Bimodal modulation of *in vitro* angiogenesis by photoactive polymer nanoparticles

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Supporting Information

Table S1. Mean diameters and standard deviation obtained from lognormal approximation from DLS and TEM data.

Conjugated polymer:Pluronic F127	P3HT	PTB7	PBDB-T
Dynamic Light Scattering (DLS)			
9:1	327 ± 69	260 ± 87	273 ± 101
3:1	275 ± 127	256 ± 114	275 ± 152
1:1	244 ± 93	255 ± 123	227 ± 121
TEM			
9:1	185 ± 100	131 ± 44	127 ± 52
3:1	150 ± 49	98 ± 49	112 ± 32
1:1	137 ± 41	-	-

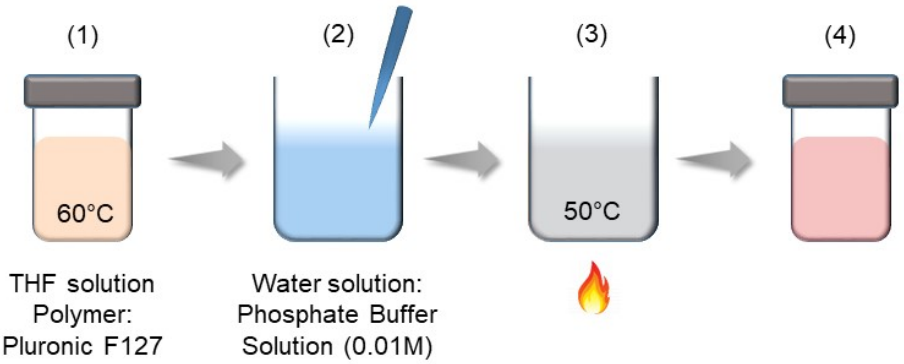

Figure S1. Scheme of flash nanoprecipitation method employed in this work to generate the nanoparticles: (1) the mix of polymers is solved in THF, (2) 1 mL of the polymer solution is injected into 10 mL of PBS solution in water, (3) evaporation of THF from the water, (4) final colloid of spherical polymer nanoparticles in dispersed in water.

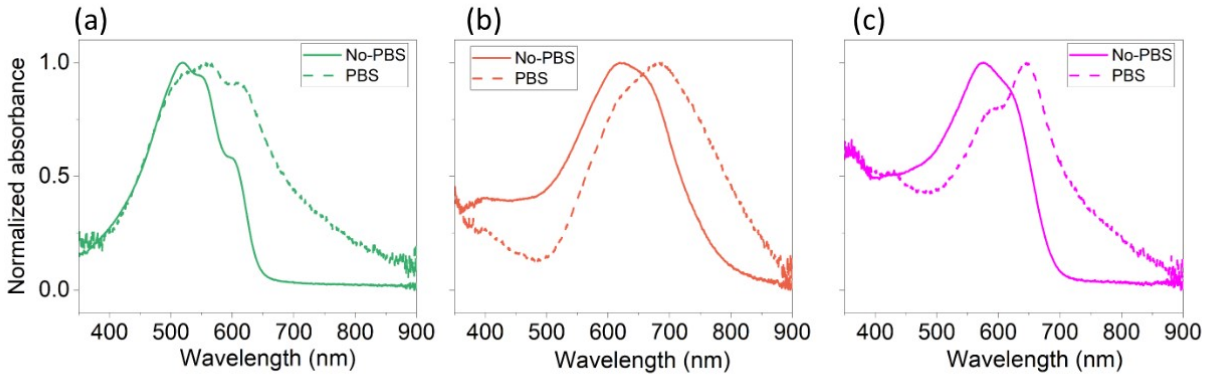

Figure S2. UV-vis absorbance spectra of (a) P3HT), (b) PTB7 and (c) PBDB-T nanoparticles prepared in water with or without PBS at 9:1 mass relation of polymer:Pluronic® F127.

