

SUPPLEMENTARY DATA

The High Mobility Group A2 protein epigenetically silences the *Cdh1* gene during epithelial-to-mesenchymal transition

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SUPPLEMENTARY TABLES

Supplementary Table S1. Oligonucleotides used in quantitative real-time PCR reactions for mRNA analysis.

| Gene | Species | Primer Sequence | Product size (bp) | Accession number |
|---------------|---------|--|-------------------|------------------|
| <i>Gapdh</i> | mouse | fwd 5'- TGTGTCCGTCGTGGATCTGA -3' rev 5'- CCTGCTTCACCACCTTCTTGA -3' | 77 | NM_008084 |
| <i>Dnmt1</i> | mouse | fwd 5'- CCAAGCTCCGGACCCTGGATGTGT -3' rev 5'- CGAGGCCGGTAGTAGTCACAGTAG -3' | 373 | NM_010066 |
| <i>Dnmt3a</i> | mouse | fwd 5'- ATGTGGTTCGGAGATGGCAAG -3' rev 5'- AGATGGCTTTGCCGTACATGG -3' | 124 | NM_007872 |
| <i>Dnmt3b</i> | mouse | fwd 5'- CAAGGAGGGCGACAACCGTCCATT -3' rev 5'- TGTTGGACACGTCCGTGTAGTGAG -3' | 221 | NM_010068 |
| <i>Cdh1</i> | mouse | fwd 5'- GACTGTGAAGGGACGGTCAAC -3' rev 5'- CCACCGTTCTCCTCCGTAGA -3' | 151 | NM_009864 |
| <i>MMP1</i> | human | fwd 5'- CCAAATGGGCTTGAAGCT -3' rev 5'- gtagcacattctgtccctaa -3' | 100 | NM_002421 |
| <i>MMP2</i> | human | fwd 5'- AGATGCCTGGAATGCCAT -3' rev 5'- GGTTCTCCAGCTTCAGGTAAT -3' | 107 | NM_004530 |
| <i>TIMP1</i> | human | fwd 5'- GGGGACACCAGAAGTCAACCAGA -3' rev 5'- CTTTTTCAGAGCCTTGGAGGAGCT -3' | 400 | NM_003254 |
| <i>TIMP3</i> | human | fwd 5'- CTACACCATCAAGCAGATGAAGAT -3' | 455 | NM_000362 |

| | | | | | |
|--------------|-------|-----|------------------------------------|-----|-----------|
| | | rev | 5'- TCCAGGGGTCTGTGGCATTGAT -3' | | |
| <i>TNC</i> | human | fwd | 5'- GCTCAACCATCACTGCCAAGT -3' | 81 | NM_002160 |
| | | rev | 5'- CAGTTTCCGACTGAACCTCAGTAG -3' | | |
| <i>KLF4</i> | human | fwd | 5'- CGACGCGCTGCTCCCATCTT -3' | 97 | NM_004235 |
| | | rev | 5'- CCGCCAGCGGTTATTCGGGG -3' | | |
| <i>CDH1</i> | human | fwd | 5'- TACGCCTGGGACTCCACCTA -3' | 101 | NM_004360 |
| | | rev | 5'- CCAGAAACGGAGGCCTGAT -3' | | |
| <i>HMGA2</i> | human | fwd | 5'- GACGTCCGGGCATTCATATAGG -3' | 105 | NM_003483 |
| | | rev | 5'- TTGGTGTTCTAAACAGAGGATTCACT -3' | | |
| <i>GAPDH</i> | human | fwd | 5'- GGAGTCAACGGATTTGGTCGTA -3' | 78 | NM_002046 |
| | | rev | 5'- GGCAACAATATCCACTTTACCA -3' | | |

Supplementary Table S2. Oligonucleotides used in quantitative real-time PCR reactions for chromatin immunoprecipitation assays or in restriction enzyme methylation assays.

| Position relative to TSS* | Primer Sequence | Product size (bp) |
|------------------------------------|--|-------------------|
| mouse <i>Cdh1</i> -108/+3 promoter | fwd 5'- GGCCCTGCAGTTCCTTGGCT -3' rev 5'- AGTGAGCAGCGCAGAGGCTG -3' | 111 |
| human <i>CDH1</i> -78/+26 promoter | fwd 5'- AGGTGAACCCTCAGCCAATCAGCG -3' rev 5'- AGGTGCTTTGCAGTTCCGACGC -3' | 105 |

