Supplemental Methods

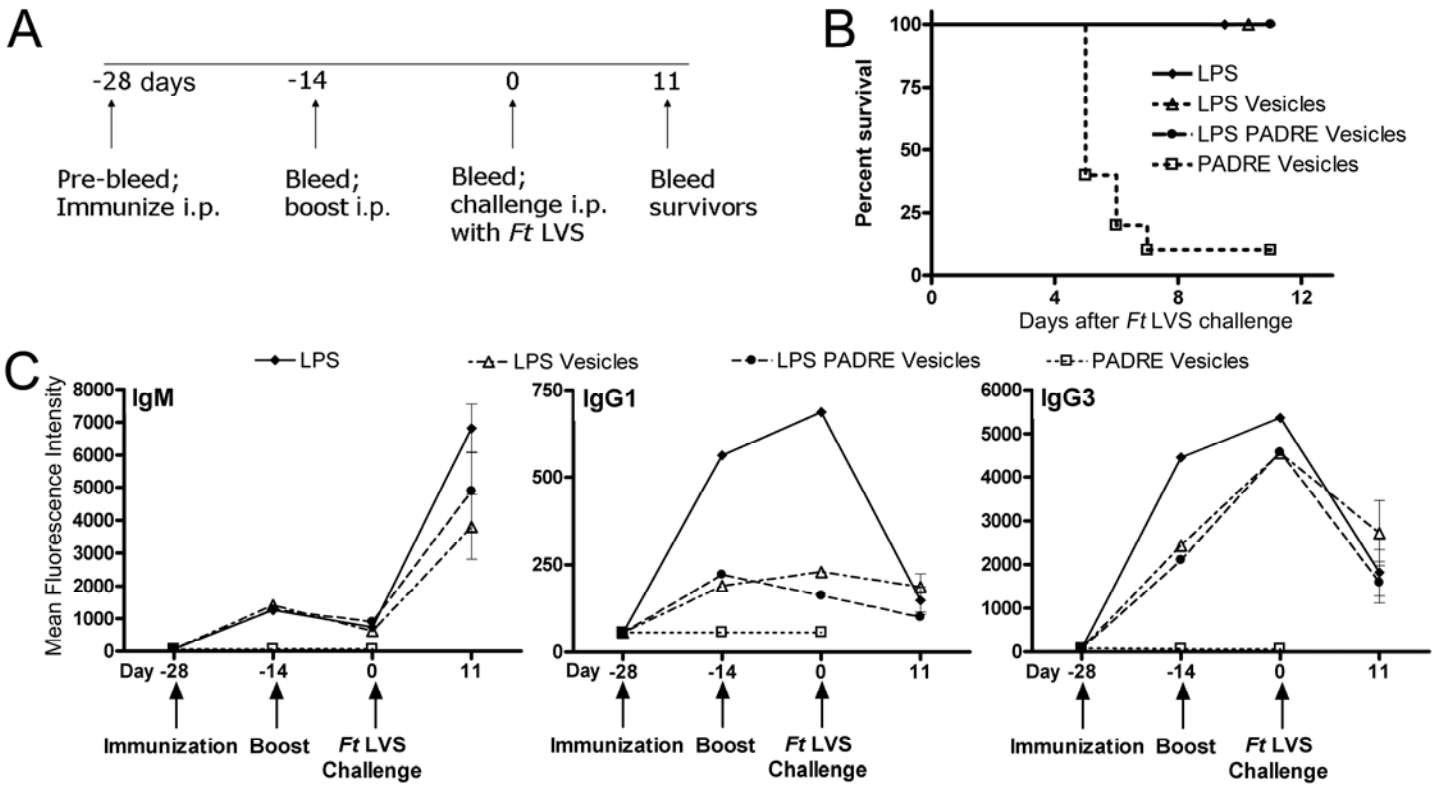
The vesicles used for immunization in Supplemental Figure 1 were functionalized as follows: The T cell epitope PADRE was conjugated to dodecanoic acid employing the N-hydroxysuccinimide methodology described below. The lipid A portion of LPS is sufficiently hydrophobic to be incorporated into the vesicle leaflet, and therefore, no modification of LPS was required. Whole LPS isolated from *Ft* LVS (1) and/or the C_{12}-modified PADRE peptide were incorporated during the assembly of catanionic vesicles as both surfactants were mixed concurrently at the same ratios as described in the text. These vesicles were administered to mice by i.p. injections twice, two weeks apart. Two weeks following the second immunization, mice were challenged with *Ft* LVS (Fig. S1) and survival of each individual mouse was followed for two weeks. Mice were bled prior to each injection and 11 days post-challenge. Antibody detection in sera were carried out by a FACS-based bead assay as previously described (2).

