SUPPORTING INFORMATION

In vivo fate of Cowpea Mosaic Virus *in situ* vaccine: biodistribution and clearance

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Supporting Methods

Stability of CPMV under simulated gastric gastric fluid (SGF) and simulated intestinal fluid (SIF)

Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to Takagi et al.[59] Briefly, pepsin (3.8 mg; Sigma Aldrich, CAS# 9001-75-6) was dissolved in 5 ml of gastric control fluid (Ricca Chemical, pH 1-1.4, 25 ºC). SIF was prepared by dissolving pancreatin (10 mg/mL; Sigma Aldrich, CAS# 8049-47-6) in intestinal control fluid (Ricca Chemical, pH 6.7-6.8, 25 ºC). Both solutions were used within the same day. Before addition of CPMV (1 mg), SGF was incubated at 37 ºC for 2 min. The tube contents were mixed by mild vortexing and the tube was immediately incubated at 37 ºC in Eppendorf® ThermoMixer® F1.5 for time points ranging from 0 to 60 min, 24h and 5 days, followed by neutralization with 70 µl of 200 mM sodium bicarbonate solution. Similarly, before addition of CPMV (1 mg), SIF was incubated at 37 ºC for 2 min and the tube contents were incubated at 37 ºC in Eppendorf® ThermoMixer® F1.5 for 120 min, 24 h or 5 days. Aliquots were then analyzed on native and denaturing gels. 1.2% (w/v) agarose gels stained with GelRed® (Gold Biotechnologies) in TAE buffer (gels were run for 30 min at 120 V and 400 mA) were used and 4-12% SDS-PAGE precast gels in 1x MOPS buffer (Thermo Fisher Scientific) run for 40 min at 200 V and 120 mA in the presence of SeeBlue Plus2 ladder size markers (Thermo Fisher Scientific). Results are shown on **Figure S3**.

