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Supplementary Materials for

Graphene quantum dots as anti-inflammatory therapy for colitis

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This PDF file includes:

Supplementary Figures

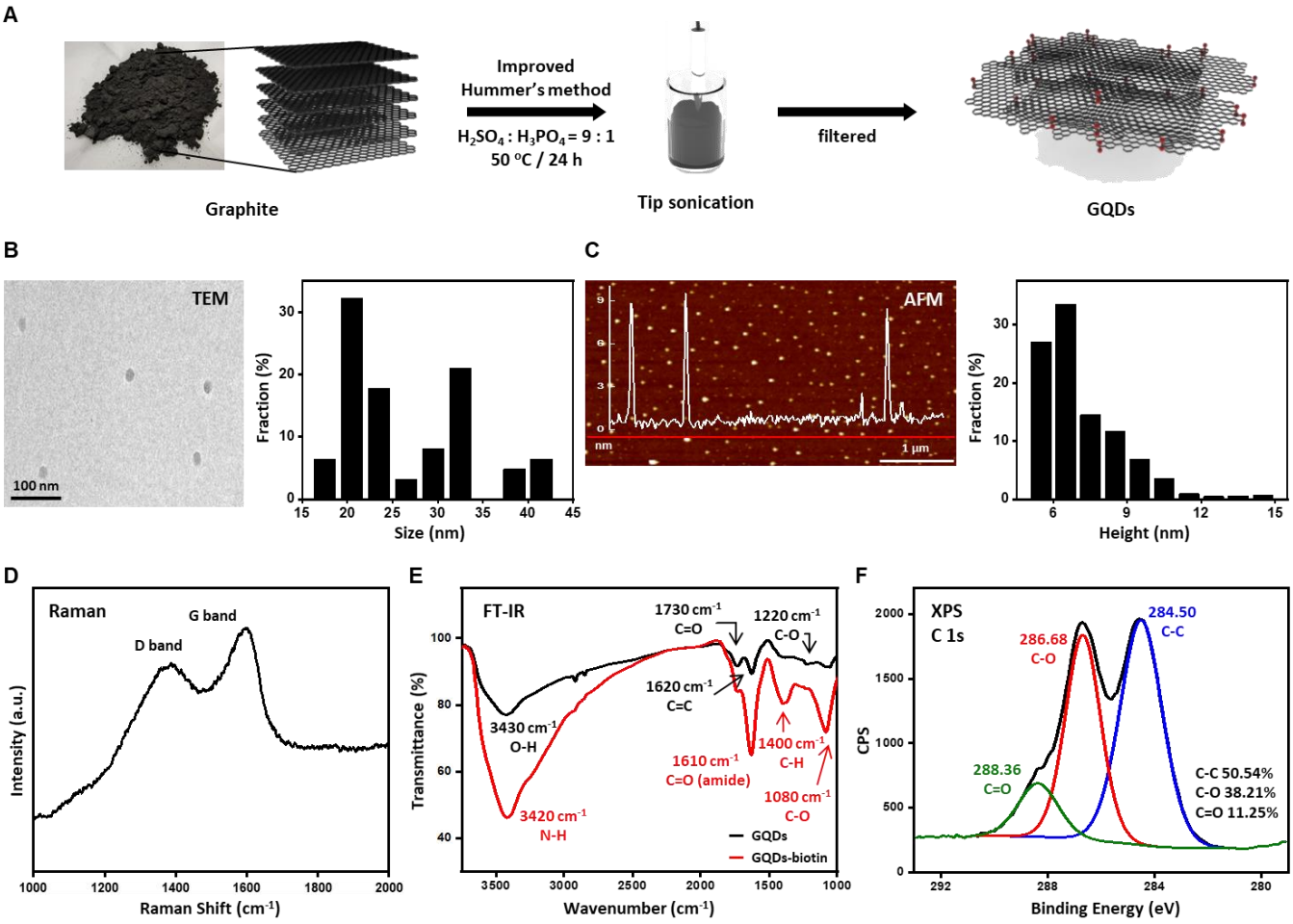

Fig. S1. Synthesis and characterization of GQDs. (**A**) Schematic representation of the synthesis process for GQDs. Photo credit: Dong Jin Kim (Program in Nano Science and Technology, Graduate School of Convergence Science and Technology, Seoul National University) (**B**) Representative TEM image of GQDs and the size distribution (*n*=62). (**C**) Representative AFM image and line profile analysis of GQDs, and the height distribution (*n*=22). (**D**) Representative Raman spectra of GQDs. (**E**) FT-IR spectra of GQDs and biotin-labeled GQDs. (**F**) XPS spectrum of GQDs, C1s.

