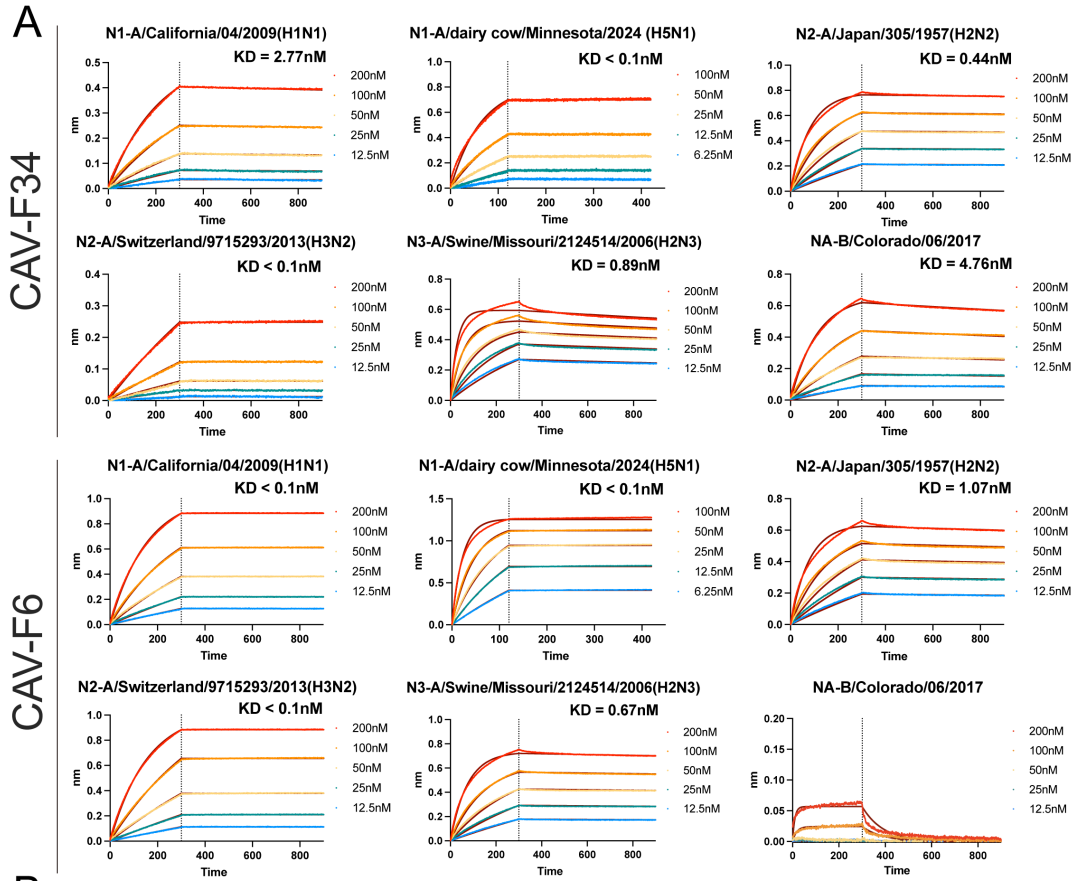


Broad Neuraminidase Antibodies Confer Protection Against Seasonal and Avian Influenza Viruses

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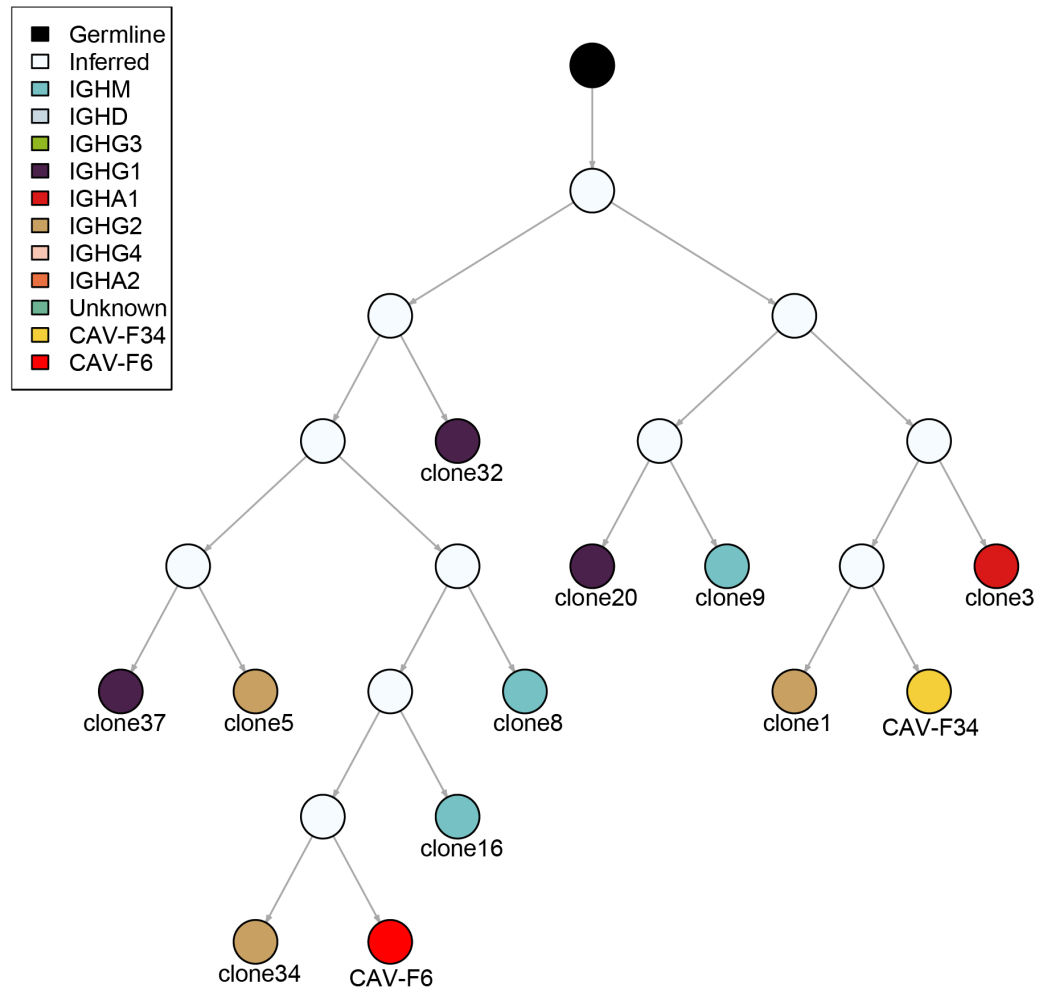
Supplementary Fig. 1-14

