

1 **Title:**

2 **Stem-like and effector peripheral helper T cells comprise distinct subsets in**
3 **rheumatoid arthritis**

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41 **Abstract**

42 Peripheral helper T (Tph) cells can play pathogenic roles in human autoimmune diseases. Tph cells
43 are proposed to be the major B cell helpers in inflamed joints of rheumatoid arthritis (RA) but whether
44 and how Tph cells are engaged in tissue inflammation remains unclear. We demonstrate that Tph cells
45 comprise two subsets in RA: stem-like Tph (S-Tph) and effector Tph (E-Tph) cells. These two subsets
46 differed in transcriptome, epigenome, B cell helper capacity, spatial localization, and cell interactions.
47 S-Tph cells displayed self-renewal capacity and were mainly found within tertiary lymphoid structures
48 (TLSs) together with B cells. S-Tph cells potently induced B cells to produce immunoglobulins. By
49 contrast, E-Tph cells expressed effector molecules and colocalized with proinflammatory macrophages
50 and CD8⁺ T cells outside TLSs. S-Tph cells could differentiate into E-Tph cells upon TCR stimulation
51 and coculture with B cells. Collectively, our study shows that S-Tph cells play a central role in
52 promoting Tph responses by undergoing self-renewal and seeding E-Tph cells.

53

54 **One Sentence Summary**

55 Stem-like peripheral helper T cells seed effector peripheral helper T cells in rheumatoid arthritis.

56 **Introduction**

57 Rheumatoid arthritis (RA) is an autoimmune disease associated with chronic arthritis symptoms, and
58 its pathogenesis includes a broad range of immune cells. Pro-inflammatory cytokines and molecules
59 expressed by synovial macrophages and fibroblast-like synoviocytes (FLSs) cause cartilage
60 degeneration and osteoclast induction, leading to joint and bone destruction (1-3). A majority of RA
61 patients display various autoantibodies, and recent studies have established peripheral helper T (Tph)
62 cells as a major B cell helper T cell subset promoting the production of autoantibodies in inflammatory
63 synovial tissue (4). Tph cells produce high amounts of CXCL13, which recruit CXCR5⁺ B cells and
64 promote their cognate interactions (4-6). Tph cells can be detected in peripheral blood, and their
65 frequency and activated phenotype in the blood correlate with autoantibody titers (7), disease activity
66 (8-10), and tissue damage (11, 12) in various autoimmune diseases. Although Tph cells were initially
67 proposed as B cell helpers in peripheral inflammatory tissues, recent evidence suggests that Tph cells
68 are composed of functionally heterogeneous populations including effector cells expressing
69 inflammatory cytokines (7, 12-15). High PD-1 expression on CXCR5⁻ CD4⁺ T cells is the broadly
70 accepted phenotypic definition of Tph cells, however, this phenotype can be observed in multiple CD4⁺
71 T cell subsets upon TCR stimulation in various conditions or settings (7). Although Tph cells are
72 considered to be involved in the pathogenesis of various human autoimmune diseases, the exact
73 features and functions of pathogenic Tph cells likely differ among diseases (7).

74 Inflamed tissues in autoimmune diseases, such as RA synovium, often generate clusters of T and B
75 cells and macrophages, called tertiary lymphoid structures (TLSs) (16). TLSs share cellular and
76 molecular features with secondary lymphoid organs (17), and several studies showed a positive
77 correlation between the presence of TLSs and the disease severity in multiple human autoimmune
78 diseases (18, 19). However, how TLSs develop in human autoimmune diseases and contribute to
79 pathogenesis remains incompletely understood. Furthermore, the relationship between B helper T cells
80 and effector T cells in inflamed RA synovium is to be elucidated.

81 Here, we performed multiomics analyses of synovial CD4⁺ T cells and showed two distinct functional
82 and spatial stages of Tph cells in RA: a self-renewing and proliferative TCF1⁺ CCR7⁺ stem-like stage
83 and a CXCR6⁺ effector stage. Stem-like Tph cells, hereafter S-Tph cells, colocalize with B cells in
84 TLSs and are capable of B cell help. By contrast, effector Tph cells, hereafter E-Tph cells, migrate out
85 of TLSs and colocalize with macrophages at inflammatory regions. These two subsets are directly
86 related, and S-Tph cells give rise to E-Tph cells.

87

88 **Results**

89 **Tph cells are composed of two subsets in RA**

90 To comprehensively analyze CD4⁺ T cells in the inflamed joints of RA, we performed single-cell
91 transcriptome analysis, along with surface molecule expression profiling (CITE-seq) and T cell
92 receptor (TCR) sequencing. We investigated CD4⁺ T cells in synovial tissue (ST, n = 10), synovial
93 fluid (SF, n = 4), and peripheral blood (PB, n = 9) obtained from RA patients exhibiting moderate to
94 high disease activity (**Fig. 1A and table S1**). Unsupervised clustering of 105,348 CD4⁺ T cells (PB:
95 44,849 cells; SF: 13,428 cells; and ST: 47,071 cells) by gene expression generated 13 transcript clusters
96 (**Fig. 1B**). Clusters 0 and 1 were characterized by high expression of *SELL*, *TCF7*, and *CCR7*. Cluster
97 0 expressed CD45RO protein and thus was central memory T cells, whereas cluster 1 expressed
98 CD45RA protein and thus was naive T cells (**Fig. 1C and fig. S1A**). Clusters 3, 8, and 9 were cytotoxic
99 CD4⁺ T cells expressing *GZMA*, *GZMB*, *GZMK*, *PRF1*, and *CCL5* at different levels (**Fig. 1C and fig.**
100 **S1B**). *TBX21*, the master regulator transcription factor of Th1 cells, was highly expressed in cluster 8
101 (**fig. S1B**). Clusters 5 and 6 were regulatory T cells expressing *IKZF2*, *FOXP3*, and *IL2RA*. Activation
102 markers, including *HLA-DRB1*, distinguished the two clusters (**Fig. 1C and fig. S1C**). Cluster 7
103 displayed Th17-related genes, including *RORC* and *CCR6* (**Fig. 1C and fig. S1D**). Cluster 10, 11, and
104 12 showed upregulation of heat shock protein genes, proliferation-related genes, and interferon
105 signature genes, respectively (**Fig. 1C and fig. S1E and fig. S2, A and B**).

106 We identified two Tph clusters (clusters 2 and 4) based on the high expression of *PDCD1*, *CXCL13*,
107 and *IL21* (**Fig. 1D and fig. S1F**), consistent with previous literature (13-15, 20). These clusters
108 exhibited the highest PD-1 expression among all the clusters both at the RNA and the surface protein
109 levels (**Fig. 1D and fig. S1F**). Tph cells in PB showed elevated expression of *PDCD1*, *CXCL13*, and
110 *IL21* compared to other clusters in PB (**fig. S2, C and D**). However, Tph cells in PB expressed these
111 genes at lower levels than those in ST and SF (**fig. S1F**), which likely reflects that blood Tph cells are
112 more at a resting state. Cluster 2 expressed more abundant *CCR7*, *IL7R*, *TCF7*, *LEF1*, and ribosomal
113 genes, suggesting their stem cell-like features (**Fig. 1E and fig. S1F**). By contrast, cluster 4 expressed
114 more effector genes, including *CXCR6*, *CCR2*, *LAG3*, *HLA-DRB1*, *PRDM1*, *GZMA*, and *IFNG* (**Fig.**
115 **1E and fig. S1, B and F**). Consistently, gene ontology analysis (21) highlighted active translation
116 processes enabling cell division in cluster 2, whereas cluster 4 was characterized by cell activation,
117 effector function, and cytokine production (**Fig. 1F**). Furthermore, gene set enrichment analysis
118 (GSEA) also demonstrated enrichment of stem-like genes and effector genes in cluster 2 and 4,
119 respectively (22) (**Fig. 1G**). Based on these findings, hereafter we annotate cluster 2 as stem-like Tph
120 (S-Tph) cells and cluster 4 as effector Tph (E-Tph) cells. Importantly, subclustering of S-Tph cluster
121 in ST identified a small subset exhibiting high expression of *CXCR5* and *BCL6* (**fig. S2, E and F**),
122 consistent with previous reports showing the presence of *CXCR5*⁺ cells within Tph clusters (15). These
123 data suggest that, although S-Tph cluster contained a minor population of *PDCD1*^{high} *CXCR5*⁺ T
124 follicular helper (Tfh) cells, *PDCD1*^{high} *CXCR5*⁻ S-Tph cells were the vast majority. Additionally, we
125 investigated whether S-Tph and E-Tph cells can be observed in non-RA inflamed joints. Two Tph
126 clusters were identified among synovial CD4⁺ T cells in patients with juvenile idiopathic arthritis (JIA)
127 (23) and with osteoarthritis (OA) (14) (**fig. S3, A to D**). Each of the two clusters demonstrated
128 enrichment of genes upregulated in S-Tph or E-Tph cells in RA (**fig. S3, E and F**). These data revealed
129 that non-RA inflamed joints contained cells that were at least transcriptionally like Tph subsets in RA.

130

131 S-Tph and E-Tph cells exhibit different epigenetic landscapes

132 To investigate whether the two Tph subsets also differ in epigenetic states, we performed single-cell
133 ATAC-seq (assay for transposase accessible chromatin sequencing) of 14,591 synovial tissue CD4⁺ T
134 cells combined with the analysis of cell surface proteins (scASAP-seq, n = 1) (24). We performed
135 unsupervised clustering solely based on open chromatin peaks and defined 7 clusters (**Fig. 1H**). We
136 used gene activity estimated by ATAC data, CITE-seq data, and reference ATAC peaks derived from
137 public scATAC/RNA-seq data for the annotation (**fig. S4, A to C**). Two clusters were identified within
138 the Tph cell population indicated by high expression of PD-1 without CXCR5 expression at the protein
139 levels (**Fig. 1, H and I**), which also showed high estimated gene activity of *CXCL13* and *IL21* (**fig.**
140 **S4A**). Within the Tph cell cluster, one subcluster expressed CCR7 and CD127 on cell surface, whereas
141 the other expressed CXCR6 and LAG3, thus representing S-Tph and E-Tph cells, respectively (**Fig. 1,**
142 **I and J, and table S2**). Motif analysis of open chromatin loci identified that motifs for TCF7, LEF1,
143 MAF, and AP-1 family genes were enriched in S-Tph cells, whereas motifs for IRF family genes,
144 including IRF4, were enriched in E-Tph cells (**fig. S4D**). Thus, S-Tph and E-Tph cells are
145 epigenetically distinct, and different families of transcription factors appear to be in play. Enriched
146 motifs for TCF7 and LEF1 in S-Tph cells suggest their stem-like feature, while enriched motifs for
147 IRF family in E-Tph cells might reflect ongoing signals via T cell receptors (TCRs) (25, 26).
148 Our scASAP-seq dataset also identified Tfh cells as cells with a CXCR5⁺ PD-1^{high} phenotype (**Fig. 1,**
149 **H to J and table S2**) (14, 15). Tfh cells comprised 2.3% of total CD4⁺ T-cell population in ST (**Fig.**
150 **1H**), a frequency consistent with previous results with flow cytometry (4). Tfh cells showed enriched
151 motifs for POU family genes, including POU2F1 and POU2F2 (**fig. S4D**), which bind to promoters of
152 *BCL6* gene (27), the master regulator of Tfh cell differentiation (28). Thus, Tfh cells are epigenetically
153 and phenotypically distinct from Tph cells in RA synovium.

154

155 CCR7 and CXCR6 identify S-Tph and E-Tph cells

Chemokine receptors play a crucial role in directing T cells to distinct sites (29, 30), and are often useful to define distinct human CD4⁺ T cell subsets (7). A comprehensive assessment of chemokine receptors demonstrated that *CCR7* in S-Tph cells and *CXCR6* in E-Tph cells most effectively distinguished between the two subsets (**Fig. 2A, fig. S5, A and B**). *CCR2* and *CCR5*, broadly recognized as chemokine receptors of Tph cells (4), were predominantly expressed by E-Tph cells (**Fig. 2A and fig. S5C**). Flow cytometry analysis confirmed mutually exclusive expression of *CCR7* and *CXCR6* on *CXCR5*⁻ PD-1^{high} Tph cells, and S-Tph and E-Tph cells were successfully distinguished as *CCR7*⁺ *CXCR6*⁻ and *CCR7*⁻ *CXCR6*⁺, respectively (**Fig. 2B and fig. S6A**). *CCR2* and *CCR5* expression was higher on E-Tph cells than S-Tph cells (**Fig. 2B**). *CCR7*⁻ *CXCR6*⁻ Tph cells moderately expressed *CCR2* and *CCR5*, suggesting their intermediate status (**fig. S5D**).

