Supporting Information for

Infection- or AZD1222 vaccine-mediated immunity reduces SARS-CoV-2 transmission but increases Omicron competitiveness in hamsters

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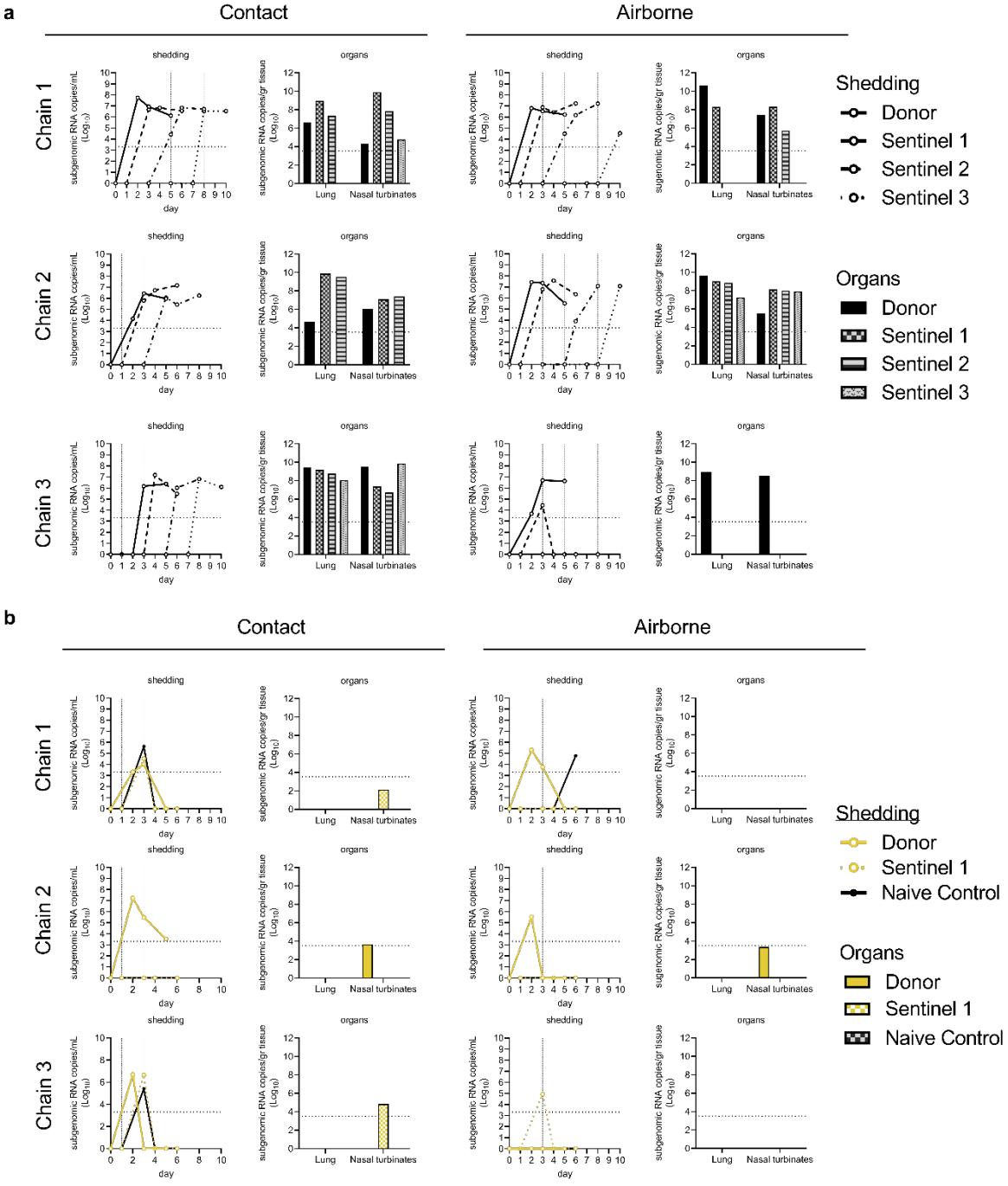
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Contact
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subgenomic RNA copies/mL (Log10)
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Shedding
Donor
Sentinel 1
Sentinel 2
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Organs
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subgenomic RNA copies/gr tissue (Log10)
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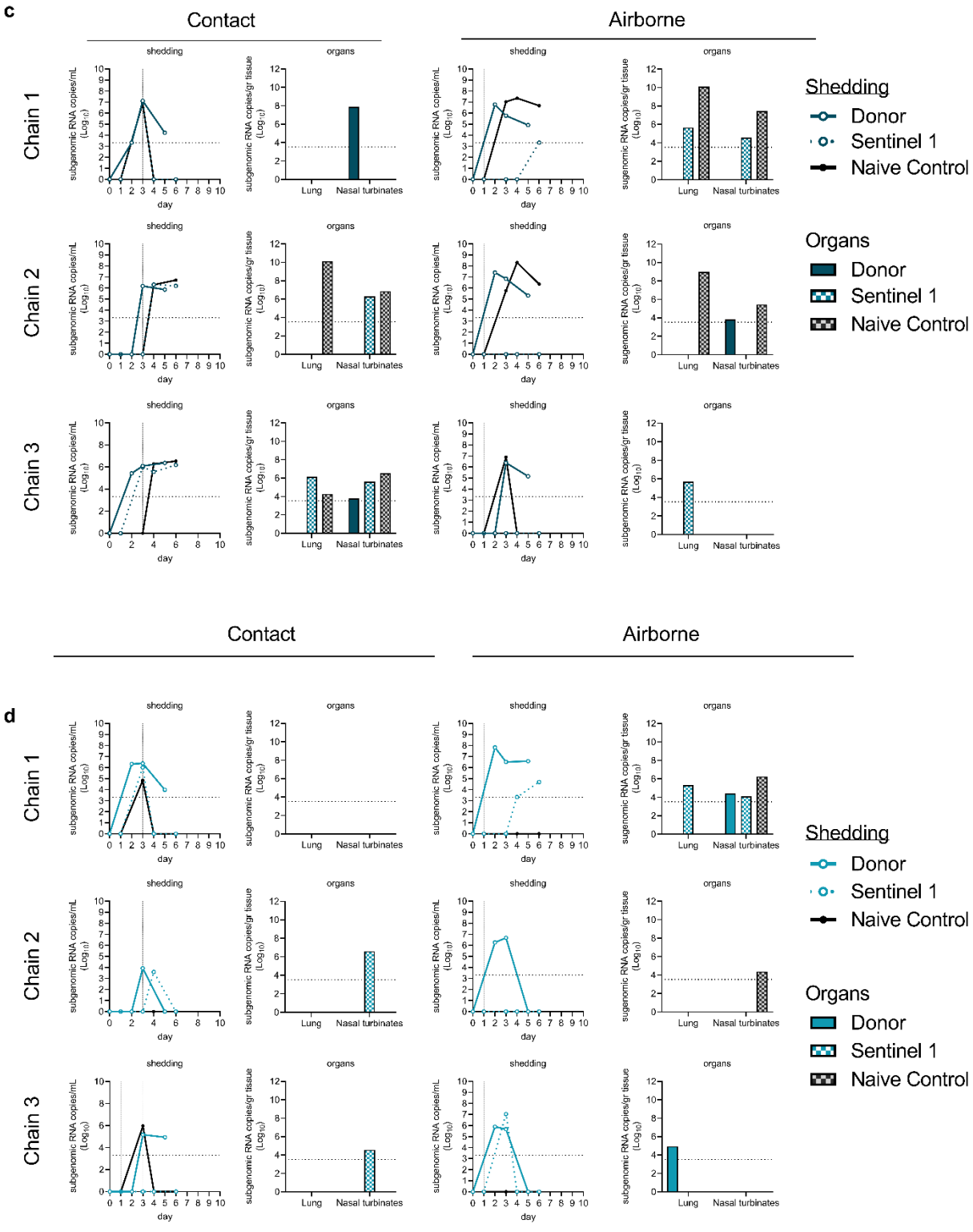

