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Supplemental Information

Human Pluripotent Stem Cell-Derived Multipotent Vascular Progenitors of the Mesothelium Lineage Have Utility in Tissue Engineering and Repair

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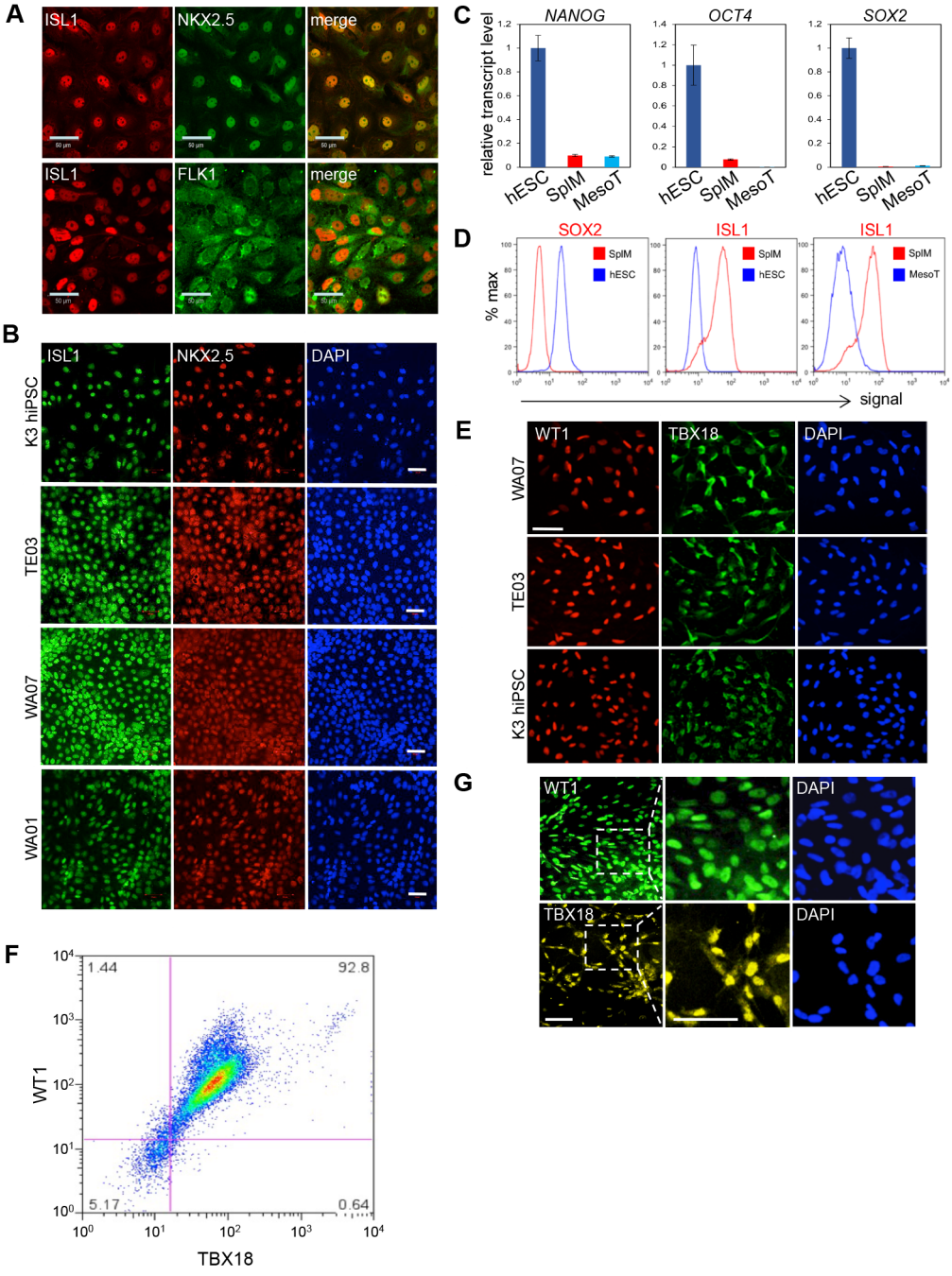
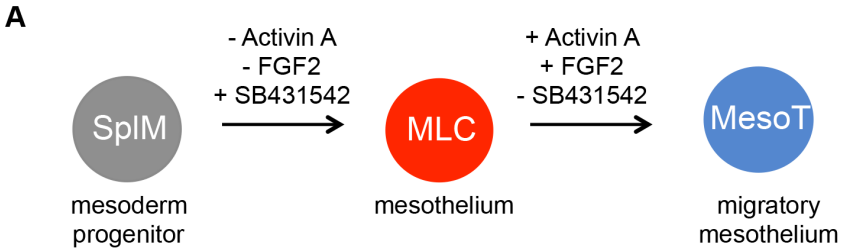
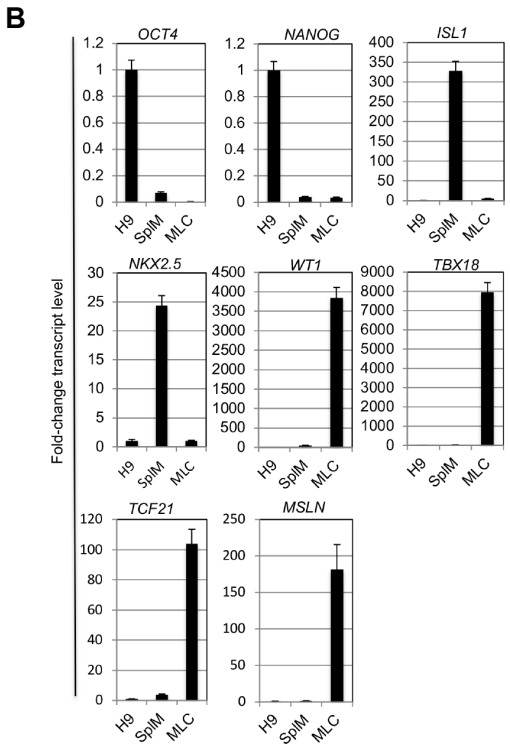