166

167 **S-Tph cells are endowed with self-renewal capacity**

Consistent with scRNA-seq data, S-Tph cells showed higher expression of *TCF1*, a protein encoded by *TCF7*, in flow cytometry (**Fig. 2C**). We confirmed that *TCF7* transcripts of scRNA-seq were more highly expressed in *CCR7*⁺ *CXCR6*⁻ Tph cells than in *CCR7*⁻ *CXCR6*⁺ Tph cells defined by protein expression of CITE-seq (**fig. S7, A and B**). We next examined whether S-Tph cells display a higher proliferative capacity than E-Tph cells. We FACS-sorted S-Tph and E-Tph cells from SF of RA patients as *CCR7*⁺ *CXCR6*⁻ and *CCR7*⁻ *CXCR6*⁺ cells (**fig. S6B**). Upon *in vitro* stimulation for 3 days with anti-CD3/CD28 coated beads, S-Tph cells displayed more proliferation and better viability than E-Tph cells (**Fig. 2, D and E**). The proliferation of E-Tph cells was only partially enhanced even with the addition of IL-2 in the culture (**fig. S7C**). S-Tph cells maintained *CCR7* expression during cell division and further enhanced *TCF1* expression (**Fig. 2, F and G**). *TCF1* has been shown to mark T cells with high proliferative capacity (31, 32). Indeed, the deletion of *TCF1* in S-Tph cells derived from SF using the CRISPR-Cas9 system significantly diminished the proliferative capacity (**Fig. 2, H and I, fig. S7D, and Data file S1**). Furthermore, a greater fraction of S-Tph cells expressed a cell-cycle marker Ki-67

181 than E-Tph cells (**Fig. 2J**), suggesting the proliferation of S-Tph cells *in situ*. Collectively, these
182 findings indicate that S-Tph cells are endowed with a self-renewal capacity at least partly mediated by
183 TCF1.

184

185 **E-Tph cells are clonally expanded**

186 Next, we compared the composition of subsets between inflammatory sites (ST and SF) and PB. Both
187 E-Tph and S-Tph cells were more abundant in ST and SF than in PB (**Fig. 3, A and B, and fig. S8, A**
188 **and B**). These Tph cells as well as Treg cells in ST highly expressed CD69 compared to those in PB
189 (**fig. S8C**), suggesting that Tph and Treg cells expressing CD69 might be retained in the inflamed
190 joints of RA. When ST and SF were compared, E-Tph cells, but not S-Tph cells, were substantially
191 enriched in SF (**Fig. 3B**), suggesting that E-Tph cells display a higher propensity to migrate to SF. The
192 frequency of E-Tph cells, but not S-Tph cells, in ST positively correlated with disease activity (**Fig.**
193 **3C and fig. S8D**). No clear impact of therapeutics was observed on the frequency of these subsets,
194 potentially due to insufficient number of samples (**fig. S8E**).

195 To gain insights into the dynamics of T cell differentiation in inflamed ST, we integrated single-cell
196 transcriptome and paired-TCR $\alpha\beta$ sequencing data (scRNA/TCR-seq, n = 80,574 cells) and analyzed
197 the clonal expansion of each subset. ST and SF contained more expanded clones than PB (**Fig. 3D and**
198 **fig. S9A**). Among CD4⁺ T cell subsets, clonal expansion of cytotoxic CD4⁺ T cells (expressing *GZMB*
199 or *GZMK*) was commonly observed in ST, SF, and PB (**fig. S9B**). However, clonal expansion of Tph
200 cells (both S-Tph and E-Tph) and activated regulatory T cells was more dominant in ST and SF than
201 in PB (**Fig. 3E and fig. S9C**). E-Tph cells contained more clonally expanded cells than S-Tph cells,
202 indicating more robust oligoclonal expansion (**Fig. 3F**).

203 Nonetheless, due to the highly diverse nature of the TCR gene, the accuracy of the analysis of expanded
204 clonotypes is an issue when the number of sequenced cells per sample is limited. To overcome this
205 issue, we restricted our analysis to CD4⁺ T cells expressing one of the 3 V β molecules out of 24 (V β 3,

206 V β 5.3, or V β 7.1), using FACS-sorted cells (**fig. S10, A and B**. n = 17,751 cells). This “TRBV usage
207 restriction” strategy allowed a more robust analysis of clonal expansion, as evidenced by a substantial
208 increase of non-singletons (i.e. cells that shared clonotypes with other cells) from 9.4% to 42.4% per
209 sample (**fig. S10C**). Of note, the frequency of cells expressing the selected 3 V β molecules (encoded
210 by *TRBV28*, *TRBV4*, and *TRBV5-5*, respectively) was comparable among all clusters at pre-sorting,
211 showing that this strategy did not cause a substantial bias in the clonotype analysis (**fig. S10, D and**
212 **E**). This strategy also demonstrated a substantially higher proportion of expanded cells in ST and SF
213 than in PB (**Fig. 3G and fig. S10F**). E-Tph cells displayed more robust clonal expansion than S-Tph
214 cells in ST and SF (**Fig. 3H and fig. S10G**). Thus, these results show that inflamed ST in RA is
215 associated with an increase of clonally expanded E-Tph cells *in situ*.

216

217 **S-Tph and E-Tph cells highly share clonotypes**

218 We wondered whether S-Tph and E-Tph cells are directly associated or emerge independently in
219 inflamed ST. We analyzed the TCR clonotype overlap between the two subsets, and a high degree of
220 clonotype sharing between S-Tph and E-Tph cells was observed both in ST and SF (**Fig. 4A and fig.**
221 **S11A**). By contrast, clonal overlaps between Tph cells and non-Tph subsets were limited, except for
222 cycling cells. These results suggest that the differentiation trajectory of S-Tph and E-Tph cells is shared
223 but largely distinct from that of non-Tph subsets. The cycling cells shared clonotypes more strongly
224 with E-Tph cells than S-Tph cells in both ST and SF (**fig. S11A**), suggesting that a fraction of S-Tph
225 cells differentiate into E-Tph cells via cell proliferation. Consistently, RNA velocity analysis (33, 34)
226 showed the trajectory of S-Tph cells into E-Tph cells (**Fig. 4B**). The “TRBV usage restriction” strategy
227 further confirmed profound clonotype overlap between S-Tph and E-Tph cells in ST and SF (**fig. S11,**
228 **B and C**), and 35.2% of S-Tph and 53.8% of E-Tph cells shared clonotypes in ST. The cycling cells
229 and E-Tph cells also strongly shared the clonotypes (**fig. S11C**). Shared clonotypes between S-Tph
230 and E-Tph cells were diverse, and some clonotypes underwent a massive expansion in E-Tph cells (**fig.**

231 **S11D).**

232 We also sought to investigate whether Tfh and Tph cells in ST shared clonotypes. Tfh cells identified
233 within the subclustered S-Tph subset showed only a minor clonal overlap with S-Tph or E-Tph cells
234 (**fig. S11E**).

235

236 **A fraction of S-Tph cells differentiate into E-Tph cells**

237 To test whether S-Tph cells can differentiate into E-Tph cells, we sorted S-Tph and E-Tph cells from
238 SF and cultured these T cells for 9 days in the presence of anti-CD3/CD28 coated beads (**Fig. 4C**).
239 Consistent with **Fig. 2D**, prolonged 9-day stimulation also resulted in higher proliferation of S-Tph
240 cells than E-Tph cells (**Fig. 4D**). However, unlike 3-day stimulation, 9-day stimulation caused S-Tph
241 cells to downregulate CCR7 and upregulate CXCR6. By contrast, E-Tph cells did not upregulate CCR7
242 (**Fig. 4E and fig. S6C**). The “converted” CCR7⁻ CXCR6⁺ cells from S-Tph cells significantly
243 upregulated the expression of E-Tph markers CCR2, CCR5, and LAG3 at the expense of TCF1 (**Fig.**
244 **4F and fig. S12A**). Using bulk RNA sequencing analysis, we confirmed that the “converted” CCR7⁻
245 CXCR6⁺ cells upregulated expression of *CCR2*, *CCR5*, *CCL4*, *CCL5*, *GZMA*, *GNLY*, *IFNG*, and *LAG3*
246 (**fig. S12B**), a gene profile of bona fide E-Tph cells (**Fig. 1E**). Furthermore, we confirmed that the gene
247 set retrieved from bona fide E-Tph cells was enriched in “converted” CCR7⁻ CXCR6⁺ cells (**fig. S12C**).
248 CCR7⁻ CXCR6⁻ cells showed intermediate expression of CCR2, CCR5, LAG3, and TCF1 (**Fig. 4F**
249 **and fig. S12A**), suggesting their intermediate state is similar to bona fide DN cells. To examine the
250 role of TCF1 in the conversion of S-Tph cells, we depleted TCF1 from S-Tph cells by CRISPR-Cas9
251 and stimulated them with anti-CD3/CD28 for 9 days. Whereas TCF1 depletion reduced cell
252 proliferation (**fig. S12D**), this modification promoted the conversion to CCR7⁻ CXCR6⁺ E-Tph cells
253 by 1.5-fold (**Fig. 4G**). Thus, S-Tph cells can differentiate into E-Tph cells upon TCR stimulation, and
254 the differentiation is facilitated by the downregulation of TCF1.

255

256 Spatial transcriptomics identifies Tph subsets in ST

257 To determine the localization of S-Tph and E-Tph cells in inflamed RA synovium together with their
258 interacting cells *in situ*, we performed spatial transcriptomics at a single-cell resolution using 3
259 synovial tissue sections from different patients (**table S1 and S3**). Cell segmentation identified
260 212,639, 165,842, and 166,051 cells in each section, and gene expression was assigned to individual
261 cells. Unsupervised clustering yielded 18 cell clusters, including FLSs, endothelial cells, mural cells,
262 macrophages, dendritic cells, T cells, and B cells (**Fig. 5A and fig. S13, A and B**). FLSs were divided
263 into two subpopulations based on their distinct localization and gene expression (**Fig. 5A and fig. S13,**
264 **B and C**): *PDPN* and *PRG4* positive FLSs in the lining layer of the synovium (lining FLSs: cluster
265 M), and *THY1* and *CXCL12* positive FLSs in the sublining connective tissue (sublining FLSs: clusters
266 N, O and P). Among the immune cells, macrophages expressing *CD14* and *CD68* were the most
267 dominant and contained *MERTK*^{high} anti-inflammatory macrophages (14, 20, 35) (clusters I and J),
268 *MERTK*^{low} inflammatory macrophages expressing *IL1B*, *S100A12* and *FCN1* (cluster H), and *SPPI*
269 positive macrophages (cluster K) (**Fig. 5A and fig. S13, B and C**). Lymphocytes were divided into
270 *CD8A*⁺ T cells (clusters A and B), *CD4*⁺ T cells (clusters C and D), a cluster containing mixed T cells
271 (cluster E), *MS4A1*⁺ B cells (cluster F) and *CD38*⁺ plasma cells (cluster G) (**Fig. 5A and fig. S 13B**).
272 Among *CD4*⁺ T cells, cluster C expressed *TCF7*, *CCR7*, *SELL*, and *IL7R*, in addition to *CXCL13*, and
273 thus represented S-Tph cells. By contrast, cluster D displayed *CXCR6*, *CCR5* and *LAG3*, and thus
274 represented E-Tph cells. The expression of *SOX4* and *PRDM1*, genes previously identified as being
275 expressed in Tph cells (4, 36), was detected in some Tph cells (**fig. S13D**). The positive rates of *SOX4*
276 and *PRDM1* tended to be higher in E-Tph cells than in S-Tph cells. By referring to the hematoxylin
277 and eosin stain of the same tissue section (**Fig. 5B**), we confirmed that *CD3E* and *MS4A1*, canonical
278 markers of T cells and B cells respectively, accumulated in the TLS regions (**Fig. 5C**). *PDPN*, a marker
279 gene for lining synoviocytes, was high on synovial lining layers, whereas *VWF*, a marker for
280 endothelial cells, was high around the vessels. Of note, the ratio of S-Tph/E-Tph was similar between

the scRNA-seq and spatial transcriptomics datasets in one tissue where we analyzed the entire section for spatial transcriptomics.

