

Supplements

Requires Working Solutions

- 1. D10 buffer:** 500 ml Dulbecco Modified Eagle Medium (Gibco, Waltham, MA), 50 mL fetal bovine serum (Gibco, Waltham, MA), 5 mL penicillin-streptomycin-glutamine [250 mg/L] (Gibco, Waltham, MA).²⁷
- 2. Digestion buffer:** 18 mL Dulbecco Modified Eagle Medium, 2.5 mL D10 buffer, 2.5 mL $\text{Ca}^{2+}\text{Mg}^{2+}$ (Sigma-Aldrich, St. Louis, MO), 100 mg collagenase type II (Gibco, Waltham, MA), 100 mg collagenase type A (Sigma-Aldrich, St. Louis, MO), 100 mg dispase (Gibco, Waltham, MA). Sterilize the digestion buffer by passing it through a 40 μm membrane filter.²⁷
- 3. Lysis buffer:** 3 mL NH_4Cl (Sigma-Aldrich, St. Louis, MO), 1 mL phosphate buffered saline (PBS) (Gibco, Waltham, MA).²⁷
- 4. Isolation buffer:** 450 mL PBS, 2.5 mL bovine serum albumin (Sigma-Aldrich, St. Louis, MO), 50 mL 6% ACD-A solution (1L double-distilled H_2O , 8 g citric acid (Sigma-Aldrich, St. Louis, MO), 22 g sodium citrate (Sigma-Aldrich, St. Louis, MO), 22.3 g glucose (Gibco, Waltham, MA)).²⁷
- 5. EGM-2 Culture Medium (Lonza Bioscience, Walkersville, MD):** Endothelial Cell Growth Basal Medium-2 (Lonza) containing 2% fetal bovine serum, hydrocortisone, heparin, ascorbic acid, human fibroblast growth factor-b, vascular endothelial growth factor, human endothelial growth factor, gentamicin sulfate-amphotericin (GA-1000), R3 insulin-like growth factor-1, and penicillin-streptomycin-glutamine (250 mg/L) at 37 C and 5% carbon dioxide (CO_2). Add small molecule SB431542, a transforming growth factor beta 1 (TGF- β 1) inhibitor (Milenyi Biotech, Gaithersburg, MD), at a concentration of 6 mg/L to the medium to inhibit further fibrogenic activation of EECs during cultivation.²⁷
- 6. Labelling buffer:** PBS with 0.5% bovine serum albumin (BSA) and 2 mM ethylenediaminetetraacetic acid (EDTA) (Gibco, Waltham, MA).

Supplementary Tables

Supplementary Table S1: Table of Materials Working Solutions

Name of Material/ Equipment	Catalog Number	Company
Ammonium chloride	#254134	Sigma-Aldrich, St. Louis, MO
Bovine serum albumin	#A4737	Sigma-Aldrich, St. Louis, MO
Calcium chloride	#C1016	Sigma-Aldrich, St. Louis, MO
Citric acid	#C0759	Sigma-Aldrich, St. Louis, MO
Collagenase type A	#10103586001	Sigma-Aldrich, St. Louis, MO
Collagenase type II	#17101015	Gibco, Waltham, MA
Dispase II	#17105041	Gibco, Waltham, MA
Dulbecco Modified Eagle Medium	#11965092	Gibco, Waltham, MA
EGM-2 Endothelial Cell Growth Medium-2 BulletKit	#CC-3162	Lonza Bioscience, Walkersville, MD
Fetal bovine serum	#A5256801	Gibco, Waltham, MA
Glucose solution	#A2494001	Gibco, Waltham, MA
Magnesium sulfate	#M7506	Sigma-Aldrich, St. Louis, MO
Penicillin-streptomycin-glutamine	#15140122	Gibco, Waltham, MA
Phosphate buffered saline	#10010023	Gibco, Waltham, MA
Sodium citrate	#6132-04-3	Sigma-Aldrich, St. Louis, MO
StemMACS SB431542	#130-106-543	Miltenyi Biotec, Gaithersburg, MD
UltraPure 0.5M EDTA	#15575020	Invitrogen, Carlsbad, CA

Supplementary Table S2: Table of Materials CD144-based Magnetic Isolation

Name of Material/ Equipment	Catalog Number	Company
Anti-Mouse IgG MicroBeads	#130-048-402	Miltenyi Biotec, Gaithersburg, MD
CD144 primary antibody	#14-1449-82	Invitrogen, Carlsbad, CA
MS column	#130-042-201	Miltenyi Biotec, Gaithersburg, MD
OCTO MACS Separator	#130-042-108	Miltenyi Biotec, Gaithersburg, MD

