Supplemental information

EXPERIMENTAL PROCEDURES

Plasmid construction – A series of luciferase reporter (pScx-Luciferase) plasmids were constructed as follows: to make the pGL3-pScx(-271) plasmid, -271 to +46 region of mouse Scx gene was amplified by PCR using the following primer set (F; 5'-CGC TGC TCA TGC CGC CTC TTT AGG TCC C-3', R; 5'-GGG CAC CAT TGT CTT GGG GCA CTA GTA GCA CC-3'). The amplified fragment was cloned to pCR2.1 vector (Invitrogen, Carlsbad, CA) by using TA-cloning system (Invitrogen) (pCR2.1-pScx). pCR2.1-pScx plasmid was cut with SacII and blunted, then cut with XhoI resulting in a fragment (-271 to -18 region). The fragment was cloned into the pGL3 plasmid (Promega) at XhoI and NcoI (blunted) sites (pGL3-pScx(-271)). The pGL3-pScx(-271) plasmid was cut with SacI to remove -271 to -159 region, then the plasmid was self-ligated (pGL3-pScx(-158)). SKA3SH8Z11NA1 plasmid that contains approximately 11k bp of mouse Scx genomic DNA (kindly given by Dr. A. Perez, CFG University at Albany) was cut with XhoI and SpeI to get the fragment (6969 bp of 5'-flanking region of the Scx gene). The fragment was cloned into the pGL3-pScx(-271) at XhoI and SpeI sites (pGL3-pScx(-7k)). The pGL3-pScx(-7k) plasmid was cut with XhoI and EcoRI to remove -7220 to -1543 region then blunted, the plasmid was self-ligated (pGL3-pScx(-1542)). The pGL3-pScx(-7k) plasmid was cut with XhoI and HindIII to remove -7220 to -2427 region then blunted, the plasmid was self-ligated (pGL3-pScx(-2426)). The pGL3-pScx(-7k) plasmid was cut with NheI to remove -7220 to -2838 region, the plasmid was self-ligated (pGL3-pScx(-2837)). The pGL3-pScx(-2426) plasmid was cut with EcoRI and SpeI to remove -1542 to -271 region then blunted, the plasmid was self-ligated (pGL3-pScx(Δ-1542~-271)). All plasmids were confirmed by sequencing (NIEHS sequencing core).

Luciferase assay – Cos1 cells were maintained in DMEM with 10% FBS, penicillin and streptomycin. The cells ($0.8x10^5$ cells/well) were plated into 24-well plates. The cells were grown in the DMEM with 10% dextran coated charcoal treated FCS (DCC-FCS) for 24h before transfection. The cells were transfected with, 0.1 μg of luciferase reporter plasmid, 0.1 μg of renilla luciferase reference plasmid (PRL-TK; Promega) and 0.05 μg of mouse ERalpha expression plasmid (pcDNA3-mERa) or empty plasmid (pcDNA3; Invitrogen). Cells were transfected using FuGene 6 (Roche) for 8 h and then washed with PBS and cultured further 18 h in the 10% DCC-FCS supplemented DMEM with or without 10^8 M of estradiol (E2). Cell extracts were prepared by passive lysis buffer for dual-luciferase reporter assay (Promega). The assay was performed following the manufacture's instruction. Luciferase activity was normalized by renilla luciferase activity.

FIGURE LEGEND

Fig. S1. Scx promoter sequence corresponding to -250 to +1 region.
The sequence between -250 to +95 region of Scx gene is shown. TATA boxs are indicated as the boxes. Transcription start sites based on UCSC genome browser are indicated as arrows (5' end of the S78079 and BC062161 cDNAs). Scx protein is translated from the ATG at +83 to +85 as indicated. Italic character suggests CMV-Scx cDNA and CMV-Scx gDNA(+1) containing Scx gene element.

Fig. S2. ER alpha-dependent positive regulatory element in the 5'-flanking region of Scx gene. (A) The location of the Bop1 exons 4 to 10 (gray boxes) and Scx gene (black boxes) is illustrated to suggest the correlation between Bop1 exonic region and the 5'-flanking region of the Scx gene fused with the luciferase reporter plasmids. Arrows suggest the direction of the gene transcription. The position of the ER alpha-dependent positive responsive element is indicating as ERE. (B) Relative luciferase activities in Cos1 cells are shown. We found a similar profile of the Scx promoter activity in SKBr3 and C2C12 cells (data not shown).