S-Tph cells are localized at the center of TLSs

To comprehensively detect TLSs in the tissues, we aimed to locate regions exceeding defined thresholds for both cellular density and area. We successfully identified 162, 94, and 167 TLS regions in each section (**Fig. 5D and fig. S13E**). Strikingly, we found a strong colocalization of *CCR7*, *TCF7*, and *CXCL13* in the TLS (**Fig. 5E**), indicating the accumulation of S-Tph cells. By contrast, *CXCR6* and *LAG3* expression were mainly outside TLSs and scattered throughout the tissue (**Fig. 5F and fig. S13F**). Immunofluorescence staining of RA synovium confirmed that $CD4^+ CCR7^+ TCF1^+$ cells were found within TLSs, while $CD4^+ CXCR6^+$ cells were sparsely scattered outside of TLSs (**fig. S13G**). Comprehensive analysis throughout the section revealed that 76.8% of S-Tph cells were found within TLSs, while only 38.5% of E-Tph cells were localized in TLSs (**Fig. 5, G and H, and fig. S13H**). The frequency of $CD4^+$ T cells co-expressing both *CXCR5* and *BCL6* was approximately 5% of S-Tph cells within TLSs, suggesting that Tfh cells are present only as a minor population in the TLSs. Furthermore, S-Tph and E-Tph cells showed distinct localization even within TLSs: S-Tph cells localized at the center of TLSs, and E-Tph cells were found near the margin (**Fig. 5G, and fig. S13H**). B cells were also localized at the center of TLSs (**Fig. 5G, and fig. S13H**). Both S-Tph and B cells in TLSs expressed *MKI67*, a marker for the cell cycle (**fig. S13I**). These observations suggest cognate interactions between S-Tph cells and B cells within TLSs. Taken together, S-Tph cells localized in TLSs with B cells, while E-Tph cells were observed at the margins and outside of TLSs. TLSs likely provide a niche for the self-renewal of S-Tph cells as well as sites for their differentiation into E-Tph cells.

CCL19⁺ FLSs are localized around blood vessels within TLSs

Previous studies show that CCL19 and CCL21, chemokine ligands of CCR7, are expressed in inflamed RA synovium (37, 38). We hypothesized that a subset of non-immune cells is in charge of the recruitment of CCR7⁺ S-Tph cells and/or their precursors in TLSs. To identify this subset, we performed scRNA-seq of 26,967 CD45⁻ non-immune cells sorted from ST of RA patients (n = 5) (**Fig. 6A and fig. S14, A to F**). Within FLS subsets, we identified one cluster exclusively expressing *CCL19* (cluster VI) (**Fig. 6, A to C**). CCL19⁺ FLS subset co-expressed *CCL21* and *CXCL13* (**Fig. 6, B and C**). Consistently, we found FLSs highly expressing *CCL19* (cluster P) in spatial transcriptomics analysis (**Fig. 5A, fig. S13B**). Importantly, *CCL19* transcripts accumulated selectively around blood vessels within TLSs (**Fig. 6D and fig. S15, A and B**). We also found a positive correlation between the proportion of CCL19⁺ FLSs and S-Tph cells in our scRNA-seq datasets, further supporting their association (**Fig. 6E**). Collectively, these data suggest that CCL19⁺ FLSs contribute to the recruitment of CCR7⁺ S-Tph cells and/or their precursors in TLSs.

E-Tph cells colocalize with macrophages and CD8⁺ T cells

To comprehensively assess cell-cell communication in inflamed ST in RA, we conducted the spatial proximity analysis (**Fig. 7A and fig. S15C**). This analysis confirmed the proximity between S-Tph and B cells (**Fig. 7A and fig. S15, C and D**). By contrast, E-Tph cells were found closer to CD8⁺ effector T cells and IL1B⁺/SPP1⁺ macrophages than B cells (**Fig. 7, A and B, and fig. S15C**). As E-Tph cells highly express CXCR6 and CCR5 (**Fig. 2, A and B**), we hypothesized that E-Tph cells were recruited to inflammatory sites via CXCL16-CXCR6 and/or CCL4/CCL5-CCR5 axes. Indeed, *CXCL16* was predominantly expressed by macrophages, and *CCL4* and *CCL5* were most highly expressed by effector CD8⁺ T cells in ST (**Fig. 5A and fig. S13B**). Spatial transcriptomics confirmed the co-localization of CXCR6 and CXCL16; and CCR5 and CCL4/CCL5 (**Fig. 7, C and D**). Thus, these data show that S-Tph and E-Tph cells colocalize with different subsets of immune cells in tissues. CXCL16-CXCR6 and/or CCL4/CCL5-CCR5 axes are associated with characteristic localization of E-Tph cells

331 at inflammatory sites.

332 We further conducted additional analysis by applying a Boolean gating strategy to define Tph subsets.

333 Specifically, Boolean-gated Tph (BG Tph) cells were defined first as *CXCL13* and/or *IL21* positive

334 cells in *CD3D* and/or *CD3E* positive T cells. Boolean-gated S-Tph (BG S-Tph) cells were further

335 defined as *CCR7* positive Tph cells without *CXCR6* expression, while Boolean-gated E-Tph (BG E-

336 Tph) cells as *CXCR6* positive Tph cells without *CCR7* expression. This Boolean-gated strategy showed

337 consistent results with clustering-based cell definition. Thus, BG S-Tph cells accumulated more in

338 TLSs than BG E-Tph cells (**Fig. 7E**). BG S-Tph cells were closer to B cells than BG E-Tph cells (**Fig.**

339 **7F**), whereas BG E-Tph cells were closer to macrophages than BG S-Tph cells (**Fig. 7G**). Thus, the

340 key findings of spatial transcriptomics analysis were validated through Boolean-gated definition of S-

341 Tph and E-Tph cells.

342

343 **Interaction between S-Tph and B cells promotes mutual differentiation**

344 As S-Tph and B cells were colocalized within TLSs, we tested whether their cognate interactions

345 induce E-Tph cell conversion from S-Tph cells and immunoglobulin (Ig) production from B cells. To

346 this end, we isolated S-Tph, E-Tph, non-Tph, and B cells from the same SF, and cultured each T cell

347 population with B cells for 7 days in the presence of superantigen SEB (**Fig. 7H**). Consistent with

348 CD3/CD28 stimulation, co-culture with B cells promoted S-Tph cells to convert to *CCR7*⁻ *CXCR6*⁺

349 E-Tph cells (**Fig. 7I and fig. S, 6D and 16A**). Furthermore, S-Tph cells induced B cells to produce

350 more IgG than control PD-1^{neg} non-Tph cells (**Fig. 7J**). Altogether, these data suggest that S-Tph cells

351 promote Ig production by B cells via cognate interactions and, in return, differentiate into E-Tph cells,

352 exit TLSs, and contribute to tissue inflammation.

353

354 **Discussion**

355 Our study demonstrates that Tph cells in inflamed tissue in RA are composed of two subsets, S-Tph

and E-Tph cells (**Fig. 8**). These two subsets differ in transcriptome, epigenome, B cell helper capacity, spatial localization, and the type of encountering cells. S-Tph cells and E-Tph cells are directly related in their differentiation. S-Tph cells have the capacity to self-renew as well as to differentiate into E-Tph cells. S-Tph cells and E-Tph cells can be isolated as CCR7⁺ CXCR6⁻ and CCR7⁻ CXCR6⁺ within CXCR5⁻ PD-1^{high} CD4⁺ T cells from tissues, respectively, and be functionally analyzed.

S-Tph cells displayed a gene signature of stem-like cells, including *CCR7*, *IL7R*, *TCF7*, and *LEF1*. Upon a short-term activation, S-Tph cells vigorously proliferated yet maintained the expression of CCR7 and TCF1, indicating their self-renewal capacity. TCF1 was integrated with this program, as TCF1 depletion significantly downregulated cell proliferation, consistent with previous studies with mouse CD8⁺ T cells (31, 32).

Importantly, our study shows that the majority of S-Tph cells localize within the TLSs in inflamed ST. Both S-Tph and B cells were located at the center of the TLSs, and the spatial proximity analysis further confirmed the proximity of S-Tph and B cells. Of note, S-Tph cells potentially induced B cells to produce Igs. S-Tph cells expressed ICOS and CD40L, whereas B cells in ST expressed CD80 and CD40, further supporting their interactions (**Fig. 5A**). Thus, the interactions between S-Tph cells and B cells in TLSs likely promote self-renewal of S-Tph cells as well as Ig production by B cells.

How Tph cells are related to TLSs has been an unresolved question (39). Our study suggests that S-Tph cells play a central role in TLSs, and CCR7 is highly integrated into this process. Mouse studies showed that a set of chemokines, CCL19 and CCL21, the ligand chemokines of CCR7, are critical for the generation of TLSs (40). Our scRNA-seq analysis identified a cluster of FLSs expressing *CCL19* and *CCL21* in addition to *CXCL13* in inflamed ST, a phenotype similar to fibroblastic reticular cells in secondary lymphoid organs (41-44). Importantly, CCL19⁺ CCL21⁺ FLSs were localized around blood vessels within TLSs in ST of RA, suggesting their recruitment of CCR7⁺ S-Tph cells and/or their precursors into TLSs via blood circulation.

E-Tph cells shared high expression of *CXCL13* and *PDCD1* with S-Tph cells but also expressed genes

381 associated with cell activation (*HLA-DRB1*, *PRDMI*, and *LAG3*). E-Tph cells were epigenetically
382 distinct from S-Tph cells, and open chromatin regions were enriched by IRF-family motifs. These
383 results suggest that IRF family molecules are activated due to TCR signals in E-Tph cells. Consistently,
384 E-Tph cells expressed effector cytokines and chemokines, including *IFNG*, *CCL4*, and *CCL5*, as well
385 as cytotoxic molecules such as *GNLY* and *GZMA*. GO analysis showed elevated effector-related gene
386 signatures in E-Tph cells, as previously reported as a feature of Tph cells (13, 15). E-Tph cells also
387 expressed CCR2 and CCR5, the chemokine receptors typical of Tph cells (4, 45). Furthermore,
388 whereas oligoclonal expansion was reported to be one of the features of Tph cells (15), we found that
389 E-Tph cells were more clonally expanded than S-Tph cells. Notably, the proportion of E-Tph cells, but
390 not S-Tph cells, positively correlated with clinical disease activity. Thus, some previously described
391 Tph features are more attributed to E-Tph cells.

392 Nonetheless, whereas Tph cells expressing CCR2 and CCR5 are widely considered to be B cell helpers
393 in inflammatory sites (4, 45), our spatial transcriptomic analysis shows that E-Tph cells expressing
394 CCR2, CCR5, and CXCR6 were in close proximity to inflammatory macrophages and CD8⁺ T cells
395 rather than B cells. Consistently, *CXCL16* was predominantly expressed by macrophages, and *CCL4*
396 and *CCL5* were most highly expressed by effector CD8⁺ T cells outside TLSs. Only 38.5% of E-Tph
397 cells were localized within but near the margin of TLS. E-Tph cells colocalized with IL1B⁺/SPP1⁺
398 macrophages, which induce the secretion of matrix metalloproteinases from lining FLSs (3, 46).
399 Therefore, the major function of E-Tph cells is not to support antibody production but rather to
400 augment the inflammation circuit formed by macrophages and FLSs.

401 S-Tph and E-Tph cells were directly related, as their clonotypes were robustly shared. The
402 differentiation seems unidirectional from S-Tph to E-Tph cells, as S-Tph cells were able to differentiate
403 into E-Tph cells upon TCR stimulation and coculture with B cells, but not the opposite. The different
404 localization of S-Tph (center) and E-Tph (margin) cells in TLSs suggests that such differentiation is
405 accompanied by the egress of E-Tph cells from TLSs. Consistent with our findings, recent studies

revealed the existence of stem-like T cell population that gives rise to effector cells in autoimmunity of mice and humans (47-51). The unique feature of S-Tph cells in RA, however, is their potent B cell helper function in addition to their stem-like functions.

Our current study is limited by the cohort consisting solely of female patients. Thus, further studies are warranted to generalize the results of this study across sex. Additionally, we performed unsupervised clustering and annotation differently among the datasets. For scRNA-seq analysis, we used transcriptomic data for both clustering and annotation. Tph clusters were identified based on high expression of *PDCDI*, *CXCL13*, and *IL21* transcripts. By contrast, clustering was performed based on ATAC peaks in scASAP-seq analysis. The annotation of Tph subsets were done with 1) the cell surface phenotype of CXCR5⁺ PD-1⁺ and 2) gene activity estimated by ATAC peaks, including *CXCL13* and *IL21*. As the methods in scASAP-seq were largely different from scRNA-seq and spatial transcriptomics, we surmise that Tph clusters in scASAP-seq may have contained a broader range of cells. Further investigation into the epigenomic features of Tph cells, along with better integrative approaches across different sequencing techniques, will enhance our understanding in the future.

Collectively, we propose that TLSs in inflamed ST in RA have two major roles: 1. To sustain Tph responses by promoting self-renewal of S-Tph cells and their differentiation into E-Tph cells, and 2. To promote autoantibody production mainly due to interactions between S-Tph cells and B cells in the TLSs. The sustained Tph response causes a flux of E-Tph cells that promote tissue inflammation.

Given that S-Tph cells are positioned to be precursors of E-Tph cells, we anticipate that therapeutic strategies targeting S-Tph cells may reduce global Tph responses and TLS formation. Indeed, inhibition of cognate interactions between S-Tph and B cells might be one of the modes of action of CTLA4-Ig, which inhibits the interactions between CD28 and CD80/CD86. Successful CTLA4-Ig treatment was shown to decrease B cells in inflamed synovial tissue (52), which might be caused by blocking the interactions between S-Tph and B cells. Our discovery of two stages of Tph cells, S-Tph and E-Tph, may pave the way for a new therapeutic strategy for RA.

