

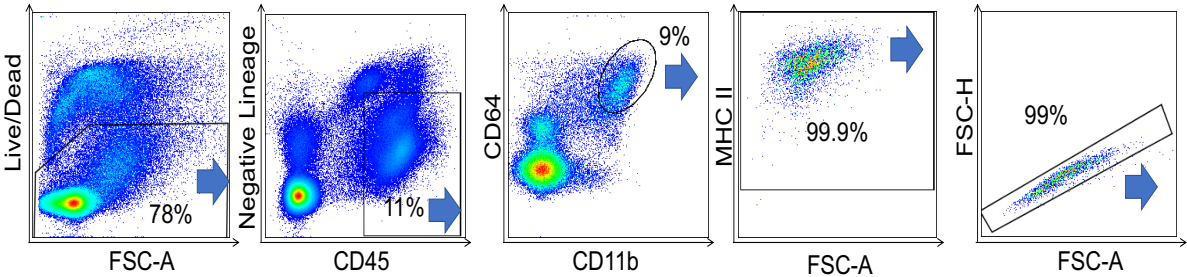
Supplementary Figure 1

a Details of antibodies used for STM phenotyping and for STMs and FLS sorting

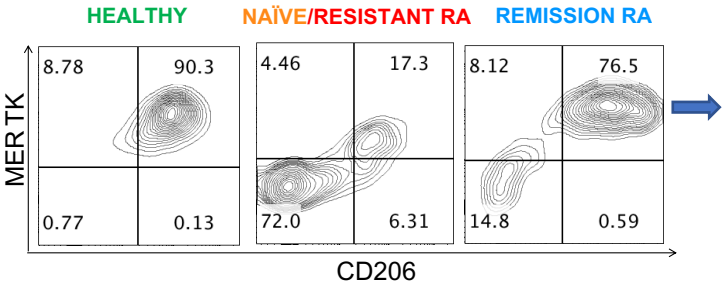
	Antibody	Cat No./ Source
1	Brilliant Violet 711 anti-human CD45 Antibody	304050/ Biolegend
2	Alexa Fluor 700 anti-mouse/human CD11b	101222/ Biolegend
3	Brilliant Violet 510 anti-human CD64	305028/ Biolegend
4	Brilliant Violet 421 anti-human CD206 (MMR)	321126/Biolegend
5	Brilliant Violet 785 anti-human HLA-DR	307642/Biolegend
6	PE anti-human MERTK	367608/Biolegend
7	PerCP/Cy5.5 anti-human CD163	326512/ Biolegend
8	APC anti-human Folate Receptor-β	391706/ Biolegend
9	PE-Cy7 recombinant-human TREM2	NPB1-07101/ Novus
10a	FITC anti-human CD15 (SSEA-1) dump channel	323004/ Biolegend
10b	FITC antihuman CD19 dump channel	302206/ Biolegend
10c	FITC anti-human CD117 (c-kit) dump channel	313232 Biolegend
10d	FITC antihuman CD3 dump channel	300440 Biolegend
10e	FITC anti-human CD56 (NCAM) dump channel	304604/Biolegend
10f	FITC anti-human CD1c dump channel	331518/Biolegend
10g	FITC anti-human CD20 dump channel	302304/Biolegend
10h	FITC anti-human CD21 dump channel	354910/Biolegend
11	PE-Cy7-anti-human TIM4	354005/Biolegend
12	APC-anti-human Podoplanin (NZ 1.3)	17-9381-42/Life Technologies

STM: synovial tissue macrophages, FLS: fibroblasts like synoviocytes

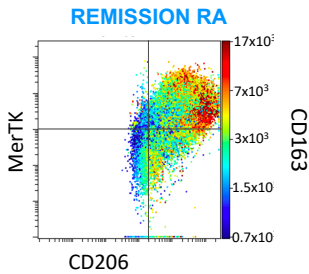
b



c

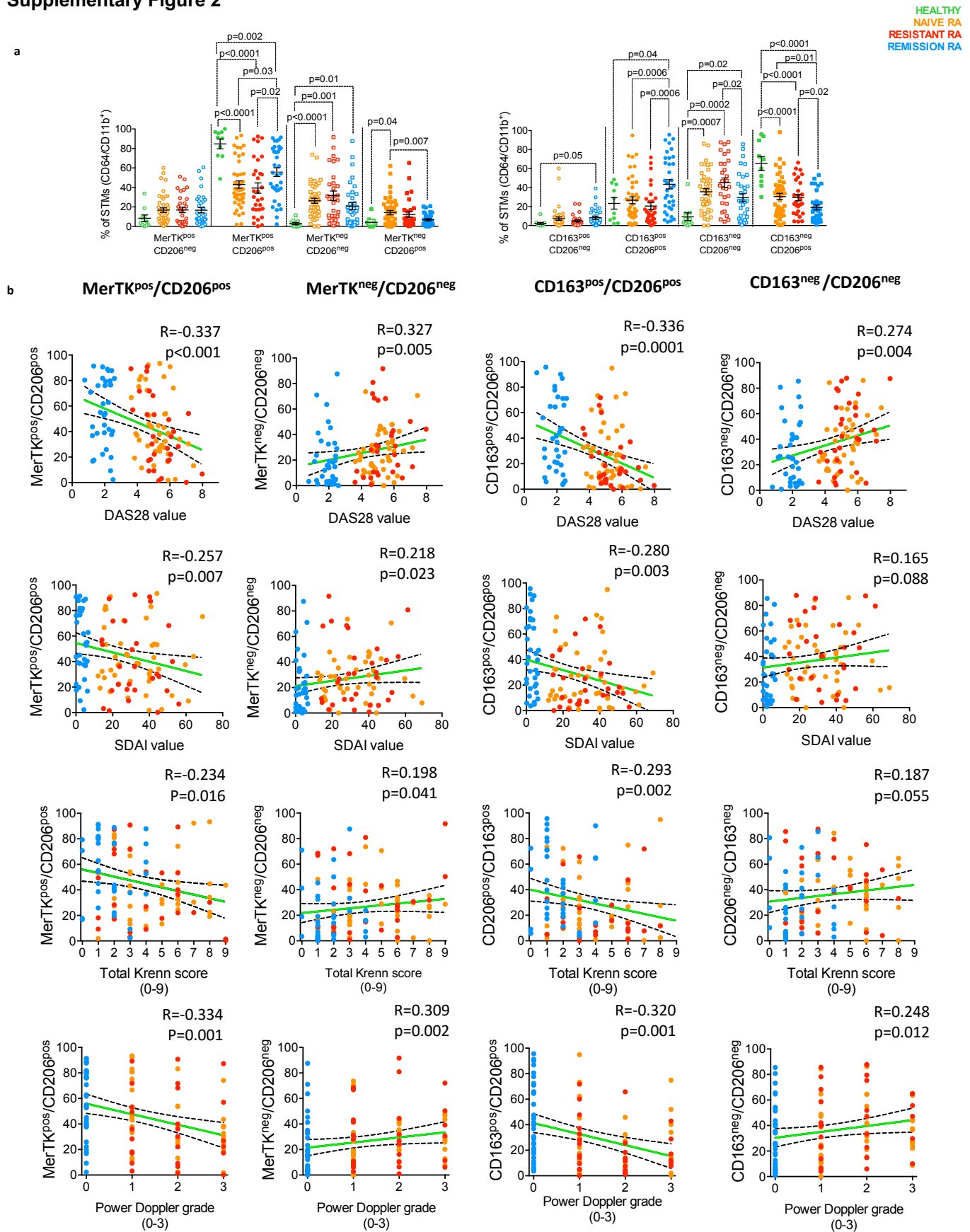


d



Supplementary Figure 1. Strategy for the analysis of STMs. (a) A list of antibodies used for gating STMs. (b) A gating strategy for STM analysis. (c) Representative expression of MerTK and CD206 on STMs from healthy, RA patients with active disease (naïve-to-treatment or resistant-to-treatment) and patients in disease remission. (d) Illustration (Cytobank software) of CD163 expression on STM populations defined by MerTK/CD206 expression showing that CD163 is exclusively expressed on MerTK/CD206^{pos}. CD163 expression is presented as arbitrary units.

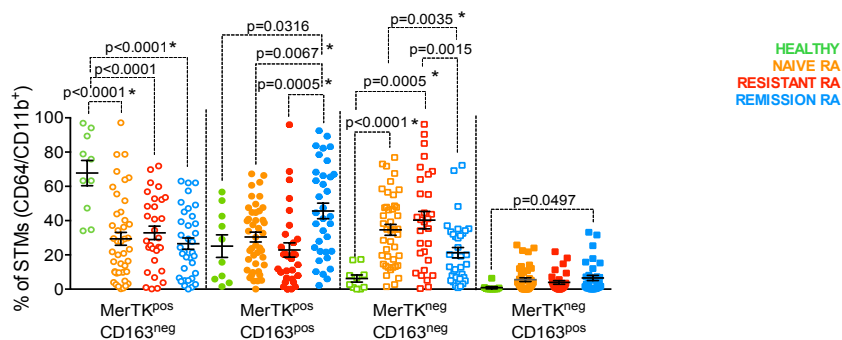
Supplementary Figure 2



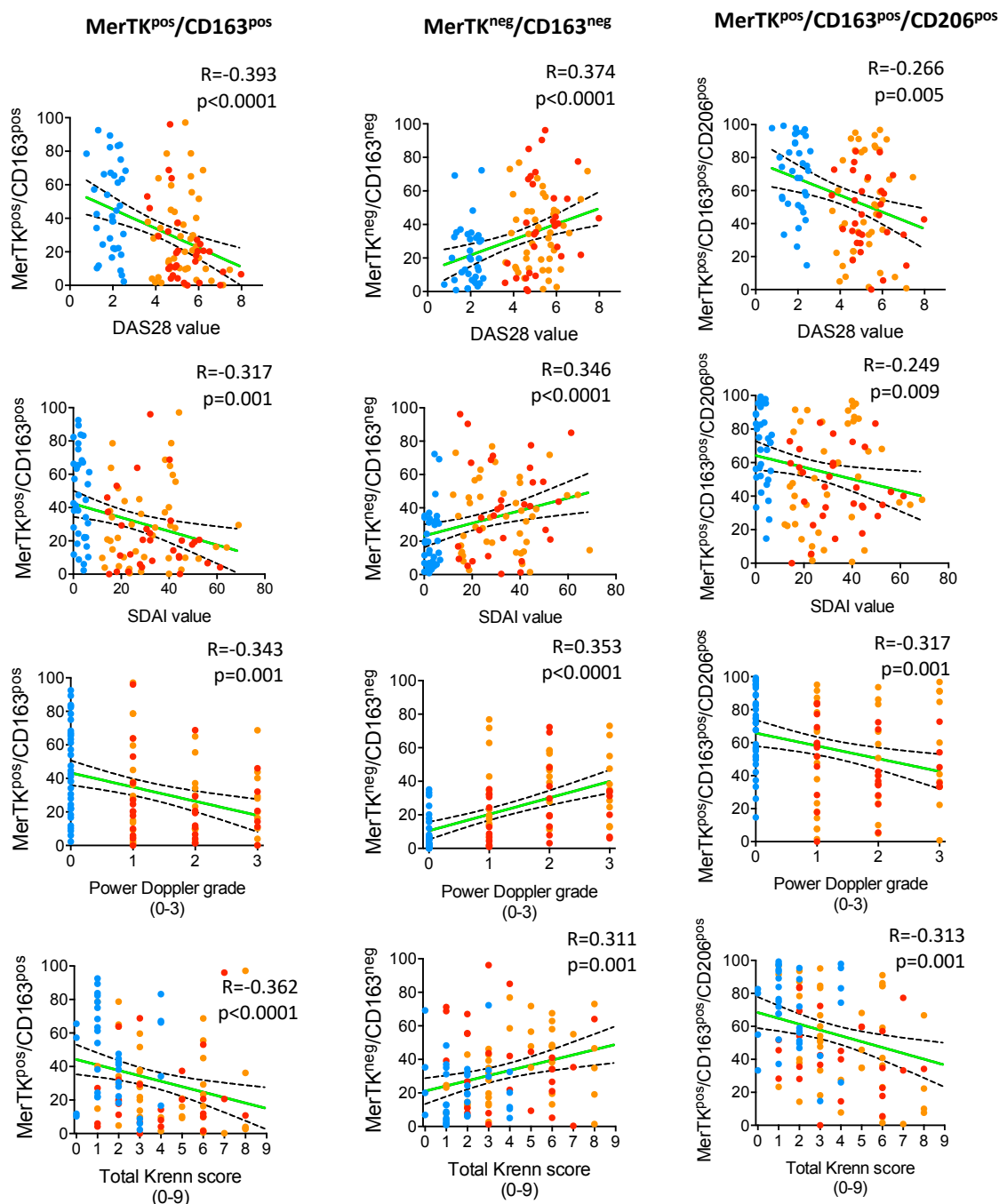
Supplementary Figure 2. Distribution of different STM populations in healthy and RA synovium and their correlations with clinical parameters. Healthy donors (n=10) are shown in green, treatment-naïve RA are shown in orange (n=43), treatment-resistant RA are shown in red (n=30) and RA in sustained clinical and ultrasound remission are shown in blue (n=36). **(a)** Distribution of distinct STM populations defined by MerTK, CD163 and CD206 is presented. **(b)** Spearman correlation analyses between Disease Activity Score over 28 joints (DAS28-CRP) or semiquantitative synovitis score (Krenn score) or Simplified Disease Activity Index (SDAI) or power doppler grade and the frequencies of RA MerTK/CD206^{pos}, MerTK/CD206^{neg}, CD163/CD206^{pos}, CD163/CD206^{neg} cells are presented. Data in (a) are mean $\pm$ s.e.m.; p-values are provided on the graphs or marked with * (≤ 0.05). The difference in individual STM populations between different joint conditions were evaluated using one-way ANOVA with Tukey correction for multiple comparison or two-tailed nonparametric unpaired Mann-Whitney test if 2 groups were compared.

