1358 **Figure S1: Expression of Wnts family members by ACs.**

1359 (A-E) Cultivated ACs at p0 and p1 were subjected to RT-PCR analysis and showed
1360 that several Wnts (A) are expressed as well as Fzd receptors (B), LRP co-receptors
1361 (D) and Sfrps inhibitors (C). G6pdx (A, E) was used as housekeeping gene and RNA
1362 from E14.5 embryo was used as positive control (c). (F) qPCR confirm the ACs as main
1363 source of Wnt factors in the whole brain compared to MBMECs. (G) qPCR analysis on
1364 same samples reveals differential expression of Frizzled Receptors in ACs and
1365 MBMECS. (H) qPCR analysis on same samples shows expression of the non-
1366 conventional ligand *Ndp* and the receptors mainly in ACs compared to MBMECs, n=3.

1367

1368 **Figure S2: Recombination efficiency.**

1369 (A) Representative images depicting the specific recombination in CNS tissue of the
1370 constitutive driver line hGFAP-cre used for Evi$^{fl/fl}$ recombination in our study crossed
1371 with the ROSA26-mTmG reporter strain. (B) Recombination in ACs (GFAP, red) is
1372 supported by co-localisation of β-galactosidase (green) expressed together with cre-
1373 recombinase. Insets show individual ACs without and with β-galactosidase
1374 expression in Evi^{Ctrl} and Evi$^{\Delta AC}$ mice, respectively.

1375

1376 **Figure S3: Vascular density is not modified in Evi$^{\Delta AC}$ animals.**

1377 (A-H) Quantification of (A, C, E, G) vascularized area and (B, D, F, H) branching points
1378 in (A-D) 10-week and (E-H) 40-week-old animals. (I-J) Details of the regions included
1379 for the analysis at (I, for results in A, B, E and F) bregma -1.82mm and (J for results in
1380 C, D, G and H) bregma 0.98mm. Asterisks point out area for multiple picture acquisition
1381 in large regions such as cortex, SVZ, piriform cortex and LSN, n=3 for each age and
1382 genotype.

1383

1384 **Figure S4: Western Blot analysis on vessels fragments.**

1385 (A) Representative Western Blot for junction proteins (Cdh5 and Cldn5) and
1386 transporters (Slc2a1 and Abcb1a) in cortical vessel fragments of 10-week-old Evi^{Ctrl}
1387 and Evi$^{\Delta AC}$ animals. Beta-actin was used for normalization. (B) Quantification of
1388 junction proteins (Cdh5 and Cldn5) and transporter (Slc2a1 and Abcb1a) in cortical
1389 vessel fragments of 10-week-old Evi^{Ctrl} and Evi$^{\Delta AC}$ animals. n=4 except for Abcb1a for

which n=3. (C) Immunofluorescent staining for Glut1 (magenta) combined with Podxl (white) as luminal vessel marker and DAPI (blue) nuclear staining of cortical vessels in 10- and 40-week-old Evi^{Ctrl} and $Evi^{\Delta AC}$ mice. Representative images of n=3 mice.

Figure S5: Quantification of junctional protein localization distribution.

(A) Legend of the evaluation criteria for junction protein distribution. Protein localization and junctional staining was evaluated on 10µm sections for intensity, continuity and pattern quality. (B) Analysis of claudin-5 (Cldn5) junctional staining. (C) Analysis of VE-cadherin (Cdh5) junctional staining (n=3).

Figure S6: Immunofluorescence staining of Lama2, Lama4 and Lama5 ECM components.

(A) Immunofluorescent staining on 10µm sections for Lama2 (green) combined with Cdh5 (white) as vessel marker and DAPI (blue) as nuclear staining of cortical vessels in 10- and 40-week-old Evi^{Ctrl} and $Evi^{\Delta AC}$ mice. (B) Immunofluorescent staining for Lama4 (white) combined with Cldn5 (magenta) as vessel marker and DAPI (blue) as nuclear staining of cortical vessels in 10- and 40-week-old Evi^{Ctrl} and $Evi^{\Delta AC}$ mice. (C) Immunofluorescent staining for Lama5 (magenta) combined with Cldn5 (green) as vessel marker and DAPI (blue) as nuclear staining of cortical vessels in 10- and 40-week-old Evi^{Ctrl} and $Evi^{\Delta AC}$ mice. Representative images of n=3 mice.

Figure S7: MACE analysis on FACS isolated cortical ECs.

(A) Steps of the simple protocol used to isolate endothelial cells from brain of Evi^{Ctrl} and $Evi^{\Delta AC}$ animals. (B) Gating strategy for the isolation of brain EC. (C-D) Histogram representative of the GO terms describing junction (C) or vesicle (D) terms. (E-H) Volcano plots of single GO term including genes belonging to amino acid transport (E), glucose transport (F), endothelial cell differentiation (G) and extracellular matrix (H) groups. Red dots represent significantly regulated genes, green dots indicate significantly regulated genes belonging also to Wnt canonical pathway, and blue dots genes of interest investigated in this study.

Figure S8: Pericyte coverage remains unchanged while cell body number is decreased in several regions in young Evi$^{\Delta AC}$ adults.

(A) Representative pictures of vessels (CD31, magenta) covered by pericytes (CD13/Anpep, white) in cortex of 10-week-old Evi^{Ctrl} and Evi$^{\Delta AC}$ animals. Green arrowheads point out pericyte cell bodies. (B-I) Quantification of (B, C, F, G) pericyte volume and (D, E, H, I) pericyte cell bodies in (B-E) 10-week and (F-I) 40-week-old animals. n=3 for each age and genotype.

