nature materials

SUPPLEMENTARY INFORMATION

DOI: 10.1038/NMAT2784

Supplementary Figure 1

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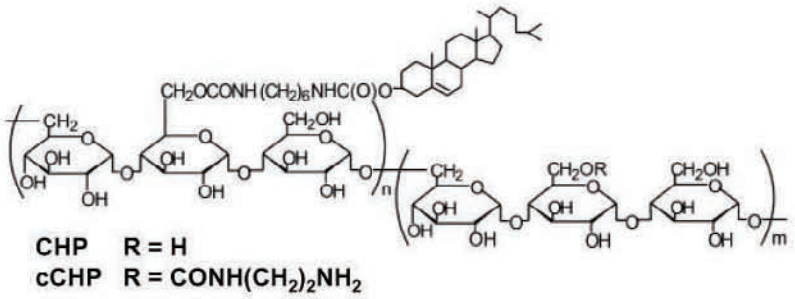

Supplementary Fig. 1. Chemical structure of cationic type of cholesteryl group–bearing pullulan (cCHP). cCHP nanogel contains 15 amino groups per 100 glucose units.

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Supplementary Figure 2

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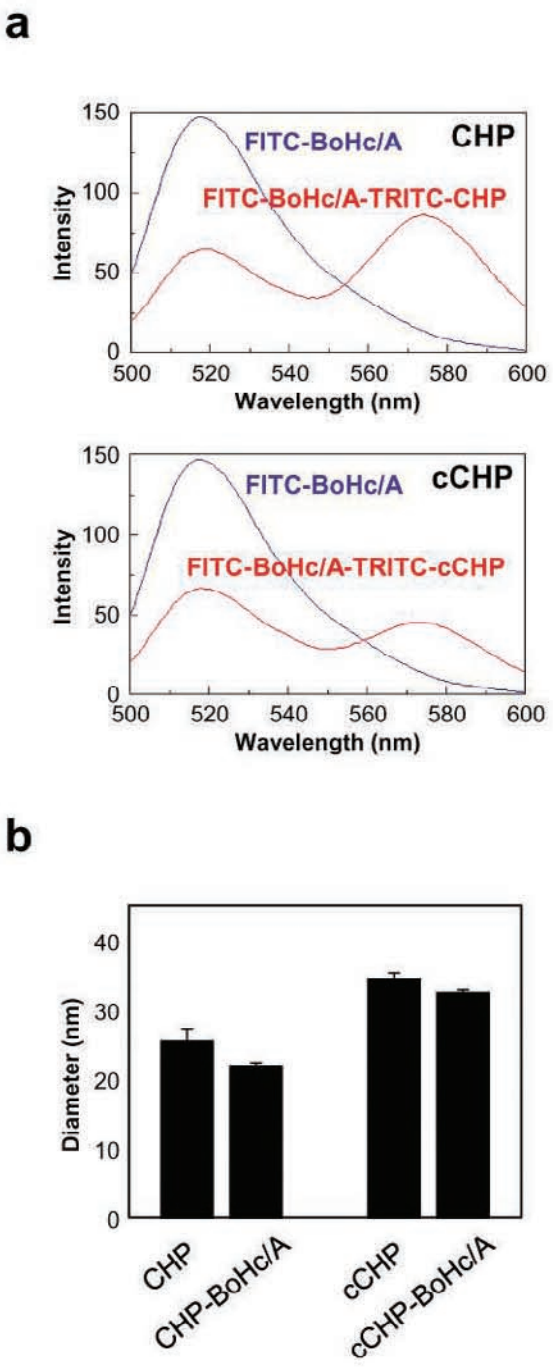

Supplementary Fig. 2. CHP and cCHP nanogels form nanosize particles carrying vaccine antigen. Fluorescence response energy transfer was detected from TRITC-conjugated CHP or TRITC-conjugated cCHP carrying FITC-conjugated BoHc/A, but not from FITC-conjugated naked BoHc/A (**a**). Dynamic light scattering analysis showed that CHP or cCHP nanogel was still of uniform size after the incorporation of BoHc/A (**b**).

