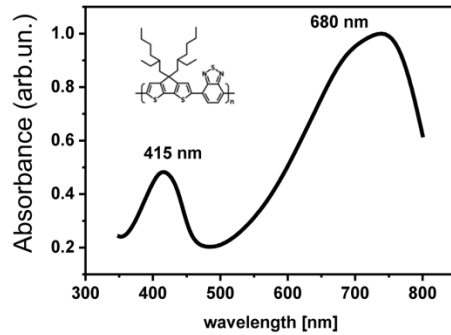


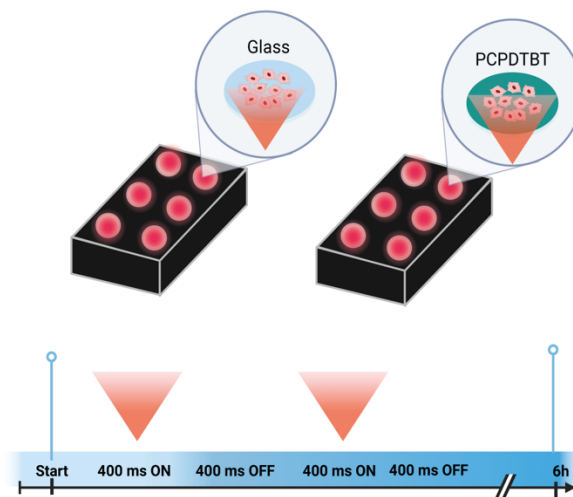
1 **Supplementary Information**

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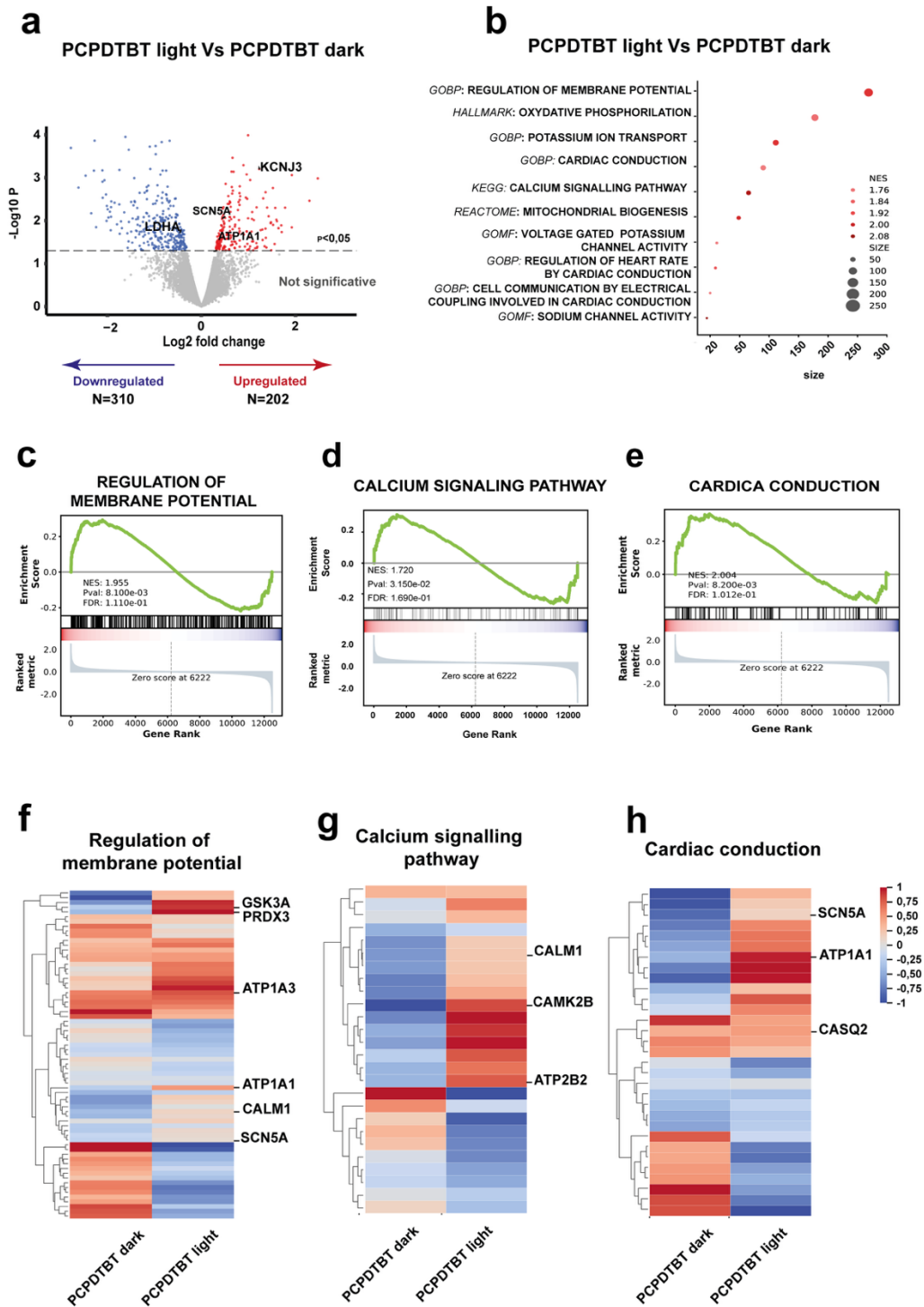


