Supporting Information

Modular Polymers as a Platform for Cell Surface Engineering: Promoting Neural Differentiation and Enhancing the Immune Response

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S1. Experimental section

S1.1 Chemical Reagents. N-Methacryloyl chloride, Oligo(ethylene glycol)methacrylate (OEGMA, Mn = 360 g mol^{-1}), 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CTA), fluorescein O-methacrylate (FluMA) and 1-adamantanamine hydrochloride were from Sigma-Aldrich Chemical Co. (St. Louis, USA). D-Glucosamine hydrochloride, cholesterol (CHO), 1-adamantanecarbonyl chloride, 2,2'-azobis(isobutyronitrile) (AIBN) and N -hydroxysuccinimide (NHS) were from TCI Development Co., Ltd. (Shanghai, China). AIBN was recrystallized from methanol before use. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was from Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China). 11-mercaptoundecanoic acid was from yuanye Bio-Technology Co., Ltd (Shanghai, China). 2-deoxy-2-(methacrylamido) glucopyranose (MAG)[1] (Figure S1b), oligo(ethylene glycol)-1-adamantanecarbonyl methacrylate (OEGMA-Ada)[2] (Figure S1c), a red fluorescent β-CD derivative of rhodamine B isothiocyanate (CD-RBITC)[3] (Figure S1d), β-cyclodextrin decorated with seven sulfonate groups (CD-S)[4] (Figure S1e)and β-cyclodextrin decorated with seven mannose units (CD-M)[3] (Figure S1f) were synthesized as reported previously. All other organic solvents were from Sinopharm Chemical Reagent Co. (Shanghai China). Deionized water with resistivity 18.2 MΩ·cm was prepared using a Millipore water purification system.

S1.2. Biological Reagents. Roswell Park Memorial Institute (RPMI) 1640 medium, Dulbecco's modified Eagle's medium (DMEM) high-glucose, 10× phosphate buffer saline (PBS) and 0.25% (1×) trypsin were from HyClone (Logan, USA). Nonessential amino acids (NEAA), fetal bovine serum (FBS) and penicillin-streptomycin solution and were from Gibco (Grand Island, USA).

Plasmocin™ prophylactic was from InvivoGen Co., Ltd (San Diego, USA). Leukemia inhibitory factor (LIF) was from Millipore (Temecula, USA). Paraformaldehyde, bovine serum albumin (BSA), gelatin, triton X-100 and heparin sodium were from Sigma-Aldrich Chemical Co. (St. Louis). 4',6-diamidino-2-phenylindole (DAPI) was from Invitrogen (Waltham, MA). Rabbit anti-mouse anti-β3-tubulin antibody was got from Cell Signaling Technology Co., Ltd. (Danvers, MA, USA). FITC-labeled goat anti-rabbit antibody was obtained from Abcam Co. (Massachusetts, USA). Recombinant human basic FGF was obtained from Cell Signaling Technology (Danvers, MA). Polymerase chain reaction (PCR) primers were from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The RNA simple total RNA kit was from Tiangen Biotech Co., Ltd. (Beijing, China). RevertAid First Strand cDNA Synthesis Kit, PE anti-human CD86 and CD206 antibodies, APC anti-mouse CD80 antibody and PE anti-mouse CD86 antibody were from ThermoFisher Scientific (Lithuania, EU). Fast SYBR Green Master Mix was from Applied Biosystems (Vilnius, Lithuania). Human, IL-12p70 and IL-10 enzyme linked immunosorbent assay (ELISA) kits were from Neobioscience Biotechnology Co., Ltd. (Shenzhen, China).

S1.3. Characterization. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were taken with an Agilent 400 MHz NMR spectrometer. Number-average molecular weights (Mn) and polydispersities (PDI) of the polymers were determined using an Agilent PL-GPC 50 gel permeation chromatography (GPC) system (mobile phase DMF with 0.05 mol/L LiBr, flow rate 0.8 mL/min, PMMA as standards). Fluorescence images were obtainted using SP5II confocal and Olympus IX71 fluorescence microscopes. The qPCR was performed on a StepOnePlus real-time PCR system using SYBR Green master mix with a ROX reference dye (ABI). Flow cytometry

carried out using a BD FACSVerse™ system. UV-vis spectra and fluorescence spectra were collected using a versatile microplate reader (Thermo Fisher Scientific Inc.). Quartz crystal microbalance-dissipation (QCMD) measurements were carried out by a Q-Sense-E4 instrument (Q-Sense, Sweden) with control software (Resonant Probes GmbH, Goslar, Germany).

