Supplementary Information

Aberrant B cell differentiation with reduced hypermutation limits humoral response maintenance and breadth in cancer patients

Ki Seong Lee et al.

File description:

This PDF contains Supplementary Methods, Supplementary Figures 1–9 with corresponding legends, and Supplementary Table 1.

This Supplementary Information provides detailed experimental and computational methods, additional analyses of vaccine-induced B cell and antibody responses, single-cell transcriptomic and BCR repertoire analyses, and individual-level demographic and clinical characteristics.

File format: PDF

SUPPLEMENTARY METHODS

Blood sample processing and storage

Peripheral blood was collected into an ethylenediaminetetraacetic acid (EDTA) tube (BD Biosciences, 367525). From the samples, peripheral blood mononuclear cells (PBMCs) were purified by density gradient centrifugation using Lymphocyte separation medium (Corning, 25-072-CV). Purified PBMCs were cryopreserved in fetal bovine serum (FBS, Corning, 35-015-CV) supplemented with 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich, D2650) until use. Plasma samples were collected by centrifuging peripheral blood at 1800 rpm (revolutions per minute) for 10 minutes, then aliquoted and stored at -80°C.

Tetramer production

Biotinylated SARS-CoV-2 WT Spike Trimer, WT RBD, BA.1 RBD, BA.5 RBD, EG.5 RBD, and BA.2.86 RBD proteins (ACROBiosystems, SPN-C82E9; SPD-C82E9; SPD-C82E4; SPD-C82Ew; SPD-C82Q5; SPD-C82Q6) were mixed with fluorescently labeled streptavidin at a 4:1 molar ratio. Biotinylated WT Spike Trimer was conjugated to streptavidin-AF647 (Invitrogen, S21374) or streptavidin-BV650 (BD Biosciences, 563855). Biotinylated WT, BA.1, BA.5, EG.5, and BA.2.86 RBD proteins were each conjugated to streptavidin-BV421 (BD Biosciences, 563259), streptavidin-PE (Invitrogen, 12-4317-87), streptavidin-PE-Cy7 (BD Biosciences, 557598), streptavidin-BV786 (BD Biosciences, 563858), streptavidin-BV711 (BD Biosciences, 563262), respectively. A decoy reagent was generated by tetramerizing free biotin with streptavidin-AF647 R-Phycoerythrin (Invitrogen, S20992). Excess biotin was added immediately after generating the tetramer and decoy solutions, in order to prevent

unintended binding.

Flow cytometry

To analyze the antigen-specific B cells, PBMC samples were stained with 100 μL of Zombie Aqua Fixable Viability Kit (1:500; BioLegend, 423102) and Fc block (1:200; Miltenyi Biotec, 130-059-901) in DPBS at 4°C for 15 minutes. Following staining, 4 mL of DPBS were added to the samples, and filtered through a 35 μm filter cap tube (Falcon, 352235) to exclude aggregated dead cells. After filtering, samples were washed (centrifuged at 600g for 5 minutes) and stained with 25 nM decoy reagent in fluorescence-activated cell sorting (FACS) buffer (1X DPBS, 1% FBS) at room temperature for 20 minutes. Without washing, tetramers were directly added to the samples to achieve a final concentration of 0.5 nM, followed by incubation at 4°C for 30 minutes. After washing, samples were incubated in FACS buffer with the following anti-human antibodies at a 1:100 dilution at 4°C for 30 minutes: anti-CD27-BUV395 (BD Biosciences, 563815), anti-CD19-BUV496 (BD Biosciences, 612938), anti-IgG-BUV563 (BD Biosciences, 741396), anti-CD73-BUV615 (BD Biosciences, 751369), anti-CD38-BUV661 (BD Biosciences, 612969), anti-CD21-BUV737 (BD Biosciences, 612788), anti-CD45RB-BUV805 (BD Biosciences, 748735), anti-IgM-BV605 (BD Biosciences, 562977), anti-IgA-FITC (Miltenyi Biotec, 130-113-475), anti-IgD-PerCP-Cy5.5 (BD Biosciences, 561315), anti-CD71-RB780 (BD Biosciences, 755839), anti-CD95-PE-CF594 (BD Biosciences, 562395), anti-CD11c-AF700 (BioLegend, 337220), and anti-CD20-APC-Cy7 (BioLegend, 302314). The stained cells were fixed with 1% paraformaldehyde (Biosesang, PC2031-100-00) for 15 minutes at 4°C. Samples were analyzed using FACSymphony A3 Cell Analyzer (BD Biosciences) and FACSymphony A5 Cell Analyzer (BD Biosciences). Data were analyzed using FlowJo software.

IgG ELISpot assay for antigen-specific memory B cells

IgG ELISpot assays were performed to quantify antigen-specific memory B cell responses. Cryopreserved PBMCs were thawed and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). To induce differentiation of memory B cells into antibody-secreting cells (ASCs), PBMCs were stimulated with R848 (1 μg/mL; Mabtech, 3650-2A) and recombinant human IL-2 (10 ng/mL; Mabtech, 3650-2A) for 72 hours at 37°C in a humidified incubator with 5% CO_2.

ELISpot plates (PVDF membrane; Millipore, MAIPSWU10) were pre-treated with 35% ethanol, washed with sterile water, and coated overnight at 4°C with anti-human IgG capture antibody (15 μg/mL; Mabtech, 3850-2A) in phosphate-buffered saline (PBS). Plates were washed and blocked with RPMI 1640 containing 10% FBS for at least 30 minutes at room temperature. After stimulation, cells were harvested, and culture supernatants were collected and stored for subsequent analysis. Cells were washed and resuspended in complete medium, then plated at 1–3 × 10^5 cells in 100 μL per well and incubated for 16 hours at 37°C with 5% CO_2 to allow antibody secretion.

For detection of antigen-specific IgG-secreting cells, 100 μL/well of biotinylated SARS-CoV-2 RBD proteins, including WT RBD (ACROBiosystems, SPD-C82E9) and BA.1 RBD (ACROBiosystems, SPD-C82E4), at a final concentration of 1 μg/mL, were added and incubated for 2 hours at room temperature. After washing, plates were incubated with streptavidin–alkaline phosphatase (Mabtech, 3850-2A) diluted 1:1000 in PBS containing 0.5% FBS for 1 hour at room temperature. Spots were developed using BCIP/NBT substrate (Mabtech, 3650-10) until distinct spots appeared, and the reaction was stopped by rinsing with water.

Single-cell RNA sequencing and library preparation

Cryopreserved PBMCs from 11 healthy controls and 13 patients with solid cancers were thawed in RPMI supplemented with 10% fetal bovine serum and DNase I. Cell viability was assessed after thawing and exceeded 85% in all samples. Switched memory B cells were enriched using a human Memory B Cell Isolation Kit (Miltenyi Biotec, 130-093-617). Non-target cells obtained during enrichment were separately collected and subjected to whole-exome sequencing to enable genotype-based demultiplexing.

Cells were stained with fluorochrome-conjugated antibodies and tetramers and sorted using a BD FACSAria Fusion cell sorter (BD Biosciences). The antibody panel included anti-CD3 BV421 (clone UCHT1, BD Biosciences, 562426), anti-IgM BV605 (clone G20-127, BD Biosciences, 562977), anti-IgD PerCP-Cy5.5 (clone IA6-2, BD Biosciences, 561315), anti-CD20 PE-Cy7 (clone 2H7, BioLegend, 302312), anti-CD19 AF700 (clone HIB19, BioLegend, 302226), and 7-AAD (BD Biosciences). SARS-CoV-2 Spike tetramers corresponding to the WT, BA.1, and BA.5 variants were used in two fluorophore channels (BV650 and AF647), resulting in six tetramers in total (BV650-WT Spike, BV650-BA.1 Spike, BV650-BA.5 Spike, AF647-WT Spike, AF647-BA.1 Spike, and AF647-BA.5 Spike), together with decoy tetramers.

