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Supplemental Information

Human ISL1^{+} Ventricular Progenitors Self-Assemble into an *In Vivo* Functional Heart Patch and Preserve Cardiac Function Post Infarction

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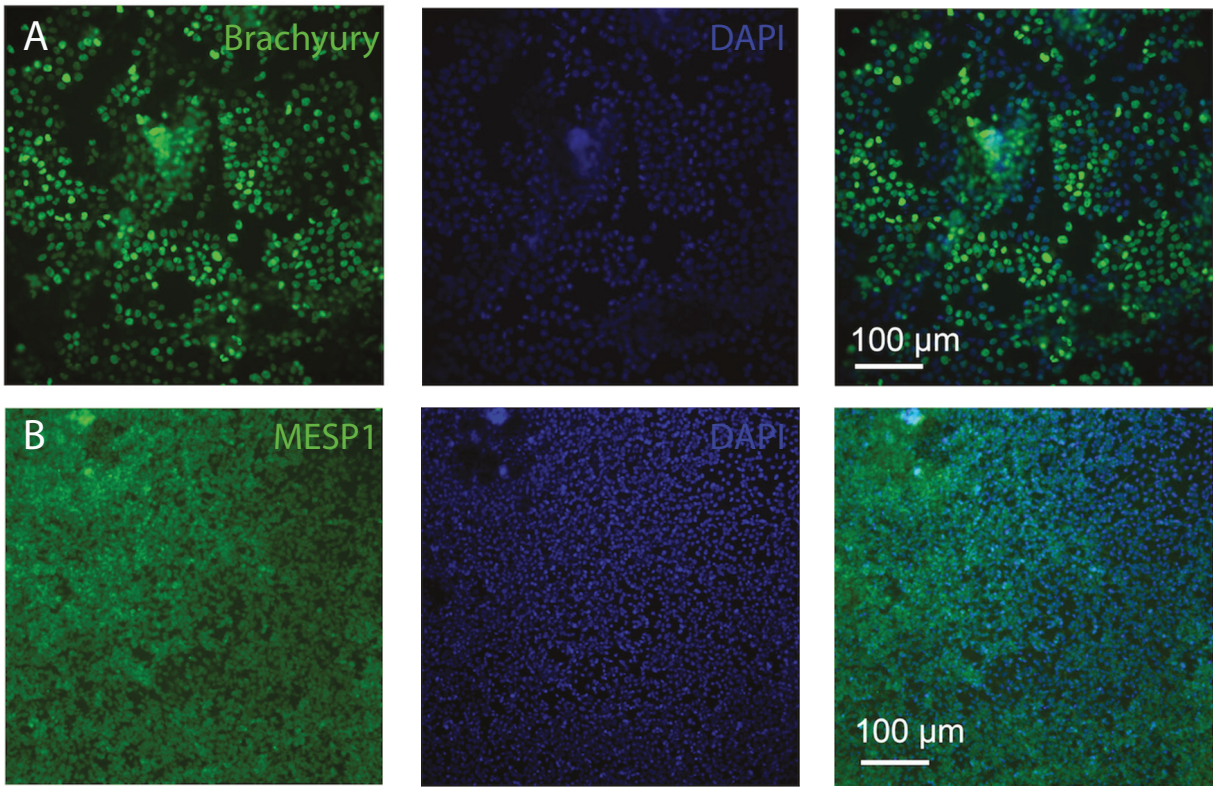
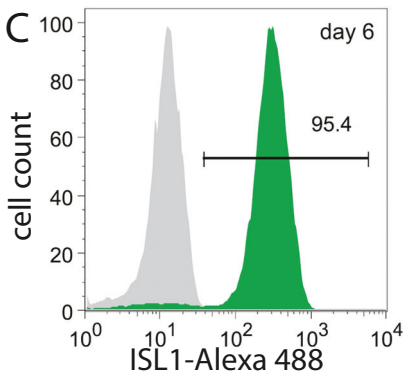
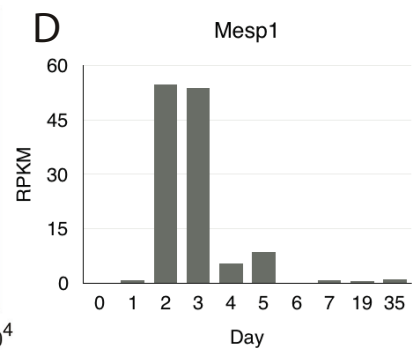
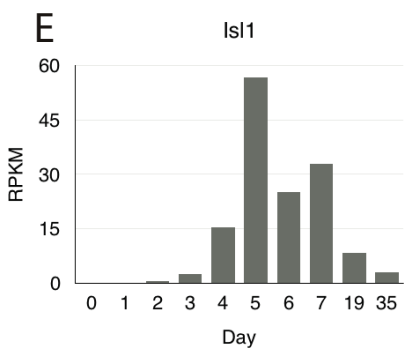
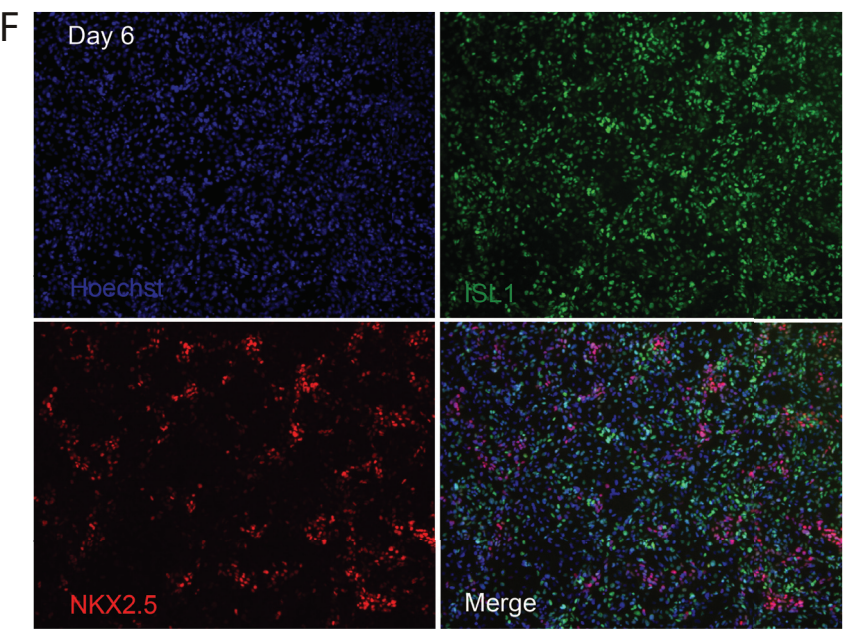
Fig. S1

