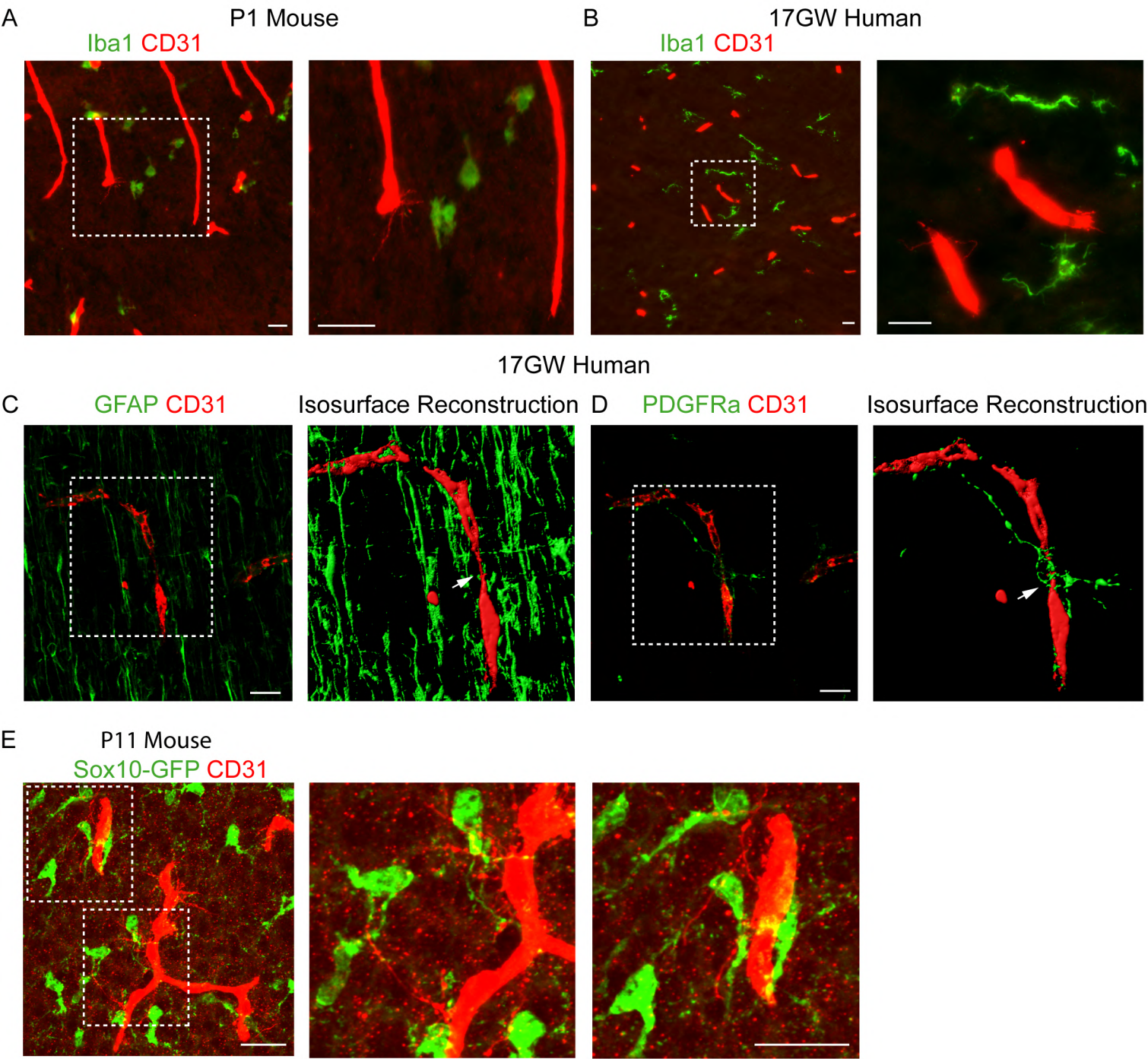
Supplementary Figure 1



Supplementary Figure 1: Characterization of Glial-Endothelial Tip Cell Interactions. (Related to Figure 1)

