LIST OF SUPPLEMENTARY MATERIALS

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Supplementary Table 1. Influenza strains aligned for design of quantitative reverse transcription polymerase chain reaction primers and probes to subtype-specific conserved regions

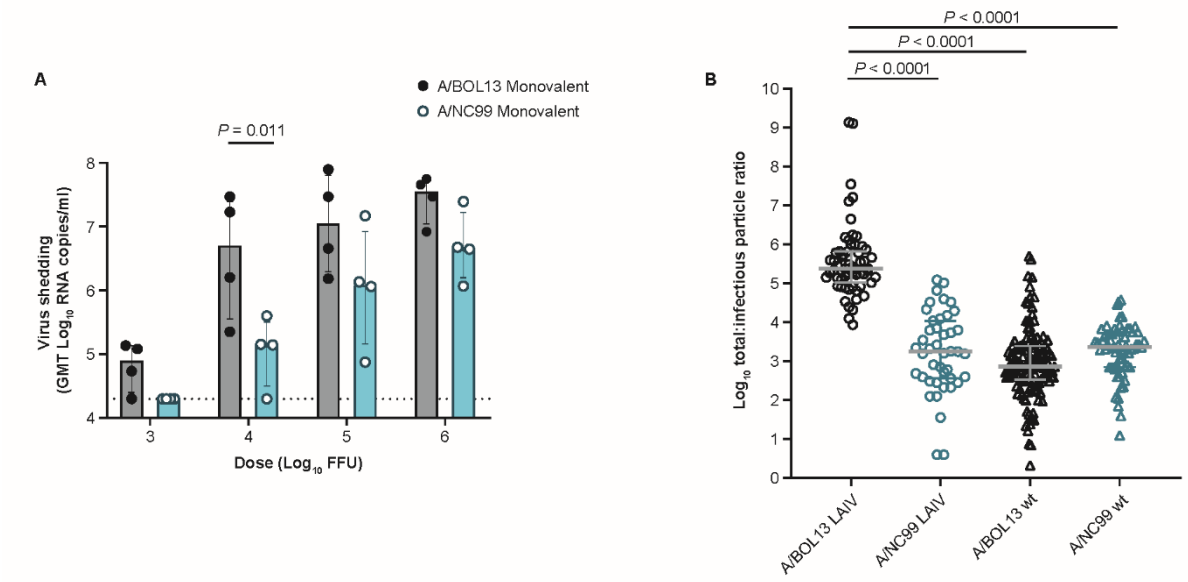
Year of Isolation	Strain	Subtype
2009	A/California/07/2009	H1N1
	A/Perth/16/2009	H3N2
	A/Victoria/210/2009	H3N2
	A/Hong Kong/26560/2009	H3N2
2010	A/Brisbane/10/2010	H1N1
	A/Perth/10/2010	H3N2
	A/Iowa/19/2010	H3N2
	B/Wisconsin/01/2010	B-Yam
2011	A/Christchurch/28/2011	H3N2
	A/Victoria/361/2011	H3N2
	A/Berlin/93/2011	H3N2
	B/Nevada/03/2011	B-Vic
	B/Texas/06/2011	B-Yam
2012	A/Hawaii/22/2012	H3N2
	A/Texas/50/2012	H3N2
	A/Ohio/2/2012	H3N2
	A/New York/39/2012	H3N2
	B/Massachusetts/02/2012	B-Yam
	A/Washington/24/2012	H1N1
2013	A/Dominican Rep/7293/2013	H1N1
	A/Bolivia/559/2013	H1N1
	A/New Hampshire/04/2013	H1N1
	A/South Africa/3626/2013	H1N1
	A/American Samoa/4786/2013	H3N2