Conjugation of PADRE to a C_{12} hydrophobic tail. A hydrophobic tail for the PADRE peptide was attached to the amino terminus as follows: A solution of dodecanoic acid (0.539 mmol, 0.108 g), N-hydroxysuccinimide (0.573 mmol, 0.0660 g), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl) (0.574 mmol, 0.110 g) in dimethylformamide (DMF) was stirred at room temperature for 4 h. The solution was diluted with ethyl acetate (20 mL) and washed with H_2O (20 mL), saturated aqueous $NaHCO_3$ (20 mL), and H_2O (20 mL x 2). The organic layer was dried over $MgSO_4$, filtered, and concentrated

in vacuo. Purification after recrystallization (diethyl ether/hexane) resulted in 0.0977 g (58%) of N-dodecanoylsuccinimide ester as white, shiny crystals: R_f = 0.85 (diethyl ether/hexane); mp 78 – 79 °C (lit.(3) 78-81°C); IR (thin film, NaCl) 2929 (m), 2852 (m), 1744 (s); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.84 (s, 4H), δ 2.61 (t, *J* = 8 Hz, 2H), δ 1.75 (m, *J* = 8 Hz, 2H), δ 1.41 (m, 16H), δ 0.89 (t, *J* = 8 Hz, 3H).

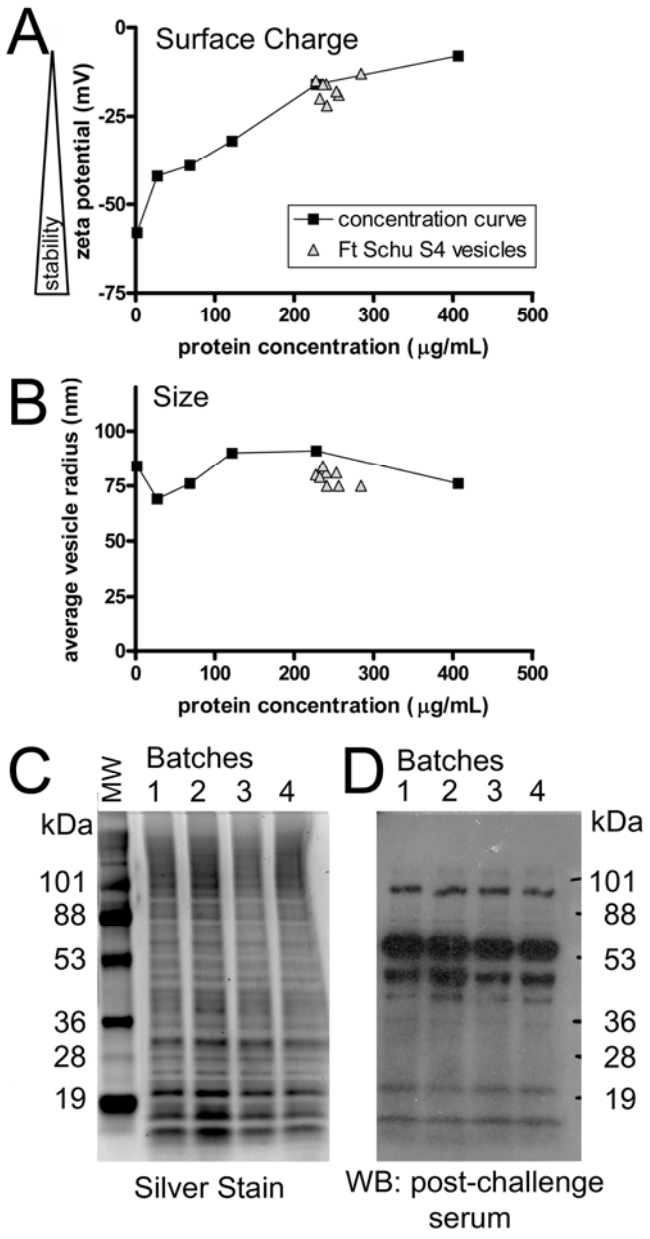
To a solution of PADRE peptide (GenScript) (1.445 μmol, 1.950 mg, 2.890 μM) in 0.5 mL of DMSO-d_6 (deuterated) was added a solution of N-dodecanoylsuccinimide (1.445 μmol, 0.430 mg, 2.89 μM) in 0.5 mL of DMSO-d_6 followed by a solution of diisopropylethylamine (50 μL, 1.44 μmol, 28.7 mM) in DMSO-d_6. The resulting solution was mixed well by vortexing and stirred at room temperature for 24 h. ^{1}H NMR analysis of the reaction mixture indicated that approximately 85% of the N-hydroxysuccinimide C_{12}-ester was reacted with PADRE by comparing the integration of starting material to product. The reaction mixture was concentrated *in vacuo* and stored at -20 °C.

