SUPPLEMENTAL MATERIAL

Table S1. Major Resources.

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Sheep	New England Ovis	Polypay	M	http://neosheep.com/index.html
Sheep	New England Ovis	Polypay	F	http://neosheep.com/index.html

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male					
Parent - Female					

Antibodies

Target antigen	Vendor or Source	Catalog #	Dilution, [ab]	Lot #	Persistent ID / URL
CDH5 middle region	Aviva Systems Biology	ARP60108_P050-FITC	0.008 mg/ml	QC32476-200711	https://www.avivasysbio.com/cdh5-antibody-middle-region-fitc-arp60108-p050-fitc.html
Rabbit IgG Isotype Control	Southern Biotechnology	0111-02	0.005 mg/ml	A0316-X056J	https://www.southernbiotech.com/PolyclonalDetails.aspx?catno=0111-02#&panel2-1
alpha smooth muscle Actin [E184]	Abcam	ab209435	0.001 mg/ml	GR3274389-1	https://www.abcam.com/alpha-smooth-muscle-actin-antibody-e184-pe-ab209435.html?productWallTab=ShowAll
Rabbit IgG PE-conjugated Antibody	R&D Systems	IC1051P	0.0005 mg/ml	AESF0116091	https://www.rndsystems.com/products/rabbit-igg-pe-conjugated-antibody_ic1051p
FOXM1	Novusbio	NBP130961	1:300	42613	https://www.novusbio.com/products/foxm1-antibody_nbp1-30961
αSMA	Dako	M0851	1:150	20049711	https://www.agilent.com/en/product/immunohistochemistry/antibodies-controls/primary-antibodies/actin-(smooth-muscle)-(concentrate)-76542
Ki67 (IF)	Abcam	156956	1:50	GR271158-47	https://www.abcam.com/ki67-antibody-oti5d7-ab156956.html#lb
Ki67 (IHC)	Abcam	15580	1:100	GR3268067-1	https://www.abcam.com/ki67-antibody-ab15580.html
β-Catenin (IF)	Abcam	22656	1:100	GR3238275-2	https://www.abcam.com/beta-catenin-antibody-12f7-ab22656.html
CD31	Novusbio	NB10001642	1:50	E-3	https://www.novusbio.com/products/cd31-pecam-1-antibody-mec-746_nb100-1642
Phospho-β-Catenin (Ser552)	Cell Signaling	9566	1:3000	2	https://www.cellsignal.com/products/primary-antibodies/phospho-b-catenin-ser552-antibody/9566
β-Catenin	Cell Signaling	9562	1:3000	13	https://www.cellsignal.com/products/primary-antibodies/b-catenin-antibody/9562
Slug (A-7)	Santa Cruz	Sc-166476	1:500	A2120	https://www.scbt.com/p/slug-antibody-a-7

FOXM1 [EPR17379]	Abcam	ab207298	1:1000	GR32533330-3	https://www.abcam.com/foxm1-antibody-epr17379-ab207298.html
VE-cadherin	Invitrogen	36-1900	1:500	SA245292	https://www.thermofisher.com/antibody/product/VE-cadherin-Antibody-Polyclonal/36-1900
αSMA	Millipore Sigma	A5228	1: 12000	099M4848V	https://www.sigmaaldrich.com/catalog/product/sigma/a5228?lang=en&region=US
rabbit IgG, HRP-linked	Cell Signaling	7074	1:5000	29	https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074
Alexa Fluor® 488 goat anti-rabbit IgG (H+L)	Invitrogen	A-11034	2 ug/ml	662488	https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11034
Alexa Fluor™ 546 donkey anti-rabbit IgG (H+L)	Invitrogen	A10036	2 ug/ml	1832039	https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A10040
Alexa Fluor anti-rabbit 647	Fisher Scientific	A21244	1:500	2086730	https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21244
Alexa Fluor anti-mouse 488	Fisher Scientific	A11001	1:500	2090562	https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11001
Alexa Fluor anti-rat 594	Fisher Scientific	A11007	1:500	2107787	https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11007

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
mitral valve endothelial cells (mitral VEC)	Ovine mitral valve tissue	unknown	
carotid artery endotelial cells (CAEC)	Ovine carotid artery	unknown	

