Supplemental figures

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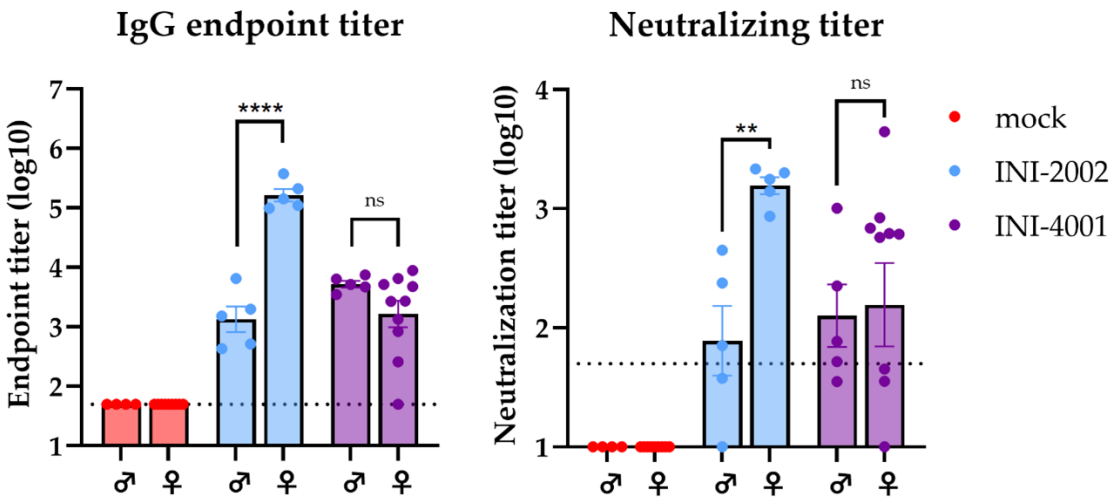
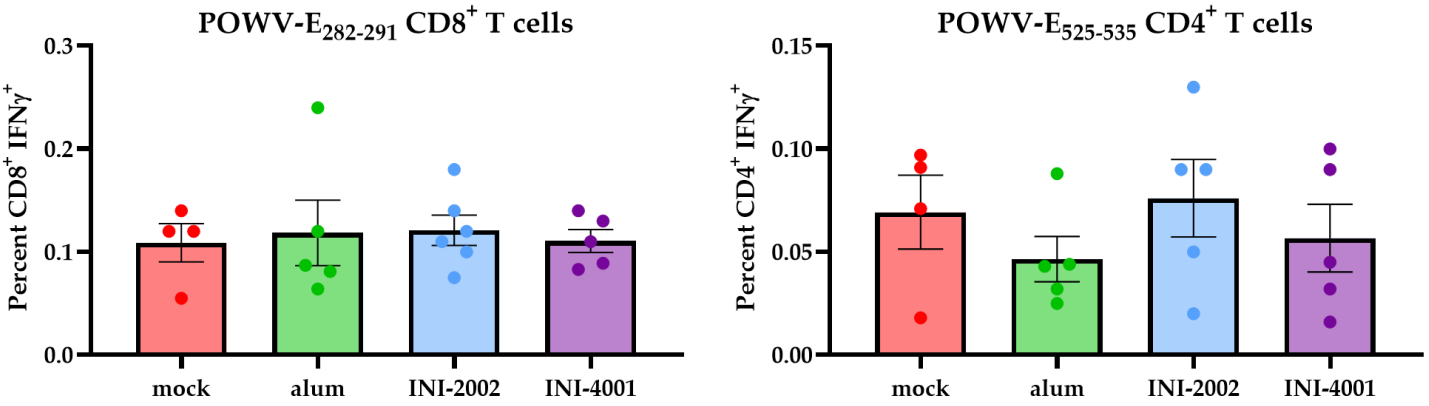
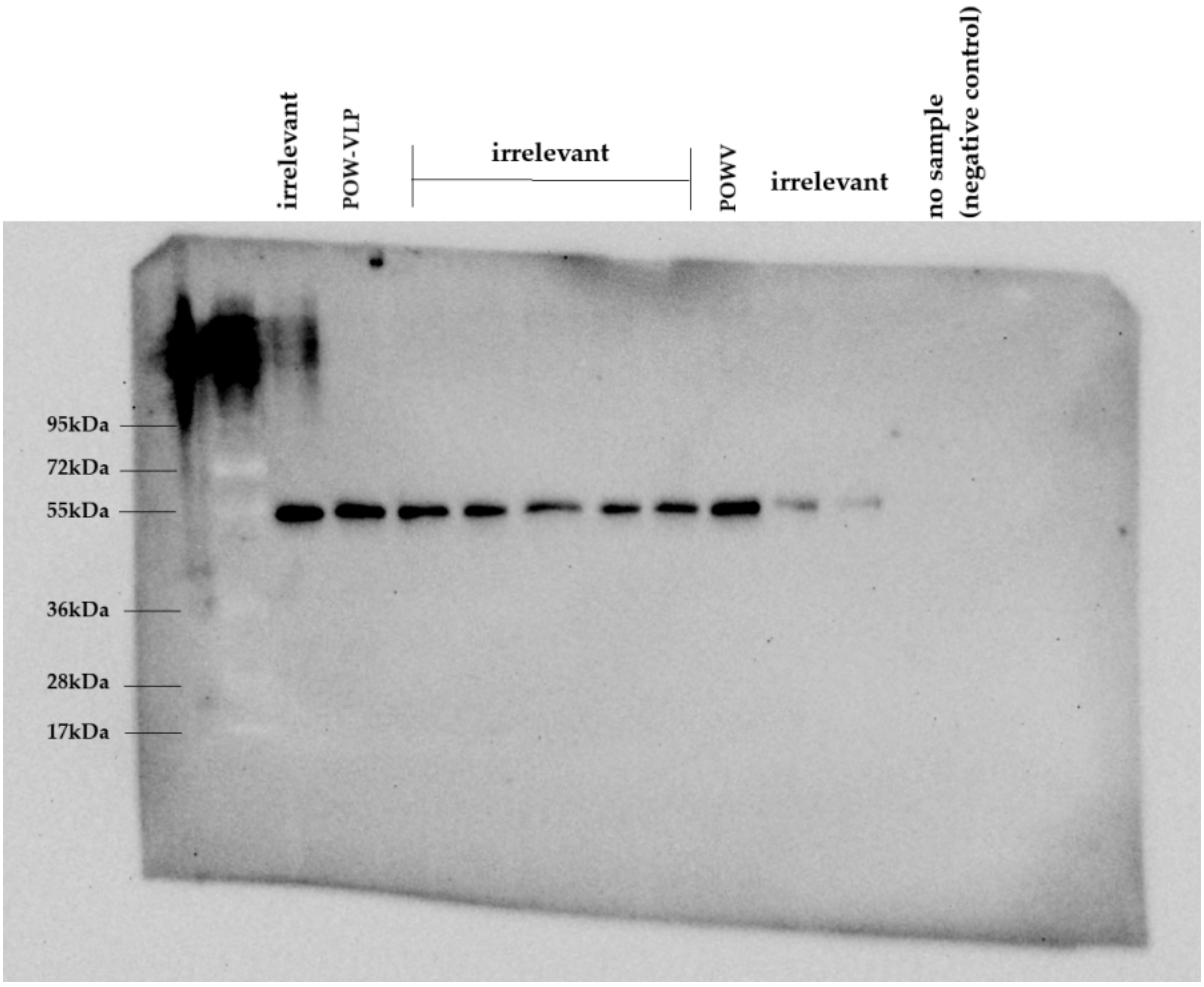


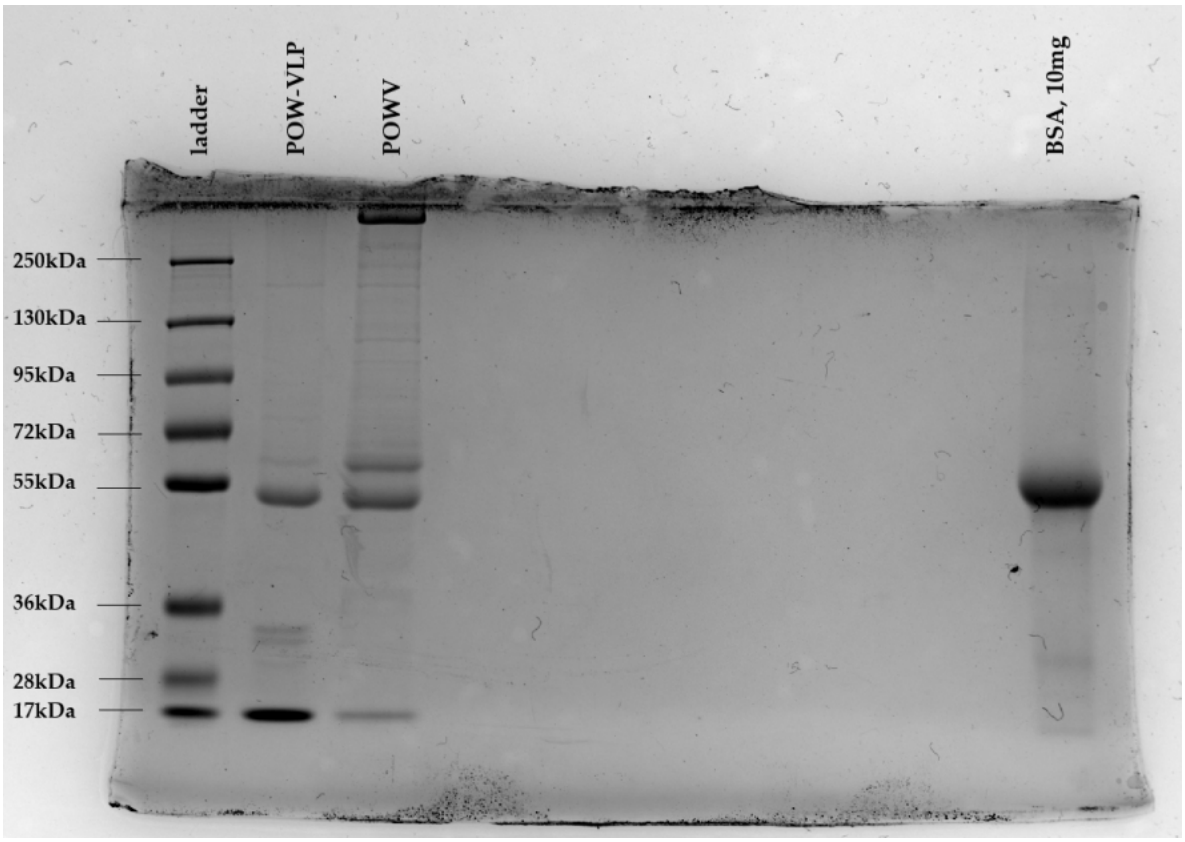
Supplementary figure 1. The antibody response to INI-2002 is higher in female mice than males. (A-B) Male and female mice prime-boost vaccinated 3 weeks apart *sc* with 10^6 FFUe of VLP adjuvanted with 1nmol INI-2002 or 10nmol INI-4001. Mock group injected with PBS vehicle alone. n=4-10. Serum collected 3 weeks post-boost and analyzed by whole-virus ELISA (A) or FRNT (B). Data are reported as log-transformed mean endpoint titer ± SEM for IgG titer and reciprocal FRNT50 for neutralizing titer. Dotted line represents least dilute sera tested (1:50). Data represent three independent experiments. (A-B) Statistical significance determined by one-way ANOVA and Šídák's multiple comparisons to compare male to female within treatment groups after log transformation. ns = not-significant, ** = $p < 0.01$, **** = $p < 0.0001$.



