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Supplemental Information

Prospective Isolation of ISL1^{+} Cardiac Progenitors from Human ESCs for Myocardial Infarction Therapy

Zaniar Ghazizadeh, Faranak Fattahi, Mehdi Mirzaei, Delger Bayersaikhan, Jaesuk Lee, Sehyun Chae, Daehee Hwang, Kyunghee Byun, Mehdi Sharifi Tabar, Sara Taleahmad, Shahab Mirshahvaladi, Parisa Shabani, Hananeh Fonoudi, Paul A. Haynes, Hossein Baharvand, Nasser Aghdami, Todd Evans, Bonghee Lee, and Ghasem Hosseini Salekdeh

Supplemental Experimental Procedures

List of primary antibodies and working dilutions for Immunofluorescence staining and flow cytometry. All antibodies used are listed below.

Antibody	Catalog number	Source	Dilution
CD49c	343803	Biolegend	1:200
ALCAM	343904	Biolegend	1:200
CD276	331606	Biolegend	1:200
ISL1	39.4D5	DSHB	1:200
Mef2c	Ab64644	Abcam	1:1000
Cardiac Actin	A9357	Sigma	1:200
VE-cadherin (*CDH5*)	ab33168	Abcam	1:200
SMA	A5228	Sigma	1:1000
MLC-2V	310111	Synaptic systems	1:200
MYH6	Ab15	Abcam	1:200
Connexin43	C6219	Sigma	1:200
vWF	A008229-5	DAKO	1:150
Akt	9272S	Cell Signaling	1:1000
pAkt	9271S	Cell Signaling	1:1000
ERK1/2	9102S	Cell Signaling	1:1000
pERK1/2	4377S	Cell Signaling	1:1000
SAPK/JNK	9252S	Cell Signaling	1:1000
SAPK/JNK	9251S	Cell Signaling	1:1000
PDGFR-α	sc21789	Santa Cruz	1:200
SSEA-1	11-0159-71	eBiosciences	1:200
SIRPA	Ab8120	Abcam	1:200
VCAM1	551147	BD	1:200
CD13	F0831	DAKO	1:200

Evaluation of infarct size. Two or four weeks after cell transplantation, all rats were sacrificed by perfusion through the right carotid artery with PBS and ice-cold 4% paraformaldehyde (Sigma-Aldrich). The heart was isolated and processed for dehydration, clearing and embedding in paraffin in transverse plane. The heart tissues were sectioned into 7 μm thickness and stained with Hematoxylin & Eosin and Masson's trichrome for morphological analysis. The size of the infarct area and other parameters were measured on the middle horizontal sections between the point of ligation and the apex of the heart. The calculation formula used for the infarct size was: % infarct size = (infarct areas/total left ventricle (LV area)) X 100

TUNEL assay. Heart tissues were washed three times in 1XPBS and incubated with permeabilization solution (0.1% Trinton X-100, 0.1% Sodium citrate, freshly prepared) 2 mins on ice. The tissues were then rinsed three times with 1XPBS. TUNEL reaction mixture (Roche) was added on tissues and incubated in a humidified atmosphere for 1 hr at 37°C in the dark. Tissues were washed again in 1XPBS three times and coverslips were mounted onto glass slides using the Vectashield mounting medium (Vector Laboratories). Apoptotic cells were analyzed under fluorescent microscope (Zeiss Imager.Z1).

Immunoconfocal staining and vessel analysis. Seven-micrometer paraffin sections were prepared from left ventricles of each experimental animal at 2 or 4 weeks after transplantation. Tissues were deparaffinized in Xylene for 10 mins and hydrated in serial graded alcohol (100% 5 mins, 90 % 3 mins, 80% 2 mins, 75% 2 mins). Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide for 20 mins. The slides were washed with PBS and incubated for 1 hr in blocking solution (5% bovine serum diluted in 1X PBS) and then incubated overnight at 4°C with a-SMA (1:150, Abcam) or vWF (1:100, Dako) as primary antibodies. Tissues were incubated with Alexa Fluor 488 donkey anti-rabbit antibody (1:500, Invitrogen) as a secondary antibody for 1 hr at room temperature. Capillaries and arterioles were counted at a magnification of 200X, confocal microscopic images were recorded, and the average number of the vessels in the infarct area or the peri-infarction area was analyzed using Images.Z1 on a Zeiss 710 microscope.

