Supplemental Materials for Capitano et al.

Material and Methods

Preparation of Lin$^-$ mouse BM cells and mouse BM/spleen Ly6G$^+$ neutrophils. Mouse BM was isolated from the femurs and tibias of C57BL/6 mice immediately prior to use by flushing with PBS. Lineage depletion of BM was performed using a mouse lineage cell depletion kit following the manufacturer's protocol (Miltenyi Biotec; catalog #130-110-470). Purity of mouse BM Lin$^-$ cells was 92-96%. BM Lin$^-$ cells were cultured in RPMI-1640 media (Lonza) with 10% FBS (Fisher Scientific), 100 ng/mL recombinant mouse stem cell factor (rmSCF; R&D Systems; catalog 455-MC-010), 100 ng/mL recombinant mouse thrombopoietin (rmTPO; R&D Systems; catalog 488-TO055/CF), and 100 ng/ml recombinant mouse Flt3 ligand (rmFlt3L; BioLegend; catalog 550706) overnight at 37°C at 5% CO_2 and 5% O_2 in a humidified chamber prior to use in chemotaxis assays. For Ly6G$^+$ cell isolation, mouse BM and splenocytes from C57BL/6 mice were pooled together and positive selection was performed using an anti-Ly-6G MicroBeads Ultra Pure isolation kit and following the manufacturer's protocol (Miltenyi Biotec; catalog #130-120-337). Purity of Ly-6G cells was 90-94%.

Percent migration of BM HSC/HPC and Ly6G$^+$ cells. To calculate percent migration of cells (after careful removal of the insert from the transwell plates), media containing migrated cells (650 µL) were transferred into individual flow tubes. Cell counts were determined by running flow tubes for 3 minutes at medium speed on an LSR II flow cytometer (BD Biosciences). For input cell counts, flow tubes were prepared with 1 x 10^5

BM Lin^- or $Ly6G^+$ cells placed into 650 µL IMDM in triplicate, and cell counts determined by running the tubes for 3 minutes at medium speed. Percent migration was determined as follows: [(experimental well cell count - average cell count for media alone wells)/ average cell count for input] x 100. To assess HSC and HPC populations, the flow tubes were spun down, the supernatant removed, cell pellets resuspended in fluorescently-conjugated anti-mouse antibody cocktail and incubated for 15 minutes at room temperature, washed in PBS and fixed using 1.5% formaldehyde. Samples were analyzed on an LSR II flow cytometer (BD Biosciences). Single-color compensation and isotype controls were included for each experiment. Data analysis was performed using FlowJo 7.6.3 software (Tree Star). Gates were determined using fluorescence minus one controls. Mouse phenotyping antibodies used were FITC-mouse lineage cocktail (CD3, Gr-1, CD11b, CD45R, Ter119; BioLegend, catalog 133302 and 133310), PE-CF594-anti-Ly6A/E (also known as Sca-1; clone D7; BD Biosciences), APC-H7-anti-CD117 (c-Kit; clone 2B8; BD Biosciences), BV421- or PE-anti-CD150 (clone Q38-480; BD Biosciences) and APC-anti-CD135 (also known as Flt3; clone A2F10.1; BD Biosciences). For mouse BM populations LSK cells= Lin^- $Sca\text{-}1^+$ $c\text{-}Kit^+$; LK cells= Lin^- $Sca\text{-}1^-$ $c\text{-}Kit^+$, long-term (LT)-HSCs= LSK $Flt3^-$ $CD150^+$, short-term (ST)-HSCs as LSK $Flt3^+$ $CD150^+$, multipotential progenitors (MPPs) as LSK $Flt3^+$ $CD150^-$. Population percentages were used to calculate migration of HSC/HPC populations for each well/input tube.

Supplemental Figure Legends

Figure S1. DEK induces migration of hematopoietic stem and progenitor-enriched mouse LSK cells in a time- and dose-dependent manner. Lineage-depleted (Lin^-)

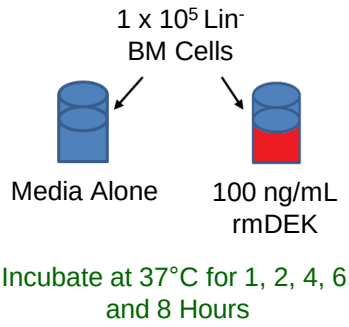
bone marrow (BM) cells from C57BL/6 mice were utilized in transwell migration assays. Percent migration of LSK (Lin^- $Sca\text{-}1^+$ $c\text{-}Kit^+$) cells was determined by using the following formula [(experimental well LSK cell count - average LSK cell count for media alone wells)/ average LSK cell count for input] x 100. (**A**) Migration of LSK cells towards 100 ng/mL of recombinant mouse (rm)DEK was determined after 1, 2, 4, 6 and 8 hours. (**B**) Migration of LSK cells towards 0, 6.25, 12.5, 25, 50, 100 and 200 ng/mL of rmDEK was determined after 4 hours. (**C**) Migration of LSK cells towards 0, 6.25, 12.5, 25, 50, 100 and 200 ng/mL of rmDEK was determined after 8 hours. (**A-C**) Each experiment point was performed in triplicate. Data represent the mean ± standard deviation (SD). Each panel is the representative of two separate experiments.

Figure S2. Experimental schema for the checkerboard chemotaxis assays. (**A**) Experimental setup for Table 1. (**B**) Experimental setup for Table 2. (**C**) Experimental setup for Table 3. (**D**) Experimental setup for Table 4.

