Supplementary Information

Materials and Methods

The Jak2, Jak3, Akt, and phospho-Akt antibodies were purchased from Cell Signaling Technology, Inc. The α-23p3 antibody has been previously described (Devireddy et al, 2001). The 24p3R cDNA has been previously described (Devireddy *et al*, 2005).

References

Devireddy LR, Teodoro JG, Richard FA, Green MR (2001) Induction of apoptosis by a secreted lipocalin that is transcriptionally regulated by IL-3 deprivation. *Science* **293:** 829-834

Devireddy LR, Gazin C, Zhu X, Green MR (2005) A cell-surface receptor for lipocalin 24p3 selectively mediates apoptosis and iron uptake. *Cell* **123**: 1293-1305

Supplementary Figure Legends

Figure S1 32D/BCR-ABL cells contain very high levels of activated Jak1. 32D and 32D/BCR-ABL cells, treated in the presence or absence of IL-3, were monitored for expression of phosphorylated (P) or total (T) Jak1. The results show that activated Jak1 levels are very high in 32D/BCR-ABL cells.

Figure S2 32D/BCR-ABL cells contain activated Jak2 and Jak3. 32D/BCR-ABL cells, treated in the presence or absence of imatinib, were monitored for expression of phosphorylated Jak2 and Jak3 by immunoblot analysis. Actin was monitored as a loading control.

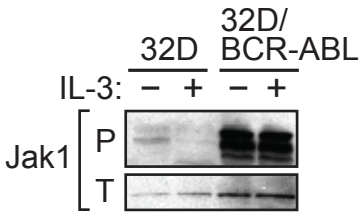
Figure S3 Imatinib does not alter Runx protein levels in 32D/BCR-ABL(T315I) cells. Immunoblot analysis monitoring levels of Runx1, Runx3, phosphorylated (P) Erk and total (T) Erk in 32D/BCR-ABL(T315I) cells treated in the presence or absence of imatinib. Actin was monitored as a loading control.

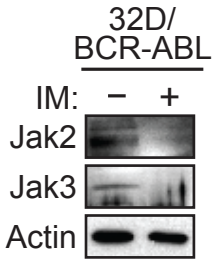
Figure S4 Over-expression of *24p3R* increases sensitivity of 32D/BCR-ABL 24p3R knockdown cells to imatinib. 32D/BCR-ABL cells, stably transfected with either a non-silencing (NS) or 24p3R shRNA, were transiently transfected with a plasmid expressing 24p3R cDNA or, as a control, empty vector. Twenty-four hours later, cells were treated with 5 μM imatinib for 24 hours, and apoptosis was assessed by annexin V staining. The difference between the groups was statistically analyzed using the Student's t-test; the p-value for groups 1 and 2 was 0.0004, and for groups 2 and 3 was 0.04.

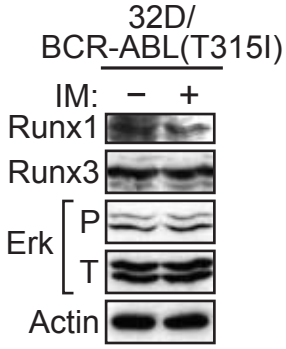
Figure S5 An anti-24p3 antibody partially protects 32D-BCR/ABL cells from imatinib-induced apoptosis. 32D/BCR-ABL cells were treated in the presence or absence of an anti-24p3 antibody, with or without 5 μM imatinib, and 24 hours later cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, following addition of MTT, cell density was monitored by measuring the absorbance at 490 nm (OD_{490}). The results, expressed as percent cell viability, were normalized to the value obtained in the absence of imatinib and anti-24p3 antibody, which was arbitrarily set to 100%. The difference between the groups was statistically analyzed using the Student's t-test; the p-value for group 1 was 0.07, and for group 2 was 0.02.

Figure S6 Titration experiments. (**A**) 32D/BCR-ABL cells were treated with various concentrations of Jak inhibitor I (Jak-I), as indicated. The results show that 10 μM Jak-I is the minimal concentration required to obtain greater than 75% inhibition of Stat5 phosphorylation. (**B**) 32D/BCR-ABL cells were treated with various concentrations of LY294002, as indicated. The results show that 25 μM LY294002 is the minimal concentration required to obtain greater than 75% inhibition of Akt phosphorylation. (**C**) 32D/BCR-ABL cells were treated with various concentrations of U0126 as indicated. The results show that 10μM U0126 is the minimal concentration required to obtain greater than 75% inhibition of Erk phosphorylation.

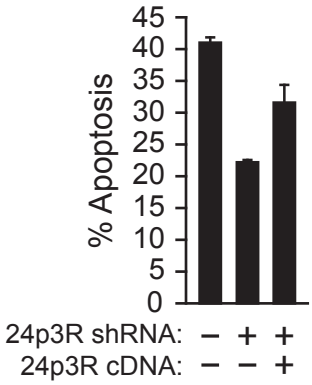
Supplementary Figure S1



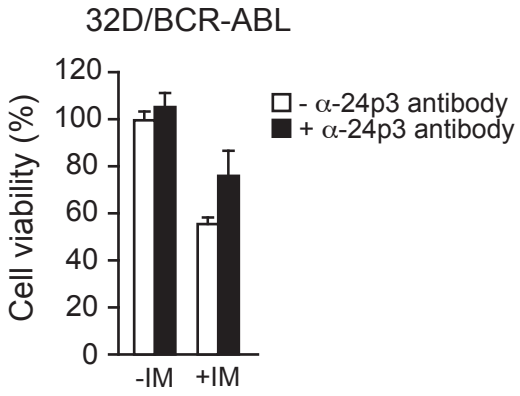

32D/
BCR-ABL
IM: − +
Jak2
Jak3
Actin

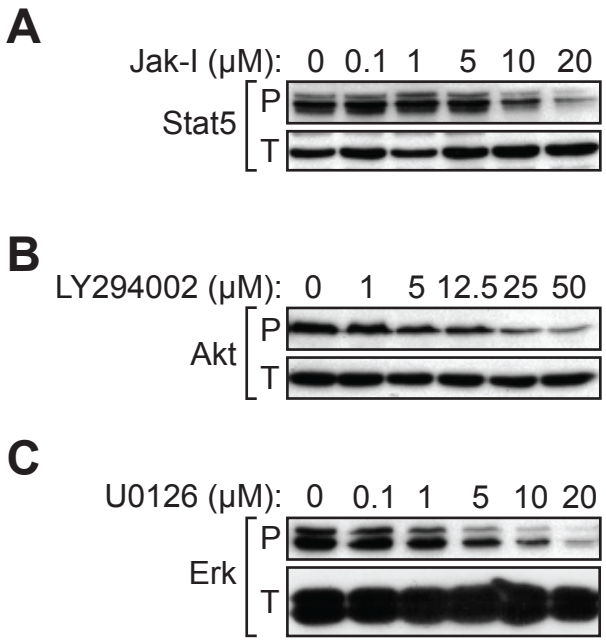

32D/
BCR-ABL(T315I)
IM: − +
Runx1
Runx3
Erk
P
T
Actin

Supplementary Figure S4



Supplementary Figure S5




A
Jak-I (μM): 0 0.1 1 5 10 20
Stat5
P
T
B
LY294002 (μM): 0 1 5 12.5 25 50
Akt
P
T
C
U0126 (μM): 0 0.1 1 5 10 20
Erk
P
T