SI MATERIALS and METHODS

Culture Conditions. For assays characterizing the resistance of cells to cytotoxic compounds, cells were seed at a density of 8 x 10^3 per well of 96-well plates. After 24 hours, medium was replaced with fresh growth medium (100 µl/well) containing the indicated concentrations of Paclitaxel (Sigma) dissolved in DMSO. Cell viability was assessed after 96 hours using the CellTiter96 Aqueous One Solution Cell Proliferation Assay (Promega).

Mammosphere cultures were performed as described [1], with the exception that culture medium contained 1% methylcellulose to reduce cell aggregation [2]. One thousand cells were plated per well into 96-well plates and cultured for 7-10 days, with fresh medium replaced every 3 days. Following incubation, mammospheres were photographed and spheres with a diameter greater than 75 µm were counted. To test effects of sunitinib, 1000 cells were plated in 96-well low attachment plate in 100 µl mammosphere media. At 24 hours and 96 hours, 100 µl of mammosphere media containing 10 µM sunitinib was added to each well. Spheres were quantified after 7 days.

To determine the effects of sunitinib on cell growth, 2 x 10^5 cells were placed in a 6-well tissues culture dish. After 24, hours the media was replaced with growth media containing 2.5 µM sunitinib, and the media was replace every 2 days. On the sixth day of treatment, viable cells per well was quantified using Beckman-Coulter Vi-Cell Viability Analyzer.

Cell type specific media used for breast cancer cell lines:

Cell Line	**Basal Media**	**Additives***
MCF-7	DMEM/F12	10% FBS
ZR75B	DMEM/F12	10% FBS

MDA-MB-468	RPMI 1640	5% FBS
MDA-MB-231	RPMI 1640	5% FBS
BT-20	RPMI 1640	10% FBS
HCC38	RPMI 1640	10% FBS
Hs578T	DMEM	10% FBS
SUM149	F12	5% FBS/Ins/Hyd
SUM159	F12	5% FBS/Ins/Hyd
MCF10A	DMEM/F12	5% Horse serum/Ins/Hyd cholera toxin
MDA-MB-436	DMEM	10% FBS
T47D	RPMI 1640	10% FBS
BT549	RPMI 1640	10% FBS
BT474	RPMI 1640	10% FBS
MDA-MB-435	DMEM	10% FBS

* All media contains penicillin (50 units/ml) and streptomycin (0.1 ug/ml).

Ins = Insulin; Hyd = Hydrocortisone

Western Blotting and Immunofluorescence. For western blotting proteins were isolated by lysing cells in ice-cold radio immunoprecipitation (RIPA) buffer containing protease and phosphatase inhibitors (Roche). Protein was quantified using the Bradford Assay (BioRad) and 50 µg of total protein was resolved using 4-12% Bis-Tris SDS-PAGE gels (NuPage, Invitrogen) and transferred to PVDF membranes. Membranes were probed with primary antibodies. Following incubation with horseradish peroxidase-conjugated secondary species specific antibodies, immunoreactive proteins were detected using chemiluminescence (ECL Plus, GE Healthcare). Immunofluorescent staining of cells was performed as previously described [2]. For 3D lrECM cultures, following fixation in methanol+acetone 1:1 cells were labeled with LamininV primary antibody (Millipore Cat #AB13012) and detected with an Alexa-594 secondary antibody. Nuclei were visualized with DAPI and slides were mounted with DAKO (#S3023). All the images were acquired through an Olympus DSU spinning disc confocal microscope and analyzed at MDA Flow Cytometry and Cellular Imaging Core Facility (NCI # CA16672).

Plasmids and Viral Transduction. HMLE derived cell lines; HMLE-Snail, HMLE-Twist, HMLE-GSC and HMLE-TGF-β1 were generated using retroviral transduction of cells using the pWZL-Blast construct encoding the relevant cDNA and selection in 4 µg/ml of blasticidin (Invitrogen). HMLER-FOXC2 and HMLER-Snail cells were generated in two steps, via which 1) HMLER cells were first generated by the transformation of HMLE cells by infection with the retroviral MSCV-H-RasV12-IRES-GFP vector (Addgene plasmid #18780) and FACS isolation of GFP-positive cells after 2 weeks of culture; and 2) stable expression of human FOXC2 and Snail cDNA following infection of HMLER cells with pWZL-Blast-FOXC2 and pWZL-Blast-Snail vectors and selection with 4 µg/ml blasticidin to generate HMLER-FOXC2 and HMLER-Snail cell lines, respectively. To suppress FOXC2 expression the shRNA-expressing pLKO lentivirus system was used (OpenBiosystems). The FOXC2 shRNA targeting sequences is

CCTGAGCGAGCAGAATTACTA (pLKO5) and GCGGGAGATGTTCAACTCCCA (pLKO4). The shRNA sequences targeting firefly luciferase (shCntrl) or GFP in the pLKO vector were used as controls. The stable suppression of target genes was achieved by selection of cells in 2 μg/ml of puromycin.

