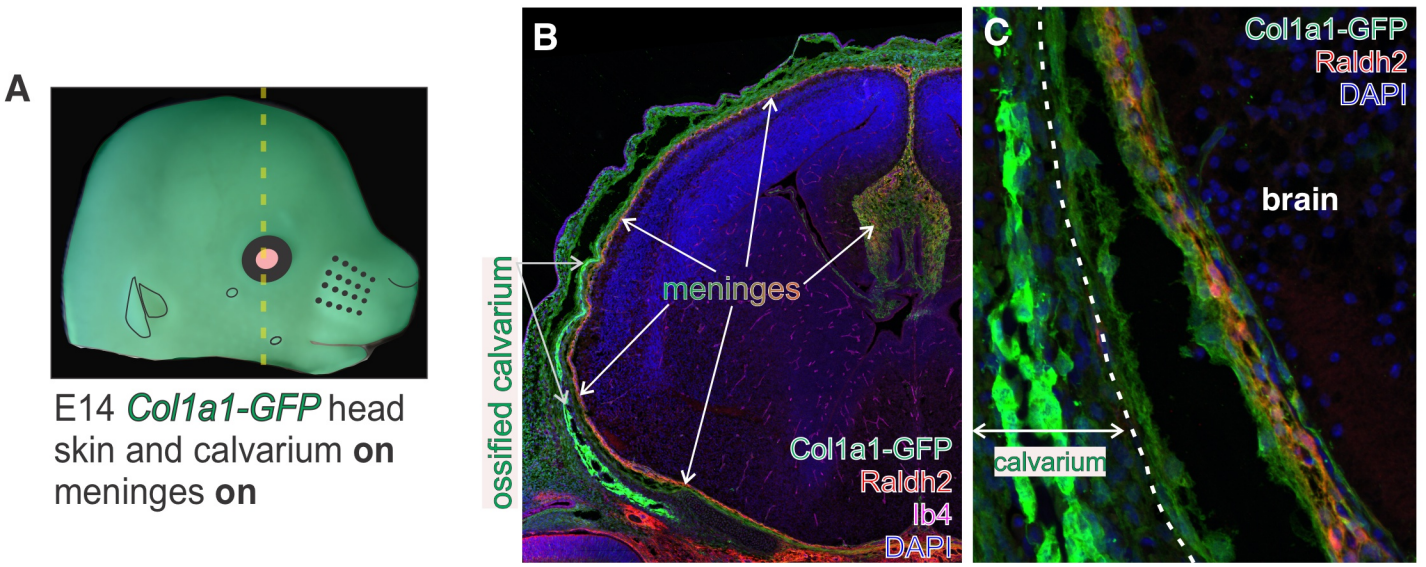
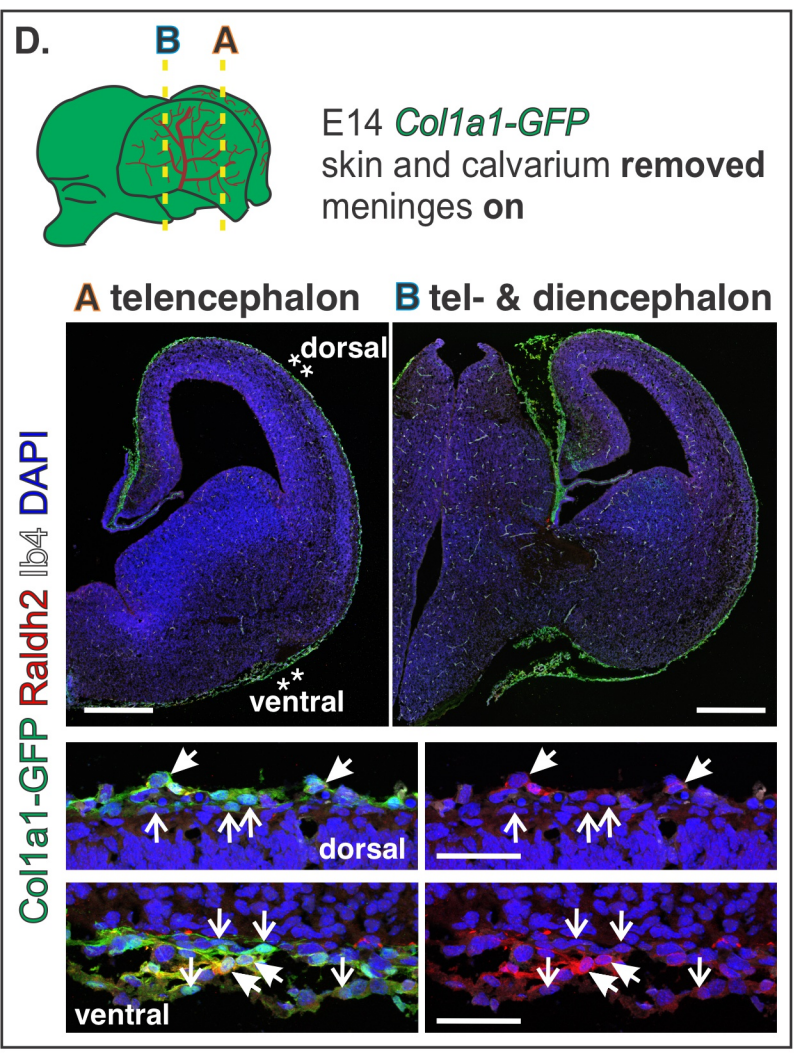
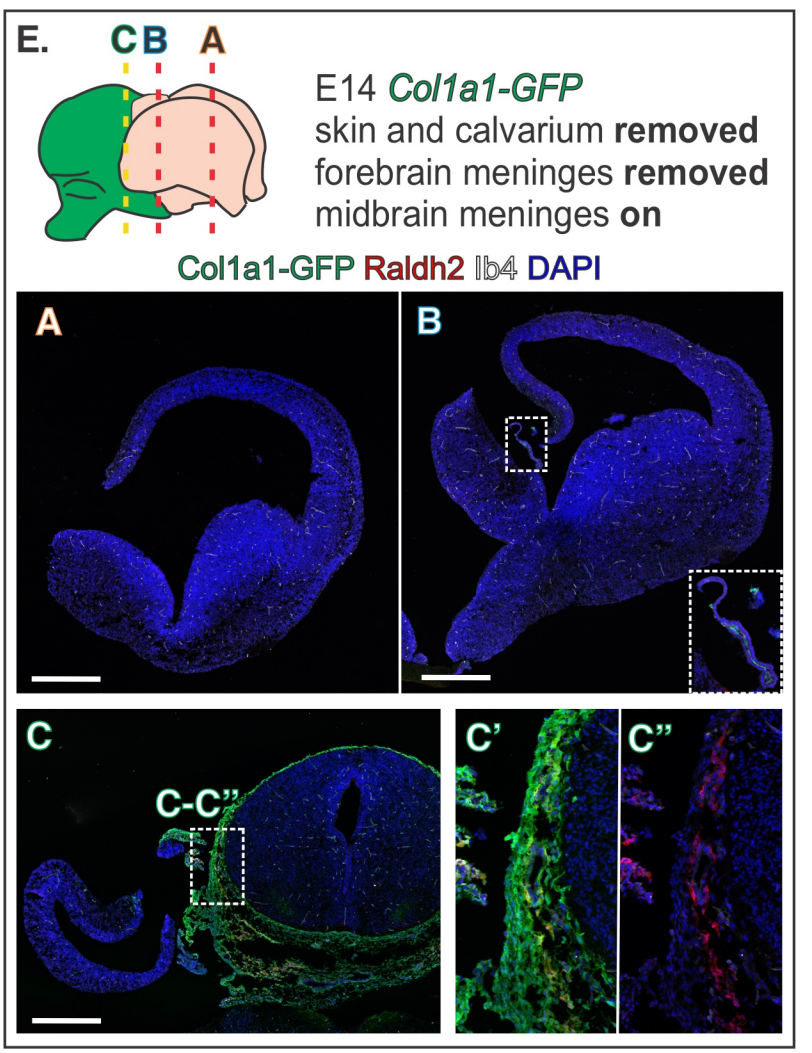