Data & Code Availability

Description	Source / Repository	Persistent ID / URL

Other

Description	Source / Repository	Persistent ID / URL

Fixation Buffer	Biolegend, Cat# 420801	https://sandbox.biolegend.com/en-us/products/fixation-buffer-1496
Intracellular Staining Permeabilization Wash Buffer (10X)	Biolegend, Cat# 421002	https://www.biolegend.com/en-us/global-elements/pdf-popup/intracellular-staining-permeabilization-wash-buffer-10x-1497?filename=Intracellular%20Staining%20Permeabilization%20Wash%20Buffer%2010X.pdf&pdfgen=true
Human TruStain FcX™, Fc Receptor Blocking Solution	Biolegend, Cat# 422301	https://sandbox.biolegend.com/en-us/products/human-trustain-fcx-fc-receptor-blocking-solution-6462
Ficoll-Paque™ PLUS Media	GE Healthcare, Cat#17-1440-02	https://www.sigmaaldrich.com/catalog/product/sigma/ge17144002?lang=en&region=US#:~:text=Ficoll%2DPaque%20Plus%20is%20a,simple%20and%20rapid%20centrifugation%20procedure.
Liberase™ TL Research Grade	MilliporeSigma, Cat# 5401020001	https://www.sigmaaldrich.com/catalog/product/roche/05401020001?lang=en&region=US
EBM-2 medium	Lonza Inc; #CC-3156	https://bioscience.lonza.com/lonza_bs/US/en/Primary-and-Stem-Cells/p/000000000000185299/EBM-2-Endothelial-Cell-Growth-Basal-Medium-2%2C-500-mL
Basic Fibroblast Growth Factor, human (hbFGF)	MilliporeSigma #11123149001	https://www.sigmaaldrich.com/catalog/product/roche/hbfgfro?lang=en&region=US
Human TGF-beta 1 Protein	R&D Systems, # 100-B-001	https://www.rndsystems.com/products/human-tgf-beta-1-protein_100-b
Difco ™ Gelatin	BD, REF# 214340	https://us.vwr.com/store/product/21080742/difcotm-gelatin-granulated-bd
iScript™ Reverse Transcription Supermix for RT-qPCR	Bio-RAD, 1708840	https://www.bio-rad.com/en-us/product/iscript-reverse-transcription-supermix-for-rt-qpcr?ID=M87EVMKG4
Kapa Sybr Fast ABI Prism 2x qPCR Master Mix	KAPA BioSystems, # KK4604	https://www.sigmaaldrich.com/catalog/product/ROCHE/SFABIKB?lang=en&region=US
Ovine Cytokine Array C1	Ray Biotech; # AAO-CYT-1-2	https://www.raybiotech.com/ovine-cytokine-array-c1/
Recombinant Human sFRP-3 Protein	R&D Systems, #7584-SF-025	https://www.rndsystems.com/products/recombinant-human-sfrp-3-protein_7584-sf
Ovine sFRP-3 ELISA	RayBiotech #ELO-sFRP3-1	https://www.raybiotech.com/ovine-sfrp-3-elisa/

Cell Lysis Buffer (10X)	Cell Signaling, #9803	https://www.cellsignal.com/products/buffers-dyes/cell-lysis-buffer-10x/9803?Ntk=Products&Ntt=9803
Protease/Phosphatase Inhibitor Cocktail (100X)	Cell Signaling, #5872	https://www.cellsignal.com/products/buffers-dyes/protease-phosphatase-inhibitor-cocktail-100x/5872?Ntk=Products&Ntt=5872
4–20% Criterion™ TGX™ Precast Midi Protein Gel	BIO-RAD, #5671094	bio-rad.com/en-us/sku/5671094-4-20-criterion-tgx-precast-midi-protein-gel-18-well-30-ul?ID=5671094
Trans-Blot Turbo RTA Midi 0.2 µm Nitrocellulose Transfer Kit	BIO-RAD, #1704271	https://www.bio-rad.com/en-us/sku/1704271-trans-blot-turbo-rta-midi-0-2-um-nitrocellulose-transfer-kit-for-40-blots?ID=1704271
6.5 mm Transwell® with 8.0 µm Pore Polycarbonate Membrane Insert, Sterile	Corning, #3422	https://ecatalog.corning.com/life-sciences/b2c/US/en/Permeable-Supports/Inserts/Transwell%C2%AE-Permeable-Supports,-Polycarbonate-(PC)-Membrane/p/3422
Thermo Scientific™ Richard-Allan Scientific™ Three-Step Stain Kit and Components	Thermo Scientific, #22-050-272	https://www.fishersci.com/shop/products/richard-allan-scientific-three-step-stain/p-4530230
Clarity Western ECL Substrate	BIO-RAD, #1705061	https://www.bio-rad.com/en-us/sku/1705061-clarity-western-ecl-substrate-500-ml?ID=1705061
ProLong® Gold Antifade Reagent with DAPI	Cell Signaling, #8961	https://www.cellsignal.com/products/buffers-dyes/prolong-gold-antifade-reagent-with-dapi/8961#:~:text=ProLong%C2%AE%20Gold%20Antifade%20Reagent%20with%20DAPI%20offers%20enhanced%20resistance,saved%20for%20months%20after%20mounting.

Table S2. Primer pairs used in this study for qPCR analysis.

