
Figures and figure supplements

Neural tube-associated boundary caps are a major source of mural cells in the skin

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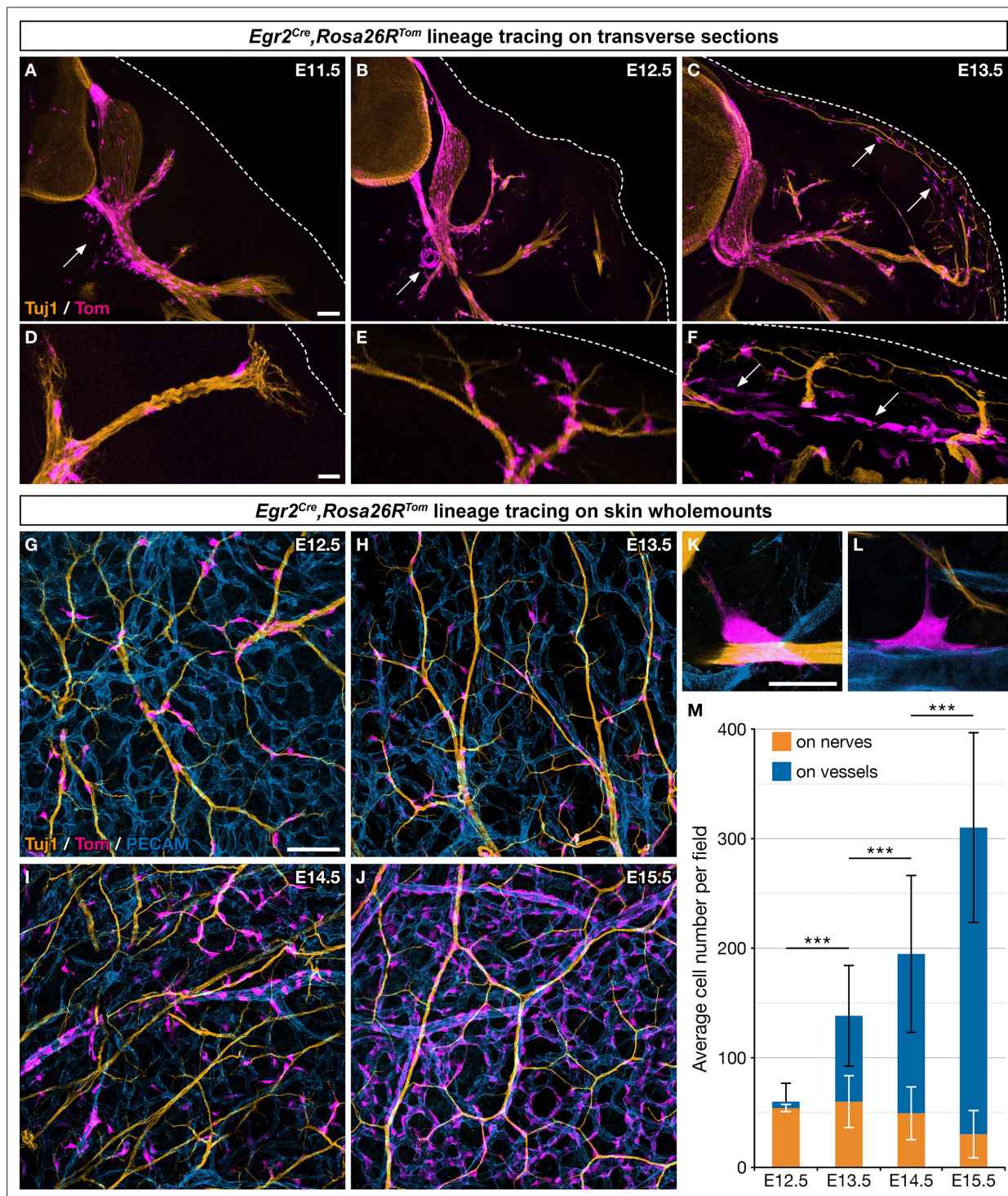


Figure 1. Boundary cap (BC) cell derivatives migrate along the nerves and incorporate into the vascular plexus. (A–F) Trunk transverse sections from *Egr2^{Cre/+}, Rosa26R^{Tom}* embryos at the indicated stages, and labeled with antibodies against Tomato (magenta) and Tuj1 (orange). Cells detached from the nerves (arrows) appear first in the vicinity of the ventral roots (A,B) and later close to the skin, as indicated by the dotted line (C,F). (G–J) Whole-mount dorsal skin from *Egr2^{Cre/+}, Rosa26R^{Tom}* embryos at the indicated stages, labeled with antibodies against Tomato (magenta), Tuj1 (orange), and PECAM (blue). (K,L) Higher magnifications showing cells in contact with both nerve and vessel at embryonic day (E) 13.5. (M) Quantification of the number of labeled cells associated with nerve or vessel per field at the indicated stages (n=3 embryos per stage). Statistical analyses of the “on nerve”/“on vessels” ratio between time points were carried out using a Mann-Whitney U test. Scale bars, 100 μ m (A–C,G–J) and 20 μ m (D–F,K–L). Error bars, one standard deviation (M). ***=p < 0.001.