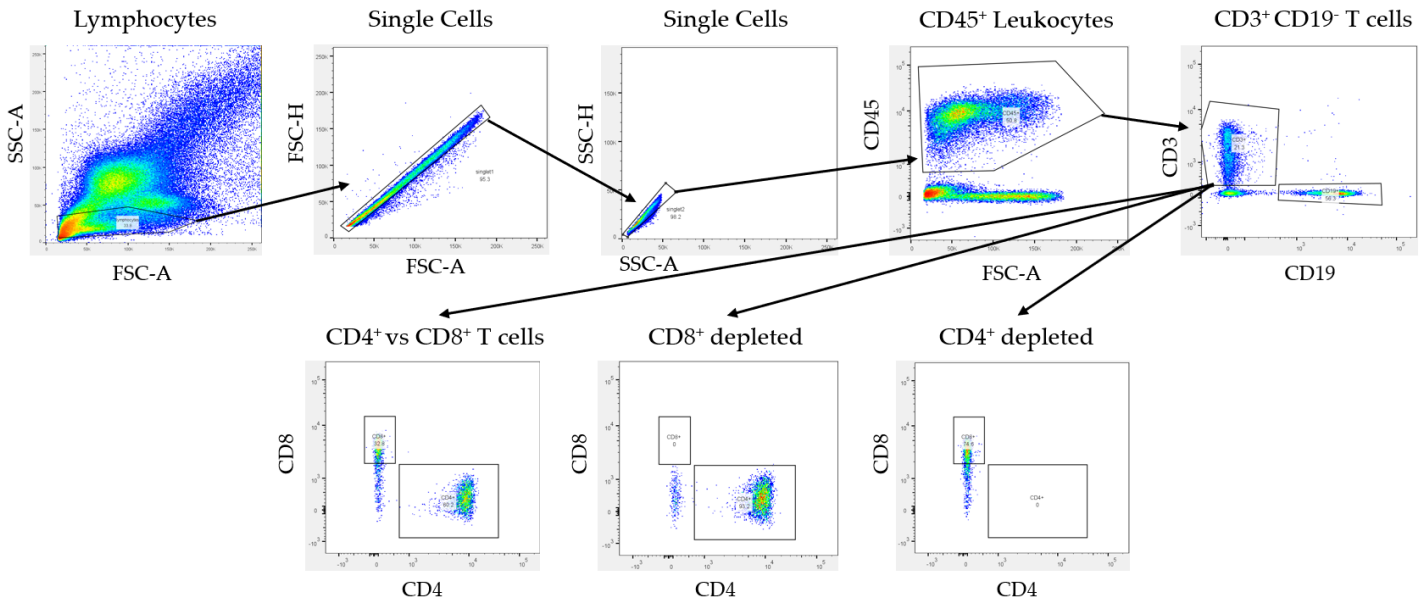
Supplementary figure 2. Vaccination does not induce measurable CD8⁺ or CD4⁺ T cell responses. (A-B) Mice prime-boost vaccinated 4 weeks apart *sc* with 10^6 FFUe of VLP alone or adjuvanted with 11µmol alum, 1nmol INI-2002, or 10nmol INI-4001. Mock group injected with PBS vehicle alone. n=4-5. Spleens collected 4 weeks post-boost, and splenocytes incubated with POWV-$E_{282-291}$ (A) or POWV-$E_{525-535}$ (B) for $CD8^+$ and $CD4^+$ T cells, respectively. Cells were then stained and gated on CD19- CD4+/CD8+ and intracellularly stained for IFNγ. Data are reported as percentage of $IFN\gamma^+$ T cells either within the $CD8^+$ (A) or $CD4^+$ (B) compartments ± SEM. Data represent one experiment. Statistical significance determined by one-way ANOVA and Šídák's multiple comparisons test to compare treatments to mock; no statistically significant differences were found.



