Supporting Information

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SI Materials and Methods

Identification of Cleaved PC1 Tail from Patient Tissue by Mass Spectrometry. The 15-kDa regions of Coomassie-stained SDS/PAGE gels were cut, washed with water, and dehydrated in acetonitrile before reduction with DTT and alkylation with iodoacetamide. In-gel proteolytic digestion was carried out overnight at room temperature using a modified, sequencing grade trypsin (Promega). The peptides that are formed were extracted from the gel, evaporated to dryness, and reconstituted in 30 μL of 1% acetic acid for LC/MS analysis using a Finnigan LCQ deca ion trap mass spectrometer system (ThermoFinnigan) equipped with a nanospray ionization source (Protana). The HPLC column was a self-packed 8 cm × 75 μm i.d. Phenomenex Jupiter C18 reversed-phase capillary chromatography column. Two-microliter volumes of the extract were injected and the peptides eluted from the column by an acetonitrile/0.05 M acetic acid gradient at a flow rate of 200 nL/min. The control PKD1-GST protein digest data were analyzed by using all peptide molecular weights and collision-induced dissociation (CID) spectra collected in the experiment to search the NCBI non-redundant database with the search program Mascot. All matching spectra were verified by manual interpretation. From this analysis, marker peptides were identified and used in the selective reaction monitoring experiments on the patient samples. In these experiments, the mass spectrometer was used to record the product ion spectra of molecular ions of the marker peptides that were characterized in the mapping experiment. Detection of the appropriate peptide was verified by the CID spectrum that was recorded at the correct retention time.

Cell Cycle Analysis. Confluent MDCK cells stably expressing the constructs were seeded in 12-well plates at low density. Forty-eight hours later, cells were trypsinized and 300,000 cells per condition were resuspended in 250 μL ice-cold PBS + 1% BSA with 50 μL of ice-cold cell cycle buffer (0.11% citrate, 0.1% Triton-100) and 10 μL of RNAseA (10 mg/mL). A total of 10 μL of propidium iodide (1 mg/mL; Sigma) was added after 4 h of incubation on ice. After 18–20 h incubation at 4 °C, samples were analyzed by flow cytometry (EasyCyte 96; Guava Technologies) using a minimum of 10,000 gated events per condition.

Immunoprecipitation. Cells were washed once with cold PBS and scraped with lysis buffer (50 mM Hepes, 50 mM potassium acetate, and 0.5% Triton X-100), containing protease inhibitior mixture (Sigma), PMSF, 200 μM sodium orthovanadate, and 0.5% BSA. Lysates were rotated at 4 °C for 30 min and precleared with CL-2B sepharose. Lysates were then incubated with either anti-STAT1 antibody or control IgG overnight. Preblocked protein-A-sepharose beads (Amersham) with BSA were used in the pull down for 1 hr at 4 °C. The beads were washed with lysis buffer three times and samples were analyzed by Western blotting.

JAK2 Binding. HEK293T cells were cotransfected with JAK2-HA and PC1-GST constructs. Cells were lysed in 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% TX-100, 5 mM DTT, and protease inhibitors. Cleared lysates were precipitated with glutathione agarose. After extensive washing, precipitates were analyzed by immunoblot.

Microscopy. Images were captured using an Olympus IX-81 inverted microscope, equipped with a Q-image Retiga EXi camera and Q Capture Pro software (QImaging).

IFNγ Mouse Injections. Two groups of bpk mice were given i.p. injections of murine IFNγ or vehicle (sterile saline/murine serum albumin). The low-dose group received injections of 1.2 μg IFNγ or 100 μL vehicle on day P19 and P21; the high-dose group received daily injections of 3 μg IFNγ or 100 μL vehicle from days P16–P20. Both groups were killed on day P21 and dissected tissues stored at −80 °C until use. Experiments were approved by the Institutional Animal Care and Use Committee of University of California at Santa Barbara.

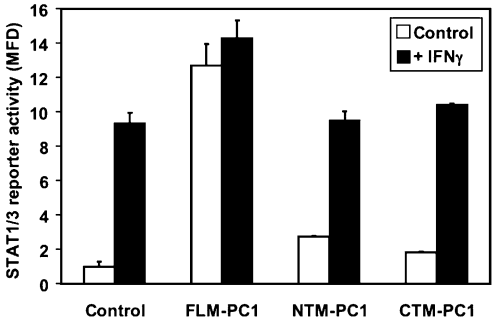

Fig. S1. STAT1/3 luciferase assay using HEK293T cells transfected with the STAT1/3 luciferase reporter and either control GFP or membrane-anchored PC1 tail constructs.

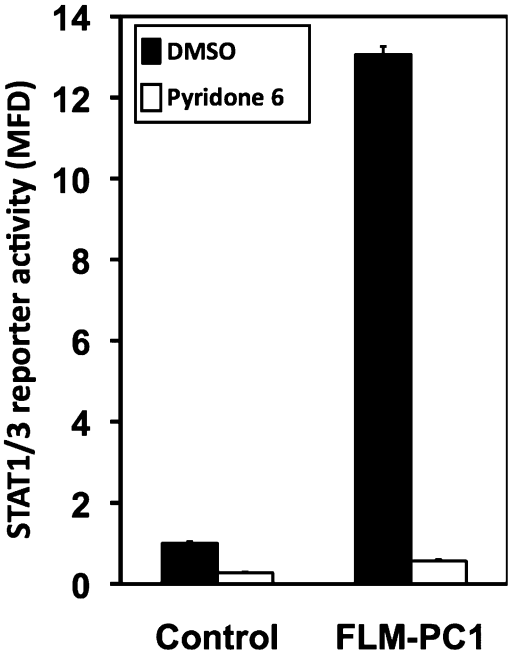

Fig. S2. STAT1/3 luciferase reporter assay in the presence or absence of the pan-JAK inhibitor pyridone 6 (0.5 μM). Pyridone 6 eliminates FLM-PC1 luciferase activity compared with DMSO control.

