Supporting Information

Interaction of Cardiomyocytes from CCND2-overexpressing Human Induced Pluripotent Stem Cells with Electrically Conductive Hydrogels

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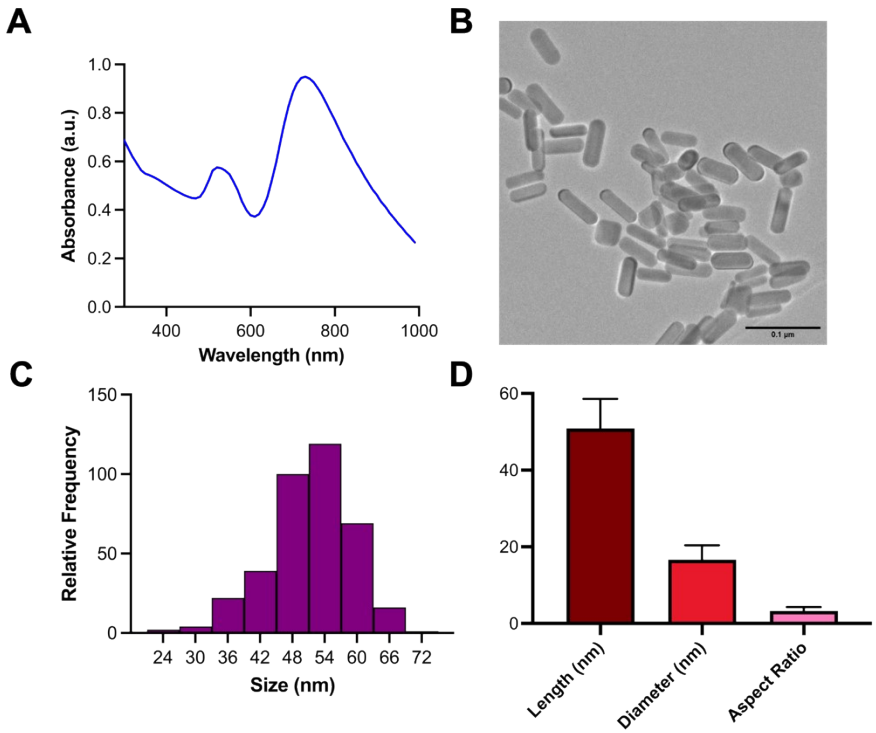

Figure S1. Synthesized GNR Size Measurement Analysis. (A) UV-Vis spectral scanning of the synthesized GNR. **(B)** TEM image of the GNRs. **(C)** Histogram of the size distribution of the GNRs. **(D)** The average length, diameter, and aspect ratio were found to be 50.9 ± 7.7 nm, 16.7 ± 3.7 nm, and 2.7. The error bar represents S.D.

