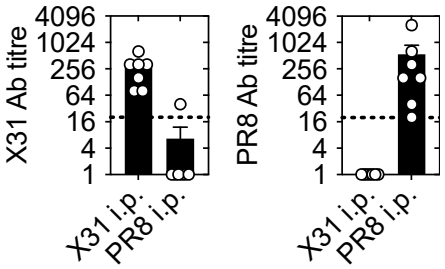
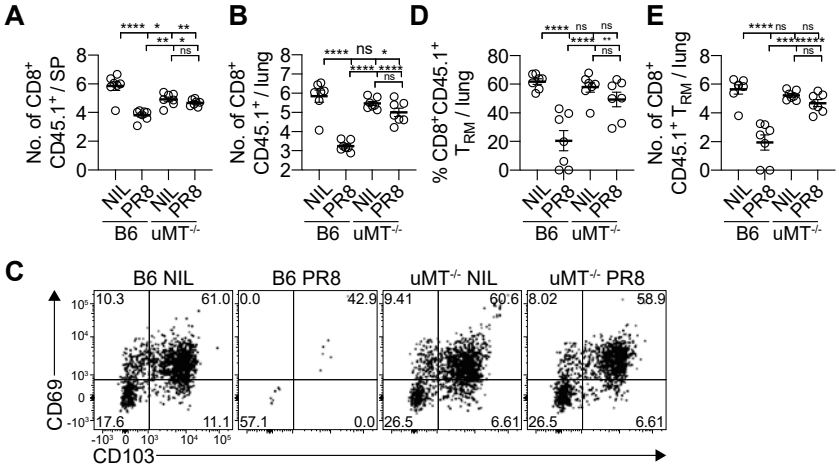
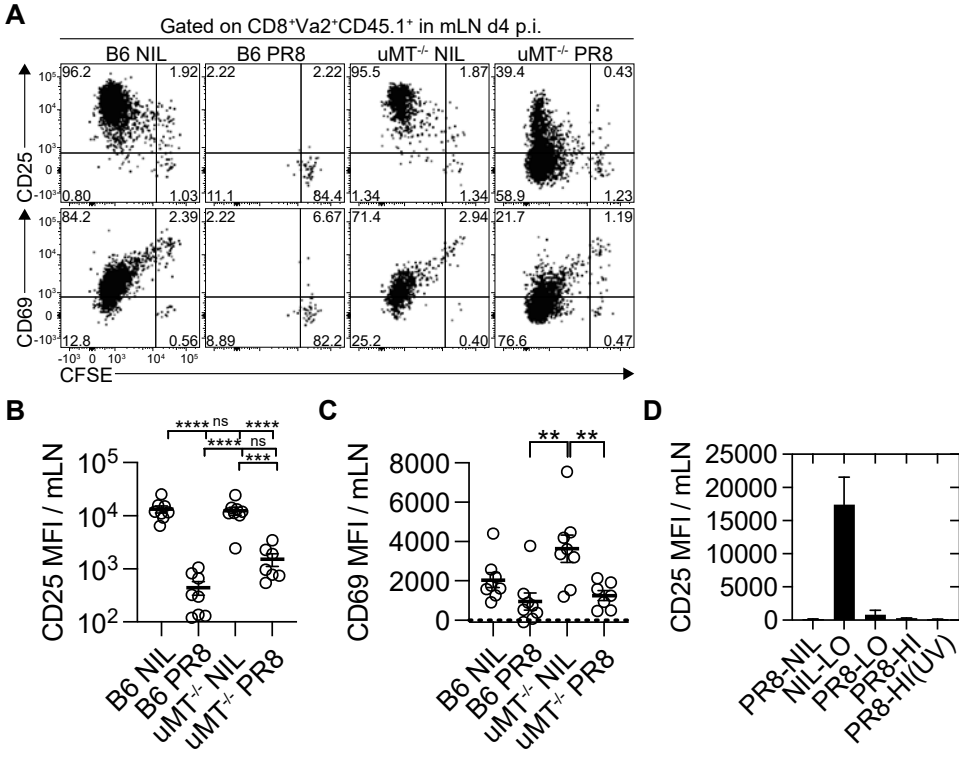


