Supplementary Data and Methods

Downregulation of epithelial cadherin is required for the metastatic outgrowth of breast cancer

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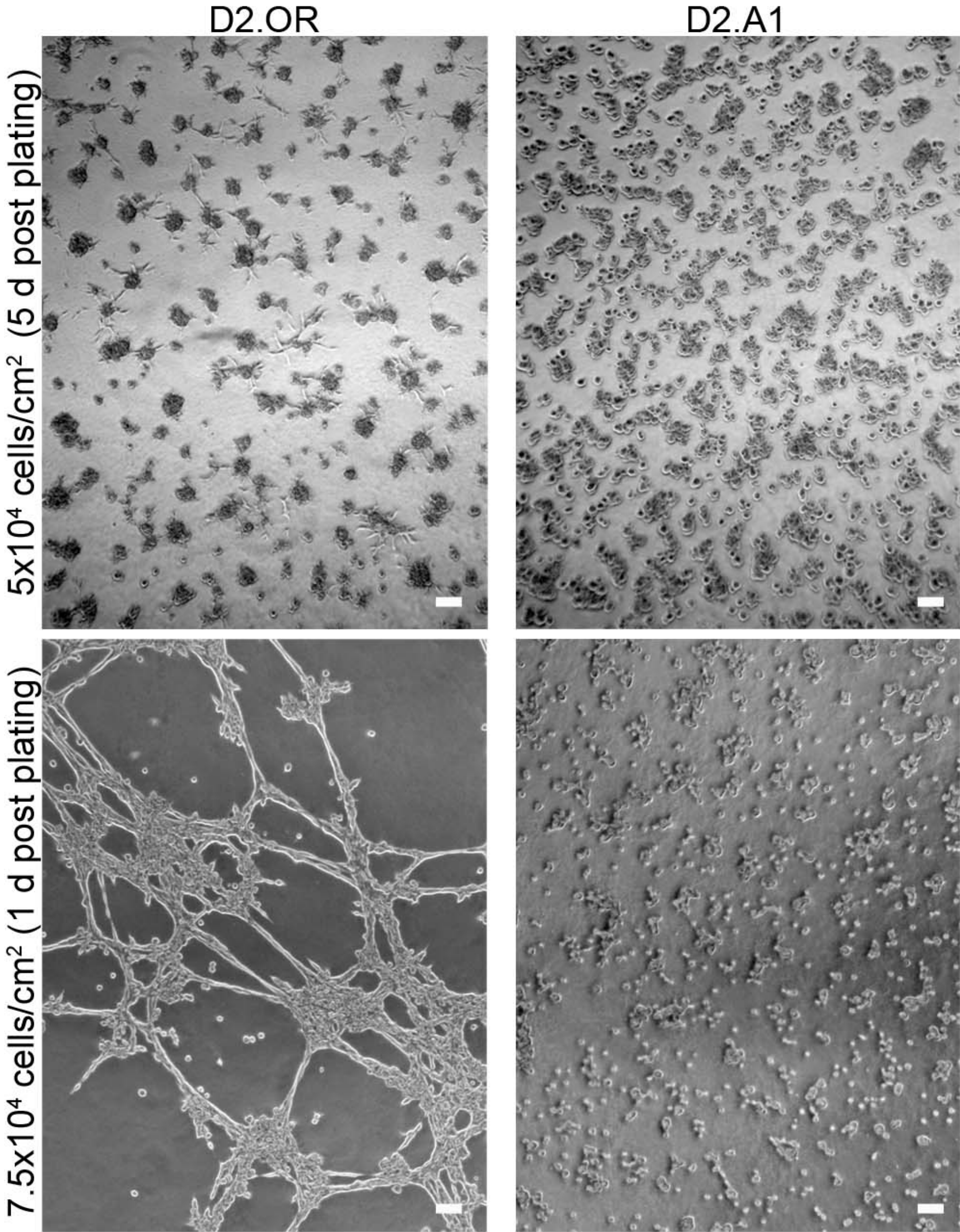
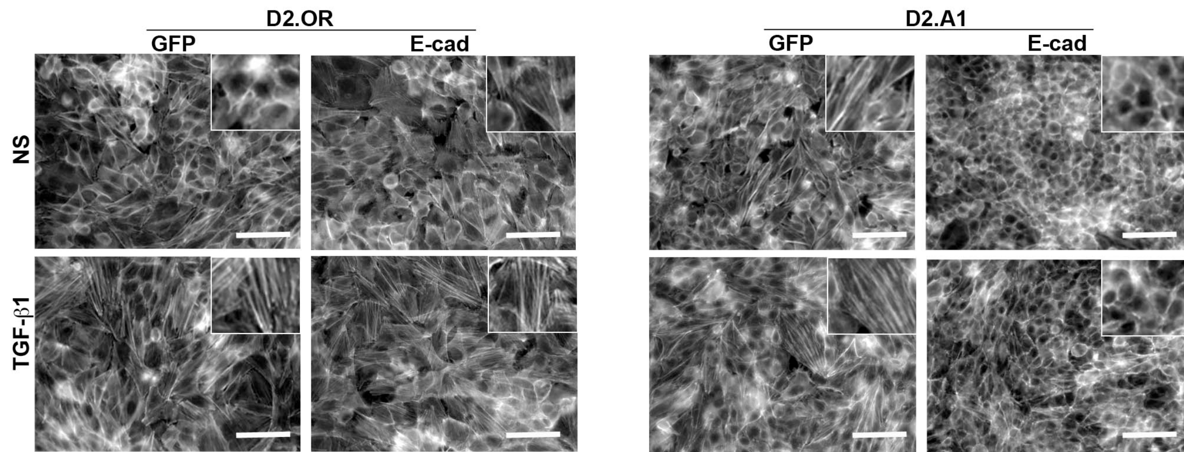
SUPPLEMENTARY FIGURE S1: Wendt *et al*



