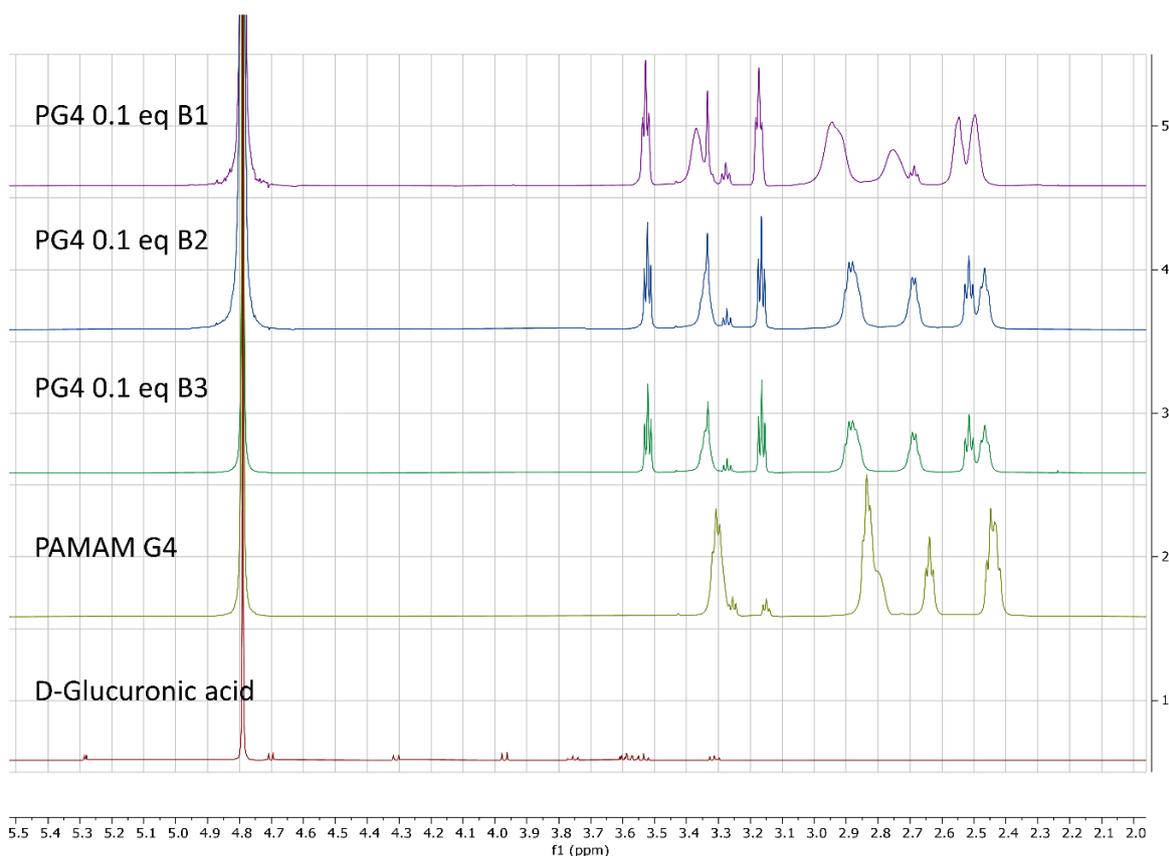
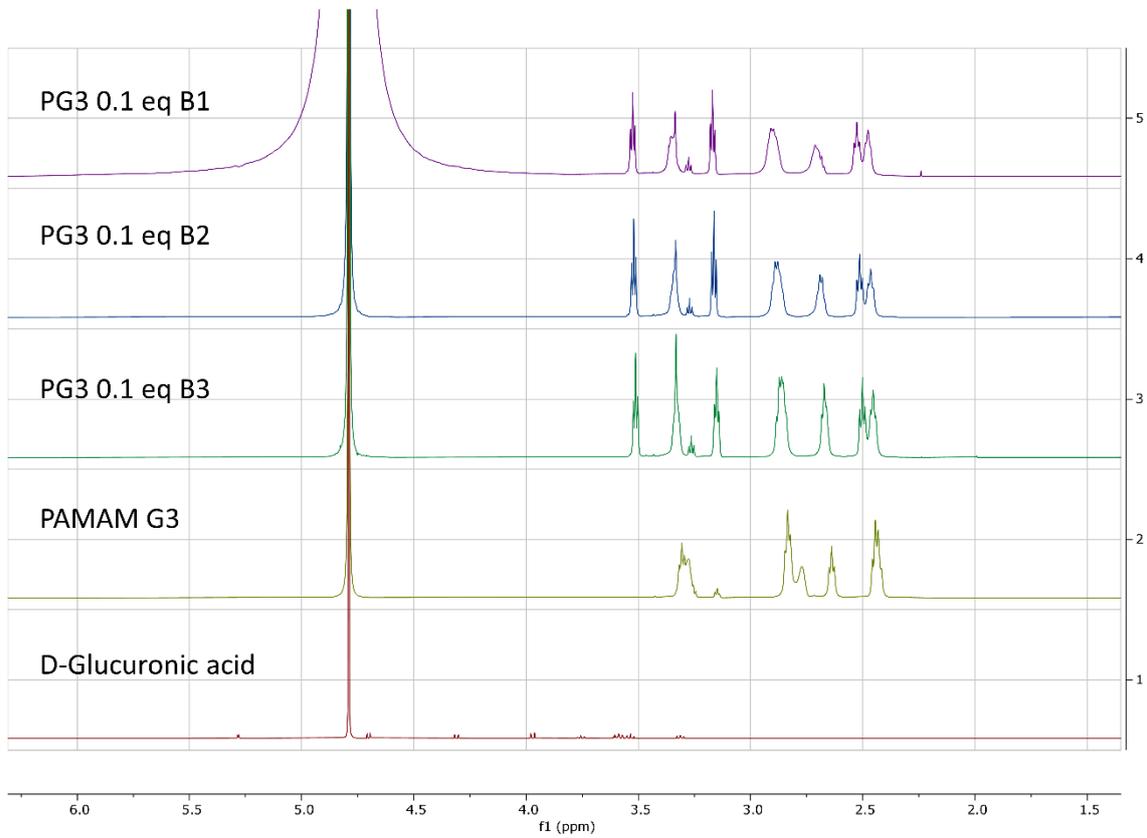


