Supporting Information for

Gold Nanoparticles as a Vaccine Platform: Influence of Size and Shape on Immunological Responses *In Vitro* and *In Vivo*

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UV-vis spectra of AuNPs

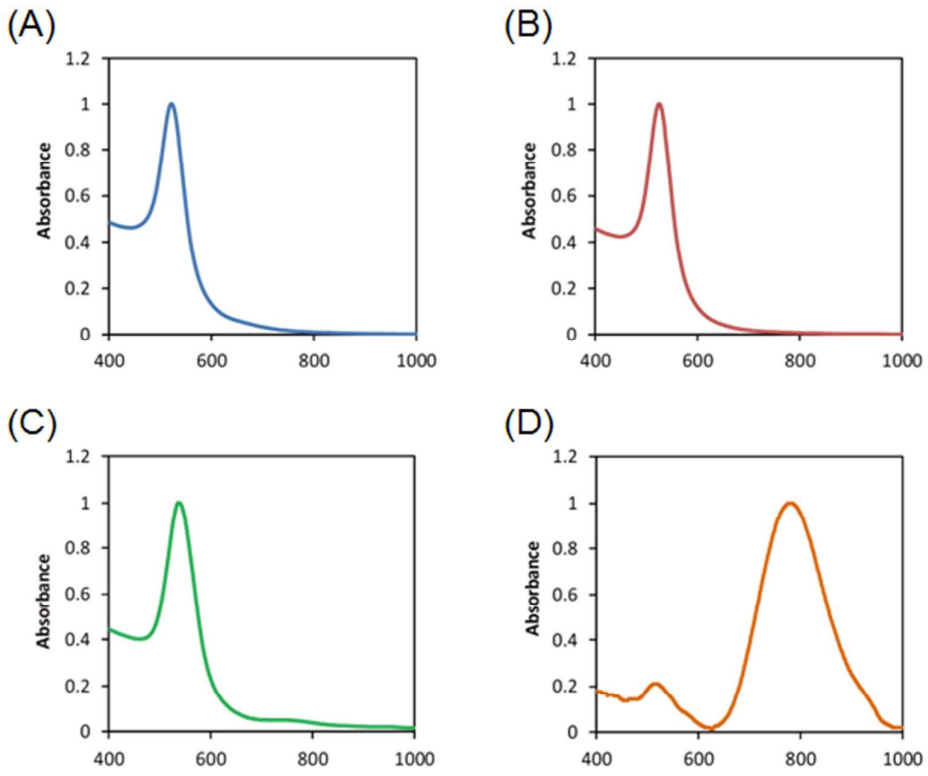

Figure S1. Absorption spectra of (A) Sphere20, (B) Sphere40, (C) Cube, and (D) Rod

Protein modification of AuNPs

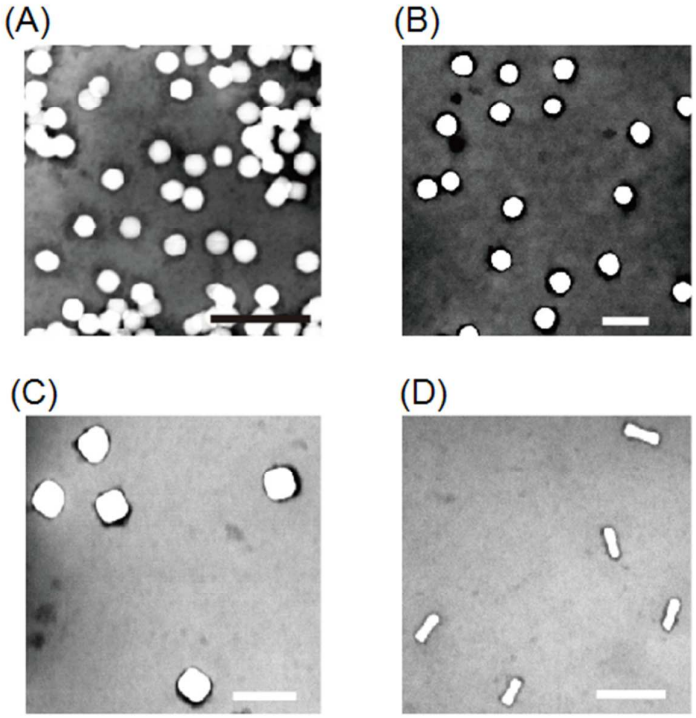

Figure S2. Dark-field TEM images of (A) Sphere20-E, (B) Sphere40-E, (C) Cube-E, and (D) Rod-E NPs. The samples were observed after negative staining with 2% phosphotungstic acid. Scale bar represents 100 nm.