Supplementary Figure 3

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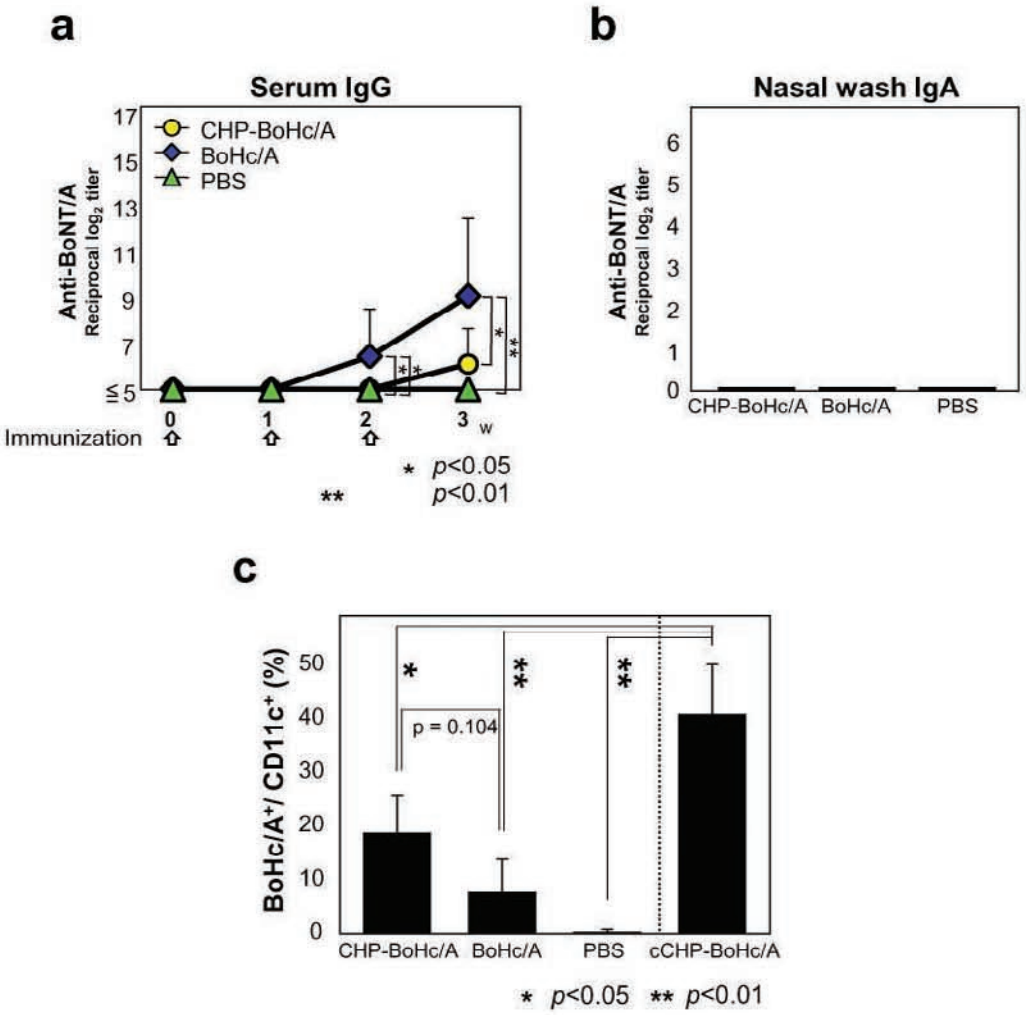

Supplementary Fig. 3. CHP nanogel does not act as a delivery vehicle for intranasal vaccine. Levels of BoNT-specific serum IgG (**a**) and nasal IgA (**b**) antibody were no greater in mice intranasally administered with CHP-BoHc/A than in mice administered with naked BoHc/A or control PBS. (**c**) BoHc/A administered with CHP nanogel was not effectively delivered to DCs in nasal tissue when compared with naked BoHc/A. cCHP-BoHc/A was used as a positive control.

