# Electronic Supplementary Material

# Orthogonal modular biosynthesis of nanoscale conjugate vaccines for vaccination against infection

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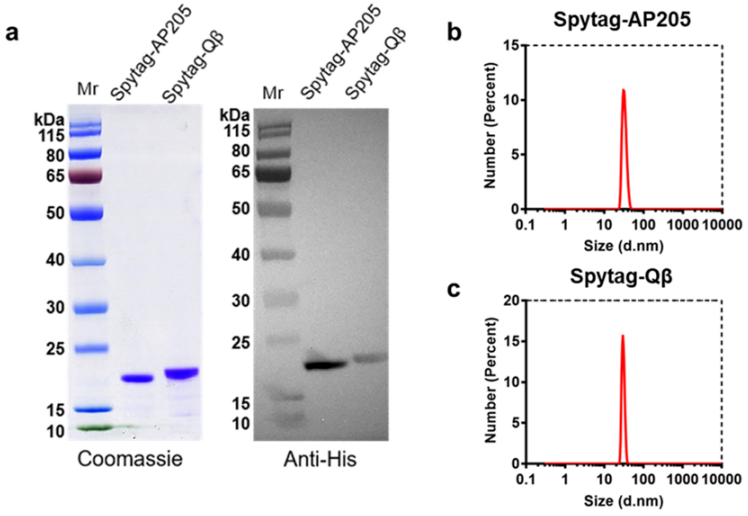


**Figure S1** Purification and characterization of SpyTag-VLPs. (a) Expression of SpyTag-AP205 and SpyTag-Qβ was induced in *Escherichia coli*. The protein was purified by affinity chromatography. The purified particles were separated by 12% SDS-PAGE and detected using Coomassie blue staining and western blotting with antibodies against the $His_6$ tag. (b, c) Dynamic light scattering determination of the hydrodynamic radii of SpyTag-AP205 (b) and SpyTag-Qβ (c).

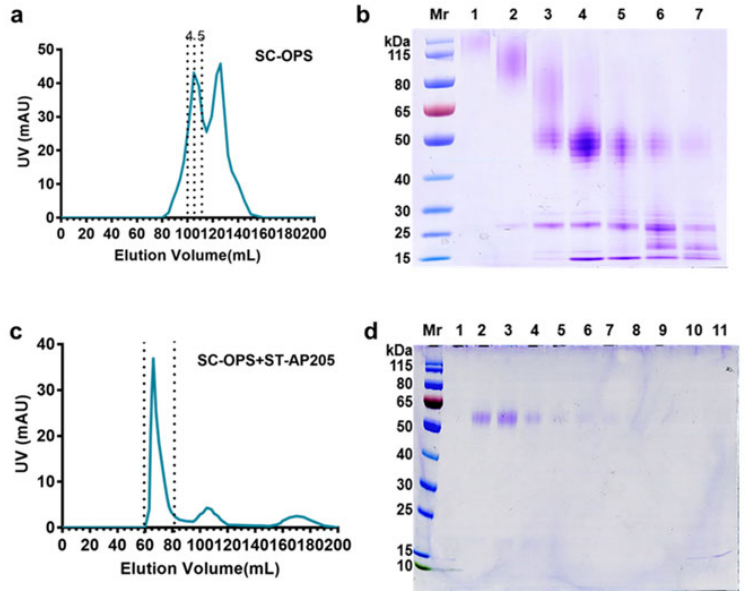


**Figure S2** Purification of SpyCatcher (SC)-O antigen polysaccharide (OPS) and SC-OPS+SpyTag (ST)-AP205 by size exclusion chromatography. Size exclusion chromatography of SC-OPS (a) and SC-OPS+ST-AP205 (c), samples were injected into a column containing 200 mL of Superdex-200 at a flow rate of 1.5 mL $min^{-1}$. Fractions of 5 mL were collected starting at a volume of 60 mL. Samples were assessed by 12% SDS-PAGE, SC-OPS (b) and SC-OPS+ST-AP205 (d) samples were analysed by Coomassie Blue staining. The retention time of SC-OPS+ST-AP205 was shorter than that of SC-OPS, indicating that unreacted SC-OPS could be effectively removed.