Cytotoxity of AuNP-Es to RAW264.7 cells

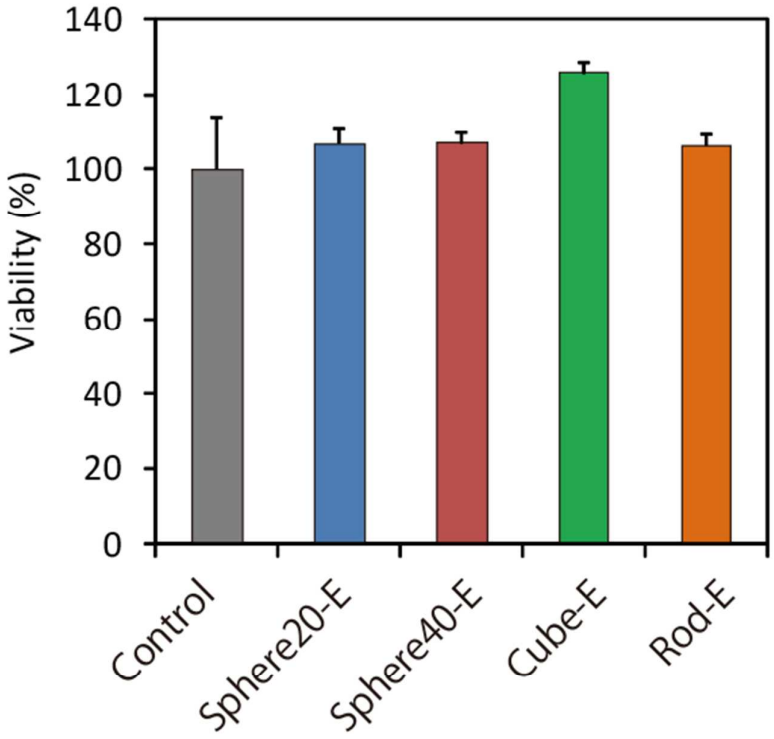

Figure S3. Cytotoxicity of AuNP-E to RAW264.7 macrophage cells treated with 5 x 10^{10} NPs/mL AuNP-Es for 1.5 hr.