Fig. S1_Guérit et al.

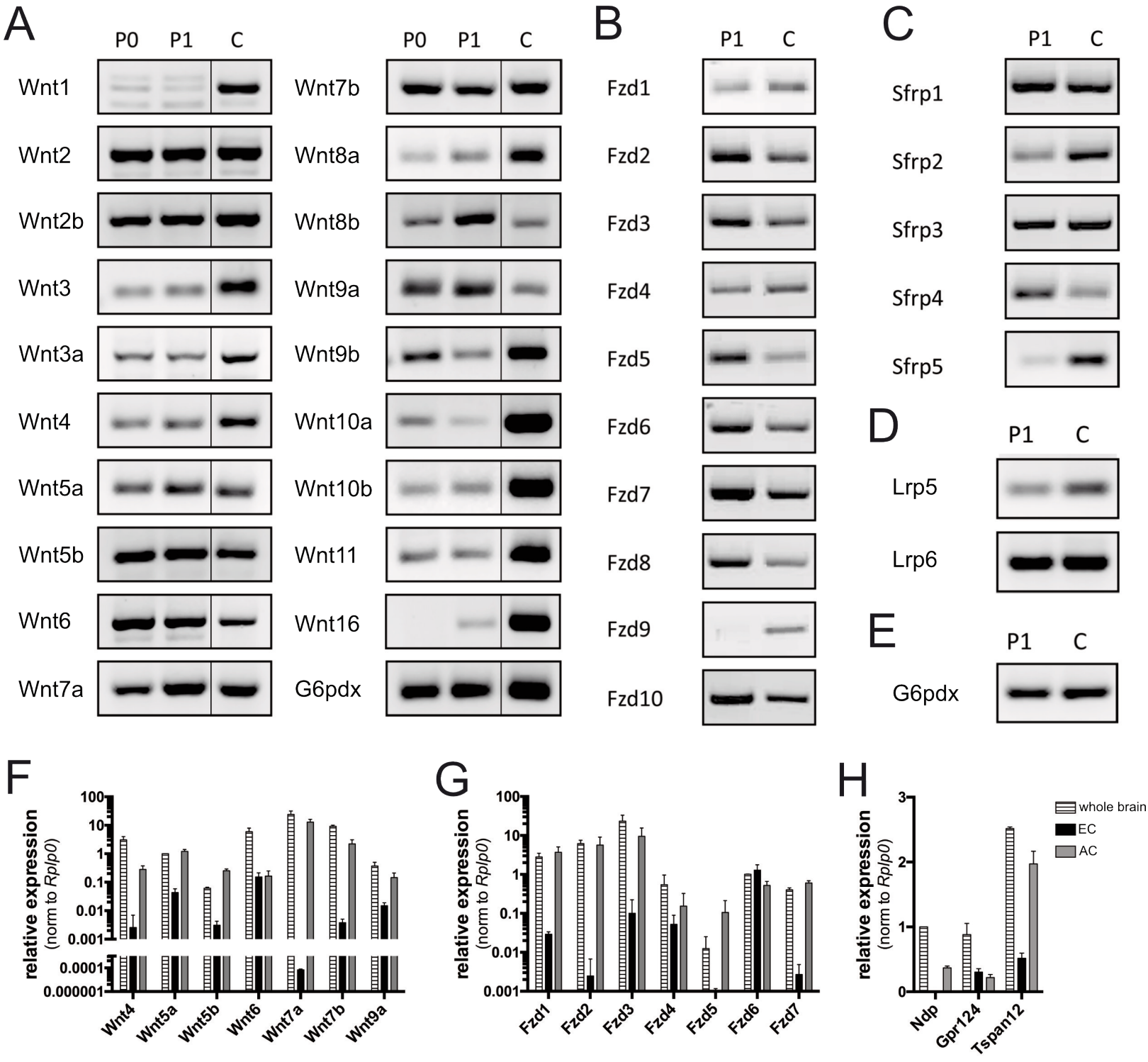


Fig. S2_Guérit et al.

