SUPPORTING INFORMATIONS

A Polymer Bio-photo-electrolytic Platform for electrical signal measurement and for light modulation of ion fluxes and proliferation in a neuroblastoma cell line.

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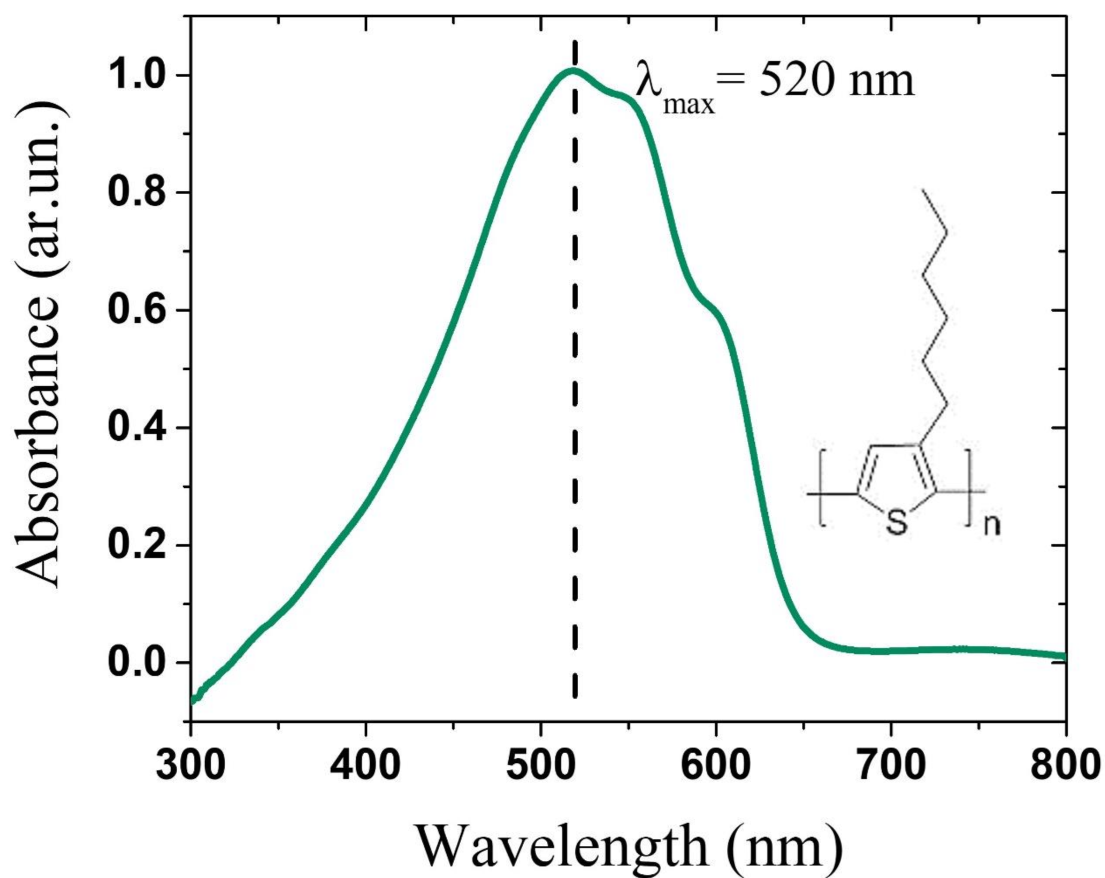

Figure S1: UV-vis absorption spectra of P3HT thin film. Maximum absorption peak at 520 nm. P3HT chemical structure is shown.

