Supporting Information

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SI Results

Sumoylation of PR Represses Basal Proliferation in Breast Cancer Cells. Distinct from MTT assays, progestins are clearly pro-survival and mitogenic to cells growing in soft agar (5). However, only cells expressing wt or K388R PR-B were sensitive to added progestin; cells expressing transcriptionally repressed (i.e., sumoylated) S294A PRs formed few colonies and were resistant to added ligand (Fig. S1). PR-B receptors that are capable of undergoing sumoylation (wt PR and S294A PR) repress breast cancer cell proliferation, whereas desumoylated (K388R) receptors promote increased proliferation.

Sumoylated PR-B Regulates Gene Expression via Altered Cofactor Recruitment. To first examine whether STC1 expression was sensitive to chromatin remodeling/HDAC activity we treated cells with an HDAC inhibitor (TSA) and assessed STC1 and β-actin transcript levels (Fig. S5*A*). STC1 expression was increased in all cell lines in response to the pan-HDAC inhibitor, TSA. However, when these data are normalized to account for the differences in basal STC1 expression levels between cell lines, only cells expressing wt PR-B exhibited a significant (23-fold) increase in STC1 expression in the presence of TSA relative to PR-null cells or cells expressing K388R PR.

SI Materials and Methods

Gene Silencing. STC1 small hairpin RNA (shRNA) constructs were generated using BD Bioscience online shRNA oligo design tool and BD Knockout RNAi Clone and Confirm Kit (Clontech Laboratories, Inc., Palo Alto, CA). Retroviral infections to generate stable cell lines were carried out as previously described (41). siRNA pools designed toward HDAC3, SRC-1, or nontargeting control siRNA (Dharmacon Inc., Lafayette, CO) were transiently transfected into HeLa (PR-null or stably expressing Flag tagged wt PR-B) or T47D K388R PR-B cells seeded at 500,000 cells/well in six-well plates using Dharmafect 1 reagent (Dharmacon Inc.). Cells were starved for 72 h and RNA harvested for real-time PCR (see below).

RT-PCR and Real-Time Quantitative PCR. T47D cell variants were plated at 6×10^5 cells per 60-mm dish, serum starved for 48 h, and/or treated with 10 nM R5020,100 nM RU486, or 10 μM TSA (Sigma-Aldrich) for 6 to 48 h. HeLa cells were plated at 2.5×10^5 cells per well in six-well plates, transfected with vector control, wt or K388R PR-B and either pSG5 or Cdk2-TY, and serum starved.

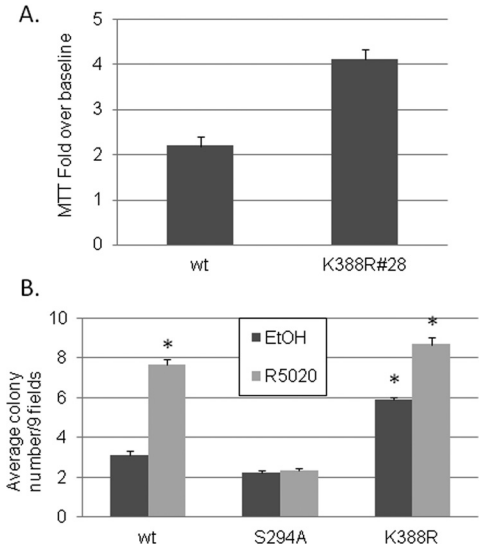

Fig. S1. Sumoylated PR represses breast cancer cell growth in the absence of progestins. (*A*) PR sumoylation represses breast cancer cell growth. Triplicate cultures of T47D cells stably expressing wt or K388R (clone 28) were subjected to an MTT assay under steroid hormone-free conditions supplemented with vehicle control (EtOH) or R5020 for 6 days. Bars (*y* axis) represent a fold increase in viable cell number over baseline (±SD). (*B*) PR sumoylation represses breast cancer cell anchorage independent growth. Triplicate cultures of T47D cells stably expressing wt, S294A, or K388R (clone 7) PR-B were plated under steroid stripped serum conditions in soft agar for 21 days. Colonies were counted and expressed as average colony number per nine fields (±SD, $*P < 0.002$). Asterisk denotes statistical significance relative to starved wt cells as determined by unpaired student t-tests.

