Supplemental Document 1

Statistical Methods

Raw mRNA counts for each experiment from the nCounter platform were normalized first to the geometric mean of onboard positive controls, followed by subtraction of the mean of onboard background controls and then normalized to the geometric mean of the housekeeping genes to adjust for sample content. Samples that failed quality control diagnostics, specifically normalization factors more than three standard deviations from the mean, were repeated and statistical methods were applied to the log-transformed data.

Gene expression following treatment was measured as mRNA counts and analysis of variance models were fit to estimate treatment effect. For each target gene, tests for treatment effect and pairwise comparisons were made at each time point within each cell line and, when appropriate, between cell lines. For each hypothesis tested across all target genes, p-values were adjusted controlling for a 5% false discovery (1).

Analysis of variance models were fit to the mRNA counts from the HCI-013 xenografts for each target gene to compare means between groups. Main effects were tested for time (Day 2 and Day 5) and treatment (+E2 and –E2) and an interaction term was included if significant. In the absence of Day 10 (+E2) data, exploratory pairwise comparisons were performed between Day 10 (-E2) and Day 5 (-E2) and between Day 10 (-E2) and Day 5 (+E2). Similar methods were applied to the Ki67 proliferation index values; two subjects in the –E2 groups, one at Day 2 and one at Day 10, were excluded from the analysis due to poor tissue quality.

Tumor growth rates from the HCI-013 xenografts were modeled using a linear mixed model to account for repeated measures within subject. Tumor volume measurements were log-transformed and growth curves were fit from the time of measurable tumor until time of sacrifice. In addition, time until measurable tumor was compared between groups by analysis of variance methods. One subject in the +E2/intact group was excluded from the analysis due to lack of tumor growth.

Statistical analysis was performed using R version 2.15.2 (2012) (2) and the normalization of nCounter data was performed with R package NanoStringNorm (3).

PDX HCI-013

In addition to the primary ILC tumor, this patient had also developed metastases to the bone, liver, lung, brain and pericardium. Prior therapy included: leuprolide, letrozole, exemestane, tamoxifen, and zoledronic acid (2004-2007); cyclophosphamide, methotrexate, and 5-fluorouracil (2008); paclitaxel, and doxorubicin (2009); carboplatin, and gemcitabine (2010). Taqman-based copy number analyses (Hs01904554_cn, Hs06220320; Life Technologies) demonstrate that HCI-013 does not carry an amplification at the FGFR1 locus (data not shown).

IHC was performed on FFPE sections of xenograft tumors by Magee-Womens Hospital Histology using standard clinical methods. To assess Ki67, stained sections were blinded, and three fields per slide were imaged with a Nikon Eclipse 90i microscope at 20X. Automated counting using NIS-Elements AR (v3.22.11, Nikon) gated color intensity for Ki67 positive cells or all cells. Non-tumor cells were gated using size filters.

Supplemental Methods

Microarray data processing

Raw intensity data were normalized by the Robust Multi-array Average (RMA) method (4) using the 'affy' package in R-Bioconductor. Student's t-tests were performed in R–Bioconductor to identify genes differentially expressed between Vehicle-treated samples and E2-treated samples at each time-point. Probe level expression values were condensed to individual genes by choosing the individual probe with the lowest q-value per timepoint. The list of genes significantly differentially expressed (fdr 5%) at each of the time points was further filtered to remove genes with less than 50 units of mean expression in both sample groups. Additionally, probes not annotated to known or putative genes were not considered in downstream analyses; updated annotation was downloaded from the Affymetrix website in July 2012.

Immunoblotting

Cells were lysed in 5% SDS and sonicated for Western blot analyses. Primary antibodies used were: ERα (6F11, Novocastra/Leica, Germany), E-cadherin (HECD-1, Life Technologies), FGFR1 (sc-121, Santa Cruz Biotechnology), Actin (A5441, Sigma). Blots were imaged using an Odyssey Imaging System (LI-COR, Lincoln, NE).

Binding site motif analyses

ER binding peaks for MCF-7 and MM134 were assessed for enriched motifs by MEME-ChIP (5) using JASPAR (6) and TRANSFAC (7) databases as the reference. The E-value significance threshold of motif discovery was set to 0.05 searching for enriched motifs in a width range of 9-25bp; motifs were only reported that had q-values for matching known motifs of <0.05.

