SUPPLEMENTARY FIGURE LEGENDS

Supp. Figure 1: Nodal promotes invasion and migration in MCF7 breast cancer cells. (A) MCF7 cells were seeded in Transwell chambers and treated with 0, 50 or 100 ng/mL of rhNodal for 24 hours to assess cellular migration. Cells exhibited a significant dose-dependent up-regulation of cellular migration in response to rhNodal ($n=4$, $p<0.05$). (B) MCF7 cells were seeded in Matrigel-coated Transwell chambers and treated with 0, 50 or 100 ng/mL of rhNodal for 24 hours to assess cellular invasion. Cells exhibited a significant up-regulation of cellular invasion at 100 ng/mL rhNodal ($n=3$, $p=0.004$).

Supp. Figure 2: Nodal promotes EMT-like phenotypes in breast cancer cell lines. (A) Real time RT-PCR analysis of EMT markers in MCF7 cells treated with 0, 50 or 100 ng/mL of rhNodal for 48 hours. 100 ng/mL Nodal caused a decrease in *ESR1* expression ($n=5$, $p=0.008$), and an increase in *TWIST1* ($n=5$, $p=0.036$) and *VIM* expression ($n=5$, $p=0.008$) compared to untreated cells. *CDH2* (*N-Cadherin*) and *CDH1* (*E-Cadherin*) expression did not change ($n=5$, $p>0.05$). (B) Real time RT-PCR analysis of EMT markers in T47D cells treated with 0, 50 or 100 ng/mL of rhNodal for 48 hours. 100 ng/mL Nodal caused a decrease in *ESR1* expression ($n=4$, $p=0.029$), and an increase in *TWIST1* ($n=4$, $p=0.029$) and *VIM* expression ($n=4$, $p=0.02$) compared to untreated cells. *CDH2* and *CDH1* expression did not change ($n=4$, $p>0.05$). (C) Immunofluorescence (IF) showing localization of E-Cadherin (green) in MCF7 cells or (D) T47D cells treated with 0, 50 or 100 ng/mL of rhNodal for 48 hours. Nuclei are stained with DAPI (blue) and bars equal 20 μm. All IF was performed ≥3 times. Data in A and B are presented as mean ± S.E.M. for replicate values. Asterisks (*) indicate a significant difference compared to control cells as specified. Expression levels are normalized to *HPRT1*.

Supp. Figure 3: Densitometric analysis of pERK1/2 in cells expressing Nodal ± the ALK4/5/7 inhibitor SB431542. Densitometric analysis for all replicate experiments corresponding to Figure 3E (A) and 3F (B). ImageJ was used to calculate band density of P-ERK1/2 relative to total ERK1/2. Data are presented as mean fold change ± S.E.M. Different letters indicate a significant difference compared to controls ($n=4$, $p<0.05$).

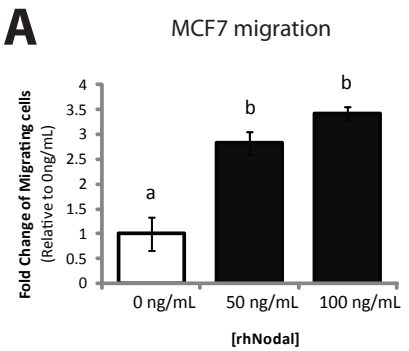
Supp. Figure 4: Cripto, ALK4 and ALK7 levels in breast cancer and choriocarcinoma cells. (A) RT-PCR and real time RT-PCR (B) analyses of *Cripto*, *ALK4* and *ALK7* mRNA expression levels in T47D+EV, T47D+Nodal, BwWo+EV, BeWo+Nodal, MDA-MB-31+shControl and MDA-MB-231+shNodal cells. All cell lines transcribe these members of the Nodal receptor complex and alterations in Nodal expression variably affect receptor expression in a cell type, and transcript dependent manner. *HPRT1* is used as a loading control in (A). Expression levels in B are normalized to *RPLPO*.

