

Supplemental Figure Titles and Legends

Figure S1. Generation and Characterization of Human Induced Pluripotent Stem Cell-Derived Vascular Smooth Muscle Cells. Related to Figure 2.

(A) Schematic illustration of VSMC differentiation of hiPSCs using an embryoid body (EB)-based approach. Briefly, floating EBs were generated by resuspending EDTA (0.5 mM) dissociated single hiPSCs in media gradually transitioned from mTeSR1 to EB differentiation medium in a 6-day low attachment suspension culture. EBs were then seeded on a gelatin-coated culture dish for six days with EB differentiation medium. The adherent EB-derived cells were next cultured in SmGM-2 VSMC growth medium for 7-10 days, resulting in the production of hiPSC-VSMCs-P. hiPSC-VSMCs-M were derived by culturing hiPSC-VSMCs-P in a maturation medium containing 1% FBS and 1 ng/ml TGF- β 1 for seven days. (B) Approaches for deriving EBs from hiPSCs. Representative images of EBs derived from hiPSCs on day 0, 2 and 4 of differentiation, generated via either the current EDTA-mediated dissociation of hiPSC colonies coupled with a 4-day medium transition from the mTeSR to the EB differentiation medium, or the previous approach which included dispase-mediated dissociation of hiPSC colonies coupled with a 2-day medium transition. Scale bar: 200 μ m. (C) Immunostaining of VSMC (α -SMA, CNN1, and MYH11), ECM (COL1 and ELN) and pluripotency (OCT4) markers in hiPSC-VSMCs-P, hiPSC-VSMCs-M, primary VSMCs-P, primary VSMCs-M, and undifferentiated hiPSCs. DNA (nuclear) was counterstained by DAPI. Scale bar: 200 μ m. (D) The percentage of cells (hiPSC-VSMCs-P, hiPSC-VSMCs-M, primary VSMCs-P, primary VSMCs-M, and undifferentiated hiPSCs) positive for VSMC (α -SMA, CNN1, and MYH11), ECM (COL1 and ELN) and pluripotency (OCT4) markers from immunostaining (One-way ANOVA with Tukey's multiple comparisons test; Mean values and S.E.M indicated by the error bars are shown; n=3; ***: p<0.001; ****: p<0.0001; N.S: not significant). (E) Quantification of fluorescence intensity of α -SMA, CNN1, MYH11, COL1, ELN and OCT4 in hiPSC-VSMCs-P, hiPSC-VSMCs-M, primary VSMCs-P, primary VSMCs-M and undifferentiated hiPSCs. Three independent experiments were completed for each cell type, and 100 cells or above were quantified in each experiment. Values in y-axis represent fold changes of average fluorescence intensity (gray value) per cell relative to that of

undifferentiated hiPSCs (One-way ANOVA with Tukey's multiple comparisons test; Mean values and S.E.M indicated by the error bars are shown; n=3; *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001; N.S: not significant). (F) Contractility of hiPSC-VSMCs-P, primary VSMCs-P, hiPSC-VSMCs-M, and primary VSMCs-M in response to treatment (before and after 20 minutes) of 1 mM carbachol. Representative cells were indicated by the blue (basal) and red (contracted) lines before and after carbachol treatment, respectively. Scale bar: 200 μ m. (G) Quantification of reduced cell area of hiPSC-VSMCs and human primary VSMCs in proliferation and maturation medium in response to 1 mM carbachol or vehicle control (PBS) (One-way ANOVA with Tukey's multiple comparisons test; Mean values and S.E.M indicated by the error bars are shown; n=3; *: p < 0.05; **: p < 0.01; ****: p < 0.0001; N.S: not significant). (H) Schematic illustration of derivation of hiPSC-VSMCs via previously established approach (Gen 1 hiPSC-VSMCs), or currently optimized approach with expansion in SmGM medium (Gen 2 hiPSC-VSMCs-SmGM or hiPSC-VSMCs-P) or DMEM containing 10% FBS (Gen 2 hiPSC-VSMCs-10% FBS, or primed hiPSC-VSMCs). (I) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to monitor the proliferation of Gen 1 hiPSC-VSMCs, Gen 2 hiPSC-VSMCs-SmGM, or Gen 2 hiPSC-VSMCs-10% FBS (One-way ANOVA with Tukey's multiple comparisons test; Mean values and S.E.M indicated by the error bars are shown; n=3; ***: p<0.001; ****: p<0.0001; N.S: not significant). (J) qRT-PCR analysis of relative mRNA transcript amounts of VSMC (α -SMA, *CNN1*, *MYH11*, and *SMTH*) and ECM (*COL1*, *COL3*, and *ELN*) markers in Gen 1 hiPSC-VSMCs, Gen 2 hiPSC-VSMCs-SmGM, Gen 2 hiPSC-VSMCs-10% FBS, or the control HUVECs for seven days. Values in the y axis represent fold changes relative to human GAPDH expression. Gene expression in hiPSC-VSMCs was normalized to that of in HUVECs (One-way ANOVA with Tukey's multiple comparisons test; Mean values and S.E.M indicated by the error bars are shown; n=4; *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001; N.S: not significant). (K) MTT assay indicating the proliferation capability of neuroectodermal (NE), lateral plate mesodermal (LM) and paraxial mesodermal (PM) hiPSC-VSMCs and hiPSC-VSMCs derived from EB-based approach (One-way ANOVA with Tukey's multiple comparisons test; Mean values and S.E.M indicated by the error bars are shown; n=3; **: p<0.01; ****: p<0.0001). (L) Quantification of fluorescence intensity of α -SMA and MYH11 in hiPSC-VSMCs in engineered vascular tissues

