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Supporting Information

Polythiophene-mediated light modulation of membrane potential and calcium signalling in human adipose-derived stem/stromal cells

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Figure S1 summarizes the features of the hASC populations of the study. The characterization includes the following procedures and/or assays:

i) Calculation of doubling time. DT (hours) = δ_{time} x ln2/ln (no. of collected cells/no. of plated cells[1]
ii) Clonogenicity assessment by colony forming units-fibroblasts. CFU-F (%) = no. of colonies/no. of plated cells[2]
iii) Immunophenotype by cytofluorymetry[2]
iv) Collagen deposition by Sirius Red staining[3]
v) Calcified extracellular matrix (ECM) production by Alizarin Red staining[3]
vi) Lipid vacuoles production by Oil-Red O staining[3]

For the assessment of hASC multi-differentiative potential, the following culture conditions were implemented:

- CTRL = High glucose DMEM plus 10%FBS, 2mM l-glutammine, 50U/ml penicillin and 50µg/ml streptomycin
- Osteoinduction = CTRL plus 10nM dexamethasone, 10mM glycerol-2-phosphate, 150µM L-ascorbic acid-2-phosphate and 10nM cholecalciferol for 14 days or 21 days for collagen and calcified ECM evaluation respectively
- Adipoinduction = CTRL plus 1µM dexamethasone, 10µg/ml insulin, 500µM 3-isobutyl-1-methyl-xanthine and 200µM indomethacin for 14 days

A

hASC features	
Doubling time (h)	121±41
CFU-F (%)	10±6
Immunophenotype	% of positive events
CD90	98±3
CD14	3±4
CD73	100±1
CD45	2±3
Differentiated vs CTRL hASC	% of increase
Collagen deposition	136±142
Calcified ECM production	199±166
Lipid vacuoles production	70±84

