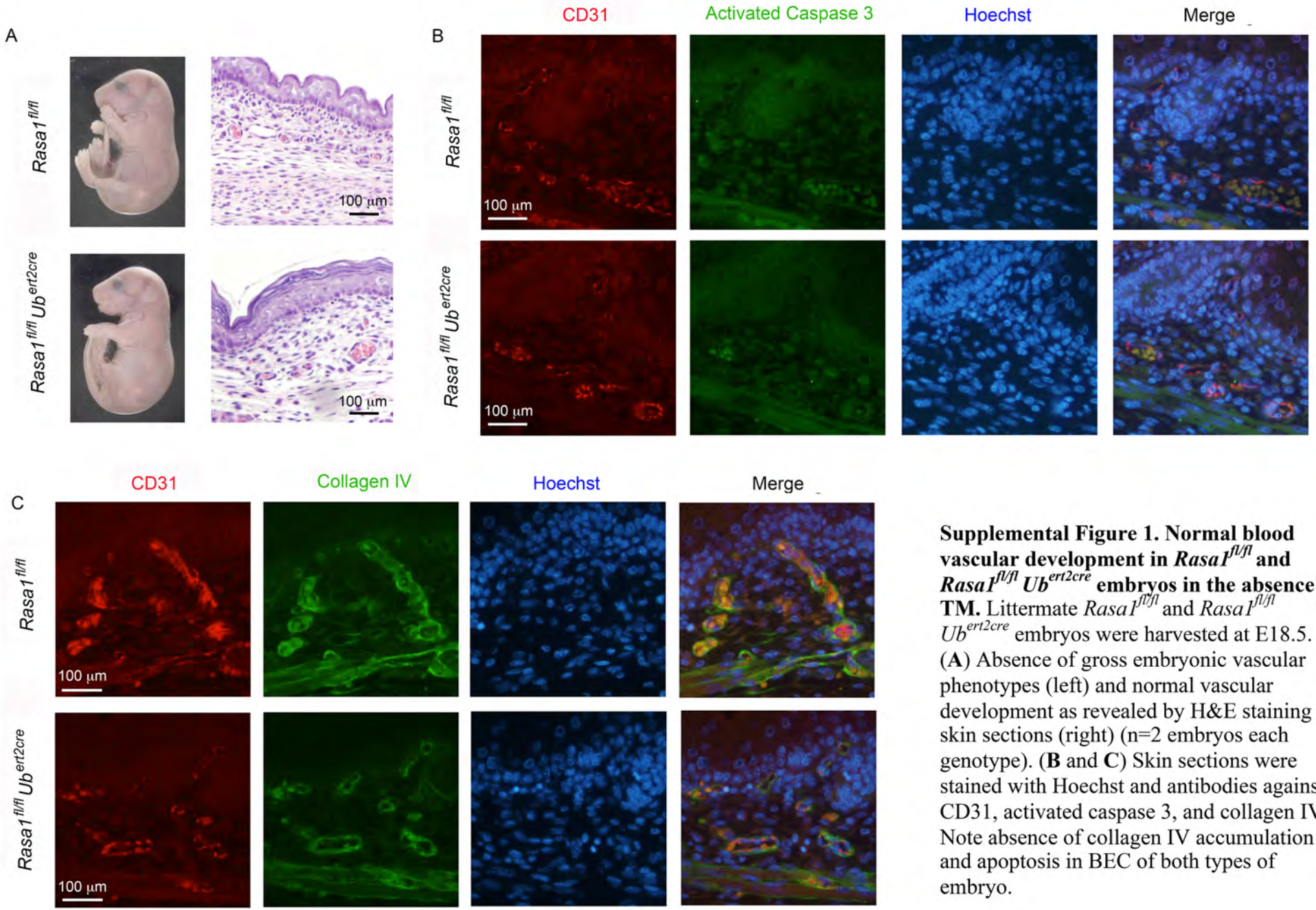
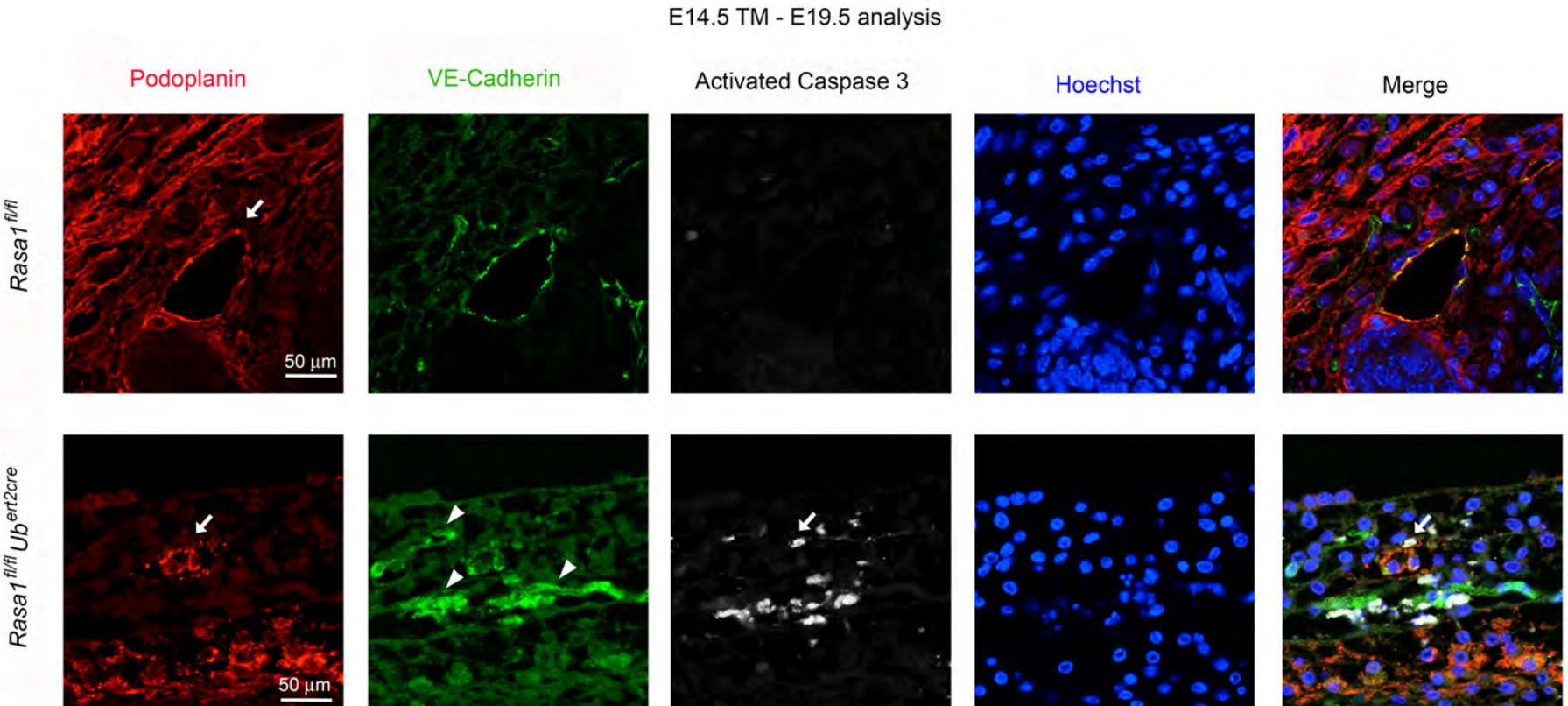
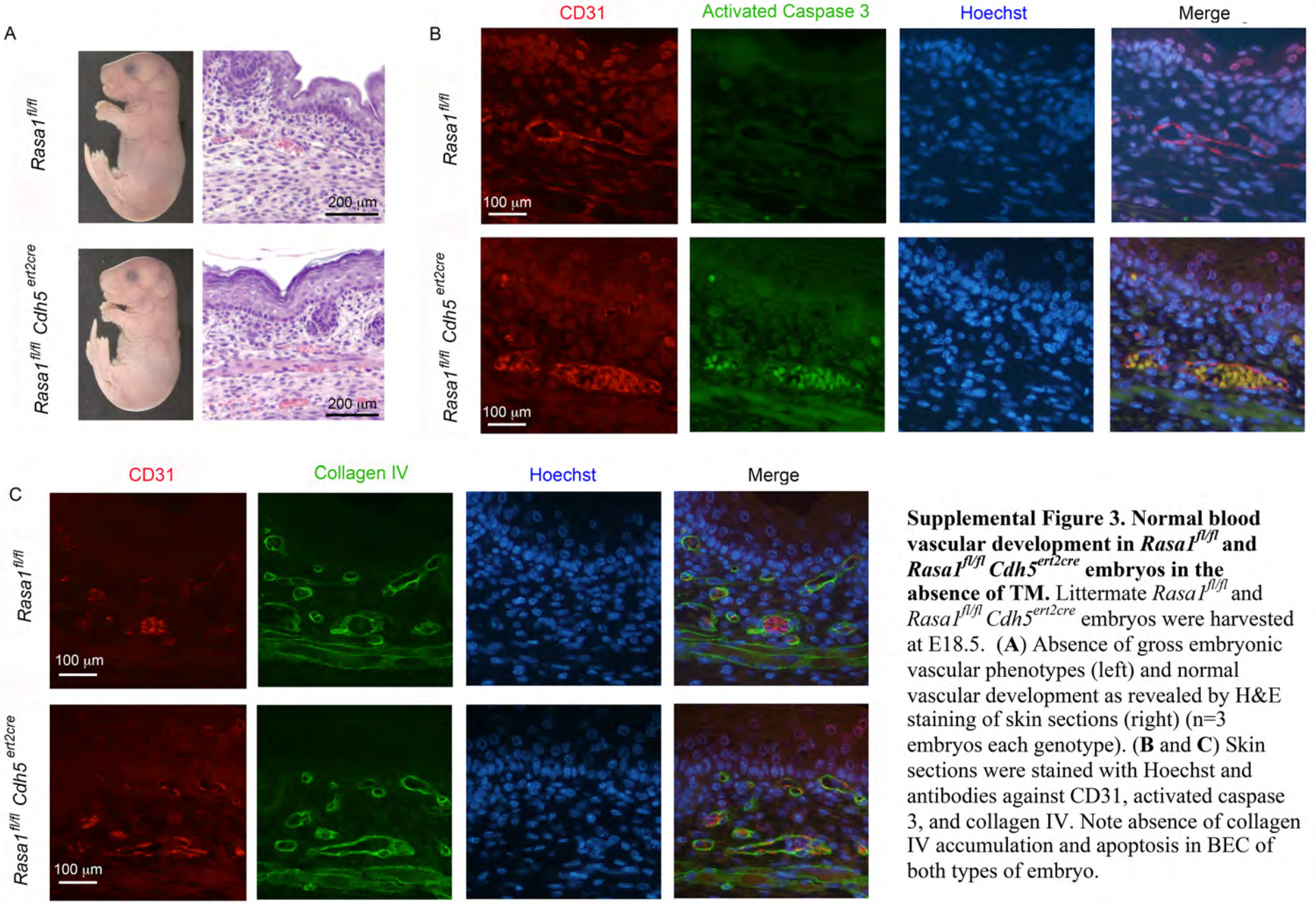


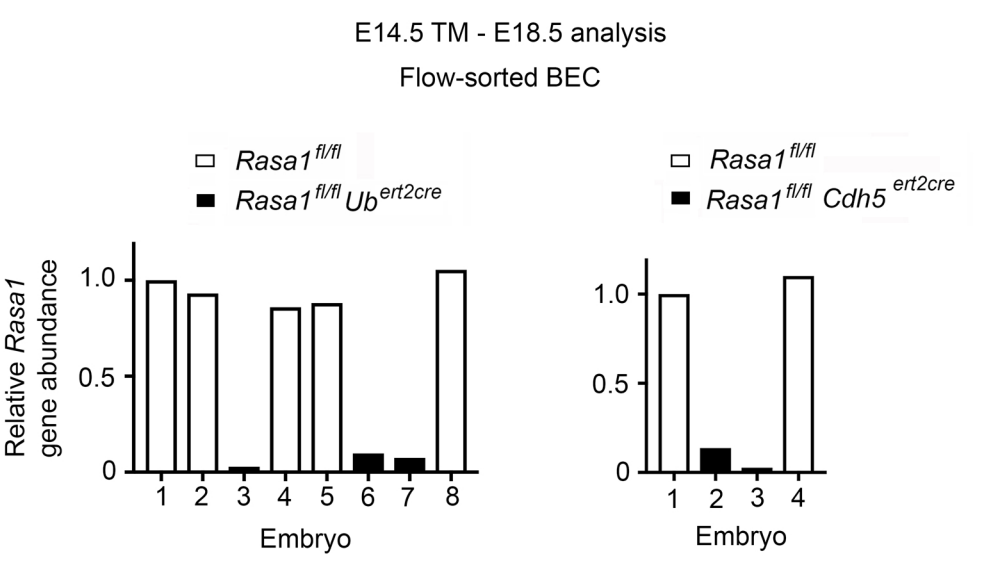
Supplemental Figure 1. Normal blood vascular development in *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos in the absence of TM. Littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos were harvested at E18.5. (**A**) Absence of gross embryonic vascular phenotypes (left) and normal vascular development as revealed by H&E staining of skin sections (right) (n=2 embryos each genotype). (**B** and **C**) Skin sections were stained with Hoechst and antibodies against CD31, activated caspase 3, and collagen IV. Note absence of collagen IV accumulation and apoptosis in BEC of both types of embryo.



