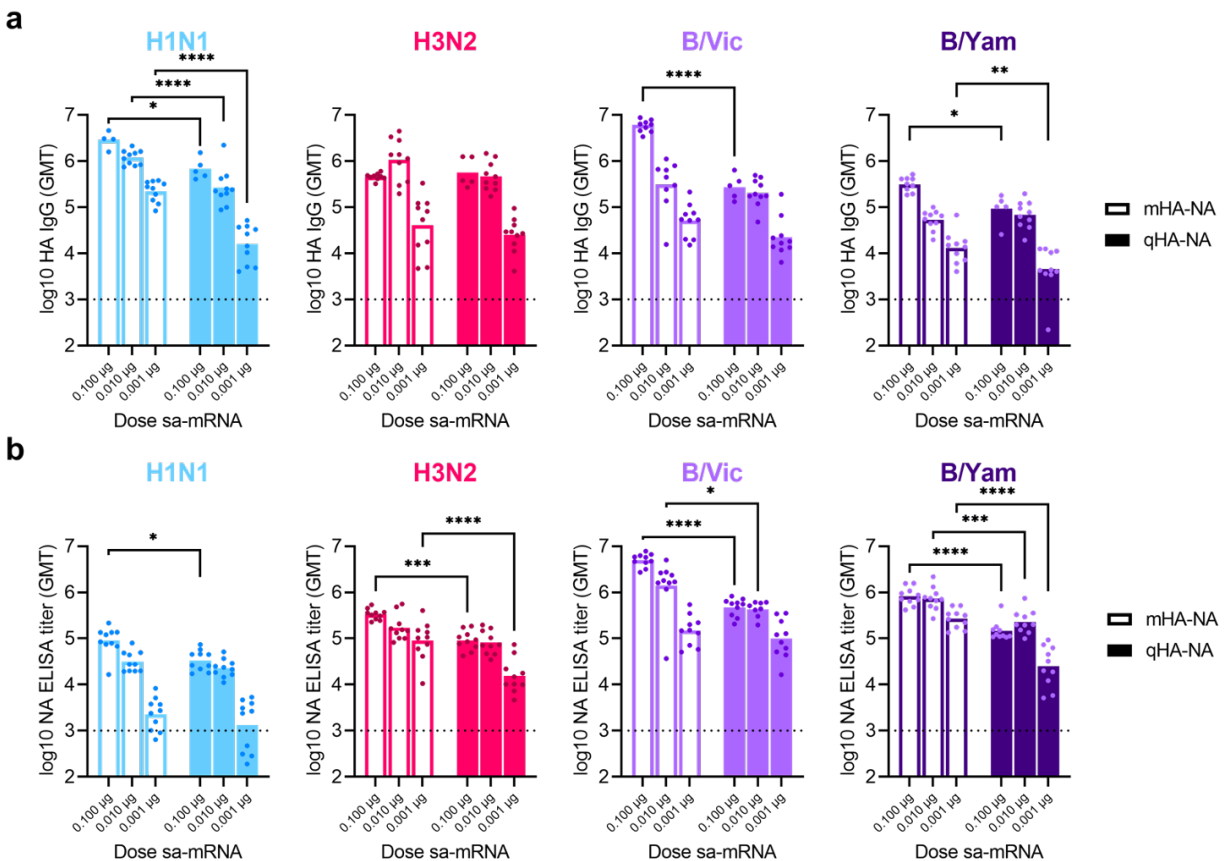


1 **Supplemental figures for - Self-amplifying mRNA seasonal influenza vaccines elicit mouse**  
 2 **neutralizing antibody and cell-mediated immunity and protect ferrets**

3  
 4 Michael Cheung<sup>1#</sup>, Cheng Chang<sup>1#</sup>, Raveen Rathnasinghe<sup>1</sup>, Evan Rossignol<sup>1</sup>, Yunfei Zhang<sup>1</sup>,  
 5 Annette Ferrari<sup>1</sup>, Harsh Patel<sup>1</sup>, Yanjun Huang<sup>1</sup>, Michelle Sanchez Guillen<sup>1</sup>, Tina Scalzo<sup>1</sup>,  
 6 Changkeun Lee<sup>1</sup>, Gillis R. Otten<sup>1</sup>, Ethan C. Settembre<sup>1</sup>, Nedzad Music<sup>1</sup>, Giuseppe Palladino<sup>1</sup>,  
 7 Yingxia Wen<sup>\*</sup>

