Science Advances
AAAS

Supplementary Materials for

Label-Free High-Density Mapping Reveals Sustained Reentrant Activity in iPSC-Derived Atrial Cardiomyocytes from Brugada Syndrome Patients

Wener Li *et al.*

*Corresponding author. Email: kaomei.guan@tu-dresden.de; wener.li@tu-dresden.de

This PDF file includes:

Supplementary Text
Figs. S1 to S5
Tables S1 to S2
Movies S1 to S2

Other Supplementary Materials for this manuscript include the following:

Movies S1 to S2

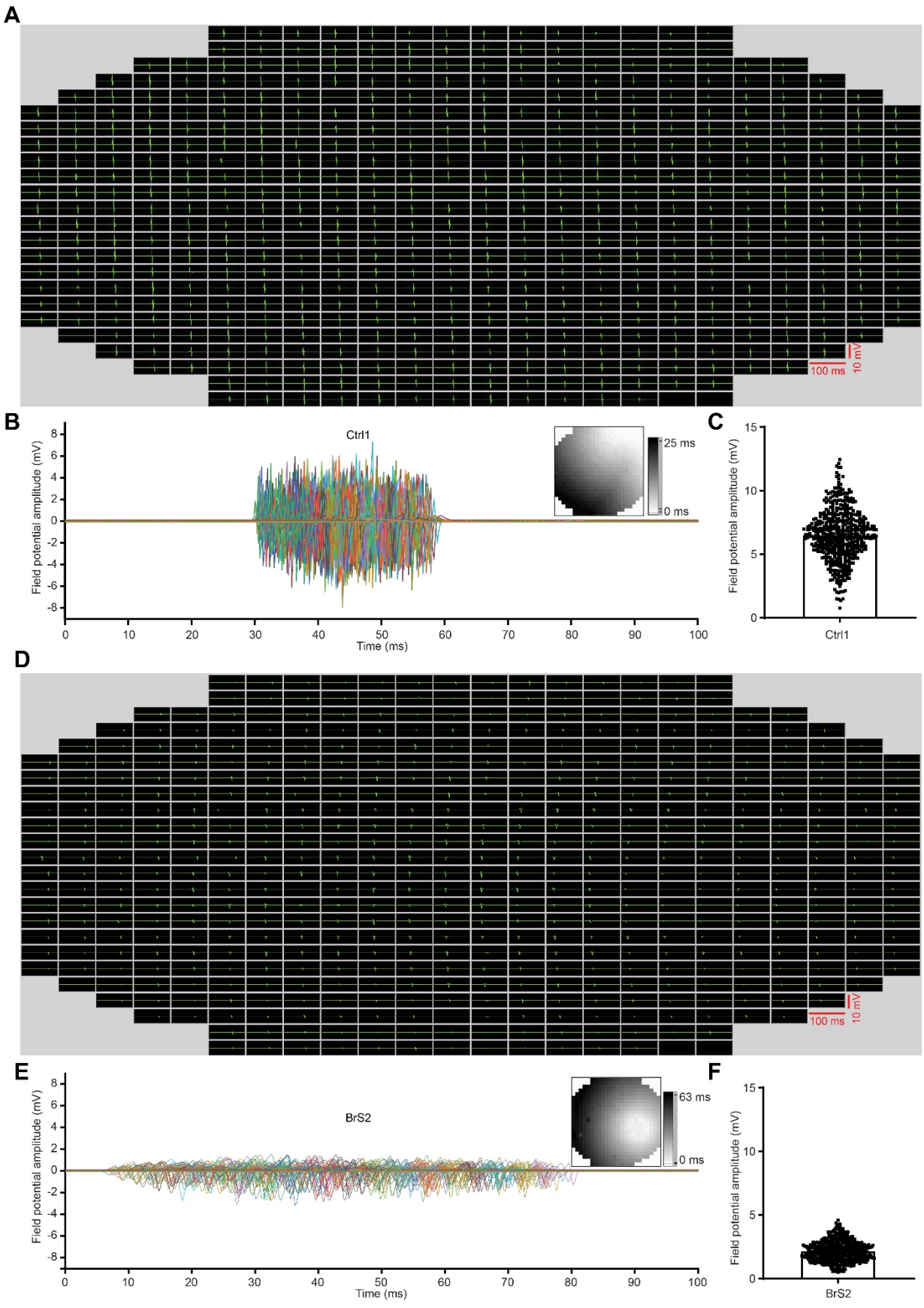
A
100 ms
10 mV
B
Ctrl1
25 ms
0 ms
Field potential amplitude (mV)
Time (ms)
C
Field potential amplitude (mV)
Ctrl1
D
100 ms
10 mV
E
BrS2
63 ms
0 ms
Field potential amplitude (mV)
Time (ms)
F
Field potential amplitude (mV)
BrS2

Fig. S1.