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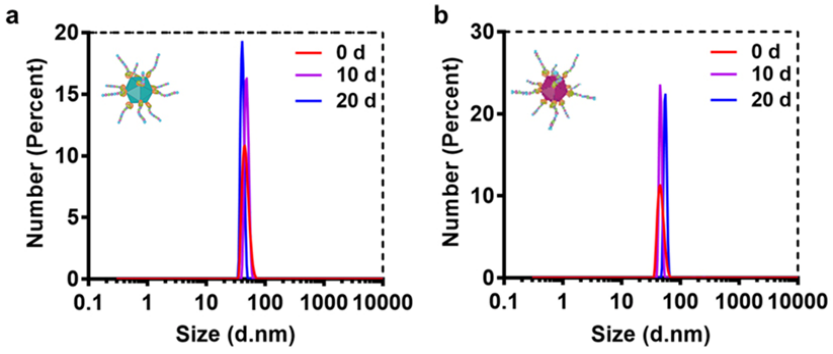


**Figure S3** Stability of nanovaccines during long-term storage. The hydrodynamic radii of AP205-O antigen polysaccharide (OPS) (a) and Qβ-OPS (b) during storage at 30 °C were determined by dynamic light scattering.

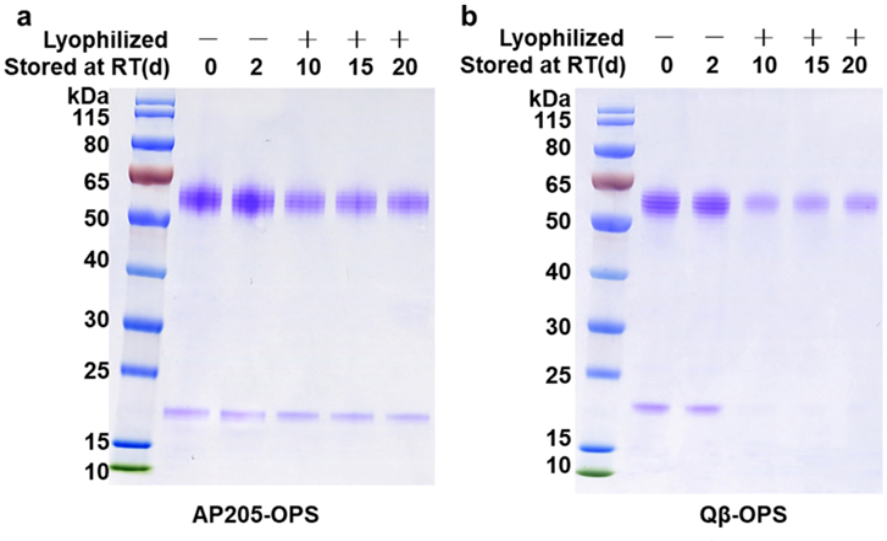


**Figure S4** Tolerance of nanovaccines to lyophilization. AP205-O antigen polysaccharide (OPS) (a) and Qβ-OPS (b) were lyophilized and stored for the indicated times at 25 °C. The nanovaccines were then reconstituted in PBS and analyzed by SDS-PAGE and Coomassie staining.

