

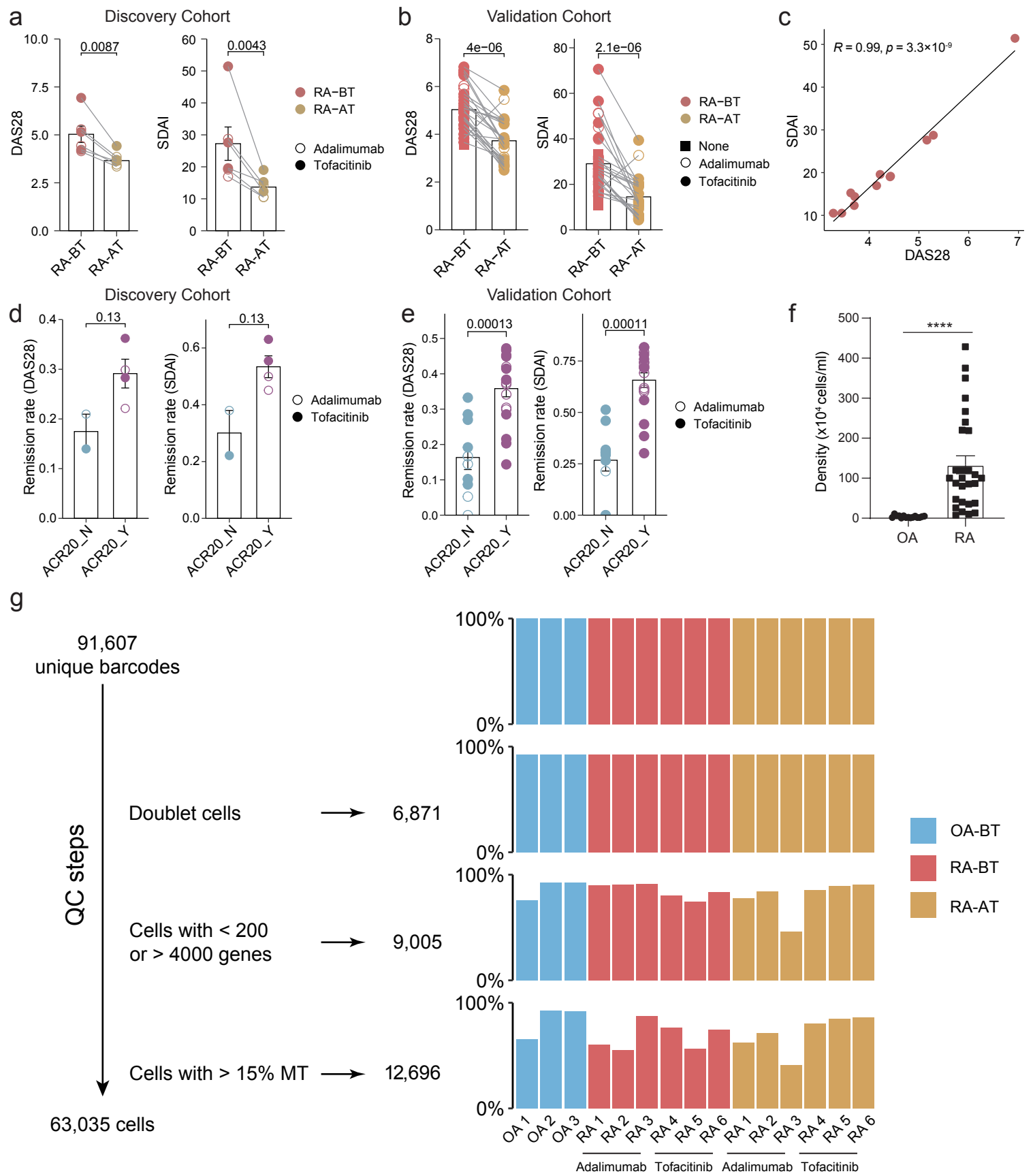


Fig. S1 Clinical information of the patients and quality control of the single-cell transcriptome profile. **a-b** DAS28/SDAI index of patients included in discovery and validation cohorts. The p -values were calculated by the two-sided Wilcoxon test. The discovery cohort consisted of 3 samples of OA-BT, 6 samples of RA-BT, and matched 6 samples of RA-AT. The validation cohort consisted of 38 individuals with RA-BT and 24 individuals with RA-AT. **c** Pearson's correlation analysis of DAS28 and SDAI index in discovery cohort. **d-e** The remission rates of ACR20_N and ACR20_Y in both our discovery and validation cohorts. The p -values were calculated by the two-sided Wilcoxon test. **f** Cell density for each SF sample. p -values were calculated by the two-sided Wilcoxon test. **g** Quality control pipeline for scRNA-seq of SF. The Left: the aggregate count of cells both before and after filtration for all samples, as well as the quantity of cells eliminated under the corresponding filtration parameters. Right: the proportion of cells that remain in each sample after the respective filtering condition, represented as a percentage of the total number of cells.

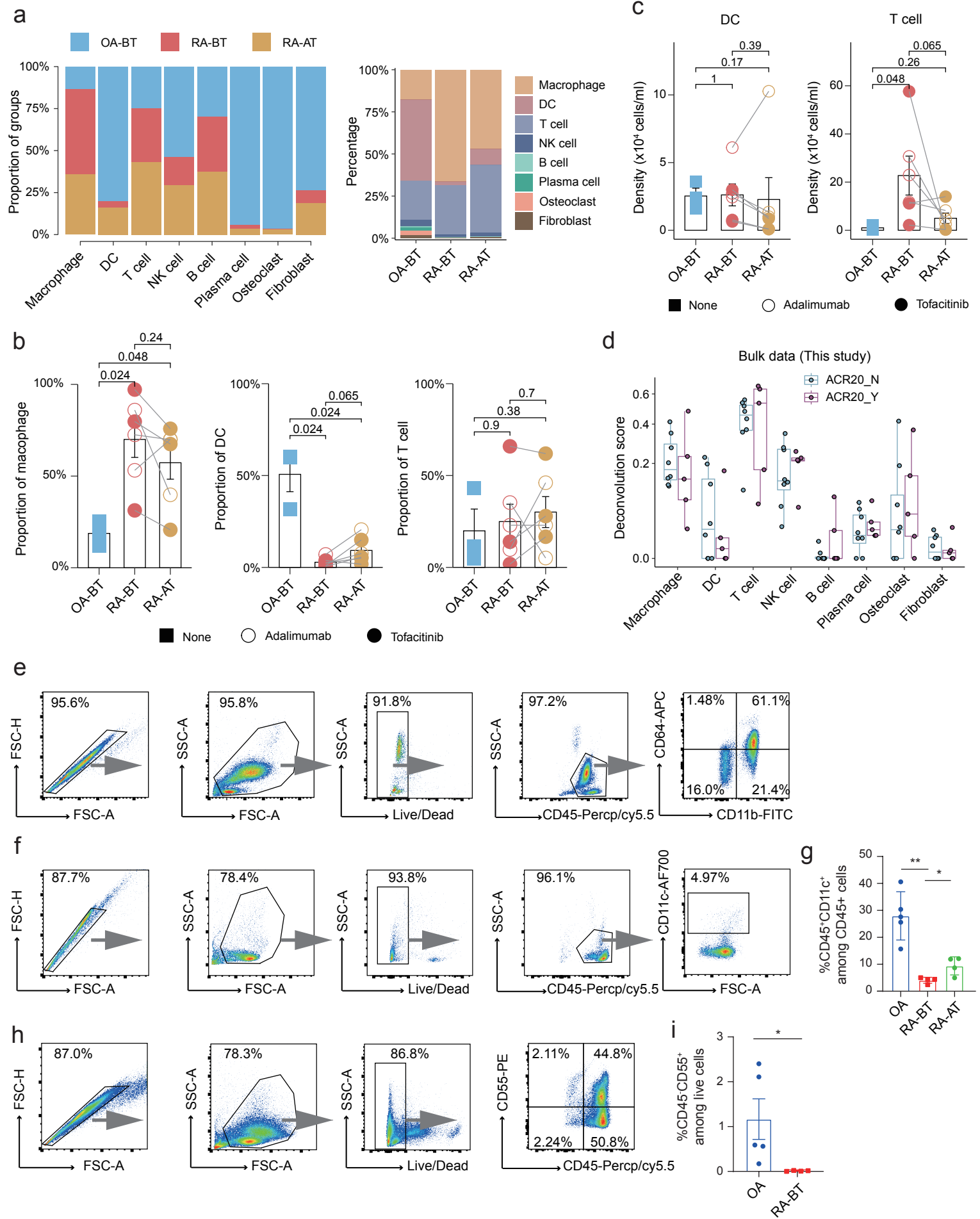


Fig. S2 Proportion of cell clusters and gating strategy. **a** Proportional analysis. Left: proportions of various groups inside the primary cell clusters; Right: proportions of distinct primary cell clusters within each group. **b-c** Proportions and density of macrophage, DC, and T cell clusters among different groups. *p*-values were calculated by the two-sided Wilcoxon test. The density was determined by multiplying the cell concentration in the samples' SF by the proportion of the DC/T cell cluster in the samples. solid squares, patients didn't receive b/tsDMARDs treatment; hollow circles, adalimumab treated; solid circles, tofacitinib treated. **d** Deconvolution of in-house bulk RNA-seq data for 8 main cell clusters between ACR20_N and ACR20_Y based on canonical marker genes. The *p*-values were calculated by the two-sided Wilcoxon test. **e-f** Gating strategy for the study of macrophages and DCs separately. **g** Comparison of FACS-based DC proportions among OA, RA-BT and RA-AT. Data are presented as mean $\pm$ SD. *p*-values are determined by Student's t-test. **p*<0.05, ***p* < 0.01. **h** Gating strategy for FLS analysis. **i** Comparison of FACS-based FLS proportion in OA with that in RA-BT patients. Data are presented as mean $\pm$ SD. *p*-values are determined by Student's t-test, **p* < 0.05.

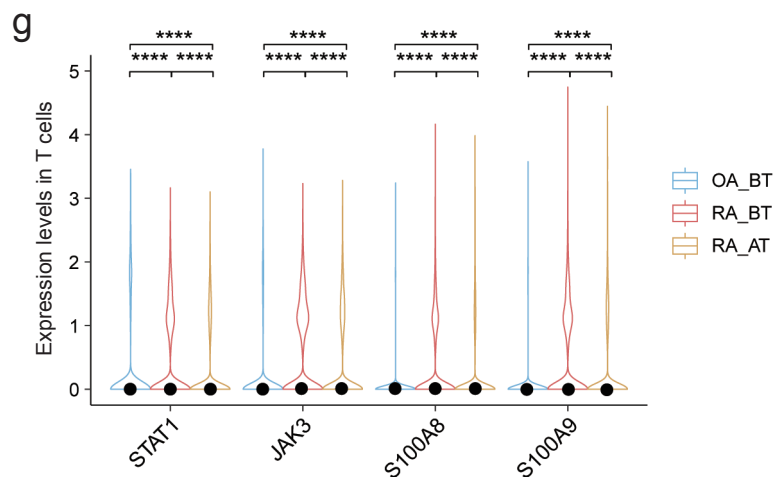
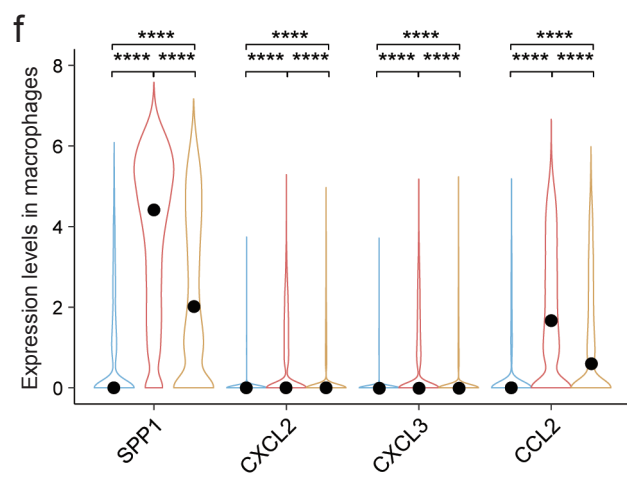
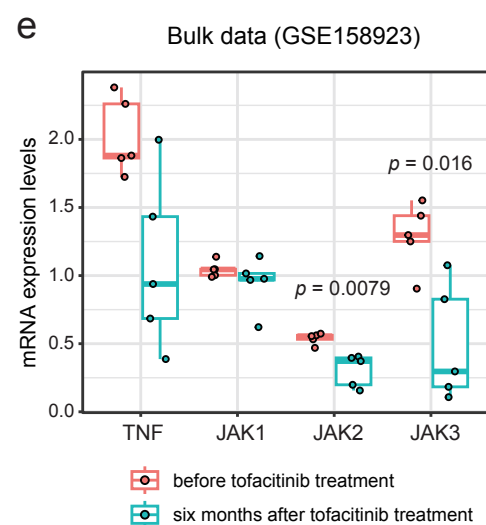
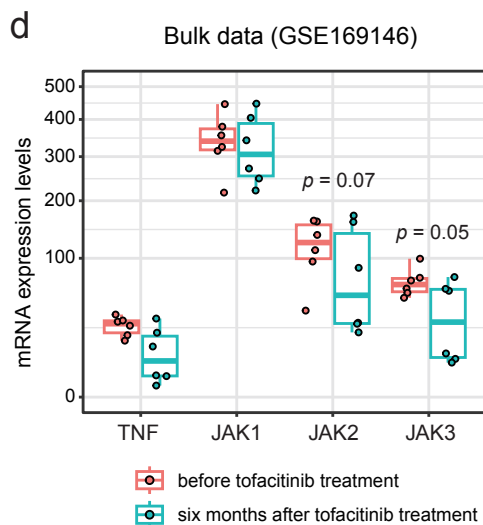
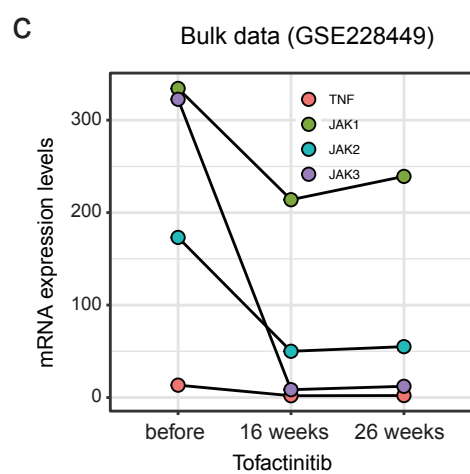
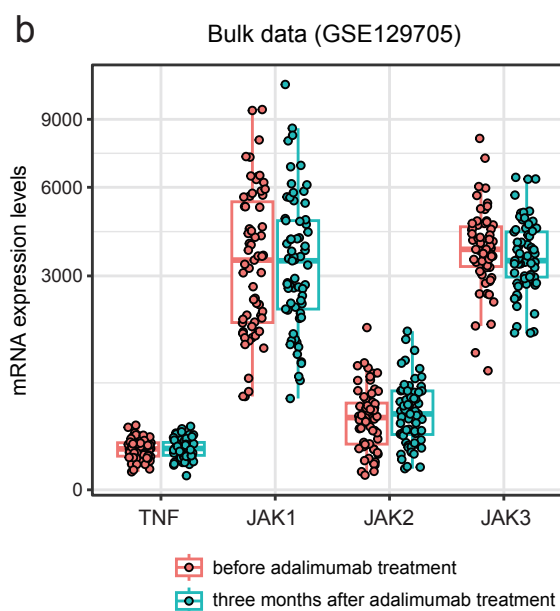
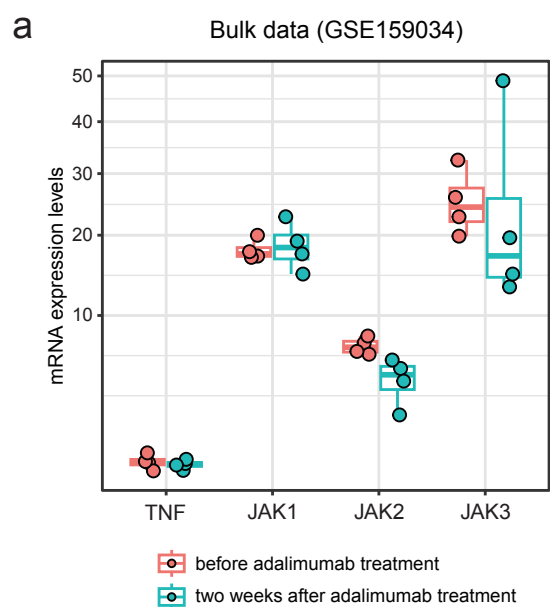