Supplementary Figure 3

a

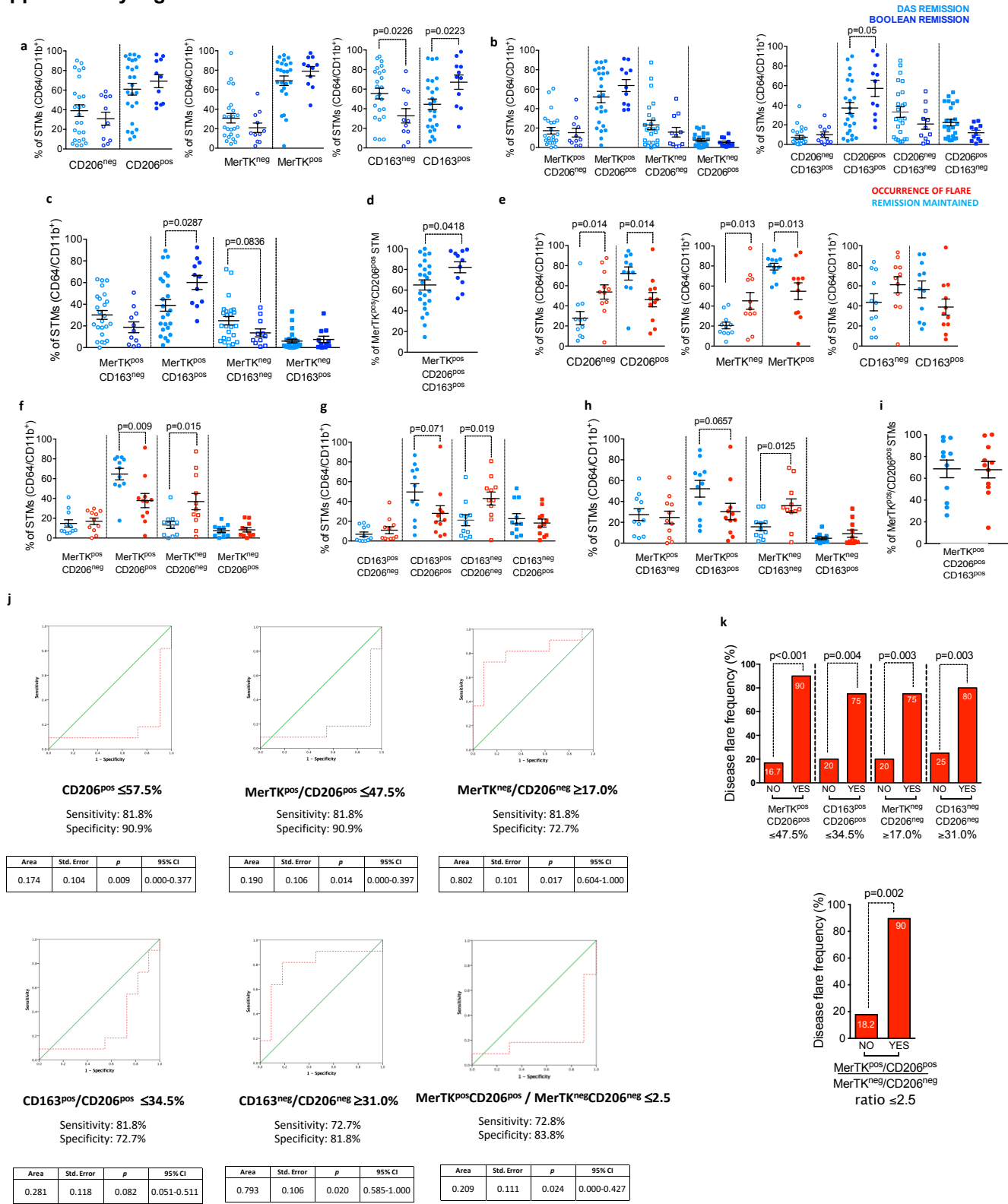


b



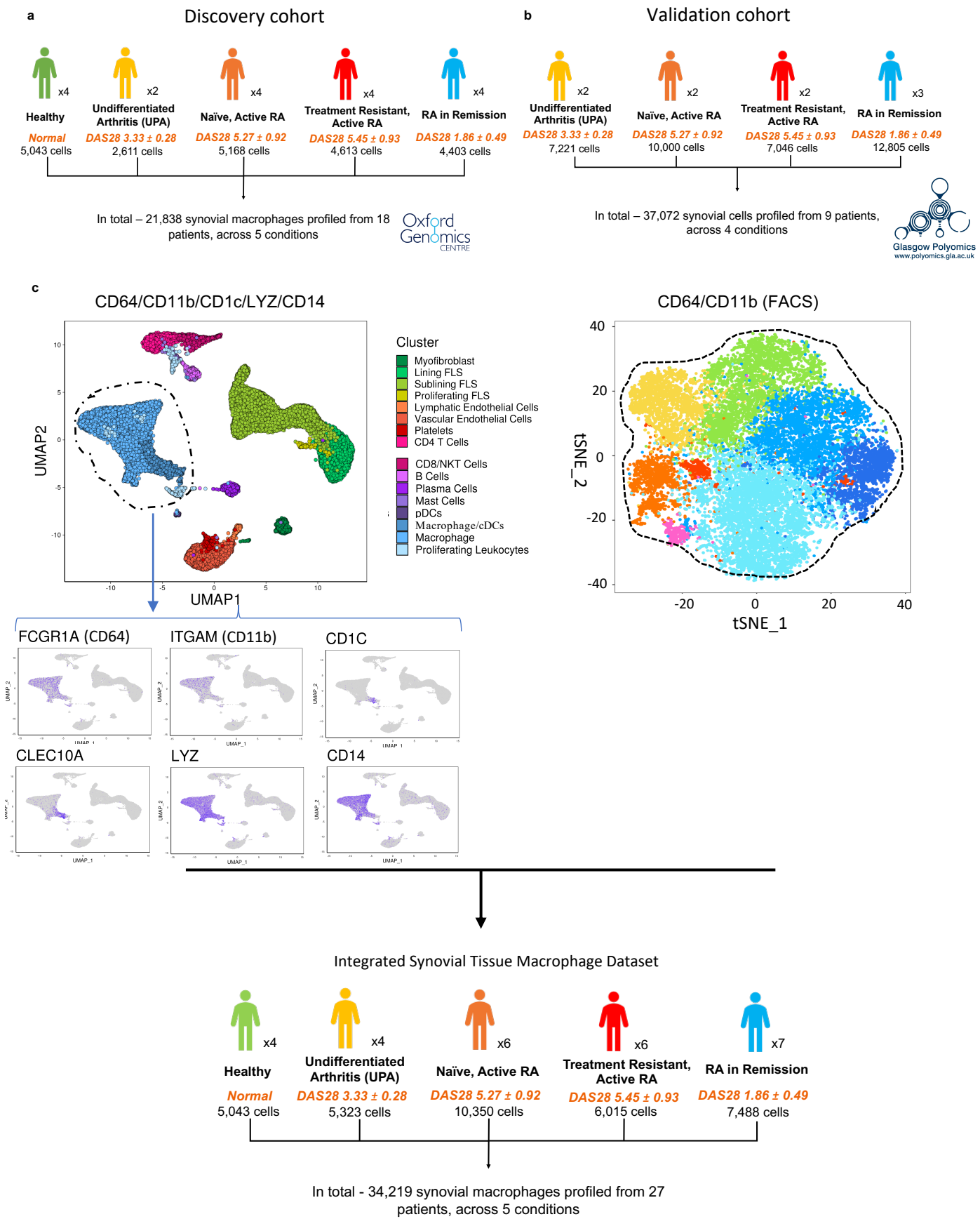
Supplementary Figure 3. Distribution of MerTK/CD163 positive and negative STM populations in healthy and RA synovium and their correlations with clinical parameters. Healthy donors (n=10) are shown in green, treatment-naïve RA are shown in orange (n=43), treatment-resistant RA are shown in red (n=30) and RA in sustained clinical and ultrasound remission are shown in blue (n=36). **(a)** Distribution of STM populations defined by MerTK, CD163 and CD206 is presented. **(b)** Spearman correlation analyses between Disease Activity Score over 28 joints (DAS28-CRP) or semiquantitative synovitis score (Krenn score) or Simplified Disease Activity Index (SDAI) or power doppler grade and the frequencies of RA MerTK/CD163^{pos}, MerTK/CD163^{neg}, and MerTK/CD163/CD206^{pos} are presented. Data in (a) are mean $\pm$ s.e.m.; p-values are provided on the graphs or marked with * (≤ 0.05). The difference in individual STM populations between different joint conditions were evaluated using one-way ANOVA with Tukey correction for multiple comparison or two-tailed nonparametric unpaired Mann-Whitney test if 2 groups were compared.

Supplementary Figure 4



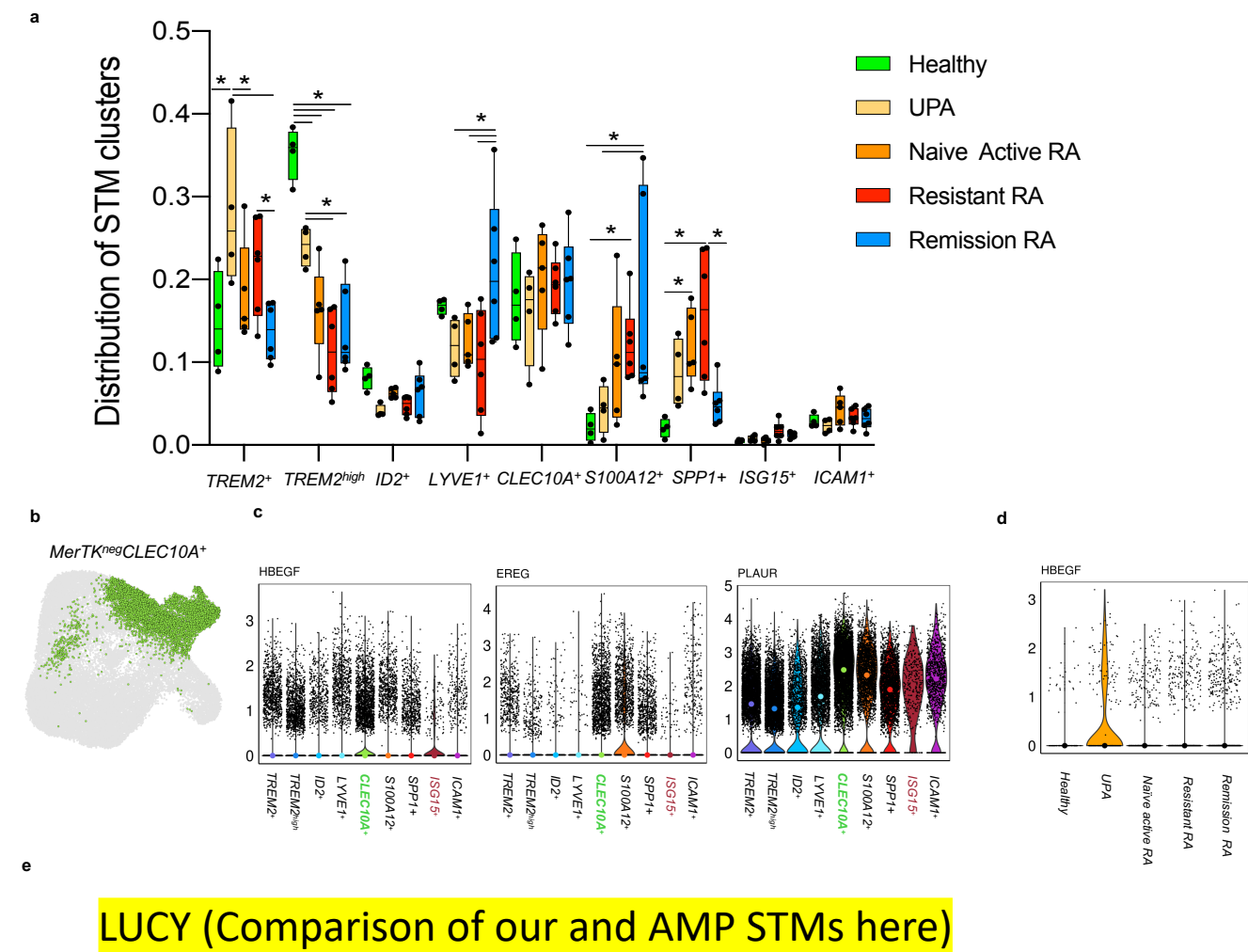
Supplementary Figure 4. Distinct distribution of STM populations defined by CD163, MerTK and CD206 between different types of remission. (a-d) Comparison of STMs distribution between patients with disease remission defined by DAS28 (n=24 RA patients) and those who met Boolean remission criteria (n=11). The analyses include single marker positive or negative STMs in (a), MerTK/CD206, CD163/CD206 positive and negative STMs in (b), MerTK/CD163 positive and negative STMs in (c) and MerTK/CD163/CD206^{pos} STMs in (d). Comparison of STMs distribution between RA patients in sustained clinical and US remission who flared (n=11) or maintained in disease remission (n=11) after treatment discontinuation. The analyses include CD163, MerTK and CD206 single marker positive and negative STMs in (e), MerTK/CD206 positive and negative STMs in (f), CD163/CD206 positive and negative STMs in (g), MerTK/CD163 positive and negative STMs in (h) and MerTK/CD163/CD206^{pos} STMs in (i). (j) ROC curves for cut-off values of distribution data for CD206^{pos}, MerTK/CD206^{pos}, MerTK/CD206^{neg}, CD163/CD206^{pos}, CD163/CD206^{neg} STM and MerTK/CD206^{pos} to MerTK/CD206^{neg} ratio in patients described in e-i. (k) Comparison of frequencies of occurrence of flare in RA patients who discontinued treatment based on STM distribution. Patients are stratified on the bases of the odds ratio cut-off value for different STM populations. Data in (a-i) are mean ± s.e.m.; p value are provided on the graphs. The difference in individual STM populations between different remission states were evaluated using two-tailed nonparametric unpaired Mann-Whitney test.

Supplementary Figure 5

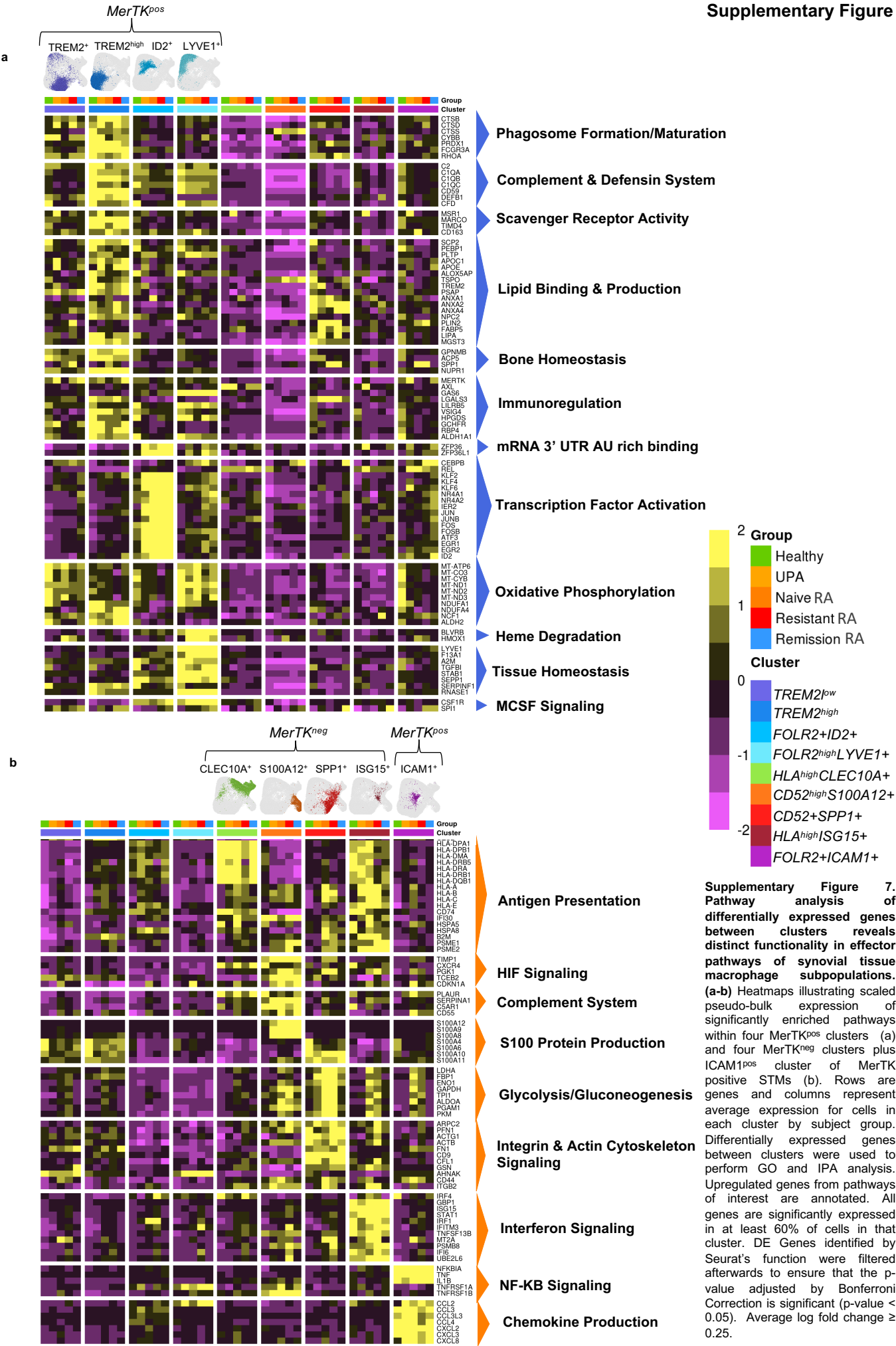


Supplementary Figure 5. Integration of scRNAseq data. (a) Metadata for CD64/CD11b positive synovial tissue macrophages sequenced in the Discovery Cohort at Oxford Genomics Centre. (b) Metadata for all synovial cell types sequenced in the Validation Cohort at Glasgow Polyomics. (c) Myeloid cells sequenced in the Validation cohort are separated computationally (based on positive expression of CD14, MARCO and LYZ) and integrated with synovial macrophages sequenced in the Discovery cohort.