	A/Costa Rica/4700/2013	H3N2
	A/Switzerland/9715293/2013	H3N2
	B/Texas/2/2013	B-Vic
	B/Phuket/3073/2013	B-Yam
2014	A/Palau/6759/2014	H3N2
	A/Hong Kong/4801/2014	H3N2
	A/New Caledonia/71/2014	H3N2
	A/Laos/1187/2014	H1N1
	A/New Caledonia/58/2014	H1N1
	A/Tasmania/24/2014	H1N1
	B/Hawaii/24/2014	B-Vic
2015	A/South Australia/9/2015	H3N2
	A/Alaska/232/2015	H3N2
	A/Michigan/45/2015	H1N1
	A/St Petersburg/61/2015	H1N1
	A/Lisboa/32/2015	H1N1
	A/Singapore/GP1908/2015	H1N1
	A/Shanghai-Putuo/1860/2015	H1N1
	A/Slovenia/2903/2015	H1N1
	B/Indiana/25/2015	B-Vic
	B/Florida/78/2015	B-Vic
	B/Maryland/12/2015	B-Yam
	B/California/12/2015	B-Yam
	B/Arizona/10/2015	B-Yam
2016	A/Idaho/33/2016	H3N2

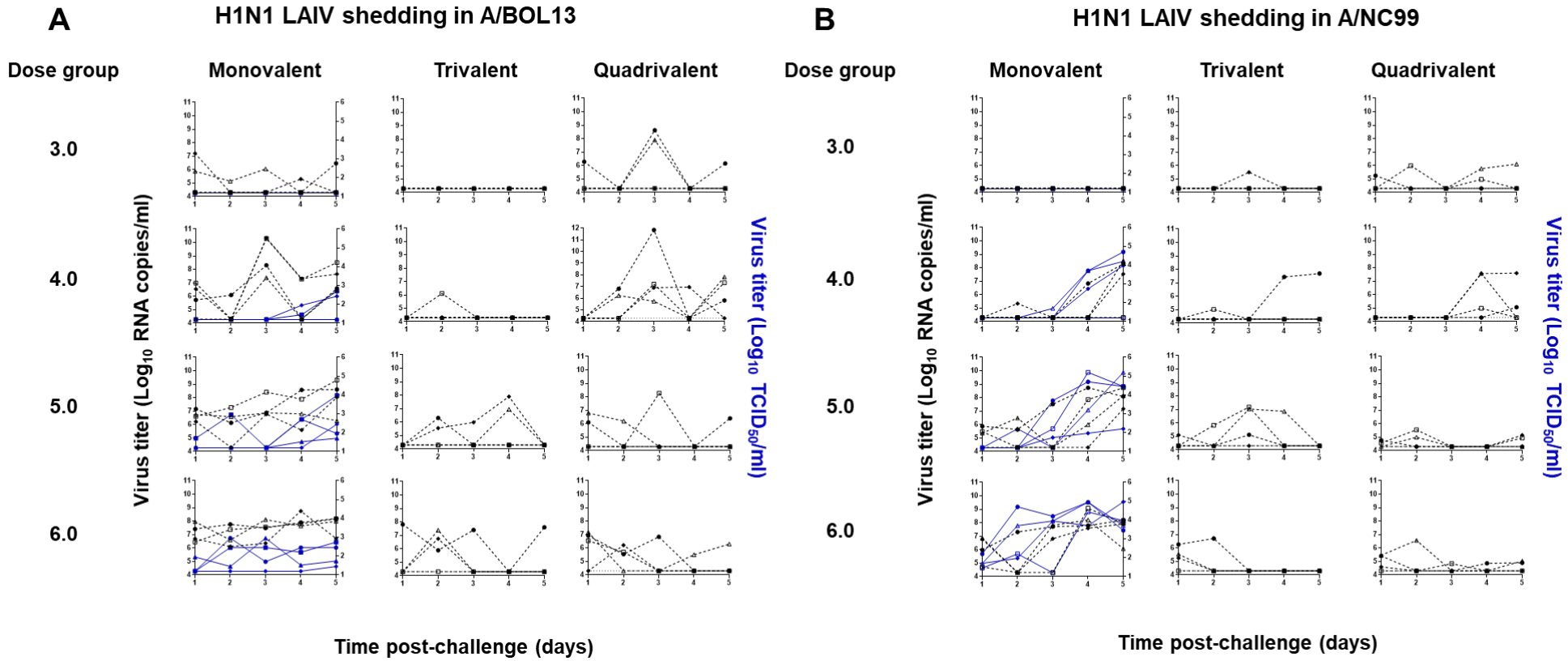
Supplementary Table 2. Primers and probes for RT-qPCR analysis

