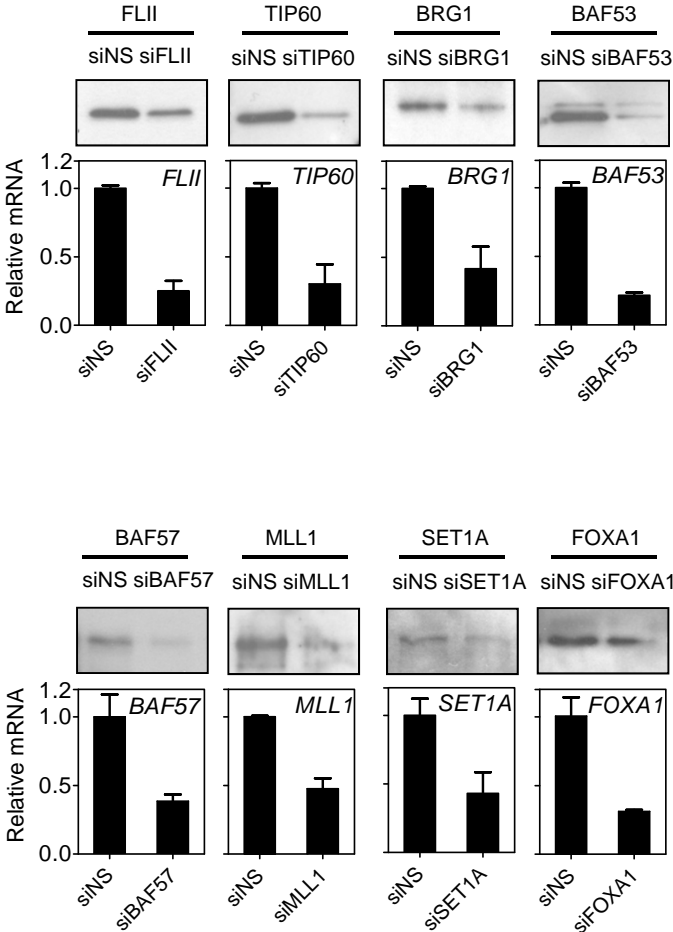
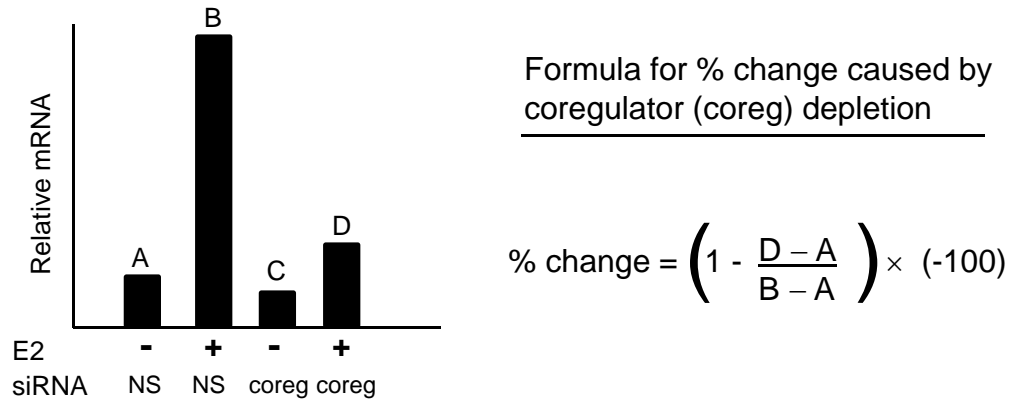


Supplemental Figure 1. Estrogen receptor-dependent expression of *PgR*. A, Time course induction of *PgR* expression in MCF-7 cells upon E2 treatment. MCF-7 cells were treated with E2 (10 nM) for different times as indicated. Total RNA was analyzed for *PgR* mRNA by qRT-PCR and normalized to the level of *GAPDH* mRNA. B, The indicated pre-mRNA levels in MCF-7 cells were measured by qRT-PCR using primers spanning the 3' end of exon 1 of *PgR-A* and the 5' end of intron 1. Cells were treated for the indicated time with 10 nM E2. C, Effect of ER α antagonist on E2-induced *PgR* expression. MCF-7 cells were treated with ICI 182,780 (100 nM) for 1 hour before adding E2 and during treatment with 10 nM E2 for 16 hours. Level of *PgR* mRNA was determined as in A. D, Comparison of estrogen effect on *PgR* expression in ER α positive (MCF-7) and ER α negative (MDA-MB231) breast cancer cells. After incubation in hormone-free medium for 3 days, cells were treated with 10 nM E2 for 16 hours before harvesting cells and determining *PgR* mRNA level as in A.