Fig. S1. Shedding and organ titers of individual animals in each chain. Animals were grouped into either airborne or contact transmission chains by immune state (N = 3). Chains consisted of either a donor and three consecutive sentinels (naïve chains) or a donor and two

sentinels, one with previous immunity and one naïve control (IM, IN, and PI chains). Donors were infected with 1 x 10^4 $TCID_{50}$ SARS-CoV-2 at a 1:1 ratio of Omicron and Delta and 24 hours later exposed to either a sentinel and naïve animal (IM, IN, and PI chains) or to the first sentinel (naïve chains) for 48 hours. Naïve chains continued in a similar fashion over four total animals. Replicating virus was measured via oropharyngeal swabs at 2, 3, and 5 DPI/DPE, and in lung and nasal turbinate samples at 5 DPI/DPE. Viral shedding is depicted in oropharyngeal swabs and viral replication in lung and nasal turbinate samples of all animals in contact (left) and airborne (right) chains. Viral shedding and replication are displayed as $\log_{10}$ (sgRNA copies/mL) in swabs and $\log_{10}$ (sgRNA copies/gram of tissue) in tissues. Horizontal lines indicate limit of detection. Vertical lines indicate exposure events in the chain. A. Naïve chains. B. Previously infected chains, C. Intramuscularly vaccinated chains. D. Intranasally vaccinated chains. Colours refer to legends on the right. Source data are provided as a Source Data file.

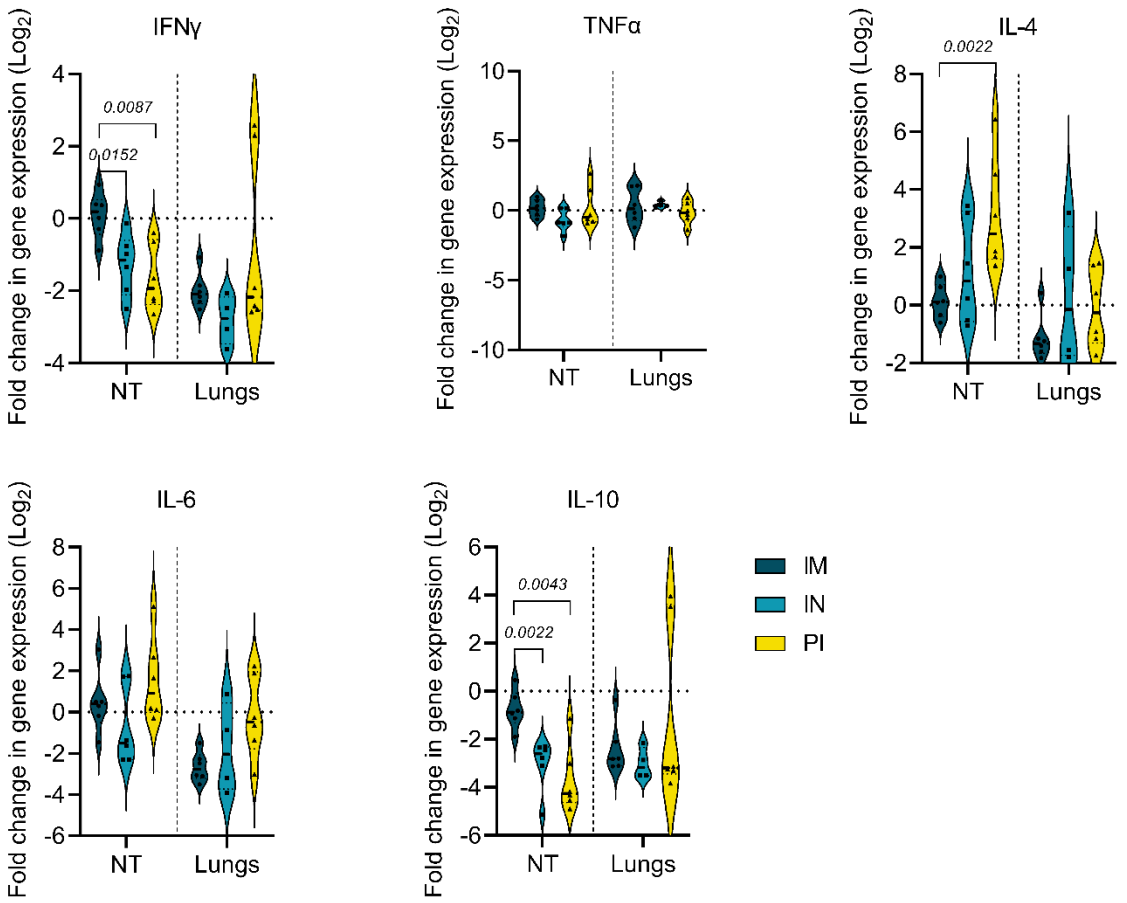

Fig. S2. Changes in cytokine gene expression post-challenge. Nasal turbinate (NT) and lung samples were collected at 5 DPI and the fold-change in mRNA expression was calculated for challenged animals with pre-existing immunity over challenged naïve animals. Violin plots depicting median, quantiles, and individual values, N = 6 (lungs IN = 4). Dark blue = IM vaccinated, light blue = IN vaccinated, yellow = PI. Kruskal-Wallis followed by Mann Whitney if statistically significant; p-values stated were significant (<0.05). Source data are provided as a Source Data file.