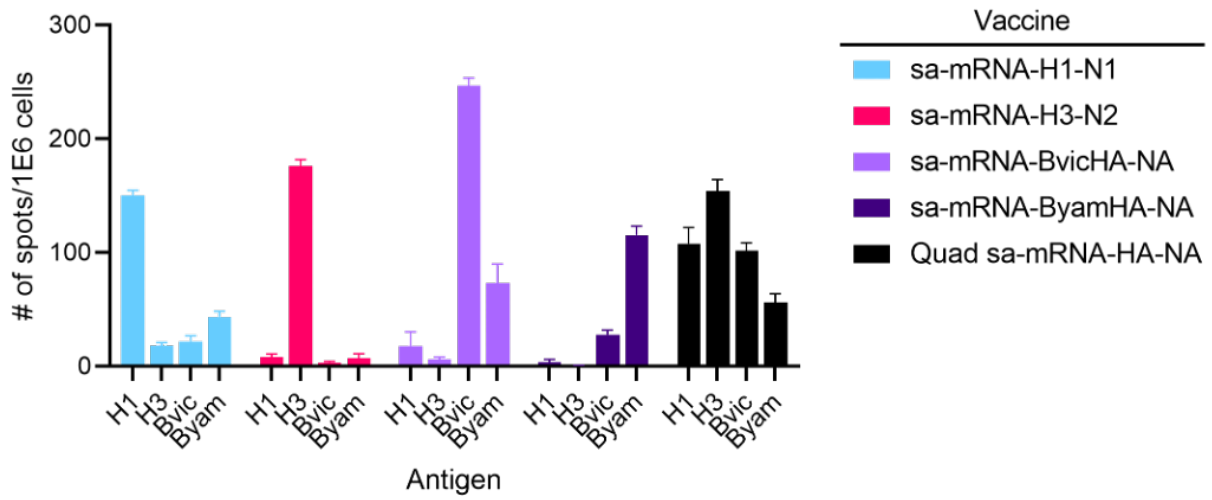
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 9 <sup>1</sup>CSL Seqirus, 225 Wyman Street, Waltham, MA 02451, USA  
 10 <sup>#</sup>Contributed equally  
 11

12 **Supplementary Fig. 1. Bicistronic sa-mRNA vaccines induce both anti-HA and anti-NA**  
 13 **IgG. a.** HA-specific IgG antibodies induced by monovalent and quadrivalent sa-mRNA-HA-NA  
 14 vaccines were measured by ELISA in vaccine strain monobulks. **b.** NA-specific IgG antibodies  
 15 were measured by ELISA for recombinant NA proteins from each vaccine strain. mHA-NA and  
 16 qHA-NA compared by 2-way ANOVA, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .



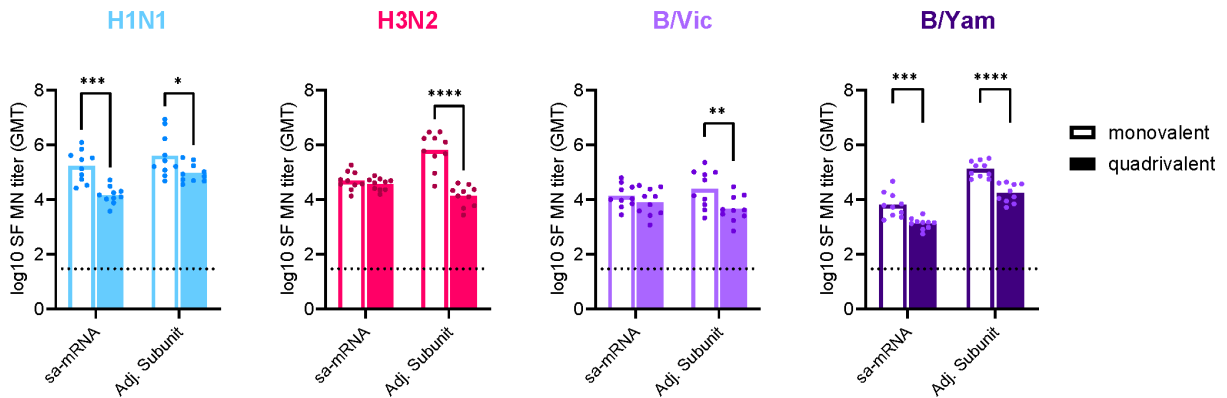
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19 **Supplementary Fig. 2. sa-mRNA vaccines establish vaccine-specific memory B cells in**  
 20 **monovalent and quadrivalent formulations, as shown by the ELISPOT assay.** Vaccine-  
 21 specific memory B cells were detected in mice immunized with sa-mRNA vaccine (0.1  $\mu\text{g}$  dose)  
 22 by quantifying vaccine-specific antibody secreting cells in *ex vivo*-stimulated splenocytes 3  
 23 weeks following the second immunization.



