Supplementary Information for

Human induced pluripotent stem cell line with genetically encoded fluorescent voltage indicator generated via CRISPR for action potential assessment post-cardiogenesis

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This file includes:

Supplementary Figures S1 to S6

Supplementary Table S1 to S4

Other Supplementary Materials for this manuscript includes the following:

Supplementary Movie S1 to S9

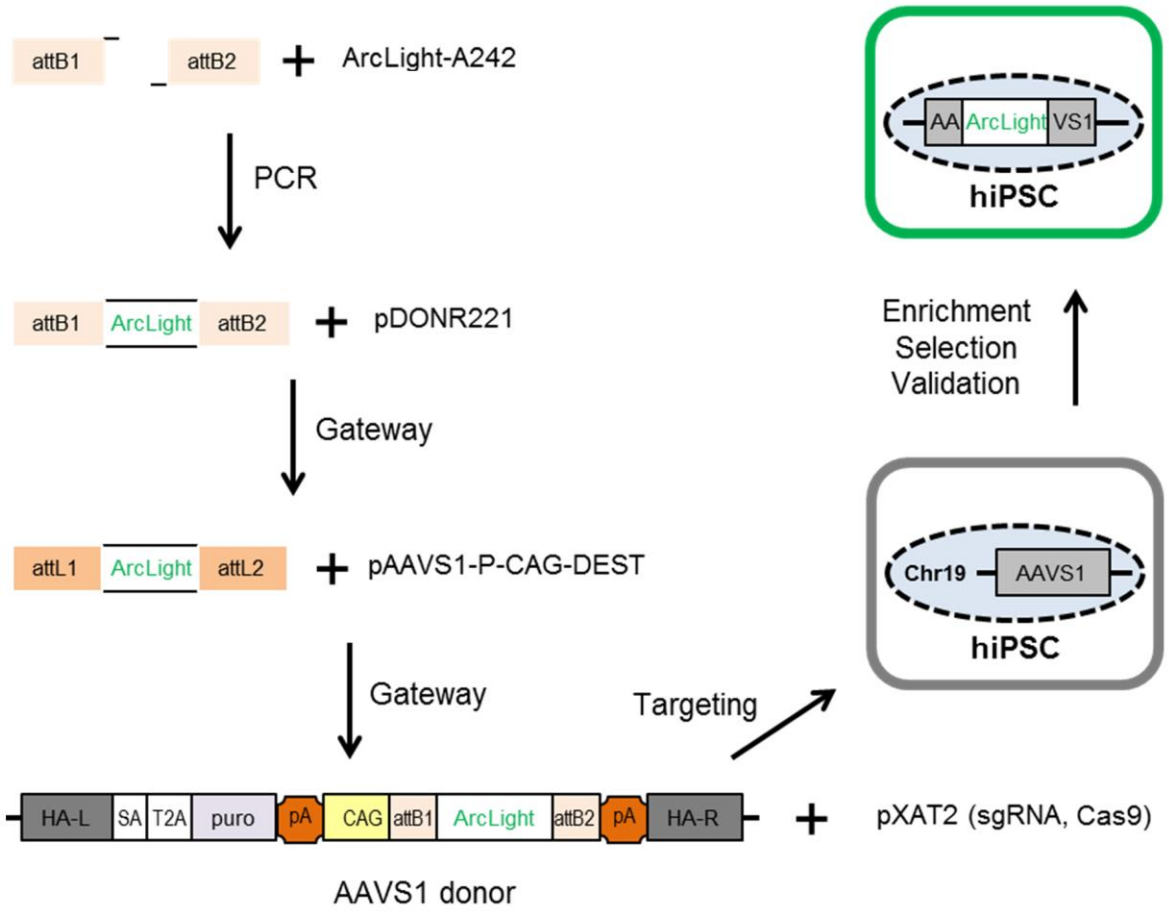

Figure S1. A schematic showing overall strategy and flowchart to generate ArcLight reporter line in human iPSCs. The cloning process was achieved in three main steps. First, Gateway (Invitrogen) cloned ArcLight reporter gene [23] into an AAVS1 destination vector [35]. Second, CRISPR/Cas9 mediated targeted ArcLight reporter gene to the AAVS1 locus [44]. Third, puromycin selection and enrichment by FACS were performed, followed by PCR screening and verification by Sanger sequencing.