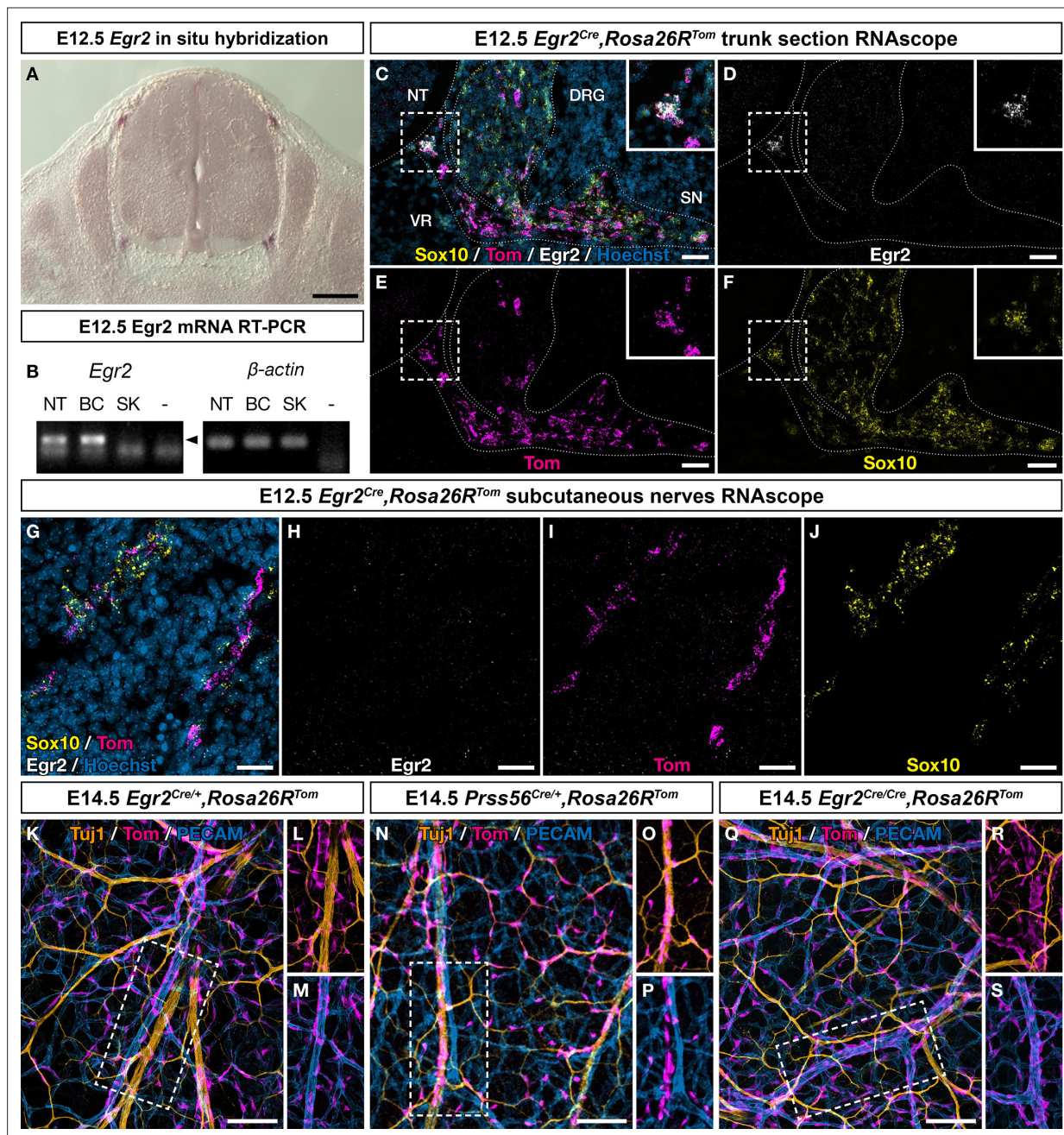


Figure 1—figure supplement 1. Traced cells, apart from boundary cap (BC) cells themselves, do not express *Egr2* at embryonic day (E) 12.5 and behave differently from *Prss56*-positive BC cell derivatives. (A) *Egr2* in situ hybridization on an E12.5 embryo section. BC cells at dorsal entry and ventral exit points express *Egr2*. In contrast, *Egr2* mRNA is not detected along the nerves or in the skin. (B) RT-PCR for *Egr2* mRNA performed on RNA extracted from dissociated E12.5 dorsal skin (SK), as compared to neural tube (NT) and BCs, used as positive controls. The arrowhead indicates the position of the predicted band corresponding to *Egr2* amplification. Trunk transverse (C–F) and embryonic skin (G–J) sections from *Egr2*^{Cre/+}, *Rosa26R*^{Tom} embryos at E12.5 were analyzed by RNAscope in situ hybridization for *Tomato* (magenta), *Sox10* (yellow), and *Egr2* (white), confirmed that *Egr2* mRNA was restricted to BC. (D) Whole-mount E14.5 embryonic dorsal skin from *Egr2*^{Cre/+}, *Rosa26R*^{Tom} (K–M), *Prss56*^{Cre/+}, *Rosa26R*^{Tom} (N–P), and *Egr2*^{Cre/Cre}, *Rosa26R*^{Tom} (Q–S) embryos, immunostained for Tuj1 (yellow), *Tomato* (purple), and PECAM (blue). In contrast to *Egr2*-traced derivatives that mostly reside on the vascular plexus (K–M), all *Prss56*-traced cells are attached to Tuj1-positive nerves and not to PECAM-positive blood vessels (N–P). *Egr2* inactivation does not affect the association of *Egr2*-traced derivatives to the vascular plexus (Q–S). Scale bars, 200 μm (A) and 50 μm (C–S).

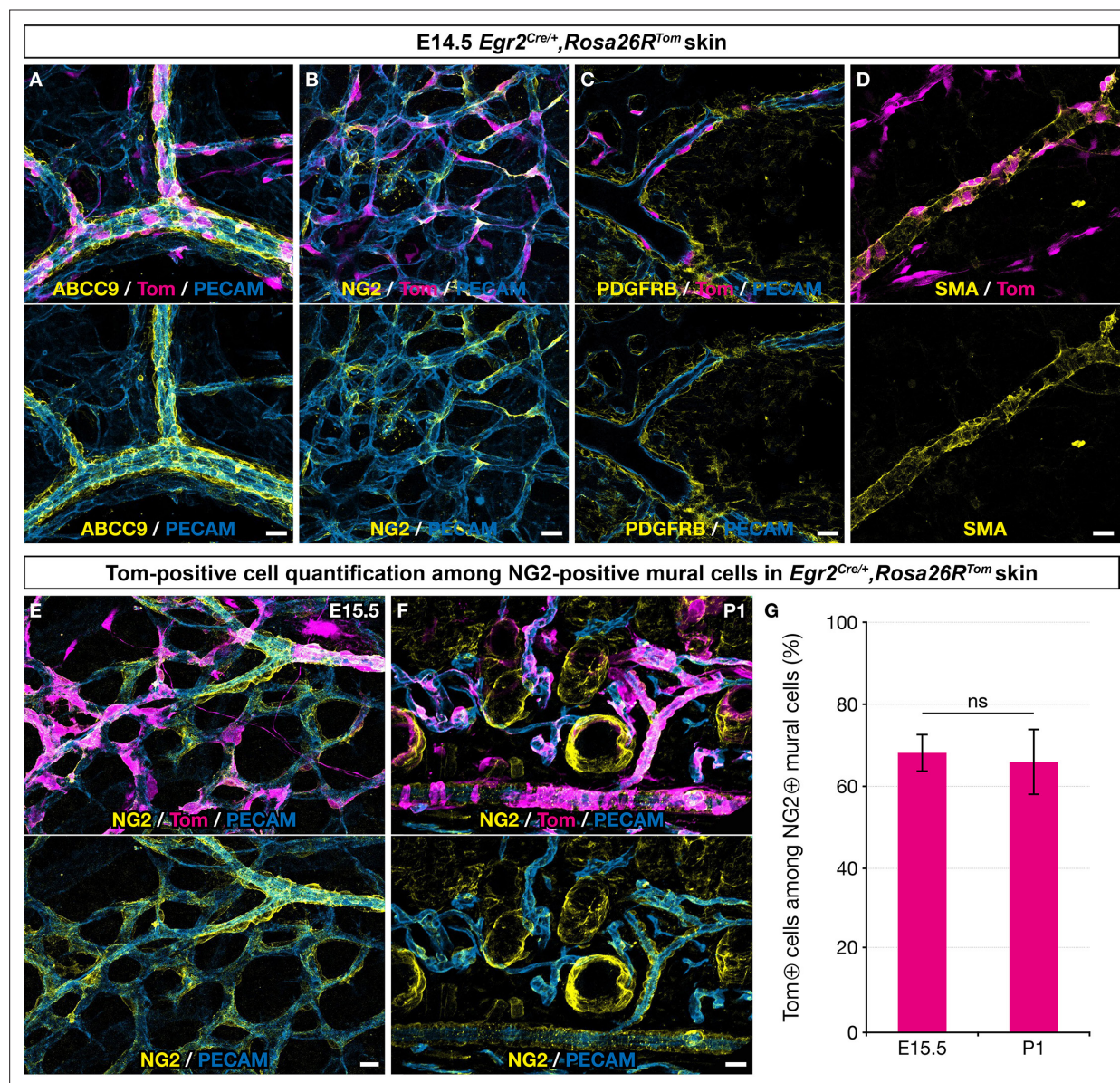


Figure 2. Boundary cap (BC) cell derivatives give rise to the majority of skin mural cells (MCs). Whole-mount embryonic dorsal skin at the indicated stages (A–E) or transverse section of newborn skin (F), labeled with antibodies against Tomato (magenta), PECAM (blue), and ABCC9, NG2, PDGFR β , or smooth muscle actin (SMA) (yellow). (A–D) In the vascular plexus, Tomato-positive cells express MC markers: ABCC9 (A), NG2 (B), PDGFR β (C), and SMA (D). (E–G) Tomato-positive cells account for approximately two thirds of NG2-positive MCs in the skin at embryonic day (E) 15.5 (E,G; n=3) and postnatal day (P) 1 (F,G; n=3). Tomato labeling is omitted in the lower images (A–F). Statistical analysis of the “NG2 and Tomato-positive”/“NG2-positive” ratio between time points was carried out using a Mann-Whitney U test. Scale bars, 20 μ m. ns, non-significant (G).