Synthesis, Formulation and Characterization of Immunotherapeutic Glycosylated Dendrimer/cGAMP Complexes for CD206 Targeted Delivery to M2 Macrophages in Cold Tumors

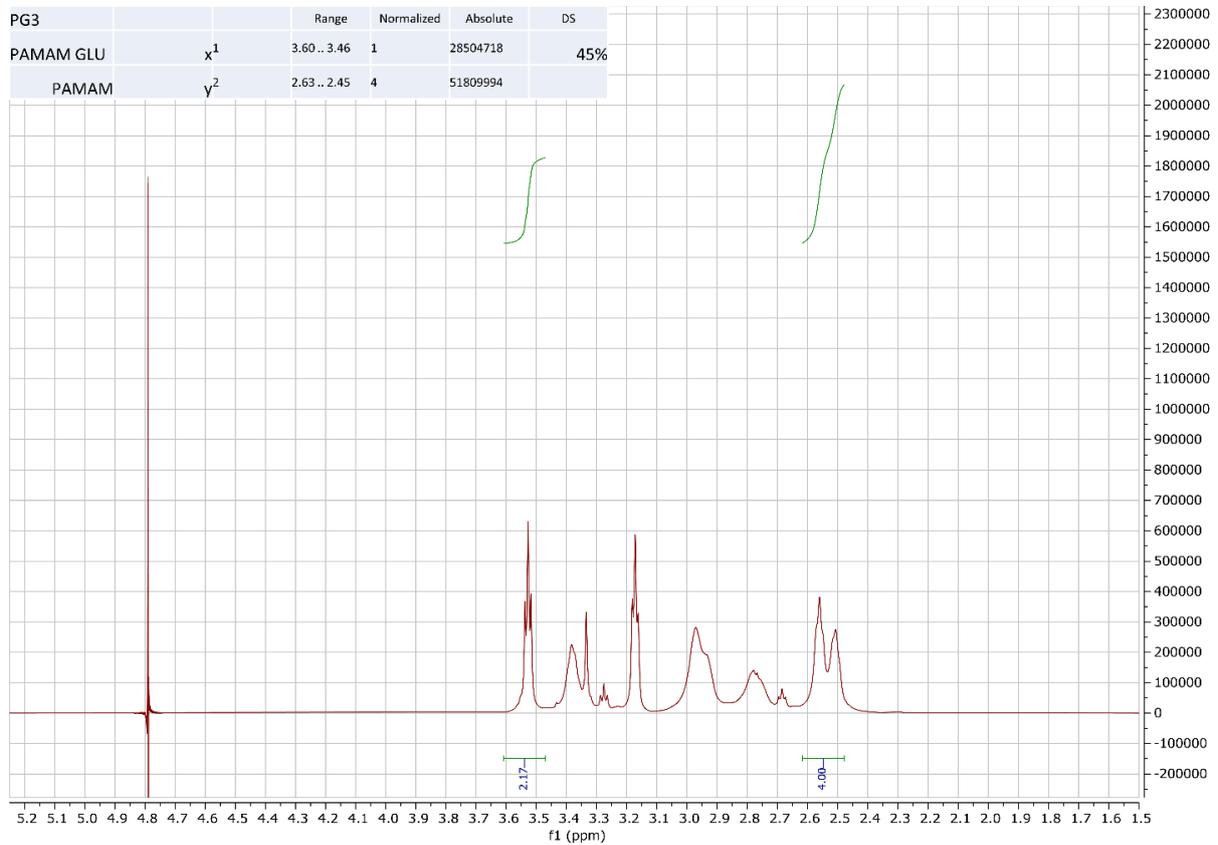
Supplementary data:



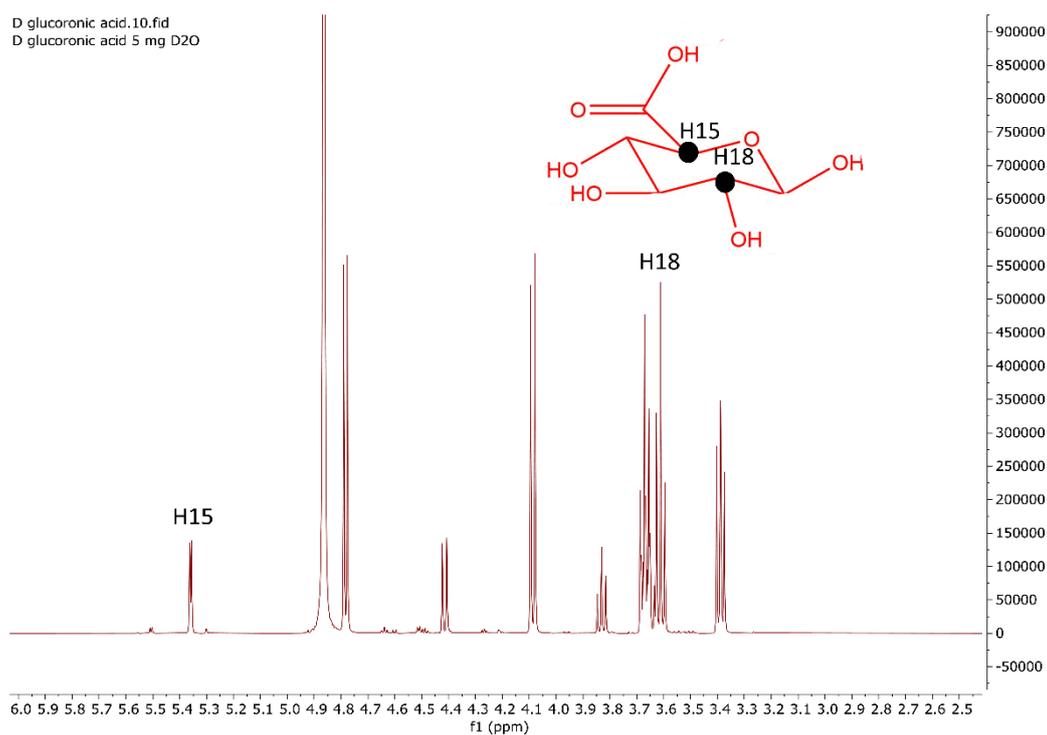
(a)



(b)



(c)



(d)

Figure S1. NMR graphs and integration of (a) PG4 0.1 eq batches overlap (b) PG3 0.1 eq batches overlap and (c) PG3 0.1 eq integration example (d) D-Glucuronic acid assignment.