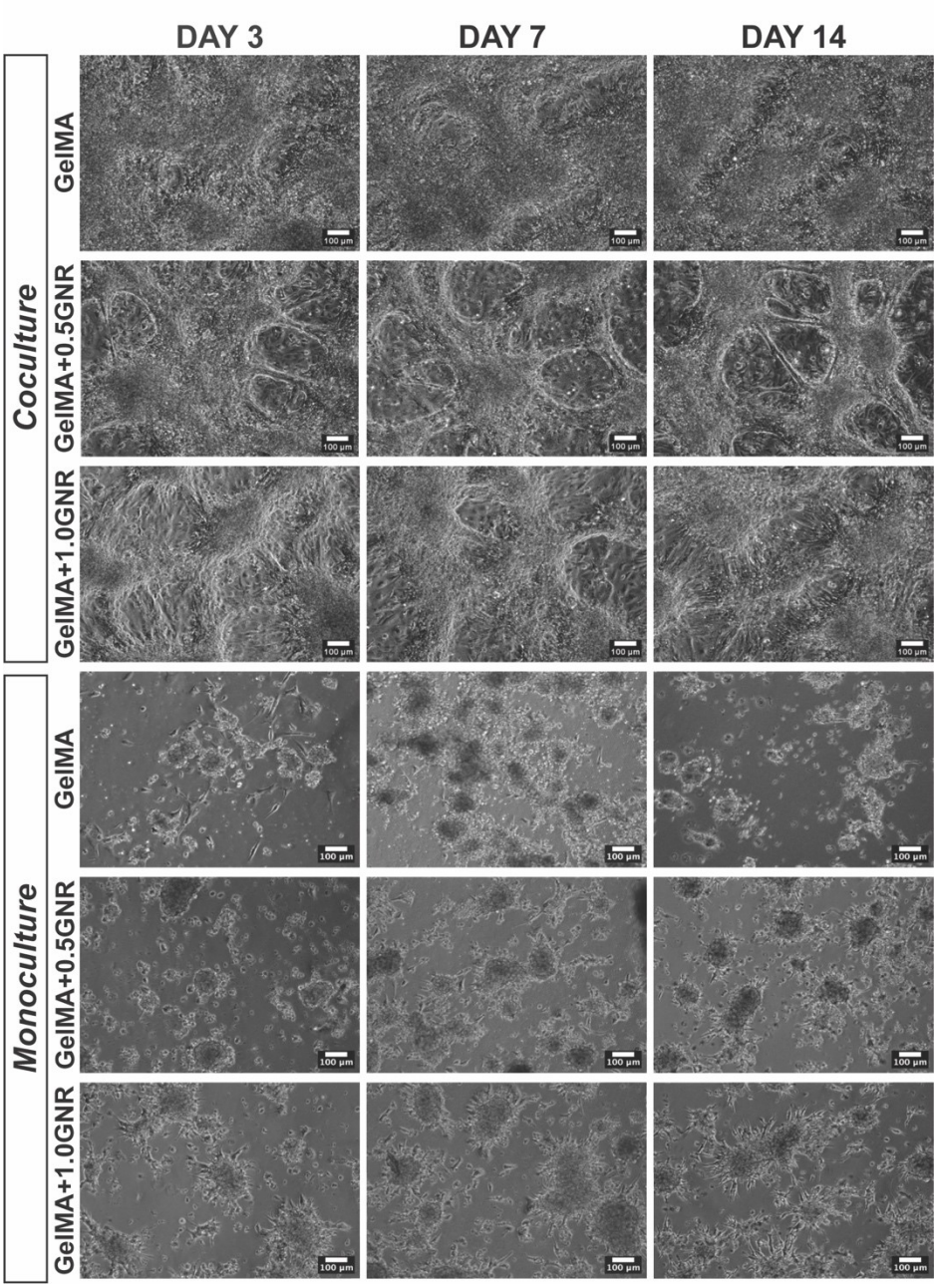

Figure S2. Engineered Cardiac Tissue Morphology. 3 x 3 Tile phase contrast images of the cardiac tissues formed on GelMA, GelMA+0.5GNR, GelMA+1.0GNR at days 3, 7, and 14 of culture. Scale bar: 100 µm.