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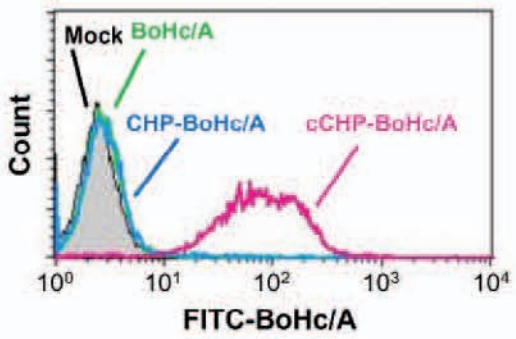
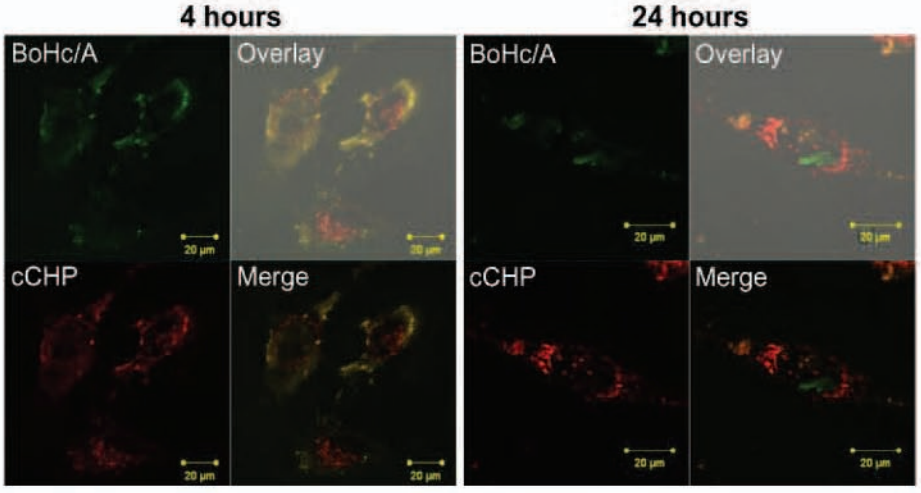
Supplementary Figure 4 T. Nochi, et al.

a

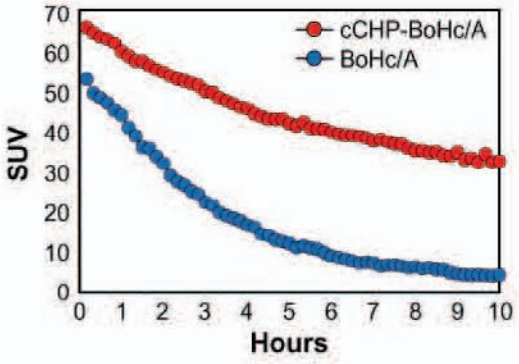


b



Supplementary Fig. 4. cCHP but not CHP nanogel delivers vaccine antigen into cells *in vitro*. Unlike CHP-BoHc/A, cCHP-BoHc/A interacted strongly with HeLa cells (**a**) and was immediately taken up into the cells by endocytosis (**b**). BoHc/A was released from the cCHP nanogel in a controlled manner (**b**).

Supplementary Figure 5 T. Nochi, et al.



Supplementary Fig. 5. Antigen intranasally administered with cCHP nanogel is effectively delivered to nasal mucosa. Quantitative analysis of positron emission tomography imaging experiments showed that approximately 50% of the BoHc/A was retained in the nasal cavity 10 h after intranasal administration with cCHP nanogel. SUV, standardized uptake value.