Live (7-AAD$^-$) CD3$^-$CD19$^+$CD20$^+$ B cells were first gated, and class-switched memory B cells were defined as IgM$^-$IgD$^-$ cells. Spike-specific B cells were identified as decoy-negative cells double positive for Spike tetramers. Non–antigen-specific class-switched memory B cells were collected separately. To enable genotype-based demultiplexing of Spike-specific populations, cells were loaded into two independent GEM reactions with a cross-mixed design: GEM1 contained antigen-specific B cells from cancer patients together with non–antigen-specific switched memory B cells from healthy controls, whereas GEM2 contained antigen-specific B cells from healthy

controls together with non–antigen-specific cells from cancer patients.

Single-cell libraries were prepared using the Chromium GEM-X Single Cell 5′ Reagent Kits v3 (10x Genomics) according to the manufacturer's protocol. Gene expression and V(D)J libraries were sequenced on an Illumina NovaSeq X Plus platform. Sequencing generated hundreds of millions of reads per library with Q30 scores exceeding 95%, indicating high sequencing quality.

Single-cell RNA-sequencing data processing

Raw sequencing data were processed using the Cell Ranger pipeline (v10.0.0, 10x Genomics) and aligned to the GRCh38 human reference genome. Donor demultiplexing was performed using genotype information derived from matched whole-exome sequencing (WES) data. WES reads were aligned and processed using BWA, SAMtools, and GATK to generate donor genotype VCF files. This genotype information was used together with Cell Ranger BAM files as input to Demuxlet, which assigned each cell barcode to a donor and identified potential doublets. The resulting demultiplexing output was integrated with the gene expression matrix in R by matching cell barcodes.

Downstream analyses were performed in R (v4.1.2) using the Seurat (v5.0.0)[1]. Cells with >20% mitochondrial gene expression, >45% ribosomal protein gene expression (RPS or RPL genes), fewer than 250 detected features, or more than 5,000 detected features were excluded from further analysis. Gene expression values were log-normalized using the *NormalizeData* function. Highly variable genes were identified using the *FindVariableFeatures* function (top 2,000 genes). Immunoglobulin genes were excluded from the variable feature selection except for heavy chain constant region genes (IGHM, IGHD, IGHG1, IGHG2, IGHG3, IGHG4, IGHA1, IGHA2,

and IGHE), which were retained to preserve isotype information.

Gene expression values were scaled using the *ScaleData* function, followed by dimensionality reduction using the *RunPCA* function. For dataset integration, the *SelectIntegrationFeatures* function was used to identify 2,000 shared variable genes across datasets, excluding mitochondrial genes, ribosomal protein genes, and immunoglobulin genes except for heavy chain constant genes. Batch effects were corrected using reciprocal PCA–based integration. The first 30 principal components were used for neighbor graph construction with *FindNeighbors*, and clustering was performed using *FindClusters* with a resolution of 0.3. Cells were visualized in two dimensions using Uniform Manifold Approximation and Projection (UMAP) generated with *RunUMAP*.

Differentially expressed genes (DEG) between clusters were identified using the *FindAllMarkers* or *FindMarkers* functions. Genes with an average log fold change > 0.25, expressed in at least 10% of cells within a given cluster, and an adjusted P value ≤ 0.05 were considered differentially expressed.

B cell receptor sequencing data processing

Single-cell B cell receptor (BCR) sequences were assembled using the Cell Ranger V(D)J pipeline. Filtered contig FASTA files and corresponding 10x contig annotation tables were processed using the Immcantation toolkit (v4.5.0)[2]. V(D)J gene assignments were performed using IgBLAST with IMGT human germline references through the AssignGenes.py script. IgBLAST outputs were converted to Change-O database format using MakeDb.py with the --10x option. Heavy (IGH) and light (IGK/IGL) chain sequences were separated using ParseDb.py.

Clonal lineages were defined on heavy chains using DefineClones.py with a

Hamming distance model. The distance threshold for clone assignment was determined using the *distToNearest* function implemented in the SHazaM package and set to 0.274. Clonotypes were defined as BCR sequences sharing identical CDR3 amino acid sequences. Heavy and light chain pairing was refined using light_cluster. Germline sequences were reconstructed using CreateGermlines.py in both full and D-masked modes.

Somatic hypermutation (SHM) was calculated by comparing each rearranged immunoglobulin sequence with its inferred germline sequence and determining the number of nucleotide substitutions across the aligned sequences of heavy chain. Only productive paired heavy and light chain sequences were included in downstream analyses.

Gene Set Enrichment Analysis (GSEA)

Gene set enrichment analysis (GSEA) was performed using the fgsea R package (v1.20.0) on a pre-ranked gene list. Differentially expressed genes were identified using the *FindMarkers* function in Seurat (v5.0.0), applying thresholds of average log fold change > 0.25 and adjusted P value ≤ 0.05. Genes were ranked according to the average log fold change values obtained from the differential expression analysis. Gene sets were obtained from the Molecular Signatures Database (MSigDB, v7.5.1, human)[3], including the Hallmark[4], C5: Gene Ontology Biological Process (C5:BP)[5], and C7: Immunologic Signature collections[6].

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA was performed to quantify antibodies binding to various SARS-CoV-2 receptor-binding domain (RBD) proteins, including WT (Wuhan-Hu-1) RBD (SPD-C52H1), Delta (B.1.617.2) RBD (SPD-C52Hh), BA.1 (B.1.1.529) RBD (SPD-C522e), BA.5 (or BA.4) RBD (SPD-C522r), BA.2.86 RBD (SPD-C5247), and BQ.1 RBD (SPD-C5243), all obtained from ACROBiosystems. Clear flat-bottom Immuno 96-well plates (NUNC, 439454) were coated with 50 µL per well of a 1 µg/ml solution of the indicated protein in Dulbecco's phosphate-buffered saline (DPBS) overnight at 4°C.

The next day, the plates were incubated with 200 µL per well of blocking buffer (1X DPBS with 5% FBS) for 1 hour at room temperature. After blocking, serially diluted plasma samples (starting dilution at 1:66, followed by 9 additional three-fold serial dilutions) in blocking buffer were added and incubated for 1 hour at room temperature. Plates were then washed three times with washing buffer (1X PBS with 0.05% Tween-20, Biosesang, TR1027-500-00) and subsequently incubated with biotinylated anti-human IgG (SouthernBiotech, 2040-08) in blocking buffer at a 1:1,000 dilution for 1 hour at room temperature. Following this, plates were washed five times with washing buffer and incubated with Streptavidin-HRP (horseradish peroxidase, BD Biosciences, 554066) in blocking buffer at a 1:1,000 dilution for 1 hour at room temperature. Plates were washed another five times with washing buffer and then incubated with 50 µL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate for 5 minutes. The reaction was stopped with 50 µL of 1M H_2SO_4, and absorbance was measured at 450 nm using an ELISA microplate reader (SpectraMax Mini, Molecular Devices) with SoftMax Pro software for analysis.

The endpoint plasma dilution was calculated using curve fit analysis of optical density (OD) 450 values for serially diluted plasma, with a cut-off value set to three

times the background signal, using GraphPad Prism V10.1.1.

SARS-CoV-2-pseudotyped reporter virus

Pseudoviruses expressing the spike protein of SARS-CoV-2 WT, Delta, BA.1, and BA.5 variants were generated through the transfection of Lenti-X 293T cell lines (Takara, 632180) using Polyethyleneimine (PEI; Polysciences, 23966) and the following vectors: pLV-Spike WT (Invivogen, pLV-Spike), pLV-Spike Delta (Invivogen, pLV-SpikeV8), pLV-Spike BA.1 (Invivogen, pLV-Spike-V11), pLV-Spike BA.5 (Invivogen, pLV-SpikeV13), pLV-eGFP, pRSV-Rev, and pMDLg/pRRE. The transfection was performed by mixing PEI and DNA at a 3:1 ratio.

