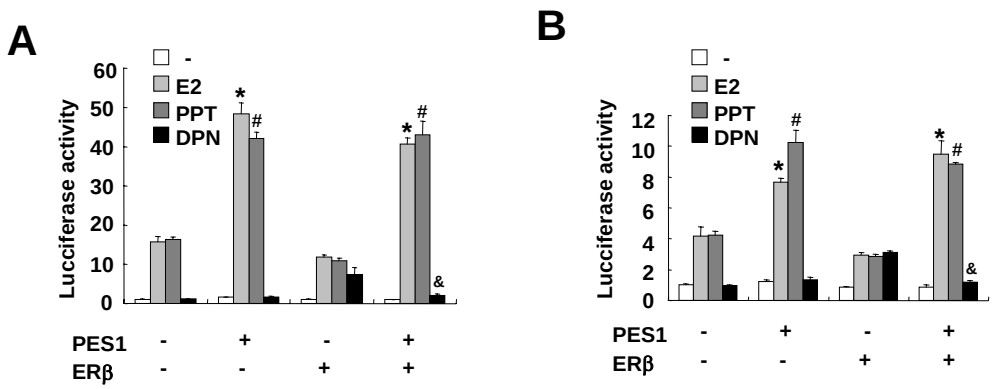
Supplemental Figure 1

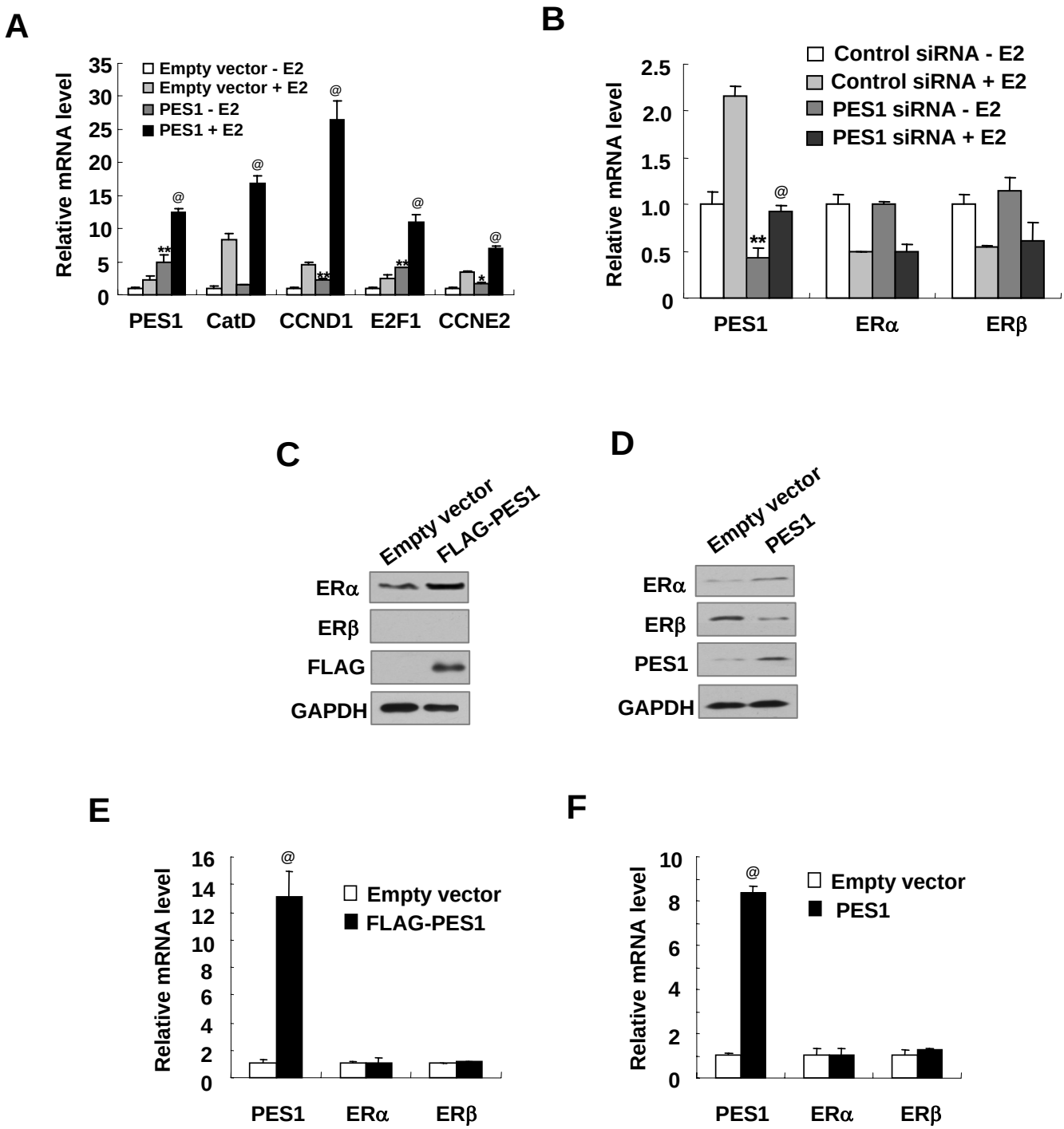


Supplemental Figure 1. PES1 differentially modulates ER α and ER β transcriptional activity in breast cancer cells. Luciferase reporter assays in ER α -positive and ER β -negative ZR75-1 (**A**) and T47D (**B**) cells transiently transfected with the reporter ERE-LUC, ER β , and PES1, and treated with or without 10 nM 17 β -estradiol (E2), 1 nM PPT (ER α -specific agonist), or 1 nM DPN (ER β -specific agonist) for 24 h. The results shown are mean ± SD of three independent experiments. $^{*}P < 0.01$, $^{\#}P < 0.01$ and $^{\&}P < 0.01$ versus empty vector in the absence or presence of ER β with E2, PPT and DPN, respectively.

Supplemental Figure 2

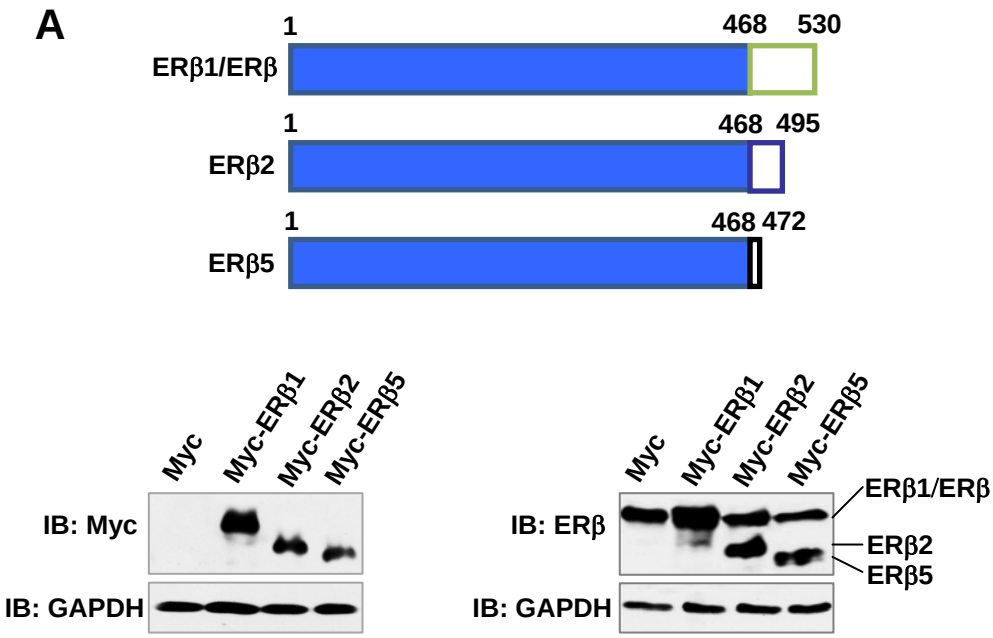
Supplemental Figure 2. Hierarchical cluster analysis of genes that are differentially expressed in PES1 knockdown MCF7 cells. MCF7 cells stably transfected with control siRNA or PES1 siRNA were treated with or without 10 nM E2 for 24 h. Each column corresponds to a single cell line, and each row to a single gene. All cell lines were compared with MCF7 cells transfected with control siRNA in the absence of 10 nM E2. Red indicates up-regulation, green indicates down-regulation, and black indicates no change.

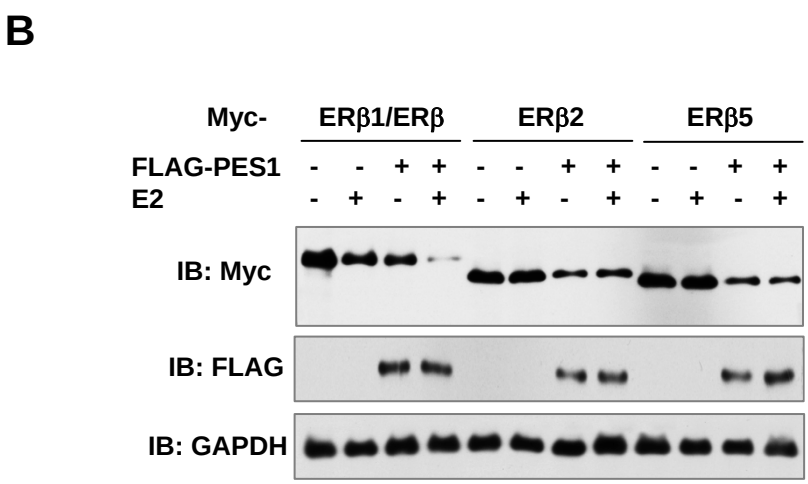
Supplemental Figure 3



Supplemental Figure 3. PES1 increases estrogen-responsive gene transcription and regulates ER α and ER β expression at the protein level. (**A**) Real-time RT-PCR analysis of estrogen-responsive genes in MCF7 cells transfected with PES1 or empty vector in the presence or absence of 10 nM E2 for 24 h. (**B**) Real-time RT-PCR assays of the expression of ER α , ER β , and PES1 in MCF-7 cells stably transfected with control siRNA or PES1 siRNA in the presence or absence of 10 nM E2 for 24 h. (**C** and **D**) Western blot analysis of lysates from ZR75-1 cells transfected with FLAG-PES1 (C) or from normal human mammary epithelial cells (HMECs) infected with recombinant virus vector for PES1 (D) and treated with 10 nM E2 for 24 h. (**E** and **F**) Real-time RT-PCR assays of the expression of ER α , ER β , and PES1 in the above-mentioned ZR75-1 cells and HMECs. All values shown are mean ± SD of triplicate measurements and have been repeated 3 times with similar results. **P* < 0.05, ***P* < 0.01 versus empty vector or control siRNA without E2. @*P* < 0.01 versus empty vector or control siRNA with E2.

