

## Supplementary Materials for

- **Meningeal macrophages regulate fibroblasts to influence meningeal lymphatic function following TBI**

Xiaoming Guo *et al.*

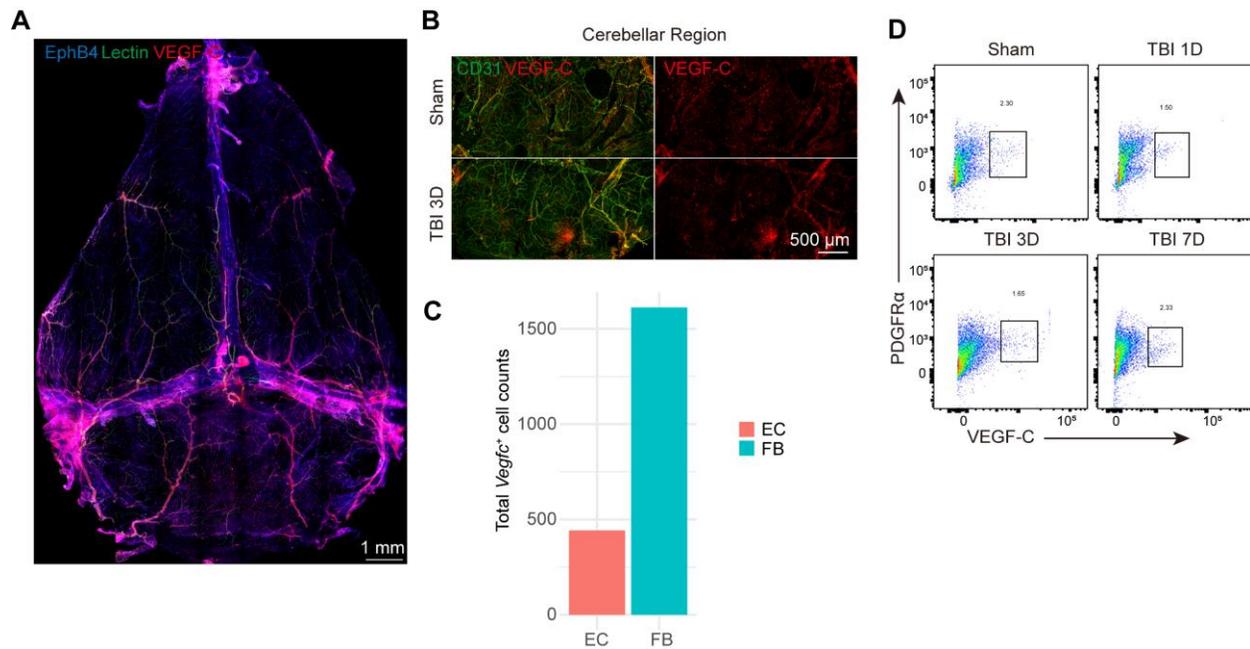
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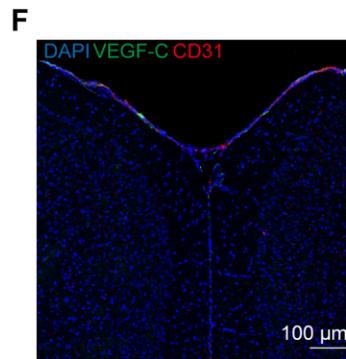
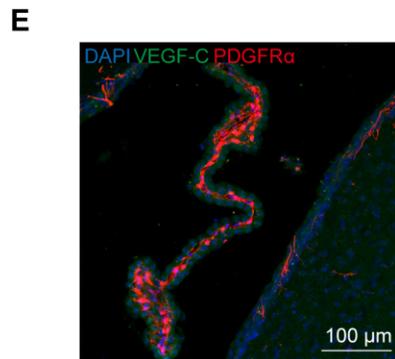
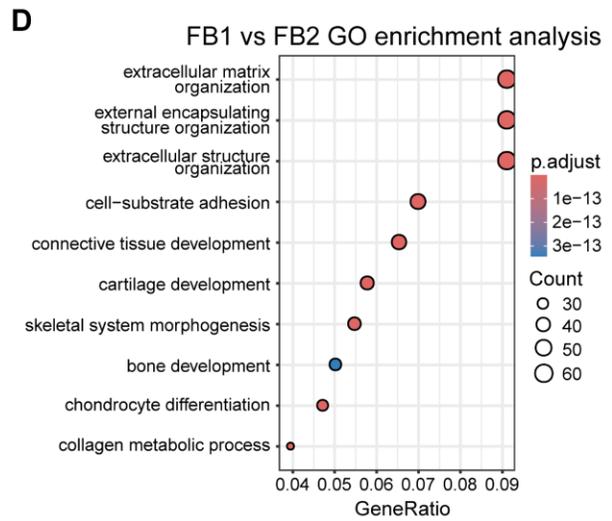
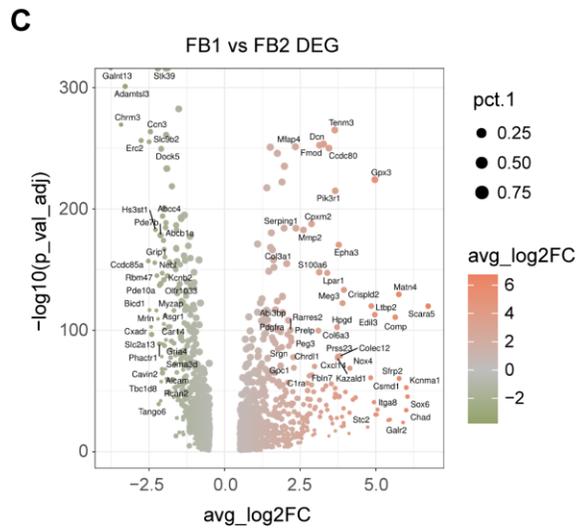
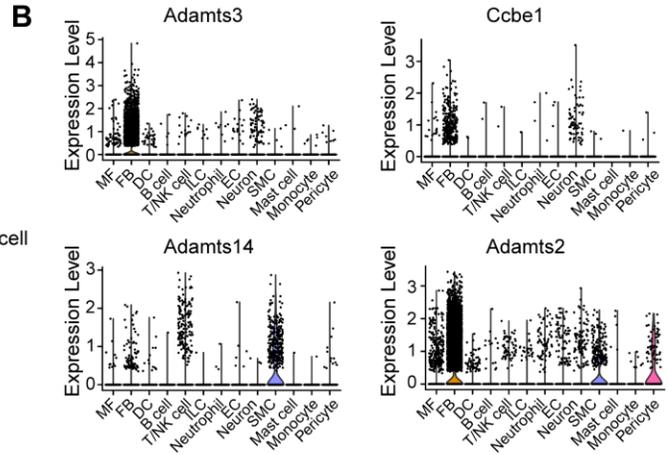
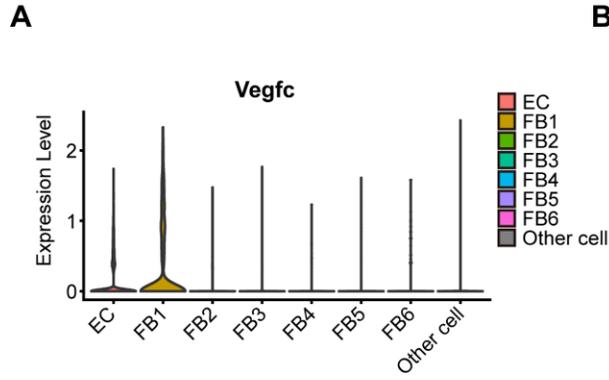
Figs. S1 to S7

