Distinct fibroblast subsets drive inflammation and damage in arthritis

Adam P. Croft1,2, Joana Campos1, Kathrin Jansen1, Jason D. Turner1, Jennifer Marshall1, Moustafa Attar1, Loriane Savary1, Corinna Wehmeyer1,4, Amy J. Naylor1, Samuel Kemble1, Jenefa Begum1, Kerstin Dürholz1,5, Harris Perlman6, Francesca Barone1, Helen M. McGettrick1, Douglas T. Fearon7, Kevin Wei8, Soumya Raychaudhuri8, Ilya Korsunsky8, Michael B. Brenner8, Mark Coles3, Stephen N. Sansom3,11, Andrew Filer1,2,9,10,11 & Christopher D. Buckley1,2,3,10*

The identification of lymphocyte subsets with non-overlapping effector functions has been pivotal to the development of targeted therapies in immune-mediated inflammatory diseases (IMIDs)1,2. However, it remains unclear whether fibroblast subtypes with non-overlapping functions also exist and are responsible for the wide variety of tissue-driven processes observed in IMIDs, such as inflammation and damage3-5. Here we identify and describe the biology of distinct subsets of fibroblasts responsible for mediating either inflammation or tissue damage in arthritis. We show that deletion of fibroblast activation protein-α (FAPα+) fibroblasts suppressed both inflammation and bone erosions in mouse models of resolving and persistent arthritis. Single-cell transcriptional analysis identified two distinct fibroblast subsets within the FAPα− population: FAPα+ THY1+ immune effector fibroblasts located in the synovial sub-lining, and FAPα+ THY1− destructive fibroblasts restricted to the synovial lining layer. When adoptively transferred into the joint, FAPα+ THY1− fibroblasts selectively mediate bone and cartilage damage with little effect on inflammation, whereas transfer of FAPα+ THY1+ fibroblasts resulted in a more severe and persistent inflammatory arthritis, with minimal effect on bone and cartilage. Our findings describing anatomically discrete, functionally distinct fibroblast subsets with non-overlapping functions have important implications for cell-based therapies aimed at modulating inflammation and tissue damage.

Non-haematopoietic, tissue-resident fibroblasts contribute to the pathogenesis of many diseases and are known to develop epigenetically imprinted, site- and disease-specific phenotypes6-8. Rheumatoid arthritis (RA) is a prototypic IMID9 in which synovial fibroblasts contribute to both joint damage2,8 and inflammation9. We found that expression of FAPα, a cell-membrane dipeptidyl peptidase10, was significantly higher in both synovial tissue and cultured synovial fibroblasts isolated from patients who fulfilled classification criteria for RA compared to patients in whom joint inflammation resolved (Fig. 1a–c), suggesting that FAPα expression may associate with a pathogenic fibroblast phenotype.

To map the expression of FAPα+ expressing cells in the RA synovium, we used mass cytometry (CyTOF), together with a combination of podoplanin (PDPN) and thymus cell antigen 1 (THY1, also known as CD90) to discriminate sub-lining layer (SL, THY1−) from lining layer (LL, THY1+) fibroblasts, as in previous studies5,11. FAPα colocalized with PDPN in both the LL and SL cells (Fig. 1d). A small subset of pericytes (defined as CD45− PDPN+ and THY1−) also expressed FAPα. These findings were confirmed by confocal analysis in RA synovial tissue (Fig. 1e).

To determine the role of FAPα+ synovial fibroblasts in arthritis, we used serum transfer induced arthritis (STIA)12 in a transgenic FAPα luciferase-DTR reporter mouse13. FAPα expression (bioluminescence) increased during the course of arthritis (Fig. 1f, g) and correlated with the severity of ankle joint swelling (Fig. 1h). Synovial expression of FAPα was either low or undetectable under resting conditions (Extended Data Fig. 1a) but increased in synovial membrane and focal areas of pannus tissue invading cartilage and bone during inflammation (Fig. 1i, j, Extended Data Fig. 1a). FAPα expression was restricted to mesenchymal cells (CD45−) (Extended Data Fig. 1b–f) and the number of FAPα+ fibroblasts increased during inflammation and returned to baseline levels with resolution of inflammation (Fig. 1k, Extended Data Fig. 1c, d), confirming that FAPα is a biomarker of tissue inflammation (Fig. 1f–k, Extended Data Fig. 1a, c, d).

In the mouse synovium, THY1 expression also distinguished SL from LL fibroblasts, whereas FAPα was expressed in both cellular compartments (Extended Data Fig. 1e–g). Induction of Pdpn, Pdpn and Thy1 mRNA was significantly higher in the inflamed synovial membrane (Fig. 1l), and expression positively correlated with joint swelling (Fig. 1m). A significant increase in the proliferation of both THY1− FAPα− (LL) and THY1+ FAPα+ (SL) cells was observed during inflammation, with very little change in the number of FAPα− expressing pericytes (Fig. 1n). The severity of joint inflammation positively correlated with the total number of FAPα+ THY1− cells but not FAPα+ THY1+ cells (Extended Data Fig. 1h). The extent of cartilage damage did however correlate with the number of FAPα+ THY1+ cells (Extended Data Fig. 1i), whereas the severity of bone erosion positively correlated with the number of FAPα+ THY1− cells and not FAPα+ THY1− cells (Extended Data Fig. 1j). Collectively, these data suggest that the expansion of a potentially pathogenic population of synovial fibroblasts is marked by expression of PDPN, FAPα and THY1.

To determine the functional role of FAPα+ fibroblasts in vivo, we selectively depleted FAPα+ cells during arthritis (Extended Data Fig. 1k–m). Depletion of these cells led to a significant reduction in the cellularity of the synovial membrane (Extended Data Fig. 1n–p), attenuated synovial inflammation and accelerated resolution in both the resolving (Fig. 2a) and persistent models of STIA (Fig. 2b), with the same effect observed regardless of the stage of arthritis at the time of deletion (Extended Data Fig. 2a, b). However, deletion before induction of arthritis had no effect on joint thickness (Extended Data Fig. 2c), an observation consistent with the low numbers of FAPα+ cells in the synovial membrane under resting conditions (Extended Data Fig. 1a, c).

1Rheumatology Research Group, Institute for Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham, Queen Elizabeth Hospital, Birmingham, UK. 2Versus Arthritis Centre of Excellence in the Pathogenesis of Rheumatoid Arthritis, College of Medical and Dental Sciences, University of Birmingham, Queen Elizabeth Hospital, Birmingham, UK. 3The Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK. 4Musculoskeletal Medicine, University of Muenster, Muenster, Germany. 5Department of Medicine, Division of Rheumatology, Northwestern University, Feinberg School of Medicine Chicago, Evanston, IL, USA. 6Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA. 7Division of Rheumatology, Immunology, and Allergy, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA. 8University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK. 9MRC and Versus Arthritis Centre for Musculoskeletal Ageing Research (DMAR), College of Medical and Dental Sciences, University of Birmingham, Queen Elizabeth Hospital, Birmingham, UK. 10MRC Centre for Genomic Mayo and Arthritis Research at Cytokinetics (GMA), University of Oxford, Oxford, UK. 11These authors contributed equally: Stephen N. Sansom, Andrew Filer. *e-mail: c.d.buckley@bham.ac.uk
Deletion of FAPα⁺ cells reduced structural joint damage (cartilage and bone damage), inflammatory bone remodelling (Fig. 2c, d and Extended Data Fig. 2f–i), osteoclast numbers (Fig. 2d, Extended Data Fig. 2g, h) and expression of osteoblast and osteoblast bone markers in whole joint tissue (Extended Data Fig. 2l).

FAPα⁺ cell deletion led to reduced leukocyte infiltration (Fig. 2e), negatively correlated with the severity of joint inflammation (Fig. 2f) and was associated with a reduction in the number of both LL and SL fibroblasts, with no significant change in pericyte numbers (Fig. 2g). Circulating blood monocyte number and phenotype were unchanged (Extended Data Fig. 3a), excluding any potential indirect effects of myelosuppression. Accompanying these changes was a marked reduction in the number of synovial leukocytes, specifically neutrophils, macrophages, CD11b⁺ dendritic cells and monocytes, but not eosinophils (Extended Data Fig. 3b for gating strategy, Fig. 2h for resolving STIA model data and Extended Data Fig. 3c for persistent model), and a reduction in the percentage of major histocompatibility complex (MHC) class II-expressing macrophages (persistent model: Extended Data Fig. 3c; resolving model: Extended Data Fig. 3e). There were very few remaining macrophages in the synovial membrane following deletion of FAPα⁺ cells (Extended Data Fig. 3d) and those remaining had a more anti-inflammatory phenotype (Extended Data Fig. 3f). These cellular changes in the synovium were accompanied by a marked reduction in pro-inflammatory chemokines, cytokines, RANKL and matrix metalloproteases (MMPs) (Fig. 2i), demonstrating that synovial FAPα⁺ cells are a major source of these proteins.

