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Supporting Information for

Viral Nanoparticle Vaccines Against S100A9 Reduce Lung Tumor Seeding and Metastasis

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1. Materials

Potassium phosphate monobasic and dibasic anhydrates were purchased from Fisher. Phosphate buffered saline (PBS) was purchased from both Corning and G Biosciences. Sodium phosphate was purchased from Thermo Fisher Scientific, sodium chloride was purchased from Fisher Scientific, and ethylenediaminetetraacetic acid (EDTA) was purchased from Sigma-Aldrich. Tris acetate EDTA (TAE) and morpholinepropanesulfonic (MOPS) acid buffer were both purchased from Thermo Fisher Scientific. Tween-20 was purchased from Thermo Fisher Scientific, and bovine serum albumin (BSA) fraction V was purchased from Millipore Sigma. Pierce 1-Step Ultra 3,3',5,5'-tetramethylbenzidine (TMB) solution was purchased from Thermo Fisher Scientific. Ethanol (EtOH) was purchased from Sigma-Aldrich while bleach was purchased from Clorox. Chloroform was purchased from Fisher Scientific and 1-butanol was purchased from Sigma-Aldrich. PEG-8000 was purchased from Fisher. Magic Media was purchased from Thermo Fisher Scientific. Maleimide-polyethylene glycol$_8$-succinimidyl ester (SM(PEG)$_8$) was purchased from Sigma-Aldrich, and dimethyl sulfoxide was purchased from VWR. The S100A9 peptide (sequence: CGSGRGHGHSHGKG) was purchased from Genscript.

B16F10 (CRL-6475) and 4T1-Luc (CRL-2539-LUC2) cells were both purchased from ATCC. B16F10 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin (P/S). 4T1-Luc cells were grown in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% (v/v) FBS and 1% (v/v) P/S. DMEM and RPMI-1640 were purchased from Corning while FBS was purchased from R&D Systems. P/S was purchased from Cytiva. The cells were grown at 5% CO_2 and 37 °C.

Goat anti-mouse IgG horseradish peroxidase (HRP) secondary antibodies (Invitrogen A16072, RRID AB_2534745) and goat anti-mouse F_c IgG HRP secondary antibodies (Invitrogen A16090, RRID AB_2534764) were purchased from Thermo Fisher Scientific. Goat anti-mouse IgG2a HRP secondary antibodies were purchased from Thermo Scientific (A-10685, RRID AB_2534065) while goat anti-mouse IgG2b (ab97250, clone # unknown) and IgG2c (ab97255, clone # unknown) HRP secondary antibodies were purchased from Abcam. Goat anti-mouse IgE HRP secondary antibodies (Invitrogen PA184764, RRID AB_931454) were purchased from Fisher Scientific. Goat anti-mouse IgM HRP (ab97230, clone # unknown) and goat anti-mouse IgA (ab97235, clone # unknown) HRP secondary antibodies were purchased from Abcam.

2. Preparation of S100A9-Subunit Vaccines

Cowpea mosaic virus (CPMV) nanoparticles were propagated in black eyed pea No. 5 plants and purified as previously reported(1). Qβ virus like particles (VLPs) were expressed in Bl21 (DE3) (New England BioLabs), and purified as previously reported(2). CPMV was stored in 0.1 M potassium phosphate buffer (from here on out referred to as KP buffer) (pH 7.2) while Qβ was stored in 1x PBS pH 7.2. Both nanoparticles were stored at 4°C until further use.

Before conjugation, the buffers of CPMV and Qβ VLPs were exchanged to 10 mM KP buffer using 100 kDa, 0.5 mL molecular weight cut off (MWCO) spin filters (EMD Millipore), as instructed by the manufacturer. CPMV and Qβ VLPs were then modified by adding 10 molar equivalents per CP of the hetero-bifunctional linker, SM(PEG)$_8$, and the reaction was run for 2 hours at room temperature (RT). For the CPMV, the excess SM(PEG)$_8$ was removed using ultracentrifugation at 52,000 g for 1 h at 4°C with a 30% (w/v) sucrose cushion. The SM(PEG)$_8$ in the Qβ was removed using PD MidiTrap G-25 columns (Cytiva), as instructed by the manufacturer. The final volume of 1.5 mL was reduced down to 500 μL using the same 100 kDa

MWCO spin filters from above. The S100A9 peptide (sequence: CGSGRGHGHSHGKG) was added to both the CPMV-SM(PEG)$_8$ and the Qβ-SM(PEG)$_8$ at 1 molar equivalent per CP and allowed to react at RT for 2 hours. The excess peptide from the CPMV solution was filtered out using the same PD MidiTrap G-25 column while excess peptide from the Qβ solution was removed by dialyzing with a 12-14 kDa MWCO dialysis membrane (Avantor) in 10 mM KP. The resulting vaccine candidates CPMV-S100A9 and Qβ-S100A9 were kept at 4°C until further use.

3. Characterization of CPMV-S100A9 and Qβ-S100A9 Particles

Concentration

Native CPMV and CPMV-S100A9 particle concentrations were calculated using ultraviolet-visible (UV-VIS) spectroscopy (Nanodrop 2000) and Beer's Law. CPMV has an extinction coefficient of 8.1 mL mg^{-1} cm^{-1} at 260 nm; a ratio of 1.8 at A260/280 nm indicates intact and pure CPMV preparations. Qβ and Qβ-S100A9 concentration was carried out using a Pierce™ BCA Assay (Thermo Scientific) according to the manufacturer's protocol.

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE)

CPMV, Qβ, and S100A9-conjugated samples were loaded with 4x lithium dodecyl sulfate Sample Buffer (Life Technologies). In the samples with Qβ, an additional 10x reducing agent (Invitrogen) was added to break disulfide bonds between CPs. The particles were denatured at 95 °C for 5 min, loaded onto a 12% NuPAGE gel (ThermoFisher Scientific), and run at 200 V, 120 mA, and 25 W for 40 min in 1x MOPS buffer. The gels were then visualized using GelCode™ Blue Safe Protein Stain (ThermoFisher Scientific), and imaged on an AlphaImager System (Protein Simple). The number of peptides conjugated to each CP of CPMV and Qβ was calculated using densitometry analysis on ImageJ.

Agarose gel electrophoresis

The samples were stained with 6x Gel Loading Purple dye (Biolabs) before loading onto a 0.8% (w/v) agarose gel stained with 1 μL of GelRed nucleic acid gel stain (Gold Biotechnologies) in 1x TAE buffer. The gels were run for 30 min at 120 V and 400 mA. RNA was imaged under UV light using the AlphaImager System, and the protein bands were imaged using a 0.25% (w/v) Coomassie Blue stain.

Transmission electron microscopy (TEM)

All samples (10 μg protein) were mounted onto Formvar carbon film coated TEM grids (VWR International) and stained with 2% (w/v) uranyl acetate in deionized water prior to imaging using a FEI Tecnai Spirit G2 BioTWIN TEM.

Size exclusion chromatography (SEC)

SEC was performed by fast protein liquid chromatography using an Äkta Pure (Cytiva) with a Superose 6 Increase 10/300 GL column (dimensions: 10 x 300 mm with exclusion limit of 4 x 10^7 M_r). Samples were diluted to 0.1 mg mL^{-1} in their respective buffers, and absorbance was measured at 260 and 280 nm with an isocratic elution profile.

Dynamic light scattering (DLS)

DLS measurements were carried out on a Zetasizer Nano ZSP/Zen5600 (Malvern Panalytical). The samples were diluted to 0.1 mg mL^{-1} in 10 mM KP and measured at RT.

4. Animal Immunization

All animal experiments were carried out in accordance with the guidelines set out by the IACUC of the University of California, San Diego. All mice were purchased from Jackson Labs and housed at the Moores Cancer Center. The mice were granted unlimited food and water at all times.

