Stem Cell Reports, Volume *10*

Supplemental Information

Breast Cancers Activate Stromal Fibroblast-Induced Suppression of Progenitors in Adjacent Normal Tissue

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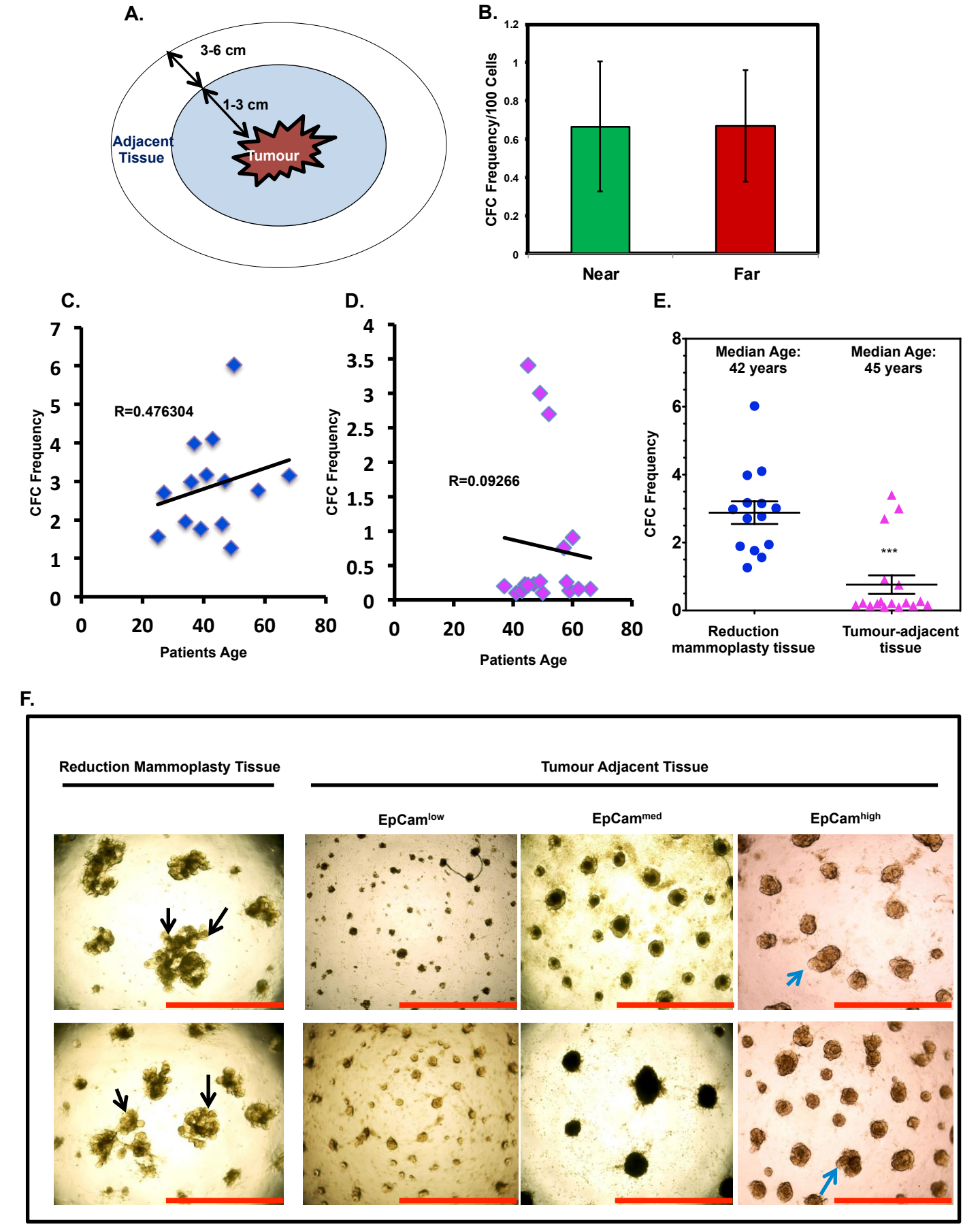
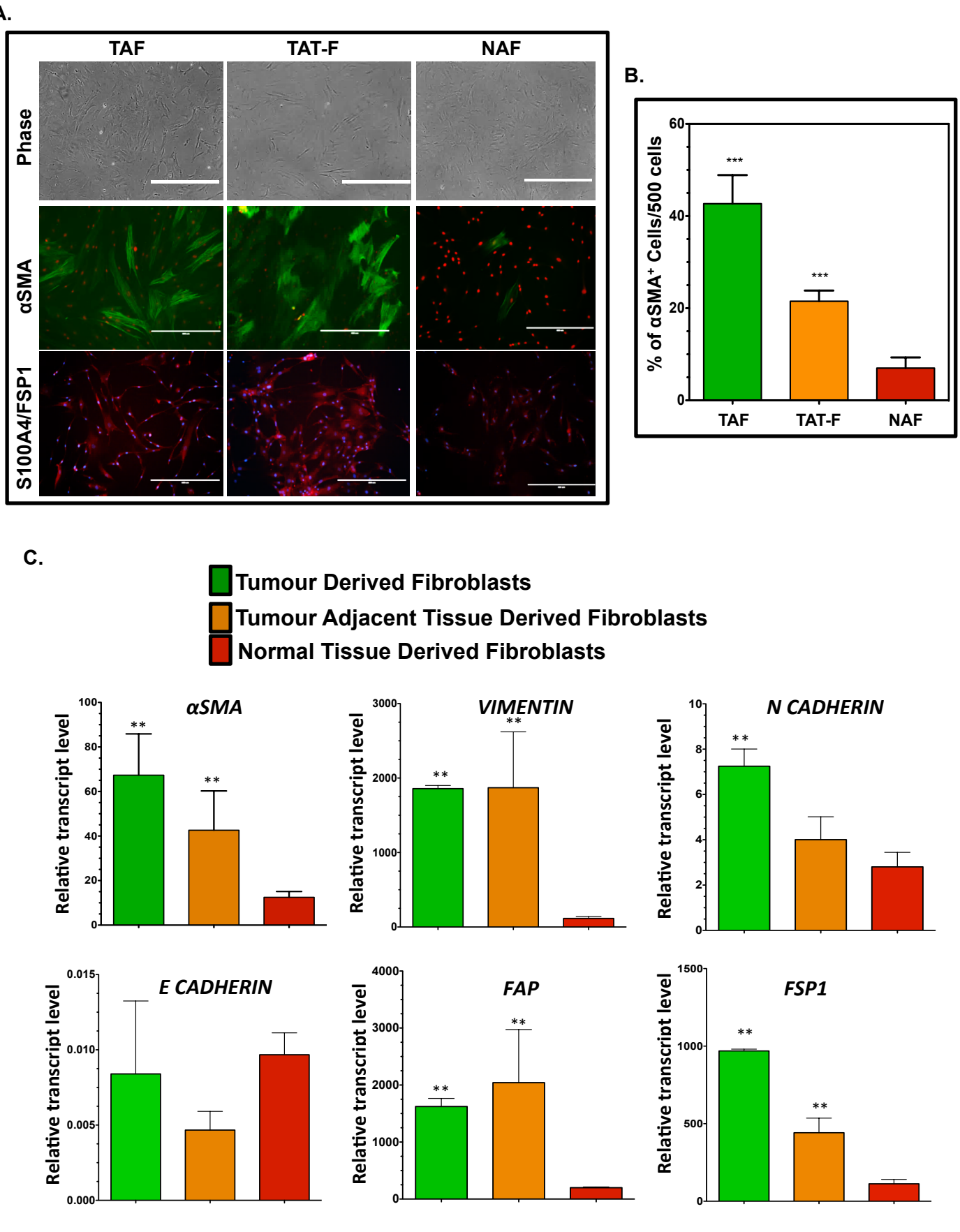
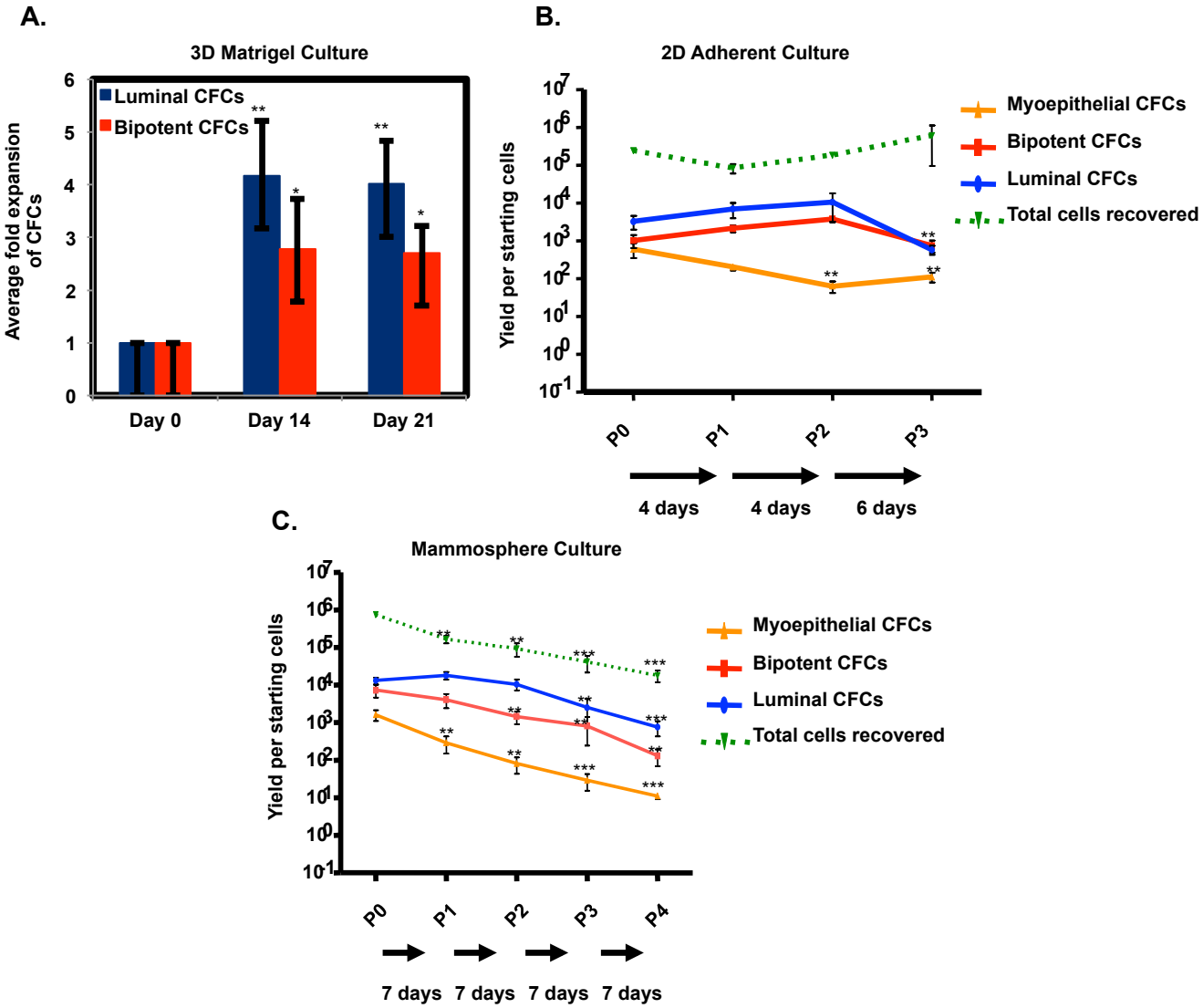
Supplementary Figure 1 (A-F) related to Figure 1



