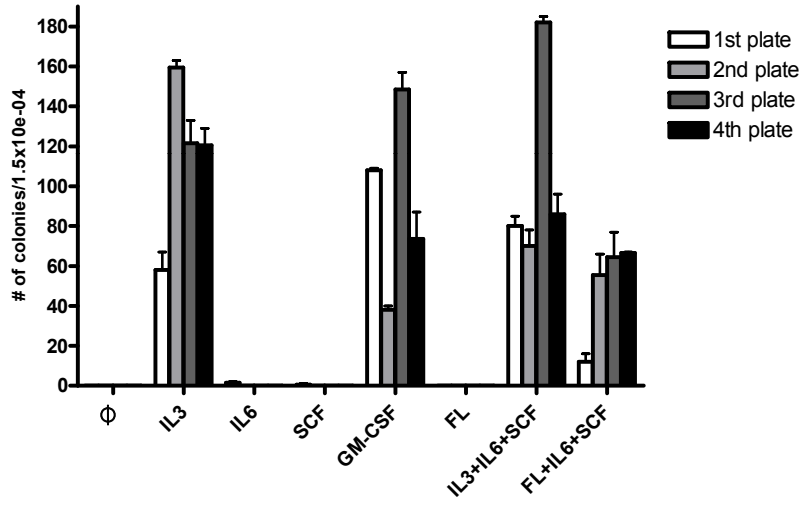
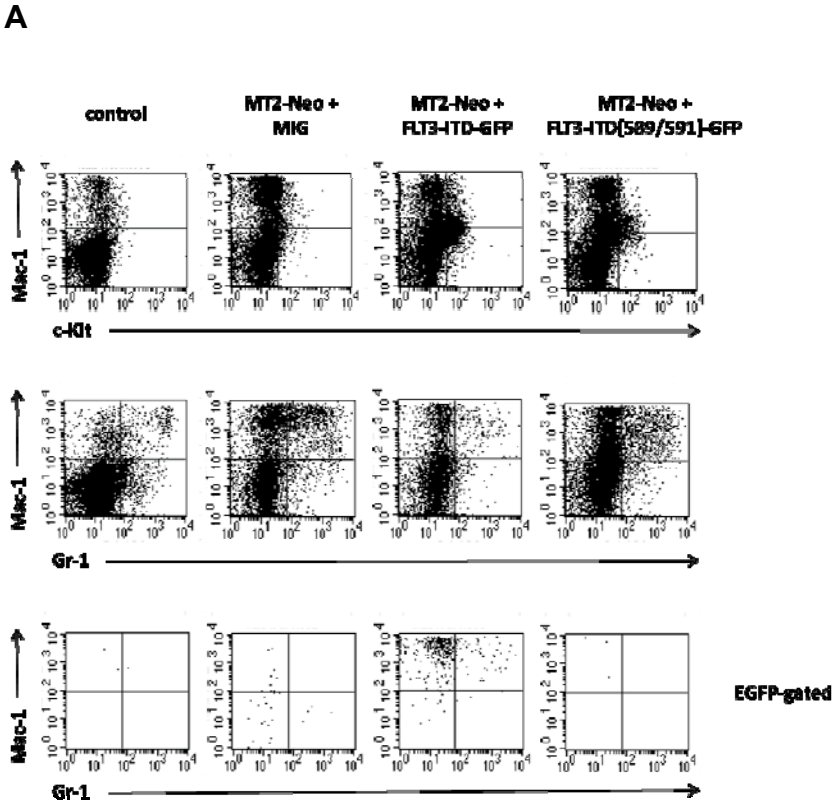
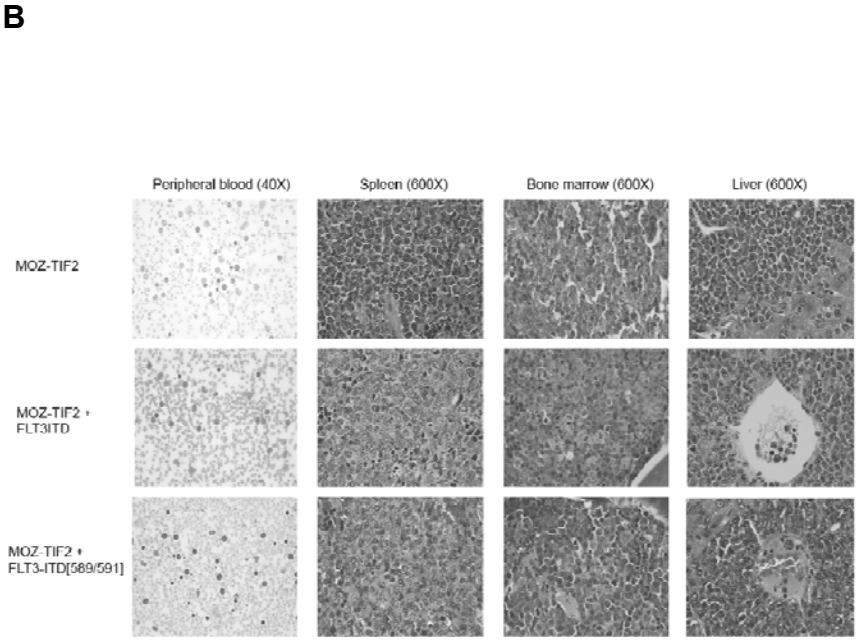
Supplementary Figure 1

