SUPPLEMENTAL FIGURES AND TABLES: A Novel Manganese Glycerophosphate Vaccine Gel Elicits Broad and Durable Immunity Across An Aged and Pox Virus Model

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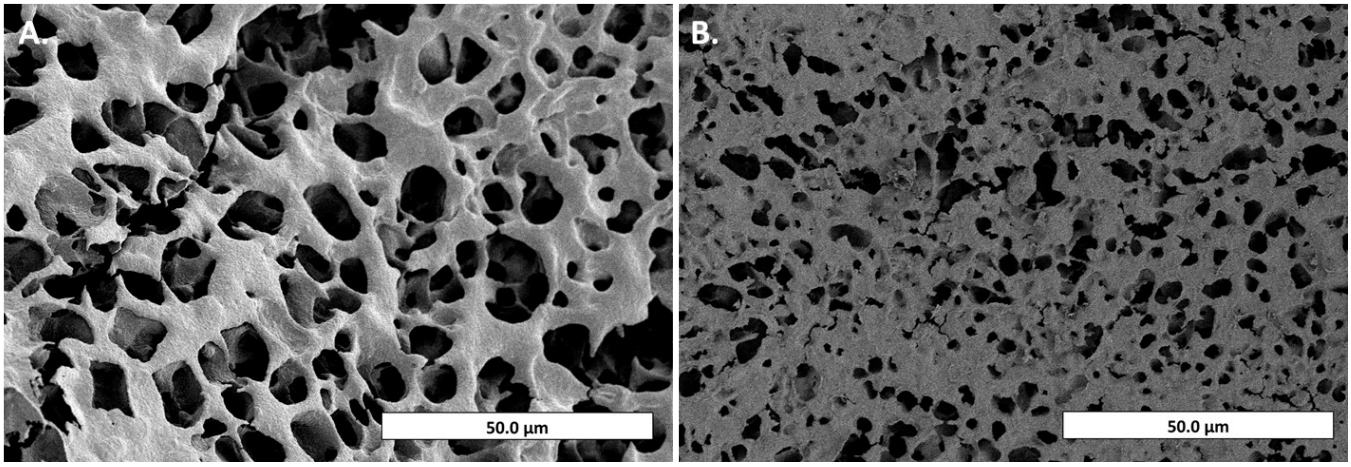


Figure S1. Comparison of MnGp gels fabricated with or without sucrose. SEMs of **(A)** 350 mg/mL MnGp with 150 mg/mL sucrose and **(B)** without sucrose.

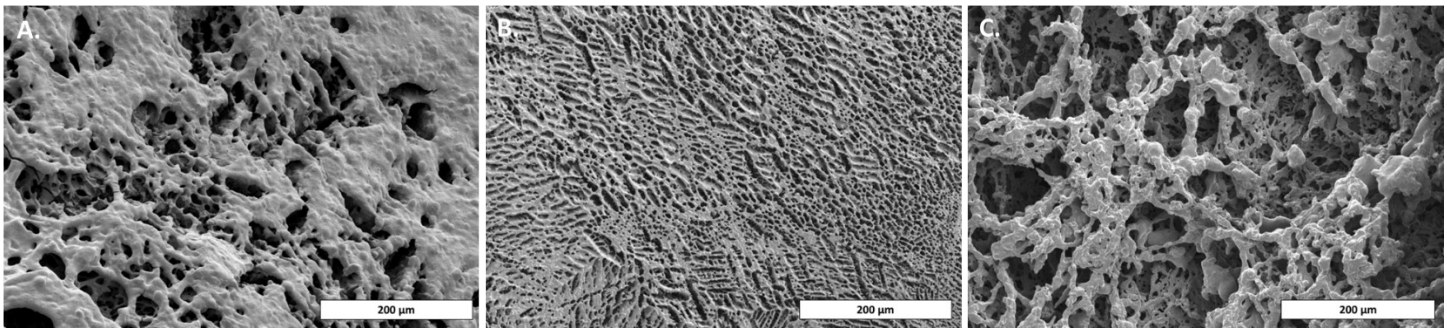


Figure S2. Comparison of MnGp gels at different concentrations. SEMs of **(A)** 250 mg/mL, **(B)** 100 mg/mL, and **(C)** 25 mg/mL. No gel-like material was observed for the 5 mg/mL.

