SUPPLEMENTARY MATERIAL

Figure S1 Single letter amino acid sequence of Ag insert.

The signal sequence is underlined. The 10x histidine tag is italicized. The ovalbumin-derived SIINFEKL peptide is bold faced. The SIINFEKL epitope is embedded in a larger peptide to facilitate Ag processing.

Figure S2 Gating strategy for detecting Ag-specific CD8⁺ T cells in mice challenged with LM-OVA.

Mice were immunized with equimolar amounts of MC-Ag, FL-Ag or VC DNA three times, three days apart (on d0, d3, d6). One day after each immunization, mice were injected subcutaneously with 10µg polyICLC. Peripheral blood and spleens were harvested on d11 from LM-OVA challenged mice that were immunized with the indicated DNA. The contour plots below were generated from lymphocytes, based on their forward and side scatter profiles. The gate indicates cells binding the anti-CD8-APC antibody and SIINFEKL-K^b-PE tetramer.

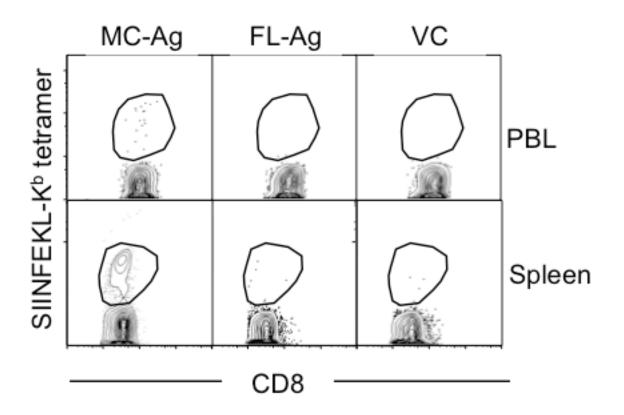


Figure S3. Protection against lower dose LM-OVA challenge elicited by MC-Ag or FL-Ag immunization.

Mice were immunized with equimolar amounts of MC-Ag, FL-Ag or VC DNA three times, three days apart (on d0, d3, d6). One day after each immunization, mice were injected subcutaneously with 10μg polyICLC. Five days after the last immunization (d11), each mouse was injected intravenously with 4.9x10⁴ LM-OVA bacteria. Five days later (d16), the mice were euthanized and bacterial burden was measured as the number of CFUs/spleen. Mice immunized with MC-Ag were significantly protected from LM-OVA challenge when compared to the VC control (P**<0.01; Kruskal-Wallis test corrected with a Dunn's multiple comparison test). There was a trend towards decreased bacterial burden among the FL-Ag immunized mice but the group's mean bacterial burden was not significantly less than the burden in the VC cohort. LOD, limit of detection.

