

## **SUPPLEMENTAL DATA**

### **Progesterone Receptor Activation Downregulates GATA3 by Transcriptional Repression and Increased Protein Turnover Promoting Breast Tumor Growth**

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Figure S1

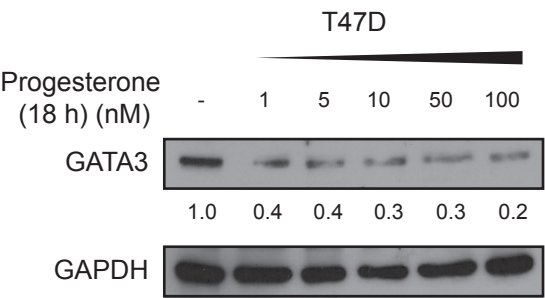


Figure S1. T47D cells were treated or not with the indicated concentrations of progesterone for 18 h. Total protein lysates were prepared and GATA3 expression was measured by western blot (WB). GATA3 bands underwent densitometry and values were normalized to GAPDH protein bands setting the value of untreated cells as 1.0.

Figure S2

ATTGAGCCCTGATTTCAAACAAACATCAGATTTAAGAAGGTGAGGACTCCAAAGAAGATCCAAACGCGAG  
ACCAGGAAGATTACGTTTCCACTACTTCTTCCAATACATTTCACTAATCTTTCTACAGGGATAGTGTTT  
TAAACACGACGACCCTCACACACCAAGGTGAAGTTGAATATTGTTGTTGTTCCCTTACCCGCATGAAAGG  
**PRE primer forward**  
AATCCTTCAGAACTTACTTTTCAAGGACGGCT [TCTTCT] ATCTCGAATTTTGCATTGTAGGGAATTTT  
**PRE**  
ATGGGGTAGAGGAGCGAAGAGGGAACAATGTCTCCAATTCAAATAAAACCCCAAATTAAGCTCTCAGCCC  
**PRE primer reverse**  
CATCGGTGGGATAGCCTGCGGGGAACCCACTCCCTCTCTCTGGCAGCATTGCCCTGGGGGCGAGCAGAGA  
GTGGATTTGGAGTCTTCTTTTCCCGCTGACCCCTGCTCGCCCGAGCAGCATTTCGGGCCAGCGGCTGC  
AGCCACCCGGGCGCCGCTAGGGGATGCAGTGGCTCAAAGGACCGAGCGGGCGGTGCAGGTTGGAACCCGC  
GGGGCGGACCAATCGCGGCTCGGCCACAGCCTCGCCCGCTGATTGGTCCCTCCAGGCCCCGCCCCGCTC  
GCCCCGCCCCCTCTCGCTGGGGCGCCTCGGAGCCGCGTGCCCTCCGCCCCGGGGTGCCCATTCGCGCAGAGC  
GTGGCCTGGAGACCCGCGAGCCGGGAAGGTGCGCGTGGAGTCCCGACCAGAGGCCGGGGTTGGGGTTCGG  
TGCAGACCGAGGGCTGGTTTCTTGTACTGTGGGAGAAACGCCGGGAGCCGGAGTAAGTAGGGCTCCGGGC  
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CTCCCAAACACCCTGCATTAGATCCTAATAATGATCCATGCGTGCTATTTTTTTAAAAGTCTGAAAAAGA  
AAATTCTGCCCATCGAAATGAACTTCATGAATGGGGCAGGCTGGCTGCACCGGGACGGAATCGTCCACCC  
GACCCGAATGAATTGGCAGGAGCCGCGGCCACATTTAAAGGGCCAGAGCGCGGTTCCCTCCCGTCCGCC  
CCCAAGCCCCGCGGGCCTCGCCACCCCTGCCCCGCGCCCTCCGCGGCGGCGCCCTCTGCGGCGCCCC  
TTTCCGGTCAGTGGAGGGGCGGGAGGAGGGGCGGGGGTGCGCGGGGCGGGGGAGAAGTCTGGAGCGGG  
TTTGGGTTGCAGTTTCTTGTGCGGGGATCCTGTCCCTACTCGCCAGCGCCAGGCTCCTCCCCCCCCG  
CGCGGATGACACTAGAACCTCCTTAAGTTGCGTCGCGCCACAGCTGTCTGCGAACACTGAGCTGCCTGGC  
GCCGTCTTGATACTTTTCAAGAAAGATGCATTCCCTGT