cultured in different TEVG media (T/P, T/-, -/P, and -/-). Values in the y axis represent fold changes of average fluorescence intensity (gray value) per cell relative to that of hiPSC-VSMCs in vascular tissue cultured in -/- medium. Three independent vascular tissue constructs were generated and immunostained, and at least 90 cells were analyzed for each group in each batch (One-way ANOVA with Tukey's multiple comparisons test; Mean values and S.E.M indicated by the error bars are shown; n=3; *: p<0.05; **: p<0.01; ***: p<0.001; N.S: not significant). **(M)** Contractility of hiPSC-VSMCs (primed) in the TGF- β 1-containing TEVG medium (T/-) in response to the treatment (before and after 20 minutes) of 1 mM carbachol. Representative cells were indicated by the blue (basal) and red (contracted) lines before and after carbachol treatment, respectively. Scale bar: 200 μ m. **(N)** Quantification of reduced cell area of hiPSC-VSMCs (primed) in TEVG medium (T/-) in response to 1 mM carbachol or vehicle control (PBS) (Two-tailed unpaired Student's T-test; Mean values and S.E.M indicated by the error bars are shown; n=3; ***: p <0.001).

Figure S2. Characterization of hiPSC-VSMCs Cultured under Static or Stretched Condition in the Previously Reported or Currently Optimized TEVG Medium. Related to Figures 2 and 3.

(A) qRT-PCR analysis of relative mRNA transcript amounts of VSMC (α -SMA, *CNN1*, and *MYH11*), extracellular matrix (*COL1*, *COL3*, and *ELN*), focal adhesion (vinculin), adherens junction (N-cadherin), and metabolism-associated (*GLUT1*, *GLUT4*, *CS*, and *PGC1 α*) markers in hiPSC-VSMCs, which were cultured in the previously reported TEVG medium (T/P) or currently optimized TEVG medium (T/-) under static or stretched (uniaxial cyclic stretching: 2.5% distention at 2.75 Hz) conditions with a FlexCell FX-6000T™ Tension System for 2 days, respectively. Y-axis values represent fold changes relative to human GAPDH expression. The gene expression in hiPSC-VSMCs of experimental groups is normalized to that of hiPSC-VSMCs cultured in the absence of stretching with the previously reported TEVG medium (static, T/P). Interaction between the effect of medium and mechanical treatment on gene expression was evaluated via two-way ANOVA with Tukey's multiple comparisons test to assess differences among independent groups. Gene expression was synergistically upregulated by optimized culture medium (T/-) and stretching

