Supplemental materials

Table S1. Timing of mass vaccination and sampling points

Sampling point	0	Mass vaccination	1	2	3	4	Mass vaccination	5	6	7
Timing	Before vaccination	BNT162b2 monovalent	3 weeks after the second dose; 3 wks aft 2nd	8 weeks after the second dose; 8 wks aft 2nd	3 months after the second dose; 3 mos aft 2nd	6 months after the second dose; 6 mos aft 2nd	BNT162b2 monovalent	3 weeks after the third dose; 3 wks aft 3rd	8 weeks after the third dose; 8 wks aft 3rd	6 months after the third dose; 6 mos aft 3rd
Time period	16/02/2021 -09/03/2021	1st dose 05/03/2021 -12/03/2021 & 2nd dose 26/03/2021 -02/04/2021	15/04/2021 -28/04/2021	20/05/2021 -02/06/2021	28/06/2021 -09/07/2021	28/09/2021 -08/10/2021	3rd dose 20/12/2021 -28/12/2021	11/01/2022 -21/01/2022	14/02/2022 -25/02/2022	28/09/2021 -08/10/2021
RBD-IgG	✓		✓	✓	✓	✓		✓	✓	✓
NA-Omicron								✓		
QFN	✓			✓		✓			✓	✓

Abbreviations:

RBD-IgG, anti-receptor binding domain IgG titer measured using SARS-CoV-2 IgG II Quant reagents and Alinity Analyzer (Abbott Laboratories, Abbott Park, IL, USA)；NA-Omicron, Neutralizing antibody titer for Omicron variants measured using clinically isolated Omicron (B.1.1.529) variant strain; QFN, T-cell immunity assessed by QuantiFERON SARS-CoV-2 tests (Qiagen, Hilden, Germany)

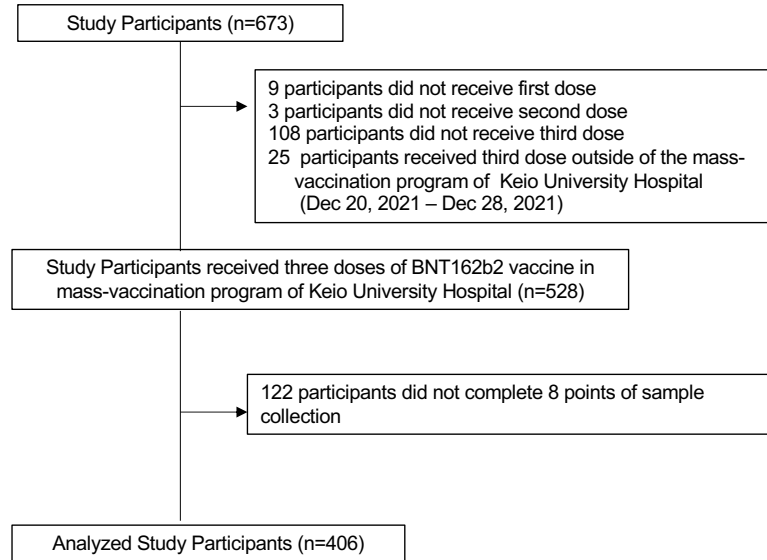**Fig S1. Flow chart of the participants included in the study**

```
┌─────────────────────────────────┐
│  Study Participants (n=673)      │
└─────────────────────────────────┘
         │
         │      ┌──────────────────────────────────────────────────────┐
         ├─────>│ 9 participants did not receive first dose            │
         │      │ 3 participants did not receive second dose           │
         │      │ 108 participants did not receive third dose          │
         │      │ 25  participants received third dose outside of the  │
         │      │     mass-vaccination program of  Keio University     │
         │      │     Hospital (Dec 20, 2021 – Dec 28, 2021)           │
         │      └──────────────────────────────────────────────────────┘
         │
┌───────────────────────────────────────────────────────┐
│ Study Participants received three doses of BNT162b2    │
│ vaccine in mass-vaccination program of Keio University │
│ Hospital (n=528)                                       │
└───────────────────────────────────────────────────────┘
         │
         │      ┌──────────────────────────────────────────────────────┐
         ├─────>│ 122 participants did not complete 8 points of sample  │
         │      │ collection                                            │
         │      └──────────────────────────────────────────────────────┘
         │
┌─────────────────────────────────────┐
│ Analyzed Study Participants (n=406)  │
└─────────────────────────────────────┘
```

Table S2. Demographics of the analyzed participants.

