SUPPLEMENTARY MATERIALS

Cell lines and culturing. MCF 10A, MCF-7, ZR-75-1, T-47D, CAL-51, MDA-MB-231, MDA-MB-468, MDA-MB-231-LM2.4^{Luc}, SK-BR-3, BC3-WT TP53, BC3-shTP53 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM 10-013-CV Corning Cellgro) supplemented with 10% fetal bovine serum (FBS 10437-028 gibco), penicillin (50 IU/ml), and streptomycin (50 g/ml). All cell lines were grown at 37°C, under 5% CO_2, in a humidified incubator. MCF 10A cells were maintained in DMEM/F12 with L-Glutamine (Invitrogen #11330-032) medium (500ml) containing 5 % Horse Serum (Invitrogen), 10µg/mL insulin (Sigma), 20 ng/mL Epidermal growth factor (EGF) (Peprotech) 0.5 µg/mL Hydrocortisone (Sigma), 0.1µg/mL Cholera toxin (Sigma) and penicillin and streptomycin (Gibco). MCF-7 and MDA-MB-231 cells were obtained from ATCC, and MDA-MB-231-LM2.4^{Luc+}cells were a kind gift from the laboratory of Dr. John Ebos. SK-BR-3 and CAL-51 cells were provided by Drs. Boyko Atanassov and Muthusami Thangaraju, respectively. BC3-WT TP53, BC3-shTP53 cells were a kind gift from Dr. Helen Piwnica-Worms. All cell lines were authenticated by short tandem repeat (STR) analysis.

siRNA transfections. Specific siRNAs were transiently transfected using Lipofectamine 2000 and 3000 as per the manufacturer's instructions (ThermoFisher Scientific). Cells were transfected with a final concentration of 120 nM stealth control or *ESR2*-specific siRNA (Invitrogen #12935-300) or TP73-specific siRNA (80nM). Cells were harvested 48 hours post-siRNA-transfection for further analysis. Sequence information of siRNAs is provided in Supplementary Table 1.

Antibodies and reagents. See Supplementary Table 2.

Plasmid constructs. Wild type human TP53 (pRc/CMV hp53) and pCR3.1/hERα expression plasmids were kindly provided by Drs. A.J. Levine and C. Smith, respectively. The pCR3.1/p53 construct was generated by cloning full-length p53 cDNA (HindIII & XbaI fragment) from pRc/CMV hp53 plasmid into pCR3.1 vector. The human ERβ1cDNA clone was obtained from Dr. Massami Muramatsu .The full length ERβ was amplified and cloned into BamHI and XhoI restriction sites of pCR3.1 vector. For

truncated ERβ mutants, PCR products of respective sizes were cloned into pCR3.1 vector, followed by sequence verification by dideoxy sequencing. N-terminally-FLAG-tagged-human-*ESR2* full-length plamid was provided by Dr. John Hawse, Mayo Clinic). HA-tagged *ESR2* full-length expression vector was generated by cloning HA-tagged human *ESR2* cDNA into the pLVX-Tight-PuroTet-regulated vector (Clontech). The vector was constructed via a PCR approach using high-fidelity reverse transcriptase, Accuscript™ (Agilent Technologies; 600180), high-fidelity thermostable polymerase, Phusion™ (New England Biolab, F-530), and specific primers (Supplementary Table 1) that were designed to amplify the full length sequence of human *ESR2* (NM_001437). A 2X HA-tag-encoding sequence (1x = YPYDVPDYA) was incorporated into the reverse primer to add a C-terminal tag. Total RNA isolated from MCF-7 cells was used as template for the PCR reaction. The amplified PCR products were cloned into the *Not*I and *Mlu*I sites of pLVX-Tight-Puro Vector. The integrity of the selected clone was verified by sequencing.

Transient transfections. For transient transfection of plasmid constructs, Xtremegene 9 (Roche) was used, as per the manufacturer's instructions. Cells were harvested 48 hours post-transfection for analysis. Empty vectors corresponding to the transfected cDNA expression plasmids were used as controls.

Generation of inducible ESR2 shRNA stable cells. The SMART inducible lentiviral shRNA VECTOR was transduced into MCF-7 and MDA-MB-231 cells as per the manufacturer's protocol (Dharmacon, USA). Lentiviral Particles titrations were performed on MCF-7 and MDA-MB-231 cells with serial 3 fold dilutions of lentiviral particles (V3SH7675-01EG2100-SMART vector inducible Human ESR2 mCMV-TurboGFPshRNA, Dharmacon USA) with 4 µg/mL polybrene concentrations. Combination of three shRNA sequences (A+B+C=10µl) was mixed with transduction medium. 25 µL volumes of each dilution were transfer to cells and incubated overnight (16-20hrs). After 20hrs, 75µl DMEM (10%FBS) medium was added to cells without removing old medium. 1µg/mL freshly dissolved doxycycline containing DMEM was added into cells after 24 hrs and further incubated for 48 hours. Vector titer was determined

based on the positive GFP population. Positive GFP cell population was further grown to get individual clones that were used for the final experiments. Sequence information for the three shRNAs used are as follows:

SMART vector inducible Human ESR2 mCMV-TurboGFPshRNA

A. TGCTGGGAATGCTGTAATT

B. CAGCGCAGAAGTGAGCATC

C. GGATATTCATGGTGGCTGT

RNA isolation, cDNA synthesis, semi-quantitative end-point PCR, and quantitative real time PCR (qRT-PCR) assays. Total cellular RNA was isolated using TRizol reagent (Invitrogen) as per the manufacturer's protocol, and quantified using a Nanodrop 8000 spectrophotometer. Isolated RNA was treated with DNAse I (Amplification grade, Invitrogen) and used for cDNA synthesis. The first strand cDNA pool was synthesized using 1 µg of total RNA and BioRad'siScript™ cDNA synthesis kit (170-8891) in a 20 µl reaction volume. A portion of the first strand cDNA products (5-10%) were amplified using target-specific primers (for primer sequences see Supplementary Table 1). For semi-quantitative analysis of relative transcript levels reactions were carried out using AccuPrime™ Taq DNA Polymerase system (Invitrogen,12339-016) in BioRad's iCycler thermocycler for 20-30 cycles with the cycling parameters of: Initial denaturation, 94°C x 2 minutes, Denaturation, 94°C x 30 minutes, Annealing, 58°C x 45 minutes, Extension, 68°C x 45 minutes, Final extension, 72°C x 5 minutes. qRT-PCR was performed using either FastStart Universal SYBR Green mastermix (Roche) or iScript™ Reverse Transcription Supermix for RT-qPCR (BIO-RAD) in a 10µl reaction in Applied Biosystem's ABI Prism 7300 Real time PCR machine. 50°C for 15s (1 cycle); 95°C for 10 min (1 cycle); 95°C for 15 s, followed by 60°C for 45s (35 cycles). Dissociation curves were used to confirm the detection of a single amplicon. Primer efficiencies were calculated from a dilution curve and determined to be with the acceptable range (90-110%). Data acquisition and analysis was carried out by ABI's 7000 system sequence detection software V1.4. The relative target levels were determined by the ΔΔCT method (1) using β-actin mRNA as reference gene endogenous controls. All experimental and control groups were performed in

biological and technical triplicates. Error bars are representative of standard deviation, as indicated in the figure legends. Statistical analyses were performed by two tailed student t- test using 'Graphpad Prism 7.0'.

Glutathione S-Transferase (GST) Pull-Down assay. The GST and wild type and mutant GST-ESR1 and GST-TP53 constructs were expressed in BL21 (DE3) pLySs bacteria (Stratagene) and immobilized on Glutathione-Sepharose 4B GST-tagged protein purification resin (GE Healthcare Biosciences, Piscataway, NJ) as previously described (2). Full length and truncated ESR2 proteins were *invitro* translated and [^{35}S] methionine-labeled using TNT T_7 Quick coupled transcription translation system (Promega). [^{35}S]- labeled proteins were incubated with ~4µg of GST (negative control) or GST proteins bound to sepharose beads in 500µl of NENT buffer (20mm Tris-HCl at pH 8.0, 100mm NaCl, 1mM EDTA, 0.5% NP-40 containing 0.5% non-fat dry milk) for 2h at 4^{o}C.The beads were washed four times with NENT buffer and bound proteins eluted with SDS-sample buffer were separated by SDS-PAGE. The gels were fixed, dried and subjected to fluorography.

Co-Immunoprecipitation (Co-IP) assay. Cells were rinsed twice with phosphate-buffered saline (PBS) and lysed in NENT buffer (20mM Tris-HCl at pH 8.0, 100mM NaCl, 1mM EDTA, 0.5% NP-40) for 30 min by end-over-end mixing at 4°C. cOmplete™protein inhibitor cocktail (Roche,11873580001) or Halt protease inhibitor cocktail (ThermoFisher Scientific, 78420) and phosphatase inhibitors (1 mM sodium orthovanadate and 10 mM sodium fluoride) were included in the lysis and wash buffers. For Co-IP with TP53, extracts were cleared by centrifugation at 12,000g for 15 min at 4^{o}C and further pre-cleared by rocking for 2h at 4^{o}C with agarose-conjugated mouse IgG (Santa Cruz Biotechnology). Precleared extract was immunoprecipitated with agarose conjugated anti-TP53 (DO-1AC; Santa Cruz Biotechnology) or equivalent amount of mouse IgG overnight at 4^{o}C. The agarose beads containing bound proteins were washed three times with lysis buffer and boiled in SDS-sample buffer. For all other Co-IPs, the extracts were cleared by centrifugation for 10 min at 10,000 rpm at 4 °C and lysate was pre-cleared with Recombinant Protein G Agarose beads (Invitrogen, 15920-010) for 1 hour at 4°C with rotation. 2.5 mg of pre-cleared lysate was immunoprecipitated with 5 µg of the indicated antibody or

control IgG overnight at 4 °C with rotation. The antigen-antibody complexes were captured with protein G-Agarose beads for 3 h at 4°C with rotation, washed 4 times in NENT buffer, eluted with SDS-PAGE sample buffer. Immunoprecipitated proteins were resolved by SDS-PAGE-western blotting, using the ECL Chemiluminescent method (ThermoFisher Scientific 32106).

Cell cycle analysis. Cells were subjected to cell cycle distribution analysis 48 hours post-transfection. Propidium iodide (PI) (Sigma Aldrich-P4170) was used for staining total cellular DNA. Flow cytometry was performed on a FACScan cytometer (Pharmingen), and the data were analyzed using ModFit software.

Apoptosis analysis. APC Annexin V (BD Pharmingen; 550474) and FITC Annexin V (Invitrogen V13242) kits were used for flow cytometric analysis of cells undergoing apoptosis. For analyzing effect of *ESR2* knockdown, Cells were transiently transfected with either *ESR2* siRNA or non-specific (NS) siRNA for 48 hours. In the case of cells stably expressing *ESR2*shRNA, cells were treated with or without doxycycline for 48 hours to induce shRNA expression. For overexpression of ESR2, cells were transiently transfected with FLAG-*ESR2* for 48 hours. Cells were subsequently washed with PBS followed by Annexin and PI staining as per manufacturer protocol. Stained cells were analyzed by FACS Calibur flow cytometer and CellQuest Pro software (BD Bioscience) and Winlist3D 8.0.

Clonogenic growth assay. 48 hours post-transfection, approximately 2000 cells/cm^2 were seeded and allowed to grow for 8-10 days. At this point, cells were fixed with 10% formaldehyde solution for 15 minutes at room temperature. Subsequently, cells were stained with 0.1% crystal violet solution for 15 minutes at room temperature. Unabsorbed crystal violet dye was washed away by submerging the plate several times in running water. Plates were completely air dried at ambient temperature for several hours and photographed. For quantitation purposes, the absorbed dye, which is proportional to cell mass, was extracted with 10% acetic acid solution. 100 µl of extract per sample was loaded into 96-well plates, and the absorbance at 595 nm was measured using a Synergy 2 (BioTek) plate reader.