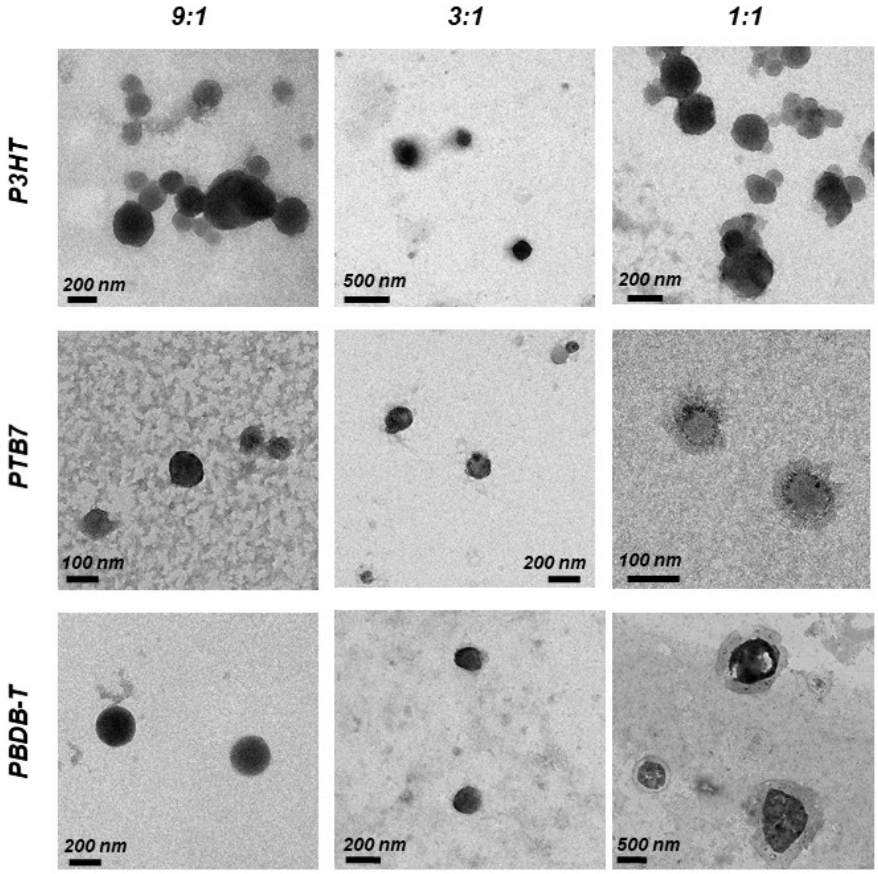

Figure S3. TEM images of the conjugated polymer NPs prepared at different Polymer:Pluronic® F127 mass ratios.