Gene	Forward	Reverse
RSP9	5’-CGACCAAGAGCTGAAGCTGA-3’	5’-TTCATCTTGCCCTCGTCCAG-3’
VE-cadherin	5’-ACATCCGTGGTTCTGGACTC-3’	5’-AGATGGGGAAGTTGTCGTTG-3’
αSMA	5’-AGCTTCGTGTTGCTCCTGAA-3’	5’-GTGGGTGACACCATCTCCAG-3’
Slug	5’-GGACGCACACCTTACCTTGT-3’	5’-CGAGAAGGTTTTGGAGCAAC-3’
MMP2	5’-GAGACTCCCACTTCGACGAC-3’	5’-AACACCAGAGGAAACCATCG-3’
TGFβ1	5’-CAATTCCTGGCGCTACCTCA-3’	5’-GGTTCATGCCGTGAATGGTG-3’
TGFβ2	5’-ACCCTCGGAAAATGCCATCC-3’	5’-TTCGTGAACAGCATCGGTGA-3’
TGFβ3	5’-ACAGTGATGATGATCCGGGC-3’	5’-CAATGTAGAGAGGGCGCACA-3’
FOXM1	5’-CTCTGGTCTGGATAGGGGGTC-3’	5’-AGGAGTATGGGGGTCGTTCA-3’
CCNB1	5’-CCCCATGCCTCAAGACAGATT-3’	5’- TGATGGAGCTGTTTGCAGTGA-3’

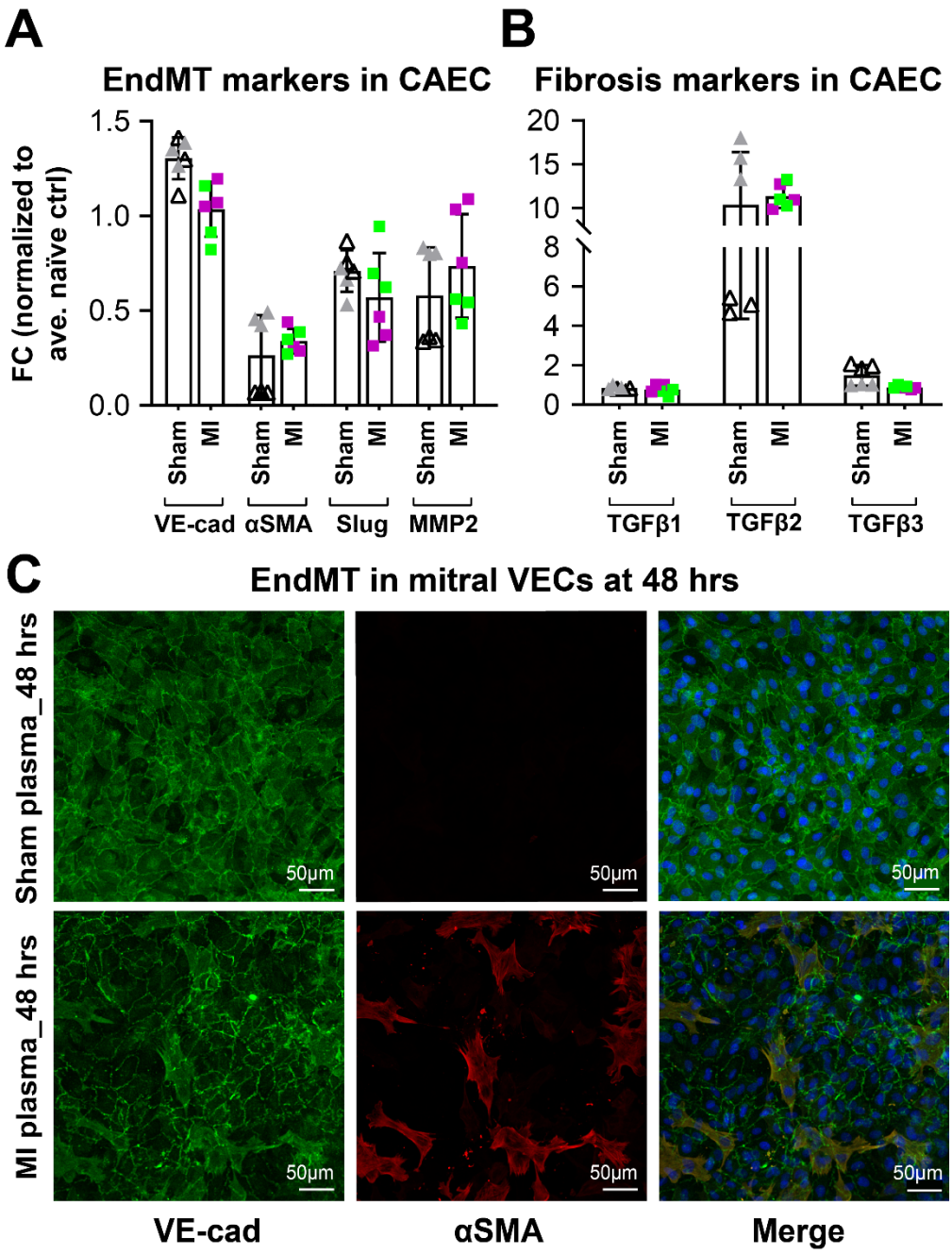

Figure S1. Carotid artery endothelial cells (CAEC) were exposed to plasma from two different naïve, two different sham (open and grey triangles), and two different inferior MI (green and purple squares) animals for 24 hours prior to qPCR analysis. The data from naïve animals (n=2) were used for normalization of sham and MI samples and is not shown in the graphs. Normalized mRNA levels of the indicated