Supplementary Figure 6



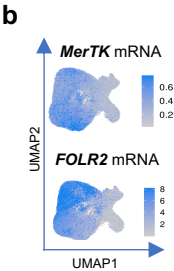
Supplementary Figure 6. Changes in the STMs clusters in health, synovitis and resolution of synovitis (a) Bar plot illustrating the change in cluster distribution (clusters are ordered here as in the heatmap on Figure 2b) across conditions. Statistical differences are highlighted and were calculated by two-way ANOVA with correction for multiple comparisons (Tukey test). (b) CLEC10A-positive STM cluster localization on the STM UMAP. (c) Expression of the top markers of HBEGF positive synovial myeloid cells described by Kuo et al. (2019) indicating that this population might be embedded in the MerTK-negative CLEC10A and ISG15 positive synovial tissue resident dendritic cells. (d) Expression of HBEGF is increased in CLEC10A positive cells in early undifferentiated arthritis. (e) Comparison of single-cell transcriptional profiling of our human STM with synovial myeloid cells published by Zhang et al. (Nat Immunol 2019)



Supplementary Figure 8 (FACS representative plot per marker per condition)

a Details of antibodies used for validation of scRNAseq STM clusters with flow cytometry

Antibody	Clone	Supplier	Cat num	Concentration	Panel
PE anti-human MERTK Antibody	590H11G1E3	Biologend	367608	1:100	1 (MerTK pos)
Anti-Human TREM-2 Antibody (2B5) [PE/Cy7]	2B5	Novus Biologicals	NBP1-07101PECY7	1:100	1 (MerTK pos)
LYVE-1 Antibody (ALY7) [Alexa Fluor® 405]	ALY7	Novus Biologicals	NBP1-43411AF405	1:50	1 (MerTK pos)
PE/Cy7 anti-human MERTK	590H11G1E3	Biologend	367610	1:100	2 (MerTK neg)
PE anti-human CD301 (CLEC10A)	H037G3	Biologend	354704	1:100	2 (MerTK neg)
Biotin anti-human CD9	HI9a	Biologend	312112	1:500	2 (MerTK neg)
Brilliant Violet 421™ Streptavidin	-	Biologend	405226	1:500	2 (MerTK neg)
APC anti-human Folate Receptor β (FR-β)	94b	Biologend	391706	1:200	Both
PE-CF594 Mouse Anti-Human CD48	TU145	BDBiosciences	562717	1:100	Both
PerCP/Cy5.5 anti-human CD206 (MMR)	15-2	Biologend	321122	1:100	Both

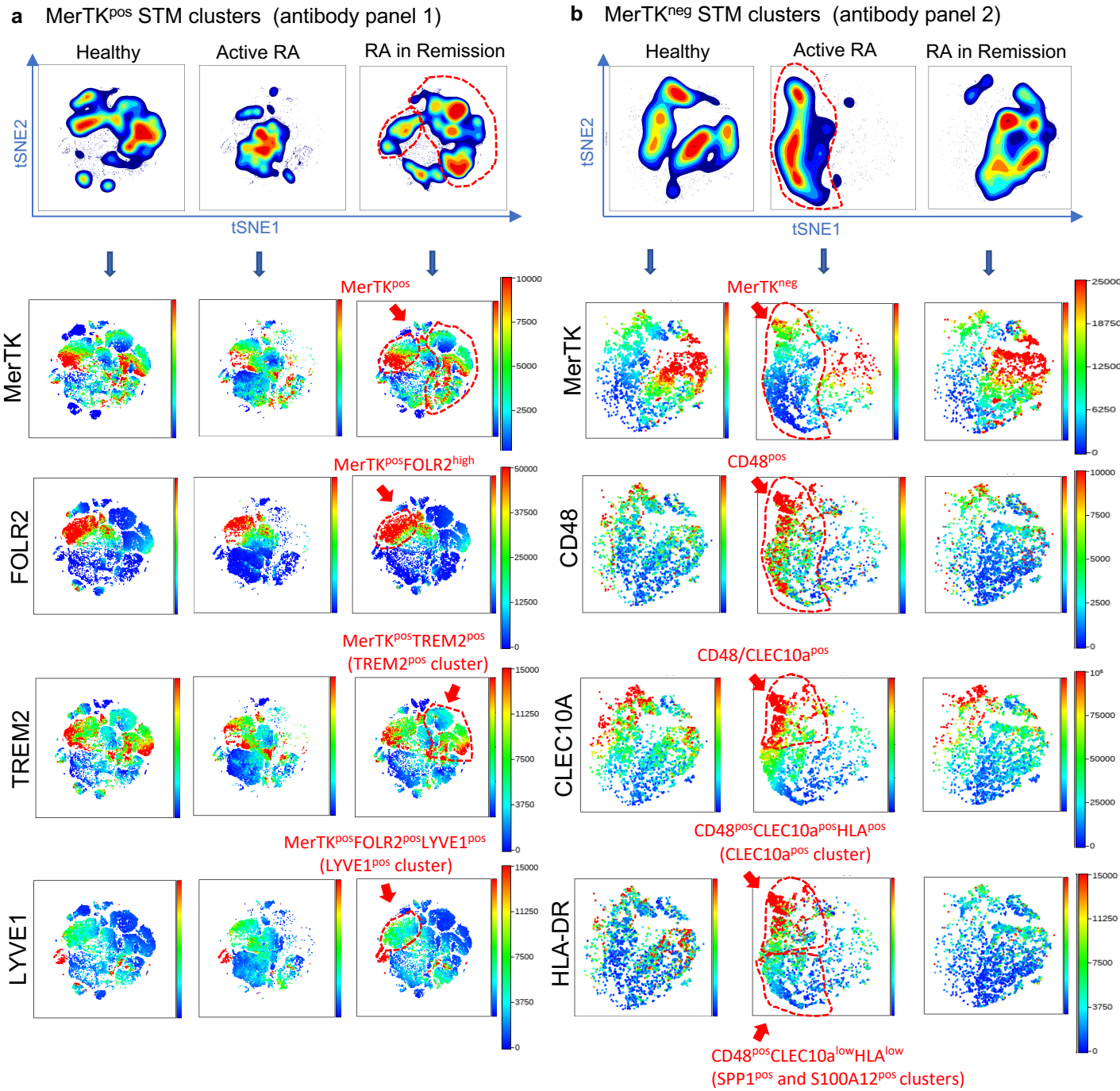


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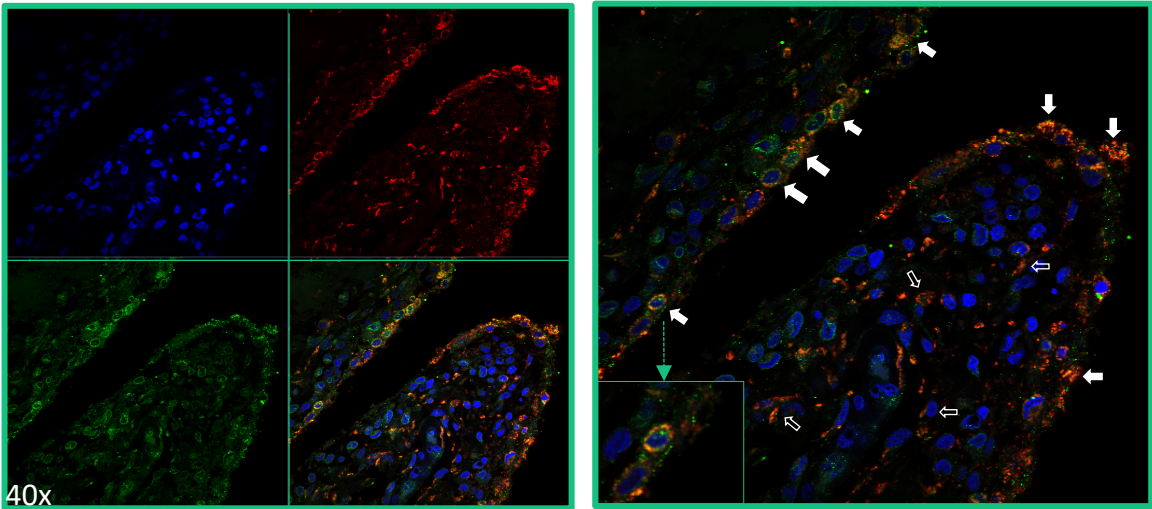
Supplementary Figure 8. Validation of scRNAseq identified STM clusters with flow cytometry. (a) Antibody panel 1 used in conjunction with dump panel described in Suppl Fig.1a to identify MerTK positive clusters, and Antibody panel 2 used in conjunction with dump panel to identify MerTK negative clusters. (b) scRNAseq data shows that mRNA expression of MerTK and FOLR2 overlaps. (c-d) Single cell synovial tissue digests from n=8-10 healthy controls and n=12-17 patients with active RA and n=7-14 patients in disease remission were stained with antibody panel 1 or panel 2. (c) Representative gating strategy for TREM2, FOLR2, LYVE1 positive STMs in conjunction with MerTK expression in health, active RA and RA in disease remission. The TREM2^{pos} cluster is defined by the positive expression of MerTK and TREM2, and the LYVE1^{pos} cluster is defined by the positive expression of LYVE1, MerTK and high expression of FOLR2. (d) Representative gating strategy for CD48, CD9 and CLEC10a in conjunction with MerTK expression in health, active RA and RA in disease remission is shown. MerTK^{neg}S100A12^{pos} cluster is defined by CD48^{pos}/CD9^{neg}/CLEC10a^{neg} ; MerTK^{neg}SPP1^{pos} cluster is defined by CD48^{pos}/CD9^{pos}/CLEC10a^{neg} and MerTK^{neg}CLEC10a^{pos} cluster is defined by CD48^{pos}/CD9^{pos}/CLEC10a^{pos}.

Supplementary Figure 9

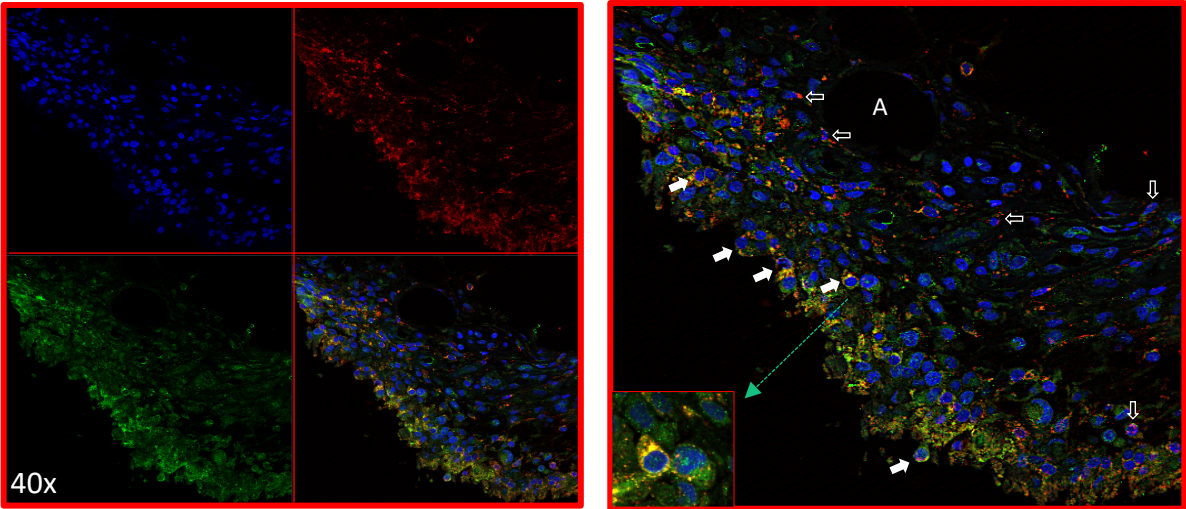


Supplementary Figure 9. STMs in healthy and RA in sustained disease remission show enrichment in MerTK positive clusters (TREM2^{pos} and FOLR2^{high}LYVE1^{pos}) while patients with active RA show a decrease in these clusters and an increase in MerTK negative clusters (CLEC10a^{pos} and SPP1^{pos} and S100A12^{pos} clusters). a-b, Single cell synovial tissue digests from n=10 healthy controls and n=12 patients with RA in disease remission and n=14 patients with active RA were stained with a panel 1 of 9 antibodies (plus dump panel) to identify MerTK positive clusters (a) or with a panel 2 of 9 antibodies (plus dump panel) to identify MerTK negative clusters (b). viSNE plots of clustered total STMs (CD64^{pos}/CD11b^{pos}/lineage^{neg}) are displayed for MerTK positive and MerTK negative STMs, showing cell density of clusters and changes between conditions. The number of cell per condition were normalized to 25K. Bars represent individual expression scale for each marker.

