

Supplemental information

**Optotermination of spiral wave reentry
by a membrane-targeted phototransducer**

Chiara Florindi, Kevin Shani, Vito Vurro, Yongjun Jang, Paola Moretti, Maksymilian Prondzynski, Yashasvi Tharani, Vassilios J. Bezzerides, William T. Pu, Chiara Bertarelli, Guglielmo Lanzani, Antonio Zaza, Kevin Kit Parker, and Francesco Lodola

Supplementary Information

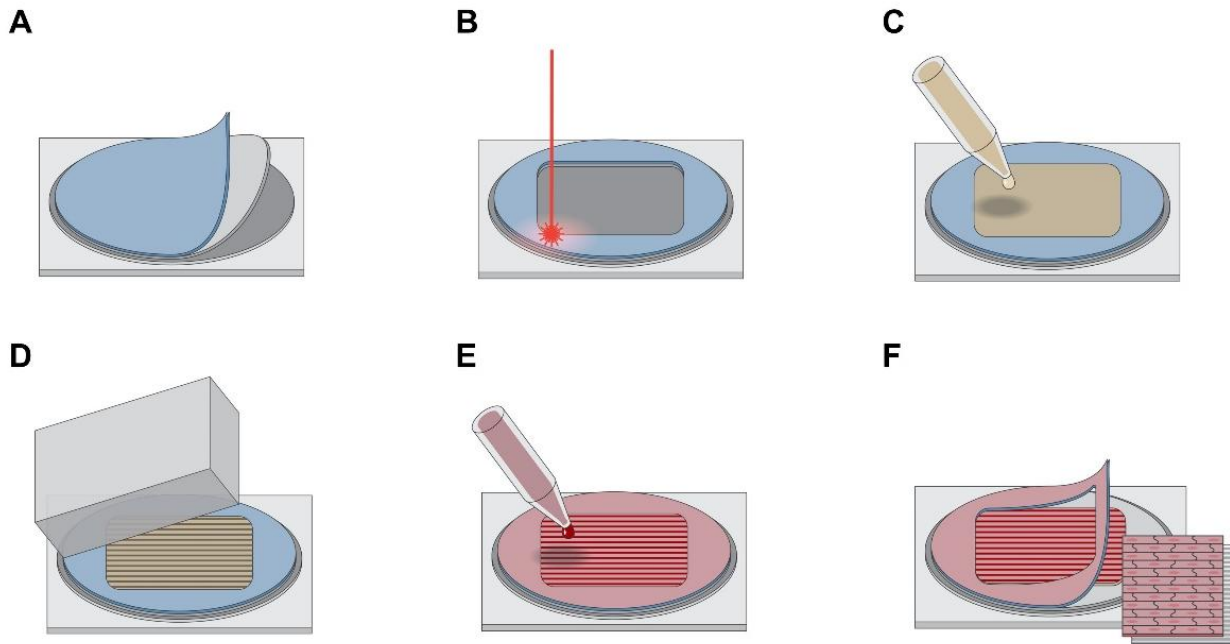


Figure S1: Diagram of the laminar cardiac tissue preparation process. **A.** The process begins with the placement of two overlapping layers of paper tape onto an acetate base. **B.** Both paper layers are then patterned using laser ablation to define the desired geometry. **C.** A gelatin solution is applied onto the patterned substrate, covering the exposed areas. **D.** The gelatin layer is shaped and cured using a PDMS stamp featuring a linear pattern, which helps define the tissue architecture. **E.** Once the gelatin structure is in place, cardiac cells are seeded onto the prepared surface. **F.** Finally, the top layer of paper tape is removed, revealing the final geometry of the engineered tissue (see inset).

