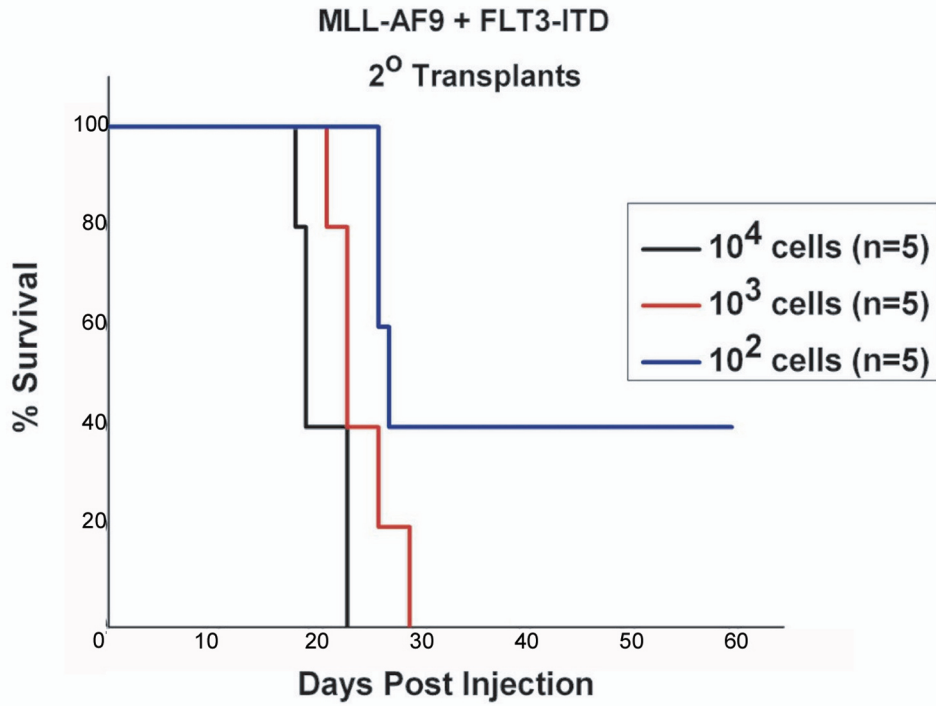
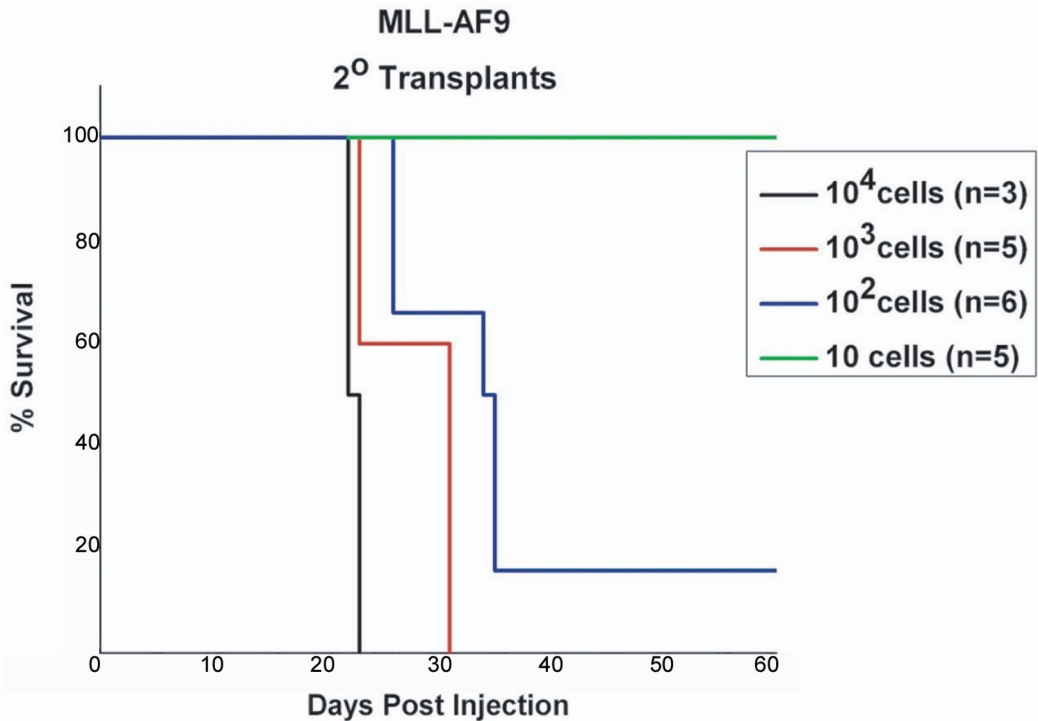