Fig. S3 Effects of adalimumab and tofacitinib on gene expression patterns. a-e

Line graph and box plots showing the effect of adalimumab and tofacitinib on the expression levels of the corresponding target genes estimated by using published bulk RNA-seq data (GSE159034, GSE129705, GSE228449, GSE169146, and GSE158923). **f-g** Violin plots displaying the expression levels of specific genes that exhibited a convergence towards OA after treatment in macrophages and T cells obtained from RA patients, respectively.

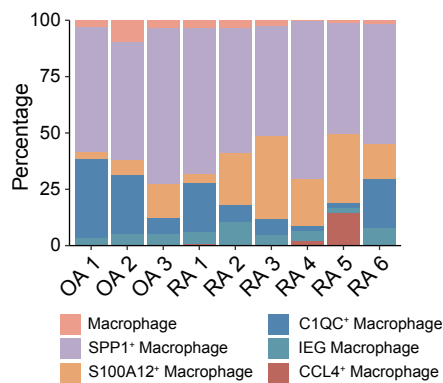
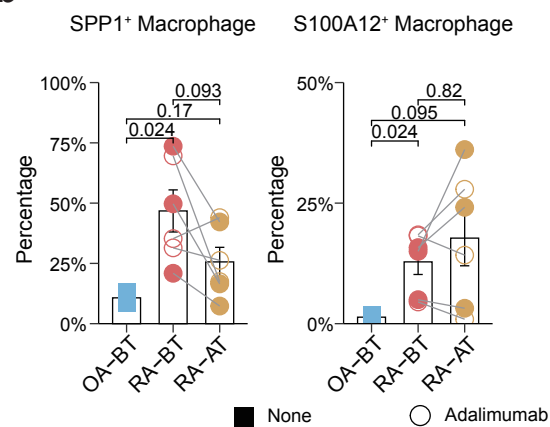
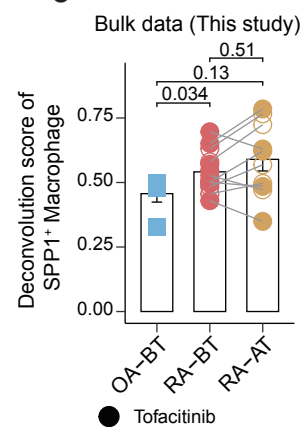
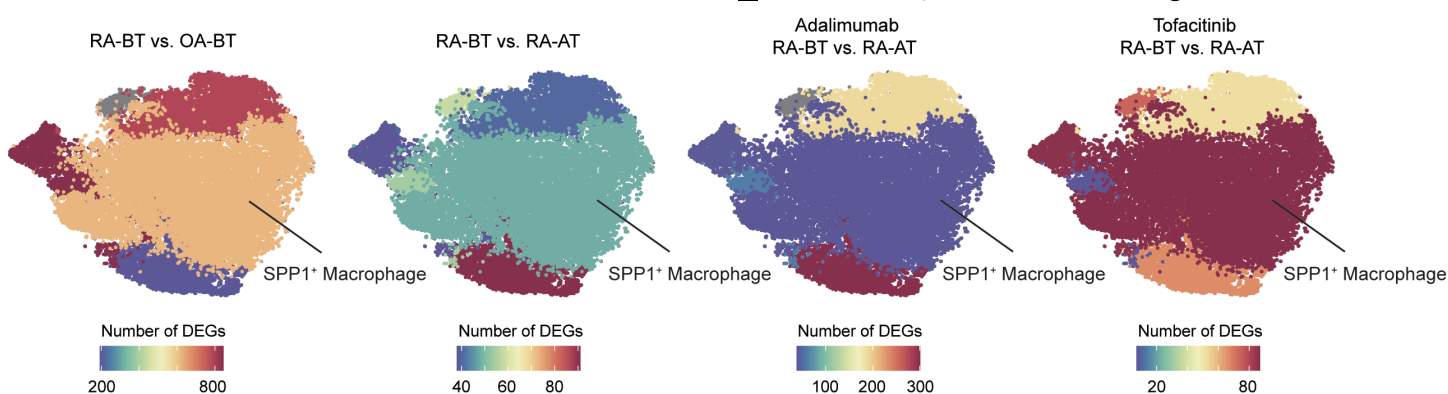
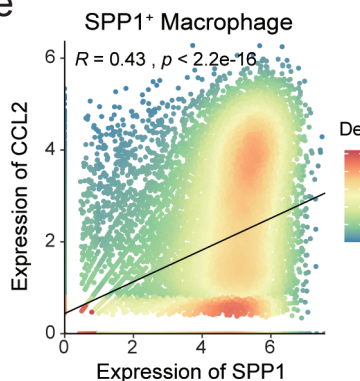
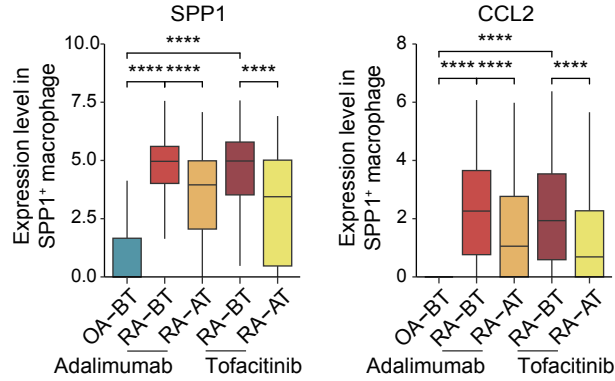
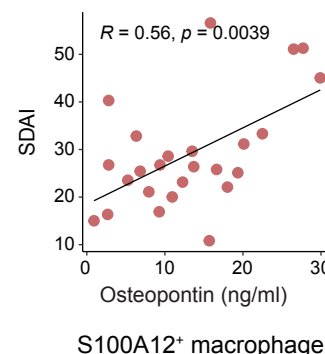
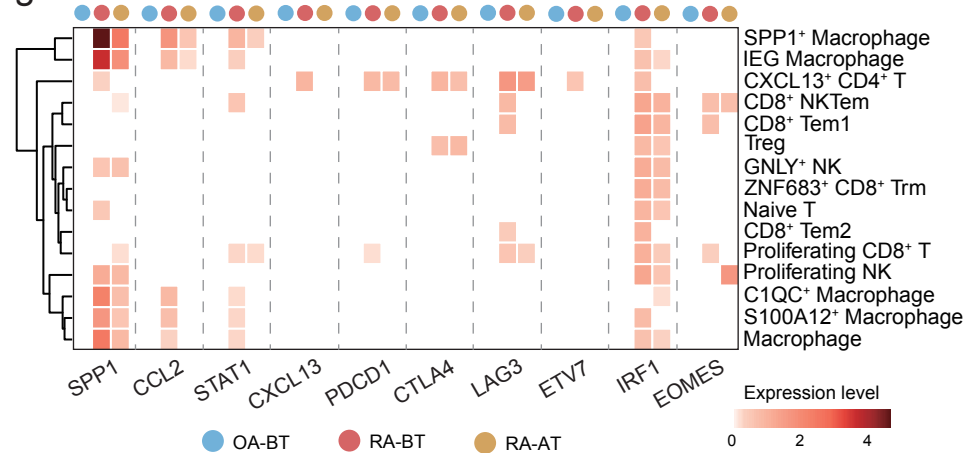
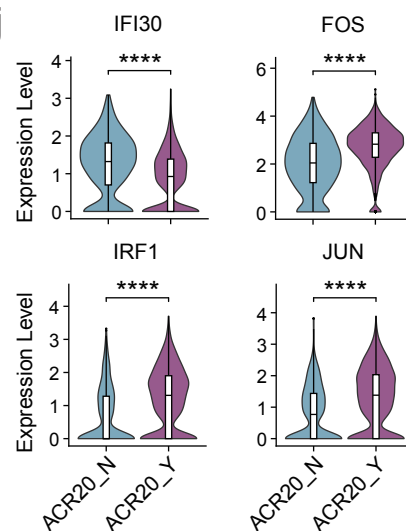
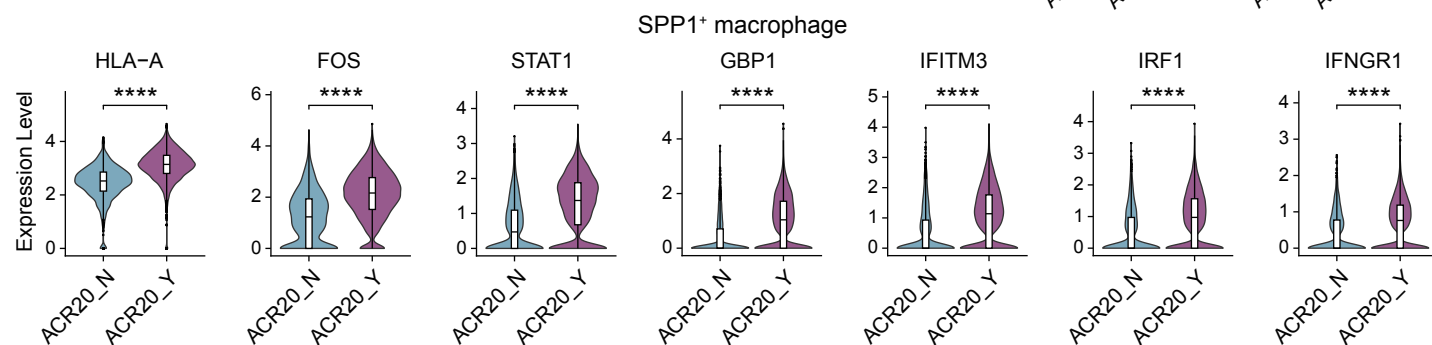
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Fig. S4 Expression characteristics of *SPP1*⁺/*S100A12*⁺ macrophage. **a** Histogram illustrating the proportions of macrophage subclusters among each patient with OA and RA. **b** Proportions of *SPP1*⁺/*S100A12*⁺ macrophage among different groups. The *p*-values were calculated by the two-sided Wilcoxon test. solid squares, patients didn't receive b/tsDMARDs treatment; hollow circles, adalimumab treated; solid circles, tofacitinib treated. **c** Deconvolution analysis of in-house bulk RNA-seq data for *SPP1*⁺ macrophages among groups based on marker genes. The *p*-values were calculated by the two-sided Wilcoxon test. **d** Feature plots displaying the number of DEGs in each macrophage subcluster among different samples. *p*-values were calculated by the two-sided Wilcoxon test. **e** Pearson's correlation analysis of *SPP1* and *CCL2* expression in *SPP1*⁺ macrophage. The hue signifies the level of density. **f** Box plots illustrating the varying levels of *SPP1* and *CCL2* expression across different groups. **g** Heatmap for expression levels of key genes in different cell subclusters. **h** Pearson's correlation of the expression level of osteopontin with the SDAI. **i-j** Violin plots depicting the expression levels of DEGs between ACR20_N and ACR20_Y in *SPP1*⁺ and *S100A12*⁺ macrophages.

