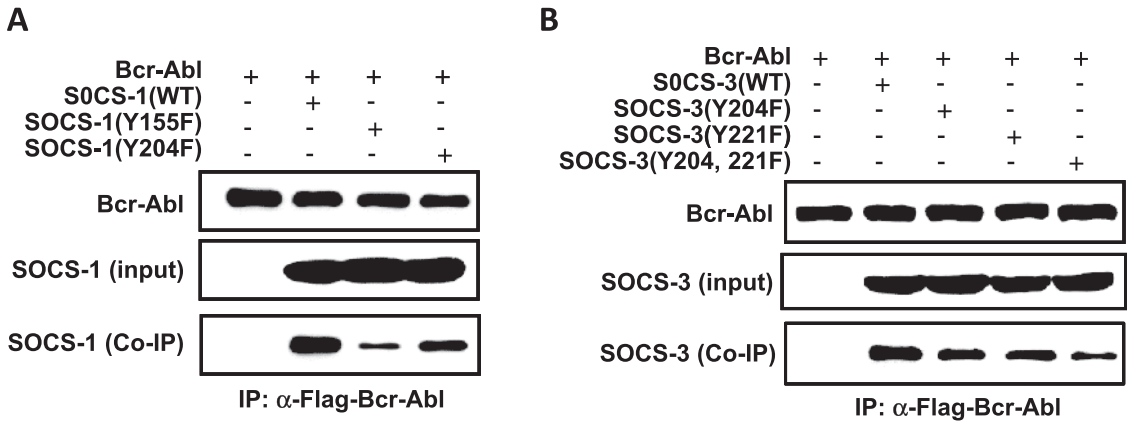

Figure W1. The interaction between Bcr-Abl and SOCS proteins. 293T cells were cotransfected with Flag-tagged Bcr-Abl and either wild-type or mutant SOCS-1 (A), wild-type or mutant SOCS-3 (B). Bcr-Abl protein was then immunoprecipitated with anti-Flag antibody (Sigma). Precipitated proteins were examined for SOCS proteins by Western blot analysis. Shown are immunoblots probed with indicated antibodies.

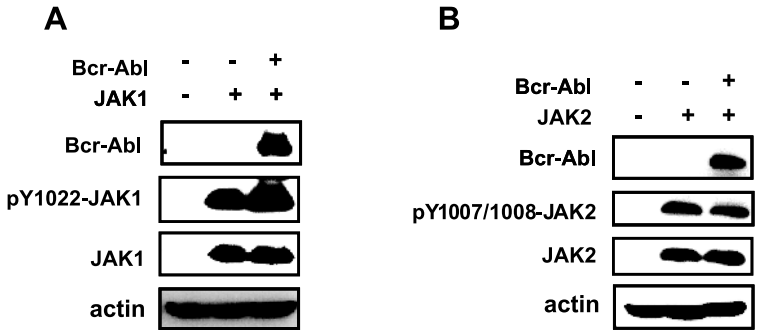

Figure W2. When overexpressed in 293T cells, JAK1 and JAK2 become activated independently of Bcr-Abl oncoprotein. Bcr-Abl was coexpressed with either JAK1 (A) or JAK2 (B) in 293T cells. Cell lysates were examined for JAK1 and pJAK1 (A) and JAK2 and pJAK2 (B) as indicated by Western blot analysis.

