Supporting Information

Enhanced Immune Adjuvant Activity of Aluminum Oxyhydroxide Nanorods through Cationic Surface Functionalization

Bingbing Sun^{a,b,*}, Zhaoxia Ji^c, Yu-Pei Liao^b, Chong Hyun Chang^c, Xiang Wang^b, Justine Ku^d, Changying Xue^e, Vahid Mirshafiee^b, and Tian Xia^{b,*}

^aState Key Laboratory of Fine Chemicals, School of Chemical Engineering, Dalian University of Technology, 2 Linggong Road, 116024, Dalian, China

^bDivision of NanoMedicine, Department of Medicine, University of California, Los Angeles, CA 90095, United States;

^cCalifornia NanoSystems Institute, University of California, Los Angeles, CA 90095, United States;

^dDepartment of Ecology and Evolutionary Biology, University of California, Los Angeles, CA 90095, United States:

^eSchool of Life Science and Biotechnology, Dalian University of Technology, 2 Linggong Road, 116024, Dalian, China;

^{*}Address correspondence to txia@ucla.edu and bingbingsun@dlut.edu.cn.

Figure S1

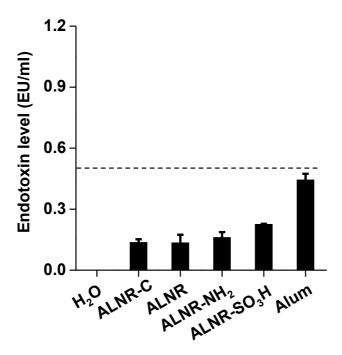


Figure S1. Endotoxin levels of ALNRs. The endotoxin levels in 25 μ g of ALNRs (resuspended at 250 μ g/mL) were determined using a Limulus Amebocyte Lysate assay kit (Lonza).

Figure S2

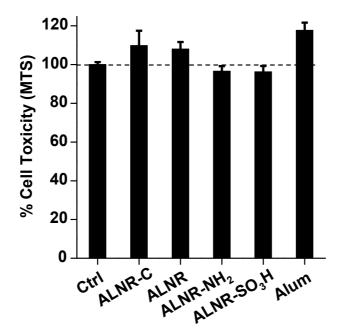


Figure S2. Cytotoxicity of ALNRs. Cell viability of THP-1 cells after exposure to ALNRs was determined using a MTS assay. The cell viability of the nanoparticle-treated cells was normalized according to the value of non-treated control cells, for which the vitality was regarded as 100%.

Figure S3

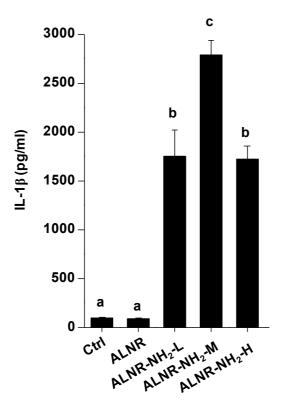


Figure S3. IL-1β production induced by ALNRs with different degree of NH₂ functionalization in THP-1 cells. (A) PMA-differentiated THP-1 cells were exposed to ALNRs with different degree of NH₂ functionalization (L, Low; M, Medium; H, High) at 500 μ g/mL in the presence of LPS (10 ng/mL) for 6 h. IL-1β production was quantified by ELISA. Statistical analysis was performed using Tukey's test. Values that do not share the same letter indicate statistical differences at p<0.01.

Figure S4

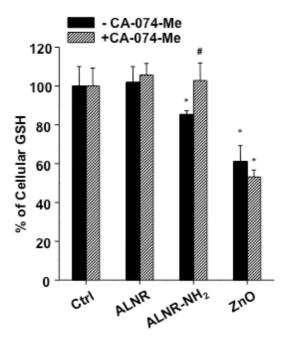


Figure S4. Cathepsin B inhibitor reduced oxidative stress induced by ALNR-NH₂ in THP-1 cells. PMA-differentiated THP-1 cells were pre-treated with 20 μM inhibitor for 30 min, and then exposed to particles in the presence of LPS (10 ng/mL) for 24 h. Intracellular GSH level was determined using a GSH-Glo assay. Zinc oxide (ZnO) (50 μg/mL) was used as a positive control. *p <0.05 compared to THP-1 cells without CA-074-Me treatment; *p <0.05 compared to control.

Figure S5

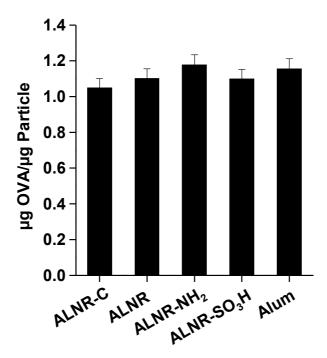


Figure S5. OVA binding efficiency to ALNRs. 100 μ L of ALNRs (100 μ g/mL in PBS) was mixed with 100 μ L of OVA (100 μ g/mL in PBS), and the mixture was incubated for 30 min at room temperature. The particles were centrifuged and the supernatant was collected to analyze the OVA concentration with a BCA protein assay.

Table S1. Peak assignments of FTIR analysis of aluminum oxyhydroxide nanorods with various surface functionalization.

Wavenumber (cm ⁻¹)	Assignments
3300	v _{as} (Al)O-H stretching vibration of OH groups
3095	v _s (Al)O-H stretching vibration of OH groups
1558	Deformation vibration of N-H bond
1539	N-H bending vibration
1413	$v_{as}(SO_2)$ stretching vibration
1335	$v_{as}(SO_2)$ stretching vibration
1156	(v _{as} Al-O-H) OH deformation
1067	(v _s Al-O-H) OH deformation