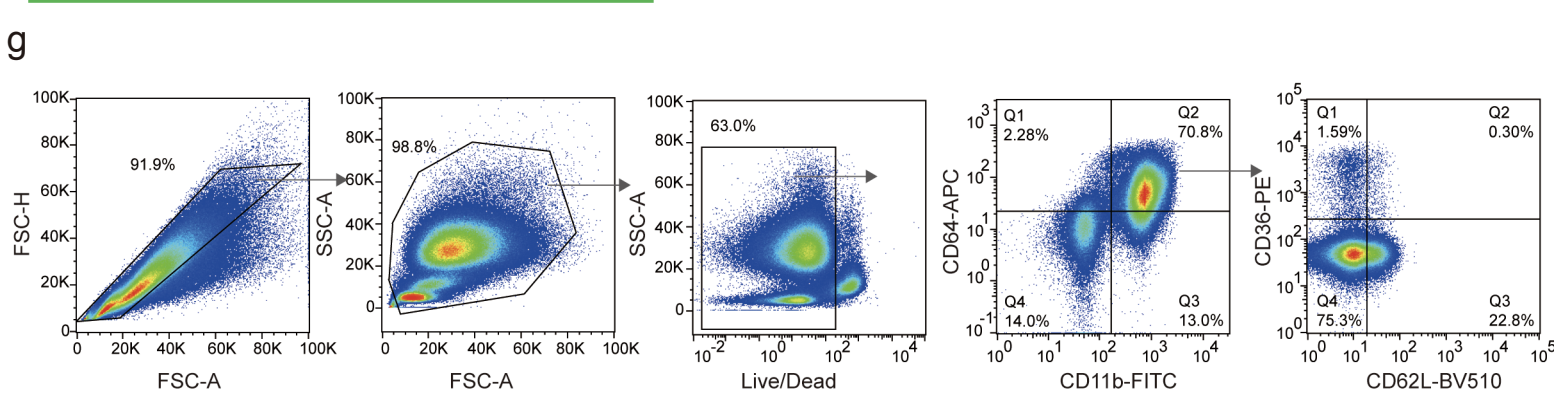
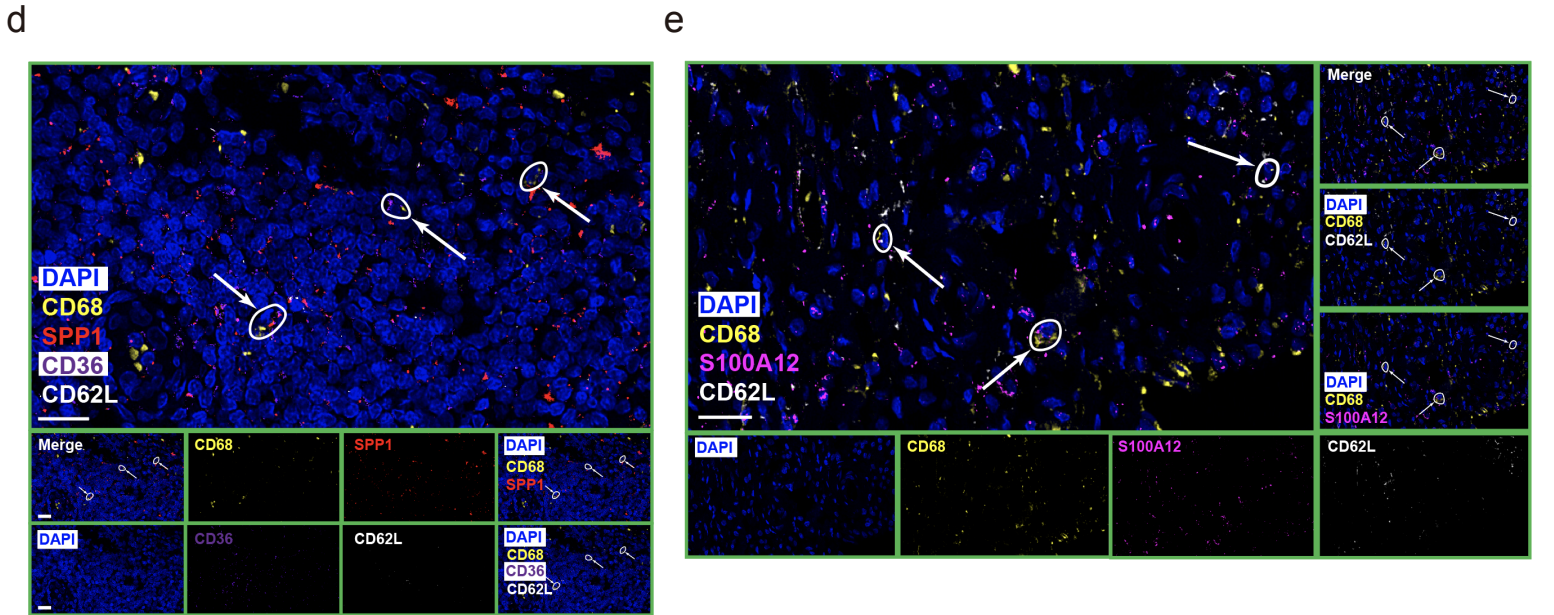
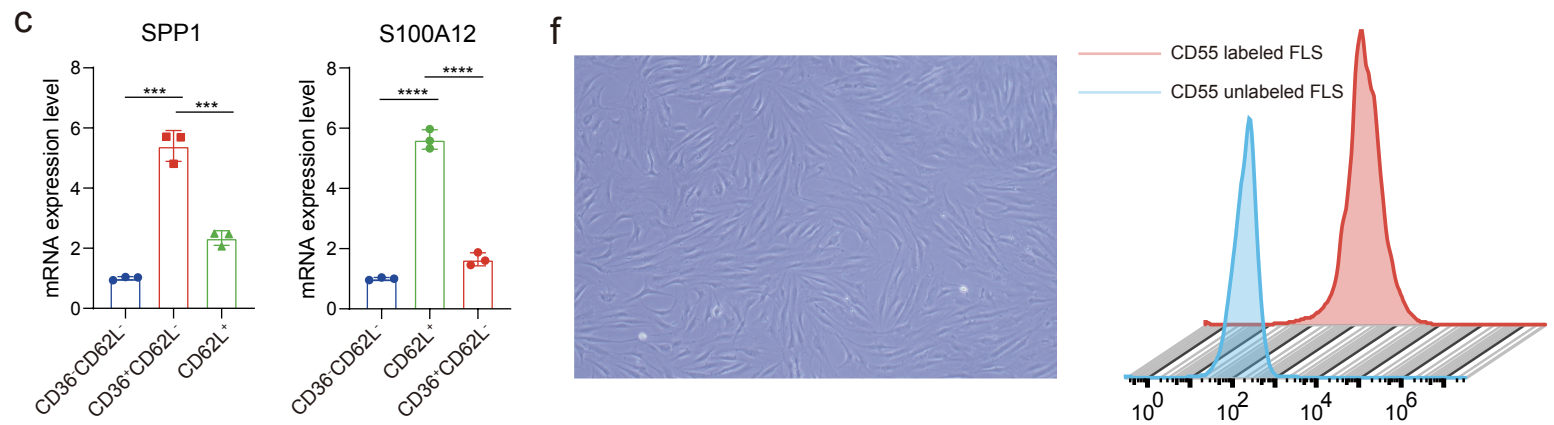
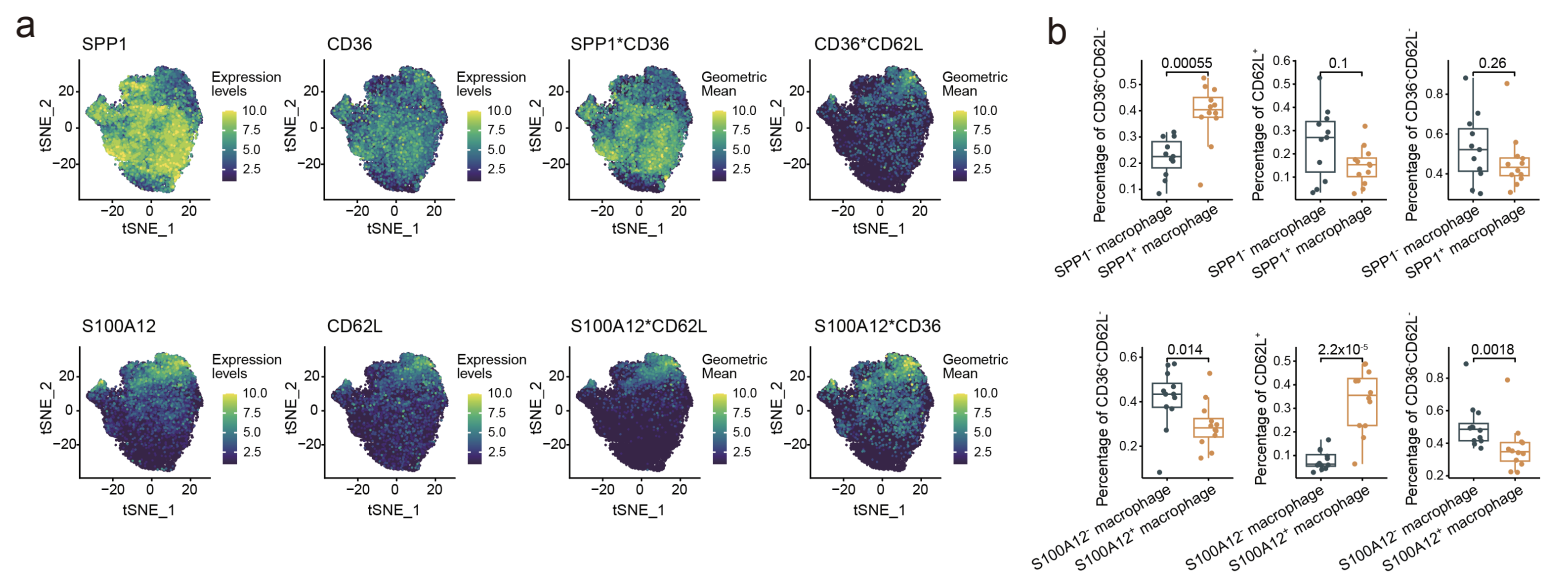


Fig. S5 Flow cytometry marker selection and validation. **a** Feature plots displaying gene expression levels and geometric means for selection of flow cytometry markers, based on scRNA data of SF macrophages. **b** Box plots showing the precision of flow cytometry marker selection based on scRNA data of SF macrophages. The x-axis represents *SPP1*⁺ and *SPP1*⁻ macrophages, indicating macrophage with *SPP1* expression greater than 0 and equal to 0, respectively. The same definition applies to *S100A12*⁺ and *S100A12*⁻ macrophages. **c** Box plots illustrating the mRNA expression levels of *SPP1* and *S100A12* (measured by qPCR) in cells sorted from the SF of RA patients using flow cytometry. The x-axis represents *SPP1*⁺ (CD11b⁺CD64⁺CD36⁺CD62L⁻), *S100A12*⁺ (CD11b⁺CD64⁺CD62L⁺) and double negative (CD11b⁺CD64⁺CD36⁻CD62L⁻) macrophages. **d-e** Co-localization of *SPP1*⁺ and CD36⁺CD62L⁻ macrophages (CD68⁺), as well as *S100A12*⁺ and CD62L⁺ macrophages (CD68⁺) in the ST of patient with RA by immunofluorescence, indicated by the white arrows. The color of gene labels is consistent with the fluorescence staining of each protein and blue represents DAPI. Scale bars: 20 μm. **f** Identification of ST-derived FLS (passage 3) from RA patients: morphological analysis (left) and CD55 expression assessment via flow cytometry (right). **g** Gating strategy for *SPP1*⁺/*S100A12*⁺ macrophages in the SF of RA patients.

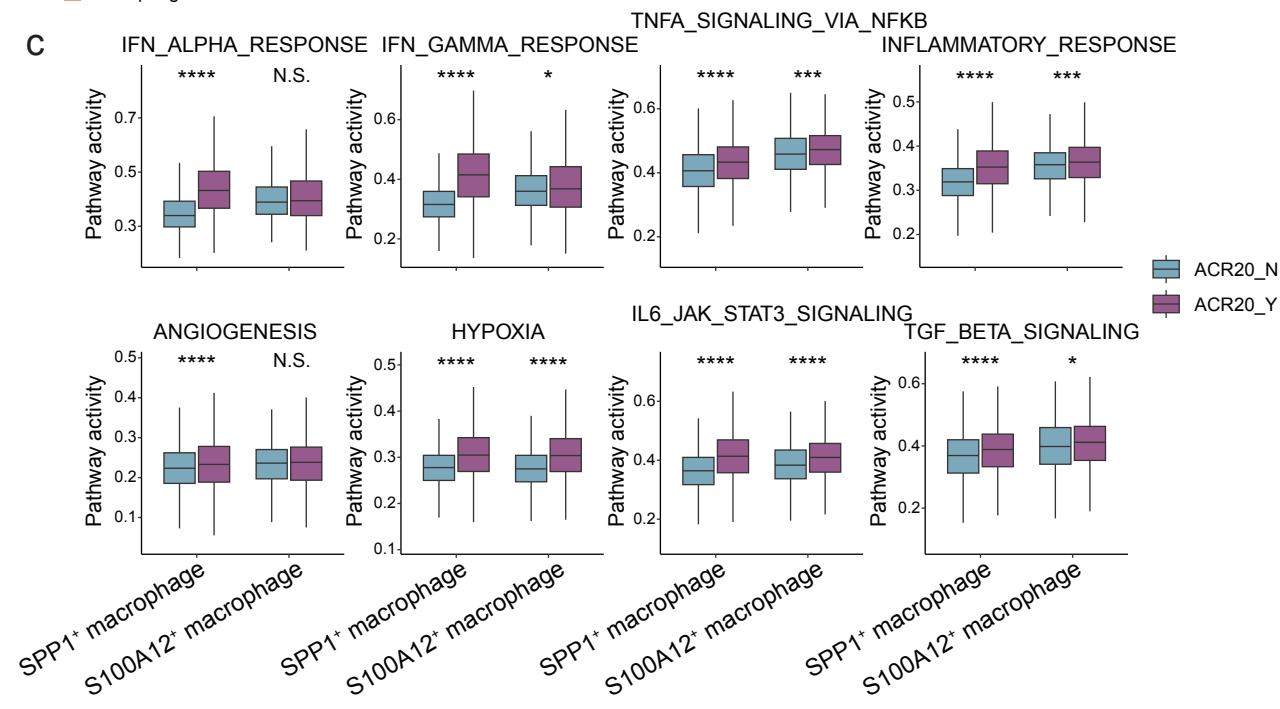
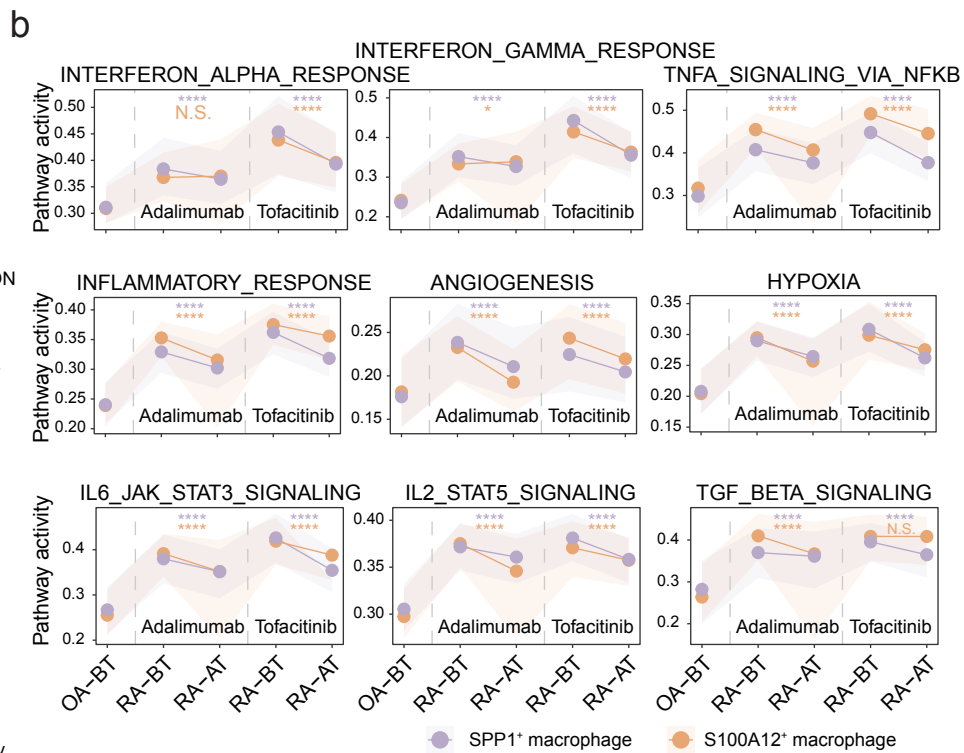
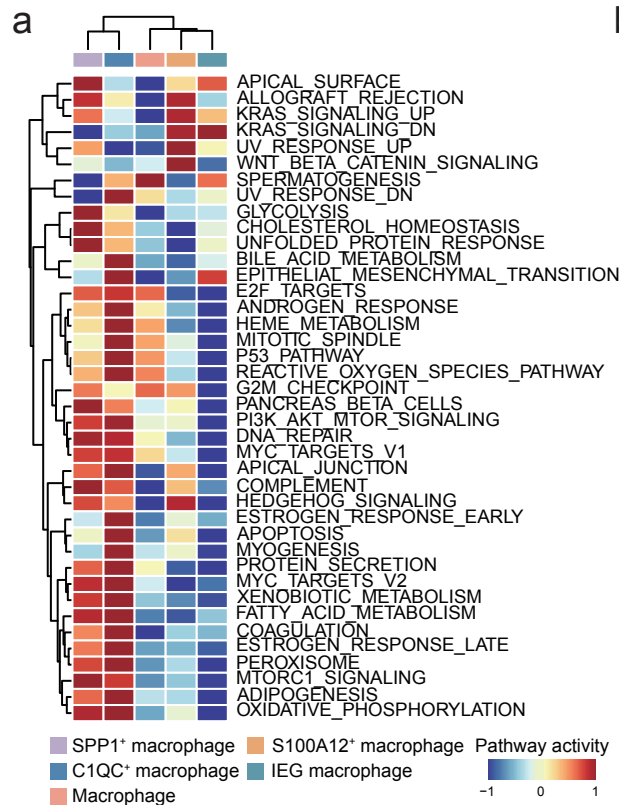


