Supplementary Data

Supplementary Methods

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Supplementary Methods

Short-term estrogen deprivation (STED) experiments

MCF7 cells were prepared by plating 1.5 x 10^5 cells/well in a 24-well plate. The next day, the parental media was removed, the cell were washed twice with DPBS, and LTED media was added. The cells were fed every two or three days with LTED media. Cells were lysed on the appropriate day with the lysis buffer from the Quick mRNA Miniprep kit (Zymo Research, Irvine, CA, R1055).

5-azacytidine Treatment

MCF7 cultures were prepared by plating 3 x 10^5 MCF7 cells/well in a 6-well plate. The following day, the media was replaced and 5-azacytidine (Sigma-Aldrich, St. Louis, MO, A1287-1VL, dissolved in MCF7 media) was added to each well at a final concentration of 10 uM. Fresh media and 5-azacytidine were added to the cells each day. Cells were lysed on the appropriate day with the lysis buffer from the Quick mRNA Miniprep kit (Zymo Research R1055).

Methyl-Screen

Methyl-screen analysis was performed as in Holemon *et al.*[32] with slight modification. Genomic DNA was mock digested, AciI (NEB) digested, McrBC (NEB) digested, or digested with both AciI and McrBC. Real-time quantitative PCR was performed with *PTGER4*-specific primers: 5'-GCAGCTTTGTCTCTCTTC-3' and 5'-TACCGAGACCCATGTTG-3'. Unmethylated control gDNA was produced by whole genome amplification of MCF7 gDNA with the REPLI-g kit (Qiagen, Hilden, Germany). Methylated control DNA was produced by treating amplified gDNA with M.SssI (Zymo Research).

The Cancer Genome Atlas data analysis

Infinium 450k methylation, RNA-seq expression and clinical data were downloaded from the The Cancer Genome Atlas (TCGA) data portal. P-values were computed using Mann-Whitney U.

Supplementary Tables

Supplementary Table S1: Genes with differential expression and DNA methylation. Fold-change expression is MCF7-LTED / MCF7.

GeneID	Gene	Log2 Expr. Fold-Change	Description	Locus (hg18)
Up-regulated				
ENSG00000171522	**PTGER4**	4.56	PTGER4 prostaglandin E receptor 4, subtype EP4	chr5:40715789-40729594
ENSG00000131016	AKAP12	3.38	AKAP12 A kinase (PRKA) anchor protein 12 isoform 2	chr6:151602827-151721390
ENSG00000119514	GALNT12	3.21	UDP-N-acetyl-alpha-D-galactosamine:polypeptide	chr9:100609802-100652184
ENSG00000141756	FKBP10	2.04	FK506 binding protein 10, 65 kDa	chr17:37222488-37232995
ENSG00000114993	RTKN	2.03	rhotekin isoform c	chr2:74506496-74521218
Down-regulated				
ENSG00000064205	**WISP2**	-5.11	WNT1 inducible signaling pathway protein 2	chr20:42777299-42789866
ENSG00000132329	RAMP1	-3.29	receptor activity-modifying protein 1 precursor	chr2:238432926-238485498

Supplementary Table S2: ChIP-qPCR and RT-qPCR primer sequences.

Locus	Purpose	Forward Primer Sequence	Reverse Primer Sequence
pS2	ChIP-qPCR	GGCCATCTCTCACTATGAATCACTTCTGC	GGCAGGCTCTGTTTGCTTAAAGAGCG
PGR1	ChIP-qPCR	GCCTGACCTGTTGCTTCAAT	GCAGGACGACTTCTCAGACC
chr8q24	ChIP-qPCR	AATGCTGGGCTTCCAAGGA	GACCTTGGTGACTGTTGAGGAAAC
PTGER4	RT-qPCR	AGGACAAGGTGAAAGCAGG	GAGTGGACATGATAGTGGCTG
RPL0	RT-qPCR	AGACTGGAGACAAAGTGGGA	CAGACAGACACTGGCAACA
CARM1	RT-qPCR	TCGCCCTCTACAGCCATGA	CACACGGCTGCACTCTGTCT

Supplementary Figure Legends

Figure S1. Genome-wide methylation analysis of MCF7 and MCF7-LTED cells. (a) Percentage of differentially methylated CpGs found in specific genomic compartments. (b) Raw sequencing statistics from Methyl-MAPS analysis of MCF7 and MCF7-LTED cells. (c) Number of CpGs retained in analysis at different levels of coverage in Methyl-MAPS data.

