Supplement Material

TEA Domain Transcription Factor 1 (TEAD1) Promotes Smooth Muscle Cell Proliferation through Up-regulating SLC1A5-mediated Glutamine Uptake

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Running title: Role of TEAD1 in neointima formation

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Supplemental Methods

Mouse breeding and genotyping. TEAD1 floxed allele mice were generated as we recently reported [1]. To generate TEAD1 heterozygous mice, first we crossed TEAD1$^{F/W}$ female mice with male mice ubiquitously expressing Cre (CMV-Cre, Jackson lab, stock#: 006054) [2] that is under the transcriptional control of a human cytomegalovirus (CMV) minimal promoter. The resultant heterozygous offspring mice were then bred with C57BL/6 mice to obtain heterozygous TEAD1 mice and littermate WT control mice for vascular wire injury. SM MHC-CreER^{T2+} transgenic mice were originally generated by Dr. Stefan Offermanns lab as previously published [3] and obtained from Dr. Joseph Miano at the University of Rochester. Only male mice were used in this study because SM MHC-CreER^{T2} transgene is only localized in the Y chromosome [3]. To generate smooth muscle-specific tamoxifen-inducible TEAD1 knockout mice, we first generated SM MHC-CreER^{T2}/TEAD1$^{F/W}$ mouse line, by crossing SM MHC-CreER^{T2+} male mice with TEAD1$^{F/F}$ female mice. Subsequently SM MHC-CreER^{T2+}/TEAD1$^{F/W}$ male mice were bred with TEAD1$^{F/F}$ female mice to generate SM MHC-CreER^{T2+}/TEAD1$^{F/F}$ mice. 10-week old SM MHC-CreER^{T2+}/TEAD1$^{F/F}$ male mice were randomly assigned to two groups. These two groups of mice were then intraperitoneally injected with either sunflower oil (control) or tamoxifen (iKO; 1 mg/mouse/IP; SM-specific TEAD1 inducible KO), respectively, for 2 rounds of 5 days each, with 2 days' break in-between. After the last tamoxifen administration, a 2-week washout period was allowed before mice were used for wire injury. All mice used in this study were maintained on a C57BL/6 background. Genotyping of these mice was performed as previously described [1] and primer information is further provided in Online Table II. The use of experimental animals has been approved by the Institutional Animal Care and Use Committee and Biosafety committee at Augusta University in accordance with NIH guidelines.

Mouse femoral artery wire injury. Mouse wire injury was performed as previously described [4, 5]. Briefly, the injury was induced by inserting a straight spring wire (0.38 mm in diameter; Cook Medical) from an exposed muscular branch artery into the left femoral artery (LFA) for > 5 mm toward the iliac artery. The wire was left in place for 1 minute to denude and dilate the femoral artery. Then, the wire was removed, and the proximal portion of the muscular branch artery was secured with silk sutures followed by restoration of blood flow in the LFA. The right femoral artery (RFA) was not subjected to wire injury and therefore served as a contralateral control.

Rat carotid artery balloon injury. Rat carotid artery balloon injury was performed as we previously described [6-8]. Briefly, male Sprague-Dawley rats (350 g; Taconic Farms, Germantown, NY) were anesthetized with ketamine (70 mg/kg) and xylazine (4.6 mg/kg) via intraperitoneal injection. Following a midline cervical incision and muscular tissues separation, the left common carotid artery was exposed and blunt dissection was performed alongside the artery by dull forceps to expose the carotid artery bifurcation into the internal/external branches. Blood flow cessation was achieved by arterial clamps and a small arteriotomy was made in the external carotid artery. A 2F Fogarty balloon embolectomy (Edwards) was inserted through the small cut and passed into the common carotid artery. After balloon inflation at 1.6-2.0 atmospheric pressure, the catheter was partially withdrawn and reinserted 3 times. After the catheter was removed, the external carotid artery was secured with silk sutures, and the blood flow in the common carotid artery and its internal branch was restored by releasing arterial clamps. The right intact carotid artery served as a contralateral control.

Sections, Hematoxylin/Eosin (HE) staining, Verhoeff-van Gieson (EVG) staining, morphometric analysis of femoral artery and immunofluorescence staining (IF). Mice were euthanized by an overdose of 4% Isoflurane *via* inhalation, then systemically perfused with PBS *via* the left ventricle. Isolated femoral arteries were fixed with 4% paraformaldehyde in PBS over-

night at 4°C, washed 3 times with PBS, then kept in 30% sucrose in PBS over-night at 4°C. Fixed tissues were embedded in optimal cutting temperature compound (OCT) and kept at -80°C till cryo-sectioning. Sections were cut at 8-μm thickness and HE or EVG staining was performed following standard protocol as previously described [6, 9]. HE- and EVG-stained images were captured using an Olympus BX51 inverted microscope. Sections were analyzed blindly by an independent investigator for neointimal areas and neointima-to-media ratios using Image J software. The neointimal area was calculated by subtraction of the luminal area from the area enclosed by the internal elastic lamina. The medial area was calculated by subtraction of the area enclosed by the internal elastic lamina from the area enclosed by the external elastic lamina. For IF, cryo-sections were air-dried for 15 minutes and antigen retrieval was performed by heating at 98°C for 10 minutes in citric acid buffer (10 mM, pH 6.0). Sections were blocked and permeabilized with goat serum (10%, Thermo Fisher Scientific) plus 0.1% Tween for 30 minutes, then incubated with TEAD1 (Abcam, ab133533, rabbit, 1:250), SM α-actin (Sigma, A2547, mouse, 1:80), p4EBP1 (Cell signaling, #2855, rabbit, 1:100), pS6 (Cell signaling, #4858, rabbit, 1:100), Ki67 (Thermo Scientific, RM-9106, rabbit, 1:30), SM MHC (Biomedical Technologies Inc, BT-562, rabbit, 1:50), or SM22α (Abcam, ab14106, rabbit, 1:50) antibodies over-night at 4°C. After washing with PBS, sections were incubated with secondary antibodies (488 nm-conjugated anti-mouse secondary antibody or 594 nm-conjugated anti-rabbit secondary antibody, 1:250 dilution, Thermo Fisher Scientific) diluted in blocking buffer for 1 hour at room temperature. Sections incubated without primary antibodies but with secondary antibodies only served as a negative control. Following wash with PBS for three times, sections were mounted with ProLong Gold antifade reagent with DAPI (Thermo Fisher Scientific) and imaged using confocal microscopy (780 upright, Zeiss). For IF staining of TEAD1 in sections, a TSA kit (TSA plus DIG, Perkin Elmer, cat. #: NEL748001KT) was used following the protocol provided by the manufacturer.