Three Dimensional (3D) Laminin-rich Extracellular Matrix (lrECM) On-Top Cultures. Cells in culture were trypsinized and seeded at a density of 2.5 x 10^3 cells per well on top of a thin gel of Engelbreth-Holm-Swarm tumor extract (Matrigel; BD Biosciences) in 8-well chamber slides (Falcon), with cells suspended in propagation medium containing 5% Matrigel. The propagation medium for HMLE derived cells was MEBM containing insulin and hydrocortisone (pituitary extract was not included) and for SUM159 cells was F12 media containing 5% FBS, insulin and hydrocortisone. Every 4 days, the top layer was replaced with fresh propagation medium containing 20 ng/ml PDGF-BB (BD Biosciences) and 2.5% Matrigel. Cultures were maintained for 10-14 days, after which point cells were fixed and stained as previously published.

Fluorescence-Activated Cell Sorting. The PE- and APC-conjugated anti-CD44 (clone G44-26) and FITC-conjugated anti-CD24 (clone ML5) antibodies used for FACS analysis were obtained from BD Biosciences. The PECy7-conjugated anti-CD24 (clone ML5) was purchased from BioLegend. In all instances, the antibodies were used for FACS analysis in accordance with the manufacturer's protocols. Briefly, 1 x 10^6 cells in PBS + 2% FBS (FACS buffer) were stained with the indicated antibodies for 30 minutes on ice. Following extensive washing, cells were resuspended in 500 μl of FACS buffer and analyzed on a BD FACSCanto II Flow Cytometer.

Quantitative Reverse Transcription PCR (qRT-PCR). Total RNA was isolated using the RNeasy Plus kit (Qiagen) according to manufacturer's instructions. Complementary DNA was synthesized using Moloney Murine Leukemia virus reverse transcriptase (Invitrogen) following the manufacturer's instructions. The specific primers sequences used for SYBR Green qRT-PCR analysis are detailed below. Quantitative RT-PCR was performed on an Applied Biosystems 7900HT Sequence Detection System (Perkin-Elmer) equipped with a 96-well optical reaction plate. All qRT-PCR experiments were run in triplicate and a mean value was used for the determination of mRNA levels. Relative quantifications of the mRNA levels was performed using the comparative Ct method with GAPDH as the reference gene and with the formula $2^{-\Delta\Delta Ct}$.

Primers used for qRT-PCR:

Gene	**Forward Primer**	**Reverse Primer**
hGAPDH	ACCCAGAAGACTGTGGATGG	TCTAGACGGCAGGTCAGGTC
hFOXC2	GCCTAAGGACCTGGTGAAGC	TTGACGAAGCACTCGTTGAG
hE-cadherin (CDH1)	TGCCCAGAAAATGAAAAAGG	GTGTATGTGGCAATGCGT TC
hN-cadherin (CDH2)	ACAGTGGCCACCTACAAAGG	CCGAGATGGGGTTGATAATG
hFibronectin (FN1)	CAGTGGGAGACCTCGAGAAG	TCCCTCGGAACATCAGAAAC
hVimentin (VIM)	GAGAACTTTGCCGTTGAAGC	GCTTCCTGTAGGTGGCAATC
hSnail (SNAI1)	CCTCCCTGTCAGATGAGGAC	CCAGGCTGAGGTATTCCTTG

Transwell Migration and Invasion Assays. Cells were serum starved for 24 hr, trypsinized and seeded at a density of 5 x 10^4 cells/well into the upper well of 24-well Transwell inserts in serum-free medium. Cells were allowed to migrate for 16 hrs using 10% FBS or PDGF-BB (20 ng/ml) in serum-free media in the lower well chamber. Following incubation, non-migrated cells were removed from the upper membrane surface and migrated cells on the lower side were fixed and stained using Diff Quick (IMEB INC Cat# K7128) and quantified by counting cells from 5 captured images per well.