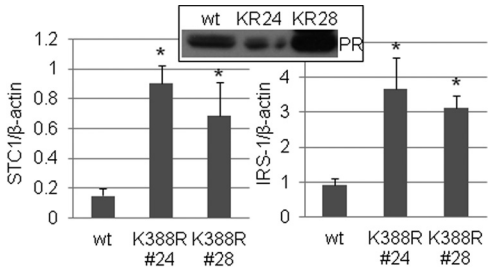

Fig. S2. Multiple clones expressing SUMO-deficient K388R PR-B display increased STC1 and IRS-1 levels. Desumoylated PR induces IRS-1 and STC1 in the absence of ligand. T47D cells stably expressing wt or K388R (clones 24 and 28) PR-B were plated in triplicate cultures, starved for 48 h and RNA was harvested. Real-time PCR was performed using primers specific for IRS-1, STC1, and β-actin. Values are expressed as target gene, STC1 (*left panel*) or IRS-1 (*right panel*), normalized to β-actin ($\pm$SD, $^*P < 0.02$).

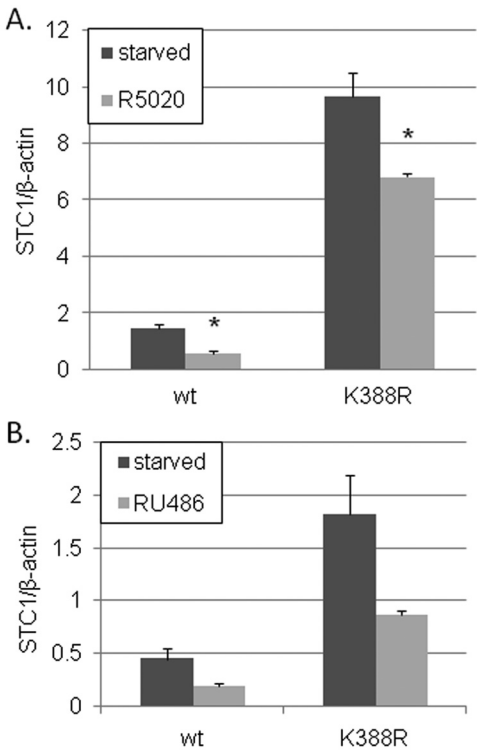

Fig. S3. Progestins mediate decreased STC1 expression. (*A*) STC1 regulation by R5020. T47D cells stably expressing wt or K388R PR-B were serum starved for 24 h and treated for 6 h with R5020 (10^{-8} M). Triplicate cultures were harvested, RNA was isolated, and real-time PCR was performed using primers specific for STC1 and β-actin; bars are expressed as STC1 mRNA normalized to β-actin ($\pm$SD, $P < 0.02$). (*B*) PR antagonist regulation of STC1. T47D cells stably expressing wt or K388R PR-B were serum starved for 24 h and treated for 6 h with RU486 (10^{-7} M). Triplicate cultures were harvested, RNA was isolated, and real-time PCR was performed using primers specific for STC1 and β-actin; bars are expressed as STC1 mRNA normalized to β-actin ($\pm$SEM).

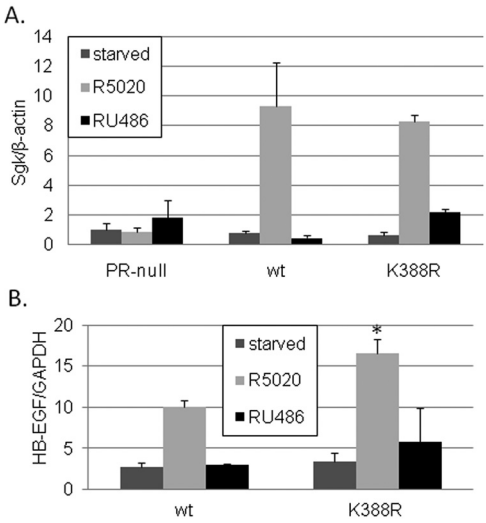

Fig. S4. PR sumoylation regulates the expression of a subset of PR target genes. (*A*) PR regulation of SGK. T47D cells stably expressing wt, K388R, or S294A PR-B were serum starved for 24 h and treated for 6 h with R5020 (10^{-8} M) or RU486 (10^{-7} M). Triplicate cultures were harvested, RNA was isolated, and real-time PCR was performed using primers specific for SGK and β-actin; bars are expressed as SGK mRNA normalized to β-actin ($\pm$SD). (*B*) HB-EGF regulation by PR. T47D cells stably expressing either wt or K388R PR-B were plated in triplicate cultures, starved for 24 h, and treated for 6 h with R5020 (10^{-8} M) or RU486 (10^{-7} M). RNA was harvested and real-time PCR was performed using primers specific for HB-EGF and GAPDH; bars represent HB-EGF mRNA normalized to GAPDH ($\pm$SD, $^*P < 0.02$). Asterisks denote statistical significance relative to wt cells as determined by unpaired Student *t* tests. Unless otherwise noted K388R PR-B cells are clone 7.

