SUPPLEMENTAL DATA

Fig S1 : Control analysis of reciprocal bleed-through fluorescence from the red and green channels.

COS-7 cells were transiently transfected with the expression vector encoding HA-SRC-1. Cells were fixed after 40 h and immunolabeled overnight at 4°C with either the primary rat anti-HA (upper panel) or the rabbit anti-S7/Rpt1antibodies (lower panel). First antibody was followed by a 30 min incubation with a mix of the two corresponding secondary antibodies Dylight 549 anti-rat (red) and alexa 488 anti-rabbit (green). Cells were analyzed by confocal microscopy as described in Methods section. Note the lack of overlap of both fluorescences. DIC: Differential Interferential Contrast.

Fig S2 : Colocalization analyses of SRC-1 with antigens and organelles.

A and B, COS-7 cells were transiently transfected with vectors encoding HA-SRC-1. Cells were fixed after 40 h and immunolabeled with the anti-PML (A) or anti-SC-35/SRp30 spliceosome component (B) antibodies. Colocalization analysis was performed by confocal microscopy. Note the partial colocalization of SRC-1 with PML nuclear bodies and the absence of colocalisation with SC-35.

C and D, COS-7 cells were transiently transfected with vector encoding GFP-SRC-1. After 40 h, cells were incubated with a mitochondrial (C) or lysosomial tracker (D) (Invitrogen), followed by fixation and observation by confocal microscopy. No significant colocalization was observed.

E, COS-7 cells were cotransfected with the vector encoding HA-SRC-1 and the pEGFP-Peroxy vector (Clontech). Immunodetection was performed with the anti-HA antibody and cells were analyzed by confocal microscopy. No significant colocalization was observed.

Fig S3: **Epoxomicin increases SRC-1 expression level.**

COS-7 cells were transfected with the vector encoding SRC-1 and incubated during 15 h in the presence of epoxomicin (500 nM) or vehicle (DMSO, -). Expression of SRC-1 was analyzed by Western blot using anti-HA monoclonal antibody. The bands were quantified as described in "Materials and Methods".

Fig S4: The Nuclear Export Signal deletion of SRC-1 decreases its turnover rate.

COS-7 cells were transfected with the wt SRC-1 or the Δ(NES) encoding vector (deleted of the Nuclear Export Signal). Seventy-two hours after transfection, cells were treated with cycloheximide (100 µg/ml) during 1, 4 or 6 h. Whole cell extracts were analyzed by electrophoresis on 7.5% SDS-PAGE and immunoblotted with the indicated antibodies. Band intensities (right panel) were quantified as described in "Material and Methods". Note the decrease in kinetics degradation rate of the nuclear mutant Δ(NES) compared to wt SRC-1.

Fig S5 : PRB is degraded in the presence of R5020 but not RU486.

Ishi PR-B cells were treated during 24 h with vehicule (control, -), the agonist R5020 (10 nM) or the antagonist RU486 (10 nM) and western blot analysis was performed with the corresponding whole cell extracts as described in "Materials and Methods". PR-B was detected using monoclonal antibody Let126 and loading control protein α-tubulin was detected using anti-α-tubulin antibody.

Fig S6 : R5020 or RU486 does not modify endogenous SRC-1 mRNA levels.

Ishi PR-B cells were treated during 24 h with vehicle (EtOH, -), R5020 (10 nM) or RU486 (10 nM). Total RNA was extracted and relative expression of SRC-1 mRNA levels were quantified by quantitative RT-PCR as described in "Materials and Methods". Results were normalized by the 18S RNA and are expressed as mean ± SEM of six independent determinations.

Fig S7 : Coactivator specificity in the ligand-induced down-regulation.

Ishi PR-B cells were transfected as indicated, with either the SRC-1, SRC-2 or SRC-3 encoding vector. Forty-eight h after transfection, cells were cultured for 24 h either in the absence of ligand (control vehicule, -H) or in the presence of R5020 (10 nM). Whole cell extracts were analyzed by electrophoresis on 7.5% SDS-PAGE and immunoblotted with the indicated antibodies.

<u>Fig S8</u> : Epoxomicin and MG132 do not increase expression levels of the two non degradable Δ(PEST) and Δ(bHLH) mutants.

COS-7 cells were transfected with Δ(PEST) and Δ(bHLH) encoding vectors and incubated in the absence (vehicule, -) or presence of either epoxomixin (500 nM) or MG132 (5 μM) for 15 h. Expression level of SRC-1 mutants was analyzed by Western blot using the indicated antibodies. Note the absence of accumulation of both mutants in the presence of both proteasome inhibitors.