5-ethynyl-20-deoxyuridine (EdU) incorporation assay. EdU incorporation assay was performed using the Click-iT EdU imaging kit (Thermo Fisher Scientific, cat. #: C10339) according to the manufacturer's instructions. Briefly, mice were injected intraperitoneally with EdU at a dose of 50 mg/g body weight 2 hours before sacrifice. Isolated RFA and LFA were collected, fixed, and sectioned as described above. For EdU incorporation assays in human coronary artery SMCs (HCASMCs), cells were incubated with EdU (10 mM) in vascular cell basal medium supplemented with smooth muscle growth supplement with 5% FBS (SMGS, Thermo Fisher Scientific) overnight. EdU-positive nuclei were detected following the manufacturer's protocol and imaged using a confocal microscopy (780 upright, Zeiss).

Cell culture. Mouse aortic endothelial cells (MAECs) were purchased from CellBiologics (cat. #: C57-6052). Human umbilical vein aortic endothelial cells (HUAECs, cat. #: PCS-100-013), human aortic endothelial cells (HAECs, cat. #: PCS-100-011) and RAW264.7 cells (cat. #: TIB-71) were purchased from ATCC. All commercial cells described above were cultured following the venders' protocols and plated for assays at 70-80% confluency. Mouse and rat primary aortic smooth muscle cells were isolated as we previously reported [6]. Mouse bone marrow-derived macrophages or mouse peritoneal macrophages were prepared following standard protocols. HCASMCs (Gibco, cat. #: C-017-5C; Lot #: 1130140 and Lot #: 1689414), vascular cell basal medium (cat. #: M231500) and smooth muscle growth supplement with 5% FBS (SMGS, cat. #: S00725) were purchased from Thermo Fisher Scientific. HCASMCs (passages 3-5) were incubated in vascular cell basal medium containing SMGS (complete medium) and 5.5 mM D-glucose along with antibiotic/antimycotic solution in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. Sub-confluent VSMCs were trypsinized, centrifuged, and seeded onto petri-dishes or multi-well plates. In some experiments, VSMC quiescence was induced by substituting the complete medium with vascular cell basal medium without SMGS for at least 48 hours before treatments.

Cell counts. Sub-confluent HCASMCs were equally plated onto 6-well plates in complete medium in the absence or presence of the respective treatments for 3 or 5 days as described in the figure legends. During the treatment period, the media was replaced every 48 hours with a fresh complete medium containing the indicated concentrations of treatments. VSMCs were then trypsinized and the changes in cell counts were determined using a hemocytometer.

WST-1 proliferation assay. The assay was done according to manufacturer's instructions as we previously reported [6, 8]. Briefly, sub-confluent HCASMCs were equally plated onto 96-well plates in complete medium with or without the respective treatments for the indicated period of time as described in the figure legends. WST-1 reagent (Sigma-Aldrich, cat. #: 5015944001, 10 μl/100 μl of media) was added to the culture medium and cells were kept at 37°C for 2 hours. Afterwards, the absorbance at 450nm, as an indirect measure of cell proliferation, was measured using a microplate reader.

Quantitative real time PCR (qRT-PCR) analysis. For aortic tissue samples, mice were euthanized by 4% Isoflurane *via* inhalation, then systemically perfused with PBS. Dorsal aortae were rapidly dissected and cleared of perivascular fat and connective tissues under a stereoscope. Then, aortic tissues were rapidly frozen in liquid nitrogen and stored at -80°C till subsequent analysis. Total RNA from aortic tissues or HCASMCs was extracted with TRIzol reagent (Thermo Fisher Scientific). 1 μg of RNA was reverse transcribed to cDNA using the High Capacity RNA-to-cDNA Kit and random hexamer primers (Thermo Fisher Scientific). qRT-PCR was performed with the respective gene-specific primers as listed in Online Table II. All samples were amplified in duplicate and all experiments were repeated at least 3 independent times. Relative gene expression was determined using the $2^{-\triangle\triangle CT}$ method (CT, comparative threshold cycle). CT values were normalized to the internal control hypoxanthine phosphor ribosyl transferase 1 (HPRT) for mouse tissue samples, and β2 microglobulin (B2M) for human SMC samples where $\triangle\triangle CT = (CT_{\text{experimental gene}} - CT_{\text{experimental HPRT or B2M}}) - (CT_{\text{control gene}} - CT_{\text{control HPRT or B2M}})$.

Next generation sequencing. Aortic tissues were isolated from oil- or tamoxifen-treated mice as described above. Total RNA was then extracted from the aortic tissues using TRIzol reagent (Thermo Fisher Scientific) and subjected to whole transcriptome RNA-seq analysis. Differential gene expression analysis was performed at the Genome Technology Access Center at Washington University. Following amplification of the input RNA by Sigma SeqPlex RNA amplification kit, the library was prepared and single-end sequencing in 50-bp length was performed on a HiSeq 3000 system (Illumina). Adaptor sequence and low-quality reads were removed using Trimmomatic 0.32 [10]. Pass-filtered reads were then mapped to Ensembl mouse reference genome mm10 using TopHat version 2.1.0 [11]. The genes annotated in Ensembl GRCm38 were quantified with HTSeq 0.6.1 [12] and only those with count ≥ 10 in at least one group were considered as expressed genes and used for subsequent analysis. Differential expression analysis was performed with the R package edgeR (https://www.r-project.org) [13]. Cutoff values of fold change greater than 1.2 and false discovery rate (FDR) less than 0.1 were considered statistically significant between control and iKO groups (2 samples each group). Fragments Per Kilobase of exon per Million fragments mapped (FPKM), which corrects for variations in contig length and read depth between samples, was calculated from raw counts for each sample (Online Table I) [14]. Volcano plot was generated using R program. Heat map was generated by MeV (MultiExperiment Viewer) program. GO (Gene Ontology) analysis was carried out by Metascape (http://metascape.org) [15]. The RNA-seq data generated in this study have been deposited in the Sequences Read Archive at the NCBI under accession number PRJNA492803.