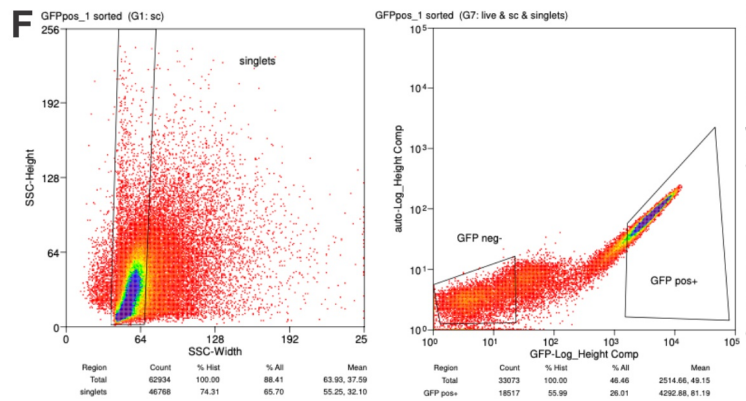
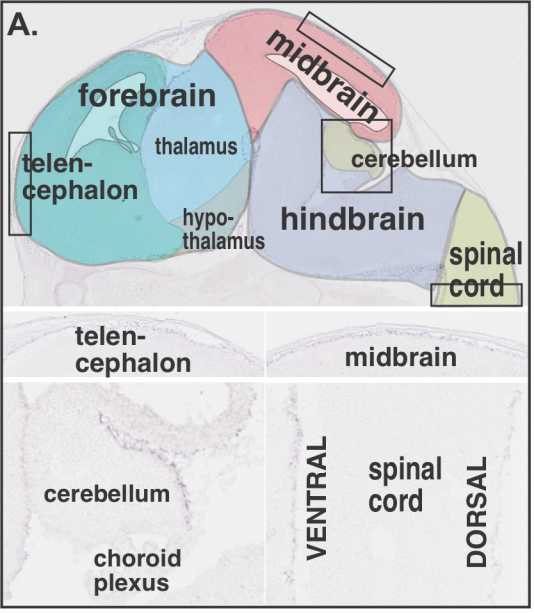
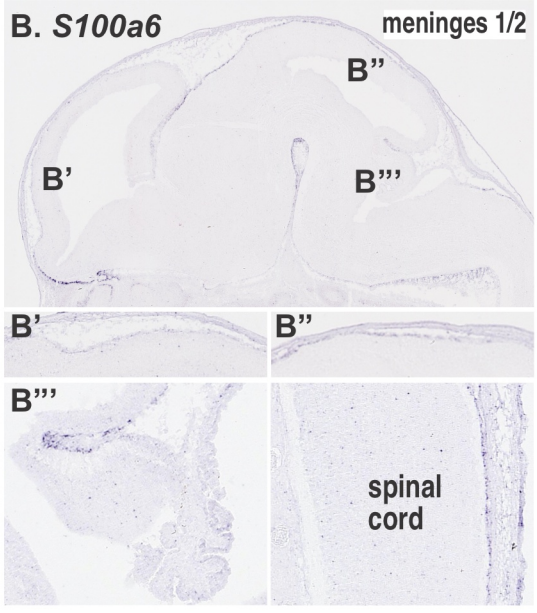
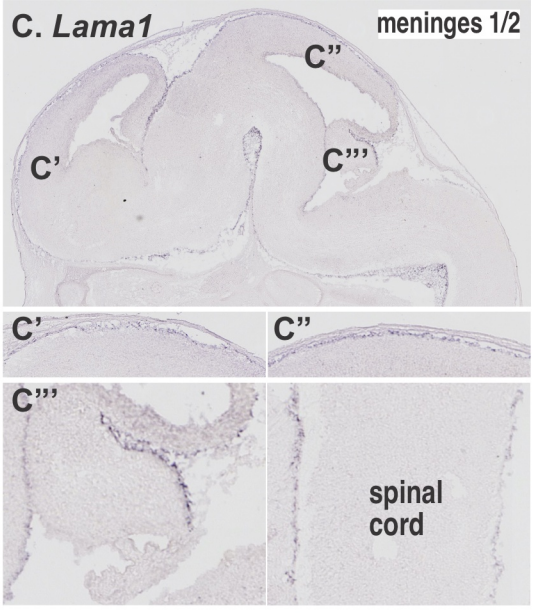