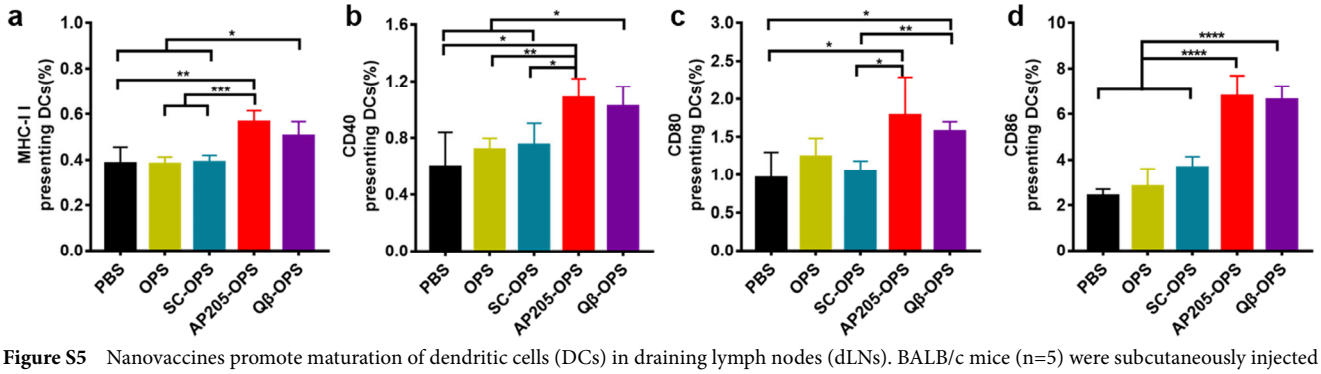


**Figure S5** Nanovaccines promote maturation of dendritic cells (DCs) in draining lymph nodes (dLNs). BALB/c mice (n=5) were subcutaneously injected with the indicated formulations. Expression of MHC-II and co-stimulatory markers (CD40, CD80 and CD86) on DCs (MHC-II$^+$, CD40$^+$, CD80$^+$ or CD86$^+$ cells among the CD11c$^+$ cell population) in dLNs 24 h post-vaccination was assessed. Percentages of MHC-II$^+$ (a), CD40$^+$ (b), CD80$^+$ (c), and CD86$^+$ (d) cells are shown.