# Supplemental Figure 1

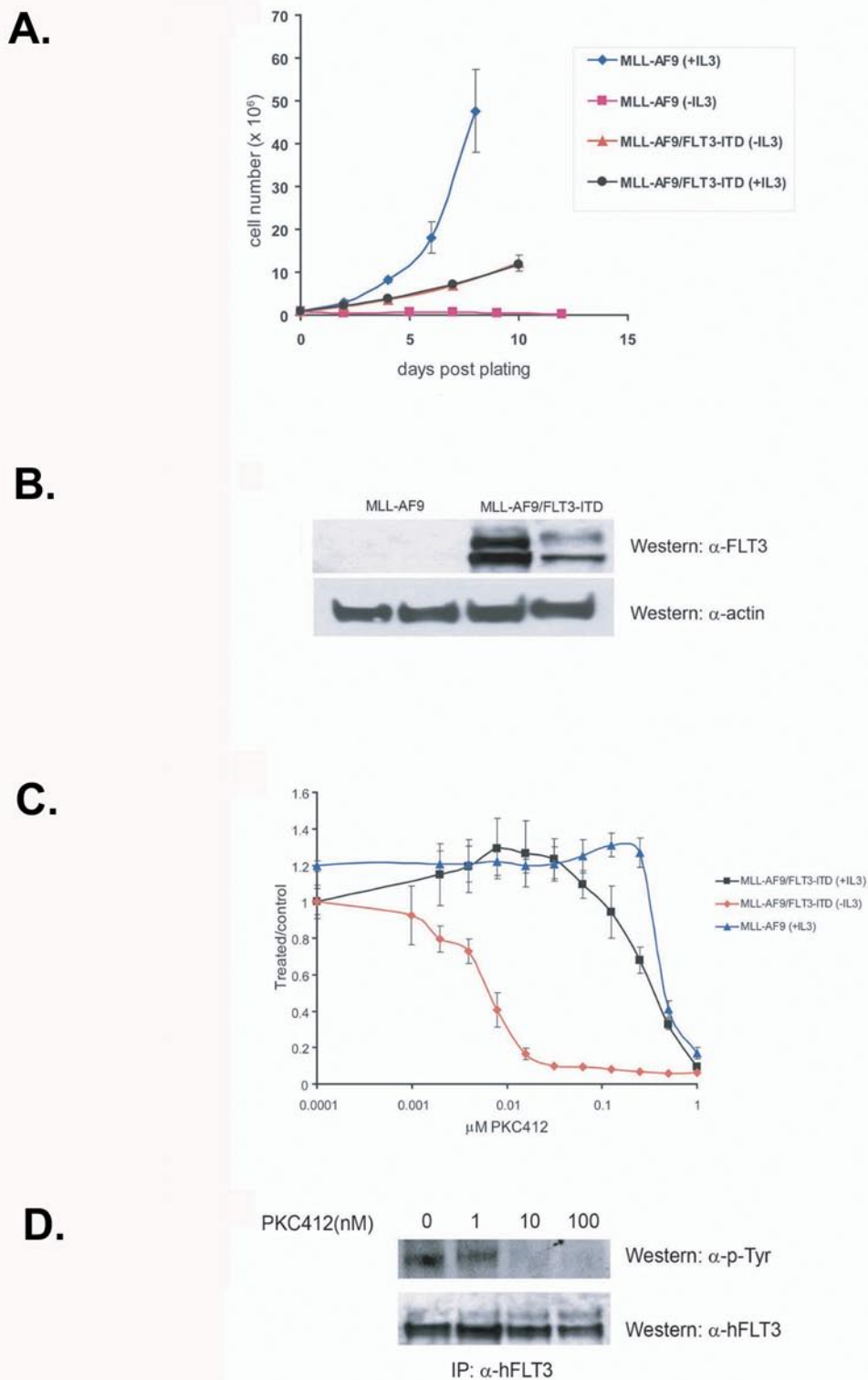
**A.**



**B.**



# Supplemental Figure 2



## Supplemental Data:

### **Supplemental Figure 1: AML from MLL-AF9 and MLL-AF9 + FLT3-ITD mice are similarly transplantable.**

**A and B.**  $10^4$ ,  $10^3$ ,  $10^2$ , or 10 spleen cells were taken from mice with leukemia expressing either MLL-AF9 or MLL-AF9/FLT3-ITD and injected into sublethally irradiated recipients. Kaplan-Meier curves are shown for mice injected with the indicated leukemia. AML can be transferred to recipient mice with as few as 100 cells.

### **Supplemental Figure 2: FLT3-ITD is necessary for cytokine independent cell growth and is inhibited PKC412 in vitro.**

**A.** Primary cells isolated from spleen or bone marrow of MLL-AF9 and MLL-AF9/FLT3-ITD mice were grown in culture in the presence or absence of IL3, and counted manually. MLL-AF9/FLT3-ITD cell lines expanded without IL3, whereas MLL-AF9 cells could not grow in the absence of IL3. This figure represents 3 different MLL-AF9 cell lines from 2 mice, and 2 different MLL-AF9/FLT3-ITD cell lines from 2 mice. **B.** Whole cell extracts were made from  $2.5 \times 10^6$  cells from the primary cell lines, and Western analyses performed. Shown is a blot probed with an  $\alpha$ -hFLT3 antibody as well as  $\alpha$ -pan-actin antibody as a protein loading control. **C.** MLL-AF9 and MLL-AF9/FLT3-ITD cells were plated at a density of  $10^5$  cells per 100 $\mu$ l media into 96-well microtiter plates and incubated in serial dilutions of PKC412 with or without IL3. MTT assays were then performed. Shown is the graph of cell growth, as expressed in MTT colorimetric units, in the presence of various concentrations of PKC412. FLT3-ITD expressing cell lines are extremely sensitive to PKC412, having an  $IC_{50}$  of roughly 8nM. PKC412 sensitivity is alleviated in the presence of IL3. **D.** Immunoprecipitations were performed on whole cell lysates made from cells treated with varying concentrations of PKC412 and FLT3 was

immunoprecipitated with an  $\alpha$ -hFLT3 antibody. Western blots were performed using  $\alpha$ -phosphotyrosine and  $\alpha$ -hFLT3 (to show equivalent protein loading). PKC412 inhibits phosphorylation of FLT3-ITD at concentrations of 10nM and greater.