SUPPLEMENTAL MATERIAL

Gata4-dependent differentiation of c-Kit^{+} derived endothelial cells underlies artefactual cardiomyocyte regeneration in the heart

Bryan D. Maliken, BA[1]; Onur Kanisicak, PhD[1]; Jason Karch, PhD[1]; Hadi Khalil, PhD[1]; Xing Fu, PhD[1]; Justin G. Boyer, PhD[1,2]; Vikram Prasad, PhD[1]; Yi Zheng, PhD[1]; Jeffery D. Molkentin, PhD[1,2]

[1] University of Cincinnati and Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA.
[2] Howard Hughes Medical Institute, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, USA.

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Supplemental Methods

Experimental animals:

All experiments involving mice were approved by the Institutional Animal Care and Use Committee (IACUC) at Cincinnati Children's Hospital Medical Center. Targeted *Kit*-Cre (*Kit*^{Cre}) and *Kit*-MerCreMer (*Kit*^{MCM}) mice were previously described[1]. LoxP-targeted *Gata4* (*Gata4*^{fl}) and *Gata6* (*Gata6*^{fl}) mice were described previously[2,,3]. *Rosa26* reporter mice (*R26*^{eGFP}) were purchased from the Jackson Laboratories (Stock# 012429)[4]. Fusion studies were conducted utilizing the dual reporter *B6.129(Cg)-Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J* (Jackson laboratories Stock # 007676). The endothelial specific CreERT2 transgenic mouse model is described elsewhere (Zheng, Y et al, submitted). Briefly, a Tie2-CreERT2 transgene comprising a version of Tie2 promoter with a β-globulin intron sequence as a fusion with CreERT2 cDNA and a polyadenylation signal (pA) signal and Tie2 enhancer sequence was used for *C57Bl/6* pronuclear micro-injection to generate transgenic mouse lines. *C57Bl/6* mice and *Rosa26-TD-Tomato* mice (*B6; 129S6-Gt (ROSA) 26Sortm9(CAG-tdTomato)Hze/J* stock number: 007905) were from Jackson Lab. No procedures causing pain or distress to the mice were used in this study. All animal protocols were written prospectively with defined primary and secondary endpoints specified.

Randomization and Blinding

Mice were included in the study based on genotype and no mice of the correct genotype were excluded. Experimental animals were matched for species, sex, age, strain, diet, housing, and source. No randomization was required for genetic experiments in mice because as all interventions were identical and all mice were of the same genotype and same environment and age within a study. However, we used a 50/50 mix of male and female mice in all studies. The primary author managed the mouse colony, determined genotype, and provided tamoxifen treatment. For experiments, tissues were harvested and randomly assigned blinded identifiers

by a co-author and distributed among other authors for blinded data collection. Experimental groups were then unblinded and analyzed accordingly.

Tamoxifen treatment:

To activate the inducible MerCreMer or the CreERT2 protein, experimental mice were administered tamoxifen citrate containing mouse food (TD.130859 Envigo) used at a dosage of 400 mg/kg, or via 5 consecutive daily intraperitoneal injections with pharmaceutical grade tamoxifen (100 mg/kg body weight) dissolved in 95% peanut oil/ 5% ethanol. Control animals were treated with control food without the tamoxifen. Mice were either sacrificed by CO_2 asphyxiation or by excision of the heart under deep isoflurane sedation.

5-ethynyl-2'-deoxyuridine (EdU) incorporation for in vivo proliferation analysis:

Experimental mice were administered a total of 8, weekly I.P injections of EdU (Thermo Fisher Scientific - Molecular Bioproducts A10044) at a dosage of 50 mg/kg body weight. Harvested hearts were processed as described above and EdU was detected using the Click-iT Plus Alexa Fluor 647 Picolyl Azide Toolkit (Thermo Fisher Scientific Cat # C10643).

Histology and immunostaining:

Isolated hearts and skeletal muscles were fixed for 3.5 hours at 4°C in pre-chilled freshly diluted paraformaldehyde (PFA) (Electron Microscopy Sciences Cat# 15714) in Phosphate Buffered Saline (PBS). Tissues were then rinsed with PBS 3 times 15 minutes each and cryoprotected by immersion in PBS containing 30% sucrose overnight before embedding in OCT (Tissue-Tek Cat # 4583). Tissues were sectioned using Leica CM1850 Cryostat and 10 µm cryosections were collected. Sections were incubated in blocking solution containing 5% goat serum, 2% bovine serum albumin, 0.1%Triton X-100 in PBS for 45 minutes. Primary antibodies against CD31 (BD Biosciences 553370), CD45 (BD Biosciences 553076), and Gata4 (Abcam ab84593) were all diluted at 1:200 dilution in blocking solution directly applied on slides and sections were

incubated overnight at 4°C in a humidity chamber. Sections were washed three times for 5 min each in PBS and incubated with Alexa Fluor 568-conjugated secondary antibody (Life Technologies A11077 or A11011) diluted at 1:500 in blocking solution for 90 minutes at room temperature. Finally, sections were washed three times for 5 min each in PBS, counter-stained with 4',6-diamidino-2-phenylindole (DAPI) at a 0.1 µg/ml concentration in PBS for 5 minutes at room temperature and cover slips applied using VECTASHIELD aqueous mounting medium (Vector Laboratories H-1000). Direct eGFP fluorescence produced from the recombined state of the *Rosa26*-containing loxP-dependent reporter allele was detected. Images were acquired using an inverted Nikon A1R confocal microscope system using the NIS Elements AR 4.13 software.

eGFP$^+$ and mTomato$^+$ cardiomyocytes were quantified using a Nikon Ti-E Inverted microscope with fluorescence capabilities and a 20X objective. Cardiomyocytes were identified by characteristic morphology and were quantified on multiple sections from each heart at various sectional planes, typically 10 or more. eGFP$^+$ cardiomyocytes were presented as a percentage of the total myocardium, calculated by dividing eGFP$^+$ or mTomato$^+$ cardiomyocytes by the total cardiomyocytes in a section which were identified either by Wheat Germ Agglutinin staining or endogenous membrane Tomato signal (such as dual reporter mice in Figure 1G-H or bone marrow transplant experiment in Figure 3A-F).