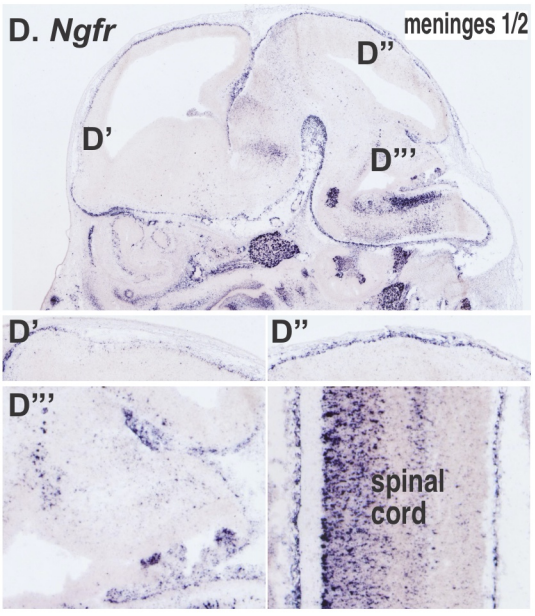
Figure S1 related to Figure 1. E14 embryo *Col1a1-GFP* meninges isolation validation

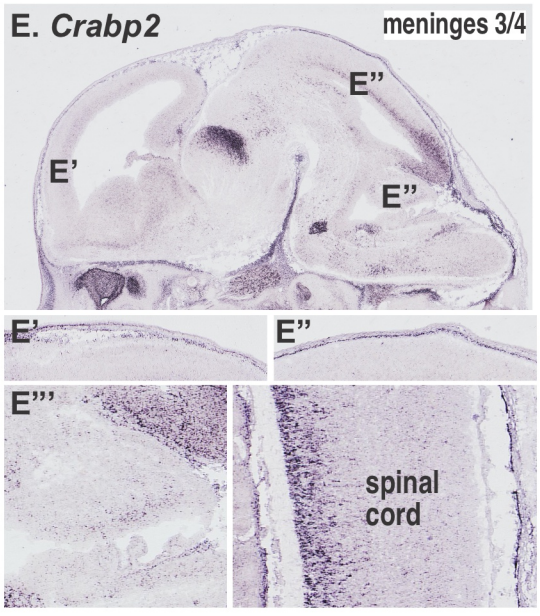
(A-C) Yellow dash line in A indicates area depicted in B, C. *Col1a1-GFP* with Raldh2 IF and Ib4 (vasculature) in B, arrows indicate meninges, a subset of which are Raldh2+ (C). GFP signal is detected in other meningeal layers and in calvarium. (D) Depiction of *Col1a1-GFP* brain removed from head. GFP+ meninges are observed around forebrain structures. High magnification of dorsal/ventral telencephalon show GFP+/Raldh2+ cells (closed arrows) between layers of GFP+/Raldh2- cells (open arrows), more prominent in ventral regions. (E) *Col1a1-GFP* brain with forebrain meninges removed. No GFP+ cells are seen around forebrain structures expect in choroid plexus (inset in **B**). GFP+ meninges, some of which are Raldh2+, are observed around midbrain (**C**). (F) representative scatter plots of gating for singlets (left) and GFP+ (right) cells from *Col1a1-GFP* dissociated meninges. Scale bars = 500 µ m (D, E low mag) and 50 µm (D, dorsal and ventral high mag)