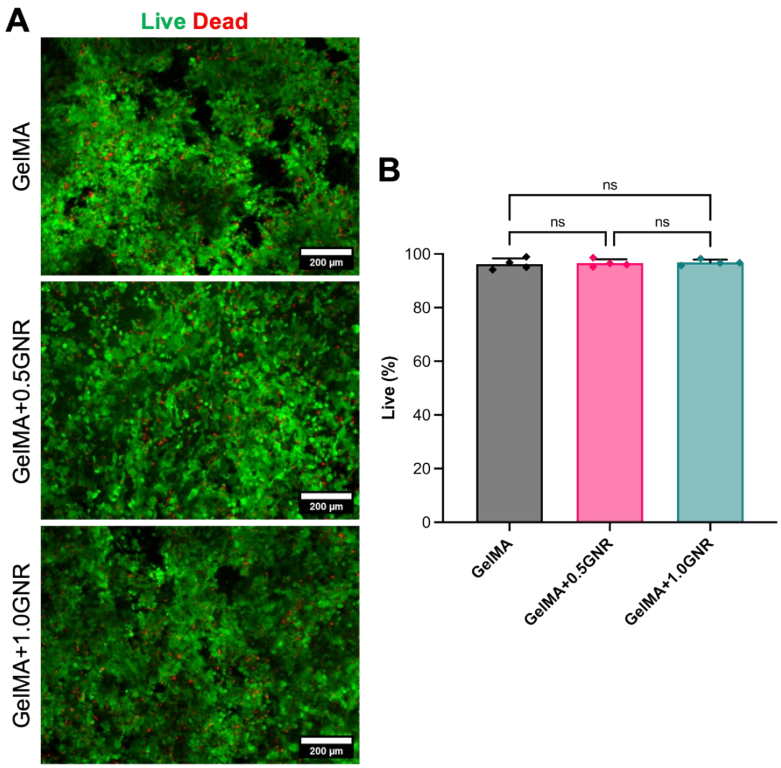

Figure S3 Cell Viability. (A) Live/Dead assay of cardiac tissue cultured on GelMA, GelMA+0.5GNR, and GelMA+1.0GNR hydrogels imaged on Day 3 (Scale bar: 200 µm). **(B)** Quantification of live cells percentage on Day 3 for GelMA, GelMA+0.5GNR, and GelMA+1.0GNR conditions (one-way ANOVA with a Tukey's posthoc test, n = 4). Data was expressed as mean ± standard deviation. ns (not significant).

