Supplemental Figures

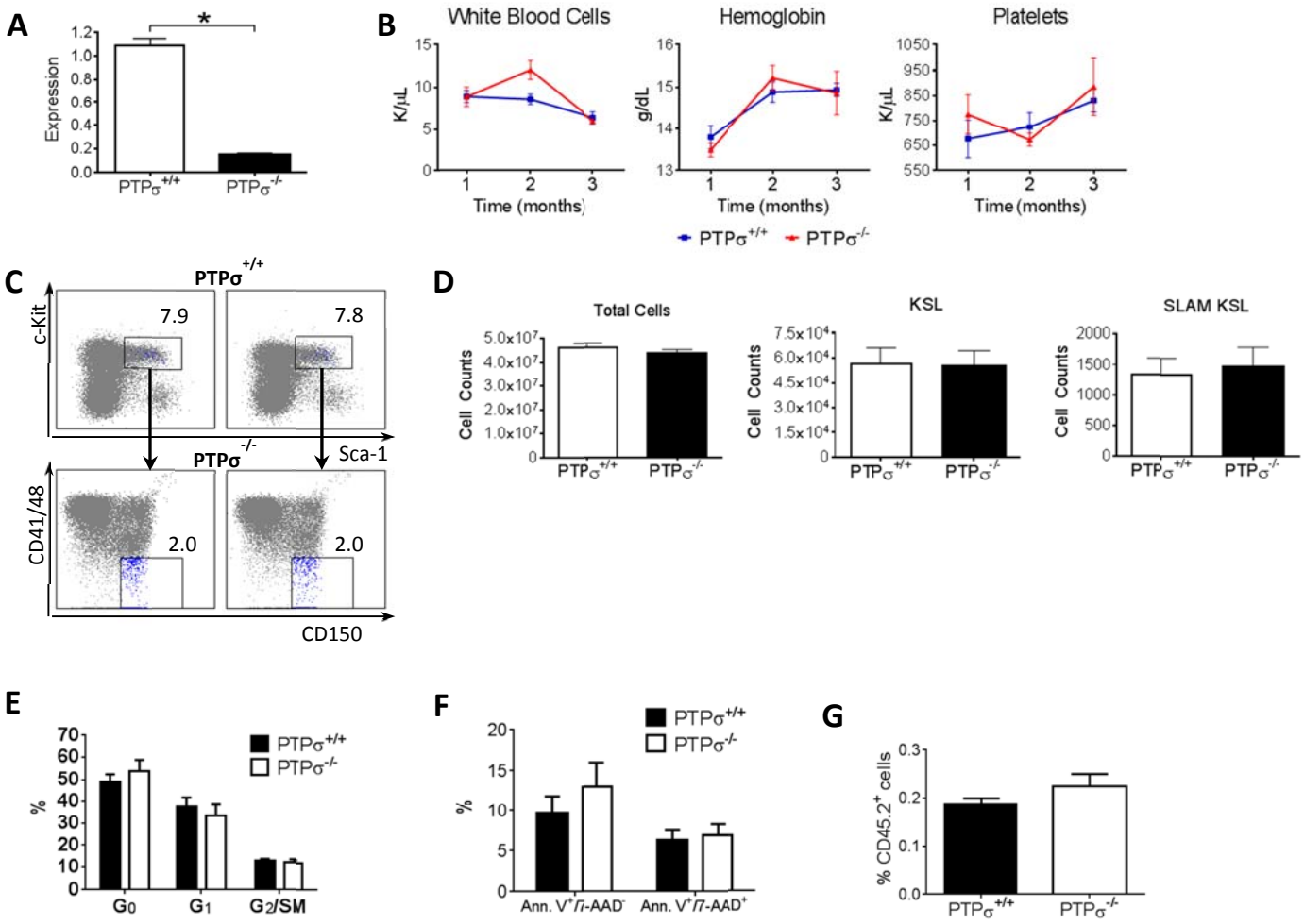

Figure S1. Baseline hematologic profile of adult PTPσ$^{-/-}$ mice versus PTPσ$^{+/+}$ mice. (**A**) Expression of PTPσ in BM KSL cells in PTPσ$^{+/+}$ and PTPσ$^{-/-}$ mice by qRT-PCR (n=6/group, *P<0.0001). (**B**) Peripheral blood complete blood counts in 8-12 week old PTPσ$^{+/+}$ and PTPσ$^{-/-}$ mice (n = 6-14 mice/group). (**C**) Representative flow cytometric analysis of BM KSL cells and SLAM^{+} KSL cells in PTPσ$^{+/+}$ and PTPσ$^{-/-}$ mice. Numbers represent the percentages of KSL cells and CD150^{+}CD41/48^{-}KSL cells in each group. (**D**) Mean numbers of total BM cells, KSL cells,

