Supplementary Figure Legend

Supplementary Fig. 1 Validation of shRNA constructs. Lentiviral vectors carrying shRNAs targeting *GFP* (control), *JAK1*, *JAK2*, *JAK3*, or *TYK2* were infected into JURKAT cells. Following selection by puromycin, whole cell extracts were harvested after three days of infection and subjected to immunoblot analysis using antibodies specific for **(A)** JAK1, **(B)** JAK2, **(C)** JAK3, or **(D)** TYK2. Lanes with shRNAs marked in red showed effective target silencing and, thus, were used for functional studies in Fig. 1. In each case one of the other JAK proteins not being targeted was used as an internal control to show equal loading volume and specificity of each shRNA. **(E)** The three *TYK2*-targeting lentiviral constructs were introduced into RPMI-8402, HPB-ALL, and LOUCY cells to confirm equivalent knockdown in these cells.

Supplementary Fig. 2 Cell cycle distribution after *TYK2* knockdown in T-ALL cells. A, JURKAT, HPB-ALL or RPMI-8402 cells harboring *GFP* (control) or *TYK2* shRNAs were analyzed for cell cycle distribution after five days of lentiviral infection by flow cytometric analysis of propidium iodide incorporation into permeabilized cells. **B,** Values are means ± standard error of the mean (s.e.m) of triplicate experiments. * $P<0.05$ by two-sample, two-tailed t-test.

Supplementary Fig. 3 Protein expression levels and phosphorylation of candidate signaling molecules in T-ALL cell lines A, Nineteen T-ALL cell lines were cultured for 24 hrs without any stimuli and lysed in buffers containing phosphatase inhibitors, and whole cell extracts were subjected to immunoblot analysis with antibodies specific for total or phospho-proteins as indicated. Antibody against α-tubulin was used as an internal loading control. Arrowhead indicates STAT2 protein. **B,**

JURKAT cells were stimulated with IFN-β (40 ng/ml) or IFN-γ (10 ng/ml) and whole cell extracts were subjected to immunoblot analysis using an antibody specific for phopho-STAT2 (Y689).

Supplementary Fig. 4 Knockdown efficiency and BCL2 expression in JURKAT cells. **A, B,** shRNAs were individually transduced into JURKAT cells by lentivirus infection. Total RNA was harvested after three days of infection. Quantitative RT-PCR analysis was performed for each gene as well as *GAPDH* (internal control). Gene expression was normalized to that of *GAPDH* and shown as percent mean relative to control shRNA(s) ± s.e.m. of duplicate experiments. BCL2 expression was also analyzed **(B)**. Of note, IL6R and IL23R were not examined because these genes were not expressed in JURKAT cells.

Supplementary Fig. 5 Confirmation of CD3 expression on OP9 DL1 outgrowth cells. A, B, Outgrowth cells on OP9 DL1 stroma were stained with antibody specific for murine CD3 and analyzed by flow cytometry. Dot plots show stained cells are positive for both CD3 and GFP, while unstained cells are only positive for GFP. Histogram of CD3 staining intensity is shown in panel B.

Supplementary Fig. 6 Expressions of IL-10 and IL-10 receptors, and effect of an anti-IL10 neutralizing antibody on growth of T-ALL cell lines. A, mRNA expression of the *IL-10* gene in three TYK2-dependent T-ALL cell lines (HPB-ALL, JURKAT and MOLT-4) and one TYK2-indepdent line (LOUCY) was measured by NanoString. Values are mean ± s.e.m (n=2). **B,** mRNA expressions of the IL-10 receptor genes (*IL10RA* and *IL10RB*) in the same cell lines were measured by microarray analysis. **(c)** JURKAT and HPB-ALL transduced with an empty vector or *TYK2 E957D* cDNA were cultured with

graded concentrations of an anti-IL10 antibody for three days, at which time the cell viability was evaluated. Values are percent mean relative to cells cultured in the absence of drug ± s.e.m (n=2).

Supplementary Fig. 7 Effect of JAK kinase inhibitors with varying TYK2-specificity on growth and viability of T-ALL cells. Three TYK2-dependent T-ALL cell lines (JURKAT, MOLT-4 and RPMI-8402; red) and one independent line (LOUCY; blue) were cultured with graded concentrations of AG490 **(A)** or CP-690550 **(B)** for three days, at which time the cell viability was evaluated. Values are percent mean relative to cells cultured in the absence of drug ± s.e.m. (n = 6).