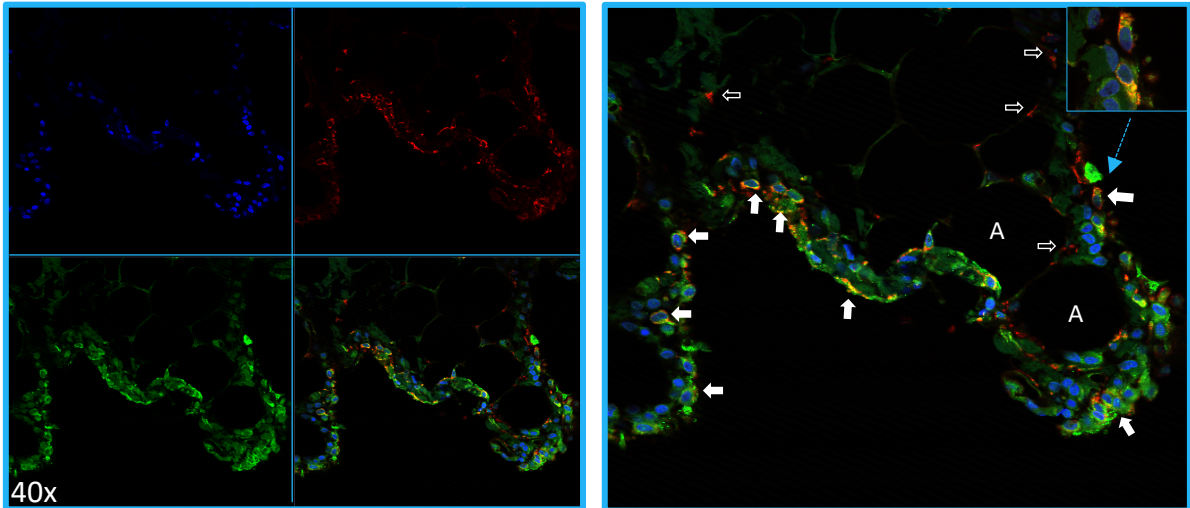
a Healthy (TREM2/CD68/Nuclei)



b Active (TREM2/CD68/Nuclei)

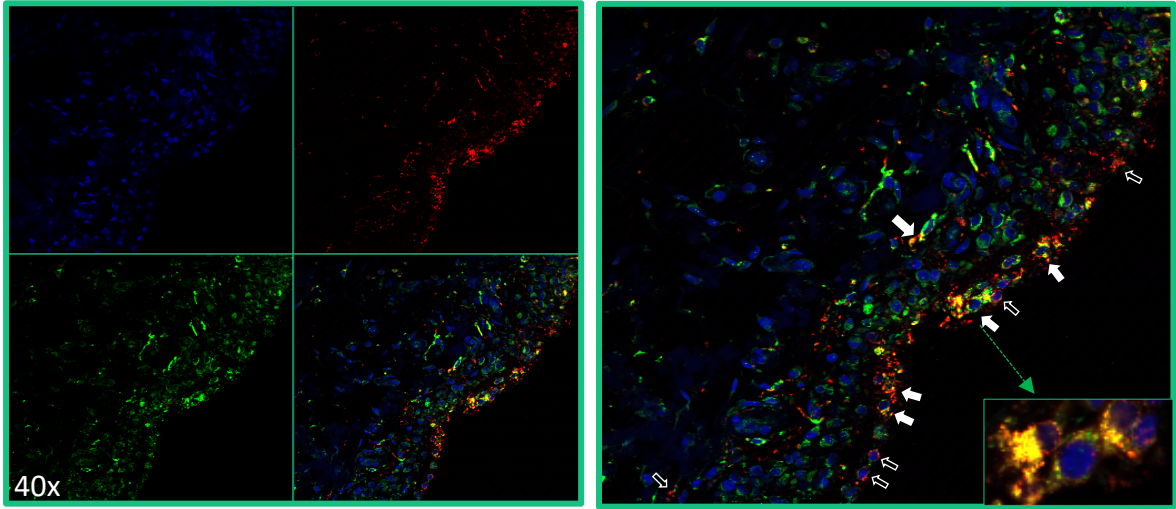


c Remission (TREM2/CD68/Nuclei)

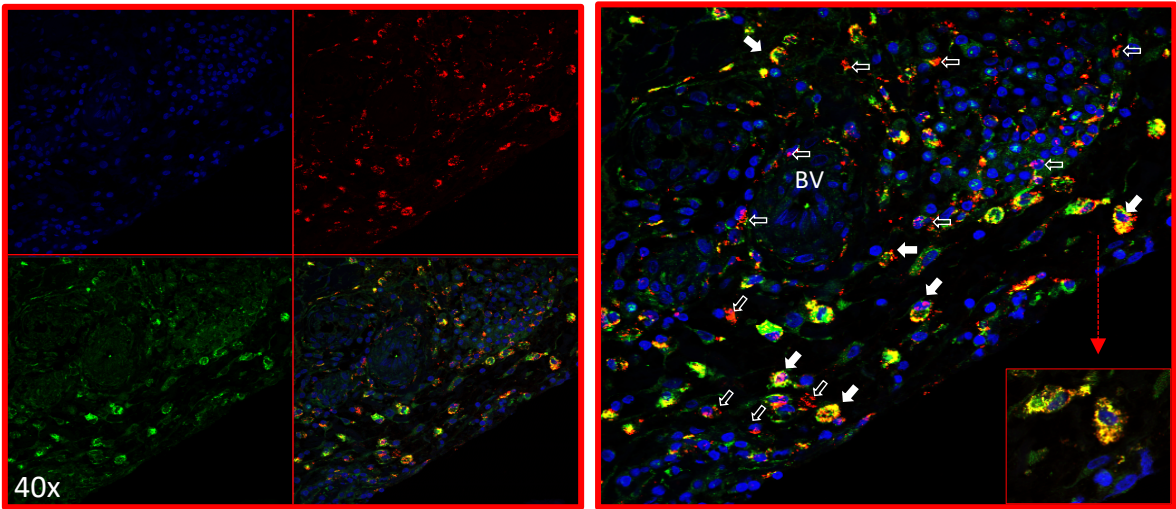


Supplementary Figure 10. TREM2 positive STMs form lining layer in healthy synovium and in synovium from RA patients in sustained disease remission. Representative confocal microscopy pictures of TREM2 (green) and macrophage marker staining (CD68, red) in healthy synovium (a), synovium from patients with active RA (b) and RA in remission (c), showing double positive (TREM2/CD68⁺) macrophages and TREM2 negative CD68⁺ macrophages. These pictures are representative of n=3 healthy, n=6 active RA and n=6 RA in remission **synovial tissues**. The solid white arrows indicate double TREM2/CD68⁺ macrophages while hollow arrows indicate TREM2 negative macrophages (CD68⁺). The corner pictures show double positive cells under 63x magnification. The nuclei are stained with DAPI (blue). A, adipocyte.

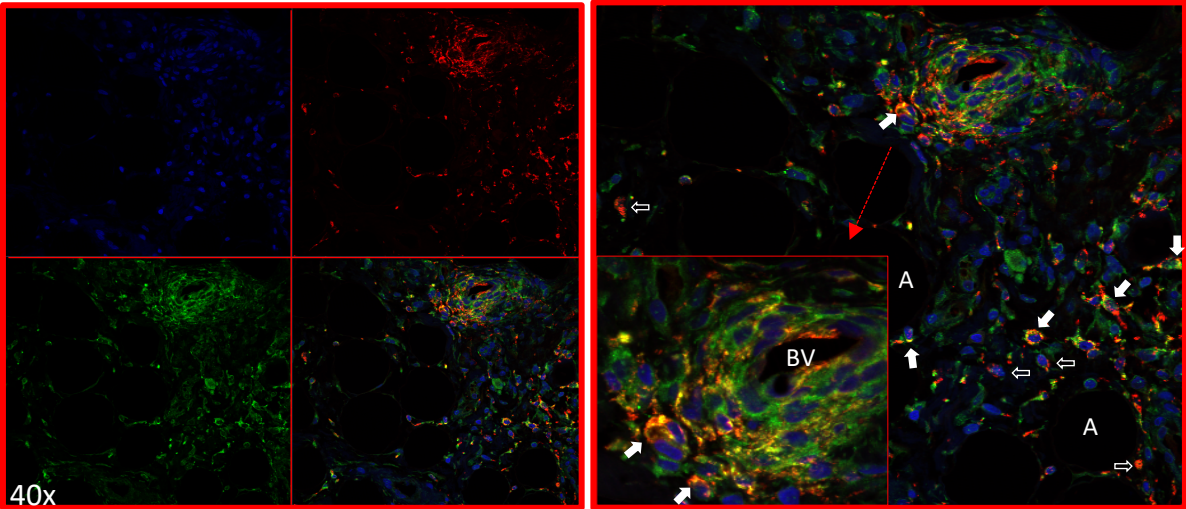
a Healthy (LYVE1/CD68/Nuclei)



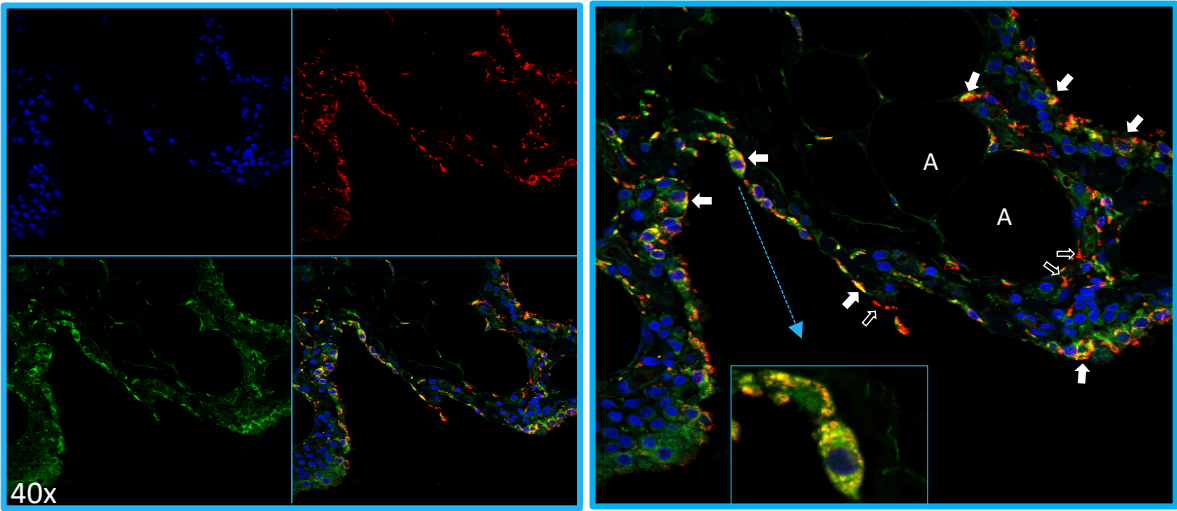
b Active ⌘ 1 (LYVE1/CD68/Nuclei)



c Active ⌘ 2 (LYVE1/CD68/Nuclei)

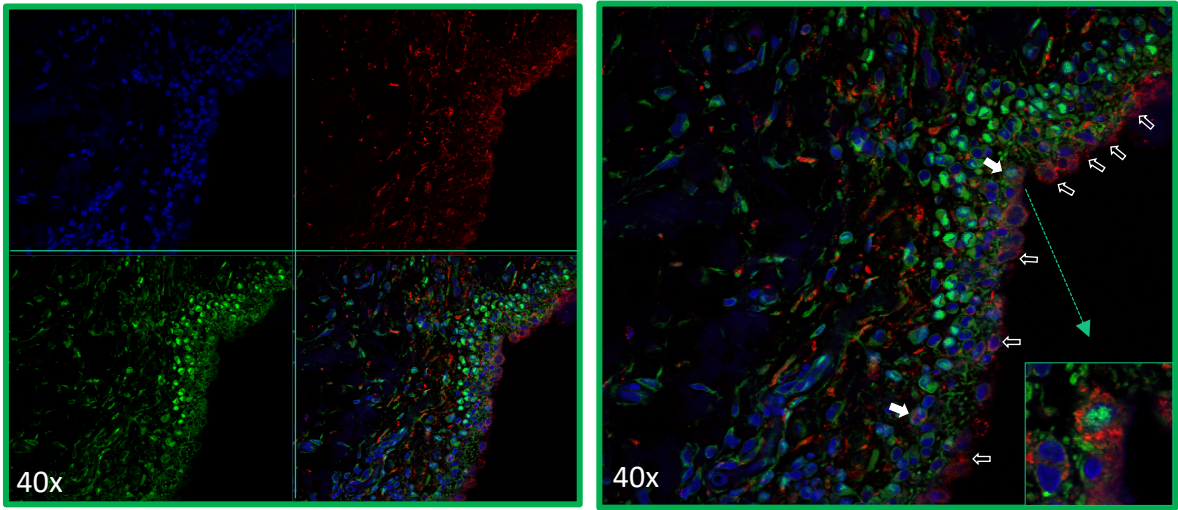


c Remission (LYVE1/CD68/Nuclei)

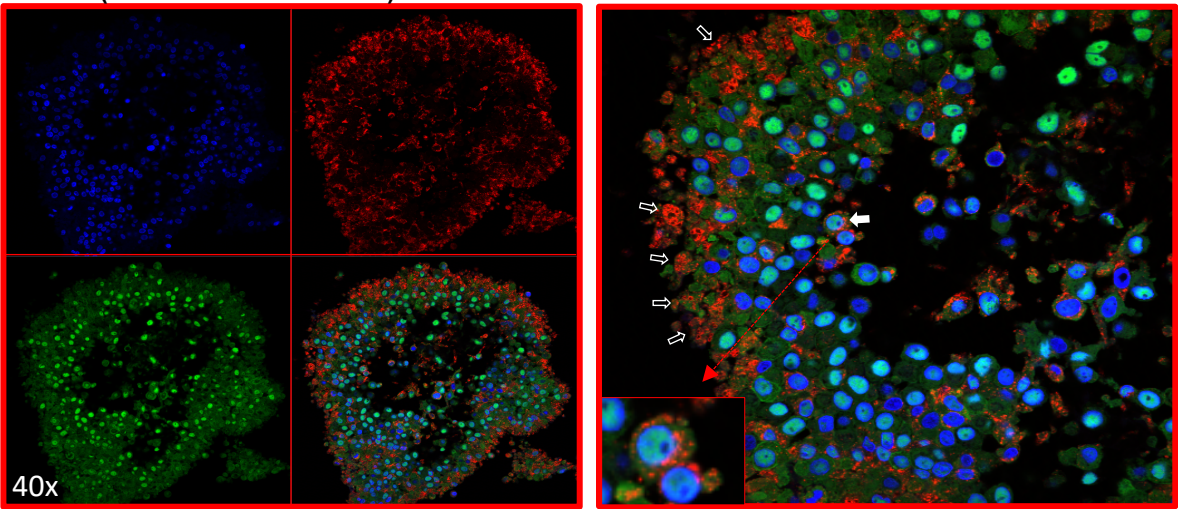


Supplementary Figure 11. LYVE1 positive STMs are present in lining layer and interstitial space in healthy and RA in remission synovium while mostly in interstitial space in active RA synovium. Representative confocal microscopy pictures of LYVE1 (green) and macrophage marker staining (CD68, red) in healthy synovium (a), synovium from patients with active RA (b-c) and RA in remission (d), showing double positive (LYVE1/CD68⁺) macrophages and LYVE1 negative CD68⁺ macrophages. These pictures are representative of n=3 healthy, n=6 active RA and n=6 RA in remission synovial tissues. The solid white arrows indicate double LYVE1/CD68⁺ macrophages while hollow arrows indicate LYVE1 negative macrophages (CD68⁺). The corner pictures show double positive cells under 63x magnification. The nuclei are stained with DAPI (blue). A, adipocyte; BV, blood vessel.

