

Supplementary Table

Table 1s. Sequences of primers used.

Primer	Sequence
mITD-5F	TGC AGA TGA TCC AGG TGA CT
mITD-3R	CTC TCG GGA ACT CCC ACT TA
NPM874F	GGT TCT CTT CCC AAA GTG GAA GC
MRP8R	GAG GTA TTG ATG ACT TTA TTA TTC TGC AGG
NPMexon 1F	ATG TCT CTT GTC AGC TGT CTT TC
NPM500R	CAG ATA TAC TTA AGA GTT TCA CAT C
HPRT1	CCT GCT GGA TTA CAT TAA AGC ACT G
HPRT2	GTC AAG GGC ATA TCC AAC AAC AAA C
mFLT3-RT-5F	GCT CTG TCT CCC CTT CAT TG
mFLT3-RT-3R	CCA GGA CCT TCC CAA ACT CT
mSTAT5-5F	AAG TTC ACA GTC CTG TTT GAG TC
mSTAT5-3R	GGT GGC AGT AGC ATT GTG G

Supplementary Methods

Complete peripheral blood cell count and cytology

Peripheral blood was collected from cardiac incision immediately after the animals were euthanized. CBC was performed on the sample using a Hemavet950 hematology system (Drew Scientific, Oxford, CT).

Histopathology and cytology

Murine tissues were prepared and stained with hematoxylin and eosin solution (H&E) as described previously [1]. Images were captured at room temperature with a Zeiss Axioskop upright microscope with Achromplan 5×/0.16 NA, 10×/0.3 NA, and

40×/0.6 NA objectives and were photographed with an AxioCam camera (Zeiss) and Axiovision 4.0 software (Zeiss Peabody, MA)).

Immunofluorescence

For immunofluorescence, BM cytopins were prepared then fixed in 4% paraformaldehyde. Alexa Fluor350 conjugated wheat germ agglutinin (WGA) (Molecular Probes/Invitrogen, Carlsbad, CA) was used to stain the cell membrane, 4',6-diamidino-2-phenylindole (DAPI) to stain the nucleus and polyclonal anti-Flag antibody produced in rabbit (Sigma) to bind the Flag-tagged NPM protein. Alexa Fluor 488 conjugated anti-rabbit secondary antibody was used to detect the Flag-tagged NPM protein. Immunofluorescent images were acquired at room temperature on a Nikon ECLIPSE-Ti inverted microscope (Nikon Instruments, Melville, NY) with Hoffman Modulations Contrast (HMC) 40x ELWD Plan Fluoro/0.60 na objective and HMC Model G1 0.5 na, 55mm WD condenser (Modulations Optics Inc., Rochester, NY). Fluorescent images were captured with Nikon Digital Sight DS-Qi1 digital microscope camera (Nikon instruments). The images were collected and converted to JPEG format using NIS-Elements AR 3.0 software (Nikon Instruments).

Immunohistochemistry

For immunohistochemistry, a monoclonal NPM antibody conjugated to APAAP complex (clone 376, M7305, Dako, Glostrup, Denmark) was used. Images were captured at room temperature with a Zeiss Axioskop upright microscope with

Achroplan 5×/0.16 NA, 10×/0.3 NA, and 40×/0.6 NA objectives and were photographed with an AxioCam camera (Zeiss) and Axiovision 4.0 software (Zeiss Peabody, MA)).

Supplementary Reference:

1. Baldwin BR, Li L, Tse KF, Small S, Collector M, Whartenby KA, Sharkis SJ, Racke F, Huso D, Small D. Transgenic mice expressing tel-FLT3, a constitutively activated form of FLT3, develop myeloproliferative disease. *Leukemia*. 2007 Apr;21(4):764-71.

Supplementary Figure Legends

Figure 1s: Histopathologic images of leukemic mice BM, peripheral blood (PB), spleens and other infiltrated organs compared to wt. H&E staining of BM cytopspins (i-iii), PB smears (iv-vi), spleen (vii-ix), kidney (x-xi), and meninges (xii-xiii) of a representative wt mouse (column 1), a representative mouse with T cell ALL (column 2) and a representative mouse with AML (column 3). Bar scale (i-vi = 10µM; vii, x = 50µM; viii, ix, xi,xiii=100µM).

