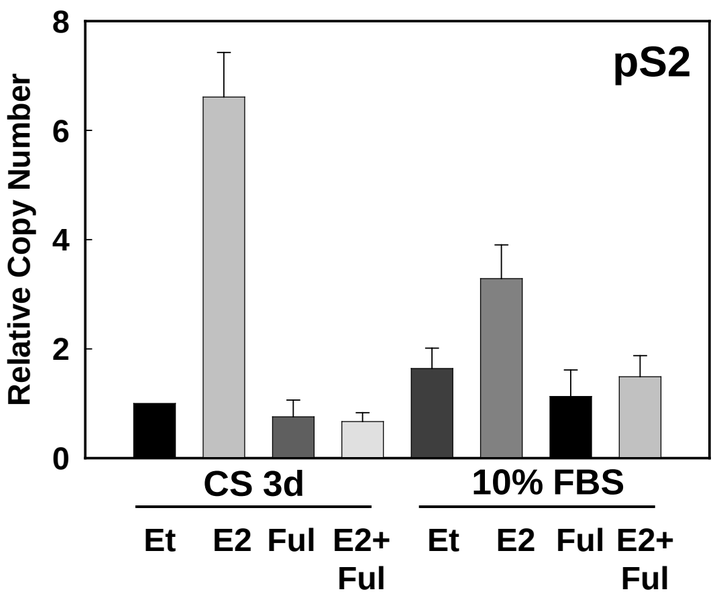
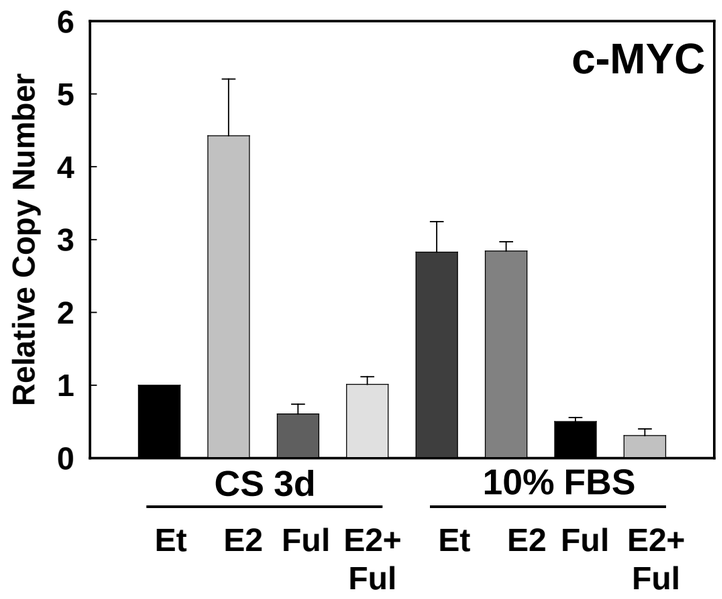
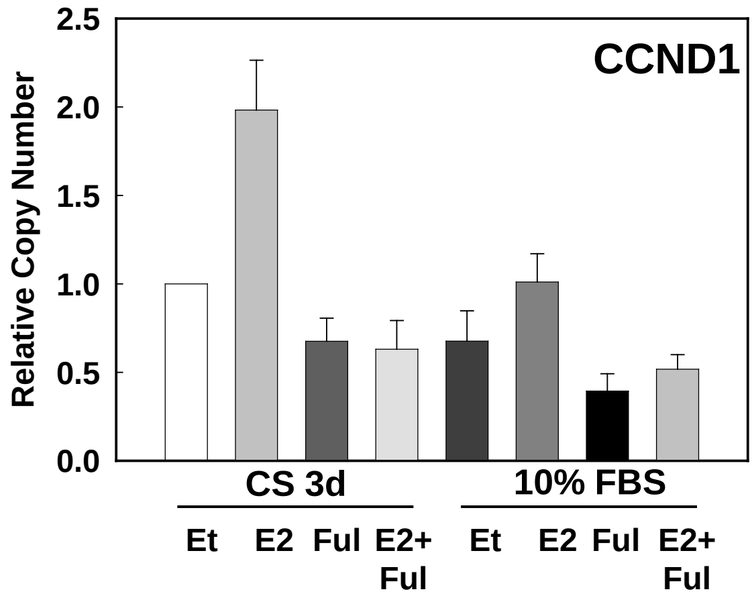
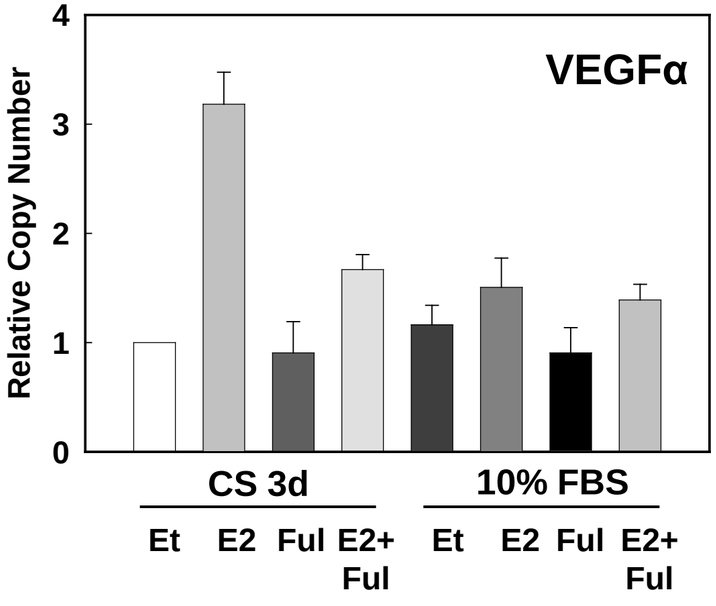
Supplemental Figure 1









