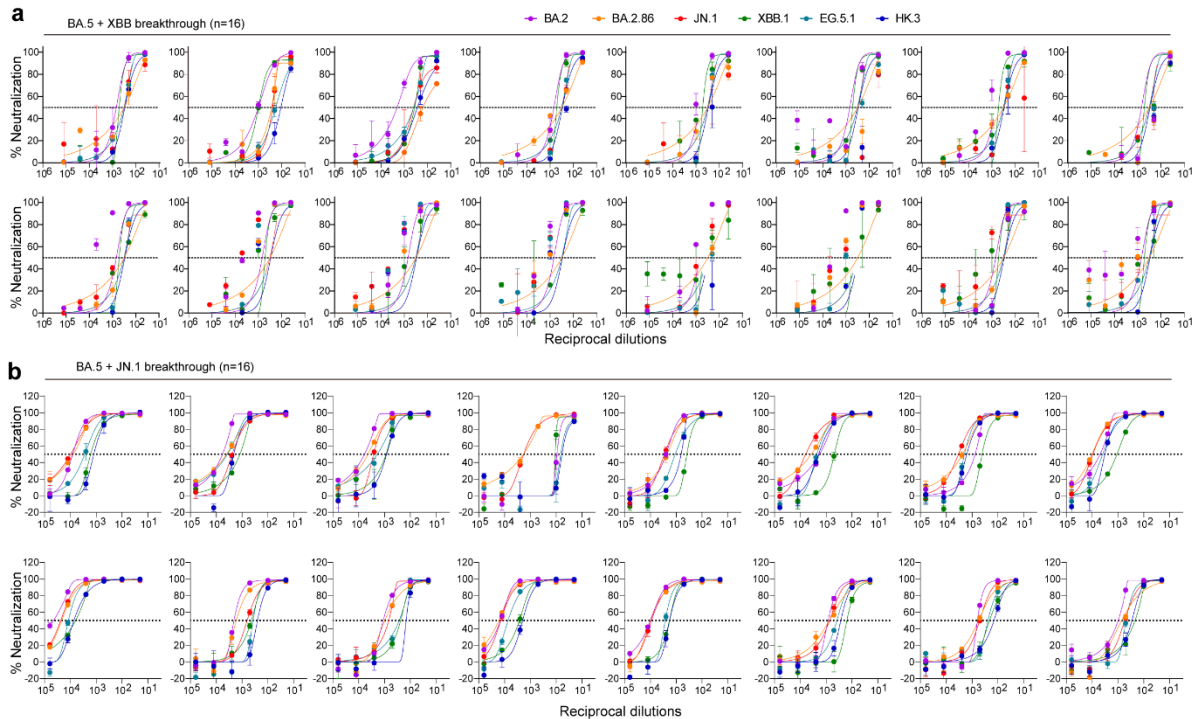**SUPPLEMENTARY INFORMATION**

Lineage-specific pathogenicity, immune evasion, and virological features of SARS-CoV-2 BA.2.86/JN.1 and EG.5.1/HK.3

Yuanchen Liu[1,*], Xiaoyu Zhao[2,3,*], Jialu Shi[1,*], Yajie Wang[4,*], Huan Liu[1,*], Ye-Fan Hu[5,*], Bingjie Hu[1], Huiping Shuai[1], Terrence Tsz-Tai Yuen[1,6], Yue Chai[1], Feifei Liu[1], Hua-Rui Gong[7], Jiayan Li[3], Xun Wang[3], Shujun Jiang[8], Xiang Zhang[4], Yanliang Zhang[8], Xiangnan Li[9], Lei Wang[1], Madeline Hartnoll[1], Tianrenzheng Zhu[1,6], Yuxin Hou[1,6], Xiner Huang[1,6], Chaemin Yoon[1], Yang Wang[1], Yixin He[1], Minmin Zhou[1], Lianzhao Du[1], Xiaojuan Zhang[1], Wan-Mui Chan[1], Lin-Lei Chen[1], Jian-Piao Cai[1], Shuofeng Yuan[1,6,10], Jie Zhou[1,6], Jian-Dong Huang[11,12], Kwok-Yung Yuen[1,6,10,13,14,15], Kelvin Kai-Wang To[1,6,10,14,15], Jasper Fuk-Woo Chan[1,6,10,13,14,15,#], Bao-Zhong Zhang[7,#], Lei Sun[4,#], Pengfei Wang[3,#], Hin Chu[1,6,10,12,16,#]

Supplementary Figure 1. Non-spike mutations of the evaluated Omicron subvariants.

Summary of the non-spike mutations of SARS-CoV-2 lineages BA.2, BA.2.86, JN.1, XBB.1, EG.5.1, and HK.3 compared to the ancestral SARS-CoV-2 spike protein. Color represents the proportion of each mutation in each variant.
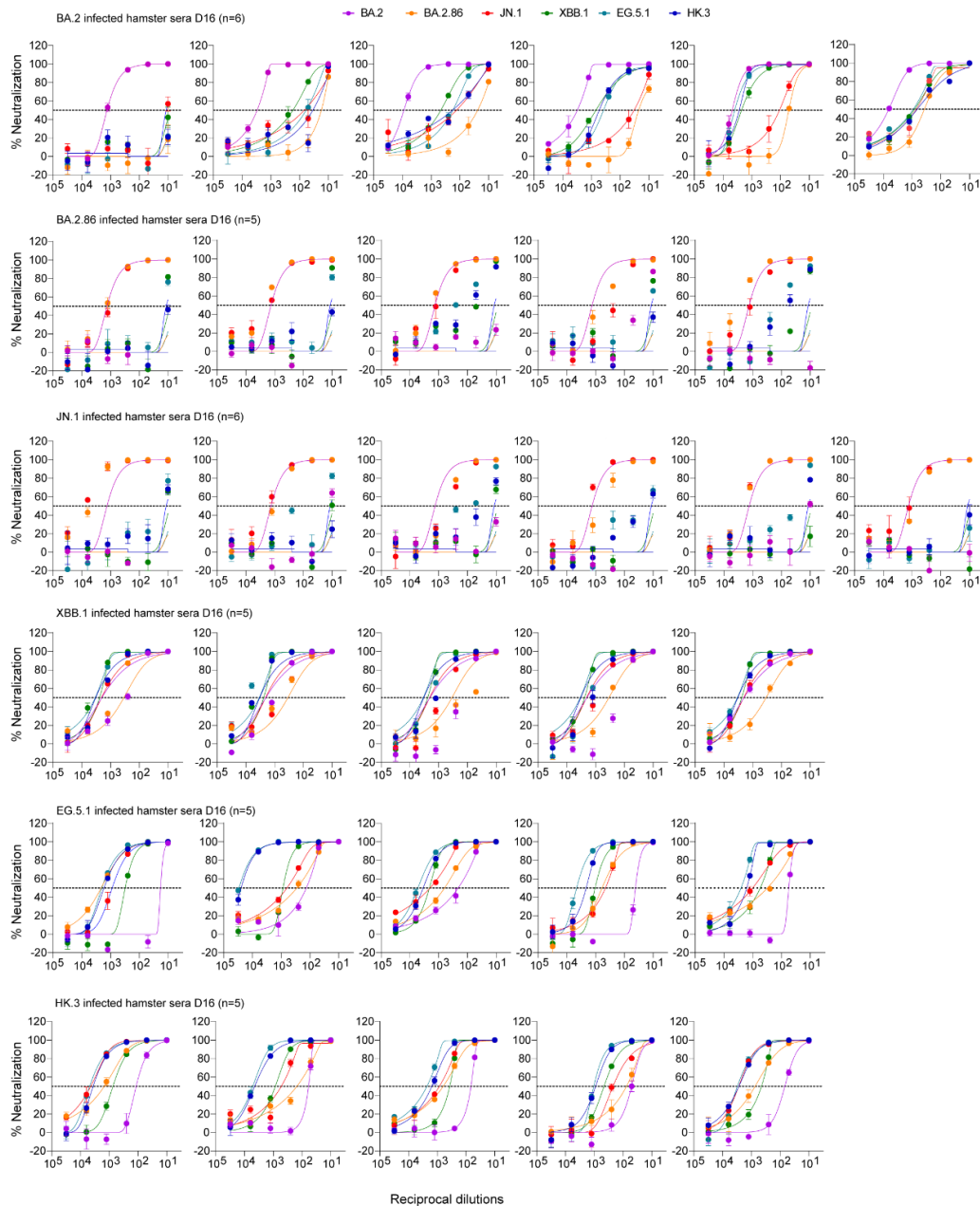
Supplementary Figure 2. Neutralization curves of human sera against BA.2, BA.2.86, JN.1, XBB.1, EG.5.1, and HK.3 pseudoviruses.