for expression of α -SMA (interaction $p=0.0005$), *CNN1* (interaction $p=0.001$), *COL1* (interaction $p=0.01$), and *COL3* (interaction $p=0.04$). Expression of *ELN* was independently upregulated by optimized culture medium (T/-) and stretching ($p=0.003$ for culture medium and $p<0.0001$ for stretching; interaction between factors was not significant, $p=0.2$). Additionally, stretching alone increased the expression of vinculin, N-cadherin, *GLUT1*, *GLUT4*, *CS*, and *PGC1 α* independent of culture medium utilized. Mean values and S.E.M indicated by the error bars are shown; $n=3$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$; N.S: not significant. **(B)** Immunostaining of ECM markers (collagen type 1 and elastin), filamentous actin (phalloidin), and cell-cell adhesion marker (N-cadherin) in hiPSC-VSMCs cultured in the previously reported (T/P) or currently optimized (T/-) TEVG medium under static or stretched conditions. White double arrows show stretch directionality. DNA (nuclear) was counterstained by DAPI. Scale bar: 200 μ m. **(C)** Quantification of collagen type I, elastin, phalloidin, and N-cadherin staining intensity relative to cell number in hiPSC-VSMCs under static or stretched conditions with the previously reported (T/P) or currently optimized (T/-) TEVG medium. Values in the y axis represent fold change of fluorescence intensity (gray value) per cell relative to that of cells cultured in the T/P medium under a static condition (static, T/P). Two-way ANOVA showed that optimized culture medium (T/-) and mechanical stretching synergistically upregulated the expression of collagen I (interaction $p<0.0001$) and filamentous actin bundles (phalloidin) (interaction $p=0.03$). Expression of *ELN* was upregulated by optimized culture medium (T/-) and stretching independently. Additionally, stretching alone increased the expression of N-cadherin independent of culture medium utilized. Three independent experiments were completed for the experimental groups, and 100 cells or above were quantified for each group in each experiment. Mean values and S.E.M indicated by the error bars are shown; $n=3$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$; N.S: not significant. **(D)** Collagen weight per hiPSC-VSMC cultured in the previously reported (T/P) or currently optimized (T/-) TEVG medium under static or stretched conditions via a hydroxyproline assay. Two-way ANOVA showed that optimized culture medium (T/-) and mechanical stretching promoted collagen deposition independently. Mean values and S.E.M indicated by the error bars are shown; $n=3$; *: $p<0.05$; ****: $p<0.0001$. **(E)** Glucose consumption rates of hiPSC-VSMCs cultured in the previously reported (T/P) or

currently optimized (T/-) TEVG medium under static or stretched conditions. Two-way ANOVA showed that stretching alone increased glucose consumption independent of culture medium utilized. Mean values and S.E.M indicated by the error bars are shown; n=3; ***: p<0.001. **(F)** Cellular ATP concentration of hiPSC-VSMCs cultured in the previously reported (T/P) or currently optimized (T/-) TEVG medium under static or stretched conditions. Two-way ANOVA showed that optimized culture medium (T/-) and mechanical stretching synergistically increased cellular ATP production (interaction p=0.01). Mean values and S.E.M indicated by the error bars are shown; n=3; **: p<0.01, ***: p<0.001, ****: p<0.0001; N.S: not significant.

Figure S3. Histological Characterization and Physical Properties of Human Umbilical Arteries, Porcine Coronary Arteries and hiPSC-TEVGs. Related to Figures 3 and 4.

(A-D) Histological examination (H&E staining [A], Masson's trichrome staining [B], Alizarin Red staining [C] and EVG staining [D]) of HUAs were performed. Asterisk indicates the lumen of the graft. Scale bar: 100 μ m. **(E-H)** Immunostaining of sections of HUAs **(E-G)**. Sections were stained for VSMC markers α -SMA, CNN1, and MYH11. DNA (nuclear) was counterstained by DAPI. Asterisk indicates the lumen of the graft. Scale bar: 100 μ m; **(H)** Quantification of fluorescence intensity (gray value) of α -SMA, CNN1, and MYH11 per cell in three representative sections (>100 cells/section) in each HUA (Mean values and S.E.M indicated by the error bars are shown; n=3). **(I-J)** Immunostaining of KI67 in HUAs **(I)**. DNA (nuclear) was counterstained by DAPI. Asterisk indicates the lumen of the graft. Scale bar: 100 μ m; **(J)** Quantification of percentage of KI67-positive cells from three representative sections (>100 cells/section) in each HUA (Mean values and S.E.M indicated by the error bars are shown; n=3). **(K-O)** A representative photograph of porcine coronary arteries **(K)**, a representative stress-strain plot **(L)**, and mechanical parameters, including maximum modulus **(M)**, ultimate tensile stress **(N)** and failure strain **(O)** of the tissue rings sectioned from porcine coronary arteries (Mean values and S.E.M indicated by the error bars are shown in Panel M-O; Dots indicate the values for individual porcine coronary arteries; n=3). **(P)** Representative photograph of a tissue ring sectioned from a hiPSC-TEVG mounted onto micromanipulators and immersed in the temperature-controlled perfusion bath containing Tyrode's solution for contractility assay. Green arrows indicate the tissue ring, and red arrows