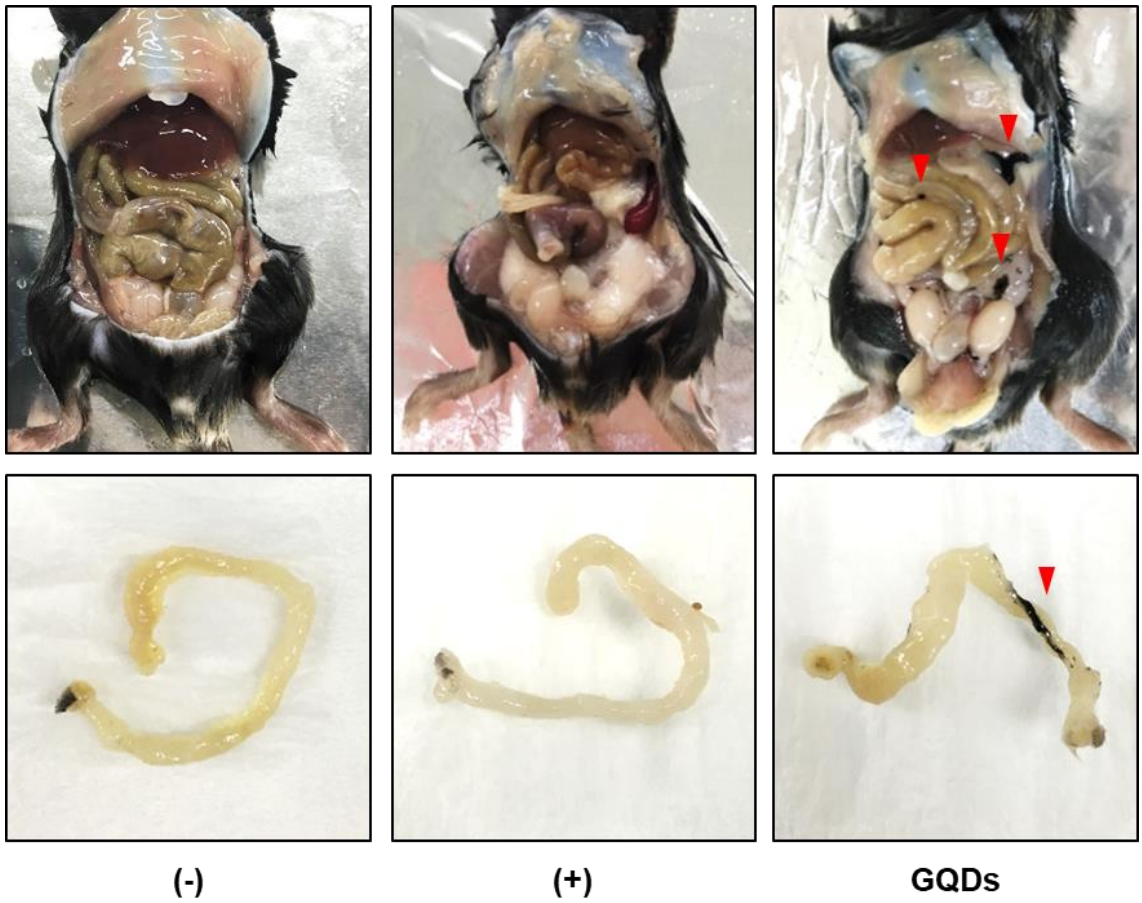

Fig. S2. GQDs accumulate in the abdominal cavity. Mice infused with GQDs were sacrificed 14 days after the induction of acute colitis with DSS. Photo credit: Byung-Chul Lee (Adult Stem Cell Research Center and Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University)

