Appendix 1: Experimental manipulation of cellular response

For detected ASC and cTfh level, cell surface staining was performed on PBMC after 4 hours' resting at room temperature for 15 min with the following anti-human monoclonal antibodies: BUV395-CD3 (clone: UCHT1), PE-CD19 (clone: HIB19), APC-CD27 (clone: M-T271), BV786-CD38 (clone: HIT2), BV510-CD4 (clone: SK3), APC-R700-CXCR5 (clone: RF8B2), and BUV737-CD45RA (clone: HI100). Fixable viability dye Fixable Viability Stain 440UV was stained at room temperature for 15 min to exclude dead cells. Cytofix Solution (BD Biosciences) was used to fix cells.

To acquire SARS-CoV-2 specific $CD4^+$ T and $CD8^+$ T and memory subset information, we conducted the ICS procedure. In brief, one million cells per well were cultured in 96-well round-bottom plates at 37 ℃ for 14 hours in the presence of 200 nM SARS-CoV-2 peptide pool (15 mer, 5 mer overlapping) from ancestral strain structural proteins including spike (S), nucleocapsid (N), membrane (M), envelope (E), and brefeldin A (BD Biosciences, 1:1,000 final) or brefeldin A only (control group). The cells were then labeled for cell surface markers at room temperature for 15 min with the following anti-human monoclonal antibodies: BUV396-CD3 (clone: UCHT1), BV510-CD4 (clone: SK3), BV786-CD8 (clone: RPA-T8), BV650-CD56 (clone: NCAM16.2), APC-R700-CXCR5 (clone: RF8B2), BB515-CD45RA (clone: HI100), PE-Cy7-CCR7 (clone: G043H7), then stained with fixable viability dye Zombie Nir (APC-Cy7) at room temperature for 15 min to exclude dead cells, subsequently fixed and permeabilized with Cytofix/Cytoperm Solution (BD Biosciences) at 4 ℃ for 30 min, finally stained with anti-intracellular cytokine/protein antibodies: BV421-IFN-γ (clone: B27), BV605-TNF (clone: MAb11), PE-CD40L (clone: 24-31) at 4 ℃ for 30 min.