Supplementary Table. 1-7

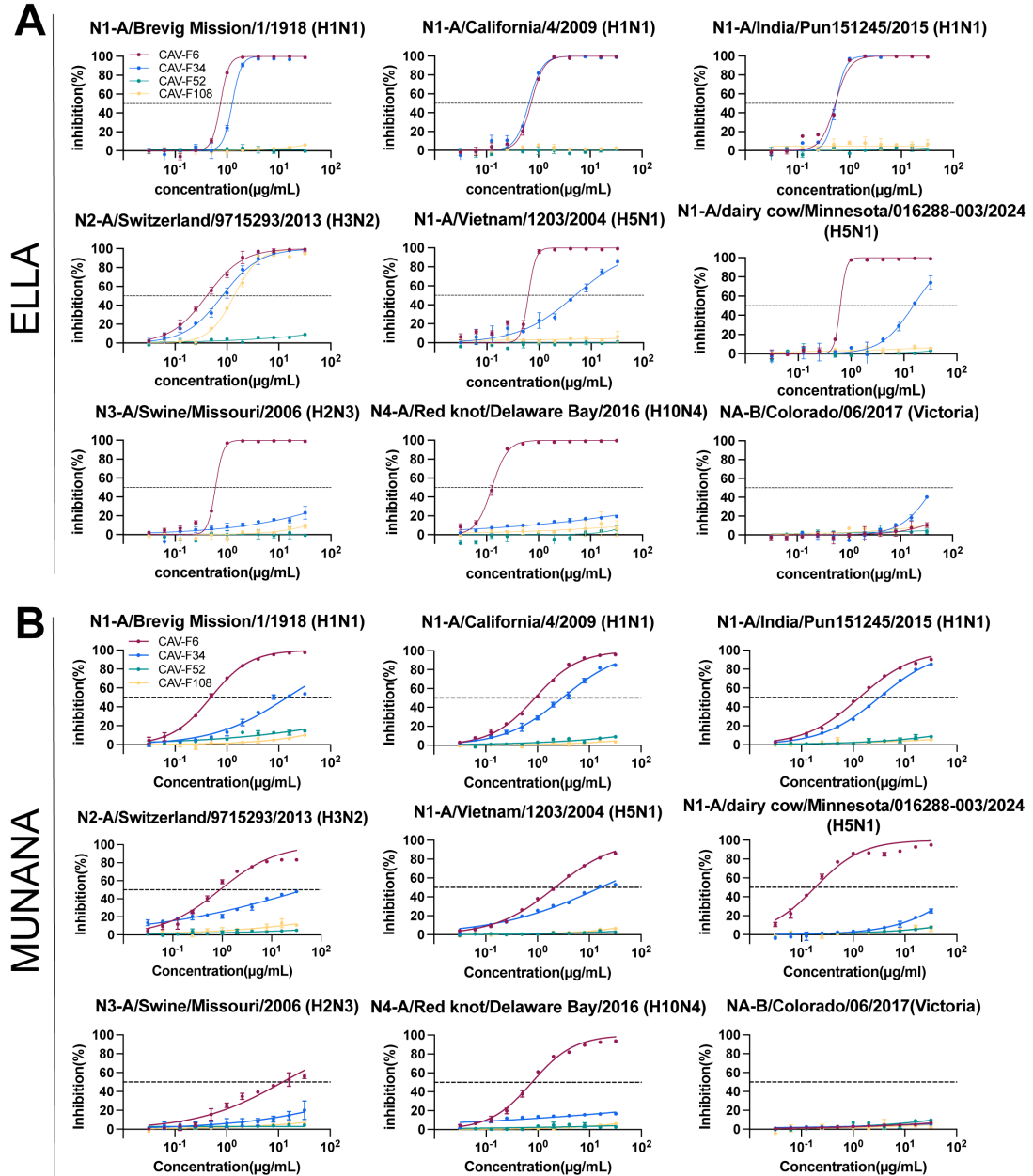


Antibody	Antigen	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	KD (nM)	Full R ²
CAV-F34	N1-A/California/04/2009 (H1N1)	2.35×10^4	6.51×10^{-5}	2.77	0.9992
	N1-A/dairy cow/Minnesota/2024 (H5N1)	7.62×10^4	5.93×10^{-7}	<0.1	0.9989
	N2-A/Switzerland/9715293/2013 (H3N2)	8.98×10^3	3.88×10^{-7}	<0.1	0.9989
	N2-A/Japan/305/1957 (H2N2)	6.19×10^4	2.69×10^{-5}	0.44	0.9984
	N3-A/Swine/Missouri/2124514/2006 (H2N3)	1.71×10^5	1.52×10^{-4}	0.89	0.9900
	NA-B/Colorado/06/2017 (Victoria)	4.24×10^4	2.02×10^{-4}	4.76	0.9990
CAV-F6	N1-A/California/04/2009 (H1N1)	3.29×10^4	4.23×10^{-7}	<0.1	0.9996
	N1-A/dairy cow/Minnesota/2024 (H5N1)	3.49×10^5	4.49×10^{-7}	<0.1	0.9918
	N2-A/Switzerland/9715293/2013 (H3N2)	2.42×10^4	3.72×10^{-7}	<0.1	0.9997
	N2-A/Japan/305/1957 (H2N2)	6.51×10^4	6.94×10^{-5}	1.07	0.9966
	N3-A/Swine/Missouri/2124514/2006 (H2N3)	6.73×10^4	4.49×10^{-5}	0.67	0.9989
	NA-B/Colorado/06/2017 (Victoria)	4.48×10^5	1.23×10^{-2}	-	0.9676

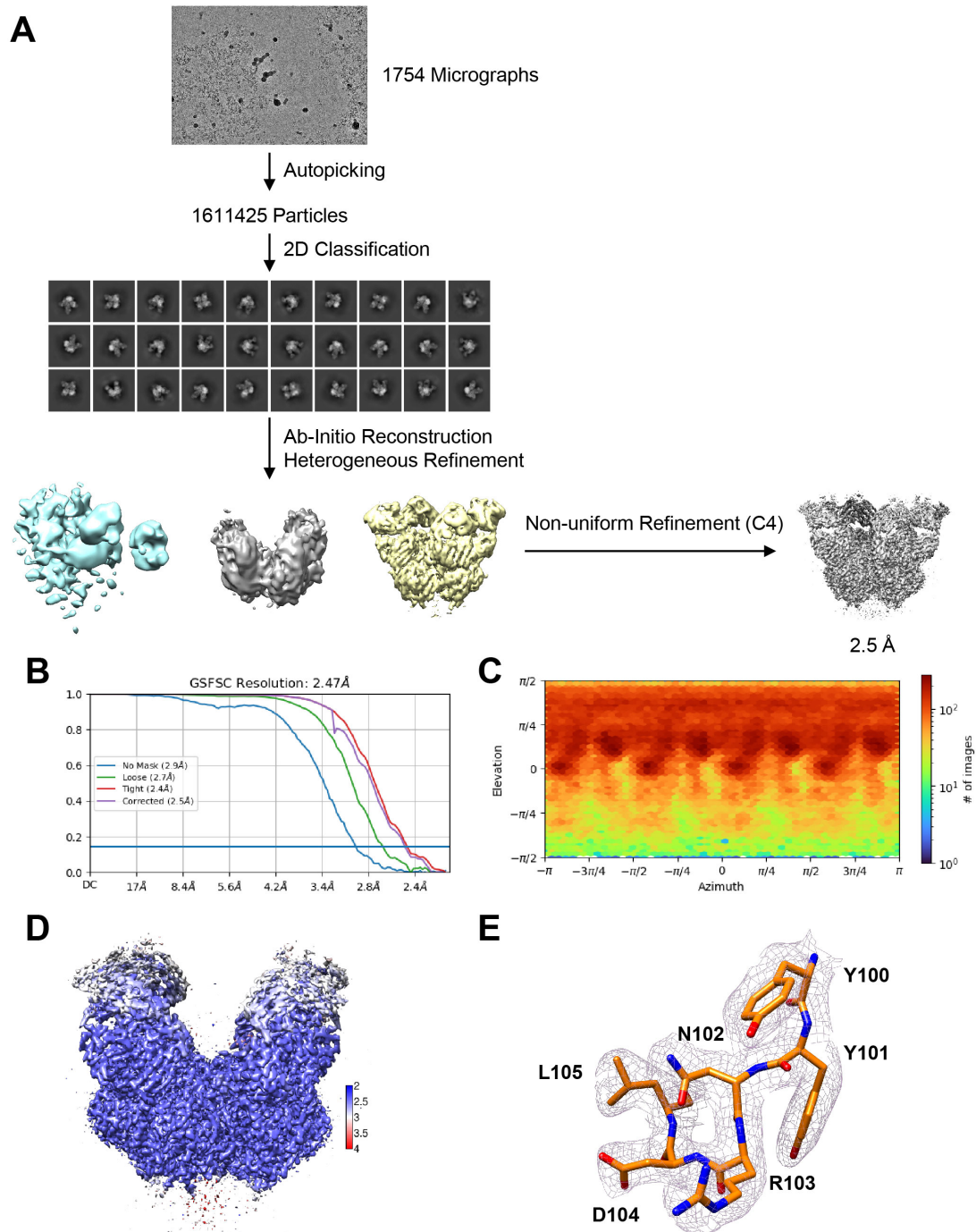
Supplementary Fig. S1 Binding affinity measured by BLI with recombinant NA proteins and full IgG. Binding profiles (A) and kinetic parameters (determined by 1:1 interaction modeling) (B) of CAV-F34 and CAV-F6 to NA proteins from the following strains (assayed via BLI): A/California/04/2009 (H1N1), A/dairy cow/Minnesota/24_016288-003/2024 (H5N1), A/Switzerland/9715293/2013 (H3N2), A/Japan/305/1957 (H2N2), A/Swine/Missouri/2124514/2006 (H2N3) and B/Colorado/06/2017 (Victoria).