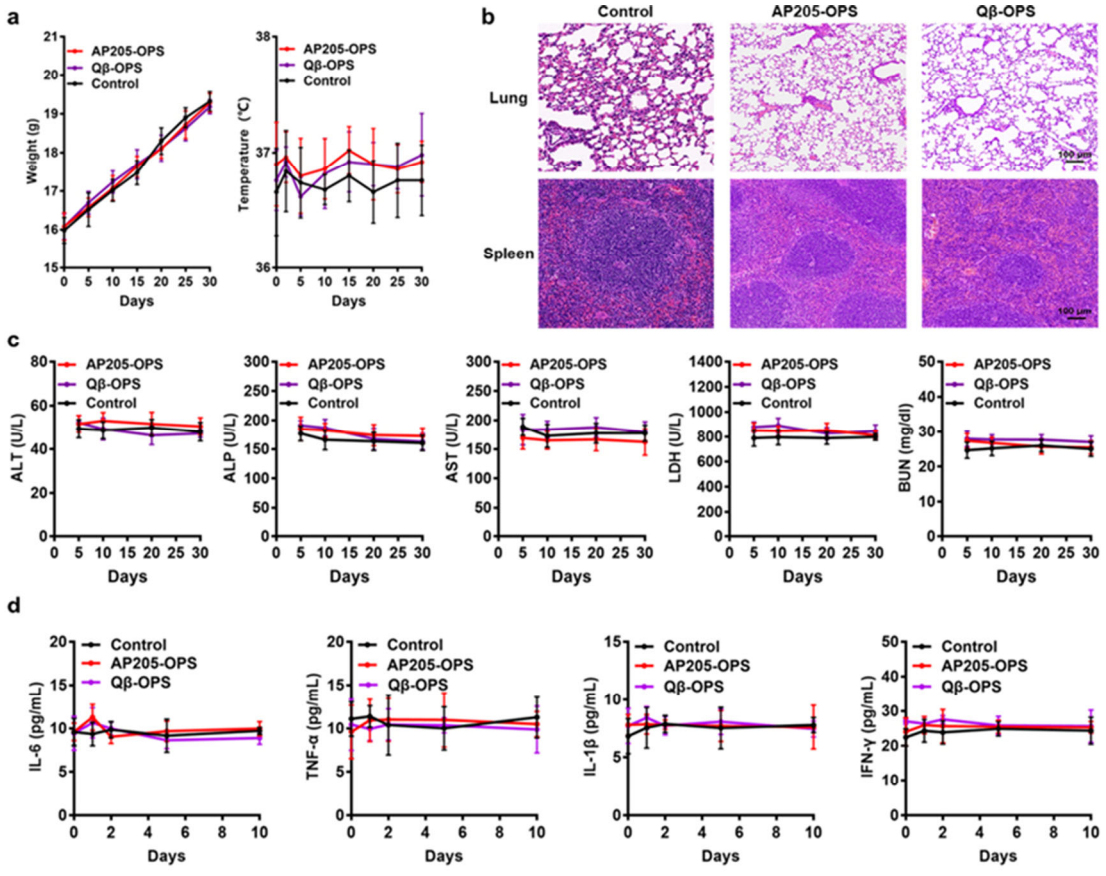


**Figure S6** Safety assessment of the nanovaccines. (a) Weight and temperature changes in mice immunized with AP205-O antigen polysaccharide (OPS) and Qβ-OPS containing 25 μg of polysaccharide. No changes in body weight or temperature were observed in the AP205-OPS and Qβ-OPS groups compared with the control group. (b) Hematoxylin and eosin staining of pathological sections of organs 20 days after immunization. (c) Serum biochemical indexes were detected at different time points after immunization. Levels of alanine aminotransferase (ALT), aminotransferase (AST), alanine aminotransferase (ALP), lactate dehydrogenase (LDH), and blood urea nitrogen (BUN) were evaluated. The results were all within normal ranges, indicating the excellent biocompatibility of AP205-OPS and Qβ-OPS. (d) Cytokine profiles (interleukin [IL]-6, tumor necrosis factor-α, interferon-γ and IL-1β) at different time points after immunization. No differences were observed between AP205-OPS, Qβ-OPS and the control groups, indicating that AP205-OPS and Qβ-OPS had no systemic toxicity.

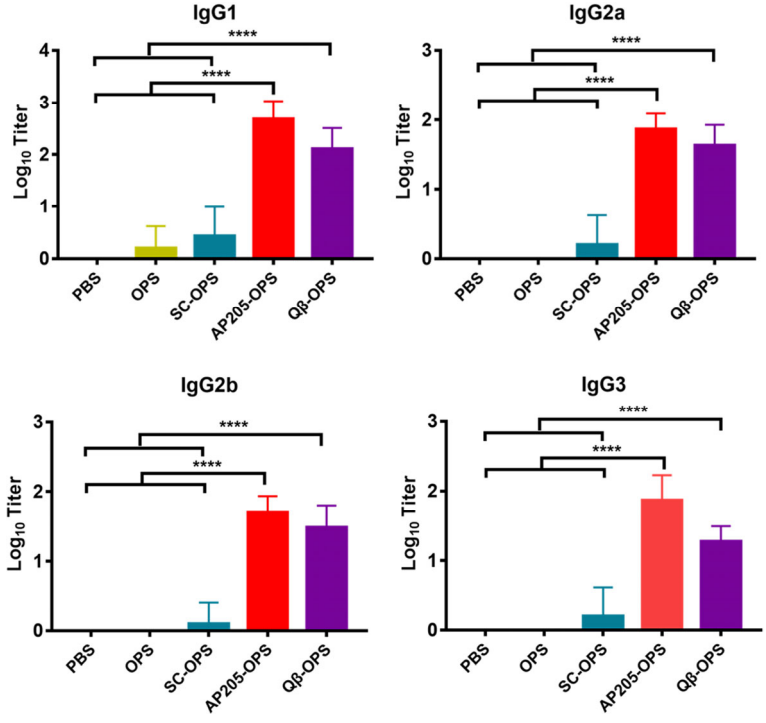


**Figure S7** Anti-LPS IgG subclass profiling performed at 42 days after first immunization. The IgG subtype titers against *S. flexneri* 2a strain 301 LPS were measures in the serum samples of BALB/c mice immunized with PBS, OPS, SC-OPS, AP205-OPS or Qβ-OPS (n=10).