S1.4. Cell Culture. HeLa cells were cultured in DMEM medium. L929, 4T1, U937 and DC2.4 cells were cultured in RPMI-1640 medium. Both media were supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. mESCs were maintained in a T25 culture flask on a feeder layer of 10 μg/mL mitomycin C-inactivated mouse embryonic fibroblasts (mEFS) in mESC maintenance medium (DMEM medium supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, 1000 U/mL LIF, 0.1 mM NEAA and 0.1 mM β-mercaptoethanol). The cells were incubated at 37 °C with 5% CO_2 in a water-saturated chamber. The medium was changed every second day and passage operation was carried out every 4 days.

S1.5. FGF2 Binding Assay. pACGF-S were prepared by mixing the polymers (10 μL, 100 μM) and CD-S solution (2.25 μL, 46.8 μM) overnight. mESCs were seeded in a gelatinized 48-well plate with a density of 4×10^5 cells per well in mESC maintenance medium overnight. Then, the mESCs were washed twice with PBS and incubated with the pACGF and pACGF-S solution in DMEM (1 μM) and heparin solution in DMEM (5 μg/mL) at 37 °C for 30 min. the control group was mESCts incubated in the normal DEME medium. Then the cells were washed three times with PBS to remove the unbound polymers. After that, the FGF2 binding abilities were characterized by immunofluorescence staining.

S1.6. Measurement the interaction between polymers and CD206 by QCM-D. At first,

pACGF1 and CD-M were mixed in water for host-gust interaction overnight, and then excess CD-M was removed by centrifugation using an ultrafiltration tube and the pACGF1-M solid was obtained by freeze-drying. The QCM-D chips were immersed in the solution of 11-mercaptoundecanoic acid (0.2M) overnight. NHS (0.1M) and EDC (0.1M) were used to activate carboxyl group on QCM-D chips for 1h. Then the chips were put into chambers and pumped with PBS at a flow rate of 10 μL min^{-1} until the base line keep stable. The freshly CD206 solution (10 μg/mL, PBS) were pumped in with a flow rate of 10 μL/min until to obtain the adsorption plateau. The chips were pumped with pACGF1 and pACGF1-M solution (1 mg/mL), respectively, after washed by pumping PBS. Finally, the chips were washed with PBS to remove loosely attached polymers.