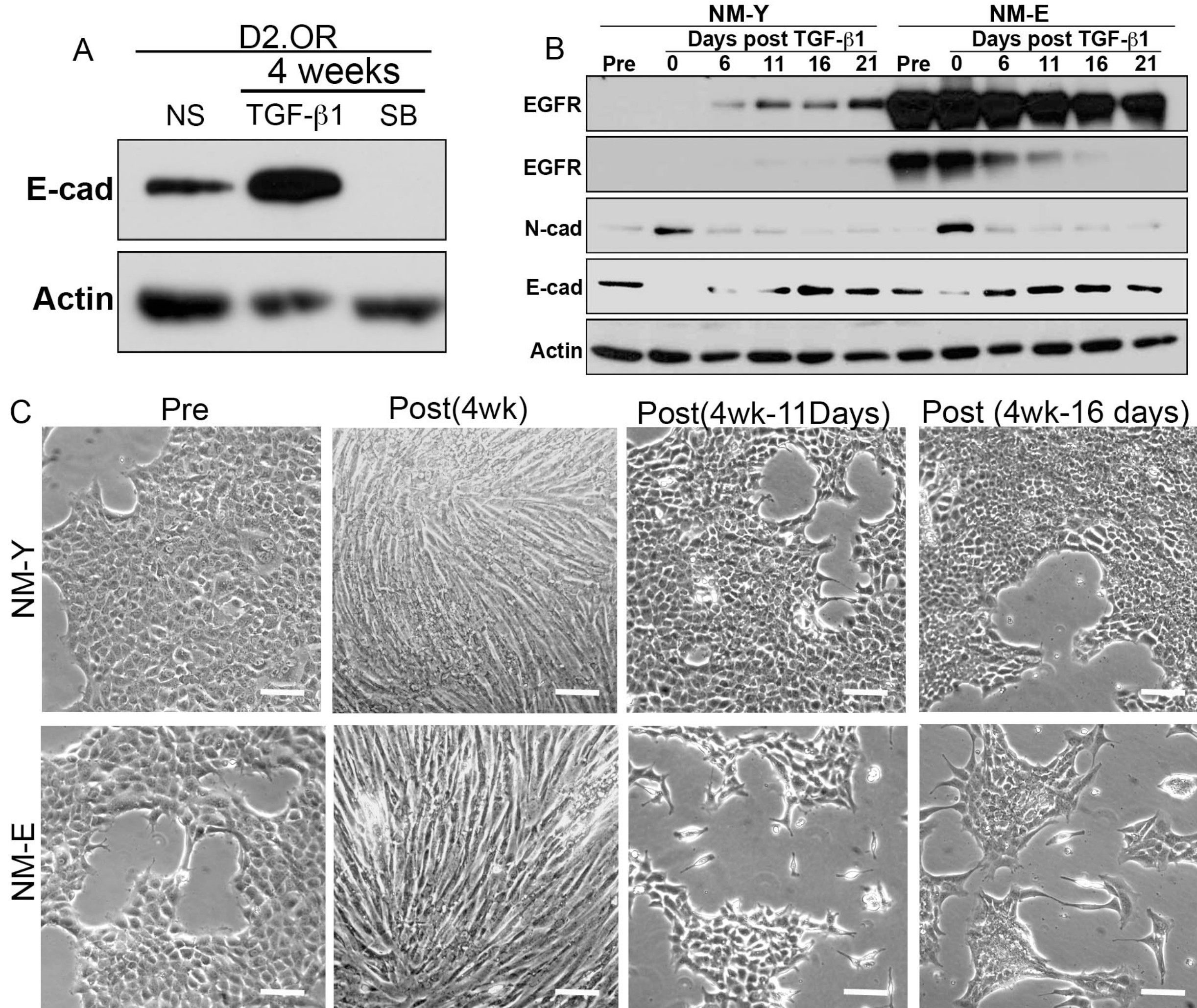
SUPPLEMENTARY FIGURE S1: 3D morphologies of the D2.OR and D2.A1 cells. D2.OR and D2.A1 cells were seeded into 3D cultures at the indicated cell densities. The morphology of the cellular structures was imaged under Phase contrast microscopy (40x) at the indicated time points.