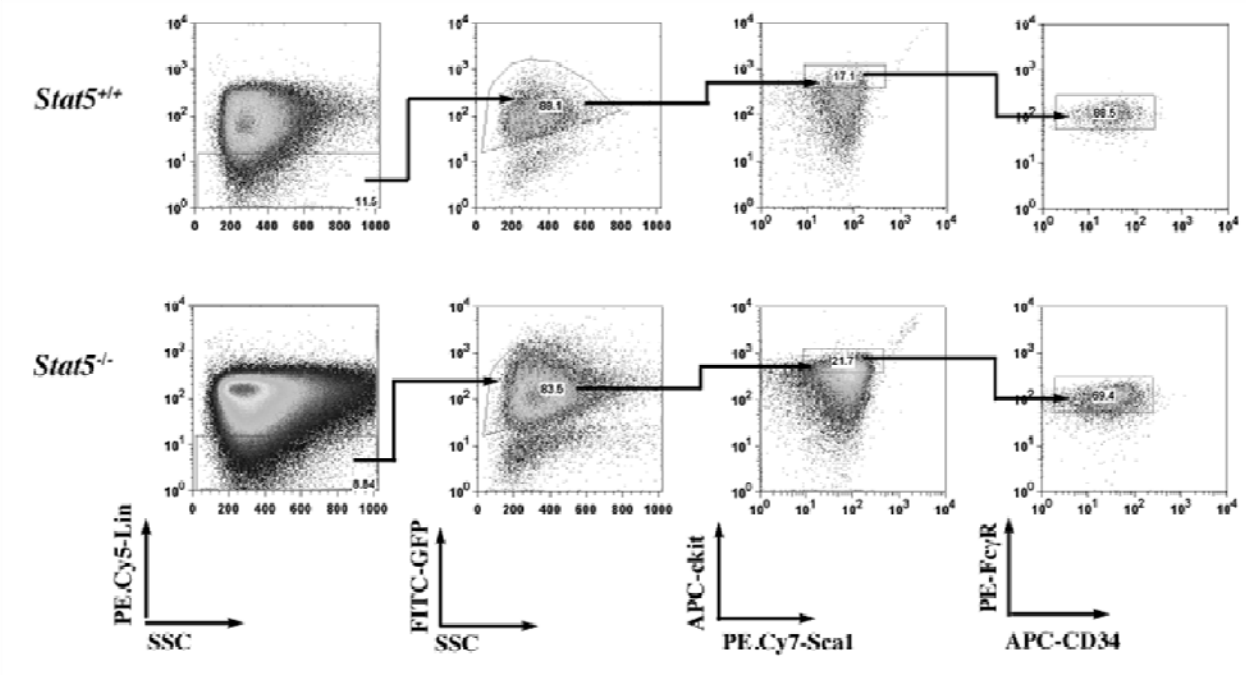


Supplementary Figure 2

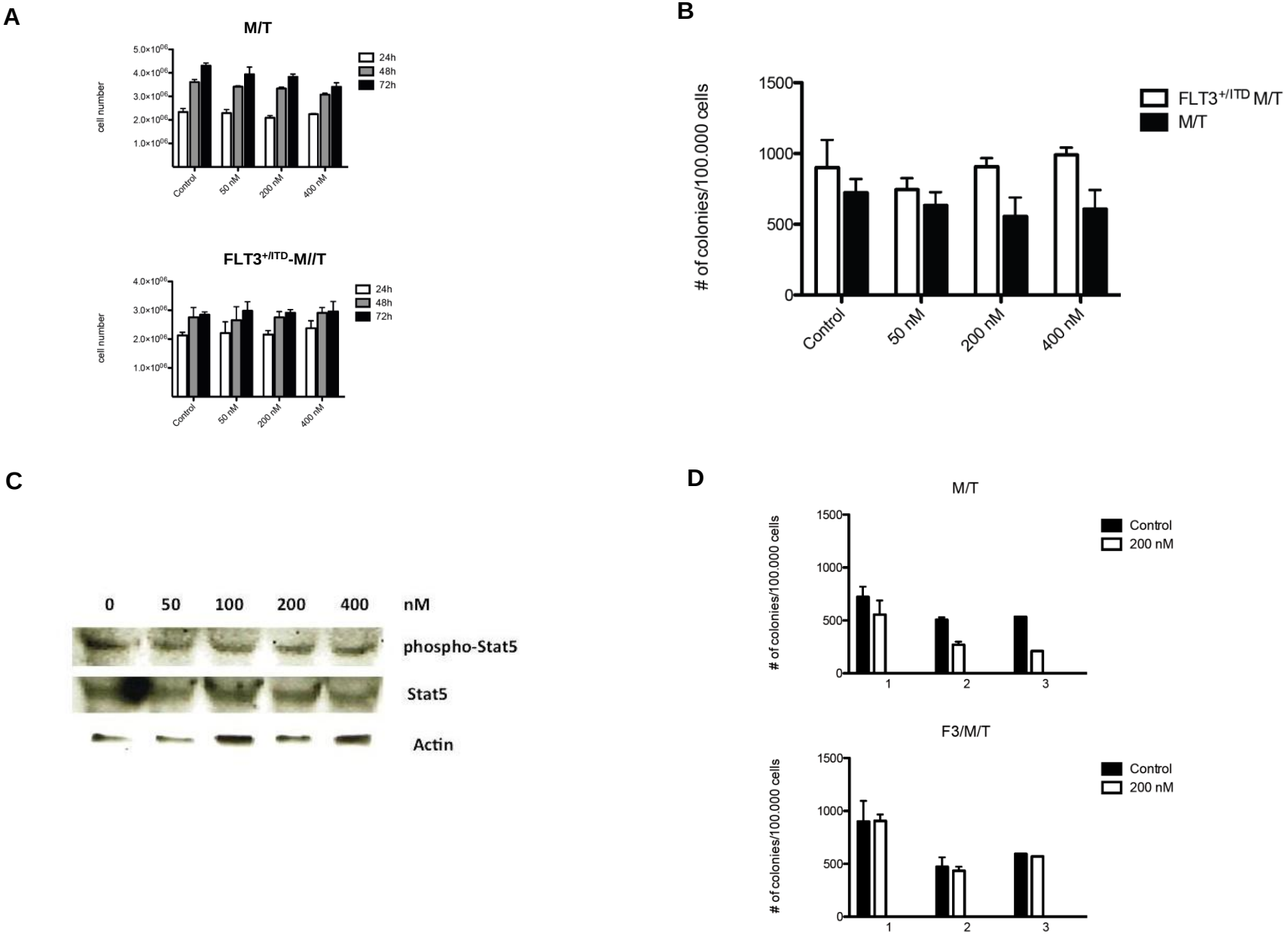




Supplementary Figure 3



Supplementary Figure 4



Supplementary Table 1

Summary of serial transplant experiments with *Stat5*$^{+/+}$ or *Stat5*$^{-/-}$ fetal liver cells expressing MOZ-TIF2-GFP.

Constructs	No. of diseased mice/transplant mice	Disease Latency days [median]	Spleen wt. mg [median]	Liver wt. mg [median]	WBC x10^6 cells/ml [median]
1st-Stat5$^{+/+}$	10/10	32-45 [34]	200-340 [260]	720-1300 [1111]	7.0-26.1 [15.9]
1st- Stat5$^{-/-}$	10/10	38-67 [53]	110-800 [270]	780-2710 [1115]	2.2-56.0 [31.4]
2nd-Stat5$^{+/+}$	10/10	24-51 [34]	30-310 [200]	500-1320 [935]	3.9-27.1 [14.1]
2nd- Stat5$^{-/-}$	10/10	70-82 [80]	110-800 [270]	1100-2370 [130]	7.6-118.0 [16.8]
3rd-Stat5$^{+/+}$	8/8	35-45 [35]	110-370 [180]	790-1090 [1000]	5.1-47.3 [29.2]
3rd- Stat5$^{-/-}$	7/8	93-(130) [97]	150-600 [260]	1440-2040 [1750]	2.8-56.0 [34.9]

