

**Supplemental Information**

**Human ISL1<sup>+</sup> Ventricular Progenitors Self-  
Assemble into an *In Vivo* Functional Heart Patch  
and Preserve Cardiac Function Post Infarction**

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Fig. S1

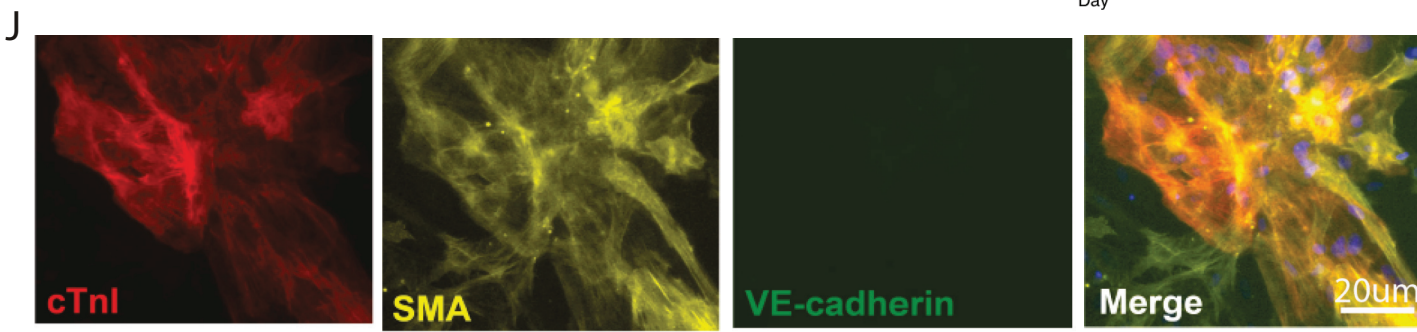
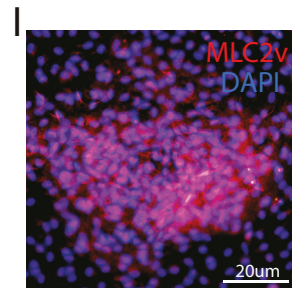
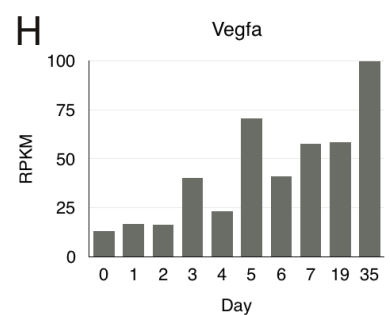
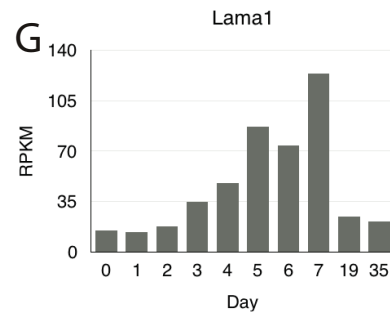
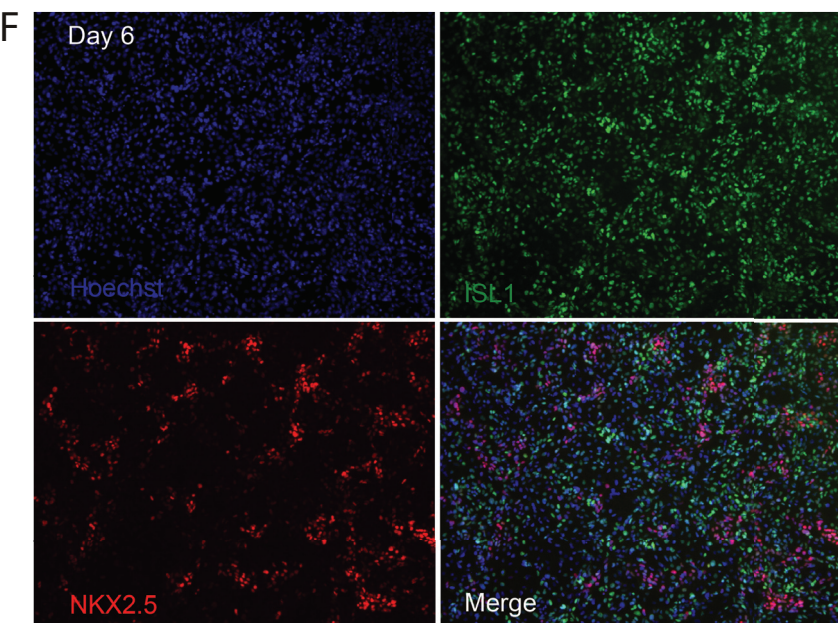
