Supplementary Information

Supplementary Figure 1: GSK3β expression in HSPCs. (A) Representative histograms of GSK3β expression in Lin^+ cells, Lin^-cKit^+ (LK) cells, $Lin^-Sca\text{-}1^+cKit^+$ (LSK) cells and $CD34^-Lin^-Sca\text{-}1^+cKit^+$ ($CD34^-$LSK) cells from the BM. Background represents staining with secondary antibody alone. (B) Mean Fluorescence Intensity (MFI) levels of GSK3β expression in LK cells, LSK cells and $CD34^-$LSK cells at ZT5 (5 hrs after initiation of light) as compared to ZT13 (1 hr after initiation of darkness); n=3-5. * $p<0.05$, ** $p<0.01$ between ZT5 and ZT13 time points.

Supplementary Figure 2: Phospho-GSK3β expression in HSPCs. Ser9-phosphorylated GSK3β (pS9-GSK3β) or Tyr216-phosphorylated GSK3β (pY216-GSK3β) expression levels (fold change or in histogram) in LK cells in the BM. (A) pS9-GSK3β and pY216-GSK3β expression at ZT5 as compared to ZT13; n=3-7. (B) pS9-GSK3β and pY216-GSK3β expression following administration of 10 mg/kg NE or PBS for 20 min; n=3. (C) pS9-GSK3β and pY216-GSK3β expression following stimulation or not with 200ng/ml CXCL12 for 30sec or 5min – representative histograms are shown; n=5. Background represents staining with secondary antibody alone. (D) pS9-GSK3β expression following administration of PBS or IGF-1 for 7 consecutive days, 5 µg/mouse/day; n=4. (E) pS9-GSK3β and pY216-GSK3β expression following exposure in vitro to 100 ng/ml SCF for 4 hr; n=5. * $p<0.05$, ** $p<0.01$ in comparison to control.

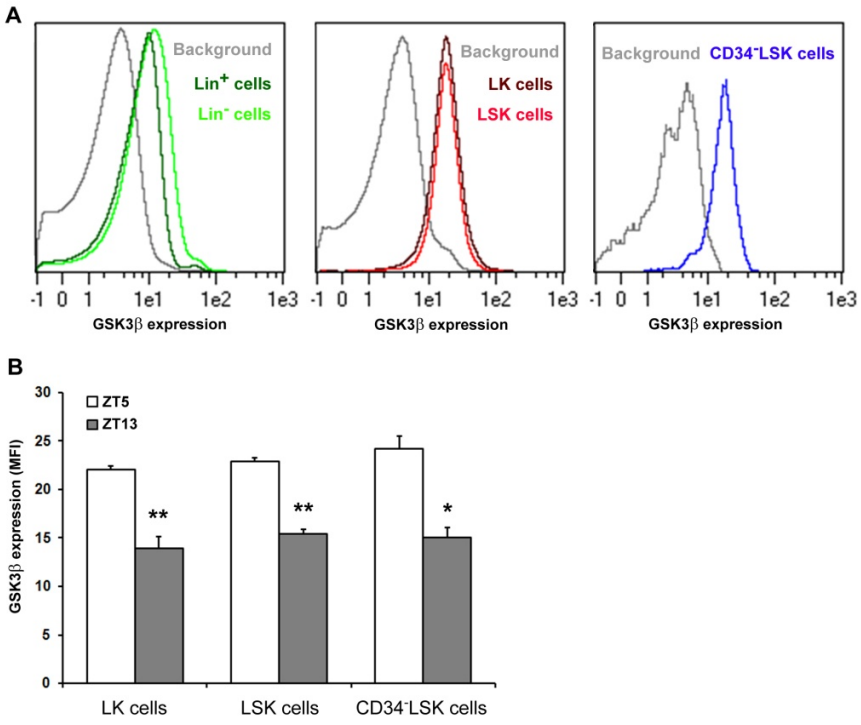
Supplementary Figure 3: GSK3β inhibition reduces HSPC egress. (A) Mice were injected once with 0.6 mg/kg BIO-A or equivalent DMSO, or injected twice with 0.6 mg/kg BIO-A or equivalent DMSO, 60 min and 30 min before administration of 10 mg/kg norepinephrine (NE). One hr post the last injection, mice were sacrificed and PB was obtained to measure circulating $Lin^-Sca\text{-}1^+cKit^+$ (LSK) cells (as shown in Figure 3A and B). Representative FACS dot plots are shown here ($Sca\text{-}1^+cKit^+$ out of Lin^- cells per million PB MNC). (B) Long-term repopulation assay by PB HSCs. Donor mice were treated with 0.6 mg/kg BIO-A or equivalent DMSO, and after 1 hr, the PB was collected. Congenic recipient mice were lethally irradiated (1000 rad) 24hr prior to transplantation. Each recipient mouse was transplanted with 500 µl of donor PB together with $200x10^3$ host-type competitive BM cells. Donor chimerism in the recipient BM was evaluated 4 months following transplantation (as shown in Figure 2H). Representative FACS dot plots are shown here, where CD45.1 marks the host leukocytes and CD45.2 marks the donor leukocytes. (C) Mice were injected with 10 mg/kg PQ401 (IGF-1R antagonist), together with 0.6 mg/kg BIO-A or equivalent DMSO. After 1hr, mice were sacrificed and PB was obtained to