The median survival times are taken from the Kaplan-Meier survival plots shown in Fig. 6A.

Supplementary Methods

Retroviral transduction

For transduction of primary bone marrow cells, donor mice were primed with intraperitoneal injection of 5'-flurouracil (150 mg/kg, Sigma, St. Louis, MO) and subsequently sacrificed by CO_2 asphyxiation 6 days later. Bone marrow cells were flushed from femurs and tibias, and red blood cells were lysed with Red Blood Cell Lysis buffer (Sigma, St. Louis, MO). The collected bone marrow cells were incubated overnight with transplant medium [RPMI 1640 supplemented with 15% FBS, 6 ng/ml IL-3, 10 ng/ml IL-6, and 10 ng/ml stem cell factor (R & D systems, Minneapolis, MN)] before transduction. Two rounds of retroviral transduction were conducted by spin-infection at 24 hours and 48 hours after harvesting. One ml of equivalent titers of viral supernatant was added to $4x10^6$ bone marrow cells in a 6-well culture plate with 3 ml transplant media containing 5 μg/ml Polybrene and 7.5 mM HEPES buffer. The plate was centrifuged at 1800 rpm for 90 minutes at 30ºC. After two rounds of transduction, cells were washed in PBS and used for further experiments.

Phospho-flow analysis

For the analysis of intracellular phosphorylated STAT5, freshly isolated splenic or bone marrow cells from transplant mice were cultured in RPMI 1640 with 1% BSA for 5 hours. Cells were then washed and fixed at room temperature in 10% freshly prepared paraformaldehyde (PFA) for 10 minutes, followed by a rinse in ice-cold methanol. The washed cells were stained with PE-conjugated anti-phosphorylated STAT5 antibody (Becton Dickinson) according to the manufacturer's instructions.

Supplementary Legends

Supplementary Figure 1. Serial replating assay of MOZ-TIF2-transduced progenitors with different cytokines.