indicate the arm of the force transducer. **(Q)** Contractility changes (Pascal) of tissue rings sectioned from pre-implant or explanted hiPSC-TEVGs in response to the vasoconstrictor carbachol (1mM) for 30 min (Two-tailed unpaired Student's T-test; Mean values and S.E.M indicated by the error bars are shown; n=3; N.S: not significant). **(R)** The percentage of human cells (HLA-A+ cells) positive for VSMC markers (α -SMA, CNN1 or MYH11) in hiPSC-TEVGs 30 days after implantation. The sections were co-stained for HLA-A and α -SMA, CNN1, or MYH11 from three representative sections (>80 cells/section) in each hiPSC-TEVG (Mean values and S.E.M indicated by the error bars are shown; n=3).

Figure S4. Generation of Engineered Tissue Using a Gene-Edited hiPSC Line and Characterization of hiPSC-TEVGs in a Nude Rat Model 60 Days Post-Operation. Related to Figure 4.

(A) Schematic illustration of the strategy of generating HLA-C-retained hiPSCs (Xu et al., 2019). **(B)** Immunostaining of VSMC (α -SMA, CNN1, and MYH11), ECM (COL1 and ELN), pluripotency (OCT4), and human surface antigen (HLA-A) markers in HLA-C-retained hiPSC-VSMCs in proliferation medium (HLA-C-retained hiPSC-VSMCs-P) or maturation medium (HLA-C-retained hiPSC-VSMCs-M). Wild type hiPSC-VSMCs were stained for HLA-A as positive control. DNA (nuclear) was counterstained by DAPI. Scale bar: 200 μ m. **(C)** Contractility of HLA-C-retained hiPSC-VSMCs in proliferation medium (HLA-C-retained hiPSC-VSMCs-P) or maturation medium (HLA-C-retained hiPSC-VSMCs-M) in response to treatment (before and after 20 minutes) of 1 mM carbachol. Representative cells were indicated by the blue (basal) and red (contracted) lines before and after carbachol treatment, respectively. Scale bar: 200 μ m. **(D)** Quantification of reduced cell area of HLA-C-retained hiPSC-VSMCs in proliferation medium (HLA-C-retained hiPSC-VSMCs-P) or maturation medium (HLA-C-retained hiPSC-VSMCs-M) in response to 1 mM carbachol or vehicle control (PBS) (One-way ANOVA with Tukey's multiple comparisons test; Mean values and S.E.M indicated by the error bars are shown; n=3; *: p<0.05; ***: p<0.001). **(E-H)** H&E staining (**E** and **F**) and Masson's Trichrome (**G** and **H**) staining of the engineered tissues derived from "primed" HLA-C-retained hiPSC-VSMCs seeded onto PGA scaffolds and cultured for three weeks. Green (**F**) and red (**H**) arrow heads indicate PGA remnants. Scale bars: 500 (**E** and

G) and 100 (**F** and **H**) μm . (**I**) Immunostaining of the engineered tissues from hiPSC-VSMCs. Section of engineered tissues derived from HLA-C-retained hiPSC-VSMCs was stained for α -SMA and MYH11. DNA (nuclear) was counterstained by DAPI. White arrow heads indicate PGA remnants. Scale bar: 100 μm . (**J**) Collagen weight per mesh of engineered tissue generated using “primed” HLA-C-retained or wild type hiPSC-VSMCs via hydroxyproline assay (Two-tailed unpaired Student's T-test; Mean values and S.E.M indicated by the error bars are shown; n=3; N.S: not significant). (**K**) A representative image of an explanted TEVG graft on day 60 post-implantation. The dashed line indicates the position of sectioning. (**L-O**) Histological analysis (H&E staining [**L**], Masson's trichrome staining [**M**], Alizarin Red staining [**N**] and EVG staining [**O**]) of an explanted TEVG graft on day 60 post-implantation. Asterisk indicates the lumen of the graft. The dashed line suggests the boundary between the human cells and host rat cells [**O**]. Green arrows indicate limited, discontinuous, extracellular elastin fibers in the medial layers of hiPSC-TEVGs. Also note the existence of immature, disorganized ELN fibers below the dashed line potentially generated by rat host cells. Scale bar: 100 μm . (**P** and **Q**) Immunostaining of sections of explanted TEVG graft on day 60 post-implantation. The section was stained for VSMC marker α -SMA (**P**, **P'**, **P''**), MYH11 (**Q**, **Q'**, **Q''**) and human surface antigen HLA-A (green pseudo-color). DNA (nuclear) was counterstained by DAPI. Asterisk indicates the lumen of the graft. The white dashed line indicates the boundary between the human cells and host rat cells. Scale bar: 100 μm .