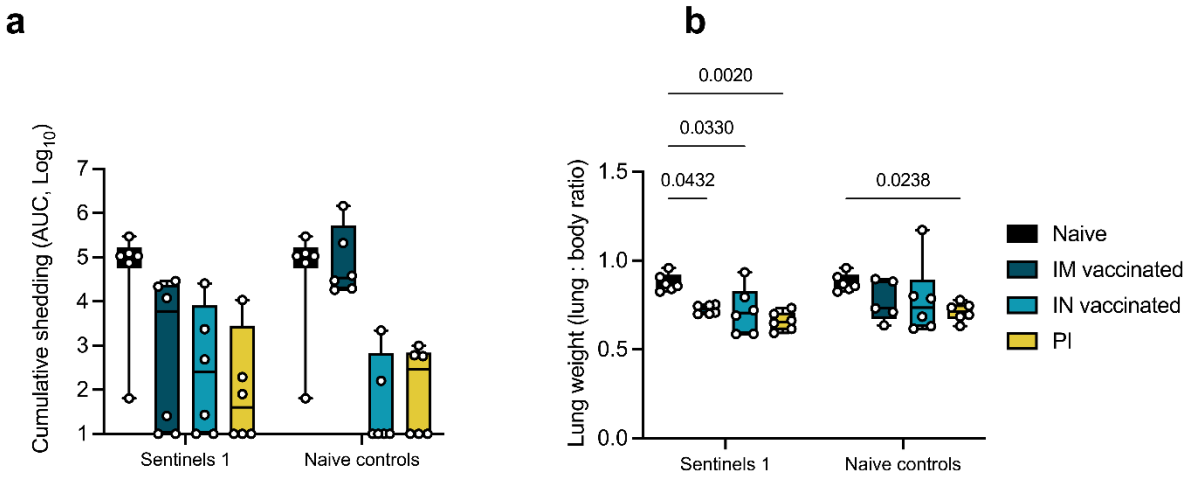

Fig. S3. Reduction of disease severity and shedding in sentinels. A. Cumulative shedding. Area under the curve (AUC) of sgRNA measured in oral swabs taken on 2, 3, and 5 DPE (sgRNA copies/rxn/experiment). Whisker-plots depicting median, min and max values, and individual values, N = 6, ordinary two-way ANOVA, followed by Šídák's multiple comparisons test. B. Lung samples were collected at 5 DPE for sentinels and naïve controls. Lung weights (lung:body weight ratio). Whisker-plots depicting median, min and max values, and individual values, N = 6, ordinary two-way ANOVA, followed by Šídák's multiple comparisons test. Black = naïve, dark blue = IM vaccinated, light blue = IN vaccinated, yellow = PI. P-values stated were significant (<0.05). Naïve sentinels 1 are depicted twice in each graph for visualization purposes only. Source data are provided as a Source Data file.

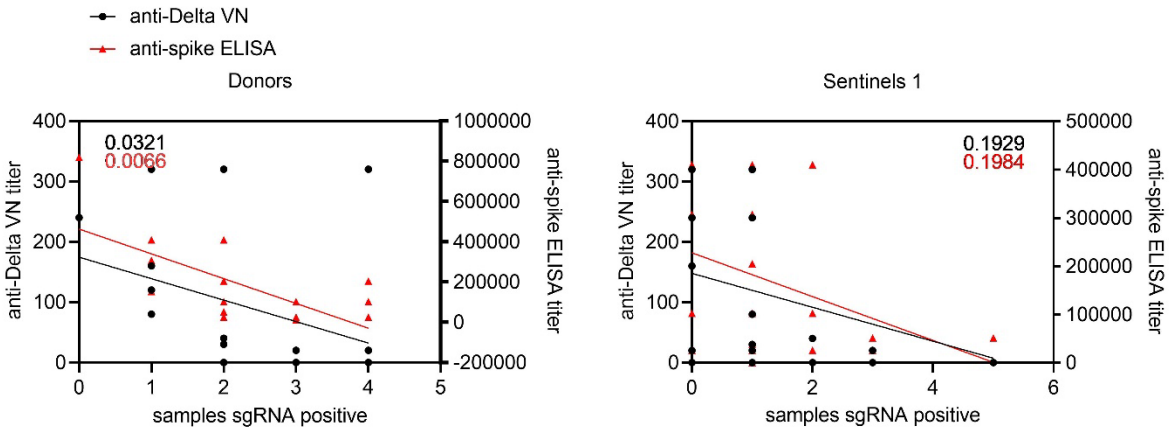

Fig. S4. Correlation between humoral immune response and protection from infection. Serum was collected at least 21 days post vaccination against Lineage A or infection with Delta. Correlation between anti-spike IgG response (Lineage A spike), measured by ELISA or individual neutralizing antibody titers against Delta and the amount of positive sgRNA swab or tissue samples (>10 copies/rxn) after challenge (donors) or exposure (sentinels 1). Individuals are depicted, as well as a linear regression line. N = 18, Spearman's correlation, p -values indicated. Source data are provided as a Source Data file.

