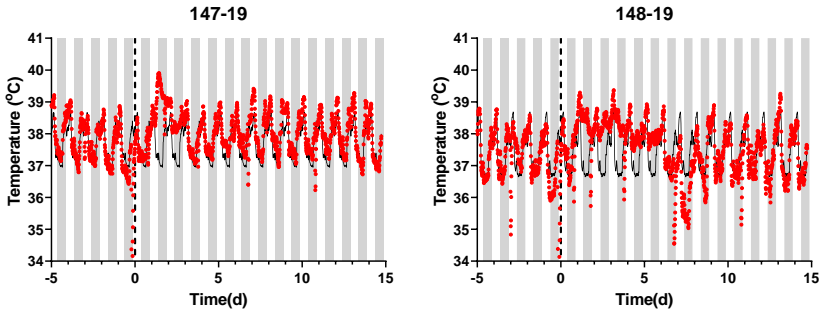


## **Supplemental information**

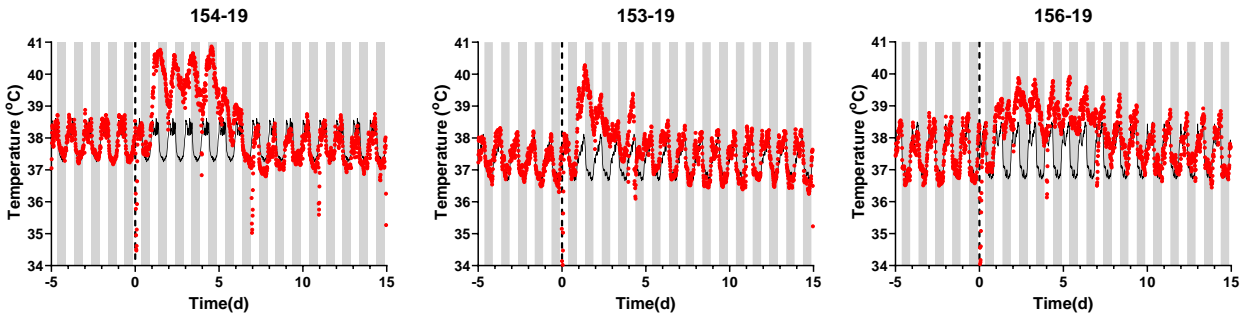
### **Refined semi-lethal aerosol H5N1 influenza model in cynomolgus macaques for evaluation of medical countermeasures**

**Masaru Kanekiyo, Rebecca A. Gillespie, Morgan Midgett, Katherine J. O'Malley, Connor Williams, Syed M. Moin, Megan Wallace, Luke Treaster, Kristine Cooper, Hubza Syeda, Gwenddolen Kettenburg, Hasala Rannulu, Tabitha Schmer, Lucia Ortiz, Priscila Da Silva Castanha, Jacqueline Corry, Mengying Xia, Emily Olsen, Daniel Perez, Gabin Yun, Barney S. Graham, Simon M. Barratt-Boyes, and Douglas S. Reed**