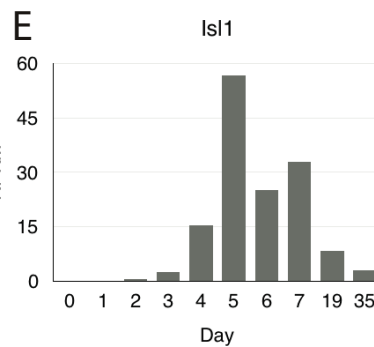
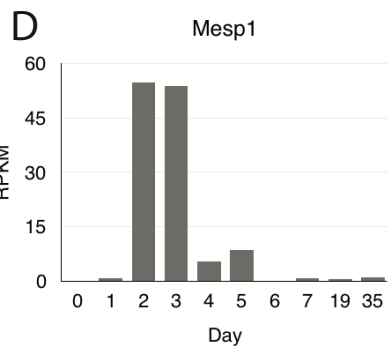
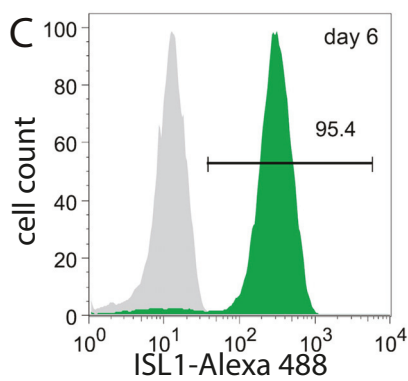
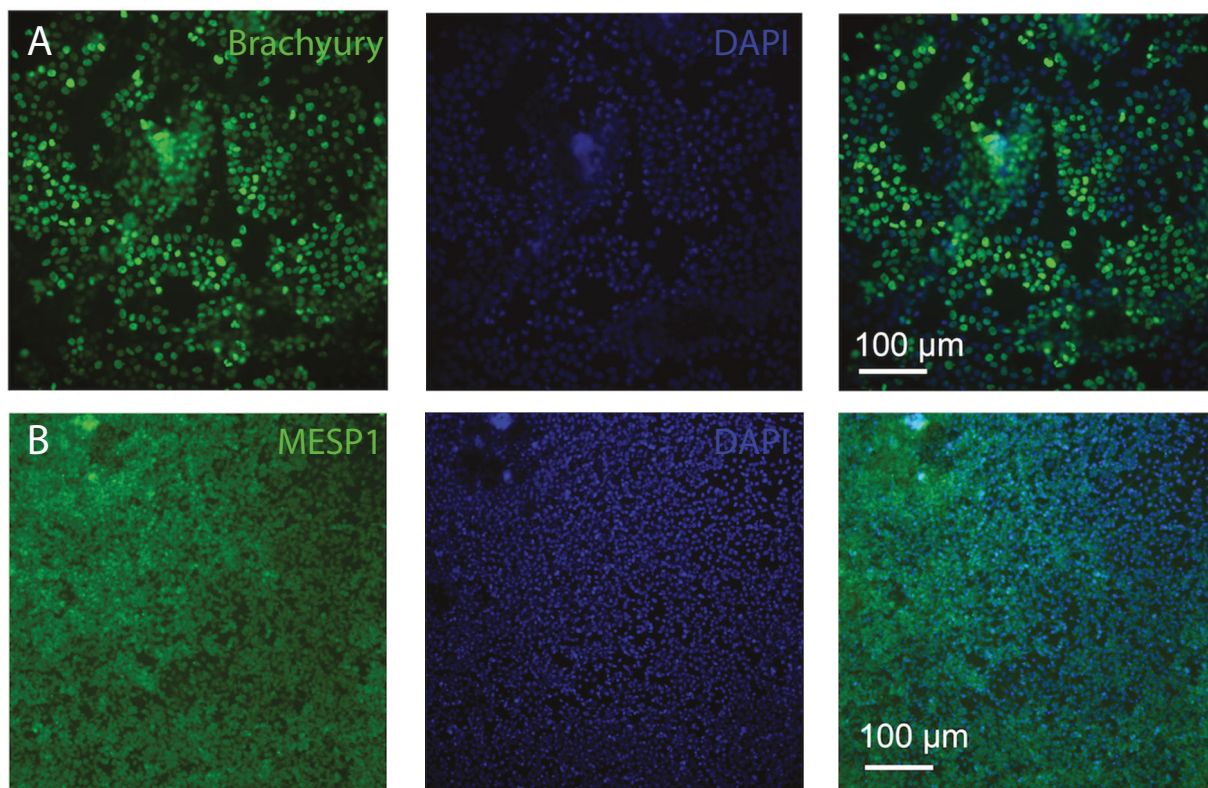
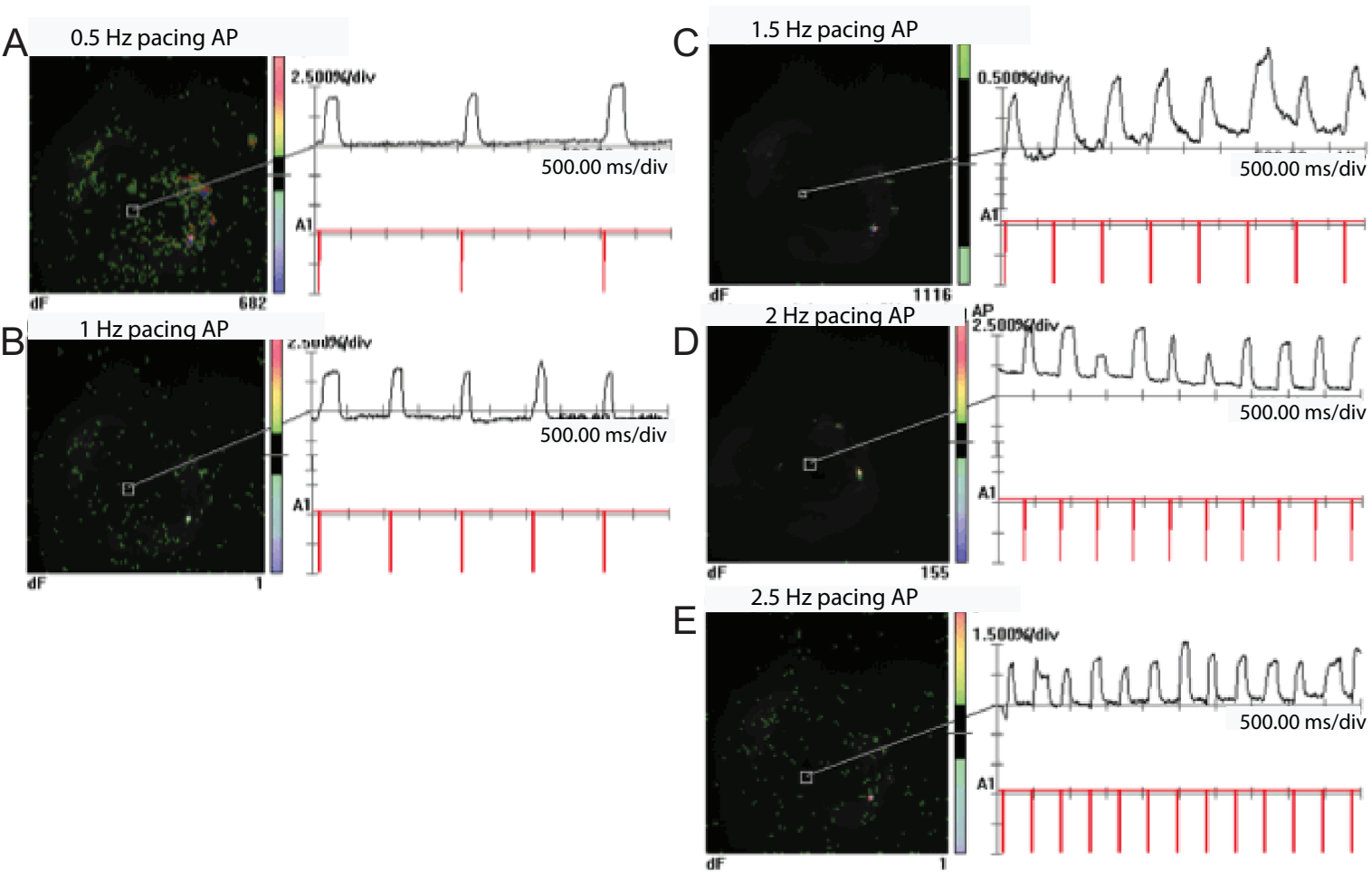




