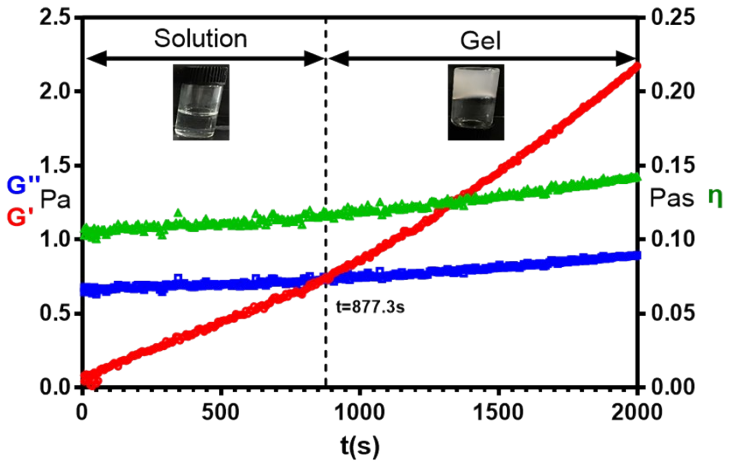
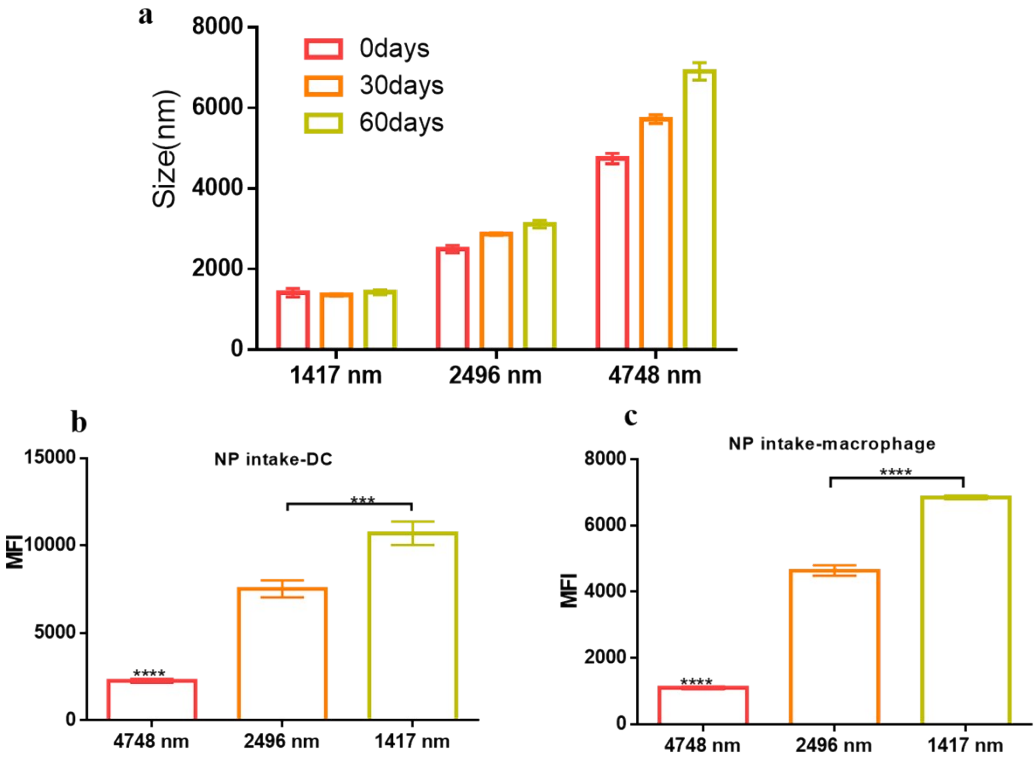
Supplementary Materials

