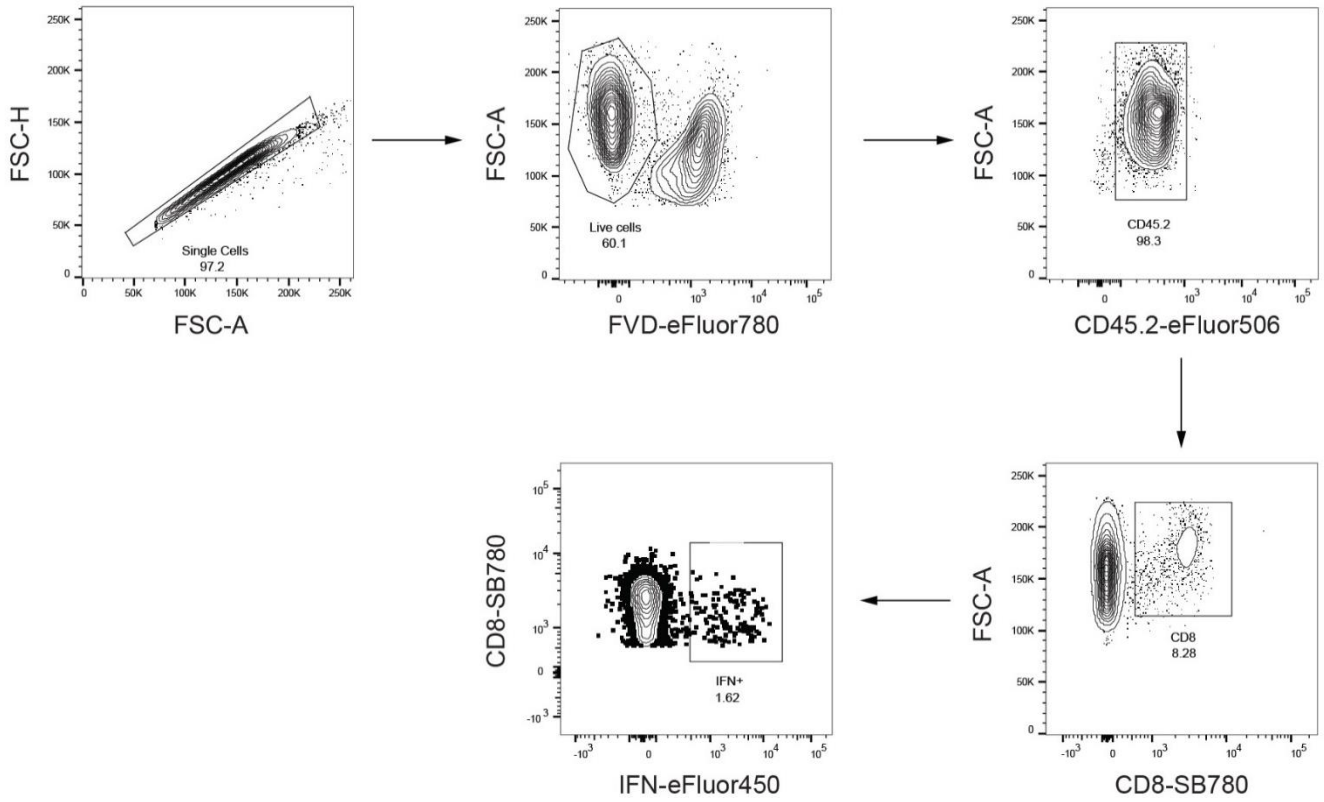
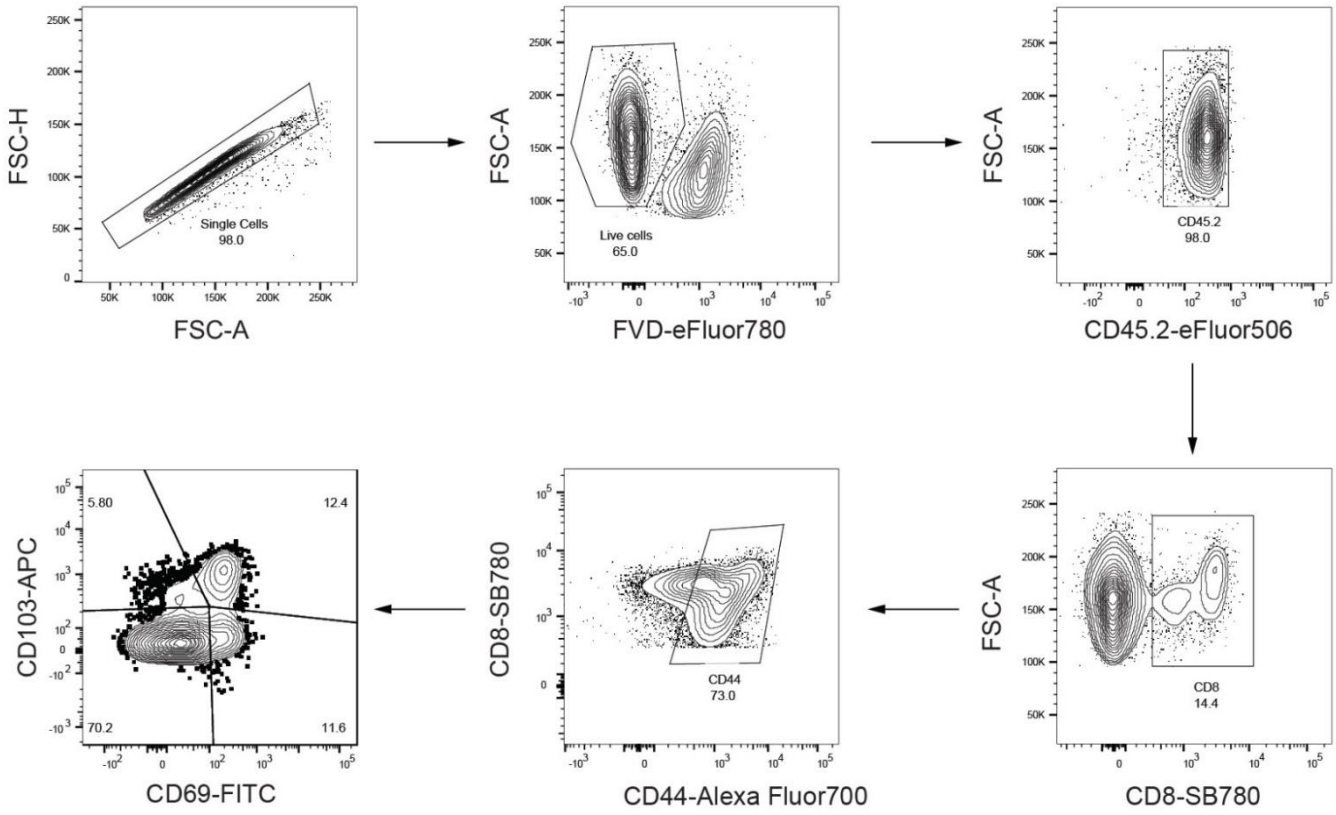
Supporting information Figure 1