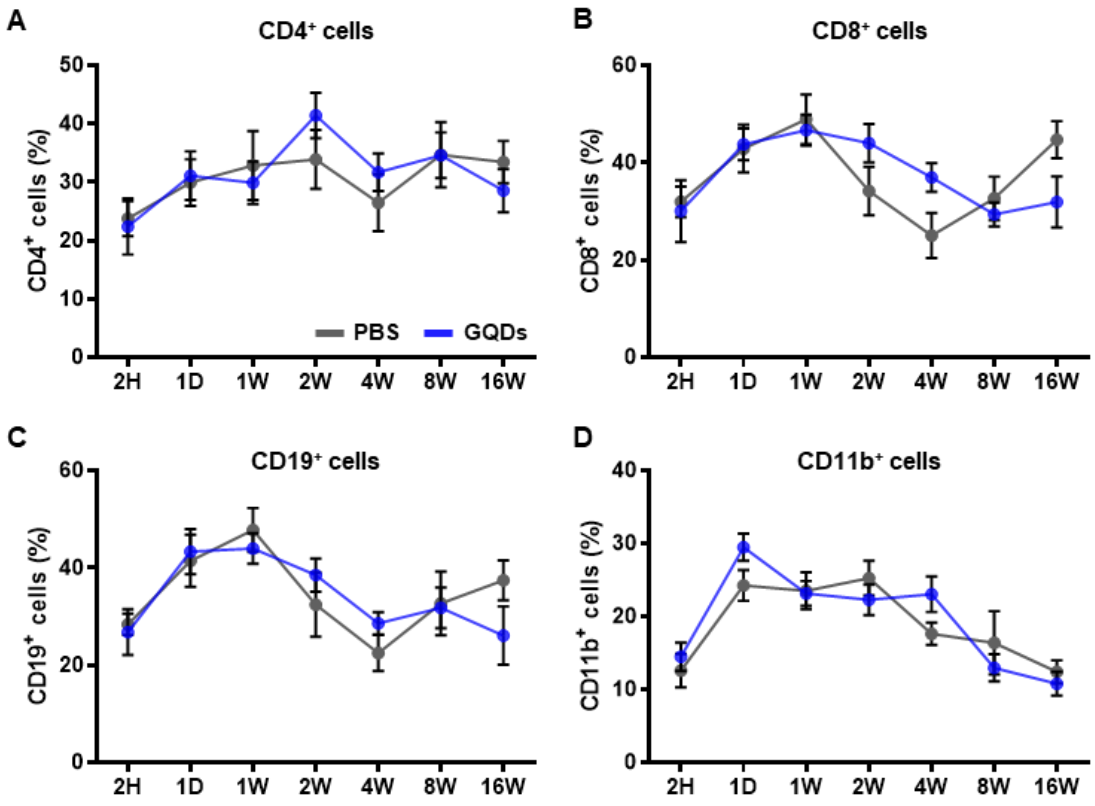

Fig. S3. Determination of immune cell proportions. Biotin-labeled GQDs were administered to normal mice without DSS induction, and mice were monitored for 16 weeks (*N*=5 mice/group). Cells from spleens of mice were stained for the indicated CD markers: (**A**) CD4, (**B**) CD8, (**C**) CD19 and (**D**) CD11b, and assessed by flow cytometric analysis. Results are shown as the mean ± SD.

