SUPPLEMENTAL MATERIAL (Supplemental Figures 1 to 8)

"Molecular and Genetic Analysis of Collagen Type IV Mutant Mouse Models of Spontaneous Intracerebral Hemorrhage Identify Mechanisms for Stroke Prevention"

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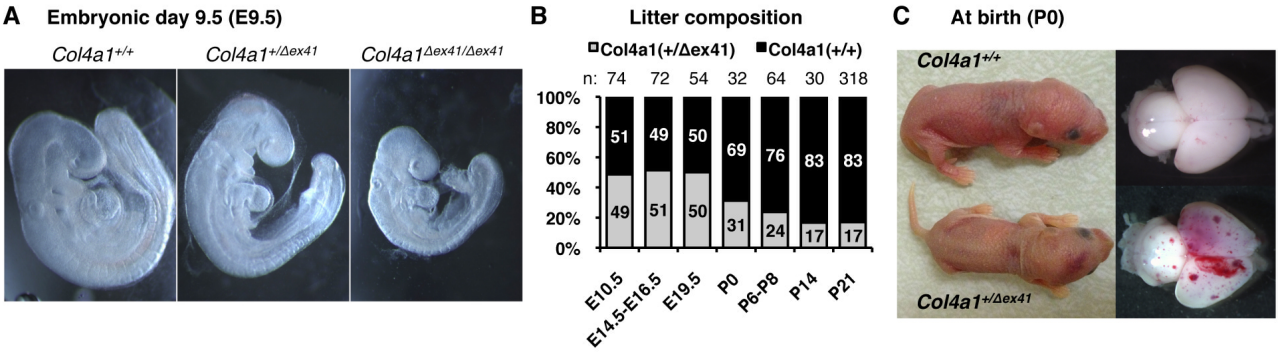
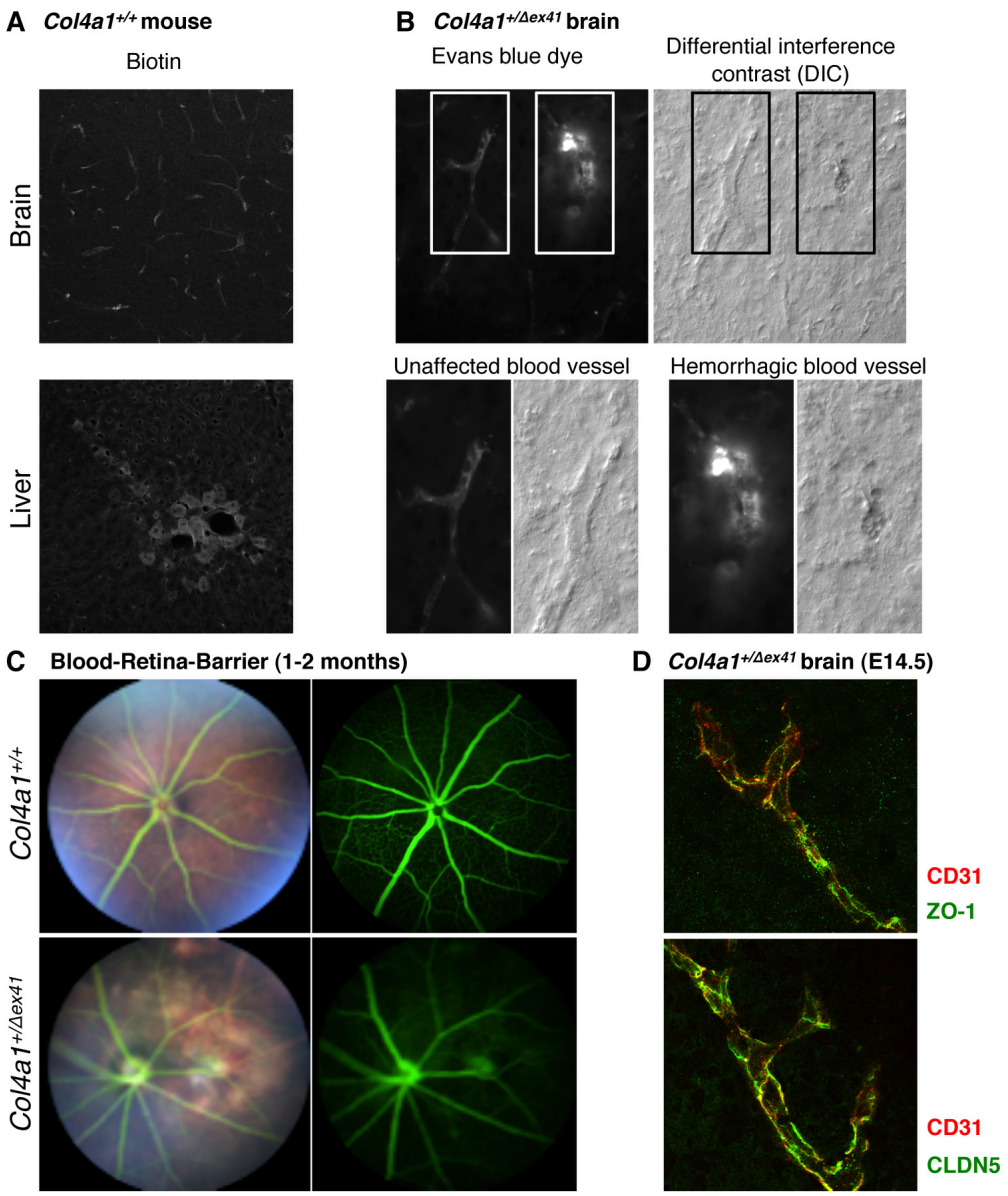
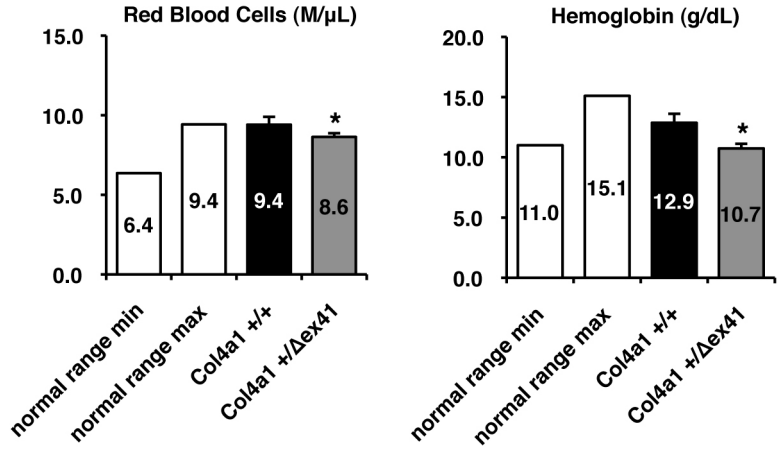


