Electronic Supplementary Material

Hydroxyapatite nanoparticles drive the potency of Toll-like receptor 9 agonist for amplified innate and adaptive immune response

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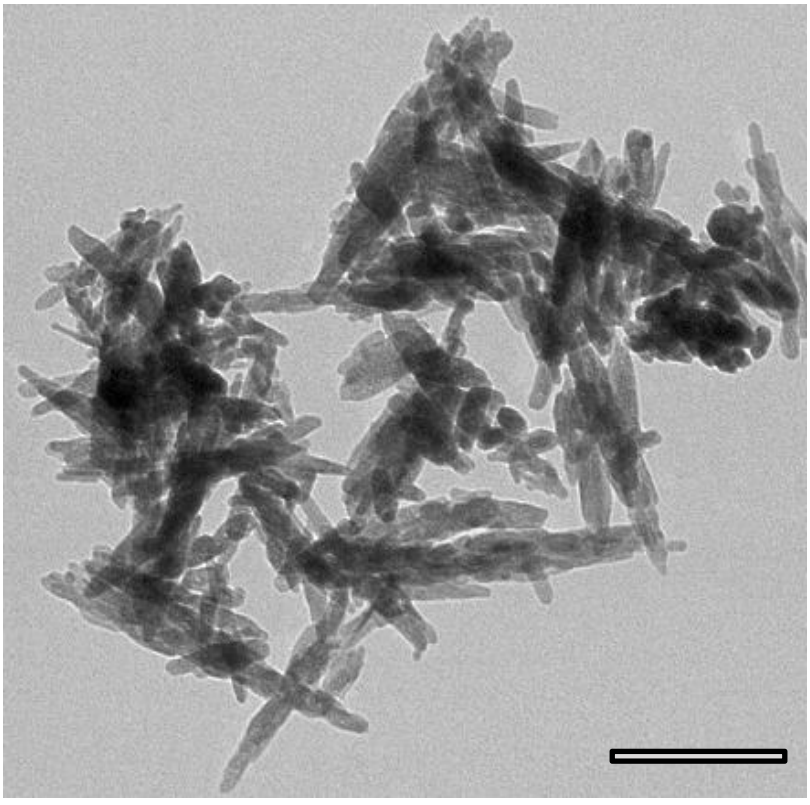

Figure S1 The TEM (HT7800, HITACHI) image of HANPs after sterilization at 200 °C for 2 h. scan bar = 200 nm.

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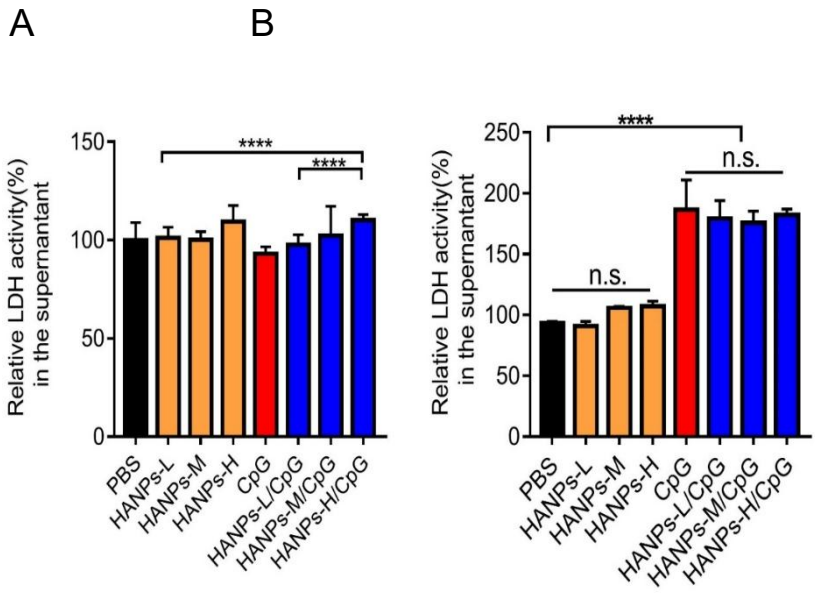

Figure S2 Relative LDH activity in the cell culture supernatant after macrophages **were** treated with PBS, HANPs at varying concentrations (50, 250 and 1000 μg/mL), CpG alone (0.5 μg/mL) or HANPs and CpG co-stimulation for 24 h (**A**) or 72 h (**B**). One-way ANOVA with Tukey's multiple comparisons test was performed for statistical analysis with ** ($p<0.01$), *** ($p<0.001$), **** ($p<0.0001$). n.s. indicated no significance.