Purpose	Gene Target	Subtype	Seq 5'-3'	Manufacturer
Reference standard synthesis	NA	H1N1 & H3N2	***TAATACGACTCACTATAGGG****AGTAGAAACAAGGAGTTTTT*[1]	IDT
		B Yamagata	***TAATACGACTCACTATAGGG****AGTAGTAACAAGAGCATTTTTCAG**	IDT
	HA	B Victoria	***TAATACGACTCACTATAGGG****TAGTAGTAACAAGAGCATTTTTC*[1]	IDT
RT-qPCR	NA	H1N1 Pre-Pandemic	*TACTAAATCAATAGAGTTGAATGCACC*	IDT
			ACTGGATTACAGCTGCCCTCTCC	IDT
			FAM-TCAAATAGGATACATCTGCAGTGGG-MGBNFQ	ThermoFisher
		H1N1 Post-Pandemic	*AGTCAAATCAGTCGAAATGAAAGCC*	IDT
			TACTGGACCACAACTGCCTGTCT	IDT
			FAM-TCAGATGGGATACATATGCAGTGGG-MGBNFQ	ThermoFisher
		H3N2	*CGTTCATACTAGCACATTGTCAGGA*	IDT
			CCAAACAATGGCTACTGCTGGAGC	IDT
			VIC-TGTTTCCAGTTATGTGTGTTCAGGA-MGBNFQ	ThermoFisher
		B Yamagata	*CAAGATTGGAAGGTGGTACTCTCG*	IDT
			TCTATCCCAATACAGGGGACATC	IDT
			*ROX-AC**T**GACAG**T**GAAGCCC**TT**GCTCT-Iowa black 3IAbRQSp*[2]	IDT
	HA	B Victoria	*GGAGGTCAATGTGACTGGTGT*	IDT
			TTTTGTTCTGTCGTGCATTATAGG	IDT
			NED-TGGGCAGACCAAAATGCAC-MGBNFQ	ThermoFisher
Sample normalization	GAPDH	Not applicable	*TGCGGCCAAGGCAGTAG*	IDT
			AGGCCATGCCAGTGAGCTT	IDT
			VIC-CTGAGCTGAATGGGAAG-MGBNFQ	IDT

Primer and probe sequences outside the date ranges of 2009–2016, as described in Materials and Methods: RNA extraction and multiplex RT-qPCR, are underlined for each RT-qPCR primer/probe set. [1]T7 sequence highlighted in bold text, [2]Super T bases highlighted in bold text.
GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HA, hemagglutinin; IDT, Integrated Device Technology, Inc.; NA, neuraminidase; RT-qPCR, quantitative reverse transcription polymerase chain reaction.



