SUPPLEMENTAL MATERIAL

DETAILED METHODS

Mice

Brg1-floxed mice (*Brg1*$^{fl/fl}$)[1,2] and transgenic *Cdh5(PAC)-Cre*^{ERT2} mice[3] were maintained on a mixed genetic background at the Oklahoma Medical Research Foundation animal facility. All animal use protocols were approved by the Institutional Animal Care and Use Committee. For induction of the *Cdh5(PAC)-Cre*^{ERT2} transgene, pregnant females were injected intraperitoneally with 1 mg tamoxifen (T5648; Sigma-Aldrich) for three consecutive days prior to embryo dissection. Tamoxifen was prepared as previously described.[4] *Brg1*-floxed and *Cdh5(PAC)-Cre*^{ERT2} mice were genotyped as previously described.[4,5]

Hematoxylin and eosin staining

Hematoxylin and eosin (H&E) staining was performed on paraffin sections as previously described.[5]

Primary antibodies

The following commercial primary antibodies were used for immunostaining purposes: PECAM-1 (Cat #550247, Lot SS0274, BD Pharmingen or Cat #AF3628, R&D Systems); Endomucin (Cat # 65495, Lot E0813, Santa Cruz Biotechnology); MRTF-A (Cat # 390324, Lot G3014, Santa Cruz Biotechnology); β-Actin (Cat #4970, Lot 14, Cell Signaling); SRF (Cat # sc-13029, Lot J0808, Santa Cruz Biotechnology); Ki67 (Cat # 9106-S0, Lot 9106S1607K, Thermo Scientific); ZO-1 (Cat # 402200, Life Technologies); VE-Cadherin (Cat # 550548, BD Biosciences); MRTF-B (Cat # 47282; Lot C1716; Santa Cruz Biotechnology); BRG1 (Cat # 17796; Lot G3013; Santa Cruz Biotechnology).

Whole mount immunostaining

For whole mount PECAM-1 staining, limb buds were dissected from E12.5 embryos and fixed in 4% PFA overnight at 4°C. Samples were then washed with PBS (2 times, 5 min each) and dehydrated for 5 min in each of the following solutions at room temperature with gentle shaking: 25% MeOH in PBT (0.2% BSA and 0.1% Triton X-100 in PBS), 50% MeOH in PBT, 75% MeOH in PBT, and 100% MeOH twice. Samples were then rehydrated for 5 min in each of the following solutions at room temperature with gentle shaking: 75% MeOH in PBST (0.5% Triton X-100 in PBS), 50% MeOH in PBST, and 25% MeOH in PBST. Next, samples were washed in PBST (3 times, 5 min each) at room temperature and were blocked in PBSMT (1% non-fat dry milk and 0.1% Triton X-100 in PBS) twice (1 hr each) at room temperature. Samples were then incubated with PECAM-1 antibody (1:100, BD Pharmingen, in PBSMT) at 4°C for 2 days with gentle shaking followed by washing with PBSMT (4 times, 30 min each) at 4°C with gentle shaking. Samples were next incubated with Cy3-anti-rat IgG (1:500 in PBSMT; Cat #712-166-150; Jackson ImmunoResearch) overnight at 4°C with gentle shaking and were washed again in PBSMT (4 times, 30 min each) at 4°C with gentle shaking.

Section immunostaining

Co-immunostaining for endomucin and either MRTF-A, β-Actin, SRF, BRG1, or ZO-1 was performed as follows: cryosections were permeabilized with 0.1% TritonX-100 for 15 min at room temperature followed by blocking with 3% BSA in PBS for one hr at room temperature. Primary antibodies against endomucin (1:100) and either MRTF-A (1:100), β-Actin (1:100), SRF (1:100), Ki67 (1:100) or ZO-1 (1:100) were added to each section. VE-Cadherin (1:100) was co-incubated with PECAM-1 (1:100, R&D Systems), rather than endomucin. Antibodies were incubated overnight at 4°C in blocking buffer. The following day, cryosections were washed twice with ice cold 1% BSA and incubated with fluorescent species-specific secondary antibodies (Jackson ImmunoResearch) for one hr at room temperature. Following the secondary antibody incubation, TO-PRO-3 (Cat # T3605; Life Technologies) was added to sections in blocking buffer (1 μM final) for 10 min at room temperature. Sections were washed and allowed to dry before being mounted in Prolong Gold mounting media (Cat # P36930; Life Technologies) and sealed with fingernail polish. MRTF-B (1:100) and endomucin primary antibodies were added to cryosections overnight at room temperature, but were otherwise immunostained as described above. Lastly, cryosections immunostained for BRG1 (1:100) were blocked with 3% BSA in PBS for 30 min at room temperature followed by a 1 hr incubation with a mouse IgG blocking reagent (Mouse On Mouse Kit, Cat# BMK-2202, Vector Laboratories), according to the manufacturer's instructions. BRG1 was co-incubated with endomucin overnight at 4°C. Sections were then incubated with biotinylated anti-mouse IgG for 1 hr at room temperature followed by strepavidin-conjugated Alexa 488 (Cat # S11223; Life Technologies) and incubated for 5 min at room temperature (15 μg /ml).

