

Supp. Table 1:

a

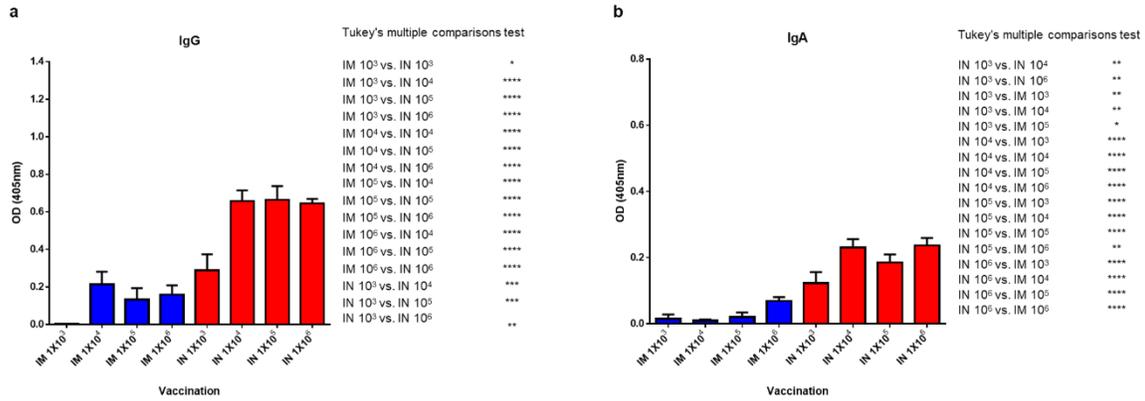
IgG - Tukey's multiple comparisons test	
10³ IN+IN vs. 10³ IM+IM	***
10 ³ IN+IN vs. 10 ⁴ IM+IM	**
10³ IN+IN vs. 10³ IM+IN	**
10 ³ IN+IN vs. 10 ⁴ IM+IN	*
10 ⁴ IN+IN vs. 10 ³ IM+IM	****
10⁴ IN+IN vs. 10⁴ IM+IM	****
10 ⁴ IN+IN vs. 10 ⁶ IM+IM	**
10 ⁴ IN+IN vs. 10 ³ IM+IN	****
10⁴ IN+IN vs. 10⁴ IM+IN	***
10 ⁴ IN+IN vs. 10 ⁵ IM+IN	*
10 ⁴ IN+IN vs. 10 ⁶ IM+IN	*
10 ⁵ IN+IN vs. 10 ³ IM+IM	****
10 ⁵ IN+IN vs. 10 ⁴ IM+IM	***
10 ⁵ IN+IN vs. 10 ³ IM+IN	***
10 ⁵ IN+IN vs. 10 ⁴ IM+IN	**

b

IgA - Tukey's multiple comparisons test	
10 ³ IN+IN vs. 10 ⁵ IN+IN	**
10 ⁴ IN+IN vs. 10 ³ IM+IM	**
10⁴ IN+IN vs. 10⁴ IM+IM	*
10 ⁵ IN+IN vs. 10 ³ IM+IM	****
10 ⁵ IN+IN vs. 10 ⁴ IM+IM	**
10⁵ IN+IN vs. 10⁵ IM+IM	*
10 ⁵ IN+IN vs. 10 ³ IM+IN	**
10 ⁵ IN+IN vs. 10 ⁴ IM+IN	**
10⁵ IN+IN vs. 10⁵ IM+IN	**