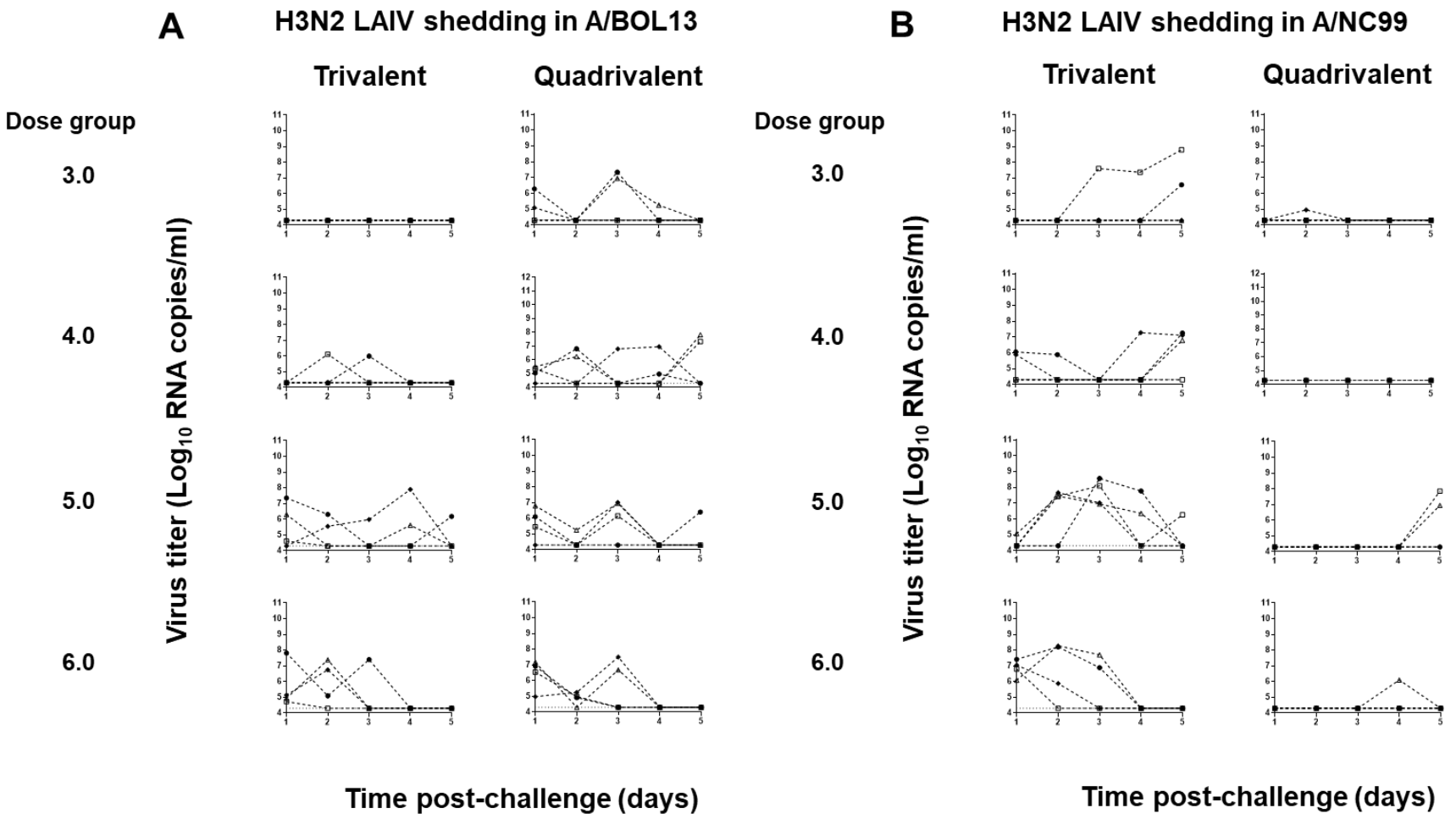
Supplementary Fig. 1. Assessment of non-infectious particle shedding by H1N1 strains. a A/BO13 versus A/NC99 monovalent LAIV geometric mean Days 1–5 shedding measured by RT-qPCR. Columns and error bars show median and interquartile range of four animal groups. **b** Ratio of monovalent LAIV and wt total virus (RT-qPCR copy number) to infectious virus ($TCID_{50}$) shedding. Symbols represent individual nasal swab samples from Days 1–5 post-monovalent LAIV vaccination or Days 1–3 post-wt challenge. Dotted lines indicate lower limit of detection; values below this were plotted as 0.5x the lower limit of detection. A/BOL13, A/Bolivia/559/2013; A/NC99, A/New Caledonia/20/1999; FFU, fluorescent focus units; GMT, geometric mean titer; LAIV, live attenuated influenza vaccine; RT-qPCR, quantitative reverse transcription polymerase chain reaction; $TCID_{50}$, tissue culture infectious dose 50%; wt, wild-type.



Supplementary Fig. 2. H1N1 LAIV shedding kinetics in individual animals. Days 1–5 post-vaccination, measured by RT-qPCR (left y-axis, dashed black lines) and $TCID_{50}$ (monovalent LAIV animals only, right y-axis, blue lines). Graphs show values from individual animals (symbols). **a** A/BOL13 LAIV shedding in monovalent, trivalent, quadrivalent (right column). **b** A/NC99 LAIV

shedding in monovalent, trivalent, and quadrivalent. Dose groups are aligned vertically, from 3.0–6.0 $\log_{10}$ FFU.

