SUPPLEMENTAL METHODS

Tamoxifen treatment of HIF1α KO mice

To conditionally delete HIF1α, $Cre^{+}HIF1\alpha^{Flox/Flox}$ mice and $Cre^{-}HIF1\alpha^{Flox/Flox}$ littermate controls were treated i.p. with 1mg of Tamoxifen resuspended in sunflower oil for 3 consecutive days. The mice were rested for 3 days and subsequently treated for 3 additional days. Fourteen days later, BM was harvested for experimental analysis and HIF1α knockdown was confirmed using qRT-PCR.

$dmPGE_2$ and DMOG Pulse-exposure

Pulse-exposure experiments with 1uM 16-16 dimethyl prostaglandin E_2 ($dmPGE_2$) (Cayman Chemical, Ann Arbor, MI) and dimethyloxalyglycine (DMOG) (Cayman Chemical) were performed as previously described[3]. Briefly, mouse BMC from femurs and tibias were isolated and $lineage^{pos}$ cells were depleted using MACS microbeads (Miltenyi Biotec, Auburn, CA). The resulting $lineage^{neg}$ cells were pulsed with either 1uM $dmPGE_2$ or various concentrations of DMOG for 2 hours at 37°C. The cells were washed and resuspended in RPMI with 10% HI-FBS for subsequent experiments. For experiments involving SNP treatment, 5uM SNP (EMD Millipore, Billerica, MA) was added to cells concomitantly with $dmPGE_2$ or DMOG.

HSPC Flow cytometry and functional analysis

Bone marrow was harvested from the femurs and tibias of mice and single-cell suspensions were prepared in PBS with 2% HI-FBS (Thermo Scientific HyClone, Logan, UT). $Lineage^{neg}$ $Sca\text{-}1^{pos}$ $ckit^{neg}$ (SKL) and CXCR4 staining and analysis were performed as described previously[3].

Chemotaxis to SDF-1 was determined using a two-chamber Costar Transwell (Cambridge, MA) system as previously described[24].

Analysis of HSPC Homing

Donor bone marrow from CD45.2 (C57Bl6) mice was harvested from the femurs and tibias of mice and single-cell suspension were prepared in PBS with 2% HI-FBS. Lineage^{neg} cells were obtained using a mouse Lineage cell depletion kit (Miltenyi Biotec) per manufacturer's instructions. The resulting lineage^{neg} cells were treated with 1uM dmPGE$_2$, 5uM DMOG or vehicle. The cells were washed and 2x10^6 cells were transplanted into lethally irradiated (1100 cGys [Mark I, JL Shepherd & Assoc., San Fernando, CA]) CD45.1 (BoyJ) recipient mice via warmed tail vein injection. Twenty-four hours after transplant, femurs and tibias were removed from recipient mice and flushed in PBS with 2% HI-FBS, and the mononuclear cell fraction was isolated using Lympholyte-M (CedarLane Labs, Burlington, ON). CD45.2 SKL events were quantitated by FACS after gating on live cells via Forward scatter/Side scatter as well as LIVE/DEAD stain exclusion to eliminate any bias associated with changes in cell viability. To evaluate the role of CXCR4 in homing, donor cells were treated with dmPGE$_2$, DMOG, or vehicle with or without the selective CXCR4 receptor antagonist AMD3100 (Genzyme, Cambridge, MA) 10 minutes prior to transplant.

Head to head competitive limiting dilution transplants

Whole bone marrow cells from CD45.1 and CD45.2 mice were treated with either 1uM dmPGE$_2$, 5uM DMOG or vehicle for 2 hours at 37°C. After treatment, cells were washed twice and mixed with 2x10^5 CD45.1/CD45.2 F1 competitor bone marrow cells at ratios of 1:1, 0.5:1, 0.25:1 and 0.075:1 and transplanted into lethally irradiated (1100 cGys, split dose [Mark I, JL

Shepherd & Assoc.]) CD45.1/CD45.2 recipients. The proportion of CD45.1, CD45.2 and CD45.2 F1 cells in peripheral blood was determined at 16 and 24 months post-transplant for DMOG-treated groups. For secondary transplants, $2x10^6$ WBM from previously transplanted CD45.1/CD45.2 mice were transplanted into lethally irradiated CD45.1/CD45.2 recipients and chimerism evaluated at 16 weeks post-transplant.

Culture of HIF1β mutant cells

Mouse hepatoma cells with mutant or wild-type HIF1β were a generous gift from Dr. Mircea Ivan (Indiana University School of Medicine, Indianapolis, IN) and were cultured in DMEM plus 10% HI-FBS (Thermo Scientific HyClone, Logan, UT) with penicillin-streptomycin at 37°C, 5% CO_2.

Quantitative RT-PCR

RT-PCR was performed using Platinum SYBR Green qPCR supermix UDG with ROX (Invitrogen, Carlsbad, CA) in an MxPro-3000 (Agilent Technologies) thermocycler. Dissociation curves were obtained for each primer set to confirm only one PCR product. HPRT expression was used as an internal normalization control. Primers sequences for SYBR Green RT-PCR were as follows: CXCR4 (Forward) 5′ - CTCGCTATTGTCCACGCCAC - 3′, (Reverse) 5′ - CCCTGACTGATGTCCCCCTG - 3, Adm (Forward) 5' - CACCCTGATGTTATTGGGTTCA - 3, (Reverse) 5' - TTAGCGCCCACTTATTCCACT - 3', GLUT1 (Forward) 5' - CTCTGTCGGCCTCTTTGTTAAT - 3, (Reverse) 5' - CCAGTTTGGAGAAGCCCATAAG - 3 HIF1α (Forward) 5' - ACCTTCATCGGAAACTCCAAAG - 3, (Reverse) 5' - ACTGTTAGGCTCAGGTGAACT - 3, HPRT (Forward) 5′ - TTGCTGACCTGCTGGATTAC – 3, (Reverse) 5′ - TATGTCCCCCGTTGACTGA - 3′.

Western Blot Analysis

Lineage^{neg} cells treated with 1uM dmPGE$_2$ or 5uM DMOG were cultured for 6 hours at 37°C, 5% CO_2, cell lysates resolved by SDS-Page gels, transferred onto a PVDF membranes, and incubated with a polyclonal anti-HIF1α (C-Terminal) antibody (Cayman Chemical). Anti-β-actin (Cell Signaling Technology, Danvers, MA) was used as a loading control.

Luciferase Reporter Assays

One million human embryonic kidney (HEK) cells were cultured in EMEM with 10% HI-FBS (Thermo Scientific HyClone, Logan, UT) to ~75% confluency at 37°C, 5% CO_2, and were transfected with 2ug of pGL2-CXCR4-Luc vector containing portions of the CXCR4 promoter (kind gifts from Dr. Wilhelm Krek, Swiss Federal Institute of Technology, Zurich) and 6ul FUGENE 6 HD Reagent (Promega, Madison, WI). Transfected cells were incubated for 24 hours, washed, trypsinized and split in equal numbers to normalize for transfection efficiency. Cells were incubated for adherence 6 hours, treated with 1uM dmPGE$_2$ or vehicle and incubated overnight at 37°C, 5% CO_2. After incubation, cells were harvested and 2 ul lysates were used to measure luciferase activity using the Firefly Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

Statistical Analysis

Data are expressed as mean ± SEM and compared by two-tailed *t* tests or One-Way ANOVA with Bonferroni post-hoc analysis as appropriate. *P* values less than 0.05 were considered statistically significant.