a Neutralization curves for sera collected from individuals received two-to-three doses of inactivated vaccines before experiencing a XBB breakthrough infection (n=16) against the indicated Omicron-spike pseudoviruses.
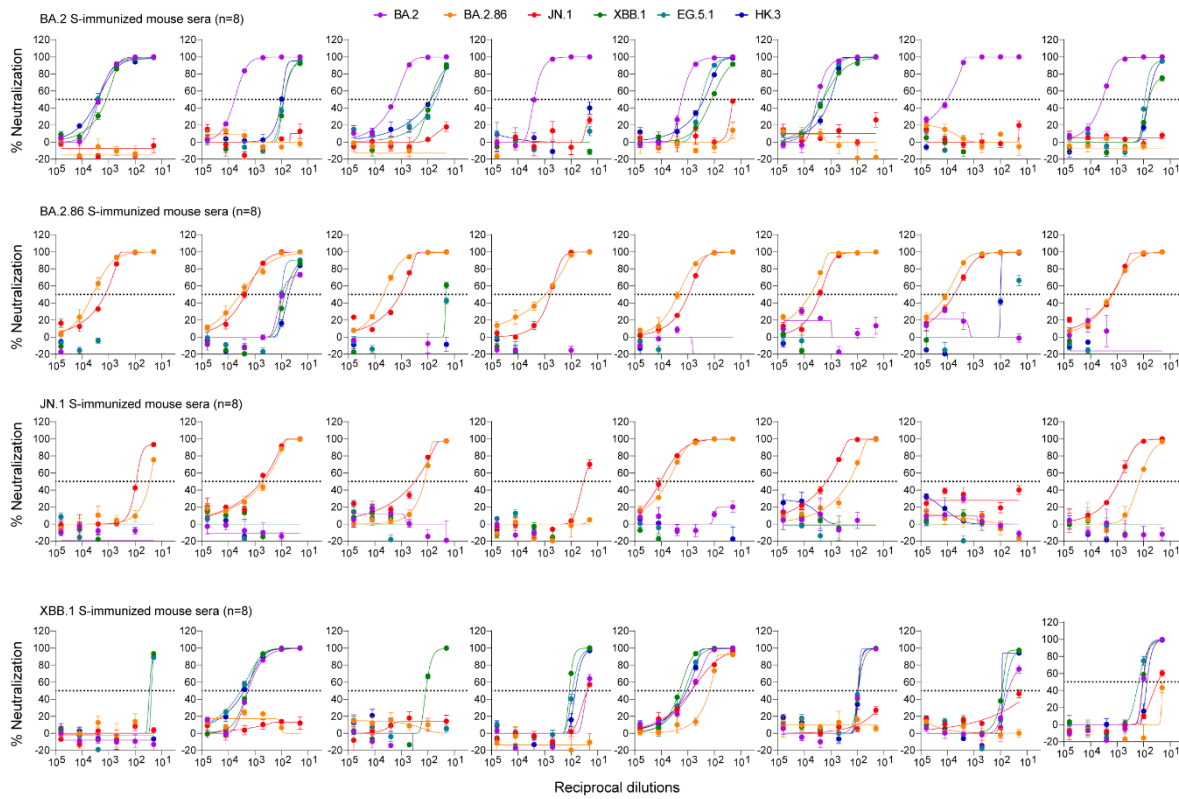
b Neutralization curves for sera collected from individuals received three doses of inactivated vaccines before experiencing a JN.1 breakthrough infection (n=16) against the indicated Omicron-spike pseudoviruses.

The data are presented as mean ± SEM.

Supplementary Figure 3. Neutralization curves of infected hamster sera against BA.2, BA.2.86, JN.1, XBB.1, EG.5.1, and HK.3 pseudoviruses.

Neutralization curves for sera collected from hamsters infected with Omicron subvariants, including BA.2 (n=6), BA.2.86 (n=5), JN.1 (n=6), XBB.1 (n=5), EG.5.1 (n=5), and HK.3 (n=5), against the indicated Omicron-spike pseudoviruses. The data are presented as the mean ± SEM.

Supplementary Figure 4. Neutralization curves of vaccinated mouse sera against BA.2, BA.2.86, JN.1, XBB.1, EG.5.1, and HK.3 pseudoviruses.