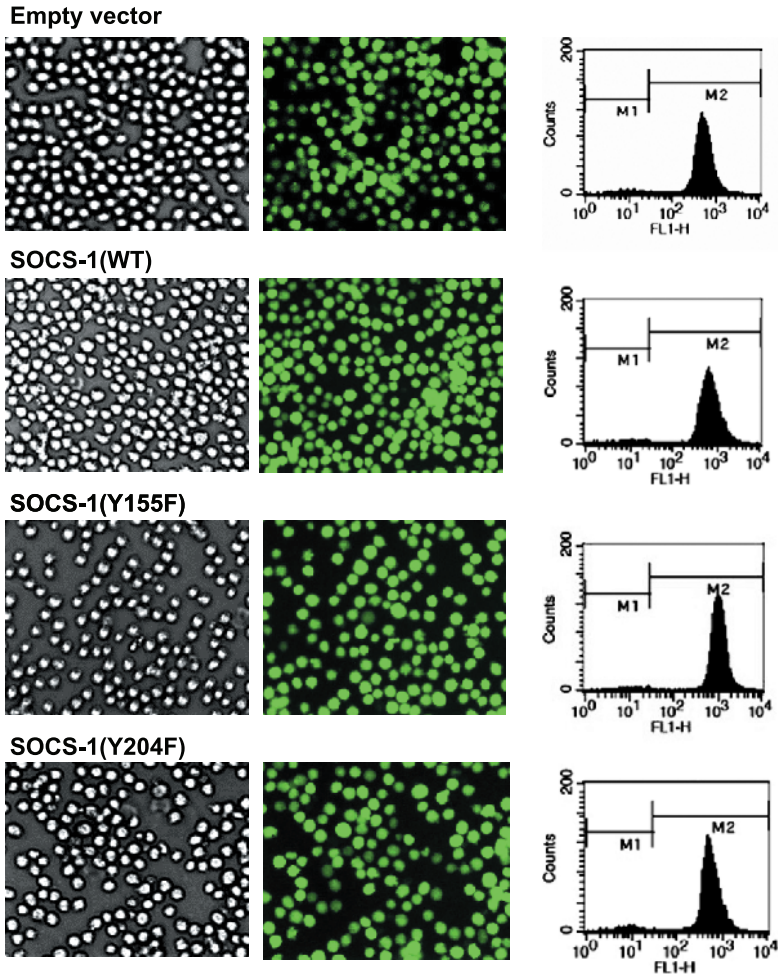

Figure W3. Generation of K562 cell lines stably expressing wild-type or mutant SOCS-1. Bicistronic retroviruses encoding GFP alone or GFP and SOCS-1(WT) or its mutants were produced in 293T cells. Cell culture supernatants containing retroviruses were collected and filtered through a 0.22-μm MCE membrane (Millipore). K562 cells were infected with these retroviruses by spin infection at 2000 rpm at 32°C for 120 minutes. After additional culture for 48 hours, GFP-positive K562 cells were sorted by flow cytometry and cultured in RPMI 1640 containing 10% fetal bovine serum. Shown are micrographs obtained from fluorescent microscope (Axiovert 200M; Zeiss, Oberkochen, Germany).

