SUPPLEMENTARY MATERIAL

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Pathology of Early Arthritis Cohort

The Pathology of Early Arthritis Cohort (PEAC) (http://www.peac-mrc.mds.qmul.ac.uk) is a cohort with contribution from many independent centers and universities including Barts and the London school of medicine and dentistry, University of Glasgow, University of Oxford, University of Birmingham, Cardiff University and more institutions. All PEAC samples are collected with full approval of the relevant research ethics committees and full consent of the tissue donor and all samples are coded. RNA-seq and histology data for 87 treatment-naïve RA patients were obtained from PEAC and analyzed for this study.

RNA-sequencing of synovial biopsies in early rheumatoid arthritis

87 synovial samples were acquired through a minimally invasive US-guided synovial biopsy [1] from patients presenting with early RA naïve to therapy from the Pathobiology of Early Arthritis Cohort (PEAC). Ethical approval was granted by the King's College Hospital Research Ethics Committee (REC 05/Q0703/198). Synovial biopsies were analyzed by histology and immunohistochemistry as previously reported [2], and categorized into Lymphoid, Myeloid and pauci-immune Fibroid pathotypes.

RNA from homogenized synovial tissue was extracted in Trizol. 1µg total RNA was used as input material for library preparation using TruSeq RNA Sample Preparation Kit v2 (Illumina). Generated libraries were amplified with 10 cycles of PCR. Library size was confirmed using 2200 TapeStation and High Sensitivity D1K screen tape (Agilent Technologies) and concentration was determined by qPCR based method using Library quantification kit (KAPA). Libraries were multiplexed (five per lane) and sequenced on Illumina HiSeq2500 (Illumina) to generate 50 million paired-end 75 base pair reads.

Transcript abundance was derived using Kallisto v0.43.0 with GENCODE v24/GRCh38 as reference [3]. Transcript abundances as TPM (transcripts per kilobase million) were summarized over transcript isoforms using Bioconductor package tximport 1.4.0. Imported abundances were processed using DESeq2 1.14.1 and transformed as regularized log expression (RLE) [4]. Statistical analysis of gene-gene correlations was performed using Pearson correlation. P values were adjusted using false discovery rate (FDR) correction (Benjamini-Hochberg). RNA-Seq data have been deposited in ArrayExpress under Accession code E-MTAB-6141.

Antibodies

The anti-Pez G-20 antibody (sc-66616) was from Santa Cruz, anti-TGFβRI (ab31013) was from Abcam, all other antibodies were from Cell Signaling. The anti-mouse IgG and anti-rabbit IgG were from GE Health Sciences, the anti-goat IgG was from Santa Cruz, the Alexa Flour 647, Alexa Flour 555 anti-rabbit antibodies were from Thermo.

FLS culture and stimulation

FLS were cultured in Dulbecco's modified Eagle's medium (DMEM; Mediatech) with 10% fetal bovine serum (FBS, Omega Scientific), 2 mM L-glutamine, 50 µg/ml gentamicin, 100 units/ml penicillin, and 100 µm/ml streptomycin (Life Technologies) at 37°C in a humidified atmosphere containing 5% CO_2. For all experiments, FLS were used between passages 4 and 10, and the cells were synchronized in 0.1% FBS (serum-starvation medium) for 24h before analysis or functional assays.

For stimulations, FLS were stimulated with 50 ng/ml human TNFα (Peprotech, cat# 300-01A), 2 ng/ml human IL-1β (eBioscience, cat# 14-8018), or 50 ng/ml human TGFβ1 (TGFβ, eBioscience,

cat# 14-8348-62) for 24h before subsequent analysis. For small molecule inhibition, FLS were pre-treated with either DMSO, 50 µM SB505124 (TGFβ receptor inhibitor, Sigma) or 1 µM RepSox and were harvest 24 hours after the treatment for subsequent analysis (TGFβ receptor inhibitor, Selleck Chem). For small molecule inhibition, FLS were pre-treated with either DMSO or 25 µM SB505124 (TGFβ receptor inhibitor, Sigma) for 1 hour before stimulation with 50 ng/ml human TGFβ1. 24h after stimulation the cells were harvested for subsequent analysis. For the YAP inhibition experiments, FLS were treated with DMSO or 1 µM verteporfin (YAP inhibitor, MedKoo Bioscience) and stimulated with 50 ng/ml human TNFα. 24h after stimulation the cells were harvested for subsequent analysis.