Supplemental Figures

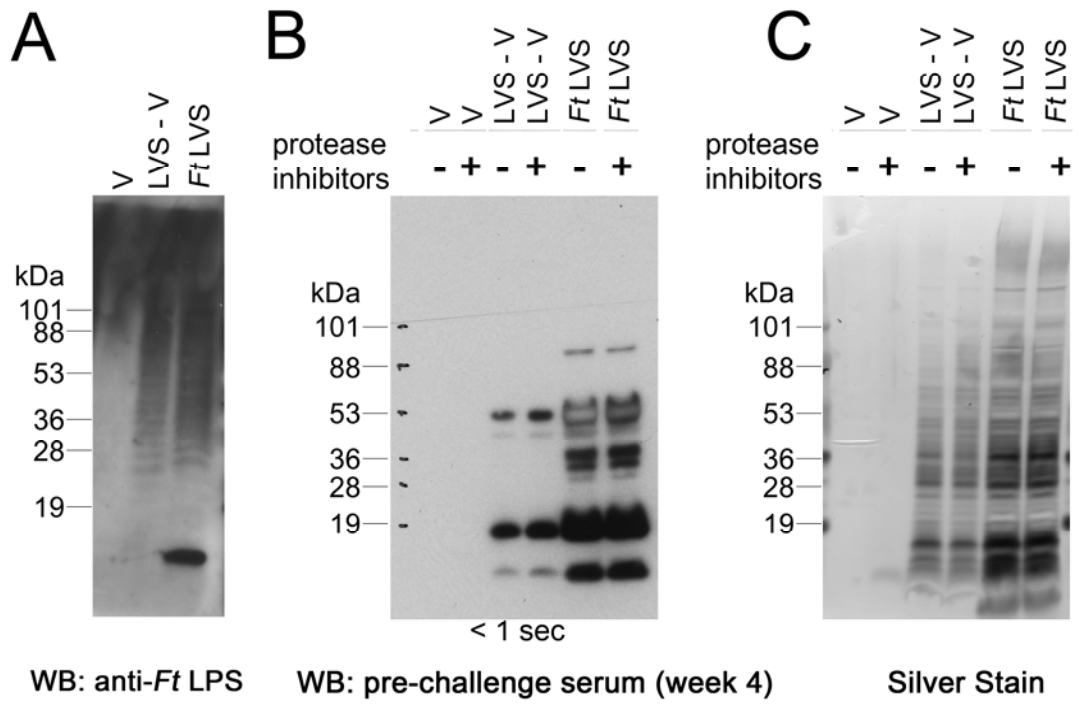


Supplemental Figure 1

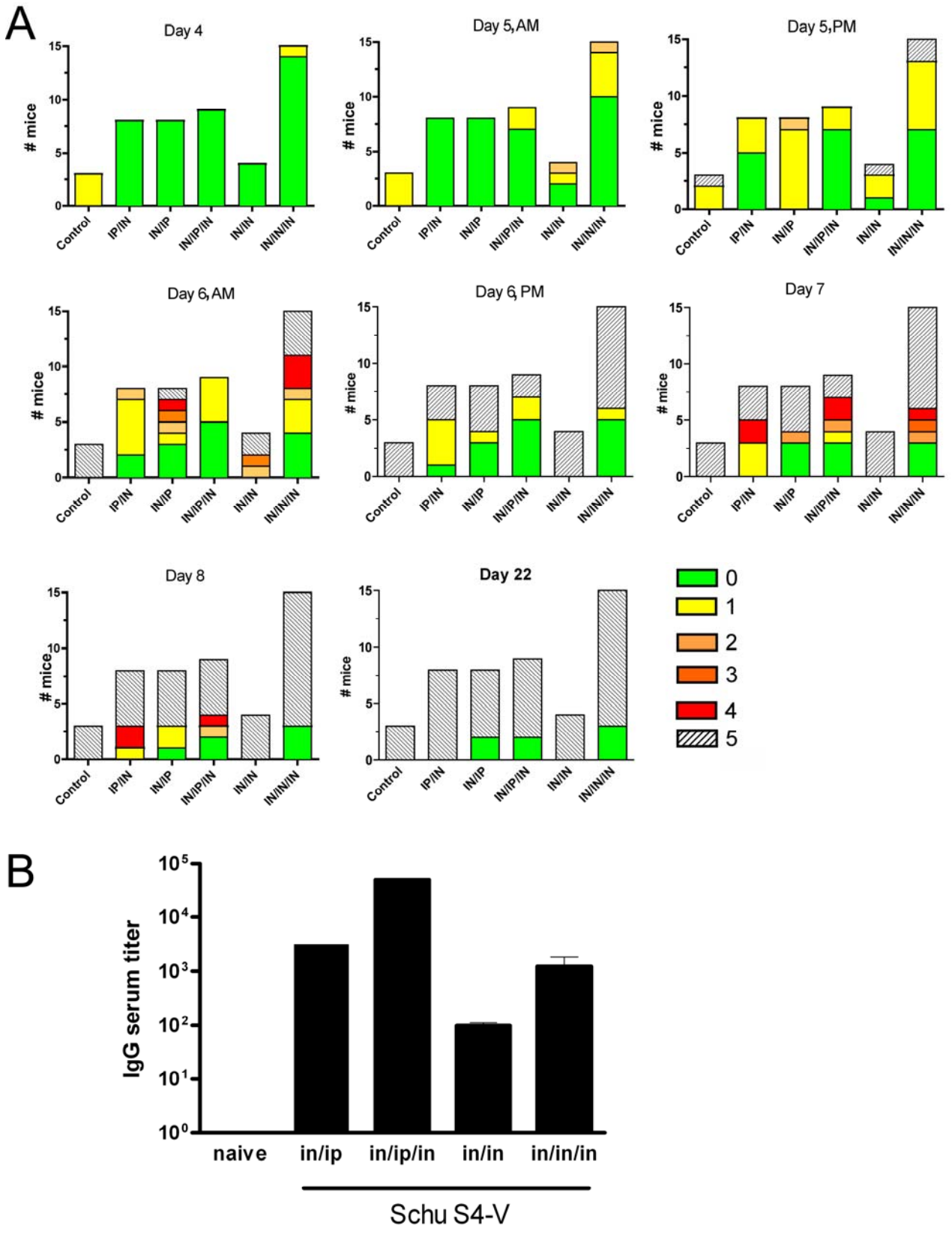
Supplemental Figure 1. Functionalized catanionic vesicles displaying *Ft* LVS LPS as the only *Ft* component are protective against *Ft* LVS challenge, but fail to induce robust IgG antibody responses. (A.) Schematic of experimental protocol. Mice were injected twice, two weeks apart (D -28, D -14), i.p., with either purified *Ft* LVS LPS in its soluble form (LPS), purified *Ft* LVS LPS included in surfactant vesicles (LPS-vesicles), the T cell epitope, C_{12}-PADRE, covalently coupled to surfactant vesicles (PADRE-vesicles), or purified *Ft* LVS LPS and C_{12}-PADRE covalently coupled to vesicles (LPS-PADRE-vesicles). Two weeks after the second immunization, all mice were challenged i.p. with ~ 6000 CFU *Ft* LVS per mouse. (B.) Survival of individual mice after the *Ft* LVS challenge. (C.) Sera were collected prior to each immunization, prior to challenge, and on the 11^{th} day following challenge. Sera from each time point were pooled, except for serum from the last time point in which each survivor was bled individually. Antibody titers to whole *Ft* LVS were measured by FACS-based bead assay utilizing isotype-specific secondary antibodies for detection as previously described (2). Error bars represent standard error of the mean for 5 mice. The results are representative of one of two separate experiments with similar outcomes.



