Supporting Information

Gold Nanoparticles Coimmobilized with Small Molecule Toll-Like Receptor 7 Ligand and α-Mannose as Adjuvants

Hiroyuki Shinchi[*,†], Toru Yamaguchi[†], Toshiro Moroishi[‡,§,¶], Masaharu Yuki[†], Masahiro Wakao[†], Howard B. Cottam[⁑], Tomoko Hayashi[⁑], Dennis A. Carson[⁑], Yasuo Suda[*,†,#]

[†] Department of Chemistry, Biotechnology and Chemical Engineering, Graduate School of Science and Engineering, Kagoshima University, 1-21-40 Korimoto, Kagoshima 890-0065, Japan

[‡] Department of Molecular Enzymology, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto 860-8556, Japan

[§] Center for Metabolic Regulation of Healthy Aging, Faculty of Life Sciences, Kumamoto University, Kumamoto 860-8556, Japan

[¶] Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency (JST), Kawaguchi 332-0012, Japan

[⁑] Moores Cancer center, University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093-0695, United States

[#] SUDx-Biotec Corporation, 1-42-1 Shiroyama, Kagoshima 890-0013, Japan

E-mail: hshinchi@eng.kagoshima-u.ac.jp, Phone: (+81)99-285-7843 (for Dr. Shinchi)

E-mail: ysuda@eng.kagoshima-u.ac.jp, Phone: (+81)99-285-8369 (for Dr. Suda)

Table S1. Calculation with the reagents needed for the preparation of 1V209-αMan-GNPs

Order	Reagents	μmol	eq.	Volume (μL)	Solvent
1	$HAuCl_4$	2.5	1	1375	H_2O
2	$NaBH_4$	12.5	5	250	H_2O
3	DMSO	-	-	625	-
4	Ligand conjugates [a]	0.375	0.15	250	50% DMSO [b]

[a] The molar ratio of a TLR7 ligand and Manα1-6Glc-mono was 1:9, 1:19 or 1:39. [b] The final volume of DMSO in the reaction mixture was 30%.

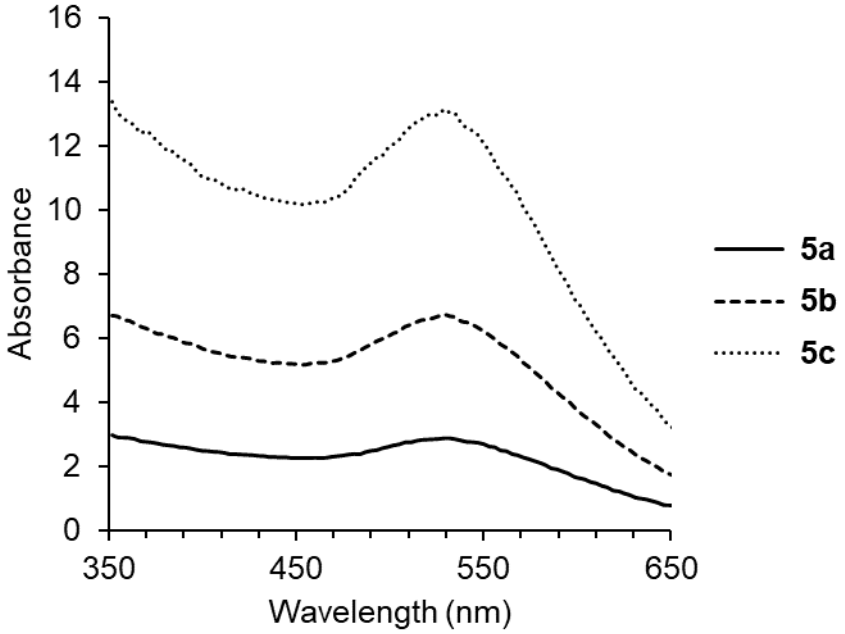

Figure S1. Representative UV-Vis spectrum of 1V209-αMan-GNPs (**5a-c**, based on 1V209 derivative at 8 μM).

