# Supporting Information

## Self-Encapsulating Poly(Lactic-*co*-Glycolic Acid) (PLGA) Microspheres for Intranasal Vaccine Delivery

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EXPERIMENTAL

**Materials**

RPMI 1640 medium, heat-inactivated FBS, L-glutamine, penicillin/streptomycin, 2-mercaptoethanol, and ACK lysis buffer were purchased from Invitrogen (Thermo Fisher Scientific Inc., USA). PMA/ionomycin (Cell Stimulation Cocktail) was purchased from eBioscience (USA).

**Distribution of $CaHPO_4$ Gel within Microspheres**

To view the distribution of $CaHPO_4$ gel within the particles after microsphere preparation, the gel was pre-loaded with a fluorescent OVA conjugate. $CaHPO_4$ gel was incubated with an Alexa Fluor 647-OVA conjugate at a ratio to meet the capacity of the gel for the OVA. The mixture was incubated at 37°C for 2 h under mild agitation on a table-top shaker. Following loading of the protein onto the gel, the gel was then used in the inner water phase for microsphere preparation. Lyophilized microspheres were resuspended in $ddH_2O$ and placed on a glass side and covered with a coverslip. The sample was viewed using a Nikon A-1 spectral confocal microscope with NIS Elements software (Nikon Instruments).

**Evaluation of OVA Stability During Self-Encapsulation**

OVA stability in the conditions for self-healing encapsulation was investigated by incubating OVA solutions at the different temperatures required for the encapsulation process. OVA solutions (0.5 mg/mL) in 10 mM MOPS buffer, pH 7.4, were incubated under mild agitation on a rotator. One sample each was incubated at 4°C for 24 h, 25°C for 24 h, 42°C for 48 h, and 4°C for 24 h + 25°C for 24 h + 42°C for 48 h. A separate sample was incubated at 80°C for 24 h to induce denaturation. The samples were then analyzed by SE-HPLC to examine for OVA aggregation.

**Cytokine Response**

On day 42, mouse spleens were collected under sterile conditions. Splenocytes were isolated by gentle disruption of the spleen through a mesh filter, and red blood cells were lysed with ACK lysis buffer and washed. Cells were plated at $5 \times 10^5$ cells/well in a 96-well tissue culture plate (Corning Inc., USA) with RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 55 µM 2-mercaptoethanol. Cells were tested in duplicate and treated with medium (negative control), 2

µL/mL PMA/ionomycin (positive control), or 25 µg/mL whole OVA. After 96 hours of incubation (37°C, 5% $CO_2$), the well supernatants were collected and stored at -80°C until analysis. Samples were submitted to the Immunology Core at the University of Michigan Cancer Center for ELISA analysis of cytokine response.

FIGURES

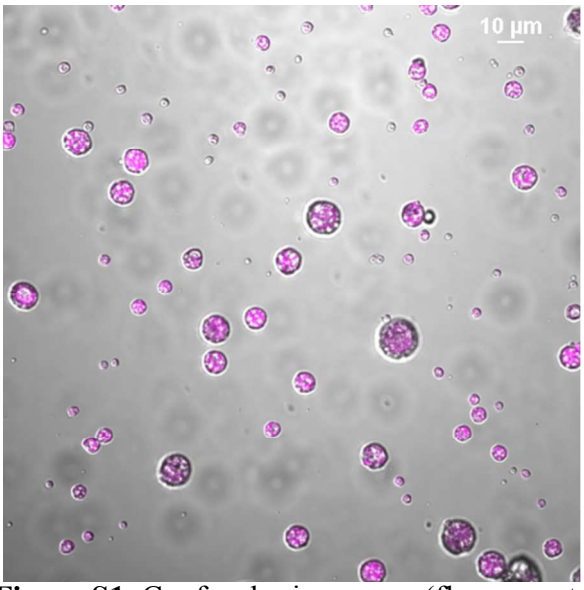


**Figure S1.** Confocal microscopy (fluorescent and brightfield image overlay) of the distribution of calcium phosphate gel (violet) in microspheres. The gel was pre-loaded with Alexa Fluor 647-OVA prior to incorporation in the inner water phase for microsphere production.

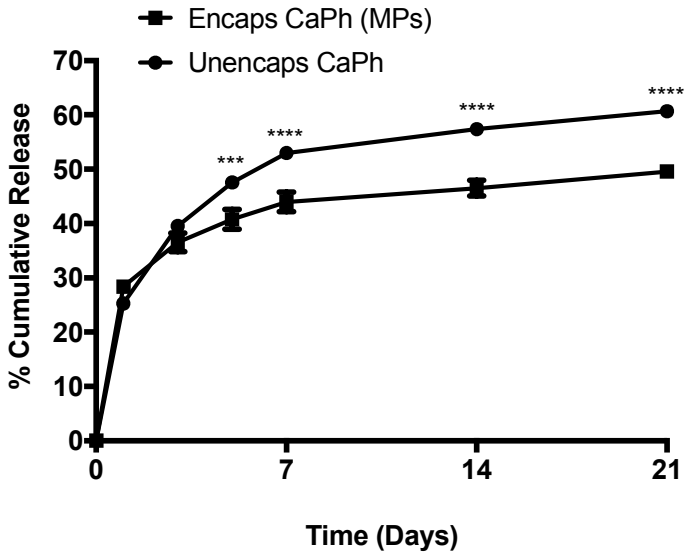


**Figure S2.** Cumulative release of OVA from PLGA self-encapsulated microspheres compared with that from unencapsulated calcium phosphate (CaPh) gel. Data represent mean ± SEM ($n$ = 3). All groups were compared using two-way ANOVA followed by Bonferroni's post-test (***$p \leq 0.001$, and ****$p \leq 0.0001$).

