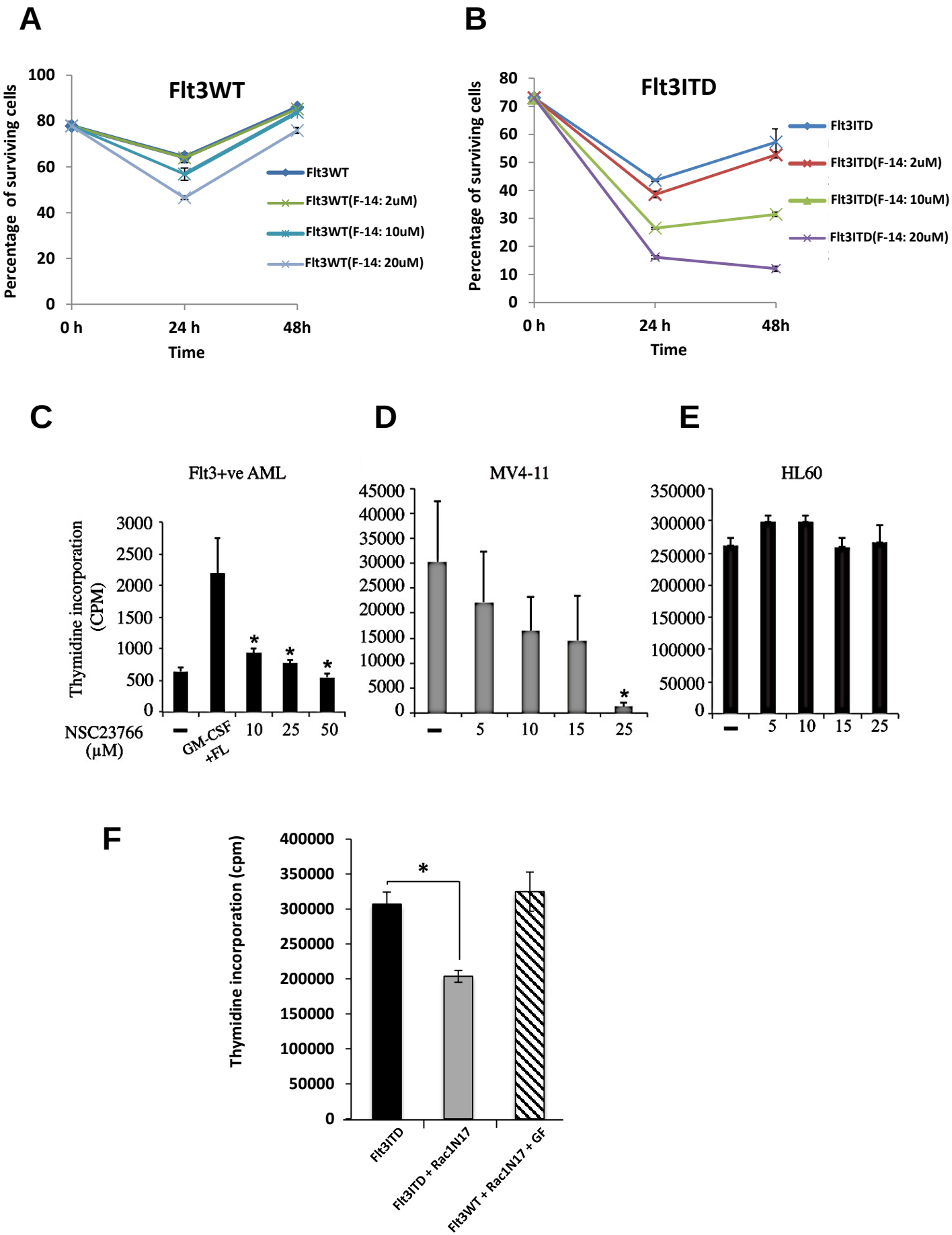

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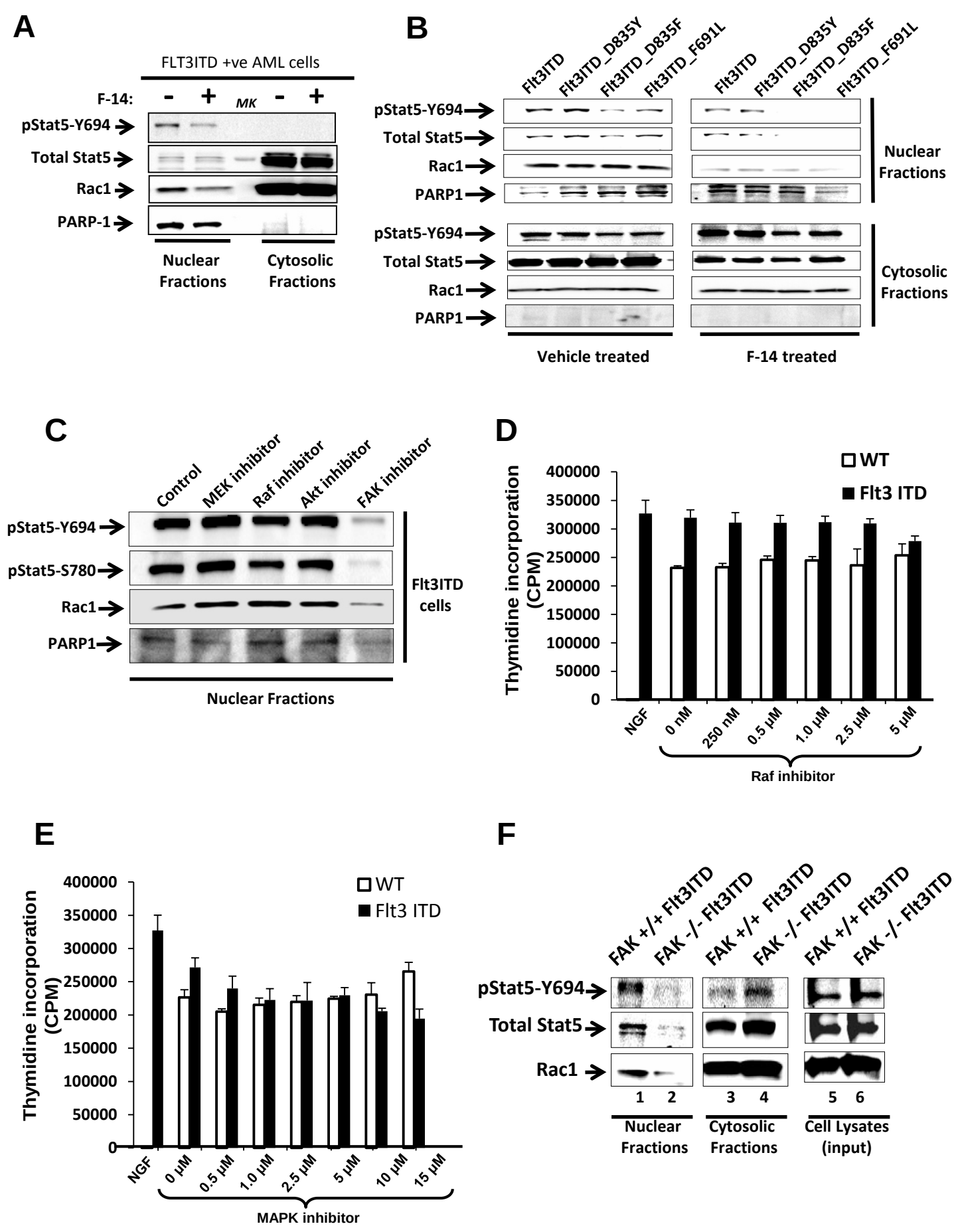