Protein extraction and western blotting. Isolated mouse tissues or HCASMCs were

homogenized in RIPA buffer (Thermo Fisher Scientific) plus 1% protease/phosphatase inhibitor cocktail (Thermo Fisher Scientific). After sonication and centrifugation of the tissue or cell lysates, proteins in the supernatant were quantified by BCA assay (Thermo Fisher Scientific) and resolved on a 7.5%, 10%, or 12.5% SDS-PAGE gel at 5-20 μg per lane as appropriate. Primary antibodies used in this study were: TEAD1 (Abcam, ab133533, rabbit, 1:1000), eNOS (BD Biosciences, cat. #: 610296, mouse, 1:1000), Mac-2 (also known as galectin-3, Santa Cruz, H-5, sc-374253, mouse, 1:1000,), YAP (Sigma, WH0010413M1, mouse, 1:1000), pS6 Ser235/236 (Cell signaling, #4858, rabbit, 1:1000), S6 (Cell signaling, #2217, rabbit, 1:1000), pH3 (Millipore, 06-570, rabbit, 1;1000), PCNA (Santa Cruz, sc-56, mouse, 1:100), SM MHC (Biomedical Technologies Inc, BT-562, rabbit, 1:1000), MLCK (Abcam, ab76092, rabbit, 1;5000), calponin (Sigma, C2687, mouse, 1:1000), SM22α (Abcam, ab10135, goat, 1:2000), β-actin (Sigma, A5316, mouse, 1:2000), Hic-5 (BD, 611164, mouse 1:5000), SM α-actin (Sigma, A2547, mouse,1:5000), HSP90 (Cell Signaling, #4874, rabbit, 1:1000), troponin T (Cell signaling, #5593, rabbit, 1:1000), troponin I (Cell signaling, #4002, rabbit, 1:1000), vinculin (Sigma, V4505, mouse,1:5000), SLC1A5 (Cell signaling, #5345, rabbit, 1:1000), SLC7A5 (Cell signaling, # 5347, rabbit, 1:1000), SLC38A1 (Cell signaling, #36057, rabbit, 1:1000), α-tubulin (Cell signaling, #2144, rabbit, 1:1000), p27 (Cell signaling, #3686, rabbit, 1:1000), pp70S6K Thr389 (Cell signaling, #9234, rabbit, 1:1000), p70S6K (Cell signaling, #2708, rabbit, 1:1000), p4EBP1 Thr37/46 (Cell signaling, #2855, rabbit, 1:1000), 4EBP1 (Cell signaling, #9644, rabbit, 1:1000), pAkt Thr308 (Cell signaling, #2965, rabbit, 1:1000), Akt (Cell signaling, #4691, rabbit, 1:1000), pERK1/2 Thr202/Tyr204 (Cell signaling, #4370, rabbit, 1:1000), ERK1/2 (Cell signaling, #4695, rabbit, 1:1000), myc (Thermo Scientific, R950-25, rabbit, 1:1000). Secondary antibodies conjugated with horseradish peroxidase were then used to visualize the target proteins on blots. Images were acquired by ImageQuant LAS 4000 Imaging Station (GE) and band densities were quantified using the Image J software.

siRNA transfection. Scrambled control siRNA duplex (#4390843), human TEAD1 siRNA duplex (#s13961), human TEAD2 siRNA duplex (#s16077), human TEAD3 siRNA duplex (#s13968), human TEAD4 siRNA duplex (#s13966) and human SLC1A5 siRNA (#s12918) were purchased from Thermo Fisher Scientific (Ambion). Delivery of siRNA into HCASMCs was done using Neon transfection system (Thermo Fisher Scientific) essentially following the manufacturer's protocol and as described in our previous report [6]. After transfection, VSMCs were equally plated on 6-well plates for different time points, as indicated in the figure legends, for cell count assays, qRT-PCR, or western blot analysis. For WST-1 proliferation assays, VSMCs were equally plated on 96-well plates.

Adenoviral construction and cell infection. Adenovirus encoding TEAD1 was generated as described previously [9, 16]. To generate human myc-tagged SLC1A5 adenovirus, a cDNA clone encoding human SLC1A5 was purchased from OriGene (cat. #: RC200305) and sub-cloned into AdTrack shuttle vector. As this vector contains an independent cytomegalovirus promoter-driven transcription cassette for green fluorescent protein (GFP), the efficiency of transduction was directly monitored by visualization of GFP expression. Transferring the human SLC1A5 and GFP expression cassettes into AdEasy viral backbone, viral packaging, and cell infection was performed as we previously reported [6, 16]. The adenovirus expressing GFP alone served as control.

Luciferase reporter assay. Cloning of WT or mutant SLC1A5 promoter-driven luciferase reporters and dual luciferase assays were performed as described in our recent report [9]. Briefly, a 1379-bp fragment spanning human SLC1A5 proximal promoter region (human chr19:46,788,319-46,789,697) was amplified by PCR with primers harboring KpnI and XhoI restriction enzyme sites (primer sequences are listed in the Online Table II) by using human genomic DNA as template (Promega). The PCR product was first cloned into pSC-B blunt vector (Stratagene), then sub-cloned into pGL2b luciferase reporter vector (Promega). A conserved

MCAT element within SLC1A5 gene promoter was identified by a sequence alignment among mouse and human at the UCSC genome browser (http://genome.ucsc.edu). Mutation of the MCAT element in the SLC1A5 promoter was carried out with QuickChange Site-Directed Mutagenesis kit (Stratagene) using the primers listed in Online Table II. All plasmids were sequenced to verify the integrity of the insert (Genewiz). Mammalian expression plasmid for TEAD1 was generously provided by Dr. Kun-liang Guan, UCSD [17]. Transfection was carried out with X-tremeGENE 9 transfection reagent (Roche) essentially following manufacturer's protocol. The promoter activity was evaluated by measurement of the firefly luciferase activity relative to the internal control TK-renilla luciferase activity using the Dual Luciferase Assay System as described by the manufacturer (Promega). A minimum of six independent transfections was performed and all assays were replicated at least twice.