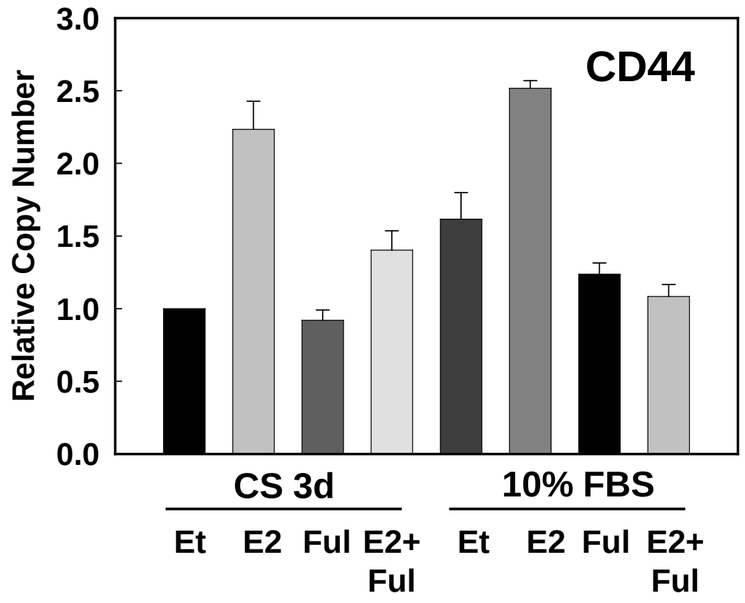


Figure S1. E2 induces expression of VEGFα, CD44, cyclin D1, c-MYC and pS2. MCF-7 cells were grown in regular RPMI medium containing 10%FBS, or in phenol red-free RPMI containing 10%DCC-FBS (CS) for a total of 3 days. Cells were treated with 5nM of E2 for 4hours (or Ethanol control), or 1μM Fulvestrant for 24 hours, or the combination prior to harvest. The mRNA levels of the target genes were measured by Real-time RT-PCR. Data were expressed as relative copy number after normalization to internal control of 18s rRNA. $*P \leq 0.001$.

Supplemental Figure 2

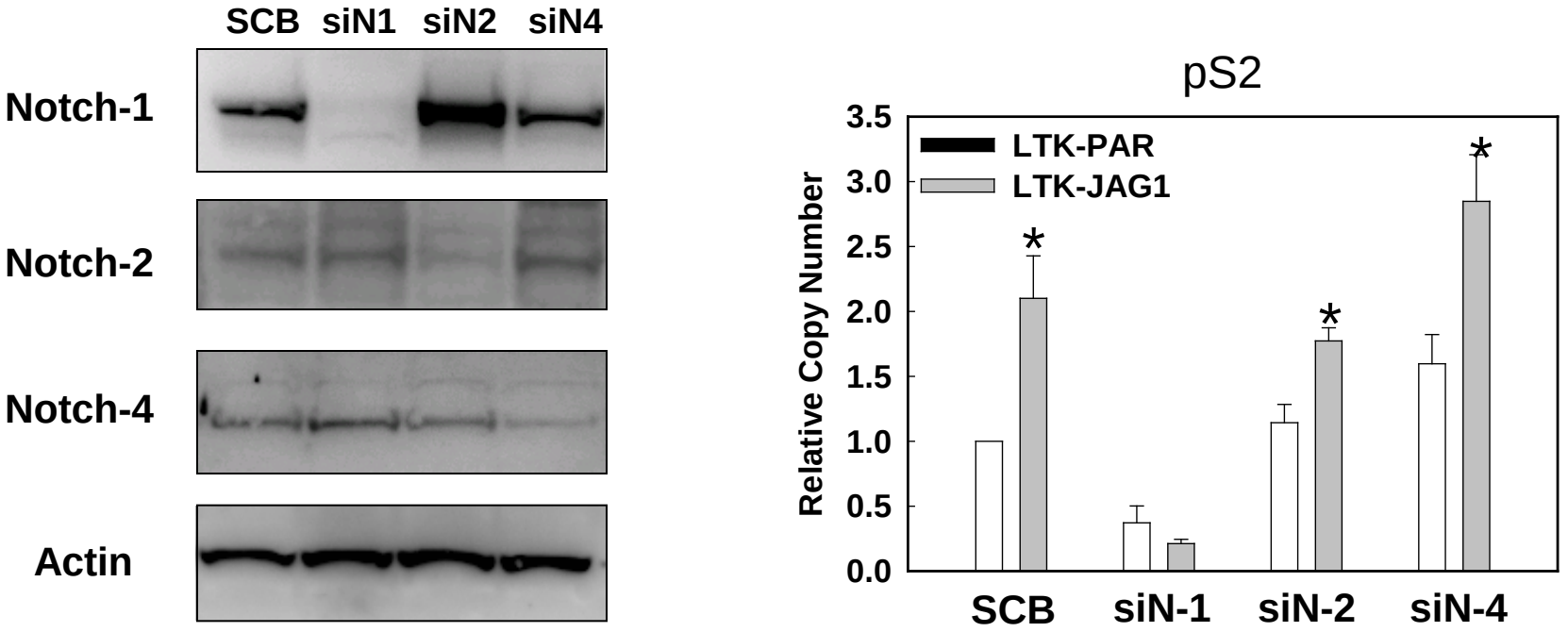


Figure S2 The induction of pS2 transcription by Jagged-1 is mediated by Notch-1 and not Notch-2 or Notch-4. A. MCF-7 cells were grown in charcoal-stripped medium for 3 days, and transfected with Notch-1, Notch-2, or Notch-4 siRNA (or scrambled control). Western blots show efficient down-regulation of specific Notch paralogs. B. pS2 Real time RT-PCR from cells transfected with different siRNAs in charcoal stripped medium (as described in A) and co-cultured with LTK cells for 12 hours. Data expressed as relative copy number after normalization to internal control RPL13aR mRNA level. $^{*}P \leq 0.001$.