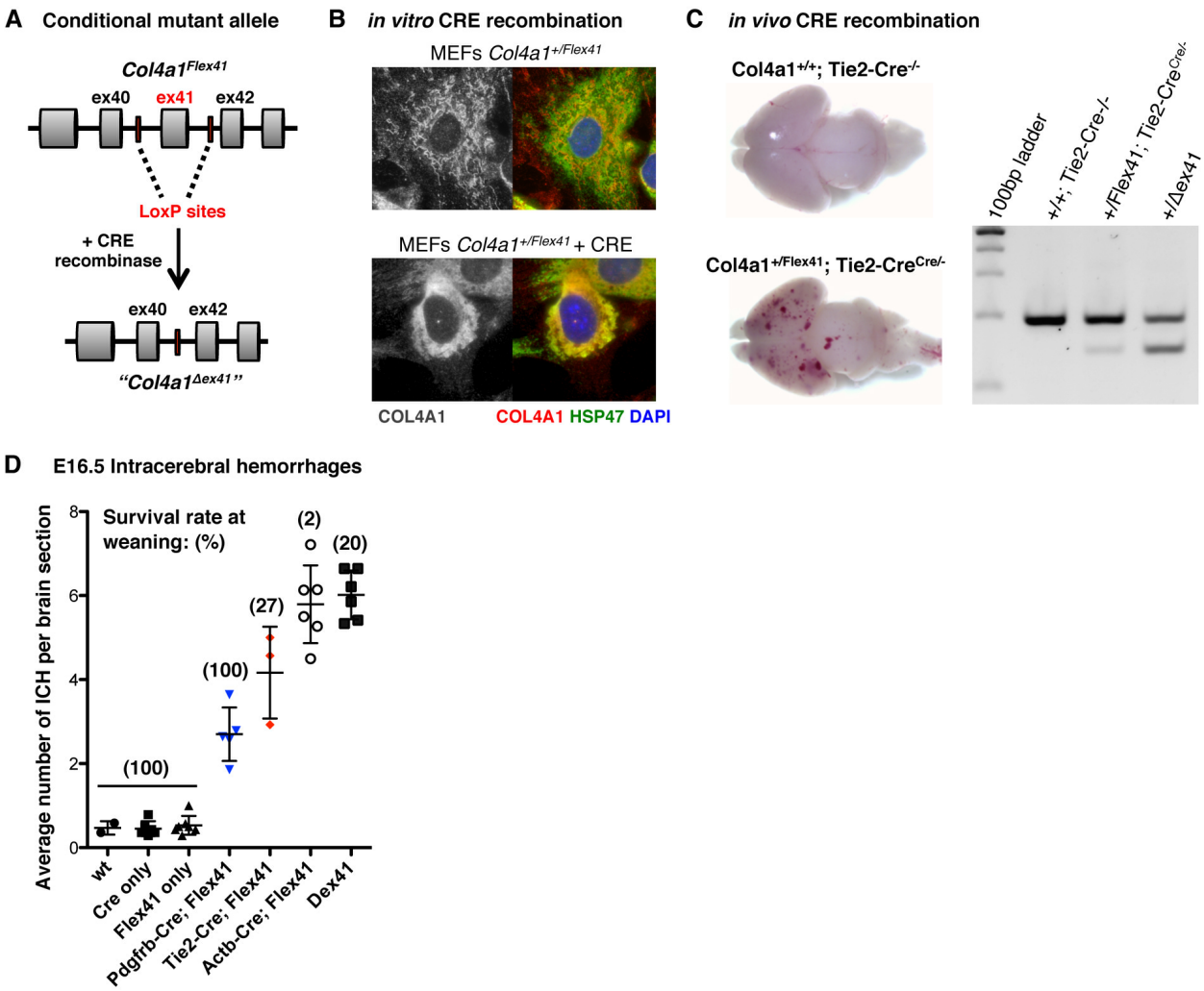
Supplemental Figure 1: *Col4a1*$^{\Delta ex41}$ causes embryonic growth retardation, reduced viability and multifocal hemorrhages (**A**) Growth retardation is observed in *Col4a1*$^{+/\Delta ex41}$ mutants as early as embryonic day (E) 9.5 and is more pronounced in homozygous *Col4a1*$^{\Delta ex41/\Delta ex41}$ mutants, which do not survive after mid-gestation. (**B**) In backcross matings, mutant embryos are represented at expected ratios of 1:1 until birth when *Col4a1*$^{+/\Delta ex41}$ mice have reduced viability (n: number of animals for each time point). (**C**) At birth (postnatal day 0: P0), *Col4a1*$^{+/\Delta ex41}$ pups have multi-focal subcutaneous hematomas and intraparenchymal hemorrhages.