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4 **Figure S1. Experimental set up and biocompatibility assessment.** **a)** Chemical structure and
5 normalized optical absorption spectrum of the PCPDTBT polymer. **b)** Schematic representation of
6 the experimental setup and protocol used for PCPDTBT optical stimulation. PCPDTBT thin films
7 were deposited by spin coating from a solution in organic solvent. Subsequently, polymer films were
8 thermally sterilized, adhesion protein layer was deposited on top of the polymer surface and hPSC-
9 CMs were seeded on top of the fibronectin-coated polymer for the illumination.



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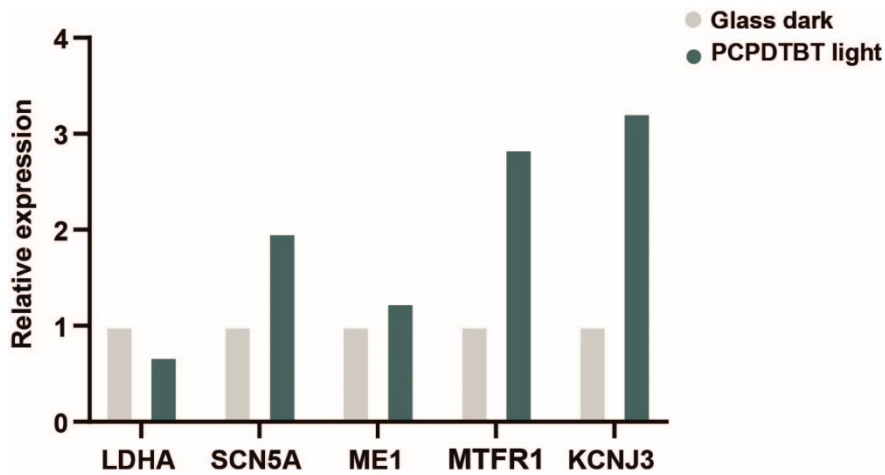
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Figure S2. Effect of polymer-mediated photostimulation on hPSC-CM transcriptional profile.
 (a) Volcano plot showing differentially expressed genes between PCPDTBT light and PCPDTBT dark conditions. Significantly upregulated and downregulated genes (FDR < 0.05) are highlighted in red and blue respectively; non-significant genes are shown in grey. Some of the key genes indicative of hPSC-CM maturation are shown in bold. (b) Dot plot of the most significantly enriched pathways (both up- and downregulated) in the PCPDTBT light vs PCPDTBT dark comparison, with relevance to cardiomyocyte maturation and functionality (c, d, e) Representative GSEA enrichment plots of

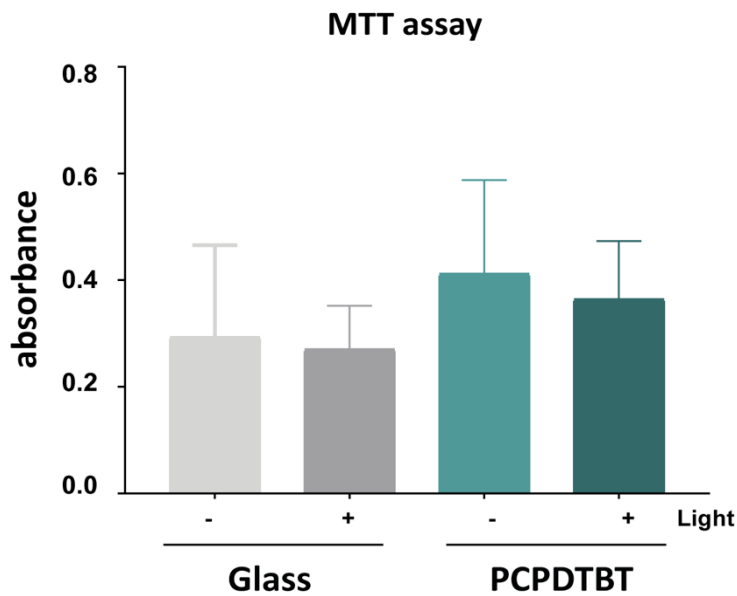
19 selected pathways. (f, g, h) Heatmaps displaying expression of leading-edge genes from significantly
20 upregulated pathways. Some relevant genes are shown in bold. RNA-sequencing was performed
21 on n = 3 independent hPSC-CM batches.

