Supporting Information

The Unique Potency of Cowpea Mosaic Virus (CPMV) *In Situ* Cancer Vaccine

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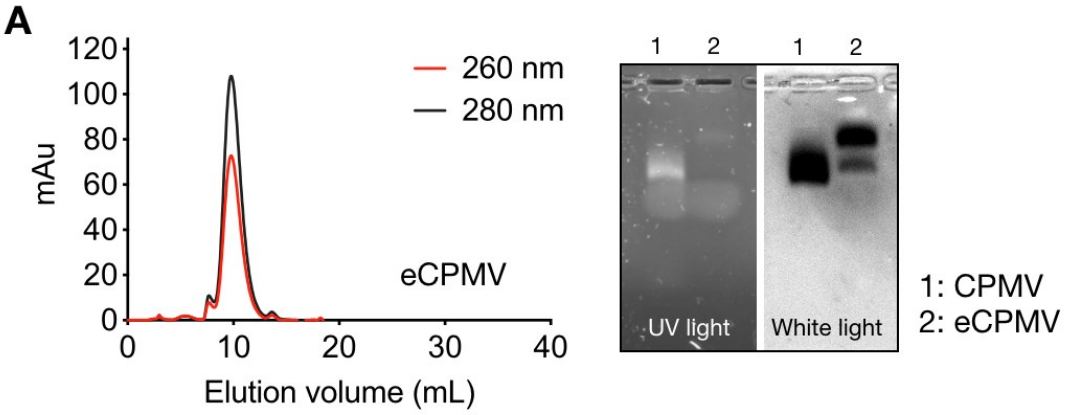


Figure S1: Characterization of eCPMV: (A, B) eCPMV was characterized by size exclusion chromatography using a Superose 6 column and native gel electrophoresis (1.2% (w/v) agarose in TBE buffer). The inverted 260:280 ratios (<1, compared to RNA-containing CPMV) confirmed absence of encapsidated nucleic acid; this was corroborated by lack of nucleic staining and reduced electrophoretic mobility of the eCPMV.

