Supplementary Material

Photoactive Poly(3-hexylthiophene) Nanoweb for Optoelectrical Stimulation to Enhance Neurogenesis of Human Stem Cells

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Supplementary Figures

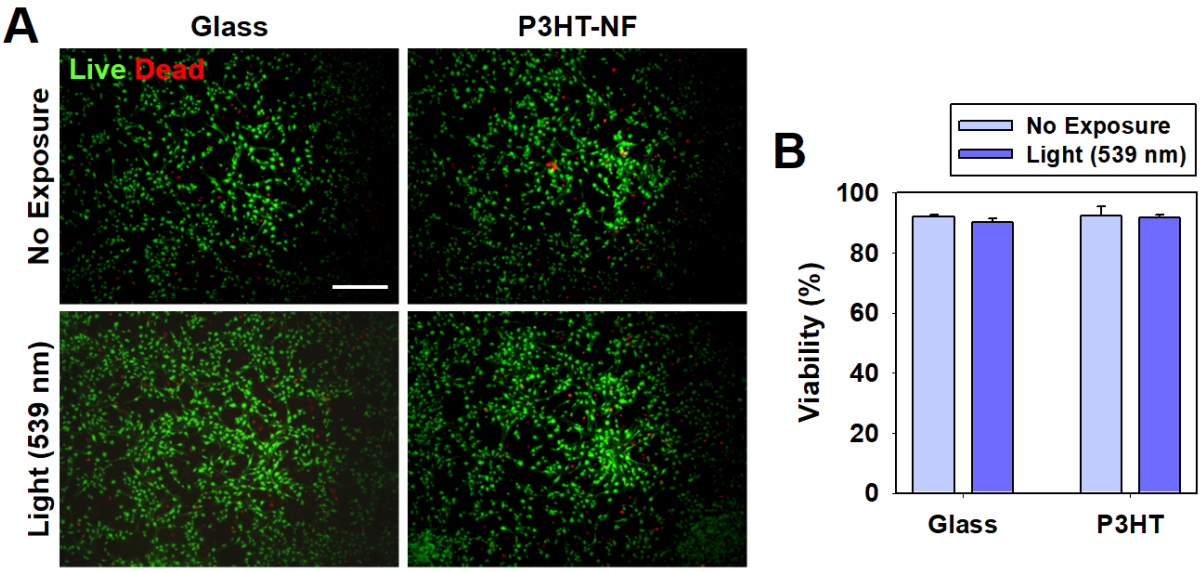

Figure S1. Live/dead staining of hfNSCs on glass and P3HT-NF substrates with or without 539 nm light stimulation after 2 day of culture. (A) Live cells were labeled by green fluorescent staining, while dead cells were labeled with red fluorescence, scale bar = 100 μm. (B) The viability of hfNSCs was determined by quantifying the percentage ratio of live cells (green) to total cell population in the live/dead stained images (n = 3).

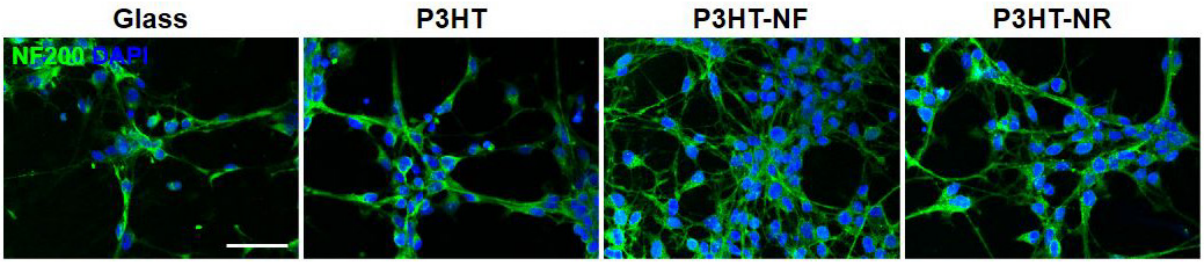

Figure S2. Immunofluorescence staining for NF200 in hfNSCs differentiated on each substrate after 7 days of culture, scale bar = 50 μm.

