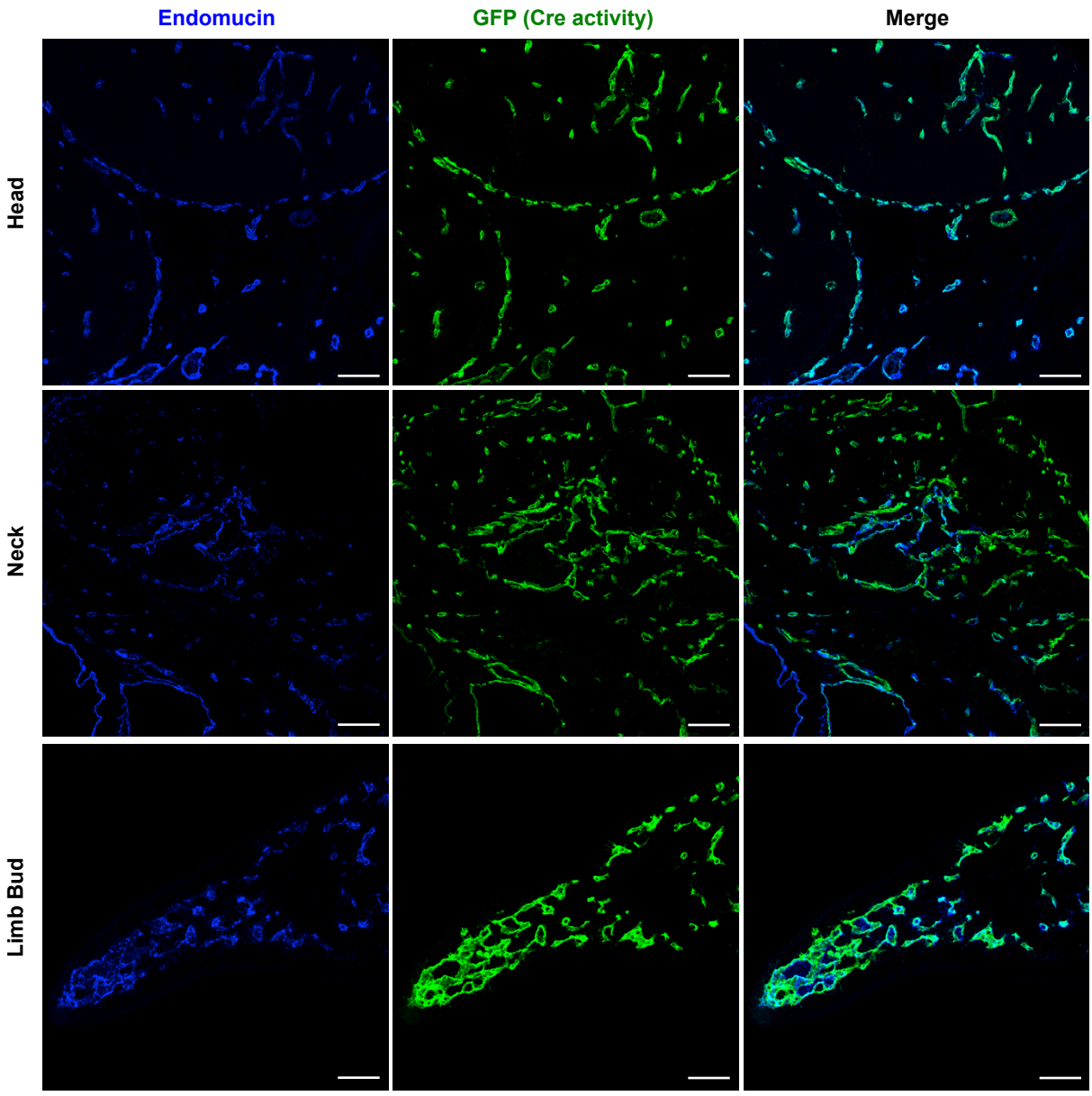

Figure I: Tamoxifen-induced activation of *Cdh5(PAC)-Cre^{ERT2}* generates widespread endothelial cell Cre activity throughout the E12.5 embryo. Pregnant females carrying *ROSA$^{mT/mG}$;Cdh5(PAC)-Cre^{ERT2}* embryos were injected with 1 mg tamoxifen at E9.5, E10.5, and E11.5. The *ROSA$^{mT/mG}$* reporter converts ubiquitous membrane-targeted Tomato (mT) to membrane-targeted green fluorescent protein (mG) upon Cre-mediated excision (Muzumdar et al., 2007). Embryos were dissected at E12.5, sectioned, and stained for the endothelial cell marker endomucin (blue) for analysis of Cre reporter activity (green) in endothelial cells. At least 3 embryos from independent litters were analyzed, and representative images of embryonic head vasculature (A), neck vasculature (B), and limb bud vasculature (C) are shown with largely uniform endothelial cell Cre activity. Scale bars: 100 μm.

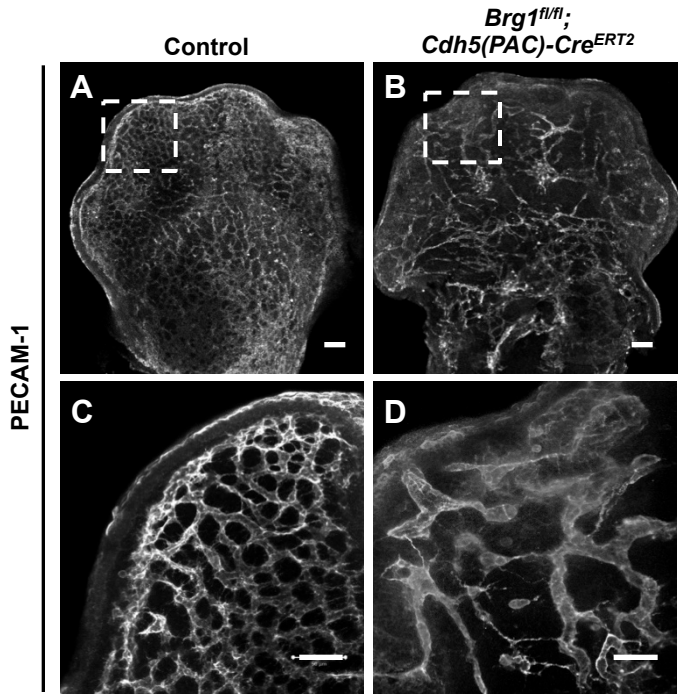

Figure II: *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* limb bud capillaries are disorganized and dilated. Limb buds from E12.5 littermate control (*Brg1$^{fl/fl}$*) and *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* embryos were whole-mount immunostained for the blood vessel endothelial cell marker PECAM-1 and imaged by confocal microscopy. PECAM-1 staining reveals disorganized and dilated capillaries in *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* limb buds (B and D). C and D are magnified views of the boxed regions in A and B, respectively. Scale bars: 100 μm (A,B), 50 μm (C,D).