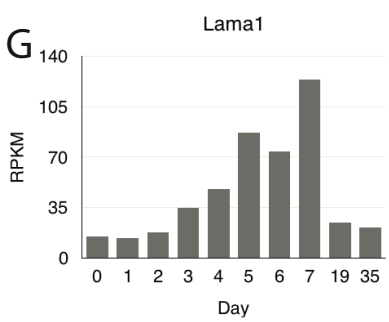

A
Brachyury
DAPI
100 μm
B
MESP1
DAPI
100 μm

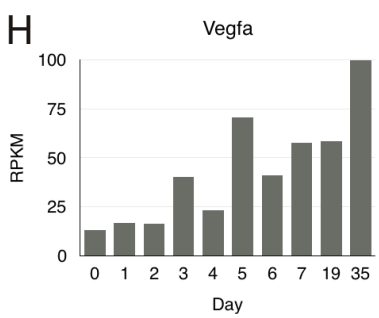

C
day 6
95.4
cell count
ISL1-Alexa 488

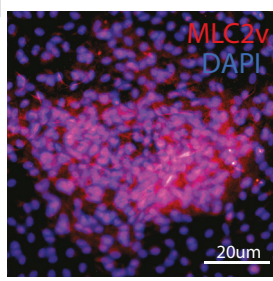

D
Mesp1
RPKM
Day


E
Isl1
RPKM
Day


F
Day 6
Hoechst
ISL1
NKX2.5
Merge


G
Lama1
RPKM
Day


H
Vegfa
RPKM
Day


I
MLC2v