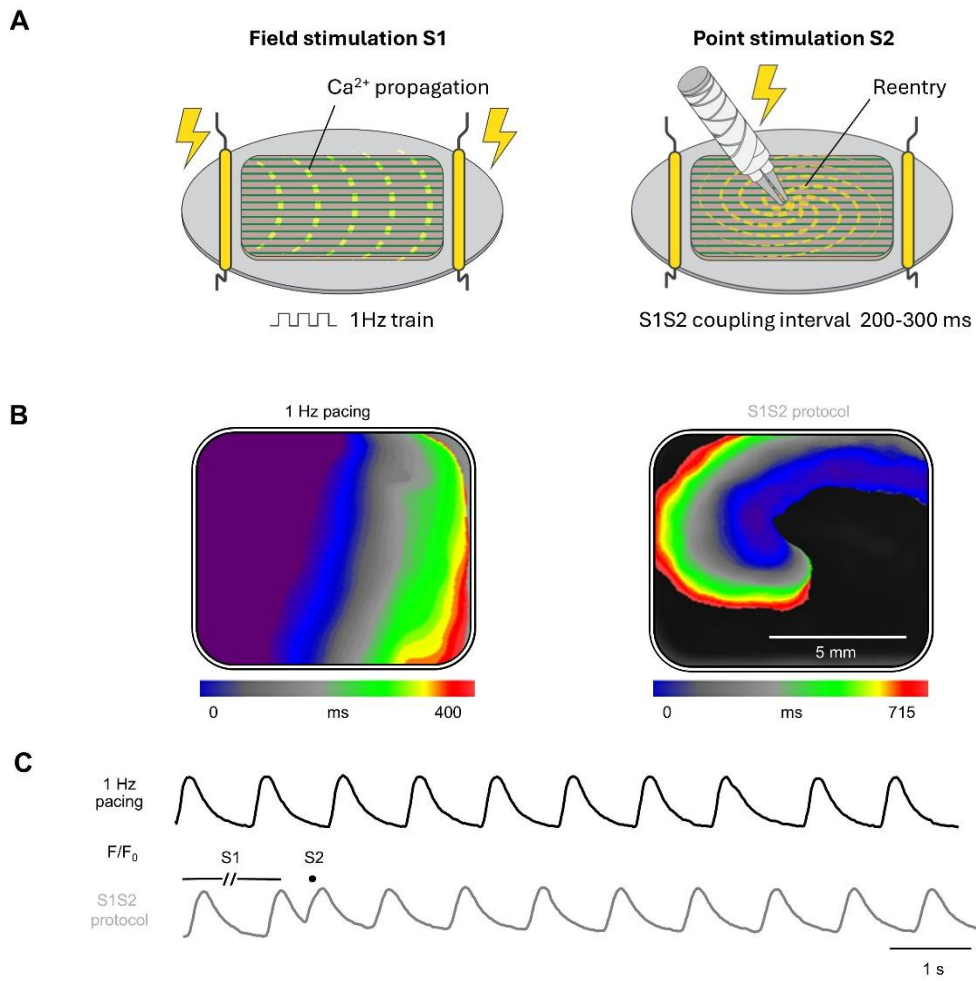


Figure S2: Induction of reentrant activity in WT tissues using the S1S2 Protocol. **A.** Schematic representation of the electrode configuration used for S1S2 stimulation and induction of reentrant activity in anisotropic cardiac tissue (see methods for details). **B.** Representative isochrone maps displaying the reentrant activity induced at the center of the platform after applying the S1S2 arrhythmia induction protocol. **C.** Representative Ca²⁺ traces recorded in WT tissues, showing the transition from regular pacing to arrhythmic activity initiated by the S1S2 protocol.