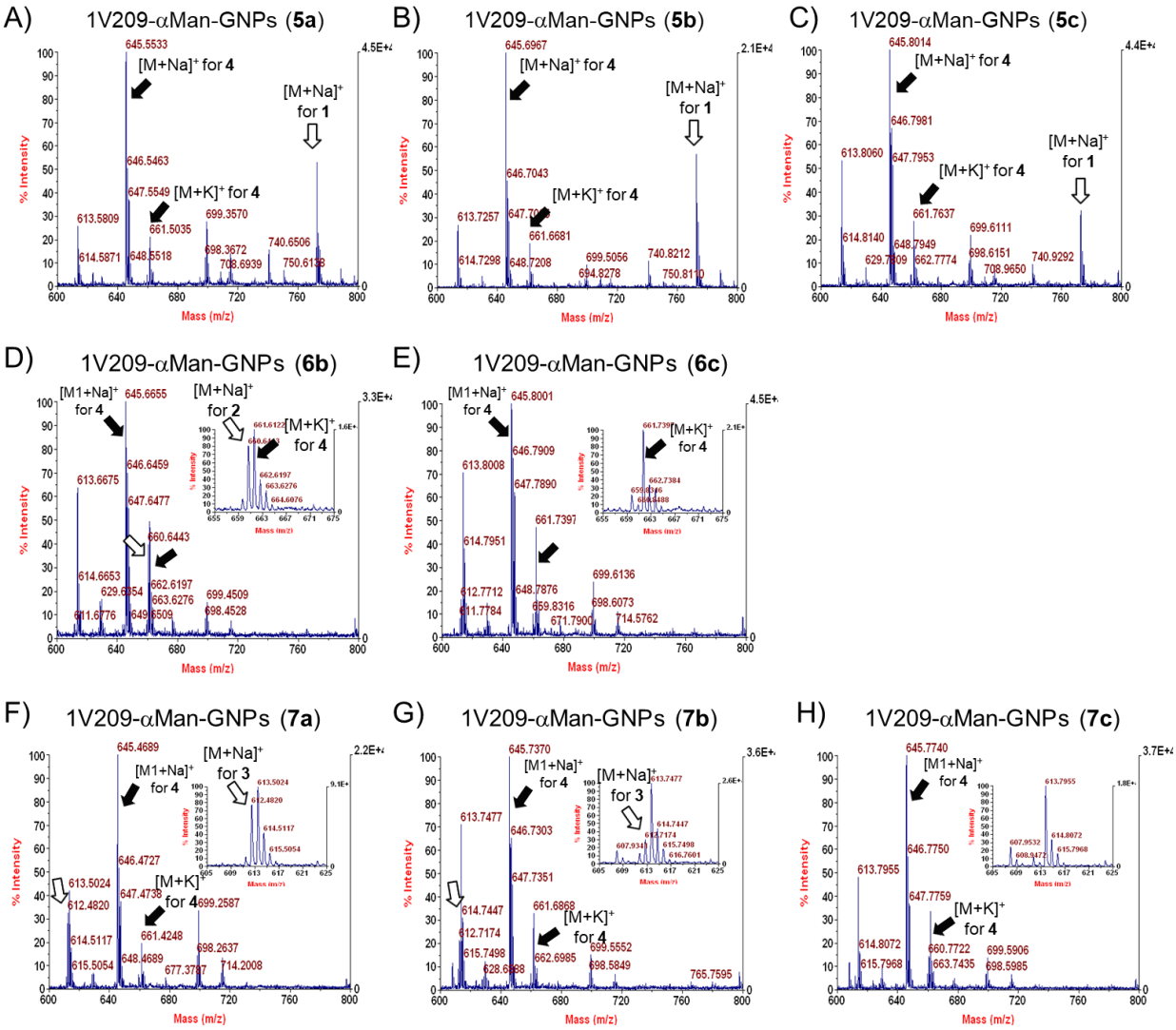

Figure S2. MALDI-TOF MS spectra of (A) **5a**, (B) **5b**, (C) **5c**, (D) **6b**, (E) **6c**, (F) **7a**, (G) **7b** or (H) **7c**. White and Black arrows in the spectrum indicate 1V209 derivative (**1**, **2** or **3**) and Manα1-6Glc-mono **4**, respectively. MALDI-TOF MS spectral were measured by reflector and in positive ion mode.