Quantitative chromatin immunoprecipitation (ChIP) assay. After transduction with adenovirus expressing TEAD1 for 72 hours, HCASMCs were washed, fixed with formaldehyde, and ChIP assays were performed using anti-TEAD1 antibody (Abcam, ab133533) or rabbit IgG control as described by the manufacturer (Active Motif) and in our previous reports [9, 18]. ChIP assays were repeated 3 times and each IP was duplicated. Equal amount of chromatin was used for IP with anti-TEAD1 antibody or IgG. The genomic DNA purified from the precipitated genomic DNA or from input were amplified by qPCR. Primers for quantitative evaluation of enrichment of TEAD1 at the SLC1A5 promoter MCAT region and exon 8 region are listed in Online Table II. Data were expressed as relative binding by using the $2^{-\triangle\triangle CT}$ method against the IgG control samples (set to 1) where $\triangle\triangle CT = (CT_{IP\ TEAD1} - CT_{input\ TEAD1}) - (CT_{IP\ control\ IgG} - CT_{input\ control\ IgG})$.

Statistical analysis. All data are expressed as means ± SE of at least three independent experiments. For *in-vivo* wire-injury experiments, sample size was calculated based on power analysis to achieve Power of 0.8 at a p=0.05. Statistical analysis of data involving more than two groups was performed using one-way or two-way analysis of variance (ANOVA), where appropriate, followed by Bonferroni t-test. Statistical analysis of data involving two groups was performed using unpaired two tailed t-test for samples with normal distribution (Shapiro-Wilk test was initially used to test for Normality). Otherwise, unpaired non-parametric Mann Whitney test was performed (GraphPad). Values of $p \leq 0.05$ were considered statistically significant.

Supplemental References

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Supplemental Figure Legends

Online Figure I. TEAD1 is readily detectable in VSMCs. A. qRT-PCR analysis was performed to examine the relative abundance of TEAD1-4 genes in cultured human coronary artery SMCs (HCASMCs), (**B**) human aortic endothelial cells (HAECs), and (**C**) mouse peritoneal macrophages (PMs). **D.** Western blot was performed to determine TEAD1 protein expression in cultured VSMCs from human, rat, and mouse aorta, in cultured endothelial cells and macrophage cells. HUVEC, human umbilical vein endothelial cells; MAEC, mouse aortic endothelial cells; BMM, mouse bone marrow-derived macrophages; RAW, mouse RAW264.7 macrophage cell line. Calponin, smooth muscle marker; eNOS, EC marker; Mac-2, macrophage marker.

Online Figure II. Heterozygous deletion of TEAD1 attenuates neointima formation. A. Aortic or (**B**) heart tissues were harvested from adult WT mice or TEAD1 heterozygous mice (HET) and analyzed by western blot. N=6 per group. **C.** Control RFAs were harvested from adult WT or TEAD1 HET mice for HE staining. **D.** Medial layer thickness were measured as shown in "C" and quantified. The relative medial layer thickness in control mice was set to 1. **E.** Wire-injured LFA were harvested from adult WT or TEAD1 HET mice at day 14 post-injury and analyzed by HE staining. N=7 per group. The boxed area was magnified and shown on the right. Yellow arrowheads denote the internal elastic lamina. **F.** Relative neointimal area, (**G**) neointima-to-media ratio and (**H**) the relative medial area in injured LFA as shown in "E" were quantified and plotted. *p<0.05.

Online Figure III. Characterization of smooth muscle-specific inducible TEAD1 KO mouse. A. 10-week old SM MHC-CreER^{T2+}/TEAD1$^{F/F}$ mice were intraperitoneally injected with sunflower oil (control) or tamoxifen (iKO). DNA was extracted from brain, heart, and aortic tissues and subjected to genotyping analysis. The deleted TEAD1 allele can only be detected in aortic tissues of iKO mice. N=3 per group. **B**. qRT-PCR analysis was performed to examine TEAD1 mRNA expression level in aortic tissues harvested from control or iKO mice. *p<0.05. **C.** Western blot analysis of aortic or (**D**) heart tissue lysates harvested from control or iKO mice. N=6 per group. **E.** IF staining for TEAD1 (red) or SMA (green) in RFA isolated from control or SM-specific TEAD1 iKO mice. The boxed area by a yellow rectangle was magnified and shown on the right. Yellow arrowheads point to TEAD1 positive endothelial cells and arrows point to TEAD1 positive adventitial cells, respectively. N=4 per group. **F.** Relative number of TEAD1 and SMA double positive cells was calculated and plotted. The total TEAD1^{+}/SMA^{+} cells in RFA from control mice was set to 100. *p<0.05. **G.** By co-staining with DAPI and SMA, total VSMC numbers were counted per section of RFAs from both control and iKO mice.

Online Figure IV. Effects of smooth muscle-specific inducible TEAD1 KO on smooth muscle marker expression. Control or SM-specific TEAD1 iKO mice were subjected to LFA wire injury. At 14 days post-injury, RFA or LFA were harvested and analyzed by IF staining with antibodies for (**A**) SM MHC or (**B**) SM22α. Nuclei were counter-stained with DAPI (blue). N=3 per group.

Online Figure V. Role of TEAD2-4 genes in regulation of SLC1A5 expression. **A**. HCASMCs were transfected with scrambled control siRNA (siControl) or TEAD2 siRNA (siTEAD2), (**B**) TEAD3 siRNA (siTEAD3), or (**C**) TEAD4 siRNA (siTEAD4) for 72 hours. Subsequently total RNA was isolated for qRT-PCR analysis to determine mRNA expression as indicated. *p<0.05.

Online Figure VI. TEAD1 expression is induced in balloon-injured rat carotid arteries and positively correlates with SLC1A5 expression. A. Control right carotid arteries (RCA) or balloon-injured left carotid arteries (LCA) were harvested from adult male rats at 7 days post-