Immunohistoenzymic 3,3'-diaminobenzidine staining. Seven-micrometer paraffin slides were washed in 1XPBS five times and immersed into 0.3% H_2O_2 for 15 mins at room temperature to reduce endogenous peroxidase activity. Tissues were washed in 1XPBS three times and incubated

overnight at 4°C with Akt (1:1,000, Cell Signaling), pAkt (1:1,000, Cell Signaling), ERK1/2 (1:1,000, Cell Signaling), pERK1/2 (1:1,000, Cell Signaling), SAPK/JNK (1:1,000, Cell Signaling), or pSAPK/JNK (1:1,000, Cell Signaling) as primary antibodies. The tissues were washed again with 1XPBS. After washing with 1XPBS, biotinylated secondary antibody was incubated for 1 hr at room temperature and ABC solution (Vector laboratories) was added to tissues and incubated at room temperature for 1 hr. Tissues were washed again in 1XPBS three times and incubated with DAB (Sigma-Aldrich) solution at room temperature for 1 min. Finally tissues were washed in a graded ethanol series (70%, 30 sec; 80%, 30 sec; 95%, 30 sec; 100%, 30 sec and 100% xylene, 5 mins). The stained slides were mounted with DPX mounting medium (Sigma-Aldrich) for microscopic image analysis.

Nanoflow liquid chromatography – tandem mass spectrometry dataset analysis. To compensate for null values and allow log transformation of the NSAF data prior to statistical analysis, a spectral fraction of 0.5 was added to the total spectral counts for each protein in the data set. Statistical analysis was performed on log-transformed NSAF data using the Statistics Software Package (Student's t-test) and $p<0.05$ was considered to indicate a statistically significant difference. The resulting sets of up- and down-regulated (>1.5 fold) proteins were then functionally annotated using Ingenuity Pathway Analysis (IPA) software from Qiagen. Heatmaps were generated using the heatmap.2 function from the gplots library in the statistical software R. The mass spectrometry proteomics data have been deposited to the ProteomeXchange consortium via the PRIDE partner repository with the dataset identifier PXD007720.
We identified at least two unique peptides for each of the proteins in the dataset. The unique peptide sequences for candidate surface makers are listed in table S3.

Cine MRI imaging methods. MRI image were taken by Bruker 4.7 T (Bruker, Ettlingen, Germany) with horizontal bore magnet transmitter / receiver coil with internal diameter of 72 mm. For imaging, all the rats were anesthetized with isoflurane 1.5% inhalation. Respiration, heart beating and body temperature were monitored by animal monitoring-gating system (SA instruments, Stony Brook, NY, USA) on warm bed. All images were obtained after application of respiratory gating and cardiac gating. MRI was performed on 2 rats per group and 3 planes in each animal were used for quantification.

Gadolinium contrast media (Gd-DTPA, Magnevist, Bayer Healthcare) was injected into the tail vein of rat. T1W transverse plane images were obtained at TR = 100 ms, TE = 3 ms, FA = 30°, matrix size = 256 * 256, FOV = 50 mm, NEX = 8 using FLASH before and after 2 minutes and after 13 minutes. Measurement of infarct size, myocardium thickness, ejection fraction through T1W was performed with Segment (Medviso, Sweden).

CINE_ BLACK BLOOD MRI imaging were obtained as transverse plane at TR = 40 ms, TE = 2.5 ms, FA = 20°, matrix size = 192 * 192, FOV = 50 mm, NEX = 6, FPS = 15, Inversion time = 50 ms using FLASH_Black blood sequence.

