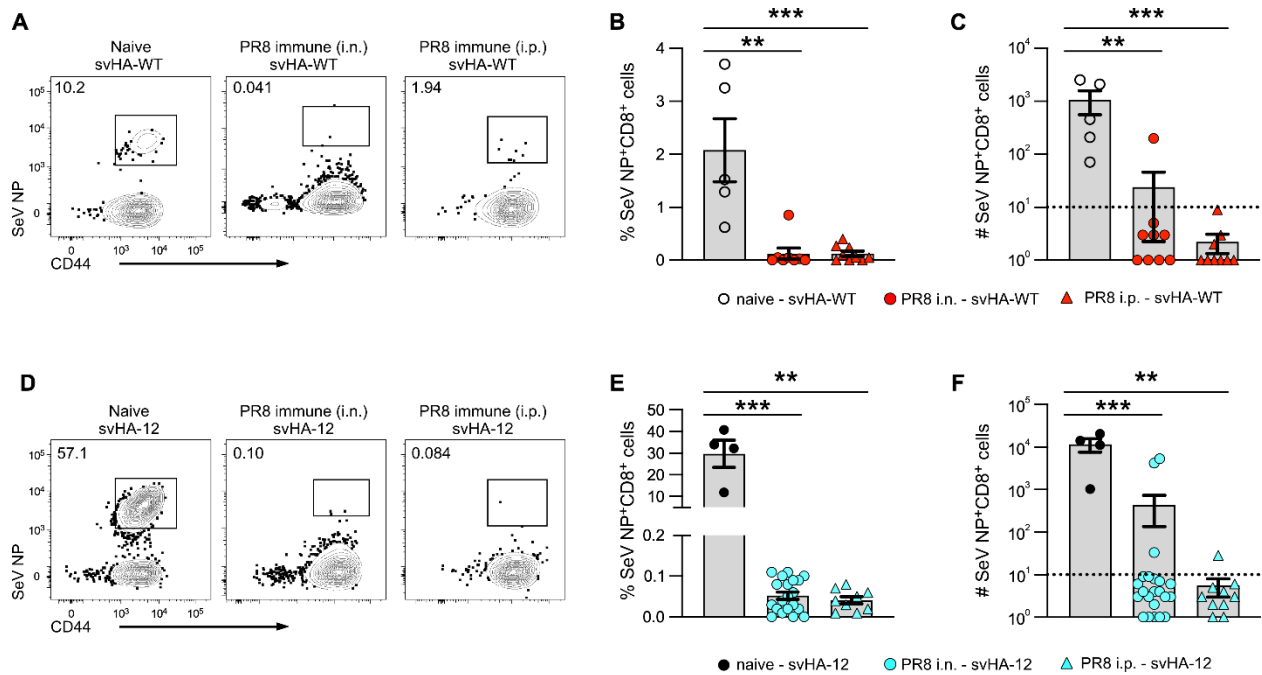