S2. Supporting Results

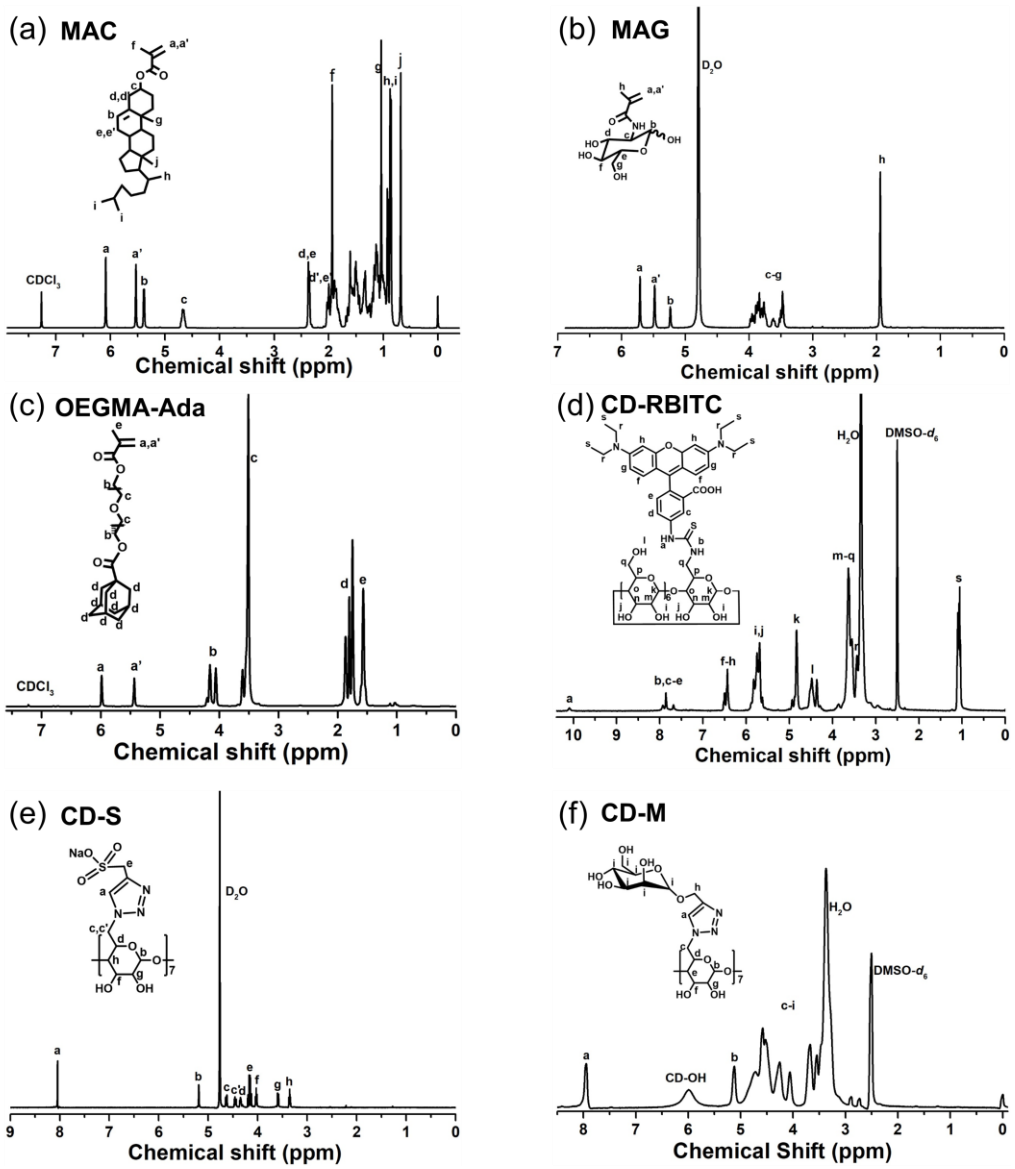

Figure S1.[1]H NMR spectra of monomers (MAC, MAG and OEGMA-Ada) and CD-X (CD-RBITC, CD-S and CD-M).

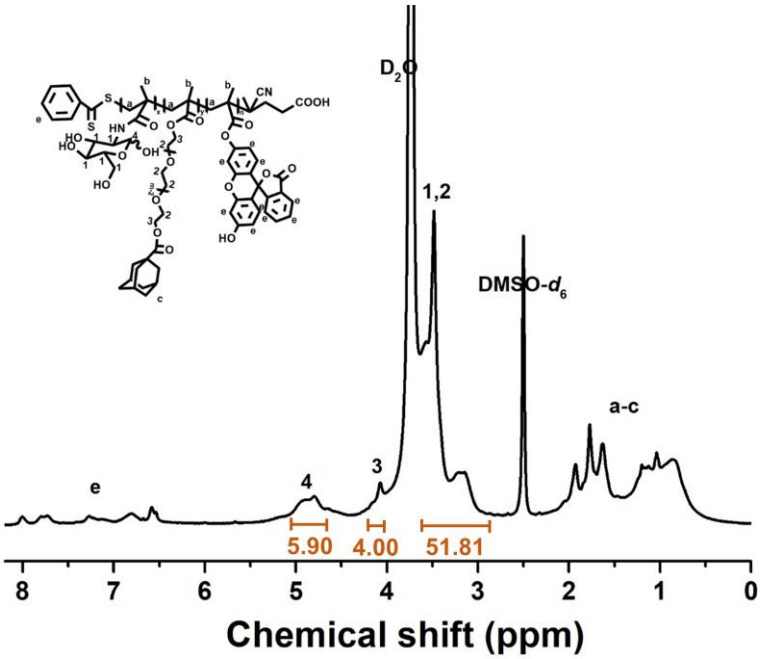

Figure S2.[1]H NMR spectrum of pACGF1 (solvent: DMSO-*d*6 with 10% D_2O).

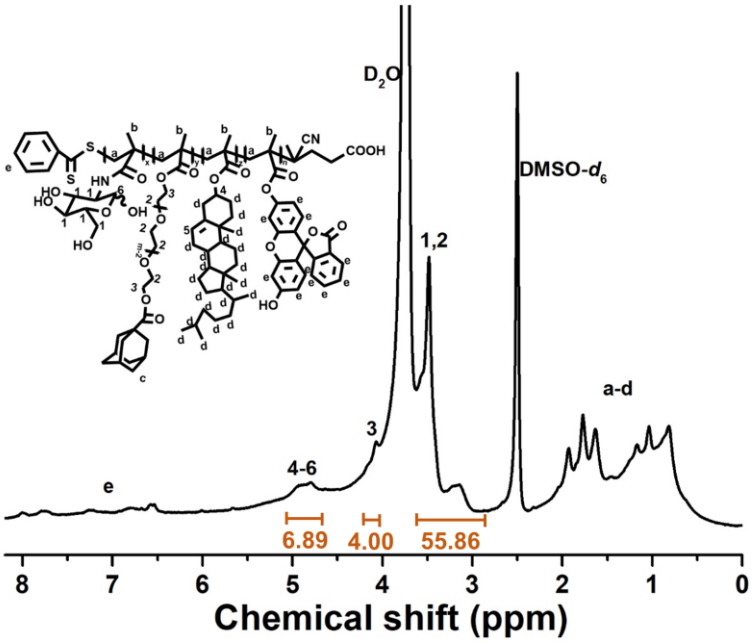

Figure S3.[1]H NMR spectrum of pACGF2 (solvent: DMSO-*d*6 with 10% D_2O).