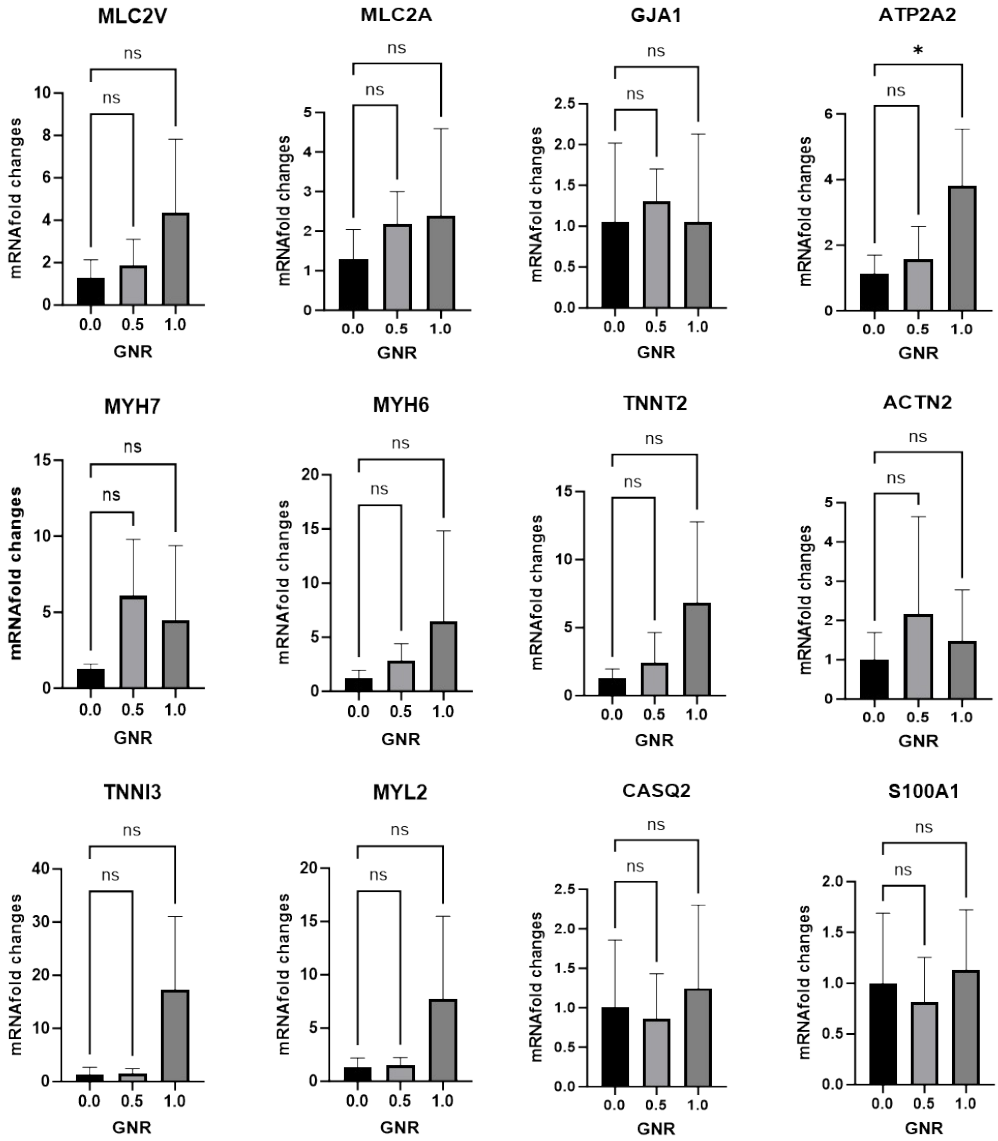

Figure S4. Quantitative PCR analysis of gene expression. Total RNA was extracted from cells, reverse-transcribed to cDNA, and analyzed using quantitative real-time PCR. Expression of genes was normalized to 18S and presented relative to the GelMA, which was set to 1. Data are shown as mean ± SEM from n = 4 biological replicates, each run-in technical triplicate. Statistical significance was assessed by unpaired t-test, $p < 0.05$. *ns, Not significant*.