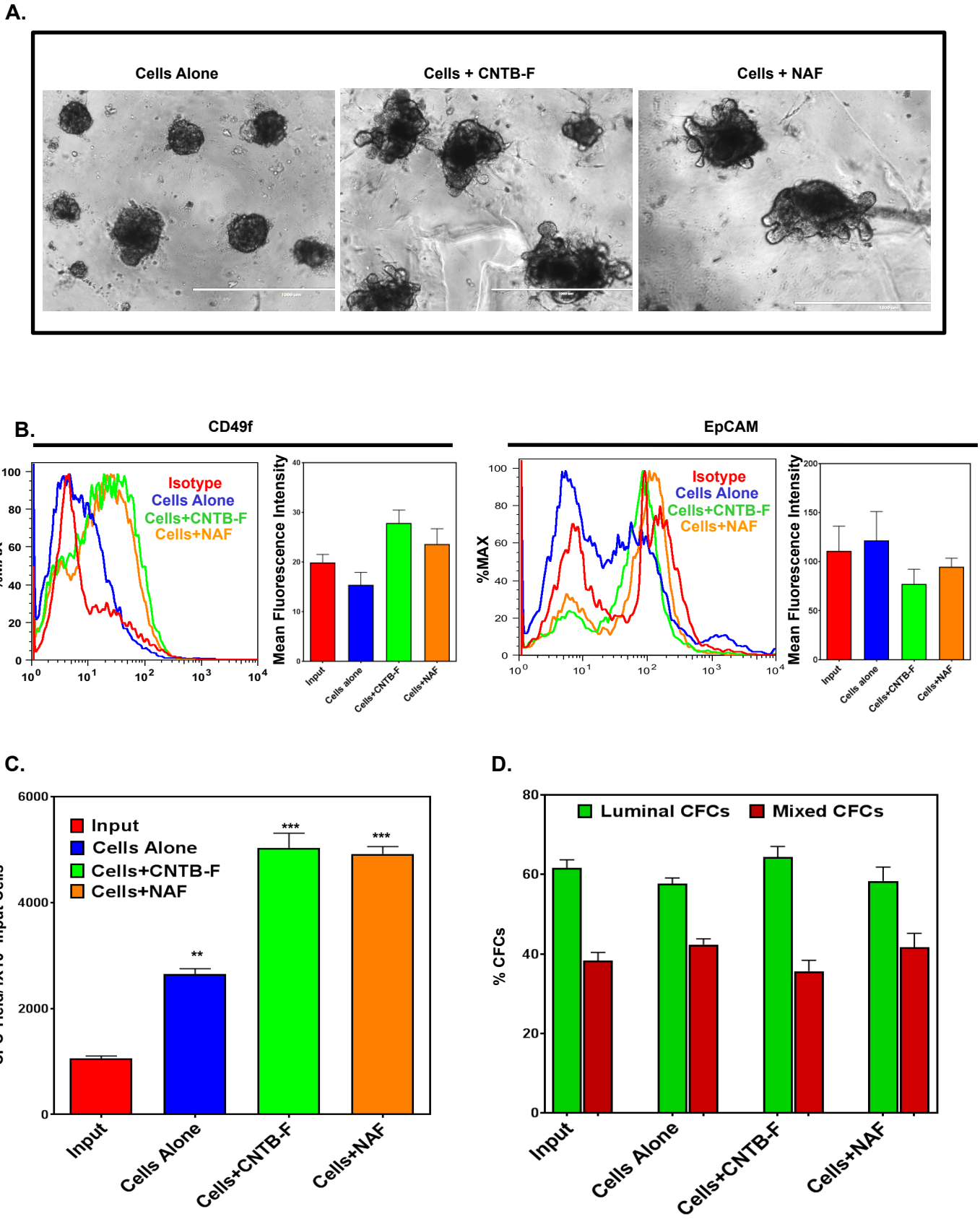
Supplementary Figure 2 (A-C) related to Figure 2