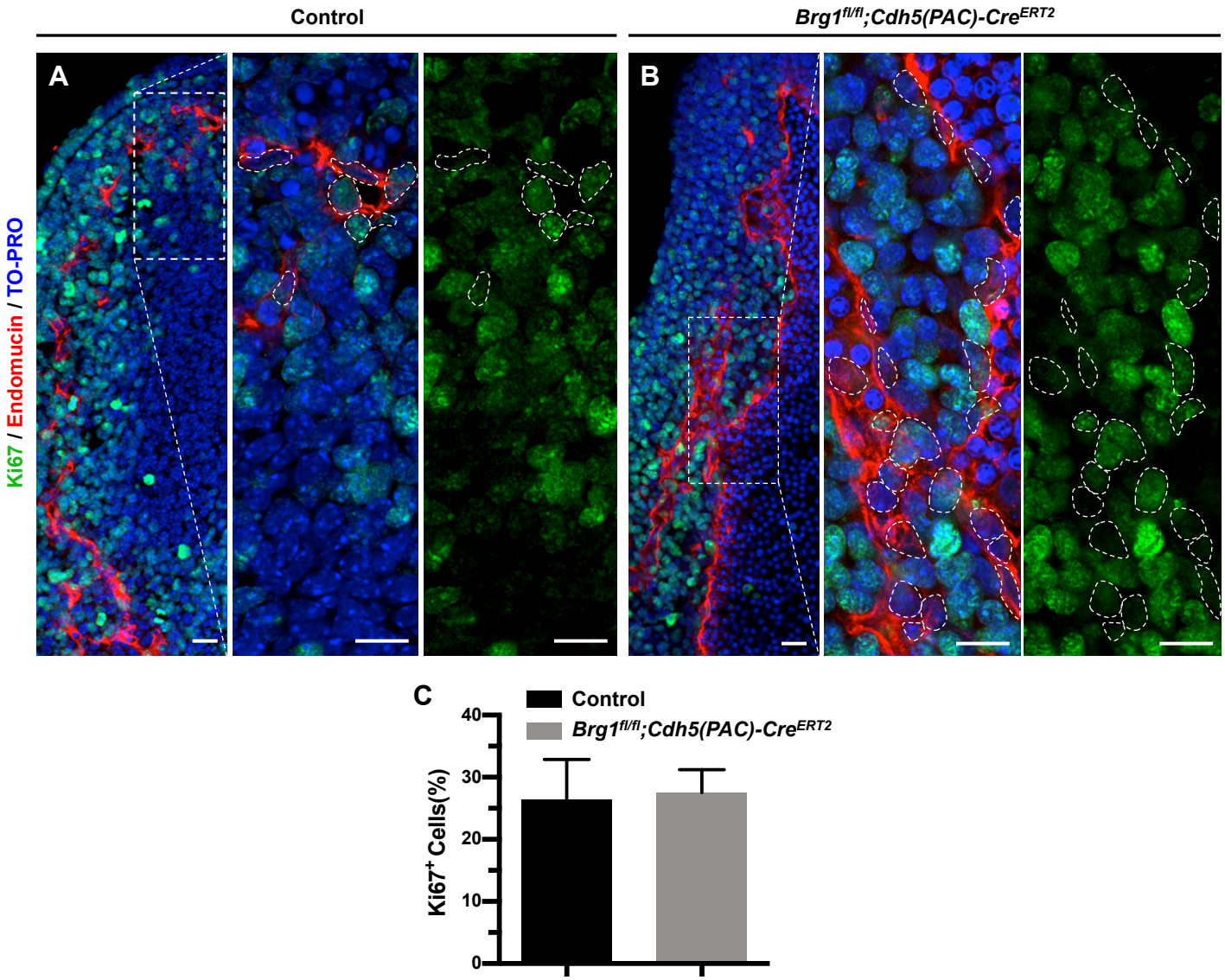

Figure III: Capillary endothelial cell proliferation is comparable in control and *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* embryos. (**A,B**) E12.5 littermate control (*Brg1$^{fl/fl}$*) and *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* embryos were sectioned and immunostained for the vascular marker endomucin (red) and for the cell proliferation marker Ki67 (green). All sections were counterstained with the nuclear marker TO-PRO (blue). Images of immunostaining in the limb buds are representative of results obtained from 3 different sets of littermate control and mutant embryos. Boxed regions are shown magnified in merged and single channel Ki67^{+} panels. Individual capillary endothelial cell nuclei are outlined with dotted lines to indicate cells assessed for Ki67 staining. Scale bars: 10μm. (**C**): Quantification of Ki67^{+} capillary endothelial cells based on images such as those shown in A and B. Data represent Ki67+ cells out of total capillary endothelial cells counted (as %age) for control and mutant samples. Data were compiled from 2 vessels in 3 images taken from 4 different sets of littermate control and mutant embryos; error bars represent S.D. Statistical calculations were performed using a two-tailed Student's t test, and no significant difference was seen between Ki67^{+} capillary endothelial cells in control and mutant samples.