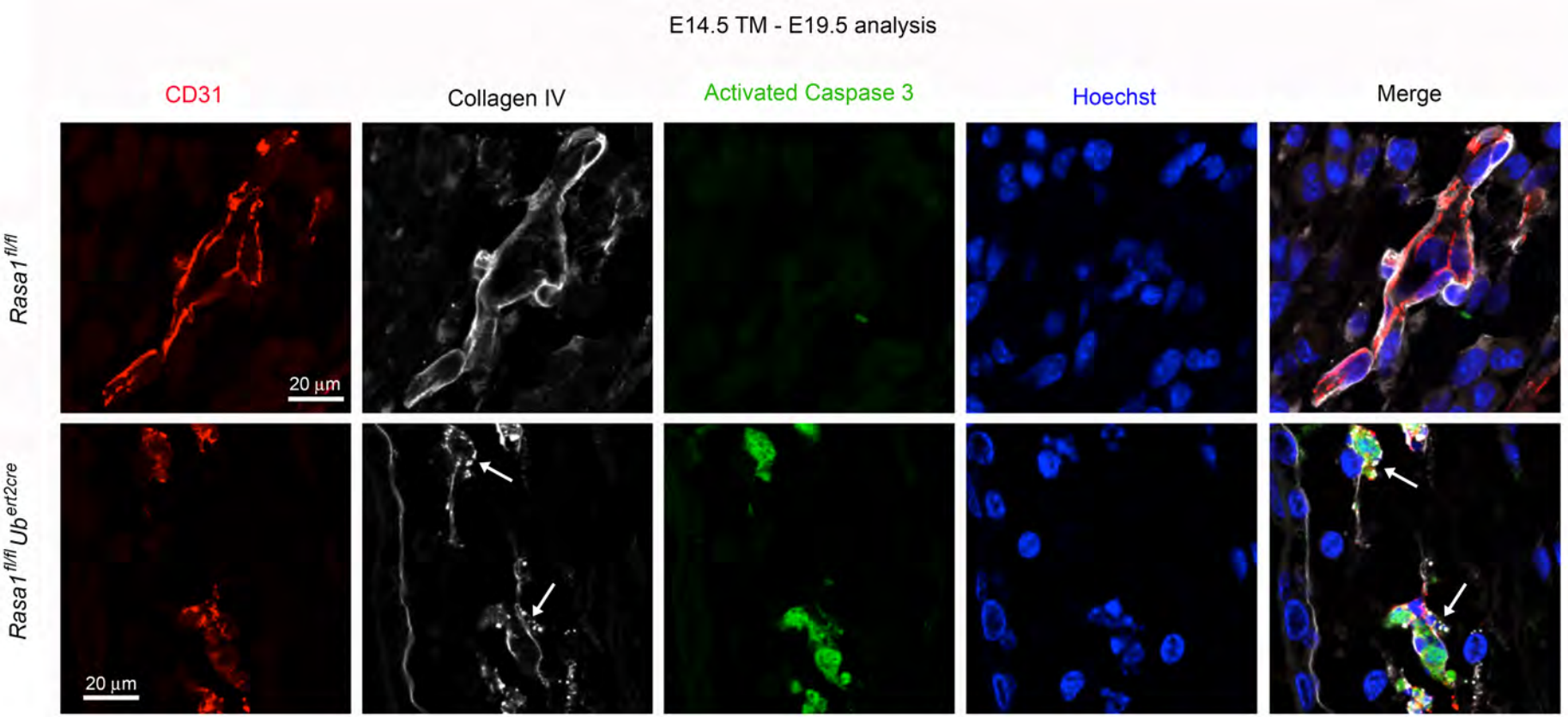
Supplemental Figure 2. LEC apoptosis following disruption of *Rasa1* during developmental angiogenesis. TM was administered to littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos at E14.5 and embryos were harvested at E19.5. Skin sections from embryos were stained with Hoechst and antibodies against podoplanin, VE-Cadherin and activated caspase 3. LV (podoplanin+, VE-Cadherin+) and BV (podoplanin-, VE-Cadherin+) are indicated by arrows and arrowheads respectively. Note apoptotic LEC as well as BEC in *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos.



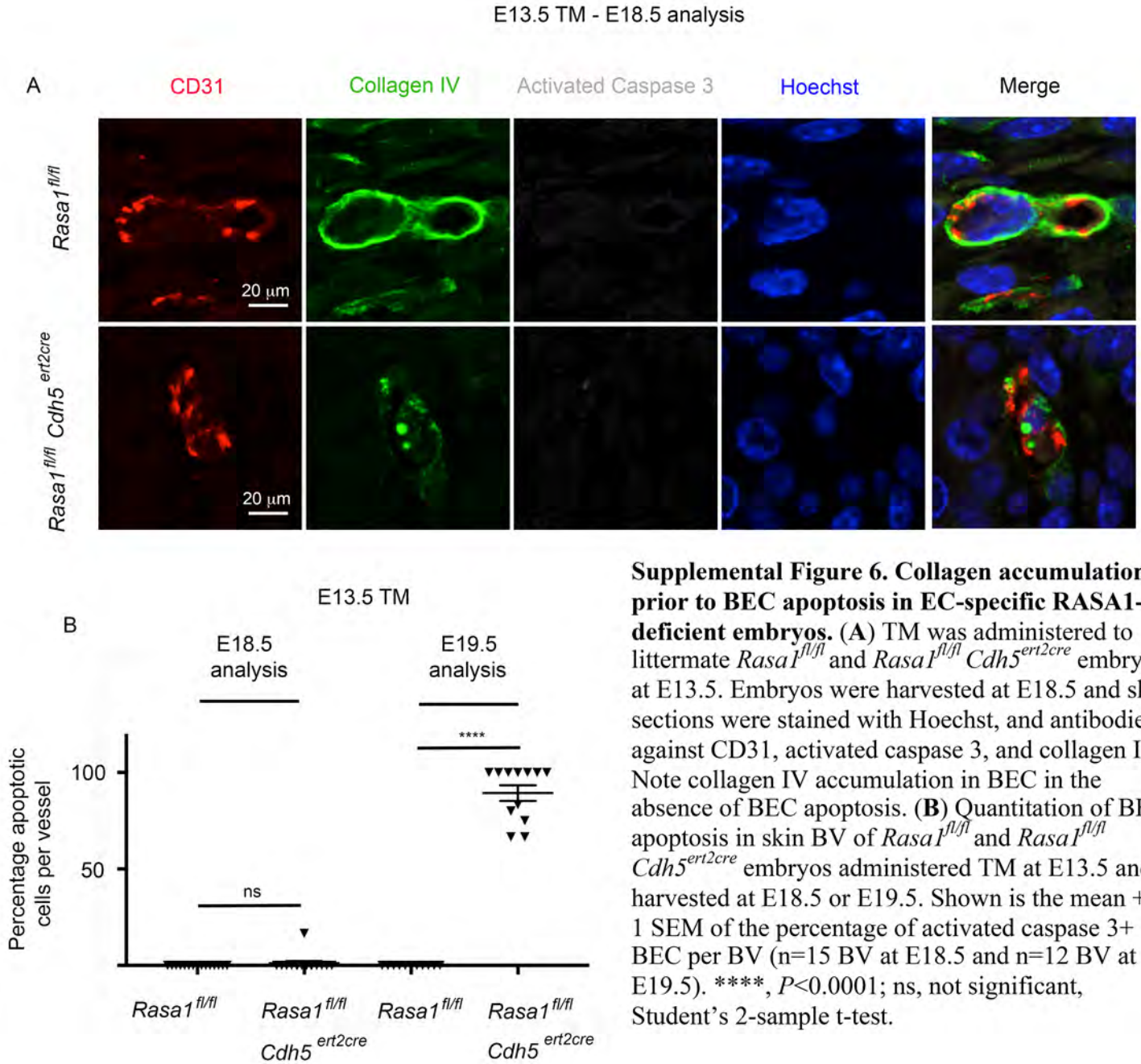
Supplemental Figure 3. Normal blood vascular development in *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} embryos in the absence of TM. Littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} embryos were harvested at E18.5. (**A**) Absence of gross embryonic vascular phenotypes (left) and normal vascular development as revealed by H&E staining of skin sections (right) (n=3 embryos each genotype). (**B** and **C**) Skin sections were stained with Hoechst and antibodies against CD31, activated caspase 3, and collagen IV. Note absence of collagen IV accumulation and apoptosis in BEC of both types of embryo.



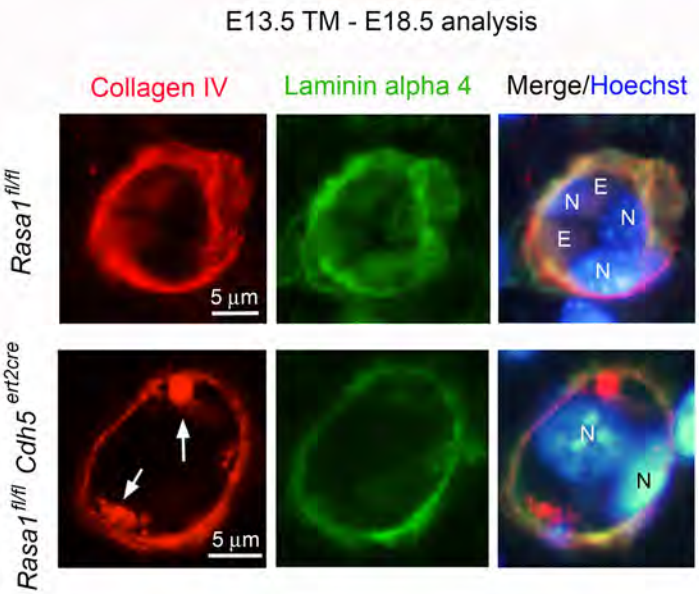
Supplemental Figure 4. *Rasa1* gene disruption efficiency in BEC. TM was administered to littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} and littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} embryos at E14.5 and embryos were harvested at E18.5. Skin BEC (CD31+ CD45- LYVE1-) were purified by flow cytometry and analyzed for *Rasa1* gene abundance by real time qPCR. Shown are results from different litters. Results are normalized to the first Cre- embryo in each litter.



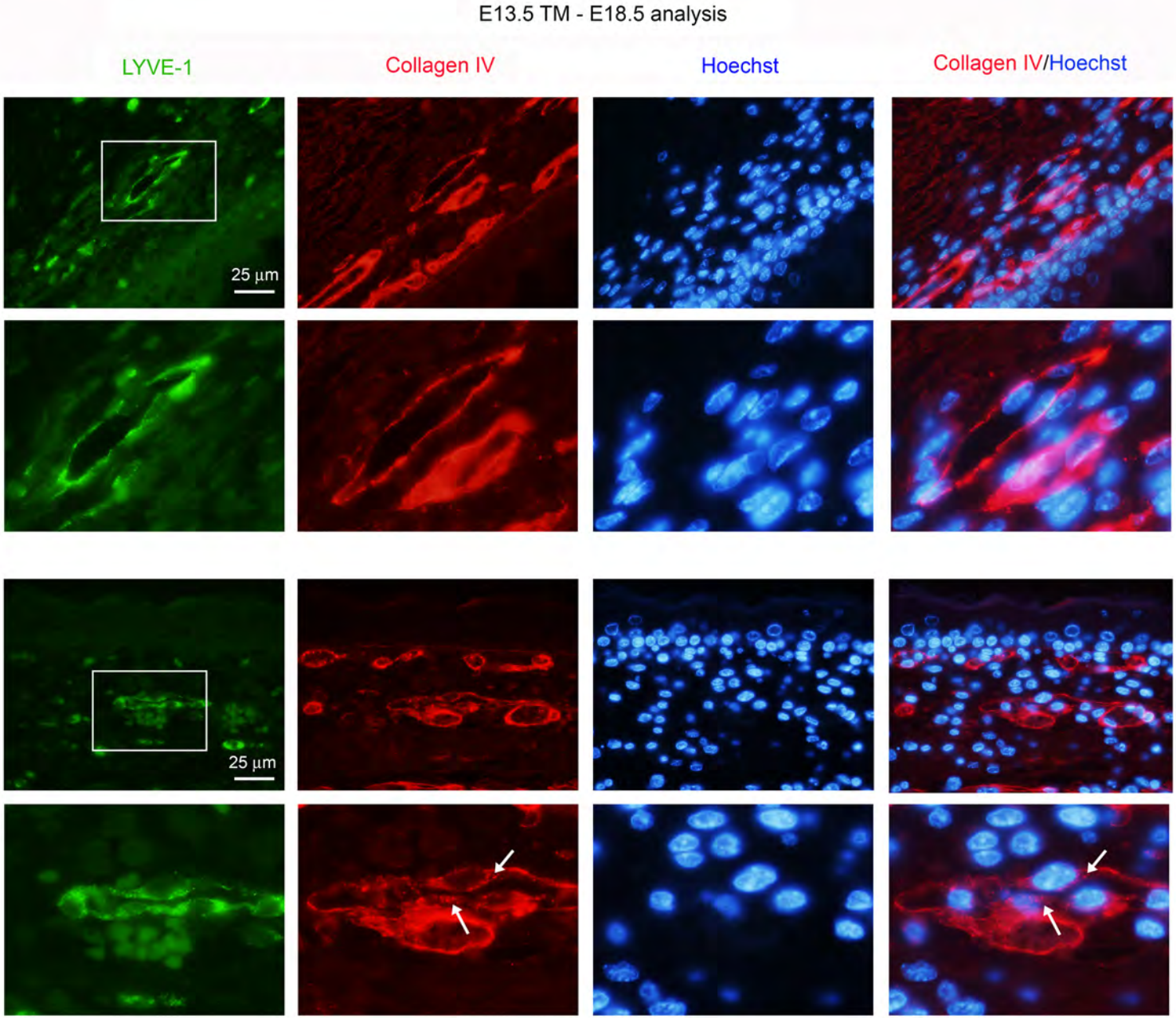
Supplemental Figure 5. Accumulation of collagen IV within BEC and BEC apoptosis following induced disruption of *Rasa1* during developmental angiogenesis. Skin sections from E19.5 littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos administered TM at E14.5 were stained with Hoechst and antibodies against CD31, collagen IV and activated caspase. Collagen IV deposition in BV BM was discontinuous in *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos and collagen IV was frequently observed in discrete punctae within BEC (arrows). BEC with intracellular accumulation of collagen IV were frequently apoptotic.



