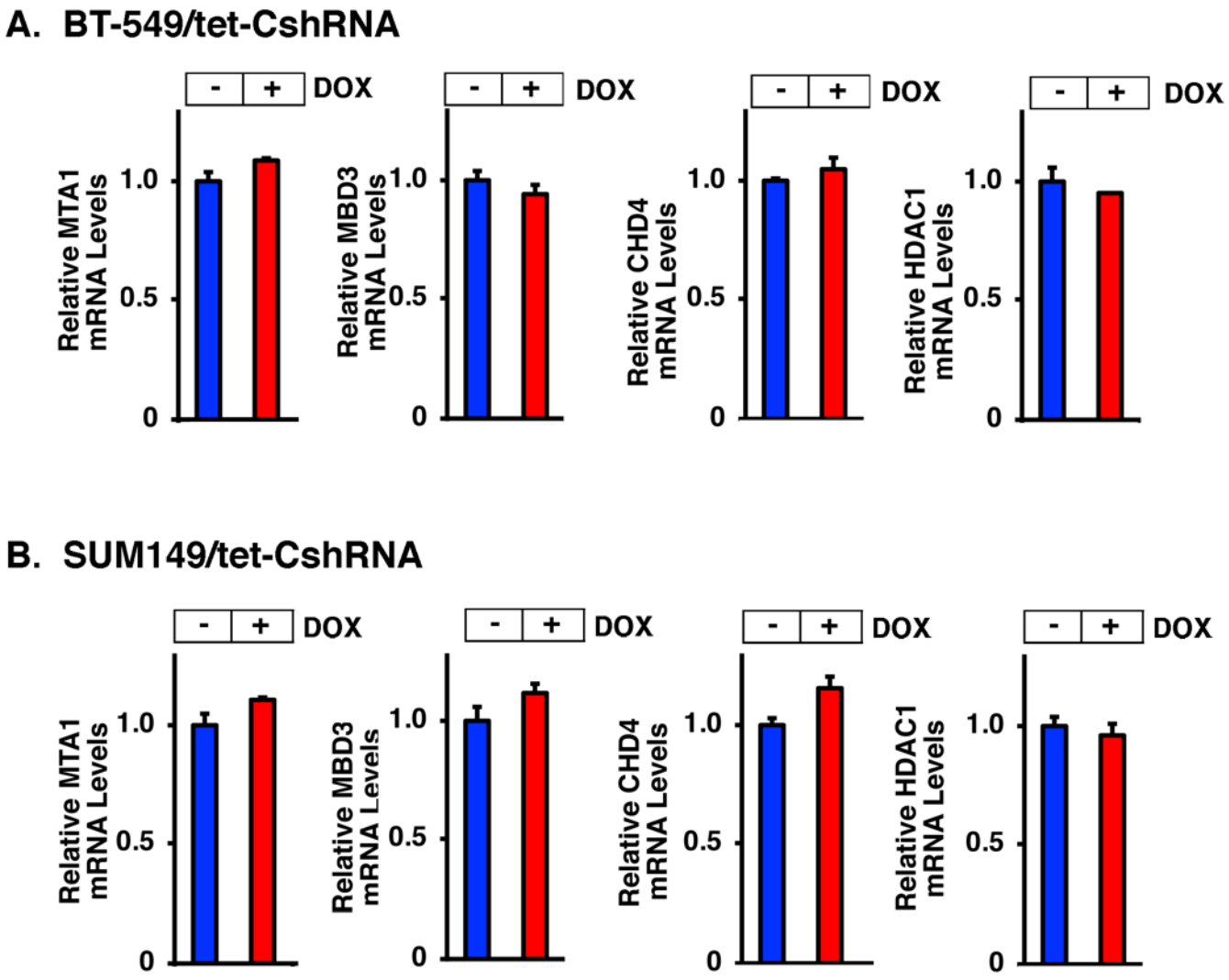
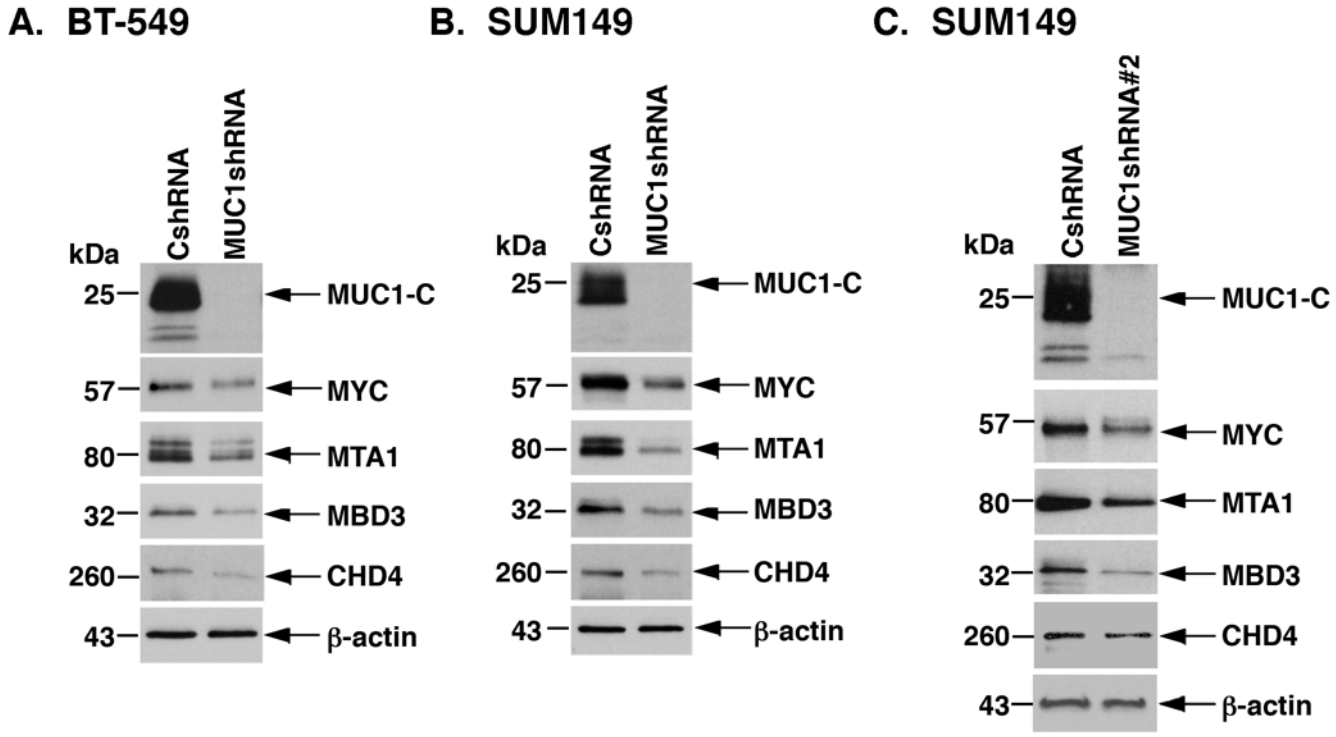
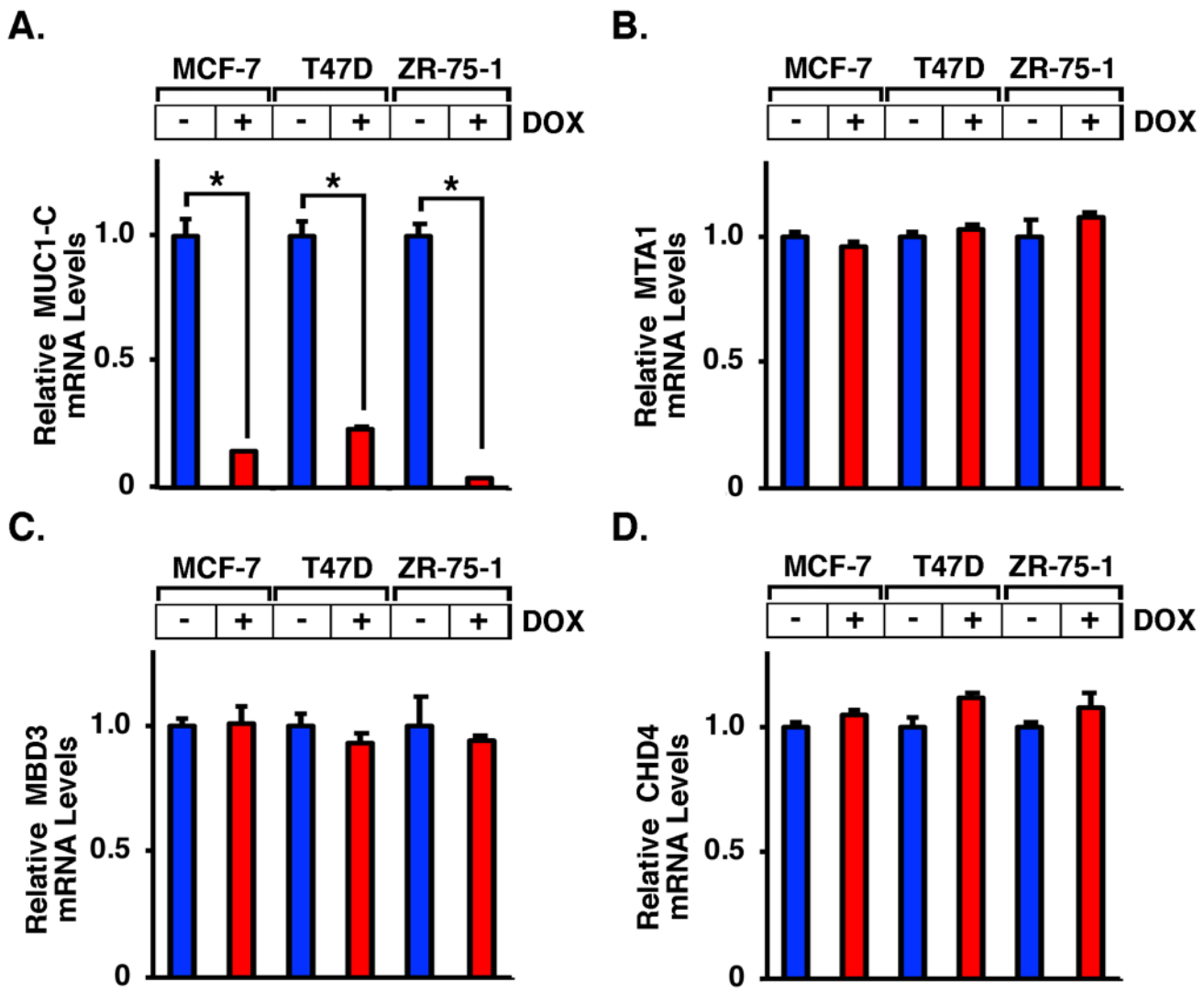