	N=406	
Age		
Median age, years (IQR)	47	(39 – 54)
≥50 years-old (%)	167	(41.1)
<50 years-old (%)	239	(58.9)
Sex		
Male (%)	104	(25.6)
Female (%)	302	(74.4)
Median BMI, kg/m^2 (IQR)	21.5	(19.7 – 23.9)
History before vaccination		
Immunosuppressant use (%)	14	(3.4)
Malignancy (%)	7	(1.7)
Diabetes (%)	3	(0.7)
COVID-19 (%)	5	(1.2)
Geometric mean RBD-IgG titer before vaccination, AU/mL (95%CI)	3.08	(2.38 – 3.80)
Geometric mean of QFN results		
IFN for Ag1, IU/mL (95%CI)	0.02	(0.02 – 0.03)
IFN for Ag2, IU/mL (95%CI)	0.02	(0.02 – 0.02)

Abbreviations: IQR, interquartile range; BMI, body mass index; RBD-IgG, Anti-receptor binding domain IgG; QFN, QuantiFERON; IFN, interferon gamma; Ag, antigen.

Table S3 Participants with laboratory confirmed breakthrough infections.

Age	Sex	Severity	RBD-IgG titer, AU/mL (The time periods of breakthrough infection episodes are demonstrated as "Star")						
			3wks aft 2nd	8wks aft 2nd	3mos aft 2nd	6mos aft 2nd	3wks aft 3rd	8wks aft 3rd	6mos aft 3rd
60's	F	Asymptomatic	13,457.0	4,860.6	2,426.5	844.7	15,840.3	11,095.8	★ 45,863.7
50's	F	Mild	15,838.9	6,091.3	3,280.5	2,282.5	10,916.9	6,962.3	★ 85,181.3
50's	F	Mild	13,720.5	5,135.1	2,536.1	860.8	17,842.9	★ 136,802.0	72,933.0
30's	M	Mild	10,630.2	4,179.5	1,918.9	737.5	15,661.5	7,974.8	★ 44,178.8
30's	F	Mild	39,878.3	16,044.1	7,565.1	2,264.9	29,523.7	19,863.9	★ 91,193.3
30's	F	Mild	6,603.2	2,367.1	1,008.3	574.2	15,758.2	8,184.5	★ 30,962.8
40's	F	Mild	18,811.6	8,738.3	5,614.8	2,339.3	29,329.0	17,878.1	★ 52,224.9
40's	F	Asymptomatic	12,826.5	4,604.3	1,487.1	253.7	24,832.2	16,709.7	★ 9,279.5
30's	M	Mild	6,960.0	3,192.5	1,727.6	709.9	8,692.9	8,000.8	★ 70,420.8
40's	F	Mild	13,912.9	4,919.1	2,414.8	900.3	25,224.9	★ 65,290.4	37,240.3
40's	F	Mild	35,454.8	15,811.5	7,118.4	1,335.0	36,027.6	19,181.4	★ 38,348.4
40's	F	Mild	7,169.9	2,924.2	1,522.3	518.7	7,254.3	4,248.6	★ 11,905.6
40's	F	Mild	26,145.8	8,917.6	3,983.5	794.0	27,534.3	13,588.2	★ 17,304.0
40's	M	Mild	3,827.1	1,514.4	766.2	277.0	7,289.3	★* 5,985.2	21,684.7
50's	F	Asymptomatic	14,127.6	5,697.4	2,287.1	657.6	19,111.7	★ 12,207.4	3,652.7
40's	F	Asymptomatic	7,947.0	2,555.9	1,346.9	359.8	12,850.3	10,307.8	★ 35,359.3
20's	F	Mild	29,500.0	8,766.7	4,412.9	1,191.3	25,160.6	15,500.4	★ 53,450.4
40's	F	Mild	15,777.1	6,725.2	3,116.1	1,011.6	15,890.5	10,963.3	★ 36,764.8
20's	F	Mild	21,306.7	9,084.4	4,225.0	1,622.2	17,422.0	10,237.1	★ 30,779.9
50's	F	Mild	29,254.3	13,839.3	10,055.7	3,920.2	35,839.9	18,557.3	★ 29,194.5
40's	F	Mild	20,958.5	8,789.1	4,509.3	1,073.3	31,373.8	14,440.3	★ 74,296.5
40's	F	Mild	17,364.3	6,953.2	3,924.4	666.8	12,897.0	9,346.6	★ 73,406.2
50's	F	Mild	9,454.2	6,310.2	4,621.6	★ 38,653.5	27,120.6	24,100.7	14,016.7
30's	F	Mild	50,183.2	17,769.2	7,915.8	1,997.1	32,135.6	22,632.1	★ 30,907.3
60's	M	Mild	24,801.5	11,430.1	6,550.6	1,809.6	16,760.7	15,984.8	★ 66,078.6
30's	F	Mild	9,408.9	3,956.5	1,808.3	780.0	12,850.0	9,386.4	★ 37,430.8
30's	M	Mild	9,052.5	3,108.1	1,481.2	★ 530.1	20,863.3	15,366.1	5,388.3
30's	F	Mild	4,223.3	1,533.9	662.6	208.1	6,554.8	4,070.4	★ 27,965.5

