SUPPLEMENTAL MATERIAL

METHODS

Mice

Male and female mice were housed in ventilated cages of up to five animals, given *ad libitum* access to chow food and water, on a 12 h light/dark cycle. Experimental mice were 12-16 weeks old and euthanized by CO_2 asphyxia. C57BL/6J mice were used throughout, unless specified otherwise. Wnt-GFP reporter mice (TCF/Lef:H2B/GFP, Jax #013752) were used for flow cytometric analysis of synovial cells after joint injury and for isolation of primary synovial fibroblasts (SFs). To induce joint injury and PTOA, C57BL/6J or Wnt-GFP reporter mice were subjected to Sham (anesthesia and analgesia only) or tibial compression-based, non-invasive anterior cruciate ligament rupture (ACLR), as reported previously (1) and based on a modified protocol from Christiansen *et al* (2). Briefly, mice under inhaled isoflurane anesthesia were placed prone on a custom fixture on a materials testing system (Electroforce 3300, TA Instruments). The right knee was flexed to 100^o and the paw was mounted in 30^o dorsiflexion. After preloading and preconditioning, a 1.5 mm displacement was rapidly applied to the paw (10 mm/s), causing tibial subluxation and ACL rupture. Mice were then administered a single subcutaneous dose of carprofen (5 mg/kg). For R-spondin 2 delivery, mice were given intra-articular injections of recombinant mouse R-spondin 2 (500 ng, R&D Systems) or vehicle (phosphate-buffered saline, PBS) for five consecutive days using a 33G needle and microsyringe (Hamilton) in a total volume of 5 µL. Hair was removed from the hindlimbs using an electronic shaver and hair removal cream, and injections were carried out under isoflurane anesthesia. All procedures were performed according to approved IACUC protocols.

Knee hyperalgesia testing

Prior to pain testing, mice were acclimated to handling and application of the measurement device. Knee hyperalgesia was measured in a blinded fashion using a Randall-Selitto device (IITC Life Science) modified for pressure application on a mouse knee, as described previously(1). The convex tip of the pressure applicator was applied to the medial knee joint until a vocal or physical response occurred. The average reading of triplicate applications was calculated for each limb.

Near-infrared live imaging

To assess extracellular matrix remodeling in the joint, we employed live near-infrared imaging using the matrix metalloproteinase (MMP) activatable fluorescent probe MMPSense680 (Perkin Elmer). The day prior to imaging, hair was completely removed from the hindlimbs. Under isoflurane anesthesia, bilateral intra-articular injection of MMPsense680 (3) probe (4 µL per knee) was carried out using a 33G needle and microsyringe (Hamilton). Mice were recovered from anesthesia, permitted two hours of cage activity, and then near-infrared imaging of hindlimbs was performed under anesthesia using a Pearl Impulse Imaging System (LI-COR). MMPsense signal intensity was analyzed in ImageJ using a consistent defined region of interest over the right and left knee to calculate raw integrated density value

Histology

Limbs from mice injected with vehicle or R-spondin 2 were harvested 28 d after the first injection, fixed for 48 h in 10% neutral-buffered formalin, rinsed with water, decalcified for two weeks in 10% EDTA, then processed in paraffin. Sagittal sections (5 µM) spanning the medial joint, spaced ~100 µM apart, were cut and stained with Safranin-O/Fast Green (SafO) and Hematoxylin. Sections were imaged at 10x magnification on a Nikon Eclipse Ni E800 microscope with a Nikon DS-Ri2 camera. Qualitative OA and synovitis grading was performed by two blinded observers according to established grading schemes (4, 5), with the addition of a fibrosis sub-score for synovitis grading, given the recognized histological and clinical importance of fibrosis in OA (6, 7). Grading criteria for PTOA and synovitis scores can be found in

Supplementary Tables 1 and 2. Scores were averaged across sections within each specific compartment of interest, for each limb (minimum of three sections per limb) to obtain mean limb scores for each independent grader. Then the mean scores were averaged across graders to obtain aggregate compartment scores for each limb.

Immunohistochemistry

Whole hindlimbs were collected from Sham or ACLR mice, fixed for 48 h in 10% neutral-buffered formalin, rinsed with water, subjected to decalcification for two weeks using 10% EDTA, and embedded in paraffin. Sagittal sections (5 µm) spanning the medial joint were cut and mounted. Epitopes were retrieved in 10 mM sodium citrate buffer (pH 6.0) for 30 min at 70°C. After permeabilization and blocking, sections were incubated overnight at 4°C with primary antibody, then with secondary antibody for 1 h at room temperature. Sections were counterstained with Hoechst 33342 (Invitrogen), then incubated in 0.2% Sudan black for 30 min and mounted with ProLong Gold (Invitrogen). Slides were imaged at 10x (at least three sections per limb) on a Lionheart FX (Biotek) for three mice per condition. For qualitative grading of R-spondin 2 staining, the following scale was used: no expression (0), <25% expression (1), 25-50% expression (2), >50% expression (3). All antibody information can be found in Supplementary Table 3.

Immunocytochemistry

Primary SFs were plated on coverslips and fixed using 4% paraformaldehyde. Cells were permeabilized in 0.1% TBST, blocked for 1 h, then incubated overnight at 4°C in primary antibody. The following day, secondary antibody was added for 1 h at room temperature, before counterstaining with Hoechst 33342 (Invitrogen) and mounting of coverslips onto slides with ProLong Gold (Invitrogen). For staining of SFs on soft substrate dishes, cells were permeabilized with 0.3% Triton-X100 before 2 h of blocking in normal goat serum and overnight incubation with primary antibody. Images were acquired on a Lionheart FX (Biotek). All antibody information can be found in Supplementary Table 3.

Primary synovial fibroblast isolation and culture

SFs were isolated from C57BL/6J or Wnt-GFP reporter mice at 12-16 weeks of age. Hindpaw SFs were derived from the mouse hindpaw based on previously reported methods (8). Briefly, claws and skin were removed from hindpaws, muscles and tendons excised, then longitudinal incisions were made with a scalpel along the hindpaw. Both dissected hindpaws were digested for up to 50 min at 37°C and vortexed every 5 min in a 4.5 mL volume of synovial digestion medium (DMEM with 400 µg/mL collagenase IV, 400 µg/mL liberase, 400 µg/mL DNaseI). After digestion, cells were centrifuged at 500 x *g* for 5 min then plated in DMEM containing 10% fetal bovine serum (FBS), 1x L-glutamine, and 1x antibiotic/antimycotic (Invitrogen). Knee-derived SFs were derived from mouse knee synovium as reported previously (1). Briefly, knee synovium was dissected from the anterior, medial, and lateral compartments, including the fat pad (9), keeping the joint space hydrated with PBS throughout. Surrounding capsule and muscle was carefully removed or avoided. The posterior synovium and posterior fat pad were not collected. Synovia were digested for up to 35 min while shaking at 1000 rpm at 37°C, with vortexing at 0, 15 and 30 min. Up to two synovia were digested together in a 1.5 mL volume of synovial digestion listed above. Cells were pelleted by centrifugation after digestion then plated in culture medium as listed above for hindpaw-derived SFs. Both hindpaw-derived and knee-derived SFs were used for experiments between passage 4 to 6. Prior to treatment, SFs were serum-starved overnight and treated in the absence of serum. To reduce basal Wnt signaling activity, SFs were seeded atop soft polydimethylsiloxane (PDMS) substrates (1.5 kPa µ-Dish ESS, 35 mm diameter, Ibidi, Martinsreid, Germany) coated with fibrinogen (10). In brief, 0.8 mg/mL fibrinogen in 1 M Tris-HCl was overlaid on the substrates for 2 h. Cells were seeded at a density of 16,500 cells/cm^2. SFs were serum starved (1% FCS) the night before treatment.

Primary bone marrow-derived macrophage (BMDM) isolation and culture

BMDM were grown from whole mouse bone marrow based on previously reported protocols (1, 11). Briefly, femoral and tibial bone marrow from C57BL/6J mice aged 12-16 weeks was flushed and grown in DMEM containing 10% FBS, 1x L-glutamine, 1x antibiotic/antimycotic (Invitrogen), and 20 ng/mL recombinant M-CSF (Gibco). BMDM were lifted and seeded into experimental plates for treatment at passage 1.

ATDC5 culture and differentiation

ATDC5 cells were kindly provided by Dr. Rhima Coleman (University of Michigan). Cells were cultured in DMEM containing 10% FBS, 1x L-glutamine and 1x antibiotic/antimycotic (Invitrogen). For chondrogenic differentiation, ATDC5 cells were grown to 90% confluency then switched to differentiation media (DMEM high glucose, 10 ng/mL TGFβ, 100 nM dexamethasone, 40 µg/mL ascorbic acid 2-phosphate, 1 mM sodium pyruvate, 40 µg/mL L-proline, and 1x ITS pre-mix containing 6.25 µg/mL insulin, 6.25 µg/mL transferrin, 6.25 µg/mL selenous acid, 5.33 µg/mL linoleic acid, 1.25 mg/mL bovine serum albumin). Cells were differentiated for 21 days, with media replaced every 3 days.

Gene expression analysis

Transcript levels were analyzed in whole synovium, ATDC5 cells, BMDM, hindpaw-derived SFs and knee-derived SFs. Synovium were subjected to homogenization in CK28 PreCellys tubes (Bertin Technologies) containing TRIzol (Invitrogen) at 4°C for two to three cycles on the soft tissue setting (5800 rpm, 2 x 15 s, 30 s rest). Debris was then removed by centrifugation at 10,000 x *g* for 10 min before proceeding with phenol-chloroform RNA isolation, as with cell culture samples. Nucleic acid concentration and purity was determined using a Nanodrop spectrophotometer (ThermoFisher) then a standard amount of RNA subjected to reverse transcription using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative real-time PCR (qPCR) was employed to assess gene expression using Power SYBR Master Mix (Applied Biosystems) on a QuantStudio5 Real-Time PCR System (Applied Biosystems). Genes of interest were normalized to expression of a stable housekeeping gene and control samples set to 1 using the $2^{-\Delta\Delta Ct}$ method. All primer sequences can be found in Supplementary Table 4. For calculation of the M1:M2 macrophage polarization score, based on Paige *et al* (12), we used a composite of M1 (*Il1b*, *Il6* and *Nos2*) and M2 (*Mrc1*, *Il10* and *Arg1*) gene expression. Expression levels of each gene were first normalized to prevent bias arising from differential baseline expression. The M1:M2 score was then calculated as a ratio of the sum of normalized M1 gene expression values to the sum of normalized M2 gene expression values.

siRNA knockdown of *Sox5*

Hindpaw SFs were seeded into 12-well plates at a density of 20,000 cells/well. At ~75% confluency, cells were transfected with 25 µM siRNA or were left untransfected (Sham). The following siRNAs were all purchased from Invitrogen: Silencer™ Select Negative Control No. 1 siRNA (#4390843), Silencer™ Select Sox5 siRNA n377426, and Silencer™ Select Sox5 siRNA n377427. 25 nM of siRNA was transfected per reaction, using DharmaFECT 1 Transfection Reagent (according to manufacturer's instructions) in Opti-MEM reduced serum media (Invitrogen). After 24 h, the transfection media was replaced with DMEM media containing 10% FCS, 1x L-glutamine and 1x antibiotic/antimycotic (Invitrogen). After another 24 h (48 h post-transfection), cells were harvested in TRIzol (Invitrogen) for RNA isolation.

Protein quantification

Conditioned medium was harvested from cell cultures, centrifuged at 500 x *g* for 5 min to remove debris, then stored at -80°C. Synovial fluid was collected from mouse knees based on the protocol reported by Seifer *et al* (13). Briefly, the joint capsule was carefully opened and an alginate microsponge inserted for 30 s to recover synovial fluid. The soaked sponge was then removed and placed into a cryovial for flash freezing and storage at -80°C. Upon thawing, alginate lyase

was added for incubation at 34°C for 30 min to digest the sponge. At completion, sodium citrate was added prior to vortexing.

To quantify the concentration of total protein in synovial fluid samples, a Coomassie Plus Assay Kit (based on the Bradford assay) was used in accordance with manufacturer's instructions. This permitted normalization of target analyte concentration to total protein amount in synovial fluid, given the variability of fluid recovery from mouse to mouse. To measure the concentration of R-spondin 2 in synovial fluid or cell culture conditioned medium, a mouse R-spondin 2 ELISA kit (CusaBio) was used according to manufacturer's instructions.

Flow cytometry

Multicolor flow cytometry was used to assess synovial cell types based on expression of surface-based or intracellular proteins, to measure cell viability, to assess endogenous reporter signal, or to evaluate fibroblast markers on the surface of cultured hindpaw SFs. Synovial tissue was dissected and digested as described above, resulting in a single cell suspension. Hindpaw SFs were lifted with 0.25% trypsin-EDTA at passage 2 or 4, immediately prior to staining. For viability testing, cells were either pre-stained with a fixable viability dye (eFluor660, eBioscience) or post-stained with a nuclear viability dye (TOPRO3, Invitrogen). Cold FACS buffer (PBS containing 2% FBS, 1 mM EDTA) was used for all staining and preparation steps, and for running samples on the cytometer. For all experiments, non-specific binding was blocked using TruStain FcX (Biolegend). For intracellular detection of R-spondin 2, cells were fixed and permeabilized using the Cyto-Fast Fix-Perm Buffer Set (Biolegend), and an anti-R-spondin 2 antibody (ProteinTech) or Rabbit IgG Isotype Control (Abcam) were used with a secondary anti-rabbit BV421. CD31-PECy7 (Biolegend) and CD45-BV650 (Biolegend) were used for surface staining to identify major synovial cell types; Podoplanin-FITC (Biolegend), CD90.2-PE/Dazzle594 (Biolegend), and CD55-PE/Cy7 (Biolegend) were used as fibroblast surface markers, and CD11b-BV605 (Biolegend) as a macrophage marker; and the Wnt-GFP fluorescent reporter was used to assess canonical Wnt signaling activity based on TCF/Lef transcriptional activity. Antibodies and their conjugates are listed in detail in Supplementary Table 3. In addition to fully stained samples, all experiments included an unstained cells control, single-stained controls comprised of cells or UltraComp eBeads (Invitrogen), and fluorescence-minus-one (FMO) or isotype controls to determine population gating. Compensation and acquisition were performed using a BD LSRFortessa cytometer with FACSDiva software (BD Biosciences), then data analysis was performed using FlowJo v10 (TreeStar/BD Biosciences).

Single-cell RNA-sequencing (scRNA-seq)

For scRNA-seq, two biological replicates of the following conditions were used: Sham (no injury, anesthesia/analgesic only), 7d ACLR, and 28d ACLR, for a total of six samples. Each biological replicate was comprised of a synovium from one male and from one female mouse. For Sham, one biological replicate was comprised of cells from mice harvested 7d after Sham, and the other replicate from mice harvested 28d after Sham. Both Sham samples showed high correlation of cell clustering (Figure S1D). Synovia were dissected and digested as described above, to yield a single cell suspension at >90% viability. Single cells were immediately submitted to the University of Michigan Advanced Genomics Core for loading onto the 10x Genomics pipeline (Chromium Next GEM Single Cell 3' Kit v3.1) for barcoding and library preparation. Pooled libraries were then submitted to BGI for paired-end (100bp+100bp) sequencing on the DNBseq G400 system (MGI Tech), generating ~350 million reads per sample (>50 000 reads per cell). Raw data were aligned to the GRCm38 (mm10) reference mouse transcriptome using STAR (14) and underwent pre-processing in Cell Ranger v3.1.0 (10x Genomics).

Quality control, filtering, clustering, annotation, and analysis of scRNA-seq data

Pre-processed aligned data were read from Cell Ranger into Seurat (R, v4.1.0). Cells with less than 100 detected genes and genes expressed in less than 10 cells were excluded from Seurat object generation. Next, low-quality cells were filtered out based on number of expressed features (i.e. genes), with remaining cells having 200 < *nFeature* < 6000. Cells in which > 5% of all genes were mitochondrial-derived (percent.mt > 0.05) were also excluded. Canonical correlation analysis, which performs anchor-based integration (15), was used to integrate data across conditions (Sham, 7d ACLR, 28d ACLR) using the top 3000 variable features. In tandem, we performed Spearman correlation analysis of average expression for all genes between integrated biological replicates of each condition. *ScaleData()* was used to perform linear transformation and to regress out unwanted variation due to cell cycle and mitochondrial gene expression. Linear dimensionality reduction was performed using principal component analysis, and elbow plots and principal component heatmaps were used to determine the number of dimensions used in subsequent nonlinear dimensionality reduction via Uniform Manifold Approximation and Projection (UMAP). Dimensions 1-25 were used in the object representing all conditions, all biological replicates, and all cells. Unsupervised clustering was performed using *FindNeighbors* (dims = 1:25) and *FindClusters* (resolution = 0.3*)* to derive distinct cell clusters.

Cluster markers were identified using *FindAllMarkers*. Genes expressed in greater than 80% of cells in the cluster of interest and in less than 20% of cells in all other cells were focused on, as were genes with positive differential expression relative to other clusters, as opposed to negative markers. To identify and functionally annotate cell clusters, the genes outputted by *FindAllMarkers* for each cluster were submitted to Gene Ontology (GO) pathway analysis (GO: Biological Process) using PantherDB (16, 17). We further utilized Cluster Identity Predictor (CIPR) (18) and published synovial scRNA-seq datasets at the Single Cell Portal (19, 20) to compare gene marker profiles to known cell annotations. The Mouse Brain Atlas (mousebrain.org) (21) was used to identify Schwann cells. Violin plots and gene feature plots were generated using the Seurat functions *VlnPlot()* and *FeaturePlot()*, respectively. Bubble plots of GO terms were made using ggplot2 (22). Global gene expression differences between conditions or groups were assessed by identifying the top 25 differentially expressed genes (DEGs) between conditions with most positive and most negative log2FC using a pseudo-bulk analysis approach and DESeq2 (23). Read counts of genes were then subset by sample, and then averaged for all cells across each sample. Global z-scores of average read count for each gene were computed and plotted using *pheatmap*.

To identify fibroblast subsets, all fibroblast-like cells (not including pericytes) were subset from the “all synovial cells” Seurat objects within each condition and re-clustered (dims = 1:25, resolution = 0.13). Cluster markers and functional annotation were performed as described above. To identify conserved functions across all fibroblast subsets, the *gsfisher* R package was used to test for GO overrepresentation, and the gene universe (i.e. background list of expressed genes) was defined as genes expressed in 25% of cells in the cluster of interest or 25% of all remaining cells. To identify unique functions across fibroblast subsets, the statistically significant ($Q < 0.05$, where Q is the P value adjusted for false discovery rate) genes and their Log2FC from *FindAllMarkers()* were submitted to GO (Biological Processes) or Reactome analysis via PantherDB. Pathway terms were manually curated and grouped into categories of conserved themes based on the following criteria: $Q < 0.05$ (expressed as $\log_{10}$(FDR)), only found in a single cluster, rich ratio (higher number of DEGs in proportion to the total genes in term), and pathway term similarity. Fibroblast subsets were identified, defined, and named based on these categories and known, functionally relevant cluster markers.

For comparison of fibroblast subsets to human OA and RA, scRNA-seq data from two studies, Wei *et al*, Nature, 2020 (20) and Zhang *et al*, Nat Immunol, 2019 (19), were downloaded from the Single Cell Portal (singlecell.broadinstitute.org/single_cell) and feature plots generated using ggplot2 (22).

Intercellular communication analysis

The CellChat R package (CellChat 1.1.3) (24) was used to infer and quantify cell-cell communication networks. In the analysis of all synovial cells, macrophages and dendritic cells were combined into a single “myeloid” group, and vascular endothelial cells and lymphatic endothelial cells were combined into a single “endothelial” group to focus on major cell group signaling. Consistent with recommendations by the CellChat toolbox to curate expanded ligand-receptor sets for focused analysis of signaling axes of interest, an “Rspo” pathway of known ligand-receptor interactions between R-spondins 1-4 and Lgr receptors 4-6 was added to the pathway interaction database. Over-expressed genes and ligand-receptor interactions were identified with default *identifyOverExpressedGenes()* and *identifyOverExpressedInteractions()* functions. Data were projected against the provided PPI.mouse dataset to reduce dropout effects of signaling genes. Probabilities of cell-cell communications were inferred with *computeCommunProb()*. The method for calculating average gene expression per cell group was *triMean* for all synovial cells, and *truncatedMean* with 10% trim for SFs. River plots were generated with pattern numbers at which cophenetic and silhouette measure scores decreased simultaneously, and conservative cutoff values at which all patterns were present. Circle plots and contribution plots for pathways of interest were constructed using the *netVisual_aggregate()* and *netAnalysis_contribution()* functions.

Trajectory and Transcription Factor Binding Motif Analyses

Cellular trajectory analysis was performed using Monocle3 (v1.0.0) (25) in R. First, the Seurat object representing all fibroblasts across all conditions was imported into Monocle3 using the Seurat-Monocle wrapper. Data was preprocessed with the same number of dimensions as the Seurat-based analysis, and nonlinear dimensionality reduction was performed using UMAP. Trajectories were reconstructed using the *learn_graph()* function, and cells were ordered along pseudotime using the *order_cells()* function. The reconstructed trajectory identified a terminal node in the Dpp4+ cluster, which was selected as the trajectory root. Next, the trajectory branch originating in the Dpp4+ cluster and terminating in the $Prg4^{hi}$ cluster was subset for focused pseudotime-dependent analyses of $Prg4^{hi}$ lining fibroblasts. Unsupervised preprocessing, dimensionality reduction, and clustering of this trajectory was performed, and *learn_graph()* identified a trajectory from Dpp4+ cells to $Prg4^{hi}$ cells, via αSMA+ cells. The trajectory node in the most proximal aspect of the Dpp4+ cluster was again selected as a root, and pseudotime was calculated. To find genes that vary as a function of pseudotime along a trajectory, gene read count data was fitted to a quadratic spline function, similar to the *plot_genes_in_pseudotime()* function in Monocle3. Genes with quadratic $r^2 > 0.15$ and with trajectory-wide average read count values > 0.1 (to exclude noise) were then analyzed for co-regulation using the *find_gene_modules()* function, which groups genes into modules depending on their variance patterns along pseudotime using Louvain community analysis. Modules with high degree of composite expression in the $Prg4^{hi}$ cluster (Module 2 in Figure S10A), representing genes that either increase or decrease with similar variance patterns indicative of co-regulation, were selected for subsequent transcription factor (TF) binding analysis to identify the TFs regulating lineage specification of $Prg4^{hi}$ lining cells. These genes were then input to the RcisTarget (26) R package for DNA binding motif analysis. *mm9-tss-centered-10kb-7species.mc9nr.feather* was applied as a database with motifs using the "directAnnotation” parameter. SOX5 was predicted as a key TF enriched in the $Prg4^{hi}$ lining cluster that regulates *Rspo2* and other co-regulated genes along the differentiation trajectory. TF gene regulatory network for SOX5 was then visualized using Cytoscape (v3.91) (27).

Sample sizes and statistical analyses

Microsoft Excel was used for tabulation of raw data. GraphPad Prism 9 was used for statistical analyses and generation of figures. All data are presented as mean ± SEM and $P<0.05$ was considered statistically significant. Shapiro-Wilk tests were used to assess normal distribution. For two-group comparisons of normally distributed data, parametric two-tailed student’s t-tests were used, in a paired or unpaired manner dependent upon the experimental design. For comparisons of data across three or more independent groups/conditions, one-way or two-way ANOVA with post-hoc Tukey testing were

used for normally distributed data. For scRNA-seq analyses, we used default statistical tests built into R packages Seurat, Monocle, RcisTarget, and CellChat, that employ false discovery rate testing to account for multiple comparisons in large datasets.

REFERENCES

1. Rzeczycki P, Rasner C, Lammlin L, Junginger L, Goldman S, Bergman R, et al. Cannabinoid receptor type 2 is upregulated in synovium following joint injury and mediates anti-inflammatory effects in synovial fibroblasts and macrophages. Osteoarthritis and Cartilage. 2021;29(12):1720-31.
2. Christiansen BA, Anderson MJ, Lee CA, Williams JC, Yik JHN, Haudenschild DR. Musculoskeletal changes following non-invasive knee injury using a novel mouse model of post-traumatic osteoarthritis. Osteoarthritis and cartilage. 2012;20(7):773-82.
3. Leahy AA, Esfahani SA, Foote AT, Hui CK, Rainbow RS, Nakamura DS, et al. Analysis of the trajectory of osteoarthritis development in a mouse model by serial near-infrared fluorescence imaging of matrix metalloproteinase activities. Arthritis Rheumatol. 2015;67(2):442-53.
4. Jackson MT, Moradi B, Zaki S, Smith MM, McCracken S, Smith SM, et al. Depletion of protease-activated receptor 2 but not protease-activated receptor 1 may confer protection against osteoarthritis in mice through extracartilaginous mechanisms. Arthritis and Rheumatology. 2014;66(12):3337-48.
5. Little CB, Barai A, Burkhardt D, Smith SM, Fosang AJ, Werb Z, et al. Matrix metalloproteinase 13-deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. Arthritis Rheum. 2009;60(12):3723-33.
6. Kalson NS, Borthwick LA, Mann DA, Deehan DJ, Lewis P, Mann C, et al. International consensus on the definition and classification of fibrosis of the knee joint. Bone Joint J. 2016;98-B(11):1479-88.
7. Remst DFG, Blaney Davidson EN, van der Kraan PM. Unravelling osteoarthritis-related synovial fibrosis: a step closer to solving joint stiffness. Rheumatol. 2015;54(11):1954-63.
8. Armaka M GV, Kontoyiannis D, Kollias G. A standardized protocol for the isolation and culture of normal and arthritogenic murine synovial fibroblasts. Protoc Exch. 2009.
9. Haubruck P, Colbath AC, Liu Y, Stoner S, Shu C, Little CB. Flow Cytometry Analysis of Immune Cell Subsets within the Murine Spleen, Bone Marrow, Lymph Nodes and Synovial Tissue in an Osteoarthritis Model. J Vis. Exp. 2020(158):e61008.
10. Gandhi JK, Knudsen T, Hill M, Roy B, Bachman L, Pfannkoch-Andrews C, et al. Human Fibrinogen for Maintenance and Differentiation of Induced Pluripotent Stem Cells in Two Dimensions and Three Dimensions. Stem Cells Transl Med. 2019;8(6):512-21.
11. Knights AJ, Liu S, Ma Y, Nudell VS, Perkey E, Sorensen MJ, et al. Acetylcholine-synthesizing macrophages in subcutaneous fat are regulated by beta2 -adrenergic signaling. EMBO J. 2021;40(24):e106061.
12. Paige JT, Kremer M, Landry J, Hatfield SA, Wathieu D, Brug A, et al. Modulation of inflammation in wounds of diabetic patients treated with porcine urinary bladder matrix. Regen Med. 2019;14(4):269-77.
13. Seifer DR, Furman BD, Guilak F, Olson SA, Brooks SC, 3rd, Kraus VB. Novel synovial fluid recovery method allows for quantification of a marker of arthritis in mice. Osteoarthr. Cartil. 2008;16(12):1532-8.
14. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013;29(1):15-21.
15. Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM, et al. Comprehensive Integration of Single-Cell Data. Cell. 2019;177(7):1888-902.e21.
16. Mi H, Thomas P. PANTHER pathway: an ontology-based pathway database coupled with data analysis tools. Methods Mol Biol. 2009;563:123-40.
17. Mi H, Ebert D, Muruganujan A, Mills C, Albou L-P, Mushayamaha T, et al. PANTHER version 16: a revised family classification, tree-based classification tool, enhancer regions and extensive API. Nucleic Acids Res. 2021;49(D1):D394-D403.
18. Ekiz HA, Conley CJ, Stephens WZ, O'Connell RM. CIPR: a web-based R/shiny app and R package to annotate cell clusters in single cell RNA sequencing experiments. BMC Bioinform. 2020;21(1):191.
19. Zhang F, Wei K, Slowikowski K, Fonseka CY, Rao DA, Kelly S, et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. Nat Immunol. 2019;20(7):928-42.
20. Wei K, Korsunsky I, Marshall JL, Gao A, Watts GFM, Major T, et al. Notch signalling drives synovial fibroblast identity and arthritis pathology. Nature. 2020;582(7811):259-64.
21. Zeisel A, Hochgerner H, Lönnerberg P, Johnsson A, Memic F, Van Der Zwan J, et al. Molecular Architecture of the Mouse Nervous System. Cell. 2018;174(4):999-1014.e22.
22. Hadley W. Ggplot2: Elegant Graphics for Data Analysis: Springer; 2016. 1 online resource p.
23. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.
24. Jin S, Guerrero-Juarez CF, Zhang L, Chang I, Ramos R, Kuan C-H, et al. Inference and analysis of cell-cell communication using CellChat. Nat Commun. 2021;12(1):1088.
25. Cao J, Spielmann M, Qiu X, Huang X, Ibrahim DM, Hill AJ, et al. The single-cell transcriptional landscape of mammalian organogenesis. Nature. 2019;566(7745):496-502.
26. Aibar S, Gonzalez-Blas CB, Moerman T, Huynh-Thu VA, Imrichova H, Hulselmans G, et al. SCENIC: single-cell regulatory network inference and clustering. Nat Methods. 2017;14(11):1083-6.

27. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-504.