Tables S1 to S2

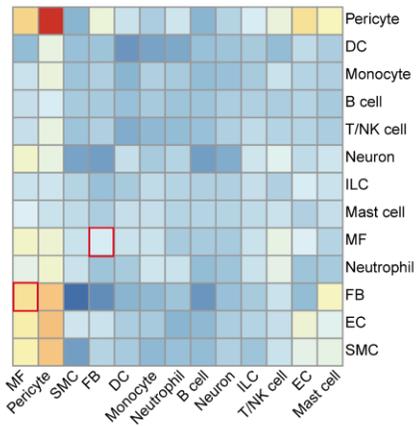
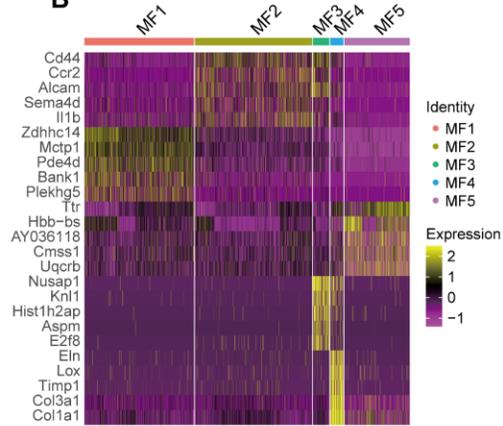
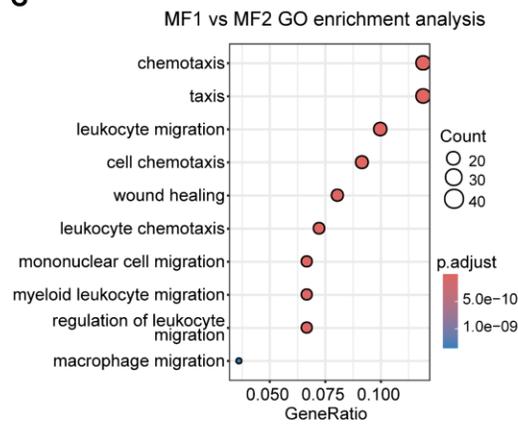
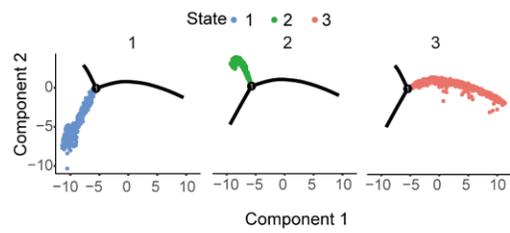
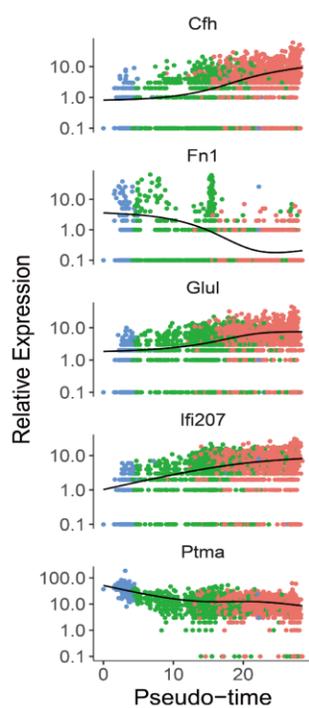
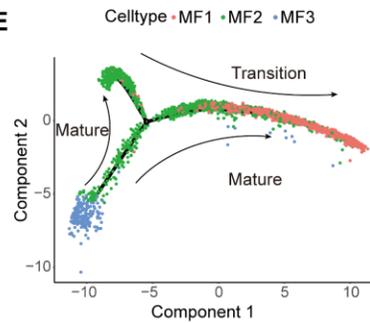
**Figure S1 VEGF-C<sup>+</sup> cells are present in the meninges. Related to Figure 1. (A)** Immunofluorescence showing the overall view of the meninges, with VEGF-C<sup>+</sup> cells located around arteries (Lectin) and veins (EphB4). **(B)** Representative images of cerebellar meninges isolated from sham and 3 days post-TBI groups, staining for VEGF-C and CD31. **(C)** Bar plots of sc-RNA analyses showing *Vegfc* expression in EC and FB. EC endothelial cells, FB fibroblast. **(D)** Representative pseudocolor flow cytometry plots of CD45<sup>-</sup>CD31<sup>-</sup>VEGF-C<sup>+</sup> meningeal cells from sham and 1, 3, 7 days post-TBI mice. FB: fibroblast, EC: endothelial cell.



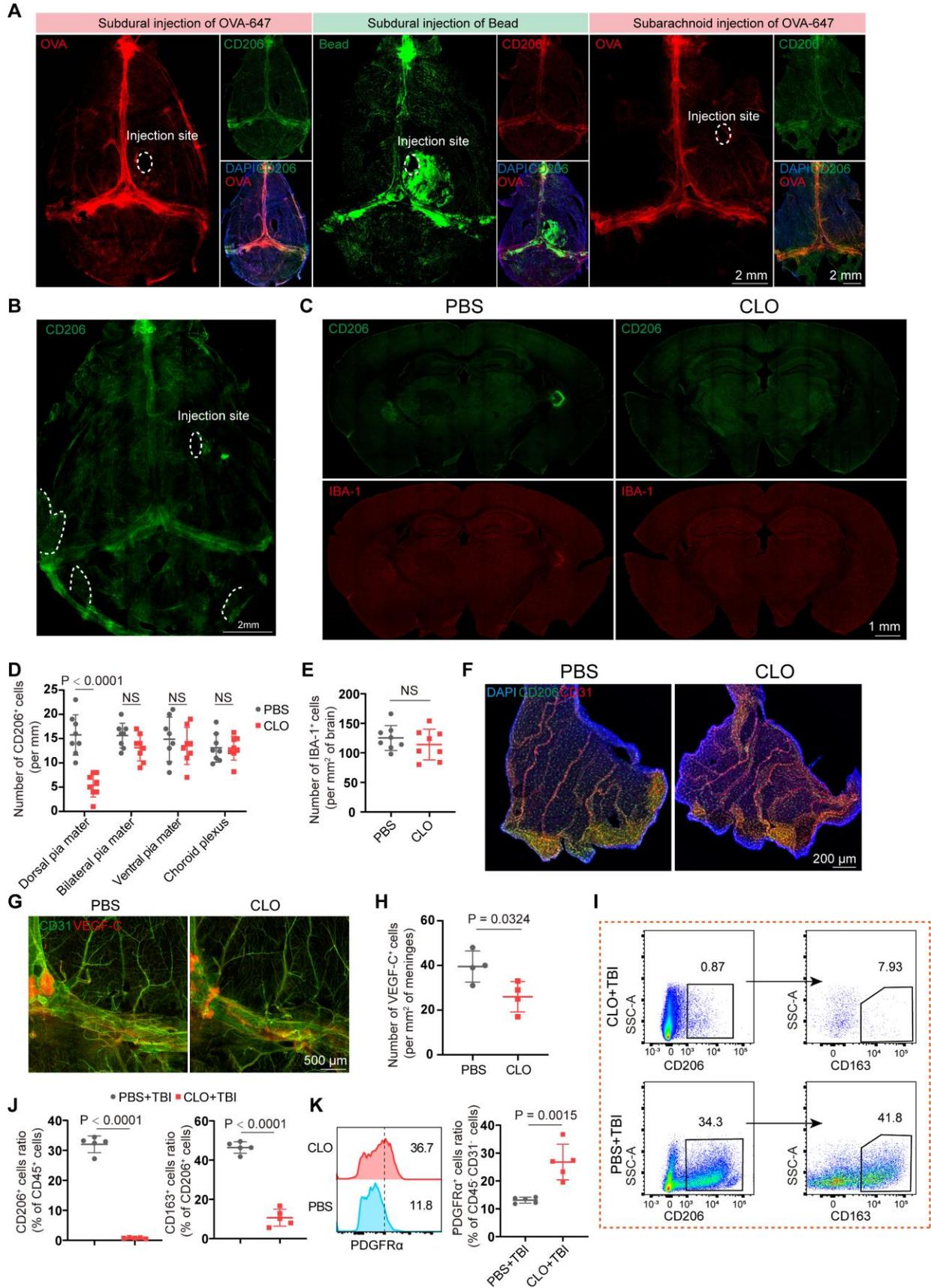
**Figure S2 FB1 is the primary cell population responsible for VEGF-C production in the meninges. Related to Figure 2.** (A) Violin plots show *Vegfc* expression in FB subsets and EC. FB, fibroblast, EC, endothelial cell. (B) Violin plots showing the expression of *Adamts3*, *Ccbe1*, *Adamts14*, and *Adamts2* in different cell types of the meninges. (C) Volcano plot showing differentially expressed genes between FB1 and FB2. (D) Dot plot showing functional differences in GO enrichment analysis between FB1 and FB2. (E, F) Representative immunofluorescence images showing the absence of VEGF-C<sup>+</sup> cells in the choroid plexus, while a small number of VEGF-C<sup>+</sup> cells are present in leptomeninges. FB: fibroblast.



