1   **SUPPLEMENTARY INFORMATION**

2   **mRNA vaccines and hybrid immunity use different B cell germlines against Omicron BA.4 and BA.5**

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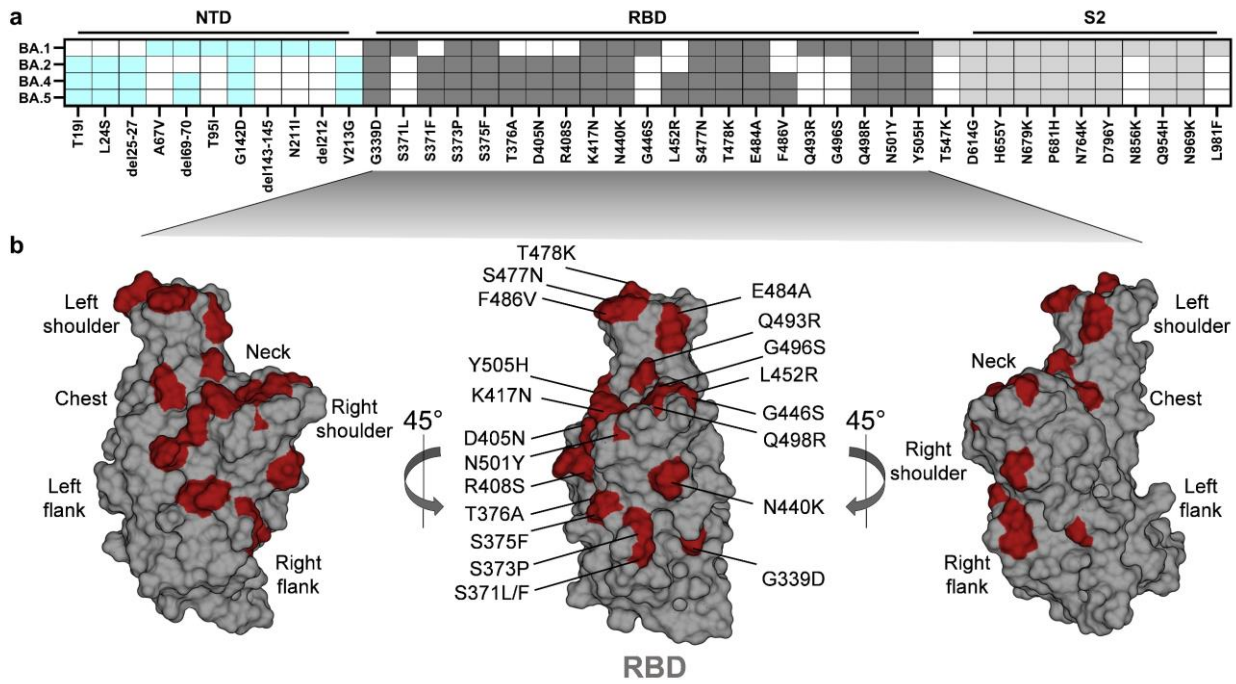11   [5]Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy

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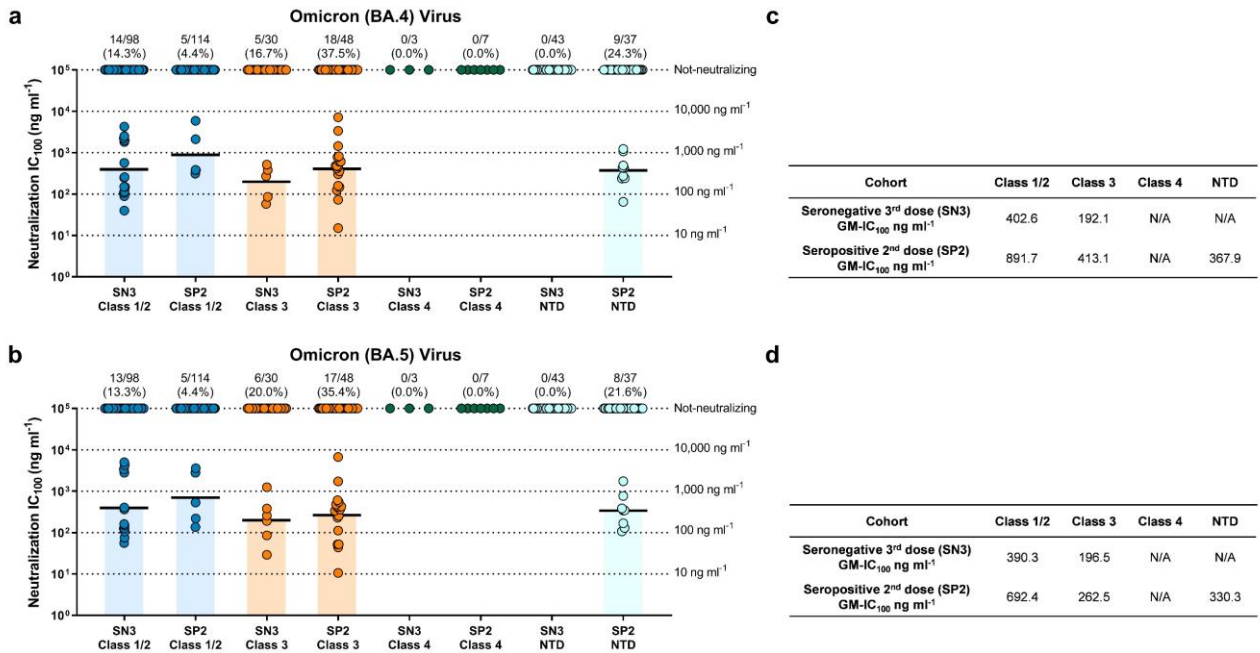15   [8]Fondazione Biotecnopolo di Siena, Siena, Italy

