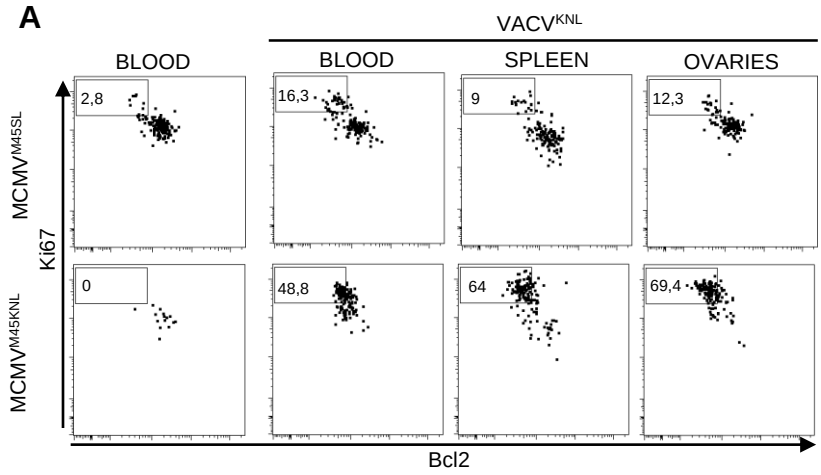
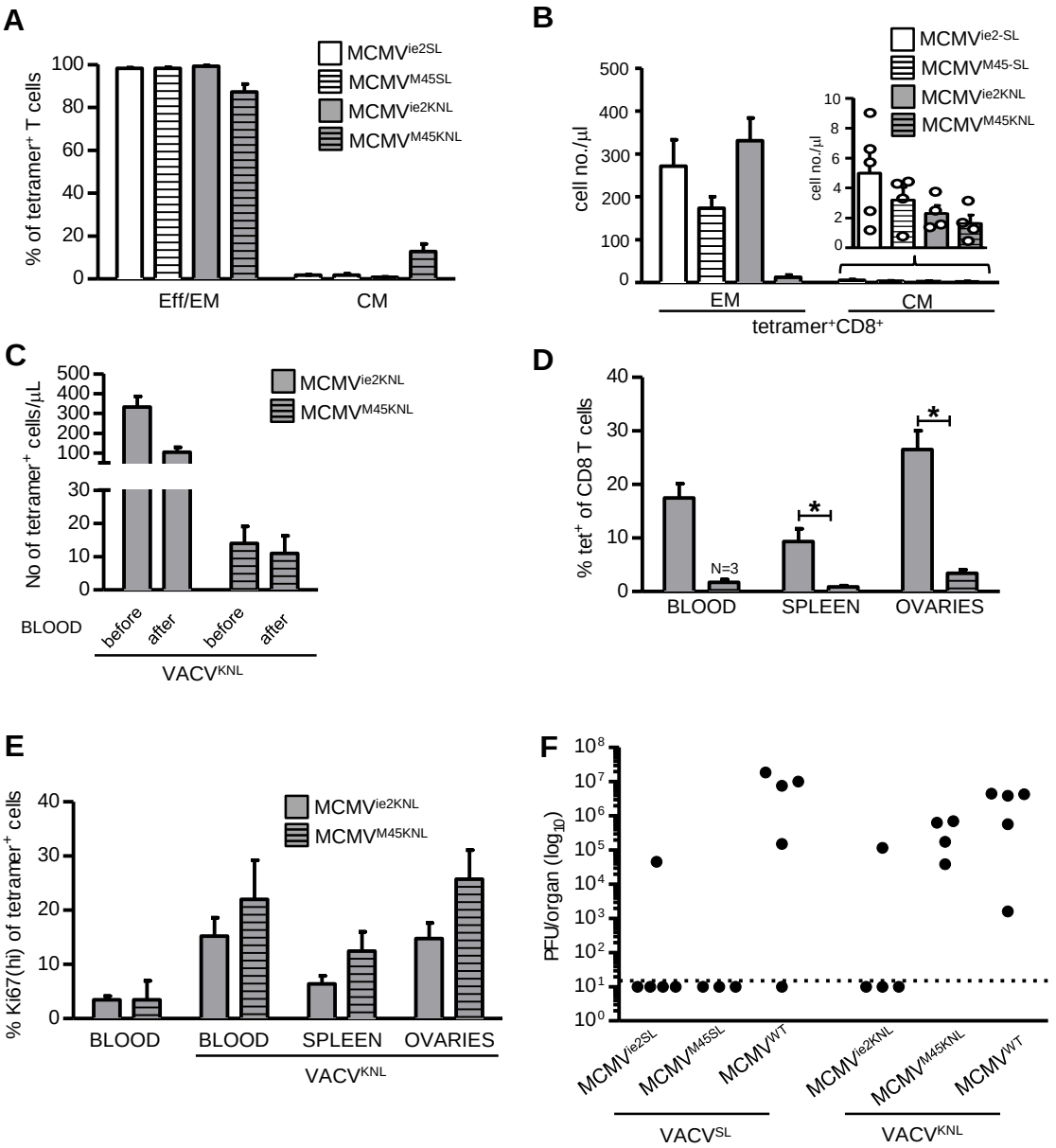
Supplementary Figure 1