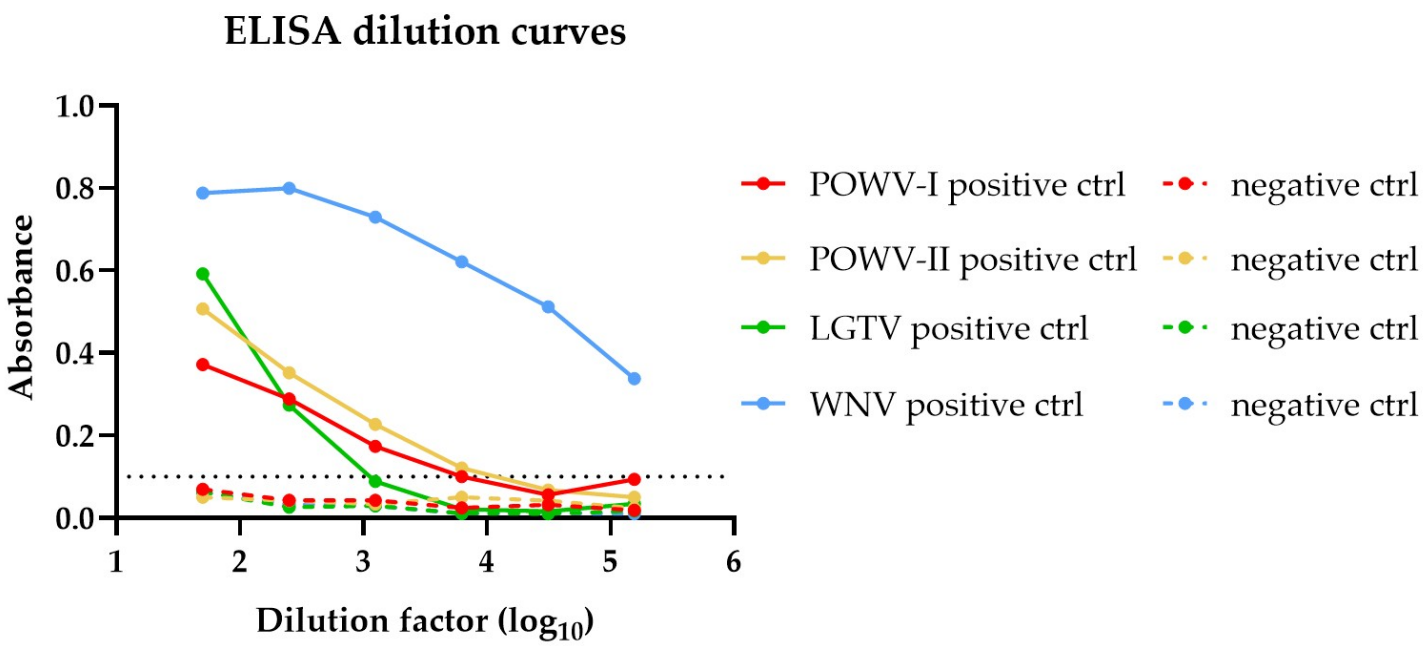
Supplementary figure 3. Western blot. Uncropped western blot from Figure 1B.



Supplementary figure 4. Uncropped Coomassie Blue stained SDS-PAGE gel from Figure 1C.



Supplementary figure 5 Flow gating strategy. Gating strategy to determine CD4+ and CD8+ T cell depletion began with initial gates for lymphocytes and two gates for isolating single cells using forward- and side-scatter. T cells were then isolated by sequentially gating for CD45+ cells followed by CD3+ CD19-. CD45+ CD3+ CD19- cells were then gated as either CD4+ or CD8+ T cells.



Supplementary figure 6. ELISA dilution curves for assay validation. High-binding Costar 96-well plates were coated with 10^5 FFU of POWV-I, LGTV, or WNV or $3.2X10^4$ FFU of POWV-II in final volumes of 100uL PBS per well. Representative positive (unbroken lines) and negative (dotted lines) serum controls tested for anti-flavivirus binding are shown. Dotted line at y = 0.1 represents baseline for determining endpoint titers. Positive control for: POWV-I and -II is convalescent serum from a mouse that twice-survived infection with POWV-I without vaccination; LGTV is serum from mouse prime-boost vaccinated 3 weeks apart with low-dose POW-VLP adjuvanted with INI-4001 and bled 3 weeks post-boost; WNV is convalescent serum from a WNV-immune mouse. Negative controls are all PBS-vaccinated mice.