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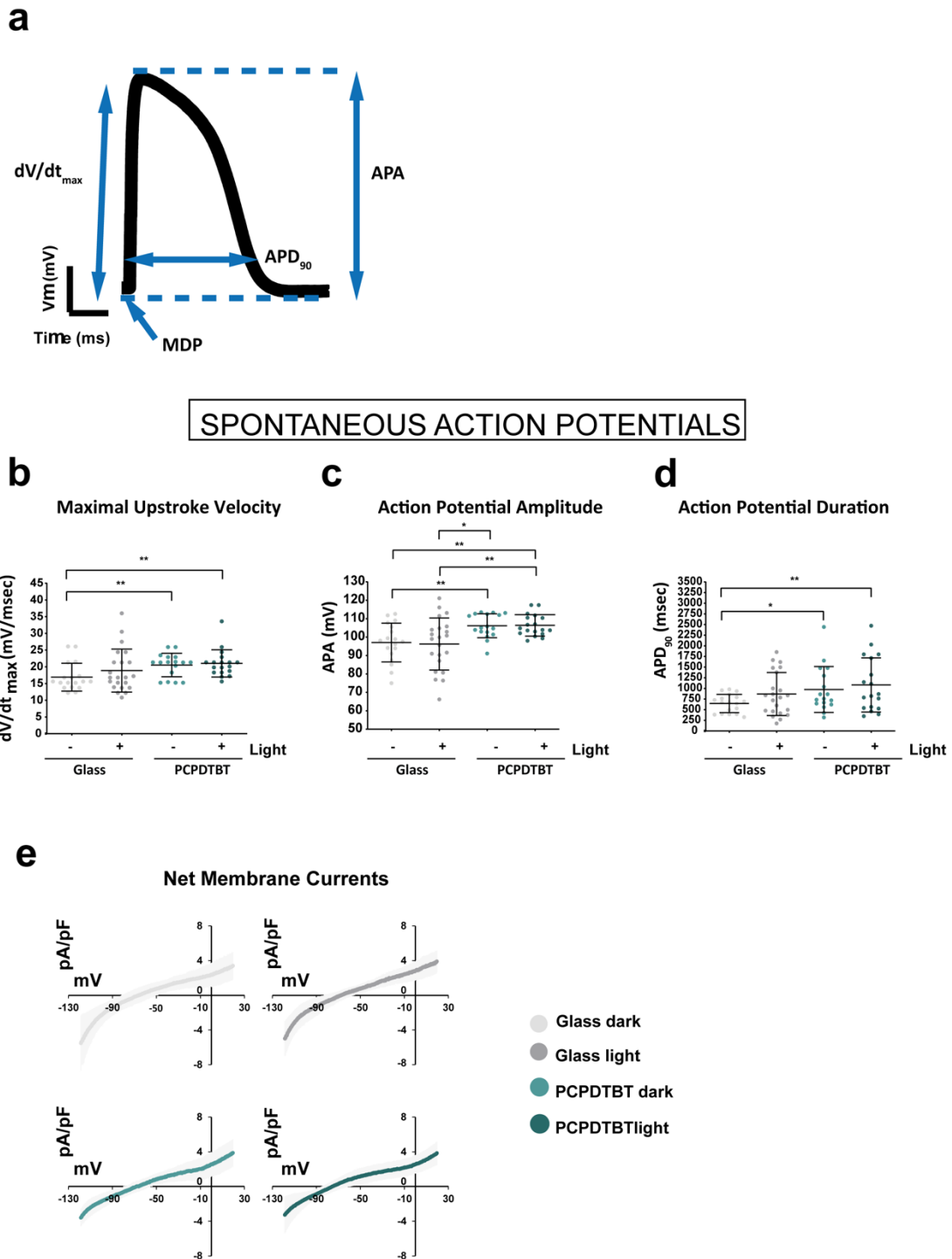
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Figure S3. RNA-sequencing validation. Relative expression of *LDHA*, *SCN5A*, *ME1*, *MTFR1* and *KCNJ3* genes, measured via Rt-qPCR and normalized to 18S gene analysed in hPSC-CMs under two experimental conditions (glass dark and PCPDTBT light). Data are represented relative to the glass dark condition.



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Figure S4. MTT assay. MTT assay performed of hPSC-CMs seeded on control glass and PCPDTBT thin film in dark condition and following light stimulation protocol. N=3. Not significant (Unpaired t-test).