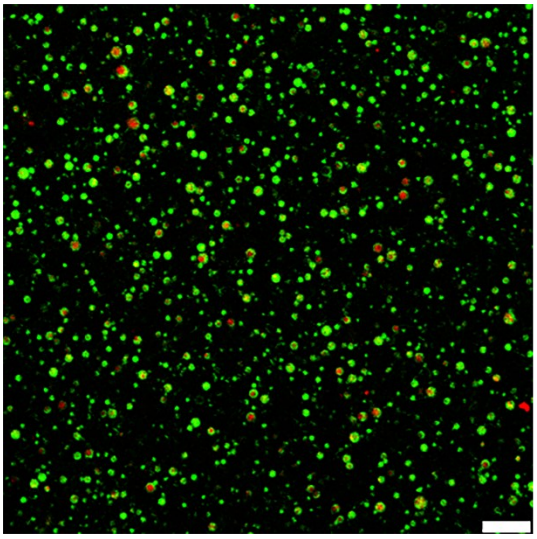


Supplementary Figure 1 Apparent viscosity (green), viscous modulus G" (blue) and elastic modulus G' (red) of CS-GP hydrogel. Gelation time at 37 ℃ was determined by the critical point of sol-gel transition when G"= G'.

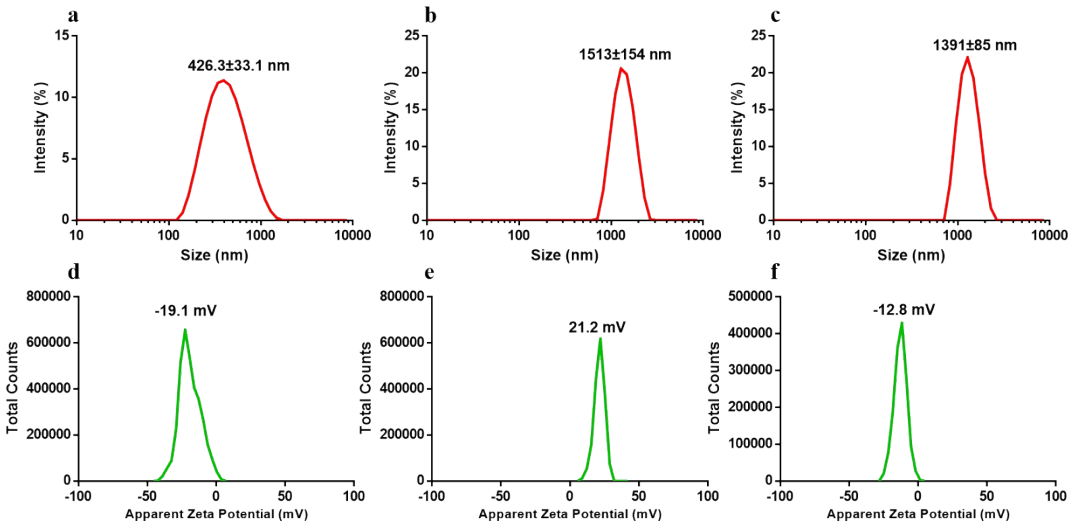


Supplementary Figure 2 (a) CSSE with different sizes and corresponding stability. Stability was tested with three parallel samples each in an incubator at 25 °C. (b-c) The mean fluorescence intensity (MFI) of chitosan nanoparticles in various CSSE intaking by APCs. Flow cytometry