Fluorescence microscopy of RAW264.7 cells treated with AuNP-Es

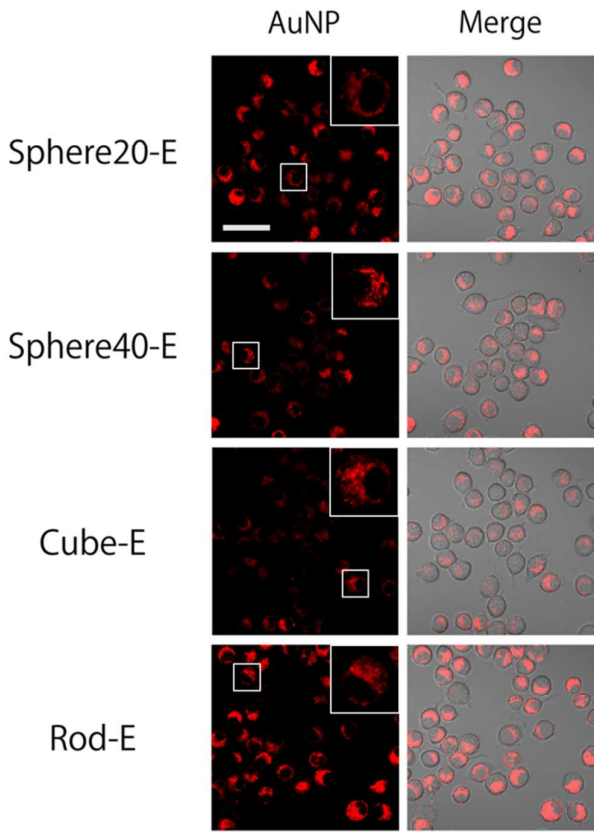

Figure S4. CLMS images of RAW264.7 macrophage cells treated with 5 x 10^{10} NPs/mL AuNP-Es conjugated with Alexa Fluor 647 for 1.5 hr. Scale bar represents 40 μm.

Effect of protein coating on cellular uptake by RAW264.7 cells

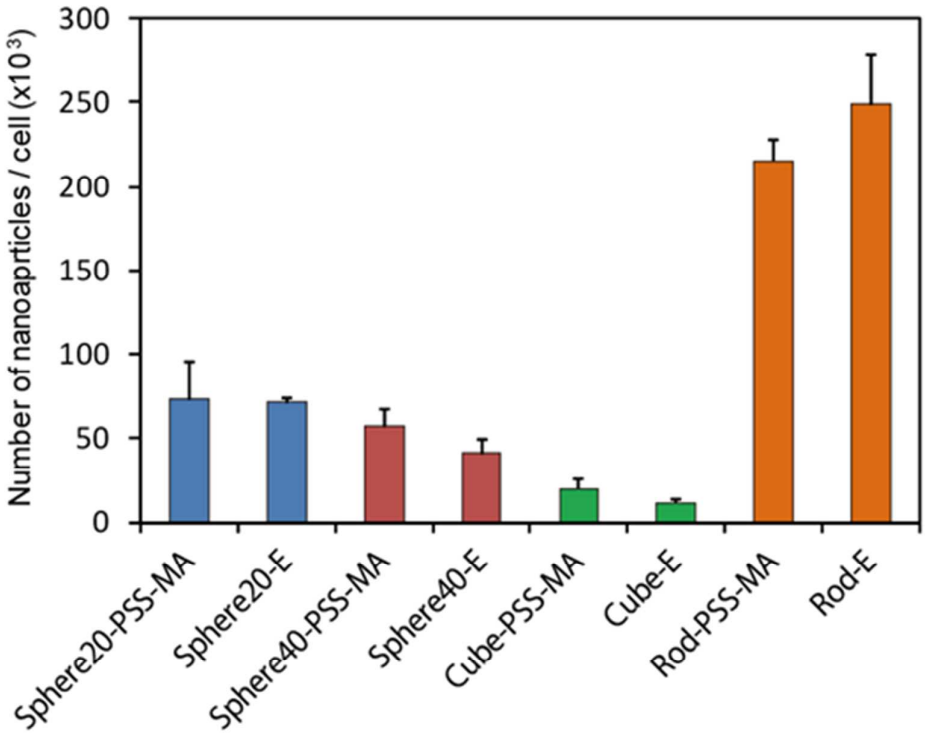

Figure S5. Uptake of AuNPs by RAW264.7 cells treated with 5 x 10^{10} NPs/mL AuNPs coated with or without WNVE protein (AuNP-E and AuNP-PSS-MA, respectively) for 1.5 hr (means ± SEM, n = 3).