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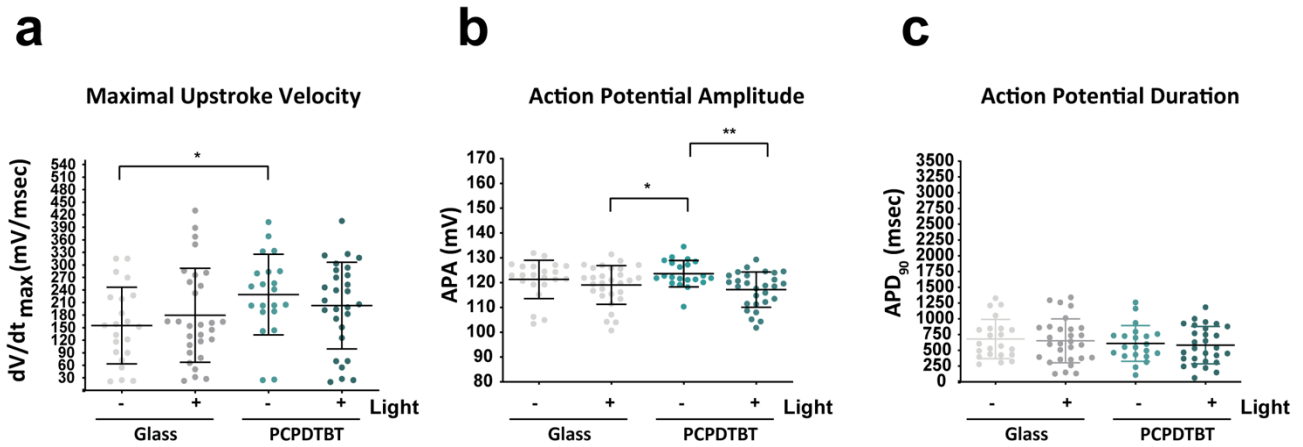
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40 **Figure S5. (a)** Schematic representation of key action potential parameters analysed in hPSC-CMs:
 41 maximal diastolic potential (MDP), action potential amplitude (APA), maximal upstroke velocity
 42 (dV/dt_{max}) and action potential duration at 90% repolarization (APD_{90}). **(b-d)** Quantification of
 43 spontaneous action potential parameters in hPSC-CMs cultured on glass or PCPDTBT substrates,
 44 with or without chronic red-light stimulation: (a) maximal upstroke velocity (dV/dt_{max}), (b) action
 45 potential amplitude (APA), and (c) action potential duration at 90% repolarization (APD_{90}). Glass dark
 46 $n= 18$, Glass light $n= 22$, PCPDTBT dark $n= 17$, PCPDTBT light $n= 18$. **(d)** Net membrane current

47 (NMC) recordings obtained using a voltage ramp protocol in hPSC-CMs grown on the indicated
48 substrates and under the specified conditions. Currents are normalized to cell capacitance (pA/pF);
49 shaded areas indicate SD.

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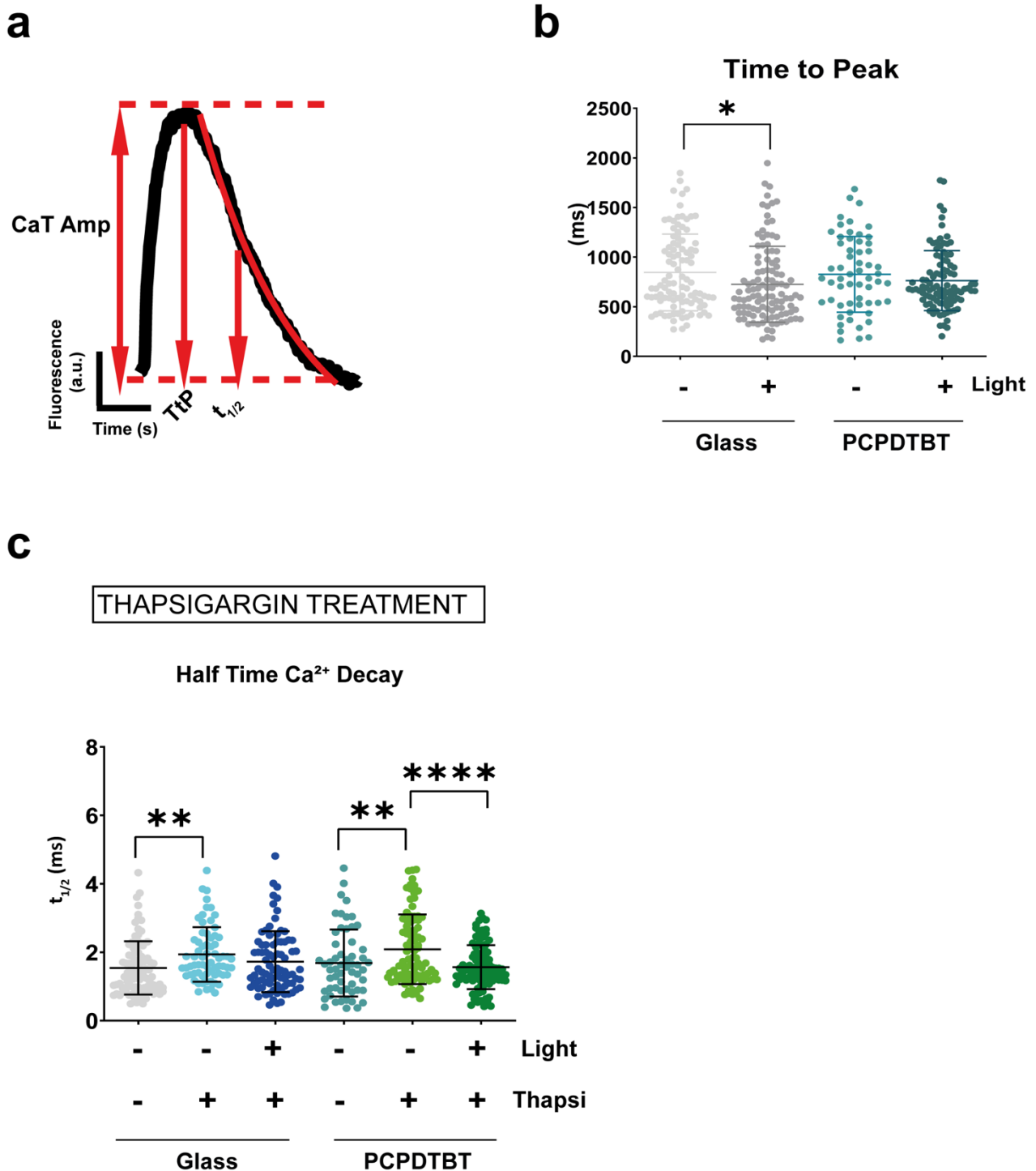
EVOKED ACTION POTENTIALS