Supplemental Figure 3

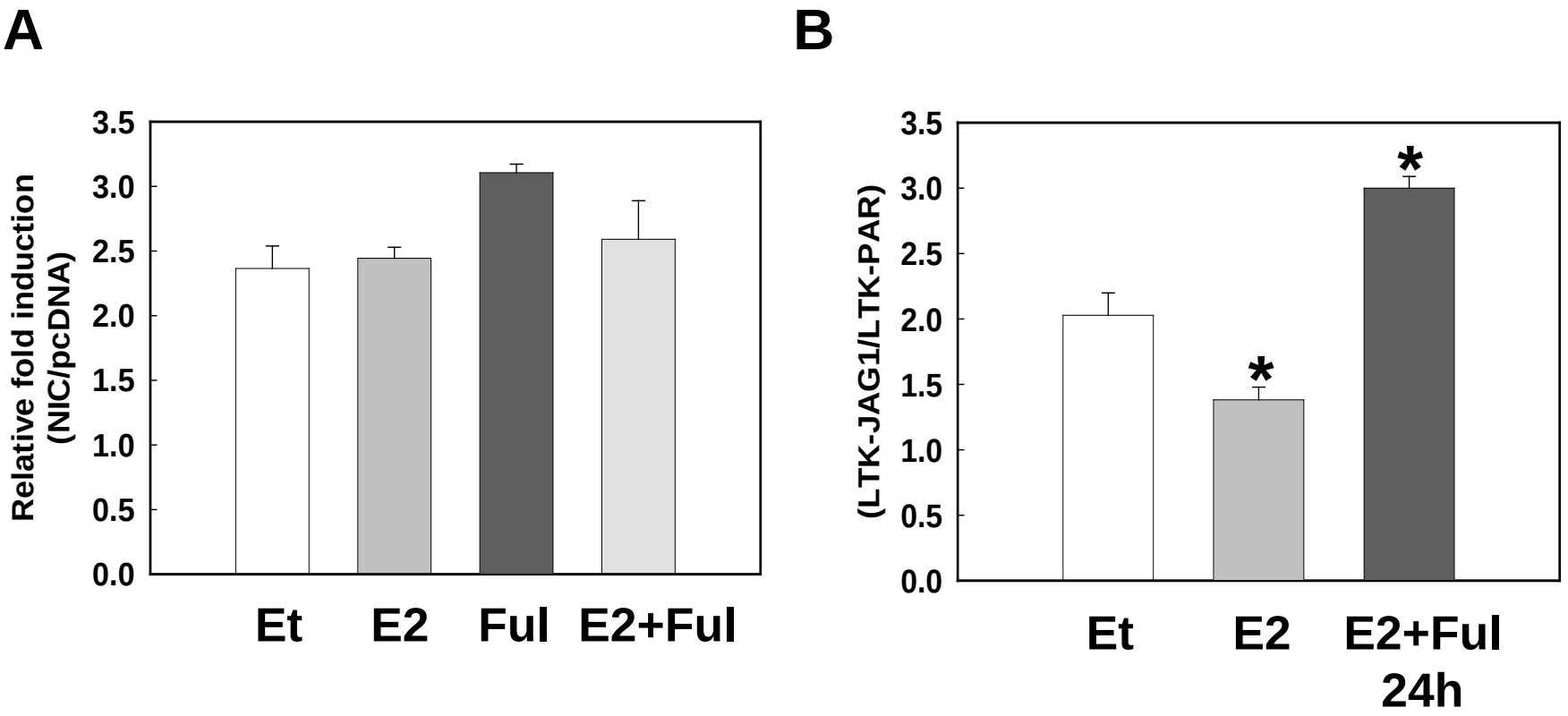


Figure S3. E2 or fulvestrant had no effect on Notch-induced HEY1 expression. HEY-1 real-time RT-PCR. MCF-7 cells were grown in charcoal stripped medium for 3 days prior to harvest. A. 12 h after serum starvation, MCF-7 cells were transfected with Notch-1IC (NIC) or pcDNA vector control. Cells were treated with 5nM E_2 (4 h), 1μM fulvestrant (24 h) or the combination before harvest. Data are expressed as relative fold induction by NIC over pcDNA after normalization to the internal control 18S rRNA. * $P \leq 0.001$. B. MCF-7 cells were treated with E_2 alone or in combination with fulvestrant, and co-cultured with Jagged-1 expressing LTK fibroblasts for 12 h. Data are expressed as relative fold induction by LTK-Jagged-1 over LTK-Parental after normalization to the internal control RPL13a. * $P \leq 0.001$.