*TSS = transcription start site

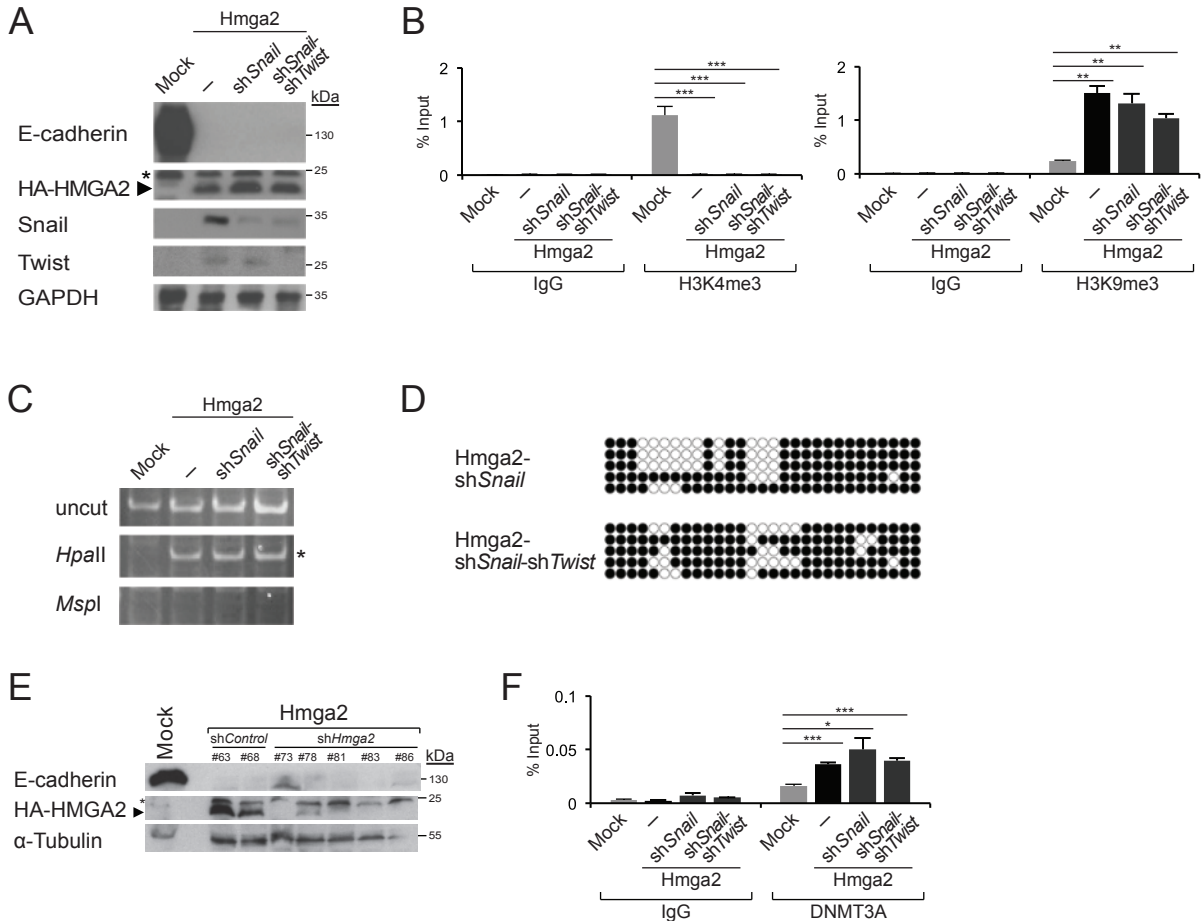


Figure S1: Epigenetic repression of the *Cdh1* promoter by HMGA2 is independent of Snail and Twist. (A) Immunoblots for E-cadherin, HA-tagged HMGA2, Snail and Twist in NM-Mock and NM-Hmga2 and its derivative stable clones (sh*Snail* and sh*Snail*-sh*Twist*). GAPDH serves as loading control. (B) ChIP-qPCR assays of active H3K4me3 and repressive H3K9me3 histone marks on the *Cdh1* promoter in cells described in panel A. (C) *HpaII*-*MspI* digestion-methylation assay of the *Cdh1* promoter in cells described in panel A. The PCR product was subjected to agarose gel electrophoresis and bands observed after *HpaII*-digestion indicate that the amplified DNA was methylated (asterisk). (D) DNA methylation status of the *Cdh1* promoter in NM-Hmga2 cells depleted of Snail or both Snail and Twist, was analysed by bisulphite sequencing of the *Cdh1* promoter region shown in Figure 1A, and 5 out of 10 clones of each cell line are shown here. White and black circles represent unmethylated and methylated CpG sites respectively. (E) Immunoblots for E-cadherin, HA-HMGA2 (arrowhead) and α -tubulin in NM-Hmga2 cells depleted of HMGA2. Asterisk indicates an unspecific band. (F) ChIP-qPCR analysis with IgG or DNMT3A antibody on the *Cdh1* promoter in cells described in panel A.

