Supporting Information

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SI Materials and Methods

Human Equivalent Dose Translation. An animal dose of 1.4 mg/kg teriflunomide every other day was extrapolated to human equivalent dose of 0.114 mg/kg using body surface area conversion calculation (1). This conversion equates to a dose of 6.84 mg for a 60-kg person, which is within the 5- to 20-mg daily clinical dose range for rheumatoid arthritis patients (2, 3).

Immunohistochemistry. Four micrometer sections from formalin-fixed paraffin-embedded tissue were stained with H&E or Masson's trichrome or immuno-stained for phosphorylated (PY)-signal transducer and activator of transcription-6 (STAT6). Briefly, sections were deparaffinized in xylene, rehydrated through a series of alcohols, and then subjected to antigen retrieval by microwaving for 4 × 5 min in 10 mM sodium citrate, pH 6.0. Sections were incubated with blocking buffer (10% normal goat serum, 5% normal mouse serum, 0.5% fish skin gelatin, 1% BSA in Tris-buffered saline with 0.1% tween-20) and incubated with primary antibody or IgG control overnight. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide in Tris-buffered saline and endogenous biotin was blocked using the Avidin/Biotin blocking kit (Vectastain Labs). Primary antibody was detected using the Rabbit IgG kit (Vectastain Labs) and DAB.

Microarray and Quantitative RT-PCR. ***U133 Plus 2.0 oligonucleotide DNA microarrays and data analysis.*** Renal cyst samples of varying size were obtained from five polycystic kidney disease-1 (PKD1) patients and include small cysts (SC) less than 1 mL ($n = 5$), medium cysts (MC) between 10 and 25 mL ($n = 5$), and large cysts (LC) greater than 50 mL ($n = 3$). Minimally cystic tissue (MCT) ($n = 5$) from the same patients and normal renal cortical tissue ($n = 3$) were used as controls. All tissues were dissected within 30 min of nephrectomy, snap frozen, and stored at −80 °C. Total RNA was extracted from each sample using Absolutely RNA RT-PCR Miniprep Kit (Stratagene) with an on-column DNA digestion step to minimize genomic DNA contamination. Microarray samples were prepared according to the Affymetrix Two-Cycle Target Labeling Reagents for small sample (http://www.affymetrix.com/support/). A labeled cRNA sample was hybridized onto GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix). Scanned raw data images were processed with Genechip Operating Software 1.4. Probeset signal intensities were extracted and normalized by the robust multiarray average algorithm (4), which can be found in the R package *affy* that can be downloaded from the Bioconductor project Web site (http://www.bioconductor.org). Significance analysis of microarrays was used to identify genes whose expression was statistically significantly different in cyst versus MCT by setting the false-discovery rate at 0.5% (5). Pathway analysis was done by using Gene Set Enrichment Analysis (GSEA) (6). GSEA is a supervised analysis method to identify differentially expressed gene sets (groups of genes that share common biological function or coregulation) between two classes (using NOM P value < 0.01).

Validation by quantitative real-time PCR on selected genes of interest. For the quantitative PCR (qPCR) studies, we used an expanded cyst sample set: SC ($n = 16$); MC ($n = 19$); LC ($n = 3$), and MCT ($n = 16$) from the same patients and normal renal cortical tissue ($n = 4$). Total RNA was extracted using the same methods as detailed above. cDNA was generated with SuperScript II reverse transcriptase (Invitrogen). qPCR assay was performed with the ABI PRISM 7900 Sequence Detection System, using Power SYBR Green PCR Master Mix (Applied Biosystems). Primer sets for IL4Rα, IL13Rα1, and IL13Rα2 were designed to the exon sequences using the Primer Express software (Applied Biosystems). A standard curve of relative concentration was calculated for each reaction run using serial dilutions of human blood genomic DNA; absolute transcript copy numbers were calculated using standard curves for each primer set (primer sequences in Table S1). The geNorm software (http://medgen.ugent.be/~jvdesomp/genorm/) determines the most stable housekeeping genes (7). Gene expression normalization factor based on the geometric mean of three housekeeping genes *EEFIA1*, *B2M*, and *PPIA* were calculated. The resulting calculated copy numbers were normalized against the qPCR results and are expressed as mean ± SE. GraphPad Prism 3.0 (GraphPad Software) was used for statistical analysis of the results. Comparisons between cysts and control were done by one-way ANOVA with Tukey's multiple comparison posttest. The statistical significance was defined as $P < 0.05$.