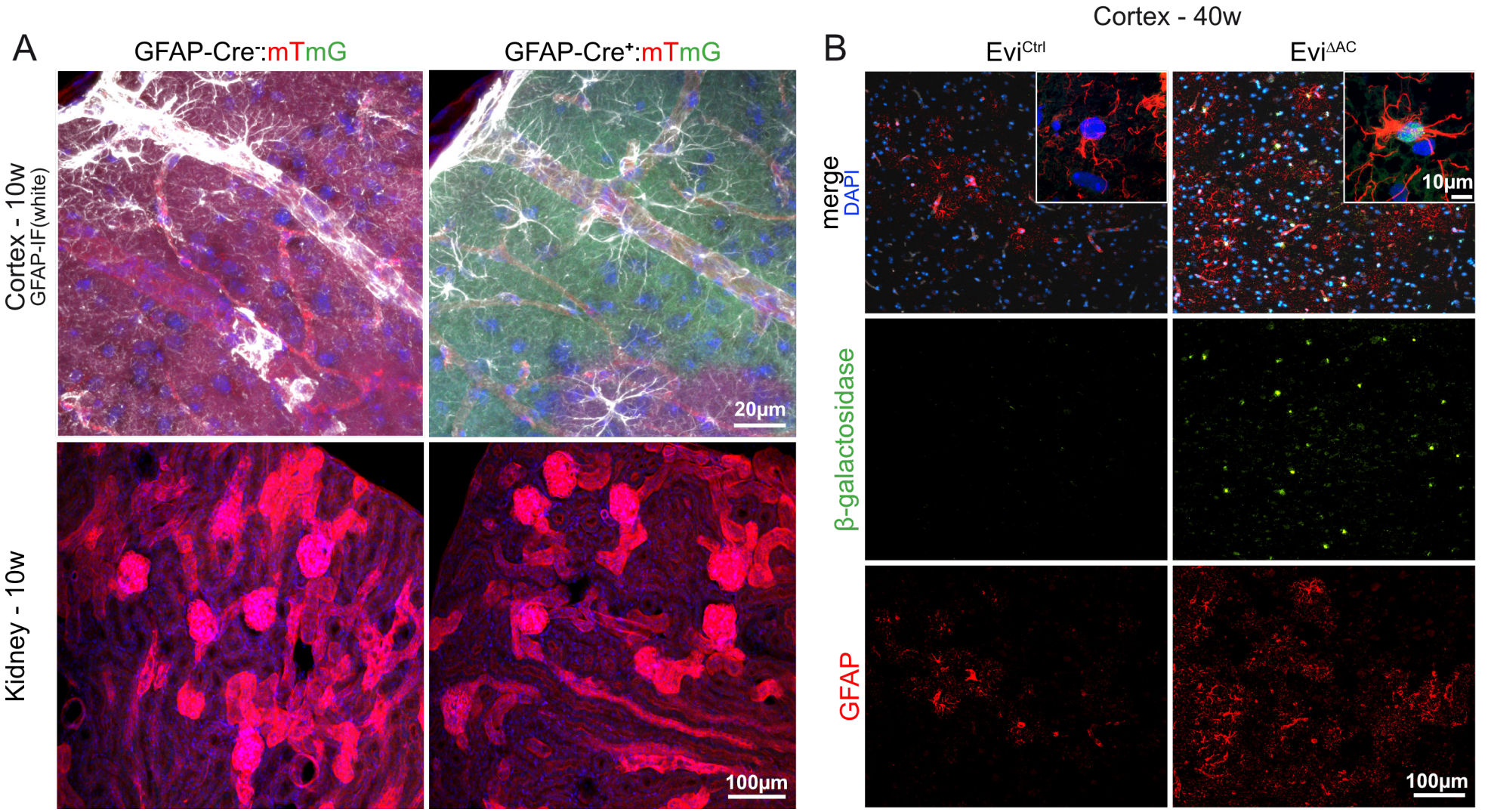
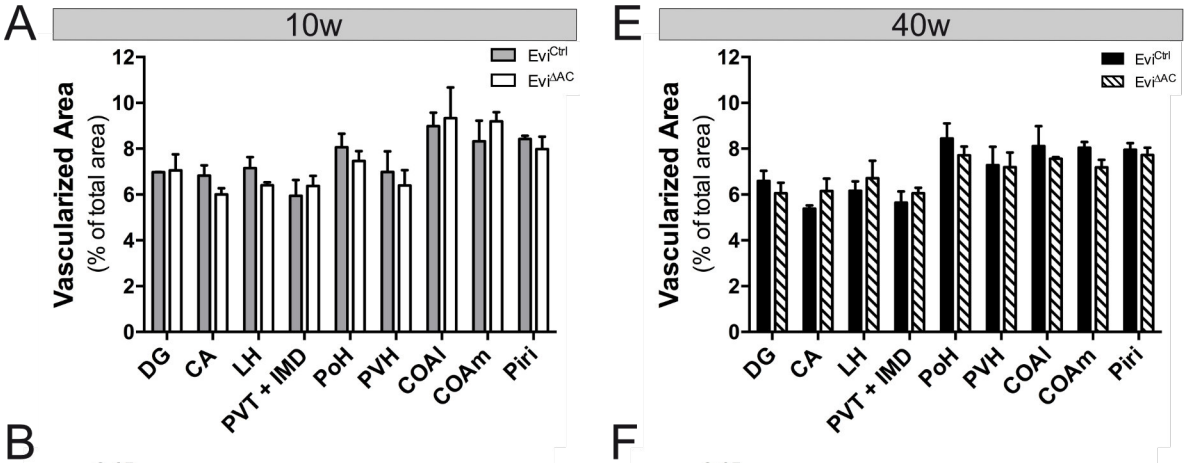
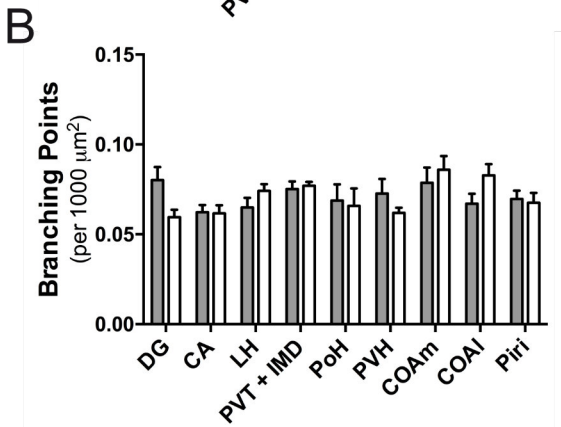
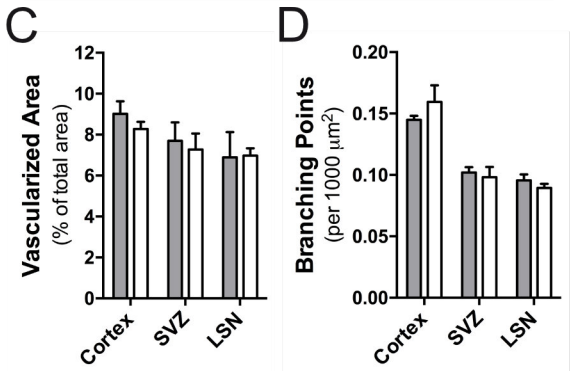
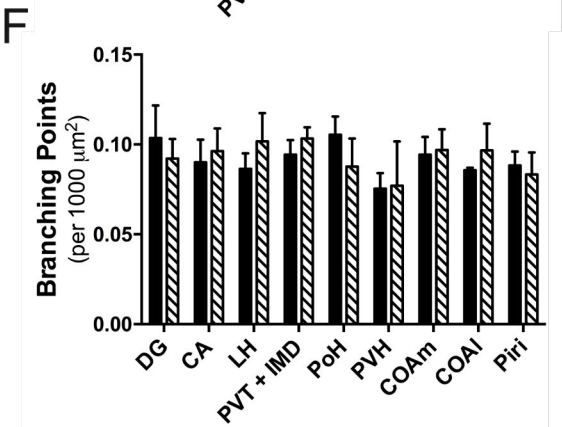