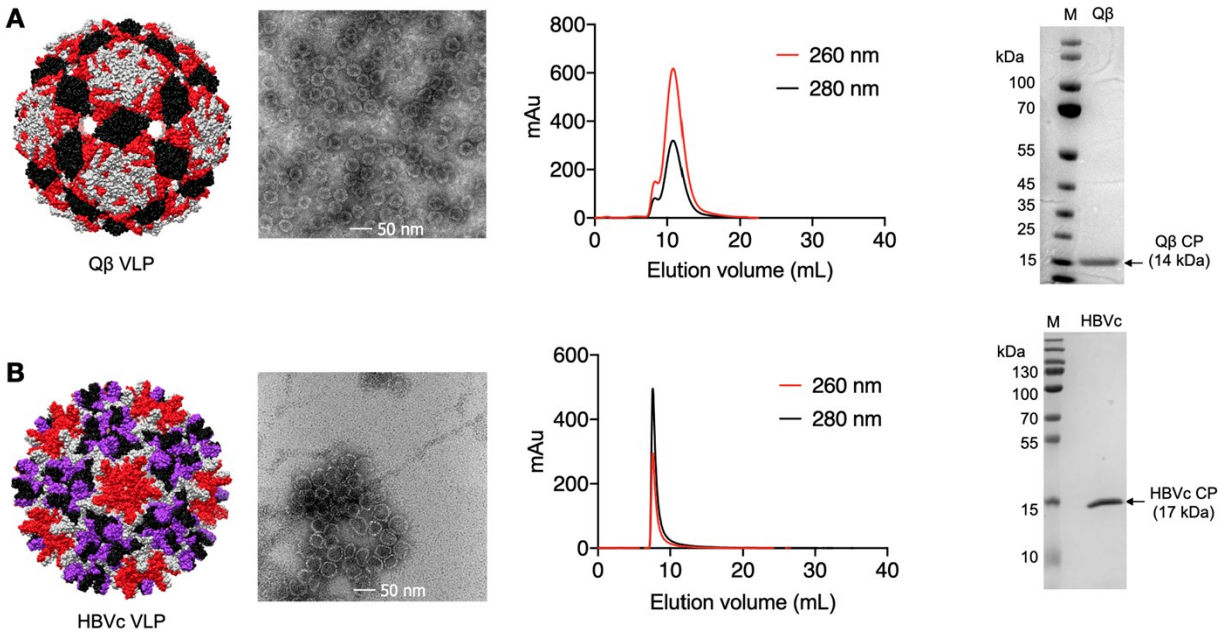
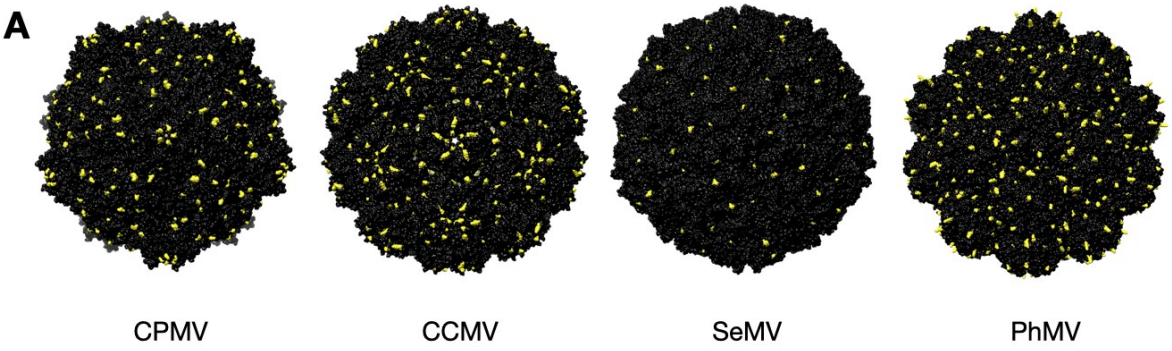
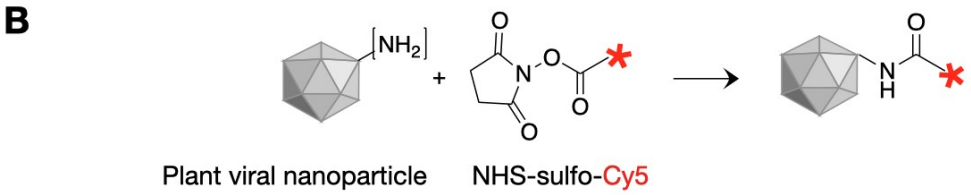


Figure S2: **Characterization of Qβ and HBVc VLPs**: Qβ and HBVc VLPs were analyzed by transmission electron microscopy (TEM), size exclusion chromatography (SEC) using a Superose 6 column and SDS-PAGE (using a 12% Nu-PAGE gel). TEM and SEC data indicate intact VLPs and SDS-PAGE showed the coat proteins; 14 kDa for Qβ and 17 kDa for the HBVc.





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Plant virus	Molecular weight	Size of CP	No. of Lys/capsid	Cy5/capsid	FI
CPMV	5.6 x 10^6 g/ mol	42, 24 kDa (LCP, SCP)	300	28	41893
CCMV	3.78 x 10^6 g/ mol	21 kDa	1080	39	38845
SeMV	5.4 x 10^6 g/ mol	29 kDa	180	16	46263
PhMV	3.78 x 10^6 g/ mol	21 kDa	720	28	42823