Primers for qPCR and ChIP-PCR

qPCR Primers	Fwd	Rev
GREB1a	AAATCGAGGATGTGGAGTG	TCTCACCAAGCAGGAGGAG
MYC	GCTGCTTAGACGCTGGATTT	GAGTCGTAGTCGAGGTCATAGT
IGFBP4	ACGAGGACCTCTACATCATCC	GTCCACACACCAGCACTTG
PDZK1	GGTAGACAAAGAGACGGACAAC	GACTTCCAGAGAAGTGGGAGTA
CXCR4	AGCAGGTAGCAAAGTGACG	CCTCGGTGTAGTTATCTGAAGTG
CCNG2	GTTTGGATCGTTTCAAGGCG	CCTCTCCACAACTCATATCTTCAC
TFF1	GTGCAAATAAGGGCTGCTGTT	CAGATCCCTGCAGAAGTGTCTA
CXCL12	CCAAACTGTGCCCTTCAGAT	CCAGGTACTCCTGAATCCACTT
CA12	CTCCTGCTGGTGATCTTAAAGG	GGGTACTTCTTGGACCAGCTAT
NEDD9	TGTAGGAAAACGGCTCAACC	CCCTGTGTTCTGCTCTATGAC
RPLP0	TAAACCCTGCGTGGCAATC	TTGTCTGCTCCCACAATGAAA

ChIP-PCR primers	Fwd	Rev	Genomic loci (hg18)
NFERE	AACCTCGCTATGCTCCCTTC	TGTGTGCCTTTCATCTGGAG	chr22:27413218-27413359
GREB1	GTGGCAACTGGGTCATTCTGA	CGACCCACAGAAATGAAAAGG	chr2:11590101-11590213
TFF1	CCGGCCATCTCTCACTATGAA	CCTCTTTCCCATGGGAGTCTC	chr21:42659943-42660068
WNT4	GAATTCCACATCACCTCCGT	TCCAATGGCTGTTGCTATGA	chr1:22340715-22340832

TFCP2L1	GTCACAGAGCCAGGAGAAAG	TGGTTGCAGCAGCAGAT	chr2:121734643-121734740
PDE4B	AGCAGGAGACAGAGTTGTATTG	GCACCTTAGGTCTTCGATGTTA	chr1:66504630-66504723
NR3C2	TGCAGCCTTCAACAAGGG	CGGAGGGAAATAGAAGGCTAGA	chr4:149355581-149355674

References

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Supplemental Figure Legends

Supplemental Figure 1. E2 treatment modulates expression of canonical ER target genes in ILC cells. (A), Cells were treated as described for Figure 1 +/- 1μM ICI. Gene expression was assessed by qPCR; data shown as mean log2 fold change for E2 vs vehicle treatment of 3 biological replicates. Red, increased expression; Green, decreased expression. **(B)**, MM134 cells were hormone deprived and treated with increasing concentrations of E2 as indicated for 24 hours. Gene expression was assessed by qPCR. Points represent average of biological duplicate +/- SD.

Supplemental Figure 2. Motif analysis of ER binding sites in MCF-7 versus MM134 cannot identify co-factor sites in distal ILC-specific binding sites. (A-B), Motif output for indicated group of ER binding sites. Only sites with q-values for enrichment and identification <0.05 were considered. Q-value for enrichment is indicated. **(A)**, Motif enrichment in all identified sites. **(B)**, Motif enrichment in distal sites only, i.e. those >20kb away from a gene body. **(C)**, Motif enrichment in MM134 ER binding sites within 20kb of an ILC-specific E2-regulated gene.

Supplemental Figure 3. Tamoxifen and tamoxifen metabolites 4OHT and endoxifen act as agonists specifically in ILC cells. (A-B), Breast cancer cells grown in full serum conditions (10% FBS) were treated using 1μM of the indicated anti-estrogen (see bottom of heatmap). Heatmap values shown as log2 fold change versus d0 untreated control. Samples and genes were clustered by Pearson complete correlation. Boxes represent the mean of biological triplicate. **(A)**, MCF-7, **(B)**, MM134. Dashed line on sample key (bottom) indicates separation of highest level cluster separation on sample tree (top). Genes denoted with a '+' are commonly E2-regulated target genes. **(C)**, Gene expression in MM134 (log2 fold change versus d0 control) per treatment and time point for all genes in indicated clusters. Points represent mean expression of n genes +/- SD. Indicated p-value calculations represent treatment effect by two-way ANOVA.

Supplemental Figure 4. Induction of cell death in specific to FGFR1 inhibitors. (A), Breast cancer cells grown in standard conditions were assessed by Western blot. **(B-C)**, Hormone-deprived MM134 cells were treated with 0.1% EtOH vehicle, 1μM 4OHT, or 100pM E2 +/- increasing concentrations of kinase inhibitor. Cell proliferation was assessed at 6 days post-treatment. Points represent mean of 6 biological replicates +/- SEM. Dashed line represents growth observed in hormone-deprived, vehicle-treated cells (i.e. –E2). **(D)**, MM134 cells were hormone-deprived and treated with 0.1% EtOH vehicle, 1μM 4OHT, or 100pM E2 +/- 10μM lapatinib. Cell death was assessed by repeated reads of 6 biological replicates at the indicated time post-treatment. Points represent mean +/- SEM. **(E),** Hormone-deprived MCF-7 and MM134 cells treated with 0.1% EtOH vehicle, 1μM 4OHT, or 100pM E2 +/- 1μM CDK4/6 inhibitor PD0332991. Cell proliferation was assessed at 6 days post-treatment.