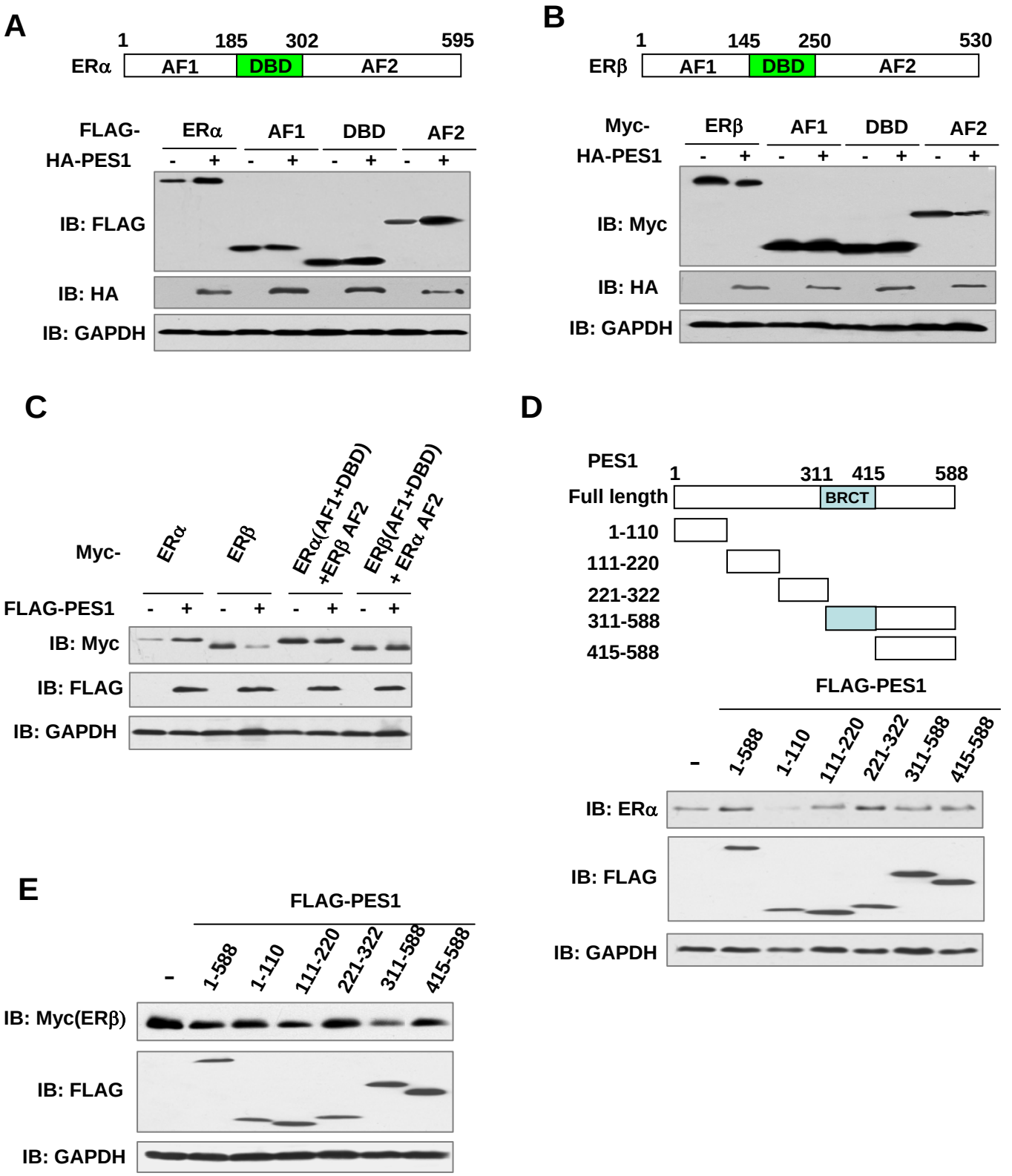
Supplemental Figure 4





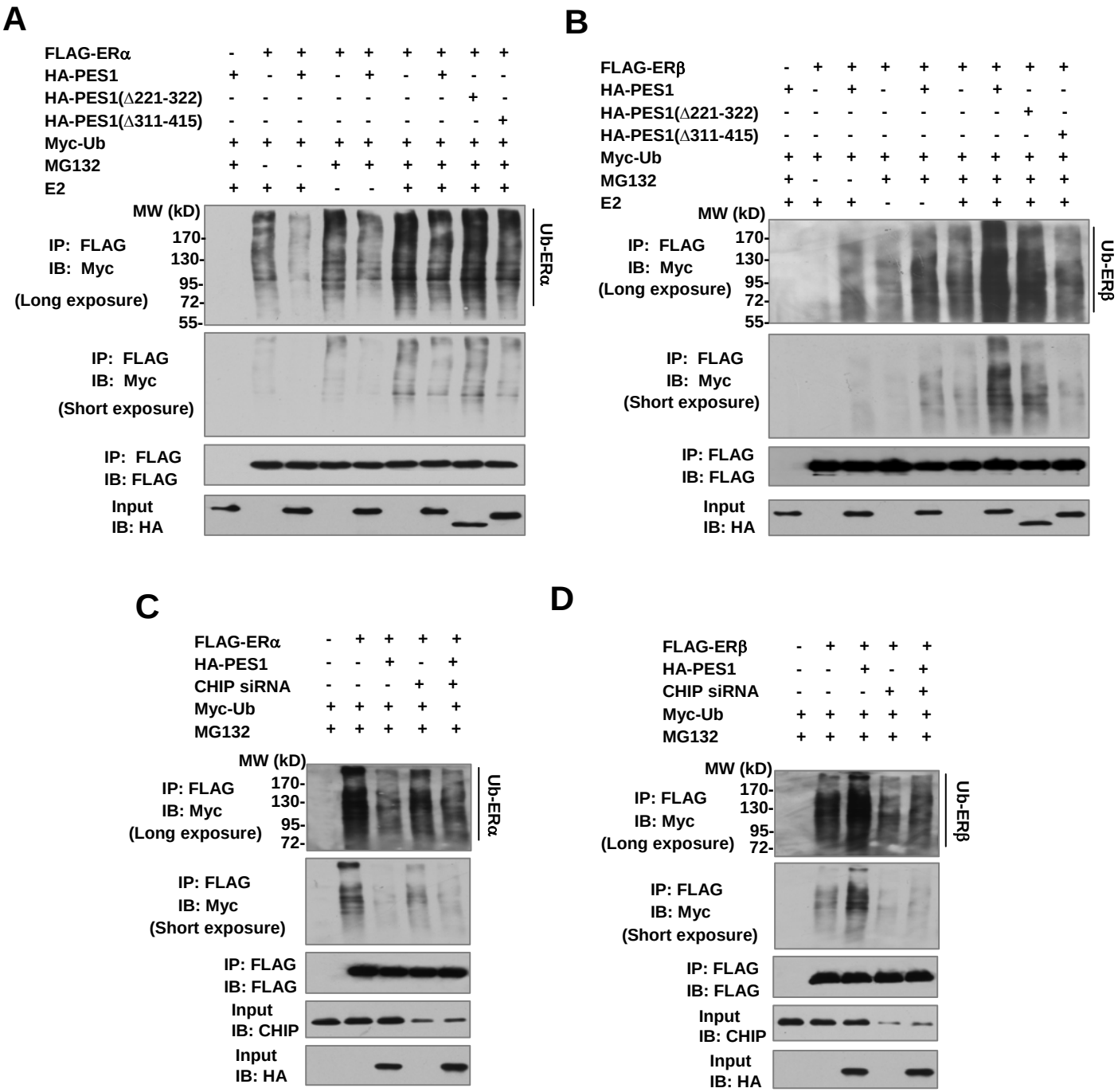
Supplemental Figure 4. PES1 decreases the expression of ERβ1, ERβ2 and ERβ5. (**A**) Immunoblot analysis of lysates from MCF7 cells transfected with Myc-tagged ERβ1, ERβ2 and ERβ5 using anti-Myc (left panel) or anti-ERβ (right panel). Also presented is a schematic diagram of ERβ1, ERβ2 and ERβ5 proteins. (**B**) Immunoblot analysis of lysates from MCF7 cells transfected with FLAG-tagged PES1 and Myc-tagged ERβ1, ERβ2 or ERβ5 and treated with 10 nM E2 for 24 h.

Supplemental Figure 5



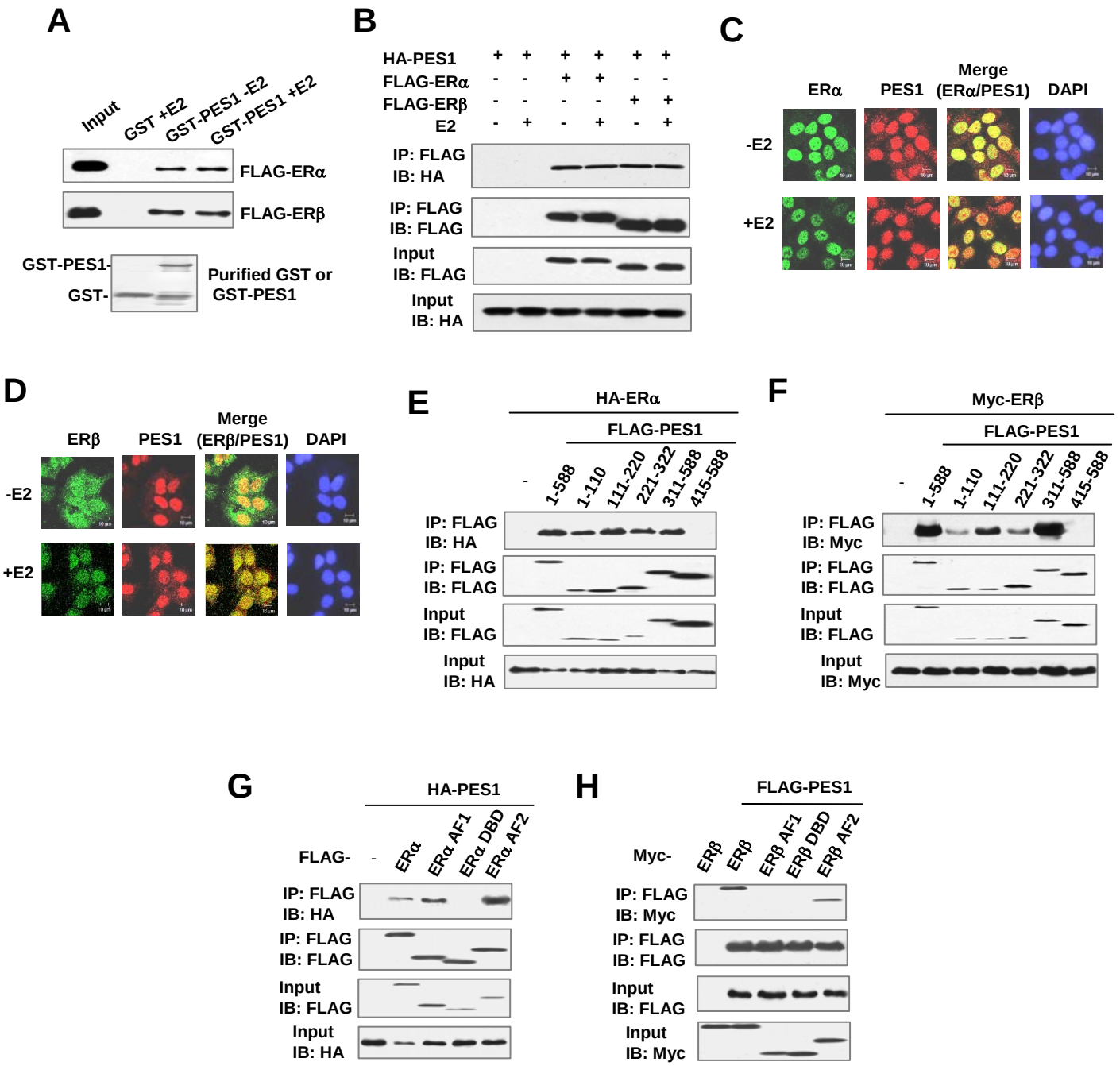
Supplemental Figure 5. Effects of PES1 on the protein levels of ER α and ER β domains and effects of different PES1 regions on ER α and ER β protein expression. (**A** and **B**) Immunoblot analysis of lysates from HEK293T cells transiently transfected with FLAG or Myc-tagged AF1, DBD, and AF2 domains of ER α (A) or ER β (B) in the presence or absence of HA-tagged PES1. Also shown at top of the graph is a schematic diagram of the ER α (A) or ER β (B) protein, illustrating the locations of various domains. (**C**) Immunoblot analysis of lysates from HEK293T cells transiently transfected with Myc-tagged ER α or ER β or chimeric constructs in which the AF2 domain of ER α was switched with the AF2 domain of ER β with or without FLAG-PES1. (**D** and **E**) Immunoblot analysis of lysates from HEK293T cells transiently transfected with ER α (**D**) or Myc-tagged ER β (**E**), and FLAG-tagged PES1 or its deletion mutants as indicated. Schematic diagram of the PES1 deletion constructs used is shown at the top (D).

