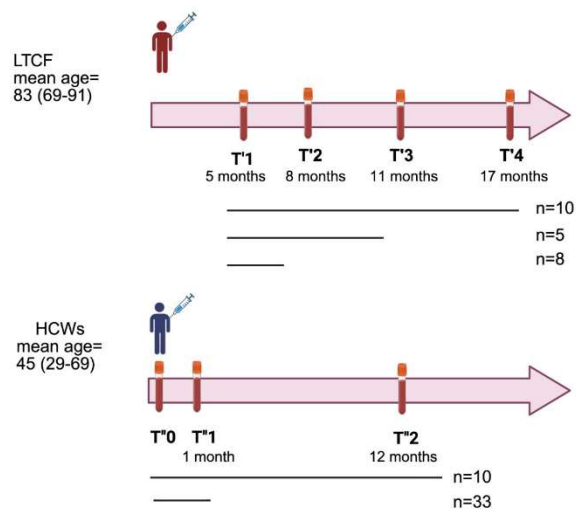


1 **Supplementary Figures and Tables**



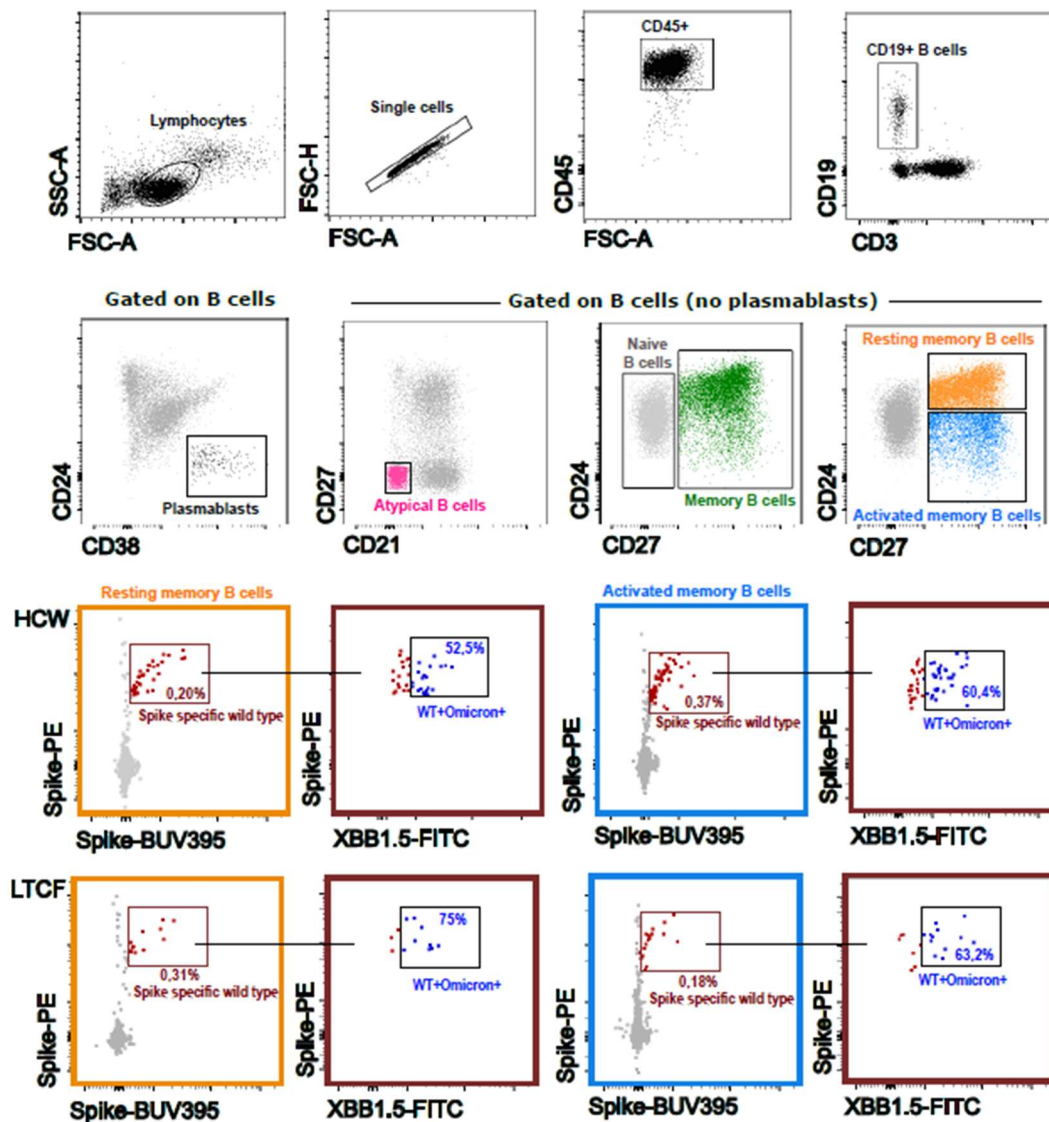
2

3 **Supplementary Figure 1. Schematic representation of the longitudinal follow-up for**
4 **participants enrolled in the B-cell characterization analysis.**