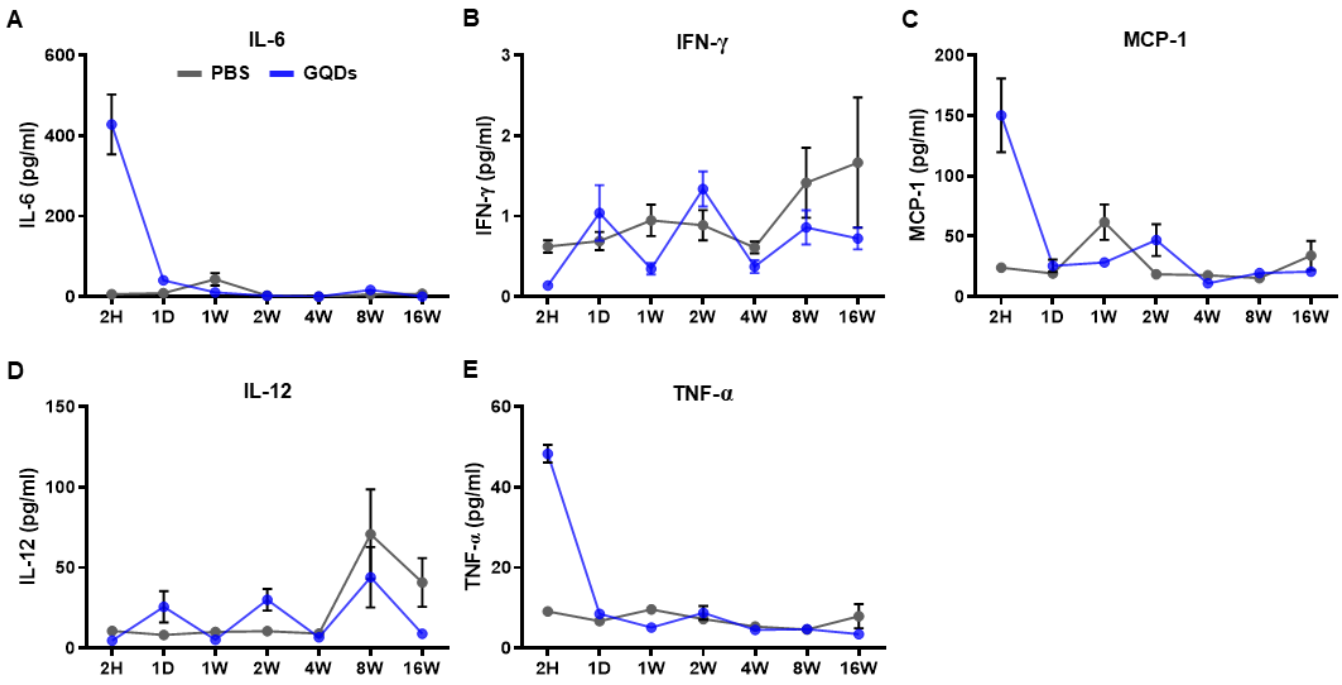

Fig. S4. Analysis of serum cytokines. Biotin-labeled GQDs were administered to normal mice without DSS induction, and mice were monitored for 16 weeks (*N*=5 mice/group). The indicated cytokines: (**A**) IL-6, (**B**) IFN-γ, (**C**) MCP-1, (**D**) IL-12 and (**E**) TNF-α in the serum of mice were analyzed by CBA analysis at the indicated time points. Results are shown as the mean ± SD.