markers of EndMT (A), and fibrosis (B) from three technical replicates are shown. (C) Mitral VECs treated for 48 hours with sham-operated (top row) or post-MI plasma (lower row) were stained for VE-cadherin and α-SMA (scale bar: 50 μm).

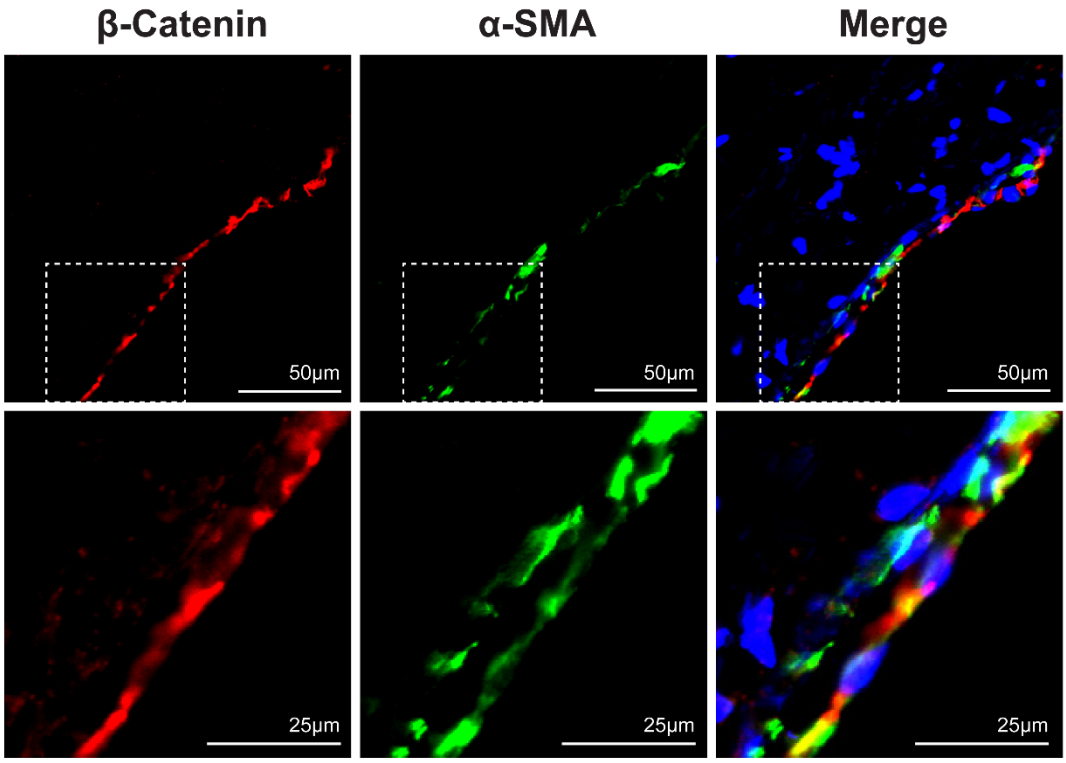

Figure S2. Double antibody-stained MV leaflet from 8–10-day post MI animal shows co-expression of β-Catenin and α-SMA along the MV endothelium (scale bar: 50 μm). Boxed areas are enlarged in the lower row.

