Supplementary materials

List of rHAs used in the antibody forensics assay in this study

H3 antibody forensics panel: A/URUGUAY/716/2007, A/VICTORIA/361/2011, A/HONGKONG/4801/2014, A/SINGAPORE/INFIMH-16-0019/2016, A/BRISBANE/1059/2017, A/ETHIOPIA/1877/2017, A/KENYA/105/2017, A/MISSOURI/37/2017, A/MIYAZAKI/89/2017, A/OSORNO/60580/2017, A/SAPPORO/46/2017, A/SHANGHAIXUHUI/1373/2017, A/SYDNEY/1093/2017, A/SYDNEY/1013/2017, A/AKSARAY/4048/2016, A/ALBANIA/7165/2016, A/ANKARA/4110/2016, A/BRETAGNE/2836/2016, A/BRISBANE/1009/2016, A/CALIFORNIA/168/2016, A/CHIBA/33/2016, A/CHRISTCHURCH/513/2016, A/GUANGXIQIXIN/328/2016, A/HAWAII/67/2016, A/JORDAN/J16420301NT/2016, A/KAWASAKI/142/2016, A/KHMELNITSKY/719/2016, A/LAOS/F2884/2016, A/LINKOU/0051/2016, A/LISBOA/NIEVA063/2016, A/MARTINIQUE/531/2016, A/MARYLAND/24/2016, A/MEKNES/168/2016, A/MICHIGAN/84/2016, A/PORTUGAL/MS68/2016, A/SAUDIARABIA/192150/2016, A/SHANDONGLAICHENG/1763/2016, A/SINGAPORE/GP2366/2016, A/TASMANIA/97/2016, A/TOWNSVILLE/51/2016.

H1 antibody forensics panel: A/CALIFORNIA/07/2009, A/BAYERN/69/2009, A/HONGKONG/34079/2009, A/HONGKONG/33597/2009, A/LVIV/N6/2009, A/MONTPELLIER/2051/2009, A/CHRISTCHURCH/16/2010, A/ANKARA/TR40/2011, A/ASTRAKHAN/1/2011, A/HONGKONG/3934/2011, A/GOTEBORG/1/2011, A/MEXICO/2208/2011, A/HONG/KONG/5659/2012, A/STOCKHOLM/25/2012, A/ISRAEL/Q504/2015, A/MICHIGAN/45/2015, A/HUNGARY/12/2016, A/BRATISLAVA/342/2016.

Table S1. Severity score and score definitions of the peak body weight loss, the peak of body temperature rise and the AUC for virus shedding.

Severity score	Peak body weight loss (%)	Peak temperature rise (°C)	AUC virus shedding
0 (Normal)	Weight increase or less than –3.42	<0.7	<8.375
1 (Mild)	[3.42–5.56]	[0.7–0.9]	[8.375–13]
2 (Marked)	[5.56–7.46]	[0.9–1.4]	[13–18.5]
3 (Severe)	>7.46	>1.4	>18.5

AUC, area under the curve

Figure S1. Immunogenicity of A/Singapore/INFIMH-16-0019/2016 N2 and A/Michigan/45/2015 N1 rTET-NA, LVNA and IIV preparations was tested in naïve mice. Female BALB/c mice (n=8 per group) were immunized twice intramuscularly and terminally bled 2 weeks after second immunization as shown in Figure 2A. Sera were collected two weeks after booster vaccination; sera pools from two animals were created and in turn tested via ELISA to assess NA-binding antibody titers using A/Singapore/INFIMH-16-0019/2016 N2 rTET-NA (A) or A/Michigan/45/2015 N1 rTET-NA (B) as coating antigens, respectively. ○ symbol represents EC_{50} antibody titers without AF03 addition and ■ represents groups with AF03 addition. The dashed line indicates the starting serum dilution used for testing. *p <0.05; ** p<0.01; *** p<0.001 (the red text/symbols are comparisons vs adjuvanted PBS/control). For comparisons between adjuvanted and non-adjuvanted matched dose and formulation, all were significant with p <0.001 (except for LVNA 0.2µg [p<0.05] and IIV 1 µg [p <0.01] A/Singapore/2016 N2). LLOD: Lower limit of detection