Microscopy and image acquisition

Gross embryo images and light microscopy were obtained with a Nikon SMZ800 stereomicroscope and Nikon DS-Fi1 camera. Confocal images for whole mount PECAM-stained limb buds were obtained with a Zeiss LSM-710 confocal head mounted on an Axio Observer Z1 stand running Zen 2012 SP2 software. Epifluorescent images were obtained with a Nikon Eclipse Ti-E inverted microscope and an Andor Zyla 4.2 Plus sCMOS camera using NIS-Elements AR4.5 (Nikon) software. For electron microscopy, embryonic limb buds were fixed by immersion in a mixture of 2% PFA and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (1 hr), followed by postfixation in 1% osmium tetroxide (90 min) and 1% tannic acid (1 hr). Samples were subsequently dehydrated in a graded ethanol series and embedded in epoxy resin (Electron Microscopy Sciences). Semithin (500 nm) and ultrathin sections (70 nm) were obtained using an ultramicrotome (RMC MT 7000, RMC Boeckeler) equipped with a diamond knife. Semithin sections were stained with Epoxy Tissue Stain (Electron Microscopy Sciences) and analyzed with a Nikon Eclipse E800M microscope equipped with a Nikon DX1200 digital color camera and Nikon ACT-1 image acquisition software. Ultrathin sections were stained with uranyl acetate and lead citrate before analysis with a Hitachi H-7600 electron microscope equipped with a 4 megapixel digital monochrome camera and AMT-EM image acquisition software (Advanced Microscopy Techniques).

Immunostaining quantification
Immunofluorescent images were quantified using the NIS-Elements AR4.5 (Nikon) software. Briefly, mean fluorescence intensity (MFI) was measured from manually traced blood vessels using the imaging software. This was performed on at least two vessels per section (at least three regions per vessel), with two sections per embryo; control and mutant littermates from 3 different litters were analyzed. Statistical analysis was performed in GraphPad Prism 7 using the column statistics function after normalizing MFIs from control embryos. Two-tailed Student's t-tests were used to compare differences between the normalized control and mutant MFIs.

Endothelial cell isolation
Primary endothelial cells from E12.5 yolk sacs were isolated as previously described.[6]

Cell culture and transfections
The murine C166 yolk sac-derived endothelial cell line (Cat #CRL-2581; Lot 63121587; ATCC) was maintained as described.[6] For BRG1 knockdown, C166 cells were transfected with BRG1 Silencer Select or nontargeting control siRNA oligonucleotides (40 nM final concentration; Cat #M-031135-01 and #D-001210-01-05, respectively; Thermo Fisher) using Lipofectamine 2000 (Invitrogen) in reduced-serum OptiMEM (Invitrogen). After 4 hr, DMEM (Cat #12604F; Lonza) with 10% FBS (Cat #AWC99353; Hyclone) was added, and cells were cultured for an additional 20 hr. For overexpression studies, C166 cells were transfected as above with 2 µg (total) of murine MRTF-A, MRTF-B, and/or SRF expression plasmids or with a corresponding empty pcDNA3.1+ vector (per 0.5 X 10^6 cells). The expression vector for SRF was constructed by shuttling *mSrf* cDNA (from IMAGE clone 6404515; Thermo Fisher) into pcDNA3.1+. The expression vector for MRTF-B was constructed by shuttling *mMrtfb* cDNA (from a vector gifted by William Berry and James Tomasek) into pcDNA3.1+ with the NEBuilder HiFi DNA Assembly Cloning Kit (Cat #E5520; New England Biolabs). The expression vector for MRTF-A was constructed by amplifying *mMrtfa* cDNA from C166 cell cDNA and inserting it into pcDNA3.1+ with the NEBuilder HiFi DNA Assembly Cloning Kit.

For BRG1 overexpression studies, HEK293T cells (gift of Lijun Xia) were transfected as above with 2 µg of a human BRG1-expressing plasmid (Addgene plasmid #17873; generated by Jerry Crabtree) or with a corresponding empty pBJ5 vector (per 0.5 X 10^6 cells). HEK293T cells were also transfected similarly with the murine MRTF-A, MRTF-B, and SRF expression vectors to confirm by immunoblot that these plasmids could generate relevant proteins.

Immunoblots
Total protein was harvested from transfected HEK293T cells. Five micrograms of protein lysate was fractionated on 9% polyacrylamide gels and transferred to a PVDF membrane for immunoblotting with antibodies against MRTF-A (Cat # sc-390324; Lot G3014; Santa Cruz Biotechnology), MRTF-B (Cat # 14613; Cell Signaling Technology; gift of William Berry and James Tomasek), SRF (Cat # sc-335; Lot J0808; Santa Cruz Biotechnology), and GAPDH (Cat # G9645; Sigma). Horseradish peroxidase-

conjugated secondary antibodies were applied and detected with ECL Western Blotting Detection Reagent (GE Healthcare) and X-ray film (Cat # 34090; Thermo Fisher).