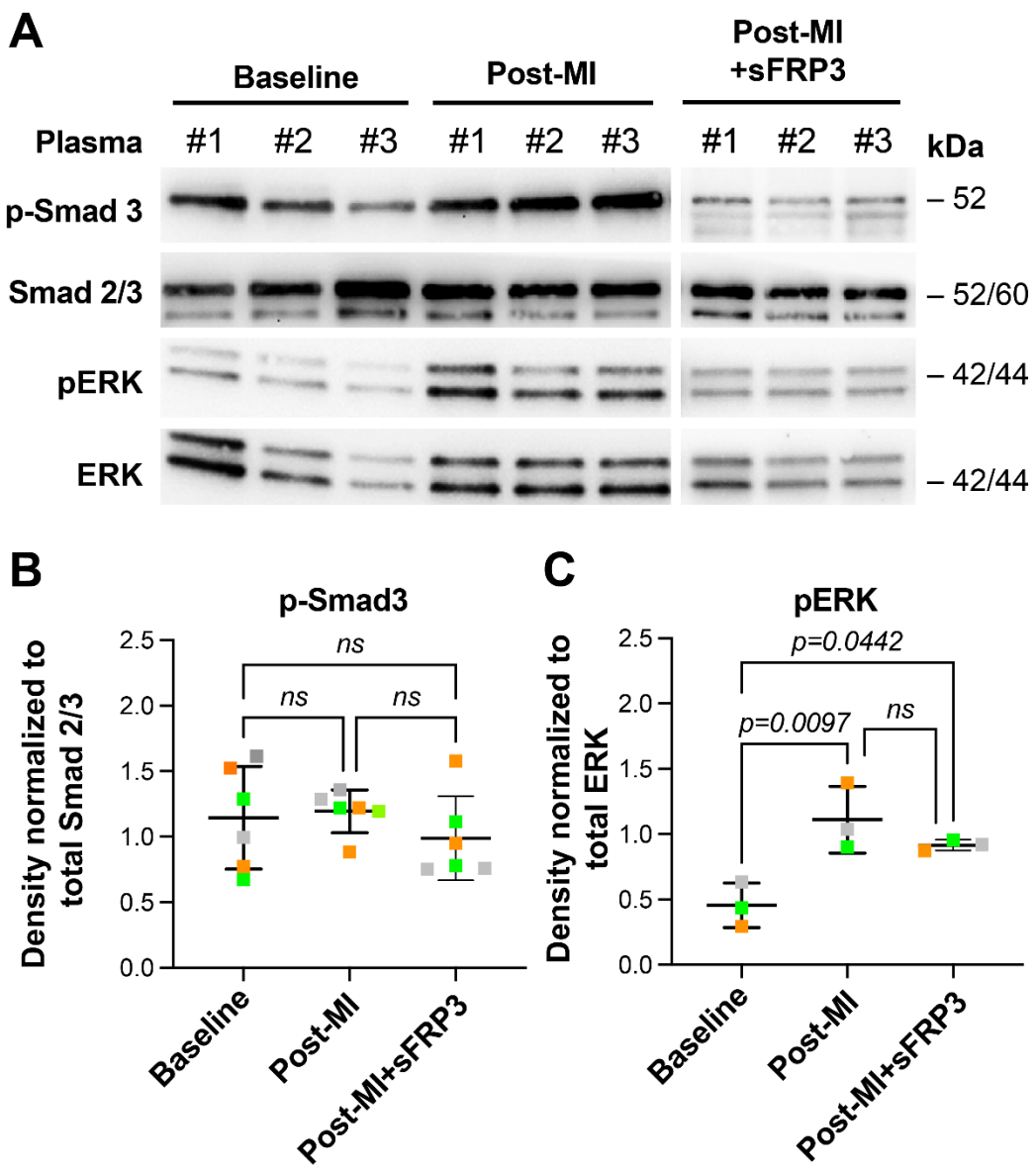

Figure S3. (A) Mitral VECs were treated with three individuals ovine before MI (baseline), post-MI, sFRP3 supplemented post-MI plasma for 10 minutes and lysates were subjected to western blot analysis. (B) Band densities were quantified and subjected to one-way ANOVA (F (2,15) =0.7333; *p*=0.04968) with Tukey's multiple comparisons test which revealed non-significant (ns) results for all conducted analysis. (C) Band densities were quantified and subjected to one-way ANOVA (F (2,6) =10.7;

p=0.0105) with Tukey's multiple comparisons test with *p* values indicated on the graph for each comparison.