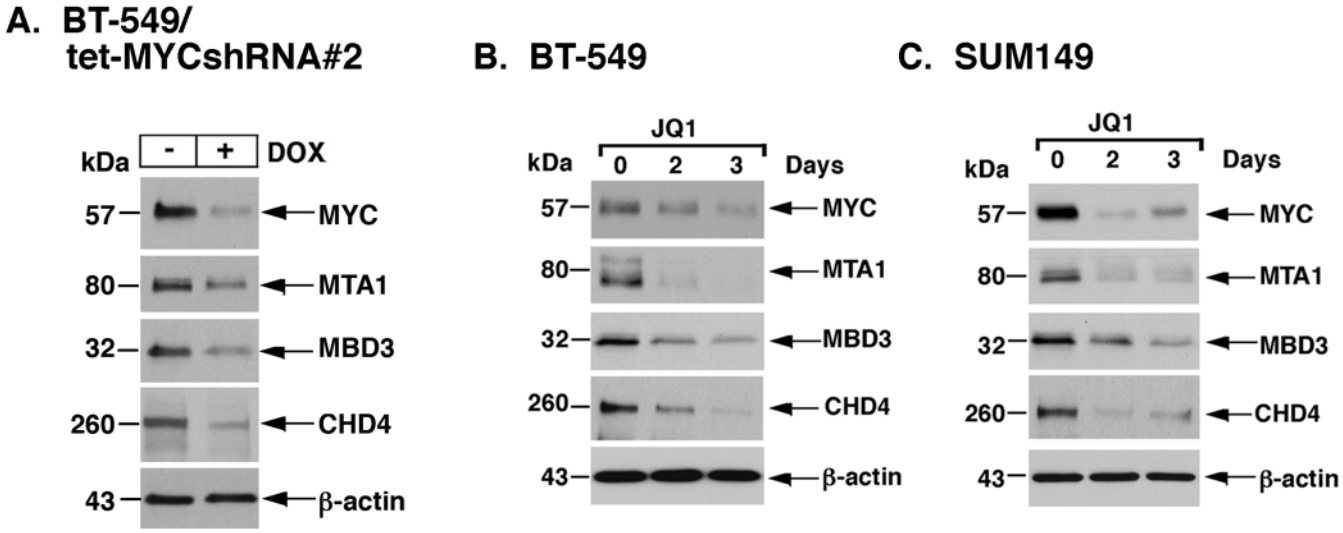
Supplemental Figure S1. Treatment of BT-549/tet-CshRNA and SUM149/tet-CshRNA cells with DOX has no apparent effect on MTA1, MBD3, CHD4 or HDAC1 expression. A-B. BT-549/tet-CshRNA (A) and SUM149/tet-CshRNA (B) cells were left untreated or treated with 500 ng/ml DOX for 7 days. Cells were analyzed for MUC1-C, MTA1, MBD3, CHD4 and HDAC1 mRNA levels using primers listed in Supplemental Table S2. The results (mean±SD) are expressed as relative mRNA levels compared to that obtained for untreated cells (assigned a value of 1).



