Supplemental Figure S1. Analysis of ATAC-seq libraries. A. DU-145/tet-MUC1shRNA (left) and BT-549/tet-MUC1shRNA (right) cells were treated with vehicle or DOX for 7 days. Lysates were immunoblotted with the indicated antibodies. B. Fragment size distribution of ATAC-seq libraries showing phasing of mono-, di- and tri-nucleosomal regions. C. Presentation of ATAC-seq read distributions as an average plot depicting signal enrichment at gene start sites. D. Principal Component Analysis (PCA) depicting data variability and clustering of samples by group. E and F. Chromosomal localization of opening (E) and closing (F) DARs in MUC1-C-silenced DU-145 (left) and BT-549 (right) cells.
Supplemental Figure S2. Cistromes in MUC1-C-induced DARs. DARs from DU-145 (left) and BT-549 (right) were queried against all publicly available ChIP-seq datasets collected in the Cistrome DB. The x and y-axis show the giggle-scores measuring the significance of overlaps between either opening or closing DARs and the top 1000 peaks from each ChIP-seq dataset. The red dots highlight the top10 hits for either opening or closing DARs.
A. Opening DARs

DU-145

BT-549

B. Opening DARs, Up-regulated DEGs

DU-145

BT-549

C. Closing DARs

DU-145

BT-549

D. Closing DARs, Up-regulated DEGs

DU-145

BT-549

E. T-test

| Gene      | TF   | p-value  | Gene      | TF   | p-value  |
|-----------|------|----------|-----------|------|----------|
| MC00456   | JUND | 3.46e-05 | MC00456   | JUND | 4.03e-08 |
| MC00321   | JUN  | 6.33e-04 | MC00321   | JUN  | 6.45e-07 |
| MC00371   | JUNB | 1.50e-03 | MC00371   | JUNB | 7.48e-08 |
| MS00336   | NFE2 | 1.41e-03 | MS00336   | NFE2 | 1.21e-04 |
| MC00351   | FOSL1| 9.46e-04 | MC00351   | FOSL1| 8.95e-07 |
| MC00330   | FOS  | 3.33e-03 | MC00330   | FOS  | 7.54e-07 |
Supplemental Figure S3. Associations of DARs and corresponding DEGs with GO BIOLOGICAL PROCESSES. A. Correlations of opening DARs with up- and down-regulated DEGs in DU-145 (left) and BT-549 (right) cells. B. Associations of opening DARs and upregulated DEGs with GO BIOLOGICAL PROCESSES in DU-145 (left) and BT-549 (right) cells. C. Correlations of closing DARs with up- and down-regulated DEGs in DU-145 (left) and BT-549 (right) cells. D. Associations of closing DARs and the indicated DEGs with GO BIOLOGICAL PROCESSES in DU-145 (left) and BT-549 (right) cells. E. Associations of DARs and DEGs with motifs recognized by the indicated AP-1 family members in DU-145 (left) and BT-549 (right) cells.
Supplemental Figure S4. Associations of MUC1 and JUN/AP-1 gene signatures. A and B. Lysates from DU-145/tet-MUC1shRNA cells (A) and BT-549/tet-MUC1shRNA (B) cells treated with vehicle or DOX for 7 days were immunoblotted with antibodies against the indicated proteins. C and D. RNA-seq was performed in triplicate on DU-145/tet-MUC1shRNA (C) and BT-549/tet-MUC1shRNA (D) cells treated with vehicle or DOX for 7 days. The datasets were analyzed with GSEA using the AP-1 Q6 gene signature. E and F. Overlap of down- and up-regulated genes in DU-145 and BT-549 cells with MUC1-C silencing obtained from GSEA of the AP-1 Q4 (E) and AP-1 Q6 (F) target gene signatures. G and H. Overlap of down- and up-regulated genes in DU-145 cells with MUC1-C and ARID1A silencing obtained from GSEA of the AP-1 Q4 (G) and AP-1 Q6 (H) target gene signatures.
**Supplemental Figure S5. MUC1-C forms a complex with JUN and effects of silencing MUC1-C, JUN and ARID1A.**

**A.** Soluble chromatin from DU-145 cells was precipitated with a control IgG or anti-JUN (ChIP) and then reprecipitated with a control IgG or anti-MUC1-C (Re-ChIP). The DNA samples were amplified by qPCR with primers for the NOTCH1 PELS. The results (mean±SD of 3 determinations) are expressed as fold enrichment relative to that obtained with the IgG control (assigned a value of 1).