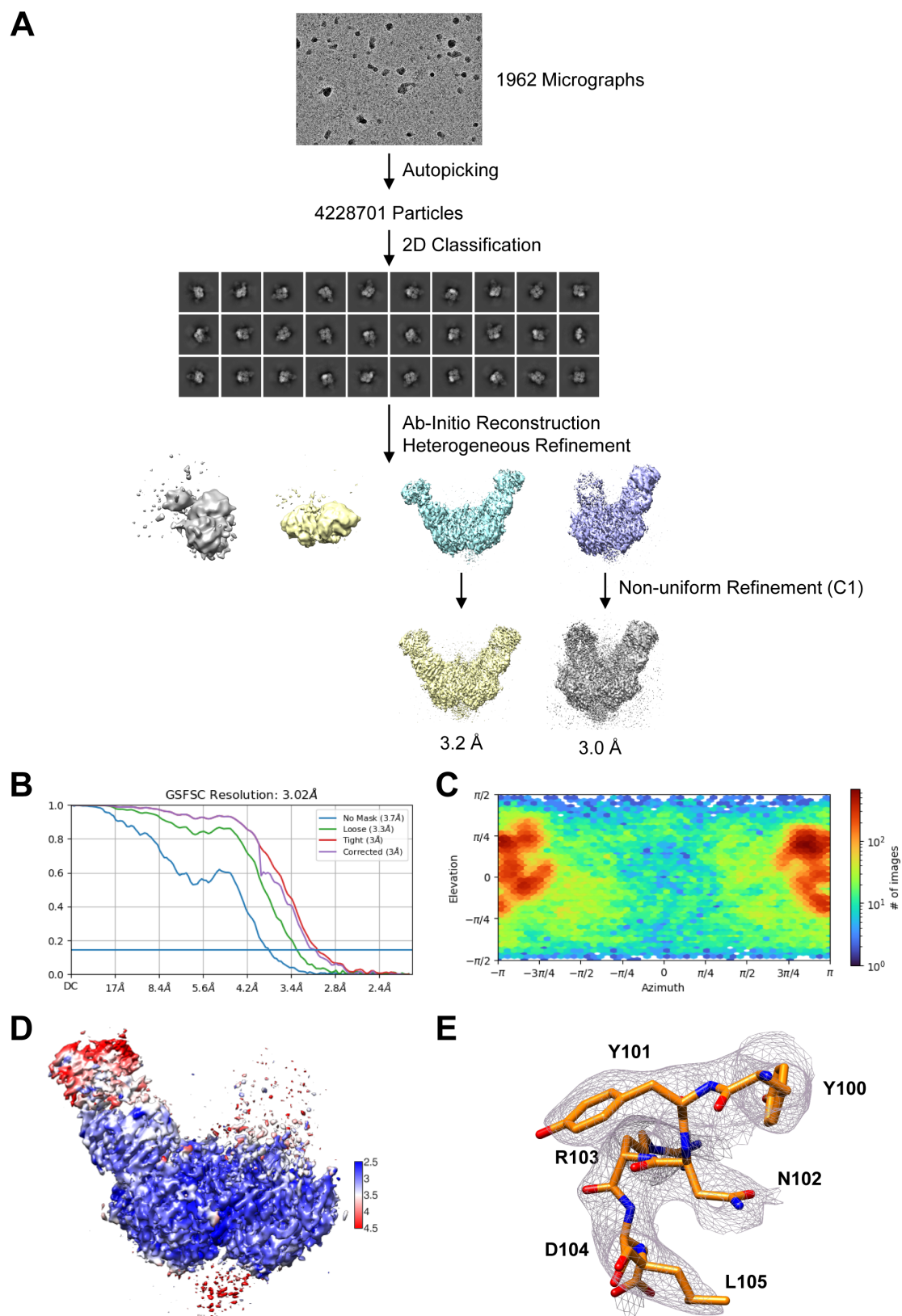
Supplementary Fig. S2 Phylogenetic tree depicting the clonal evolution and isotype class switching of VH3-48 antibodies. Phylogenetic tree illustrating the genetic relationships among VH3-48 antibody heavy chain sequences, with nodes colored by isotype. The reconstructed germline sequence is shown in black, while sequences inferred using the Dowser package are represented in white. CAV-F34 and CAV-F6 are highlighted in orange and red, respectively. The tree's structure depicts the diversification process of antibody clones and their evolutionary relationships with the germline.



Supplementary Fig. S3 Enzymatic inhibition ability of CAV-F6, CAV-F34, CAV-F52 and CAV-F108. The enzyme inhibitory ability of antibodies against NA proteins in ELLA assay (**A**). The enzyme inhibitory ability of antibodies against NA proteins in MUNANA assay (**B**). Data represents one of three independent experiments, shown as mean $\pm$ SD of three technical replicates.