Supplementary Figures

Figure S1. Characterization of rH5-isl1-Hygro cell line and enriched ISL1+ progenitors. A, Schematic representations of the hygromycin selection construct. **B,** Immunofluorescent staining of rH5-isl1-Hygro cell line for pluripotency markers. **C,** ISL1 percentage at day 8 post differentiation without antibiotic selection. **D,** Western blot quantification of ISL1 expression at day 8 of differentiation in rH5-isl1-Hygro cell line with/without hygromycin treatment. **E,** qRT-PCR for expression of cardiac transcription factors following cardiac differentiation of rH5-isl1-Hygro with/without hygromycin treatment. **F,** Immunofluorescence staining and quantification of cTnT in beating clusters at day 25. **G,** Immunofluorescence staining of cardiomyocyte markers in differentiated Hygro. treated at day 25 **H,** Multi-electrode array (MEA) experiment paradigm. Cultures of hESC-derived cardiomyoctes in MEA chambers (top panel). Electrophysiological characteristics were re-evaluated following treatment with 100 nM isoprenaline (bottom panel). **I,** MEA characterizations, including field potential duration, peak amplitude, interspike interval and beating rate properties of differentiated ISL1 enriched population at day 20. n=3-5 independent experiments. Scale bars = 100 μm. *p<0.05, **p<0.01, ***p<0.001.

Figure S2. Proteomic characterization and molecular phenotype of purified ISL1+ progenitor cells. A, Heatmap of ≥1.5 fold differentially expressed proteins in ISL1+ versus ISL1+/- populations. **B,** Heatmaps of differentially expressed transcriptional regulators in ISL1+/- and ISL1+ populations. **C,** Heatmaps of differentially expressed signaling pathway proteins in

ISL1+/- and ISL1+ populations. **D,** Heatmaps of differentially expressed cardiovascular development proteins in ISL1+/- and ISL1+ populations. **E,** Heatmaps of differentially expressed cardiovascular disease proteins in ISL1+/- and ISL1+ populations **F,** Western blot confirmation of selected proteins from proteomics data. proteins in ISL1+/- and ISL1+ populations; 1,4: hESC, 2,5: ISL1+/-, 3,6: ISL1+. **G,** Immunofluorescence staining of ISL1 in CD49C positive and negative cardiac progenitor populations derived from hESCs. **H,** Immunofluorescence staining of ISL1 in CD276 positive and negative cardiac progenitor populations derived from hESCs. Scale bar = 50 μm.

Figure S3. ALCAM expression in hPSC-derived cardiac progenitors. A, Quantification of Immunolabeling for MYH6, MLC-2v, c-Actin, CX43, SMA and VE-cadherin in ALCAM+ and ALCAM- sorted populations. n=3-5 independent experiments. **B,** Time-course flow cytometry of ALCAM in differentiating H9 hESC line. **C,** Time-course flow cytometry of ALCAM in differentiating hiPSC line. **D,** ALCAM co-expression with PDGFR-alpha, SIRPA, SSEA1, VCAM, CD13, and ISL1. **E,** Time-course qPCR analysis of *SIRPA* mRNA expression. n=3-4 independent experiments. *$p<0.05$, **$p<0.01$, ***$p<0.001$

Figure S4. ALCAM+ cells promote tissue repair and angiogenesis. A, Immunofluorescence staining for human specific marker Hu-Nu in AMI hearts transplanted with ALCAM+ cells. **B,** Hematoxylin and Eosin (H&E) staining and Masson's Trichrome (MT) staining in sham or AMI rat hearts in control, medium only and ALCAM-, ALCAM-/+, or ALCAM+ cell transplanted groups, 2 weeks post-transplantation. Insets are high magnification images of the fibrotic area. **C,** TUNEL (apoptotic cell marker, red) staining in sham or AMI rat hearts in control, medium only and ALCAM-, ALCAM-/+, or ALCAM+ cell transplanted groups, 2 and 4 weeks post transplantation. **D,E**, SMA staining in infarct or border area in sham or AMI rat hearts in control, medium only and ALCAM-, ALCAM-/+, or ALCAM+ cell transplanted groups, 2 weeks (top panels) and 4 weeks (bottom panel) post transplantation. **F,G,** vWF staining in infarct or border area in sham or AMI rat hearts in control, medium only and ALCAM-, ALCAM-/+, or ALCAM+ cell transplanted groups, 2 weeks (top panels) and 4 weeks (bottom panel) post transplantation. Scale bar = 1 mm in b and 100 μm in c-g.

Figure S5. MRI images for Sham, AMI, and medium and ALCAM+ injected rat hearts. A, Cine MRI image of blackblood in Sham, AMI, and medium and ALCAM+ treated rats. **B,** Short axis images of Cine MRI of normal Sham, AMI, and medium and ALCAM+ treated rats at Early Gadolinium Enhancement (EGE, 2mins) and Late Gadolinium Enhancement (LGE, 13 mins). Representative two images are shown at each group.