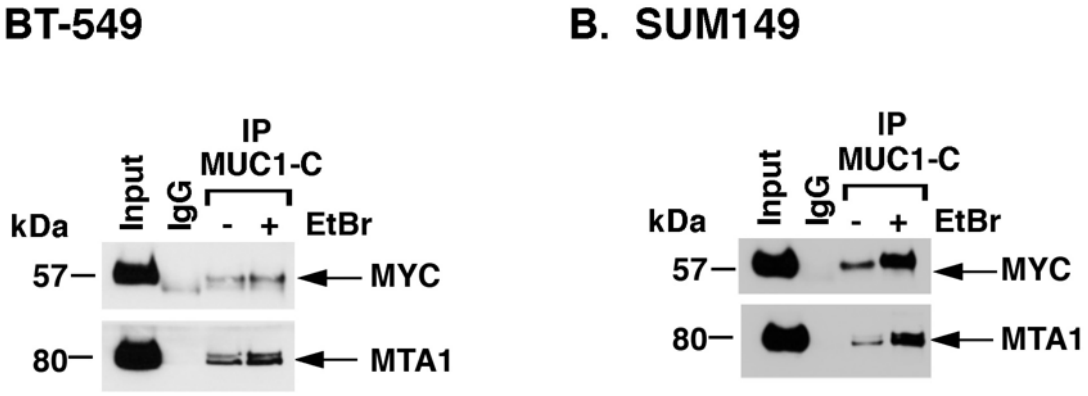
Supplemental Figure S2. Targeting MUC1-C with a different shRNA is associated with downregulation of MTA1, MBD3 and CHD4 expression. A-B. Lysates from BT-549 (A) and SUM149 (B) cells stably expressing a CshRNA or MUC1shRNA were immunoblotted with antibodies against the indicated proteins. C. Lysates from SUM149 cells stably expressing a CshRNA or a different MUC1shRNA#2 were immunoblotted with antibodies against the indicated proteins.



