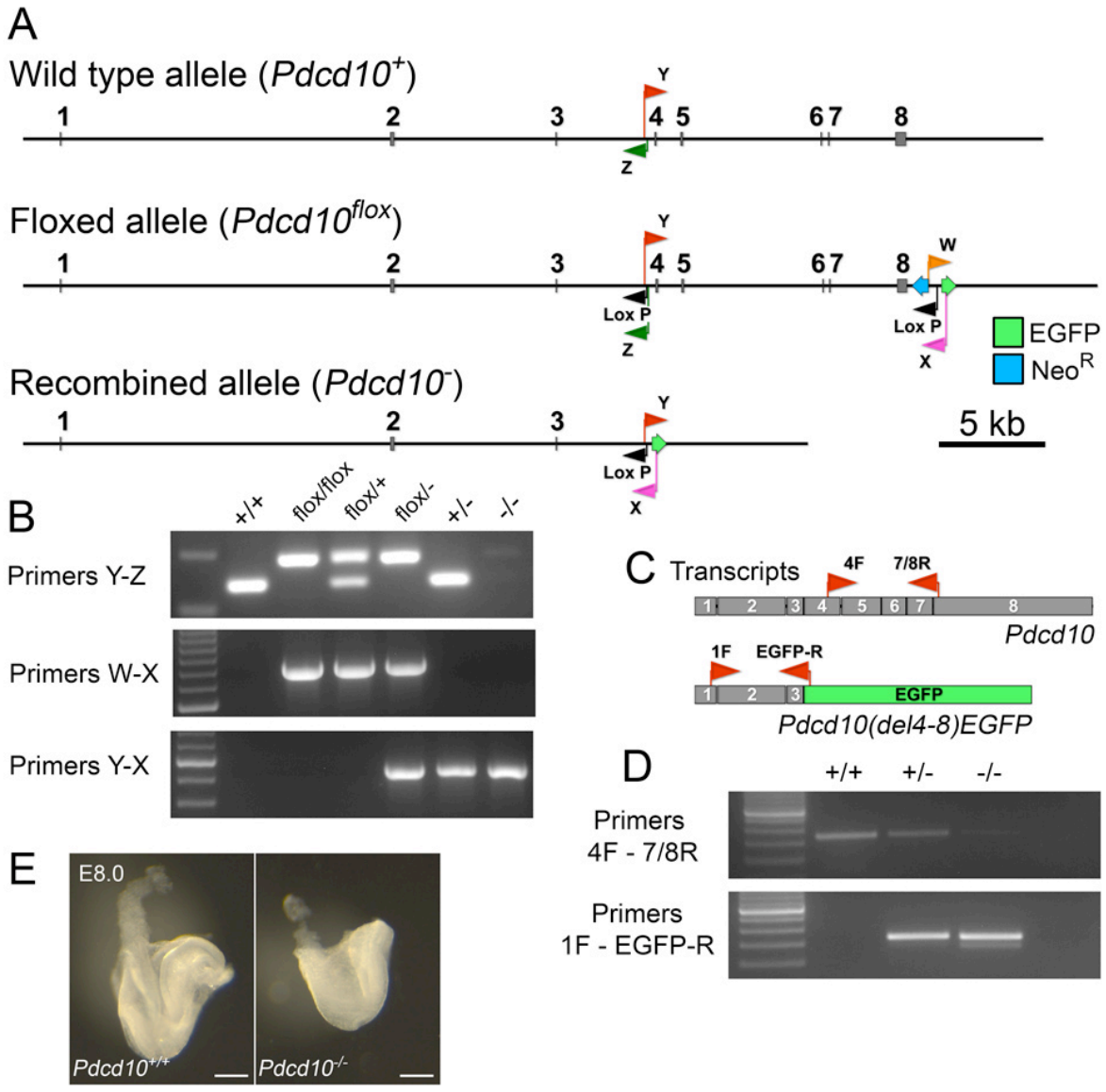
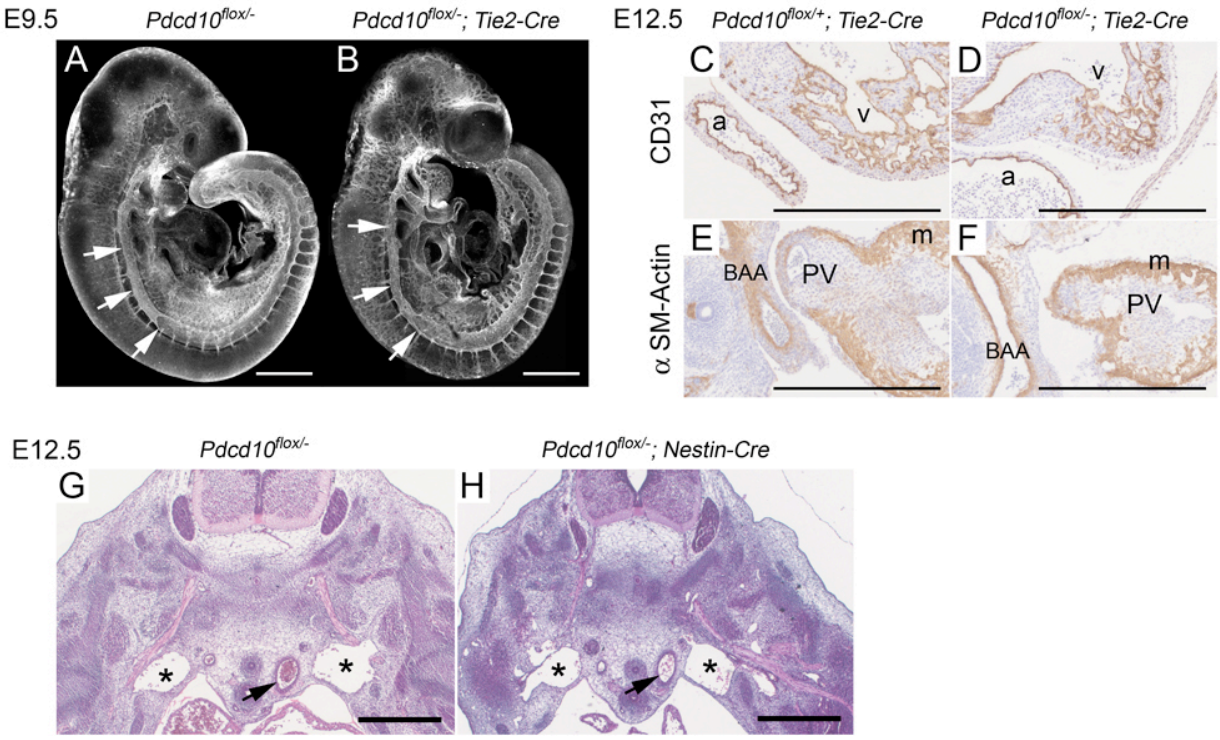