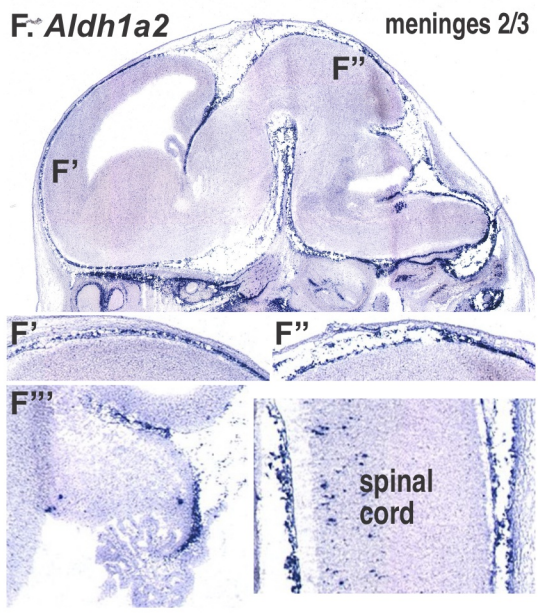


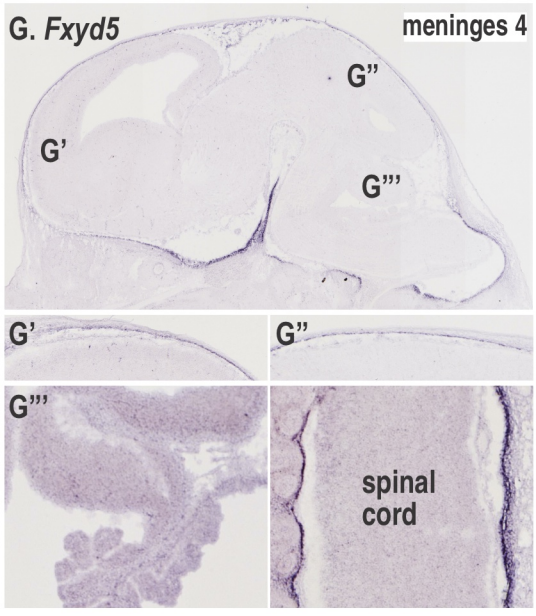












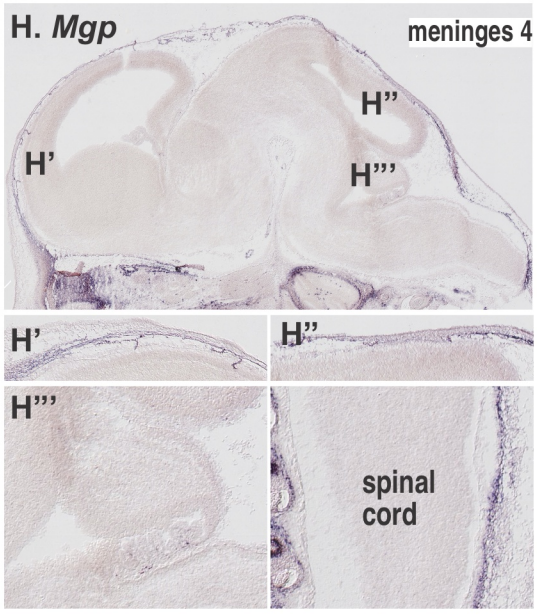


Figure S2 related to Figure 2. *In situ* expression of genes enriched in M1-4 .

(A) Schematic diagram of CNS regions depicted in B-H (top). Boxes in top image indicate magnified areas of the anterior telencephalon, dorsal midbrain, cerebellum/4th ventricle choroid plexus and spinal cord depicted in the bottom panels. (B-H) *In situ* images of E14.5 sagittal sections depicting gene expression patterns of gene enriched in M1-4 principal clusters. Images obtained from GenePaint. Letters on low magnification image indicate areas depicted in high magnification panels. High magnification image image of anterior telencephalon are rotated 90 degrees clockwise. Images of spinal cord are not depicted in low magnification image. These are from adjacent whole embryo section that includes the spinal cord.

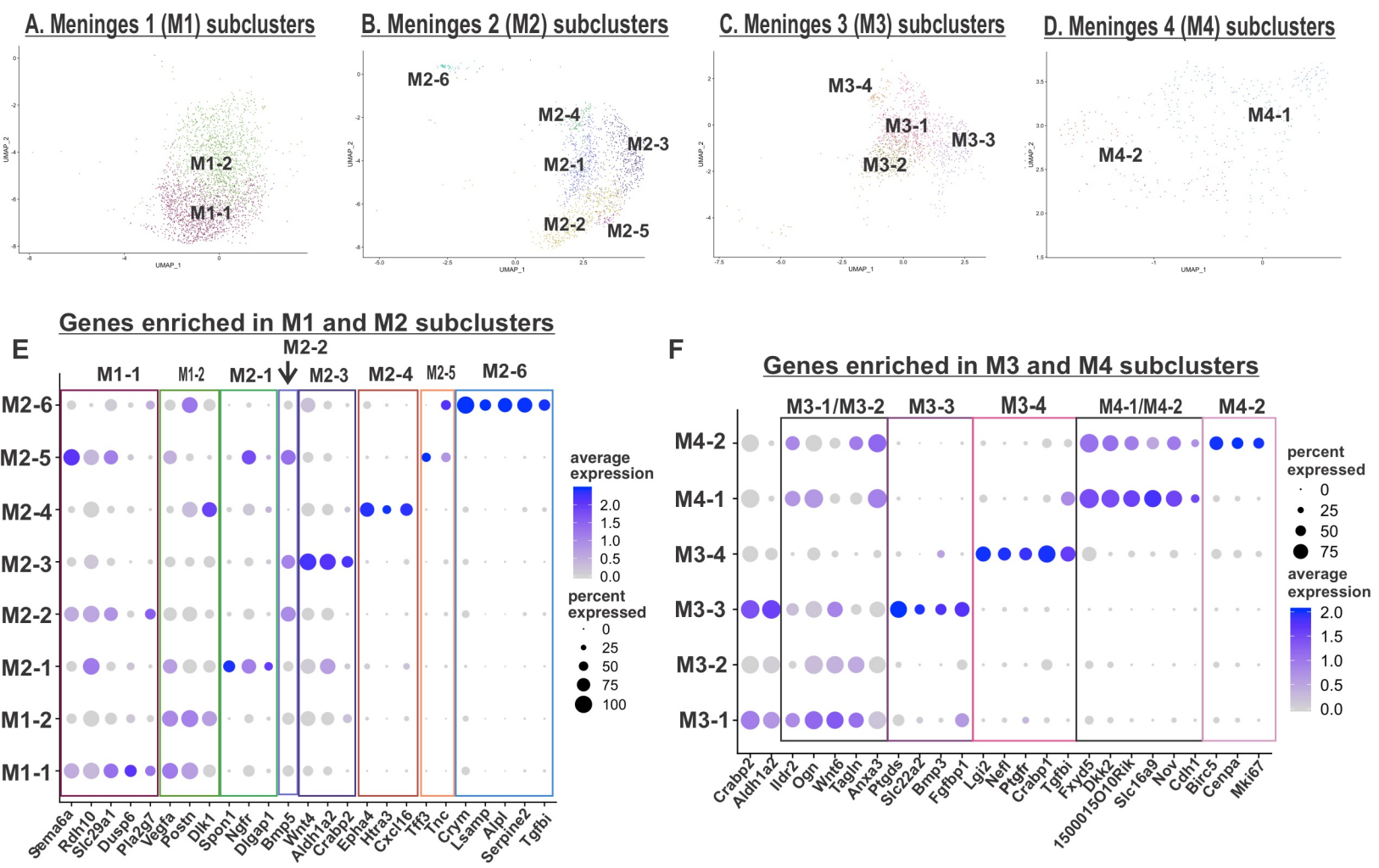


Figure S3 related to Figure 3 M1-M4 subcluster UMAPs and dot plots with enriched genes.
(A) UMAP plot of M1 subclusters M1-1 (1410 cells) and M1-2 (1461 cells). (B) UMAP plot of M2 subclusters M2-1 (519 cells), M2-2 (472 cells), M2-3 (451 cells), M2-4 (103 cells), M2-5 (58 cells), and M2-6 (43 cells). (C) UMAP plot of M3 subclusters M3-1 (583 cells), M3-2 (393 cells), M3-3 (245 cells) and M3-4 (59 cells). (D) UMAP plot of M4 subcluster M4-1 (269 cells) and M4-2 (122 cells). (E) Dot plot depicting enriched gene in M1 and M2 subclusters curated from the top 30 enriched genes ranked on adjusted p-value. (F) Dot plot depicting enriched gene in M3 and M4 subclusters curated from the top 30 enriched genes ranked on adjusted p-value.