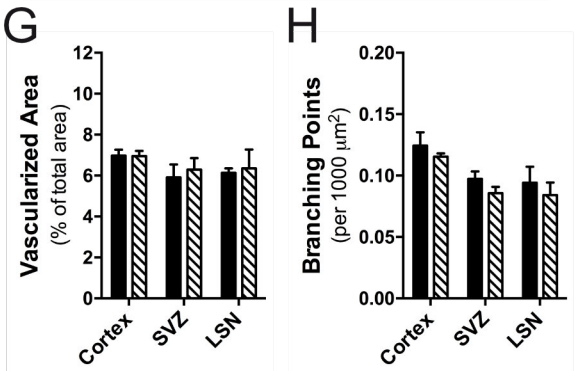
Fig. S3_Guérit et al.

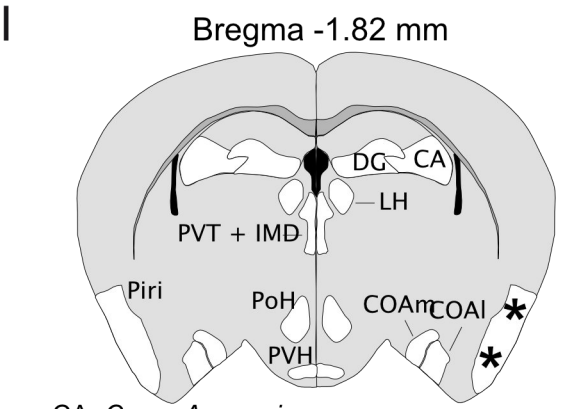




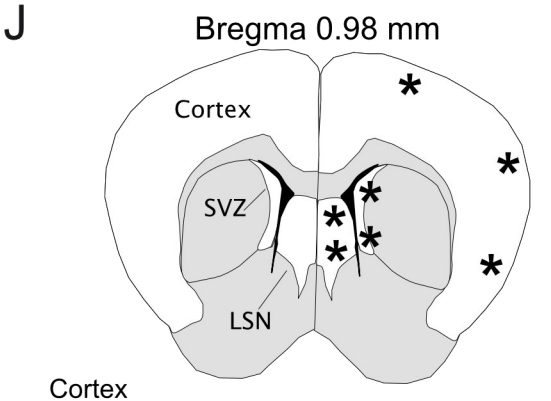






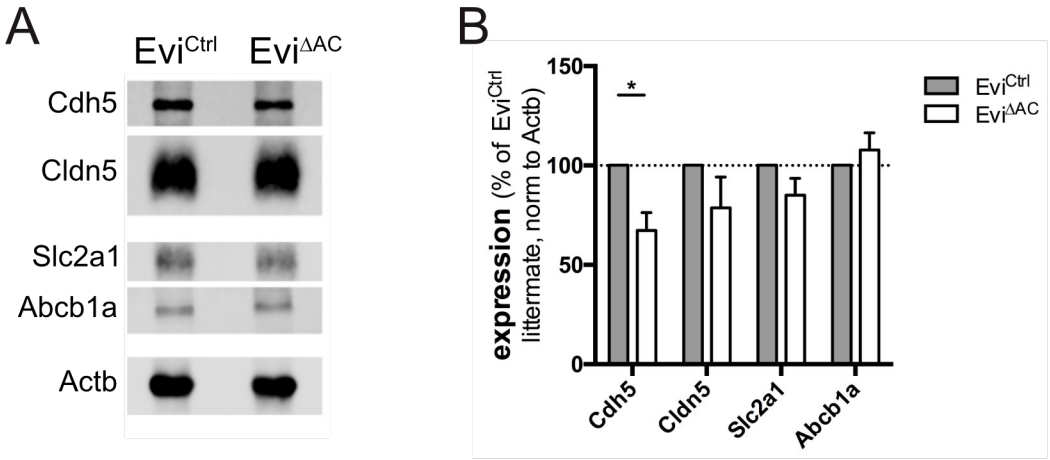


CA: *Cornu Ammonis* area
COAl: cortical amygdalar area lateral
COAm: cortical amygdalar area medial
DG: dentate gyrus
LH: lateral habenula
Piri: piriform cortex
PoH: posterior hypothalamic nucleus
PVH: periventricular hypothalamic nucleus
PVT + IMD: periventricular thalamus + intermediodorsal thalamic nucleus



Cortex
SVZ: Subventricular zone
LSN: Lateral septal nucleus

Fig. S4_Guérit et al.