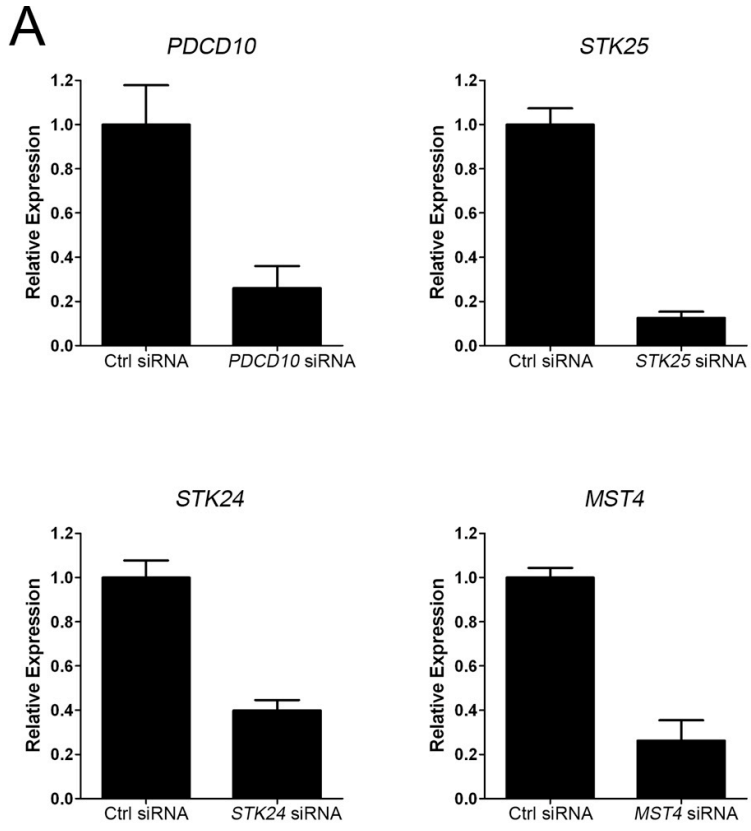
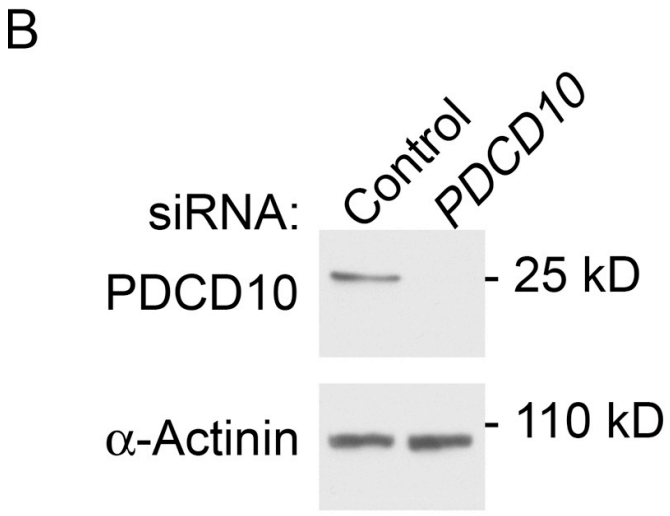