Ninety-six hours after transfection, supernatants were harvested and concentrated using a Lenti-X concentrator (Clontech, 631232), and stored at -80°C. Before conducting the assay, the 100% infection titer of the pseudovirus was titrated by infecting hACE2 (human angiotensin-converting enzyme 2) 293T cells (Takara, 631289) with serially diluted pseudovirus in DMEM (Dulbecco's Modified Eagle Medium, Corning, 10-013-CVRC) for 48 hours. Subsequently, the percentage of eGFP (enhanced green fluorescent protein)-expressing hACE2 293T cells was analyzed using flow cytometry (LSR II, BD Biosciences). The titer with eGFP-expressing hACE2 293T cells exceeding 90% was determined as the 100% infection titer. All cell lines used were reviewed and approved by Takara Bio USA Quality Department and were tested negative for *Mycoplasma* contamination.

Pseudovirus neutralization assay

Plasma samples were heat-inactivated for 30 minutes at 56°C before the assay. After inactivation, serially diluted plasma samples (starting dilution at 1:3, followed by

5 additional three-fold serial dilutions in DMEM) were incubated at 37°C for 1 hour with the 100% infection titer of pseudovirus at a 1:1 ratio in a storage plate (SPL, 34396). After incubation, samples were loaded onto hACE2 293T cells (1x10^4 cells/well in a 96-well plate; Corning, 3603) and incubated for 48 hours.

Each plate included three wells of positive controls and three wells of negative controls. Positive controls were incubated with DMEM and pseudovirus at a 1:1 ratio, while negative controls were incubated without the virus, using only DMEM. After 48 hours, plates were washed twice with DPBS and then supplemented with 200 μL of DPBS. Subsequently, eGFP signals were detected using a microplate reader (SpectraMax Mini, Molecular Devices).

The inhibition rate was calculated using the following equation:

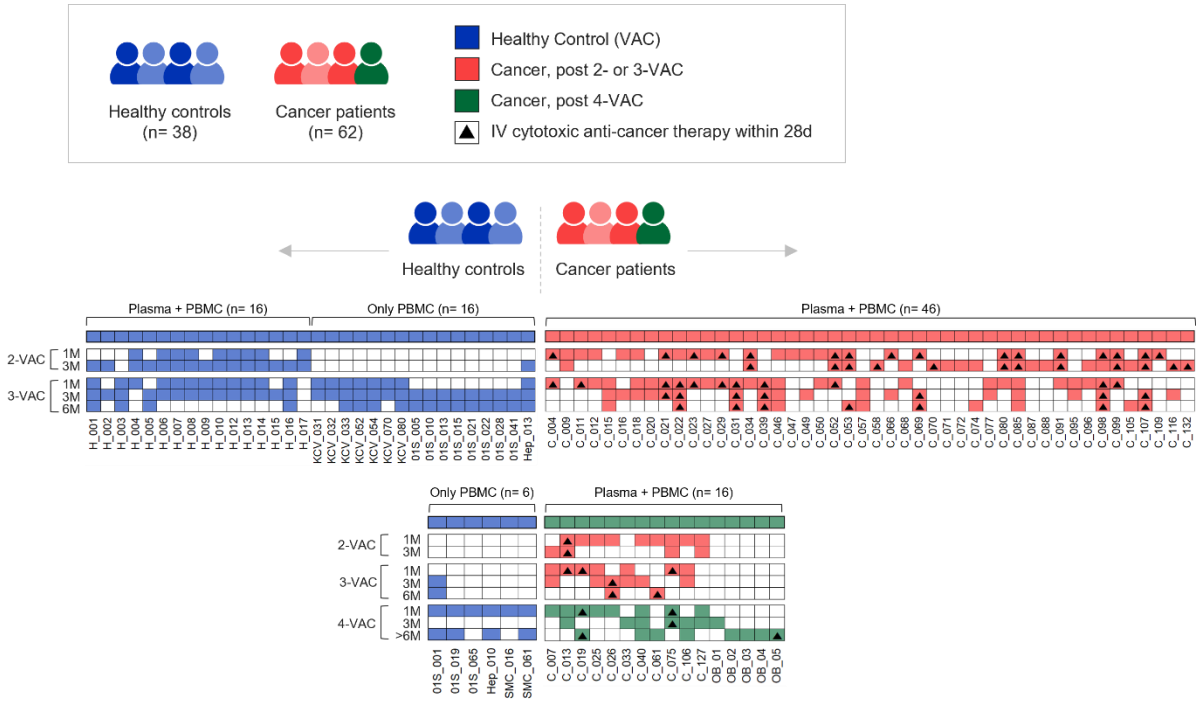
$$\text{Inhibition rate}\ (\%)=\left(1-\frac{\text{Sample}-\text{Negative control}}{\text{Virus only positive control}-\text{Negative control}}\right)\times 100\%$$

50% neutralizing titer (NT_{50}) values were calculated by performing curve fit analysis on the inhibition rate data using GraphPad Prism V10.1.1.

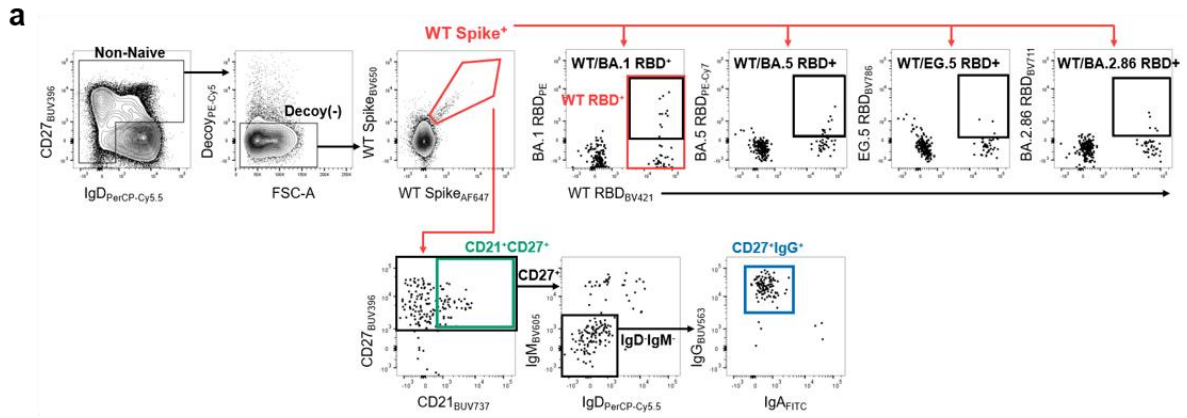
References

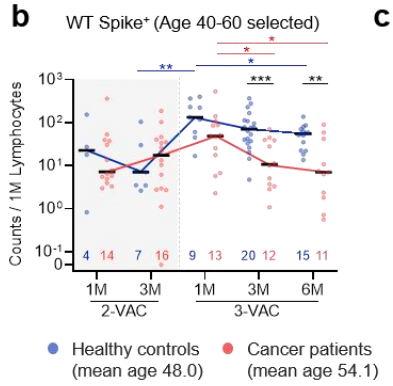
1 Hao, Y. *et al.* Integrated analysis of multimodal single-cell data. *Cell* **184**, 3573-3587 e3529 (2021).
2 Gupta, N. T. *et al.* Change-O: a toolkit for analyzing large-scale B cell immunoglobulin repertoire sequencing data. *Bioinformatics* **31**, 3356-3358 (2015).
3 Liberzon, A. *et al.* Molecular signatures database (MSigDB) 3.0. *Bioinformatics* **27**, 1739-1740 (2011).
4 Liberzon, A. *et al.* The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst* **1**, 417-425 (2015).
5 Gene Ontology, C. The Gene Ontology resource: enriching a GOld mine. *Nucleic Acids Res* **49**, D325-D334 (2021).
6 Godec, J. *et al.* Compendium of Immune Signatures Identifies Conserved and Species-Specific Biology in Response to Inflammation. *Immunity* **44**, 194-206 (2016).