DAPI
20um

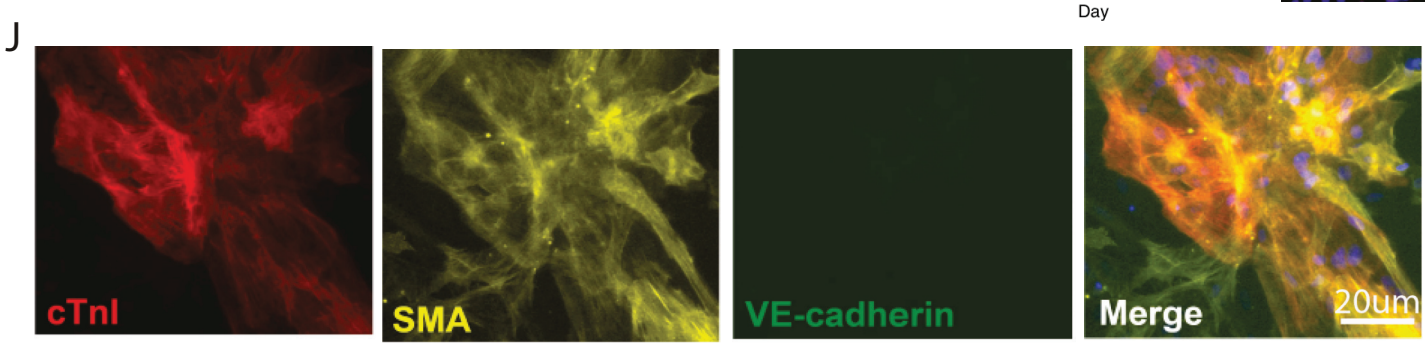

J
cTnI
SMA
VE-cadherin
Merge
20um

Fig. S2

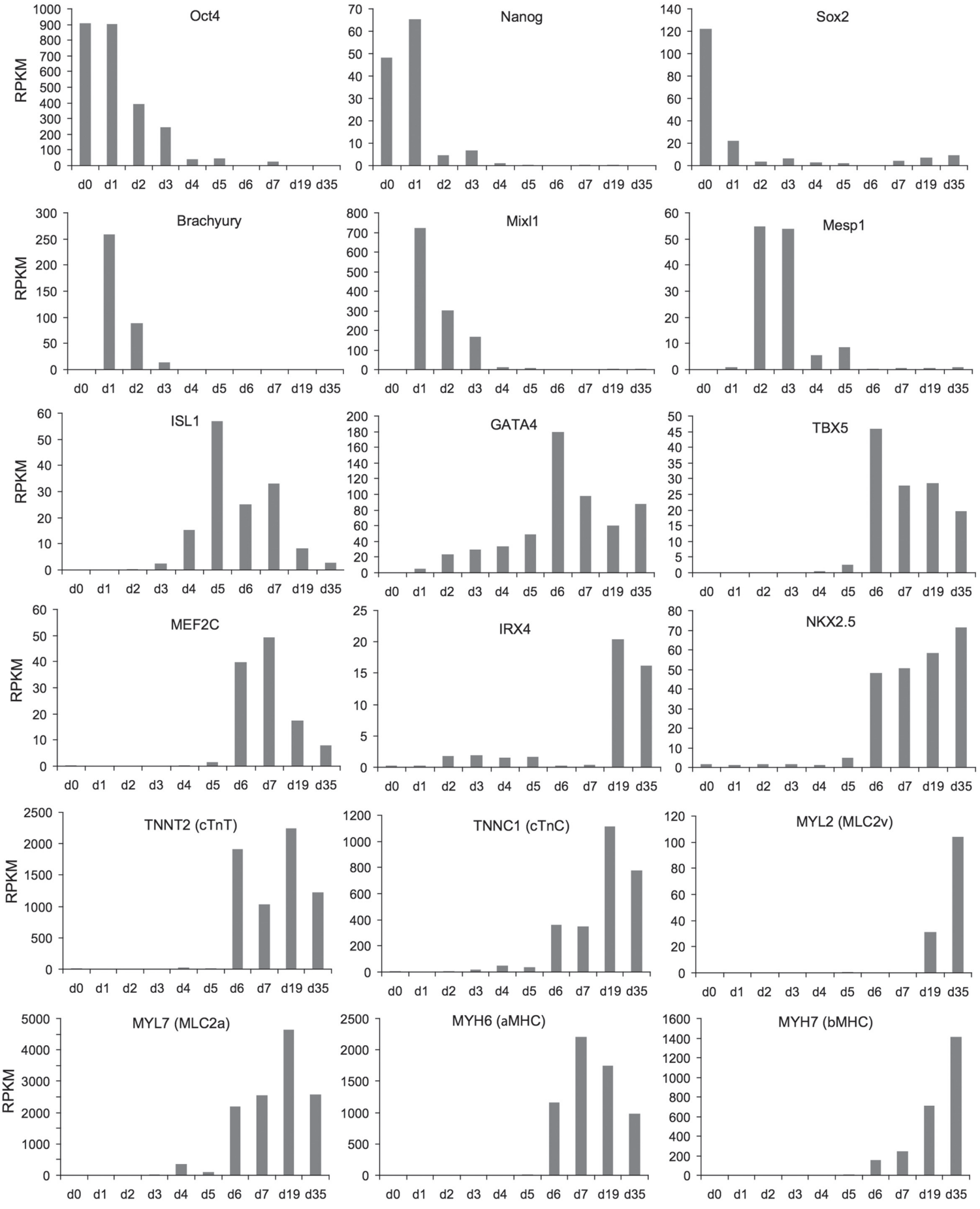

Oct4
Nanog
Sox2
Brachyury
Mixl1
Mesp1
ISL1
GATA4
TBX5
MEF2C
IRX4
NKX2.5
TNNT2 (cTnT)
TNNC1 (cTnC)
MYL2 (MLC2v)
MYL7 (MLC2a)
MYH6 (aMHC)
MYH7 (bMHC)
RPKM
d0 d1 d2 d3 d4 d5 d6 d7 d19 d35