Supplemental Figure 1. Knockout design for *Pdcd10*. Ubiquitous deletion of *Pdcd10* results in early embryonic death. (**A**) Alleles of *Pdcd10* are shown. The floxed allele resulting from the targeting strategy includes loxP sites flanking exons 4-8 and places the enhanced green fluorescent protein (EGFP) in position to splice onto exon 3 in a fusion transcript resulting from the recombined allele. Genotyping primers are indicated by the letters W, X, Y and Z. (**B**) Genotyping by PCR using combinations of primers W, X, Y, and Z distinguishes the possible genotypes. (**C**) The wildtype and fusion transcripts are illustrated with exons outlined. The location of primers for RT-PCR are shown. (**D**) Results of RT-PCR performed on cDNA from embryos pooled by genotype that resulted from a mating of $Pdcd10^{+/-}$ vs. $Pdcd10^{+/-}$ mice. (**E**) Gross photos of E8.0 mouse embryos on dissection. Wildtype is shown in (**E**, left panel), *Pdcd10* knockout in (**E**, right panel). Even at this early stage, the knockout embryo has growth arrested and is smaller than its wildtype littermate. Scale bars = 200 μm.

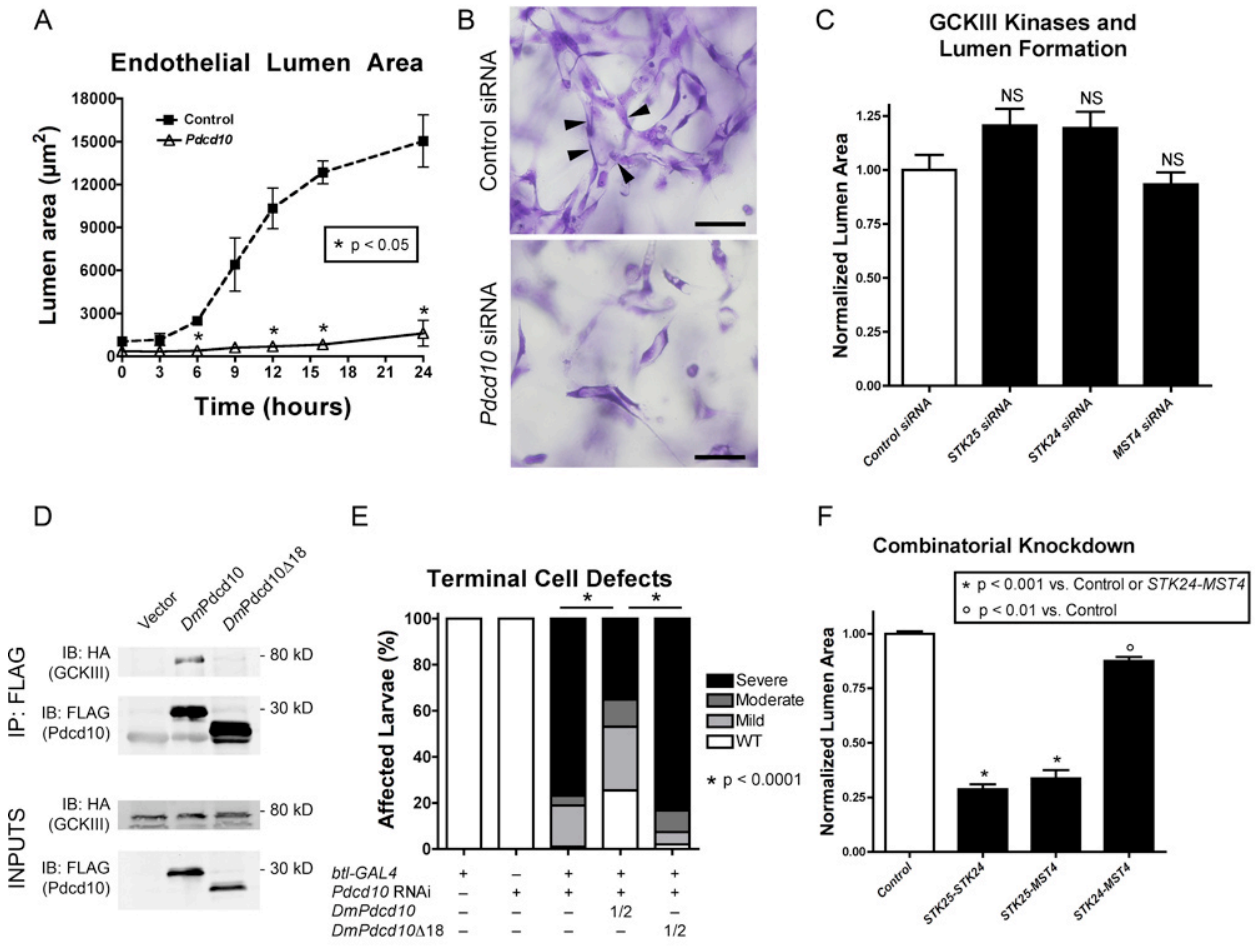


Supplemental Figure 2. Endothelial knockout of *Pdcd10* does not affect establishment of circulation or cardiac structure. Neural knockout of *Pdcd10* is viable. (**A-B**) Whole-mount fluorescent staining for CD31 in E9.5 *Pdcd10*$^{flox/-}$ (**A**) and *Pdcd10*$^{flox/-}$*; Tie2-Cre* (**B**) embryos. Arrows denote the dorsal aorta. (**C-D**) Hematoxylin and CD31 staining in E12.5 *Pdcd10*$^{flox/+}$*; Tie2-Cre* (**C**) and *Pdcd10*$^{flox/-}$*; Tie2-Cre* (**D**) embryos. (**E-F**) Hematoxylin and α-smooth muscle actin staining in E12.5 *Pdcd10*$^{flox/+}$*; Tie2-Cre* (**E**) and *Pdcd10*$^{flox/-}$*; Tie2-Cre* (**F**) embryos. a, atrium; v, ventricle; BAA, branchial arch artery; m, myocardium; PV, pulmonary valve. (**G-H**) Hematoxylin and eosin staining in E12.5 *Pdcd10*$^{flox/+}$*; Nestin-Cre* (**G**) and *Pdcd10*$^{flox/-}$*; Nestin-Cre* (**H**) embryos. Asterisks denote the cardinal veins. Arrows denote the dorsal aorta. Scale bars = 500 μm.