Severity is defined according to the COVID-19 practice manual by Ministry of Health, Labour and Welfare, Japan. Asymptomatic cases were defined as patients who had positive COVID-19 diagnostic test (antigen test or molecular test) results without COVID-19 symptoms. Mild cases were defined as symptomatic COVID-19 patients without any signs of pneumonia or oxygen demands.

*The onset of breakthrough infection was at the same day the samples of 8wks aft 3rd were collected.

Fig S2. Comparison of RBD-IgG and QFN between the participants with laboratory confirmed breakthrough infections and those without infections.
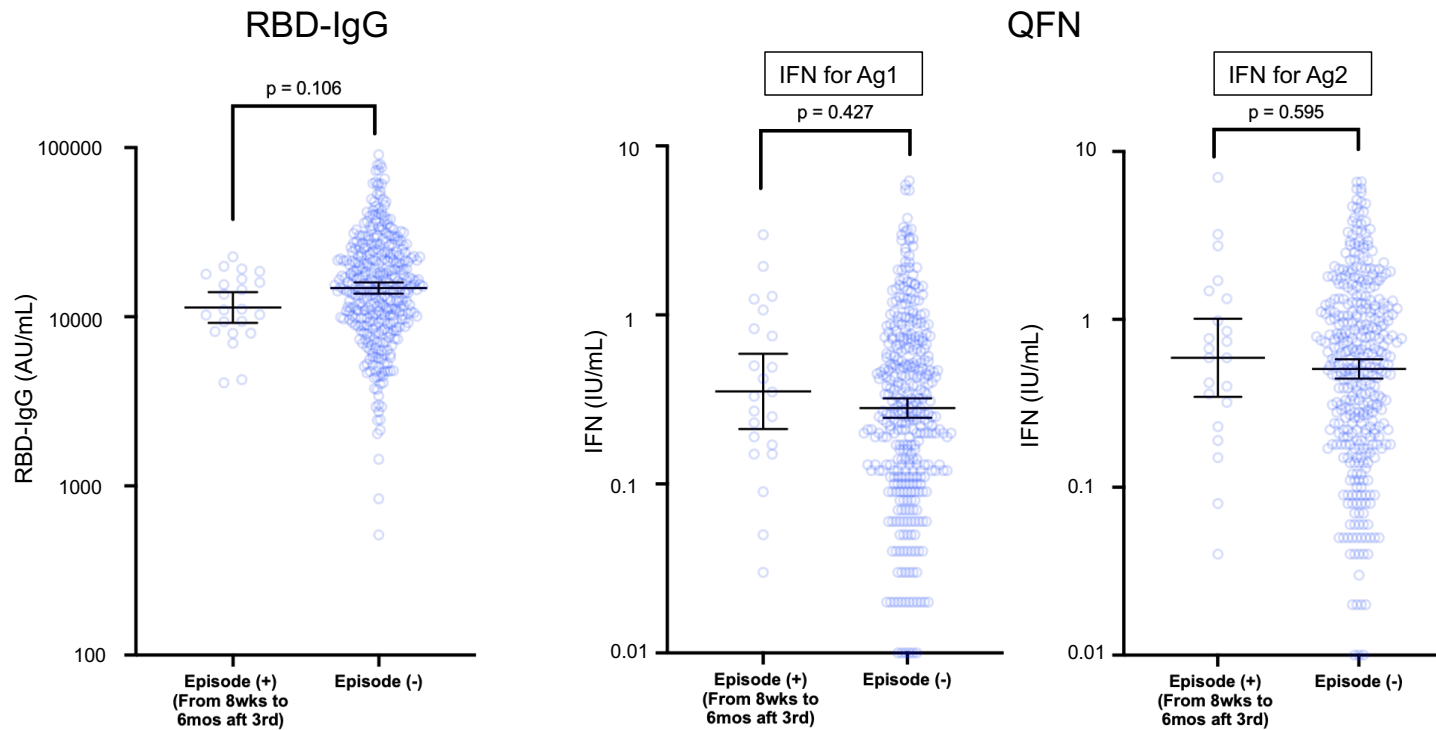