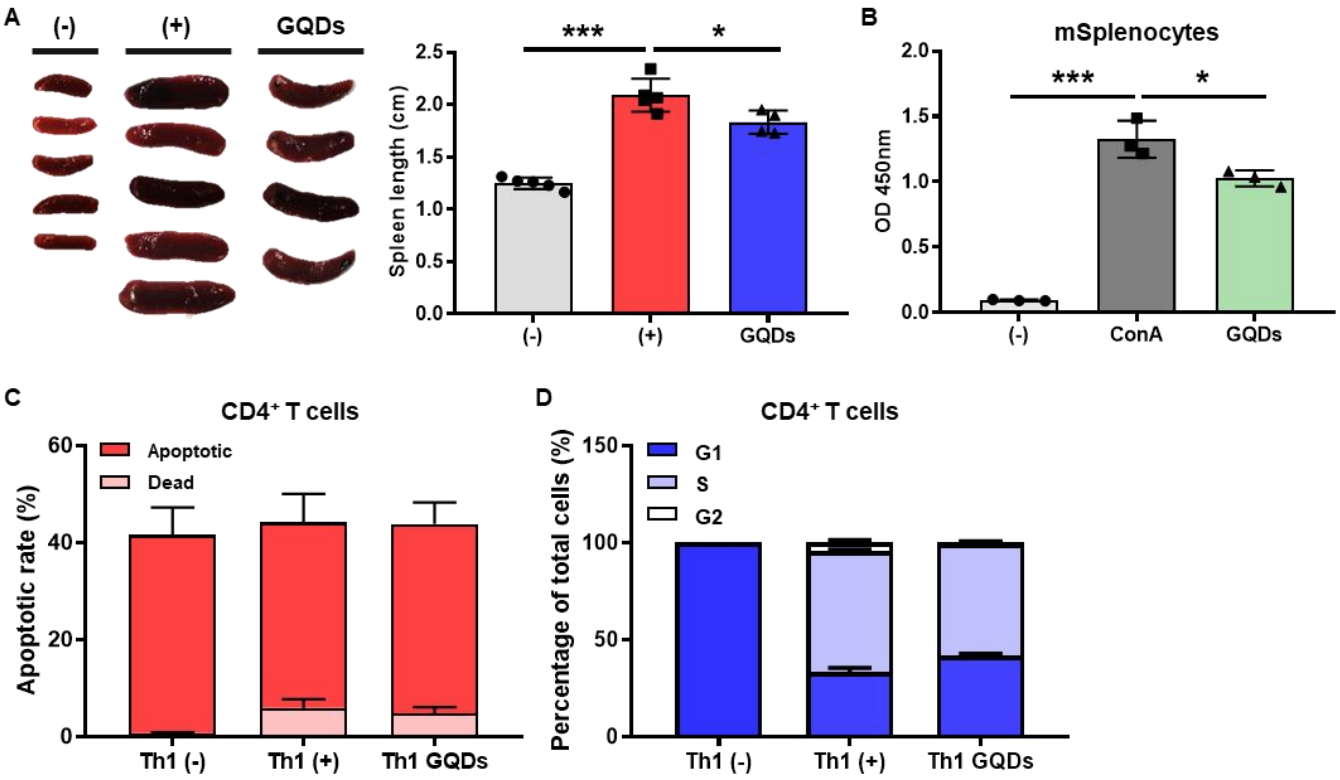

Fig. S5. GQDs suppress the proliferation of CD4⁺ T cells. (**A**) After sacrifice of chronic colitis-induced mice, spleens were collected for *ex vivo* analysis (*N*=4-5 mice/group). Left: Representative images of spleens from mice. Right: Quantification of spleen lengths. Photo credit: Byung-Chul Lee (Adult Stem Cell Research Center and Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University) (**B**) *Ex vivo* proliferation of splenocytes isolated from mice in the presence of GQDs was assessed by BrdU assay. **C-D**, primary CD4⁺ T cells were isolated from human cord blood and cultured in the presence of anti-CD3/28 microbeads and GQDs for 2 days. (**C**) Apoptosis and (**D**) cell cycle status of GQD-treated cells were determined by flow cytometric analysis. (*n*=3). *P<0.05, ***P<0.001. Results are shown as the mean ± SD.