SLAM^{+}KSL cells in PTPσ$^{+/+}$ and PTPσ$^{-/-}$ mice are shown (± SEM, n=6) (**E**) Cell cycle analysis of BM KSL cells from PTPσ$^{+/+}$ and PTPσ$^{-/-}$ mice is shown. Mean values +/- SEM (n = 5/group). (**F**) Mean levels of apoptotic cells (Annexin V^{+}/7AAD^{-}) and necrotic cells (Annexin V^{+}/7AAD^{+}) are shown (n=7). (**G**) Mean percentage donor CD45.2^{+} cell engraftment is shown at 18 hours post-injection of 9 x 10^{4} BM Sca-1^{+}lin^{-} cells from PTPσ$^{+/+}$ and PTPσ$^{-/-}$ mice into CD45.1^{+} recipients (n = 4/group).

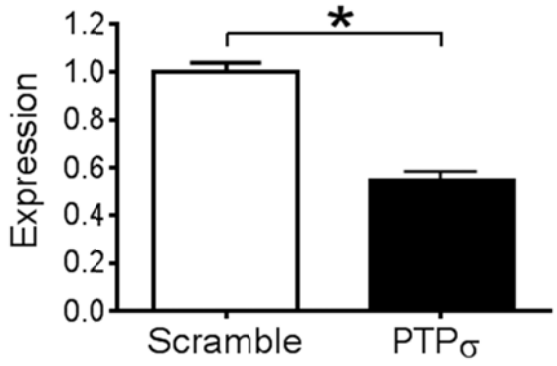

Figure S2. Expression of PTPσ in BM KSL cells at 48 hours after treatment with PTPσ-shRNA (PTPσ) or scramble shRNA (n=6/group), *P<0.0001.