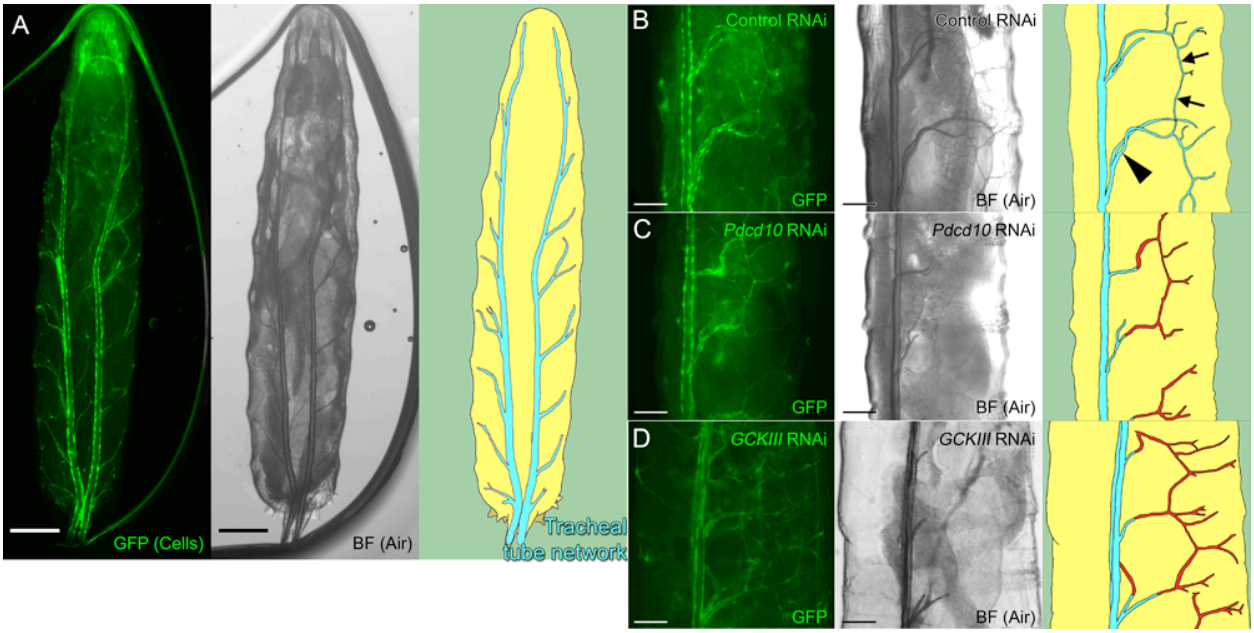




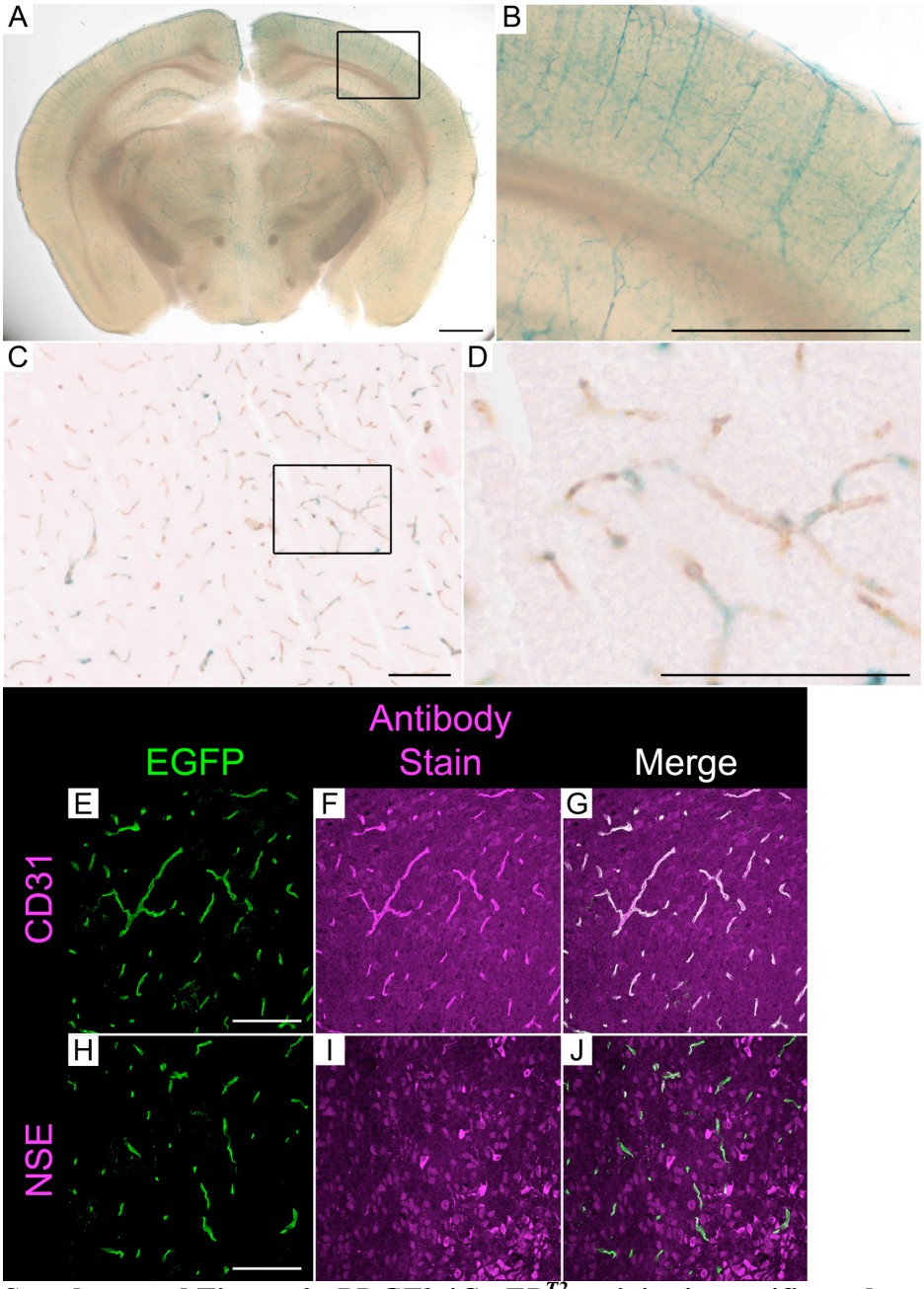
Supplemental Figure 3. siRNA effectively reduces levels of *PDCD10*, *STK25*, *STK24*, and *MST4*. (**A**) Quantitative PCR to detect *PDCD10*, *STK25*, *STK24*, and *MST4* was performed on cDNA made from the RNA of human endothelial cells to assess knockdown. (**B**) Western blot for PDCD10 to confirm knockdown.



