfsd2a* |
| | *Plvap* |
| | *Rgcc* |
| | *Sgk1* |
| | *Sparc* |

## Table S4.

## The top 15 up-regulated genes in ischemic limb tissues of *Trail*$^{EC-/-}$ *mice.*

| Gene | Fold Change | *P* value | Function | Reference |
|---|---|---|---|---|
| *Csmd1* | 16.17723187 | 4.44054E-58 | Tumour suppressor; inhibits proliferation, migration and invasion of cancerous cells | *(68)* |
| *Kcnt2* | 15.30062718 | 9.53328E-66 | Voltage-gated potassium channel subunit; involved in blood pressure regulation | *(69)* |
| *Pcdh9* | 13.97038328 | 1.07846E-66 | Protocadherin, mediated cell-cell adhesion in neural tissue | *(70)* |
| *Kcnab1* | 11.69655966 | 8.23554E-46 | Voltage-gated potassium channel subunit; involved in blood pressure regulation | *(71)* |
| *Tpm3-rs7* | 10.42682927 | 5.01724E-85 | Pseudogene | |
| *Thsd4* | 10.03501363 | 7.15917E-61 | Variants associated with thoracic aortic aneurysm predisposition | *(72)* |
| *Sox6* | 9.040404717 | 2.97456E-48 | Tumour suppressor; inhibits inducible EC proliferation and tubule formation | *(73)* |
| *Efna5* | 8.390461672 | 4.60505E-53 | Involved in retinal neovascularisation | *(74)* |
| *Grm8* | 7.584277003 | 2.20594E-40 | Mediates EC proliferation and tubule formation | *(75)* |
| *B3galt1* | 7.518566799 | 5.21455E-50 | Involved in brain development | *(76)* |
| *Lsamp* | 7.332219299 | 4.24164E-74 | Polymorphisms associated with coronary artery disease | *(77)* |
| *Tenm3* | 6.926393728 | 6.24619E-38 | Transmembrane protein; involved in ERK/AKT/SAT3 activation | *(78)* |
| *Esr1* | 6.874832485 | 1.45671E-42 | Involved in VEGFA regulation in adipose tissue | *(79)* |
| *Slc8a1* | 6.846967715 | 5.84893E-76 | $Na^+/Ca^{2+}$ exchanger, maintains $Ca^{2+}$ homeostasis; stimulates angiogenesis | *(80)* |
| *Rgs6* | 6.43223378 | 3.23921E-55 | Required for normal cardiovascular development | *(81)* |

## Table S5.

## The top 15 down-regulated genes in ischemic limb tissues of *Trail*$^{EC-/-}$ *mice.*

| Gene | Fold Change | *P* value | Function | Reference |
|---|---|---|---|---|
| *Hba-a1* | -3.248921374 | 0.005029777 | Haemoglobin chain; acts as a nitrite reductase in ECs | *(82)* |
| *Higd1b* | -2.216554847 | 1.44602E-07 | Pericyte marker | *(83)* |
| *Ramp3* | -2.093268791 | 2.50109E-08 | Involved in lymphatic EC migration | *(84)* |
| *Il31ra* | -2.083654763 | 2.219E-164 | IL-31 receptor; Regulates immunity, hematopoiesis, proliferation, apoptosis | *(84)* |
| *Jak3* | -2.047762948 | 6.67371E-21 | Marker of bone marrow endothelium | *(85)* |
| *Hlx* | -2.039091723 | 6.08803E-33 | Negative regulation of endothelial cell sprouting | *(86)* |
| *Gm42418* | -1.932766499 | 0 | Pseudogene | |
| *Ctla2a* | -1.914428933 | 3.3819E-99 | Inhibits EC proliferation and angiogenesis | *(87)* |
| *Wbp1* | -1.882749838 | 5.5348E-12 | Binds tumour suppressor WWOX | *(88)* |
| *2310039H08Rik* | -1.878559416 | 4.70367E-13 | Predicted gene | |
| *Srxn1* | -1.877820154 | 1.95222E-06 | Inhibits smooth muscle cell proliferation and migration | *(89)* |
| *Gngt2* | -1.872098727 | 2.19706E-54 | Marker in esophogeal cancer | *(90)* |
| *Cmss1* | -1.864645757 | 0 | Part if the ribosome unit; binds mRNA | *(91)* |
| *Cyp26b1* | -1.858356536 | 3.31131E-29 | Monooxygenase; interacts with retinoic acid; increased expression induces angiogenesis in infarcted hearts | *(92)* |
| *Slc2a8* | -1.857274319 | 1.80318E-06 | Glucose transporter; induces autophagy in hepatocytes | *(93)* |

