Electronic Supplementary Material (ESI) for Food & Function.

SUPPORTING INFORMATION

Allium tuberosum-derived nanovesicles with anti-inflammatory properties prevent DSS-induced colitis and modify the gut microbiome

Minkyoung Kang[1], Minji Kang[1], Juyeon Lee[1], Jiseon Yoo[1], Sujeong Lee[1] and Sangnam Oh[1, 2*]

[1]Department of Environmental Science and Biotechnology, Jeonju University, Jeonju 55069, Republic of Korea

[2]Department of Food and Nutrition, Jeonju University, Jeonju 55069, Republic of Korea

*To whom correspondence should be addressed: osangnam@jj.ac.kr

Supplementary Figures.

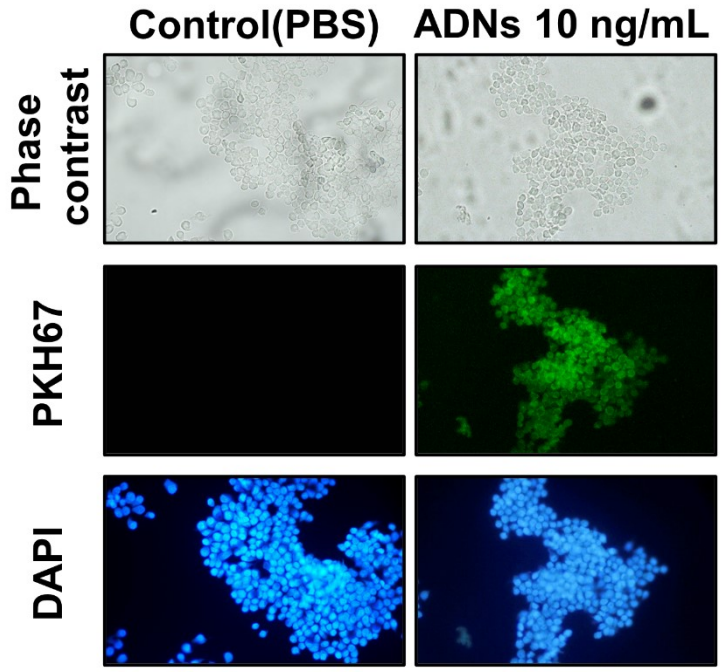

Figure S1. Uptake of *Allium tuberosum*-derived nanovesicles (ADNs) by RAW 264.7 Cells. The experimental setup used to confirm the absorption of ADNs into RAW 264.7 cells. 250 μL of 100 μg/mL ADNs suspended in PBS was diluted 1:1 (v/v) with PKH67 (1 μL PKH67 + 250 μL Diluent C, Sigma-Aldrich, Saint Louis, Mo, #MINI67) and labeled at room temperature for 5 min. The mixture was then diluted with 500 μL of 1% bovine serum albumin (BSA), and 9 mL of PBS was added. Residual unbound PKH67 was removed using a 100 kDa Vivaspin filter, followed by two PBS washes. RAW 264.7 cells (0.65×10^4/mL in 8 well slide, SPL Life Sciences, Pocheon, Republic of Korea, #20018) were treated with the labeled ADNs at a final concentration of 10 ng/mL for 24 h. Cell nuclei were stained with DAPI (Sigma-Aldrich, Saint Louis, Mo, USA; # D9542) to identify and locate the cells.

