

### Supplementary Fig. 1. Gating strategy for T cell analyses

CD8<sup>+</sup>CD4<sup>-</sup> T cells were gated within the CD3+ population and progressively gated for primed cells (CD11<sup>hi</sup> and CD44<sup>hi</sup>) and primed subsets, including  $T_{EFF}$  (CD62L<sup>lo</sup>),  $T_{TDE}$  (CD62L<sup>lo</sup>KLRG1<sup>hi</sup>),  $T_{EM}$  (CD62L<sup>lo</sup>KLRG1<sup>lo</sup>) or  $T_{CM}$  (CD62L<sup>hi</sup>KLRG1<sup>lo</sup>) subpopulations, which were further sub-gated for the antigen-specific subpopulations based on tetramer labeling.



### Supplementary Fig. 2. T cell immunity upon vaccination

CD8+CD4- among CD3+ T cells and  $T_{CM}$  (CD62L<sup>hi</sup>KLRG1<sup>lo</sup>) subpopulations are shown. Each symbol indicates an individual mouse and the median of biological replicates was shown. One MCMV<sup>S</sup>-vaccinated animal with poor immune responses is marked with an X throughout and marked with black arrows. Kruskal-Walis tests were used for the statistical analyses.



# Supplementary Fig. 3. Protection against body weight loss upon MCMV<sup>s</sup> vaccination

Loss of body weights were daily observed for 21 days after challenge with the D614G variant (n=6).



## Supplementary Fig. 4. Viral loads upon the challenge with D614G strain after short-term vaccination

Viral RNA loads in the representative organs at the indicated time points. The horizontal lines indicate the median of biological replicates (MCMV<sup>S</sup>: n=7 for dpi 3, n=8 for dpi 7, n=6 for dpi 21; MCMV<sup>WT</sup>: n=9 for dpi 3, n=10 for dpi 5-7; PBS: n=10 for dpi 3, n=9 for dpi 5-7). Organs from animals that reached humane endpoints earlier than 7 dpi were collected on the day of euthanasia. Pooled data from two independent experiments are shown (except for the 21 days data set, which was generated once). The outlier animal with poor immunity is marked with an X (X-marked red dots). Samples with ct values >33 were considered negative determined by uninfected controls and positive SARS-CoV-2 N RNA and plotted in the area of detection limits (grey-shaded).



# Supplementary Fig. 5. Viral loads upon the challenge with D614G strain after long-term vaccination

Viral RNA loads in the representative organs at the indicated time points. The horizontal lines indicate the median of biological replicates (n=4 for MCMV<sup>S</sup>, MCMV<sup>WT</sup>, n=3 PBS for dpi 3; n=5 each for dpi 5-7). Organs from animals that reached humane endpoints earlier than 7 dpi were collected on the day of euthanasia. Samples with ct values >33 were considered negative determined by uninfected controls and positive SARS-CoV-2 N RNA and plotted in the area of detection limits (grey-shaded).



#### Supplementary Fig. 6. Clinical scoring after the challenge with the Omicron variant

(A) Percentage of body weight monitored for 3 days after the Omicron variant infection. The median values of biological replicates with 95% confidence interval are shown (n=4 MCMV<sup>s</sup>, n=5 MCMV<sup>WT</sup>). (B) Accumulative clinical scores. Representative symbols represent the values of summed scores up to 3 dpi. (C) Percentage of body weight monitored for 3 days after the Omicron virus challenge. The median values of biological replicates with 95% confidence interval are shown (n=12 each). (D) Accumulative clinical scores. Representative symbols represent the values of an animal summed scores up to 3 dpi. Medians (bar graphs) are shown with 95% confidence interval. Two-tailed Mann-Whitney tests were used for statistical analyses. 1 month (A-B) or 5 months (C-D) after vaccination.