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Supplementary Figure 6

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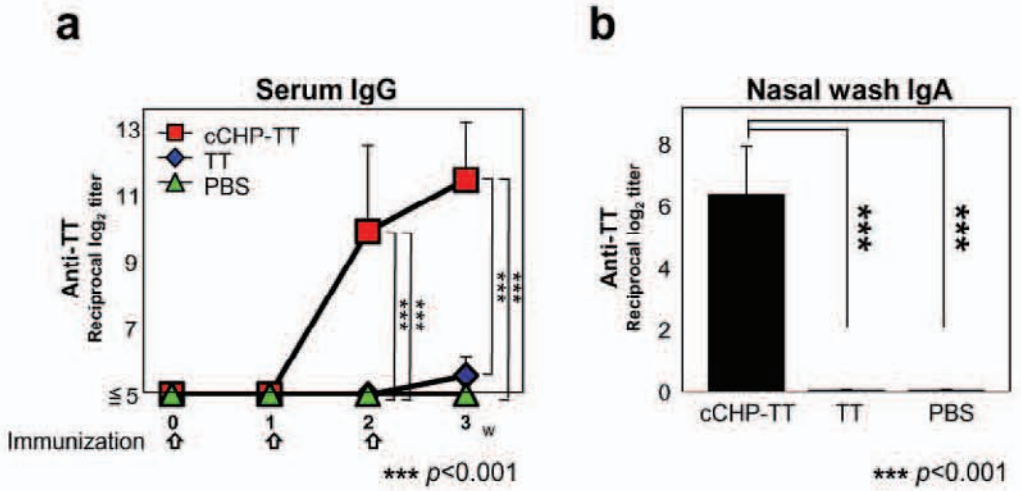
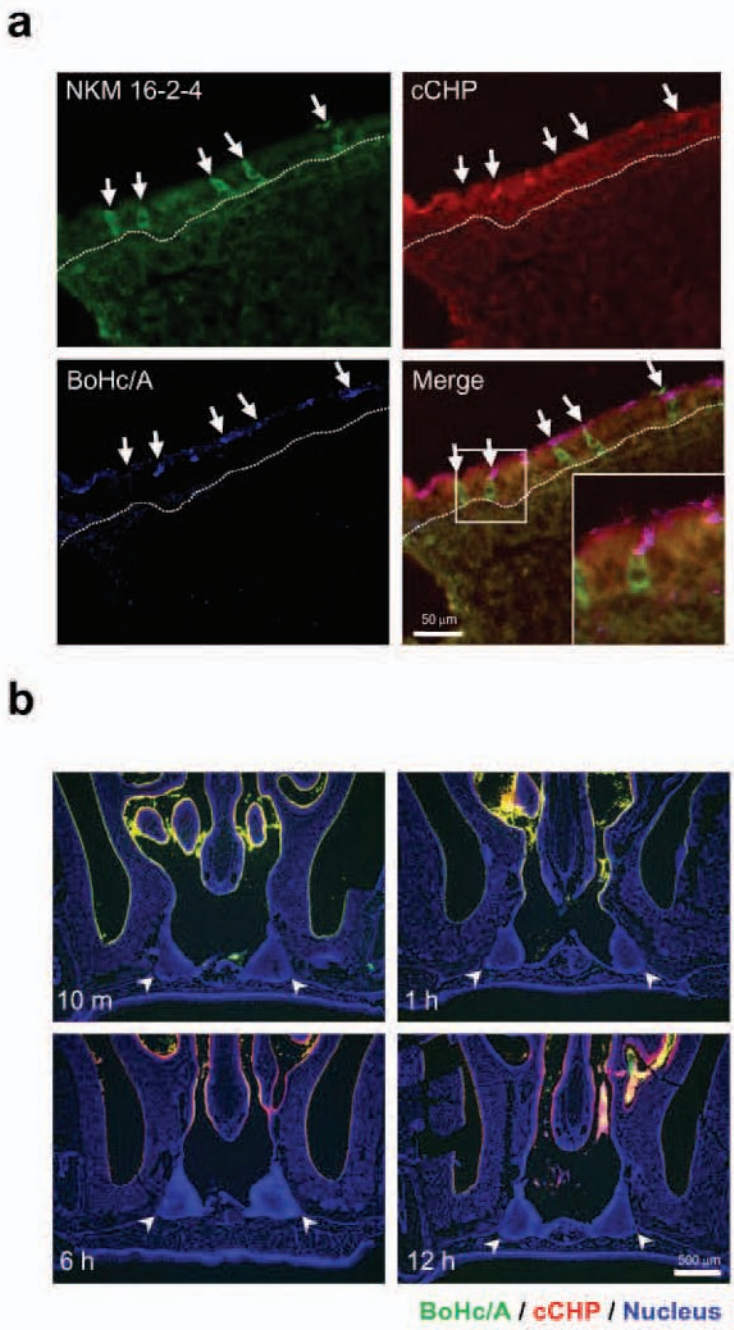


Supplementary Fig. 6. cCHP nanogel acts as a universal delivery vehicle for intranasal vaccines. As in the immunization study with cCHP-BoHc/A, mice intranasally immunized with cCHP-TT showed high levels of TT-specific serum IgG (**a**) and nasal IgA (**b**) antibody responses, unlike mice administered intranasally with naked TT or control PBS.

Supplementary Figure 7 T. Nochi, et al.