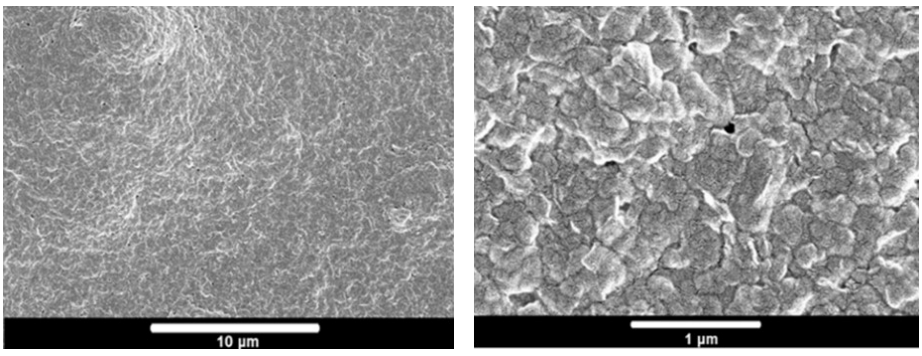
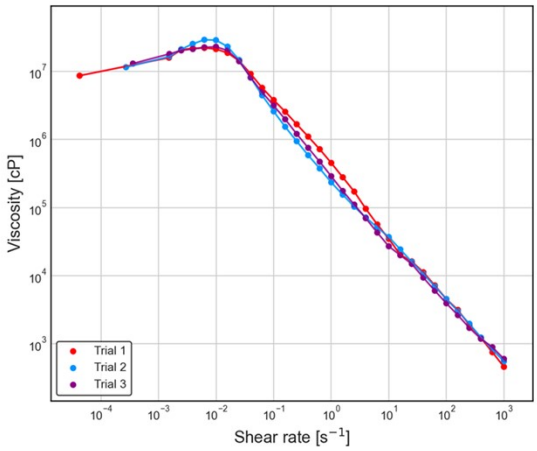


Figure S3. 250 mg/mL MnGp gel loaded with OVA.

A.



B.

Reported Values	Value	Standard Deviation	Units
Power Law Index (*n*)	0.0633	0.045	dimensionless
Consistency Index (*K*)	325.185	74.288	Pa*s^(n)
R^2	0.998		

Figure S4. (A) Viscosity of 250 mg/mL MnGp gel at different shear rates. **(B)** Shear thinning behavior of MnGp gel using the Ostwald–de Waele power-law model.

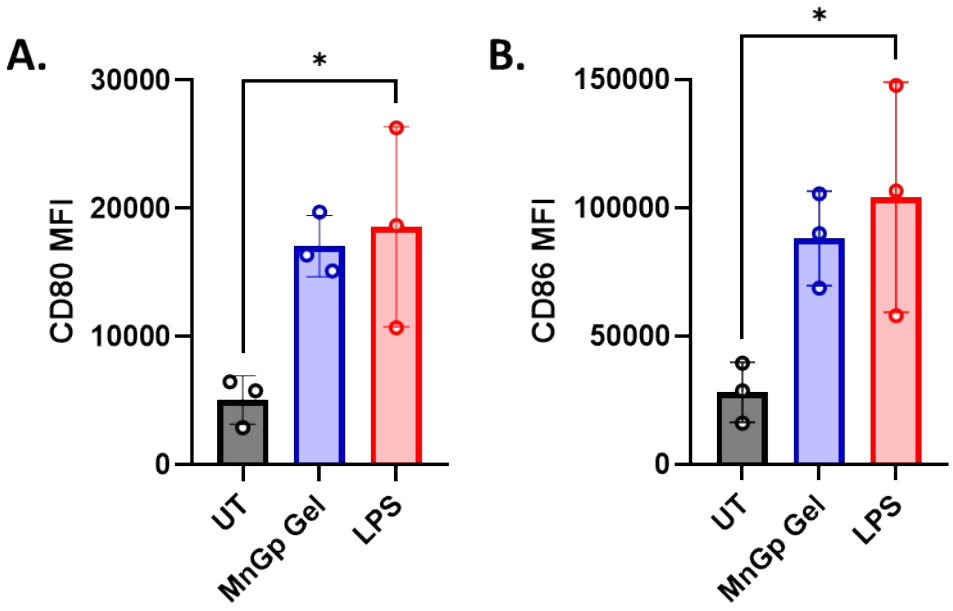


Figure S5. Enhanced co-stimulatory molecule expression in BMDCs in response to the MnGp gel. BMDCs were cultured for 12 hours with media (UT), 100 mg/mL MnGp or 100 ng/mL LPS. MFIs of **(A)** CD80 and **(B)** CD86 were assessed via flow cytometry. Data is presented as mean ± standard deviation. Statistical significance is presented as *p<0.05 for an ordinary one-way ANOVA with Tukey's multiple comparisons test.

