Supplemental Figure 1

Representative flow cytometric plots of Spike-specific CD4+ IL-2+ TNF-α+ T cells in one naïve (upper row) and one COVID-19 recovered (lower row) subject before vaccination, before second injection and after 1 and 8 months following the complete vaccination cycle.
Supplemental Figure 2. Gating strategy for the identification of spike-specific CD4+ T cells

Lymphocytes were gated based on physical parameters (FSC-SSC), then doublets were removed using FSC-A and FSC-H parameters. Dead cells were excluded using viability stain 780. T cells were identified as CD3+. We then identified CD4+ T cells. Among these, we identified CD154 expressing cells. CD4+CD154+ T cells were then evaluated for IFN-γ, TNF-α and IL-2 expression.
Supplemental Figure 3: Gating strategy for the identification of spike-specific B cells

Lymphocytes were gated based on physical parameters (FSC-SSC), then doublets were removed using FSC-A and FSC-H parameters. PerCP was used as dump channel for the exclusion of dead cells (7AAD), CD3+ T cells and CD14 monocytes. B cells were identified as CD19+. B cells binding PE- and PE Vio770-conjugated spike protein were then identified as spike-specific. Among spike-specific B cells we evaluated CD27 expression associated to IgG, IgM and IgA.
Supplemental Table S1. Basic demographic and clinical data of 30 healthcare workers who received BNT162b2 mRNA COVID-19 vaccine and were followed up to 8 months.

| Subject ID | Age (years) | Gender | COVID-19 severity | Time from COVID-19 diagnosis° to first vaccine dose (days) |
|------------|-------------|--------|-------------------|----------------------------------------------------------|
| R1         | 46          | male   | mild              | 214                                                      |
| R2         | 35          | female | mild              | 58                                                       |
| R3         | 51          | female | severe            | 294                                                      |
| R4         | 52          | female | mild              | 286                                                      |
| R5         | 60          | male   | moderate          | 302                                                      |
| R6         | 65          | male   | mild              | 312                                                      |
| R7         | 53          | female | mild              | 289                                                      |
| R8         | 37          | male   | moderate          | 297                                                      |
| R9         | 54          | female | mild              | 293                                                      |
| R10        | 29          | female | mild              | 289                                                      |
| R11        | 39          | male   | mild              | 72                                                       |
| R12        | 61          | male   | critical          | 288                                                      |
| R13        | 64          | male   | critical          | 292                                                      |
| R14        | 51          | female | mild              | 222                                                      |
| R15        | 44          | female | mild              | 306                                                      |
| Mean±SD    | -           | 49±11  | -                 | 254±82                                                    |

**COVID-19 recovered individuals**

| Subject ID | Age (years) | Gender | COVID-19 severity | Time from COVID-19 diagnosis° to first vaccine dose (days) |
|------------|-------------|--------|-------------------|----------------------------------------------------------|
| N1         | 63          | male   | na                | na                                                       |
| N2         | 41          | female | na                | na                                                       |
| N3         | 32          | male   | na                | na                                                       |
| N4         | 44          | female | na                | na                                                       |
| N5         | 35          | female | na                | na                                                       |
| N6         | 61          | female | na                | na                                                       |
| N7         | 41          | male   | na                | na                                                       |
| N8         | 37          | female | na                | na                                                       |
| N9         | 32          | male   | na                | na                                                       |
| N10        | 28          | male   | na                | na                                                       |
| N11        | 34          | male   | na                | na                                                       |
| N12        | 29          | female | na                | na                                                       |
| N13        | 46          | female | na                | na                                                       |
| N14        | 24          | female | na                | na                                                       |
| N15        | 53          | male   | na                | na                                                       |
| Mean±SD    | -           | 40±12  | -                 | -                                                        |

*na denotes not applicable

°first positive test on nasopharyngeal swab
**Supplemental Table S2.** Basic demographic and clinical data of 125 healthcare workers who received BNT162b2 mRNA COVID-19 vaccine and were followed up to 6 months.

|                          | COVID-19 recovered individuals | Naïve individuals |
|--------------------------|---------------------------------|-------------------|
| **No. subjects**         | 39                              | 86                |
| **Age (years)**          |                                 |                   |
| Mean±SD                  | 50.4±11.3                       | 45.5±14.3         |
| **Gender**               |                                 |                   |
| - Male, n (%)            | 12 (31.6)                       | 39 (45.3)         |
| - Female, n (%)          | 26 (68.4)                       | 47 (54.6)         |
| **COVID-19 severity**    |                                 |                   |
| - Asymptomatic/mild, n (%)| 31 (79.5)                       |                   |
| - Moderate, n (%)        | 2 (5.1)                         |                   |
| - Severe, n (%)          | 2 (5.1)                         |                   |
| - Critical, n (%)        | 4 (10.3)                        |                   |
| **Time from COVID-19 diagnosis* to first vaccine dose (days)** | 230.4±93.6 | na |