TSS  
GATA3

PRE: -1498 bp to -1504 bp from TSS

Figure S2. The genomic sequence upstream of the GATA3 transcriptional start site is shown. The putative PRE detected by *in silico* analysis is highlighted in red, and the sequences that match the primers used (PRE primer set) are shown. The position of the putative PRE relative to the TSS is detailed below.

Figure S3

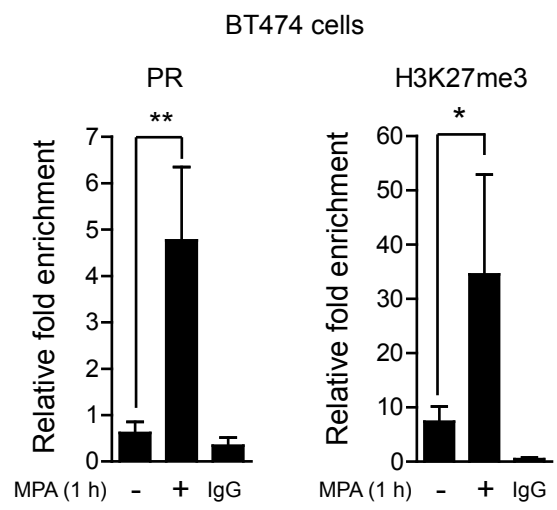


Figure S3. MPA induces PR recruitment to GATA3 promoter in BT474 cells. Protein recruitment to the GATA3 promoter was analyzed by ChIP in cells treated with MPA as indicated. Immunoprecipitated DNA was amplified by q-PCR using primers flanking the potential PRE located at position -1504 bp. Each sample was normalized to the input. Data are expressed as n-fold chromatin enrichment over isotype control (\* $P < 0.05$ , \*\* $P < 0.01$ , One-way ANOVA).

Figure S4

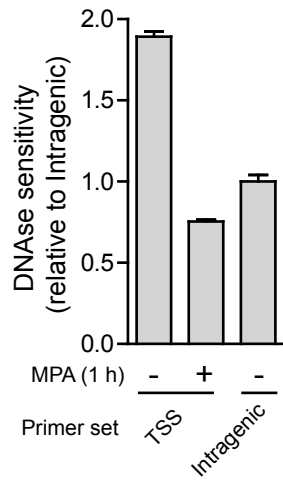


Figure S4. DNase sensitivity assay was performed as described in the materials and methods section using primers spanning the region of *GATA3* transcriptional start site (TSS) or an intragenic region as control. Sensitivity was calculated as  $2^{(C_{\text{tcut}} - C_{\text{tuncut}})}$  for each treatment. One representative experiment of two is shown.

Figure S5

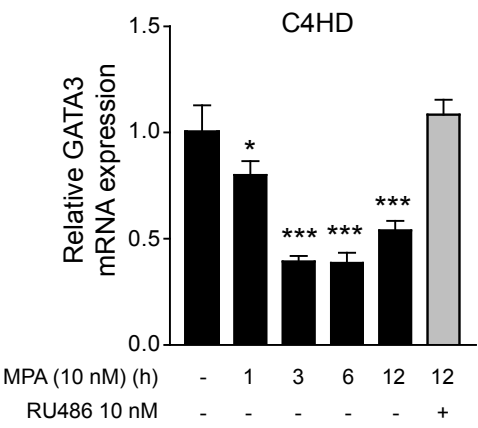


Figure S5. C4HD cells were treated as indicated and GATA3 mRNA expression levels were determined by RT-qPCR. The fold change of mRNA expression levels upon MPA treatment for the indicated times was calculated by normalizing the absolute levels of GATA3 mRNA to GAPDH levels, which were used as an internal control, and setting the value of untreated cells as 1.0. \*\*P<0.01 and \*\*\*P<0.001 (One-way ANOVA).