Figure S2. Validation of PTGER4 methylation and expression changes in MCF7-LTED cells. (a) PTGER4 methylation was assessed by Methyl-Screen. MCF7 and MCF7-LTED cells are compared to 0% and 100% methylated control DNA. (b) RT-qPCR analysis of *PTGER4* expression shows a 68-fold increase in MCF7-LTED cells compared to MCF7 cells. Error bars are standard deviation for three technical replicates.

Figure S3. Genome-wide methylation and expression at WISP2 in MCF7-LTED cells. Genome browser view of Methyl-MAPS methylation and RNA-seq expression data for *WISP2*. Red and blue lines indicate coverage of methylated and unmethylated fragments, respectively. Individual CpG sites are noted by tics in black at the top track.

Figure S4. Methylation analysis of EP4 in TCGA data. (a) Genome browser view of EP4 (*PTGER4*) showing CpG sites and Infinium probe locations. The green line indicates the correlation coefficient between methylation and expression across each probe for 730 TCGA normal and carcinogenic breast samples. The orange line indicates the mean methylation difference between 632 carcinogenic and 98 normal breast samples at each probe. The arrow marked (i) indicates the most differentially methylated probe and the arrow marked (ii) indicates

the most anti-correlated probe. (b) Boxplots of methylation levels (mCG/CG) at each Infinium probe in tumor and normal TCGA breast samples. Most differentially methylated probe (i) and most anti-correlated probe (ii) are indicated. (c) Boxplots comparing methylation and expression data for normal versus tumor tissue.

Figure S5. EP4 expression in responders and non-responders to AI-therapy. Expression data from 42 responders and 14 non-responders to neoadjuvant AI-therapy. Data from Miller et al. 2011.

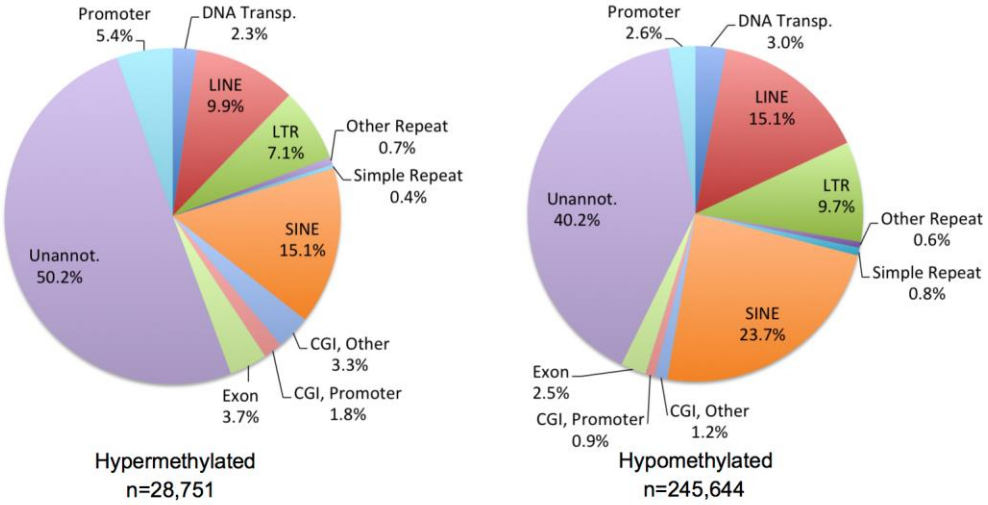
Figure S6. Gene set enrichment analysis of ER target genes in MCF7-LTED cells. Differentially expressed genes are ranked from highest to lowest expression change. ER-response genes are marked.

Figure S7. Expression analysis of *PTGER4* in MCF7 cells after withdrawal of estrogen. RT-qPCR expression data for *PTGER4* in MCF7 cells grown in LTED media for 30-days. Expression data is relative to the sample from MCF7 parental cells on Day 0 before changing to LTED media. Error bars are the standard deviation of triplicates.

Figure S8. 5-azacytidine treatment increases *PTGER4* expression. RT-qPCR data for *PTGER4* expression in MCF7 cells treated with 10 uM 5-azacytidine for four days. Expression data is relative to the untreated sample from Day 0. Error bars represent the stand deviation of triplicates.