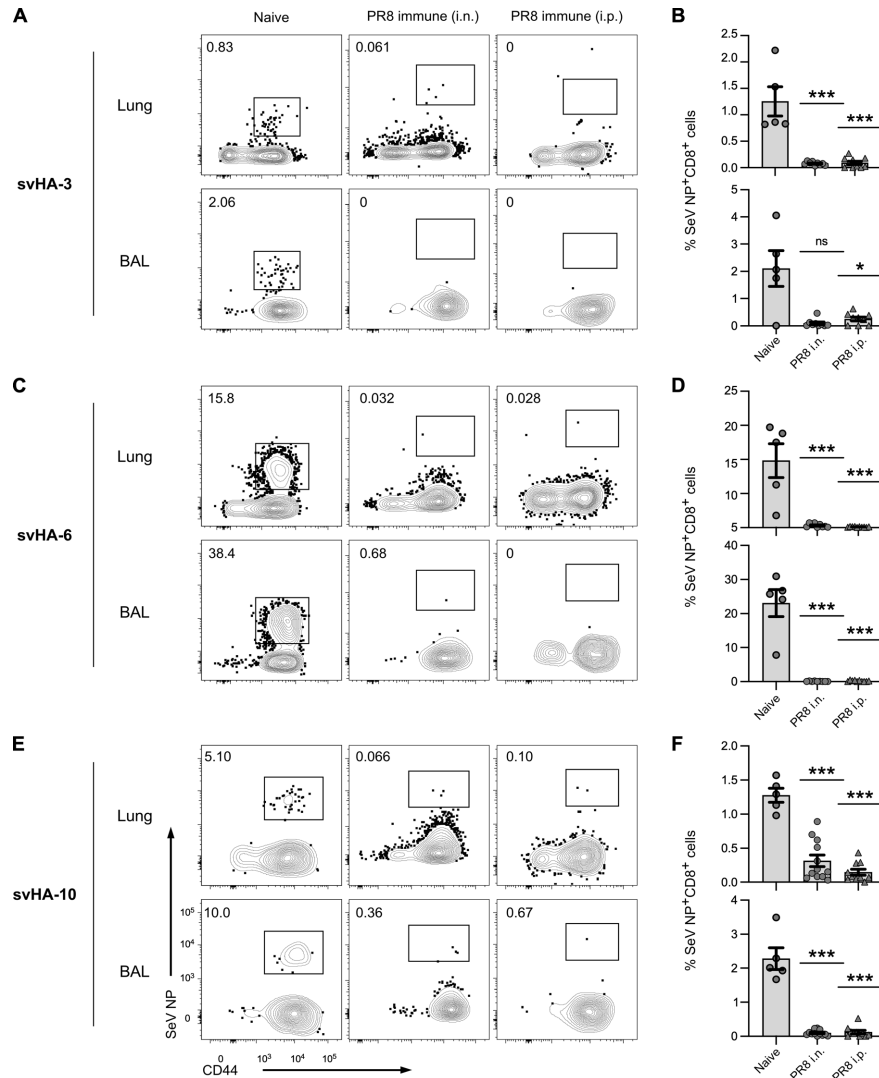
Supplemental data, Lobby et al.

