

Supporting Information

Utilization of Click Chemistry to Study the Effect of Poly(ethylene) Glycol Molecular Weight on the Self-Assembly of PEGylated Gambogic Acid Nanoparticles for the Treatment of Rheumatoid Arthritis

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Table S1 Primer sequences used for RT-PCR

Gene	Primer	Sequence
TNF- α	Forward primer	5'-CCCTCACACTCAGATCATCTTCT-3'
	Reverse primer	5'-GCTACGACGTGGGCTACAG-3'
GAPDH	Forward primer	5'-AGGTCGGTGTGAACGGATTTG-3'
	Reverse primer	5'-TGTAGACCATGTAGTTGAGGTCA-3'

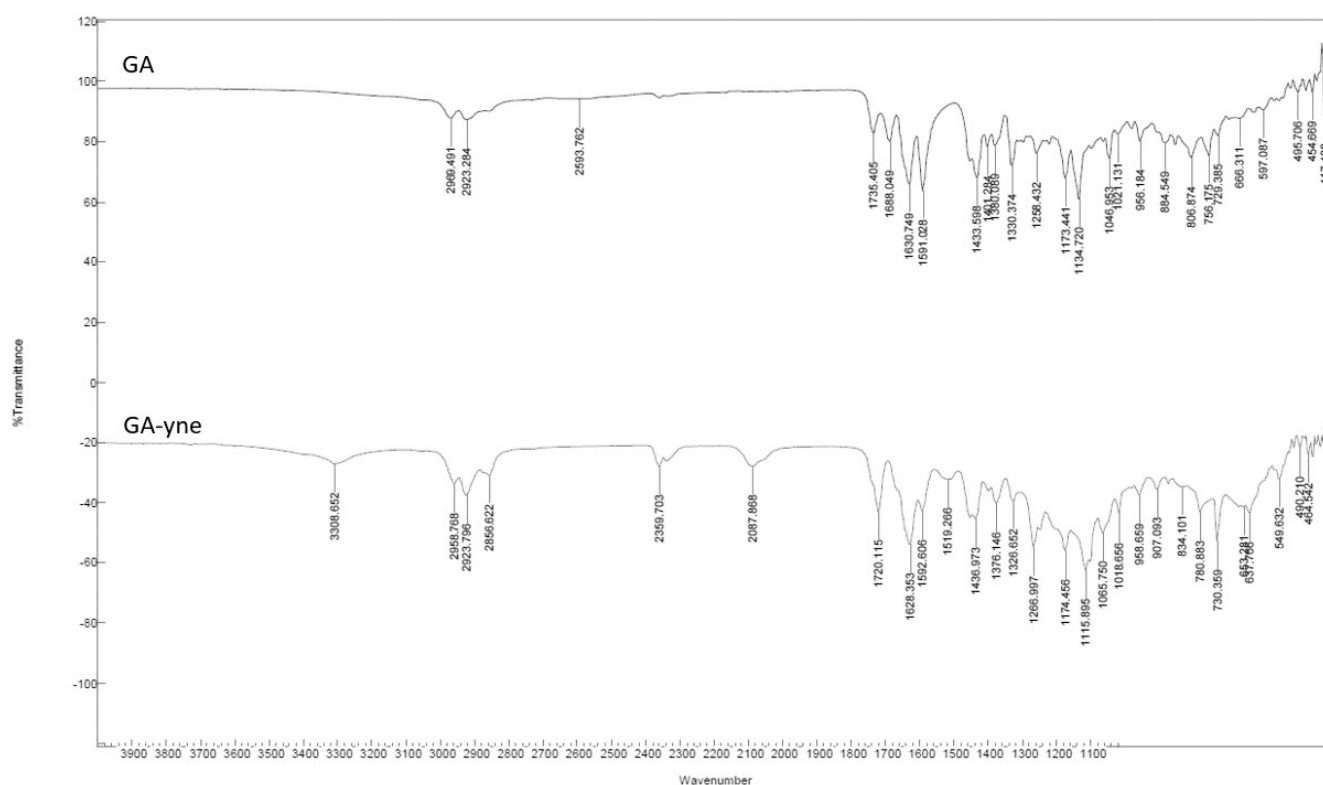


Figure S1 FTIR spectra of GA and GA-yne: ν (cm^{-1}) = 3308 (terminal alkyne), 2359 (C-C alkyne), 1720 (C=O amide)