**B.** DU-145 cells expressing a CshRNA or JUNshRNA were analyzed for JUN mRNA levels by qRT-PCR (left). The results (mean±SD of 3 determinations) are expressed as fold enrichment relative to that obtained with the IgG control (assigned a value of 1).

**C.** DU-145 cells expressing a CshRNA or ARID1AshRNA were analyzed for ARID1A mRNA levels by qRT-PCR (left). The results (mean±SD of 3 determinations) are expressed as fold enrichment relative to that obtained with the IgG control (assigned a value of 1).

**D.** BT-549/tet-MUC1shRNA cells were treated with DOX and analyzed for NOTCH1 mRNA levels by qRT-PCR (left). The results (mean±SD of 3 determinations) are expressed as fold enrichment relative to that obtained with the IgG control (assigned a value of 1).

**E.** BT-549 cells expressing a CshRNA and ARID1AshRNA were analyzed for ARID1A mRNA levels by qRT-PCR (left). The results (mean±SD of 3 determinations) are expressed as fold enrichment relative to that obtained with the IgG control (assigned a value of 1).

**F.** BT-549 cells expressing a CshRNA or JUNshRNA were analyzed for NOTCH1 mRNA levels by qRT-PCR (left). The results (mean±SD of 3 determinations) are expressed as fold enrichment relative to that obtained with the IgG control (assigned a value of 1).

**G.** BT-549 cells expressing a CshRNA and ARID1AshRNA were analyzed for NOTCH1 mRNA levels by qRT-PCR (left). The results (mean±SD of 3 determinations) are expressed as fold enrichment relative to that obtained with the IgG control (assigned a value of 1).