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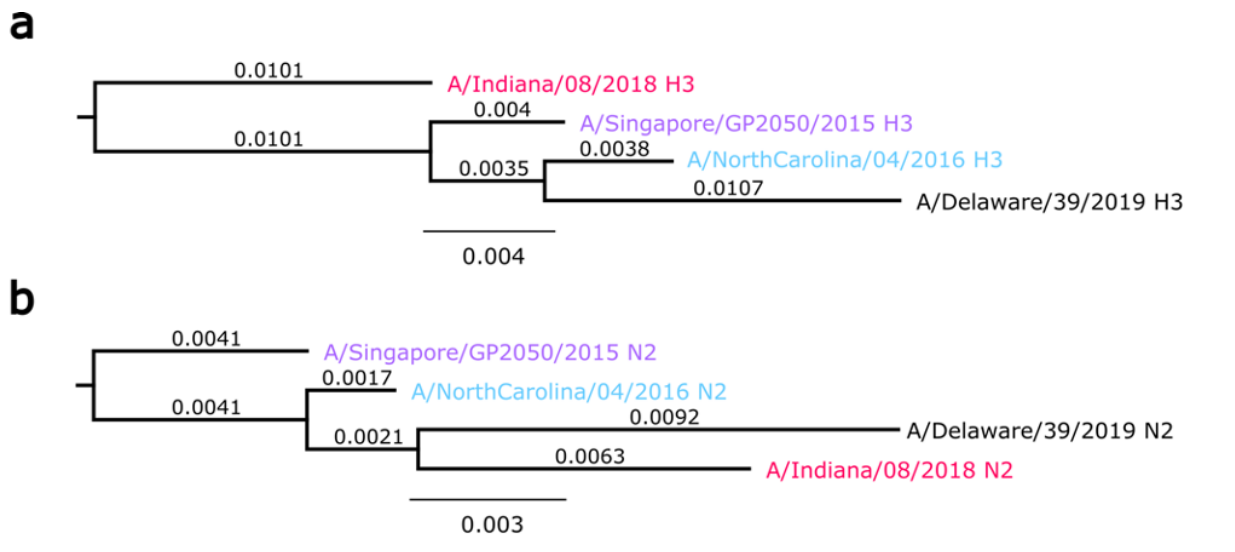
26 **Supplementary Fig. 3. Quadrivalent formulations of either sa-mRNA or adjuvanted**  
27 **subunit vaccines result in lower titers than monovalent vaccines.** The HA-specific  
28 neutralizing antibody response to 1  $\mu$ g monovalent (open bars) and quadrivalent (filled bars) sa-  
29 mRNA-HA or adjuvanted subunit vaccines was measured by a short-form microneutralization  
30 (MN) assay. 2-way ANOVA, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .  
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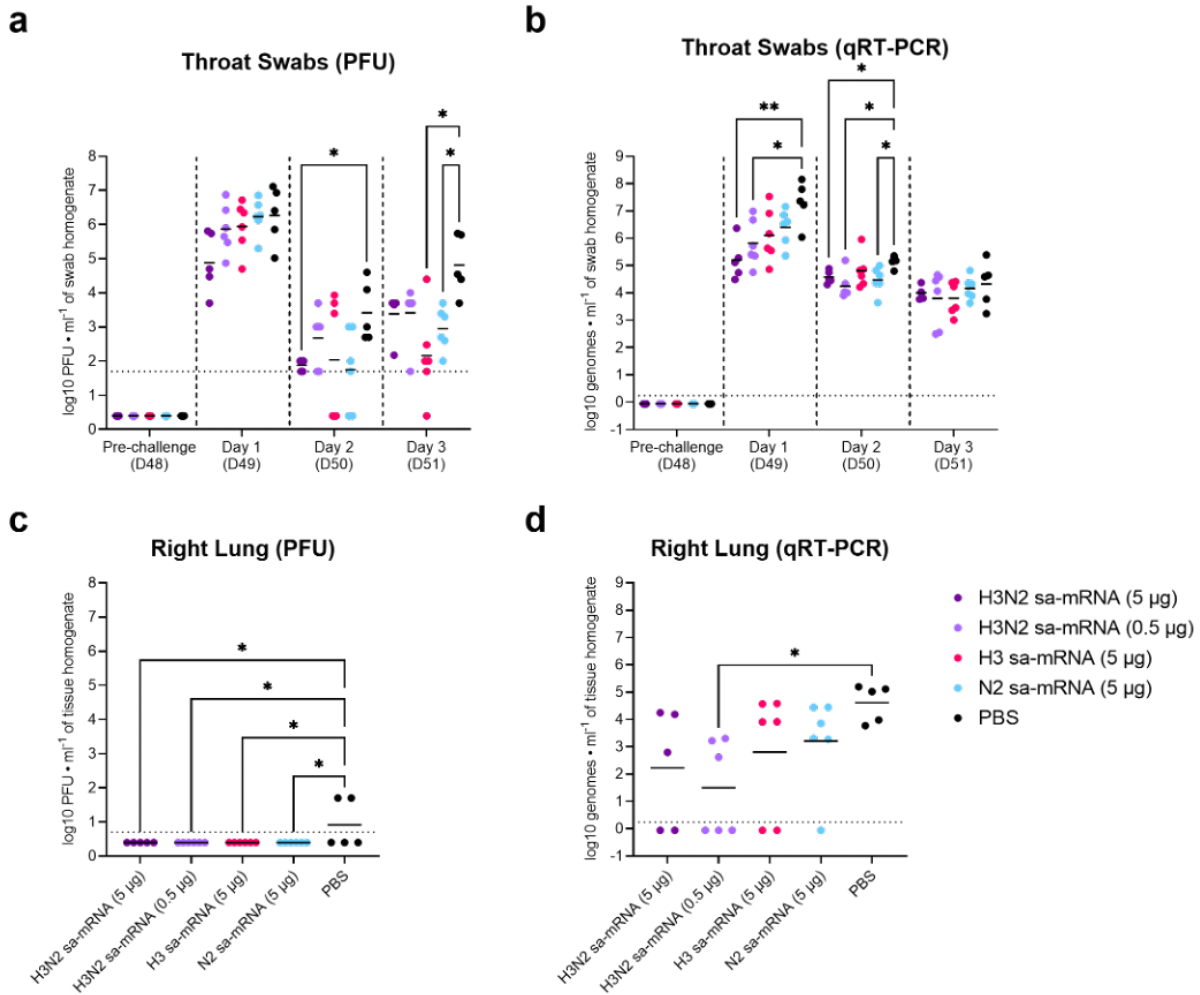
34 **Supplementary Fig. 4. Phylogenetic distance of HA and NA in recent A(H3N2) vaccine**  
35 **strains. a.** A phylogenetic tree of H3 for the four A(H3N2) strains in Fig. 5 was generated using  
36 Geneious Prime software. **b.** A similar phylogenetic tree was created for N2.