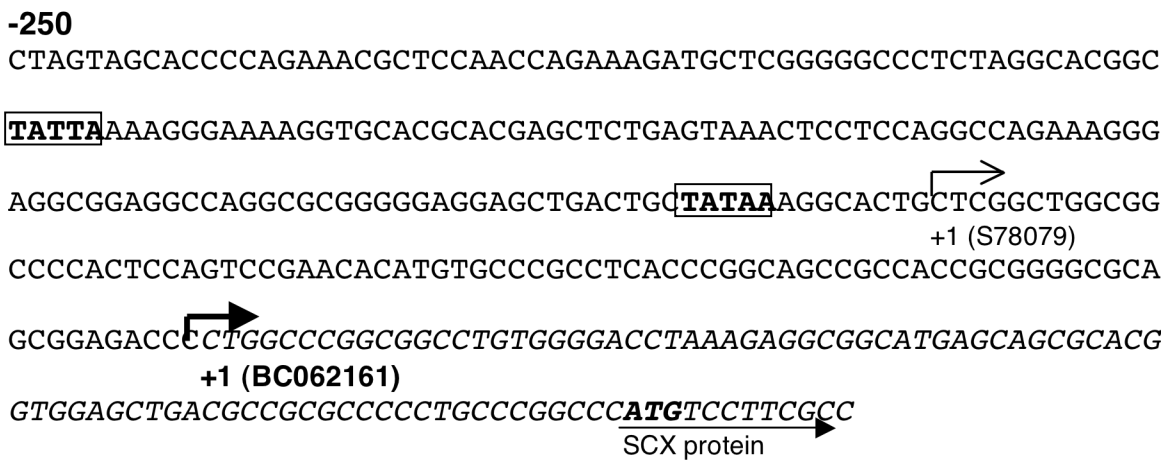

Fig. S1 Arao et al.

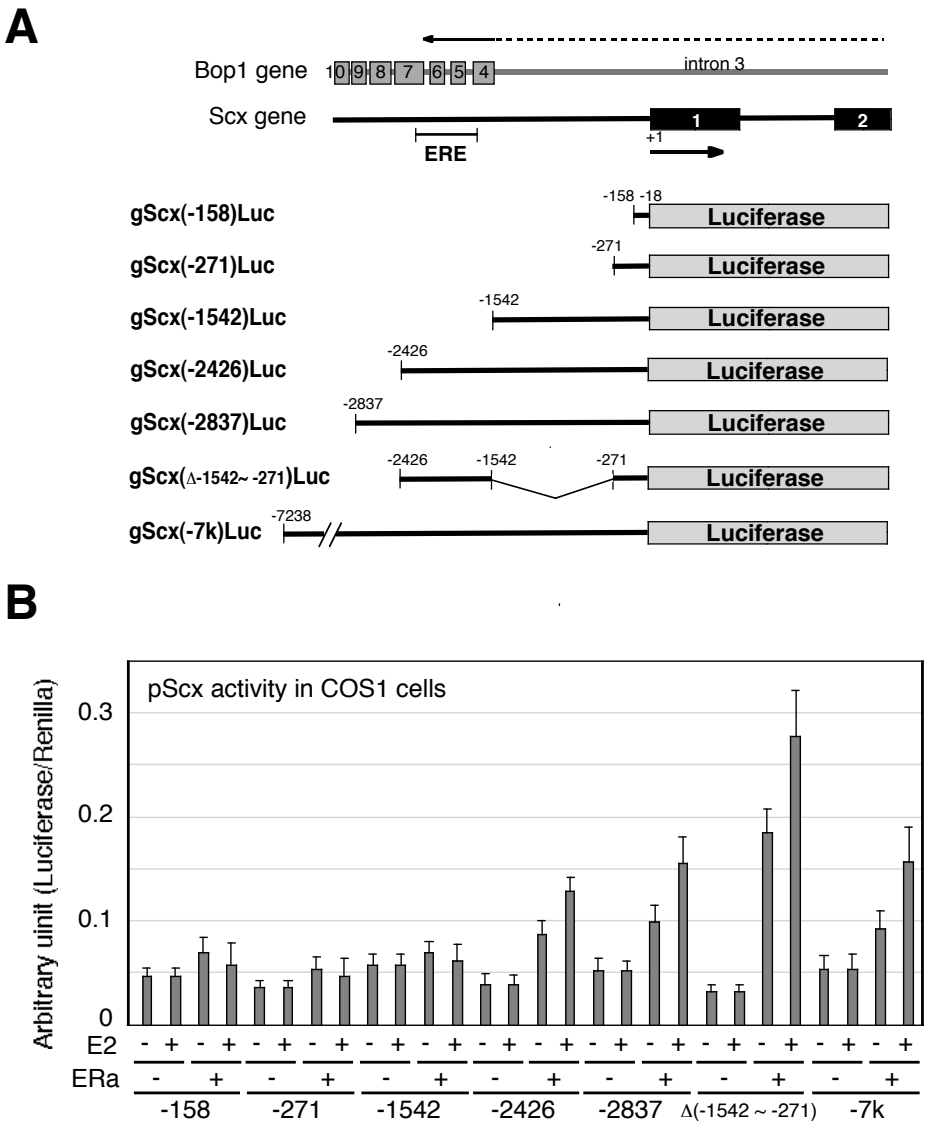

Fig. S2 Arao et al.