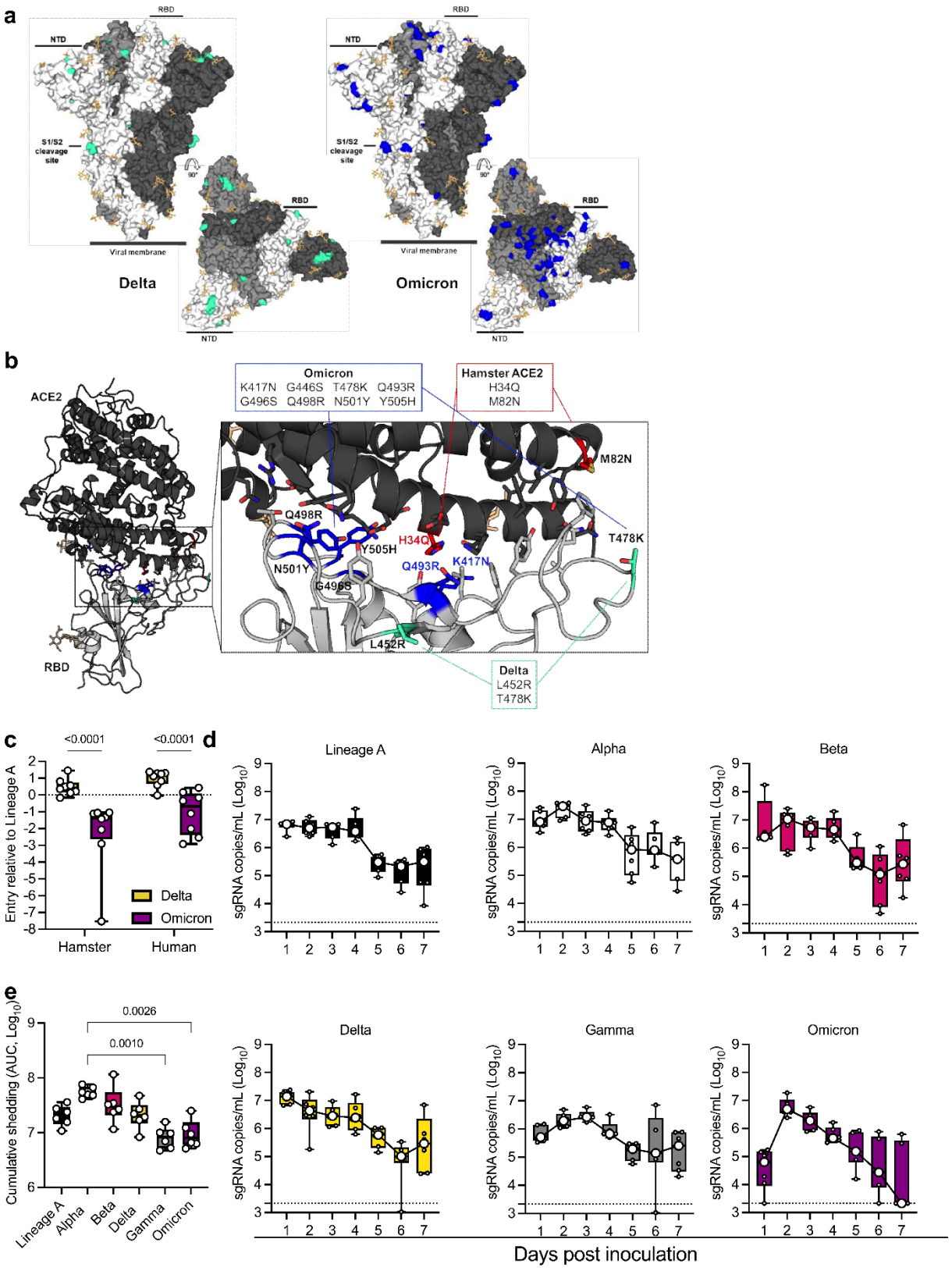

Fig. S5. Comparison of SARS-CoV-2 variants Omicron and Delta infection in the Syrian hamster. A. Mutations observed in the SARS-CoV-2 Delta and Omicron VOCs are highlighted

on the structure of SARS-CoV-2 spike (compared to Lineage A, PDB 6ZGE, [1]). The spike trimer is depicted by surface representation with each protomer colored a different shade of gray. The residues at the positions of the spike protein mutations observed in the Delta and Omicron SARS-CoV-2 VOCs are colored teal green (Delta) and blue (Omicron). The receptor binding domain (RBD), N-terminal domain (NTD), and cleavage site are annotated. N-linked glycans are shown as light, orange-colored sticks. B. The structure of the Alpha VOC RBD and human ACE2 complex (PDB 7EKF, [2]) is depicted with cartoon representation. ACE2 is colored dark gray and the RBD is colored light gray. N-linked glycans are shown as light, orange-colored sticks. A box reveals a close-up view of the RBD-ACE2 binding interface. Side chains of the residues participating in the interaction, as identified and described by Lan, et al [3] are shown as sticks. The residues within the RBD that are mutated in the Delta and Omicron VOCs are colored teal green (Delta) and blue (Omicron). Residue T478 is mutated in both Delta and Omicron VOCs but is colored teal green in the figure. Though they do not participate directly in the ACE2 interface, the sidechains of residues L452 and T478 are also shown. The residues that differ between human and hamster ACE2 within the interface are colored red. C. BHK cells expressing either human ACE2 or hamster ACE2 were infected with pseudotyped VSV reporter particles with the spike proteins of Delta or Omicron. Relative entry to a Lineage A control is depicted. Whisker-plots depicting median, min and max values, and individual values, N = 8, ordinary two-way ANOVA, followed by Šídák's multiple comparisons test. D. Viral load as measured by sgRNA in oropharyngeal swabs collected at 1-7 days post intranasal 1,000 $TCID_{50}$ inoculation with Lineage A, Alpha, Beta, Delta, Gamma, or Omicron. Whisker-plots depicting median, min and max values, and individual values, N = 6 (3 males and 3 females). E. Cumulative sgRNA shedding for each variant. Area under the curve for data shown in D (sgRNA copies/ml/experiment). Kruskal-Wallis test. P-values stated were significant (<0.05). Source data are provided as a Source Data file.

Table S1: Pathological assessment of IN, IM vaccinated or PI Syrian hamsters on day 5 post challenge. nsf = no significant findings. y = yes. n = no. Nucleoprotein reactivity score: 0 = none, 1 = rare/few, 2 = scattered, 3 = moderate, 4 = numerous, 5 = diffuse.

Group	Naïve						IM vaccinated						IN vaccinated						Previously infected					
Lesions visible grossly	y	y	n	y	y	y	y	y	y	?	y	n	n	n	n	N	n	n	n	n	?	n	n	n
Lung			nsf							nsf			nsf	nsf	nsf	nsf		nsf		nsf	nsf	nsf	nsf	
% total area affected	60	60		60	70	80	30	<10	20		20	<10					10		<10					<10
Interstitial Pneumonia																								
Syncytial Cell	y	y	n	y	y	y	y	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
Alveolar inflammation or exudate	y	y	n	y	y	y	y	y	y	n	y	y	n	n	n	n	n	n	n	n	n	n	n	n
Endotheleitis, vasculitis	y	y	n	y	y	y	y	y	y	n	y	n	n	n	n	n	n	n	n	n	n	n	n	n
Type II pneumocyte hyperplasia	n	n	n	n	n	n	y	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
Hemorrhage, fibrin and or edema	y	y	n	y	y	y	n	n	y	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
Bronchiolar hyperplasia	y	y	n	y	y	y	y	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
SARS-CoV-2 IHC bronchioles	3	2	0	2	2	2	0	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
SARS-CoV-2 IHC alveoli	4	3	0	3	4	4	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Positive pixel analysis CD3	16	17	7	14	14	16	6	10	10	9	9	10	7	8	8	8	6	9	8	7	8	7	8	6
Positive pixel analysis PAX5	3	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	1	1	2	2	2	2

Table S2: Shedding and tissue titers for each transmission chain (donors and sentinels 1). IM = intramuscularly vaccinated, IN = intranasally vaccinated, PI = Previously infected, BDL = Below qRT-PCR detection limit, sgRNA = sub-genomic RNA. Swab days = 2, 3 and 5. Samples with < 10 sgRNA copies/rxn (approx. > ct = 36) were excluded from sequencing analysis. No data provided if sequencing did not pass quality control or produced no results.