To exclude an indirect effect of systemic deletion of FAPα⁺ cells, we delivered diphtheria toxin locally to the joint. This resulted in cell deletion in the synovial membrane but not in draining or distant lymph nodes (Extended Data Fig. 3g) and did not result in systemic cachexia (Extended Data Fig. 3h), as reported previously after systemic FAPα⁺ cell deletion.13 Local deletion had the same effect on joint inflammation and bone damage with no effect observed in non-injected joints or following administration of diphtheria toxin in non-arthritic mice (Extended Data Fig. 3i–k).

We next investigated whether both THY1⁺ FAPα⁺ (LL) and THY1⁺ FAPα⁺ (SL) fibroblast populations contribute equally to inflammation and bone damage. We first performed single-cell RNA sequencing of CD45⁻ non-hematopoietic cells from inflamed mouse synovium. After assigning identities to all cell clusters (Fig. 3a, Extended Data Figs. 4a–d, 5, Supplementary Table 1), targeted reanalysis of the fibroblast populations on the basis of expression of known fibroblast marker genes revealed the existence of five distinct subgroups that we designate F1–F5 (Fig. 3b, Extended Data Figs. 6, 7, Supplementary Table 1). Gene ontology (GO) analysis of significant cluster marker genes suggested a diversification of function between the subsets. F1 fibroblast marker genes were over-represented in categories related to bone, cartilage and extra-cellular matrix formation. F2 cells were strongly characterized by expression of inflammatory genes, including those involved in ‘cytokine production’ and ‘regulation of leukocyte chemotaxis’. Meanwhile, F3 fibroblasts showed an enrichment for genes involved in the ‘complement activation’ and ‘vasculogenesis’ biological processes. F4 fibroblasts expressed genes characteristic of an actively cell cycling population. Finally, F5 fibroblasts displayed a phenotype that included a distinctive over-representation of genes associated with ‘acid secretion’ and ‘hydrogen transport’ (Fig. 3c, Supplementary Table 2).

Examination of the top cluster marker genes allowed us to easily differentiate these five subsets at the mRNA expression level (Fig. 3d). Whereas Pdnp and Fap were expressed by all of the fibroblast subsets, Thy1 was expressed selectively by F1–F4 fibroblasts but not F5 fibroblasts (Fig. 3d, Extended Data Fig. 6), suggesting that we could use THY1 as a marker to discriminate the LL F5 subset from the four SL clusters (F1–F4). We also examined the expression of other known fibroblast markers across the subsets as well as the specific expression of selected chemokines (Extended Data Fig. 7a–c).

We next examined the potential developmental relationship between the different fibroblast subsets using a diffusion map (Fig. 3e),
Application of the pseudotime algorithm Slingshot, identified two single-branch trajectories comprising of F1–F2–F3–F4 and F1–F2–F5. Existence of the F1–F2–F5 trajectory is consistent with the topology of the diffusion map, and analysis of genes that are differentially expressed along this trajectory (Extended Data Fig. 8a) showed that cells of cluster F2 have overlapping profiles with those of F1 and F5.

To investigate the existence of homologous fibroblast subsets in human arthritis, we selectively reanalyzed data from synovial biopsies from people with RA. This analysis identified five distinct subpopulations (Extended Data Fig. 8b–e). Correlation of orthologous (one-to-one) cluster markers from the human and mouse datasets identified three homologous populations of fibroblasts (Fig. 3f) that share distinctive gene expression profiles (Fig. 3g). The homologous clusters consisted of (i) LL fibroblasts (mouse STIA F5, human RA F4), (ii) Ccl34 SL fibroblasts (mouse STIA F3, human RA F5) and (iii) Ccl11at SL fibroblasts (mouse STIA F1, human RA F2).

To confirm the validity of using PDNP, THY1 and FAPα as a cassette of cell surface markers to discriminate LL fibroblasts and SL fibroblasts, we performed ultra-low-input RNA sequencing on purified PDNP+, FAPα+ and THY1+ cell populations (Extended Data Fig. 9a–d, Supplementary Tables 3–6). Principal component analysis of transcriptional differences confirmed that the subsets defined by expression of THY1 represented transcriptionally distinct populations with the most obvious separation between THY1+ versus THY1− populations, regardless of FAPα expression (Extended Data Fig. 9b). The THY1+ cell population showed expression of many chemokines and cytokines and expressed F1–F4 subset-specific genes (Extended Data Fig. 9c, d). By contrast, THY1− cell gene expression was consistent with F5 fibroblast-associated genes such as Ptgs2, Clec9a and Tip71 as well as genes associated with cartilage and bone erosion. Therefore, the greatest determinant of the transcriptional profile of synovial fibroblasts appeared to be their anatomical location in the synovial membrane (as defined by THY1 expression).

As predicted from the single-cell transcriptome analysis, FAPα−/THY1+ subsets had an immune-effector profile with higher expression of chemokines as well as cytokines including: Ifn, Il1f, Il13 and Il34. By contrast, FAPα+/THY1− subsets had higher expression of Ccl9 and Tnfsf11, both potent inducers of osteoclast activity, as well as Mmp3, Mmp9 and Mmp13, which are MMPs involved in cartilage degradation (Extended Data Fig. 9d). These findings were validated, when possible, for protein expression (Fig. 4a). FAPα+/THY1− cells also expressed receptor activator of nuclear factor-kappa B ligand (RANKL) on their surface, secreted higher levels of RANKL, exhibited a significantly increased RANKL:osteoprotegerin (OPG) ratio (Fig. 4b, c) and stimulated osteoclast differentiation and activation in vitro, leading to significantly more resorption of hydroxyapatite matrix in vitro (Fig. 4d). Together, these results support a model in which THY1− and THY1+ cells might perform distinct non-overlapping functions in vivo.

To test this hypothesis directly, we injected PDNP+ FAPα+/THY1− or PDNP− FAPα+/THY1+ cells into the inflamed ankle joint of mice.
during STIA. Injection of PDNP⁺FAP⁺THY1⁺ cells resulted in more severe and sustained joint swelling (Fig. 4e) with higher levels of leukocyte infiltration (Fig. 4f), but had little effect on bone and cartilage destruction (Fig. 4g, h). By contrast, injection of PDNP⁺FAP⁺THY1⁻ cells had no effect on the severity or temporal dynamics of joint inflammation (Fig. 4e, f), but did result in increased osteoclast activity and increased structural joint damage (Fig. 4g, h). Similar effects were observed following the injection of each cell population into an inflamed ankle joint of mice with collagen induced arthritis (CIA) (Extended Data Fig. 10a, b). In this case, injection of PDNP⁺FAP⁺THY1⁻ cells also resulted in increased effector CD4⁺ T cells, reduced FOXP3⁺ T regulatory cells, and a global increase in neutrophil and macrophage cell infiltration (Extended Data Fig. 10c).

Following joint injection, cells engrafted into the synovial membrane remained largely at the site of injection and were detectable up to 14 days after injection (Extended Data Fig. 10d). They maintained their original cell phenotype with regards to THY1 expression (Extended Data Fig. 10e) but did not preferentially localize to any specific anatomical compartment of the synovial membrane. No significant difference in the level of engraftment or viability between injected cell populations was observed (Extended Data Fig. 10f). Collectively, these data suggest that in pathological conditions, PDNP⁺FAP⁺THY1⁺ cells assume an immune effector role capable of sustaining inflammation through the production of a distinct repertoire of chemokines and cytokines, whereas PDNP⁺FAP⁺THY1⁻ cells are bone effector cells that mediate joint damage.

In support of this conclusion, and as validation of the relevance of our findings to human disease, we identified an expanded population of PDNP⁺FAP⁺THY1⁺ immune effector fibroblasts in the synovia of patients with RA, in whom joints are persistently inflamed, compared to patients with osteoarthritis, which is a disease characterized predominantly by cartilage damage rather than inflammation (Fig. 4i). The expansion of PDNP⁺THY1⁺FAP⁺ cells positively correlated with markers of systemic and tissue inflammation (Fig. 4j).

In summary, we have identified and described the pathological significance of fibroblast heterogeneity in RA, an IMID in which inflammation and damage play key pathogenic roles. We describe discrete, anatomically distinct subsets of fibroblasts with non-overlapping effector-cell functions, including joint and cartilage damage (production of MMPs and induction of osteoclastogenesis) and immuno-inflammatory regulation (production of inflammatory cytokines and chemokines). These findings provide the underlying justification for the development of therapies that selectively target deletion or replacement of different mesenchymal subpopulations in a wide range of diseases.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-1263-7.

Received: 22 July 2018; Accepted: 2 May 2019;
Published online 29 May 2019.