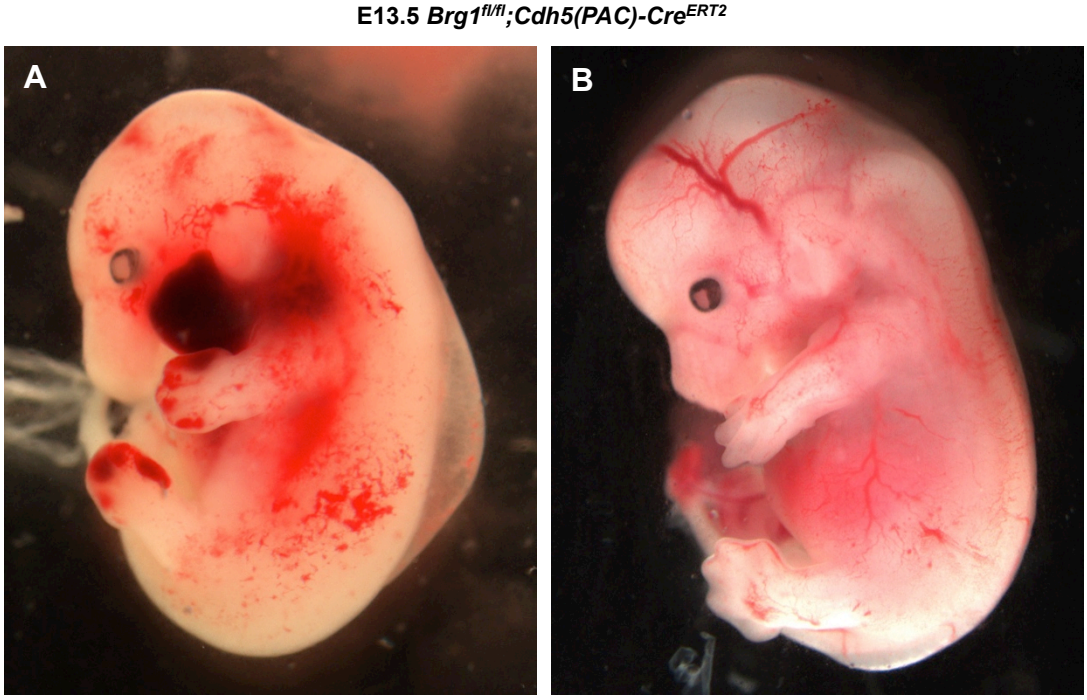

Figure IV: *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* vascular phenotypes are dependent on the time of Cre induction. (**A**) When pregnant females carrying *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* mutant embryos were induced daily with 1 mg tamoxifen at E9.5-11.5, mutant embryos displayed exacerbated neck and limb bud aneurysms and lethal hemorrhage by E13.5. (**B**) However, when pregnant females were induced daily with 1 mg tamoxifen at E10.5-12.5, *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* mutant embryos were indistinguishable from littermate *Brg1$^{fl/fl}$* control embryos at E13.5. Representative images from at least four independent litters for each induction scheme are shown.