Supplementary Table S3: Table of Materials Miscellaneous

Name of Material/ Equipment	Catalog Number	Company
Click-iT™ Plus EdU Alexa Fluor™ 647 Flow Cytometry Assay Kit, 50 rxn(s)	# C10634	Invitrogen, Carlsbad, CA
Conical tube, 15 mL	#339650	Thermo Fisher Scientific, Waltham, MA
Conical centrifuge tube, 50 mL	#339653	Thermo Fisher Scientific, Waltham, MA
Cover Slip, Round, 12 mm Diameter	#CLS-1763-012	Chemglass Life Sciences, Vineland, NJ
Fix & Perm / Cell Fixation & Permeabilization Kit (Flow Cytometry)	#ab185917	Abcam, Waltham, MA
Filter, 40 µm membrane	#352340	Corning, Corning, NY
Ibidi µ-Slide VI 0.4 slides IbiTreat	#80606-90	ibidi, Fitchburg, WI, USA
MicroAmp Optical 96-Well Reaction Plate with Barcode	#4306737	Applied Biosystems, Carlsbad, CA
MicroAmp Optical Adhesive Film	#4311971	Applied Biosystems, Carlsbad, CA
Normal goat serum	#31872	Invitrogen, Carlsbad, CA
OCTO MACS Separator	#130-042-108	Miltenyi Biotec, Gaithersburg, MD
Paraformaldehyde	#J19943.K2	Thermo Fisher, Waltham, MA
Porcine gelatin	#G1890	Sigma-Aldrich, St. Louis, MO
RNeasy Micro Kit	#74004	QIAGEN, Germantown MD
RT-PCR grade water	#AM9935	Invitrogen, Carlsbad, CA
Stainless Steel Blades	#371210	Aspen Surgical Products, Caledonia, MI
TaqMan 1-Step Multiplex Master Mix (No ROX)	#A28523	Applied Biosystems, Carlsbad, CA
HEK293-derived Recombinant Human TGF-beta 1 (Human Cell-expressed) Protein (7754-BH)	#240-B-010/CF	R&D Systems, Minneapolis, MN, USA
Triton X-100	#X100	Sigma-Aldrich, St. Louis, MO
Trypsin Neutralizer Solution	#R002100	Gibco, Waltham, MA
Trypsin-EDTA, 0.05%	#25300054	Gibco, Waltham, MA

Supplementary Table S4: Antibodies Flow Cytometry

Antibody	Catolog Number	Company	Dilution
APC Mouse IgG1, Isotype Control	#555751	BD Biosciences, San Diego, CA	1:50
Fibroblast, APC, REAfinity	#130-123-827	Miltenyi Biotec, Gaithersburg, MD	1:50
NFATc1 Antibody (7A6)	#sc-7294 AF647	Santa Cruz Biotechnology, Dallas, TX	1:50
PE anti-human CD31	#303106	BioLegend, San Diego, CA	1:100
PE Mouse IgG1, Isotype Control	#559320	BD Biosciences, San Diego, CA	1:100
REA Control Antibody, human IgG1, APC, REAfinity	#130-113-446	Miltenyi Biotec, Gaithersburg, MD	1:50
Vimentin Monoclonal Antibody (VI-RE/1), PE	#MA1-19656	Invitrogen, Carlsbad, CA	1:100