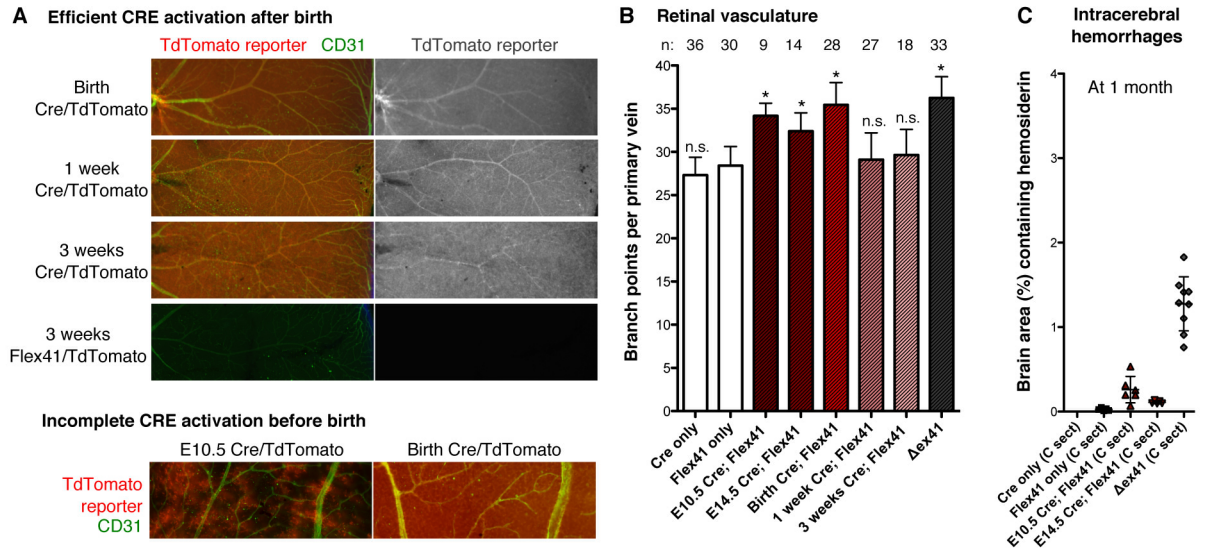
Supplemental Figure 2: Blood-brain-barrier and blood-retina-barrier are not compromised in *Col4a1*$^{+/\Delta ex41}$ animals (**A**) As a positive control for the experiment presented in Figure 1 (D), we show that biotin diffuses from the blood vessel into the liver (bottom) whereas it stays inside the cerebral vasculature (top) because of the presence of the blood-brain-barrier in a *Col4a1*$^{+/+}$ mouse (**B**) In *Col4a1*$^{+/\Delta ex41}$ mice, intracardiac perfusion of biotin revealed that the blood-brain-barrier is intact (unaffected blood vessel) until the vasculature ruptures, leading to hemorrhage and leakage of biotin in the surrounding parenchyma (hemorrhagic blood vessel) (**C**) Intra-peritoneal injection of sodium fluorescein reveals no general leakage of the *Col4a1*$^{+/\Delta ex41}$ blood-retina-barrier on ocular fundus examination. (**D**) Blood-brain-barrier proteins such as zona occludens protein 1 (ZO-1) and Claudin 5 (CLDN5) (labeled in green) are expressed in *Col4a1*$^{+/\Delta ex41}$ cerebral blood vessels (labeled with CD31, red) as early as E14.5.



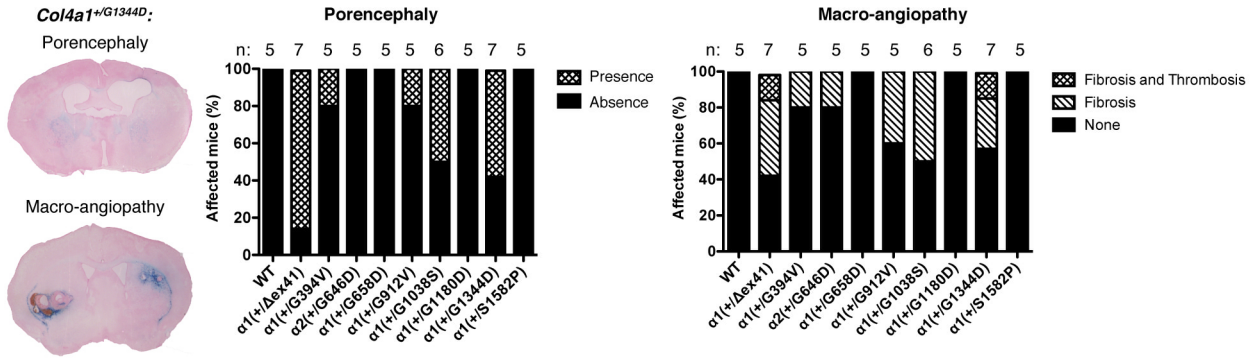
Supplemental Figure 3: *Col4a1*$^{+/\Delta ex41}$ mice have anemia. Blood analysis at 4 months shows decreased number of red blood cells and abnormally low hemoglobin level in *Col4a1*$^{+/\Delta ex41}$ (n=5) compared to *Col4a1*$^{+/+}$ mice (n=9). Data are reported as mean + standard deviation. *: $p<0.05$ compared to *Col4a1*$^{+/+}$ mice by Student's t-test.