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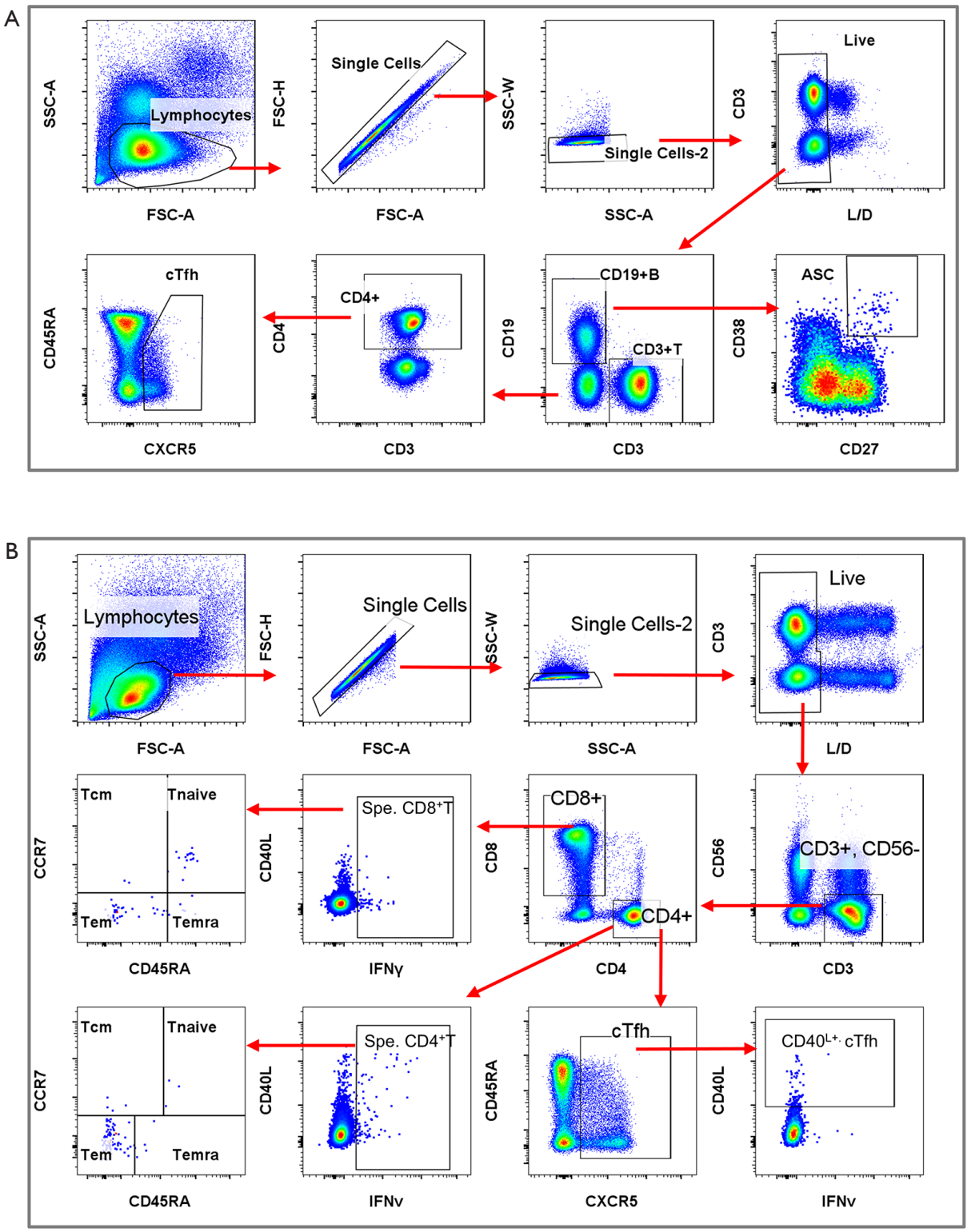

Figure S1 Gating strategies for surface (A) and intracellular staining (B) of recovered PBMC. Intracellular staining was carried out after stimulation with 200 nM peptide pool from spike, nucleocapsid, membrane, envelope protein of ancestral SARS-CoV-2 and brefeldin A. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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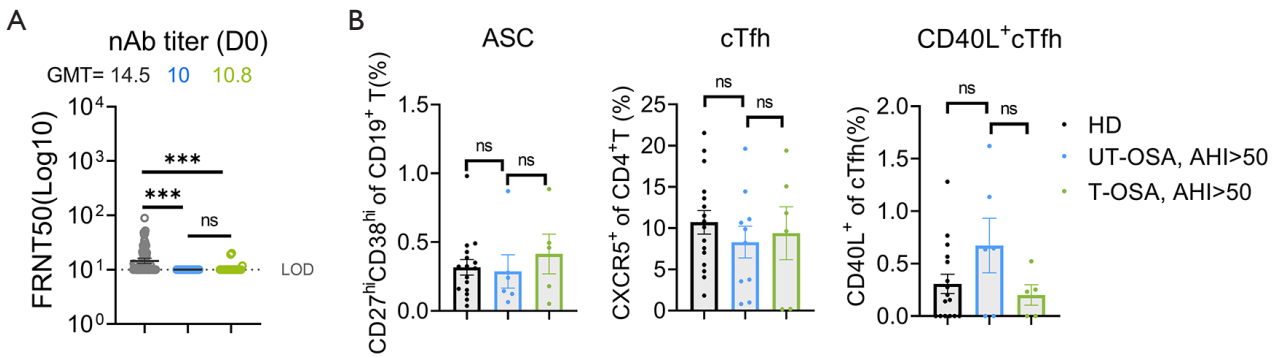

Figure S2 Pre-booster vaccination immune status. (A) Live virus nAb titer against ancestral SARS-CoV-2. (B) cellular immune response against spike, nucleocapsid, membrane, envelope protein of ancestral SARS-CoV-2. UT-OSA, OSA patients without any symptomatic treatment before and during our follow-up period (blue); T-OSA, OSA patients who start PAP treatment before their third vaccination (green). nAb titer was showed as a geometric mean with 95% CI, and cellular response data were shown as a mean with SEM. Unpaired and nonparametric Mann-Whitney test was performed between the two groups. *, P<0.05; **, P<0.01, two tailed, ns, not significant. CI, confidence interval; nAb, neutralizing antibody; OSA, obstructive sleep apnea; PAP, positive airway pressure; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SEM, standard error of the mean.