SUPPLEMENTARY FIGURES



Supplementary Fig. 1 | Cohort composition and sample availability. Colored boxes indicate available samples, and alphanumeric labels below correspond to patient IDs. Colors represent samples obtained under different conditions: cancer patients after the second or third vaccine dose (red), fourth vaccine dose (monovalent or bivalent; green), and healthy controls (blue). Both plasma and peripheral blood mononuclear cells (PBMCs) were obtained from cancer patients, whereas only plasma or peripheral blood mononuclear cells were available for some samples from healthy controls. Triangles (▲) indicate samples obtained from patients who received intravenous cytotoxic chemotherapy within 28 days before SARS-CoV-2 vaccination. M, months; VAC, vaccination; IV, intravenous.

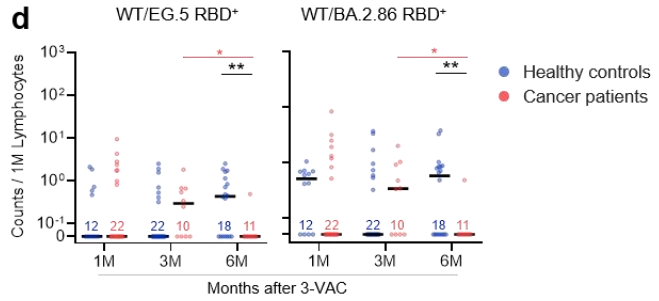


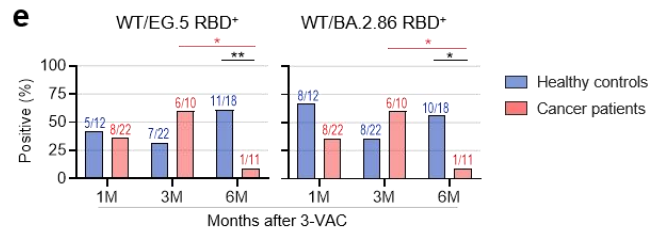


c

Multiple linear regression (WT Spike+ B cell counts following 3-VAC)

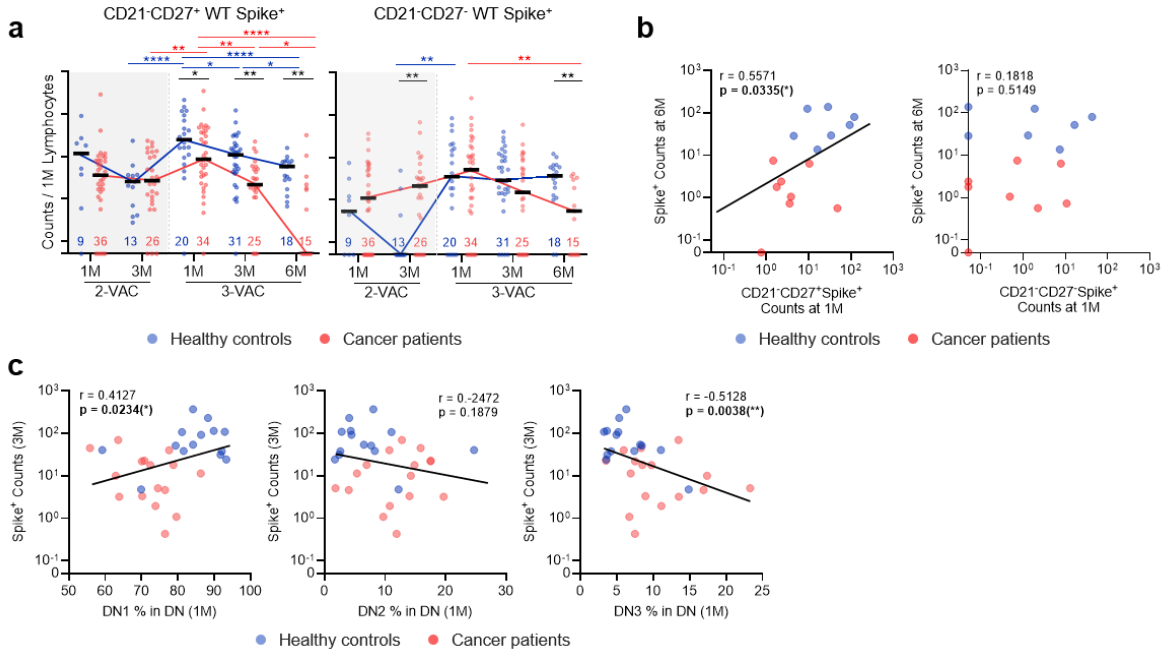
	Parameter	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	Summary
3-VAC 1M	β0	Intercept	1.924	0.5782	0.7629 to 3.085	3.328	0.0016	**
	β1	group[Pt]	-0.1504	0.2799	-0.7125 to 0.4117	0.5374	0.5934	ns
	β2	age	-0.007156	0.008915	-0.02506 to 0.01075	0.8027	0.426	ns
	β3	sex[F]	0.09547	0.1766	-0.2592 to 0.4501	0.5407	0.5911	ns
3-VAC 3M	β0	Intercept	0.6742	0.4818	-0.2926 to 1.641	1.399	0.1676	ns
	β1	group[Pt]	-0.6933	0.2067	-1.108 to -0.2785	3.354	**0.0015**	**
	β2	age	0.007899	0.007724	-0.007600 to 0.02340	1.023	0.3112	ns
	β3	sex[F]	0.04117	0.145	-0.2499 to 0.3322	0.2839	0.7776	ns
3-VAC 6M	β0	Intercept	2.264	0.5187	1.203 to 3.324	4.364	0.0001	***
	β1	group[Pt]	-0.6805	0.2075	-1.105 to -0.2561	3.279	**0.0027**	**
	β2	age	-0.01288	0.01122	-0.03583 to 0.01006	1.148	0.2602	ns
	β3	sex[F]	0.01395	0.181	-0.3562 to 0.3841	0.07707	0.9391	ns