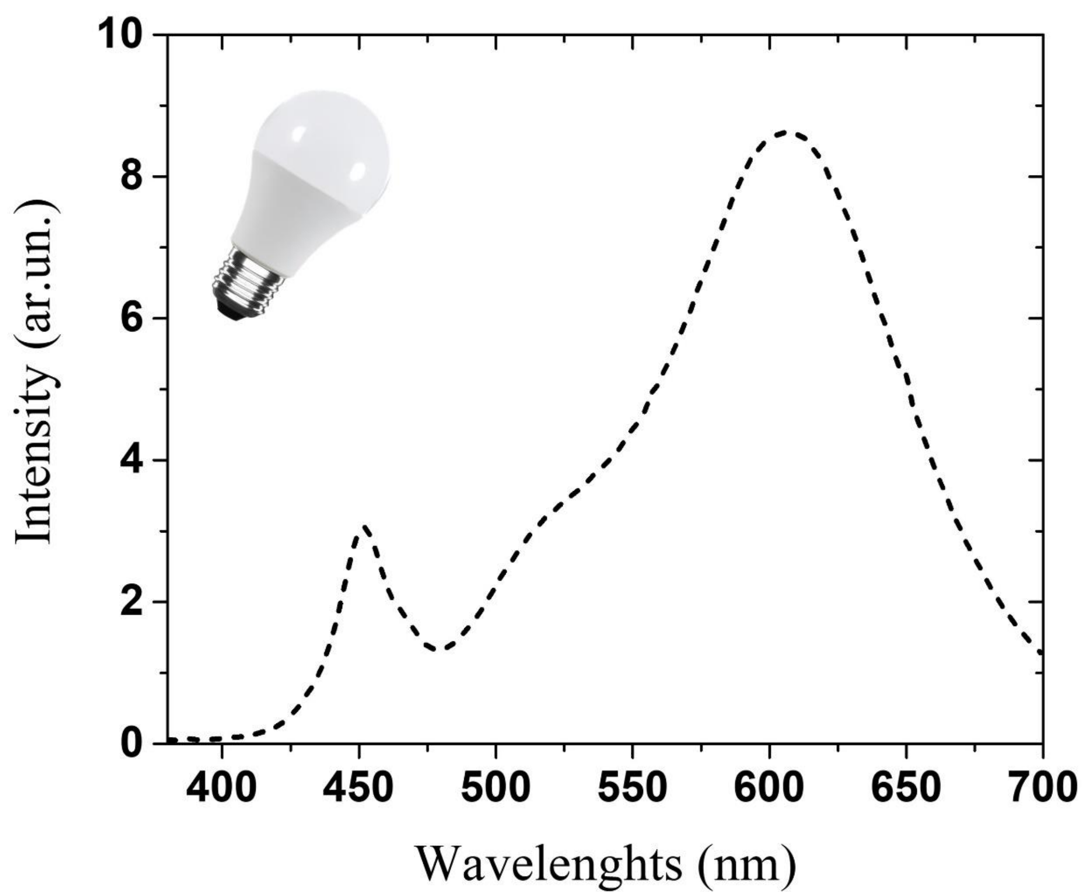

Figure S2: Standard warm white LED light spectrum. LED used for the light-stimulation protocol (LS) of cells cultured on the bio-photo-electrolytic platform P3HT-photo electrode. Light power was 1.26 mW cm^{-2}.