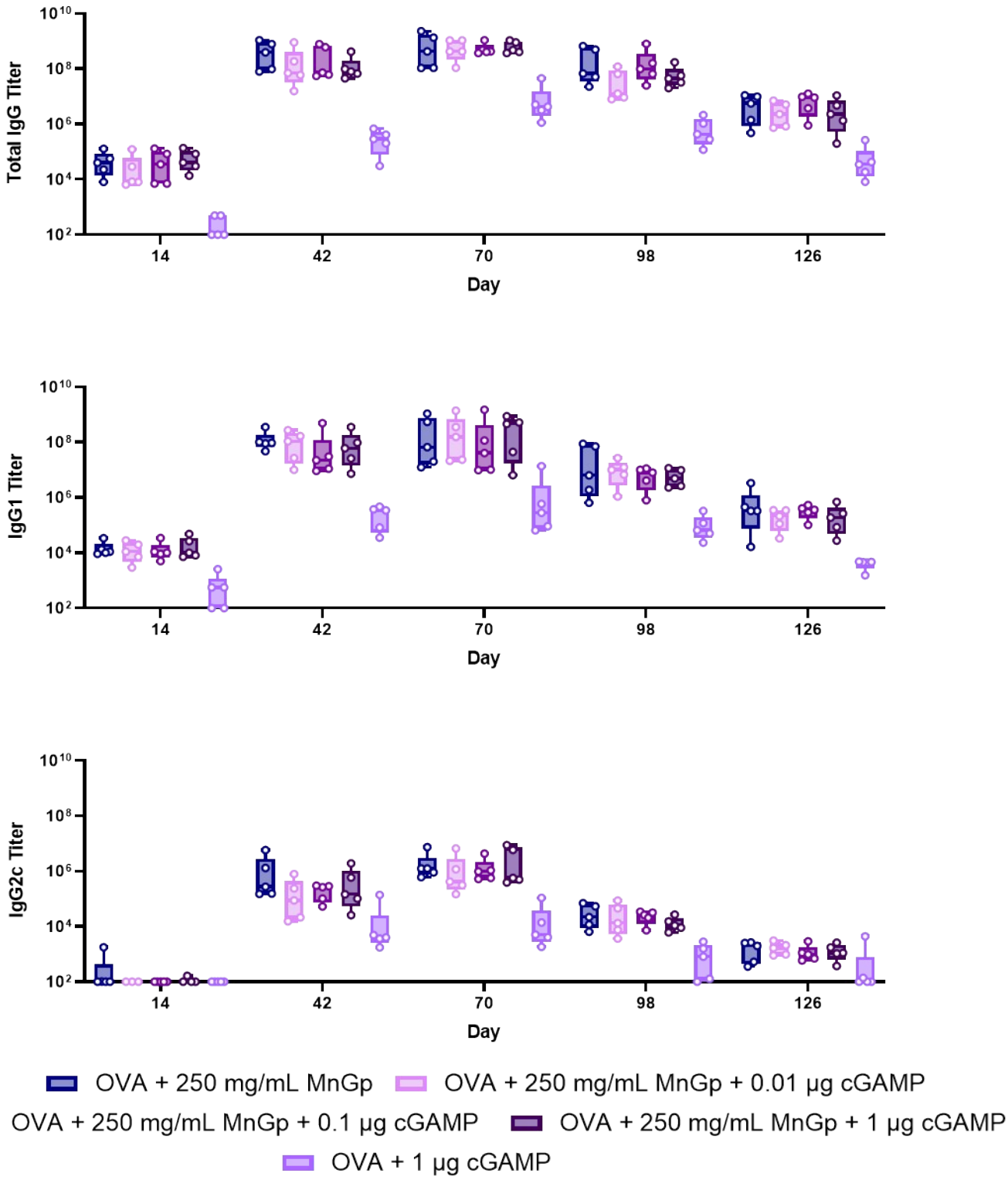


Figure S6. OVA-specific serum antibody titers following prime-boost immunization schedule. C57BL/6 mice (n = 5) were immunized intramuscularly on days 0 and 28 with OVA + 250 mg/mL MnGp, OVA + 1 µg cGAMP, OVA + 250 mg/mL MnGp + 0.01 µg cGAMP, OVA + 250 mg/mL MnGp + 0.1 µg cGAMP, or OVA + 250 mg/mL MnGp + 1 µg cGAMP. Sera were collected at the indicated timepoints and assessed for OVA-specific **(A)** total IgG, **(B)** IgG1, and **(C)** IgG2c titers via ELISA. Data is presented as median ± range.