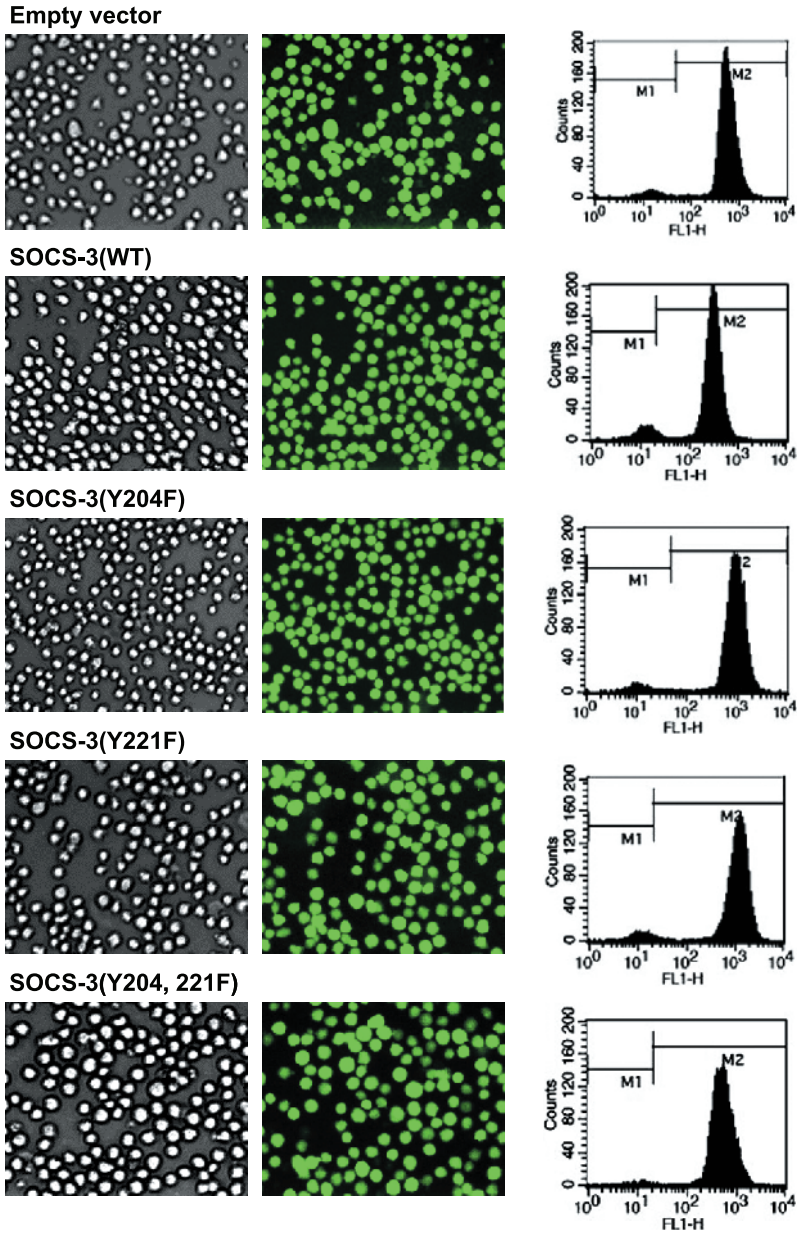

Figure W4. K562 cell lines stably expressing wild-type or mutant SOCS-3 were generated in this study. Experiments were performed as described in Figure W2. Bicistronic retroviruses encoding GFP alone or GFP and SOCS-3(WT) or its mutants were generated in 293T cells. K562 cells were then infected with these retroviruses and GFP-positive K562 cells stably expressing GFP alone, GFP and SOCS-3(WT), or its mutants were sorted by flow cytometry. Shown are micrographs obtained from fluorescent microscope (Axiovert 200M; Zeiss).

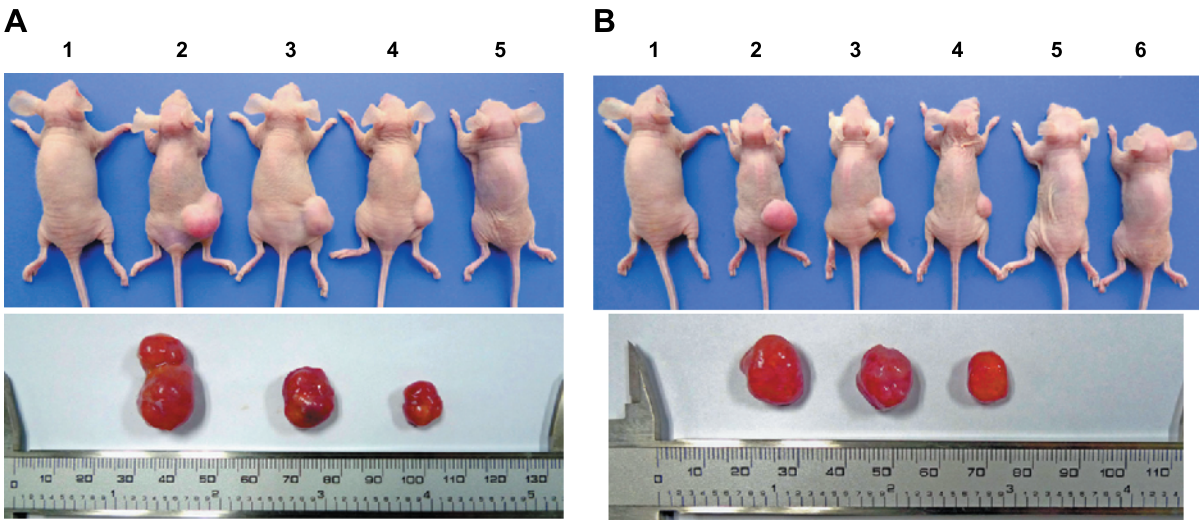

Figure W5. Disrupting Bcr-Abl–mediated tyrosine phosphorylation of SOCS-1 or SOCS-3 blocks tumor formation caused by K562 cells in nude mouse model. Nude mice inoculated with K562 cells expressing GFP alone, wild-type, or mutant SOCS-1 (A) and wild-type or mutant SOCS-3 (B) were killed 1 day after the last measurement of tumor growth and tumors were excised from nude mice. Shown are representative images from at least three independent experiments with similar results.