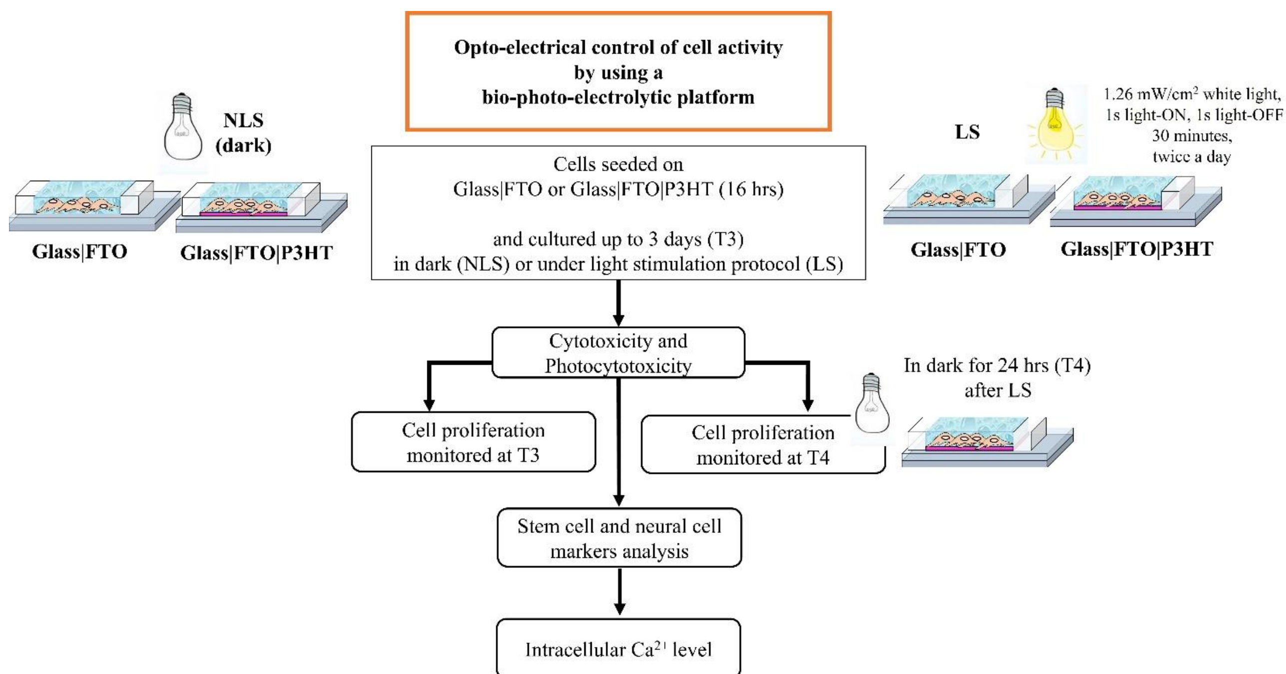

Figure S3: Experimental flow chart. Cells were seeded on substrates (Glass|FTO and Glass|FTO|P3HT) and let to adhere for 16 hours, then subjected to light stimulation protocol (LS) or kept in dark condition (NLS). Cytotoxicity and photo-cytotoxicity, cell proliferation and cell differentiation (stem cell and neural cell markers), and intracellular Ca^{2+} level were evaluated at the indicated time points.

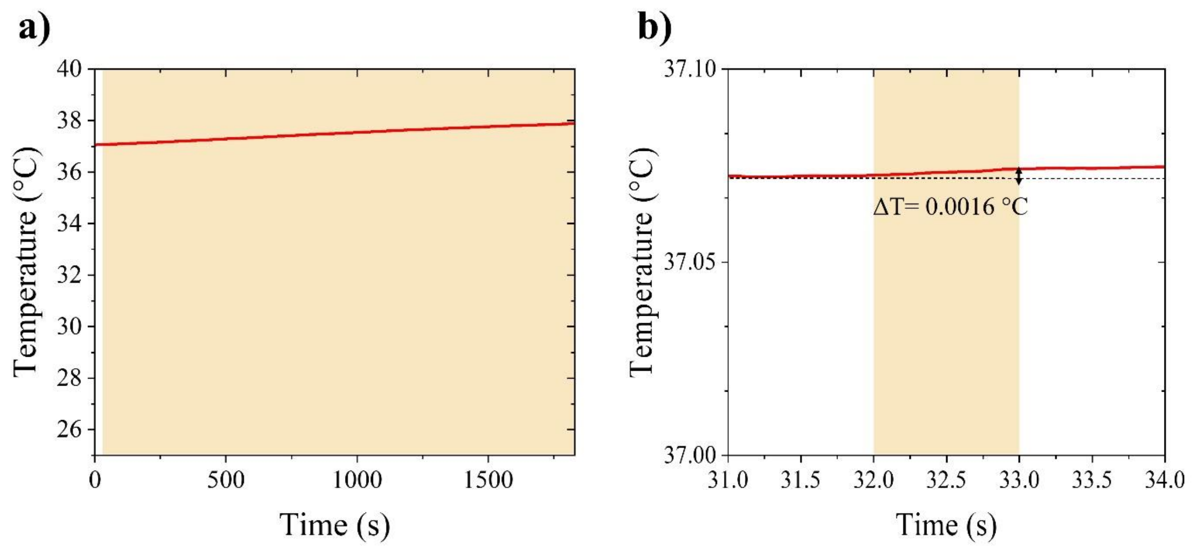

Figure S4: Temperature variation (ΔT= 0.52 °C) of the extracellular bath upon light stimulation protocol over 30 minutes (a), 1s on and 1 s off, as a function of time. Temperature variation over 1 s of photoexcitation (b). The experiment is performed by using a thermocouple K placed on the P3HT thin film surface, immersed in cell culture medium, inside incubator (37°C, 5% CO2) with a photoexcitation density of 1 mW cm-2 (warm white LED).