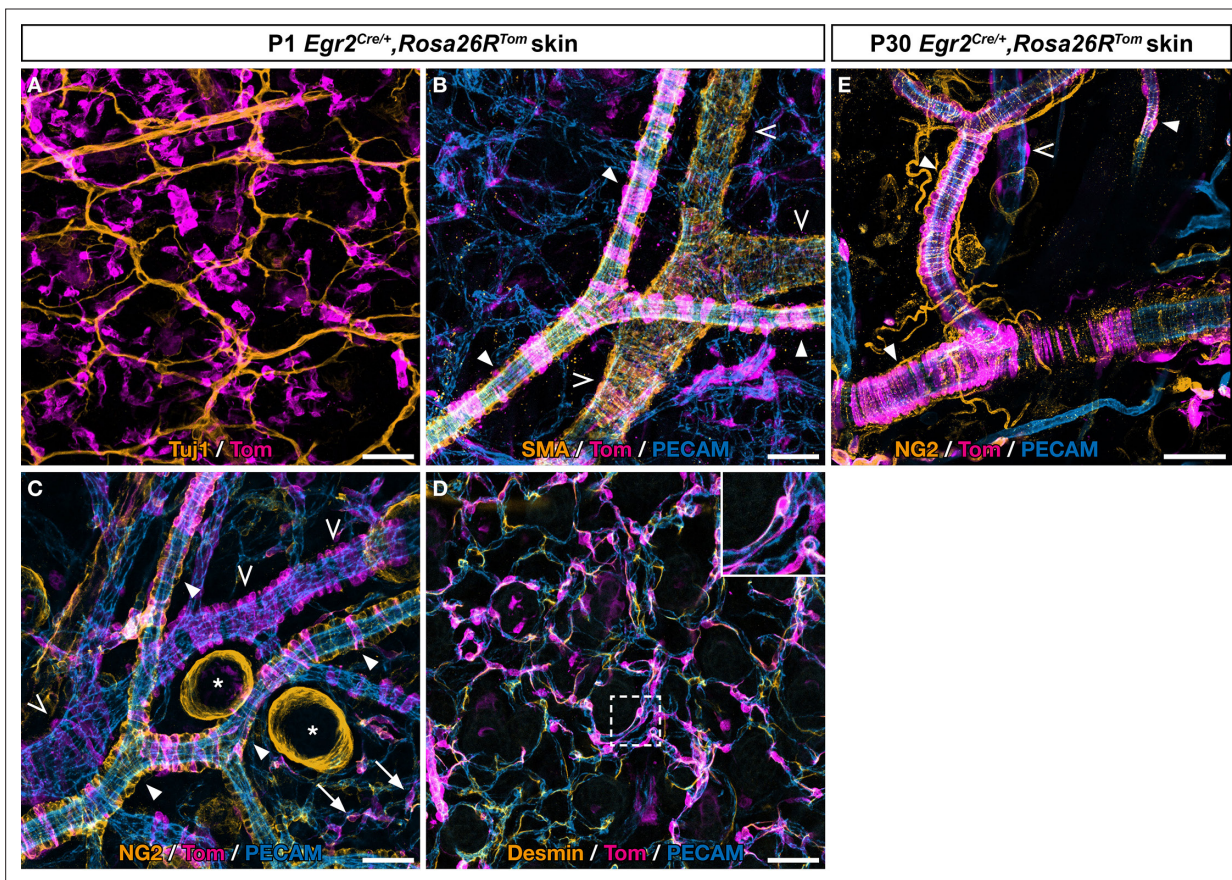


Figure 2—figure supplement 1. Boundary cap (BC) cells give rise to skin mural cells (MCs), including pericytes, arterial and venous vascular smooth muscle cells (VSMCs). *Egr2^{Cre/+}; Rosa26R^{Tom}* newborn (A–D) and postnatal day (P) 30 (E) dorsal skin was analyzed by whole-mount immunostaining with various antibodies (see color code on each picture). (A) In newborn animals (P1), most Tom-positive cells are not associated with Tuj1-positive nerves. (B–C) On P1 venules (empty arrowheads) and arterioles (arrowheads), which differ by diameter and shape, traced cells express smooth muscle actin (SMA), whereas they only express NG2 when associated with arterioles. Labeled cells also include capillary NG2-positive pericytes (arrows in C). (D) P1 traced capillary pericytes also express Desmin. (E) Numerous Tom-positive MCs expressing NG2 are visible in the adult skin on arterioles (arrowheads) and venules (empty arrowhead). Please note the presence of NG2-positive hair follicles (asterisks in C). Scale bars, 50 μm.

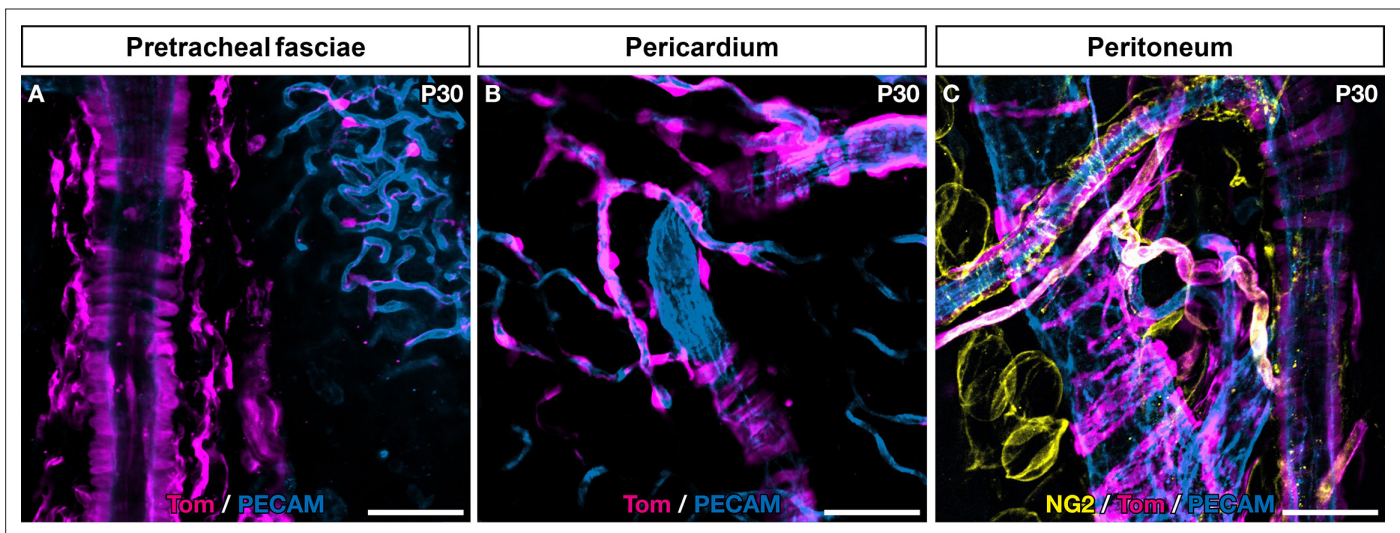


Figure 2—figure supplement 2. Numerous traced mural cells (MCs) are present in serous membranes from various organs. Serous membranes from *Egr2^{Cre/+};Rosa26^{Tom}* adult mice (A–C) were analyzed by whole-mount immunostaining with antibodies directed against Tomato (magenta), PECAM (blue), and NG2 (yellow). Numerous tomato-positive, BC-derived MCs were visible on arteries, veins, and capillaries, interspaced with non-traced cells. Scale bars, 50 μm.