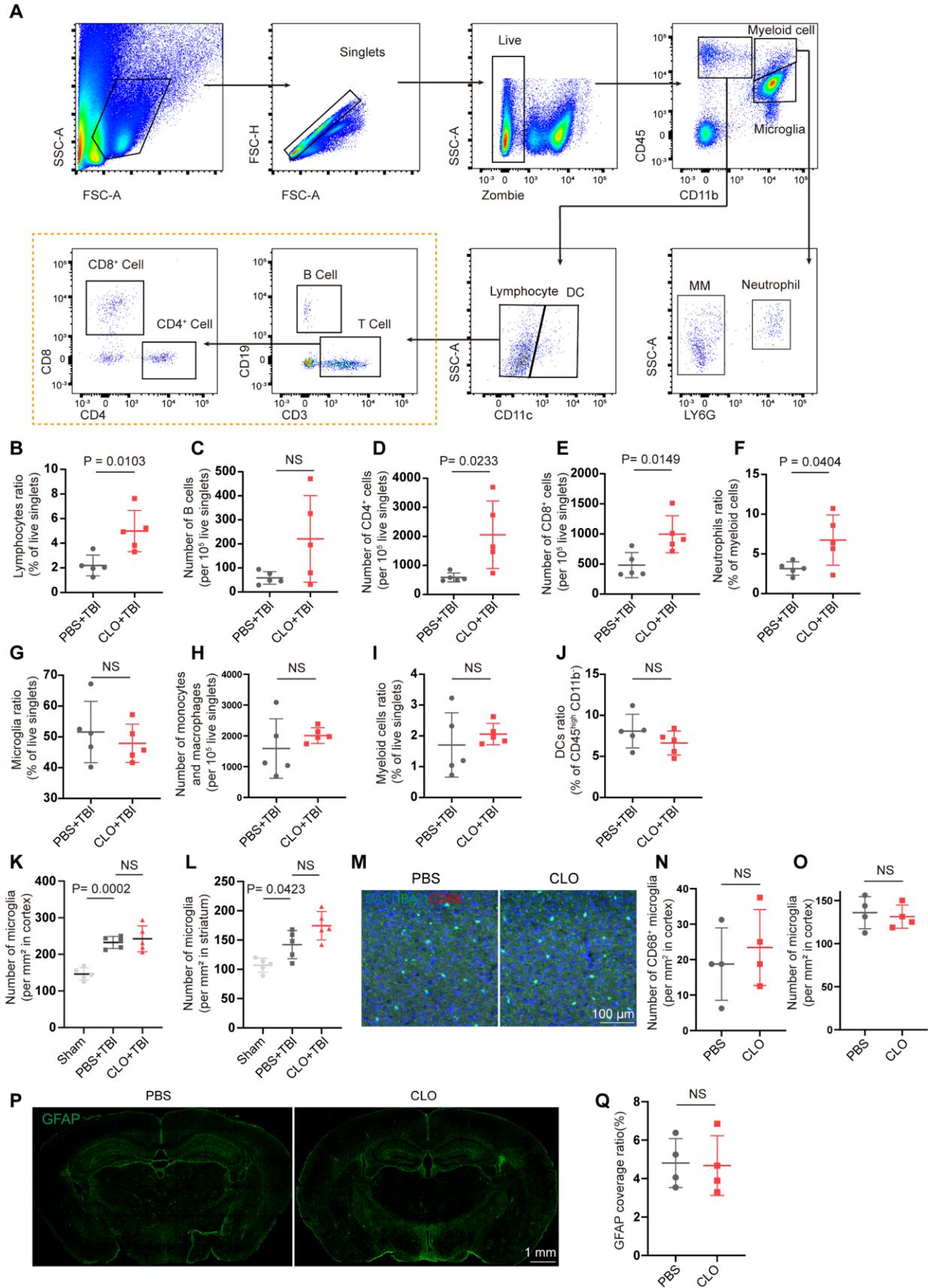
**Figure S3 Multiple macrophage subsets are present in the meninges, which undergo changes following TBI. Related to Figure 3.** (A) CellChat analysis showing cell communication between macrophages and fibroblasts. (B) Heatmap showing the top 5 genes of macrophage subsets (MF1-5). (C) Dot plot showing functional differences in GO enrichment analysis between MF1 and MF2. (D, E) Pseudotime trajectory of macrophage subclusters. (F) Gene expression changes in the pseudotime analysis.