5 The timelines illustrate the scheduled blood sampling intervals (indicated by tube icons) for Long-
6 Term Care Facility (LTCF) residents and Healthcare Workers (HCWs) from sixth dose vaccination
7 with XBB.1.5 (T0). For the LTCF cohort, longitudinal monitoring began at 5 months (T1) and follow-
8 up extended to 17 months (T4), whereas for HCWs, sampling was performed up to 12 months (T2).
9 Mean age and age ranges are provided for each group. Horizontal lines indicate the number of
10 participants available at each time point.

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14 **Supplementary Figure 2:** Representative FACS plots illustrating the gating strategy used to identify
 15 B cell populations in peripheral blood using antibodies against CD19, CD24, CD27, CD38, and
 16 CD21. Within the CD19⁺ B cell gate, plasmablasts (PBs; black) are defined as CD24⁻CD38⁺⁺ cells.
 17 After exclusion of plasmablasts (not-plasmablast gate), naïve B cells were gated as CD27⁻ (grey) and
 18 memory B cells are identified as CD27⁺ cells (green). Within the CD27⁺ compartment, resting
 19 memory B cells (orange) are distinguished from activated memory B cells (blue). Among both resting
 20 and activated memory subsets, spike-specific wild-type (WT) B cells are identified (dark red). Among
 21 these, XBB1.5 (Omicron-specific) B cells are further delineated

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24 **Supplementary Table 1. Reagents and antibodies used for B-cell phenotyping and SARS-CoV-**
25 **2 antigen-specific B-cell identification.**

Antibodies and Fluorochrome	Manufacturer
IgD (IA6-2) BUV737	Beckton Dickinson
IgG (G18-145) RB613	Beckton Dickinson
IgM (Polyclonal) APC	Jackson ImmunoResearch
CD27 (L128) RB705	Beckton Dickinson
CD45 (2D1) APC-H7	Beckton Dickinson
CD3 (UCHT1) BUV496	Beckton Dickinson
CD21 (1048) BUV805	Beckton Dickinson
CD38 (HIT2) BV421	Beckton Dickinson
CD19 (SJ25C1) BV510	Beckton Dickinson
CD24 (ML5) BV711	Beckton Dickinson
FCRL5 (509F6) BV786	Beckton Dickinson
CD5 (UCHT2) RB780	Beckton Dickinson
CD11c PE-Cy7	Immunological Sciences
Streptavidin PE	Beckton Dickinson
Streptavidin BUV395	Beckton Dickinson
Streptavidin FITC	Beckton Dickinson
Protein	
Biotinylated SARS-CoV-2 S protein (D614G) trimer	Acro Biosystem
Biotinylated SARS-CoV-2 Spike trimer protein (XBB.1.5)	Acro Biosystem

26
27 Reagents and antibodies used for flow cytometry are listed with their respective clone names (in
28 parentheses), fluorochrome conjugates, and manufacturers. SARS-CoV-2 antigen-specific B-cells
29 were identified using biotinylated Spike (S) protein trimers (D614G and XBB.1.5 variants)
30 conjugated to different streptavidin-fluorochromes for multimeric detection.

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32 **Supplementary Table 2. Baseline clinical conditions and comorbidities**

Comorbidity	Cases (%)	Non vaccinated	Vaccinated	p-value
Arterial Hypertension	227 (58.5%)	102	125	0.67
Cognitive Decline / Dementia	198 (51%)	70	128	<0.001
Ischemic Heart Disease	136 (35%)	56	80	0.372
Osteoarthritis	126 (32.4%)	48	78	0.086
Depression	110 (28.3%)	48	62	0.88
Diabetes Mellitus	82 (21.1%)	41	41	0.22
Heart Failure	73 (18.8%)	27	46	0.17
COPD	68 (17.5%)	23	45	0.056
Peripheral Artery Disease	66 (17%)	24	42	0.16
Stroke	66 (17%)	21	45	0.025
Chronic Liver Disease	62 (16%)	24	38	0.34
Solid or Hematologic Malignancy	34 (8.7%)	13	21	0.47
Obesity	25 (6.4%)	11	14	0.99
Chronic Kidney Disease	20 (5.1%)	8	12	0.70
Malnutrition	20 (5.1%)	5	15	0.076
Inflammatory Bowel Disease	16 (4.1%)	6	10	0.39
Immunopathies	11 (2.8%)	3	9	0.17
Mobility Status				0.026
<i>Independent</i>				
Walks independently	71 (21.1%)	32	39	0.79
<i>Assistive devices required</i>				
Walks with a cane	21 (6.3%)	14	7	0.039
Walks with a walker	68 (20.2%)	38	30	0.044
Moves with a wheelchair	76 (22.6%)	24	51	0.009
<i>Highly limited / Bedbound</i>				
Non-ambulatory (assisted transfer to chair)	66 (20.2%)	32	34	0.53
Generally bedbound (occasionally sits up)	22 (6.6%)	8	14	0.39
Entirely bedbound (unable to sit independently)	10 (3%)	3	7	0.33

