SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Quantitative Real-Time RT-PCR (qPCR)

Cells were stimulated as indicated for 24 hr or left unstimulated. RNA was extracted using RNeasy Kits (Qiagen, Valencia, CA). cDNA was synthesized using the SuperScript® III First-Strand Synthesis SuperMix for qRT-PCR (Life Technologies, Carlsbad, CA). qPCR was performed using a Roche Lightcycler 480 (Indianapolis, IN), with primer assays and SYBR® Green qPCR Mastermix from SABiosciences/Qiagen. Primer assay efficiencies were guaranteed by the manufacturer to be greater than 90%. Each reaction was measured in triplicate and data was normalized to the expression levels of the house-keeping gene RNA Polymerase II (*RPII*)[1], also measured in triplicate.

Cell Lysis for Western Blotting and RPTPκ Immunoprecipitation

Cells were lysed in RIPA buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) containing 1 mM phenylmethanesulfonyl fluoride, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 10 µg/ml soybean trypsin inhibitor, 1-10 mM sodium orthovanadate, 5 mM sodium fluoride and 2 mM sodium pyrophosphate. Protein concentration of cell lysates was determined using the Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL).

Immunohistochemistry of Synovial Tissue

Paraffin embedded slides of human RA synovial tissues were obtained from the UCSD CTRI Biorepository. Slides were deparaffinated, rehydrated and pretreated for 10 min with boiling citrate antigen retrieval buffer (1.9 mM citric acid, 10 mM Tris-sodium citrate pH 6.0) before being treated with 3% H_2O_2 for 10 min. Slides were then blocked with 5% goat serum for 1 hr at room temperature. Rabbit anti-RPTPκ antibody or negative control rabbit IgG (1:100 in 5% bovine serum albumin [BSA]) were incubated with the slides overnight at 4°C. The slides were then washed and incubated with SignalStain Boost IHC Detection reagent (HRP, rabbit) (Cell Signaling Technologies) for 30 minutes, and then incubated for 5 min with 3,3'-

diaminobenzidine substrate (Sigma-Aldrich). Slide images were obtained using an Eclipse 80i microscope (Nikon, Melville, NY).

Spot Migration Assay

The spot migration assays were performed as in[2]. Following treatment with ASO, RA FLS were resuspended at 1×10^6 cells/50 µl in DMEM. Cells (2 µl) were mixed 1:1 in 4 mg/ml growth factor–reduced BD Matrigel Matrix (BD Biosciences), pipetted as a spot in a 24-well tissue culture dish, and incubated at 37°C for 15 min. Serum-starvation medium with or without PDGF-BB (10 ng/ml) was added. Cell movement concentrically was monitored after 2 d. Cells were fixed and stained using the Hemacolor staining kit (EMD Millipore). Images were acquired from 4 fields per spot, and migrated cells were counted using ImageJ software.

FLS Spreading and Adhesion Assay

Following treatment with ASO, equal RA FLS numbers were resuspended in FLS medium containing 5% FBS and allowed to adhere onto circular coverslips coated with 20 µg/ml fibronectin (FN) at 37ºC for 15 min (adhesion assays) or 15, 30 and 60 min (spreading assays). Following the incubation period, cells were fixed in 4% para-formaldehyde for 5 min, permeabilized in 0.2% Triton X-100 for 2 min, and stained with 5 U/ml Alexa Fluor® 568 (AF 568)-conjugated phalloidin and 2 µg/ml Hoechst for 20 min each (Life Technologies). Samples were imaged with an Olympus FV10i Laser Scanning Confocal microscope (Olympus, Center Valley, PA). Using the FV10i acquisition software, each circular coverslip of cells was separated into four nine-paneled mega-images. Each panel (1024x1024) was optically acquired with a 10x objective using the FV10i acquisition software and stitched together, through a 10% overlap, with the Olympus FluoView 1000 imaging software. Total cell number and cell areas for each panel were calculated using Image Pro Analyzer software (Media Cybernetics, Rockville, MD).

FLS Survival and Apoptosis Assay

Following treatment with ASO, RA FLS were washed and incubated for an additional 24 h in serum-starvation media. Adherent and non-adherent cells were collected and stained with

Annexin V-Alexa Fluor® 647 (AF 647) and propidium iodide according to the manufacturer's instructions (Biolegend, San Diego, CA). Cell fluorescence was assessed by FACS using a BD LSR-II (BD Biosciences).