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Materials and Methods

Study design

We designed this study to evaluate how the diverse functions of Tph cells are established by performing multi-modal analyses and *in vitro* validation. 10 patients with RA were recruited, and matched ST, SF, and PB samples were obtained. Transcriptome, epigenome, TCR clonotype and spatial RNA expression analyses at the single cell level were conducted. Inferred function and differentiation of Tph cells were validated by *in vitro* TCR stimulation and coculture assays. This research protocol was approved by Kyoto University institutional review boards and ethics committees (G1287). All patients gave written informed consent.

Sample collection and processing

All patients met the 2010 ACR/EULAR criteria for RA diagnosis. Clinical data collected included age, sex, ESR, rheumatoid factor titer, anti-CCP antibody titer, treatments and DAS28-ESR (**table S1**). ST, SF, and PB samples from patients were collected at Kyoto University Hospital. ST samples were obtained from surgical procedures. SF samples were obtained either from surgical procedures or from arthrocentesis. ST was mechanically and enzymatically dissociated in RPMI-1640 containing LiberaseTL (100 µg/ml, Roche) and DNaseI (100µg/ml, Roche) at 37°C for 30 minutes using MACSmix Tube Rotator (Miltenyi Biotec). Synovial fluid mononuclear cells (SFMCs) and PBMCs were isolated by Lymphocyte Separation Solution 1.077 (Nacalai Tesque). ST, SFMCs, and PBMCs were immediately used for single-cell library construction or cryopreserved in Cryostor CS10 (STEMCELL Technologies) for further analyses. Details of samples used for sequencing analyses are provided in **table S4**.

456 **Cell isolation for scRNA-seq**

457 T lymphocytes and non-immune cells were isolated either by magnetic selection or by fluorescence-
458 activated cell sorting (FACS Melody, BD). For magnetic selection, CD3⁺ T lymphocytes were isolated
459 with EasySep Human CD3 Positive Selection Kit II (STEMCELL Technologies), and CD45⁻ non-
460 immune cells with EasySep Release Human CD45 Positive Selection Kit (STEMCELL Technologies)
461 in accordance with the protocol. For FACS sorting, cells were stained with fluorescent-conjugated
462 antibodies (**table S5**) at 4°C for 15 minutes. CD3⁺ T lymphocytes were sorted with FACS Melody
463 (BD) as viability dye⁻ CD45⁺ CD3⁺ cells, and CD45⁻ non-immune cells as viability dye⁻ CD45⁻ cells.
464 For TRBV gene usage restriction, CD4⁺ T lymphocytes were gated as viability dye⁻ CD3⁺ CD4⁺ cells
465 and TCR Vβ3⁺, TCR Vβ7.1⁺, and TCR Vβ5.3⁺ CD4⁺ T cells were sorted.

466

467 **Library construction and sequencing of scRNA/TCR-seq and CITE-seq**

468 Isolated T cells were stained with TotalSeqTM-C antibodies (Biolegend) in the presence of TruStain
469 FcX (BioLegend) as per the protocol (**table S6**). Following 10x Genomics Chromium single cell 5' kit
470 protocol, cells were loaded into a Chromium Next GEM Chip G or K (10x Genomics). cDNA libraries
471 for gene expression, feature barcode and TCR VDJ were synthesized and amplified, and were
472 sequenced on NovaSeq 6000 (Illumina) to obtain a read depth of 30,000 reads for gene expression,
473 2,500 reads for TCR VDJ sequences, and of 5,000 reads for feature barcode per cell. Following
474 sequencing, the pooled GEX, VDJ, and ADT libraries were demultiplexed to generate FASTQ files for
475 each sample using bcl2fastq (v2.20.0.422). The raw FASTQ data were processed with CellRanger
476 (v7.0.1) using GRCh38 annotation.

477

478 **scRNA-seq data analysis**

479 The output matrix from CellRanger was analyzed by R (v4.0.5) with the Seurat package (v4.3.0). For
480 each sample, doublet cells were defined by scDblFinder (v1.4.0) and cells with high percentage of

mitochondrial genes (>10%) and doublet cells were removed. Among CD3⁺ T cells, CD4⁺ T cells were extracted based on the positive CD4 gene expression and/or surface protein expression. Data of CD4⁺ T cells from ST, SF, and PB samples were merged and normalized, and top 2000 highly variable genes were identified with the vst method. We then integrated samples using canonical correlation analysis (CCA) and performed scaling, dimensionality reduction (30 principal components), and UMAP visualization. For clustering, we set the resolution parameter to be 0.4. FindAllMarkers function was used to obtain differentially expressed genes (DEGs) specific to each cluster. Data of CD45⁻ non-immune cells were separately merged and followed the same process mentioned above, except for the resolution parameter being set to 0.5.

Gene ontology analysis

Gene ontology analysis was performed online by Metascape (21). Upregulated genes meeting the criteria of both average log2 fold-change > 0.5 and p-value < 0.05 in S-Tph cells compared to E-Tph cells and vice versa were used for input. Top 5 GO biological processes were shown in the figures.

Gene set enrichment analysis

Publicly available data (22) were utilized to obtain gene sets for stem-like and effector CD4⁺ T cells in mice. Stem-like and effector CD4⁺ T cells were identified according to the analysis workflow provided in the method section of the article (22). Other gene sets were obtained from the data generated in this study. Upregulated genes meeting the criteria of both average log2 fold-change > 0.5 and p-value < 0.05 were extracted as gene sets. The analysis was performed by GSEA (v4.2.3: UC San Diego and Broad Institute).

Cell-cycle scoring and regression

The CellCycleScoring function in Seurat was used to assign a cell-cycle score to each cell of the

506 scRNA-seq dataset, based on its expression of G2/M and S phase markers. Then, the ScaleData
507 function subtracted these scores and generated corrected gene expression data. Downstream analysis
508 of UMAP visualization was done as mentioned above.

509

510 **RNA velocity**

511 The BAM files generated by CellRanger were processed with Velocyto (v0.17.17) using the GRCh38
512 reference genome. Output files of Velocyto and UMAP of the integrated Seurat object were loaded
513 into scVelo version 0.2.4 in the Python (v3.6.13) and the direction of cell differentiation was estimated
514 with the steady state mode.

515

516 **CITE-seq data analysis**

517 The output matrix from CellRanger was analyzed by R (v4.0.5) with the Seurat package (v4.3.0). For
518 the visualization using the FeaturePlot function, the count data was normalized with the CLR method
519 across cells. When expression levels were visualized using the FeatureScatter function, count data
520 were log1P transformed after adding random values ranging between -0.4 and 0.4 to reduce
521 overlapping points and improve visualization.

522

523 **TCR clonotype analysis**

524 TCR sequences were extracted from fastq files for gene expression and VDJ expression using TRUST4
525 (v1.0.8) (53). Gene usage of TRAV and TRBV and nucleotide sequences of CDR3 from
526 barcode_report.tsv files were imported to the metadata of Seurat objects. Cells with both TCR alpha
527 and TCR beta sequence information were used for clonotype analyses. Cells with the identical
528 sequences for both CDR3 alpha and beta chains at the nucleotide level were defined to share the same
529 clonotype. Horn index was calculated to investigate the clonotype overlap between different clusters
530 by vegdist function in vegan (v2.6-4) with the method being horn. Honeycomb plot was generated

531 using enclone (10x Genomics) to visualize shared clonotypes and cell numbers in each clonotype.
532 Permutation tests (1,000 permutations) were conducted for statistical analyses of samples with TRBV
533 gene usage restriction. Cells from different groups were pooled and the identities were randomly
534 reassigned. In each simulation, the expected percentages of cells in expanded clones were calculated.
535 The P value was determined by counting the number of simulations in which the expected percentage
536 was either 1) lower than the observed percentage of the lower condition between the two in the actual
537 data or 2) higher than the observed percentage of the higher condition.

538

539 **Library construction, sequencing, and data analysis of Bulk RNA-seq**

540 Sorted cells were washed with PBS and were resuspended in Buffer RLT (Qiagen) supplemented with
541 2-mercaptoethanol (Nacalai). Cells were vortexed for 1 minute and were stored at -80 °C. RNA from
542 the cells was collected using Agencourt RNAClean XP beads (Beckman Coulter). Subsequent library
543 construction was performed following SMART-Seq mRNA LP protocol (Takara Bio USA). cDNA
544 libraries were synthesized and amplified, and were sequenced on NovaSeq 6000 (Illumina) to obtain
545 a read depth of 60 million reads per sample. The quality check of the raw FASTQ data were performed
546 using FastQC (v0.12.1). Adapter trimming was performed using Trim Galore (v0.6.10), followed by
547 the alignment of the reads to GRCh38 using STAR (v2.7.10b). ribosomal RNA reads were excluded
548 for further analysis. Gene expression was quantified using featureCounts (v2.0.3). Normalization of
549 the gene expression data was performed using edgeR installed in iDEP (v0.96).

550

551 **scASAP-seq**

552 CD45⁺ lymphocytes from enzymatically digested ST were obtained using EasySep Release Human
553 CD45 Positive Selection Kit (STEMCELL Technologies) and stained with TotalSeqTM-B antibodies
554 (**table S7**) and antibodies conjugated with fluorochromes (**table S5**). CD4⁺ T cells were sorted with
555 FACS Melody (BD) as viability dye⁻ CD4⁺ cells. scASAP-seq was performed as previously described

(24). Briefly, sorted cells were fixed with 1% formaldehyde for 10 minutes at room temperature, quenched by glycine at the final concentration of 0.125 M, washed twice with cold PBS, and incubated with OMNI lysis buffer for 3 minutes. After washes for two times, cells were processed according to Chromium Next GEM Single Cell ATAC Reagent Kits v2 (10x Genomics) except that bridging oligo at the final concentration of 8.3 nM was added at step 2.1 and samples were preincubated at 40°C for 5 minutes at step 2.5. ATAC and feature barcode libraries were sequenced on NovaSeq 6000 (Illumina) to obtain a read depth of 25,000 reads for ATAC libraries and of 3,000 reads for feature barcode libraries per cell. The raw FASTQ for scATAC-seq data were processed with CellRanger ATAC (v2.1.0) using GRCh38 annotation. Feature barcodes were quantified with salmon (v1.10.2) and alevin-fry (0.8.2).

The output peak matrix and fragment files from CellRanger and salmon were analyzed by R (v4.2.2) with Signac (v1.10.0) and Seurat package (v4.0.3). Cells with nCount_peaks between 3,000 and 6,000, pct_reads_in_peaks more than 15, blacklist_ratio less than 0.05, nucleosome_signal less than 5, and TSS.enrichment more than 3 were used for downstream analyses. After normalization with RunTFIDF function, UMAP and cell clusters were defined using second through 24th dimensions of signal value decomposition for top features extracted with FindTopFeatures function. Clusters with negative surface protein expression of CD3 were removed. Gene activity was quantified by assessing the chromatin accessibility at each gene locus. Specifically, the GeneActivity function was used to count the number of sequenced fragments spanning the gene body and its promoter region. Reference ATAC peaks for naive and central memory CD4⁺ T cells were obtained by utilizing publicly available scATAC/RNA-seq data of human PBMCs (10k Human PBMCs, Multiome v1.0, Chromium X from 10x Genomics). Differential ATAC peaks were calculated using the FindMarkers function where min.pct was set to 0.2. DNA sequence motif information required for motif analyses was obtained from JASPAR 2022 transcription factor binding profile database. Using peak matrix as input, RunChromVAR function (54) was performed to identify motifs associated with variability in

581 chromatin accessibility across individual cells and to calculate motif deviation scores that estimate the
582 activity of corresponding transcription factors.

583

584 **Spatial transcriptomics data acquisition**

585 10x Xenium in situ analysis was conducted utilizing the pre-designed Human Multi-Tissue and Cancer
586 Panel (377 genes) with an add-on custom-designed gene panel (100 genes) (**table S3**). Data acquisition
587 was conducted according to the protocol. Briefly, formalin fixed paraffin embedded ST was sliced at
588 5 μm and mounted onto the Xenium slide. The section was deparaffinized and hybridized with probes,
589 followed by post-hybridization washes, ligation and amplification. Autofluorescence of the section
590 was quenched and nuclei staining was performed before probe signals were detected by 10x Xenium
591 Analyzer instrument. After the signal detection, Hematoxylin and Eosin (H&E) staining of the tissue
592 was performed and the digitalized image was obtained. Cell segmentation was performed by the
593 pipelines available within 10x Xenium Analyzer instrument. Xenium Explorer (10x Genomics, v1.3.0)
594 was used to visualize the distribution of transcripts on the H&E tissue image.