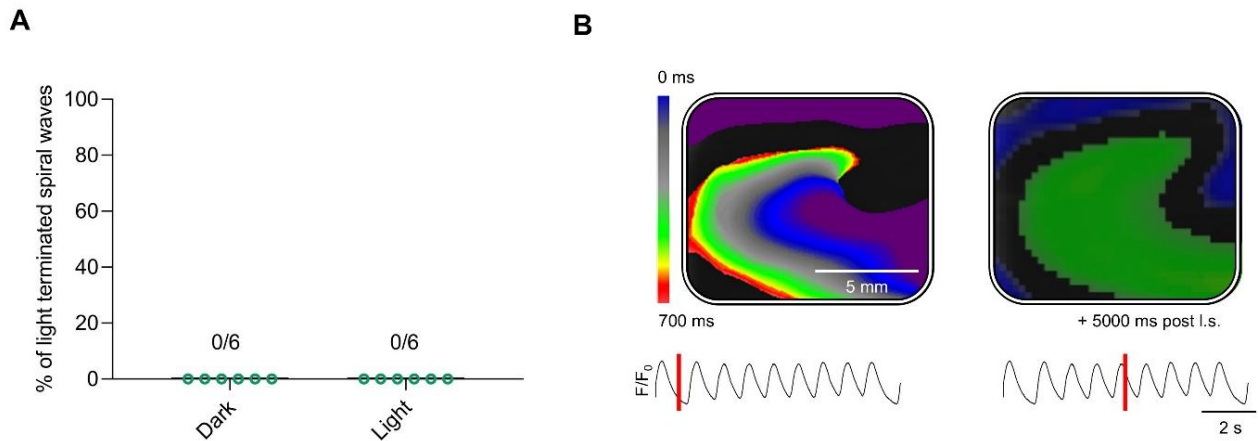


Figure S3: Vehicle-treated tissues maintain persistent reentry after optical stimulation. A. DMSO-loaded cultures failed to terminate reentrant activity in all 6 tissues from 3 WT-Cas9 hiPSC-CMs differentiations. Statistical analysis was performed using the Fisher's exact test ($p = 1$). **B.** The figure shows the isochrone maps at the top, with the corresponding Ca^{2+} transients acquired at a given time (indicated by the red bar on the traces) below from WT tissues treated with vehicle (DMSO) and subjected to the same optical stimulation protocol used for Ziapin2-incubated tissues. Reentry was induced via an electrical S1S2 protocol.

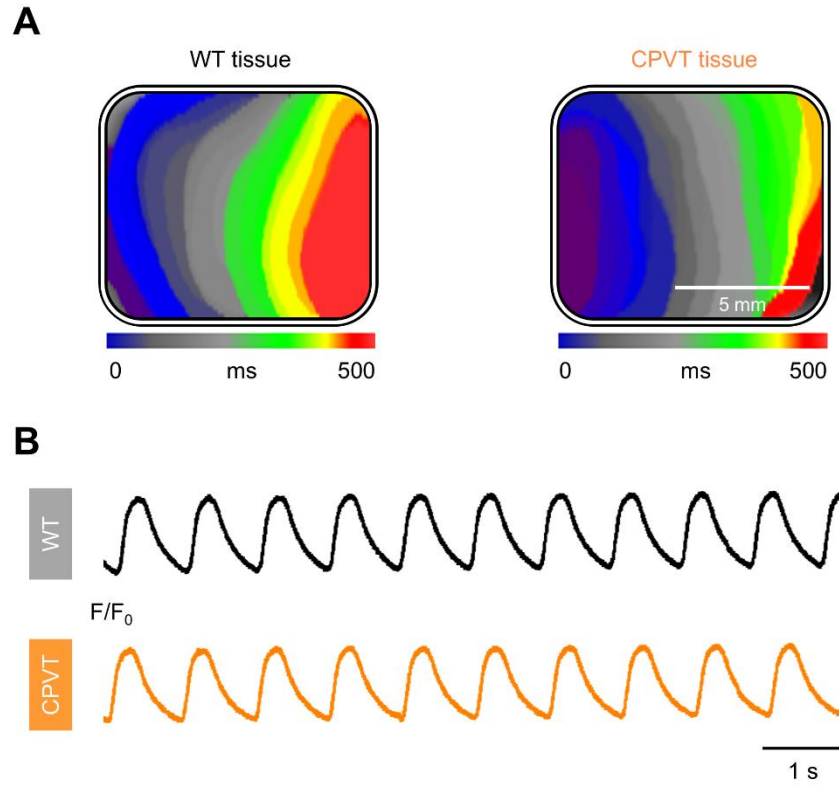


Figure S4: Preserved tissue excitability following optotermiation. Isochrone maps illustrating activation patterns (**A**) and corresponding Ca^{2+} transients (**B**) recorded in WT (black) and CPVT (orange) engineered tissues during 1 Hz field electrical stimulation after optical termination of reentrant arrhythmias.