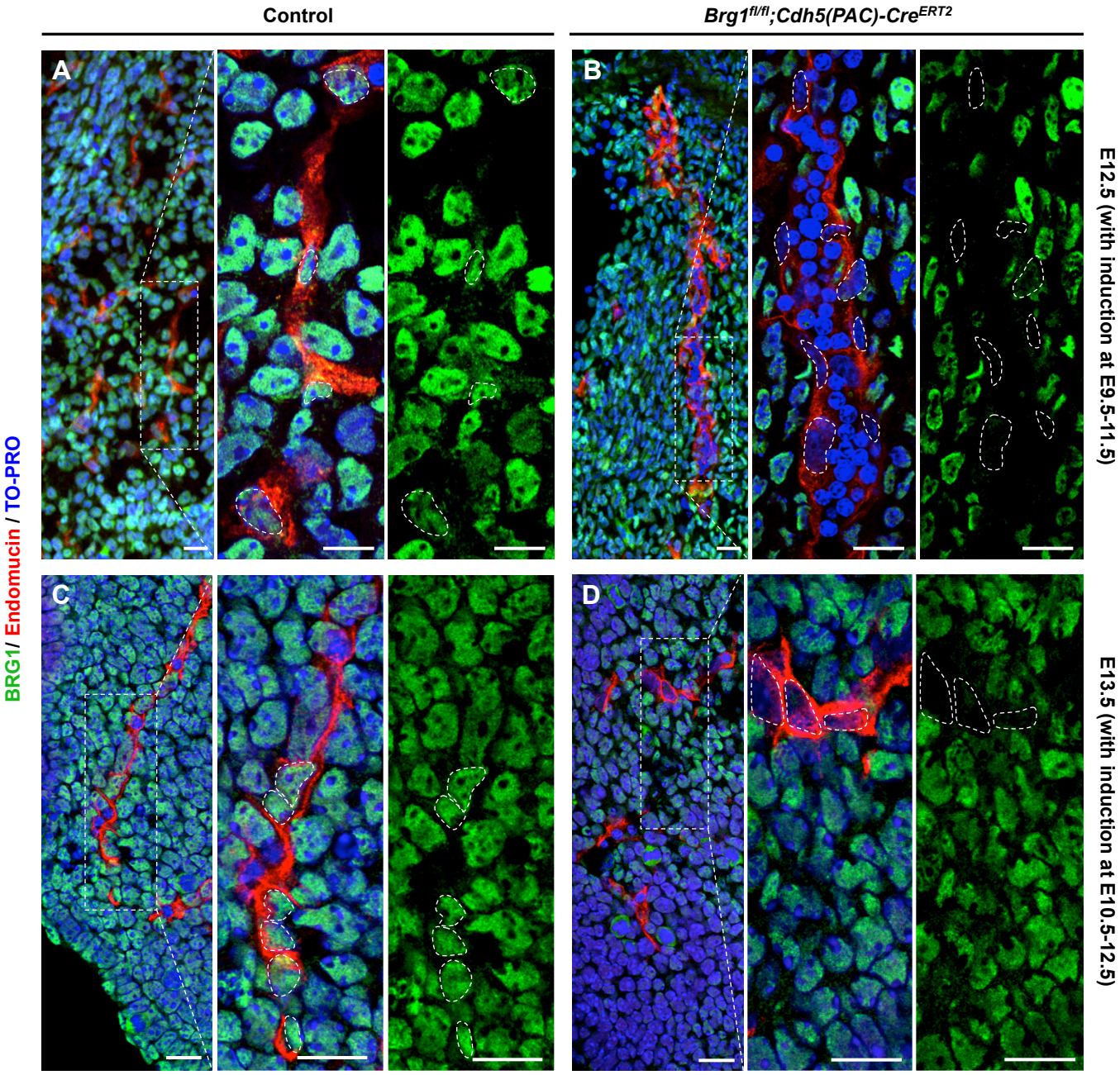

Figure V: Tamoxifen-induced activation of *Cdh5(PAC)-Cre^{ERT2}* at E9.5-11.5 or E10.5-12.5 efficiently excises *Brg1* in endothelial cells. (**A,B**): Pregnant females carrying control (*Brg1$^{fl/fl}$*) and *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* embryos were injected with 1 mg tamoxifen at E9.5, E10.5, and E11.5. Littermate embryos were dissected at E12.5, and cryosections were immunostained with antibodies against BRG1 (green) and the endothelial cell marker endomucin (red) and were counterstained with the nuclear marker TO-PRO (blue). Images of immunostaining in the limb buds are representative of results obtained from 3 different sets of littermate control and mutant embryos. Boxed regions are shown magnified in merged and single channel BRG1^{+} panels. Individual capillary endothelial cell nuclei are outlined with dotted lines to indicate cells assessed for BRG1 staining. (C,D): Similar analyses were performed on sections obtained from E13.5 embryos in which pregnant females were injected with 1 mg tamoxifen at E10.5, E11.5, and E12.5. Scale bars: 10μm.

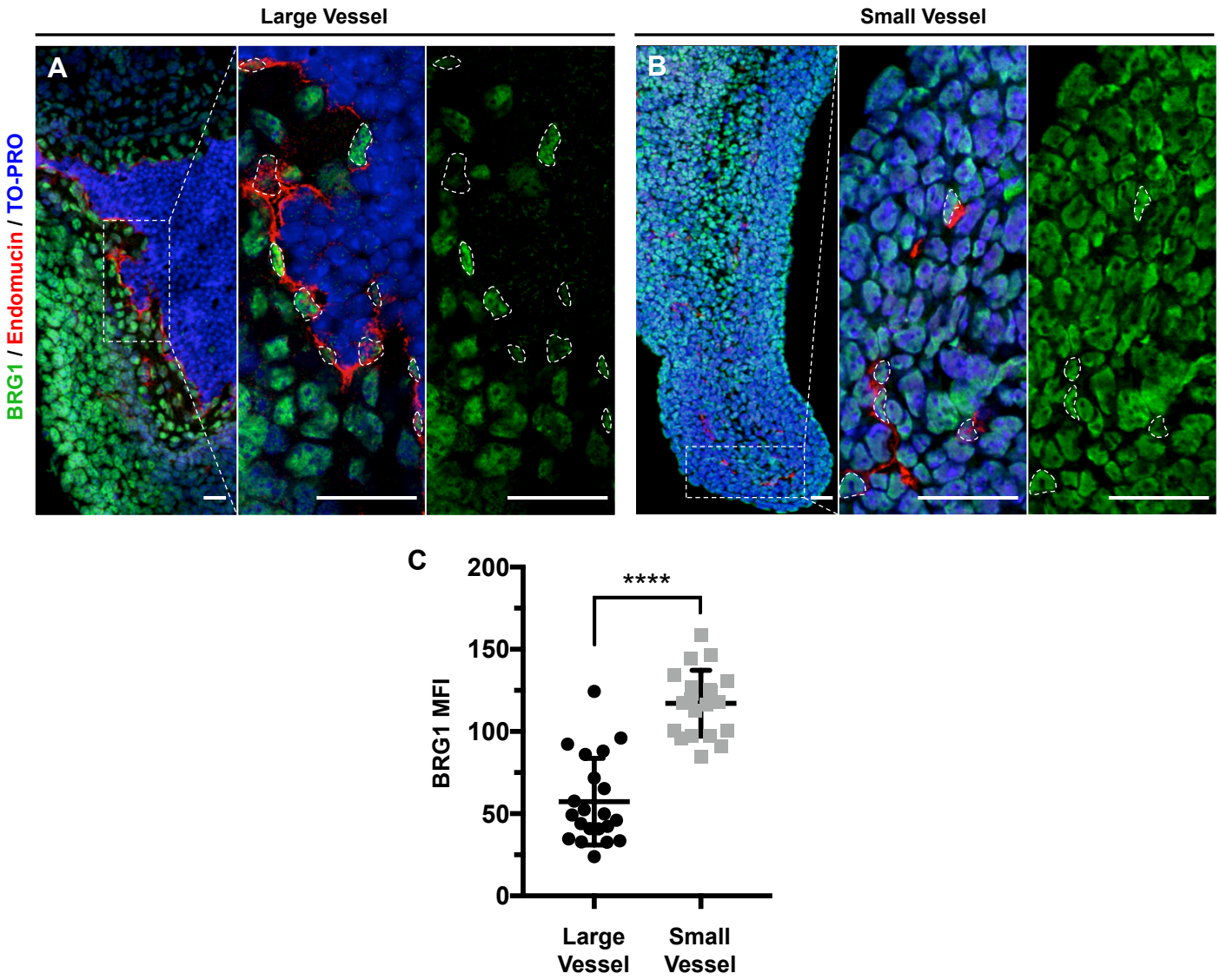

Figure VI: BRG1 expression levels are higher in endothelial cells lining small caliber vessels versus large caliber vessels at E12.5. (**A,B**) E12.5 wildtype embryos were sectioned and immunostained for BRG1 (green) and the vascular marker endomucin (red) and were counterstained with the nuclear marker TO-PRO (blue). Images are representative of results obtained from 3 different sets of littermate control and mutant embryos. Boxed regions are shown magnified in merged and single channel BRG1^{+} panels. Individual endothelial cell nuclei are outlined with dotted lines to indicate cells assessed for BRG1 staining in large caliber vessels (A) and small caliber (i.e. capillary) vessels (B). Scale bars: 20μm. (**C**): Quantification of BRG1 mean fluorescent intensity (MFI) in large and small caliber vessels, based on images such as those shown in A and B. Data were compiled from 2 vessels in 2 images taken from 3 different embryos; error bars represent S.D. Statistical calculations were performed using a two-tailed Student's t test (****, P<0.0001).