Quantitative real-time PCR (qPCR)
Total RNA from yolk sac-isolated endothelial cells, whole limb buds, or C166 cultured endothelial cells was collected and purified using the E.Z.N.A. Total RNA Kit I (Omega). cDNA was prepared using the iScript cDNA synthesis kit (BioRad), and real-time quantitative PCR was performed using 2x SYBR green qPCR master mix (Life Technologies) and a CFX96 Real Time System (Bio-Rad). Transcripts of interest were normalized to *Gapdh* and *Rn18s*. See Table I in the online-only Data Supplement for a complete list of qPCR primer sequences.

Chromatin immunoprecipitation (ChIP)
ChIP assays were performed as previously described[7] with slight modifications. Briefly, C166 cells ($1x10^6$/sample) were fixed on culture dishes with 1% PFA (5 min) at room temperature. Glycine (125 µM final) was then added to stop fixation. Cells were scraped, pelleted, and re-suspended in 200 µl lysis buffer [1% SDS, 10 mM EDTA, 50 mM Tris Base pH 8.1, and protease inhibitors (Cat #P8349; Sigma) in water and incubated on ice (15 min). Lysates were sonicated (four 10 sec pulses at an amplitude of 50) using a Misonix S-4000 sonicator. Following sonication, 1.4 ml of dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris Base pH 8.1, 166.5 mM NaCl, and protease inhibitors in water) was added to each sample. Samples were then pre-cleared using protein A/G-conjugated agarose beads (Calbiochem) for 3 hr at 4°C with gentle shaking. Agarose beads were pelleted, and 10 µg of anti-BRG1 (Cat. # 17796; Lot G3013; Santa Cruz) or rabbit IgG (Cat. # 12-370; Lot 2426484; Millipore) antibodies were added to supernatants and rotated overnight at 4°C. Samples were next incubated with 60 µl of protein A/G agarose beads for 3 hr at 4°C with rotation. Antigen complexes were pelleted by centrifugation and washed (2 times each) with 750 µl of low salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 40 mM Tris HCl pH 8.1, 150 mM NaCl), LiCl buffer (1 mM EDTA, 250 mM LiCl, 1% Na Deoxycholate) and TE buffer (1 mM EDTA, 5 mM Tris pH 7.4) and rotated at 4°C for 5 min. Antigen was eluted by incubating with 500 µl of elution buffer (1% SDS and 100 mM $NaHCO_3$) under rotation at room temperature for 30 min. Crosslinks were reversed by incubating samples in 0.2 M NaCl at 65°C overnight. DNA was purified by Proteinase K treatment (20 mg/mL), phenol-chloroform extraction, and ethanol precipitation. DNA was resuspended in 25 µl of TE and PCR amplified. Primers used for ChIP-PCR are listed in Table II in the online-only Data Supplement. Amplicon density was determined from four independent experiments using ImageJ software (NIH). Band density of the immunoprecipitated samples was normalized to that of the input samples and divided by the IgG negative control bands.

Luciferase reporter assays
Fragments of the *Mrtfa* (1.5 kb), *Mrtfb* (2.5 kb), and *Srf* (2.5 kb) promoters were amplified from C166 genomic DNA and inserted using the NEBuilder HiFi DNA Assembly Cloning Kit (Cat #E5520; New England Biolabs) into a pCEP9 chromatinizable luciferase reporter expression vector.[8] HEK 293T cells were transfected

with the indicated constructs (1 µg per 5x10^5 cells) using Polyethylenimine "Max" DNA transfection reagent (7 µg per 1 µg of DNA; Cat #24885; Polysciences, Inc.) in DMEM. Forty-eight hours post-transfection, media was replaced with DMEM containing G418 (1 µg/ml; EMD Millipore) for selection of transfected cells. Once established, stable cell lines were transfected with (40nM final concentration) BRG1 or nontargeting siRNA oligos or with 500 ng of an hBRG1 expression plasmid (Addgene plasmid # 17873) or corresponding empty pBJ5 vector. Twenty-four hours post-transfection, cells were harvested and analyzed with the Luciferase Assay Reagent from the Dual-Luciferase Reporter Assay System (Promega) using a Glomax 20/20 Luminometer (Promega). Three independent luciferase assays were performed in triplicate.

Statistical analysis

All statistical differences were calculated in GraphPad Prism 7. A two-tailed Student's *t*-test or one-way ANOVA was used to compare the means of independent groups for qPCR, ChIP-PCR, immunofluorescence quantification, and luciferase assays. Error bars represent the mean ± S.D. *P* values of less than or equal to 0.05 were considered statistically significant for all analyses.

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