Neutralization curves for sera collected from mice immunized with the indicated spike trimer, including BA.2 (n=8), BA.2.86 (n=8), JN.1 (n=8), and XBB.1(n=8), against the indicated Omicron-spike pseudoviruses. The data are presented as the mean ± SEM.
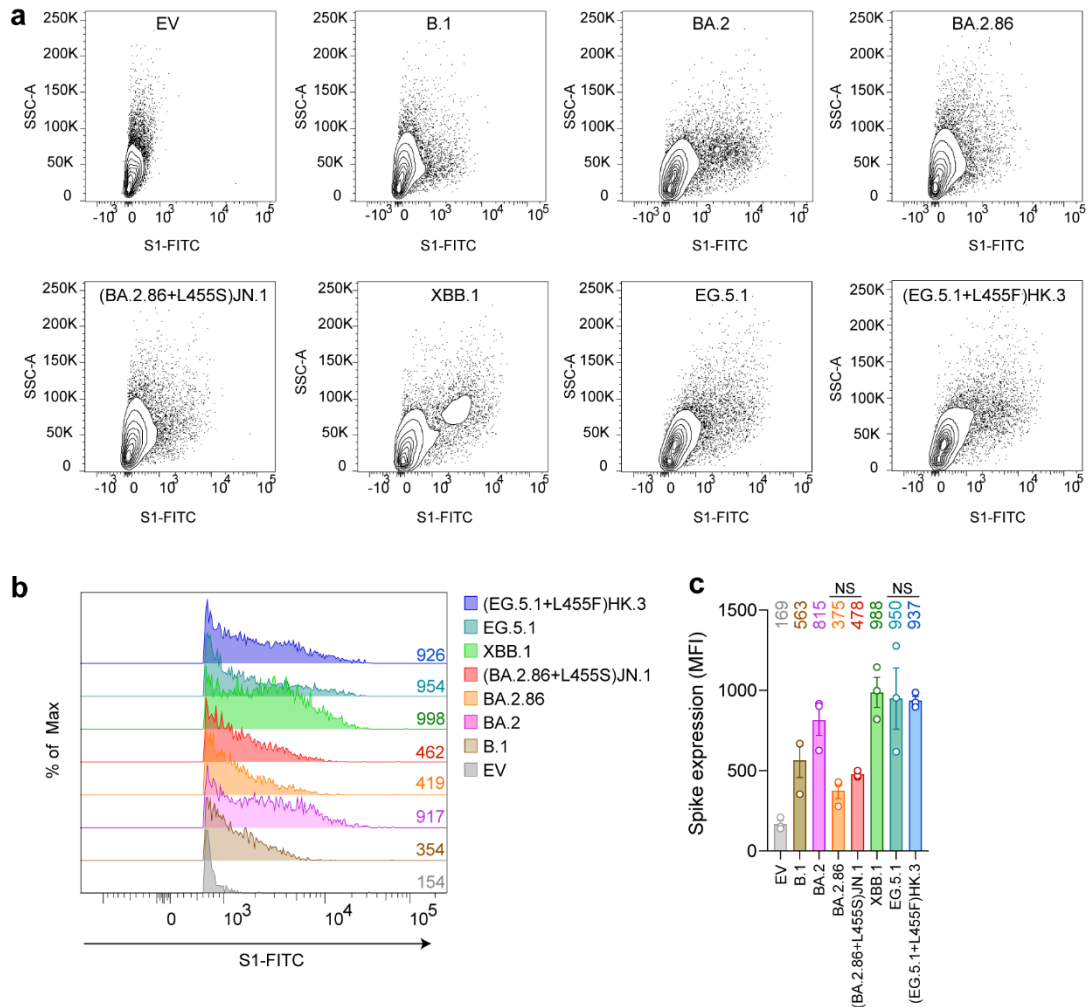
Supplementary Figure 5. Surface expression of B.1, BA.2, BA.2.86, JN.1, XBB.1, EG.5.1, and HK.3 spike protein on 293T cells.
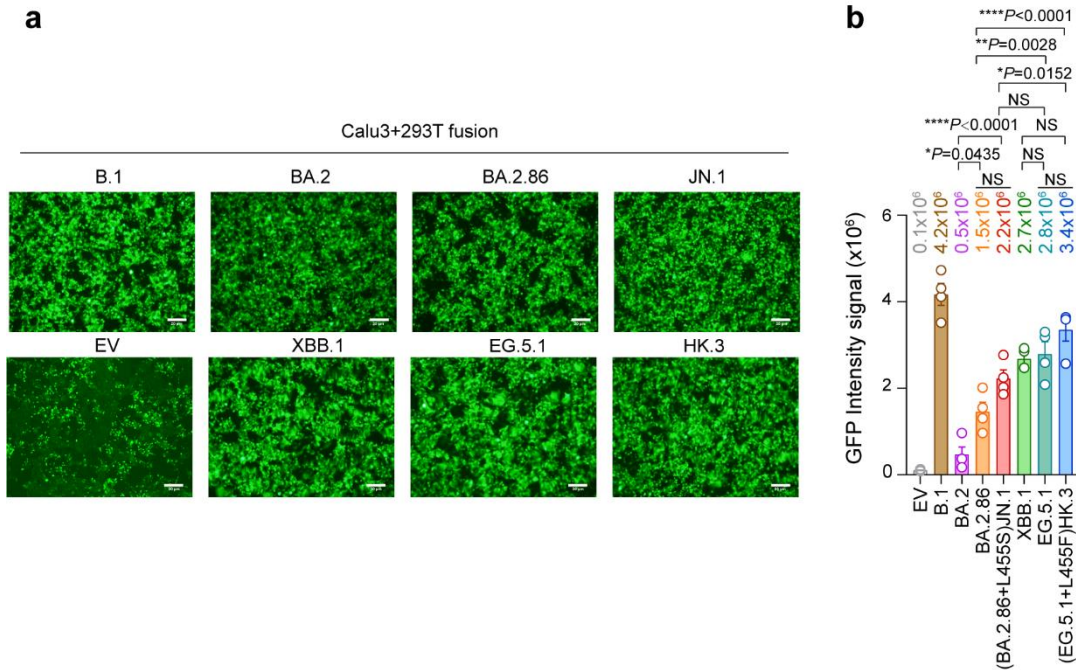
293T cells were transfected with B.1, BA.2, BA.2.86, JN.1, XBB.1, EG.5.1, and HK.3 spike expression plasmids. Surface expression of spike protein was evaluated at day 2 post transfection with flow cytometry.

a Representative contour plots of the spike plasmid-transfected 293T cells.

b Representative histograms of the spike plasmid-transfected 293T cells.

c The summary plot of the S1 mean fluorescence intensity (MFI) of three biological replicates (n =3). Data represent mean ± SEM. Statistical significance in **c** was determined with one-way ANOVA. NS, not statistically significant.

Supplementary Figure 6. B.1, BA.2, BA.2.86, JN.1, XBB.1, EG.5.1, and HK.3 spike protein mediated cell-cell fusion in Calu3 cells.

293T cells were co-transfected with the indicated spike-expressing plasmids and a GFP-expressing plasmid before co-cultured with Calu3 cells. The cells were fixed at 24 h after co-culture. GFP signal intensity was quantified by ImageJ.
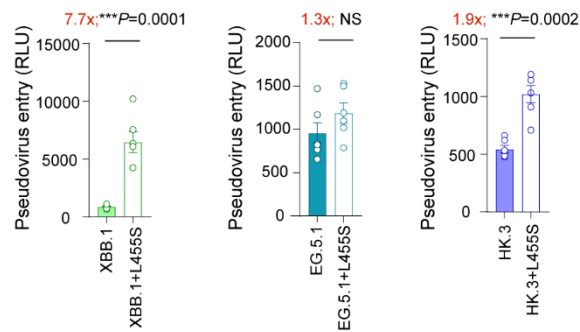
a Representative image for each variant.

b Summary of the quantified GFP intensity of four biological replicates (n=4).