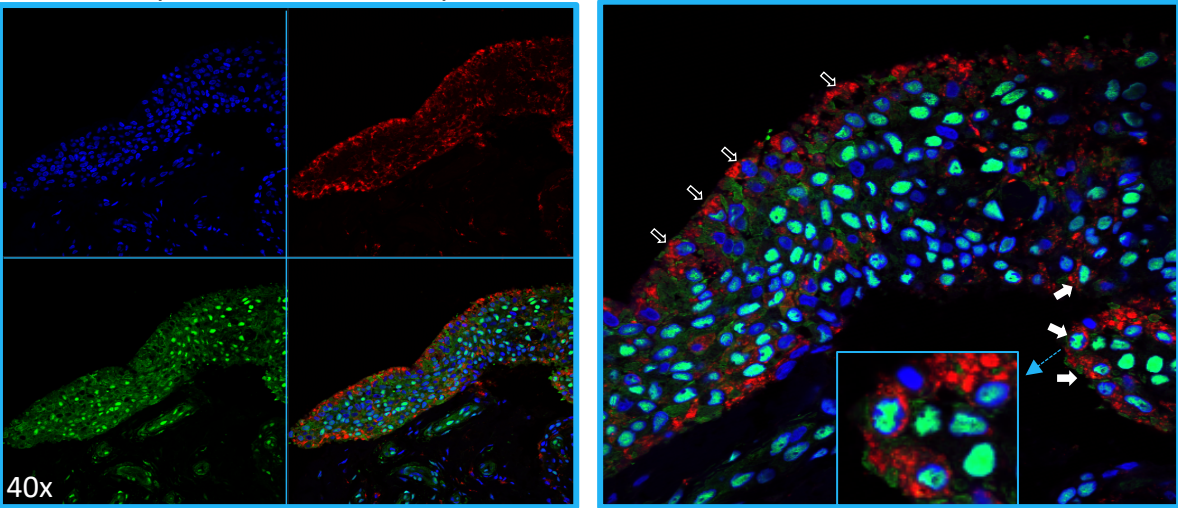
a Healthy (CLEC10a/CD68/Nuclei)



b Active (CLEC10a/CD68/Nuclei)

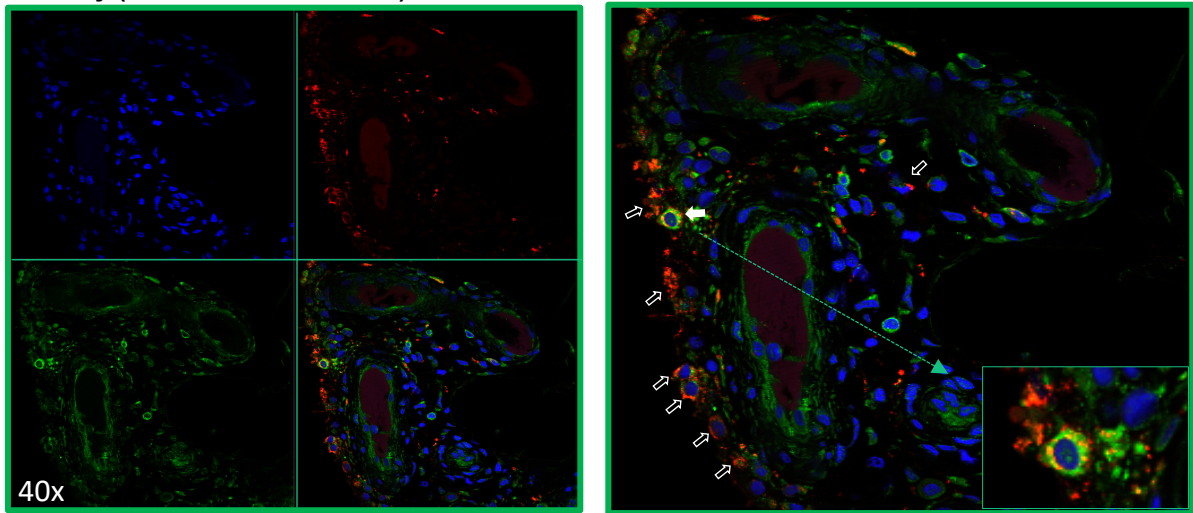


c Remission (CLEC10a/CD68/Nuclei)

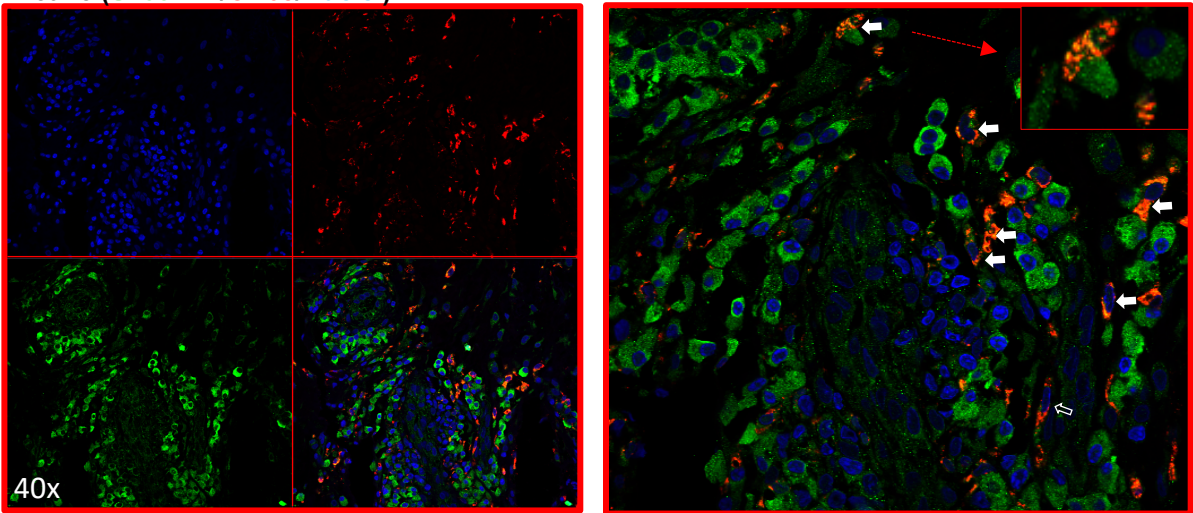


Supplementary Figure 12. CLEC10a positive STMs are mostly present in interstitial space of synovial tissue. Representative confocal microscopy pictures of CLEC10a (green) and macrophage marker staining (CD68, red) in healthy synovium (a), synovium from patients with active RA (b) and RA in remission (c), showing double positive (CLEC10a /CD68⁺) macrophages, CLEC10a negative CD68⁺ macrophages and CLEC10a cells which are not macrophages (CD68 negative). These pictures are representative of n=3 healthy, n=6 active RA and n=6 RA in remission synovial tissues. The solid white arrows indicate double positive CLEC10a /CD68⁺ macrophages while hollow arrows indicate CLEC10a negative macrophages (CD68⁺). The corner pictures show double positive cells under 63x magnification. The nuclei are stained with DAPI (blue).

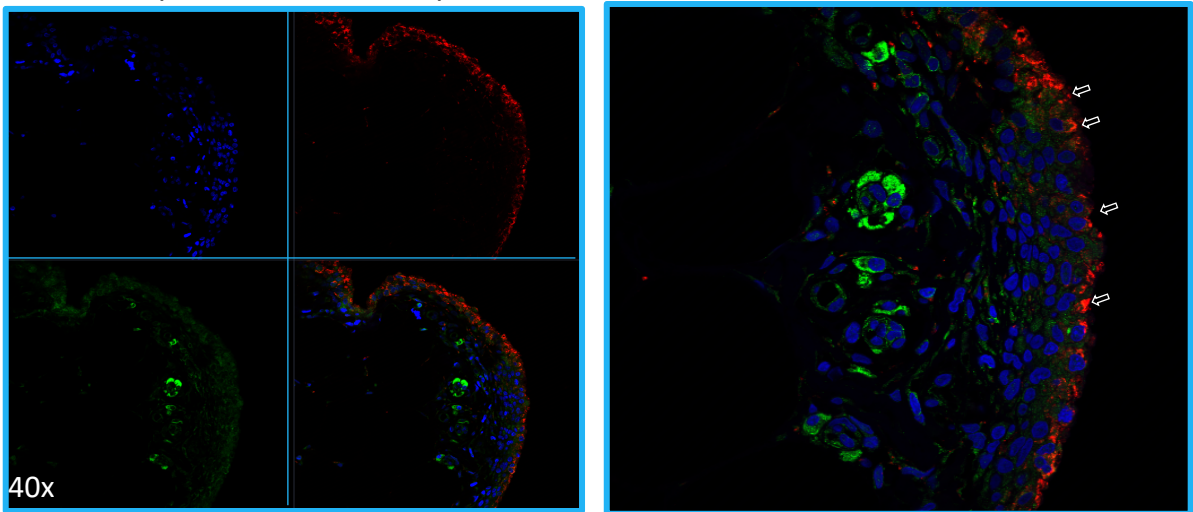
a Healthy (S10012A/CD68/Nuclei)



b Active (S10012A/CD68/Nuclei)

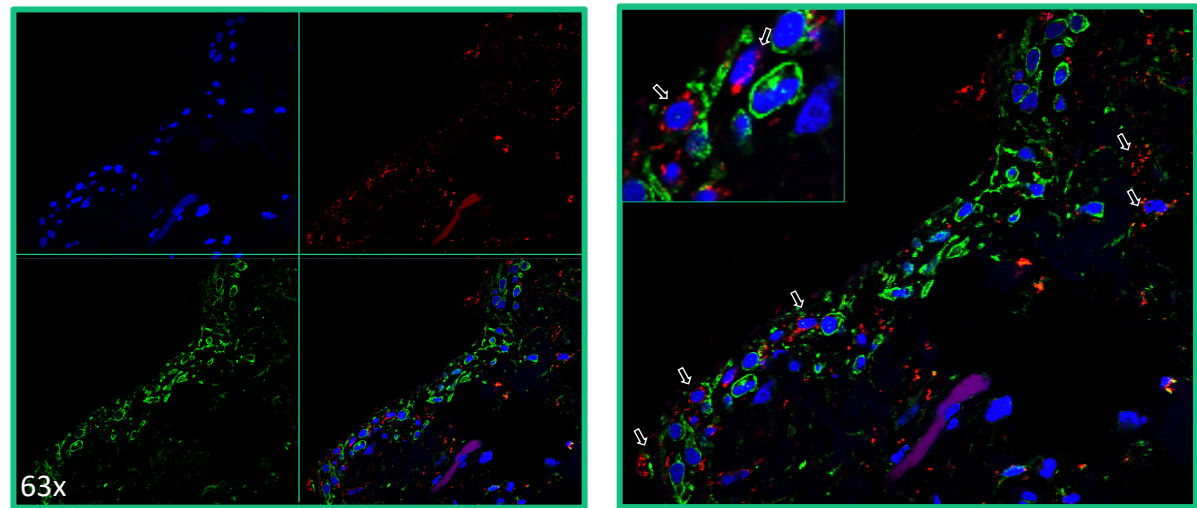


c Remission (S10012A/CD68/Nuclei)

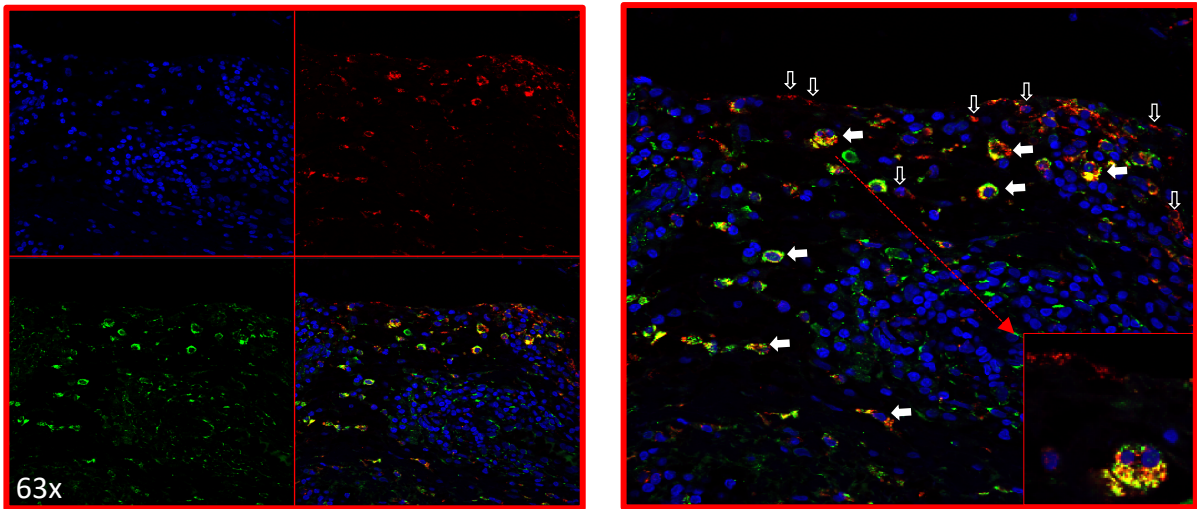


Supplementary Figure 13. S10012A positive STMs are mostly absent in the healthy and RA in remission synovium and increased in sublining layer of synovium from patients with active RA. Representative confocal microscopy pictures of S10012A (green) and macrophage marker staining (CD68, red) in healthy synovium (a), synovium from patients with active RA (b) and RA in remission (c), showing double positive (S10012A /CD68⁺) macrophages only in active RA, S100A12A negative CD68⁺ macrophages and S100A12A cells which are not macrophages (CD68 negative) in all types of tissues. These pictures are representative of n=3 healthy, n=6 active RA and n=6 RA in remission synovial tissues. The solid white arrows indicate double positive S100A12 /CD68⁺ macrophages while hollow arrows indicate S10012A negative macrophages (CD68⁺). The corner pictures show double positive cells under 63x magnification. The nuclei are stained with DAPI (blue).

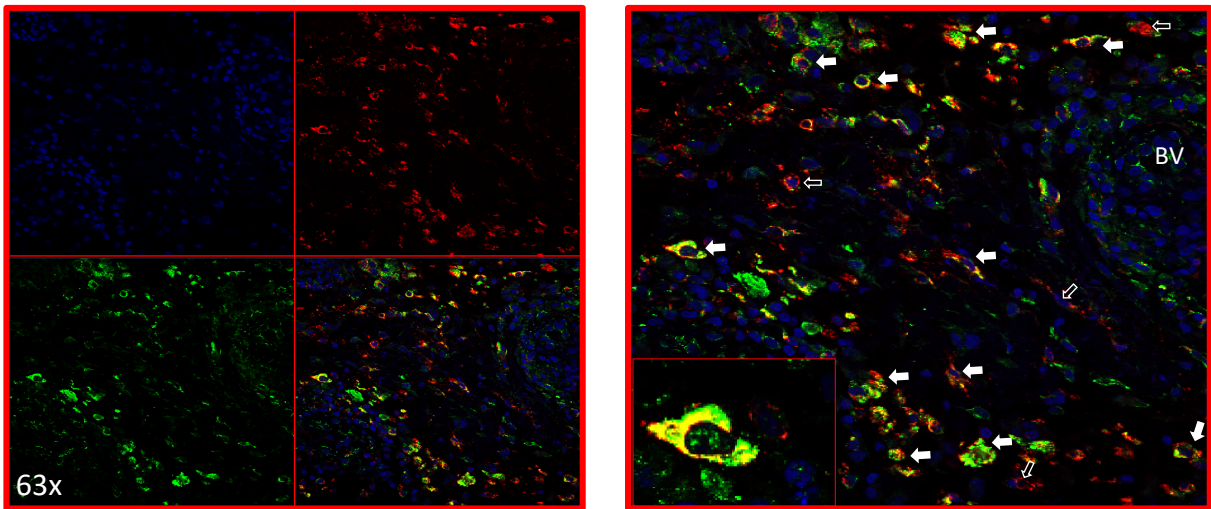
a Healthy (SPP1/CD68/Nuclei)