Bone marrow transplant:

To determine the altered infiltration, fusion, and potential of cardiomyocyte differentiation of bone marrow derived cells (BMCs), we performed BM transplantations (BMT) from 6-8 weeks old donor mice (various experimental mice were used as described in each experiment) into recipients. Donor BMCs were isolated by aseptically flushing femurs and tibiae with Hanks Balanced Salt Solution (HBSS) (Fisher Scientific SH30588.01) using a 25 gauge needle attached to a syringe containing 10 ml of ice cold HBSS supplemented with 2% bovine growth serum (Fisher Scientific SH3054103). BMT recipient mice first received lethal irradiation (12 Gy

in a divided dose 4 hours apart) and then immediately received a tail vein injection of BMCs at ~50 million per mice under isoflurane anesthesia to effect. No anesthesia was used prior to irradiation and no pain medications were administered following the procedure as it is nonpain generating procedure. Mice were housed in the barrier facility associated with the CCHMC irradiator suite for 10 days following the procedure for maximum survival (100% survival in all BMT experiments was observed). Tissues were isolated and processed after 8 weeks of recovery and processed as described above.

Preparation of Retinal Flat Mounts:

To observe the superficial vascular plexus to assess endothelial developmental defects, retinal flat mounts were prepared as described earlier[5]. Briefly, p0 mice were given intraperitoneal injections of 200 µg tamoxifen (Sigma T5648; dissolved in 5 µl ethanol and 95 µl peanut oil) for gene inactivation and Tie2 lineage tracing. Mice were euthanatized and eyes were carefully harvested at age p8 and lightly fixed in 4% PFA for 15 minutes at 4°C and the cornea, lens, uvea, and sclera were removed under dissection microscope. The retinas were then radially cut and flat mounted on slides and cover slips placed with aqueous mounting media. Flat mounts were imaged and eGFP fluorescence was captured using a Nikon Ti-E Inverted microscope with fluorescence capabilities.

Whole mount imaging:

Imaging of whole mount femurs for endothelial and bone marrow eGFP signal detection was achieved using a M165FC Leica stereomicroscope with fluorescence capability. Timed matings were established by setting sexually mature mice overnight and detection of post-coitum plug was referred to as embryonic day (ED) 0. Experimental mice were isolated at ED11.5 and fixed in 4% PFA for 3 hours at 4°C and images were captured with a M165FC Leica stereomicroscope.

Tail vein injection of AAV-Vegfa:

The cDNA for VEGF-A (Accession #BC061468) was cloned into the pAAV-MCS Expression Vector (Cell Biolabs Inc., # VPK-410) with the In-Fusion HD Cloning Plus Kit (Clontech, # 638910). The insertion of the VEGF-A cDNA was confirmed by sequencing and the plasmid was amplified overnight in DH5α cells (ThermoFisher, # 18265017), purified using the PureYield Plasmid Maxiprep System (Promega, #A2392) and sent to VigeneBioscience for packaging and large scale purification. Adult C57BL/6 mice were administered AAV9-VEGF-A by tail vein injection at concentration of $1x10^{12}$ viral particles in 200 µl of sterile PBS under inhaled isoflurane anesthesia to effect.

Cell isolation:

FACS analysis was performed on cardiac interstitial cells and bone marrow cells as described in detail before[1,6,7]. We isolated bone marrow cells by flushing femurs and tibiae with 2% BGS/HBSS as described above. Cardiac interstitial cells were isolated by digesting ventricles with collagenase type 2 (Worthington LS004177) as described before[6]. Isolated cells were spun at 10 g for 5 minutes to remove large debris and the supernatant containing the cell suspension was then spun at 400 g for 10 minutes at 4 ^{0}C and pellets were re-suspended in 2% BGS/HBSS. Isolated cells were kept on ice during the rest of the process for flow cytometry, FACS isolation or RNA extraction.

Flow cytometry analysis and FACS isolation:

Flow cytometry analysis was performed using a BD FACSCanto II running FACSDiva software (BD Biosciences). Analysis was performed using FlowJo vX (FlowJo). BMCs and cardiac interstitial cells were stained with surface markers using APC conjugated antibodies against surface markers such as CD45 (BD Biosciences 559864); CD31 (eBioscience17-0311-82); CD133 (Biolegend 141207). To determine optimum gating strategy, laser voltages and viability analyses of isolated cells, separate flow cytometry experiments with single-labeled controls from

wild-type mice along with viability dyes such as 7-Aminoactinomycin D (7-AAD) (Life TechnologiesA1310) or Calcein Blue, AM (Thermo Fisher Scientific Cat. # C1429) were used. For fluorescence-activated cell sorting (FACS), BD FACS Aria Instrument was utilized and endothelial lineage cells that were $eGFP^+$, $CD31^+$, and $7AAD^-$ (live) cells were collected in to pre-chilled 2% BGS/HBSS. Sorted cells were then either put into 96-well plates for culture, mixed in cooled Matrigel for injections, or processed for RNA isolation.