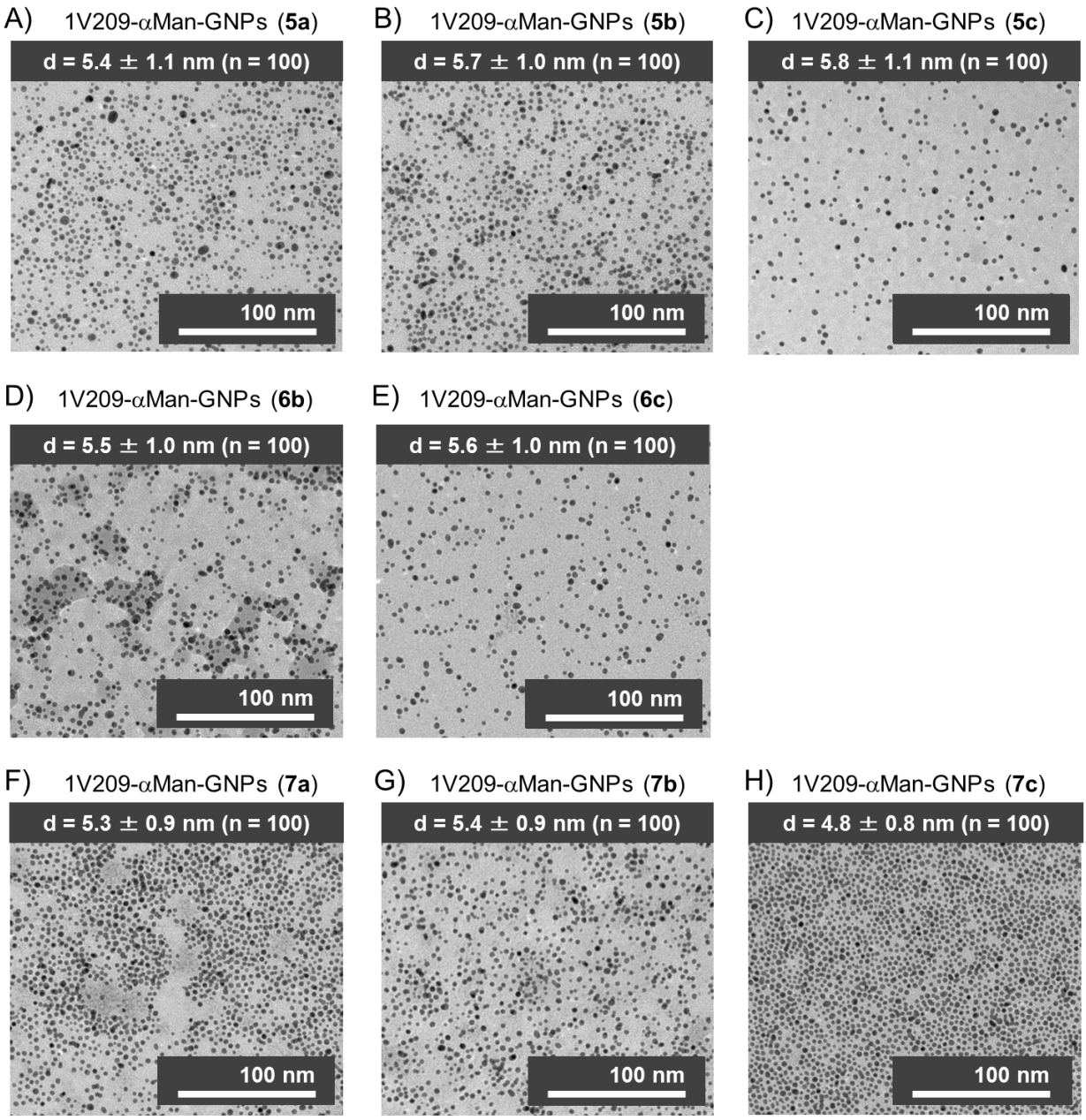

Figure S3. Representative TEM images of (A) **5a**, (B) **5b**, (C) **5c**, (D) **6b**, (E) **6c**, (F) **7a**, G) **7b** or (H) **7c**. The particle sizes were determined using *Image J* and the average diameters were mean of randomly selected one hundred particles.