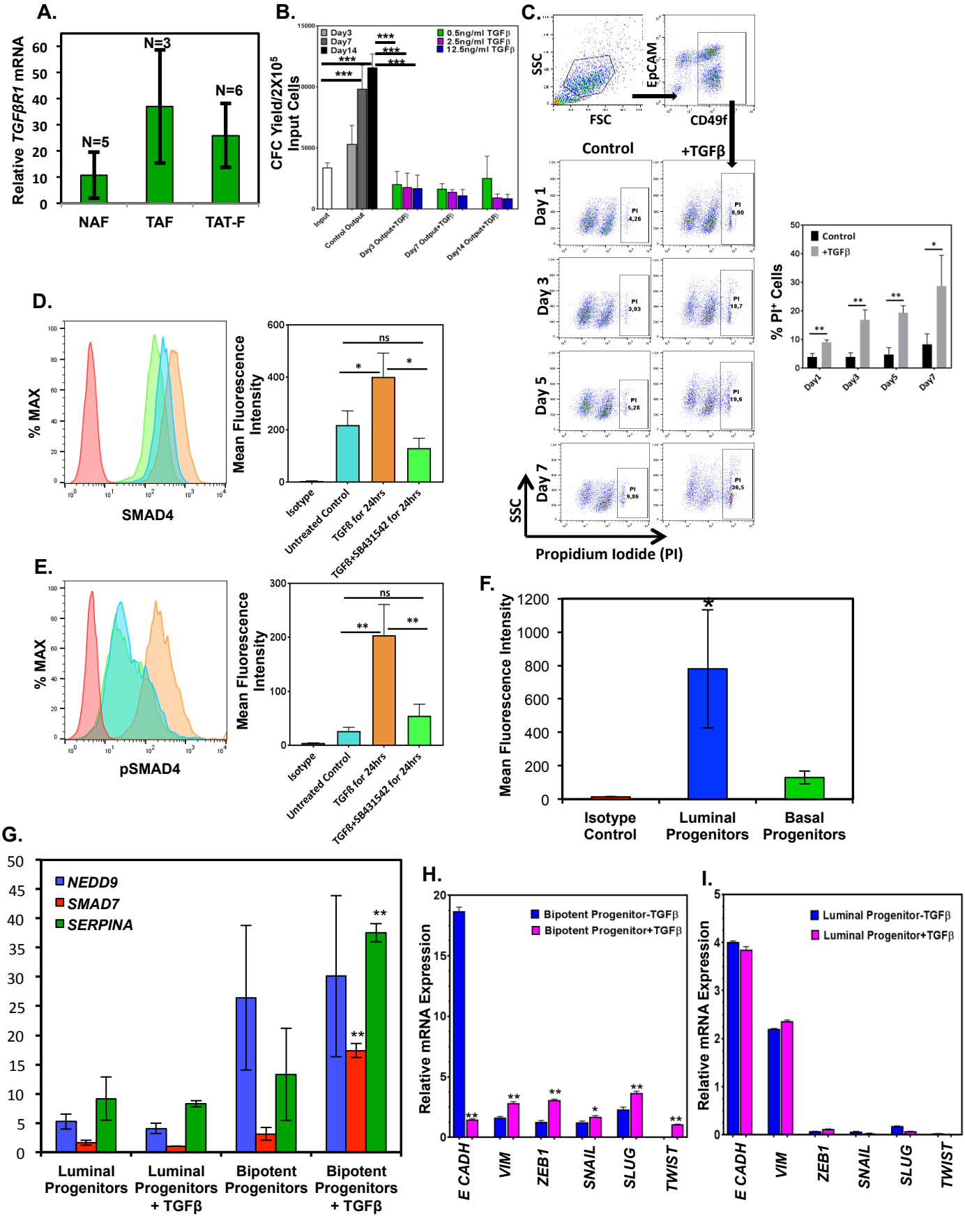
Supplementary Figure 3 (A-C) related to Figure 2



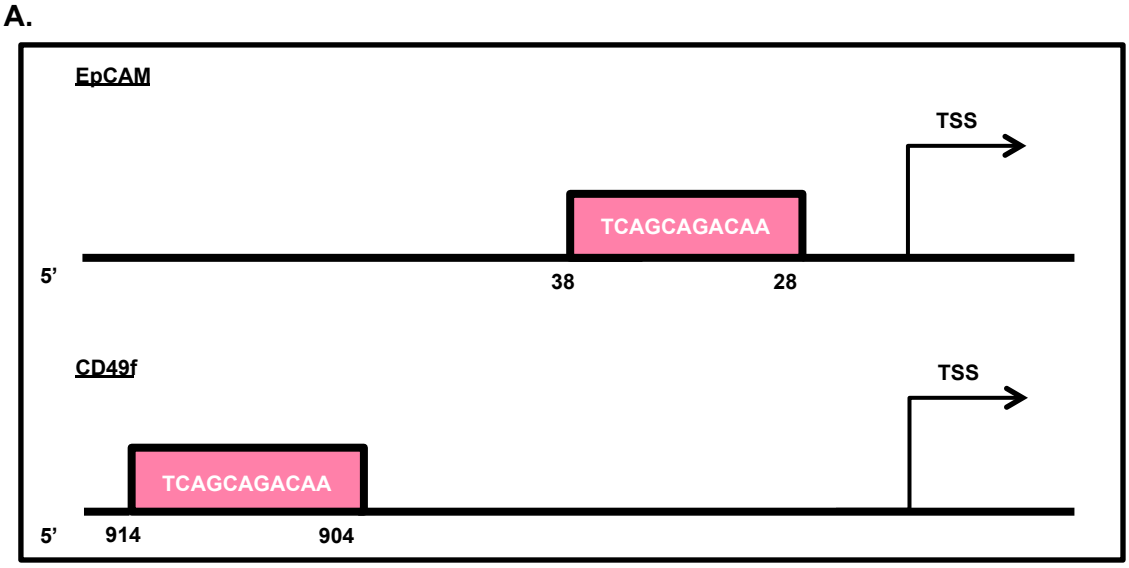
Supplementary Figure 4 (A-D) related to Figure 2