Supplementary Fig. 7. Uptake of cCHP-BoHc/A by M cells in nasopharynx-associated lymphoid tissues (NALT). Although some of the BoHc/A administered intranasally with cCHP nanogel was taken up by NKM 16-2-4$^+$ M cells (arrows) in the follicle-associated epithelium of NALT within 1 h of administration (**a**), the fluorescence signals were lower in NALT (**b**) than that detected in nasal epithelium (see Figure 3A). Basal layer of epithelium in A and NALT in B are shown by dotted line and arrowheads, respectively.

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Supplementary Figure 8

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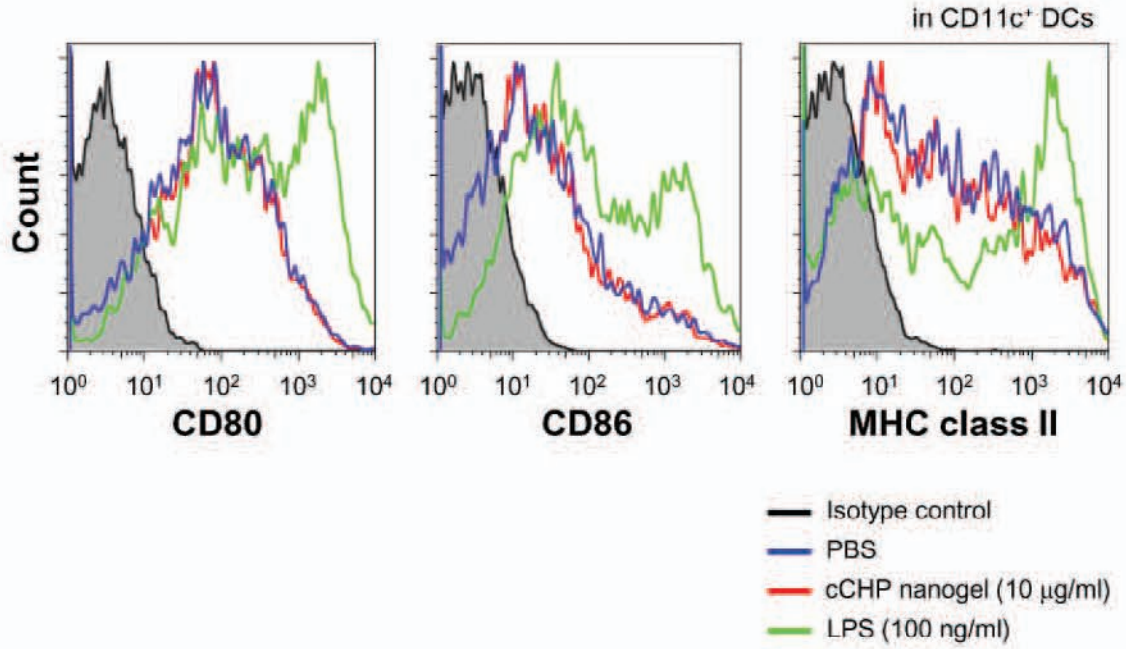
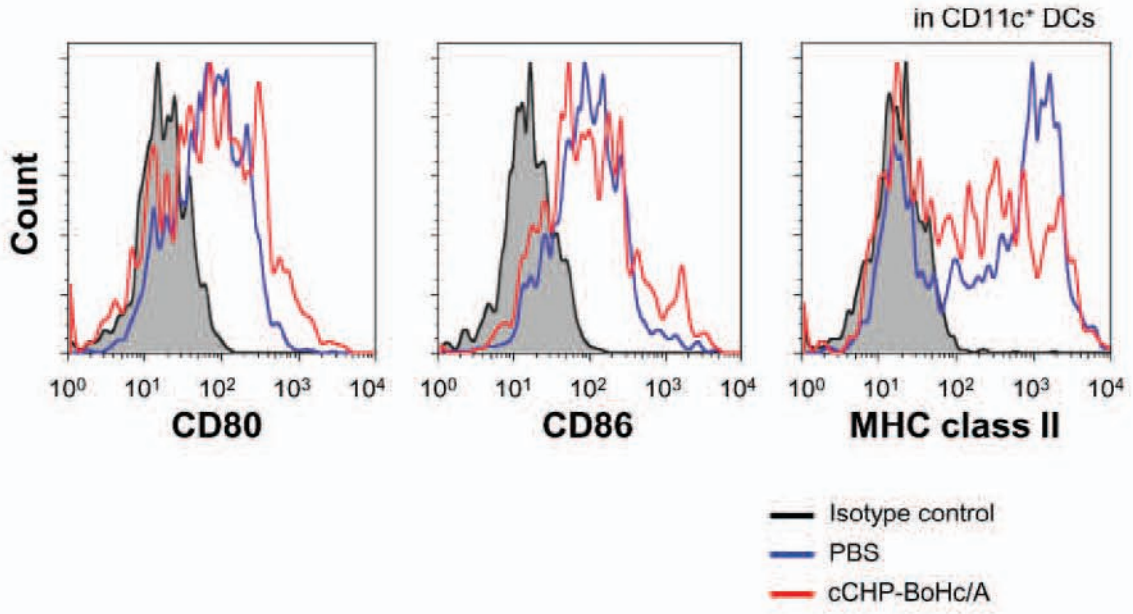