Supplemental Figure 4

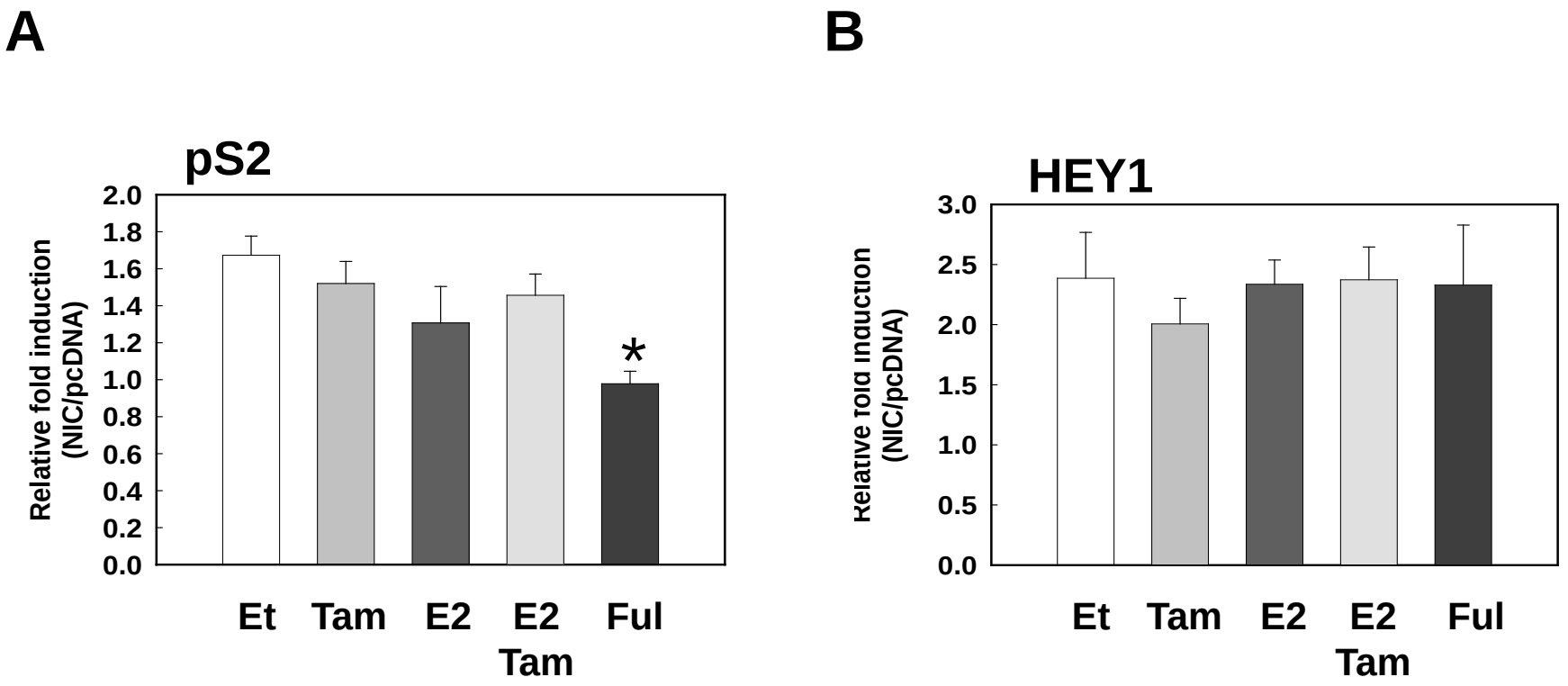
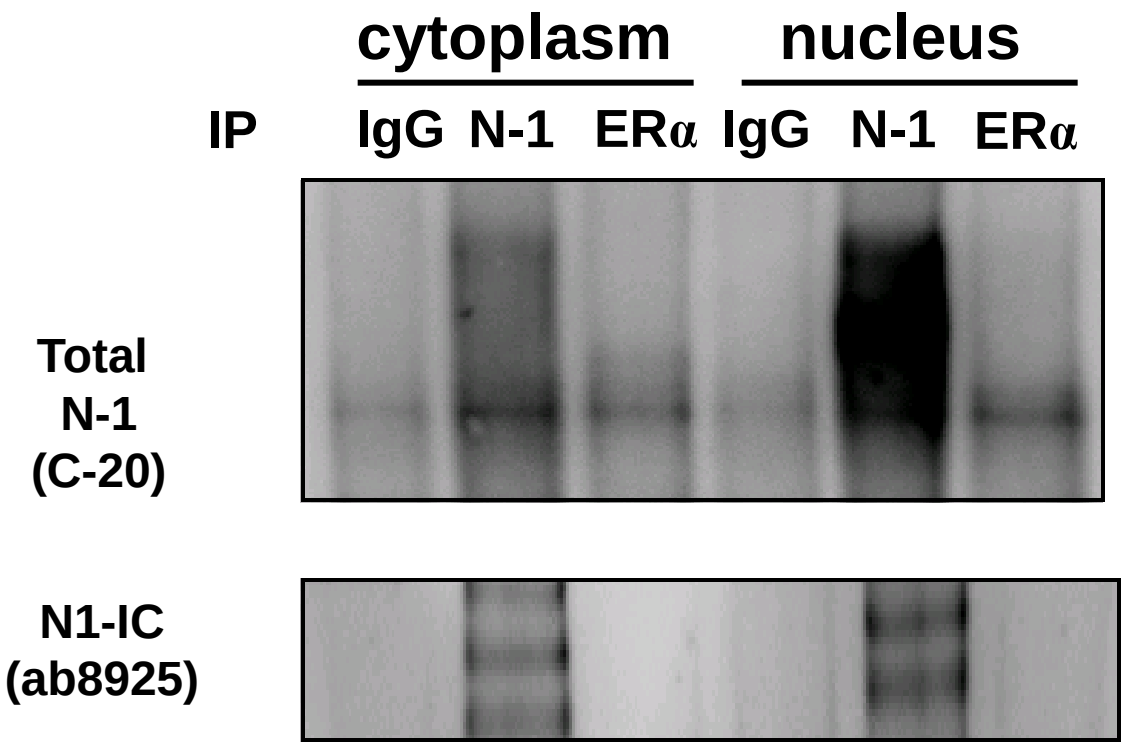


Figure S4. Tamoxifen did not affect pS2 or HEY1 induction by Notch-1IC. In all experiments, MCF-7 cells were grown in charcoal stripped medium for a total of 3 days. Cells were transfected with NIC or empty vector pcDNA 48 h prior to harvest. Cells were treated with either 1μM 4-hydroxytamoxifen, 1nM E_2, or the combination, or 1μM fulvestrant 24 h prior to harvest. pS2 (A) or HEY1 (B) mRNA levels were measured by real-time RT-PCR. Data were expressed as relative fold induction by NIC over pcDNA after normalization to internal control 18S rRNA. *$P \leq 0.001$.

Supplemental Figure 5



The Notch-1-ERα interaction is DNA-dependent. IP-Western with cytoplasmic or nuclear extracts treated with 50μg/mL ethidium bromide for 30 min on ice before immunoprecipitation.