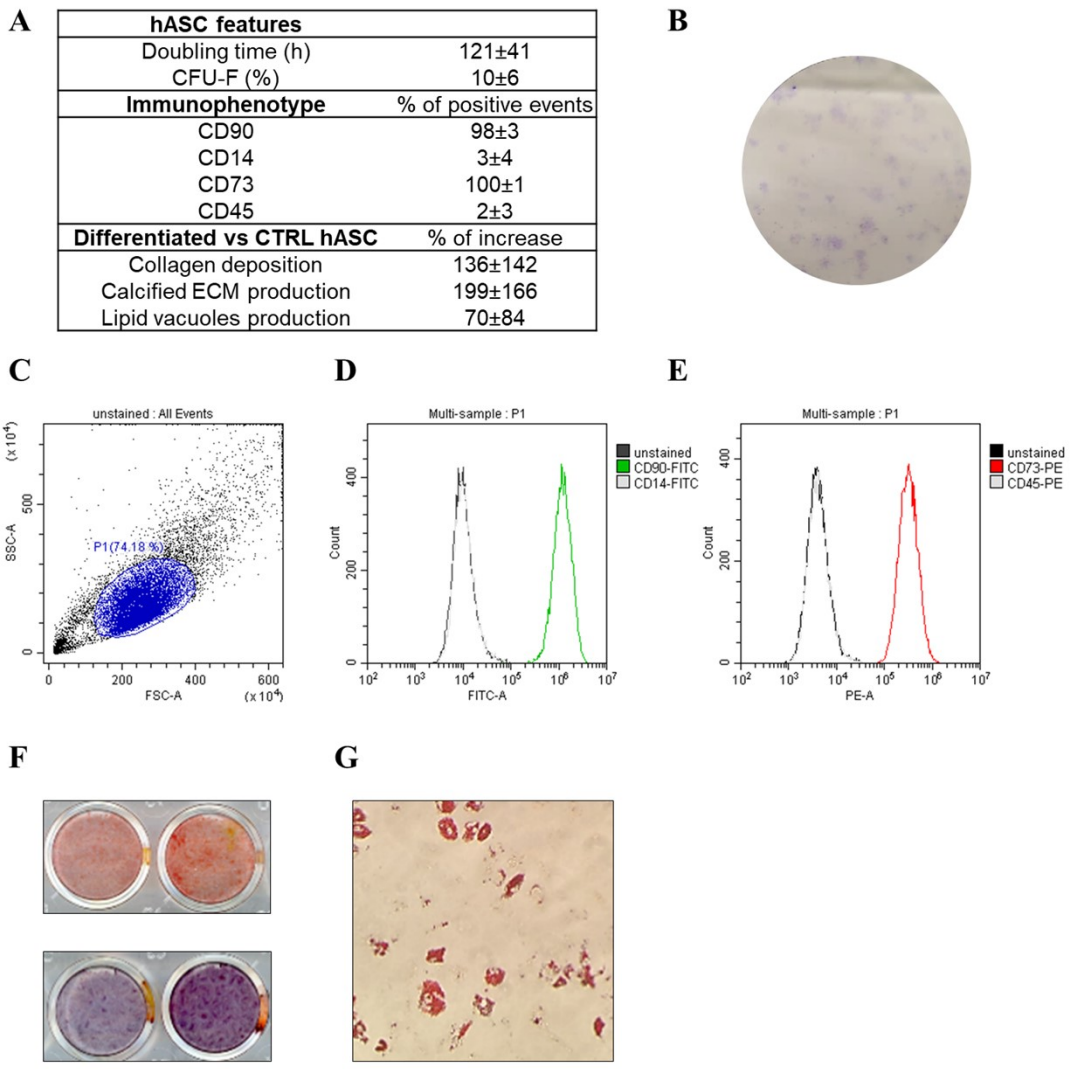

Figure S1 - hASC characterization. A – Summary of hASC features in terms of growth kinetics, clonogenicity, immunophenotype and differentiative potential. Data are expressed as mean ± SD of 47 hASC populations. **B** - CFU-F colonies stained by Crystal Violet ($5x10^2$ hASC were initially plated). **C-E** – Cytofluorimetric analysis of hASC for the expression of the typical positive (CD90 and CD73) and negative (CD14 and CD45) surface markers. **F –** Stainings to evaluate the osteogenic commitment of differentiated hASC (right wells) with respect to undifferentiated ones (left wells). Upper row: collagen deposition by Sirius Red. Lower row: calcified ECM matrix by Alizarin Red. **G** – Microphotograph of adipoinduced hASC. Lipid vacuoles are colored with Oil Red-O. 200X magnification.

	Donor characteristics			Experiments					
Population ID	Gender	Age	Type of surgery	Cell proliferation	ALP activity	Ca^{2+} imaging 20/200	Ca^{2+} imaging 400/4000	Ca^{2+} imaging (Ca^{2+} free medium) 20/200	Ca^{2+} imaging (Ca^{2+} free medium) 400/4000
ASC1	m	56	prosthetic	X	X			X	X
ASC2	f	26	aesthetic	X	X				
ASC3	f	22	aesthetic	X	X				
ASC4	f	35	aesthetic			X	X	X	X
ASC5	f	46	aesthetic			X	X		
ASC6	f	56	prosthetic				X		
ASC7	f	70	prosthetic			X			

Table S1 – Detail of cell populations. For each line, the donor characteristics are reported, as well as the experiments in which they were employed.

Responsivity evaluation

For our measurements we developed a macro to automatically find and characterize the Ca^{2+} peaks. Thus, we implemented a composite threshold system, in order to remove cells that show no appreciable dynamics. After various measurements, we assessed the usual shapes and dimensions of the Ca^{2+} fluxes in hASCs, and we used this knowledge to define not responsive cells. In particular, we had a simple 3-steps threshold. The first step was a constraint on risetime, using both upper (120 s) and lower (1.5 s) limits for it to be acceptable. If the risetime was longer than 120 s, it was most likely not a real Ca^{2+} dynamics, but instead an effect due to background drift; on the other hand, if the risetime resulted shorter than 1.5 s, it was ascribed to a random oscillation. Those constraints were pretty much conservative, as the usual risetimes for our dynamics were of tens of seconds. The second step was a threshold on the peak amplitude: with our acquisition settings, peaks with amplitude lower than 20 counts were ascribed to background effects. Again, this was a highly conservative threshold, as most of our Ca^{2+} peaks had amplitudes of hundreds to thousands counts. Finally, the peaks that were not cut out during the previous two thresholding are averaged, giving the distribution of the population calcium peaks. Since these distributions were broad, we considered as non-responsive cells also those whose peak was below 10% of the population average. Overall, this last step did not significantly modify the difference between mean and modal, giving a good representation of the population.