C57BL/6J mice were subject to a prime and double-boost vaccine regimen with the injections spaced two weeks apart. The initial groups tested were CPMV, CPMV-S100A9, Qβ, Qβ-S100A9, PBS, and S100A9 peptide only delivered through subcutaneous (s.c.) injection. The viruses were diluted to 1 mg mL^{-1} in PBS and a total of 200 μL was administered (200 μg per dose); the S100A9 peptide concentration was determined through densitometry analysis of peptide-conjugation efficiency from SDS-PAGE gels of the Qβ-S100A9 vaccine. Two weeks after the last boost (Week 6), the mice were injected intravenously (i.v.) through the tail vein with either 50,000 or 100,000 B16F10 melanoma cells (in 100 μL of PBS) per mouse. The blood of the mice was also collected by subjecting mice to retroorbital (r.o.) bleeding every two weeks starting from the first injection all the way to Week 8. Serum was isolated from the blood by spinning down the blood at 2000 x g for 10 min at 4°C and collecting the clear supernatant. Samples were stored at -80°C until further use.

BALB/C mice received the same double-boost regiment at the same dose (200 μg) through s.c. injection. In BALB/C mice, only the Qβ, Qβ-S100A9, PBS, and S100A9 peptide groups were tested. Two weeks after the last boost (Week 6), 50,000 4T1-Luc cells that express luciferase were injected i.v. through the tail vein in 100 μL of PBS. Serum was also collected from the mice at two-week intervals until Week 6. Samples were stored at -80°C until further use.

5. Antibody Titers against S100A9 Peptide

Sera was analyzed by ELISA against the S100A9 peptide. Maleimide-activated plates (Thermo Fisher Scientific) were utilized for ELISA analysis due to the terminal cysteine in the linker of the S100A9 peptide (sequence: CGSGRGHGHSHGKG) in accordance with the manufacturer's protocols. Briefly, plates were first washed with 200 μL of PBS + 0.1% (v/v) Tween-20 (PBST) three times before coating with 100 μL of 25 μg mL^{-1} of peptide diluted in 0.1 M sodium phosphate, 0.15 M sodium chloride, 10 mM EDTA, pH 7.2 (binding buffer). The peptide was incubated at 4°C overnight. Excess peptide was washed away with three 200 μL washes of PBST. The plates were blocked with 100 μL of 10 μg mL^{-1} L-cysteine (Sigma-Aldrich) in binding buffer for 1 hr at RT followed by 3 washes with PBST. The sera from the mice were diluted in binding buffer at a starting dilution of 1:200 followed by 2-fold dilutions all the way down to a final dilution of 1:25,600. Qβ-S100A9 samples from the C57BL/6J mice started at a dilution of 1:10,000 all the way down to a final dilution of 1:1,280,000. The sera collected from Section 4 were then added to the wells and incubated for 1 hr at RT. Following three PBST wash steps, goat anti-mouse horseradish HRP IgG secondary antibodies specific to the Fc region were diluted in PBST (1:5,000 dilution), and 100 μL of the antibody solution was added and incubated at RT for 1 hr. After one last wash step, 100 μL of 1-Step Ultra TMB was added and incubated at RT for 5 minutes (C57BL/6J samples) or 1 minute (BALB/C samples) before 100 μL addition of 2N H_2SO_4 to quench the reaction. Absorbance was read at 450 nm on a Tecan microplate reader, and endpoint titers were calculated as the dilution at which the absorbance was greater than twice the absorbance of the blank.

Antibody Isotyping

The CPMV-S100A9 and Qβ-S100A9 samples were further analyzed by ELISA as before except that at the sera addition step, the sera from five mice at each time point were pooled at a final concentration of 1:1000, which was then run in triplicate. At the secondary antibody step, isotype-specific HRP antibodies (IgG_{total}, IgG1, IgG2a, IgG2b, IgG2c, IgA, IgM, and IgE) were used instead. All secondary antibodies were used at 1:5000 dilutions except for IgG2a and IgE antibodies, which were diluted 1:1000. The IgG2b $IgG1^{-1}$ and IgG2c $IgG1^{-1}$ ratio was calculated for C57BL/6J mice, and a ratio < 1 was considered to be a Th2 response. For BALB/C mice, the IgG2b $IgG1^{-1}$ and IgG2a IgG^{-1} ratio was utilized.

6. Antibody Titers Against VNP/VLPs

The protocol for testing antibody titers against CPMV and Qβ was modified slightly from Section 5. During the coating step, 20 μg mL^{-1} of CPMV or Qβ in PBS was used to coat Microlon 200 plates (Greiner Bio-One) before overnight incubation at 4°C. Blocking was carried out by adding 100 μL of 3% (w/v) BSA in PBS for 1 hr at RT. Sera and secondary antibody addition steps remained unchanged; however, TMB was reacted with the HRP secondary antibody for 8 min before quenching the reaction with 2N H_2SO_4. CPMV titers were only tested in C57BL/6J mice while Qβ titers were examined in both C57BL/6J and BALB/C mice.

7. Western Blot (WB) and Dot Blot (DB) against Full-Length S100A8 and S100A9

WB and DB assays were used to test the ability of the antibodies in the mice sera in binding full-length S100A9 without cross-reacting with S100A8. First, for the WBs, 10 μg of full-length S100A8 and S100A9 proteins (Sino Biological) were run through SDS-PAGE like before, and the gels were transferred over to nitrocellulose paper (VWR) for 1 hr at 25 V, 160 mA, and 17 W. The nitrocellulose was blocked with 10% (w/v) skim milk (Research Product International) diluted in PBS for 1 hr at RT. The paper was then washed 3 times with PBS with 5 min soaks between washes. The sera from five mice at the Week 6 timepoint of the CPMV-S100A9, Qβ-S100A9, and S100A9 peptide only samples were pooled and diluted 1:100 in PBS and incubated with the nitrocellulose paper for 1 hr at RT. The paper was then subject to the same wash steps and incubated with a goat anti-mouse HRP secondary antibody (1:5000 dilution in PBS) for 1 hr at RT. Following washing, a 3,3'-diaminobenzidine (DAB) substrate (Vector Laboratories) was added for 2 min, washed away with PBS, and then imaged on the AlphaImager system.

For the DBs, 4.5 μg of the full-length S100A8 or S100A9 protein was directly added onto the nitrocellulose paper. The paper was blocked with 10% (w/v) skim milk in PBS for 30 min at RT and washed 3 times with PBS. The mice sera from each of the CPMV, Qβ, CPMV-S100A9, Qβ-S100A9, PBS, and S100A9 peptide only groups were pooled separately and diluted 1:100 in PBS before incubation onto the nitrocellulose paper overnight at 4°C. The paper was washed 3 times with PBS, and a goat anti-mouse HRP secondary antibody (1:5000 dilution in PBS) was incubated for 1 hr at RT. After another round of washing, the DAB substrate was added for 2 min before the reaction was stopped by washing away the substrate with PBS and imaging on the AlphaImager system.

8. Lung Tumor Seeding B16F10 and 4T1-Luc Model

Naïve and vaccinated mice were challenged by i.v. injection using B16F10 melanoma cells (50,000 or 100,000 cells) or 4T1-Luc TNBC cells (50,000 cells) as described in Section 4. In the animals injected with 50,000 B16F10 cells, the lungs were harvested after three weeks and moved into 10 mL of 10% (v/v) neutral-buffered formalin solution (Sigma-Aldrich). The lungs of

animals injected with 100,000 B16F10 cells were collected after two weeks. The next day, the lungs were moved from the formalin into 70% (v/v) EtOH and tumor nodules were manually counted.

The mice that were injected with 4T1-Luc were analyzed *via* luminescence imaging. The mice were injected intraperitoneally (i.p.) with 150 mg kg^{-1} of D-luciferin (Gold Biotechnologies) and imaged using the in vivo imaging system (IVIS) (Xenogen) every two days starting four days post tumor injection (PTI). The luminescence of the lungs was measured through region of interest (ROI) measurements using the Living Image 3.0 software, and the weight of the mice was also measured during imaging. Two weeks PTI, the lungs were harvested and stored in 10 mL of Bouin's solution (Sigma-Aldrich) overnight and moved to 70% EtOH the next day. The tumor nodules were then manually counted.