RBD-IgG antibody titer and QFN results at 8 weeks after 3rd dose administration were compared between the participants with or without breakthrough. The participants who had an episode of breakthrough infection from 8 weeks to 6 months after 3rd dose administration were shown as Episode (+) (N = 22), while those who had no episodes of breakthrough infections during the study period were as Episode (-) (N = 378). Geometric means and 95% confidence intervals were demonstrated. A parametric test of the logarithm was used for comparison.

Abbreviations: QFN, QuantiFERON; RBD-IgG, Anti-receptor binding domain IgG; IFN, interferon gamma; Ag, antigen.

Table S4 Participants who received the fourth dose of COVID-19 vaccine.

| Age*	Sex	The fourth dose of COVID-19 vaccine		8wks aft 3rd				6mos aft 3rd			
		Date of administration	Brand†	Date of sample collection	Antibody RBD-IgG (AU/mL)	QFN IFN for Ag1 (IU/mL)	IFN for Ag2 (IU/mL)	Date of sample collection	Antibody RBD-IgG (AU/mL)	QFN IFN for Ag1 (IU/mL)	IFN for Ag2 (IU/mL)
60's	F	22-Jun	Comirnaty	16-Feb	512.7	0.00	0.00	6-Jul	5,204.4	0.03	0.02
60's	F	23-Jun	Spikevax	15-Feb	7,038.1	0.10	0.09	7-Jul	14,431.8	0.07	0.17
60's	F	23-Jun	Spikevax	14-Feb	11,095.8	0.49	0.32	1-Jul	45,863.7	0.73	0.68
60's	F	24-Jun	Spikevax	17-Feb	14,202.3	0.89	0.93	7-Jul	17,117.4	0.19	0.30
60's	M	24-Jun	Spikevax	18-Feb	31,439.4	0.14	0.51	4-Jul	37,989.0	1.08	1.82
60's	M	11-Jun	Comirnaty	15-Feb	11,898.5	0.01	0.01	1-Jul	146,518.7	0.01	0.01
60's	M	23-Jun	Spikevax	18-Feb	24,102.1	0.74	1.05	1-Jul	39,255.2	1.53	1.24
60's	F	23-Jun	Spikevax	22-Feb	6,967.3	0.01	0.02	6-Jul	20,134.3	0.09	0.13
60's	M	18-Jun	Spikevax	15-Feb	9,380.5	0.20	0.18	1-Jul	31,555.4	0.73	0.76
60's	M	24-Jun	Spikevax	22-Feb	33,983.1	0.66	1.07	8-Jul	44,413.4	1.31	1.64
60's	F	8-Jun	Comirnaty	15-Feb	35,141.2	0.48	0.46	28-Jun	56,935.4	0.35	0.40
60's	F	23-Jun	Spikevax	16-Feb	10,269.2	0.02	0.06	30-Jun	21,639.9	0.38	0.51
60's	F	24-Jun	Spikevax	24-Feb	2,939.2	0.11	0.15	4-Jul	10,248.9	0.17	0.17
60's	F	23-Jun	Spikevax	22-Feb	21,579.5	0.06	0.05	1-Jul	35,701.6	0.10	0.08
30's	F	11-Jun	Comirnaty	24-Feb	840.8	0.20	0.27	1-Jul	4,410.7	1.25	2.07
60's	F	18-Jun	Comirnaty	14-Feb	23,902.6	0.26	0.83	29-Jun	29,680.0	1.13	1.03
60's	F	24-Jun	Spikevax	18-Feb	5,757.9	0.07	0.09	8-Jul	10,999.3	0.31	0.43
50's	M	24-Jun	Spikevax	21-Feb	10,385.6	0.17	0.54	30-Jun	18,481.0	0.58	0.38
60's	F	23-Jun	Spikevax	16-Feb	30,273.3	0.70	0.73	30-Jun	37,424.1	2.60	1.80