				sgRNA Ct-value					% Delta reads					% Omicron reads				
Transmission route	Chain	Chain link	Immune status	Day 2 swab	Day 3 swab	Day 5 swab	Lung	Nasal turbinates	Day 2 swab	Day 3 swab	Day 5 swab	Lung	Nasal turbinates	Day 2 swab	Day 3 swab	Day 5 swab	Lung	Nasal turbinates
air	1	Donor	IM	25	28	32	BDL	BDL	82	94	95			17	0	0		
air	1	Naïve Control	IM	24	23	26	15	23	99	100	100	100	100	0	0	0	0	0
air	1	Sentinel 1	IM	BDL	BDL	37	30	32					97					0
contact	1	Donor	IM	40	24	34	BDL	22		98	98		99		0	0		0
contact	1	Naïve Control	IM	25	BDL	BDL	BDL	BDL	94					0				
contact	1	Sentinel 1	IM	24	BDL	BDL	BDL	BDL	98					0				
air	2	Donor	IM	23	25	30	BDL	35	86	92	100		100	13	7	0		0
air	2	Naïve Control	IM	28	20	27	19	30	100	99	99	100	100	0	0	0	0	0
air	2	Sentinel 1	IM	BDL	BDL	BDL	BDL	BDL										
contact	2	Donor	IM	BDL	27	28	BDL	BDL		99	99				0	0		
contact	2	Naïve Control	IM	BDL	27	25	16	26		100	99	100	100		0	0	0	0
contact	2	Sentinel 1	IM	BDL	27	27	BDL	28		100	99		100		0	0		0
air	3	Donor	IM	BDL	26	31	BDL	BDL		99	99				0	0		
air	3	Naïve Control	IM	25	BDL	BDL	BDL	BDL	99					0				
air	3	Sentinel 1	IM	BDL	BDL	BDL	28	BDL										
contact	3	Donor	IM	30	27	27	BDL	35	94	99	100			0	0	0		
contact	3	Naïve Control	IM	BDL	27	26	34	31		100	100		100		0	0		0
contact	3	Sentinel 1	IM	28	29	27	27	29	19	100	99	100	100	80	0	0	0	0
air	1	Donor	IN	21	26	26	BDL	33	81	92	98		81	18	7	0		18
air	1	Naïve Control	IN	BDL	BDL	BDL	BDL	27					100					0
air	1	Sentinel 1	IN	BDL	36	32	31	35			100		100			0		0

contact	1	Donor	IN	27	26	35	BDL	BDL	98	95	98			0	0	0		
contact	1	Naïve Control	IN	32	BDL	BDL	BDL	BDL	100					0				
contact	1	Sentinel 1	IN	28	BDL	BDL	BDL	BDL	100					0				
air	2	Donor	IN	26	25	BDL	BDL	BDL	84	93				15	0			
air	2	Naïve Control	IN	BDL	BDL	BDL	BDL	33					100					0
air	2	Sentinel 1	IN	BDL	BDL	BDL	BDL	BDL										
contact	2	Donor	IN	BDL	35	BDL	BDL	BDL		76					23			
contact	2	Naïve Control	IN	BDL	BDL	BDL	BDL	BDL										
contact	2	Sentinel 1	IN	BDL	36	BDL	BDL	27		100					0			
air	3	Donor	IN	28	29	BDL	31	BDL	63	84				36	15			
air	3	Naïve Control	IN	BDL	BDL	BDL	BDL	BDL										
air	3	Sentinel 1	IN	24	BDL	BDL	BDL	BDL	75					24				
contact	3	Donor	IN	BDL	31	32	BDL	BDL		99	100				0	0		
contact	3	Naïve Control	IN	28	BDL	BDL	BDL	BDL	97					0				
contact	3	Sentinel 1	IN	BDL	BDL	BDL	BDL	32					100					0
air	1	Donor	Naïve	25	26	27	14	23	83	92	99	100	99	16	7	0	0	0
air	1	Sentinel 1	Naïve	24	26	24	20	21	100	90	84	100	99	0	9	15	0	0
air	1	Sentinel 2	Naïve	32	27	24	BDL	29	100	99	100		100	0	0	0		0
air	1	Sentinel 3	Naïve	BDL	BDL	33	BDL	BDL			100					0		
contact	1	Donor	Naïve	22	25	27	25	33	93	98	98	100	97	0	0	0	0	0
contact	1	Sentinel 1	Naïve	25	25	26	18	16	96	99	99	99	100	0	0	0	0	0
contact	1	Sentinel 2	Naïve	33	25	25	23	21	83	100	99		100	16	0	0		0
contact	1	Sentinel 3	Naïve	BDL	26	26	BDL	32		100	100		100		0	0		0
air	2	Donor	Naïve	23	23	30	16	29	82	85	99	99	98	17	14	0	0	0
air	2	Sentinel 1	Naïve	25	22	27	19	22	87	100	100	99	100	12	0	0	0	0
air	2	Sentinel 2	Naïve	BDL	34	24	20	23		100	100	100	100		0	0	0	0
air	2	Sentinel 3	Naïve	BDL	BDL	24	24	22			99		100			0		0
contact	2	Donor	Naïve	34	26	28	32	27	100	97	97	100	99	0	0	0	0	0

contact	2	Sentinel 1	Naïve	29	25	24	15	24	100	99	99	100	99	0	0	0	0	0
contact	2	Sentinel 2	Naïve	28	30	27	17	23	100	100	99	100	100	0	0	0	0	0
air	3	Donor	Naïve	36	25	26	19	20	100	99	99	100	99	0	0	0	0	0
air	3	Sentinel 1	Naïve	33	BDL	BDL	BDL	BDL	100					0				
air	3	Sentinel 2	Naïve	BDL	BDL	BDL	BDL	BDL										
contact	3	Donor	Naïve	BDL	25	28	21	16		100	99	100	100		0	0	0	0
contact	3	Sentinel 1	Naïve	BDL	27	27	17	17		99	99	99			0	0	0	
contact	3	Sentinel 2	Naïve	BDL	24	30	18	25		97	99	100	100		0	0	0	0
contact	3	Sentinel 3	Naïve	BDL	28	25	20	27		100	99	100	100		0	0	0	0
air	1	Donor	PI	32	35	BDL	BDL	BDL	0	0				93	95			
air	1	Naïve Control	PI	BDL	BDL	32	BDL	BDL			100					0		
air	1	Sentinel 1	PI	BDL	BDL	BDL	BDL	BDL										
contact	1	Donor	PI	37	35	BDL	BDL	BDL		100					0			
contact	1	Naïve Control	PI	29	BDL	BDL	BDL	BDL										
contact	1	Sentinel 1	PI	33	BDL	BDL	BDL	39	86					13				
air	2	Donor	PI	29	BDL	BDL	BDL	37	0					95				
air	2	Naïve Control	PI	BDL	BDL	BDL	BDL	BDL										
air	2	Sentinel 1	PI	BDL	BDL	BDL	BDL	BDL										
contact	2	Donor	PI	23	30	37	BDL	35	95	96	57		95	0	0	42		0
contact	2	Naïve Control	PI	BDL	BDL	BDL	BDL	BDL										
contact	2	Sentinel 1	PI	BDL	BDL	BDL	BDL	BDL										
air	3	Donor	PI	BDL	BDL	BDL	BDL	BDL										
air	3	Naïve Control	PI	BDL	BDL	BDL	BDL	BDL										
air	3	Sentinel 1	PI	32	BDL	BDL	BDL	BDL	91					8				
contact	3	Donor	PI	25	BDL	BDL	BDL	BDL	100					0				
contact	3	Naïve Control	PI	30	BDL	BDL	BDL	BDL	100					0				
contact	3	Sentinel 1	PI	25	BDL	BDL	BDL	32	100					0				