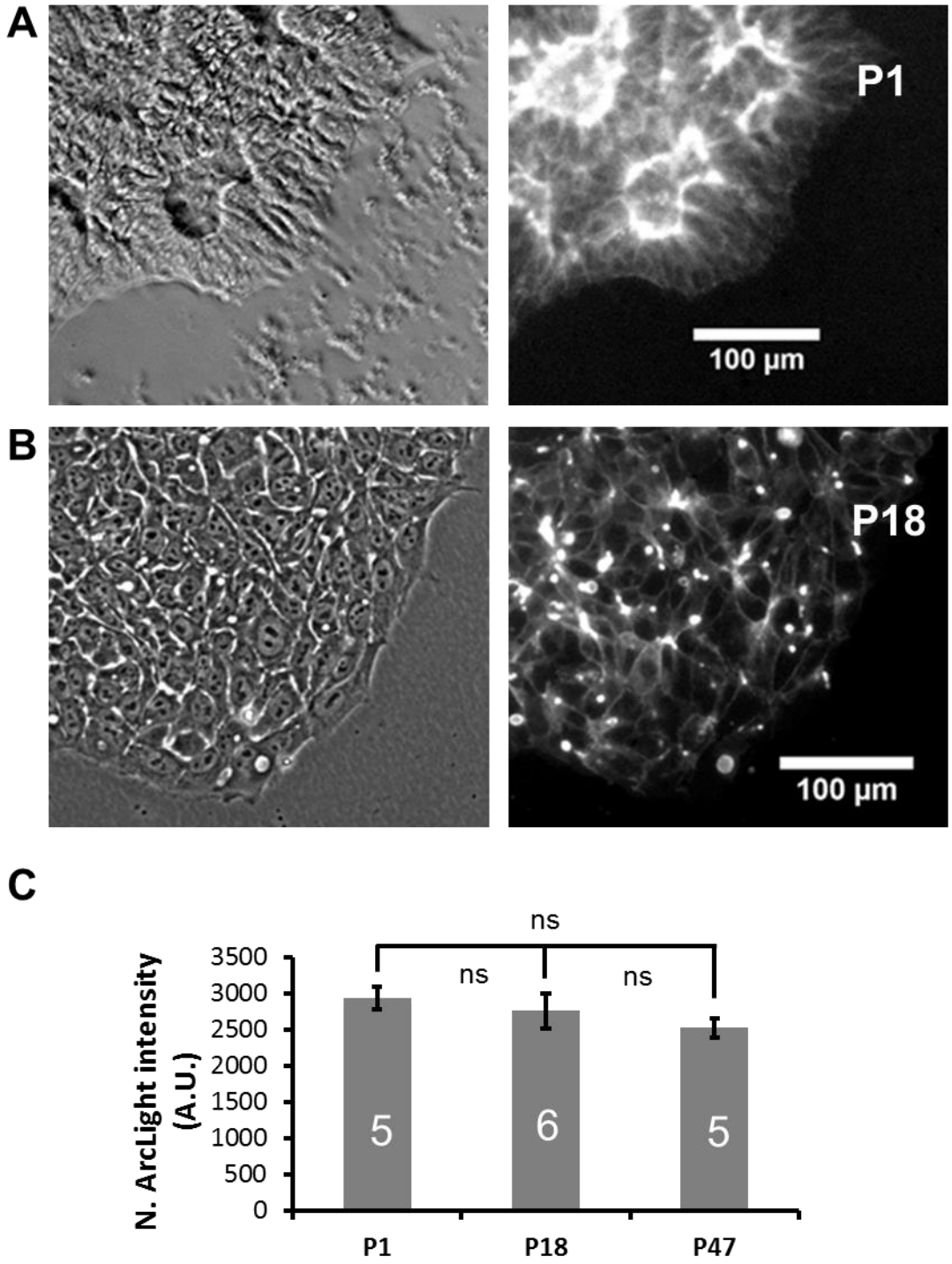

Figure S2. Long-term expression of ArcLight in ArcLight-hiPSCs. (A) Representative phase contrast and ArcLight fluorescent images of an ArcLight-hiPSC colony of the first passage (P1) after colony selection. **(B)** Representative phase contrast and ArcLight fluorescent images of ArcLight-hiPSCs that were maintained for 18 passages without puromycin selection. **(C)** Quantification of ArcLight expressing