595

596 **Spatial transcriptomics analysis**

597 Giotto Suite 3.2 was used for downstream analyses. Data of transcript counts and polygons indicating
598 nuclei and cell boundaries were loaded and Giotto object was created. Transcript data were assigned
599 to each polygon and centroid coordinates for each polygon were calculated. Cells containing less than
600 5 transcripts and genes detected in less than 3 cells were removed from the further analyses. The
601 normalization function normalized the feature expression measurements for each cell by dividing by
602 the total expression, scaled the result by a factor of 5,000, and log-transformed the result. Principal
603 component analysis was performed using all filtered genes, followed by UMAP visualization with 10
604 dimensions being used. Louvain clustering was done with the resolution set to 0.9. Canonical marker
605 genes and differentially expressed genes in each cluster were used for cell-type annotation. Spatial

606 network analysis across clusters was performed using library-based functions, named
607 createSpatialNetwork and cellProximityEnrichment functions. The createSpatialNetwork function
608 computationally identifies neighboring cells in the spatial data. The cellProximityEnrichment function
609 calculates the spatial proximity enrichment between pairs of cell types by leveraging the data of
610 neighboring cells. The enriched interaction was visualized in network diagram. Overlaid figures of
611 spatial gene expression were generated using ImageJ (v1.53k).

612

613 **TLS extraction**

614 TLS regions were defined according to cell density calculated by kernel density estimation of
615 densityarea package (v0.1.0) in R using probs = 0.25, h = 80, and n = 300. Regions smaller than 1000
616 micrometer square were removed. Validation of these TLS regions was performed by H&E staining of
617 the tissue. Cells inside or outside of TLS regions were extracted using st_intersects function of sf
618 package (v1.0-16) in R.

619

620 **Flow cytometry**

621 SFMCs were stained with Zombie UV™ Fixable Viability Kit (Biolegend), followed by staining for
622 cell surface molecules with fluorescent-conjugated antibodies at 4 °C for 15 minutes. Cells were then
623 fixed (4 °C, 20 min) and permeabilized with Cytofix/Cytoperm™ Fixation/Permeabilization Kit (BD),
624 and were stained for intracellular molecules with fluorescent-conjugated antibodies at 4 °C for 30
625 minutes. Acquisition was performed with Aurora (Cytek Biosciences) (**fig. S6A**) and data were
626 analyzed using FlowJo (BD, v10.8.1). Details of antibodies used for flow cytometry analysis and cell
627 sorting are shown in **table S8**.

628

629 **Proliferation assay**

630 Cryopreserved SFMCs were thawed in water bath at 37 °C and washed twice with RPMI-1640. Debris

631 was removed by filtering cell suspension through 40µm cell strainer (Greiner Bio-one). CellTrace™
632 Violet Cell Proliferation Kit, for flow cytometry (Thermo Fisher Scientific) was used for *in vitro*
633 labeling of cells to trace cell division according to the protocol. Briefly, cells were suspended in PBS
634 at the concentration of 1×10^7 cells/ml and CellTrace Violet was subsequently added at the final
635 concentration of 125 nM. Cells were then placed in 5% CO₂ incubator at 37 °C for 20 minutes. For
636 FACS sorting, cells were stained with fluorescent-conjugated antibodies at 4 °C for 15 minutes. Tph
637 cells were gated as viability dye⁻ CD4⁺ CD8⁻ CD14⁻ CD16⁻ CD56⁻ PD-1^{high} cells, and S-Tph (CCR7⁺
638 CXCR6⁻ Tph) and E-Tph cells (CCR7⁻ CXCR6⁺ Tph) were sorted using FACS Melody (**fig. S6B**).
639 Cells were centrifuged and resuspended in complete RPMI-1640 (RPMI-1640 (Wako) supplemented
640 with 10% fetal bovine serum (Sigma-Aldrich), 1% non-essential amino acids (Thermo Fisher
641 Scientific), 0.1% 2-Mercaptoethanol (Thermo Fisher Scientific), HEPES buffer (25mM, nacalai
642 tesque), sodium pyruvate (1mM, Thermo Fisher Scientific), streptomycin (50 µg/ml, Thermo Fisher
643 Scientific) and penicillin (50 units/ml, Thermo Fisher Scientific). Live cell count was performed,
644 followed by seeding 2×10^4 cells per well in a 96-well U-bottom plate. Dynabeads™ Human T-
645 Activator CD3/CD28 (Thermo Fisher Scientific) were added according to the protocol at the bead-to-
646 cell ratio of 1:1. After 3 days of incubation in 5% CO₂ incubator at 37 °C, cells were counted or were
647 stained with fluorescent-conjugated antibodies and were acquired on Aurora (**fig. S6C**).

648

649 **Differentiation assay**

650 SFMCs were thawed and FACS sorting was performed as mentioned above. Contamination of
651 CXCR6⁺ cells in sorted CCR7⁺ CXCR6⁻ cell was less than 1%. Cells were centrifuged and resuspended
652 in complete RPMI-1640 supplemented with low dose of recombinant IL-2 (30 units/ml, Miltenyi
653 Biotec). Live cell count was performed, followed by seeding 1.5×10^4 cells per well in a 96-well U-
654 bottom plate. CD3/28 dynabeads were added at the bead-to-cell ratio of 1:1. Half of the culture medium
655 was replaced with fresh medium every 3 days. After 9 days of incubation, cells were stained with

656 fluorescent-conjugated antibodies and were acquired on Aurora or sorted on Melody (viability dye⁻
657 CCR7⁺ CXCR6⁻ cells and viability dye⁻ CCR7⁻ CXCR6⁺ cells) (**fig. S6C**).

658

659 **Gene knockout using CRISPR-Cas9 system**

660 Single guide RNA (sgRNA) targeting TCF7 (AGAGAGTTGGGGGACACCGT) and non-target
661 control sgRNA (ATCGTTTCCGCTTAACGGCG) were purchased from Sigma-Aldrich. sgRNAs
662 were reconstituted in nuclease free duplex buffer (Integrated DNA Technologies) for the concentration
663 of 80 μ M. sgRNAs were mixed with TrueCut Cas9 Protein v2 (30 μ M, Thermo Fisher Scientific), and
664 were incubated at 20 °C for 20 minutes to generate crRNP. SFMCs were thawed as mentioned above.
665 CD4⁺ T lymphocytes were isolated with CD4⁺ T Cell Isolation Kit (Miltenyi Biotec) as per the protocol
666 and were washed with PBS. crRNPs (6 μ M sgRNA, 3 μ M Cas9) were nucleofected into 5x10⁵ cells
667 per cuvette using P3 Primary Cell 4D-Nucleofector™ X Kit S (Lonza). After 6 hours of incubation,
668 FACS sorting was performed as mentioned above. Cells were centrifuged and resuspended in complete
669 RPMI-1640 supplemented with low dose of recombinant IL-2. Live cell count was performed,
670 followed by seeding 1.5x10⁴ cells per well in a 96-well U-bottom plate. CD3/28 dynabeads were
671 added at the bead-to-cell ratio of 1:1. Half of the culture medium was replaced with fresh medium
672 every 3 days. Cells were counted on day 6 to assess proliferation and were stained with fluorescent-
673 conjugated antibodies and acquired on Aurora on day 9 to assess differentiation (**fig. S6C**).

674

675 **T-B coculture**

676 SFMCs were thawed as mentioned above. CD19⁺ B lymphocytes were isolated with CD19
677 MicroBeads (Miltenyi Biotec) as per the protocol. CD19⁻ cells were stained with fluorescent-
678 conjugated antibodies at 4 °C for 15 minutes. FACS Melody was used to sort S-Tph, E-Tph, and non-
679 Tph cells (**fig. S6B**). Cells were centrifuged and resuspended in complete RPMI-1640. Live cell count
680 was performed, followed by seeding 1.0x10⁴ B cells and either 1.0x10⁴ S-Tph, E-Tph, or non-Tph

681 cells per well in a 96-well U-bottom plate. Staphylococcal enterotoxin B (1 µg/ml, Sigma-Aldrich)
682 was added to the culture medium. After 7 days of incubation, culture supernatant was collected, and
683 cells were stained with fluorescent-conjugated antibodies and were acquired on Aurora (**fig. S6D**).
684

685 **IgG measurement**

686 The concentrations of total IgG in the culture supernatant were determined using enzyme-linked
687 immunosorbent assay (ELISA). 96-well flat bottom plates were coated with mouse anti-human-IgG
688 monoclonal antibody (Bio-rad, product code: MCA2531) overnight at 4°C. Plates were washed with
689 wash buffer (0.05% Tween-20 in PBS) and were blocked for 1 hour at room temperature with diluent
690 buffer (Biolegend). Samples were added and incubated for 1 hour at room temperature. After washing,
691 plates were incubated with goat anti-human-IgG polyclonal antibody conjugated with HRP (Bio-rad,
692 product code: 5172-2504) for 1 hour at room temperature. Plates were washed and TMB solution
693 (Thermo Fisher Scientific) was added. The reaction was stopped by sulfuric acid (Nacalai). Optical
694 density at 405 nm was measured by Spectra Max iD3 (Molecular Devices). IgG from human serum
695 (Sigma-Aldrich) was used to draw the standard curve.
696

697 **Protein immunoblot**

698 Naïve CD4⁺ T cells were isolated with Naïve CD4⁺ T Cell Isolation Kit II, human (Miltenyi Biotec)
699 from PBMCs of healthy individuals. Gene knockout using CRISPR-Cas9 system was performed as
700 described above. The cells were incubated with CD3/28 dynabeads for 3 days. Total protein extracts
701 were prepared by mixing cells with RIPA buffer (Nacalai) supplemented with protease inhibitors
702 (Nacalai). For immunoblotting, extracted proteins were subjected to 5-20% polyacrylamide gel
703 (ATTO) and transferred onto a nitrocellulose membrane with Power Blotter Select Transfer Stacks
704 (Thermo). After blocking with 5% skim milk, the membrane was probed with anti-TCF1 rabbit
705 monoclonal antibody (2203, Cell Signaling Technology; 1:1000) followed by incubation with

706 horseradish protein (HRP)-conjugated anti-rabbit IgG antibody (7074, Cell Signaling Technology;
707 1:3000), or the membrane was probed with HRP-conjugated anti-human/mouse β -actin antibody
708 (A3854, Sigma Aldrich; 1:10000). The membrane was visualized using ECL Prime Western Blotting
709 Detection Reagent (GE Healthcare). Full size blot image is found in **Data file S1**.

710

711 **Immunofluorescence**

712 Paraffin-embedded synovial tissues of RA were sectioned, deparaffinized, and antigen retrieved by 20-
713 minute heating with 0.01M citric acid of pH 6.0 using an autoclave machine. Immunofluorescence
714 staining was performed with CD4 antibody (Clone 1F6, mouse monoclonal, Leica Microsystems,
715 1:100), CCR7 antibody (Clone EPR23192-57, rabbit monoclonal, Abcam, 1:500), TCF1 antibody
716 (Clone 2203, rabbit monoclonal, Cell Signaling Technology, 1:200), and CXCR6 antibody (Clone
717 EPR24686-113, rabbit monoclonal, Abcam, 1:1000), and was detected with TSA Plus Fluorescence
718 Kit (PerkinElmer, Inc.). Fluorescence imaging analysis was performed using the BZ-X800
719 Fluorescence Microscope (KEYENCE).

720

721 **Statistical analysis**

722 Data were analyzed and visualized using GraphPad Prism (v10.1.1) or R (v4.2.2). Statistical tests
723 performed are indicated in the figure legends.

724

725 Supplementary Materials include:

726 Figure S1-S16

727 Table S1-S8

728 Data file S1-S2

729 MDAR Reproducibility checklist

730

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919

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921 Conceptualization: Y.Masuo, H.U., and H.Y. Methodology: Y.Masuo, H.U., and H.Y. Investigation:
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924 and H.Y. Supervision: H.U. and H.Y. Writing – original draft: Y.Masuo, H.U., and H.Y. Writing –
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926

927 **Competing interests**

928 The authors declare that they have no competing interests.

929

930 **Data and materials availability**

931 Raw sequencing data and processed data have been deposited to National Bioscience Database Center
932 (NBDC) with the accession number JGAS000727 and JGAS000774. Tabulated data underlying the
933 figures are available in data file S2. All reagents and materials used in this study are commercially
934 available.