Illustration of the analysis of field potential amplitude. (**A**) The screenshot of the original recording of one Ctrl1-aCM culture. For all 512 channel windows, the time scale was 100 ms and the voltage scale was 10 mV. (**B**) Time-based overlay plotting of all channels for the Ctrl1-aCM culture. Inset shows a spatial plot of activation time to visualize the time it takes for the signal to propagate from its starting point to its end point. (**C**) Scatter plot of field potential amplitude for the Ctrl1-aCM culture. (**D**) The screenshot of the original recording of one BrS2-aCM culture. For all 512 channel windows, the time scale was 100 ms and the voltage scale was 10 mV. (**E**) Time-based overlay plotting of all channels for the BrS2-aCM culture. Inset shows a spatial plot of activation time to visualize the time it takes for the signal to propagate from its starting point to its end point. (**F**) Scatter plot of field potential amplitude for the BrS2-aCM culture.

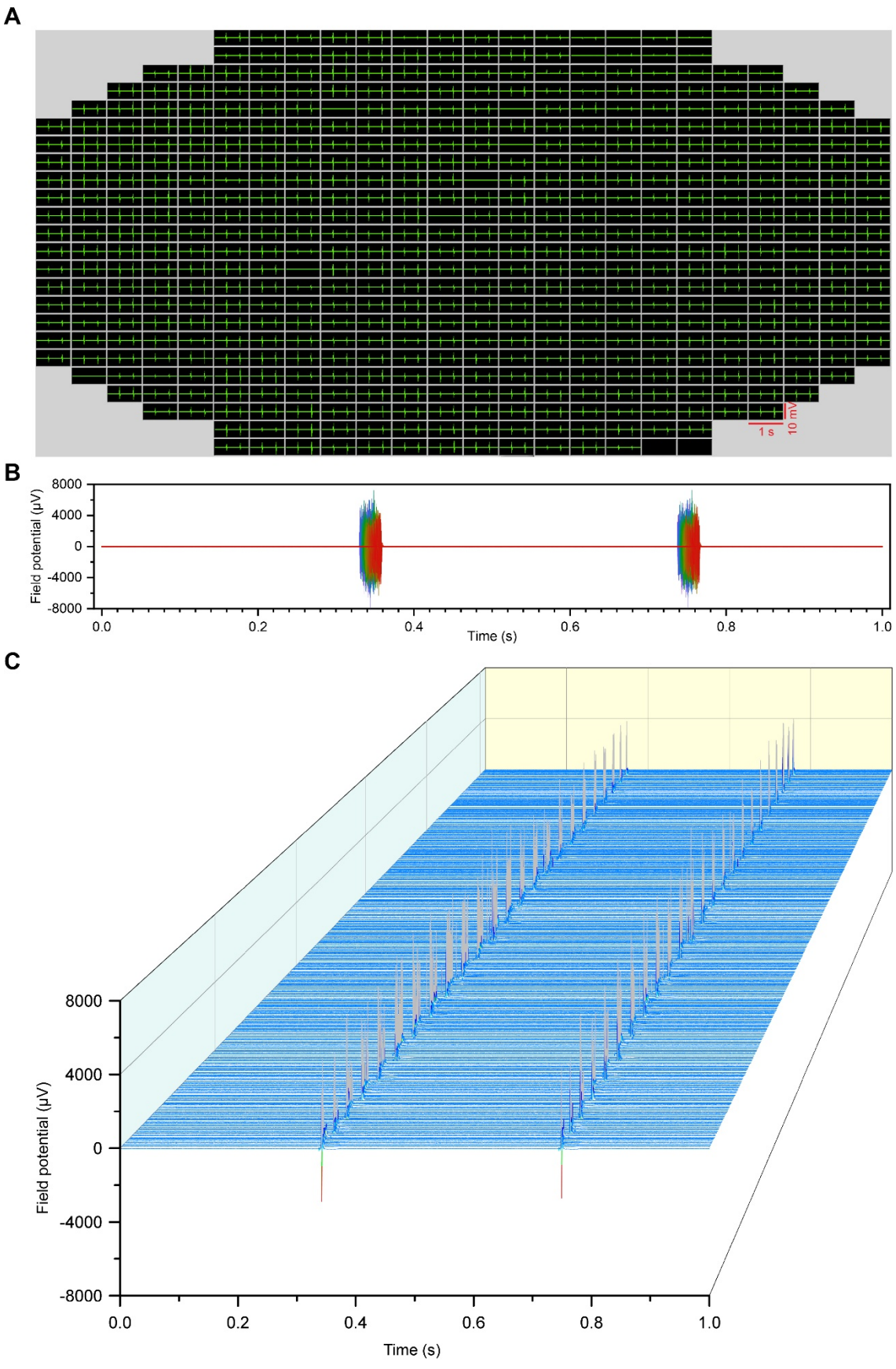
A
1 s
10 mV
B
Field potential (μV)
8000
4000
0
-4000
-8000
0.0
0.2
0.4
0.6
0.8
1.0
Time (s)
C
Field potential (μV)
8000
4000
0
-4000
-8000
0.0
0.2
0.4
0.6
0.8
1.0
Time (s)