Supporting Data

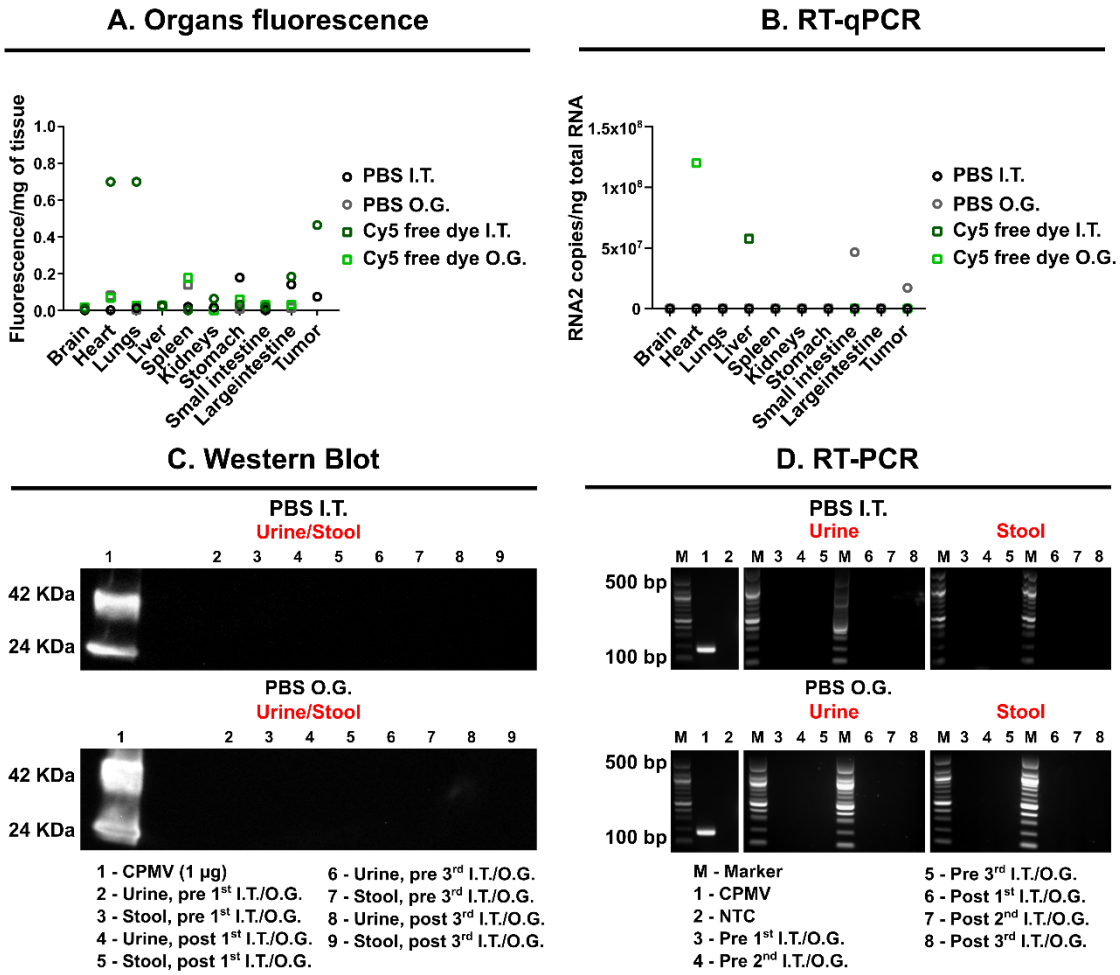

Figure S1. Biodistribution of free Cy5 and PBS (control groups). I.T. or O.G. administration of CPMV using B16F10 tumor-bearing or healthy C57BL/6 mice. **(A)** Fluorescence signals of homogenized tissues was quantified 24 h post the final (third) administration. **(B)** Western blot and **(C)** RT-PCR for detection of CPMV protein and in urine and stool samples pre and post administration. For RT-PCR a 100 bp DNA ladder was used as the marker (M).

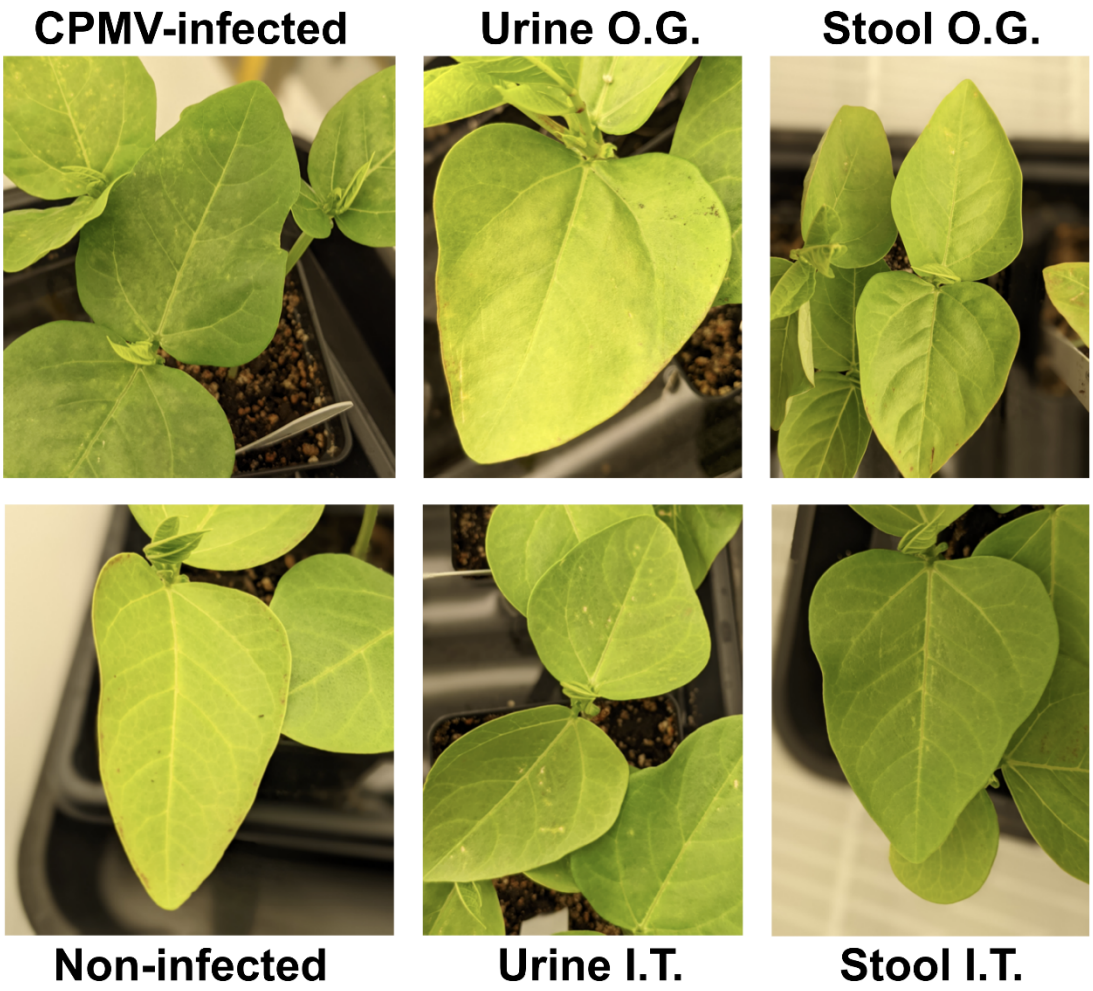

Figure S2. Analysis of the infectivity of urine and stool collected from mice receiving I.T. or O.G. dosing of CPMV; the natural host *V. unguiculata* (black eyed pea No. 5) and a mechanical inoculation protocol was used. Photographs of plants 7 days post mechanical inoculation. Non-infected leaf (negative control) had no symptoms of infection, while CPMV infected leaf (positive control) shows visual symptoms of infection. Plants inoculated with stool or urine samples did not show evidence of disease.