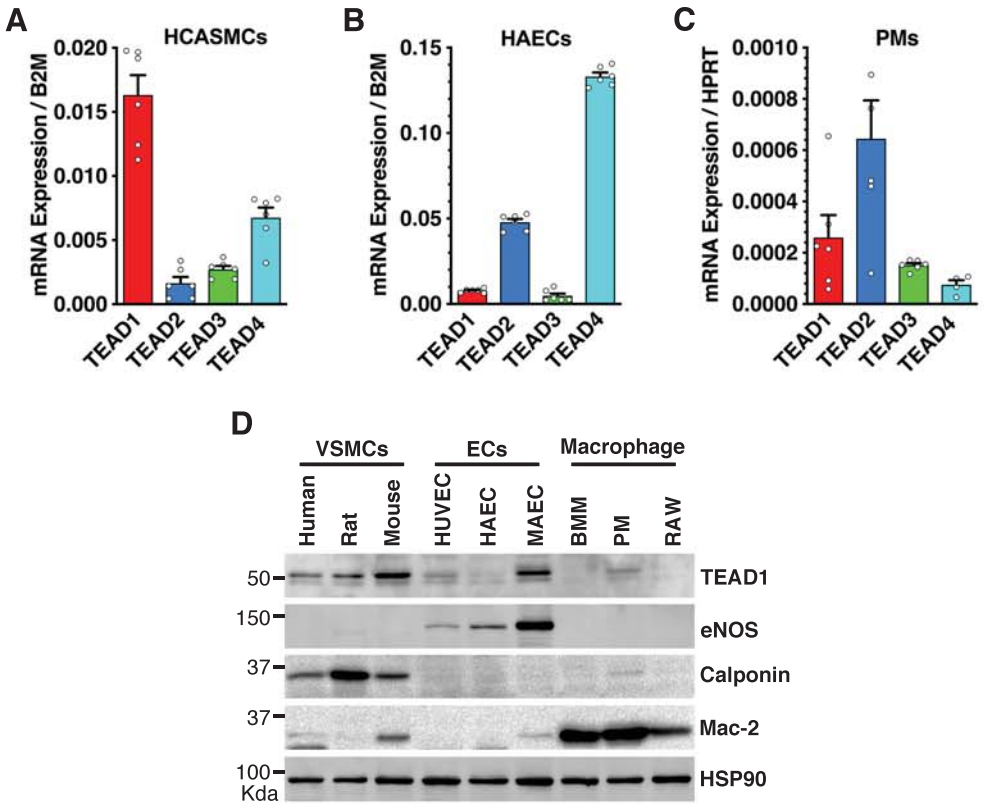
injury and analyzed by western blot. **B.** Densitometric analysis of protein expression in "A" normalized to the loading control (α-tubulin) and expressed relative to signals from the uninjured control RCA (set to 1, red line). *p<0.05.

Online Figure VII. Time-dependent effects of PDGF-BB in HCASMCs. HCASMCs were cultured in the absence or presence of PDGF-BB (50ng/ml) for 2 or 5 days, respectively, and then harvested for western blot analysis as indicated.

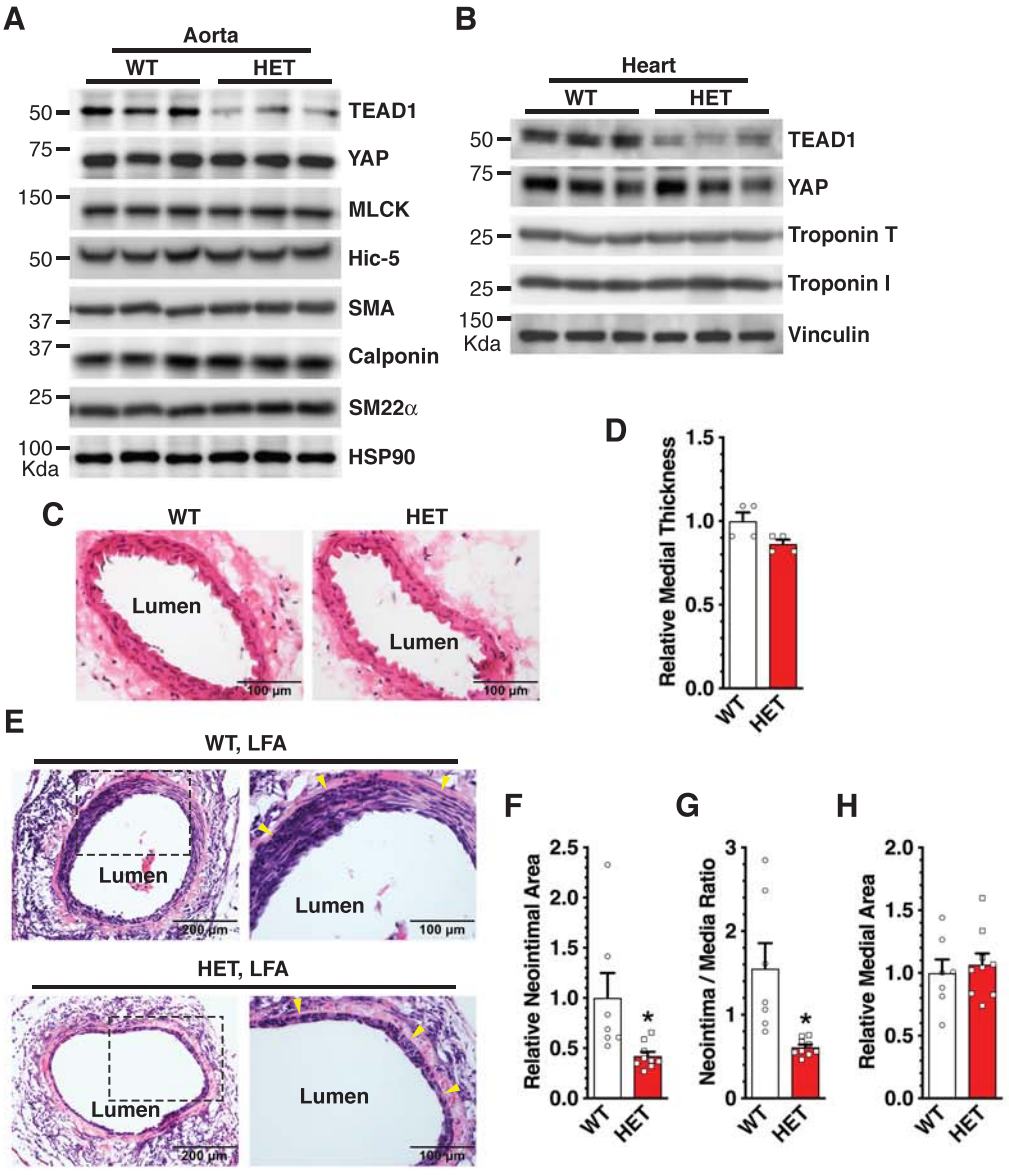
Online Figure VIII. TEAD1 up-regulates SLC1A5 and activates mTORC1 signaling in an independent isolate of HCASMCs. HCASMCs derived from an independent human donor (Gibco, cat. #: C-017-5C; Lot #: 1689414) were transduced with GFP or TEAD1 adenovirus for 48 hours, then harvested for western blot analysis. S: short exposure; L: long exposure. N=3 per group.