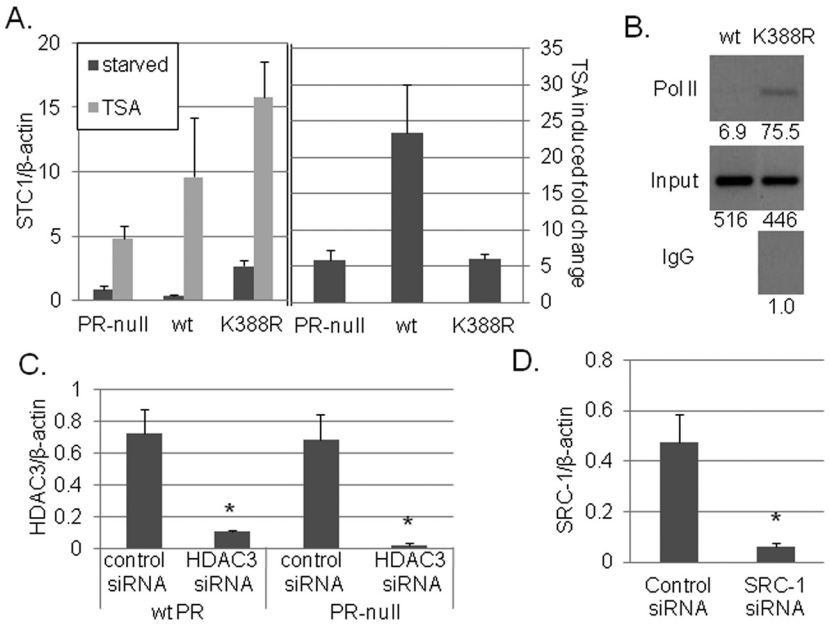

Fig. S5. PR sumoylation alters the recruitment of cofactors to PR target promoters. (*A*) HDAC inhibition relieves PR transcriptional repression by sumoylation. PR-null T47D cells or T47D cells stably expressing either wt or K388R PR-B were plated in triplicate cultures and treated for 24 h with vehicle or TSA (10 μM) under serum starved conditions. (*Left panel*) Real-time PCR was performed to evaluate STC1 transcript levels normalized to β-actin ($\pm$SD). (*Right panel*) Bars represent TSA induced fold change of STC1 mRNA levels over vehicle controls ($\pm$SEM). (*B*) RNA Polymerase II associates with STC1 DNA in K388R PR-B–expressing cells. T47D cells expressing wt or K388R PR-B were serum starved for 48 h and harvested for a ChIP assay using an antibody against Pol II or normal mouse IgG for an isotype control. PCR was performed using primers specific for STC1 Exon 1. Densitometry values are listed below each band. (*C*) Transient knockdown of HDAC3. PR-null HeLa cells and HeLa cells stably expressing wt PR-B were transfected with HDAC3 or negative control siRNA, starved for 72 h and harvested for RNA. Real-time PCR was performed using primers specific for HDAC3 and β-actin; bars represent HDAC3 mRNA normalized to β-actin ($\pm$SD, $^*P < 0.006$). (D) Transient knockdown of SRC-1. T47D cells stably expressing K388R PR-B were transfected with SRC1 or negative control siRNA, starved and harvested for RNA. Real-time PCR was performed using primers specific for SRC1 and β-actin; bars represent SRC1 mRNA normalized to β-actin ($\pm$SD, $^*P < 0.005$). Asterisks denote statistical significance relative to control siRNA as determined by unpaired Student *t* tests.

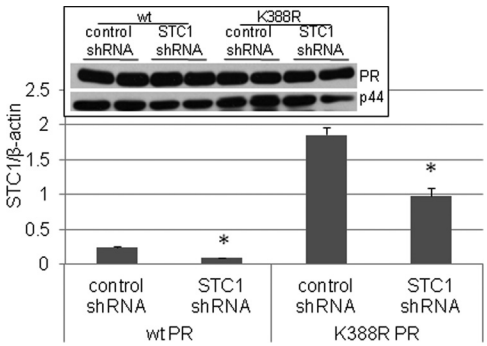

Fig. S6. Stable knockdown of STC1 in T47D breast cancer cells. (*A*) Stable STC1 knockdown by shRNA. T47D cells stably expressing either wt or K388R PR-B and either control or STC1 specific shRNA were plated in triplicate cultures. Cells were starved for 48 h and harvested for RNA isolation. Real-time PCR was performed to assess the levels of STC1 transcript relative to β-actin ($\pm$SD, $*P < 0.0006$). (*Inset*) T47D cells stably expressing wt or K388R PR-B and either control or STC1 specific shRNA were plated in duplicate and harvested for Western blot using PR and p44 MAPK specific antibodies.