Fig. S6 Expression modules for macrophage subtypes. **a** Heatmap displaying the ssGSEA scores of the hallmark gene sets from the MSigDB database across the five macrophage subclusters. **b-c** Dynamic activity of hallmark pathways in *SPP1*⁺ and *S100A12*⁺ macrophages among groups categorized by treatment status and ACR20 status, respectively. N.S. $p > 0.05$, * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

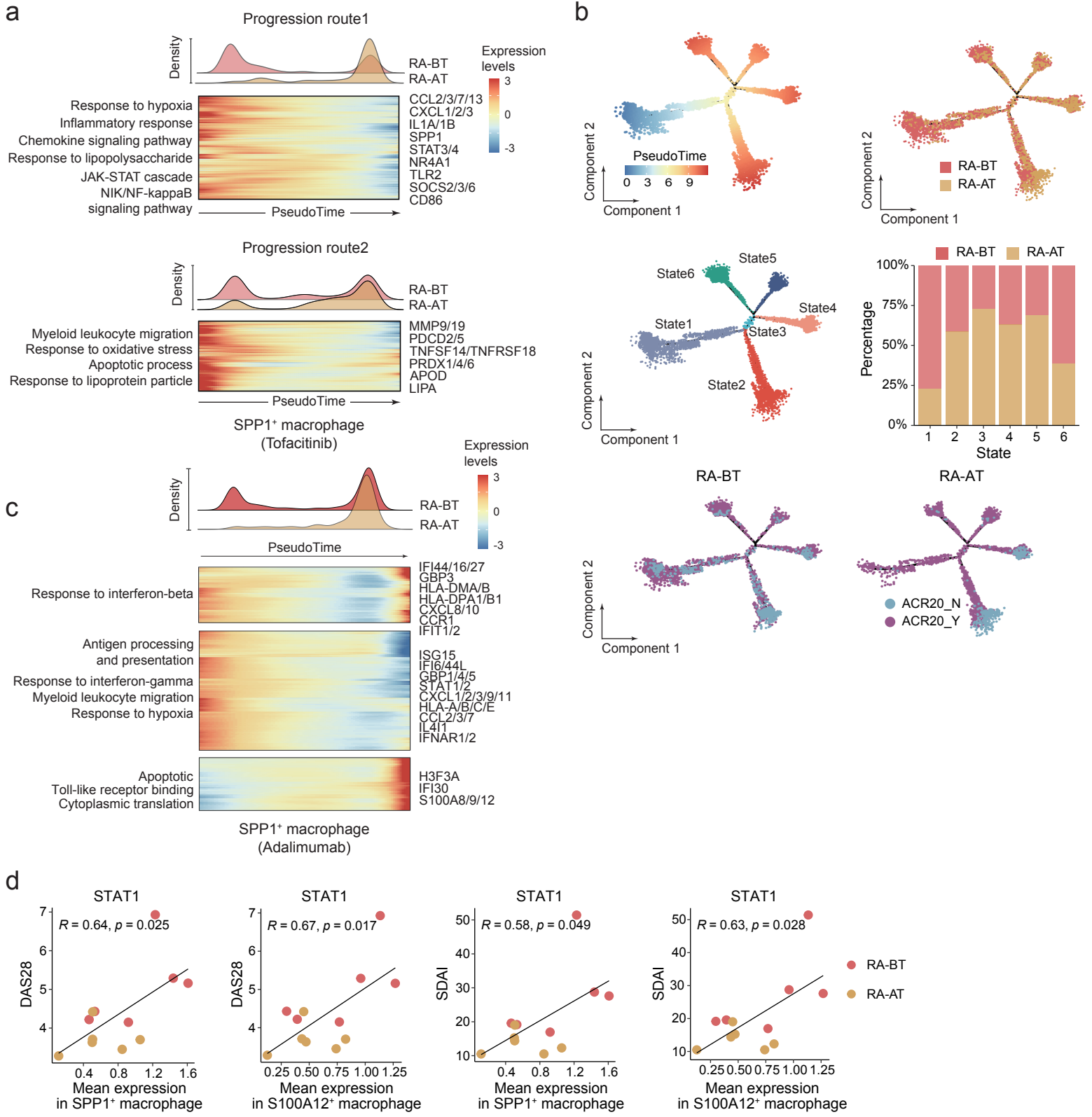


Fig. S7 Evolutionary trajectory of *SPP1*⁺ macrophage in RA patients. **a** Fluctuating genes (in rows, q-value < 10⁻¹⁰) along different progression routes of *SPP1*⁺ macrophages before and after treatment with tofacitinib were hierarchically clustered into three subclusters, enriched in the representative genes and GO-based pathways. **b** The evolutionary trajectory of *SPP1*⁺ macrophage in RA patients treated with adalimumab. Upper panel: pseudotime curve and activation trajectory of *SPP1*⁺ macrophage; Middle panel: six states of trajectory with histography for the various sources in each state; Lower panel: distinct dynamic pathways of evolution observed between ACR20_Y and ACR20_N. **c** Fluctuating genes (in rows, q-value < 10⁻¹⁰) along different progression routes of *SPP1*⁺ macrophages before and after treatment with adalimumab were hierarchically clustered into three subclusters, enriched in the representative genes and GO-based pathways. **d** Pearson's correlation analysis of *STAT1* and DAS28/SDAI index in *SPP1*⁺/*S100A12*⁺ macrophages.

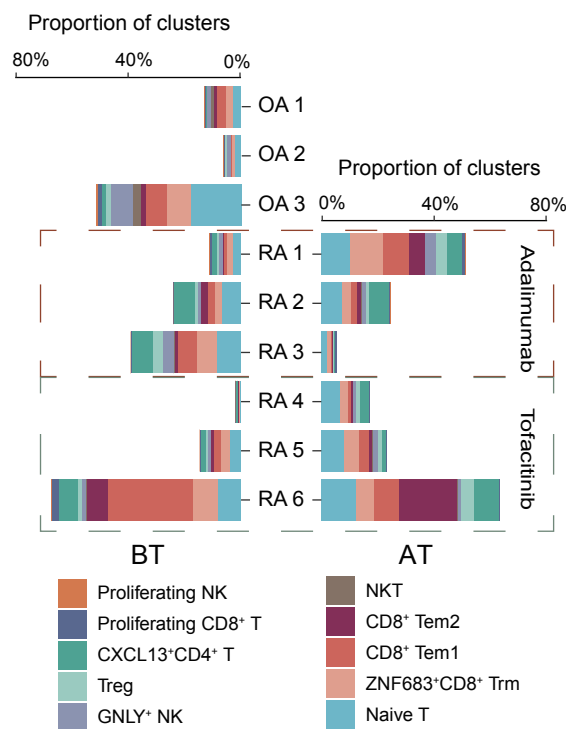
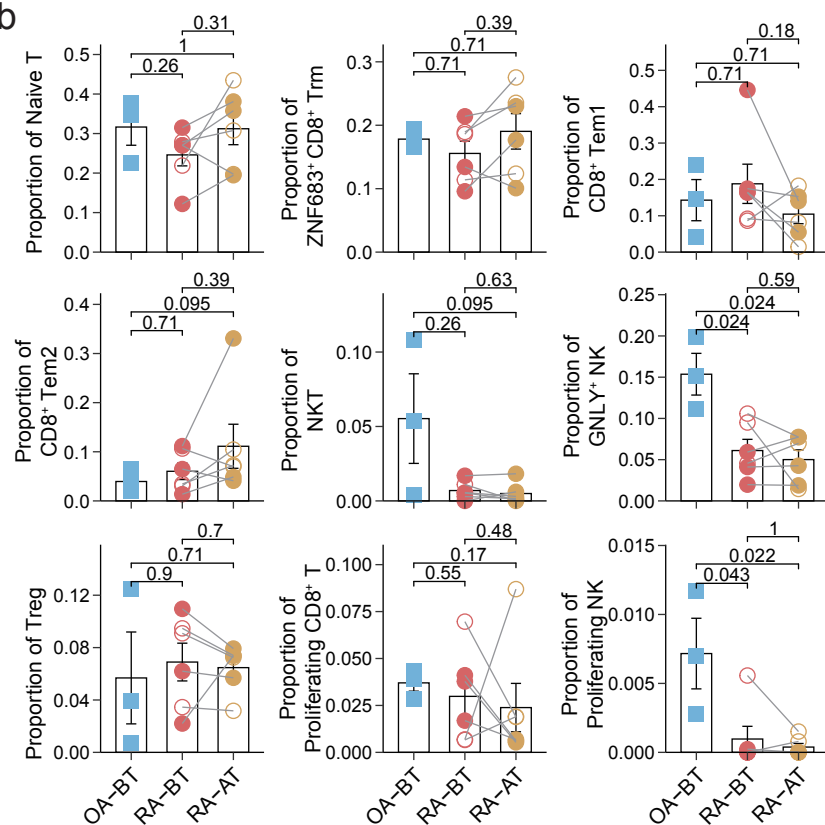
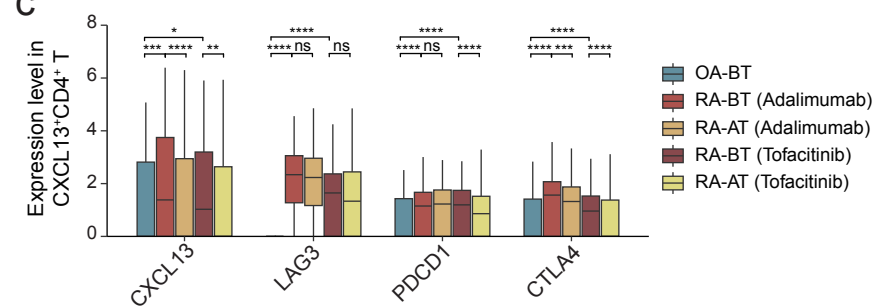
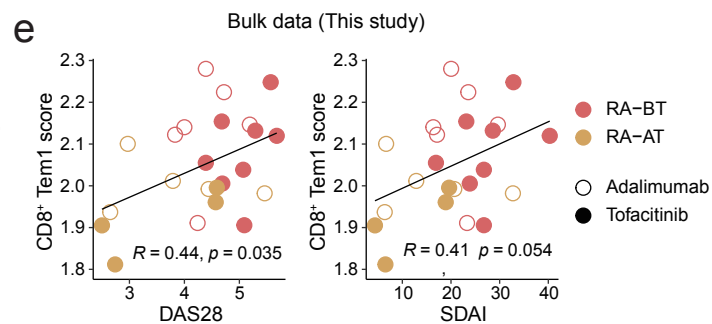
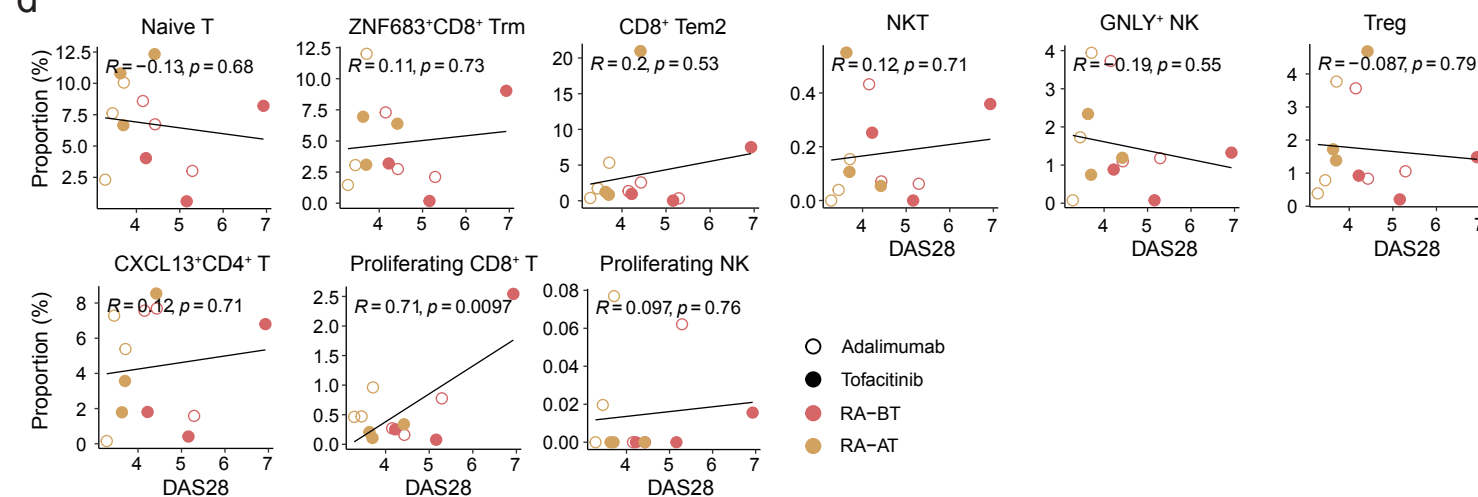
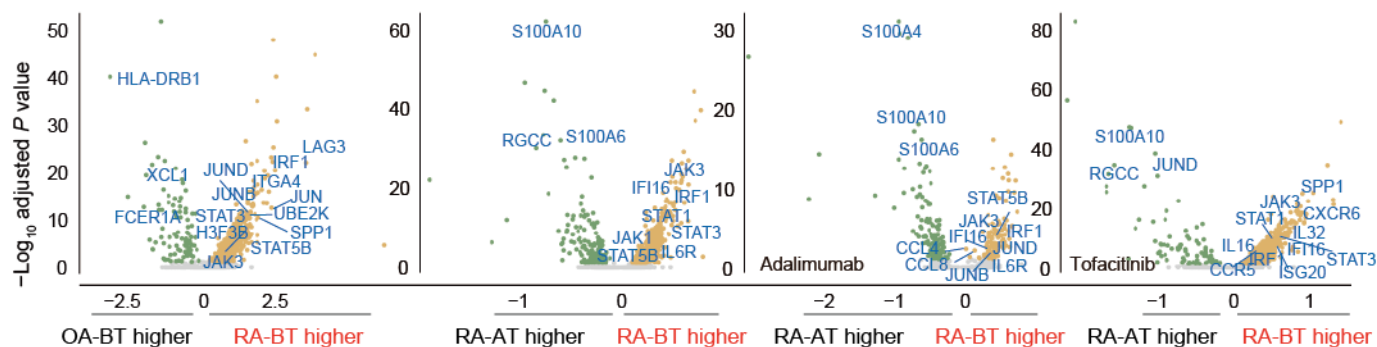
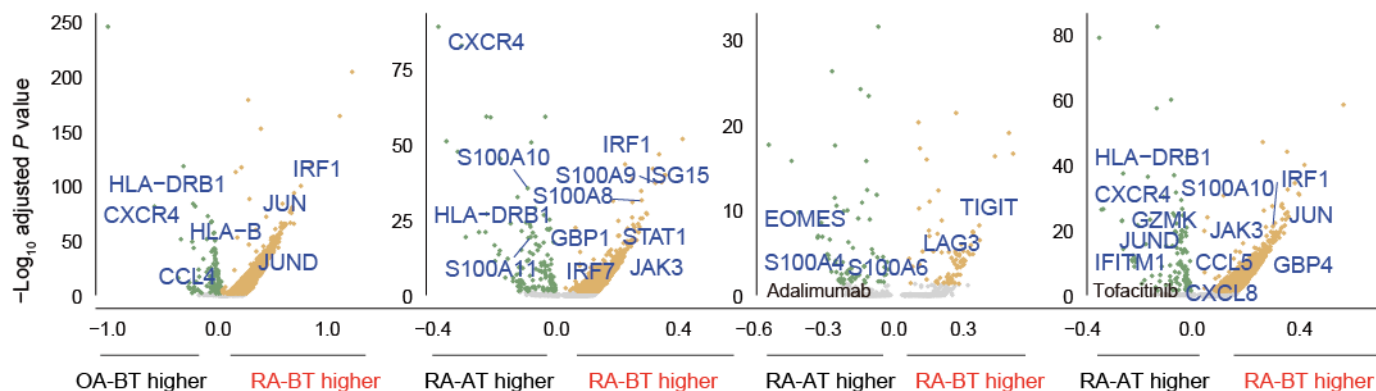
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Fig. S8 Analysis of percentage in T cell subtypes. **a-b** Proportions of each T cell subcluster across different samples and groups. **c** Expression levels of the dysfunction markers (*CXCL13*, *LAG3*, *PDCD1*, *CTLA4*) in *CXCL13*⁺*CD4*⁺ T. The *p*-values were calculated by the two-sided Wilcoxon test. ns *p* > 0.05, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001. **d** Pearson's correlation analysis of the proportion of each T cell subclusters and the DAS28 index. **e** Pearson's correlation analysis of *CD8*⁺ Tem1 deconvolution score and DAS28/SDAI of RA patients. hollow circles, adalimumab treated; solid circles, tofacitinib treated.