**H.** BT-549 cells expressing a CshRNA and NOTCH1shRNA were analyzed for NOTCH1 mRNA levels by qRT-PCR (left). The results (mean±SD of 3 determinations) are expressed as fold enrichment relative to that obtained with the IgG control (assigned a value of 1).
3 determinations) are expressed as relative mRNA levels compared to that obtained for the CshRNA cells (assigned a value of 1). Lysates were immunoblotted with antibodies against the indicated proteins (right). C. DU-145 cells expressing a CshRNA or ARID1AshRNA were analyzed for ARID1A mRNA levels by qRT-PCR (left). The results (mean±SD of 3 determinations) are expressed as relative mRNA levels compared to that obtained for the CshRNA cells (assigned a value of 1). Lysates were immunoblotted with antibodies against the indicated proteins (right). D. BT-549/tet-MUC1shRNA cells treated with vehicle or DOX for 7 days were analyzed for NOTCH1 mRNA levels by qRT-PCR (left). The results (mean±SD of 3 determinations) are expressed as relative mRNA levels compared to that obtained for control cells (assigned a value of 1). Lysates were immunoblotted with antibodies against the indicated proteins (right). E. DU-145/CshRNA, DU-145/JUNshRNA and DU-145/ARID1AshRNA cells were analyzed for NOTCH1 mRNA levels by qRT-PCR. F. BT-549/CshRNA, BT-549/JUNshRNA and BT-549/ARID1AshRNA cells were analyzed for NOTCH1 mRNA levels by qRT-PCR. The results (mean±SD of 3 determinations) are expressed as relative mRNA levels compared to that obtained for CshRNA cells (assigned a value of 1). G. Lysates from BT-549/CshRNA and BT-549/NOTCH1shRNA cells were immunoblotted with antibodies against the indicated proteins. H. BT-549/CshRNA and BT-549/NOTCH1shRNA cells (5000/well) were assayed for tumorsphere formation at 10 days (left). Scale bar: 100 um. The results (mean±SD of 3 biological replicates) are expressed as the number of mammospheres (right).
Supplemental Figure S6. MUC1-C activates the EGR1 pELS and dELS in BT-549 cells. A. Soluble chromatin from BT-549 cells was precipitated with a control IgG, anti-MUC1-C, anti-JUN, anti-ARID1A and anti-PBRM1. B. Soluble chromatin from BT-549/tet-MUC1shRNA cells treated with vehicle or DOX was precipitated with a control IgG, anti-MUC1-C, anti-JUN and anti-ARID1A. C. Soluble chromatin from BT-549/CshRNA and BT-549/JUNshRNA cells was precipitated with a control IgG, anti-MUC1-C, anti-JUN and anti-ARID1A. D. Soluble chromatin from BT-549/CshRNA and BT-549/ARID1AshRNA cells was precipitated with a control IgG, anti-MUC1-C, anti-JUN and anti-ARID1A. E. Soluble chromatin from BT-549/tet-MUC1shRNA cells treated with vehicle or DOX was precipitated with a control IgG, anti-EP300, anti-H3K27ac, anti-H3K4me1 and anti-H3K4me3. The DNA samples were amplified by qPCR with primers for the EGR1 pELS (left) and dELS (right). The results (mean±SD of 3 determinations) are expressed as fold enrichment relative to that obtained with the IgG control (assigned a value of 1). F and G. BT-549/tet-MUC1shRNA cells were treated with vehicle or DOX for 7 days. Genome browser snapshots of ATAC-seq data from the EGR1 pELS and dELS (F). Chromatin was analyzed for accessibility by nuclease digestion (G). The results (mean±SD of 3 determinations) are expressed as % untreated chromatin. H. DU-145/tet-MUC1shRNA (left) and BT-549/tet-MUC1shRNA (right) cells treated with vehicle or DOX for 7 days were analyzed for EGR1 mRNA levels by qRT-PCR. I and J. The indicated DU-145 (I) and BT-549 (J) cells expressing a CshRNA, JUNshRNA or ARID1AshRNA were analyzed for EGR1 mRNA levels by qRT-PCR. The results (mean±SD of 3 determinations) are expressed as relative mRNA levels compared to that obtained for control cells (assigned a value of 1).
Supplemental Figure S7. MUC1-C activates the LY6E pELS and dELS in BT-549 cells. A. Soluble chromatin from BT-549 cells was precipitated with a control IgG, anti-MUC1-C, anti-JUN and anti-ARID1A. The DNA samples were amplified by qPCR with primers for the LY6E pELS (left), PLS (middle) and dELS (right). B. Soluble chromatin from BT-549/tet-MUC1shRNA cells treated with vehicle or DOX was precipitated with a control IgG, anti-MUC1-C, anti-JUN and anti-ARID1A. C. Soluble chromatin from BT-549/CshRNA and BT-549/JUNshRNA cells was precipitated with a control IgG, anti-MUC1-C, anti-JUN and anti-ARID1A. D. Soluble chromatin from BT-549/CshRNA and BT-549/ARID1AshRNA cells was precipitated with a control IgG, anti-MUC1-C, anti-JUN and anti-ARID1A. E. Soluble chromatin from BT-549/tet-MUC1shRNA cells treated with vehicle or DOX was precipitated with a control IgG, anti-EP300, anti-H3K27ac, anti-H3K4me1 and anti-H3K4me3. The DNA samples were amplified by qPCR with primers for the LY6E PLS (left) and dELS (right). The results (mean±SD of 3 determinations) are expressed as fold enrichment relative to that obtained with the IgG control (assigned a value of 1). F and G. BT-549/tet-MUC1shRNA cells were treated with vehicle or DOX for 7 days. Genome browser snapshots of ATAC-seq data from the LY6E pELS, PLS and dELS (F). Chromatin from the PLS and dELS was analyzed for accessibility by nuclease digestion (G). The results (mean±SD of 3 determinations) are expressed as % untreated chromatin. H. DU-145/tet-MUC1shRNA (left) and BT-549/tet-MUC1shRNA (right) cells treated with vehicle or DOX for 7 days were analyzed for LY6E mRNA levels by qRT-PCR. I. DU-145/CshRNA, DU-145/JUNshRNA and DU-145/ARID1AshRNA cells were analyzed for LY6E mRNA levels by qRT-PCR. J. BT-549/CshRNA, BT-549/JUNshRNA and BT-549/ARID1AshRNA cells were analyzed for LY6E mRNA levels by qRT-PCR. The results (mean±SD of 3 determinations) are expressed as relative
mRNA levels compared to that obtained for CshRNA cells (assigned a value of 1).
Table S1. Primers used for qRT-PCR.