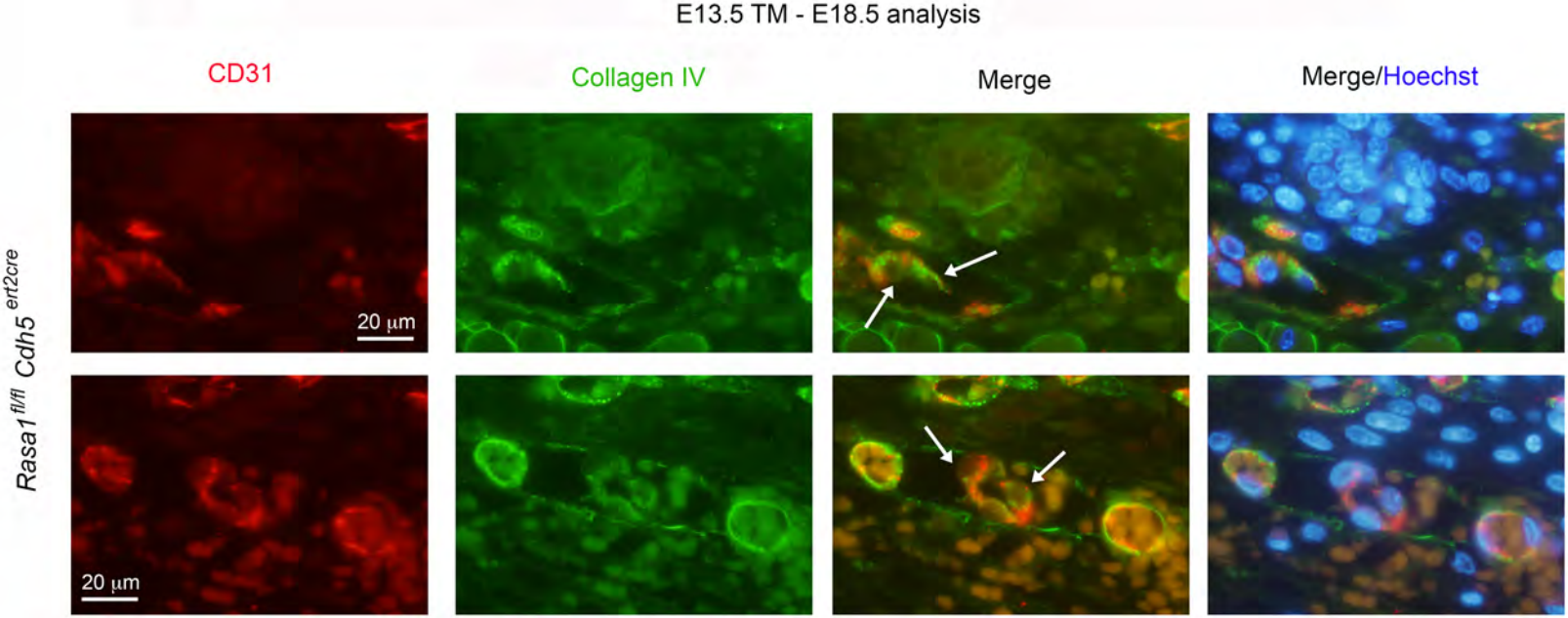
Supplemental Figure 6. Collagen accumulation prior to BEC apoptosis in EC-specific RASA1-deficient embryos. (**A**) TM was administered to littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} embryos at E13.5. Embryos were harvested at E18.5 and skir sections were stained with Hoechst, and antibodies against CD31, activated caspase 3, and collagen IV. Note collagen IV accumulation in BEC in the absence of BEC apoptosis. (**B**) Quantitation of BEC apoptosis in skin BV of *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} embryos administered TM at E13.5 and harvested at E18.5 or E19.5. Shown is the mean +/- 1 SEM of the percentage of activated caspase 3+ BEC per BV (n=15 BV at E18.5 and n=12 BV at E19.5). ****, $P<0.0001$; ns, not significant, Student's 2-sample t-test.



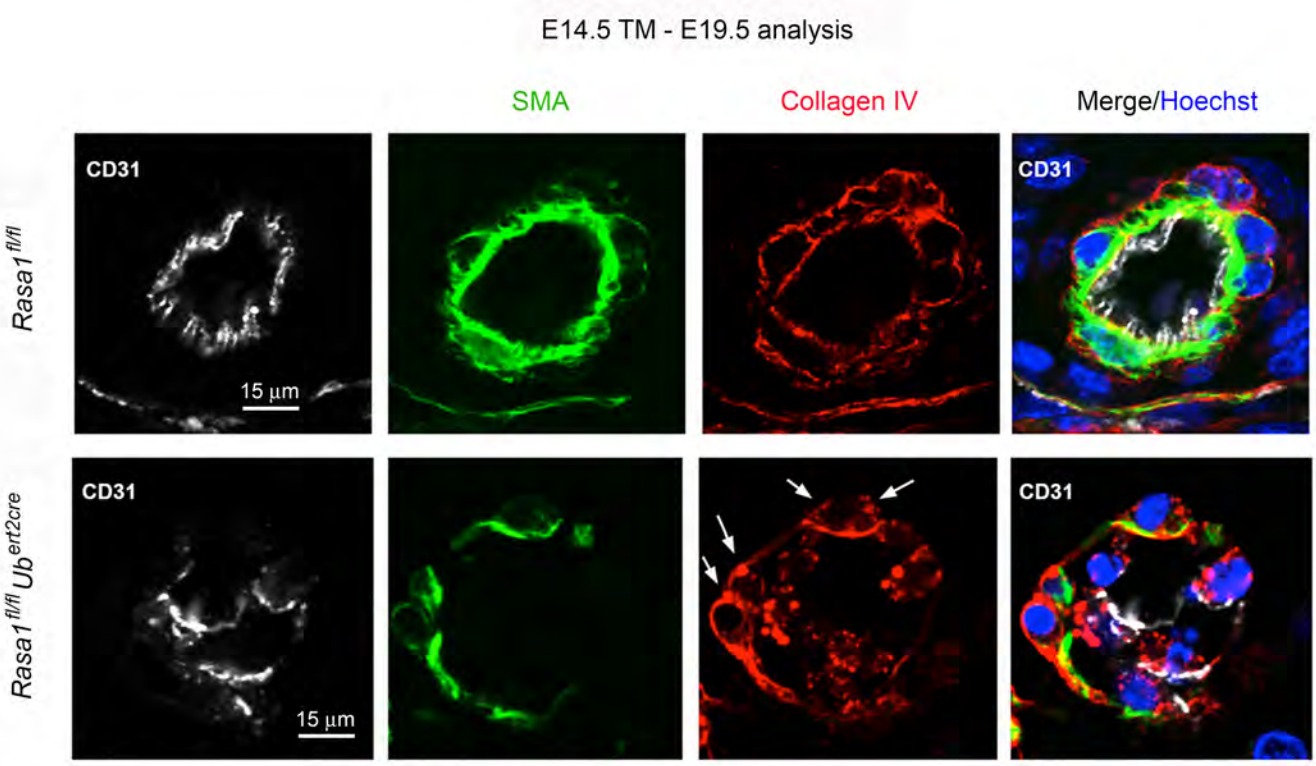
Supplemental Figure 7. Specific accumulation of collagen IV within BEC following induced loss of RASA1 during developmental angiogenesis. Skin sections from E18.5 littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} embryos administered TM at E13.5 were stained with Hoechst and antibodies against collagen IV and laminin alpha 4. Shown are representative small cutaneous BV. Note intracellular accumulation of collagen IV (arrows) but not laminin alpha 4 in BEC of *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} embryos. N, nucleus; E, erythrocyte.



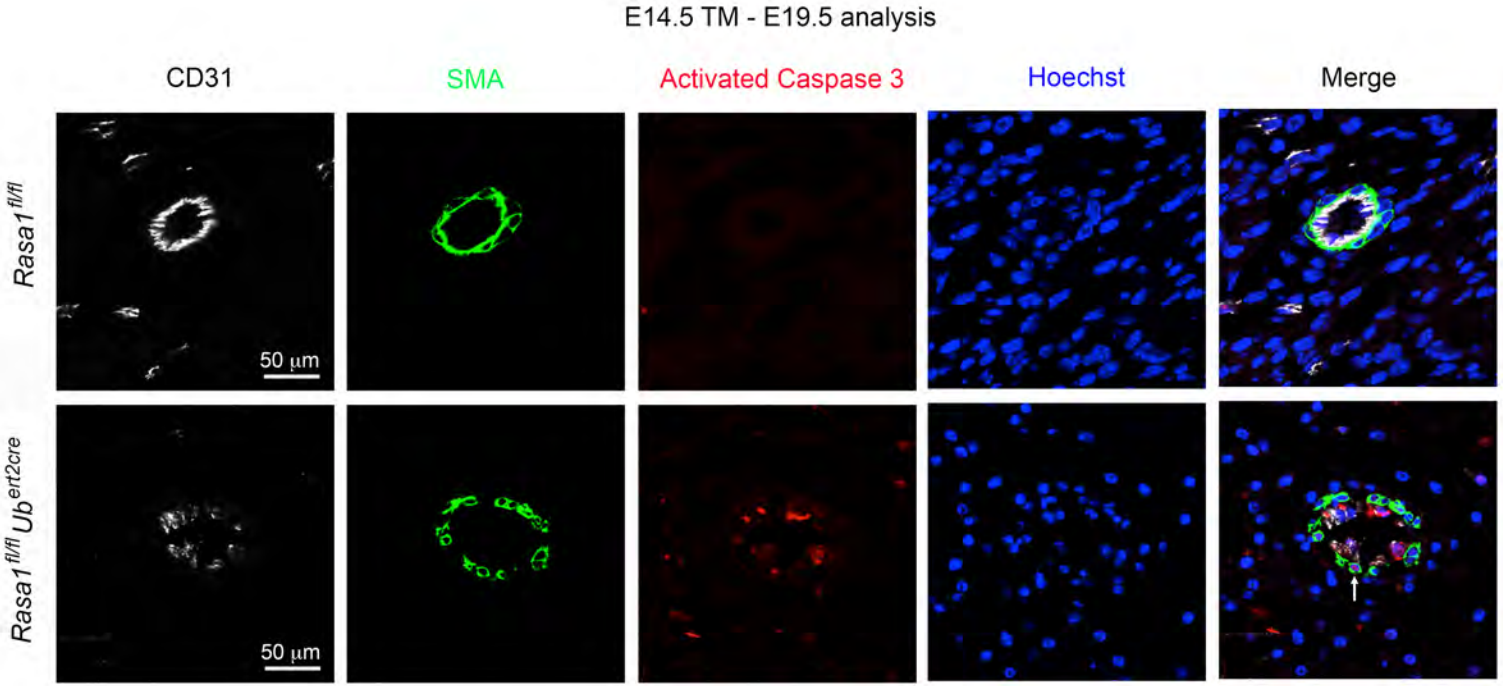
Supplemental Figure 8. Accumulation of collagen IV within LEC following induced loss of RASA1 during developmental angiogenesis. Skin sections from E18.5 littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} embryos administered TM at E13.5 were stained with Hoechst and antibodies against collagen IV and LYVE-1. Lower power images are shown in top rows. Higher power images of boxed areas containing LYVE-1+ LV are shown below. Note accumulation of collagen IV in LEC of *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} embryos (arrows).