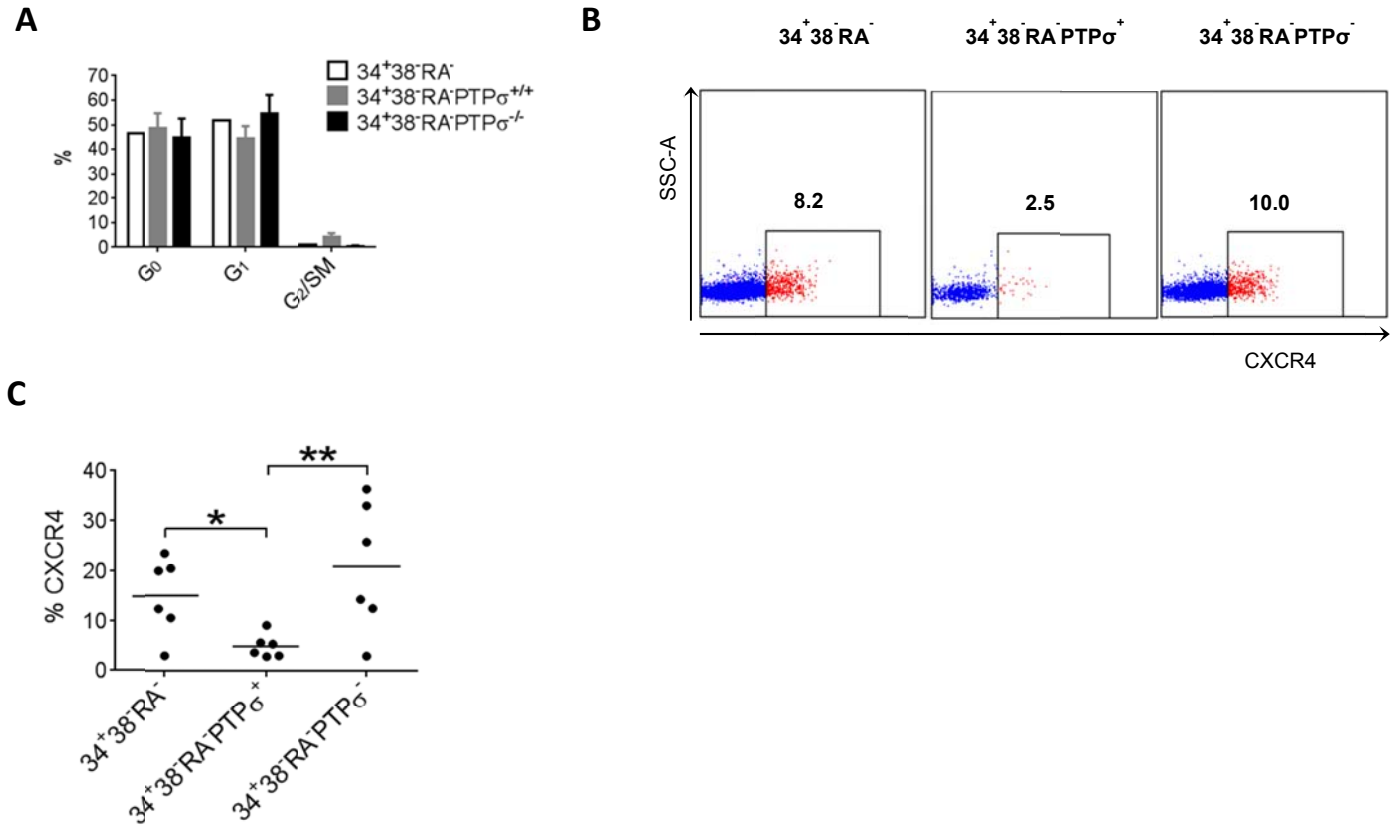

Figure S3. Cell cycle status and CXCR4 expression on human CB cells based on PTPσ expression. (**A**) Mean percentages of cells in G_0, G_1 and G_2SM phase of cell cycle within the cell populations shown (n=5). (**B**) Representative flow cytometric analysis of surface CXCR4 expression on the human CB cell populations shown. Numbers represent the percentages of $CXCR4^+$ cells in each group. (**C**) The scatter plot shows %$CXCR4^+$ cells within CB $CD34^+CD38^-CD45RA^-lin^-$ cells ($34^+38^-RA^-$), $CD34^+CD38^-CD45RA^-lin^-PTP\sigma^+$ cells ($34^+38^-RA^-PTP\sigma^+$) or $CD34^+CD38^-CD45RA^-lin^-PTP\sigma^-$ cells ($34^+38^-RA^-PTP\sigma^-$). Horizontal bars represent mean values for each group. **P*=0.01, ***P*=0.01 (n=6/group, t test)