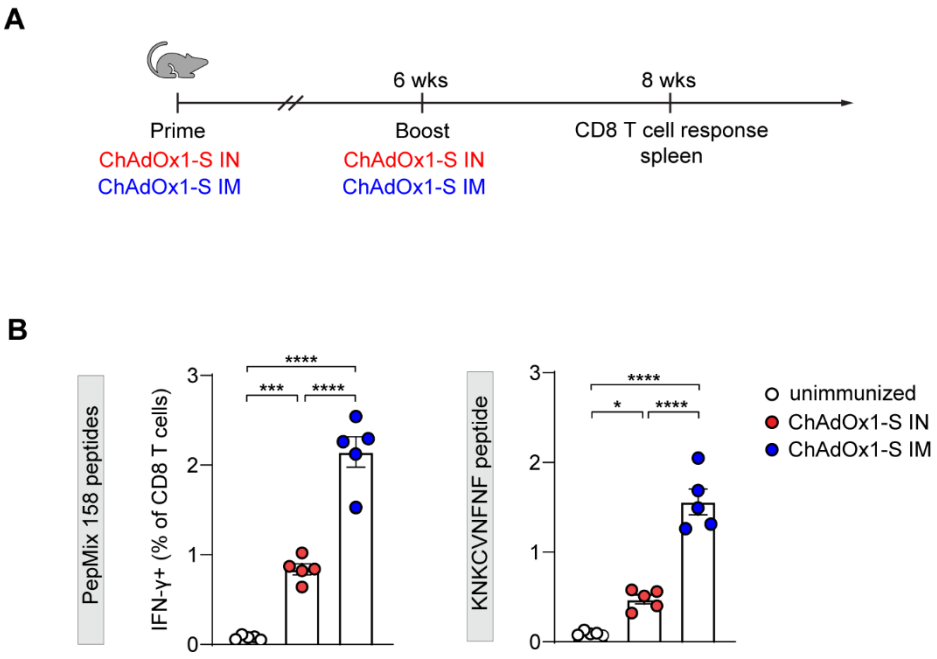
Supporting information Figure 1 (related to Figure 2 and Figure 3). Gating strategy used to detect IFN-γ producing cells in the lungs and spleen of BALB/c mice. Depicted is the gating path in a spleen sample from ChAdOx1-S IM immunized animal.