Table S1. List of ≥1.5 fold differentially expressed proteins in ISL1+ versus ISL1+/- populations.

Table S2. List of differentially expressed genes (DEGs) in ALCAM-, ALCAM-/+ and ALCAM+ cells versus hESCs.

Table S3. Unique peptide sequences for hit surface markers.

CD49c	SETVLTCATGR; QLDPGGGQGPPPVTLAAAKK; AKSETVLTCATGR
CD276	NPVLQQDAHSSVTITPQR; QLVHSFTEGR; SPTGAVEVQVPEDPVVALVGTDATLR
ALCAM	SSPSFSSLHYQDAGNYVCETALQEVEGLK; TIHSEQAVFDIYYPTEQVTIQVLPPK; CLGNGNPPPEEFLFYLPGQPEGIR; FVCMLVTEDNVFEAPTIVK; SVQYDDVPEYKDR; ESLTLIVEGKPQIK

Supplementary Movie 1. Spontaneous beating in cardiomyocytes generated from ISL1+ cells.

Supplementary Movie 2. Cine MRI videos of blackblood in Sham, AMI, and medium and ALCAM+ treated rats.

Figure S1 (Ghazizadeh et al.,)

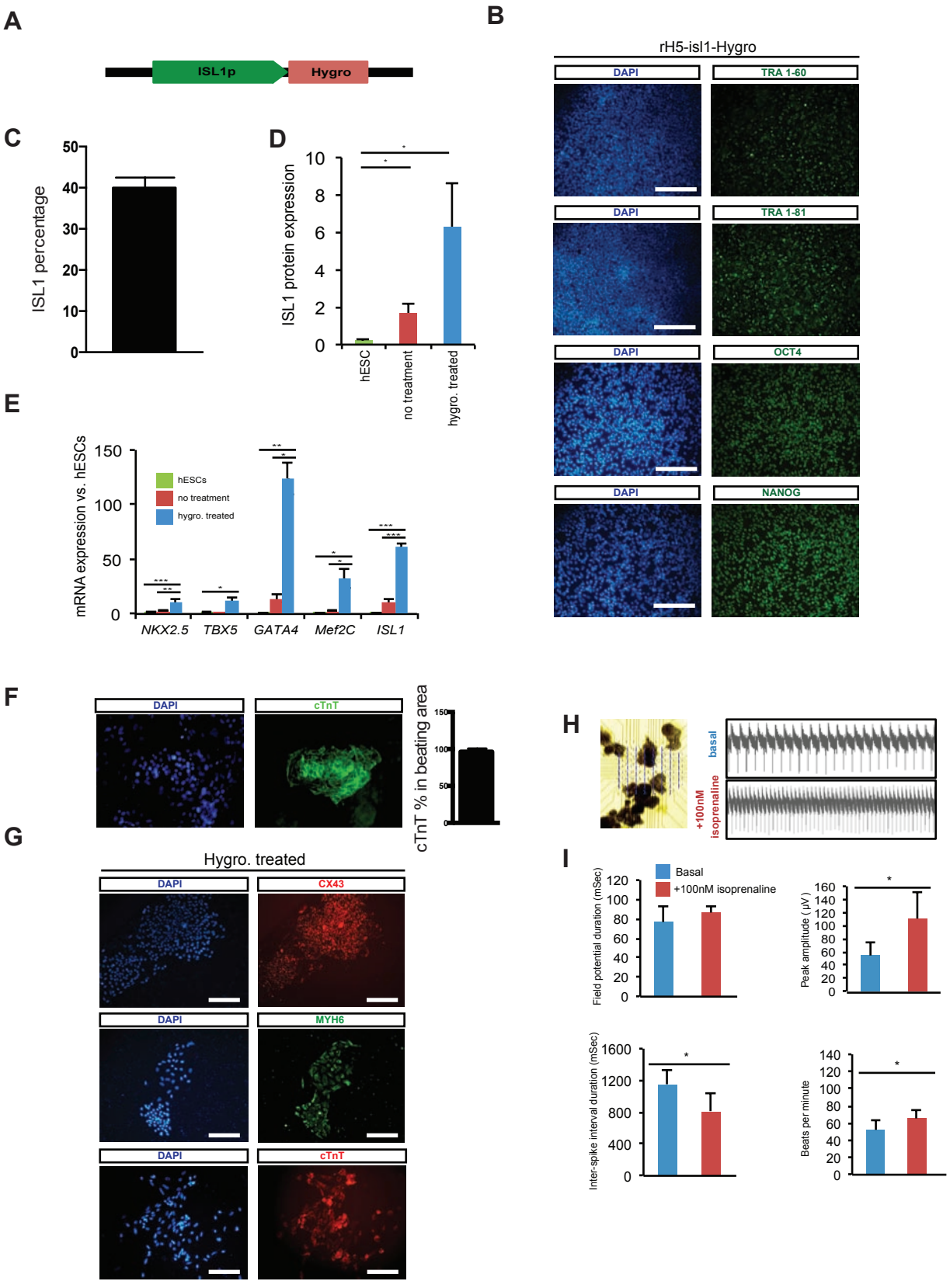

Figure S2 (Ghazizadeh et al.)