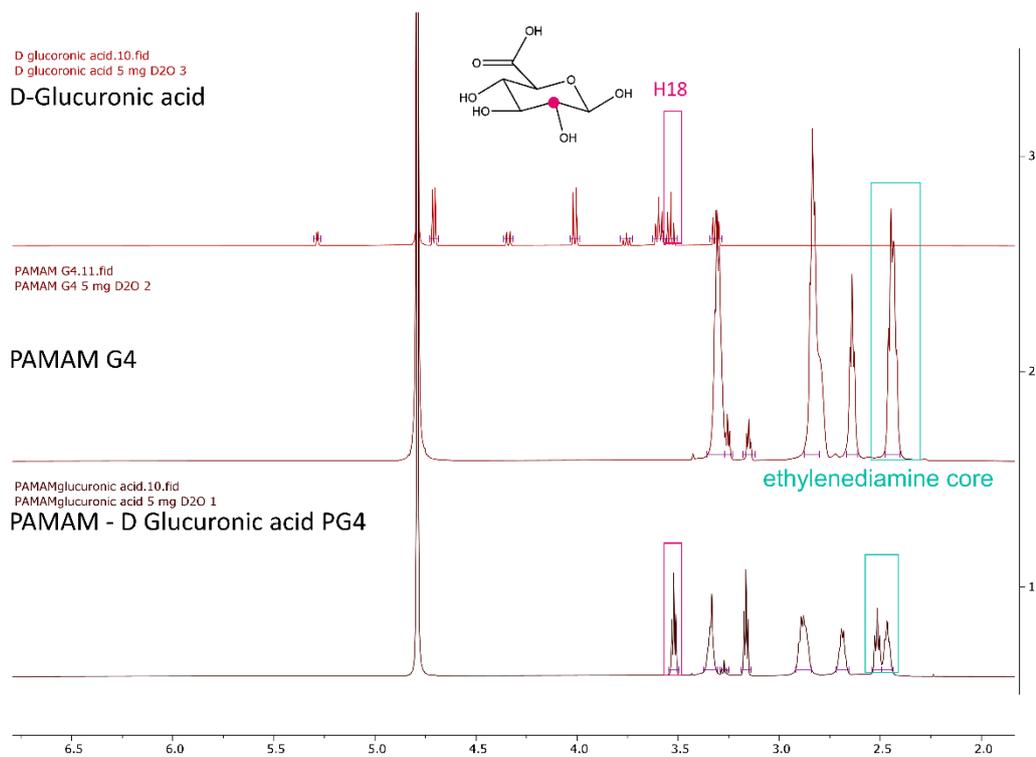


Figure S2. PAMAM G4 grafted with D-Glucuronic acid (PG4 0.1 and 0.3 eq) synthesis and characterization: NMR peak representation.

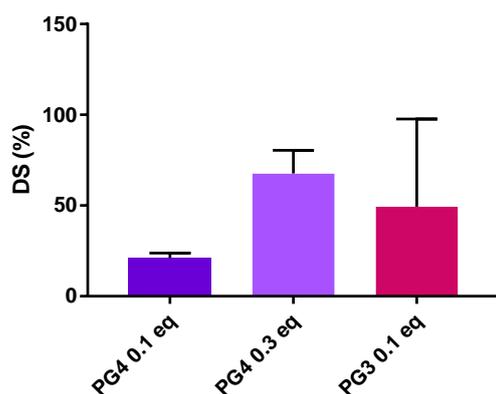
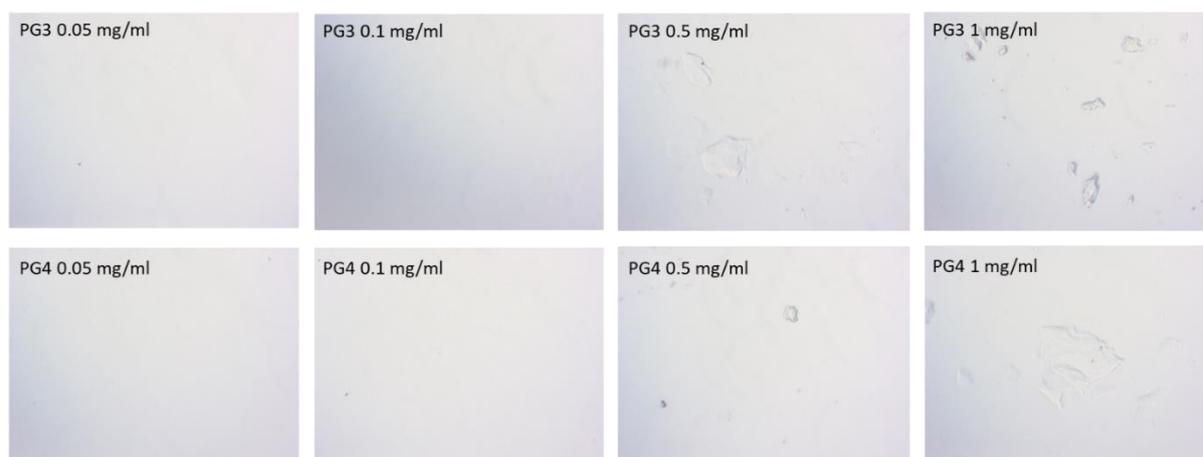


Figure S3. PAMAM amidation by Glucuronic acid; Gravimetry - DS was calculated by gravimetry and the results were $68 \pm 13\%$ for PG4 0.3, $21 \pm 3\%$ for PG4 0.1 and $49 \pm 48\%$ for PG3 0.1 eq. Results are presented as a mean \pm SD. n = 3.