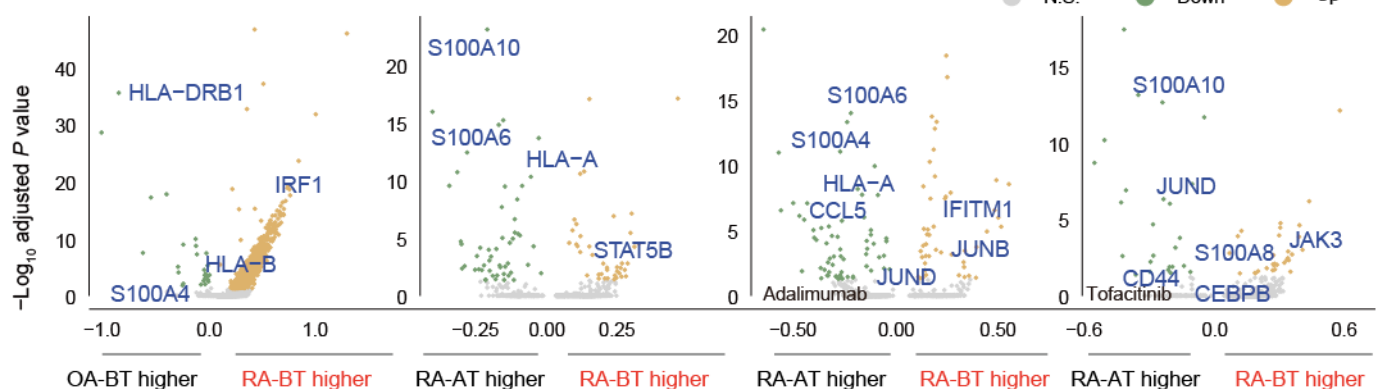
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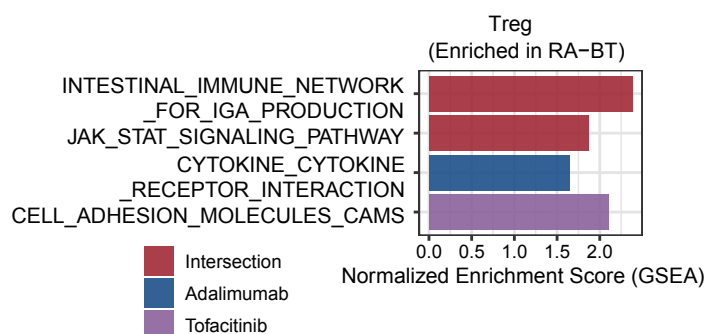
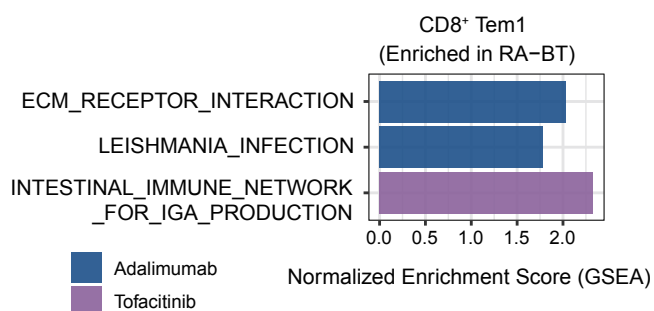


Fig. S9 DEG and GO enrichment analysis. a-c Volcano maps displaying DEGs of *CXCL13*⁺*CD4*⁺ T cell, *CD8*⁺ Tem1, and Treg among pairwise comparisons. The maps showing the fold change (Log2 transformed, x-axis) and FDR corrected *p*-value (Log10 transformed, y-axis) for each DEG. The *p*-values were calculated by the two-sided Wilcoxon test. N.S., *p* > 0.05, Up and Down represent the DEGs significantly up- and down-regulated in RA-BT group (*p* < 0.05), respectively. **d** Positively enriched GSEA pathways in RA-BT group for *CD8*⁺ Tem1 and Treg. The enriched pathways in the RA-BT were divided into three groups: red, blue, and purple, corresponded to the shared, adalimumab-specific, and tofacitinib-specific, respectively.

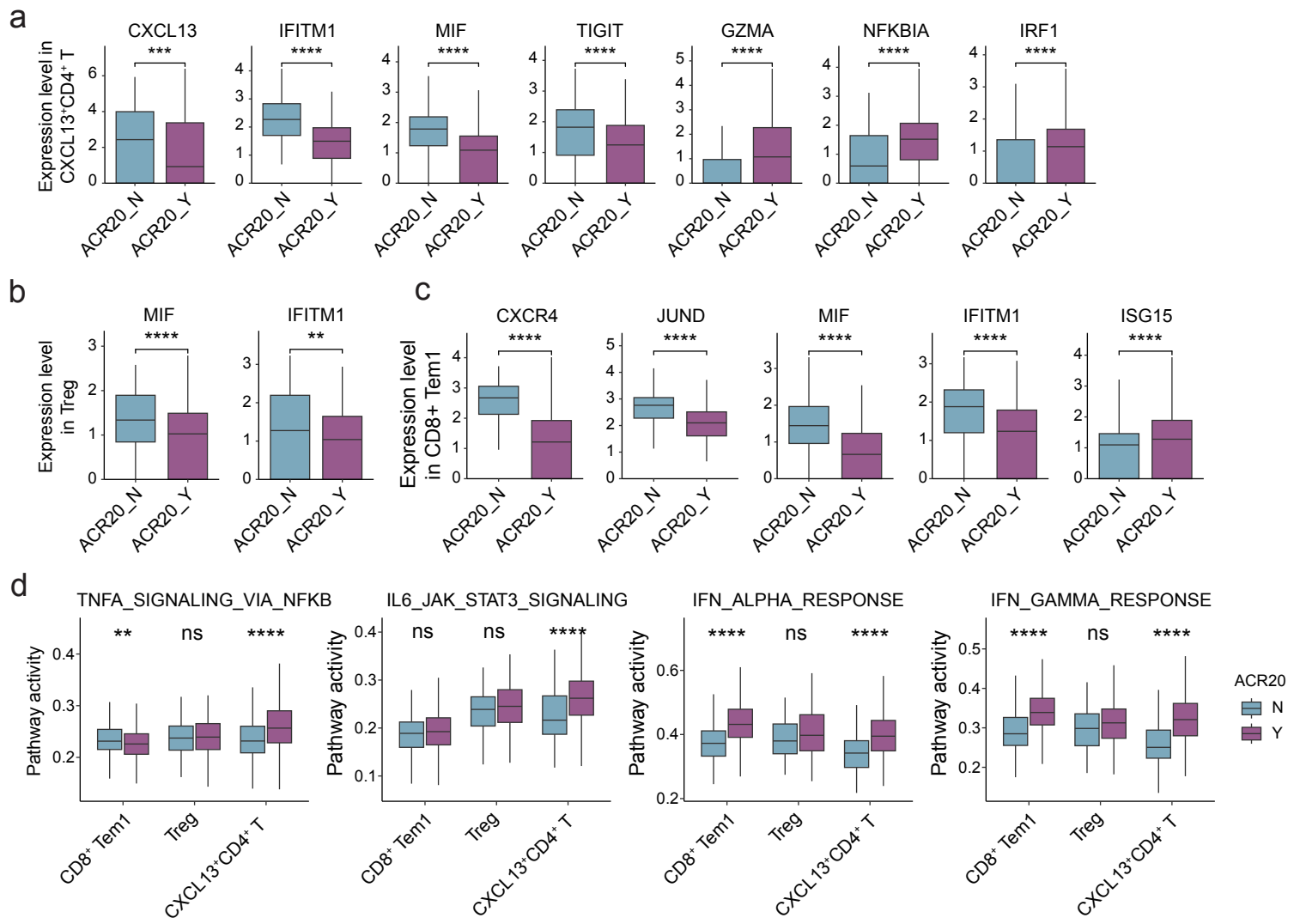


Fig. S10 DEG and hallmark enrichment analysis. **a-c** Representative DEGs between ACR20_N and ACR20_Y in *CXCL13*⁺*CD4*⁺ T, Treg, and *CD8*⁺ Tem1. *p*-values were calculated by the two-sided Wilcoxon test. **d** Different activities of the hallmark pathways between ACR20_Y and ACR20_N in pathogenic T cell subclusters. *p*-values were calculated by the two-sided Wilcoxon test. ns *p* > 0.05, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