in ArcLight-hiPSCs of different passages after colony selection. Normalized fluorescent intensities are present as Mean ± SE in arbitrary units (A.U.). Sampling sizes are indicated inside each bar. ns: no significant difference by unpaired Student's *t*-test.

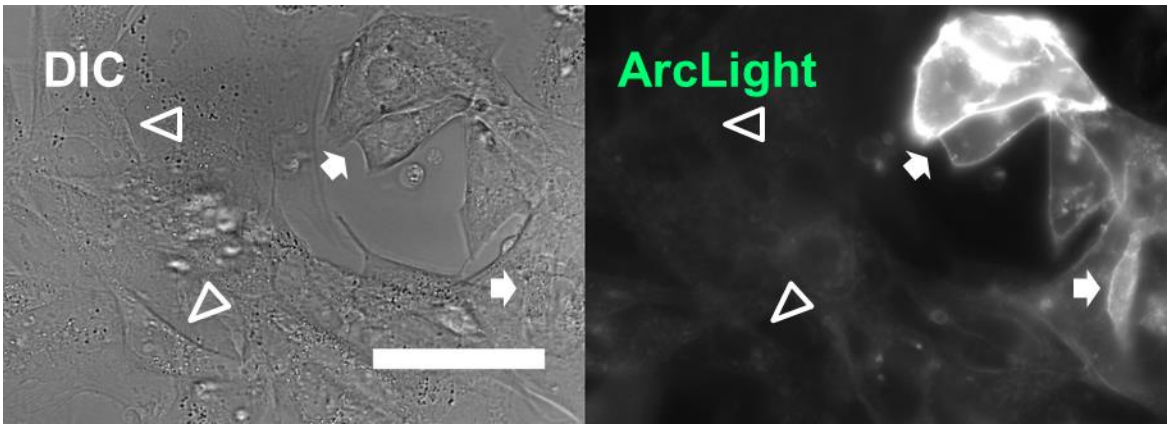

Figure S3. Differential interference contrast (DIC) and ArcLight fluorescence images of live CMs and non-CMs. Differentiated CMs (arrows) exhibited higher ArcLight intensity than non-CMs (arrowheads). Scale bar, 50 μm.