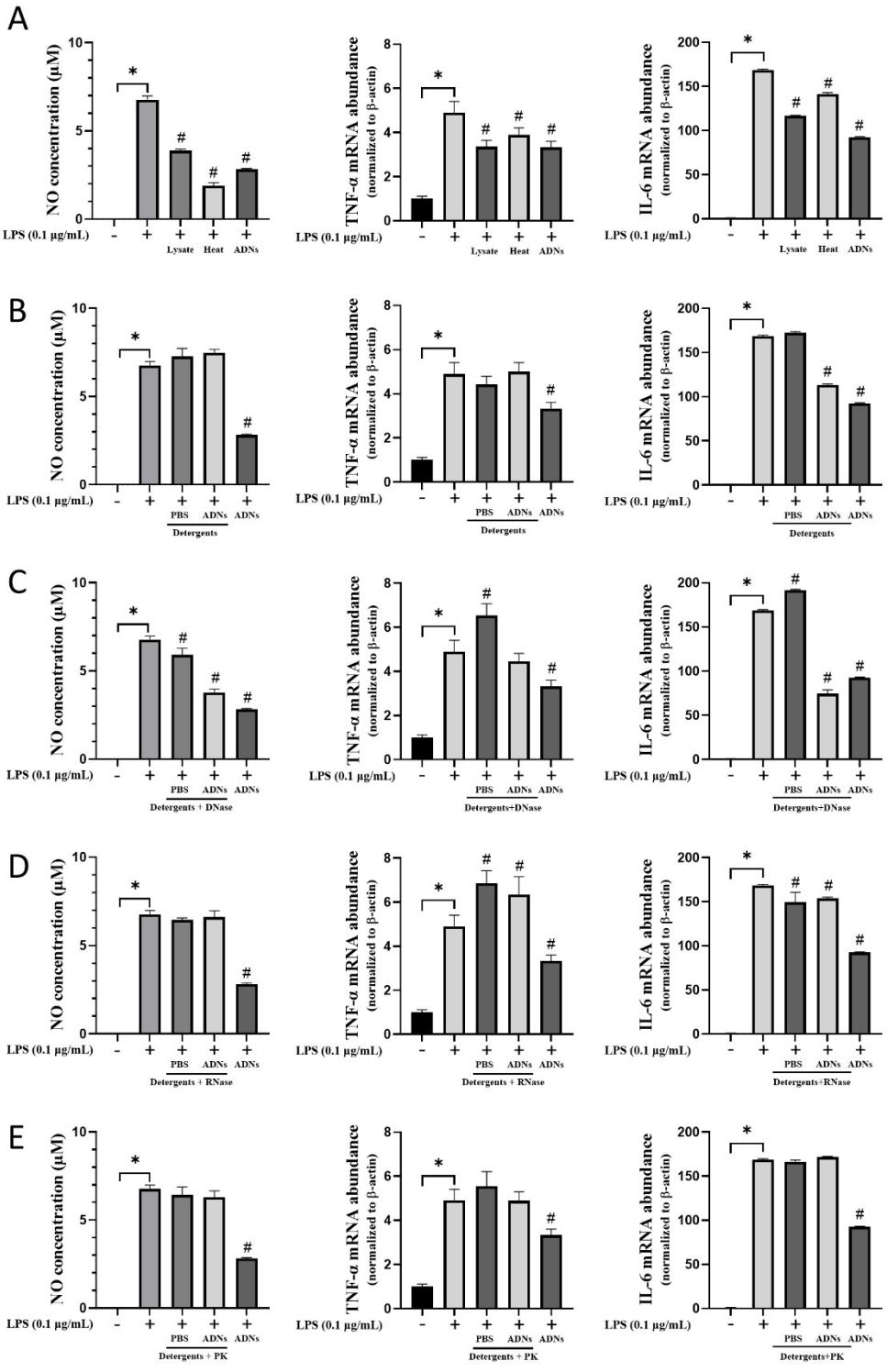

Figure S2. Figure 2. Characterization of *Allium tuberosum-derived* nanovesicles (ADNs) and Their Specific Components. (A) Lysate treatment involved sonication at 200 W for 10 min and heating at 80°C for 30 min to disrupt vesicles. (B) Detergent treatment exposed

ADNs to 0.02% Triton X-100 and 0.1% SDS for 30 min at room temperature to permeabilize the membranes. (C) DNase I treatment (3 μg/mL at 37°C for 30 min) followed by detergent treatment to degrade DNA with inactivation by 10 mM EDTA. (D) RNase A treatment (6 μg/mL at 37°C for 30 min) was used to degrade the RNA, followed by inactivation with 10 mM EDTA. (E) Protease K treatment (100 μg/mL at 37°C for 30 min) degraded proteins, which were inactivated by pH adjustment using 6N HCl. The nitric oxide and mRNA levels of IL-6 and TNF-α were measured after each treatment to determine the anti-inflammatory efficacy and presence of specific components within the ADNs.