A
ISL1
NKX2.5
merge
ISL1
FLK1
merge
B
ISL1
NKX2.5
DAPI
K3 hiPSC
TE03
WA07
WA01
C
NANOG
OCT4
SOX2
relative transcript level
hESC
SplM
MesoT
D
SOX2
ISL1
ISL1
SplM
hESC
MesoT
% max
signal
E
WT1
TBX18
DAPI
WA07
TE03
K3 hiPSC
F
1.44
92.8
5.17
0.64
WT1
TBX18
G
WT1
DAPI
TBX18
DAPI

Figure S1. Related to Figure 1.
(A) hESC-derived (WA09) splanchnic mesoderm (SplM) cells generated after 4 days of culture in CDM supplemented with Wnt3a (25 ng/ml) and BMP4 (100 ng/ml) were fixed and stained with antibodies for ISL1, NKX2.5 and FLK1. Scale bars, 50 μm.
(B) Immunofluorescence analysis of K3 hiPSCs and hESCs (TE03, WA07 and WA01) cultured and stained as in **(A)**. Nuclei were counter stained with DAPI. Scale bars, 50 μm.
(C) qRT-PCR data showing fold-change of transcript levels for pluripotency markers (*NANOG*, *OCT4* and *SOX2*) in SplM and MesoTs relative to hESCs. TaqMan assays for each transcript were performed in technical triplicate and fold-change shown relative to hESCs (WA09) after normalization with 18S RNA.
(D) Flow cytometry data of untreated hESCs (WA09), SplM, and MesoT showing the absence of the pluripotent marker SOX2 (left plot) and presence of lineage specific marker ISL1 (middle plot) in SplM. As cells transition to MesoT, ISL1 is downregulated (right plot).
(E) Immunofluorescence of WA07, TE03 and K3 hiPSC lines after differentiation of SplM to MesoT followed by probing with WT1 and TBX18 antibodies. Nuclei were counter stained with DAPI. Scale bar, 50 μm.
(F) Flow cytometry pseudocolor plot of MesoT cells probed with antibodies for WT1 and TBX18.
(G) WA09-derived MesoT cells were fixed and probed with antibodies for lineage specific markers WT1 and TBX18. Nuclei were counterstained with DAPI. Right hand side is a magnification of the insets from left. Scale bars, 50 μm.
Error bars ± standard deviation.


A
- Activin A
- FGF2
+ SB431542
+ Activin A
+ FGF2
- SB431542
SpIM
MLC
MesoT
mesoderm progenitor
mesothelium
migratory mesothelium


B
Fold-change transcript level
OCT4
NANOG
ISL1
NKX2.5
WT1
TBX18
TCF21
MSLN
H9
SpIM
MLC

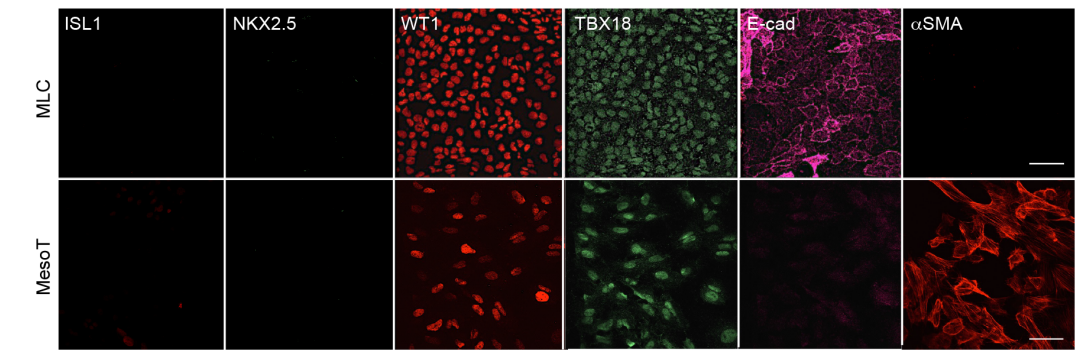

C
ISL1
NKX2.5
WT1
TBX18
E-cad
αSMA
MLC
MesoT

Figure S2. Related to Figure 1.

