(Supporting Information)

Biomineralization improved the thermostability of foot-and-mouth disease virus-like particles and protective immune response

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Name	VLPs concentration after biomineralization (µg/mL)	VLPs concentration in supernatant (µg/mL)	Primary concentration of VLPs (µg/mL)	Biomineralized efficacy (%)
VLPs	26.2	14.3	48	66
VLPs-W6	8	9	48	80
VLPs-N6P	21.38	15.1	48	64
VLPs-NW	27.04	10.35	48	75





Fig. S1 Cellular uptake of VLPs and VLP–W6 or their biomineralized particles as quantified through Western blot analysis. A. BHK-21 cells were treated with 5 μ g of different VLPs for 1, 2, 3, and 4 h for the analysis of time-dependent effects on cellular uptake. B. BHK-21 cells were treated with 0.1, 1, 5, and 10 μ g of different VLPs for 1 h for the analysis of concentration-dependent effects on cellular uptake.



Fig. S2 Biomineralization VLPs and VLPs-W6 enter into immunity cell Raw264.7 varied with concentration and infectious times determined by western-blotting. A. immunity cell raw264.7 were infected with 5µg different antigen for 1, 2, 3 and 4h, to analysis the time-dependent effects. B. BHK-21 cells were infected with 0.1, 1, 5 and 10 µg different antigen for 1h, to analysis the concentration-dependent effects. The experiments repeated independent for three occasions. were Merge DAPI Cell Cell VLPs DAPI VLPs Merge 1h 1h 2h 2h 4h 4h

Fig. S3 Fluorescence microscopy images of the intracellular delivery of native and biomineralization VLPs. The nucleus was stained by DAPI (blue), cells were showed by light field (gray). Anti-VP1was conjugated with green fluorescent protein.

VLPs-W6-CaP

VLPs-W6



Fig. S4 DCs treated with PBS, VLPs, LPS, VLPs–W6, or VLPs–W6–Si at 37 °C were stained with FITC–dextran and analyzed with flow cytometry . Samples treated at 4 °C were used as negative controls. Results are representative of three independent experiments. Error bars indicate SD. *P < 0.05; **P < 0.01, ***P < 0.001.



Fig. S5 Biomineralization VLPs and VLPs-W6 taken up by imDCs varied with concentration and infectious times determined by western-blotting. A. imDCs treated with 0.1, 1, 5 and 10 μ g different antigen for 1h, to analysis the concentration-dependent effects. B. imDCs treated with 5 μ g different antigen for 1, 2, 3 and 4h, to analysis the time-dependent effects. The experiments were repeated for three independent occasions.



Fig. S6 A representative BMDC activation experiment analyzed by flow cytometry following 24 hours incubation with VLPs, LPS, VLPs-W6, VLPs-CaP and VLPs-W6-CaP (5 μ g/ml equivalent concentration). Rows display different surface markers, and columns correspond to the different compounds added (imDCs = immature DCs). Events to the right of the red bar are considered positive for the specific marker.



Fig. S7 CFSE-labeled DCs treated by VLPs, VLPs-W6, VLPs-CaP and VLPs-W6-CaP, PBS, VLPs transplanting to recipients were measured for DC migration 24 h later. The experiments were repeated for three independent occasions. Error bars mean standard deviation (SD).