

Supplementary Table 1. Ventricular arrhythmia characteristics

Subject	Re-entrant arrhythmia inducible at terminal EPS	Automatic arrhythmia present at terminal EPS	CMR assessed scar size (%)	Longest run of arrhythmia identified on telemetry analysis (hours)
<i>Sham</i>				
<i>Sham #1</i>	No	No	0	n/a
<i>Sham #2</i>	No	No	1.2	n/a
<i>Vehicle</i>				
Vehicle #1	Yes	No	10.9	n/a
Vehicle #2	Yes	No	7	n/a
Vehicle #3	Yes	No	14.7	n/a
Vehicle #4	No	No	1.3	n/a
Vehicle #5	No	No	0	n/a
<i>Vehicle + AA</i>				
Vehicle + AA #1	Yes	No	16.0	n/a
Vehicle + AA #2	No	No	13.6	n/a
Vehicle + AA #3	Yes	No	11.8	n/a
Vehicle + AA #4	No	No	0.8	n/a
<i>PSC-CM</i>				

PSC-CM #1	Yes	Yes	7.1	24
PSC-CM #2	Yes	Yes	11.8	7
PSC-CM #3	No	Yes	0.2	17
PSC-CM #4	Yes	No	15.4	12
PSC-CM #5	Yes	Yes	15.2	22
<i>PSC-CM + AA</i>				
PSC-CM + AA #1	Yes	No	19.8	3
PSC-CM + AA #2	No	No	9.8	4
PSC-CM + AA #3	Yes	No	25.2	1
PSC-CM + AA #4	Yes	No	22.8	1
PSC-CM + AA #5	No	No	6.0	1
<i>RA-PSC-CM</i>				
RA-PSC-CM #1	n/a	n/a	n/a	72
RA-PSC-CM #2	n/a	n/a	n/a	24
RA-PSC-CM #3	Yes	No	n/a	66

Supplementary Table 2. CMR scar size and volumes for Phase 1 large animal experiments.

Subject	Timepoint	Scar Size (% of LV mass)	LVEDV (mL)	LVESV (mL)	LVSV (mL)	LVEF (%)	RVEF (%)
<i>Sham</i>							
Sham #1	Baseline	0	77	31	46	59	56
	Follow-up	0	86	35	51	59	56
Sham #2	Baseline	1.2	77	34	43	57	58
	Follow-up	1.6	85	37	48	57	58
Mean (± SEM)	Baseline	0.6 (0.6)	77 (0)	32.5 (1.5)	44.5 (1.5)	58 (1.0)	57 (1.0)
	Follow-up	0.8 (0.8)	85.5 (0.5)	36 (1.0)	49.5 (1.5)	58 (1.0)	57 (1.0)
<i>Vehicle</i>							
Vehicle #1	Baseline	8.9	95	53	42	44	45
	Follow-up	10.9	109	61	48	44	44
Vehicle #2	Baseline	6.6	71	39	32	46	61
	Follow-up	7.0	100	55	45	46	52
Vehicle #3	Baseline	14.7	102	63	39	39	52
	Follow-up	13.1	123	74	49	40	56
Mean (± SEM)	Baseline	10.1 (2.4)	89.3 (9.4)	51.7 (7.0)	37.7 (3.0)	43 (2.1)	52.7 (4.6)