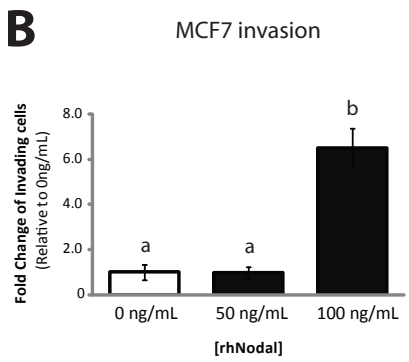
Supp. Figure 5: Nodal-induced invasion and migration in MCF-7 cells is dependent on active ERK signaling. (A) MCF-7 cellular invasion through a Matrigel-coated Transwell chamber increased in response to 100 ng/mL rhNodal ($n=3$, $p=0.005$), and this effect was mitigated by the addition of 10 μM U0126. (B) MCF-7 cellular migration through a Transwell chamber (without Matrigel) increased in response to 100 ng/mL rhNodal ($n=3$, $p=0.005$), and this effect was mitigated by the addition of 10 μM U0126. (C) MCF-7 viability assay demonstrating equal cell viability after 24 hours between treatments corresponding to (A) and (B). Data are presented as mean ± S.E.M. for replicate values. Different letters indicate a significant difference as specified.

Supp. Figure 6: Nodal-induced EMT in MCF-7 cells is dependent on active ERK signaling. (A) Real time RT-PCR analysis of EMT markers in MCF-7 cells exposed to 100 ng/mL rhNodal alone, or 100 ng/mL rhNodal + 10 μM U0126 (48 hours). In response to 100 ng/mL of Nodal, MCF-7 cells displayed a decrease in *ESR1* expression ($n=5$, $p=0.008$), and an increase in *TWIST1* ($n=5$, $p=0.036$) and *VIM* expression ($n=5$, $p=0.008$) compared to controls. *CDH2* and *CDH1* expression did not change ($n=5$, $p>0.05$). Treatment of MCF-7 cells with U0126 prior to treatment with rhNodal rescued *TWIST1* and *VIM* expression back to control levels, but did not rescue *ESR1* expression ($n=5$, $p<0.001$). All PCR data are presented as mean ± S.E.M. for replicate values. Different letters indicate a significant difference compared to control treatments/genes as specified. (B) Immunofluorescence (IF) showing localization of E-Cadherin (green) in MCF-7 cells treated with 100 ng/mL rhNodal, or 100 ng/mL rhNodal + 10 μM U0126 (compared to vehicle controls). Nuclei are stained with DAPI (blue) and bars equal 20 μm. All IF was performed 3-4 times.

Supp. Figure 7: Inhibition of the Nodal type 1 receptor in aggressive Hs578t breast cancer cells causes reduced invasion and migration *in vitro*. (A) Hs578t cellular invasion through a Matrigel-coated Transwell chamber was reduced in response to 1-10 μM SB431542 ($n=5$, $p<0.05$) compared to vehicle controls. (B) Hs578t cellular migration through a Transwell chamber (without Matrigel) was reduced in response to 1-10 μM SB431542 ($n=5$, $p<0.001$) compared to vehicle controls. Data are presented as mean ± S.E.M. for replicate values. Different letters indicate a significant difference as specified.

Quail, Supplementary Figure 1.





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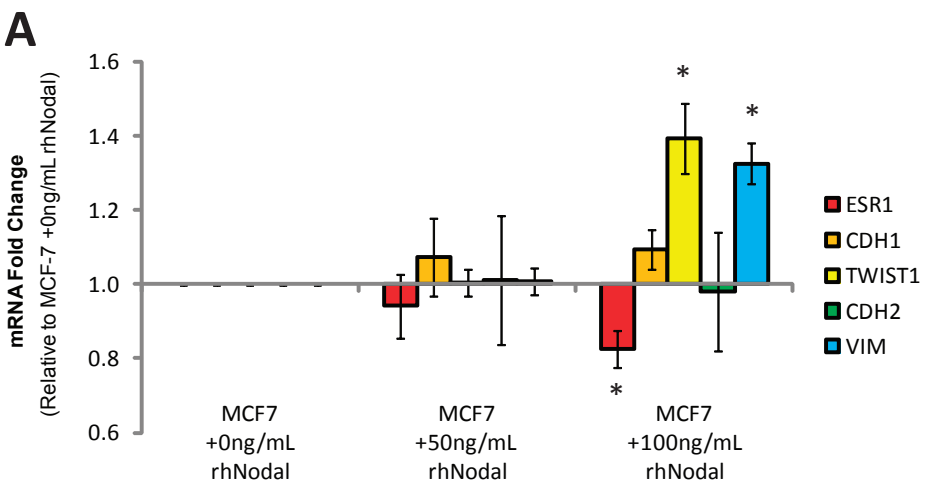

