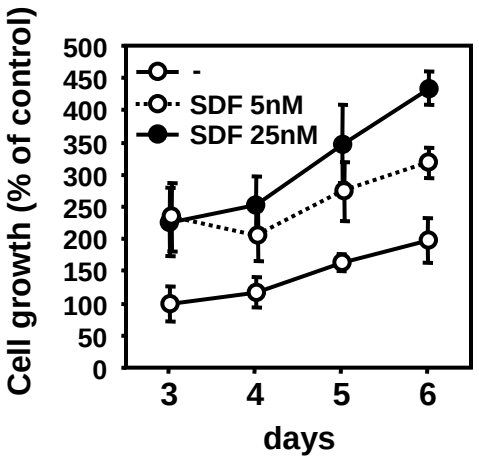
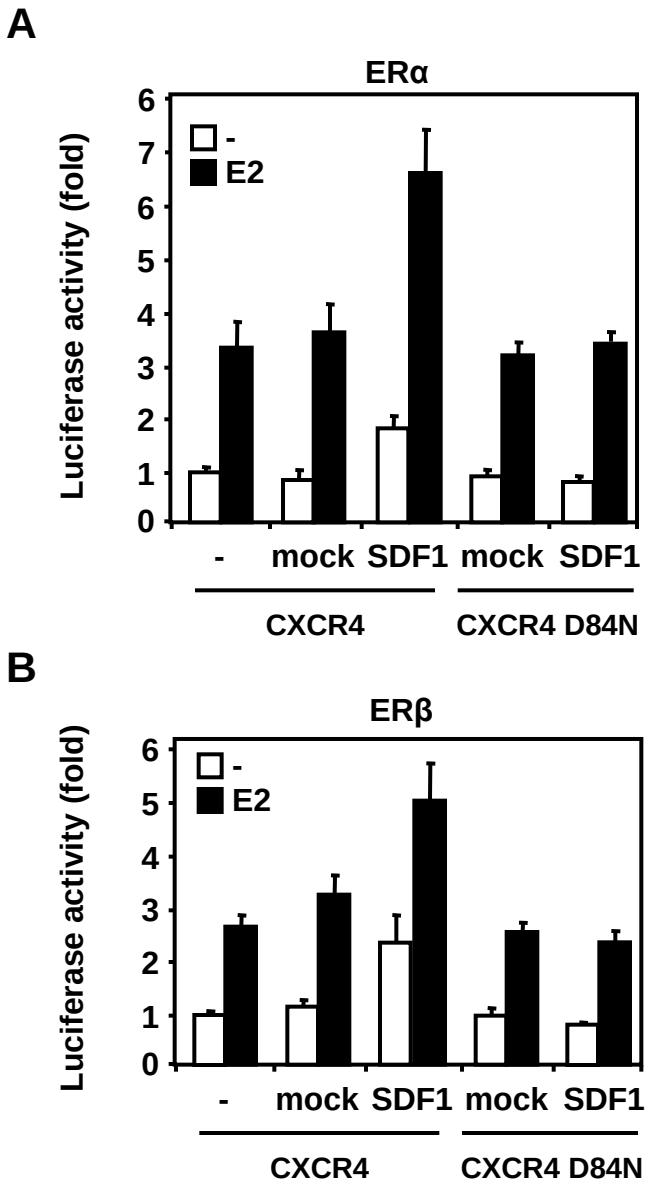
Fig. S1



Supplementary Figure 1. SDF-1 promotes the growth of MCF-7 cells. MTT proliferation assay on MCF-7 cells treated with 5 or 25nM SDF-1 over the indicated time period. Results are expressed as % change from untreated cells which is set at 100%.