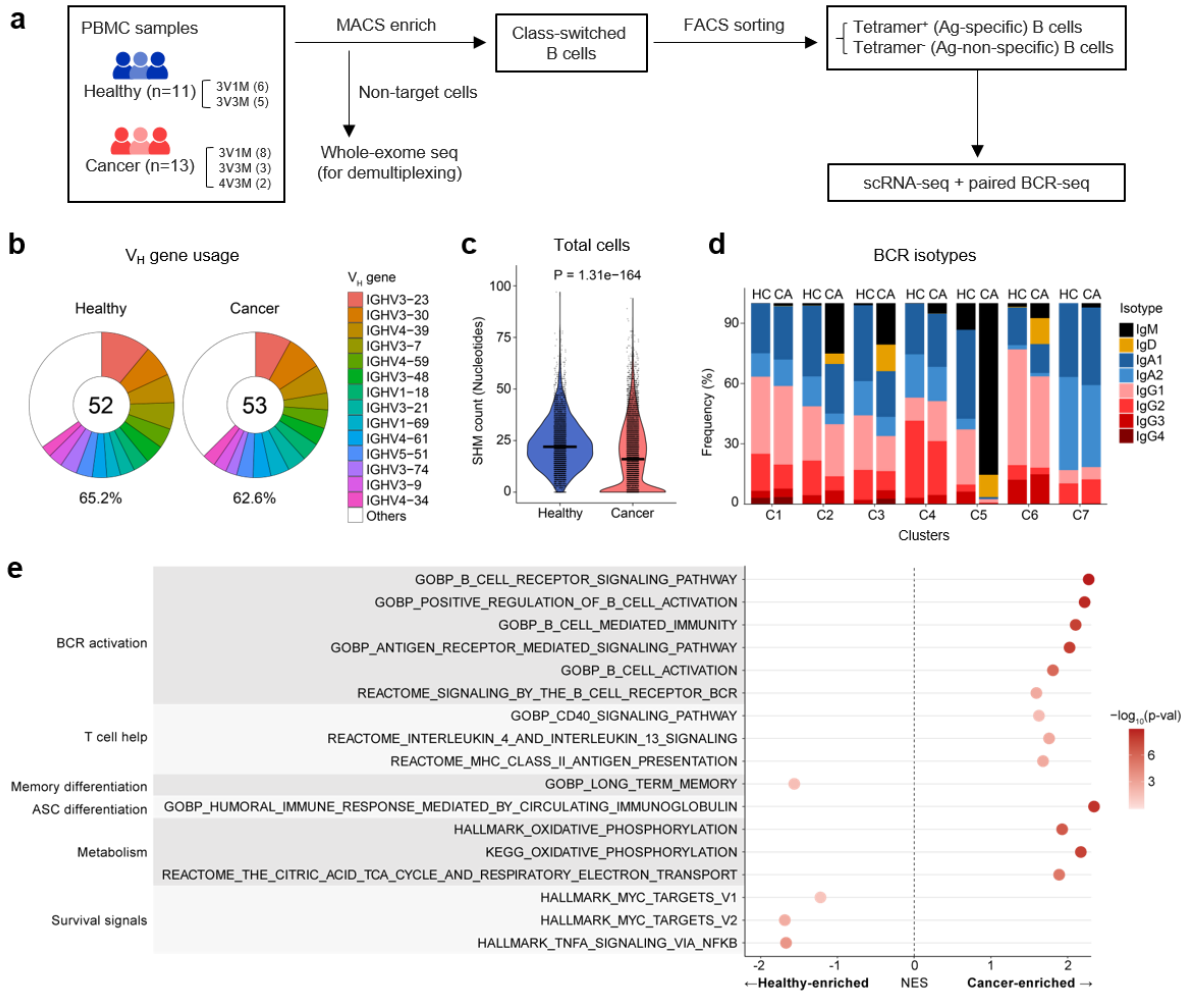


Supplementary Fig. 2 | Identification of SARS-CoV-2–specific B cells and additional analyses of WT Spike⁺ and variant cross-reactive B cells. a Gating strategy for identifying SARS-CoV-2–specific B cells. Live CD19⁺CD20⁺ B cells were analyzed after exclusion of naïve (IgD⁺CD27⁻) and decoy-binding cells. WT Spike⁺ B cells were defined by binding to WT Spike tetramers, and RBD-binding cells were further identified using WT and variant RBD tetramers (BA.1, BA.5, EG.5, and BA.2.86). Expression of CD21, CD27, and immunoglobulin isotypes (IgD, IgM, IgG) was evaluated within the WT Spike⁺ population. **b** WT Spike⁺ B cell counts per 10^6 lymphocytes in subset of individuals aged 40–60 years (mean ages: healthy 48.0 years; cancer 54.1 years). **c** Multiple linear regression analysis of WT Spike⁺ B cell counts at one, three, and six months after the third vaccination. **d, e** Cross-reactive B

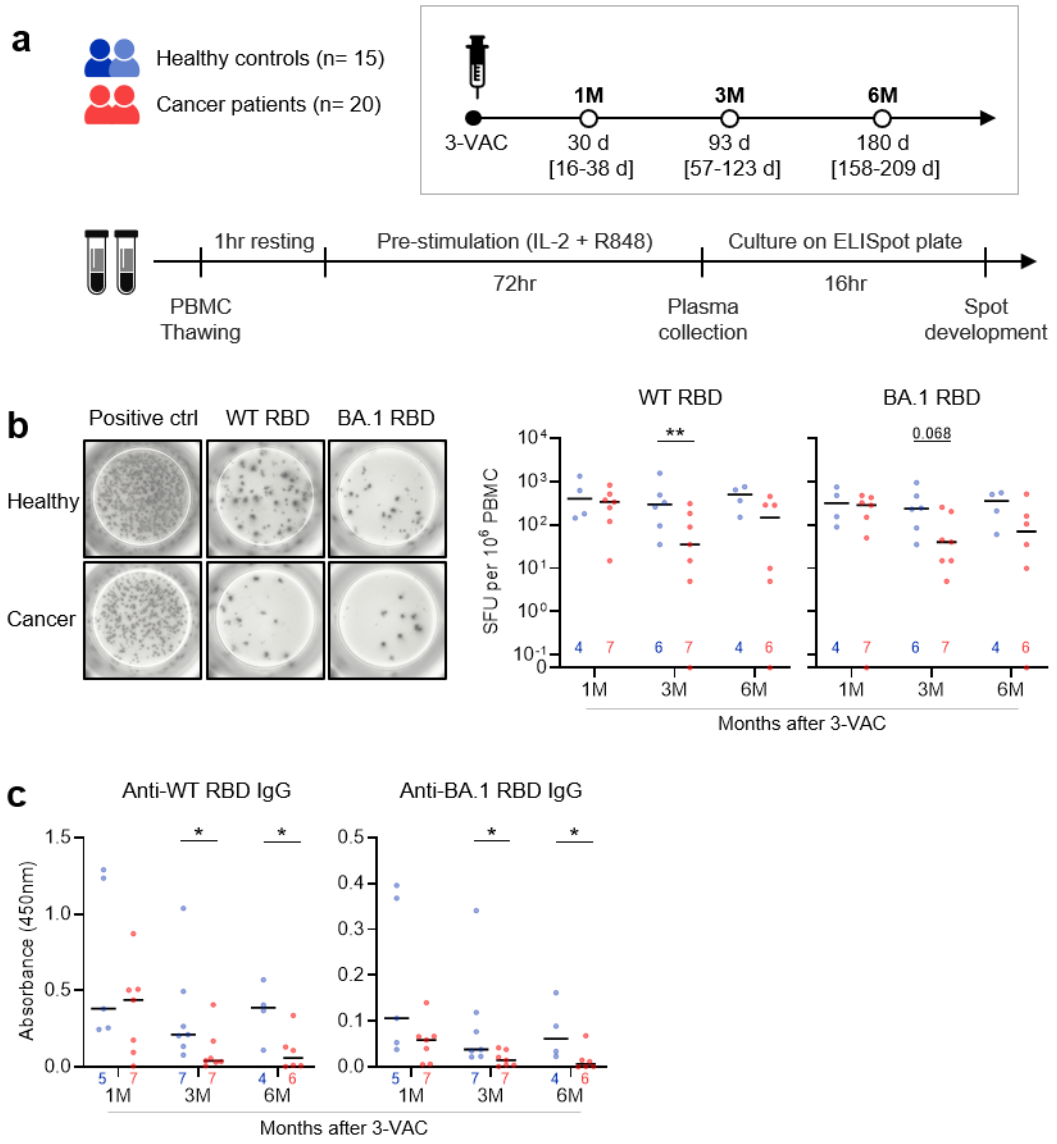
cell responses against emerging variants (EG.5 and BA.2.86) after the third vaccination. B cell counts per 10^6 lymphocytes (**d**) and proportion of samples with detectable B cells (**e**). Sample sizes are shown above the x-axes; lines indicate medians where applicable. Statistical comparisons were performed using the Mann–Whitney U test, and positive rates were analyzed using Fisher's exact test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001. M, months; VAC, vaccination.