Fig. S2.

Data visualization of pacemaker activity recorded from Ctrl1-aCM. (**A**) Screenshot showing 1 s of raw field potential recordings from all 512 electrodes. (**B**) Superimposed field potential traces from all 512 channels plotted on the same x–y axes; individual colors represent different electrodes. (**C**) Three-dimensional waterfall plot of the same data, with color mapping along the z-axis illustrating field potential amplitudes.

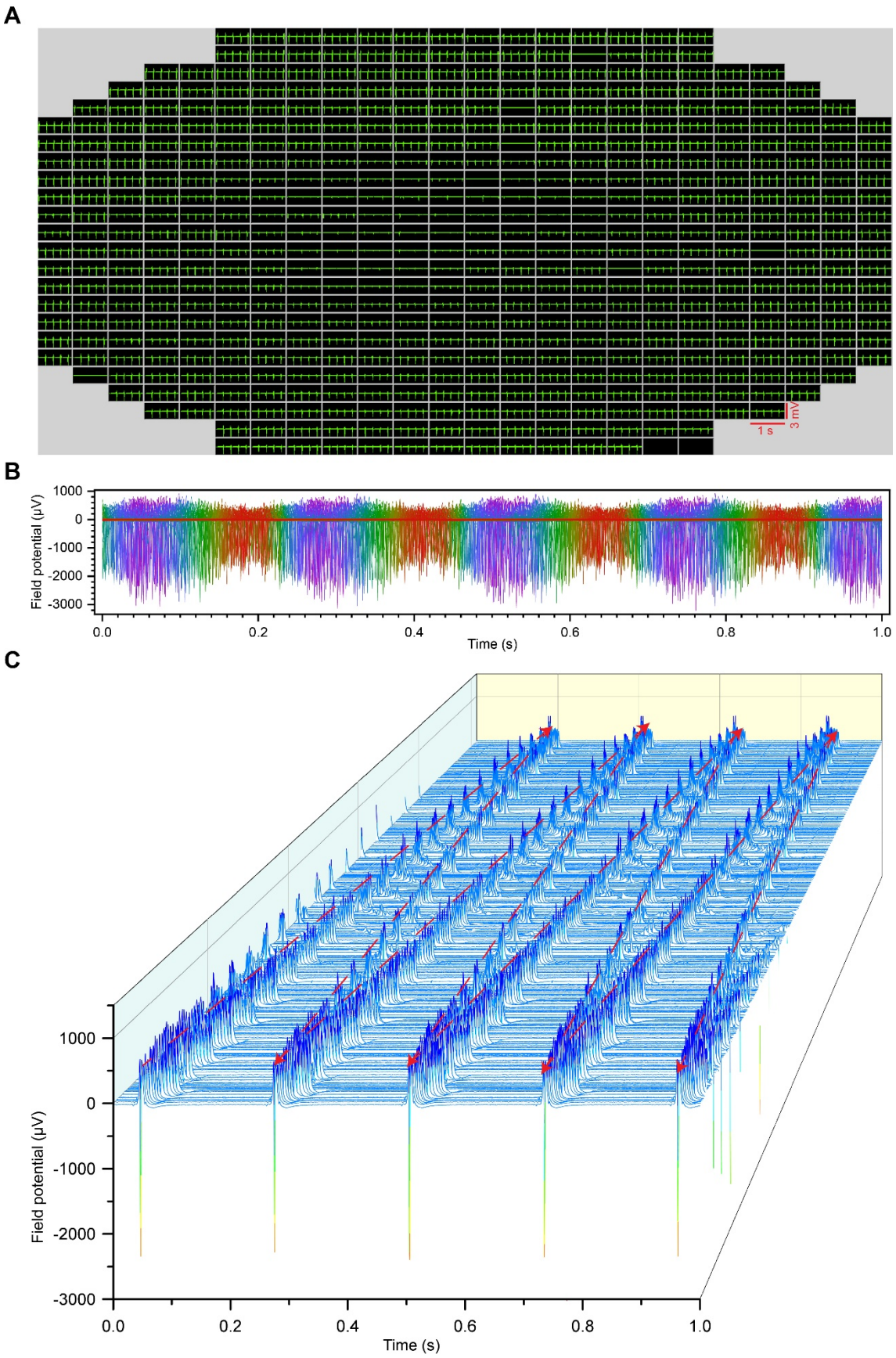
A
1 s
3 mV
B
Field potential (μV)
1000
0
-1000
-2000
-3000
0.0
0.2
0.4
0.6
0.8
1.0
Time (s)
C
Field potential (μV)
1000
0
-1000
-2000
-3000
0.0
0.2
0.4
0.6
0.8
1.0
Time (s)