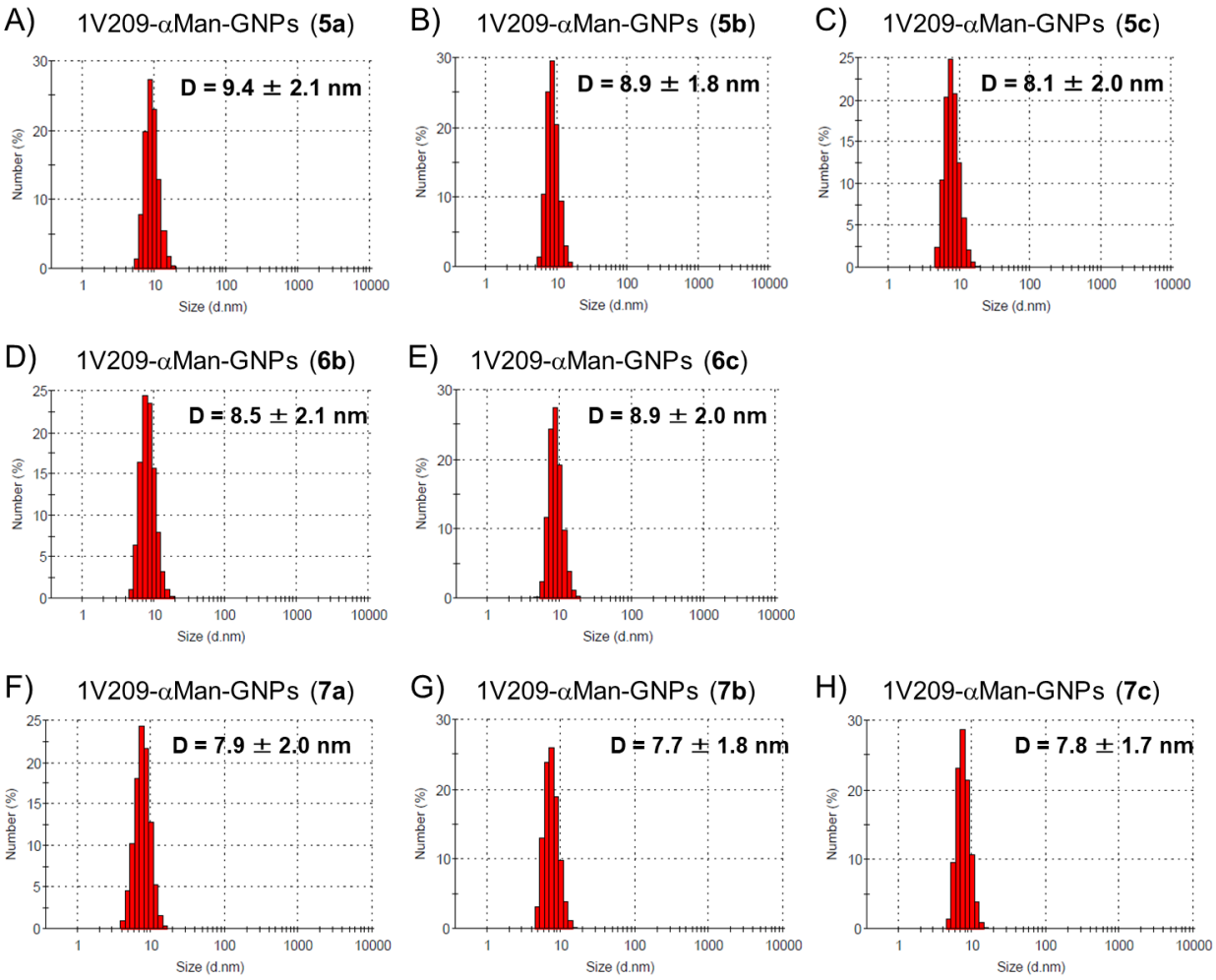

Figure S4. Representative plots for Hydrodynamic diameter of (A) **5a**, (B) **5b**, (C) **5c**, (D) **6b**, (E) **6c**, (F) **7a**, (G) **7b** or (H) **7c** measured by DLS.

