

## Supplementary information

**Supplementary Table S1: Cohort characteristics**

<b>Cohort</b>	<b>n</b>	<b>Female (n)</b>	<b>Age (median)</b>	<b>Age (min)</b>	<b>Age (max)</b>	<b>Days since last vacc./inf. (median)</b>	<b>Days since last vacc./inf. (min)</b>	<b>Days since last vacc./inf. (max)</b>
<b>2 x Vacc.- monov.</b>	11	9	40	20	81	20	15	34
<b>3 x Vacc.- monov.</b>	14	11	45.5	27	64	27.5	15	40
<b>4 x Vacc.- biv. (BA.1/WT)</b>	9	4	52	38	62	21	20	28
<b>4 x Vacc.- biv. (BA.5/WT)</b>	13	8	50	32	57	21	21	30
<b>WT infection</b>	18	4	48	22	77	19.5	11	25
<b>Prepandemic control</b>	30	15	23	19	55	/	/	/

*Age in years, Days since last vacc./inf.: Days since last vaccination (vaccinated cohorts) or infection (WT infection cohort). /: not applicable.*

**Supplementary Table S2: Vaccination history**

<b>Cohort</b>	<b>Vaccination history</b>	<b>n</b>
<b>2 x Vacc.-monov.</b>	P + P	10
	P + P	1
<b>3 x Vacc.-monov.</b>	P + P + P	11
	P + P + M	3
<b>4 x Vacc.-biv. (BA.1/WT)</b>	P + P + P + P-BA.1	8
	AZ + AZ + P + P-BA.1	1
<b>4 x Vacc.-biv. (BA.5/WT)</b>	P + P + P + P-BA.5	9
	J + P + P + P-BA.5	3
	AZ + AZ + P + P-BA.5	1
<b>WT infection</b>	not vaccinated	18
<b>Prepandemic control</b>	not vaccinated	30

AZ: Astra-Zeneca “Vaxzevria” ChAdOx1; P: Monovalent Biontech/Pfizer “Comirnaty” BNT162b2; P-BA.1: Biontech/Pfizer Bivalent (WT/BA.1) BNT162b2 BA.1; P-BA.5: Biontech/Pfizer Bivalent (WT/BA.5) BNT162b2 BA.5, M: Moderna “Spikevax” mRNA-1273. n: number.

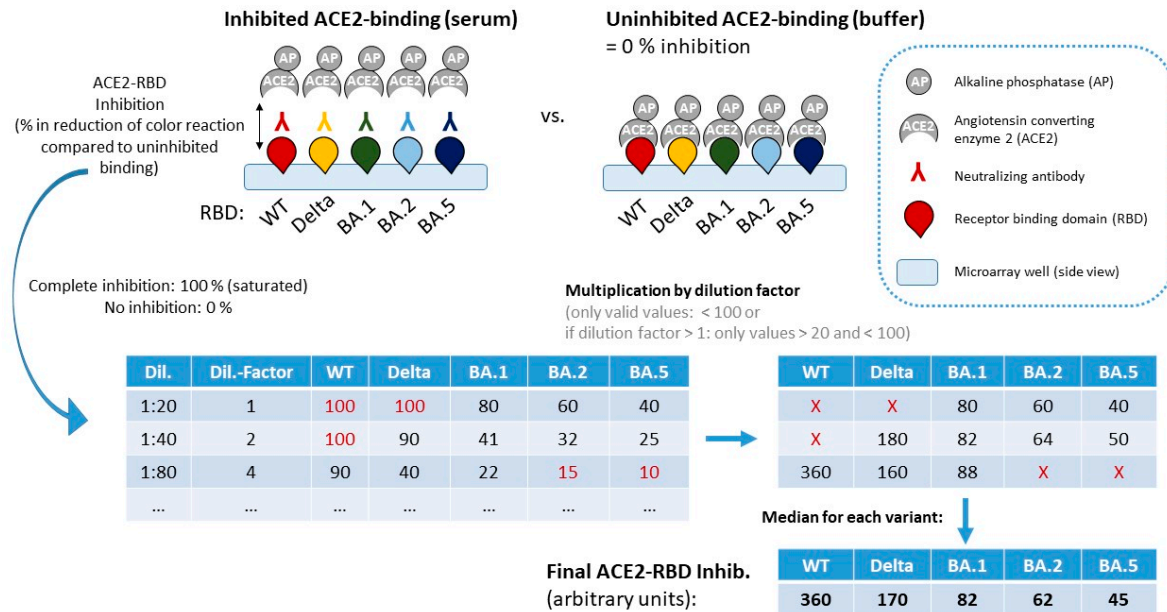
**Supplementary Table S3: sVNT cutoff values obtained by ROC and Youden’s Index**