## Table S6.

## Primer sequences

| | Human | |
|---|---|---|
| | **Forward** | **Reverse** |
| **TRAIL** | ACCAACGAGCTGAAGCAGAT | CAAGTGCAAGTTGCTCAGGA |
| **TRAIL-R1** | GTCCTGCTGCAGGTCGTACC | GATGTCACTCCAGGGCGTAC |
| **TRAIL-R2** | CCAACAAGACCTAGCTCCCCAGC | AAGACTACGGCTGCAACTGTGACTCC |
| **VEGF** | ATGAACTTTCTGCTGTCTTGGGTG | TCACCGCCTCGGCTTGTCACAT |
| **NOX4** | CTGGAGGAGCTGGCTCGCCAACGAAG | GTGATCATGAGGAATAGCACCACCACCATGCAG |
| **HBEGF** | ATCGTGGGGCTTCTCATGTTT | TTAGTCATGCCCAACTTCACTTT |
| **ERBB2** | TGTGACTGCCTGTCCCTACAA | CCAGACCATAGCACACTCGG |
| **EGFR** | AGGCACGAGTAACAAGCTCAC | ATGAGGACATAACCAGCCACC |
| **β-ACTIN** | AGCCATGTACGTAGCCATCC | CTCTCAGCTGTGGTGGTGAA |
| | **Mouse** | |
| | **Forward** | **Reverse** |
| **TRAIL** | AAGCTGAAGACGCTTCCAAGAT | CCGGATAGCTGGTGTACTTGTA |
| **TRAIL-R** | GCAGAGAGGGTATTGACTACA | GCATCGGGTTTCTACGACTTT |
| **CD31** | CTGCCAGTCCGAAAATGGAAC | CTTCATCCACCGGGGCTATC |
| **NG2** | ACCATGCTACTCCGCAACAG | CCGGTGAACATCTATGTGTACG |
| **RGS5** | GGGTTGCCTGTGAGAATTACA | TGAAGTGGTCAATGTTCACCTCT |
| **PDGFRβ** | GTGGTCCTTACCGTCATCTCTC | GTGGAGTCGTAAGGCAACTGCA |
| **HBEGF** | CGGGGAGTGCAGATACCTG | TTCTCCACTGGTAGAGTCAGC |
| **ERBB2** | GCTAGAGCGGCTTCTGAGAAA | ACCACAGGGTCTACCACTTCC |
| **EGFR** | CAATGTTCCCATCGCTGTCGT | TGTCTTTGCATGTGGCCTCAT |
| **LAMC1** | TGCCGGAGTTTGTTAATGCC | CTGGTTGTTGTAGTCGGTCAG |
| **ITGα1** | GACAGCCCTTGGAATAGACAC | GTTGTCATGCGATTCTCCATCA |
| **ITGβ1** | ACTGTGATGCCGTATATTAGCAC | GATATGCGTTGCTGACCAACA |
| **β-ACTIN** | AGCCATGTACGTAGCCATCC | CTCTCAGCTGTGGTGGTGAA |
| **HPRT** | GTTAAGCAGTACAGCCCCAAA | AGGGCATATCCAACAACAAACTT |

