

Supplemental Figure 1. Screening of Ad4 seropositive individuals.

Prevalence of Ad4 seropositive participants (n=198). ID₅₀ (Green) and ID₈₀ (Purple) are shown. Horizontal dotted line indicates cut off of 100 ID₈₀.

Demographics	Oral	Tonsillar*	Intranasal*	Comparisons (P values) [†]		
	(N=10)	(N=25)	(N=28)	Oral v. Tons.	Oral v. Intran.	Tons. v. Intran.
Age (Median Yrs, range)	30.5 (23, 44)	31 (19, 49)	28 (20, 49)	-	-	-
Female/Male, #	5/5	9/16	10/18	-	-	-
Ethnicity/Race, # (% total N)						
Non-Hispanic Black	2 (20%)	16 (64%)	9 (32%)	0.03	-	0.03
Non-Hispanic White	8 (80%)	8 (32%)	9 (32%)	0.02	0.02	-
Non-Hispanic Asian	0	1 (4%)	7 (25%)	-	-	0.05
Hispanic Black	0	0	1 (4%)	-	-	-
Hispanic Mixed	0	0	2 (7%)	-	-	-

Supplemental Table 1. Participant demographic.

*One Tonsillar vaccine recipient and two Intranasal vaccine recipients received both lower (10^3 or 10^4) and higher doses (10^7 or 10^8) of their respective vaccines but were counted only once in each sub-group.

N=total number of Ad- and Ad4+ study subjects who received the study agent.

[†]Only p values ≤ 0.1 are shown.