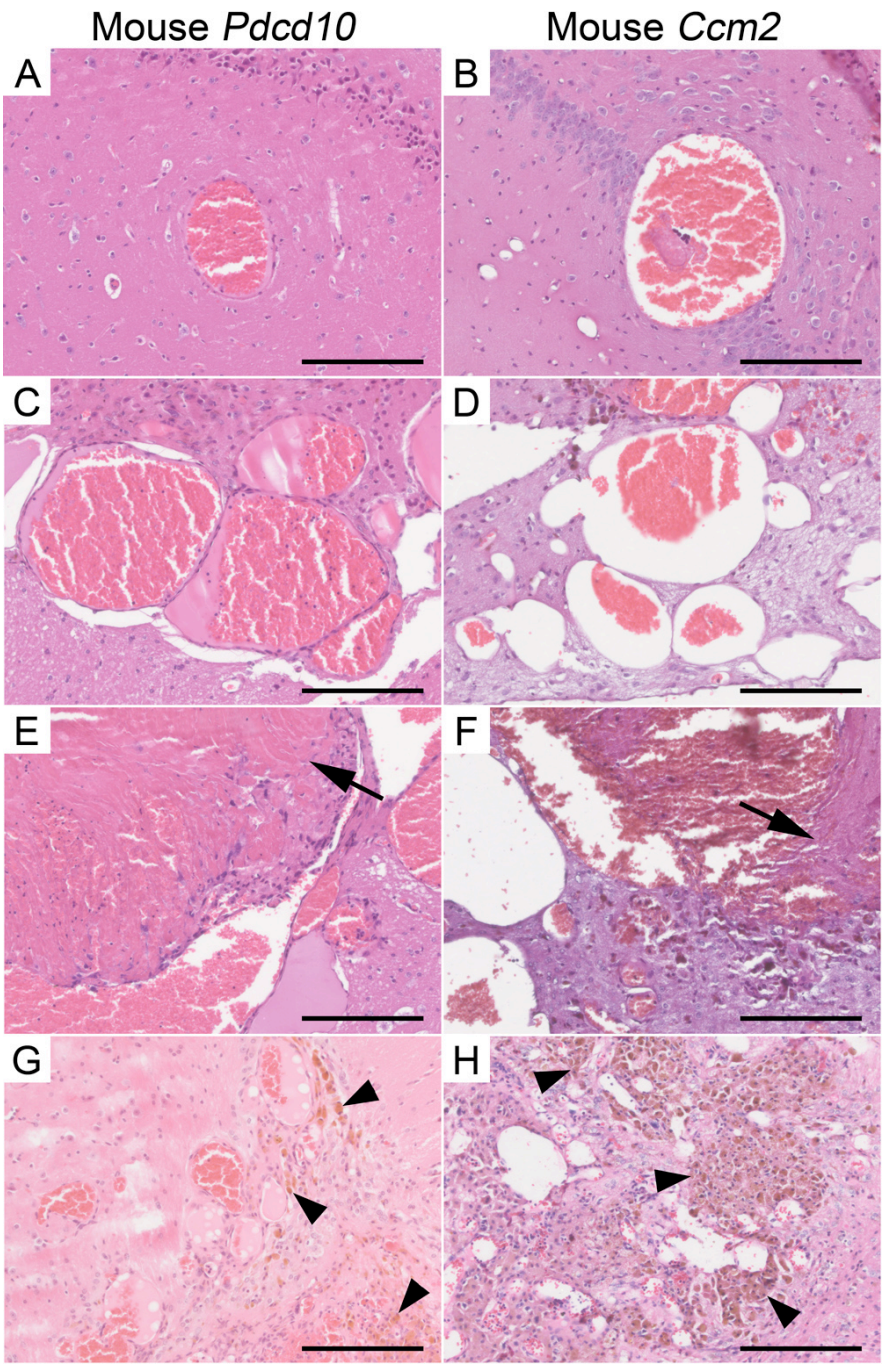
Supplemental Figure 4. Pdcd10 signals through GCKIII kinases in lumen formation. (**A**) Quantification of lumen area over time for human umbilical vein endothelial cells after treatment with *PDCD10* siRNA or control siRNA directed against Luciferase. (**B**) Toluidine blue staining of lumen formation assay. Lumens are shown with arrowheads. (**C**) Quantification of lumen area at 24 hours for cells treated with siRNA directed against each of the GCKIII kinases or Luciferase control (5 fields per time point in 3 independent experiments). (**D**) Immunoprecipitation for HA-tagged *Drosophila* GCKIII using full length *Drosophila* Pdcd10 (*Dm*Pdcd10) or *Drosophila* Pdcd10 with 18 amino acid deletion (*Dm*Pdcd10Δ18). Results are representative of 3 independent experiments. (**E**) Quantification of tracheal tube lumen formation defects in *Drosophila* with RNAi knockdown of *Pdcd10* with or without rescue constructs (N ≥ 64 for each genotype). Note: for rescue experiments only ~50% of all larvae contained the corresponding rescue transgene since they were generated by mating rescue heterozygous males (*btl-GAL4*, *UAS-GFP/+*; *UAS-Rescue/+*) to homozygous *UAS-RNAi* virgin females. (**F**) Quantification of 3D endothelial cell lumen area with combinatorial knockdown of human GCKIII kinases (5 fields per time point in 3 independent experiments). Data indicate mean ± SD. Scale bars = 50 μm.