Synovial Micromass Organ Cultures

Synovial organ cultures were prepared as described in[3]. RA FLS were suspended in ice-cold Matrigel Matrix (BD Biosciences) at 2×10^6 cells/ml. Twenty-five-µl droplets of the suspension were placed onto culture dishes coated with poly-2-hydroxyethyl-methacrylate (Aldrich Chemical Co., Milwaukee, WI) and allowed to gel for 30 minutes at 37°C. Gels were overlaid with FLS culture medium supplemented with nonessential amino acid solution, ITS (Insulin-Transferrin-Selenium, Life Technologies) and 0.1 mM ascorbic acid. The floating three-dimensional culture was maintained for 3 weeks, with the medium replaced twice per week. After 2 weeks, 2.5 µM Ctl or PTPRK ASO was added to the medium to induce knockdown (KD) of PTPRK expression during the final week of culture. Micromasses were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 micron-thick sections, and stained with hematoxylin and eosin.

Beta-Catenin and SMAD3 Subcellular Localization Assays

RA FLS were plated on glass coverslips. Cells were pre-treated with ASO for 5 days, and synchronized in the presence of ASO for an additional 2 days. Cells were then stimulated with 50 ng/ml TGFβ1 for 30 min or 24 hr, or left unstimulated, and then fixed in 4% para-formaldehyde for 5 min, permeabilized in 0.2% Triton X-100 for 2 min, and stained for 1 hr each with anti-SMAD3 antibody (Cell Signaling Technology) and anti-beta-catenin antibody (BD Biosciences), and AF 488 goat anti-rabbit secondary antibody and AF 647 goat anti-mouse secondary antibody (Life Technologies). Cells were then stained with 5 U/ml AF 568-conjugated phalloidin and 2 µg/ml Hoechst for 20 min each. Samples were imaged with an Olympus FV10i Laser Scanning Confocal microscope (Olympus, Center Valley, PA). Using the FV10i acquisition software, each circular coverslip of cells was imaged by using 3 random fields of

view and acquiring a stitched nine paneled mega-image. Each panel (1024 x1024) was optically acquired with a 60x objective using the FV10i acquisition software and stitched together, through a 10% overlap, with the Olympus FluoView 1000 imaging software. Each mega-image was then further processed, post stitching, using Image Pro Analyzer software (Media Cybernetics, MD). Using Image Pro, the original mega images were used to first automatically define nuclear localization by masking the Hoechst nuclear signal then isolating the fluorescence of either SMAD3 or beta-catenin signal localized within the nucleus mask. Cytoplasmic signals were defined by removing the masked nuclear signal from the images and thus quantifying the remaining cytoplasmic signal of SMAD3 or beta-catenin. Total area of nuclear and cytoplasmic fluorescence label of either SMAD3 or beta-catenin were calculated.

COS-1 Transfection

cDNA encoding C-terminally HA-tagged wild type (WT) or catalytically inactive (C1100S) RPTPκ (NM_008983.2) was cloned into the pcDNA3.1(+) vector. COS-1 cells were cultured in DMEM with 10% FBS, 100 units/ml of penicillin and 100 µg/ml streptomycin. Cells were transfected with Lipofectamine 3000 (Life Technologies) according to the manufacturer's protocol, and harvested after 2 d.

***In vitro* Pull-Down Assay**

HA-tagged WT RPTPκ was overexpressed in COS-1 cells. Cells were lysed in TNE buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM EDTA pH 8.0 and 1% NP-40) containing 10 μg/mL aprotinin, 10 μg/mL leupeptin, 10 μg/mL soybean trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride. RPTPκ was immunoprecipitated using anti-HA antibody (Covance, Princeton, NJ). RA FLS were lysed in RIPA buffer containing 10 μg/mL aprotinin, 10 μg/mL leupeptin, 10 μg/mL soybean trypsin inhibitor, 1 mM phenylmethylsulfonyl fluoride and 5 mM iodoacetamide. Lysates were treated with 10 mM dithiothreitol for 10 min, and then diluted 10-fold in buffer containing 25 mM Tris-HCl pH 7.6, 150 mM NaCl and 1 mM EDTA**.**

Immunoprecipitates were incubated with RA FLS lysates for 3 hr at 4°C, washed in the dilution buffer, and subjected to Western blotting.