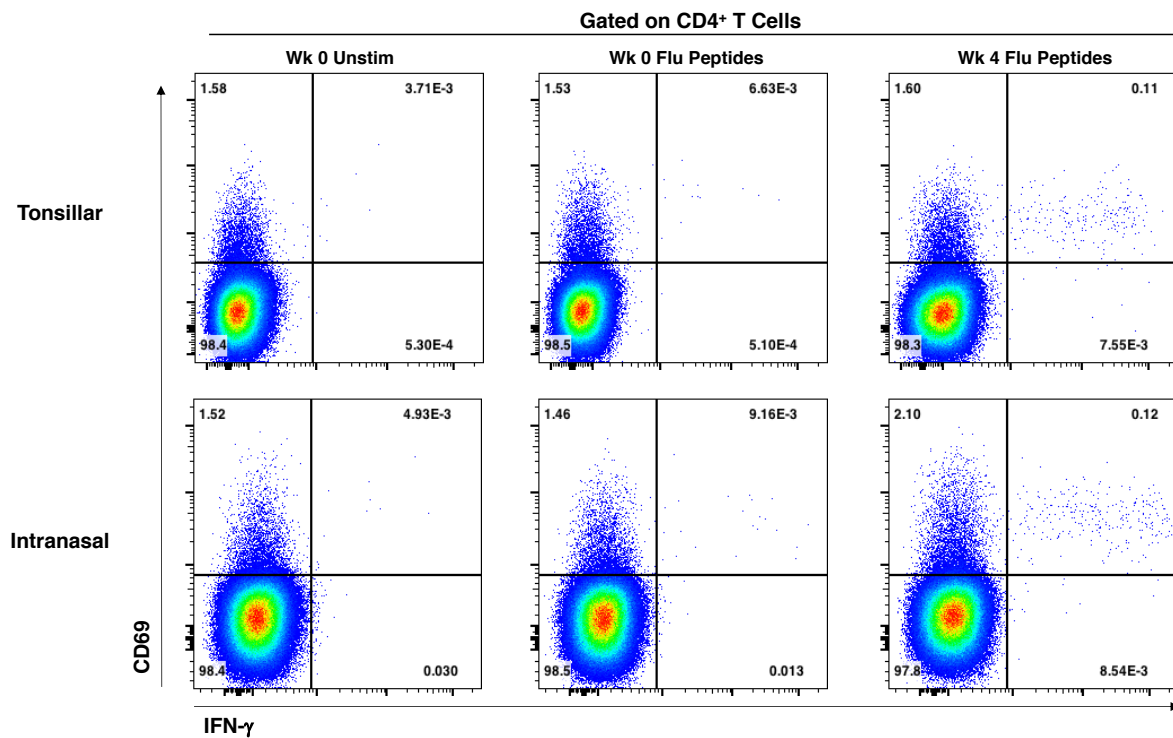
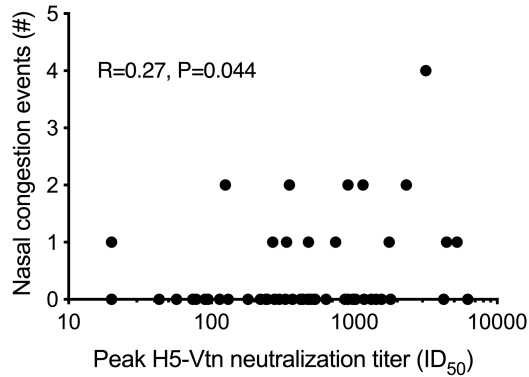
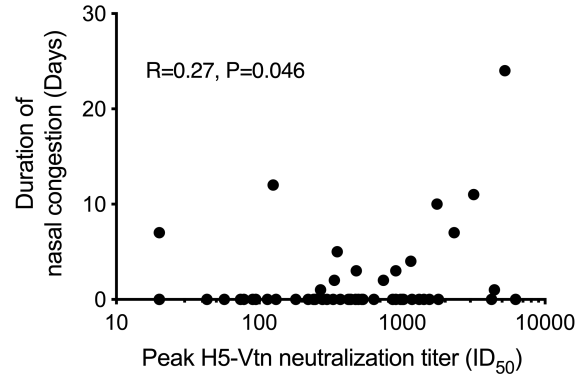
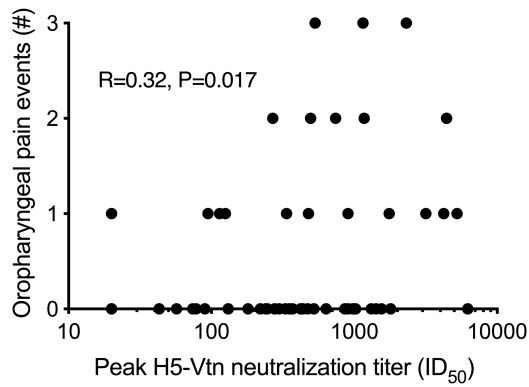
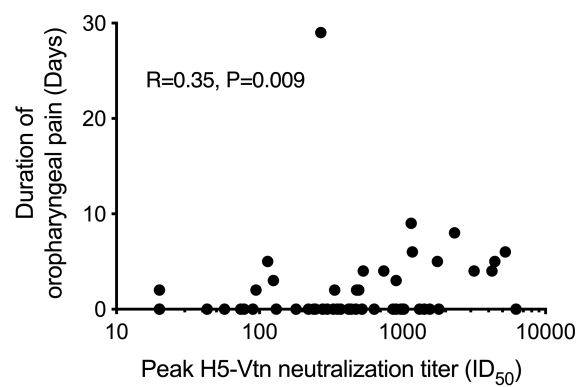