HEK 293T cell culture

HEK 293T cells from ATCC were cultured in DMEM with 4.5 g/L glucose, 4.5 g/L L-glutamine, and 4.5 g/L sodium pyruvate purchased from Corning (10-013) supplemented with 10% FBS (Omega) with 100 units/ml of penicillin, 100 µg/ml streptomycin from Thermo Fisher Scientific at 37°C in a 5% CO_2 humidified atmosphere.

Transwell migration assays

5.0 x 10^4 cells were then seeded into the upper chamber of the transwell (pore size 8.0 µm, Corning #3422) and left to migrate for 24h at 37°C in response to chemoattractant-rich media (DMEM + 5% FBS) or assay media (0.5% BSA) below. Following incubation, the cells were fixed in 100% methanol for 5 minutes and stained with 0.2% crystal violet for 30 minutes. Four images per transwell were taken with a Nikon 80i microscope to count the cells that migrated through the transwell. 2 chambers were counted for each condition. The percent of cell migrated is calculated as the number of cells that migrated through vs the number of control cells that migrated through with 5% FBS at the bottom well.

Invasion assays

5.0 x 10^4 cells were seeded into the upper chamber of matrigel invasion chambers (pore size 8.0 µm; Corning #354483) and left to invade for four days in response to chemoattractant-rich media (DMEM + 5% FBS) or assay media (0.5% BSA) below. Following incubation, the cells were fixed in 100% methanol for 5 minutes at room temperature and stained with 0.2% crystal violet for 30 minutes. Four images per chamber were taken with a Nikon 80i microscope to count cells that invaded through the chambers. 2 chambers were counted for each condition.

Cartilage attachment assays

Before the assay, bovine cartilage explants (Animal Technologies) were incubated with 2 ng/mL human IL-1 β in cell culture media (DMEM + 10% FBS) for 24h at 37°C as described in [5]. After IL-1β treatment, serum-starved control or PTPN14 knock down RA FLS lines (n = 3) were stimulated with 50 ng/ml human TNFα for 24h at 37°C. Following stimulation, the cells were lifted off with 1mM EDTA at 37°C for 15 min and stained with 2.5 µi cell tracker green (Thermo Fisher) in assay media (DMEM+ 0.5% BSA) for 40 minutes at room temperature, protected from light. The cells were then washed and re-suspended in culture media (DMEM + 10% FBS). 1.5 x 10^5 cells were seeded onto each cartilage explant and rotated on an orbital shaker at 37°C for 2h followed by incubation for 16h at 37°C. Subsequently the cells were fixed in 4% formaldehyde for 20 minutes at room temperature. Three images per cartilage explant were taken with a Nikon 80i microscope and numbers of attached cells were counted. Two explants were assessed for each condition.

Quantitative polymerase chain reaction (qPCR)

Mini or micro RNeasy kits (Qiagen, Cat # 74134 or 74034) were used to extract RNA from human FLS followed by cDNA synthesis using SuperScript III First-Strand Synthesis kits (Thermo Fisher).

qPCR was run using a LightCycler 480 (Roche) or CFX384 Touch (Bio-rad). RT^2 qPCR primer assays and SYBR Green qPCR Master Mix were purchased from Qiagen. For each condition, triplicate were run, and data were normalized to the expression levels of housekeeping genes *GAPDH*. $2^{-\Delta Ct}$ or $2^{-\Delta\Delta Ct}$ methods were used for comparisons of gene expression.