SUPPLEMENTARY FIGURE S2: Wendt *et al*



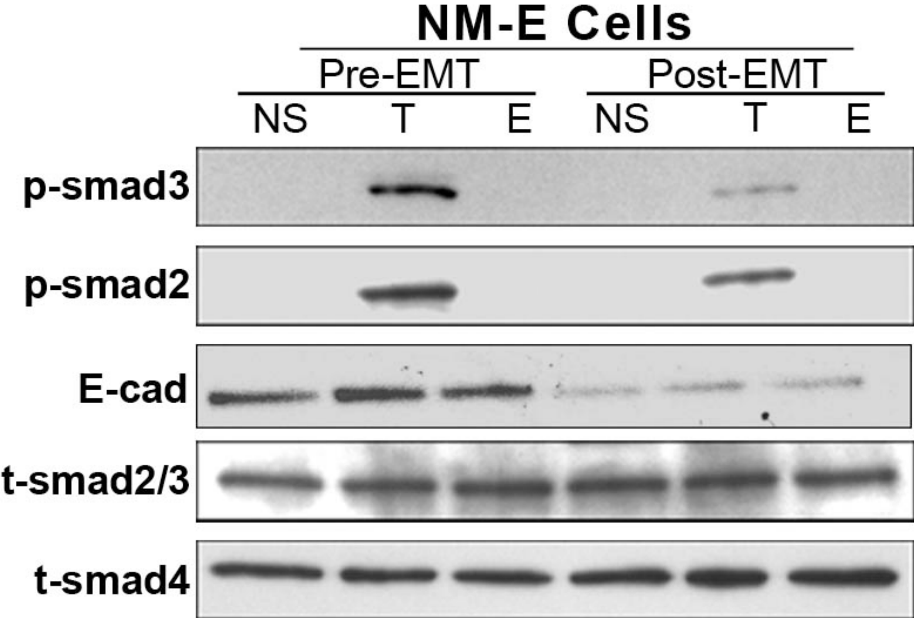
SUPPLEMENTARY FIGURE S2: D2.A1 and D2.OR cells were stably transduced with E-cad or control GFP vectors, and subsequently were stimulated with TGF-β1 (5 ng/ml) for 48 h prior to being fixed and stained with fluorescently-labeled phalloidin to visualize the actin cytoskeleton (400x).

SUPPLEMENTARY FIGURE S3: Wendt *et al*



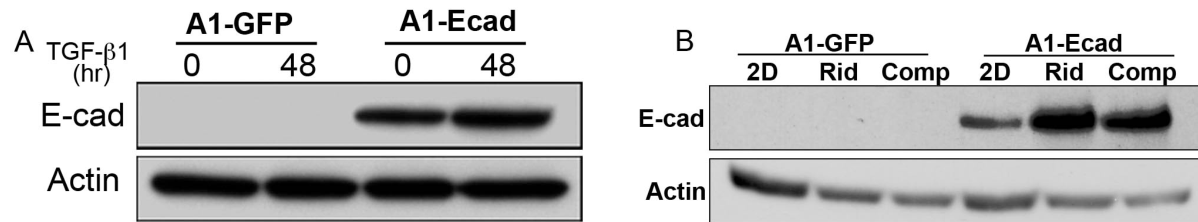
SUPPLEMENTARY FIGURE S3: Chronic TGF-β treatment of D2.OR cells fails to downregulate E-cad expression. (A) D2.OR cells were cultured in the presence of TGF-β1 (5 ng/ml) or the TβR-I inhibitor, SB431543 (10 μM) for 4 weeks, at which point the cells were analyzed for the presence of E-cad and Actin as a loading control. Data are representative of 2 independent experiments. (B) YFP (NM-Y)- and EGFR (NM-E)-expressing NMuMG cells were continuously cultured with TGF-β1 (5 ng/ml) for 4 weeks (Post 4wk), and subsequently were withdrawn from TGF-β1 and cultured for varying lengths of time as indicated. Lysates were prepared at the indicated times before and after TGF-β withdrawal and compared to "TGF-β naïve" cells (Pre). Cell extracts were immunoblotted for the expression of EGFR (shown at two different exposures), N-cadherin (N-cad), E-Cadherin (E-cad), and Actin as a loading control. (C) Representative photomicrographs (100x) of Pre- and Post-EMT cells described in *Panel B*.