*In Japan, 4th dose of the COVID-19 vaccine were recommended for all people aged over 60 years-old and adult people with underlying illness from May 25th, 2022 to July 23th, 2022. †Comirnaty vaccine was BNT162b2 monovalent vaccine (Pfizer, NY, USA) and Spikevax vaccine was mRNA-1273 monovalent vaccine (Moderna, MA, USA).
Abbreviations: QFN, QuantiFERON; RBD-IgG, Anti-receptor binding domain IgG; IFN, interferon gamma; Ag, antigen.

Fig S3. Relationship between RBD-IgG and NA-Omicron.
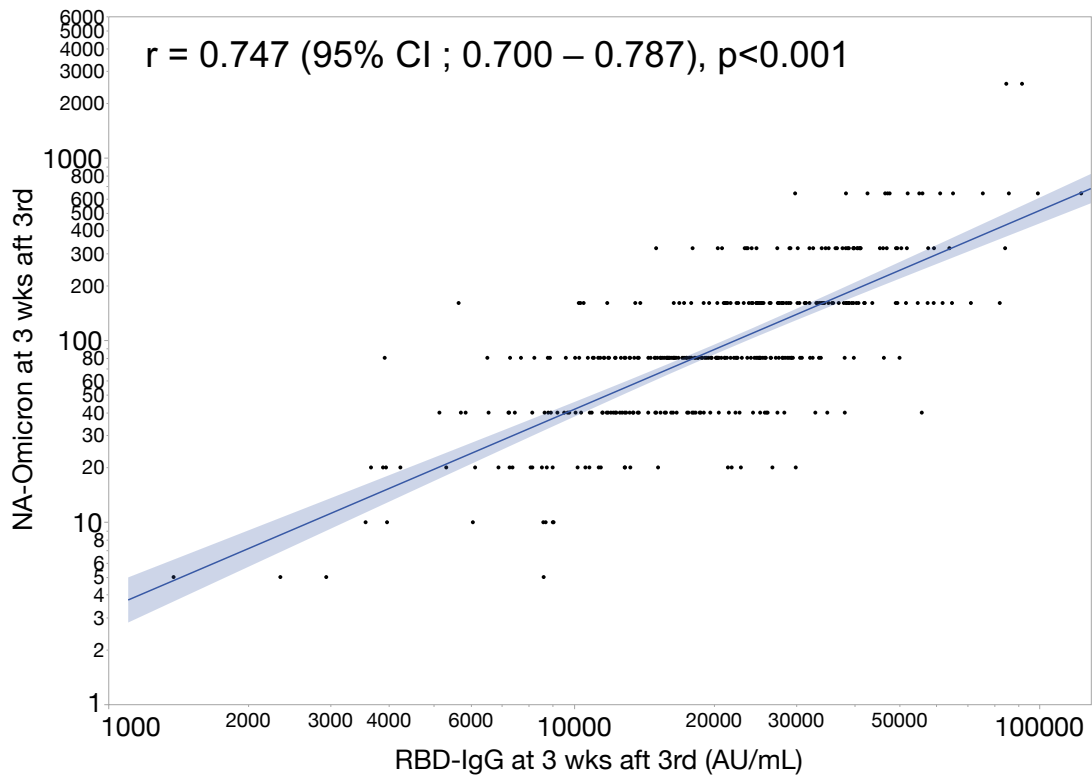
*RBD-IgG: anti-receptor binding domain IgG titer measured using SARS-CoV-2 IgG II Quant reagents and Alinity Analyzer (Abbott Laboratories, Abbott Park, IL, USA). NA-Omicron: Neutralizing antibody titer for Omicron variants measured using clinically isolated Omicron (B.1.1.529) variant strain.