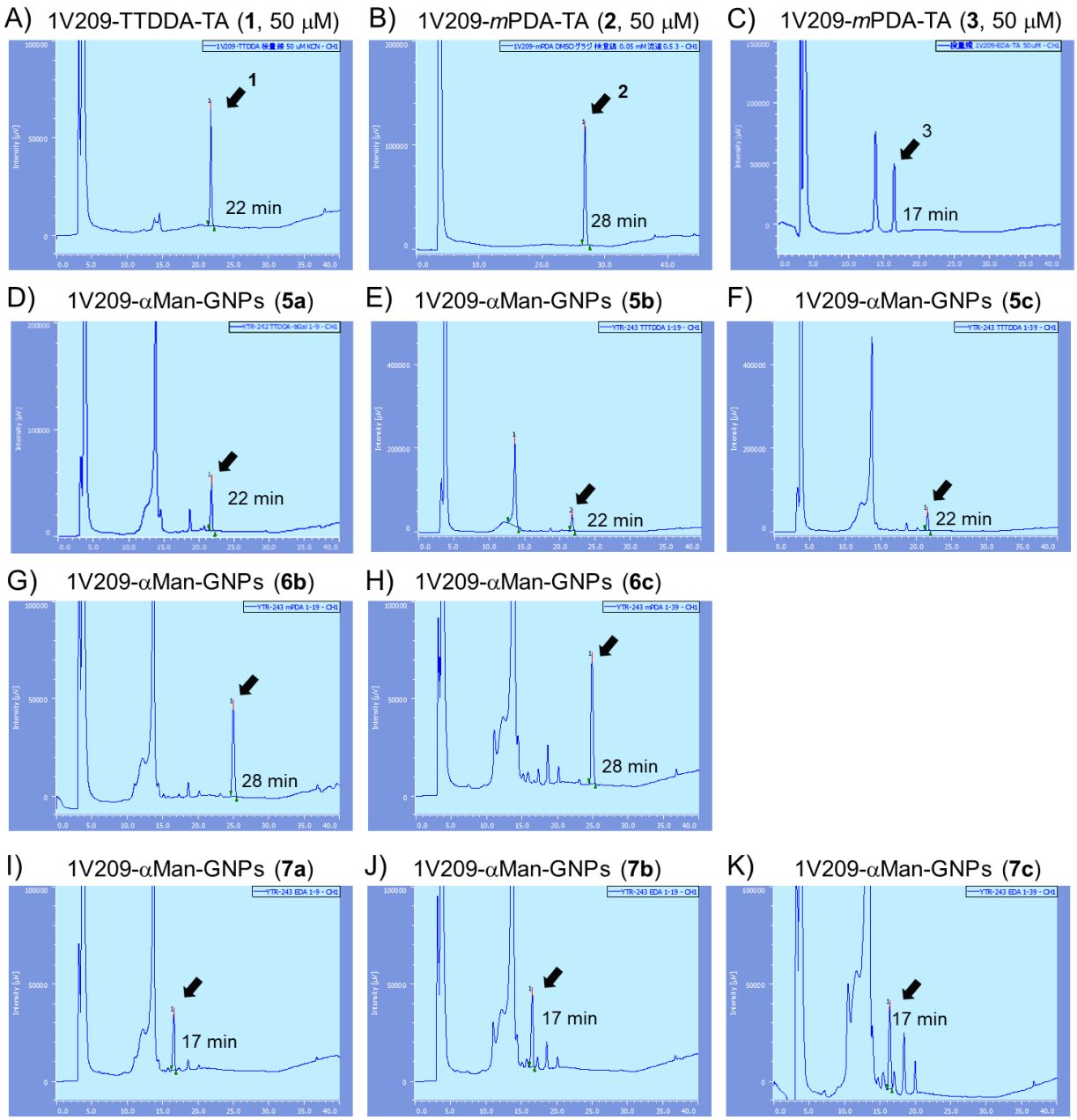

Figure S5. HPLC spectra of (A) 50 μM 1V209-TTDDA-TA, (B) 1V209-*m*PDA-TA, (C) 1V209-EA-TA, (D) **1a**, (E) **1b**, (F) **1c**, (G) **2b**, (H) **2c**, (I) **3a**, (J) **3b** or (K) **3c** after treatment with potassium cyanide. HPLC analysis was performed using Inertsil® ODS-3 (Particle size: ϕ4.6 mm, column length: 150 mm) by monitoring the absorbance at 230 nm (flow rate: 0.5 mL/min, eluent: 0-5 min;50% methanol aq., 5-15 min; 50% methanol aq. to 70% methanol aq., 15-25 min; 70% methanol aq., 25-35 min; 70% methanol aq. to methanol, 35-40 min; methanol).