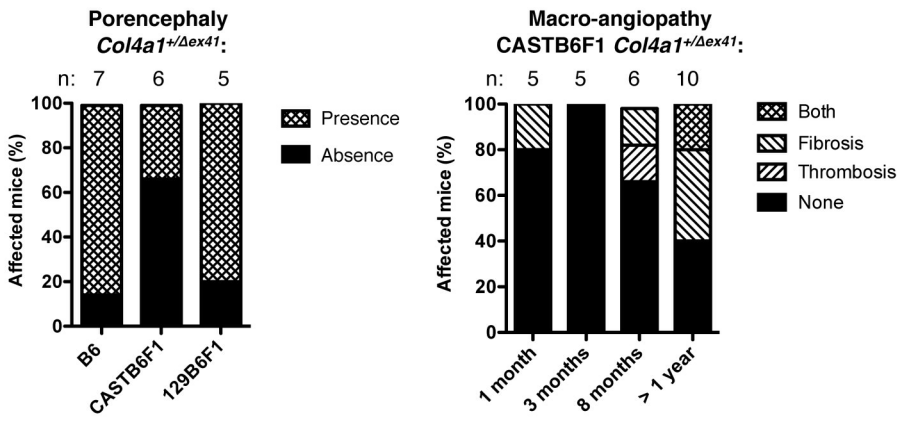
Supplemental Figure 4: The *Col4a1*^{Flex41} conditional mutant allele in presence of active CRE recombinase mimics the *Col4a1*$^{\Delta ex41}$ mutation (**A**) The *Col4a1*^{Flex41} conditional mutant allele has exon 41 flanked by *LoxP* sites. In the presence of active CRE recombinase, exon 41 is removed, mimicking pathogenic effects of the *Col4a1*$^{\Delta ex41}$ mutation such as (**B**) intracellular accumulation of collagen in the endoplasmic reticulum (ER) (MEFs: primary mouse embryonic fibroblasts, HSP47: ER marker). (**C**) Compared to littermate controls that did not have hemorrhage, double heterozygous *Col4a1*$^{+/Flex41}$; *Tie2-Cre*$^{CRE/-}$ (endothelial-specific expression of CRE) P0 pups showed perinatal multi–focal intracerebral hemorrhage (ICH). cDNA from whole brain amplified with primers in *Col4a1* exons 40 and 42 detected expression of transcripts missing exon 41 (51 nucleotides) in *Col4a1*$^{+/Flex41}$ mice when CRE is present (lower band). (**D**) Average number of ICHs per brain section in E16.5 embryos. *Col4a1*$^{+/Flex41}$; *Actb-Cre*$^{CRE/-}$ embryos, expressing CRE recombinase ubiquitously have as many ICH as *Col4a1*$^{+/\Delta ex41}$ embryos. The survival rate at weaning age is indicated (%). Data are reported as mean ± standard deviation.



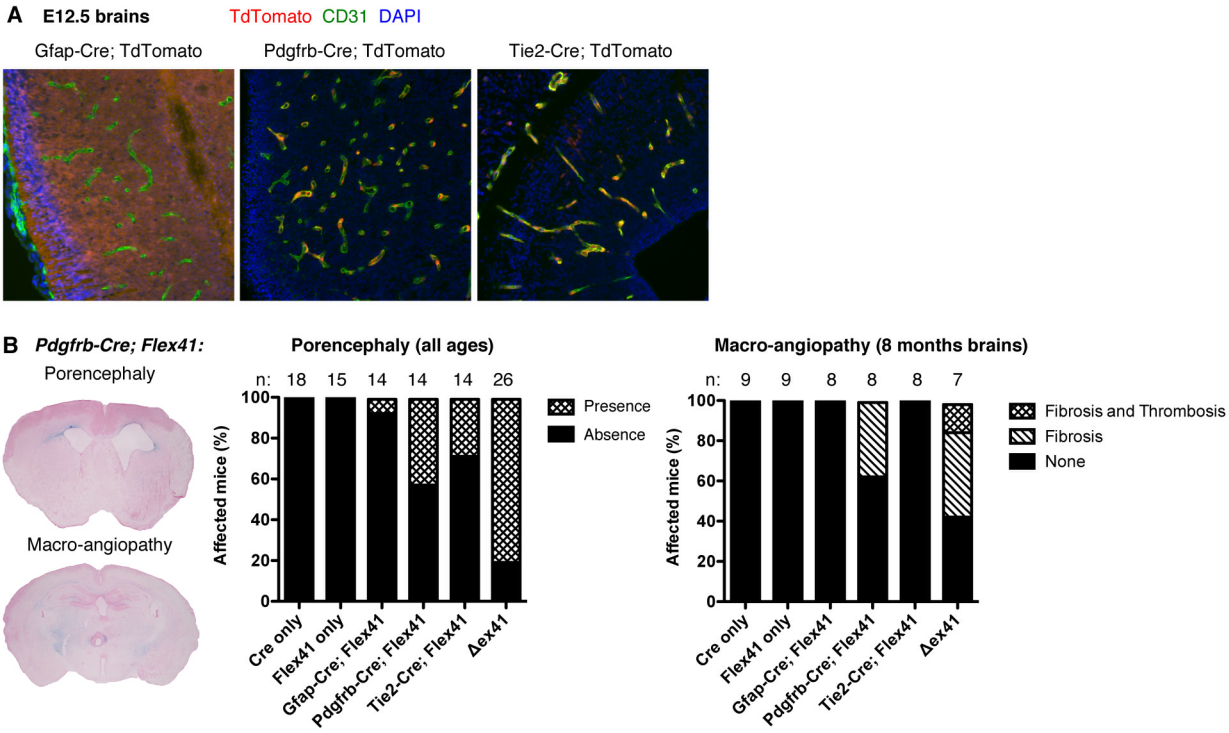
Supplemental Figure 5: Tamoxifen induces ubiquitous CRE activation when injected after birth, but only partial CRE activation when injected during gestation (**A**) We evaluated the efficiency of CRE activation by tamoxifen using the TdTomato reporter mouse strain. Postnatal tamoxifen injection achieved ostensibly complete recombination whereas prenatal tamoxifen injection did not (TdTomato in red, CD31 in green) (**B**) Quantification of retinal vein branch points showed excess branching when tamoxifen was delivered before or at birth but not after one week (when retinal vascular development is completed) confirming that tamoxifen delivery