Supplementary Methods

Cells

Human primary normal hematopoietic CD34+ cells, normal stromal cells and CML-BC CD34+ cells were obtained from consenting donors, through Stem Cell Core facility, using guidelines approved by the Institutional Review Board of the University of Pennsylvania. Primary cells were cultured in Isocov Modified Dulbecco media (IMDM) supplemented with 20% fetal bovine serum (catalog #SH30071.03, HyClone), 1% Penicillin/Streptomycin (#15140122, Invitrogen), 1% GlutaMax (#35050061, Invitrogen). Mo7e cells (# ACC 104, DSMZ) were cultured in IMDM with 10% FBS, 1% Pen/Strep, 1% GlutaMax, and GM-CSF (Leukine, 50ng/mL). K562 cells were cultured in IMDM with 10% FBS, 1% Pen/Strep, 2% GlutaMax and 2% $NaHCO_3$ (#25080-094, Invitrogen).

siRNA

We used 4 short interfering RNAs targeting human Lyn and 4 nonspecific siRNA, as a negative control (Dharmacon, Lafayette, CO). Methodology as well as Lyn and control siRNA sequences are described in detail in the online Supplemental Methods of Ptasznik et al[1].

Antibodies

The antibodies used for flow cytometry and their suppliers were as follows: anti-human integrin β2 (CD18) (catalog #MAB1730, R&D), purified mouse IgG1 (#349040, BD Pharmingen), monoclonal antibody (mAb) 24 for the active conformation of human β2 integrins was kindly provided by Dr. Nancy Hogg (London Research Institute), CD184 (#555976, BD Pharmingen), mouse IgG2a (#340757, BD Pharmingen), anti-human β1 integrin active conformations monoclonal antibody (#MAB2079Z, Chemicon), anti-human CD11b (Mac-1) activation epitope (#12-0113, eBioscience), mouse IgG1(#555749, BD Pharmingen), goat F(ab')2 anti-mouse IgG (#M35004-1, Caltag Laboratories). The antibodies used for western blotting and their suppliers were following: anti-human Abl SH3 domain (#06-466) and anti-Lyn (#06-207) from Upstate Biotechnology, anti-b-actin (#4970) and anti-GAPDH (#2118) from Cell Signaling, anti-human CXCR4 (#OPA1-01101) from Affinity BioReagents.

Retroviral production and Mo7e infection protocol

p210 $^{BCR\text{-}ABL}$-eGFP and eGFP retroviral vectors were generous gifts from Dr. Catherine Verfaillie (Stem Cell Institute, Leuven). 293T cells were first transfected with p210 $^{BCR\text{-}ABL}$-eGFP or eGFP alone with viral envelope using FuGENE 6 (#11815091001, Roche). The viral supernatant was collected at 48 h after transfection and Mo7e cells were transduced by spin-infection. Infected cells were sorted based on low and high GFP (>95% purity) and then the result was confirmed by BCR-ABL western blotting normalized to housekeeping gene Actin.

FACS analysis
mAb24 staining was performed exactly as previously described[2]. We used in our experiments both irrelevant mouse IgG1 (#349040, BD Pharmingene) or secondary antibody alone (goat F(ab')2 anti-mouse IgG, Caltag Laboratories, #M35004-1), as controls. For surface and total CXCR4 staining, 400,000 cells were first stained by CXCR4 antibody according to manufacture protocols and then split into 2 tubes: one (surface CXCR4) was then fixed with 1% paraformaldehyde. The other (total CXCR4) was treated with 0.75 ml of fixation/permabilization buffer (eBioscence, cat.#00-5223-56) at room temperature for 10 min in the dark and then washed twice with FACS Buffer (1% FBS in PBS) at 1800 rpm for 6 min. The sample was then stained again with CXCR4 antibody described in manufacture protocols. We used mouse IgG2a, as isotope controls (#340757, BD Pharmingene)

Migration assay
The assay was performed as we previously described[3] with minor changes. 1×10^5 cells in IMDM with 2% FBS were placed in the upper chamber of a Transwell (#3421, Corning), and SDF-1α (#300-28A, PeproTech Inc) was added to the lower compartment at the final concentration of 100 ng/mL. The plate was incubated for 3 h at 37°C. Cells were then collected from the lower compartment and counted by FACS at top speed for 1min. The chemotactic index was determined as follows: (number of cells migrating to SDF-1 chemokine)/(number of cells migrating to medium alone). The migration assay was performed both on bare filters (polycarbonate membrane inserts) and coated with ICAM-1 filters (10µg/ml, 50µl per filter, incubation in 37C for 1h).

Adhesion assay
The assay was performed as previously described with minor changes[3]. Briefly, cells stained with 12.5 µM Calcein AM (#C3099, Molecular Probes) according to manufacture protocol, were added to 96 well plates coated with recombinant human ICAM-1 (#ADP-4, R&D) or human plasma fibronectin (#33016-015, GIBCO) plates (10 μg/mL, 50 μL/well) or human bone marrow stromal cells derived from healthy individuals. Cells were cultured with or without SDF-1 α (#300-28A, PeproTech Inc) (100 ng/mL) for 30 min at 37°C. After incubation, measure the "before wash" fluorescence of the samples using a fluorescence plate reader at excitation wavelength 485 nm and emission wavelength 520 nm. Wash away non-adherent cells for 3 times and then measure the "after wash" fluorescence. Calculate the average percent adhesion using the following formula: $[(RFU_{\text{after wash}}) / (RFU_{\text{before wash}})] \times 100$.

Data analysis
We used Student's t-test. Results are presented as mean ± s.e.m. A probability value of <0.05 was considered to be statistically significant.

References

1. Ptasznik A, Nakata Y, Kalota A, Emerson SG, Gewirtz AM. Short interfering RNA (siRNA) targeting the Lyn kinase induces apoptosis in primary, and drug resistant, BCR-ABL(+) leukemia cells. Nature Med. 2004; 10(11):1187-1189.

2. Bouaouina M, Blouin E, Halbwachs-Mecrelli L, Lesavre P, Rieu P. TNF-induced beta2 integrin activation involves Src kinases and a redox-regulated activation of p38 MAPK. J. Immunol., 2004; 15;173:1313-1320.

3. Nakata Y, Tomkowicz B, Gewirtz AM, Ptasznik A. Integrin inhibition through Lyn-dependent cross talk from CXCR4 chemokine receptors in normal human CD34+ marrow cells. Blood. 2006; 107(11): 4234-4239.