Substrate-Trapping Pull-down Assay

Substrate-trapping is a well-established technique to identify substrates of PTPs[4]. 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. cDNA (codon optimized for expression in *E. coli* [Genscript, Piscataway, NJ]) encoding a substrate-trapping mutant of the catalytic domain of RPTPκ (iPTPκ-D1051A, aa865-1156 of NP_002835.2) was cloned into the pET30c vector. S-tagged-iPTPκ-D1051A was isolated from *E. coli* using S-protein agarose (EMD Millipore). RA FLS were lysed in RIPA buffer containing 10 μg/mL aprotinin, 10 μg/mL leupeptin, 10 μg/mL soybean trypsin inhibitor, 1 mM phenylmethylsulfonyl fluoride and 5 mM iodoacetamide. Lysates were treated with 10 mM dithiothreitol for 10 min, and then diluted 10-fold in buffer containing 25 mM Tris-HCl pH 7.6, 150 mM NaCl and 1 mM EDTA**.** Agarose-bound S-tagged-iPTPκ-D1051A was incubated with RA FLS lysates for 3 hr at 4°C, washed in the dilution buffer, and subjected to Western blotting.

***In vitro* PTP Assay**

HA-tagged WT and catalytically inactive mutant C1100S (C/S) RPTPκ were overexpressed in COS-1 cells. Cells were lysed in HNE buffer (50 mM Hepes, pH 7.4, 150 mM NaCl, 5 mM EDTA and 1% Triton-X) containing 10 μg/mL aprotinin, 10 μg/mL leupeptin, 10 μg/mL soybean trypsin inhibitor, 1 mM phenylmethylsulfonyl fluoride. RPTPκ was immunoprecipitated using anti-HA antibody. Immunoprecipitates were washed extensively in 50 mM Bis-Tris pH 6., and then incubated with 50 mM Bis-Tris pH 6.0 and 5 mM DTT for 30 min at 4°C. Immunoprecipitates were divided into triplicate reactions and incubated with 0.2 mM phospho-SRC Y527 peptide [H-TSTEPQ-pY-QPGENL-OH] (Anaspec, Fremont, CA) in 50 mM Bis-Tris pH 6.0, 5 mM DTT and

0.005% Tween-20 at 37°C for 30 min. Reactions were stopped with the addition of Biomol Green (Enzo Life Sciences, Plymouth Meeting, PA), and absorbance of the solution was measured at 620 nm using a Tecan M1000 plate-reader (Tecan Systems, San Jose, CA). PTP activity was plotted as absorbance following subtraction of absorbance from the blank reactions (control anti-HA immunoprecipitations from lysates of cells transfected with empty vector), also measured in triplicate.

***In vivo* Invasion Assay**

The *in vivo* invasion assay was performed as described in[5]. Skin inflammation was induced in athymic nude mice by subcutaneously injecting 120 µg complete Freund's adjuvant (CFA) in each flank. The next day, $5x10^5$ RA FLS pretreated with Ctl or PTPRK ASO were intradermally implanted 1.2 cm distance from the CFA injection sites (each mouse was injected with Ctl ASO-treated cells in one flank and PTPRK ASO-treated cells in the contralateral flank). After 5 days, the skin regions between the 2 injection sites was harvested, and FLS invasion from the implantation site towards the inflammation site was assessed by immunohistochemical staining with an anti-HLA Class I antibody (Abcam, Cambridge, MA). Skin samples were frozen in optimal cutting temperature compound (OCT), and 2 cryosections from immediately adjacent the CFA injection site were obtained from each skin sample. Cryosections were fixed in 4% para-formaldehyde for 10 min and pretreated for 10 min with boiling citrate antigen retrieval buffer (1.9 mM citric acid, 10 mM Tris-sodium citrate, pH 6.0) before being treated with 3% H_2O_2 for 10 min. Slides were blocked with 5% BSA overnight at 4°C, then incubated with anti-human HLA Class I antibody (1:200 in 5% BSA) for 1 hr at room temperature. The slides were washed and incubated with peroxidase-conjugated anti-mouse IgG from Vector Laboratories for 30 min at room temperature, then incubated for 5 minutes with 3,3'-diaminobenzidine substrate and stained with hematoxylin. Samples were then imaged using a BZ-9000E microscope (Keyence, Itasca, IL). The numbers of invaded FLS, recognized by staining with the anti-human HLA Class I antibody, in each 20X field were manually counted.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. RPTPκ is expressed in the RA synovium. Immunohistochemical staining of RA synovial section using control IgG antibody.