Figure S1

a.



b.

Sample	Library	F3 Raw Reads	R3 Raw Reads	AAA Pairs
MCF7	McrBC	72,138,738	71,799,998	34,427,093
	RE	80,625,832	80,235,306	37,871,732
MCF7-LTED	McrBC	100,022,230	99,469,120	36,963,847
	RE	92,579,974	92,095,440	36,860,673

c.

Sample	MR Sites with 1x Coverage	MR Sites with 7x Coverage	% Coverage at 7x	MR Sites with 10x Coverage	% Coverage at 10x
MCF7	8,171,301	7,402,593	88.7%	6,895,024	82.6%
MCF7-LTED	8,160,032	7,234,459	86.7%	6,698,537	80.3%

Figure S2

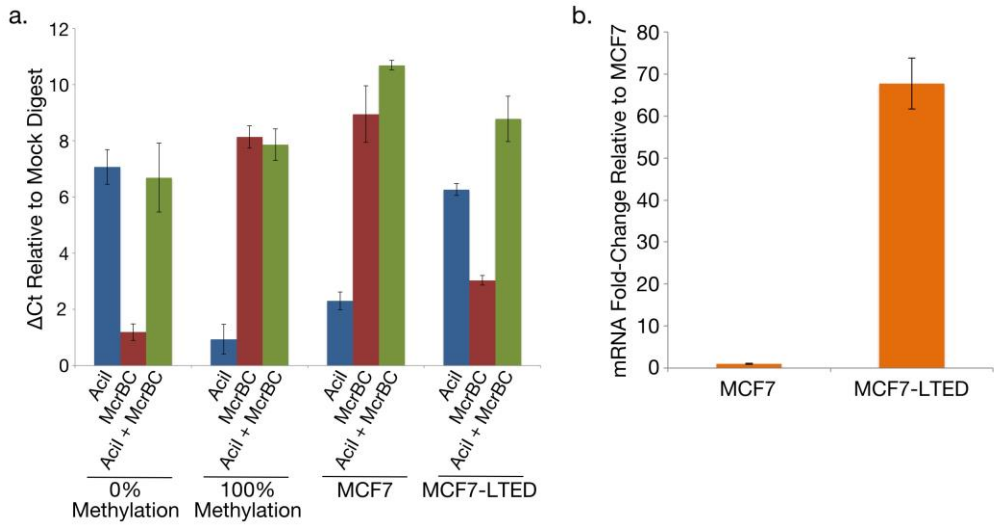

a.
ΔCt Relative to Mock Digest
0
2
4
6
8
10
12
AciI
McrBC
AciI + McrBC
0% Methylation
100% Methylation
MCF7
MCF7-LTED
b.
mRNA Fold-Change Relative to MCF7
0
10
20
30
40
50
60
70
80
MCF7
MCF7-LTED

Figure S3

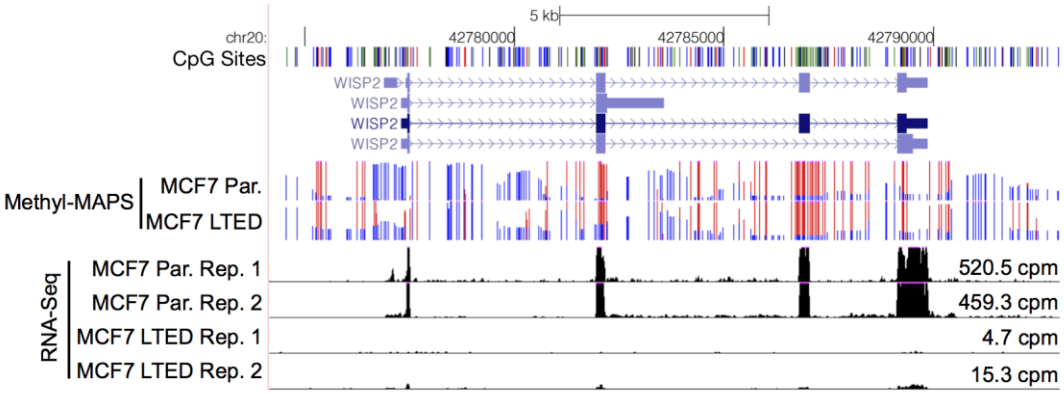

5 kb
chr20:
42780000
42785000
42790000
CpG Sites
WISP2
WISP2
WISP2
WISP2
Methyl-MAPS
MCF7 Par.
MCF7 LTED
RNA-Seq
MCF7 Par. Rep. 1
MCF7 Par. Rep. 2
MCF7 LTED Rep. 1
MCF7 LTED Rep. 2
520.5 cpm
459.3 cpm
4.7 cpm
15.3 cpm