Supporting information Figure 2



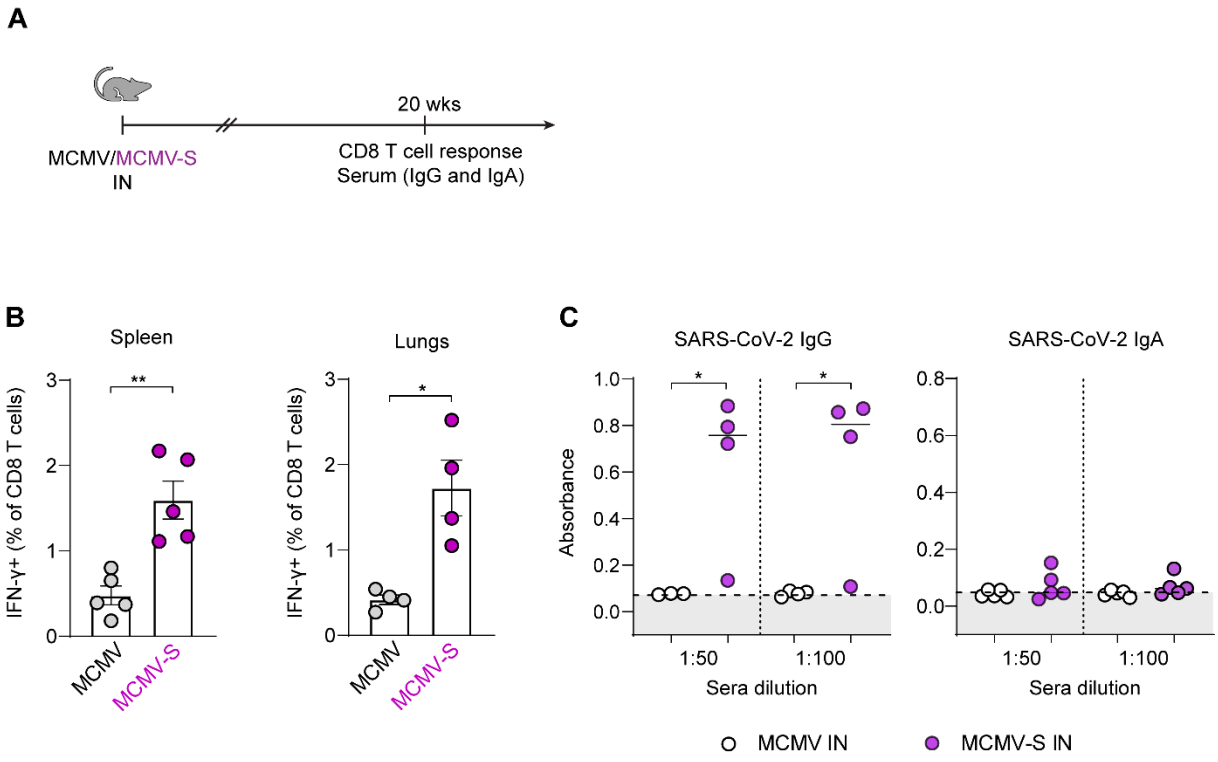
Supporting information Figure 2 (related to Figure 2). Gating strategy used to detect tissue-resident (T_{RM}) CD8 T cells in the lungs of BALB/c mice. Depicted is the gating path in a lung sample from ChAdOx1-S IN immunized animal.