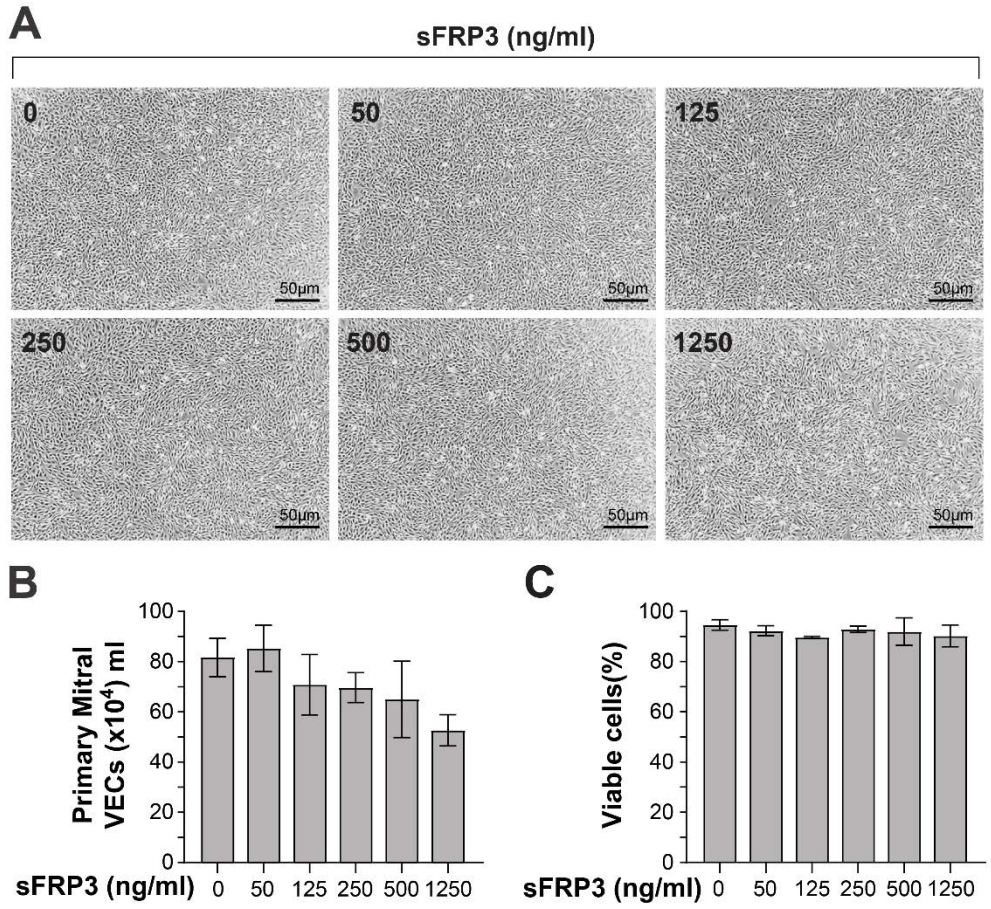

Figure S4. (A) Primary mitral VECs were treated with 0, 50, 125, 250, 500, and 1250 ng/ml of recombinant sFRP3 for 24 hours and imaged by phase contrast microscopy (scale bar: 50 μm). Total cells (B) and percent viable cells (C) were determined after trypsinization. Data from three independent assays were graphed.