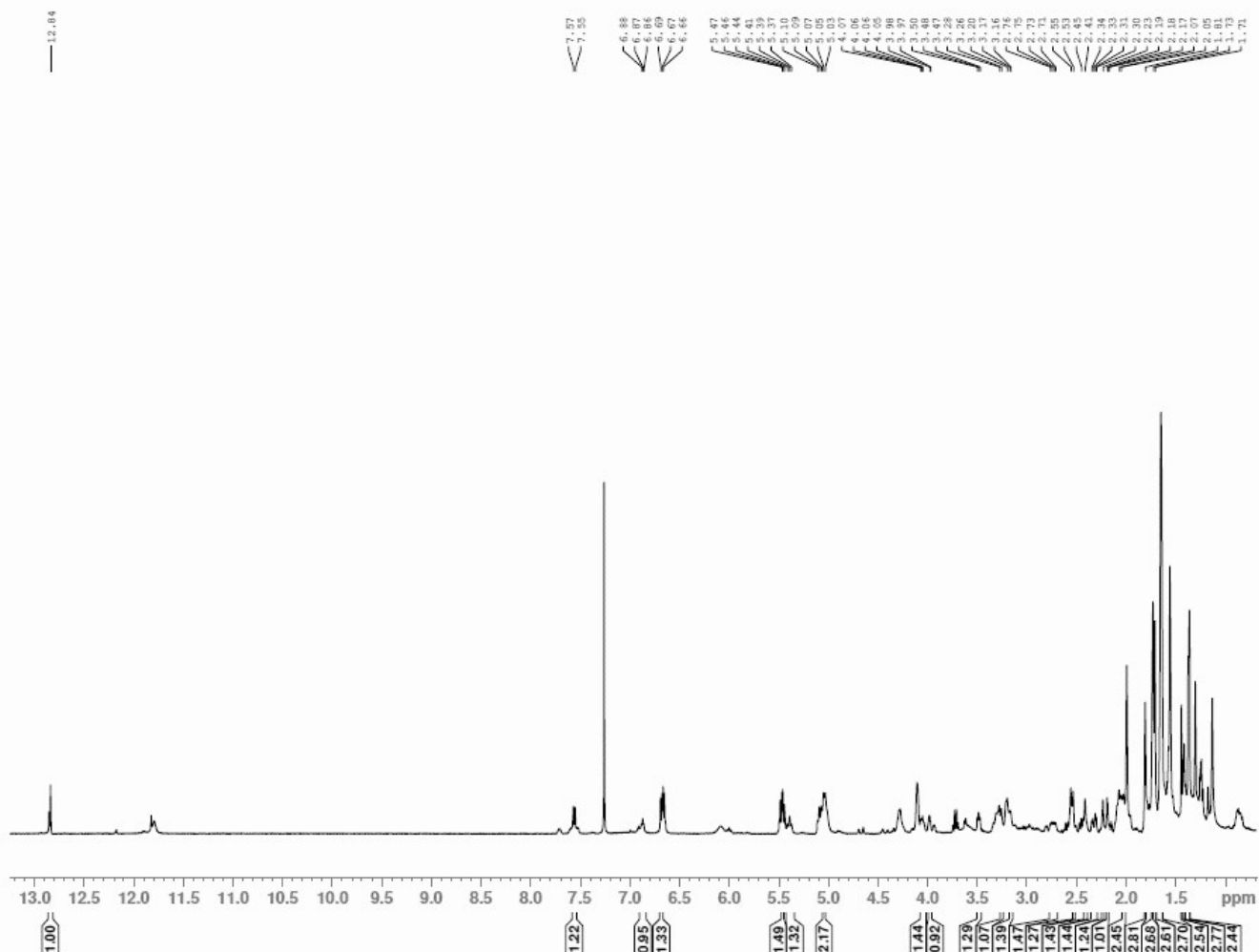


Figure. S2 ^1H -NMR spectrum of GA-yne: ^1H -NMR (400Hz, CDCl_3 , δ , ppm): 12.84 (s, 1H), 7.57 (d, 1H), 6.89 (t, 1H), 6.68 (m, 1H), 5.47 (m, 1H), 5.39 (m, 2H), 5.04 (m, 2H), 4.06 (m, 1H), 3.98 (m, 1H), 3.48 (t, 1H), 3.28 (m, 1H), 3.16 (m, 1H), 2.71 (m, 1H), 2.56 (d, 1H), 