Supplemental Figure 2. Depletion of coregulators by siRNA. MCF-7 cells were transfected with siRNA against the indicated coregulators or non-specific siRNA (siNS). Levels of the indicated proteins were assessed by immunoblotting; actin was used as a loading control (not shown). Coregulator mRNA levels were determined by qRT-PCR and expressed relative to *GAPDH* mRNA, as described in Fig. 1A. This is an independent experiment from the one shown in Fig. 1.

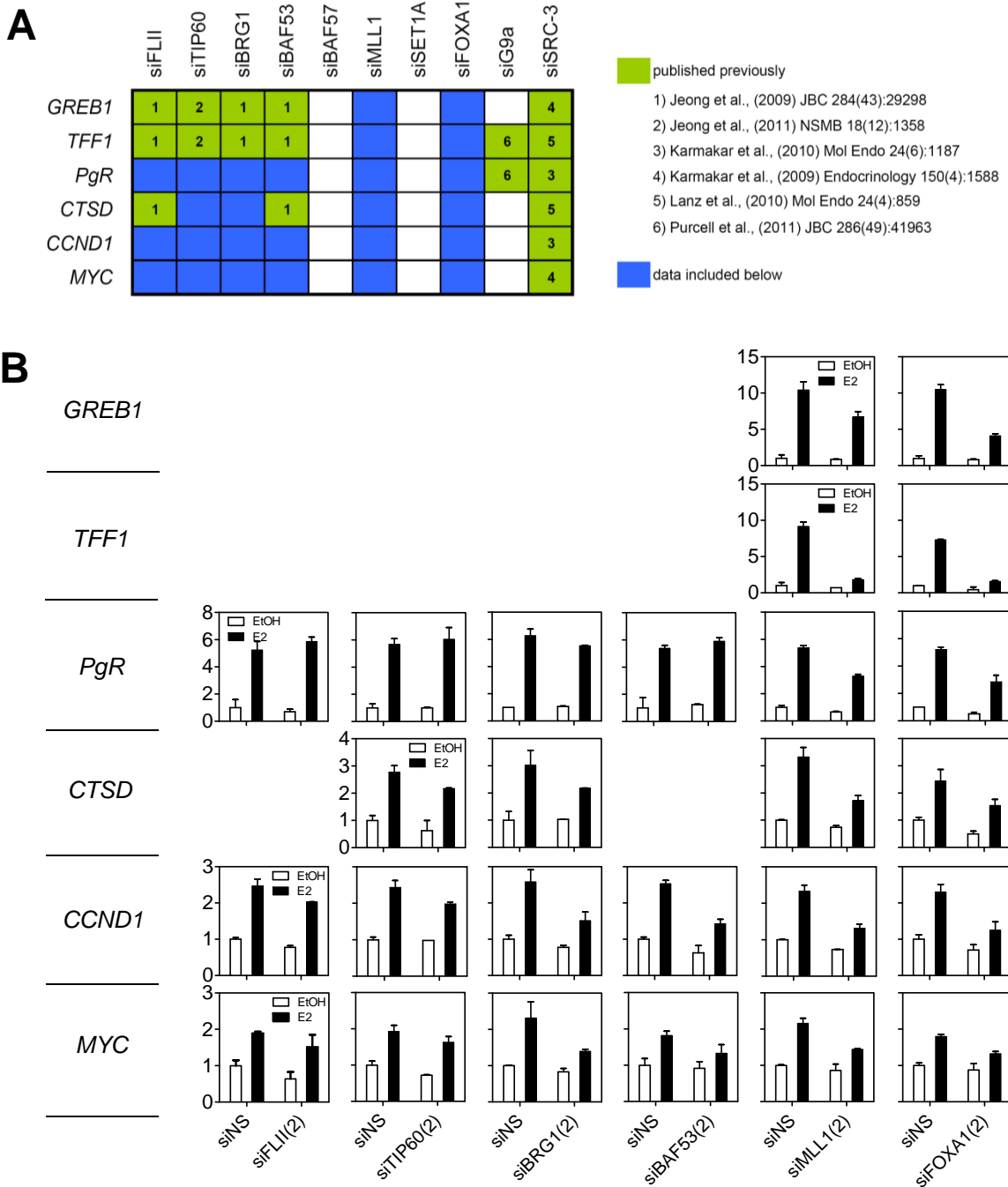
Supplemental Figure 3



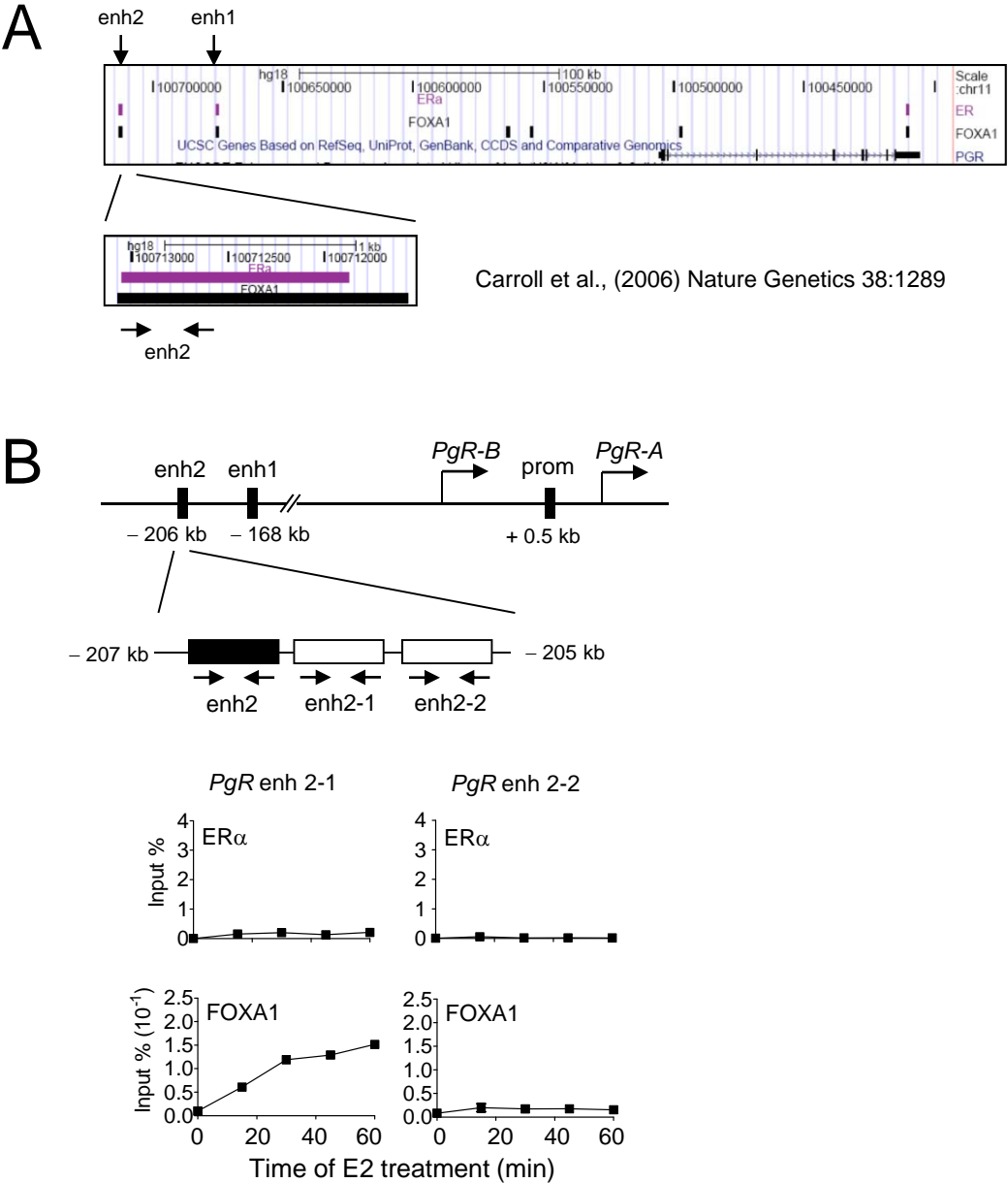
Supplemental Figure 3. Method for calculating the percent change in mRNA level caused by coregulator depletion. This formula was used to calculate the percent change in the E2-induced mRNA levels of the six target genes, caused by depletion of a specific coregulator. B – A represents the increase or “value added” in the target gene mRNA level caused by E2 in cells transfected with siNS. The formula assesses the effect of coregulator depletion on the B – A value. The physiological response (i.e. the level of target gene protein produced) depends upon the absolute mRNA level in the presence of E2 rather than the fold change in the mRNA level caused by E2. Therefore, the formula focuses on the difference between B and D rather than the difference between B/A versus D/C. If B > D, then a negative % change (decrease in mRNA level caused by coregulator depletion) is calculated. If B < D, then a positive % change (increase in mRNA level caused by coregulator depletion) is calculated.