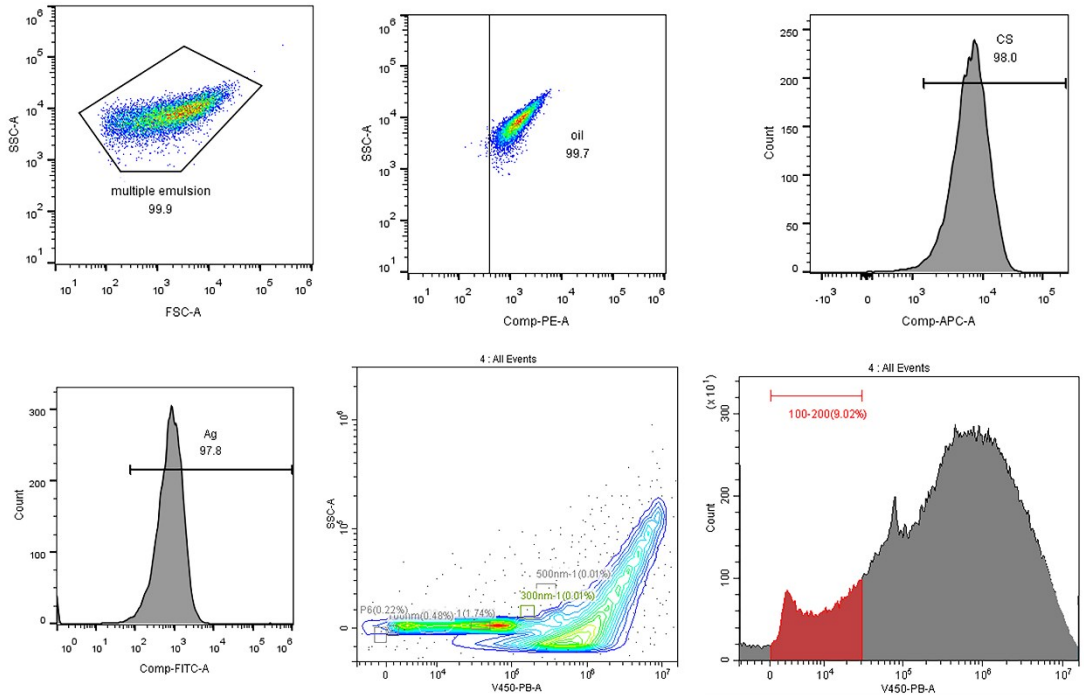
assessment of CSSE internalization by evaluating the fluorescence intensity of Cy5-chitosan. (b) Cells were stained with CD11c-ef450. The diagram showed the Cy5 intensity within the gated $CD11c^+$ population. (c) Cells were stained with CD11b-ef450 and $Cy5^+$ $CD11b^+$ population was gated.



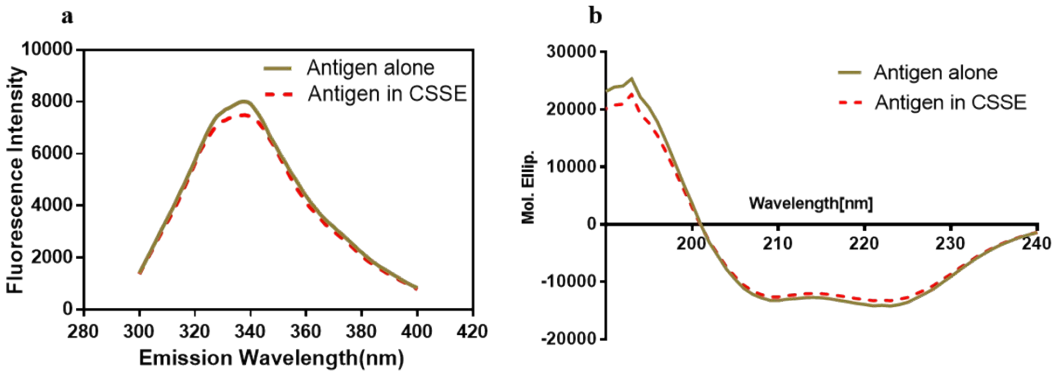
Supplementary Figure 3 Confocal image of CSSE droplets by labelling White oil (green) and chitosan (red). Scale bar=10 μm.



Supplementary Figure 4 Sizes and zeta potentials of various control vaccines. (a-c) Size of ISA206 (a), CMP (b), and W/O/W emulsion (c). (d-f) Zeta potential of ISA206 (d), CMP (e), and W/O/W emulsion (f).