2.41 (m, 1H), 2.34 (m, 1H), 2.18 (m, 1H), 2.05 (m, 2H), 1.81 (s, 3H), 1.73 (s, 3H), 1.71 (s, 3H), 1.65 (s, 3H), 1.44 (s, 3H), 1.41 (s, 3H), 1.36 (s, 3H), 1.30 (s, 3H), 1.23 (s, 3H)

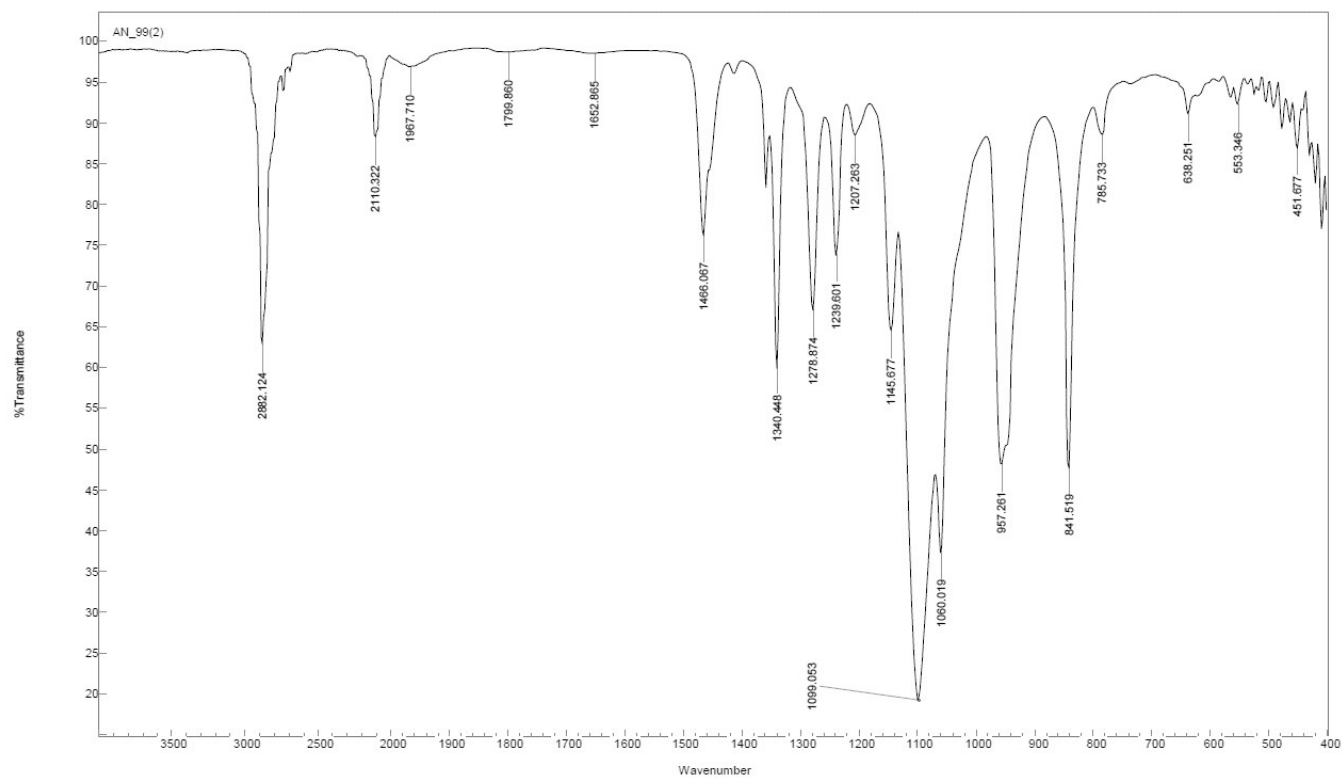


Figure. S3 FTIR spectrum of mPEG_{2K}-N₃: ν (cm⁻¹) = 2882 (C-H alkane), 2110 (N=N=N azide)