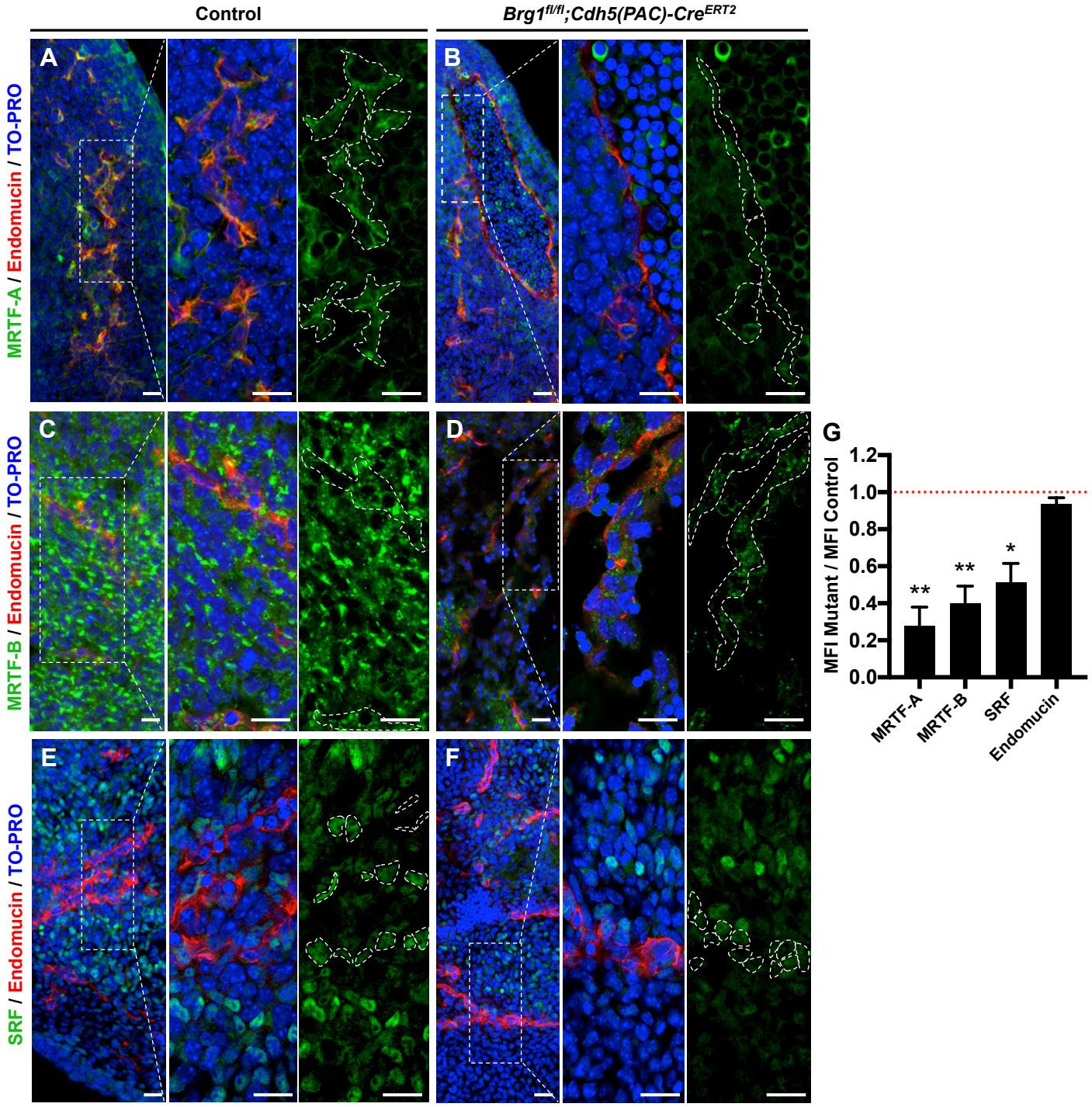

Figure VII: MRTF-A, MRTF-B, and SRF protein expression are downregulated in *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* capillary endothelial cells. (**A-F**): E12.5 littermate control (*Brg1$^{fl/fl}$*) and *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* embryos were sectioned and immunostained for the vascular marker endomucin (red) and for MRTF-A (green, A,B) or MRTF-B (green, C,D) or SRF (green, E,F). All sections were counterstained with the nuclear marker TO-PRO (blue). Images of immunostaining in the limb bud or neck capillary beds are representative of results obtained from 3 different sets of littermate control and mutant embryos. Boxed regions are shown magnified in merged and single channel panels. Individual capillary endothelial cells or nuclei are outlined with dotted lines in the single channel panels to indicate cells for which immunostaining was quantified. Scale bars: 10µm. (**G**): Quantification of immunostaining for MRTF-A, MRTF-B, SRF, and endomucin in capillary endothelial cells based on images such as those shown in A-F. Data represent average mean fluorescent intensities (MFI) of mutant over normalized control endothelial cells (dotted line). Data were compiled from 2 vessels in 2 images taken from 3 different sets of littermate control and mutant embryos; error bars represent S.D. Statistical calculations were performed using a two-tailed Student's t test (**, $P<0.01$; *, $P<0.05$). The same quantification data are also summarized in Figure 3.