Supplemental Figure 4

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Supplemental Figure 4. Effect of coregulator depletion by a second siRNA on the E2-regulated expression of ER α target genes. A, Fig. 1A (main manuscript) shows the effect of a specific coregulator-directed siRNA on the expression of a specific ER α target gene. Supp. Fig. 4A summarizes the use of second siRNAs directed against different sequences within the same coregulator mRNAs to validate Fig. 1A. The matrix on the left provides a summary of the sources of data obtained with the second siRNA. Green squares indicate that the data on the second siRNA has been published, and the number indicates which source reference on the right contains the data. Blue squares indicate that the data on the second siRNA is given below in B. White squares indicate that a second siRNA has not been tested. B, MCF-7 cells were transfected with siRNA against the indicated coregulator or non-specific siRNA (siNS). The siRNAs used were directed against a different sequence in the same mRNAs as those used in Fig. 1A. Target gene mRNA levels were determined by qRT-PCR and expressed relative to *GAPDH* mRNA, as described in Fig. 1A. The sequences of siRNAs are given in Supplemental Table 1.



Supplemental Figure 5. E2-induced FOXA1 occupancy of sites near enh2 of the *PgR* gene.

A, Upper panel, View from the UCSC Genome Browser of the *PgR* gene, indicating the transcription unit and binding regions for ER α and FOXA1 described by Carroll et al (2006) Nature Genetics 38:1289. The locations of enh1 and enh2 are indicated by vertical arrows at the top of the panel. Lower panel, expanded UCSC Genome Browser view of the extent of the ER α and FOXA1 binding footprints in the region of enh2. B, ChIP was performed as in Fig. 2B, using the indicated primers. Primer sequences are: *PgR* enh2-1, 5'-TCTTTTCTGGGAATGCTGCT-3' (forward) and 5'-GAAGGCTTTTGGTCCATGTC-3' (reverse); *PgR* enh2-2, 5'-CATGAGACACAGCACCTTCAA-3' (forward) and 5'-GATCCAAGGGGAAATGAACA-3' (reverse).

Sequences of siRNAs

siFLII(2)

5'-GCUGGAACACUUGUCUGUGdTdT-3' (sense)

5'-CACAGACAAGUGUCCAGCdTdT-3' (anti-sense)

siTIP60 (#2)

5'-CGUCCAUUACAUUGACUUCdTdT-3' (sense),

5'-GAAGUCA AUGUAAUGGAUGdTdT-3' (anti-sense)

siBRG1 (#2)

5'-CCGUGGACUUCAAGAAGAUdTdT-3' (sense)

5'-AUCUUCUUGAAGUCCACGGdTdT-3' (anti-sense)

siBAF53(2)

5'-GGUACUUCAAGUGUCAGAUdTdT-3' (sense)

5'-AUCUGACACUUGAAGUACCDdTdT-3' (anti-sense)

siMLL1(2)

5'-GCACUGUUAAACAUCCACdTdT-3' (sense)

5'-GUGGAAUGUUUACAGUGCdTdT-3' (anti-sense)

siFOXA1(2)

5'-GCGAAGUUUAAUGAUCCACdTdT-3' (sense)

5'-GUGGAUCAUUAAACUUCGCdTdT-3' (anti-sense)