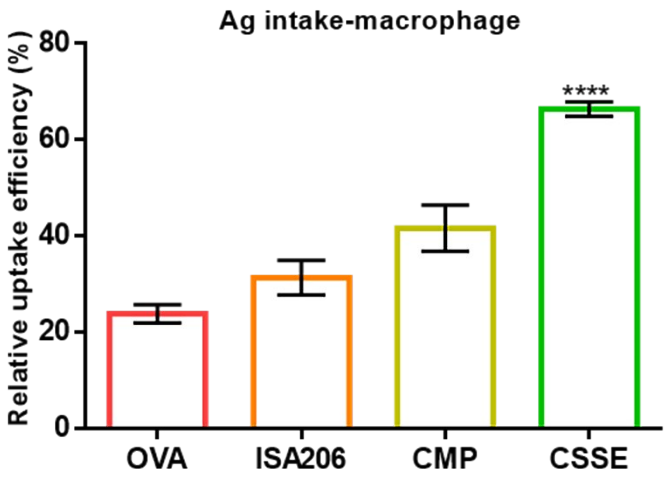


Supplementary Figure 5 Gate strategies of the compositions of CSSE emulsions. (a-d) Mineral oil, antigens and chitosan were stained with DiI, FITC and Cy5, respectively. And the representative percentage of encapsulated nanoparticles and antigens was detected by BD LSRFortessa. (e-f) Beckman Coulter CytoFlex was set to calculate side scatter using 405 nm for the nanoparticle detection. After standard beads of 100 nm to 500 nm were calibrated, representative graph of frequency of 100-200 nm chitosan nanoparticles in all particles was shown.

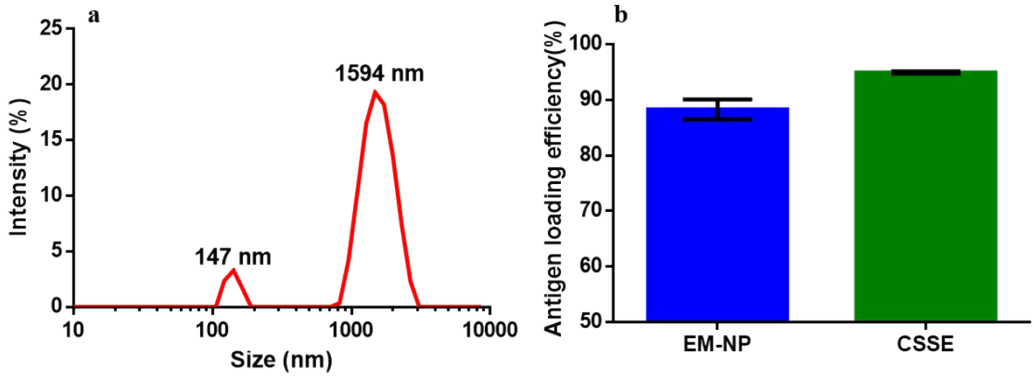


Supplementary Figure 6 The characterization of antigen integrity. Results were representative of three replicate tests. (a) The fluorescent intensity of antigens. Analysis showed that the maximum emission wavelength within the acceptable range of 331~342 nm, which suggested that the tertiary structure of antigens was not essentially changed. (b) The CD spectra of antigens. Each spectrum

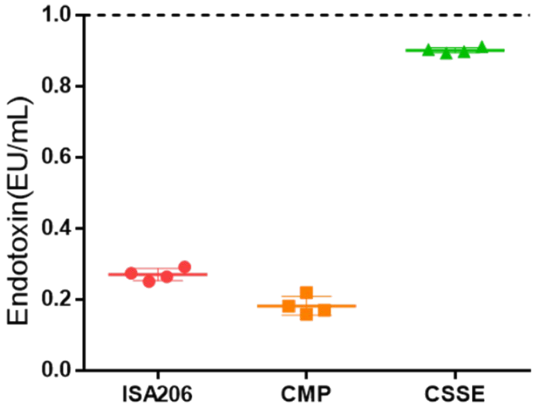
had two minima at 208 and 222 nm, which represented the a-helix. All these results suggested that the fabrication process of CSSE had little influence on the antigen integrity.