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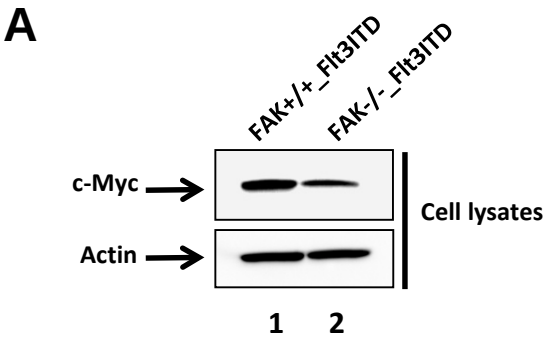

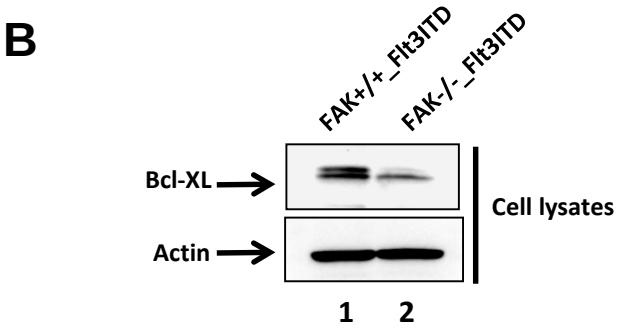

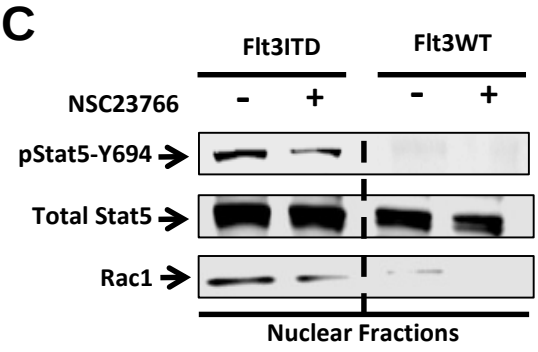

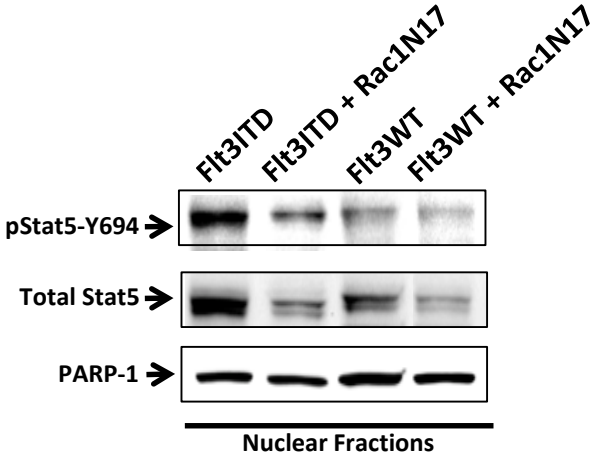

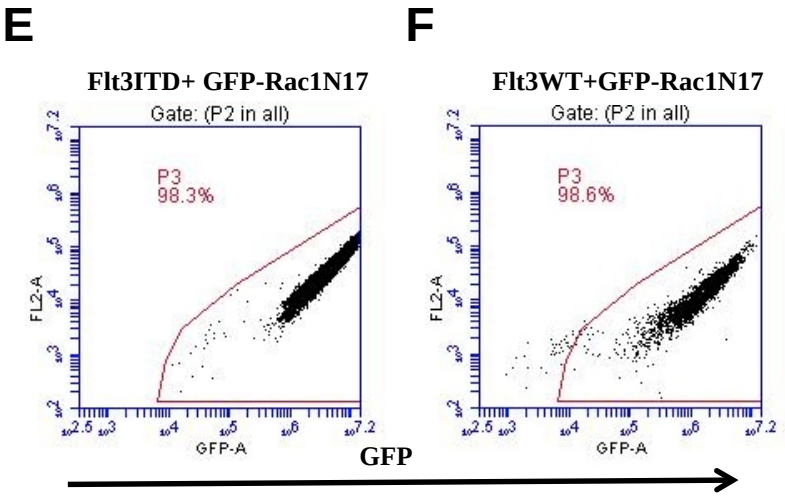

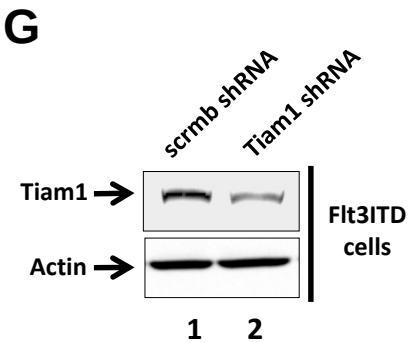

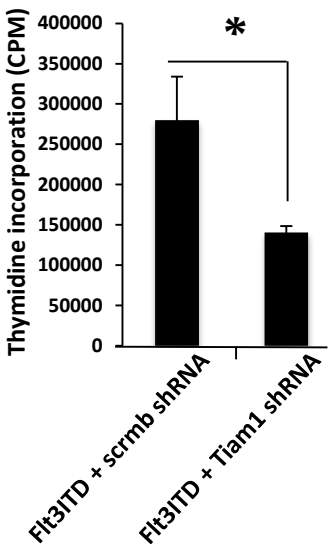

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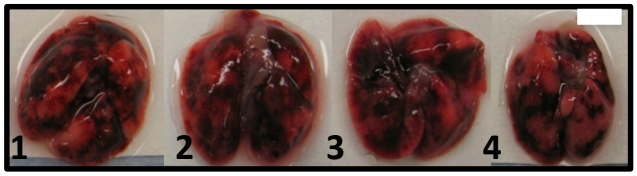
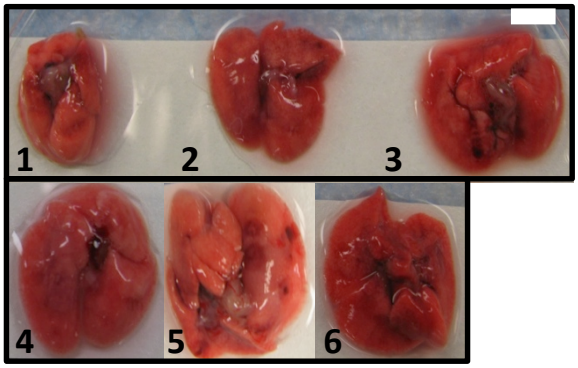
A

DMSO Treated (lungs)