PTPN14 substrate-trapping assays

Substrate-trapping is a well-established technique to identify substrates of PTPs [6]. PTP substrate-trapping involves mutation of a residue, typically an aspartic acid essential for catalysis, in the catalytic domain of the PTP. Substrates can bind the catalytic cleft, but catalysis is not completed, leading to formation of a complex in which the substrate is "trapped' by the PTP. The catalytic domain of PTPN14 (aa. 895 to 1184) was cloned into the *E. coli* expression vector pMCSG10, encoding an N-terminal His-GST-TEV tag. WT and double mutant D1079A/C1121S PTPN14 catalytic domain was expressed in BL21 cells and purified by affinity chromatography on nickel beads. RA FLS were pretreated with pervanadate for 30min and then lysed in lysis buffer containing 150mM NaCl, 50mM HEPES, 1% Triton X-100, 10% Glycerol, 1mM EDTA. Lysates were treated with 5mM iodoacetic acid and protease inhibitor. Purified PTPN14 catalytic domain WT and D1079A/C1121S mutant were incubated with RA FLS lysates for 1 hr at 4°C, and then incubated with protein G beads for another hour at 4°C, to pull down GST-tagged proteins. The pull-down was washed with HNTG wash buffer containing 150mM NaCl, 20mM HEPES, 0.1% Triton X-100, 10% Glycerol and subjected to Western blotting.

PTPN14/YAP Co-IP

Perhaps because of its binding to cytoskeleton proteins [7], in our experience PTPN14 only solubilizes in buffers containing high detergent (1% SDS). Thus for PTPN14 IPs, RA FLS were lysed in IP buffer containing 1% SDS (25 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP-40, 1% SDS, 0.5% sodium deoxycholate; with protease inhibitor) which was subsequently diluted down to 0.1% SDS with IP buffer (25 mM Tris-HCl pH 7.5, 150mM NaCl, 1% NP-40; with protease inhibitors) to enable PTPN14 refolding. PG sepharose beads (GE) were added to capture the antibody/protein complex for 2h at 4°C. The beads were washed before fresh RA FLS lysate (in IP buffer) was added and incubated overnight rotating at 4°C. The next day, the beads were washed before boiling in Laemmli buffer for 5 min at 95°C for Western blotting.

Western blotting

The cells were lysed in radioimmunoprecipitation assay buffer (RIPA) buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1% NP40, 5 mM EDTA, 0.5% sodium deoxycholate, 0.1% SDS) with protease and inhibitor cocktails tablets (Roche). The protein concentration in cell lysates was determined using a Pierce bicinchoninic acid protein assay kit (Thermo Scientific). Nuclear and cytoplasmic fractions of total cell lysates were isolated using NE-PER kit (Thermo) following manufacturer's instructions.

Immunofluorescence microscopy

For synovial specimen IF, fresh-frozen sections of human OA and RA synovium, obtained from the UC San Diego Clinical and Translational Research Institute Translational Research Technology Division (UC San Diego IRB #140175), were embedded in optimal cutting temperature compound (*OCT) from Fisher Scientific. 4 μM* slides were blocked with 5% goat serum for 1 hour at room temperature and incubated overnight at 4°C with goat anti-PTPN14 antibody clone G20 from Santa Cruz Biotechnology (1:50 dilution in immunofluorescence antibody dilution buffer from Cell Signaling Technology). Slides were then washed and incubated for 1 hour with Alexa Fluor 488-conjugated donkey anti-goat IgG from BioLegend (1:100 dilution in immunofluorescence antibody dilution buffer from Cell Signaling Technology). Nuclei were

stained with Vectashield antifade mounting medium with DAPI from Vector Laboratories. Images were obtained using a Fluoview FV10i confocal microscope from Olympus (Tokyo, Japan).

For RA FLS IF, RA FLS were grown on coverslips until 90% confluent before knock down treatment. Following 24h serum-starvation, RA FLS were stimulated with 50 ng/ml TGFβ1 for 30 minutes and then fixed in 4% formaldehyde for 15 minutes at room temperature. RA FLS were washed in PBS, then permeabilized with 1% BSA, 0.4% Triton-X in PBS for 20 minutes. Coverslips were incubated overnight at 4°C with anti-SMAD3 antibodies (Cell Signaling), or anti-YAP1 antibodies (Santa Cruz), then stained with fluorescent-conjugated secondary Alexa Fluor 555 anti-rabbit antibodies (Thermo) and 5 µg/ml Hoescht 33342 (Thermo). Coverslips were mounted with gold anti-fade reagent (Thermo Fisher) and analyzed with a FluoView FV10i confocal microscope (Olympus). Image J was used to quantify the fluorescence intensity of 4 cells per field. Three fields per RA lines from 3 independent experiments using different RA FLS lines were assessed for each study

Flow cytometry

RA FLS were trypsinized and prepared into single cell suspensions. Cells were blocked with human Fc Block (BD) and then stained with Alexa Fluor 647-conjugated anti-human TGFβR rabbit antibodies. Data were acquired on a ZE5 cell analyzer (Bio-rad) with EVO software (Bio-rad). Analysis of flow cytometry data was performed using FlowJo Software (TreeStar).