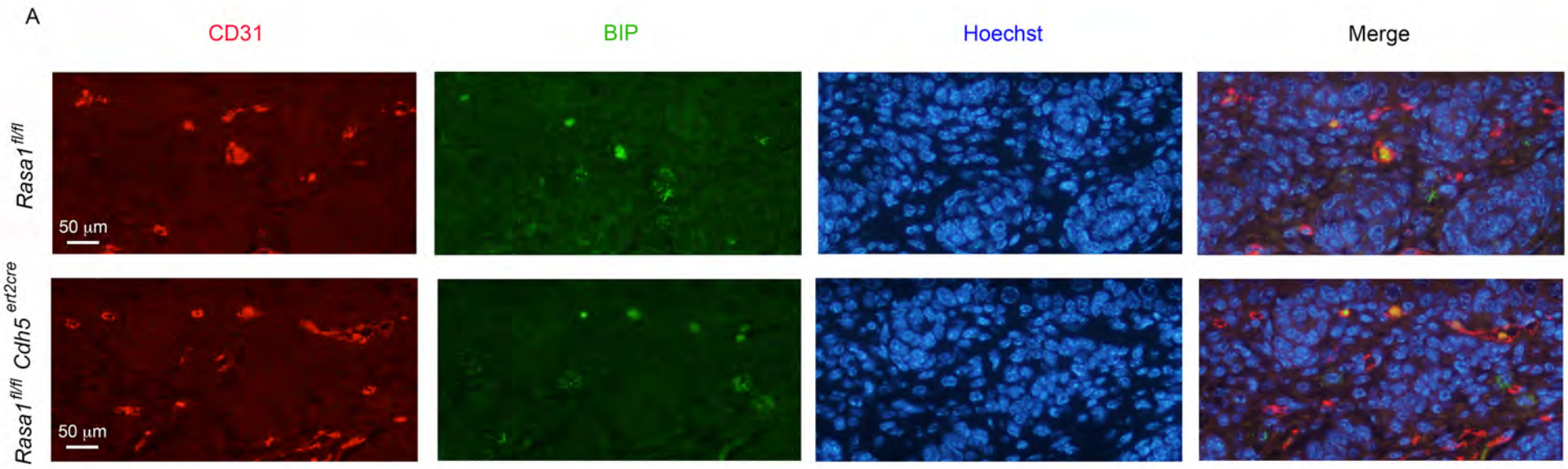
Supplemental Figure 9. Evidence of BEC anoikis in EC-specific RASA1-deficient embryos. *Rasa1*$^{Ji/Ji}$ *Cdh5*^{ert2cre} embryos were administered TM at E13.5 and harvested at E18.5. Skin sections were stained with Hoechst and antibodies against CD31 and collagen IV. Note detached or detaching BEC with intracellular collagen IV in lumens of vessels (arrows).

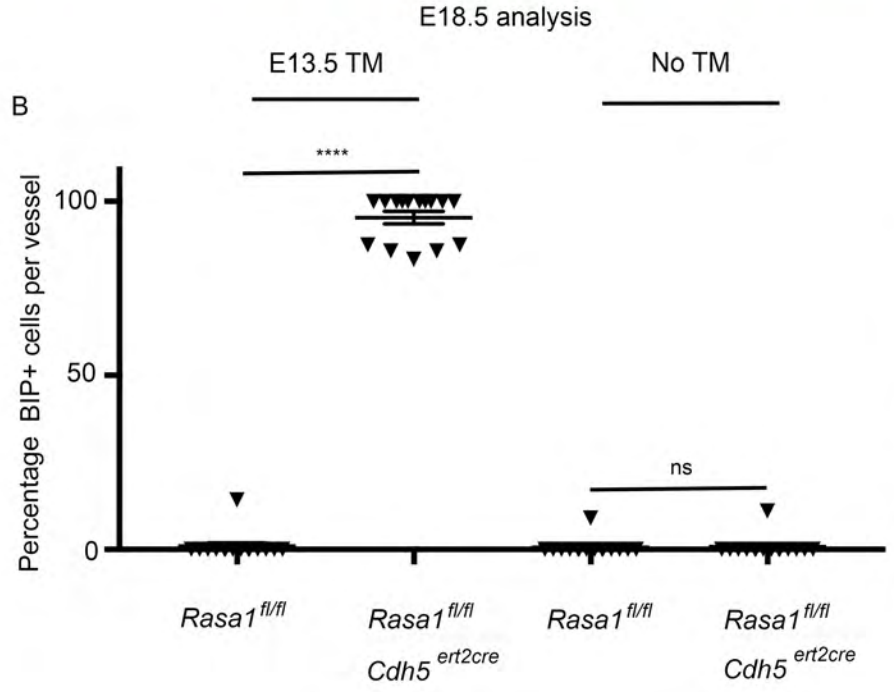


Supplemental Figure 10. Accumulation of collagen IV within vascular smooth muscle cells following induced loss of RASA1 during developmental angiogenesis. Skin sections from E19.5 littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos administered TM at E14.5 were stained with Hoechst and antibodies against CD31, smooth muscle actin (SMA) and collagen IV. Note intracellular accumulation of collagen IV in vascular smooth cells of *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos (arrows).

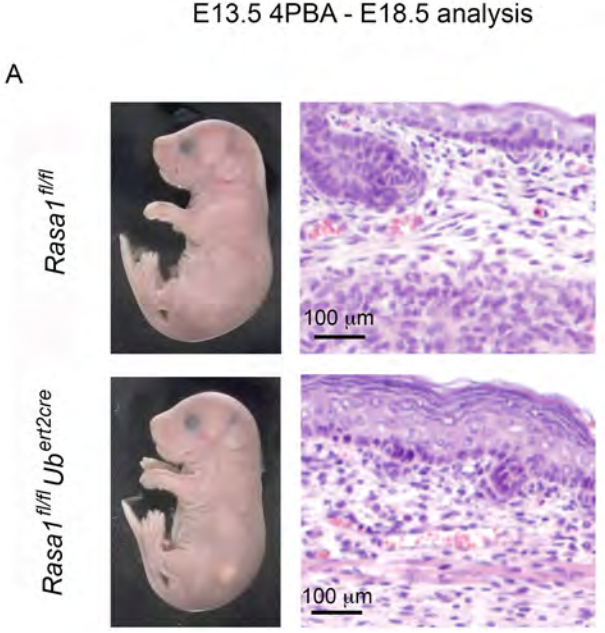


Supplemental Figure 11. VSMC apoptosis in RASA1-deficient embryos. Skin sections from E19.5 littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos administered TM at E14.5 were stained with Hoechst and antibodies against CD31 and activated caspase 3. Note evidence of VSMC apoptosis in *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos (arrow).

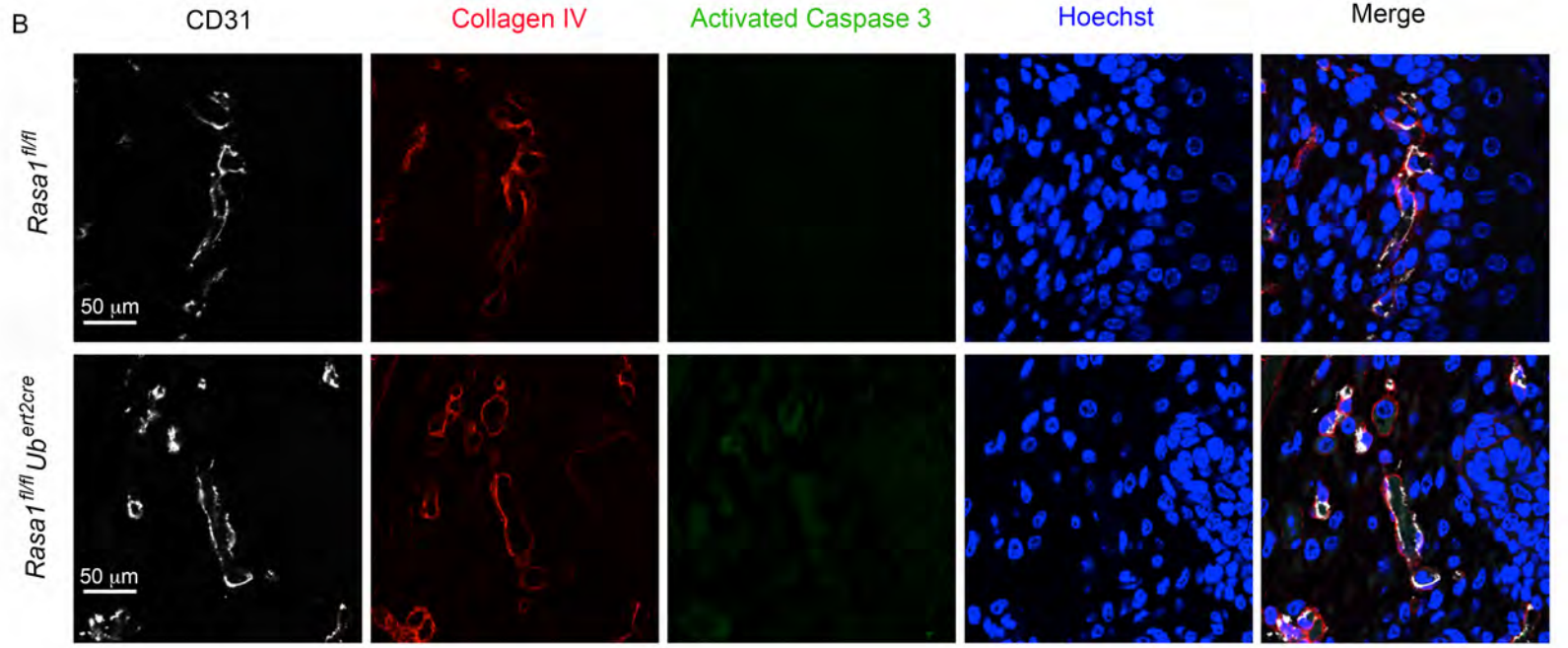


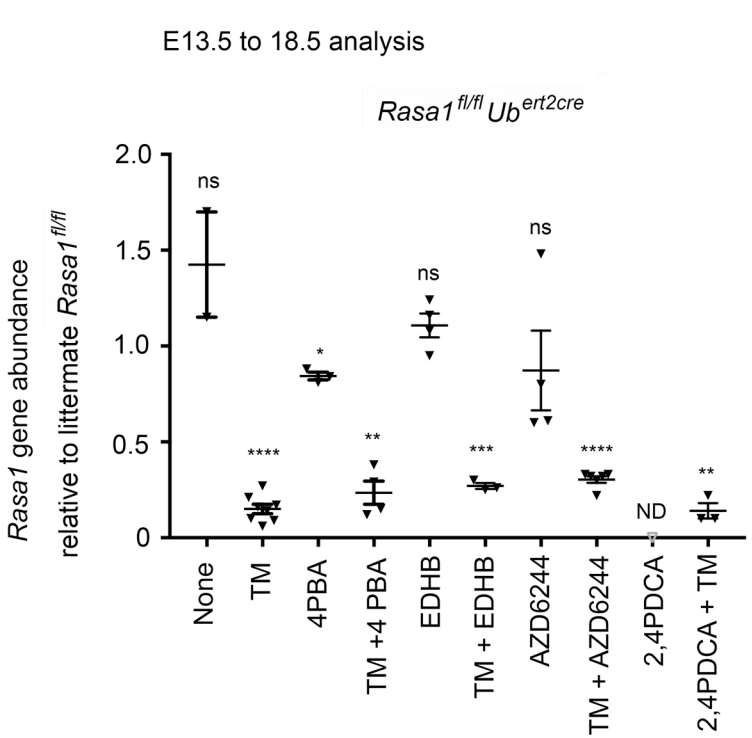


Supplemental Figure 12. No increased BIP in BEC of *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} embryos in the absence of TM. (**A**) Skin sections from littermate E18.5 *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} embryos were stained with Hoechst and antibodies against CD31 and BIP. Note lack of detectable BIP in BEC both embryos. (**B**) Mean +/- 1 SEM of the percentage of BIP+ BEC per BV in skin sections of *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} embryos at E18.5 treated or not with TM at E13.5 (n=15 BV each genotype and condition). ****, $P<0.0001$; ns, not significant, Student's 2-sample t-test.