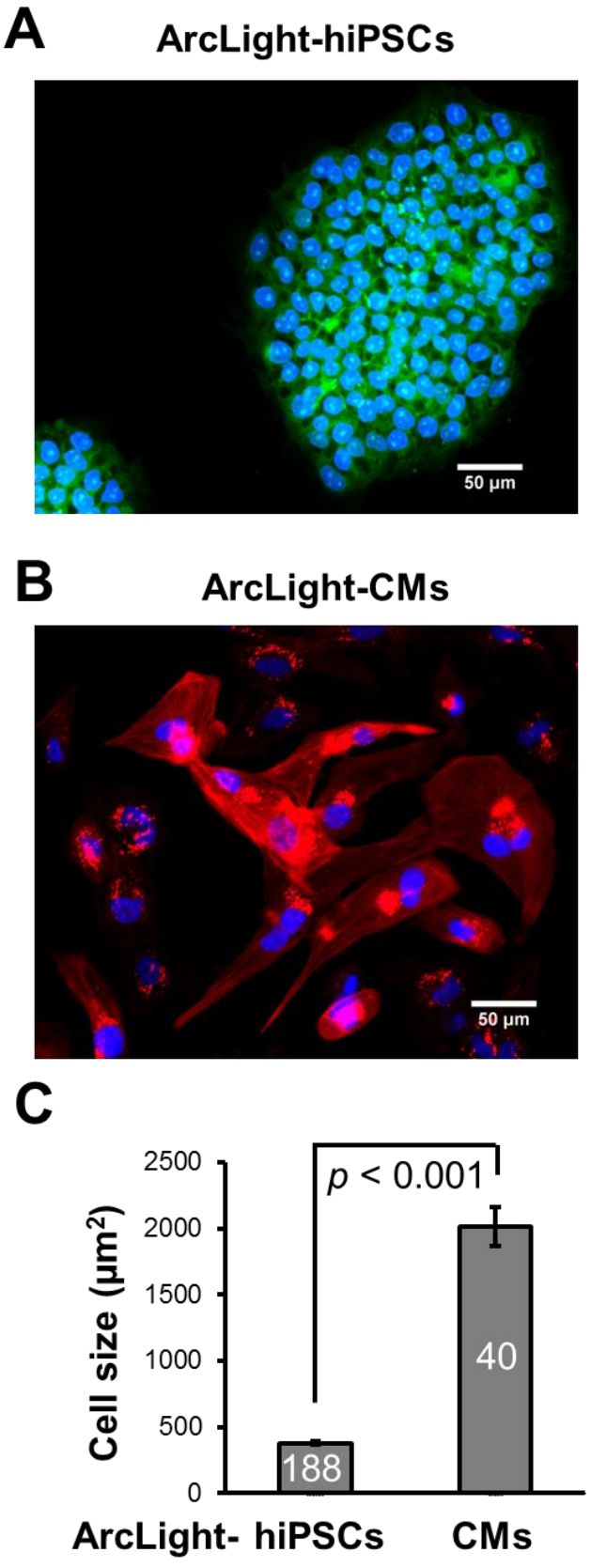
A
ArcLight-hiPSCs
50 μm
B
ArcLight-CMs
50 μm
C
p < 0.001
Cell size (μm²)
2500
2000
1500
1000
500
0
188
40
ArcLight- hiPSCs
CMs

Figure S4. Cell size comparison between ArcLight-hiPSCs and ArcLight-CMs. (A) A representative image of hiPSCs expressing ArcLight (green). Nuclei were stained with Hoechst 33342 (blue). Scale bar, 50 μm. **(B)** A representative image of ArcLight-CMs showing sarcomere structure labeled by anti-cTnT (red). Nuclei were stained with Hoechst 33342 (blue). Scale bar, 50 μm. **(C)** Images of ArcLight-hiPSCs or ArcLight-CMs of D40 after differentiation were used to quantify cell size by thresholding ArcLight or cTnT signal with particle analysis tool in ImageJ. ArcLight-CMs are significantly larger than ArcLight-hiPSCs. Number of quantified cells is indicated inside each bar. Data is presented as Mean ± SE. Statistic difference was analyzed by unpaired two-tail Student's *t*-test.