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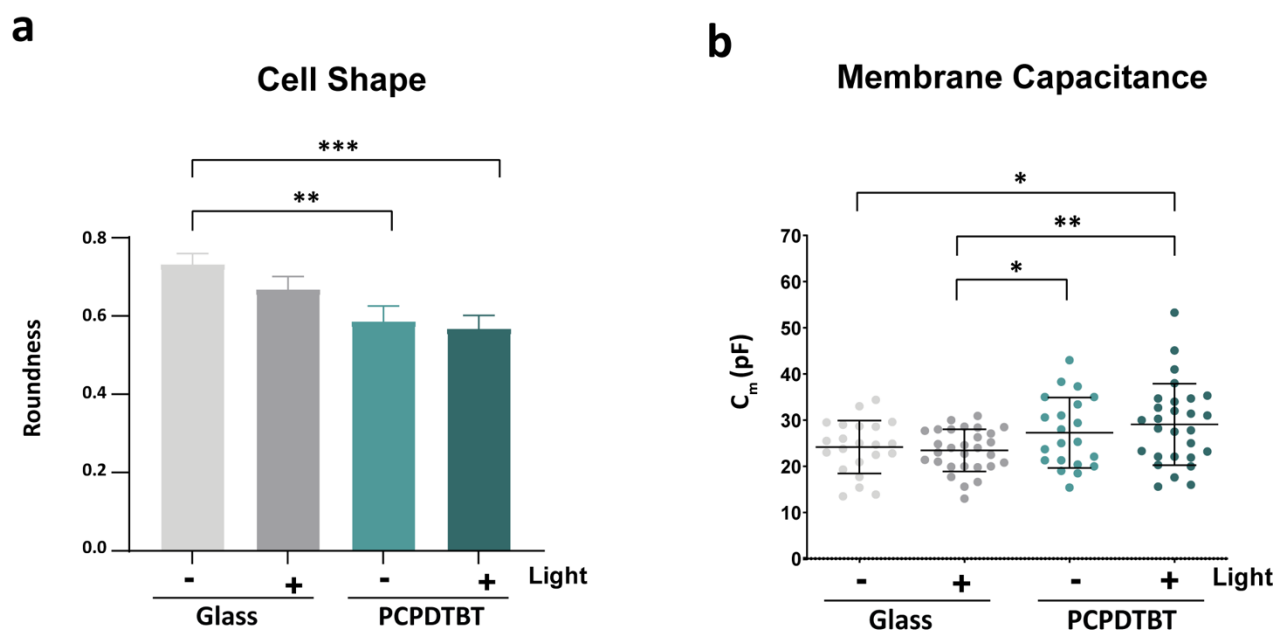
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54 **Figure S6. AP parameters measurements in evoked hPSC-CM action potentials. 1 (a–c)**
 55 Quantification of evoked action potential parameters in hPSC-CMs cultured on glass or PCPDTBT
 56 substrates, with or without chronic red-light stimulation: (a) maximal upstroke velocity (dV/dt_{max}), (b)
 57 action potential amplitude (APA), and (c) action potential duration at 90% repolarization (APD_{90}).
 58 Data are presented as mean \pm SD; $p^* < 0.05$, $p^{**} < 0.005$ (unpaired t-test). Glass dark $n = 22$, Glass
 59 light $n = 29$, PCPDTBT dark $n = 21$, PCPDTBT light $n = 28$.