	Follow-up	10.3 (1.8)	110.7 (6.7)	63.3 (5.6)	47.3 (1.2)	43.3 (1.8)	50.7 (3.5)
<i>Vehicle + AA</i>							
Vehicle + AA #2	Baseline	16.0	82	49	33	40	61
	Follow-up	12.7	109	57	52	47	71
Vehicle + AA #3	Baseline	13.6	134	90	44	33	67
	Follow-up	20.0	143	90	53	37	61
Vehicle + AA #4	Baseline	11.8	94	43	51	55	65
	Follow-up	6.7	131	74	57	59	68
Mean (± SEM)	Baseline	13.8 (1.2)	103.3 (15.7)	60.7 (14.8)	42.7 (5.2)	42.7 (6.5)	64.3 (1.8)
	Follow-up	13.1 (3.8)	127.7 (10.0)	73.7 (9.5)	54 (1.5)	47.7 (6.4)	66.7 (3.0)
<i>PSC-CM</i>							
PSC-CM #1	Baseline	12.1	89	59	30	33	54
	Follow-up	7.1	105	60	45	42	59
PSC-CM #2	Baseline	10.6	105	60	45	43	48
	Follow-up	11.8	126	70	56	44	61
PSC-CM #4	Baseline	13.4	109	68	41	38	60
	Follow-up	15.4	134	80	54	41	73

PSC-CM #5	Baseline	15.2	99	58	41	42	55
	Follow-up	13.9	138	67	71	51	56
Mean ($\pm$ SEM)	Baseline	12.8 (1.0)	100.5 (4.3)	61.3 (2.8)	39.3 (4.5)	39.0 (2.3)	54.3 (2.4)
	Follow-up	11.8 (1.8)	125.8 (7.4)	69.2 (5.8)	56.3 (3.4)	44.5 (2.3)	62.3 (3.7)
<i>PSC-CM + AA</i>							
PSC-CM + AA #1	Baseline	22.2	108	76	32	31	51
	Follow-up	19.8	133	87	46	35	60
PSC-CM + AA #2	Baseline	9.8	106	50	56	53	59
	Follow-up	9.8	113	37	76	67	64
PSC-CM + AA #3	Baseline	20.3	149	108	41	28	45
	Follow-up	25.2	215	142	73	34	53
PSC-CM + AA #4	Baseline	22.8	121	74	47	39	58
	Follow-up	n/a	162	86	76	47	54
PSC-CM +AA #5	Baseline	6.0	85	49	36	42	58
	Follow-up	1.6	118	45	73	62	64
Mean ($\pm$ SEM)	Baseline	16.2 (3.5)	113.8 (10.5)	71.4 (10.8)	42.4 (4.2)	38.6 (4.4)	54.2 (2.7)
	Follow-up	14.1 (5.2)	148.2 (18.8)	79.4 (18.7)	68.8 (5.7)	49.0 (6.8)	59.0 (2.4)

<i>Excluded Subjects (Scar size < 1.5%)</i>							
Vehicle #4	Baseline	1.3	108	60	48	44	56
Vehicle #5	Baseline	0	77	36	41	53	64
Vehicle + AA #1	Baseline	0.8	66	28	38	58	64
PSC-CM #3	Baseline	0.2	83	45	39	47	57
Mean (± SEM)	Baseline	0.6 (0.3)	83.5 (8.9)	42.2 (6.9)	41.5 (2.3)	50.5 (3.1)	60.3 (2.2)

Supplementary Table 3. Antibodies used for high-parameter flow cytometry experiments.

Company	Marker	Clone	Fluorophore	Dilution	Significance
	GCaMP		eGFP		Genetically encoded, intracellular calcium transient marker ¹
					hPSC-CM lineage marker ²
BD Biosciences	VCAM-1	51-10C9	PerCP-Cy5.5	1:100	SIRPA ⁺ /VCAM1 ⁺ phenotype indicates committed CM ³
Biolegend	SIRPα	SE5A5	PECy5	1:100	hPSC-CM marker ^{3, 4}
ThermoFisher Scientific	Desmoglein 2	CSTEM28	PE	1:200	Desmosomal protein, highly expressed in CMs ⁵
Biolegend	CD31	WM59	PECy7	1:100	Endothelial cell marker ⁶
BD Biosciences	CD235a	GA-R2(HIR2)	BUV395	1:100	Early marker of mesoderm that gives rise to ventricular CM's ⁷
Biolegend	CD90	5E10	BV650	1:100	Cardiac fibroblast marker, although non-specific (also expressed on endothelium, mesenchymal stem cells) ⁸