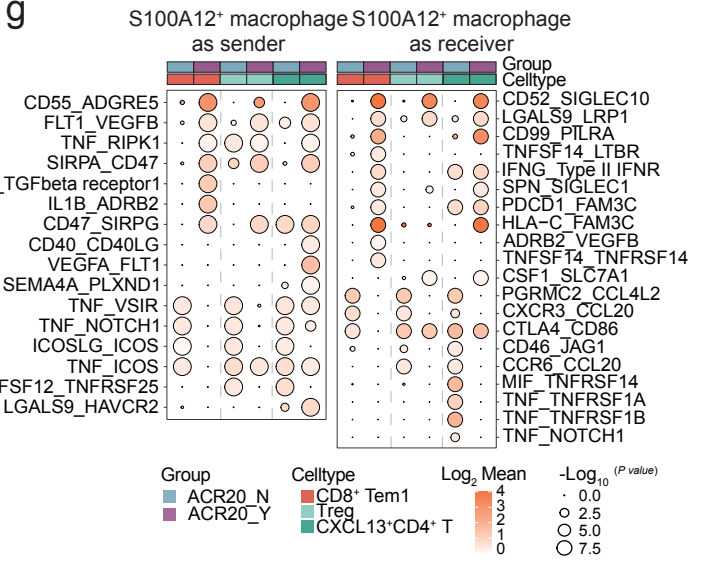
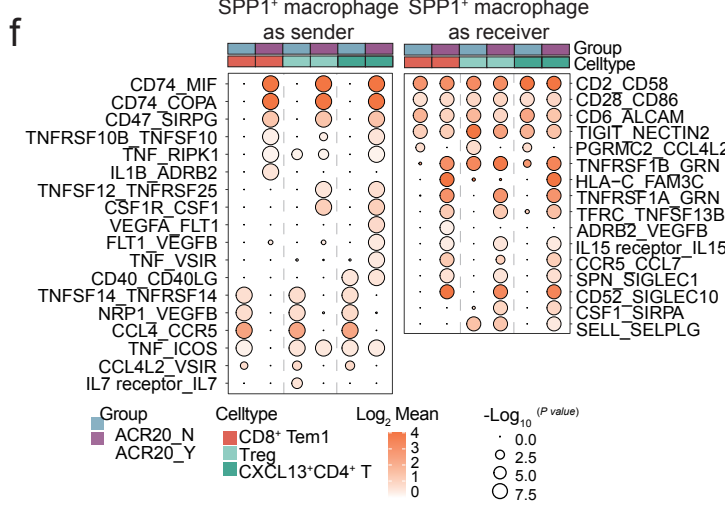
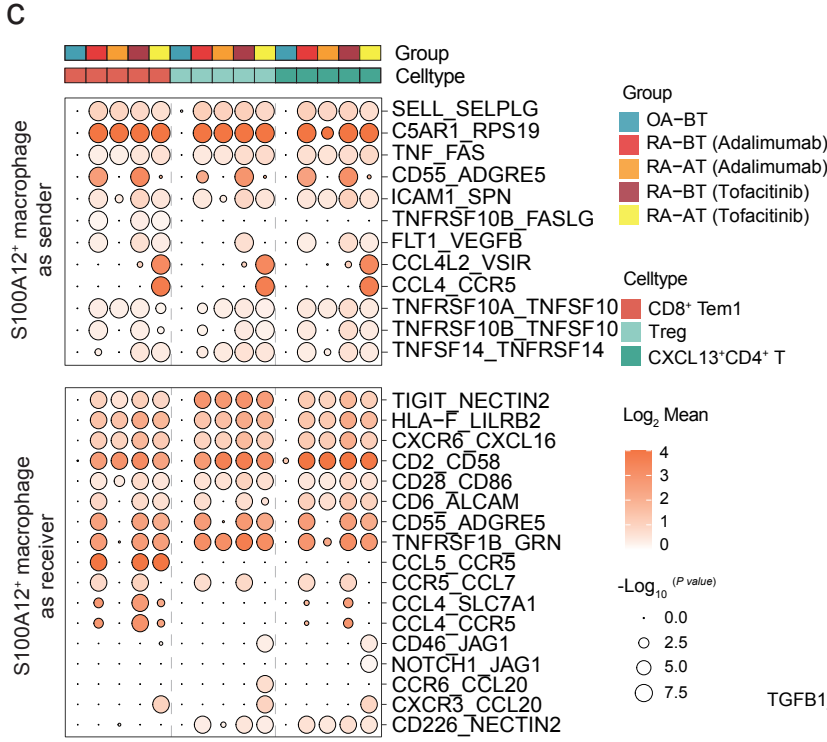
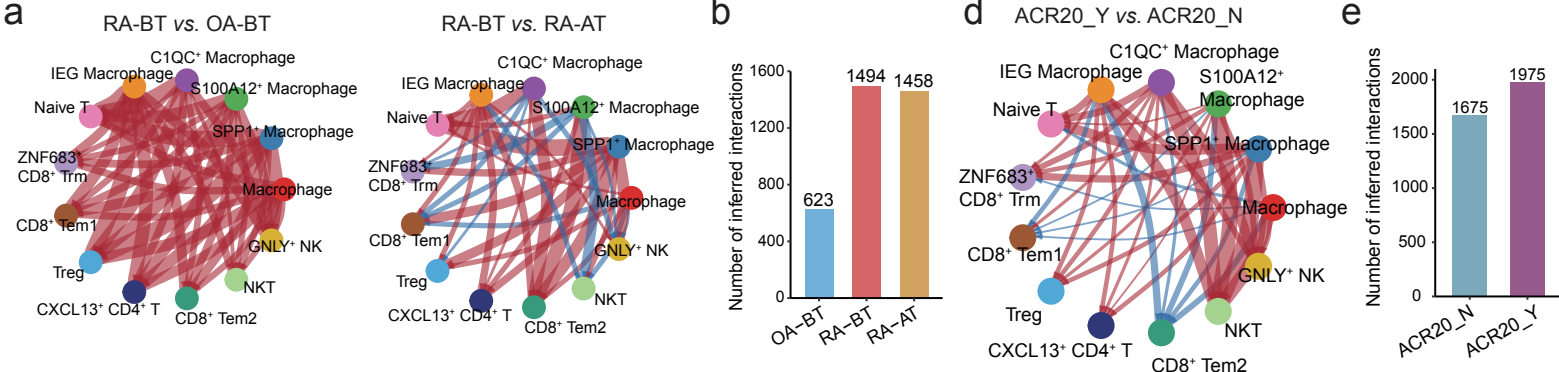


Fig. S11 Communications between focused subtypes of macrophage and T cell.

a Circle plots displaying the pairwise comparisons (RA-BT vs. OA-BT and RA-BT vs. RA-AT) of Cellchat-based interactions within the intercellular communication network among different cell clusters. Greater line thickness indicates more intense interactions, red and blue colors indicate heightened and diminished signaling in RA-BT relative to OA-BT/RA-BT, respectively. **b** Histogram displaying the number of Cellchat-inferred interactions among different groups. **c** Predicted and detail CellphoneDB-based ligand-receptor communications of *S100A12*⁺ macrophage with pathogenic T cell subclusters. Dot size and color spectrum indicate the *p*-value ($-\log_{10}$ transformed) and mean expression ($\log_2$ transformed) of ligand and receptor, respectively. **d** Circle plots displaying the pairwise comparisons (ACR20_Y vs. ACR20_N) of Cellchat-based interactions within the intercellular communication network among different cell clusters. Greater line thickness indicates more intense interactions, red and blue colors indicate heightened and diminished signaling in ACR20_Y relative to ACR20_N, respectively. **e** Histogram illustrating the number of Cellchat-based interactions in ACR20_Y and ACR20_N. **f-g** Comparison of the predicted and detail CellphoneDB-based ligand-receptor communications of pathogenic T and macrophage subclusters between ACR20_N and ACR20_Y in *SPP1*⁺/*S100A12*⁺ macrophage. Dot size and color spectrum indicate the *p*-value ($-\log_{10}$ transformed) and mean expression ($\log_2$ transformed) of ligand and receptor, respectively.

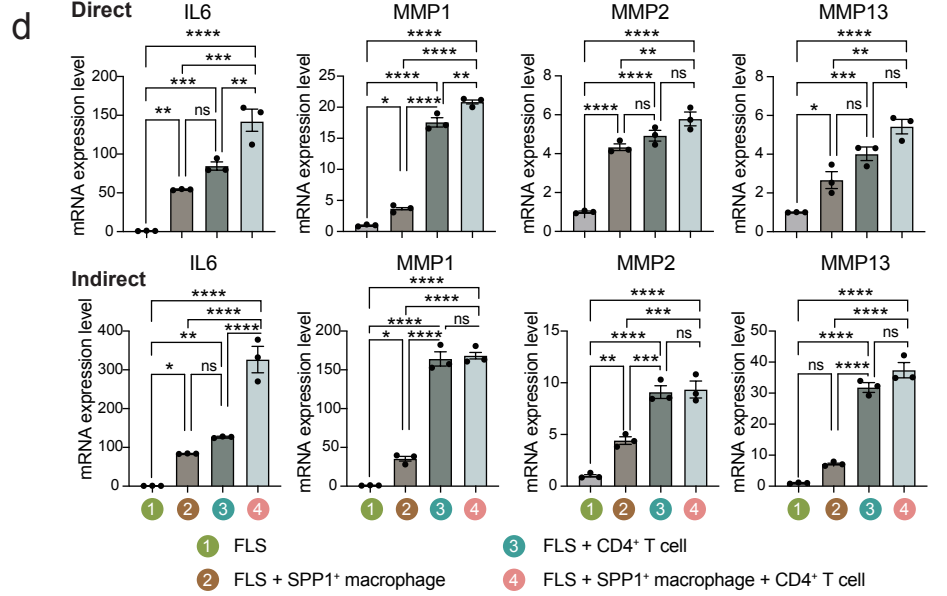
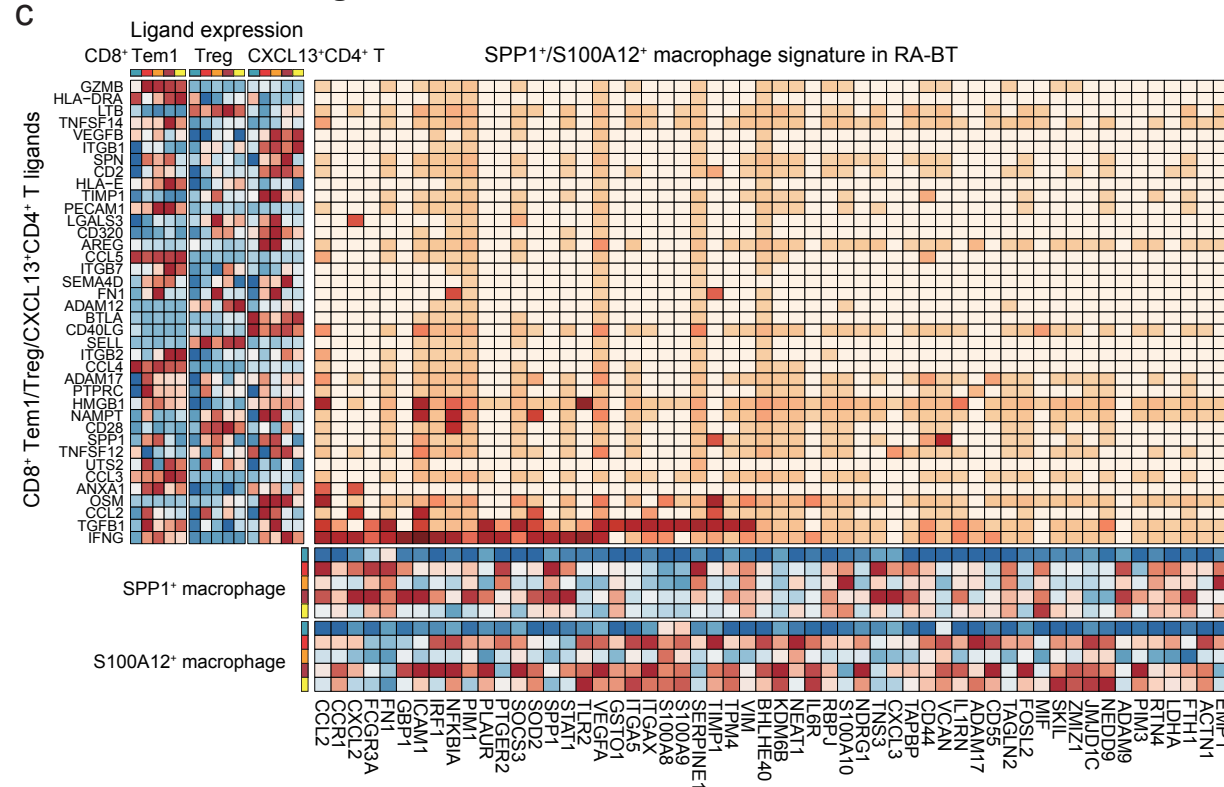
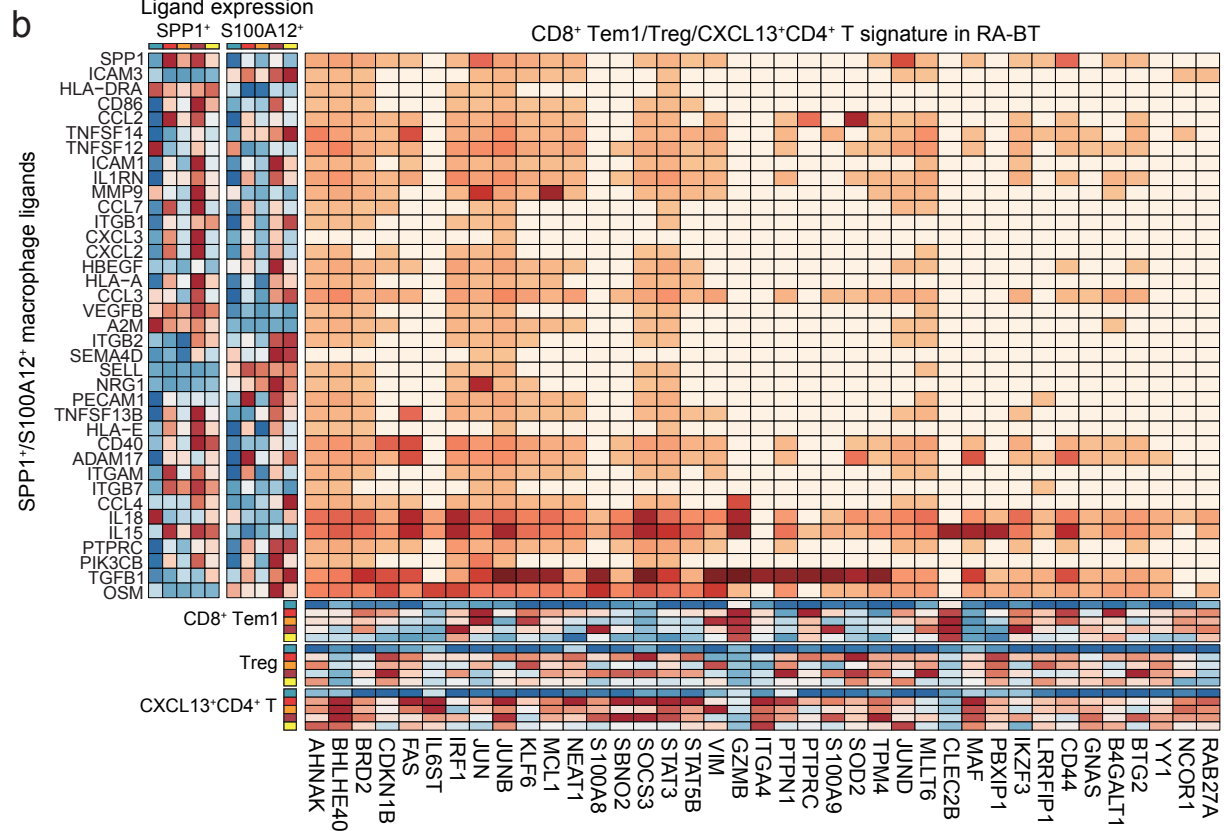
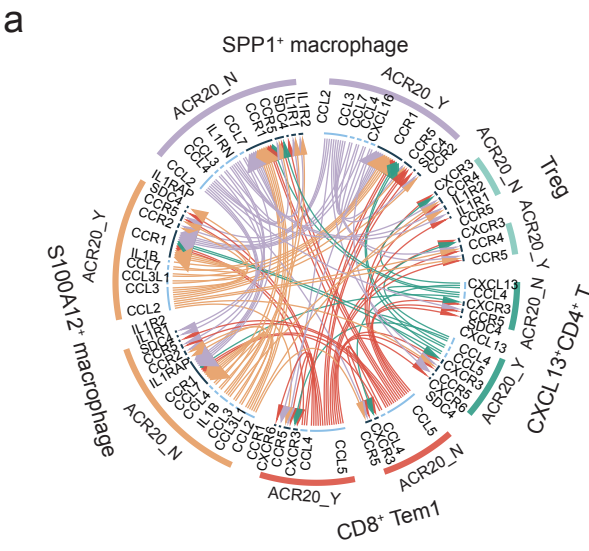