Figure 2s: A subset of mice with both mutations develop a T cell ALL characterized by CD3⁺/CD8⁺/CD4⁻ lymphoblasts. Flow cytometry plots demonstrating the characteristic phenotype of a wild-type mouse (first row) compared to a mouse with T cell ALL with CD3⁺/CD8⁺/CD4⁻ lymphoblasts (second row) infiltrating the bone marrow and thymus.

Figure 3s: Flag-NPMc+ and Flt3/ITD are expressed in leukemia cells and NPMc+ localizes to the cytoplasm. A) RT-PCR of RNA extracted from BM cells of leukemic mice and a wild-type mouse. Lane 2 demonstrates a mouse with loss of heterozygosity of the wild-type *Flt3* allele and therefore lacks the smaller, wild-type *Flt3* band. B) Detection of Flt3/ITD and NPMc+ transcript in other infiltrated organs by RT-PCR. C) **Quantitative RT-PCR performed on whole bone marrow isolated from 2-month-old mice demonstrates the expected increase in HoxA9 and HoxA10 attributable to NPMc+ expression. Assays done in triplicate, error bars represent standard deviation.** D) IF was performed using Dapi nuclear stain (blue), polyclonal anti-Flag antibody with AF488 (green) secondary antibody. BM cytopspins from transgenic Flag-NPMwt mice (row 1). Aggregates of Flag-NPMwt are visible in the nuclei of the BM cells. IF of BM cytopspins from leukemic ITD/NPMc+ mice (rows 2 and 3) in which the Flag-NPMc+ signal localizes to the cytoplasm with no aggregates visualized in the nuclei. **(left 3 columns at high power, far right column at low power)** E) Immunohistochemistry staining of NPM in NPMwt cells and NPMc+ cells showing aberrant expression of NPM in the cytoplasm only in murine cells with NPMc+ (arrows).

Figure 4s: NPMc+ is aberrantly expressed in T cells of NPMc+ transgenic mice. RT-PCR performed on T cells isolated via magnetic-bead separation from the spleens of 2-month-old mice of each genotype. Flag-NPMc+ expression is seen in mice the T cells of NPMc+ mice and ITD/NPMc+ mice.

Figure 5s: Trend towards expansion of myelomonocytic lineage in the peripheral blood of mice with ITD, NPMc+ and both mutations prior to overt disease development. Box and whisker plots showing number of peripheral blood monocytes and neutrophils in wild-type (wt, N=3), mice with Flt3/ITD alone (ITD, N=6), mice with NPMc+ (N=6) alone, and mice with both mutations (NPMc+/ITD, N=9).

ACCEPTED MANUSCRIPT

Supplementary Figures:

Figure 1s

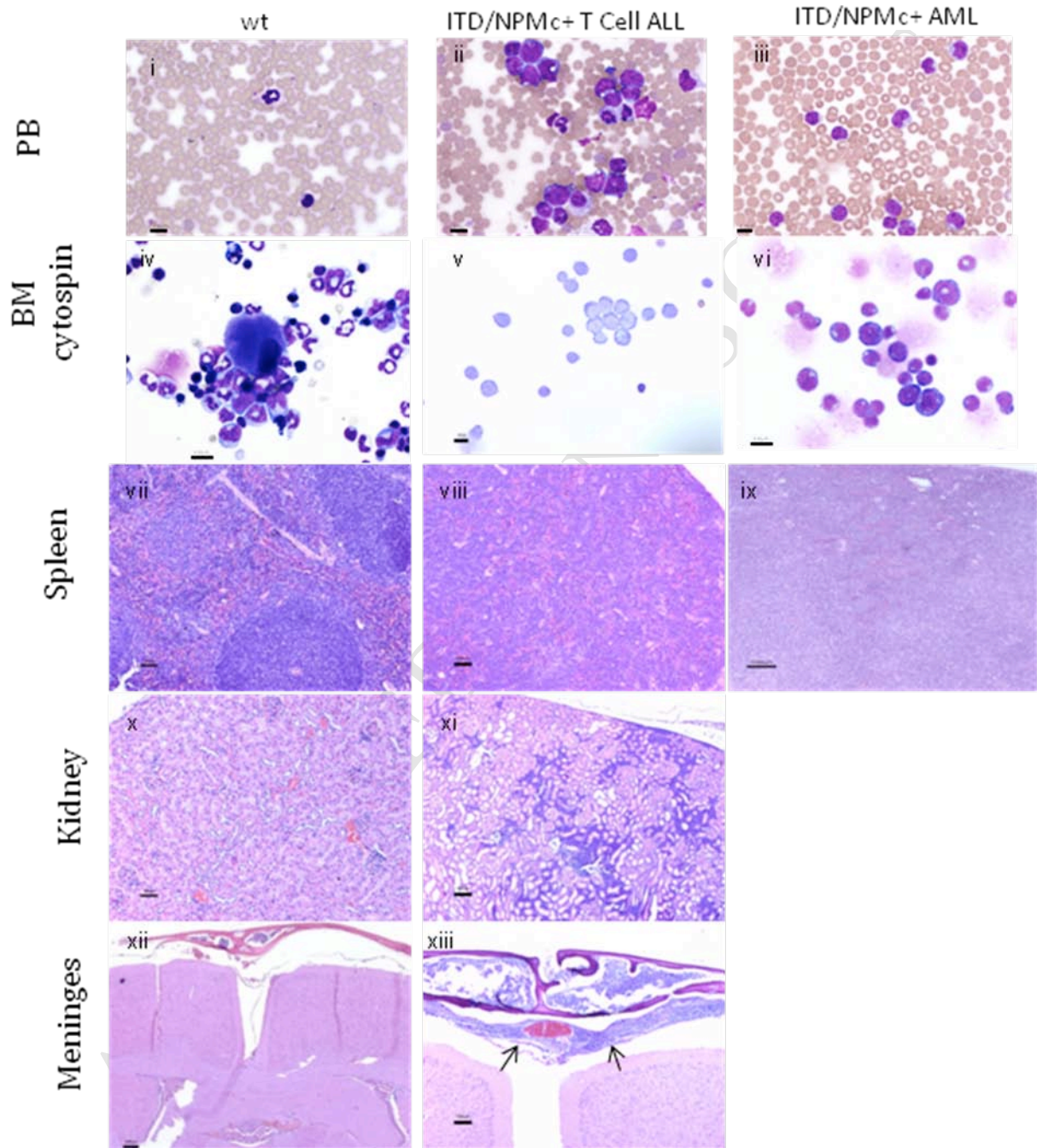


Figure 2s

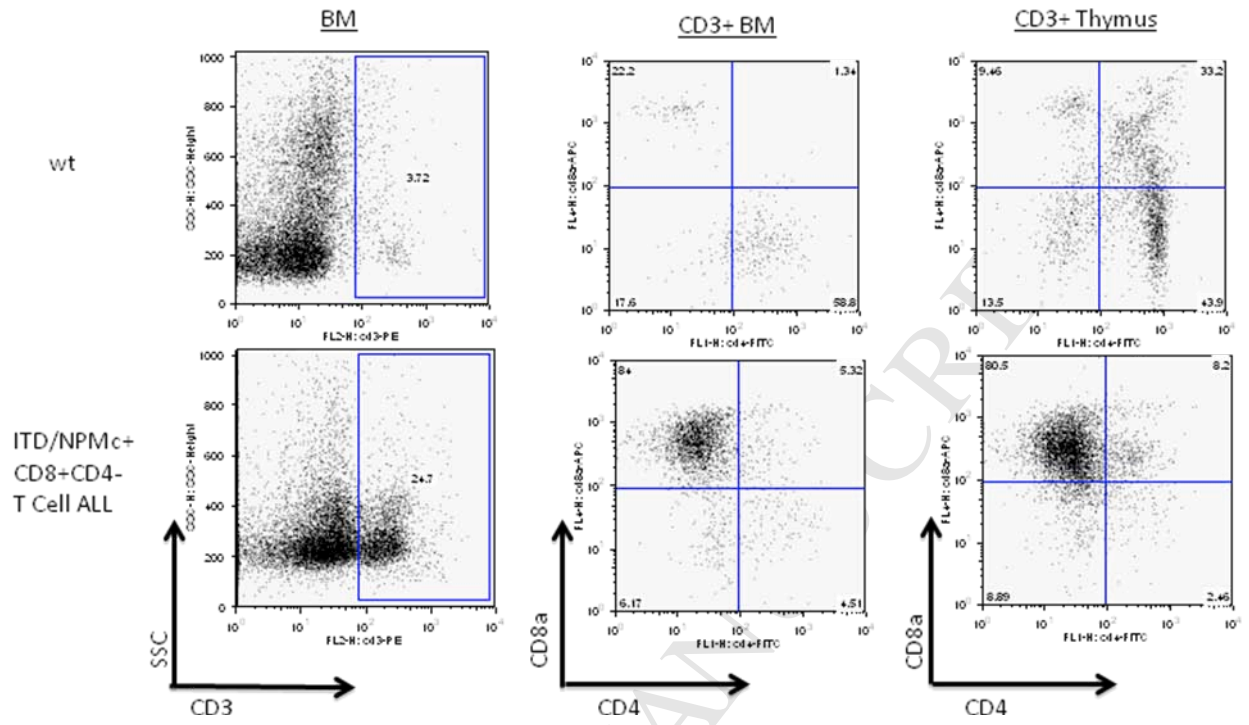