Chromatin immunoprecipitation (ChIP) assay. ChIP assay was carried out as previously described (3). Briefly, cells were fixed with 1.5% Formaldehyde for 10 minutes at 37˚C. Cell lysis was done using 1 ml ChIPlysis buffer per $5x10^6$ cells. Chromatin shearing was carried out in batch using cup-horn attachment in MisonixSonicator 3000 (Misonix, NY)set at medium power for total of 20 minutes (intermittent pulses of 10 seconds ON 20 seconds OFF). Chromatin fragments equivalent of $1\text{-}2X10^6$ cells and 2.5-5 µg of either species matched control-IgG or specific primary antibody was used per ChIP reaction. Chromatin-bound protein-antibody complex was harvested using Protein-A-agarose/salmon sperm DNA (Millipore; 16-157). Immunoprecipitated chromatin samples were analyzed through real-time q-PCR as stated above. A region downstream of *CDKN1A/p21* promoter sequence was used as endogenous control for the relative quantitation of immunoprecipitated chromatin fragments (Details of the primers are provided in the Supplementary Table-1). The promoter occupancy by transcription factor (s) of interest was analyzed as qRT-PCR.

Proximity ligation assay (PLA). *In situ* PLA was carried out using the Duolink II reagent kit (Olink Bioscience) and supplier's protocol. Briefly, 10,000 cells were seeded on 12 mm coverslips (Thermo Fisher Scientific) in 24-well plates. After 12-24 h, cells were fixed with freshly prepared 2% paraformaldehyde (Sigma) solution in PBS, pH 7.4 at room temperature for 20 minutes. Subsequently, the coverslips were washed twice with 1 ml of PBS, blocked and permeabilized with a solution containing 2% BSA and 0.1% Triton-X-100 in PBS, pH 7.4, for 1 hour at room temperature, followed by nuclear permeabilization using a buffer containing 1% BSA and 0.1% NP40 in PBS, pH 7.4, for 15 minutes at room temperature. Mouse and rabbit primary antibodies were diluted appropriately in antibody dilution buffer (supplied in the kit) and were applied to the coverslips in an open droplet manner and incubated at room temperature for 1 hour in a humidified chamber. The remainder of the protocol, which included secondary probe hybridization, ligation, and amplification, were carried out as per the manufacturer's instructions. Coverslips were mounted with the supplied mounting media containing DAPI. Photographs were taken with an AXIOSKOP (Carl Zeiss, Germany) fluorescent microscope fitted with a Hamamatsu

3CCD digital camera and ImagePro Plus Software. Subsequently, Blob-Finder image analysis software (developed by Centre for Image Analysis, Uppsala University) was used to quantify the PLA signals.

For PLA on archived TNBC tissue, a harmonized antigen retrieval protocol for both TP53 and ESR2 (determined by immunohistochemistry with TP53 antibody FL393 and ESR2 antibody, MC10) was developed. Antigen retrieval was conducted for 40 minutes in steamer with EDTA buffer followed by a 20 minute cooling at ambient temperature. Endogenous peroxidase was quenched with aqueous 3% H2O2 for 10 minutes and washed with PBS/T. Serum free protein block (Dako) was applied for 5 minutes. Primary antibodies for TP53 and ESR2 were added and incubated at room temp for one hour followed by washing with PBS/T. Rest of the PLA protocol and image analysis were as described above.

Generation of TP53 knockout cells using CRISPR/Cas9. Mutant *TP53* knockout (MDA-MB-231-TP53KO) cells were generated in collaboration with the Genome Engineering and iPSC center at Washington University School of Medicine. MDA-MB-231-LM2.4^{Luc+} cells were nucleofected with Cas9 and a *TP53*-specific sgRNA (5'-TCCTCAGCATCTTATCCGAGNGG-3'). Cells were single cell-sorted and genomic DNA was extracted. DNA was amplified using the following forward and reverse primers: 5'-CCATGAGCGCTGCTCAGAT-3' and 5'-TCATGGGGTTATAGGGAGGTCA-3'. Clones were screened for frameshifts by sequencing the target region with IlluminaMiSeq at approximately 500x coverage. After genomic screening, two clones which showed indels resulting in a frameshift mutation were additionally screened for mutant TP53 protein, confirming a complete knock out of mutant *TP53* in both clones. One clone was selected to be used in the current experiments. Lack of TP53 protein expression was confirmed by immunoblotting.

Immunohistochemical staining and scoring of the human breast cancer tissue micro array (TMA).

TMA Construction: Three 1-millimeter tissue cores from each formalin-fixed paraffin embedded donor blocks were precisely arrayed into a new recipient paraffin block that included tumor specimens and controls. Eligible patients had surgeries performed between 1995 and 2008 at Roswell Park Comprehensive Cancer Center (Roswell) Buffalo, NY. Specimens for controls within the TMA consisted

of multiple cores of normal tissue from 10 different organs including heart, colon, kidney, adrenal, ovary, myometrium, brain, thyroid, lung, and prostate thereby representing more than 20% of all the cores in a TMA. Appropriate Institutional Review Board approval consistent with federal, state and local requirements was obtained for this project and clinical and outcome data was de-identified.

Immunohistochemistry (IHC): For antigen retrieval slides containing serial sections of triple negative breast cancer (TNBC) TMA wetre heat-treated and endogenous peroxidase was quenched with aqueous 3% H2O2 for 10 minutes. Slides were then loaded on a Dako autostainer where they were treated with serum free protein block (DAKO, cat #X0909) for 5 minutes followed by reaction with primary antibodies against TP53 (FL-293 or CM1) and ESR2 (14C8) for one hour. Secondary antibodies, HRP-conjugated anti-rabbit (DAKO, cat # K4003) and anti-mouse (Leica,cat # PV-6114) were used for 30 minutes. Slides were developed by the DAB chromagen (DAKO,cat # K3468) for 10 minutes and finally, counterstained with hematoxylin, dehydrated, cleared and cover-slipped.

Aperio Slide Scanning and Image Analysis: TMA slides were digitally scanned using AperioScanscope (Aperio Technologies, Inc., Vista, CA) with 20x bright-field microscopy. These images are then accessible using Spectrum (Aperio Technologies, Inc., Vista, CA), a web-based digital pathology information management system. Slide images are automatically associated to a digital slide created in the Digital Slide table in Spectrum. Once slides are scanned, Aperio Image Scope version 11.2.0.780 (Aperio Technologies, Inc., Vista, CA) was used to view images for image analysis. Slide image data field were populated, images were examined for quality and were amended as necessary. An annotation layer was created for each core of interest in the TMA. Invasive tumor cell-only regions were identified and annotated to appropriately represent the heterogeneity of staining of each TMA core for image analysis. Care was taken to avoid including areas of carcinoma in situ and regions with staining artifacts. When possible, representative areas of tumor were selected for analysis with a minimum target of 30 cells per TMA core. Areas of target cells for image analysis were circled using the free form pen tool and areas to be excluded were marked using the negative free form pen to reduce cells irrelevant regions from image analysis calculations. Image analysis data was exported from Spectrum as a tab delimited .csv file and converted to a .xls file and formatted using Microsoft Excel 2010.

Quantitative scoring of nuclear ESR2 and TP53 Immunohistochemistry signals: The nuclear segmentation factor parameter was set to account for brown stain in the nuclear compartment and the nuclear threshold type was set to adaptive, in which allows the algorithm to adjust thresholds based on the strength of the staining. The Nuclear Algorithm detects the positive (DAB) nuclear staining for the individual tumor cells and quantifies their staining intensity. Cell nuclei are individually classified as 0 - none, 1+ - weak, 2+- moderate, and 3+ -strong. The algorithm uses color de-convolution that separates the hematoxylin and DAB stains, thereby providing stain separation. The analysis results provided the total number of detected cells, the percentage of cells per class (0, 1+, 2+ and 3+) and the percentage of positive stained cells along with the average staining intensity of the positive nuclei as a score of 0, 1+, 2+ and 3+. The H-score score equals = 1*(%1+) + 2*(%2+) + 3*(%3+) with the score is between 0 and 300, where 300 represents 100% of cells being 3+. The counterstain hematoxylin, a blue stain, was applied for morphologic detail of the surrounding tissue to help identify nuclear and cytoplasmic compartments of cells for analysis.

Statistical analyses. Statistical analyses of experiments in cell models: Statistical analyses were performed using the two-tailed Student's t-test comparisons of relative gene expression, cell growth, cell cycle and apoptosis assays. *p* values less than 0.05 were deemed statistically significant throughout, with no adjustment for multiplicity.

Statistical analysis of METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) data: All statistical analyses for determining clinical significance *ESR2/TP53* combination in METABRIC were performed using WinSTAT (http://www.winstat.com/). Breast cancer-specific survival (BCSS), defined as the time of diagnosis to the time of a breast cancer-related death was used for the survival analyses, and statistical significance was tested using Univariate cox analysis (supplementary table 3). For analyzing the Roswell cohort of TNBC patient tumors, differences in clinical characteristics between the expression classes were tested using Fisher's Exact and Wilcoxon Rank Sum tests as appropriate. All *P* values are two sided, and are interpreted as described in 2016 ASA *P* Value Statement (4). Statistical significance of comparison of relative gene expression, cell growth, cell cycle and apoptosis assays were determined

by two-tailed Student's t-test. *p* values less than 0.05 were deemed statistically significant throughout, with no adjustment for multiplicity.

Statistical analysis of TMA data: Up to three tissue samples per patient were obtained from three separate Tissue Micro Arrays (TMA). Marker expression levels in the samples were obtained as described above. Sample records with no cells staining positive were considered to contain no tumor tissue, and were excluded from the analysis. Sample-level expression was quantified as the H-Score, the weighted arithmetic mean of the intensity level [0,1,2&3] in the sample, weighted by the percentage of cells at each intensity. Patient-level expression was quantified as the arithmetic mean of the available sample-level scores. The High/Low expression classification was determined by the median of the observed patient-level H-Scores. Differences in clinical characteristics between the expression classes were tested using Fisher's Exact and Wilcoxon Rank Sum tests as appropriate. Overall Survival (OS) was defined as the number of months between diagnosis and death from any cause. Progression Free Survival (PFS) was defined a time from diagnoses to documentation of disease progression or death, whichever came first. Patients with no event were censored at the date of last follow up. Differences in OS and PFS between the expression classes were tested with a log rank test, and displayed using Kaplan-Meier plots. Median follow up time was about 77 months, estimated by the Reverse Kaplan Meier method (5, 6). All *P* values are two sided, and are interpreted as described in 2016 ASA *P* Value Statement (4). *P* values less than 0.05 were deemed statistically significant throughout, with no adjustment for multiplicity.

Samples were first stratified at the median TP53 Nuclear H Score. Within the TP53 groups, the samples were further stratified at the median ESR2 Nuclear H-Score.

Tumor size: TP53-high (surrogate for mutant TP53) breast cancer patients stratified at the median ESR2 nuclear H-Score

Size		ESR2 H Score		All
		Low	High	
	N	12	11	23
	mean	3.71	1.80	2.80
	Std	2.61	0.46	2.11
	Med	2.80	1.60	2.00
	Min	1.50	1.30	1.30
	Max	11.00	3.00	11.00

Tumor stage: TP53-high (surrogate for mutant TP53) breast cancer patients stratified at the median ESR2 nuclear H-Score

Stage	ESR2 H Score				All	
	Low		High			
	n=	%	n=	%	n=	%
1	1	8.33	9	81.82	10	43.48
2	10	83.33	2	18.18	12	52.17
3	1	8.33	.	.	1	4.35

References associated with Supplementary Materials

1. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001;25(4):402-8.
2. Liu W, Konduri SD, Bansal S*, et al.* Estrogen receptor-alpha binds p53 tumor suppressor protein directly and represses its function. *J Biol Chem* 2006;281(15):9837-40.
3. Liu W, Swetzig WM, Medisetty R*, et al.* Estrogen-mediated upregulation of Noxa is associated with cell cycle progression in estrogen receptor-positive breast cancer cells. *PLoS One* 2011;6(12):e29466.
4. Wasserstein RL, Lazar Na. The ASA's statement on p-Values: Context, process, and purpose. *The American Statistician* 2016;70(2):129-133.
5. Shuster JJ. Median follow-up in clinical trials. *J Clin Oncol* 1991;9(1):191-2.
6. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. *Control Clin Trials* 1996;17(4):343-6.