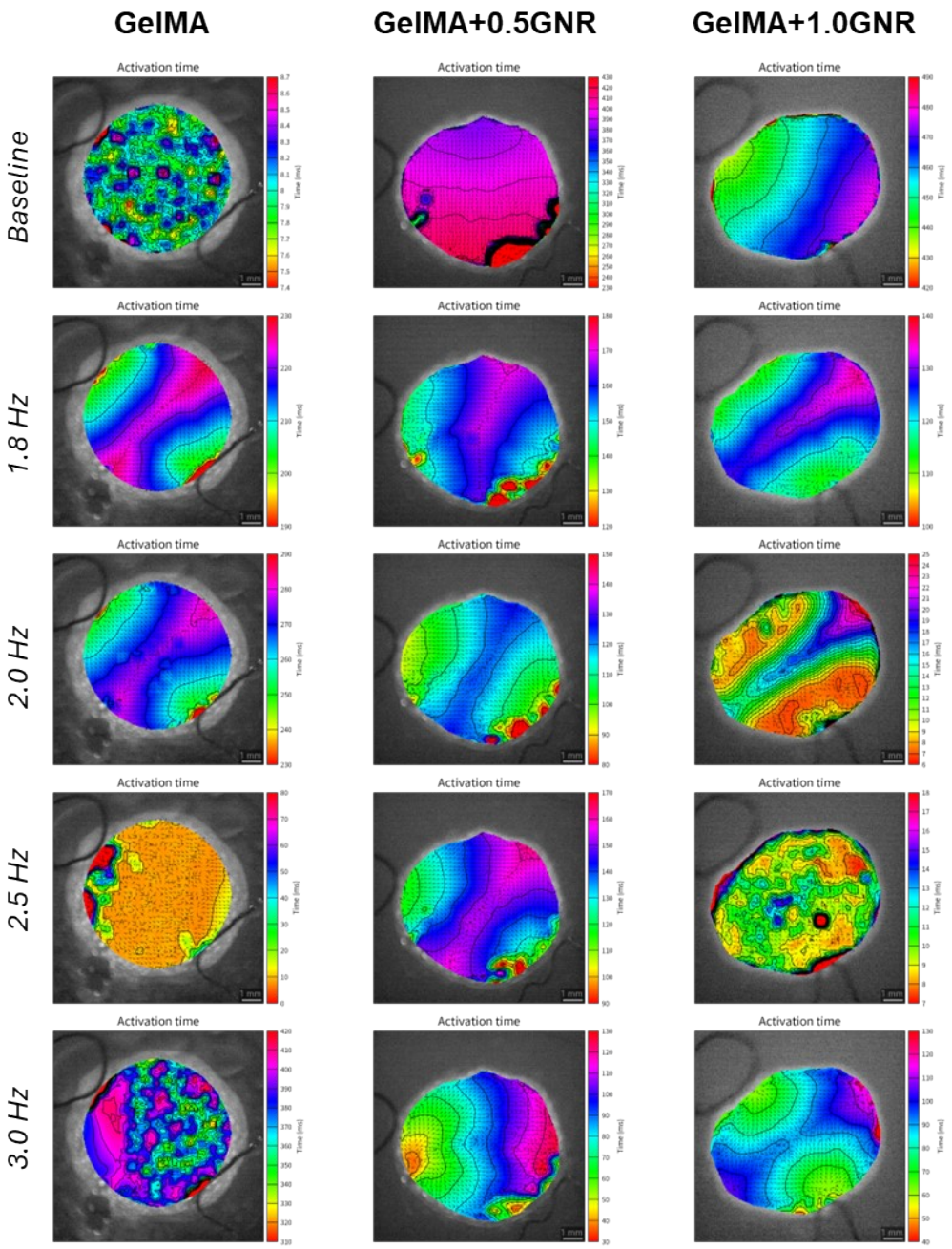

Figure S5. Representative activation maps of EHTs. Activation maps generated through WB software. Colors represent the activation time of each specific pixel. The vectors represent the direction of the electrical wave propagation.

Table S1. List of the used reagents.

Reagents	Manufacturer	Cat #
Nanomaterial synthesis		
Gold (III) chloride trihydrate (HAuCl4)	Sigma Aldrich	520918-1G
Sodium borohydride (NaBH4)	Sigma Aldrich	213462-25G
Silver nitrate (AgNO3)	Sigma Aldrich	S6506-5G
hexadecyltrimethylammonium bromide (CTAB)	VWR	97062-432
L-Ascorbic acid	Sigma Aldrich	A4544-25G
Silica Nanoparticles (40 nm)	General Engineering and Research	N/A
GelMA synthesis and hydrogel fabrication		
Gelatin from porcine skin	Sigma Aldrich	G1890-500G
Methacrylic anhydride (MA)	Sigma Aldrich	276685-500ML
3-(Trimethoxysilyl)propyl methacrylate (TMSPMA)	Sigma Aldrich	440159-100ML
2-Hydroxy-4'-(2 -hydroxyethoxy)-2- methylpropiophenone	Sigma Aldrich	410896-50G
Claritex Supa Mega Slide	CellPath	MAC-1400-02A
hiPSC maintenance and differentiation		
mTeSR	StemCell Technologies	100-0274
Matrigel Matrix (hESC-qualified)	Corning	354277
EDTA	Corning	46-034-Cl
TrypLE™ Express	Gibco	12604-021
RPMI 1640 (1X)	Corning	10-040-CM
B27 supplement (50X)	Gibco	17504-001
B27 minus insulin (50X)	Gibco	A18956-02
EZSolution™ CHIR99021	BioVision	1748-5
IWP2	Tocris	3533
RPMI 1640 (1X) without glucose	Corning	10-043-CV
Sodium DL-Lactate solution	Sigma Aldrich	L4263-100ML
IF staining		
Paraformaldehyde (PFA)	Thermo scientific	J19943-K2
Goat serum	ImmunoReagents	SP-004-VX2
Mouse monoclonal anti-Troponin T	Invitrogen	MA5-12960
Rabbit monoclonal anti-vimentin	Cell signaling	5741
Alexa Fluor 488 Phalloidin	Invitrogen	A12379
Mouse monoclonal anti-integrin β1	Abcam	ab30394
Mouse monoclonal anti-sarcomeric alpha actinin	Thermo Fisher	MA1-22863
Rabbit polyclonal anti-connexin 43	Abcam	ab11370
4',6-diamidino-1-phenylindole (DAPI)	Tocris	5748
Alexa Fluor 488 goat anti-rabbits	Life Technologies	A11008
Alexa Fluor 647 goat anti-rabbits	Life Technologies	A21244
Alexa Fluor 488 goat anti-mouse	Invitrogen	A11001
Alexa Fluor 647 goat anti-mouse	Life Technologies	A21235
Live/Dead assay		
Live/Dead assay kit	Biotium	30002-T
RNA extraction, cDNA synthesis, and qPCR		
Quick-RNA™ MicroPrep	Zymo Research	R1050
qScript™ cDNA Supermix	Quantobio	95048-100
iTaq Universal SYBR Green Supermix	Bio-Rad	1725120

Table S2. List of the used primers.