Data represent mean ± SEM. Statistical significance was determined with one-way ANOVA. *p < 0.05, **p < 0.01, ****p < 0.0001. NS, not statistically significant. Scale bar = 20 μm.
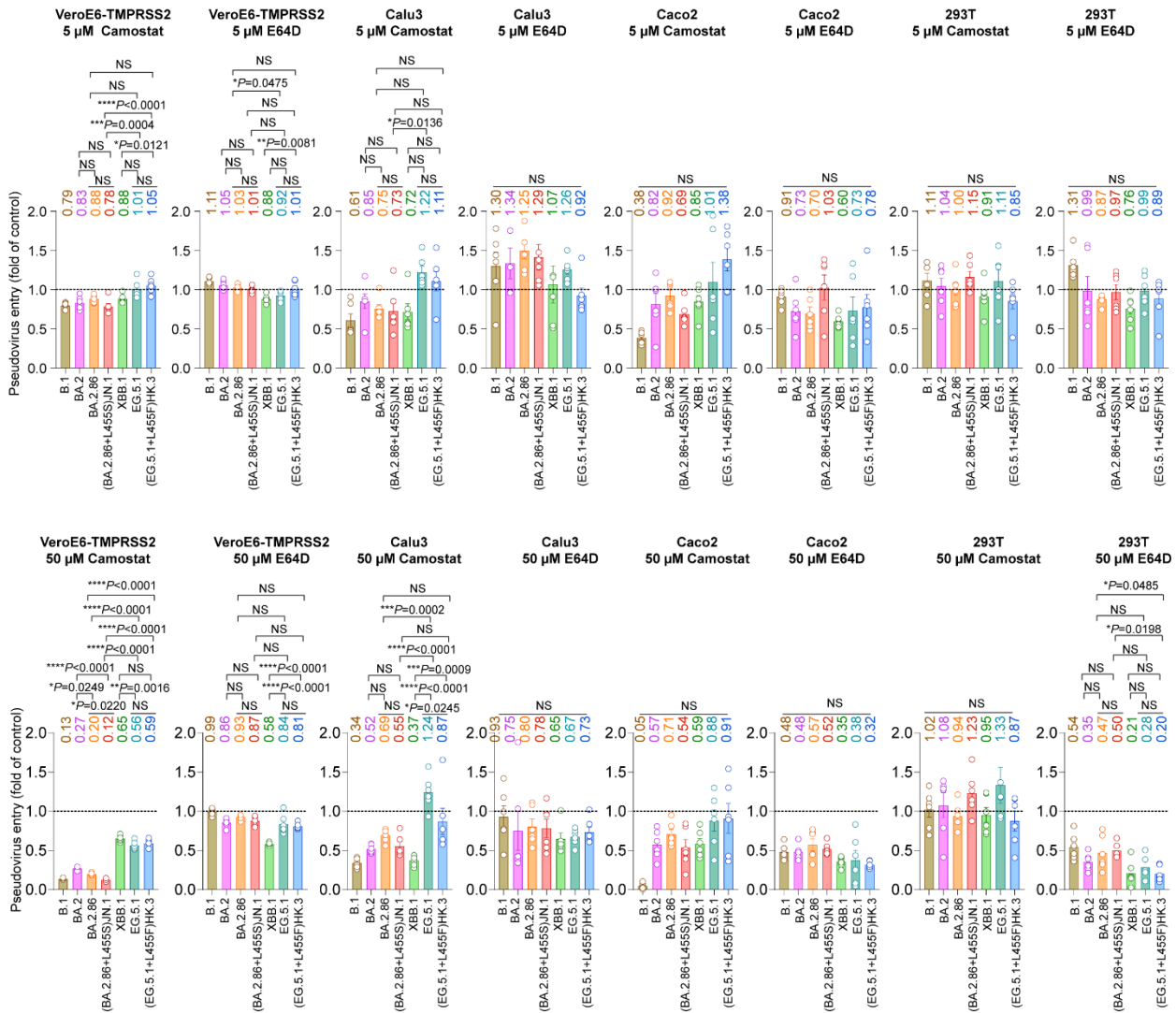
Supplementary Figure 7. Protease usage by pseudoviruses in cell lines.

Protease usage by pseudoviruses in cell lines. VeroE6-TMPRSS2, Calu3, Caco2, and 293T cells were pre-treated with 5 μM and 50 μM Camostat or E64D for 2 h followed by transduction with B.1-, BA.2-, BA.2.86-, JN.1-, XBB.1-, EG.5.1-, and HK.3-spike pseudoviruses. At 24 h post-transduction, the level of pseudovirus entry was quantified by measuring the luciferase signal. The fold change was normalized to the mean luciferase readout of cells treated with DMSO for each variant (n=6). The n number represents biological replicates. Data represent mean ± SEM. Statistical significance was determined with one-way ANOVA. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. NS, not statistically significant.

Supplementary Figure 8. Pseudovirus entry of XBB.1, EG.5.1, and HK.3 with the L455S mutation in hNECs.

The hNECs were transduced with XBB.1-, EG.5.1-, HK.3-, XBB.1+L455S-, EG.5.1+L455S-, or HK.3+L455S pseudoviruses. At 24 h post-transduction, the level of pseudovirus entry was quantified by measuring the luciferase signal (n=6). The n number represents biological replicates. Data represent mean ± SEM. Statistical significance was determined with two-tailed Student's t-tests. ***p < 0.001. NS, not statistically significant.
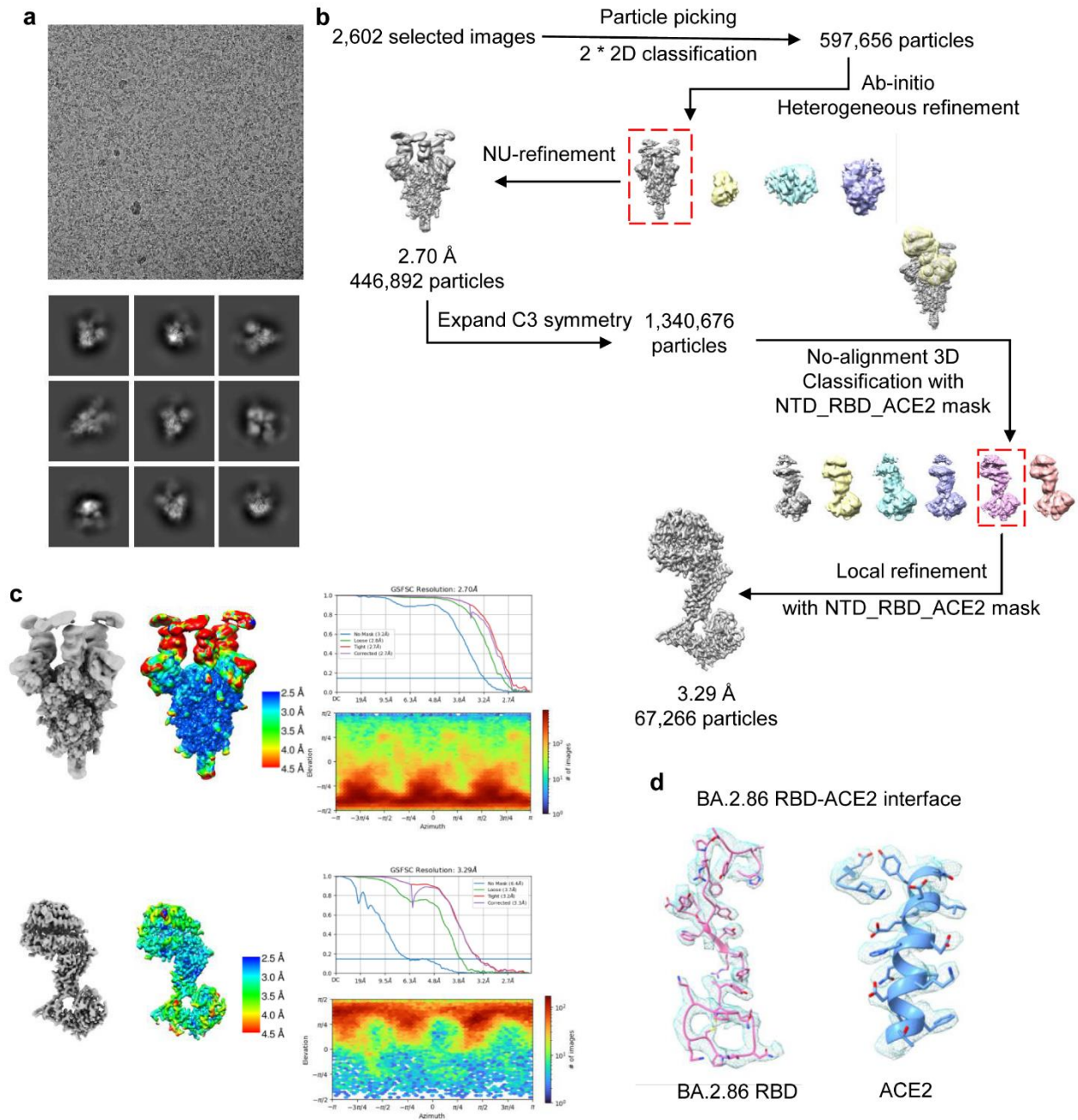
Supplementary Figure 9. Cryo-EM data processing of the BA.2.86 spike in complex with ACE2.