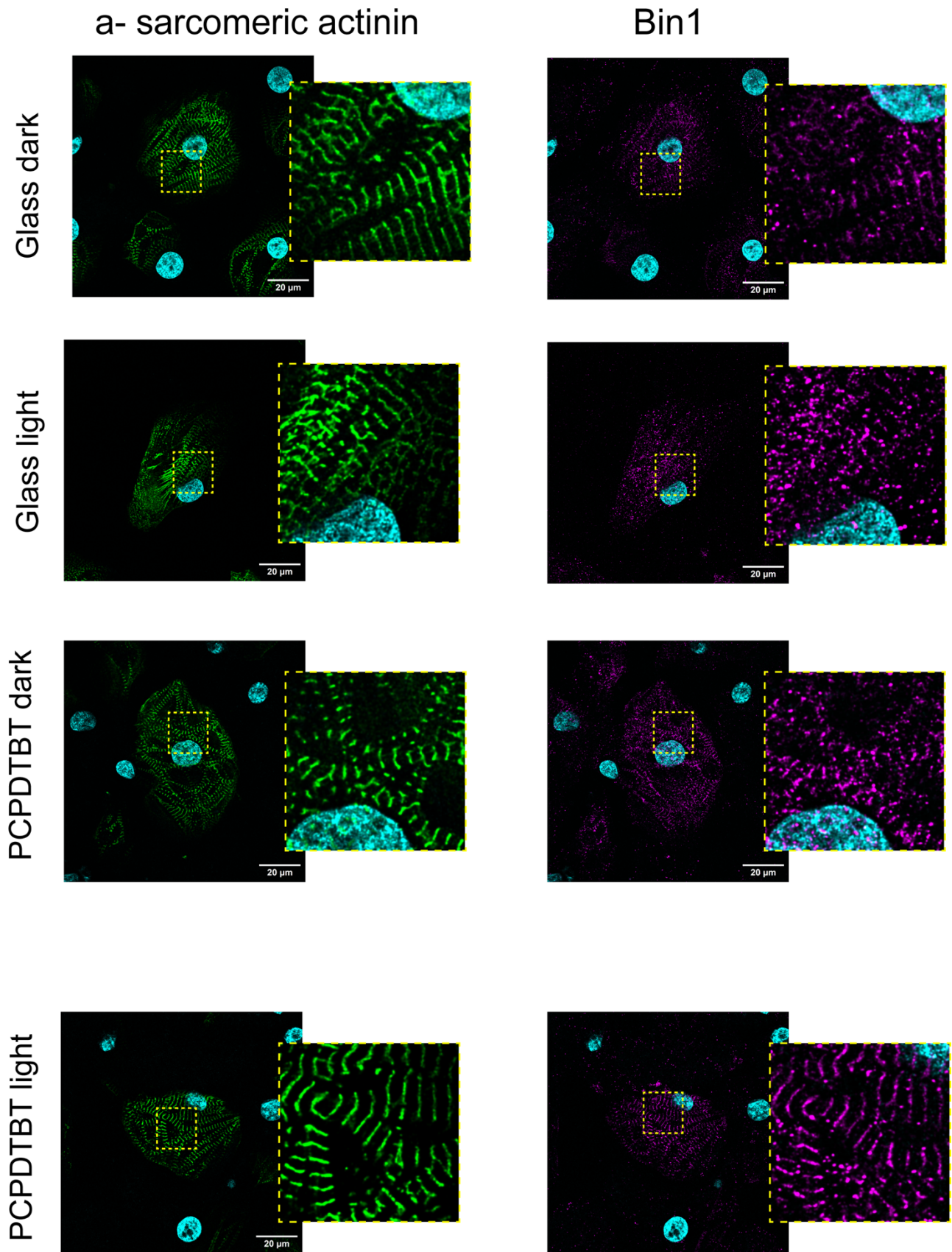
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61 **Figure S7. Ca^{2+} dynamics.** (a) Schematic representation of the different Ca^{2+} parameters
 62 measured. (b) Time to Peak (TtP) of hPSC-CMs seeded on control glass and PCPDTBT in dark or
 63 light conditions (c) Half Time Ca^{2+} decay of hPSC-CMs seeded on control glasses and PCPDTBT in
 64 dark or light conditions and treated with Thapsigargin (Thapsi). Glass dark n= 99, glass light + Thapsi
 65 n= 75, Glass light + Thapsi n= 86; PCPDTBT dark n=58, PCPDTBT dark + Thapsi=95, PCPDTBT
 66 light + Thapsi=96. Data are presented as dot plot with mean \pm SD; $p^* < 0.05$, $p^{**} < 0.005$, $p^{****} <$
 67 0.0001 (unpaired t-test).