Biolegend	CD77	5B5	BV510	1:100	CD77 ⁺ /CD200 ⁻ suggests ventricular CM phenotype ^{9, 10}
Biolegend	CD13	WM15	BV786	1:100	Early cardiac mesoderm marker ¹¹
Biolegend	CD200	OX-104	BV421	1:100	CD77 ⁺ /CD200 ⁻ suggests ventricular CM phenotype ^{9, 10}
Biolegend	CD34	581	AF700	1:100	Endothelial cell and progenitor marker ^{3, 6}
Abcam	Vinculin	EPR8185	AF647	1:200	Adhesion marker, links CM contractile apparatus to adherens junctions (intracellular) ¹²
BD Biosciences	Cardiac Troponin T	13-11	BV421	1:20	A regulatory protein of the cardiomyocyte contractile apparatus and pan-CM marker (intracellular) ¹³
Biolegend	Zombie Viability		NIR	1:1000	Live/dead stain

Supplementary Table 4. Radiofrequency ablation parameters for catheter ablation subjects.

<i>PSC-CM + CA #1 (Day 13 post-injection)</i>					
Ablation number	Impedence – start (Ω)	Impedence – end (Ω)	Power (W)	Duration (sec)	Notes
1	170	162	30	40	
2	180	165	30	30	
3	175	160	30	60	VF induced – 3x 250J shocks required, successfully defibrillated to EA with 3 rd shock
4	165	200	30	24	
5	170	205	30	18	
6	170	204	30	24	
7	170	158	30	30	
8	168	150	30	21	Sinus rhythm restored
<i>PSC-CM + CA#3 (Day 5 post-injection)</i>					
1	n/a	231	40	21	
2	n/a	153	40	18	VF induced – successfully defibrillated with 200J shock

3	205	199	40	28	VF induced – successfully defibrillated with 200J shock
4	175	202	40	22	VF induced – successfully defibrillated with 200J shock
5	179	178	40	15	
6	170	158	40	19	VF induced – successfully defibrillated with 200J shock
7	180	158	40	15	VF induced – successfully defibrillated with 200J shock
8	170	156	40	9	VF induced – successfully defibrillated with 200J shock
9	190	171	40	8	
10	170	160	40	13	
11	180	167	40	20	VF induced – successfully defibrillated with 200J shock
12	175	165	40	16	
13	160	168	40	19	

14	195	147	40	18	
15	155	196	40	18	VF induced – successfully defibrillated with 200J shock
16	155	169	40	16	VF induced – successfully defibrillated with 200J shock
17	165	165	40	14	
18	166	152	30	23	
19	155	163	30	15	VF induced – successfully defibrillated with 200J shock
20	160	143	30	20	VF induced – successfully defibrillated with 200J shock
21	156	143	30	20	
22	155	145	30	26	
23	168	152	30	12	VF induced – successfully defibrillated with 200J shock
24	166	143	30	22	VF induced – successfully defibrillated with 200J shock

25	155	156	30	26	VF induced – successfully defibrillated with 200J shock
<i>RA-PSC-CM #3 – Catheter Ablation 1 for EA 1 (Day 10 post-injection)</i>					
1	220	237	40	15	Impedance cut-off
2	190	165	40	30	
3	200	165	40	30	
4	220	157	40	30	VF induced – successfully defibrillated with 250J shock back to EA1
5	158	242	40	25	
6	161	259	40	14	
7	170	246	40	12	
8	160	249	40	27	
9	206	197	40	30	
10	167	240	40	17	
11	164	247	40	17	
12	173	156	40	30	
13	153	158	40	14	