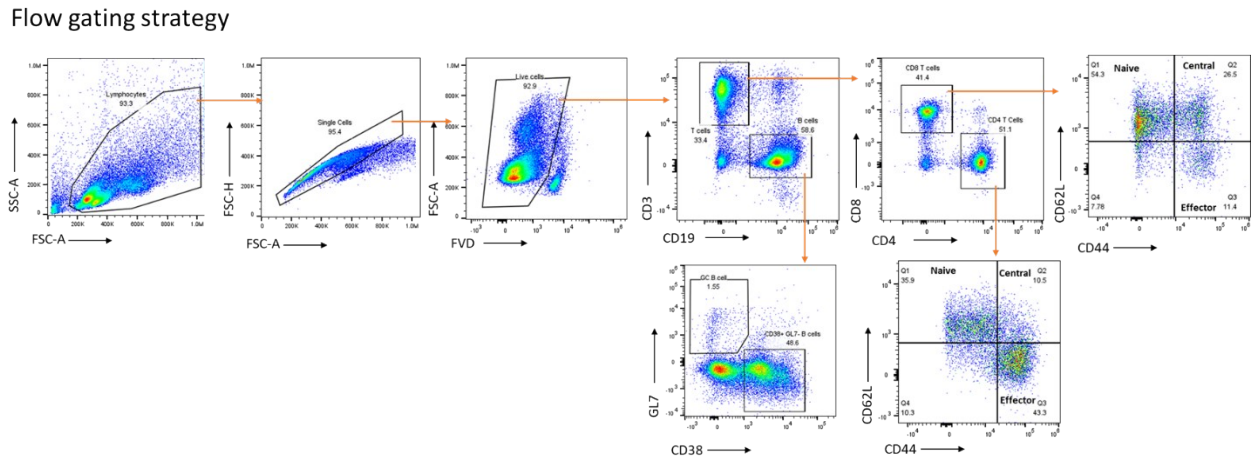


Figure S7. Flow cytometry gating strategy to characterize T and B cell phenotypes in the spleens and lymph nodes of immunized mice. Example is shown from the spleen of a mouse immunized with OVA + 250 mg/mL MnGp.