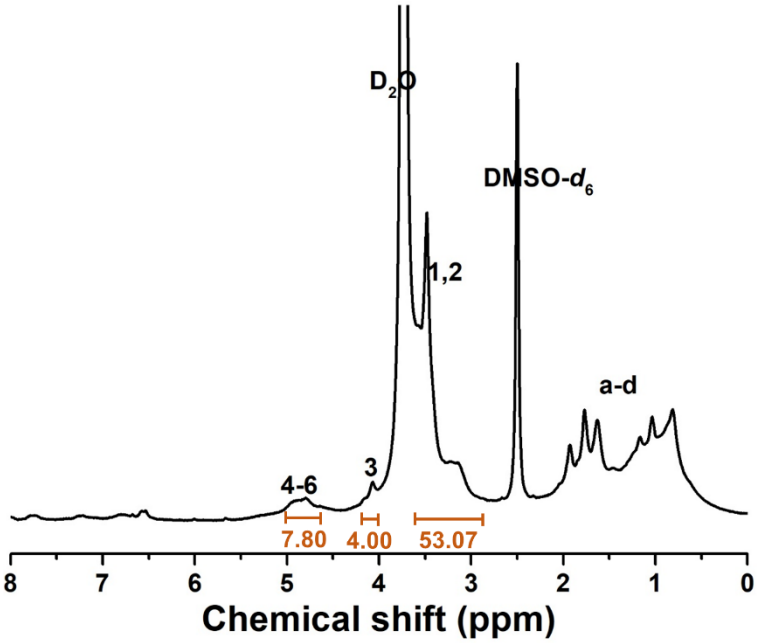

Figure S4.[1]H NMR spectrum of pACGF3 (solvent: DMSO-*d*6 with 10% D_2O).

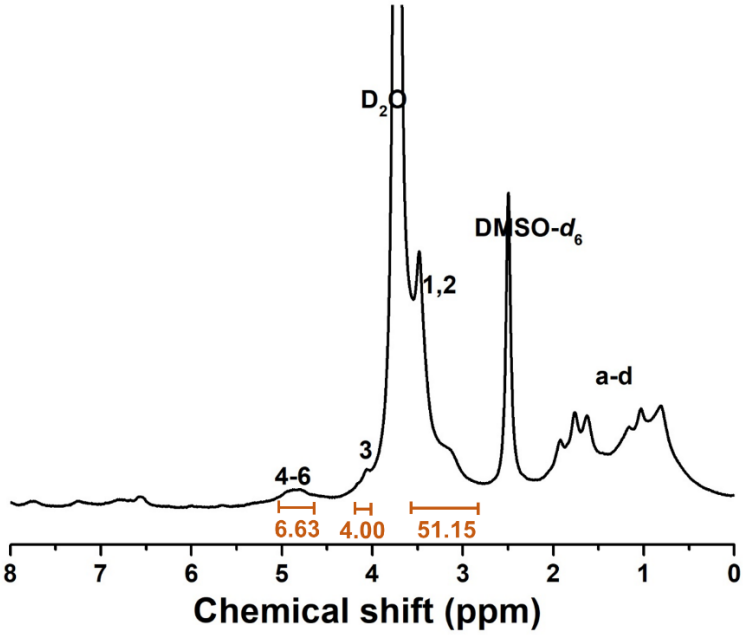

Figure S5.[1]H NMR spectrum of pACGF4 in (solvent: DMSO-*d*6 with 10% D_2O).