Figure 3s

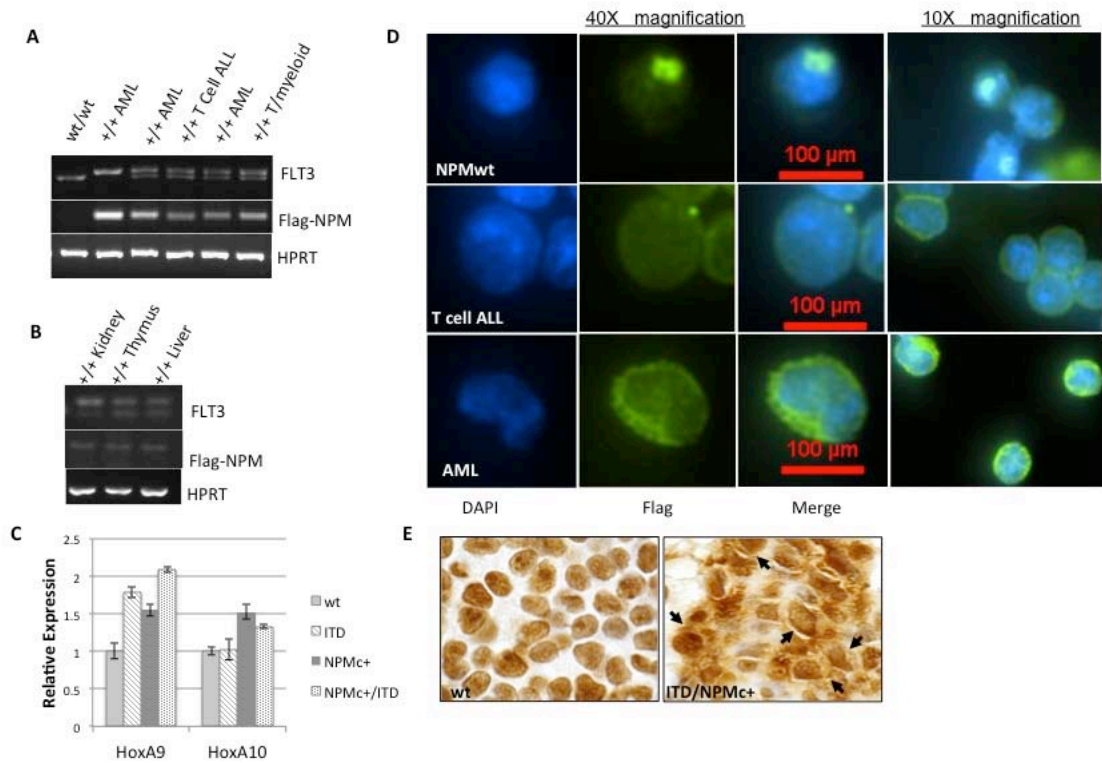


Figure 4s

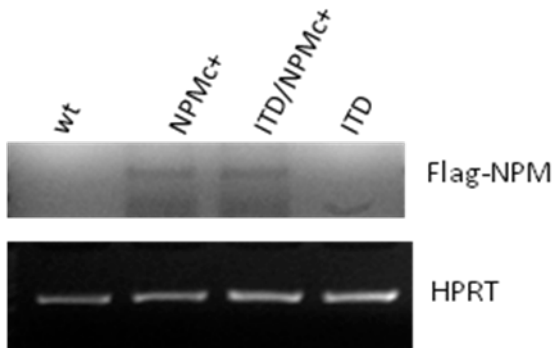


Figure 5s