Online Figure IX. Glutamine uptake is critical for mTORC1 activation and VSMC proliferation. A. HCASMCs were counted after cultured in the medium with (control) or without (W/O) glutamine for 3 days. *p<0.05; N=3. **B.** HCASMCs treated with or without GPNA (3mM), an inhibitor of SLC1A5-mediated glutamine uptake, were subjected to western blot to assess mTORC1 activation or cell proliferation by (**C**) WST1 assay and (**D**) cell number counting. *p<0.05; N=3 per group.

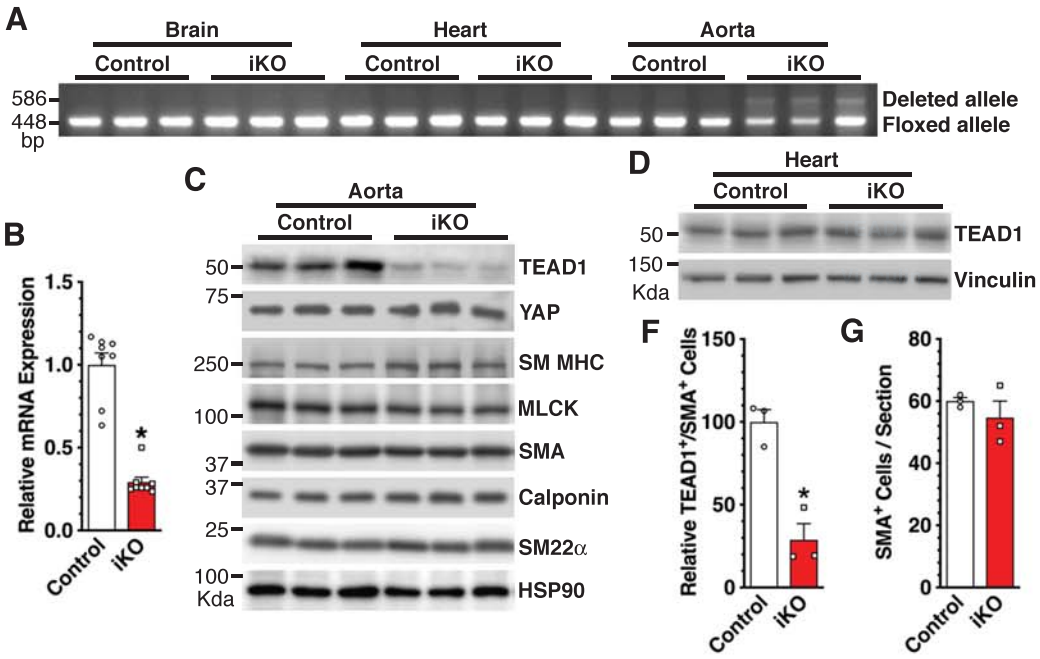
Online Figure I

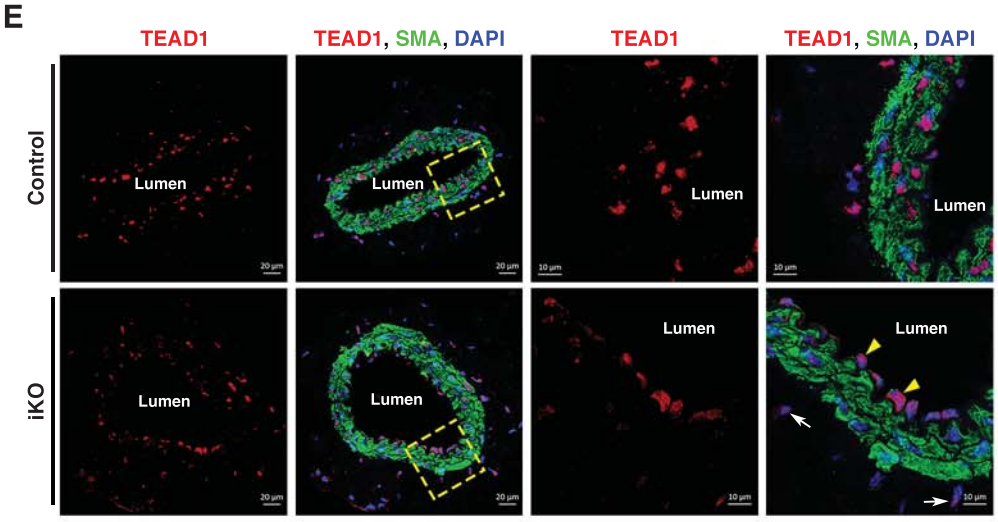


Online Figure II

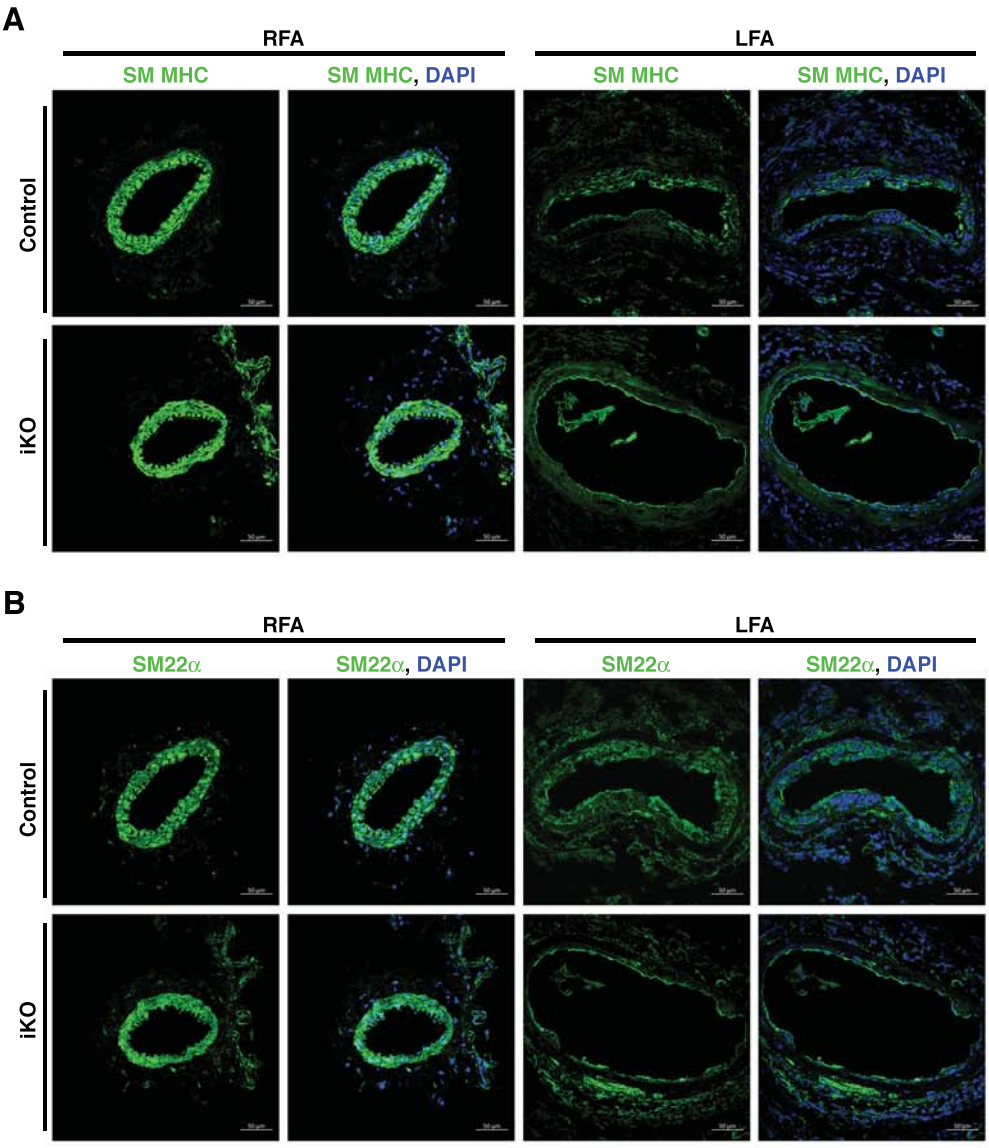


Online Figure III

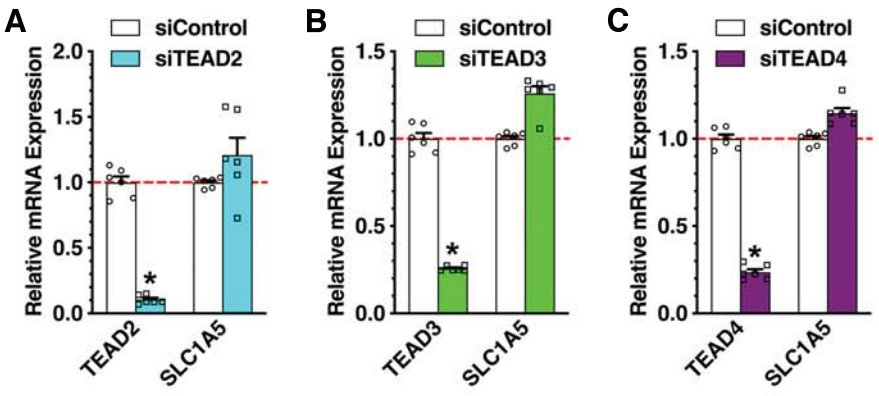




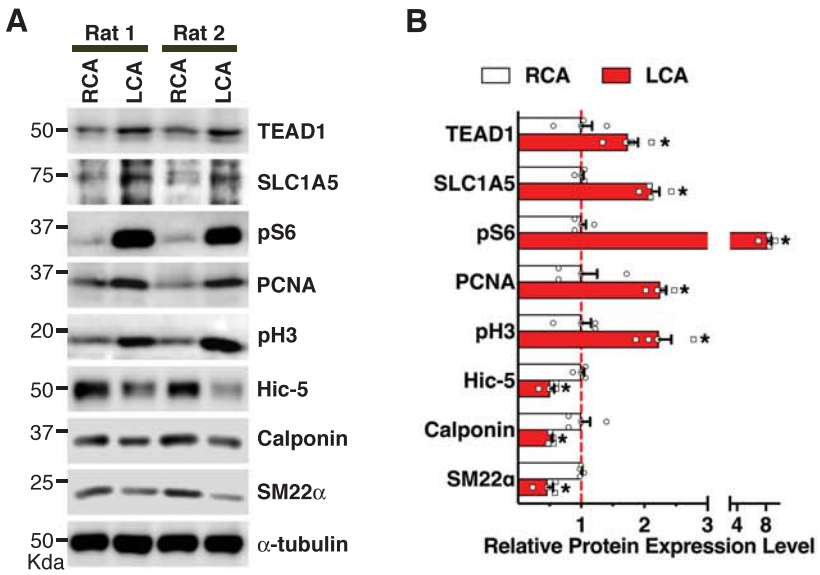
Online Figure IV



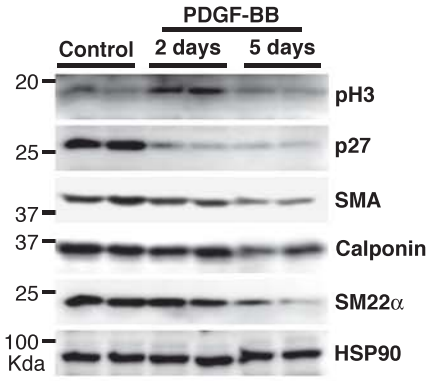
Online Figure V



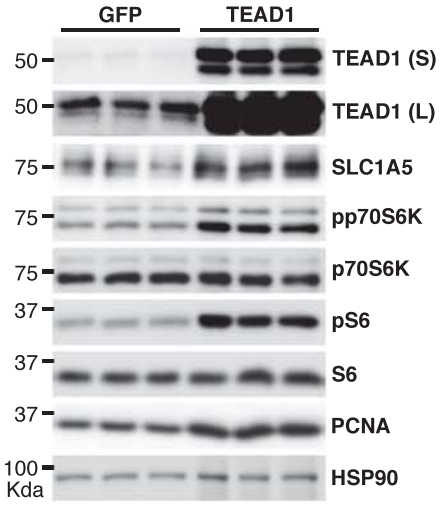
Online Figure VI



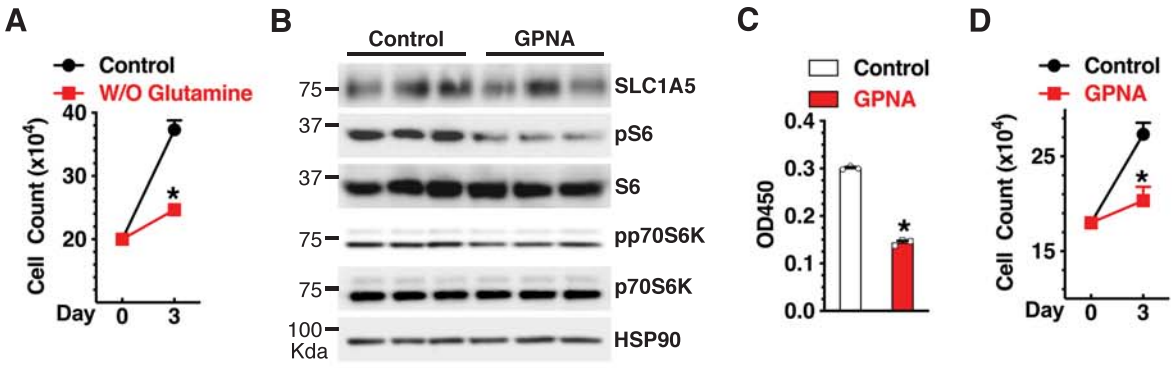
Online Figure VII



Online Figure VIII



Online Figure IX



Online Table II. List of oligonucleotides used in the study.

A. Primers used for quantitative RT-PCR (F: forward; R: reverse)

Genes	*Species*	*Sequence (5'-3')*
TEAD1	Mouse	F: AGCCAGATACATCAAACTCAGGACG
	Mouse	R: CTTAATGGCGGCTTGAATTTCTCGAAC