Table S1. The residual moisture levels after lyophilization were determined by the Karl-Fisher method.

<i>Products</i>	<i>Residual Moisture Level [%]</i>
<i>PAMAM G4</i>	8.2–5.6
<i>PAMAM G3</i>	7.8
<i>PG4 0.1 eq</i>	3.2
<i>PG4 0.3 eq</i>	6.8
<i>PG3 0.1 eq</i>	4.6



(a)

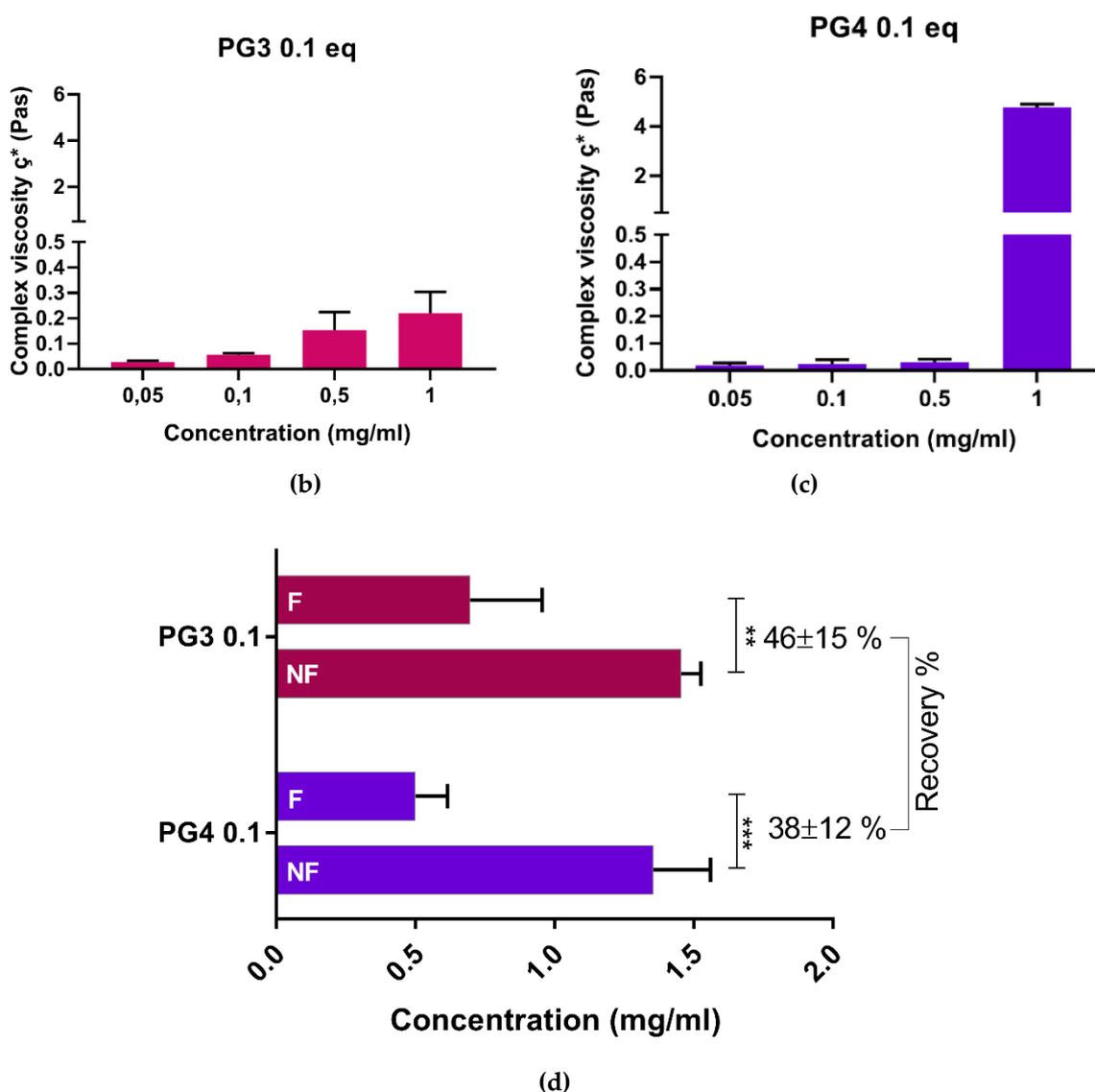
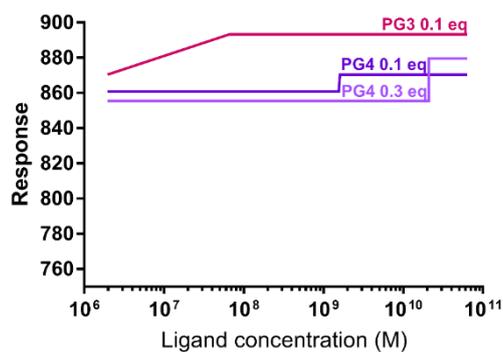
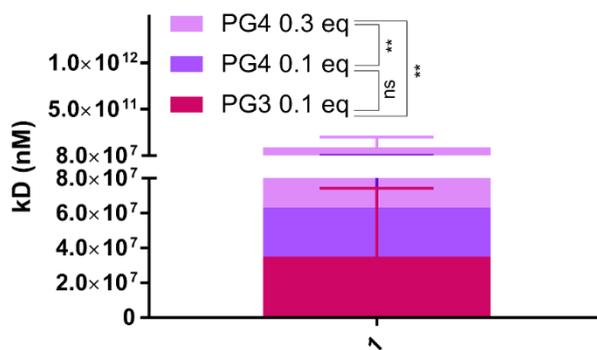


Figure S4. It was observed that both PG3 and PG4 tend to form gels at concentrations higher than 0.5 mg/ml (a) Light microscopy - images confirmed the presence of gel domains with the formation of a network due to the aggregation and the interactions of modified dendrimers at 0.5 mg/ml and 1 mg/ml for both polymers. (b,c) Rheological behaviors, expressed as complex viscosity at 0.05, 0.1 and 0.5 mg/ml (i.e., 0.02 Pa·s) and more than 20 times higher at 1 mg/ml. PG3 0.1 eq showed different behavior with an increase of viscosity from 0.02 to 0.2 Pa·s linked too to concentration (d) comparison on PG recovery at 1 mg/ml, non-filtered (NF) and filtered over 0.45 μ m filter (F) after lyophilization (optical microscopy, 10X). Recovery (%) was calculated as a weight difference between F and NF Concentration of F sample was considered as gelation concentration and further work was performed below this concentration. Results are presented as a mean \pm SD. n = 3. ** p < 0.01; *** p < 0.001.