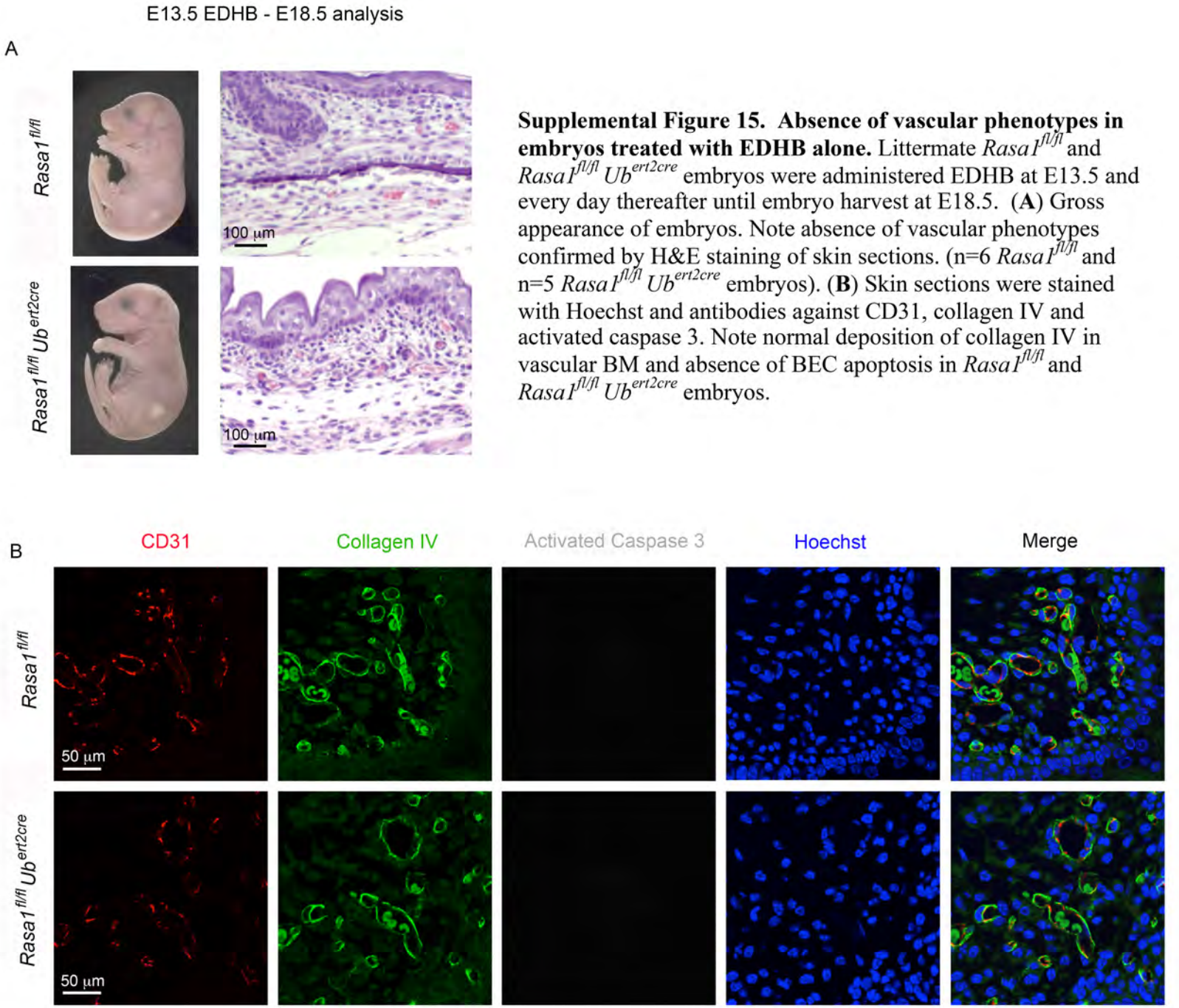


Supplemental Figure 13. Absence of vascular phenotypes in embryos treated with 4PBA alone. Littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos were administered 4PBA at 13.5 and every day thereafter until embryo harvest at E18.5. (**A**) Gross appearance of embryos. Note absence of vascular phenotypes confirmed by H&E staining of skin sections. (n=3 embryos each genotype). (**B**) Skin sections were stained with Hoechst and antibodies against CD31, collagen IV and activated caspase 3. Note normal deposition of collagen IV in vascular BM and absence of BEC apoptosis in *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos.

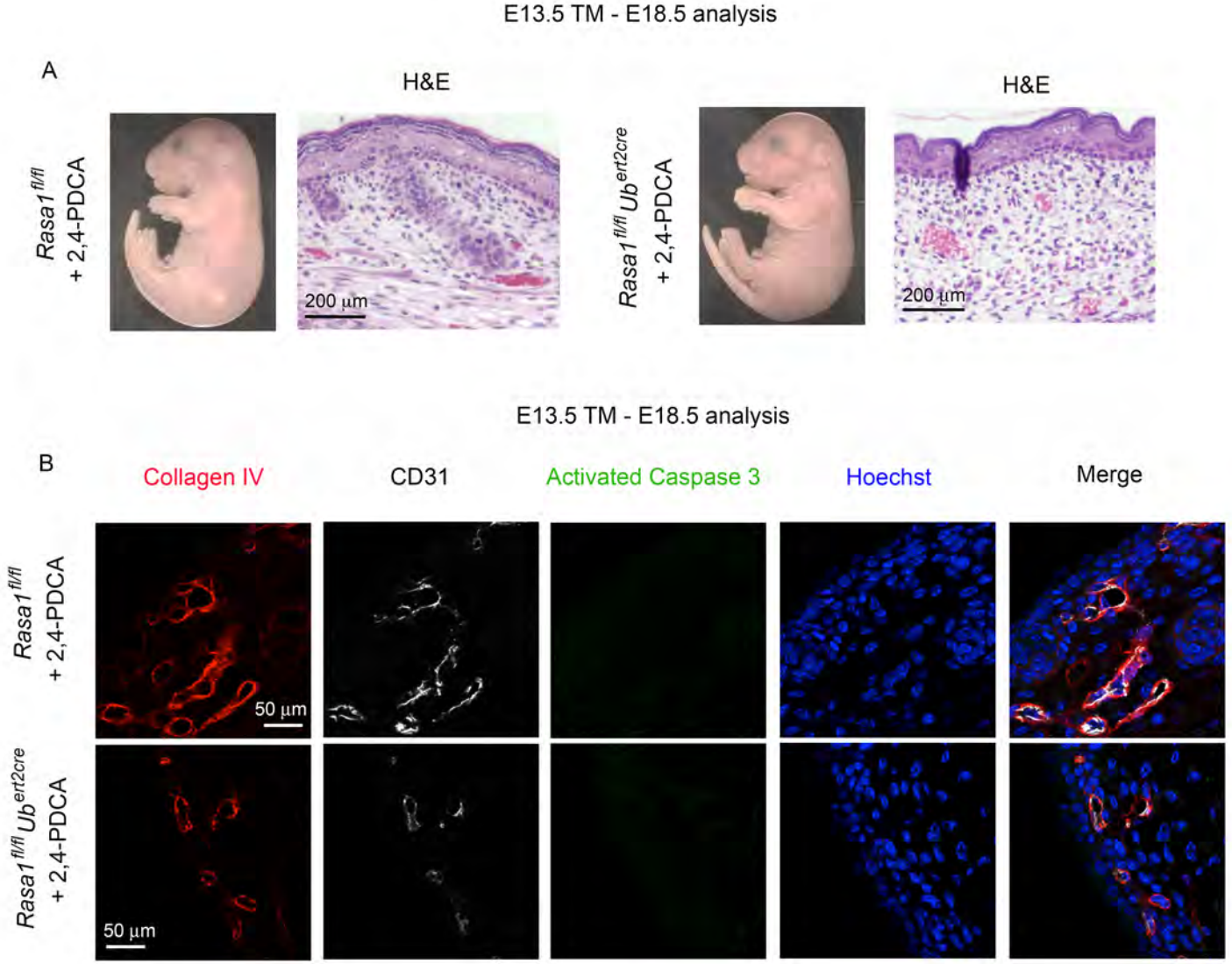




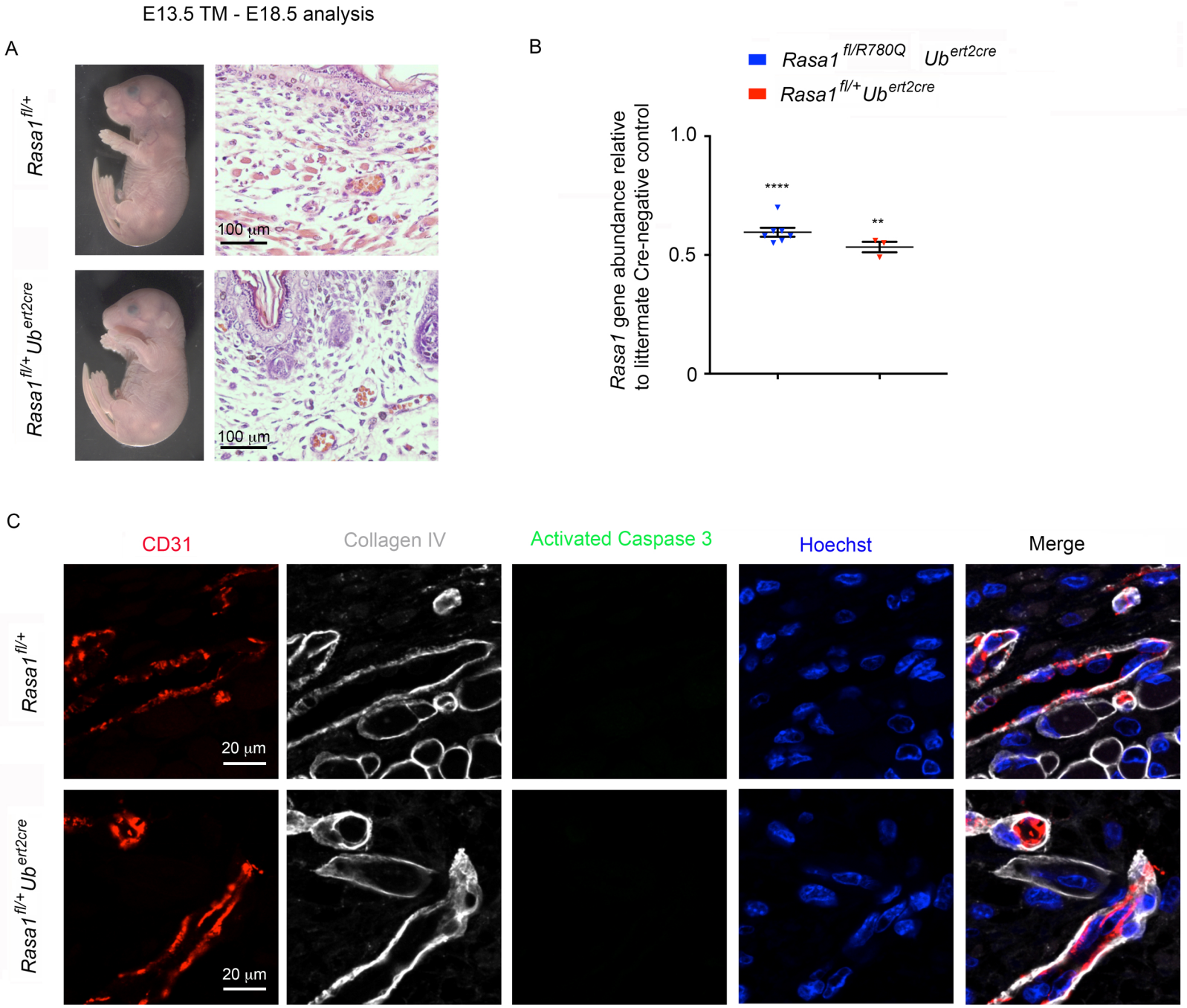
Supplemental Figure 14. *Rasa1* gene disruption efficiency in *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos after treatment with different drugs. *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos were administered the indicated drugs starting at E13.5 and for the same number of days thereafter as indicated in other figures. Embryos were harvested at E18.5 and *Rasa1* gene abundance was determined by real time qPCR using tail genomic DNA as a template. Results are expressed as mean +/- 1 SEM of *Rasa1* gene abundance relative to *Rasa1*$^{fl/fl}$ embryos in the same litter (mean value). n=2, 8, 3, 4, 4, 3, 4, 6, 0 and 3 for None, TM, 4PBA, TM+4PBA, EDHB, TM+EDHB, AZD6244, TM+AZD6244, 2,4PDCA (ND, not determined) and 2,4PDCA+TM respectively. *, $P<0.05$, **, $P<0.01$; ***, $P<0.001$, ****, $P<0.0001$; ns, not significant, Student's 1-sample t-test.



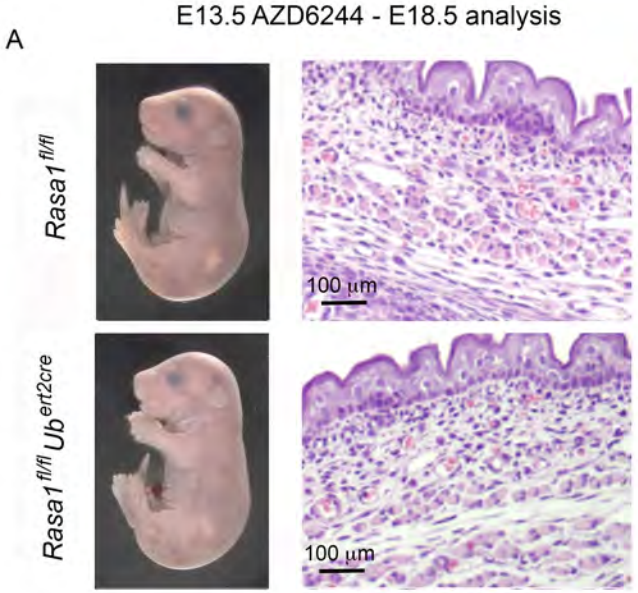
Supplemental Figure 15. Absence of vascular phenotypes in embryos treated with EDHB alone. Littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos were administered EDHB at E13.5 and every day thereafter until embryo harvest at E18.5. (**A**) Gross appearance of embryos. Note absence of vascular phenotypes confirmed by H&E staining of skin sections. (n=6 *Rasa1*$^{fl/fl}$ and n=5 *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos). (**B**) Skin sections were stained with Hoechst and antibodies against CD31, collagen IV and activated caspase 3. Note normal deposition of collagen IV in vascular BM and absence of BEC apoptosis in *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos.