935

936 **Figure legends**

937 **Fig. 1. Single-cell transcriptomic and epigenomic analyses identify stem-like and effector Tph**
938 **cells.**

939 (A) Overview of the multi-modal single-cell analysis in this study. (B) UMAP of scRNA-seq data for
940 CD4⁺ T cells from synovial tissue (ST; n=10), synovial fluid (SF; n=4) and peripheral blood (PB; n=9)
941 combined. (C) Heatmap of the differentially expressed genes among clusters. (D) Violin plots of

942 *PDCD1* and *CXCL13* expression. (E) Scatter plot comparing gene expression between S-Tph cells
 943 (cluster 2) and E-Tph cells (cluster 4). Selected genes with higher expression in S-Tph or E-Tph cells
 944 are shown in red or blue dots, respectively. CPM, count per million. (F) Enriched gene ontology terms
 945 for upregulated genes in S-Tph and E-Tph cells. (G) GSEA showing the enrichment of the mouse
 946 stem-like CD4⁺ T upregulated gene set and effector CD4⁺ T upregulated gene set in S-Tph and E-Tph
 947 cells, respectively. NES, normalized enrichment score; FDR, false discovery rate. (H) UMAP of
 948 scASAP-seq data for CD4⁺ T cells from synovial tissue (n=1). (I) Feature plots showing surface protein
 949 expression of characteristic genes of S-Tph, E-Tph and Tfh cells. (J) Dot plot of the differentially
 950 expressed surface proteins among clusters. Color and size of the dots indicate average expression and
 951 percent expressed, respectively.

952
 953 **Fig. 2. CCR7⁺ S-Tph cells demonstrate self-renewal potential.**

954 (A) Scatter plot comparing chemokine receptor gene expression between S-Tph and E-Tph cells from
 955 synovial tissue (ST) and synovial fluid (SF) in scRNA-seq. Chemokine receptor genes with higher
 956 expression in S-Tph or E-Tph cells are shown in red or blue dots, respectively. (B) Flow cytometry of
 957 CCR2 and CCR5 expression on S-Tph and E-Tph cells (n=8). A gating strategy with representative
 958 plots of CCR2 and CCR5 expression, and summary plots showing positive rates are shown. (C) TCF1
 959 expression in S-Tph and E-Tph cells (n=6). Representative histogram and a plot showing positive rates
 960 are shown. (D) Proliferation of S-Tph and E-Tph cells on day 3 detected using CellTrace™ Violet.
 961 Representative dot plots and a plot showing average cell division numbers are shown. (E) Viability of
 962 S-Tph and E-Tph cells on TCR stimulation on day 3. Representative dot plots and a plot showing
 963 frequency of viable cells are shown. (F and G) CCR7 (F) and TCF1 (G) expression during cell division.
 964 Representative plots and summary plots are shown. (H) Experimental scheme for I. Gene-editing was
 965 performed on sorted CD4⁺ T cells from SF. S-Tph cells were sorted and cultured under TCR
 966 stimulation, followed by cell count on day 6. (I) Proliferation of gene-edited S-Tph cells on TCR

stimulation. Fold changes in cell numbers post-stimulation are shown. KO, knockout; CTRL, control. (J) Flow cytometry of Ki-67 expression in S-Tph and E-Tph cells (n=8). Representative density plots and a summary plot showing positive rates are shown. Each dot represents a sample in B, C, and J. Representative data from one of five (D-G) or two (H-I) experiments using different samples are shown (n = 3 (H-I) or 4 (D-G) technical replicates). Means are shown in D-G and I. ** $p < 0.01$, *** $p < 0.001$; two-tailed paired t test (B, C, and J); two-tailed unpaired t test (D, E, and I); one-way ANOVA with Dunnett test (G).

Fig. 3. E-Tph cells are clonally expanded in inflamed joints.

(A) Bar graphs showing frequency of each cluster in synovial tissue (ST), synovial fluid (SF) and peripheral blood (PB). (B) Frequency ratios between sites in each cluster. (C) Scatter plots showing the association between disease activity (ESR) and the frequency of E-Tph or S-Tph cells in ST. ESR, erythrocyte sedimentation rate. (D) Bar graphs showing the extent of clonal expansion in ST, SF and PB for samples without TRBV gene usage restriction. (E) Differences of the extent of clonal expansion of each cluster between sites without TRBV gene usage restriction. (F) Plots showing the extent of clonal expansion of S-Tph and E-Tph cells in ST and SF for samples without TRBV gene usage restriction. (G) Bar graphs showing the extent of clonal expansion in ST, SF and PB for samples with TRBV gene usage restriction. (H) Bar graphs showing the extent of clonal expansion of S-Tph and E-Tph cells in ST and SF for samples with TRBV gene usage restriction. Each dot represents a sample in C and F. p values and r by Pearson's correlation analysis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; two-tailed paired t test (F); permutation test with Bonferroni correction (G); permutation test (H).

Fig. 4. S-Tph cells can differentiate into E-Tph cells *in vitro*.

(A) Clonotype repertoire similarity (Horn index) to S-Tph cells (upper row) or E-Tph cells (lower row) in each cluster of synovial tissue (ST, left column) and synovial fluid (SF, right column). The dark grey

clusters indicate the references. Color in each cluster indicates the extent of similarity. **(B)** RNA velocity in ST. Arrows indicate the predicted future state of respective cells. **(C)** Experimental scheme for D-F. S-Tph and E-Tph cells were sorted from SF and were cultured under TCR stimulation, followed by flow cytometry analysis on day 9. **(D)** Proliferation of S-Tph and E-Tph cells on TCR stimulation. Fold changes in cell numbers post-stimulation are shown. **(E)** Phenotypic changes of S-Tph and E-Tph cells post-stimulation. Representative plots and summary plots showing the percentage and the number of cells that converted their phenotypes. **(F)** Expression of key markers in CCR7⁺ CXCR6⁻, CCR7⁻ CXCR6⁻, and CCR7⁻ CXCR6⁺ cells originating from S-Tph cells. Representative histograms and summary plots showing positive rates are shown. **(G)** Phenotypic changes of gene-edited S-Tph cells post-stimulation. Gene-editing was performed on CD4⁺ T cells sorted from SF. S-Tph cells were sorted and cultured under TCR stimulation, followed by flow cytometry analysis on day 9. Representative plots and summary plots showing the percentage of cells that converted their phenotypes are shown. KO, knockout; CTRL, control. Representative data from one of three (C-F) or two (G) experiments using different samples are shown (n = 3 technical replicates). Means are shown in D-G. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns, not significant two-tailed unpaired t test (D and E); one-way ANOVA with Tukey test (F); two-tailed paired t test (G).

Fig. 5. S-Tph and E-Tph cells exhibit distinct localization.

(A) Heatmap of the differentially expressed genes among clusters in spatial transcriptomics. **(B)** Hematoxylin and Eosin (H&E) staining of RA synovium. Inset indicates a magnified tertiary lymphoid structure (TLS). **(C)** Spatial RNA expression of canonical marker genes. Insets indicate a TLS (*CD3E*, *MS4A1*), synovial lining layers (*PDPN*), or vessels (*VWF*) on H&E staining. **(D)** Computational identification of TLS regions. Each black dot indicates a cell. Areas enclosed by pink lines are TLSs defined by cell density. An enlarged view of a rectangle is shown on the right. **(E and F)** Distribution of transcripts related to S-Tph (E) or E-Tph (F) cells expressed by cells with detectable levels of both

CD3D/E and CD4. Enlarged views of white squares are shown on the right. Dotted circles indicate a TLS region. **(G)** Spatial distribution of Tph and B cells. Pie charts describe the proportion of the indicated cells inside or outside TLSs (upper row). Cells in each subset are shown in black dots with TLSs (middle row). Enlarged views of a TLS region and cells (bottom row). **(H)** Summary plot showing the percentage of S-Tph cells within TLSs among all S-Tph cells, compared with that of E-Tph cells. A-G show data from RA02 tissue section. Each dot represents a patient in H. Scale bars, 100 μ m (insets of B-C, E-F), 200 μ m (inset of D and G) or 1 mm (B-G). * $p < 0.05$; two-tailed paired t test (H).

1025

Fig. 6. Accumulation of S-Tph cells in TLS is regulated by CCL19⁺ FLS.

(A) UMAP of scRNA-seq data for CD45⁻ non-immune cells from synovial tissue (n=5). FLS, fibroblast-like synoviocytes; Mt, mitochondria. **(B)** Feature plots showing expression of selected chemokines. **(C)** Dot plot of the differentially expressed genes among clusters. Color and size of the dots indicate average expression and percent expressed, respectively. **(D)** Spatial distribution of *CCR7*, *CCL19* and *VWF* transcripts surrounding vessels in a TLS. **(E)** Scatter plot showing the correlation between S-Tph cell frequency in total CD4⁺ T cells and CCL19⁺ FLS frequency in total FLSs. Each dot represents a patient in E. p values and r by Pearson's correlation analysis. Scale bar, 100 μ m (D).

1034

Fig. 7. E-Tph cells interact with inflammatory macrophages and effector CD8⁺ T cells.

(A) Network visualization of cellular proximity in spatial transcriptomics. Red lines represent spatial proximity enrichment of cell type pairs. Higher enrichment is shown with a thicker line. **(B)** Cell-cell interaction of E-Tph cells. Indicated cells are shown in colored polygons. Enlarged views of white squares are shown on the right. **(C and D)** RNA expression of a chemokine ligand-receptor pair related to E-Tph cells. Enlarged views of white squares are shown on the right. **(E)** Summary plot showing the percentage of Boolean-gated S-Tph cells within TLSs among all Boolean-gated S-Tph cells,

1042 compared with that of Boolean-gated E-Tph cells. **(F)** Summary plot showing the proximity index of
1043 Boolean-gated S-Tph and E-Tph cells to B cells. The average number of B cells adjacent to each T cell
1044 is shown. **(G)** Summary plot showing the proximity index of Boolean-gated S-Tph and E-Tph cells to
1045 macrophages. The average number of macrophages adjacent to each T cell is shown. **(H)** Experimental
1046 scheme for I and J. S-Tph, E-Tph or non-Tph cells and autologous CD19⁺ B cells were isolated from
1047 synovial fluid (SF) and cocultured, followed by flow cytometry analysis and measurement of IgG
1048 levels in culture supernatant on day 7. **(I)** Phenotypic changes of S-Tph and E-Tph cells post-coculture.
1049 Representative plots and summary plots showing the percentage of cells that converted their
1050 phenotypes are shown. **(J)** IgG concentration in culture supernatant quantified by ELISA. A-D show
1051 data from RA02 tissue section. Each dot represents a patient in E-G. Scale bar, 50 μ m (insets of B),
1052 100 μ m (insets of C and D) or 1 mm (B to D). Representative data from one of two (H-J) experiments
1053 using different samples are shown (n = 3 technical replicates). Means are shown in E-G and I-J. * p <
1054 0.05, ** p < 0.01, *** p < 0.001; ns, not significant; two-tailed paired t test (E-G); two-tailed unpaired
1055 t test (I); one-way ANOVA with Dunnett test (J).

1056

1057 **Fig. 8. Dynamic functional and spatial transition of peripheral helper T cells in rheumatoid**
1058 **arthritis.** Stem-like Tph (S-Tph) and effector Tph (E-Tph) cells differ in transcriptome, epigenome, B
1059 cell helper capacity, spatial localization, and cell interactions. S-Tph cells express genes associated
1060 with stem-like features of T cells, including CCR7 and TCF1. S-Tph cells mainly localize within
1061 tertiary lymphoid structures (TLSs) and interact with B cells. Perivascular CCL19⁺ fibroblast-like
1062 synoviocytes (FLSs) are suggested to recruit S-Tph cells and/or their precursors into TLSs via blood
1063 circulation. Interaction between S-Tph and B cells in TLSs likely promote self-renewal and
1064 differentiation of S-Tph cells as well as antibody production by B cells. By contrast, E-Tph cells
1065 express genes related to cell activation, cytokines, and cytotoxic molecules. CXCR6 in addition to
1066 CCR2 and CCR5 are also expressed. E-Tph cells egress from TLSs and mainly localize outside of