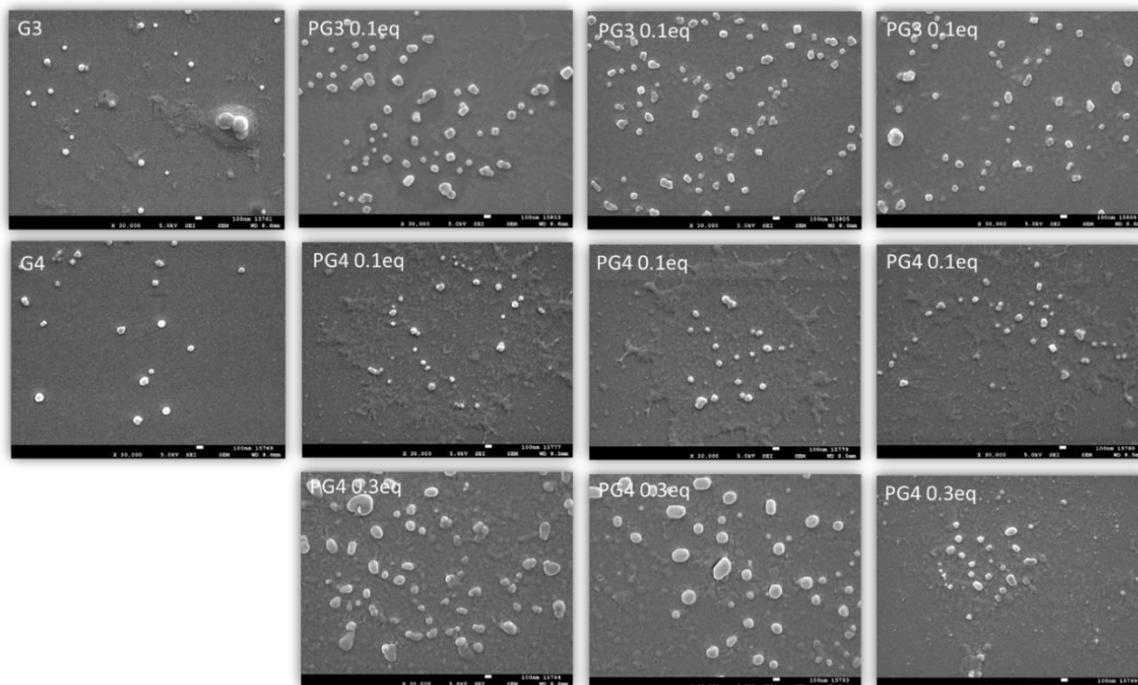


(a)

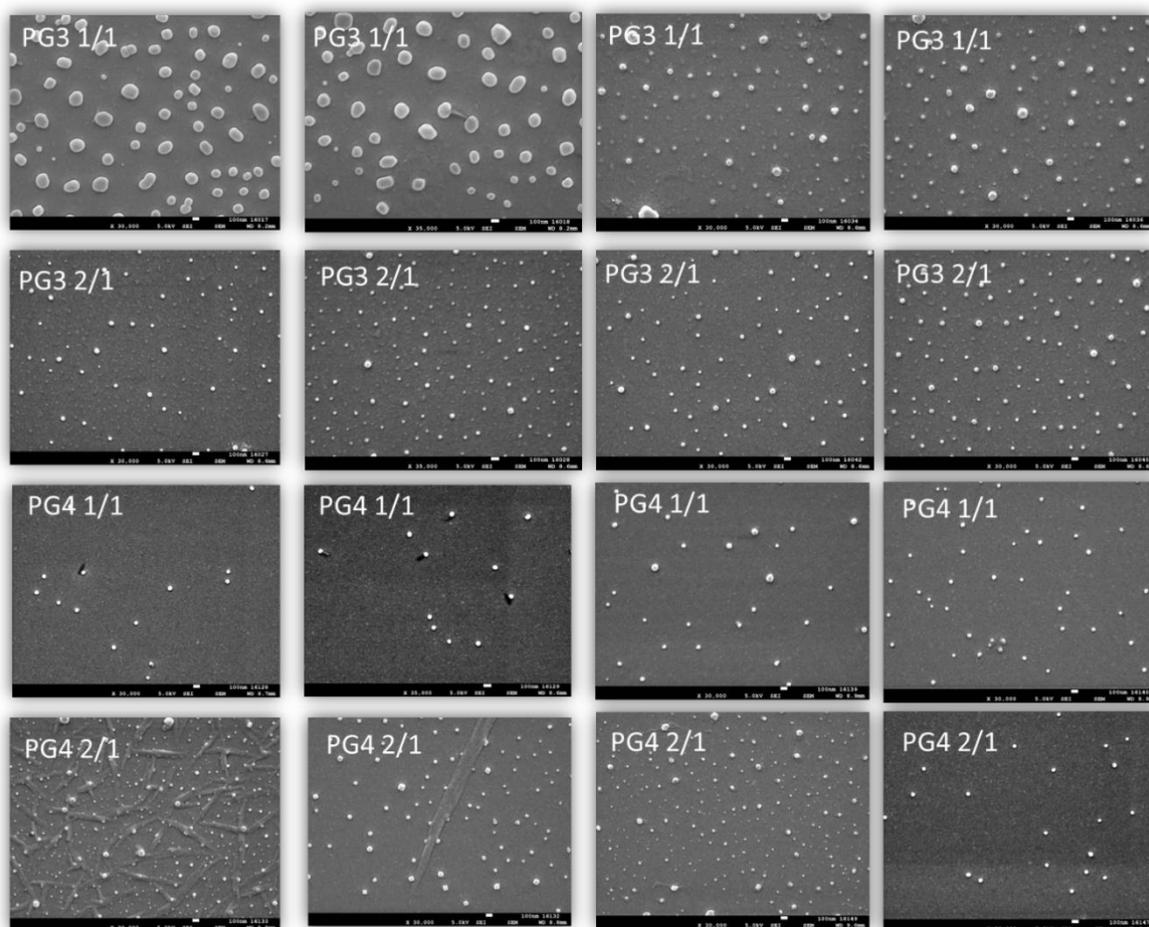


(b)