Primary endothelial cell culture and enzyme-linked immunosorbent assays (ELISAs)

Primary endothelial cells (Tie2 lineage-traced as $eGFP^+$, $CD31^+$, $7AAD^-$) were isolated from the cardiac interstitium as described above and sorted into 96-well plates at a concentration of 10,000 cells/well. Cells were cultured in Endothelial Cell Basal Medium-2 (Lonza CC-3156) supplemented with 10% bovine growth serum (Fisher Scientific, SH3054103) and in the presence of penicillin-streptomycin (ThermoFisher Scientific, 30–002 CI). Cell morphology was captured using Nikon Ti-E Inverted microscope with fluorescence capabilities. Cell culture supernatant was collected at various time points and analyzed for secreted factors VEGF-A and angiopoietin-2 by ELISA (R&D Systems, Quantikine ELISA kits MMV00 and MANG20, respectively) following manufacturer protocol. Ninety-six-well ELISA plates were read for absorbance using a microplate reader (Biotek Synergy 2) at 570 nm and angiokine concentrations were calculated using a standard curve.

Matrigel angiogenesis assay:

Approximately 50,000 FACS isolated cells were embedded in 50 μl of cold, sterile Matrigel basement membrane (Corning #354234) and injected subcutaneously into surrogate recipient mice. Matrigel plugs were harvested with a fluorescent stereomicroscope after 2 weeks of growth and fixed in in 4% PFA for 60 minutes at 4°C. Z-stack images of vasculature networks were obtained using an inverted Nikon A1R confocal microscope system using the NIS Elements AR 4.13 software.

Western blot:

Western blot was performed as described previously[8]. Briefly, isolated cells were homogenized in RIPA buffer (50 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, 1% NP-40) containing protease inhibitor cocktail (Sigma-Aldrich P8340), resolved on 10% SDS–polyacrylamide gel electrophoresis gels, and transferred to PVDF membranes. Membranes then immunoblotted for antibodies against Gata4 (Abcam ab84593) at 1:500, VEGF-A (Abcam ab46154) at 1:500 and Gapdh (Fitzgerald 10R-G109a) at a 1:20,000 dilutions, and then incubated with the appropriate alkaline phosphate-linked secondary antibody. Membranes were imaged using hemifluorescence (Amersham) on a Gel Doc™ XR+ System (BioRad).

PCR analysis for DNA recombination:

PCR was performed to detect recombined and non-recombined Gata4 loxP-targeted alleles using primers 5'-CCCAGTAAAGAAGTCAGCACAAGGAAC-3' 5'-AGACTATGGATCCCGGAGTGAACATT-3'. PCR conditions were 96 °C for 2 min, followed by 34 cycles of amplification (96 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s) and a 5-min elongation step at 72 °C. PCR products were visualized on ethidium bromide-stained agarose gels using a UV molecular imager (Bio-Rad).

RNA-Seq and bioinformatics analysis:

Total RNA was amplified using the Ovation RNA-Seq System v2 (NuGEN) according to the manufacturer's protocol. The libraries were prepared with the Nextera XT DNA Sample Preparation kit (Illumina Technologies Cat# FC-131-1024). The purified cDNA was captured on an Illumina flow cell for cluster generation. Libraries were sequenced on the Illumina HiSeq2500 following the manufacturer's protocol. Differential gene expression was determined using the DESeq2 method[9] with the R/Bioconductor package. Analysis was performed using standard parameters with the independent filtering function enabled to filter genes with low mean

normalized counts. Differential expression was computed with the application of the Benjamini-Hochberg false discovery rate (FDR) method to adjust the p-values from multiple testing. Genes were considered differentially expressed if they passed a statistical cutoff of FDR $p<0.05$ and if they contained an absolute $\log_2$ fold change greater than or equal to 1 or less than or equal to -1. RT-PCR validation was performed on the majority of differentially expressed genes shown in Figure 7, as well as several selected genes with biological relevance that were determined to be nearly significant by DESeq2 analysis. The RNA sequencing data were deposited with the GEO database group and given accession number GSE109661.

Reverse transcriptase polymerase chain reaction (RT-PCR):

Cardiac endothelial cells were isolated as described above and RNA was isolated using Qiashredder homogenization and the RNAeasy kit (Qiagen Cat# 74104) according to the manufacturer's instructions. Total RNA was reverse transcribed using random oligo-dT primers and a Verso cDNA synthesis kit (Thermofisher AB1453) according to manufacturer protocol. Real-time PCR was performed using Sso Advanced SYBR Green (Biorad) and Rpl7 expression was used for normalization. The following primers were used for qPCR to identify the listed mRNA products: Cadherin 5 5'-CACTGCTTTGGGAGCCTTC and 5'GGGGCAGCGATTCATTTTTCT, Platelet And Endothelial Cell Adhesion Molecule 1 5'-CTGCCAGTCCGAAAATGGAAC and 5'-CTTCATCCACCGGGGCTATC, Von Willebrand Factor 5'-CTTCTGTACGCCTCAGCTATG and 5'-GCCGTTGTAATTCCCACACAAG, Apolipoprotein L Domain Containing 1 5'-CGCTTCCAAGGATTGCTGC and 5'-CTGAGTGACAACCCCACGAT, Hyaluronan Synthase 3 5'-GTGGGCACCAGTCTGTTTG and 5'-CCACTGAACGCGACCTCTG, Early Growth Response 3 5'-CCGGTGACCATGAGCAGTTT and 5'-TAATGGGCTACCGAGTCGCT, Endothelial Cell Specific Molecule 1 5'-CTGGAGCGCCAAATATGCG and 5'-TGAGACTGTACGGTAGCAGGT, E2F Transcription Factor 7 5'-GCATACGGCCAGATCCGAG and 5'-GACCCTTGTCTTTCTCCCTGT, Matrix Metallopeptidase 2 5'-CAAGTTCCCCGGCGATGTC and 5'-TTCTGGTCAAGGTCACCTGTC,