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70 **Figure S8. PCPDTBT photostimulation effects of hPSC-CMs structure.** a) Cell roundness
 71 quantification. b) Dot plot with membrane capacitance (C_m) data recorded from hPSC-CMs
 72 seeded on control glasses and PCPDTBT in dark or light conditions. glass dark n=43, glass light
 73 n=29, PCPDTBT dark n=32, PCPDTBT light n=38). * $p < 0,05$, **** $p < 0,0001$ (Unpaired t-test).



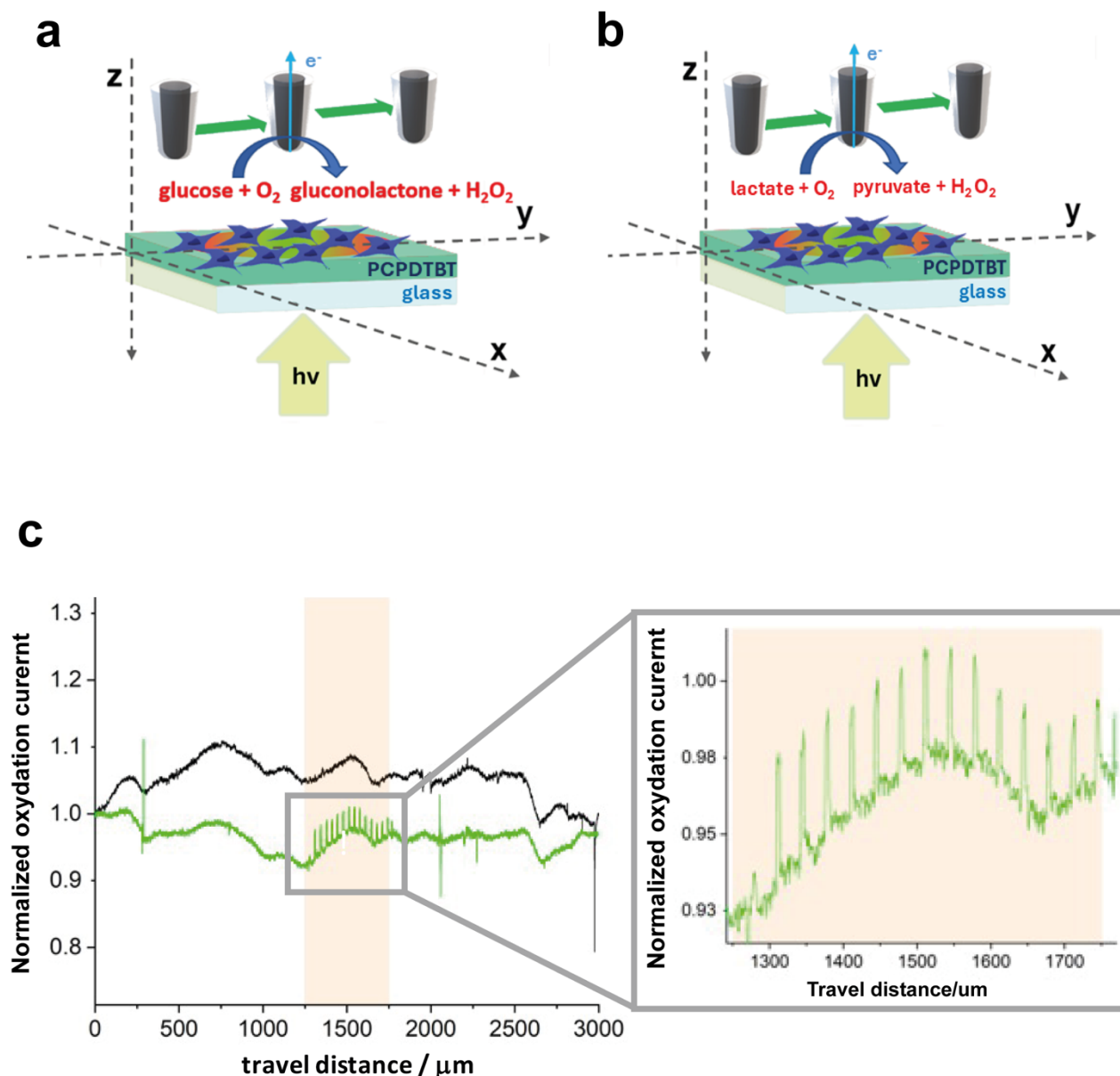
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76 **Figure S9. Effect of PCPDTBT photostimulation of Bin-1 expression in hPSC-CM. (a-d)**