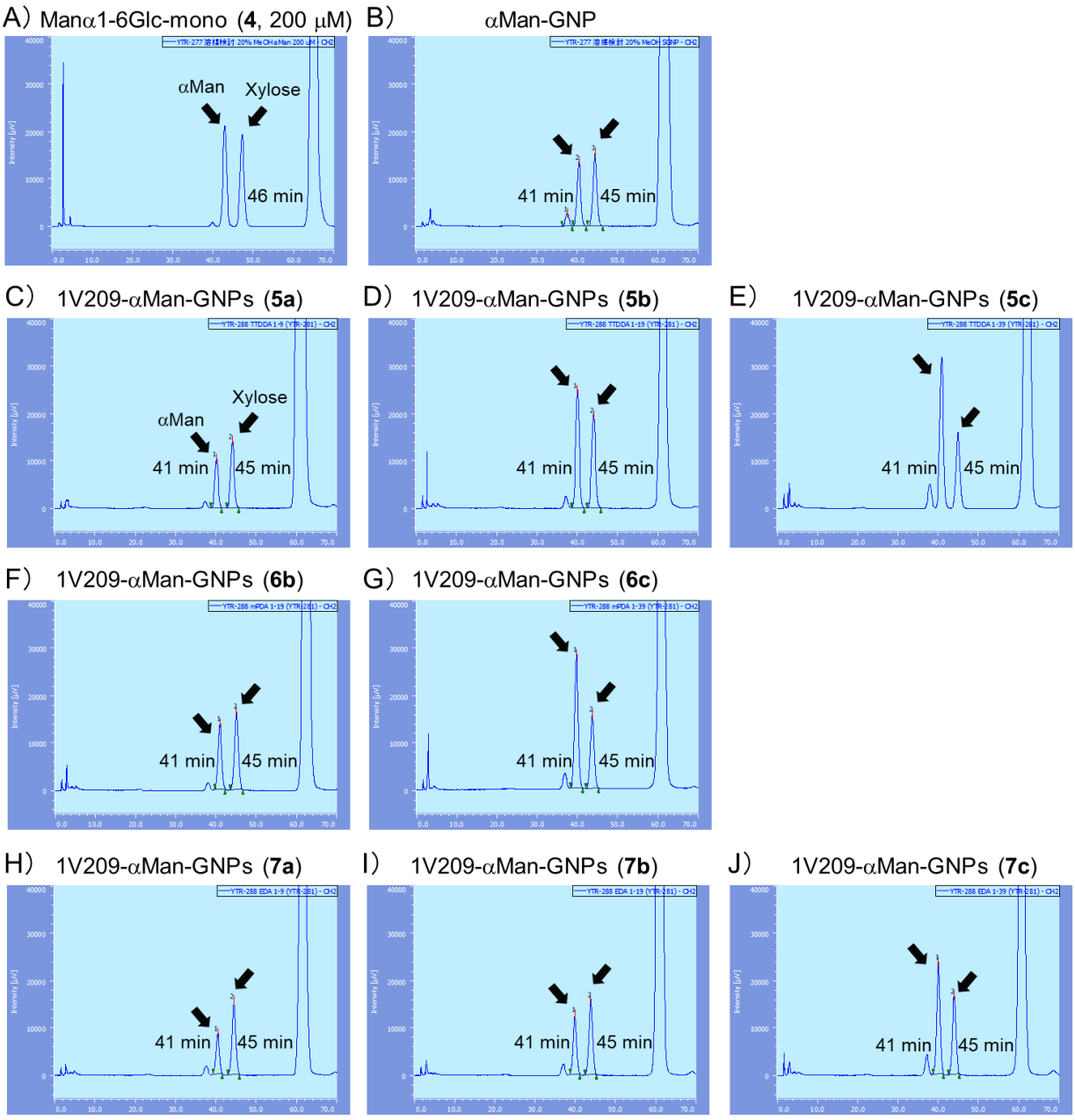

Figure S6. HPLC spectra of α-mannose labeled with *p*-aminobenzoic acid ethyl ester (ABEE) after hydrolysis of Manα1-6Glc-mono on (B) αMan-GNPs, (C) **1a**, (D) **1b**, (E) **1c**, (F) **2b**, (G) **2c**, (H) **3a**, (I) **3b** or (J) **3c** using trifluoroacetic acid. Xylose (100 μg/mL) was added for the internal standard. HPLC analysis was performed using Inertsil® ODS-3 (Ex.: 305 nm, Em.: 360 nm, Particle size: ϕ4.6 mm, column length: 150 mm, flow rate: 1.0 ml/min, eluent: 20% methanol aq.).