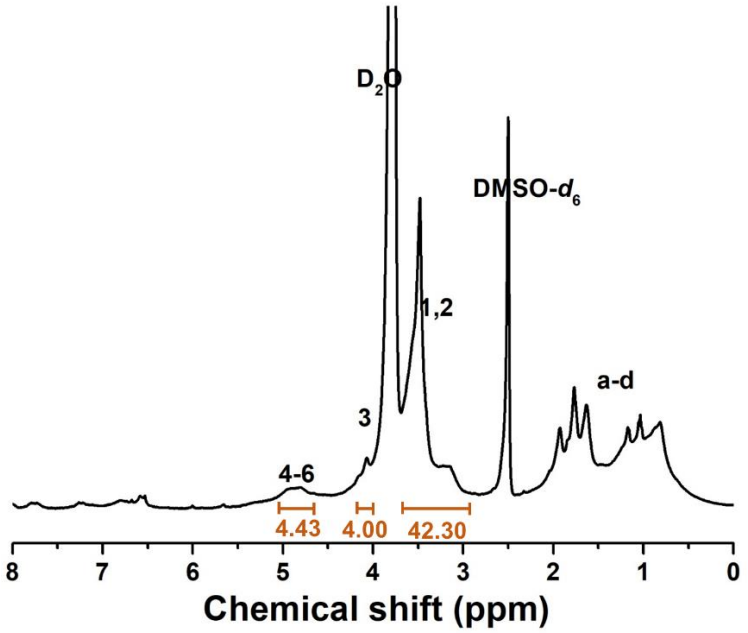

Figure S6.[1]H NMR spectrum of pACGF5 in (solvent: DMSO-*d*6 with 10% D_2O).

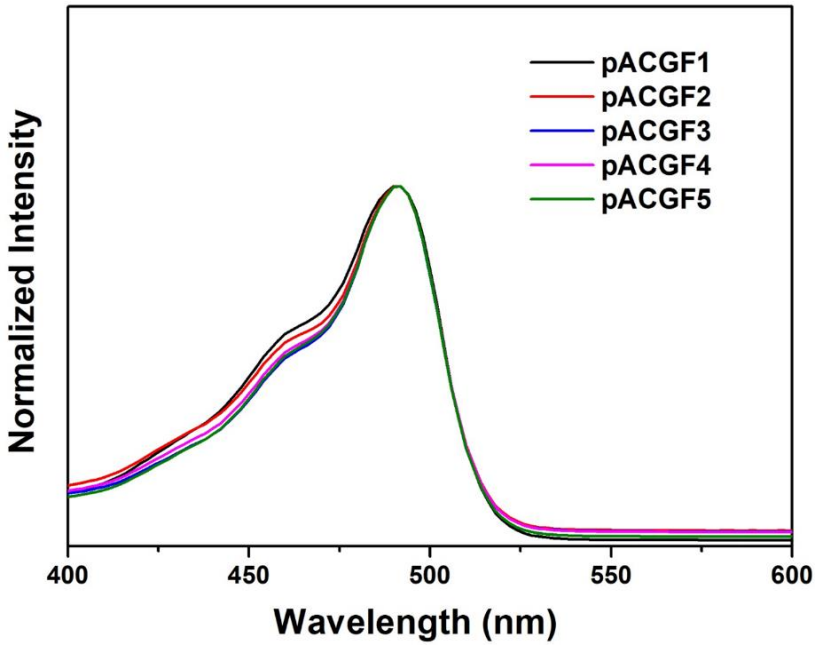

Figure S7. UV-visible spectra of pACGFs.

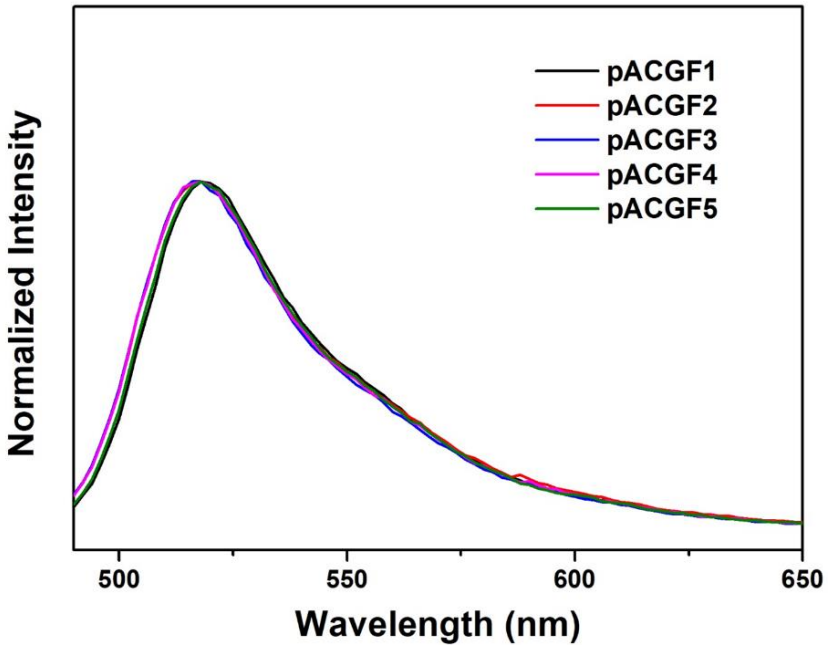

Figure S8. Fluorescence spectra of pACGFs excited with 460nm radiation.

