

Supporting Information for Virus-Like Particles Encapsidating Respiratory Syncytial Virus M and M2 Proteins induce robust T cell responses

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SUPPORTING FIGURES

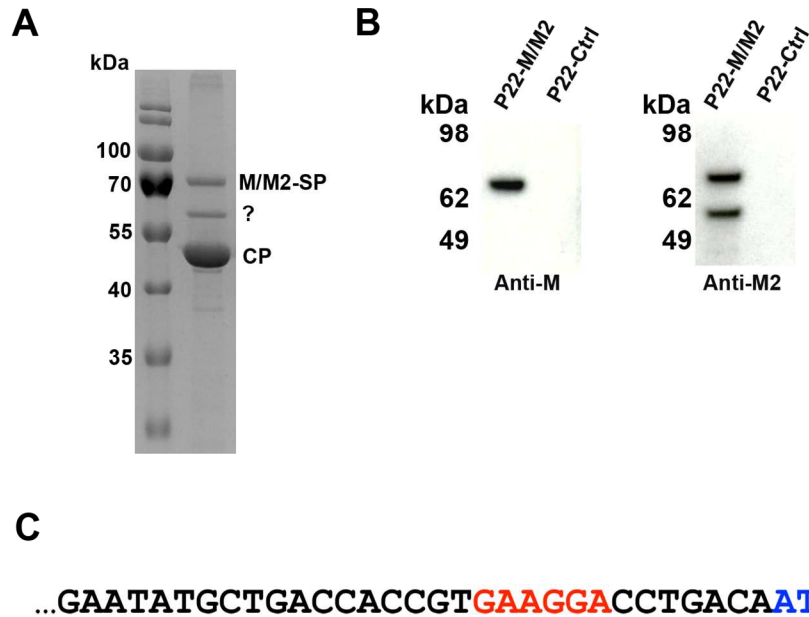


Figure S1: A ~60 kDa alternative product is encapsidated with the expected ~70 kDa M/M2-SP. (A) SDS-Page showing the presence of the expected CP (46 kDa) and M/M2-SP (70 kDa) but also a strong band at ~60 kDa. (B) Western blot of P22-M/M2 with anti-M (left) or anti-M2 (right) indicates that the unknown band at ~60 kDa binds anti-M2 but not anti-M. (C) Sequence analysis of the M/M2-SP gene shows a canonical Shine-

Dalgarno sequence (red) 7 base pairs upstream of the M134 codon (blue). Expression of this product would result in a 55.351 kDa protein.

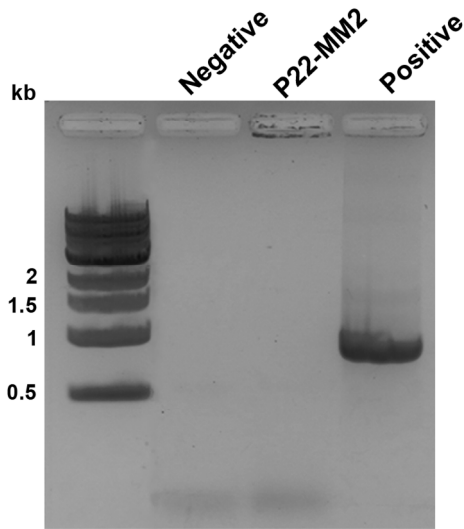


Figure S2: The P22-M/M2 construct does not contain nucleic acid, so intracellular production of M/M2 could not occur as an explanation for the robust induction of CD8 T cells. A primer set, which amplifies the section of the M/M2-SP gene from base pair 201-1115, was used to search for the presence of the M/M2-SP gene in the P22-M/M2 sample. Negative control contains no template and the positive control contains ~50 ng of the P22-M/M2 expression vector.

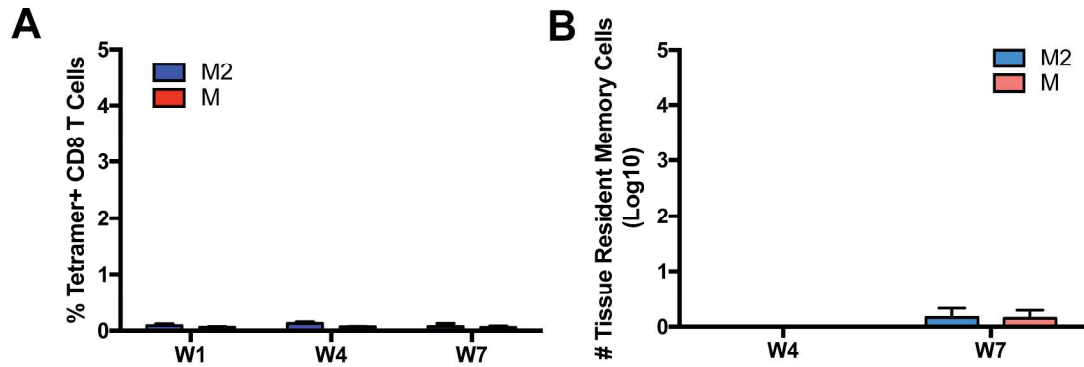


Figure S3. P22-Ctrl does not induce RSV-specific CD8 T cell responses. A) Percent M2 and M-specific CD8 T cells in the lungs of mice vaccinated with P22-Ctrl at weeks 1, 4 and 7. B) Number of M2-specific and M-specific CD8 T cells with a tissue-resident memory phenotype following vaccination with P22-Ctrl. Bars represent mean $\pm$ SEM with 5 mice per group.

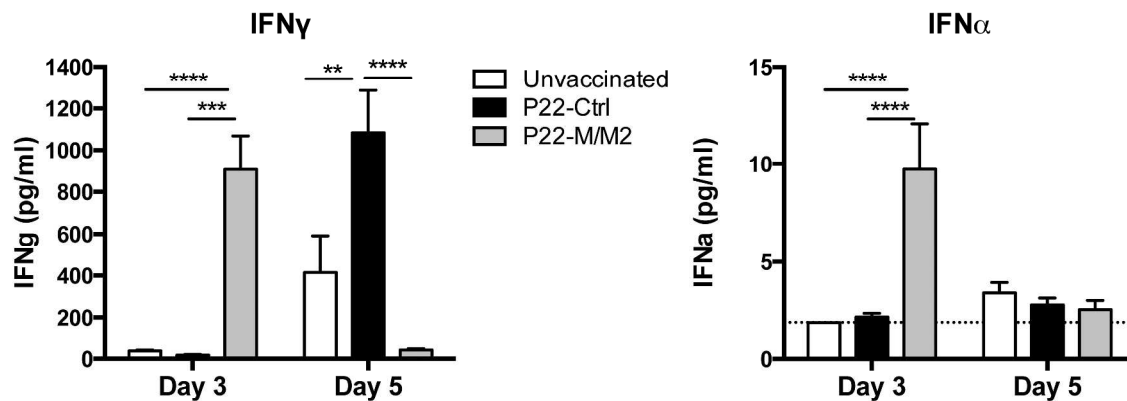


Figure S4: Cytokine levels following RSV challenge. Three and 5 days post-challenge, cytokine concentrations in the lungs were determined by bead-based multiplex assay. Dashed line indicates lowest limit of quantification. Bars represent mean $\pm$ SEM with 5 mice per group. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$ by 2-way ANOVA.

CONSTRUCT SEQUENCES

M/M2-*Bam* HI-thrombin cleavage site-Cys-SP142-303

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M/M2 M134A-Bam HI-thrombin cleavage site-Cys-SP142-303

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