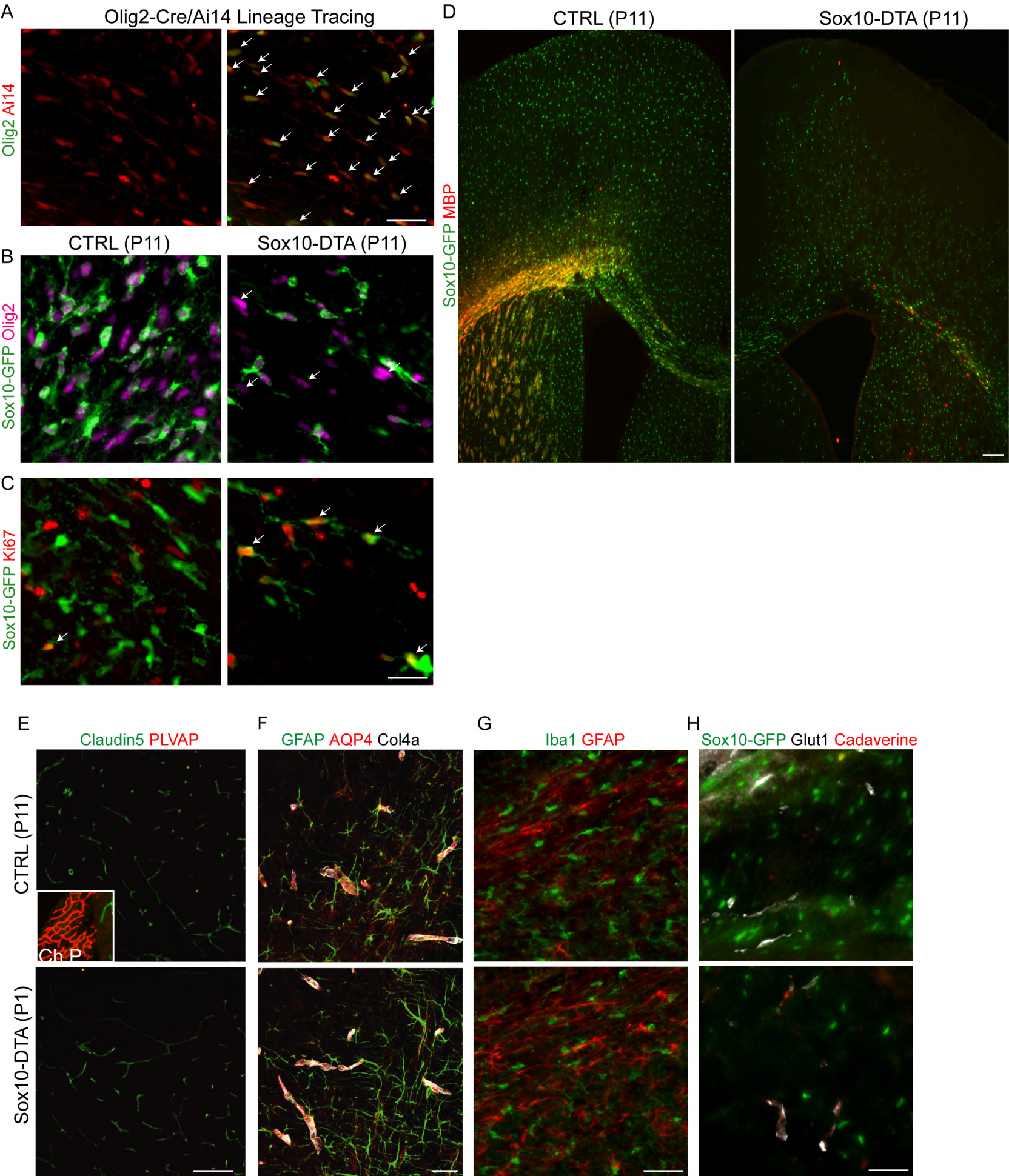
(A) Representative images from P1 mouse brain labelled with Iba1 and CD31 show that microglial cells (Iba1+ cells) don't frequently contact endothelial tip cells in developing white matter. Scale bar: 25μm.

(B) Representative images from 17GW human brain labelled with Iba1 and CD31 show that microglial cells (Iba1+ cells) don't frequently contact endothelial tip cells in developing deep cortical regions adjacent to the ventricle. Scale bar: 25μm.

(C-D) Representative images from 17GW human brain coronal section labelled with GFAP (C), PDGFRa (D) and CD31. Isosurface reconstruction of highlighted region shows that PDGFRa+ OPCs but not GFAP+ astroglial cell processes make complex contacts with endothelial tip cells at the sites where neighboring vessels fuse. Scale bar: 25μm.

(E) Representative images from P11 *Sox10-GFP* transgenic mouse labelled with CD31 show that OPC-endothelial tip cell contacts persist into late postnatal stages in white matter. Scale bar: 25μm.

Supplementary Figure 2



Supplementary Figure 2: Characterization of *Olig2-Cre/Sox10-DTA* model. (Related to Figure 2)

(A) Lineage tracing analysis reveals that ~75% of oligodendroglial cells in the white matter are targeted by *Olig2-Cre* line. Scale bar: 50μm.

(B) Representative images from P1 Control and *Sox10-DTA* white matter shows oligodendroglial targeting by *Sox10-DTA* strategy. Scale bar: 50 μm.

(C) Oligodendroglial cells that escape recombination by either *Olig2-Cre* or *Sox10-DTA* strategy proliferate in an effort to repopulate the brain. Scale bar: 50 μm.

(D) Coronal brain sections from Control and *Sox10-DTA* mice at P11 shows repopulation of oligodendroglial cells. *Sox10-DTA* animals remain hypomyelinated at P11. Scale bar: 100 μm.