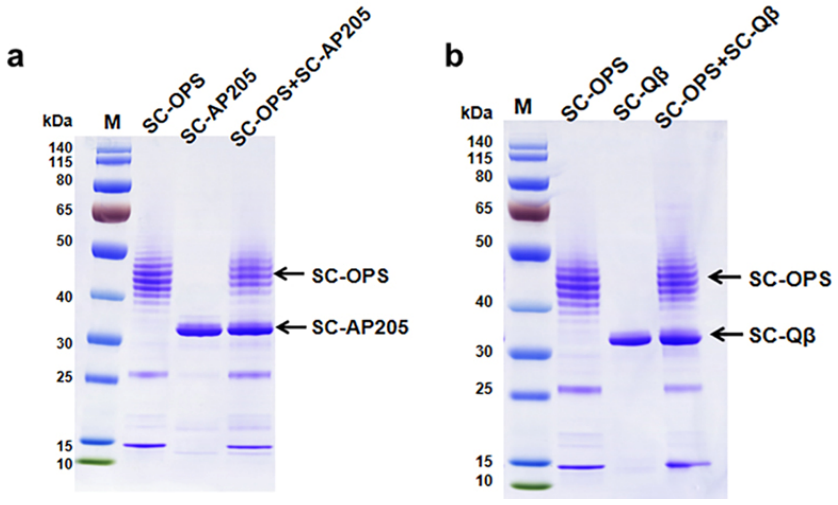


**Figure S8** SpyCatcher (SC)-O antigen polysaccharide (OPS) and SC-VLPs failed to form an isopeptide bond. SC-OPS were mixed with SC-AP205 (a) or SC-Qβ (b) and analyzed by SDS-PAGE with Coomassie staining.

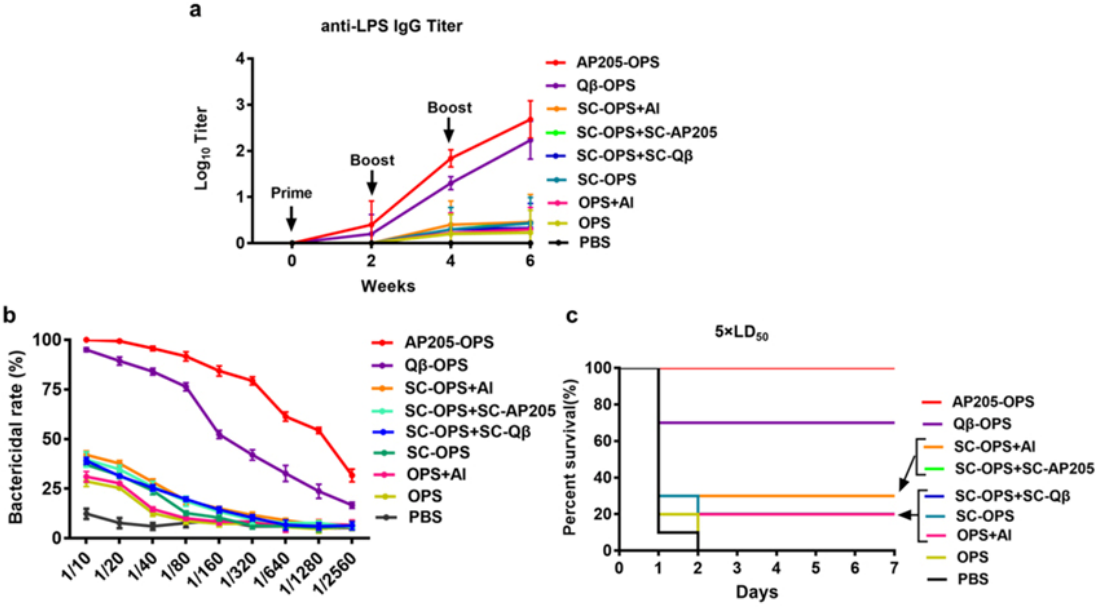


**Figure S9** Nanovaccines elicited robust prophylactic immunity. (a) Groups of 10 mice were immunized on days 0, 14, and 28 with different vaccines. Total serum IgG titers against *S. flexneri* 2a strain 301 LPS were assessed in serum samples collected on days 14, 28, and 42. (b) Bactericidal activity in serum samples collected on day 42 was determined. (c) Survival of different groups of mice challenged with $4.35\times10^7$ CFU per mouse ($5\times LD_{50}$) of *S. flexneri* 2a strain 301 cells on day 42. Survival was monitored over the subsequent 7 days (n = 10).