	<b>Cutoff value</b>	<b>Sensitivity</b>	<b>Specificity</b>
<b>WT</b>	26	>99 %	>99 %
<b>Delta</b>	43	>99 %	>99 %
<b>BA.1</b>	24	92 %	78 %
<b>BA.2</b>	9	91 %	87 %
<b>BA.5</b>	28	88 %	92 %

**Supplementary Table S4: Pairwise cohortwise-comparisons of variant/WT ratios (Wilcoxon test)**

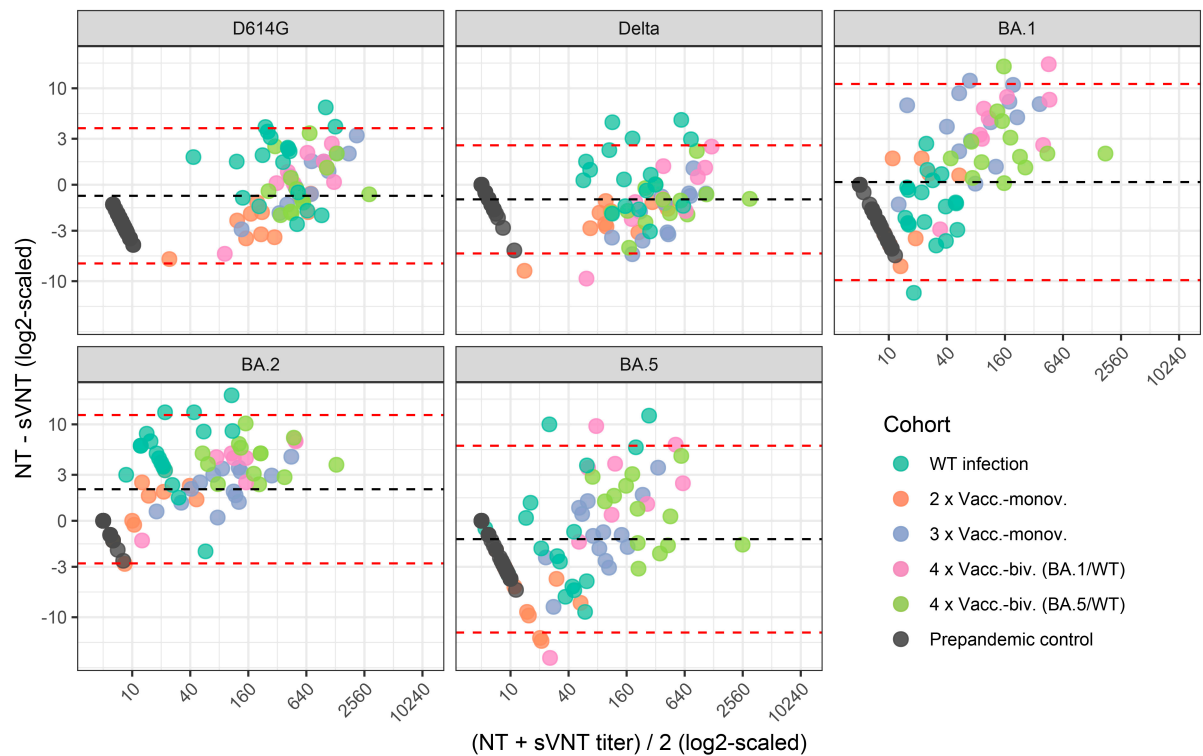
<b>Ratio</b>	<b>Cohort</b>	<b>WT infection</b>	<b>2 x Vacc.- monov.</b>	<b>3 x Vacc.- monov.</b>	<b>4 x Vacc.-biv. (BA.1/WT)</b>
<b>Delta/WT</b>	<b>2 x Vacc.- monov.</b>	n.S.			
	<b>3 x Vacc.- monov.</b>	***	****		
	<b>4 x Vacc.-biv. (BA.1/WT)</b>	**	***	n.S.	
	<b>4 x Vacc.-biv. (BA.5/WT)</b>	**	***	n.S.	n.S.
<b>BA.1/WT</b>	<b>2 x Vacc.- monov.</b>	**			
	<b>3 x Vacc.- monov.</b>	n.S.	n.S.		
	<b>4 x Vacc.-biv. (BA.1/WT)</b>	n.S.	***	*	
	<b>4 x Vacc.-biv. (BA.5/WT)</b>	*	****	**	n.S.
<b>BA.2/WT</b>	<b>2 x Vacc.- monov.</b>	n.S.			
	<b>3 x Vacc.- monov.</b>	****	****		
	<b>4 x Vacc.-biv. (BA.1/WT)</b>	****	****	n.S.	
	<b>4 x Vacc.-biv. (BA.5/WT)</b>	****	****	n.S.	n.S.
<b>BA.5/WT</b>	<b>2 x Vacc.- monov.</b>	n.S.			
	<b>3 x Vacc.- monov.</b>	n.S.	n.S.		
	<b>4 x Vacc.-biv. (BA.1/WT)</b>	n.S.	n.S.	n.S.	
	<b>4 x Vacc.-biv. (BA.5/WT)</b>	***	***	**	n.S.