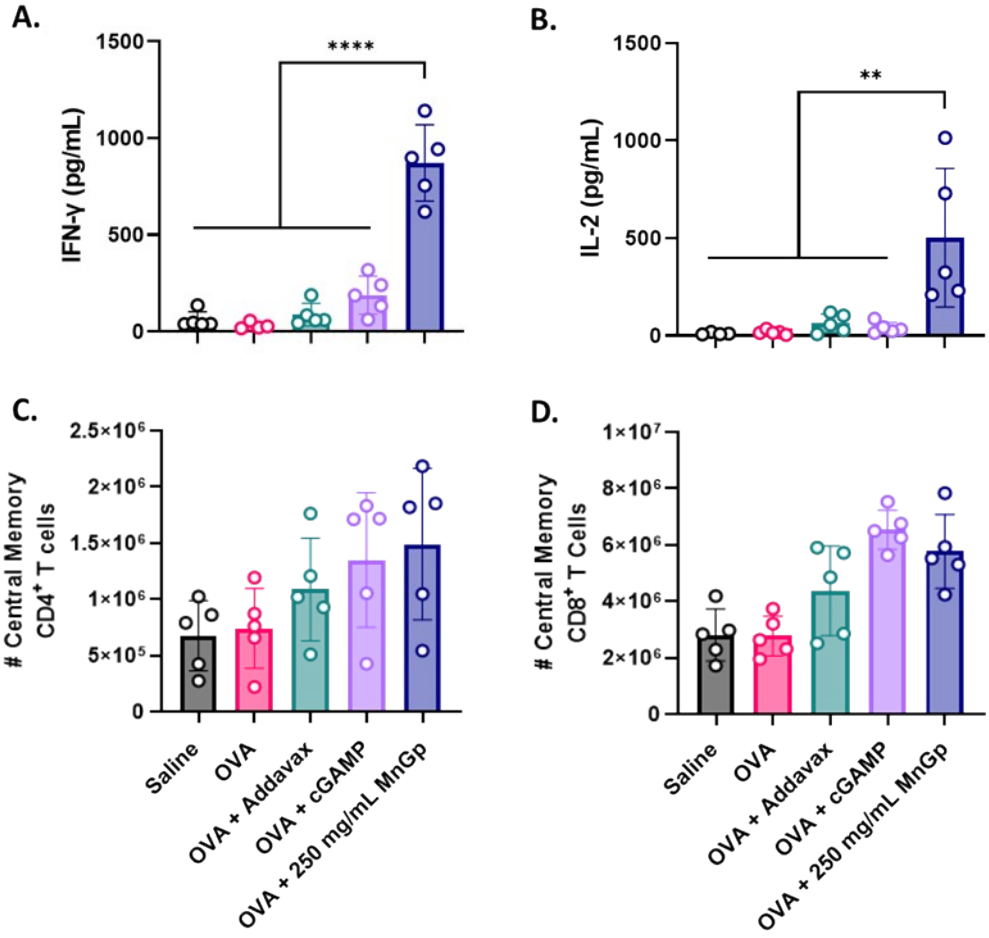


Figure S8. IFN-γ, IL-2 production and counts of central memory T cell in spleens following a prime-boost-boost immunization. C57BL/6 mice (n = 5) were immunized intramuscularly on days 0, 28, and 140 with saline, unadjuvanted OVA, OVA + Addavax, OVA + cGAMP, or OVA + 250 mg/mL MnGp. On day 151, splenocytes were collected and re-stimulated with OVA to measure the release of **(A)** IFN-γ and **(B)** IL-2. Splenocytes were stained to measure **(C)** central memory $CD4^+$ T cells ($CD4^+CD44^+CD62L^+$) and **(D)** central memory $CD8^+$ T cells ($CD8^+CD44^+CD62L^+$). Data is presented as mean ± standard deviation. Statistical significance is presented as **p<0.01, ****p<0.0001 for an ordinary two-way ANOVA with Tukey's multiple comparisons test.