Supplemental Figure 2. Surface charge, but not size, of vesicles is affected by the *Ft* protein content of the nanoparticles. The vesicles prepared from different sized bacterial pellets (as described in the Methods) were analyzed for (A.) zeta potential and (B.) average radius (solid squares). In addition, the same analysis was carried out for multiple individually prepared preparations of Schu S4-V (gray triangles). Each data point represents a distinct batch of vesicle vaccine. (C.) Silver stain and (D.) Western analysis of four independent batches of Schu S4-V.



Supplemental Figure 3. Catanionic vesicles associate with bacterial LPS in addition to the proteins, and addition of protease inhibitors does not significantly affect the components incorporated into the vesicles. *Ft* LVS molecules were incorporated into surfactant vesicles (V) to form LVS-vesicles (LVS-V). Lysates derived from a sampling of the bacterial colonies prior to the addition of surfactants was also included for comparison (*Ft* LVS). (A.) Samples were separated by SDS-PAGE on a 10-20% gradient gel and were subjected to Western analysis with rabbit antibodies directed against *Ft* LVS LPS. (B.-C.) V, LVS-V, and *Ft* LVS lysates were produced in the absence or presence of Roche Complete protease inhibitor cocktail. Samples were separated by SDS-PAGE on a 4-20% gradient gel and compared by (B.) Western analysis with mouse sera of LVS-V-immunized mice harvested two weeks after the second immunization, prior to Ft LVS challenge. (C.) Samples were also compared by silver staining.



Supplemental Figure 4

Supplemental Figure 4. Mice immunized with Schu S4-V show a delay in clinical symptoms after i.n. challenge with *Ft* Schu S4 and the route of immunization affects IgG titer. (A.) Clinical scoring of individual mice (see methods for criteria) on indicated days: Green (0) – healthy, Yellow (1) – mild illness, Light Orange (2) – moderate illness, Dark orange (3) – severe illness, Red (4) – moribund or dead, Black Hatch (5) – cumulative total of dead mice in each group. These results represent a single representative experiment in which all mice were assessed twice daily. (B.) *Ft*-specific IgG titers from pooled sera obtained one week after the second and third immunizations (mean and SEM).

Supplemental References

1. **Cole LE, Elkins KL, Michalek SM, Qureshi N, Eaton LJ, Rallabhandi P, Cuesta N, Vogel SN.** 2006. Immunologic Consequences of *Francisella tularensis* Live Vaccine Strain Infection: Role of the Innate Immune Response in Infection and Immunity. J. Immunol. **176:**6888-6899.
2. **Cole LE, Yang Y, Elkins KL, Fernandez ET, Qureshi N, Shlomchik MJ, Herzenberg LA, Herzenberg LA, Vogel SN.** 2009. Antigen-specific B-1a antibodies induced by *Francisella tularensis* LPS provide long-term protection against *F. tularensis* LVS challenge. Proc. Natl. Acad. Sci. U.S.A. **106:**4343-4348.
3. **Rothman ES, Serota S, Swern D.** 1964. Enol esters. II. N-Acylation of amides and imides. J. Org. Chem. **29:**646-650.