Supplementary Fig. 3 | Memory B cell subset composition associated with durability of vaccine-induced WT Spike⁺ B cells. a $CD21^{-}CD27^{+}$ and $CD21^{-}CD27^{-}$ WT Spike⁺ B cell counts per 10^6 lymphocytes after two or three vaccine doses in healthy controls and cancer patients. **b** Correlation between $CD21^{-}CD27^{+}$ and $CD21^{-}CD27^{-}$ WT Spike⁺ B cell subset counts at one month after the third vaccination (x-axis) and WT Spike⁺ B cell counts at six months (y-axis, n = 15). **c** Correlation between DN1, DN2, and DN3 proportions within DN B cells at one month after the third vaccination (x-axis) and WT Spike⁺ B cell counts at three months (y-axis, n = 30). Sample sizes are shown above the x-axes; lines indicate medians where applicable. Correlations were analyzed using Spearman's correlation with best-fit lines generated by simple linear regression, and statistical comparisons were performed using the Mann–Whitney U test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001. M, months; VAC, vaccination.

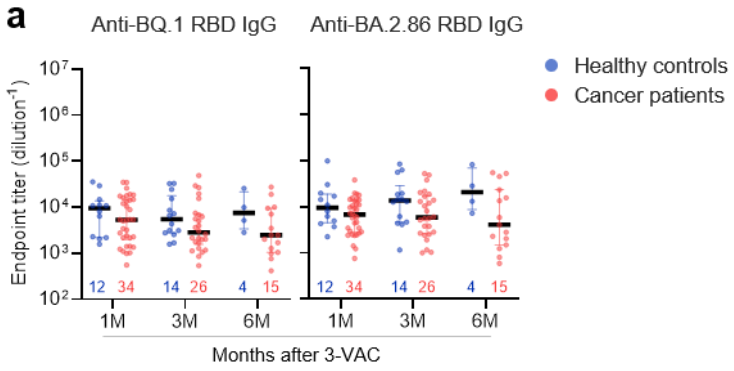


Supplementary Fig. 4 | Single-cell transcriptomic and BCR repertoire analysis of class-switched B cells in healthy controls and cancer patients. a Experimental workflow for single-cell RNA sequencing and paired BCR sequencing. PBMCs from healthy controls and cancer patients were enriched for B cells by MACS, followed by FACS sorting of class-switched B cells. Antigen-specific B cells were identified using Spike tetramers, and both tetramer-positive and tetramer-negative B cells were subjected to scRNA-seq with paired BCR sequencing; non-target cells were processed for whole-exome sequencing for sample demultiplexing. **b** VH gene usage frequencies showing the distribution of the most expanded VH genes. **c** Somatic hypermutation (SHM) counts in immunoglobulin heavy chain sequences comparing healthy controls and cancer patients. **d** Immunoglobulin isotype distribution based on BCR sequencing. **e** Gene set enrichment analysis (GSEA) comparing transcriptional programs between healthy controls and cancer patients, shown as a dot plot with normalized enrichment scores (NES) and corresponding *P* values. Statistical comparisons were performed using the Mann–Whitney U test.

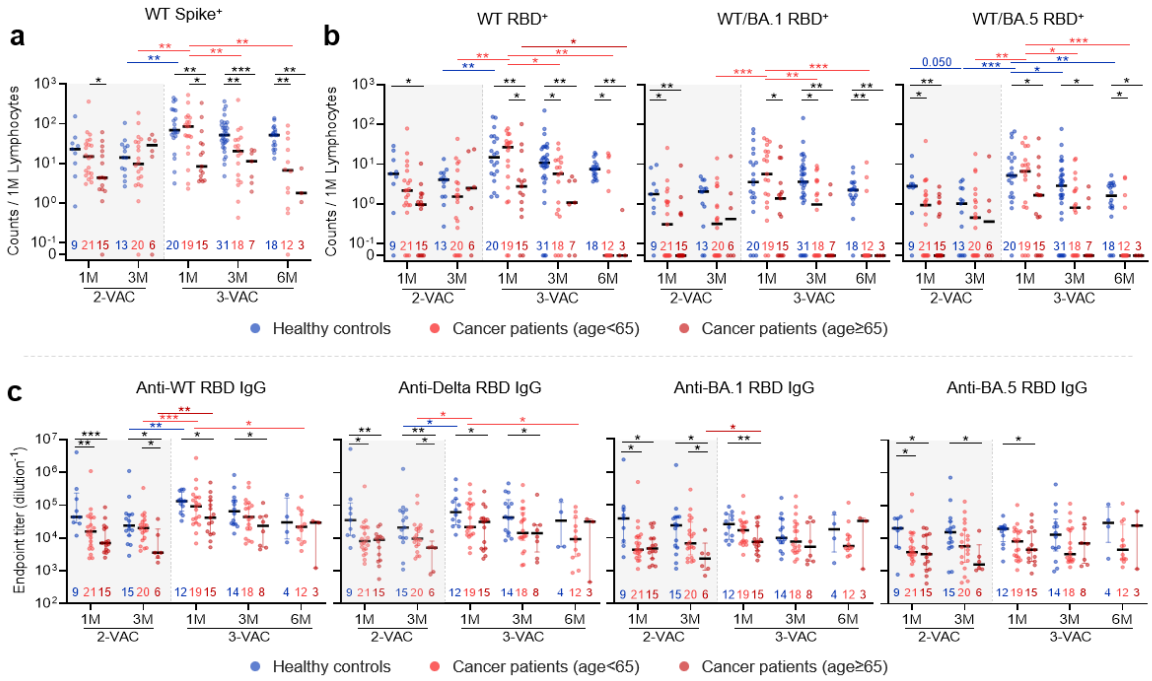


328

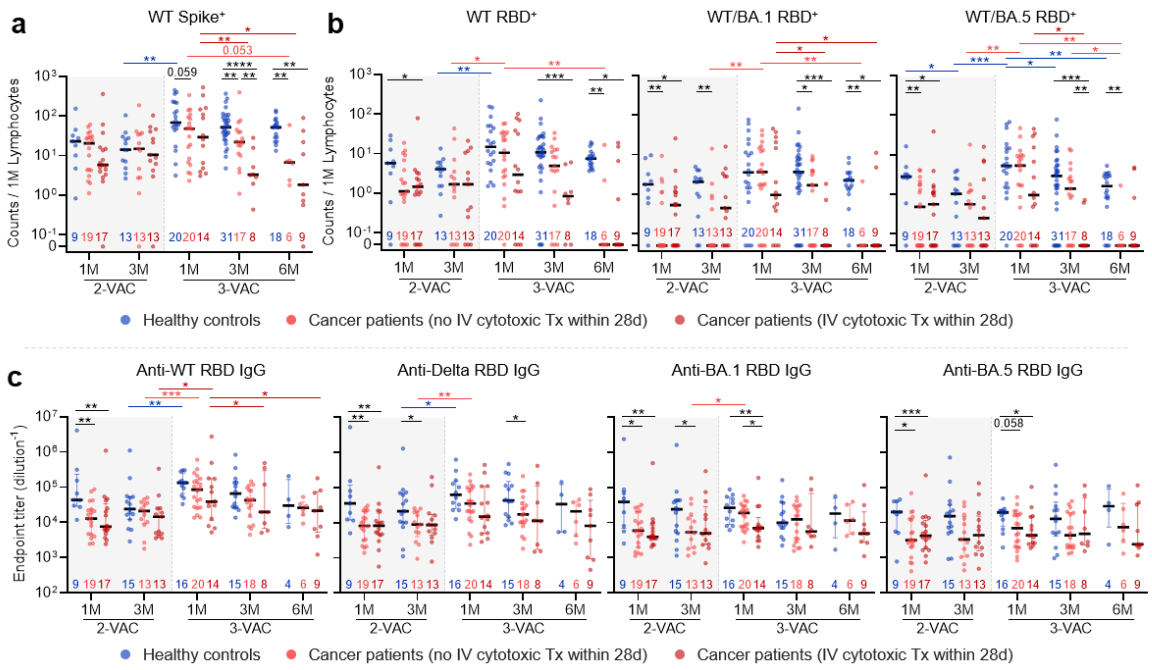
329 **Supplementary Fig. 5 | Reduced functional responsiveness of memory B cells**
330 **following SARS-CoV-2 vaccination in cancer patients. a** Schematic overview of
331 the IgG B cell ELISpot assay. PBMCs were stimulated with IL-2 and R848 for 72 hours,
332 after which supernatants were collected for antigen-specific IgG quantification and
333 cells were plated to assess antigen-specific antibody-secreting cell (ASC) formation.
334 **b** WT RBD– and BA.1 RBD–specific spot-forming units (SFUs) at one, three, and six
335 months after the third vaccination in healthy controls and cancer patients. **c** Antigen-
336 specific IgG levels in culture supernatants after IL-2/R848 stimulation at one, three,
337 and six months after the third vaccination for WT RBD and BA.1 RBD. Sample sizes
338 are shown above (**b**) and below (**c**) the x-axes; lines indicate medians where
339 applicable. Statistical comparisons were performed using the Mann–Whitney U test.
340 *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. M, months; VAC, vaccination.