Morphometric Analysis. H&E-stained kidney sections were used for quantitative analysis of tubule density and cyst diameter. In Adobe Photoshop CS, a blinded investigator used 80 × 80-μm regions of interest (ROI) to measure cysts and count tubules, $n = 6$ ROI per section (cortex and medulla), $n = 4$, 5, 8, and 4 animals per genotype, respectively. For cysts fully contained within or that had a portion of the cyst within the ROI, the cyst diameters were measured from epithelial to epithelial layer through the midpoint of the cyst along the longest axis of each cyst. Proliferation was determined by counting the number of cyst-lining Ki-67 positive cells as a percentage of the total number of cyst-lining cells per high-powered field, $n = 4$ fields per section, $n = 4$ animals per genotype. Apoptosis was determined by counting the total number of cleaved caspase 3-positive cells per field (kidney section), $n = 3$ animals per genotype. Cystic index was determined as described previously (8), $n = 6$ fields per section, $n = 7$, 4, and 4 animals per genotype, respectively.

1. Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. *FASEB J* 22:659–661.
2. Chan V, Charles BG, Tett SE (2005) Population pharmacokinetics and association between A77 1726 plasma concentrations and disease activity measures following administration of leflunomide to people with rheumatoid arthritis. *Br J Clin Pharmacol* 60:257–264.
3. van Roon EN, et al. (2005) Therapeutic drug monitoring of A77 1726, the active metabolite of leflunomide: Serum concentrations predict response to treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 64:569–574.
4. Irizarry RA, et al. (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 4:249–264.
5. Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* 98:5116–5121.
6. Subramanian A, et al. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 102:15545–15550.
7. Vandesompele J, et al. (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 3(7): RESEARCH0034.
8. Shillingford JM, et al. (2006) The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. *Proc Natl Acad Sci USA* 103:5466–5471.

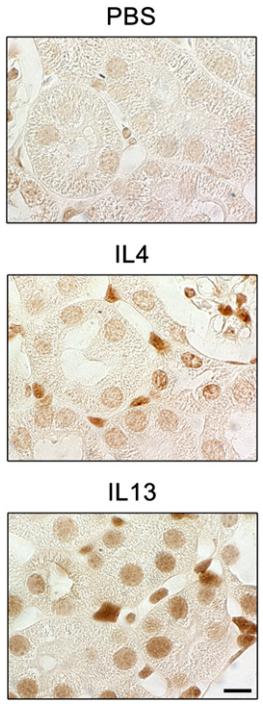

Fig. S1. PY-STAT6 is activated following acute stimulation with IL4 or IL13. Wild-type animals were injected intraperitoneally with cytokine or PBS, and kidneys harvested after 1 h. Immunostaining of renal tissue sections with PY-STAT6 reveals a prominent nuclear signal in the epithelial cells and positive staining of interstitial cells following IL13 stimulation. (Scale bar, 10 μm.)

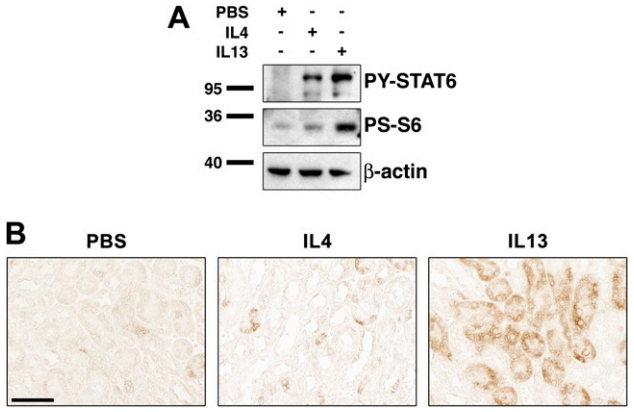

Fig. S2. Mammalian target of rapamycin (mTOR) pathway activation following injection of IL13. Immunoblot (*A*) and immunohistochemistry (*B*) for PS235/236-S6 ribosomal protein indicate that the mTOR pathway is activated in tubule epithelial cells from mouse kidney tissue after acute stimulation with IL13 for 1 h. (Scale bar, 50 μm.)

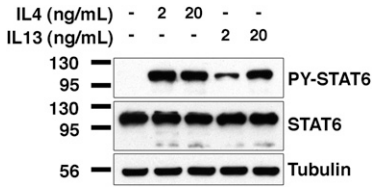

Fig. S3. Madin Darby canine kidney (MDCK) cells are capable of responding to IL4 and IL13 by phosphorylating STAT6. Subconfluent MDCK cells were treated with the indicated concentrations of recombinant canine IL4 or human IL13 for 30 min, lysed and analyzed by immunoblotting for PY-STAT6, STAT6, and tubulin.