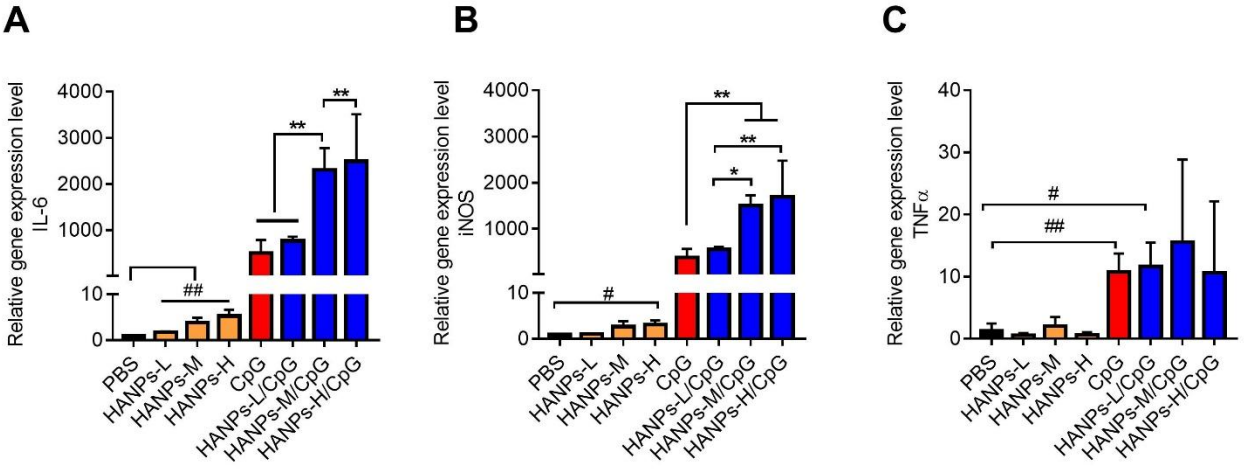

Figure S3 qRT-PCR analysis for mRNA expressing TNF-α (**A**), IL-6 (**B**) and INOS (**C**) by macrophage after the cells were treated with PBS, CpG ODN alone (0.5 μg/mL), HANPs at varying concentration (50, 250, and 1000 μg/mL), or co-stimulators of CpG ODN and HANPs for 24 h. One-way ANOVA with Tukey's multiple comparisons test was performed for statistical analysis with * ($p<0.05$), ** ($p<0.01$). Student's t-test was used to test compare two groups with $^{\#}$($p<0.05$), $^{\#\#}$ ($p<0.01$).

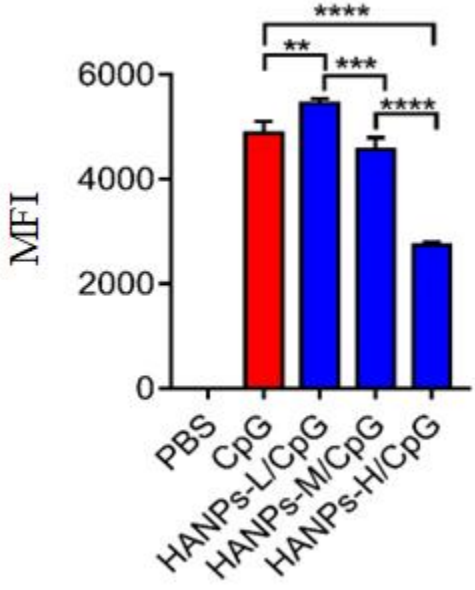

Figure S4 Mean fluorescence intensity (MFI) of Cy5-CpG in macrophage following the cells received treatments of PBS, CpG (0.5 μg/mL) in the presence or absence of HANPs at varying concentrations (50, 250, 1000 μg/mL). One-way ANOVA with Tukey's multiple comparisons test was performed for statistical analysis with ** ($p<0.01$), *** ($p<0.001$), **** ($p<0.0001$).

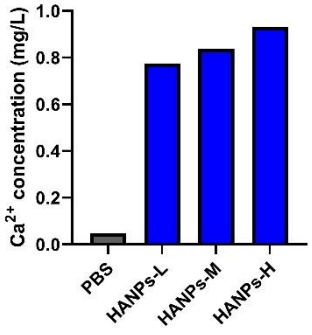

Figure S5 The release of Ca^{2+} after HANPs with varying concentration were incubated in PBS (pH 7.4) for 24 hours.