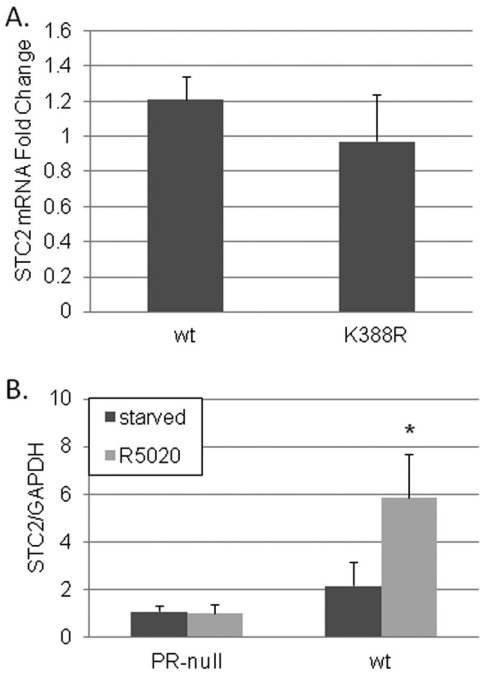

Fig. S7. STC2 mRNA levels are unaltered in STC1 knockdown cells and induced by progestin. (*A*) STC2 mRNA levels in stable STC1 knockdown cells. T47D cells stably expressing either wt or K388R PR-B and either control or STC1 specific shRNA were plated in triplicate cultures. Cells were starved for 48 h and harvested for RNA isolation. Real-time PCR was performed to assess the levels of STC2 transcript relative to β-actin; bars represent STC2 levels in STC1 shRNA expressing cells normalized to cells expressing control shRNA ($\pm$SD). (*B*) STC2 mRNA levels are induced by progestin. PR-null or T47D cells stably expressing wt PR-B were starved for 48 h and treated for 6 h with R5020 (10^{-8} M). Triplicate cultures were harvested and real-time PCR was performed to asses mRNA levels of STC2 relative to β-actin ($\pm$SD, $P < 0.04$).

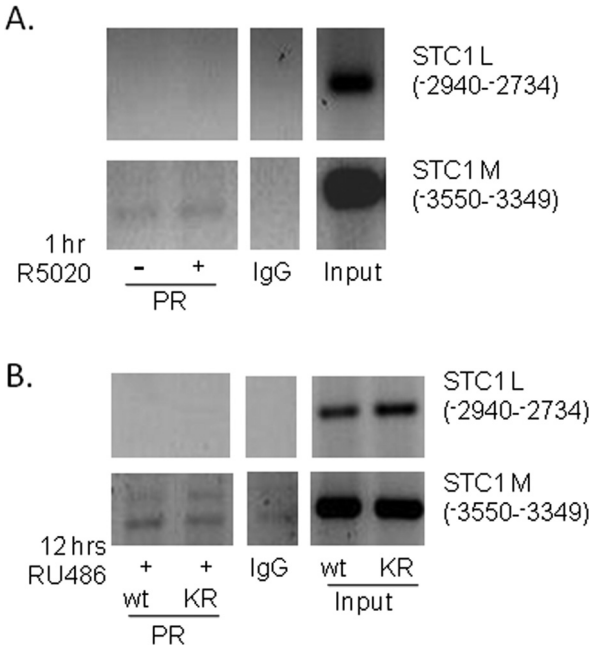

Fig. S8. PR remains bound to the STC1 promoter in the presence of progestins. (*A*) Liganded PR binds the STC1 promoter. T47D cells expressing wt PR-B were serum starved for 48 h, treated for 1 h with vehicle or R5020 (10^{-8} M), and harvested for a ChIP assay using an antibody against PR or normal mouse IgG (isotype control). PCR was performed using primers specific for the STC1 promoter. (*B*) PR binds the STC1 promoter in the presence of RU486. T47D cells expressing wt or K388R PR-B were serum starved for 48 h, treated for 12 h with RU486 (10^{-7} M), and harvested for a ChIP assay using an antibody against PR or normal mouse IgG (isotype control). PCR was performed using primers specific for the STC1 promoter.