**A****B****C****D****F****E**

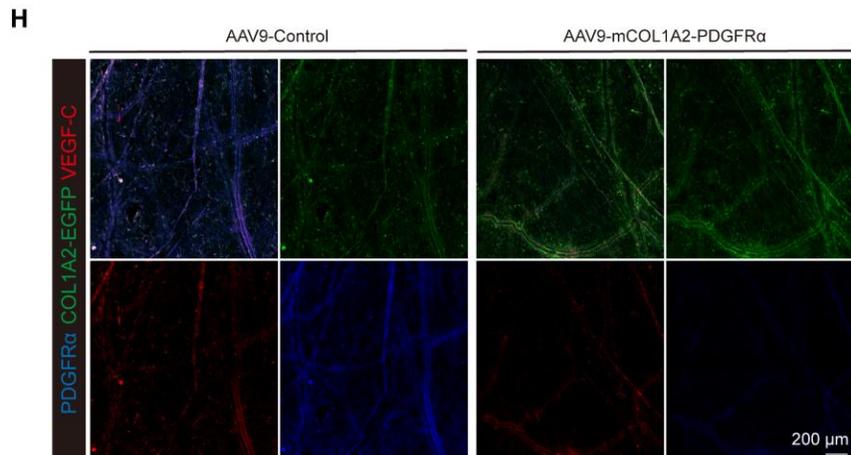
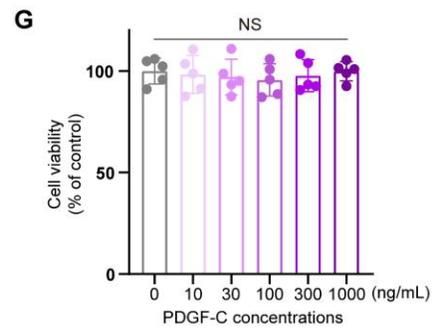
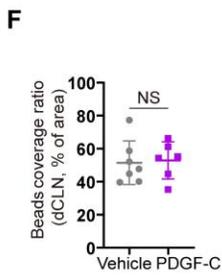
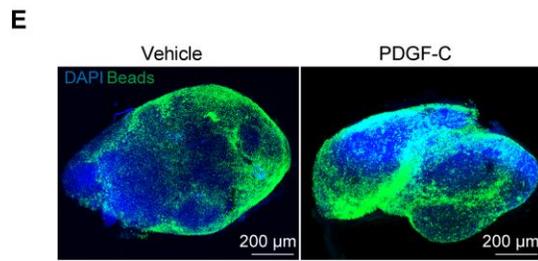
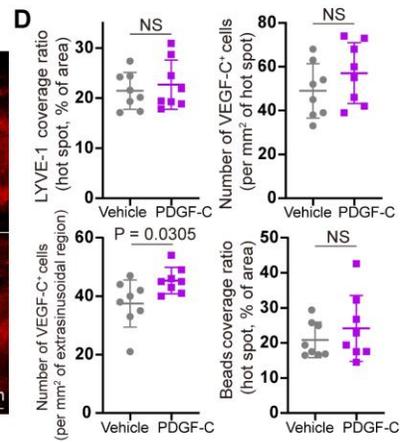
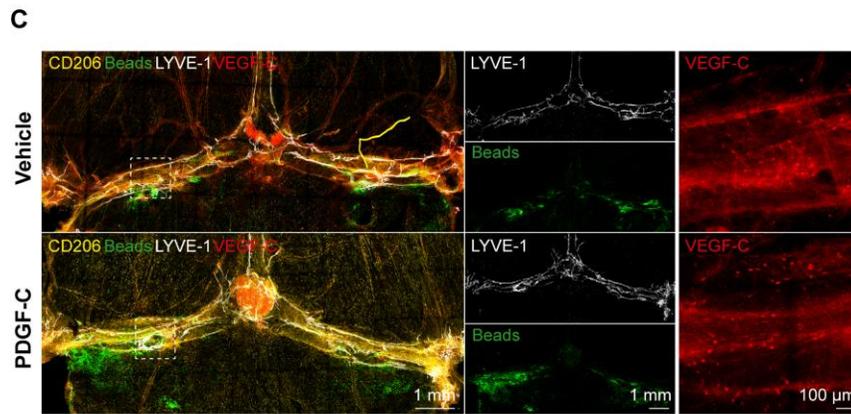
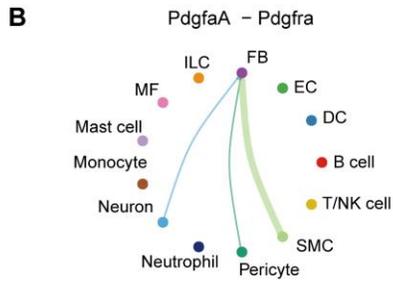
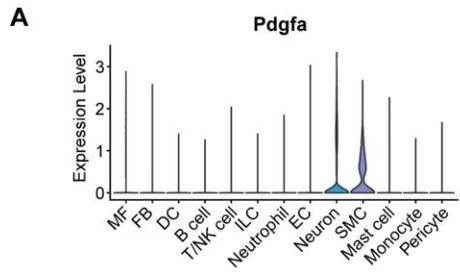
**Figure S4 Subdural injection serves as an effective technique for targeted intervention in the dura mater, while exhibiting minimal impact on brain tissue. Related to Figure 4.** (A) Representative images showing subdural injection of OVA-647 and fluorescent beads, and subarachnoid injection of OVA-647. (B) Representative images showing depletion of meningeal macrophages following subdural injection of CLO, with only a few residual macrophages remaining (dashed lines in the images). (C-E) Overview of fluorescent images and quantitative analysis of parenchymal macrophages (CD206, green) and microglia (IBA-1, red) changes after PBS and CLO treatment in the brain. (F) Immunofluorescence images showing no changes of macrophages in the choroid plexus (CD206, green) after PBS and CLO treatment. (G, H) Representative images and quantification of VEGF-C<sup>+</sup> cell changes after PBS and CLO treatment. (I, J) Representative flow cytometry pseudocolor plots and quantification of meningeal macrophages for CLO+TBI and PBS+TBI groups. (K) Flow cytometry histogram and quantitative analysis of changes in PDGFR $\alpha$  expression for CLO+TBI and PBS+TBI groups.