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Supplementary Fig. 1. Distribution of omicron mutations on SARS-CoV-2 S protein. a, the heatmap shows

the SARS-CoV-2 S protein mutations in the NTD (cyan), RBD (dark gray) and S2 domain (light gray) of omicron

BA.1, BA.2, BA.4 and BA.5. **b,** Representation of the RBD with all omicron mutations highlighted in red.

Omicron (BA.4) Virus (a)

Column labels: SN3 Class 1/2 (14/98, 14.3%), SP2 Class 1/2 (5/114, 4.4%), SN3 Class 3 (5/30, 16.7%), SP2 Class 3 (18/48, 37.5%), SN3 Class 4 (0/3, 0.0%), SP2 Class 4 (0/7, 0.0%), SN3 NTD (0/43, 0.0%), SP2 NTD (9/37, 24.3%)

Omicron (BA.5) Virus (b)

Column labels: SN3 Class 1/2 (13/98, 13.3%), SP2 Class 1/2 (5/114, 4.4%), SN3 Class 3 (6/30, 20.0%), SP2 Class 3 (17/48, 35.4%), SN3 Class 4 (0/3, 0.0%), SP2 Class 4 (0/7, 0.0%), SN3 NTD (0/43, 0.0%), SP2 NTD (8/37, 21.6%)

c

Cohort	Class 1/2	Class 3	Class 4	NTD
Seronegative 3rd dose (SN3) GM-IC$_{100}$ ng ml^{-1}	402.6	192.1	N/A	N/A
Seropositive 2nd dose (SP2) GM-IC$_{100}$ ng ml^{-1}	891.7	413.1	N/A	367.9

d

Cohort	Class 1/2	Class 3	Class 4	NTD
Seronegative 3rd dose (SN3) GM-IC$_{100}$ ng ml^{-1}	390.3	196.5	N/A	N/A
Seropositive 2nd dose (SP2) GM-IC$_{100}$ ng ml^{-1}	692.4	262.5	N/A	330.3

Supplementary Fig. 2. Distribution and neutralization potency of RBD and NTD-targeting nAbs against omicron BA.4 and BA.5. a-b, dot charts compare the distribution of nAbs isolated from subjects belonging to the SN3 and SP2 cohorts against omicron BA.4 (**a**) and BA.5 (**b**) omicron variants. The number, percentage and GM-IC$_{100}$ (black lines and colored bars) of nAbs are denoted on each graph. **c-d**, table summarizing the GM-IC$_{100}$ of Class 1/2, Class 3, Class 4 and NTD nAbs against BA.4 (**c**) and BA.5 (**d**). Source data are provided as a Source Data file.

29   **SUPPLEMENTARY TABLES**

30   **Supplementary Table 1. Numbers and frequency of nAbs in the SN3 cohort.**

Subject	Vaccine	Wuhan – Neutralizing antibodies	Omicron BA.1 – Neutralizing antibodies (%)	Omicron BA.2 – Neutralizing antibodies (%)	Omicron BA.4 – Neutralizing antibodies (%)	Omicron BA.5 – Neutralizing antibodies (%)
VAC-001	BNT162b2	106	22 (20.8)	24 (22.6)	11 (10.4)	9 (8.5)
VAC-002	BNT162b2	57	15 (26.3)	17 (29.8)	9 (15.8)	9 (15.8)
VAC-008	BNT162b2	36	7 (19.4)	9 (25.0)	4 (11.1)	5 (13.9)
VAC-010	mRNA-1273	7	1 (14.3)	2 (28.6)	0 (0.0)	0 (0.0)
	Total	**206**	**45 (21.8)**	**52 (25.2)**	**24 (11.6)**	**23 (11.2)**