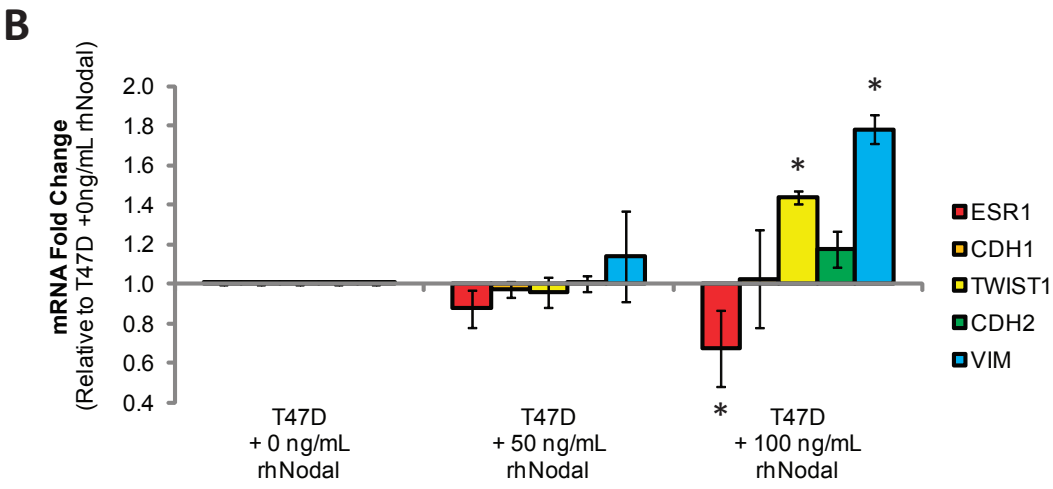

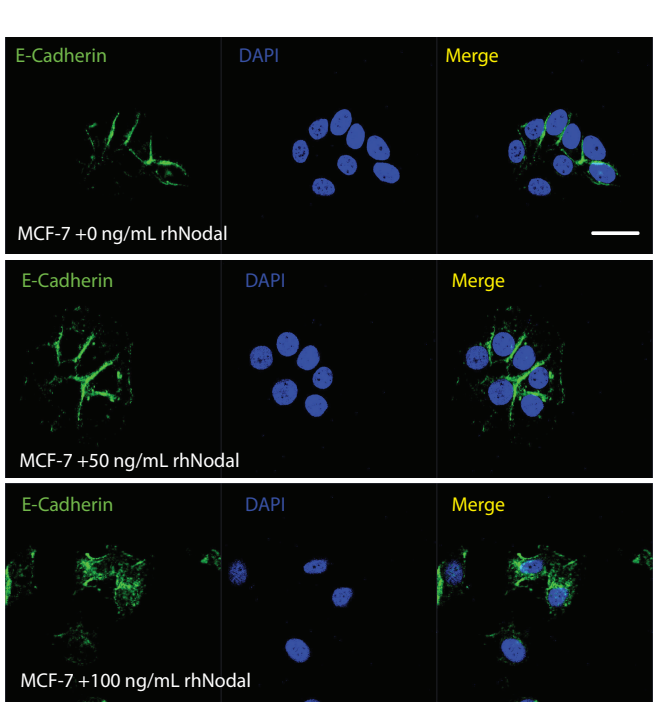

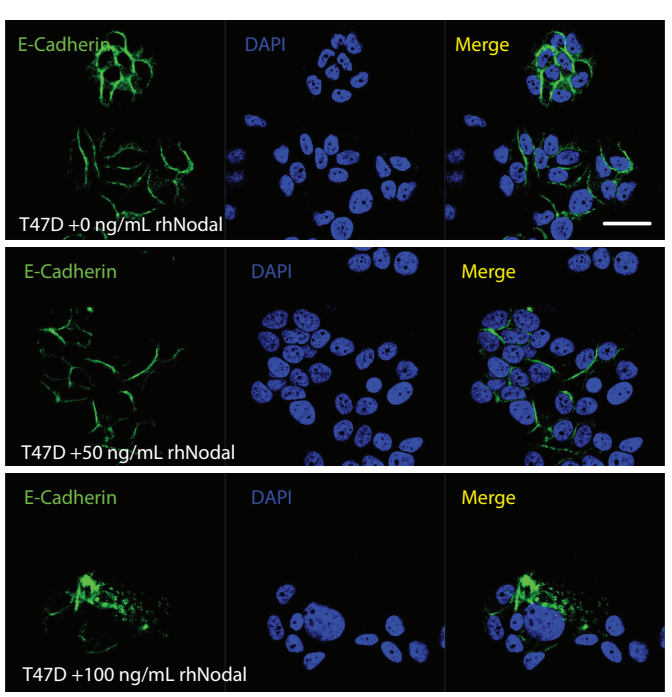

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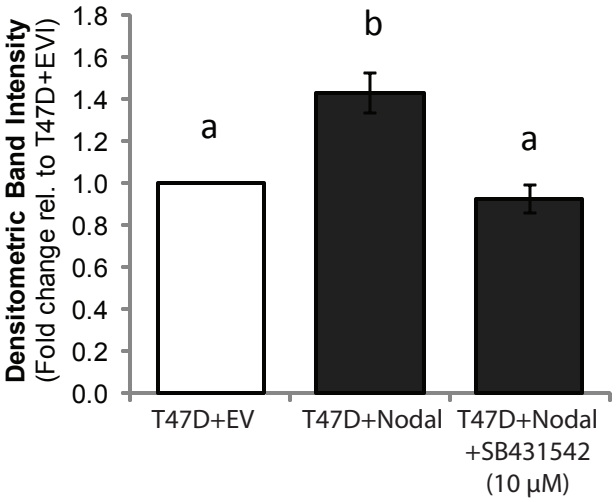