(A) Schematic showing the progression of splanchnic mesoderm (SplM) to mesothelium-like cells (MLCs) and then MesoTs. Removal and addition of growth factors and inhibitors are indicated above the arrows for each stage.

(B) qRT-PCR data showing fold-change of transcript levels for pluripotency (*OCT4, NANOG*), SplM (*ISL1* and *NKX2.5*) and mesothelium (*WT1, TBX18*, *TCF21 and MSLN*). TaqMan assays for each transcript were performed in technical triplicate and fold-change shown relative to untreated hESCs (WA09) after normalization with 18S RNA.

(C) Immunofluorescence analysis of MLCs and MesoTs directly derived from MLCs. After EMT induction of MLCs **(A)**, cells become migratory but retain expression of mesothelial lineage markers. Cells were fixed and stained with antibodies against ISL1, NKX2.5, WT1, TBX18, E-cadherin (epithelial marker) and alpha smooth muscle actin (αSMA). Scale bars, 50 μm. Error bars ± standard deviation.

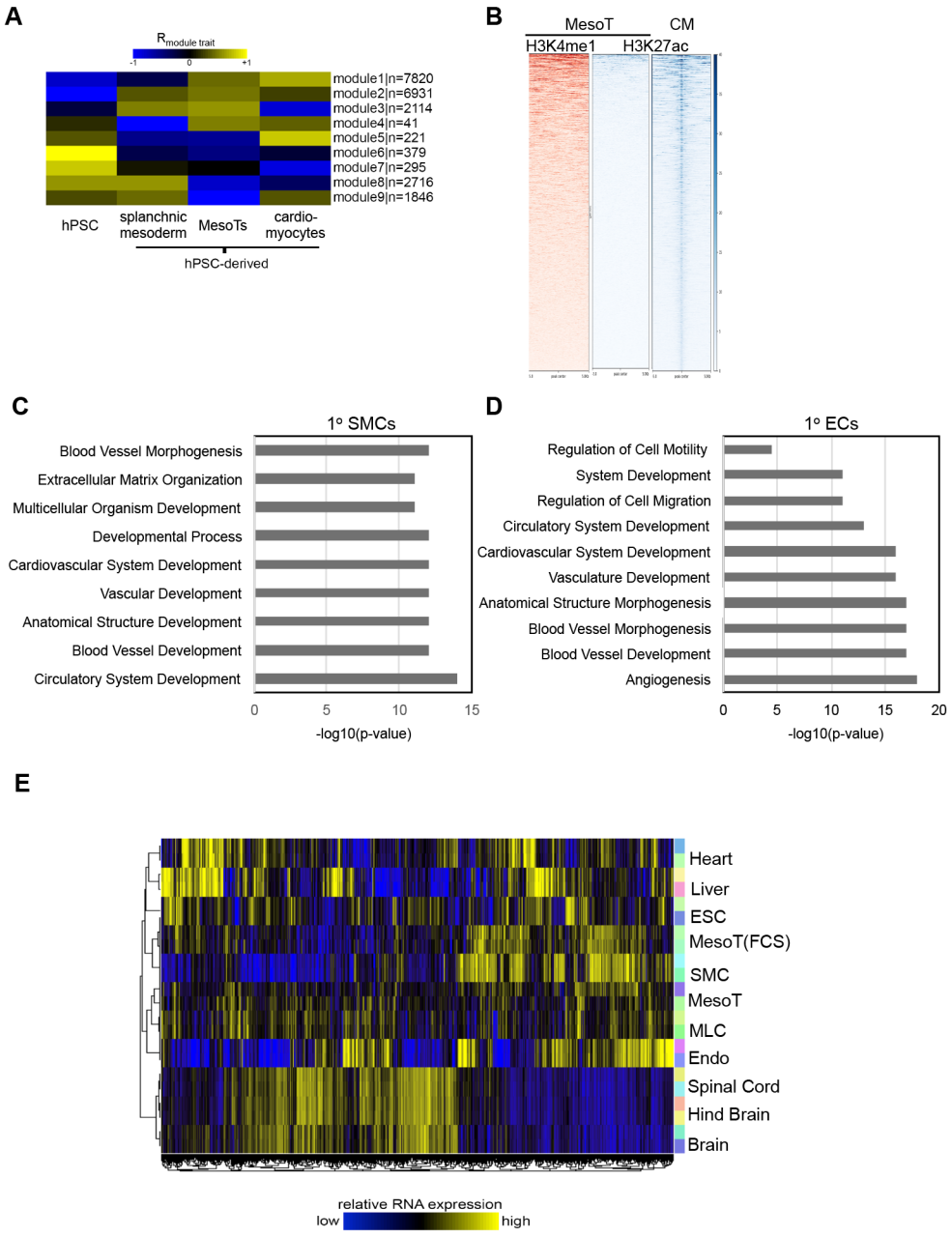


Figure S3. Related to Figure 2.