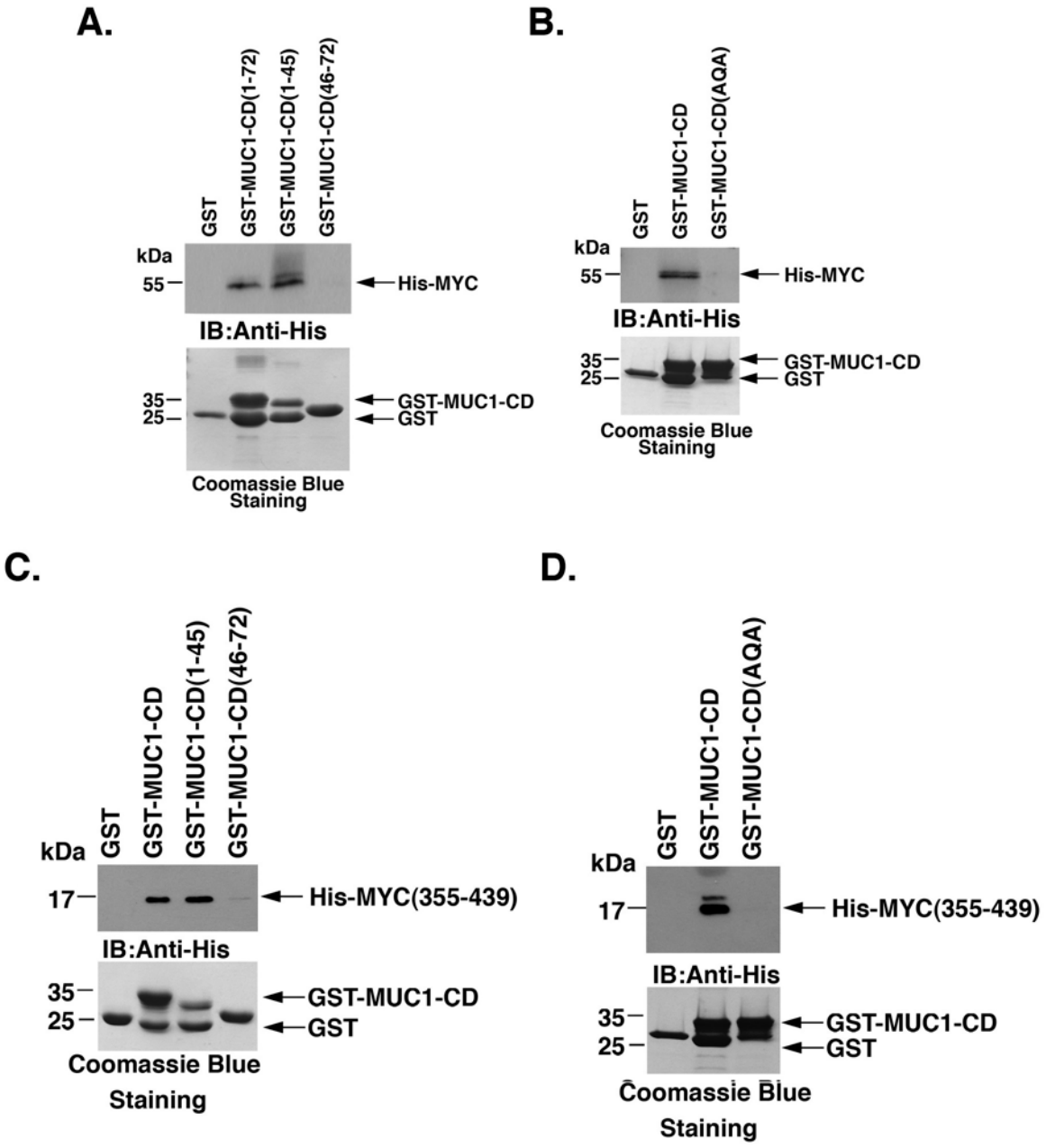
Supplemental Figure S3. Targeting MUC1-C in luminal MCF-7, T47D and ZR-75-1 BC cells has no detectable effect on MTA1, MBD3 or CHD4 expression. A-D. MCF-7/tet-MUC1shRNA, T47D/tet-MUC1shRNA and ZR-75-1/tet-MUC1shRNA cells left untreated or treated with 500 ng/ml DOX for 7 days were analyzed for MUC1-C (A), MTA1 (B), MBD3 (C) and CHD4 (D) mRNA levels. The results (mean±SD) are expressed as relative mRNA levels compared to that obtained for untreated cells (assigned a value of 1).



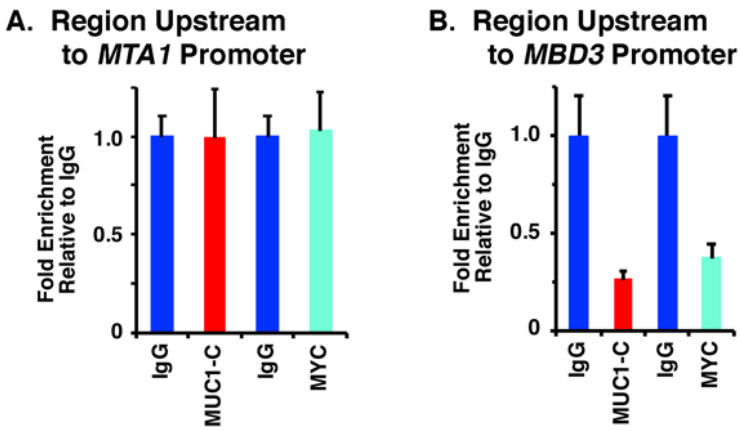
Supplemental Figure S4. Targeting MYC with a different shRNA or treatment with the JQ1 inhibitor is associated with downregulation of MTA1, MBD3 and CHD4 expression. A. Lysates from BT-549 cells stably expressing a different tet-MYCshRNA#2 left untreated or treated with 500 ng/ml DOX for 7 days were immunoblotted with antibodies against the indicated proteins. B-C. BT-549 (B) and SUM149 (C) cells were treated with 5 μM JQ1 for the indicated days. Lysates were immunoblotted with antibodies against the indicated proteins.