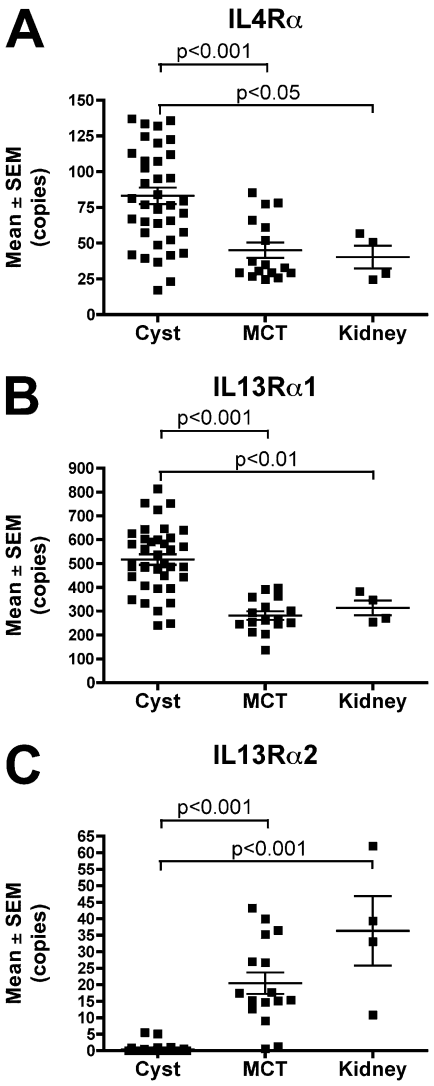

Fig. S4. qPCR validation of microarray results for IL4Rα (*A*), IL13Rα1 (*B*), and IL13Rα2 (*C*) using an expanded sample set of microdissected cysts from autosomal-dominant polycystic kidney disease (ADPKD) patients, MCT, and normal human kidney.

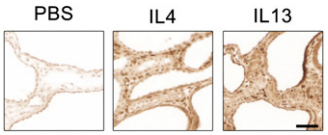

Fig. S5. Polycystic kidneys of bpk/bpk mice are hyperresponsive to activation with IL4 or IL13. Immunostaining of renal tissue sections with PY-STAT6 reveals increased signal in the epithelial cells and positive staining of interstitial cells following stimulation. (Scale bar, 50 μm.)

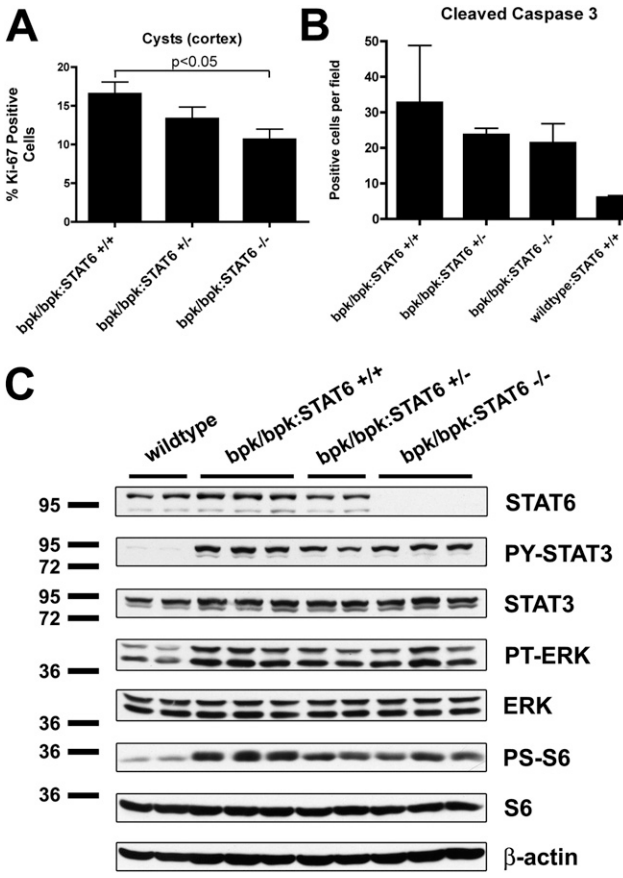

Fig. S6. Proliferation, apoptosis and signaling pathways in Bpk-STAT6 crosses. (*A*) Percentage of Ki-67 positive cortical cyst-lining cells out of total cortical cyst-lining cells of bpk/bpk:STAT6$^{+/+}$, bpk/bpk:STAT6$^{+/-}$, and bpk/bpk:STAT6$^{-/-}$ kidney tissue sections. (*B*) Number of cleaved caspase 3-positive cells per field (kidney section). n = 3 per genotype. (*C*) Kidney tissue lysates were analyzed by immunoblotting for STAT6, PY-STAT3, STAT3, PT-ERK, ERK, PS-S6, S6, and β-actin.