Supplemental Methods

PTPσ Gene Expression Analysis in Murine Hematopoietic Cell Subsets

BM cells were sorted by FACS as previously described (13). Briefly, femurs were collected from 12-14 week female C57BL/6 ($CD45.2^+$) mice into IMDM with 10% FBS and 1% penicillin-streptomycin. Red blood cell lysis was performed using ACK Lysis buffer (Sigma Aldrich). Viable BM cells were quantified using Trypan blue, followed application of a lineage depletion column (Miltenyi Biotec, Auburn, CA). BM cells and lineage-depleted cells were isolated during the purification process. Cells were incubated with antibodies to c-Kit, Sca-1 and a lineage antibody cocktail (BD Biosciences, San Jose, CA) and sorted for the hematopoietic stem/progenitor cell subsets. cDNA was generated using RNeasy kit (Qiagen, Valencia, CA). Real-time PCR was performed using Taqman probes for glyceraldehyde-3-phosphate (GAPDH) and the various RPTPs (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol.

Hematopoietic progenitor cell assays

BM cells were collected as described above. Cells were then incubated with antibodies to c-Kit, Sca-1, a Lineage antibody, CD41, CD48 and CD150 (BD) to measure KSL and $SLAM^+KSL$ cells by flow cytometry. For CFCs, whole BM or Lineage-depleted cells were plated into methylcellulose (StemCell Technologies, Vancouver, BC, Canada), and colonies were scored on day 14. Complete blood counts were performed on a HemaVet 950 (Drew Scientific, Dallas, TX). Homing assays were performed with $Sca\text{-}1^+Lin^-$ BM cells as previously described (31). Briefly, $PTP\sigma^{+/+}$ or $PTP\sigma^{-/-}$ mice (CD45.2) were sorted by FACS for $Sca\text{-}1^+Lin^-$ BM cells ($9x10^4$ cells per recipient mouse) and transplanted via tail-vein injection into lethally irradiated $CD45.1^+$ recipients. Donor cell homing to the BM was assessed at 18 hours post-transplant by flow cytometry.

Cell Cycle, Apoptosis and CXCR4 Expression Analysis

Cell cycle analysis and measurement of apoptosis were performed using flow cytometry, as previously described (5). Briefly, BM cells were collected and stained for surface expression of ckit, sca-1 and lineage markers. Cells were then fixed with 2.5% paraformaldehyde and 2% FBS in 1x PBS and permeabilized with 0.25% saponin, 2% FBS in PBS and labeled with antibodies to Ki67-FITC (1% dilution, catalog number 556026, BD Biosciences) and 7-AAD (BD Biosciences). Analysis for apoptosis and necrosis was performed using the BD Annexin V Apoptosis Detection Kit Protocol (No. 556547, BD). For analysis of CXCR4 expression, murine BM cells were labeled with antibodies to ckit, sca-1 and lineage markers and CCXR4 (BD) and analyzed by flow cytometry. Human CB cells were lineage depleted and labeled with antibodies to CD34, CD38, CD45RA, PTPσ and CXCR4 (BD). Cells were then analyzed by flow cytometry.

shRNA Experiments and Rac1-GTP Analysis

BM KSL cells were cultured overnight in X-VIVO 15 serum-free media (Lonza, Basel Switzerland) with thrombopoietin (R & D), stem cell factor (R & D) and Flt-3 ligand (R & D), each at 100 ng/mL, and 50 uM 2-mercaptoethanol (Sigma Aldrich). Following overnight incubation, cells were treated with 10 ug/mL polybrene (Sigma Aldrich) and transduced with a PTPσ-lentiviral shRNA or a scrambled lentiviral vector expressing green fluorescent protein (GFP) under the control of a murine stem cell virus (MSCV) promoter for an additional 48 hours. GFP^+ cells were isolated by FACS and re-stained for KSL. Cells were then fixed in 4% paraformaldehyde, permeabilized with 0.25% saponin in PBS and stained with a 1% solution of Rac1-GTP antibody (NewEast Biosciences, King of Prussia, PA), followed by flow cytometric analysis.

