Supplementary Materials

Supplemental Figure legends

Figure S1. Western blotting analysis of classical markers for EMT in MCF7 cells treated with TGF-β1 or control vehicle for 4 days.

Figure S2. ChIP-qPCR analysis of Smad2/3 at the promoter regions of AC026904.1 and UCA1 in MDA-MB-231 cells and MCF7 cells treated or untreated with TGF-β1 for 72 hours. Immunoglobulin G (IgG) was used as a control. ChIP DNA was analyzed by qPCR (mean ± SEM, n = 3).

Figure S3. Western blot analysis of phosphorylated ERK1/2 in MCF7 cells pretreated with 10 μM SB431542 or 1 μM U0126 for 60 minutes, followed by TGF-β1 for 24 hours. Total ERK1/2 was used as loading control.

Figure S4. Western blot analysis of Slug, Vimentin and E-cadherin protein levels in various breast cancer cell lines. Blots were probed with an antibody against GAPDH to ensure equal loading.

Figure S5. Left panel: relative RNA levels of AC026904.1 (mean ± SEM, n = 3) in MDA-MB-231 cells untreated or treated with si-Control, or si-AC026904.1 #1, or si-AC026904.1 #2. Right panel: relative RNA levels of UCA1 (mean ± SEM, n = 3) in MDA-MB-231 cells untreated or treated with si-Control, or si-UCA1 #1, or si-UCA1 #2.

Figure S6. Phase-contrast images of stable AC026904.1 or UCA1 knockdown MCF7 cells treated with TGF-β1 (10 ng/mL) for 4 days. Scale bar: 50 μm.

Figure S7. Relative RNA levels of UCA1 (mean ± SEM, n = 3) in stable UCA1 knockdown MDA-MB-231 cells treated with miR-1 inhibitor or miR-203a inhibitor, or both.

Figure S8. Cell proliferation curves of MDA-MB-231 cells with stable knockdown of AC026904.1 or UCA1. The experiment was carried out in triplicate wells and repeated at least twice.

Figure S9. Transwell migration assay of BT-549 cells with stable knockdown of AC026904.1 or UCA1, or with concurrent overexpression of Slug.

Figure S10. Transwell migration assay of MDA-MB-231 cells treated with si-Control, or si-AC026904.1, or both.

Figure S11. Transwell migration assay of MCF7 cells overexpressing UCA1 or its antisense control (AS) or empty vector (vec).

Figure S12. Primary tumor volume (mean ± SEM) in nude mice injected with MDA-MB-231-luc-D3H2LN cells with stable knockdown of either AC026904.1 or UCA1 orthotopically. Tumor volumes were calculated by using the following formula: V (mm^3) = $a \times b^2/2$, where a is the largest diameter and b is the perpendicular diameter.

Figure S13. Upper panel: Relative RNA levels of miR-1 and miR-203a in metastatic and non-metastatic breast cancer (n = 30 per group). Each data point represents an individual breast cancer sample. Lower panel: Correlation between UCA1 RNA levels and miR-1 or miR-203a levels. Each data point represents an individual breast cancer sample, and a coefficient of determination (R^2) is shown.

Figure S14. Left panel: Phase-contrast images of MCF10A cells treated with TGF-β1 (10 ng/mL) or control vehicle for 4 days. Scale bar: 50 μm. Right panel: Relative RNA levels of AC026904.1 and UCA1 (mean ± SEM, n = 3) in MCF10A cells treated with TGF-β1 (10 ng/mL) or control vehicle for 4 days.

Figure S15. Western blotting analysis of Snail, ZEB2, Twist1 and FOXC2 protein levels in MDA-MB-231 cells treated with si-Control or si-AC026904.1 or si-UCA1 for 48 hours. Blots were probed with an antibody against GAPDH to ensure equal loading.

Figure S16. UCSC genome browser tracks (http://genome.ucsc.edu/) of the *AC026904.1* locus with data for H3K27ac modifications in NHLF, HSMM, NHEK, and H1-hESC cell lines.