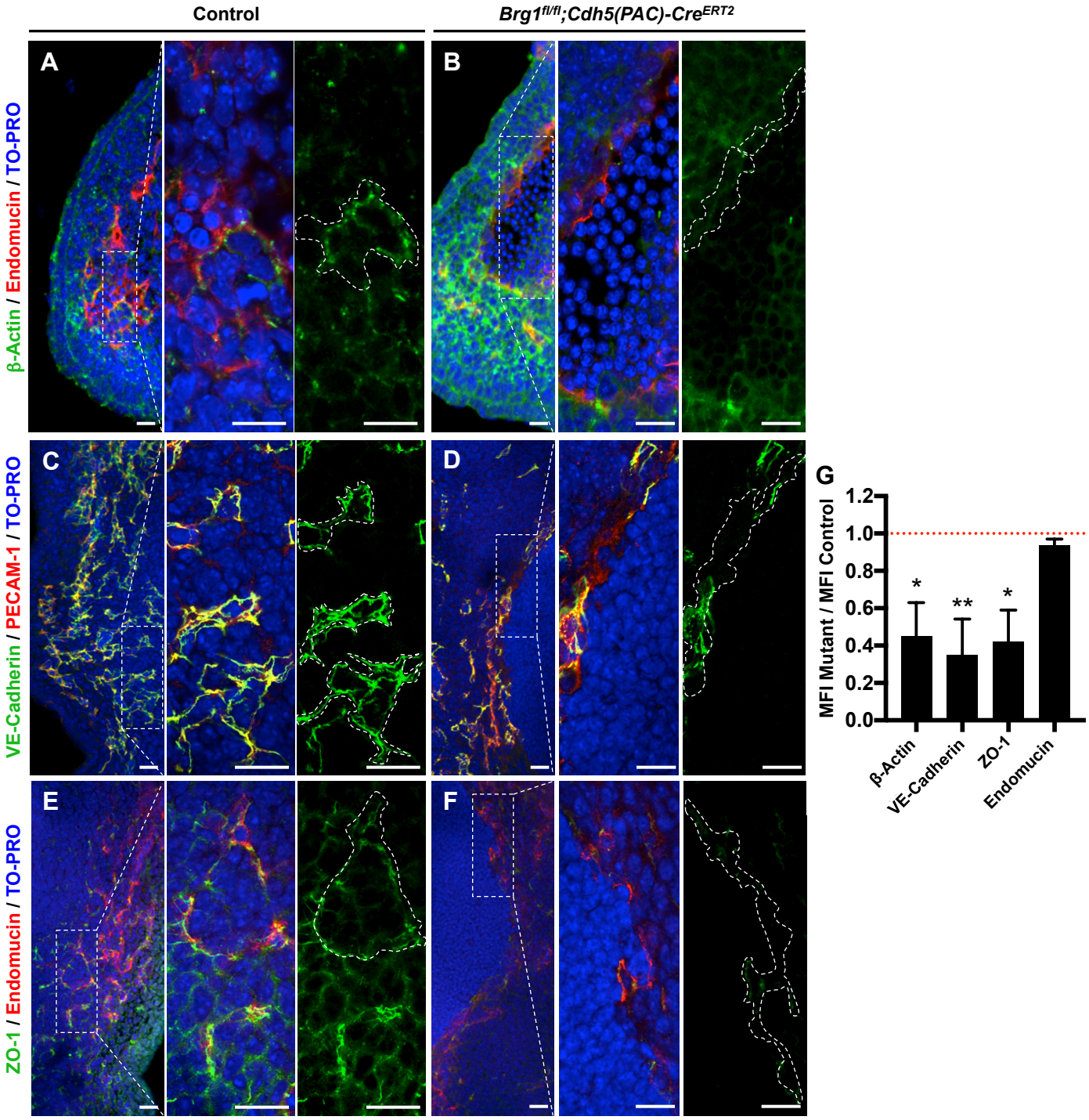

Figure VIII: The SRF target gene products β-actin, VE-Cadherin, and ZO-1 are downregulated in *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* capillary endothelial cells. (**A-F**): E12.5 littermate control (*Brg1$^{fl/fl}$*) and *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* embryos were sectioned and immunostained for the vascular markers endomucin or PECAM-1 (red) and for β-actin (green, A,B) or VE-Cadherin (green, C,D) or ZO-1 (green, E,F). All sections were counterstained with the nuclear marker TO-PRO (blue). Images of immunostaining in the limb bud or neck capillary beds are representative of results obtained from 3 different sets of littermate control and mutant embryos. Boxed regions are shown magnified in merged and single channel panels. Individual capillary endothelial cells are outlined with dotted lines in the single channel panels to indicate cells for which immunostaining was quantified. Scale bars: 10μm. (**G**): Quantification of immunostaining for β-actin, VE-Cadherin, ZO-1, and endomucin in capillary endothelial cells based on images such as those shown in A-F. Data represent average mean fluorescent intensities (MFI) of mutant over normalized control endothelial cells (dotted line). Data were compiled from 2 vessels in 2 images taken from 3 different sets of littermate control and mutant embryos; error bars represent S.D. Statistical calculations were performed using a two-tailed Student's t test (**, $P<0.01$; *, $P<0.05$). The same quantification data are also summarized in Figure 3.