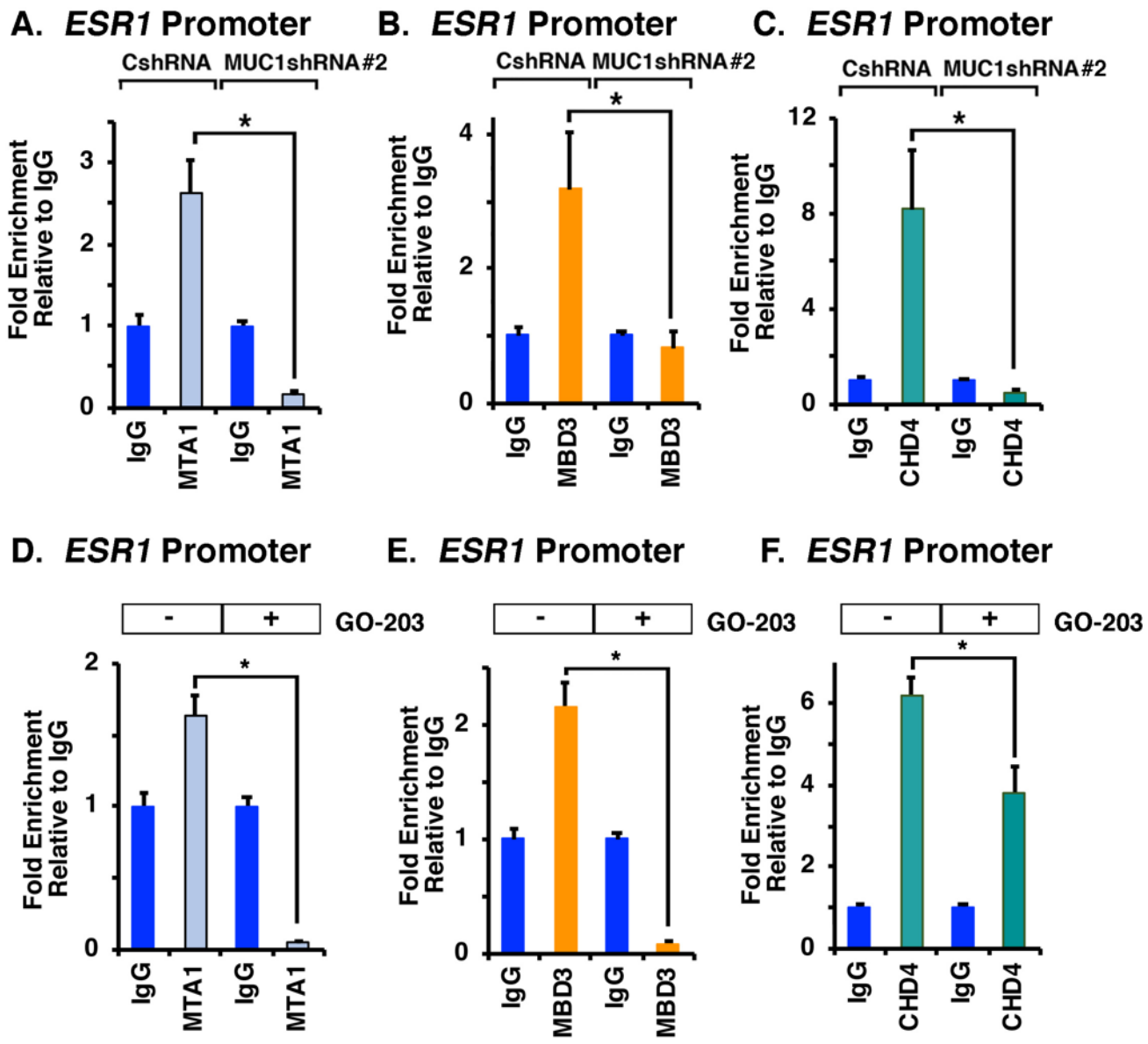
Supplemental Figure S5. Association of MUC1-C with MYC and MTA1 in the presence of ethidium bromide to inhibit non-specific association of proteins and nucleic acids. A-B. Nuclear lysates from BT-549 (A) and SUM149 (B) cells were incubated with anti-MUC1-C or a control IgG in the absence and presence of ethidium bromide (EtBr). The input and precipitates were analyzed by immunoblotting with anti-MYC and anti-MTA1.



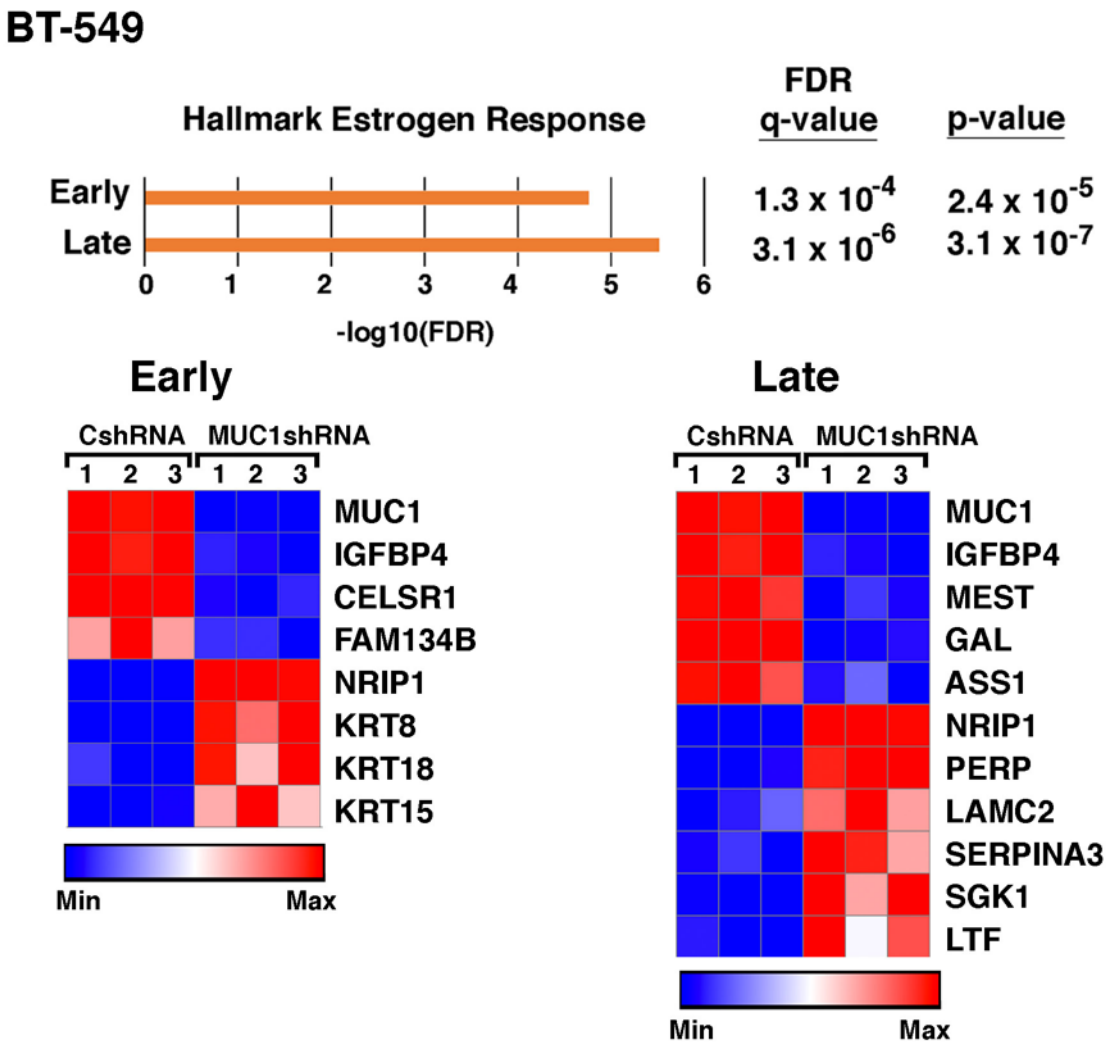
Supplemental Figure S6. MUC1-C CQC motif binds directly to the MYC HLH-LZ domain. A-B. GST and the indicated GST-MUC1-CD fusion proteins were incubated with His-MYC. The adsorbates were immunoblotted with anti-His. Input of the GST proteins was assessed by Coomassie Blue staining. C-D. GST, GST-MUC1-CD (full length; 1-72), GST-MUC1-CD(1-45), GST-MUC1-CD(46-72) and GST-MUC1-CD(AQA) were incubated with His-tagged MYC(355-439). The adsorbates were immunoblotted with anti-His. Input of the GST proteins was assessed by Coomassie Blue staining.