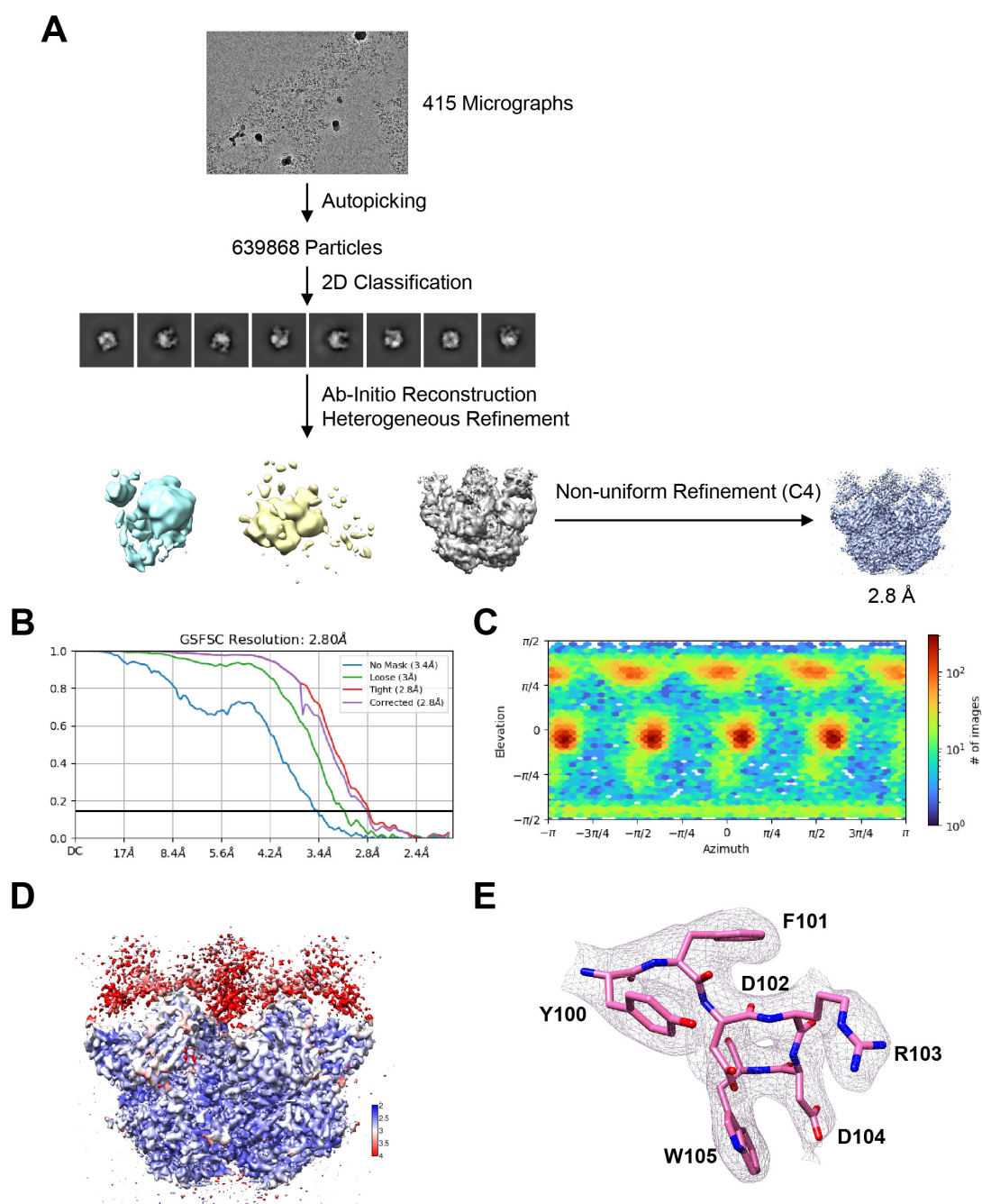


Supplementary Fig. S4 Cryo-EM structure validations of the A/California/04-CIP100_RGCM_1868/2009 H1N1 NA/CAV-F34 complex. A-D, Data processing workflow (A), Gold-standard Fourier Shell Correlation (GSFSC) curves of the final density map (B), particle orientation distribution (C), and local resolution map (D) of the A/California/04-CIP100_RGCM_1868/2009 H1N1 NA/CAV-F34 complex. The final resolution of this complex is 2.5 Å. E, Representative density map of the HCDR3 of CAV-F34.

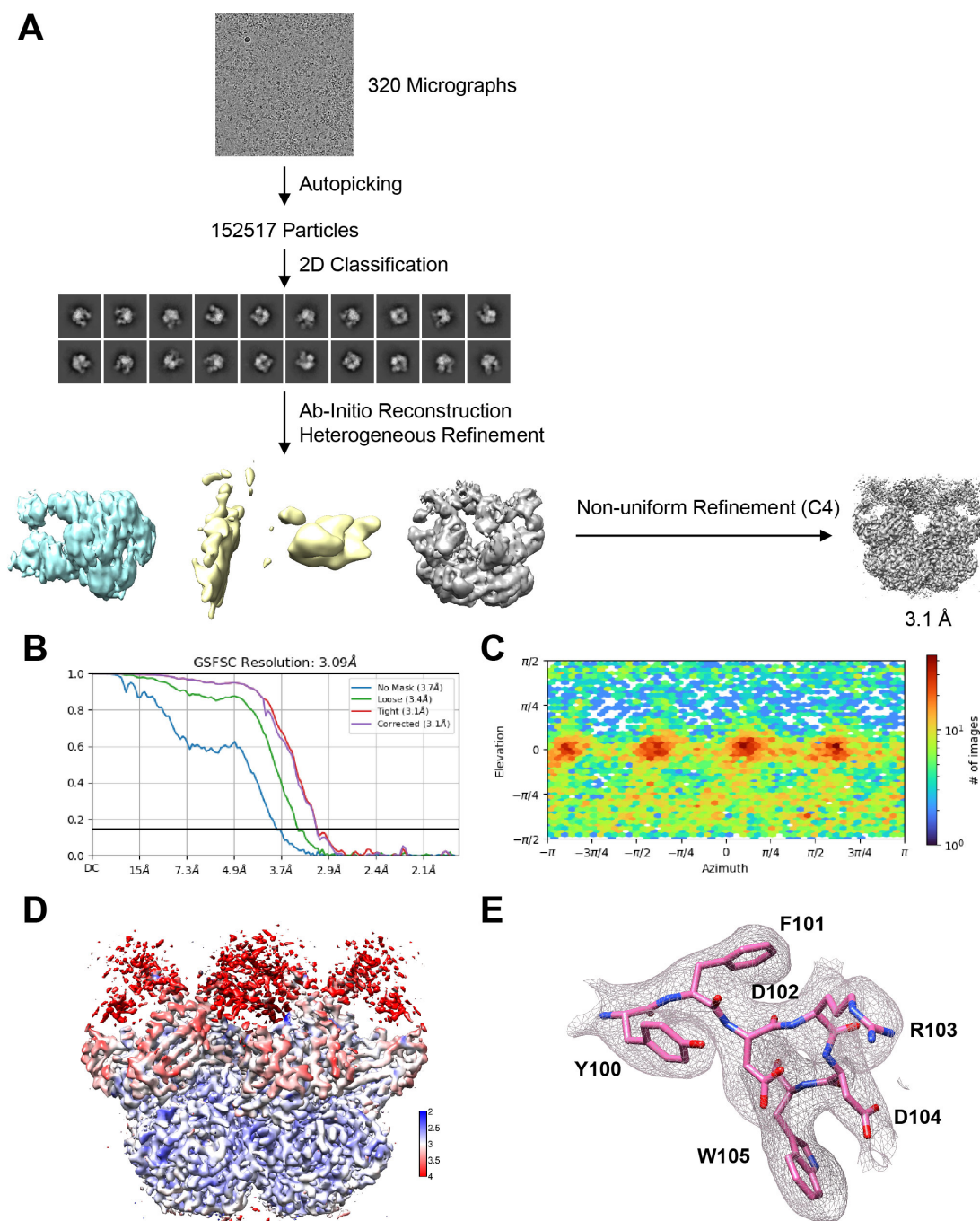


Supplementary Fig. S5 Cryo-EM structure validations of the A/Switzerland/9715293/2013 H3N2 NA/CAV-F34 complex. A-D, Data processing workflow (A), GSFSC curves of the final density map (B), particle orientation distribution (C), and local resolution map (D) of the