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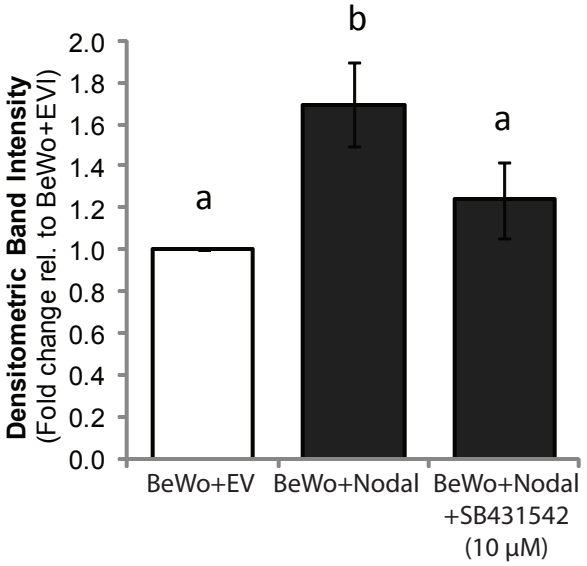

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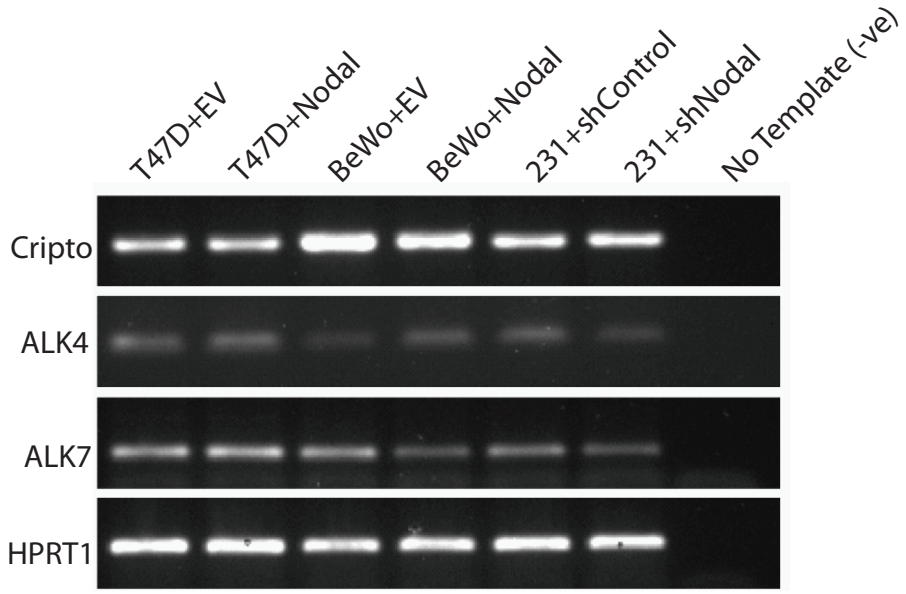