Supplemental Figure 1



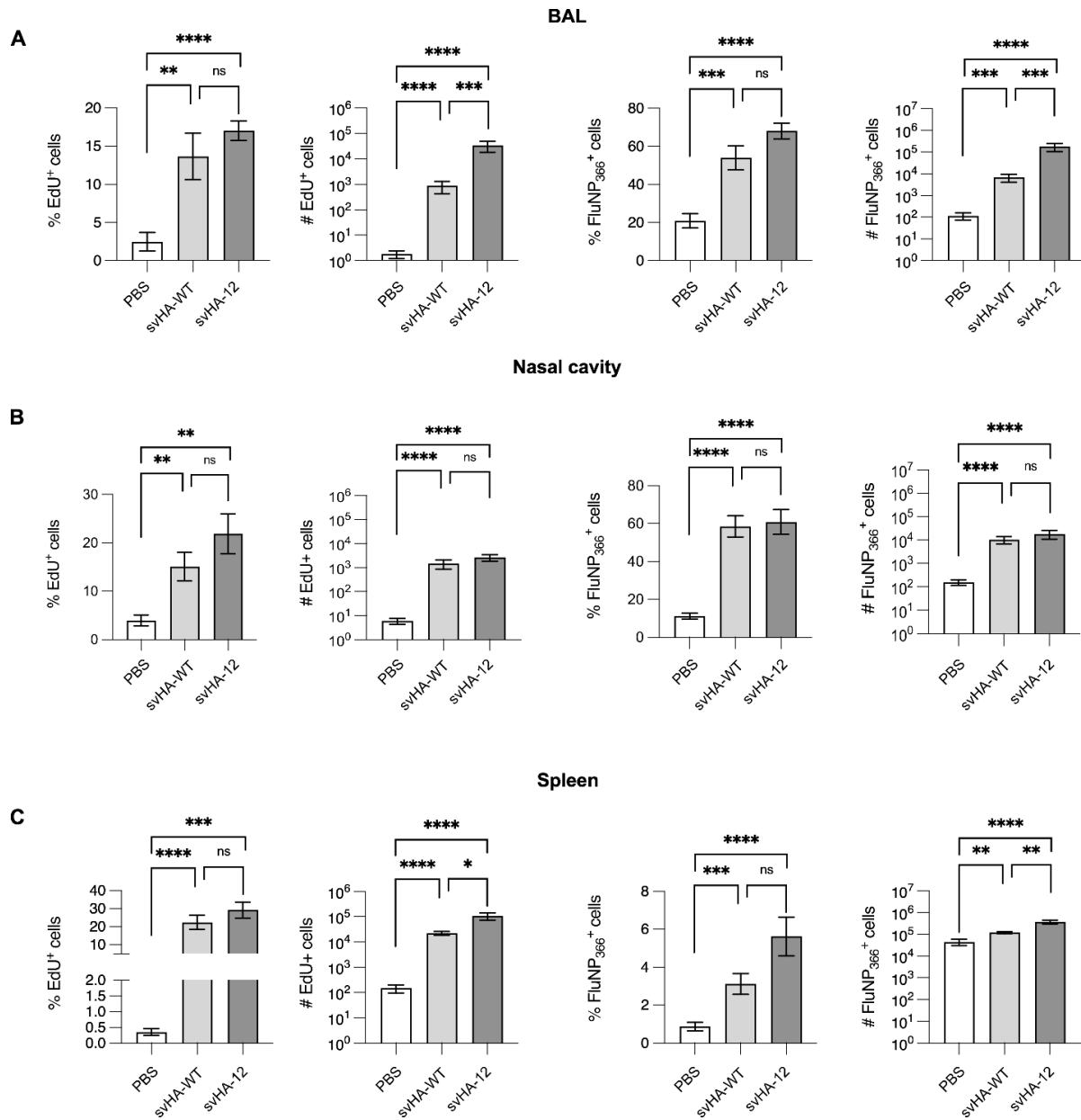
Supplemental Figure 1. LAIV immunization of PR8-immune mice fails to generate *de novo* CD8⁺ T_{RM} in the airways. (A) Example staining for SeV NP⁺ CD8⁺ T_{RM} in the BAL of naïve or PR8-immune (i.n. or i.p. administration) mice 30 d.p.i. with svHA-WT. (B, C) Frequency (B) and number (C) of SeV NP⁺ CD8⁺ BAL T_{RM} in naïve or PR8-immune mice 30 d.p.i. with svHA-WT. (D-F) Same as A-C except with svHA-12. For naïve animals, n = 5 mice from 2 experiments. For PR8-immune animals for each immunization strain, n = 2 independent experiments with 5-11 mice per experiment. LOD indicated by dotted line at 10¹.