Figure S1

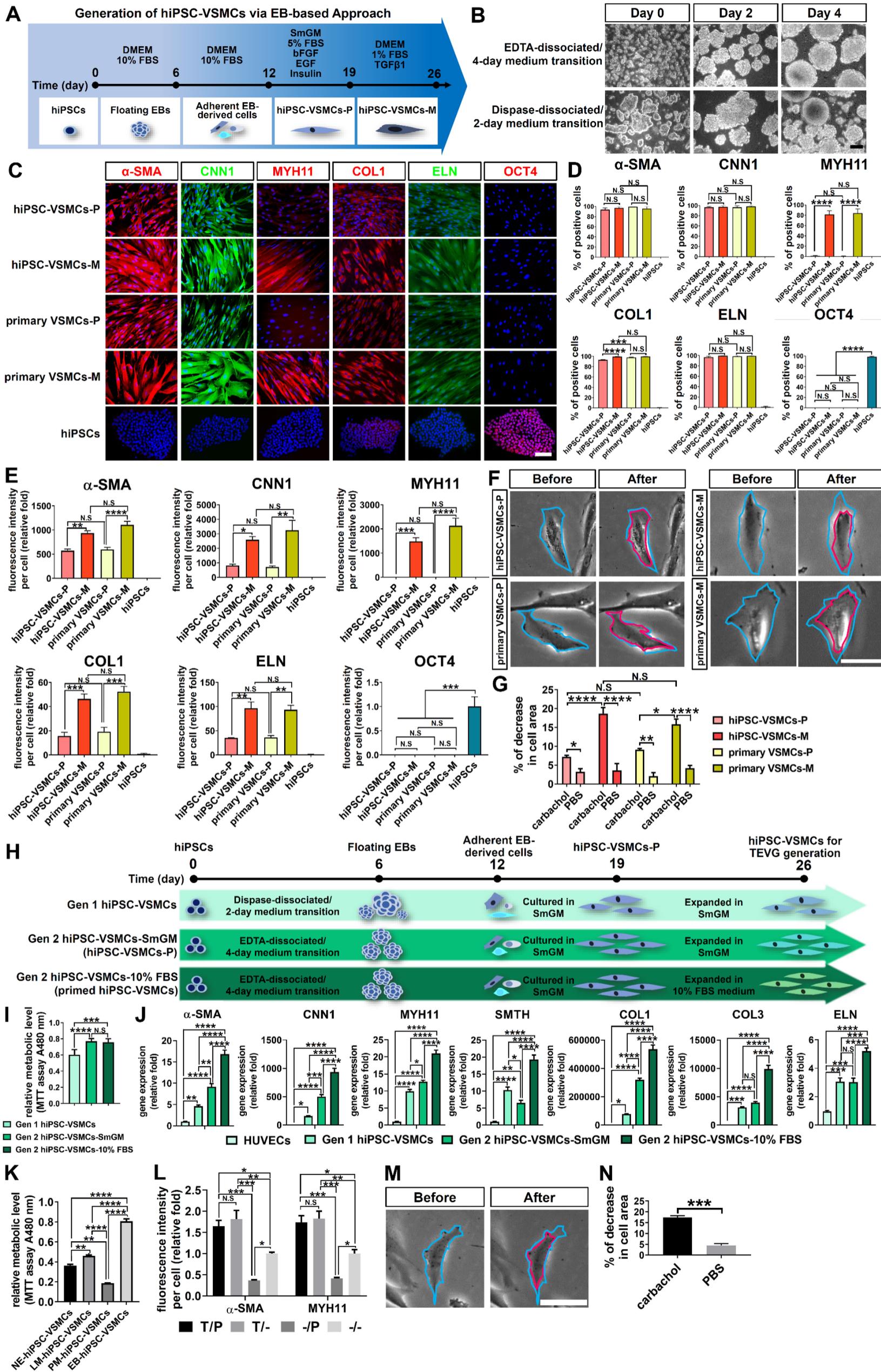


Figure S2

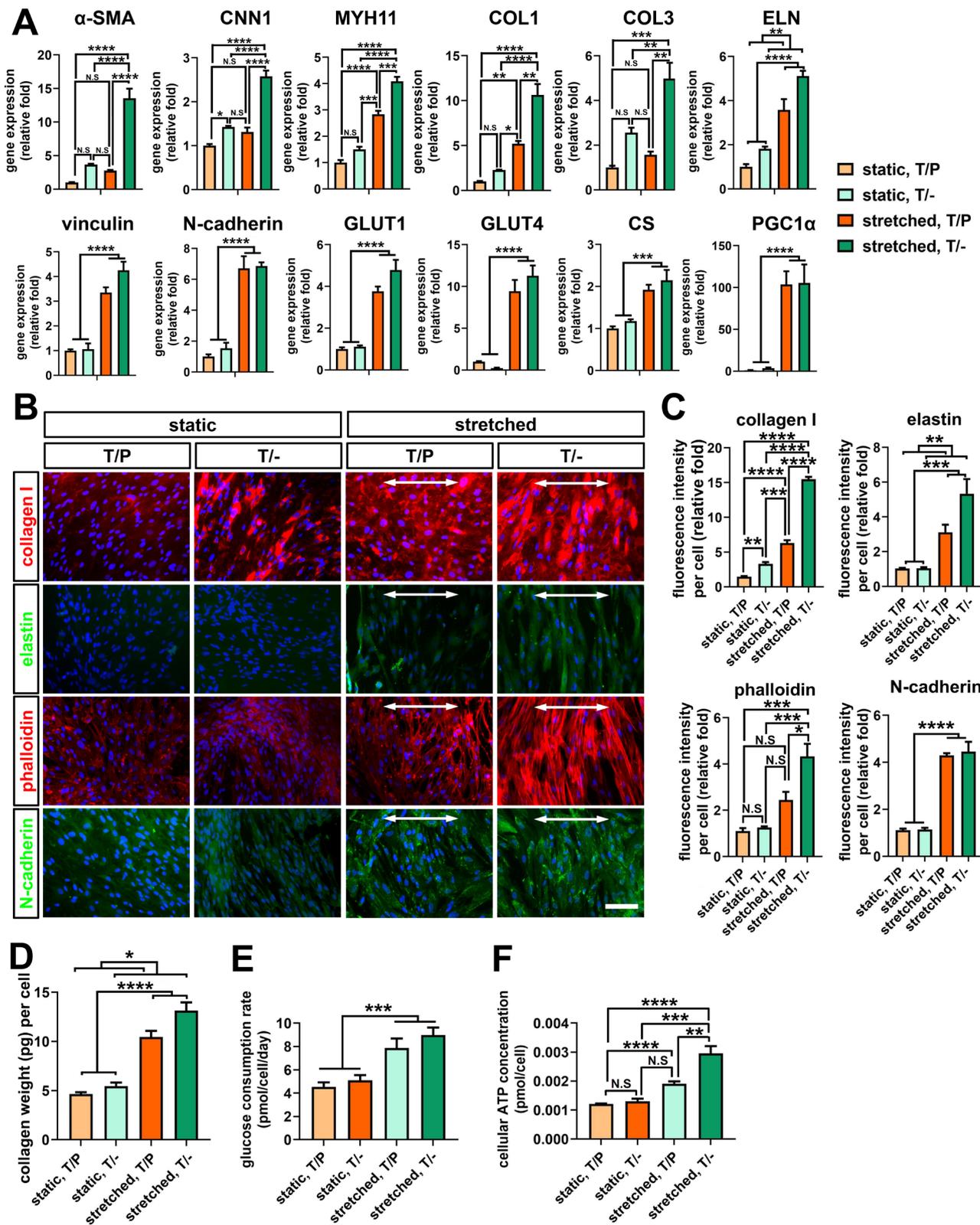


Figure S3

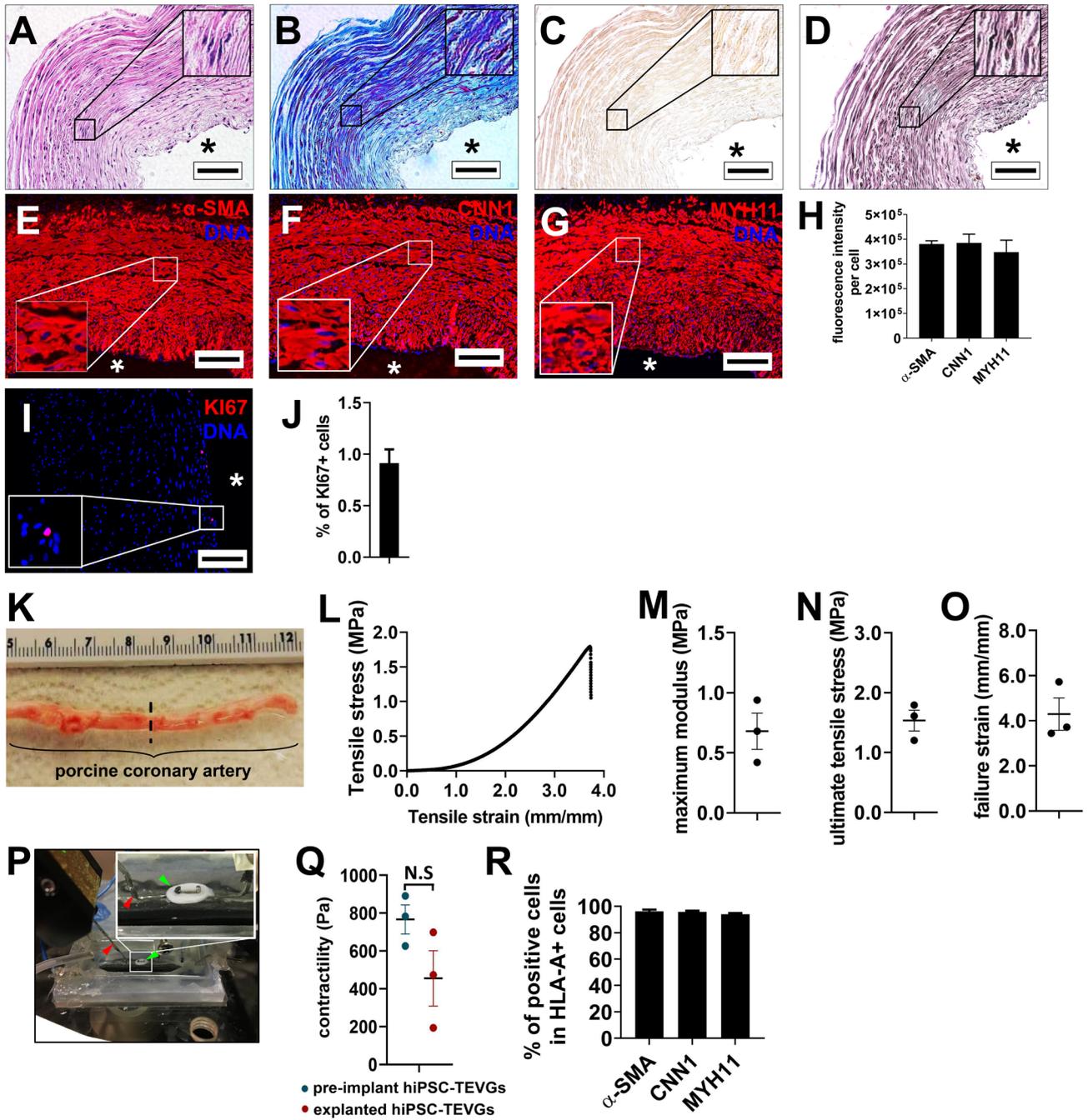


Figure S4

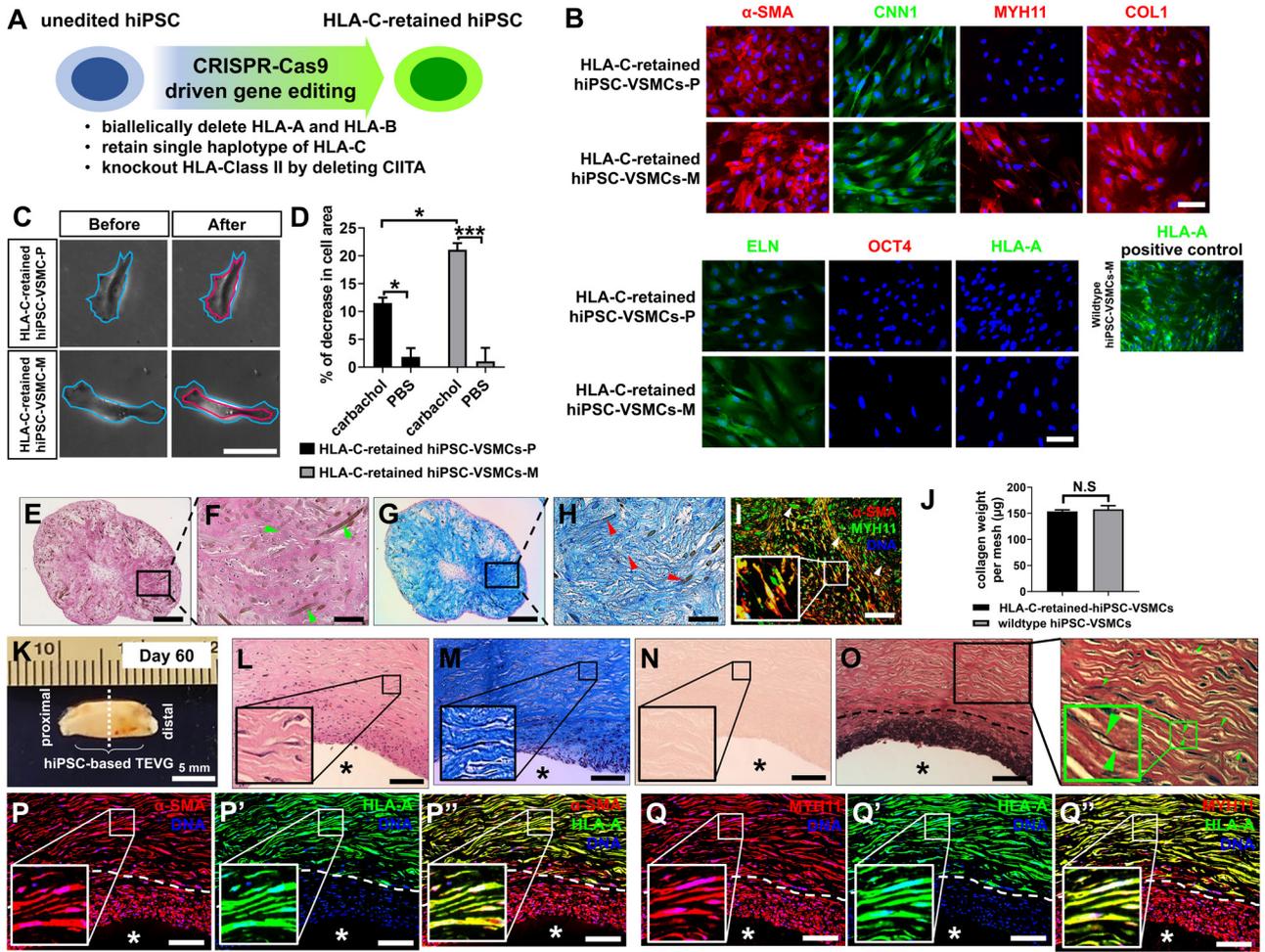


Table S1. List of Primers for qRT-PCR. Related to “Quantitative Reverse Transcription PCR” in the STAR Methods.