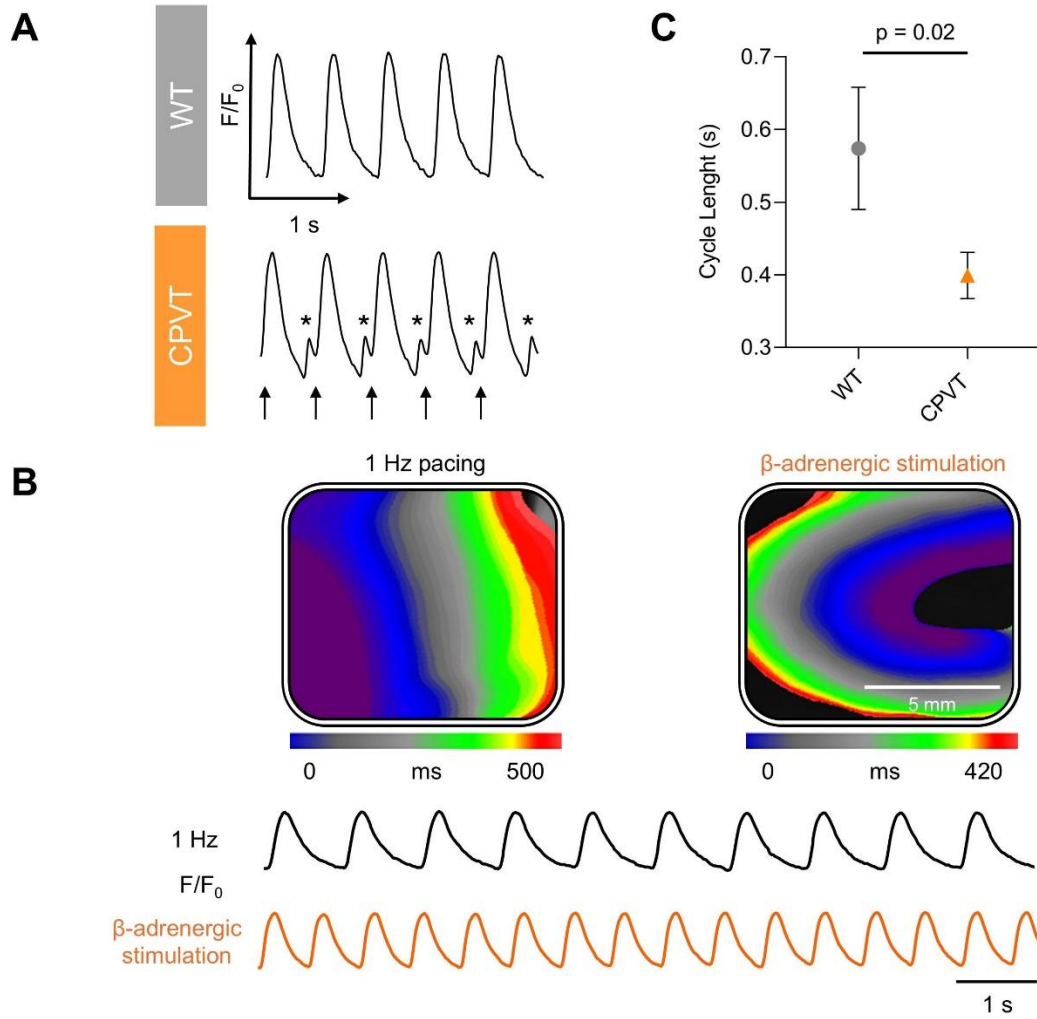


Figure S5: Phenotypic assessment and reentrant activity in CPVT Ziapin2-loaded tissues. A. Representative Ca^{2+} transients for WT and CPVT tissues. Asterisks indicate spontaneous Ca^{2+} release events (i.e. uncontrolled, untriggered releases of Ca^{2+} from the sarcoplasmic reticulum into the cytosol of cardiomyocytes, occurring independently of normal excitation-contraction coupling), and the upward arrow marks electrical stimulation. **B.** Representative isochrone maps (top) and relative Ca^{2+} traces (bottom) displaying the reentrant activity in response to rapid pacing (> 2 Hz) and $1 \mu\text{M}$ isoproterenol, showing the transition from regular pacing to arrhythmic activity initiated by β -adrenergic stimulation. **C.** Cycle length of reentry in WT ($N = 6$ tissues from 3 differentiations) and CPVT ($N = 8$ tissues from 1 differentiation) hiPSC-CMs tissues. Data are represented as mean \pm SEM. Statistical analysis was performed using the Mann–Whitney test.