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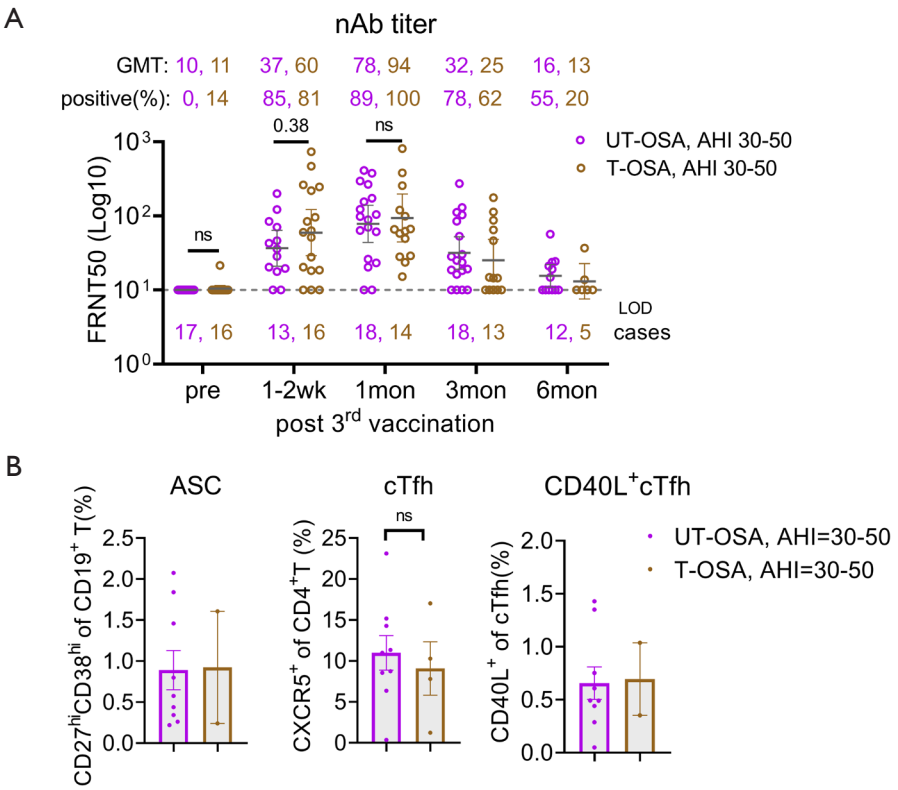

Figure S3 Immune responses in OSA patients with AHI =30–50 events/hour after PAP treatment. (A) Live virus nAb titer against ancestral SARS-CoV-2 at different stages post booster vaccination. Serum conversion ratio and GMT were displayed above and cases number involved were marked below. (B) Frequency of ASC, cTfh, and CD40L^{+} cTfh at 1 month post booster vaccination. UT-OSA, OSA patients without any symptomatic treatment before and during our follow-up period; T-OSA, OSA patients who start PAP treatment before their third vaccination. Not every individual's nAb titer and cellular response were detected, and each data point represents an individual case. nAb titer was showed as a geometric mean with 95% CI, and other data were shown as a mean with SEM. Unpaired and nonparametric Mann-Whitney test was performed between two groups at the same. *, P<0.05; **, P<0.01, two tailed, ns, not significant. AHI, apnea-hypopnea index; ASC, antibody-secreting cell; CI, confidence interval; cTfh, circulating T follicular helper; FRNT50, 50% focus reduction neutralization titer; GMT, geometric mean titer; nAb, neutralizing antibody; OSA, obstructive sleep apnea; PAP, positive airway pressure; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SEM, standard error of the mean.

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