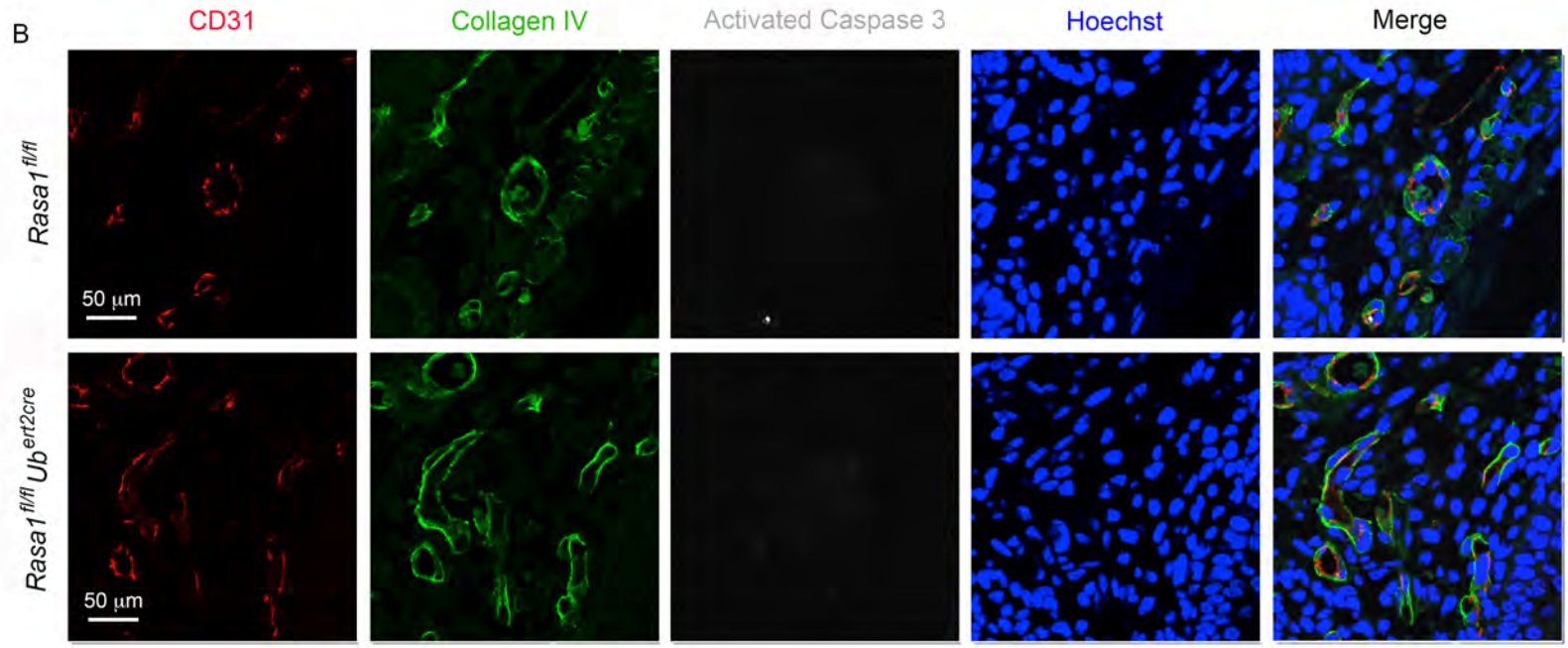
Supplemental Figure 16. Rescue of developmental angiogenesis defects in induced RASA1-deficient mice with the 2OG dependent oxygenase inhibitor, 2,4PDCA. TM was administered to littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos at E13.5. 2,4PDCA was co-administered with the TM and was also administered to embryos on consecutive days thereafter until embryo harvest at E18.5. (**A**) Gross appearance of embryos. Note absence of hemorrhage and edema in *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos that was confirmed by H&E staining of skin sections. (**B**) Skin sections were stained with Hoechst and antibodies against CD31, collagen IV and activated caspase 3. Note normal deposition of collagen IV in vascular BM and absence of BEC apoptosis in *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos.

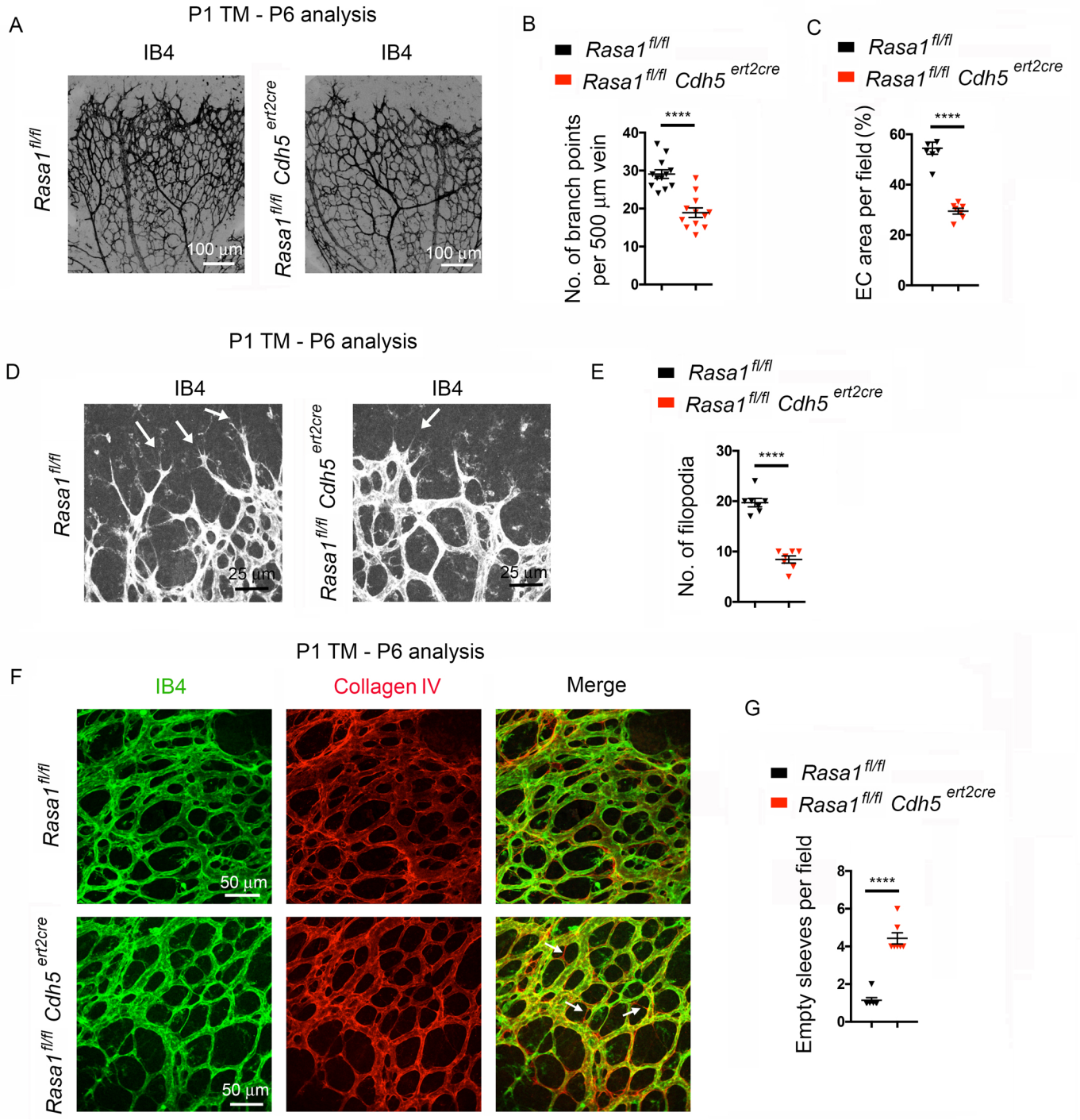


Supplemental Figure 17. Absence of vascular phenotypes in induced *Rasa1* heterozygous embryos. Littermate *Rasa1*$^{fl/+}$ and *Rasa1*$^{fl/+}$ *Ub*^{ert2cre} embryos were administered TM on E13.5 and harvested at E18.5. (**A**) Gross appearance of embryos. Note absence of hemorrhage and edema in *Rasa1*$^{fl/+}$*Ub*^{ert2cre} embryos that was confirmed by H&E staining of skin sections. (**B**) *Rasa1* gene abundance of embryos in (A) and Fig. 9 was determined by real time qPCR using tail genomic DNA as a template. Results are expressed as mean +/- 1 SEM of *Rasa1* gene abundance relative to *Rasa1*$^{fl/R780Q}$ or *Rasa1*$^{fl/+}$ embryos in the same litter (mean value). n=7 and 3 for *Rasa1*$^{fl/R780Q}$ *Ub*^{ert2cre} and *Rasa1*$^{fl/+}$*Ub*^{ert2cre} embryos respectively. (**C**) Skin sections from embryos in (A) were stained with Hoechst and antibodies against CD31, collagen IV and activated caspase 3. Note normal deposition of collagen IV in vascular BM and absence of BEC apoptosis in *Rasa1*$^{fl/+}$*Ub*^{ert2cre} embryos. **, $P<0.01$; ****, $P<0.0001$, Student's 1-sample t-test.

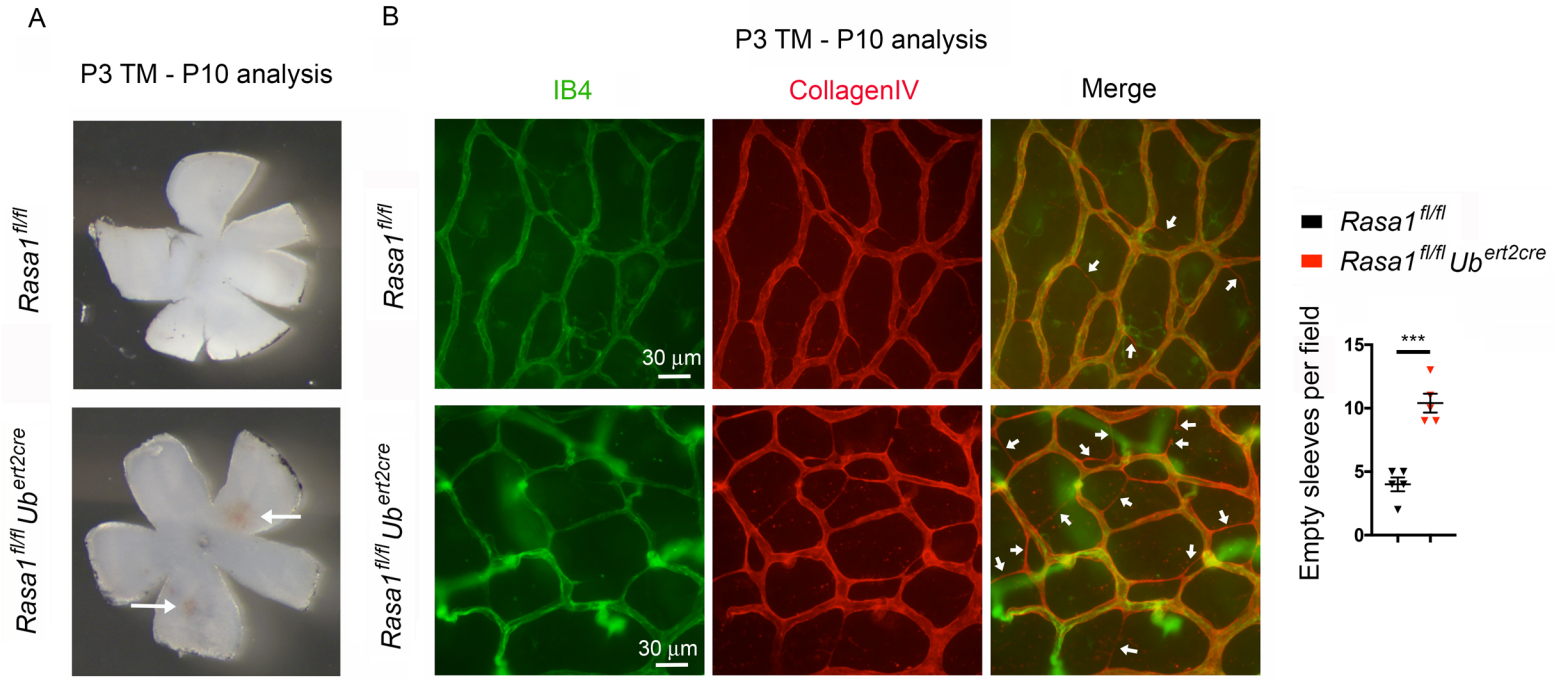


Supplemental Figure 18. Absence of vascular phenotypes in embryos treated with AZD6244 alone. Littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos were administered AZD6244 at E13.5 and every day thereafter until embryo harvest at E18.5. (**A**) Gross appearance of embryos. Note absence of vascular phenotypes confirmed by H&E staining of skin sections. (n=1 *Rasa1*$^{fl/fl}$ and n=4 *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos). (**B**) Skin sections were stained with Hoechst and antibodies against CD31, collagen IV and activated caspase 3. Note normal deposition of collagen IV in vascular BM and absence of BEC apoptosis in *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos.