SMAD reporter assays

The full-length PTPN14 expression construct has been already described [7]. PTPN14 mutants Y570F/Y732F and C1121S expression constructs were generated via the Mutagenex's site-directed mutagenesis service. The YAP1 expression construct with the SV40 large T antigen NLS added to the N-terminus was from Invitrogen. Empty pcDNA3.1 vector was used as control. HEK 293 T cells were serum-starved (DMEM + 0.1% FBS) for 24h before transfection with protein expression constructs along with the Cignal reporter plasmid using the Lipofectamine 3000 kit (Thermo). 10h after transfection, the cells were stimulated with 50 ng/ml TGFβ1 (eBioscience) overnight then lysed for analysis. Luciferase activity was measured with a Tecan Infinite M1000 plate reader.

SCID cartilage engraftment assays

Bovine cartilage was cut into ~8 mm^3 pieces, which were then placed in ~80 mm^3 pieces of GelFoam surgical sponge (Pfizer Inc.). The cartilage/sponge implants were then soaked with 100 microl FLS media containing 500,000 RA FLS. Implants were then surgically placed under the skin on the back of male SCID mice (Jax). One day after the surgery, mice were injected intraperitoneally with 15 mg/kg verteporfin in DMSO or DMSO every other day for 34 days. After 36 days, the implants were removed and frozen in Tissue Tek O.C.T compound (VWR) for histological analysis after hematoxylin and eosin (H&E) staining. Three fields per implant were counted.

K/BxN passive serum-transfer arthritis model

On the day of acute arthritis induction, eight-week old female Balb/c mice were injected intra-peritoneally (i.p.) with 100 µl serum from K/BxN mice and injected i.p. daily with 50 mg/kg verteporfin or vehicle containing DMSO starting on the day of arthritis induction. Arthritis was scored as described in [5] and ankle thickness was measured daily using a digital caliper.

Histology

Right ankles from verteporfin treated mice were harvested, fixed in 10% neutral buffered zinc-formalin from Thermo Fisher Scientific for 48 h, decalcified in Shandon TBD1 decalcifier from

Thermo Fisher Scientific for 3 days, and embedded in paraffin using La Jolla Institute histology facility. Six micron sagittal sections were cut and stained with safranin-O fast-green or haematoxylin and eosin. Images were obtained using an AxioScan Z1 slide scanner from Zeiss.

Genome-wide multidimensional clustering by EpiSig (landscape)

EpiSig is a program written with C++ for aligning sequences and pattern discovery. All sequencing datasets were first processed, normalized and filtered. Then the datasets were input in EpiSig to identify clusters. For details about patients, IRB approval for the epigenetic landscape study, and the methods for RNA-seq, ATAC-seq and Chip-seq processing, the reader is referred to [8].

Analysis of differences in epigenetic marks

After defining the overall epigenomic landscape for FLS, we compared the relative intensity of each epigenetic mark between RA FLS and 10 OA FLS. DMERs of ChIP-seq markers (H3K4me1, H3K27ac, H3K4me3, H3K9me3, H3K27me3 and H3K36me3) and ATAC-seq (open chromatin regions) were determined by DiffBind package in R with q value < 0.05. For WGBS, DMLs were first determined and differentially methylated regions were defined by callDMR function of DSS package in R with delta beta of DMLs > 0.05 and q value < 0.0015. For RNA-seq, differentially expressed genes are defined as fold-change > 2 and q value < 0.05.

Ingenuity pathway analysis

The Hippo signaling pathway was generated through the use of QIAGEN's Ingenuity Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity) [9].