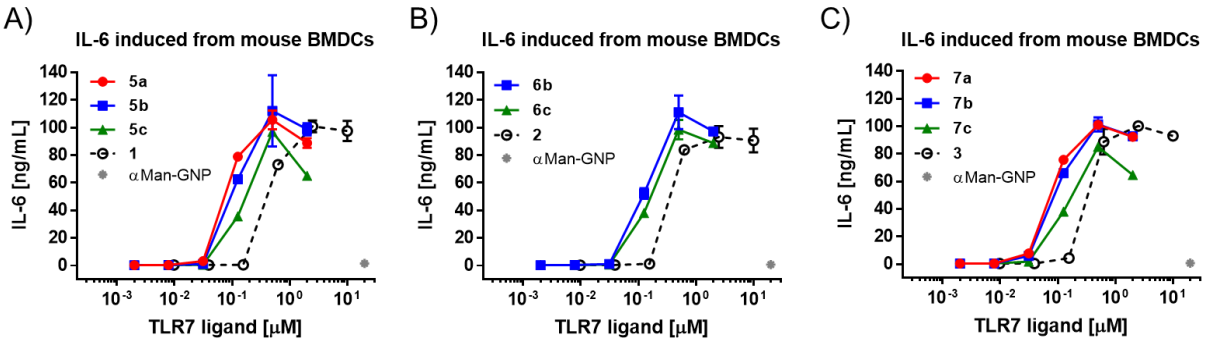

Figure S7. *In vitro* cytokine induction by 1V209-αMan-GNPs in mouse BMDC. (A-C) Mouse BMDCs (1×10^5 cells/well) were plated and incubated with serially diluted 1V209-αMan-GNPs, which were immobilized with (A) 1V209-TTDDA-TA(**1**), (B) 1V209-*m*PDA-TA (**2**) or (C) 1V209-EDA-TA (**3**), or unconjugated 1V209 derivative for 18 h. The control cells were incubated with αMan-GNPs whose concentration was equivalent to that contained in **1a**, **1b** or **1c**. IL-6 released in the culture supernatants were determined by ELISA. All data shown are means ± SD of triplicate and representative of three independent experiments. TLR7 ligand concentrations in the 1V209-αMan-GNPs are equivalent to unconjugated 1V209 derivative.

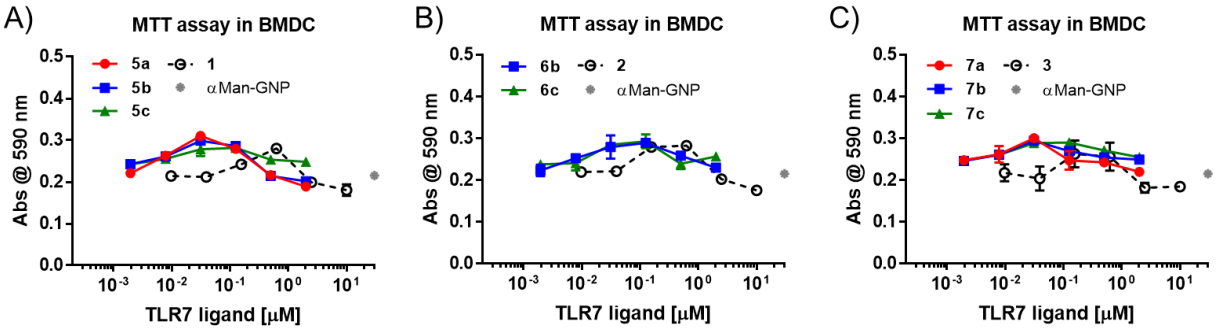

Figure S8. Cellular cytotoxicity of 1V209-αMan-GNPs in mouse BMDCs. (A-C) mouse BMDC (1×10^5 cells/well) were plated and incubated with serially diluted 1V209-αMan-GNPs which were immobilized with (A) 1V209-TTDDA-TA(**1**), (B) 1V209-*m*PDA-TA (**2**) or (C) 1V209-EDA-TA (**3**), or unconjugated 1V209-TTDDA-TA (**1**) for 18 h. The control cells were incubated with αMan-GNPs whose concentration was equivalent to that contained in **1c**. Formazan converted from MTT by mitochondrial metabolism in BMDCs was dissolved in DMSO and the absorbance at 590 nm was measured. All data shown are means ± SD of triplicate and representative of three independent experiments. TLR7 ligand concentrations in the 1V209-αMan-GNPs are equivalent to unconjugated 1V209 derivative.

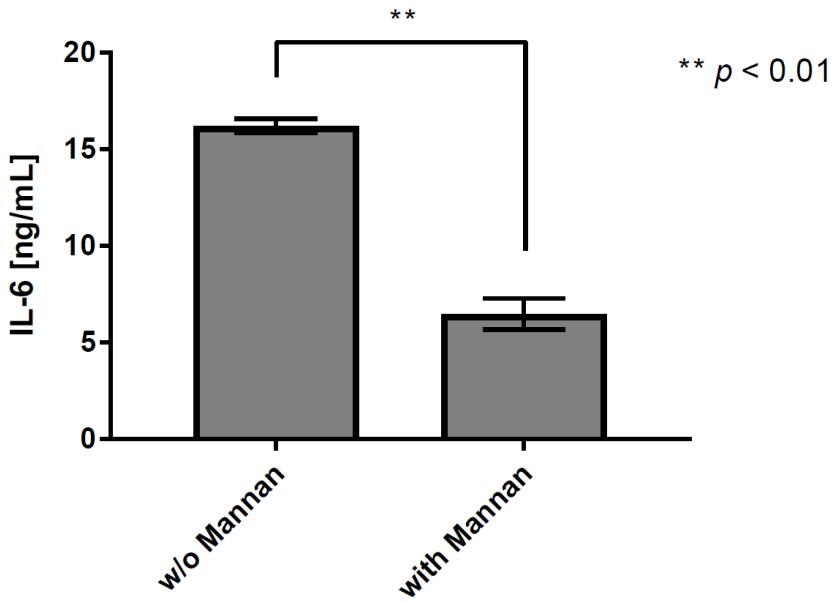

Figure S9. *In vitro* cytokine induction by 1V209-αMan-GNPs (**5a**) in mouse BMDCs in the presence or absence of mannan. Mouse BMDCs (1×10^5 cells/well) were preincubated with or without mannan (1 mg/mL) for 1 h. The cells were then incubated with **5a** for 8 h. IL-6 released in the culture supernatants were determined by ELISA. The data shown are means ± SD of triplicate in single experiment. TLR7 ligand concentration in **5a** is equivalent to unconjugated 1V209 derivative.

