

1 Supplementary information for
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3 **Immunodominant antibodies against hemagglutinin in ferrets following infection**
4 **with 2018 to 2019 influenza vaccine strains**

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6 Stevens^{1,✉}

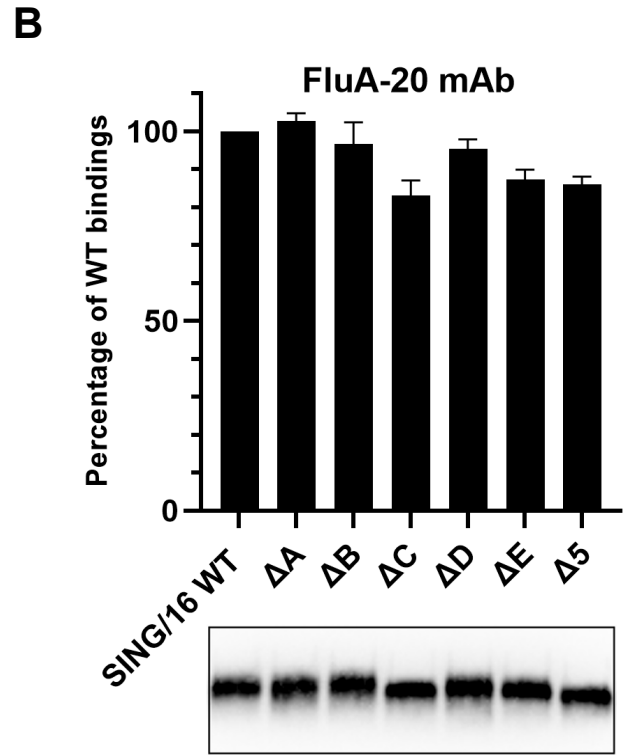
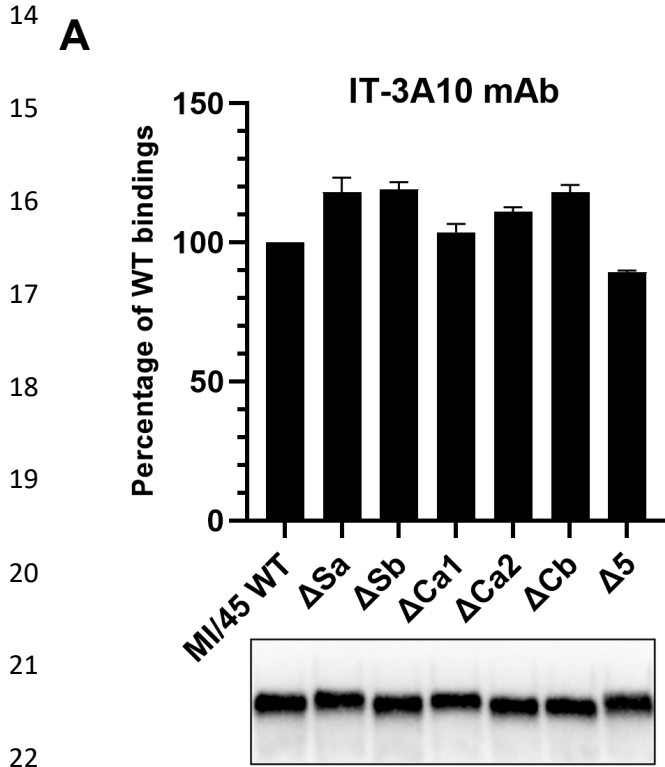
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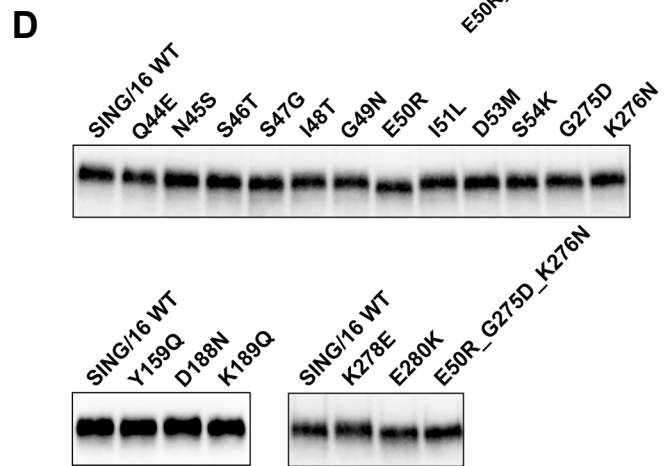
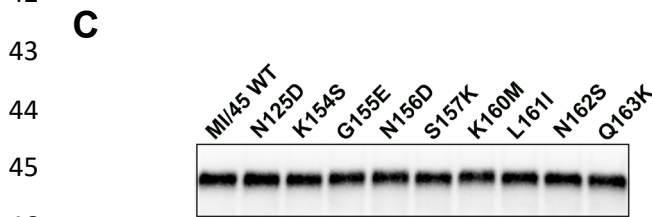
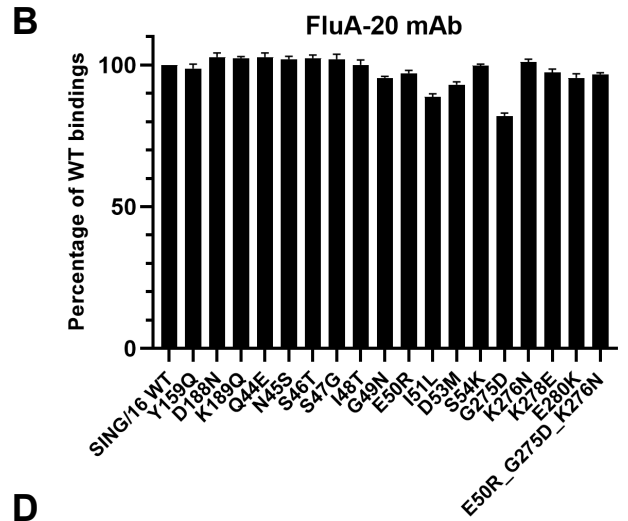
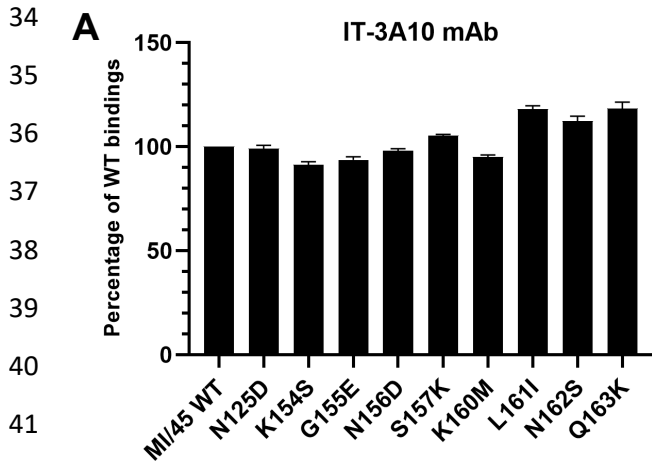
12 Supplementary Figures 1 to 5