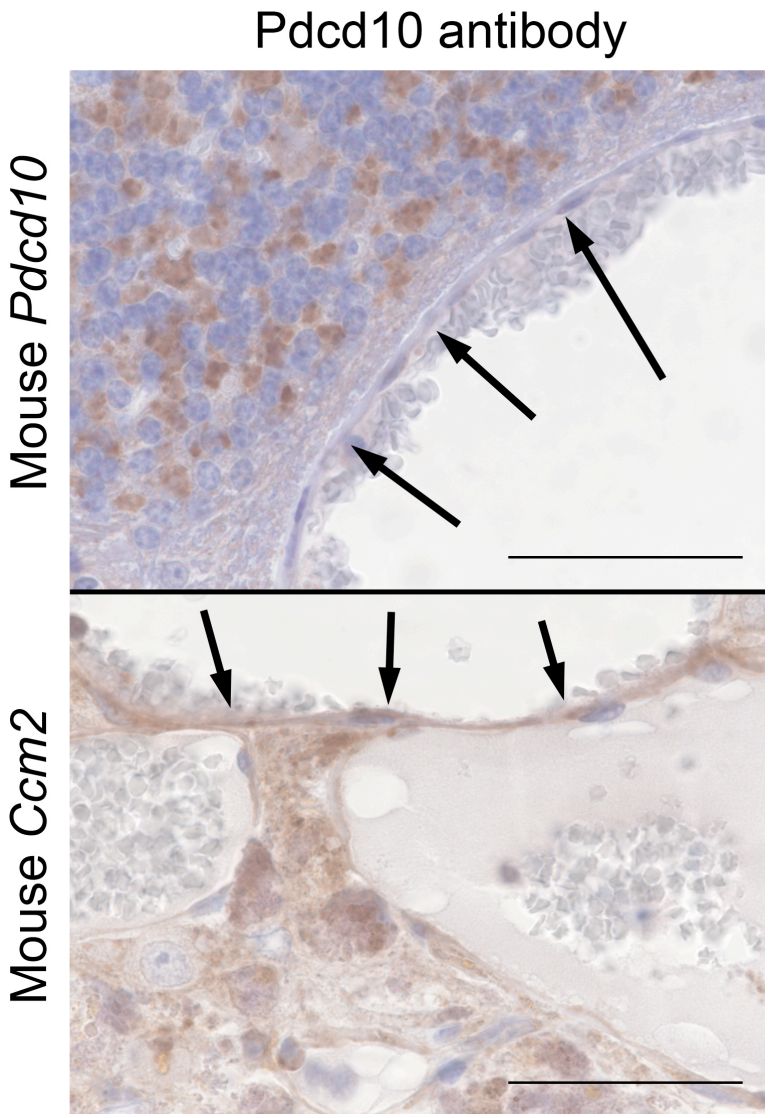
Supplemental Figure 5. Loss of Pdcd10 or GCKIII results in failure of tracheal tube lumenization in *Drosophila*. (**A**) The tracheal network of a *Drosophila* larva. A *UAS-GFP* transgene was used to label tracheal cells (left panel). Brightfield images of the same larva show air filling in lumens (middle panel). A cartoon representing the lumenized tube network (cyan) appears in the rightmost panel. (**B-D**) Tracheal tube lumen formation in flies expressing control RNAi (**B**), RNAi directed against *Pdcd10* (**C**), or RNAi directed against *GCKIII* (**D**) under the control of the tracheal-specific *btl-GAL4* driver. Arrowhead (in cartoon panel of **B**) indicates the primary trunk of a tracheal tube (where lumen truncation would indicate a severe phenotype), and arrows indicate the fine terminal branches (where lumen truncation would indicate a mild phenotype). Loss of *Pdcd10* or *GCKIII* results in similar air-filling defects. GFP expressing tracheal cells that lack air-filled lumens are outlined in red on the cartoon panels of **C-D**. Scale bars in (**A**) = 500 μm. Scale bars in (**B-D**) = 200 μm.



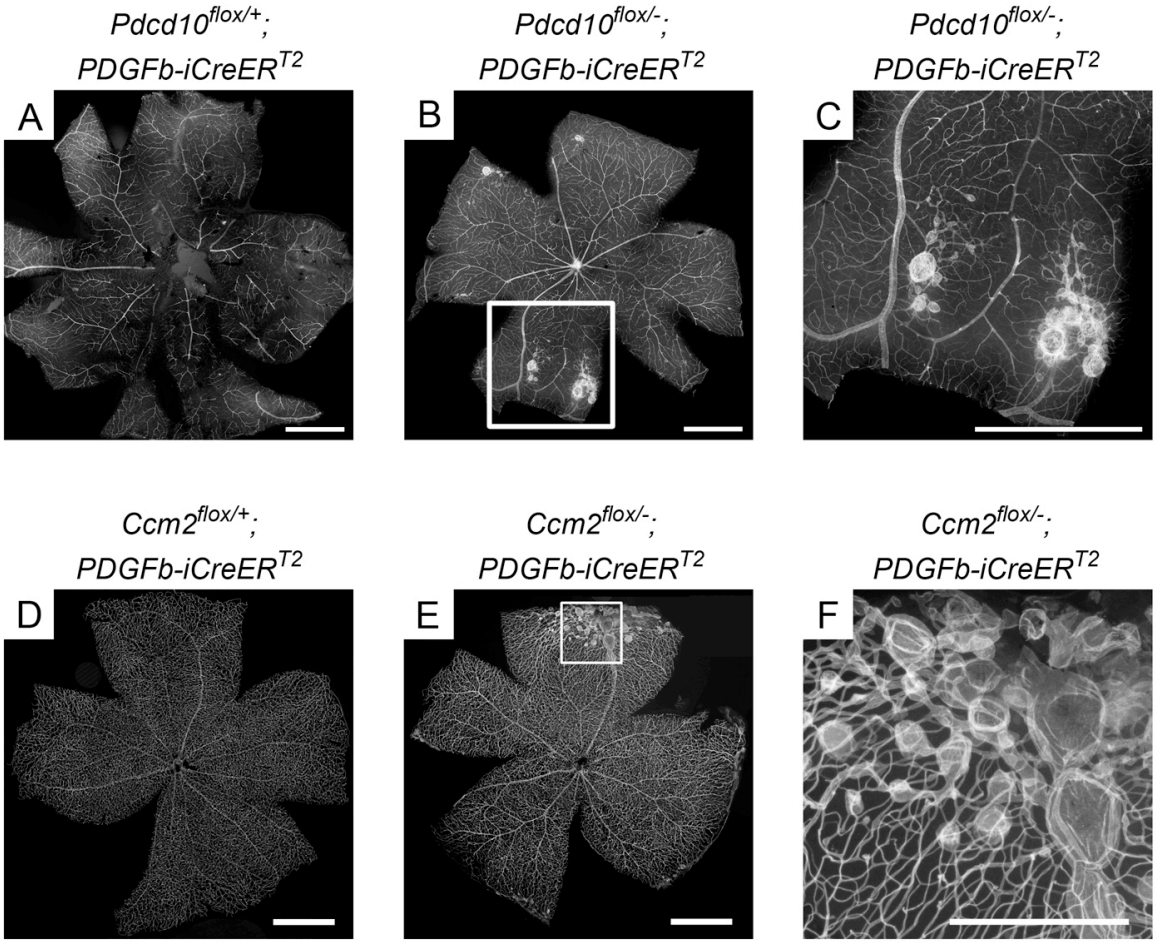
Supplemental Figure 6. *PDGFb-iCreER^{T2}* activity is specific to the endothelium. (**A**) X-gal staining (blue) of brain from a 6 month old *PDGFb-iCreER^{T2}; Rosa26-LacZ* mouse that was given tamoxifen at birth. (**B**) Close-up of the boxed area in panel (**A**). (**C-D**) X-gal (blue) and CD31 (brown) staining of brain from a 6 month old *PDGFb-iCreER^{T2}; Rosa26-LacZ* mouse that was given tamoxifen at birth. (**E-G**) CD31 (magenta) staining of brain from a 12 day old *PDGFb-iCreER^{T2}; Rosa26-ACTB-tdTomato,-EGFP* mouse that was given tamoxifen at birth. Cre activation converts ubiquitous tomato expression (not shown) to EGFP expression (green). (**H-J**) Neuron-specific enolase (NSE, magenta) staining of brain from a 12 day old *PDGFb-iCreER^{T2}; Rosa26-ACTB-tdTomato,-EGFP* mouse that was given tamoxifen at birth. Cre activation converts ubiquitous tomato expression (not shown) to EGFP expression (green). Scale bars in (**A-B**) = 1 mm. Scale bars in (**C-J**) = 100 μm.