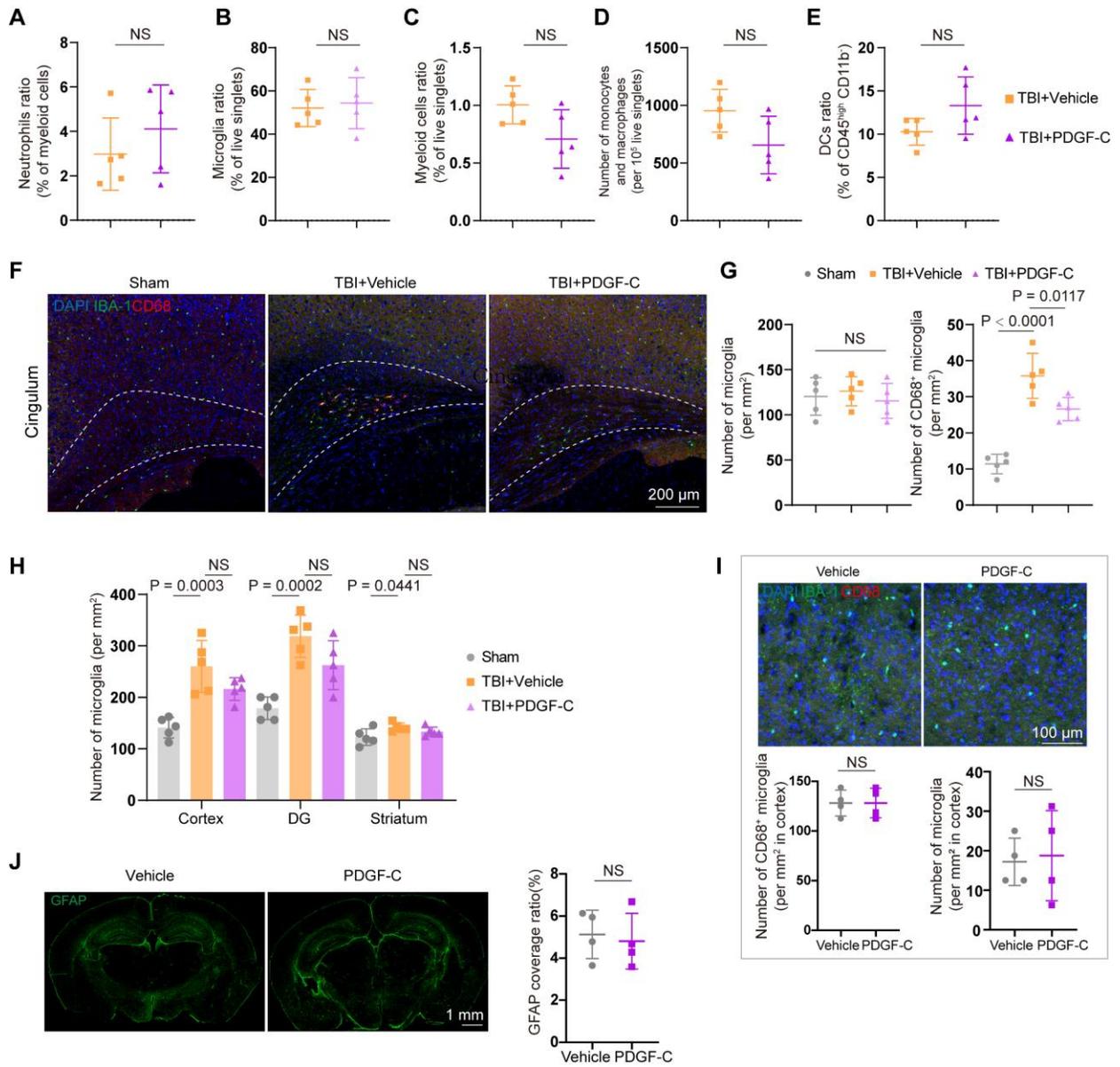
**Figure S5 Effects of meningeal macrophage depletion on intracerebral inflammation. Related to Figure 4.** (A) Flow cytometry gating strategy for immune cells in the brain. (B-J) Quantification of immune cells in PBS+TBI and CLO+TBI groups. (K, L) Quantification of microglia in the cortex and striatum in the sham, PBS+TBI, and CLO+TBI groups. (M-O) Immunofluorescence images and quantification showing that subdural PBS or CLO injection does not alter cortical microglia or CD68<sup>+</sup> microglia in healthy mice (n = 4). (P, Q) Immunofluorescence images and quantification showing that subdural PBS or CLO injection does not alter GFAP coverage (n = 4).