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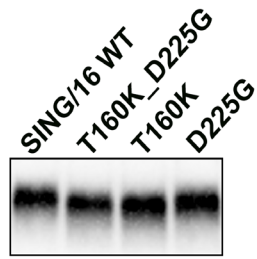


23 **Supplementary Figure 1. Characterization of antigenic site mutants of MI/45 and**
 24 **SING/16 HA1.** The proper folding of antigenic site mutants of MI/45 and SING/16 rHA1
 25 was assessed using the f-AbBA-2 with the anti-MI/45 HA1 mAb, IT-3A10 (**A**) and the pan-
 26 H3 anti-HA1 mAb, FluA-20 (**B**), respectively. Binding results for each rHA1 mutant were
 27 normalized against corresponding WT controls. Each bar represents the median and
 28 standard deviation from three independent experiments. Equivalent amounts of rHA1s
 29 used in the assay were confirmed by Western blot.

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Supplementary Figure 2. Characterization of rHA1 mutants carrying single or multiple substitutions within dominant antigenic sites of MI/45 and SING/16. The proper folding of MI/45 and SING/16 rHA1 mutants was assessed using the f-AbBA-2 with the anti-MI/45 HA1 mAb, IT-3A10 (**A**) and the pan-H3 anti-HA1 mAb, FluA-20 (**B**), respectively. Binding results for each rHA1 mutant were normalized against corresponding WT controls, with each bar representing the median and standard deviation from three independent experiments. Equivalent amounts of rHA1s used in the assay were confirmed by Western blot (**C & D**).



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67 **Supplementary Figure 3. Characterization of SING/16 rHA1 mutants carrying single**
68 **or multiple egg-adaptive substitutions.** Equivalent amounts of rHA1s used in the assay
69 were confirmed by Western blot.