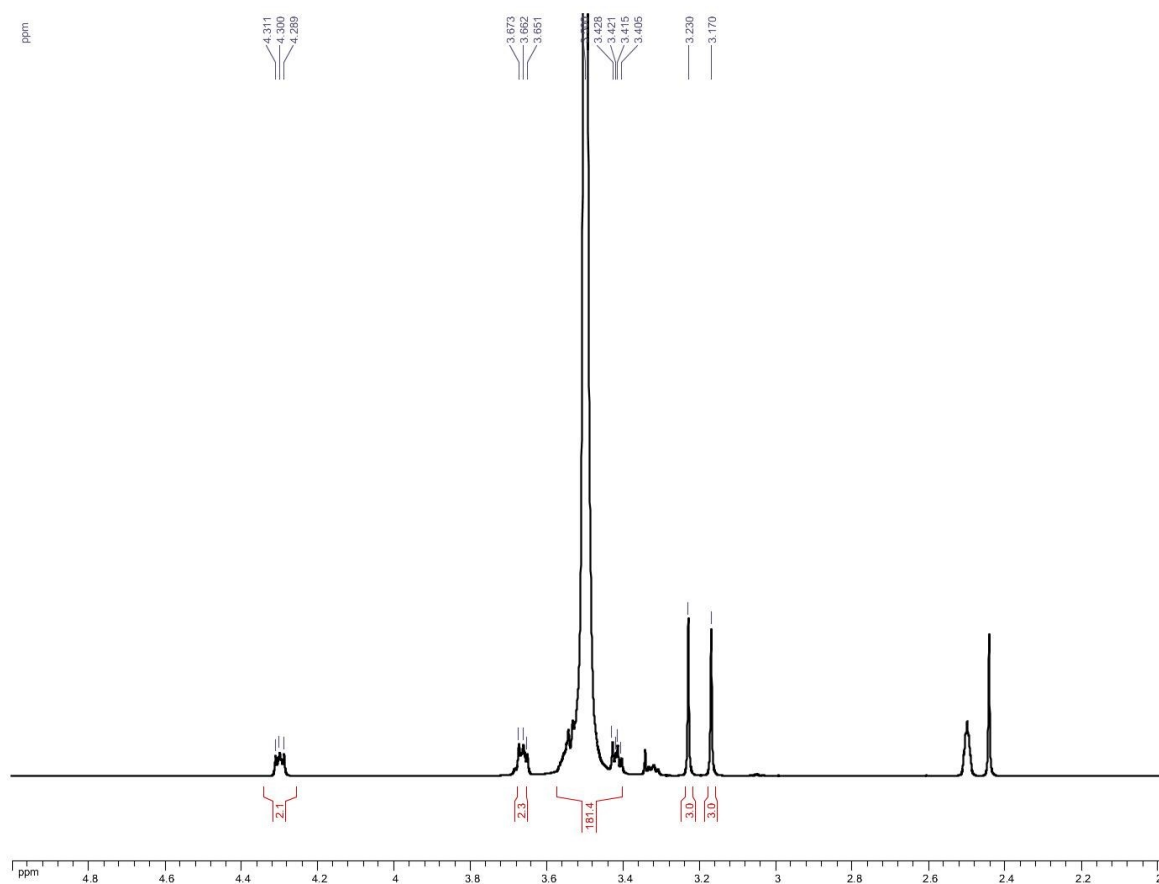


Figure. S4 ^1H -NMR spectrum of mPEG_{2K}-OMs: ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$, δ , ppm): 4.31-4.29 (*m*, 2H), 3.68-3.65 (*m*, 2H), 3.99 (*m*, $\sim n \times 4\text{H}$), 3.23 (*s*, 3H), 3.17 (*s*, 3H)