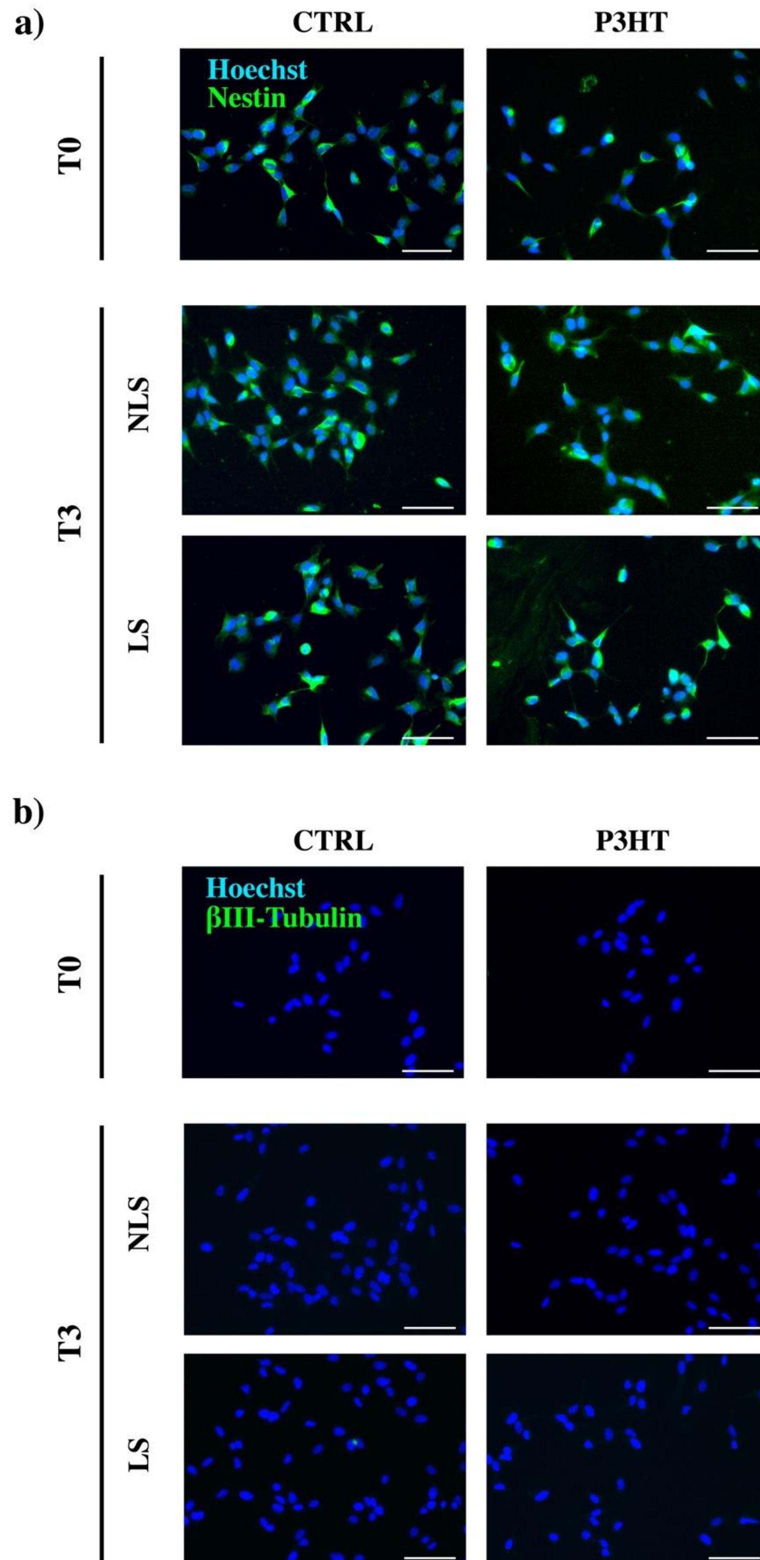

Figure S5: Representative micrographs of SH-SY5Y cell cultures analysed at the beginning (T0) and after three days of culture (T3) by immunofluorescence for a) Nestin (green) and b) βIII-Tubulin (green). In both panels, cell nuclei were stained with Hoechst (blue). Scale bar = 50 μm.