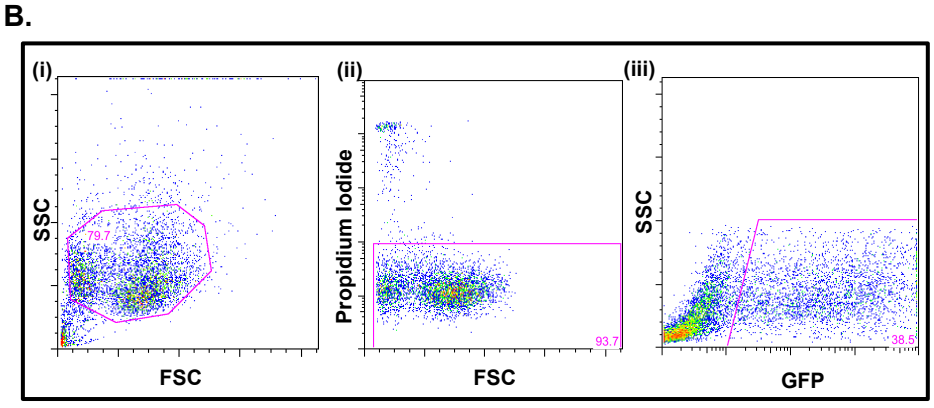


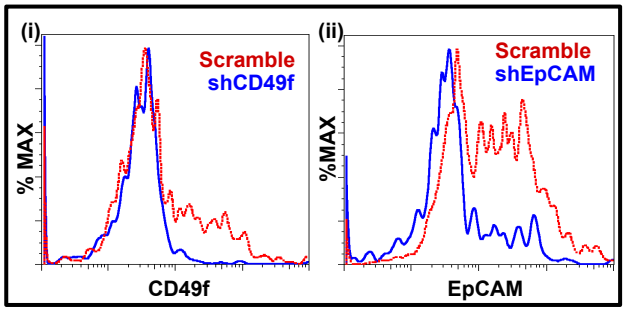
Supplementary Figure 5 (A-I) related to Figure 3, 4 and 5