CAFC and Transendothelial Migration Assays

CAFCs were performed as previously described (32). Briefly, M2-10B4 stromal cells were cultured to confluence in 96-well flat bottom plates and irradiated at 4000 cGy. At 24 hours post irradiation, wells were seeded with dilutions of BM cells from PTPσ$^{+/+}$ or PTPσ$^{-/-}$ mice (81,000 – 333 cells per well). CAFCs were scored at week 5. Transendothelial migration assays were performed as previously described (15). Briefly, VeraVec™ mouse spleen endothelial cells (Angiocrine Bioscience, New York NY) were cultured to confluence in 8μM pore transwells (Corning Incorporated, Corning NY). Transwells were seeded with 200,000 BM cells in IMDM with 10%FBS, 1%penicillin-streptomycin, 125ng/mL stem cell factor, 50ng/mL Flt-3 ligand and 20ng/mL thrombopoietin with or without 25 μM Rac inhibitor, EHT1864 (R&D Systems, Minneapolis, MN). 100 ng/mL SDF-1α (R&D) or media was added to the bottom chamber of the transwell. 16 hours post culture, cells in the bottom chamber were collected and CFCs were set up with the migrated cells.

Cell cycle Analysis

For cell cycle analysis, BM was immunostained for KSL cells and the cells were then fixed with 2.5% paraformaldehyde in 2% FBS. Cells were permeabilized with 0.25% saponin (Calbiochem, La Jolla, CA) and labeled with antibodies to Ki67-FITC and 7-AAD (BD).

Competitive Repopulation Assays

BM cells from PTPσ$^{+/+}$ or PTPσ$^{-/-}$ mice (CD45.2), along with 2 x 10^5 BM competitor cells (CD45.1) were injected via tail-vein into lethally irradiated (700-800 cGy), congenic CD.45.1 Balb/c mice. Multi-lineage hematopoietic reconstitution in the PB was measured at 4 - 16 weeks post-transplant by flow cytometry, as previously described (33, 34). Animals were considered to be engrafted if $\geq$ 1% donor CD45.2$^+$ cells were detected. BM cells of recipient mice were also

analyzed at 16 weeks by flow cytometry. Secondary competitive repopulation assays were performed using BM cells from primary mice, as previously described (5).

Human Cord Blood Transplantation Assays

Human CB units were obtained according to a protocol approved by the Duke Institutional Review Board or the UCLA Institutional Biosafety Committee. For the xenotransplantation assays into NSG mice, CB cells were FACS-sorted for $CD34^{+}CD38^{-}CD45RA^{-}Lin^{-}$ cells, $CD34^{+}CD38^{-}CD45RA^{-}Lin^{-}PTP\sigma^{-}$ cells or $CD34^{+}CD38^{-}CD45RA^{-}Lin^{-}PTP\sigma^{+}$ cells (PTPσ antibody, R & D). 200 purified cells were transplanted via intrafemoral injections in 8-12 week old female NSG recipients preconditioned with 275 cGy, as previously described (13, 27). Prior to transplantation, NSG mice were anesthetized using 20mg/mL Avertin (Sigma-Aldrich). Engraftment was monitored in the PB as described above. Mice were considered as engrafted if ≥ 0.5% human $CD45^{+}$ cells were detected in the bone marrow of NSG recipients.

References

31. Christopherson KW, Hangoc G, Mantel C, Broxmeyer HE. Modulation of hematopoietic stem cell homing and engraftment by CD26. *Science*. 2004;305(5686):1000-1003.
32. Brandt J, et al. Ex vivo expansion of autologous bone marrow CD34(+) cells with porcine microvascular endothelial cells results in a graft capable of rescuing lethally irradiated baboons. *Blood*. 1999;94(1):106-113.
33. Doan P, et al. Tie2+ bone marrow endothelial cells regulate hematopoietic stem cell regeneration following radiation injury. *Stem Cells*. 2013;31:327-337.
34. Salter AB, et al. Endothelial progenitor cell infusion induces hematopoietic stem cell reconstitution in vivo. *Blood*. 2009;113(9):2104-2107.