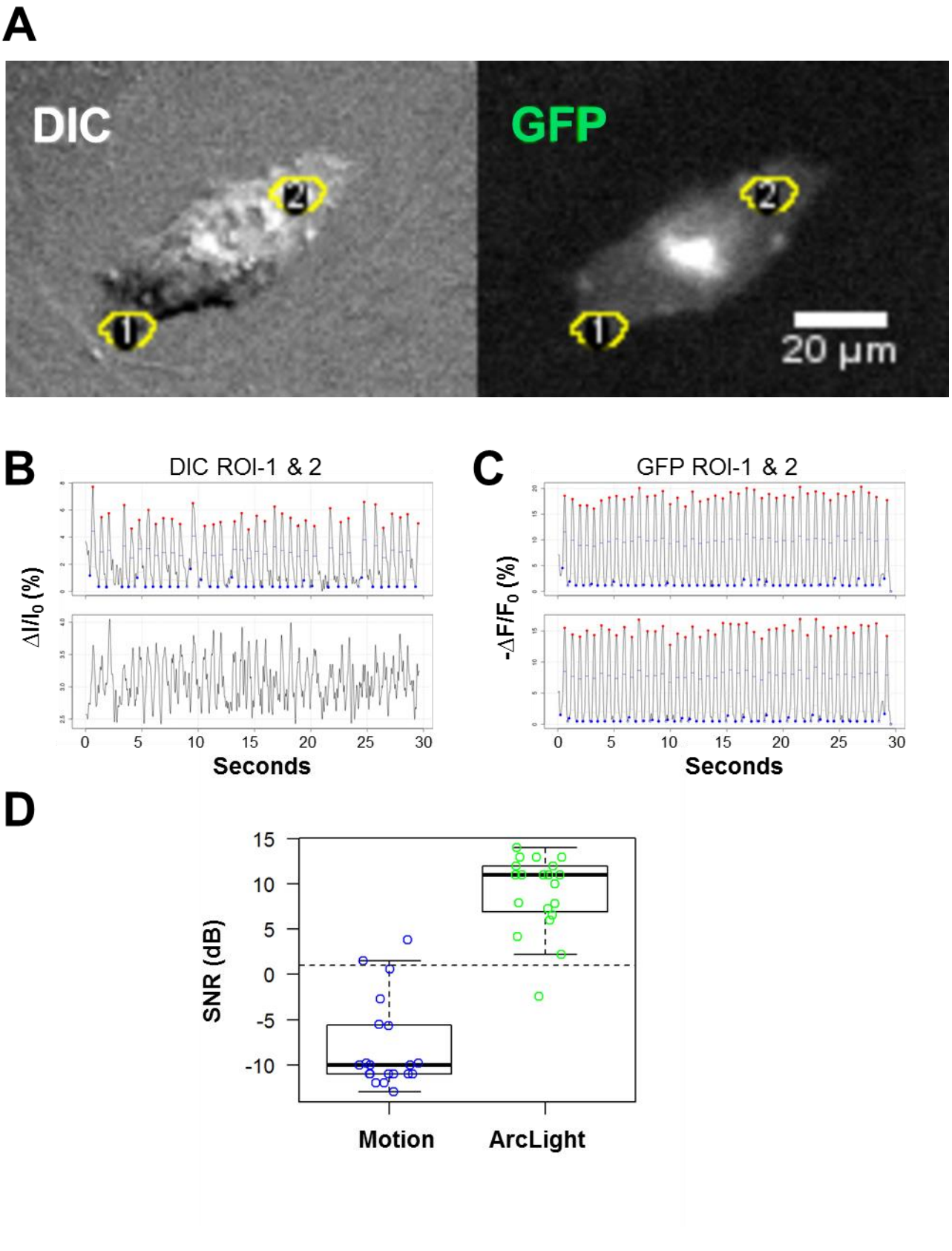
A
DIC
GFP
2
1
20 µm
B
DIC ROI-1 & 2
ΔI/I0 (%)
Seconds
C
GFP ROI-1 & 2
-ΔF/F0 (%)
Seconds
D
SNR (dB)
Motion
ArcLight

Figure S5. Minimization of motion artifacts on optical recording of ArcLight. (A) DIC and GFP fluorescence images of the same field showing two region of interest (ROI) in a beating ArcLight-CM. Scale bar, 20 µm. **(B)** Traces based on mean intensity changes ($\Delta I/I_0$) were processed with Spikemapping software (https://amlab.shinyapps.io/spikemap/). Motion effects were detected in ROI-1 that included a contrast cell boundary but absent in ROI-2 that covers a relatively uniform area within the cell. **(C)** Traces of ArcLight intensity changes ($-\Delta F/F_0$) showing nearly identical AP profiles in both ROI-1 and -2. **(D)** Signal-to-noise ratio (SNR) of motion relative to ArcLight recording (n = 20).