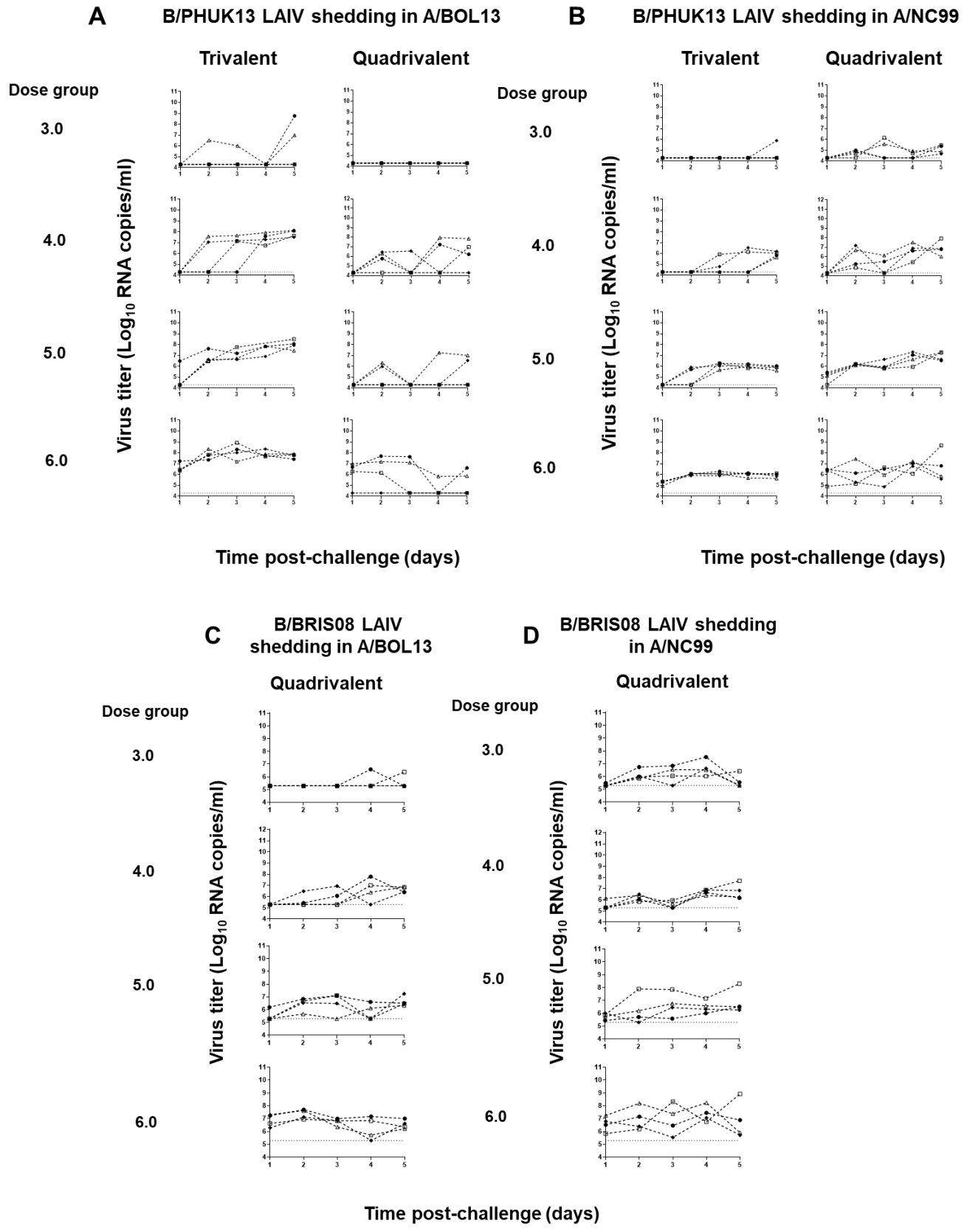
A/BOL13, A/Bolivia/559/2013; A/NC99, A/New Caledonia/20/1999; FFU, fluorescent focus units; LAIV, live attenuated influenza vaccine; RT-qPCR, quantitative reverse transcription polymerase chain reaction; $TCID_{50}$, tissue culture infectious dose 50%.