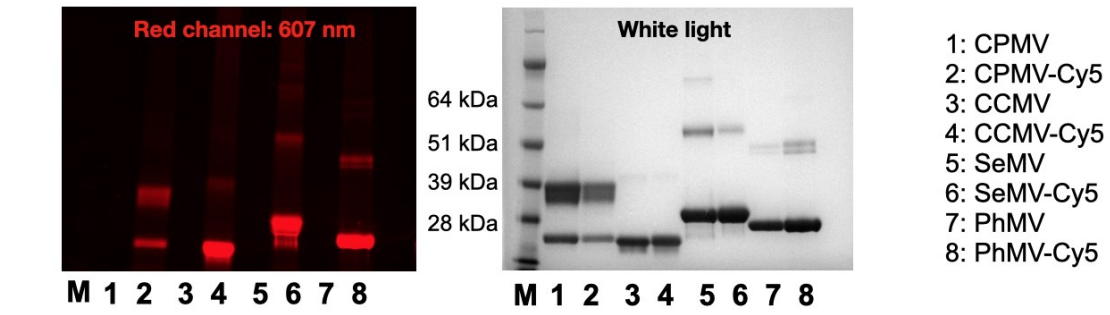

Figure S3: **Synthesis and characterization of fluorescent plant viral nanoparticles:** Cy5-labeled viral nanoparticles were synthesized by conjugating NHS-sulfo-Cy5 to the solvent exposed Lys residues on viral capsids (A, B). All four particles offer variable number of reactive Lys residues; therefore the conjugation protocols were carefully adjusted yielding fluorescent viral particles with comparable fluorescence intensities (FI, C). The FI of the Cy-labeled viral nanoparticles measured using a particle concentration of 0.1mg/ mL and 635/655 excitation/emission. (D) SDS-PAGE confirmed presence of fluorescent coat proteins (D).