Vascular Cell Adhesion Molecule 1 5’-AGTTGGGGATTCGGTTGTTCT and 5’-CCCCTCATTCCTTACCACCC, C-Fos Induced Growth Factor (Vascular Endothelial Growth Factor D) 5’-TTGAGCGATCATCCCGGTC and 5’-GCGTGAGTCCATACTGGCAAG, Dickkopf WNT Signaling Pathway Inhibitor 3 5’-CTCGGGGGTATTTTGCTGTGT and 5’-TCCTCCTGAGGGTAGTTGAGA, T-Box 20 5’-AAACCCCTGGAACAATTTGTGG and 5’-CATCTCTTCGCTGGGGATGAT, Zinc Finger Protein, FOG Family Member 2 5’-ACCAGGAGAGCTAGAAGTGTTT and 5’-GGACCTGAGCCTTCGTCTT, Myosin Light Chain 7 5’-GGCACAACGTGGCTCTTCTAA and 5’-TGCAGATGATCCCATCCCTGT, Endothelin 1 5’-TTTCCCGTGATCTTCTCTCTGC and 5’-CGCCTACCTGTTTCTGGAGC, Sphingosine-1-Phosphate Receptor 1 5’-ATGGTGTCCACTAGCATCCC and 5’-CGATGTTCAACTTGCCTGTGTAG, BTG Anti-Proliferation Factor 2 5’-ATGAGCCACGGGAAGAGAAC and 5’-GCCCTACTGAAAACCTTGAGTC, Early Growth Response 1 5’-TCGGCTCCTTTCCTCACTCA and 5’-CTCATAGGGTTGTTCGCTCGG, Early Growth Response 2 5’-GCCAAGGCCGTAGACAAAATC and 5’-CCACTCCGTTCATCTGGTCA, FOS Like 2, AP-1 Transcription Factor Subunit 5’-CCAGCAGAAGTTCCGGGTAG and 5’-GTAGGGATGTGAGCGTGGATA, Polo Like Kinase 3 5’-GCACATCCATCGGTCATCCAG and 5’-GCCACAGTCAAACCTTCTTCAA, Fibulin 1 5’-CCGCCAAGAGAAAACAGACAC and 5’-CGGGTGAACTCTCGAAAGGTG, Fibromodulin 5’-AGCAGTCCACCTACTACGACC and 5’-CAGTCGCATTCTTGGGGACA, Integrin Subunit Beta 4 5’-GCAGACGAAGTTCCGACAG and 5’-GGCCACCTTCAGTTCATGGA, L1 Cell Adhesion Molecule 5’-CAGTGCTTCAGGATGAACGAT and 5’-TCTGGGGCTTTAACCTGTAGG, Matrix Metallopeptidase 3 5’-ACATGGAGACTTTGTCCCTTTTG and 5’-TTGGCTGAGTGGTAGAGTCCC, Protocadherin 7 5’- CAGCCATTTCGTAGAGTGACG and 5’-CTTGGTGTTTCTGACTCCTCC, Plexin Domain Containing 1 5’-CAACCATAACTACTACGTGTCCC and 5’-AGGTCCACCCACAGATCCTG, Plexin Domain Containing 2 5’-GCCGCAGCAGGAGTTATGTTA and 5’-TTCATTCCAAGGAAAAGCGTTTG, TIMP Metallopeptidase Inhibitor 2 5’-TCAGAGCCAAAGCAGTGAGC and 5’-

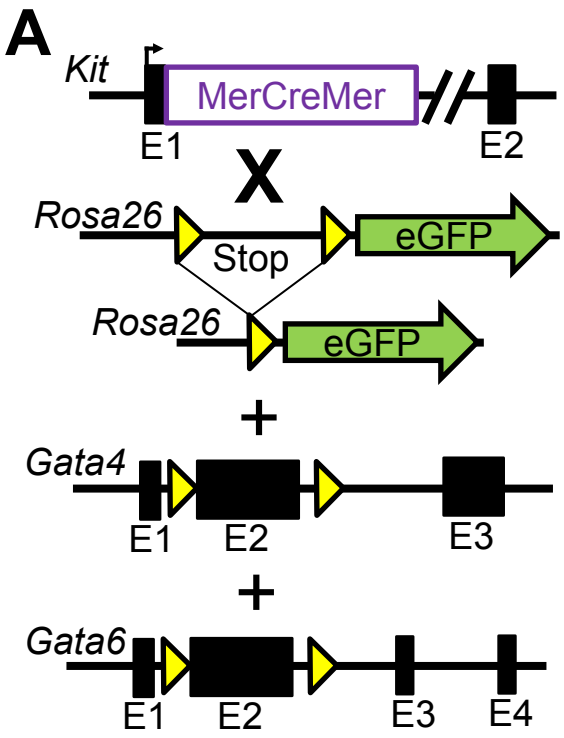
GCCGTGTAGATAAACTCGATGTC, Versican 5'-ACTAACCCATGCACTACATCAAG and 5'-ACTTTTCCAGACAGAGAGCCTT, Heart And Neural Crest Derivatives Expressed 2 5'-GCAGGACTCAGAGCATCAACA and 5'-AGGTAGGCGATGTATCTGGTG, Kruppel Like Factor 15 5'-GAGACCTTCTCGTCACCGAAA and 5'-GCTGGAGACATCGCTGTCAT, SRY-Box 9 5'-GAGCCGGATCTGAAGAGGGA and 5'-GCTTGACGTGTGGCTTGTTC, and Ribosomal Protein L7 5'- ACCGCACTGAGATTCGGATG and 5'- GAACCTTACGAACCTTTGGGC.