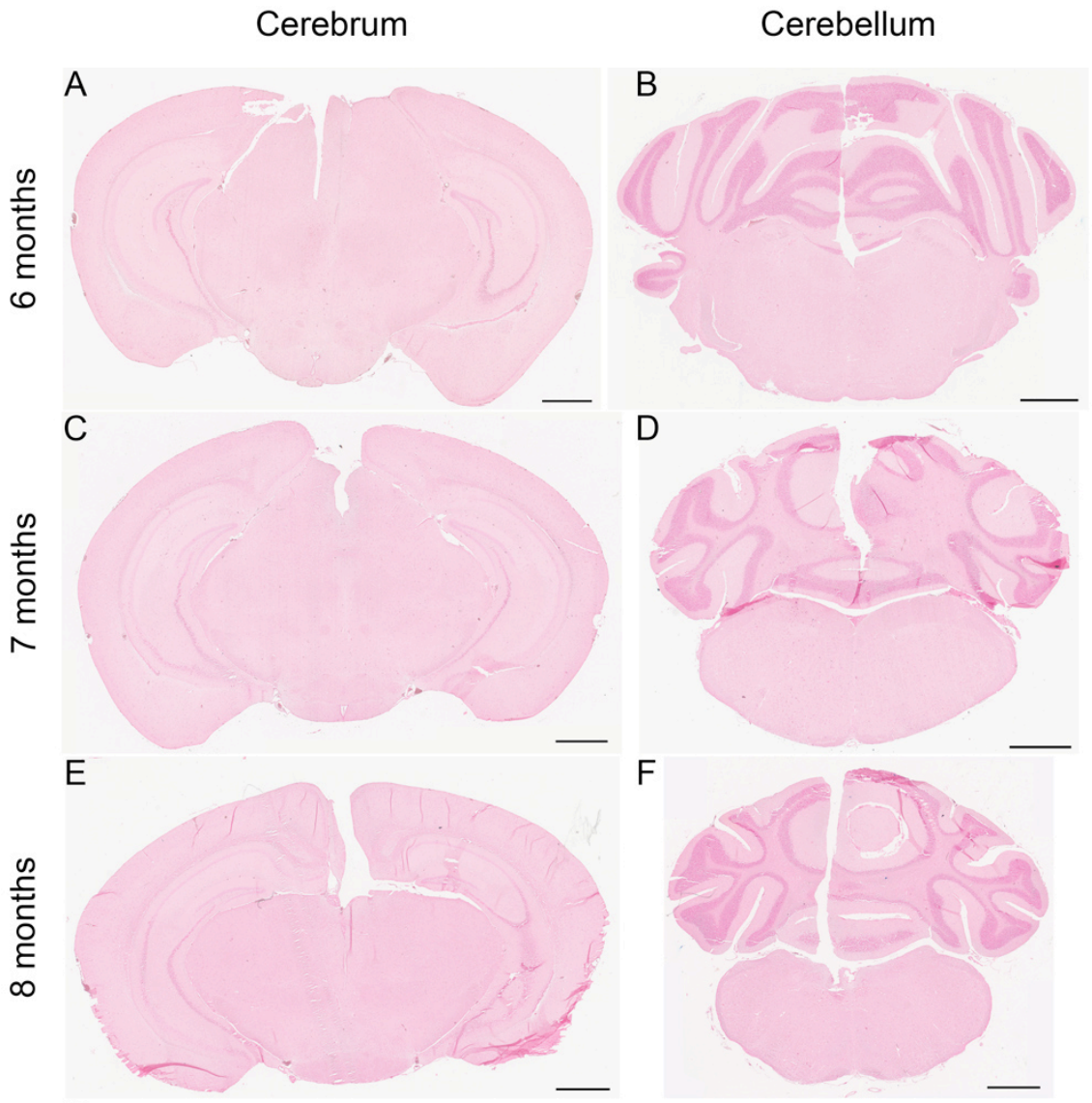
Supplemental Figure 7. LOH of either *Ccm2* or *Pdcd10* results in a range of vascular malformations. Both mouse models of CCM exhibit the same spectrum of pathology. Both the *Pdcd10* (**A**, **C**, **E**, and **G**) and *Ccm2* (**B**, **D**, **F**, and **H**) induced endothelial knockout models develop vascular lesions that exhibit the previously described spectrum of CCM pathology. Examples shown here include lesions consistent with solitary telangiectasias (**A-B**), multichannel "pristine" caverns (**C-D**), complex multichannel lesions with organizing thromboses (arrows in **E-F**), and multiple small caverns associated with heavy hemosiderin staining (arrowheads in **G-H**). Scale bars = 200 μm.



Supplemental Figure 8. Loss of Pdcd10 protein from *Pdcd10* (but not *Ccm2*) vascular lesions. An antibody against Pdcd10 does not stain endothelial cells of a CCM lesion from a *Pdcd10* induced knockout mouse (upper panel) but does stain the endothelial cells of a CCM from a *Ccm2* induced knockout mouse (bottom panel). Arrows indicate endothelial cells. Scale bars = 50 μm.