1067 TLSs. E-Tph cells localize close to CXCL16⁺ inflammatory macrophages and CCL4⁺ CCL5⁺ CD8⁺ T
1068 cells.

Fig. 1

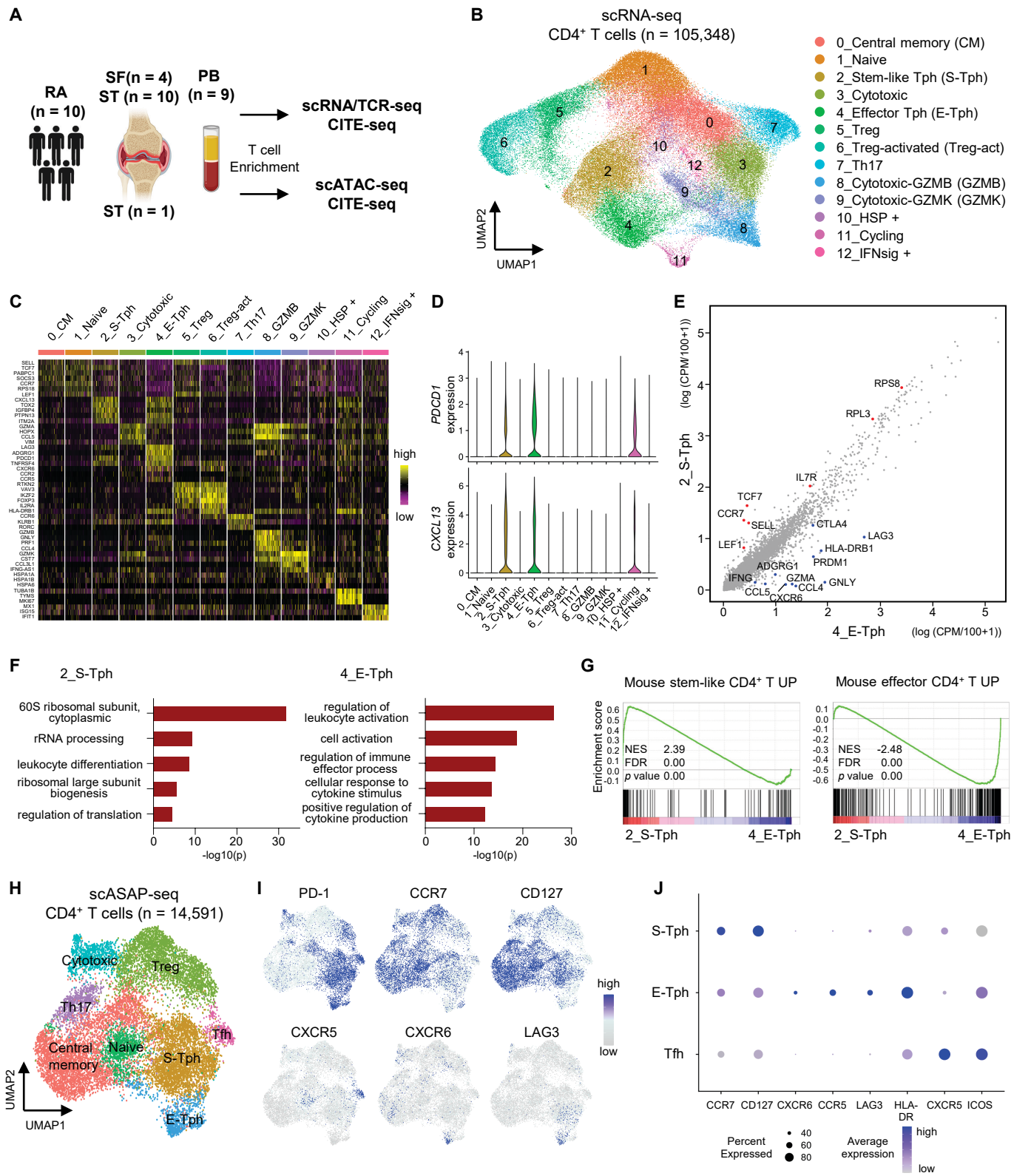


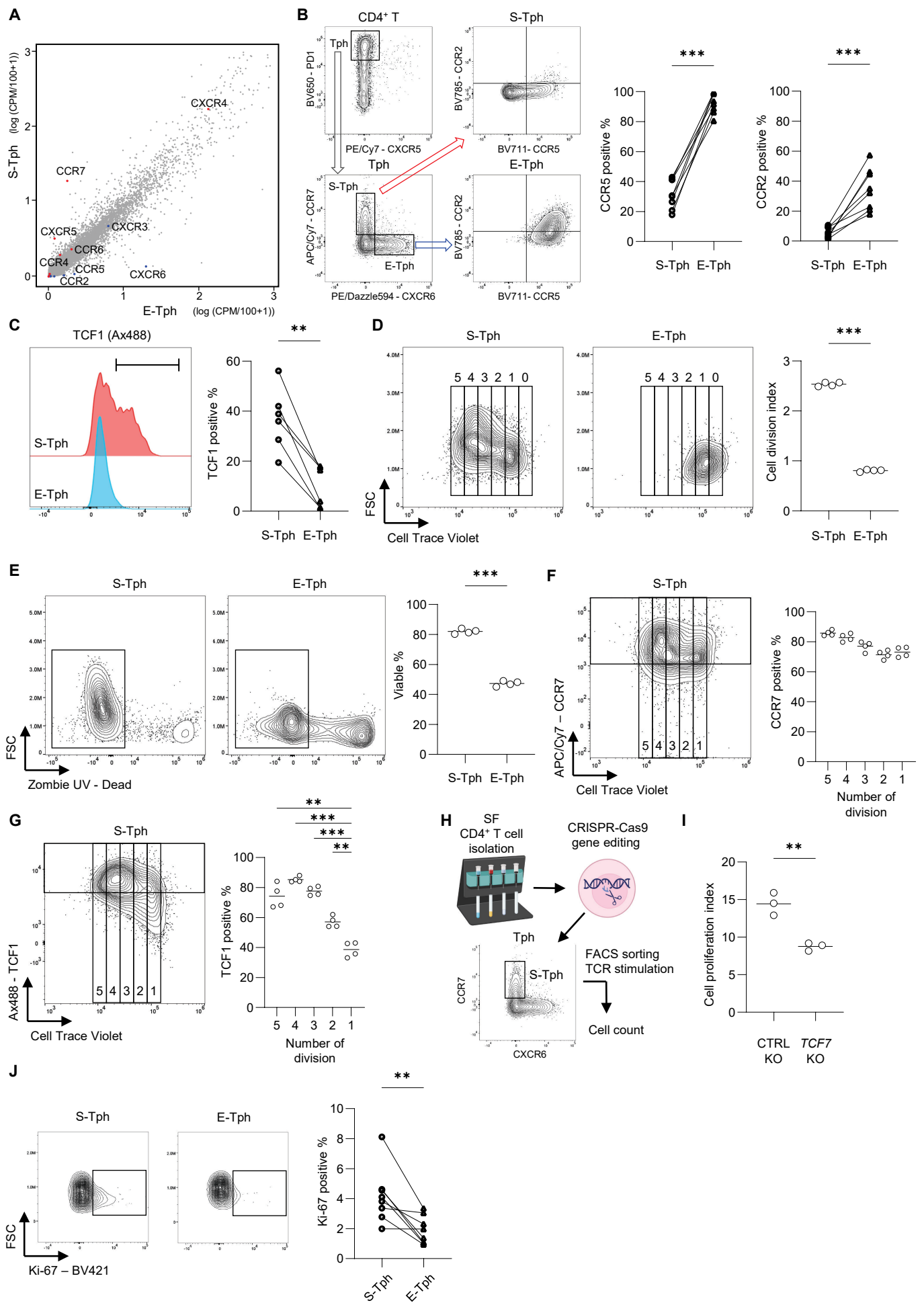
Fig. 2

Fig. 3

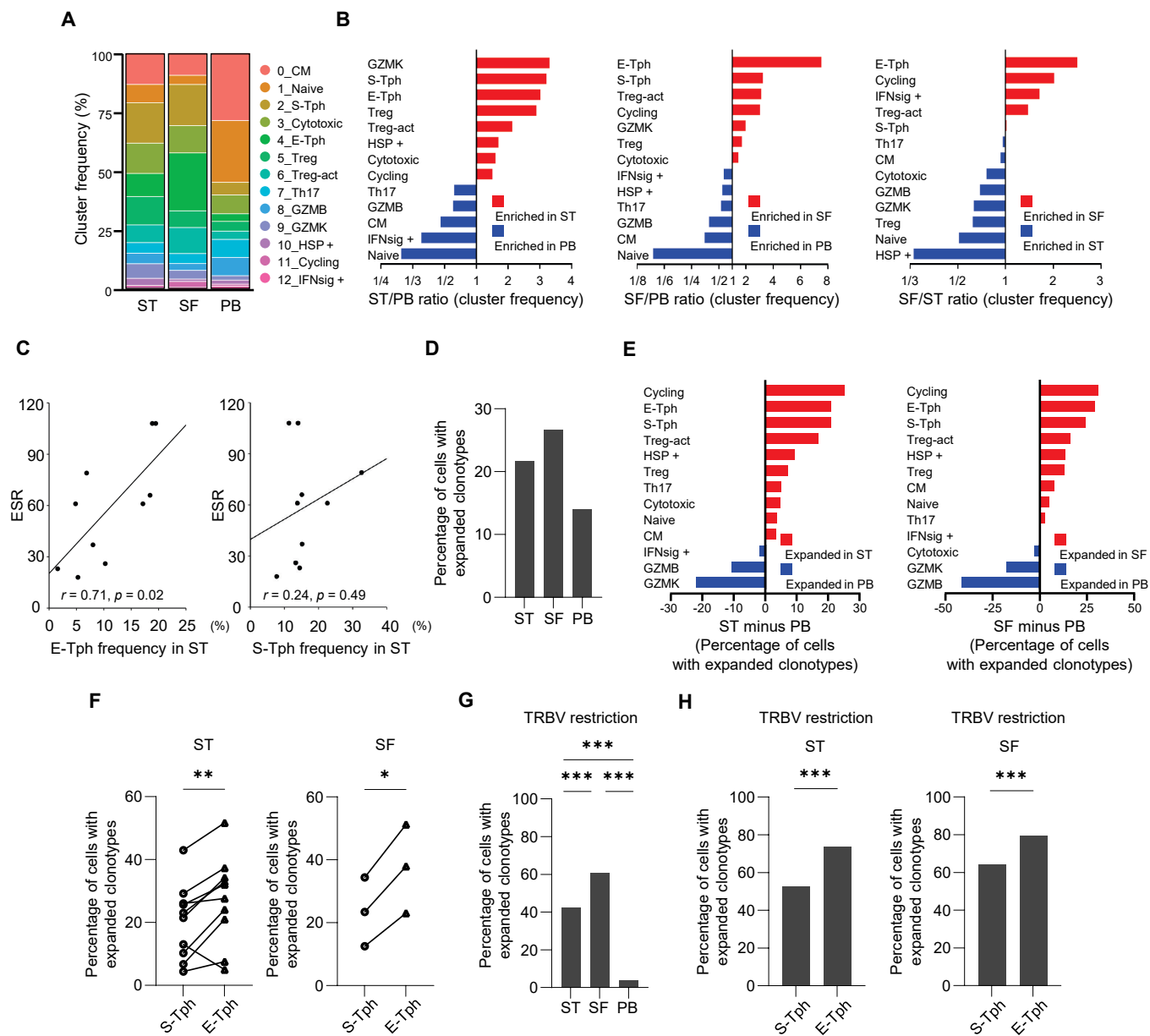


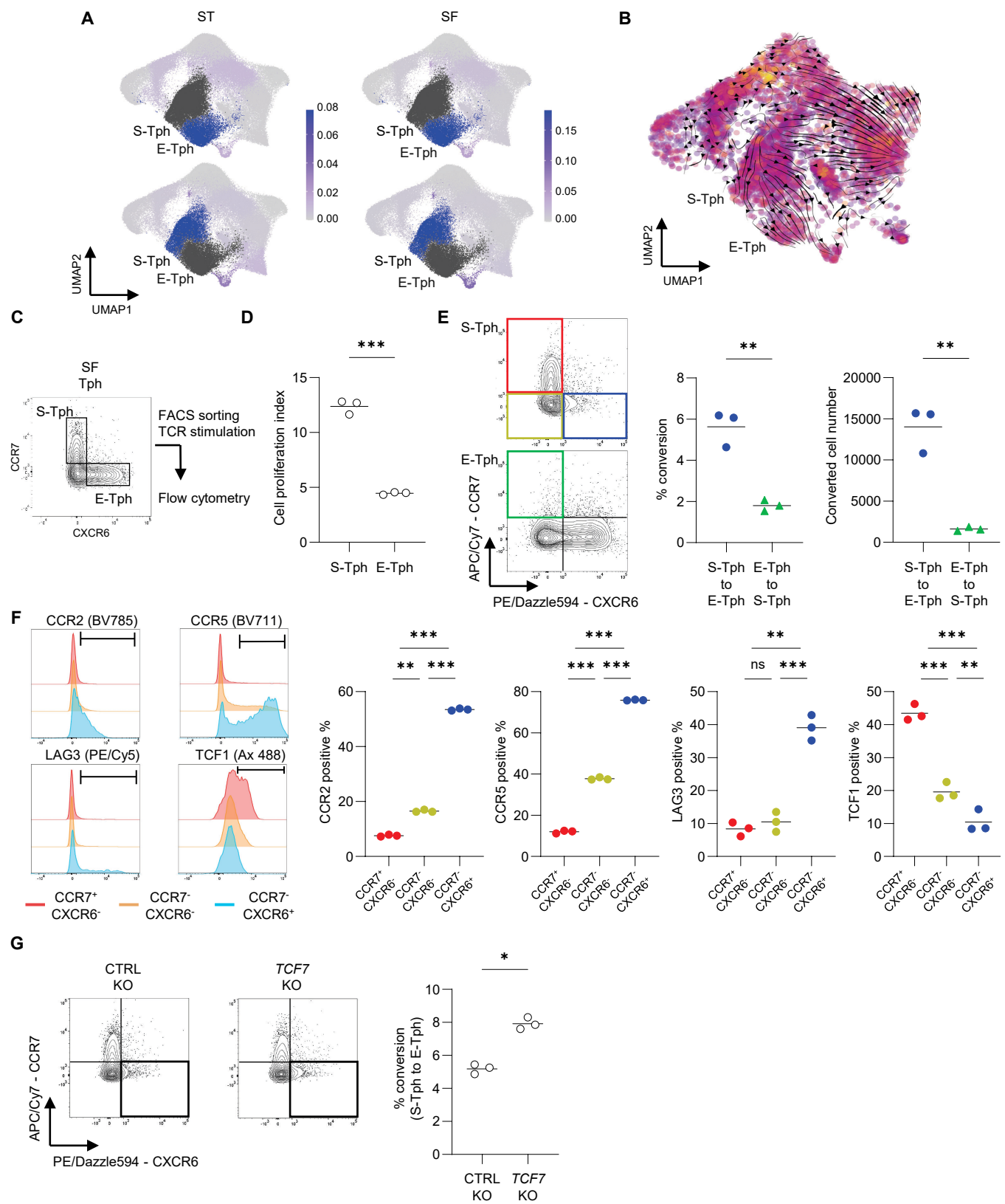
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Fig. 5