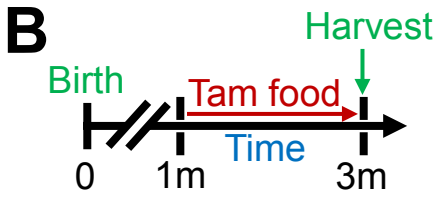
Statistics:

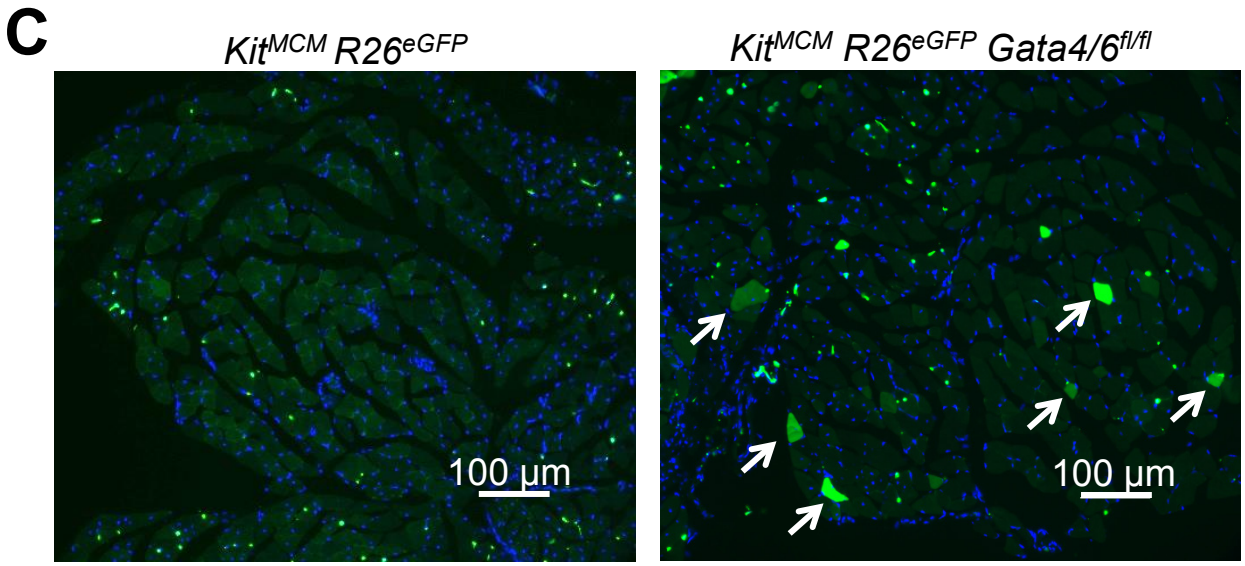
Data are shown as mean +/- SEM. One-way analysis of variance (ANOVA) was used to determine statistical significance for experiments with more than two groups and one independent variable, while two-way ANOVA was used in experiments with more than two groups and multiple independent variables. Statistically significant pairwise differences were then further determined by Tukey methods (Prism software). For experiments in which only 2 groups were compared, unpaired t-tests were used to determine statistical significance. P-values <0.05 were considered statistically significant and all tests performed were two-sided. To determine sample sizes for the proposed experiments the desired power was established as 80% and the criterion for significance (α) was 0.05. The probability of a type II error (ß = 0.2) for any null findings is reported in terms of the study power (1 – ß = 0.8). In a pilot study, a mean effect size for endogenous lineage tracing experiments was determined to be 4.7 $eGFP^{+}$ cardiomyocytes per section. Hearts were analyzed N=3 per group with 10 cardiac cross sections observed per mouse (mean = 1.3 vs. 6.0 and standard deviation = 0.26 vs. 1.33 in Kit^{MCM} $R26^{eGFP}$ versus Kit^{MCM} $Gata4/6^{fl/fl}$ $R26^{eGFP}$, respectively). From this data, a sample size of N=4 was calculated for endogenous lineage tracing experiments. We did not have preliminary data to inform our sample size calculation prior to the design of the bone marrow transplant experiments, so we assumed a similar distribution as in the endogenous lineage tracing experiments. To properly power the experiment we conservatively assumed that the mean difference between groups would be reduced by 50% (effect size = 2.4 cardiomyocytes/section)