14	162	156	40	30	
15	160	149	40	30	
16	180	154	40	30	Sinus rhythm restored
<i>RA-PSC-CM #3 – Catheter Ablation 2 for EA 2 and EA 3 (Day 14 post-injection)</i>					
1	209	182	40	20	
2	182	171	40	40	VF induced – successfully defibrillated with 250J shock back to EA2
3	237	188	40	40	Sinus rhythm restored however onset of new EA (EA3) shortly after
4	246	159	40	16	VF induced – successfully defibrillated with 250J shock back to EA3
5	167	153	40	40	Sinus rhythm restored
6	160	185	40	19	Empiric ablation
7	160	152	40	38	Empiric ablation

Supplementary Table 5. Characterisation of working cell bank

Characterisation	Method	Result
Pluripotency	Flow cytometry	>90% positive for each of the markers Oct4, Sox2, Tra-1-60 and SSEA4
	Immunofluorescence	>80% positive for Oct4, Nanog and Tra-1-60
Mycoplasma	Lonza Mycoalert Mycoplasma detection assay	Negative
Karyotype	Stem Cell Technologies Human Pluripotent Stem Cells Genetic Analysis Kit (Product No. #07550)	Negative for 8 common deletions and duplications found in human pluripotent cells
Quantitative evaluation of differentiation capacity to cardiomyocytes	Suspension based differentiation using small molecules	Positive detection (>80%) ^ of CM specific cardiac troponin using flow cytometry and ICC.
Post thaw viability	N/A	Confluent T75 5 days after thaw of cryopreserved vial.

^ one-off measurement of cardiac troponin to establish suitability of working cell bank for cardiomyocyte differentiation.

Supplementary Table 6. Genes and primers used for qPCR experiments.

Gene	Forward Primer	Reverse Primer
OCT3/4	AGCGATCAAGCAGCGACTAT	AGAGTGGTGACGGAGACAGG
NANOG	ACCTTCCAATGTGGAGCAAC	GAGAATTTGGCTGGAAGTGC
DNMT3B	TCCTCAAAGAGTTGGGCATAA	TTTGATATTCCCCTCGTGCT
HAND1	GCCTAGCCACCAGCTACATC	ATCCGCCTTCTTGAGTTCAG
GATA6	AGAGCACCAATCCCGAGAAC	GCACGGAGGACGTGACTT
EOMES	ACCCCCTTCCATCAAATCTC	CCATGCCTTTTGAGGTGTCT
MESP1	AGCCCAAGTGACAAGGGACAAC	AAGGAACCACTTCGAAGGTGCTGA
Brachyury (T)	CCTTGCTCACACCTGCAGTAGC	GGCCAACTGCATCATCTCCA
ISL1	GAAGGTGGAGCTGCATTGGTTTGA	TAAACCAGCTACAGGACAGGCCAA
SIRPA	ACCTGGCTCAGGCTAGTTCCAAAT	TGTGCACACGTATGTGCTGTCTCT
CTNT	TTCACCAAAGATCTGCTCCTCGCT	TTATTACTGGTGTGGAGTGGGTGTGG
MYH6	TCAGCTGGAGGCCAAAGTAAAGGA	TTCTTGAGCTCTGAGCACTCGTCT
MYL7	ACATCATCACCCATGGAGACGAGA	GCAACAGAGTTTATTGAGGTGCCC

MYH7	TCGTGCCTGATGACAAACAGGAGT	ATACTCGGTCTCGGCAGTGACTTT
NXK2-5	TTTGCATTCACTCCTGCGGAGACCTA	ACTCATTGCACGCTGCATAATCGC
MYL2v	TGTCCCTACCTTGTCTGTTAGCCA	ATTGGAACATGGCCTCTGGATGGA
NPPA	GGGTCTCTGCTGCATTTGTGTCAT	AGAGGCGAGGAAGTCACCATCAAA
KCNJ3	TCATCAAGATGTCCCAGCCCAAGA	CACCCGGAACATAAGCGTGAGTTT
HCN4	TCTTCCTCATTGTGGAGACACGCA	TGAGGATCTTCGTGAAGCGGACAA
SHOX2	AAGAGGATGCGAAAGGGATG	TGAGTTGTTCCAGGGTGAAAT