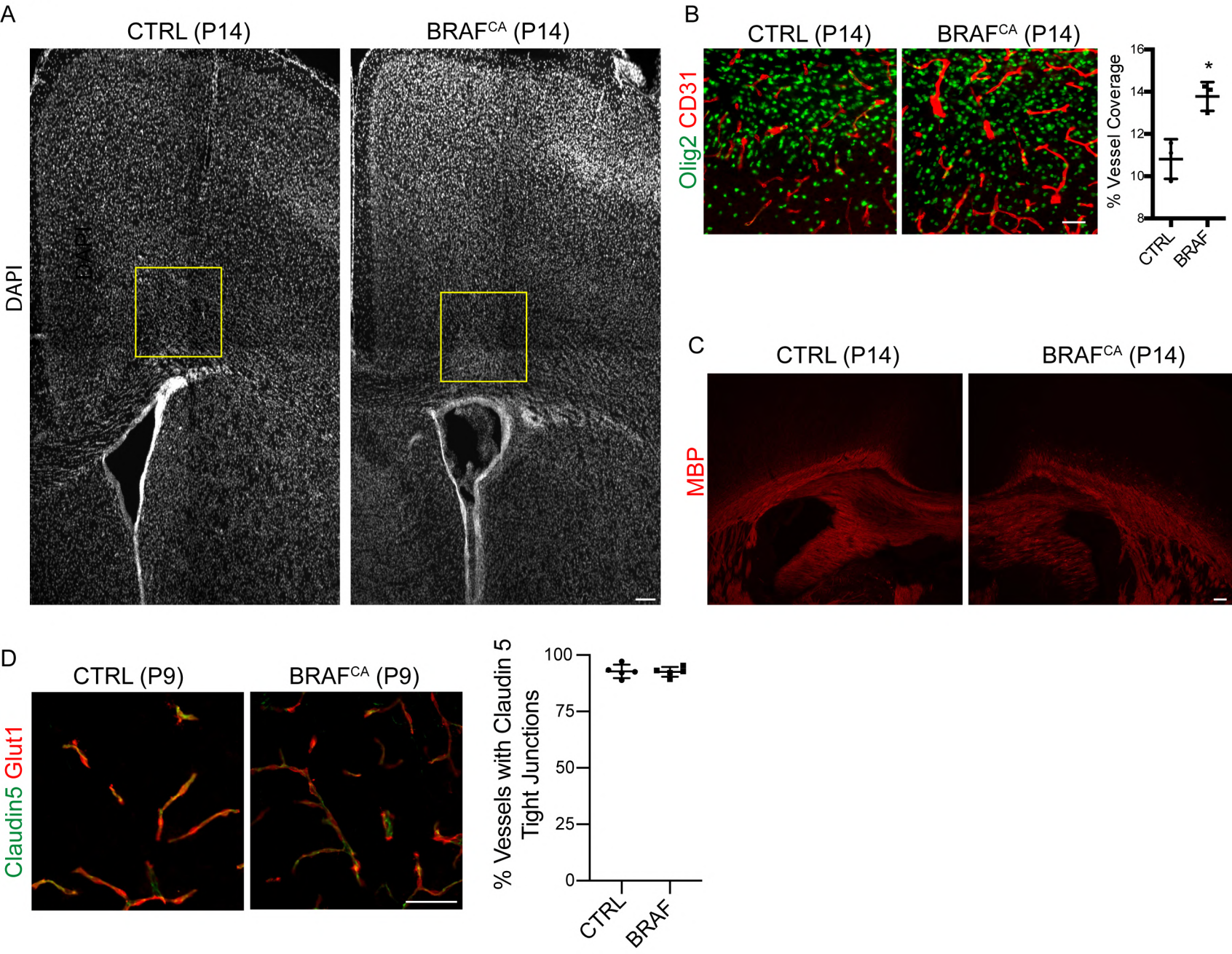
(E) Oligodendroglial ablation in *Sox10-DTA* did not cause BBB disruptions as revealed by lack of Claudin5 negative PLVAP positive endothelial cells. Inset in top panel represents positive control for PLVAP labelling, showing PLVAP+ vessels in choroid plexus from the same section. Scale bar: 50μm.

(F) Astrocyte endfeet coverage abnormalities were not observed in *Sox10-DTA* mice. Scale bar: 50μm.

(G) No major microglial (Iba1+) or astroglial reactivity (GFAP+) were observed in *Sox10-DTA* white matter. Scale bar, 50μm.

(H) Representative images from corpus collosum region of Cadaverine-555 injected Control and *Sox10-DTA* mice labelled with Glut1. Note no leakage of cadaverine-555 in *Sox10-DTA* animals, indicating no damage to BBB. Scale bar: 50μm.

Supplementary Figure 3



Supplementary Figure 3: Characterization of *BRAF^{CA}* Forebrain White Matter. (Related to Figure 3)

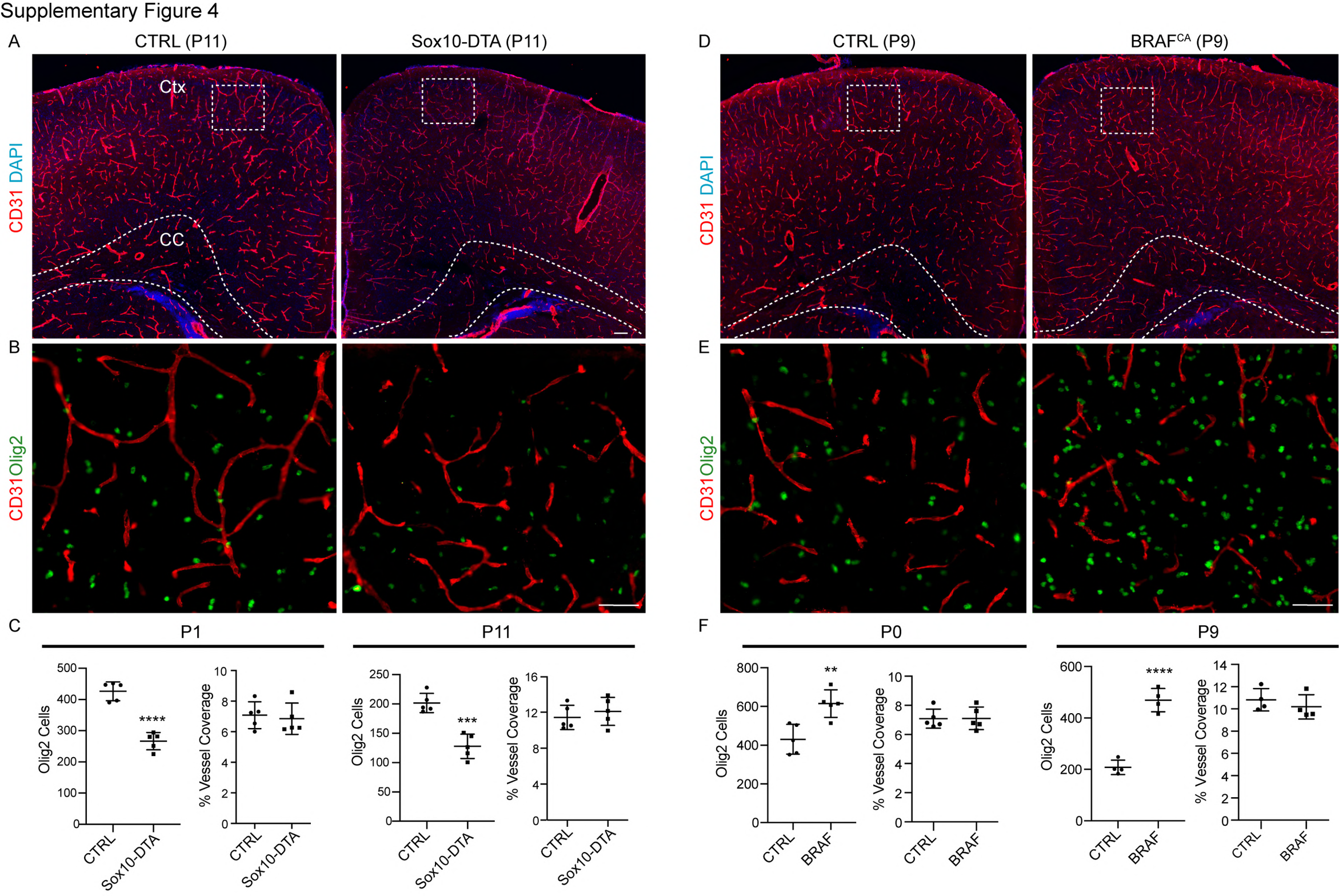
(A) DAPI stained coronal brain sections from *BRAF^{CA}* mice. Scale bar: 100 μm.

