

Supplementary information

Epitope mapping and kinetics of CD4 T cell immunity to pneumonia virus of mice in the C57BL/6 strain

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Inventory

Supplementary Figure S1, related to Figure 1

Supplementary Figure S2, related to Figure 2

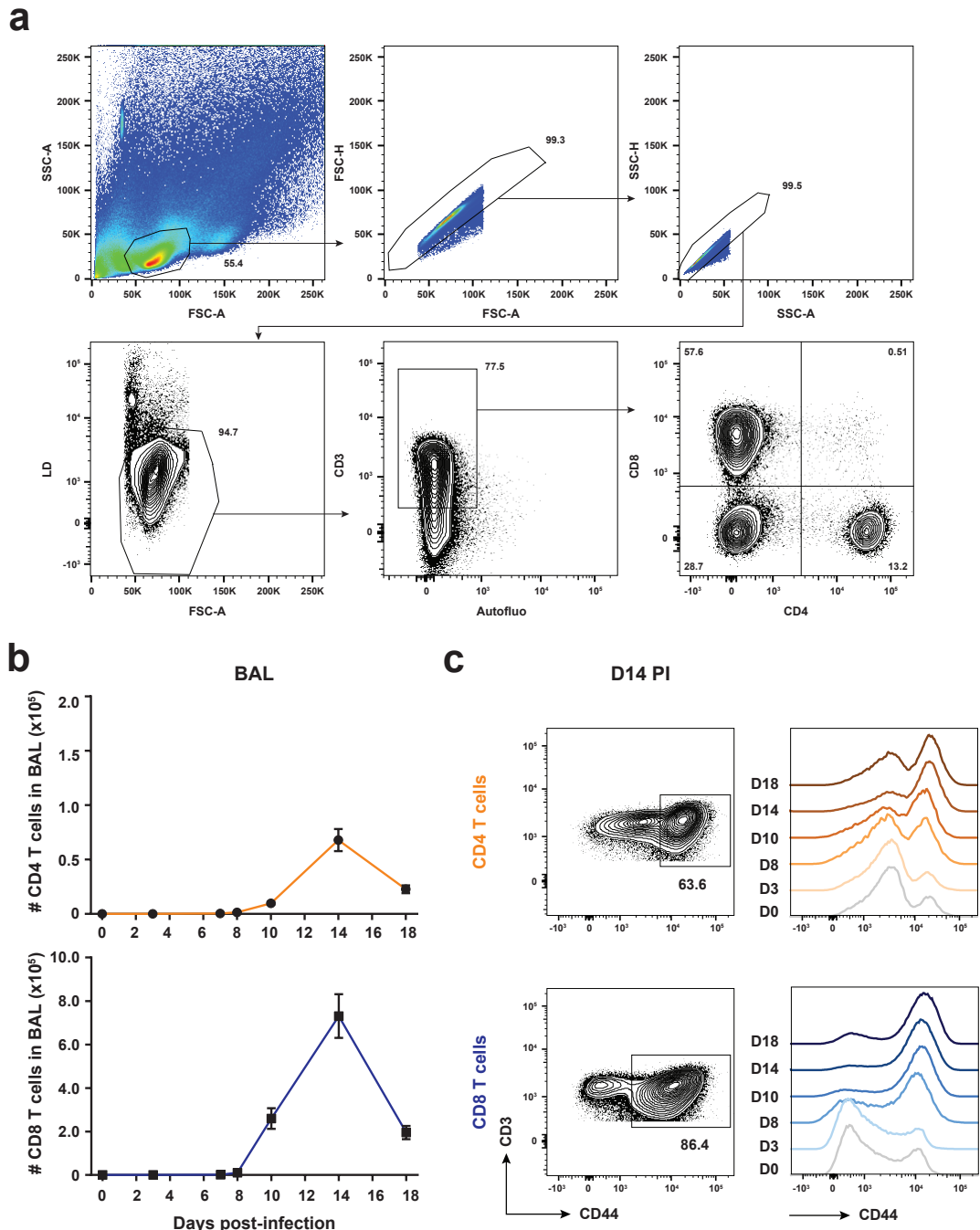
Supplementary Figure S3, related to Figure 3