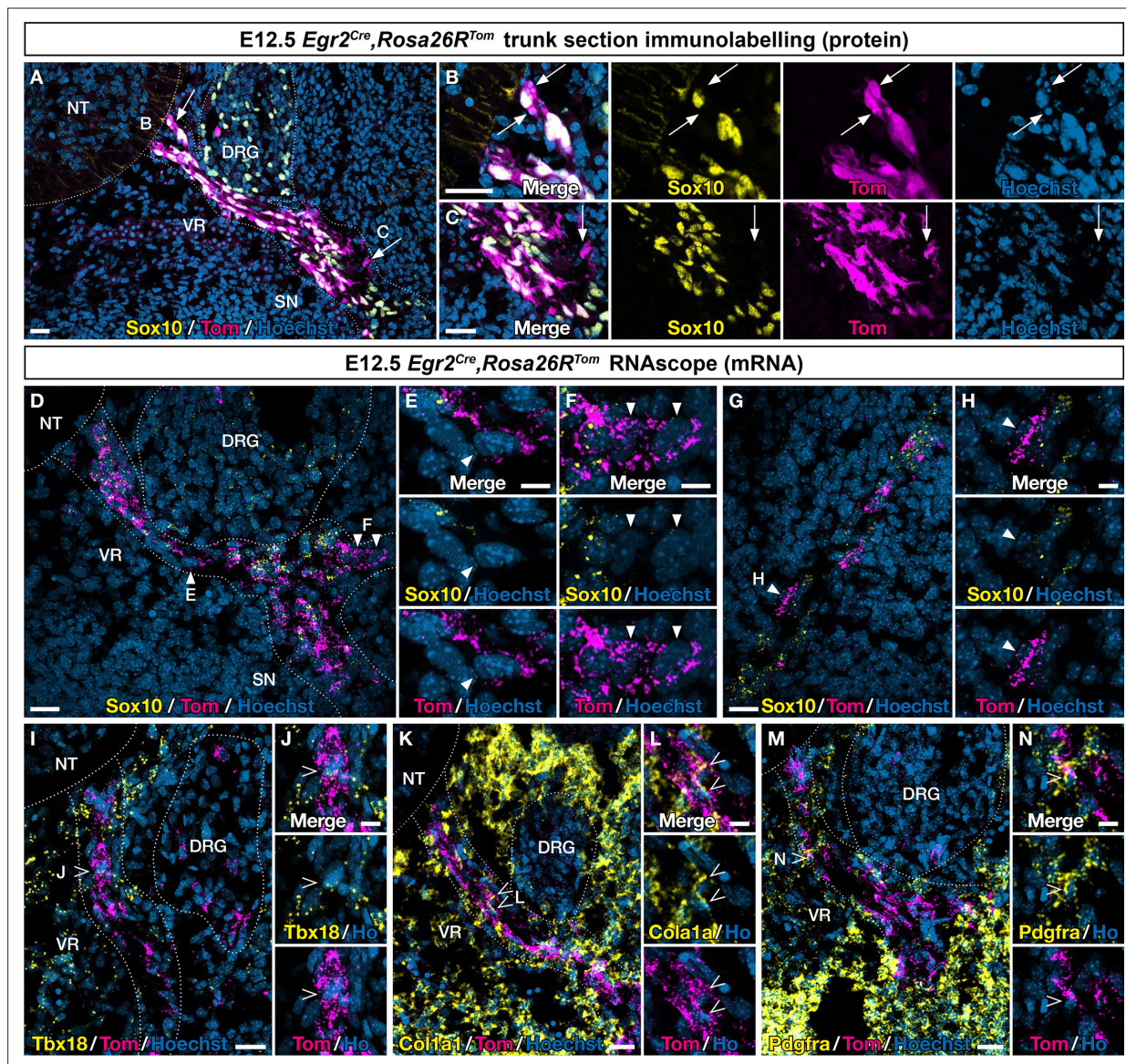


Figure 3. An *Egr2*-expressing boundary cap (BC) cell subpopulation displays mesenchymal identity before migrating to the periphery. Trunk transverse sections (A–F, I–N) and skin sections (G,H) from *Egr2^{Cre/+},Rosa26R^{Tom}* embryos at embryonic day (E) 12.5. Sections were labeled with antibodies (A–C) against Tomato (magenta) and Sox10 (yellow), or analyzed by RNAscope in situ hybridization (D–H) for Tomato (magenta) and Sox10 (yellow). Detailed analysis revealed that some Tomato-positive cells on ventral roots did not express Sox10 at the protein (arrows) and RNA level (arrowheads). RNAscope in situ hybridization of *Tbx18* (I,J), *Col1a1* (K,L), and *Pdgfra* (M,N) expression in ventral roots from E12.5 embryos. Note that some tomato-positive cells on ventral roots express these markers (empty arrowheads). Scale bars, 25 µm (A,D,G,I,K,M) or 10 µm (B,C,E,F,H,J,L,N).