Figure S1

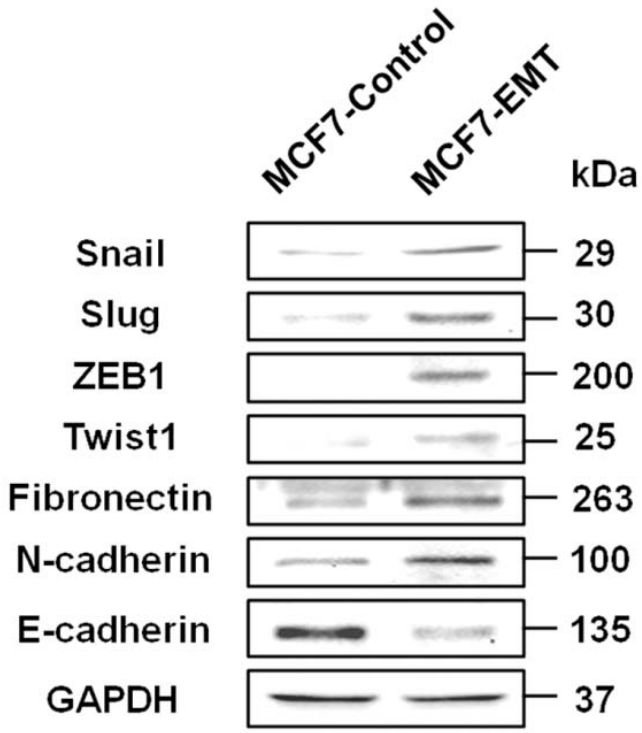

Figure S2

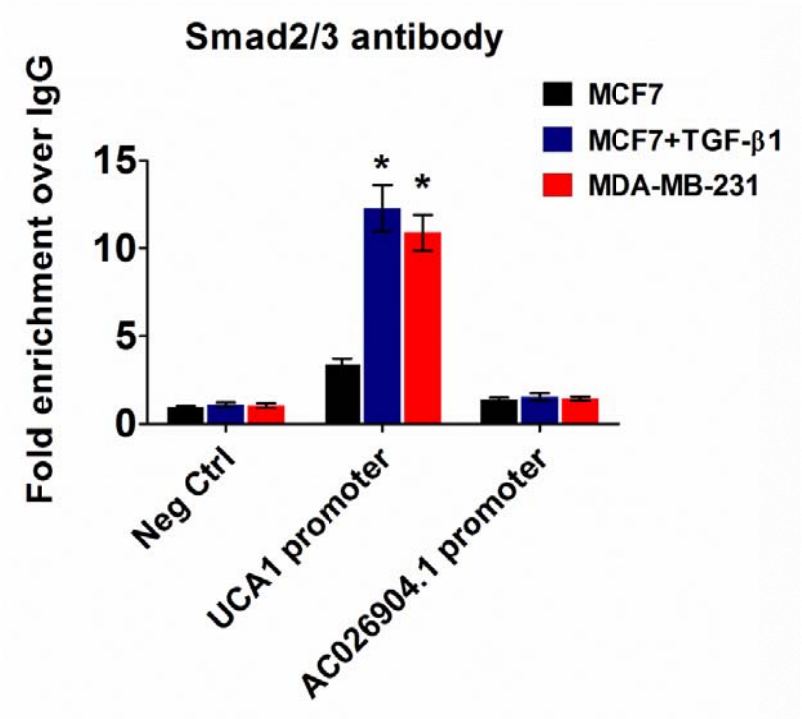

Figure S3

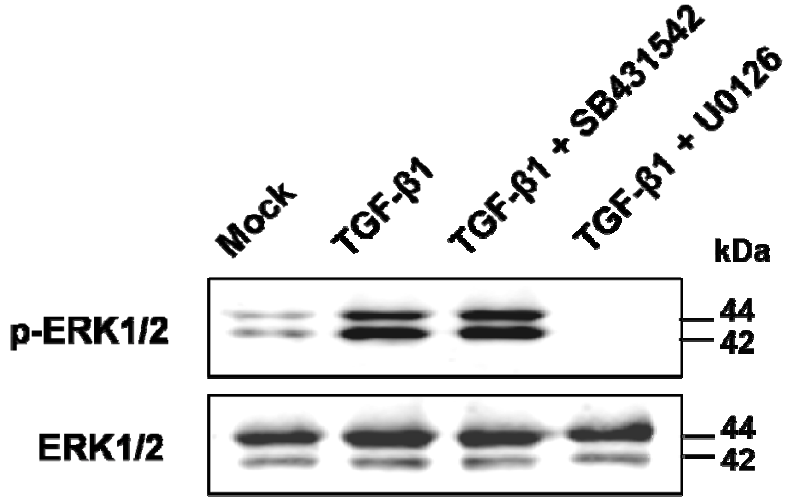

Figure S4

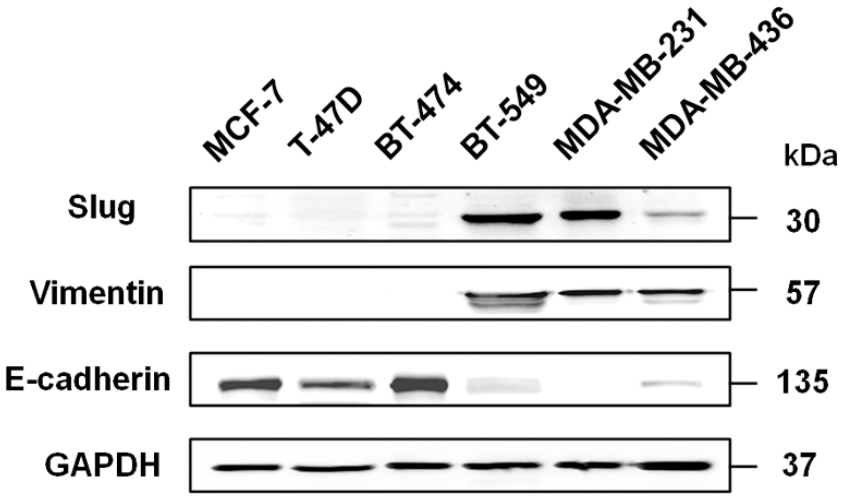

Figure S5

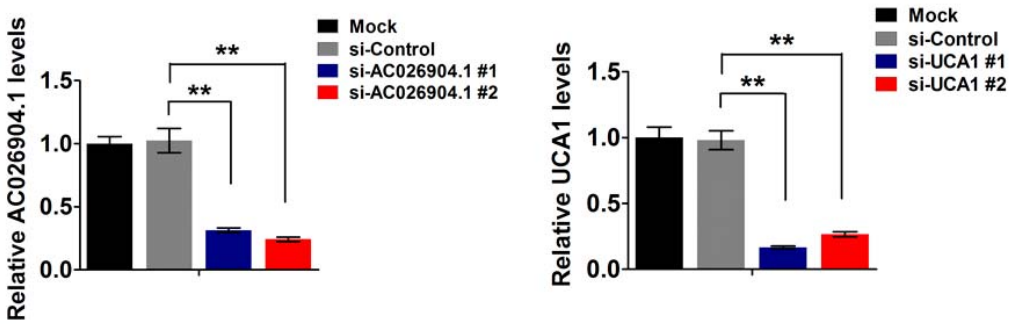

Figure S6

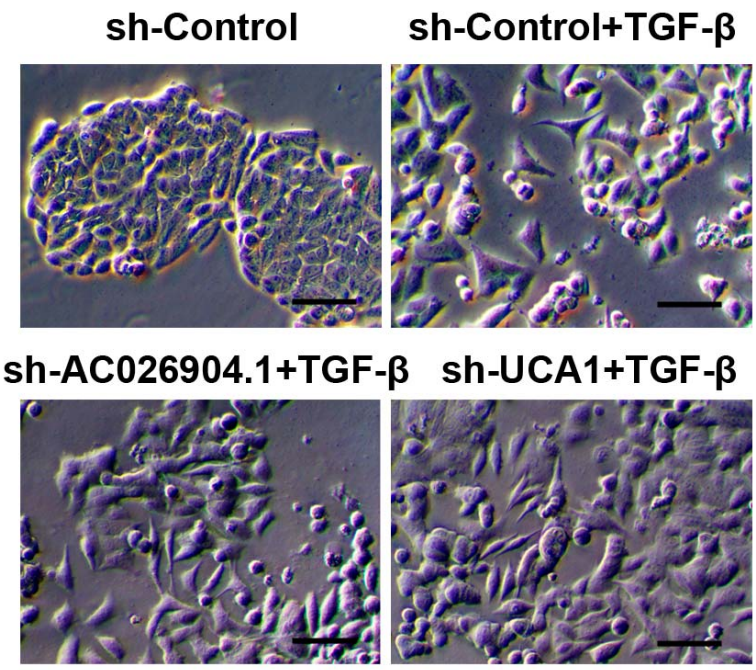

Figure S7

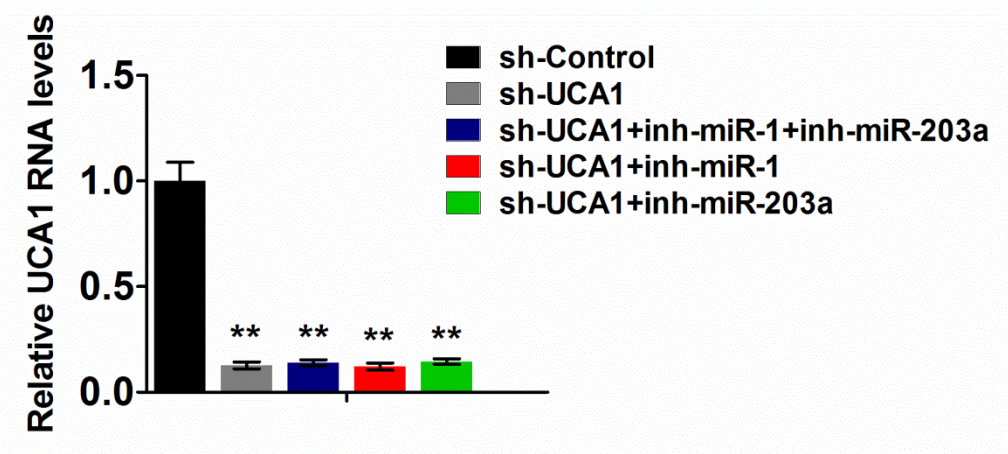

Figure S8

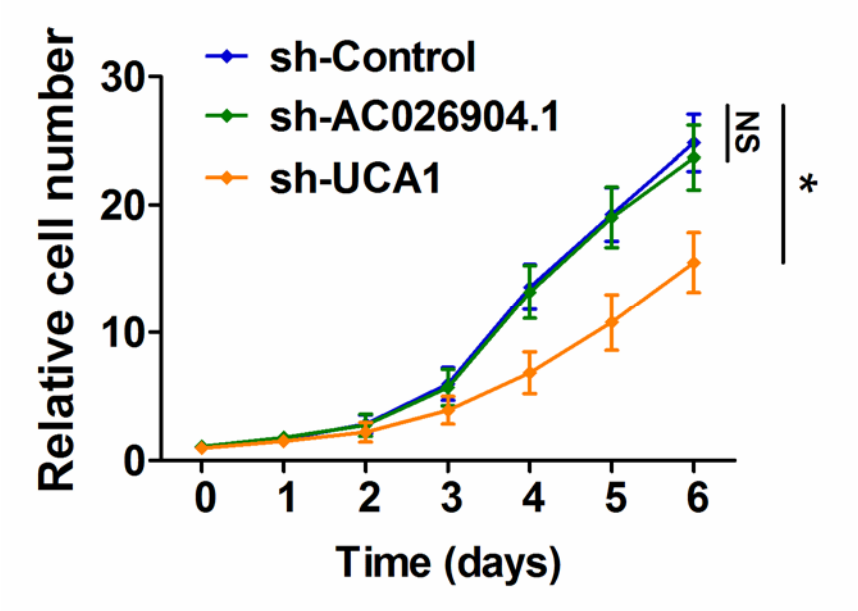

Figure S9