Supplemental Figure 2



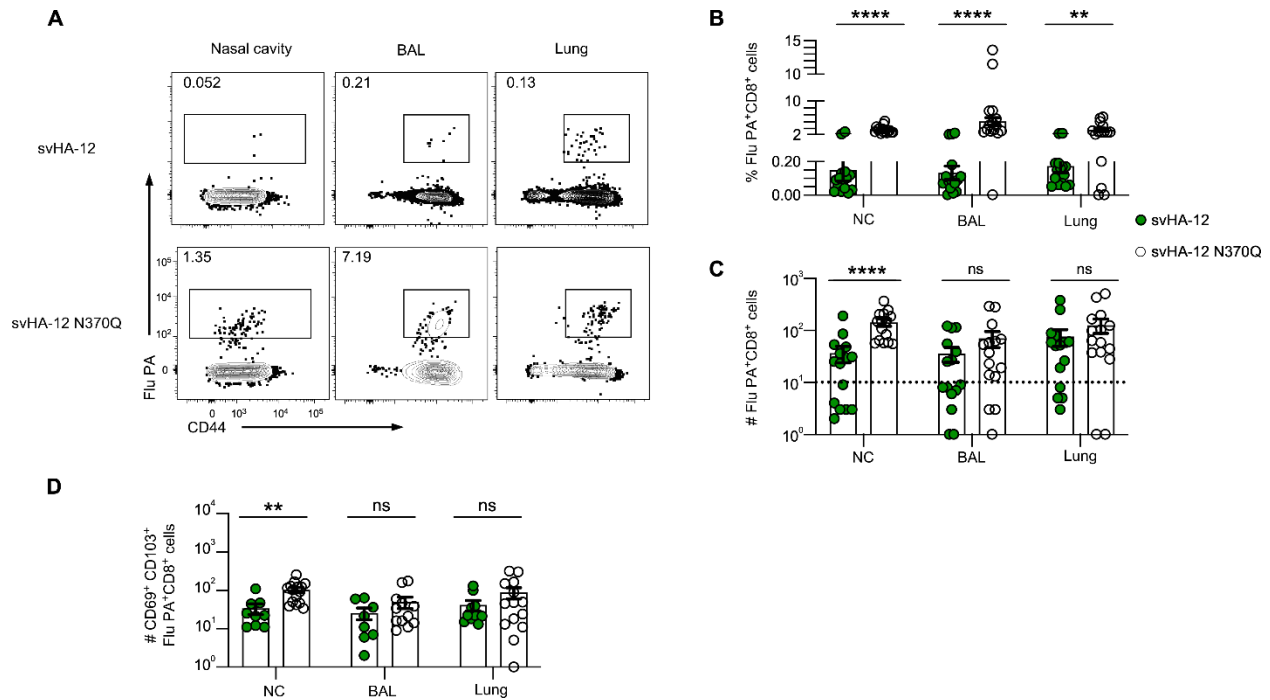
Supplemental Figure 2. Drifted LAIV immunization of PR8-immune mice fails to generate *de novo* CD8⁺ T_{RM}. (A) Example staining for SeV NP⁺ CD8⁺ T_{RM} in the lung and BAL of naïve or PR8-immune mice 30 d.p.i. with svHA-3. (B) Frequency of SeV NP⁺ CD8⁺ T_{RM} in the lung and BAL of naïve or PR8-immune mice 30 d.p.i. with svHA-3. (C-F) Same as A & B except with svHA-6 (C, D) and svHA-10 (E, F). Indicated significance is for comparison to naïve mice. For naïve animals, n = 5 mice from 2 experiments. For PR8-immune animals, n = 2 independent experiments with 4-6 mice per experiment.

Supplemental Figure 3



Supplemental Figure 3. Pre-existing CD8⁺ T_{RM} in other tissues undergo expansion upon LAIV immunization. Frequency and number of EdU⁺ FluNP₃₆₆⁺ CD8⁺ T cells and total frequency and number of FluNP₃₆₆⁺ CD8⁺ T cells in the BAL (A), nasal cavity (B), or spleen (C) of PR8-immune mice (i.p. injection) 30 d.p.i. with svHA-WT, svHA-6, or svHA-12. n = 2 independent experiments with 5 mice per experiment, per immunization strain.

Supplemental Figure 4



Supplemental Figure 4. Loss of the immunodominant Flu NP epitope improves boosting of pre-existing Flu PA-specific CD8⁺ T cells in the nasal cavity. Example staining (A), frequency (B) and number of total IV- (C) or CD69⁺ CD103⁺ IV- (D) Flu PA⁺ CD8⁺ T cells in the NC, BAL, and lung following immunization of PR8-immune mice with svHA-12 or svHA-12 N370Q LAIV. Data are from 30 d.p.i after LAIV immunization. Data represent 3 independent experiments with $n = 5$ mice per group, per experiment. LOD indicated with dotted line at 10^1 .