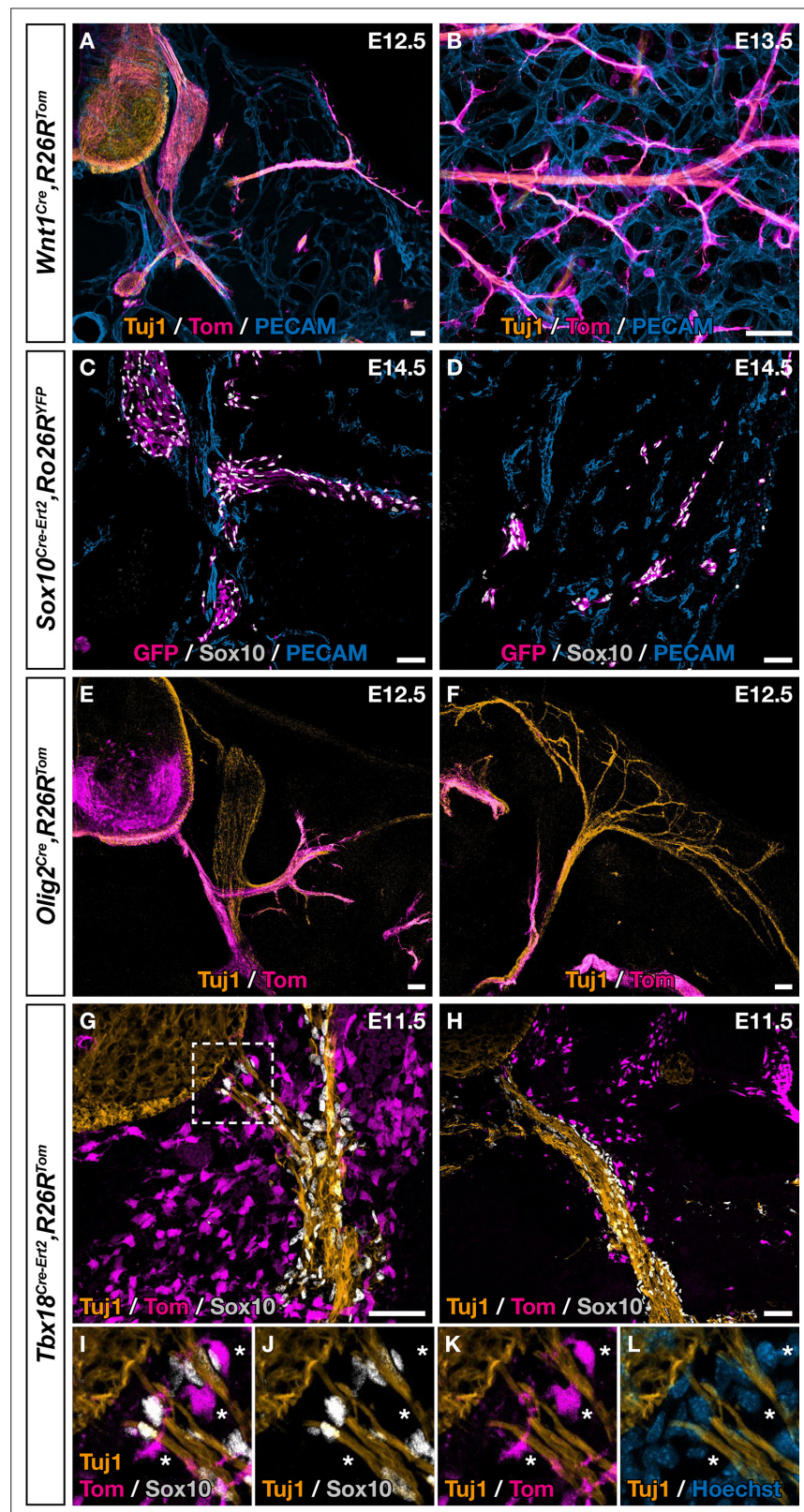


Figure 4. Ventral boundary caps (BCs) have a dual embryonic origin. Trunk transverse sections (A,C,E–L), dorsal skin whole-mount (B), and skin section (D) at the indicated embryonic stages. Genetic fate mapping of neural crest (NC) using *Wnt1*^{Cre/+},*Rosa26R*^{Tom} (A,B) and *Sox10*^{Cre-Ert2/+},*Rosa26R*^{Tom} (C,D) embryos and of neuroepithelial cells and their derivatives using *Olig2*^{Cre/+},*Rosa26R*^{Tom} embryos (E,F) did not reveal any tomato-positive cells attached to the

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vascular plexus, neither close to ventral roots nor to nerves in the skin. (**G,L**) Genetic fate mapping of developing pia matter using *Tbx18*^{Cre-Ert2/+}, *Rosa26*^{Tom} embryos revealed the presence of numerous tomato-positive, Sox10-negative cells in close contact with the nerve (asterisks, **I-L**). Tamoxifen was delivered to pregnant females at embryonic day (E) 9.5 (**C,D,G-L**). Scale bars, 50 μ m.

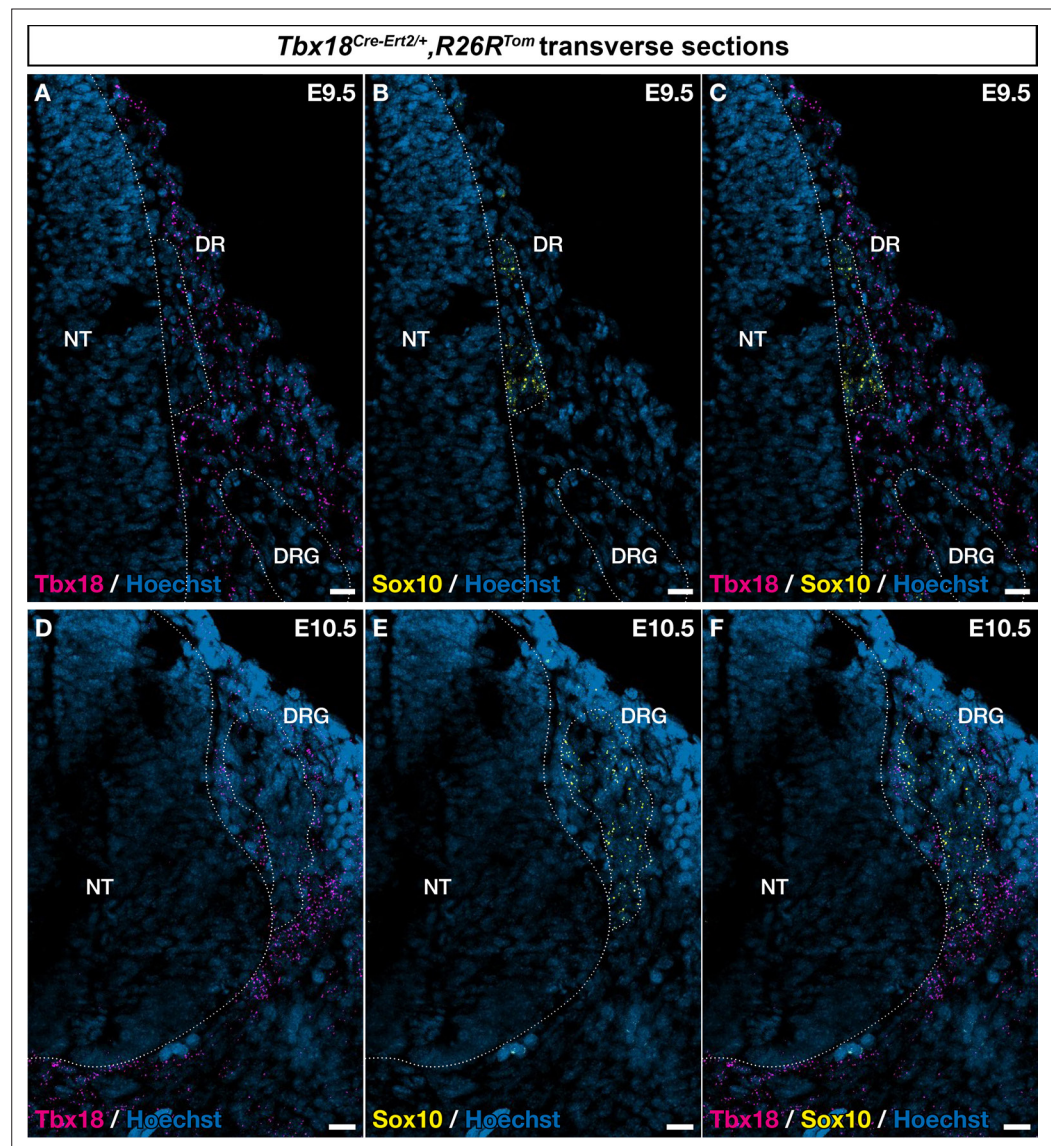


Figure 4—figure supplement 1. *Tbx18* is expressed in cells around the developing neural tube. Trunk transverse sections of embryos at embryonic day (E) 9.5 (A–C) and E10.5 (D–F) were analyzed by RNAscope in situ hybridization for *Tbx18* (magenta) and *Sox10* (yellow). *Tbx18*-expressing cells were observed around the neural tube (NT), dorsal roots (DR), and dorsal root ganglia (DRG), in the area that corresponds to the developing pia matter. Scale bars, 20 μm.