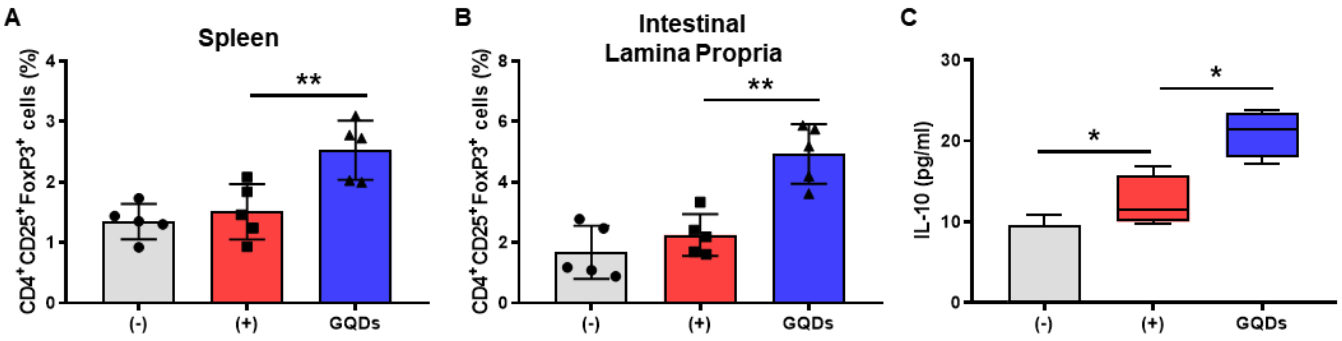

Fig. S6. *Ex vivo* analysis of Tregs. Mice received intraperitoneal injections of GQDs after acute DSS-colitis induction. On day 14, mice were sacrificed for further investigation (*N*=5 mice/group). Proportions of Tregs were assessed in (**A**) the spleen and (**B**) the intestinal lamina propria by flow cytometric analysis. (**C**) Levels of secreted IL-10 in the serum were measured by CBA analysis. *P<0.05, **P<0.01. Results are shown as the mean ± SD.