# Table S7.

## Quality control and clustering steps for mouse scRNA-seq data set.

| **Sample** | **QC steps -clean up**<br>**for doublet discrimination** | **nPcs and resolution for clustering** | **Expected % doublets**<br>**According to 10X recommendation** | **DF results** |
|---|---|---|---|---|
| ***Trail*$^{EC-/-}$ control**<br>Also called EC_TKO_Control | Started with 14,092 cells.<br>nFeature RNA > 200 & nFeature RNA < 8000 & percent.mt < 18.03(1sd)<br>11,178 cells<br>removed cluster 4,6,9, 13-15, 23-25, 29<br>8,265 cells | dims = 1:30, resolution = 0.8 | 0.8*8.265~6.2% | Doublet Singlet<br>471 7794 |
| ***Trail*$^{EC-/-}$ HLI**<br>Also called EC_TKO_HLI | Started with 20,000 cells.<br>nFeature_RNA > 200 & nFeature_RNA < 7000 & percent.mt < 20.23 (1sd)<br>17,625 cells<br>Removed cluster - 1,4,29<br>14,516 cells<br>Removed clusters 2,12<br>12, 900 cells<br>Removed clusters 2,16,25,28<br>11,540 cells | dims = 1:30, resolution = 0.8 | 0.8*11.540 ~8.6% | Doublet Singlet<br>931 10609 |
| ***Trail*$^{EC+/+}$ control**<br>Also called EC_WT_Control | Started with 22,046 cells.<br>nFeature RNA > 100 & nFeature RNA < 9000 & percent.mt < 23.08 (1sd)<br>17,893 cells<br>removed clusters - 2,5,11,15,16,17,18,21,22,26,28<br>12,460 cells<br>Removed clusters- 3,13,14<br>10,773 cells | dims = 1:30, resolution = 0.8 | 0.8*10.773~8.2% | Doublet Singlet<br>822 9951 |
| ***Trail*$^{EC+/+}$ HLI**<br>Also called EC_WT_HLI | Started with 12,862 cells.<br>nFeature_RNA > 100 & nFeature_RNA < 9000 & percent.mt < 11.41(1sd)<br>11,045 cells<br>Removed clusters- 4,12<br>9,963 cells<br>Removed cluster - 8<br>9,541 cells<br>Removed cluster -8<br>9,125 cells | dims = 1:30, resolution = 0.8 | 0.8*9.125~7% | Doublet Singlet<br>595 8530 |

# Table S8.

## Quality control and clustering steps for human scRNA-seq data set.

| Sample | QC steps -clean up<br>for doublet discrimination | nPcs and resolution for clustering | Expected % doublets<br>According to 10X<br>recommendation | DF results |
|---|---|---|---|---|
| **Non-ishc** | Started with 33,697 cells.<br>1. nFeature RNA > 200 & nFeature_RNA < 6000 & percent.mt < 39.39(1sd)<br>28,387 cells<br>1. removed clusters 0, 14 23,235 cells<br>2. removed clusters 10, 20, 25, 28 21,918 cells<br>3. removed cluster 15, 23 21,081 cells<br>4. removed clusters 21, 23 20, 945 cells<br>5. removed cluster 3, 7, 8, 11 *after running doublet finder | dims = 1:40,<br>resolution = 0.8<br>dims = 1:35,<br>resolution = 0.8 | 0.8*20.945~16.8% | Doublet Singlet<br>1592 19353 |
| **Isch** | Started with 28,870 cells.<br>1. nFeature_RNA > 200 & nFeature_RNA < 7500 & percent.mt < 25.00(1sd) 27,242 cells<br>2. removed clusters 0, 10 23,321 cells<br>3. removed clusters 19, 20 22,648 cells<br>4. removed cluster 15, 21, 26, 31 21,760 cells<br>5. removed clusters 1, 22, 23 17,813 cells<br>6. removed clusters 22, 23 17,639 cells | dims = 1:40,<br>resolution = 0.5<br>dims = 1:40,<br>resolution = 0.8<br>dims = 1:30,<br>resolution = 0.8 | 0.8*17.639~14.1% | Doublet Singlet<br>1341 16298 |

# Appendix Data S1: Supporting Data Values

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