Figure S6

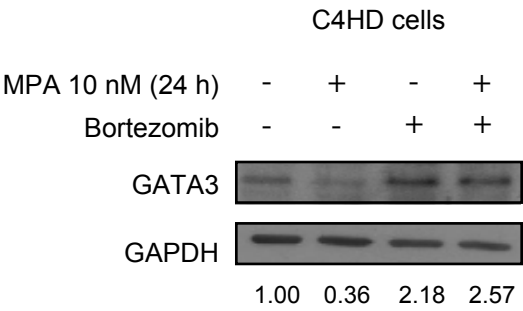


Figure S6. C4HD cells were treated or not with MPA and/or Bortezomib as indicated, protein cell lysates were prepared and GATA3 expression was measured by WB. GATA3 bands underwent densitometry and values were normalized to GAPDH protein bands setting the value of untreated cells as 1.0.

Figure S7

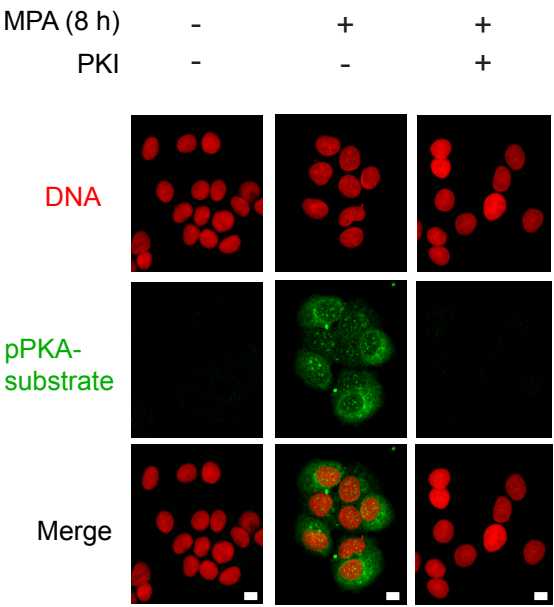


Figure S7. T47D cells were treated or not with MPA and/or the PKA inhibitor PKI for 8 h. Cells were then fixed and incubated with the indicated antibodies. Nuclei were stained with propidium iodide and confocal microscopy analysis was performed. Scale bar = 10  $\mu$ m.