Supplemental Figure 2. *PTPRK* expression in OA FLS is induced by cell stimulation with TGFβ1. *PTPRK* and *PTPRM* mRNA expression in OA FLS was measured following cell stimulation with 50 ng/ml TGFβ1 for 24 hr. Median±IQR is shown. *, $p<0.05$, Mann-Whitney test.

Supplemental Figure 3. *PTPRK* expression in RA FLS is not affected by cell stimulation with TNF or IL-1. The expression of *PTPRK* and as a positive control, *IL6*, was measured by qPCR in 8 RA and 8 OA FLS lines following cell stimulation with 50 ng/ml TNF or 2 ng/ml interleukin 1 (IL-1β) for 24 hr. Panel shows median±IQR. Significance was calculated using the Wilcoxon matched-pairs signed rank test. *, $p<0.05$.

Supplemental Figure 4. Knockdown of PTPRK does not affect the expression of *TGFB1* in RA FLS. (A-B) Cell-permeable antisense oligonucleotide (ASO) enables efficient knockdown of *PTPRK* expression in RA FLS. RA FLS were treated with 2.5 μM control (Ctl) or PTPRK ASO for 7 days. (A) After 6 days of ASO treatment, cells were stimulated with 50 ng/ml TGFβ1, or left unstimulated, in the presence of ASO for 24 hr. Panels show Western blotting of lysates with anti-RPTPκ or anti-Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibodies. Data is representative of 5 independent experiments in different FLS lines. (B) *PTPRK* expression was assessed by qPCR, normalized to the housekeeping gene *RPII*, and plotted relative to the *PTPRK* expression in Ctl ASO-treated cells. Data from 5 independent experiments in different FLS lines is shown. Significance was calculated using the Wilcoxon matched-pairs signed rank test. *, $p<0.05$. (C) *PTPRK* expression does not affect the expression of *TGFB1* in RA FLS. Following treatment of RA FLS for 7 days with 2.5 μM Ctl or PTPRK ASO, *TGFB1* expression was assessed by qPCR, normalized to the housekeeping gene *RPII*, and plotted relative to the

TGFB1 expression in Ctl ASO-treated cells. Panel shows median±IQR. Data from 3 independent experiments in different FLS lines is shown.

Supplemental Figure 5. Knockdown of RPTPκ, but not RPTPμ, reduces RA FLS invasiveness. (A) PTPRK_2 ASO enables efficient knockdown of RPTPκ. RA FLS were treated with 2.5 μM Ctl or PTPRK_2 ASO for 7 days. *PTPRK* expression was assessed by qPCR, normalized to the housekeeping gene *RPII*, and plotted relative to the *PTPRK* expression in Ctl ASO-treated cells. Panel shows mean±range. Data from 2 independent experiments in different FLS lines is shown. (B) PTPRM ASO enables efficient knockdown of *PTPRM*. RA FLS were treated with 2.5 μM Ctl or PTPRM ASO for 7 days. *PTPRM* expression was assessed by qPCR, normalized to the housekeeping gene *RPII*, and plotted relative to the *PTPRM* expression in Ctl ASO-treated cells. Panel shows mean±range. Data from 2 independent experiments in different FLS lines is shown. (C) Following treatment with 2.5 μM Ctl or PTPRM ASO for 7 d, RA FLS were allowed to invade through Matrigel-coated transwell chambers in response to 50 ng/ml PDGF-BB for 24 hr. Graph shows median±IQR % maximum number of cells per field. Data from 3 independent experiments in different FLS lines is shown. Significance was calculated using the Mann-Whitney test. *, $p<0.05$.

Supplemental Figure 6. RPTPκ knockdown does not affect RA FLS survival. Following treatment with Ctl or PTPRK ASO and cell synchronization, RA FLS were serum-starved (FLS medium with 0.1% FBS) for an additional 24 hr. Cells were collected and stained with Annexin V and PI, and cell fluorescence was assessed by FACS. (A-B) Graphs show gating strategy to detect early apoptotic (Annexin V^+PI^-) and necrotic/late apoptotic (Annexin V^+PI^+) cells following treatment with Ctl (A) or PTPRK (B) ASO. (C) Graph shows median±IQR percentage of early apoptotic (Annexin V^+PI^-) and necrotic/late apoptotic (Annexin V^+PI^+) cells. Data from 5 independent experiments in different FLS lines is shown. Significance was calculated using the Wilcoxon matched-pairs signed rank test.