Gene	Sequence
ACTNT2	FW: GGCACCCAGATTGAGAACAT
	RV: CCTGAATAGCAAAGCGAAGG
GJA1	FW: TACCAAACAGCAGCGGAGTT
	RV: TGGGCACCACTCTTTTGCTT
MYH6	FW: TCCTGCGGCCCAGATTCTTC
	RV: TCTTCCTTGTCATCGGGCAC
MYH7	FW: CACAGCCATGGGAGATTCGG
	RV: CACAGCCATGGGAGATTCGG
TNNT2	FW: GACAGAGCGGAAAAGTGGGA
	RV: CTCCTTGGCCTTCTCCCTCA
TNNI3	FW: CCTGCGGAGAGTGAGGATCT
	RV: CCGGTTTTCCTTCTCGGTGT
MLC2V	FW: GGCGCCAACTCCAACGTGTT
	RV: ACGTTCACTCGCCCAAGGGC
MLC2A	FW: GAGGACAAGGTCAACACCCT
	RV: CGCACCTTCTTCTCTTGCTC
ATP2A2	FW: CATCAAGCACACTGATCCCGT
	RV: CCACTCCCATAGCTTTCCCAG
CASQ2	FW: GTTGCCCGGGACAATACTGA
	RV: CTGTGACATTCACCACCCCA
S100A1	FW: AGACCCTCATCAACGTGTTC
	RV: CACAAGCACCACATACTCCT
18S	FW: GTAACCCGTTGAACCCCATT
	RV: CCATCCAATCGGTAGTAGCG

Table S3. Summarized CV and Standard Deviation

CV						
Stimulation Frequency	**GelMA**		**GelMA+0.5GNR**		**GelMA+1.0GNR**	
	Mean	SD	Mean	SD	Mean	SD
Baseline	14.19	2.35	17.10	2.00	14.44	3.33
1.8 Hz	13.83	2.32	17.11	2.67	14.90	3.65
2.0 Hz	13.38	2.77	14.67	2.23	14.17	4.61
2.5 Hz	12.33	3.47	13.01	1.61	13.06	5.46
3.0 Hz	10.78	2.26	12.55	4.97	10.31	1.89

Table S4. Summarized APD50 and Standard Deviation

APD50						
Stimulation Frequency	**GelMA**		**GelMA+0.5GNR**		**GelMA+1.0GNR**	
	Mean	SD	Mean	SD	Mean	SD
Baseline	111.50	93.12	160.20	84.41	191.60	68.08
1.8 Hz	129.10	68.42	153.00	95.16	148.10	80.01
2.0 Hz	115.80	59.88	174.70	72.92	134.30	75.74
2.5 Hz	61.08	29.26	173.30	61.29	131.90	54.42
3.0 Hz	89.47	43.69	135.10	66.25	129.90	56.04

Table S5. Summarized APD80 and Standard Deviation

APD80						
Stimulation Frequency	**GelMA**		**GelMA+0.5GNR**		**GelMA+1.0GNR**	
	Mean	SD	Mean	SD	Mean	SD
Baseline	129.40	109.10	220.90	92.76	230.40	76.34
1.8 Hz	159.40	70.85	181.50	109.10	205.80	80.04
2.0 Hz	147.10	71.81	257.30	5.04	164.20	89.63
2.5 Hz	76.11	40.99	163.40	76.70	159.50	69.62
3.0 Hz	81.12	15.59	169.40	80.25	160.00	69.50

Movie S1. Spontaneous beating responses of GelMA cardiac tissues at day 7 of culture.

Movie S2. Spontaneous beating responses of GelMA cardiac tissues at day 14 of culture.

Movie S3. Spontaneous beating responses of GelMA+0.5GNR cardiac tissues at day 7 of culture.

Movie S4. Spontaneous beating responses of GelMA+0.5GNR cardiac tissues at day 14 of culture.

Movie S5. Spontaneous beating responses of GelMA+1.0GNR cardiac tissues at day 7 of culture.

Movie S6. Spontaneous beating responses of GelMA+1.0GNR cardiac tissues at day 14 of culture.