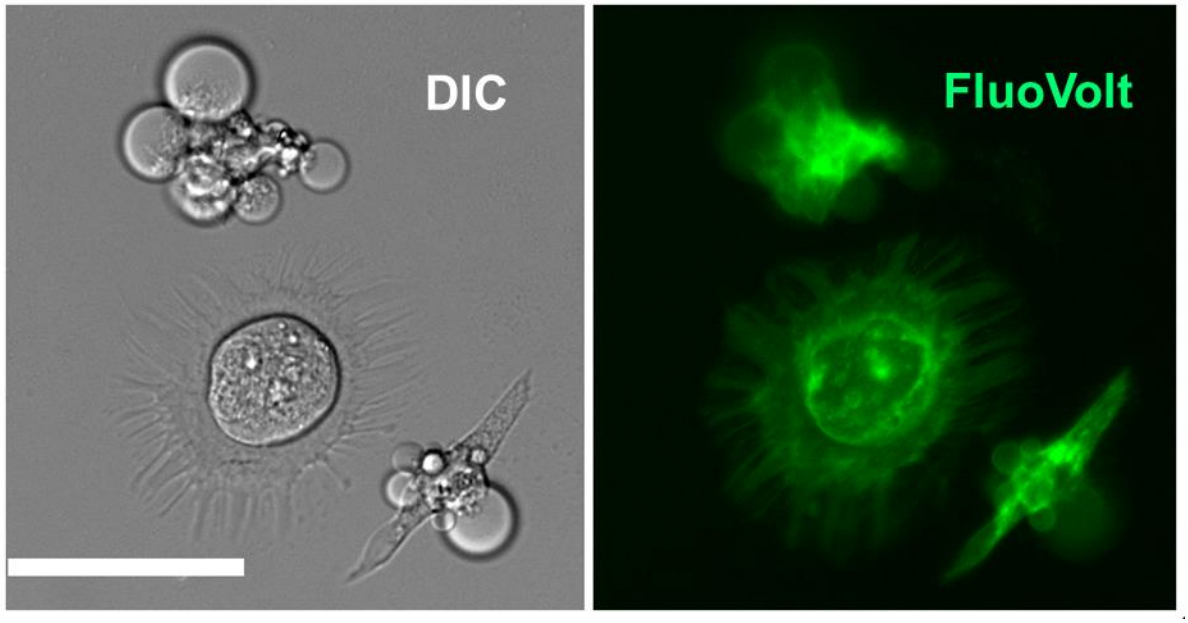

Figure S6. Prolonged exposure to excitation light induced phototoxicity in hiPSC-CMs loaded with FluoVolt. A representative DIC/FluoVolt image of hiPSC-CMs showing retraction of cell leading edge and blebbing of the cell membrane in response to excessive illumination. Scale bar, 50 µm.

Table S1. Primers used in this study

Primer	Sequence (5' to 3')	Description and Reference
ArcLight-F	GGGGACAAGTTTGTACAAAAAA GCAGGCTACCATGGAGGGATTC GACGGTTCAG	Forward primer with attB1 to amplify ArcLight cDNA from ArcLight A242 [23]
ArcLight-R	GGGGACCACTTTGTACAAGAAA GCTGGGTTCATTTGTATAGTTCA TCCATGCCATG	Reverse primer with attB2 to amplify ArcLight cDNA from ArcLight A242 [23]
803	TCGACTTCCCCTCTTCCGATG	Forward primer to amplify targeted AAVS1 region from edited hiPSC genomic DNA [35]
804	GAGCCTAGGGCCGGGATTCTC	Reverse primer in T2A to amplify the junction region from edited hiPSC genomic DNA [35]
182	CCCCTTACCTCTCTAGTCTGTGC	Insertion sequence primer [35]
183	CTCAGGTTCTGGGAGAGGGTAG	Combine with WT-F to amplify wild type or mutated allele of AAVS1 [35]
WT-F	ATGGCCTTCTCCGACGGATGTC TC	Forward primer to amplify targeted or normal AAVS1. Also used for sequencing
pCAG-F	GCAACGTGCTGGTTATTGTG	Forward sequence primer of ArcLight destination vector (Addgene)
BglobpA-R	TTTTGGCAGAGGGAAAAAGA	Reverse sequence primer of ArcLight destination vector (Addgene)