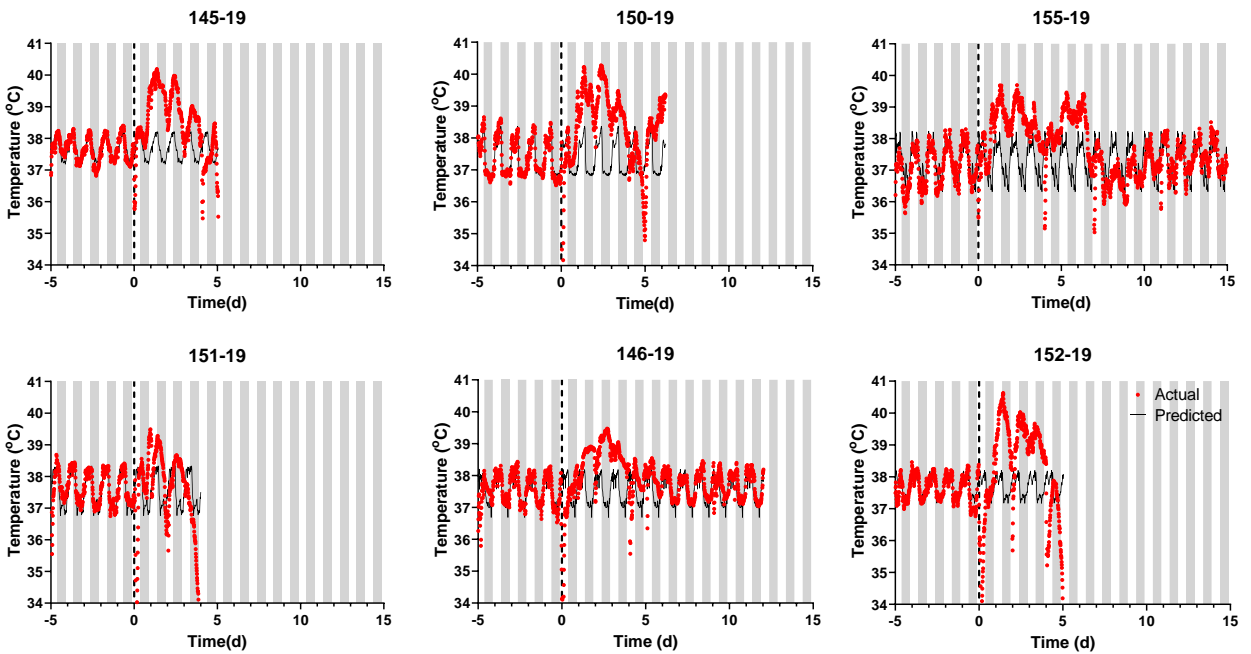
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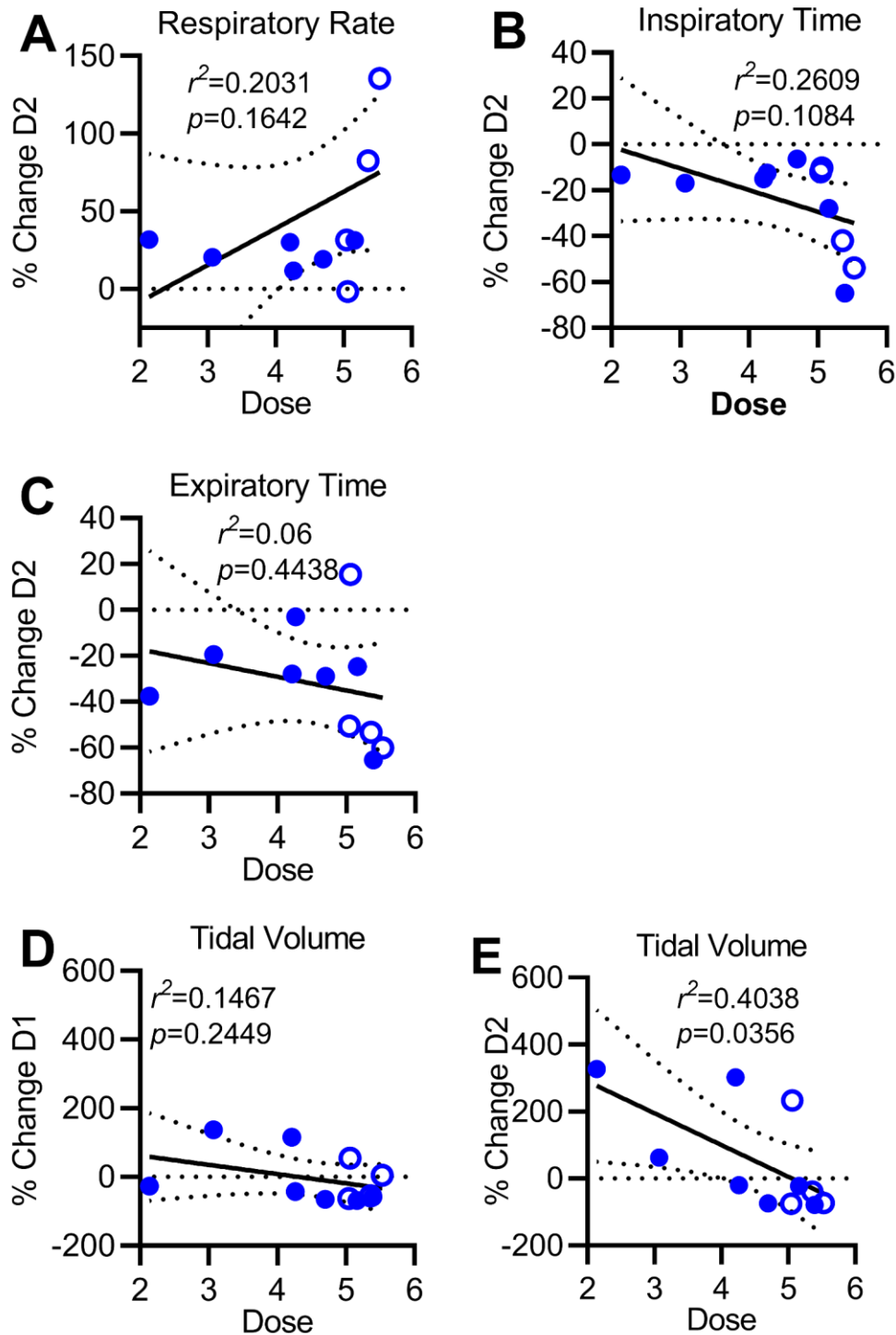
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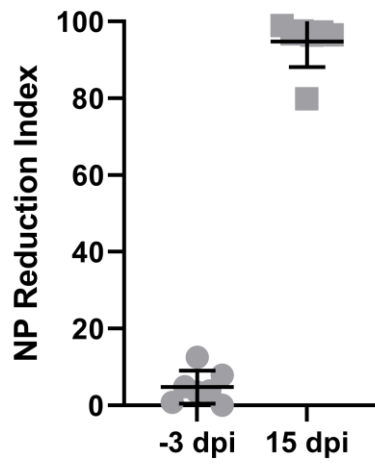
5-5.5 log<sub>10</sub>