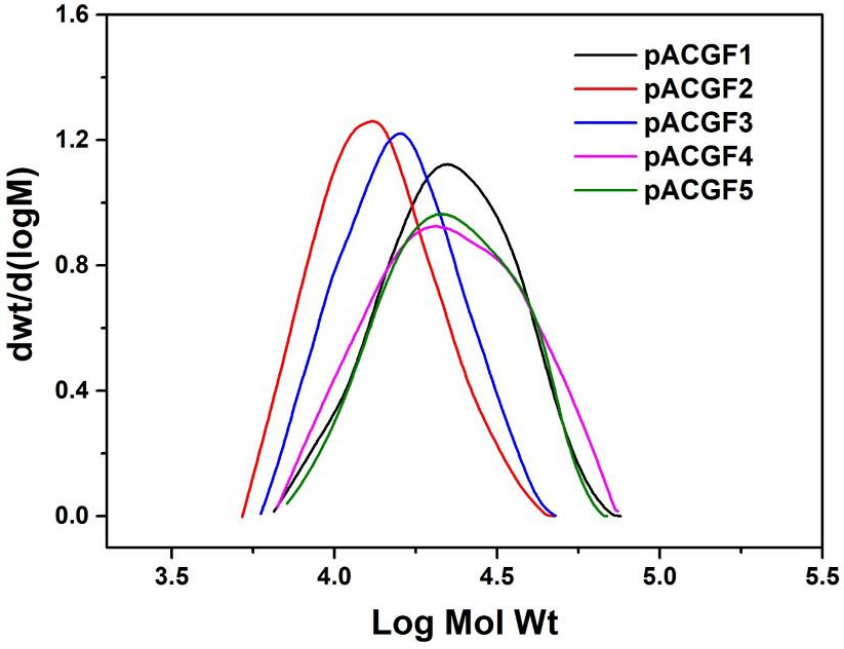

Figure S9. GPC elution profiles of pACGFs using DMF with 0.05 mol/L LiBr solution as mobile phase.

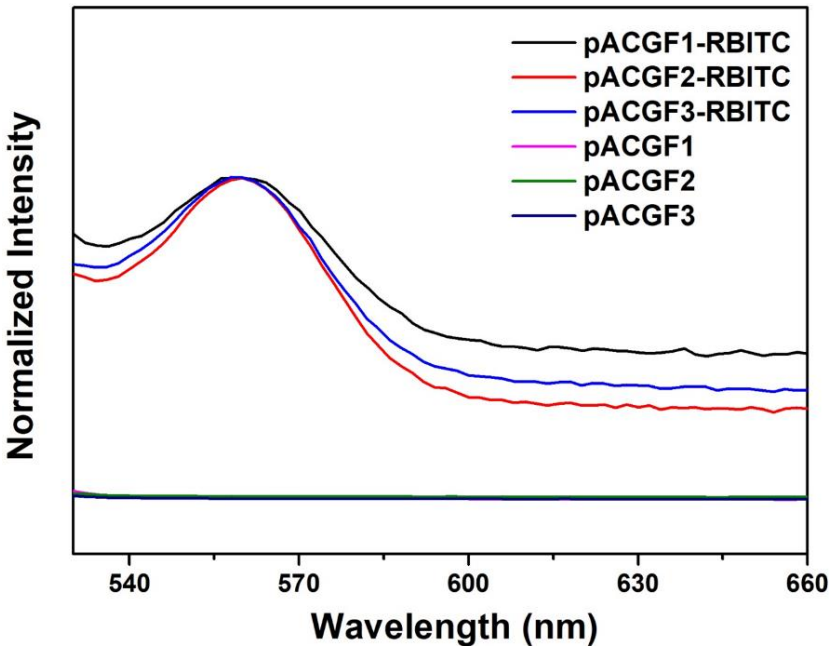

Figure S10. UV-visible spectra of pACGF-RBITC complexes and pACGFs.

