**Supplemental Methods**

**Quantitative real-time PCR.** Total RNA was extracted using RNeasy Mini Kit (Qiagen). cDNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Quantitative RT-PCR was performed with SsoAdvanced SYBR Green Supermix (Bio-Rad) on a CFX96 Real-Time PCR Detection System (Bio-Rad). Data were normalized to *GUS* and fold-change in gene expression was determined by ΔΔCt method. For primer sequences see Supplemental Table 3.

**{Khorashad, 2015 #197}Tetracycline-inducible shRNA.** Four shRNA sequences targeting *RAN* (three from the shRNA library, one not), and two shRNA sequences targeting *RANBP2* (one from the shRNA library, the other not) were individually inserted into a tetracycline-inducible vector (pRSIT12-U6Tet-CMV-TetR-2A-TagRFP-2A-Puro, Cellecta) containing wild-type tetracycline repressor (tetR), which blocks transcription in the absence of doxycycline (Dox). Constructs were lentivirally packaged for transduction. Transduced cells were sorted for RFP+ cells 72 hours post infection. For shRNA sequences, see Supplemental Table 4.

**Characterization of HEL-R cells.** Flow cytometry was used to analyze HEL-R cells for signaling pathway activation. HEL parental, HEL parental with 7 µM CYT387 for 3 hours, and HEL-R (always in 7 µM CYT387) were incubated with Aqua fixable dead cell stain then fixed with 4% paraformaldehyde, permeabilized with cold methanol, and incubated in human Fc block and 0.5% bovine serum albumin in PBS with the flow cytometry antibodies shown in Supplemental Table 5. Only live cells (Aqua negative) were gated for analysis. The median fluorescent intensity (MFI) is shown. Data was collected on a BD FACSCanto and analyzed with FlowJo (Ashland, OR).

**Immunoblot.** Cells were harvested and washed once with cold PBS, then lysed in radioimmunoprecipitation assay (RIPA) buffer (Cell Signaling Technology, 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na3VO4, 1 µg/ml leupeptin; with 1 mM PMSF added immediately before use). Equal amounts of protein were separated by SDS-PAGE (Bio-Rad) and transferred to nitrocellulose membrane. For detailed antibody information, see Supplemental Table 5. The images were obtained using a Bio-Rad ChemiDoc or a Li-Cor Odyssey (Lincoln, NE).

**Preparation of inhibitors for in vivo administration.** For in vivo studies, ruxolitinib was dissolved in DMSO (final DMSO concentration 2% (v/v)) then in 0.5% methylcellulose (Spectrum Chemical, New Brunswick, NJ) with 5% dimethylacetamide (Sigma-Aldrich, St. Louis, MO). Ruxolitinib was prepared weekly and stored at -20 ºC. KPT-330 was prepared in 0.6% Pluronic F-68, 0.6% PVP-K29/32 (both from Thermo Fisher) and water weekly, stored at 4ºC, according to the manufacturer’s protocol.

**Supplemental Figure Legends**

**Supplemental Figure 1. Schematic of the shRNA library screen.**

**Supplemental Figure 2. Effect of NCT inhibition on cell viability is JAK2V617F-independent. (A)** Colony formation assay ofHEL and SET-2 cells transduced with the shRNA empty vector (pRSIT12) then treated with or without Dox. **(B)** The effect of KPT-330 and KPT-8602 on UT7 and TF1 cells after 72 hours, measured by MTS. Data were from at least three independent experiments with triplicate in each experiment.

**Supplemental Figure 3. Characterization of HEL cells resistant to momelotinib (CYT387) (HEL-R cells).** HEL-R cells were maintained in medium containing 7 µM momelotinib. **A.** Flow cytometric analysis of pJAK2, pSTAT3, and pSTAT5 of HEL-R cells. The HEL-R cells show increases in pJAK2, pSTAT3, and pSTAT5 compared to parental HEL cells (3 hours in 7 µM CYT387). **B.** HEL-R cells show increased apoptosis (Annexin V) upon RAN knockdown.

**Supplemental Figure 4**. **Inhibition of NCT led to nuclear retention of some but not all previously identified cargo proteins in MF.** Whole cell (WCL), cytoplasmic (CYT), and nuclear (NUC) lysates of HEL cells treated with either DMSO vehicle (-) or KPT-330 (100 nM) for 24 hours were analyzed for subcellular localization of tumor suppressors, as indicated (**A**). FOXO3A showed reduced nuclear retention upon inhibition of NCT, while PP2A was not detected in the nucleus, and only a minimal amount of the total CIP2A was observed in the nucleus without or with inhibition. NPM1 was abundant in the nucleus but not altered by KPT-330 treatment. IκBα was retained in the nucleus following treatment. Fractionation markers tubulin and lamin B are shown in Figure 6A. Two different primary MF patient samples (**B, C**) of different genotypes and treatment responses showed variable responses with regards to NPM1 while IκBα was not detected in the nucleus in B or C. Lamin B, tubulin, and p53 for C is shown in Figure 6C.

**Supplemental Figure 5. Green fluorescent protein (GFP) positive cells are comparable in pretreatment groups.** Three weeks after bone marrow transplant, mice were randomized to vehicles, KPT-330, ruxolitinib, or ruxolitinib plus KPT-330 treatment. The percentage of GFP+ cells in the blood was similar in each group prior to treatment initiation.

**Supplemental Figure 6. KPT-330 did not normalize bone marrow pathology. (A)** H&E stained bone marrow sections are shown at 10x and the treatment is indicated. The bar on the healthy image represents 100 µm. **(B)** Bone marrow reticulin fibers are shown at 20x and the treatment is indicated. The bar on the healthy image represents 100 µm. **(C)** Fibrosis was scored on a scale from 0 (none) to 3 (severe) and the averages for three mice (with three images per mouse) per treatment are shown. (**D**) Average myeloid:erythroid ratios and (**E**) bone marrow cellularity are shown for 3 mice/treatment group.