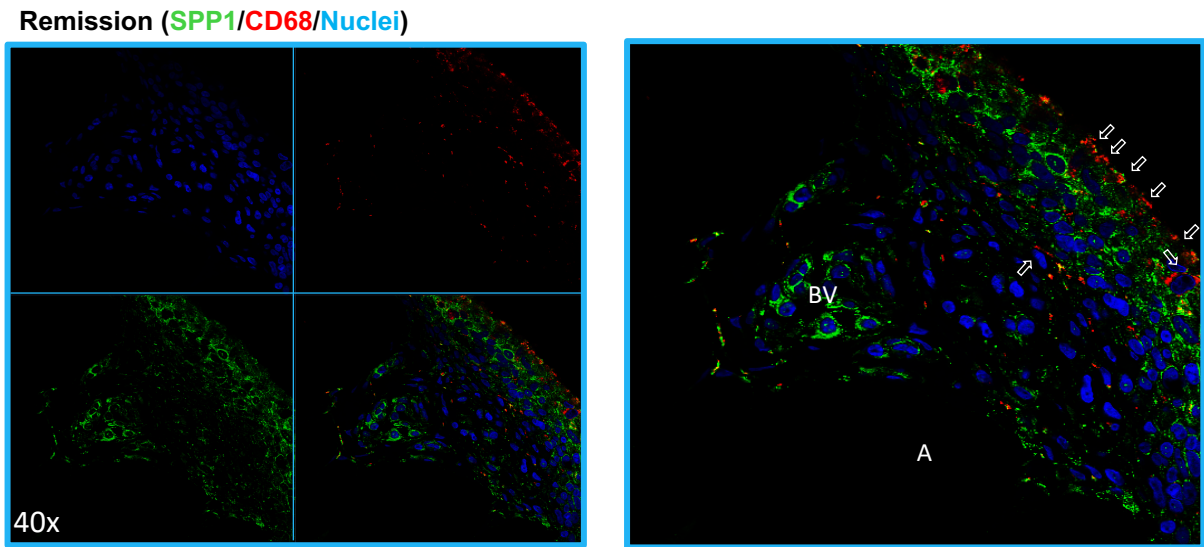


b Active ♂ 1 (SPP1/CD68/Nuclei)



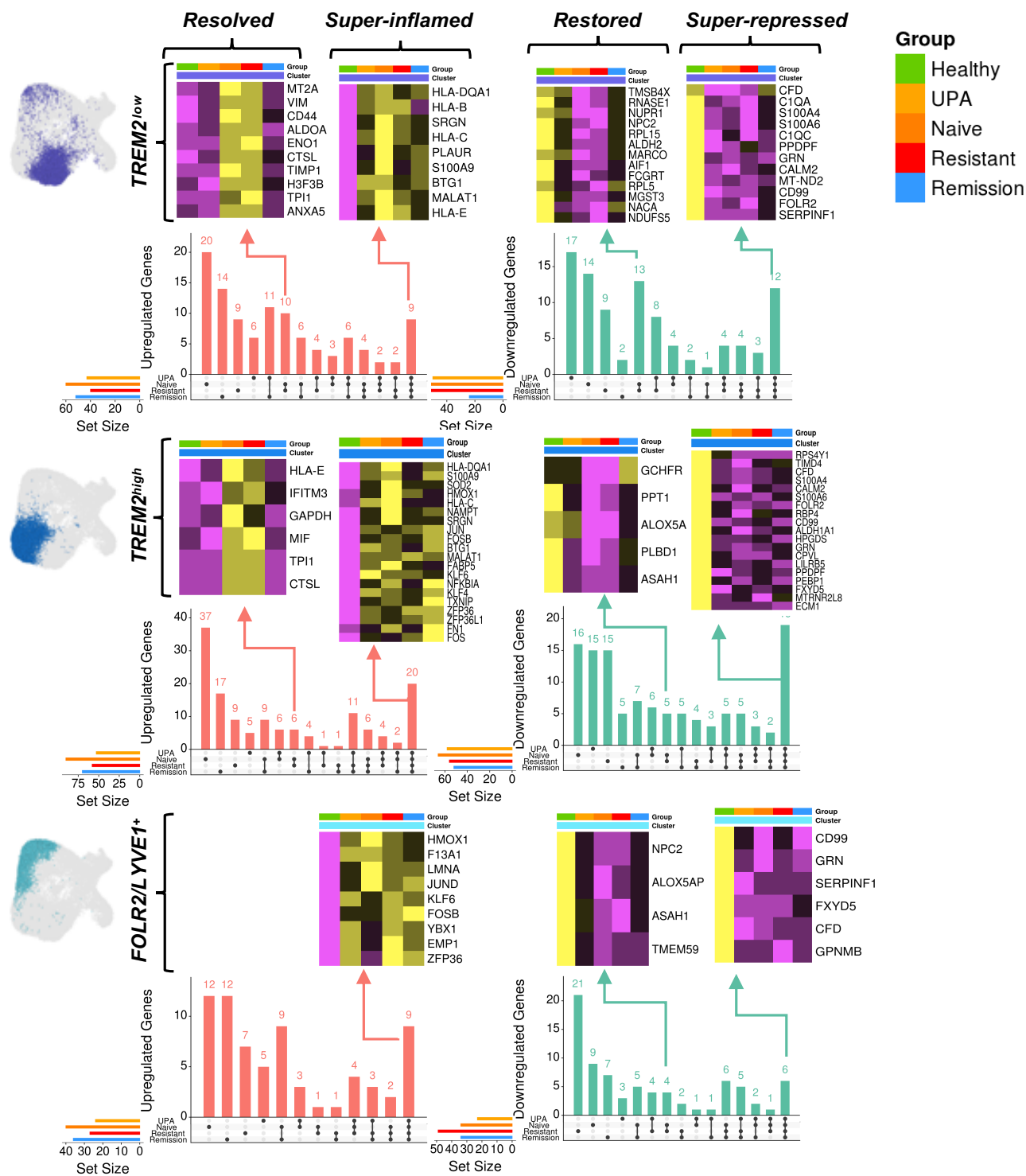
c Active ♂ 2 (SPP1/CD68/Nuclei)





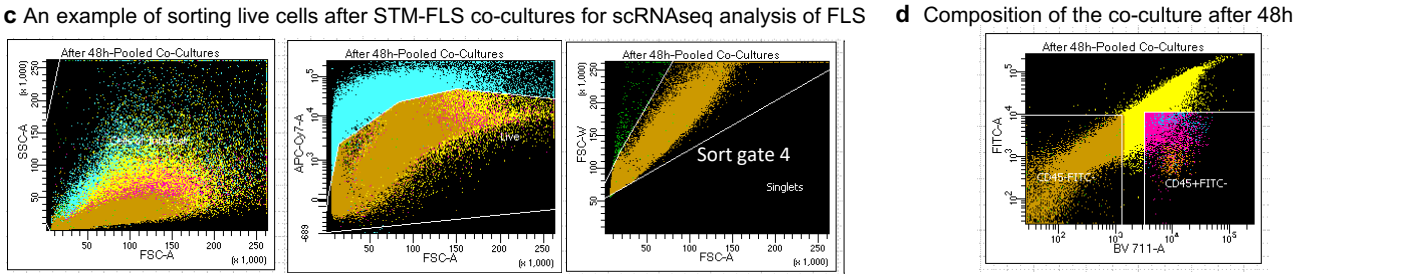
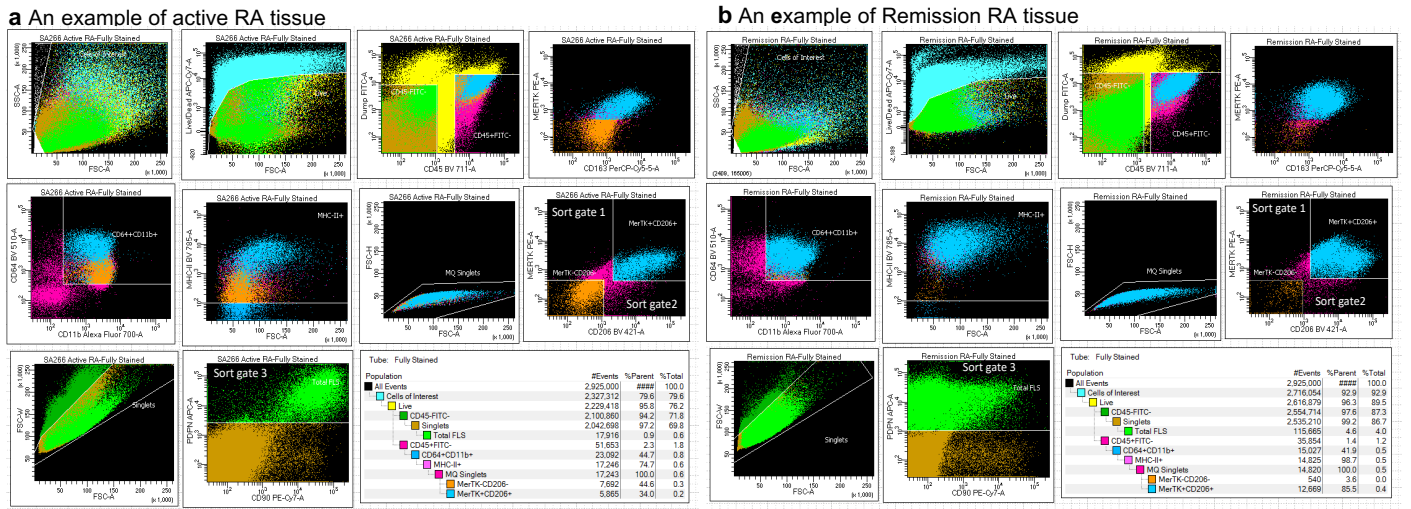
Supplementary Figure 14. SPP1 positive STMs are mostly absent in the healthy and RA in remission synovium and increased in sublining layer of synovium from patients with active RA. Representative confocal microscopy pictures of SPP1(green) and macrophage marker staining (CD68, red) in healthy synovium (a), synovium from patients with active RA (b-c) and RA in remission (d), showing double positive (SPP1/CD68+) macrophages and SPP1 negative CD68+ macrophages. These pictures are representative of n=3 healthy, n=6 active RA and n=6 RA in remission synovial tissues. The solid white arrows indicate double SPP1/CD68+ macrophages while hollow arrows indicate SPP1 negative macrophages (CD68+). The corner pictures show double positive cells under 63x magnification. The nuclei are stained with DAPI (blue). A, adipocyte; BV, blood vessel.

Supplementary Figure 15



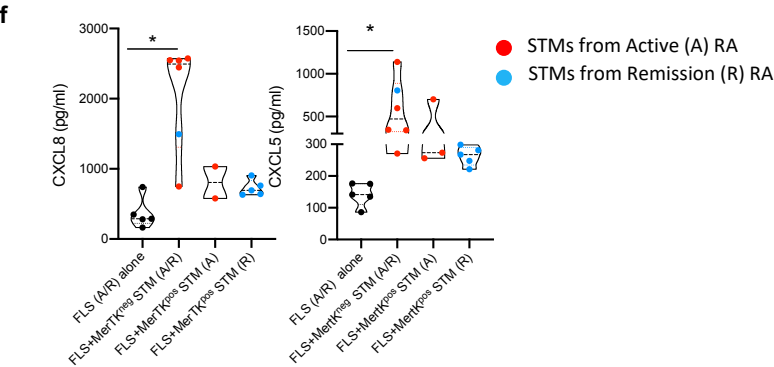
Supplementary Figure 15. MerTK positive clusters in RA patients in disease remission show unique gene expression pattern. Bar plots illustrating the number of intersecting genes differentially expressed between healthy and UPA, healthy and naïve active RA, healthy and resistant RA, and healthy and RA in remission for each MerTK^{pos} clusters of interest (TREM2^{low}, TREM2^{high}, and FOLR2/LYVE1^{pos}). Red bar plots represent common upregulated genes and green bar plots represent common downregulated genes. Expression of genes identified as resolved (upregulated in active RA and downregulated in disease remission), super-inflamed (upregulated in active RA and in disease remission), restored (downregulated in active RA and restored to normal in disease remission), super-repressed (downregulated in active RA and in disease remission) are illustrated per cluster as a heatmap displaying the pseudo-bulk expression per group. Gene expression pattern was dissected using R package.

Supplementary Figure 16



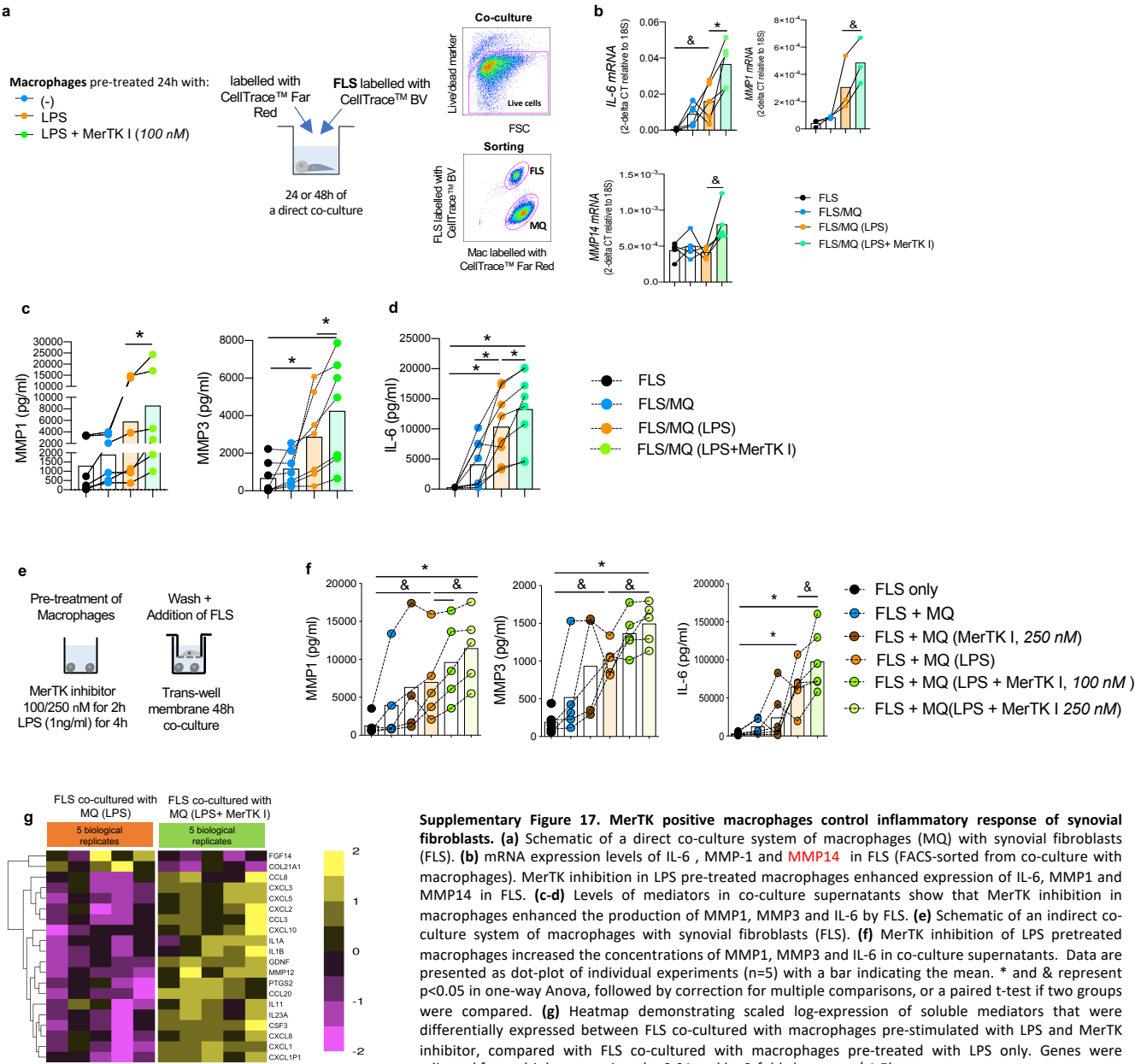
e Exclusion of STMs from FLS analysis

(LUCY) Total co-culture cell clustering; identification of STMs and removal from the analysis, Illustration of CD14 and col1a expression that differentiate STM from FLS to assure the reader