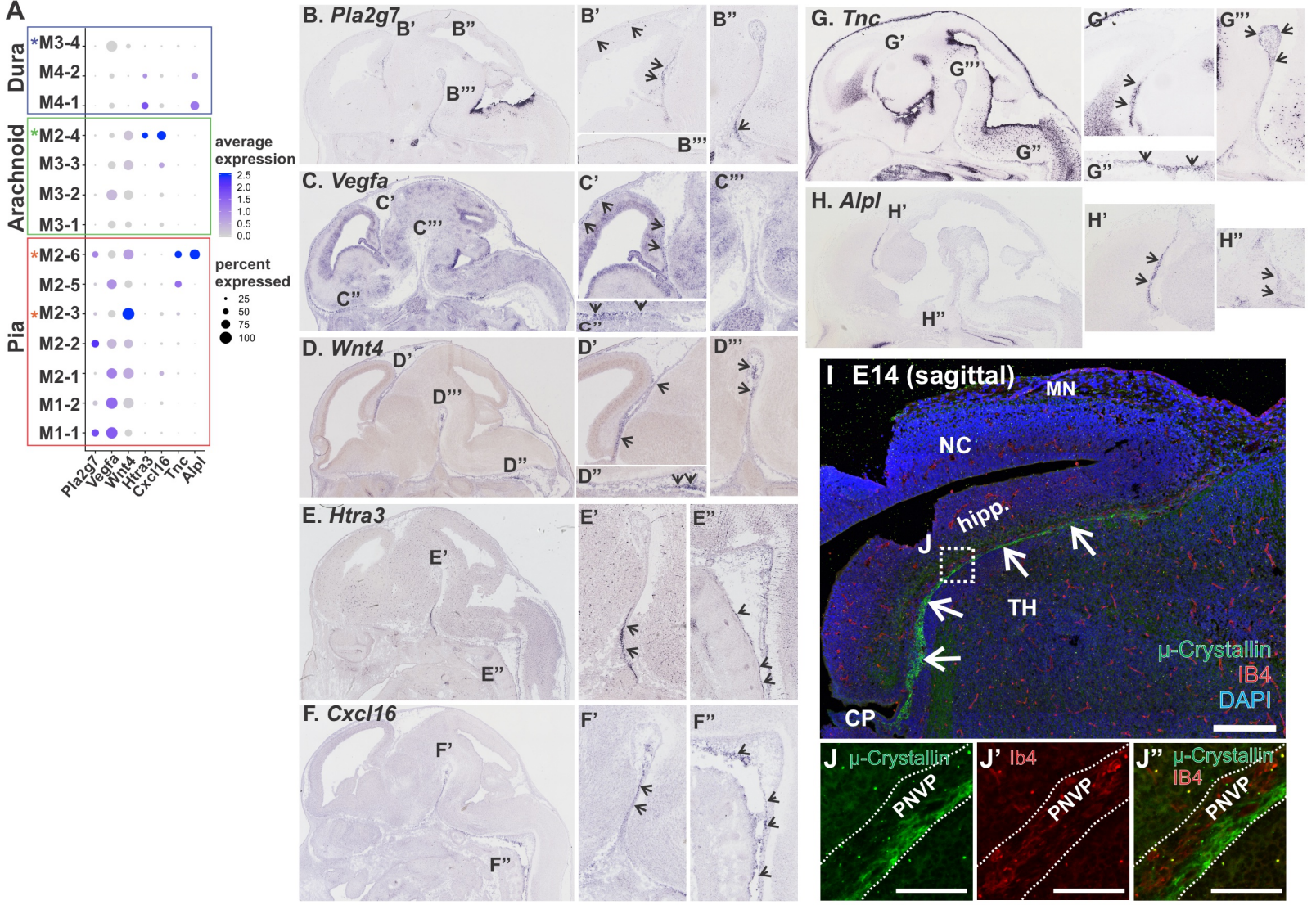


Figure S4 related to Figure 4. *In situ* validation of genes enriched in M1 and M2 subcluster.
(A) Dot plot depicting genes enriched in M1 and M2 subclusters. (B-H) *In situ* images of E14.5 sagittal brain sections depicting genes enriched in M1 and M2 subclusters. Letter annotation in low magnification image indicates magnified areas. Open arrows in magnified images indicate meninges-located signal. Images obtained from GenePaint. (I) E14 sagittal image shows μ-Crystallin antibody labeling (arrows) in the meninges between the future hippocampus (hipp.) and thalamus (TH). No μ-Crystallin antibody labeling is seen in the meninges (MN) overlaying the neocortex (NC). (J-J") Magnified area in 'I' shows μ-Crystallin antibody labeling immediately adjacent to the surface of the thalamus and separate from the meningeal located perineural vascular plexus (PNVP) labeled with isolectin-B4 (IB4). CP: choroid plexus. Scale bars = 200 μm (I) and 50 μm (J-J").