Supplemental Figure 6



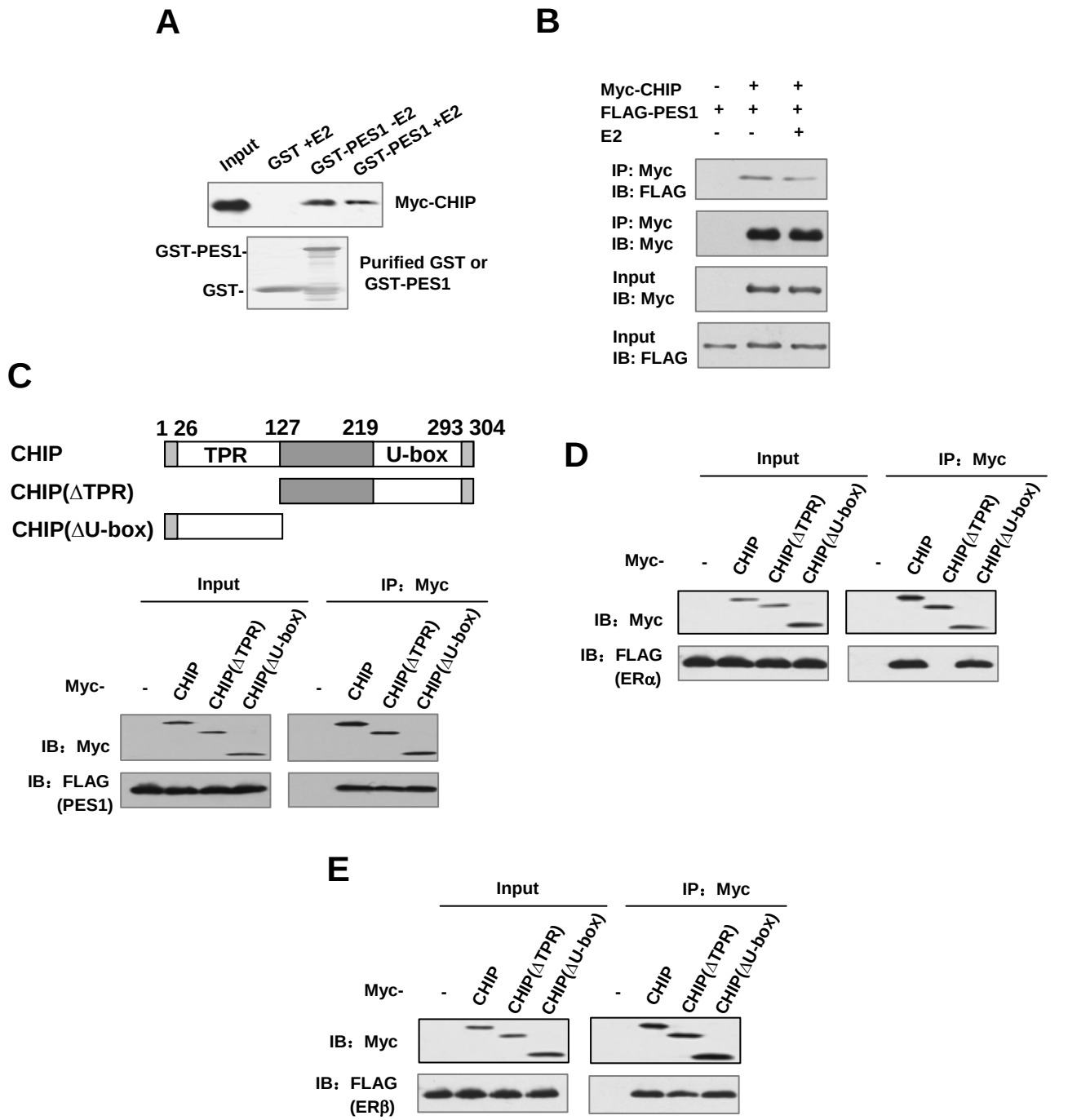
Supplemental Figure 6. CHIP is required for PES1-mediated ER α and ER β ubiquitination. (**A** and **B**) Effects of PES1 and its mutants on ER α and ER β ubiquitination. HEK293T cells transiently transfected with Myc-ubiquitin (Myc-Ub), FLAG-ER α (**A**), or FLAG-ER β (**B**), and HA-PES1, HA-PES1(Δ221–322), or HA-PES1(Δ311–415) were immunoprecipitated with anti-FLAG, followed by immunoblotting with the indicated antibodies. MW, molecular weight. (**C** and **D**) Effects of PES1 on CHIP-mediated ubiquitination of ER α and ER β . HEK293T cells transiently transfected with Myc-Ub, CHIP siRNA, HA-PES1, and FLAG-ER α (**C**) or FLAG-ER β (**D**) were immunoprecipitated with anti-FLAG followed by immunoblotting with the indicated antibodies.

Supplemental Figure 7



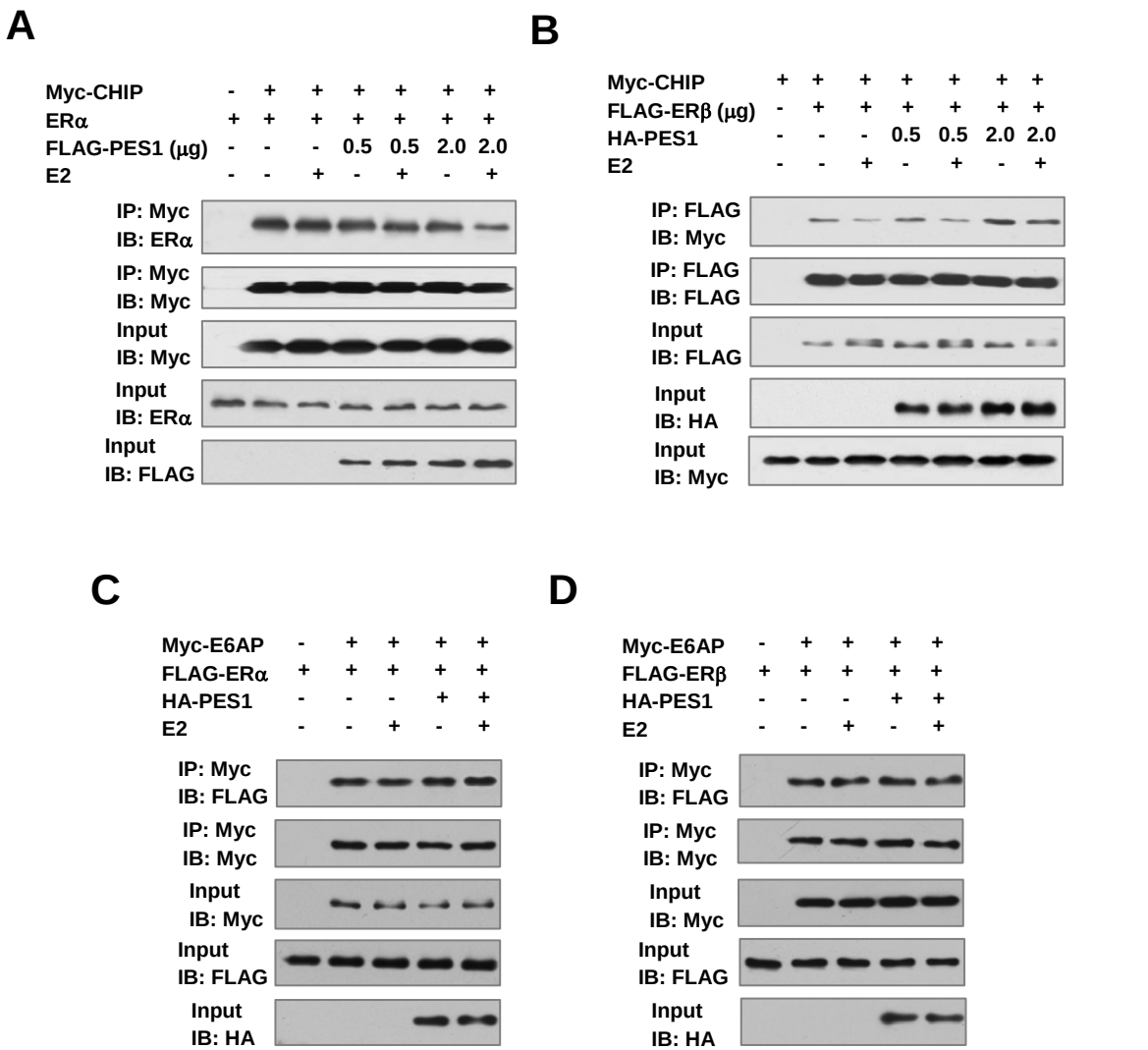
Supplemental Figure 7. PES1 interacts with and co-localizes with ERα and ERβ. (**A**) GST pull-down analysis of purified GST or GST-PES1 fusion protein incubated with lysates of HEK293T cells expressing FLAG-tagged ERα or ERβ in the presence or absence of 10 nM E2. (**B**) HEK293T cells transiently transfected with HA-tagged PES1 and FLAG-tagged ERα or ERβ in the presence of 10 nM E2 were immunoprecipitated with anti-FLAG followed by immunoblotting with the indicated antibodies. (**C** and **D**) PES1 co-localized with ERα (**C**) or ERβ (**D**) predominantly in the nucleus. MCF7 cells treated with 10 nM E2 for 2 h were stained with anti-PES1 and anti-ERα (**C**) or anti-ERβ (**D**) before visualization by confocal microscopy. The nuclei were stained with DAPI (blue). Scale bar, 10 μm. (**E** and **F**) Mapping of the regions of PES1 that interact with ERα (**E**) or ERβ (**F**). The binding ability of the indicated FLAG-tagged PES1 deletion mutants was analyzed in HEK293T cells by co-immunoprecipitation assays. (**G** and **H**) Mapping of the PES1 interaction domains of ERα (**G**) and ERβ (**H**). The indicated FLAG-tagged deletion mutants of ERα (**G**) or Myc-tagged deletion mutants of ERβ (**H**) were analyzed as in (**E** and **F**).

Supplemental Figure 8



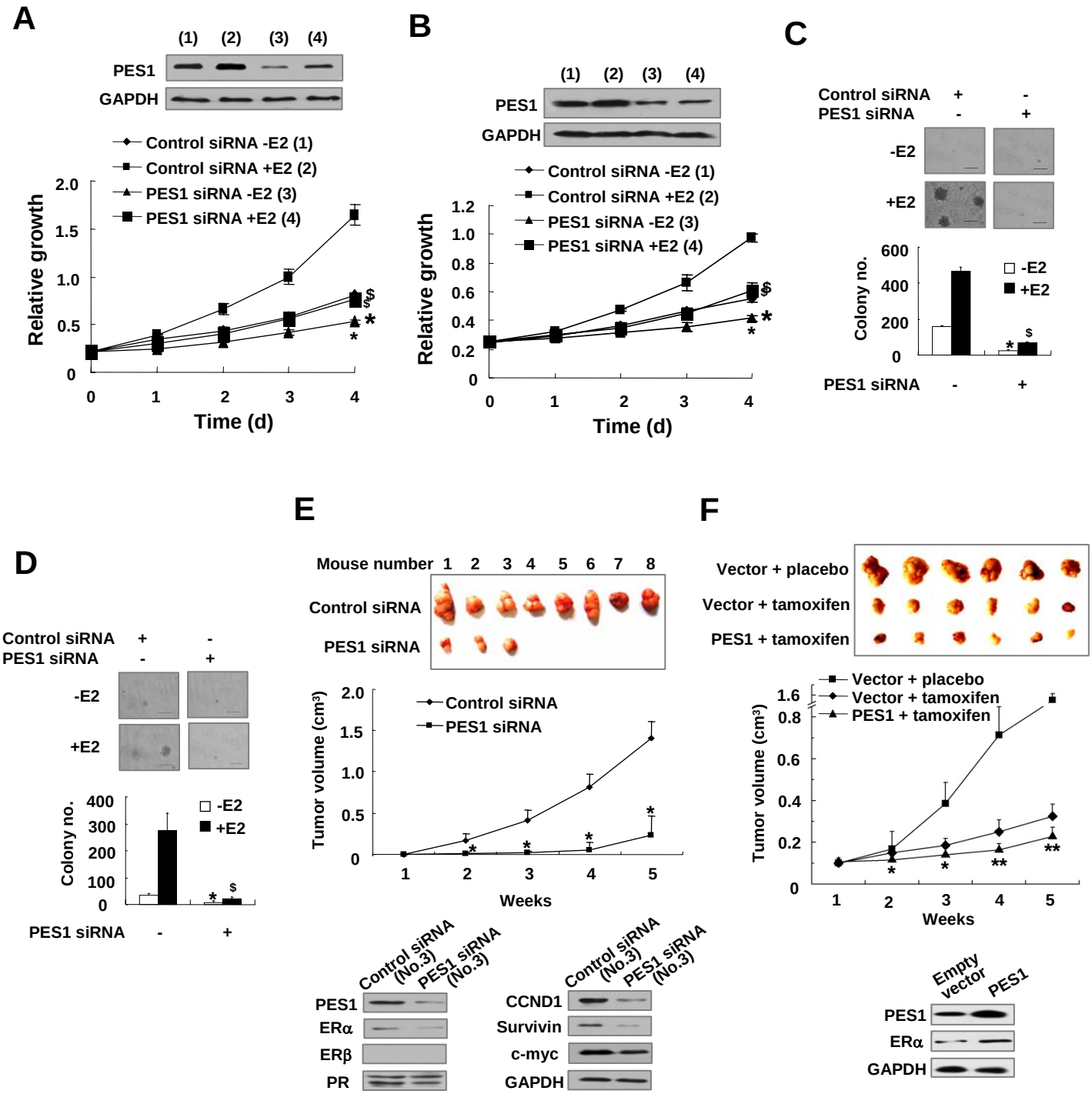
Supplemental Figure 8. CHIP interacts with PES1 as well as ERα and ERβ. (**A**) Immunoblot analysis of bound proteins in lysates of HEK293T cells expressing CHIP following incubation with Sepharose beads coupled to GST alone or a fusion protein of GST and PES1 (GST-PES1). (**B**) Immunoblot analysis of HEK293T cells co-transfected with vectors expressing FLAG-PES1 and Myc-CHIP. Lysates were immunoprecipitated with anti-FLAG and then analyzed by immunoblotting. (**C**-**E**) Mapping of the CHIP domains responsible for interaction with PES1 (**C**), ERα (**D**), and ERβ (**E**). A Myc-tagged CHIP(1-126) mutant in which the U-box domain was deleted and a Myc-tagged CHIP(127-304) mutant in which the TPR domain was deleted were analyzed for their binding ability in HEK293T cells by co-immunoprecipitation assays. Schematic diagram of the CHIP deletion constructs used is shown at the top (C).