Supplementary Table 1. Details of Oligonucleotide Primers Used

Gene Primer		Sequence (5'- 3')/Reference
ESR2-1(ERβ1) Cloning Primers	CtHA-NotI-Forward	CCATAGCGGCCGCCACCATGGAT ATA AAA AC TCACCATCTAGC
	CtHA-MluI-Reverse	GGTCA ACG CGT TCA AGC GTA ATC TGG AAC ATC GTA TGG GTA AGC GTA ATC TGG AAC ATC GTA TGG GTA CTG AGA CTG TGG GTT CTG GGA GC
RT-PCR Primers	Pan-ERβ-RT-Forward1	CCT ATG TAG ACA GCC ACC ATG AAT
	Pan-ERβ-RT-Reverse1	CCC ACC TCC CAA GTT AGT GAC ATT
	HA-ERβ-RT-Forward	GCT TTG GTT TGG GTG ATT GCC A
	HA-ERβ-RT-Reverse	CGT TCA AGC GTA ATC TGG AAC
	ERβ1,2,4,5-RT- Forward and Reverse 'Triple Primers': Forward ERβ1-RT-Reverse ERβ2,4,5-RT-Reverse	CGATGCTTTGGTTTGGGTGAT GCCCTCTTTGCTTTTACTGTC CTTTAGGCCACCGAGTTGATT
	β-Actin-RT-Forward	ATG GGT CAG AAG GATTCC TAT GT
	β-Actin-RT-Reverse	AAG GTC TCA AAC ATG ATCTGG G
	p21-RT-Forward	GAG ACT CTC AGG GTC GAA AAC G
	p21-RT-Reverse	GAT GTA GAG CGG GCC TTT GA
	PUMA-RT-Forward	ATG CCT GCC TCA CCTTCA TC
	PUMA-RT-Reverse	TCA CAC GTC GCT CTCTCT AAA CC
	BTG2-RT-Forward	GTG AGC GAG CAG AGGCTT AAG
	BTG2-RT-Reverse	GAGCCCTTGGACGGCTTT
	NOXA-RT-Forward	ATG AAT GCA CCT TCA CAT TCC T
	NOXA-RT-Reverse	TCC AGC AGA GCT GGA AGT CGA
	CD44-RT-Forward	CCA CGT GGA GAA AAA TGG TC
	CD44-RT-Reverse	CAT TGG GCA GGT CTG TGA C
	VEGFα-RT-Forward	AAC ACA GAC TCG CGT TGC AA
	VEGFα-RT-Reverse	CGG CTT GTC ACA TCT GCA AGT
ChIP Primers	ChIP-Nonspecific (NS)-Forward	CAG AGT GAG ACC TTG TCT GTC TCC
	ChIP-Nonspecific (NS)-Reverse	CAG AAG ATG CAT GCA ACA GCA CCT TG
	p21-ChIP-Forward	CAG GCT GTG GCT CTG ATT GG
	p21- ChIP-Reverse	CCT CAC CTG AAA ACA GGC AGC
	PUMA-ChIP-Forward	GCGAGACTGTGGCCTTGTGT
	PUMA-ChIP-Reverse	CGT TCC AGG GTC CAC AAA GTC
siERβ#1	ESR2	CCC UGC UGU GAU GAA UUA CAG CAU U
siERβ#2	ESR2-HSS103380, ThermoFisher	CCU UUA GUG GUC CAU CGC CAG UUA U
siTP73	siTP73- AM16708, ThermoFisher	GGG ACU UCA ACG AAG GAC Att

Supplementary Table 2. Details of Antibodies Used

Protein	Antibodies	Dilutions			Company (Cat.no)
		IHC★	PLA / IF †	IB‡	
ERβ	H-150	–	1:300	1:5000	Santa Cruz (SC-8974x)
	68-4	–	–	1:5000	Millipore (05-824)
	14C8	1:300	1:250	–	Abcam (ab288)
	MC10	–	1:300	–	Reference §
TP53	DO1		1:250	1:7000	Santa Cruz (SC-126)
	FL393	1:300	1:250	1:7000	Santa Cruz (SC-6243)
	CM1	1:300	–	–	Leica Biosystem (NCL-p53-CM1)
TP73	H-79	–	1:300	–	Santa Cruz (SC-7957x)
	5B429	–	–	1:7000	Imgenex (IMG-246)
	Anti-p73	–	1:300	1:5000	Bethyl Laboratories (A300-126A)
	p73 (D3G10)	–	1:250	1:5000	Cell Signaling (14620)
FLAG	Anti-FLAG	–	1:250	1:5000	Sigma (F1804)
HA	HA.11	–	1:250	1:5000	Covance (MMS-101P)
p21	Anti-p21	–	–	1:7000	Santa Cruz (SC-397)
	p21 Waf1/Cip1 (12D1)	–	–	1:5000	Cell Signaling (2947)
PUMA	Anti-PUMA	–	–	1:7000	Sigma (P4618)
	H-136	–	–	1:7000	Santa Cruz (SC-28226)
β-actin	Anti-β-actin	–	–	1:10000	Sigma (A5441)
PARP	Anti-PARP	–	–	1:7000	Cell Signaling (9542)
BIM	Anti-Bim	–	–	1:5000	Cell Signaling (2933)
Caspase 3	Anti-Caspase-3	–	–	1:5000	Cell Signaling (9662)
BID	Anti-Bid	–	–	1:5000	Cell Signaling (2002)
BAX	Bax (D2E11)	–	–	1:5000	Cell Signaling (50230)
Secondary antibodies	HRP-anti-rabbit	–	–	1:10000	Millipore (12-348)
	HRP-anti-mouse	–	–	1:10000	Millipore (12-349)
Secondary antibodies	Alexa Fluor 488 anti-mouse	–	1:300	–	Invitrogen (A11001)
	Alexa Fluor 594 anti-rabbit	–	1:300	–	Invitrogen (A11012)
Secondary antibodies	HRP-conjugated anti-rabbit HRP-conjugated anti-mouse	1:300	–	–	DAKO (K4003) Leica (PV-6114)
IgG negative controls	Normal mouse IgG	–	1:250	–	Millipore (12-371)
	Normal rabbit IgG	–	1:250	–	Millipore (12-370)

★ IHC= Immunohistochemistry, † PLA= Proximity Ligation Assay , IF=Immunofluorescence

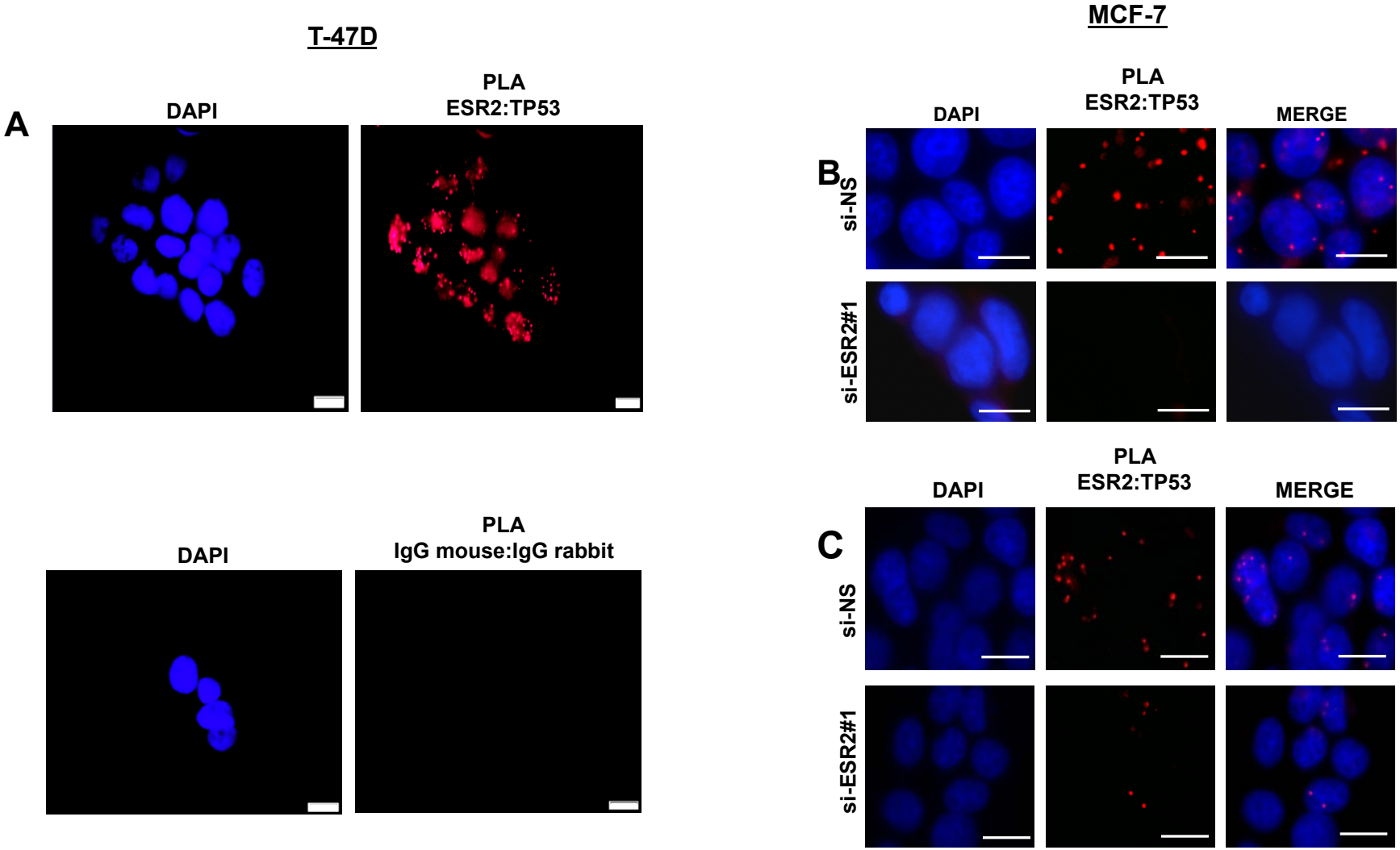
‡ IB = Immunoblotting, § Wu, X et al., J Cellular Biochem. 2012. 113: 711-723

– Not used for the particular assay

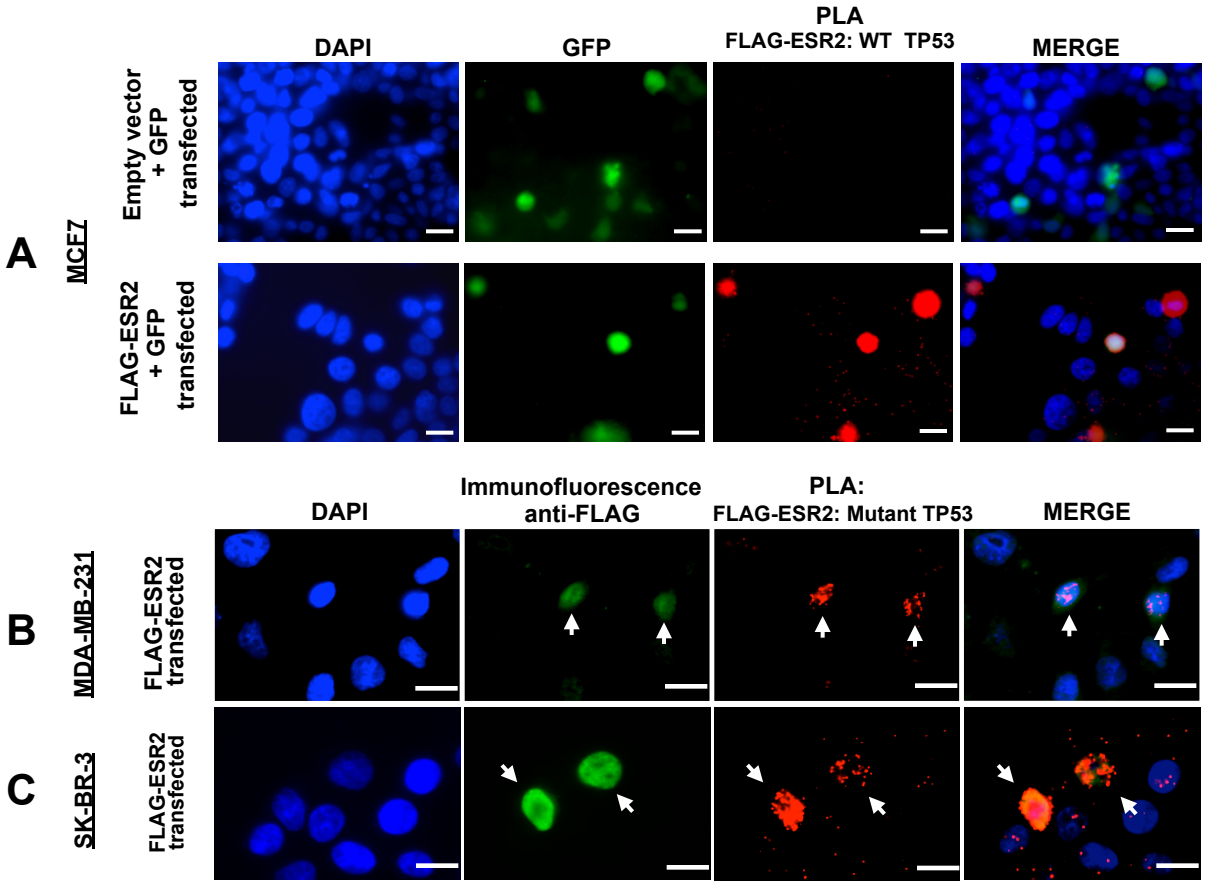
Supplementary Table 3. Univariate cox Regression analysis of ESR2 expression in TP53 mutated Basal-like subtypes