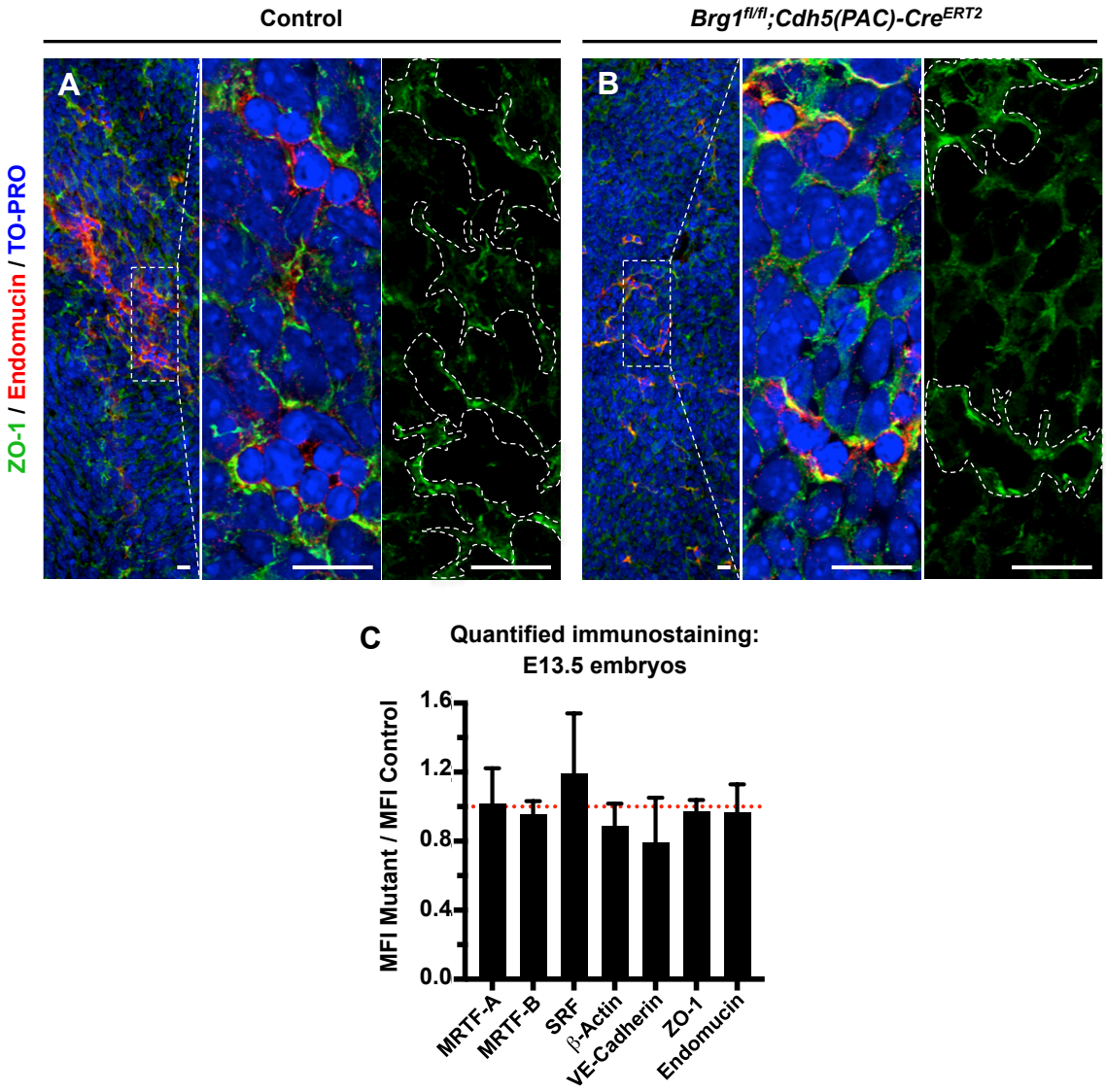

Figure IX: MRTFA/B, SRF, and SRF target gene products are expressed at normal levels in capillary endothelial cells of E13.5 *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* embryos following E10.5-12.5 tamoxifen induction. (**A,B**): E13.5 littermate control (*Brg1$^{fl/fl}$*) and *Brg1$^{fl/fl}$;Cdh5(PAC)-Cre^{ERT2}* embryos that had undergone tamoxifen induction at E10.5-12.5 were sectioned and immunostained for the vascular marker endomucin (red) and for ZO-1 (green). All sections were counterstained with the nuclear marker TO-PRO (blue). Images of immunostaining in the limb bud or neck capillary beds are representative of results obtained from 3 different sets of littermate control and mutant embryos. Boxed regions are shown magnified in merged and single channel ZO-1 panels. Individual capillary endothelial cells are outlined with dotted lines in the single channel panels to indicate cells for which ZO-1 immunostaining was quantified. Scale bars: 10μm. (**C**): Quantification of immunostaining for MRTF-A, MRTF-B, SRF, β-actin, VE-Cadherin, ZO-1, and endomucin in capillary endothelial cells, based on images such as those shown for ZO-1 staining in A and B. Data represent average mean fluorescent intensities (MFI) of mutant over normalized control endothelial cells (dotted line). Data were compiled from 2 vessels in 2 images taken from 3 different sets of littermate control and mutant embryos; error bars represent S.D. Statistical calculations were performed using a two-tailed Student's t test, and no significant differences in expression of any of the proteins assessed were detected. This stands in contrast to the significant downregulation of MRTF-A, MRTF-B, SRF, β-actin, VE-Cadherin, and ZO-1, which was seen in E12.5 embryos following tamoxifen induction at E9.5-11.5 (see Figures 3 and VII and VIII in the online only Data Supplement).

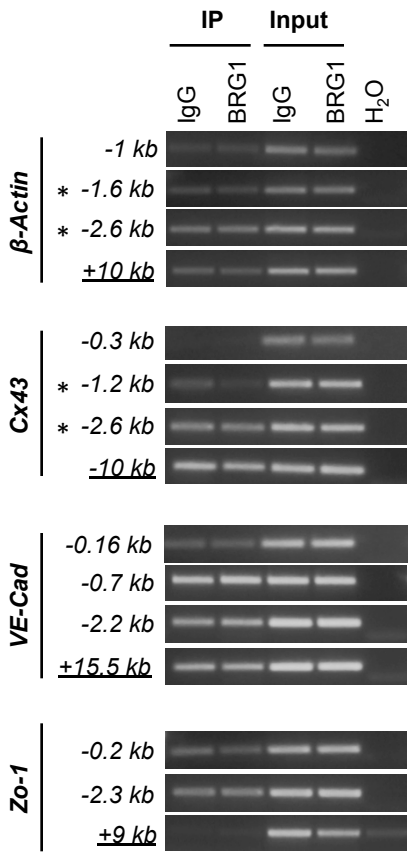

Figure X: BRG1 does not interact with the promoters of SRF target genes in endothelial cells. ChIP assays were performed in C166 endothelial cells using antibodies against BRG1 or rabbit IgG as a negative control. Immunoprecipitated (IP) DNA was isolated and amplified by PCR to determine whether BRG1 bound conserved regions of the promoters of the SRF target genes *β-actin*, *Cx43*, *VE-Cadherin*, and *Zo-1*. Input DNA was isolated from chromatin prior to immunoprecipitation and was used to confirm PCR efficiency. H_2O was amplified instead of DNA as a negative control for PCR. PCR amplicons were designed at designated distances from each gene's transcription start site. Negative control amplicons (underlined) were selected based on their greater distances from each transcription start site. Asterisks (*) denote amplicons that contain a CArG box (SRF-binding motif). Images shown are representative of at least three independent experiments.