a Representative motion-corrected micrograph and representative reference-free 2D class averages generated in cryoSPARC.

b Data processing flowchart of BA.2.86 spike-ACE2 complex.

c Local resolution of the cryo-EM maps and their Golden Standard Fourier Shell Correlation (GSFSC) curve, and the angular distribution of the particles used in the final reconstruction.

d Electron density maps for secondary structure elements in the BA.2.86 RBD-ACE2 interface. Cryo-EM data processing of the BA.2.86 spike in complex with ACE2.

Supplementary Figure 10. Cryo-EM data processing of the JN.1 spike in complex with ACE2.

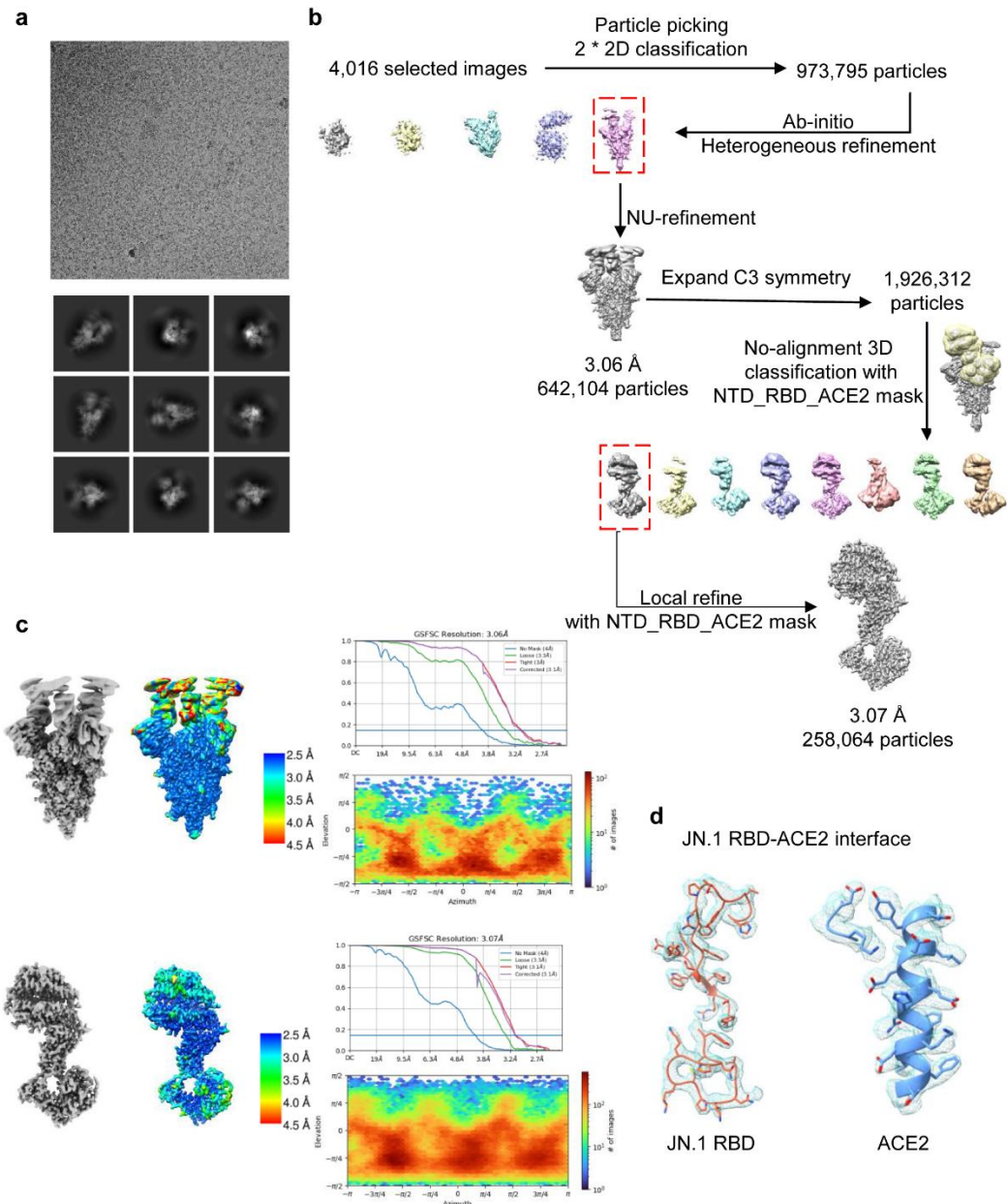**a** Representative motion-corrected micrograph and representative reference-free 2D class averages generated in cryoSPARC.

b Data processing flowchart of JN.1 spike-ACE2 complex.

c Local resolution of the cryo-EM maps and their GSFSC curve, and the angular distribution of the particles used in the final reconstruction.