SUPPLEMENTARY FIGURE S4: Wendt *et al*



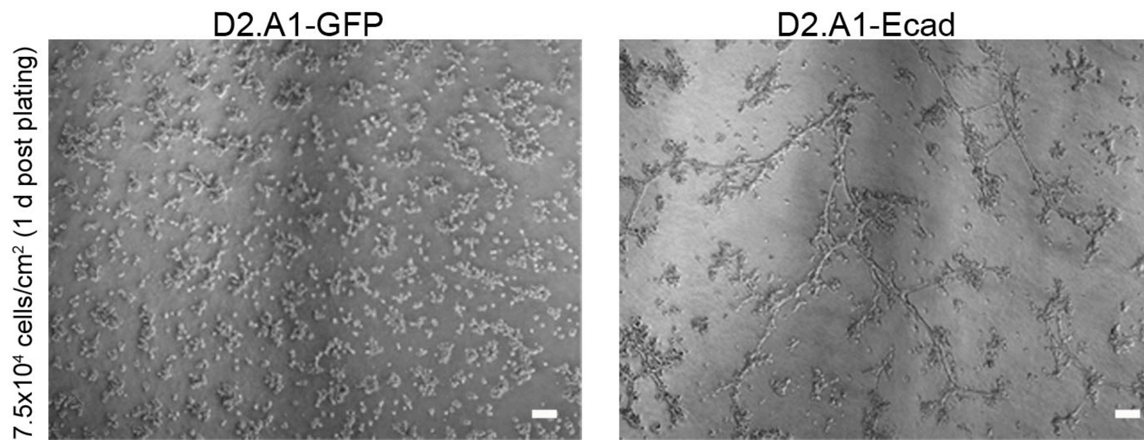
SUPPLEMENTARY FIGURE S4: TGF-β-induced EMT decreases subsequent activation of Smad2/3 by TGF-β. NM-E cells were cultured in the presence of TGF-β1 (5 ng/ml) over a span of 4 weeks to induce a mesenchymal state (Post-EMT). Quiescent Pre- and Post-EMT cells were stimulated with TGF-β1 (5 ng/ml) for 30 min and analyzed for the presence of phosho-Smad2 (p-smad2), phospho-Smad3 (p-smad3), E-cad, Smad4 (t-smad4) and Smad2/3 (t-smad2/3) as a loading control. Shown are representative immunoblots from 2 independent experiments.

SUPPLEMENTARY FIGURE S5: Wendt *et al*



SUPPLEMENTARY FIGURE S5: Recombinant expression of E-cad in D2.A1 cells is resistant to TGF-β and 3D culture. (A) D2.A1 cells described in transduced with E-cad or GFP as a control were stimulated with TGF-β1 (5 ng/ml) for 48 h and analyzed for the presence of E-cad, while Actin is shown as a loading control. (B) D2.A1 cells described in *Panel A* were grown in 2D, or in compliant (comp) or rigid (Rid) 3D cultures for 5 days, at which point cell lysates were harvested and analyzed for the presence of E-cad and Actin as a loading control. All data are representative of at least 3 independent experiments.

SUPPLEMENTARY FIGURE S6: Wendt *et al*



SUPPLEMENTARY FIGURE S6: Expression of E-cad induces a branched morphology in the metastatic D2.A1 cells. Control (GFP) or E-cad expressing D2.A1 cells were grown in 3D cultures for 1 day. The resulting cellular structures were imaged by phase contrast microscopy (40x). Images are representative of 3 independent experiments.

SUPPLEMENTARY MOVIE S1: Systemically dormant cells form intricate 3D branching structures. Nonmetastatic D2.OR cells were grown for 10 days in compliant 3D culture. Movie is a Z-series (400x) that runs from the top to the bottom of the organoid at a depth of 452 μm that is comprised of 3.2 μm sections.

SUPPLEMENTARY MOVIE S2: D2.OR cells migrate to form multicellular structures under 3D culture conditions. D2.OR cells (7.5×10^4 cells/cm^2) were plated under 3D culture conditions. The time-lapse movie (50x) was acquired over a span of 18 h, with individual frames captured at 10 min intervals.