9. 4T1-Luc Primary Cancer Surgical Removal Metastasis Study

BALB/C mice were injected s.c. in the left flank with 200,000 4T1-Luc cells in 100 μL of PBS. Three days PTI, the mice were injected with either PBS, Qβ, Qβ-S100A9, or S100A9 peptide only (200 μg $mouse^{-1}$) in a prime and double-boost vaccine regimen as described in Section 4. Between the first and second injection, at day 14 PTI, the tumors were surgically removed, and the skin was sutured using Vetbond tissue adhesive (3M). Following surgery, mice were given the anesthetic lidocaine (Vet One). The mice were then subjected to luminescence imaging as in Section 8, and ROI measurements of the lungs were taken to assess lung metastasis between groups.

10. S100A8/9 Levels in the Lungs and Serum Following Vaccination

A mouse S100A8/9 detection kit (R&D Systems) was used to determine the levels of S100A8/9 in the lungs and sera; vaccinated and unvaccinated groups were studied pre- and post-tumor challenge using 50,000 B16F10 or 4T1-Luc cells (i.v. in 100 μL) in C57BL/6J or BALB/C mice. The lungs of the C57BL/6J mice were harvested at weeks 0, 2, and 3 post-tumor challenge; the lungs of the BALB/C mice were harvested at weeks 0, 1 and 2 and stored at -20 °C until further use. The lungs were thawed and then homogenized with a LabGEN 125 homogenizer (Cole-Parmer) in 1 mL of PBS. The homogenate was centrifuged at 10,000 g for 10 min, and the supernatants were stored at -80°C until S100A8/9 detection by ELISA as instructed by the manufacturer (R&D Systems). In brief, Maxisorp plates (Thermo Scientific) were coated with 100 μL of the capture antibody at 4 μg mL^{-1} and incubated at 4°C overnight on a platform shaker. The next day, the plates were washed 3 times with PBST and blocked with 1% (w/v) BSA in PBS for one hour at RT. After a wash step, the lung homogenates were diluted 1:200 in PBST and serially diluted to a final dilution of 1:25600 before incubation for 1.5 h at RT. The plates were washed, and 100 μL of a 40 ng mL^{-1} detection antibody solution was incubated for 1.5 h at RT. Following washing, 100 μL of streptavidin-HRP (diluted 40 x from the stock) was incubated for 20 min at RT. The excess streptavidin-HRP was washed away and 100 μL of TMB was added for 20 min followed by 100 μL addition of 2N H_2SO_4. The plates were then read at 450 nm on a Tecan microplate reader.

Sera was collected from both vaccinated and unvaccinated mice at the same timepoints as with the lung S100A8/9 detection, and the sera was isolated and investigated for S100A8/9 levels using ELISA according to the manufacturer's protocols (R&D Systems). The lungs of the mice were also collected, and the tumor burden within the lungs at the last timepoint (week 2 for 4T1-Luc, week 3 for B16F10) were compared to correlate tumor burden with S100A8/9 sera levels.

11. Bacterial Load in Lungs Following Vaccination

BALB/C mice were vaccinated as before, and 2 weeks post-vaccination, the lungs were harvested. Lungs from unvaccinated age-matched mice were also collected. The lungs were then homogenized in 5 mL of PBS, and 20 μL of the solution was plated onto an agar plate. Following incubation at 37°C, the number of bacterial colonies were counted.

12. Cytokine Analysis of Lungs

The lungs of vaccinated and unvaccinated BALB/C and C57BL/6J mice were harvested and analyzed for expression of IL-6, IL-10, IL-12, TGFβ, and IFNγ through ELISA according to the manufacturer's protocols (ThermoFisher). In unvaccinated mice, BALB/C and C57BL/6J mice were injected i.v. with 50,000 4T1-Luc and B16F10 cells in 200 μL of PBS, respectively. The lungs of mice injected with 4T1-Luc were harvested 1 and 2 weeks post tumor inoculation, and the lungs of B16F10-inoculated mice were harvested 2 and 3 weeks post tumor inoculation. In vaccinated mice, the same injection and lung harvesting schedule was followed. Following lung harvesting, the lungs were dipped immediately into liquid nitrogen and stored at -80°C until further use. The lungs were then individually weighed, and 10 mL per gram of tissue extraction reagent II (ThermoFisher) supplemented with a protease inhibitor cocktail (ThermoFisher) and 10 mM PMSF was added. The lungs were homogenized, and incubated in the tissue extraction buffer for 2 h at 4°C followed by centrifugation at 10,000 x g at 4°C. The supernatant was collected and analyzed by ELISA according to the manufacturer's protocols.

13. Flow Cytometry of Lungs

The lungs of unvaccinated and vaccinated BALB/C and C57BL/6J mice were analyzed using flow cytometry at the same timepoints as with the cytokine analysis. At each timepoint, the lungs were harvested and immediately digested using a lung dissociation kit (Miltenyi Biotec) according to the manufacturer's protocol. In brief, each lung was added to 2.4 mL of 1x buffer S, 100 μL of enzyme D, and 100 μL of enzyme A in a gentleMACS C tube (Militenyi Biotec). The lungs were digested using the 37C_m_LDK_1 protocol with a gentleMACS dissociator and then centrifuged at 500 x g for 5 min at 4°C. The pellet was resuspended in 2 mL of RPMI and then strained over a 70 μm cell strainer. The solution was centrifuged again at 300 x g for 10 min at 4°C, and red blood cells were lysed with 1x red blood cell lysis buffer (eBioscience) for 5 min. Following centrifugation, the cells were counted and diluted to 1 x 10^7 cells mL^{-1} in 100 μL of PBS.

The isolated cells were then added to a 96-well V-shape bottom plate and spun down at 500 x g for 5 min at 4°C. The supernatant was removed, and the cells were stained with LIVE/DEAD Aqua (Thermo Scientific) diluted 1:1000 in PBS for 20 min at RT. The cells were washed once with 100 μL of FACS buffer (48.15 mL of 1x PBS + 100 μL of 0.5M EDTA + 500 μL of FBS + 1.25 mL of 1M HEPES) and blocked in 1 μg mL^{-1} of Fc block (Biolegend) solution (101301, [93]) in FACS buffer for 20 min at 4°C. The cells were washed twice, and stained with the following antibodies (all purchased from Biolegend except for the Ly6G antibody, which was purchased at ThermoFisher) at a 1:500 dilution for 1 hr at RT: Pacific Blue CD45 (103125, [30-F11]), SuperBright 645-CD11b (101207, [M1/70]), PE-eFluor610-Ly6G (61-9668-82, [1A8-Ly6G]), and PE/Cy7-Ly6C (128017, [HK1.4]). The cells were washed twice with FACS buffer and fixed with 1x BD fixative solution diluted in deionized water for 10 min at RT. The cells were washed twice with FACS buffer and then kept and stored in FACS buffer at 4°C until further use. Flow cytometry was done using a BD FACSCelesta, and data analysis was done using FlowJo.

14. Statistical Analysis

All analyses were done on GraphPad Prism. The tumor nodule counts were compared using one-way ANOVA and Tukey's multiple comparisons test. S100A8/9 levels, cytokine levels, and MDSC populations were analyzed using Student's T-test