Figure S8

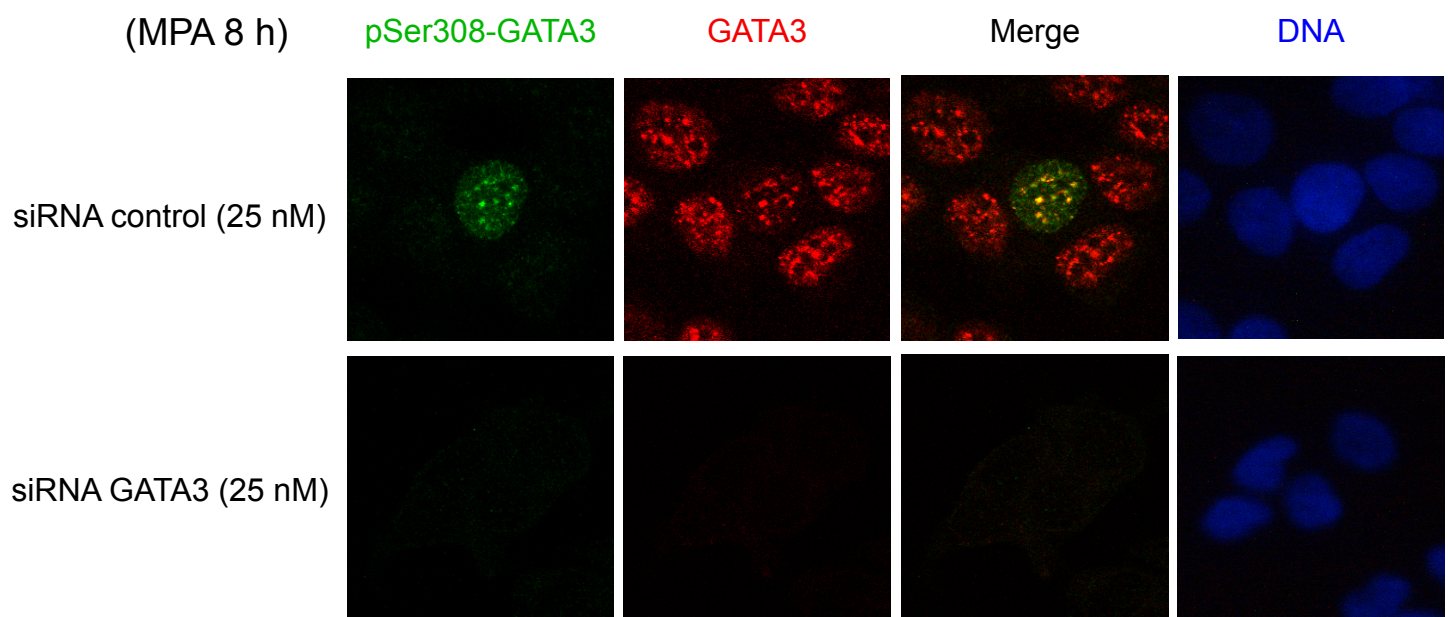


Figure S8. T47D cells were transfected with an unspecific siRNA as control or with a siRNA targeting GATA3 and treated with MPA for 8 h. Cells were fixed and pSer308-GATA3 and total GATA3 were analyzed by confocal microscopy. Nuclei were detected by DAPI staining.

Figure S9

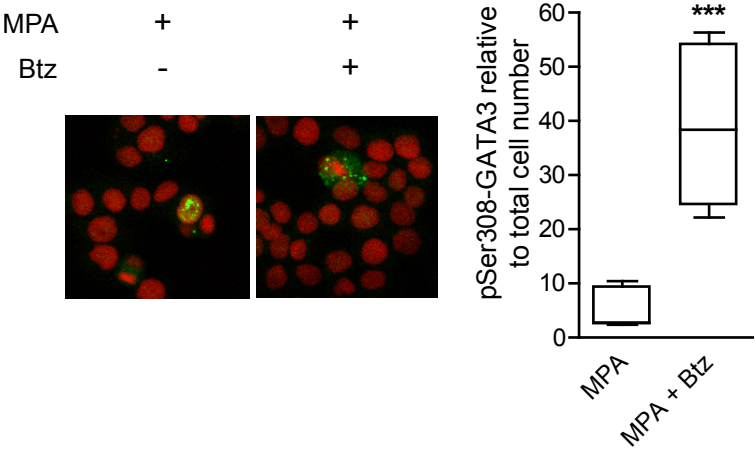


Figure S9. T47D cells were treated or not with MPA and Btz or 8 h. Cells were fixed and pSer308-GATA3 was analyzed by confocal microscopy. Nuclei were stained with propidium iodide. Scale bar = 10  $\mu$ m. \*\*\* $P < 0.001$  (Student's  $t$ -test).