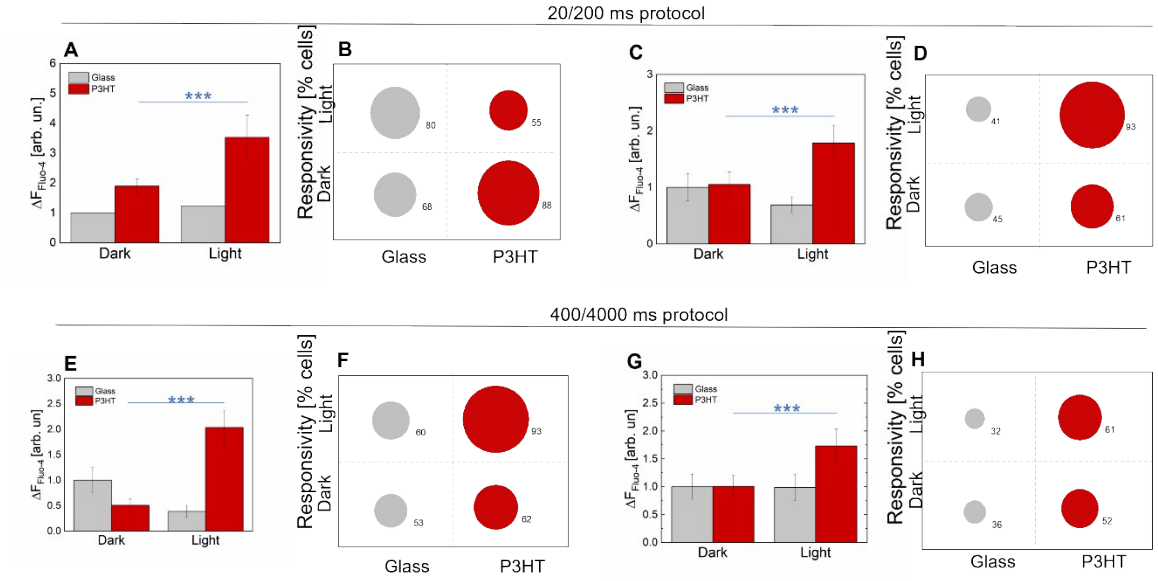

Figure S2 – Biological replicas of Calcium imaging experiment. A, C, E, G report the average calcium peak amplitude for cells grown on glass and P3HT substrates in dark and light conditions. Statistical significance is indicated as *** for $p<0.001$, the other differences are not statistically significant. **B, D, F, H** report the responsivity of the related average peak amplitude on the same substrates.

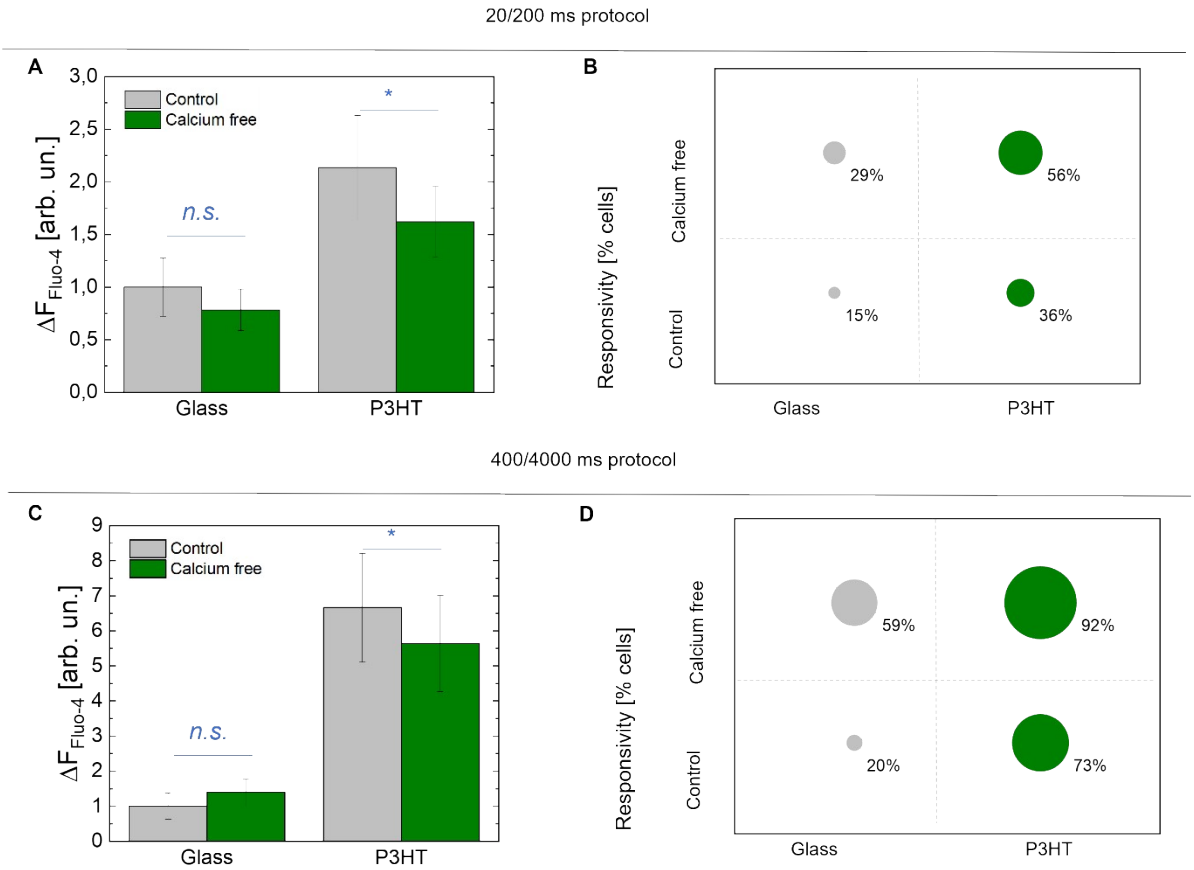

Figure S3 – Biological replicas of Calcium imaging experiment in Calcium free medium. A, B – average calcium peak amplitude and responsivity, respectively, of cells grown on P3HT and glass substrates upon exposure to 20/200 ms protocol. **C, D** – average calcium peak amplitude and responsivity, respectively, of cells grown on P3HT and glass substrates upon exposure to 400/4000 ms protocol. In both cases, the statistical significance is indicated as * for $p<0.05$ and n.s. as non-significant difference.

References

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