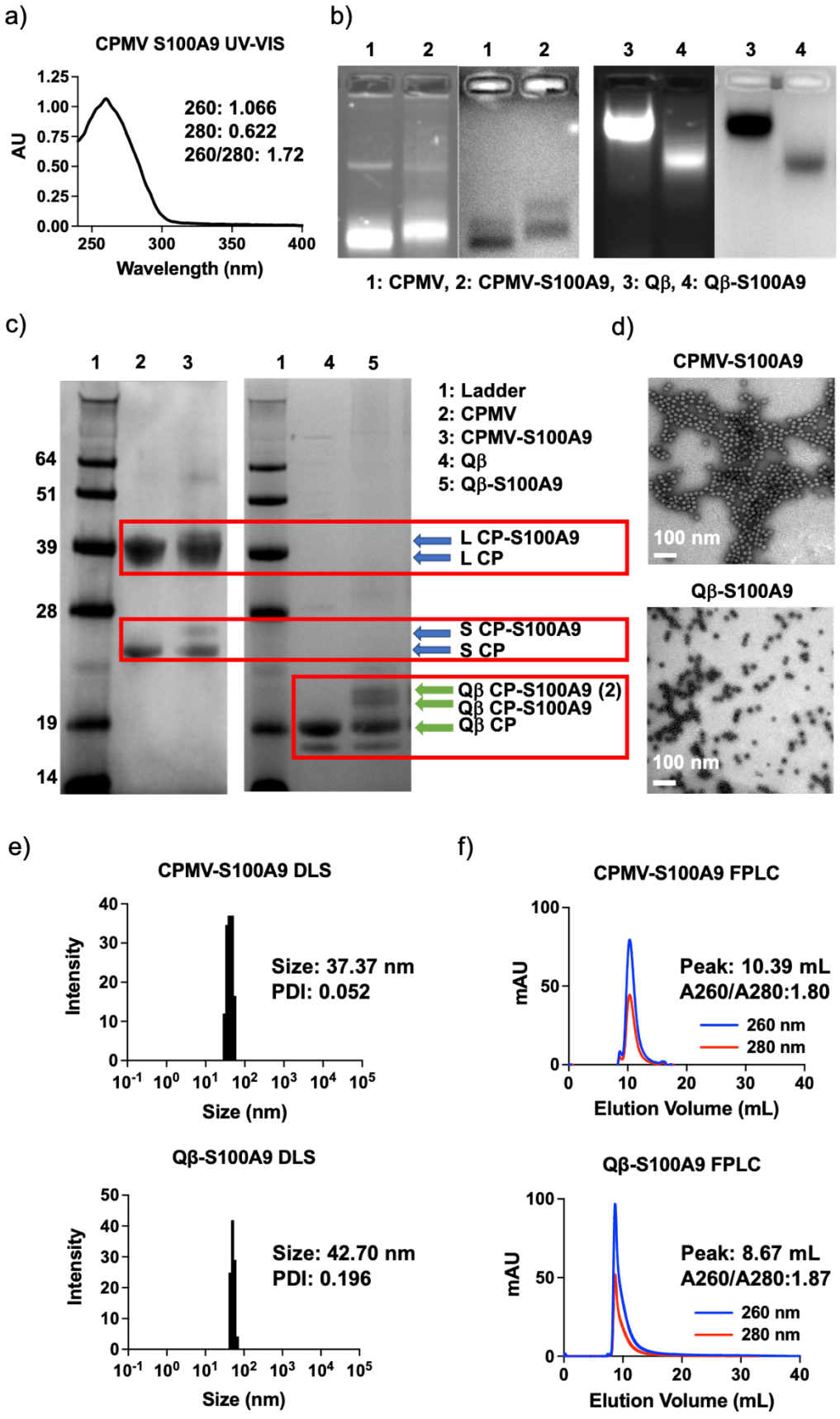

a)
CPMV S100A9 UV-VIS
AU
1.25
1.00
0.75
0.50
0.25
0.00
250
300
350
400
Wavelength (nm)
260: 1.066
280: 0.622
260/280: 1.72
b)
1 2 1 2 3 4 3 4
1: CPMV, 2: CPMV-S100A9, 3: Qβ, 4: Qβ-S100A9
c)
1 2 3 1 4 5
64
51
39
28
19
14
1: Ladder
2: CPMV
3: CPMV-S100A9
4: Qβ
5: Qβ-S100A9
L CP-S100A9
L CP
S CP-S100A9
S CP
Qβ CP-S100A9 (2)
Qβ CP-S100A9
Qβ CP
d)
CPMV-S100A9
100 nm
Qβ-S100A9
100 nm
e)
CPMV-S100A9 DLS
Intensity
Size: 37.37 nm
PDI: 0.052
Size (nm)
Qβ-S100A9 DLS
Intensity
Size: 42.70 nm
PDI: 0.196
Size (nm)
f)
CPMV-S100A9 FPLC
mAU
Peak: 10.39 mL
A260/A280:1.80
260 nm
280 nm
Elution Volume (mL)
Qβ-S100A9 FPLC
mAU
Peak: 8.67 mL
A260/A280:1.87
260 nm
280 nm
Elution Volume (mL)

Figure S1: Characterization of CPMV-S100A9 and Qβ-S100A9 nanoparticles. a) UV-VIS spectra of CPMV-S100A9. The inset indicates the absorbance at 260 and 280 nm as well as the absorbance ratio (A260/A280). b) Agarose gel electrophoresis of CPMV-S100A9 and Qβ-S100A9 particles. The left gels are the nucleic acid stains while the right gels are protein stains. Qβ encapsulates host RNA during capsid formation, which can be stained. c) SDS-PAGE of CPMV-S100A9 and Qβ-S100A9. The appearance of slower mobility bands above the CPs of CPMV-S100A9 and Qβ-S100A9 demonstrate successful conjugation of peptides to the CPs. S CP = small coat protein, L CP = large coat protein. d) TEM of CPMV-S100A9 and Qβ-S100A9. e) DLS of CPMV-S100A9 and Qβ-S100A9. The inset shows the measured average size of the particles as well as the PDI. f) SEC elution profiles of CPMV-S100A9 and Qβ-S100A9. The inset shows the elution peak of the particles as well as the absorbance ratio of 260 to 280 nm at that peak.

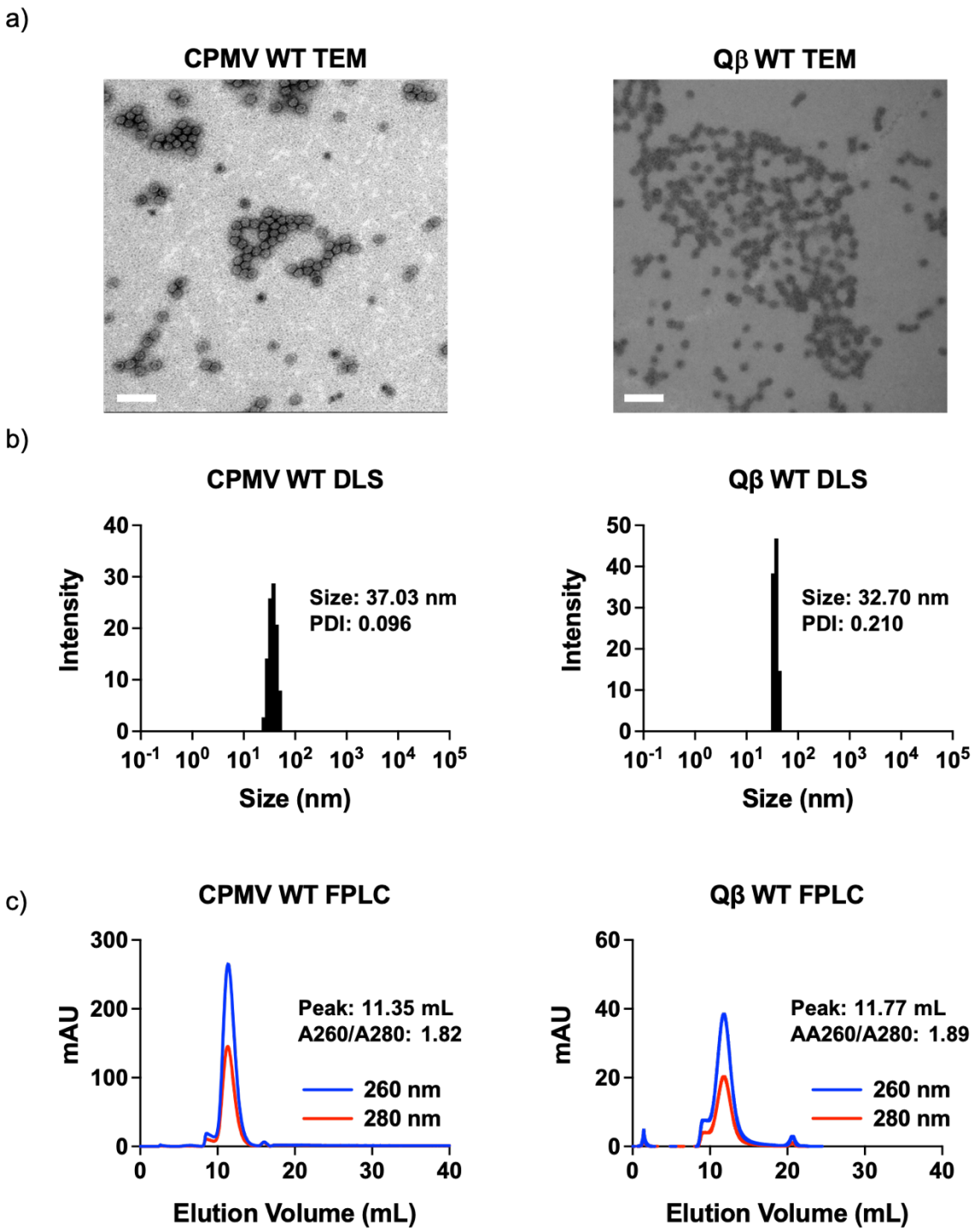


Figure S2: Characterization of WT CPMV and Qβ particles. a) TEM. The scale bar represents 100 nm. b) DLS. The inset is showing the measured size as well as the polydispersity index of the particles. c) FPLC. The inset is showcasing the elution peak of the particles through an SEC as well as the ratio of the absorbances at 260 and 280 nm at the elution peak.