Table S2. Plasmids used in this study

Plasmid	Description	Reference or Source
ArcLight A242	Ci-VSP with super ecliptic pHluorin driven by CMV promotor	https://www.addgene.org/36857/ [23]
pXAT2	AAVS1 sgRNA-T2 and mammalian CRISPR/Cas9 co-expression vector	https://www.addgene.org/80494/ [35]
pAAVS1-P-CAG-DEST	Gateway Destination vector for AAVS1 targeting with puromycin selection	https://www.addgene.org/80490/ [35]
pDONR221	Gateway Donor vector	(Invitrogen)
pArcLight-DNOR	ArcLight Donor by BP recombination	(This work)
pArcLight-DEST	ArcLight Destination by LR recombination	(This work)

Table S3. Summary of optical APs in ArcLight-CMs

Parameters	Fast (Mean ± SE)	Slow (Mean ± SE)	*t*-test (*p* value)
Number of cells	50	35	
Amplitude (% change)	-14 ± 0.83	-20 ± 1.83	0.011
APD_{50} (ms)	203 ± 6.96	323 ± 21.31	0.000
APD_{90} (ms)	348 ± 8.82	509 ± 25.09	0.000
APD_{50}/APD_{90}	0.579 ± 0.01	0.620 ± 0.01	0.015
Frequency (bpm)	110 ± 3.13	46 ± 3.97	0.000
V_{max} (%/s)	115 ± 6.65	137 ± 12.25	0.120
SNR (dB)	6.6 ± 0.41	7.4 ± 0.72	0.353

Table S4. Comparison of optical APs recorded with ArcLight or FluoVolt

Parameters	ArcLight (Mean ± SE)	FluoVolt (Mean ± SE)	*t*-test (*p* value)
Number of cells	87	83	
Amplitude (% change)	-16 ± 0.92	17 ± 0.46	0.748
APD_{50}/APD_{90}	0.595 ± 0.01	0.587 ± 0.01	0.948
Frequency (bpm)	87 ± 4.68	69 ± 3.10	0.004
V_{max} (%/s)	124 ± 6.31	129 ± 3.81	0.457
SNR (dB)	6.9 ± 0.38	7.7 ± 0.21	0.089

Movie S**1:** An example of beating ArcLight-CM (related to Fig. 5A) recorded with a conventional epifluorescence microscope by monitoring relative fluorescent intensity changes of ArcLight. Movie plays in real time. Timestamp in ms. Scale bar, 50 μm.

Movie S**2:** Motion effects on optical recording of ArcLight (related to Fig. S5). Time-lapse images of DIC and GFP channels are combined. Movie plays in real time. Timestamp in ms. Scale bar, 20 μm.

Movie S**3:** Optical recording of an ArcLight-CM on differentiation day 21. Time-lapse images of DIC and GFP channels are combined. Movie plays in real time. Timestamp in ms. Scale bar, 50 μm.

Movie S**4:** Continuous optical recording of an ArcLight-CM for 10 minutes with full excitation power. Movie plays in 60× speed. Timestamp in mm:ss. Scale bar, 50 μm.

Movie S**5:** Long-term monitoring of the same ArcLight-CM over 8 days. Movie combined from time-lapse images plays in real time. Timestamp in ms. Scale bar, 50 μm.

Movie S**6:** Movie of time-lapsed images for ArcLight-CM recorded in Fig. 5B. A fast beating ArcLight-CM with pacemaker-like AP profile. Movie plays in real time. Timestamp in ms. Scale bar, 50 μm.

Movie S**7:** Movie of time-lapsed images for ArcLight-CM recorded in Fig. 5C. A slow beating ArcLight-CM with working CM-like AP profile. Movie plays in real time. Timestamp in ms. Scale bar, 50 μm.

Movie S8: A representative optical recording of WT hiPSC-CMs loaded with FluoVolt. Movie plays in real time. Timestamp in ms. Scale bar, 50 μm.

Movie S**9:** Comparison of photobleaching of ArcLight and FluoVolt. Time-lapse images were recorded for 10 minutes with full excitation power. Combined-movie plays in 60× speed. Timestamp in mm:ss. Scale bar, 50 μm.