Supplemental Figure 6

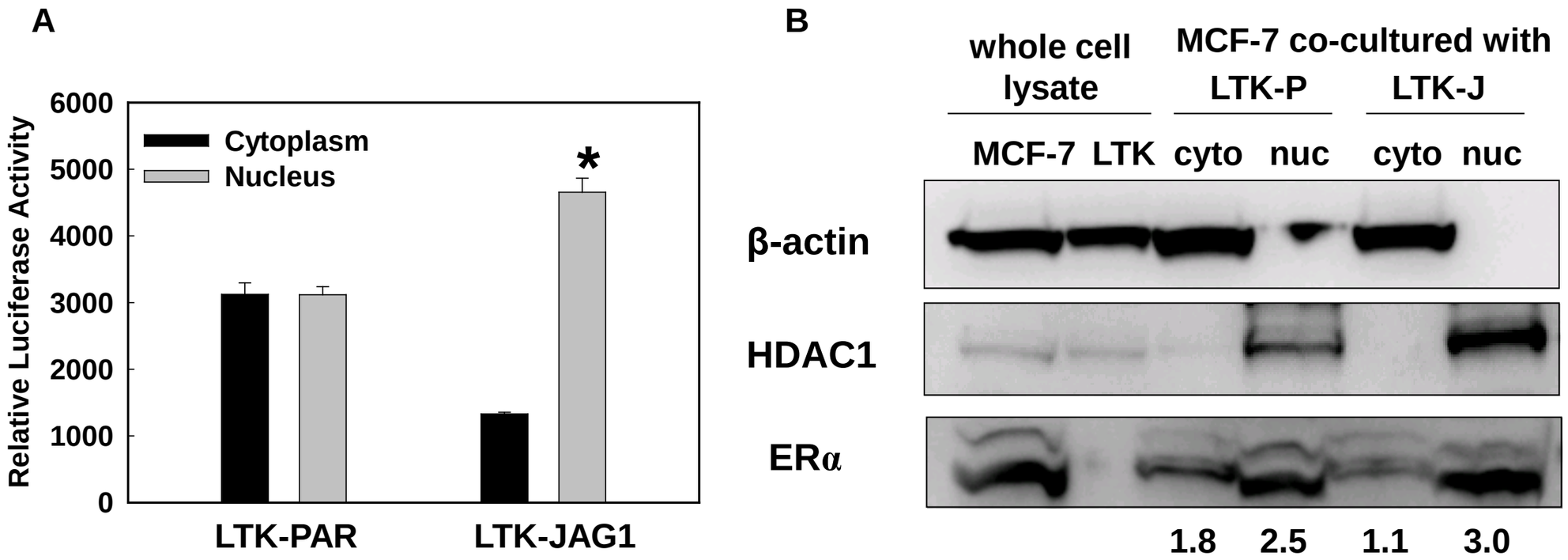


Figure S6. Activation of Notch increases the nuclear localization of ERα. MCF-7 cells were transfected with ER-RL construct and grown in charcoal-stripped medium for 3 days before co-cultured with LTK-Jagged1 (or LTK-Parental) cells for 45 minutes. Nuclear extraction was performed. A. Renilla luciferase activity of cytoplasmic lysates or nuclear extracts was measured. B. Western blot for β-actin, HDAC1 and ERα with MCF-7 or LTK whole cell lysates, and the cytoplasmic (cyto) and nuclear (nuc) fractions after co-culture. Numbers indicate densitometric scanning of ERα bands divided by loading controls. Note that the ratio between nuclear and cytoplasmic ERα increased from 1.39 (2.5/1.8) to 2.7 (3/1.1), consistent with the ER-RL data in panel A.

Supplemental Figure 7

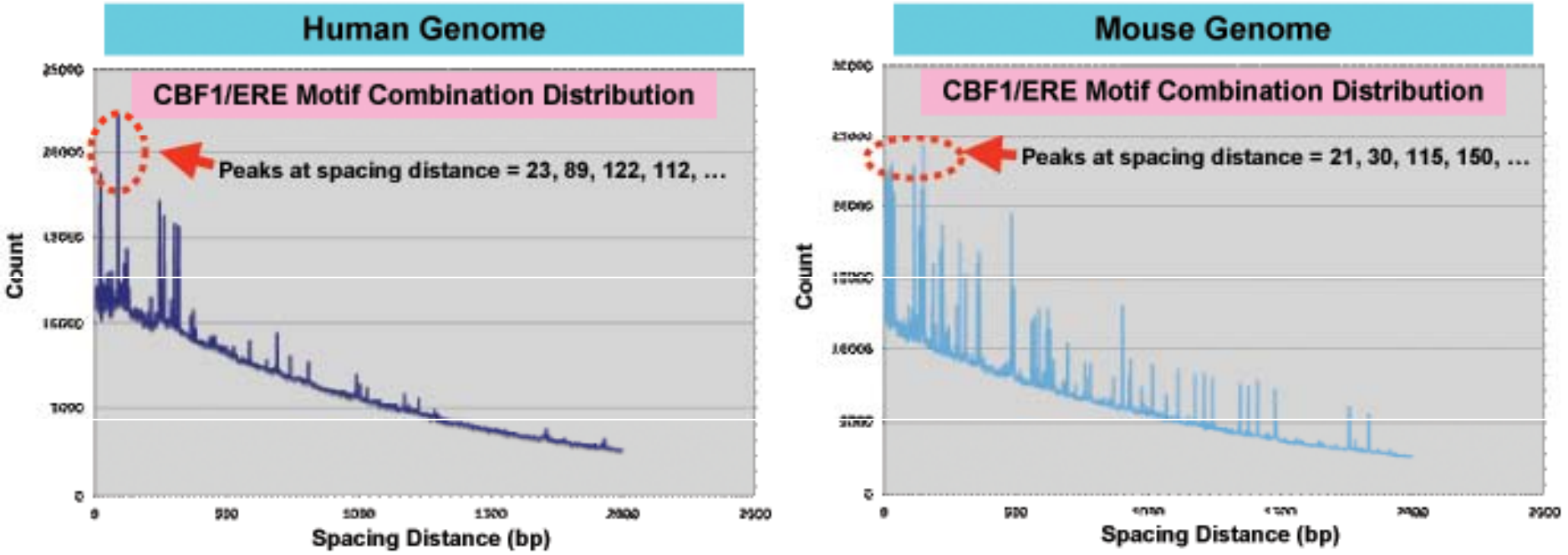


Figure S7. Human and Mouse CBF1/ERE Motif Combination Distributions. Peaks indicate spacing distances at which motifs occur with significantly non-random frequencies (P-value < 1.0E-303 in all cases) with respect to the specific spacing distance between motifs. The distances were defined as the number of nucleotides between the last nucleotide of the first motif and the first nucleotide of the next motif in a motif pair.