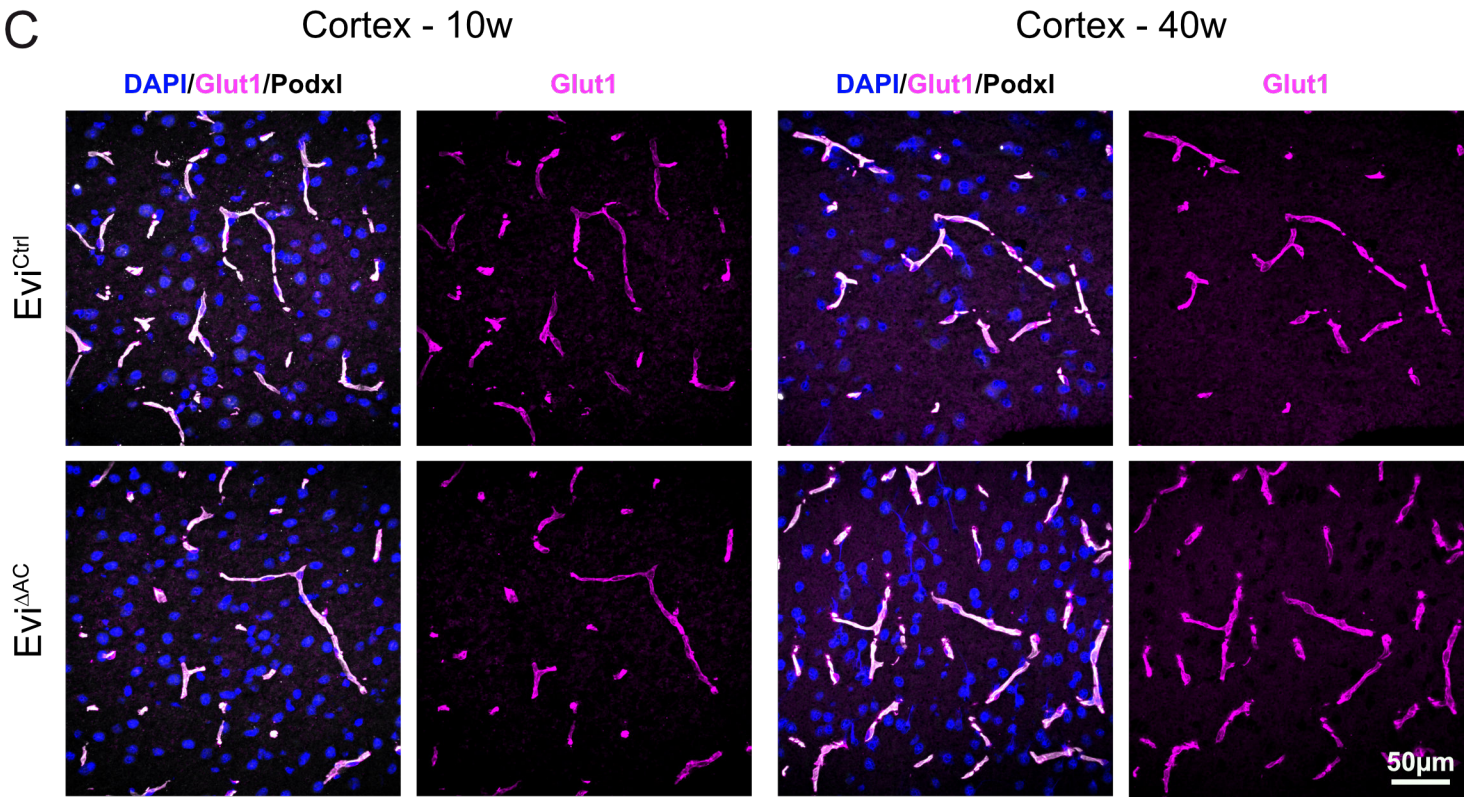


Fig. S5_Guérit et al.

A

intensity

grade 0: weak
grade 1: middle/weak
grade 2: middle
grade 3: middle/strong
grade 4: strong
grade 5: strong +

continuity score

grade 1: 0–24%
grade 2: 25–49%
grade 3: 50–74% (exple)
grade 4: 75–99%
grade 5: 100%

pattern quality

grade 0 tinny line(s), no tortuosity, continuous junctions

grade 1 broad/blurry line(s)

grade 2 tortuous, discontinuous, dots, loops

10w adults
Evi^{Ctrl}
Evi$^{\Delta AC}$

40w adults
Evi^{Ctrl}
Evi$^{\Delta AC}$

B

Claudin-(Cldn)5

intensity | continuity | pattern

Cldn5 intensity score (a.u.) | Cldn5 continuity score (a.u.) | Cldn5 pattern score (a.u.)

cortex, SVZ, LSN

COAm, COAl, DG, CA, PoH, PVH, Piri, LH, PVT + IMD

C

VE-Cadherin

intensity | continuity | pattern

VECad intensity score (a.u.) | VECad continuity score (a.u.) | VECad pattern score (a.u.)

cortex, SVZ, LSN

COAm, COAl, DG, CA, PoH, PVH, Piri, LH, PVT + IMD

Fig. S6_Guérit et al.