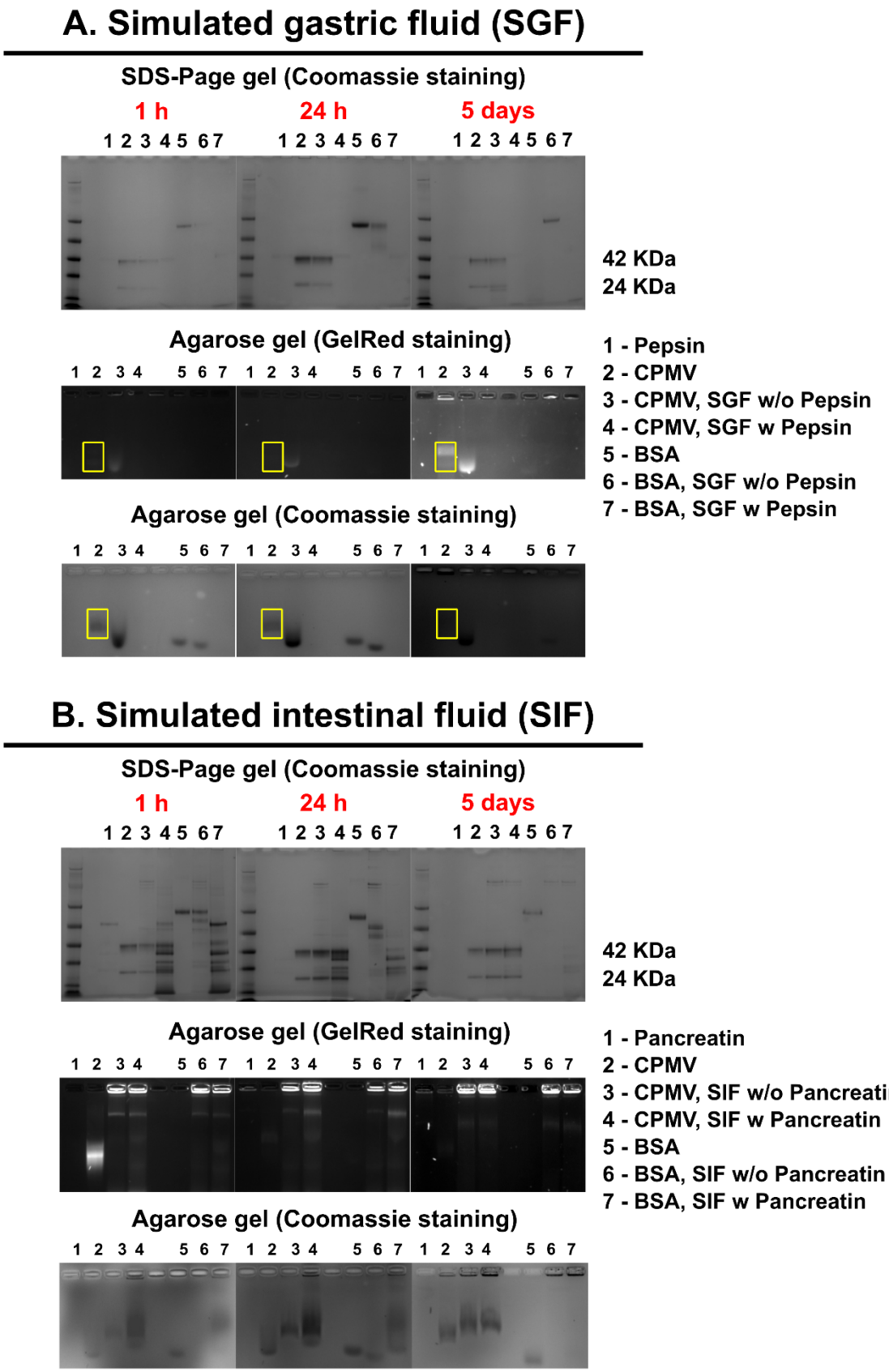

Figure S3. CPMV stability under simulated gastric and intestinal fluids (SGF and SIF, respectively). CPMV was incubated for 1 h, 24 h or 5 days in either PBS (control) or SGF/SIF with or without the digestive enzymes (pepsin for SGF and pancreatin for SIF), respectively. BSA was used as a protein control. CPMV exposure to SGF (**A**) and SIF (**B**): SDS-PAGE (top) and native agarose gel electrophoresis stained with

GelRed® nucleic acid stain (middle) and Coomassie Brilliant Blue (bottom); gels were imaged under UV light and white light using a FluorChem R imager. SDS-PAGE gels were stained with GelCode ™ Blue Safe protein stain.