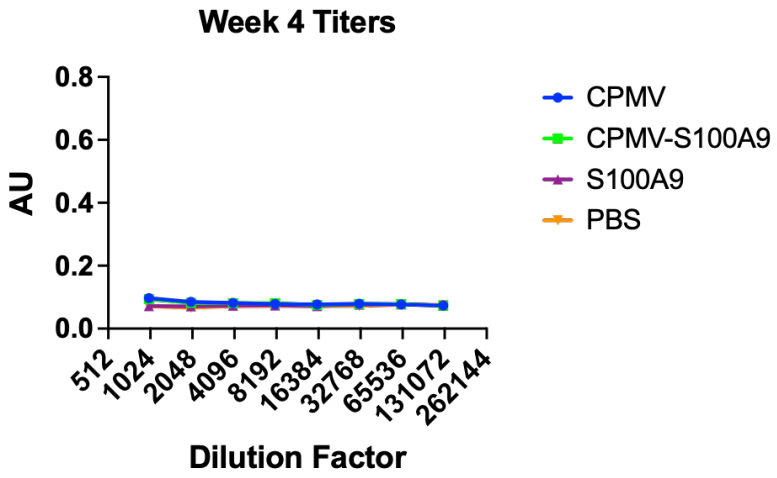
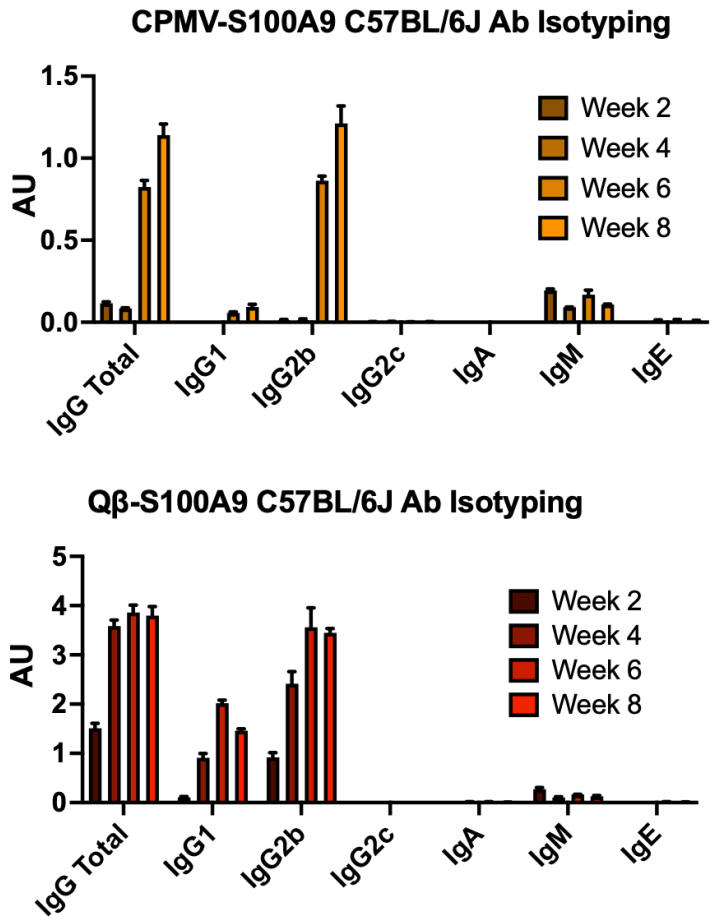


Figure S3: Titers against the S100A9 peptide in BALB/C mice vaccinated with CPMV-S100A9. CPMV-S100A9 was unable to generate any titers by week 4 in BALB/C mice (n = 2-3).

a)



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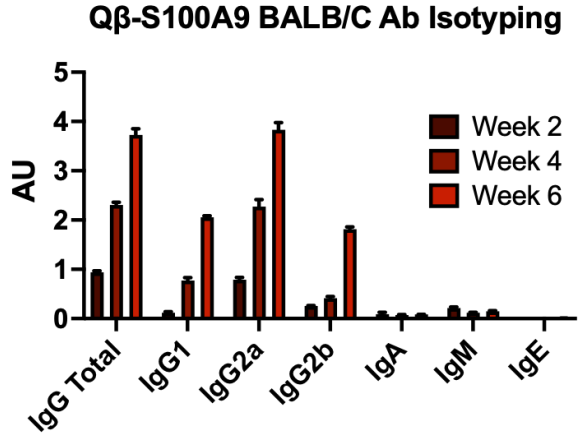


Figure S4: Complete isotyping of the CPMV and Qβ-S1009 vaccines. a) Isotyping of the sera in C57BL/6J mice injected with CPMV and Qβ-S100A9. b) Isotyping of the sera in BALB/C mice injected with Qβ-S100A9. All samples were run in duplicate or triplicate, and the error bars represent the standard deviation.