Endpoint	Hazard Ratio(HR)	lower.95	upper.95	Pr(>|z|)
Breast Cancer-Specific Survival	0.321	0.082	1.260	0.103
Overall Survival	0.257	0.079	0.838	0.024

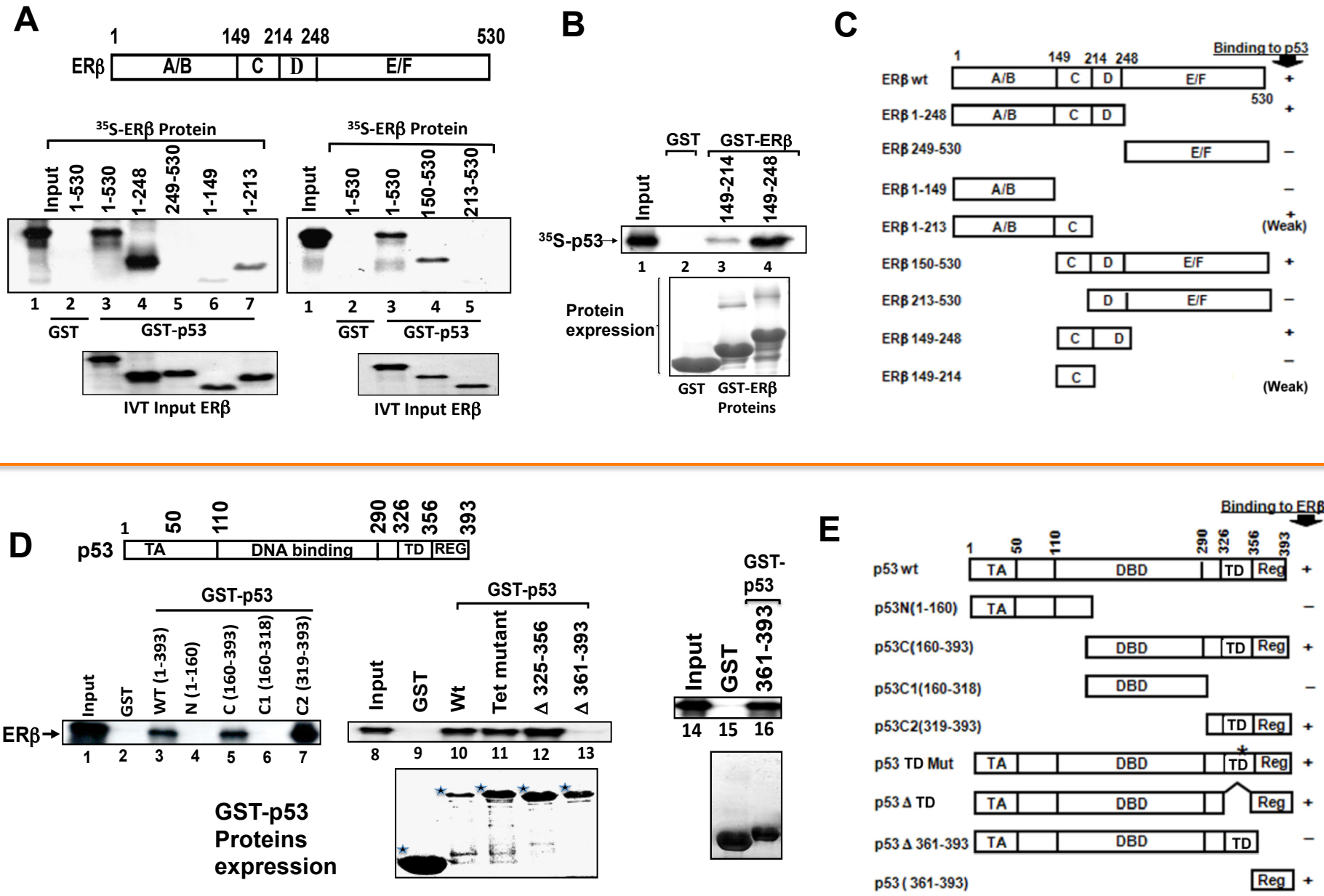
*n= 259 cases, number of death events=134, number of disease-specific death events=97. Log2 expression levels of ESR2 were treated as a continuous variable. P-values are two-sided.


T-47D
A
DAPI
PLA
ESR2:TP53
DAPI
PLA
IgG mouse:IgG rabbit
MCF-7
B
si-NS
si-ESR2#1
DAPI
PLA
ESR2:TP53
MERGE
C
si-NS
si-ESR2#1
DAPI
PLA
ESR2:TP53
MERGE

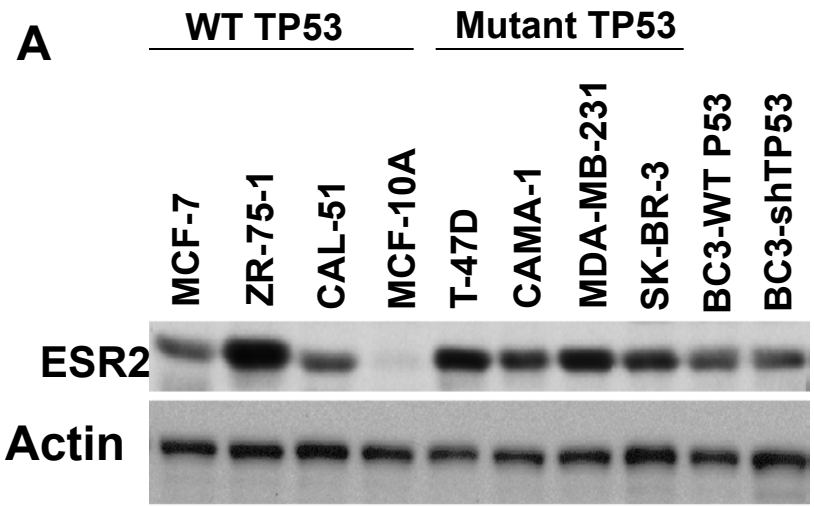
Supplementary Figure 1. Additional PLA analyses of ESR2-TP53 interaction. (**A**) Top panel: PLA for interaction between endogenous ESR2 and mutant TP53 was performed in T-47D cells. Antibodies against ESR2 (clone 14C8, GeneTex) and TP53 (FL-393, Santa Cruz Biotechnology) were used. Bottom panel: Negative control for PLA in T-47D cells with mouse IgG and rabbit IgG are shown. (**B-C**) MCF-7 cells were transfected with either si-NS or si-ESR2 #1 and were processed for PLA with (**B**) Anti-ESR2 mouse antibody (clone 14C8, GeneTex) and anti-TP53 rabbit antibody (FL393, Santa Cruz Biotechnology) as primary antibodies, and (**C**) with additional antibodies anti-ESR2 rabbit (H-150, Santa Cruz) and anti-TP53 mouse (DO-1, Santa Cruz) to further validate the specificity of the PLA signal. si-NS = non-specific siRNA; si-ESR2 #1 = ESR2-specific siRNA# 1; PLA = Proximity Ligation Assay. Scale bars (A)=10 μm; Scale bars (B,&C) = 20 μm.

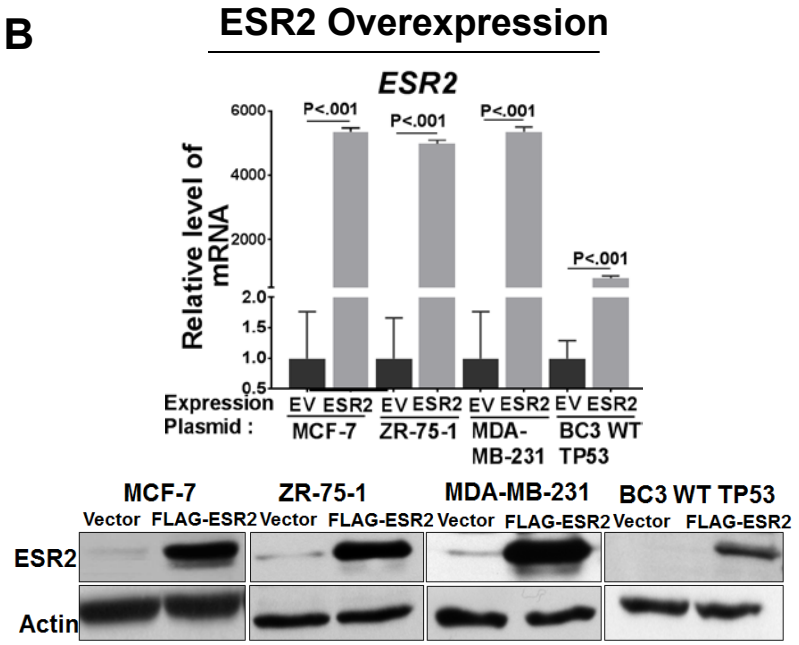

A
MCF7
Empty vector + GFP transfected
FLAG-ESR2 + GFP transfected
DAPI
GFP
PLA
FLAG-ESR2: WT TP53
MERGE
B
MDA-MB-231
FLAG-ESR2 transfected
C
SK-BR-3
FLAG-ESR2 transfected
DAPI
Immunofluorescence anti-FLAG
PLA:
FLAG-ESR2: Mutant TP53
MERGE

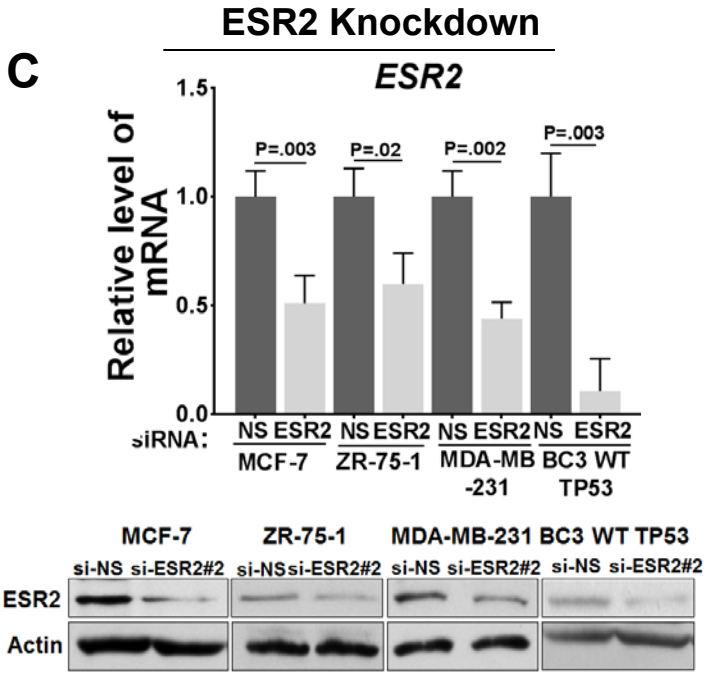
Supplementary Figure 2. Analysis of interaction between endogenous WT and mutant TP53 and exogenously overexpressed FLAG-ESR2-overexpressing cells. (**A**) PLA for interaction between ESR2 and WT TP53 was performed in MCF7 cells. Cells were transfected with 1 µg of empty vector or Flag-ESR2 plasmid DNA along with 0.1 µg of a GFP expression construct (pBabe-eGFP). At 24 hour post-transfection cells were trypsinized and counted and were re-plated. At 48 hour post-transfection cells were fixed, permeabilized and subjected to *in situ* PLA. DAPI was used as a nuclear stain. (**B**) *In situ* PLA for ESR2-TP53 interactions in MDA-MB-231 cells transfected with 1 µg of empty vector or FLAG-ESR2 plasmid DNA. At 24 hours post-transfection cells were trypsinized and counted and were re-plated. At 48 hour post-transfection cells were fixed, permeabilized and subjected to *insitu* PLA with anti-FLAG and anti-TP53 antibodies (Anti-FLAG^{M} + anti-TP53^{R}). An additional staining for ESR2 (using mouse anti-FLAG-Alexa^{488}) was incorporated during the amplification stage of PLA to delineate transfected from un-transfected cells. Both immunofluorescence and PLA signals are indicated by white arrows. DAPI was used as a nuclear stain. (**C**) Similar experiment as (A) in SK-BR-3 cells. WT=Wild type; PLA= Proximity Ligation Assay; M = mouse and, R = rabbit, Scale bar = 20 µm.