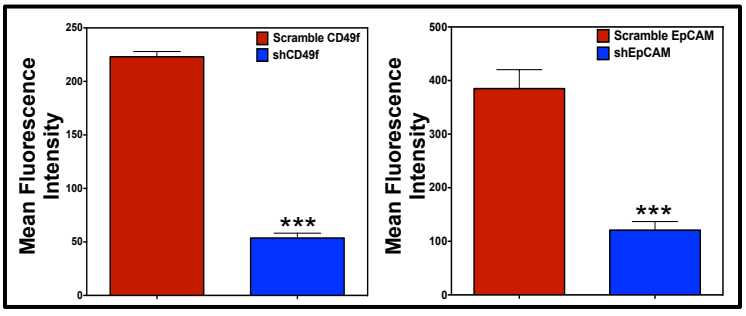


Supplementary Figure 6 (A-D) related to Figure 6

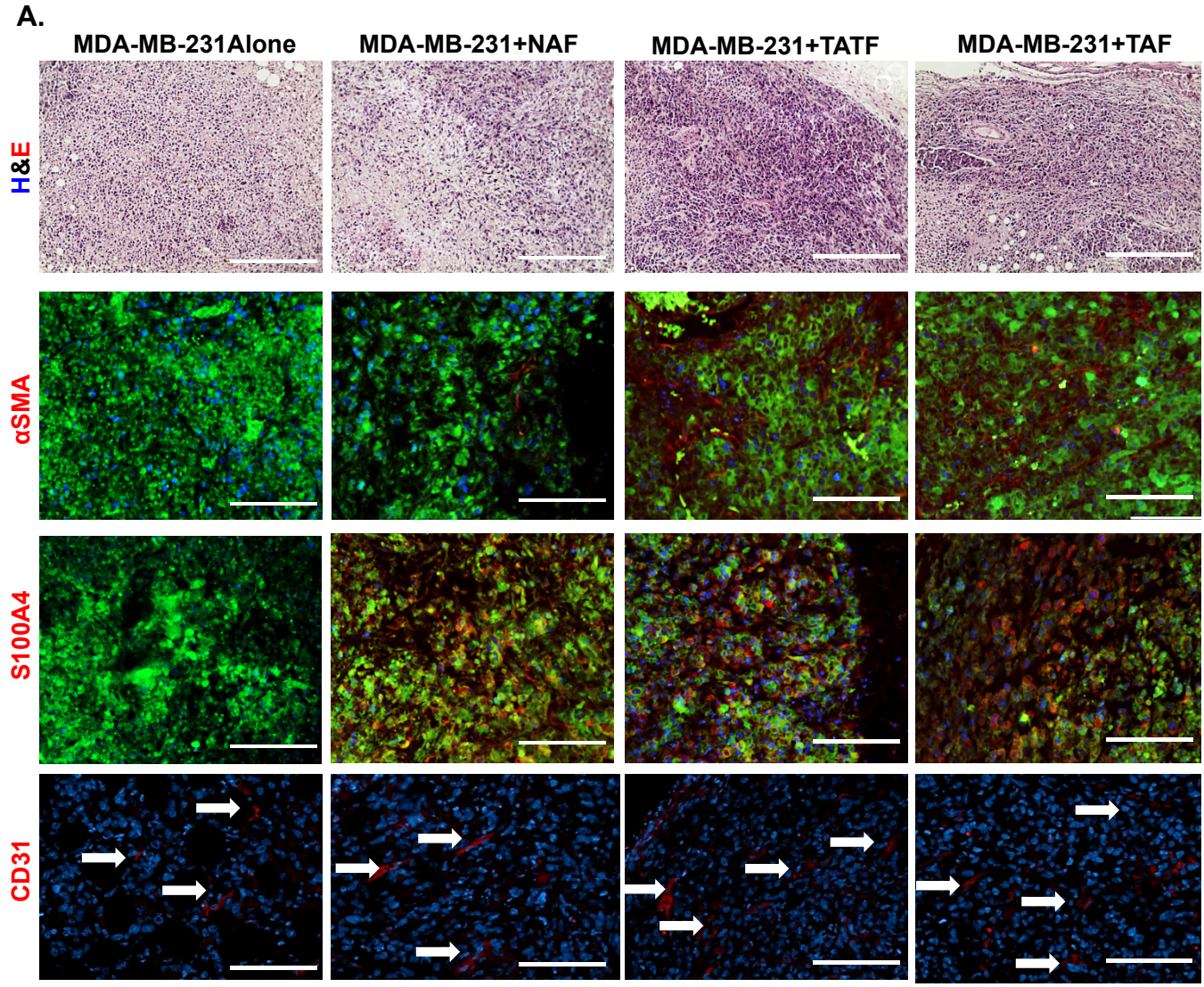


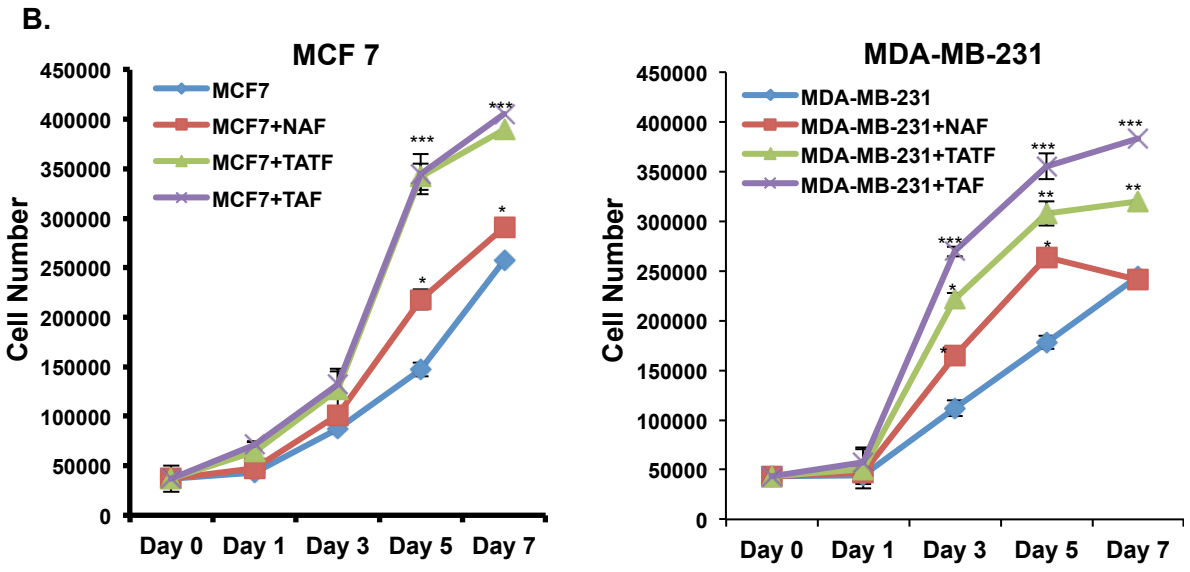






Supplementary Figure 7 (A-B) related to Figure 7





Supplementary Figure Legends

Supplementary Figure 1 (A-F) related to Figure 1. Tissue collection and characterization (A) Tumour and tumour-adjacent breast tissue (TAT) samples were obtained from patients undergoing mastectomy (**Supplementary Table 1**) according to the geographic tissue mapping described by Gao et al. (Gao et al., 2010). (B) Comparison of the CFC frequencies in Lin^- TAT-near and TAT-far cells from patients#7, 8, 9, 10 and 14 (Supplementary Table 1). Values shown are the mean±SEM. (C) The colony forming cell frequency in 14 Lin^- reduction mammoplasty samples (A) and 15 Lin^- TAT samples (D) were plotted against the patient's age. The best fit line was used to show correlation between CFC frequencies and patient's age. (E) Average CFC frequencies from (A) and (B). Values shown are the mean±SEM (*** = $p<0.0005$). (F) $EpCAM^{low}$ and $EpCAM^{high}$ expressing cells from Lin^- reduction mammoplasty (RM) and the TAT samples were placed in matrigel cultures for 14 days. The black arrows show multi-branched rudimentary breast structures and the blue arrows show very basic multi-branched structures. Photomicrographs are representative of 3 different patient samples and the red bars represent 1000 µm.

Supplementary Figure 2 (A-C) related to Figure 2. TAT-derived fibroblasts show activated phenotype. (A) photomicrographs of different sources of fibroblasts (3 normal and 3 matched TAT and tumour samples from patients#3, 4 and 6, Supplementary Table 1) grown in sub-confluent conditions. Expression of alpha smooth muscle actin (αSMA) and the Fibroblast-specific Protein 1 (S1004/FSP1) proteins are shown using immunofluorescent antibody staining. The cell nuclei were visualized using DAPI staining (scale bar represents 400µm). The representative pictures from 3 independent experiments with 3 independent fibroblast samples for each group are shown. Fibroblasts from remaining 2 TAT samples (Patients#9 and 10, Supplementary Table 1) also showed the activated phenotype (Data not shown) (**B**) Frequency of αSMA^+ cells in cells from (A) is shown. Values shown are the mean±SEM (**C**) Transcript expression for *αSMA*, *VIMENTIN*, *N CADHERIN*, *E CADHERIN*, Fibroblast activation protein (*FAP*) and *FSP1* was measure via qPCR. Average transcript expression normalized to the *GAPDH* expression and values shown are the mean±SEM. All values are compared to the transcript expression in the NAFs (* = $p<0.05$, ** = $p<0.005$, *** = $p<0.0005$).

Supplementary Figure 3 (A-C) related to Figure 2. Bipotent and luminal progenitors expand in the 3-D matrigel cultures. (**A**) Lin^- reduction mammoplasty (RM) samples were placed in co-cultures with 3T3 fibroblasts in 3D matrigels (A), 2D culture plates (B) or in mammosphere cultures (C). Day 0 or passage 0 indicate input CFC numbers and out CFCs were obtained on the indicated days. Values shown are the mean±SEM obtained from 3 RM samples. In (A), input CFC = 1.

Supplementary Figure 4 (A-D) related to Figure 2. T**he contralateral non-tumour bearing breast fibroblasts show normal characteristics. (A)** Photomicrograph of matrigel cultures initiated with Lin^- reduction mammoplasty (RM) samples with the contralateral non-tumour bearing breast fibroblasts (CNTB-F) obtained from patients#4, 6 and 9 (Supplementary Table 1) and 3 NAFs are shown (Scale bar represents 1000µm). (**B**) Expression of CD49f and EpCAM in the Matrigel cultures from (A) are measured via Flow cytometry. Representative histograms are shown and average mean fluorescent intensities (MFI) are plotted in the bar graph. Values shown are the mean±SEM obtained from 3 RM samples. (**C**) The progenitor cell expansion was measured by comparing output CFCs from matrigel cultures in (A) to the input CFCs. Values shown are the mean±SEM obtained from 3 RM samples. (D) Shows the frequencies of CFC subtypes in the Lin^- CNTB samples assessed in (C). Values shown are the mean±SEM (** = $P<0.005$, ***$P<0.0005$).