Fig. S3.

Data visualization of reentry recorded from BrS1-aCM culture. (**A**) Screenshot showing 1 s of raw field potential recordings from all 512 electrodes showing high frequency sustained-reentry field potential activity. (**B**) Superimposed field potential traces from all 512 channels plotted on the same x–y axes; individual colors represent different electrodes. (**C**) Three-dimensional waterfall plot of the same data, with color mapping along the z-axis illustrating field potential amplitudes. The red dashed line indicate the field potential propagation direction.

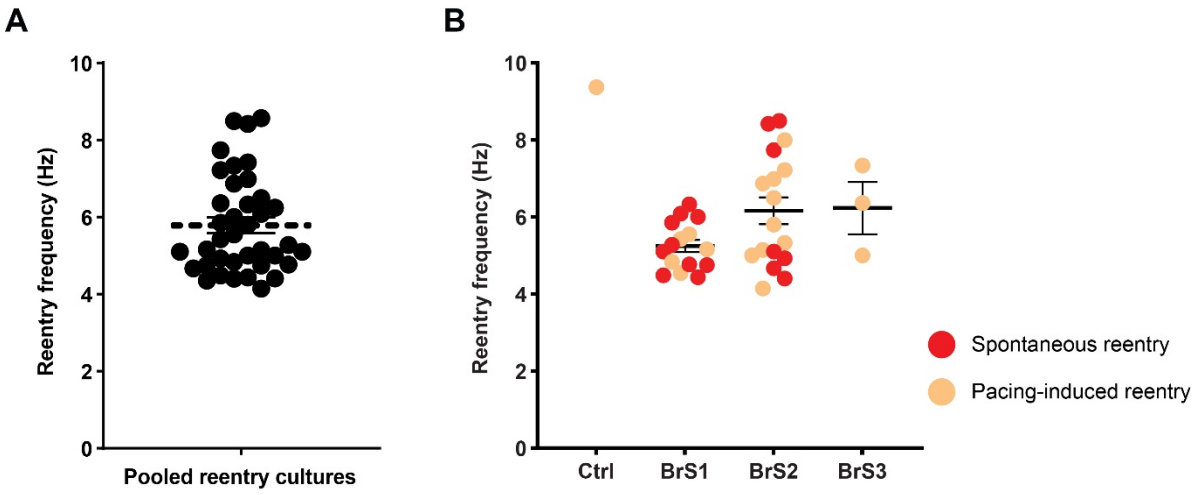

Fig. S4.

Quantification of reentry frequency in Ctrl- and BrS-aCM cultures. (**A**) Distribution of reentry frequencies pooled from all BrS1/2/3-aCM reentry-positive cultures, showing a mean frequency of approximately 6 Hz. (**B**) Comparison of reentry frequencies among Ctrls and three independent BrS-aCM patients (BrS1, BrS2 and BrS3). Each data point represents one culture exhibiting either spontaneous (red) or pacing-induced (orange) reentry. Horizontal bars indicate mean ± SEM.