Supplemental Figure 7. RPTPκ knockdown inhibits RA FLS spreading but not adhesion on an extracellular matrix. (A) RPTPκ knockdown does not affect RA FLS adhesion on fibronectin (FN). Graph shows median±IQR cell number of cells from Figure 2F. Data from 3 independent experiments in different FLS lines is shown. Significance was calculated using the Mann-Whitney test. (B) Panel shows representative images of cells from (A) and Fig. 2F 60 min after plating on FN.

Supplemental Figure 8. Cadherin-11 and beta-catenin are not substrates of RPTPκ in RA FLS. (A) RPTPκ knockdown does not affect tyrosine phosphorylation of cadherin-11. Following treatment with 2.5 μM Ctl or PTPRK ASO for 7 days, RA FLS lysates were subjected to immunoprecipitation with anti-cadherin-11 (CDH11) antibody. Panels show Western blotting with the indicated antibodies. (B) RPTPκ knockdown does not affect formation of synovial lining in FLS micromass organ cultures. FLS micromass organ cultures were prepared in Matrigel as described in[3]. Two weeks after plating, cultures were treated with 2.5 μM Ctl or PTPRK ASO for an additional week. (B) Panels show histological images of micromasses stained with hematoxylin and eosin. (C) Following treatment with 2.5 μM Ctl or PTPRK ASO for 7 days, RA FLS were stained with anti-beta-catenin antibody, phalloidin and Hoechst and imaged by immunofluorescence microscopy. Graph shows proportions of beta-catenin in cytosolic and nuclear fractions. Data from 3 independent experiments in different FLS lines is shown. Significance was calculated using the Mann-Whitney test.

Supplemental Figure 9. Treatment of RA FLS with chemical inhibitors of SRC and PLCγ1 does not affect RA FLS survival. (A-B) RA FLS were synchronized and then serum-starved (FLS medium with 0.1% FBS) for an additional 24 hr in the presence of DMSO or 20 μM SRC inhibitor PP2 (A) or 1 μM PLCγ1 inhibitor U73122 (B). Cells were collected and stained with Annexin V and PI, and cell fluorescence was assessed by FACS. Graphs show gating strategy to detect early apoptotic (Annexin V^+PI^-) and necrotic/late apoptotic (Annexin V^+PI^+) cells.

Supplemental Figure 10. TGFβ signaling in RA FLS is unaffected by knockdown of RPTPκ. Following treatment with Ctl or PTPRK ASO for 7 days, RA FLS were stimulated with 50 ng/ml TGFβ1 for 5, 15, 30 or 60 min, or left unstimulated. Panels show Western blotting of cell lysates with anti-phospho-SMAD3 and anti-SMAD3 antibodies. (B) Following treatment with Ctl or PTPRK ASO for 7 days, RA FLS were stimulated with 50 ng/ml TGFβ1 for 30 min, 24 hr, or left unstimulated. Cells were stained with anti-SMAD3 antibody, phalloidin and Hoechst and imaged by immunofluorescence microscopy. Graph shows proportions of SMAD3 in cytosolic and nuclear fractions. Data from 3 independent experiments in different FLS lines is shown. Significance was calculated using the Mann-Whitney test.

Supplemental Figure 11. RPTPκ promotes IL-1 signaling in RA FLS. Following treatment with Ctl or PTPRK ASO for 7 days, RA FLS were stimulated with 2 ng/ml IL-1β for 24 h or left unstimulated. Graph shows median±IQR mRNA expression levels relative to the Ctl ASO-treated, IL-1β-stimulated samples from the same FLS line. Data from 4 independent experiments in different FLS lines is shown. *, $p<0.05$, Mann-Whitney test.

Supplemental Figure 12. FAK activity promotes JNK activation in RA FLS. RA FLS were pre-treated for 20 min with 5 μM FAK inhibitor PF573228 or DMSO, and then stimulated with 50 ng/ml TNFα for 15 min or left unstimulated. Western blotting of cell lysates in shown. Data is representative of 3 independent experiments in different FLS lines.

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