Supplementary Figure 5 (A-I) related to Figure 3, 4 and 5. TGFβ signaling targets both bipotent and the luminal progenitors. (A) The TGFβ receptor-1 (*TGFβR1*) transcript expression in 5 normal-associated (NAF), 3 tumour-associated (TAF) (from patients#3, 4 and 6, Supplementary Table 1), and the 6 tumour-adjacent tissue (TAT-F) fibroblasts (from patients#3, 4, 6, 9, 10 and 15, Supplementary Table 1) was quantified using qPCR. Transcript levels relative to *GAPDH* levels are shown. Values shown are the mean±SEM. (B) Matrigel cultures were initiated with 3 different Lin^- reduction mammoplasty (RM) samples and treated with various doses of TGFβ or vehicle control. On the indicated days output CFCs were obtained and compared to the input CFCs. Values shown are the mean±SEM based on 3 samples. (C) Matrigel cultures were initiated with 3 different Lin^- reduction mammoplasty (RM) samples and treated with various doses of TGFβ or vehicle control. On the indicated days % of (propidium iodide) PI^+ cells in the $CD49f^+EpCAM^+$ and $CD49f^+EpCAM^{-/low}$ populations were determined by flow cytometry. Values shown are the mean±SEM based on 3 samples. (D-E) Total and phosphorylated SMAD4 protein

expressions were measured by intracellular flow cytometry in 3 different Lin^- reduction mammoplasty (RM) samples treated with either TGFβ or TGFβ and SB431542 for 24 hours. Values shown are the mean±SEM based on 3 samples. (**F**) Expression of TGFβ receptor 1 (TGFβR1) in the normal bipotent and the luminal progenitors was examined using flow cytometry. Mean fluorescent intensities (MFI) from progenitors obtained from 3 reduction mammoplasty (RM) samples are shown as the mean±SEM. (**G-I**) Matrigel assays were initiated with the luminal and bipotent progenitor-enriched sub-populations of RM cells and exposed to TGFβ for 14 days. The transcript expression of TGFβ-target genes (G) or epithelial to mesenchymal (EMT) marker genes (H-I) were ascertained using qPCR. Values shown are the mean±SEM of the transcript expression relative to the *GAPDH* based on 3 experiments (* = $p<0.05$, ** = $p<0.005$, *** = $p<0.0005$).

Supplementary Figure 6 (A-D) related to Figure 6. Lenti shCD49f and lenti shEpCAM virus successfully knockdown CD49f and EpCAM protein expression in primary cells. (A) Graphical representation of SMAD4 consensus DNA binding site in the proximal promoter/enhancer regions of CD49f and EpCAM genes. TSS = Transcription Start Site. (**B**) 3 different Lin^- reduction mammoplasty (RM) samples were infected with shCD49f and shEpCAM lenti virus and the expression of CD49f and EpCAM proteins in the transduced (GFP+) cells was obtained using flow cytometry. (C) A representative histogram showing the mean fluorescent intensity (MFI) levels of CD49f and EpCAM proteins. (D) Values shown are the mean±SEM based on 3 experiments (*** = $p<0.0005$).

Supplementary Figure 7 (A-B) related to Figure 7. Fibroblasts are present in xenografted breast cancer tumours. (**A**) photomicrographs (scale bar represents 400μm) of histological sections prepared from MDA-MB-231 (MDA231) tumour xenografts generated in immunodeficient mice with fibroblasts from different sources (3 normal and 3 matched TAT and tumour samples from patients#3, 4 and 6, Supplementary Table 1). Expression of a *αSMA*, Fibroblast Specific Protein 1 (*FSP1*/*S1004*) or *CD31* was determined immunofluoroscently. DAPI staining was used to stain the nucleus. White arrows show $CD31^+$ cells inside the tumours. (**B**) GFP+ MCF7 or MDA231 cells were placed in *in vitro* co-cultures with fibroblasts from the different sources (3 normal and 3 matched TAT and tumour samples from patients#3, 4 and 6, Supplementary Table 1) and number of GFP+ cells was determined on the indicated days using flow cytometry. Values shown are the mean±SEM based on 3 experiments (* = $p<0.05$, ** = $p<0.005$, *** = $p<0.0005$).

Supplementary Table 1. Collection of Primary Breast Tumour and Tumour Adjacent Tissues from Patients undergoing Reconstructive Breast Surgery following Mastectomy

Patient ID	Age	Histological Type and Grade	Estrogen Receptor Status	Progesterone Receptor Status	HER2 Receptor Status	Lymph Node Metastasis Status
Patient#1	41	Invasive ductal carcinoma grade 3 Stage: pT3N3	Negative	Negative	Equivocal	Positive
Patient#2	37	Invasive ductal carcinoma grade 3 Stage: mpT2N1a	Negative	Negative	Positive	Positive
Patient#3	50	Invasive ductal carcinoma. Grade 3 Stage: pT2N0.	Negative	Negative	Negative	Negative
Patient#4	43	Invasive ductal carcinoma grade 2 Stage: pT3N2a.	Positive	Positive	Positive	Positive
Patient#5	59	Invasive ductal carcinoma grade 3 Stage: pT2pN3.	Positive	Positive	Negative with some overexpression	Positive
Patient#6	44	Invasive ductal carcinoma grade 2 Stage: pT3N0sn	Positive	Positive	Positive	Negative
Patient#7	47	Invasive ductal carcinoma grade 2 Stage: pT2N1	Negative	Negative	Negative	Negative
Patient#8	49	Invasive ductal carcinoma grade 3 Stage: pT1cN1a.	Positive	Positive	Positive	Positive
Patient#9	60	Invasive lobular carcinoma grade 2 Stage: pT3N2a	Positive	Positive	Negative	Positive
Patient#10	66	Invasive ductal carcinoma grad 3 Stage: pT1cN0sn	Negative	Negative	Negative	Positive
Patient#11	58	Invasive lobular carcinoma grade 3 Stage: pT2N3	Positive	Positive	Positive	Positive
Patient#12	45	Invasive ductal carcinoma grade 3 Stage: pT3N3	Positive	Positive	Negative	Positive
Patient#13	62	Invasive ductal carcinoma grade 3 Stage: pT3N3	Positive	Positive	Negative	Positive
Patient#14	49	Invasive ductal carcinoma. Grade 2 Stage: pT3N1c	Positive	Positive	Positive	Positive
Patient#15	57	Invasive ductal carcinoma grade 3 Stage: pT2N0	Positive	Positive	Positive	Positive

Supplementary Experimental Procedures:

Tissue sample collection

Breast tumour tissue and TAT samples were obtained from patients undergoing mastectomy procedures who had not received any neoadjuvant chemotherapy and/or radiation treatment. All TAT samples were examined histologically by a certified pathologist and were deemed to be disease-free and histologically benign. TAT samples were obtained from 15 patients with invasive cancers (Supplementary Table 1). In the cases where the primary breast tumour was >2 cm, a sample of the breast tumour was also obtained. TAT samples were categorized as ER^+ or ER^- according to the pathology reports of the associated tumour tissue. Contralateral tumour-free breast tissue was also obtained from 3 patients with ER^+ tumours undergoing prophylactic bilateral mastectomies. Reduction mammoplasty tissue obtained from 14 healthy pre- and postmenopausal women was used as a source of normal mammary cells. In all cases, tissues samples were placed in transport media (1:1 vol/vol, Ham's F12: Dulbecco's modified eagle's medium [DMEM] supplemented with 5% bovine serum, insulin and penicillin/streptomycin) within 1 hour of surgery and transported on ice to the laboratory for processing. TAT samples removed 3-6 cm away from the primary tumour margin are referred to as "far", and from 1-3 cm as "near" (Supplementary Figure 1). All samples were obtained with written informed patient consent according to protocols approved by the University of Manitoba's Research Ethics Board.