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39 **Supplementary Fig. 5. Bicistronic and monocistronic H3 and N2 sa-mRNA vaccines**  
 40 **reduced viral load in throat and lung tissue of A(H3N2)-challenged ferrets.** Viral load was  
 41 quantified for daily throat swabs by plaque assay (a) and qRT-PCR (b). Viral load in  
 42 homogenized lung lobes 3 days post challenge were determined by plaque assay (c) and by qRT-  
 43 PCR (d). Each dot represents an animal and data are presented as geometric means. Data for  
 44 daily throat swabs were log-transformed and analyzed using two-way ANOVA with multiple  
 45 comparisons using Dunnett's adjustment. Lung tissue data was log-transformed and analyzed  
 46 using a one-way ANOVA with multiple comparisons using Dunnett's adjustment. PBS,  
 47 phosphate-buffered saline. \* $p < 0.05$ , \*\* $p < 0.01$ .



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 49

50 **Supplementary Fig. 6. Bicistronic and monocistronic N2-containing sa-mRNA vaccines**  
 51 **induced cross-neutralizing antibodies in ferrets. a.** N2-specific neutralizing antibody response  
 52 was measured by a long-form microneutralization (LF MN) assay. **b.** Cross-neutralizing  
 53 response against heterologous A(H3N2) viruses are expressed as percent homologous (H3N2  
 54 A/Delaware/39/2019) LF MN titer (mean  $\pm$  SEM). Titers against heterologous strains compared  
 55 to A/Delaware/39/2019 by 2-way ANOVA, \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