Figure S5. (a) Affinity of glu-modified dendrimers for CD206: MST graphical and (b) bar comparison for PG3 0.1 eq, PG4 0.1 eq and 0.3 eq affinity towards CD206. Results are presented as a mean ± SD. n = 3. ** *p* < 0.01.



(a)



(b)

Figure S6. (a) SEM of comparison of three batches of PG3 0.1 eq, PG4 0.1 and 0.3 eq (b) NCs of 1/1 and 2/1 0.1 eq PG3 and PG4. Magnification 30 000 X, cf 100 nm bar.

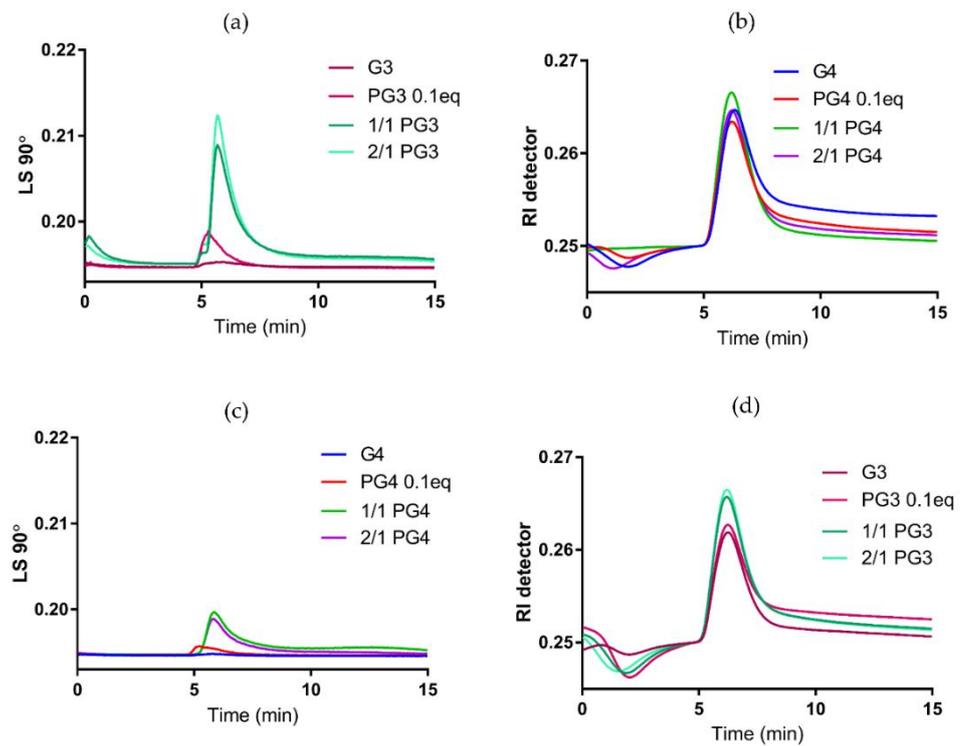


Figure S7. (a,c) AF4-MALS signal on different NCs (b,d) AF4-RI on different NCs.

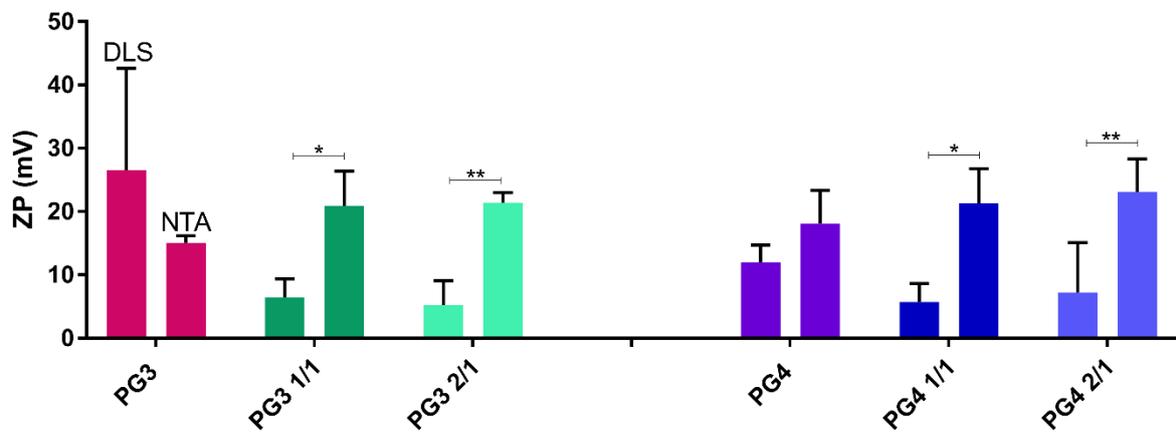


Figure S8. Comparison of zeta potential as measured by two technique (DLS and NTA) for control compounds PG3 and PG4 and their corresponding NCs Results are presented as a mean \pm SD. n = 3. * $p < 0.05$; ** $p < 0.01$.