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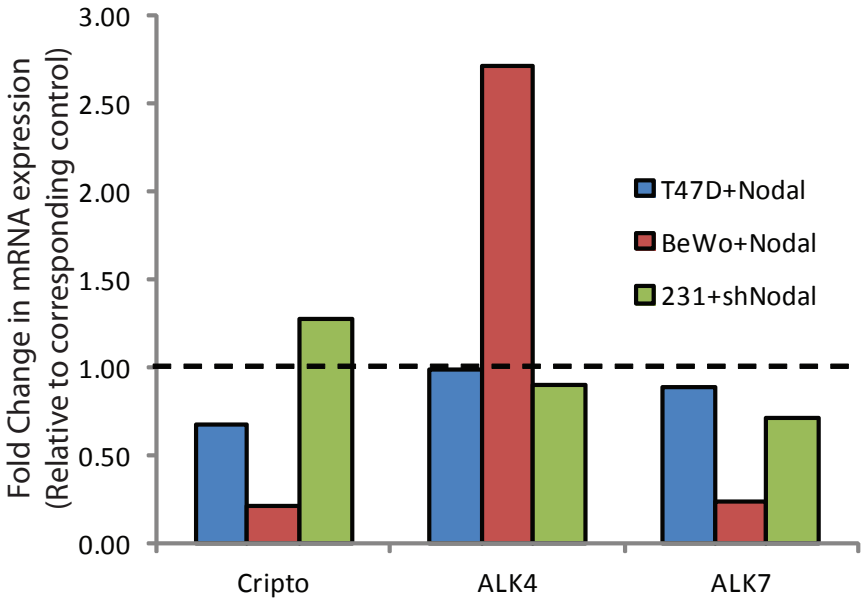

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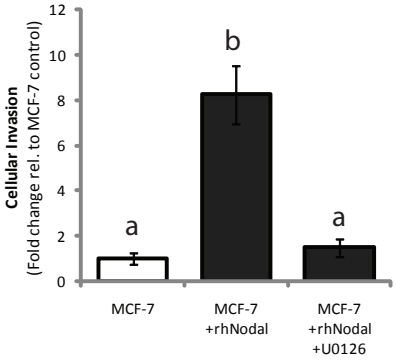

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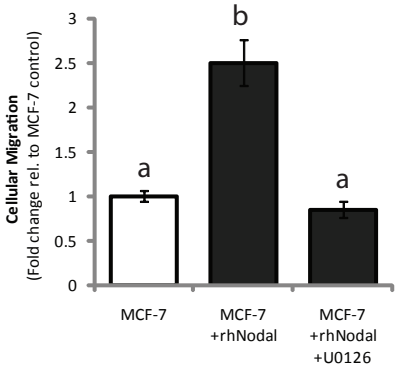

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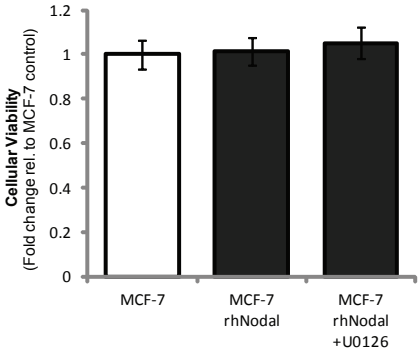

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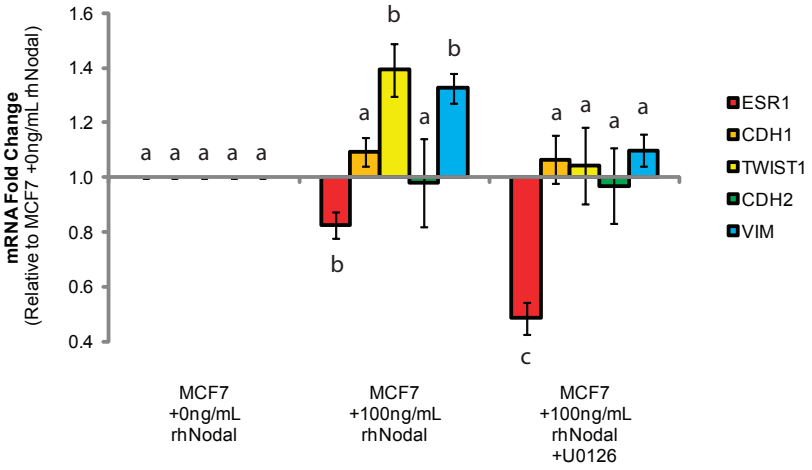

