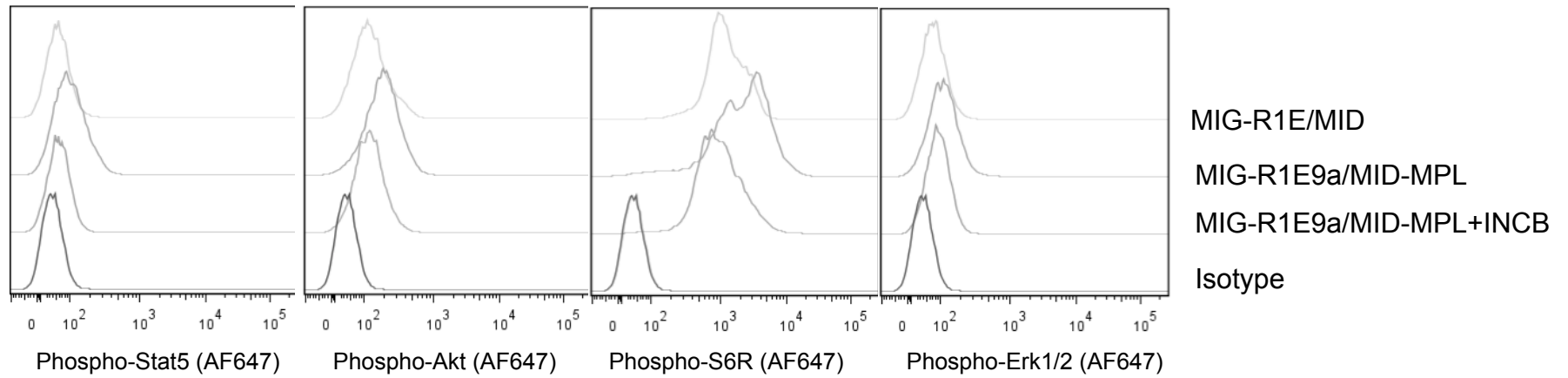


Supplementary Figure 1. R1E and endogenous Runx1 are expressed in leukemic cells. Western blot analysis of R1E and Runx1 expression in MIG-R1E/MID and MIG-R1E/MID-MPL leukemic cells, All samples were run in the same gel, and immunoblotted using Runx1 antibody (Ab-2; Calbiochem).



Supplementary Figure 2. Intracellular flow cytometry analysis of STAT5a/b(Tyr 694), Akt1/2/3(Ser473), S6R(Ser235/236), and Erk1/2 (Thr202/Tyr204) phosphorylation in cells harvested from leukemic mice (see Figure 2D), and isotype control.

Supplementary Material and Methods:

Intracellular flow cytometry analysis: Single-cell suspensions were prepared from spleen of leukemic mice, and fixed, permeabilized and stained following manufacturer's instructions. Briefly, cells were fixed in 4% paraformaldehyde in PBS at 10^7 cells/mL for 10 minutes at 37°C. Cells were washed in FACS buffer (PBS supplemented with 0.2% BSA), resuspended at 10^8 cells/mL and permeabilized by slowly adding ice-cold methanol while vortexing to a final concentration of 90% methanol. Samples were then incubated for 20 minutes on ice and stored at -20°C. Samples were washed twice with FACS buffer, resuspended at 10^7 cells/mL and blocked in a 1:100 dilution of rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) in FACS buffer for 10 minutes at room temperature. Samples were washed twice, resuspended at 10^7 cells/mL in FACS buffer containing AlexaFluor647-conjugated phospho-specific antibodies against Akt1/2/3-Ser473 (Cell Signaling, Danvers, MA), Erk1/2-Thr202/Tyr204 (BD Biosciences, San Jose, CA), S6R-Ser235/236 (Cell Signaling, Danvers, MA) and Stat5a/b-Tyr 694 (BD Biosciences, San Jose, CA) for 1 hour at room temperature. The samples were washed once in FACS buffer, resuspended at 0.5×10^7 cells/mL in FACS buffer and immediately analyzed on an LSRII (BD Biosciences, San Jose, CA). Data analysis was done gating on GFP(+) cells, using FlowJo software (TreeStar Inc., Ashland, OR).