Figure S10

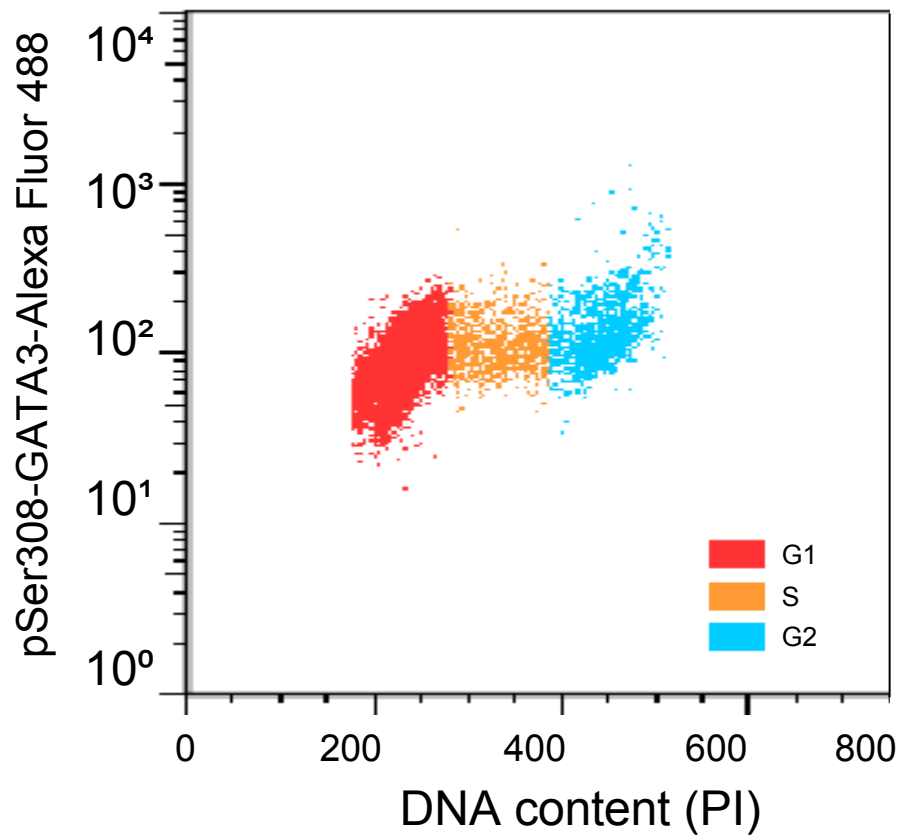


Figure S10. T47D cells treated with MPA for 8 h were stained with pSer308-GATA3-Alexa Fluor 488 and propidium iodide (PI) and were analyzed by flow cytometry. The different stages of the cell cycle were determined by PI. The experiment shown is representative of a total of three.