Supplemental Figure 9



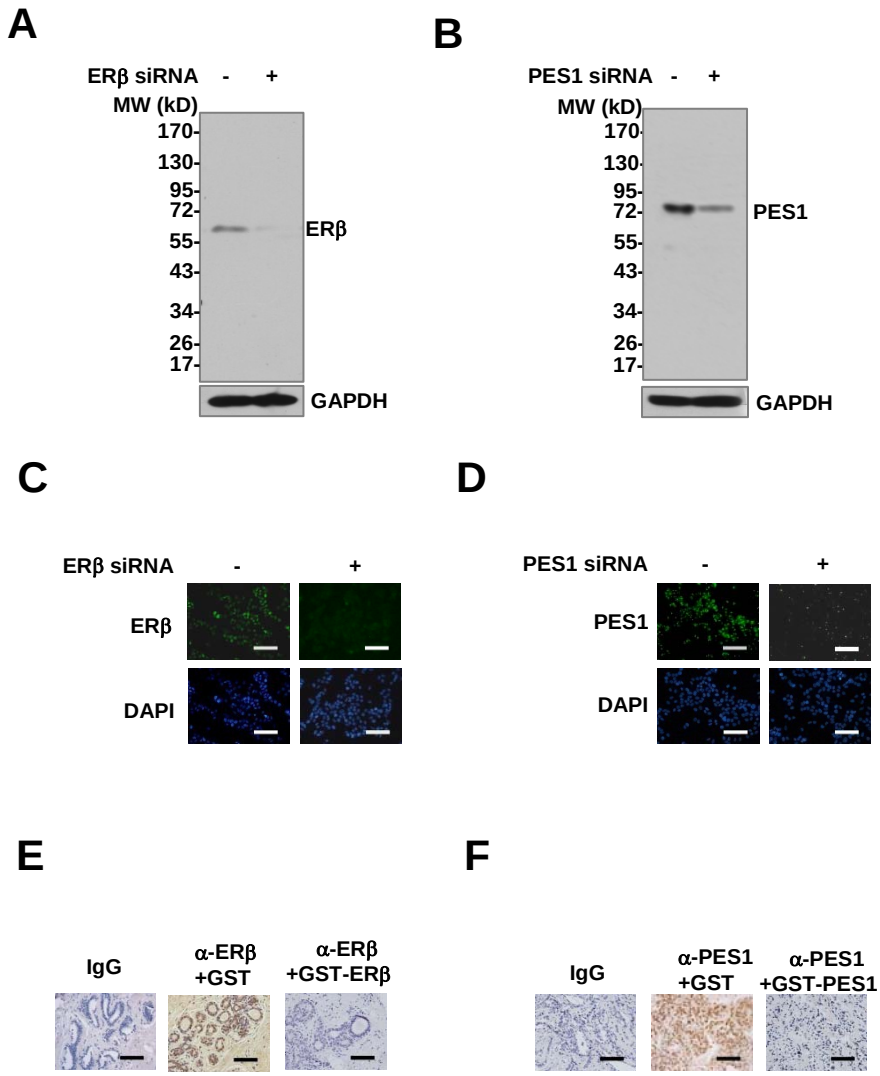
Supplemental Figure 9. PES1 changes the interaction of ER α and ER β with CHIP but not with E6AP. (**A** and **B**) Effects of PES1 on the interaction of CHIP with ER α and ER β. HEK293T cells transiently transfected with Myc-CHIP, ER α (**A**) or FLAG-ER β (**B**) and increasing amounts of FLAG-PES1 (**A**) or HA-PES1 (**B**) in the presence of 10 nM E2 were immunoprecipitated with anti-Myc (**A**) or anti-FLAG (**B**), followed by immunoblotting as indicated. (**C** and **D**) PES1 does not affect the interaction of E6AP with ER α and ER β. Co-immunoprecipitation assays were performed in HEK293T cells transiently transfected with Myc-E6AP, PES1, and either FLAG-ER α (**C**) or FLAG-ER β (**D**).

Supplemental Figure 10



Supplemental Figure 10. PES1 regulates breast cancer cell growth. (**A** and **B**) Anchorage-dependent growth in ZR75-1 (**A**) or T47D (**B**) cells stably transfected with control siRNA or PES1 siRNA. Cell viability was measured at the indicated times. Data are presented as mean ± SD of three independent experiments. $^{*}P < 0.01$ versus control siRNA without E2 on day 4. $^{\$}P < 0.01$ versus control siRNA with E2 on day 4. The PES1 levels in the stable cell lines were determined by immunoblotting with anti-PES1 (Upper panel). (**C** and **D**) Anchorage-independent growth assays in ZR75-1 (**C**) or T47D (**D**) cells stably transfected as in (**B**). Scale bar, 50 μm. Data are presented as mean ± SD of three independent experiments. $^{*}P < 0.01$ versus control siRNA without E2. $^{\$}P < 0.01$ versus control siRNA with E2. (**E**) Volume of xenograft tumors derived from ZR75-1 cells expressing control siRNA or PES1 siRNA. Data are presented as mean ± SD (n=8 for control siRNA and n=3 for PES1 siRNA due to absence of visible tumors in the other five mice). $^{*}P < 0.01$ versus control siRNA. Representative tumor tissues were used for immunoblotting with the indicated antibodies. (**F**) Volume of xenograft tumors derived from control or PES1-overexpressing ZR75-1 cells upon treatment with placebo or tamoxifen. Data are shown as mean ± SD (n=6). $^{*}P < 0.05$, $^{**}P < 0.01$ versus empty vector with tamoxifen. Western blot showed the expression of PES1 and ERα in PES1-transfected ZR75-1 cells (lower panel).

Supplemental Figure 11



Supplemental Figure 11. Validation of antibody specificity to ERβ and PES1. (**A** and **B**) Immunoblot analysis of lysates from MCF7 cells transfected with ERβ siRNA or PES1 siRNA using antibodies specific for ERβ (**A**) or PES1 (**B**). MW, molecular weight. (**C** and **D)** Immunofluorescence staining of ERβ (**C**) and PES1 (**D**) in MCF-7 cells transfected as in (**A**, **B**). Original magnification, ×20. Scale bar, 100 μm. (**E** and **F**) Immunohistochemical staining of breast cancer specimens incubated with normal IgG, anti-ERβ, or anti-PES1. To validate antibody specificity, the anti-ERβ (**E**) and anti-PES1. (**F**) antibodies were pre-incubated with recombinant GST-ERβ (1-144) and GST-PES1 proteins for 1 h prior to applying to tissue. Original magnification, ×20. Scale bar, 100 μm.

Supplemental Table 1. The list of 256 genes that are differentially expressed in PES1 knockdown MCF7 cells in response to E2.

Gene*	Ratio**	Gene description	NCBI Accession No.
TPM3	0.0654	Tropomyosin alpha-3 chain	NM_001043351.1
PES1	0.1076	Pescadillo homolog 1	NM_014303.2
CXCL12	0.1367	Stromal cell-derived factor 1 precursor (SDF-1)	NM_000609.4
KRT13	0.1844	Keratin, type I cytoskeletal 13 (Cytokeratin-13)	NM_153490.1
RHGXX_HUMAN	0.2148	Putative Rho GTPase-activating protein FLJ46335 precursor	NM_144967.2
CAV1	0.2180	Caveolin-1	NM_001753.3
MCM10	0.2189	minichromosome maintenance protein 10 isoform 2	NM_018518.3
KRT10	0.2198	Keratin, type I cytoskeletal 10 (Cytokeratin-10)	NM_000421.2
PDZK1	0.2304	PDZ domain-containing protein 1	NM_002614.3
RRM2	0.2442	Ribonucleoside-diphosphate reductase M2 subunit	NM_001034.1
GLA	0.2508	Alpha-galactosidase A precursor	NM_000169.1
KIAA1794	0.2531	KIAA1794 (KIAA1794)	NM_018193.2
MGP	0.2583	Matrix Gla-protein precursor	NM_000900.2
EXO1	0.2594	Exonuclease 1	NM_006027.3
AREG	0.2668	Amphiregulin precursor	XM_001125684.1
MYB	0.2669	Myb proto-oncogene protein	NM_005375.2
CCNE2	0.2673	G1/S-specific cyclin-E2	NM_057749.1
TAZ	0.2710	Tafazzin	NM_181314.1
KLK11	0.2771	Kallikrein-11 precursor	NM_006853.2
PRSS23	0.2808	Serine protease 23 precursor	NM_007173.4
MCM4	0.2892	DNA replication licensing factor MCM4	NM_182746.1
PSMC3IP	0.2995	TBP-1 interacting protein isoform 1	NM_016556.1
POLA2	0.3016	DNA polymerase subunit alpha B	NM_002689.2
GINS2	0.3024	DNA replication complex GINS protein PSF2	NM_016095.1
UGT2B17	0.3063	UDP-glucuronosyltransferase 2B17 precursor	NM_001077.2
PBK	0.3136	T-lymphokine-activated killer cell-originated protein kinase	NM_018492.2
KIAA0101	0.3155	PCNA-associated factor (p15PAF)	NM_014736.4
MCM2	0.3175	DNA replication licensing factor MCM2	NM_004526.2
TYMS	0.3176	Thymidylate synthase	NM_001071.1
FKBP4	0.3176	FK506-binding protein 4	NM_002014.2
MLF1IP	0.3196	Centromere protein U	NM_024629.2
RAB31	0.3197	Ras-related protein Rab-31 (Rab-22B)	NM_006868.2
MCM6	0.3198	DNA replication licensing factor MCM6	NM_005915.4
PCNA	0.3202	Proliferating cell nuclear antigen	NM_182649.1
DEPDC6	0.3254	DEP domain containing 6	NM_022783.1
RAD51	0.3278	DNA repair protein RAD51 homolog 1	NM_002875.2
CHAF1A	0.3287	Chromatin assembly factor 1 subunit A	NM_005483.2
PPIF	0.3300	"Peptidyl-prolyl cis-trans isomerase, mitochondrial precursor	NM_005729.3
ABHD2	0.3349	Abhydrolase domain-containing protein 2	NM_152924.3
SFXN2	0.3355	Sideroflexin-2	NM_178858.3
NPY1R	0.3403	Neuropeptide Y receptor type 1 (NPY1-R)	NM_000909.4
ATAD2	0.3432	ATPase family AAA domain-containing protein 2	NM_014109.2
EME1	0.3444	Crossover junction endonuclease EME1	NM_152463.1
SPBC25	0.3461	Kinetochore protein Spc25	NM_020675.3
E2F2	0.3480	Transcription factor E2F2 (E2F-2)	NM_004091.2
RAMP3	0.3491	Receptor activity-modifying protein 3 precursor	NM_005856.2
TMPRSS3	0.3493	"Transmembrane protease, serine 3	NM_032401.1
TACC3	0.3560	Transforming acidic coiled-coil-containing protein 3 (ERIC-1)	NM_006342.1
WISP2	0.3563	WNT1-inducible-signaling pathway protein 2 precursor	NM_003881.2
UHRF1	0.3577	E3 ubiquitin-protein ligase UHRF1	NM_013282.3
SGK3	0.3627	Serine/threonine-protein kinase Sgk3	NM_001033578.1
PAQR4	0.3630	Progestin and adipoQ receptor family member 4	NM_152341.2
CELSR2	0.3643	Cadherin EGF LAG seven-pass G-type receptor 2 precursor	NM_001408.1
UBE2T	0.3683	Ubiquitin-conjugating enzyme E2 T	NM_014176.1
RECQL4	0.3697	ATP-dependent DNA helicase Q4	NM_004260.2
RAD51AP1	0.3716	RAD51-associated protein 1	NM_006479.2
DHFR	0.3722	Dihydrofolate reductase	XM_932666.2
SGOL1	0.3725	Shugoshin-like 1	NM_001012410.1
SLC22A5	0.3731	Organic cation/carnitine transporter 2	NM_003060.2
SUSD4	0.3734	Sushi domain-containing protein 4 precursor	NM_017982.2
MCM7	0.3737	DNA replication licensing factor MCM7	NM_182776.1
PRIM1	0.3737	DNA primase small subunit	NM_000946.2
HIST1H4C	0.3738	Histone H4	NM_003542.3
CDT1	0.3749	DNA replication factor Cdt1	NM_030928.2