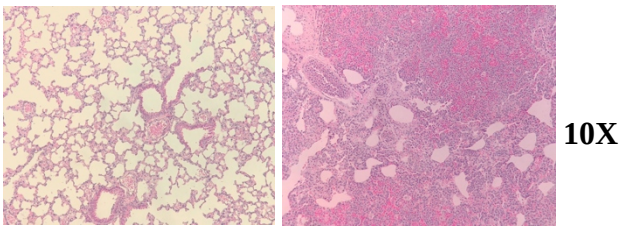
B

FAK inhibitor F-14 Treated (Lungs)



C

F-14 Treated (Lungs) DMSO Treated (Lungs)



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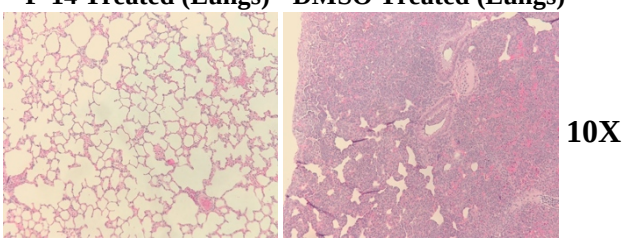

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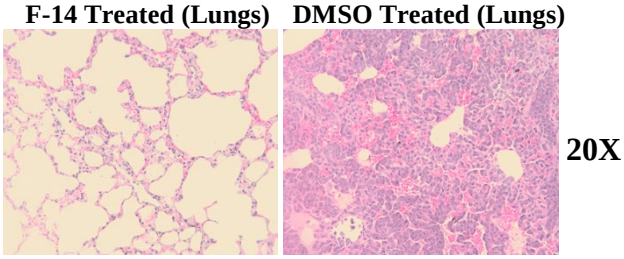

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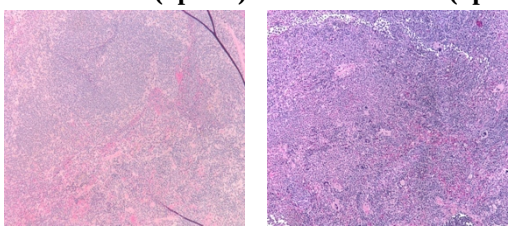
D

F-14 Treated (Spleen) DMSO Treated (Spleen)



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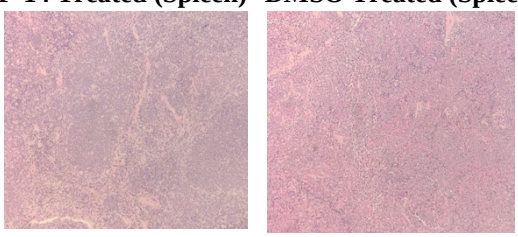
F-14 Treated (Spleen) DMSO Treated (Spleen)

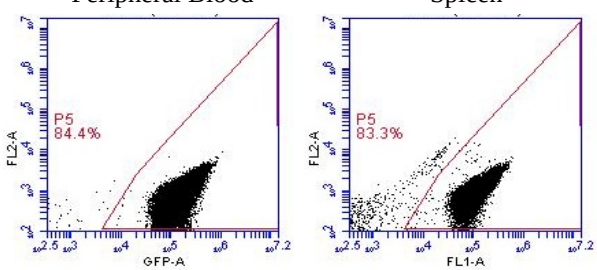


10X

E

Vehicle treated

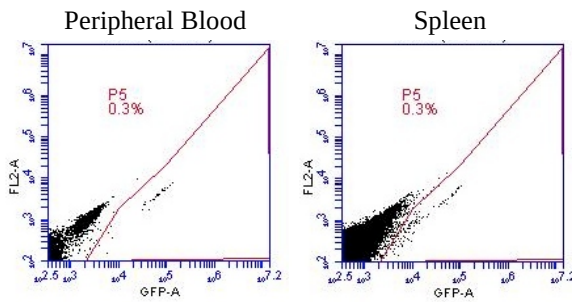
Peripheral Blood Spleen



F

F14 treated

Peripheral Blood Spleen



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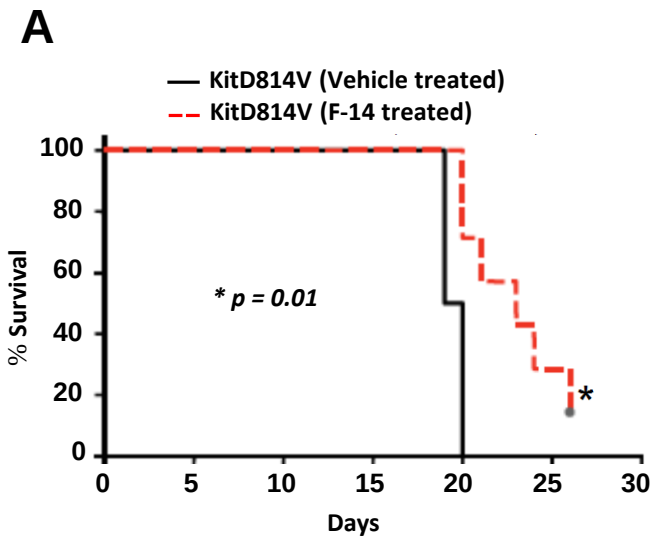