Fig. S12 Communications between macrophage and T cell focusing subtypes. a

Comparison of iTALK-based cytokines-chemokines communications across pathogenic macrophage and T cell subtypes between ACR20_N and ACR20_Y. **b** NicheNet-based potential ligands screening. The left heatmap displays the level of expression of the possible ligands in *SPP1*⁺/*S100A12*⁺ macrophages treated with adalimumab or tofacitinib. The lower heatmap presents the expression scale of the potential target genes in three pathogenic T subtypes of patients treated with adalimumab or tofacitinib. The center graph indicates the strength of ligand-mediated regulation over target genes. **c** NicheNet-based potential ligands screening. The left heatmap displays the expression of possible ligands in *CXCL13*⁺*CD4*⁺ T, Treg, and *CD8*⁺ Tem1 cells treated with adalimumab or tofacitinib. The lower heatmap presents the expression scale of the potential target genes in *SPP1*⁺/*S100A12*⁺ macrophage subclusters of patients treated with adalimumab or tofacitinib. The center graph indicates the strength of ligand-mediated regulation over target genes. **d** Box plots for mRNA expression of *IL-6*, *MMP-1*, *MMP-2* and *MMP-13* in FLS, FLS+*SPP1*⁺ macrophage, FLS+*CD4*⁺ T cells, FLS+*SPP1*⁺ macrophage+*CD4*⁺ T cells through direct coculture system and Trans-well-based coculture system (n = 3); Data were presented as mean ± SEM. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparisons test for multi-group comparisons. ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. FLS, fibroblast-like synoviocytes.

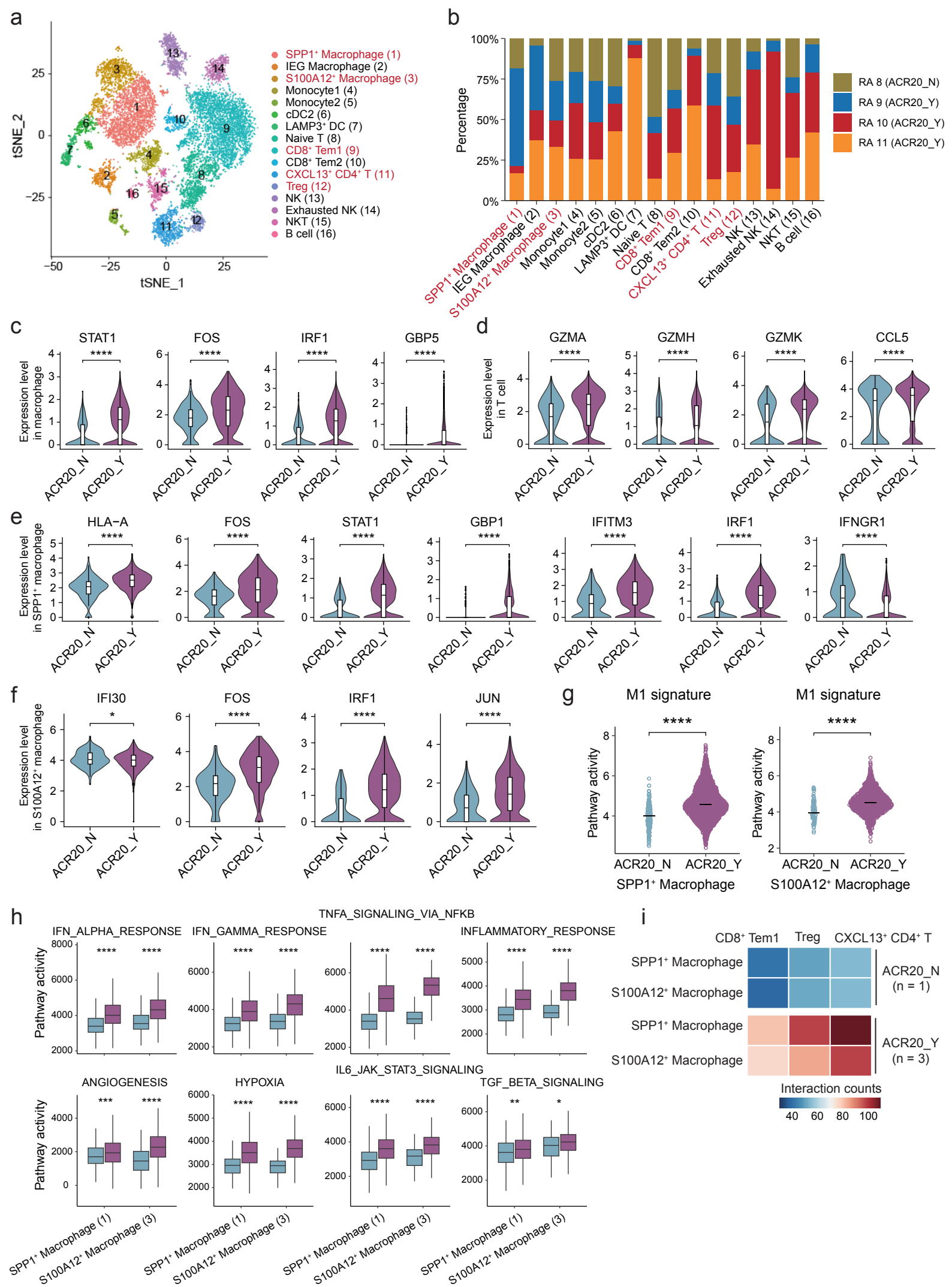
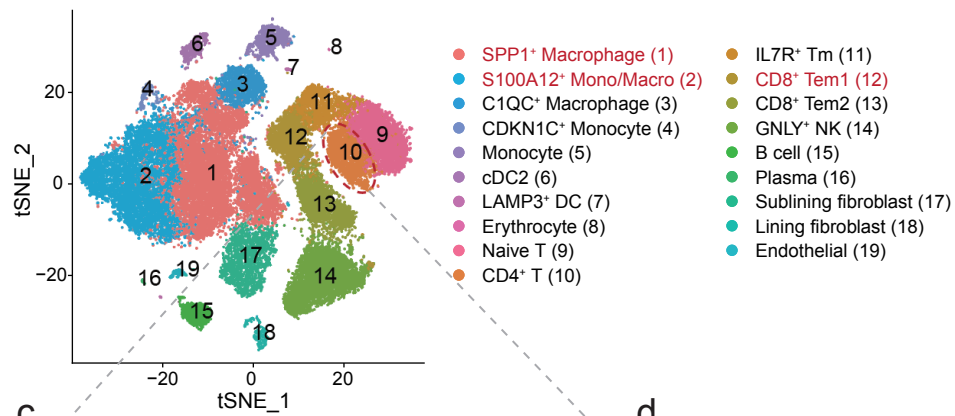
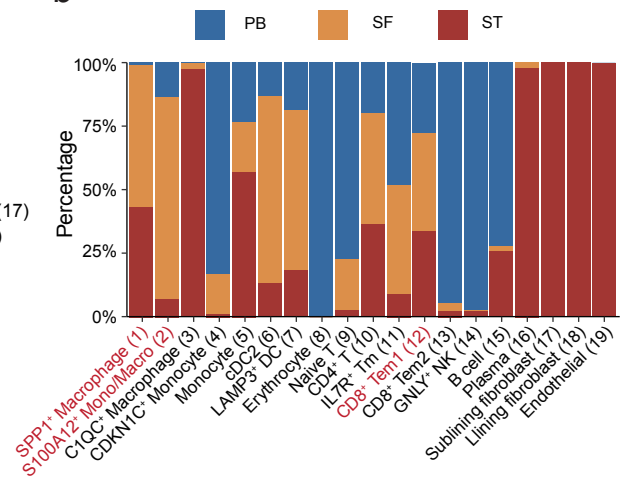


Fig. S13 Single-cell analysis of SF from the validation cohort. **a** tSNE-based visualization of sixteen clusters for scRNA-seq analysis in validation cohort with 4 RA-BT SF samples. Pathogenetic cell clusters identified in the discovery cohort were highlighted in red. **b** Histogram depicting the distribution of each patient among different subclusters. **c-f** Validation of DEGs between ACR20_N and ACR20_Y in macrophage, T cell, *SPP1*⁺ and *S100A12*⁺ macrophages in the validation cohort. **g-h** Validation of different M1 signature scores and MSigDB-based hallmark pathway activity in *SPP1*⁺/*S100A12*⁺ macrophages in the validation cohort. *p*-values were calculated by the two-sided Wilcoxon test, *****p* < 0.0001. **i** Validation of CellphoneDB-based ligand-receptor communication difference between ACR20_N and ACR20_Y among pathogenetic cell types in the validation cohort.

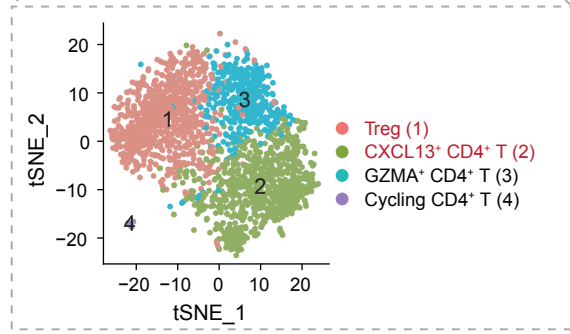
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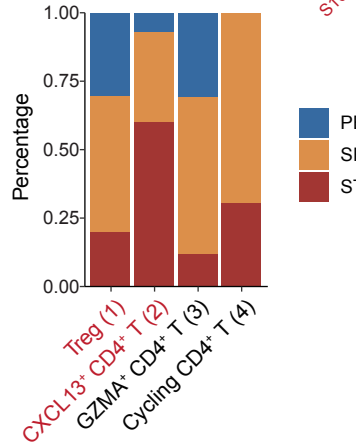
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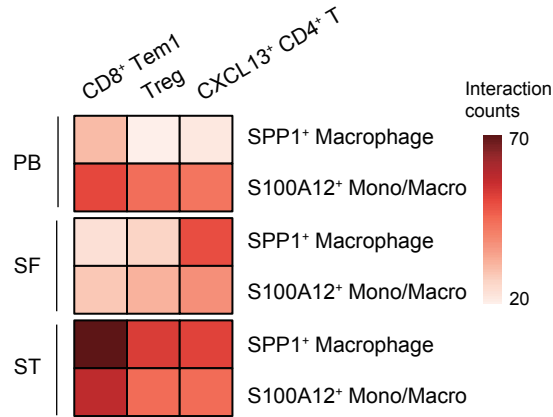
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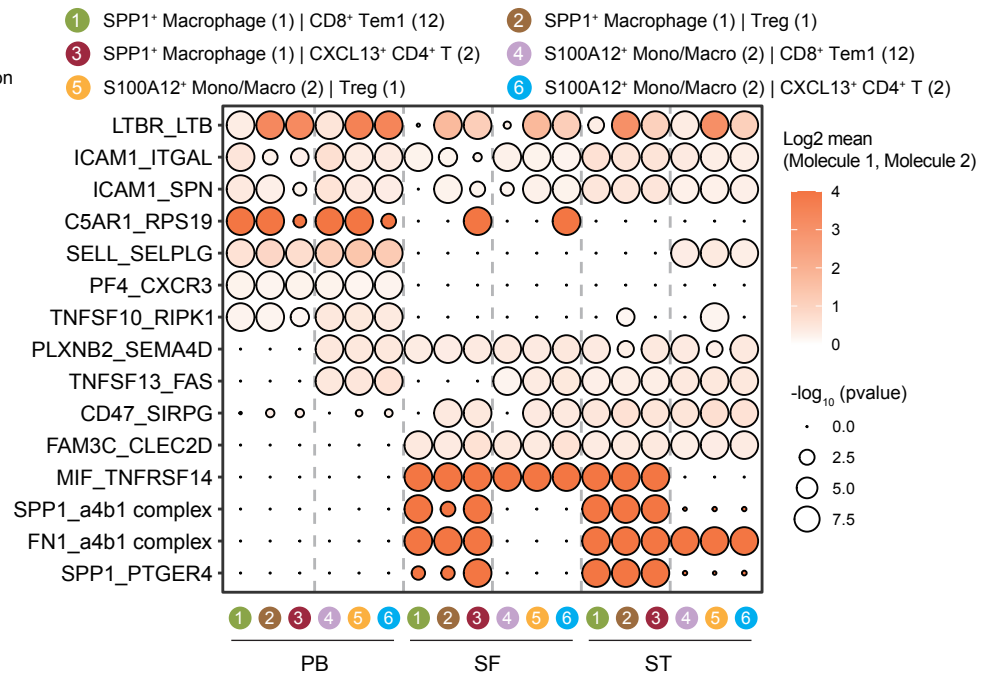


Fig. S14 Single-cell analysis of matched SF, ST, and peripheral blood from RA patients. **a** tSNE-based visualization of nineteen clusters for scRNA-seq analysis in validation cohort with matched SF, ST and blood samples, pathogenetic cell clusters identified in the discovery cohort are highlighted in red. **b** Distribution of sample types in each cell cluster. **c** Four subclusters of $CD4^+$ T (cluster 10) on a tSNE visualization. Pathogenetic cell clusters identified in the discovery cohort were highlighted in red. **d** Distribution of sample types in each $CD4^+$ T cell subcluster. **e** Heatmap illustrating the number of interactions between key subclusters of macrophages and T cells in blood, SF, and ST. **f** Predicted and detailed communications among pathogenetic macrophage and T cell clusters in blood, SF, and ST. Dot size and color spectrum indicate the p -value ($-\log_{10}$ transformed) and mean expression ($\log_2$ transformed) of ligand and receptor, respectively. SF, synovial fluid; ST, synovial tissue; PB, peripheral blood.

