Supporting Information

Supplementary materials and methods

Cell culture

Ba/F3 cells were grown in RPMI medium supplemented with 10% FBS and 1 ng/ml interleukin-3 (IL-3). To generate Ba/F3 cells stably expressing wild-type STAT3 ($STAT3^{WT}$), $STAT3^{D661V}$ and $STAT3^{Y640F}$, Ba/F3 cells were transduced with MSCV-IRES-GFP-based retroviruses expressing $STAT3^{WT}$, $STAT3^{D661V}$ and $STAT3^{Y640F}$ and the infected cells were sorted for GFP. To assess for cell proliferation, Ba/F3 cells expressing $STAT3^{WT}$, $STAT3^{D661V}$ and $STAT3^{Y640F}$ were plated in RPMI + 10% FBS in the absence or presence of 1 ng/ml IL-3. Viable cells were counted by trypan blue exclusion every 24 hours for 4 days.

Flow cytometry

Single-cell suspensions were prepared from BM and spleen, and red cells were lysed with red cell lysis solution. Cells were washed and resuspended in PBS plus 2% FBS and stained for 20 minutes on ice with directly conjugated (either PE or APC) monoclonal antibodies specific for Ter119, CD71, CD41, CD61, Mac-1, Gr-1, B220, or Thy-1 and CD3, CD4, CD8 and NK1.1. All antibodies were purchased from either eBioscience, or BioLegend. Flow cytometry was performed with an LSRII (BD Biosciences) and analyzed using FlowJo software (TreeStar, Ashland, OR).

Retroviral transduction and transplantation

High-titer retroviral stocks of MSCV-STAT3^{WT}-IRES-GFP, MSCV-STAT3^{D661V}-IRES-GFP and MSCV-STAT3^{Y640F}-IRES-GFP were prepared by transient transfection of 293T cells as described previously (Yan *et al*, 2012). Bone marrow cells from 5-fluorouracil (5-FU)-primed C57BL/6 wild type (WT) mice were transduced with retroviruses expressing STAT3^{WT}, STAT3^{D661V} and STAT3^{Y640F} by two rounds of spin infection. Transduced bone marrow cells (1X10^6) were injected into retro-orbital veins of lethally irradiated (2X 450 cGy) C57BL/6 recipient mice. Mice were maintained on acidified water. Animal studies were performed in accordance with approved guidelines of the Institutional Animal Care and Use Committee of SUNY Upstate Medical University.

Blood and tissue analysis

Peripheral blood counts were measured using Hemavet 950FS (Drew Scientific). For histopathologic analysis, mouse tissue specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections (4 μm) were stained with hematoxylin and eosin (H&E) stain.

Statistical analysis

Results are expressed as mean ± SEM, and statistical significance was determined by Student's *t*-test. $P<0.05$ was considered to be statistically significant.

Supplementary figure legends

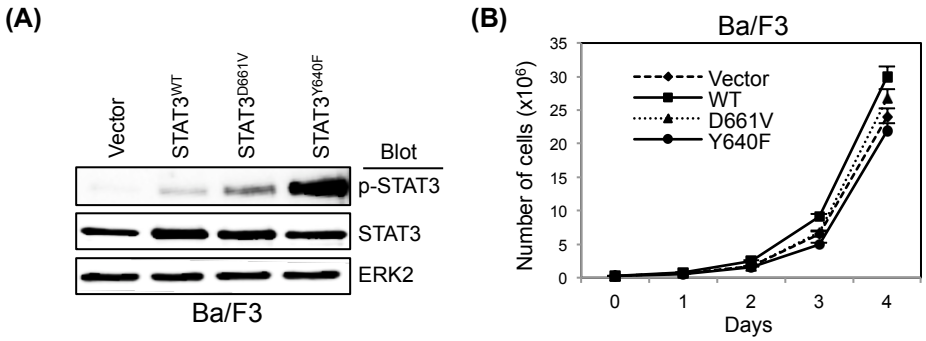
Fig. S1. (A) Immunoblot analysis showed increased phosphorylation of STAT3 in Ba/F3 cells expressing $STAT3^{D661V}$ and $STAT3^{Y640F}$ mutant compared with vector or $STAT3^{WT}$. Immunoblotting was performed using phospho-specific (Tyr705) or total antibodies against STAT3. ERK2 was used as a loading control. (B) Cell proliferation assay. Ba/F3 cells expressing vector, $STAT3^{WT}$, $STAT3^{D661V}$ and $STAT3^{Y640F}$ were cultured in the presence of IL-3 and cell proliferation was measured by viable cell counts every 24 hours over a 4-day period.