TEAD2	Mouse	F: GGGAAGACGAGAACGCGAAA
	Mouse	R: GGCCTTGTCCTTGGAGACTT
TEAD3	Mouse	F: ATACAGGTTCTAGCTCGGAAGAAGGT
	Mouse	R: CAGAGACGATTTGGGCAGACGACAT
TEAD4	Mouse	F: TTGAACGGGGGCCCTCTAAT
	Mouse	R: GGCTGGAGACCCCATAGAAG
SLC1A5	Mouse	F: CATCAACGACTCTGTTGTAGACC
	Mouse	R: CGCTGGATACAGGATTGCGG
PCNA	Mouse	F: CTCTGAGGTACCTGAACTTTTTCACA
	Mouse	R: ATACTTTAAGTGTCCCATGTCAGCAAT
SM α-actin	Mouse	F: ATGCTCCCAGGGCTGTTTTCCCAT
	Mouse	R: GTGGTGCCAGATCTTTTCCATGTCG
calponin	Mouse	F: AACTGGCACCAGCTGGAGAACATAG
	Mouse	R: GAGTGGACTGAACTTGTGTATGGTTG
HPRT	Mouse	F: TCTTTGCTGACCTGCTGGATTACA
	Mouse	R: AGTTGAGAGATCATCTCCACCAAT
TEAD1	Human	F: TTTGTGCAGCAGGCCTACCCCATC
	Human	R: GGCGAAGCTTGGTTGTGCCAATGGA
TEAD2	Human	F: TGAGCTTTTCCAGTTTTGGTCTG
	Human	R: TTCCACGAAGGCTGAGAACT
TEAD3	Human	F: ACCAGCACAATAGCGTCCAA
	Human	R: TGGAAGCTCTGCTCGATGTC
TEAD4	Human	F: TACCTCGAAGCCGTGGACAT
	Human	R: AGAGATCCTTGAGTCCACCC
SLC1A5 PS1	Human	F: AAGATCATCACCATCCTGGTCAC
	Human	R: CCAGAGCGTCACCTTCTACATT
SLC1A5 PS2	Human	F: GAGCTGCTTATCCGCTTCTTC
	Human	R: GGGGCGTACCACATGATCC
SLC7A5	Human	F: GGGAAGGGTGATGTGTCCAAT
	Human	R: GGTTGATCATTTCCTCTGTGACG
SLC38A1	Human	F: AGGAACTCAAAGAATTTGGGCTG
	Human	R: AAGTGGTGAGAGATTGCTGATGT
B2M	Human	F: GGGTTTCATCCATCCGACA
	Human	R: ACACGGCAGGCATACTCATC