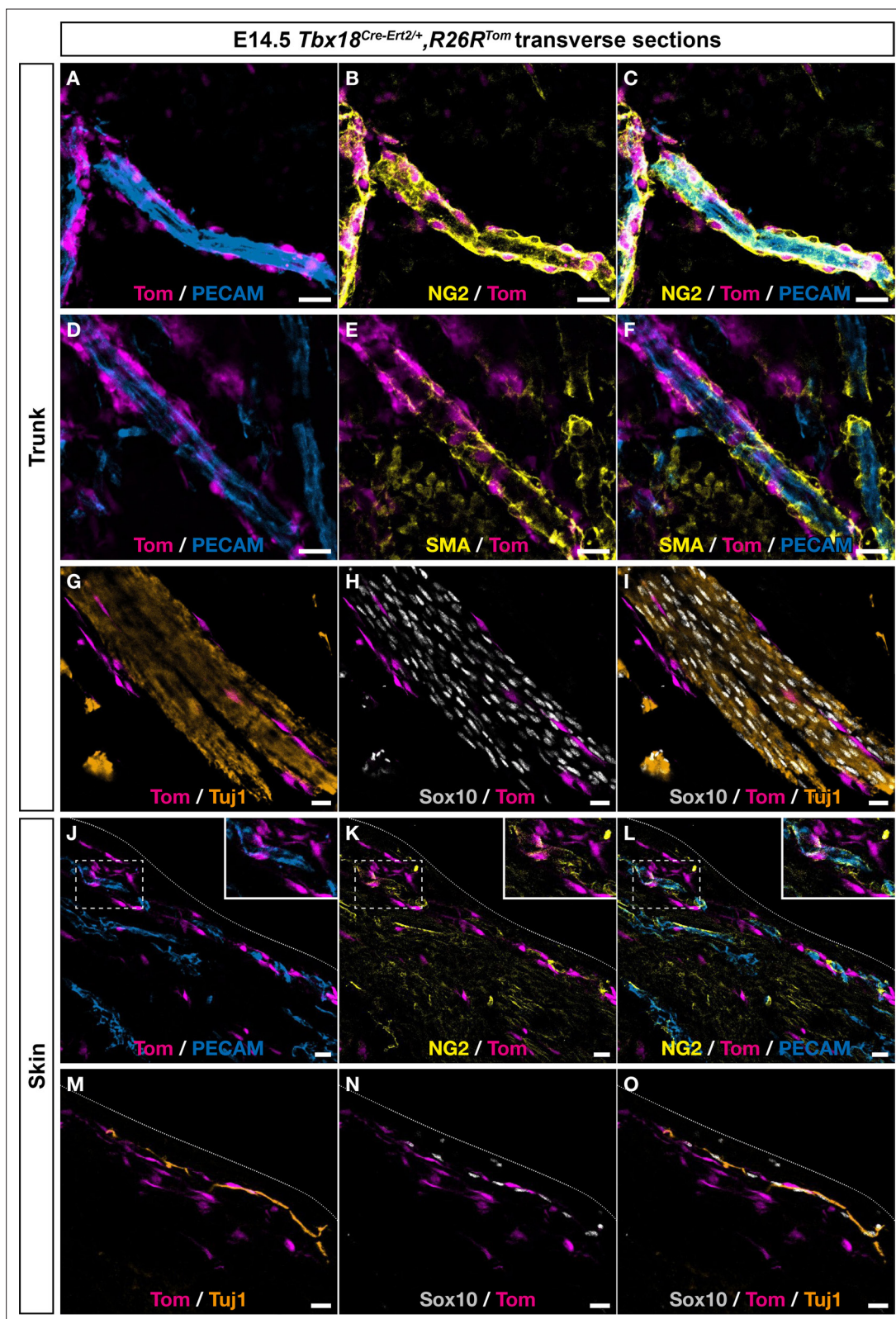


Figure 4—figure supplement 2. *Tbx18*-expressing cells contribute to mural cells (MCs) in the skin and around the neural tube. Immunostaining on trunk transverse sections (**A–I**) and skin section (**J–O**) from *Tbx18*^{Cre-Ert2/+}, *Rosa26R*^{Tom} embryos at embryonic day (E) 14.5, labeled with antibodies against tomato, PECAM, Tuj1, NG2, smooth muscle actin (SMA), or Sox10 (see color code on each picture). Numerous tomato-positive cells expressing MC markers NG2 and SMA were present along blood vessels near the neural tube and along and within spinal nerve (**A–I**). No tomato-positive cell

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expressed Sox10 (**G–I**). In the skin (**J–O**), tomato-positive cells expressed MC marker NG2 but remained negative for Sox10 (**J– O**). Tamoxifen was delivered to pregnant females at E9.5. Scale bars, 20 μ m.