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32   **Supplementary Table 2. Frequency of predominant germlines against omicron variants.**

Seronegative 2nd doses (SN2)					
Germlines	Wuhan Virus (%)	Omicron (BA.1) (%)	Omicron (BA.2) (%)	Omicron (BA.4) (%)	Omicron (BA.5) (%)
IGHV1-69;IGHJ4-1	2 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
IGHV3-30;IGHJ6-1	2 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
IGHV3-53;IGHJ6-1	6 (13.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)
IGHV3-66;IGHJ4-1	3 (6.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tot. nAbs	46	1	4	0	0
Tot. germline specific nAbs	13 (28.3)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)
Seronegative 3rd doses (SN3)					
Germlines	Wuhan Virus (%)	Omicron (BA.1) (%)	Omicron (BA.2) (%)	Omicron (BA.4) (%)	Omicron (BA.5) (%)
IGHV1-58;IGHJ3-1	12 (6.8)	5 (13.9)	4 (9.5)	1 (4.5)	0 (0.0)
IGHV1-69;IGHJ3-1	10 (5.7)	4 (11.1)	5 (11.9)	1 (4.5)	3 (13.6)
IGHV1-69;IGHJ4-1	16 (9.1)	9 (25.0)	9 (21.4)	4 (18.2)	4 (18.2)
IGHV3-66;IGHJ4-1	9 (5.1)	0 (0.0)	1 (2.4)	2 (9.1)	3 (13.6)
IGHV3-66;IGHJ6-1	10 (5.7)	3 (8.3)	4 (9.5)	4 (18.2)	3 (13.6)
Tot. nAbs	176	36	42	22	22
Tot. germline specific nAbs	57 (32.4)	21 (58.3)	23 (54.8)	12 (54.6)	13 (59.1)
Seropositive 2nd doses (SP2)					
Germlines	Wuhan Virus (%)	Omicron (BA.1) (%)	Omicron (BA.2) (%)	Omicron (BA.4) (%)	Omicron (BA.5) (%)
IGHV1-24;IGHJ6-1	5 (2.4)	0 (0.0)	3 (6.1)	3 (10.0)	3 (10.3)
IGHV1-58;IGHJ3-1	10 (4.8)	7 (18.9)	5 (10.2)	0 (0.0)	0 (0.0)
IGHV2-5;IGHJ4-1	9 (4.3)	2 (5.4)	6 (12.2)	6 (20.0)	6 (20.7)
Tot. nAbs	208	37	49	30	29
Tot. germline specific nAbs	24 (11.5)	9 (24.3)	14 (28.6)	9 (30.0)	9 (31.0)

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34   **Supplementary Table 3. Neutralization potency of predominant germlines against omicron variants.**

Germlines	Wuhan Virus GM-IC$_{100}$ ng ml^{-1}	Omicron (BA.1) GM-IC$_{100}$ ng ml^{-1}	Omicron (BA.2) GM-IC$_{100}$ ng ml^{-1}	Omicron (BA.4) GM-IC$_{100}$ ng ml^{-1}	Omicron (BA.5) GM-IC$_{100}$ ng ml^{-1}
Seronegative 2nd doses (SN2)					
IGHV1-69;IGHJ4-1	191.63	N/A	N/A	N/A	N/A
IGHV3-30;IGHJ6-1	382.71	N/A	N/A	N/A	N/A
IGHV3-53;IGHJ6-1	79.58	N/A	25.60	N/A	N/A
IGHV3-66;IGHJ4-1	66.42	N/A	N/A	N/A	N/A
Seronegative 3rd doses (SN3)					
IGHV1-58;IGHJ3-1	93.79	157.36	192.01	4261.36	N/A
IGHV1-69;IGHJ3-1	50.21	155.79	124.13	39.67	616.76
IGHV1-69;IGHJ4-1	212.38	987.84	1069.88	1318.53	1927.94
IGHV3-66;IGHJ4-1	327.61	N/A	66.17	253.58	578.35
IGHV3-66;IGHJ6-1	272.30	295.43	223.63	494.97	453.70
Seropositive 2nd doses (SP2)					
IGHV1-24;IGHJ6-1	314.04	N/A	130.20	413.36	463.99
IGHV1-58;IGHJ3-1	52.19	852.49	982.36	N/A	N/A
IGHV2-5;IGHJ4-1	28.10	379.68	197.85	264.09	166.37

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