d Electron density maps for secondary structure elements in the JN.1 RBD-ACE2 interface.
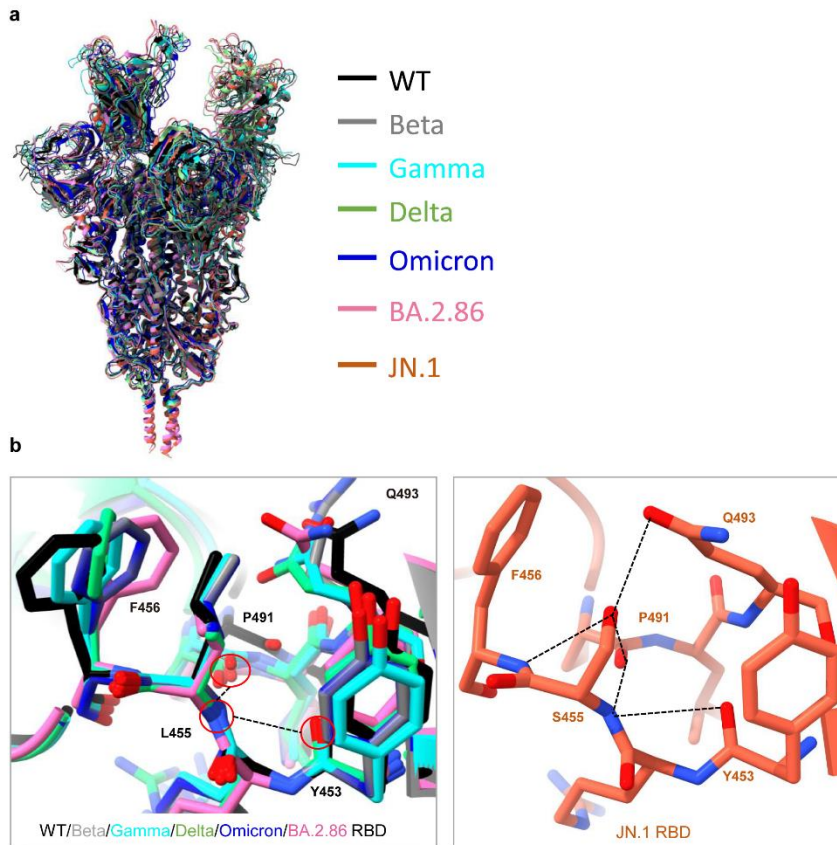
Supplementary Figure 11. Structural comparation of variants at spike residue 455.

a Structural comparation of various variants, including WT S (PDB:7A98), Beta S (PDB:7V7Z), Gamma S (PDB:7V82), Delta S (PDB:7V89) and Omicron S (PDB:7T9K).

b Detailed interactions in local regions of RBD$^{455L/S}$.
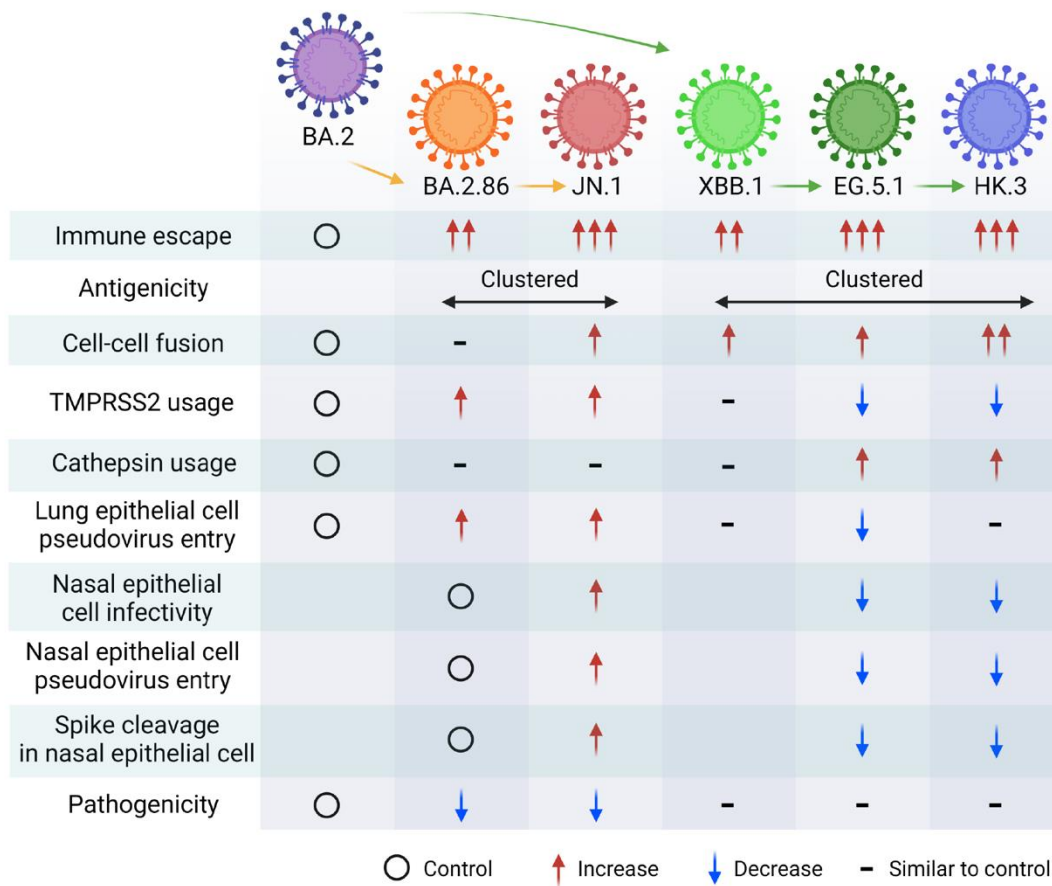
Supplementary Figure 12. Schematic of findings of the study. This Figure was created with BioRender.com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license.
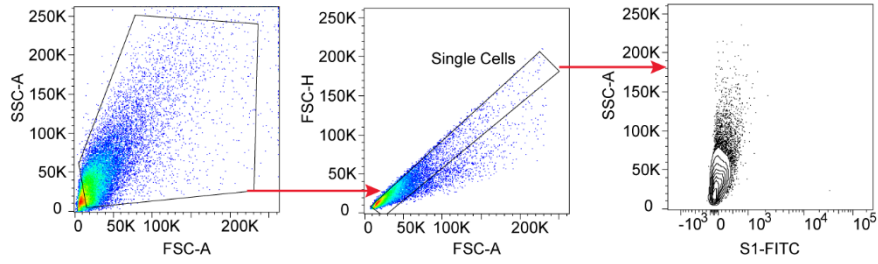
Supplementary Figure 13. Gating strategy for the flow cytometry panels presented in supplementary Figure 5.

The main 293T cell population was gated with SSC-A vs FSC-A. The selected cells were gated with FSC-H vs FSC-A for single cells. From that, spike expression positive cells were gated with SSC-A vs S1-FITC. 293T cells transfected with EV and staining with S1-antibody were used as the control for gating.
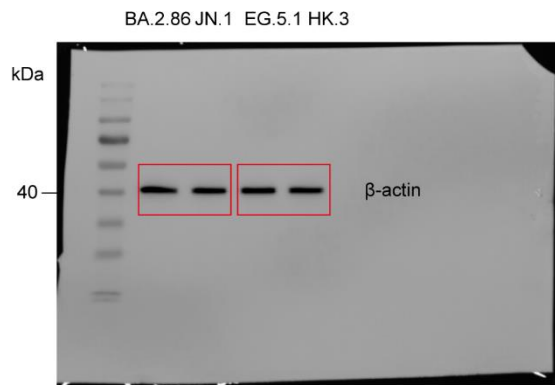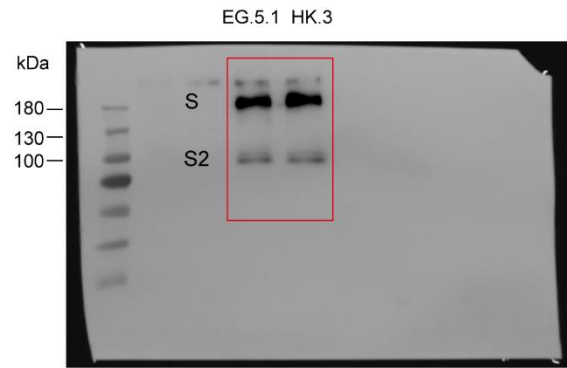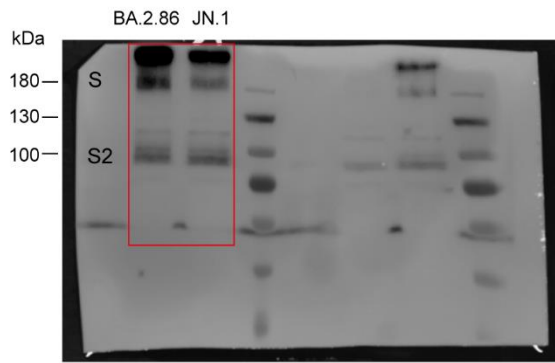
Red box indicates how the gels were cut