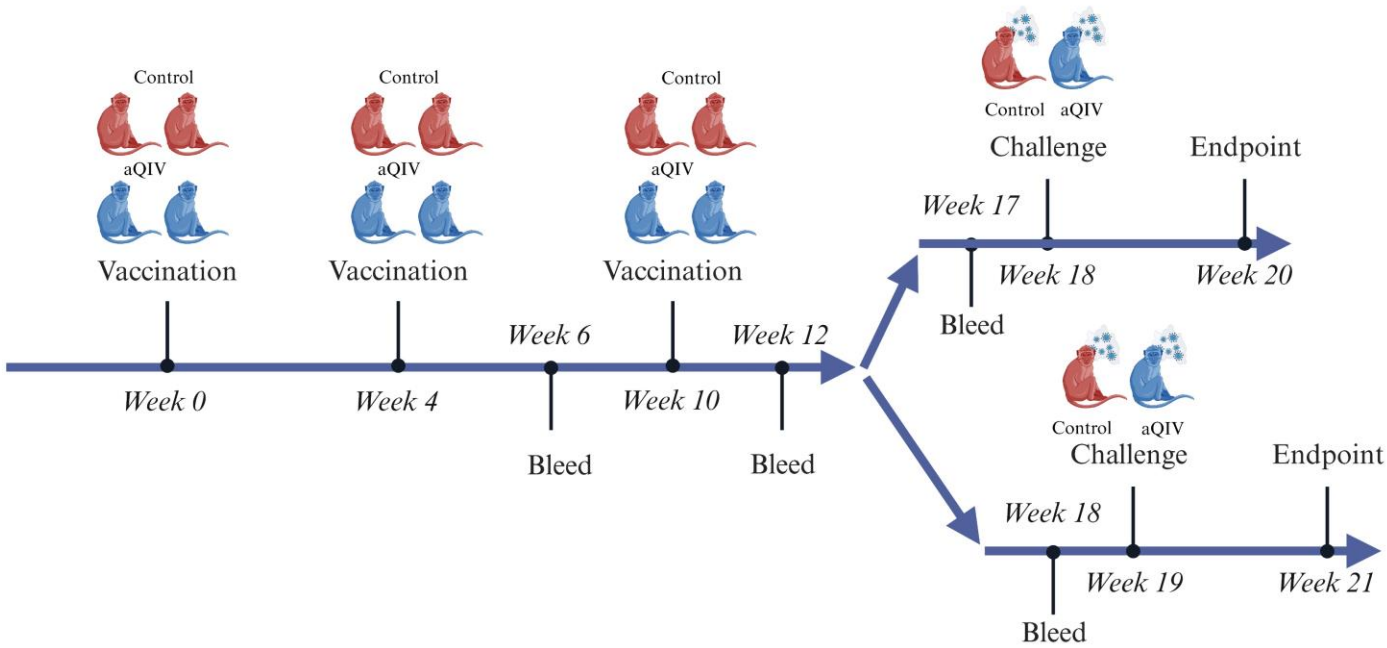
**Figure S1. Actual vs predicted temperatures for each macaque, related to Figure 1.** White/gray shaded areas indicate day/night cycle (6 AM – 6 PM for day, 6 PM – 6 AM for night). Black lines indicate predicted body temperature based on analysis of pre-exposure body temperatures. Red circles are actual temperatures. Macaques are sorted by dose range.