Figure S4

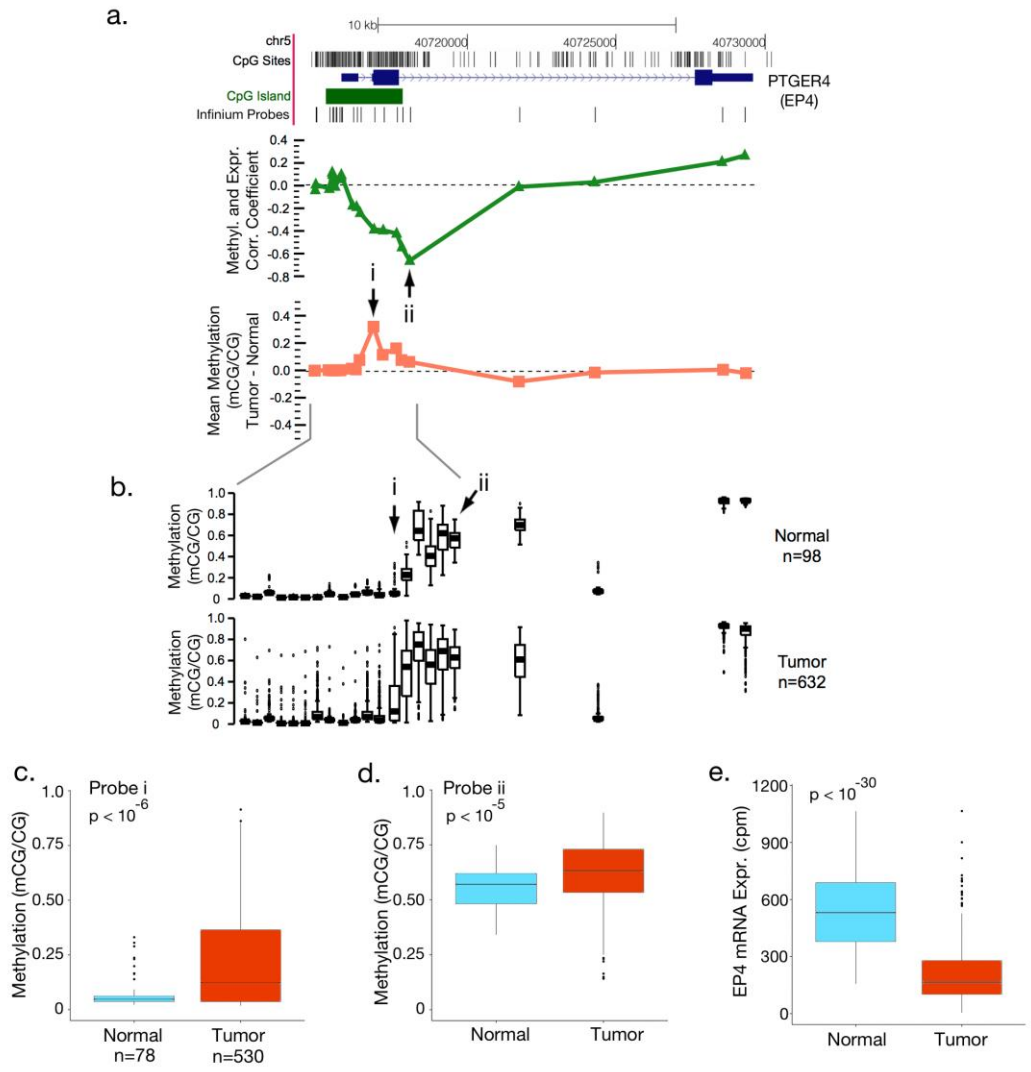

a.
10 kb
chr5
40720000
40725000
40730000
CpG Sites
CpG Island
Infinium Probes
PTGER4
(EP4)
Methyl. and Expr.
Corr. Coefficient
Mean Methylation
(mCG/CG)
Tumor - Normal
i
ii
b.
Methylation
(mCG/CG)
Normal
n=98
Tumor
n=632
c.
Probe i
p < 10-6
Methylation (mCG/CG)
Normal
n=78
Tumor
n=530
d.
Probe ii
p < 10-5
Methylation (mCG/CG)
Normal
Tumor
e.
p < 10-30
EP4 mRNA Expr. (cpm)
Normal
Tumor