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34 Data are presented as absolute numbers and percentages N (%). The study population is stratified by
35 vaccination status (Vaccinated vs. Non-vaccinated). P-values indicate statistical differences between
36 the two groups.

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Supplementary Table 3. Baseline demographic, clinical, and immunological characteristics between participants who completed follow-up and those who did not.

	Non-completers (n=138)	Completers (n=250)	Total (n=388)	p-value
Age, mean (SD)	85.166 (8.30)	83.632 (8.86)	84.178 (8.69)	0.096
Number of chronic conditions, mean (SD)	4.083 (2.16)	3.991 (1.94)	4.021 (2.01)	0.696
Number of SARS-CoV-2 vaccine doses, mean (SD)	4.595 (0.99)	4.352 (0.91)	4.434 (0.94)	0.024
logS _{T0} , mean (SD)	3.735 (0.650)	3.710 (0.58)	3.719 (0.608)	0.708
Sex (1 = female*)	42 (30.7%)	72 (28.8%)	114 (29.5%)	0.702
Anti-N IgG positive T0	91 (65.9%)	169 (67.6%)	260 (67.0%)	0.74
COVID diagnosis last 6 months	1 (0.7%)	10 (4.0%)	11 (2.8%)	0.063
COVID diagnosis last 12 months	0 (0.0%)	1 (0.4%)	1 (0.3%)	0.458
Ischemic heart disease	36 (31.6%)	100 (44.2%)	136 (40.0%)	0.024
Obesity	3 (2.6%)	22 (9.7%)	25 (7.4%)	0.018
Malnutrition	12 (10.5%)	8 (3.5%)	20 (5.9%)	0.01

Values are presented as mean (standard deviation) for continuous variables and number (percentage) for categorical variables. p-values were obtained using t-test, Wilcoxon rank-sum test, chi-square test, or Fisher's exact test as appropriate.

Note: Due to missing or incomplete medical records at baseline for selected participants, the sample size for individual chronic conditions varies slightly across categories. Percentages are calculated based on the available data within each column.

Supplementary Table 4. Association between LTCF residents characteristics and longitudinal anti-Spike IgG titers at follow-up timepoints in LTCF residents.

Variable	T1	T2	T3
Sex (p-value)	0.051	0.800	0.498
Age (Spearman ρ)	0.004	0.006	0.036
Age (p-value)	0.944	0.914	0.575
Comorbidity burden (p-value)	0.425	0.704	0.966

Associations between longitudinal anti-Spike IgG titers and sex, age, and comorbidity burden were assessed at T1 (3 months), T2 (6 months), and T3 (12 months) follow-up visits. Sex differences were evaluated using the Mann-Whitney U test. Age was assessed using Spearman rank correlation. Comorbidity burden was assessed by stratifying residents into two groups (≤ 4 vs > 4 chronic conditions) and comparing anti-Spike IgG titers using the Mann-Whitney U test. No statistically significant associations were identified at any timepoint.

59 **Supplementary Table 5. Demographic characteristics of the B-cell analysis study populations.**

Variable	LTCF residents (N=25)	HCWs (N=33)	p-value
Age, years (Mean $\pm$ SD)	85.3 $\pm$ 8.7	42.2 $\pm$ 14.3	<0.001
Female, n (%)	19 (76.0%)	21 (63.6%)	0.39
Male, n (%)	6 (24.0%)	12 (36.4%)	
Vaccine doses received (Mean $\pm$ SD)	5.5 $\pm$ 0.5	4.4 $\pm$ 0.7	<0.001

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61 Data are presented as mean $\pm$ standard deviation (SD) for continuous variables and as absolute
62 numbers (percentages) for categorical variables. The study cohort for B-cell analysis is stratified by
63 population type: LTCF residents and HCWs. P-values indicate statistical differences between the
64 two groups.