Supporting information Figure 3



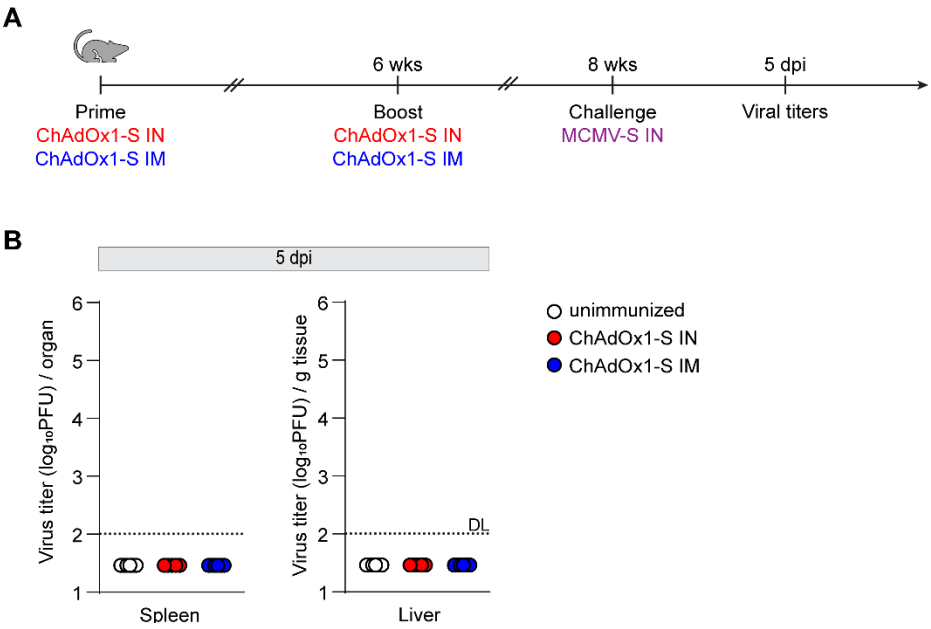
Supporting information Figure 3 (related to Figure 2). IFN-γ production by CD8 T cells in the spleen of BALB/c mice after peptide stimulation. (A) BALB/c mice were vaccinated via intranasal (IN) or intramuscular (IM) route with ChAdOx1-S. (B) Eight weeks after the first immunization spleen homogenates were restimulated with Spike-specific peptide pool (left panel) and peptide KNKCVNFNF (S535-543) (right panel). The responding CD8 T cells were identified by intracellular staining for accumulated IFN-γ. Bars represent group means overlaid with individual data points (n=5). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test; p values indicate significant differences (*p < 0.05, ***p < 0.001, ****p < 0.0001).