(B) Olig2 and CD31 labelling of highlighted regions from (A) reveal hypervascularization of *BRAF^{CA}* forebrain white matter at P14. Scale bar: 50μm.

(C) Mild decrease in myelin levels (MBP) were observed in *BRAF^{CA}* forebrain white matter at P14, when they begin to display motor abnormalities and increase in mortality. Scale bar: 100μm.

(D) Representative images of white matter region from Control and *BRAF^{CA}* brain sections labelled with Claudin 5 and Glut1. Note the absence of Claudin 5 and Glut1 negative vessels, indicating no blood brain barrier (BBB) damage. No significant changes in Claudin 5+ vessel coverage in white matter, indicating no tight junction disruptions. Scale bars: 50μm. Data are represented as mean $\pm$ S.D. and analyzed by two-tailed unpaired Student's t test. n=3 animals/genotype for data shown in (B) and n=5 for (D). Significance between two groups are shown as *$p<0.05$.

Supplementary Figure 4



Supplementary Figure 4: OPC Density Does Not Alter Forebrain Cortical Vascular Coverage. (Related to Figures 2 and 3)

(A) Representative images from P11 - Control and *Sox10-DTA* coronal brain sections labelled with CD31 showing no alterations in cortical vascular coverage. Scale bar: 100μm Ctx: Cortex, CC: Corpus Collosum.

(B) Cortical regions (highlighted in A), showing a decrease in Olig2+ cell numbers but not vascular coverage. Scale bar: 50μm.

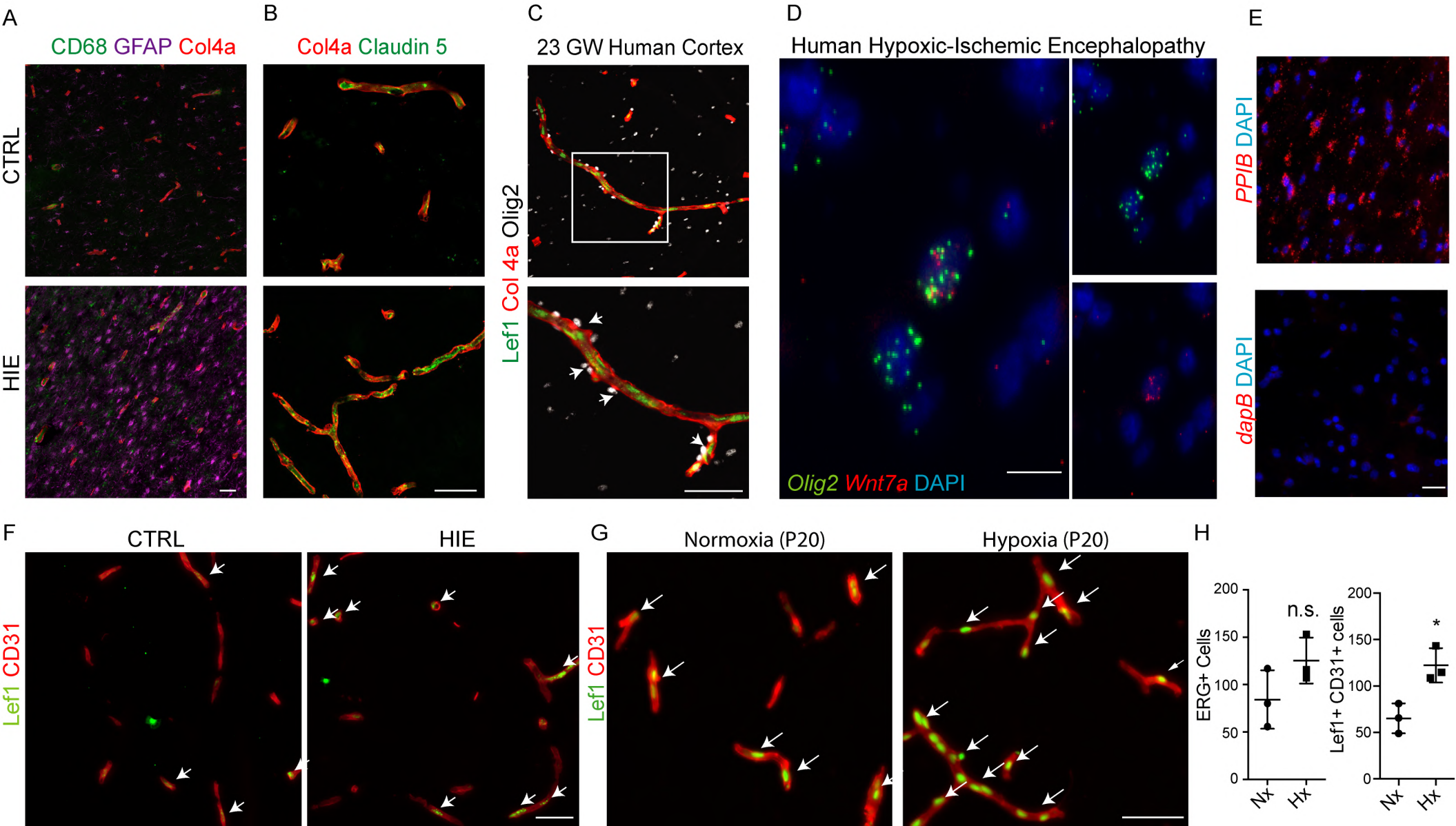
(C) Quantification of Olig2+ cell numbers and vascular coverage from forebrain cortical regions of P1 and P11 CTRL and *Sox10-DTA* mice.