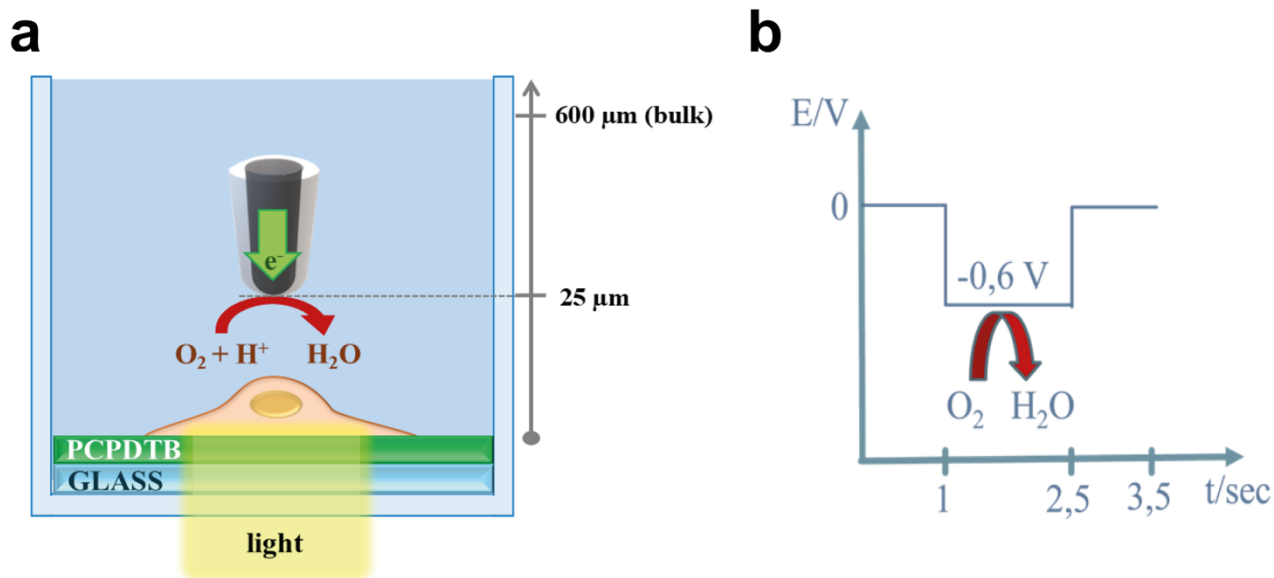
77 Representative immunofluorescence images for α -sarcomeric actinin (green) and Bin1 (magenta) of

78 hPSC-CMs seeded on control glass and PCPDTBT both in dark and light conditions. Nuclei were
79 marked with DAPI (cyan).
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83 **Figure S10 SECM apparatus and rationale of SECM measurements to quantify changes of**
 84 **glucose uptake and lactate release in hPSC-CMs upon chronic illumination of PCPDTBT**
 85 **films. (a-b)** Schematic representations of SECM horizontal Probe-Scan Curves (PSCs) at fixed
 86 height from the surface of the PCPDTBT films. Scheme of the measurement strategy employing
 87 modified platinum microelectrodes for glucose detection **(a)** and lactate release **(b)**. **(c)**
 88 Representative horizontal PSCs, before (black trace) and during (green trace) illumination. In this
 89 region, spikes, which are due to transient local hydrogen peroxide production as a consequence of
 90 PCPDTBT film illumination, are visible.



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93 **Figure S11 SECM apparatus and rationale of SECM measurements to quantify oxygen**
 94 **consumption changes in hPSC-CMs upon PCPDTBT chronic photostimulation**

95 **(a-b)** Schematic representation of the analytical strategy for oxygen consumption determination with
 96 SECM. Potential scan pulses at -0.6 V vs Ag/AgCl (KCl 3 M) were programmed and performed as
 97 represented in panel **b** at two distances from PCPDTBT film surface, 25 and 600 μm, as showed in
 98 panel **c**. Potential pulses were performed at - 0.6 V vs Ag/AgCl (KCl 3M) for 0.5 s to measure oxygen
 99 reducing currents, which can be directly related to local oxygen concentration in the bulk, 600 μm,
 100 and in the cell proximity, 25 μm.

Primers	Sequences
LDHA FWD	5' TTGTCTCTGGCAAAGTGGAT 3'
LDHA REV	5' CTCCATGTTCCCCAAGGACC 3'
ME1 FWD	5' TGTTTCGGAAGCCAAGAGGT 3'
ME1 REV	5' CATCCCTCCGCAAGCTGTAT 3'
MTFR1 FWD	5' GCGGCCGAGTGTGTTTT 3'
MTFR1 REV	5' AGGCGCTTAATCCAGCCAA 3'
KCNJ3 FWD	5' CAAGCTGCTCAAAGGATGACT 3'
KCNJ3 REV	5' AAGGGGACGACATGAGAAGC 3'

101

102 **Table S1.** List of PCR primer sequences used for RNA-seq validation