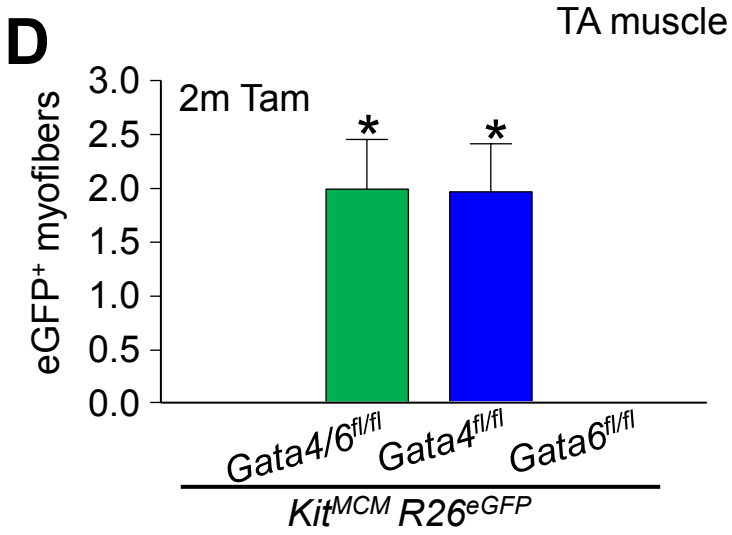
and following these assumptions, the necessary sample size was determined to be N=6 per group. However, some secondary exploratory experiments used 3 or fewer mice as we considered these analyses to be hypothesis-generating, or given the highly-reliable nature of the assays used (RNAseq, some Western blots, FACS plots, etc) the results were obvious and consistent with all the data in the greater manuscript. For statistical consideration of the RNAseq data see "***RNA-Seq and bioinformatics analysis"*** section above.

Supplemental Figure S1









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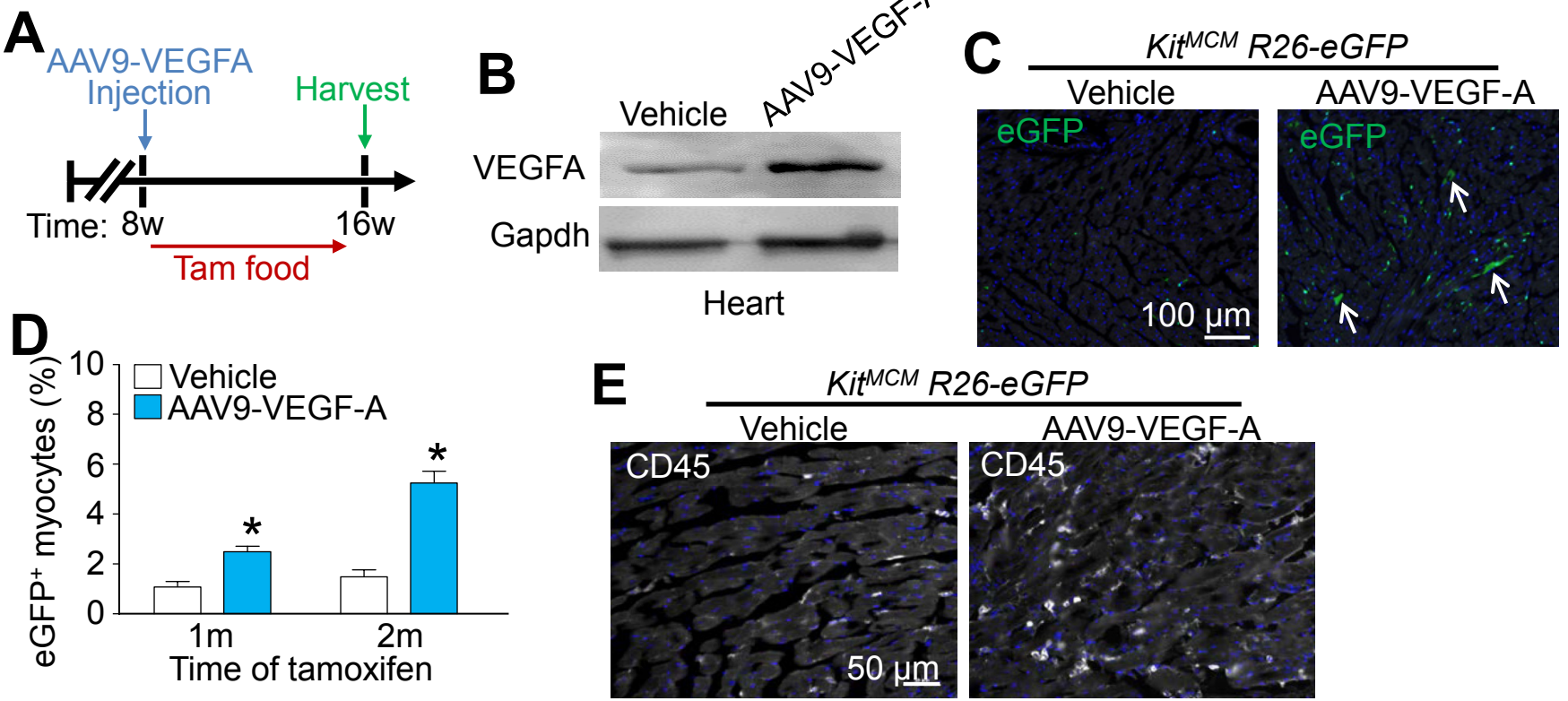