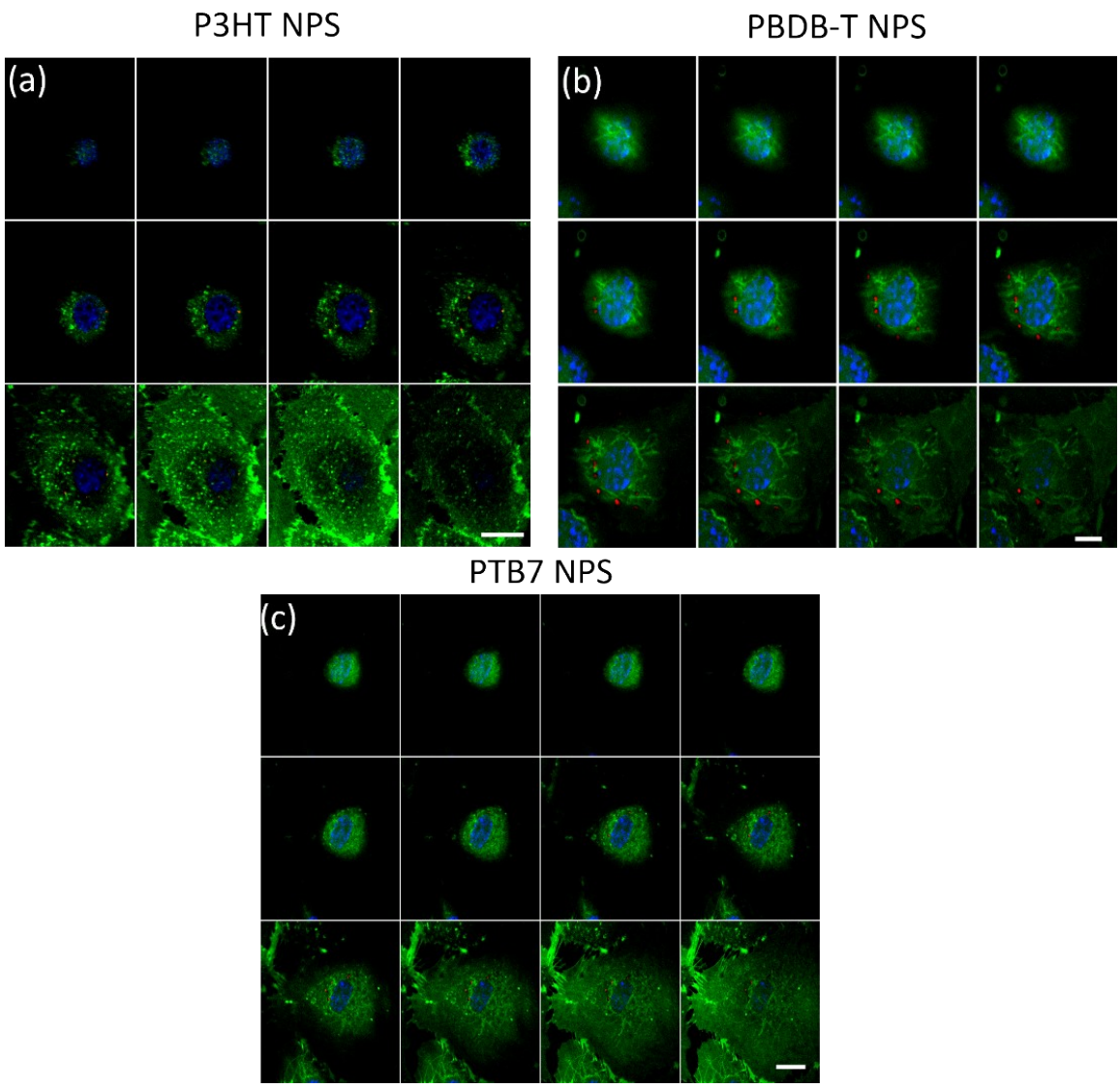

Figure S4. Confocal optical sections depicting HUVEC cells treated with P3HT, PBDB-T and PTB7 NPs. Cells are stained with Cell mask green (membrane, green) and Hoechst 33342 (nuclei,blue), Nps emission is depicted in red. Focal planes are acquired from the top interface with the extracellular bath (upper left) to the bottom of the cells (lower right). Scale bars 5 µm.

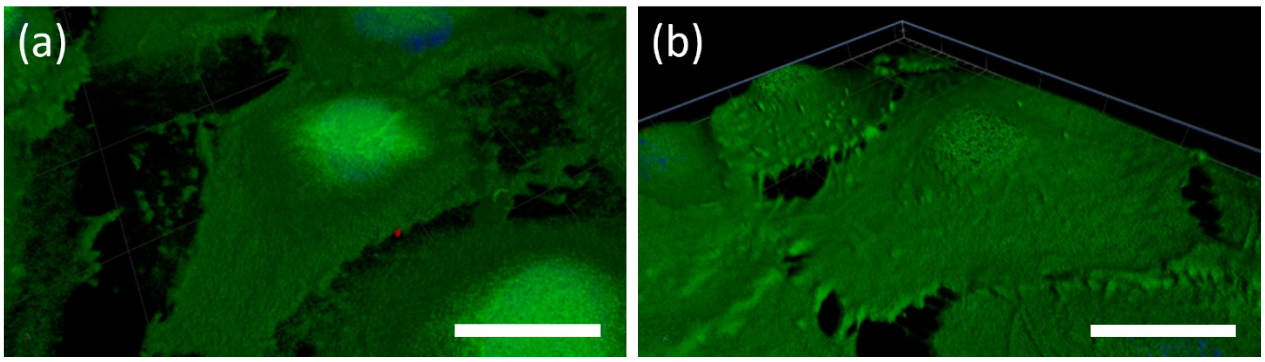

Figure S5. 3D reconstruction of confocal images depicting HUVECs treated with PBDB-T (a) and PTB7 NPs (b). Cells are stained with Cell mask green (membrane, green) and Hoechst 33342 (nuclei, blue). Scale bars 10 µm. Arivis software was employed for the analysis.