Fig. S3

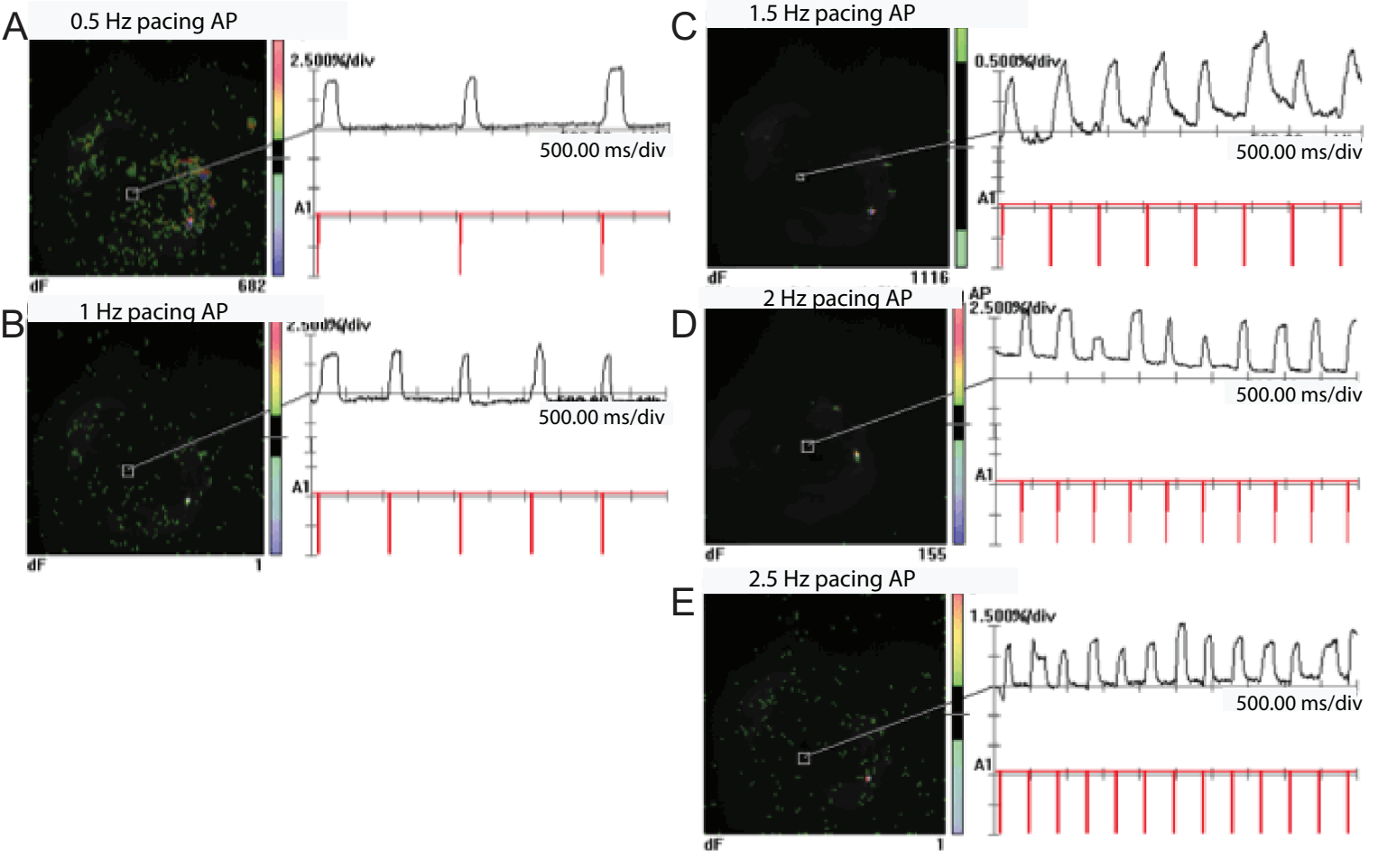

A
0.5 Hz pacing AP
2.500%/div
500.00 ms/div
A1
dF
682
B
1 Hz pacing AP
500.00 ms/div
A1
dF
1
C
1.5 Hz pacing AP
0.500%/div
500.00 ms/div
A1
dF
1116
D
2 Hz pacing AP
AP
2.500%/div
500.00 ms/div
A1
dF
155
E
2.5 Hz pacing AP
1.500%/div
500.00 ms/div
A1
dF
1

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 = 100μm). (**B**) ES03 hESCs differentiated as illustrated in Fig. 1a and dissociated on day 3 showed Mesp1 expression by immunostaining. Scale bar = 100μm. (**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 $ISL1^+$ 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 μm.

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+ 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

Pharmacological agent	Concentration (uM)	Conduction velocity (cm/s)	% Conduction Velocity change	APD90 (ms)
Isoproterenol (n=2)	0	4		57 ± 18
	0.01		167 ± 230	590 ± 115
	0.1		43 ± 7	562 ± 51
	1		149 ± 99	521 ± 68
Acetylcholine (n=2)	0	1 ± 0.6		281 ± 33
	0.01		-52 ± 33	322 ± 117
	0.1		-70 ± 20	208 ± 56
	1		-81 ± 31	200 ± 65
Nifedipine (n=3)	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 ±1 50
Heptanol (n=3)	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 β-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.