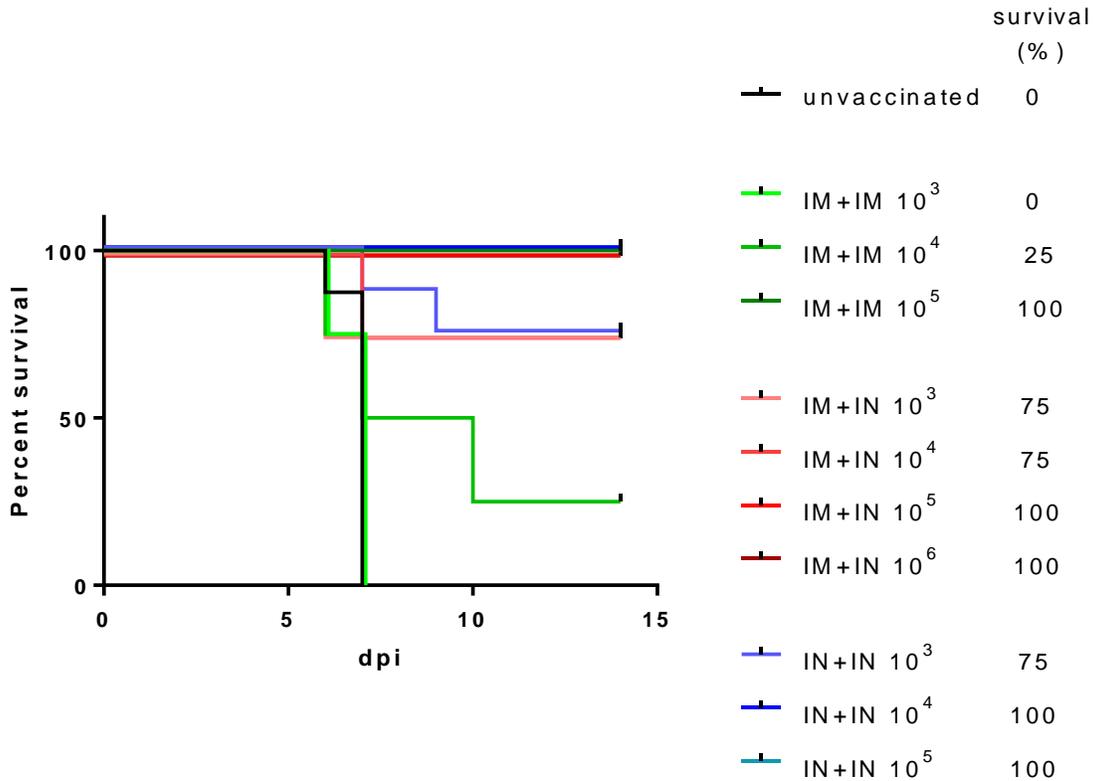
Supplementary table S1: Statistical significance of humoral immunity following mucosal or muscular vaccination: (a) Statistical analysis of induction of IgG by IM+IM vaccination, IM+IN vaccination, or IN+IN vaccination, at various doses. (b) Statistical analysis of IgA by IM+IM vaccination, IM+IN vaccination, or IN+IN vaccination, at various doses. Analysis was performed using One-way ANOVA, with Tukey's Post Hoc test, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Matched doses are in Bold.

Supp. Fig. S1:



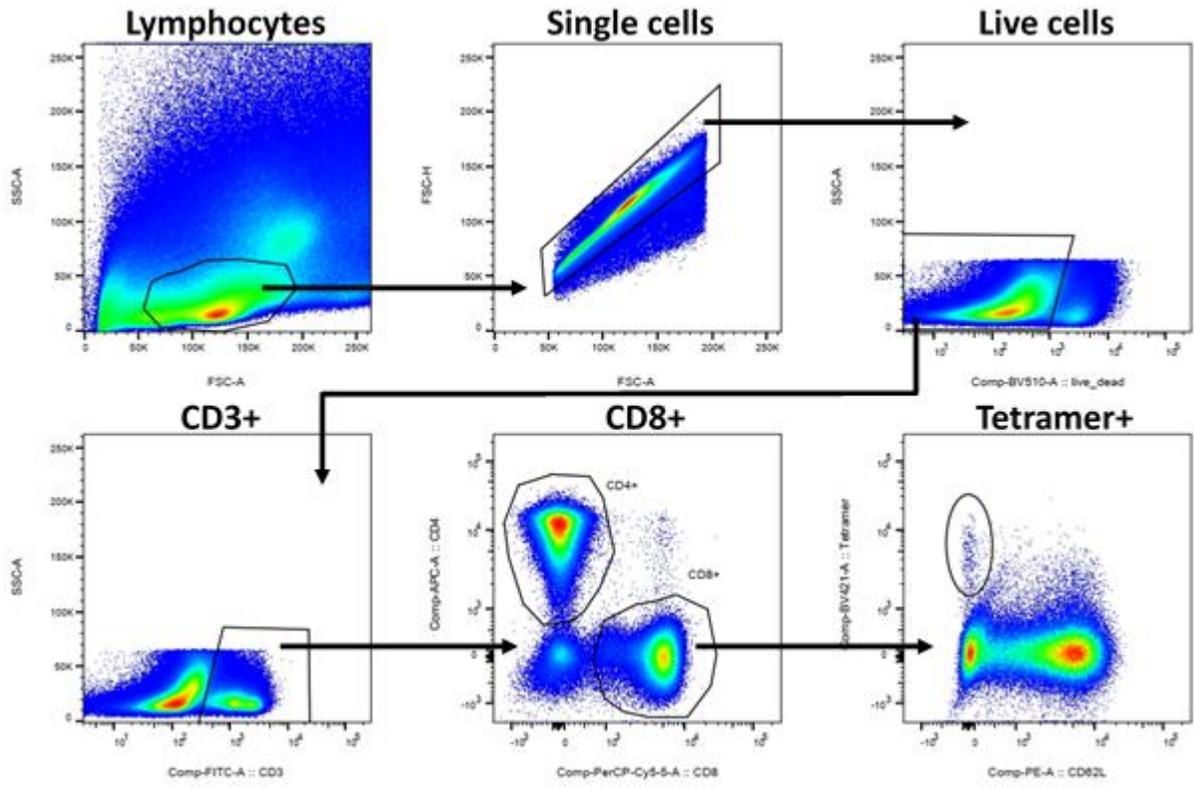
Supplementary Figure S1: Induction of humoral immunity following a prime only mucosal or muscular vaccination: (a) Induction of IgG by IM or IN vaccination at various doses. Each color represents route of administration. (b) Induction of IgA by IM or IN vaccination at various doses. Each color represents route of administration. Data is presented as mean \pm SEM. n=8 for each group. Statistical analysis for each panel is presented. Analysis was performed using One-way ANOVA, with Tukey's Post Hoc test, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Supp. Fig. S2:



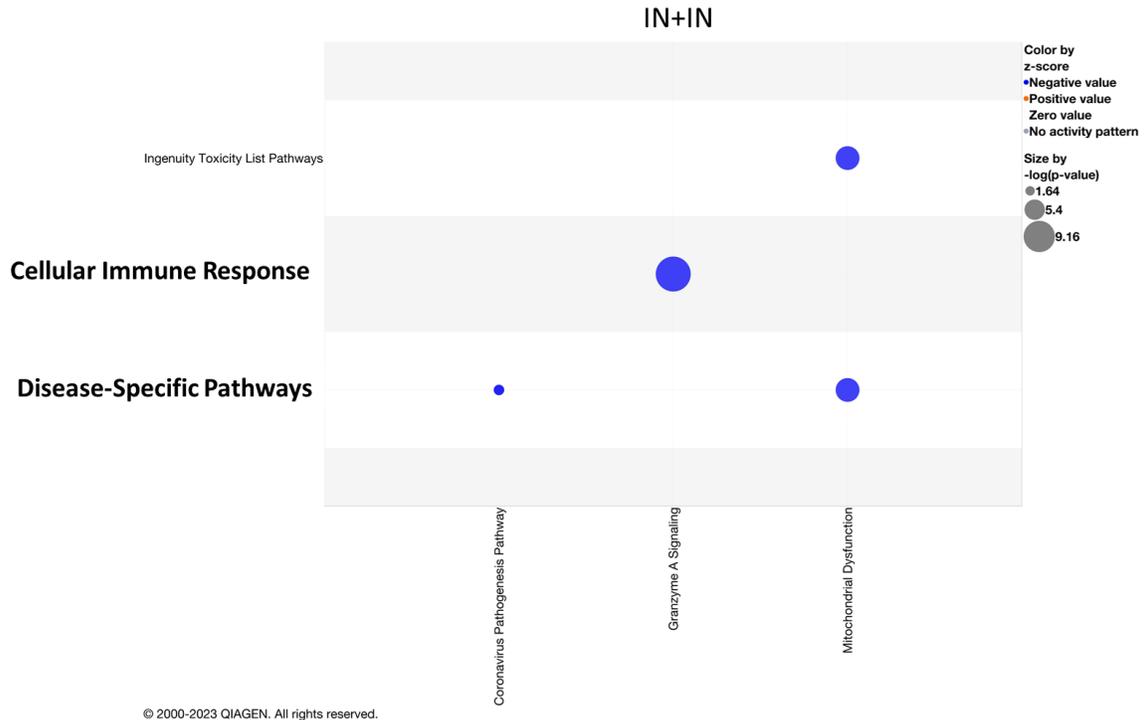
Supplementary Figure S2: Protection of mice from lethal SARS-CoV-2 challenge by mucosal or muscular vaccination: Survival curve of K18-hACE2 mice vaccinated by IM+IM, IM+IN, or IN+IN regimens at doses ranging between 10³-10⁶ pfu, or unvaccinated, challenged by IN instillation with Delta variant of SARS-CoV-2. Statistical analysis was performed using One-way ANOVA, with Tukey's Post Hoc test. Significance is indicated next to figure legend. n=8 for unvaccinated and IN+IN vaccinated groups, n=4 for IM+IM and IM+IN groups.

Supp. Fig. S3:



Supplementary Figure S3: Gating strategy for evaluation of effector, spike specific CD8+ T-cells in the Lung.

Supp. Fig. S5:



Supplementary Figure S5: Reduced enrichment of cellular and humoral immune responses, cytokine signaling and disease and pathogen influenced signaling by homologous IN vaccination: Ingenuity pathway analysis bubble plot for IN+IN vaccinated K18-hACE2 mice brains at 4 days post delta variant SARS-CoV-2 infection. Enriched pathways arranged by categories of interest: cellular immune response, cytokine signaling, disease-specific pathways, humoral immune response and pathogen-influenced signaling (y-axis), and the enriched canonical pathways that map to each category (x-axis), arranged in an alphabetical order. Blue predicts decreased activation, bubble size represents $-\log(p\text{-value})$. IPA was set to a cutoff of $-\log(p\text{-value})$ of 1.3 (threshold), and adjusted p-value <0.05 . $n=3$. The significance of the association between the data set and specific canonical pathways was accessed using a right-tailed Fisher's Exact test. A Benjamini-Hochberg multiple comparisons correction test was applied.