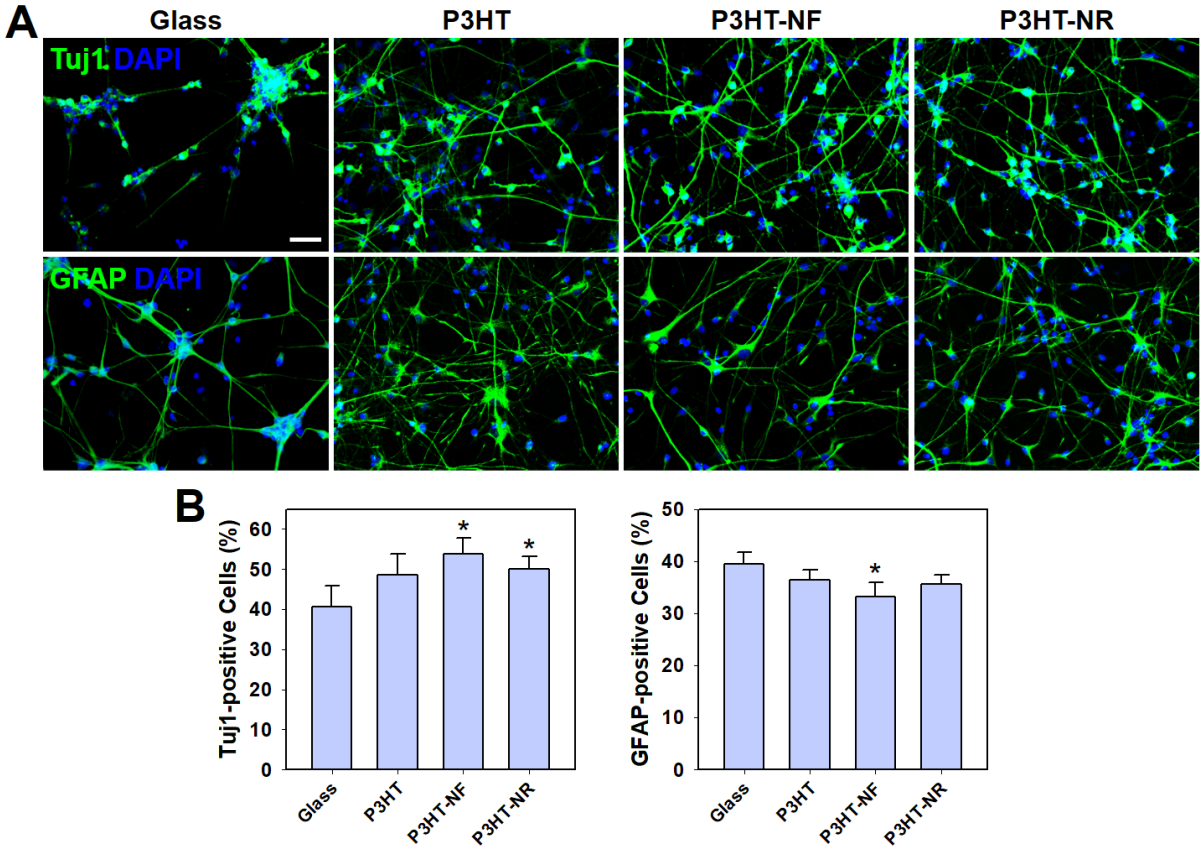

Figure S3. Promoted neuronal differentiation of hfNSCs on P3HT substrates with 539 nm light stimulation. (A) Immunofluorescent staining of hfNSCs that differentiated into neuronal (Tuj1) and astrocyte (GFAP) lineages on each substrate after 7 days of culture with 539 nm light stimulation, scale bar = 50 μm. (B) The percentage ratio of Tuj1- or GFAP-positive cells to total population on each substrate (n = 3, *; $p < 0.05$, compared to glass substrate with light stimulation).