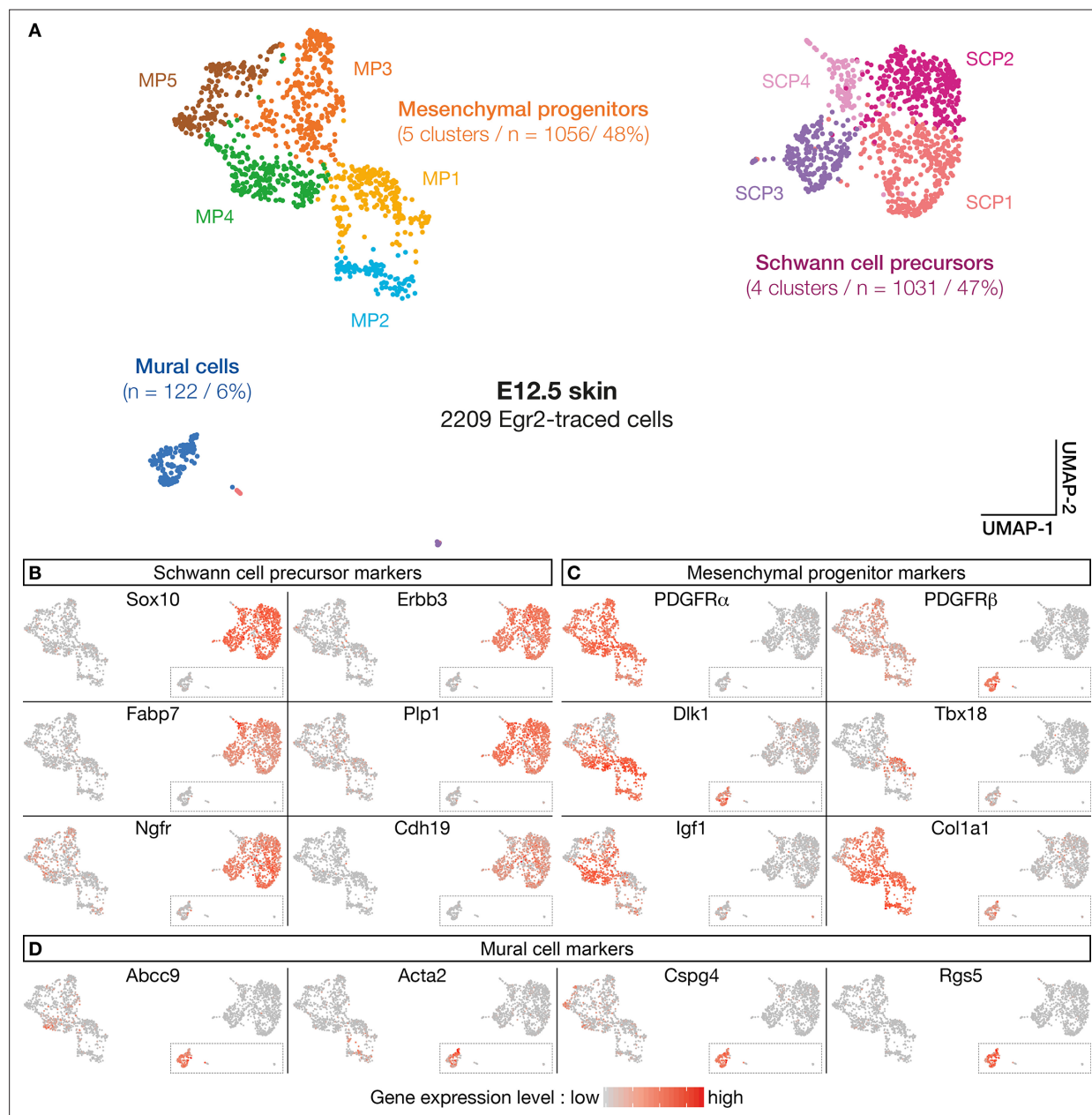


Figure 5. Numerous molecular features reveal diversity in cell identity in embryonic day (E) 12.5 boundary cap (BC) derivatives. **(A–D)** 10 clusters can be delineated on the basis of single-cell RNA sequencing (scRNA-seq) experiments performed on traced cells from E12.5 dorsal skin. They can be organized in three supra-clusters based on their molecular signatures: Schwann cell precursors, mesenchymal progenitors, and mural cells. In **(B–D)**, cells are color-coded, with color intensities (from gray to red) reflecting the relative levels of expression of each gene.

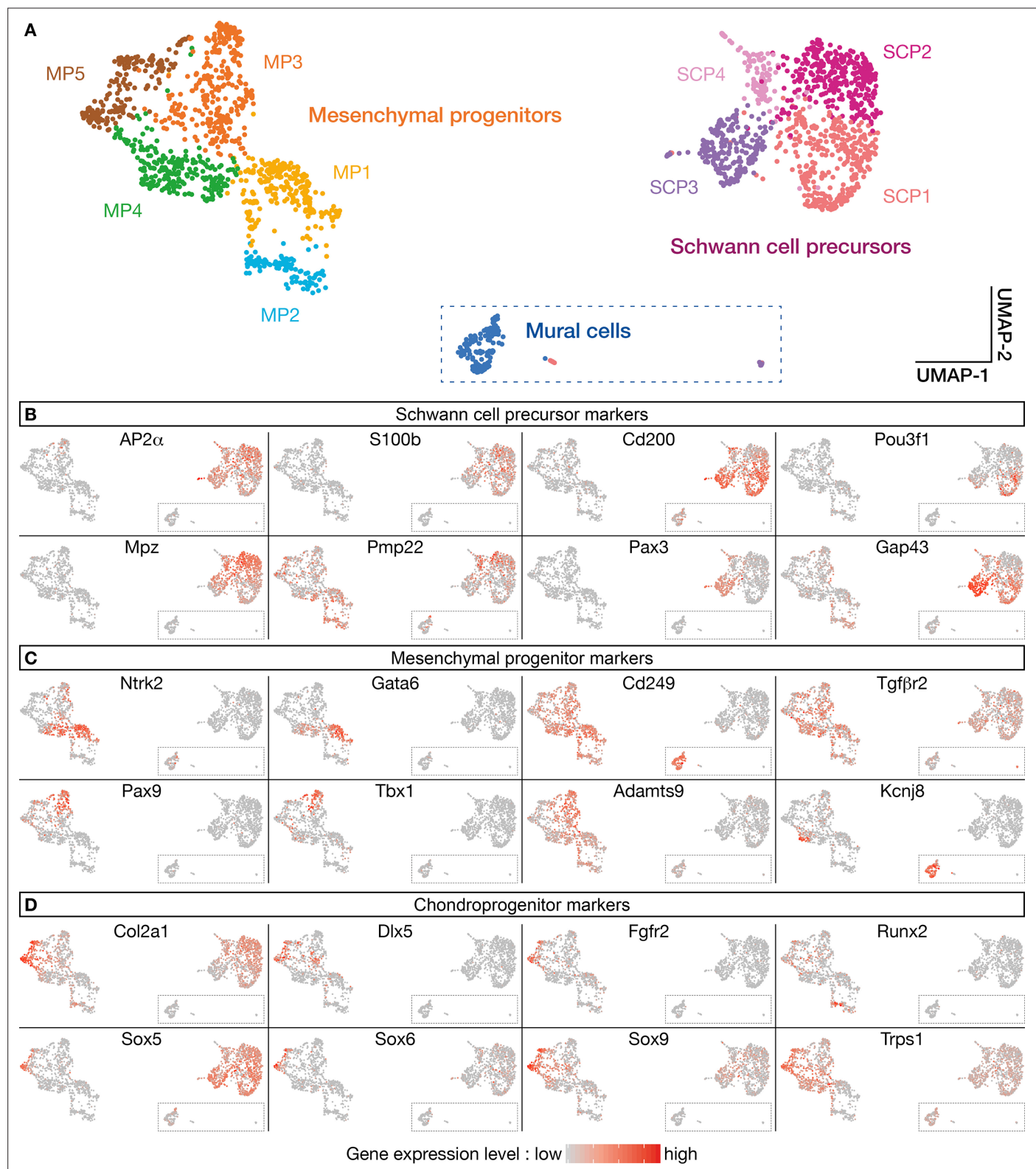


Figure 5—figure supplement 1. Heterogeneity of the SPC and mesenchymal progenitor (MP) supra-clusters. Analysis of the supra-clusters (A) derived from traced cell single-cell RNA sequencing (scRNA-seq) data with respect to some SPC (B), MP, (C) or chondroprogenitor (D) markers illustrates their heterogeneity. Cells are color-coded according to gene expression levels (gray = low, red = high).