(A) Heatmap showing the relationship between cell type-specific DNA methylation modules (WA09 hPSCs, hPSC-derived splanchnic mesoderm, MesoTs and hPSC-derived cardiomyocytes). Module 9 comprises 1846 cytosines and is characteristic of MesoTs.

(B) Heatmap showing highly enriched H3K27ac (blue) lineage specific sites in hESC-derived cardiomyocytes (CM) and the absence of H3K4me1 in corresponding sites for MesoT.

(C and D) Gene Ontology analysis of genes analyzed in **Figure 2D**.

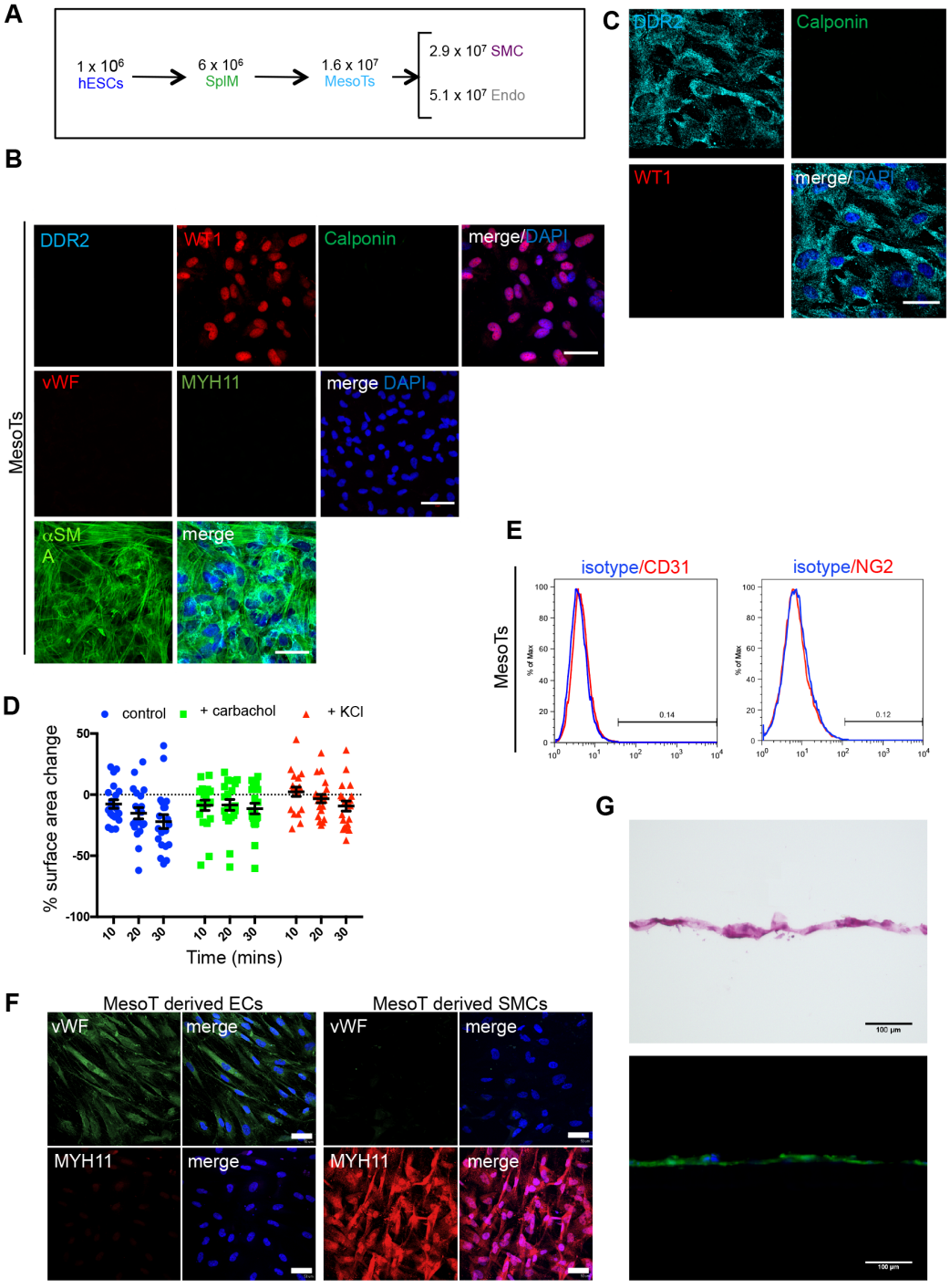

A
1 x 10^6 hESCs
6 x 10^6 SplM
1.6 x 10^7 MesoTs
2.9 x 10^7 SMC
5.1 x 10^7 Endo
B
MesoTs
DDR2
WT1
Calponin
merge/DAPI
vWF
MYH11
merge DAPI
αSMA
merge
C
DDR2
Calponin
WT1
merge/DAPI
D
control
+ carbachol
+ KCl
% surface area change
Time (mins)
E
MesoTs
isotype/CD31
isotype/NG2
% of Max
0.14
0.12
F
MesoT derived ECs
MesoT derived SMCs
vWF
merge
MYH11
G
100 µm