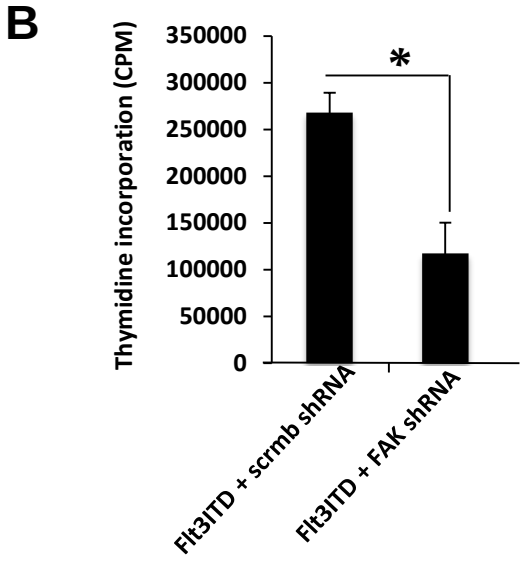

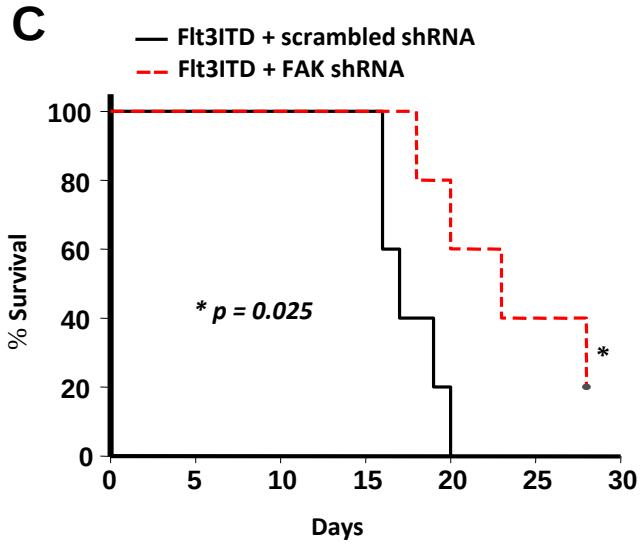

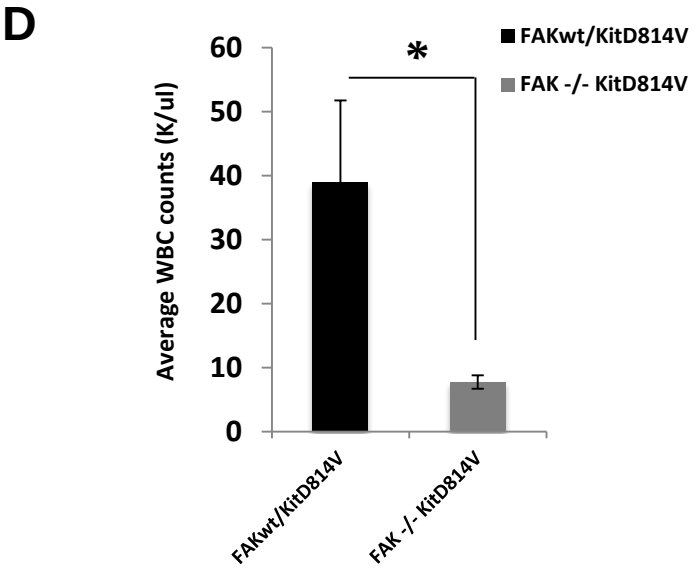

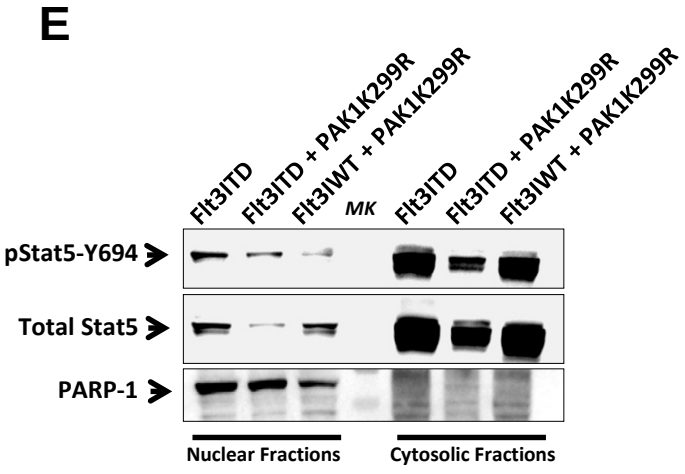

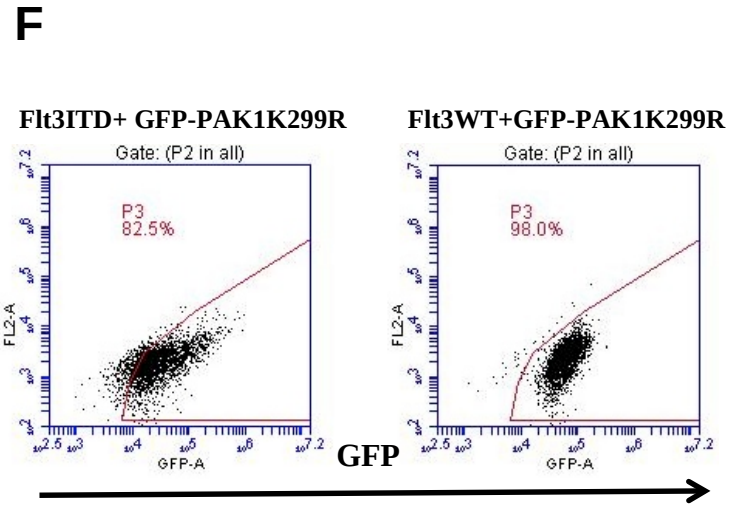

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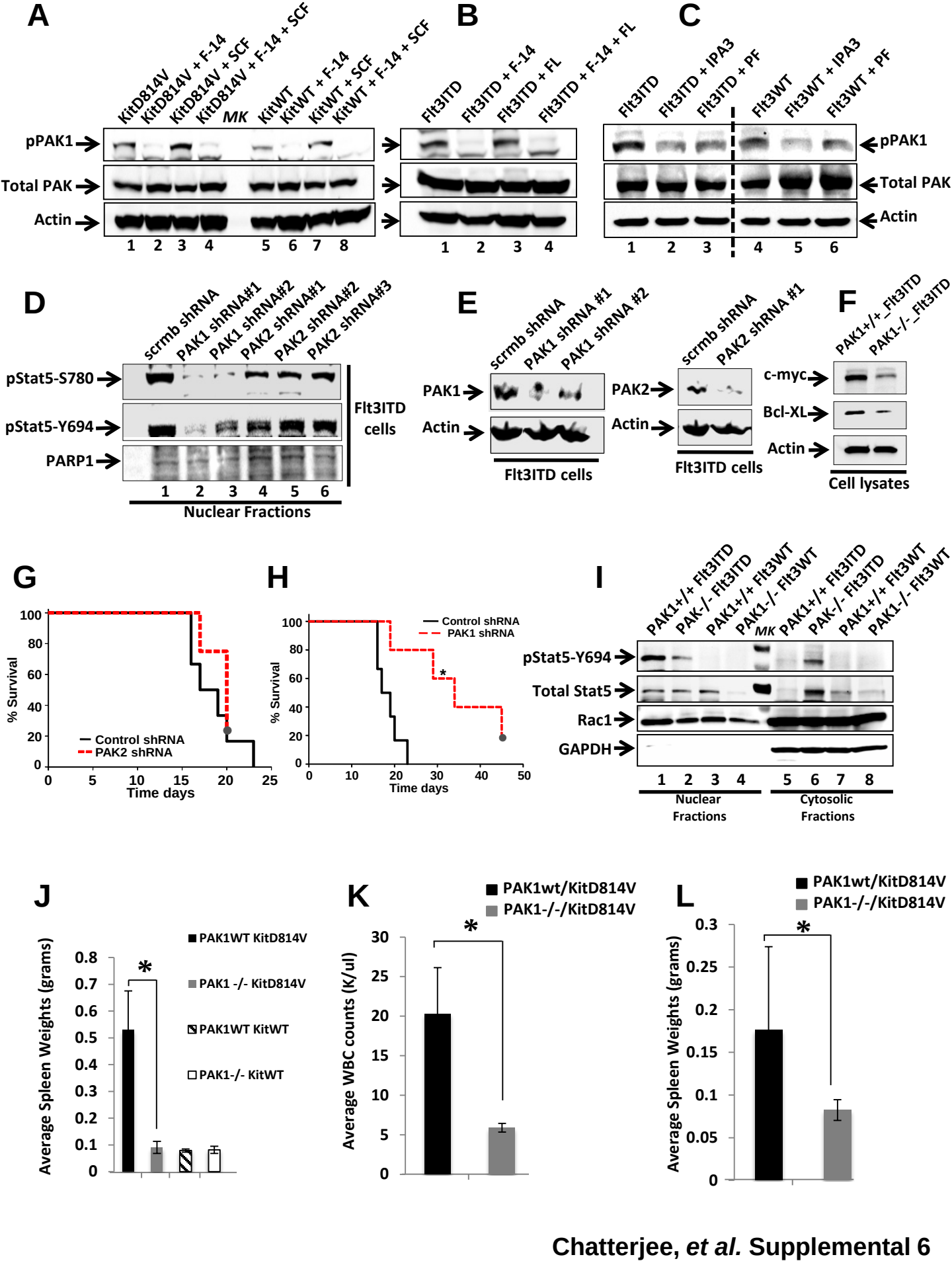