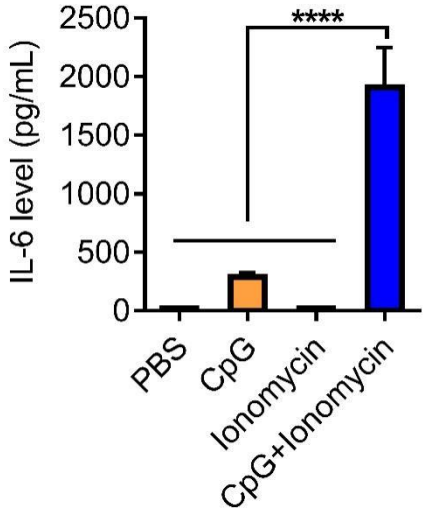

Figure S6 The level of IL-6 secreted by RAW 264.7 exposed to PBS, CpG (0.5 μg/mL), Ionomycin (1 μM) and co-stimulation of CpG and Ionomycin for 48 h. One-way ANOVA with Tukey's multiple comparisons test was performed for statistical analysis with **** ($p<0.0001$).

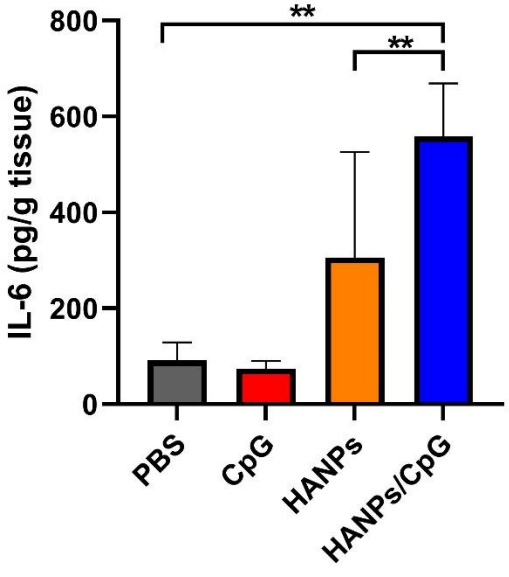

Figure S7 The level of IL-6 in the injection tissue. C57BL/6 mice were injected subcutaneously with PBS, CpG (5 μg), HANPs (5 mg), or a mixture of HANPs and CpG. 7 days later, the injection site was excised and homogenized, and IL-6 was determined by ELISA.