Supporting information Figure 4



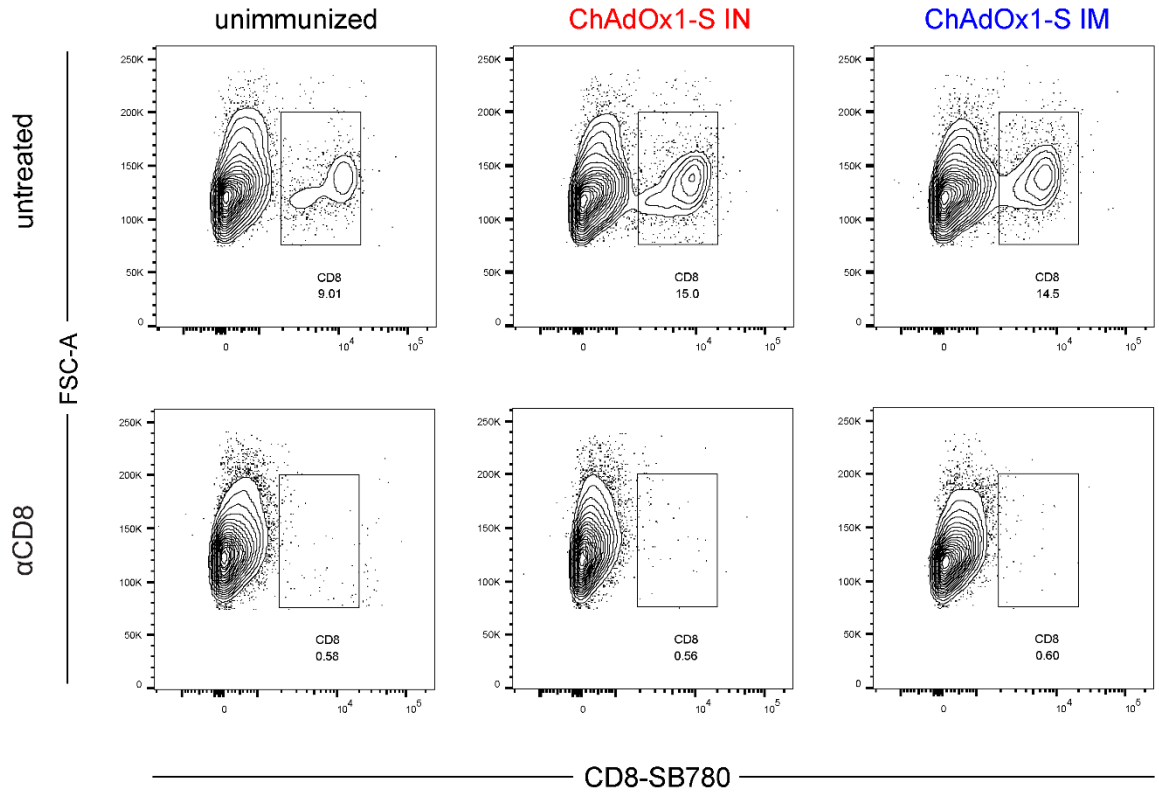
Supporting information Figure 4 (related to Figure 3). Cellular and humoral immune response after intranasal MCMV-S infection. (A) BALB/c mice were infected intranasally with MCMV or MCMV-S (2×10^5 PFU/mouse). (B) Twenty weeks after intranasal immunization spleen (left panel) and lung (right panel) homogenates were restimulated with Spike-specific peptide pool. The responding CD8 T cells were identified by intracellular staining for accumulated IFN-γ. Bars represent group means overlaid with individual data points (n=5). (C) Spike-specific IgG and IgA were assessed by ELISA (n=3-5). Dotted lines indicate the median absorbance value of MCMV immunized mice sera. Data were analyzed by Mann-Whitney U test; p values indicate significant differences (*$p < 0.05$; **$p < 0.01$).

Supporting information Figure 5



Supporting information Figure 5 (related to Figure 3). Virus titers in the organs of BALB/c mice after intranasal MCMV-S challenge. (A) BALB/c mice were vaccinated via intranasal (IN) or intramuscular (IM) route with ChAdOx1-S. Six weeks after the first immunization, mice were boosted and two weeks later challenged with intranasal (IN) inoculation of MCMV-S (2×10^5 PFU/mouse). (B) Five days after the intranasal challenge,tissues were harvested and viral titers determined in spleen and liver homogenates (n=4-7). Titers in organs of individual mice are shown (circles). Dotted lines indicate the detection limit of the assay (DL).

Supporting information Figure 6



Supporting information Figure 6 (related to Figure 3). Efficacy of in vivo CD8 T cell depletion in BALB/c mice. CD8 T cells were depleted systemically by intraperitoneal injection of 500 µg of α-CD8α antibody one day before intranasal MCMV-S challenge. Leukocytes from spleen and lungs were analyzed by flow cytometry on day 5 post-depletion. Representative flow cytometry panels of CD8 T cells in the lungs are shown.