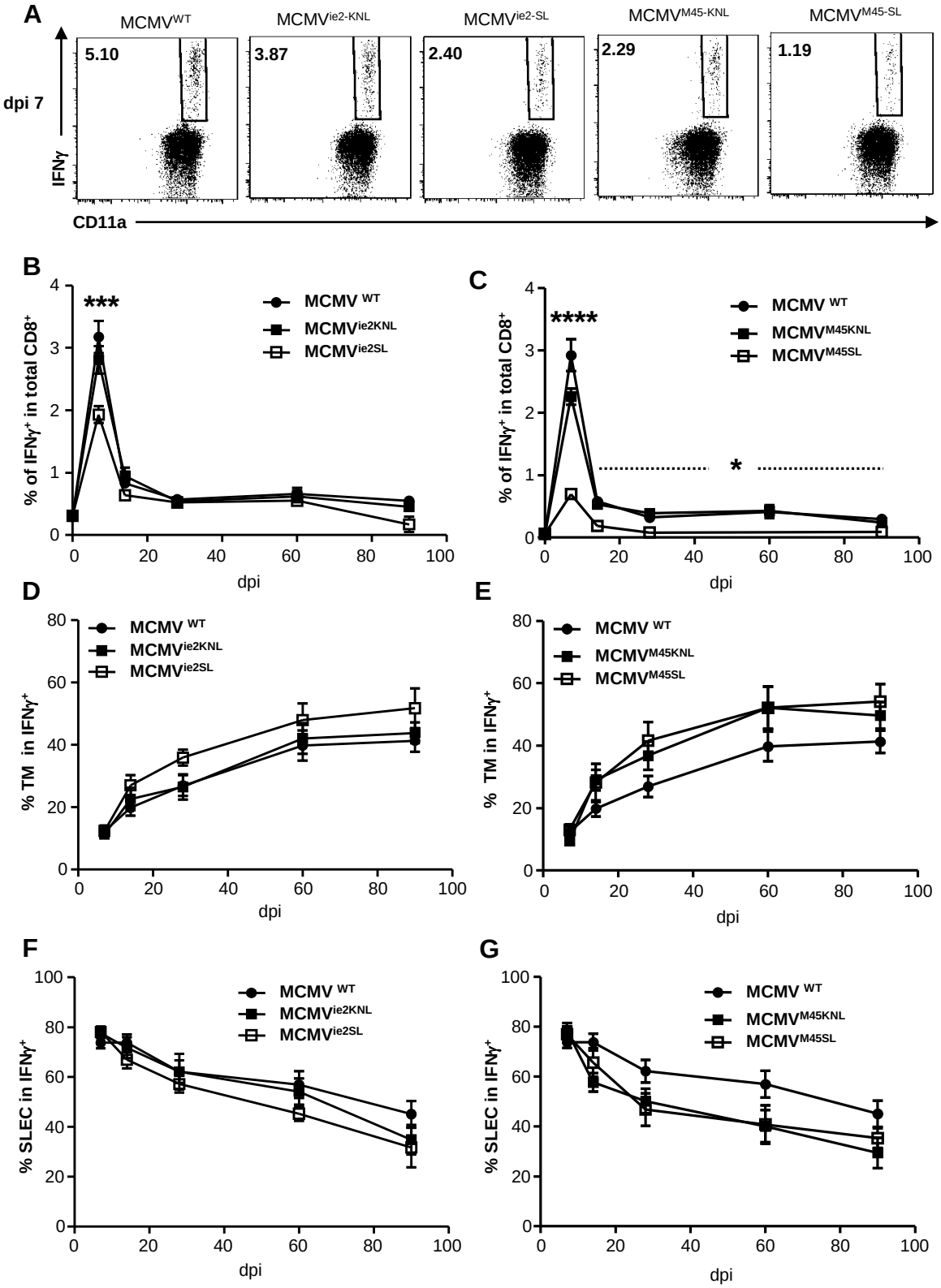
Supplementary Figure 1. Gating strategy used to identify Ki67^{hi}Bcl2^{low} cells. 129S2/SvPas females were infected intraperitoneally with 2x10^5 PFU of MCMV^{ie2KNL} or MCMV^{M45KNL}. Three months later animals were bled for analysis and three days later challenged intraperitoneally with 10^6 PFU VACV^{KNL}. Isolates from blood, spleen and ovaries analyzed at dpi 4. Plots are pre-gated on tetramer$^+$CD8 T cells. Representative data from one experiment using 4-5 mice per group.