Colocalization and lysosomal escape of AuNP-Es

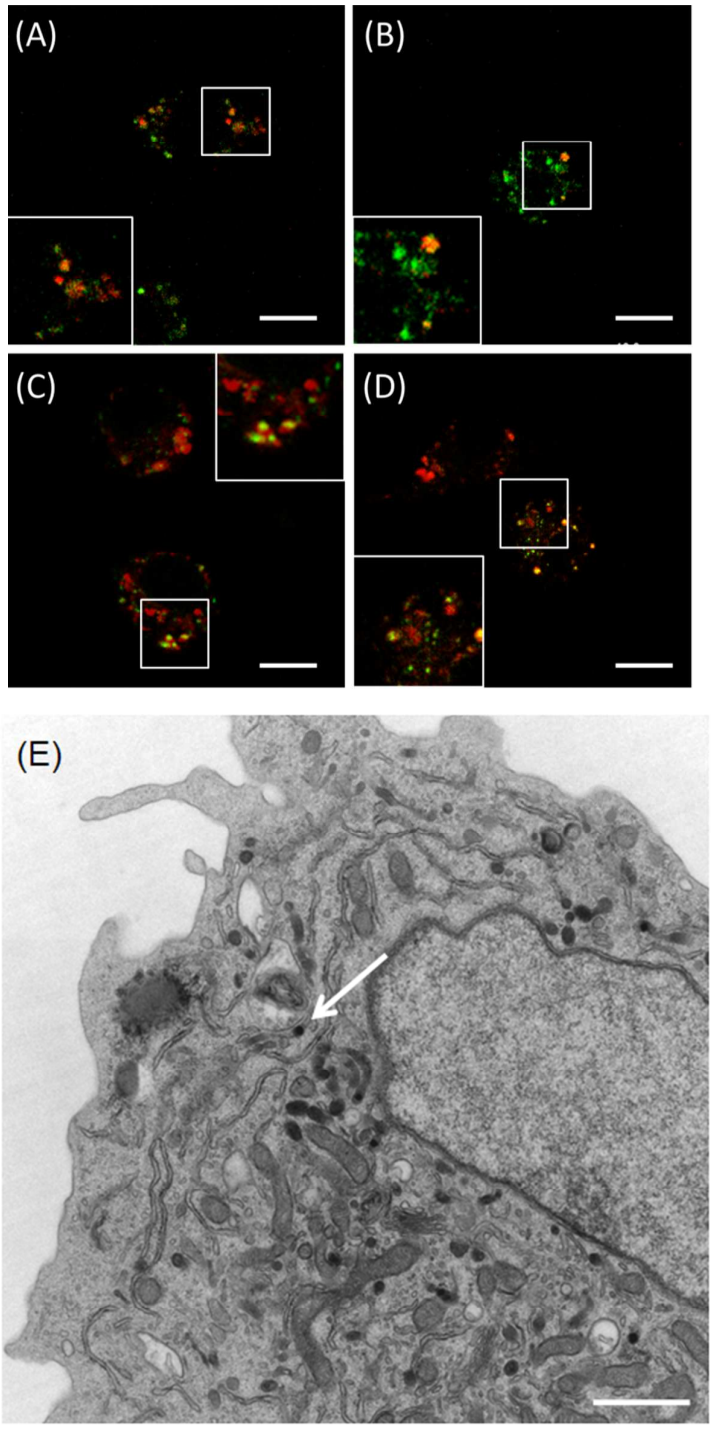

Figure S6. CLMS images of RAW264.7 macrophage cells treated with 2 μg/mL (A) Sphere20-E, (B) Sphere40-E, (C) Cube-E, and (D) Rod-E NPs conjugated with Alexa Fluor 647 for 2 hr (red). After AuNP-E treatment, lysosomes were stained with 50 nM Lysotracker Blue DND-22 for 30 min (green). Scale bar represents 10 μm. TEM images of whole cells treated with (E) Cube-Es. The white arrow indicates a nanoparticle distributed in the cytosol. Scale bar represents 1μm.