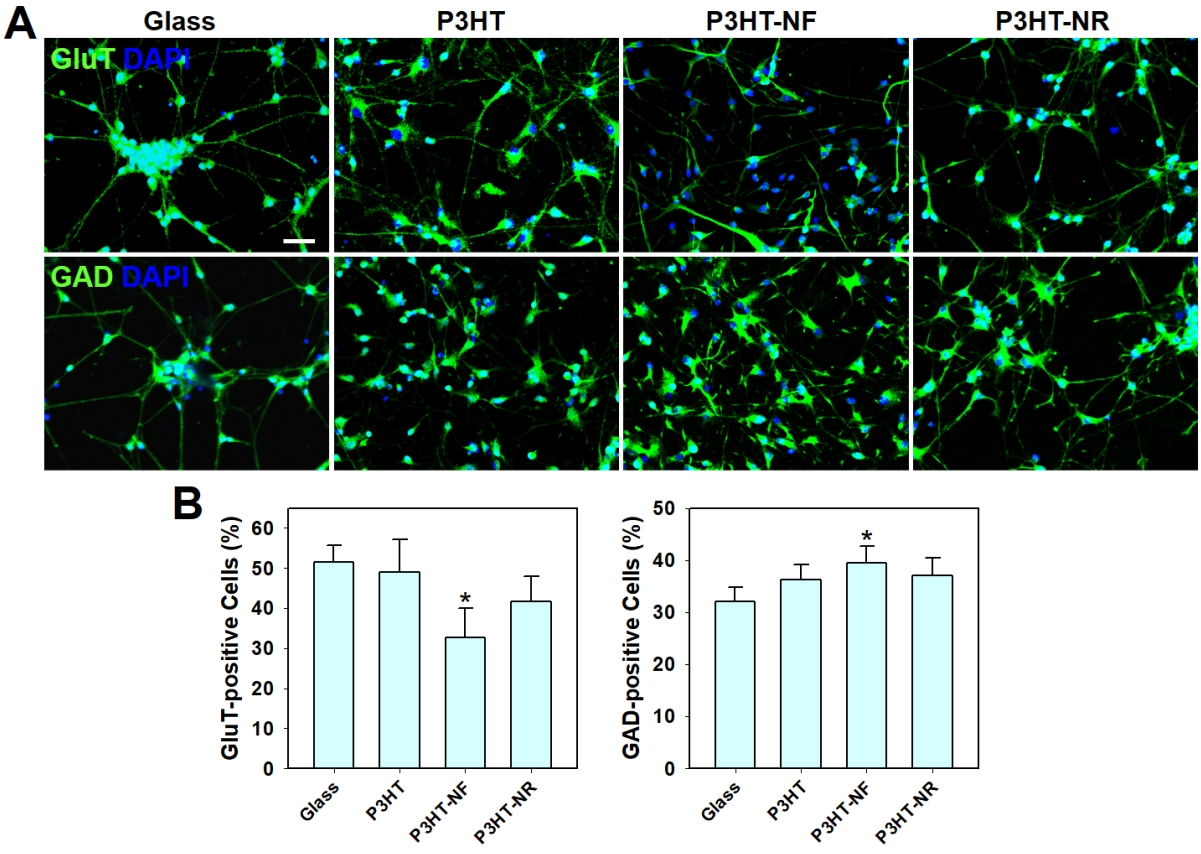

Figure S4. Guided differentiation of hfNSCs into specific neuronal subtypes by P3HT substrates with 539 nm light stimulation. (A) Immunofluorescent staining of hfNSCs differentiated on each substrate for neuronal subtype markers (GluT, glutamatergic neuron; GAD, GABAergic neuron) after 7 days in culture with 539 nm light stimulation, scale bar = 50 μm. (B) Quantification of the population of GluT- or GAD-positive cells on each substrate ($n = 3$, *; $p < 0.05$, compared to glass substrate with light stimulation).