Supplementary Table S5: Real-Time Quantitative PCR TaqMan Probes

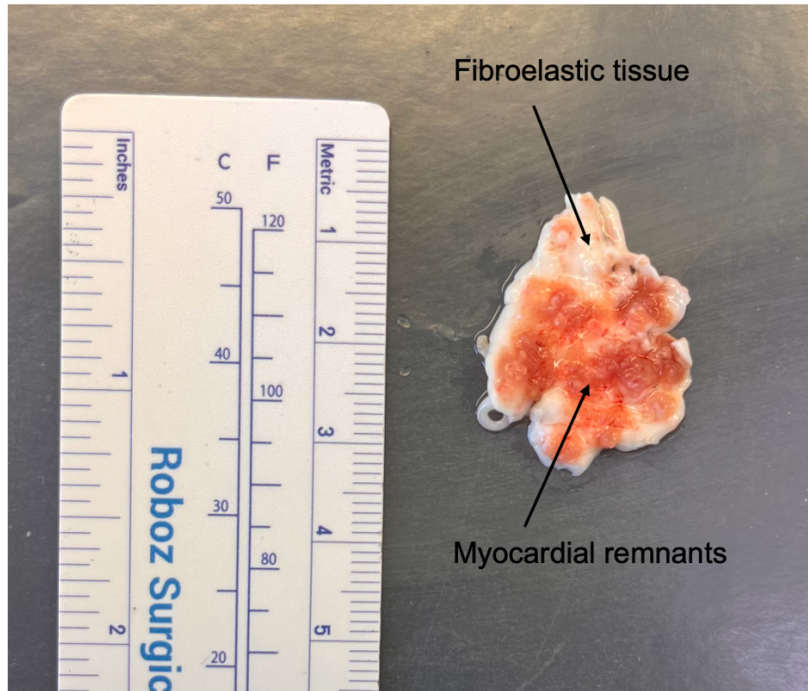
Target	Assay ID	Dye- Quencher	Catalog Number	Company
ACTA2	Hs00426835_g1	ABY-QSY	Custom	Thermo Fisher Scientific, Waltham, MA
COL1A1	Hs01076741_gH	JUN-QSY	Custom	Thermo Fisher Scientific, Waltham, MA
GAPDH	Hs02758991_g1	VIC-MGB-PL	#4448484	Thermo Fisher Scientific, Waltham, MA
NFATC1	Hs00542675_m1	FAM-MGB	#4448892	Thermo Fisher Scientific, Waltham, MA
NPR3	Hs01099013_m1	FAM-MGB	#4448892	Thermo Fisher Scientific, Waltham, MA
PECAM	Hs00169777_m1	FAM-MGB	#4331182	Thermo Fisher Scientific, Waltham, MA
SNAI2	Hs00161904_m1	ABY-QSY	Custom	Thermo Fisher Scientific, Waltham, MA
VIM	Hs00958111_m1	FAM-MGB	#4331182	Thermo Fisher Scientific, Waltham, MA

Supplementary Table S6: Antibodies Immunofluorescence Staining

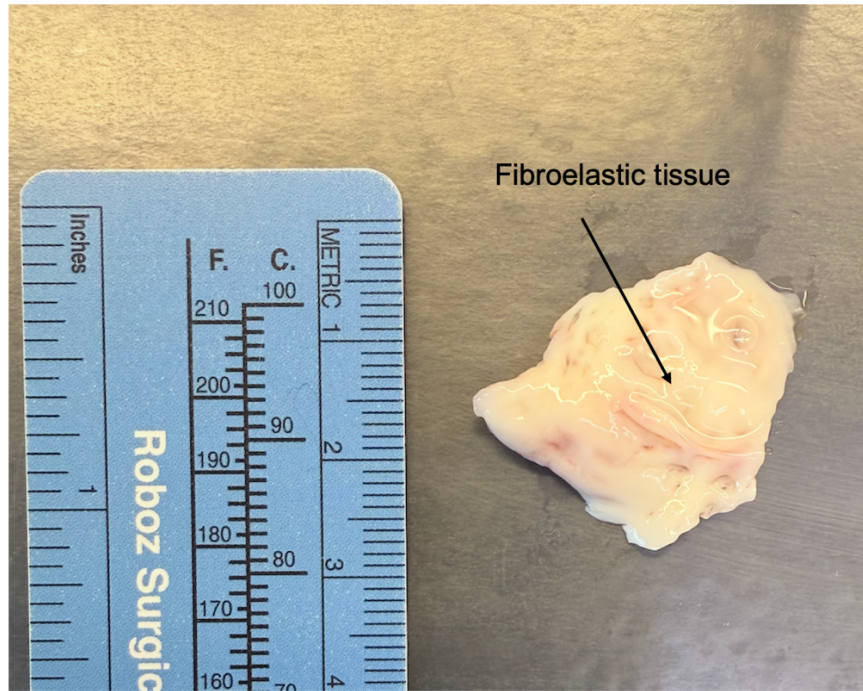
Antibody	Catolog Number	Company	Dilution
4',6-diamidino-2-phenylindole	#D1316	Invitrogen, Carlsbad, CA	1:5000
Alexa Fluor 488 Anti-alpha smooth muscle Actin antibody [EPR5368]	# ab202295	Abcam, Waltham, MA	1:500
CD31	#ab32457	Abcam, Waltham, MA	1:100
Goat anti-Rabbit IgG Texas Red-X	#T6391	Invitrogen, Carlsbad, CA	1:200
NPR3	#PA5-85282	Invitrogen, Carlsbad, CA, USA	1:100
Vimentin	#MA5-11883-A488	Invitrogen, Carlsbad, CA	1:100