Fluorescence microscopy of BMDCs treated with AuNP-Es

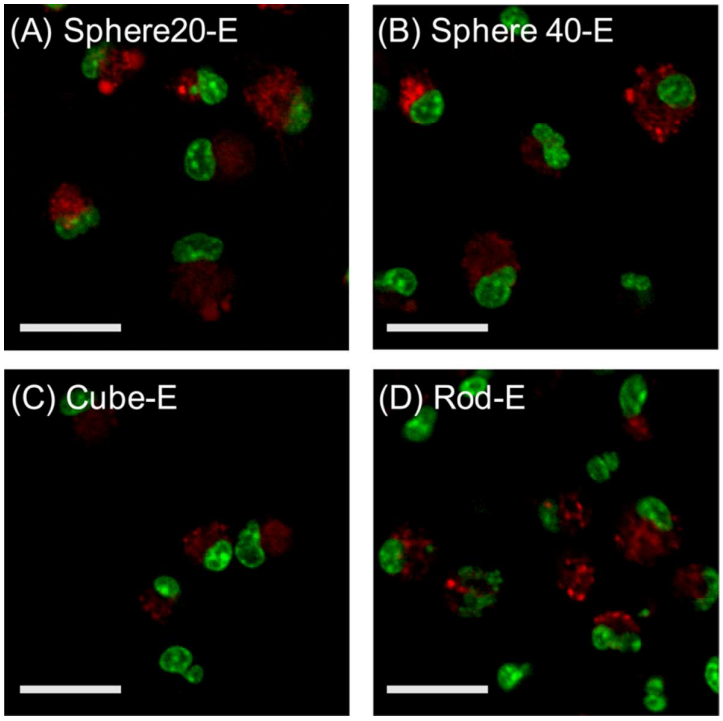

Figure S7 CLMS images of BMDCs treated with 10 μg/mL (A) Sphere20-E, (B) Sphere40-E, (C) Cube-E, and (D) Rod-E NPs conjugated with Alexa Fluor 647 for 24 hr. Scale bar represents 20 μm.

Cellular uptake of AuNP-Es by BMDCs determined by ICP-AES.

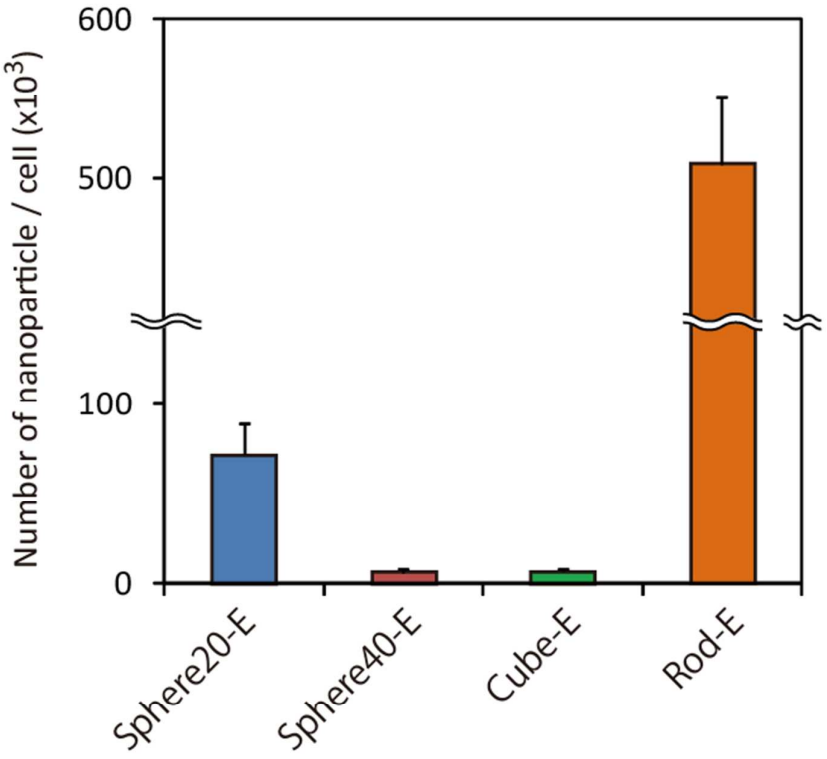

Figure S8. Uptake of AuNP-Es by BMDCs treated with 10 μg/mL AuNP-Es for 24 hr (means ± SEM, n = 3).

Table S1. Physicochemical parameters of AuNP-E

	ζ-potential (mV)	Surface area (nm^2)	Number of protein /particle	Specific surface area (nm^{-1})
Sphere20-E	-24 ± 3	1100	9.7 ± 3	0.32
Sphere40-E	-23 ± 1	5800	74 ± 4	0.14
Cube-E	-11 ± 0.5	10100	52 ± 6	0.15
Rod-E	-9.9 ± 0.3	1300	46 ± 2	0.46