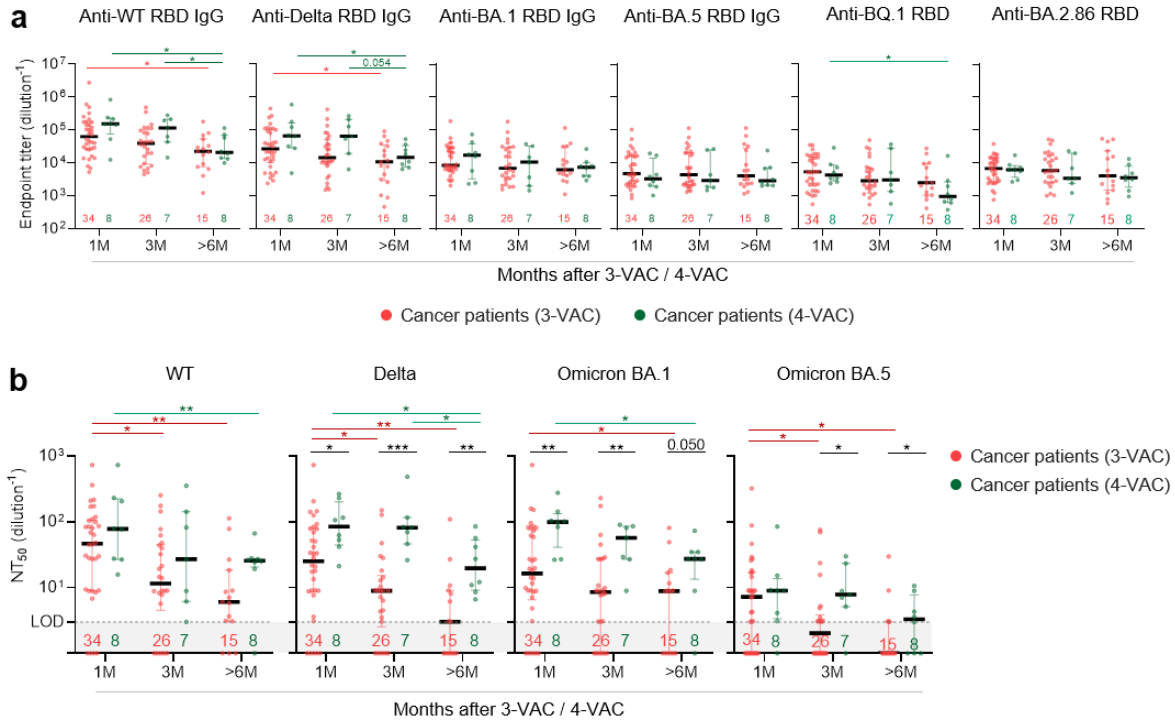
Supplementary Fig. 6 | Antibody responses to emerging SARS-CoV-2 variants following the third vaccination. a Anti-BQ.1 RBD and anti-BA.2.86 RBD IgG titers (endpoint titers) at one, three, and six months after the third vaccination in healthy controls and cancer patients. Sample sizes are shown above the x-axes; lines indicate medians with interquartile ranges. Statistical comparisons were performed using the Mann–Whitney U test.



Supplementary Fig. 7 | Effects of age on B cell and antibody responses following SARS-CoV-2 vaccination. a, b Total WT Spike⁺ (**a**) and WT RBD⁺, WT/BA.1 RBD⁺, and WT/BA.5 RBD⁺ (**b**) B cell counts per 10^6 lymphocytes after two or three vaccine doses in healthy controls, cancer patients aged <65 years, and ≥65 years. **c** Anti-RBD IgG titers against WT, Delta, Omicron BA.1, and BA.5 after two or three vaccine doses. Sample sizes are shown above the x-axes; lines indicate medians with interquartile ranges where applicable. Statistical comparisons were performed using the Mann–Whitney U test. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. M, months; VAC, vaccination.



Supplementary Fig. 8 | Effects of recent IV cytotoxic chemotherapy on B cell and antibody responses following SARS-CoV-2 vaccination. a, b WT Spike$^+$ (**a**) and WT RBD$^+$, WT/BA.1 RBD$^+$, and WT/BA.5 RBD$^+$ (**b**) B cell counts per 10^6 lymphocytes after two or three vaccine doses in healthy controls, cancer patients without IV cytotoxic therapy within 28 days before vaccination, and those who received IV cytotoxic therapy within 28 days before vaccination. **c** Anti-RBD IgG titers against WT, Delta, Omicron BA.1, and BA.5 after two or three vaccine doses. Sample sizes are shown above the x-axes; lines indicate medians with interquartile ranges where applicable. Statistical comparisons were performed using the Mann–Whitney U test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$. M, months; VAC, vaccination; IV, intravenous; Tx, therapy.



Supplementary Fig. 9 | Antibody responses following an additional fourth vaccine dose in cancer patients. a Anti-RBD IgG titers against WT, Delta, Omicron BA.1, BA.5, BQ.1, and BA.2.86 in cancer patients after three or four vaccine doses. **b** Neutralization titers (NT_{50}) against WT, Delta, Omicron BA.1, and BA.5 after the third or fourth vaccination in cancer patients. Sample sizes are shown above the x-axes; lines indicate medians with interquartile ranges. Statistical comparisons were performed using the Mann–Whitney U test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001. M, months; VAC, vaccination.