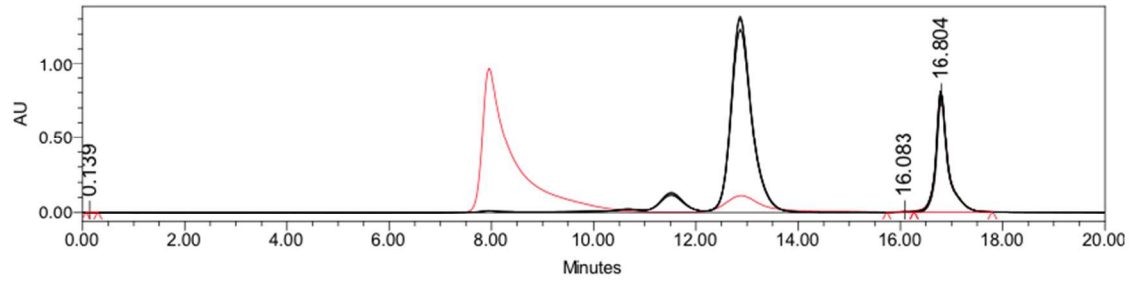


**Figure S3.** HP-SEC chromatograms of OVA incubated in MOPS buffer at different temperatures. The overlaid black curves represent OVA incubated at the temperatures of the self-encapsulation protocol individually and as one process (4°C for 24 h, 25°C for 24 h, 42°C for 48 h, and 4°C (24 h) + 25°C (24 h) + 42°C (48 h)). The red curve represents OVA treated at 80°C for 24 h to denature the protein.

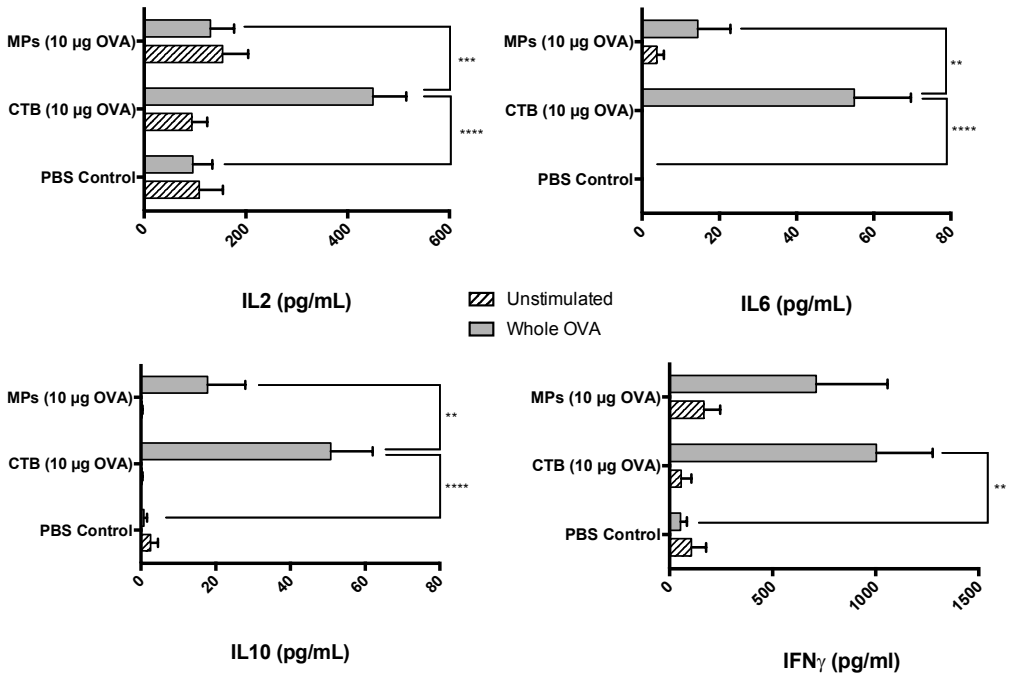


**Figure S4.** Analysis of cytokines produced from mouse splenocytes restimulated *ex vivo* with whole OVA protein. Splenocytes from intranasally-immunized mice were collected three weeks post booster immunization. Data represent mean ± SEM ($n = 5$). All groups were compared using two-way ANOVA followed by Bonferroni's post-test (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, and ****$p \leq 0.0001$).