Bone marrow cells from 5-FU primed BALB/c mice were transduced with MSCV-*MOZ-TIF2*-pgk-*puro* retroviral vector and plated on methylcellulose M3434 plates with 1µg/ml puromycin for clonal selection, together with IL3, IL6, SCF, GM-CSF and erythropoietin. Cells were pooled 7 days later and 1.5 x 10^4 cells were plated on separate M3234 methylcellulose plates containing no cytokines (lane 1), 10ng/ml IL3 (lane 2), 10ng/ml IL-6 (lane 3), 50ng/ml SCF (lane 4), 50ng/ml GM-CSF (lane 5), 50ng/ml FLT3 ligand (FL) (lane 6), IL3+IL6+SCF (lane 7), or FL+IL6+SCF (lane 8). Cells were then counted, pooled and replated on methylcellulose M3234 plates with fresh cytokines every week for three more weeks. The results shown are the representatives of duplicate measurements of at least two independent experiments.

Supplementary Figure 2. Ruxolitinib treatment of M/T and $FLT3^{ITD/+}$-M/T blast cells.

(A) Bone marrow cells derived from leukemic WT-M/T (left) or $FLT3^{+/ITD}$-M/T (right) mice were resuspended in RPMI1640 media without cytokines and treated with the Jak1/Jak2-inhibitor Ruxolitinib as indicated. Cell viability was analysed after 24h (white bars), 48h (grey bars) and 72h (black bars) of treatment by trypane exclusion. The results shown represent duplicate measurements of three independent experiments.

(B) Colony forming unit assay of $FLT3^{+/ITD}$-M/T (white bars) and B6-M/T (black bars) bone marrow cells. Leukemic blasts were plated in M3434 at 1x10^5 cells/plate and treated with Ruxolitinib as indicated. Colonies were counted on day 7. The results shown represent duplicate measurements of two biological replicates.

(C) Immunoblot analysis of extracts prepared from B6-M/T leukemic blasts. M/T blasts were maintained in M3434 and treated with Ruxolitinib as indicated. After 7 days lysates were prepared and immunoblot analysis using an anti-phospho-STAT5-antibody was performed. The blot was stripped and reprobed with an anti-STAT5- or an anti-Actin antibody as indicated.

Supplementary Figure 3. FLT3-ITD requires STAT5 for cooperation with MOZ-TIF2 in vivo.

(A) Immunophenotype analysis of hematopoietic cells from the transplant mice. Representative flow cytometric analysis of cells from the spleen of a healthy control BALB/c mouse (1st column); and mice transduced with retroviral vectors expressing MOZ-TIF2-neo, together with GFP (2nd column); FLT3-ITD-GFP (3rd column); FLT3-ITD-589/591-GFP (4th column). Cells were depleted of red blood cells, followed by staining with 7-AAD and the antibodies indicated in the dot plots. First and second panels show dot plots gated on live cells only. Third panels show plots gated on EGFP, as well as live cells.

(B) Histopathology of the transplant mice. Wright-Giemsa stained peripheral blood (40X) and hematoxylin/eosin-stained sections (x 600) of bone marrow, spleen, and liver from representative mice transplanted with cells expressing i) MOZ-TIF2; ii) MOZ-TIF2 and FLT3ITD; iii) MOZ-TIF2 + FLT3-ITD-589/591 are shown.

Supplementary Figure 4. Separation of leukemic GMPs

Leukemic-granulocyte monocyte progenitors (L-GMP) were sorted from mice transplanted with either *Stat5* deficient fetal liver cells (*Stat5*$^{-/-}$) (lower panel) or WT control cells (*Stat5*$^{+/+}$)

(upper panel), expressing MOZ-TIF2-GFP. L-GMP population was sorted from the spleen cells gated on $Lin^{-}GFP^{+}ckit^{hi}Fc\gamma R^{hi}$.