(D) Representative images from P9 - Control and $BRAF^{CA}$ coronal brain sections labelled with CD31 showing no alterations in cortical vascular coverage. Scale bar: 100μm

(E) Cortical regions (highlighted in D), showing an increase in Olig2+ cell numbers but not vascular coverage. Scale bar: 50μm.

(F) Quantification of Olig2+ cell numbers and vascular density from forebrain cortical regions of P0 and P9 CTRL and $BRAF^{CA}$ mice. Data are represented as mean $\pm$ S.D. and analyzed by two-tailed unpaired Student's t test. n=4-5 animals/genotype for data shown in C and F. Significance between two groups are shown as **p<0.01, ***p<0.001, **** p<0.0001. Ctx: Cortex, CC: Corpus Collosum.

Supplementary Figure 5



Supplementary Figure 5: Characterization of Hypoxic Lesions in Human and Ferret Brain. (Related to Figure 4)

(A) Increase in astrogliosis and macrophage infiltration in HIE lesions revealed by GFAP and CD68 immunostaining. Scale bar: 50μm.

(B) Claudin 5+ junctional coverage on the vessels was not affected in the HIE white matter lesions. Scale bar: 50μm.

(C) Higher numbers of Lef1+ endothelia in vessels that are in apposition to Olig2+ cells in 23 Gestational Weeks (GW) developing human brain. Scale bar: 50μm.

(D) Multiplex smFISH of *Wnt7a* and *Olig2* in HIE white matter lesions. Scale bar: 10μm.

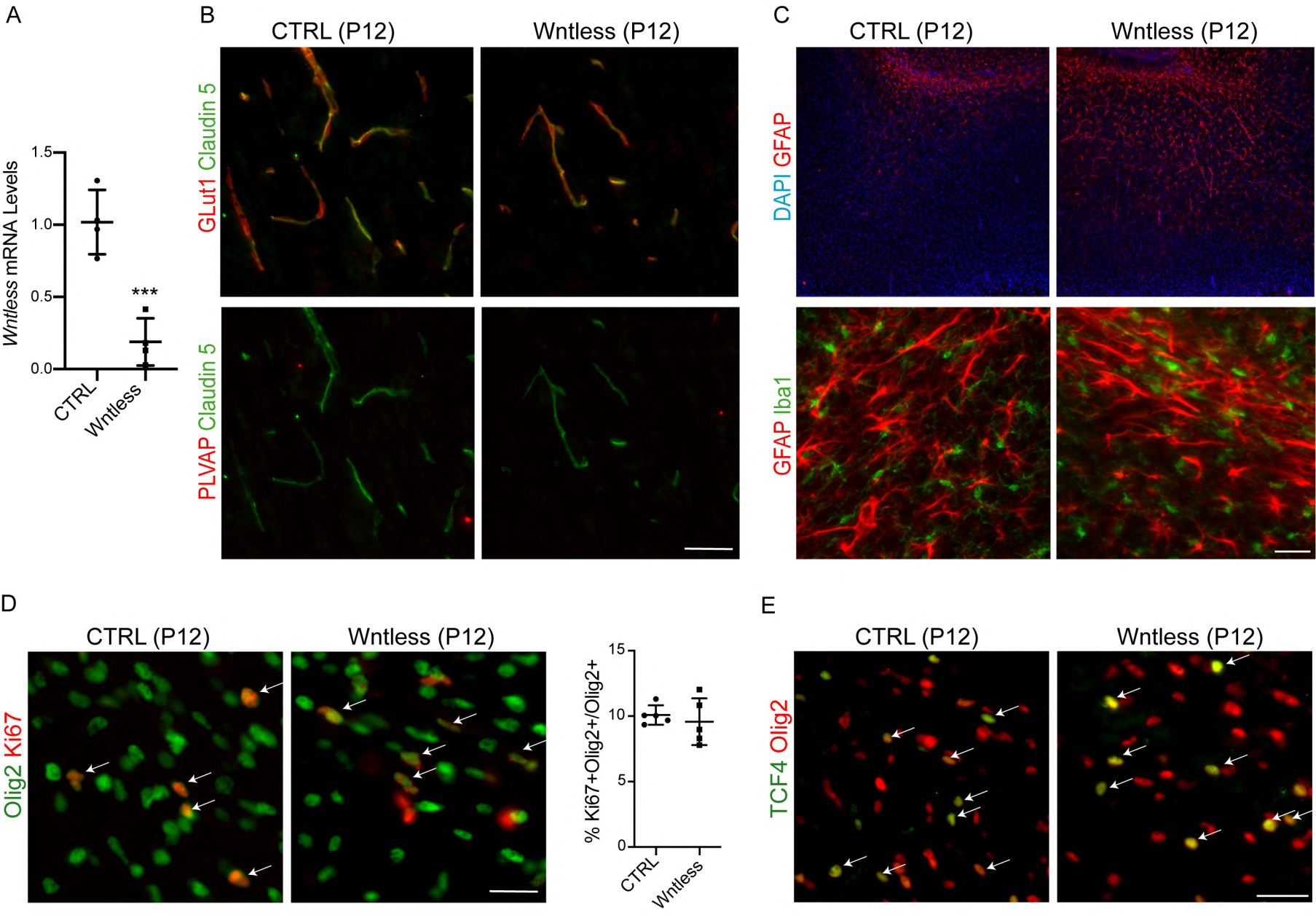
(E) RNAScope positive (*PPIB;* top panel) and negative (*dapB;* bottom panel) controls from human brain tissue. Scale bar: 25μm.