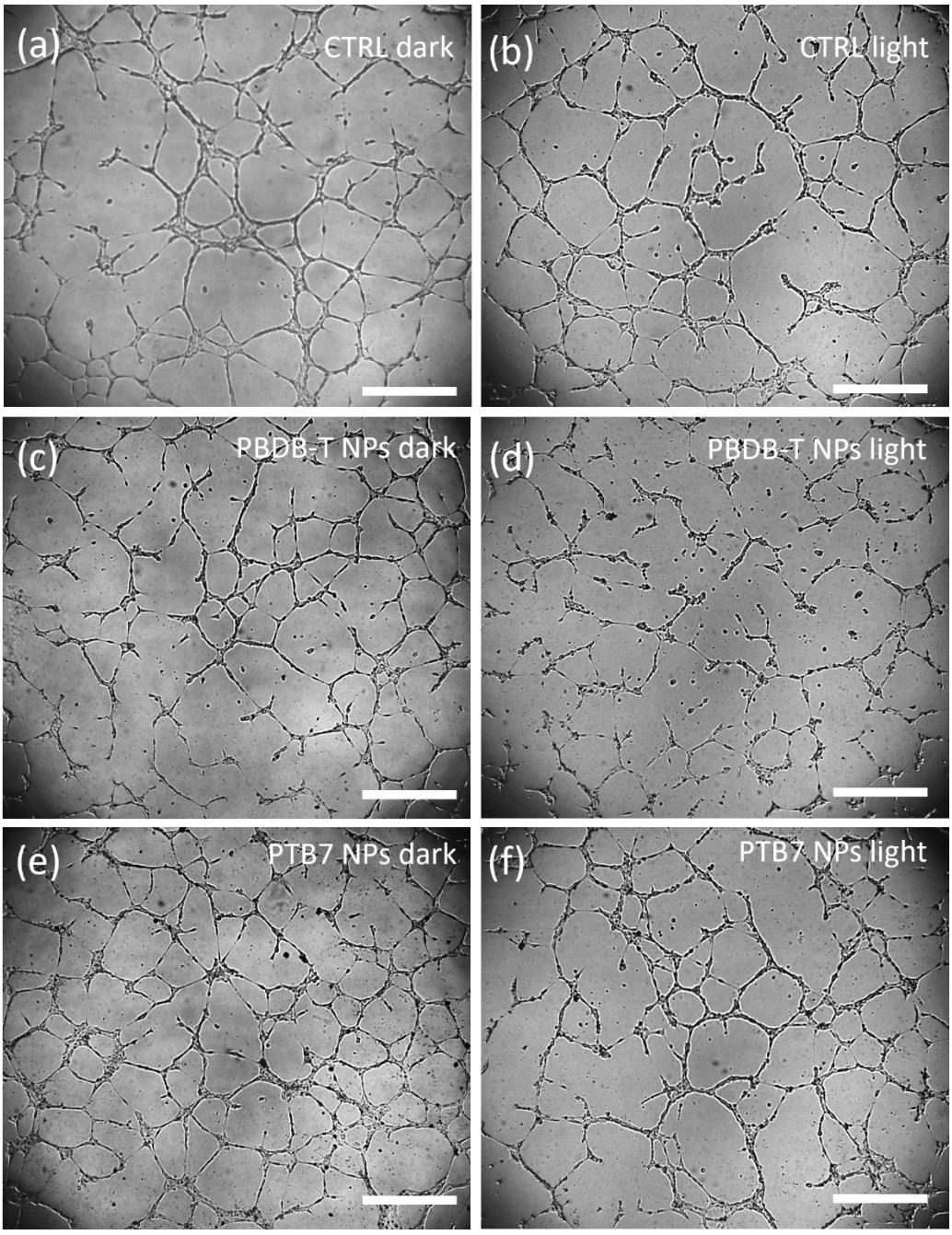

Figure S6. Representative bright field images depicting the capillary-like network formed by HUVECs during the *in vitro* angiogenesis assay in all the considered conditions. Scale bars 500 µm.