1. Baker, K. F. & Isaacs, J. D. Novel therapies for immune-mediated inflammatory diseases: What can we learn from their use in rheumatoid arthritis, spondyloarthritides, systemic lupus erythematosus, psoriasis, Crohn’s disease and ulcerative colitis? *Ann. Rheum. Dis.* 77, 175–187 (2018).
2. Smolen, J. S. & Aletaha, D. Rheumatoid arthritis therapy reappraisal: strategies, opportunities and challenges. *Nat. Rev. Rheumatol.* 11, 276–289 (2015).
3. Croft, A. P. et al. Rheumatoid synovial fibroblasts differentiate into distinct subsets in the presence of cytokines and cartilage. *Arthritis Res. Ther.* 18, 270 (2016).
4. Mizoguchi, F. et al. Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. *Nat. Commun.* 9, 789 (2018).
5. Stephenson, W. et al. Single-cell RNA-seq of rheumatoid arthritis synovial tissue using low-cost microfluidic instrumention. *Nat. Commun.* 9, 791 (2018).
6. Gerlag, D. M., Norris, J. M. & Tak, P. P. Towards prevention of autoantibody-positive rheumatoid arthritis: from lifestyle modification to preventive treatment. *Rheumatology* 55, 607–614 (2016).
7. Pap, T., Müller-Ladner, U., Gay, R. E. & Gay, S. Fibroblast biology, *Role of synovial fibroblasts in the pathogenesis of rheumatoid arthritis.* *Arthritis Res. Ther.* 20, 361–367 (2000).
8. Osbelt, C. & Gay, S. The role of resident synovial cells in destructive arthritis. *Best Pract. Res. Clin. Rheumatol.* 22, 239–252 (2008).
9. McGuckin, H. M., Butler, L. M., Buckley, C. D., Rainger, G. E. & Nash, G. B. Tissue stroma as a regulator of leukocyte recruitment in inflammation. *J. Leukoc. Biol.* 91, 385–400 (2012).
10. Choi, L. Y. et al. Stromal cell markers are differentially expressed in the synovial tissue of patients with early arthritis. *PloS ONE* 12, e0182751 (2017).
11. Filer, A. The fibroblast as a therapeutic target in rheumatoid arthritis. *Curr. Opin. Pharmacol.* 13, 413–419 (2013).
12. Kollias, G. et al. Animal models for arthritis: innovative tools for prevention and treatment. *Ann. Rheum. Dis.* 70, 1357–1362 (2011).
Fig. 4 | Fibroblast subsets are responsible for different aspects of disease pathology. a, FAPα−/THY1− and FAPα+/THY1+ cells isolated from day 9 STIA synovia were stimulated and analysed using luminex. P ≤ 0.0001, except: CXCL12, P = 0.0002; CCL2, P = 0.0001; CCL7, P = 0.0028; CXCL12, P = 0.0016; IL-6, P = 0.0013; MMP9, P = 0.0019 (n = 6 mice per group). b, c. Secretion of RANKL and OPG (b, n = 6 mice per group; RANKL, P = 0.007 and Ratio, P = 0.001) and quantification of the synovial RANKL+THY1−/+ cells by flow cytometry (c; numbers, percentage of cells; n = 8 mice per group; P ≤ 0.0001). d. Effect of FAPα−/THY1− and FAPα+/THY1+ cells on the number of osteoclasts (N. Oc; n = 4 mice per group; P ≤ 0.0001) and matrix degradation as assessed by resorption area (black) of Osteo Assay plates (left) and quantified as percentage degradation (n = 8 mice per group; P = 0.053). e. Effect of intra-articular (IA) injection of PDPN−/FAPα+ THY1− or PDPN−/FAPα+ THY1+ cells into the ankle joints of day 3 STIA mice compared to contra-lateral sham-injected joints, with AUC analysis (n = 8 mice per group; P ≤ 0.0001). f. Flow cytometric analysis of absolute number of leukocyte subsets isolated from the digested synovia of injected joints at day 12 (n = 8 mice per group). Leukocytes THY1− versus THY1+, P = 0.0002; THY1+ versus sham, P ≤ 0.0001; neutrophils THY1+ versus THY1− and THY1+ versus sham, both P ≤ 0.0001; macrophages THY1+ versus sham, P = 0.0133. g, h. Representative images at day 12 (g) and quantification of micro-CT data and histomorphometric analysis of osteoclast number, cartilage damage, bone erosion and synovial pannus (h; n = 16 mice per group; area destained cartilage THY1− versus sham, P = 0.0026, all others P ≤ 0.0001). Scale bar, 100 μm. i. Mass cytometry (CyToF) analysis of PDPN−/FAPα+ and THY1+/− cells in synovium of patients with osteoarthritis (OA) and RA (n = 15 OA, n = 8 RA patient samples). j. Spearman’s correlation between THY1−/FAPα− cells in RA and serum erythrocyte sedimentation rate (ESR; P = 0.006) and synovial Krenn score (P = 0.008). n = 8 RA patient samples. Statistics: two-tailed paired Student’s t-test (a–d, i); one-way ANOVA with Dunnett’s post hoc test compared to sham injected (e, f); two-tailed paired Student’s t-test (AUC, h). Data are mean ± s.d., except c, d, f, i and AUC analysis in e, which are shown as box plots (centre line, median; box limits, upper and lower quartiles; whiskers, maximum and minimum values).
13. Roberts, E. W. et al. Depletion of stromal cells expressing fibroblast activation protein-α from skeletal muscle and bone marrow results in cachexia and anemia. J. Exp. Med. 210, 1137–1151 (2013).

14. Street, K. et al. Slingshot: cell lineage and pseudotime inference for single-cell transcriptomics. BMC Genomics 19, 477 (2018).

15. Zhang, F. et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. Preprint at https://www.biorxiv.org/content/10.1101/351130v1 (2018).

16. Butler, A., Hoffman, P., Smibert, P., Papalexi, E. & Satija, R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. Nat. Biotechnol. 36, 411–420 (2018).

Acknowledgements A.P.C. was supported by a Wellcome Trust Clinical Career Development Fellowship no. WT104551MA; A.F. was supported by Arthritis Research UK Clinician Scientist Fellowship no. 18547; F.B. was supported by an Arthritis Research UK Senior Fellowship; H.M.M. was supported by an Arthritis Research UK Career Development Fellowship (19899); C.W. was supported by a Deutsche Forschungsgemeinschaft (DFG) Fellowship (ref. 319464273); A.J.N was supported by a Versus Arthritis Career Development Fellowship no. 21743; K.W. was supported by Rheumatology Research Foundation Scientist Development Award; K.J. was supported by a Wellcome Trust PhD studentship; S.N.S. and M.A. are supported by the Kennedy Trust for Rheumatology Research. K.D. was supported by a Deutsche Forschungsgemeinschaft (DFG) award CRC1181. This work was supported by the Arthritis Research UK Rheumatoid Arthritis Pathogenesis Centre of Excellence no. 20298 (RACE); The National Institutes of Health Accelerating Medicines Partnership in RA/SLE and Arthritis Research UK programme grant no. 19791 (to C.D.B.). This paper presents independent research supported by the NIHR Birmingham Biomedical Research Centre at the University Hospitals Birmingham NHS Foundation Trust and the University of Birmingham. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, our funding bodies or the Department of Health.

Reviewer information Nature thanks Jason Cyster, Thomas A. Wynn and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Author contributions A.P.C. conceived the project, performed experiments, analysed data and wrote the manuscript. J.C. performed experiments, analysed data and helped to write the manuscript. K.J. analysed the single-cell RNA sequencing data and helped to write the manuscript. J.D.T. performed flow cytometry on human synovial biopsy tissue. J.M. performed immunofluorescence microscopy. M.A. performed single cell capture and library preparation. L.S. performed tissue histology and microscopy. C.W. and A.J.N. performed osteoclast differentiation assays. S.K. assisted with the CIA experimental arthritis model. J.B. performed micro-CT analysis. K.D. performed flow cytometry from CIA mouse joints. H.P. generated serum from KBxN mice. F.B. and H.M.M. helped in the design and interpretation of experimental mouse data. D.T.F. generated the FAPα-DTR mouse. K.W. performed and analysed mass cytometry of human synovial biopsy tissue. S.R. and I.K. helped generate, analyse and interpret human single-cell transcriptomic data. M.B.B. and M.C. provided critical interpretation of experimental data. S.N.S. supervised the design, execution, analysis and interpretation of the single-cell transcriptomics experiments and helped to write the manuscript. A.F. participated in study design, patient recruitment, sample acquisition and review of the data. C.D.B. conceived the project, supervised the work, analysed data and co-wrote the manuscript. All authors discussed the results and commented on the manuscript.

Competing interests The authors declare no competing interests.

Additional information Extended data is available for this paper at https://doi.org/10.1038/s41586-019-1263-7.

Supplementary information is available for this paper at https://doi.org/10.1038/s41586-019-1263-7.

Reprints and permissions information is available at http://www.nature.com/reprints.

Correspondence and requests for materials should be addressed to C.D.B.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2019
METHODS

Data reporting. For mice experiments, assessors were blinded to allocation, and experiments were randomized. Statistics were performed to determine sample size; see Reporting Summary for further details.

Human subjects research. Human subjects research was performed according to the Institutional Review Boards at Partners HealthCare, Hospital for Special Surgery and the West Midlands and Black Country Research Ethics Committee via approved protocols with appropriate informed consent. The study was compliant with all relevant ethical regulations. Sympathetic tissues and clinical outcome data of patients included in the early arthritis patient cohort in Birmingham (BEACON) were used in this study.10 All patients were naïve to treatment with disease-modifying anti- rheumatic drugs (DMARDs) and corticosteroids at inclusion. Control sympathetic tissue was obtained from uninflected joints of patients with joint pain but normal imaging studies undergoing exploratory arthroscopy. Samples from patients with osteoarthritis were obtained from arthroplasty procedures and tissue from patients with RA obtained by ultrasound guided synovial biopsy as previously described10,15.

Human synovial tissue processing, histological analysis and immunofluorescence staining. Sympathetic tissue samples were frozen in Tissue-Tek OCT medium (Miles) or formalin fixed and paraffin embedded (FFPE).

For immunohistochemistry, antigen retrieval was performed at pH 9 on FFPE sections using Tris-EDTA, 0.05% Tween 20 (10 mM Tris Base, 1 mM EDTA Solution, 0.05% Tween 20). Sections were stained using anti-FAPα (R&D Systems) and anti-goat horseradish peroxidase (HRP) (Dako). HRP staining was developed using the ImmPACT DAB Peroxidase HRP Substrate (Vector Labs). Images were acquired using an Axioscan and analysed with Zen lite 2012 software (Zeiss). Number of pixels was quantified and divided by a manually defined tissue area and the average number of pixels per unit area was calculated.

For immunofluorescence, acetone-fixed frozen sections were incubated with anti-FAPα (F11-24, ebioscience), anti-PDNP (NZ-1.3, ebioscience) and anti-THY1 (Thy-1A1, R&D Systems). These were detected with goat anti-mouse IgG1 FITC, anti-mouse IgG2a TRITC and anti-mouse IgG2b Cy5 (all Southern Biotechnology). To increase signal from FITC-channel, goat anti-FITC Alexa-488 anti-body (Invitrogen) was used. Images were acquired using a Zeiss LSM 510 confocal microscope and ZEN pro 2011 imaging software.

Human fibroblast cell culture. Primary human fibroblasts were isolated as described17 and cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Sigma-Aldrich) with 2% fetal bovine serum (FBS) (Gibco), 2 mM L-glutamine, antibiotics (penicillin and streptomycin), and essential and non-essential amino acids (Life Technologies). Fibroblast lines at passage 3 or 4 were used for in vitro experiments.

Enzymatic digestion of human synovial tissue. Sympathetic tissue samples were digested down to single cell suspension as previously described17. Sympathetic tissue fragments were separated using Liberase TL (100 μg/ml; Roche) with 2% fetal bovine serum (FBS; Gemini), 2 mM l-glutamine, antibiotics (penicillin and streptomycin), and essential and non-essential amino acids (Life Technologies). Fibroblast lines at passage 3 or 4 were used for in vitro experiments.

Mass cytometry on human synovial cells. Cryopreserved disaggregated human synovial cells were thawed into RPMI + 10% FBS (HyClone). Viability was assessed and cells were stained with primary antibody cocktails at 1:100 dilution (CD45, metal 89Y, clone HI30; PDNP, metal 156Gd, clone NC-08; FAP, metal 147Sm, Poly; THY1, metal 162Dy, clone SE 10). All antibodies were obtained from the Longwood Medical Area CYTOF Antibody Resource Core. Cells were fixed and permeabilized using the ebioscience Transcription Factor Fix/Perm Buffer followed by staining for intracellular markers. Cells were re-fixed in formalin (Sigma-Aldrich), washed with Milli-Q water, and analysed on a Helios (Fluidigm).

Mass cytometry data were normalized using EQ Four Element Calibration Beads (Fluidigm) viaSNe analyses were performed on cytometry data, using the Barnes-Hut SNE implementation on Cytobank (www.cytobank.org). All biaxial gating was performed using FlowJo v.10.0.7.

Histological analysis of human synovial biopsy tissue. Haematoxylin and eosin (H&E)-stained sections of synovial biopsy tissue samples were scored for the severity of inflammatory infiltrate using the inflammatory component of the Krenn synovitis score19. Inflammatory infiltrates were graded from 0 to 3 (0, no inflammatory infiltrate; 1, few mostly perivascular situated lymphocytes or plasma cells; 2, numerous lymphocytes or plasma cells sometimes forming follicle-like aggregates; 3, dense band-like inflammatory infiltrate or numerous large follicle-like aggregates). Sections were scored by two blind individuals and then provided consensus.

Mice. All animal experiments were approved by the UK Home Office and conducted in accordance with the UK’s Animals (Scientific Procedures) Act 1986 and the UK Home Office Code of Practice. The project and experimental protocols were approved by the University of Birmingham Animal Ethics Review Committee, who provided ethical oversight of the study.

C57BL/6 mice were purchased from Charles River and DBA/1 mice from Envigo. FAPα-DTR transgenic (Tg) embryos were a gift from D. Fearon, generated as previously described13. ROSA26tm1GSC mice were purchased from Jackson laboratory and bred in the unit. All mice were housed at a barrier and specific pathogen-free facility at the Biomedical Services Unit, University of Birmingham. All mice used in experimental studies were male or females aged 8-10 weeks. Single animals were considered as experimental units.

In vivo imaging. FAPα-DTR mice were injected intraperitoneally with 150 μg/g body weight o-Lucifer (PerkinElmer) and serially imaged using IVIS (Xenogen). The count data was normalized and expressed as radiance units of photons/second/cm²/sr (normalized bioluminescence) using Living Image software v.4.7 (PerkinElmer) and presented as percentage change from baseline signal.

Diphtheria toxin-mediated deletion of FAPα-expressing cells. Diphtheria toxin (List Biological Laboratories) was administered by intraperitoneal injection of FAPα-DTR mice (25 ng/g), twice a day, both in prophylactic (at day −7 and −5 before STIA) and in therapeutic regimes (at days 3 and 5 or at days 7 and 9 at days 10 and 12 after STIA). For the persistent inflammatory arthritis model, diphtheria toxin injections (25 ng per g body weight) were performed on day 3 and day 5 initially and then once a week. For local deletion of FAPα Tg cells, diphtheria toxin (5ng per g body weight) was administered by intra-articular injection into the tarsal-tibial joint at day 4 and 6 after STIA. Sterile water was used as vehicle control for injections.

Mouse models of inflammatory arthritis. STIA was induced by intravenous injection of 100 μl arthritogenic serum from KRN mice (K/BxN)12. Ankle or wrist joint thickness was monitored using callipers and reported as the change from baseline. In the persistent model of arthritis, mice were administered 100 μl arthritogenic serum intravenously at day 0 and then 50 μl once a week. Severity of joint swelling was quantified using the UAI analysis of serial measurements.

For BrdU incorporation, mice were injected with 100 μl of 10 mg/ml BrdU in PBS and then kept on BrdU-containing drinking water (0.8 mg/ml).

For collagen-induced arthritis model DBA/1 mice were immunised with 100 or 200 μl of rat CII emulsified 1:1 in complete Freund’s adjuvant (CFA; Difco, containing Mycobacterium butyricum, 0.5 mg/ml or Freund’s incomplete adjuvant (IFA) containing Mycobacterium tuberculosis H37Ra (Difco; 3.33 mg/ml). Mice were boosted 3 weeks later with 100 μg CII in IFA.

Mouse synovial tissue digestion. Bones with intact joints were dissected and transferred into RPMI-1640 (+ 2% FCS) containing 0.1 g/ml collagenase D (Roche), 0.01 g/ml of DNase I (Sigma-Aldrich). Samples were incubated at 37 °C, 40 min, followed by incubation with medium containing 0.1 g/ml collagenase dispase (Roche) and 0.01 g/ml DNase I at 37 °C for 20 min.

Flow cytometry and cell sorting. Cells were stained at 4 °C and dead cells excluded using Zombie Yellow staining (BioLegend). Peripheral blood was collected by cardiac puncture into EDTA tubes, centrifuged and red cell lysis performed before staining. Antibodies were used to anti-CD45 (30-F11), anti-THY1 (53-2.1), anti-podoplanin (8.1.1, streptavidin–APC (17–4317–82), anti-CD31 (900), anti-CD11b (M1/70), anti-SiglecF (1RNM44N), anti-CD11c (N418), anti-ITGA7 (334908), anti-αCTLA4 (F11-24, eBioscience), anti-PDPN (NZ-1.3, eBioscience) and anti-CD132 (2H7, eBioscience) for cell surface markers immediately post sorting. Purity was determined by reanalysis for the target population based on cell surface markers immediately post sorting. Purity was >99% for each target population.

Generation and analysis of droplet-based single-cell RNA sequencing data. Following sorting, CD45 live synovial cells isolated from hind limbs of day 9 STIA inflamed mouse joints (n = 3 biological replicate samples, each consisted of cells isolated from the joints of three animals) were captured with the 10x Genomics Chromium system. Sequencing libraries were generated using the 10x Genomics Single Cell 3 Solution (version 2) kit and subjected to Illumina sequencing (HiSeq 4000, read 2 sequence to 75 bp). Alignment, quantitation and aggregation of sample count matrices was performed using the 10x Genomics Cell Ranger pipeline (v2.1.0) and mouse reference sequences (v2.1.0), retaining a median of 59,900 reads per cell (mapped-read depth normalization applied). To circumvent known index-hopping issues with the HiSeq 4000 platform20 cell barcodes common to both the samples were removed from the aggregated count matrix. The UMI count data was transferred to a common median number of per-cell counts between the samples. Downstream analysis was performed using the Seurat package (v2.3.0) as follows. Cells with greater than 5% mitochondrial reads or fewer than 500 genes were excluded from the analysis. Cells were downscaled to a
In order to amplify small amounts of cDNA without introducing amplification bias, TaqMan PreAmp Master Mix was used according to manufacturer’s instructions for flow-sorted cell samples.

Reverse transcription with Quantitative PCR (RT–qPCR) was performed using Taqman assays and Taqman universal Mastermix (from Applied Biosystems) on a real-time PCR detection system (CFX96 Touch Real-Time PCR Detection System).

Expression levels were normalized to an internal housekeeping gene (RPLP0) for human. Fold changes were calculated as $2^{-\Delta\Delta C_{\text{target}}}$, where $\Delta\Delta C_{\text{target}}$ refers to the cycle threshold for the target gene amplification and $C_{\text{norm}}$ refers to the cycle threshold for the housekeeping reference gene. TaqMan primer or probes (Applied Biosystems) used were Fap (human; Hs00990791_m1; mouse; Mm01329771_m1), Pdpx (Mm01348912_g1), Thy1 (Mm00493681_m1), Cd34 (Mm00519283_m1), Cd248 (Mm00547485_s1), Cdh11 (Mm00515566_m1), Pdgfra (Mm00440701_m1), Vandalone (Mm01320970_m1), Gdf5 (Mm00433113_m1), Csf2 (Mm00290962_g1), Csf7r (Mm00424124_m1), Ctsk (Mm01302427_m1), Cd11f (Mm02519783_m1), Csf5 (Mm00442160_m1), Cd11f (Mm00442185_s1), Csf5 (Mm00446499_m1), Il18 (Mm00434226_m1), Ptgs2 (Mm00478374_m1), Ptg (Mm00451205_m1), Pdgf (Mm01284582_m1), Tnfsf11 (Mm00441906_m1), Mpn3 (Mm00440295_m1), Mmp9 (Mm00442991_m1), Mmp13 (Mm00439941_m1), Csk6 (Mm00484039_m1), Runx2 (Mm00510584_m1), Spp1 (Mm00346767_m1), Acp5 (Mm00475698_m1), Tnfsf11a (Mm00475327_m1), Sost (Mm00470479_m1), Bglap (Mm03431826_m1), Dmp1 (Mm01208363_m1), Il6 (Mm00446190_m1), Tnf (Mm00434228_m1), Il10 (Mm01288386_m1), Inos (Mm00440502_m1) and Arg1 (Mm00475988_m1).

Mouse tissue histology and immunofluorescence staining. Mouse legs were fixed for 24 h in 10% formalin solution (Sigma-Aldrich) and decalcified in 10% EDTA (pH 7.4). Samples were embedded in paraffin, sectioned and H&E staining was performed at the Royal Orthopaedic Hospital Pathology Laboratories according to the standard protocol. Safranin O staining for cartilage was performed as previously described.

Antigen retrieval was performed on FFPE tissue sections; 0.05% trypsin-EDTA for PDPP, FAPs and cathepsin K staining at 37 °C, or citrate buffer pH 6 (Dako) at 58 °C for F4/80 staining. Sections were stained with primary antibodies: PDPP (eBioscience), FAPs (R&D), F4/80 (BioRad), CD146 (Abcam) or cathepsin K (Abcam). Secondary antibodies used were: biotin goat anti-hamster IgG antibody (Vector Labs); biotin rabbit anti-sheep IgG antibody (BioLegend); biotin rabbit anti-sheep IgG (Vector Labs) biotin goat anti-rabbit IgG (Vector Labs). Tissue sections were imaged using the AxioScanz.I slider scanner and analysed using Zen light 2012 software. Quantification by pixel counts was performed using Imaging J. Tissue sections were fixed with 10% buffered Formalin and embedded in paraffin for histological processing.

For immunofluorescence, limbs were placed in a cryomold, embedded in OCT, and sectioned at 4 μm. Sections were stained with primary antibodies (anti-F4/80, anti-vimentin, anti-S100, anti-Ctsk, anti-Mmp3, anti-Mmp9, anti-Mmp13) using the Dako Envision+ detection kit (Dako). Sections were counterstained with DAPI. Images were acquired using a Zeiss LSM780 confocal microscope and ZEN pro 2011 imaging software.

Histomorphometry scoring. Analysis and cell counting of H&E and Safranin O was performed on images from whole joint tissue sections as previously described.22 Leukocyte infiltration was scored 0–3 (0, normal; 1, minimal infiltration; 2, moderate infiltration; 3, marked infiltration). Osteoclasts were identified by both morphology and cathepsin K staining. Scoring and measurements were performed by two independent blinded assessors on a consistent region of the ankle joint and on three different cutting levels and expressed as a mean of these measurements.

Adoptive transfer of fibroblasts. Live sorted, purified cells (500,000, from day 9 STIA) were injected into the inflamed talo-tibial joint of day 3 STIA recipient mice. The contralateral joint was injected with PBS. For CIA, cells were sort-purified and injected into the talo-tibial joint of DBA/1 mice with CIA at the first sign of arthritis, under the same experimental conditions described above.

Tracking of adoptively transferred cells in vivo. Cells were sort-purified from digestively naïve of day 9 STIA ROSA26LacZ mice and injected as described above. Collected tissue was mounted in OCT, frozen, sectioned and fixed in acetone. For immunofluorescence, cells were labelled with a cell line dye (Invitrogen) according to the manufacturer’s instructions before injection.

Micro-CT analysis. Hind limbs were imaged using a SkyScan 1172 micro-CT scanner (Bruker) using settings and reconstruction algorithms using MeshLab, v.1.3.2.
Micro-CT meshes were divided into three regions: heel (compromising the calcaneus, centrale, distal tarsals, tibulae and astagalus and distal tibia and fibula), metatarsals and phalanges (excluding the claws). Each region was scored for erosion (0, normal; 1, roughness; 2, pitting; 3, full thickness holes) and the extent of the area affected (0, none; 1, a few small areas; 2, multiple small–medium areas; 3, multiple medium–large areas). The two scores were then multiplied together for each region. With the exception of local deletion and intra-articular cell transfer studies, micro-CT scores from both the front and hind limb were combined as an average for each mouse.

**Statistical analysis.** Statistical analysis was performed as described in each section using Prism 8 software. Unless otherwise stated, data are presented as mean ± s.d. from data obtained from at least two independent experiments. Parametric and non-parametric analyses were used where appropriate based on testing for a normal distribution using the D’Agostino–Pearson omnibus normality test. Differences were considered to be significant when $P < 0.05$. Multiple testing corrections were applied where appropriate.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability**
STIA single cell and bulk fibroblast RNA sequencing data that support the findings of this study have been deposited in Gene Expression Omnibus (GEO) with the accession codes GSE129087 and GSE129451. Source Data for Figs. 1, 2, 4 and Extended Data Figs. 1–3, 10 are provided with the online version of the paper.

**Code availability**
The source code repository of the computational pipeline for single-cell data analysis and integration is located at https://www.github.com/sansomlab/tenx/.

17. Filer, A. et al. Identification of a transitional fibroblast function in very early rheumatoid arthritis. *Ann. Rheum. Dis.* **76**, 2105–2112 (2017).
18. Donlin, L. T. et al. Methods for high-dimensional analysis of cells dissociated from cryopreserved synovial tissue. *Arthritis. Res. Ther.* **20**, 139 (2018).
19. Krenn, V. et al. Synovitis score: discrimination between chronic low-grade and high-grade synovitis. *Histopathology* **49**, 358–364 (2006).
20. Sinha, R. et al. Index switching causes ‘spreading-of-signal’ among multiplexed samples in Illumina HiSeq 4000 DNA sequencing. Preprint at https://doi.org/10.1101/125724 (2017).
21. Tirosh, I. et al. Single-cell RNA-seq supports a developmental hierarchy in human oligodendrogioma. *Nature* **539**, 309–313 (2016).
22. Ashburner, M. et al. Gene Ontology: tool for the unification of biology. *Nat. Genet.* **25**, 25–29 (2000).
23. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21 (2013).
24. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
25. Luo, W., Friedman, M. S., Shedd, K., Hankenson, K. D. & Woolf, P. J. GAGE: generally applicable gene set enrichment for pathway analysis. *BMC Bioinformatics* **10**, 161 (2009).
26. Ross, E.A., et al. Treatment of inflammatory arthritis via targeting of tristetraprolin, a master regulator of pro-inflammatory gene expression. *Ann. Rheum. Dis.* **76**, 612–619 (2017).
27. Wehmeyer, C. et al. Sclerostin inhibition promotes TNF-dependent inflammatory joint destruction. *Sci. Transl. Med.* **8**, 330ra35 (2016).
Extended Data Fig. 1 | Mouse synovial FAPα expression.

a. Expression of PDPN and FAPα by immunohistochemistry in ankle joints (representative of n = 8 mice). b–e. Flow cytometry of digested synovia. Gating strategy for synovial fibroblasts in digested synovia (b) representative expression of PDPN and FAPα during STIA (c), corresponding absolute numbers of FAPα+ cells (n = 10 mice per group) (d), and plot of FAPα expression in THY1− (LL, blue) or THY1+ (SL, red) PDPN+ cells and PDPN−/THY1+ pericytes (black) in day 9 STIA synovia (e). Each plot is representative of n = 12 mice; numbers represent the percentage of cells. f. Quantification of Fap transcript expression in sort-purified cells (day 9 STIA synovia, n = 12 mice). g. Immunofluorescence staining for FDPN, FAPα and THY1 expression in day 9 STIA ankle joints (representative of n = 12 mice). h–j. Spearman’s correlation analysis between the total (black), LL (blue) and SL (red) FAPα expressing cells quantified by flow cytometry and the change in ankle joint thickness (h), cartilage destruction (i) and bone erosion (j; cartilage destruction and bone erosion assessed by histology) (n = 40 mice). k, l, Representative bioluminescence of in vivo imaging of FAPα-DTR+ mice treated with diphtheria toxin or vehicle (Veh) (k) and quantification of bioluminescence (l; n = 8 mice per group). m. Quantification of synovial FAPα+ cells following administration of either diphtheria toxin or vehicle (n = 8 mice per group). n. Immunohistochemistry staining of FAPα (red) expression in ankle joints following diphtheria toxin (representative of n = 8 mice). o. Total number of CD45−/CD31− cells by flow cytometry in day 9 STIA synovia compared to non-arthritis (control) mice following diphtheria toxin (n = 7 mice per group). p. H&E staining and quantification of cellularity following diphtheria toxin treatment. Arrow indicates synovial membrane. Data are expressed as the average number of cells per quantified per histological section (n = 8 mice per group).

Statistics: two-way ANOVA with Tukey’s post hoc test (f, m, o, p). Data are mean ± s.d., except in f, m, o, which are shown as box plots (centre line, median; box limits, upper and lower quartiles; whiskers, maximum and minimum values).
Extended Data Fig. 2 | Effects of FAPα cell deletion. a–c. Change in wrist and ankle joint thickness during STIA with AUC analysis following treatment with diphtheria toxin (FAPα cell deletion) at days 5 and 7 (a), and days 10 and 12 (b), or prophylactically before the induction of STIA (c) (all, n = 8, mice per group). d. Representative time-course analysis of structural joint damage assessed by micro-CT following treatment with diphtheria toxin (FAPα cell deletion) at days 3 and 5 following induction of STIA. e. Quantification of bone erosion and new bone formation (e; n = 8 mice per group), combined for front and hind paws. f. Histological examination of ankle joint tissue sections at day 12 STIA with quantification of bone erosion, pannus formation and bone formation (all by H&E) and cartilage destruction (by safranin O staining) (n = 12 mice per group). g. Representative images of cathepsin K immunohistochemical staining of osteoclasts (brown) in the ankle joints of day 12 STIA mice. h. Number of osteoclasts (cathepsin K positive) per tissue section in DTR− versus DTR+ mice at day 12 STIA compared to non-arthritis control mice (n = 12 mice per group). i. Expression of bone turnover markers including osteoclast and osteoblast markers in whole paw tissue analysed by RT–qPCR (n = 8 mice per group, data are expressed as mean fold change in expression compared to expression in non-arthritis mice). Statistics: Mann–Whitney test (a–c); two-way ANOVA with Tukey’s post hoc test (e); one-way ANOVA with Tukey’s post hoc test (f, h). Data are mean ± s.d., except AUC analysis in a–c, which are shown as box plots (centre line, median; box limits, upper and lower quartiles; whiskers, maximum and minimum values).
Extended Data Fig. 3  | Effect of FAPα cell deletion on leukocyte infiltration.  
a. Flow cytometry plot of peripheral blood monocytes (numbers, percentage of positive cells) with quantification at day 9 STIA following diphtheria toxin at day 3 and day 5 (n = 6 mice per group).  
b. Flow cytometry gating strategy for leukocyte populations in digested synovia.  
c. Numbers of leukocytes and percentage of MHC class II expressing F4/80 cells (M1) in hind limb joints of day 28 persistent STIA mice analysed by flow cytometry (n = 13 mice per group).  
d. Representative immunohistochemical staining of macrophages (F4/80+, brown; nuclei, blue) in the ankle joint tissue sections at day 12 STIA mice following diphtheria toxin at day 3 and 5 (representative of n = 6 mice).  
e. Percentage of F4/80+ macrophages staining positive for MHC class II as detected by flow cytometry in day 12 STIA digested synovia from DTR−/− mice following diphtheria toxin at day 3 and 5 (n = 13 mice per group).  
f. Expression of functional macrophage markers detected by RT-qPCR in sort-purified macrophages (CD45+CD11b+F4/80) isolated from the synovia of day 12 STIA mice following diphtheria toxin at day 3 and 5 (n = 7 mice per group).  
g. Number of FAPα expressing cells quantified by flow cytometry from digested synovia (n = 8 mice), popliteal (draining) and mesenteric (non-draining) lymph nodes (n = 6 mice) following intra-articular administration of diphtheria toxin into the ankle joint during STIA (harvested day 14) (g) and daily change in weight from baseline (expressed as percentage of original body weight) in STIA mice compared to non-arthritic mice (h) (n = 6 mice).  
i. Effect of local deletion of synovial FAPα-expressing cells (by intra-articular injection of diphtheria toxin) on ankle joint thickness in the resolving model of STIA model when compared to the wrist joints on the same mouse (non-deleted limbs) (n = 8 mice) and to non-arthritic DTR−/− and DTR−/− injected mice (n = 6 mice per group) with AUC analysis.  
j. Representative micro-CT images of day 14 STIA and non-arthritic control mice following intra-articular injection of diphtheria toxin and quantification of bone erosion and new bone formation (STIA DTR− n = 10, STIA DTR− n = 13, DTR− and DTR− n = 8).  
k. Quantification of the number of fibroblasts and leukocytes in digested synovia of day 9 STIA mice analysed by flow cytometry following intra-articular administration of diphtheria toxin (n = 8 mice).  
Statistics: two-way ANOVA with Tukey’s post hoc test (a, c, e, j, k), two-tailed paired Student’s t-test (f, g), one-way ANOVA with Tukey’s multiple comparison tests (i, j). Data are mean ± s.d., except in a, c, e, f, g, k and AUC analysis in i, which are shown as box plots (centre line, median; box limits, upper and lower quartiles; whiskers, maximum and minimum values).
Extended Data Fig. 4 | 10X Chromium single-cell RNA sequencing (droplet-based single-cell) analysis of CD45− cell populations from inflamed synovium. a, t-SNE projection of non-haematopoietic stromal cells from the inflamed mouse joint (n = 3 biological replicates, day 9 STIA) showing the initial automatic cluster assignments from Seurat (projection is identical to that shown in Fig. 3a). b, The same t-SNE plot coloured by biological replicate. c, The bar plot shows the number of cells in each cluster stratified by replicate. d, Cluster cell identification: the five panels of violin plots show expression (normalized, log-transformed counts of the cells from all of the n = 3 biological replicates, y axes) of known cell-type marker genes (for fibroblasts, LL fibroblasts, osteoblasts, chondrocytes and vascular cells) in each of the automatically assigned clusters (x axes). The colours of violin plots correspond to those shown in Extended Data Fig. 4a.
Extended Data Fig. 5 | Continued cluster identification analysis. The four panels of violin plots show expression (normalized, log-transformed counts of the cells from all of the $n = 3$ biological replicates, $y$ axes) of known cell-type marker genes (for pericytes, muscle cells, erythrocytes and the cell cycle) in each of the automatically assigned clusters ($x$ axes). The colours of violin plots correspond to those shown in Extended Data Fig. 4a.
Extended Data Fig. 6 | Differential gene expression between fibroblast clusters. The heat map shows the (row-scaled) expression of the top-20 (by P value) discovered significant, conserved marker genes for each cluster (Benjamini–Hochberg adjusted $P < 0.1$ in separate tests of cells from each of the $n = 3$ biological replicate samples, two-sided Wilcoxon tests). Each column represents a single fibroblast and each row shows the given gene. The cluster identification is indicated for each column. LL fibroblasts correspond to F5 and are PDPN$^+$ THY1$^-$, and SL fibroblasts correspond to F1–F4 fibroblast subsets and are PDPN$^+$ THY1$^+$. 
Extended Data Fig. 7 | Differential gene expression in specific fibroblast clusters. 

a, A set of violin plots showing gene expression (normalized, log-transformed counts of the cells from all of the $n = 3$ biological replicates, $x$ axes) of additional fibroblast markers in each of the F1–F5 fibroblast clusters ($y$ axes) (corresponds to Fig. 3b).

b, t-SNE projection of fibroblasts from the inflamed mouse joint coloured by replicate (corresponds to Fig. 3b).

c, A set of violin plots showing gene expression (normalized, log-transformed counts of the cells from all of the $n = 3$ biological replicates, $x$ axes) of known markers for chemokines in each of the F1–F5 fibroblast clusters ($y$ axes) (corresponds to Fig. 3b).

d, Number of cells in each cluster stratified by replicate.
Extended Data Fig. 8 | Trajectory analysis and identification of fibroblast subpopulations from human RA patients. a, The heat map shows genes most strongly up- or downregulated across the inferred F1–F2–F5 trajectory in the mouse fibroblasts from the STIA model (as determined by Slingshot\(^4\)). b–e, Reanalysis of CEL-Seq2 single-cell RNA sequencing dataset from 20 RA patients\(^5\). b, t-SNE projection of the RA patient fibroblasts indicating the automatic cluster assignments from Seurat. c, The sets of violin plots show expression (normalized, log-transformed counts, cells from all \(n = 20\) RA patients, \(x\) axes) of cluster marker genes in each of the RA patient fibroblast clusters (\(y\) axes). The violin plots are grouped into six sets comprising ‘other markers’ (known markers or markers reported by Zhang et al.\(^6\)) or of markers characteristic of each of the human RA F1–F5 clusters, as indicated. d, The same t-SNE plot as in b, coloured by patient ID. e, The bar plot shows the number of patients represented in each assigned cluster.
Extended Data Fig. 9 | Bulk RNA sequencing of sort-purified FAPα expressing LL and SL cell populations from the inflamed hind limb joints of day 9 STIA mice. a, Gating strategy for flow cytometry based cell sorting from day 9 STIA digested synovia gated on CD45−CD31− live cells. Coloured gates correspond to each sort-purified population and the percentage gated cell population is indicated. b, Principal component analysis reveals that each population clusters according to either a SL phenotype or a LL phenotype. Each dot presents a single biological replicate sample and is coloured according to the gating strategy outlined in a. c, The heat map shows the differential expression of the 50 most-significant genes (by $P$ value) for each population (Benjamini-Hochberg adjusted $P < 0.1$) and reveals distinct transcriptional profiles between THY1+ and THY1− cell populations. d, Expression of specific genes RNA sequencing in PDPN$^+$FAPα$^+$THY1− versus PDPN$^+$FAPα$^+$THY1+ sort-purified cells. For each heat map, a column represents a single biological replicate, coloured according to the gating strategy in a. Biological replicates represent cells isolated and purified from the digestion of synovia from the hind limbs of a single mouse ($n = 10$ THY1$^-$FAPα$^+$, $n = 5$ THY1$^-$FAPα$^-$, $n = 6$ THY1$^+$FAPα$^-$ and $n = 12$ THY1$^+$FAPα$^+$ samples).
Extended Data Fig. 10 | Effect of intra-articular injection of fibroblast subsets. a, Effect on ankle joint thickness of intra-articular injection of 500,000 sort-purified PDPN⁺FAP⁺⁺THY1⁻ (blue) or PDPN⁺FAP⁺⁺THY1⁺ (red) cells into the ankle joint of CIA mice at the first sign of joint inflammation (day 0) compared to contralateral sham injected joints (n = 8 mice per group). b, Representative images of micro-CT analysis and quantification of bone erosion and new bone formation (n = 8 mice per group). c, Flow cytometric analysis of leukocytes in the digested synovia of injected joints, 7 days post injection (n = 8 mice per group). d, Representative confocal microscopy of ankle joint tissue of mice injected with tomato red-expressing PDPN⁺FAP⁺⁺THY1⁻ or PDPN⁺FAP⁺⁺THY1⁺ isolated from day 9 STIA cells and injected into day 3 STIA recipient mice (representative images from n = 6 mice, 14 days post injection). e, Flow cytometric analysis of digested synovia, 14 days after injection of cell trace labelled cells (isolated from day 9 STIA mice and injected into the ankle joint of day 3 STIA recipient mice), gated on the CD45⁻CD31⁻PDPN⁺ cell fraction. Percentage of THY1⁺ (red) or THY1⁻ (blue) cell in this gated cell fraction is indicated (representative of n = 6 per group). f, Percentage of engraftment and viability of injected cells 14 days after injection into the ankle joints of STIA mice (n = 8 per treatment group, two tailed Student’s t-test). Engraftment is expressed as the percentage recovery of the original injected cell number. Statistics: one-way ANOVA with Bonferroni post hoc test (b, c) and AUC analysis (a), paired two-tailed Student’s t-test (f). Data represented as mean ± s.d., except in c, e and AUC analysis in a, which are shown as box plots (centre line, median; box limits, upper and lower quartiles; whiskers, maximum and minimum values).
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- **n/a**
- **Confirmed**

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

   Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- For flow cytometry BD FACS Diva software version 4.0 was used to acquire the data

Data analysis

- Zen lite 2012
- Zen Pro 2011
- FlowJo 10.0.7, 10.5.3
- Living Image 4.7
- Prism 8
- Zen 2010
- Adobe Photoshop 19.1.6
- ImageJ 1.51
- MeshLab 1.3.2
- Cell Ranger 2.1.0
- Seurat R package 2.3.0
- STAR 2.5.2b24
- The 10x data was analysed using the 10x pipeline (www.github.com/sansomlab/tenx/tree/master/pipelines), which utilises the cellranger (v2.1.0) and Seurat packages (v2.3.0; R v3.4.2).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
STIA single cell and bulk fibroblast RNA sequence data that support the findings of this study have been deposited in Gene Expression Omnibus (GEO) with the accession codes GSE129087 and GSE129451. Source data for figures 1, 2, 4 and extended data 1, 2, 3 and 10 are provided with the paper.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | We designed our experiments to ensure that minimum number of mice are used to obtain biologically significant results. Pilot experiments used small numbers of animals, eg 2-3 mice. For designed experiments, the level of significance was set at 5%, and the power will be set at 80%. The least practicable difference between groups was set at no less than 25%. Generally, the expected coefficient of variation (ie standard deviation/mean) was 15%. For a 4-group experiment, this means a group size of about 6 animals/group. In our pilot experiments we found an n=6 to give reproducible and significant differences between control and experimental groups. |
| Data exclusions | No data was excluded from analyses |
| Replication | All findings were successfully replicated in at least two independent experiments performed under identical conditions. |
| Randomization | Mice were randomly assigned to treatment groups by a technician blinded to the experimental design and as below the assessor was blinded to the treatment allocation group until after the data analysis stage. |
| Blinding | Investigators were blinded to the clinical scoring of mice during arthritis and blinded to the scoring of microCT images and histological analysis and scoring. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
| ☑ ☑ Antibodies |
| ☑ ☐ Eukaryotic cell lines |
| ☐ ☐ Palaeontology |
| ☐ ☑ Animals and other organisms |
| ☑ ☑ Human research participants |
| ☑ Geological samples |
| ☐ ☑ Clinical data |

Methods

| n/a | Involved in the study |
| ☑ ☑ ChIP-seq |
| ☜ Flow cytometry |
| ☑ ☑ MRI-based neuroimaging |

Antibodies

| Antibodies used |
| Antibody/Fluorochrome/Supplier/Catalogue # Clone Dilution |
| CD45 APC-eFluor 780 ebioscience 47-0451-82 / 30-F11 / 1:600 |
| CD45 PerCP-Cyanine5.5 eBioscience 45-0451-82 30-F11 1:600 |
| Thy1.2 APC-eFluor 780 eBioscience 47-0902-82 1:1000 |
| Podoplanin PE eBioscience 12-5381-82 eBio8.1.1 1:1000 |
| FAPα Unconjugated R&D systems AF3175 Polyclonal 1:50 |
| CD31 PE-Cyanine7 eBioscience 25-0311-82 1:400 |

**Validation**

Antigen Format/Fluorochrome Supplier Validation

CD45 APC-eFluor 780 eBioscience https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/47-0451-82.

CD45 PerCP-Cyanine5.5 eBioscience https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/45-0451-82.

CD11b PerCP-Cyanine5.5 eBioscience https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/45-0112-82.

Ly-6G AlexaFluor 594 BioLegend https://www.biolegend.com/fr-fr/products/alexa-fluor-594-anti-mouse-ly-6g-antibody-9634.

Ly-6G Brilliant Violet 510 BioLegend https://www.biolegend.com/fr-fr/products/brilliant-violet-510-anti-mouse-cd68-antibody-8726.

Ly-6C Brilliant Violet 510 BioLegend https://www.biolegend.com/fr-fr/products/brilliant-violet-510-anti-mouse-ly-6c-antibody-8726.

Siglec-F eFluor 660 eBioscience https://www.thermofisher.com/antibody/product/CD170-Siglec-F-Antibody-clone-1RNM44N-Monoclonal/50-1702-82.

CD64 PE/Cy7 BioLegend https://www.biolegend.com/en-gb/products/pe-cy7-anti-mouse-cd144-csf-1r-antibody-7788.

CD115 BV421 BioLegend https://www.biolegend.com/en-gb/products/brilliant-violet-421-anti-mouse-cd115-csf-1r-antibody-8971.

MHCII Brilliant Violet 711 BioLegend https://www.biolegend.com/fr-fr/products/human-cd90-thy1-antibody-thy-1a1-mab2067.

CD11c FITC eBioscience https://www.thermofisher.com/antibody/product/CD11c-Antibody-clone-N418-Monoclonal/11-0114-82.

Ki67 AlexaFluor 488 BioLegend https://www.biolegend.com/en-gb/products/alexa-fluor-488-anti-mouse-human-ki-67-antibody-12889.

CD140b PE BioLegend https://www.biolegend.com/fr-fr/products/pe-anti-mouse-cd40-antibody-6256.

ITGAV FITC eBioscience https://www.thermofisher.com/antibody/product/ITGAV-Antibody-clone-334908-Monoclonal/MA5-23653.

CD11b PerCP-Cyanine5.5 eBioscience 45-0112-82 M1/70 1:200

Ly-6G AlexaFluor 594 BioLegend 127636 1A8 1:800

Ly-6C Brilliant Violet 510 BioLegend 128033 HK1.4 1:600

Siglec-F eFluor 660 eBioscience 50-1702-82 1RMN44N 1:100

CD64 PE/Cy7 BioLegend 139314 X54-5/7.1 1:100

F4/80 PE BioLegend 123110 BM8 1:400

CD43 APC BioLegend 143208 S11 1:800

CD115 BV421 BioLegend 135513 AFS98 1:800

MHCII Brilliant Violet 711 BioLegend 107643 M5/114.15.2 1:800

CD11c FITC eBioscience 11-0114-82 N418 1:100

RANKL PE BioLegend 510006 IK22/5 1:100

Ki67 AlexaFluor 488 BioLegend 151204 11F6 1:50

CD43 APC BioLegend 143208 S11 1:800

CD115 BV421 BioLegend 135513 AFS98 1:800

F4/80 PE BioLegend 123110 BM8 1:400

CD43 APC BioLegend 143208 S11 1:800

CD115 BV421 BioLegend 135513 AFS98 1:800

CD31 PE-Cyanine7 eBioscience https://www.thermofisher.com/antibody/product/CD31-PECAM-1-Antibody-clone-390-Monoclonal/25-0311-82.

CD11b PerCP-Cyanine5.5 eBioscience https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/45-0112-82.

Ly-6G AlexaFluor 594 BioLegend https://www.biolegend.com/fr-fr/products/alex-fluor-594-anti-mouse-ly-6g-antibody-9634.

Ly-6C Brilliant Violet 510 BioLegend https://www.biolegend.com/fr-fr/products/human-cd68-antibody-8726.

CD45 PerCP-Cyanine5.5 eBioscience https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/45-0451-82.

Ly-6C Brilliant Violet 510 BioLegend https://www.biolegend.com/fr-fr/products/brilliant-violet-510-anti-mouse-ly-6c-antibody-8726.

Siglec-F eFluor 660 eBioscience https://www.thermofisher.com/antibody/product/CD170-Siglec-F-Antibody-clone-1RMN44N-Monoclonal/50-1702-82.

CD64 PE/Cy7 BioLegend https://www.biolegend.com/fr-fr/products/pe-cy7-anti-mouse-cd64-fcgammari-antibody-10062.

F4/80 PE BioLegend https://www.biolegend.com/fr-fr/products/pe-anti-mouse-f4-80-antibody-4068.

CD11b PerCP-Cyanine5.5 eBioscience https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/45-0112-82.

Ly-6G AlexaFluor 594 BioLegend https://www.biolegend.com/fr-fr/products/alex-fluor-594-anti-mouse-ly-6g-antibody-9634.

Ly-6C Brilliant Violet 510 BioLegend https://www.biolegend.com/fr-fr/products/human-cd68-antibody-8726.

CD45 PerCP-Cyanine5.5 eBioscience https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/45-0451-82.

CD11b PerCP-Cyanine5.5 eBioscience https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/45-0112-82.

Ly-6G AlexaFluor 594 BioLegend https://www.biolegend.com/fr-fr/products/alex-fluor-594-anti-mouse-ly-6g-antibody-9634.

Ly-6C Brilliant Violet 510 BioLegend https://www.biolegend.com/fr-fr/products/human-cd68-antibody-8726.

CD11c FITC eBioscience https://www.thermofisher.com/antibody/product/CD11c-Antibody-clone-N418-Monoclonal/11-0114-82.

RANKL PE BioLegend https://www.biolegend.com/fr-fr/products/pe-anti-mouse-cd254-trance--rankl-antibody-309.

BrdU FITC BD Biosciences http://www.bdbiosciences.com/eu/applications/research/intracellular-flow/intracellular-kits-sets-and-cocktails/fcitc-mouse-anti--brdu-set/p/556028.

Ki67 AlexaFluor 488 BioLegend https://www.biolegend.com/en-gb/products/alexa-fluor-488-anti-mouse-human-ki-67-antibody-12889.

CD45 APC-eFluor 780 eBioscience https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/47-0451-82.

CD45 PerCP-Cyanine5.5 eBioscience https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/45-0451-82.

Thr1.2 APC-eFluor 780 eBioscience https://www.thermofisher.com/antibody/product/CD90-2-Thy-1-2-Antibody-clone-53-2-1-Monoclonal/47-0902-82.

Podoplanin Unconjugated BioRad https://www.bio-rad-antibodies.com/monoclonal/human-podoplanin-antibody-d2-40-mca2543.html?f=concentrate.

Podoplanin Unconjugated BioRad https://www.bio-rad-antibodies.com/monoclonal/human-podoplanin-antibody-cl-a3-1-mca497.html?f=purified.

FAPα Unconjugated Abcam https://www.abcam.com/fibroblast-activation-protein-alpha-antibody-ab53066.html.
Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals

The study involved the following laboratory animals:
- C57BL/6 mice (purchased from Charles River).
- DBA/1 mice (purchased from Envigo).
- FAP-DTR transgenic mice (embryos were a gift from Prof Douglas Fearon and mice were bred in house).
- ROSAmT/mG mice (purchased from Jackson laboratory and bred in the unit).
All mice used in experimental studies were male or females aged 8-10 weeks old weighing between 20-22g.

Wild animals

The study did not involve the use of wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

The animal experiments were approved by the U.K. Home Office and conducted in accordance with the U.K.’s Animals (Scientific Procedures) Act 1986 and the U.K. Home Office Code of Practice. The project and experimental protocols were also approved by the University of Birmingham Animal Ethics Review Committee who provided ethical oversight of the study.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics

For immunohistochemistry and confocal immunofluorescence studies using human synovial biopsy tissue the following patients were included: control n=8 patients (mean age 41 years, range 23-49 years, females, n=3 (38%); resolving (mean age 44 years, range 32-55 years, females n=3 (27%); rheumatoid arthritis n=11 (mean age 53 years, range 43-70 years, females n=4 (36%))). For mass cytometry (CyToF) studies the following patients were included: patients with osteoarthritis n=15 (mean age 71 years, range 64-81 years, females n=10 (67%)) and rheumatoid arthritis n=8 (mean age 56 years, range 51-71 years, females n=5 (63%)). In addition, in the sub-analysis of human RNA single cell sequencing data the following patients were included in the dataset: patients with rheumatoid arthritis n=20 (mean age 58 years, range 38-78 years, females n=17 (85%)).

All patients were naïve to treatment with disease modifying anti-rheumatic drugs (DMARDs) and corticosteroids at inclusion. Diagnostic and prognostic outcomes were assigned after 18-months of follow-up. Patients were classified as having RA according to the 2010 ACR/EULAR classification criteria for RA or were classified as having self-limiting resolving disease if they had no clinical evidence of synovial swelling and had not taken DMARDs or received glucocorticoid treatment in any form in the preceding 3 months.

Recruitment

Synovial tissues and clinical outcome data of patients included in the early arthritis patient cohort in Birmingham (BEACON) were used in this study. BEACON is an inception cohort recruiting patients from unselected early arthritis clinics in Birmingham UK to which patients are directly referred for rapid review by primary care physicians. Patients with clinical synovitis present in at least one synovial joint (of 66 joints examined) were recruited to the cohort if symptom duration, defined as any symptom attributed by the assessing rheumatologist to inflammatory joint disease (pain, stiffness and/or swelling), was no greater than 3 months. All studies were approved by local medical ethical committees and patients gave written, informed consent to participate.

Ethics oversight

Human subjects research was performed according to the Institutional Review Boards at Partners HealthCare, Hospital for Special Surgery and the West Midlands and Black Country Research Ethics Committee via approved protocols

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

For mice, single cell samples were created from digested synovial membrane (collagenase digestion, outlined in methods) or from whole blood obtained by cardiac puncture and treated with EDTA. Red cell lysis was performed and staining performed on the remaining single cell suspension. For human tissue, single cell suspensions were acquired by enzymatic digestion of synovial tissue obtained by ultra-sound guided biopsy.
Instrument | For cell sorting MoFlo Astrios EQ machine (Beckman Coulter) was used. For flow cytometry BD LSR Fortessa machine.
Software | Data was analysed using FloJo version 10.5.
Cell population abundance | For post sort populations purity was determined by re-analysis for the target population immediately post sorting based on cell surface markers. Purity was >99% for each target population.
Gating strategy | Gating strategies for cell populations is shown in the manuscript supplementary data for each cell population. Gates were set to Floresence-1 (FMO controls).

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.