Supplementary Figure 14. Uncropped Western blot images.

Supplementary Table 1. Baseline characteristics of enrolled participants.

	BA.5+XBB individuals (n=16)	BA.5+JN.1 individuals (n=16)
Age (years), median (range)	33.62 (18-69)	37.13 (23-60)
Male, n (%)	7 (43.75%)	8 (50.00%)
BMI (kg/m$^{2)}$), mean (SD)	21.89 (2.45)	22.99 (2.54)
Breakthrough infections days after the last Coronavirus vaccines, median (range)	498.5 (159-793)	651.5 (415-965)
Number of Coronavirus vaccines doses	2-3	3
Days after the last Coronavirus vaccines, median (range)	436.75 (174-607)	652.12 (416-965)
Coronavirus infection times	2	2
Comorbidities (%)	0 (0%)	0 (0%)
Any, n (%)	0 (0%)	0 (0%)
HTN, n (%)	1 (6.25%)	1 (6.25%)
CAD, n (%)	0 (0%)	0 (0%)
DM, n (%)	0 (0%)	0 (0%)
NAFLD, n (%)	0 (0%)	0 (0%)
Hyperlipidemia, n (%)	0 (0%)	0 (0%)
Obesity (%)	0 (0%)	0 (0%)
Arrhy, n (%)	0 (0%)	0 (0%)
Asthma, n (%)	0 (1%)	0 (1%)
Rhinitis, n (%)	0 (2%)	0 (2%)
Urticaria, n (%)	0 (3%)	0 (3%)

BMI, body mass index. CAD, coronary artery disease. HTN, hypertension. DM, diabetes mellitus. Arrhy, arrhythmia, NAFLD, non-alcoholic fatty liver.

Supplementary Table 2. Pseudovirus entry efficiency in cell lines.

(1) VeroE6-TMPRSS2   Luminescence (RLU)

Pseudo viruses	VSV-G	EV	B.1	BA.2	BA.2.86	JN.1	XBB.1	EG.5.1	HK.3
A	1.30E+08	1.71E+03	5.67E+07	1.35E+07	1.14E+07	1.99E+07	1.85E+07	5.30E+06	5.80E+06
B	1.49E+08	2.76E+03	5.30E+07	1.21E+07	1.05E+07	1.77E+07	1.88E+07	4.74E+06	5.42E+06
C	1.48E+08	2.87E+03	5.63E+07	1.15E+07	1.08E+07	1.74E+07	1.81E+07	4.51E+06	4.97E+06
D	1.49E+08	2.91E+03	5.50E+07	1.20E+07	1.08E+07	1.74E+07	1.67E+07	5.23E+06	5.57E+06
E	1.54E+08	2.86E+03	5.25E+07	1.18E+07	1.03E+07	1.75E+07	1.61E+07	4.74E+06	5.50E+06
F	1.44E+08	2.80E+03	5.27E+07	1.11E+07	9.89E+06	1.78E+07	1.64E+07	4.82E+06	5.14E+06
G	1.42E+08	1.85E+03		1.24E+07	1.09E+07	1.80E+07	1.82E+07	5.30E+06	5.53E+06

* Relative Luminescence Units (RLU)

(2) VeroE6   Luminescence (RLU)

Pseudo viruses	VSV-G	EV	B.1	BA.2	BA.2.86	JN.1	XBB.1	EG.5.1	HK.3
A	5.90E+07	3.26E+04	3.23E+06	2.33E+06	2.24E+06	6.55E+06	5.66E+06	2.98E+06	4.76E+06
B	6.50E+07	4.01E+04	3.40E+06	2.11E+06	1.66E+06	5.29E+06	3.79E+06	2.94E+06	3.93E+06
C	6.44E+07	4.02E+04	3.18E+06	2.15E+06	1.73E+06	5.65E+06	3.76E+06	2.62E+06	3.78E+06
D	6.72E+07	4.44E+04	3.85E+06	1.94E+06	1.63E+06	5.24E+06	3.88E+06	2.68E+06	3.45E+06
E	6.84E+07	4.43E+04	3.79E+06	1.95E+06	1.44E+06	5.45E+06	3.83E+06	3.12E+06	3.92E+06
F	6.44E+07	4.82E+04	4.01E+06	2.09E+06	1.48E+06	5.31E+06	4.10E+06	3.01E+06	4.06E+06
G	6.36E+07	4.33E+04		2.05E+06	1.77E+06	5.84E+06	4.43E+06	3.06E+06	4.62E+06

(3) Calu3 Firefly Luminescence (RLU)

Pseudo viruses	VSV-G	EV	B.1	BA.2	BA.2.86	JN.1	XBB.1	EG.5.1	HK.3
A	5.70E+06	2.37E+02	3.97E+04	2.11E+04	1.86E+04	1.52E+04	8.57E+03	3.12E+03	5.78E+03
B	5.12E+06	2.90E+02	2.24E+04	1.47E+04	1.48E+04	3.09E+04	1.66E+04	2.36E+03	3.11E+04
C	5.65E+06	2.90E+02	2.16E+04	2.31E+04	2.43E+04	2.30E+04	1.71E+04	4.03E+03	2.51E+04
D	5.73E+06	3.60E+02	2.15E+04	2.53E+04	3.50E+04	3.62E+04	1.84E+04	4.96E+03	2.17E+04
E	5.39E+06	3.92E+02	1.62E+04	1.41E+04	2.24E+04	1.91E+04	5.63E+03	1.86E+03	1.39E+04
F	4.96E+06	5.21E+02	3.98E+04	1.75E+04	3.18E+04	3.26E+04	9.32E+03	3.54E+03	1.18E+04
G	5.54E+06	4.70E+02		1.88E+04	1.02E+04	1.34E+04	1.19E+04	6.46E+03	1.44E+04

(4) Caco2   Luminescence (RLU)