A
1
149 214 248
530
ERβ
A/B
C
D
E/F
35S-ERβ Protein
Input
1-530
1-530
1-248
249-530
1-149
1-213
1 2 3 4 5 6 7
GST
GST-p53
IVT Input ERβ
35S-ERβ Protein
Input
1-530
1-530
150-530
213-530
1 2 3 4 5
GST
GST-p53
IVT Input ERβ
B
GST
GST-ERβ
Input
149-214
149-248
35S-p53
1 2 3 4
Protein expression
GST
GST-ERβ Proteins
C
Binding to p53
1
149 214 248
530
ERβ wt
A/B
C
D
E/F
+
ERβ 1-248
A/B
C
D
+
ERβ 249-530
E/F
–
ERβ 1-149
A/B
–
ERβ 1-213
A/B
C
+
(Weak)
ERβ 150-530
C
D
E/F
+
ERβ 213-530
D
E/F
–
ERβ 149-248
C
D
+
ERβ 149-214
C
–
(Weak)
D
p53
1
50
110
290
326
356
393
TA
DNA binding
TD
REG
GST-p53
Input
GST
WT (1-393)
N (1-160)
C (160-393)
C1 (160-318)
C2 (319-393)
35S ERβ
1 2 3 4 5 6 7
GST-p53
Input
GST
Wt
Tet mutant
Δ 325-356
Δ 361-393
8 9 10 11 12 13
GST-p53 Proteins expression
GST-p53
Input
GST
361-393
14 15 16
E
Binding to ERβ
1
50
110
290
326
356
393
p53 wt
TA
DBD
TD
Reg
+
p53N(1-160)
TA
–
p53C(160-393)
DBD
TD
Reg
+
p53C1(160-318)
DBD
–
p53C2(319-393)
TD
Reg
+
p53 TD Mut
TA
DBD
TD
Reg
+
p53 Δ TD
TA
DBD
Reg
+
p53 Δ 361-393
TA
DBD
TD
–
p53 (361-393)
Reg
+

Supplemental Figure 3. Mapping of interaction domains in ERβ (ESR2) and p53 (TP53). (**A-B**) *In vitro* interaction and mapping of domains of ESR2 that interact with TP53. (**A**) Schematic diagram of the full length ESR2 protein containing 530 amino acids is shown (top panel). GST or GST-TP53 proteins expressed in *E.coli.* were bound to glutathione-sepharose beads and allowed to interact with *in vitro* translated ^{35}S-labeled ESR2 wild type (WT) or various mutant proteins synthesized by a TNT kit. The bound complexes were subjected to SDS-PAGE, followed by fluorography. Input lane represents 5% of total radiolabeled protein used in the pull-down. The expression of each *in vitro* translated protein (IVT) as analyzed by SDS-PAGE and autoradiography is shown in the bottom panel. (**B**) Bacterially expressed and immobilized GST-ESR2 (149-214aa; C domain) and (149-248aa; C & D domains) were incubated with full-length ^{35}S-labeled TP53 and subjected to GST-pull down. Coomassie stained GST-ESR2 proteins are shown in the lower panel. (**C**) Schematic representation of domains in ESR2 required for interaction with TP53. (D-E) Mapping of domain of TP53 required for ESR2 interaction. (**D**) Schematic diagram of the full-length TP53 consisting of 393 amino acids is shown at the top. GST-WT TP53 and different GST-TP53 mutants, GST-TP53 (1-160), (160-395), (160-318), (319-393), (teramerization mutant), (Δ325-356), (Δ361-393), and (361-393) were expressed in bacteria and bound to glutathione-sepharose beads as described. These GST-tagged proteins were allowed to interact with *in vitro* translated ^{35}S-labeled ESR2 wild type or various mutant proteins synthesized by a TNT kit. The bound complexes were subjected to SDS-PAGE, followed by fluorography. Bottom panels show GST-TP53 fusion proteins expression as monitored by SDS-PAGE followed by staining with coomassie blue. (**E**) Schematic representation of domains in TP53 required for interaction with ESR2. GST = Glutathione S-Transferase; TA= Transactivation domai; DBD= DNA binding domain; TD= Tetramerization domain; REG= Regulatory domain; *point mutation; − = no binding; + = strong binding. Numbers on the top of rectangular schematic representation of ESR2 and TP53 proteins indicate amino acid residues.

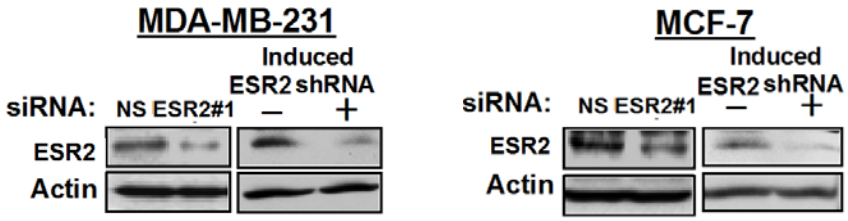




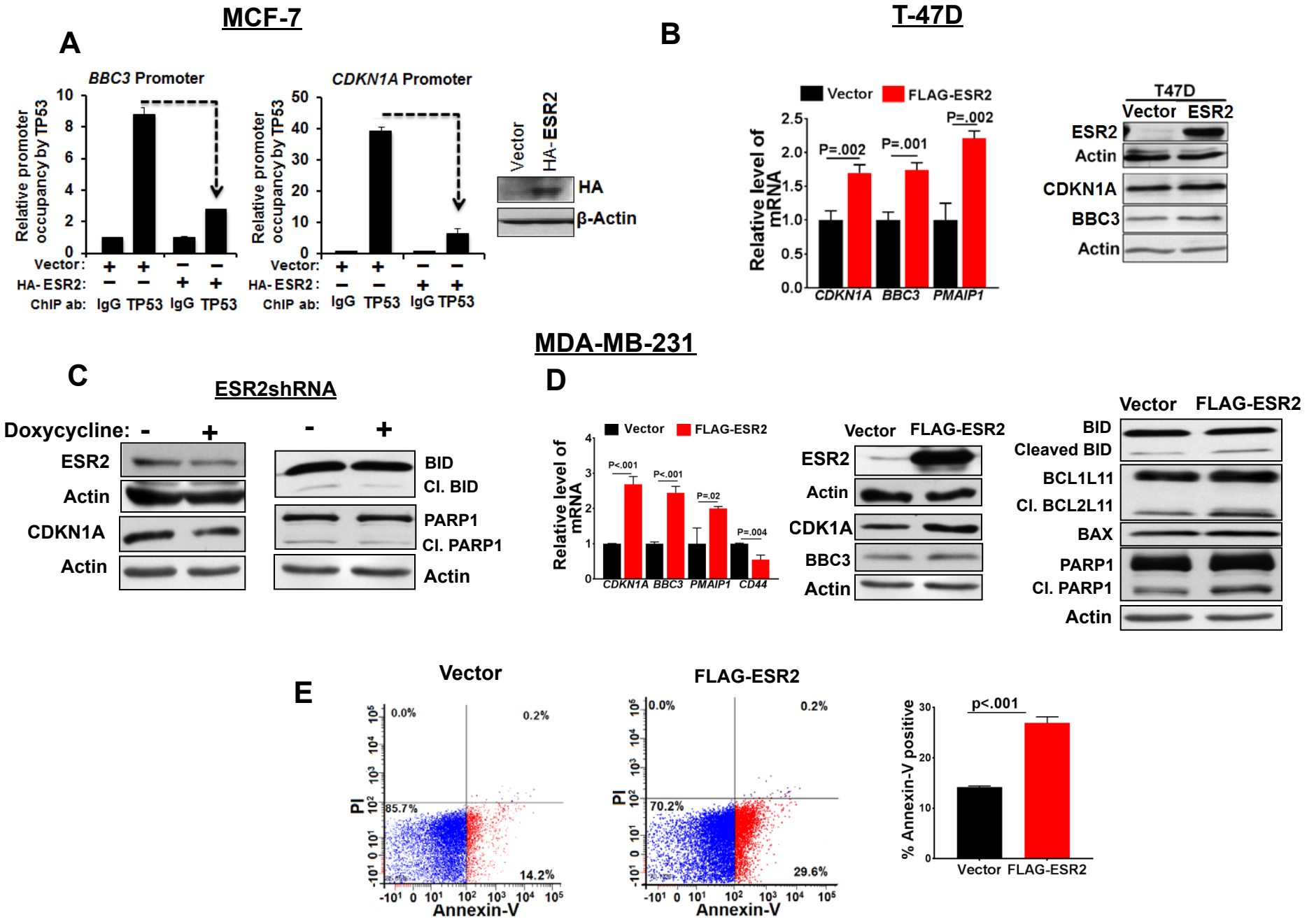


D

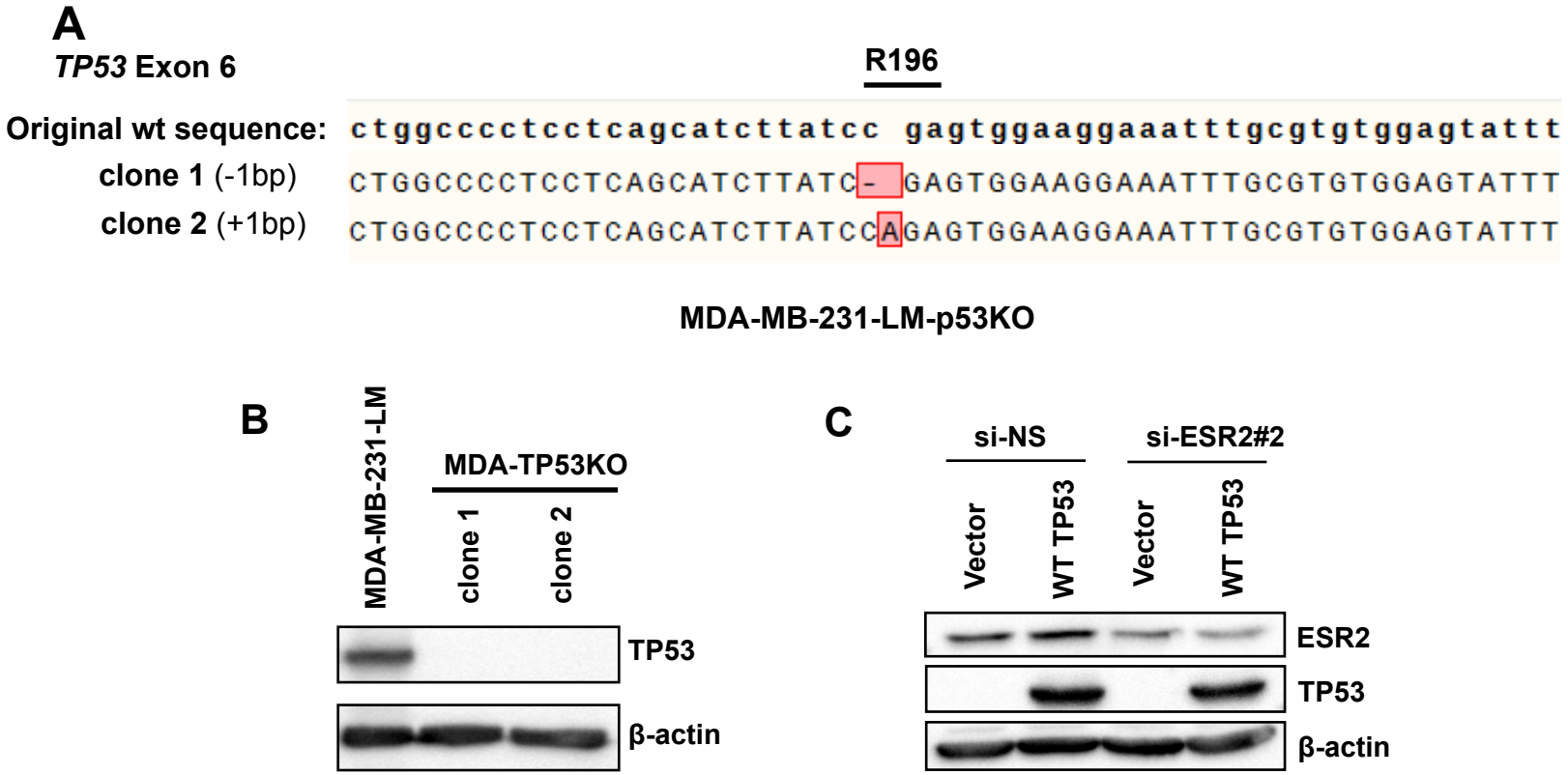
Cell lines	si-NS	siESR2 knockdown	Vector	ESR2 Overexpression
MCF-7	29.05 ±0.12	30.44 ± 0.13	29.15 ± 0.80	15.20 ± 3.22
ZR-75-1	28.13 ±0.13	30.19 ±0.14	28.74 ± 0.66	19.61 ± 2.14
MDA-MB-231	25.17 ±0.12	28.14 ± 0.18	28.17 ± 0.76	19.50 ± 3.10
BC3 WT TP53	28.41 ±0.20	30.68 ± 0.15	29.21 ± 0.26	18.37 ± 2.47