Tissue dissociation and cell separation

Tumour samples were minced with scalpels and dissociated enzymatically and mechanically for 16 hours in Ham's F12 and DMEM dissociation media (1:1 vol/vol F12 to DMEM supplemented with 2% wt/vol bovine serum albumin (BSA), 300 U/ml/ collagenase, 100 U/ml hyaluronidase, 10 ng/ml epidermal growth factor (EGF), 1 mg/ml insulin, and 0.5 mg/ml hydrocortisone (all from Sigma)). The dissociated cells were pelleted via centrifugation and then treated with red blood cell lysis buffer as per the manufacturer's protocol (BD Biosciences) prior to being resuspended and cryopreserved in 6% dimethylsolfoxide (DMSO)-containing fetal bovine serum (FBS)-supplemented medium and stored in liquid nitrogen. Reduction mammoplasty tissue, TAT, and matched contralateral tumour-free tissue were processed as previously described(Raouf and Sun, 2013; Stingl et al., 2005). Briefly, tissues were minced, dissociated overnight, and an organoid-rich fraction, an epithelial-endothelial enriched fraction, and a fibroblast-enriched fraction obtained by differential centrifugation. The organoid-enriched fractions were then further dissociated enzymatically as described(Raouf and Sun, 2013; Raouf et al., 2008).

Primary fibroblasts cultures

Tumour-associated fibroblasts (TAFs) were obtained from cryopreserved breast tumour samples. Briefly, cell pellets (2 from ER^+ tumours and 1 from a triple negative tumour) were thawed and single-cell suspensions obtained as for normal tissue. The cells were then filtered through a 40-µm mesh (BD Biosciences) and up to $3x10^6$ cells placed in 10 cm tissue culture plates in DMEM/F12 media supplemented with 10% FBS. After 4 hours at 37°C the medium and non-

adherent cells were removed and fresh medium added. Fibroblasts from either 5 normal mammoplasty and 5 TAT samples (3 matching the primary tumours used to derive TAFs and 2 additional fibroblast lines, one from tissue adjacent to ER^+ tumour and one from tissue adjacent to ER^- tumour) were cultured to 70-75% confluence and passaged at least twice to obtain near homogeneity, as shown by microscopic examination and immunofluorescent detection of smooth muscle actin and S100A4, and an absence of EpCAM, CD31 and CD41 expression in any cells.

Matrigel cultures

50 µl of liquid growth factor-reduced matrigel (BD Biosciences) was placed in each well of a 96-well plate and 50 µl of PBS added on top to prevent evaporation while the gels were allowed to polymerize at 37°C for 30 minutes. The un-polymerized gels were then removed, washed with PBS. $2x10^5$ cells placed on top of each gel and plates were then incubated for up to 21 days with SF7 medium plus 70 µg/ml bovine pituitary extract which was then replaced every 3 days. Matrigels were dissolved in dispase and made into single-cell suspensions. In some experiments, the medium was supplemented with 2.5 ng/ml TGFβ (Cat#T7039, Sigma,) or 10 µM of the SB431542 SMAD4 blocker (Sigma Cat#S4317), or vehicle control (PBS or DMSO, accordingly). For some experiments 10^5 single cells were mixed with 10^5 fibroblasts and placed in matrigel cultures as described(Basak et al., 2015; Makarem et al., 2013). On the indicated days, gels were dissolved (in dispase for 45 minutes at 37°C)and cell aggregates were made into single-cell suspensions as described. For sorted progenitors, $5x10^4$ luminal or bipotent progenitors were used without fibroblasts.

Flowcytometric analyses and cell separation

Single-cell suspensions from organoid-enriched fractions obtained from the RM or the TAT samples were pre-blocked in 2% FBS-containing Hank's Balanced Salt Solutions (HBSS) supplemented with 10% human serum for 15 minutes. Subsequently cells were labeled with an allophycocyanin (APC)-conjugated rat antibody to human CD49f (clone GOH3, Biolegend) and fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal antibody to human EpCAM (clone VU-1D9, StemCell Technologies). Propidium iodide (PI, 1 mg/ml, Sigma) exclusion was used to identify the dead cells and CD49f and EpCAM expression was analyzed using a Guava EasyCyte 8HT Flow cytometer (Millipore). To obtained bipotent and luminal progenitors from the reduction mammoplasty samples, pre-blocked single-cell suspensions were depleted of non-epithelial cells by removing CD31+ and CD45+ cells (Lin^-) using a negative EasySep magnetic separation kit (StemCell Technologies). The Lin^- cells were then stained with PI, anti-CD49f, and anti-EpCAM antibodies and the bipotent (PI-$CD49f^{bright}EpCAM^{low}$) and luminal (PI-$CD49f^{low}EpCAM^{bright}$) progenitor-enriched fractions isolated using a fluorescent activated cell sorter (FACS, MoFlo XDP, Beckman Coulter) as described(Basak et al., 2015).

In vitro colony-forming cell assays

Colony-forming cell (CFC) assays were performed as previously described(Basak et al., 2015; Raouf et al., 2008). Briefly, 5,000 Lin^- cells were plated together with 80,000 irradiated mouse NIH 3T3 cells in SF7 medium supplemented with 5% FBS. After 8–10 days, colonies were fixed

with a 1:1 vol/vol mixture of methanol and acetone on ice and the stained with crystal violet (Sigma). Colony types and numbers were obtained using a bright field microscope. In some cases, colonies were stained with cytokeratin 8/18 and cytokeratin 14 antibodies and detected by secondary fluorescently-conjugated antibodies to simultaneously identify mixed colonies (positive for both cytokeratins) or pure luminal colonies (positive staining for cytokeratin 8/18 only).

Secretome analysis of the primary fibroblast cells

The CM samples were concentrated with Amicon Ultra-15 Centrifugal Filter Unit with 10kDa molecular weight cut off as per manufacturer's protocol. Each sample was denatured, reduced and alkylated using an on-filter digestion procedure as described previously(Wisniewski et al., 2009) and peptides fragments were analyzed via 2-dimensional liquid chromatography (LC) mass spectroscopy (MS) using a splitless nanoflow 2D LC Ultra system. Raw spectra files were converted into Mascot Generic File format (MGF) for peptide/protein identification by X!Tandem search algorithm. Proteins that had at least two different proteotypic peptides with Log(e) < – 2.0 (i.e. 99%) peptide match score were selected for further analysis.

Breast cancer cell lines

The ER^+PR^+ MCF7 and the (ER^-PR^- $claudin^{low}$) MDA-MB-231 cells were obtained from the American Type Culture Collection (ATCC) and grown in DMEM media supplemented with 5% or 10% FBS accordingly, as per ATCC guidelines. Cells were passaged before reaching 75% confluence.

Immunofluorescent staining

For fibroblast characterization, the cells were grown in 6-well culture plates until 80% confluent and cells were fixed and permeabilized with 100% methanol in -20°C for 20 minutes. Subsequently plates were washed with PBS and blocked with Dual endogenous blocking solution (DAKO) for 30 minutes on ice. Plates were then stained with mouse anti-human Smooth Muscle Actin (αSMA) (at a 1:400 dilution, Abcam, Cat#ab5694) and rabbit anti-human S100A4 (at a 1:400 dilution Abcam, Cat#ab124805) antibodies for 1-1.5 hours at room temperature and washed with PBS. αSMA or S100A4 expression was detected via anti-mouse FITC and anti-rabbit Cy3 conjugated secondary antibodies and nuclear staining was obtained using PI or DAPI. IgG matched control antibody was used as negative control and all plates were examined using EVOS Fluorescence Microscope.