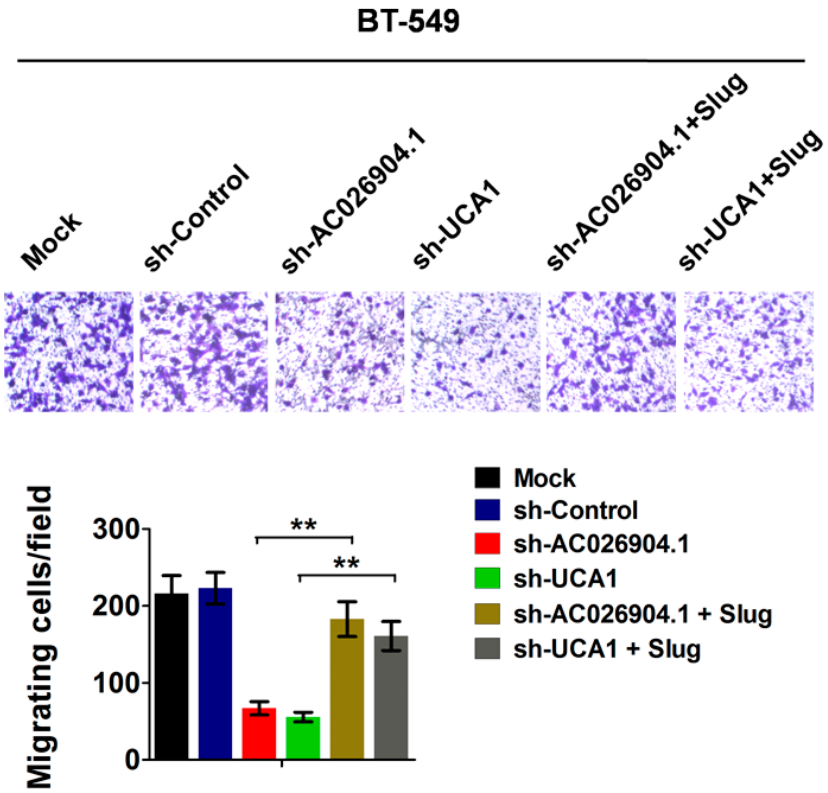

Figure S10

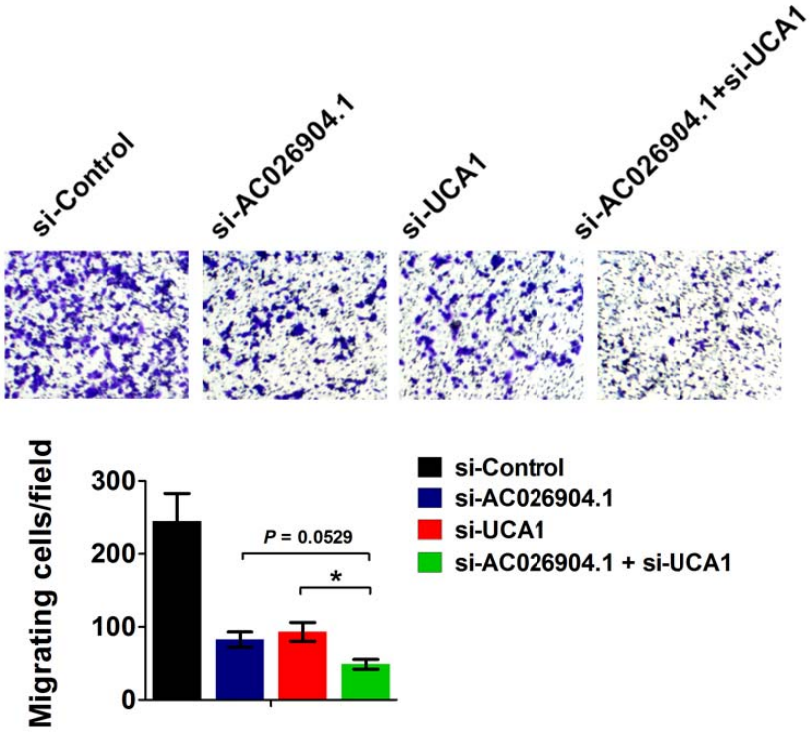

Figure S11

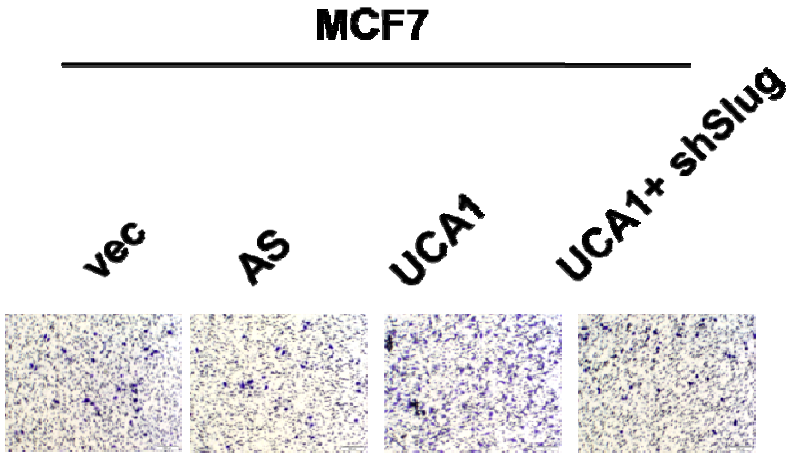

Figure S12

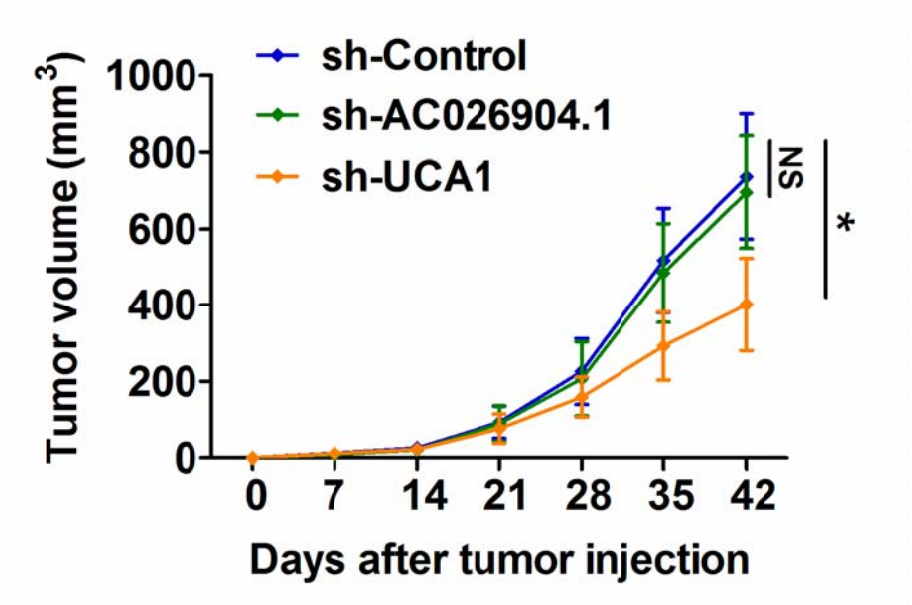

Figure S13

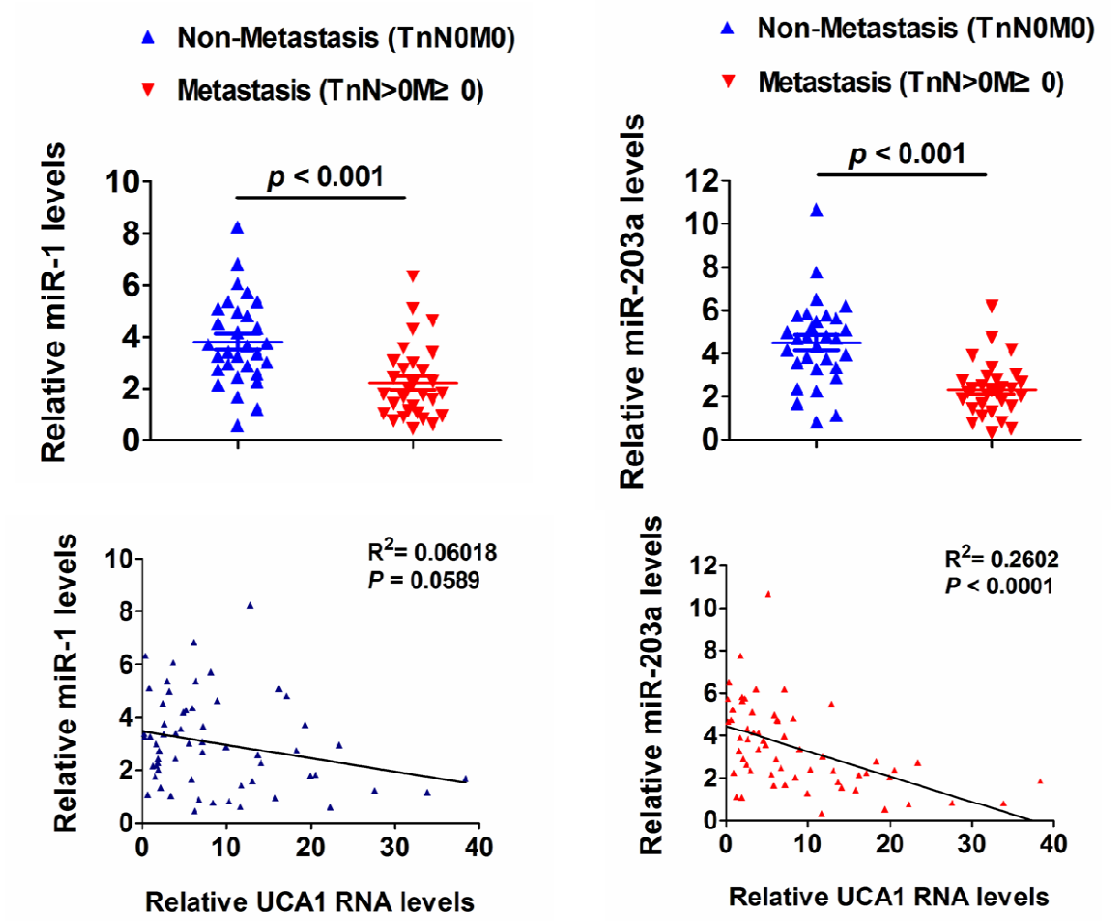

Figure S14

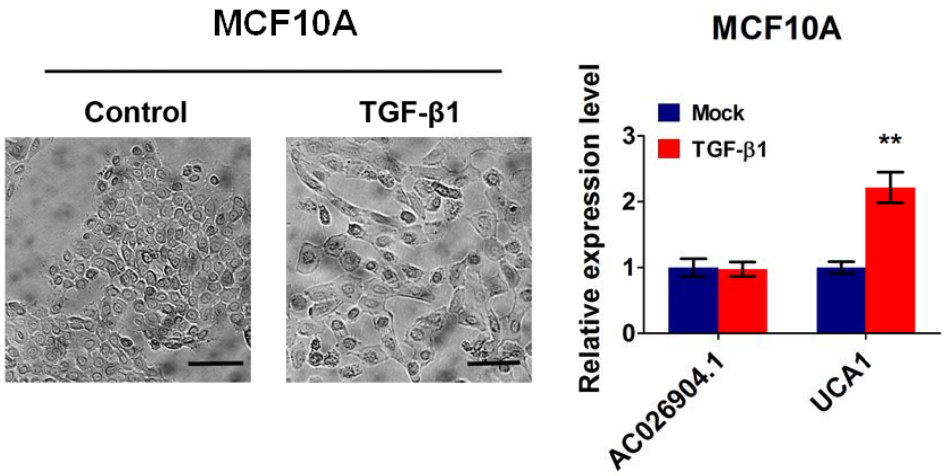

Figure S15

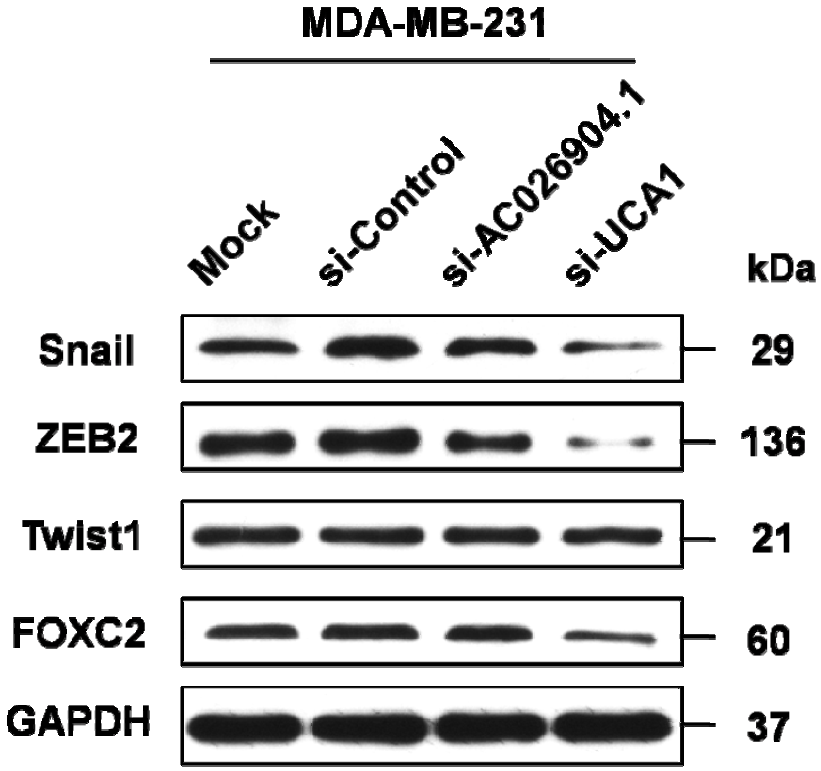

Figure S16

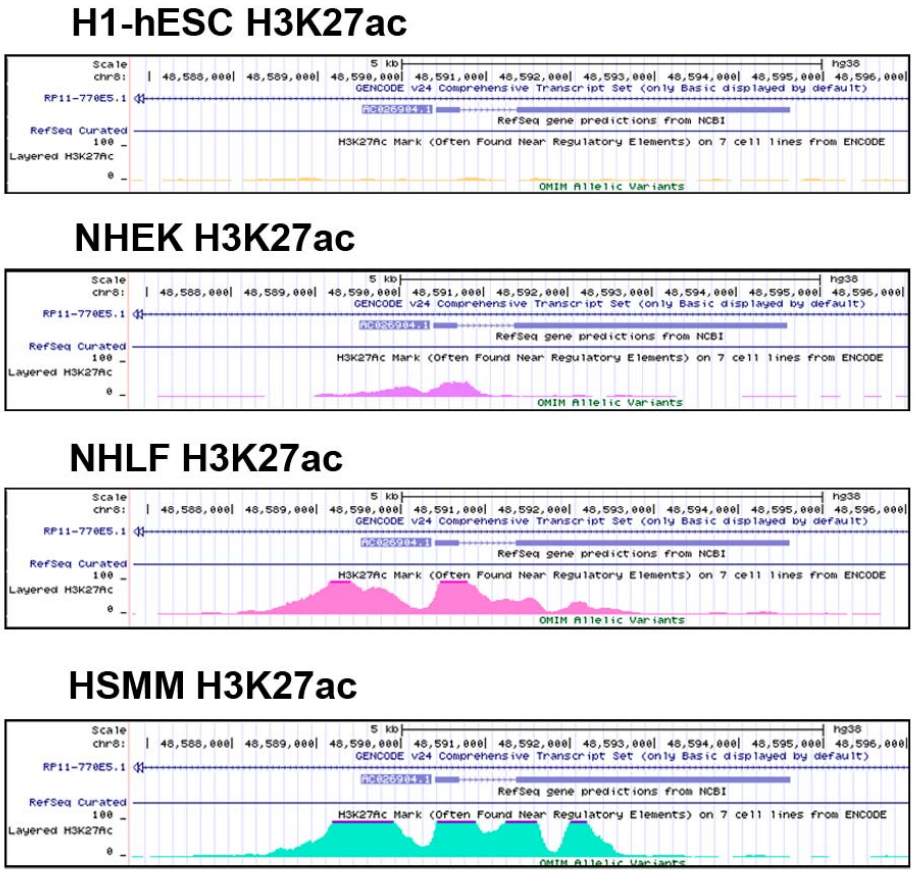

