Supplementary figures

Figure S1 Pacritinib concentration in the blood

Wild type (WT) mice and ABIN1[D485N] knock-in mice (D485N) that were eight weeks old were fed for a further 10 weeks with RM3 control diet or RM3 diet containing 150 mg/kg pacritinib. Blood was collected at the end of the experiment and the concentration of pacritinib in the blood was measured in female (A) and male (B) mice between 08.00 and 10.00 h.

Figure S2 Effect of pacritinib on body weight

WT and ABIN1[D485N] knock-in mice that were 8 weeks old were fed for a further 10 weeks with RM3 control diet or RM3 diet containing 150 mg/kg pacritinib. The mice were weighed each week and the graphs show the body weights of female (A) and male (B) mice as a percentage of their weight at the start of the experiment.

Figure S3 Lack of effect of pacritinib on monocyte and neutrophil numbers in the blood

WT and ABIN1[D485N] knock-in mice that were eight weeks old were then fed for 10 weeks on RM3 diet without (-) or with (+) 150 mg/kg pacritinib. Blood was then collected via cardiac puncture, the red blood cells were lysed and the leukocytes counted and analysed by flow cytometry. The number of patrolling monocytes (pMo) (A), inflammatory monocytes (iMo) (B) and neutrophils (C) in 50 µl blood is shown. Each symbol shows the result from a single mouse. Statistical significance was calculated using one-way ANOVA and the Tukeys post-hoc test; ** denotes $p< 0.01$ and *** denotes $p < 0.001$.

Figure S4 Pacritinib prevents infiltration of B cells in lung, kidney and liver.

(A, B, C) Representative histological images showing anti-PAX5 staining (brown) of the lung (A), kidney (B) and liver (C) of 18-week-old WT (n=10) and ABIN1[D485N] knock-in mice (n=11) after feeding for 10 weeks with RM3 diet with or without 150 mg/kg pacritinib. (D, E, F) The frequency of immunohistochemistry stained cells in the tissue were scored using a non-linear semi-quantitative grading system – (0), + (1), ++ (2), +++ (3). Plots show cumulative pathology scores for the lung (D), kidney (E) and liver (F). Each symbol shows the result from a single individual mouse. Statistical significance was calculated using the Kruskal-Wallis and the Mann-Whitney tests * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$.

Figure S5 T cell infiltration is prevented in the kidney, but not in the liver of ABIN1[D485N] knock in mice fed with pacritinib.

(A, B, C) As in Fig S4, except that histological staining was carried out using anti-CD3 antibody. (D, E, F) The frequency of immunohistochemistry stained cells in the tissue were scored using a non-linear semi-quantitative grading system – (0), + (1), ++ (2), +++ (3). Plots show cumulative pathology scores for the lung (D), kidney (E) and liver (F). Each symbol shows the result from a single individual mouse. Statistical significance was calculated using the Kruskal-Wallis and the Mann-Whitney tests * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$.

Figure S6 F4/80$^+$ cell infiltration is prevented in the kidney, but not in the liver of ABIN1[D485N] knock in mice fed with pacritinib.

(A, B, C) As in Fig S4, except that histological staining was carried out using anti-F4/80 antibody to stain macrophages and monocytes. (D, E, F) The frequency of immunohistochemistry stained cells in the tissue were scored using a non-linear semi-quantitative grading system – (0), + (1), ++ (2), +++ (3). Plots show cumulative pathology scores for the lung (D), kidney (E) and liver (F). Each symbol shows the result from a single individual mouse. Statistical significance was calculated using the Kruskal-Wallis and the Mann-Whitney tests * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$.

Figure S7 Pacritinib reduces IL-6-stimulated STAT3 phosphorylation in splenic B cells but splenic B cell numbers are not reduced in IL-6 KO mice.

(A) B cells were magnetically purified from the spleens of wild type mice. Cells (2×10^6) were incubated for 1 h without (-) or with (+) 1 μM pacritinib followed by stimulation with IL-6 (25 ng/ml) for the times indicated. The cells were lysed in 1% (w/v) SDS containing protease inhibitor cocktail (Roche) and benzonase (50 units/ml) to hydrolyse DNA, subjected to SDS-PAGE followed by transfer to PVDF membranes and immunoblotting with antibodies recognising STAT3 phosphorylated at Tyr705 or all forms of STAT3 (Total-STAT3). **(B)** Splenic cells from WT (n=3) and IL-6 KO (n=3) mice were analyzed by flow cytometry and the graph shows the total number of splenic B cells (DAPI$^-$B220$^+$). Each black circle shows the result from a single mouse.