Immunofluorescence to examine TGFβ and TGFβ receptor (TGFβR1) protein expression was performed on sections fixed in 4% buffered formaldehyde, and then dehydrated in graded alcohol concentrations, cleared in xylene, and subsequently embedded in paraffin. Deparaffinized sections were then microwaved for 20 minutes in 10 mmol/L sodium citrate (pH 6.0) and incubated with dual endogenous enzyme block (DAKO) for another 20 minutes. Next, the sections were incubated with rabbit anti-human TGFβ (1:400, Abcam) or rabbit antihuman TGFβR1 (1:100, Abcam) for 60 minutes at room temperature. The sections were then washed 3x with PBS and stained with Cy3 conjugated anti-rabbit secondary antibody and subsequently with DAPI to visualize the nucleus. As negative controls, IgG matched control antibody was used

instead of the primary antibody. For dual-color immunofluorescence staining, colonies were briefly fixed inside each culture dish with 500 µl of 1:1 vol/vol acetone and methanol and pre-blocked in Tris-buffered saline containing 5% wt/vol BSA and 10% FBS. Subsequently, plates were stained with unconjugated mouse monoclonal antibody raised against human Cytokeratin 8/18 (1:300, Abcam, Cat#ab17139) and rabbit monoclonal antibody raised against human cytokeratin 14 (1:400, Abcam, Cat#ab48576). Protein expression was obtained using anti-mouse FITC- and anti-rabbit Cy3-conjugated secondary antibodies and the nuclei visualized by DAPI staining.

Intracellular Flowcytometry

Intracellular flowcytometry was done as per protocol described (Basak et al., 2015). Briefly, cells were fixed and permeabilized using BD Perm and Fix Kit (BD-Biosciences, using the manufacture's protocol). Thereafter, cells were stained with anti SMAD4 (Aviva systems biology) and/or pSMAD4 antibodies (Aviva systems biology) and analyzed by Guava flow-cytometer (Millipore). The data was further analyzed using the FlowJo software.

Quantitative real-time PCR

Total RNA was extracted from fresh, FACS-purified or cultured cells using the Trizol reagent (Invitrogen) and cDNA prepared from 1 µg of this RNA using the Maxima cDNA synthesis kit (Thermo Fisher, Canada) which was then used as a template for PCR (CFX Connect 96, Bio-Rad). Transcript expression of specific genes was obtained using gene-specific primers. Relative expression levels of each of the test transcripts were calculated by normalizing to the *GAPDH* transcript levels.

Western blot analysis

Cells were lysed in 2% sodium dodecyl sulfate (SDS) buffer with complete protease inhibitor tablets (Roche Diagnostics). Protein lysates were then quantified using the bicinchoninic acid assay kit (Bio-Rad) and 90 µg of total protein then size fractionated and transferred onto PVDF membranes (Millipore). Specific protein levels were determined using rabbit anti-human TGFβ (1:1000; Cell Signaling, Cat#3711), anti-human TGFβR1 (1:500; Abcam, Cat#ab31013) and mouse anti-human beta actin (1:10,000; Sigma, cat#WH0000060M1) antibodies by chemiluminescence. The expression level of each protein was determined using beta actin as the loading control. The average expression of each protein was obtained from 3 independent samples.

Lentiviral infection of primary human breast epithelial cells

Single cells (10^6) from 3 different reduction mammoplasty samples were placed in 2-dimensional co-cultures with $1.6x10^5$ NIH3T3 fibroblasts as described(Basak et al., 2015) and 16-18 hours later, cells were infected with 10^7 lenti-viral particles prepared from a pool of 4 different pGIPZ-puro-GFP lenti-plasmids containing short hairpin (sh)RNAs to target CD49f and EpCAM transcripts or pGIPZ-puro-GFP expressing a scrambled shRNA fragment. Cells were allowed to

recover for 2 additional days and the GFP+ cells then isolated by FACS and placed in CFC assays as described(Basak et al., 2015; Raouf et al., 2008).

Mouse xenograft assays

10^6 MDA-MB-231 cells were mixed with 10^6 fibroblasts and re-suspended in a 1:1 (v/v) mixture (200 µl total volume) of SF7 and matrigel. Cells were then injected into the flank of 6-8 week-old female BALB/c (H-2d) $RAG2^{-/-}$ IL-$2R\gamma c^{-/-}$ immunodeficient mice(Weijer et al., 2002). As controls, 10^6 MDA-MB-231 cells or 10^6 fibroblasts were injected into the flank of other mice. Tumour growth was monitored every 3 days with digital calipers. Tumour volume (in mm^3) was calculated as length × $(width)^2$ × 0.5. Animal maintenance was performed in accordance with the animal care guidelines of the University of Manitoba, Canada. All the animal experiments were approved by the Animal Care and ethical Committee of the University of Manitoba, Canada.

qPCR primers

Gene ID	Forward Primer	Reverse Primer
FSP1	TCTTGGTTTGATCCTGACTGC	TGAGCTTGAACTTGTCACCCT
FAP	CTGACCAGAACCACGGCTTA	AGGCTTGCATCTGCATCGTT
NEDD9	TCAGTGCAGAGAAGCATTGG	GATGAGGGAGGGATGTCGTA
SMAD7	TGCTCCCATCCTGTGTGTTAAG	TCAGCCTAGGATGGTACCTTGG
SERPINA1	CACCGTGAAGGTGCCTATGATG	GGCATTGCCCAGGTATTTCATC
αSMA	CTGGACGCACAACTGGCATCGTGC	CTCGGCCAGCCAGATCCAGACGCA
VIMENTIN	CAGGAGGCAGAAGAATGGTACAAATCCAAG	CTGTCTCCGGTACTCAGTGGACTC
N-CADHERIN	GAGCAGATAGCCCGGTTTCATTTGAGG	CCTGGTGTAAGAACTCAGGTCTGTTGTC
E-CADHERIN	CTGGATAGAGAACGCATTGCCACATACAC	GGCTTGTTGTCATTCTGATCGGTTACC
ZEB1	GCACAAGAAGAGCCACAAGTA	GCAAGACAAGTTCAAGGGTTC
TWIST	GTCCGCAGTCTTACGAGGAG	TGGAGGACCTGGTAGAGGAA
SNAIL	CTCTAGGCCCTGGCTGCTACAAGG	ATCTGAGTGGGTCTGGAGGTGGGC
SLUG	GCATTTCTTCACTCCGAAGC	TGAATTCCATGCTCTTGGAG
TGFβR1	TCAGCTCTGGTTGGTGTCAG	ATGTGAAGATGGGCAAGACC
GAPDH	GCCTCCCGCTTCGCTCTC	CCGTTGACTCCGACCTTCACC

Supplementary References

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