Supplementary Fig. 8. Expression levels of co-stimulatory and antigen-presentation molecules in bone marrow-derived DCs (BM-DCs) are not changed after stimulation with cCHP nanogel. BM cells were cultured in the presence of 10 ng/ml of GM-CSF for 5 days *in vitro* for generating the BM-DCs. BM-DCs were then cultivated with 10 μg/ml of cCHP nanogel or 100 ng/ml of LPS. After 1 day of stimulation, the cells were harvested and used for flow cytometory analysis. The expression levels of CD80, CD86 and MHC class II on CD11c⁺ BM-DCs were not changed when cultivated with cCHP nanogel. In contrast, the levels were enhanced by stimulation with LPS.

Supplementary Figure 9 T. Nochi, et al.



Supplementary Fig. 9. Expression levels of co-stimulatory and antigen-presentation molecules by CD11c⁺ nasal DCs are not enhanced by intranasal administration with cCHP-BoHc/A. Nasal DCs spontaneously expressed CD80, CD86 and MHC class II, and their expression levels were not changed 1 day after intranasal administration with cCHP-BoHc/A.

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Supplementary Table 1 T. Nochi, et al.

Sample	Endotoxin levels
BoHc/A	< 50mEU / μg protein
CHP nanogel	Not detected
cCHP nanogel	Not detected

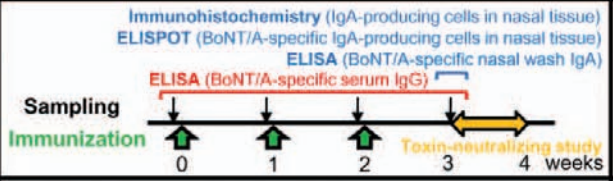
Supplementary Table 1. The amount of endotoxin contained in 10 μg, 1 μg, 100 ng, 10 ng and 1 ng of BoHc/A, CHP or cCHP nanogel were measured by Limulus J Single Test (Wako, Osaka, Japan). Detection sensitivity was ≥50 mEU / sample.

Supplementary Table 2 T. Nochi, et al.

Sample	Zeta-potential (mV)
CHP	-1.0±2.0
CHP-BoHc/A	-1.4±1.1
cCHP	+5.4±1.5
cCHP-BoHc/A	+7.0±0.5

Supplementary Table 2. The zeta-potentials of CHP, CHP-BoHc/A, cCHP, cCHP-BoHc/A were measured by Zetasizer Nano ZS instrument (Malvern Instruments, Malvern, UK).

Supplementary Table 3 T. Nochi, et al.

Exp. No	Antigen (Dose)	Delivery vehicle (Amount)	Experimental design	Figure
1	BoHc/A (10 μg)	CHP nanogel (88.9 μg)		Fig. S3a,b
	BoHc/A (10 μg)	None		
	None	None		
2	BoHc/A (10 μg)	cCHP nanogel (88.9 μg)		Fig. 2
	BoHc/A (10 μg)	None		
	None	None		
3	TT (30 μg)	cCHP nanogel (78.5 μg)		Fig. S6
	TT (30 μg)	None		
	None	None		

Supplementary Table 3. Three immunization experiments were performed in this study.