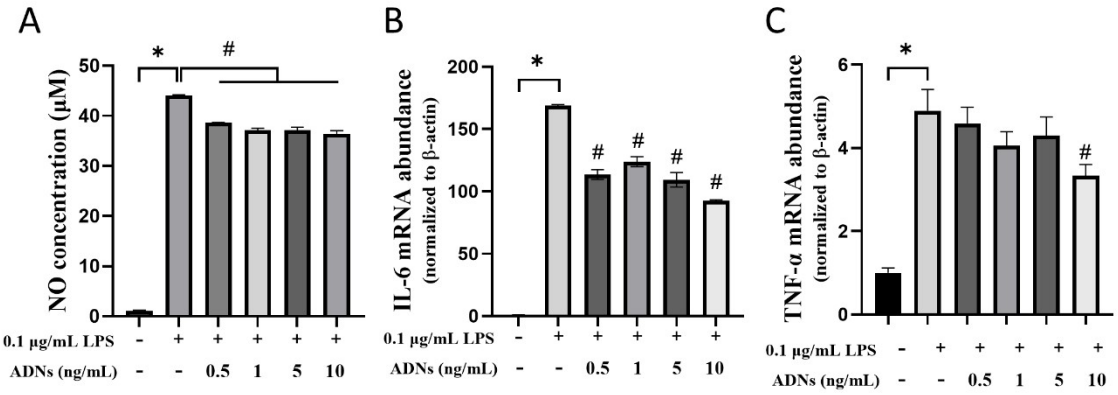

Figure S3. Effect *of Allium tuberosum*-Derived Nanovesicle (ADN) Concentrations on Nitric Oxide Production and Inflammatory mRNA Levels in RAW 264.7 Cells. RAW 264.7 cells (5×10^4/mL in 6 well plate) were pre-treated with 0.5, 1, 5, and 10 ng/mL ADNs for 24 h, followed by a 24 h co-treatment with 0.1 μg/mL LPS. After treatment, the supernatant was collected for the NO assay, and the cells were used for RNA extraction to measure mRNA levels of TNF-α and IL-6. All experiments were performed in triplicate. Statistical significance was indicated as *$p<0.0001$ for control vs. LPS, and #$p<0.01$ for LPS vs. ADNs.

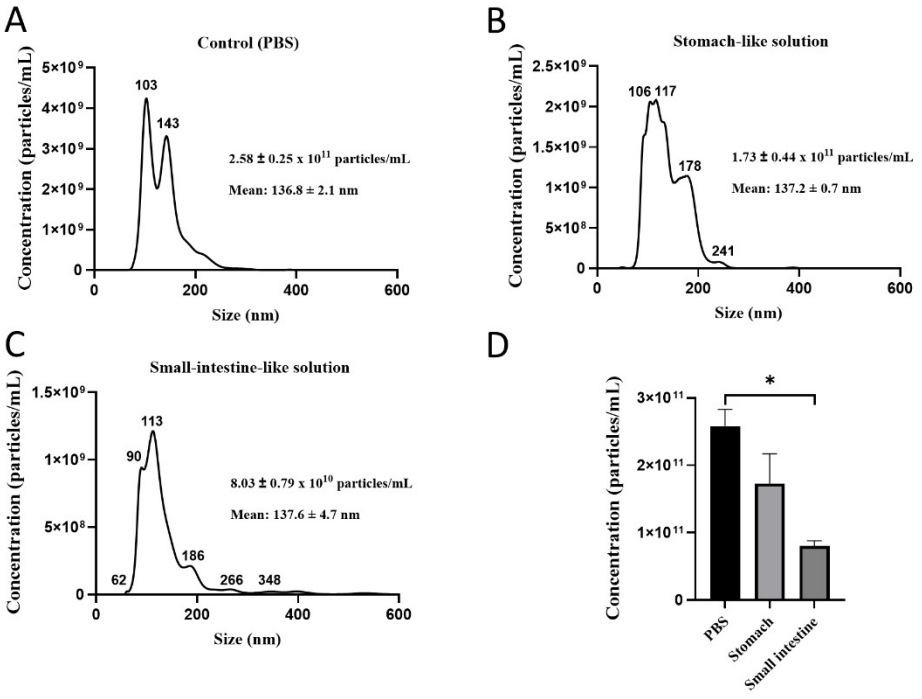

Figure S4. Stability of Allium tuberosum-Derived Nanovesicles (ADNs) in Simulated Gastrointestinal Conditions. To evaluate the stability of ADNs in a harsh gastrointestinal environment, artificial stomach-like and intestine-like solutions were prepared. For the *in vitro* stability test, (A-B) ADNs (1 mg/mL in PBS) were treated with 1.34 μL of 18.5% (w/v) HCl (pH 2.0) and 24 μL of pepsin solution (80 mg/mL in 0.1N HCl, pH 2.0) and then incubated at 37°C for 30 min to simulate gastric conditions. (C) Then 80 μL of a mixture containing 24 mg/mL bile extract and 4 mg/mL pancreatin in 0.1N NaHCO3 was added. The pH was adjusted to 6.5 with 1N NaHCO3 and the solution was incubated for 30 min at 37°C to simulate intestinal conditions. As a control, PBS was used to reach small intestinal-like conditions. The stability of ADNs was assessed by measuring changes in particle size using Nanoparticle Tracking Analysis (NTA), and (D) indicates the change in particle concentration during the digestion process.