(F) Endothelial cells in the white matter lesions of HIE cases showed higher Lef1+ expression. Scale bar: 50μm.

(G) Increase in Lef1+ endothelial cells in white matter tracts of ferrets reared in hypoxia. Scale bar: 50μm.

(H) Quantification of ERG+ and Lef1+ endothelial cells in normoxic and hypoxic ferret brain white matter tracts. Data are represented as mean $\pm$ S.D. and analyzed by two-tailed unpaired Student's t test. n=3 animals per condition for data represented in (H). Significance between two groups are shown as *$p<0.05$; n.s. not significant.

Supplementary Figure 6



Supplementary Figure 6: Additional Characterization of *Wntless* cKO Animals. (Related to Figures 5, 6 and 7)

(A) Significant decrease in *Wntless* mRNA expression levels in P3 *Wntless* cKO white matter tissue isolates.

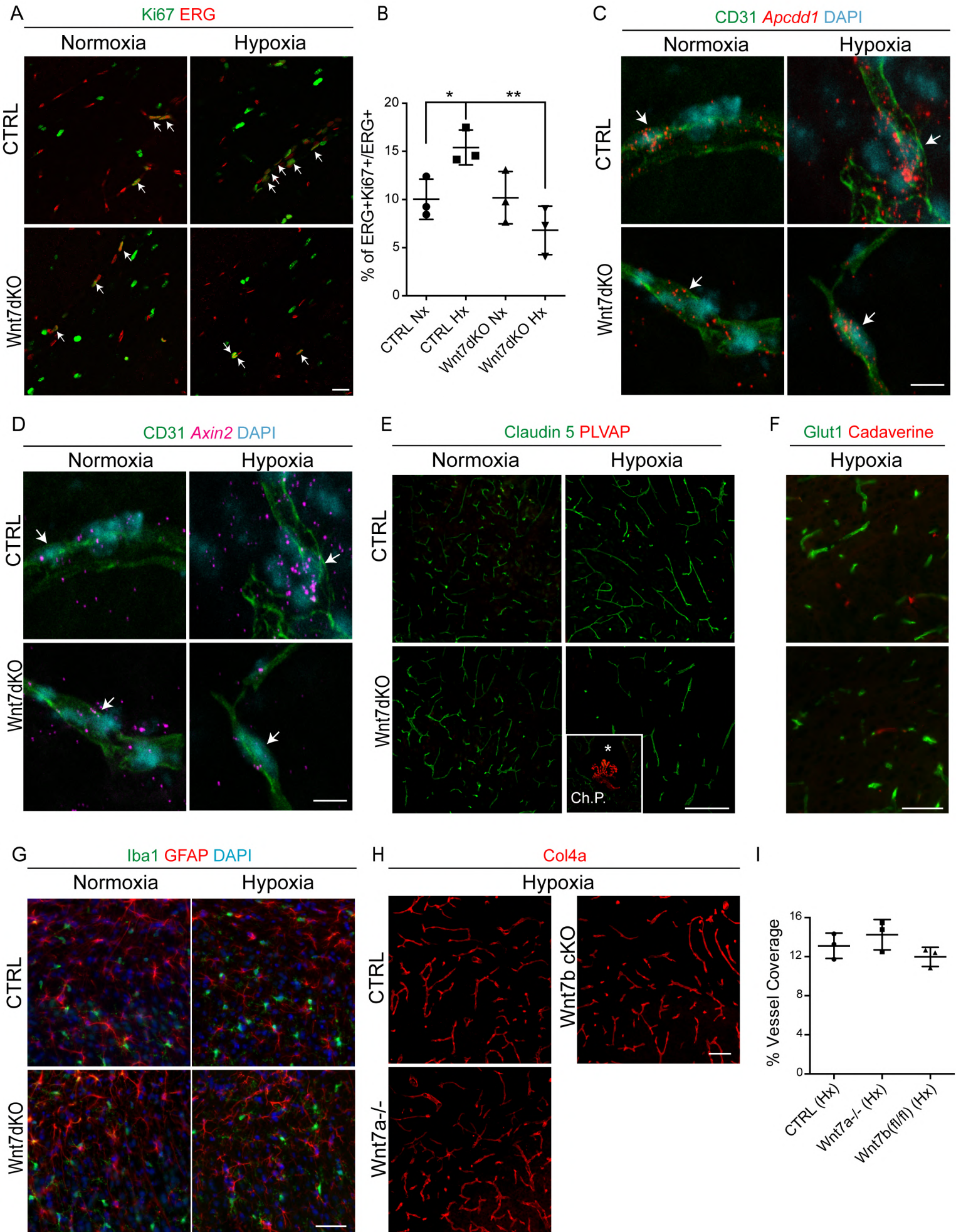
(B) Immunostaining for Claudin 5, Glut1 (top panels) and PLVAP (bottom panels) did not reveal any blood brain barrier (BBB) disruptions in *Wntless* cKO brain. Scale bar: 50μm.