Supplementary Fig. 8 Signaling and cell cycle distribution after JAK inhibitor I treatment. A, JURKAT or RPMI-8402 cells were cultured with graded concentrations of JAK Inhibitor I for 24 hrs, and whole cell extracts were subjected to immunoblot analysis with antibodies specific for TYK2, STAT1, phospho-STAT1, phospho-STAT3 and α-tubulin (loading control). **B,** JURKAT cells were cultured in the presence or absence of JAK Inhibitor I (3 μM) over a time course and whole cell extracts were subjected to immunoblot analysis with antibodies specific for PARP, phospho-STAT1 and α-tubulin. **C,** JURKAT, HPB-ALL, or RPMI-8402 cells were cultured in the presence of graded concentrations of JAK Inhibitor I for 48 hrs and cell cycle distribution was assessed by flow cytometric analysis of propidium iodide incorporation in permeabilized cells. **D,** Quantification of cells in G1, S, and G2/M phase from panel (c). Values are percent mean ± s.e.m. (n=3). * $P<0.05$ by two-sample, two-tailed t-test.

Supplementary Fig. 9 Bcl2 protein expression in wild-type and *Tyk2* knockout mice. Bcl2 protein expression was detected by immunohistochemistry in thymus samples from four independent wild-type (WT) mice and four independent *Tyk2* homozygous knockout (*Tyk2*$^{-/-}$) mice.

Supplementary Materials and Methods

Cell Viability Assays and Reagents

For drug treatment, cells were incubated in the presence of graded doses of JAK Inhibitor I, AG490 (EMD Biosciences), CP-690550 (LC Laboratories) or anti-IL10 antibody (R&D Systems; Clone # 25209) for 72 hrs. The number of viable cells was determined with the CellTiter 96 AQ_{ueous} One solution cell proliferation assay (Promega). For activation of interferon signaling pathway, cells were incubated with IFN-β (40 ng/ml) and IFN-γ (10ng/ml; PBL Interferon Source) for 20 min.

Antibodies

For immunoblot analysis, antibodies specific for JAK1, JAK2, JAK3, TYK2, phospho-TYK2 (Y1054/1055), STAT1, phospho-STAT1 (Y701), STAT2, STAT3, phospho-STAT3 (Y705), STAT5, phospho-STAT5 (Y694), ERK1/2, phospho-ERK1/2 (T202/Y204), AKT, phospho-AKT (S473), CRKL, phospho-CRKL (Y207), PARP, α-tubulin (Cell Signaling Tech), phospho-STAT2 (Y689), β-actin (Millipore), STAT4 or phospho-STAT4 (Y693; GenScript) were used.

Cell Cycle Analyses

The cells were washed in PBS, fixed in 70% ethanol, incubated with RNAse A (Qiagen) in PBS and resuspended in the buffer containing propidium iodide (Sigma-Aldrich). The percentage of cells in G1, S phase and G2/M phases were analyzed by BD FACSCalibur and ModFit software (Verity Software House).

Cytokine Profiling

Direct digital mRNA analysis of cytokines was performed using a custom designed nCounter Gene Expression CodeSet (NanoString Technologies). Oligonucleotides were synthesized by NanoString Technologies. Samples were hybridized and processed using the NanoString nCounter system following the manufacture instructions. Data presented were obtained by normalizing raw expression data via a two-step process using internal positive controls and reference housekeeping genes (ACTB, B2M, GAPDH, RPL19, and RPLP0).

Immunohistochemistry

Four independent wild-type mice and four independent *Tyk2* homozygous knockout (*Tyk2*$^{-/-}$) C57Bl/6 CO_2-euthanized mice of five weeks of age were used for thymus isolation, followed by formalin fixation. Paraffin embedded hyme were cut, attached to slides, dewaxed and rehydrated. Epitopes were retrieved by heat treatment in Tris-EDTA (pH=9). Endogenous peroxidase was blocked in 3 % H_2O_2 for 10 min. Sections were blocked in Avidin/Biotin block (Vector laboratories SP-2001), Superblock and mouse block from the Universal Mouse HRP-Kit (IDLabs IDSTM003). Primary antibody staining was done at 4°C overnight in a 1:500 dilution using an anti-Bcl2 antibody (BD, #554218) followed by anti-hamster-antibody Vector laboratories BA-9100. AEC and heamatoxylin was used as counterstain. Light microscopic images were captured with a PixeLINK camera and the corresponding acquisition software on a Zeiss Imager Z.1 (magnification: ×100, ×200 and ×400) at a color temperature of 1300 Kelvin.