measure circulating LSK cells (as shown in Figure 5C). Representative FACS dot plots are shown here (Sca-1^{+}cKit^{+} out of Lin^{-} cells per million PB MNC).

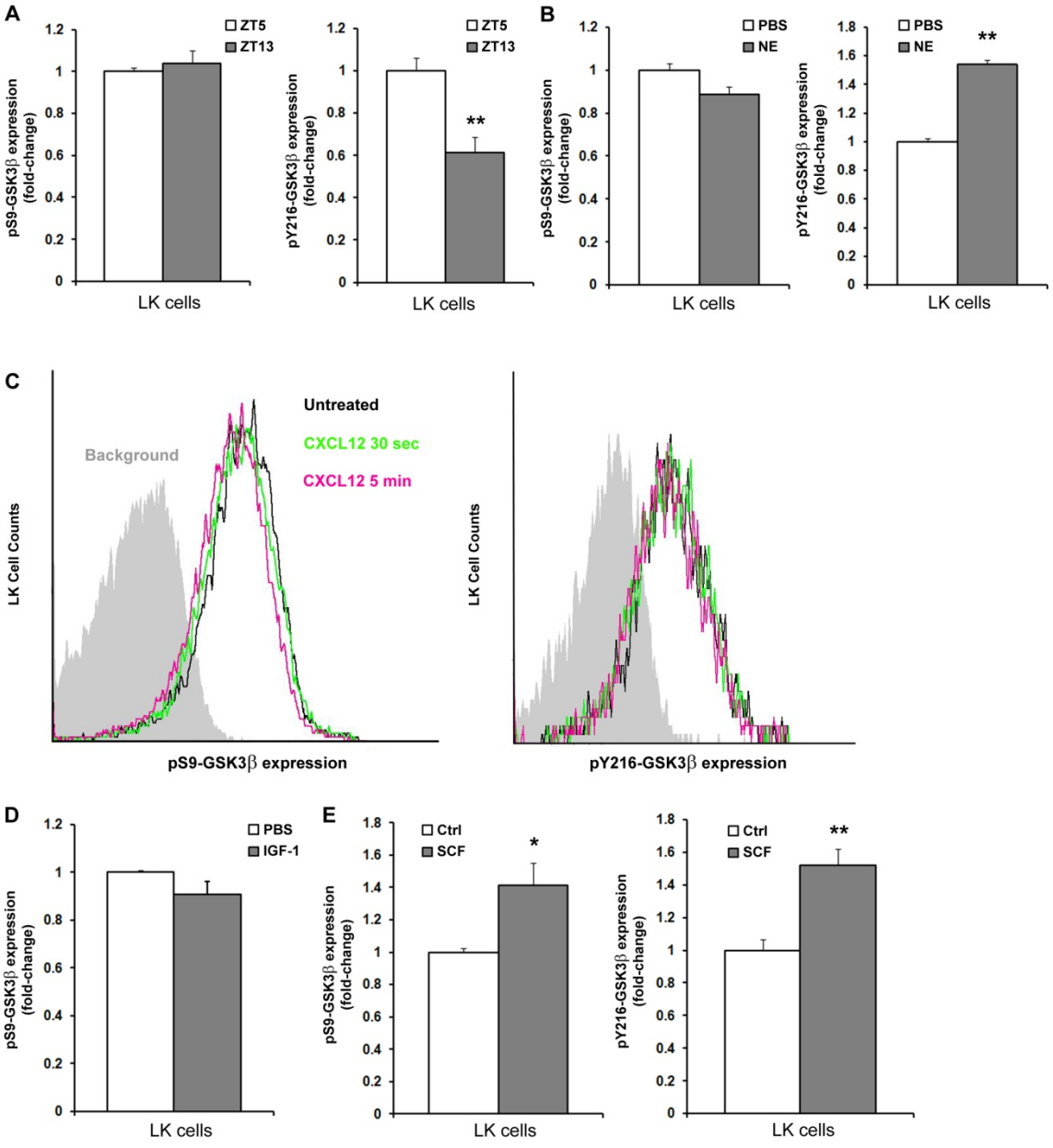
Supplementary Figure 4: GSK3β inhibition does not affect HSPC frequencies in the BM. Mice were treated with 0.6 mg/kg BIO-A, 10 mg/kg PQ401 (IGF-1R antagonist), combination of both or equivalent DMSO. After 1hr, they were sacrificed and BM was obtained to measure colony-forming unit cells (CFU-C) (A) and LSK cells (B); n=6-8. NS – non-significant.