Figure S4. Related to Figures 3 and 4.
(A) Cells numbers at different stages of differentiation are shown. Cell number was counted at each stage after plating 1 million hESCs (WA09).
(B) Immunofluorescence of MesoT cells probed with lineage specific antibodies for mesothelium (WT1), smooth muscle (calponin, MYH11), endothelium (vWF), or fibroblasts (DDR2). Nuclei were counterstained with DAPI. Scale bar, 50 μm.
(C) MesoT-derived Fibroblasts on day 12 were probed with antibodies for WT1, DDR2, calponin and counterstained with DAPI. Scale bar, 50 μm.
(D) Contraction assays as for Figure 3 except performed on WA09 hESCs. Contraction is shown as the % change in cell surface area for individual cells. Each treatment group was compared to corresponding control time point to determine statistical significance. N=20.
(E) Flow cytometry histograms of MesoT cells probed with antibodies for CD31 (endothelium), NG2 (pericyte), and isotype control.
(F) Immunofluorescence of MesoT (FBS) cells after culturing with 2% FBS +VEGF (endothelium) or +PDGF-BB (SMC). Cells were fixed and probed with antibodies against vWF or MYH11 and counter stained with DAPI. Scale bars, 50 μm.
(G) Magnified images shown in Figure 3J. Scale bar, 100 μm.

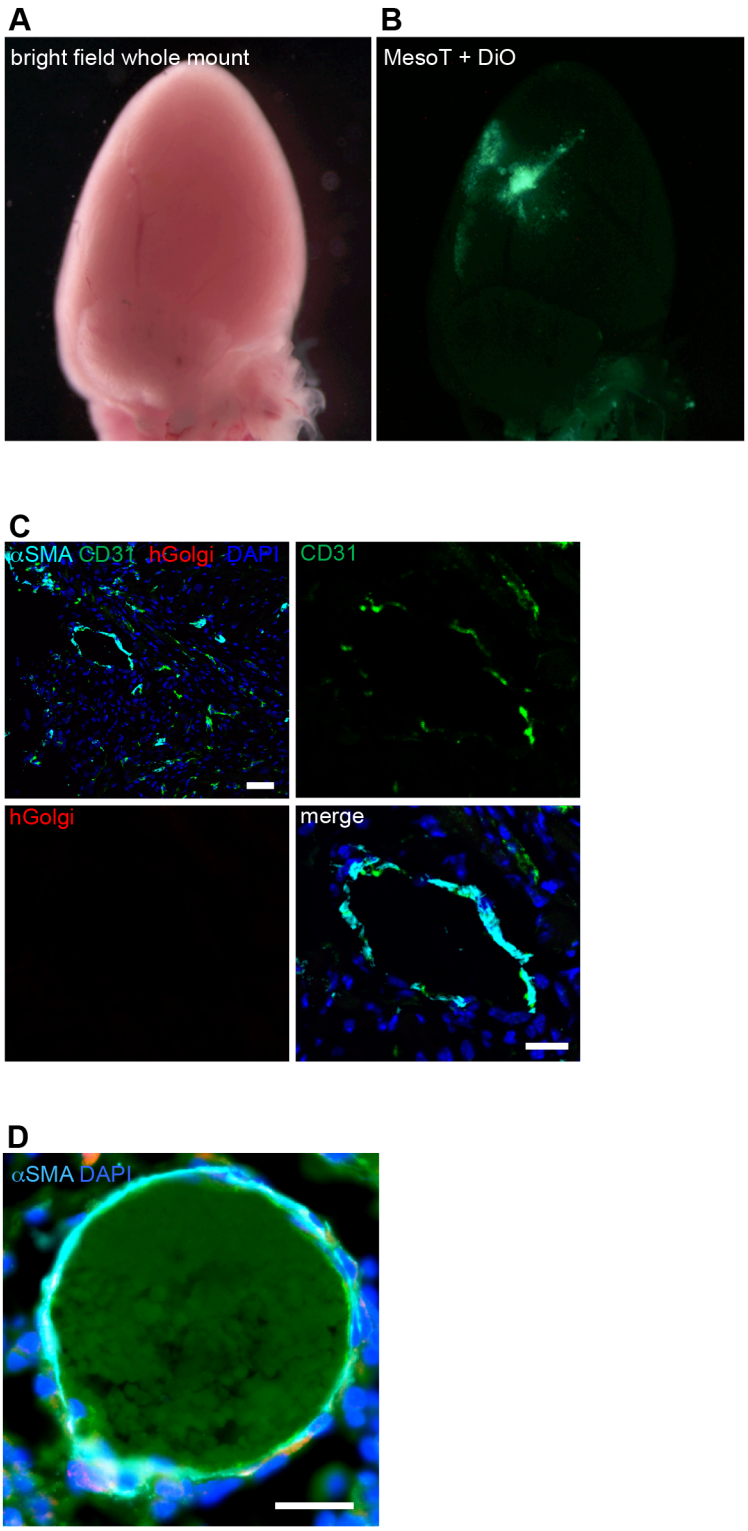

A
bright field whole mount
B
MesoT + DiO
C
αSMA CD31 hGolgi DAPI
CD31
hGolgi
merge
D
αSMA DAPI