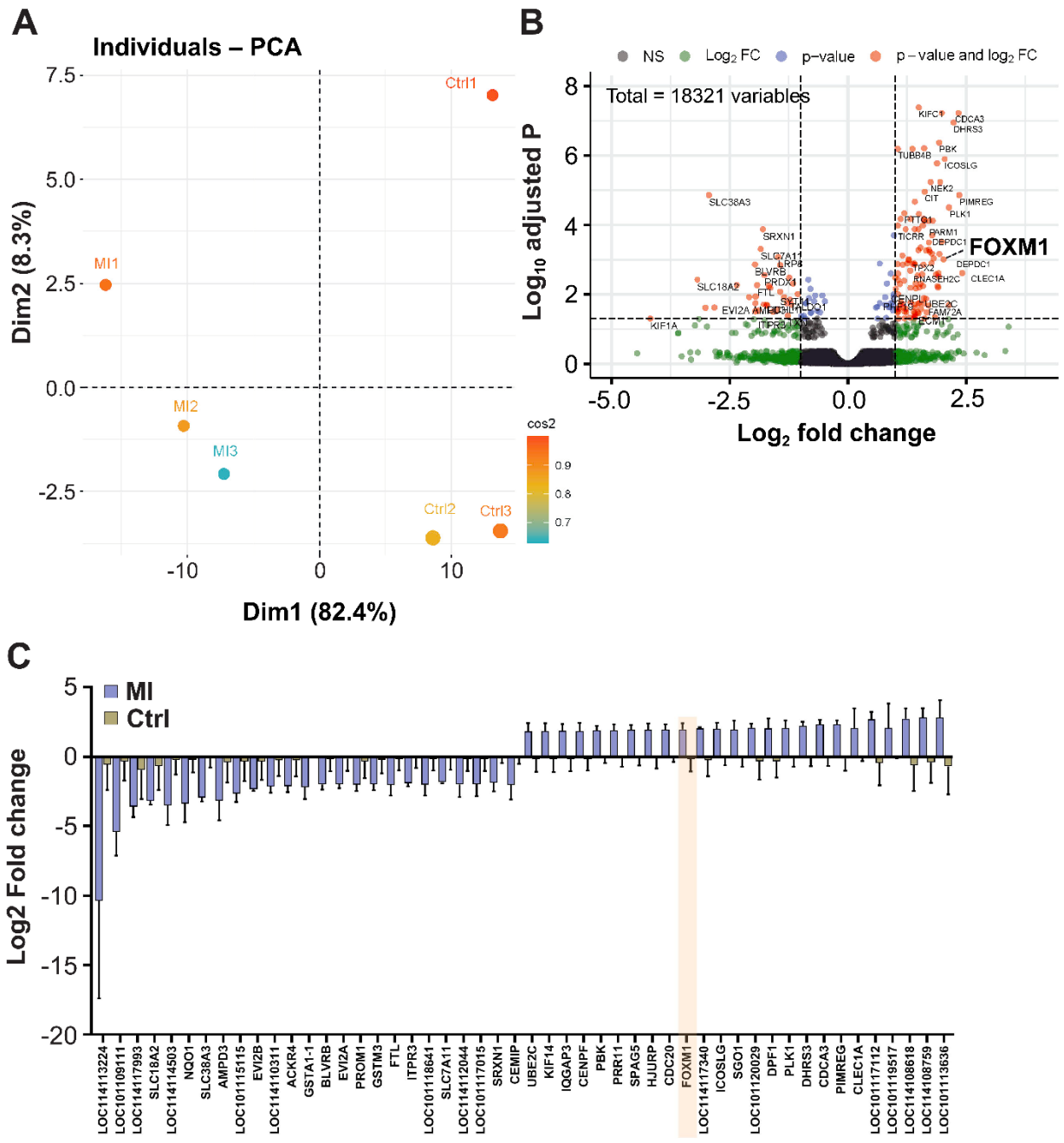

Figure S5. (A) Principal component (PC) analysis of the data in a 2D graph of PC1 and PC2. The bi-plot shows samples as labelled dots. The quality of representation of the variables on factor map is called cos2 (square cosine, squared coordinates). (B) Volcano plot of 18321 mRNAs. Differentially expressed genes are

visualized in red color. Blue dots illustrate the genes with adjusted p-value<0.05 but log2 fold change less than 1. Green dots represent non-significant genes. (C) Top 50 significantly regulated genes shown as 25 top downregulated and 25 top upregulated. Upregulated FOXM1 is highlighed in peach . Adjusted *p*-value is less than 0.05 for all of these genes.

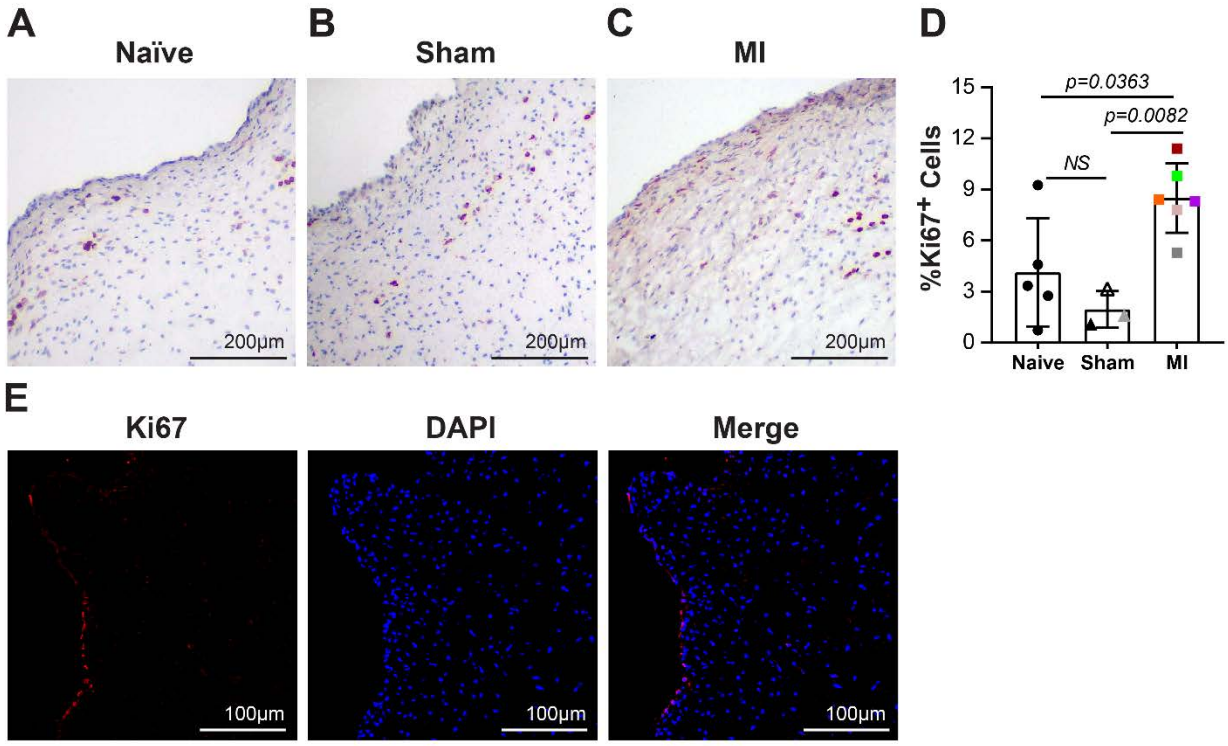

Figure S6. Mitral valve tissue from naïve (A), sham (B), and inferior MI (C) animals were stained for Ki-67 using IHC. (D) Number of cells expressing Ki-67 divided by total nuclei from 5 fields from 20X magnification per each individual section was graphed. *P* values were calculated using one-way ANOVA (p=0.0054, F=8.710) with Sidak multiple comparisons test. (E) Mitral valve tissue from an MI animal was stained for Ki-67 (red) using immunofluoresence staining. DAPI was used to stain nuclei (scale bar: 100 μm).