**Figure S6 Subdural PDGF-C injection does not alter meningeal lymphatic function in healthy mice. Related to Figure 5.** (A) Violin plots show *Pdgfa* expression across cell clusters in the meninges. (B) *Pdgfa/Pdgfra* signaling network from other cells to fibroblasts. (C, D) Immunofluorescence showing mLVs (LYVE-1), Beads, macrophages (CD206), and VEGF-C<sup>+</sup> cells in the meninges of uninjured mice after subdural injection of Vehicle or PDGF-C and quantification of bead coverage, LYVE-1 coverage, CD206<sup>+</sup> macrophages, and VEGF-C<sup>+</sup> cell numbers in Vehicle and PDGF-C groups (n = 4). (E, F) Representative images and quantification of fluorescent beads accumulation in dCLNs (n = 7 for Vehicle group, and n= 6 for PDGF-C group). (G) Histogram showing cell viability at different concentrations of PDGF-C. (H) Representative immunofluorescence showing changes in PDGFR $\alpha$  and VEGF-C expression after subdural injection of AAV9-Control and AAV9-mCOL1A2-PDGFR $\alpha$ .



**Figure S7 PDGF-C promotes meningeal lymphatic recovery and alleviates neuroinflammation after traumatic brain injury. Related to Figure 6.** (A-E) Flow cytometry histograms and quantitative analysis of changes in brain myeloid cells for TBI + Vehicle and TBI+PDGF-C groups. (F, G) Representative images and quantification of activated microglia (CD68<sup>+</sup>IBA-1<sup>+</sup>) in the cingulum (n = 5). (H) Quantification of microglia in the cortex, dentate gyrus of hippocampus and striatum in sham, TBI + Vehicle, and TBI + PDGF-C groups (n = 5). (I) Immunofluorescence images and quantification showing that subdural PDGF-C injection does not alter cortical microglia or CD68<sup>+</sup> microglia in healthy mice (n = 4). (J) Immunofluorescence images and quantification showing that subdural PDGF-C injection does not alter GFAP coverage (n = 4).