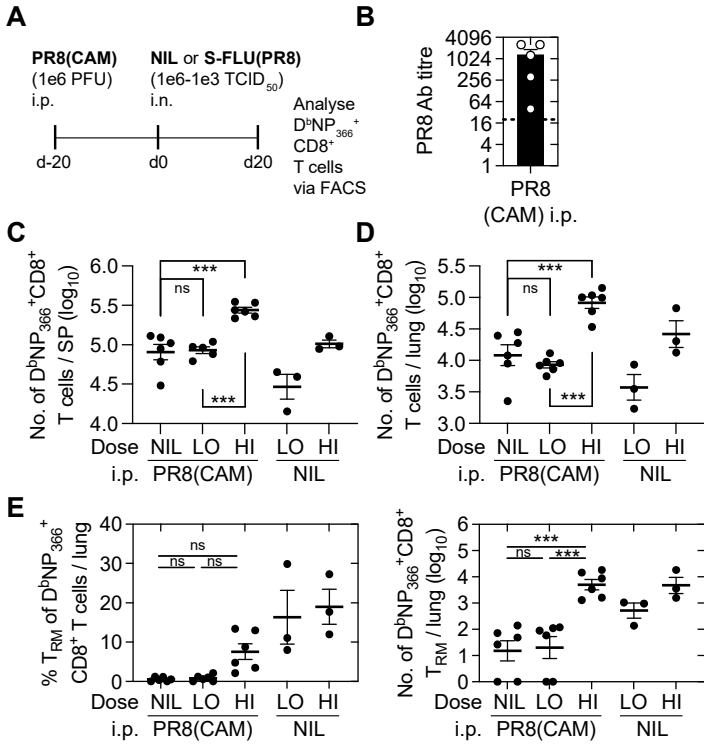
Supplementary Figure 1. Intraperitoneal influenza infection generates neutralising PR8- and X31-specific antibodies. B6 mice were infected with PR8 (1e6 PFU) or X31 (1e6 PFU) i.p., and then 20d later, the titre of X31- and PR8-specific neutralising antibodies in the serum was measured by the microneutralisation assay. Data from 2 independent experiments with n=7 mice per group. Symbols represent individual mice. Mean ± SEM.



Supplementary Figure 2. Pre-existing influenza antibody immunity impairs the development of lung CD8$^+$ Trm to a new T cell epitope. (A) CD45.2 B6 and uMT-/- mice were infected with PR8 (1e6 PFU) via the intraperitoneal route, and then twenty days later seeded with 1e6 CD45.1 CFSE-labelled OT-I CD8$^+$ T cells and subsequently intranasally infected with PR8-OVA (50 PFU). At d20 p.i., the development of CD8$^+$CD45.1$^+$ OT-Is were analysed by flow cytometry. **(A-B)** Absolute cell numbers of memory CD8$^+$CD45.1$^+$ OT-Is in the **(A)** spleen and **(B)** lung d20 p.i. **(C)** FACS profiles of lung CD69$^+$CD103$^+$ T_{RM} gated on divided CD8$^+$CD45.1$^+$ OT-I with the corresponding **(D)** frequencies and **(E)** numbers. Each symbol is a biological replicate. Mean ± SEM. Data pooled from 3 independent experiments with n=7-8 mice per group. One-way ANOVA with Tukey's multiple comparison test, comparing the mean of each cohort against every other cohort. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns non-significant.



Supplementary Figure 3. Reduced CD25 and CD69 expression on CD8$^+$ T cells in mice with prior exposure to influenza virus. (A) CD45.2 B6 and uMT$^{-/-}$ mice were infected with PR8 (1e6 PFU) via the intraperitoneal route, and then twenty days later seeded with 1e6 CD45.1 CFSE-labelled OT-I CD8$^+$ T cells and subsequently intranasally infected with PR8-OVA (50 PFU). **(A)** FACS profiles of CD25 and CD69 expression on CD8$^+$Va2$^+$CD45.1$^+$ in the mLN d4 p.i. with the corresponding mean fluorescence intensity values for **(B)** CD25 and **(C)** CD69. Data pooled from 3 independent experiments with n=7-8 mice per group. **(D)** CD45.2 B6 mice were infected with PR8 (1e6 PFU) via the intraperitoneal route, and then twenty days later seeded with 1e6 CD45.1 CFSE-labelled OT-I CD8$^+$ T cells and subsequently intranasally infected with PR8-OVA (50-1e4 PFU) or UV-inactivated PR8-OVA (1e4 PFU). At d4 p.i., the mean fluorescence intensity of CD25 for CD8$^+$CD45.1$^+$ OT-Is was analysed by flow cytometry. Mean ± SEM. Data pooled from 2 independent experiments with n=2-6 mice per group. One-way ANOVA with Tukey's multiple comparison test, comparing the mean of each cohort against every other cohort. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns non-significant.



Supplementary Figure 4. The impact of pre-existing influenza-specific antibody immunity on S-FLU vaccination. (A) B6 mice were infected with PR8(CAM) (1e6 PFU) via the intraperitoneal route, and then twenty days later re-infected with an intranasal dose of S-FLU(PR8) (1e6-1e3 TCID50). Twenty days later, the quantity of NP_{366}^{+}tetramer^{+} $CD8^{+}$ T cells was analysed by flow cytometry. **(B)** Neutralising antibody titres against PR8(CAM) in the serum of PR8(CAM) i.p. mice as measured by microneutralisation assay. Data from 2 independent experiments with n=5 mice. **(C-D)** Absolute cell numbers of NP-specific $CD8^{+}$ T cells in the **(C)** spleen, and **(D)** lung. **(E)** The frequency (top) and numbers (bottom) of NP-specific $CD69^{+}CD103^{+}$ $CD8^{+}$ T_{RM} in the lung. Each symbol is a biological replicate. Mean ± SEM. Data pooled from 2 independent experiments with n=3-6 mice per cohort. One-way ANOVA with Tukey's multiple comparison test, comparing the mean of each cohort against every other cohort. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns non-significant.