128

Table S3: Humoral immune parameters for each transmission chain (donors, sentinels). IM = intramuscularly vaccinated, IN = intranasally vaccinated, PI = Previously infected, NA = sample not available for analysis.

				spike ELISA		virus neutralization				pre-challenge variant-specific antibodies						post-challenge variant-specific antibodies					
Transmission route	Chain	Chain link	Immune status	Pre-challenge titer	Post-challenge titer	Pre-challenge titer against Delta	Pre-challenge titer against Omicron	Post-challenge titer against Delta	Post-challenge titer against Omicron	Lineage A	Alpha	Beta	Delta	Gamma	Omicron	Lineage A	Alpha	Beta	Delta	Gamma	Omicron
air	1	Naïve Control 2	IM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	225	229	201	163	187.5	174
air	1	Naïve Control 3	IM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	204	185.5	172	124.5	126.5	287
air	1	Sentinel 2	IM	12800	12800	0	10	60	10	8872	7362.5	1548.5	3906	2807.5	539.5	6475	5929	2394	4833	3782	682
air	1	Sentinel 3	IM	25600	25600	0	0	30	0	10275	6937	1868	4635.5	6003.5	981	6338	4211	1660	3990	4740	813
air	2	Naïve Control 2	IM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	134.5	143.5	126.5	88.5	110.5	122
air	2	Naïve Control 3	IM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	210	209	200	149	154	177
air	2	Sentinel 2	IM	25600	25600	0	0	40	10	14111	10683.5	3039	7358.5	3489	1064.5	9190	7314	4144	7326	5374	1868
air	2	Sentinel 3	IM	12800	25600	0	0	30	0	7051	5115	1437	3886.5	1873	628	4003	2660	1515	3005	3080	629
air	3	Donor	IM	25600	204800	0	0	320	160	15559	11207	3172.5	9197.5	3624.5	773.5	17544	14468	9299	14224	9116	3577
air	3	Naïve Control	IM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	175	194.5	214	143	175	199.5
air	3	Sentinel 1	IM	0	0	0	0	0	0	4588	3362	1151.5	1982	1673.5	469.5	1259	1088	605	922	759	308
air	1	Donor	IM	25600	307200	0	0	640	240	8599.5	7160	3077	5781.5	2785	904.5	10518	8734	5663	8850	7336	2042
air	1	Naïve Control	IM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	276.5	257	208	104	179.5	185
air	1	Sentinel 1	IM	25600	25600	0	0	80	20	15357	9581.5	2884	7039.5	3514	819.5	5988	3285	1802	3358	3388	526
air	2	Donor	IM	25600	204800	0	0	960	120	9971.5	6740.5	1563.5	5578.5	2662	395	26804	22179	11636	22517	20067	5302
air	2	Naïve Control	IM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	201.5	203.5	225	134.5	166.5	203.5
air	2	Sentinel 1	IM	25600	12800	0	0	80	0	13238.5	9276	2246.5	6619	2369.5	572.5	6419	4567	1922	4830	3073	481
contact	1	Donor	IM	25600	204800	20	0	240	80	16743.5	13326	2819	8573.5	3601	1267.5	32519	28087	17345	27140	25087	8295
contact	1	Naïve Control	IM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	184	193.5	219.5	135	135.5	177
contact	1	Sentinel 1	IM	25600	12800	20	0	20	0	18251.5	13077.5	4640	9481	7430	1339.5	6065	4194	3272	5522	4329	1159
contact	2	Donor	IM	25600	819200	0	0	1280	60	8307	5499.5	1489.5	3626.5	1513.5	345	39741	29212	16450	32467	22736	9082
contact	2	Naïve Control	IM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	165.5	149.5	165	102.5	194.5	127
contact	2	Sentinel 1	IM	25600	51200	0	0	80	0	9442.5	6783.5	2007	5154	2006.5	743	6829	5457	3030	5879	3887	1198
contact	3	Donor	IM	12800	307200	0	0	320	10	11302.5	6796	1577	3882.5	1811.5	492.5	24484	18982	11163	21790	14532	3841
contact	3	Naïve Control	IM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2725.5	214.5	203	140.5	165	181.5
contact	3	Sentinel 1	IM	51200	76800	0	0	240	0	24218.5	15132.5	3701.5	13232.5	6625	1409	11510	8410	4185	9750	7879	1739
air	1	Naïve Control 2	IN	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	202	192.5	152	97	179	142.5

