Supplementary Figure legends

Supplementary Figure S1. Pim-3 has little effect on eIF4B phosphorylation at Ser422. 293T cells were co-transfected with empty vector pcDNA3.1(-), Pim-3 wild-type or Pim-3 kinase dead (Pim-3-KD) mutant and eIF4B wild-type or eIF4BS422A mutant. Cell lysates were examined for eIF4B, eIF4B phosphorylation on Ser422, and Pim-3 by Western blotting as indicated.

Supplementary Figure S2. Expression and phosphorylation of eIF4B exhibit an Abl kinases-dependent manner in v-Abl or Bcr-Abl transformants. A, expression of eIF4B were examined in human peripheral blood mononuclear cells (Normal), SMMC-7721, mouse bone marrow cells (BMC), Jurkat, K562 and NS2 by immunoblotting. B and C, experiments were performed as described respectively in Figure 3B and C to examine the effects of imatinib treatment on eIF4B expression and phosphorylation in K562 cells. Error bars represent SEM, n=3. D and E, v-Abl transformed cells or K562 cells were treated in a time course with imatinib. Expression of eIF4B was analyzed by RT-PCR.

Supplementary Figure S3. Pim-1 and Pim-2 kinases regulate eIF4B phosphorylation in Abl transformants. A, v-Abl-transformed cells were treated in a time course with SMI-4a, an inhibitor of Pim-1 and Pim-2 kinases. Cell lysates were analyzed by immunoblotting to examine the eIF4B expression and phosphorylation as
described in Figure 4A. Levels of eIF4B expression and its phosphorylation on Ser406 and Ser422 were quantitated by densitometry and normalized to actin protein levels. The levels of eIF4B expression and phosphorylation are 100% at 0 hour. Plotted are results from three independent experiments. Error bars represent SEM, n=3. B, experiments using K562 cells were performed as described in (A). Plotted are results from three independent experiments. Error bars represent SEM, n=3. C, interference efficiency of Pim-1 in K562 cells was examined by RT-PCR. D and E, interference efficiency of S6K in v-Abl transformed cells (D) or K562 cells (E) were examined by RT-PCR. F and G, v-Abl transformed cells (F) or K562 cells (G) were treated with different concentration of rapamycin for 24 hours. Cell lysates were analyzed by immunoblotting with indicated antibodies. H, shown is bicistronic retroviral vectors constructed in this study that encode GFP alone or GFP and either Pim-1, Pim-2 or their mutants. I, K562 cells ectopically expressing either bicistronic retroviral empty vector (EV), wild-type or kinase dead mutant of Pim-1 were treated with 10 μM imatinib for indicated times. Cell lysates were probed with indicated antibodies to examine the effects of Pim-1 expression on eIF4B phosphorylation on Ser422 after imatinib treatment.

Supplementary Figure S4. Phosphorylation of eIF4B on Ser422 is regulated by Pim kinase downstream of Jak2 and Stat5 signaling. A, the interference efficiency of eIF4B-specific shRNAs in Bcr-Abl was examined by RT-PCR. B and C, survival of v-Abl-transformed cells (B) and K562 cells (C) expressing luciferase shRNA or
eIF4B-specific shRNAs was analyzed by flow cytometry after propidium iodide staining. D, lentiviral vectors encoding eIF4B or its phosphomimetic mutants were constructed in this study. Shown is an immunoblot of K562 cells ectopically expressing either empty vector, eIF4B or its phosphomimetic mutants probed with indicated antibodies. E and F, K562 cells expressing luciferase-specific shRNA, Jak2-specific shRNAs (E) or Stat-5-specific shRNAs (F) were analyzed by immunoblotting with indicated antibodies.

Supplementary Figure S5. Generation of eIF4B-knockdown transgenic mice and retroviral or lentiviral vectors used in Abl-mediated bone marrow transformation, and model of eIF4B phosphorylation and function in Abl transformants. A, the sequences used in the shRNA targeting mouse eIF4B (5’-GCCAAAGAAACCTGAGGAGAA-3’) were cloned into an expression plasmid after the H1 promoter. The eIF4B-knockdown transgenic mice were generated by the microinjection method and genotyped by PCR. Western blot analysis using anti-eIF4B antibody was performed to determine the interference efficiency. The transgenic founders with high interference efficiency were selected and maintained on a C57BL/6J genetic background. Shown is a representative photograph of eIF4B-knockdown transgenic mouse and wild-type littermate. B, shown is representative genotyping of eIF4B-knockdown transgenic mice by specific primers (5’-AAATCCTGGTTGCTGTCTCTTTATG-3’ and 5’-GGAAGGTCCGCTGGATTGA-3’) to determine the integration of eIF4B-specific
shRNA. A 350 bp fragment of the shRNA cassette was amplified, which represented integration of the transgenic DNA. Numbers 1-7 represent seven 14-days old mice. P, positive control; N, negative control. C and D, bicistronic retroviral vectors expressing v-Abl or Bcr-Abl and either GFP, eIF4B-WT or eIF4BS422A were constructed (C) and examined by immunoblotting with indicated antibodies (D). E, bicistronic retroviral vectors encoding v-Abl or lentiviral vectors encoding Bcr-Abl and either GFP, Pim-1-WT or Pim-1-KD mutant were constructed in this study. F, expression of bicistronic retroviral vectors encoding v-Abl or lentiviral vectors encoding Bcr-Abl and either GFP, Pim-1-WT or Pim-1-KD were examined by immunoblotting with indicated antibodies. G, our data suggest a model through which phosphorylation of eIF4B could be connected to cellular transformation by Abl oncogenes. Abl signaling could activate JAK/STAT/Pim pathway and PI3K/Akt/mTOR/S6K pathway. Then Pim kinases Pim-1 and Pim-2 both directly phosphorylate eIF4B mainly on Ser422 and to a lesser extent, S6K phosphorylates eIF4B on Ser422. Phosphorylation of eIF4B on Ser422 plays a critical role in Abl-mediated cellular transformation.