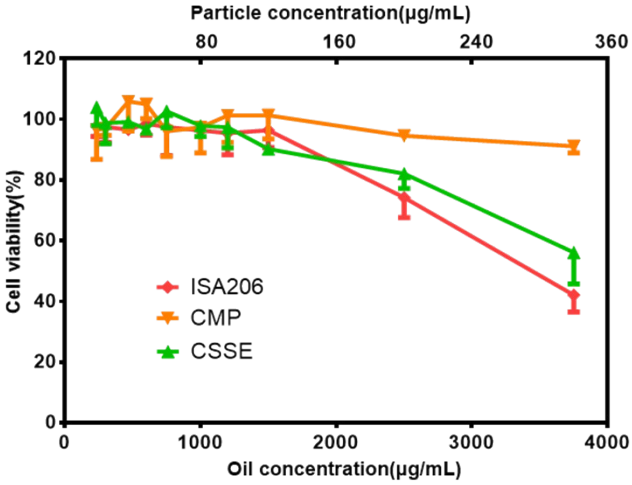
Supplementary Figure 7 Antigen uptake by the macrophages in vitro. The diagram showed the percentages of antigen-positive macrophages within the gated $CD11b^{+}$ population.



Supplementary Figure 8 The characterization of the control group EM-NP. (a) Size distribution of EM-NP. Analysis showed that one peak around 147 nm, representing the chitosan nanoparticles and another peak around 1594 nm, referring to the emulsion size. (b) Antigen loading efficiency of EM-NP. Data showed the lower loading efficiency comparing to CSSE.



Supplementary Figure 9 Endotoxin level of the immune adjuvants. The supernatants of CMP and subnatants of CSSE, ISA206 were analyzed by chromogenic LAL endotoxin assay kit. Endotoxin content of all tested samples was below 1.0 EU/mL, much lower than 50EU/each dose, the regulated limit set by the Ministry of Agriculture of the People's Republic of China. It means that the immune efficacy is completely unaffected by the endotoxin content.



Supplementary Figure 10 In vitro cytotoxicity assay showing the BMDC viability at different particle or oil concentrations using CCK8 kit.

Supplementary Table 1 Comparison of properties between FMDV-based emulsion and OVA-based emulsion. The antigen loading efficiencies were presented the mean values of three parallel data with standard error of mean.

Antigen	Size (nm)	Zeta potential (mV)	Antigen loading efficiency
OVA	1417±104	-12.0±1.2	97.99%
FMDV	1465±89	-12.6±0.67	94.94%

Supplementary Table 2 Biochemical analysis of the formulations. The results were presented the mean values of six parallel data with standard error of mean. Data showed that the vaccines containing different adjuvants caused no significant difference on the below indexes.

	ALT	ALP	LDH	BUN	AST
PBS	40.0±10.0	205.8±24.2	1167.5±272.5	8.2±2.0	78.3±8.3
Ag	41.7±6.7	206.7±13.3	1063.3±156.7	8.0±0.4	80±10.0
ISA206	41.7±6.7	200.0±20.0	940.8±109.2	8.2±1.6	85.8±14.2
CMP	40.8±9.2	203.3±23.3	1106.7±166.7	8.1±0.9	80.0±5.0
CSSE	41.7±3.3	205.0±15.0	1088.3±91.7	8.2±0.3	85.0±10.0

Supplementary Table 3 Body weight changes in each vaccination group. Each data was presented the mean values of six parallel data with standard error of mean. As shown, it was suggested that vaccination with CSSE does not affect animal weight gain.

	PBS	Ag	ISA206	CMP	CSSE
0 day	16.9±0.7	16.6±0.9	16.9±0.8	16.7±0.9	16.9±1.0
14 days	19.0±0.8	18.8±0.5	19.0±0.8	18.7±1.6	18.8±0.7
28 days	20.5±0.8	20.7±0.2	20.6±1.8	20.7±0.8	20.5±0.6