Supplemental Figure 8

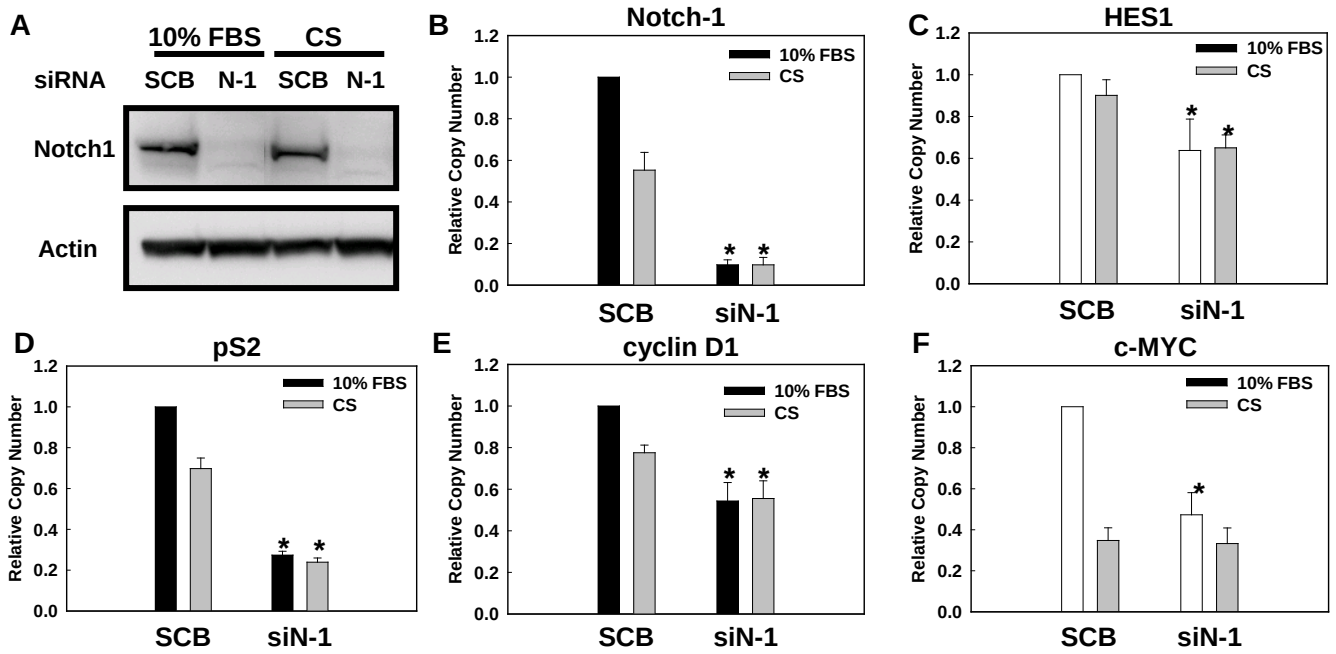


Figure S8. Notch-1 siRNA downregulates a subset of E_2-dependent genes. MCF-7 cells were grown in standard RPMI medium containing 10%FBS, or in phenol red-free RPMI containing 10% DCC-FBS (CS) for a total of 3 days. Cells were transfected with Notch-1 siRNA or scrambled (SCB) control 48 h prior to harvest. A. Western blot showing downregulation of Notch-1 expression by siRNA. Actin was used as loading control. B-F. mRNA levels of target genes were measured by real-time RT-PCR. Data are expressed as relative copy number after normalization to internal control 18S rRNA. *P ≤0.001..

Supplemental Figure 9

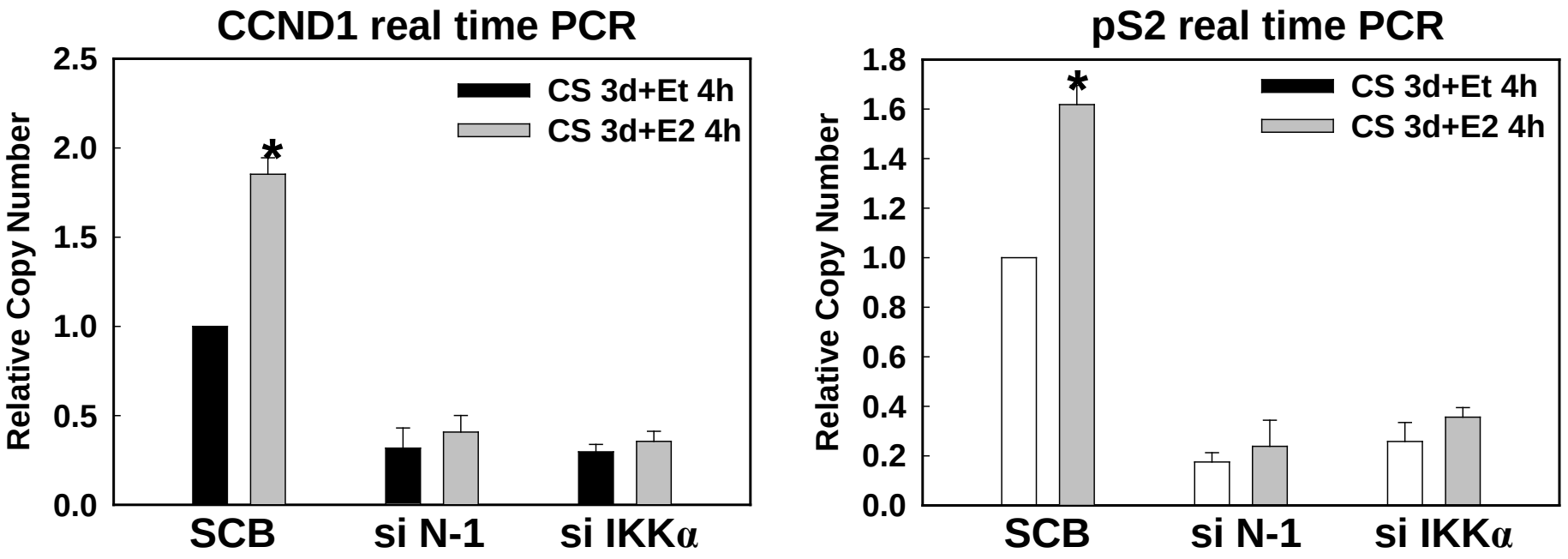


Figure S9. Notch-1 or IKKα siRNA prevents E_2 from activating cyclin D1 and pS2 transcription. MCF-7 cells were grown in charcoal-stripped medium for a total of 3 days. Cells were transfected with Notch-1 siRNA, IKKα siRNA or scrambled control. 48 h later, cells were treated with 5nM E_2 or ethanol control for 4 h. mRNA levels were measured by real time RT-PCR. Data are expressed as relative copy number after normalization to internal control 18S rRNA.