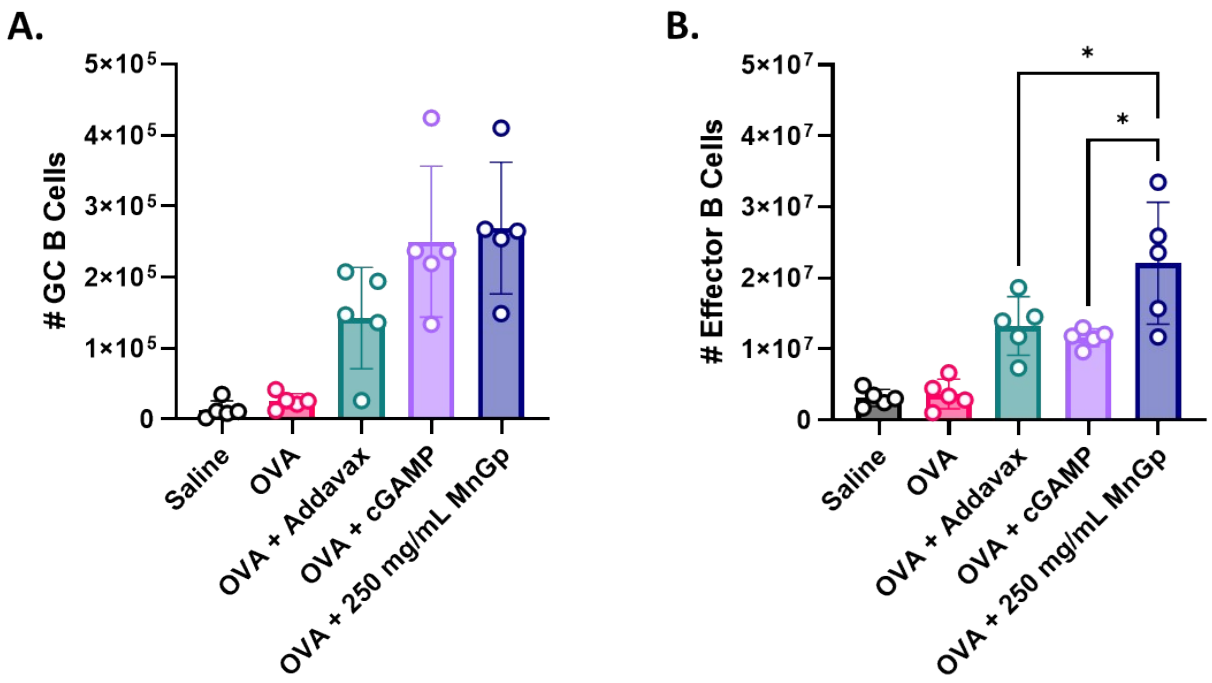


Figure S9. Counts of B cell phenotypes in the spleen following a prime-boost-boost immunization schedule. C57BL/6 mice (n = 5) were immunized intramuscularly on days 0, 28, and 140 with saline, unadjuvanted OVA, OVA + Addavax, OVA + cGAMP, or OVA + 250 mg/mL MnGp. On day 151, splenocytes were collected and stained to measure expansion of **(A)** germinal center (GC) B cells ($CD19^+GL7^+CD38^-$) and **(B)** effector B cells ($CD19^+GL7^-CD38^+$). Data is presented as mean ± standard deviation. Statistical significance is presented as *$p<0.05$ for an ordinary two-way ANOVA with Tukey's multiple comparisons test.

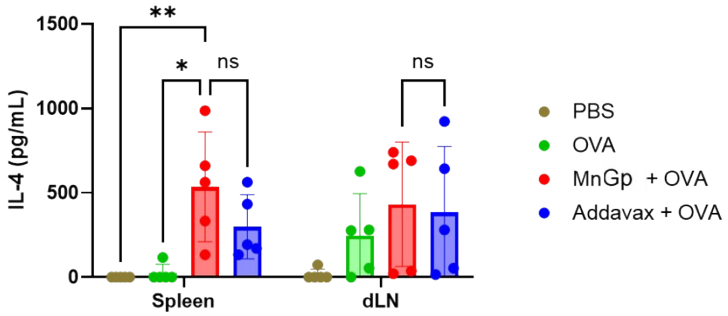


Figure S10. Spleen IL-4 production in aged mice immunized with MnGp. C57BL/6 18-month-old mice (n = 5) were immunized intramuscularly on days 0, 28 and 77 with PBS, unadjuvanted OVA, MnGp (250 mg/mL) + OVA or Addavax + OVA. IL-4 released from Splenocytes and dLN cells harvested on day 84 after a re-stimulation with OVA. Data is presented as mean ± standard deviation. Statistical significance is presented as *p<0.05 or **p<0.01 for an ordinary two-way ANOVA with Tukey's multiple comparisons test.

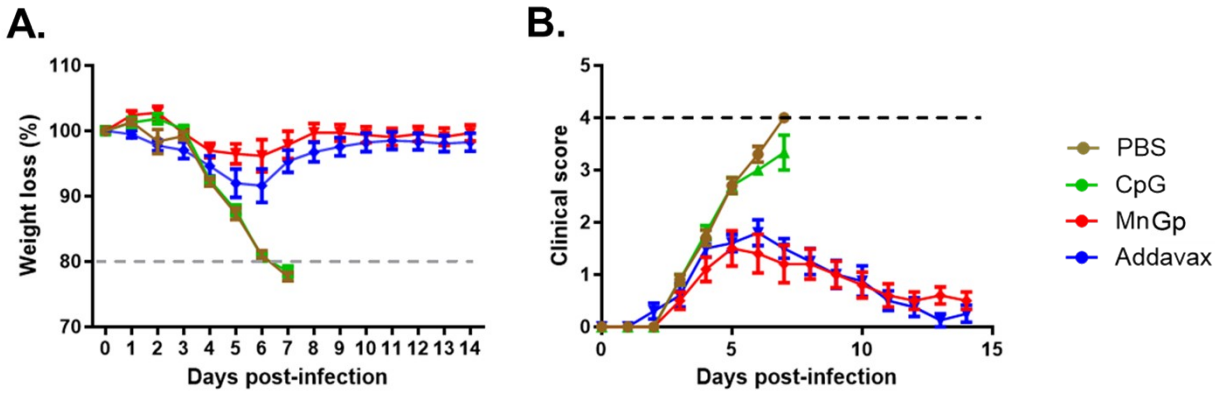


Figure S11. Weight loss and clinical score from mice immunized with MnGp + B5R and challenged with vaccinia virus. BALB/c mice (n = 10) were immunized intramuscularly on days 0 and 28 with PBS, CpG + B5R, MnGp (250 mg/mL) + B5R or Addavax + B5R. On day 56 the mice were challenged with vaccinia virus. **(A)** Weight loss of mice after challenge. **(B)** Clinical score of mice after challenge. Data is presented as mean ± standard deviation. No statistical significance was found for an ordinary two-way ANOVA with Tukey's multiple comparisons test.