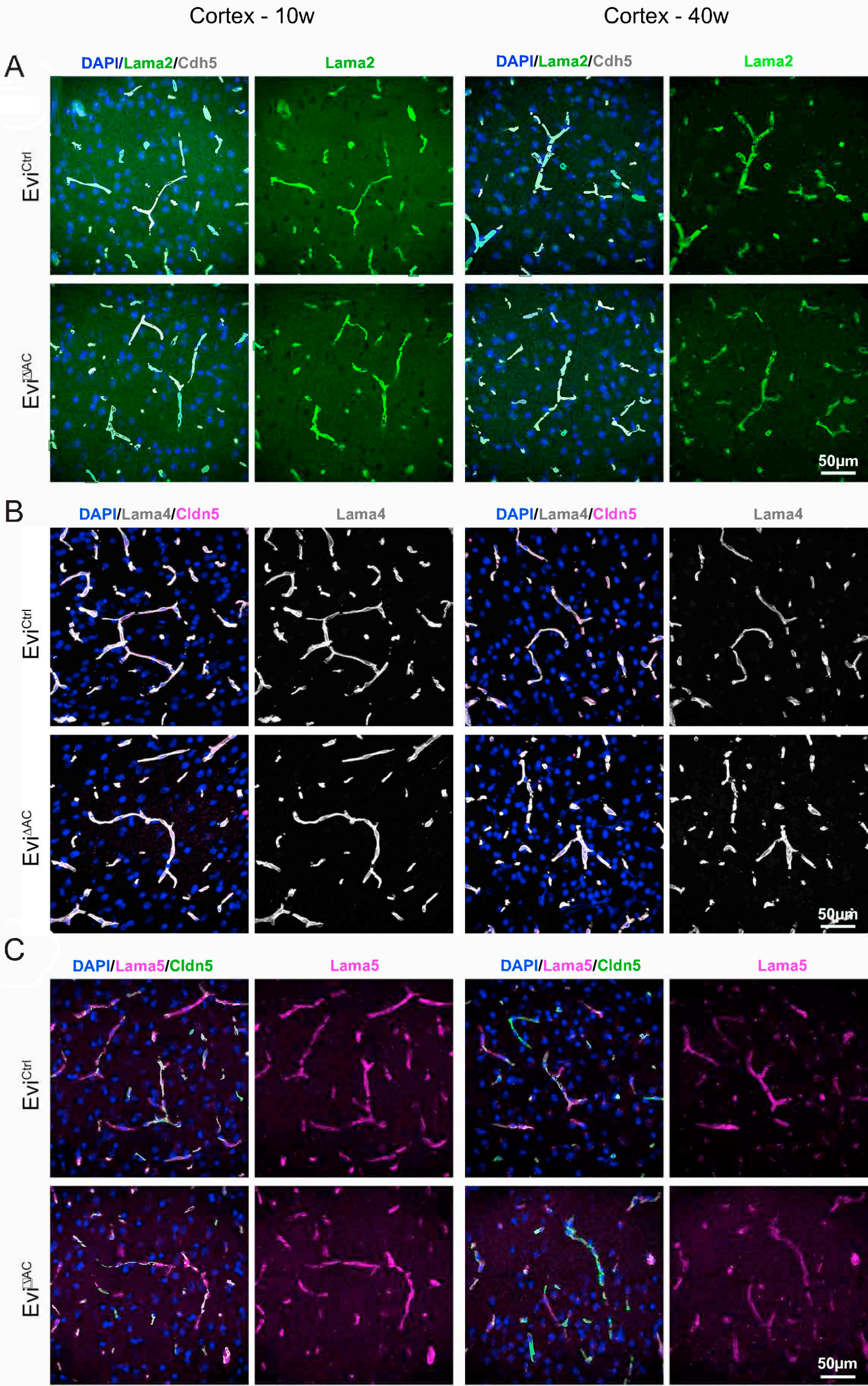


Fig. S7_Guérit et al.