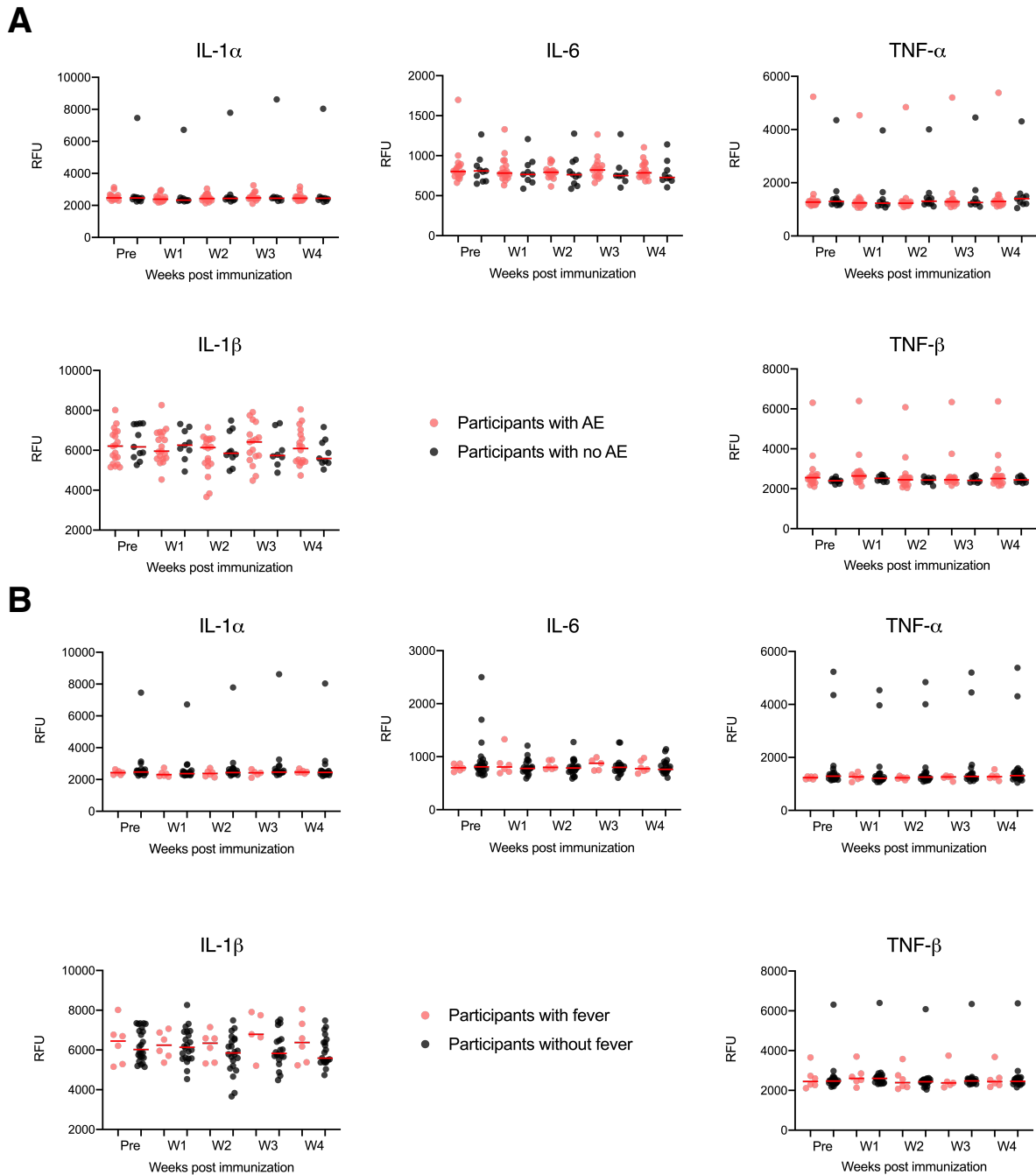
Figure S2. Representative Flow Cytometry Plots

Plots depict frequencies of CD3⁺CD4⁺ lymphocytes in PBMCs from Week 0 (left and center columns) and Week 4 post-vaccination (right column) that were CD69⁺ and IFN- γ ⁺ after 6-hour stimulation with medium alone (left column) or a 93-peptide array of mostly 17-mers spanning the hemagglutinin protein H5 (center and right columns) in a representative subject from the tonsillar (top row) or intranasal (bottom row) arms. Quadrant numbers in each plot represent percentages of gated CD4⁺ T cells.

A**B****C****D**

Supplemental figure 3. Clinical correlates of anti-H5 neutralizing antibody titers.