air	1	Naïve Control 3	IN	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	153	167.5	161	115	126.5	144
air	1	Sentinel 2	IN	102400	25600	160	0	160	0	18654.5	12321.5	5946.5	14155.5	8430	901	8092	5516	3554	6607	6090	734
air	1	Sentinel 3	IN	51200	102400	20	10	60	60	21121.5	14226.5	4345	12966.5	6829	1715.5	15642	11446	6864	10732	12066	2580
air	2	Naïve Control 2	IN	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	195.5	188	194	130.5	160.5	163.5
air	2	Naïve Control 3	IN	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	279.5	247.5	207.5	189	269	151
air	2	Sentinel 2	IN	25600	102400	20	0	120	0	13333	8894.5	2597.5	6319.5	3660	1642.5	21341	17102	8831	16033	11495	5162
air	2	Sentinel 3	IN	51200	204800	40	30	120	30	35071	26897	9294	18631.5	10813	3873	29222	23231	14650	23339	17456	7084
air	3	Donor	IN	204800	409600	30	0	1280	960	32627	26304	4571	17189	5621	2254	75480	69977	45669	60304	54882	25180
air	3	Naïve Control	IN	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	248	228	200.5	148	141.5	206
air	3	Sentinel 1	IN	307200	102400	30	20	240	80	63281.5	43021	10080	30283.5	21163.5	4397.5	25934	16953	11330	17340	20516	4159
air	1	Donor	IN	102400	819200	20	10	2560	240	17130.5	13665	4560	9842	5318	2147	77893	69821	47948	76692	64141	20137
air	1	Naïve Control	IN	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	153	183	170	117	119.5	163.5
air	1	Sentinel 1	IN	51200	51200	20	0	240	0	13633.5	7299.5	2638	6143.5	10384	779.5	12721	8875	4065	7528	9664	1137
air	2	Donor	IN	102400	819200	30	0	2560	1280	18251.5	11373.5	3365.5	10029.5	5736	992	70484	56418	39148	64785	59592	16958
air	2	Naïve Control	IN	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	246.5	260	229.5	162	235.5	201
air	2	Sentinel 1	IN	102400	102400	20	0	120	30	17617.5	14478	6240.5	11090	6330.5	1632.5	12339	10436	6460	10860	6871	2041
contact	1	Donor	IN	102400	409600	20	10	480	1280	18493	15194.5	5024.5	10525	3922.5	1419	67260	61139	46506	57046	49177	25578
contact	1	Naïve Control	IN	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	691.5	624.5	394.5	382	485	271
contact	1	Sentinel 1	IN	102400	102400	80	0	240	40	28134.5	22233	6303	16121	8088	2434.5	14841	12908	9179	11683	9760	2925
contact	2	Donor	IN	153600	819200	80	40	1280	960	56527.5	47228.5	14040	28777	18741	7338	90648	77342	60164	81921	69626	30869
contact	2	Naïve Control	IN	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	169.5	182	161.5	120	144.5	153.5
contact	2	Sentinel 1	IN	102400	153600	40	0	160	30	71702.5	47943.5	14011	36254.5	21275	5457.5	35493	29902	17418	29716	26537	6445
contact	3	Donor	IN	51200	102400	40	0	1280	640	18729.5	18111.5	3655.5	6977.5	5931.5	1819	52008	58123	40191	47389	44985	13766
contact	3	Naïve Control	IN	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	309.5	445.5	235	160	214.5	215.5
contact	3	Sentinel 1	IN	38400	51200	20	40	240	160	13843.5	9101	3469.5	6311	5563	1242.5	18167	14362	8812	14190	10875	2652
air	3	Donor	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	7072	5257.5	397.5	406.5	4179	386
air	3	Sentinel 1	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	580.5	1228	871	600	760	486.5
air	3	Sentinel 2	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	171.5	183	175.5	107.5	132.5	151.5
air	1	Donor	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	16474.5	8646.5	1713.5	1862	10746	1571.5
air	1	Sentinel 1	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	441.5	464.5	492.5	255	291	402
air	1	Sentinel 2	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	362.5	343	246	127.5	227	213.5
air	1	Sentinel 3	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6468	5445.5	3426	4065	4973.5	1328.5
air	2	Donor	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	9551.5	6714.5	747.5	866	6441	1614.5

air	2	Sentinel 1	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	219.5	196.5	180.5	112	143	165.5
air	2	Sentinel 2	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	211.5	204.5	217	131	145.5	184
air	2	Sentinel 3	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	168	178.5	184.5	133.5	133.5	150.5
contact	1	Donor	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	8912.5	6554.5	690.5	518	5060.5	454
contact	1	Sentinel 1	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1259	1026	652	650	940	389
contact	1	Sentinel 2	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	201	213	217.5	127.5	135.5	198.5
contact	1	Sentinel 3	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	345.5	334	219	227	243	184
contact	2	Donor	Naïve	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	14381.5	7911.5	1257.5	1170.5	10736	539
contact	2	Sentinel 1	Naïve	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	160.5	139	141	128	115.5	131.5
contact	2	Sentinel 2	Naïve	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	202	234	215	133.5	155	198
contact	3	Donor	Naïve	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	641	546.5	411	363	513	296.5
contact	3	Sentinel 1	Naïve	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	18064	12168.5	1101.5	1350.5	11167.5	718.5
contact	3	Sentinel 2	Naïve	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	225.5	233.5	227	164.5	179.5	211.5
contact	3	Sentinel 3	Naïve	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	232.5	238.5	200.5	159.5	188.5	205
air	1	Naïve Control 2	PI	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	240	298	251.5	177.5	180.5	252
air	1	Naïve Control 3	PI	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	183	154.5	168.5	108	139	169
air	1	Sentinel 2	PI	102400	307200	320	0	1280	320	77091.5	64443.5	27405.5	79600	25469.5	16203.5	47334.5	44303	28614	63907	26894.5	11696.5
air	1	Sentinel 3	PI	204800	204800	320	0	640	30	66655.5	48554.5	19968.5	69392.5	24370	8664.5	35060	31041.5	21082	45068	19088	6117.5
air	2	Naïve Control 2	PI	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	231	252	229.5	144.5	178	221
air	2	Naïve Control 3	PI	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	185.5	180	177.5	178.5	146.5	165.5
air	2	Sentinel 2	PI	102400	153600	320	60	960	80	67804.5	54699	24231	82264.5	31855	8200	35169	31507	20163	54281.5	18901.5	7387.5
air	2	Sentinel 3	PI	409600	409600	160	30	960	160	75900.5	64294.5	25405.5	75421.5	38502.5	15369.5	36773.5	34585.5	27677	60129.5	28448.5	10180
air	3	Donor	PI	819200	819200	240	30	2560	640	97759.5	83310.5	35128	101515.5	50381	28233	52638	51036.5	42596	75964	37616	19598.5
air	3	Naïve Control	PI	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	221	231.5	212	138	175.5	207
air	3	Sentinel 1	PI	204800	204800	240	40	1920	960	53198.5	38329.5	13905.5	79721.5	15449.5	7838.5	19330.5	17545.5	14728	28954.5	11520	5096
air	1	Donor	PI	409600	204800	320	240	1920	1920	123928.5	110427	50020.5	132427.5	68374	26803	74852	68762.5	53904	87935	57204	24813
air	1	Naïve Control	PI	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	159.5	164	153	79	120	166.5
air	1	Sentinel 1	PI	409600	102400	240	0	320	60	81121.5	66302	27641.5	88361	30078	17041	29559	26161.5	16480.5	45111	16073.5	6515
air	2	Donor	PI	307200	409600	160	0	2560	1280	71450	55514	21661	96791	28806.5	13905	80417	76805.5	52033	112139.5	52535.5	27554
air	2	Naïve Control	PI	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	283	356	317	193.5	234.5	281.5
air	2	Sentinel 1	PI	307200	102400	320	10	960	60	75529	53828	21013	73745.5	23580.5	9521	38821	32743	25025	53059.5	22210.5	8108.5
contact	1	Donor	PI	409600	614400	320	20	2560	480	91773	82524	35151.5	105404.5	40463	14481.5	70536	66229	45650	97128	45424	19512

contact	1	Naïve Control	PI	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	173	215.5	161.5	119.5	135	178
contact	1	Sentinel 1	PI	409600	204800	320	80	1280	240	103973.5	82091.5	36887	91762.5	42246	23805	45534	40183	29798.5	55114	28030	14086.5
contact	2	Donor	PI	204800	307200	320	160	1280	960	83254	67851.5	22721	87493	29854	13598.5	71475	64482.5	47832.5	84450.5	43852.5	22673.5
contact	2	Naïve Control	PI	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	614	557.5	436	399	709.5	268
contact	2	Sentinel 1	PI	409600	307200	160	0	640	160	84023	66186.5	29457.5	102057.5	34172	13539	44630.5	38414.5	29174.5	61470	26151	13332
contact	3	Donor	PI	409600	409600	120	30	1280	480	82590	68282.5	29771	83558.5	42848.5	20544.5	93009	82432	61849.5	114292	62931	31692
contact	3	Naïve Control	PI	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	155.5	148.5	170.5	99	120	161
contact	3	Sentinel 1	PI	409600	409600	480	120	1280	320	134347	115621.5	54286.5	139190	69516.5	26920.5	71184.5	61945.5	46613	85393	43008.5	17968