Table S1. List of antibodies used in this study

Antigen	Applications	Source
Slug	WB; IF	Cell Signaling (cat 9585)
Snail	WB	Cell Signaling (cat 3879)
E-cadherin	WB; IF; IHC	Cell Signaling (cat 3195)
N-cadherin	WB	Abcam (cat ab98952)
ZEB1	WB; IF	Abcam (cat ab203829)
ZEB2	WB	Abcam (cat ab138222)
Twist1	WB	Abcam (cat ab175430)
FOXC2	WB	Abcam (cat ab24340)

Vimentin	WB	Cell Signaling (cat 5741)
p-ERK1/2	WB	Cell Signaling (cat 4370)
ERK1/2	WB	Cell Signaling (cat 4695)
Smad2/3	ChIP; WB	Cell Signaling (cat 5678)
H3K27ac	ChIP	Cell Signaling (cat 8173)
H3K4me3	ChIP	Cell Signaling (cat 9727)
H3K27me3	ChIP	Cell Signaling (cat 9733)
MED1	ChIP	EMD Millipore (cat 17-10530)
MED12	ChIP	Abcam (cat ab70842)
GFP	IP	Abcam (cat ab290)
GAPDH	WB	Abcam (cat ab128915)

Table S2. List of primers used in this study

Gene	Application	Forward	Reverse
AC026904.1	qPCR	CAAGGCATTTTTGCACTCAGTAA	AACACGGCTCAGCTATGGAAA