Figure legends

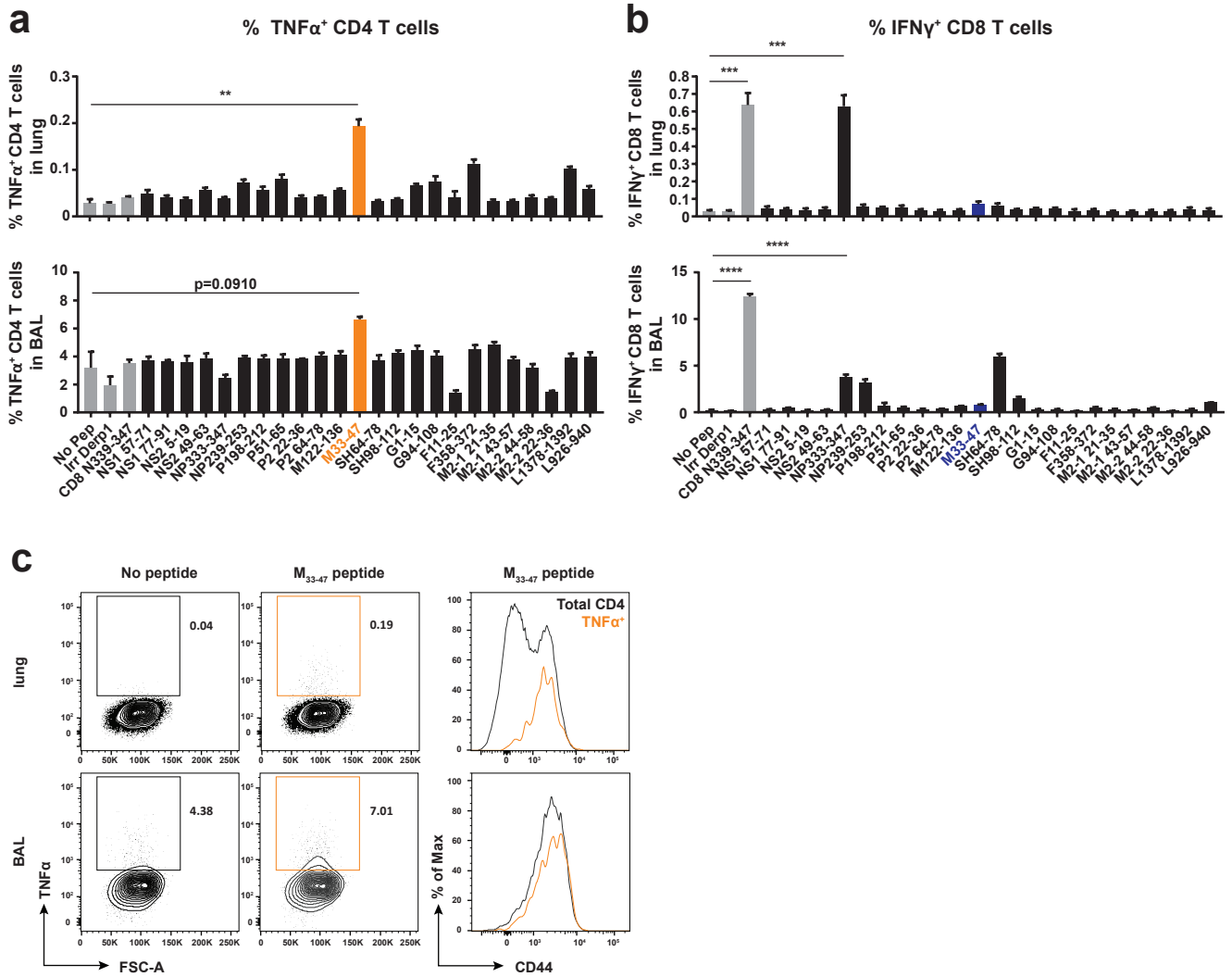
Supplementary Figure S1 | Gating strategy and dynamics of CD4 and CD8 T cell responses in PVM-infected mice. Mice were infected i.t. with a sub-lethal dose of PVM strain J3666 and sacrificed at the indicated days post-infection. (a) Representative FACS plots show gating strategy on lung cells at day 14 pi to identify CD4 and CD8 T cells by means of flow cytometry. A similar gating strategy was used to analyze MLN and BAL (data not shown). (b) Absolute numbers of CD4 (orange) and CD8 (blue) T cells in the BAL, as determined by flow cytometry. Data points are shown as mean \pm SEM (n=5 per time point). (c, left panel) Representative gating strategy on lung CD4 and CD8 T cells 14 days pi to determine the frequency of CD44⁺ T cells (n=5 per time point). (c, right panel) Histogram overlays depict MFI of CD44 expression for both CD4 and CD8 T cells of one representative mouse at the indicated days post-infection. All results are representative of three independent experiments. BAL, bronchoalveolar lavage; MFI, mean fluorescence intensity; PI, post-infection; D, day.