Supplementary Fig. 3. H3N2 LAIV shedding kinetics in individual animals. Days 1–5 post-vaccination, measured by RT-qPCR. Graphs show values from individual animals (symbols). **a–b.** A/SWITZ13 shedding in either A/BOL13 (**a**) or A/NC99 (**b**) trivalent

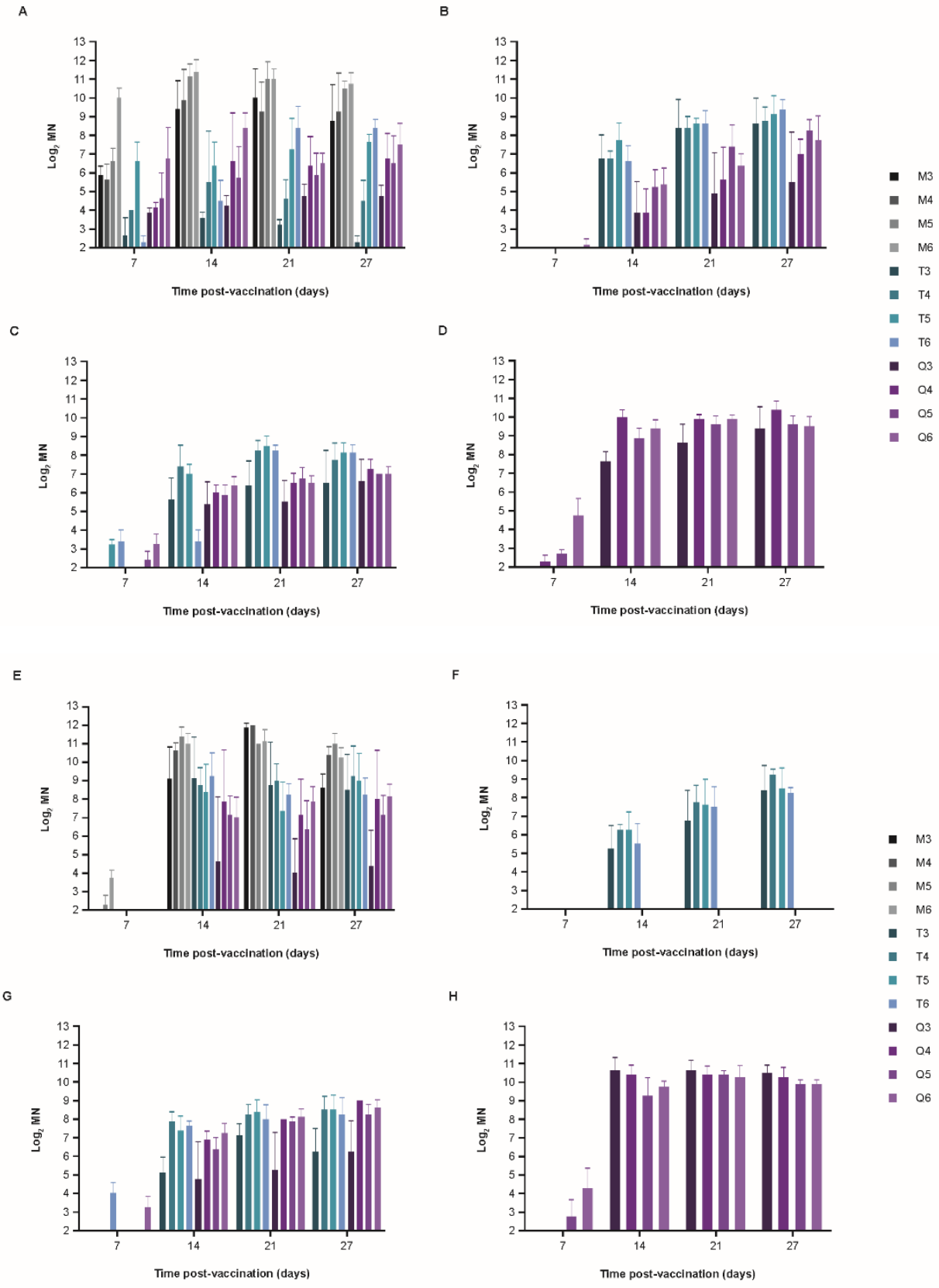
and quadrivalent LAIV. Dose groups are aligned vertically, from 3.0–6.0 $\log_{10}$ FFU.

A/BOL13, A/Bolivia/559/2013; A/NC99, A/New Caledonia/20/1999; FFU, fluorescent focus units; LAIV, live attenuated influenza vaccine; RT-qPCR, quantitative reverse transcription polymerase chain reaction; $TCID_{50}$, tissue culture infectious dose 50%.