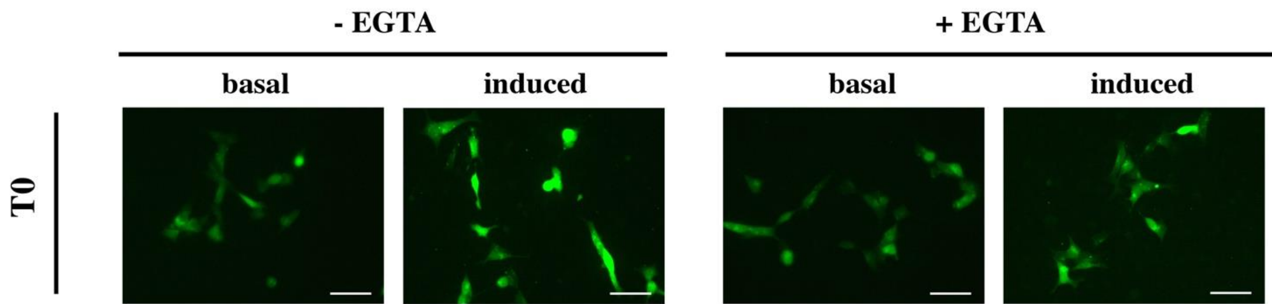

Figure S6: Calcium imaging of FLUO-4 AM-loaded SH-SY5Y cells. Representative images of randomly selected fields of cells cultured on Glass|FTO|P3HT (P3HT) analyzed at T0 and pretreated without (-EGTA) and with (+EGTA) 100 µM EGTA. Images were acquired before (basal) and after (induced) 30 min of photo-stimulation with 1s light on and 1 s light off. Scale bar = 50 µm.

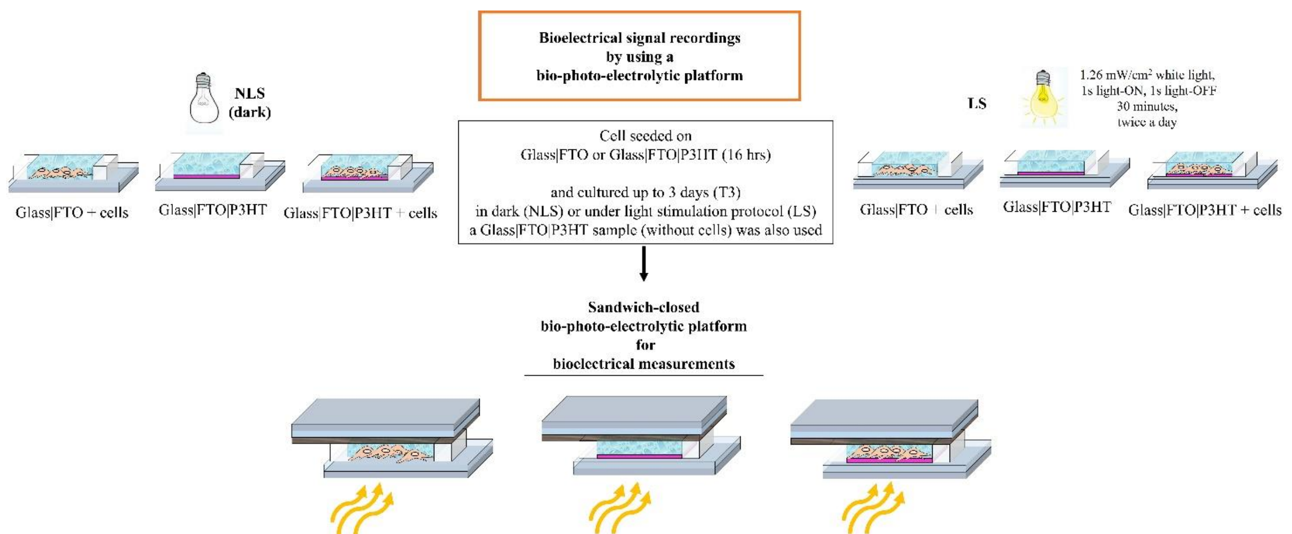

Figure S7: Flow chart illustrating the adopted experimental approach for the bioelectrical analysis. Cells were seeded on substrates (Glass|FTO and Glass|FTO|P3HT) and let to adhere for 16 hours, then kept in dark (NLS) or subjected to light stimulation protocol (LS: white LED light, 1.26 mW cm^{-2}, 1 s light-ON and 1 s light-OFF, 30 minutes twice a day for three days). Opto-electrical measurements were performed upon light irradiation (white LED light, 15 mW cm^{-2}, 300 ms duration) of the sandwich-closed platforms. Sandwich-closed platforms containing cells (Glass|FTO and Glass|FTO|P3HT) and without cells (Glass|FTO|P3HT) were analysed.