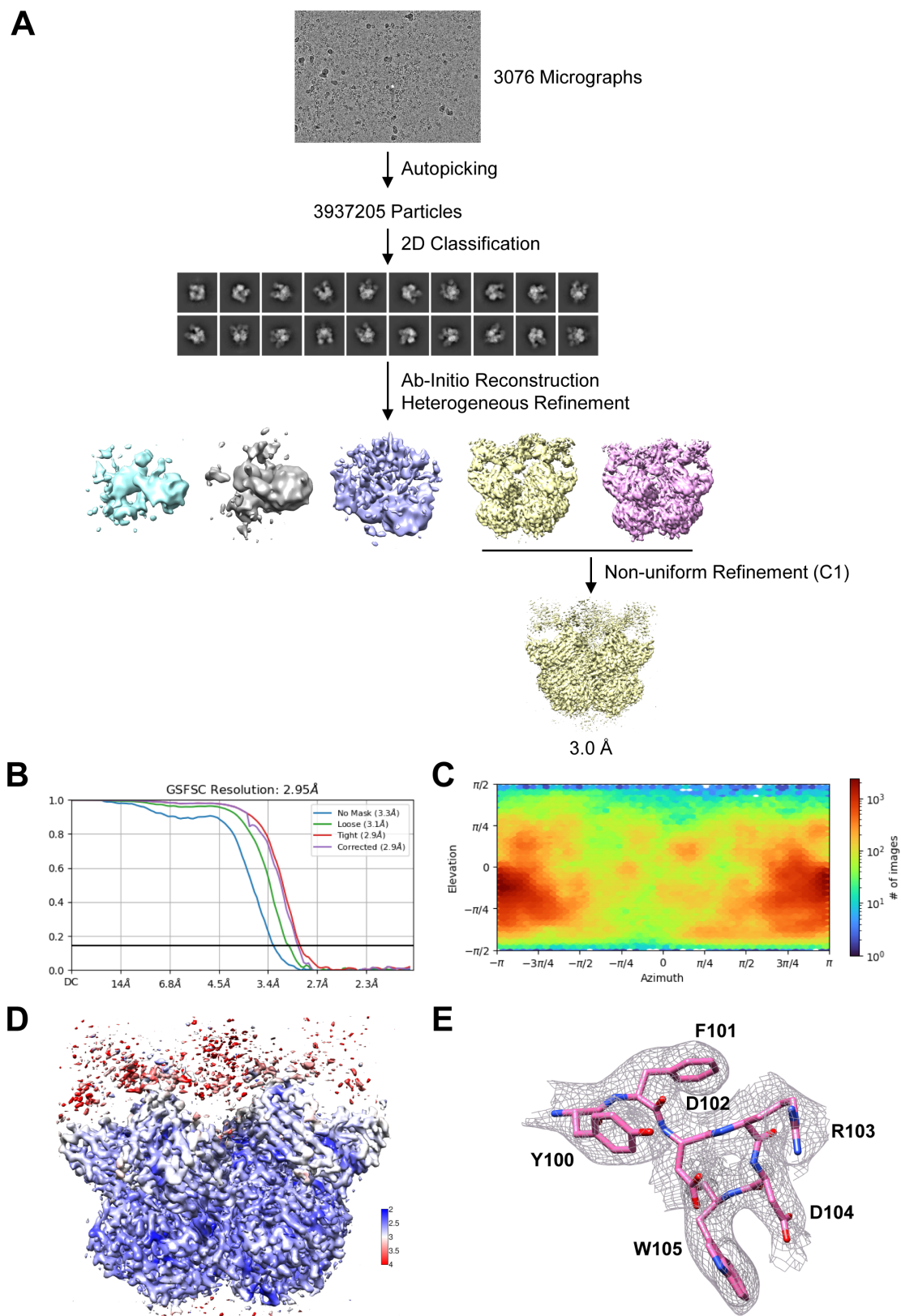
A/Switzerland/9715293/2013 H3N2 NA/CAV-F34 complex. The final resolution of this complex is 3.0 Å. **E**, Representative density map of the HCDR3 of CAV-F34.



Supplementary Fig. S6 Cryo-EM structure validations of the A/Switzerland/9715293/2013 H3N2 NA/CAV-F6 complex. A-D, Data processing workflow (A), GSFSC curves of the final density map (B), particle orientation distribution (C), and local resolution map (D) of the A/Switzerland/9715293/2013 H3N2 NA/CAV-F6 complex. The final resolution of this complex is 2.8 Å. E, Representative density map of the HCDR3 of CAV-F6.

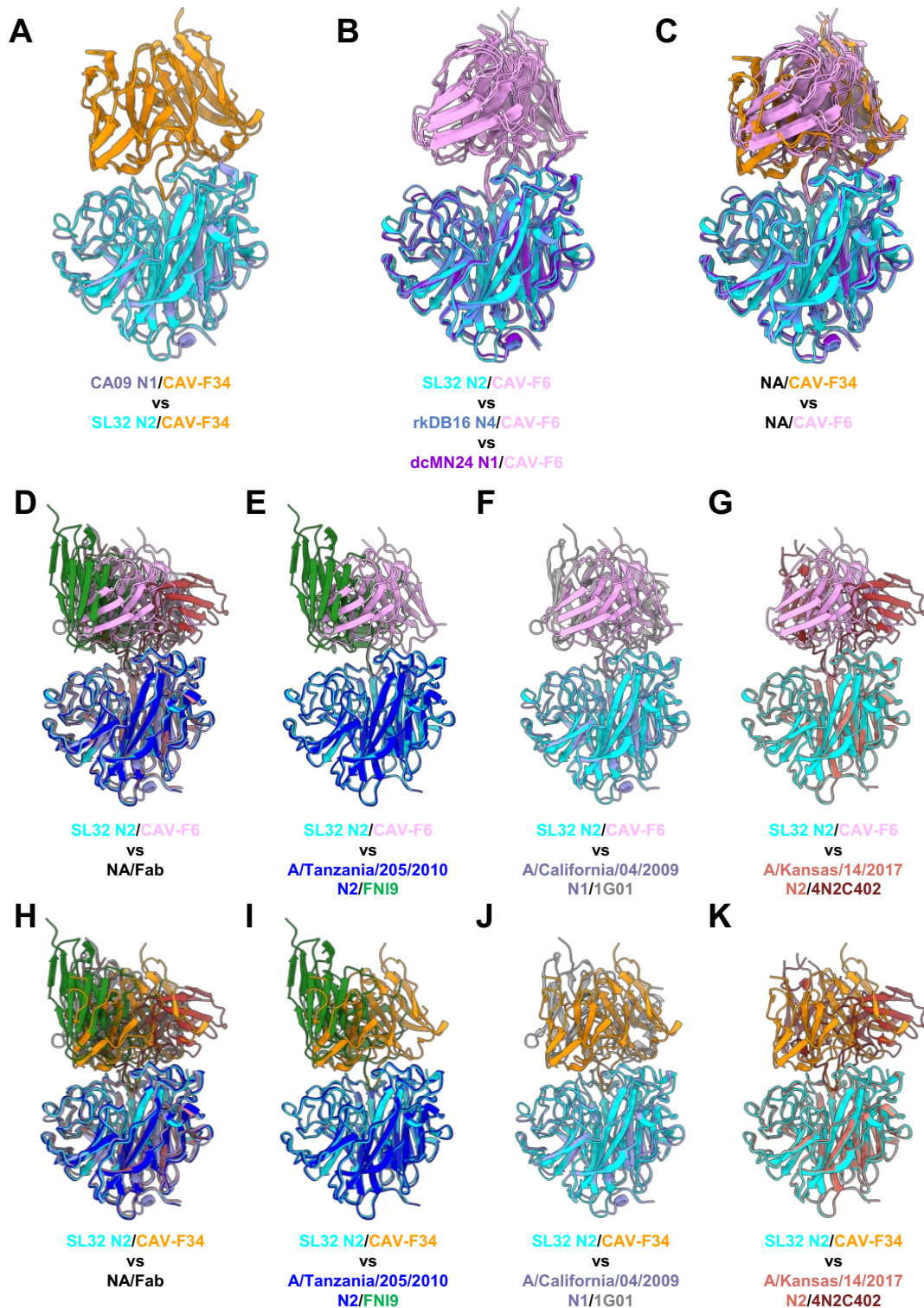


Supplementary Fig. S7 Cryo-EM structure validations of the A/Red knot/Delaware Bay/310/2016 H10N4 NA/CAV-F6 complex. A-D, Data processing workflow (**A**), GSFSC curves of the final density map (**B**), particle orientation distribution (**C**), and local resolution map (**D**) of the A/Red knot/Delaware Bay/310/2016 H10N4 NA/CAV-F6 complex. The final resolution of this complex is 3.1 Å. **E**, Representative density map of the HCDR3 of CAV-F6.



Supplementary Fig. S8 Cryo-EM structure validations of the A/dairy cow/Minnesota/24_016288-003/2024 H5N1 NA/CAV-F6 complex. A-D, Data processing workflow (A), GSFSC curves of the final density map (B), particle orientation distribution (C), and local resolution map (D) of the A/dairy cow/Minnesota/24_016288-003/2024 H5N1 NA/CAV-F6

complex. The final resolution of this complex is 3.0 Å. **E**, Representative density map of the HCDR3 of CAV-F6.