Figure S5. Related to Figure 5.
(A) Whole mount image of a mechanically injured heart 5 days after resecting part of the ventricle.
(B) DiO labeled MesoT human cells (green) were applied immediately after resection and attached to injured area. Micron bar, 1 mm.
(C) Immunohistochemistry image showing the absence of $hGolgi^{+}$ cells in the repair zone of neonatal hearts that did not receive MesoT cells following mechanical injury. The section was also probed with antibodies for a-smooth muscle actin (aSMA), CD31 and counterstained with DAPI. Scale bar, 50 µm.
(D) Blood vessel from **Figure 5D** showing the presence of erythrocytes due to autofluorescence. Scale bar, 20 µm.

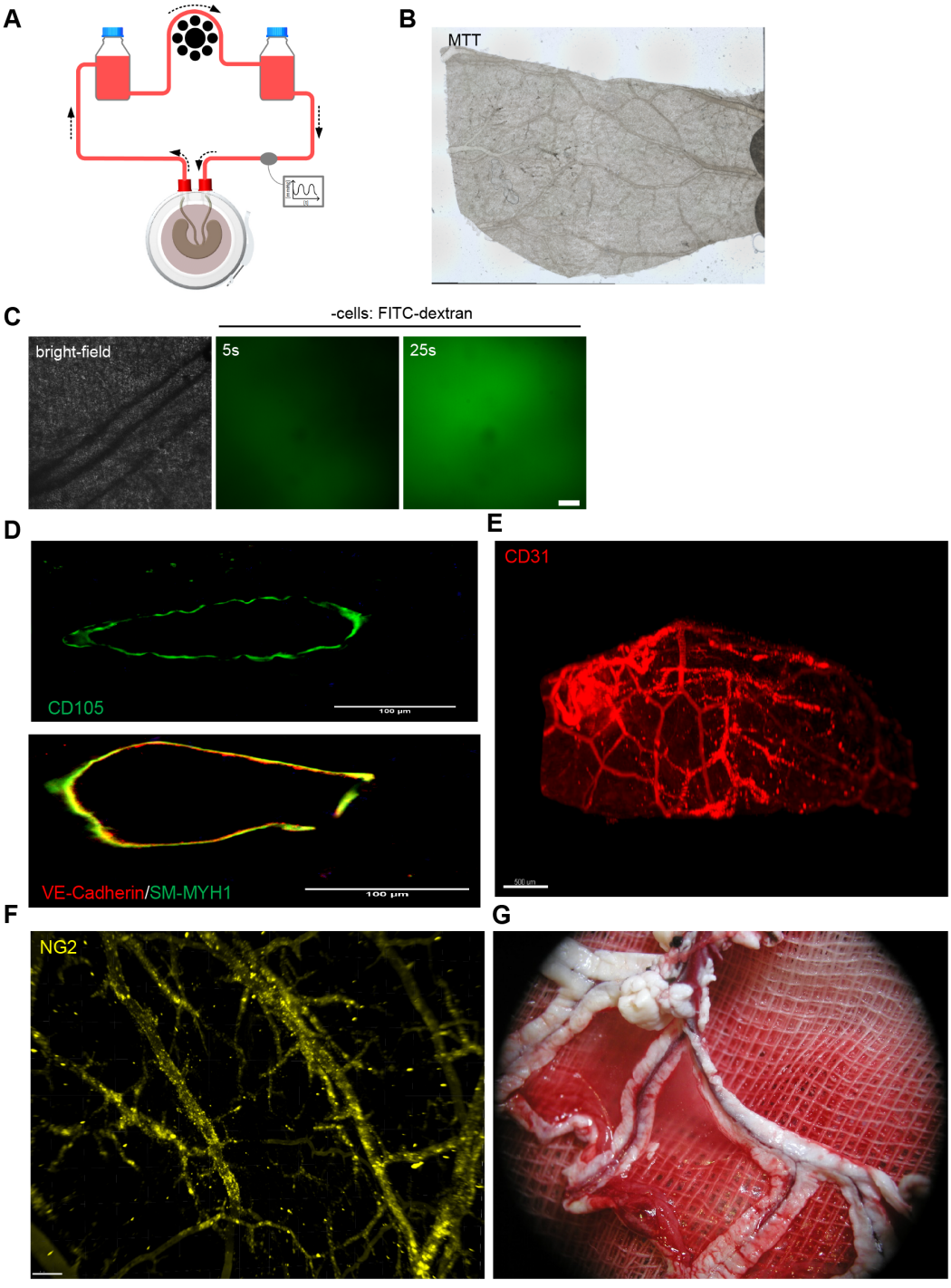

A
B
MTT
C
-cells: FITC-dextran
bright-field
5s
25s
D
CD105
100 µm
VE-Cadherin/SM-MYH1
100 µm
E
CD31
500 µm
F
NG2
G