**Figure S2. Correlation of change in respiratory parameters on days 1 and 2 with challenge dose, related to Figure 2.** Closed symbols are macaques that survived infection; open symbols are macaques that succumbed to infection. A-F) Graphs show relationship between inhaled virus dose (x axis) with A) percent change in respiratory rate on day 2, B) percent change in inspiratory time on day 2, C) percent change in expiratory time on day 2, D) percent change in tidal volume on day 1, and E) percent change in tidal volume on day 2.



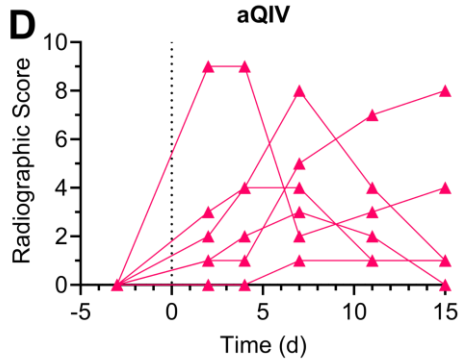
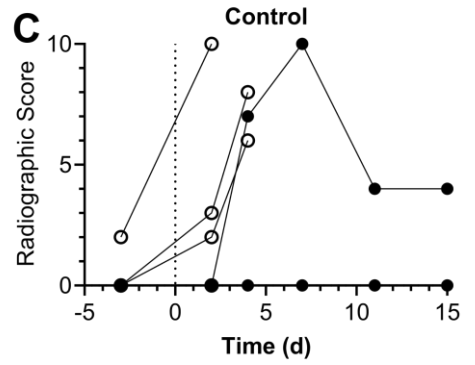
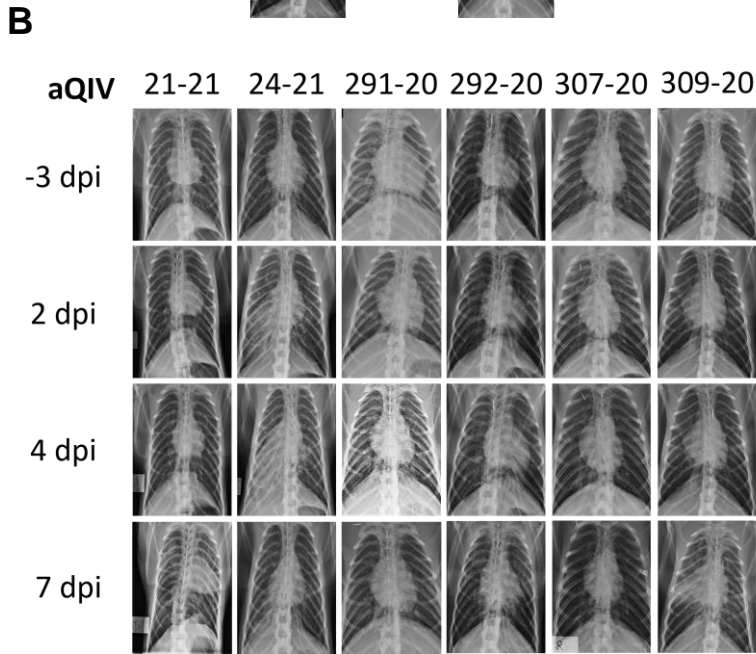
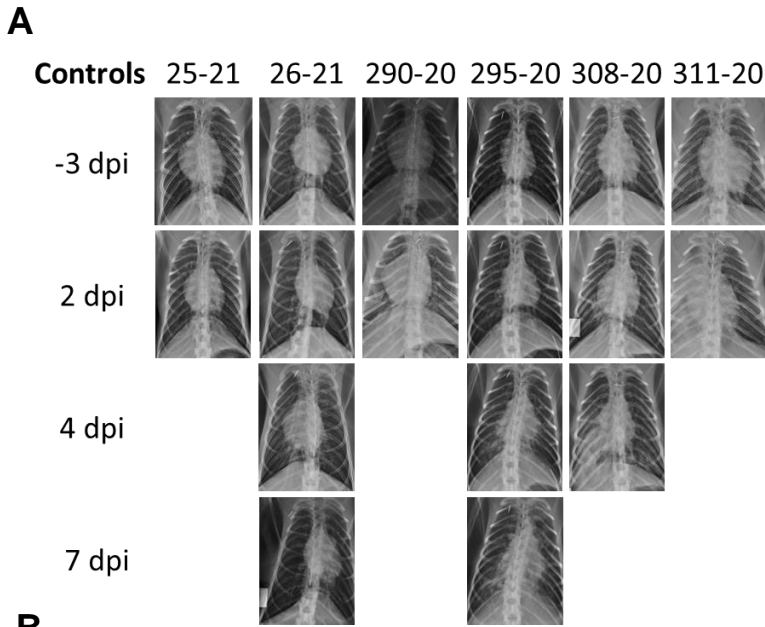
**Figure S3. Macaques that survive H5N1 infection had antibody against influenza nucleoprotein, regardless of inhaled dose, related to Figure 3.**