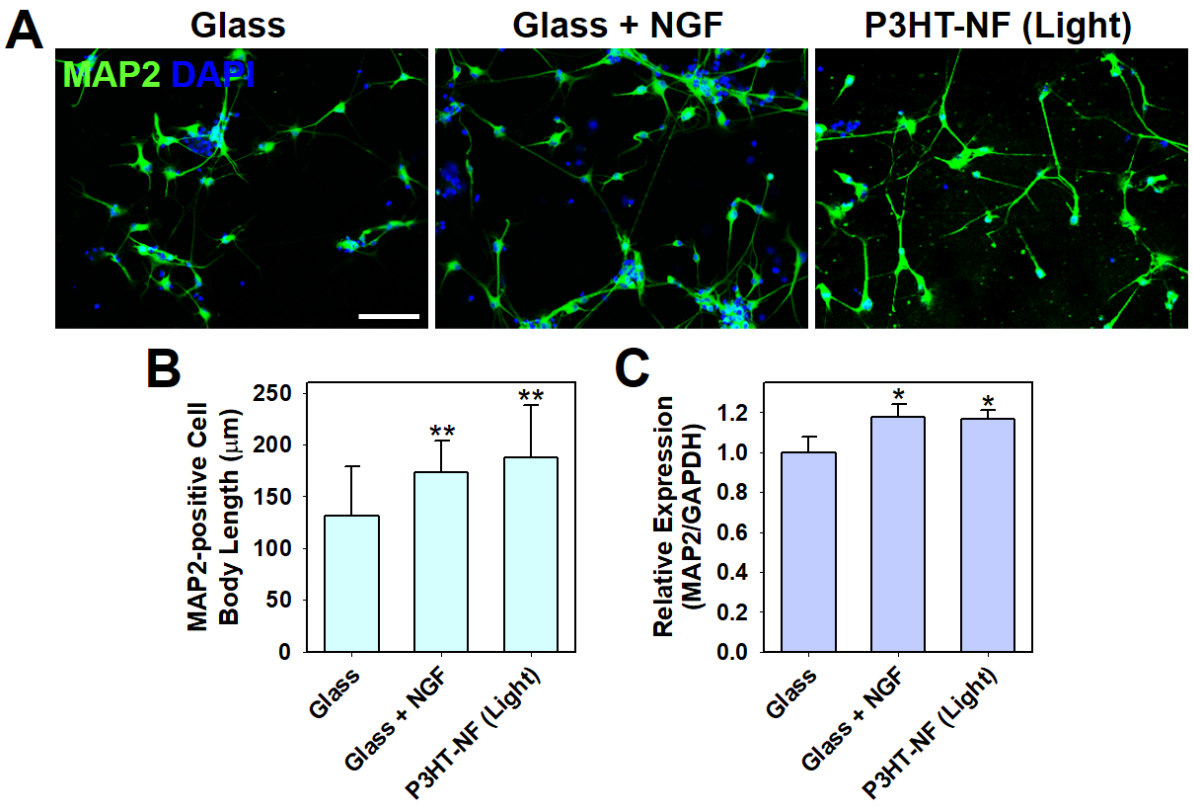

Figure S5. Comparison of hfNSC differentiation capacity between photoelectrical stimulation method and conventional culture (glass) or chemical induction method (glass + NGF). (A) Immunofluorescent staining of hfNSCs for a mature neuronal marker (MAP2) on the substrates under each condition after 7 days in culture, scale bar = 50 μm. (B) Quantification of MAP2-positive cell body length (n = 15, **; $p < 0.01$, compared to glass group) and (C) qPCR analysis of the expression of *MAP2* in hfNSCs grown on the substrates under each condition for 7 days (n = 3, *; $p < 0.05$, compared to glass group).

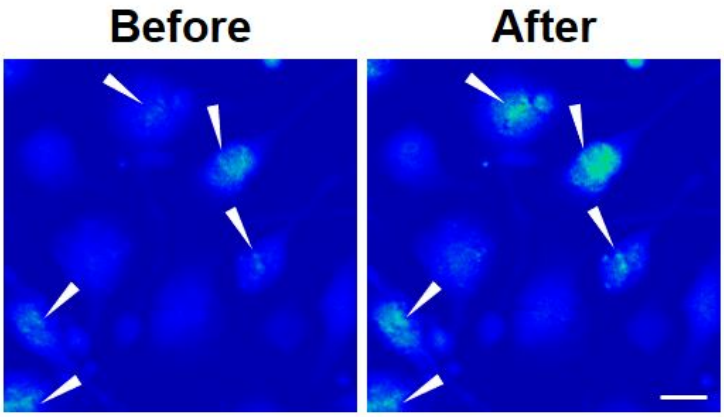

Figure S6. Calcium influx imaging of Fluo-4 AM-treated hfNSCs on P3HT-NF upon exposure to optoelectrical stimulation after 7 days of culture. White arrowheads indicate cells with increased calcium influx fluorescence, scale bar = 20 μm.