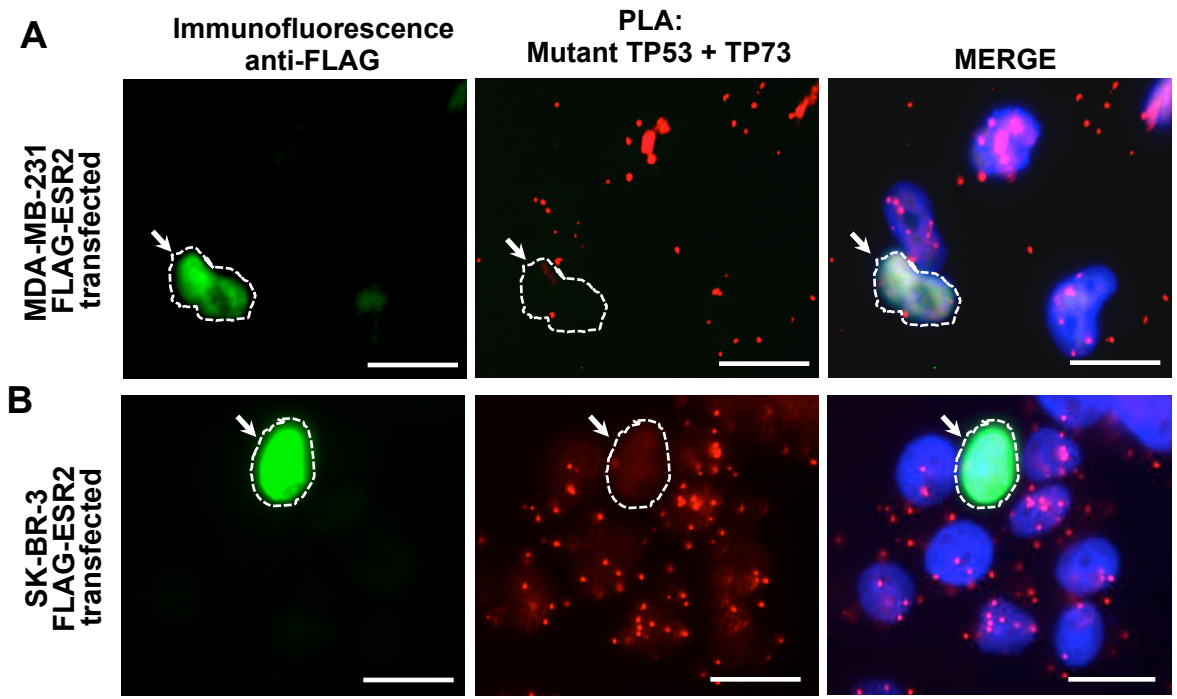
Supplementary Figure 4. Analysis of ESR2/ESR2 RNA and protein expression in multiple breast cancer cell lines. (**A**) **Expression of endogenous ESR2 protein**. MCF-7, ZR-75-1, CAL-51, MCF-10A,T-47D, CAMA-1, MDA-MB-231, SK-BR-3, BC3-WT TP53 and BC3-shTP53 cells were harvested and lysed in Radioimmunoprecipitation assay (RIPA) buffer. ESR2 protein expression was analyzed by SDS-PAGE-immunoblotting. (**B**) **Expression of Exogenous ESR2:** Upper panel: MCF-7, ZR-75-1, MDA-MB-231, and BC3-WT TP53 cells were co-transfected with either empty vector or FLAG-ESR2 plasmid for 48 hrs. After 48hrs, cells were harvested and lysed in RIPA buffer for protein and Trizol (Invitrogen) reagent for mRNA. ESR2 mRNA was quantified by real time qRT-PCR using FastStart Universal SYBER Green mastermix (Roche) in Applied Biosystem's ABI Prism 7300 Real time PCR machine. Lower panel: Protein expression measured by immunoblotting of cell lysates corresponding to the RNA samples in the upper panel. (**C**) **Knocking down of endogenous ESR2/*ESR2***: Upper panel: Knockdown of *ESR2* with si-ESR2#2 in MCF7, ZR-75-1, MDA-MB-231, and BC3-WT TP53 cells was quantified by Real time PCR. Cells were harvested and lysed in RIPA buffer for protein and Trizol reagent for mRNA. ESR2 mRNA was quantified by qRT-PCR using FastStart Universal SYBER Green mastermix (Roche) in Applied Biosystem's ABI Prism 7300 Real time PCR machine. Lower panel: Protein expression was measured by immuno blotting of cell lysates corresponding to the RNA samples in the upper panel. (**D**) Average Ct Value of *ESR2* mRNA in breast cancer cells with and without knockdown and with and without overexpression are shown. (**E**) Expression of ESR2 protein in MCF7 and MDA-MB-231 cells where *ESR2* was knocked down with si-ESR2 #1 or with doxycycline-inducible ESR2 shRNA was analyzed by immuno blotting. Error bars are represented as mean (SD) and *p* values are as per the two tailed Student's t-test. si-NS=non-specific siRNA; si-ESR2#1&2=ESR2-specific siRNAs. qRT-PCR = Quantitative real time polymerase chain reaction, Ct = Cycle Threshold.


MCF-7
A
BBC3 Promoter
CDKN1A Promoter
Relative promoter occupancy by TP53
Vector:
HA-ESR2:
ChIP ab:
IgG TP53 IgG TP53
Vector
HA-ESR2
HA
β-Actin
B
T-47D
Vector
FLAG-ESR2
Relative level of mRNA
P=.002
P=.001
P=.002
CDKN1A BBC3 PMAIP1
T47D
Vector ESR2
ESR2
Actin
CDKN1A
BBC3
Actin
MDA-MB-231
C
ESR2shRNA
Doxycycline: - +
ESR2
Actin
CDKN1A
Actin
BID
Cl. BID
PARP1
Cl. PARP1
Actin
D
Vector
FLAG-ESR2
Relative level of mRNA
P<.001
P<.001
P=.02
P=.004
CDKN1A BBC3 PMAIP1 CD44
Vector FLAG-ESR2
ESR2
Actin
CDK1A
BBC3
Actin
Vector FLAG-ESR2
BID
Cleaved BID
BCL1L11
Cl. BCL2L11
BAX
PARP1
Cl. PARP1
Actin
E
Vector
FLAG-ESR2
PI
Annexin-V
0.0%
0.2%
85.7%
14.2%
70.2%
29.6%
% Annexin-V positive
p<.001
Vector FLAG-ESR2

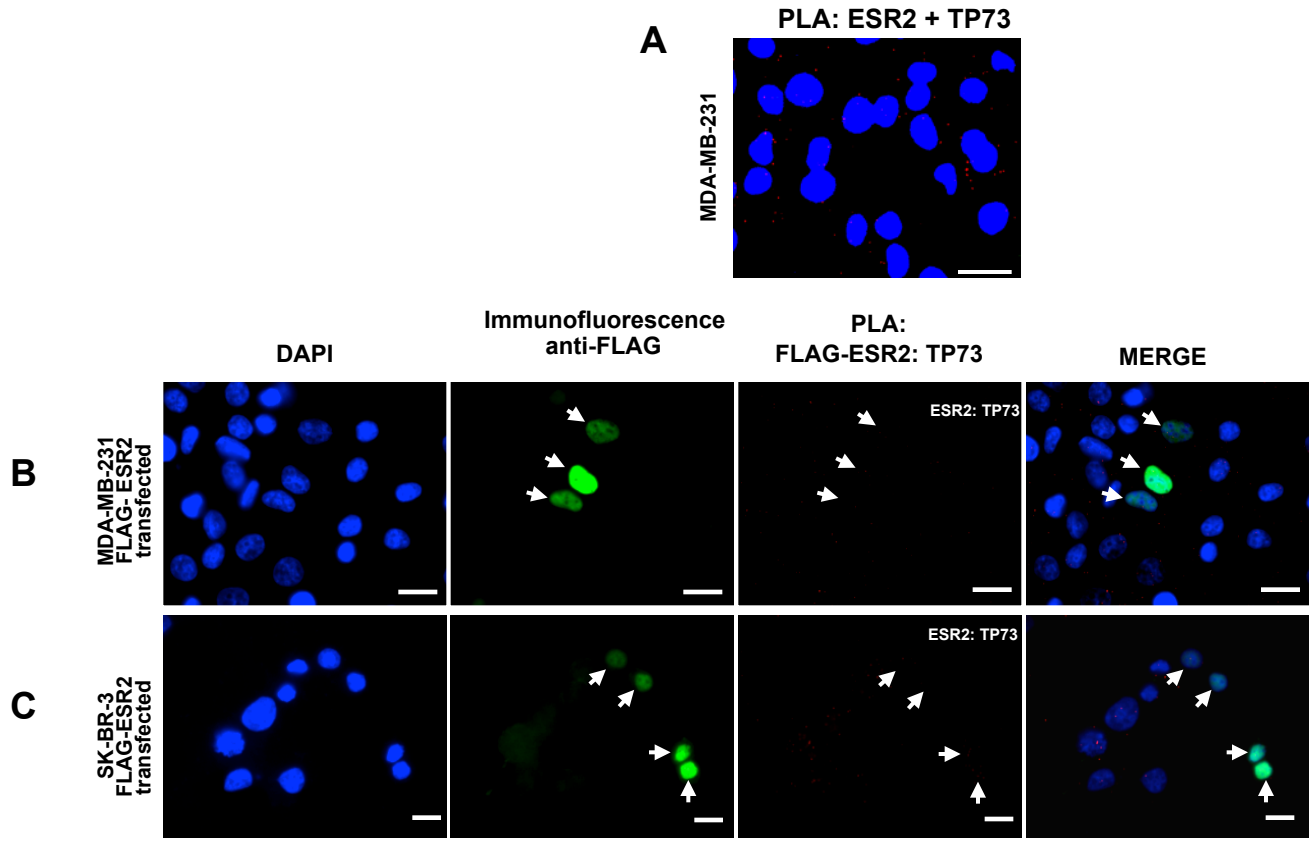
Supplemental Figure 5. Effect of ESR2 on proliferation of MCF-7 (luminal breast cancer cell line with WT TP53) and T47D (luminal breast cancer cells with mutant TP53), and in MDA-MB-231 (TNBC cell line with mutant TP53). (**A**) qChIP was performed for promoter occupancy of *CDKN1A* by TP53 in MCF-7 cells stably transfected with empty vector (control) or HA-ESR2. 1µg of total RNA was reverse-transcribed and 1/10th -1/20th of which was PCR-amplified for 25-30 cycles. Right panel: Expression of HA-ESR2 protein in stably transfected MCF-7 cells was analyzed by IB. (**B**) T-47D cells were co-transfected with either empty vector or FLAG-ESR2 plasmid for 48 hrs. After 48hrs, cells were harvested and lysed in RIPA buffer for protein and Trizol (Invitrogen) reagent for mRNA. Left panel: TP53-target gene expression in T-47D cells following ESR2 overexpression for 48 hours was determined by qRT-PCR. Right panel: Expression of ESR2, CDKN1A, and BBC3 proteins in T-47D cells following ESR2 overexpression for 48 hours was analyzed by IB. (**C**) Expression of ESR2 and CDKN1A (Left panel) and pro-apoptotic proteins (right panel) in MDA-MB-231 ESR2shRNA stable cells with or without 1 µg/ml doxycycline treatment for 48 hours to induce ESR2 shRNA was analyzed by IB. (**D**) Left panel: TP53-target gene expression in MDA-MB-231 cells with or without FLAG-ESR2 transfection for 48 hours was determined by qRT-PCR. Middle panel: Expression of ESR2, CDKN1A, and BBC3 in MDA-MB-231 cells with or without FLAG-ESR2 transfection for 48 hours was analyzed by IB. Right panel: Expression of markers of active apoptosis: cleaved BID, cleaved BCL2L11, BAX, and cleaved PARP1 proteins in MDA-MB-231 cells 48 hours post-FLAG-ESR2 transfection was analyzed by IB. (**E**) MDA-MB-231 cells were transfected with vector or FLAG-ESR2 for 48 hours. 48 hours of post-transfection cells were double-stained with Annexin V-FITC and Propidium Iodide for apoptosis assay by flow cytometry analysis. Bar graph (far right panel) shows fold change of Annexin +/PI – cells. Error bars are represented as mean (SD) and *p* values are as per the two tailed Student's t- test. qRT-PCR = Quantitative real time polymerase chain reaction; IB = Immuno blotting; Cl. BCL2L11 = Cleaved BCL2L11; Cl. PARP1: Cleaved PARP1.