Supplemental Figure 10

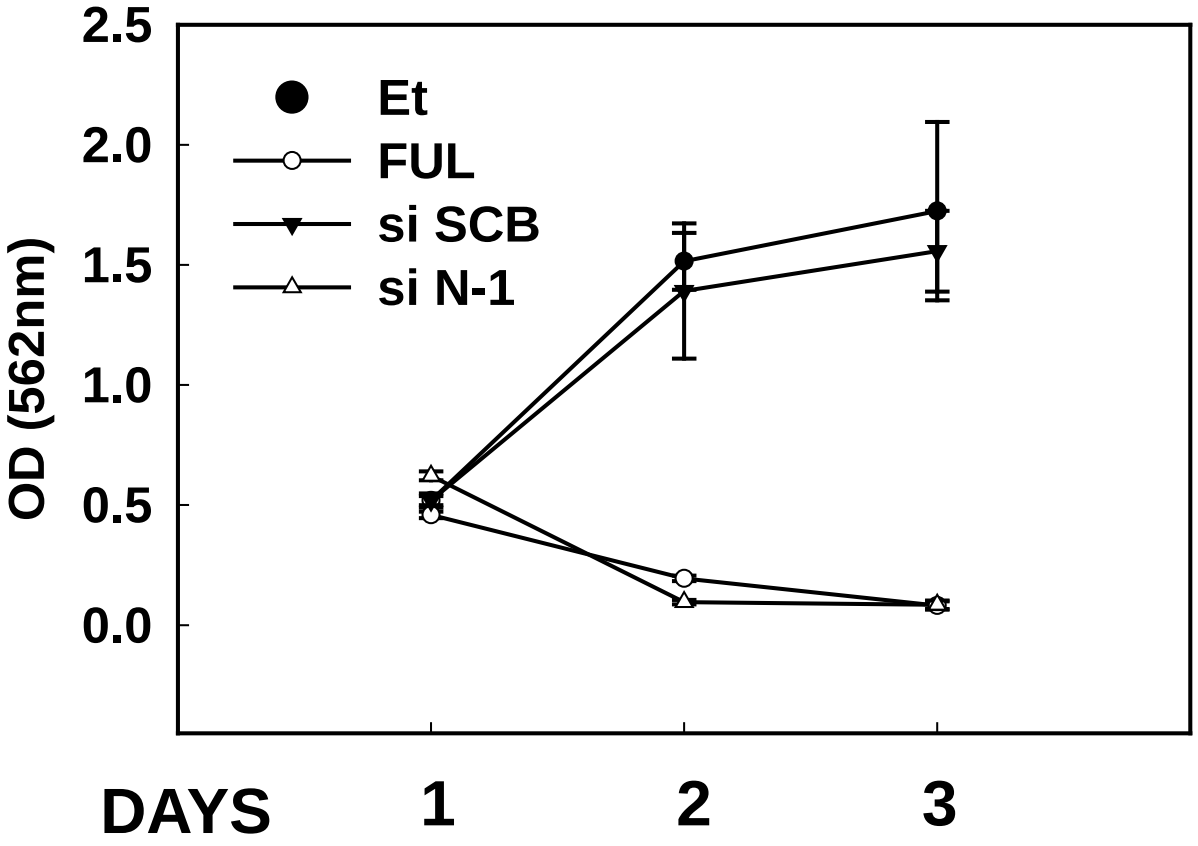


Figure S10. Notch-1 Knockdown inhibits MCF-7 cells growth. MCF-7 cells were grown in charcoal-stripped medium for total of 3 days. Cells were treated with 1μM Fulvestrant (or Et control), or transfected with Notch-1 siRNA (or scrambled control) on day 0. Cell growth were measured by crystal violet assay for 3 consecutive days. Error bars are standard deviations (n =3)

Supplemental Figure 11

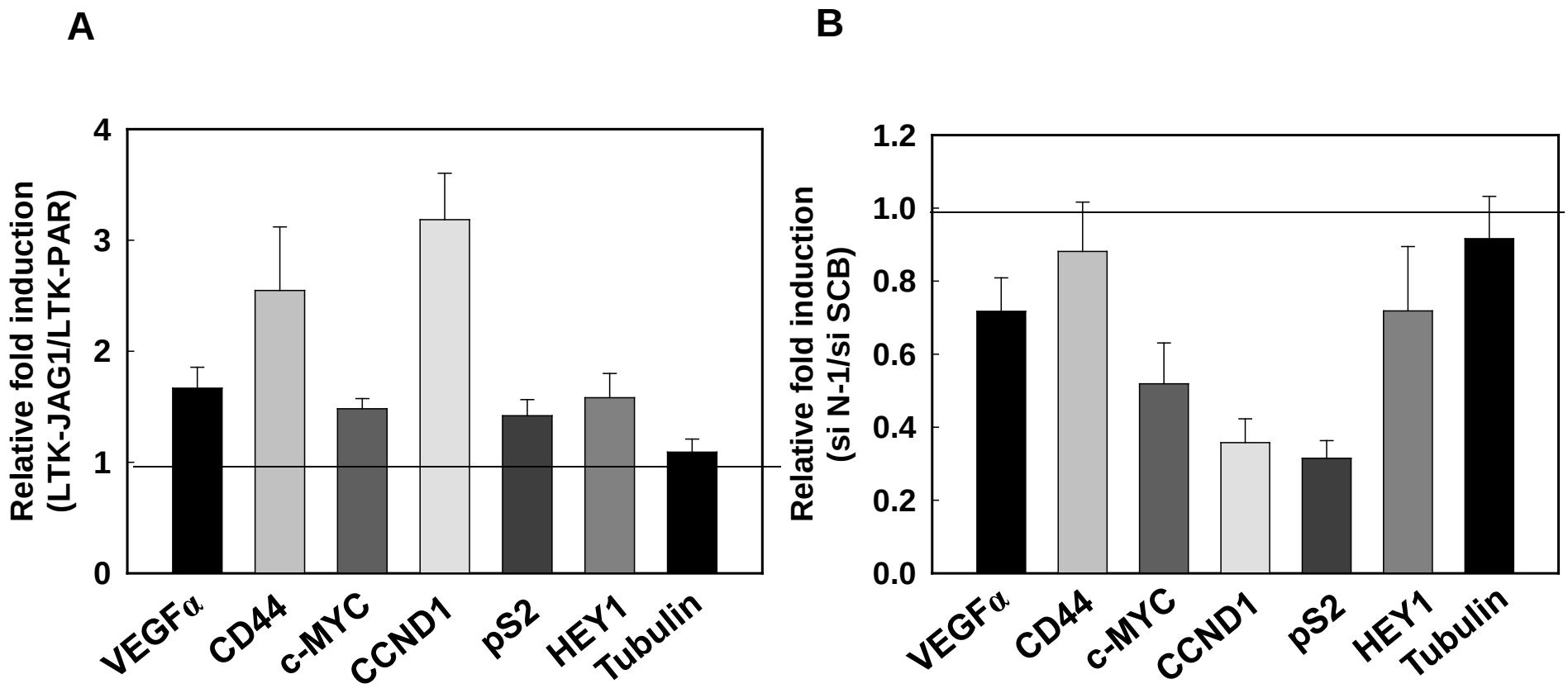


Figure S11. Notch-1 regulates ERα-targets in T47D:A18 cells in the absence of E2. In all experiments T47D:A18 cells were grown in phenol red-free RPMI containing 10% DCC-FBS for 3 days prior to harvest. (A) A18 cells were co-cultured with mouse fibroblasts expressing Jagged-1 (LTK-JAG1), or vector-transfected controls (LTK-PAR) for 12 h prior to harvest. (B) A18 cells were transfected with Notch-1 siRNA (or the scrambled control). The mRNA levels of VEGFα, CD44, c-MYC, CCND1, pS2, HEY1 and β-Tubulin were measured by real time RT-PCR with validated human-specific primers. Values are expressed as relative fold induction after internal normalization for RPL13a mRNA.