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SING/16	ELVQNSSIGE	ICDSPHQILD	GENCTLIDAL	LGDPQCDGFQ	NKKWDLFVER	SKAYSNCYPY	DVPDYASLRS	LVASSGTLEF	
KS/14	ELVQNSSIGE	ICDSPHQILD	GENCTLIDAL	LGDPQCDGFQ	NKKWDLFVER	NKAYSNCYPY	DVPDYASLRS	LVASSGTLEF	
		130	140	150	160	170	180	190	200
SING/16	KNESFNWITGV	TQNGTSSACI	RGSSSSFFSR	LNWLTHLNYT	YPALNVTMPN	KEQFDKLYIW	GVHHPGTDKD	QIFLYAQSSG	
KS/14	KNESFNWAGV	TQNGTSSSCI	RGSSSSFFSR	LNWLTHLNSK	YPALNVTMPN	NEQFDKLYIW	GVHHPGTDKD	QISLYAQSSG	
		210	220	230	240	250	260	270	280
SING/16	RITVSTKRSQ	QAVIPNIGSR	PRIRDIPSRI	SIYWTIVKPG	DILLINSTGN	LIAPRGYFKI	RSKSSSIMRS	DAPIGKCKSE	
KS/14	RITVSTKRSQ	QAVIPNIGSR	PRIRDIPSRI	SIYWTIVKPG	DILLINSTGN	LIAPRGYFKI	RSKSSSIMRS	DAPIGKCKSE	
		290	300						
SING/16	CITPNGSIPN	DKPFQNVNRI	TYGACPRY						
KS/14	CITPNGSIPN	DKPFQNVNRI	TYGACPRY						

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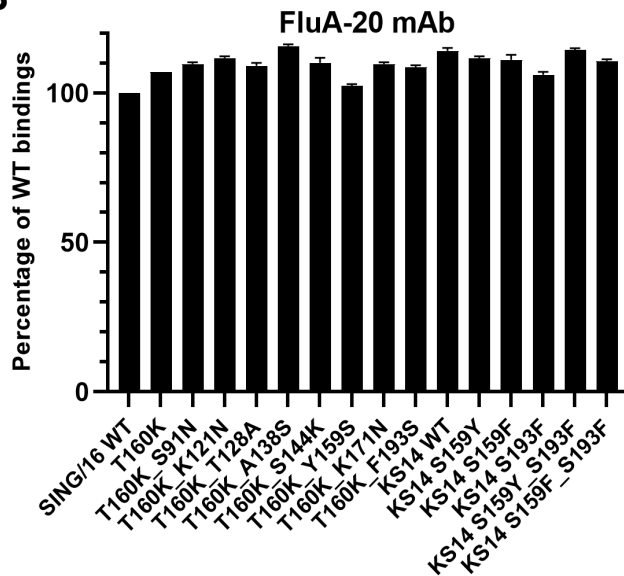
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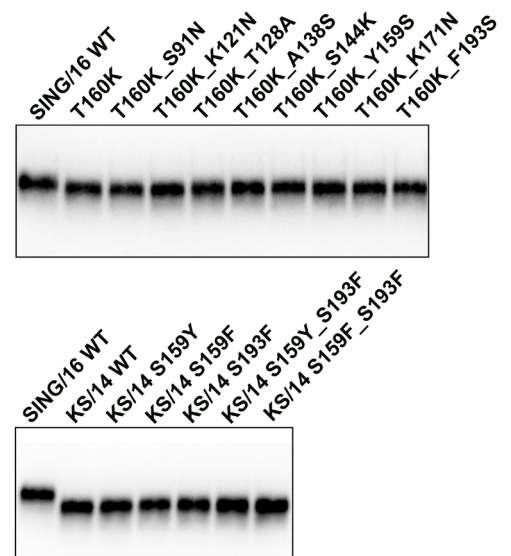
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94 **Supplementary Figure 4. Characterization of SING/16 and KS/14 rHA1 mutants used**
 95 **in SING/16 HA1 epitope mapping.** (A) Amino acid sequences of SING/16 and KS/14
 96 HA1 were aligned with substitutions highlighted. (B) The proper folding of expressed rHA1
 97 mutants from SING/16 and KS/14 was assessed using the f-AbBA-2 with the pan-H3 anti-
 98 HA1 mAb, FluA-20. Binding results for each rHA1 mutant were normalized against
 99 SING/16 WT controls, with each bar representing the median and standard deviation from
 100 three independent experiments. (C) Equivalent amounts of rHA1s used in the assay were
 101 confirmed by Western blot.