RFC2	0.3750	Replication factor C subunit 2	NM_181471.1
NKX2-5	0.3757	Homeobox protein Nkx-2.5	NM_004387.2
MKI67	0.3787	Antigen KI-67	NM_002417.3
TFF1	0.3801	Trefoil factor 1 precursor	NM_003225.2
IL17RB	0.3807	Interleukin-17 receptor B precursor	NM_018725.3
FAM25_HUMAN	0.3815	Protein FAM25	XM_926802.2
BRIP1	0.3821	Fanconi anemia group J protein	NM_032043.1
B4GALT5	0.3859	Beta-1,4-galactosyltransferase 5	NM_004776.2
FOXC1	0.3873	Forkhead box protein C1	NM_001453.2
MCM5	0.3878	DNA replication licensing factor MCM5	NM_006739.2
DSN1	0.3883	Uncharacterized protein C20orf172	NM_024918.2
EGR3	0.3890	Early growth response protein 3	NM_004430.2
CTPS	0.3915	CTP synthase 1	NM_001905.1
BIRC5	0.3931	Baculoviral IAP repeat-containing protein 5	NM_001012271.1
ATAD2B	0.3939	ATAD2B protein	XM_938907.2
CAV2	0.3985	Caveolin-2	NM_001233.3
TRIP13	0.3997	Thyroid receptor-interacting protein 13	NM_004237.2
PCP4	0.4000	Brain-specific polypeptide PEP-19	NM_006198.2
HELLS	0.4004	Lymphoid-specific helicase	NM_018063.3
RNASEH2A	0.4052	Ribonuclease H2 subunit A	NM_006397.2
TPD52L1	0.4054	Tumor protein D53	NM_001003395.1
SERPINA1	0.4061	Alpha-1-antitrypsin precursor	NM_001002236.1
GINS3	0.4062	GINS complex subunit 3	NM_022770.2
PRC1	0.4081	Protein regulator of cytokinesis 1	NM_003981.2
CCNA2	0.4083	Cyclin-A2	NM_001237.2
KNTC1	0.4086	Kinetochore-associated protein 1	NM_014708.3
MYOHD1	0.4097	myosin head domain containing 1 isoform 2	NM_001033580.1
IMPA2	0.4118	Inositol monophosphatase 2	NM_014214.1
CALCR	0.4151	Calcitonin receptor precursor	NM_001742.2
TFAP2C	0.4151	Transcription factor AP-2 gamma	NM_003222.3
ASF1B	0.4153	anti-silencing function 1B	NM_018154.2
CENPO	0.4155	Centromere protein O	NM_024322.1
CHRNA3	0.4170	Neuronal acetylcholine receptor subunit alpha-3 precursor	NM_000743.2
GINS4	0.4175	SLD5	NM_032336.1
CENPN	0.4182	Centromere protein N	NM_018455.3
AATF	0.4189	Protein AATF	NM_012138.3
CDC2	0.4201	Cell division control protein 2 homolog	NM_001786.2
BRI3BP	0.4201	BRI3-binding protein	NM_080626.5
RFC4	0.4218	Replication factor C subunit 4	NM_181573.1
ARF6	0.4226	ADP-ribosylation factor 6	XR_018939.1
NCAPG2	0.4246	Condensin-II complex subunit G2	NM_017760.5
FBXO5	0.4247	F-box only protein 5	NM_012177.2
ORC6L	0.4274	Origin recognition complex subunit 6	NM_014321.2
RAPGEFL1	0.4290	Rap guanine nucleotide exchange factor (GEF)-like 1	NM_016339.1
MYBL2	0.4292	Myb-related protein B (B-Myb)	NM_002466.2
KRT16	0.4306	Keratin, type I cytoskeletal 16	NM_005557.2
POLE2	0.4342	DNA polymerase epsilon subunit 2	NM_002692.2
RGS22	0.4343	Regulator of G-protein signaling 22	NM_015668.2
ZWINT	0.4373	ZW10 interactor	NM_001005414.1
TMPO	0.4378	Lamina-associated polypeptide 2, isoforms beta/gamma	NM_001032284.1
AURKB	0.4380	Serine/threonine-protein kinase 12	NM_004217.2
BYSL	0.4397	Bystin	NM_004053.3
PDSS1	0.4403	Decaprenyl-diphosphate synthase subunit 1	NM_014317.3
MCM3	0.4403	DNA replication licensing factor MCM3	NM_002388.3
CHRNA5	0.4411	Neuronal acetylcholine receptor subunit alpha-5 precursor	NM_000745.2
TBAK_HUMAN	0.4413	Tubulin alpha-ubiquitous chain	NM_006082.2
ZNF534	0.4423	Kruppel-like zinc finger protein isoform 1 (Fragment)	XM_496320.3
GMNN	0.4452	Geminin	NM_015895.3
IGSF1	0.4453	immunoglobulin superfamily, member 1 isoform 1	NM_001555.2
CDK2	0.4463	Cell division protein kinase 2	NM_001798.2
HNRPAB	0.4464	Heterogeneous nuclear ribonucleoprotein A/B (hnRNP A/B) (	NM_031266.2
LRRFIP2	0.4474	Leucine-rich repeat flightless-interacting protein 2	NM_006309.1
HIST1H4I	0.4493	Histone H4	NM_003495.2
RANBP1	0.4496	Ran-specific GTPase-activating protein	NM_002882.2
ESCO2	0.4498	N-acetyltransferase ESCO2	NM_001017420.2
PKMYT1	0.4501	Membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase	NM_182687.1
KIF23	0.4507	Kinesin-like protein KIF23	NM_004856.4
KLF10	0.4519	Krueppel-like factor 10	NM_001032282.1
C13orf3	0.4523	Uncharacterized protein C13orf3	NM_145061.3
CCT5	0.4539	T-complex protein 1 subunit epsilon	NM_012073.3

CDKN3	0.4548	Cyclin-dependent kinase inhibitor 3	NM_005192.2
UBE2S	0.4548	Ubiquitin-conjugating enzyme E2 S	NM_014501.2
FKBP5	0.4569	FK506-binding protein 5	NM_004117.2
VRK1	0.4577	Serine/threonine-protein kinase VRK1	NM_003384.2
CA8	0.4583	Carbonic anhydrase-related protein	NM_004056.4
FEN1	0.4585	Flap endonuclease 1	NM_004111.4
MND1	0.4588	GAJ protein	NM_032117.2
TUBB6	0.4595	Tubulin beta-6 chain	NM_032525.1
SRM	0.4596	Spermidine synthase	NM_003132.2
KNTC2	0.4618	Kinetochore protein Hec1	NM_006101.1
FAM72A	0.4628	family with sequence similarity 72, member A	NM_207418.2
UNG	0.4634	Uracil-DNA glycosylase	NM_080911.1
TMEM160	0.4636	transmembrane protein 160	NM_017854.1
DNAJC9	0.4640	DnaJ homolog subfamily C member 9	NM_015190.3
KIF11	0.4642	Kinesin-like protein KIF11	NM_004523.2
TUBB	0.4648	Tubulin beta chain	NM_178014.2
CCDC34	0.4652	coiled-coil domain containing 34 isoform 1	NM_030771.1
RFC3	0.4660	Replication factor C subunit 3	NM_181558.2
NOL5A	0.4665	Nucleolar protein Nop56	NM_006392.2
TLN2	0.4679	Talin-2	NM_015059.1
CDC20	0.4684	Cell division cycle protein 20 homolog	NM_001255.2
MAPT	0.4686	Microtubule-associated protein tau	NM_016835.2
CDH1	0.4703	Epithelial-cadherin precursor (E-cadherin)	NM_004360.2
DONSON	0.4714	Protein downstream neighbor of Son (B17)	NM_017613.2
CCDC74B	0.4730	coiled-coil domain containing 74B	NM_207310.1
NUF2	0.4733	Kinetochore protein Nuf2	NM_031423.3
IGFBP4	0.4736	Insulin-like growth factor-binding protein 4 precursor	NM_001552.2
MMD	0.4764	Monocyte to macrophage differentiation protein	NM_012329.2
PKIB	0.4775	cAMP-dependent protein kinase inhibitor beta	NM_032471.4
CDKN2C	0.4782	Cyclin-dependent kinase 6 inhibitor	NM_078626.2
COPS3	0.4796	COP9 signalosome complex subunit 3	NM_003653.2
STMN1	0.4803	Stathmin (Phosphoprotein p19)	NM_203401.1
FBXL6	0.4809	F-box/LRR-repeat protein 6	NM_024555.3
FHL2	0.4820	Four and a half LIM domains protein 2	NM_001039492.1
UBE2C	0.4827	Ubiquitin-conjugating enzyme E2 C	NM_181803.1
RERG	0.4828	Ras-related and estrogen-regulated growth inhibitor	NM_032918.1
EPS15L1	0.4843	Epidermal growth factor receptor substrate 15-like 1	NM_021235.1
FOXM1	0.4845	Forkhead box protein M1	NM_202002.1
WDR5	0.4851	WD repeat protein 5	NM_052821.3
ACAT2	0.4861	Acetyl-CoA acetyltransferase, cytosolic	NM_005891.1
C1orf9	0.4868	Membrane protein CH1	NM_016227.1
TPM1	0.4869	Tropomyosin 1 alpha chain	NM_001018006.1
SGK	0.4876	Serine/threonine-protein kinase Sgk1	NM_005627.2
MBOAT1	0.4880	Membrane-bound O-acyltransferase domain-containing protein 1	NM_001080480.1
SERPINA3	0.4885	Alpha-1-antichymotrypsin precursor	NM_001085.4
ZNF695	0.4927	Zinc finger protein 695	NM_020394.2
HIST1H4B	0.4933	Histone H4	NM_003544.2
PPIL5	0.4943	Peptidylprolyl isomerase-like 5	NM_152329.3
AGR3_HUMAN	0.4956	Anterior gradient protein 3 homolog precursor	NM_176813.3
LOC730995	0.4962	LOC730995	XM_001130291.1
DCK	0.4962	Deoxycytidine kinase	NM_000788.1
MCM8	0.4963	DNA replication licensing factor MCM8	NM_032485.4
CCNB2	0.4969	G2/mitotic-specific cyclin-B2	NM_004701.2
UGDH	0.4973	UDP-glucose 6-dehydrogenase	NM_003359.2
LIG1	0.4974	DNA ligase 1	NM_000234.1
BARD1	0.4989	BRCA1-associated RING domain protein 1	NM_000465.1
HSPA8	0.4998	Heat shock cognate 71 kDa protein	NM_153201.1
ZDHHC7	2.0018	Palmitoyltransferase ZDHHC7	NM_017740.1
TGFB3	2.0114	Transforming growth factor beta-3 precursor	NM_003239.1
AQP3	2.0325	Aquaporin-3	NM_004925.3
ASB9	2.0365	Ankyrin repeat and SOCS box protein 9	NM_001031739.1
NEDD9	2.0374	Enhancer of filamentation 1 (HEF1)	NM_006403.2
A2BP1_HUMAN	2.0377	Ataxin-2-binding protein 1	NM_145893.1
ATP1B1	2.0470	Sodium/potassium-transporting ATPase subunit beta-1	NM_001001787.1
CALCOCO1	2.0531	coiled-coil transcriptional coactivator	NM_020898.1
PBEF1	2.0563	Nicotinamide phosphoribosyltransferase	NM_005746.2
BRWD1	2.0573	Bromodomain and WD repeat domain-containing protein 1	NM_018963.3
CMYA5	2.0649	cardiomyopathy associated 5	NM_153610.3
CSTA	2.0753	Cystatin-A (Stefin-A)	NM_005213.3
ANGPTL4	2.0860	Angiopoietin-related protein 4 precursor (Angiopoietin-like 4)	NM_139314.1