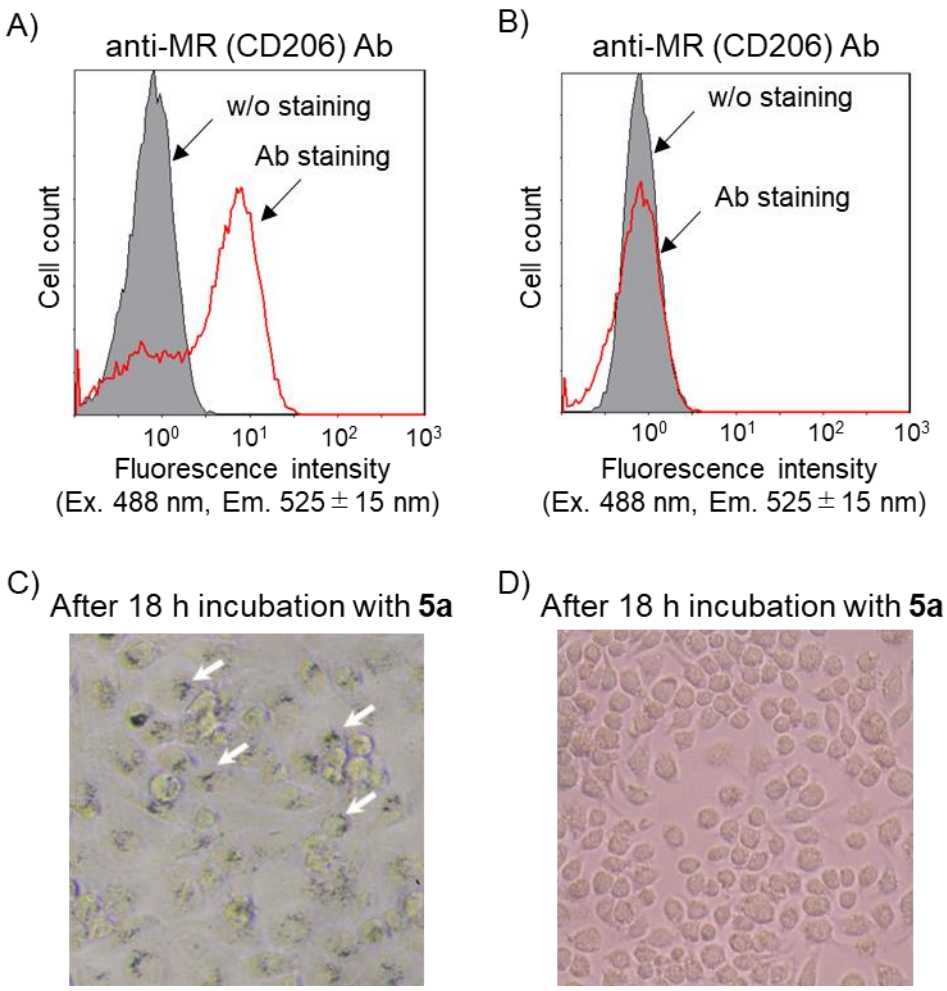

Figure S10. (A and B) Expression of mannose receptor (CD206) on mouse BMDCs or murine macrophage cell line J774A.1. (A) mouse BMDCs or (B) J774A.1 cells were stained with anti-mannose receptor IgG-FITC (1:400 dilution with PBS). The cells were analyzed by flow cytometry. The excitation and emission wavelength in the flow cytometry analysis were 488 nm and 525 ± 40 nm, respectively. (C and D) Optical microscopy images (×200) of mouse BMDCs or J774A.1 cells after 18 h incubation with 1V209-αMan-GNPs. (C) mouse BMDCs (1×10^5 cells/well) or (D) J774A.1 cells (4×10^4 cells/well) were plated and incubated with 2 μM of 1V209-αMan-GNPs (**5a**) for 18 h at 37 °C, 5% CO_2. White arrows indicate the accumulated GNPs.