Fig. S3



### Figure S1

(A) ES03 hESCs differentiated as illustrated in Fig. 1a and dissociated on day 1 showed Brachyury expression by immunostaining (staining with DAPI in middle panel, co-localization at right, scale bar = 100 $\mu$ m). (B) ES03 hESCs differentiated as illustrated in Fig. 1a and dissociated on day 3 showed *Mesp1* expression by immunostaining. Scale bar = 100 $\mu$ m. (C) ES03 hESCs differentiated as illustrated in Fig. 1a dissociated on day 6 showed ISL1 expression by flow cytometry. (D, E) RNA-seq analysis of *Mesp1* (D), and ISL1 (E) expression during HVP differentiation. (F) Immunostaining analysis of ISL1 and NKX2.5 expression in cells differentiated from ES03 at day 6. (G, H) RNA-seq analysis of the laminin subunit alpha 1 (LAMA1) (G) and vascular endothelial growth factor A (VEGFA) (H) expression during the HVP differentiation process. (I) Immunohistochemistry staining of MLC2v (red) of HVPs FACS purified for LIFR on day 6 and subsequently differentiated to day 15 *in vitro*. (J) One single day 6 ISL1<sup>+</sup> cell differentiated from ES03 cell line was added into one well of a 48-well plate and further cultured Three weeks later, cells were analyzed for cTnI, SMA, and VE-cadherin expression by immunohistochemistry. Scale bar = 20  $\mu$ m.

### Figure S2

RNA-seq analysis of expression of selected developmental genes during HVP differentiation. RNA was sampled daily from day 0 to day 7 and again on day 19. Day 35 served as a control for later stage cardiomyocytes. Analyses were performed on two parallel batches of cells undergoing simultaneous differentiation to provide two biological replicates on each day.

### Figure S3

*Ex-vivo* optical mapping of action potentials in 7 week-old HVP kidney graft attained by loading cells with voltage sensitive dye. HVP kidney patches were observed to be electrically responsive across a range of pacing frequencies: (A) 0.5Hz, (B) 1Hz, (C) 1.5Hz, (D) 2Hz, and (E) 2.5Hz.

### Movie S1

Video showing uniform beating wave of day 15 HVPs derived from the NKX2.5-GFP cell line.

### Movie S2

Video of FAC-sorted LIFR<sup>+</sup> HVPs. Cells were sorted on day 6, and subsequently cultured until day 12 when they began to beat.

### Movie S3

*In vitro* optical mapping on day 18 of HVP differentiation. Cells were observed to exhibit spontaneous action potentials, but were also responsive to electrical pacing at 1Hz.

### Movie S4

*In vitro* optical mapping on day 36 of HVP differentiation. Cells were observed to exhibit spontaneous action potentials, but were also responsive to electrical pacing at 1Hz.

**Movie S5**

Video of *in vitro* optical mapping of calcium transients in day 18 HVPs showing spontaneous calcium activity.

**Movie S6**

Video of *in vitro* optical mapping of calcium transients in day 18 HVPs, showing electrical responsiveness during 1Hz pacing.

**Movie S7**

Ultrasound video of *in vivo* contractions in 6+ week old HVP kidney graft patch. The video was recorded under respiratory gating to minimize movement artefacts caused by breathing.

**Movie S8**

Magnetic resonance imaging cine video of HVP-treated post-MI heart at 2 months following transplantation, imaged at the mid-ventricular region.

**Movie S9**

Magnetic resonance imaging cine video of placebo-treated post-MI heart at 2 months following transplantation, imaged at the mid-ventricular region.

**Table S1**

<b>Pharmacological agent</b>	<b>Concentration (uM)</b>	<b>Conduction velocity (cm/s)</b>	<b>% Conduction Velocity change</b>	<b>APD90 (ms)</b>
<b>Isoproterenol (n=2)</b>	0	4		57 ± 18
	0.01		167 ± 230	590 ± 115
	0.1		43 ± 7	562 ± 51
	1		149 ± 99	521 ± 68
<b>Acetylcholine (n=2)</b>	0	1 ± 0.6		281 ± 33
	0.01		-52 ± 33	322 ± 117
	0.1		-70 ± 20	208 ± 56
	1		-81 ± 31	200 ± 65
<b>Nifedipine (n=3)</b>	0	6 ± 4		435 ± 172
	0.01		-52.34 ± 55	425 ± 172
	0.1		-47.0 ± 2.6	366 ± 153
	1		-81.4 ± 2.0	239 ± 150
<b>Heptanol (n=3)</b>	0	2.78 ± 1.15		174 ± 12
	1000	1.42 ± 0.59	-50.6 ± 25	176 ± 23

**Comparison of AP parameters in response to pharmacological agents *in vitro*.**

Various pharmacological agents were applied *in vitro* to differentiated Day 15+ HVPs to examine their response. The  $\beta$ -adrenergic agonist isoproterenol increased conduction velocity, while acetylcholine shortened APD and decreased conduction velocity. The calcium channel blocker Nifedipine shortened APD and decreased conduction velocity, while the gap junction blocker Heptanol decreased conduction velocity.