Supplementary Figure S2 Tan *et al.*

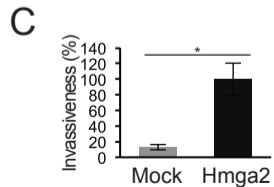
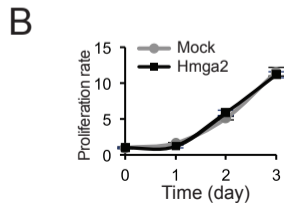
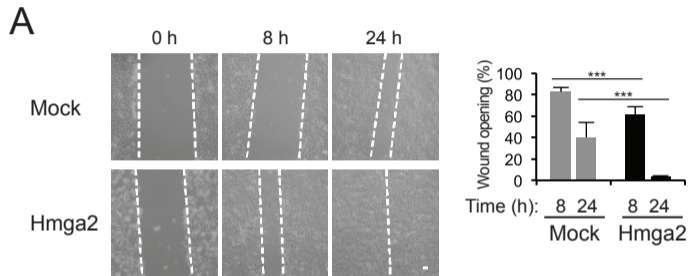


Figure S2: HMGA2 promotes migration and invasion. (A) Light microscopic images of NM-Mock and NM-Hmga2 cells in a wound healing assay at 0, 8 and 24 h after the wound. *Scale bar*, 50 μm . Bar graph shows wound opening at 24 h as a percentage of original wound opening at 0 h (right panel; mean \pm SD from 9 fields). (B) MTS assay was performed over 3 days to measure cell proliferation rate in NM-Mock and NM-Hmga2 cells (mean \pm SD from 5 replicates). The proliferation rate is measured in arbitrary units (relative, normalised values). (C) Invasion ability of NM-Mock and NM-Hmga2 cells were assayed using a Matrigel transwell assay. The bar graph shows invasion rate relative to NM-Hmga2 cells, which is set to 100% (mean \pm SD values from triplicates). The experiments were done at least twice.