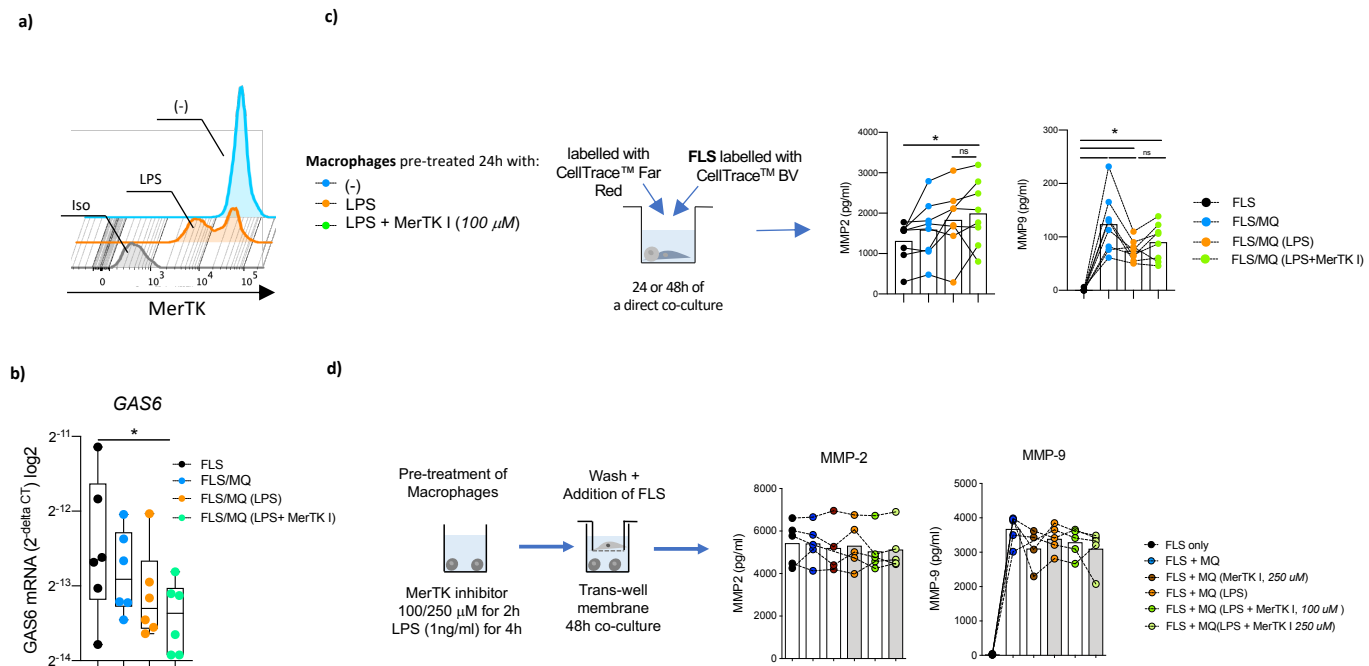
Supplementary Figure 16. The co-culture of MerTK/CD206^{neg} and MerTK/CD206^{pos} STMs with primary FLS. (a-b) The gating strategy for sorting total FLS and MerTK/CD206^{neg} and MerTK/CD206^{pos} STMs from biopsies of patients with active RA and RA in remission. Sort gate 1 illustrates sorting strategy for MerTK/CD206^{neg} while Sort gate 2 for MerTK/CD206^{pos} STMs. The gating strategy for sorting total FLS is illustrated by Sort gate 3. FLS identified by the expression of PDPN and lack of Thy expression (lining) and FLS identified by the expression of both markers (sublining) were sorted. **(c)** The gating strategy for sorting live cells for scRNAseq analysis after 48h co-culture of STM-FLS. After co-cultures cells were stained with condition unique tags, live-dead marker and anti-CD45 (BV711)-antibody. Total single live cells were sorted as illustrated by Sort gate 4. **(d)** Both FLS and STM are present in sorted live cells (Sort gate 4) after 48h as illustrated by negative (FLS) and positive (STM) CD45 staining.

(e) Co-culture supernatants of MerTK^{neg} but MerTK^{pos} STMs with FLS is enriched in CXCL8 and CXCL5 proteins. The co-cultures were set up as described in Figure 4 Legend. * p<0.05 one-way ANOVA with Dunn's test for the multiple comparison correction. A, Active RA; R, RA in remission

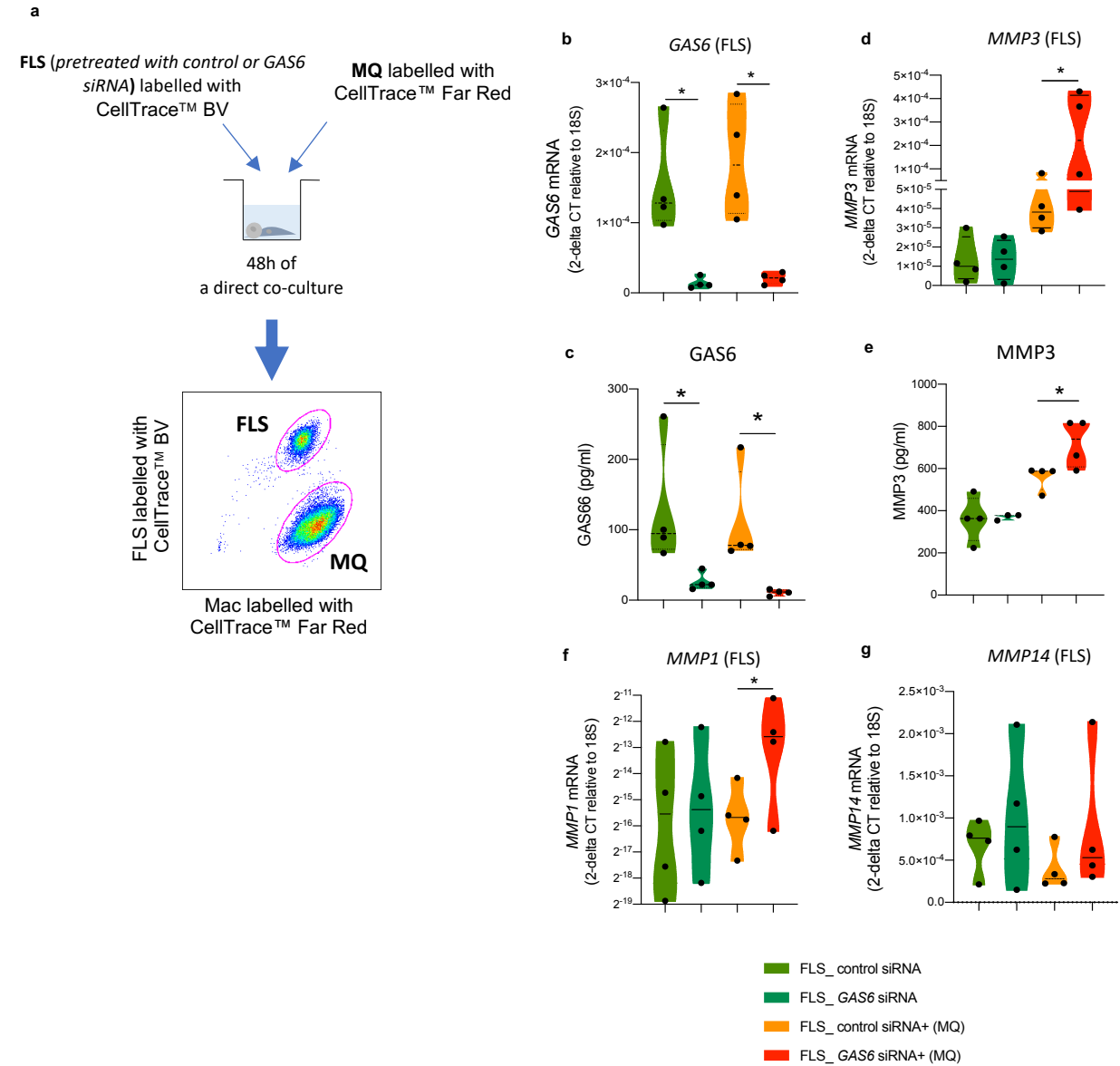


Supplementary Figure 17. MerTK positive macrophages control inflammatory response of synovial fibroblasts. (a) Schematic of a direct co-culture system of macrophages (MQ) with synovial fibroblasts (FLS). (b) mRNA expression levels of IL-6, MMP-1 and MMP14 in FLS (FACS-sorted from co-culture with macrophages). MerTK inhibition in LPS pre-treated macrophages enhanced expression of IL-6, MMP1 and MMP14 in FLS. (c-d) Levels of mediators in co-culture supernatants show that MerTK inhibition in macrophages enhanced the production of MMP1, MMP3 and IL-6 by FLS. (e) Schematic of an indirect co-culture system of macrophages with synovial fibroblasts (FLS). (f) MerTK inhibition of LPS pretreated macrophages increased the concentrations of MMP1, MMP3 and IL-6 in co-culture supernatants. Data are presented as dot-plot of individual experiments (n=5) with a bar indicating the mean. * and & represent p<0.05 in one-way Anova, followed by correction for multiple comparisons, or a paired t-test if two groups were compared. (g) Heatmap demonstrating scaled log-expression of soluble mediators that were differentially expressed between FLS co-cultured with macrophages pre-stimulated with LPS and MerTK inhibitor, compared with FLS co-cultured with macrophages pre-treated with LPS only. Genes were adjusted for multiple comparison (p<0.01 and log2-fold change > +/-1.5).