Supplemental Figure 9. Murine CCMs occur in the retinal vasculature. Lectin stained retinal flat mounts from a 5 month old *Pdcd10$^{flox/+}$; PDGFb-iCreER^{T2}* mouse (**A**), a 5 month old *Pdcd10$^{flox/-}$; PDGFb-iCreER^{T2}* mouse (**B-C**), a 7 month old *Ccm2$^{flox/+}$; PDGFb-iCreER^{T2}* mouse (**D**), and a 7 month old *Ccm2$^{flox/-}$; PDGFb-iCreER^{T2}* mouse (**E-F**). All mice were given tamoxifen at birth. (**C**) Close-up of the boxed, CCM-containing area in (**B**). (**F**) Close-up of the boxed, CCM-containing area in (**E**). Scale bars for all panels = 1 mm, except for panel (**F**), which is 500 μm.

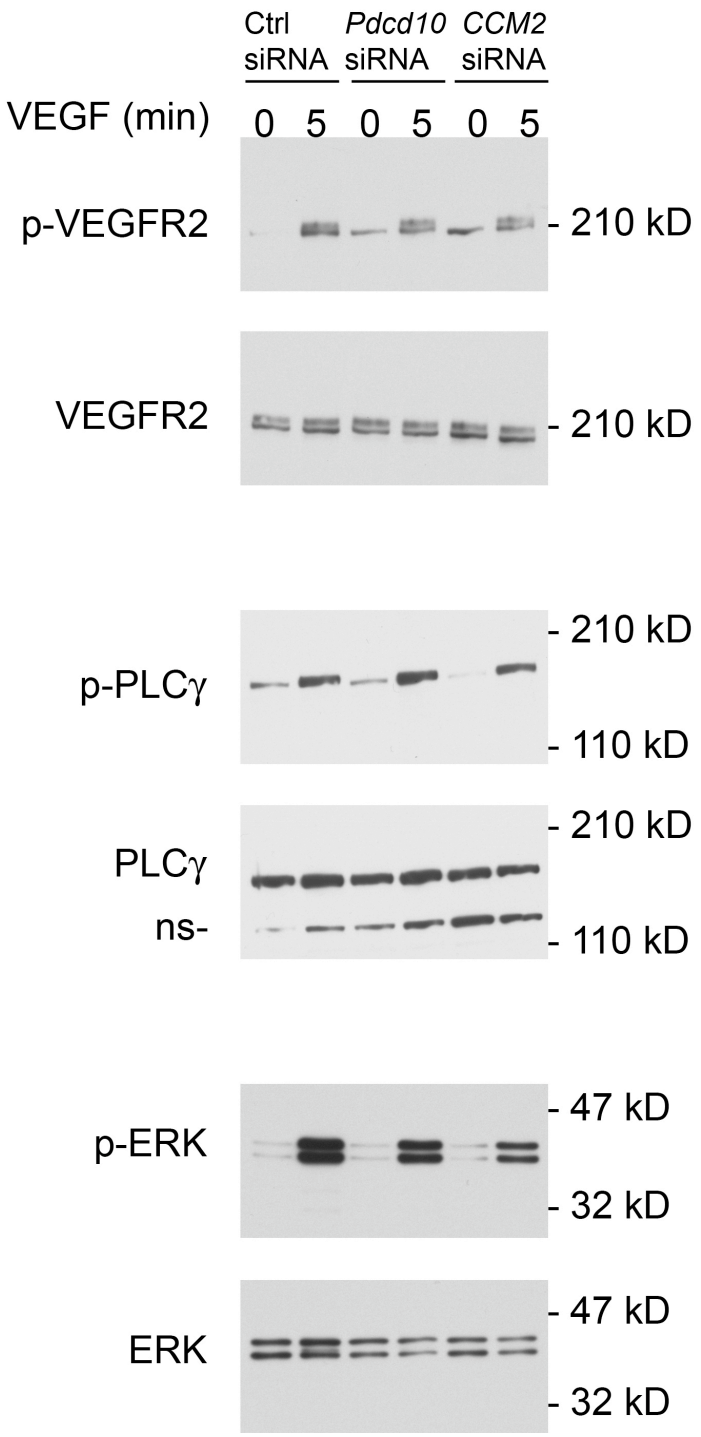


G

		CCM postive	CCM negative	Total
Ccm2$^{flox/-}$	*Nestin-Cre*	0	6	6
	PDGFb-iCreER^{T2}	16	0	16
	Total	16	6	22

Fisher's exact test – $p < 0.0001$

Supplemental Figure 10. Neural-specific deletion of *Ccm2* does not result in CCMs. (**A-F**) Histology of *Ccm2*$^{flox/-}$*; Nestin-Cre* mouse brain at 6 months (**A-B**), 7 months (**C-D**), and 8 months (**E-F**). Staining is Prussian blue for hemosiderin with nuclear fast red counterstain. No lesions are found in these brains, and no hemosiderin is apparent. Scale bars = 1 mm. (**G**) Table comparing prevalence of CCMs in neural knockout (*Nestin-Cre*) vs. inducible endothelial knockout (*PDGFb-CreER*^{T2}).



Supplemental Figure 11. Loss of PDCD10 does not affect VEGFR2-MAPK signaling. Western blot for VEGFR2, PLCγ, and ERK1/2 phosphorylation after stimulation of HMVECs with 10 ng/mL VEGF for the indicated amounts of time. ns, non-specific band.

Cross	*Pdcd10*$^{flox/flox}$ X *Pdcd10*$^{+/-}$; *Nestin-Cre*			
Genotype	*Pdcd10*$^{flox/+}$	*Pdcd10*$^{flox/-}$	*Pdcd10*$^{flox/+}$; *Nestin-Cre*	*Pdcd10*$^{flox/-}$; *Nestin-Cre*
# of progeny				
E12.5	2	7	9	8
P1	12	17	7	7

Supplemental Table 1. Table showing numbers of living offspring by genotype in matings between *Pdcd10*$^{flox/flox}$ and *Pdcd10*$^{+/-}$*; Nestin-Cre* parents.