(C) Mild astroglial reactivity was noticed in the *Wntless* cKO forebrain white matter region but microglial inflammatory response was not observed. Scale bar: 50μm.

(D) Proliferation of oligodendroglial cells is not affected in *Wntless* cKO white matter at P12. Scale bar: 25μm.

(E) Oligodendroglial TCF4 expression is not affected in *Wntless* cKO white matter. Scale bar: 25μm. Data are represented as mean $\pm$ S.D. and analyzed by two-tailed unpaired Student's t test, n=4 animals/genotype for data represented in (A) and n=5 for (D). Significance between two groups are shown as ***p<0.001

Supplementary Figure 7



Supplementary Figure 7: Additional Characterization and Controls Showing Oligodendroglial Wnt7a/b are Required for White Matter Vessel Growth. (Related to Figure 8)

(A) Hypoxia induced angiogenesis in white matter is associated with endothelial cell proliferation as revealed by increase in ERG+Ki67+ cells. Note, *Wnt7dKO* animals reared in hypoxia do not exhibit a similar proliferatory response observed in controls. Scale bar: 25μm.

(B) Quantification of endothelial cell proliferation (ERG+Ki67+) in control and *Wnt7dKO* animals reared in normoxic and hypoxic conditions.

(C-D) Multiplex smFISH to label *Apcdd1* (C) and *Axin2* (D) reveals hypoxia induced increase in downstream Wnt signaling in endothelial cells of white matter at P11. Note this response was reduced in *Wnt7dKO* animals reared in hypoxia. Scale bar: 5 μm.

(E) Immunostaining for PLVAP and Claudin 5 did not reveal any BBB disruptions in *Wnt7dKO* animals. Inset in bottom right panel shows PLVAP expression in choroid plexus vessels from the same section, confirming successful PLVAP labelling. Scale bar: 50μm.

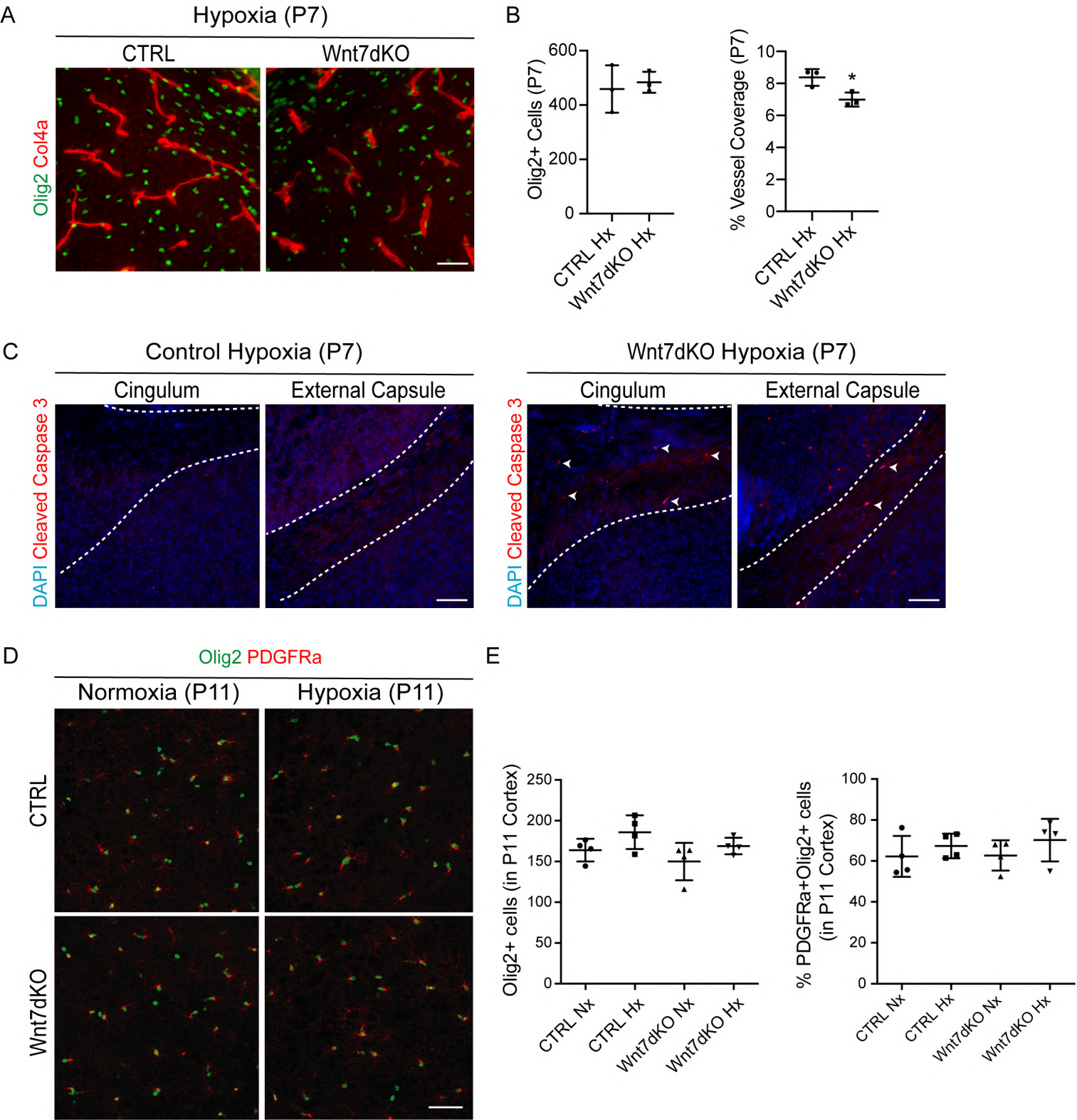
(F) Representative images from corpus collosum region of Cadaverine-555 injected control and *Wnt7dKO* animals labelled with Glut1. Note no leakage of cadaverine-555 in *Wnt7dKO* animals, indicating no damage to BBB. Scale bar: 50μm.

