

Supplementary figure 1. Whole blood analysis reveals lower CTLA4 expression on CD4 T cells in MTX-treated patients at baseline

(A-C) Relative expression intensity of indicated markers projected on different Fit-SNE maps, including (A) immune panel 1 for B cell clusters, (B) immune panel 2 for Th clusters and (C) immune panel 2 for cTfh clusters. (D) Volcano plot illustrating differentially expressed CD4 T cell populations that express PD1, CTLA4, TIM3, TIGIT, CD137, CD40L or CD38/HLA-DR, with a  $\log_2$  fold change  $>0.5$  and  $p$  value  $<0.5$ , between MTX-treated patients ( $n=18$ ) and RA controls ( $n=7$ ). Numbers in the top left and right corner of the volcano plots indicate number of differentially expressed populations. (E) Percentages and dynamics of CTLA4-expressing Th cells in HC ( $n=24$ ), MTX ( $n=18$ ) and RA controls ( $n=7$ ). Statistical significance was determined using Wilcoxon ranked sum test for unpaired and Wilcoxon ranked signed rank test for paired comparisons, with Bonferroni-Holm multiple comparison correction.

Supplementary figure 2: High-dimensional spectral flow cytometry reveals 16 B cell meta clusters.

(A) Representative flow cytometry plots of CD19+ B cell gating. (B) Representative flow cytometry plots of Spike- and RBD-specific CD19+ B cells based on dual fluorescently-labeled antigenic probes. (C) UMAP and (D) cluster identification from FlowSOM analysis including all antigen-specific B cells, based on 12 lineage markers (CD20, CD21, CD27, CD138, CD38, CD24, CD45RB, CD11c, IgM, IgA, IgG, IgD). (E) Relative expression intensity of indicated markers projected on UMAP. (F) Correlation analysis of %RBD+ B cells at V2D7 versus anti-RBD antibody titer at V2D7 or V2D28, in both MTX-treated patients and controls. Correlations were calculated using Spearman rank correlation.

Supplementary figure 3: Gating strategy and phenotyping of Spike-induced CD40L+CD137+ CD4 T cells.

(A) Representative gating strategy to identify CD40L+CD137+ CD4 T cells from PBMCs. (B) Dynamics of Spike-induced CD40L+CD137+ CD4 T cells over time. (C) Correlation analysis of Spike-induced CD40L+CD137+ CD4 T cells at V2D7 versus RBD-specific B cells at V2D7 in MTX-treated patients and controls. (D) Representative flow cytometry plots of IFN $\gamma$ , TNF $\alpha$  and IL-2 expression in Spike-induced CD4 T cells. (E) Flow cytometry overlay plots of Spike-induced CD40L+CD137+ CD4 T cells (grey) and cytokine co-expressing CD4 T cells (red). (F) Overlay of Spike-induced single-, double- and triple IL-2, TNF $\alpha$ , IFN $\gamma$  expressing CD4 T cells on CD40L vs CD137 dot plot, presented in a stacked bar graph. Statistical significance was determined using Wilcoxon ranked sum test for unpaired comparisons and Bonferroni-Holm multiple comparison correction. Correlations were calculated using Spearman rank correlation.