132
133

Table S4: SARS-CoV-2 isolates used in this study.

Virus	WHO Description	PANGO Lineage	GISAID[$]/Genbank Acc*
SARS-CoV-2/human/USA/WA-CDC-WA1/2020	Lineage A	WA1	MN985325
England/204820464/2020	Alpha	B.1.1.7	EPI_ISL_683466
USA/MD-HP01542/2021	Beta	B.1.351	EPI_ISL_890360
hCoV-19/USA/MD-HP03867/2021	Gamma	P.1	EPI_ISL_1468644
hCoV-19/USA/KY-CDC-2-4242084/2021	Delta	B.1.617.2	EPI_ISL_1823618
hCoV-19/USA/GA-EHC-2811C/2021	Omicron	B.1.1.529	EPI_ISL_7171744

[$]GISAID: https://www.epicov.org/epi3/frontend#57217d
*GenBank: https://www.ncbi.nlm.nih.gov/nuccore/MN985325

Table S5: *Cytokine gene expression primers*

Primer name	Sequence (5' -> 3')
IFNy-F	TTGTTGCTCTGCCTCACTC
IFNy-R	CCCTCCATTCACGACATCTAAG
IFNy-P	FAM-TACTGCCAGGGCACACTCATTGAA
IL10-F	AGCGCTGTCATCGATTTCTC
IL10-R	CGCCTTTCTCTTGGAGCTTAT
IL10-P	FAM-AAGGCTGTGGAACAGGTGAAGGAT
IL4-F	CCACGGAGAAAGACCTCATCTG
IL4-R	GGGTCACCTCATGTTGGAAATAAA
IL4-P	FAM-CAGGGCTTCCCAGGTGCTTCGCAAGT
IL6-F	CCACCAGGAACGAAAGACAA
IL6-R	CAGCAGTCCCAAGAAGACAA
IL6-P	FAM-AACTTCATAGCTGTTCCTGGAGGGC
TNFa-F	GGAGTGGCTGAGCCATCGT
TNFa-R	AGCTGGTTGTCTTTGAGAGACATG
TNFa-P	FAM-CCAATGCCCTCCTGGCCAACG

Methods

VOC virus shedding comparison

For the comparison of virus shedding of all VOCs, four-to-six-week-old Syrian golden hamsters

(N = 6 per group, Envigo Indianapolis) were inoculated intranasally with 40 µL containing 1 ×

10^3 $TCID_{50}$ virus in sterile DMEM. Oropharyngeal swabs were collected in 1 mL of DMEM2 on day post infection 1-7.

Pseudotype entry assay

The spike coding sequences for SARS-CoV-2 variant Lineage A, Delta, and Omicron (MN985325, EPI_ISL_2441471, EPI_ISL_6699767, respectively) were truncated by deleting 19 aa at the C-terminus. The spike (S) proteins with the 19 aa deletions of coronaviruses were previously reported to show increased efficiency regarding incorporation into virions of VSV [4, 5]. These sequences were codon optimized for human cells, then appended with a 5′ kozak expression sequence (GCCACC) and 3′ tetra-glycine linker followed by nucleotides encoding a FLAG-tag sequence (DYKDDDDK). These spike sequences were synthesized and cloned into pcDNA3.1$^+$(GenScript). Human ACE2 (Q9BYF1.2, https://www.ncbi.nlm.nih.gov/protein/Q9BYF1.2) and hamster ACE2 (GQ262794.1 https://www.ncbi.nlm.nih.gov/nuccore/GQ262794.1) were synthesized and cloned into pcDNA3.1$^+$ (GenScript). All DNA constructs were verified by Sanger sequencing (ACGT). BHK cells (kindly provided by Marshal Bloom, also available from ATCC {CCL-10, https://www.atcc.org/products/ccl-10)) were seeded in black 96-well plates and transfected the next day with 100 ng plasmid DNA encoding human or hamster ACE2, using polyethylenimine (Polysciences). All downstream experiments were performed 24 h post-transfection. Pseudotype production was carried as previously described [6]. Briefly, plates pre-coated with poly-L-lysine (Sigma–Aldrich) were seeded with 293T cells (kindly provided by Sonja Best, also available from ATCC (CRL-3216 https://www.atcc.org/products/crl-3216)) and transfected the following day with 1,200 ng of empty plasmid and 400 ng of plasmid encoding coronavirus spike or no-spike plasmid control (green fluorescent protein (GFP)). After 24 h, transfected cells were

infected with VSVΔG seed particles pseudotyped with VSV-G, as previously described [6, 7]. After one hour of incubating with intermittent shaking at 37 °C, cells were washed four times and incubated in 2 mL DMEM supplemented with 2% FBS, penicillin/streptomycin and L-glutamine for 48 h. Supernatants were collected, centrifuged at 500 x g for 5 min, aliquoted, and stored at −80 °C. BHK cells previously transfected with ACE2 plasmid of interest were inoculated with equivalent volumes of pseudotype stocks. Plates were then centrifuged at 1200 x g at 4 °C for one hour and incubated overnight at 37 °C. Approximately 18–20 h post-infection, Bright-Glo luciferase reagent (Promega) was added to each well, 1:1, and luciferase was measured. Relative entry was calculated normalizing the relative light unit for spike pseudotypes to the plate relative light unit average for the no-spike control. Each figure shows the data for two technical replicates.

Structural interaction analysis

The locations of the described spike mutations in the Delta and Omicron VOCs were highlighted on the SARS-CoV-2 spike structure (PDB 6ZGE [1]). To visualize the molecular interactions at the RBD – ACE2 binding interface, the crystal structure of the Alpha variant RBD and human ACE2 complex (PDB 7EKF [2]) was utilized. All figures were generated using The PyMOL Molecular Graphics System version 2.5.4 (https://www.schrodinger.com/pymol).

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