B. Primers for cloning human SLC1A5 gene promoter to generate the luciferase reporter

Primers	*Sequence (5'-3')*
SLC1A5 Forward (KpnI)	AGGTACCCGGCCGTATTATCTCATTTGAACCCT
SLC1A5 Reverse (XhoI)	ACTCGAGAGATGGCGGAGGTCTGCAGGAGGCTA

C. Primers for mutation of the MCAT element in SLC1A5 gene promoter

Primers	*Sequence (5'-3')*
Forward	CCCTACCCTTATCCCTGGTCAACGTATATAAACGGGCTCAGAGCCACGC
Reverse	GCGTGGCTCTGAGCCCGTTTATATACGTTGACCAGGGATAAGGGTAGGG

D. Primers for quantitative ChIP assay

Primers	*Sequence (5'-3')*
Human SLC1A5 Promoter	F: GTCGGTCCCCGCCTTCTCAAGC
	R: TGTCCGGGAGTAGCGGTTACCAG
Human SLC1A5 Exon 8	F: CTCAATGTAGAAGGTGACGCTCTG
	R: GGCAGCTCACTCTTCACTTGTATC

E. siRNA duplexes

siRNA duplex	*Sequence (5'-3')*
Human TEAD1	F: GGACAUUCGUCAGAUUUAUtt
	R: AUAAAUCUGACGAAUGUCCac
Human TEAD2	F: CGAAGGAAAUCAAGGGAAAtt
	R: UUUCCCUUGAUUUCCUUCGgg
Human TEAD3	F: GAUUGCACGCUAUAUUAAAtt
	R: UUUAAUAUAGCGUGCAAUCaa
Human TEAD4	F: CCCAUGAUGUGAAGCCUUUtt
	R: AAAGGCUUCACAUCAUGGGac
Human SLC1A5	F: GGAUGUGGGUUUACUCUUUtt
	R: AAAGAGUAAACCCACAUCCtc

F. Primers for TEAD1 knockout mouse genotyping

Primers	*Sequence (5'-3')*
TEAD1 P1 Forward	TGCCATCATGCCAAGCTATACTGG
TEAD1 P2 Reverse	AAGGCAGACTCCTTCATTGGAATGGGTG
TEAD1 P3 Reverse	CCTGCTTTATAGTCACAGCAGAGGC