Supplementary Figure S2 | Evaluation of T cell-mediated cytokine production in response to predicted MHCII-restricted PVM peptides. 8 week old C57BL/6 females were infected i.t. with a sub-lethal dose of PVM strain J3666 and sacrificed 14 days later. BAL and lung single-cell suspensions were restimulated for 6 h with each of the predicted MHCII-restricted PVM peptides (enlisted in Table 1), in the presence of Golgistop. T cells were evaluated for cytokine production by intracellular staining and flow cytometry analysis. (a) TNF α production by CD4 T cells in BAL and Lung following peptide restimulation, depicted as frequency of TNF α -producing cells among total CD4 T cells. (b) CD8 T cell-mediated IFN γ production in BAL and lung after peptide restimulation. Data are shown as frequency of IFN γ -producing cells among total CD8 T cells. As controls, cells were incubated without peptide, with an irrelevant Derp1 CD4 peptide or with a MHCI-restricted PVM N₃₃₉₋₃₄₇ peptide, as shown in gray. (c, left panel) Representative FACS plots (gated on CD4 T cells) show the percentage of TNF α ⁺ CD4 T cells in response to restimulation with or without M₃₃₋₄₇ peptide. (c, right panel) Histogram overlays depict CD44 expression levels of total CD4 T cells and gated TNF α ⁺ CD4 T cell populations (marked orange in left panel) following restimulation with M₃₃₋₄₇ peptide. Data are normalized to and depicted as the percentage of the maximum count (% of max on the Y axis). Results are shown as mean \pm SEM from 3 biological replicates. For each biological replicate 10 mice were pooled to obtain sufficient cell numbers for epitope screening. Data are representative of two independent experiments. For statistics (Student's *t* test with Welch correction in (a) or ANOVA for multiple comparisons in (b)), conditions restimulated with peptide were compared to the no-peptide control as indicated. BAL, bronchoalveolar lavage; IFN γ , interferon gamma; TNF α , tumor necrosis factor alpha; MHCI/II, major histocompatibility complex class I or II; Derp1, *Dermatophagoides pteronyssinus* peptidase 1; Irr, irrelevant.

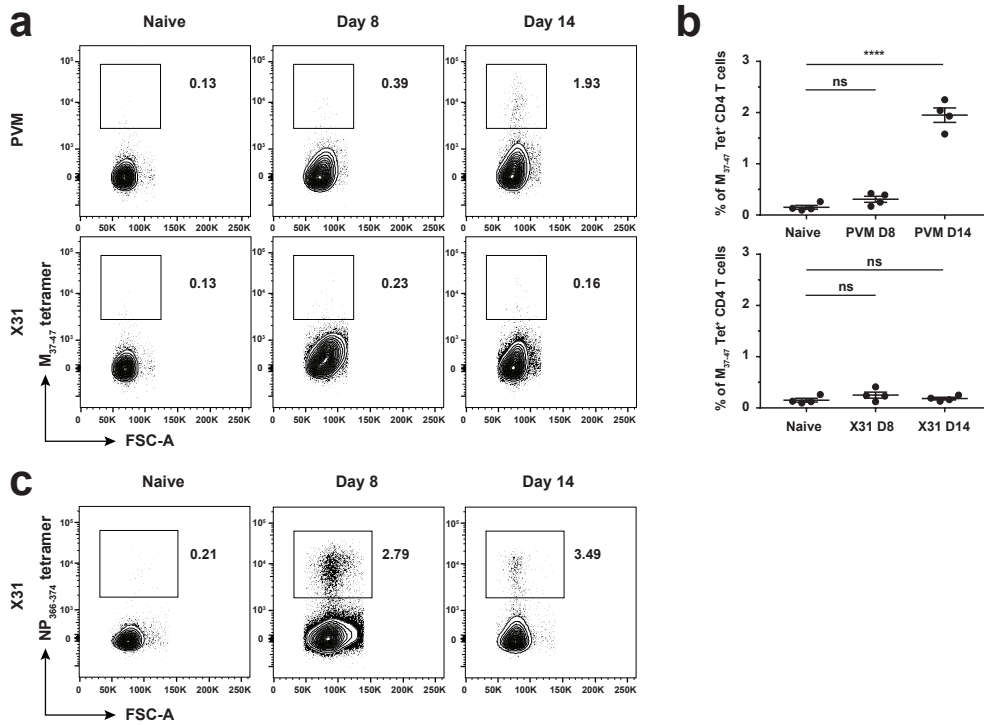
Supplementary Figure S3 | *In vivo* validation of the specificity of the M₃₇₋₄₇ MHCII tetramer in naïve mice and PVM- or Influenza X31-infected mice. 11 week old C57BL/6 females were infected i.t. with a sub-lethal dose of PVM strain J3666 or i.n. with 10³ TCID50 of Influenza virus strain X31. At the indicated timepoints post-infection (pi), PVM-specific CD4 T cells and Influenza X31-specific CD8 T cells in the lung were identified by flow cytometry, using M₃₇₋₄₇ and NP₃₆₆₋₃₇₄ peptide-loaded MHCII and MHCI tetramers. (a) Representative FACS plots of manually gated CD4 T cells show percentages of M₃₇₋₄₇-tetramer⁺ CD4 T cells from PVM-infected (upper panels) or Influenza X31-infected mice (lower panels). Naïve uninfected mice were also included as a control. (b) Same data as in a, summarized in a graph with each datapoint representing one individual mouse. Results are shown as mean \pm SEM (n=4). (c) Representative FACS plots of manually gated CD8 T cells show percentages of NP₃₆₆₋₃₇₄-tetramer⁺ CD8 T cells from naïve mice or Influenza virus X31-infected mice sacrificed at day 8 or day 14 pi. The data shown here were performed one time. For statistics, PVM-infected mice were compared to non-infected controls as indicated (ANOVA for multiple comparisons).



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Supplementary Figure S2 | Evaluation of T cell-mediated cytokine production in response to predicted MHCII-restricted PVM peptides.



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