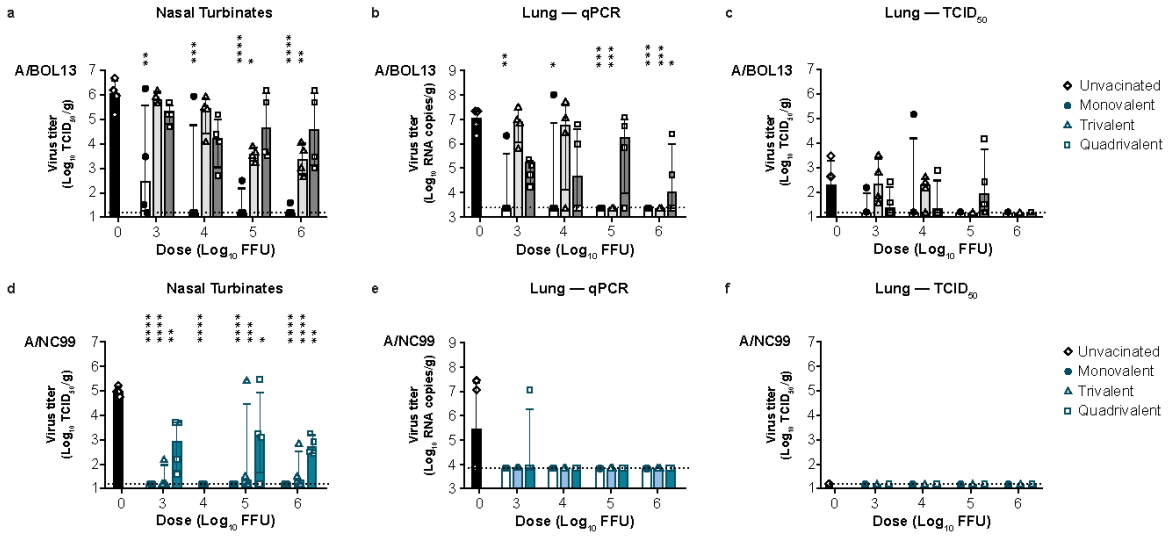
Supplementary Fig. 4. B LAIV shedding kinetics in individual animals. Days 1–5 post-vaccination, measured by RT-qPCR. Graphs show values from individual

animals (symbols). **a–b.** B/PHUK13 LAIV shedding in A/BOL13 (**a**) or A/NC99 (**b**) trivalent and quadrivalent LAIV. **c–d.** B/BRIS08 LAIV shedding in A/BOL13 (**c**) or A/NC99 (**d**) quadrivalent. Dose groups are aligned vertically, from 3.0–6.0 $\log_{10}$ FFU. A/BOL13, A/Bolivia/559/2013; A/NC99, A/New Caledonia/20/1999; B/PHUK13, B/BRIS08, B/Brisbane/60/2008; B/Phuket/3073/2013; FFU, fluorescent focus units; LAIV, live attenuated influenza vaccine; RT-qPCR, quantitative reverse transcription polymerase chain reaction; $TCID_{50}$, tissue culture infectious dose 50%.


A
B
C
D
E
F
G
H
Log2 MN
Time post-vaccination (days)
7
14
21
27
M3
M4
M5
M6
T3
T4
T5
T6
Q3
Q4
Q5
Q6