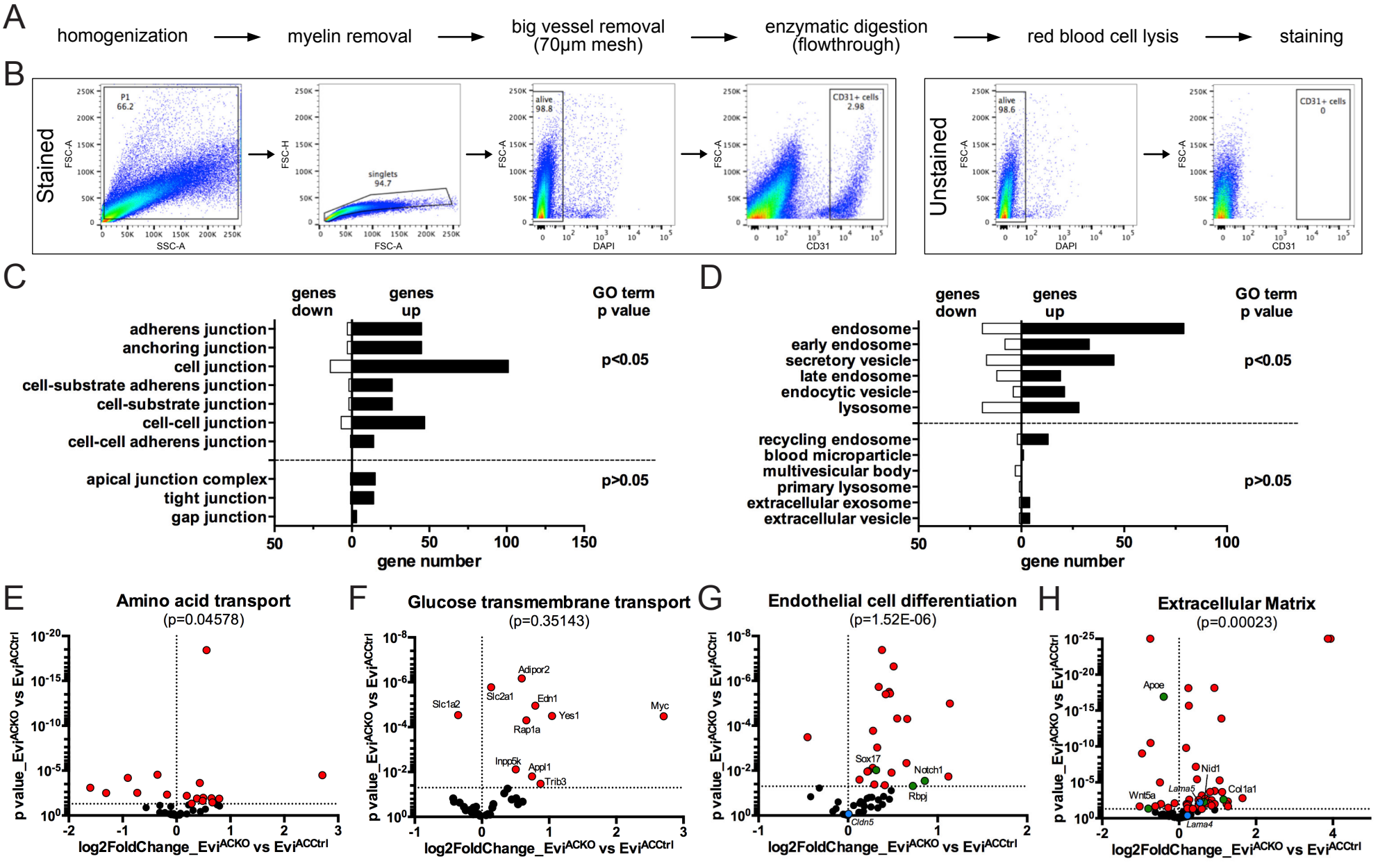
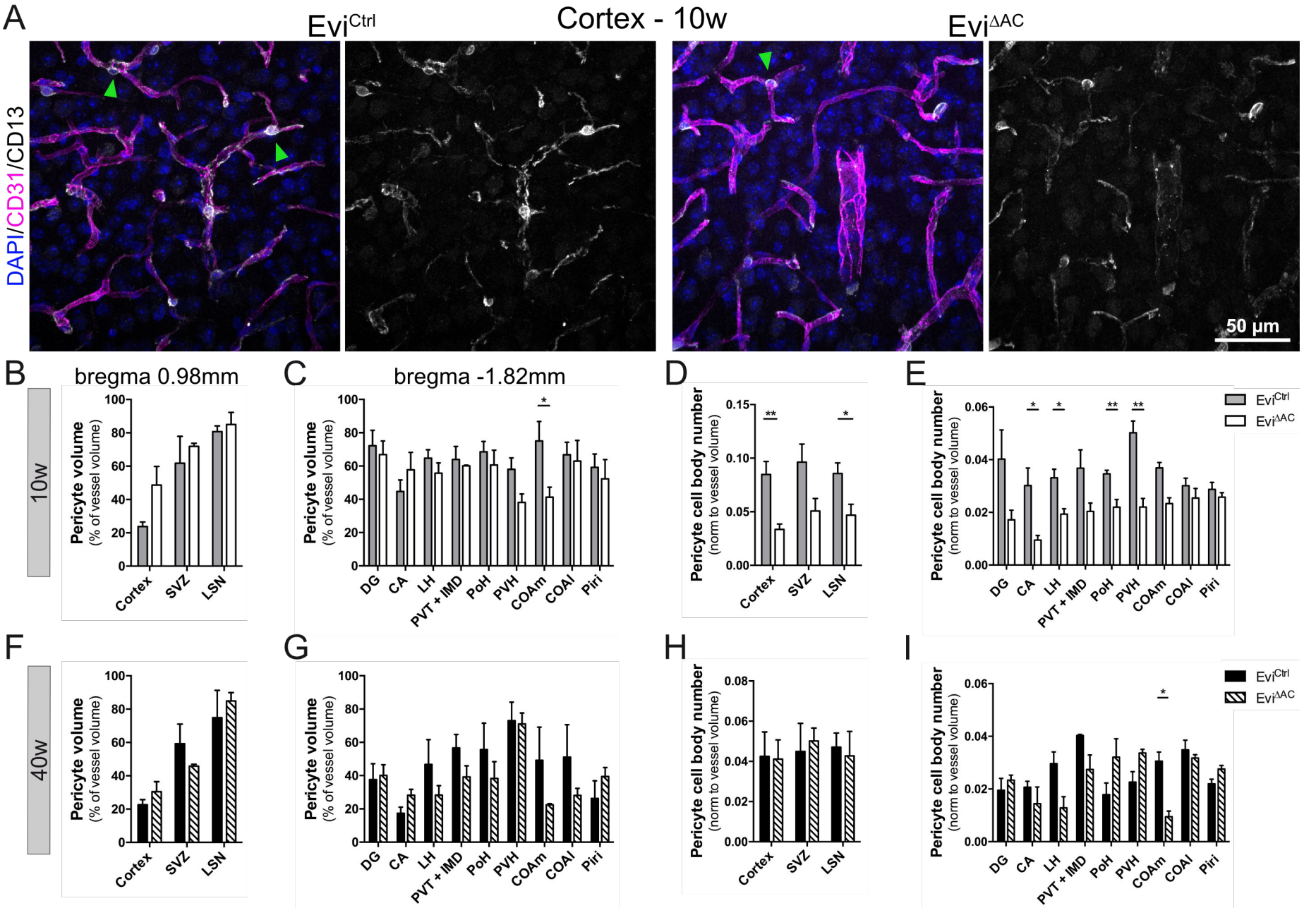