Supplemental Figure S7. ChIP studies performed on regions upstream to the *MTA1* and *MBD3* promoters. A-B. Chromatin from BT-549 cells was precipitated with anti-MUC1-C, anti-MYC or a control IgG. The DNA samples were amplified by qPCR with primers for regions ~5-kb upstream to the *MTA1* (A) and *MBD3* (B) promoters (Supplemental Table S2).

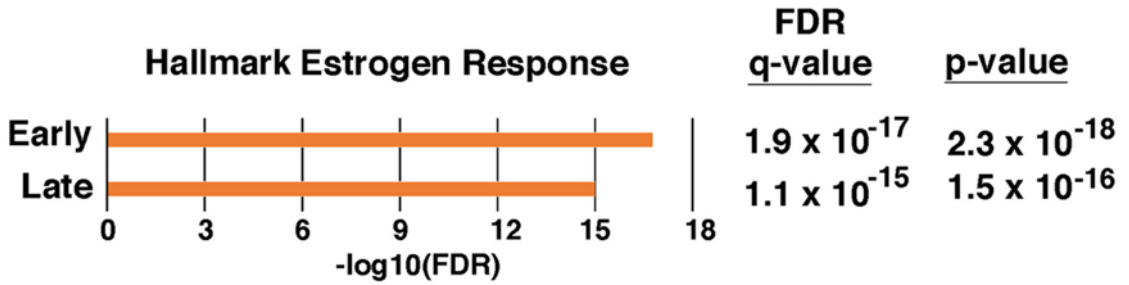


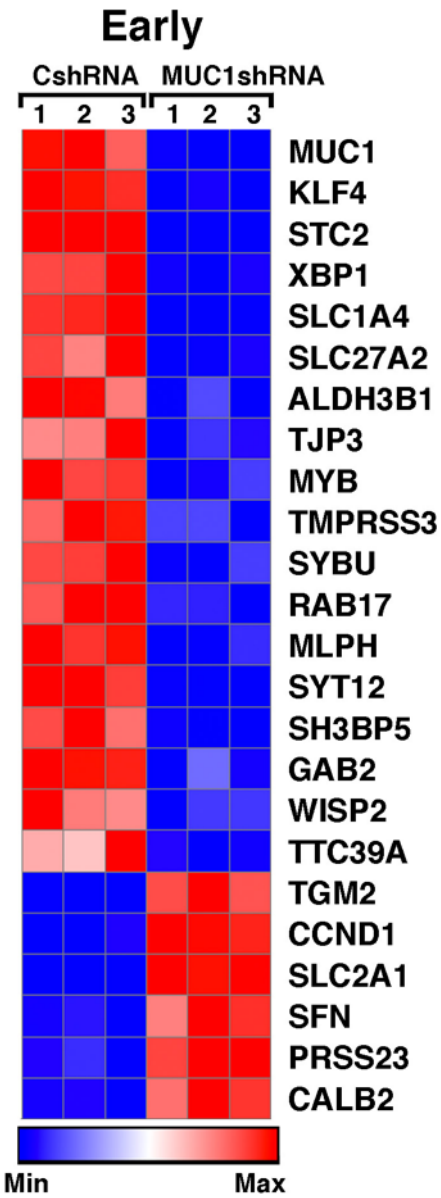
Supplemental Figure S8. ChIP studies performed on the *ESR1* promoter. A-C. Chromatin from BT-549/CshRNA and BT-549/MUC1shRNA#2 cells was precipitated with anti-MTA1 (A), anti-MBD3 (B), anti-CHD4 (C) or a control IgG. D-F. Chromatin from BT-549 cells treated with 5 μM GO-203 for 48 h was precipitated with anti-MTA1 (D), anti-MBD3 (E), anti-CHD4 (F) or a control IgG. The DNA samples were amplified by qPCR with primers for the *ESR1* promoter. The results (mean±SD of three determinations) are expressed as the relative fold enrichment compared to that obtained with the IgG control (assigned a value of 1).