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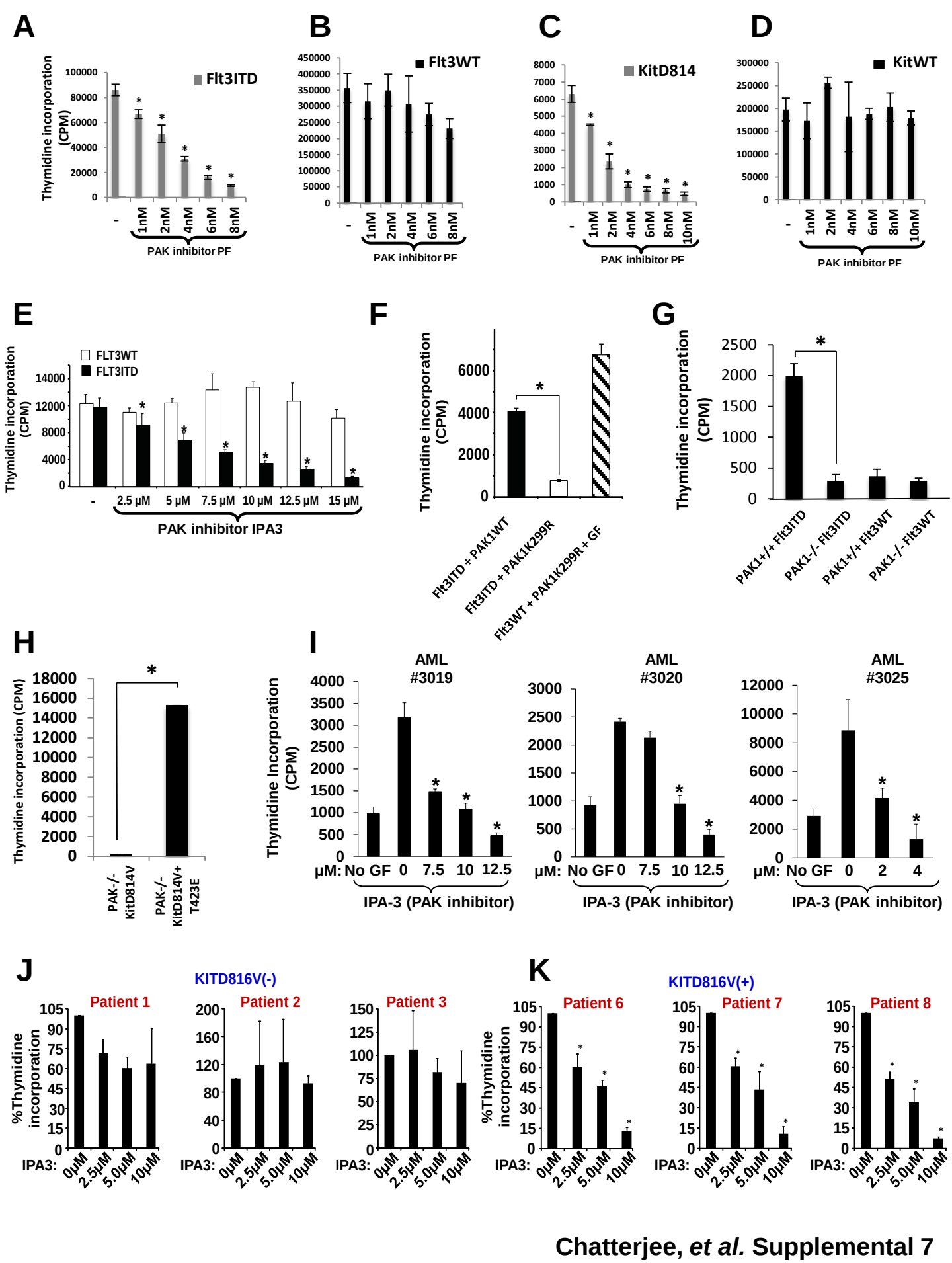

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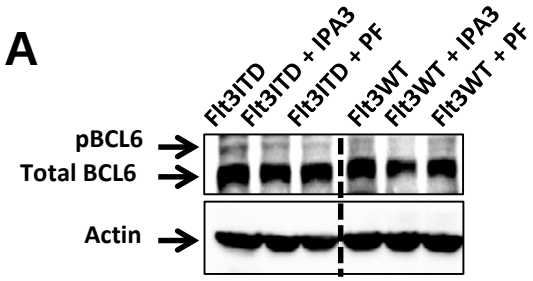