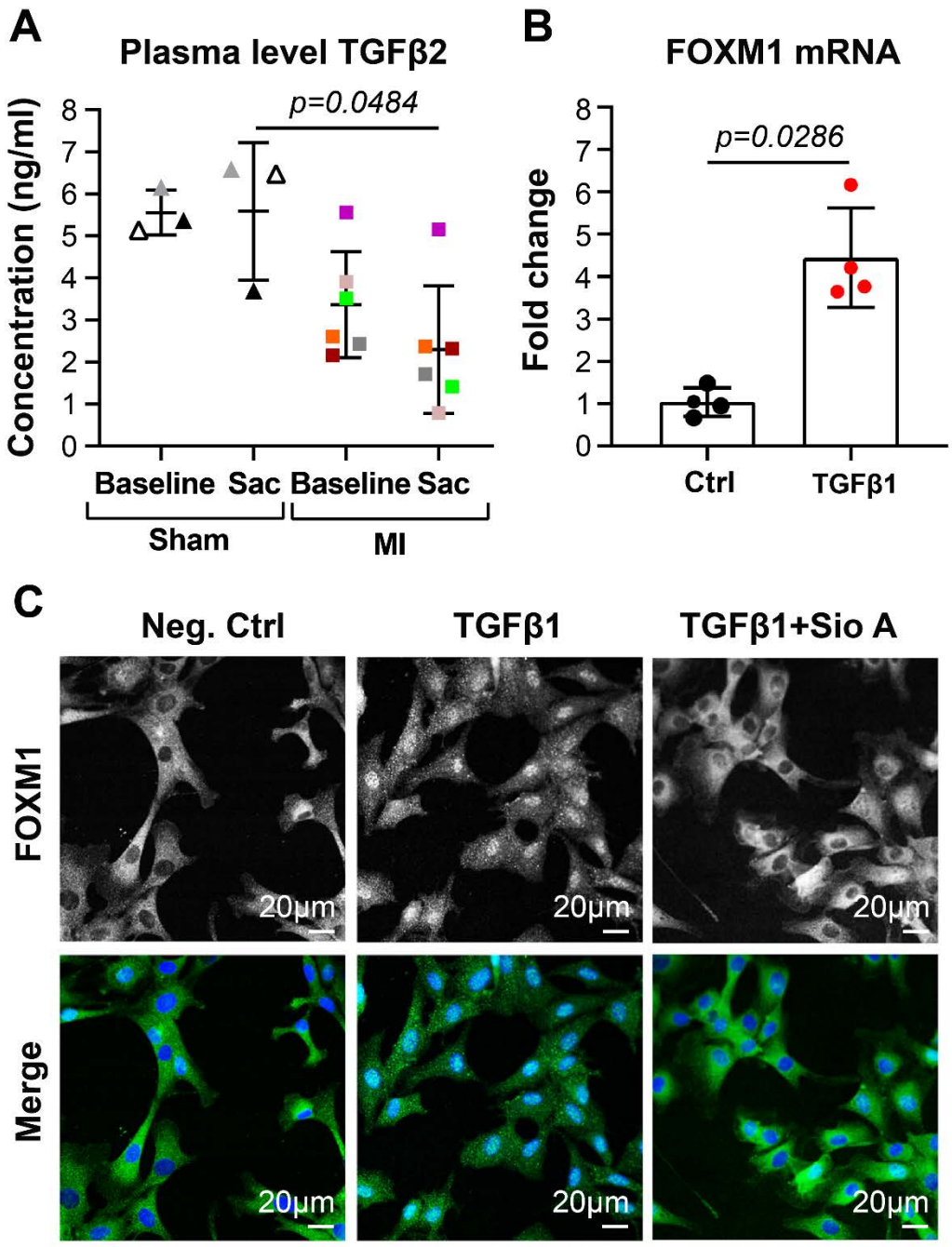

Figure S7. (A) Plasma TGFβ2 in sham (n=3) versus MI (n=6) plasma was measured by ELISA. Non-parametric Kruskal-Wallis test was performed following Dunn's multiple comparisons test. (B) Mitral VECs were treated with 2 ng/ml of TGFβ1 for 24 hours and lysates were analyzed by qPCR for FOXM1 transcripts. Values are

normalized to cells with no treatment and mean ±SD from four independent assays are presented in the graph. P values were calculated using non-parametric Mann Whitney test. (C) Mitral VECs treated for 24 hours with 1) media only (neg. ctrl), 2) TGFβ1 (2 ng/ml), and 3) TGFβ1 (2 ng/ml) +Sio A (1 μM) were stained using FOXM1 antibody (green). DAPI was used to stain nuclei (scale bar: 20 μm); FOXM1 staining is shown in black and white in the upper panel and the merge with DAPI is shown in color in the lower panel.