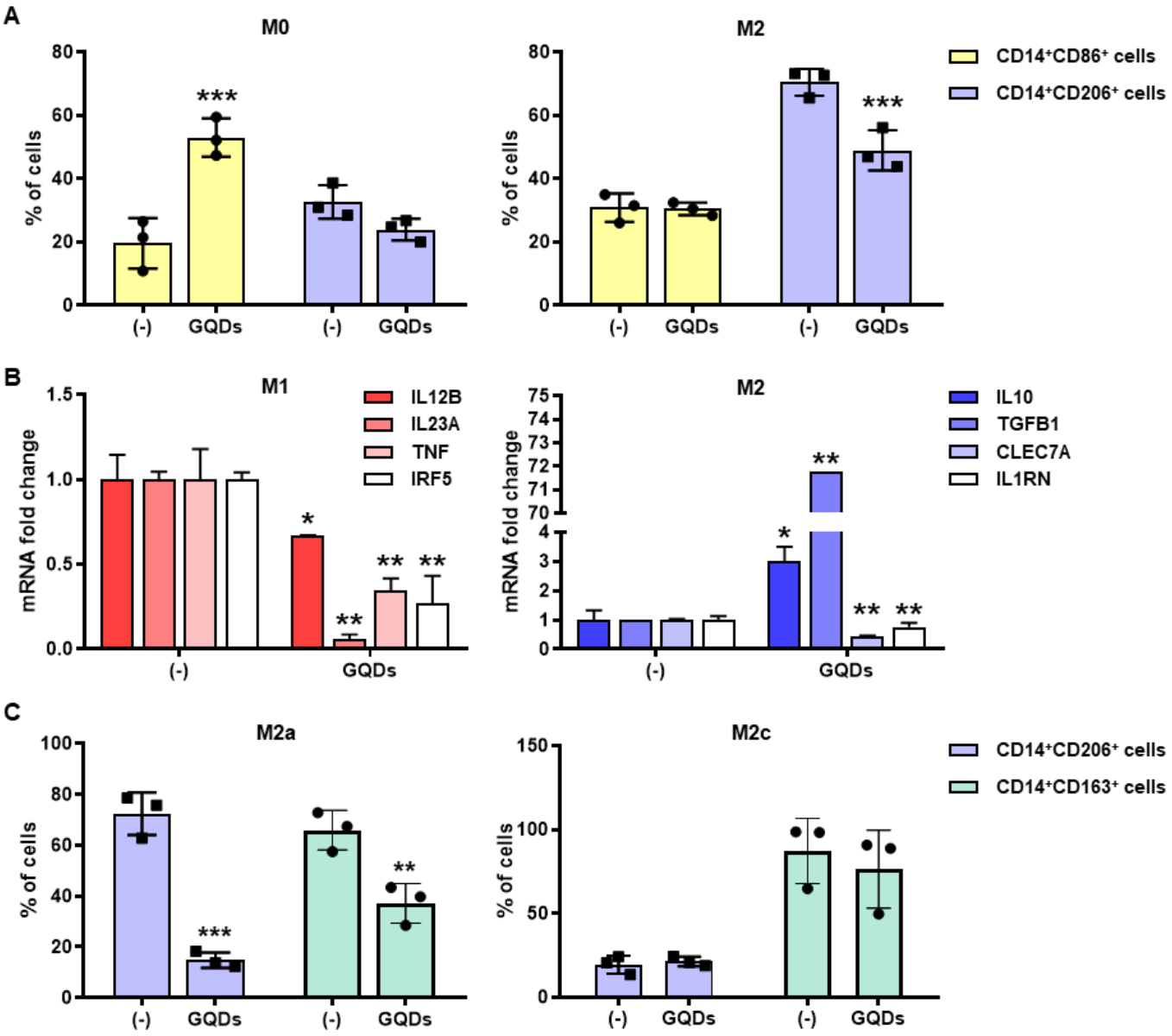

Fig. S7. GQDs have a role in determining macrophage subtypes, especially on M2 type specific polarization. (**A**) primary $CD14^+$ macrophage-like cells were isolated from human cord blood and polarized into M0 and M2 types with type-specific inducer cytokines in the presence of GQDs. M0 and M2 polarized macrophages were analyzed by assessment of the type-specific cell surface CD markers CD86 and CD206 by flow cytometric analysis. (**B**) mRNA expression levels in M1 macrophage cells in the presence of GQDs were investigated for the indicated M1 and M2 specific markers. (**C**) Primary $CD14^+$ macrophage-like cells were cultured with subtype specific cytokines for M2a and M2c, and expression levels of the CD markers CD163 and CD206 were detected by flow cytometric analysis. (n=3). * P<0.05, ** P<0.01, *** P<0.001. Results are shown as the mean ± SD.