and mutant *Col4a1* induction was successful (9≤n≤36). n.s.: p>0.05, *: p<0.05 compared to Flex41 only (*Col4a1*$^{+/Flex41}$; *R26CreER*$^{-/-}$) mice by Student's *t*-test. (**C**) Intracerebral hemorrhage (ICH) quantification of brains from 1-month old mice showed that mutant *Col4a1* induction at E10.5 or E14.5 caused ICH and that earlier induction led to more ICH. Tamoxifen administration compromised natural birth so all liters were surgically delivered (C sect). The observation that E10.5 induction did not phenocopy *Col4a1*$^{+/Δex41}$ mice likely reflects the reduced recombination efficiency shown in A. Data are reported as mean + (B) or ± (C) standard deviation.



Supplemental Figure 6: Porencephaly and macro-angiopathy in an allelic series of *Col4a1* and *Col4a2* mutations. We analyzed 27 regularly spaced coronal cryo-sections per brain (n=5-7 mice per genotype) to determine the frequencies of porencephaly and macro-angiopathy in mice from the allelic series.



Supplemental Figure 7: CASTB6F1 genetic context prevents or delays cerebrovascular disease caused by *Col4a1*$^{\Delta ex41}$ mutation. The penetrance of porencephaly in *Col4a1*$^{+/\Delta ex41}$ mice was reduced by CASTB6F1 but not 129B6F1 genetic context. CASTB6F1 also prevents or delays macro-angiopathy in *Col4a1*$^{+/\Delta ex41}$ mice. C57BL/6J (B6), CAST/EiJ (CAST), 129S6/SvEvTac (129) (n: number of animals in each cohort).



Supplemental Figure 8: Cell type specific expression of mutant COL4A1 (**A**) Cell type specific CRE mouse strains were crossed with a TdTomato reporter strain to show the specificity of the CRE expression: in astrocytes, in pericytes and in vascular endothelial cells (VECs) for *Gfap-Cre*, *Pdgfrb-Cre* and *Tie2-Cre* respectively (CD31 labeled the VECs in green, TdTomato is red in cells expressing the CRE, brain sections of embryonic day 12.5 embryos) (**B**) Porencephaly and macro-angiopathy penetrance were quantified using serial coronal brain sections (n: number of animals in each cohort).