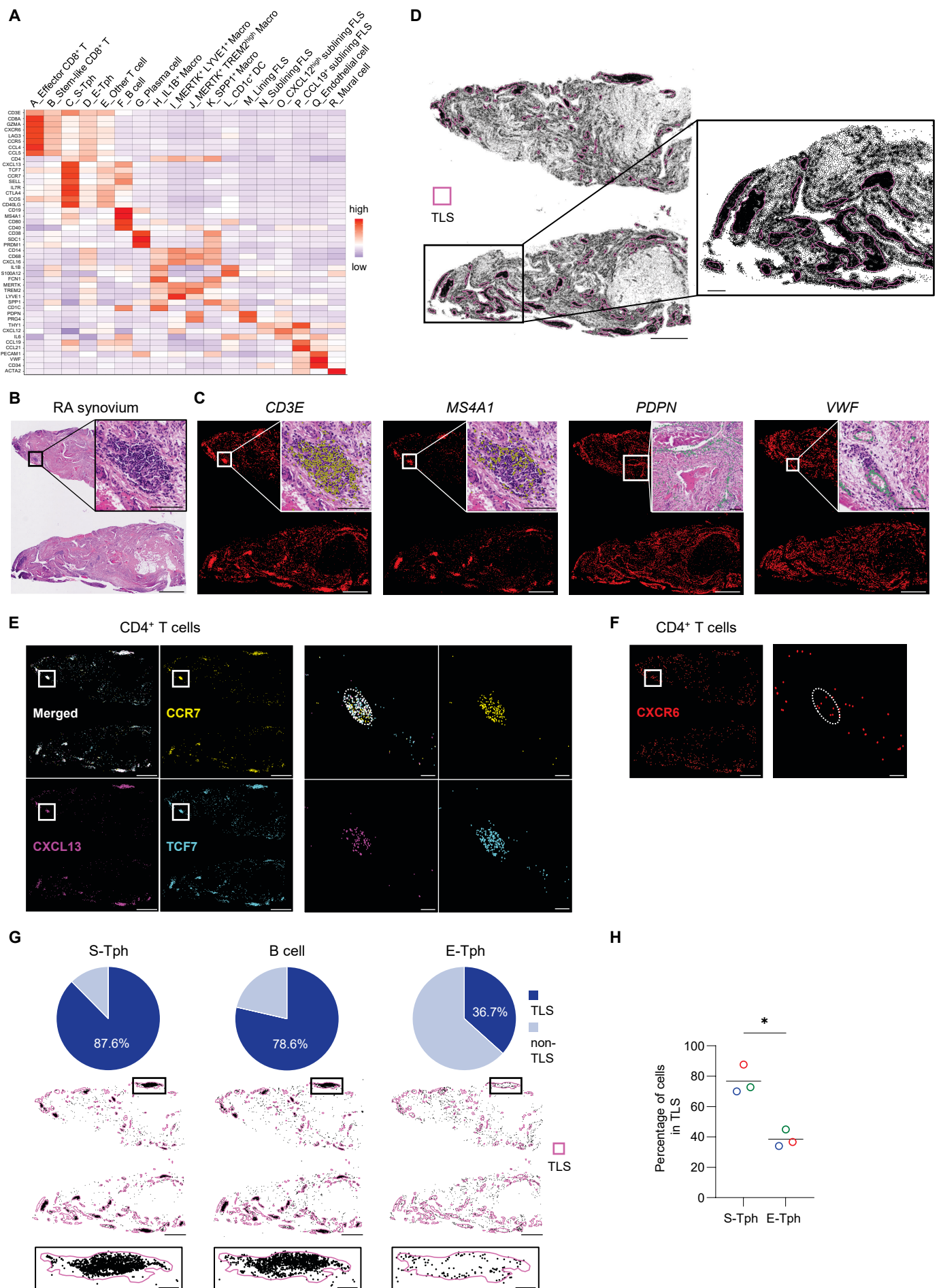


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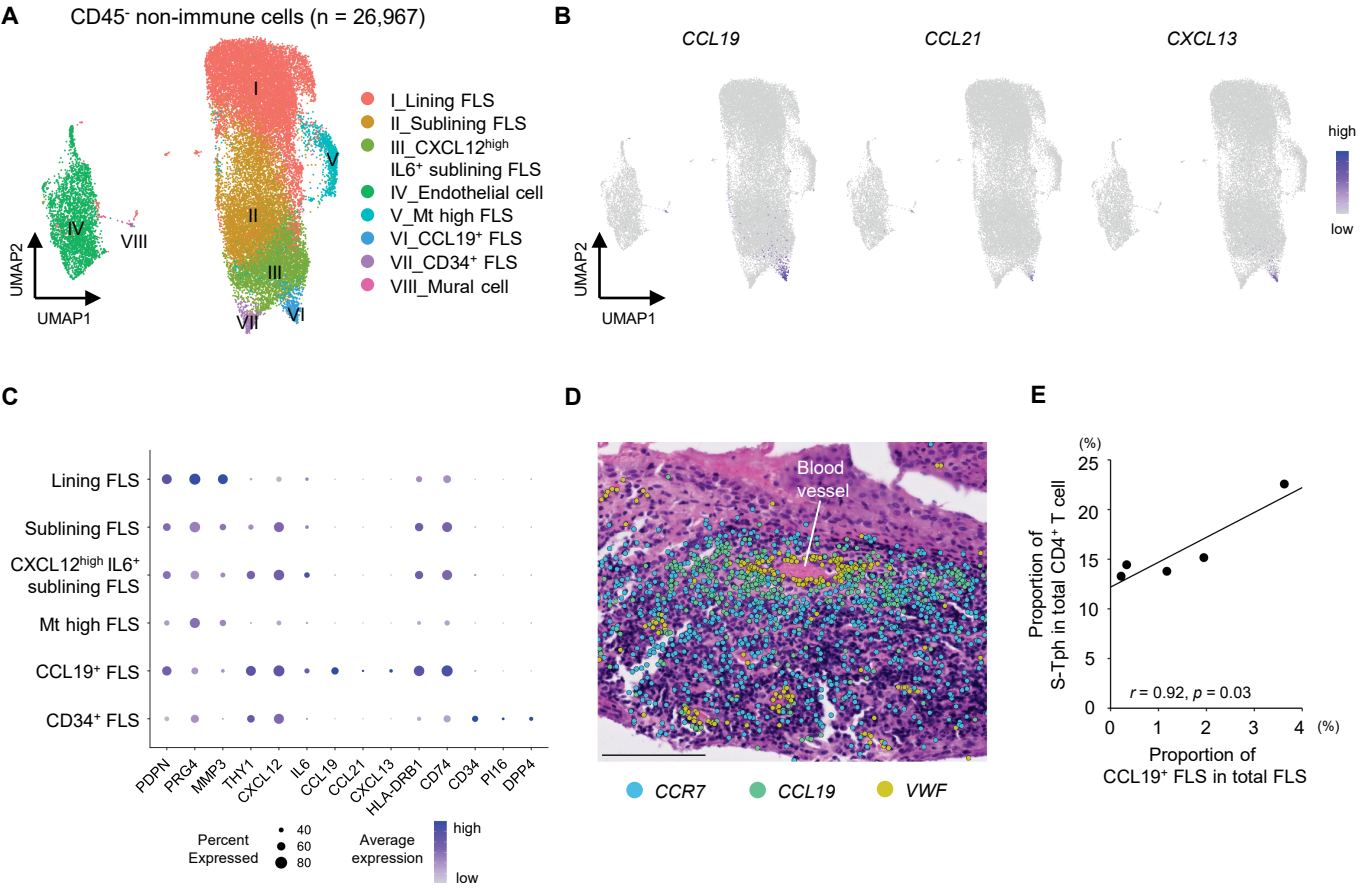


Fig. 7

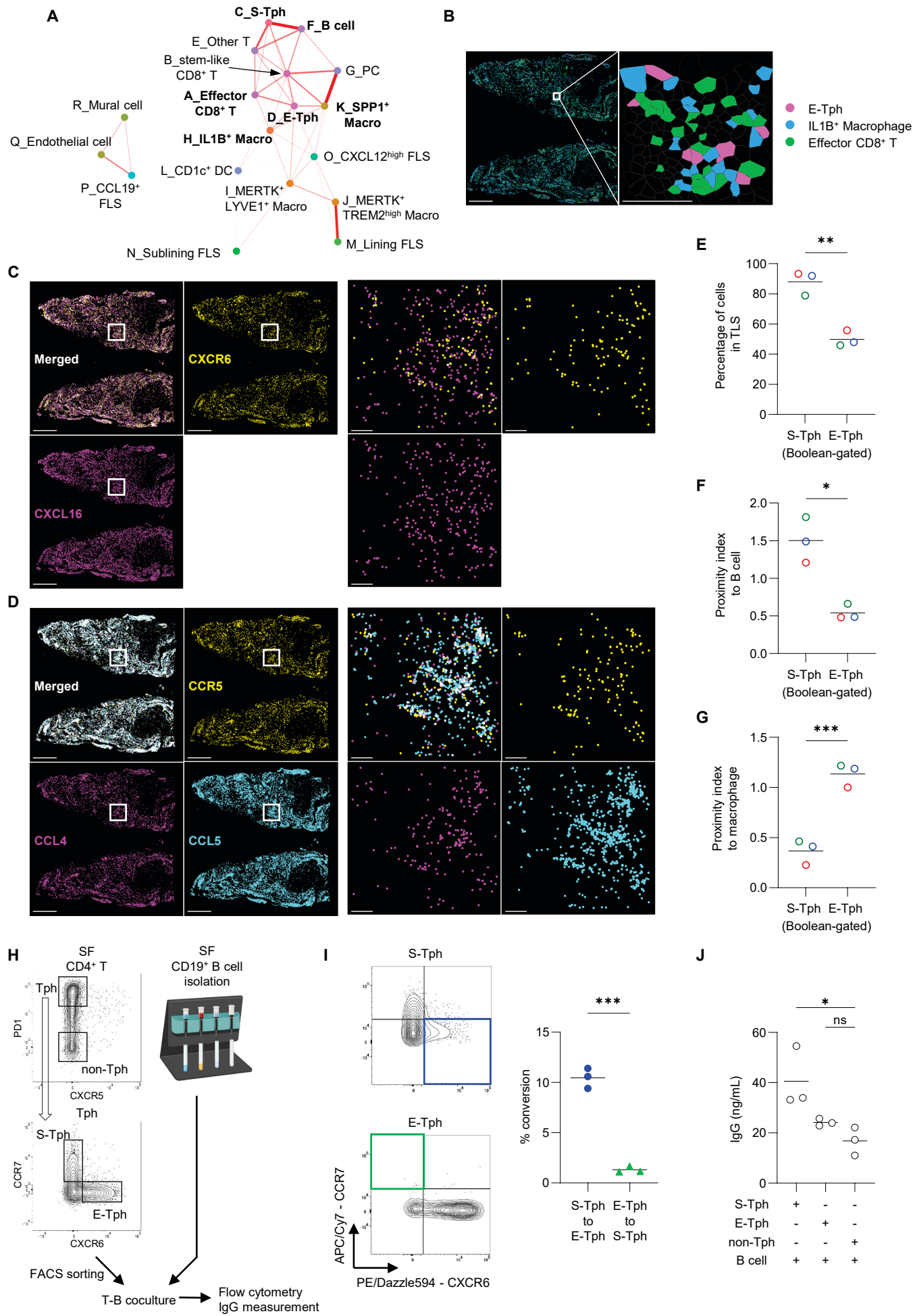


Fig. 8