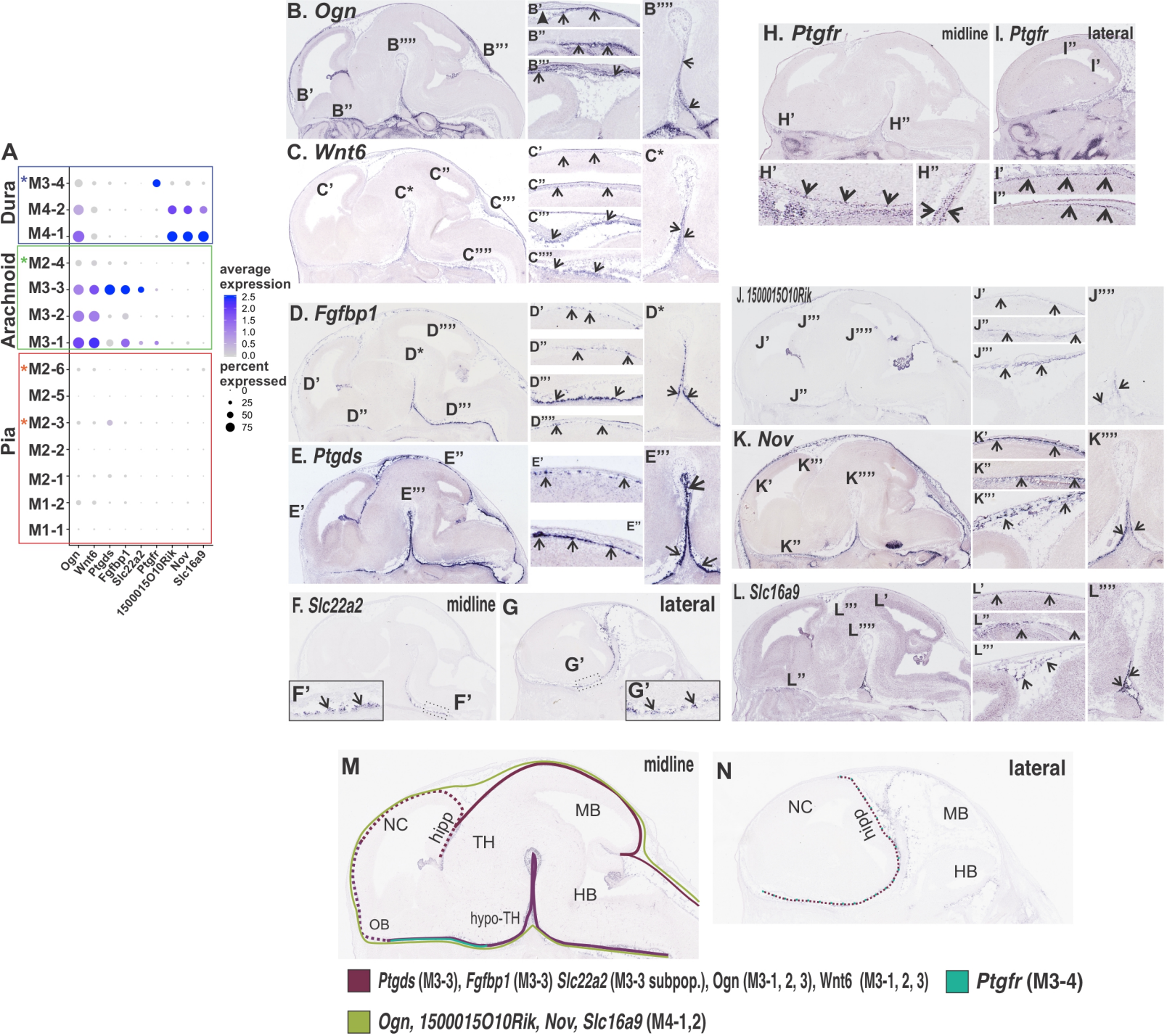


Figure S5 related to Figure 4. *In situ* validation of genes enriched in M3 and M4 subcluster.
(A) Dot plot depicting genes enriched in M3 and M4 subclusters. (B-L) *In situ* images of E14.5 sagittal brain sections depicting genes enriched in M3 and M4 subclusters. Letter annotation in low magnification image indicates magnified areas. Open arrows in magnified images indicate meninges-located signal. Closed arrows in B' indicate signal in outer dural layer and open arrows indicate signal in arachnoid layer. Images obtained from GenePaint. (M, N) Summary diagrams of the regions of the meninges where M3 and M4 subcluster enriched genes are detected. Dotted line in M indicates patchy expression of *Ptgds* and *Fgfbp1* over the dorsal telencephalon. In N, diagram depicts overlapping expression areas of *Slc22a2* (M3-3) and *Ptgfr* (M3-4) in lateral/posterior telencephalon.