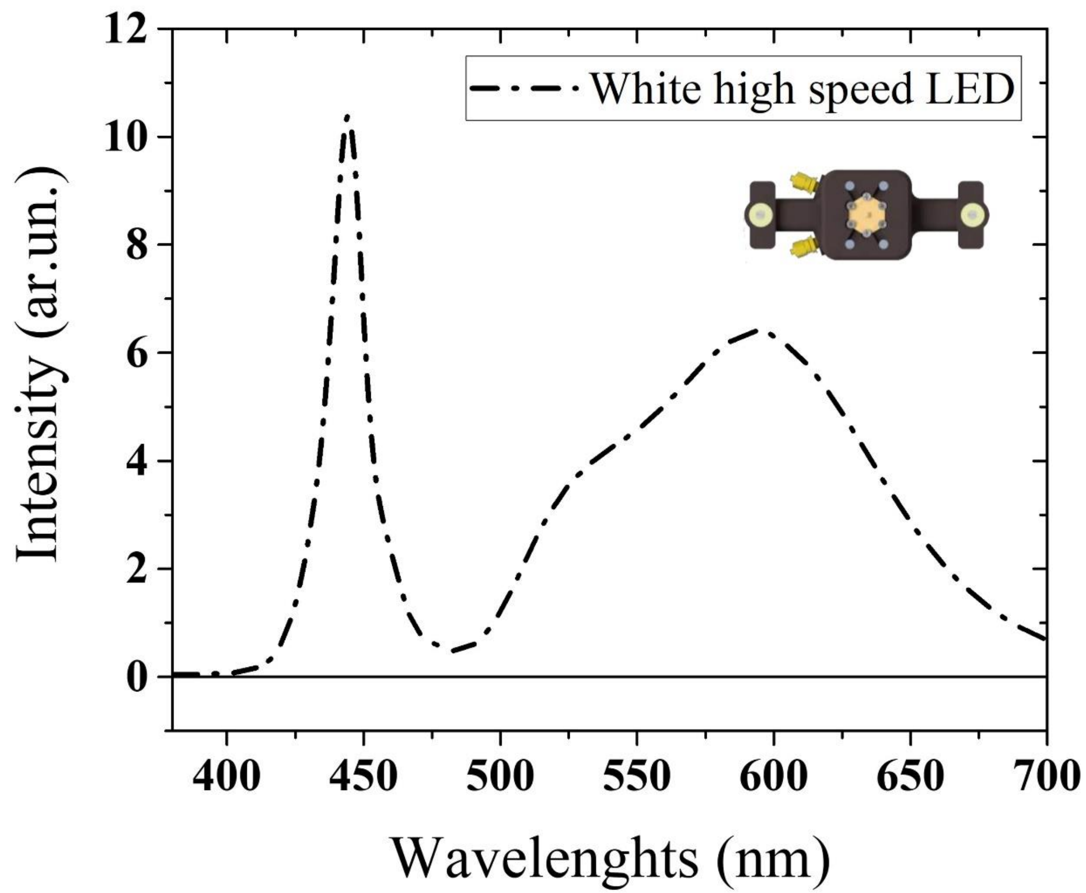

Figure S8: White high-speed LED spectrum. LED used for light stimulation embedded in the used measurement ARKEO set-up.