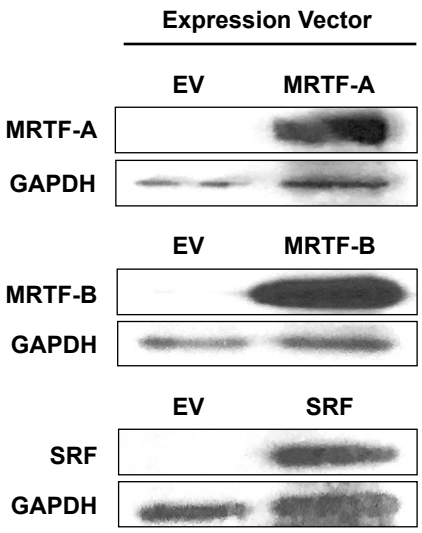

Figure XI: Validation of MRTF-A/B and SRF overexpression plasmids. HEK293T cells were transfected with equivalent quantities of empty vector (EV) or with murine MRTF-A, MRTF-B, or SRF expression plasmids for 24 hr. Cell lysates were collected and analyzed by immunoblot for expression of the relevant proteins or GAPDH (loading control). These overexpression plasmids were subsequently used for the experiment shown in Fig. 5C.

Table I

qPCR primers (murine)

Gene Name	Forward (5' to 3')	Reverse (5' to 3')
Srf	CACCTACCAGGTGTCGGAAT	GCTGTCTGGATTGTGGATTGTGGAGGT
Mrtfa	CCTAAGCAGCAGGAAAATGG	GCTGCTGGAGGTGACTTTTC
Mrtfb	ACCCTGCTCTTCAATCCAGA	GGTGGATCTTTTGTCTTCGTG
Zo-1	AGCAAGCCTTCTGCACATCT	CAGCATCAGTTTCGGGTTTT
Cx43	GGACTGCTTCCTCTCACGTC	GAGCGAGAGACACCAAGGAC
VE-Cad	CTTCAAGCTGCCAGAAAACC	ATTCGGAAGAATTGGCCTCT
β-actin	TGTTACCAACTGGGACGACA	GGGGTGTTGAAGGTCTCAAA
Gapdh	TCAACGGCACAGTCAAGG	ACTCCACGACATACTCAGC
Rn18s	CCCGAAGCGTTTACTTTGAAA	CGCGGTCCTATTCCATTATTC

Table II

ChIP-PCR primers (murine)

Gene Name	Forward (5' to 3')	Reverse (5' to 3')
Mrtfa -0.5 kb	GGCAGGGACATGACAGAGTT	GCCTGGAGGTTTAATGCAAA
Mrtfa -1.1 kb	ATGCTGGGGTGCAACTTTT	CTCCTCCTTTTAGGCCACCA
Mrtfa -2.5 kb	GGGCTTTTCAGGTCTGAGG	AAGGCAGGGCATACTGGATA
Mrtfa -10.5 kb	TTTGGCTTGCTGAAGTGTTG	CAGTTGTGGATGGTTGGTTG
Mrtfb -0.2 kb	CAGCCGTTCATCTTCCTCTT	CTTCCCTTTGCCATTCCAG
Mrtfb -0.8 kb	TTCGACAAGACTCCTCATCG	CAGGCAACGTGGAAACAAGT
Mrtfb -2.0 kb	TGCTATCTCTCAGTTTTCCCATC	GGAGTAACCACAAGGCACTAATTT
Mrtfb -7.5 kb	GCCTAGAGTTTCGTGGTGGA	GCTTTGGTTTGTACCCACGTT
Srf -0.2 kb	GTTCGGCTCCACTGTTCCT	CCCGAAGGGGACACTTATTT
Srf -0.7 kb	CAAAGCTCCCCCTCCACCCC	CGAAAGAGGGCAGGGATAGA
Srf -1.0 kb	CTATGTAGGTCGGGTTGGCT	GGTGTTACAAAGCCCAGGTT
Srf +10 kb	GCGAGGACTTAATCGATGGT	GGTAGTCCGGTGTGAAGCAT
β-actin -1.0 kb	GCTTGTCACTCCCAGAATCC	TGGGCCGTTAGCTAGTGTCT
β-actin -1.6 kb	CAAACTGCCTGAGCAACTGA	GCAGTCACCAACCCTGAGAG
β-actin -2.6 kb	TGGGTGCCTGACAGTAACAG	GGGGAAAAGAGGAAGTGGAG
β-actin +10 kb	CTTCCTGCCTCCACTTTCTG	GGAGTGGGGATGTAGTTCA
Cx43 -0.3 kb	TCCTGAAGGAATGACCCATC	GCAGAAGAAGCAACCTGGTC
Cx43 -1.2 kb	CCTAGTGAGAGGGTGCTTCG	GGCTTGTGATAGGGCGATAA
Cx43 -2.6 kb	CTCGTCCACTCTTTCCTTGC	TGGTGTGAGCAGCTATCCTG
Cx43 -10 kb	AGATGGGAGGGGAGGTACAG	CATGTGGTTGCTGAGATTGG
VE-Cad -0.16 kb	TTGAATACCCCAGGCAGGT	CTCGGGATGGTTTCCTGTTA
VE-Cad -0.7 kb	GCTGACTCAGACCTATGGCTA	TGGCCCATCTCACTTTACAA
VE-Cad -2.2 kb	CAGAGTTCGCTAGCCTAGGTG	TGCCAGGAAGACAGGAAAC
VE-Cad +15.5 kb	CACTTCTCCTCACCCCTCTG	CTCTGACTCTCCTGCCTGCT