Figure S5

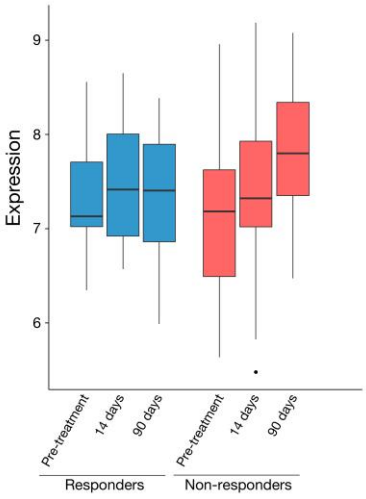

Expression
9
8
7
6
Pre-treatment
14 days
90 days
Pre-treatment
14 days
90 days
Responders
Non-responders

Figure S6

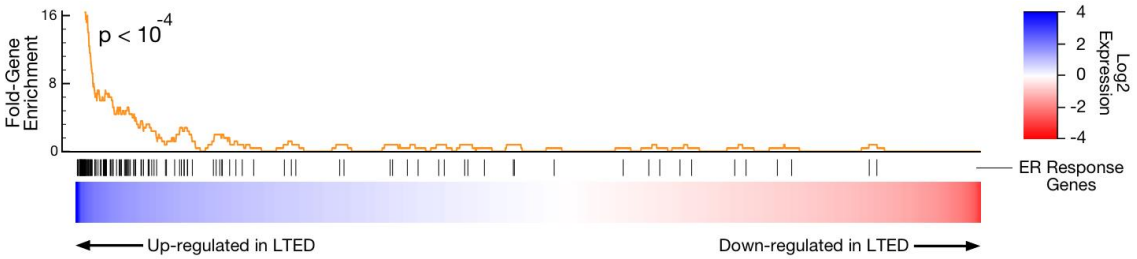

16
8
0
Fold-Gene Enrichment
p < 10-4
4
2
0
-2
-4
Log2 Expression
ER Response Genes
Up-regulated in LTED
Down-regulated in LTED

Figure S7

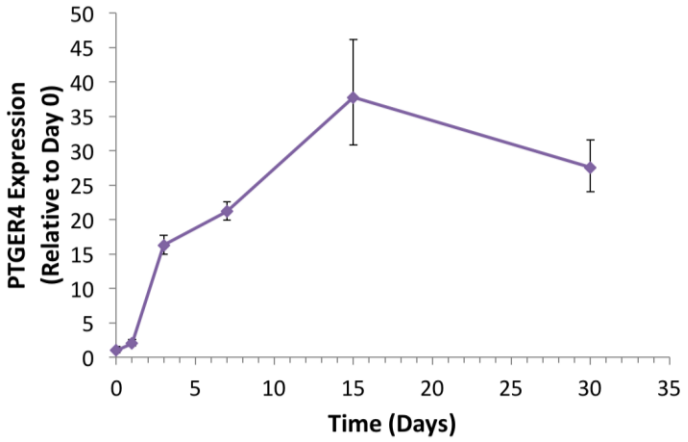
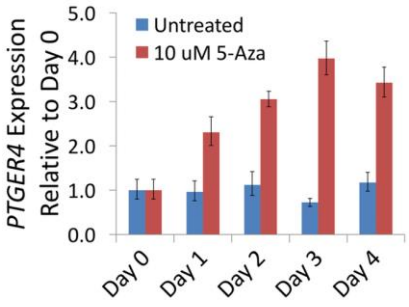

PTGER4 Expression
(Relative to Day 0)
50
45
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
Time (Days)

Figure S8


PTGER4 Expression Relative to Day 0
5.0
4.0
3.0
2.0
1.0
0.0
Untreated
10 uM 5-Aza
Day 0
Day 1
Day 2
Day 3
Day 4