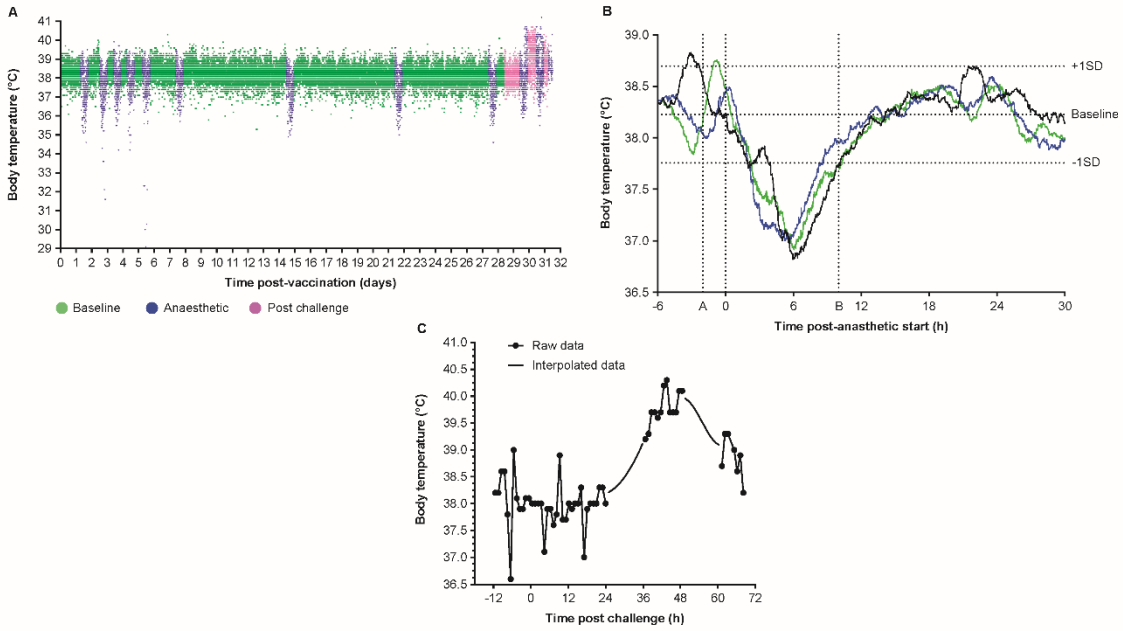
Supplementary Fig. 5. Serum MN responses to vaccination. MN responses were measured on Days 7, 14, 21, and 27 post-vaccination. a**–d.** A/BOL13 formulations: **a** A/BOL13; **b** A/SWITZ13; **c** B/PHUK13; **d** B/BRIS08. **e–h** A/NC99 formulations: **e** A/NC99; **f** A/SWITZ13; **g** B/PHUK13; **h** B/BRIS08. Columns and error bars show geometric mean and geometric standard deviation. Grey bars: monovalent LAIV 3.0–6.0 $\log_{10}$ doses (M3–M6), blue bars: trivalent LAIV 3.0–6.0 $\log_{10}$ doses (T3–T6) and purple quadrivalent LAIV 3.0–6.0 $\log_{10}$ doses (Q3–Q6).

A/BOL13, A/Bolivia/559/2013; A/NC99, A/New Caledonia/20/1999; A/SWITZ13, A/Switzerland/9715293/2013; B/BRIS08, B/Brisbane/60/2008; B/PHUK13, B/Phuket/3073/2013; LAIV, live attenuated influenza vaccine; MN, microneutralization.



Supplementary Fig. 6. A/BOL13 provides reduced protection against wt challenge virus replication in respiratory tissues. Levels of wt challenge virus replication at Day 3 post-challenge in lung and NT tissues. **a, d**. wt virus titers in NT by $TCID_{50}$. **b, e**. wt virus titers in lungs by RT-qPCR. **c, f**. wt virus titers in lungs by $TCID_{50}$. Data shown are geometric mean virus titers for individual animals (symbols), with 4 animals per dose group. Columns and error bars for all virus titer data show group median and interquartile range. Statistical comparison between groups was performed by two-way analysis of variance with Sidak's post-test correcting for multiple comparisons. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Dotted lines indicate lower limit of detection; values below this were plotted as 0.5x the lower limit of detection.

A/BOL13, A/Bolivia/559/2013; A/NC99, A/New Caledonia/20/1999; FFU, fluorescent focus units; GMT, geometric mean titer; NT, nasal turbinate; RT-qPCR, quantitative reverse transcription polymerase chain reaction; $TCID_{50}$, tissue culture infectious dose 50%; wt, wild-type.



Supplementary Fig. 7. Quantitative analysis of ferret fever development. Intraperitoneal telemetry monitors were used to measure ferret core temperature 1 hourly for study duration. **a** Example of complete raw temperature data set for wt A/BOL13 challenge (52 animals). Green: normal body temperature. Blue: anesthetic hypothermia. Pink: post-challenge period. **b** Example of removal (A to B) of anesthetic hypothermia artefact, with three representative temperature traces shown (50-point moving average, all animals). **c** Example of quadratic spline interpolation of data to repair excluded hypothermia artefact in post-challenge temperature trace. A/BOL13, A/Bolivia/559/2013; h, hour; SD, standard deviation; wt, wild-type