SUPPLEMENTARY MOVIE S3: D2.A1 cells are non-migratory under 3D culture conditions. D2.A1 cells (7.5×10^4 cells/cm^2) were plated under 3D culture. The time-lapse movie (50x) was acquired over a span of 18 h, with individual frames captured at 10 min intervals.

SUPPLEMENTARY TABLE S1:

Antibody	Dilution	Supplier
β-Catenin	1:1000	BD Biosciences
E-Cadherin	1:5000	BD Biosciences
β3 Integrin	1:1000	Cell Signaling Technologies
Vimentin	1:1000	BD Biosciences
FAK	1:1000	Santa Cruz Biotechnologies
Pyk2	1:1000	Cell Signaling Technologies
EGFR	1:1000	Cell Signaling Technologies
TβR-II	1:1000	Santa Cruz Biotechnologies
β-actin	1:1000	Santa Cruz Biotechnologies
Phospho-P38 MAPK	1:500	Cell Signaling Technologies
Total P38 MAPK	1:1000	Santa Cruz Biotechnologies
Phospho-Smad3	1:500	Cell Signaling Technologies
Phospho-Smad2	1:1000	Cell Signaling Technologies
Total Smad2/3	1:1000	Cell Signaling Technologies
*Total Smad2/3	1:250	BD Biosciences
Phospho-Erk1/2	1:2000	Cell Signaling Technologies
Total-Erk1/2	1:1000	Cell Signaling Technologies
Twist	1:250	Abcam
Snail	1:1000	Cell Signaling Technologies
β1 Integrin	1:1000	Cell Signaling Technologies

* Denotes antibody used for immunofluorescence.

SUPPLEMENTARY TABLE S2:

Target	Application	Sequence (5' to 3')
E-Cadherin	PCR-Sense	5’-CCCTACATACACTCTGGTGGTTCA
E-Cadherin	PCR-Antisense	5’-GGCATCATCATCGGTCACTTTG
β3-Integrin	PCR-Sense	5’- GTCCGCTACAAAGGGGAGAT
β3-Integrin	PCR-Antisense	5’-TAGCCAGTCCAGTCCGAGTC
GAPDH	PCR-Sense	5’-CAACTTTGGCATTGTGGAAGGGCTC
GAPDH	PCR-Antisense	5’-GCAGGGATGATGTTCTGGGCAGC
Vimentin	PCR-Sense	5’- CAAGTCCAAGTTTGCTGACCTCTC
Vimentin	PCR-Antisense	5’ - CTCTTCCATCTCACGCATCTGG
FAK	PCR-Sense	5’-GTGTAAAATTGGGAGACT
FAK	PCR-Antisense	5’-GTAGCCTGTCTTCTGGAT
Pyk2	PCR-Sense	5’- GGACTATGTGGTGGTGGTGA
Pyk2	PCR-Antisense	5’- GGACTATGTGGTGGTGGTGA
EGFR	PCR-Sense	5’- GAGAGGAGAACTGCCAGAA
EGFR	PCR-Antisense	5’ - GTAGCATTTATGGAGAGTG
E-Cadherin	shRNA#1	CCGGGCTGGAATCTTTGTCCATGTA**CTCGAG**TACATGGACAAAGATTCCAGCTTTTTG
E-Cadherin	shRNA#2	CCGGCGGGACAATGTGTATTACTAT**CTCGAG**ATAGTAATACACATTGTCCCGTTTTTG
E-Cadherin	shRNA#3	CCGGGCCTCATATCATCACCATCTT**CTCGAG**AAGATGGTGATGATATGAGGCTTTTTG
E-Cadherin*	shRNA#4	CCGGCCGAGAGAGTTACCCTACATA**CTCGAG**TATGTAGGGTAACTCTCTCGGTTTTTG
E-Cadherin	shRNA#5	CCGGCCACGACCAATGATGGCATTT**CTCGAG**AAATGCCATCATTGGTCGTGGTTTTTG
β1-Integrin	shRNA#1	CCGGGCACGATGTGATGATTTAGAA**CTCGAG**TTCTAAATCATCACATCGTGCTTTTTG
β1-Integrin	shRNA#2	CCGGCCAAGTTTCAAGGGCCAACTT**CTCGAG**AAGTTGGCCCTTGAAACTTGGTTTTTG

* Denotes E-cad-targeted shRNA sequence used in the current study

Underlined text denotes complimentary RNA targeted sequence, while **bold text** denotes hairpin sequence.