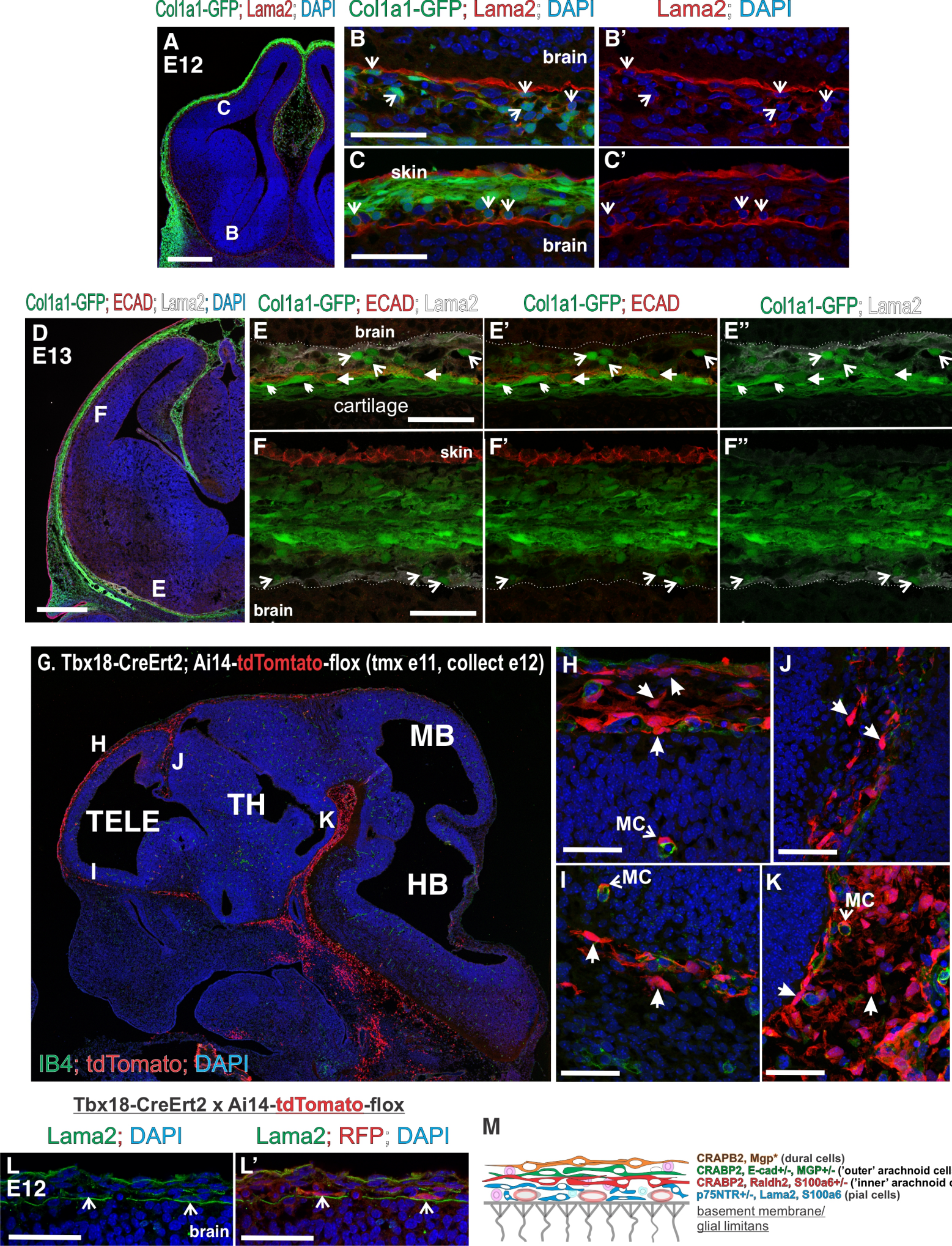

Col1a1-GFP; Lama2; DAPI
Col1a1-GFP; Lama2; DAPI
Lama2; DAPI
A
E12
C
B
B
brain
B'
C
skin
brain
C'
Col1a1-GFP; ECAD; Lama2; DAPI
Col1a1-GFP; ECAD; Lama2
Col1a1-GFP; ECAD
Col1a1-GFP; Lama2
D
E13
F
E
E
brain
cartilage
E'
E''
F
skin
brain
F'
F''
G. Tbx18-CreErt2; Ai14-tdTomtato-flox (tmx e11, collect e12)
H
J
MB
TH
TELE
K
I
HB
IB4; tdTomato; DAPI
H
MC
J
I
MC
K
MC
Tbx18-CreErt2 x Ai14-tdTomato-flox
Lama2; DAPI
Lama2; RFP; DAPI
L
E12
brain
L'
M
CRAPB2, Mgp* (dural cells)
CRABP2, E-cad+/-, MGP+/- ('outer' arachnoid cells)
CRABP2, Raldh2, S100a6+/- ('inner' arachnoid cells)
p75NTR+/-, Lama2, S100a6 (pial cells)
basement membrane/
glial limitans

Figure S6 related to Figure 6. Developmental emergence of meningeal fibroblast layer markers
(A-C) Low and high magnification images of E12 *Col1a1-GFP/+* IF labeling with Laminin-α2. Open arrows in B and C indicate Laminin α2+/GFP+ cells in ventral (B) and lateral (C) telencephalon. (D-F) Low and high magnification images of E13 *Col1a1-GFP/+* IF labeling of E-cadherin (E-cad) and Laminin α2 (Lama2). E and F depicts magnified ventral and lateral areas of the telencephalon, respectively. Open arrows indicate GFP+/Laminin α2+, closed arrows indicate E-cad+/GFP+ and carets in (B) indicate GFP+/Laminin α2-/E-cad- cells in the outer dura layer of the meninges. (G) Low magnification images of E12 *Tbx18-CreErt2; Ai14-fl/+* (tamoxifen or tmx E11) depicting tdTomato+ cells in the meninges of the forebrain (telencephalon or TELE, thalamus or TH) and regions of the midbrain (MB) and hindbrain (HB), though recombination is not detected in the meninges overlaying anterior midbrain and dorsal hindbrain areas. (H-K) Magnified areas of meninges overlaying different brain regions shows tdTomato+ recombined cells in the brain and meninges are perivascular mural cells (MC) (open arrows in H, I, & K) and non-vascular associated cells, adjacent to and slightly away from the brain surface consistent with meningeal fibroblast identity. (L) Laminin α2 IF in E12 *Tbx18-CreErt2; Ai14-fl/+* (tmx E11) depicts Laminin α2+/tdTomato+ cells at the brain surface, consistent with a pial fibroblast (arrows).(M) Refined model of marker expression in E14 embryonic meninges with E-cad+ cells in outer arachnoid. Scale bars = 500 μm (A, D) and 50 μm (B, C, E, F, L), 25 μm (H-K).