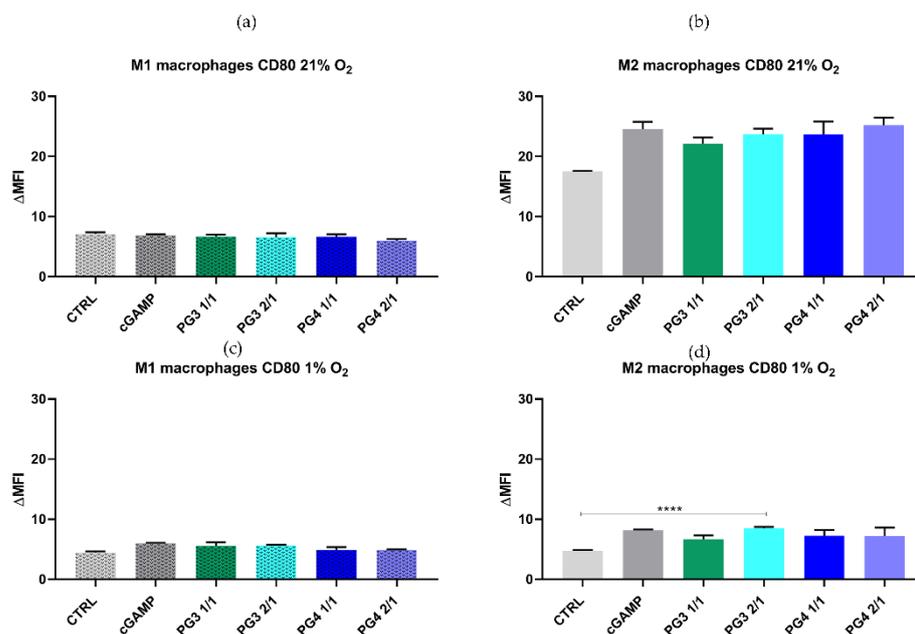


Figure S9. Surface expression of costimulatory molecule CD80 on murine BMDM cells. Polarized M1 and M2 BMDMs were stimulated in vitro with corresponding PAMAM NCs (PG3 1/1, PG3 2/1, PG4 1/1 or PG4 2/1), cGAMP (25 μ g/condition) or with media under normoxic (a,b) or hypoxic (c,d) conditions for 24 h. The cells were stained with fluorophore-conjugated antibodies against the CD86 markers and analyzed by flow cytometry. Error bars are SD of three independent experiments. **** $p < 0.0001$.

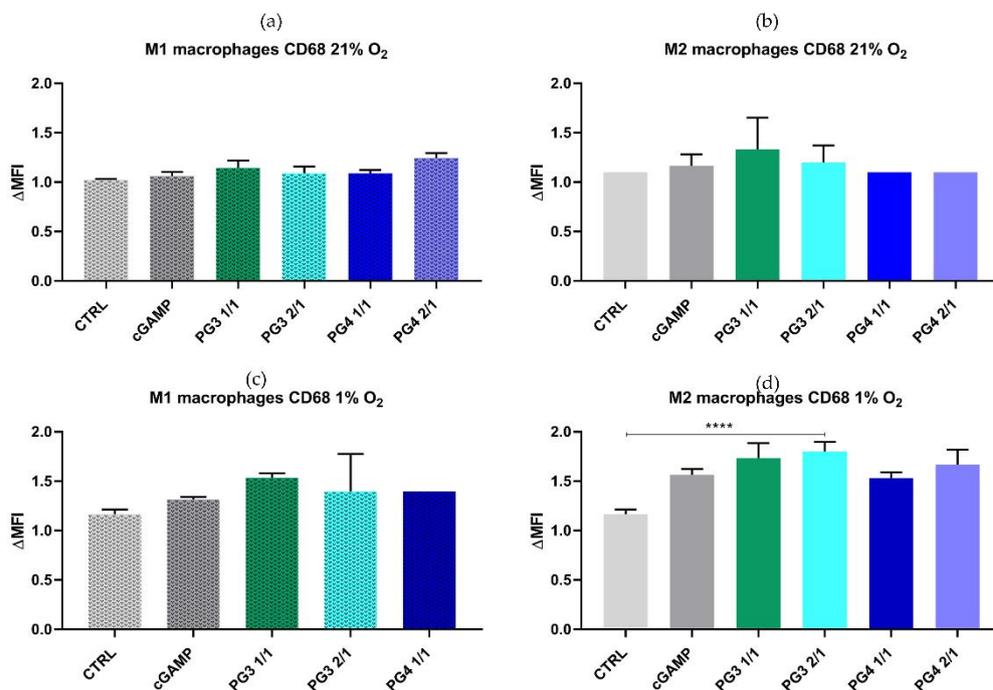


Figure S10. Surface expression of costimulatory molecule CD68 on murine BMDM cells. Polarized M1 and M2 BMDMs were stimulated in vitro with corresponding PAMAM NCs (PG3 1/1, PG3 2/1, PG4 1/1 or PG4 2/1), cGAMP (25 μ g/condition) or with media under normoxic (a,b) or hypoxic (c,d) conditions for 24 h. The cells were stained with fluorophore-conjugated antibodies against the CD86 markers and analyzed by flow cytometry. Error bars are SD of three independent experiments. **** $p < 0.0001$.