A
TP53 Exon 6
R196
Original wt sequence: ctggcccctcctcagcatcttatcc gagtggaaggaaatttgcgtgtggagtattt
clone 1 (-1bp) CTGGCCCCTCCTCAGCATCTTATC- GAGTGGAAGGAAATTTGCGTGTGGAGTATTT
clone 2 (+1bp) CTGGCCCCTCCTCAGCATCTTATCCA GAGTGGAAGGAAATTTGCGTGTGGAGTATTT
MDA-MB-231-LM-p53KO
B
MDA-MB-231-LM
MDA-TP53KO
clone 1
clone 2
TP53
β-actin
C
si-NS
si-ESR2#2
Vector
WT TP53
Vector
WT TP53
ESR2
TP53
β-actin

Supplementary Figure 6. CRISPR/Cas9 method to knockout of mutant TP53 in MDA-MB-231-LM-4^{LUC+} cells. (**A**) Next generation/Massively parallel sequencing was performed to confirm the presence of indels. Mutations at single base pair deletion (-1bp) in clone 1 and single base pair insertion (+1bp) in clone 2 are shown in red shade. (**B**) Expression of TP53 protein in MDA-MB-231-LM-4^{LUC+}-TP53KO (also called MDA-MB-231-TP53KO) cells and in 2 CRISPR/Cas9 TP53 knockout clones was analyzed by IB. (**C**) MDA-MB-231-LM-4^{LUC+}-TP53KO cells transfected with and without combination of WT TP53 and knockdown of ESR2 with si-ESR2 RNA#2 for 48 hr. After 48 hours transfection, expression of ESR2 and TP53 proteins was analyzed by IB. si-NS = non-specific siRNA; si-ESR2#2 =ESR2 specific siRNA; IB = Immuno blotting.



Supplementary Figure 7. Effect of overexpression of ESR2 on mutant TP53-TP73 interaction. MDA-MB-231 cells (**A**) and SKBR-3 cells (**B**) were transfected with FLAG-ESR2 were subjected to PLA using a mouse anti-TP53 antibody (DO-1) and a rabbit anti-TP73 antibody (H-79). An additional staining for ESR2 (using mouse anti-FLAG-Alexa^{488}) was incorporated during the amplification stage of PLA to delineate transfected from un-transfected cells. White dotted line delineates the boundary of ESR2 overexpressing cells (also indicated with white arrowhead). PLA = Proximity Ligation Assay. Scale bar = 20 μm.

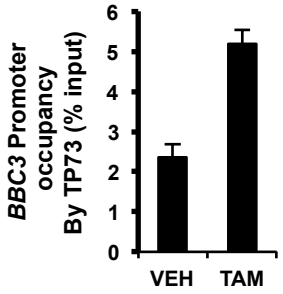


Supplementary Figure 8. Analysis of interaction between ESR2 and TP73 in MDA-B-231. (**A**) PLA for interactions between ESR2 and TP73 (antibodies: α-ESR2^{M} & α-TP73^{R}). (**B)** PLA analysis (with anti-FLAG^{M} + anti-TP73^{R}) was performed to test interaction between exogenously over-expressed ESR2 and endogenous TP73 in MDA-MB-431. Mouse anti-Flag-Alexa^{488} was used as an additional secondary fluorophore to monitor ESR2 expressing cells (white arrow heads). (**C**) Similar experiment as in (B) in SK-BR-3 cells. PLA = Proximity Ligation Assay; M = mouse and, R = rabbit. Scale bar=20 µm.