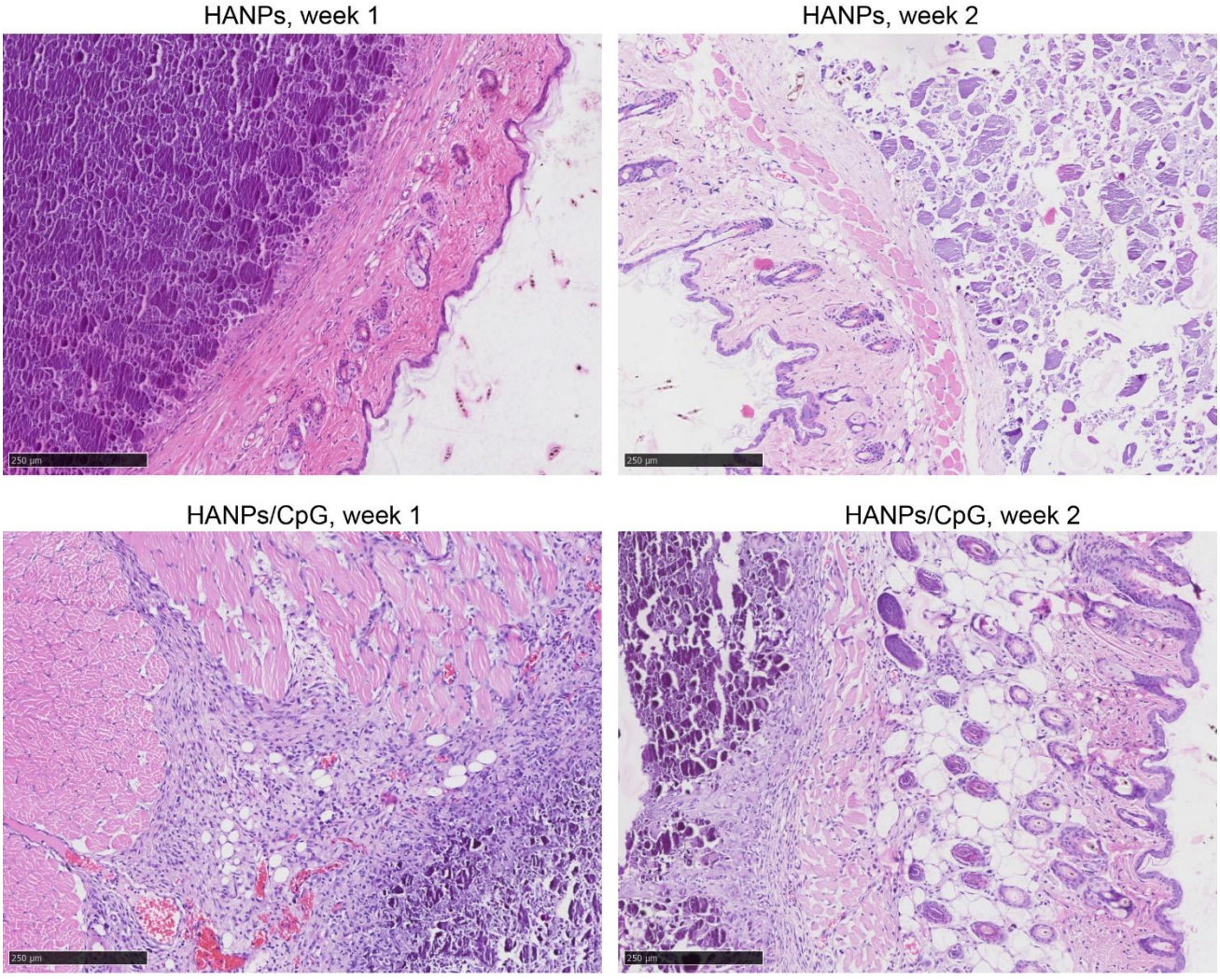

Figure S8 H&E staining of the surrounding tissues together with the biomaterials after HANPs or HANPs/CpG were subcutaneously injected into the mice for 1 and 2 weeks. Scale bar = 250 μm.

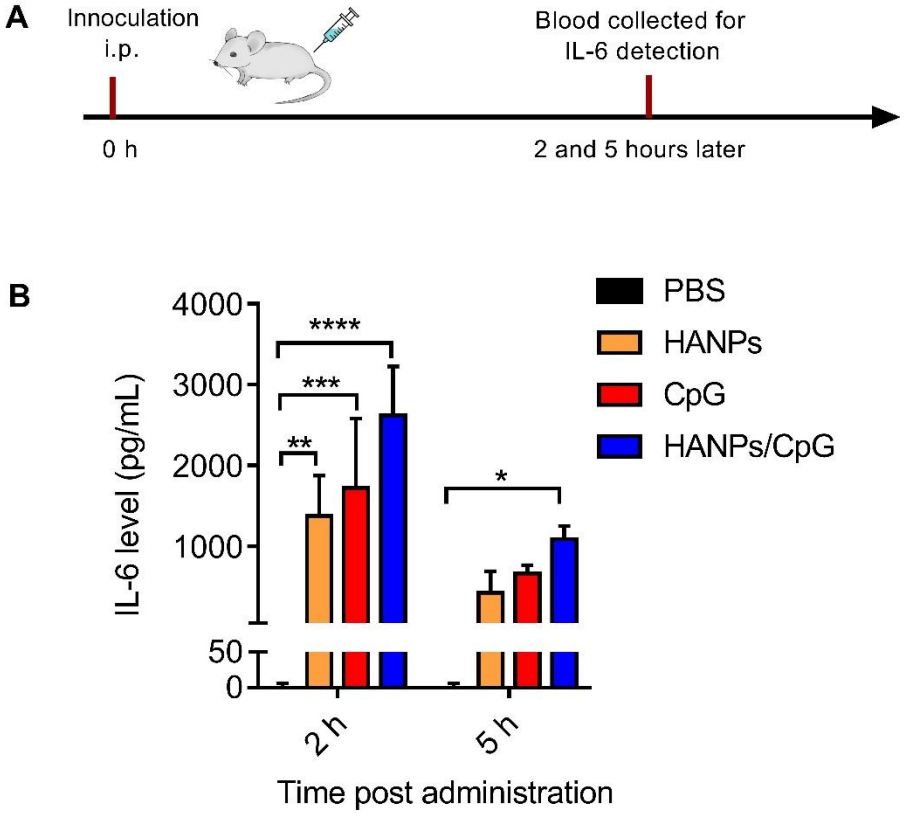

Figure S9 The schematic of administration and serum collection. Serum IL-6 level of mice following intraperitoneal (i.p.) treatment with PBS, HANPs, CpG alone or HANPs and CpG co-stimulation for 2 and 5 h (B). One-way ANOVA with Tukey's multiple comparisons test was performed for statistical analysis with ** ($p<0.01$), *** ($p<0.001$), **** ($p<0.0001$).

Table 1 qPCR primer sequences for mRNA encoding INOS, TNFα and IL-6

Gene	Primer sequences
TNFα	F: 5'-TGGGAGTAGACAAGGTACAACCC -3'
	R: 5'-CATCTTCTCAAAATTCGAGTGACAA-3'
IL-6	F: 5'-GAGGATACCACTCCCAACAGACC-3'
	R: 5'-AAGTGCATCATCGTTGTTCATACA-3'
INOS	F: 5'-GTGACGGCAAACATGACTT-3'
	R: 5'-TCGATGCACAACTGGGT-3'