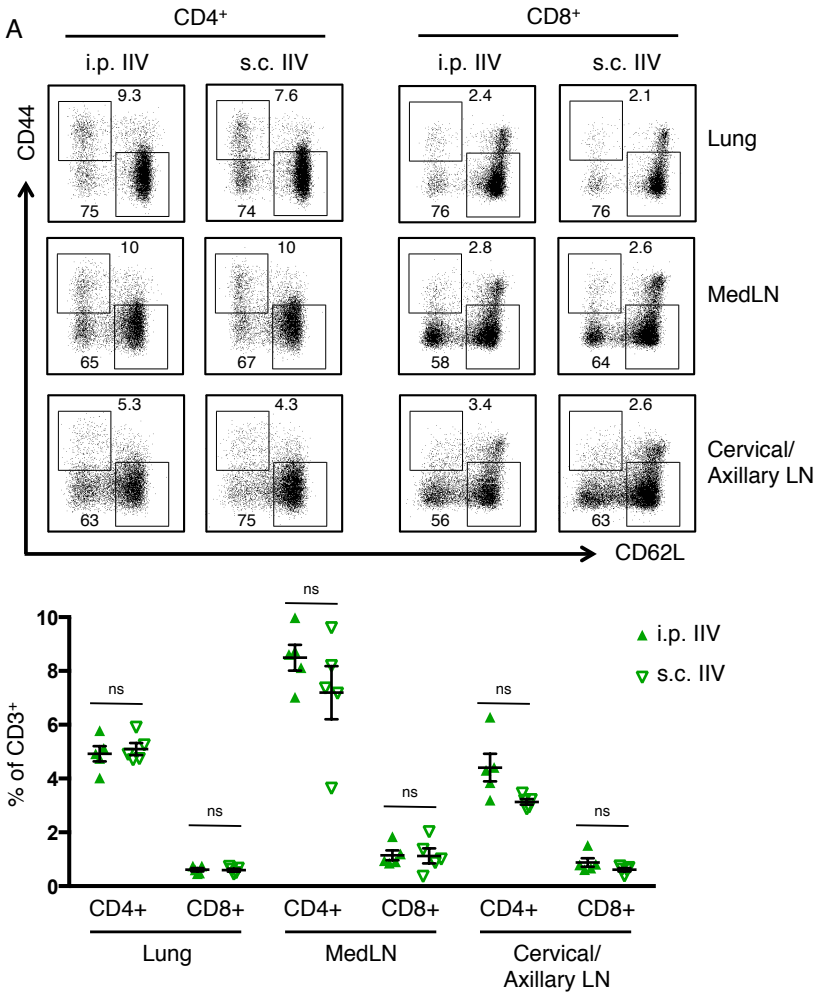
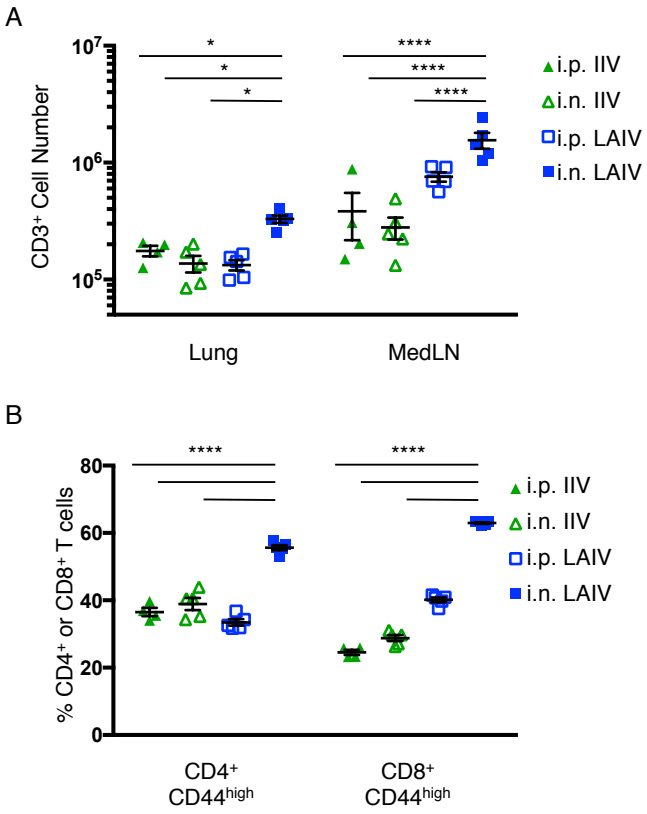
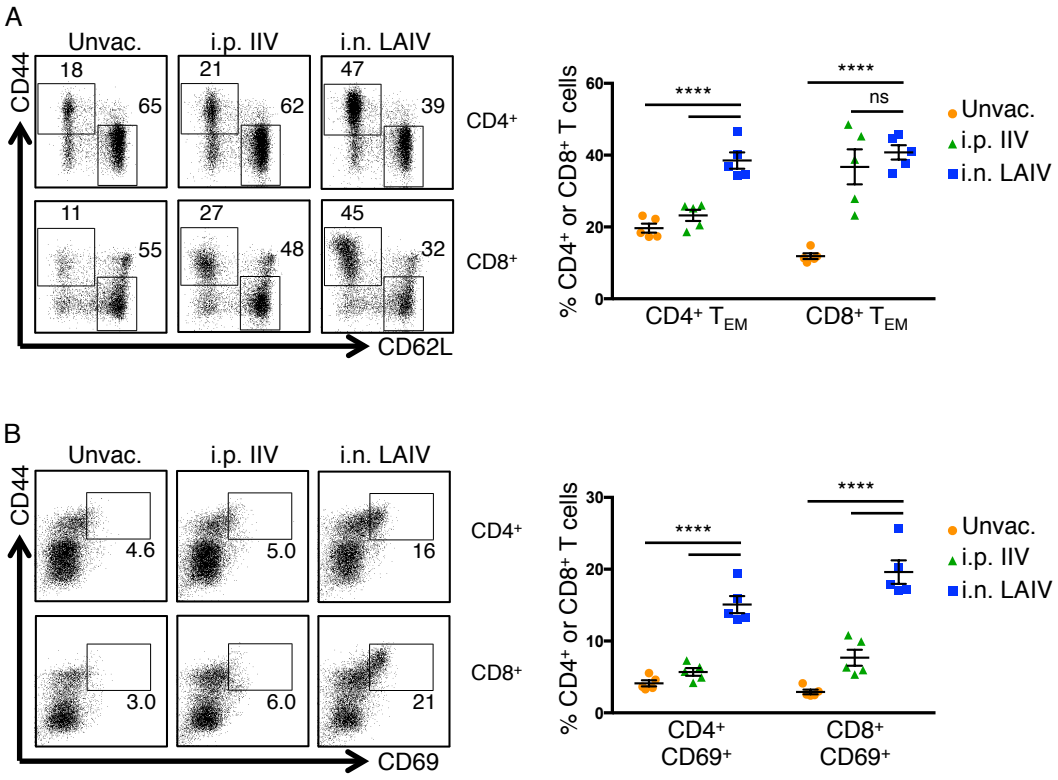
SUPPLEMENTARY FIGURES



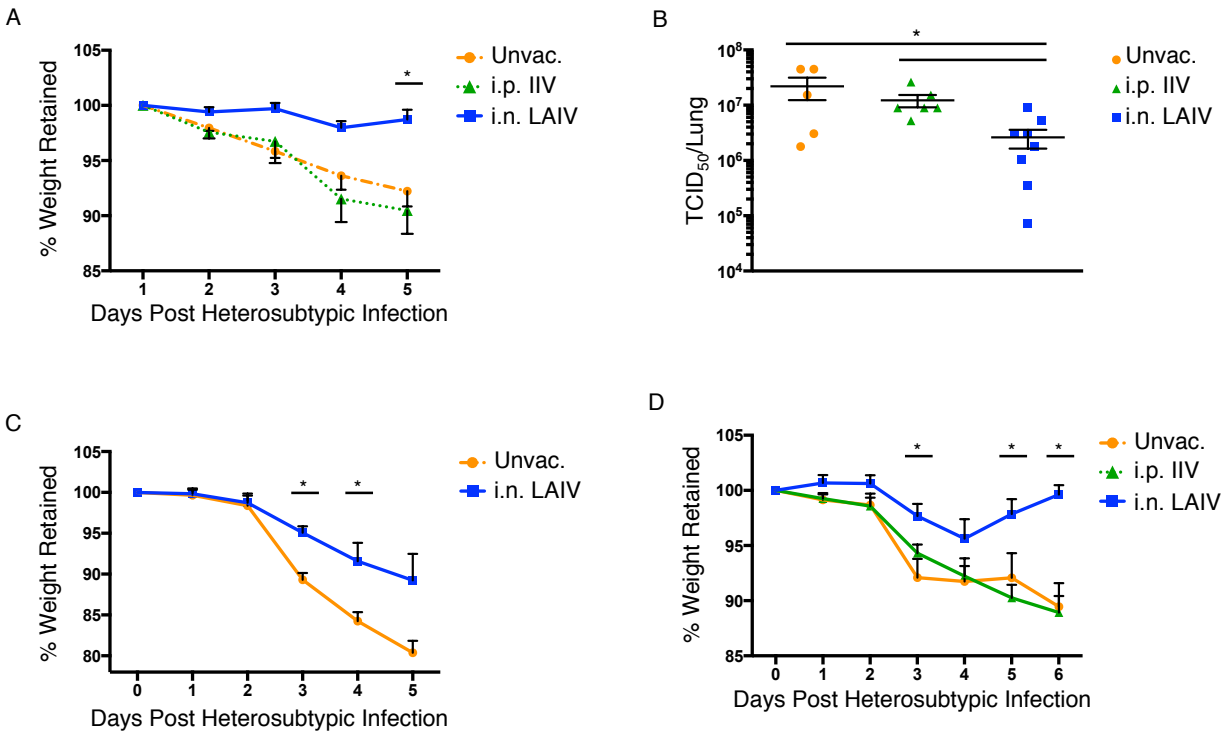
Supplementary Figure 1. Similar primary T cell responses following vaccination with intraperitoneal (i.p.) or subcutaneous (s.c.) IIV. (a) Flow cytometric analysis of $CD4^+$ and $CD8^+$ T cells 10 days post-vaccination in the lung, medLN and pooled cervical/axillary lymph nodes of mice vaccinated intraperitoneally (i.p.) or subcutaneously (s.c.) at the base of the neck with 2014-2015 IIV. Top: Representative percentages of cells with naïve ($CD44^{lo}CD62L^{hi}$) and effector/memory ($CD44^{hi}CD62L^{lo}$) phenotypes. Bottom: Individual percentages of effector/memory phenotype in each tissue ± SEM. (n=5 mice per group; significance determined by two-way ANOVA with Holm-Sidak's multiple comparisons test, ns $P>0.05$).