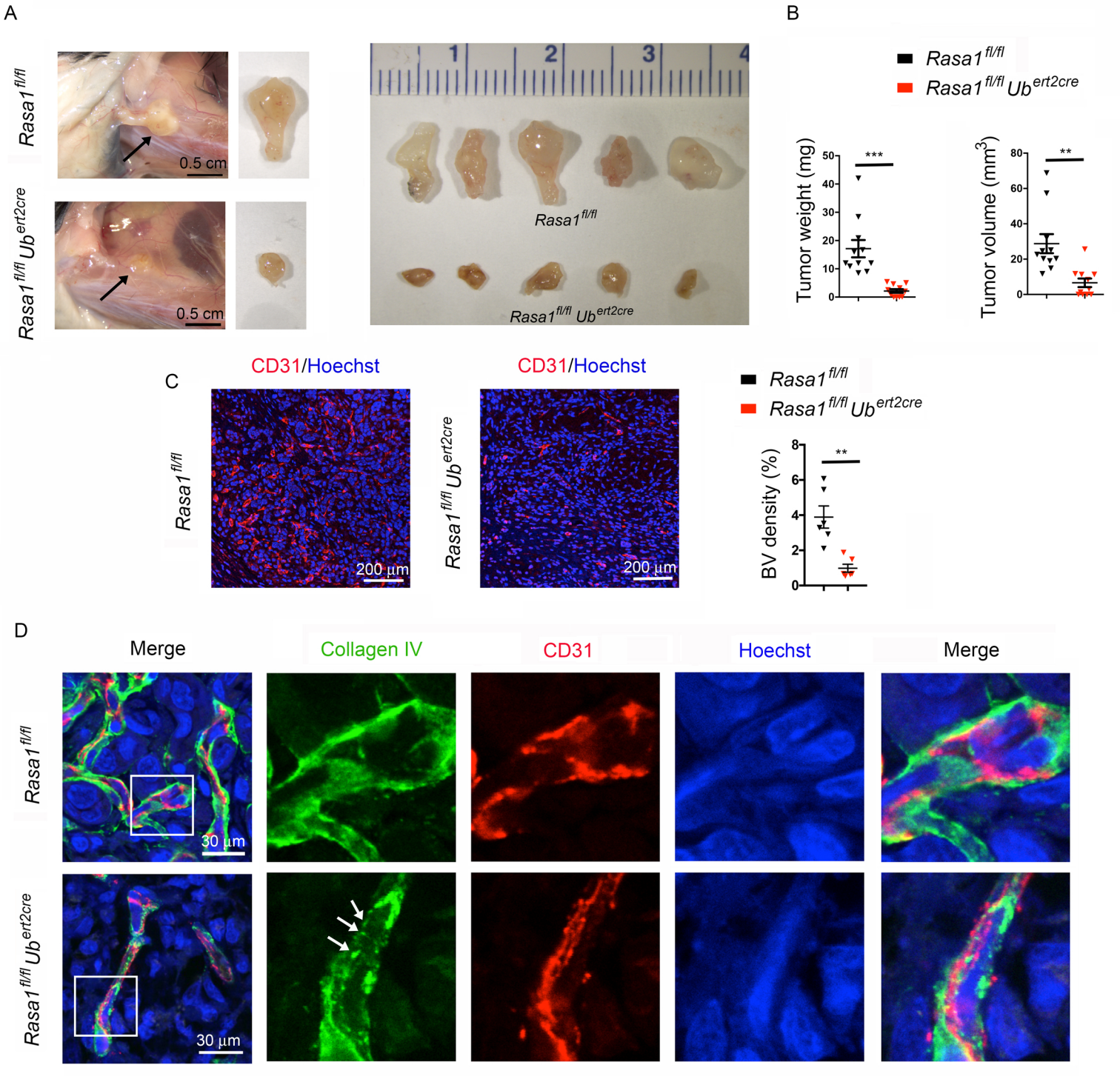




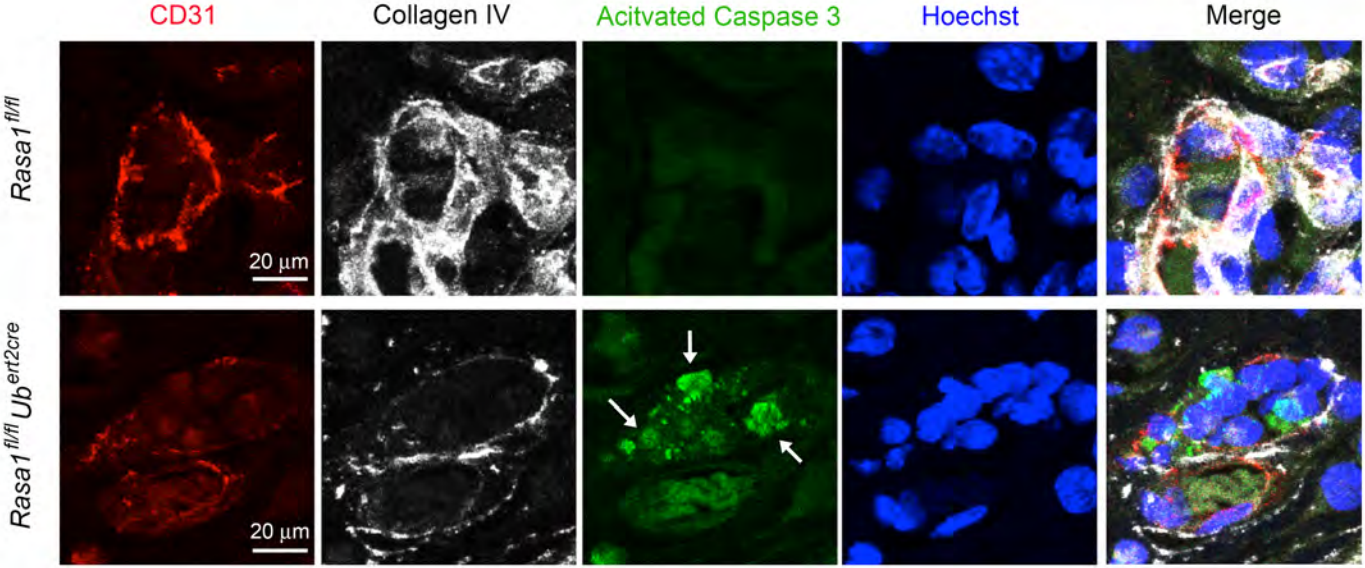
Supplemental Figure 19. Impaired retinal angiogenesis in neonatal induced RASA1-deficient mice. TM was administered to littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} mice at P1 and retinas were harvested at P6. (**A-E**) Retinas were stained with isolectin B4 (IB4) to identify BV. (**A**) Shown are representative images of IB4 staining. (**B**) Graph shows mean +/- 1 SEM of the number of branch points from veins (n=12 retinas each genotype). (**C**) Graph shows mean +/- 1 SEM of percentage coverage of retinas with BEC (n=7 retinas each genotype). (**D**) Representative higher power images of IB4 staining showing filopodia of BEC at edge of vascular fields (arrows). (**E**) Graph shows mean +/- 1 SEM of the number of filopodia per field (n=7 retinas each genotype). (**F**) Retinas were stained with IB4 and an antibody against collagen IV. Shown are representative images of staining. Note empty collagen IV sleeves in retinas of *Rasa1*$^{fl/fl}$ *Cdh5*^{ert2cre} mice (arrows). (**G**) Graph shows mean +/-1 SEM of the number of empty collagen sleeves (n=7 retinas each genotype). ****, $P<0.0001$, Student's 2-sample t-test.



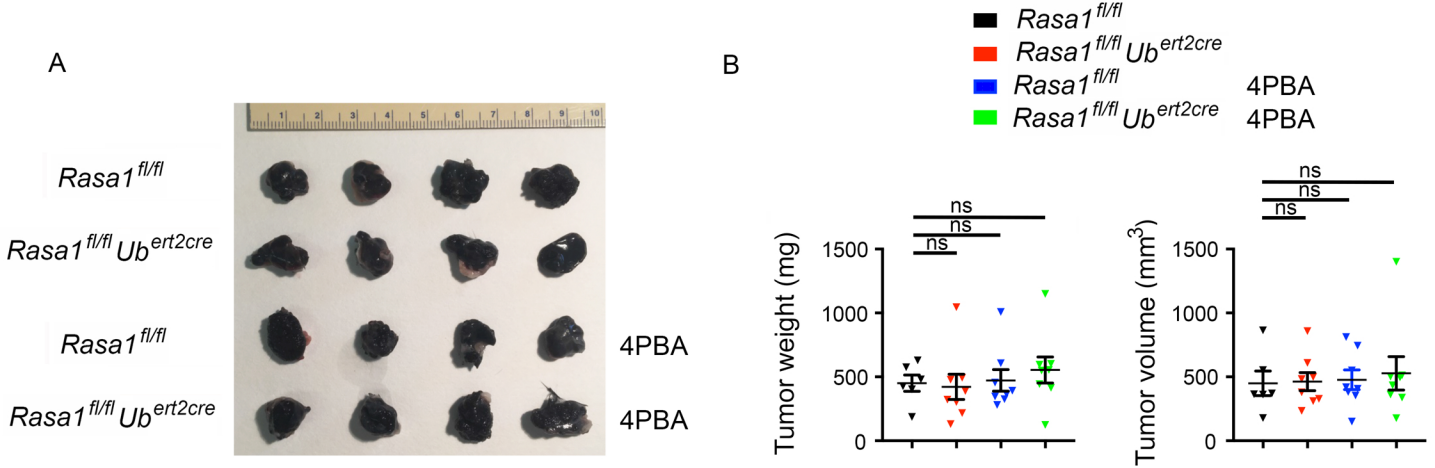
Supplemental Figure 20. Hemorrhage and impaired angiogenesis in retinas of neonatal induced RASA1-deficient mice. TM was administered to littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} mice at P3 and retinas were harvested at P10. (**A**) Gross appearance of representative retinas. Note areas of hemorrhage in retinas of *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} mice (arrows). (**B**) Retinas were stained with isolectin B4 (IB4) and an antibody against collagen IV. Collagen IV empty sleeves are indicated (arrows). Graph at right shows mean +/- 1 SEM of the number of empty collagen sleeves (n=5 retinas each genotype). ***, $P<0.001$, Student's 2-sample t-test.



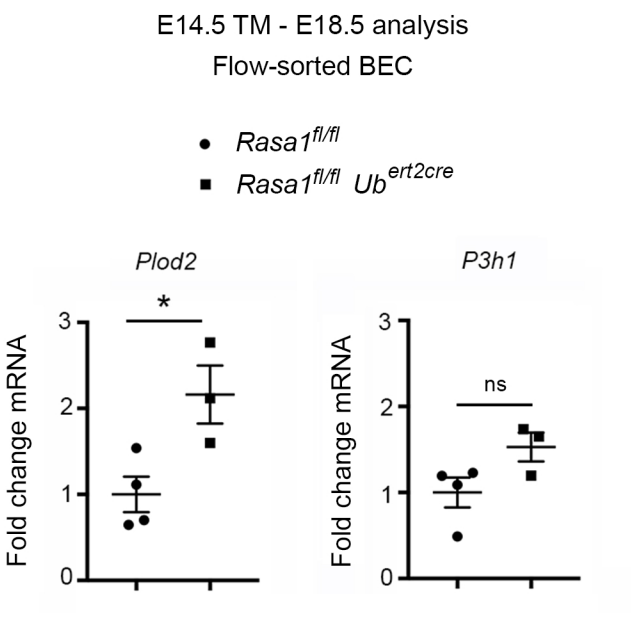
Supplemental Figure 21. Disruption of *Rasa1* in adult mice inhibits pathological angiogenesis in an ovarian tumor model. Littermate adult female *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} mice were administered TM before subcutaneous injection of ID8 ovarian tumor cells into flanks one week later. After 6 weeks, mice were euthanized and tumor growth was assessed. (**A**) Representative images of tumors before and after excision from indicated recipients are shown. (**B**) Graphs show mean +/- 1 SEM of tumor weight and volume (n=11 tumors from mice of each genotype). (**C**) Sections of tumors were stained with Hoechst and CD31 antibodies. Representative images show reduced BV density in tumors from *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} mice. Graph at right shows mean +/- 1 SEM of percentage coverage of fields with BV (n=6 tumors from mice of each genotype). (**D**) Tumors were stained with Hoechst and antibodies against collagen IV and CD31. Representative images are shown. Note accumulation of collagen IV in BEC of tumors from *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} mice. **, $P<0.01$; ***, $P<0.001$, Student's 2-sample t-test.



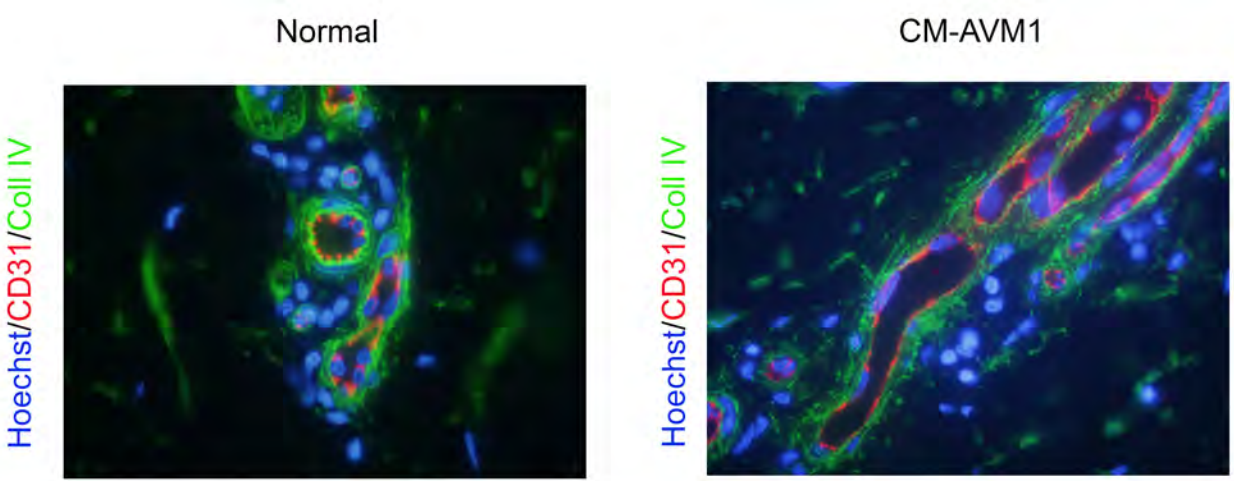
Supplemental Figure 22. Accumulation of collagen IV in BEC and BEC apoptosis in BV of ID8 tumors from induced RASA1-deficient mice. ID8 tumors from *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} mice in Supplemental Figure 21 were sectioned and stained with Hoechst and antibodies against CD31, collagen IV and activated caspase 3. Note accumulation of collagen IV in BEC and BEC apoptosis (arrows) in tumors from *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} mice.