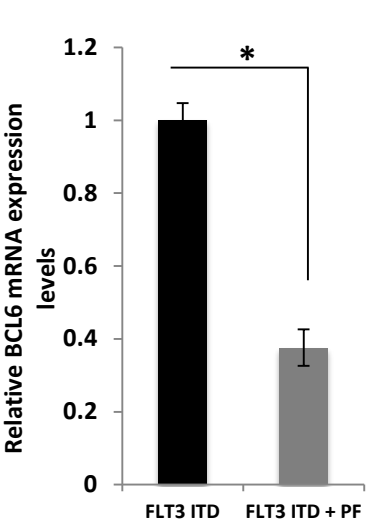

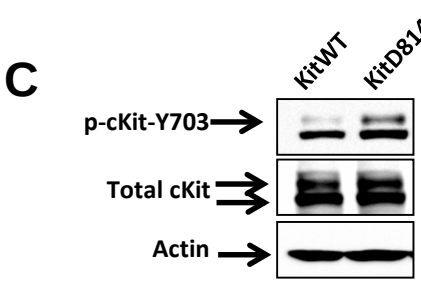

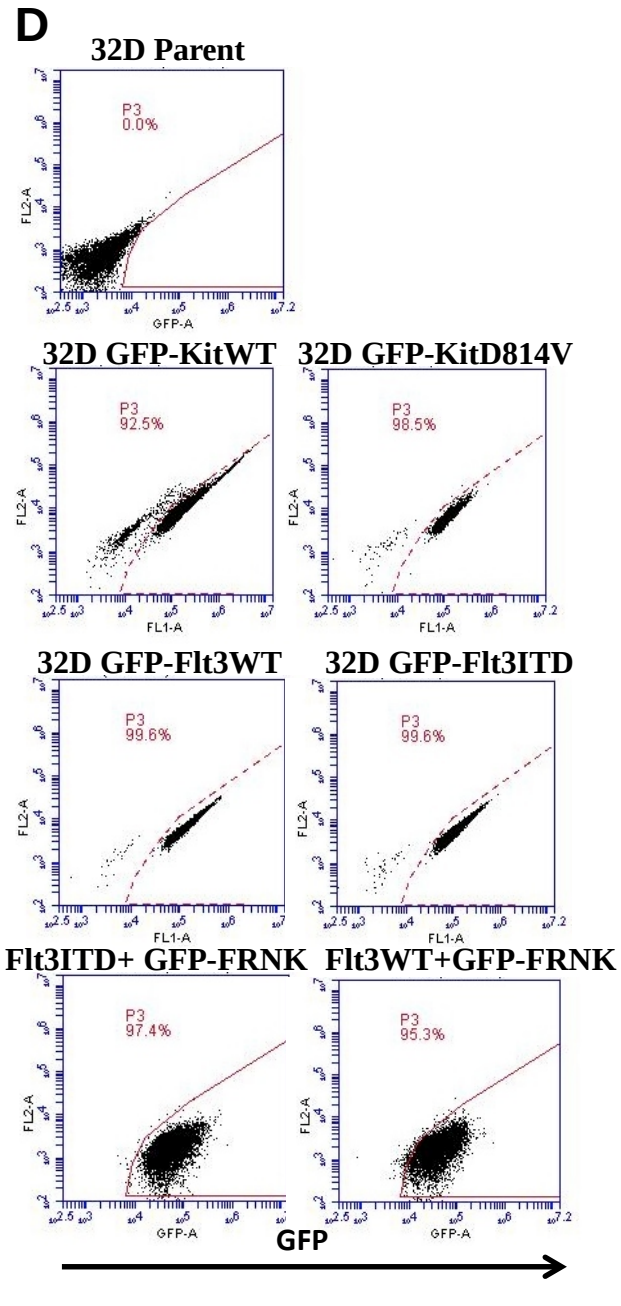

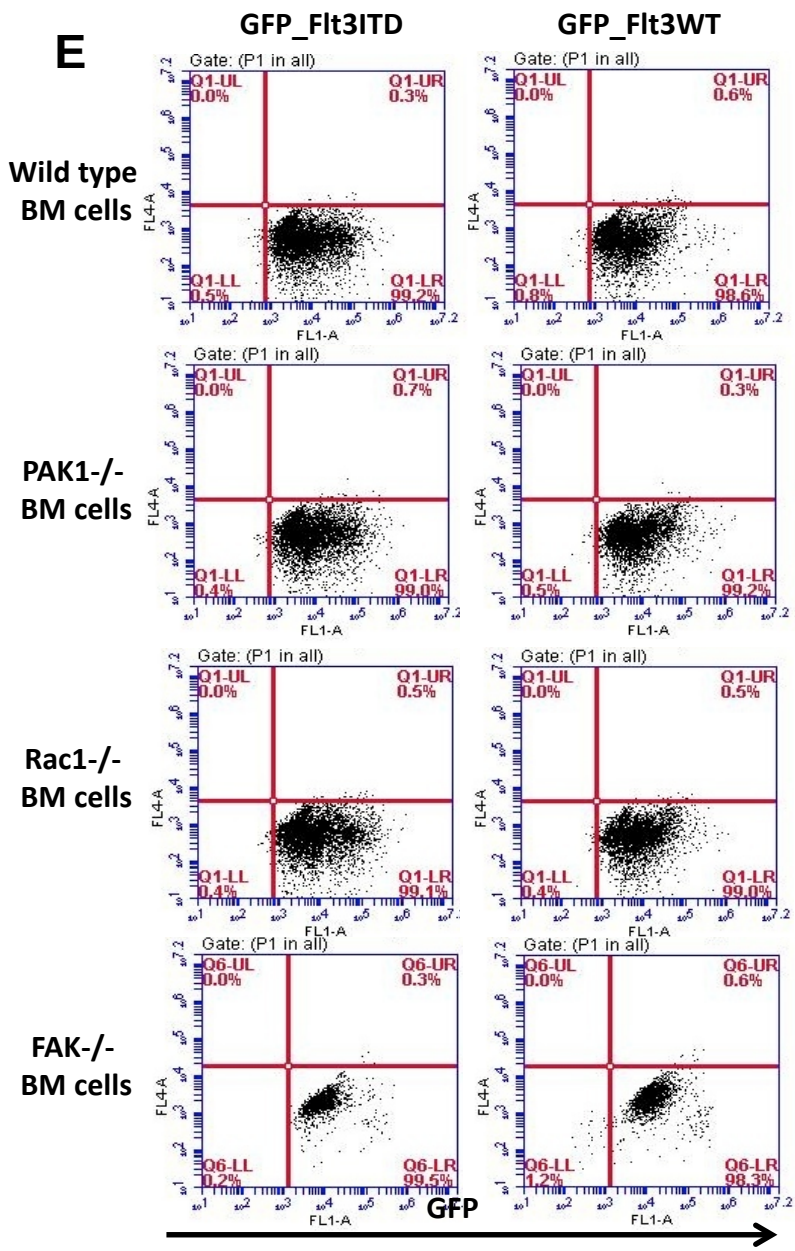

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Supplementary Figure legends

Fig. S1: Inhibition of FAK and Rac1 represses constitutive growth of FLT3ITD expressing cells by inducing cell death. Related to Figures 2,4. Apoptosis assays were preformed as described in materials and methods using BaF3 cells expressing FLT3ITD or Flt3WT receptors. Cells were incubated under varying concentrations of FAK inhibitor F-14 (2, 10, 20 μM) as indicated. Apoptosis was measured at different time points (0, 24, 48 hours). Results indicate a dose dependent induction of apoptosis in (A) FLT3ITD bearing cells with no effect on cells bearing the (B) WT FLT3 receptor (n=2), *p<0.05. (C) FLT3ITD +ve AML patient sample were treated with Rac1 inhibitor NSC23766 in doses indicated. Cells were untreated in absence of growth factors (-), in presence of growth factors (GM-CSF, FL), and in presence of varying doses of NSC23766 + growth factors (GM-CSF, FL), (D) human leukemia derived MV4-11 and (E) HL60 cells expressing endogenous levels of FLT3ITD or FLT3WT respectively, were treated with varying doses of Rac1 inhibitor NSC23766, and were subjected to thymidine incorporation proliferation assays. (F) BaF3 cells co-expressing dominant negative Rac1N17 with FLT3ITD or FLT3WT, or expressing FLT3ITD + vector were subjected to thymidine incorporation proliferation assay. '+GF' indicates IL-3 was added to the WT receptors. Thymidine incorporation is depicted on y-axis as ± SD, *p<0.05.