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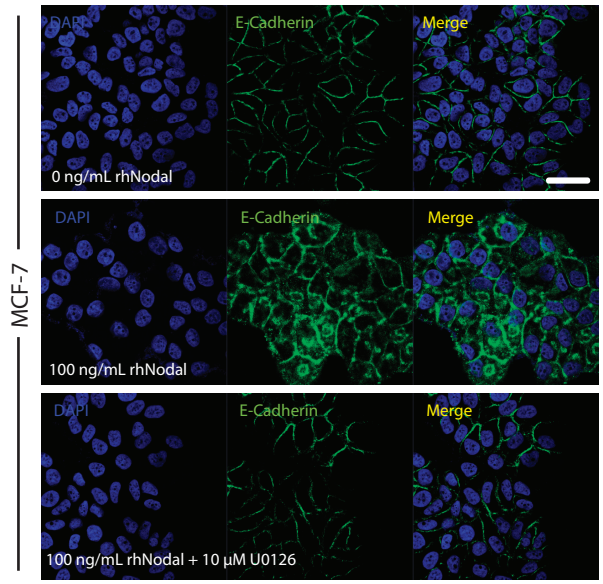

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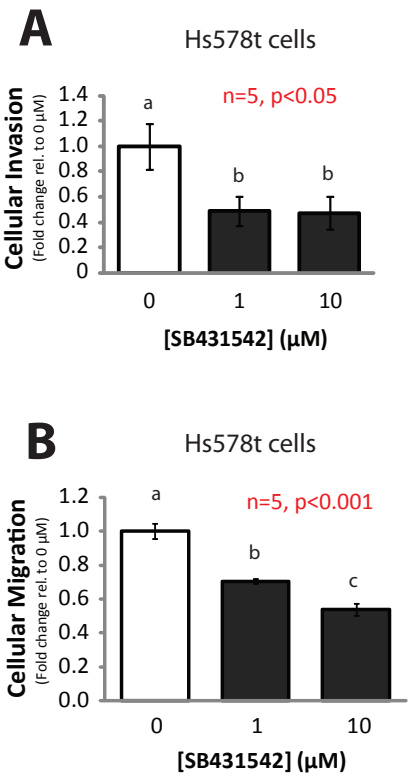

Primary Antibody	Clone	Company	Dilution & Use
Monoclonal mouse anti-Nodal	WS65	Santa Cruz	1:500 WB
Monoclonal mouse anti-hNodal	5C3	AbCam	1:500 IHC
Monoclonal rabbit anti-phospho-SMAD2	A5S	Millipore	1:1000 WB
Polyclonal rabbit anti-SMAD2/3		Millipore	1:1000 WB
Monoclonal mouse anti-β-Actin	C4	Santa Cruz	1:5000 WB
Monoclonal rabbit anti-E-Cadherin	24E10	Cell Signalling	1:1000 WB 1:100 IF
Polyclonal rabbit anti-p44/42 (ERK1/2)		Cell Signalling	1:1000 WB
Monoclonal mouse anti-p44/42 (ERK1/2)	E10	Cell Signalling	1:1000 WB
Polyclonal rabbit anti-N-Cadherin		Cell Signalling	1:1000 WB
Monoclonal rabbit anti-Vimentin	D21H3	Cell Signalling	1:1000 WB
Monoclonal mouse anti-Twist	Twist2C1a	AbCam	1:1000 WB

Supplementary Table 1: Primary antibodies used for Western blot (WB), Immunofluorescence (IF) and Immunohistochemistry (IHC).

Gene	ID
CDH1 (E-Cadherin)	Hs00170423_A1
ESR1 (Estrogen Receptor)	Hs00174860_A1
TWIST1	Hs01675818_S1
CDH2 (N-Cadherin)	Hs00983056_m1
VIM (Vimentin)	Hs00185584_A1
ALK-4 (ACVR1B)	Hs00244715_m1
ALK-7 (ACVR1C)	Hs00377065_m1
Cripto-1	Hs02339499_g1
RPLPO	4333761-0707014
HPRT1	4333768-0904021

Supplementary Table 2: Primer/Probes used for real-time PCR (Applied Biosystems/Invitrogen).