Fig. S2. Total numbers of myeloid ($Gr\text{-}1^+/Mac\text{-}1^+$), erythroid ($Ter119^+/CD71^+$), megakaryocytic ($CD61^+/CD41^+$), B-cell ($B200^+$) and T-cell ($Thy\text{-}1^+$) precursors in the BM and spleens of transplanted animals expressing $STAT3^{WT}$, $STAT3^{D661V}$ and $STAT3^{Y640F}$ are shown in bar graphs as mean ± SEM (n=4).

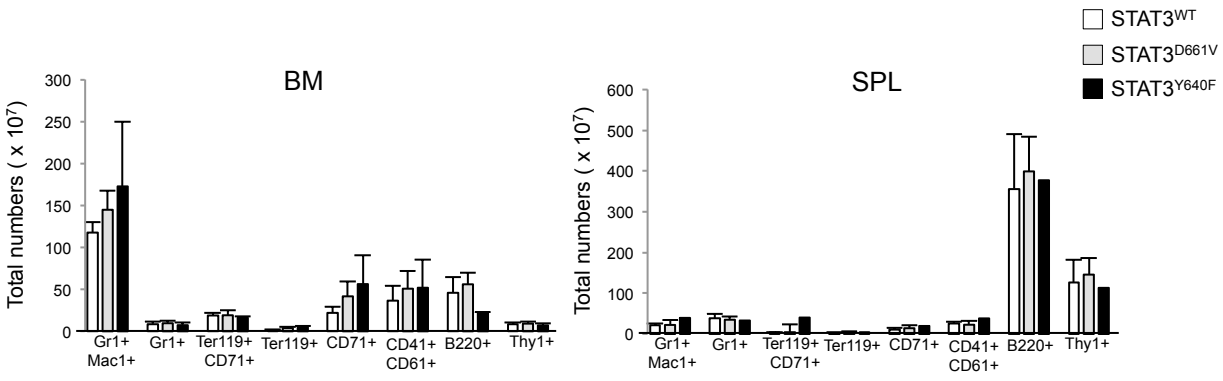
Fig. S3. (A, B) Flow cytometric analysis of $CD3^+CD8^+$ cells (A) and $CD3^-NK1.1^+$ cells (B) in the thymus of transplanted animals expressing $STAT3^{WT}$, $STAT3^{D661V}$ and $STAT3^{Y640F}$ at 16-20 weeks after BMT are shown in histograms as mean ± SEM (n=4).

Supplementary Reference

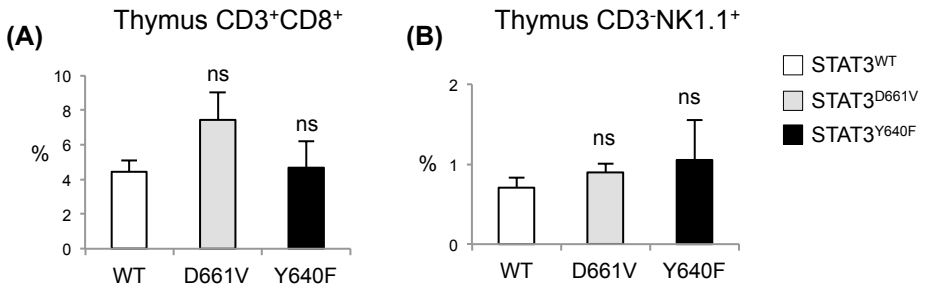
Yan, D., Hutchison, R.E. & Mohi, G. (2012) Tyrosine 201 is required for constitutive activation of JAK2V617F and efficient induction of myeloproliferative disease in mice. *Blood,* **120,** 1888-1898.



Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3