\*\*\*\* p < 0.0001, \*\*\* p < 0.001, \*\* p < 0.01, \* p < 0.05, n.s.: not significant (p > 0.05) in pairwise Wilcoxon signed rank tests comparing the variant / WT ratios between cohorts for each variant (multiplicity adjusted using Bonferroni-Holm within each variant).



### Supplementary Figure S1: Schematic overview of the sVNT result calculation

ACE2-RBD inhibition was obtained by measuring the reduction in binding of ACE2-AP to recombinant variant RBDs in the presence/absence of serum neutralizing antibodies relative to the control well (buffer). A value of 100 indicated total inhibition (i.e. no binding and no color reaction). This was repeated at serial two-fold dilutions of 1:20 up to 1:360 for each serum, and, additionally up to 1:2560 in some cases. Next, all valid values (i.e. not oversaturated (= 100) for all dilutions and not below 20 for all dilutions except 1:20) were corrected for the dilution relative to 1:20 (i.e. multiplication by the dilution factor relative to 1:20). Invalid values are indicated in red font. Finally, the median of all valid values was calculated for each variant. Due to the dilution correction, values above 100 can occur; thus, the results are given in arbitrary units instead of % inhibition. ACE2-AP: Angiotensin converting enzyme 2 bound to alkaline phosphatase, Dil.: dilution; Dil.-Factor: dilution factor; RBD: receptor binding domain, WT: wildtype.



### Supplementary Figure S2: Bland-Altman analysis

The panels display the virus variants against which neutralizing activity was measured using a sVNT and NT. The x-axis shows the mean result (“titer”) of the two measurement methods (mean calculated after log2-transformation). The y-axis shows the difference between the two measurement methods (also calculated after log2-transformation). Each serum is represented by one dot (color coded for the cohorts). The black dotted line indicates the mean and the red lines indicate the 95% agreement intervals. biv.: bivalent; NT: neutralization test; sVNT: surrogate virus neutralization test; Vacc.: vaccinated; WT: wildtype.