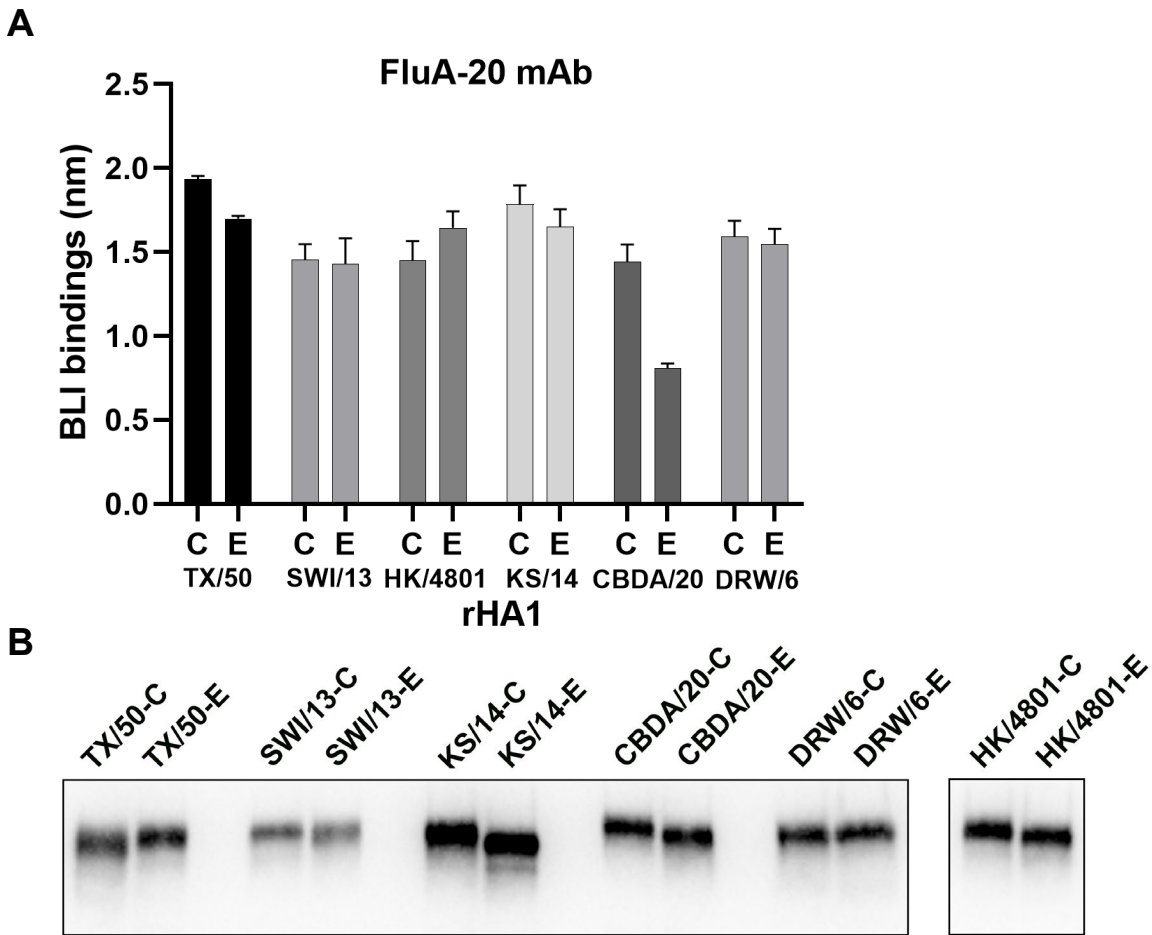
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Supplementary Figure 5. Characterization of rHA1s of A(H3N2) vaccine viruses from recent flu seasons. (A) The proper folding of expressed rHA1s from cell-grown or egg-grown A(H3N2) vaccine viruses was assessed using the f-AbBA-2 with the pan-H3 anti-HA1 mAb, FluA-20. Each bar represents the median BLI binding with standard deviation from three independent experiments. “C” denotes cell-version rHA1 from cell-grown virus, while E denotes egg-version rHA1 from egg-grown virus. **(B)** Equivalent amounts of each rHA1 pair used in the assay were confirmed by Western blot.