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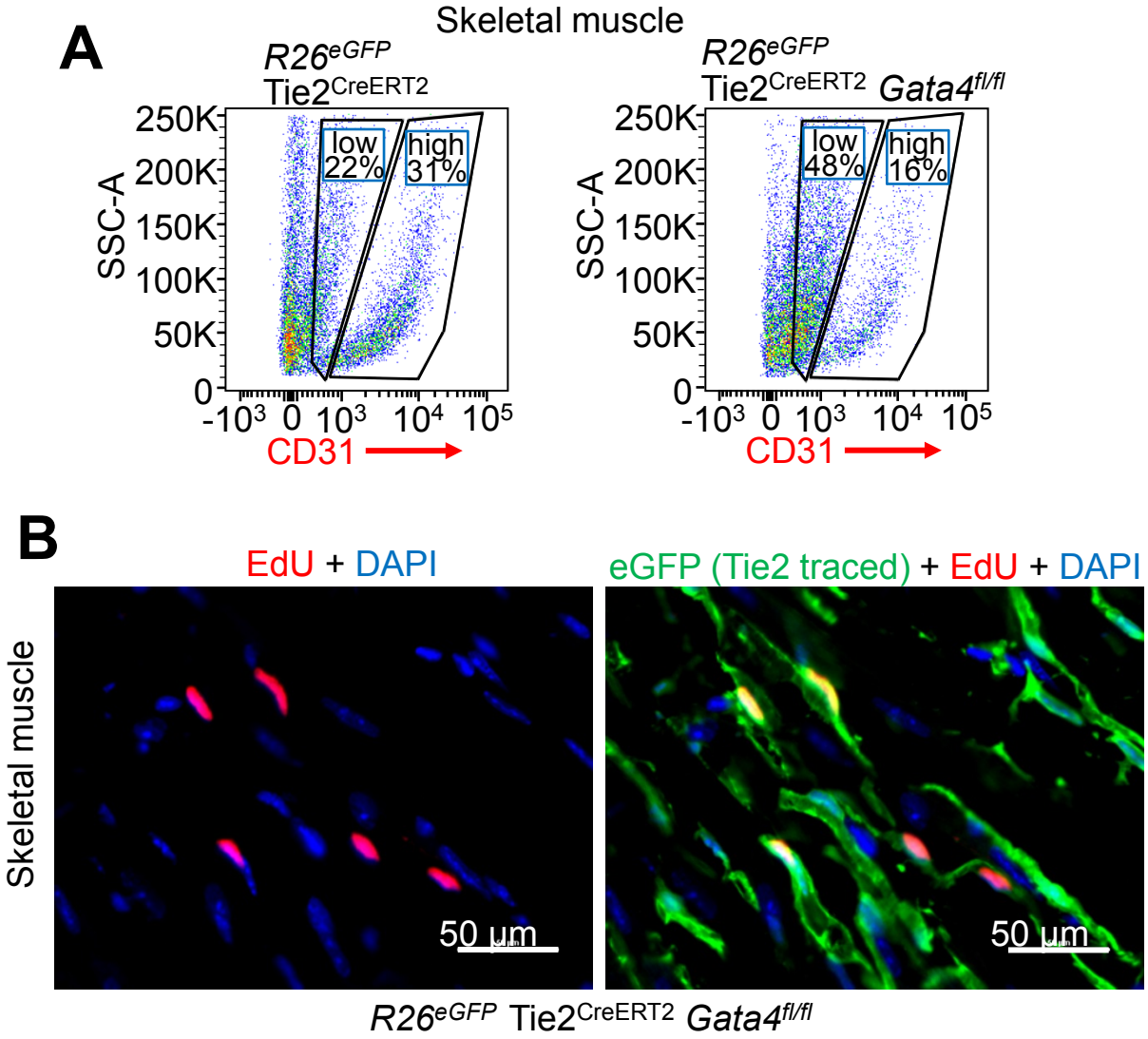

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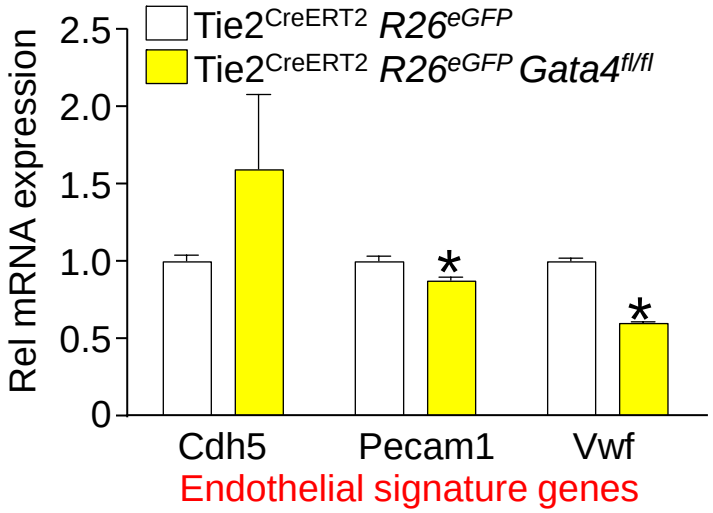