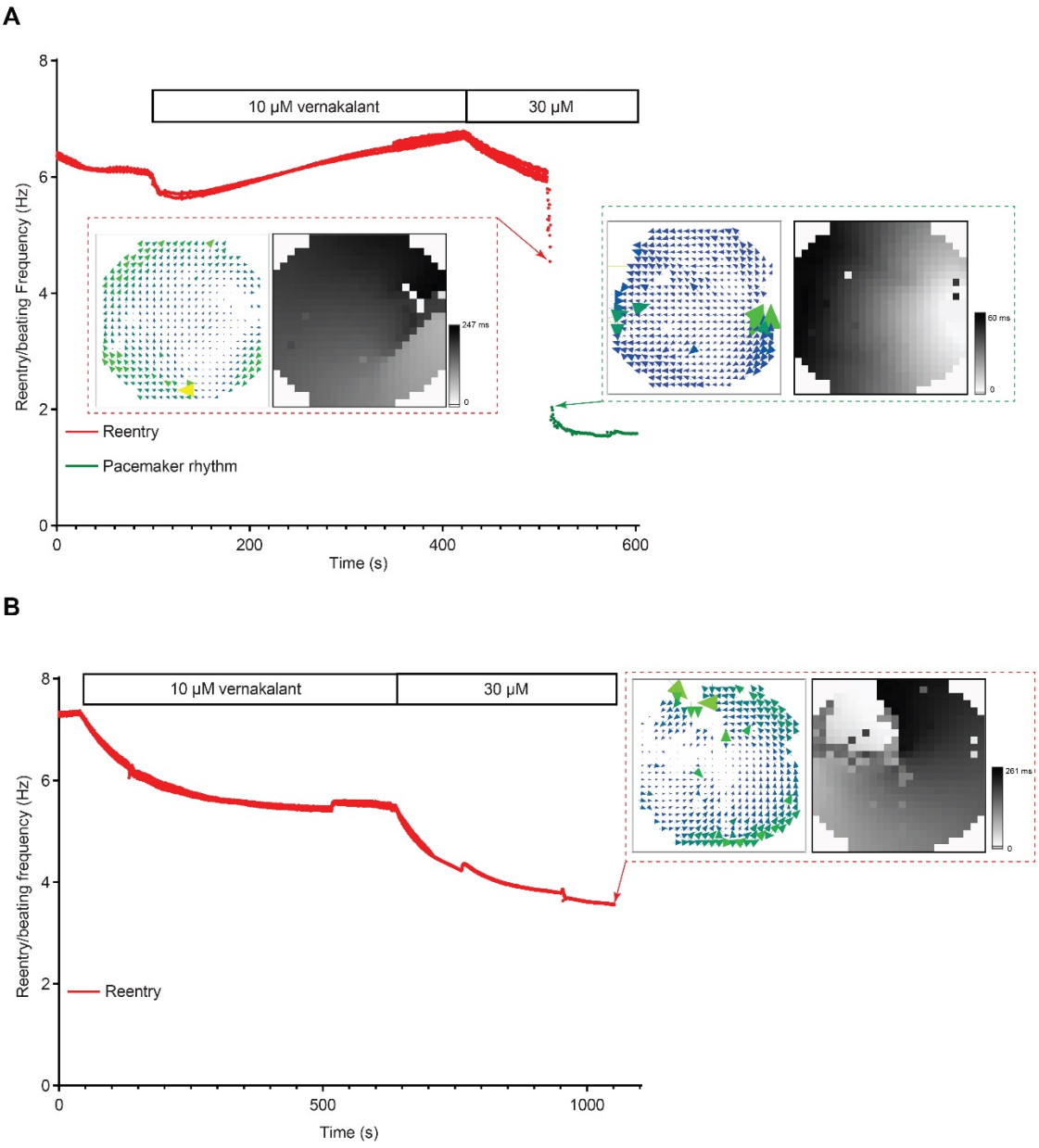

Fig. S5.

Pharmacological cardioversion testing in pacing-induced reentry of BrS3-aCMs. (**A**) Reentry/beating frequency trace from a BrS3-aCM culture exhibiting pacing-induced sustained reentry. Application of vernakalant (30 μM) successfully terminated the reentrant driver, restoring a stable pacemaker rhythm. The conversion point is illustrated in the inserted dash rectangles. (**B**) Reentry/beating frequency trace from a second BrS3-aCM culture where pacing-induced reentry proved resistant to vernakalant treatment. The reentrant arrhythmia persisted with high-frequency rotation despite identical drug exposure.