Fig. S2: Inhibition of FAK inhibits nuclear localization of active Stat5 in AML patients, and also in AC220 resistant "driver" mutations of FLT3, while FLT3ITD oncogene bearing cells do not utilize the MAPK, Raf or Akt signaling pathways to facilitate nuclear translocation of active Stat5. Related to Figure 5. Cellular fractionation assays were carried out in (A) FLT3ITD+ve AML patient sample, (B) BaF3 cells expressing FLT3ITD and AC220 resistant mutations (FLT3ITD+D835Y, D835V, F691L) were subjected to cellular fractionation assays upon treatment with FAK inhibitor F-14 or vehicle (DMSO). Nuclear and cytosolic

fractions were analyzed by western blotting showing levels of active Stat5 (pY694), total Stat5 and total Rac1 in corresponding fractions. PARP-1 was used as a loading control and nuclear marker. 'MK' denotes lane with protein ladder. (C-E) 32D cells expressing FLT3ITD were treated in presence or absence of MEK inhibitor (PD98059), Raf (PLX4720), Akt (124005) or FAK inhibitor F-14 and subjected to cellular fractionation (C), and proliferation assays (D,E). Nuclear and cytosolic fractions (C) were analyzed by western blotting showing levels of active Stat5 (pY694), Stat5 (pS780), total Rac1 in corresponding fractions. PARP-1 was used as a loading control and nuclear marker. (F) WT (*FAK*+/+) and FAK deficient (*FAK*-/-) primary bone marrow (BM) cells expressing FLT3ITD were subjected to fractionation assay and nuclear (left panel) and cytosolic fractions (middle panel) were analyzed as described earlier. Total protein levels are shown in the right side panels, n=2. The total proteins levels are from the same experiment depicted in Fig.6B. Nuclear and cytosolic fractions were analyzed by western blotting showing levels of active Stat5 (pY694), total Stat5 and total Rac1 in corresponding fractions.

Fig. S3: Genetic ablation, pharmacological inhibition and knockdown of FAK, Rac1 or Tiam1 rescues Flt3ITD mediated transformation by inhibiting the nuclear translocation of Stat5 and repression of Stat5 responsive genes c-Myc and Bcl-XL. Related to Figures 5,6. (A-B) Low density BM cells from wild type *FAK*+/+ and *FAK*-/- deficient mice were transduced with FLT3ITD as described in materials and methods. Cells were sorted to homogeneity and subjected to whole cell lysate analysis. Equal amounts of samples were probed via western blotting with antibodies against (A) c-Myc and (B) Bcl-XL as indicated. Actin was used as a loading control. (C) Cellular fractionation assay was performed using BaF3 cells expressing FLT3ITD or FLT3WT in presence or absence of Rac1 inhibitor NSC23766. Nuclear fractions were probed for active Stat5 (pStat5Y694), and total Stat5 and Rac1 as indicated; unrelated lanes were removed indicated by dotted lines; (D) cells co-expressing FLT3ITD or FLT3WT and

dominant negative Rac1(N17) was subjected to cellular fractionation and analyzed as described above; unrelated lanes were removed. Treatment with either Rac1 inhibitor NSC23766 or expression of dominant negative Rac1 results in inhibition in nuclear translocation of active Stat5. (E,F) BaF3 cells co-expressing Rac1N17 with FLT3ITD or FLT3WT showing expression of GFP-Rac1N17. (G) 32D cells bearing expressing FLT3ITD were transduced with shRNA targeting Tiam1 as per manufacturers' instructions (Origene). Transduced cells were selected by puromycin. Equal amount of protein were loaded and analyzed via western blotting showing downregulation of total Tiam1 levels. Actin was used as a loading control. (H) Proliferation assay of 32D cells expressing FLT3ITD and transduced with either Tiam1 shRNA or scrambled shRNA (control). Thymidine incorporation is depicted on y-axis as mean ± SD, *$p<0.05$ (n=2).

Fig. S4: In vivo inhibition of FAK delays the progression of FLT3ITD induced MPN in mice. Related to Figure 7. C3H/HeJ mice transplanted with GFP-FLT3ITD expressing 32D cells were treated with F-14 or vehicle as described in materials and methods. Fig.S4A-C Moribund mice were euthanized and lesions in lungs were analyzed as depicted. (A) Vehicle treated mice (panels 1-4) showed lesions in the lungs, while mice treated with FAK inhibitor F-14 (B) (panels 1-6) demonstrated significant reduction in development of lesions in lungs. White bar represents 0.5 cm. (C) Histopathological analysis of F-14 and DMSO treated lungs were analyzed. Shown are representative tissue sections from corresponding mice cohorts (panels in A,B), that were fixed in 10% buffered formalin, sectioned, and stained with H&E. Fig.4C demonstrates collapse of alveolar architecture in DMSO treated mice as compared to F-14 treated mice, and presence of leukemic KITD814V cells in place of normal erythroid and myeloid components. Magnifications shown are 10X and 20X as indicated beside panels. (4D) Histopathological analysis of spleen from F-14 and DMSO treated mice as described in Fig.4A-C. F-14 treated mice show normal splenic architecture, while DMSO treated mice depicts replacement of normal erythroid and myeloid components by leukemic KITD814V cells.

(E-F) C3H/HeJ mice were transplanted with GFP-FLT3ITD expressing 32D cells, and treated with FAK inhibitor F-14 as described in materials and methods. Moribund mice were sacrificed and peripheral blood and spleen cells were analyzed for the presence of GFP +ve cells. Fig.4E shows representative data of GFP content in vehicle treated mice, while Fig.4F represents F-14 treated mice. The percentage of GFP +ve cells is indicated inside the gated quadrant.

Fig.S5: Inhibition of FAK represses the development of MPN mediated by FLT3ITD and KITD814V oncogenes, while dominant negative mutant of PAK1 (K299R) inhibits Stat5 nuclear localization and represses ligand independent growth in FLT3ITD oncogene bearing cells. Related to Figures 7,8. (A) Survival curve of C3H/HeJ mice transplanted with 32D cells expressing KITD814V were treated with FAK inhibitor F-14 (n=7) or vehicle (n=4) as described in materials and methods, (*p<0.01). (B) FLT3ITD cells expressing FAK shRNA used for syngenic transplants described below in (C) were subjected to proliferation assays. Thymidine incorporation is depicted on y-axis as ± SD, *p<0.05. (C) Survival curve of C3H/HeJ mice transplanted with 32D cells co-expressing FLT3ITD and FAK shRNA (n=5) or with control (scrambled) shRNA (n=5), *p<0.025.

(D) 5'FU-treated primary BM transplants using GFP tagged KITD814V receptor in a WTFAK (*FAK*+/+) (n=9) or FAK deficient (*FAK-/-*) (n=9) background was performed as described in materials and methods. Mice were bled at regular intervals and peripheral blood counts were analyzed for WBC (K/ul) levels, *p<0.05. (E) BaF3 cells co-expressing GFP-PAK1(K299R), a dominant negative version of PAK1, and either FLT3ITD or WTFLT3 were analyzed by cellular fractionation assay. 'MK' denotes lane with protein ladder.

(F) Expression levels of GFP-PAK1(K299R), a dominant negative version of PAK1, in BaF3 FLT3ITD and FLT3WT cells.