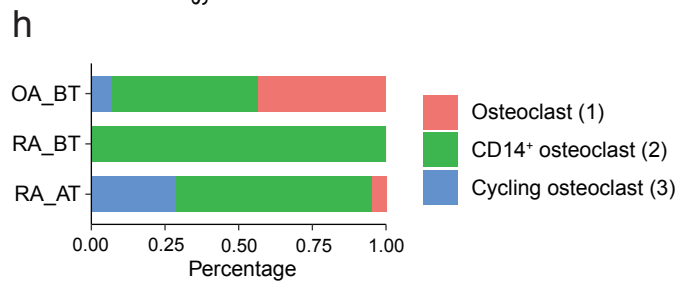
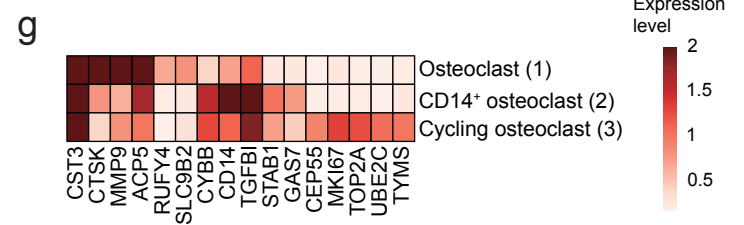
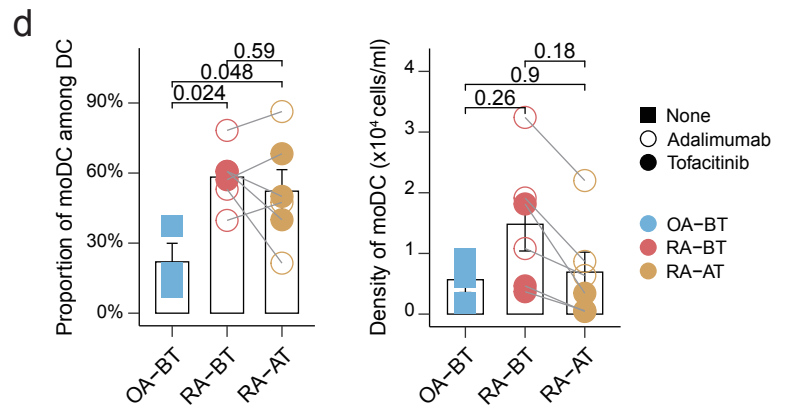
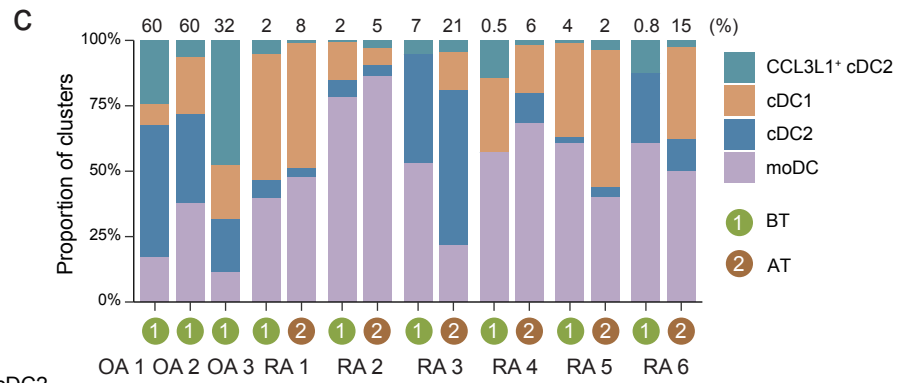
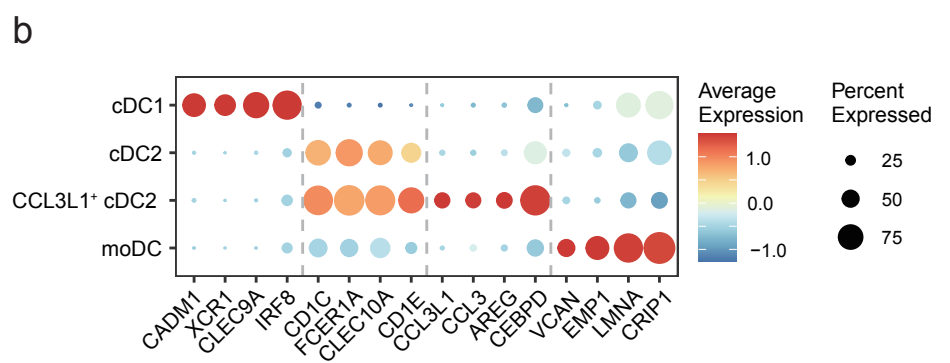
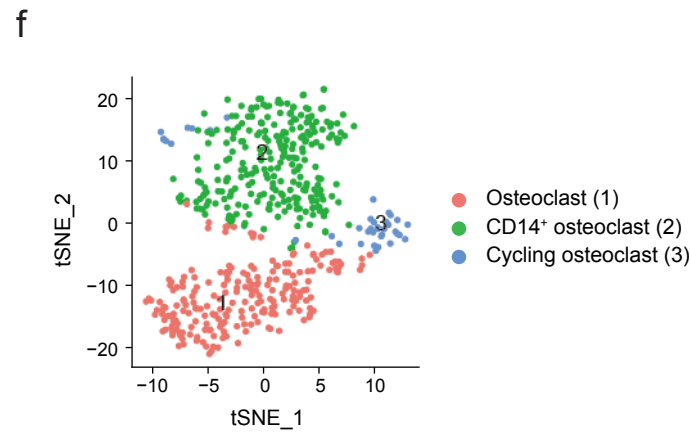
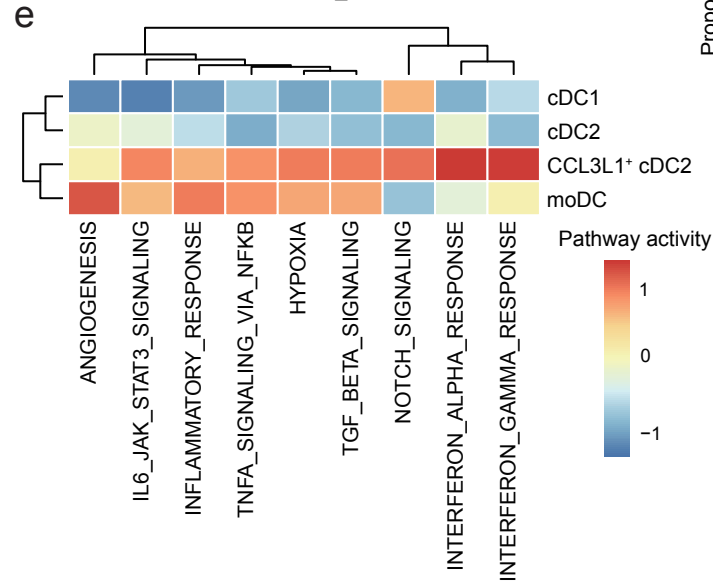
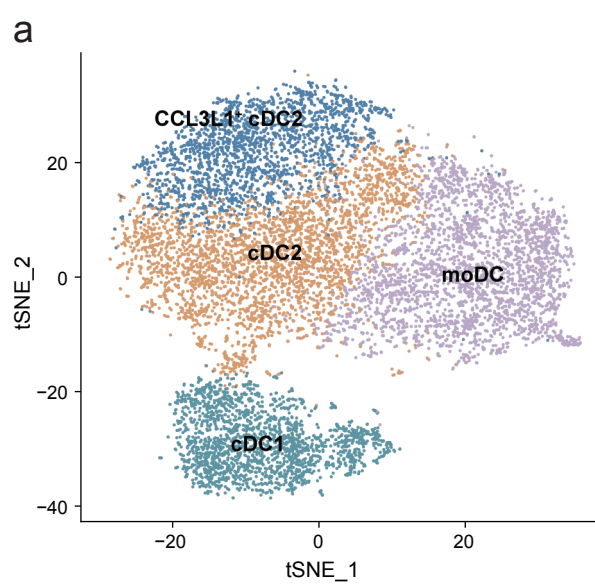


Fig. S15 Subcluster analysis for DC and osteoclast. **a** tSNE visualization of four subclusters of DCs. **b** Dot plot of the marker gene expression for each DC subcluster. The dots' size and color spectrum indicate positive percentage and average expression (log1p transformed) of particular markers genes in each cell type, respectively. **c** Proportions of each subcluster in relation to the total number of cells across different samples. **d** Proportion and density of moDC among different groups. *p*-values were calculated by the two-sided Wilcoxon test. solid squares, patients didn't receive b/tsDMARDs treatment; hollow circles, adalimumab treated; solid circles, tofacitinib treated. **e** Heatmap displaying the ssGSEA scores of the MSigDB-based hallmark pathway activity across the DC subclusters. **f** tSNE visualization of three osteoclast subclusters. **g** Heatmap illustrating the expression levels of specific markers for each subcluster. **h** Proportions of each subcluster among different groups.

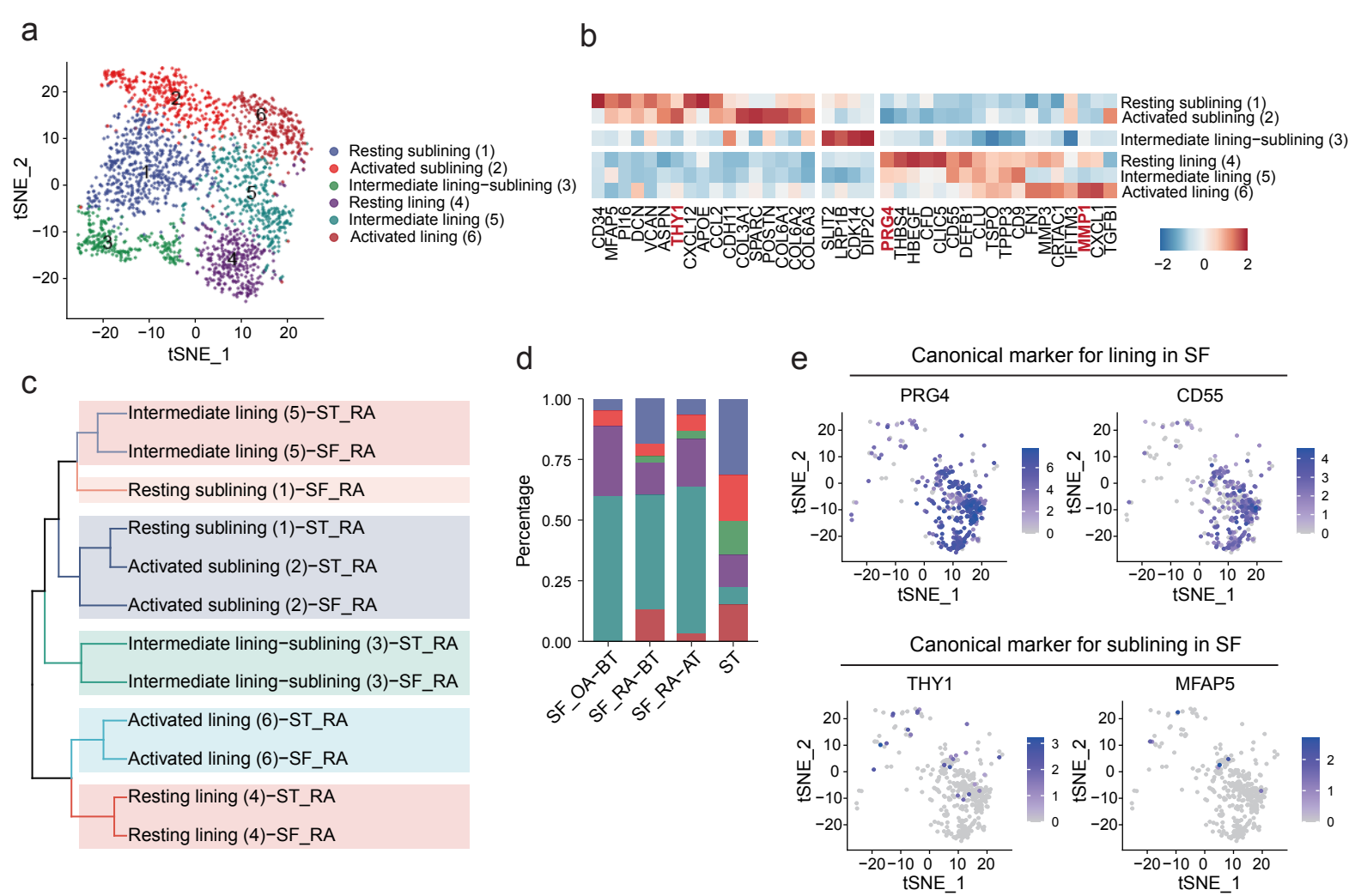


Fig. S16 Subpopulation analysis of fibroblasts in SF and ST. **a** tSNE visualization of Six subclusters of fibroblasts. **b** Heatmap illustrating the expression levels of markers genes of each subcluster. Tag genes were highlighted in red. **c** Hierarchical clustering analysis on fibroblast subclusters of SF and ST samples from RA-BT. **d** Distribution of each subcluster among different samples. **e** Feature plots displaying the expression of conventional markers for lining and sublining in SF.