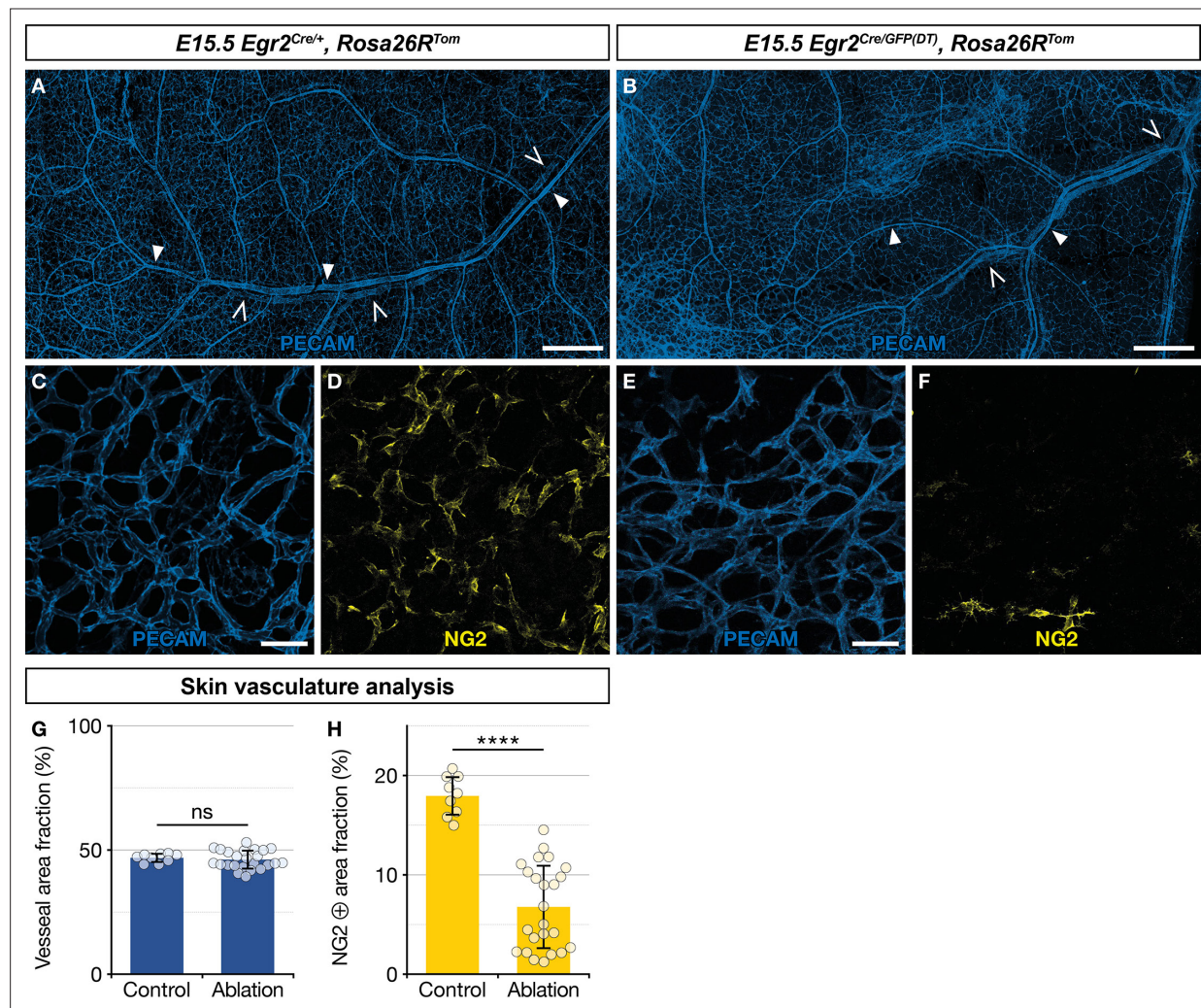


Figure 6. Ablation of *Egr2*-expressing boundary caps (BCs) impacts mural cell (MC) development in the skin. Whole-mount embryonic dorsal skin at embryonic day (E) 15.5, labeled with antibodies against PECAM (blue) and NG2 (yellow). Compared with littermate *Egr2*^{Cre/+}, *Rosa26R*^{Tom} control mice (A), vascular network development appeared normal at low magnification in mutant mice (B), in which BC cells and their derivatives were eliminated (arterioles and venules are indicated by full and empty arrowheads, respectively). Capillaries from mutant mice skin (E,F) showed a significant decrease in NG2-positive MCs as compared to controls (C,D). Vessel area fractions were similar in controls and mutants (G), but the NG2-positive area fraction was significantly decreased in the mutants (H), indicating a depletion of MCs. Statistical analyses of the PECAM and NG2-positive surface areas between controls (n=3) and mutants (n=4) were carried out using a Mann-Whitney U test. Scale bars, 500 μ m (A,B), 50 μ m (C–F). Error bars, one standard deviation (G,H). ****= $p < 0.0001$. ns, non-significant.

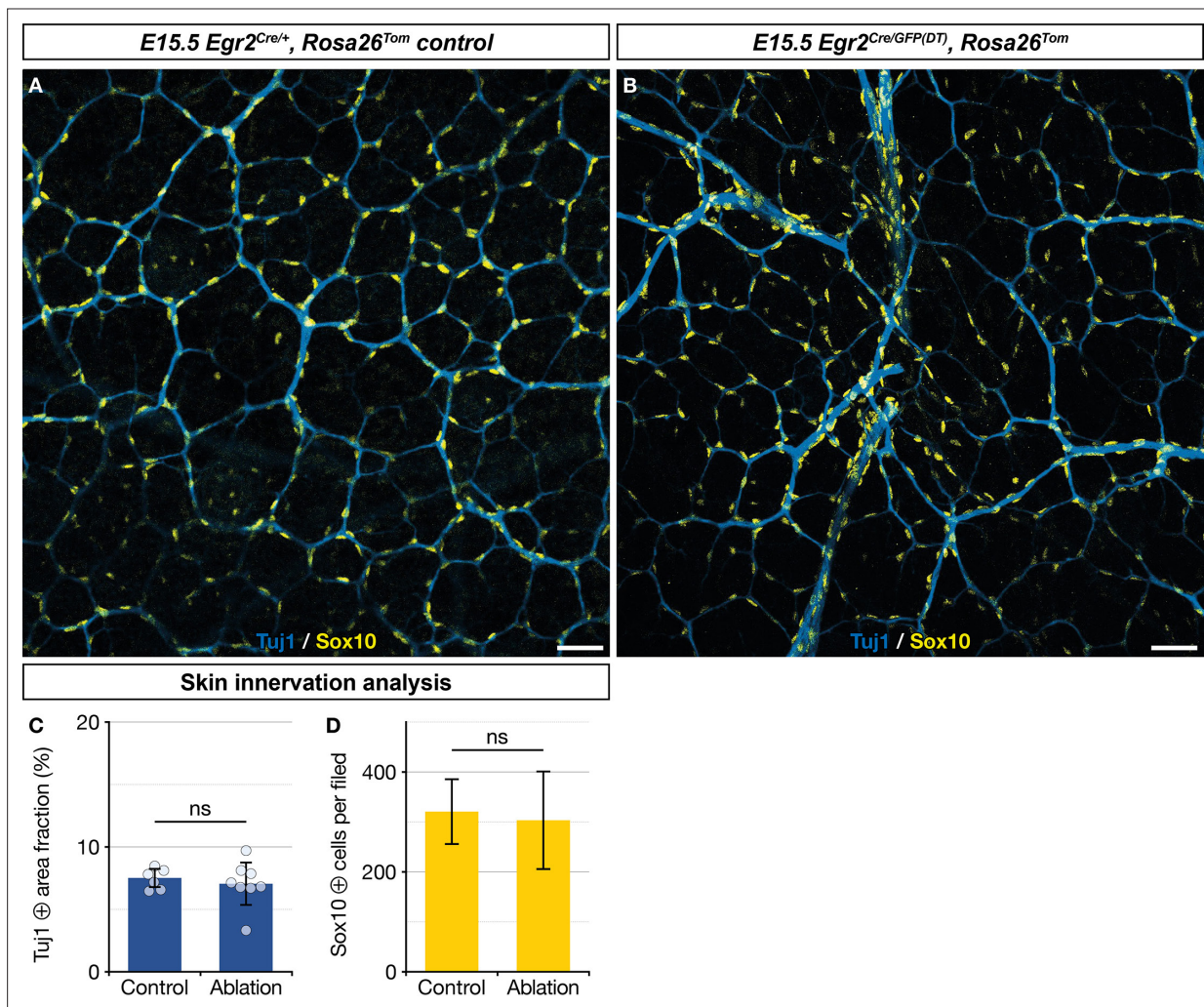


Figure 6—figure supplement 1. Ablation of *Egr2*-expressing boundary caps (BCs) does not affect normal skin innervation. Immunolabeling of whole-mount embryonic day (E) 15.5 embryonic dorsal skin with antibodies against Tuj1 (blue) and Sox10 (yellow). Compared with non-littermate *Egr2*^{Cre/+},*Rosa26*^{Tom} control mice (**A**), nerve network organization appeared normal in mutant mice (**B**). Nerve area fractions (**C**) and Sox10-positive cell counts (**D**) were similar in controls and mutant samples. Scale bars, 50 μ m (**A,B**). Statistical analyses of the Tuj1-positive surface areas and Sox10-positive cell counts between controls (n=2) and mutants (n=2) were carried out using a Mann-Whitney U test. Error bars, one standard deviation (**C,D**). ns, non-significant.