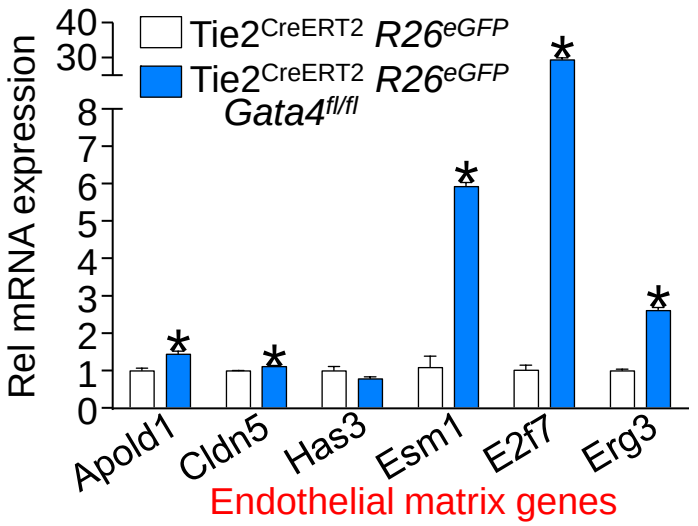

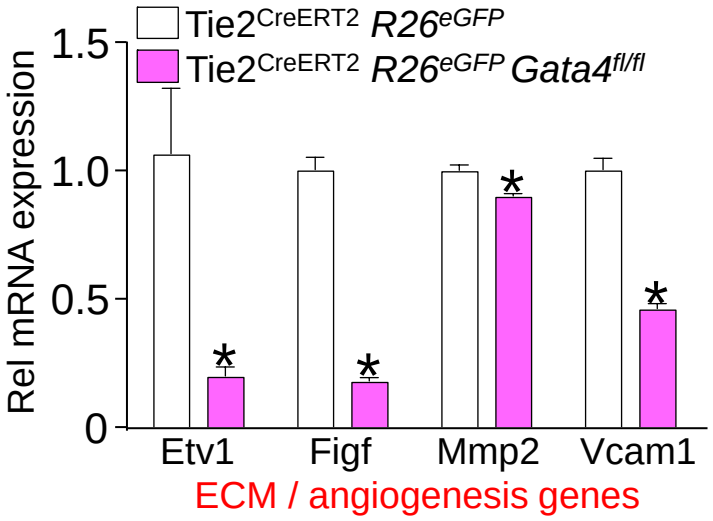

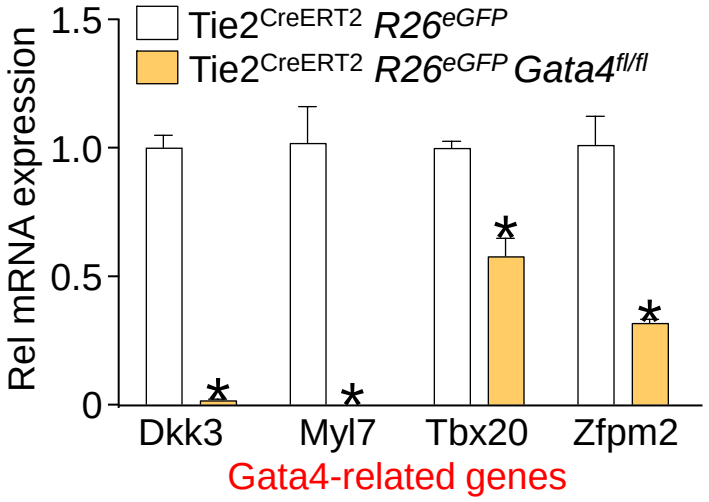

Figures legends for Supplemental Figures

Figure S1. *Kit*-lineage deletion of *Gata4/6* and *Gata4* alone leads to lineage-traced skeletal muscle fibers for the first time. **A,** Genetic models with the indicated alleles in mice that were crossed and (**B**) experimental design of the protocol used in this figure. **C.** Representative histological images of tibialis anterior muscle from *Kit* lineage-traced mice (white arrows point to *Kit* lineage eGFP$^+$ muscle fibers). **D.** Quantification of eGFP$^+$ skeletal muscle fibers in *Kit* lineage-traced mice with *Gata4, Gata6*, or *Gata4/6* deletion compared to lineage-traced *Gata4/6*-replete controls. Error bars are SEM, N=4 per group (10 sections per heart), *P<0.05 vs Kit^{MCM} $R26^{eGFP}$ for each genotype.

Figure S2. VEGF-A induced endothelial stimulation alters endothelial cell properties similar to loss of *Gata4*. **A.** Experimental design for AAV9-VEGF-A tail vein injection to Kit^{MCM} $R26^{eGFP}$ lineage tracing mice over the 8 week experimental period. **B,** Western blot measure VEGF-A from heart tissue of vehicle or AAV9-VEGF-A tail vein injected mice. Gapdh is used as a processing and loading control. **C,** Representative cardiac histological images and (**D**) quantitation of *Kit* lineage-traced cells (green) following tail vein injection and 2 months of continuous tamoxifen induction (white arrows highlight eGFP$^+$ cardiomyocytes). Error bars are SEM, N=4 per group (10 sections per heart), *P<0.05 vs vehicle treated mice at each time point. **E,** Representative cardiac histological sections showing CD45$^+$ cells (white) in the hearts of mice at baseline (vehicle) or 2 months after AAV9-VEGF-A infusion. N=4 per group (3 CD45$^+$ stained sections per heart).

Figure S3. Skeletal muscle with endothelial cell *Gata4* deletion shows reduced profile of endothelial cell maturation and greater proliferation. **A.** Representative flow cytometry plots of CD31-stained skeletal muscle interstitial cells comparing control Tie2^{CreERT2} lineage-traced mice to Tie2^{CreERT2} lineage-traced *Gata4*-deleted mice. Cells are gated to highlight CD31^{low} and CD31^{high} populations. N=3 per group. **B,** Representative skeletal muscle

histological images from Tie2^{CreERT2} lineage-traced (green) *Gata4*-deleted mice stained for EdU (red) and DAPI (blue). N=3 per group (6 EdU stained sections per heart).

Figure S4. RT-qPCR confirms altered endothelial gene profiles in *Gata4*-deleted endothelial cells. **A**, Quantitative mRNA levels for endothelial signature genes, (**B**) endothelial cell cycle and matrix genes, (**C**) ECM & angiogenesis genes, and (**D**) Gata4-associated genes. N=3 samples each, *P<0.05 versus WT by t-test. Three mRNAs that were not significantly changed are still shown because they were significantly changed in the DESeq2 analysis of the RNAseq data analysis (see Online Methods).

References for Supplemental Methods

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