Figure S11

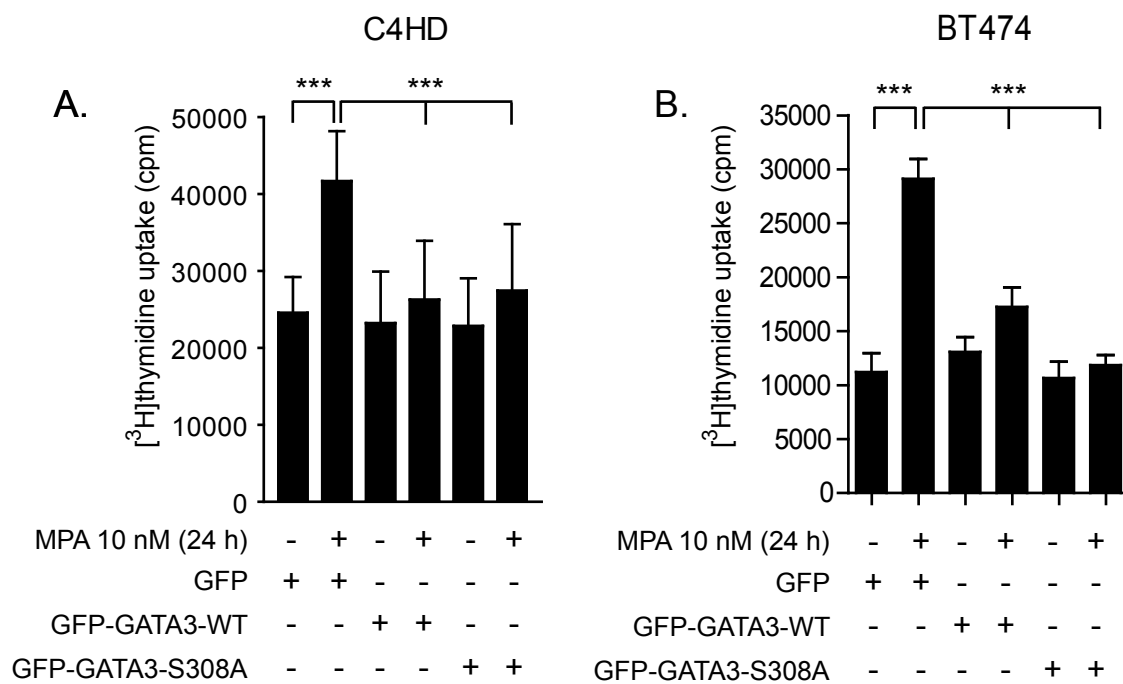


Figure S11. C4HD and BT474 cells were transfected with the indicated vectors for 48 h, starved for 24 h and treated with MPA as indicated. Incorporation of [<sup>3</sup>H]-thymidine was used as measure of DNA synthesis and cell proliferation. \*\*\*P<0.001 (One-way ANOVA).