Fig S4. Mixed effect model analysis results of RBD-IgG and QFN

a) RBD-IgG

b) QFN
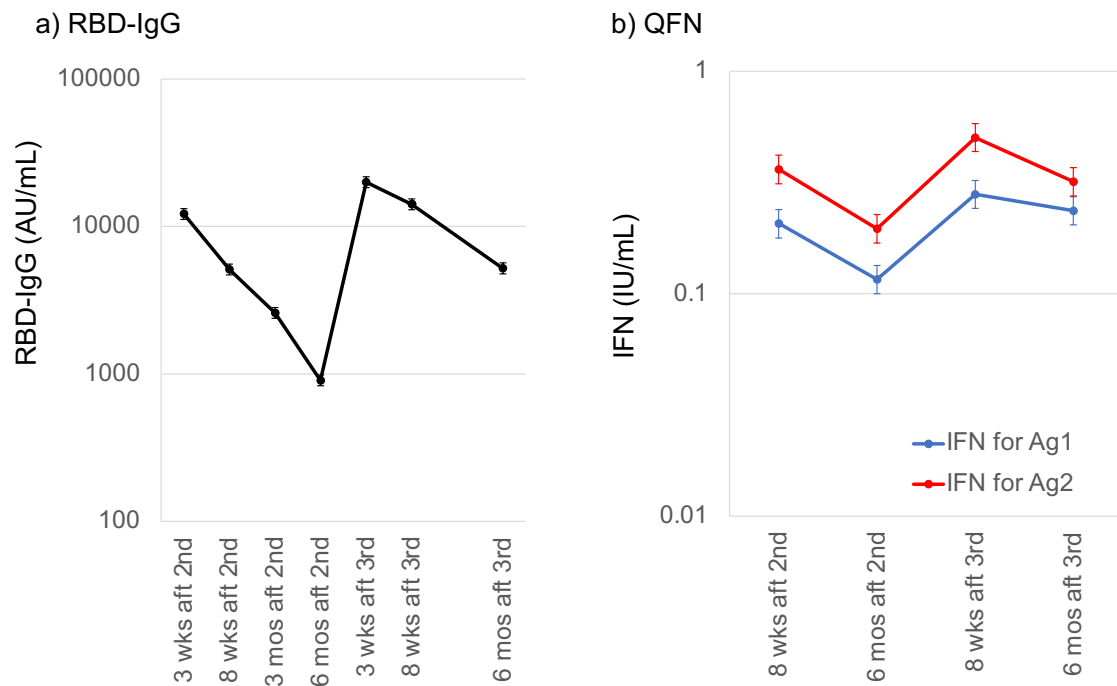
a) Mixed effects model analysis including logarithms of RBD-IgG titers at all seven blood collection points after vaccination, age groups and sex were performed. The plots demonstrated adjusted means and error bars demonstrated 95% confidence intervals. b) Mixed effects model analysis including logarithms of IFN for Ag1 or Ag2 titers at all four blood collection points after vaccination, age groups and sex were performed. The plots demonstrated adjusted means and error bars demonstrated 95% confidence intervals.

*Abbreviations: RBD-IgG, anti-receptor binding domain IgG titer；QFN, QuantiFERON SARS-CoV-2 tests (Qiagen, Hilden, Germany); IFN for Ag1, the interferon gamma levels for antigen 1；IFN for Ag2, the interferon gamma levels for antigen 2.