ALAD	2.0976	Delta-aminolevulinic acid dehydratase	NM_001003945.1
ISG15	2.1012	Interferon-induced 17 kDa protein precursor	NM_005101.1
CFLAR	2.1146	CASP8 and FADD-like apoptosis regulator precursor	NM_003879.3
NDRG1	2.1150	Protein NDRG1 (N-myc downstream-regulated gene 1 protein)	NM_006096.2
SOCS2	2.1350	Suppressor of cytokine signaling 2	NM_003877.3
BCL6	2.1423	B-cell lymphoma 6 protein (BCL-6)	NM_001706.2
BCL2L1	2.1427	Apoptosis regulator Bcl-X	NM_138578.1
FLI1	2.1431	Friend leukemia integration 1 transcription factor	NM_002017.2
REC8L1	2.1445	Meiotic recombination protein REC8-like 1	NM_001048205.1
LMTK3	2.1464	Serine/threonine-protein kinase LMTK3 precursor	NM_001080434.1
SLC29A3	2.1580	Equilibrative nucleoside transporter 3	NM_018344.3
ANGPTL6	2.1595	Angiopoietin-related protein 6 precursor	NM_031917.2
NR3C1	2.1871	Glucocorticoid receptor (GR)	NM_001018077.1
TNFRSF11B	2.2075	Tumor necrosis factor receptor superfamily member 11B precursor	NM_002546.2
MYO1B	2.2240	Myosin-Ib (Myosin I alpha)	NM_012223.2
EGLN3	2.2478	Egl nine homolog 3	NM_022073.2
MPP7	2.2480	palmitoylated membrane protein 7	NM_173496.3
TNFSF10	2.2530	Tumor necrosis factor ligand superfamily member 10	NM_003810.2
IFI27	2.2716	Interferon-alpha-induced 11.5 kDa protein (p27)	NM_005532.3
FGF13	2.2862	Fibroblast growth factor 13 (FGF-13)	NM_033642.1
TMTC4	2.2902	transmembrane and tetratricopeptide repeat containing 4 isoform 1	NM_001079669.1
KIAA1370	2.2909	"KIAA1370 (KIAA1370), mRNA	NM_019600.1
LIMA1	2.2916	LIM domain and actin-binding protein 1 (Epithelial protein lost in neoplasm)	NM_016357.3
MCCC1	2.2989	"Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial precursor	NM_020166.3
IFIH1	2.3606	Interferon-induced helicase C domain-containing protein 1	NM_022168.2
SLIT1	2.3645	Slit homolog 1 protein precursor	NM_003061.1
APOD	2.3727	Apolipoprotein D precursor	NM_001647.2
TMEM45B	2.4241	Transmembrane protein 45B	NM_138788.2
CCNG2	2.4355	Cyclin-G2	NM_004354.1
GRIK2	2.4427	Glutamate receptor, ionotropic kainate 2 precursor	NM_175768.1
SGCG	2.4658	Gamma-sarcoglycan	NM_000231.1
IRF2BP2	2.5079	interferon regulatory factor 2 binding protein 2 isoform B	NM_182972.2
TSSK2	2.5134	Testis-specific serine/threonine-protein kinase 2	NM_053006.3
RAB27B	2.5329	Ras-related protein Rab-27B (C25KG)	NM_004163.3
CEACAM3	2.5664	Carcinoembryonic antigen-related cell adhesion molecule 3 precursor	NM_001815.2
ST3GAL1	2.5778	"CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase	NM_003033.2
BTG2	2.6162	BTG2 protein (NGF-inducible anti-proliferative protein PC3)	NM_006763.2
ANG	2.6223	Ribonuclease 4 precursor	NM_194430.1
TFPI	2.6354	Tissue factor pathway inhibitor precursor (TFPI)	NM_006287.4
BCAS1	2.6611	Breast carcinoma amplified sequence 1	NM_003657.1
ARHGAP12	2.7012	Rho GTPase-activating protein 12	NM_018287.4
OAS1	2.7034	"2',5'-oligoadenylate synthetase 1 isoform 3	NM_016816.2
KRT86	2.7114	Keratin type II cuticular Hb6	NM_002284.3
PARP9	2.7375	Poly [ADP-ribose] polymerase 9	NM_031458.1
FAM107B	2.8069	Protein FAM107B	NM_031453.2
TP53INP1	2.9091	Tumor protein p53-inducible nuclear protein 1	NM_033285.2
ABCC5	3.0233	Multidrug resistance-associated protein 5	NM_005688.2
TMEM140	3.2135	"transmembrane protein 140 (TMEM140), mRNA	NM_018295.2
PARP12	5.3926	Poly [ADP-ribose] polymerase 12	NM_022750.2
PSCA	6.2991	Prostate stem cell antigen precursor	NM_005672.3
CYP1A1	6.7022	Cytochrome P450 1A1 (EC 1.14.14.1)	NM_000499.2
ITGB6	7.6411	Integrin beta-6 precursor	NM_000888.3
IFIT1	9.7797	Interferon-induced protein with tetratricopeptide repeats 1 (IFIT-1)	NM_001548.3

*Red: E2-responsive genes that have been reported in the literature

**Ratio=PES1 siRNA/Control siRNA

Supplemental Table 2. List of PES1-regulated genes categorized by Pathway analysis (*p*<0.05).

Pathway	Count	p-Value	Gene
DNA polymerase	16	9.63E-29	MCM4;POLA2;MCM2;MCM6;PCNA;MCM7;PRIM1;RFC2;MCM5;RNASEH2A;RFC4;POLE2;MCM3;FEN1;RFC3;LIG1
Cell cycle	18	1.51E-22	CCNE2;MCM4;MCM2;MCM6;PCNA;E2F2;MCM7;MCM5;CCNA2;CDC2;ORC6L;MCM3;CDK2;PKMYT1;CDC20;CDKN2C;CCNB2;TGFB3
Mismatch repair	6	7.07E-10	EXO1;PCNA;RFC2;RFC4;RFC3;LIG1
Nucleotide excision repair	6	4.57E-08	PCNA;RFC2;RFC4;POLE2;RFC3;LIG1
Pentose and glucuronate interconversions	5	8.45E-08	UGT2B17;UGT2B15;UGT2B7;UGT2B28;UGDH
Ascorbate and aldarate metabolism	5	1.04E-07	UGT2B17;UGT2B15;UGT2B7;UGT2B28;UGDH
Pyrimidine metabolism	7	2.22E-07	RRM2;POLA2;TYMS;PRIM1;CTPS;POLE2;DCK
Base excision repair	5	5.76E-07	PCNA;POLE2;FEN1;UNG;LIG1
P53 signaling pathway	6	7.08E-07	RRM2;CCNE2;CDC2;CDK2;CCNB2;CCNG2
Porphyrin and chlorophyll metabolism	5	1.12E-06	UGT2B17;UGT2B15;UGT2B7;UGT2B28;ALAD
Starch and sucrose metabolism	5	3.74E-06	UGT2B17;UGT2B15;UGT2B7;UGT2B28;UGDH
Pathogenic Escherichia coli infection - EHEC	5	4.96E-06	TUBA1B;TUBB6;TUBB;TUBB2C;CDH1
Pathogenic Escherichia coli infection - EPEC	5	4.96E-06	TUBA1B;TUBB6;TUBB;TUBB2C;CDH1
Retinol metabolism	5	1.14E-05	UGT2B17;UGT2B15;UGT2B7;UGT2B28;CYP1A1
Metabolism of xenobiotics by cytochrome P450	5	1.63E-05	UGT2B17;UGT2B15;UGT2B7;UGT2B28;CYP1A1
Androgen and estrogen metabolism	4	5.03E-05	UGT2B17;UGT2B15;UGT2B7;UGT2B28
Gap junction	5	6.45E-05	CDC2;TUBA1B;TUBB6;TUBB;TUBB2C
Drug metabolism - other enzymes	4	8.26E-05	UGT2B17;UGT2B15;UGT2B7;UGT2B28
Drug metabolism - cytochrome P450	4	3.16E-04	UGT2B17;UGT2B15;UGT2B7;UGT2B28
Pancreatic cancer	4	3.33E-04	RAD51;E2F2;TGFB3;BCL2L1
Small cell lung cancer	4	6.21E-04	CCNE2;E2F2;CDK2;BCL2L1
Purine metabolism	5	6.35E-04	RRM2;POLA2;PRIM1;POLE2;DCK
Glycosphingolipid biosynthesis - globoseries	2	0.00175065	GLA;ST3GAL1
One carbon pool by folate	2	0.002294989	TYMS;DHFR
Melanoma	3	0.004036775	E2F2;CDH1;FGF13
Chronic myeloid leukemia	3	0.004707318	E2F2;TGFB3;BCL2L1
Cytokine-cytokine receptor interaction	5	0.006707097	CXCL12;IL17RB;TGFB3;TNFRSF11B;TNFSF10
Homologous recombination	2	0.006978749	RAD51;EME1
Prostate cancer	3	0.007332649	CCNE2;E2F2;CDK2
Thyroid cancer	2	0.007473752	TPM3;CDH1
Apoptosis	3	0.00756379	CFLAR;BCL2L1;TNFSF10
MAPK signaling pathway	5	0.008173929	MAPT;STMN1;HSPA8;TGFB3;FGF13
O-Glycan biosynthesis	2	0.008509827	B4GALT5;ST3GAL1
Folate biosynthesis	2	0.009606357	DHFR;IFIH1
Focal adhesion	4	0.013862091	CAV1;CAV2;TLN2;ITGB6
Bladder cancer	2	0.015258348	E2F2;CDH1
Tryptophan metabolism	2	0.015956055	ACAT2;CYP1A1
Valine, leucine and isoleucine degradation	2	0.018129303	ACAT2;MCCC1
Glutathione metabolism	2	0.021209028	RRM2;SRM
Ubiquitin mediated proteolysis	3	0.024815446	UBE2S;CDC20;UBE2C
Nucleotide sugars metabolism	1	0.02655516	UGDH
Systemic lupus erythematosus	3	0.027171602	HIST1H4C;HIST1H4I;HIST1H4B
Neuroactive ligand-receptor interaction	4	0.028507515	NPY1R;CALCR;NR3C1;GRIK2
Complement and coagulation cascades	2	0.038441413	SERPINA1;TFPI
PPAR signaling pathway	2	0.039457958	ANGPTL4;FABP2
Synthesis and degradation of ketone bodies	1	0.039568389	ACAT2
Renal cell carcinoma	2	0.040484659	TGFB3;EGLN3

Supplemental Table 3. List of PES1-regulated genes categorized by GO analysis (*p*<0.05).