Supplemental Figure 23. Influence of 4PBA alone upon B16 melanoma growth. Littermate adult *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} mice were injected into flanks subcutaneously with B16 melanoma cells. 4PBA was administered to some mice at the same time as B16 melanoma cells and every day thereafter for the duration of the experiment. After 13 days, mice were euthanized and tumors were harvested. (**A**) Representative images of harvested tumors are shown. (**B**) Graphs show mean +/- 1 SEM of tumor weight and volume (n=6-8 tumors from mice of each genotype and treatment condition). ns, not significant, one-way ANOVA test with a Dunnett's multiple comparisons post-hoc test.



Supplemental Figure 24. mRNA levels of collagen IV-modifying enzymes in induced RASA1-deficient BEC. TM was administered to littermate *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos at E14.5. At E18.5 skin BEC were purified from embryos and mRNA expression levels of the indicated collagen IV modifying enzymes were determined by real time PCR. Each symbol represents the fold change in mRNA expression in BEC from individual *Rasa1*$^{fl/fl}$ and *Rasa1*$^{fl/fl}$ *Ub*^{ert2cre} embryos relative to the mean fold change in mRNA expression in *Rasa1*$^{fl/fl}$ embryos. Bars indicate mean +/- 1 SEM of the fold change mRNA. *, $p < 0.05$; ns, not significant, Student's 2-sample t-test.



Supplemental Figure 25. Collagen IV distribution in CM-AVM1 lesion vessels. Sections of skin from a normal subject and lesional skin from a CM-AVM1 patient with an established somatic second hit *RASA1* mutation in EC of the same lesion were stained with Hoechst and antibodies against CD31 and collagen IV. Note absence of evidence of collagen IV accumulation in EC of the CM-AVM1 vessels.

Supplemental methods

Developmental angiogenesis. Pregnant mice were given 3 i.p. injections of TM (Sigma; 0.05 mg/g body weight per injection, dissolved in corn oil) on consecutive days. The MEK inhibitor, AZD6244 (Selleckchem; 0.05 mg/g body weight per injection), the PI3K inhibitor, PX-866 (Cayman chemical; 0.015 mg/g body weight per injection), the chemical chaperone, 4PBA (Sigma; 0.25 mg/g body weight per injection), and the 2OG dependent oxygenase inhibitors, EDHB and 2,4PDCA (Sigma; 0.1 mg/g body weight per injection) were injected i.p. into mice at the same time as TM and the day following the last TM injection for AZD6244 and PX-866 or for all subsequent days of gestation until harvest for 4PBA, EDHB and 2,4PDCA. The same drug administration protocols were employed for embryos that had not been administered TM. Embryos were fixed in 3.75% formaldehyde overnight and embedded in paraffin. Five micrometer sections were dehydrated and antigen retrieval was performed with a Diva de-cloaking kit (Biocare Medical). Sections were blocked in PBS/10% donkey serum/0.3% Triton-X100 and incubated overnight with the following primary antibodies in PBS in 10% donkey serum: rat anti-CD31 (SZ31, Dianova), rabbit anti-active caspase 3 (AF835, R&D Systems), goat anti-collagen IV (1340-01, Southern Biotech), goat anti-VE-Cadherin (AF1002, R&D systems), mouse anti-SMA (1A4/asm-1, Novus Biologicals), rabbit anti-laminin alpha 4 (PAC077Mu03, Cloud clone corp.), rabbit anti-TGN46 (ab16059, Abcam), rabbit anti-LMNA1 (ab125006, Abcam), rabbit anti-calnexin (ab22595, Abcam), rabbit anti-LYVE-1 (ab14917, Abcam), rabbit anti-calreticulin (D3E6, Cell Signaling Technology), rat anti-LAMP1 (1D4B, Thermo Scientific), rabbit anti-BIP (3177, Cell Signaling

Technology), hamster anti-podoplanin (provided by Y. Hong, University of Southern California, Los Angeles, California, USA). Secondary antibodies used were species-specific anti-immunoglobulin donkey $F(ab)_2$ fragments coupled to Alexa Fluor 488 , 594 or 647 (Jackson Immunoresearch) and were incubated with tissues in PBS for 2 hours. Sections were stained with Hoechst (Invitrogen) to identify nuclei before mounting and viewing on a BX60 upright fluorescence microscope (Nikon) or a Leica SP5 X confocal microscope (Leica Microsystems).

BEC isolation. Pregnant mice were given 3 i.p. injections of TM as above starting at E14.5 and embryos were harvested at E18.5. Skin was removed from embryos and single cell suspensions were prepared by digestion in collagenase IV (Sigma; 2 mg/ml) and DNase I (Sigma; 20 U/ml) for 1 h at 37^0C. Cells were stained with CD45.2-FITC (104, BD Biosciences), CD31-BV510 (MEC13.3, BD Biosciences) and LYVE-1-Alexa Flour 700 (ALY7, Novus Biologicals) antibodies and CD45.2- CD31+ LYVE1- BEC were isolated by flow cytometry on a BD FACSAria II (BD Biosciences) and flash frozen. Purity of sorted BEC from all embryos was greater than 98%.

Cell culture and siRNA transfection

HUVEC from pooled donors (Lonza, CC-2519**)** were cultured in EBM™-2 Basal Medium (Lonza, CC-3156**)** supplemented with EGM™-2 SingleQuotsTM Supplement Pack (Lonza, CC-4176). Cells were transfected with 50 nM *RASA1* siRNA (5'-GCAGGCAGGGAAGUCUGGCAGUUAU-3', Invitrogen) using Lipofectamine RNAi MAX (Invitrogen) reagent according to the manufacturer's protocol. Stealth RNAi™

siRNA Negative Control Hi GC (Invitrogen) was used as control. Following transfection, HUVEC were recultured in the presence or absence of 2 mM 4PBA for 24 hours.

Real time PCR. Genomic DNA was prepared from sorted BEC or from embryo tails using a DNEasy Blood and Tissue kit (Qiagen) and relative *Rasa1* gene abundance was determined by real time qPCR for *Rasa1* exon 18 as described (1). Total RNA was isolated from HUVEC and sorted BEC using an RNeasy Mini Kit (Qiagen) and reverse transcribed using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions. *RASA1* gene expression in HUVEC and *Plod2* and *P3h1* gene expression in sorted BEC was assessed by real time PCR of cDNA using a SYBR Green mix (Applied Biosystems) and the following primers: *RASA1*, forward 5'-GGCCGGGAAGAAGATCCAC-3' and reverse 5'-GCAGACTTGACCAACTGTCATT-3'; human *β-actin* internal control, forward 5'- TGACCCAGATCATGTTTGAGA-3' and reverse 5'- TACGGCCAGAGGCGTACAGC-3'; *Plod2,* forward 5'-GAGAGGCGGTGATGGAATGAA-3', reverse 5'-ACTCGGTAAACAAGATGACCAGA-3'; *P3h1,* forward 5'-AACAGAAGTCGGAACGCGAAA-3', reverse 5'- TCCACGAGGGTCTCGATCTC-3'; mouse *β-actin* internal control, forward 5'- CGGTTCCGATGCCCTGAGGCTCTT-3' and reverse 5'- TCCACGAGGGTCTCGATCTC-3'.

Tissue culture immunofluorescence

HUVECs were fixed with 4% paraformaldehyde in PBS for 30 minutes at room temperature washed in PBS, permeabilized with 0.2% Triton X-100, and blocked with

10% donkey serum for 1 hour. Cells were incubated with antibodies against collagen IV (1340-01, SouthernBiotech) and calnexin (PA5-34754, Thermo Fisher) overnight, followed by Alexa Fluor®488 or 594 conjugated secondary antibodies (Jackson ImmunoResearch) for 4 hours. Cells were stained with Hoechst to identify nuclei before mounting and viewing on a Leica SP5 X confocal microscope.

BEC protein extraction and digestion. Sorted BEC were lysed in RIPA buffer (2% SDS, 150mM NaCl, 50mM Tris pH8, 1X Roche Complete) and lysates from three individual embryos of the same genotype (determined by *Cre* PCR of tail genomic DNA), representing approximately 3 $x10^5$ BEC total, were pooled. Gene disruption in Cre+ embryos exceeded 90% in all cases as determined by *Rasa1* exon 18 qPCR of tail genomic DNA. The protein concentration of the extract was determined by Qubit fluorometry. Five micrograns of each sample was processed by SDS-PAGE using a 10% Bis-Tris NuPage Mini-gel with the MES buffer system (Invitrogen). The gel was run 5cm, each gel lane was excised into twenty equally sized bands. Gel bands were processed by in-gel digestion with trypsin using a ProGest robot (DigiLab) with the following protocol: (a) washed with 25mM ammonium bicarbonate followed by acetonitrile; (b) reduced with 10mM dithiothreitol at 60°C followed by alkylation with 50mM iodoacetamide at RT; (c) digested with sequencing grade trypsin (Promega) at 37°C for 4h and; (d) quenched with formic acid and analyzed without further processing.

Mass spectrometry. Half of each digest was analyzed by nano LC-MS/MS with a Waters NanoAcquity HPLC system interfaced to a ThermoFisher Fusion Lumos mass

spectrometer. Peptides were loaded on a trapping column and eluted over a 75µm analytical column at 350nL/min using a 0.5 h reverse phase gradient; both columns were packed with Luna C18 resin (Phenomenex). The mass spectrometer was operated in data-dependent mode with the Orbitrap operating at 60,000 FWHM and 15,000 FWHM for MS and MS/MS respectively. The instrument was run with a 3 s cycle for MS and MS/MS with Advanced Peak Determination enabled. A total of 10 h of instrument time/sample was employed.

Proteomic data processing and analysis. Data were searched using a local copy of Mascot with the following parameters: Enzyme, Trypsin/P; Database: SwissProt Mouse (concatenated forward and reverse plus common contaminants); Fixed modifications: Carbamidomethyl (C); Variable modifications: Oxidation (M), Acetyl (N-term), Pyro-Glu (N-term Q), Deamidation (N,Q); Mass values: Monoisotopic; Peptide mass tolerance: 10 ppm; Fragment mass tolerance: 0.02 Da; Maximum missed cleavages: 2. Mascot data files were subsequently parsed into Scaffold (Proteome Software Inc.) and analyzed with protein and peptide thresholds set at 1% FDR, and minimum number of peptides to 2. For quantitation by spectral counting, the complete list of proteins displaying the total spectrum count was exported to excel, the Normalized Spectrum Abundance Factor (NSAF) calculated according to literature and subsequently the fold change was calculated from the NSAF values (2). Fold changes from >2 (for up-regulation) or <0.5 for down-regulation were considered significant.

Retinal angiogenesis. Newborn pups were injected with TM (0.05 mg/g body weight per injection) on three consecutive days from P1-P3 and retinas were harvested at P4 or P6. Alternatively, pups were administered TM from P3-P5 and retinas were harvested at P10. Retinas were fixed in 4% paraformaldehyde for 2 h, were blocked in PBS/10% donkey serum and incubated overnight with IB4-FITC (Sigma) and goat anti-collagen IV in PBS/10% donkey serum. Retinas were subsequently incubated for 2 h with a secondary anti-goat donkey $F(ab)_2$ coupled to Alexa 594 in PBS to detect collagen. Whole mounts were viewed on a BX60 upright fluorescence microscope. The percentage EC coverage and number of empty sleeves per field was determined in a random 50 μm x 50 μm area behind the angiogenic front in a region between an artery and a vein. The number of endothelial branch points was determined for the same vein. The number of filopodia at the angiogenic front was determined for a random 200 μm x 200 μm region.

Tumor angiogenesis. TM was administered to mice at 2 months of age (female mice for ID8 experiments and mice of either sex for B16 experiments). After 1 week, mice were injected s.c. in the flank with 1 x 10^6 ID8 cells or 0.75 x 10^6 B16F10 cells (provided by Weiping Zou, Michigan Medicine, Ann Arbor Michigan) suspended in 100 μl matrigel (Corning). In B16 experiments, 4PBA was administered to some mice at the same time as tumor and for each day thereafter (0.4 mg/g body weight per injection). ID8 and B16 tumors were harvested 6 weeks or 13 days later respectively and tumor weight and volume was determined as described (3). Tumors were fixed in 4% paraformaldehyde overnight and 5 μm sections were stained with Hoechst and antibodies against CD31, activated caspase 3 and collagen IV as above. Sections were viewed on a BX60 upright

fluorescence microscope or a Leica SP5 X confocal microscope. BV density was assessed within a random 800 μm x 800 μm region.

CM-AVM1 tissue samples and staining. CM-AVM1 patients examined in these studies have been reported previously (4, 5). Skin punch biopsy specimens from lesional areas of patients and normal controls were fixed in 3.75% formaldehyde overnight and embedded in paraffin. Five-micrometer sections were processed as above and stained with Hoechst and antibodies against CD31 (clone EP3095, LSBio) and collagen IV followed by species-specific labeled secondary antibodies (Jackson Immunoresearch). Sections were viewed on a BX60 upright fluorescence microscope.

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