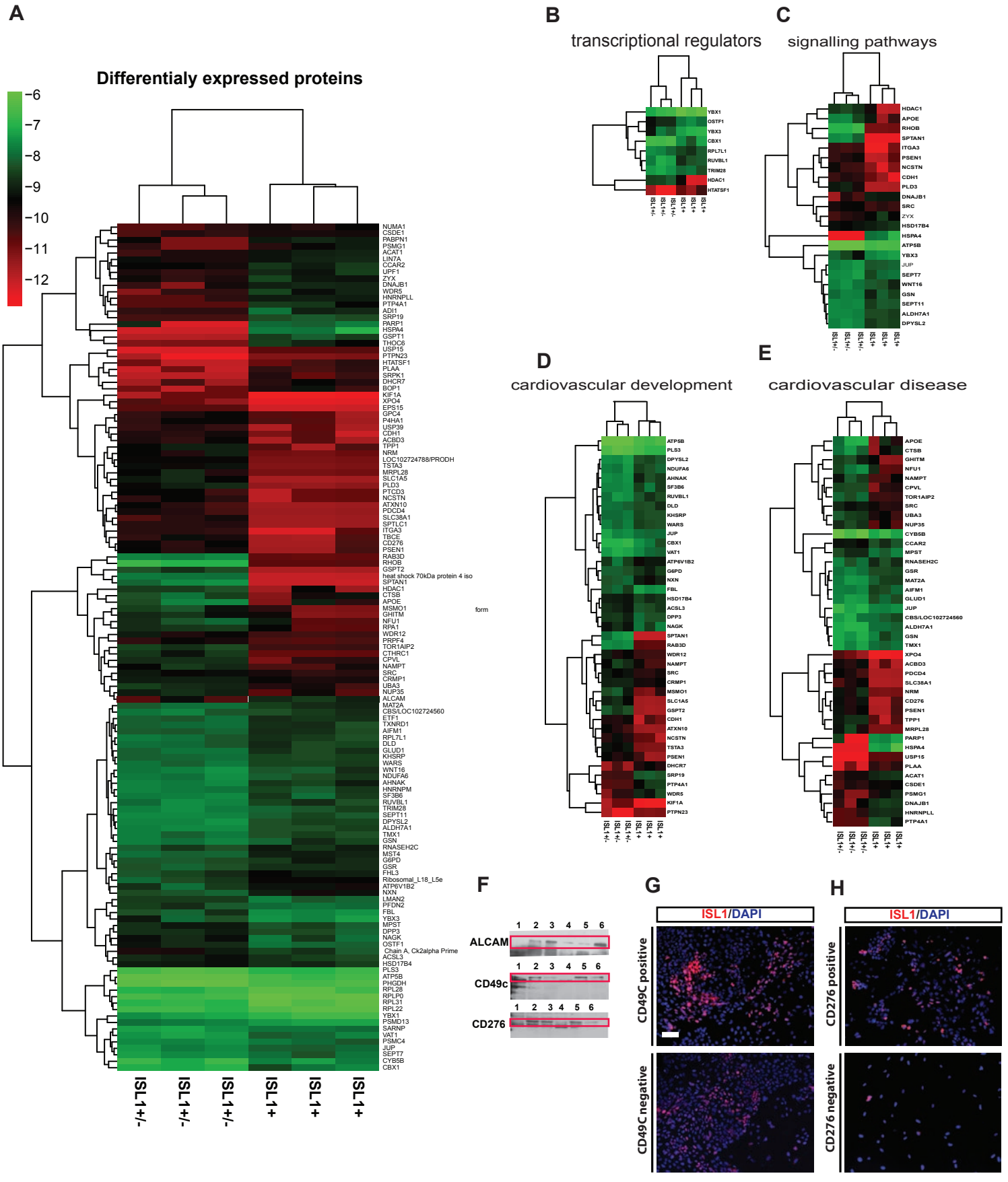

Figure S3 (Ghazizadeh et al.)