Table S1. Individual characteristics of enrolled subjects

Group	ID	Age (years)	Sex	COVID-19 history	Tumor type	Aim of treatment	Treatment	Study subgroup	
								post 2- or 3-dose VAC cohort	post 4-dose (mono+biv) VAC cohort
Cancer	C004	70	M	No	Gastric	Palliative	Cytotoxic CTx + Immunotherapy	Yes	-
Cancer	C007	78	M	No	Gastric	Palliative	Immunotherapy + Targeted therapy	Yes	Yes
Cancer	C009	77	M	Yes	Gastric	Adjuvant	Cytotoxic CTx mono	Yes	-
Cancer	C011	68	M	No	Gastric	Palliative	Cytotoxic CTx + targeted therapy	Yes	-
Cancer	C012	65	M	No	Gastric	Palliative	Cytotoxic CTx + targeted therapy	Yes	-
Cancer	C013	81	F	No	Gastric	Palliative	Targeted therapy mono	Yes	Yes
Cancer	C015	59	F	No	Breast	Palliative	Hormone + Targeted therapy	Yes	-
Cancer	C016	70	M	Yes	Gastric	Adjuvant	Cytotoxic CTx mono	Yes	-
Cancer	C018	62	M	No	Gastric	Palliative	Cytotoxic CTx + targeted therapy	Yes	-
Cancer	C019	60	M	No	Gastric	Palliative	Cytotoxic CTx mono	Yes	Yes
Cancer	C020	72	M	No	Gastric	Adjuvant	Cytotoxic CTx mono	Yes	-
Cancer	C021	72	M	No	Prostate	Palliative	Targeted therapy mono	Yes	-
Cancer	C022	50	F	No	Colorectal	Palliative	Immunotherapy + Targeted therapy	Yes	-
Cancer	C023	71	F	Yes	Gastric	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C025	62	M	No	Gastric	Adjuvant	Cytotoxic CTx mono	Yes	Yes
Cancer	C026	61	M	No	Gastric	Palliative	Cytotoxic CTx mono	Yes	Yes
Cancer	C027	68	M	Yes	Kidney	Palliative	Targeted therapy mono	Yes	-
Cancer	C029	45	F	No	Gastric	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C031	66	F	No	Gastric	Palliative	Cytotoxic CTx + Immunotherapy	Yes	-
Cancer	C033	63	M	No	Colorectal	Palliative	Cytotoxic CTx mono	Yes	Yes
Cancer	C034	74	F	No	Gastric	Palliative	Targeted therapy mono	Yes	-
Cancer	C039	64	M	No	Gastric	Palliative	Cytotoxic CTx + targeted therapy	Yes	-
Cancer	C040	61	F	No	Gastric	Palliative	Cytotoxic CTx + Immunotherapy	Yes	Yes
Cancer	C046	69	F	No	Melanoma	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C047	65	M	No	Prostate	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C049	63	M	Yes	Colorectal	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C050	73	M	Yes	Gastric	Adjuvant	Cytotoxic CTx mono	Yes	-
Cancer	C052	62	M	No	Gastric	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C053	42	F	No	Gastric	Palliative	Immunotherapy + Targeted therapy	Yes	-
Cancer	C057	58	F	No	Gastric	Palliative	Cytotoxic CTx + Immunotherapy	Yes	-
Cancer	C058	53	M	Yes	Melanoma	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C061	50	M	No	Gastric	Palliative	Cytotoxic CTx + targeted therapy	Yes	Yes
Cancer	C066	52	M	No	Gastric	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C068	62	F	No	Gastric	Adjuvant	Immunotherapy + Targeted therapy	Yes	-
Cancer	C069	57	M	No	Gastric	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C070	51	M	Yes	Gastric	Palliative	Cytotoxic CTx + Immunotherapy	Yes	-
Cancer	C071	54	M	Yes	Biliary	Palliative	Immunotherapy mono	Yes	-
Cancer	C072	44	F	Yes	Gastric	Palliative	Immunotherapy mono	Yes	-
Cancer	C074	56	F	No	Gastric	Adjuvant	Targeted therapy mono	Yes	-
Cancer	C075	74	M	No	Gastric	Palliative	Cytotoxic CTx mono	Yes	Yes
Cancer	C077	49	M	Yes	Gastric	Adjuvant	Cytotoxic CTx mono	Yes	-
Cancer	C080	38	F	No	Gastric	Palliative	Cytotoxic CTx + Immunotherapy	Yes	-
Cancer	C085	56	M	No	Melanoma	Palliative	Cytotoxic CTx + Immunotherapy + Targeted therapy	Yes	-
Cancer	C087	41	M	Yes	Melanoma	Palliative	Targeted therapy mono	Yes	-
Cancer	C088	48	M	Yes	Gastric	Adjuvant	Targeted therapy mono	Yes	-
Cancer	C091	55	F	Yes	Liposarcoma	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C095	58	M	No	Gastric	Adjuvant	Immunotherapy mono	Yes	-
Cancer	C096	58	M	Yes	Gastric	Adjuvant	Cytotoxic CTx mono	Yes	-
Cancer	C098	48	F	No	Gastric	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C099	57	M	No	Gastric	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C105	59	F	No	Gastric	Adjuvant	Cytotoxic CTx mono	Yes	-
Cancer	C106	56	M	No	Gastric	Palliative	Immunotherapy mono	Yes	Yes
Cancer	C107	48	M	No	Gastric	Adjuvant	Cytotoxic CTx mono	Yes	-
Cancer	C109	44	M	No	Pancreas	Adjuvant	Cytotoxic CTx mono	Yes	-
Cancer	C116	54	M	No	Gastric	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	C127	68	F	No	Biliary	Palliative	Cytotoxic CTx mono	Yes	Yes
Cancer	C132	61	M	Yes	Gastric	Palliative	Cytotoxic CTx mono	Yes	-
Cancer	OB01	68	F	No	Gynecologic	Palliative	Immunotherapy mono	Yes	Yes
Cancer	OB02	76	F	No	Gynecologic	Palliative	Targeted therapy mono	-	Yes
Cancer	OB03	67	F	No	Gynecologic	Palliative	Cytotoxic CTx mono	-	Yes
Cancer	OB04	55	F	No	Gynecologic	Palliative	Targeted therapy mono	-	Yes
Cancer	OB05	64	F	No	Gynecologic	Adjuvant	Cytotoxic CTx + Immunotherapy	-	Yes
Healthy	01S_001	54	M	No	-	-	-	Yes	Yes
Healthy	01S_005	40	F	No	-	-	-	Yes	-
Healthy	01S_010	49	F	No	-	-	-	Yes	-
Healthy	01S_013	44	F	No	-	-	-	Yes	-
Healthy	01S_015	51	F	No	-	-	-	Yes	-
Healthy	01S_019	29	F	No	-	-	-	-	Yes
Healthy	01S_021	49	F	No	-	-	-	Yes	-
Healthy	01S_022	51	M	No	-	-	-	Yes	-
Healthy	01S_028	47	F	No	-	-	-	Yes	-
Healthy	01S_041	42	F	No	-	-	-	Yes	-
Healthy	01S_065	38	M	No	-	-	-	-	Yes
Healthy	HC_001	31	F	No	-	-	-	Yes	-
Healthy	HC_002	47	F	No	-	-	-	Yes	-
Healthy	HC_003	31	F	No	-	-	-	Yes	-
Healthy	HC_004	28	M	No	-	-	-	Yes	-
Healthy	HC_005	51	F	No	-	-	-	Yes	-
Healthy	HC_006	26	F	No	-	-	-	Yes	-
Healthy	HC_007	35	M	No	-	-	-	Yes	-
Healthy	HC_008	55	F	No	-	-	-	Yes	-
Healthy	HC_009	27	F	No	-	-	-	Yes	-
Healthy	HC_010	32	M	No	-	-	-	Yes	-
Healthy	HC_012	40	F	No	-	-	-	Yes	-
Healthy	HC_013	44	M	No	-	-	-	Yes	-
Healthy	HC_014	25	F	No	-	-	-	Yes	-
Healthy	HC_015	30	M	No	-	-	-	Yes	-
Healthy	HC_016	41	F	No	-	-	-	Yes	-
Healthy	HC_017	45	M	No	-	-	-	Yes	-
Healthy	Hep_010	28	F	No	-	-	-	-	Yes
Healthy	Hep_013	49	F	No	-	-	-	Yes	-
Healthy	KCV_031	43	F	No	-	-	-	Yes	-
Healthy	KCV_032	30	F	No	-	-	-	Yes	-
Healthy	KCV_033	52	F	No	-	-	-	Yes	-
Healthy	KCV_052	31	M	No	-	-	-	Yes	-
Healthy	KCV_054	51	F	No	-	-	-	Yes	-
Healthy	KCV_070	36	M	No	-	-	-	Yes	-
Healthy	KCV_080	52	M	No	-	-	-	Yes	-
Healthy	SMC_016	32	F	No	-	-	-	-	Yes
Healthy	SMC_061	49	F	No	-	-	-	-	Yes