**Figure S4. Vaccine study timeline, related to Figures 4 and 5.** Timeline shows dates of vaccinations, bleeds, and challenge. After the week 12 bleed, macaques were divided into 2 groups, one which was bled week 17 and challenged week 18, the second bled week 18 and challenged week 19. Challenging two animals at a time allowed for better data and sample collection, as well as more detailed necropsies.

		Vaccination Date			Challenge	Elapsed
Group	Pitt ID	Week 0	Week 4	Week 10	Date	Time <sup>a</sup>
Control	290-20	8-Sep-20	6-Oct-20	17-Nov-20	11-Jan-21	18
	295-20	8-Sep-20	6-Oct-20	17-Nov-20	18-Jan-21	19
	308-20	29-Sep-20	27-Oct-20	8-Dec-20	1-Feb-21	18
	311-20	29-Sep-20	27-Oct-20	8-Dec-20	8-Feb-21	19
	25-21	20-Oct-20	17-Nov-20	29-Dec-20	22-Feb-21	18
	26-21	20-Oct-20	17-Nov-20	29-Dec-20	1-Mar-21	19
aQIV	291-20	8-Sep-20	6-Oct-20	17-Nov-20	11-Jan-21	18
	292-20	8-Sep-20	6-Oct-20	17-Nov-20	25-Jan-21	19
	307-20	29-Sep-20	27-Oct-20	8-Dec-20	1-Feb-21	18
	309-20	29-Sep-20	27-Oct-20	8-Dec-20	15-Feb-21	19
	24-21	20-Oct-20	17-Nov-20	29-Dec-20	22-Feb-21	18
	21-21	20-Oct-20	17-Nov-20	29-Dec-20	8-Mar-21	19

**Table S1. Vaccination and challenge schedule for aQIV vaccine study, related to Figures 4 and 5, and Table 2.** Table indicates dates on which macaques were vaccinated and then challenged. a = elapsed time (in weeks) between initial vaccination and challenge.



**Figure S5. Development of pneumonia in H5N1-infected macaques, related to Figure 5.** Digital radiographs are shown from -3 dpi through 7 dpi for Control (top, A) and QIV-vaccinated (bottom, B) macaques after challenge with aerosolized H5N1. C) and D) show change in radiographic score over time in control and QIV-vaccinated groups.