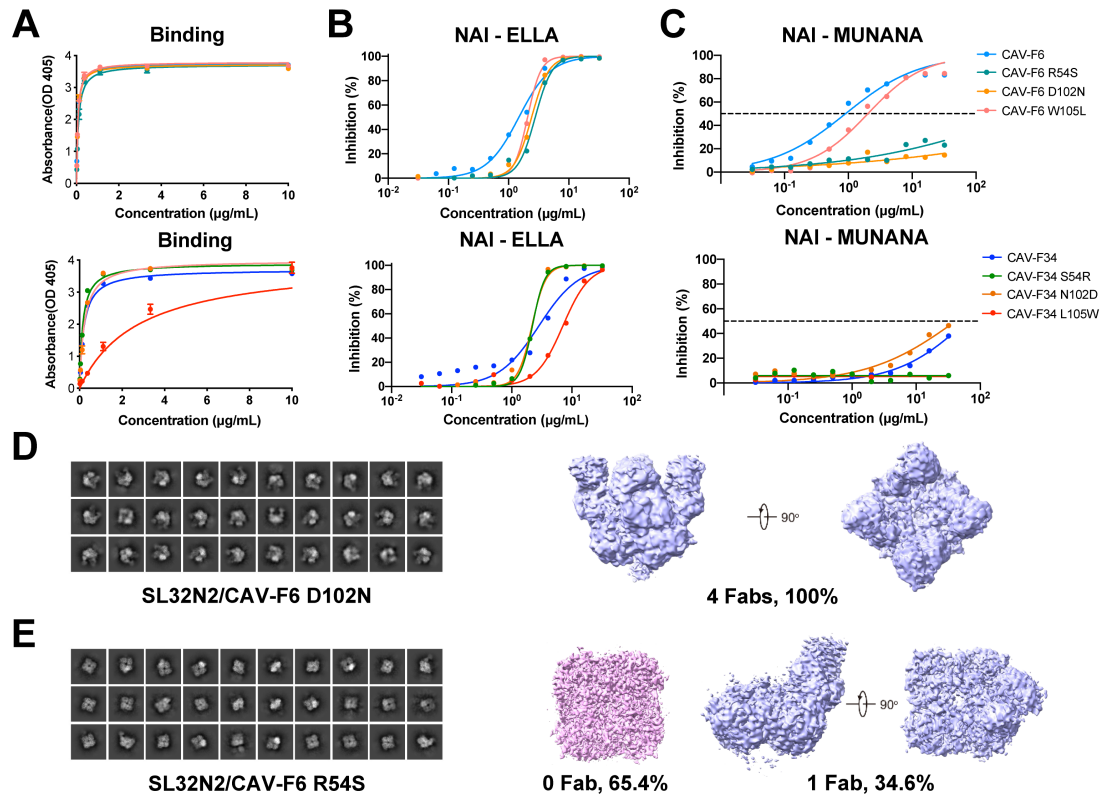


Supplementary Fig. S9 Structural alignment of NA-antibody complexes. A-C, Structures of CA09 N1 NA/CAV-F34, SL32 N2 NA/CAV-F34, SL32 N2 NA/CAV-F6, rkDB16 N4 NA/CAV-F6, and dcMN24 N1 NA/CAV-F6 complexes are superimposed to show a similar binding mode and nearly identical epitope. However, the approaching angles of CAV-F34 and CAV-F6 are slightly different. D-G, Structures of SL32 N2 NA/CAV-F6, A/Tanzania/205/2010 N2 NA/FNI9 (PDB ID:

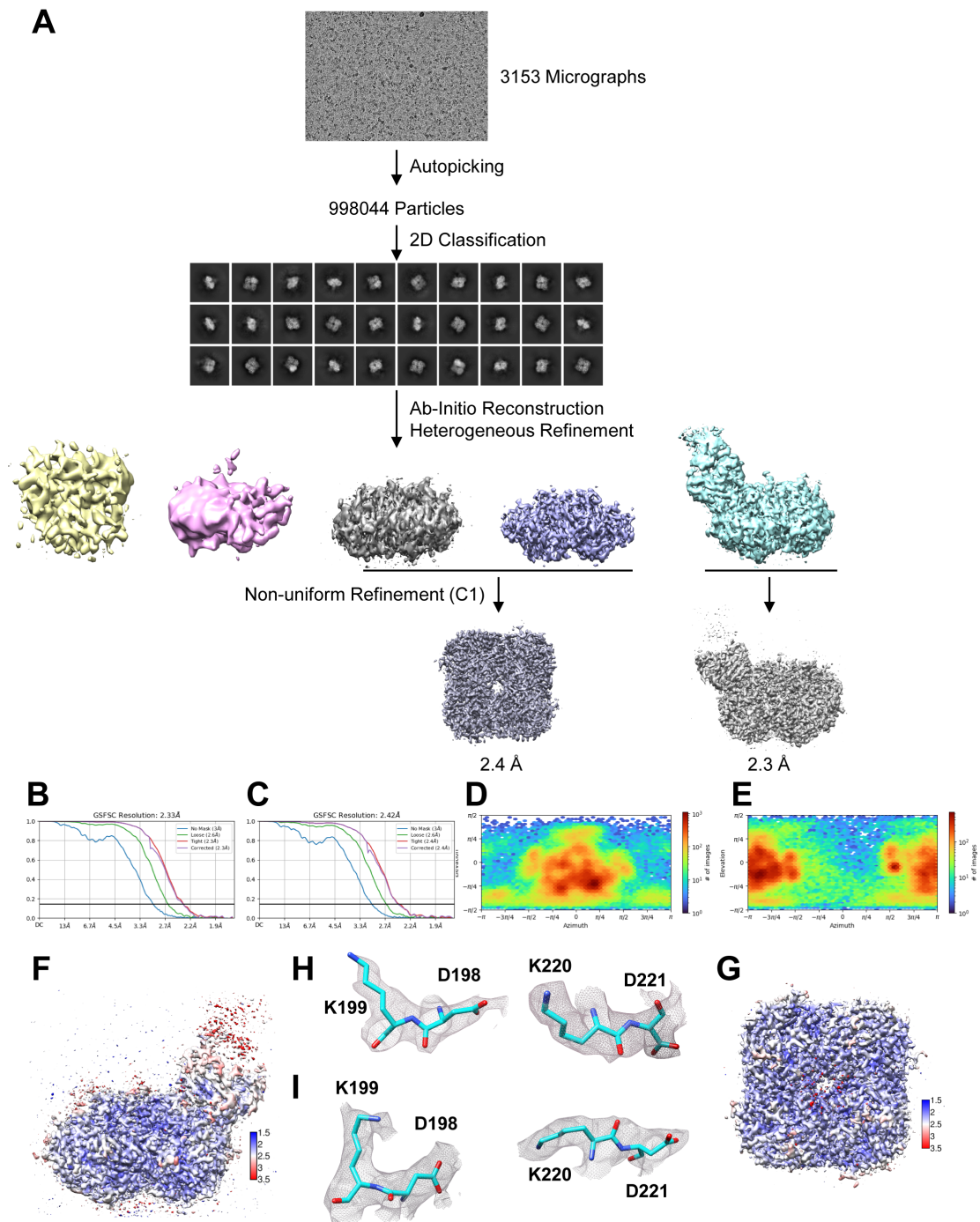
8G3N), A/California/04/2009 N1 NA/1G01 (PDB ID: 6Q23) and A/Kansas/14/2017 N2 NA/4N2C402 (PDB ID: 8ZR4) complexes are superimposed to show different approaching angles of antibodies. **H-K**, Structures of SL32 N2 NA/CAV-F34, A/Tanzania/205/2010 N2 NA/FNI9, A/California/04/2009 N1 NA/1G01 and A/Kansas/14/2017 N2 NA/4N2C402 complexes are superimposed to show different approaching angles of antibodies.