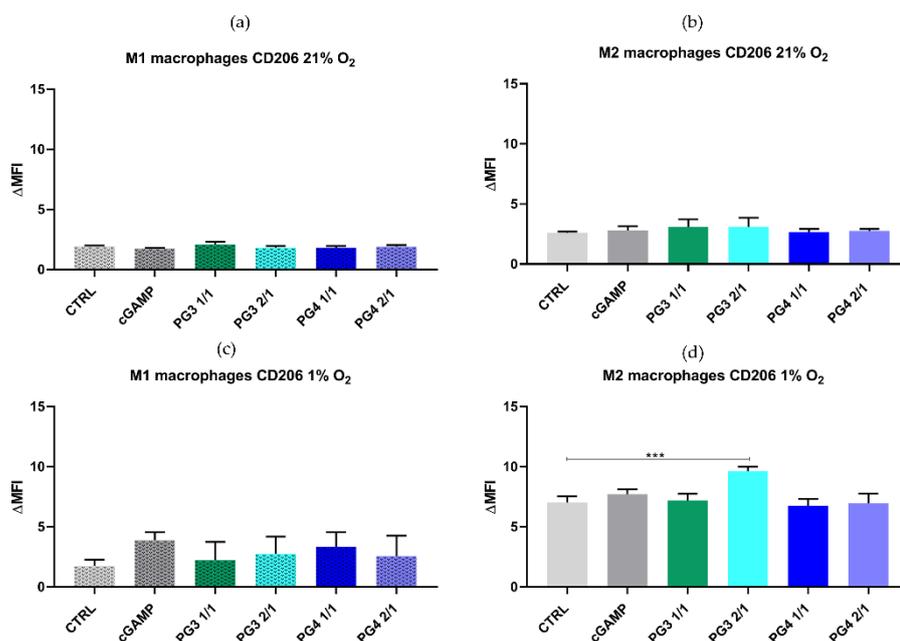


Figure S11. PAMAM up-regulates surface expression of costimulatory molecule CD80 on murine BMDM cells. Polarized M1 and M2 BMDMs were stimulated in vitro with corresponding PAMAM NCs (PG3 1/1, PG3 2/1, PG4 1/1 or PG4 2/1), cGAMP (25 μ g/condition) or with media under normoxic (a,b) or hypoxic (c,d) conditions for 24 h. The cells were stained with fluorophore-conjugated antibodies against the CD86 markers and analyzed by flow cytometry. Error bars are SD of three independent experiments. *** $p < 0.001$.

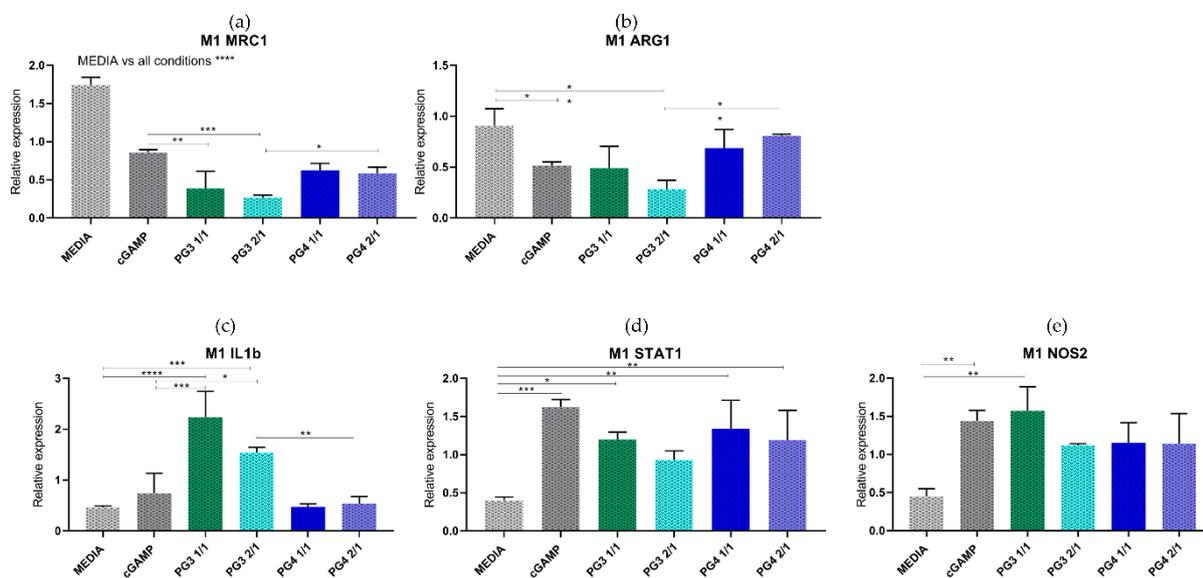


Figure S12. NC upregulates mRNA expression of pro-inflammatory (M1) genes and reduced the expression of anti-inflammatory (M2) genes in M1 polarized BMDMs. Relative mRNA expression of M2-like macrophage markers (Mrc1 (a) and Arg1 (b)) and of classically activated (M1-like) macrophage markers (IL-1b (c), STAT1 (d) and NOS2 (e)) in M2 BMDMs treated with corresponding PAMAM NCs (PG3 1/1, PG3 2/1, PG4 1/1 or PG4 2/1), cGAMP (25 μ g/condition) or with media * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

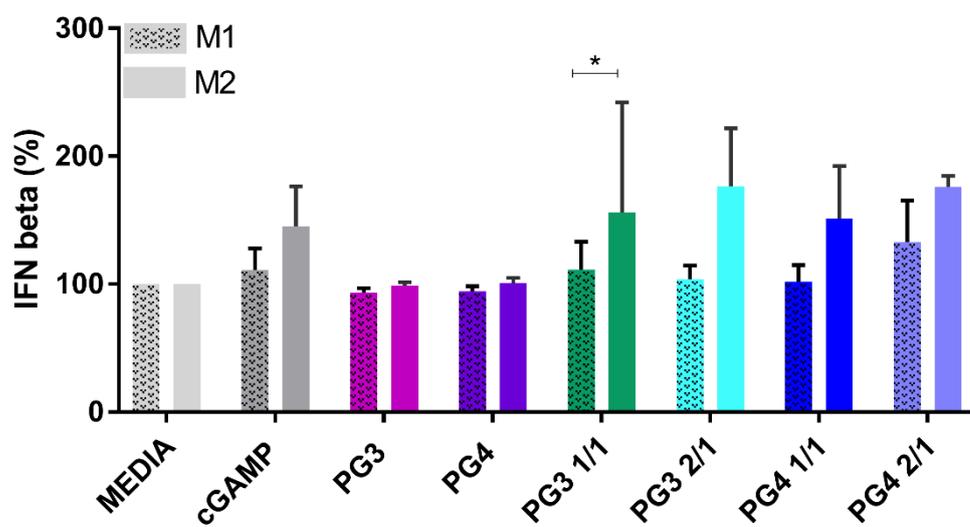


Figure S13. IFN beta level of M1 and M2 macrophages after 24h exposure of CTRL and NCs. * $p < 0.05$.