Supplementary Figure 2



Supplementary Fig 2. MCMV^{M45KNL} fails to protect against VACV^{KNL} challenge in C57BL/6 mice. C57BL/6 females were infected intraperitoneally with 10^6 PFU of MCMV^{WT}, MCMV^{ie2SL}, MCMV^{M45SL}, MCMV^{ie2KNL}, or MCMV^{M45KNL}. Three months post infection mice were bled before being challenged intraperitoneally with 10^6 PFU VACV^{SL} (MCMV^{WT}, MCMV^{ie2SL}, MCMV^{M45SL}) or VACV^{KNL} (MCMV^{WT}, MCMV^{ie2KNL}, MCMV^{M45KNL}). Four days post challenge blood, spleen, and ovaries were obtained. A) Percentage of T effector/TEM (CD62L$^-$ CD44$^+$) and TCM (CD62L$^+$ CD44$^+$) in tet$^+$ CD8 T-cells in blood prior to challenge. B) Absolute numbers of tet$^+$ T effector/TEM and tet$^+$ TCM CD8 T-cells prior to challenge with VACV. Mean (SEM) from one experiment using 4-5 mice per group. Each circle represents an animal. C) Absolute numbers of tet$^+$ CD8 T-cells in blood prior to and 4 days post challenge with VACV. Mean (SEM) from one experiment using 3-4 mice per group. D) Percentage of tet$^+$ T cells in CD8 population in blood, spleen, and ovaries on d4 post challenge. Mean (SEM) from one experiment using 3-4 mice per group. Groups were compared using the Mann-Whitney U test. * $p \leq 0.05$ E) Percentage of Ki-67^{hi} cells in the tet$^+$ CD8 T-cell population in blood, spleen, and ovaries on d4 post challenge. Mean (SEM) from one experiment using 3-4 mice per group. F) Vaccinia PFUs in ovaries on d4 post challenge. Each dot represents an animal. Dotted line indicates the detection limit of the assay.

Supplementary Figure 3



Supplementary Fig 3. High-avidity responses suppress M45-specific T-cells on dpi 7
129S2/SvPas females were infected intraperitoneally with $2x10^5$ PFU of $MCMV^{WT}$, $MCMV^{ie2SL}$, $MCMV^{M45SL}$, $MCMV^{ie2KNL}$, or $MCMV^{M45KNL}$. Blood was taken 7 days prior to infection (d0) and on dpi 7, 14, 28, 60, 90, 120, and 180 and analyzed via peptide stimulation with HGIRNASFI (M45) peptide followed by intracellular cytokine staining with antibody against IFNγ. $CD8^+$ T-cells were identified as $CD3^+$, $CD4^-$, and $CD8^+$ via antibody staining, $CD11a^+$ T-cells were considered primed. A) Representative dot plots of the M45-specific response in all viruses at dpi 7 B) Percentage of responding cells ($IFN\gamma^+$) in mice infected with MCMV recombinants with insertion of SL or KNL in the inflationary genetic context, *ie2*. *** $p \leq 0.001$ on dpi 7 comparing $MCMV^{ie2SL}$ and $MCMV^{WT}$ C) Percentage of responding cells ($IFN\gamma^+$) in mice infected with MCMV recombinants with insertion of SL or KNL in the conventional genetic context, *M45*. **** $p \leq 0.0001$ on dpi 7 and * $p \leq 0.05$ from dpi 14 onwards comparing $MCMV^{M45SL}$ and $MCMV^{WT}$. The immune phenotype of specific CD8 T-cells was analyzed via antibody staining against KLRG-1 and CD44. SLEC were identified as $CD44^+$ $KLRG\text{-}1^+$ and TM as $CD44^+$ $KLRG\text{-}1^-$. TM in antigen-specific T-cells in mice infected with $MCMV^{ie2}$ recombinants (D) or with $MCMV^{M45}$ recombinants (E). SLEC in antigen-specific T-cells in mice infected with $MCMV^{ie2}$ mutants (F) or with $MCMV^{M45}$ mutants (G). Data from three independent experiments were pooled, error bars indicate SEM. Statistical analysis was performed at each time point by Kruskal-Wallis test followed by Dunn's post hoc analysis.