Table S1.

Detailed information about the patients and healthy donors.

Donor		Cell line	*SCN5A* mutation carrier?	Remarks	Publication
Ctrl1		iWTD2-1	No	Healthy donors	W. Li, X. Luo, A. Strano, S. Arun, O. Gamm, M. S. Poetsch, M. Hasse, R.-P. Steiner, K. Fischer, J. Pöche, Y. Ulbricht, M. Lesche, G. Trimaglio, A. El-Armouche, A. Dahl, P. Mirtschink, K. Guan, M. Schubert, Comprehensive promotion of iPSC-CM maturation by integrating metabolic medium with nanopatterning and electrostimulation. Nature Communications 16, 2785 (2025).
Ctrl2		iBM76.3	No		
Ctrl3		isWTD-22	No		
BrS1	Sister and brother in one family	Na6 Na8	Yes, $Na_V1.5^{S1812X}$	Brugada syndrome patient **with atrial fibrillation**.	W. Li, M. Stauske, X. Luo, S. Wagner, M. Vollrath, C. S. Mehnert, M. Schubert, L. Cyganek, S. Chen, S. M. Hasheminasab, G. Wulf, A. El-Armouche, L. S. Maier, G. Hasenfuss, K. Guan, Disease Phenotypes and Mechanisms of iPSC-Derived Cardiomyocytes From Brugada Syndrome Patients With a Loss-of-Function SCN5A Mutation. Front Cell Dev Biol 8, 592893 (2020)
BrS2		isBrS6-Bld2 isBrS6-Bld4	Yes, $Na_V1.5^{S1812X}$	Brugada syndrome patient.	
BrS3		iBrS2-Am2 iBrS2-Am3	No. Sequencing confirmed wild-type	Brugada syndrome patient **with atrial fibrillation.**	C. C. Veerman, I. Mengarelli, K. Guan, M. Stauske, J. Barc, H. L. Tan, A. A. Wilde, A. O. Verkerk, C. R. Bezzina, hiPSC-derived cardiomyocytes from Brugada Syndrome patients without identified mutations do not exhibit clear cellular electrophysiological abnormalities. Scientific reports 6, 30967 (2016).

Table S2. Extracellular and intracellular solutions for automated and manual patch clamp.

	Automated patch clamp				Manual patch clamp	
	External solution (For cell catching)	Seal enhancer	I_{Na}		AP	
in mM			Bath under 50 mM $[Na^+]_o$	Pipette	Bath	Pipette for AP
NaCl	140	130	50	10	140	
TEA-Cl			90			
KCl	4	4			5.4	120
$CaCl_2$	2	10	2		1.8	
$MgCl_2$	1	1	1		1	1
CsCl			4	30		
CsF				90		
Glucose	5	5	5		10	
HEPES	10	10	10	10	10	10
EGTA				10		10
Nifedipine (freshly)			0.01			
Mg-ATP (freshly)				1		3
pH adjustment	7.4 with NaOH	7.4 with NaOH	7.4 with CsOH	7.2 with CsOH	7.4 with NaOH	7.2 with KOH

Movie S1.

High-density field potential recording of sustained micro-reentry in a BrS1-aCM culture. This video displays the raw field potential traces recorded across the 512-electrode array from a BrS1-aCM culture exhibiting spontaneous sustained arrhythmia. The active electrode grid allows for the direct visualization of the electrical propagation wavefront as it travels across the atrial culture. The temporal scale of the replaying (X-axis) is 100 ms per division, and the amplitude scale (Y-axis) is 500 μV per division. To resolve the rapid capture of the reentrant circuit dynamics, the recording is played back at 0.1× speed (1/10th of real-time).

Movie S2.

Dynamic magnitude and phase mapping of the reentrant rotor. This video corresponds to the recording in Movie S1 and provides a processed visualization of the reentrant driver. Left: Dynamic magnitude map (grayscale) highlighting the trajectory of the depolarization wavefront. Right: Phase map (color-coded) revealing the stable phase singularity and the core of the rotor spinning in a counter-clockwise direction. The video is replayed at 0.2× speed (1/5th of real-time) to visualize the high-frequency rotation.