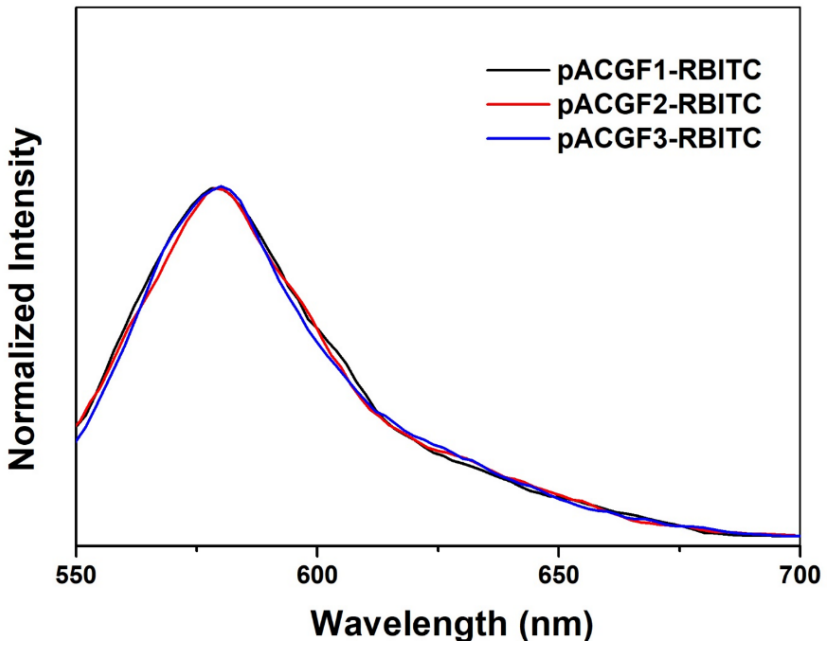

Figure S11. Fluorescence spectra of pACGF-RBITC complexes excited with 532nm radiation.

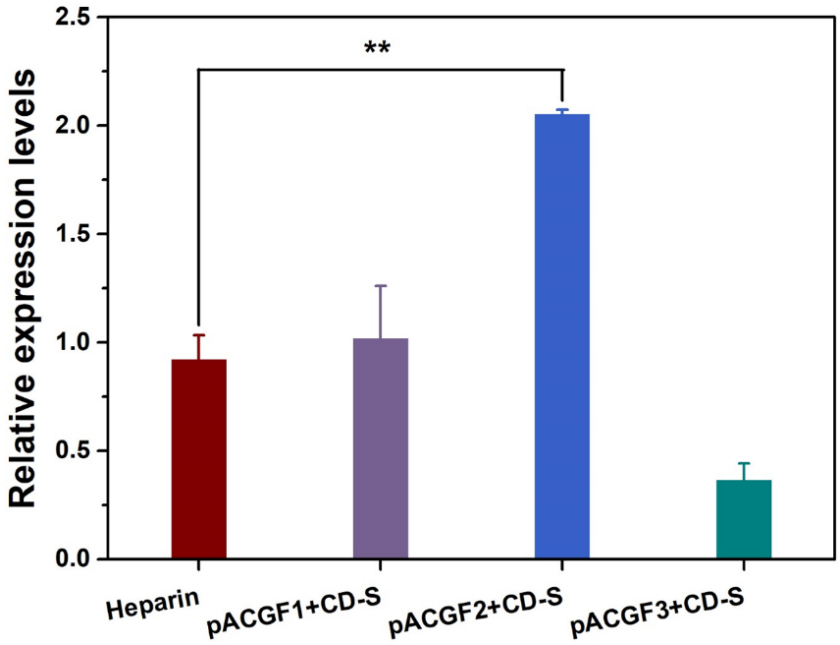

Figure S12. qRT-PCR analysis of neuron-specific marker β3-tubulin relative expressions of differentiated cells treated with heparin and pACGFs+CD-S for 7 days. Error bars represent the standard deviation of the mean (n = 3, *P < 0.05, **P < 0.01 by t test).

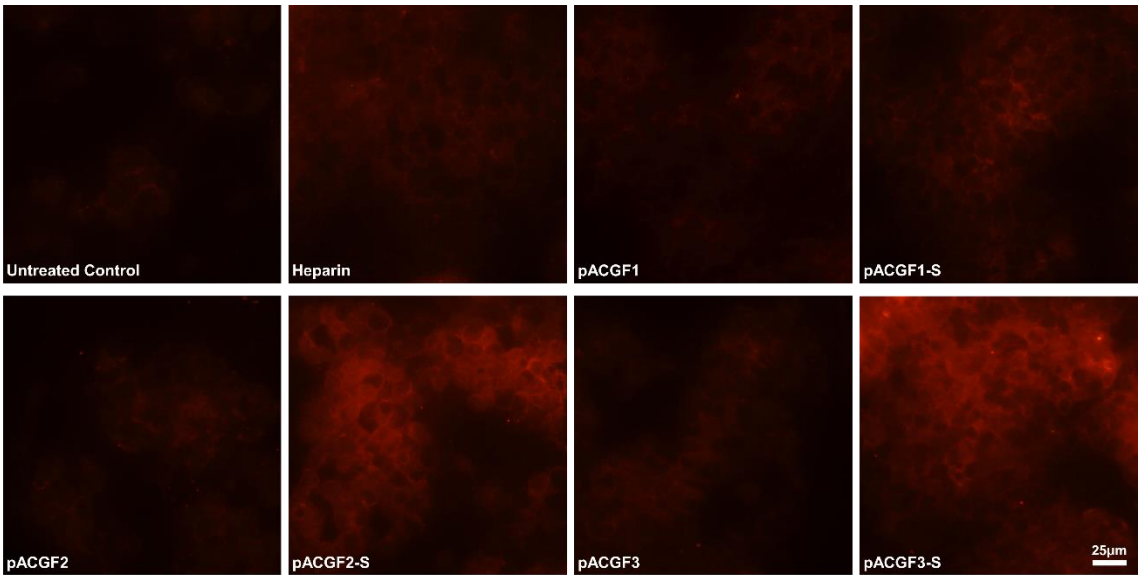

Figure S13. FGF2 (red) binding assay via inverted fluorescence microscope.