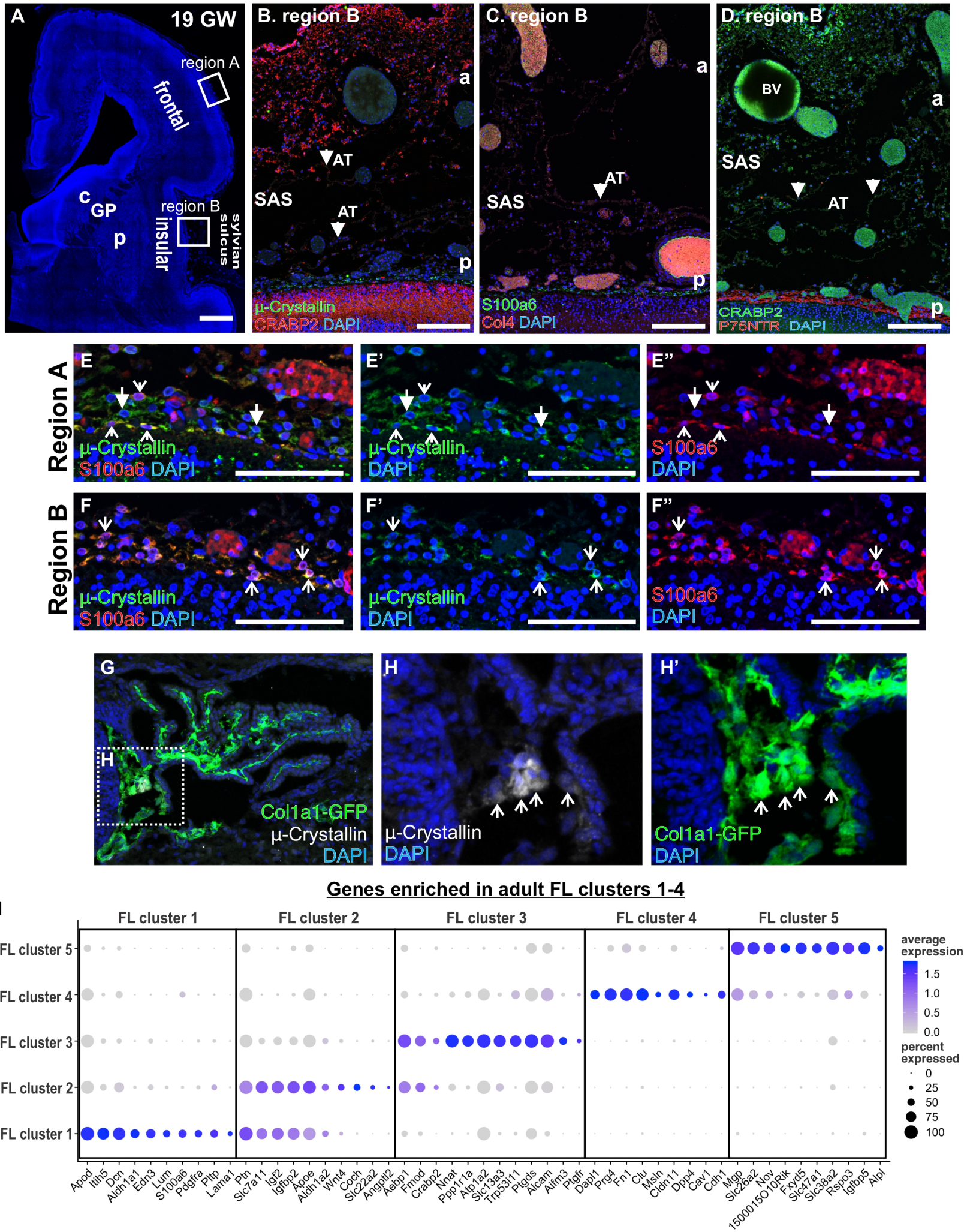

A
19 GW
region A
frontal
c
GP
p
region B
insular
sylvian sulcus
B. region B
a
AT
SAS
AT
p
μ-Crystallin
CRABP2 DAPI
C. region B
a
AT
SAS
p
S100a6
Col4 DAPI
D. region B
BV
a
SAS
AT
p
CRABP2
P75NTR DAPI
Region A
E
μ-Crystallin
S100a6 DAPI
E'
μ-Crystallin
DAPI
E''
S100a6
DAPI
Region B
F
μ-Crystallin
S100a6 DAPI
F'
μ-Crystallin
DAPI
F''
S100a6
DAPI
G
H
Col1a1-GFP
μ-Crystallin
DAPI
H
μ-Crystallin
DAPI
H'
Col1a1-GFP
DAPI
I
Genes enriched in adult FL clusters 1-4
FL cluster 1
FL cluster 2
FL cluster 3
FL cluster 4
FL cluster 5
FL cluster 5
FL cluster 4
FL cluster 3
FL cluster 2
FL cluster 1
Apod Itih5 Dcn Aldh1a1 Edn3 Lum S100a6 Pdgfra Pltp Lama1
Ptn Slc7a11 Igf2 Igfbp2 Apoe Aldh1a2 Wnt4 Coch Slc22a2 Angptl2
Aebp1 Fmod Crabp2 Nnat Ppp1r1a Atp1a2 Slc13a3 Trp53i11 Ptgds Alcam Alfm3 Ptgfr
Dapl1 Prg4 Fn1 Clu Msln Cldn11 Dpp4 Cav1 Cdh1
Mgp Slc26a2 Nov 1500015O10Rik Fxyd5 Slc47a1 Slc38a2 Rspo3 Igfbp5 Alpl
average expression
1.5
1.0
0.5
0.0
percent expressed
0
25
50
75
100

Figure S7 related to Figure 7. Meningea layer markers in human fetal meninges and adult single cell fibroblast clusters

(A) Coronal section of a 19 gestational week (GW) human telencephalon, region A is meninges overlaying the frontal cortex and region B is in the sylvian sulcus overlying insular cortex (C=caudate, GP=globus pallidus, and P=putamen). (B-D) Magnified area of Region B contained a much-expanded meninges with a distinct subarachnoid space (SAS), recognized by arachnoid trabeculae (AT) (closed arrows in B-D). The arachnoid (a) layer and some AT contained CRABP2+ cells (B, D). µ-Crystallin+ (B), S100a6+ (C) and P75NTR+ (D) cells were limited to the pia (p) adjacent to the brain surface in Region B. (E, F) S100a6+/µ-Crystallin+ pial cells (open arrows) and µ-Crystallin+/S100a6- (closed arrows) in regions A (E) and B (F). (G) P15 Col1a1-GFP 4th ventricle choroid plexus, box is magnified in H, H'. (H, H') Magnified area in G shows µ-Crystallin+ cells within the choroid plexus stroma are also GFP+ though many GFP+ in the stroma are µ-Crystallin-. (I) Dot plot depicting top enriched genes in fibroblast like (FL) clusters 1-5, curated from the top 30 genes ranked by adjusted p-value. Scale bars=1mm (A), 200 µm (B-D), 100 µm (E-F).