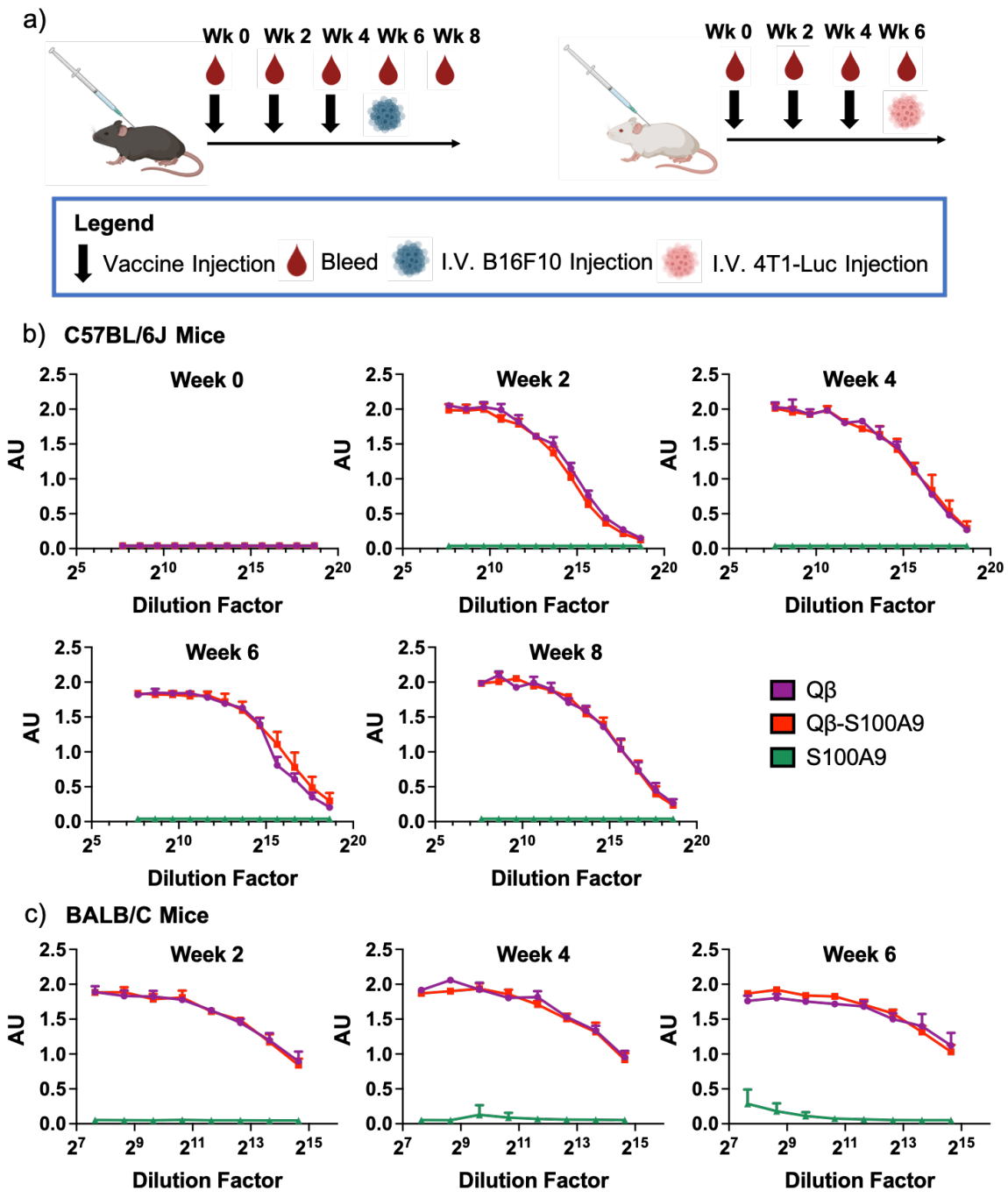


Figure S5: Generation of antibodies against Qβ in both C57BL/6J and BALB/C mice. a) Injection and bleeding schedule. b) Antibody titers against the Qβ coat protein in C57BL/6J mice (n = 3-4) injected with Qβ, Qβ-S100A9, and S100A9. c) Antibody titers against the Qβ coat protein in BALB/C mice (n = 2-3) injected with Qβ, Qβ-S100A9, and S100A9. The injection schedule schematic was created on Biorender.com. The error bars represent the standard deviation.

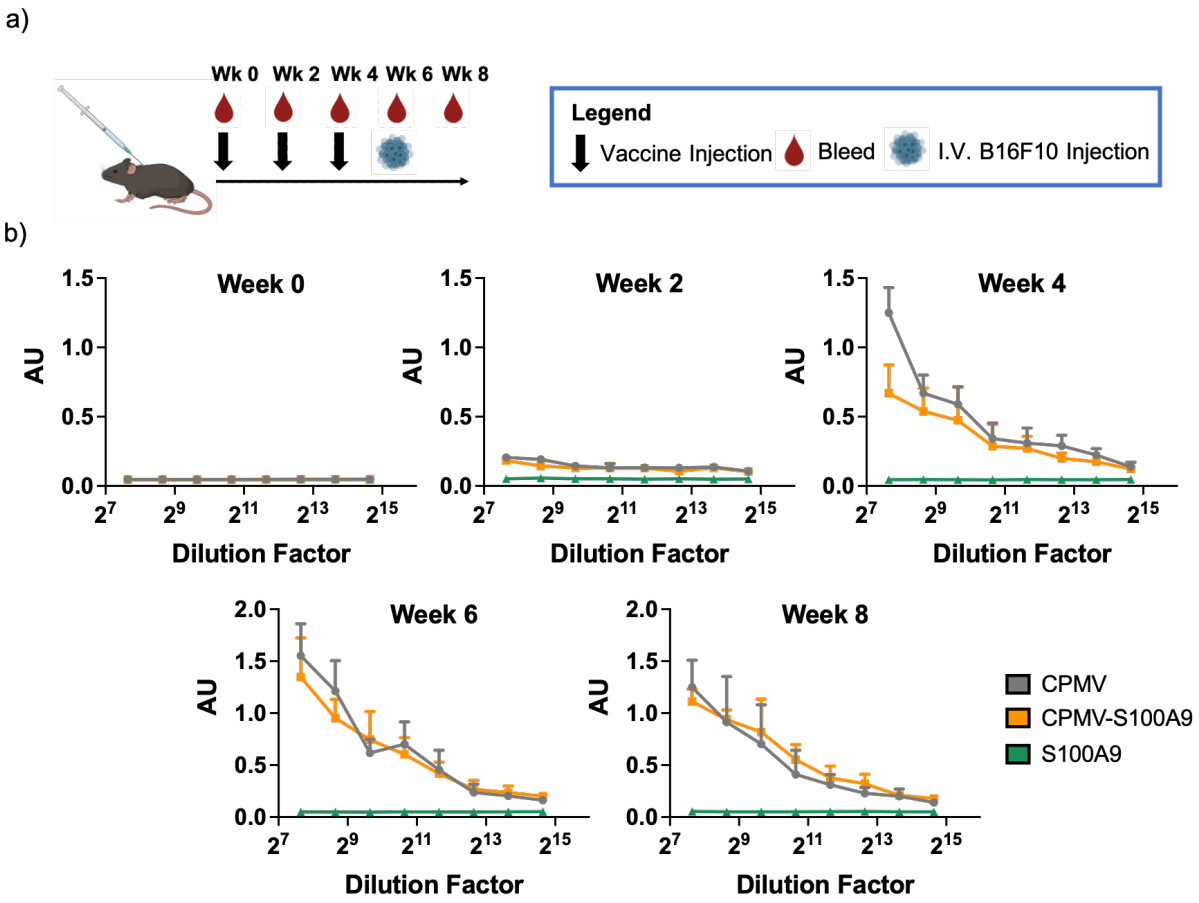


Figure S6: Generation of antibodies against CPMV in C57BL/6J mice. a) Injection and bleeding schedule. b) Antibody titers against the CPMV coat protein in mice (n = 3-4) injected with CPMV, CPMV-S100A9, and S100A9. The CPMV-S100A9 vaccine was not tested in BALB/C mice, as CPMV-S100A9 was unable to produce titers against the S100A9 peptide in BALB/C mice (**Figure S3**). The injection schedule schematic was created on Biorender.com. The error bars represent the standard deviation.

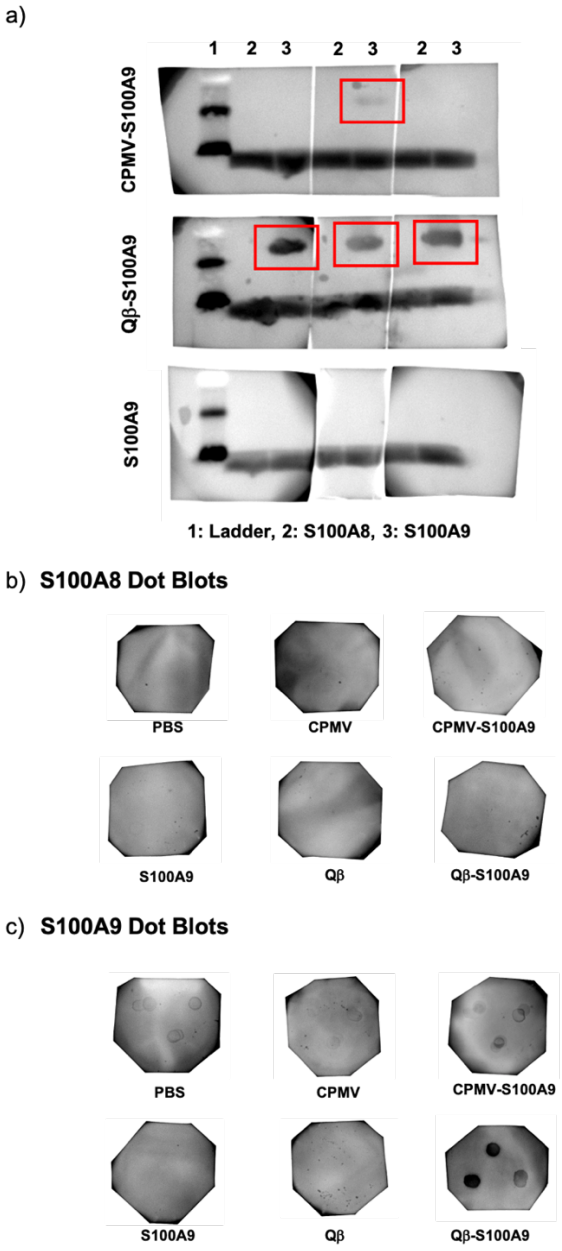


Figure S7: WBs and DBs against full-length S100A8 and S100A9. a) WBs against full-length S100A8 and S100A9 using the sera from C57BL/6J mice injected with CPMV-S100A9, Qβ-S100A9, and S100A9. The red boxes are highlighting the signals produced during the WBs. The blots show that the antibodies produced following vaccination can bind to full-length S100A9, but not S100A8, a close family member to S100A9. b) DBs against full-length S100A8 using the sera from C57BL/6J mice. For the DBs, the sera from the groups of each vaccinated mice were pooled. There is no signal in any of the DBs indicating that the antibodies produced during vaccination do not bind to S100A8. c) DBs against full-length S100A9 using pooled sera from C57BL/6J mice. The signal from Qβ-S100A9 and CPMV-S100A9 indicate that the antibodies produced can bind to full-length, native S100A9.