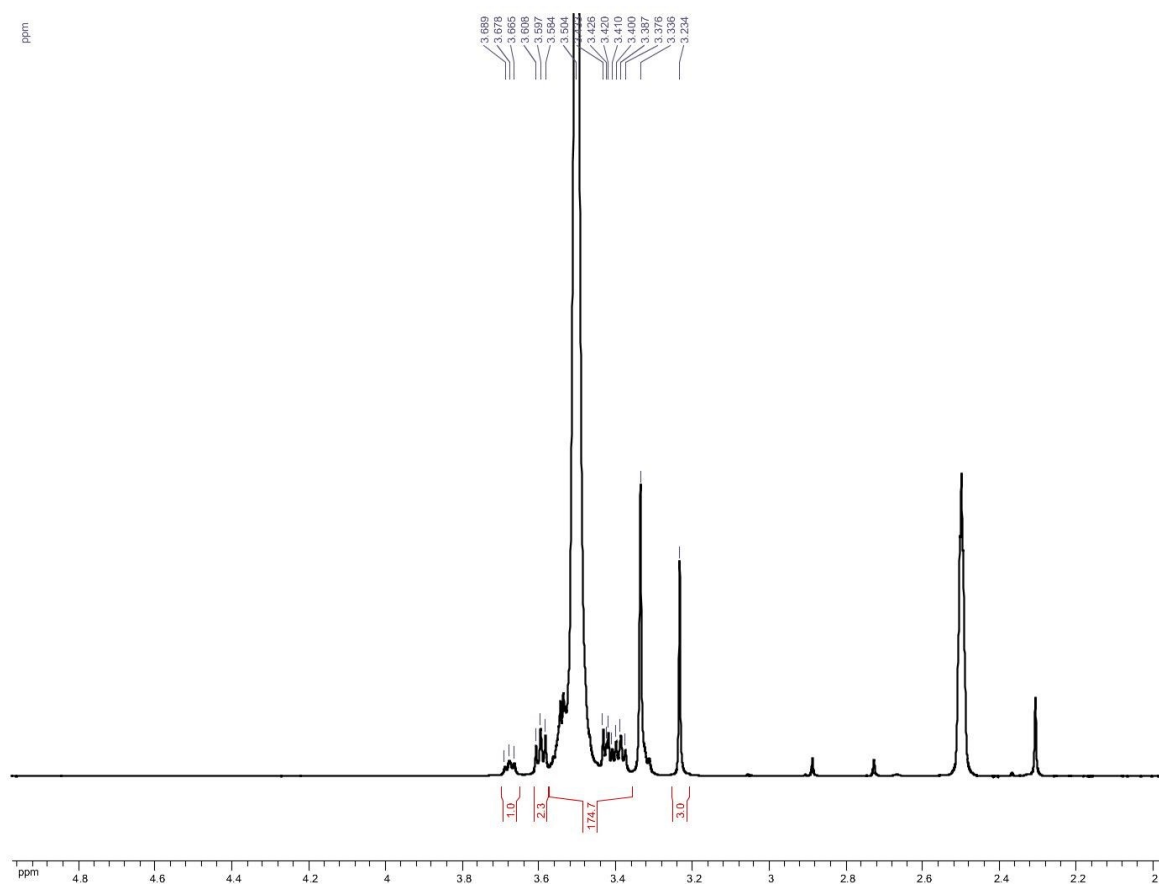


Figure. S5 ^1H -NMR spectrum of $\text{mPEG}_{2\text{K}}\text{-N}_3$: ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$, δ , ppm): 3.69-3.67 (*m*, 2H), 3.60-3.58 (*m*, 2H), 3.55-3.38 (*m*, $\sim n \times 4\text{H}$), 3.23 (*s*, 3H)