(G) No alterations were observed in inflammatory responses (Iba1) or astroglial reactivity (GFAP) in hypoxic *Wnt7dKO* animals. Scale bar: 50μm.

(H) Genetic ablation of *Wnt7a* or *Wnt7b* alone do not contribute to hinderance in hypoxia induced white matter angiogenesis as revealed by CD31 immunostaining. Scale bar: 50μm

(I) Quantification of white matter vessel coverage in Control, *Wnt7a-/-* and *Olig2-Cre/Wnt7b (fl/fl)* animals reared in hypoxia. Data are represented as mean $\pm$ S.D. and analyzed by two-tailed unpaired Student's t test. n=3 animals/genotype per condition for data represented in (B) and (I). Significance between two groups are shown as *p<0.05, ***p<0.001.

Supplementary Figure 8



Supplementary Figure 8: Additional Characterization of White Matter and Cortical Oligodendroglial Development in *Wnt7dKO* Mutants. (Related to Figure 8)
(A) Representative images from P7 white matter region of control and *Wnt7dKO* animals reared in hypoxia showing Olig2+ oligodendroglial cells and Col4a+ blood vessels. Note a decrease in blood vessel density compared to control. Scale bar: 50μm.
(B) Quantification of P7 white matter Olig2+ cell numbers and vessel coverage.
(C) Hypoxic *Wnt7dKO* animals show increase in apoptosis marker, cleaved caspase 3 in white matter regions, cingulum and external capsule at P7 compared to control animals reared in hypoxia. Scale bar: 50μm.
(D) Cortical PDGFRa+Olig2+ oligodendroglial precursor cell numbers were not affected by hypoxia in *Wnt7dKO* animals. Scale bar: 50μm.
(E) Quantification of Olig2+ oligodendroglial and PDGFRa+Olig2+ oligodendroglial precursor cell numbers in cortex. Data are represented as mean $\pm$ S.D. and are analyzed by two-tailed unpaired Student's t test and n=3 animals/genotype per condition for data represented in (B) and n=4 for (E). Significance between two groups are shown as *$p<0.05$.

Supplementary Table 1: Human postmortem cases analyzed in this study (Related to Figure 4).

Identification	Neuropathology	Gestational age	Postnatal age	Sex	Clinical Diagnosis
UCSF 2010-008	Control	36 Weeks	0 Days	F	Diaphragmatic Hernia
UCSF 2010-013	Control	37 Weeks	2 Days	F	Pneumothorax
UCSF 2010-005	Control	40 Weeks	0 Days	M	Diaphragmatic Hernia
UCSF 2010-012	Control	40 Weeks	2 Days	M	Diaphragmatic Hernia
UCSF 2017-032	Control	37 Weeks	2 Days	M	Hydronephrosis
UCSF 2012-003	Control	36 Weeks	10 Days	M	VATER malformation
UCSF 2010-019	HIE	35 Weeks	6 Days	M	Unknown/IVH
UCSF 2011-005	HIE	40 Weeks	3 Days	M	Placental abruption
UCSF 2013-008	HIE	39 Weeks	1 day	M	Fetal Bradycardia
UCSF 2010-016	HIE	37 Weeks	5 Days	F	Birth Asphyxia
UCSF 2017-027	HIE	40 Weeks	9 Days	F	Pulmonary hypertension
UCSF 2012-001	HIE	38 Weeks	3 Days	F	Birth Asphyxia
UCSF 2016-004	Control	17 Weeks	0 Days	M	Tetralogy of fallot

Supplementary Table 2: Primer sequences used in this study. (Related to Figures 5 and 6)

Plvap	F	GTTGACTACGCGACGTGAGATG
	R	AGCTGTTCCTGGCACTGCTTCT
Claudin 5	F	TGACTGCCTTCCTGGACCACAA
	R	CATACACCTTGCACTGCATGTGC
Zic3	F	CACACTGGCGAGAAACCCTTCC
	R	GTTGGCAAACCGTCTGTCACAG
Foxf2	F	CCAGCATGTCTTCCTACTCGTTG
	R	CTTTCCTGTCGCACACTGGAGT
Mfsd2a	F	GGTCTCAGAAGTTGCCAATCGC
	R	GAAGGCACAGAGGACGTAGATG

Vegfr2	F	CGAGACCATTGAAGTGACTTGCC
	R	TTCCTCACCCTGCGGATAGTCA
Dll4	F	GGGTCCAGTTATGCCTGCGAAT
	R	TTCGGCTTGGACCTCTGTTCAG
Apln	F	AGGCATAGCGTCCTCACCTCTT
	R	GGTGCAGAAACGACAAAGACGG
Vegfa	F	CTGCTGTAACGATGAAGCCCTG
	R	GCTGTAGGAAGCTCATCTCTCC
Ndp	F	CTGATGGACTCTCAACGCTGCA
	R	CTCAGAGCGTGATGCCTGGCT
Sema3e	F	CACTGTGCCTTCATCAGAGTCG
	R	CCAACTAGCGTGGACACAAAGG
Ntn1	F	GTCTGGTGTGTGACTGTAGGCA
	R	CCGAGCATGGAGGTTGCAGTTG
Wntless	F	TTGCTGTTGGCTCCTTCTGCCT
	R	GGCAGATACCTGCCACAATGATG