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. IHC characterization of surface marker expression in non-fibroid vs fibroid synovial samples. A cohort of 88 synovial samples from treatment-naïve RA patients were grouped into non-fibroid or fibroid by histological assessment. IHC was performed using antibodies for CD3 (T cells), CD20 (B cells), CD138 (plasma cells), and CD68 (macrophages). Box-and-whisker plots depict median (line within box), 25th percentile and 75th percentile (bottom and top borders), and range of minimum to maximum values (whiskers) of IHC scores. Data were analyzed using the two-tailed Mann-Whitney test.

Supplementary Figure 2. Knockdown of PTPN14 using antisense oligos in RA FLS. A. Western blotting of lysates of 3 RA FLS lines for PTPN14 expression after treatment with control antisense oligos (Ctrl ASO) or PTPN14 antisense oligos (PTPN14 ASO) for 7 days. GAPDH is shown as a loading control.

Supplementary Figure 3. Knockdown of PTPN14 using PTPN14 ASO ex2b (ASO2) reduces TNFα-stimulated MMP production in RA FLS. RA FLS (n=4) were treated with Ctrl ASO or PTPN14 ASO2 for 6 days, serum starved in the presence of ASO2 for 24h and then stimulated with 50 ng/ml TNFα for 24h. *MMP1* and *MMP13* mRNA expression levels were assessed by qPCR performed in triplicate. Results were normalized to *GAPDH*. Box-and-whisker plots depict median (line within box), 25th percentile and 75th percentile (bottom and top borders), and range of minimum to maximum values (whiskers). Data were analyzed using the Kruskal-Wallis test with one-tailed Mann-Whitney post-hoc test (left panel) or the two-tailed Mann-Whitney test (right panel).

Supplementary Figure 4. Knockdown of PTPN14 in RA FLS does not affect cell viability. RA FLS (n=3) were treated with Ctrl ASO or PTPN14 ASO for 6 days and stained with APC-annexin V and propidium iodide (PI). PI+ /annexin V+ cells were gated and analyzed by FlowJo

software. Plot shows mean±SD % PI+ /annexin V+ cells. Data were analyzed using the two-tailed unpaired t test, NS = non-significant.

Supplementary Figure 5. PTPN14 does not alter TGFβ-induced SMAD2 nuclear translocation in RA FLS. RA FLS (n=3) were treated with Ctrl or PTPN14 ASO for 7 days, stimulated with TGFβ (50 ng/ml) for 30 min, then fixed and stained with an anti-SMAD2 antibody. Nuclear/cytoplasmic ratio of SMAD2 signal was calculated using image J for 12 cells from each RA FLS line. Representative images are shown in 60X magnification. Mean±SD is shown. Data were analyzed using the Kruskal-Wallis test with two-tailed Mann-Whitney post-hoc test.

Supplementary Figure 6. YAP promotes TGFβ-induced SMAD3 nuclear translocation in RA FLS. A. Representative fields for data plotted in **Figure 3F**. Signal for YAP is depicted in green. **B.** RA FLS were incubated with 2.5 μM Ctrl ASO or antisense oligonucleotide to knockdown YAP1 expression (YAP1 ASO) for 6 days, followed by serum starvation for 24h in the presence of ASO. *YAP* mRNA expression was determined by qPCR performed in triplicate. Data were analyzed using the two-tailed unpaired t test. Results were normalized to *GAPDH*. Plot shows mean±SD. **C.** Representative fields for data plotted in **Figure 3G**. Signal for SMAD3 is depicted in red.

Supplementary Figure 7. YAP promotes RA FLS expression of TNFα-dependent genes. RA FLS (n=4) were serum-starved for 24h, then incubated with DMSO or 1μM VP for 45 min. Cells were then stimulated for 24h with 50 ng/ml TNFα in the presence of DMSO or VP. *VCAM1, IL6*, and *MMP3* mRNA expression was determined by qPCR performed in triplicate. Results were normalized to *GAPDH*. Box-and-whisker plots depict median (line within box), 25th percentile and 75th percentile (bottom and top borders), and range of minimum to maximum values (whiskers). Data were analyzed using the Kruskal-Wallis test with two-tailed Mann-Whitney post-hoc test.

Supplementary Figure 8. Verteporfin treatment protects arthritic mice from cartilage damage. Representative safranin O-stained joints from mice shown in **Figure 5B,C**. Scale bar represents 50 microns.

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