	1	10	20	30	40	50	60																	
CAV-F6-VH	EVQLVESGGGLVQPGGSLRLSCAASGFSFTTYEMNWRQAPGKGLEWVSHISSRGLVIYY																							
CAV-F34-VH	EVQLVESGGGLVEPGGSLRLSCAASGLTFSTYEMNWRQAPGKGLEWISHISSSGFAIYY																							
	CDR1										CDR2													
	70	80	90	100	110	120																		
CAV-F6-VH	ADSVKGRFTMSRDIAKNSLYLQMDSLTVA						DTAVYYCARHYFD						RDWGYSGMDLWGQGT						TVT					
CAV-F34-VH	ADSVRGRFTISRDIAKQSLDQMNSLRAE						DTAVYYCARDYFN						RDWGYSGMDVWGQGT						TVT					
	CDR3																							
CAV-F6-VH	VSS																							
CAV-F34-VH	VSS																							

Supplementary Fig. S10 Sequence alignment of CAV-F34 and CAV-F6 variable domains of heavy chains. CDRs of CAV-F34 and CAV-F6 Fabs are labeled.



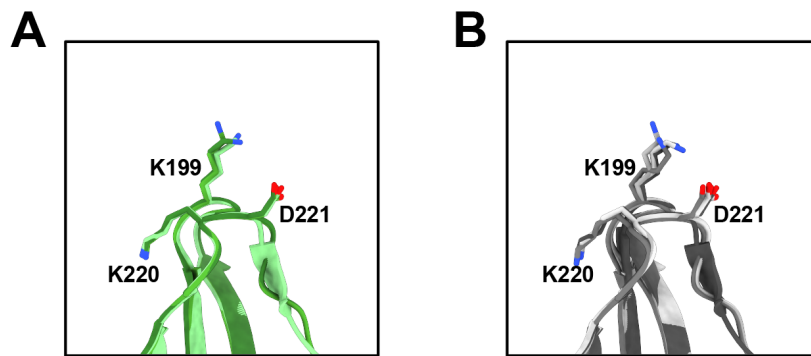
Supplementary Fig. S11 Single point mutation of Fabs leads to a different binding and NAI scenario. **A**, Binding ability of CAV-F6's single point mutant (above) and CAV-F34's single point mutant (below) against N2 protein (A/Switzerland/9715293/2013) via ELISA assay. **B**, The enzyme inhibitory ability of CAV-F6's single point mutant (above) and CAV-F34's single point mutant (below) against N2 protein (A/Switzerland/9715293/2013) via ELLA assay. **C**, The enzyme inhibitory ability of CAV-F6's single point mutant (above) and CAV-F34's single point mutant (below) against N2 protein (A/Switzerland/9715293/2013) via MUNANA assay. Data represents one of three independent experiments, shown as mean $\pm$ SD of three technical replicates. **D-E**, The 2D classification results and 3D models of the A/Switzerland/9715293/2013 (H3N2) NA/CAV-F6-R54S (**D**) and A/Switzerland/9715293/2013 (H3N2) NA/CAV-F6-D102N (**E**) complexes.



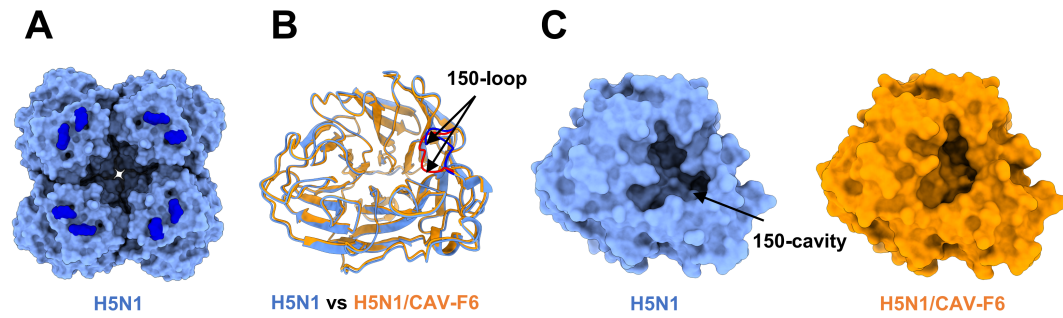
Supplementary Fig. S12 Cryo-EM structure validations of the A/Switzerland/9715293/2013 (H3N2) NA/CAV-F6-R54S complex and the A/Switzerland/9715293/2013 (H3N2) NA tetramer.

A, Data processing workflow of the A/Switzerland/9715293/2013 (H3N2) NA/CAV-F6-R54S complex and the A/Switzerland/9715293/2013 (H3N2) NA tetramer. **B-G**, GSFSC curves of the final density map (**B**), particle orientation distribution (**D**), and local resolution map (**F**) of the A/Switzerland/9715293/2013 (H3N2) NA/CAV-F6-R54S complex. The final resolution of this complex is 2.3 Å. GSFSC curves of the final density map (**C**), particle orientation distribution (**E**),

and local resolution map (**G**) of the A/Switzerland/9715293/2013 (H3N2) NA tetramer. The final resolution of this tetramer is 2.4 Å. **H**, Representative density map of the A/Switzerland/9715293/2013 (H3N2) NA/CAV-F6-R54S complex. **I**, Representative density map of the A/Switzerland/9715293/2013 (H3N2) NA tetramer.



Supplementary Fig. S13 Structural alignment of different protomers of A/Switzerland/9715293/2013 (H3N2) NA tetramer. A, Three unbounded protomers of the A/Switzerland/9715293/2013 (H3N2) NA/CAV-F6-R54S complex are superimposed to show a similar position of the K199, K220 and D221 side chains. **B,** Four unbounded protomers of the A/Switzerland/9715293/2013 (H3N2) NA tetramer are superimposed to show a similar position of the K199, K220 and D221 side chains.



Supplementary Fig. S14 Structural comparison between A/dairy cow/Minnesota/24_016288-003/2024 (H5N1) NA and A/dairy cow/Minnesota/24_016288-003/2024 H5N1 NA/CAV-F6. A, Overall structure of the A/dairy cow/Minnesota/24_016288-003/2024 (H5N1) NA tetramer (light blue). Glycans are shown in dark blue. **B,** The 150-loops in H5N1 (dark blue) and H5N1/CAV-F6 (red) adopt a different conformation. **C,** The apo H5N1 has the 150-cavity, not the H5N1/CAV-F6 complex.

Supplementary Table S1.

Variable region sequence of CAV-F6 and CAV-F34.

	Sequence
CAV-F6 VH	EVQLVESGGGLVQPGGSLRLSCAASGFSFTTYEMNWVRQAPGKGL EWVSHISSRGLVIYYADSVKGRFTMSRDTAKNSLYLQMDSLTVADT AVYYCARHYFDRDWGYSGMDLWGQGTTVTVSS
CAV-F6 VL	EVVLTQSPGTLSPGERATLSCRASQSLGTNYLAWYQHKPGQSPR LLIDGASTRAIGIPDRFSASGSGTDFTLTISRLEPEDFAVYYCQHYGN PYTFGQGTKLEIK
CAV-F34 VH	EVQLVESGGGLVEPGGSLRLSCAASGLTFSTYEMNWVRQAPGKGL EWISHISSSGFAIYYADSVRGRFTISRDIKQSLDLQMNSLRAEDTAV YYCARDYYNRDLGYSGMDVWGQGTTVTVSS
CAV-F34 VL	EIVLTQSPGTLSPGERATLSCRASQYISSMNLAWYQQKPGQAPRL LISAASSRATGIPDRFSGSGSGTDFTLTISGLEPEDFAVYYCQKYGSPY TFGQGTKVEIK

Supplementary Table S2.

