Supplemental information

B cell-derived cfDNA after primary BNT162b2 mRNA vaccination anticipates memory B cells and SARS-CoV-2 neutralizing antibodies

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Figure S1 (related to Figure 1): demographic data.

A. Age distribution in the large cohort (n=100).
B. Age distribution in the small cohort (n=29).
C. The percentage of people reporting an adverse reaction at any time point of the experiment.
Figure S2 (related to Figure 1): Methylation marker specificity and sensitivity

A. Specificity of immune-derived methylation markers.

B. Spike-in experiments demonstrating sensitivity of 12 immune-derived methylation markers. Leukocyte DNA was mixed with human 293 cells in the indicated proportion, and the frequency of each cell type was determined using methylation analysis.

C. Validation of the percentage of B cell (r=0.81), T cell (r=0.48), CD8 (r=0.61), monocyte (r=0.61-0.7), Neutrophil (0.24-0.62) and lymphocyte (r=0.54-0.7) methylation markers on gDNA extracted from whole blood compared with CyTOF and CBC. Spearman correlation.
Figure S3 (related to Figure 2): Immune cell counts following vaccination.

A. Complete blood counts among vaccinated individuals (10^9/L).
B. Fold change (log2) of cell counts normalized to individual baseline prior to 1st and 2nd vaccination. Cell counts were determined based on CBC and methylation markers in genomic DNA from whole blood.
C. Percentage of specific immune cells based on methylation among vaccinated individuals. Bars, median; error bars, CI.
D. Total and immune-derived cfDNA, presented in log2 fold change normalized to the baseline level of each individual prior to the primary vaccine and prior to the boost vaccine.
E. Percent of cfDNA from specific immune cells following vaccination. Bars, median; error bars, CI. (mixed-effects analysis)
Figure S4 (related to Figure 2): vaccination as a coordinated turnover response

A. Median Fold change of immune derived cfDNA 3 days after primary vaccine vs 3 days after booster vaccine (left) and 7 days after primary vaccine vs 7 days after the booster (right).

B. A spearman correlation matrix of cell type-specific cfDNA level (GE/ml) following vaccination. Benjamini-Hochberg-adjusted p-value to correct for multiple testing; FDR 5%.
Figure S5 (related to Figure 3): correlations between cell type-specific cfDNA and antibodies.

A heat map of the spearman’s correlation between anti-spoke IgG antibody levels and immune derived cfDNA subsets. Benjamini-Hochberg-adjusted p-value to correct for multiple testing; FDR 5%.

Figure S6 (related to Figure 4): correlations to day 28 neutralizing antibodies.

A. correlation between anti-spoke IgG (AU/ml) and day 28 neutralizing antibodies (NT50).
B. Correlation matrix of non-B immune-derived cfDNA (GE/ml) and neutralizing antibodies (NT50) on day 28, demonstrating the specificity of the correlation between B-cell cfDNA and antibodies. spearman’s correlation Benjamini-Hochberg-adjusted p-value to correct for multiple testing; FDR 5%.
A. Memory B-cells (left) and plasmablasts (right) do not change significantly after vaccination. Bars, median; error bars, CI.

B. Memory B cells (left) and plasmablasts (right) over time per age groups. Under 40 years (n=10, orange line), and over 40 years (n=19, blue line). No significant differences are observed between the two age groups (p-value: 0.35-0.99, mixed-effects analysis).

C. Spearman’s correlation between neutralizing antibodies on day 28 (y axis) and memory B-cells on days 0-42 (X axis).

D. Spearman’s correlation between age (X axis) and neutralizing antibodies on day 28 (Y axis).

E. Spearman’s correlation between memory B-cells (10^9/L) on days 0-42 (X axis) and age (Y axis).

F. Spearman’s correlation between memory B-cells on days 0-42 (X axis) and age (Y axis) corrected for age (residuals). Benjamini-Hochberg-adjusted p-value to correct for multiple testing; FDR 5%. Red background shows correlations that are statistically significant.
Supplementary Table S1 (related to star methods): methylation markers.

| Marker | Coordinate of central CpG | What defines molecules as derived from cell type X | Forward primer | Reverse primer |
|--------|---------------------------|--------------------------------------------------|----------------|----------------|
| Neut1  | chr9: 129648322           | All CpGs in block are unmethylated               | TTTTAAGAAG     | TCTAAAATACCTAA |
|        |                           |                                                  | TTTTGTGTTA     | ATACAAAACC     |
| Neut2  | chr8: 142180109           | All CpGs in block are unmethylated               | GTTTGTGTTTGA   | ATAAACATCCCTACAA |
|        |                           |                                                  | GATGTGAGAA     | ACTCACAACCAAA  |
| Neut3  | chr11: 33308345           | I CpG in block can be unmethylated               | TGTAGGTATTTTT  | AATTATCCAACTCCT |
|        |                           |                                                  | TAGATTGGGGG    | CACTCTTTA      |
| Mono1  | chr11: 32055233           | I CpG in block can be unmethylated               | TTTGTTAGGGTT   | CATCTCTCTACTTTA |
|        |                           |                                                  | AAGTAATTTG     | TAACTTCAAT     |
| Mono2  | chr10: 114911652          | All CpGs in block are unmethylated               | TGAAGGAAAT     | CCCCCCTCCCTAAAA |
|        |                           |                                                  | GAGAGAAAGAAG   | AAAACCAACCAAC  |
| B-cell1| chr11: 121440880          | All CpGs in block are unmethylated               | AGGTTGGTTTTT   | ATAAACCACAACCA |
|        |                           |                                                  | TTATTTTTTAG    | ACTACACAT      |
| B-cell2| chr17: 3493666            | All CpGs in block are unmethylated               | TTTAAAAGAAA    | ATAAACCACAACCA |
|        |                           |                                                  | GTTTTTTTTAGG   | ACTACACAT      |
| B-cell3| chr11: 34167855           | All CpGs in block are unmethylated               | ATTTTTTTTGG    | TCACAAACACAA   |
|        |                           |                                                  | TGGGATGTGT     | ACCCAA         |
| T-cell1| chr14: 61801201           | All CpGs in block are unmethylated               | GGTGTTATAG     | CCAACATTTATCATT |
|        |                           |                                                  | GTAGGGTAGA     | TTCTTCA        |
| T-cell2| chr13: 24825973           | All CpGs in block are unmethylated               | AGTATTTTTAT    | CCTACTACCTCAAAT |
|        |                           |                                                  | TGGGTTGGAT     | TAACTAAAAA     |
| CD8A   | chr2: 87012810            | All CpGs in block are unmethylated               | TTAGGTTTTTT    | CACCACAAATCA   |
|        |                           |                                                  | AGTAGGTTTTTT   | CAATACTAT      |
| CD8B   | chr: 87048747             | All CpGs in block are unmethylated               | GTTAAAGAAAT    | AAAACCCCATATTAC |
|        |                           |                                                  | TAATAGGAAA     | TCCCC          |