Microarray Gene Expression Analysis. Total RNA was isolated using the RNeasy Mini Kit (Qiagen) from HMLER-Vector and HMLER-FOXC2 cells in triplicate samples and sent to SeqWright (Houston, TX, USA) for sample processing and gene expression analysis using Affymetrix Human Genome U133 Plus 2.0 arrays as part of the SeqWright Gene Expression Service Suite. The resultant .CEL files were preprocessed and differential gene expression analysis performed using the Bioconductor package (www.bioconductor.org). To generate a FOXC2 GES we used a supervised machine learning approach described previously [3], where the expression values for the three vector replicates constituted the negative training set and the three FOXC2 replicates were the positive. Using the SIGNATURE platform [3], we scored the activation of the FOXC2 GES in a data set from 51 breast cancer cell lines (ArrayExpress accession E-TABM-157) [4] (Figure 5d), clinical breast cancer tumors (GSE18229) [5] (Figure 5c) as well as primary tumors and brain metastases for the MDA-MB-231 and CN34 xenograft models (GSE12237) [6] (Figure 5a and b). Using the same strategy, we generated GES's for Twist, Gsc, Snail, and TGF-β1 mediated EMT in HMLE cells (Figure 5f) using expression data we previously described (GEO accession GSE24202) [7]. As GSE18229 was generated on the Agilent platform, we converted the probes to Affymetrix by selecting the probes that target the

same Entrez Gene. From this data set, we selected only the breast tumor samples, leaving 337 samples.

Chromatin Immunoprecipitation. Chromatin immunoprecipitation (ChIP) was performed as previously described [8]. Antibodies against the following proteins were used for immunoprecipitation: normal sheep IgG (5μl; 12–369, Upstate), Foxc2 (10 μl; N-20, Santa Cruz). To analyze specific antibody-bound DNA fractions, quantitative real time PCR was performed using Power SYBR Green (Applied Biosystems). The percentage of the input that was bound was calculated by the formula $2\wedge(Ct1\%$ of input - Ct,IP), averaged over at least three experiments and graphed as average and standard error using GraphPad Prism v5.0 (GraphPad Software, Inc.). Subtraction of percent bound of a control IgG immunoprecipitation accounts for background of nonspecific interactions.

Supplemental Figure Legends

Supplemental Figure 1. FOXC2 does not regulate the expression of other EMT factors. Quantification of EMT marker mRNA expression by quantitative real-time PCR analysis. Data is represented as the expression of EMT markers in HMLER-FOXC2 (FOXC2) cells relative to HMLER control cells GAPDH was used as an internal normalization control. Columns indicate the mean (n = 3); error bars indicate SEM.

Supplemental Figure 2. Ectopic expression of FOXC2 induces EMT in transformed human mammary epithelial cells (HMLER). **(a)** Quantification of EMT marker mRNA expression by quantitative real-time PCR analysis. Data is represented as the expression of EMT markers in HMLER-FOXC2 (FOXC2) cells relative to HMLER-Vector control cells (Vector). GAPDH was

used as an internal normalization control. Columns indicate the mean (*n* = 3); error bars indicate SEM. **(b)** Western blot analysis of EMT marker protein expression upon FOXC2 overexpression in HMLER cells. Actin was used as a loading control. **(c)** Phase contrast and immunofluroescence images of HMLER-Vector and HMLER-FOXC2 cells. Overlayed images are shown for respective EMT markers (red) and DAPI nuclear stain (blue). Scale bar indicates 50 μm.

Supplemental Figure 3. Attenuation of FOXC2 expression leads to reduced levels of PDGFR-β. **(a)** Immunofluorescene staining for PDGFR-β (red) detected with Alexa Fluor 548 anti-mouse secondary antibody in HMLE, HMLE-Snail-shCntrl and HMLE-Snail-shFOXC2 cells. Nuclei were counterstained with DAPI and overlayed images are shown.Scale bar indicates 100 μm. **(b)** FACS analysis of PDGFR-β cell surface expression using anti- PDGFR-β-PE antibody in cells described in (a). The histogram represents the intensity of PE signal in the FL2 detector on the *x*-axis.

Supplemental Figure 4. Cells expressing endogenous FOXC2 exhibit increased sensitivity to sunitinib **(a)** Quantification of HMLE-Snail and **(b)** SUM159 cell viability expressing either shControl or shFOXC2, following culture for 96 hours in increasing concentrations of sunitinib. Cell viability was assessed using an MTS assay. **(c)** *In vitro* quantification of mammospheres formed by cells described above grown in the presence of sunitinib. Data represents the number of mammospheres formed per 1000 cells seeded after 7 days of culture. Error bars indicate SEM. * $P<0.05$.

Supplemental references:

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