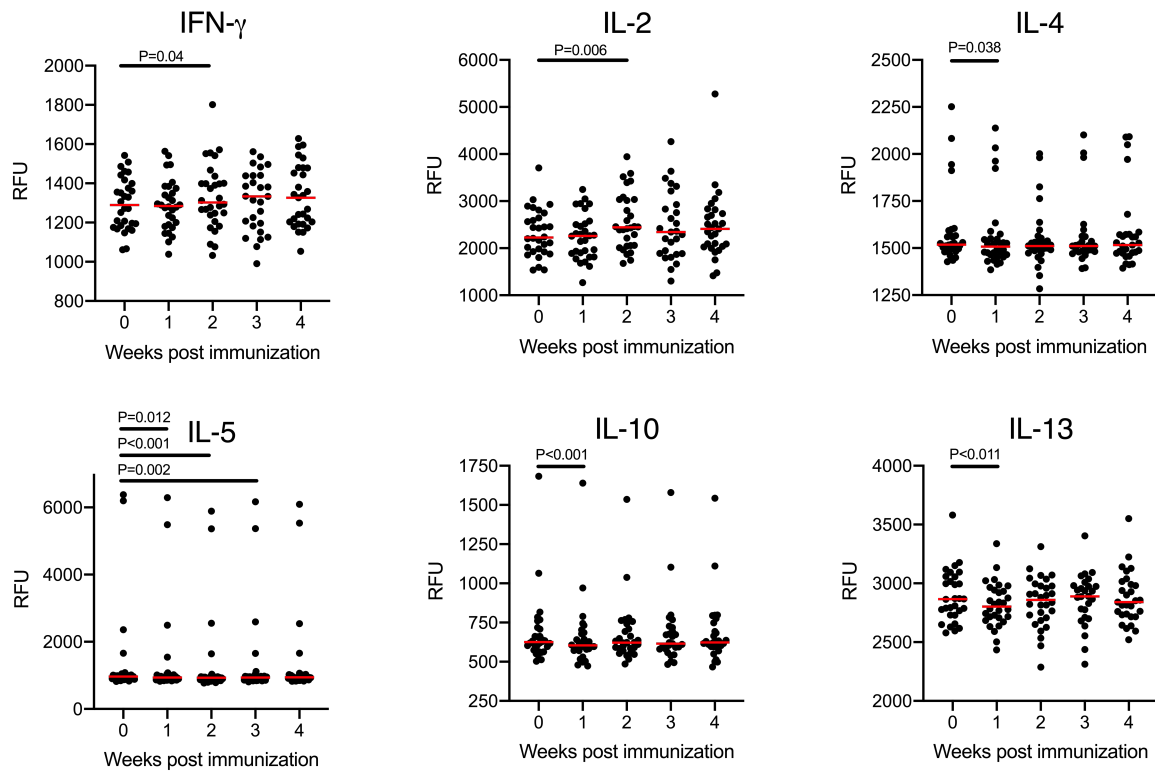
Correlations between peak H5 neutralizing antibody titer post vaccination and (A) number or (B) duration of related nasal congestion events and (C) number or (D) duration of related oropharyngeal pain events were assessed among vaccine recipients in the oral, tonsillar and intranasal arms (n=56) by the Spearman rank method.



Supplemental Figure 4. Levels of inflammatory cytokines in the serum of individuals with adverse events.