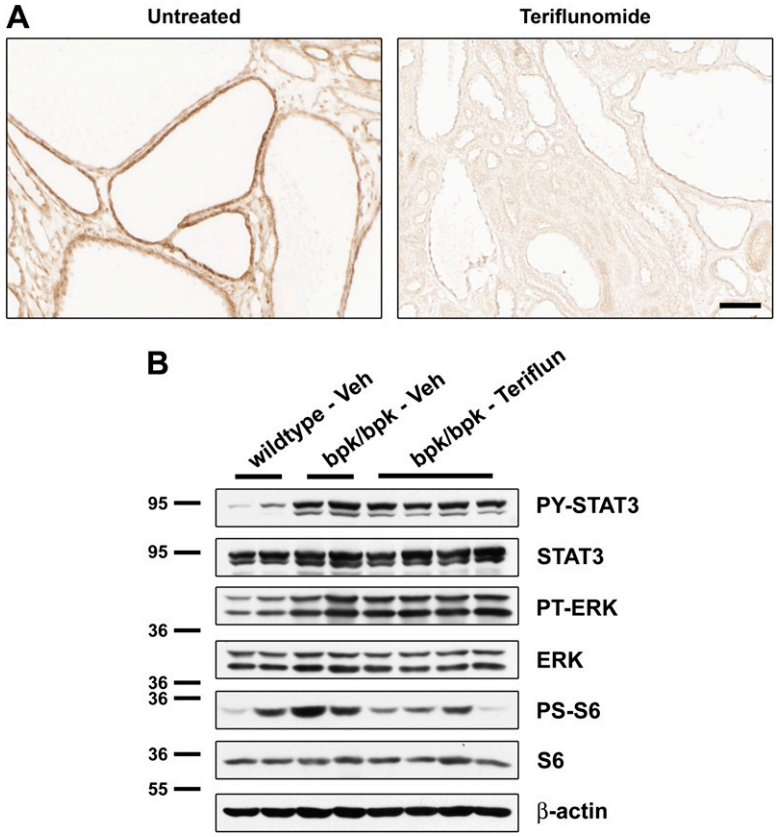

Fig. S7. Signaling pathways in leflunomide/teriflunomide-treated mice. (*A*) Immunostaining of renal tissue sections with PY-STAT6 reveals strong signal in untreated cyst-lining epithelial cells and little to no staining after teriflunomide treatment. (Scale bar, 50 μm.) (*B*) Wild-type vehicle (Veh)-treated, bpk/bpk vehicle-treated, and bpk/bpk Teriflunomide (Teriflun)-treated kidney tissue lysates were analyzed by immunoblotting for PY-STAT3, STAT3, PT-ERK, ERK, PS-S6, S6, and β-actin.

Table S1. Primers used for qPCR

Gene name	Direction	Sequence (5′→3′)	Amplicon size (bp)
IL4Rα	F	CACCTGCCTCTGTCTCACTGAA	77
	R	GGCCGCCCAAGTCATTC	
IL13Rα1	F	ATCTTTTGTTCCCATCCTCTTCT	65
	R	TTCCTTTTCCCTCCCTTTTC	
IL13Rα2	F	AACAACAAATGAAACCCGACA	85
	R	CTCACTCCAAATTCCGTCATC	
*PPIA**	F	GGTCCTGGCATCTTGTCCAT	63
	R	GATGAAAAACTGGGAACCATTTG	
*B2M**	F	GAGTGCTGTCTCCATGTTTGATGT	71
	R	AAGTTGCCAGCCCTCCTAGAG	
*EEF1A1**	F	CTGCCACCCCACTCTTAATCA	63
	R	GGCCAATTGAAACAAACAGTTCT	

Sequence are shown for forward (F) and reverse (R) primers.
*Housekeeping genes used for real-time PCR normalization.