

Supplemental Figure 1. Gating on activated CD4⁺ T cells in the LLO co-transfer model. 20,000 LLO56 and 20,000 LLO118 cells were co-transferred into a B6 recipient and infected with actA-Lm the following day. Splenocytes were harvested on day 7 post-infection. Representative flow plots are shown depicting the complete gating strategy for activated CD4⁺ T cells (live/single/CD4⁺/CD44^{hi}) within the LLO56 (CD90.1⁺), LLO118 (CD45.1⁺), and B6 (CD90.1⁻/CD45.1⁻) populations. For all plots, the numbers shown represent the frequency of the population within the drawn gate.



Supplemental Figure 2. Generation of NP-LLO^{LT}-N. a, A schematic depicting the LLOp epitope. The core residues are in purple, flanking residues in green, and the lysine that is hypothesized to bind NP and interfere with LLO56 activation is highlighted in yellow. **b**, *In vitro* T cell stimulation assays were performed with LLO56 cells in the presence of either LLO^{LT}, NP-LLO^{LT}, or NP-LLO^{LT}-N. T cells were harvested 24 hours post-stimulation and assessed by flow cytometry for upregulation of CD69. The graph shown is representative of 2 independent experiments. **c**, 20,000-500,000 naive LLO cells were co-transferred into naive B6 mice and then immunized with NP-LLO^{LT}-N to determine Tfh cell frequencies in the LLO populations at days 4, 7, and 10 post-immunization. Representative flow plots are shown for one experiment (n=5), numbers represent the cell frequencies in the Teff, pre-Tfh, and Tfh gates.



🗖 PNA 🗖 IgD 📕 Hoechst

Supplemental Figure 3. Gating strategy for NP⁺-GC B cell analysis by flow cytometry and control images for GC B cell fluorescence imaging. a, Representative flow plots depicting the gating strategy for NP⁺-GC B cells (live/single/CD19⁺/B220⁺/GL7⁺/IgD^{lo}/Fas⁺/NP⁺). Included is a control LLO^{LT}-N immunized mouse for NP-gating reference. For all plots, the numbers shown represent the frequency of the population within the drawn gate. **b**, Representative images from *Tcra^{-/-}* mice that received no T cell transfers but were immunized with NP-LLO^{LT}-N in Alhydrogel. On days 7 and 10 post-immunization spleens were harvested and prepared for immunohistochemistry. Sections were stained with PNA (yellow), IgD (white), and Hoechst (blue). Fluorescent images were taken of entire spleen sections, and images are representative of n=4 for each time-point, 3 independent experiments for day 7 and 2 independent experiments for day 10. Scale bars (bottom left corners) = 500 µm.



Supplemental Figure 4. Gating on naive CD4⁺ T cells post-CD4 enrichment. Spleens from naive Nur77-GFP mice were harvested and a CD4 enrichment was performed prior to antibody staining for flow cytometry analysis. Shown are representative flow plots depicting the complete gating strategy for a naive CD4⁺ T cell population (live/single/CD4⁺/CD62L⁺/CD25⁻/CD44^{lo}) that will be further sorted by Nur77-GFP expression, as shown in Fig. 6b. For all plots, the numbers shown represent the frequency of the population within the drawn gate.