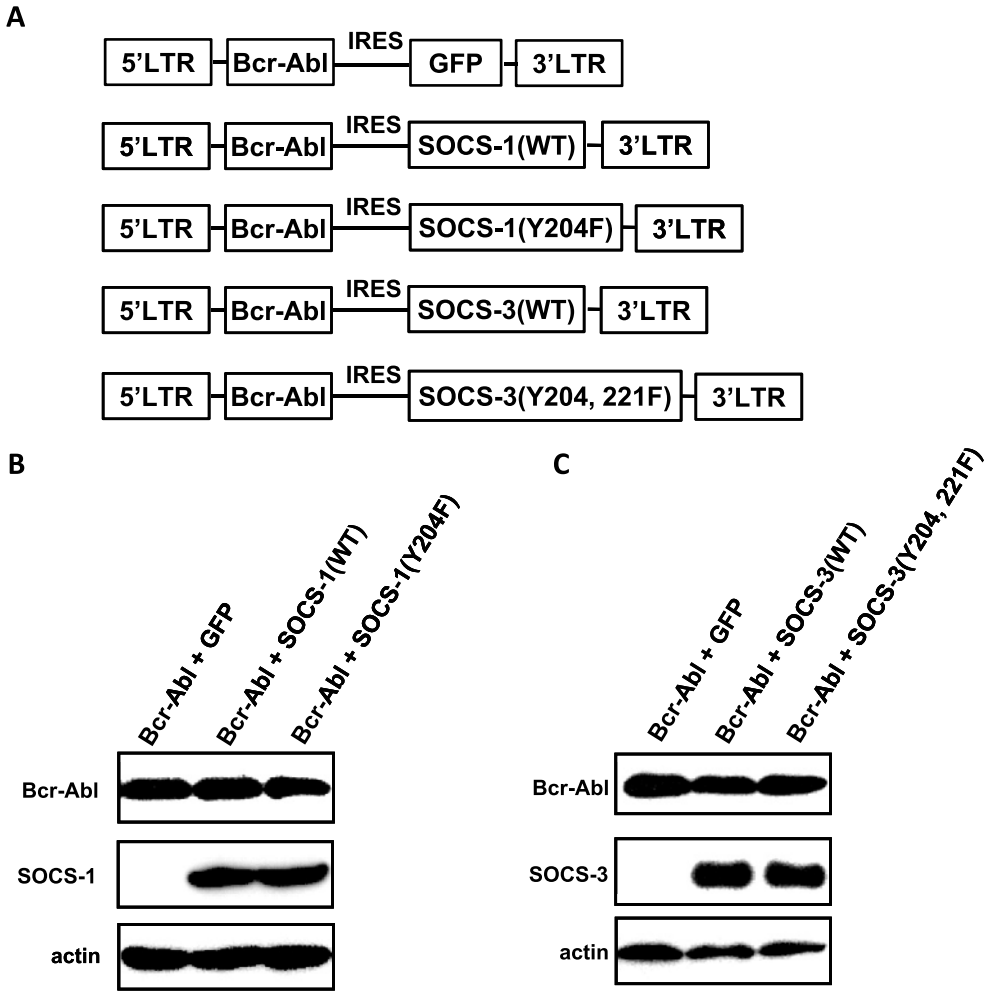

Figure W6. Shown are bicistronic retroviral vectors used in this study. (A) Bicistronic retroviral vectors encoding Bcr-Abl and GFP, SOCS-1 (WT), SOCS-1(Y204F), SOCS-3(WT), or SOCS-3(Y204, 221F) were generated. (B and C) The expression of these bicistronic retroviral vectors encoding Bcr-Abl and GFP, wild-type, or mutant SOCS-1 (B) and wild-type or mutant SOCS-3 (C) were examined in 293T cells. Shown are representative Western blots probed with indicated antibodies.

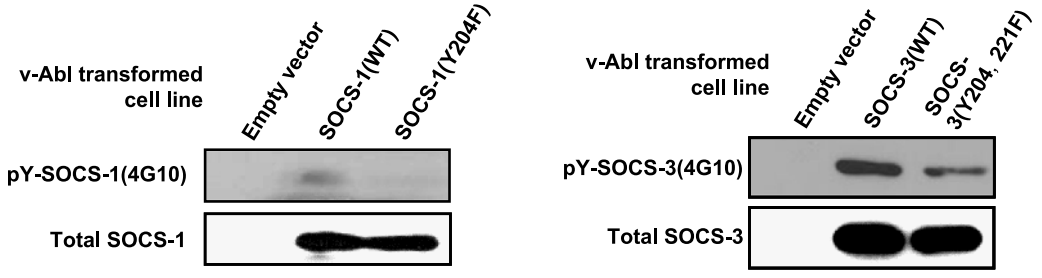

Figure W7. Tyrosine phosphorylation of SOCS-1 and SOCS-3 in a v-Abl transformed cell line ectopically expressing these SOCS proteins. v-Abl transformed cells ectopically expressing empty vector, SOCS-1 or its mutant, and SOCS-3 or its mutant were lysed and examined for SOCS proteins and their tyrosine phosphorylated forms with indicated antibodies. Shown are representative immunoblots.