Go term	Count	p-Value	Gene
Protein binding	115	2.82E-116	TPM3;PES1;CAV1;MCM10;KRT10;RRM2;FANCI;EXO1;MYB;CCNE2;MCM4;PSMC3IP;POLA2;GINS2;PBK;MCM2;MLF1IP;PCNA;DEPDC6;NPY1R;EME1;SPC25;E2F2;WISP2;UHRF1;SGK3;CELSR2;RAD51AP1;SGOL1;MCM7;PRIM1;HIST4H4;CDT1;RFC2;MKI67;BRIP1;MCM5;DSN1;CTPS;CAV2;SERPINA1;PRC1;CCNA2;KNTC1;CENPO;GINS4;AATF;CDC2;RFC4;ARF6;FBXO5;ORC6L;KRT16;AURKB;MCM3;GMNN;IGSF1;HNRNPAB;LRRFIP2;HIST4H4;RANBP1;PKMYT1;KIF23;KLF10;CDKN3;FKBP5;VRK1;CA8;FEN1;MND1;NDC80;UNG;RFC3;TLN2;CDC20;NUF2;CDKN2C;COPS3;STMN1;FBXL6;EPS15L1;FOXM1;WDR5;ACAT2;SGK1;SERPINA3;HIST4H4;PPIL5;CCNB2;BARD1;HSPA8;ZDHHC7;NEDD9;ATP1B1;CALCOCO1;BRWD1;ISG15;CFLAR;NDRG1;SOCS2;BCL6;REC8;NR3C1;TNFRSF11B;EGLN3;MPP7;FGF13;IFIH1;SLIT1;APOD;SGCG;TSSK2;RAB27B;BTG2;BCAS1;ARHGAP12;ITGB6
Nucleotide binding	50	3.35E-58	MCM4;PBK;MCM2;RAB31;MCM6;RAD51;ATAD2;SGK3;RECQL4;SLC22A5;MCM7;RFC2;MKI67;BRIP1;MCM5;ATAD2B;TRIP13;HELLS;MYO19;CDC2;RFC4;ARF6;AURKB;MCM3;TUBA1B;CDK2;HNRNPAB;PKMYT1;KIF23;CCT5;VRK1;TUBB6;KIF11;TUBB;TUBB2C;RFC3;RERG;SGK1;DCK;MCM8;LIG1;HSPA8;FOX1;LMTK3;MYO1B;MCCC1;IFIH1;TSSK2;RAB27B;ABCC5
ATP binding	42	2.02E-53	MCM4;PBK;MCM2;FKBP4;MCM6;RAD51;ATAD2;SGK3;RECQL4;SLC22A5;MCM7;RFC2;MKI67;BRIP1;MCM5;ATAD2B;TRIP13;HELLS;MYO19;CDC2;RFC4;AURKB;MCM3;CDK2;PKMYT1;KIF23;CCT5;VRK1;KIF11;UBE2C;SGK1;DCK;MCM8;LIG1;HSPA8;LMTK3;MYO1B;MCCC1;IFIH1;TSSK2;OAS1;ABCC5
DNA binding	27	3.73E-23	EXO1;MYB;MCM4;PSMC3IP;POLA2;MCM2;MCM6;PCNA;EME1;RAD51AP1;MCM7;HIST4H4;CDT1;BRIP1;MCM5;HELLS;ORC6L;POLE2;TMPO;TMPO;MCM3;HIST4H4;MND1;SERPINA3;HIST4H4;MCM8;LIG1;IFIH1;ANG
Transferase activity	23	1.55E-21	PBK;TYMS;SGK3;PRIM1;CDC2;POLE2;AURKB;PDSS1;CDK2;ESCO2;PKMYT1;VRK1;SRM;ACAT2;SGK1;MBOAT1;DCK;ZDHHC7;LMTK3;TSSK2;OAS1;PARP9;PARP12
Zinc ion binding	20	5.20E-15	MCM10;MCM2;UHRF1;RECQL4;PRIM1;EGR3;BIRC5;FBXO5;ESCO2;KLF10;CA8;FHL2;BARD1;ZDHHC7;ALAD;BCL6;NR3C1;TNFSF10;LIMA1;PARP12
Transcription factor activity	14	4.77E-14	ATAD2;E2F2;UHRF1;NKX2-5;EGR3;TFAP2C;AATF;MYBL2;HNRNPAB;KLF10;FOXM1;BCL6;FLI1;NR3C1
Metal ion binding	23	3.53E-13	MCM10;MCM2;UHRF1;PRIM1;B4GALT5;EGR3;BIRC5;RNASEH2A;FBXO5;ESCO2;KLF10;CA8;FHL2;BARD1;ZDHHC7;ALAD;BCL6;NR3C1;EGLN3;TNFSF10;LIMA1;PARP12;CYP1A1
Gtpase activity	8	4.11E-12	RAB31;ARF6;TUBA1B;TUBB6;TUBB;TUBB2C;RERG;RAB27B
GTP binding	9	1.76E-11	FKBP4;RAB31;ARF6;TUBA1B;TUBB6;TUBB;TUBB2C;RERG;RAB27B
Structural constituent of cytoskeleton	6	3.46E-11	KRT16;TUBA1B;TUBB;TLN2;MAPT;TPM1
Endonuclease activity	6	1.74E-10	EXO1;EME1;RNASEH2A;FEN1;RNASE4;ANG
Identical protein binding	9	3.33E-10	MCM6;RAD51;TRIP13;CDK2;MAPT;FHL2;TGFB3;CMYA5;ALAD
Single-stranded DNA binding	5	4.15E-10	MCM4;MCM6;RAD51;RAD51AP1;MCM7
Protein serine/threonine kinase Activity	8	2.01E-09	PBK;SGK3;AURKB;PKMYT1;VRK1;SGK1;LMTK3;TSSK2
Protein heterodimerization activity	6	2.76E-09	POLA2;NKX2-5;BIRC5;TPD52L1;PDSS1;BARD1
Hydrolase activity	14	4.01E-09	EXO1;ABHD2;ATAD2;EME1;RECQL4;BRIP1;HELLS;RNASEH2A;IMPA2;CDKN3;FEN1;IFIH1;RNASE4;ANG
DNA clamp loader activity	3	4.68E-09	RFC2;RFC4;RFC3
Nucleoside-triphosphatase activity	9	5.56E-09	MCM4;ATAD2;MCM7;RFC2;ATAD2B;TRIP13;RFC4;MCM3;MCM8
Glucuronosyltransferase activity	4	9.75E-09	UGT2B17;UGT2B15;UGT2B7;UGT2B28
Sequence-specific DNA binding	8	1.28E-08	RAD51;NKX2-5;FOXC1;HNRNPAB;FOXM1;BCL6;FLI1;NR3C1
Lligase activity	7	2.22E-08	UHRF1;UBE2T;CTPS;UBE2S;UBE2C;LIG1;MCCC1
DNA helicase activity	4	2.91E-08	MCM4;MCM6;RECQL4;MCM7
Protein homodimerization activity	6	5.78E-08	GLA;NKX2-5;BIRC5;CAV2;TPD52L1;BARD1
Ubiquitin-protein ligase activity	5	6.53E-08	UBE2T;UBE2S;FBXL6;UBE2C;BARD1
Ribonuclease H activity	3	2.12E-07	EXO1;RNASEH2A;FEN1
Actin binding	6	2.16E-07	TPM3;MYO19;TLN2;TPM1;MYO1B;ANG
Growth factor activity	5	4.01E-07	CXCL12;AREG;TFF1;TGFB3;FGF13
Acyltransferase activity	5	4.12E-07	TAZ;ESCO2;ACAT2;MBOAT1;ZDHHC7
Dihydrofolate reductase activity	2	6.06E-07	DHFRP1;DHFRL1
Magnesium ion binding	6	1.40E-06	EME1;IMPA2;AURKB;FEN1;LIG1;TSSK2
DNA-directed DNA polymerase activity	3	2.29E-06	POLA2;POLE2;ESCO2
Peptidyl-prolyl cis-trans isomerase Activity	3	2.76E-06	FKBP4;PPIF;FKBP5
Damaged DNA binding	3	6.07E-06	RAD51;ESCO2;FEN1
Lamin binding	2	9.07E-06	TMPO;TMPO
Receptor activity	9	9.57E-06	NPY1R;RAMP3;PAQR4;IL17RB;IGSF1;MMD;TNFRSF11B;GRIK2;ITGB6
Structural molecule activity	6	1.18E-05	KRT13;CAV1;TUBB6;TUBB2C;CSTA;KRT86
FK506 binding	2	1.69E-05	FKBP4;FKBP5
Double-stranded DNA binding	3	1.71E-05	RAD51;RAD51AP1;FEN1
Cytokine activity	4	1.95E-05	AREG;NAMPT;TNFRSF11B;TNFSF10
RNA binding	6	2.13E-05	RAD51AP1;RNASEH2A;BARD1;FOX1;IFIH1;OAS1

MHC class I protein binding	2	4.70E-05	TUBB;TUBB2C
Pancreatic ribonuclease activity	2	6.32E-05	RNASE4;ANG
Protease binding	2	7.22E-05	SERPINA1;CSTA
Nicotinic acetylcholine-activated	2	7.22E-05	CHRNA3;CHRNA5
Cation-selective channel activity			
Acetylcholine receptor activity	2	9.20E-05	CHRNA3;CHRNA5
Unfolded protein binding	3	9.77E-05	CHAF1A;CCT5;TUBB2C
Transcriptional activator activity	4	1.16E-04	MYB;NKX2-5;FOXC1;CDH1
Nucleotidyltransferase activity	3	1.46E-04	PRIM1;POLE2;OAS1
Isomerase activity	3	1.49E-04	FKBP4;PPIF;FKBP5
NAD+ ADP-ribosyltransferase activity	2	1.80E-04	PARP9;PARP12
Helicase activity	3	1.82E-04	BRIP1;HELLS;IFIH1
Insulin-like growth factor binding	2	1.95E-04	WISP2;IGFBP4
Cyclin-dependent protein kinase Activity	2	2.43E-04	CDC2;CDK2
Serine-type endopeptidase activity	3	2.66E-04	KLK11;PRSS23;TMPRSS3
NADP binding	2	2.78E-04	DHFRP1;DHFRL1
Iron ion binding	3	3.98E-04	RRM2;SFXN2;EGLN3
Microtubule binding	2	7.53E-04	BIRC5;MAPT
Alpha-galactosidase activity	1	7.80E-04	GLA
Single-stranded DNA specific 5'-3' Exodeoxyribonuclease activity	1	7.80E-04	EXO1
Double-stranded DNA specific 5'-3' Exodeoxyribonuclease activity	1	7.80E-04	EXO1
Thymidylate synthase activity	1	7.80E-04	TYMS
DNA polymerase processivity factor Activity	1	7.80E-04	PCNA
Calcitonin binding	1	7.80E-04	CALCR
Trans-octaprenyltranstransferase Activity	1	7.80E-04	PDSS1
Inhibin beta-B binding	1	7.80E-04	IGSF1
Histone kinase activity	1	7.80E-04	CDK2
5'-flap endonuclease activity	1	7.80E-04	FEN1
Deoxycytidine kinase activity	1	7.80E-04	DCK
UDP-glucose 6-dehydrogenase activity	1	7.80E-04	UGDH
Nicotinamide Phosphoribosyltransferase activity	1	7.80E-04	NAMPT
Porphobilinogen synthase activity	1	7.80E-04	ALAD
Glucocorticoid receptor activity	1	7.80E-04	NR3C1
Gtpase activator activity	3	8.13E-04	RANBP1;ARHGAP19;ARHGAP12
Calcium ion binding	5	8.29E-04	MGP;CELSR2;CDH1;EPS15L1;SLIT1
Heat shock protein binding	2	0.00104104	FKBP4;DNAJC9
Peptidase activity	4	0.00112108	KLK11;PRSS23;TMPRSS3;SERPINA1
Ribonuclease activity	2	0.00141312	RNASEH2A;ANG
Extracellular ligand-gated ion channel activity	2	0.00153538	CHRNA3;CHRNA5
Structural constituent of bone	1	0.00155933	MGP
Flap endonuclease activity	1	0.00155933	EXO1
Recombinase activity	1	0.00155933	RAD51
Atpase activity, uncoupled	1	0.00155933	ATAD2
Coreceptor, soluble ligand activity	1	0.00155933	RAMP3
Serum response element binding	1	0.00155933	NKX2-5
CTP synthase activity	1	0.00155933	CTPS
Trans-hexaprenyltranstransferase activity	1	0.00155933	PDSS1
Inhibin beta-A binding	1	0.00155933	IGSF1
LRR domain binding	1	0.00155933	LRRFIP2
Double-stranded DNA specific exodeoxyribonuclease activity	1	0.00155933	FEN1
Spermidine synthase activity	1	0.00155933	SRM
Apolipoprotein E binding	1	0.00155933	MAPT
Glycerol channel activity	1	0.00155933	AQP3
Protein tag	1	0.00155933	ISG15
JAK pathway signal transduction adaptor activity	1	0.00155933	SOCS2
Methylcrotonoyl-coa carboxylase activity	1	0.00155933	MCCC1
Microtubule motor activity	2	0.00170598	KIF23;KIF11
Oxidoreductase activity	4	0.00188846	RRM2;DHFRP1;UGDH;EGLN3
Atpase activity	3	0.00193292	RAD51;RFC3;ABCC5
Protein binding, bridging	2	0.00212119	FKBP4;CSTA
Single-stranded DNA-dependent atpase activity	1	0.00233809	RAD51
Pancreatic polypeptide receptor activity	1	0.00233809	NPY1R
SUMO binding	1	0.00233809	NKX2-5
Inositol-1(or 4)-monophosphatase activity	1	0.00233809	IMPA2
Calcitonin receptor activity	1	0.00233809	CALCR
Acetyl-coa C-acetyltransferase activity	1	0.00233809	ACAT2
DNA ligase (ATP) activity	1	0.00233809	LIG1
Prolactin receptor binding	1	0.00233809	SOCS2