Supplementary Table 7. Single cell RNA sequencing hashtag barcode sequences

Sample batch	Antibody	Dilution	Sequence	Cat. No.
Sample 29	Anti-human Hashtag 1 Antibody	1:100	GTCAACTCTTTAGCG	394601
Sample 32	Anti-human Hashtag 2 Antibody	1:100	TGATGGCCTATTGGG	394603
Sample 65	Anti-human Hashtag 3 Antibody	1:100	TTCCGCCTCTCTTTG	394605
Sample 66	Anti-human Hashtag 4 Antibody	1:100	AGTAAGTTCAGCGTA	394607
Sample 67	Anti-human Hashtag 5 Antibody	1:100	AAGTATCGTTTCGCA	394609
Sample 68	Anti-human Hashtag 6 Antibody	1:100	GGTTGCCAGATGTCA	394611
Sample 70	Anti-human Hashtag 7 Antibody	1:100	TGTCTTTCCTGCCAG	394613
Sample 71	Anti-human Hashtag 8 Antibody	1:100	CTCCTCTGCAATTAC	394615
Sample 73	Anti-human Hashtag 9 Antibody	1:100	CAGTAGTCACGGTCA	394617
Sample 79	Anti-human Hashtag 10 Antibody	1:100	ATTGACCCGCGTTAG	394619
Sample 88/90	Anti-human Hashtag 12 Antibody	1:100	TAACGACCAGCCATA	394623
Sample 86RA	Anti-human Hashtag 14 Antibody	1:100	CTGTATGTCCGATTG	394627
Sample 88RA	Anti-human Hashtag 15 Antibody	1:100	TAAGATTCAGAGCGA	394629

Supplementary Table 8. Antibodies used for immunohistochemistry experiments.

Company	Product	Marker (Primary) Fluorophore (Secondary)	Dilution	Antigen Retrieval (Primary) Host & Target (Secondary)
<i>Primary Antibodies</i>				
Dako	M0851	Alpha smooth muscle actin	1:200	Sodium citrate pH6
DSHB	CT3	Cardiac Troponin T	2µg/mL	Sodium citrate pH6
Invitrogen	PA5-47374	CD200	1:20	Sodium citrate pH6
Merck Millipore	AB1728	Connexin 43	1:100	Sodium citrate pH6
Cell Signalling Technology	C48E7	Ku80	1:500	Sodium citrate pH6
	91144S	Collagen, type I, alpha 1	1:200	Sodium citrate pH6
Synaptic Systems	311011	MLC-2a	1:200	Sodium citrate pH6
Abcam	ab79935	MLC-2v	1:100	Sodium citrate pH6
	ab260039	SIRPα	1:100	Sodium citrate pH6
	ab68167	Sarcomeric alpha actinin	1:500	Sodium citrate pH6

	ab28364	CD31	1:50	Sodium citrate pH6
	ab13970	GFP	1:150	Sodium citrate pH6
Sigma-Aldrich	C3865	N-Cadherin	1:225	Sodium citrate pH6
<i>Secondary Antibodies</i>				
ThermoFisher	A-11029	Alexa Fluor 488	1:500	Goat anti-mouse
	A-11034	Alexa Fluor 488	1:500	Goat anti-rabbit
	A-21245	Alexa Fluor 594	1:500	Goat anti-rabbit
	A-21245	Alexa Fluor 647	1:500	Goat anti-rabbit
	A-21236	Alexa Fluor 647	1:500	Goat anti-mouse
Abcam	ab150173	Alexa Fluor 488	1:500	Goat anti-chicken
	ab150176	Alexa Fluor 594	1:500	Goat anti-chicken
Life Technologies	A11058	Alexa Fluor 594	1:500	Donkey anti-goat