Fig. S6: Inhibition of FAK represses activation of PAK1 in FLT3ITD and KITD814V oncogene bearing cells; while in vivo inhibition of PAK1, not PAK2, delays the onset of leukemia in mice transplanted with FLT3ITD and KITD814V bearing cells. Related to Figure 8. (A) 32D KITD814V or KITWT cells were treated with FAK inhibitor F-14, KIT ligand (SCF), F-14 followed by SCF (F-14+SCF) and subjected to cell lysis. Equal amounts of samples were probed via western blotting with antibodies against active PAK1 (pPAK1) and total PAK1 as indicated. Actin was used as a loading control. (B) 32D FLT3ITD cells were treated with FAK inhibitor F-14, FLT3 ligand (FL), F-14 followed by FL (F-14+FL), and cells subjected to cell lysis, and levels of active and total PAK1 analyzed as described in (A). (C) 32D FLT3ITD and FLT3WT cells were treated with PAK inhibitors IPA-3 or PF-3758309 and analyzed for levels of active and total PAK1 as described in (A). Dotted line represent unrelated lane removed from the blot. 'MK' denotes lane with protein ladder. (D) FLT3ITD bearing cells co-expressing shRNAs against PAK1 or PAK2 or scrambled shRNA as controls, were subjected to fractionation assays as described earlier. Activated Stat5 (pY694), phospho-Stat5 (pS780) and PARP-1 are indicated in nuclear fractions. (E) 32D cells expressing FLT3ITD were transduced with shRNA targeting PAK1 and PAK2 as per manufacturers' instructions (Origene). Transduced cells were selected by puromycin. Equal amount of protein were loaded and analyzed via western blotting showing downregulation of total PAK1 and PAK2 levels as depicted. Multiple shRNA constructs were used (as shown for PAK1), and the shRNA demonstrating maximum reduction in respective protein levels was used for all other experiments. Actin was used as a loading control. (F) BM cells from wild type PAK (*PAK1+/+)* and *PAK1-/-* deficient mice were transduced with FLT3ITD as described in materials and methods. Cells were sorted to homogeneity and subjected to whole cell lysate analysis. Equal amounts of samples were probed via western blotting with antibodies against cMyc and BclXL as indicated. Actin was used as a loading control. (G,H) Kaplan-Meier survival curve of mice transplanted with 32D cells co-expressing FLT3ITD and PAK2 shRNA (n=5) (G), PAK1 shRNA

(n=5) (H) or scrambled shRNA (n=5), (*p<0.02). (I) WTPAK1 (*PAK*+/+) or *PAK1-/-* BM cells bearing FLT3ITD or FLT3WT were subjected to fractionation assay as described earlier and nuclear and cytosolic fractions were subjected to western blot analysis using corresponding antibodies against Stat5 (pY694), total Stat5, Rac1 and GAPDH as cytosolic marker and loading control, n=3. (J) Primary transplant were carried out using 5'FU treated BM cells from WTPAK1 or *PAK1-/-* deficient mice transduced with KITD814V or KITWT, and transplanted into lethally irradiated C57BL/6 mice. Four groups of mice were used: WTPAK1 KITD814V (n=8), WTPAK1 KITWT (n=5), *PAK1-/-* KITD814V (n=8), and *PAK1-/-* KITDWT (n=5). Bar graph represents the average spleen weights from mice in each group, *p<0.05. (K,L) Secondary transplants were performed using BM cells from *PAK1*+/+KITD814V (n=5) and *PAK1-/-*KITD814V (n=5) mice and transplanted into irradiated C57BL/6 mice. Mice were bled at regular intervals and peripheral blood counts were analyzed for WBC (K/ul) levels (K), and (L) represents the average spleen weights from mice in each group, *p<0.05.

Fig. S7: Inhibition of PAK1 suppresses the constitutive growth of oncogenic FLT3ITD and KITD814V bearing cells. Related to Figure 8. 32D cells expressing (A) FLT3ITD, (B) FLT3WT, (C) KITD814V or (D) KITWT were cultured for 48 hours in presence or absence PAK inhibitor PF-3758309 in replicates of four and subjected to a thymidine incorporation assay, n=2, *p<0.05. (E) 32D cells expressing FLT3ITD or FLT3WT were subjected to thymidine incorporation assay in presence of PAK inhibitor IPA-3, n=2, *p<0.05. (F) BaF3 cells co-expressing GFP-PAK1(K299R), a dominant negative version of PAK1, and either FLT3ITD or WTFLT3 were subjected to proliferation assay. '+GF' indicates IL-3 was added to the WT receptors. Thymidine incorporation is depicted on y-axis as mean ± SD, *p<0.05. (G) WTPAK1 (*PAK1*+/+) or PAK1 deficient (*PAK1-/-*) primary BM cells expressing FLT3ITD or WTFLT3 were subjected to proliferation assay in the absence of growth factors, n=2. Thymidine incorporation is depicted on y-axis as mean ± SD, *p<0.05. (H) PAK1 deficient (PAK1-/-) primary BM cells co-

expressing hCD4-KITD814V + empty vector (1st bar), or hCD4-KITD814V + GFP-PAK1(T423E) (2nd bar), a constitutively active PAK1 construct, were subjected to proliferation assay in the absence of growth factors. Thymidine incorporation is depicted on y-axis as mean ± SD, *p<0.05. (I) Representative AML patient samples grown in the presence of cytokines (FLT3 and GM-CSF) and treated with indicated amounts of PAK inhibitor, IPA-3. No GF indicates absence of cytokines. (J) Likewise KITD816V- SM (Patients 1-3), and (K) KITD816V+ SM (Patients 6-8) patient derived cells were subjected to proliferation in the presence of human SCF. After 48 hours, proliferation was evaluated. Bars denote the mean thymidine incorporation ± SD performed in triplicate or quadruplicate, *p<0.01.

Fig. S8: Inhibition of PAK1 inhibits the activation of BCL6 protein and mRNA expression. Related to Figures 1,2,5,7,8. (A) 32D FLT3ITD and FLT3WT cells were treated with PAK inhibitors IPA-3 or PF-3758309. Cells were lysed and equal amount of protein loaded and analyzed for levels of phospho-BCL6 and total BCL6 via western blotting. Actin was used as a loading control, n=2. Un-related lanes were removed as depicted by dotted line. (B) 32D FLT3ITD cells were treated with PAK inhibitor PF-3758309 and relative mRNA levels of BCL6 determined as described in materials and methods, *p<0.05, n=2.

Expression levels of proteins expressed in cell lines and bone marrow cells are shown in Figure S8C-E as follows: (C) 32D KITWT or KITD814V cells were serum starved and analyzed for levels of active KIT (pY703), total KIT and actin as loading control. 32D parent cells (D), 32D cells expressing GFP-KITWT or GFP-KITD814V (second row panels), GFP-FLT3WT or GFP-FLT3ITD (third row panels) were analyzed for GFP expression via flow cytometry. Expression of GFP-FRNK, a dominant negative version of FAK was expressed in geneticin (G418) resistant BaF3 FLT3ITD and FLT3WT cells (bottom row panels). (E) Flow cytometry analysis of retrovirally transduced GFP-FLT3ITD or FLT3WT receptors in BM cells harvested from wild type

(first row panels), *PAK1-/-* (second row panels)*, Rac1-/-* (third row panels), and *FAK-/-* (bottom row panels) deficient mice.