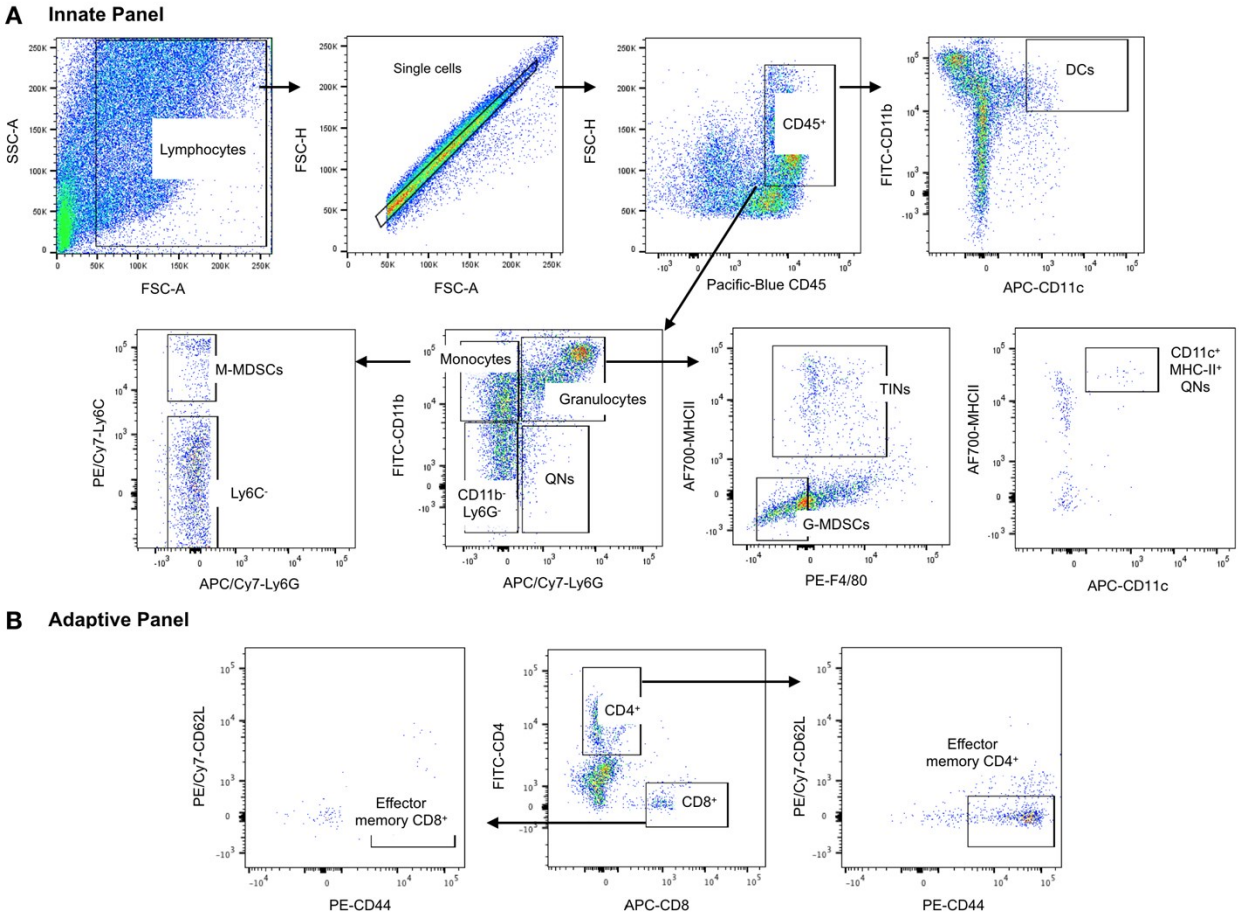

Figure S4: Gating strategy used for characterization of the immune response in the IP wash from ID8-Defb29/Vegf-a ovarian tumor bearing mice following treatment with CPMV, CCMV, SeMV and PhMV-VLP *in situ* vaccine.

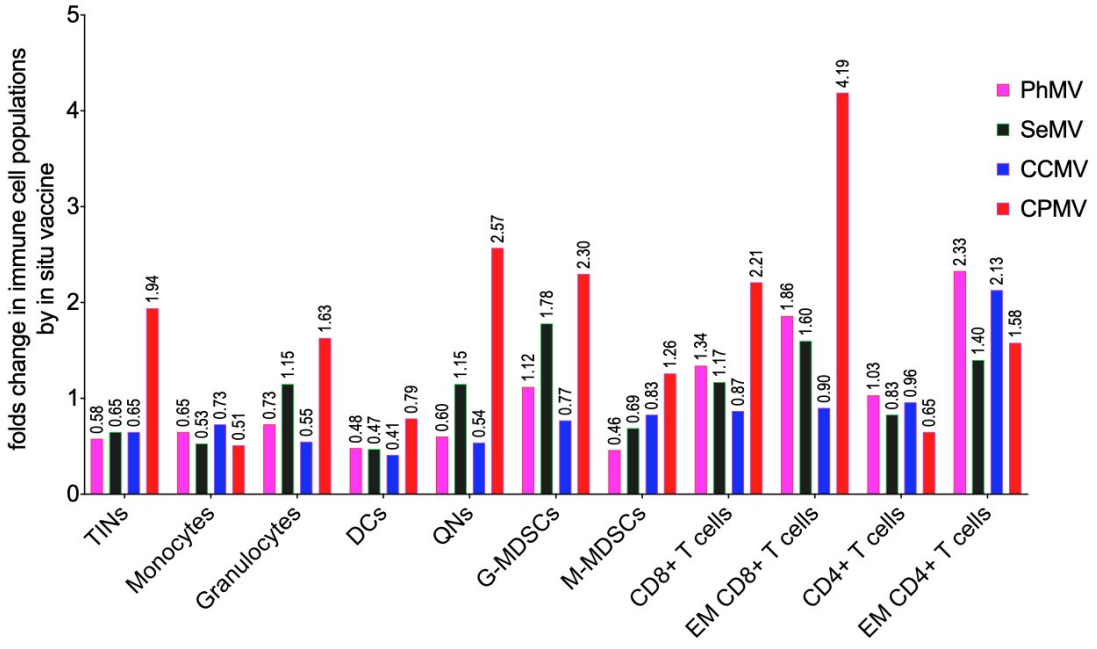

Figure S5: Comparative changes in the immune cell population of intraperitoneal tumor microenvironment by in situ vaccine using different VNPs.

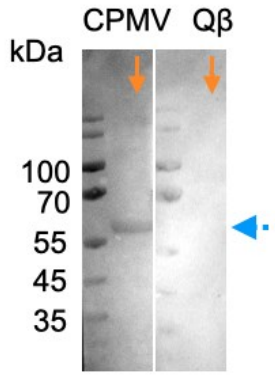

Figure S6: VOPBA assay comparing vimentin binding of CPMV and Qβ VLP.