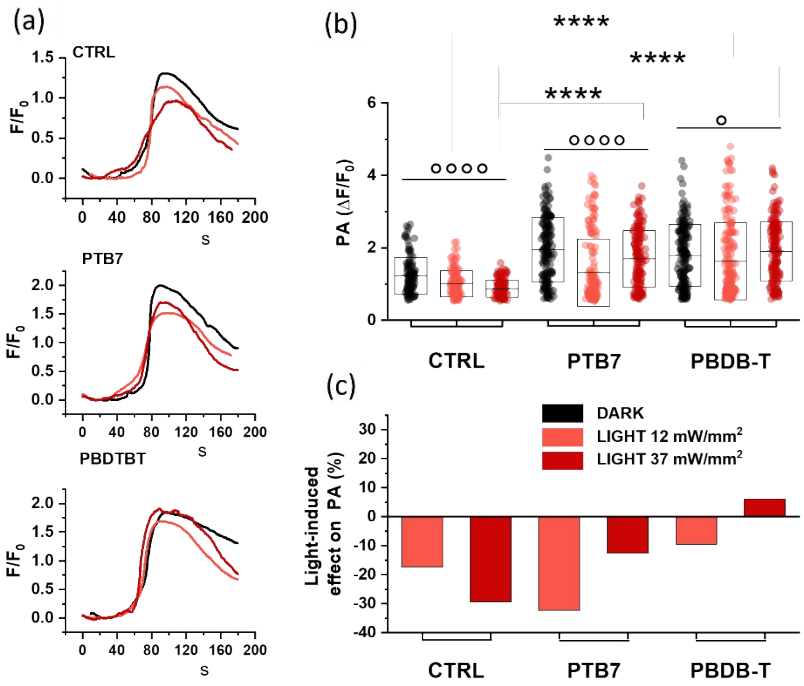

Figure S7. (a) Representative traces of Ca^{2+} dynamics in HUVEC cells, without NPs (top) and loaded with PTB7 (middle) and PBDB-T (bottom) NPs. (b) Average peak amplitude (PA) of the Intracellular Ca^{2+} responses measured in HUVECs in absence (CTRL) and in presence of PTB7 and PBDB-T NPs, in dark and upon light excitation (660nm LED, 12 and 37 mW/mm^2). Photoexcitation of the Fluo4-AM probe (Ex/Em 488/515 nm) is provided by 474 nm light source (5 mW/mm^2). (c) PA percentage variation, as induced by red light photoexcitation at two photoexcitation densities. Cells number N: CTRL N > 110, PTB7 N>170, PBDB-T N> 170; * p< 0.05 vs CTRL (Mann-Whitney test), ○ Kruskal-Wallis ANOVA.

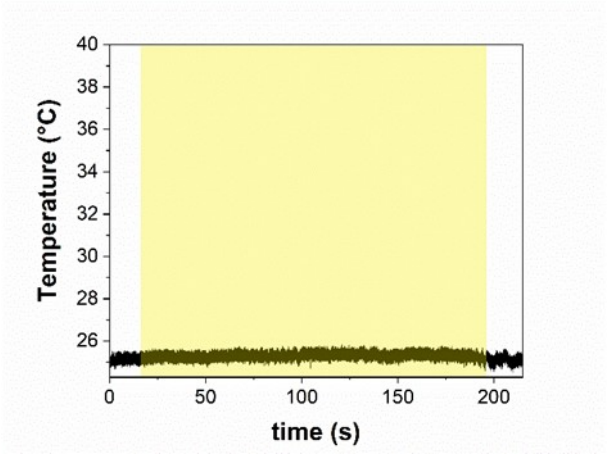

Figure S8. Local temperature measurement through the calibrated pipette method. The experiment was carried out with a patch clamp set up (Crisel instruments) in voltage clamp configuration by applying a constant current of I_0 = 4 nA at room temperature, in KRH extracellular solution. The yellow shaded area represents the light excitation (660nm, 3min, 37 mWmm^{-2}). The light-induced measured current is related to the temperature variation through the following relation:

$$T = \frac{1}{\frac{1}{T_0} - \frac{R}{E_a} log(\frac{I}{I_0})}$$

where I_0 is the current flowing through the pipette at the basal temperature T_0, R is the ideal gas constant and E_a is the activation energy. E_a was obtained by measuring the current response flowing through the pipette for a potential step of $\Delta V = 5$ mV at different values of the bath temperature, controlled with a volume-controlling chamber (Warner Instruments), and plotting the measured currents against the temperature in an Arrhenius plot.