

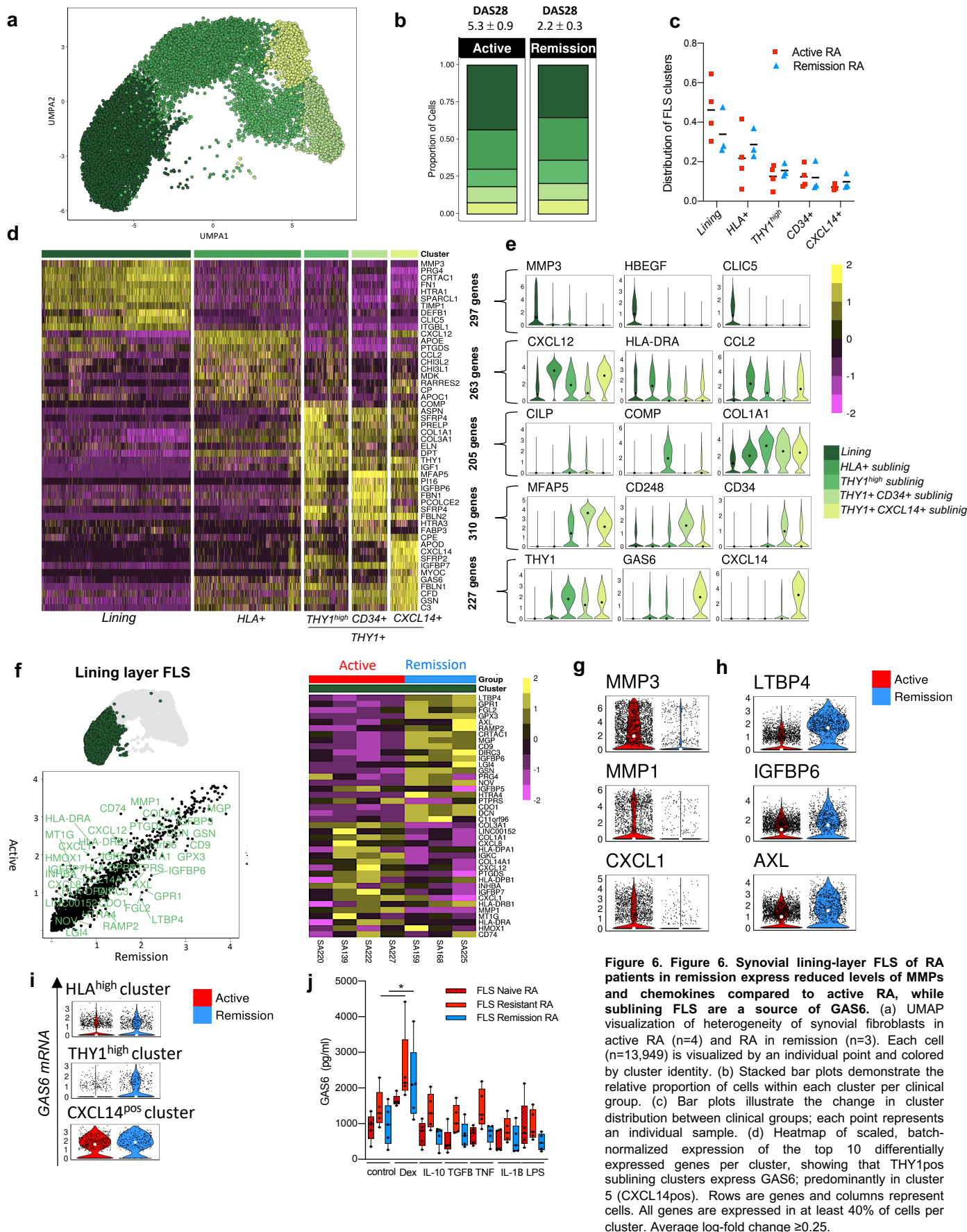


Figure 6. Synovial lining-layer FLS of RA patients in remission express reduced levels of MMPs and chemokines compared to active RA, while sublining FLS are a source of GAS6. (a) UMAP visualization of heterogeneity of synovial fibroblasts in active RA (n=4) and RA in remission (n=3). Each cell (n=13,949) is visualized by an individual point and colored by cluster identity. (b) Stacked bar plots demonstrate the relative proportion of cells within each cluster per clinical group. (c) Bar plots illustrate the change in cluster distribution between clinical groups; each point represents an individual sample. (d) Heatmap of scaled, batch-normalized expression of the top 10 differentially expressed genes per cluster, showing that THY1pos sublining clusters express GAS6; predominantly in cluster 5 (CXCL14pos). Rows are genes and columns represent cells. All genes are expressed in at least 40% of cells per cluster. Average log-fold change ≥0.25. (e) Violin plots of log-normalized expression of genes of interest, median value represented by a black dot. (f) Differentially expressed genes in the lining-layer FLS of remission RA compared to active RA. Scatterplot illustrates pseudobulk expression of each differentially expressed gene, and the top 20 genes for each condition are annotated. Heatmap shows the scaled, sample-specific pseudo-bulk expression of the top 20 differentially expressed genes, rows are genes and columns represent pseudobulk expression. All genes are expressed in at least 60% of cells per group, average log-fold change ≥0.25, p<0.05 corrected for multiple comparison. (g-h) Violin plots showing log-normalized expression of genes for MMPs, chemokines and anti-inflammatory mediators in lining-layer FLS, median value represented by white dot. (i) mRNA expression of GAS6 in distinct sublining FLS clusters comparing active and remission RA. The THY1high cluster shows greater expression of GAS6 in remission RA compared to active RA, median values represented by white dots. (j) GAS6 is produced by cultured primary FLS from biopsies of RA patients (treatment naïve, treatment resistant, and RA in remission). Each dot represents a separate FLS line (n=5 per condition) *p<0.05, two-way Anova corrected for multiple comparisons.