Supporting Information for

Cowpea Mosaic Virus Promotes the Anti-tumor Activity and Immune Memory in a Mouse Ovarian Tumor Model

Chao Wang, Steven N. Fiering, and Nicole F. Steinmetz*

Dr. C. Wang and Dr. NF. Steinmetz

Department of NanoEngineering, University of California, San Diego, La Jolla, CA 92093, United States

Department of Biomedical Engineering, Case Western Reserve University School of Medicine, Cleveland, OH 44106, United States

Dr. NF. Steinmetz

Department of Radiology, Moores Cancer Center, Bioengineering, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0448, United States

Email: <u>nsteinmetz@ucsd.edu</u>

Dr. SN. Fiering

Department of Microbiology and Immunology and Norris Cotton Cancer Center, Dartmouth University, Lebanon, NH 03756, United States



Figure S1. Cytokine secretion from peritoneal cavity wash cells after *ex vivo* CPMV stimulation. The levels of IL-1 β , IL-4, IL-6, IL-10, IL-12, TNF- α , TGF- β , GM-CSF, IFN- β , and IFN- γ were measured. Peritoneal cavity wash cells were collected on day 35 post-inoculation. Data are means \pm SEM (n=3). Statistical significance was calculated using an unpaired t-test (**p<0.01, ***p<0.0005, ****p<0.0001).



Figure S2. Innate immune cell profile from peritoneal cavity wash cells after *ex vivo* CPMV stimulation. Data are means \pm SEM (n=3). Statistical significance was calculated using an unpaired t-test (**p<0.01, ***p<0.0005, ****p<0.0001).



Figure S3. Uptake of Cy5-labeled CPMV particles. **A**, Cy5-labeled CPMV particles were injected i.p. into tumor-bearing mice and a peritoneal wash was collected 48 h later. **B**, CPMV uptake after *ex vivo* stimulation of splenocytes for 2 h.



Figure S4. Cytokine levels in the peritoneal cavity wash supernatant, collected 6 or 48 h after the final CPMV treatment. The levels of IL-6, IL-12, TNF- α , IL-10, TGF- β , IL-1 β , GM-CSF and IFN- γ were measured. Data are means \pm SEM (n=3). Statistical significance was calculated by two-way ANOVA with the Holm-Sidak test (*p<0.05, **p<0.01, ***p<0.0005; ****p<0.0001).



Figure S5. Innate immune cell profile in the peritoneal cavity wash collected 6 h after the final CPMV treatment. Statistical significance was calculated using an unpaired t-test (*p<0.05, **p<0.01, ***p<0.0005).



Figure S6. Innate immune cell profile in the peritoneal cavity wash collected 48 h after the final CPMV treatment. Statistical significance was calculated using an unpaired t-test (*p<0.05, **p<0.01, ***p<0.0005).



Figure S7. Innate and adaptive immune cell profiles of splenocytes collected 6 h after the final CPMV treatment. Statistical significance was calculated using an unpaired t-test (*p<0.05, **p<0.01, ***p<0.0005).



Figure S8. Gating strategy of innate immune cells profiles in tumor-bearing mice peritoneal cavity.

cell types	full name	Phenotype
G-MDSCs	Granulocytic myeloid-derived suppressive cells	CD45 ⁺ CD11b ⁺ Ly6G ⁺ Ly6C ⁻ MHCII ⁻
		CD86 ⁻
M-MDSCs	Monocytic myeloid-derived suppressive cells	CD45 ⁺ CD11b ⁺ Ly6G ⁻ Ly6C ⁺ MHCII ⁻
TING	Tymor infiltrating poutrophile	$\frac{55C}{CD45^{+}CD11b^{+}L_{2}CC^{+}MUCU^{+}CD96^{+}}$
	Tumor-inititating neutrophils	CD45 CD110 Lyog MHCII CD80
Activated Neutrophils	Activated Neutrophils	CD45 ⁺ CD11b ⁺ Ly6G ⁺ MHCII ⁻ CD86 ^{mid}
DCs	Dendritic cells	CD45 ⁺ CD11b ⁺ CD11c ⁺
NK	Natural killer cells	CD45 ⁺ CD11b ⁺ NK1.1 ⁺ Ly6G ⁻ Ly6C ⁻
		F4/80 ⁻
M1	Type 1 tumor associated macrophages	CD45 ⁺ CD11b ⁺ F4/80 ⁺ Ly6G ⁻ Ly6C ⁻
		MHCII ⁺ CD86 ⁺
TAMs	tumor associated macrophages	CD45 ⁺ CD11b ⁺ F/80 ⁺ Ly6G ⁻ Ly6C ⁻
CD86 ⁺ MHCII ⁺ QNs	CD86 ⁺ MHCII ⁺ Quiescent neutrophils	CD45 ⁺ MHCII ⁺ CD86 ⁺ CD11b ⁻ Ly6G ⁺
QNs	Quiescent neutrophils	CD45 ⁺ CD11b ⁻ Ly6G ⁺
CD86 ⁺ MHCII ⁺ CD11b ⁻	CD86 ⁺ MHCII ⁺ CD11b ⁻ Ly6G ⁻	CD45 ⁺ CD86 ⁺ MHCII ⁺ CD11b ⁻ Ly6G ⁻
Ly6G ⁻		
CD11b ⁻ Ly6G ⁻	CD11b ⁻ Ly6G ⁻	CD45 ⁺ CD11b ⁻ Ly6G ⁻
Granulocytes	Granulocytes	CD45 ⁺ CD11b ⁺ Ly6G ⁺
Monocytes	Monocytes	CD45 ⁺ CD11b ⁺ Ly6G ⁻
CD45	Leukocytes	CD45 ⁺

Table S1. Surface markers of each particular cell type