Figure S6. Related to Figure 6.
(A) Basic bioreactor design showing media vessels, pump, pressure sensors, and the inflow/outflow ports that circulate perfused media through the vascular bed.
(B) MTT assay of a decellularized construct showing the absence of viable cells.
(C) Left panel; bright field image before FITC-dextran perfusion. Time lapse images 5 and 25 seconds after FITC-dextran perfusion into decellularized scaffolds seeded at low density with MesoTs. Scale bar, 100 μm.
(D and E) Light sheet microscopy images as in **Figure 6J** showing $CD31^+$ endothelium lining recellularized jejunal scaffolds after 28 days. Scale bars, 500 μm.
(F) Light sheet microscopy image showing $NG2^+$ pericytes lining MesoT-seeded vessels. Scale bar, 150 μm.
(G) Gross anatomy image as in **Figure 6L** after harvesting of anastomosed tissue.

Table S2 List of Antibodies. Related to STAR Methods.

Antibody	Application	Supplier	Identifier
Goat Polyclonal anti-Islet-1	IF	R&D systems	Cat#AF1837; RRID:AB_2126324
Mouse Monoclonal anti-Nkx2.5 (Clone 259416)	IF	R&D Systems	Cat#MAB2444; RRID:AB_2151378
Goat Polyclonal anti-E-cadherin	IF	R&D Systems	Cat#AF648; RRID:AB_355504
Mouse Monoclonal anti-TBX18 (Clone 635305)	IF	R&D Systems	Cat#MAB63371; RRID:AB_10892533
Rabbit Polyclonal anti-Flk1-1	IF	Acris Antibodies GmbH	Cat# AP02618PU-S; RRID:AB_1624459
Goat Polyclonal anti-SOX2 (Clone Y-17)	IF	Santa Cruz Biotechnology	Cat#sc-17320; RRID:AB_2286684
Mouse Monoclonal anti-ZO-1 (Clone 1)	IF	BD Biosciences	Cat#610966; RRID:AB_398279
Goat Polyclonal anti-DDR2 (N-20)	IF	Santa Cruz Biotechnology	Cat#sc-7555; RRID:AB_639054
Mouse Monoclonal anti-Calponin	IF	Sigma-Aldrich	Cat#C2687; RRID:AB_476840
Mouse Monoclonal anti-smooth muscle Myosin heavy chain 11 antibody (Clone 1G12)	IF	Abcam	Cat#ab683; RRID:AB_2235569
Rabbit Polyclonal anti-von Willebrand Factor	IF	Dako	Cat#A0082; RRID:AB_2315602
Rabbit Monoclonal anti-Wilms Tumor Protein (Clone CAN-R9(IHC)-56-2)	IF, IHC	Abcam	Cat#ab89901; RRID:AB_2043201
Mouse Monoclonal anti-alpha Smooth Muscle Actin (Clone 1A4)	IF, IHC	Abcam	Cat#ab7817; RRID:AB_262054
Rabbit Polyclonal anti-alpha Smooth Muscle Actin	IF, IHC	Abcam	Cat#ab5694; RRID:AB_2223021
Rabbit Monoclonal anti-Vimentin (Clone EPR3776)	IF, IHC	Abcam	Cat#ab92547; RRID:AB_10562134
Mouse Monoclonal anti-CD31 (Clone JC70A)	IF, IHC	Dako	Cat#M0823; RRID:AB_2114471
Mouse Monoclonal anti-NG2	IF, IHC	Abcam	Cat#ab83508; RRID:AB_2087616
Donkey Polyclonal anti-Mouse IgG (H+L) Alexa Fluor 488 Conjugated	IF, IHC	Thermo Fisher	Cat#:A21202; RRID:AB_141607
Donkey Polyclonal anti-Rabbit IgG (H+L) Alexa Fluor 488 Conjugated	IF, IHC	Thermo Fisher	Cat#A21206; RRID:AB_141708
Donkey Polyclonal anti-Goat IgG (H+L) Alexa Fluor 555 Conjugated	IF, IHC	Thermo Fisher	Cat#A21432; RRID:AB_141788
Donkey Polyclonal anti-Mouse IgG (H+L) Alexa Fluor 555 Conjugated	IF, IHC	Thermo Fisher	Cat#A31570; RRID:AB_2536180
Donkey Polyclonal anti-Rabbit IgG (H+L) Alexa Fluor 555 Conjugated	IF, IHC	Thermo Fisher	Cat#A31572; RRID:AB_162543
Donkey Polyclonal anti-Goat IgG (H+L) Alexa Fluor 647 Conjugated	IF, IHC	Thermo Fisher	Cat#A21447; RRID:AB_141844
Donkey Polyclonal anti-Rabbit IgG (H+L) Alexa Fluor 647 Conjugated	IF, IHC	Thermo Fisher	Cat#A31573; RRID:AB_2536183
Donkey Polyclonal anti-Sheep IgG (H+L) Alexa Fluor 647 Conjugated	IHC	Thermo Fisher	Cat#A21448; RRID:AB_2535865