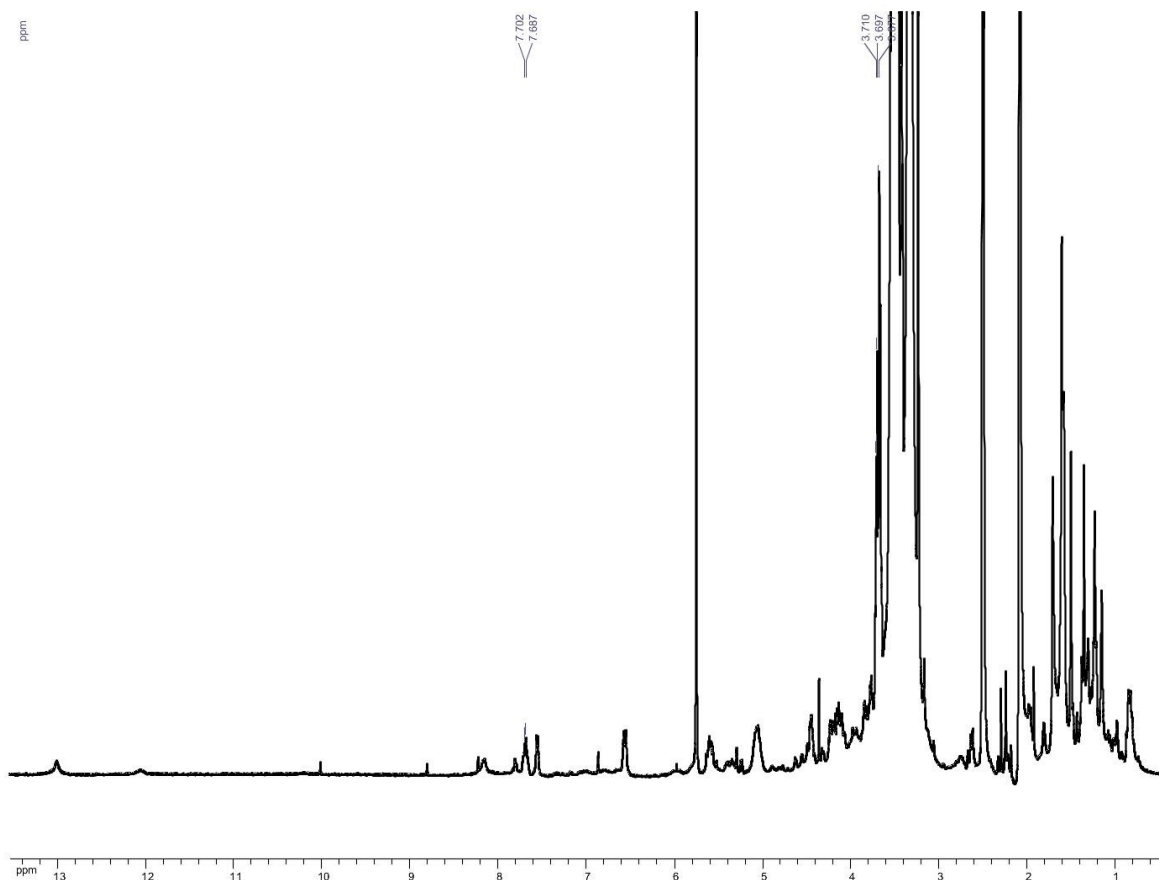


Figure. S6 ^1H -NMR spectrum of mPEG_{2K}-GA. Notable chemical shifts: ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$, δ , ppm): 7.70-7.69 (*m*, 1H), 3.71-3.68 (*m*, 2H)