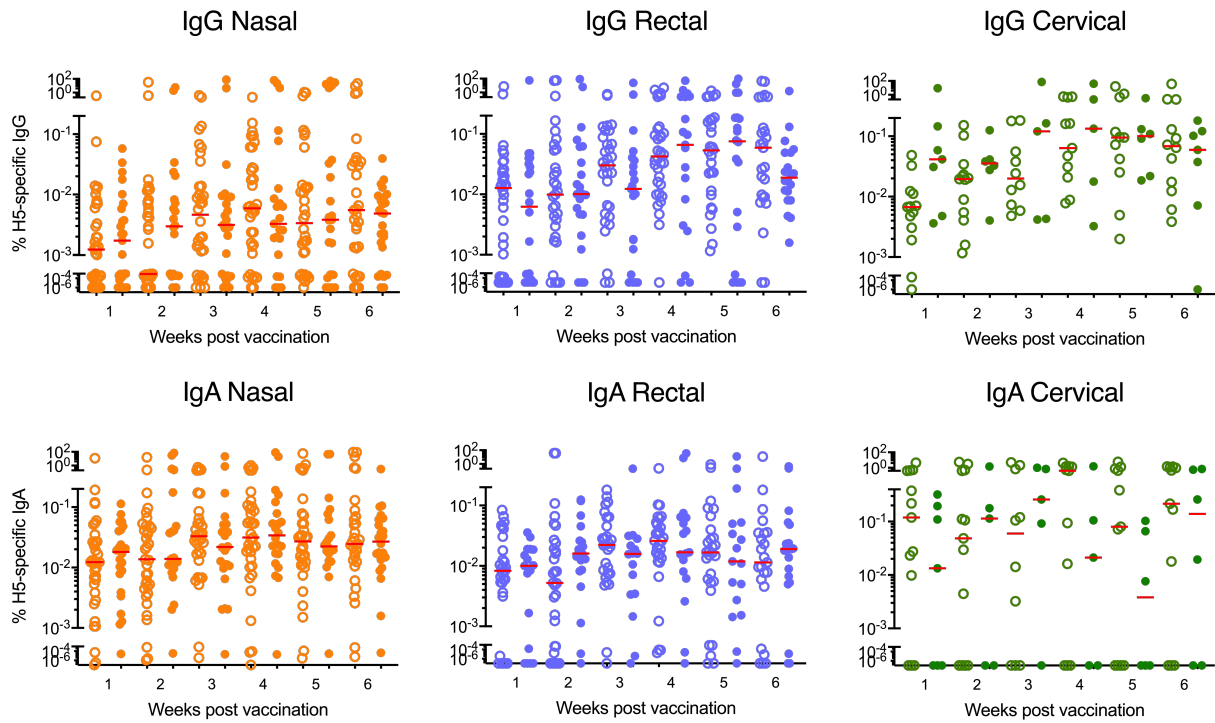
(A) IL-1 α , IL-1 β , IL-6, TNF- α and TNF- β levels expressed as relative fluorescent units (RFU) in a SOMAscan serum proteomics assay in participants with (pink; n=17-19) and without (black; n=8-11) any adverse events. Two pre-immunization timepoints are averaged for baseline timepoint. Participants with less than 4-fold increase in Ad4 neutralization titer post-immunization are excluded. Horizontal lines represent median values. Wilcoxon-Mann-Whitney (signed-rank) tests were used to calculate P values. Only significant P values are shown.

(B) IL-1 α , IL-1 β , IL-6, TNF- α and TNF- β levels in participants with (pink; n=4-6) and without (black; n=20-24) fever. Two pre-immunization timepoints are combined for baseline timepoint. The same exclusion criteria are applied as in (A). Horizontal lines represent median values. Wilcoxon-Mann-Whitney (signed-rank) tests were used to calculate P values. Only significant P values are shown.



Supplemental Figure 5. Levels of Th1/Th2 cytokines in sera post-vaccination.

IFN- γ , IL-2, IL-4, IL-5, IL-10, and IL13 levels expressed as RFU were measured by SOMAscan serum proteomics assay (n=27-30). Two pre-immunization timepoints are averaged for baseline timepoint. Participants with less than 4-fold increase in Ad4 neutralization titer post-immunization are excluded. Horizontal lines represent median values. Wilcoxon-Mann-Withney (signed-rank) tests were used to calculate P values. Only significant P values are shown.



Supplemental Figure 6. Impact of baseline Ad4 seropositivity on induction H5 specific antibodies at mucosal site.

The proportions of H5 HA specific IgG and IgA in nasal (orange), rectal (blue) and cervical (green) secretions were calculated against total IgG and IgA. Red horizontal bars indicate median values. Participants with (closed circle; nasal n=24-25, rectal n=17-22, cervical n=7) and without (open circle; nasal n=36-37, rectal n=32-36, cervical n=13) baseline Ad4 neutralizing activity are indicated side by side. Red horizontal bars indicate median values. Two-sample t-test was used to calculate the P values. Only significant P values are indicated.