

Supplementary figure legends:

Supplementary Figure 1. Glucocorticoids administration promotes surface

expression of CXCR4, facilitates SDF1/CXCR4 axis mediated chemotaxis.

(a) Quantification of mean fluorescence intensity (MFI) of surface CXCR4 of human

CB CD34⁺ cells treated with Vehicle, different doses of Flonase or Dex

(dexamethasone). Representative data from two independent experiments are

shown (n=3 cultures per group, one-way ANOVA). ***p<0.001 when compared

with Vehicle control. ###p<0.001 when compared with 10 nM Flonase group.

(b) Quantification of mean fluorescence intensity (MFI) of surface CXCR4 of human

CB CD34⁺ cells treated with Flonase for different time point.

(c,d) Quantification of mean fluorescence intensity (MFI) of surface CXCR4 of

human CB CD34⁺CD38⁻ cells and phenotypic multiple potential progenitor (MPP)

cells (CD34⁺CD38⁻CD45RA⁻CD90⁻CD49f⁻) treated with Vehicle or Flonase

(1 μM). Data pooled from three independent experiments are shown (n=9 cultures

per group).

(e,f) Human CB CD34⁺ cells were cultured in the presence of Vehicle or Flonase

(1 μM) for 16 hours. After incubation, cells were washed, resuspended in

RPMI/0.5% BSA, and allowed to migrate to human recombinant SDF-1α (50

ng/mL) for 4 hours. Total cell migration was quantified by flow cytometry. The

frequency of CD34⁺CD38⁻ cells and phenotypic multiple potential progenitor

(MPP) cells was examined by surface staining and analyzed by flow cytometry.

CD34⁺CD38⁻CD45RA⁻CD90⁻CD49f⁻ cells was characterized as MPP.

Representative data from two independent experiments are shown (n=3 cultures per group).

(g) Human CB CD34⁺ cells were cultured in the presence of Dex (dexamethasone), Medrol (methylprednisolone), cortisol for 16 hours. After incubation, cells were washed, resuspended in RPMI/0.5% BSA, and allowed to migrate to human recombinant SDF-1 α (50 ng/mL) for 4 hours. Total cell migration was quantified by flow cytometry. Representative data from two independent experiments are shown (n=3 cultures per group, one-way ANOVA).

(h,i) Histogram of GR expression in human CB CD34⁺ cells, CD34⁻ cells and HSC. Human CB mononuclear cells were used to perform surface staining with anti-human CD34, anti-human CD38, anti-human CD45RA, anti-human CD90 and anti-human CD49f. After that, cells were permeabilized and fixed. Intracellular staining was performed with anti-human glucocorticoid receptor (GR) and APC-labeled secondary antibody. MFI was examined by flow cytometry. Representative data from two independent experiments are shown (n=3 cultures per group, one-way ANOVA).

(j) Mean fluorescence intensity of surface CXCR4 in Vehicle, Flonase, RU486, Flonase+ RU486, cortisol, cortisol+ RU486, Dex (dexamethasone) and Dex+ RU486 treated human CB CD34⁺ cells was analyzed by flow cytometry. Data pooled from two independent experiments are shown (n=6 cultures per group, one-way ANOVA).

(k) Human CB CD34⁺ cells were cultured in the presence of Vehicle, Flonase, RU486, Flonase+ RU486 for 16 hours. After incubation, cells were washed, resuspended in RPMI/0.5% BSA, and allowed to migrate to human recombinant SDF-1 α (50 ng/mL) for 4 hours. Total cell migration was quantified by flow cytometry. Data pooled from two independent experiments are shown (n=6 cultures per group, one-way ANOVA). Data are shown as box-and-whisker plots (the lines indicate median values, the whiskers indicate minimum and maximum values, the boxes indicate interquartile range) in **a-g** and **i-k**. NS, not significant. **p<0.01. ***p<0.001.

Supplementary Figure 2. Glucocorticoids administration promotes homing and engraftment of human CB HSC/HPC.

- (a) 500,000 CB CD34⁺ cells treated with Vehicle or Flonase were transplanted by i.v. injection into primary NSG mouse that received 350 cGy total body irradiation one day beforehand. The percentage of human CD45⁺ cells in bone marrow was determined 24 hours after transplantation. Representative pseudocolor plot is shown.
- (b) 500,000 CB CD34⁺ cells treated with Vehicle, cortisol or Dex (dexamethasone) were transplanted by i.v. injection into NSG mouse received 350 cGy total body irradiation one day beforehand. Percentage of human CD45⁺ cells in the bone marrow was determined by flow cytometry 24 hours after transplantation. (n=5 mice per group, one-way ANOVA).
- (c) 10000 CB CD34⁺ cells treated with Vehicle or Flonase were transplanted by i.v.

injection into primary NSG mouse that received 350 cGy total body irradiation one day beforehand. The percentage of human CD45⁺ cells in bone marrow was determined 4 month after transplantation. Representative contour plot is shown.