Supplemental Figure 12

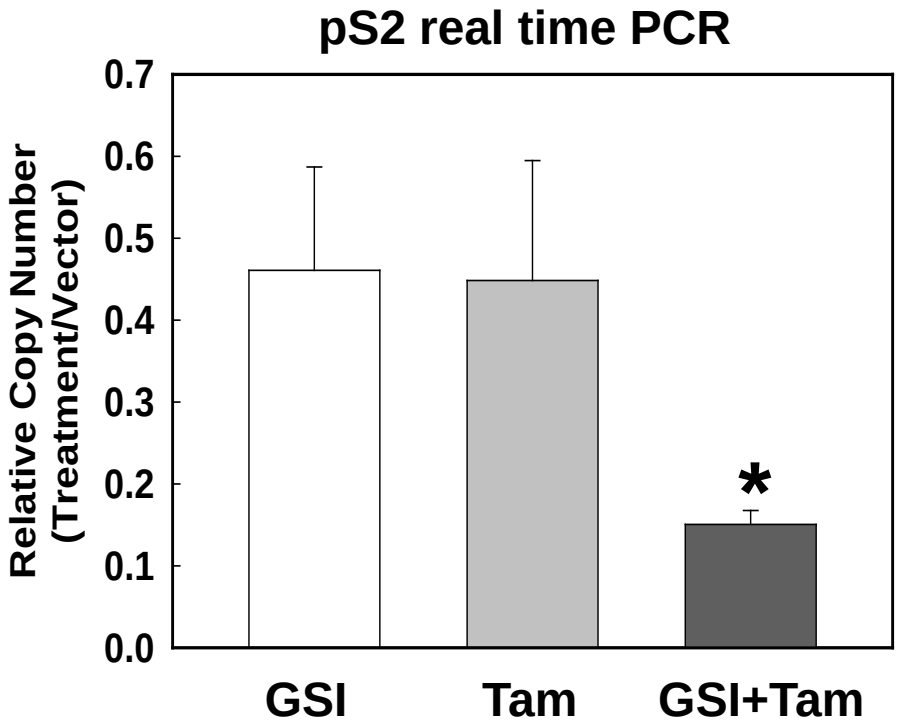


Figure S12. GSI decreases pS2 levels in T47D:A18 xenograft tumors. T47D:A18 established xenografts receiving menopausal estrogen levels were treated with GSI (MRK003 100 mg/kg orally), tamoxifen (0.5 mg tamoxifen in carboxymethylcellulose suspension) or the combination thereof. Tumors were harvested and mRNA levels were measured by real-timer RT-PCR. Either drug alone inhibited pS2 expression. The combination GSI/tamoxifen caused further downregulation of pS2 mRNA level, and was significantly different not only from controls but also from each individual treatment. * $P \leq 0.001$.

Supplemental Figure 13

The primers for real time RT-PCR measurement:

18s rRNA forward: TGA TTA AGT CCC TGC CCT TTG T
18s rRNA reverse: TCA AGT TGC ACC GTC TTC TCA G
Tubulin β forward: ACA TCC AGG CTG GTC AGT GT
Tubulin β reverse: CCA CCT GTG GCT TCA TTG TA
pS2 forward: 5’-CAC CAT GGA GAA CAA GGT GA
pS2 reverse: 5’-TCG AAA CAG CAG CCC TTA TT
HEY1 forward: CCT GGG ACT GCC ATA TTT TC
HEY1 reverse: CCA GTT CAGTGGAGGTCGTT
VEGFα forward: CCT TGC TGC TCT ACC TCC AC
VEGFα reverse: CAG TGG GCA CAC ACT CCA
CD44 forward: AGC AAC CAA GAG GCA AGA AA
CD44 reverse: GTG TGG TTG AAA TGG TGC TG
CCND1 forward: CCT GTC CTA CTA CCG CCT CA
CCND1 reverse: CCAGGT CCA CCT CCT CCT
C-MYC forward: CGA CTC TGA GGA GGA ACA AG
C-MYC reverse: TGC GTA GTT GTG CTG ATG TG
HES1 forward: CAT TGA TCT GGG TCA TGC AG
HES1 reverse: GTG CGC ACC TCG GTA TTA AC

The primers for chromatin immunoprecipitation:

RPL13a forward: CAT AGG AAG CTG GGA GCA AG
RPL13a reverse: ACA AGA TAG GGC CCT CCA AT
pS2 forward: CTA GAC GGA ATG GGC TTC ATG AGC
pS2 reverse: AGG ATT TGC TGA TAG ACA GAG ACG AC
HEY1 forward: AGC GTG GGA AAG GAT GGT TG
HEY1 reverse: CTC GCT TCA TGC TGG CTC CC
VEGFα forward: CTG GAC ACT TCC CAA AGG AC
VEGFα reverse: TTT CTG ACC TCCCAA ACA GC
CD44 forward: GAG AGG TGC CCA TTC ACA CT
CD44 reverse: TTG GAT ATC CTG GGA GAG GA
CCND1 P1 forward: CTT TCT CCT GAC CGA CCA TC
CCND1 P1 reverse: GAA GCA GGA ACT GCG GAT T
CCND1 P2 forward: CCG ACT GGT CAA GGT AGG AA
CCND1 P2 reverse: CCAAGG GGG TAA CCC TAA AA
C-MYC forward: CAT GCG GCT CTC TTA CTC TG
C-MYC reverse: CGG AGA TTA GCG AGA GAG GA

Supplemental Table 1

NCRE (CSL)	ERE
TGAGAA	GGTCA***TGACC
TGGGAA	GGTCA***CGACC
TGTGAA	GGTCG***TGACC
TTCTCA	GGTCG***CGACC
TTCCCA	GGTCA
TTCACA	GGTCG
	TGACC
	CGACC

Table SI. Sequences of core NCRE and ERE used for our analysis. Note that EREs were used as tandem sites or half-sites. NCRE, Notch-CSL response element.