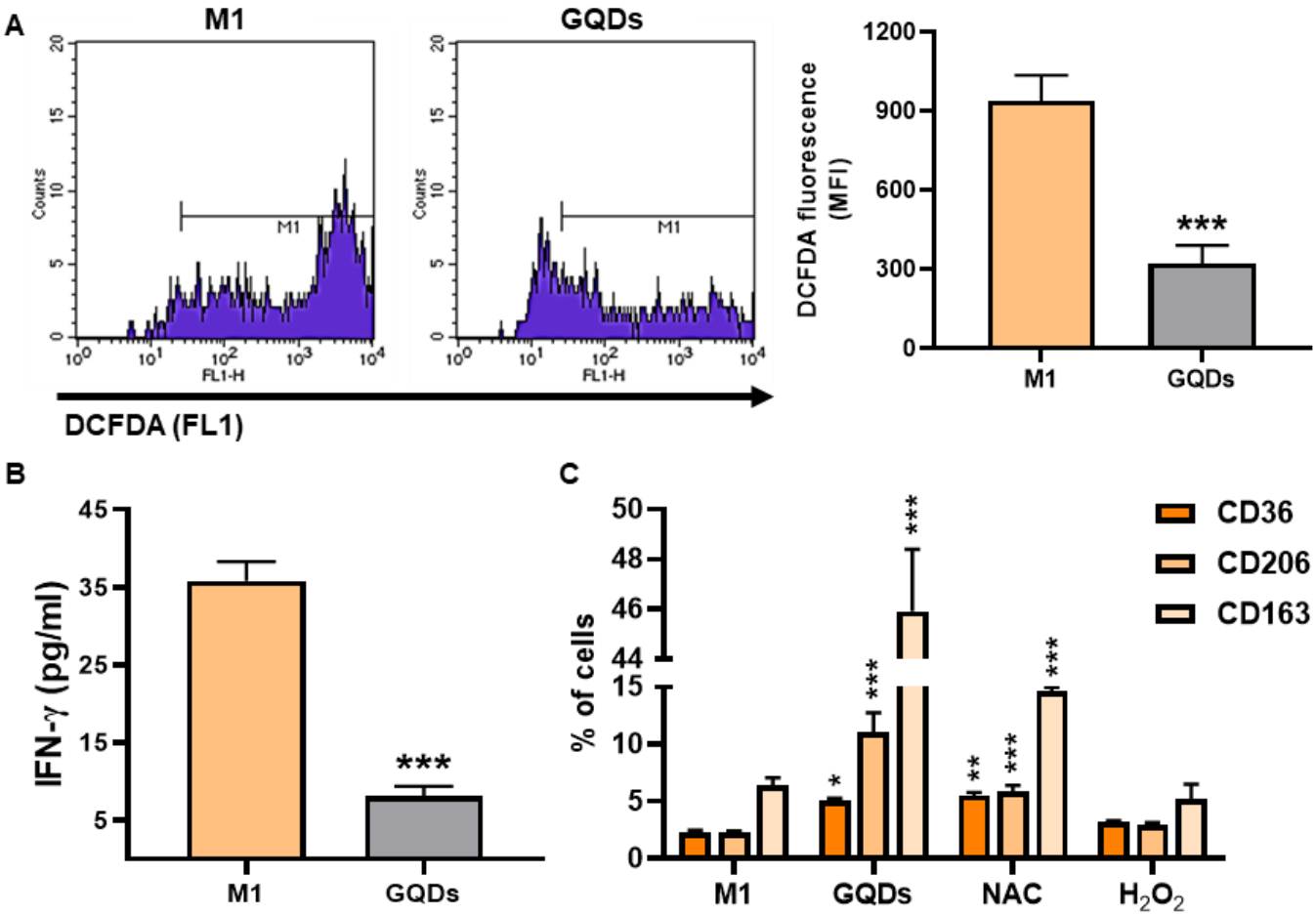

Fig. S8. GQDs regulate cellular ROS and M2 macrophage marker expressions in THP-1 macrophages. THP-1 macrophages were polarized into the M1 subtype by treating IFN-γ and LPS after PMA incubation. (**A**) Intracellular ROS levels were measured using flow cytometry with or without GQDs. (**B**) The secretion level of IFN-γ was determined by CBA analysis. (**C**) M2 specific marker expressions were investigated by flow cytometry in the presence of GQDs, N-acetyl cysteine (NAC), or hydrogen peroxide (H_2O_2). (n=3). *P<0.05, **P<0.01, ***P<0.001. Results are shown as the mean ± SD.

Supplementary Tables

Table S1. Elemental Analysis of GQDs

Element	wt%	mol%	Molar ratio
C	43.69	42.58	1
H	2.17	25.35	0.60
N	0.00	0.00	0.00
S	2.45	0.90	0.02
O	42.64	31.17	0.73

Table S2. Quantitative RT-PCR primers

Gene	Species	Forward primer	Reverse primer
TBX21	human	GGGAAGTGGGGCTCAAGAAG	AACCAAAAGCAAGACGCAGC
IFNG	human	GGCTTTATCTCAGGGGCCAA	TGGCTCAGATTGCAGGCATA
IL12RB2	human	GCCTCAGCACATCTCCCTTT	GGTTGAGGGCACACTGACTT
CXCR3	human	TCTGAGGACTGCACCATTGC	CCTCTACGCCATGCCTTGTA
IL18R1	human	CCCTACAAAAAGGGCGCATC	CCCCTCCTCCCACATCCTAT
IL12B	human	ACCGTAAGTGTCTGGAAGGC	TCTGTCTGCTTCTCACAGGG
IL23A	human	AAATCTACCACCCCAGGCAC	AATCCTCCCCAAACTGTCCC
TNF	human	CATCCAACCTTCCCAAACGC	CGAAGTGGTGGTCTTGTTGC
IRF5	human	TGCGGACTGATGTGGAGATG	CTGACCAGACCAGAGACAGC
IL10	human	ATCTTGTCTCTGGGCTTGGG	TGGGGAATGAGGTTAGGGGA
TGFB1	human	GATGGAGAGAGGACTGCGGA	CTGGTCCCCTGTGCCTTGAT
CLEC7A	human	TGTAATTGTAGGGAAACTGCTCTCA	ACTTGTTCCAATGAGGTTGGTT
IL1RN	human	CTGAGGACCAGCCATTGAGG	AATGCAGAGGCGACATGGAA
GAPDH	human	TGATGACATCAAGAAGGTGGTG	ACCCTGTTGCTGTAGCCAAAT