Beta-galactoside alpha-2,3-sialyltransferase activity	1	0.00233809	ST3GAL1
Serine-type endopeptidase inhibitor activity	2	0.00237045	SERPINA1;TFPI
Protein C-terminus binding	2	0.00307979	RAD51;FOX1
Ribonucleoside-diphosphate reductase activity	1	0.00311625	RRM2
1-acylglycerophosphocholine O-acyltransferase activity	1	0.00311625	TAZ
Glucocorticoid receptor binding	1	0.00311625	FKBP4
Carnitine transporter activity	1	0.00311625	SLC22A5
DNA primase activity	1	0.00311625	PRIM1
Uracil DNA N-glycosylase activity	1	0.00311625	UNG
Camp-dependent protein kinase inhibitor activity	1	0.00311625	PKIB
Ion channel activity	3	0.00325344	CHRNA3;CHRNA5;GRIK2
Chromatin binding	2	0.00343664	CHAF1A;HELLS
Sodium ion binding	2	0.00387615	SLC22A5;ATP1B1
Peptide YY receptor activity	1	0.00389379	NPY1R
RNA polymerase subunit kinase activity	1	0.00389379	CDC2
5'-3' exonuclease activity	1	0.00389379	FEN1
Growth hormone receptor binding	1	0.00389379	SOCS2
Biotin binding	1	0.00389379	MCCC1
Receptor binding	4	0.00390741	GLA;ANGL4_HUMAN;ANGPTL6;ANG
DNA replication origin binding	1	0.00467074	MCM2
Mutlalpha complex binding	1	0.00467074	PCNA
Sodium channel regulator activity	1	0.00467074	TMPRSS3
GDP-dissociation inhibitor activity	1	0.00467074	RANBP1
Punt binding	1	0.00467074	TGFB3
Chromatin DNA binding	1	0.00467074	BCL6
Type I transforming growth factor beta receptor binding	1	0.00544708	TGFB3
Actin monomer binding	1	0.00544708	LIMA1
Kainate selective glutamate receptor activity	1	0.00544708	GRIK2
Motor activity	2	0.00572269	MYO19;MYO1B
Nitric-oxide synthase binding	1	0.00622282	CAV1
GDP binding	1	0.00622282	RERG
Nucleoside transporter activity	1	0.00622282	SLC29A3
Manganese ion binding	2	0.00627623	B4GALT5;FEN1
Structural constituent of epidermis	1	0.00699795	KRT10
Caspase inhibitor activity	1	0.00699795	BIRC5
Estrogen receptor binding	1	0.00699795	RERG
Ran gtpase binding	1	0.00777249	RANBP1
Cyclin-dependent protein kinase inhibitor activity	1	0.00777249	CDKN2C
Transforming growth factor beta binding	1	0.00777249	TGFB3
Water channel activity	1	0.00777249	AQP3
Sodium:potassium-exchanging atpase activity	1	0.00777249	ATP1B1
DNA bending activity	1	0.00854642	FOXC1
Organic anion transporter activity	1	0.00854642	ABCC5
Peptide binding	2	0.00891029	PPIF;ANG
Transcription coactivator activity	2	0.00900525	FHL2;CALCOCO1
Phosphoprotein binding	1	0.00931975	FKBP4
Insulin-like growth factor receptor binding	1	0.01009247	SOCS2
Nucleoside kinase activity	1	0.0108646	DCK
Protein kinase activator activity	1	0.0108646	FGF13
Cholesterol binding	1	0.01240706	CAV1
Carbonate dehydratase activity	1	0.01240706	CA8
RNA polymerase II transcription factor activity	2	0.01249885	E2F2;UHRF1
Electron carrier activity	2	0.01249885	UGDH;CYP1A1
Transporter activity	4	0.01393826	SLC22A5;AQP3;FABP2;APOD
Calcium-dependent protein binding	1	0.01394712	MGP
Histone binding	1	0.01394712	ASF1B
Protease activator activity	1	0.01471626	CAV1
Glutamate-gated ion channel activity	1	0.01471626	GRIK2
Chaperone binding	1	0.01548479	BIRC5
L-ascorbic acid binding	1	0.01548479	EGLN3
Tumor necrosis factor receptor binding	1	0.01548479	TNFSF10
Retinoid binding	1	0.01548479	APOD
Rrna binding	1	0.01548479	ANG
Transcriptional repressor activity	2	0.0158711	NKX2-5;BCL6
Phosphate binding	1	0.01778682	NKX2-5
ATP-dependent DNA helicase activity	1	0.01778682	BRIP1
Transferase activity, transferring glycosyl groups	2	0.01787947	B4GALT5;NAMPT
Cell adhesion molecule binding	1	0.01931852	CDH1
Androgen receptor binding	1	0.01931852	FHL2

Cysteine protease inhibitor activity	1	0.02008348	CSTA
Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors	1	0.02008348	EGLN3
RNA polymerase II transcription factor activity, enhancer binding	1	0.02161162	FOXC1
Fatty acid binding	1	0.02161162	FABP2
Lipoprotein binding	1	0.0223748	MAPT
Galactosyltransferase activity	1	0.02389937	B4GALT5
NAD binding	1	0.02542158	UGDH
Oxygen binding	1	0.02542158	CYP1A1
Protein kinase inhibitor activity	1	0.0261818	PKIB
Scavenger receptor activity	1	0.02694143	TMPRSS3
Protein tyrosine/serine/threonine phosphatase activity	1	0.03148681	CDKN3
Actin filament binding	1	0.03148681	LIMA1
Structural constituent of muscle	1	0.03299723	TPM1
Protein serine/threonine phosphatase activity	1	0.03375156	CDKN3
Chemokine activity	1	0.03601104	CXCL12
Mrna binding	1	0.03601104	HNRNPAB
SH3/SH2 adaptor activity	1	0.03601104	SOCS2
Protein N-terminus binding	1	0.03826526	ZWINT
Hematopoietin/interferon-class (D200-domain) cytokine receptor activity	1	0.04051422	IL17RB
Lipid transporter activity	1	0.04350469	APOD
Steroid binding	1	0.04574145	NR3C1
Transcription factor binding	2	0.04626474	NKX2-5;FOXC1
Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen	1	0.047973	EGLN3

Supplemental Table 4. Oligonucleotides used for PCR. (A) Primers used for real-time RT-PCR. (B) Primers used for ChIP-PCR.

Supplemental Table 4A. Primers used for real-time RT-PCR.

Gene	Forward (5'→3')	Reverse (5'→3')
CCNA2	GCATGCACAACAGTCAATAAGAG	ACTGTACACCATATACTTTGAGTG
CCNB2	CAGCACATGGCCAAGAATGTG	AGGCAAGGTCTTTGACGGC
CCNE2	GAAGATTCCTATGGAAGACAGAC	GCACACTGGTGACAACTGTC
CDC2	CCCAAATGGAAACCAGGAAG	CGTTTGGCTGGATCATAGAT
CDC20	CACCAGTGATCGACACATTCGC	GGCCTGAGATGAGCTCCTTG
E2F2	GAGGAGGTGCAGGAGCCGG	GGATGGAGGCGGTGGGGC
MCM2	GTGACATATCAGCGCAACCG	CATCCTGAAGAGCTCACTGTC
MCM3	AGACGGCAGACTCACAGGAG	CGATTGACTGCGCATGAGCT
MCM4	TTCGGGGACAATCTGACATAG	CAAGCAGGACATGCAGGGAG
MCM5	GAGATGCTGAGCCGCATCG	TGGATGGCGTGCTCCGGGTA
MCM6	CGACTCACACACTATGATCATG	CCTCAGCTCTGGTCAGTTAC
MCM7	TGCGCCTTTCCACTGCTCTG	CTTGTCTCCTAGAAGAAGAGAGTC
MCM8	CAACTTCGGCAGATTGCCAAAG	GGCCCTAACTTGGTGAAGTC
MCM10	CAAGCACTGCAGTAACTGTG	CGGGTTATTTAAGGCTGTTCAG
MYB	TGTTCTCAAAGCATTTACAGTACC	GATGTCATCTGCTCCTCCATC
MYBL2	AGCCACTTCACGACACCTGC	CTCAGGACAAGATGAGGGTC
CXCL12	GTGCCCTTCAGATTGTAGC	GTCTTTGCCCTTTCATCTC
DHFR	AGTAGACATGGTCTGGATAGTTG	GACATCAGAGAGAACACCTGG
EGR3	CTCTTCTCCGGCAGCAGCG	CTTTCCCAAGTAGGTCACGGTC
GREB1	CAGGCTTTTGCACCGAATCT	CAAAGCGTGTCGTCTTCAGCT
HELLS	AATCTGACTGCAGCAGATACAG	TTTAGCAGCTGCTCTTTCCACA
IGFBP4	CTGCGACCGCAACGGCAACT	GCCAGCTGGTGGCAGTCCAG
KNTC2	GCTGAGAATTCCAAAGGTTATGAC	CATTCAGGAGTTCCTTAAGAGG
PCNA	CAACGGTGACACTCAGTATGTC	GAATGCCTAAGATCCTTCTTCATC
PDZK1	CCACTTGACACCCCTCCAGAT	TGTACTGTGGGCCCGTTCTT
POLA2	GACATGGCCATTGACTATGAGTC	AAGGTAGAGTCGGGCGAAGG
TFF1/pS2	CATCGACGTCCCTCCAGAAGAG	CTCTGGGACTAATCACCGTGCTG
PSMC3IP	GCTACCAATCATGTGACTCCAG	CAAAGAACTGCTTCTTGCTCTTG
RAD51	GTAGCTCAAGTGGATGGAGCAG	GTCGTAGATTTTGCAGATTCTGG
RERG	CAACAGTAAATTGTCTACCAACAC	CCATGGAAACAACTTCATCATCG
RFC3	GTATCTGAGGGAGACTGCAAATG	GTAGCAAGTTGTTCTTTGGAGGT
SURVIVIN	CTTCAAGGAGCTGGAAGGCTG	TGCTCGATGGCACGGCGCAC
TAZ	ATGCCTCTGCACGTGAAGTGG	TCACCAACGCATCAACTTCAGG
TFAP2C	GGAGGACGAAATGAGATGGCAG	GGTAATCAGGCTGAAATGAGACA
TUBB	GATCCCCAACAACGTCAAGACA	CTAGTCCATGCCCTCGCCTG
TUBB6	GTGGACTTAGAGCCAGGCACC	CCTGCAGGCAGTCGCAGTGC
WISP2	GCACACCGAAGACCCACCT	AGGTACATGGTGTCGGGCA
PCP4	CGACAAGGTGCTGGGGCAAC	CGCTGCACGTTCTGTCTCTG

OAS1	CCAGTGAGCTCCTGGATTCTG	AGGTCCAGTCCTCTTCTGCCT
PSCA	CTGCAGGTGGAGAACTGCAC	CACGTAGTAGTCCTGTGAGTC
IFIT1	CAAAACCCTGCAGAACGGCTG	ATCGTCATCAATGGATAACTCC
ITGB6	TCTGTTTCCTGCTCTCTGCAAG	ACCAGTAGCTTCCAGATGCAC
ATAD2	GAACAGCAGCAGCTCATCACTG	TATCATGGTCCTTGCGATGCCG
BRIP1	TGTGTCCAGGAACTACAGTGTC	TTGGGCTTGTGGATCTGGAAATC
AURKB	TCAAGGGAGAGCTGAAGATTGC	CACCTTCTCATTGTGCATGCG
BARD1	GCAGAAGCAGGCTCAACAGAG	GGCTTGGGCTTTCTACTGAGG
CDK2	CTGGATGAAGATGGACGGAGC	AGTCGAAGATGGGGTACTGGC
CCND1	GCTTCCTCTCCAGAGTGATC	GTCCATGTTCTGCTGGGCCT
Cathepsin D	CGCGATCACACTGAAGCTGG	ACAGTGTAGTAGCGGCCGATG
E2F1	CAGATCCCAGCCAGTCTCTAC	CCGGAGAAGTCCTCCCGCAC
c-FOS	CAAATGCCGCAACCGGAGGA	GCTGCCAGGATGAACTCTAG
ERα	CCACCAACCAGTGCACCATT	GGTCTTTTCGTATCCCACCTTTC
ERβ	AGAGTCCCTGGTGTGAAGCAAG	GACAGCGCAGAAGTGAGCATC
PES1	TCAACAAGTTCCGTGAATACAAG	GATGTGGTCGAGTTTGTAGTTG
β-actin	ATCACCATTGGCAATGAGCG	TTGAAGGTAGTTTCGTGGAT

Supplemental Table 4B. Primers used for ChIP-PCR.

Gene	Forward (5'→3')	Reverse (5'→3')	Reference
Cathepsin D-PP*	TCCAGACATCCTCTCTGGAA	GGAGCGGAGGGTCCATTC	1
Cathepsin D-DE#	CCTCCTCAACTGCTCTTGCA	GCGGCTGAGATGCTGAGTCA	2
CCND1-PP*	AACAAAACCAATTAGGAACCTT	ATTTCCTTCATCTTGTCCTTCT	3
CCND1-DE#	CAGTTTGTCTTCCCGGGTTA	TCATCCAGAGCAAACAGCAG	4
E2F1-PP*	AAAGTCCCGGCCACTTTTAC	CGCGTTAAAGCCAATAGGAA	5
CCNE2-PP*	TGACACCCCCGAAATCCA	CCTGGCTCGCGCATCT	6
β-actin-PP*	TGCACTGTGCGGCGAAGC	TCGAGCCATAAAAGGCAA	7

*PP, proximal promoter. #DE, distal enhancer.

References

1. Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell.* 2001;103(6):843-852.
2. Bretschneider N, Kangaspeska S, Seifert M, Reid G, Gannon F, Denger S. E2-mediated cathepsin D (CTSD) activation involves looping of distal enhancer elements. *Mol. Oncol.* 2008;2(2):182-190.
3. Weitsman GE, et al. Estrogen receptor-α phosphorylated at Ser118 is present at the promoters of estrogen-regulated genes and is not altered due to HER-2 overexpression. *Cancer Res.* 2006;66(20):10162-10170.
4. Eeckhoute J, Carroll JS, Geistlinger TR, Torres-Arzayus MI, Brown M. A cell-type-specific transcriptional network required for estrogen regulation of cyclin D1 and cell cycle progression in breast cancer. *Genes Dev.* 2006;20(18):2513-2526.
5. Frietze S, Lupien M, Silver PA, Brown M. CARM1 regulates estrogen-stimulated breast cancer

growth through up-regulation of E2F1. *Cancer Res.* 2008;68(1):301-306.
6. Rodríguez-Paredes M, Ceballos-Chávez M, Esteller M, García-Domínguez M, Reyes JC. The chromatin remodeling factor CHD8 interacts with elongating RNA polymerase II and controls expression of the cyclin E2 gene. *Nucleic Acids Res.* 2009;37(8):2449-2460.
7. Gao Z, et al. Coactivators and Corepressors of NF-kb in Ikb α gene promoter. *J Biol Chem.* 2005;280(22):21091-21098.