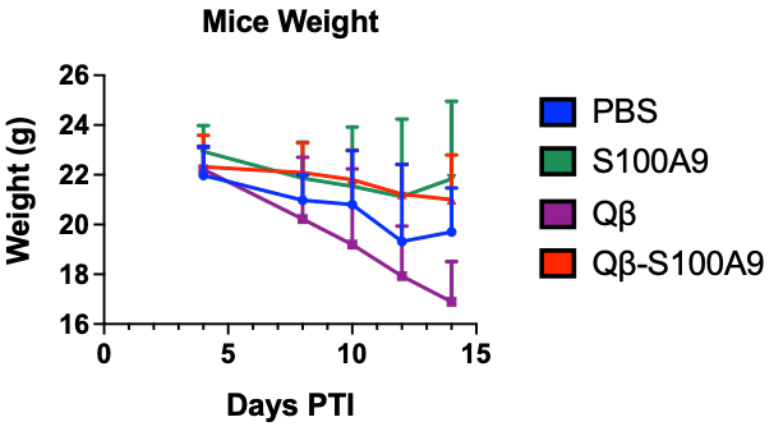
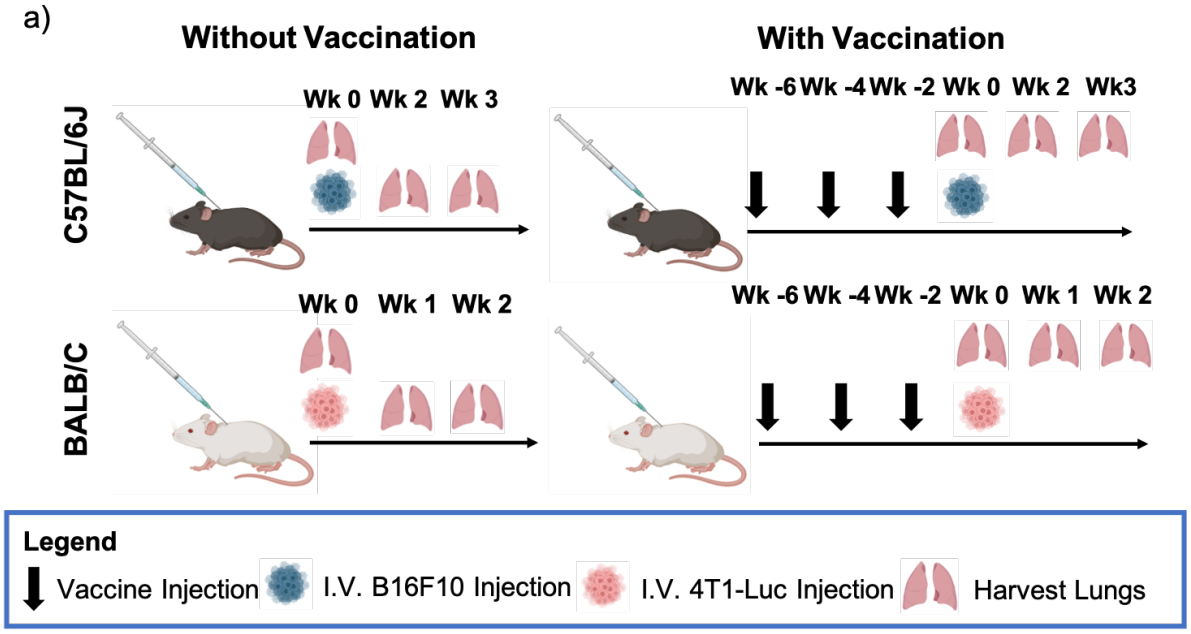


Figure S8: Weight measurements from the BALB/C mice used in Figure 3. The weight of the mice in the Qβ and PBS mice fell continuously until the last measurement. The data is only shown until day 14 as the lungs were harvested on day 14 for tumor nodule counting. The error bars represent the standard deviation (n = 4-10).



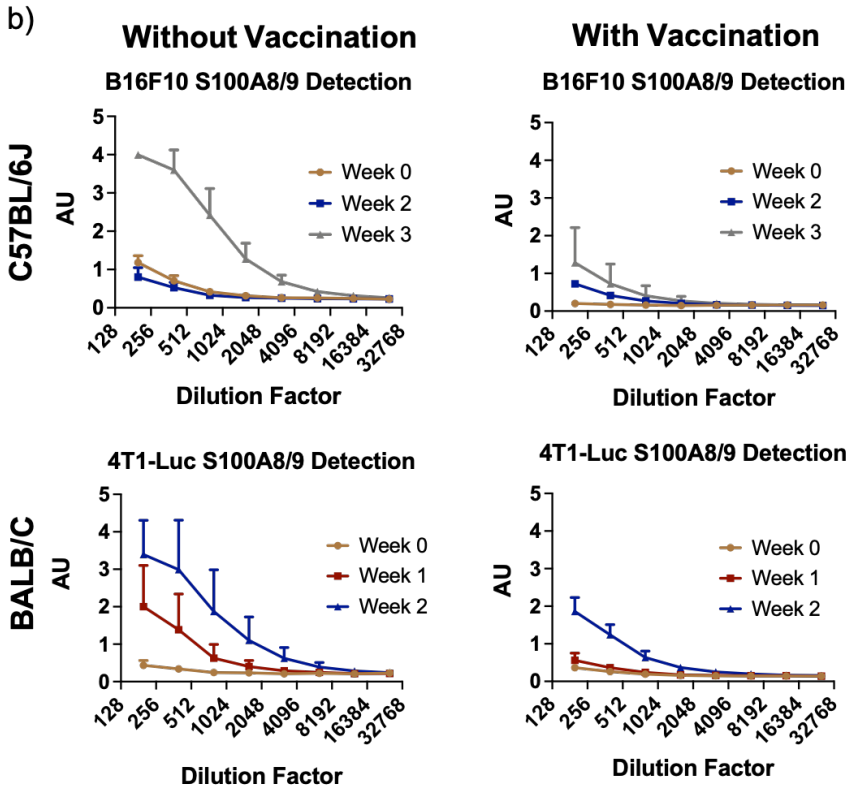


Figure S9: Complete ELISAs of the S100A8/9 measurements within the lungs of mice. a) Injection and lung harvesting schedule. b) ELISA measurements of S100A8/9 within the lungs of vaccinated and unvaccinated C57BL6/J and BALB/C mice (n = 3) using an S100A8/9 detection kit (R&D Systems). The error bars represent the standard deviation. The injection schedule schematic was created on Biorender.com.

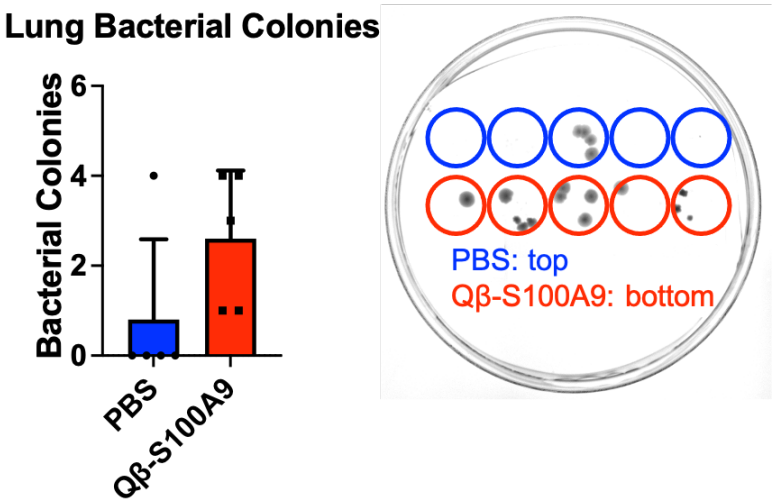


Figure S10: Bacterial load within lungs of vaccinated mice. The left graph is a quantitative measure of the number of bacterial colonies on the right (n = 5). The error bars represent the standard deviation.