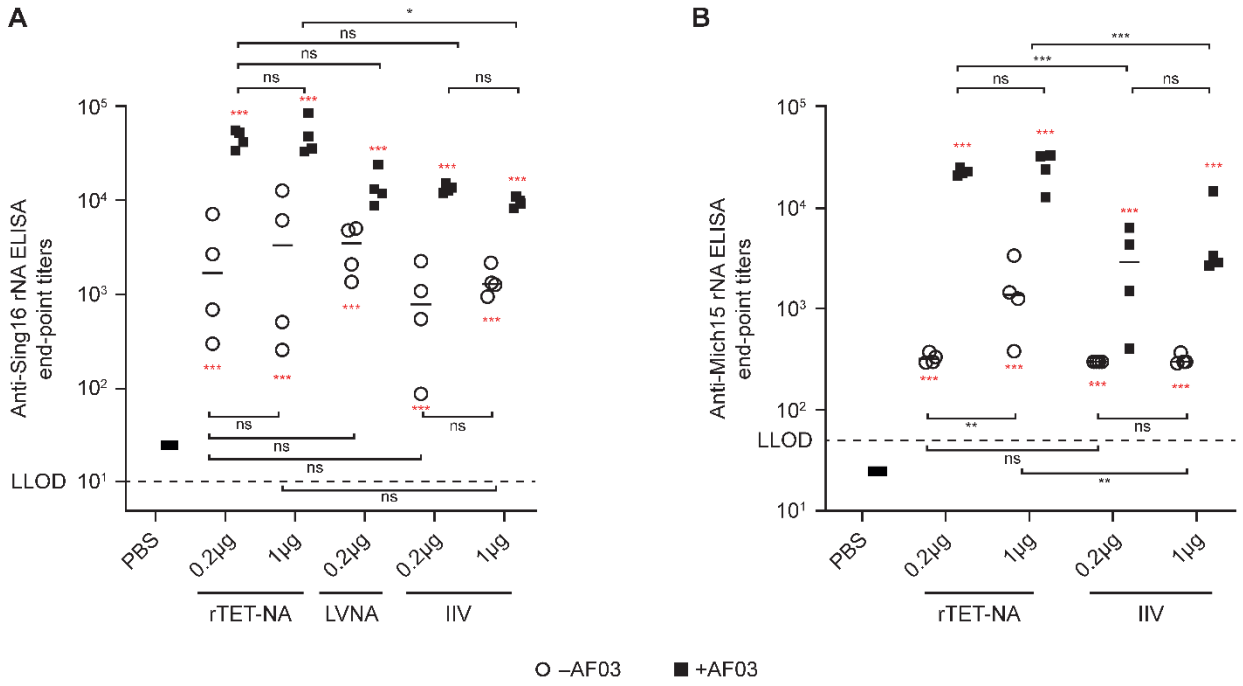


Figure S2. rTET-NA retains its immunogenicity following octavalent HA and NA vaccination in ferrets. NA-binding antibody titers against vaccinal N2, N1, B/Victoria-like and B/Yamagata-like strains were determined in sera from vaccinated ferrets three (3) weeks after each immunization via ELISA using corresponding rTET-NA as coating antigens. For all comparisons between 1 dose and 2 doses, all NA containing groups had similar or higher NA-binding antibody titers after 2 doses, while no difference or higher NA-binding titers were observed upon addition of 4x HA, statistical analysis pending. NT: Not tested. LLOD: Lower limit of detection