*first positive test on nasopharyngeal swab

na denotes not applicable
Supplemental Table S3. Basic demographic and clinical data of 14 healthcare workers who received booster BNT162b2 mRNA COVID-19 vaccine injection and were followed up to one week.

| COVID-19 recovered individuals receiving booster dose | Subject ID | Age (years) | Gender | COVID-19 severity |
|-----------------------------------------------------|------------|-------------|--------|------------------|
| RB1                                                 | 52         | female      | severe |
| RB2                                                 | 53         | female      | mild   |
| RB3                                                 | 65         | male        | critical |
| RB4                                                 | 37         | male        | moderate |
| RB5                                                 | 55         | female      | mild   |
| RB6                                                 | 62         | male        | critical |
| RB7                                                 | 54         | female      | mild   |
| Mean±SD                                             | -          | 54±9        | -      | -                |

| Naïve individuals receiving booster dose            | Subject ID | Age (years) | Gender | COVID-19 severity |
|-----------------------------------------------------|------------|-------------|--------|------------------|
| NB1                                                 | 45         | female      | na     |
| NB2                                                 | 56         | female      | na     |
| NB3                                                 | 36         | male        | na     |
| NB4                                                 | 36         | female      | na     |
| NB5                                                 | 29         | male        | na     |
| NB6                                                 | 31         | male        | na     |
| NB7                                                 | 42         | male        | na     |
| Mean±SD                                             | -          | 39±9        | -      | -                |

*na* denotes *not applicable*
**Supplemental Table S4.** Basic demographic and clinical data of 14 individuals who were hospitalized for COVID-19 between March and April 2020 and followed up to 12 months from discharge.

| Subject ID | Age (years) | Gender | COVID-19 severity |
|------------|-------------|--------|-------------------|
| U1         | 56          | male   | moderate          |
| U2         | 61          | male   | moderate          |
| U3         | 58          | male   | severe            |
| U4         | 75          | female | severe            |
| U5         | 48          | male   | moderate          |
| U6         | 62          | male   | severe            |
| U7         | 58          | male   | critical          |
| U8         | 73          | male   | critical          |
| U9         | 73          | female | severe            |
| U10        | 56          | female | critical          |
| U11        | 56          | female | moderate          |
| U12        | 54          | female | moderate          |
| U13        | 57          | female | moderate          |
| U14        | 69          | male   | critical          |
| Mean±SD    | -           | 61±8   | -                 |
### Supplemental Table S5. List of all fluorochrome mAbs used for flow cytometric analysis of antigen specific T cells.

| Antigen   | Fluochrome     | Clone       | Company          |
|-----------|----------------|-------------|------------------|
| TNF-α     | FITC           | 6401.1111   | BDBioscience     |
| CD154     | PE             | TRAP1       | BDBioscience     |
| CD3       | PerCP          | SK7         | BDBioscience     |
| CD4       | PE-Cy7         | SK3         | Invitrogen       |
| CD8       | Super Bright 600 | SK1       | eBioscience™     |
| IL-2      | APC            | MQ1-17H12   | BDBioscience     |
| IFN-γ     | Pacific Blue   | B27         | BioLegend        |
| L/D       | Fixable Viability Stain 780 |          | BDBioscience     |
Supplemental Table S6. List of all fluorochrome mAbs used for flow cytometric analysis of antigen specific B cells.

| Antigen | Flurochrome | Clone | Company          |
|---------|-------------|-------|------------------|
| CD19    | APC-Vio770  | LT19  | Miltenyi Biotech |
| CD27    | VioBright FITC | M-T271 | Miltenyi Biotech |
| IgA     | VioGreen    | IS11-8E10 | Miltenyi Biotech |
| IgG     | VioBlue     | IS11-3B2.2.3 | Miltenyi Biotech |
| CD14    | PerCP       | TÜK4  | Miltenyi Biotech |
| IgM     | APC         | PJ2-22H3 | Miltenyi Biotech |
| CD3     | PerCP       | BW264/56 | Miltenyi Biotech |
| 7AAD    |             |       | Miltenyi Biotech |
| Spike   | PE          |       | Miltenyi Biotech |
| Spike   | PE-Vio770   |       | Miltenyi Biotech |