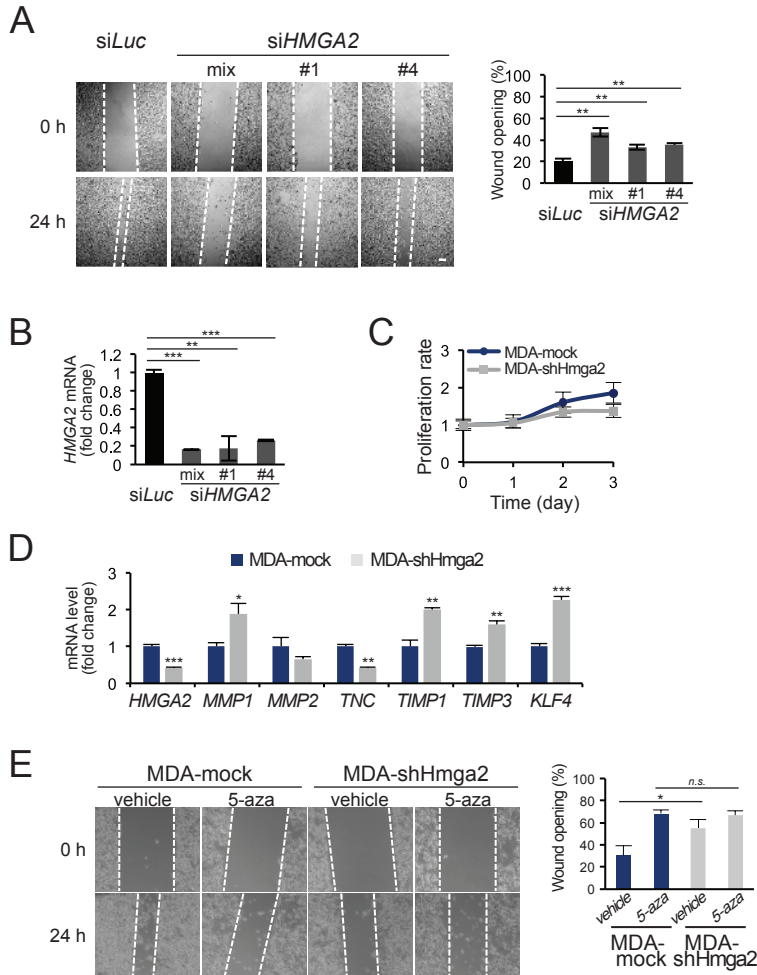


Figure S3. HMGA2 promotes cell migration in breast cancer cells. (A) Wound healing assay in MDA-MB-231 cells transiently transfected with control siRNA (*siLuc*) or two distinct siRNAs against HMGA2 (#1, #4) and their combination (mix). Bar graph represents the percentage of wound opening at 24 h as a percentage of original wound opening at 0 h (right panel; mean \pm SD from three independent measurements). (B) Knockdown efficiency of HMGA2 in cells used in the wound healing assay in panel A and analysed by qPCR (mean \pm SD from triplicate values). (C) MTS assay was performed over the course of 3 days, as described in Supplementary Figure S2B, to measure cell proliferation rate in MDA-mock and MDA-shHmga2 cells (mean \pm SD from 5 replicates). (D) Expression levels of genes, *HMGA2*, *MMP1*, *MMP2*, *TNC*, *TIMP1*, *TIMP3* and *KLF4*, normalised to *GAPDH* mRNA levels in MDA-mock and MDA-shHmga2 cells. Expression levels in MDA-mock cell are normalised to 1. (E) Wound healing assay of MDA-mock and MDA-shHmga2 cells treated with vehicle or 5-aza, as described in Figure 2C. Bar graph shows wound opening at 24 h as a percentage of original wound opening at 0 h (right panel; mean \pm SD from 9 fields).

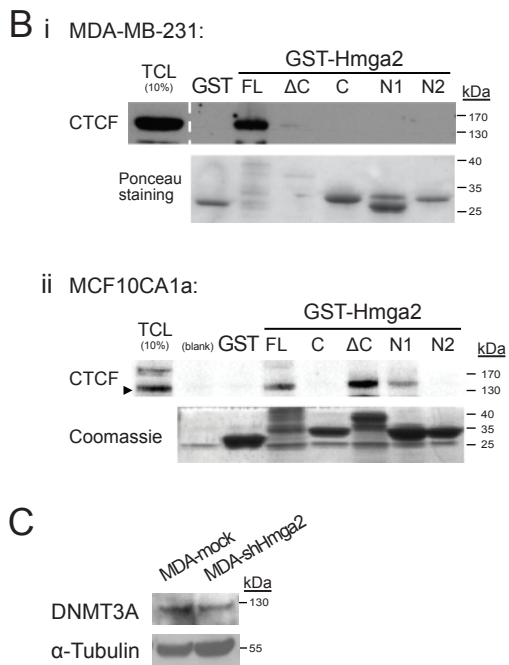
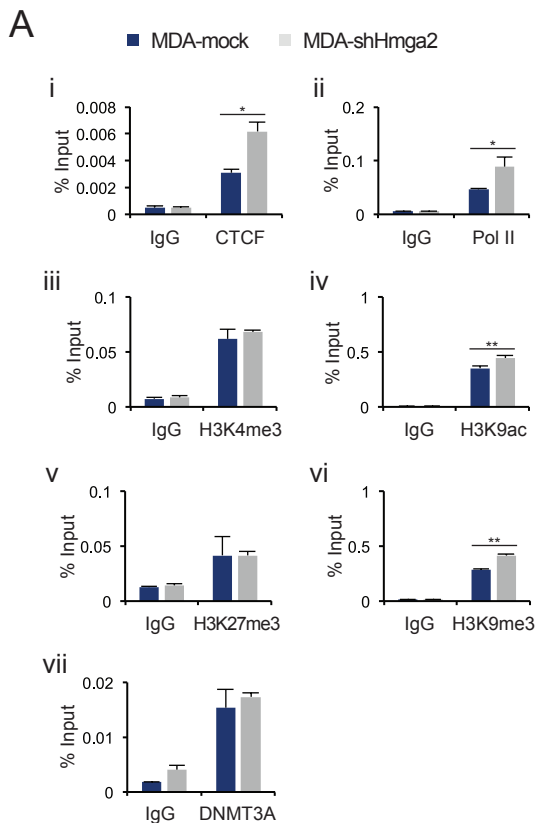


Figure S4. HMGA2 interacts with CTCF in breast cancer cells. (A) ChIP-qPCR analyses of CTCF (*i*); Pol II (*ii*); H3K4me3 (*iii*); H3K9ac (*iv*); H3K27me3 (*v*); H3K9me3 (*vi*); and DNMT3A (*vii*) binding on the *CDH1* promoter in MDA-mock and MDA-shHmga2 cells. (B) GST-HMGA2 and its deletion mutants were used in a pull-down assay with parental MDA-MB-231 (*i*) or MCF10CA1a (*ii*) cell extracts and immunoblotted for CTCF. Input of GST fusion proteins used in the pull-down were visualised by Ponceau-S or coomassie brilliant blue staining. *FL*, full-length; ΔC , deletion of C-terminal; *N1*, N-terminal 1; *N2*, N-terminal 2; *C*, C-terminal; *TCL*, total cell lysate. (C) DNMT3A and GAPDH protein levels in MDA-control and -shHmga2 cells.