Corresponding author(s): Michael M. Brenner
Soumya Raychaudhuri

Last updated by author(s): Apr 5, 2020

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	*Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.*
Data analysis	All code required to do analyses and make figures for this manuscript are available at http://github.com/immunogenomics/notch.

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.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

RNA-sequencing data supporting this publication is available at ImmPort (https://www.immport.org) under study accession SDY1599, and at the Gene Expression Omnibus (GEO) under accessioncode GSE145286 for mouse studies. Normalized read counts, UMAP projections and metadata for single cell RNAseq datasets has been made available for browsing at the Broad Institute's single cell portal: https://portals.broadinstitute.org/single_cell/study/SCP469/synovial-fibroblast-positional-identity-controlled-by-inductive-notch-signaling-underlies-pathologic-damage-in-inflammatory-arthritis.

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	No sample size was calculated. This study applied single cell analyses were applied to a cohort of patients. Sample size was determined based on the total of number of patients recruited during the time of the study. Since the goal of the study was to explore heterogeneity among stromal cells, , the total number of patients recruited here was considered sufficient for the sample size.
Data exclusions	Data were excluded from analyses based on specific quality control criteria as described in detail in the manuscript for each data sets. For single cell data, we discarded cells with low gene count and we also discarded cells that had more than 25% of molecules coming from mitochondrial genes. For bulk RNA-seq experiments, samples with low quality as determined by gene reads were excluded from subsequent analysis.
Replication	We used publicly available data from phase 1 of the Accelerating Medicines Partnership-Rheumatoid arthritis/Systemic lupus erythematosus , an independent data set generated from a separate study, to validate NOTCH activation score in RA synovial fibroblasts. No experimental replication were formed for sRNAseq studies.
Randomization	No randomization was performed due to the cross-sectional nature of the study
Blinding	No blinding was performed in this study due to the cross-sectional nature of the study

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
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Flow cytometry studies: CD45 (HI30), CD235a (KC16), CD31 (WM59), THY1 (5E10), podoplanin (NZ1.3), CD146 (P1H12), CD34 (4H1), NOTCH3 (MHN3-21), JAG1 (MHJ1-152) were purchased from Biolegend or BD biosciences. Human tissue Imaging studies: PRG4 (IgG1, clone 9G3, Merck Millipore), 525 MCAM (IgG2a, clone SHM-57, Biolegend), VWF (rabbit polyclonal, Dako), Notch3 526 (MHN3-21, BioLegend), PODXL (rabbit, clone EPR9518, Abcam), CD55 (IgG1, clone 527 143-30, Bio-Techne), GGT5 (HPA008121, Sigma), PDPN (NZ-1.3, eBioscience), THY1 528 (IgG2a, clone Thy-1A1 Bio-Techne), and CD31 (IgG1, JC70A, Dako), NOTCH3-ICD (NICD3, V1662, Genentech) Mouse tissue imaging studies: NOTCH3 (clone AF1308, R&D systems), CD45 (clone D3F8Q, Cell Signaling) Western Blot study: NOTCH3-ICD (NICD3, V1662, Genentech), GAPDH (14C10, Cell signaling) In vivo studies: IgG2A (Bio-XCell), Anti-Notch3 blocking antibody (anti-NRR3) and anti-NOTCH1 blocking antibody (anti-NRR1) was generated by C.W. Siebel (manuscript under preparation)
Validation	All commercial antibodies used for flow cytometry and cell sorting experiments were validated for flow cytometric analysis of human cells according to manufacturer's production information. Additional validation on synovial fibroblasts for cell type

specificity were performed as described in Mizoguchi and Slowikowski et al., Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. Nat Commun. 2018 Feb 23;9(1):789. For antibodies used in immunofluorescence microscopy experiments, all antibodies were tested for IF studies on human tissues and cells based on manufacturer's product description.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	human synovial fibroblasts (FLS) cell lines were derived from human synovial tissues. Human umbilical cord endothelial cells (HUVECs) were purchased from Lonza.
Authentication	gene expression analysis (qPCR) was performed every 3 passages to confirm expression of mesenchymal genes (FLS) and endothelial cells (HUVECS).
Mycoplasma contamination	Commercially obtained HUVECs tested negative for mycoplasma contamination according to vendor (Lonza)
Commonly misidentified lines (See ICLAC register)	none

Animals and other organisms

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

Laboratory animals	C57BL/6 mice (Jackson Laboratory): Male, 6-10 weeks old. Notch3-/- mice (B6;129S1-Notch3tm1Grid/2J, Jackson Laboratory): Male, 6-10 weeks. C57BL6/129 mixed background (Jackson Laboratory): male, 6-9 weeks.
Wild animals	*Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	Study was approved by BWH IACUCU

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	Rheumatoid arthritis: male and female, patients with RA fulfilled the ACR 2010 Rheumatoid Arthritis classification criteria. Osteoarthritis: male and female, patients undergoing joint replacement procedures
Recruitment	Informed consent was obtained from patients for human single cell genomic studies. For other studies, including flow cytometric analysis and in vitro cell line studies of synovial tissues, surgically discarded synovial tissue were obtained from de-identified patients underoing joint replacement procedures.
Ethics oversight	Experiments involving human subjects was performed according to the Institutional Review Boards at Partners HealthCare.

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	disaggregated human synovial cells were stained with antibodies against CD45 (HI30), CD235a (KC16), CD31 (WM59), THY1 (5E10), podoplanin (NZ1.3), CD146 (P1H12), CD34 (4H1) in 1% BSA in Hepes-Buffered Saline (HBS,20 mM HEPES, 137 mM NaCl, 3mM Kcl, 1mM CaCl2) for 30 minutes. DAPI or LIVE/DEAD (Invitrogen) viability dye was added to cell suspensions and cells were passed through a 100µm filter.

Instrument	BD FACSAria Fusion and BD Fortessa
Software	Flowjo
Cell population abundance	Stromal cells were gated on markers PDPN, CD146, CD31 and CD90 as depicted in Extended Data Figure 1e. For cell sorting experiments, cells were isolated >99% purity
Gating strategy	Synovial stromal cells were gated on markers PDPN, CD146, CD31 and CD90 as depicted in Extended Data Figure 1e. For in vitro fibroblast and endothelial cell co-culture experiments, fibroblasts were gated on CD31- CD90+ cells as shown in Extended Data Figure 5f.

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