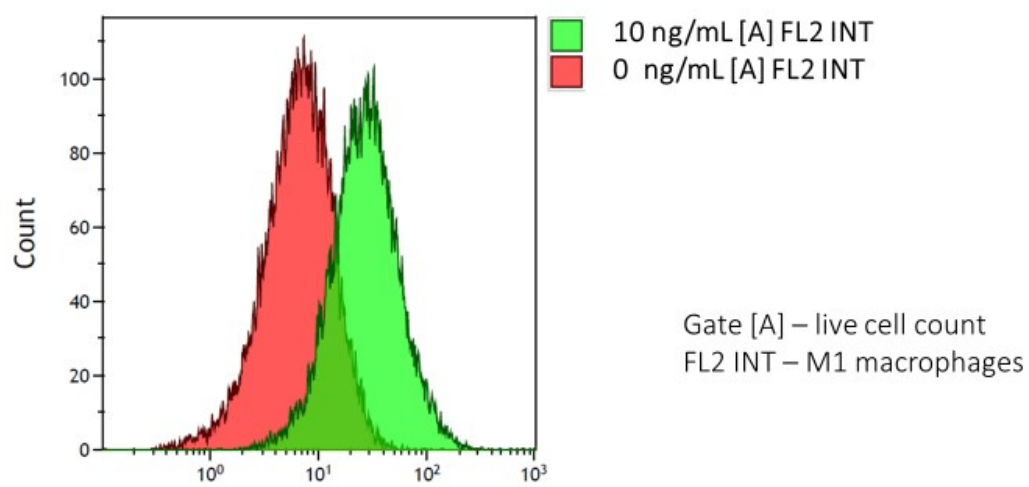


Figure. S7 CD86 (M1 marker expression). Green: vehicle control; red: 10 ng/mL LPS

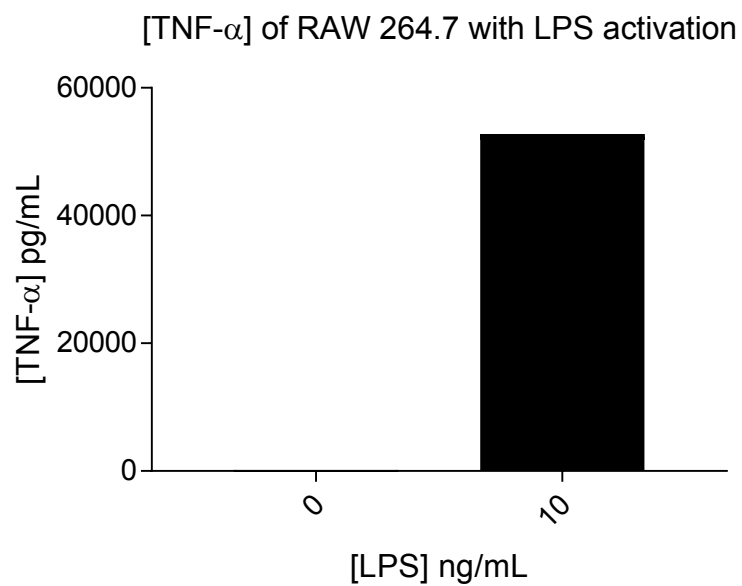
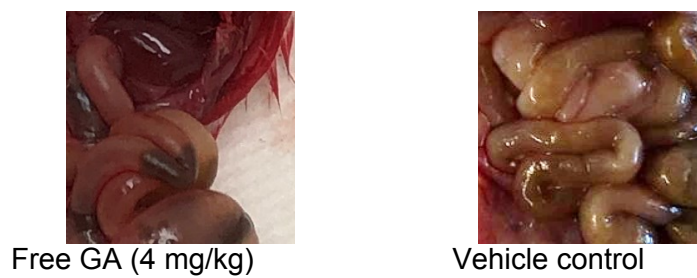


Figure. S8 ELISA study of TNF- α secretion from RAW 264.7 cells treated with 10 ng/mL of LPS.

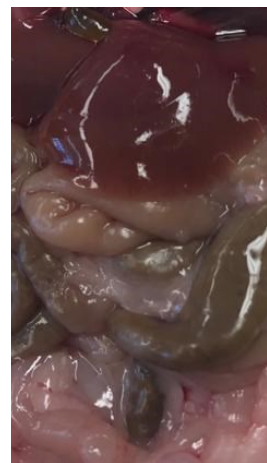




NP-2000 (4 mg GA/kg)



NP-2000 (8 mg GA/kg)



NP-2000 (20 mg GA/kg)

Figure. S9 Peritoneum of mouse treated with free GA, vehicle control, NP-2000. Irritation indicated by an arrow in mouse treated with free GA.