Fig. S8_Guérit et al.



Suppl Table 1: primers for RT-PCR

target	forward	reverse
Fzd1	TGTCCCTTGCCCAGTGTCTTT	TTTAGCCTCTCCCAACCCACA
Fzd2	TCGCCTACAACCAGACCATC	CATTGGAAGCCGAACTTGT
Fzd3	GGGTTGGAAGCAAAAAGACA	CTCCCTGCTTTGCTTCTTTG
Fzd4	CAACCTGTGTGATTGCCTGT	TGTGTGTGGGCTGAAGTGTT
Fzd5	GGCAGGCTTCGTGTCACTCTT	GCTGGCACCGTGTAGAGCAG
Fzd6	GGCTGAAGGTCATTTCCAAG	TGAACAGGCAGAGATGTGGA
Fzd7	GAAGCTGGAGAAGCTGATGG	ATCTCTCGCCCCAAATCTCT
Fzd8	CGGTGGTCTTTCTCCTTGTC	TAGAAAAGGCAGGCGACAAC
Fzd9	AGAGCCTGTGCTACCGAAAA	CCCCCTGTGTCTCACTTGTC
Fzd10	GACACCTGACTGCCTGATGA	ACAACCAGCCAACCAAGAAA
G6pdx	AGACCTAAGCTGGAGGAGTT	GGTGACTGCTTCATAGACTG
Lrp5	AGCCATTGTGTTGCACCCTGT	ACCAGGACATGCCGATCTCTC
Lrp6	CGAACTGCCAGGACAAATCAG	AACTGTGTTGGTGGCTTGTGG
Sfrp1	GGTACAACCGTGTGTCCTCCA	GCCGCTTCAGCTCCTTCTTCT
Sfrp2	CGACATCATGGAAACCCTTTG	AGCCACAGCACGGATTTCTTC
Sfrp3	CATCTCTCCTGAGGCCATCGT	TTTAGCCCGGATGACATAGTTG
Sfrp4	CAATTCCTCCTGCCAGTGTCC	TCCTGAAGCCTCTCTTCCCACT
Sfrp5	CTGGACAACGACCTCTGCATC	CGCTGTGCTCCATCTCACACT
Wnt1	ATCCATCTCTCCCACCTCCT	AGCAACCTCCTTTCCCACTT
Wnt2	AGAGTGCCAACACCAGTTCC	TACAGGAGCCACTCACACCA
Wnt2b	ATCTCGTCAGCAGGAGTGGT	CCATCATATCGCCTCCTCAG
Wnt3	GGGGCGTATTCAAGTAGCTG	GTAGGGACCTCCCATTGGAT
Wnt3a	GGCGGCTGTAGTGAGGACATT	GTTCGCAGAAGTTGGGTGAGG
Wnt4	CGAGGAGTGCCAATACCAGT	GTCACAGCCACACTTCTCCA
Wnt5a	CCCAGTCCGGACTACTGTG	TTTGACATAGCAGCACCAGTG
Wnt5b	TCTCCGCCTTACAAAAGTCT	CACAGACACTCTCAAGCCCA
Wnt6	TTCGGGGATGAGAAGTCAAG	CGGCACAGACAGTTCTCCTC
Wnt7a	GACAAATACAACGAGGCCGT	GGCTGTCTTATTGCAGGCTC
Wnt7b	GCAGTGTGGATGGATGTTGA	TGGAAGATTGGCTGTCCTCT
Wnt8a	CTGACTACTGCAACCGCAAC	TGACAGTGCAACACCACTGA
Wnt8b	CCAGAGTTCCGGGAGGTAG	GAGATGGAGCGGAAGGTGT
Wnt9a	CCCCTGACTATCCTCCCTCT	GATGGCGTAGAGGAAAGCAG
Wnt9b	GGGTGTGTGTGGTGACAATC	TCCAACAGGTACGAACAGCA
Wnt10a	GCGCTCCTGTTCTTCCTACT	ATGCCCTGGATAGCAGAGG
Wnt10b	TCAGTCGGGCTCTAAGCAAT	TGGTGCTGACACTCGTGAAC
Wnt11	TGCTTGACCTGGAGAGAGGT	AGCCCGTAGCTGAGGTTGT
Wnt16	CCCTCTTTGGCTATGAGCTG	TACTGGACATCATCCGAGCA