Gene	Primer sequence
GAPDH-F	TGTTGCCATCAATGACCCCTT
GAPDH-R	CTCCACGACGTACTIONCAGCG
α -SMA-F	CTGGGACGACATGGAAAA
α -SMA-R	ACATGGCTGGGACATTGA
CNN1-F	AGCATGGCGAAGACGAAAGGAA
CNN1-R	CCCATCTGCAGGCTGACATTGA
MYH11-F	AGAGACAGCTTCACGAGTATGAG
MYH11-R	CTTCCAGCTCTCTTTGAAAGTC
SMTH-F	CCTGGATACAGAGGACATGG
SMTH-R	CAGGTGGTTGTAGAGCGACT
COL1-F	CCTGTCTGCTTCCTGTAAACTC
COL1-R	G TTCAGTTTGGGTTGCTTGTC
COL3-F	GCTCTGCTTCATCCCACTATTA
COL3-R	CTGGCTTCCAGACATCTCTATC
ELN-F	AAGATGGTGCAGACACTTCC
ELN-R	AGAGCGAATCCAGCTTTGAG
vinculin-F	AATGGTCCAGCAAGGGCAAT
vinculin-R	GAATGAGTGCCCGCTTGGA
N-cadherin-F	GGGAAATGGAACTTGATGGCA
N-cadherin-R	CAGTTGCTAACTTCACTGAAAGGA
GLUT1-F	TGGCATCAACGCTGTCTTCT
GLUT1-R	AGCCAATGGTGGCATAACA
GLUT4-F	GGTTCTTTCATCTTCGCCGC
GLUT4-R	TCCCATCTTCGGAGCCTAT

CS-F	CTCAACTCAGGACGGGTTGT
CS-R	GGGGTCATTAGGCAGGTGTT
PGC1 α -F	CTACGGCTCCTCCTGGGAAA
PGC1 α -R	CAGTCCAGGGGCAGAAAAGT