	ChIP-qPCR (E1)	CCCTGAGATTGGGTTGCTCC	TGCATTAGGCACGCAGTCAT
	ChIP-qPCR (E2)	GCACCACACGGGGTTTCTAT	AGACCCCCTGGGATGAATGT
	ChIP-qPCR (C1)	AGGTCCCCAGAGTTTCCTACT	GAGCATGCAGCTGAGCAGTC
UCA1	qPCR	CTCTCCATTGGGTTCACCATTC	GCGGCAGGTCTTAAGAGATGAG
	ChIP-qPCR	TGACGGAGGGAGATACCAGG	TCTGAGATGCCCACAAGCTG
Slug	qPCR	CATGCCTGTCATACCACAAC	GGTGTCAGATGGAGGAGGG
	ChIRP	CAGAGTCCCAGGAGAGCGTC	GCCAGCCTCTGGTGTTAATG
Snail	qPCR	ACCACTATGCCGCGCTCTT	GGTCGTAGGGCTGCTGGAA

ZEB1	qPCR	ACTCTGATTCTACACCGC	TGTCACATTGATAGGGCTT
E-cadherin	qPCR	GCCCCATCAGGCCTCCGTTT	ACCTTGCCTTCTTTGTCTTTGTT GGA
miR-1	qPCR	TGGAATGTAAAGAAGTATGTAT	Universal Primer (QIAGEN)
miR-203a	qPCR	GTGAAATGTTTAGGACCACTAG	Universal Primer (QIAGEN)
miR-21	qPCR	TAGCTTATCAGACTGATGTTGA	Universal Primer (QIAGEN)
18S	qPCR	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
GAPDH	qPCR	TCGGAGTCAACGGATTTGGT	TCGCCCCACTTGATTTTGGA

Table S3. List of siRNAs used in this study

Gene	Sense (5'-3')	Antisense (5'-3')
AC026904.1 #1	GGAGAAAUGAGGAAGUAAATT	UUUACUUCCUCAUUUCUCCTT
AC026904.1 #2	CGAAAGAAGCACAGGGUGUTT	ACACCCUGUGCUUCUUUCGTT
UCA1 #1	GCAGGCUUCAUCCGUUCCUTT	AGGAACGGAUGAAGCCUGCTT
UCA1 #2	CUGGCACCUUGUUAGCUACTT	GUAGCUAACAAGGUGCCAGTT
Smad3	AAUGGUGCGAGAAGGCGGUCATT	UGACCGCCUUCUCGCACCAUUTT
ERK2	GGAAAAGCUCAAAGAACUATT	UAGUUCUUUGAGCUUUUCCTT
Negative Control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT