**Supplementary Methods**

**Immunofluorescence**

A standard immunofluorescence protocol for cells grown on ethanol-sterilized glass coverslips was used. Cells were fixed with 4% PFA for 10 minutes. PFA autofluorescence was quenched with 50 mM NH4Cl/PBS for 5 minutes, and cells were permeabilized with 0.5% Triton X-100 for 5 minutes. Blocking was carried out for 1 hour with PBS containing 3% BSA. Coverslips were then incubated in a humid atmosphere for 1 hour at room temperature, or 24 hours at 4°C, with a primary antibody, followed by extensive washing and a 1-h incubation with the secondary antibody in the dark (for the complete list of antibodies used and their sources, see Supplementary Table S3). Coverslips were mounted with DAPI-Fluoromount-G, and fluorescence images were captured with a Leica TCS-SP2 confocal microscope. The orientation distribution of extracellular fibronectin fibers was estimated from immunofluorescence images using a filtering approach as follows. A bank of filament-like filters was constructed to reflect variations in rotation, scales, as well as curvature. In total, 448 filters were used, covering 32 rotations, 7 scales, and 2 curvatures. Each filter was then digitally cross-correlated with the input image data and the angle of rotation corresponding to the largest response is chosen as the most likely fiber orientation for each pixels. Finally, the histograms shown in plot 1B only include pixels for which the cross correlation value exceeded the value of 0.6. The detailed protocol is currently submitted for publication, and the computer code available at https://www.andrew.cmu.edu/user/gustavor/software.html.

**Immunohistochemistry**

Harvested tissue samples were fixed in formalin and embedded in paraffin. Sections of 4 μm were obtained with a microtome and then subsequently dewaxed and rehydrated. Antigens were retrieved by boiling the samples in Tris/EDTA (50 mM Tris/HCl, 1 mM EDTA, and 10 mM NaCl, pH 9.0) for 15 minutes. Endogenous peroxidase activity was quenched for 15 minutes with 4% hydrogen peroxide in PBS containing 0.1% sodium azide. After several rinses with PBS, sections were incubated with PBS containing 1% BSA to block non-specific binding and then washed with PBS. Sections were incubated with the indicated antibodies (10 μg/ml of purified anti-Snail1 monoclonal antibody EC3 or 1/800 anti-αSMA antibody from SIGMA) overnight at 4°C. After several rinses with PBS, bound antibody was detected using anti-mouse or anti-rabbit Envision (Envision System Peroxidase; DAKO). Sections were counterstained with haematoxylin and mounted for microscopy analysis. Some paraffin sections were stained with a Masson´s trichrome reagent kit following the manufacturer´s instructions.

**RhoA-GTP Pull-Down Assay**

RhoA activation was quantified in cell extracts by capturing RhoA-GTP on a bead support that contained a purified RhoA-GTP–specific interacting protein (rhotekin fused to GST). To purify GST-rhotekin, LB broth supplemented with 100 μg/ml ampicillin was inoculated with a single colony of *E. coli* containing the GST-rhotekin construct and incubated overnight at 37ºC with shaking. The overnight culture was diluted 1:10 in LB broth containing 100 μg/ml ampicillin and grown to an OD600 of 0.6. Isopropyl-β-d-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM to induce protein expression. After 2 hours of incubation, the culture was centrifuged for 10 minutes, and the bacteria pellet was resuspended in PBS and sonicated 7× on ice. TX-100 was added to a final concentration of 1%, and the slurry was incubated 30 minutes at 4ºC. The lysed cells were then clarified by centrifugation at 10 000 rpm at 4ºC for 10 minutes. Glutathione-sepharose beads were added to the supernatant, and the mixture was incubated at 4ºC for 1 hour. Beads were washed at least three times with cold PBS and then resuspended in PBS with 10% glycerol. The purity of the fusion protein was assessed by SDS-PAGE.

For the GST pull-down assay, cells were starved 24 h, treated for five minutes with TGFβ1 at a final concentration of 5ng/ml, and then washed with cold PBS. Cold lysis buffer (50 mM Tris-HCl, pH 7.5, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 500 mM NaCl, 10 mM MgCl2, 10 μg/ml aprotinin, and 1 mM PMSF) was added, and the cells were rapidly scraped off the plates. Lysates were transferred to Eppendorf tubes, syringed five times, and centrifuged at 13 000 rpm for 10 minutes at 4°C. The protein amount in the supernatant was quantified, and 25 µg of lysate was separated for analysis as input, while 1 mg was incubated with 20 μg of GST-rhotekin for 1 hour at 4ºC. Beads were then washed 5× with 1 ml cold washing buffer (50 mM Tris-HCl, pH 7.5, 1% Triton X-100, 150 mM NaCl, 10 mM MgCl2, 10 μg/ml aprotinin, and 0.1 mM PMSF). SDS-PAGE sample buffer was added to each sample, and samples were heated at 95°C for 5 minutes and then loaded onto gels. Total RhoA and RhoA-GTP amounts were detected by Western blot, quantified with the Quantity One analysis software (BIORAD), and expressed as relative RhoA-GTP/total RhoA.

**Three-Dimensional Extracellular Matrices**

Three-dimensional ECMs were generated following a previously-described protocol (1). For 24 wells plates, 5 × 105 fibroblasts were seeded on gelatin cross-linked glass coverslips; for 96 wells plates, 1 × 105 fibroblasts were seeded on gelatin cross-linked cell culture plastic wells; and for Boyden Chamber inserts, 1 × 105 fibroblasts were seeded in gelatin cross-linked inserts, using 100 µl of medium to prevent media leaking through the insert pores. Cell culture media was supplemented with 50 µg/ml ascorbic acid and, where indicated, 5 ng/ml TGF. To foster ECM deposition by the plated cells, media was replaced every two days for ten days. Cultures were then washed with pre-warmed (37ºC) PBS and either fixed with 4% PFA for immunofluorescence analysis, or decellularized with 20 mM NH4OH and 0.5% (v/v) Triton X-100 in PBS for later use as a cell culture substrate.

The orientation distribution of extracellular fibronectin fibers was estimated from immunofluorescence images using a filtering approach as follows. A bank of filament-like filters was constructed to reflect variations in rotation, scales, as well as curvature. In total 448 filters were used, covering 32 rotations, 7 scales, and 2 curvatures. Each filter was then digitally cross-correlated with the input image data and the angle of rotation corresponding to the largest response is chosen as the most likely fiber orientation for each pixels. Finally, the histograms shown in plot 1B only include pixels for which the cross correlation value exceeded the value of 0.6. The protocol is in press (see ref 43 of the article).

**Migration and Invasion Assays**

Cell tracker–labeled cells (1000) were seeded on decellularized ECMs generated on 96-well plates and left to attach overnight. Cells were monitored with a CellObserverHS system (Zeiss). Bright-field and fluorescence images were taken every 10 minutes over 18 hours, and the position coordinates for 10 single cells were calculated for each time point using the ImageJ software. Plots represent the movement over time of single cells relative to their initial positions.

For invasion assays, decellularized ECMs were generated on 8 µm porous membranes of Boyden chamber inserts. Approximately 50 000 cells were then seeded in the upper part of the insert in serum-free media, while 10% serum-containing medium was placed in the well below as a chemoattractant. Cells were allowed to invade the ECM for 24 hours, fixed with 4% paraformaldehyde (PFA) in PBS, and stained with crystal violet. Non-invading cells were removed from the insert upper side with a cotton swab. Invading cells were imaged with a transmitted light microscopy.

***In Vivo* Wound Healing**

*Snai1*+/flox and *Snai1*-/flox mice were treated with tamoxifen both intraperitoneally and cutaneously. A solution of 0.2 mg tamoxifen in corn oil solution per gram of body weight was injected intraperitoneally every 48 hours for 4 times. At 24 hours after the first injection, a cutaneous application of 4-hydroxytamoxifen in acetone (200 µl of 10 mg/ml) in a shaved dorsal hair area was applied; this was repeated after every injection. Ten days after the first tamoxifen injection, mice were anesthetized with isoflurane (FORANE®, Abbot Laboratories, Abbot Park, IL, USA) for skin wounding. After cleaning the exposed skin with 70% ethanol, full-thickness excisional skin wounds were made aseptically on either side of the dorsal midline using a 6 mm biopsy punch. Two wounds were usually made on the same animal. Wounds were photographed and measured at 1, 2, and 5 days. The wound tissue and surrounding skin from the wound margin were harvested from mice at five days post-wounding, fixed in formalin, embedded in paraffin, and sectioned in 4 µM slices for immunohistological analysis. This study was approved by the Animal Experimentation Ethical Committee of the IMAS (Barcelona, Spain).

**Fibronectin Fibrillogenesis**

Glass coverslips were coated overnight with purified soluble fibronectin (2 µg/ml) in PBS. After extensively washing with PBS, cells were plated, grown for 16, 24, or 60 hours, and then fixed with 4% PFA in PBS. Background fibronectin left unpolymerized by cells was visualized by immunohistochemistry, and fibronectin fibrillogenesis was estimated using ImageJ software as the fibronectin-cleared background area (with an intensity value lower that a background threshold level) normalized by the number of cells in a field. An area affected by a minimum of 100 cells per condition was analyzed. No differences in the effects of TGF at 16, 24, or 60 hours were observed.

**Young’s Modulus (Ε)**

Rigidity of decellularized extracellular matrices was analyzed by atomic force microscopy. Complete elimination of cells from matrices was confirmed by fluorescent microscopy in parallel MEFs-labeled cultures (CellTracker™ Green CMFDA).

Decellularized ECMs were extensively washed with (37ºC) PBS, and Young’s Modulus was measured with a custom-built AFM attached to an inverted optical microscope (TE2000, Nikon, Tokyo, Japan) using a previously described method (2, 3). The matrices were probed with V-shaped pyramidal cantilevers with nominal spring constant (k) of 0.01 and 0.03 N/m (MLCT, Bruker, Mannheim, Germany). The cantilever was displaced in 3D with nanometric resolution (z) and the defection of the cantilever (d) was measured using the optical lever method. The force (F) on the cantilever was computed as F = k(d – doff) being doff the offset of the photodiode. The indentation of the sample (δ) was computed as δ = (z – zc) – (d – doff), where zc is the position of the tip-matrix contact point. ECM samples were measured at 9–13 points separated by 20 μm. At each measurement point, 5 F-δ curves were recorded (1 Hz triangular displacement, peak-to-peak amplitude of 5 μm), which were analyzed with the pyramidal Hertz model

$$F= \frac{3 E \tan(θ)}{4 (1- σ^{2})}δ^{2}$$

where θ is the semi-included angle of the tip and σ is the Poisson’s ratio, assumed to be 0.5. Nonlinear least-squares fit (Matlab, The MathWorks, Natick, MA) was used to estimate E from the loading branch of the F-δ curve for a maximum indentation of 1 µm. The average E obtained from the five force curves recorded at each measurement point was computed.

E values were presented in a box plot. Boxes represent the median and the 25%–75% interquartile range, and whiskers mark the 1.5× interquartile range. Circles denote outlying values. The asterisk indicates a statistically significant difference (P > 0.001) between groups as determined by ANOVA on ranks and Dunn's method (for the ECMs from KO–, KO+TGFß–, control–, and control+TGFß– MEFs, *n* equals 7, 38, 16, and 49, respectively).

**Cell Differentiation**

Cells for differentiation were grown on decellularized, MEF-derived ECMs that had been previously stored at 4°C. Osteogenic differentiation of MSCs was induced by adding 100 nM dexamethasone, 10 mM ß-glycerophosphate, and 50 mM L-ascorbic acid-2-phosphate to a confluent culture for 3 days. Cells were cultured in DMEM plus 10% FBS for an additional 3 or 6 days and then stained with alkaline phosphatase.

Myogenic differentiation was induced in confluent C2C12 by serum deprivation. The culture medium of the confluent cells was replaced with induction medium (IM) consisting of DMEM supplemented with 2% FCS and 1% penicillin/streptomycin at 37°C in a humidified incubator under 5% CO2, and the IM was replaced every day. Myogenin II expression was analyzed by immunofluorescence in DAPI-counterstained samples, and the percentage of myogenin-expressing cells was calculated by counting positive cells from fluorescent microscopy images. A minimum of 500 cells per condition were counted.

**Analysis of tumor samples**

Surgical resection specimens from primary breast tumors were obtained from Parc de Salut Mar Biobank (MARBiobanc, Barcelona, Spain), Fundación Jiménez Díaz Biobank (Madrid, Spain), and Valencia Clinic Hospital Biobank (València, Spain). Formalin-fixed paraffin-embedded (FFPE) tumor blocks were retrospectively selected from consecutive breast cancer patients diagnosed between 1998 and 2000, based on the following criteria: infiltrating carcinomas, operable, no neoadjuvant therapy, enough available tissue, and a clinical follow-up. TNM (tumor–node–metastasis) staging was classified using the American Joint Committee on Cancer (AJCC) staging system. Histological grade was defined according Scarff–Bloom–Richardson modified by Elston. ER and PR were determined by immunohistochemistry (IHC) (SP1 and PgR636 clones, respectively; Dako, Carpinteria, CA) establishing positivity criteria in ≥1% of nuclear tumor staining. HER2 amplification was assayed by FISH (Pathvysion; Abbott Laboratories, Abbott Park, IL). Ki-67 was studied by IHC (MIB1 clone; Dako). The study was approved by the Ethics Committees of the three hospitals. Three hundred and seventy-one infiltrating carcinomas were collected (Supplemental Tables S1 and S2). Tissue microarrays (TMA) were constructed as previously reported. Complete sections of infiltrating carcinoma (*n* = 30) from the same specimens were also assayed.

Immunostaining for Snail1 was carried out using 3 μm sections. Briefly, heat antigen retrieval was carried out in pH 9 EDTA-based buffered solution in a Dako Link platform. Endogenous peroxidase was quenched. A mouse monoclonal anti-Snail1 antibody (4) was used for 60 minutes with a1:10 dilution at room temperature, followed by incubation with a polymer coupled with peroxidase (Flex+; Dako). Sections were then visualized with 3,3'-diaminobenzidine (DAB) and counterstained with hematoxylin. Human tumors with previously-confirmed Snail1 expression were used as positive controls. Sections incubated with normal mouse immunoglobulin G2 were used as controls.

Double immunofluorescence for Snail1 and fibronectin were further assayed on selected set of 30 tumors (15 with Snail1 positive and 15 negative stroma) by multispectral analysis (Cri Nuance FX Multispectral Imaging System, Perkin-Elmer, Waltham, MA) for Snail1 and fibronectin (5), and second harmonic generation for collagen fibers (6).

Snail1 and fibronectin (rabbit polyclonal, diluted 1:200, Dako) were detected using appropriate Alexa Fluor 568 and 488 conjugated goat anti-rabbit IgG and anti-mouse IgG antibodies (Life Technologies, Paisley, UK) (diluted 1:700). Sections were counterstained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI; Abbott Molecular, Des Plaines, IL) to visualize cell nuclei. All incubations were performed at room temperature using a Dako Autostainer. Snail1 was scored when any percentage of stromal or epithelial cells was stained in the nucleus. Second harmonic generation analysis were carried out in the Advanced Light Microscopy Unit of the CRG (Centre of Genomic Research) at PRBB (Barcelona Biomedical Research Park). Fibronectin and collagen fibers alignment were considered positive when lineal fibers in desmoplastic stromal areas were found to align perpendicular to the tumor mass.

Statistical analysis was carried out with SPSS v13.0 (SPSS-IBM, Armonk, NY). To correlate Snail1 expression and clinicopathological variables, we used the Χ2 test (Fisher’s exact test). OS was defined as the time from the date of surgery to the date of death from any cause or the last follow-up. Univariate analysis was based on the Kaplan–Meier OS curves using the log-rank test; all predictors with *P* < 0.05 were used in multivariate analysis using the Cox proportional hazards model. All the statistical tests were conducted at the two-sided 0.05 level. This work was carried out in accordance with Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) guideline (7).

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