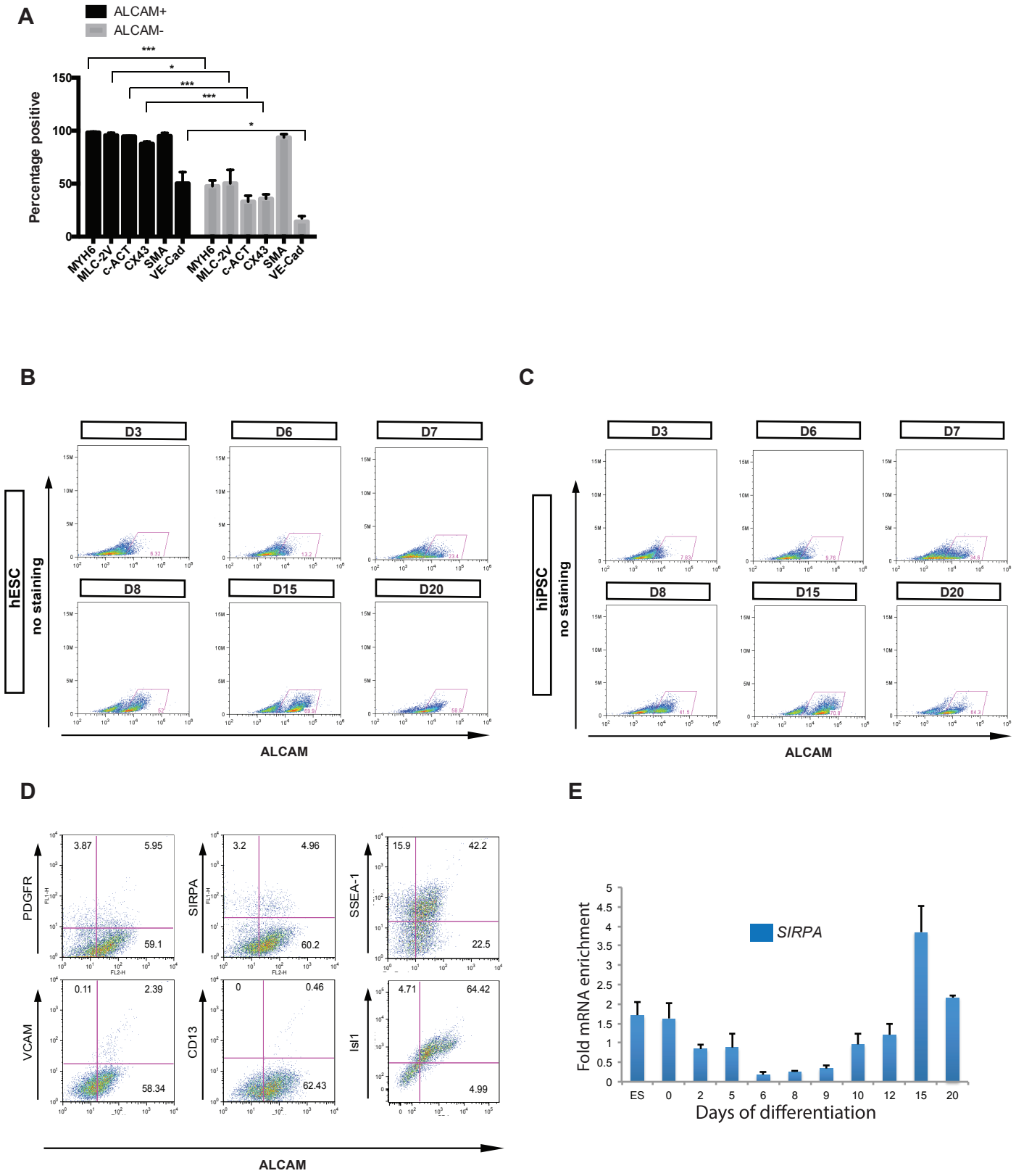

Figure S4 (Ghazizadeh et al.)