Supplementary Figure 5: Actin polarization in $CD34^{+}$LSK cells. Actin polarization was assessed in fixed/permeabilized Lin^{-} cells using Phalloidin-FITC as described in Figure 7. Representative flow-cytometry-based single cells images of $CD34^{+}$LSK cells as obtained by ImageStream. An asterisk points at a cell protrusion in response to CXCL12 stimuli. Original magnification x600.

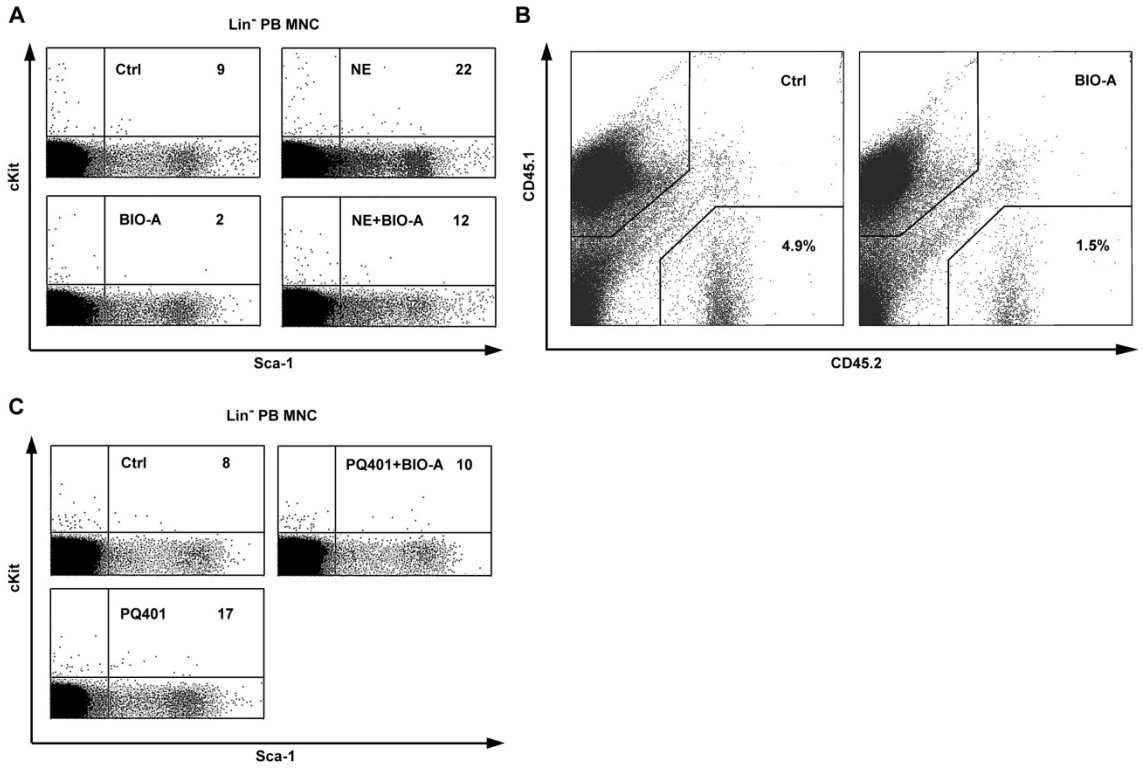
Supplementary Figure 1



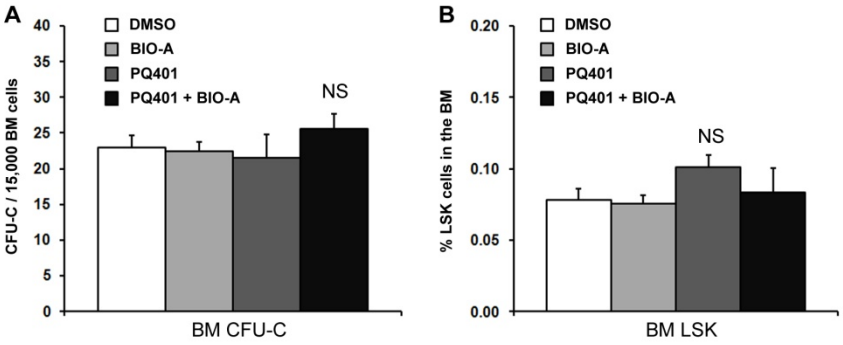
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5