List of the IC₅₀ values of enzyme activity inhibitory capacity for oseltamivir and antibodies related to Fig.1F-1I

		Enzyme activity inhibitory capacity IC ₅₀			Resistance level(fold change)		
		Oseltamivir	CAV-F6	CAV-F34	Oseltamivir	CAV-F6	CAV-F34
Recombination NA Protein	Mutations Conferring Resistance	IC ₅₀ (nM)	IC ₅₀ (μg/mL)	IC ₅₀ (μg/mL)	IC ₅₀	IC ₅₀	IC ₅₀
A/California/04/2009(H1N1)- N1	CA09-N1	0.881	0.892	3.033			
	CA09-N1-H274Y	>100	0.6493	3.117	>100	0.728	1.027
	CA09-N1-I222R	25.62	7.479	14.59	29	8.384	4.810
	CA09-N1-S246N	27.53	3.773	6.289	31	4.230	2.073
A/Switzerland/9715293/2013 (H3N2)-N2	SL32-N2	0.551	0.903	46.010			
	SL32-N2-N294S	>100	24.28	>50	>100	26.888	>100

Supplementary Table S3.

Cryo-EM data collection, refinement and validation statistics of NA-Fabs.

	A/California/04-CIP100_RGCM_1868/2009 H1N1 NA+CAV-F34 EMDB-39606 PDB-8YVL	A/Switzerland/9715293/2013 H3N2 NA+CAV-F34 EMDB-39608 PDB-8YVM	A/Switzerland/9715293/2013 H3N2 NA+CAV-F6 EMDB-39609 PDB-8YVN	A/Red knot/Delaware Bay/310/2016 H10N4 NA+CAV-F6 EMDB-39605 PDB-8YVK	A/dairy cow/Minnesota/24_016288-003/2024 H5N1 NA+CAV-F6 EMDB-62473 PDB-9KOD
Data collection and processing					
Magnification	81,000×	81,000×	81,000×	45,000×	29,000×
Voltage (kV)	300	300	300	200	200
Electron exposure (e ⁻ /Å ²)	50	50	50	50	50
Defocus range (μm)	1.3-1.8	1.3-1.8	1.3-1.8	1.3-1.8	1.3-1.8
Pixel size (Å)	1.0742	1.0742	1.0742	0.94	0.97
Symmetry imposed	C4	C1	C4	C4	C1
Initial particle images (no.)	1,611,425	4,228,701	639,868	152,517	3,937,205
Final particle images (no.)	242,813	113,270	46,554	17,312	545,144
Map resolution (Å)	2.47	3.02	2.80	3.09	2.95
FSC threshold	0.143	0.143	0.143	0.143	0.143
Map resolution range (Å)	2.47-5	3.02-5	2.80-5	3.09-5	2.95-5
Refinement					
Initial model used (PDB code)	none	none	none	none	none
Model resolution (Å)	2.47	3.02	2.80	3.09	2.95
FSC threshold	0.143	0.143	0.143	0.143	0.143
Model resolution range (Å)	2.47-5	3.02-5	2.80-5	3.09-5	2.95-5
Map sharpening <i>B</i> factor (Å ²)	100.4	99.0	93.4	105.0	136.7
Model composition					
Non-hydrogen atoms	19161	14579	19880	19164	19148
Protein residues	2468	1778	2468	2476	2468
Ligands	21	72	68	8	16
<i>B</i> factors (Å ²)					
Protein	28.80	40.07	43.04	62.76	39.81
Ligand	30.00	30.04	35.84	30.00	52.79
R.m.s. deviations					
Bond lengths (Å)	0.007	0.010	0.010	0.007	0.009
Bond angles (°)	0.899	1.541	1.301	0.998	1.137
Validation					
MolProbity score	1.27	1.89	1.80	1.62	1.73
Clashscore	3.64	8.74	7.92	4.25	7.55
Poor rotamers (%)	0.53	0.20	0.43	0.38	0.10
Ramachandran plot					
Favored (%)	97.38	93.54	94.68	93.80	95.42
Allowed (%)	2.62	6.12	5.03	6.12	4.54
Disallowed (%)	0.0	0.34	0.29	0.08	0.04

Supplementary Table S4.

Cryo-EM data collection, refinement and validation statistics of NA and CAV-F6-R54S Fab.

	A/Switzerland/9715293/2013 H3N2 NA+CAV-F6-R54S	A/Switzerland/9715293/2013 H3N2 NA	A/dairy cow/Minnesota/24_016288- 003/2024 H5N1 NA
	EMDB-60582	EMDB-60644	EMDB-62805
	PDB-8ZYY	PDB-9IJV	PDB-9L44
Data collection and processing			
Magnification	81,000×	81,000×	45,000×
Voltage (kV)	300	300	200
Electron exposure (e ⁻ /Å ²)	50	50	50
Defocus range (μm)	1.3-1.8	1.3-1.8	1.3-1.8
Pixel size (Å)	0.856	0.856	0.94
Symmetry imposed	C1	C1	C4
Initial particle images (no.)	998,044	998,044	395,348
Final particle images (no.)	170,595	154,601	43,159
Map resolution (Å)	2.33	2.42	3.02
FSC threshold	0.143	0.143	0.143
Map resolution range (Å)	2.33-5	2.42-5	3.02-5
Refinement			
Initial model used (PDB code)	8YVN	8YVN	none
Model resolution (Å)	2.33	2.42	3.02
FSC threshold	0.143	0.143	0.143
Model resolution range (Å)	2.33-5	2.42-5	3.02-5
Map sharpening <i>B</i> factor (Å ²)	76.9	80.0	
Model composition			
Non-hydrogen atoms	14,508	12,740	12,124
Protein residues	1,778	1,548	1,548
Ligands	64	64	20
<i>B</i> factors (Å ²)			
Protein	40.05	36.08	36.49
Ligand	70.44	66.29	30.00
R.m.s. deviations			
Bond lengths (Å)	0.005	0.007	0.011
Bond angles (°)	0.906	1.459	1.348
Validation			
MolProbity score	1.63	1.66	1.47
Clashscore	7.97	7.35	2.51
Poor rotamers (%)	0.46	0.30	0.15
Ramachandran plot			
Favored (%)	96.77	96.23	93.38
Allowed (%)	3.17	3.64	6.62
Disallowed (%)	0.06	0.13	0.0

Supplementary Table S5.

Overall comparison of SL32 N2 NA/CAV-F6, SL32 N2 NA/CAV-F34, A/Tanzania/205/2010 N2 NA/FN19, A/California/04/2009 N1 NA/1G01, and A/Kansas/14/2017 N2 NA/4N2C402 complexes.

Complex		SL32 N2+ CAV-F6	SL32 N2+ CAV-F34	A/Tanzania/205/ 2010 N2+FN19	A/California/04/ 2009 N1+1G01	A/Kansas/14/2017 N2+4N2C402
Interface area (Å ²)	total	1167.1	1173.2	1128.6	1025.9	960.3
	heavy chain	878.3	735.4	839.5	780.7	594.0
	light chain	288.8	437.8	289.1	245.2	366.3

Supplementary Table S6.

The germline genes of antibodies.

Antibody		CAV-F6	CAV-F34	FNI9	1G01	4N2C402
Germline	heavy chain	IGHV3-48	IGHV3-48	IGHV1-69	IGHV3-20	IGHV3-30
	light chain	IGKV3-20	IGKV3-20	IGKV3-15	IGKV1-9	IGKV2-28

Supplementary Table S7.**A full list of viruses used in this study.**

Subtype	Strain
H1N1	A/California/04/2009
H3N2	A/Guizhou/54/1989
H7N9	A/Anhui/1/2013 NIBRG-268 (HA/NA: H7N9; Backbone: A/Puerto Rico/8/1934)
H5N1	A/dairy cattle/Texas/24-008749-003-original/2024 (DCP-HA/NA: H5N1; Backbone: A/California/04/2009-DelNS1)
B/Victoria	A/Colorado/06/2017
B/Yamagata	MA-A/Florida/4/2006

*DCP: deletion of polybasic cleavage site.