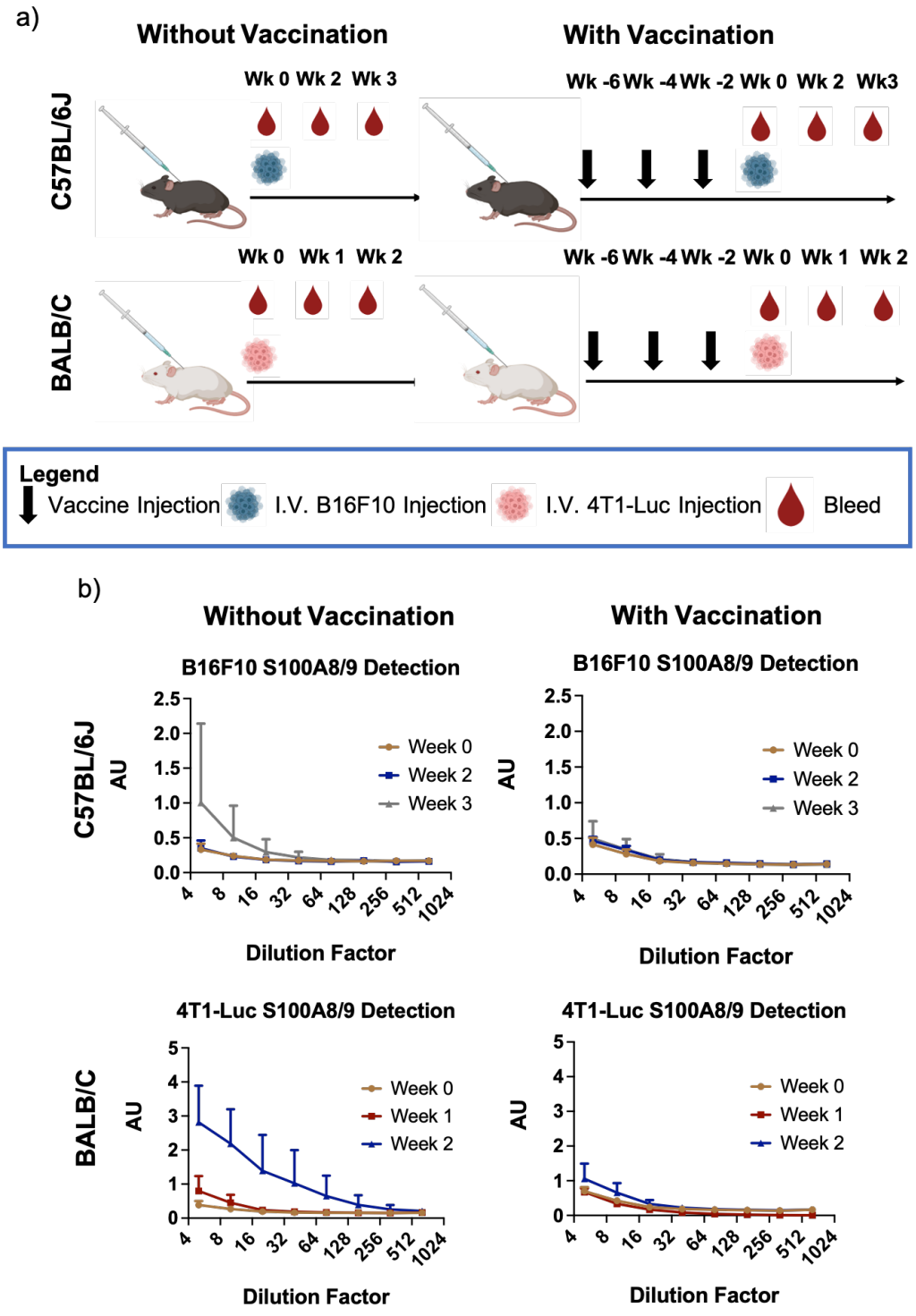


Figure S11: Complete ELISAs of the S100A8/9 measurements in the blood of mice. a) Injection and blood collection schedule. b) ELISA measurements of S100A8/9 within the blood of vaccinated and unvaccinated C57BL6/J and BALB/C mice (n = 3) using an S100A8/9 detection kit (R&D Systems). The error bars represent the standard deviation. The injection schedule schematic was created on Biorender.com.

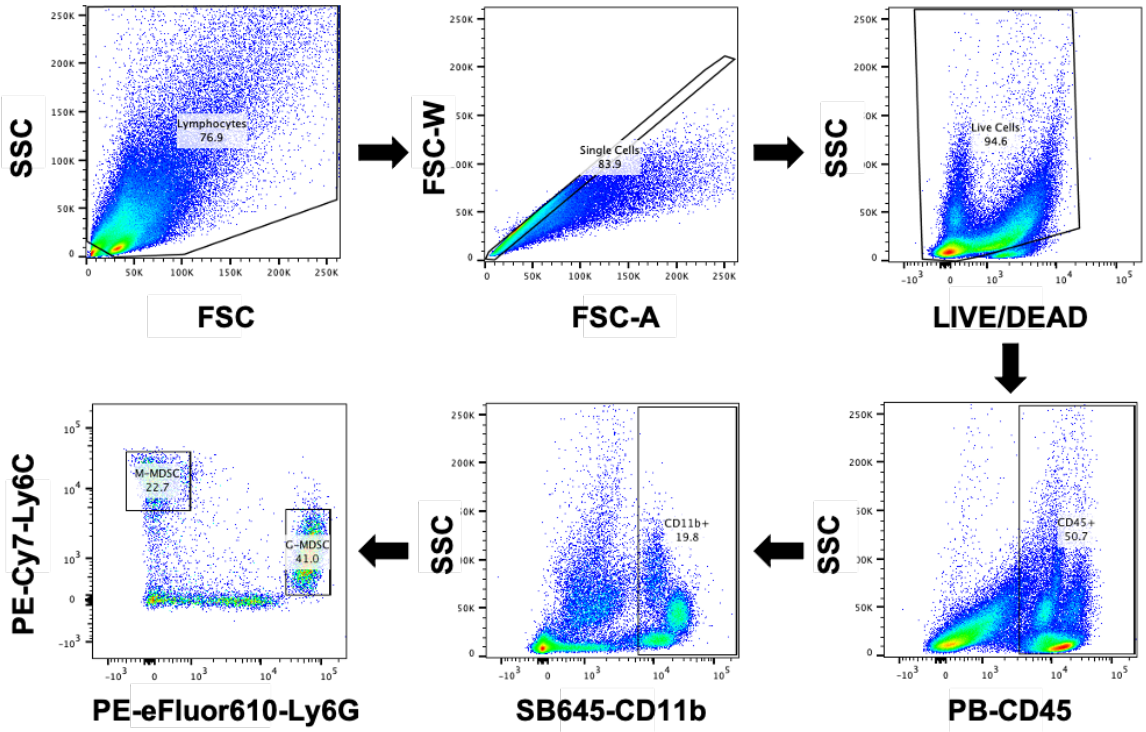


Figure S12: Flow cytometry analysis gating of homogenized lung populations of mice. A representative gating schematic is shown.

References

1. A. M. Wen, *et al.*, Viral Nanoparticles for In vivo Tumor Imaging. *J Vis Exp* (2012) https:/doi.org/10.3791/4352 (November 8, 2019).

2. O. A. Ortega-Rivera, J. K. Pokorski, N. F. Steinmetz, A Single-Dose, Implant-Based, Trivalent Virus-like Particle Vaccine against “Cholesterol Checkpoint” Proteins. *Advanced Therapeutics* **4**, 2100014 (2021).