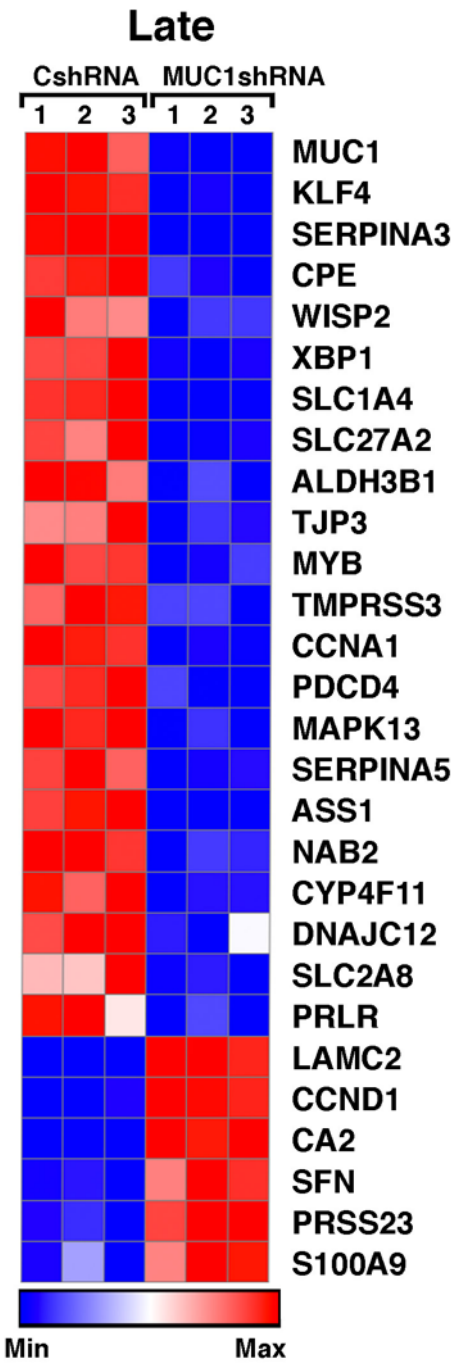


Supplemental Figure S9. MUC1-C regulates the Hallmark Estrogen Response Early and Late gene sets in BT-549 cells. A and B. RNA-seq was performed in triplicate on BT-549/CshRNA and BT-549/MUC1shRNA cells. The data sets were analyzed using Hallmark for enrichment of the Estrogen Response Early (A) and Estrogen Response Late (B) gene sets.

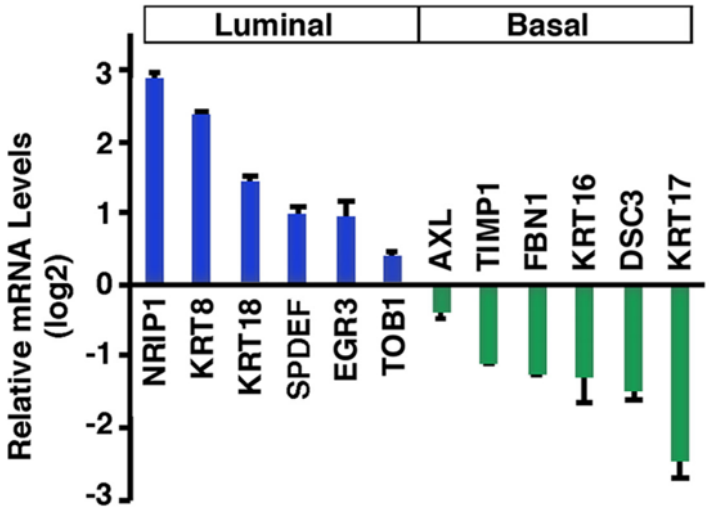
SUM149


Hallmark Estrogen Response
FDR
q-value
p-value
Early
Late
0
3
6
9
12
15
18
-log10(FDR)
1.9 x 10-17
1.1 x 10-15
2.3 x 10-18
1.5 x 10-16