**Table S1. Gene sets from GO functional categories used for AUCCell analysis**

| Functional gene sets         | Genes  |
|------------------------------|--|
| GO: lymphangiogenesis        | Vegfc, Ccbe1, Epha2, Foxc1, Ngp, Vegfa   |
| GO: wound healing            | Adipor2, Alox5, Cxcr4, Foxc2, Gata2, Hpse, Kdr, Ndnf, Serpine1, Slc12a2, Smoc2, Tafa5, Tnf, Vegfa, Vegfb, Xbp1   |
| GO: growth factor production | Ab11, Abl2, Adtrp, Aebp1, Agt, Angptl7, Antxr1, Axin2, Bmp2, Carmil2, Cflar, Chadl, Clasp1, Clasp2, Col6a1, Colgalt1, Cpb2, Cst3, Cyp2j6, Dag1, Ddr1, Ddr2, Dpp4, Efemp2, Emilin1, Ets1, Fap, Fgfr4, Fscn1, Has2, Ier3ip1, Il6, Itgb3, Lama1, Lama2, Lamb1, Lamb2, Lamc1, Lemd3, Mad2l2, Melf, Nid1, Notch1, Pdpn, Phldb1, Phldb2, Pparg, Prdm5, Rb1, Reck, Rgcc, Ric1, Sema5a, Slc2a10, Smad3, Smad4, Sox9, Tcf15, Tgfb1, Tgfb2, Tgfb1, Tgfb3, Tiel, Tnfrsf1a, Tnfrsf1b, Tnxb, Zfp469   |
| GO: fibroblast proliferation | Abcc9, Abl1, Ager, Agt, Agr2, Anxa2, Aqp1, B4galt7, BC004004, Bax, Bmi1, Bmyc, Brk1, Brpf1, Btc, C1ql4, Cav1, Ccna2, Ccnb1, Cd248, Cd300a, Cd74, Cdc6, Cdc73, Cdk1, Cdk4, Cdk6, Cdkn1a, Cks1b, Cks2, Col3a1, Creb1, Cripto, Ctc1, Dab2ip, Dach1, Dazap1, Ddr2, Dhx9, Dicer1, Dph1, E2f1, E2f8, Ecd, Ednra, Egf, Egfr, Emd, Ereg, Esr1, Fam114a1, Fbln1, Fbrs, Fbxo4, Fgf10, Fn1, Fn3, Fndc3b, Fntb, Fosl2, Fth1, Gas6, Gng2, Gpx1, Grk2, Gstp1, Hmga2, Hras, Icmt, Ifi30, Ifng, Igf1, Il13, Inca1, Ing5, Itgb3, Jun, Kat2a, Kenj8, Kenn4, Kdm8, Kmt2a, Kmt2c, Lif, Lig4, Lta, Lzts2, Med25, Med31, Men1, Meox2, Mif, Mir744, Mmp9, Morc3, Morf4l1, Myb, Myc, Nbn, Ndufs4, Nf1, Ngfr, Nlrc3, Nras, Nupr1, Parp10, Pawr, Pdgfa, Pdgfb, Pdgfc, Pdgfd, Pdgfra, Pdgfrb, Pes1, Pex2, Phip, Pla2g1b, Pmaip1, Pml, Pparg, Prdx1, Prkdc, Ptges3, Ptpv, Ptpz1, Rasal, Rasgrf1, Rnaseh2b, Rpl29, Rrn3, Serpine1, Sfrp1, Sirt6, Ski, Smarca2, Socs1, Sod2, Sp2, Sphk1, Tgfb1, Tgif1, Trim32, Trp53, Trp53inp1, Tsc2, Uts2, Uts2r, Vegfd, Wnt1, Wnt2, Wnt5a, Xrec4, Zfp469, Zmiz1, Zmpste24 |
| GO: fibroblast migration     | 2610005L07Rik, Acta2, Actr3, Adipor2, Ager, Akap12, Akt1, Apc, App11, App12, Aqp1, Arhgap4, Arid5b, Bag4, Braf, Ccn3, Cd248, Clasp2, Cln3, Coro1c, Cripto, Cygb, Ddr2, Dmtn, Fam114a1, Fer, Fgf2, Fgfr1, Fndc3b, Fut8, Gna12, Gna13, Has1, Hyal2, Ilk, Iqgap1, Itga11, Itgb1, Itgb1bp1, Itgb3, Lamtor2, Macir, Mmp1a, Mta2, Nherf1, Pak1, Pak3, Pdgfb, Pdlim1, Pip5k1a, Plec, Pml, Pmp22, Prkce, Prr51, Ptk2, Rac1, Rcc2, Rffl, Schip1, Sdc4, Sgpl1, Slc8a1, Spag6l, Tfap2a, Tgfb1, Thbs1, Tmem201, Tns1, Tsc2, Uts2, Wdpcp, Zeb2, Zfand5, Zfp640  |
| GO: ecm organization         | Aif1, Ccm2l, Vegfc, Cd59a, Fgfr1, Fgfr4, Heg1, Ptgs2, Rgcc, Rock2, Stat5b, Wnt11   |

**Table S2. The primer sequences used**

| Primer  | Sequence                        |
|---------|---------------------------------|
| Vegfc-F | 5' TGTGGGGAAGGAGTTTGGAG 3'      |
| Vegfc-R | 5' CAGTTACGGTCTGTGTCCAGTGTAG 3' |
| Gapdh-F | 5' CCTTCCGTGTTCCCTACCCC 3'      |
| Gapdh-R | 5' GATGCTGGTGCTGAGTATGRCG 3'    |