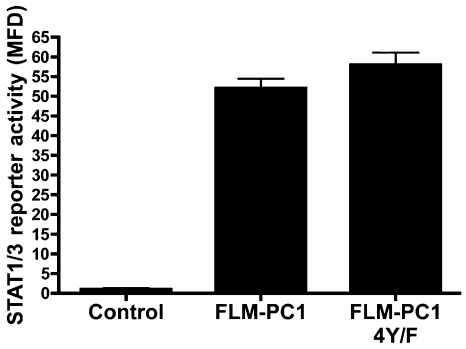

Fig. S3. STAT1/3 luciferase assay using HEK293T cells transfected with the STAT1/3 luciferase reporter and either control GFP, FLM-PC1, or FLM-PC1 in which all four tyrosine residues have been mutated to phenylalanines.

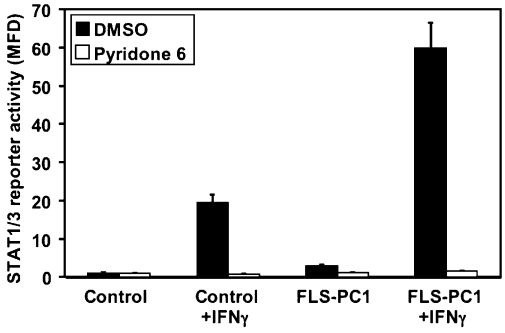

Fig. S4. STAT1/3 luciferase reporter assay in the presence or absence of the pan-JAK inhibitor Pyridone 6 (0.5μM). Pyridone 6 inhibits coactivation by FLS-PC1.

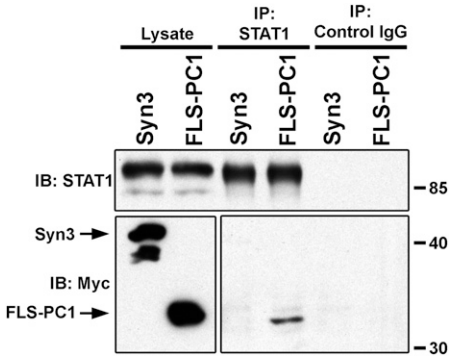

Fig. S5. FLS-PC1 interacts with STAT1. MDCK cells were transiently transfected plasmids encoding with either a myc-tagged control protein (syntaxin 3, Syn3) or myc-tagged FLS-PC1. Total lysates were immunoprecipitated with either anti-STAT1 or control rabbit IgG. STAT1 only coprecipitates with FLS-PC1.

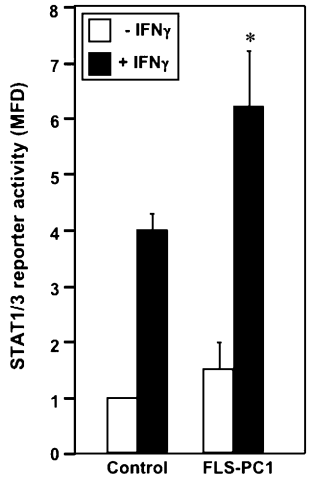

Fig. S6. FLS-PC1 does not act by a dominant-negative mechanism. MEFs with a targeted inactivation of both *pkd1* alleles were transfected with FLS-PC1 or control and treated with IFNγ as indicated. STAT1/3 luciferase reporter assay indicates coactivation by FLS-PC1 despite the lack of endogenous PC1.

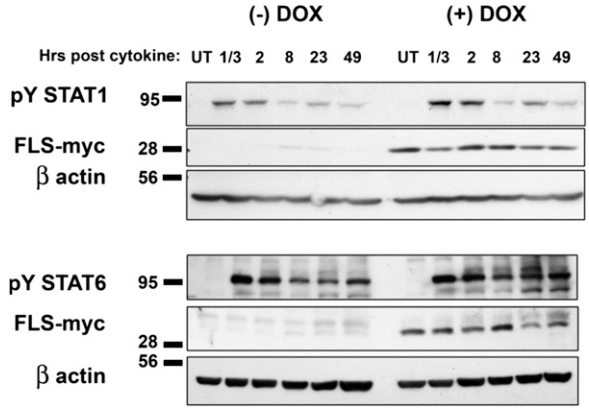

Fig. S7. As indicated, DOX was added to induce FLS-PC1 expression in stably transfected MDCK cells. Sixteen hours later, cells were washed with serum-free media and pulsed with either IFNγ (for STAT1 activation) or IL4 (for STAT6 activation) for 15 min in serum-free media. Cells were aspirated and washed twice with serum-free media to remove cytokine and then incubated for indicated times. pY-STAT1 or pY-STAT6 levels and expression of FLS-PC1 were determined by immunoblotting.