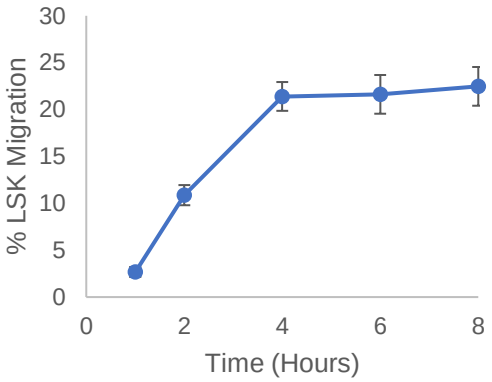
Figure S3. Microarray normalized log2 CXCR2 expression value data for mouse (A) and human (B) in the indicated cell populations.

Capitano et al., Figure S1

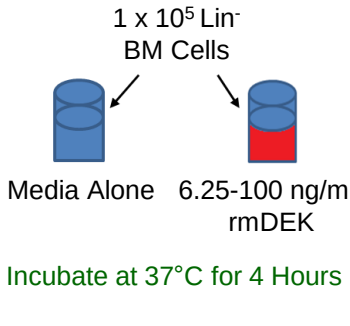
A



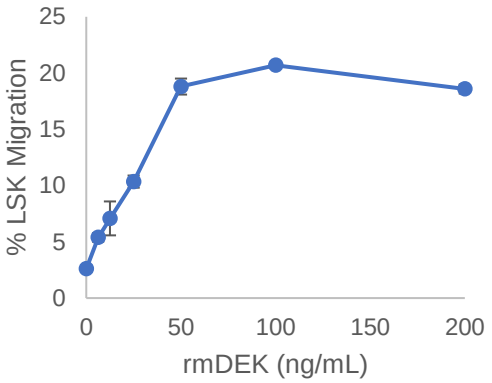
Incubate at 37°C for 1, 2, 4, 6, and 8 Hours



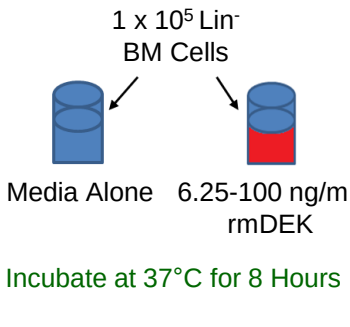
B



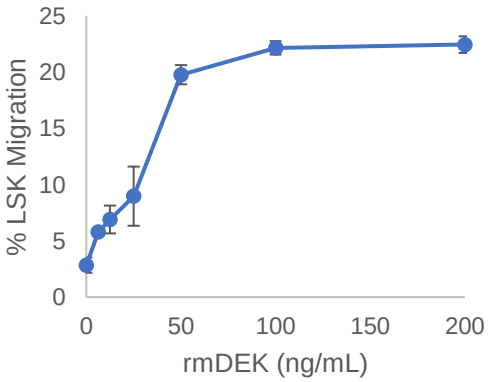
Incubate at 37°C for 4 Hours



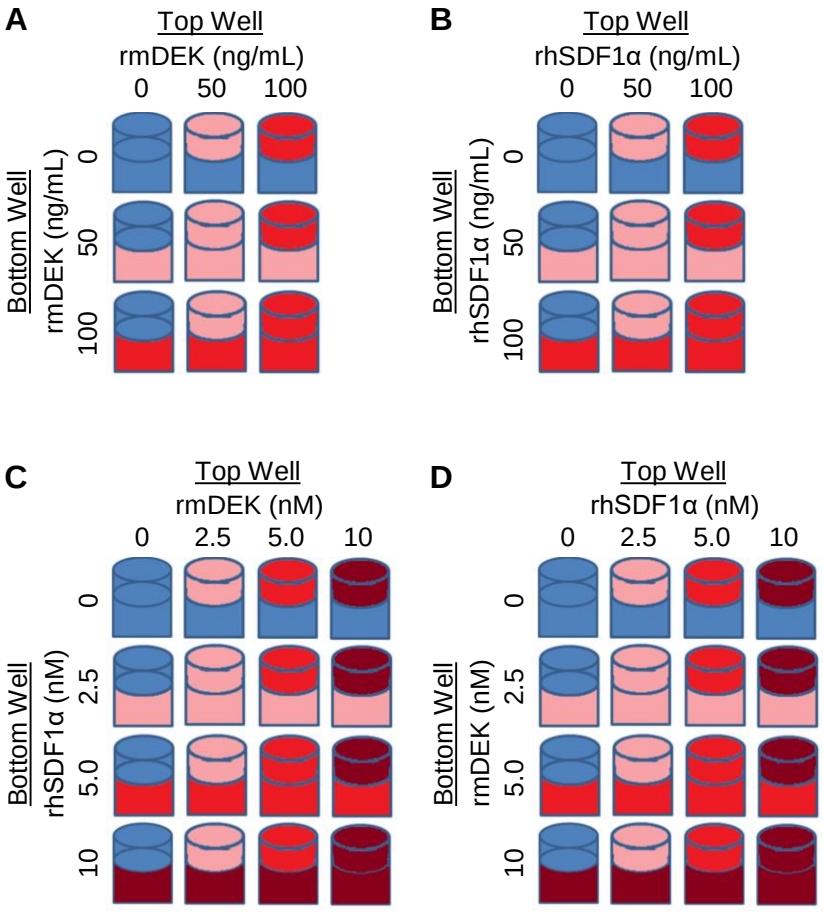
C



Incubate at 37°C for 8 Hours



Capitano et al., Fig. S2



Capitano et al., Fig. S3