Pseudo viruses	VSV-G	EV	B.1	BA.2	BA.2.86	JN.1	XBB.1	EG.5.1	HK.3
A	9.09E+07	5.87E+03	5.35E+05	3.23E+05	3.05E+05	9.06E+05	2.07E+05	1.24E+05	1.05E+05
B	8.85E+07	1.49E+04	7.18E+05	4.13E+05	4.24E+05	5.93E+05	2.07E+05	8.48E+04	1.31E+05
C	9.12E+07	1.27E+04	8.70E+05	2.32E+05	2.56E+05	4.65E+05	1.93E+05	5.04E+04	1.15E+05
D	8.84E+07	1.09E+04	6.10E+05	4.15E+05	3.70E+05	6.36E+05	3.38E+05	7.50E+04	7.98E+04
E	8.94E+07	9.71E+03	7.18E+05	4.92E+05	3.89E+05	6.10E+05	1.47E+05	7.06E+04	3.11E+04
F	9.09E+07	7.36E+03	9.28E+05	3.00E+05	4.28E+05	6.84E+05	4.36E+05	6.19E+04	1.35E+05
G	8.86E+07	1.16E+04		3.40E+05	5.71E+05	5.66E+05	3.23E+05	6.85E+04	1.07E+05

(5) A549   Luminescence (RLU)

Pseudo viruses	VSV-G	EV	B.1	BA.2	BA.2.86	JN.1	XBB.1	EG.5.1	HK.3
A	8.56E+06	1.47E+03	4.74E+04	3.10E+04	4.44E+04	2.72E+04	2.03E+04	5.39E+03	1.81E+04
B	9.59E+06	1.44E+03	6.33E+04	4.14E+04	2.94E+04	3.29E+04	1.47E+04	4.35E+03	2.15E+04
C	9.12E+06	1.59E+03	4.94E+04	5.96E+04	4.29E+04	3.10E+04	2.26E+04	6.97E+03	1.31E+04
D	9.57E+06	1.47E+03	5.54E+04	3.78E+04	3.53E+04	3.83E+04	8.67E+03	5.13E+03	1.49E+04
E	1.01E+07	1.75E+03	4.92E+04	4.95E+04	3.84E+04	3.20E+04	2.94E+04	4.04E+03	9.14E+03
F	1.01E+07	1.52E+03	5.34E+04	4.16E+04	6.04E+04	2.41E+04	1.09E+04	5.22E+03	1.95E+04
G	9.56E+06	1.15E+03		5.49E+04	2.96E+04	3.76E+04	1.28E+04	1.16E+04	9.80E+03

(6) Huh7   Luminescence (RLU)

Pseudo viruses	VSV-G	EV	B.1	BA.2	BA.2.86	JN.1	XBB.1	EG.5.1	HK.3
A	1.02E+08	1.95E+03	2.27E+05	8.67E+05	5.91E+05	7.38E+05	6.05E+05	3.48E+04	2.91E+05
B	1.01E+08	2.25E+03	1.54E+05	9.32E+05	5.96E+05	6.64E+05	3.21E+05	1.29E+04	1.67E+05
C	1.05E+08	2.22E+03	1.08E+05	3.29E+05	6.54E+05	7.48E+05	1.63E+05	1.90E+04	2.48E+05
D	1.09E+08	2.38E+03	6.38E+05	6.15E+05	8.54E+05	9.00E+05	3.61E+05	9.49E+04	9.26E+04
E	1.09E+08	2.47E+03	2.01E+05	7.47E+05	4.82E+05	3.72E+05	2.51E+05	9.59E+04	1.60E+05
F	1.10E+08	2.01E+03	1.69E+05	5.20E+05	6.97E+05	6.46E+05	4.19E+05	9.51E+04	1.23E+05
G	1.11E+08	2.51E+03		7.64E+05	6.11E+05	4.88E+05	5.19E+05	1.64E+05	4.54E+05

Pseudo viruses	VSV-G	EV	B.1	BA.2	BA.2.86	JN.1	XBB.1	EG.5.1	HK.3
				(7)293T	Luminescence (RLU)				
A	9.44E+07	1.35E+03	1.58E+05	3.47E+05	1.88E+05	2.31E+05	2.83E+05	1.70E+04	1.13E+05
B	9.75E+07	1.32E+03	9.00E+04	4.55E+05	1.61E+05	4.55E+05	2.82E+05	4.59E+04	1.35E+05
C	9.76E+07	1.76E+03	1.36E+05	3.36E+05	2.17E+05	4.27E+05	1.47E+05	4.58E+04	4.99E+04
D	9.83E+07	1.10E+03	4.69E+04	3.26E+05	1.61E+05	4.06E+05	2.56E+05	4.37E+03	3.88E+04
E	9.67E+07	1.68E+03	9.97E+04	4.67E+05	3.25E+05	5.07E+05	2.50E+05	2.69E+04	5.88E+04
F	9.59E+07	2.53E+03	1.04E+05	3.65E+05	1.34E+05	3.90E+05	1.95E+05	3.77E+04	1.20E+05
G	9.36E+07	2.02E+03		2.95E+05	2.36E+05	3.58E+05	1.59E+05	2.78E+04	1.10E+05

Supplementary Table 3. Cryo-EM data collection and refinement statistics.

	JN.1-spike-ACE2		BA.2.86-Spike-ACE2	
	Spike trimer -ACE2	RBD-ACE2	Spike trimer -ACE2	RBD-ACE2
Data collection and processing				
Magnification	105,000		105,000	
Voltage (kV)	300		300	
Total dose (e–/Å^2)	50		50	
Defocus range (μm)	-1.0 to -3.0		-1.0 to -3.0	
Pixel size (Å)	1.19		1.19	
Symmetry imposed	C1		C1	
Final particles (no.)	642,104	258,064	446,892	67,266
Map Resolution (Å)	3.06	3.07	2.70	3.29
R.m.s. deviations				
Bond lengths (Å)	0.002	0.001	0.002	0.002
Bond angles (°)	0.544	0.377	0.565	0.454
Validation				
MolProbity score	1.38	0.95	1.38	1.13
Clash score	3.21	1.83	3.00	2.07
Rotamer outlier (%)	1.95	0.86	2.23	1.58
Ramachandran plot				
Favored (%)	97.77	97.97	97.91	98.22
Allowed (%)	2.23	2.03	2.09	1.78
Disallowed (%)	0	0	0	0
EMDB	39691	39690	39689	39688
PDB	8YZE	8YZD	8YZC	8YZB

Supplementary Table 4. Sequences of Primers.

Species	Gene	Primer/Probe	Sequence 5'-3'
SARS-CoV-2	E	E_sgRNA_Forward	CGATCTCTTGTAGATCTGTTCTC
		E_sgRNA_Reverse	ATATTGCAGCAGTACGCACACA
		E_sgRNA_Probe	FAM-ACACTAGCCATCCTTACTGCG CTTCG-BBQ

Species	Gene	Primer/Probe	Sequence 5'-3'
Mice	GAPDH	(F) 5' CGACTTCAACAGCAACTCCCACTCTTCC -3'	
		(R) 5'TGGGTGGTCCAGGGTTTCTTACTCCTT -3'	

Species	Gene	Primer/Probe	Sequence 5'-3'
Hamster	β-actin	(F) 5'-ATGGCCAGGTCATCACCATTG -3'	
		(R) 5'-CAGGAAGGAAGGCTGGAAAAG -3'	