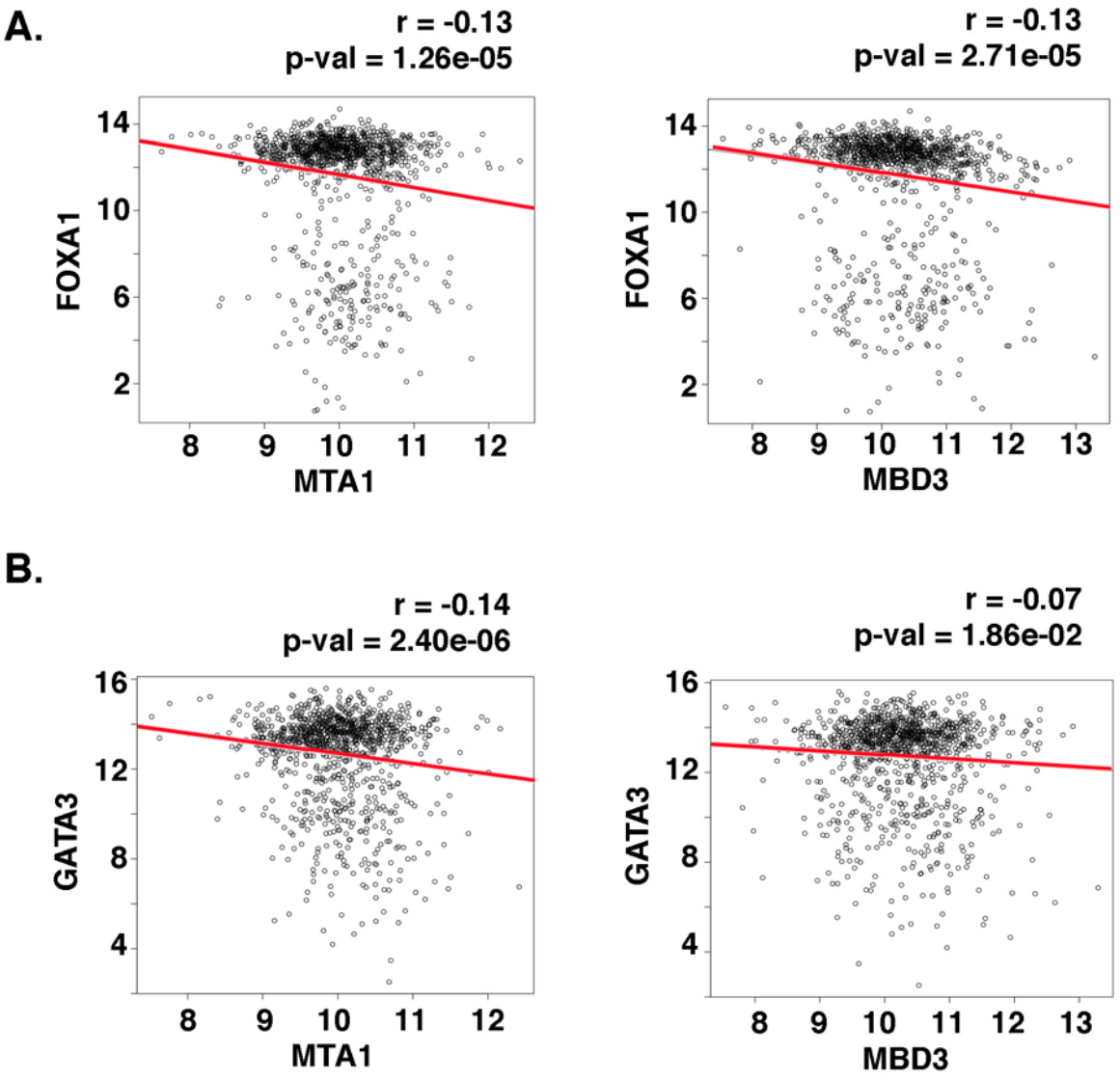

Early
CshRNA
MUC1shRNA
1 2 3 1 2 3
MUC1
KLF4
STC2
XBP1
SLC1A4
SLC27A2
ALDH3B1
TJP3
MYB
TMPRSS3
SYBU
RAB17
MLPH
SYT12
SH3BP5
GAB2
WISP2
TTC39A
TGM2
CCND1
SLC2A1
SFN
PRSS23
CALB2
Min
Max


Late
CshRNA
MUC1shRNA
1 2 3 1 2 3
MUC1
KLF4
SERPINA3
CPE
WISP2
XBP1
SLC1A4
SLC27A2
ALDH3B1
TJP3
MYB
TMPRSS3
CCNA1
PDCD4
MAPK13
SERPINA5
ASS1
NAB2
CYP4F11
DNAJC12
SLC2A8
PRLR
LAMC2
CCND1
CA2
SFN
PRSS23
S100A9
Min
Max

Supplemental Figure S10. MUC1-C regulates the Hallmark Estrogen Response Early and Late gene sets in SUM149 cells. A and B. RNA-seq was performed in triplicate on SUM149/CshRNA and SUM149/MUC1shRNA cells. The data sets were analyzed using Hallmark for enrichment of the Estrogen Response Early (A) and Estrogen Response Late (B) gene sets.



Supplemental Figure S11. Silencing MUC1-C regulates the expression of luminal and basal markers. BT-549 cells expressing a CshRNA or MUC1shRNA were analyzed for mRNA levels of the indicated genes. The results (mean±SD) are expressed as relative mRNA levels compared to that obtained for CshRNA expressing cells (assigned a value of 1) and plotted on a log2 scale.



Supplemental Figure S12. Expression of MTA1 and MBD3 correlates negatively with that of FOXA1 and GATA3 in breast cancers. A-B. Normalized, log2 transformed expression data were obtained for the TCGA-BRCA cohort and correlations were determined. Pearson correlation coefficient and significance are shown.

Supplemental Table S1. Primers used for qRT-PCR analysis.

Primer	Forward	Reverse
GAPDH	CCATGGAGAAGGCTGGGG	CAAAGTTGTCATGGATGACC
MUC1	TACCGATCGTAGCCCCTATG	CTCACCAGCCCAAACAGG
MTA1	GACCAGGCAGGCTTTCTATC	CTGTTGATGGGCAGGTAGG
MBD3	CCGCTCTCCTTCAGTAAATGTAAC	GGCTGGAGTTTGGTTTTCAGAA
CHD4	CACTTTTGAACAACAGCCTGC	TCCCGAGGTTTCTTAGGCTT
HDAC1	CTACTACGACGGGGATGTTGG	GAGTCATGCGGATTCGGTGAG
MYC	TTCGGGTAGTGGAAAACCAG	AGTAGAAATACGGCTGCACC
ESR1	GACAGGGAGCTGGTTCACAT	AGGATCTCTAGCCAGGCACA
FOXA1	CCCCTTTGTCCTCTCTACCC	CTGCAAAGCAAGAAGCAGAGT
GATA3	AGCCAGGAGAGCAGGGACG	CTGTTAATATTGTGAAGCTTGTAGTAGAG
NRIP1	AGAGCAGAGACCCAGCAAAC	CAGGAGCAACTGGAGGACAG
KRT8	CGAGGATATTGCCAACCGCAG	CCTCAATCTCAGCCTGGAGCC
KRT18	GTTGACCGTGGAGGTAGATGC	GAGCCAGCTCGTCATATTGGG
EGR3	GACATCGGTCTGACCAACGAG	GGCGAACTTTCCCAAGTAGGT
SPDEF	AGCCTACAGAAGGGCAGTGA	AACTCAGGGGTGCAGATGTC
TOB1	TCACTCTGCTGCTGTAAGCC	GGGAGAAGTACGTGCAACCT
AXL	GTGGGCAACCCAGGGAATATC	GTACTGTCCCGTGTCGGAAAG
TIMP1	AGACCTACACTGTTGGCTGTGAG	GACTGGAAGCCCTTTTCAGAG
FBN1	ATCGGGAAGGGTACTGCTTC	GTCACAGCAGCATTCCGATT
KRT16	GACCGGCGGAGATGTGAAC	CTGCTCGTACTGGTCACGC
DSC3	GACCCTCGTGATCTTCAGTC	ACCTGAAGCACTCTTCCAAA
KRT17	GGAGATTGCCACCTACCG	TGCCATCCTGGACCTCTT

Supplemental Table S2. Primers used for ChIP-PCR analysis.

Gene	Forward	Reverse
MTA1 Promoter	GAGGGATCACCAGGGAAATG	GGCCCTTCCACCAGAAC
MTA1 Upstream Region	GCATTCCTTGGCTGGTAGTT	TAGCCACAAGTGTGTTTCAGG
MBD3 Promoter	CATGGTGAAATCCCGTCTCTAC	CTCCCGGGTTCAAGTTGTTAT
MBD3 Upstream Region	CTCAGGATGGACAAGAGGAAAC	GCAGGTTTCAGTGAGGTTTAATG
ESR1 Promoter	CTAGTCAAATGGTGGTGCTAGT	ATCCGTTCTGAGTCGGTAGA
GAPDH Promoter	TACTAGCGGTTTTACGGGCG	TCGAACAGGAGGAGCAGAGAGCGA