| Gene   | FWD             | REV             |
|--------|-----------------|-----------------|
| NOTCH1 | GGGCTAACAAAGATATGCAG | ACTGAACCTGACCGTACAGTTGGCACAAGTGGTCCAG |
| JUN    | CCAAGGATAGTGCGATGTTT | CTGTCCCTCTCCACTGCAAC |
| GAPDH  | CCATGGAAAGGCTGGG | CAAAGTTGTCATGGATGACC |
| EGR1   | CTTCAACCCTCAGGCAGGACA | GAAAAGCGGGCCAGTATAGGT |
| ARID1A | ACCTCTATCGCCTCTATGCTCTGT | CTGGCAGCACTGTGCTTGATGT |
| LY6E   | CTCCAGGCAGGACGCCCATC | CGAGATTCACATGCGGCACT |
Table S2. Primers used for direct chromatin accessibility assays.

| Gene | pELS        | FWD                        | REV                        |
|------|-------------|----------------------------|-----------------------------|
| NOTCH1 | FWD       | CCTGGGACTACTTCTCGTTTG       | REV GCAAATTTTCAGTCGCCAGTTG  |
|      | REV        |                            |                             |
| EGR1  | FWD       | ATTCAGAGCTAGAGCAGGAGGAG    | REV GGTGCCAGGGAGAAGGATT     |
|      | REV        |                            |                             |
| EGR1  | dELS      | FWD AAGTGCTGGGATTACAGGC    | REV CAAAGTATGACCCCTCCATCTC |
|      | REV        |                            |                             |
| LY6E  | pELS      | FWD ATGTGTTCCTGAGTTCCC     | REV ACCCTTTTTCCCAGCAATAC    |
|      | REV        |                            |                             |
| LY6E  | PLS       | FWD GGAAGCAGGGACAAGATGAC    | REV ACGTGTTTGGTGTGAGC       |
|      | REV        |                            |                             |
| LY6E  | dELS      | FWD CTTCATGGTCTGGGTATGGG   | REV AACAATCCGGGTTCCATC      |
|      | REV        |                            |                             |
Table S3. Primers used for ChIP-qPCR.

|        | FWD                      | REV                      |
|--------|--------------------------|--------------------------|
| **NOTCH1 pELS** | CCTGGGACTACTTCTCGTTTGG   | GCAAATTTTCAGTCGCCAGTTTGG |
| **pGAPDH**     | TACTACCGGTGTACACGGGCG    | TCGAACAGGGAGGAGGAGAAGCGA |
| **EGR1 pELS**  | ATTCAGAGCTAGAGCAGGAGGAG | GGTGGCGAGGGAGAAGCGATTT   |
| **EGR1 dELS**  | AAGTGCTGGGATTACAGGC     | CAAAGTATGACCCTCCCATCTC   |
| **LY6E pELS**  | ATGTGTTTTCCCTGAGTTCCC   | ACCCTCTTTCCCAAGCAATAC    |
| **LY6E PLS**   | GGAAGCAGGGACAAGATGAC    | ACGTGTGGGTTGAC          |
| **LY6E dELS**  | CTTCAATGGTCTGGTATGAG    | AACATCCGGTTCACTC         |