Supplementary Figures

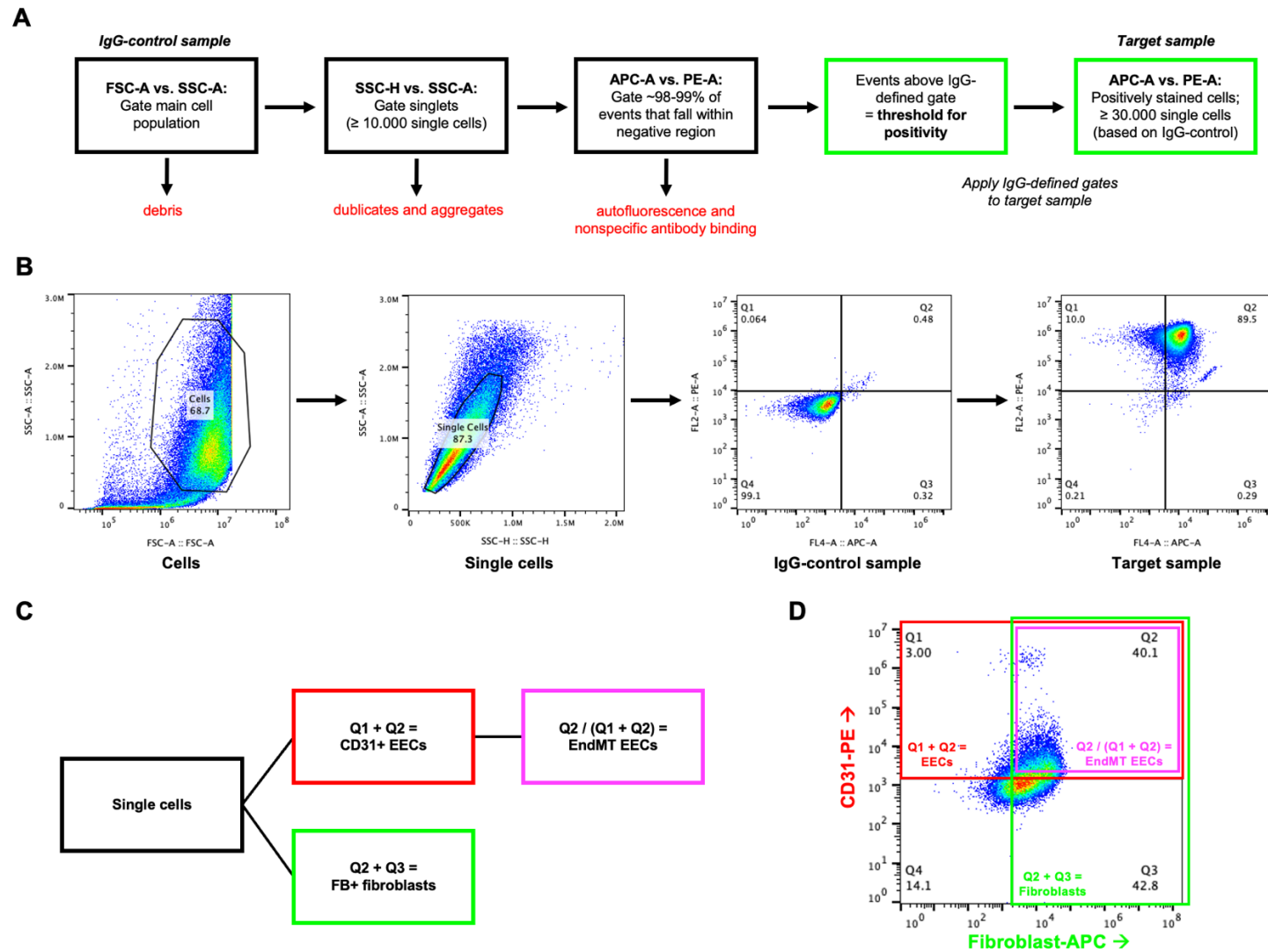
Facing myocardium



Facing left ventricular lumen



Supplementary Figure S1: Macroscopic appearance of the resected fibroelastic tissue. Tissue sample facing the myocardium (white = fibroelastic tissue, red = muscle) from a patient with hypoplastic left heart syndrome and endocardial fibroelastosis. Left: tissue sample facing the myocardium (white = fibroelastic tissue, red = muscle), right: facing toward the ventricular lumen (white = fibroelastic tissue).



Supplementary Figure S2: Gating and population selection strategy for flow cytometry. (A) Schematic overview of the gating workflow. (B) Representative sample analyzed using the described gating strategy. (C) Schematic overview of population selection according to marker expression. (D) Representative flow cytometry showing application of this selection approach.