(d-h) 10000 CB CD34⁺ cells treated with Vehicle or Flonase were transplanted by i.v. injection into NSG mouse received 350 cGy total body irradiation one day beforehand. The percentage of human CD33⁺ myeloid cell, CD19⁺ B cell and CD3⁺ T cell in bone marrow (BM) was determined 4 month after transplantation. (n=4 mice in vehicle group and n=5 mice in Flonase group). Representative pseudocolor plots are shown in **d-f**.

(i,j) The frequency of human SRCs in CB CD34⁺ cells treated with Vehicle or Flonase. Graded doses of Vehicle- or Flonase-treated CB CD34⁺ cells were transplanted into NSG mouse that received 350 cGy total body irradiation one day beforehand. The percentage of human CD45⁺ cells in bone marrow (BM) was analyzed 4 month after transplantation (n=3-5 mice per group, see **Supplementary Table 3**). HSC frequencies (line in the box) and 95% confidence intervals (box) presented as the number of SRCs in 1×10^6 CD34⁺ cells are shown in **i**. Poisson statistical analysis of data from **Supplementary Table 3** is shown in **j**. Shapes (circle or triangle in the plot) represent the percentage of negative mice for each dose of cells. The inverted triangles indicate that all tested mice were positive in this group. Solid lines indicate the best-fit linear model for each data set. Group A (black line) indicates vehicle group, Group B (red line) indicates Flonase group. Data are shown as dot plots (mean \pm s.e.m.) in **b, g, h**. *p<0.05. **p<0.01.

Supplementary Figure 3. Glucocorticoids upregulate expression of CXCR4 in human HSC/HPC and promote SDF1/CXCR4 axis mediated chemotaxis by enhancing SRC1/p300 dependent histone acetylation.

- (a) Chromatin immunoprecipitation analysis of glucocorticoid receptor (GR) enriched on CXCR4 promoter in Vehicle or Dex (dexamethasone) treated human CB CD34⁺ cells. Data are shown as box-and-whisker plots from one experiment with three replicates per group.
- (b) Acetylated H3K9 and H3K14 levels on CXCR4 in Vehicle or Flonase treated human CB CD34⁺ cells were examined by Chromatin immunoprecipitation assay. Representative data from two independent experiments are shown (n= 3 replicates per group).
- (c) Human CB CD34⁺ cells were cultured in the presence of Vehicle or Flonase for 16 hours. Cells were washed, permeabilized and fixed. Intracellular staining was performed with anti-acetyl human H4K5 or anti-acetyl human H4K16 and FITC-labeled secondary antibody. MFI was examined by flow cytometry. Representative data from two independent experiments are shown (n=3 cultures per group).
- (d,e) Knockdown of *p300* or *SRC1*, but not *CBP* suppresses increased CXCR4 expression by Flonase treatment. 6-carboxyfluorescein (6-FAM) labeled *Ctrl* siRNA, *p300* siRNA, *SRC1* siRNA or *CBP* siRNA was transfected into human CB CD34⁺ cells. 24 hours later, Vehicle or Flonase was added and cells were incubated for 16 hours. CXCR4 expression level was examined by flow

cytometry. The siRNA efficiency was determined by intracellular staining with anti-p300, anti-SRC1 and anti-CBP. Representative data from two independent experiments are shown (n=3 cultures per group, one-way ANOVA).

(f) Human CB CD34⁺ cells were cultured in the presence of Vehicle, c646 (30 μM), Flonase (10 nM), Flonase+c646 for 16 hours. After incubation, cells were washed, resuspended in RPMI/0.5% BSA, and allowed to migrate to human recombinant SDF-1α (50 ng/mL) for 4 hours. Total cell migration was quantified by flow cytometry. Data pooled from three independent experiments are shown (n=9 cultures per group, one-way ANOVA).

(g,h) Quantification of mean fluorescence intensity (MFI) of surface Cxcr4 of mouse bone marrow HSC (CD150⁺CD48⁻LSK) treated with Vehicle, Flonase, Dex (dexamethasone) or cortisol (**g**). n=3 cultures per group, one-way ANOVA. Mouse *Cxcr4* mRNA level of Vehicle or Flonase treated mouse bone marrow lineage negative hematopoietic stem and progenitor cells (HSPCs) was examined by quantitative Realtime-PCR (**h**).

(i) Mouse bone marrow HSPCs were cultured in the presence of Vehicle or Flonase for 16 hours. After incubation, cells were washed, resuspended in RPMI/0.5% BSA, and allowed to migrate to recombinant SDF-1(50 ng/mL) for 4 hours. Total cell migration was quantified by flow cytometry. n=4 cultures per group. Data are shown as box-and-whisker plots (the lines indicate median values, the whiskers indicate minimum and maximum values, the boxes indicate interquartile range) in **a-d** and **f, g** and **i**. NS, not significant. *p<0.05. **p<0.01. ***p<0.001.

Supplementary Figure 4. Uncropped western blots for Figures 2b and 2h.