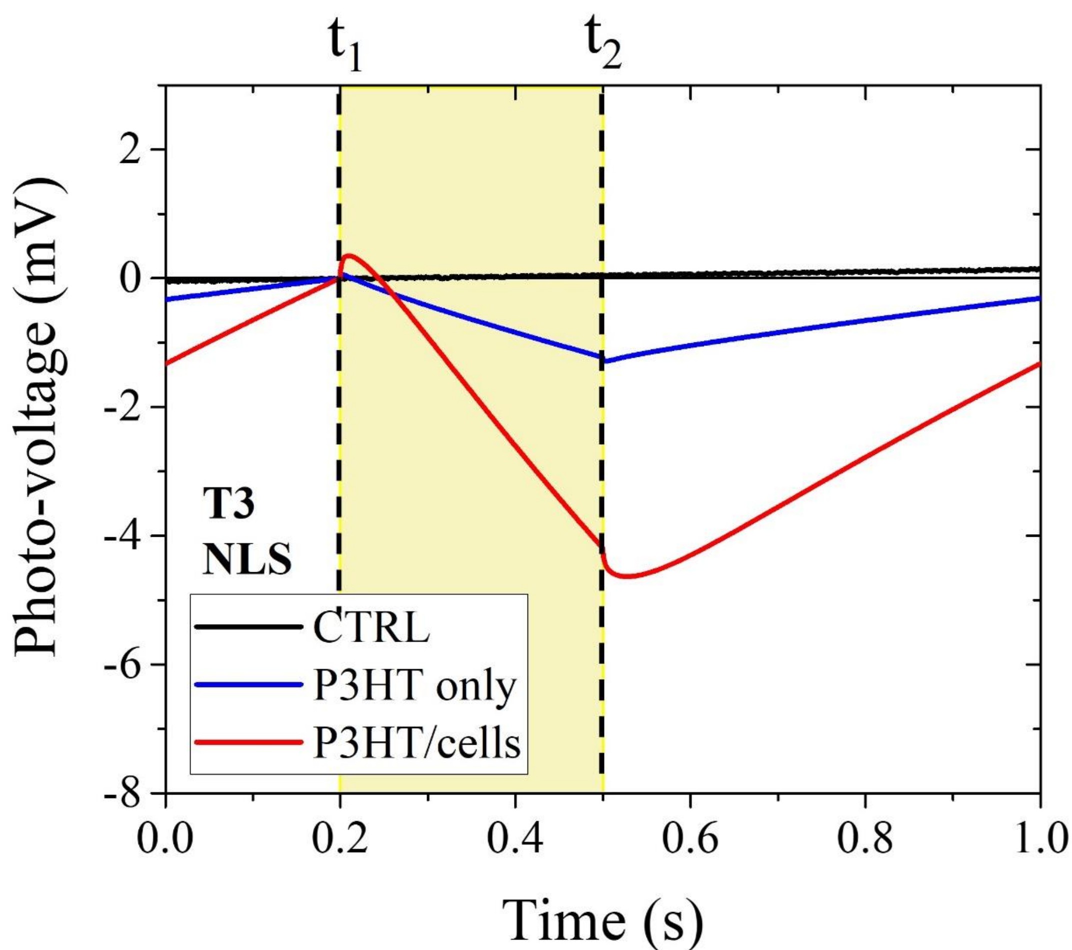

Figure S9: Optoelectrical signals measured at T3 for NLS samples. Photo-voltage generated upon light irradiation at 15.0 mW cm^{-2}, recorded at T3 from samples kept in the dark (NLS). Signals were recorded from the bio-photo-electrolytic platform, in the sandwich-closed architecture, consisting in cells cultured on Glass|FTO (CTRL, Black line), Glass|FTO|P3HT (P3HT only, Blue line), cells culture on Glass|FTO|P3HT (P3HT/cells, Red line). The shaded yellow area represents the duration of the light stimulus (300 ms). Signals are the mean on three samples. Each sample signal represents an average of twenty consecutive sweeps.

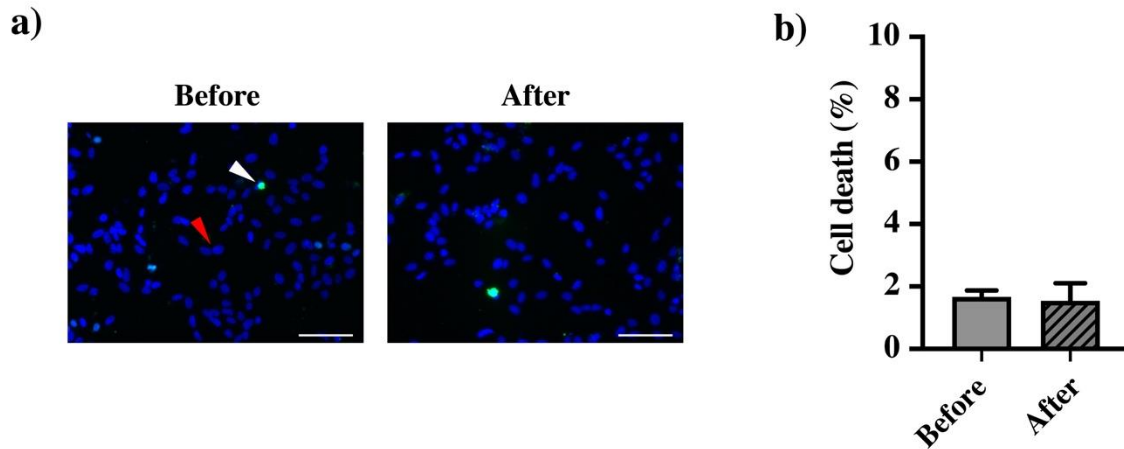

Figure S10: SH-SY5Y cells viability. a) Evaluation of cell viability by the in-situ Cell Viability Imaging Assay. Representative fluorescence micrographs of SH-SY5Y cells cultured on Glass|FTO|P3HT at the end of the culture period and before opto-electrical measurements (Before) and at the end of opto-electrical data acquisition, namely 15 minutes from when samples were taken from the incubator (After). Gelatine coating was included in all samples. White and red arrowheads indicate dead and live cells, respectively. Scale bar = 50 µm. b) Quantitative analysis of cell death expressed as percentage of positive cells on total cells: 1.67±0.21% dead cells at the end of the culture, namely T3; 1.54±0.56% dead cells at the end of opto-electrical data acquisition, namely 15 minutes from when samples were taken from the incubator.