Fig. S2

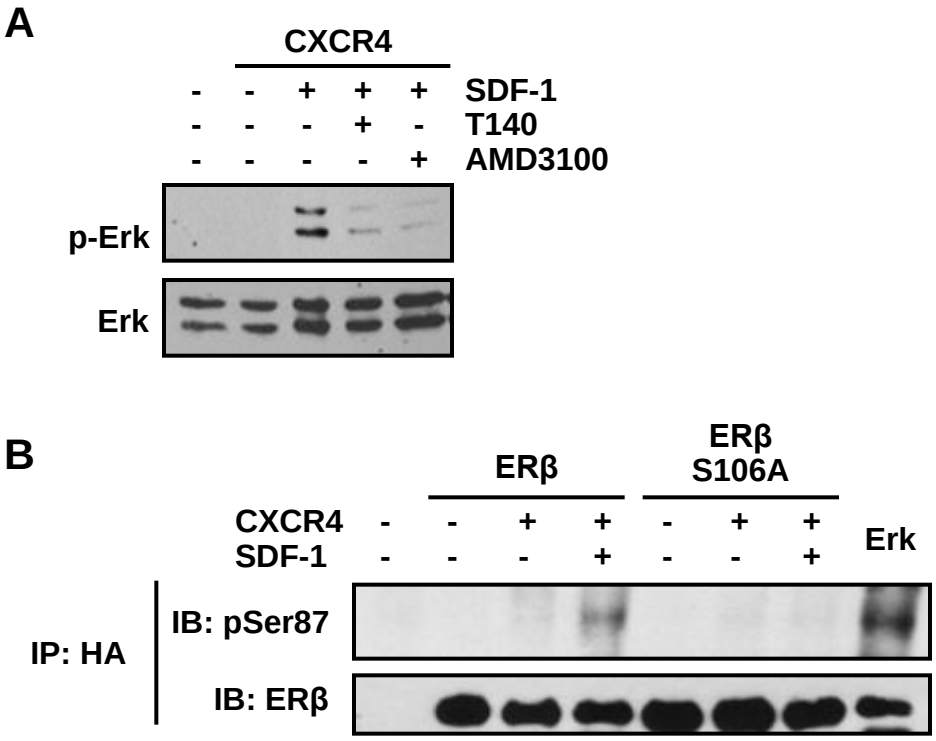


Supplementary Figure 2. Paracrine control of ERα and ERβ activation by SDF-1.

A- 293 cells were transfected with an EREtkLuc reporter and plasmids encoding ERα and CXCR4 or the inactive CXCR4 D84N variant. Separate 293 cells transfected with an empty (mock) or a plasmid coding for human SDF-1 were then added for 16h, in presence or absence of 10nM estradiol (E2). Co-cultured cells were then harvested for luciferase activity, and results expressed as fold response compared to untreated cells set at 1.0 for either wild type or mutated CXCR4 constructs used in transfection.

B- Similar experiment as in (A) except that ERβ was expressed in cells.