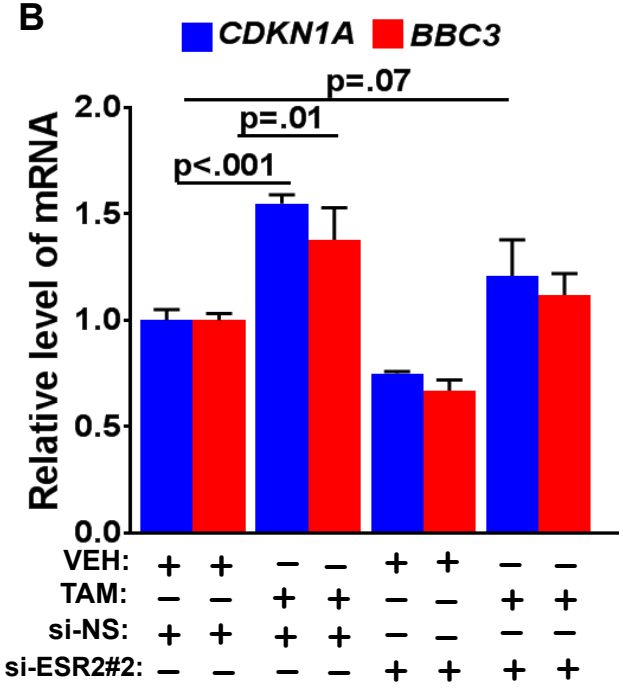
MDA-MB-231

SK-BR-3

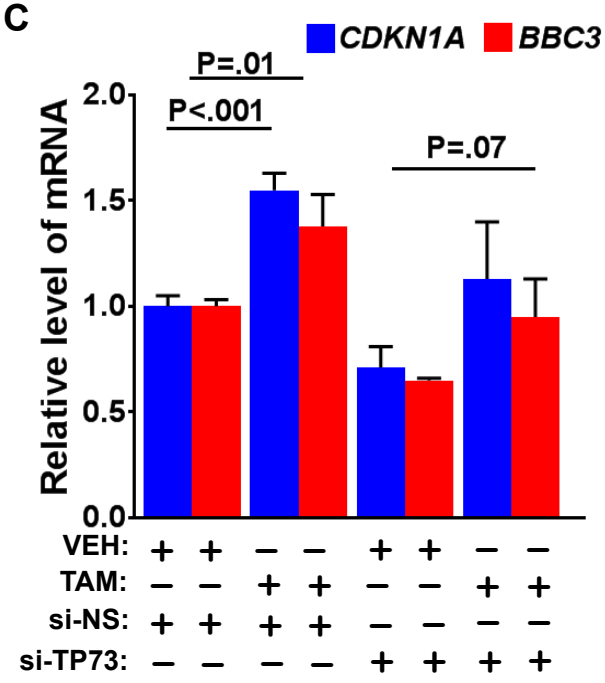
A



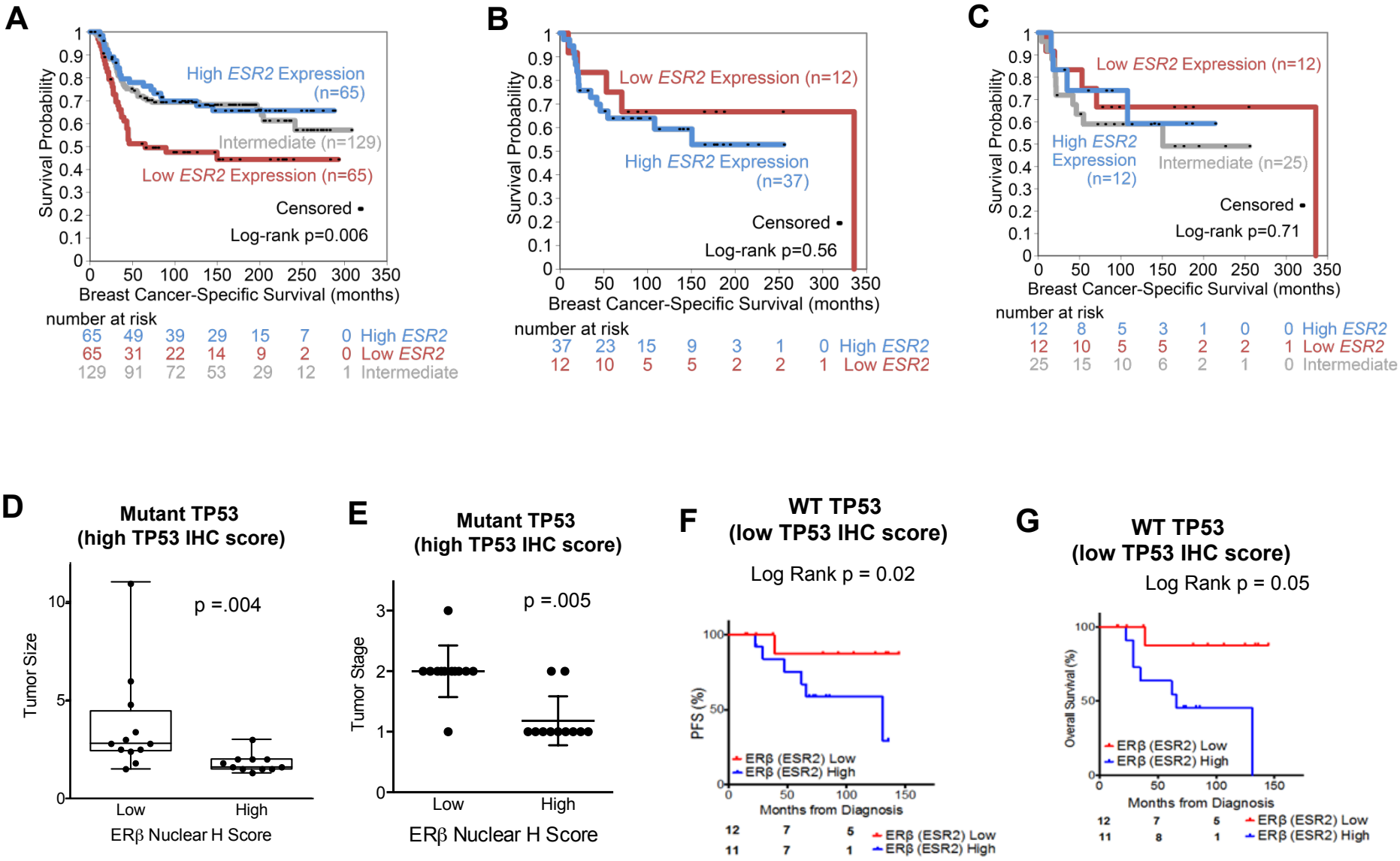
B



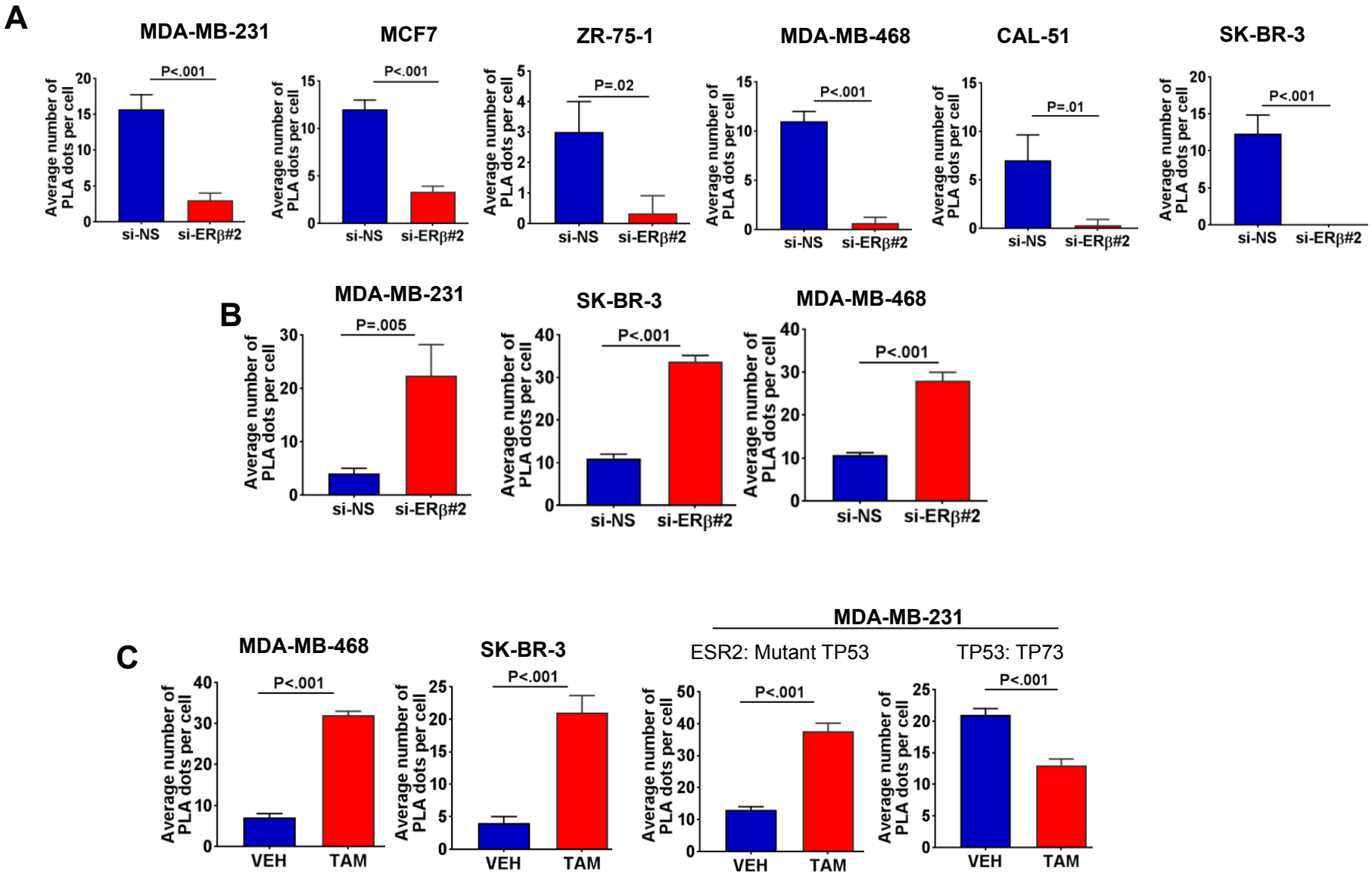
C



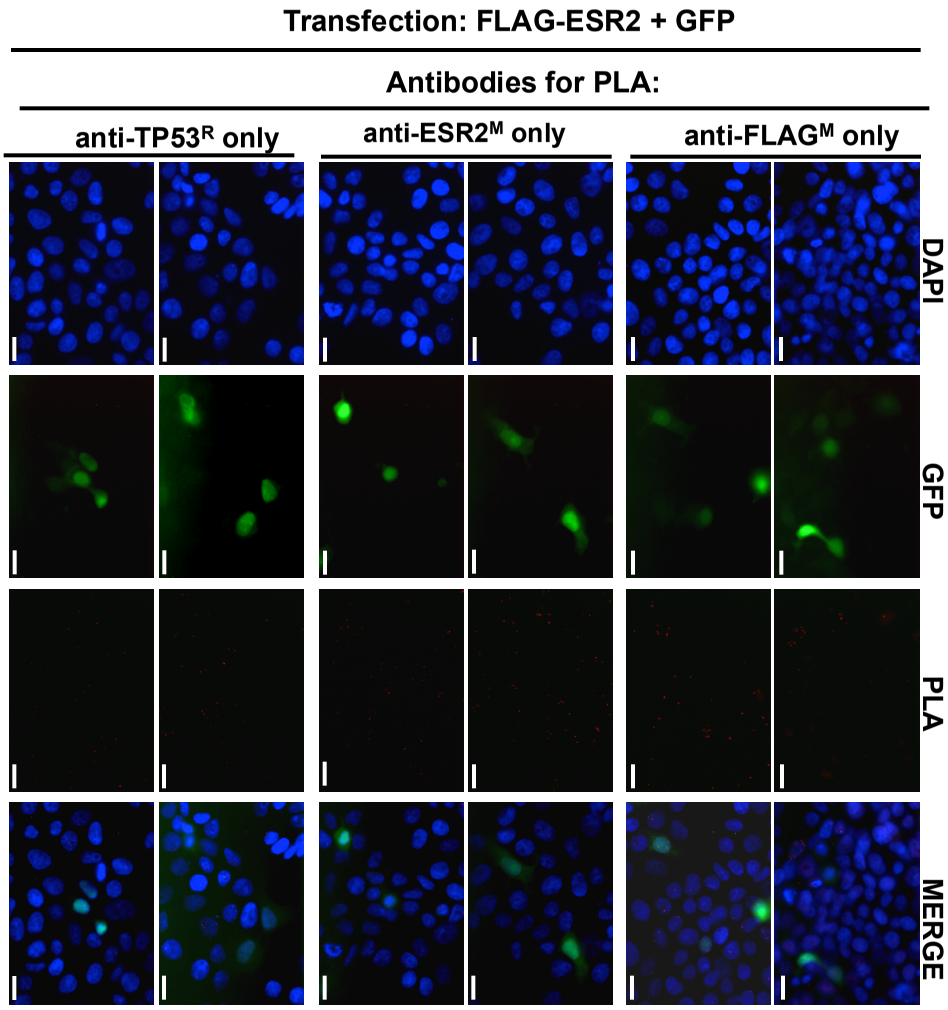
Supplementary Figure 9. Effect of ESR2 and TP73 on TNBC cells treated and not treated with tamoxifen. (**A**) Cells were treated with 5μM TAM for 24 hours. After 24 hours, BBC3 promoter occupancy by TP73 was determined using qChIP in MDA-MB-231 cells. (**B**) SK-BR-3 cells were transfected with si-NS or si-ESR2 #2 for 48 hours and treated with or without 5μM TAM for 24 hours. After 72 hours, expression of TP53 target genes (*CDKN1A* and *BBC3*) was determined using qRT-PCR. (**C**) SK-BR-3 cells were transfected with si-NS or si-TP73 for 48 hours and treated with or without 5μM TAM for 24 hours. After 72 hours, expression of TP53 target genes (*CDKN1A* and *BBC3*) was determined using qRT-PCR. Error bars are represented as mean SD of three independent experiments. All 'p' values were determined by two tailed student t-test. qRT-PCR =Quantitative real time polymerase chain reaction. si-NS = non-specific siRNA; si-ESR2#2 = ESR2 specific siRNA; si-TP73 = TP73 specific siRNA; VEH = Vehicle; TAM= 4-Hydroxytamoxifen.


A
Survival Probability
High ESR2 Expression (n=65)
Intermediate (n=129)
Low ESR2 Expression (n=65)
Censored
Log-rank p=0.006
Breast Cancer-Specific Survival (months)
number at risk
65 49 39 29 15 7 0 High ESR2
65 31 22 14 9 2 0 Low ESR2
129 91 72 53 29 12 1 Intermediate
B
Survival Probability
Low ESR2 Expression (n=12)
High ESR2 Expression (n=37)
Censored
Log-rank p=0.56
Breast Cancer-Specific Survival (months)
number at risk
37 23 15 9 3 1 0 High ESR2
12 10 5 5 2 2 1 Low ESR2
C
Survival Probability
Low ESR2 Expression (n=12)
High ESR2 Expression (n=12)
Intermediate (n=25)
Censored
Log-rank p=0.71
Breast Cancer-Specific Survival (months)
number at risk
12 8 5 3 1 0 0 High ESR2
12 10 5 5 2 2 1 Low ESR2
25 15 10 6 2 1 0 Intermediate
D
Mutant TP53
(high TP53 IHC score)
p =.004
Tumor Size
Low
High
ERβ Nuclear H Score
E
Mutant TP53
(high TP53 IHC score)
p =.005
Tumor Stage
Low
High
ERβ Nuclear H Score
F
WT TP53
(low TP53 IHC score)
Log Rank p = 0.02
PFS (%)
ERβ (ESR2) Low
ERβ (ESR2) High
Months from Diagnosis
12 7 5 ERβ (ESR2) Low
11 7 1 ERβ (ESR2) High
G
WT TP53
(low TP53 IHC score)
Log Rank p = 0.05
Overall Survival (%)
ERβ (ESR2) Low
ERβ (ESR2) High
Months from Diagnosis
12 7 5 ERβ (ESR2) Low
11 8 1 ERβ (ESR2) High

Supplementary Figure 10. Effect of TP53 status on the prognostic role of ESR2 (ERβ) in TNBC patient tumors. (**A**) Kaplan–Meier survival curves for Breast Cancer Specific Survival (BCSS) in patients with mutant *TP53*, Basal-like breast tumors of the METABRIC cohort, stratified into high, middle, and low *ESR2* expression are shown. (**B**) Kaplan–Meier survival curves for BCSS in patients with WT *TP53*, Basal-like breast tumors of the METABRIC cohort, stratified into high *ESR2* expression (expression above 25th percentile) and low *ESR2* expression (below 25th percentile) are shown. (**C**) Kaplan–Meier survival curves for BCSS in patients with WT *TP53*, Basal-like breast tumors of the METABRIC cohort, stratified into high, middle, and low *ESR2* expression are shown. (**D**) Boxplot representation of tumor size in TNBC patients with tumors of high TP53 IHC score (surrogate for mutant TP53) (n=23) stratified by ESR2 nuclear H score is shown. (**E**) Boxplot of tumor stage in TNBC patients with tumors of high TP53 IHC score (n=23) stratified by ESR2 nuclear H score is shown. (**F**) Kaplan-Meier plot of Progression-free Survival (PSF) of TNBC patients with tumors of low levels TP53 IHC score (surrogate for WT TP53) (n=23) stratified into ESR2 Low and ESR2 High protein levels (IHC score) in the Roswell patient cohort is shown. (**G**) Kaplan-Meier plot of Overall Survival of TNBC patients with tumors of low TP53 IHC score stratified into ESR2 Low and ESR2 High protein levels (IHC score) in the Roswell patient cohort is shown. METABRIC = Molecular Taxonomy of Breast Cancer International Consortium; IHC = Immunohistochemistry. All ‘p’ values are determined by Log Rank.


A
MDA-MB-231
MCF7
ZR-75-1
MDA-MB-468
CAL-51
SK-BR-3
Average number of PLA dots per cell
P<.001
P=.02
P=.01
si-NS
si-ERβ#2
B
MDA-MB-231
SK-BR-3
MDA-MB-468
P=.005
P<.001
C
MDA-MB-468
SK-BR-3
MDA-MB-231
ESR2: Mutant TP53
TP53: TP73
VEH
TAM

Supplementary Figure 11. Quantitation of PLA signals. PLA signals were counted manually as well as using Blob-Finder image analysis software (developed by Centre for Image Analysis, Uppsala University, Sweden). (**A**) Quantification of PLA data shown in the main Figure 1 is shown. **(B**) Quantification of PLA data in the main Figure 6 is shown. (**C**) Quantification of PLA data shown in the main Figure 7 is shown. Random fields (minimum three) were selected for dots counting per nucleus. Average dots/nucleus were considered for the statistical analysis. Two-tailed Student's t-test was performed using GraphPad Prism 7.0. ERβ= ESR2, PLA = proximity ligation assay; si-NS = non-specific siRNA; si-ESR2#2 = ESR2 specific siRNA; VEH = Vehicle; TAM = 4-Hydroxytamoxifen.


Transfection: FLAG-ESR2 + GFP
Antibodies for PLA:
anti-TP53R only
anti-ESR2M only
anti-FLAGM only
DAPI
GFP
PLA
MERGE

Supplementary Figure 12. PLA with single primary antibodies (negative control). PLA was performed with single primary antibodies (anti-FLAG, anti-ESR2 (MC10), and anti-TP53 (FL393) in MCF-7 cells co-transfected with either an empty vector or FLAG-ESR2 plasmid along with 1/10th amount of a GFP expression construct (pBabe-eGFP). Two sets of images are provided for each condition. DAPI was used as a nuclear stain. Antibodies used for PLA and the plasmids transfected are shown on the left side. The primary antibody species, used for PLA, are indicated in superscript, M = mouse and, R = rabbit origin; DAPI = 4′,6-diamidino-2-phenylindole; PLA = proximity ligation assay. Scale bar = 20 μm.