Table S1: SH-SY5Y neuroblastoma cell viability assay. Evaluation of cell viability by the in-situ Cell Viability Imaging Assay. Percentage of positive (i.e. dead) SH-SY5Y cells cultured on Glass|FTO (CTRL) and Glass|FTO|P3HT (P3HT) subjected to the light-stimulation protocol (LS) or kept in dark as control (NLS). Analysis was performed at T0, T1, T2, T3 and T4.

		T0 [%]	T1 [%]	T2 [%]	T3 [%]	T4 [%]
CTRL	NLS	1.25±0.39	1.15±0.16	1.36±0.38	1.60±0.20;	1.78±0.30
	LS	1.25±0.39	1.77±0.37	1.26±0.28	1.71±0.19	1.53±0.20
P3HT	NLS	1.73±0.60	1.32±0.31	1.42±0.35	1.77±0.37	1.82±0.32
	LS	1.73±0.60	1.30±0.36	1.40±0.34	2.28±0.44	1.85±0.33

Table S2: Cell density at T0, T3 and T4 of SH-SY5Y cultured on Glass|FTO (CTRL) and Glass|FTO|P3HT (P3HT) and subjected to the light-stimulation protocol (LS) or kept in dark (NLS). Data are shown as cell cm-2 ± SEM of three experiments.

		T0 [x10^4 cell cm^{-2}]	T1 [x10^4 cell cm^{-2}]	T2 [x10^4 cell cm^{-2}]	T3 [x10^4 cell cm^{-2}]	T4 [x10^4 cell cm^{-2}]
CTRL	NLS	2.6±0.2	6.9±0.8	11.7±1.1	15.6±0.3	21.6±1.5
	LS	2.6±0.2	6.4±0.2	9.2±0.5	12.8±0.6	19.4±0.2
P3HT	NLS	1.4±0.2	4.6±0.3	6.4±0.2	9.3±0.5	12.2±1.3
	LS	1.4±0.2	1.9±0.1	2.1±0.1	2.7±0.2	5.3±0.3

Table S3: Quantification of FLUO-4 AM fluorescence of cells cultured on Glass|FTO (CTRL) and Glass|FTO|P3HT (P3HT) subjected to the light-stimulation protocol (LS) or kept in dark (NLS) analyzed at T0 and T3. Data are acquired before (basal) and after (induced) 30 min of photo-stimulation. Data are shown as mean CTCF ± SEM of three experiments.

			Basal [mean CTCF]	**Induced [mean CTCF]**
T0	**NLS**	**CTRL**	4127±407	5126±386
		P3HT	4605±446	7669±391
T3	**NLS**	**CTRL**	4929±379	5804±300
		P3HT	5868±337	7600±460
	LS	**CTRL**	4694±239	5793±259
		P3HT	13521±1469	14990±835

Table S4: Optoelectrical outputs, upon light irradiation of 15.0 mW cm-2, recorded from CTRL samples (Glass|FTO|SH-SY5Y), P3HT only (Glass|FTO|P3HT), and P3HT/cells (Glass|FTO|P3HT|SH-SY5Y) samples, taken in dark (NLS) or subjected to light stimulation protocol (LS). Signals were analysed at t1, when light is switched ON (200 ms) and at t2 when light is switched OFF (after 300 ms from light switched ON, namely at 500 ms).

		V_{ph} (t_1) (photo-voltage) [mV]	**V_{ph} (t_2) (photo-voltage) [mV]**
NLS	**CTRL**	0.0±0.0	0.2±0.2
	P3HT only	0.1±0.0	1.3±0.5
	P3HT/cells	0.4±0.0	4.6±1.2
LS	**CTRL**	0.0±0.0	0.0±0.0
	P3HT only	0.0±0.0	10.4±0.9
	P3HT/cells	2.0±0.9	12.3±3.0