Fig. S3

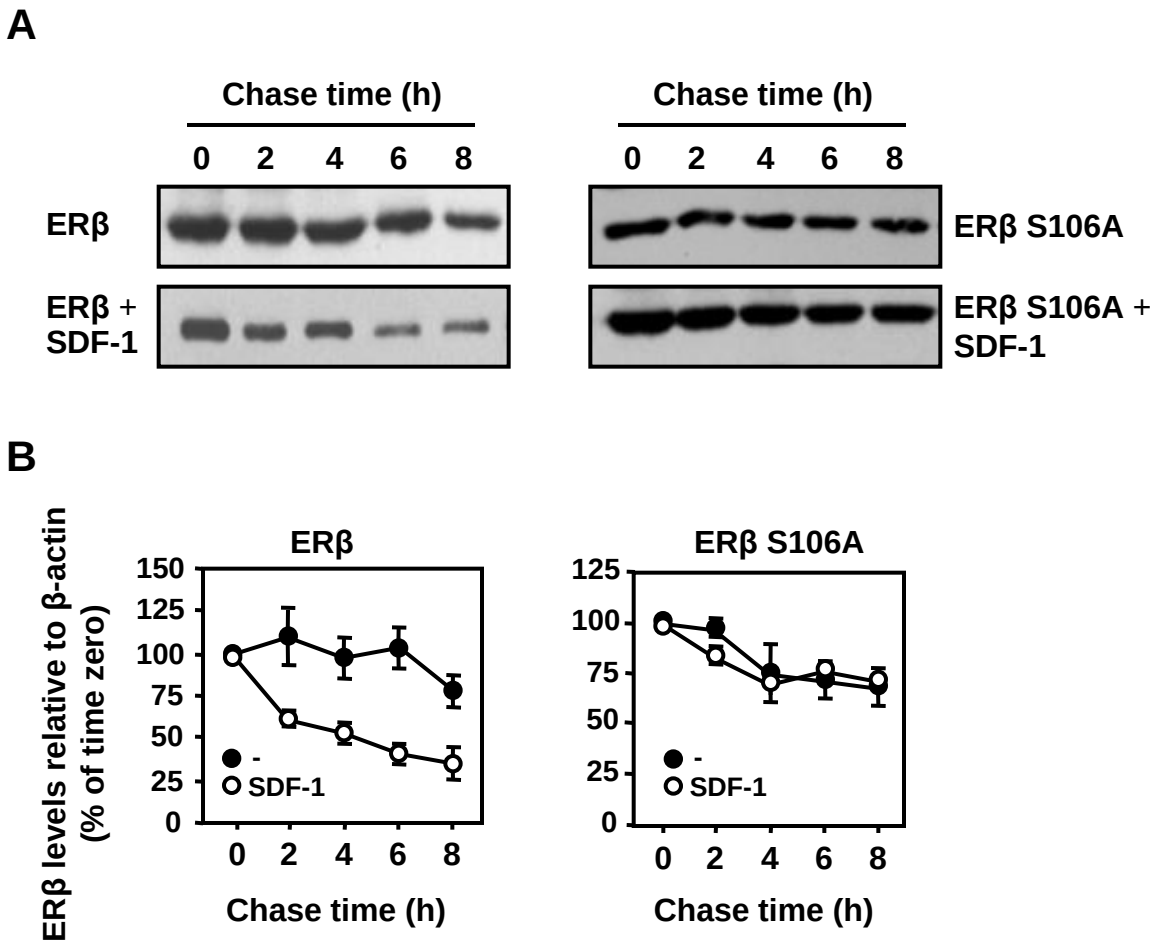


Supplementary Figure 3. The CXCR4-SDF-1 pathway signals the phosphorylation of Ser-106 of mouse ERβ.

A- CXCR4 signals to Erk. 293 cells were transfected with CXCR4 plasmid followed by treatment with 25nM SDF-1 for 15min prior to Western analysis. Cells were also treated with CXCR4 antagonists TC140 and AMD3100.

B- Ser-106 of mouse ERβ is phosphorylated upon activation of CXCR4 by SDF-1. HA-tagged ERβ was expressed in 293 cells in absence or presence of CXCR4. After transfection, cells were treated or not with 25nM SDF-1 for 15min and harvested for immunoprecipitation of ERβ using an anti-HA antibody. Western analysis was then performed on immunoprecipitates using an anti human phospho-Ser-87 (which corresponds to mouse Ser-106) ERβ antibody. The mouse S106A mutant was also tested to ensure selectivity of the pSer-87 antibody towards mouse Ser-106. As a positive control, Erk was activated by coexpressing constitutive Mek1 and Erk2 plasmids. Samples were normalized to ERβ content using an anti-ERβ antibody.

Fig. S4



Supplementary Figure 4. Ser-106 regulates mouse ERβ degradation by SDF-1.
A- 293 cells were transiently transfected with plasmids expressing HA-tagged wild-type or S106A ERβ in presence of CXCR4 plasmid. At 12 hours after transfection, cells were treated or not with 25nM SDF-1, and cycloheximide was added at a concentration of 50μM. Cells were then lysed for Western blot analysis at the indicated time points.
B- Each signal intensity was obtained from 2-3 separate experiments such as in (A) and expressed relative to β-actin levels.