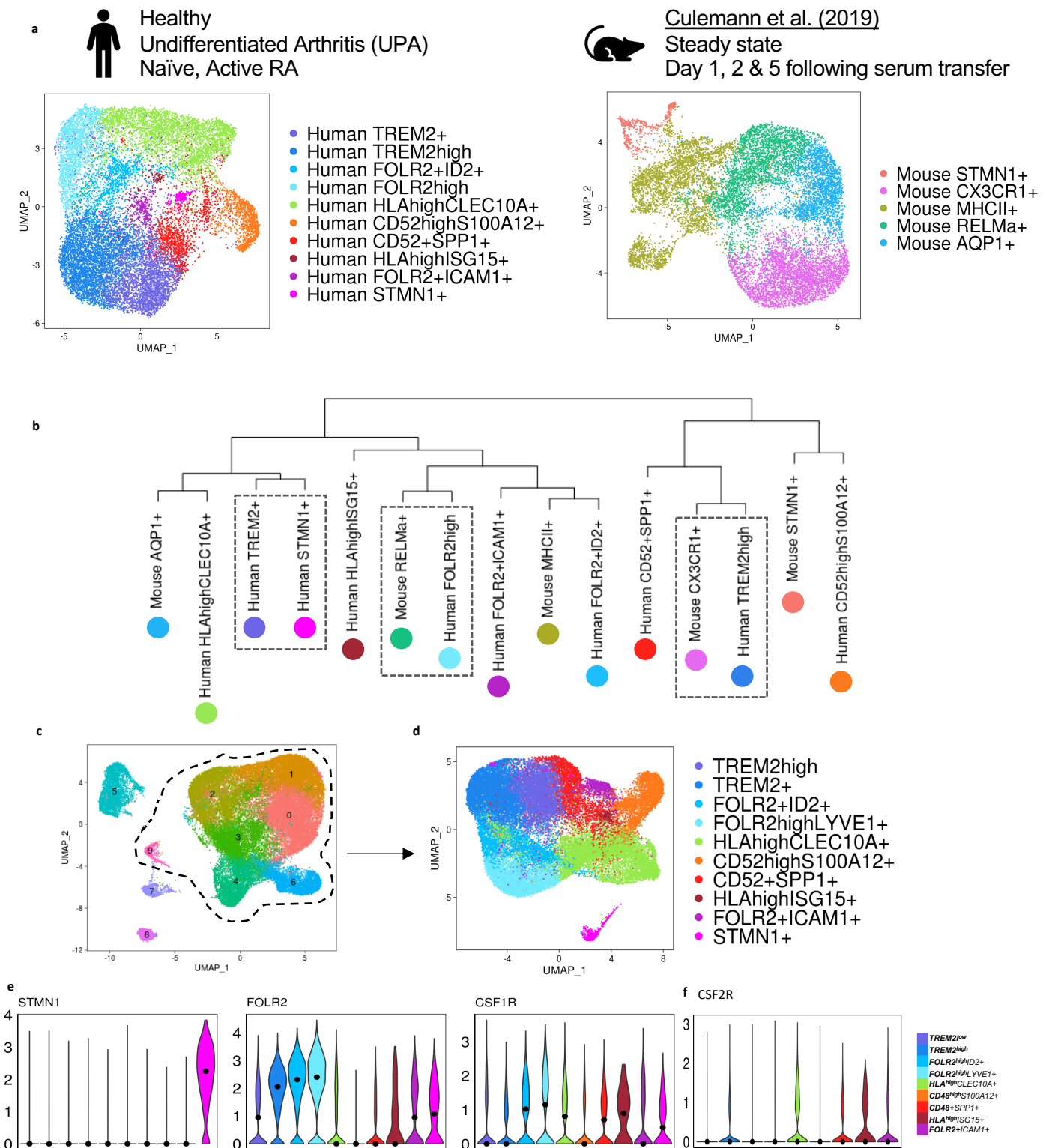
Supplementary Figure 18



Supplementary Figure 19

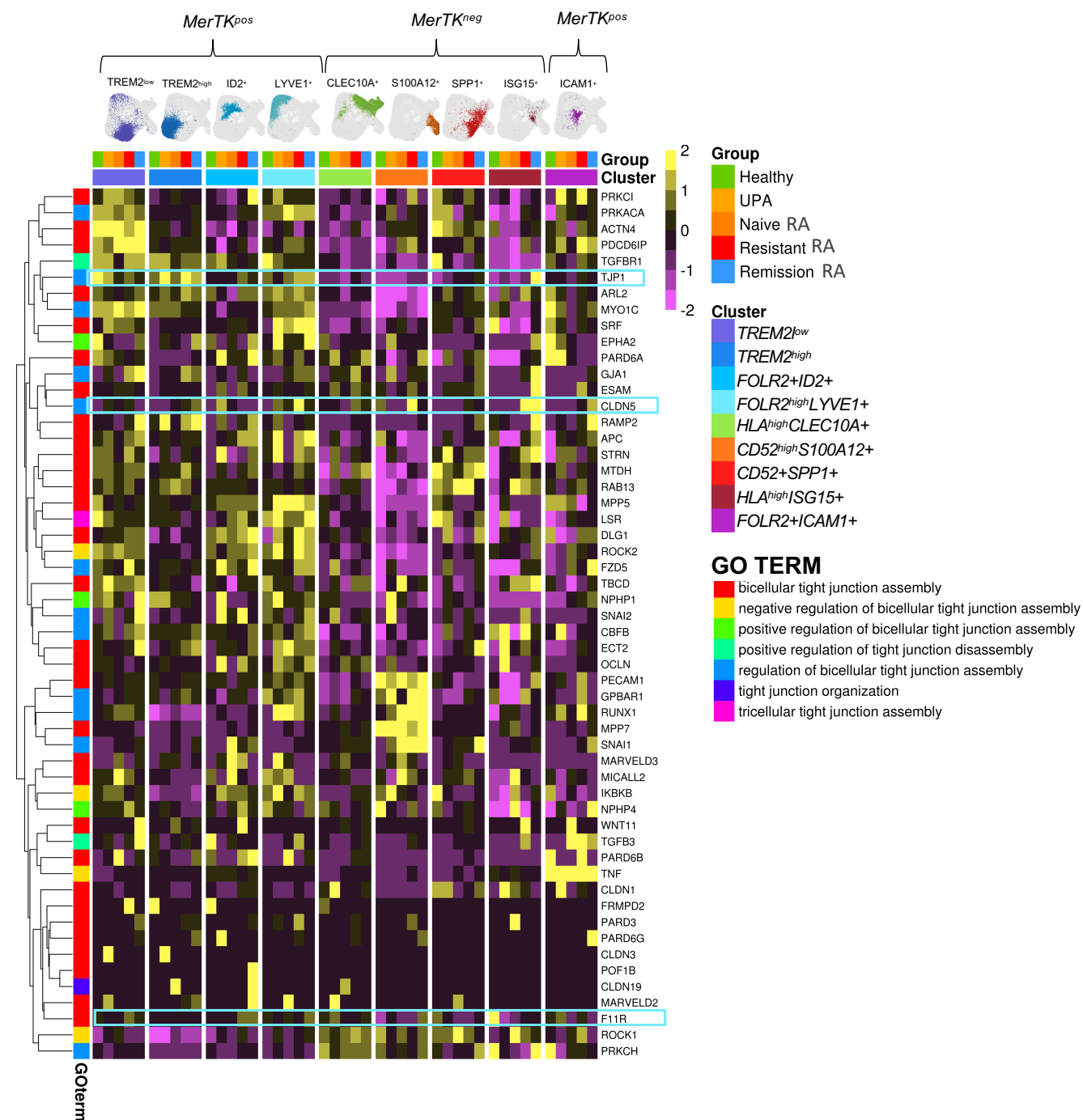


Supplementary Figure 19. Inhibition of MerTK ligand GAS6 in FLS enhances macrophage induced FLS activation. (a) Schematic of a direct co-culture system of monocyte-derived macrophages (MQ) with synovial fibroblasts (FLS). **(b)** mRNA expression levels of GAS6 in FLS transfected with control or GAS6 siRNA and co-cultured with macrophages. **(c)** Levels of GAS6 in co-cultures of FLS with macrophages, in which FLS were transfected with control or GAS6 siRNA. **(d)** Increased levels of MMP3 mRNA in FLS co-cultured with macrophages and transfected with GAS6 siRNA as compared to those transfected with control siRNA. **(e)** Increased levels of MMP3 proteins in co-cultures in which FLS were transfected with GAS6 siRNA as compared to the co-cultures in which FLS were transfected with control siRNA. **(f)** Increased levels of MMP1 mRNA in FLS co-cultured with macrophages and transfected with GAS6 siRNA as compared to those transfected with control siRNA. **(g)** Expression of MMP14 mRNA in FLS does not change upon an interaction with macrophages and upon GAS6 inhibition. Data are presented as violin plot with dots representing individual experiments (n=4). * represent p<0.05 in ratio paired t-test.

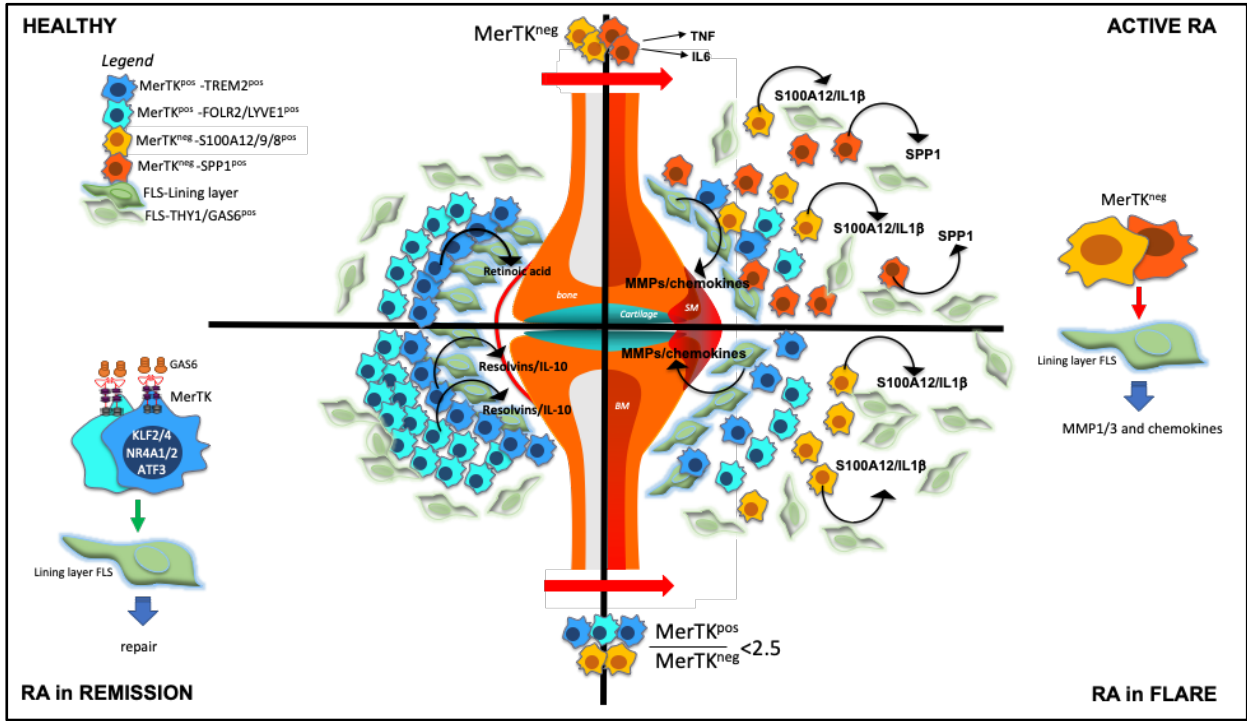


Supplementary Figure 20. Comparison of Human and Mouse (Culemann et al., 2019) single-cell transcriptional profiling of synovial macrophages. (a) UMAP projections for human and mouse scRNAseq data analyzed separately. Mouse data was clustered at a resolution of 0.3 and UMAP projection represents the top 12 PCs. Human data for this comparison included samples from healthy tissue, undifferentiated arthritis (UPA) and naïve, active RA to align with disease conditions modelled in the mouse data. (b) Dendrogram representing the relationship between human macrophage phenotypes and mouse clusters identified by Culemann et al. (2019 ref 8). This plot was generated from the hierarchical clustering of the average expression of orthologous genes by each population. (c) Original UMAP projection following the integration synovial tissue macrophages from both human sample cohorts (discovery and validation) but before the removal of doublets and contaminant non macrophage lineage positive cells. The populations selected for analysis are demarcated. For the current analysis the proliferating cells are included. (d) The UMAP projection of synovial tissue macrophages (with proliferating cells) following the merging and renaming of clusters. (e) Violin plots show expression of FOLR2 and CSF1R by the cycling macrophage population characterized by STMN1 expression (f) Violin Plots illustrating the expression of GM-CSFR (CSF2R), which is a target of recent anti-macrophage therapy in RA (mavrilimumab) in distinct human STM clusters.

Supplementary Figure 21



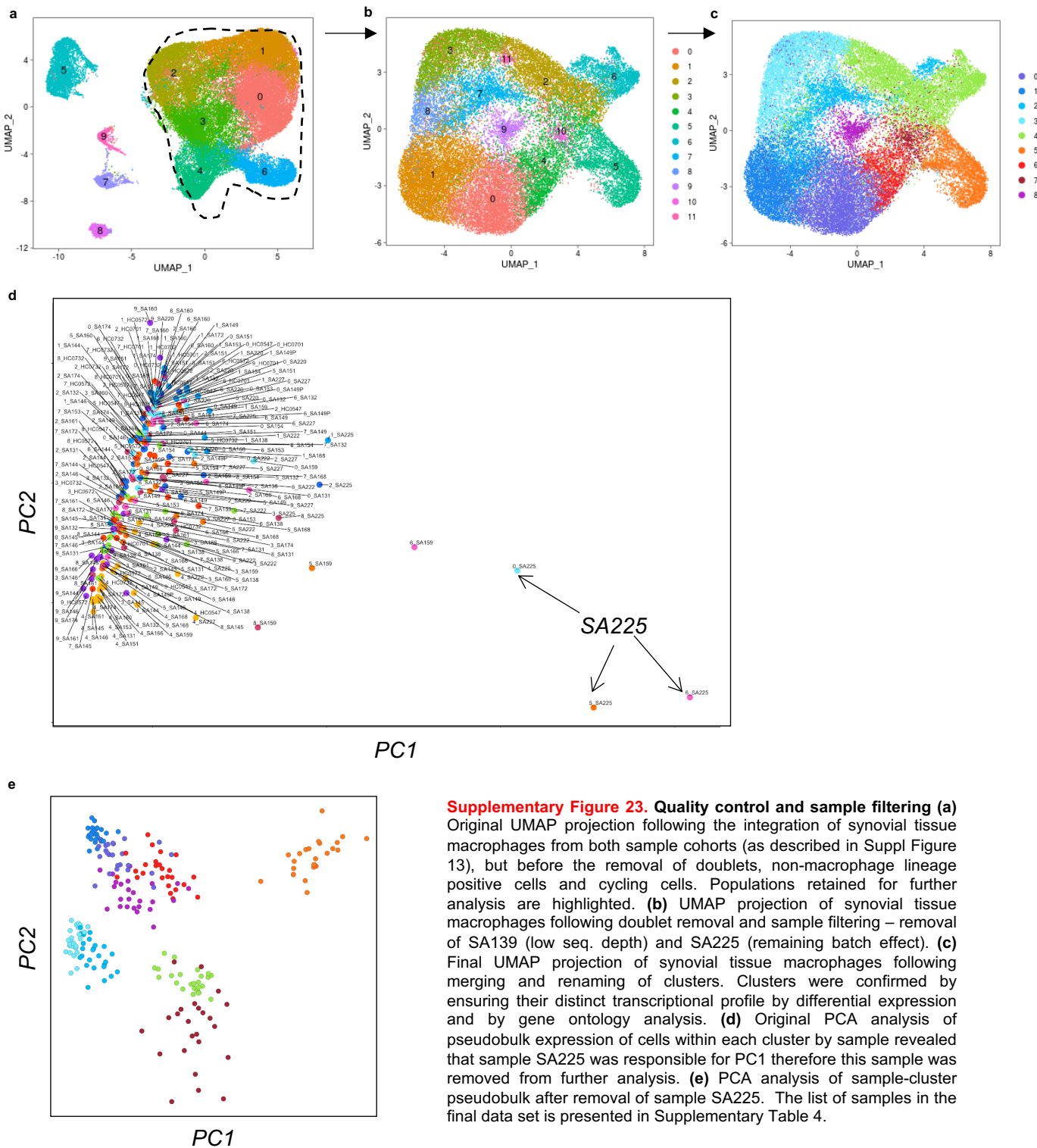
Supplementary Figure 21. Investigating potential barrier function in human synovial tissue macrophages: MerTK positive clusters are enriched in tight junction proteins. Heatmap illustrating scaled pseudo-bulk expression of significantly enriched pathways by each patient group within each of identified synovial tissue macrophage clusters. Rows represent genes with a potential contribution to synovial lining layer barrier function (GO pathway- involved tight junction assembly and organization). Columns equal average expression for cells in each cluster by subject group. Genes identified in mouse synovial lining macrophages by Culemann et al. (2019) as tight junction proteins are in blue boxes. Among them TJP1 is expressed in human MerTK/TREM2 and in MerTK/FOLR2/LYVE1 positive STM subsets.



Supplementary Figure 22. Distinct synovial tissue macrophage subsets regulate inflammation and provide a cellular and molecular mechanism for disease remission in rheumatoid arthritis. The **HEALTHY** synovial membrane mainly consists of two subsets of MerTK^{pos} STMs: TREM2^{pos} and FOLR2/LYVE1^{pos}. Their transcriptomics suggest immunoregulatory functions, e.g. production of retinoic acid or expression of B7-like inhibitory molecules (VISG4). In **ACTIVE RA** (treatment-naïve and treatment-resistant), synovial membrane is infiltrated by MerTK^{neg} CD48^{pos} STMs with two distinct phenotypes producing either pathogenic S100A alarmins (e.g. S100A12) and IL-1β, or osteopontin. Both are also the main source of pathogenic TNF and IL-6, and potent inducers of pro-inflammatory (chemokines) and destructive (MMPs) properties of synovial stromal fibroblasts (FLS). RA in **REMISSION** (maintained after treatment cessation) is characterized by restoration of the TREM2^{pos} and FOLR2/LYVE1^{pos} MerTK^{pos} subsets with transcriptome and regulatory properties that are different from those of healthy STMs; they poorly produce pro-inflammatory cytokines, which is further negatively regulated by GAS6 binding to its receptor MerTK. Instead these MerTK^{pos} STMs produce resolvins (inflammation-resolving lipid mediators) and IL-10 and induce repair response of FLSs. Their transcriptome is characterized by MerTK-dependent transcription factors that are negative-regulators of inflammation. MerTK^{pos} macrophages forestall pathogenic activation of FLS. RA patients in remission, with STMs composed of less than 47.5% MerTK^{pos}, or ratio of MerTK^{pos} to MerTK^{neg} less than 2.5 have a higher likelihood of **FLARE** after treatment cessation. The MerTK^{neg} STMs in these patients are of CD48/S100A12^{pos} phenotype and release S100A12 alarmin upon stimulation, suggesting a role in the initiation of disease flare. In summary, sustained remission of arthritis appears to be an active process maintained by tissue-resident subsets of MerTK^{pos} STM subpopulations (e.g. TREM2^{pos} and FOLR2/LYVE1^{pos}) that govern the functions of pro-inflammatory CD52/S100A12^{pos} STMs and synovial fibroblasts to reinstate and maintain homeostasis.

BM, bone marrow; SM, synovial membrane; FLS, fibroblast like synoviocytes; MMPs, metalloproteinases; SPP1, osteopontin; KLFs krueppel like factors; NR4As, nuclear receptor subfamily 4 group A; ATF3, cAMP-dependent transcription factor 3; MerTK, tyrosine-protein kinase Mer; TREM2, triggering receptor expressed on myeloid cells 2; LYVE1, lymphatic vessels endothelial hyaluronan receptor 1; FOLR2, folate receptor beta; GAS6, growth arrest-specific 6; S100A12, S100 calcium-binding protein A12; THY1, CD90.

Supplementary Figure 23



Supplementary Figure 23. Quality control and sample filtering (a) Original UMAP projection following the integration of synovial tissue macrophages from both sample cohorts (as described in Suppl Figure 13), but before the removal of doublets, non-macrophage lineage positive cells and cycling cells. Populations retained for further analysis are highlighted. (b) UMAP projection of synovial tissue macrophages following doublet removal and sample filtering – removal of SA139 (low seq. depth) and SA225 (remaining batch effect). (c) Final UMAP projection of synovial tissue macrophages following merging and renaming of clusters. Clusters were confirmed by ensuring their distinct transcriptional profile by differential expression and by gene ontology analysis. (d) Original PCA analysis of pseudobulk expression of cells within each cluster by sample revealed that sample SA225 was responsible for PC1 therefore this sample was removed from further analysis. (e) PCA analysis of sample-cluster pseudobulk after removal of sample SA225. The list of samples in the final data set is presented in Supplementary Table 4.