Figure S12

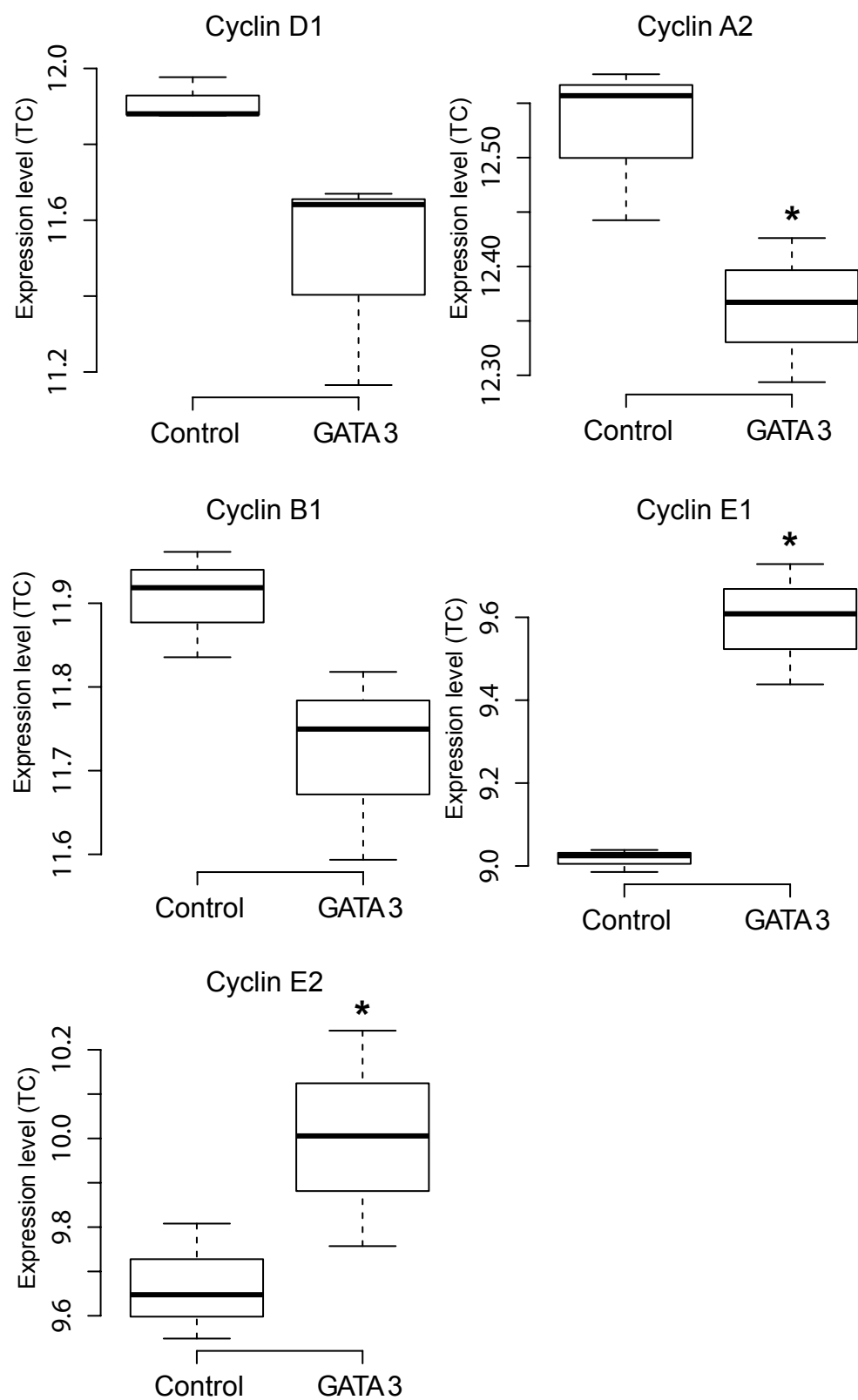


Figure S12. Data for the corresponding genes were downloaded from the publicly available Gene Expression Omnibus Data Sets (GDS4080) database and displayed as boxplots for the corresponding genes, comparing triplicate measures of transcriptional counts (TC). \*P<0.05 (Student's *t*-test).

Figure S13

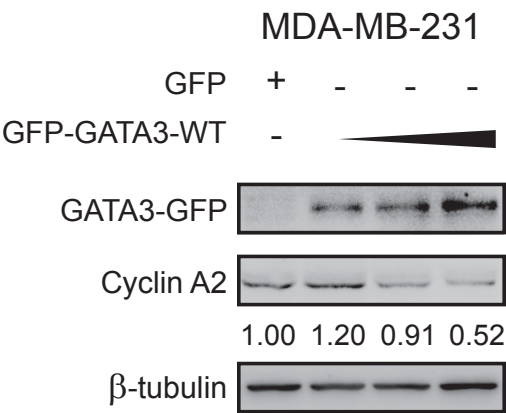


Figure S13. MDA-MB-231 cells were transfected with the indicated vectors for 48 h, protein lysates were prepared and immunoblotted for the indicated proteins. The densitometry values were obtained by normalizing cyclin A to GAPDH protein bands and setting the value of untreated cells as 1.0.

Supplementary Table 1

| Primer                     | Sequence (5' - 3')            |
|----------------------------|-------------------------------|
| PRE Forward                | GACGACCCTCACACACCAAG          |
| PRE Reverse                | TCTTCGCTCCTCTACCCCAT          |
| GATA3 Intragenic Forward   | GGGAGAGCTCGTGGTATCTG          |
| GATA3 Intragenic Reverse   | TTTCTCCCCTCCATGTTCAAG         |
| GATA3 TSS Forward          | TTGGGTTGCAGTTTCCTTGT          |
| GATA3 TSS Reverse          | CGACGCAACTTAAGGTT             |
| GATA3 mRNA (human) Forward | CAGACCACCACAACCACACTCT        |
| GATA3 mRNA (human) Reverse | GGATGCCTTCCTTCTTCATAGTCA      |
| GATA3 mRNA (mouse) Forward | CTACCGGGTTTCGGATGTAAGTC       |
| GATA3 mRNA (mouse) Reverse | GTTTACACACTCCCTGCCTTCT        |
| GAPDH mRNA Forward         | CCAGAACATCATCCCTGCAT          |
| GAPDH mRNA Reverse         | GTTTCAAGCTCTGGGATGACCTT       |
| Mutagenesis S308A Forward  | CAAGCGAAGGGCTGGCTGCAGCCAGGAG  |
| Mutagenesis S308A Reverse  | CTCCTGTGGCTGCAGCCAGCCTTCGCTTG |

Supplementary Table 2

| siRNA         | Sequence (5'-3')      |
|---------------|-----------------------|
| PR #1 (human) | CUAACAAAUCAUCGACUUA   |
| PR #2 (human) | GUAGUCAAGUGGUCUAAAU   |
| PR #1 (mouse) | CTCCACCTGTACTGCTTGAAT |
| PR #2 (mouse) | CTCCACCTGTACTGCTTGAAT |
| GATA3 (human) | AUAUCCAUCGCGUUUAGGCUU |