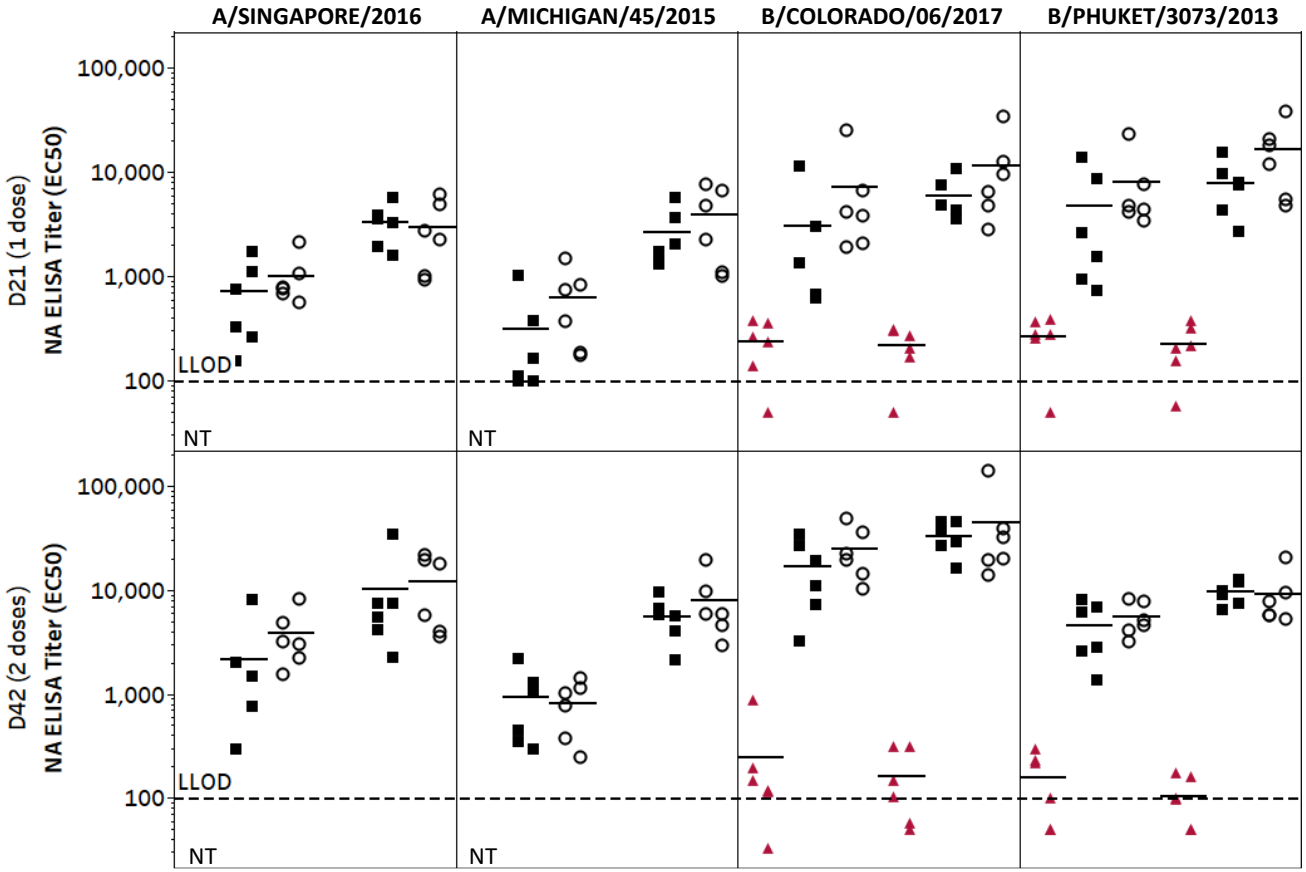
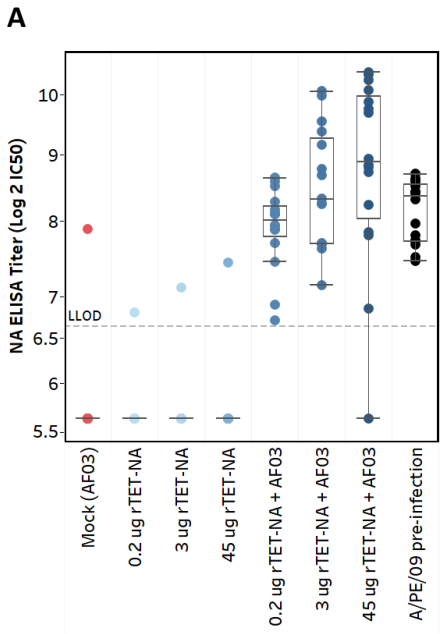
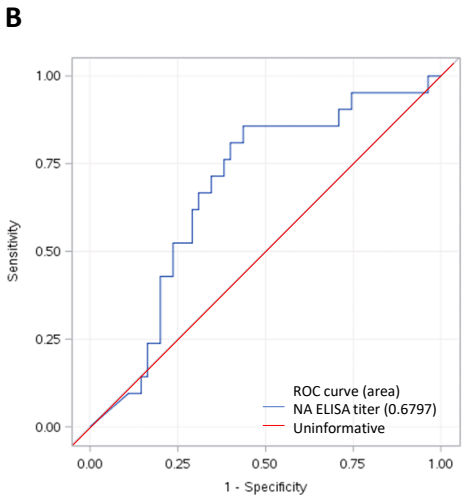
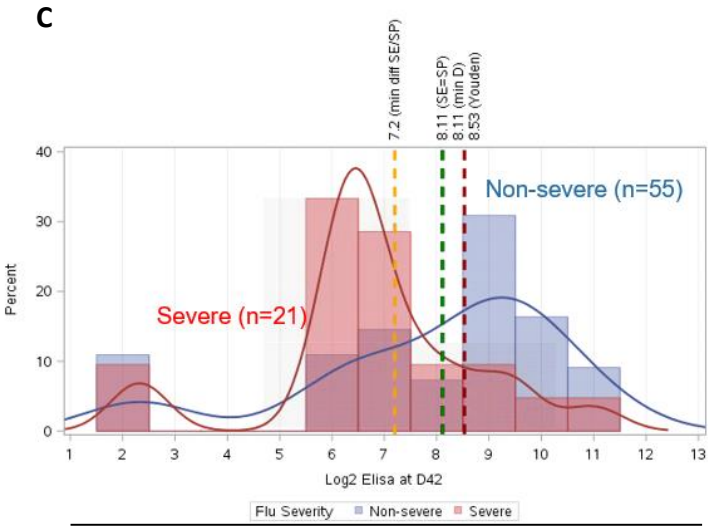


Figure S3. Post-vaccination NA-binding antibody titers are a correlate of protection in the ferret model. Pre-challenge PE09 anti-N2 NA-binding antibody titers were measured in sera collected three weeks after the second vaccination via ELISA using PE09 rTET-NA as coating antigen (A). ROC curve (B) and distribution of the pre-challenge NA ELISA titers according to severity score (C).





AUC name	AUC [95%CI]	*P*-value
NA ELISA titer	0.68 [0.55;0.81]	**0.0071(S)**
Uninformative	0.50 [0.50;0.50]	



Criterion	Criterion value	Cut-off value	Sensitivity	Specificity
Maximum Youden's index	0.42	8.53	85.71%	56.36%