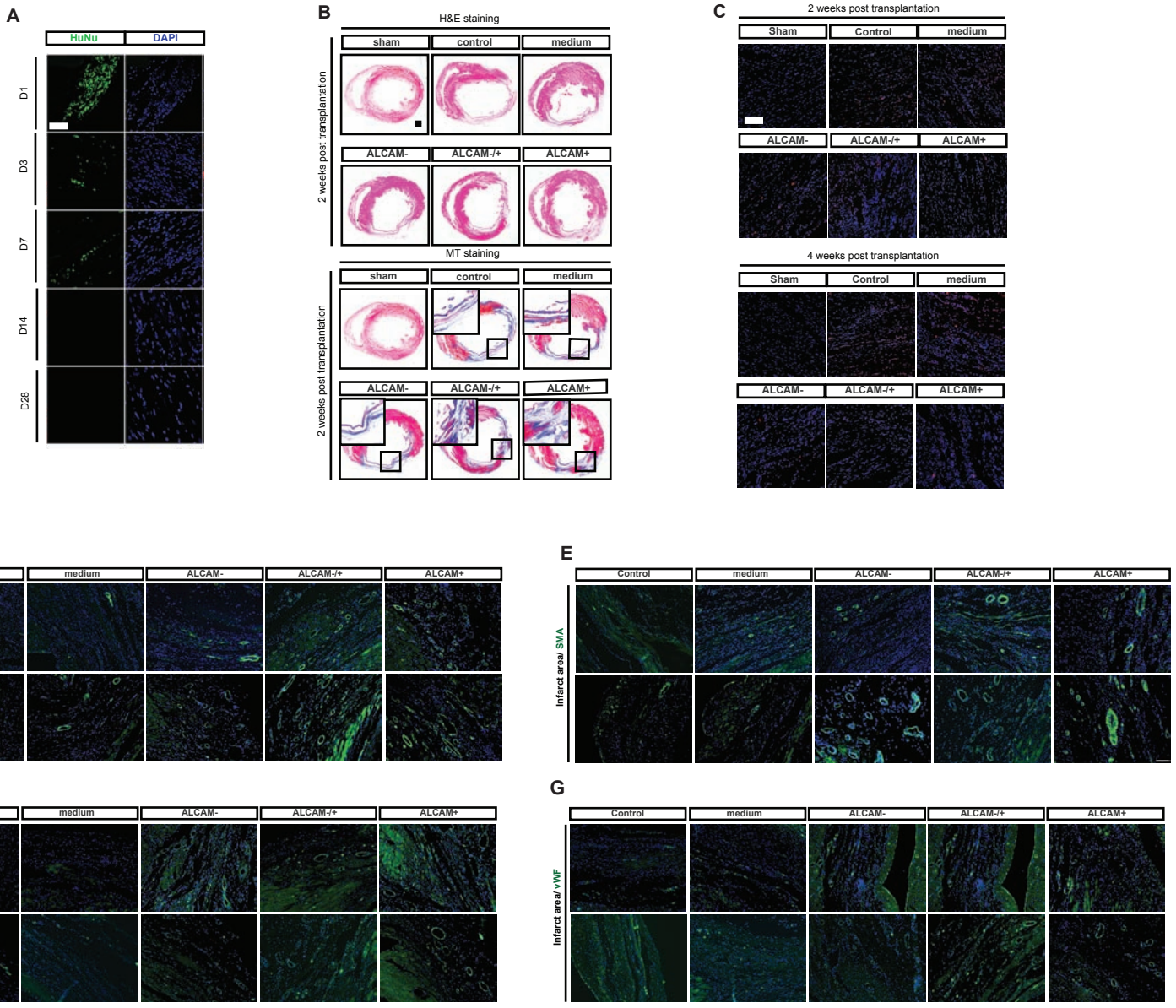

Figure S5 (Ghazizadeh et al.)