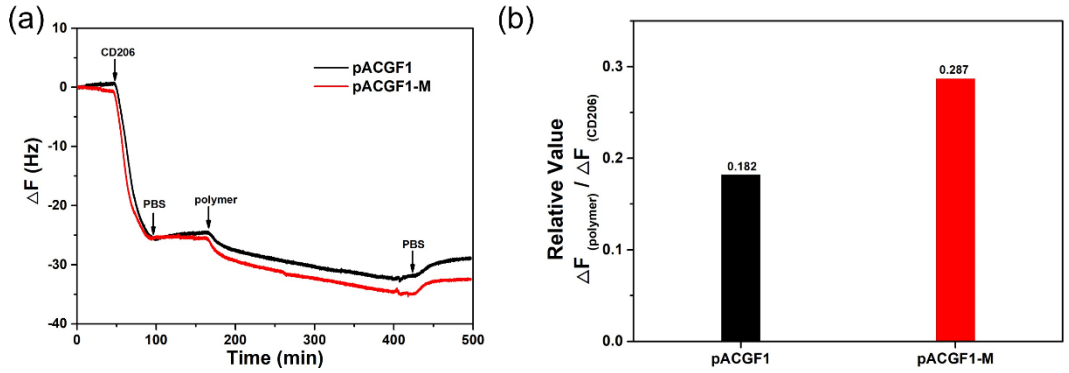

Figure S14. (a) Frequency shift with time of CD206 layers on contact with the different polymer solutions (pACGF1 and pACGF1-M); (b) Relative value of the frequency shift (ΔF) of polymers divided by ΔF of CD206.

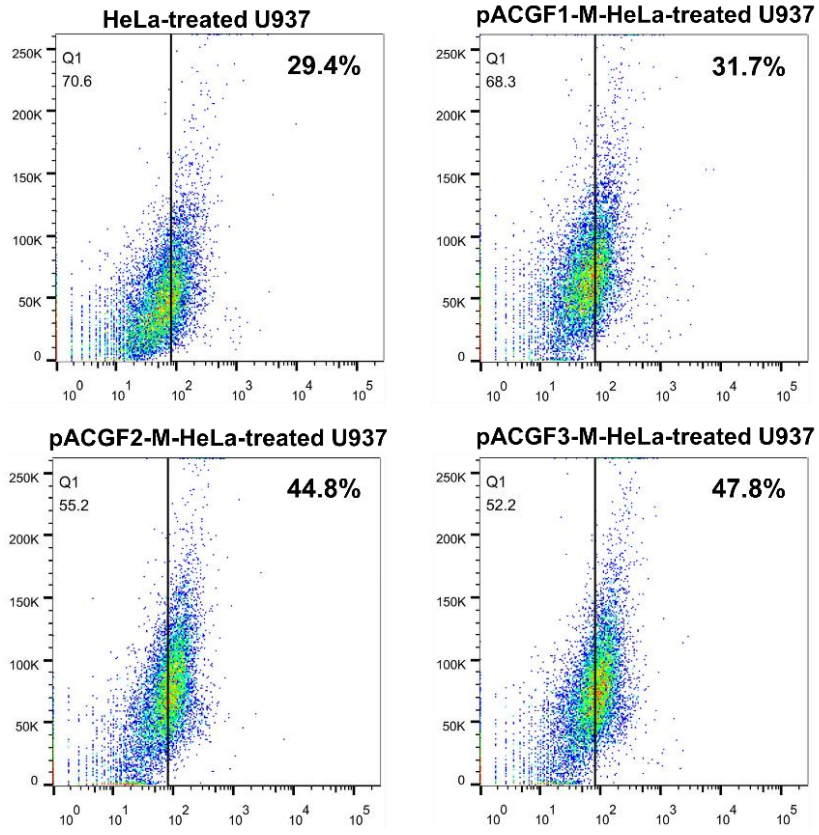

Figure S15. Flow cytometry data giving percentage of CD86-expressing cells after co-culturing with pACGF-M-remodeled Hela cells. Untreated HeLa cells were used as control.

REFERENCES

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