Supplementary Methods

Cell Culture

Human breast cancer cells (MCF-7) were maintained in DMEM (Invitrogen Life Technologies) supplemented with 5% characterized fetal bovine serum and 2mM glutamine (HyClone). C4-12 were routinely maintained in αMEM (Invitrogen Life Technologies) without phenol red supplemented with 10% charcoal/dextran-treated fetal bovine serum (Hyclone), 2 mM glutamine, 10 mM HEPES (Gibco) and 10 μg/ml Insulin. LTED cells were maintained in Improved MEM that was supplemented with 10% charcoal-dextran treated fetal bovine serum (HyClone). The cells were cultured at 37°C with 5% CO_2 in the air.

Polymerase Chain Reaction and Bisulfite Sequencing

80 ng of bisulfite modified DNA was PCR amplified using Hotstar Taq Master Mix (Qiagen) supplemented with 800nM of forward and reverse primers. Integrated DNA Technology (IDT) web-based tool was used to design primers used to amplify genomic DNA, and Methprimer web based tool was used to design all bisulfite sequencing primers. The following cycling conditions were used for PCR amplification of genomic and bisulfite converted DNA: initial denaturation 95ºC for 15mins, followed by 40 cycles at 95ºC for 20 sec, annealing temperature for 30 sec, 72ºC for 45 sec, and then finally 72ºC for 4 mins.

RNA Extraction and Quantitative Real-Time PCR (q-RT-PCR)

For cell lines, triplicate RNA samples were prepared for each experiment. Following isolation, RNA concentration of the samples was determined with the Nanodrop Spectrophotometer using an OD260/OD280 ratio. The RNA expression was measured using quantitative real-time reverse transcription-PCR (q-RT-PCR) assays using gene-specific primers (SYBR Green assay) by an ABI PRISM 7700 Sequence Detector (Applied Biosystems). Integrated DNA Technology software was used to design all the primers, which are listed in Supplementary Table 3. The SYBR Green Master Mix (Applied Biosystems) comprised of 20μM each of the forward and reverse primers, 100 nM probe, 0.025 U/μl of Taq polymerase (Invitrogen), 1× Rox Dye (Invitrogen), 125 μM each of deoxynucleotide triphosphate (Invitrogen), 5 mM $MgCl_2$ (Invitrogen), and 1× Taq polymerase buffer (Invitrogen). Cycling conditions were 50ºC for 2 minutes, followed by 95 ºC for 10 minutes, and 40 cycles at 95ºC for 15 seconds and 60ºC for 30 seconds. For each sample, q-RT-PCR (q-RT-PCR) was performed in triplicate for each gene of interest and the reference gene (β-actin for cell lines, RPS11 for tumor samples) to normalize for input cDNA.

Analysis of Cellular Proliferation

For standard growth curve and cell proliferation, prior to analysis, HIST1H2BE stable knock down cells were supplemented with 10% CSS, while stable over-expressing HIST1H2BE cells were either supplemented with 5%FBS or 10% CSS. Cells were split into 96 well dishes at a density of 4,000 cells per well. For growth curves following deprivation conditions, the HIST1H2BE stable over-expressing cells were deprived of exogenous estrogens for a period of three days by washing cells two times with serum free IMEM phenol red free (PRF) followed by incubation with IMEM and 10% CSS (PRF). Cells were split into a 96 well dish at a density of 4,000 cells per well. On day two, fresh media or DPBS was added to the wells along with either 1 nM estradiol, vehicle, or 1μM ICI treatment.

Soft Agar Assay

Anchorage independent growth was examined using a soft agar assay. A bottom layer of 0.6% bacto-agar was created by diluting autoclaved 1.2% bacto-agar with 2X DMEM (Invitrogen) through which the cells could not migrate. Bottom layers were allowed to solidify at least 45 minutes prior to plating the top 0.4% bacto-agar layer, which contained 10,000 HIST1H2BE over-expressing cells along with 100X NEAA (Gibco). Samples were plated in triplicate and incubated for a period of 14-21 days at 37°C and 5% CO_2. Following incubation, samples were treated with 0.5 mL 0.005% crystal violet (Sigma) for 15 minutes at room temperature, and then were washed and imaged. Representative images were taken at 10X magnification on an IX83 microscope with an Olympus Camera and the full dish images used for counting were taken at 0.8X on an SZX16 microscope, also with an Olympus Camera.

Selection of the housekeeping Gene RPS11, for q-RT-PCR in Human tumor samples

Selection of a housekeeping gene for qRTPCR in human tumor samples was ascertained by first determining the most stably expressed set of genes in our tumor samples. We compared Ct values of these 3 genes, UBB, GAPDH, and RPS11 (known to exhibit stable expression in many cancers, including breast cancer) in our tumor samples, and determined that RPS11demonstrated the most stable expression in all tumors, with Ct values consistently between 17 and 20. A complete list of q-RT-PCR primer sequences in listed in Supplementary Table 3