Supplementary Figure 2. Effect of vaccine formulation and administration route on lung-localized T cell responses to vaccination. (a) Absolute numbers of $CD3^+$ T cells in the lung and medLN 10 days post-vaccination in i.n. or i.p. 2014-2015 IIV-vaccinated and i.n. or i.p. 2014-2015 LAIV-vaccinated animals. Graph displays mean absolute cell numbers ± SEM. (n=4-5 mice per group; significance determined by two-way ANOVA with Holm-Sidak's multiple comparisons test, **** $P<0.0001$, * $P<0.05$). **(b)** Percentage of $CD44^+$ $CD4^+$ and $CD8^+$ T cells in the lung 10 days post-vaccination with 2014-2015 IIV given i.n. or i.p or 2014-2015 LAIV given i.n. or i.p. Graph displays individual percentages of $CD44^{hi}$ $CD4^+$ and $CD8^+$ T cells ± SEM. (n=4-5 mice per group; significance determined by two-way ANOVA with Holm-Sidak's multiple comparisons test, **** $P<0.0001$).



Supplementary Figure 3. Primary lung T cell responses following vaccination with 2015-2016 IIV or LAIV. (a) Flow cytometric analysis of lung $CD4^+$ and $CD8^+$ T cells 10 days post-vaccination with 2015-2016 IIV or 2015-2016 LAIV compared to unvaccinated mice. Left: Representative flow plots with percentages of cells with naïve ($CD44^{lo}CD62L^{hi}$) and effector/memory ($CD44^{hi}CD62L^{lo}$) phenotypes. Right: Individual percentages of lung $CD4^+$ and $CD8^+$ effector/memory phenotype T cells ± SEM. (n=5 mice per group, representative of 3 experiments; significance determined by two-way ANOVA with Holm-Sidak's multiple comparisons test, **** P<0.0001, ns P>0.05). **(b)** Frequencies of lung $CD4^+$ and $CD8^+$ T cells expressing CD69 10 days post-vaccination with 2015-2016 IIV or 2015-2016 LAIV compared to unvaccinated mice. Left: Representative flow plots with percentages of $CD44^{hi}CD69^{hi}$ cells. Right: Individual percentages of lung $CD4^+$ and $CD8^+$ $CD44^{hi}CD69^{hi}$ cells T cells ± SEM. (n=5 mice per group, representative of 3 experiments; significance determined by two-way ANOVA with Holm-Sidak's multiple comparisons test, **** P<0.0001, * P<0.05, ns P>0.05).



Supplementary Figure 4. LAIV-formulations establish long-term TRM-mediated protection against multiple viral strains. (a) Percentage weight retention following heterosubtypic PR8 (H1N1) influenza infection 12 weeks post-vaccination with 2014-2015 IIV or 2014-2015 LAIV or post-infection with X31 (H3N2) influenza, compared to unvaccinated, in animals receiving daily FTY720 treatment. Mean percentage weight retained shown ± SEM. (n=4-5 mice per group; significance determined by multiple Student's t tests comparing i.n. LAIV-vaccinated to i.p. IIV-vaccinated groups, * $P<0.05$). **(b)** Viral titers 5 days post-PR8 infection in unvaccinated animals or animals vaccinated with 2014-2015 IIV or 2014-2015 LAIV or infected with X31 influenza 12 weeks prior. Animals received daily FTY720 treatment throughout infection. Graph show individual titers ± SEM. (n=4-6 mice per group; significance determined by one-way ANOVA with Holm-Sidak's multiple comparisons test, * $P<0.05$, ns $P>0.05$). **(c)** Percentage weight retention following PR8 infection in unvaccinated animals or animals receiving 2014-2015 LAIV 45 weeks prior. Animals received daily FTY720 treatment throughout infection. Mean percentage weight retained shown ± SEM. (n=4-5 mice per group, significance determined by multiple Student's t tests comparing i.n. LAIV-vaccinated and unvaccinated groups, * $P<0.05$). **(d)** Percentage weight retention following X31 infection 6 weeks post-vaccination with 2015-2016 IIV or 2015-2016 LAIV, compared to unvaccinated, in animals receiving daily FTY720 treatment. Mean percentage weight retained shown ± SEM. (n=5-12 mice per group; significance determined by multiple Student's t tests comparing i.n. LAIV-vaccinated to i.p. IIV-vaccinated groups, * $P<0.05$).