Rabbit Polyclonal anti-CD31	IHC	Abcam	Cat#ab28364; RRID:AB_726362
Sheep Polyclonal anti-Human TGN46 (Human Golgi)	IHC	AbD Serotec	Cat#AHP500G; RRID:AB_323104
Mouse Monoclonal anti-Nuclei (Human Nuclear Antigen) (Clone 235-1)	IHC	Millipore	Cat#MAB1281; RRID:AB_94090
Mouse Monoclonal anti-NG2/MCSP Phycoerythrin Conjugated (Clone LHM-2)	FC	R&D Systems	Cat#FAB2585P; RRID:AB_2087615
Rabbit Polyclonal anti-VE-cadherin	FC	Abcam	Cat#ab33168; RRID:AB_870662
Mouse Monoclonal anti-CD31 BD Horizon™ BV421 Conjugated (Clone WM59)	FC	BD Biosciences	Cat#564089; RRID:AB_2714010
Mouse Monoclonal anti-CD73 Phycoerythrin Conjugated (Clone AD2)	FC	BD Biosciences	Cat# 550257; RRID:AB_393561
Rat Monoclonal anti-CD44 Allophyocyanin Conjugated (Clone IM7)	FC	eBioscience	Cat# 17-0441-82; RRID:AB_469390
Mouse Monoclonal anti-CD105 BD Horizon™ BV421 Conjugated (Clone 266)	FC	BD Biosciences	Cat# 563920; RRID:AB_2722606
Mouse Monoclonal IgG1 kappa Isotype Control BD Horizon™ BV421 Conjugated (Clone X40)	FC	BD Biosciences	Cat#562438; RRID:AB_11207319
Rat IgG2b kappa Isotype Control Allophycocyanin Conjugated (Clone eB149/10H5)	FC	eBioscience	Cat#17-4031-82; RRID:AB_470176
Mouse Monoclonal IgG1 kappa Isotype Control Phycoerythrin Conjugated (Clone MOPC-21)	FC	BD Biosciences	Cat#555749; RRID:AB_396091
Mouse IgG1 kappa Isotype Control Allophycocyanin Conjugated (Clone P3.6.2.8.1)	FC	eBioscience	Cat# 17-4714-42, RRID:AB_1603315
Mouse IgG1 Isotype Control Allophycocyanin Conjugated (Clone 11711)	FC	R&D Systems	Cat#IC002A; RRID:AB_357239
Anti-Histone H3 (mono methyl K4) antibody – ChIP Grade	ChIP	Abcam	Cat#ab8895; RRID:AB_306847
Mouse monoclonal anti-CD105 (clone SN6)	IF	Abcam	Cat#ab11414; RRID: AB_298019
Rabbit polyclonal anti-VE-Cadherin(CD144)	IF	Signma-Aldrich	Cat#V1514; RRID: AB_477609
Mouse monoclonal anti-fast myosin skeletal heavy chain antibody (Clone MY32)	IF	Abcam	Cat#ab51263; RRID: AB_2297993
Anti-Histone H3 (acetyl K27) antibody – ChIP Grade	ChIP	Abcam	Cat#ab4729; RRID:AB_2118291

- Key: IF – immunofluorescence, IHC – immunohistochemistry, FC – flow cytometry, ChIP – chromatin immunoprecipitation

Table S3 Taqman Primers for qRT-PCR. Related to STAR Methods.

Gene	Supplier	Identifier	Chromosome Location
POU5F1/OCT4	Thermo Fisher	Hs04260367_gH	Chr.6: 31164337 - 31170693
SOX2	Thermo Fisher	Hs01053049_s1	Chr.3: 181711924 - 181714436
NANOG	Thermo Fisher	Hs02387400_g1	Chr.12: 7789396 - 7796061
ISL1	Thermo Fisher	Hs00158126_m1	Chr.5: 51383124 - 51394730
NKX2.5	Thermo Fisher	Hs00231763_m1	Chr.5: 173232104 - 173235312
GATA4	Thermo Fisher	Hs00171403_m1	Chr.8: 11676919 - 11760002
WT1	Thermo Fisher	Hs01103751_m1	Chr.11: 32387775 - 32435535
TBX18	Thermo Fisher	Hs01385457_m1	Chr.6: 84666834 - 84764236
TCF21	Thermo Fisher	Hs00162646_m1	Chr.6: 133889121 - 133895537
MSLN	Thermo Fisher	Hs00245879_m1	Chr.16: 760746 - 768865
RNA18S5	Thermo Fisher	Hs03928985_g1	Chr.Un|NT_167214: 109078 - 110946