Supplemental Methods
Breast Cancer Research and Treatment

Patient-derived luminal subtype breast cancer xenografts contain common and unique estrogen receptor dependent gene expression signatures

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Supplemental Materials and Methods

Hierarchical clustering to determine molecular subtype

All microarray procedures including total cellular RNA isolation, amplification, and Cy3/Cy5 labeling of 1.0 µg of experimental and reference RNA, and intrinsic subtyping were done as previously described (references 37 and 38). In brief, microarray hybridizations were performed using Agilent human oligonucleotide 4x44K custom designed gene chips. Clustering analyses were performed using Cluster v2.12. Colored squares in figures containing cluster analyses indicate the relative transcript abundance, with higher expression in red and lower expression in green.

Analysis of microarray data

Partek data analysis. Data (CEL files) were imported into Partek Genomics Suite v6.6® and RMA normalized. To generate *p* values, ANOVA was used to compare PE4 (estrogen (E) vs. placebo), and PT12 (E vs. placebo, E+Tamoxifen vs. E, and EWD vs. E). Genes lists were created by filtering on $p<0.05$, and >1.5 fold up- or down- regulated, and annotated genes only. Venn comparisons were used to cross compare estrogen regulated genes in PE4 and PT12, and endocrine therapy (tamoxifen and EWD) regulated genes in PT12. Publically available data sets available in GEO database for MCF7 cells (GSE848, GSE3834) were imported into Partek. Although there are multiple reports on MCF7 gene regulation, these represent the most comprehensive experiments measuring estrogen and tamoxifen regulated genes *in vitro*, performed from two independent laboratories (references 21, 22, 38). In GSE3834, the estrogen regulated genes *in vivo* were determined by 24-48 h EWD. For GSE848, data were transposed to log2 RMA normalization. For comparison to our *in vivo* studies, only the longer timepoints (24, 48 h) were analyzed. In particular, ANOVAs were performed for MCF7 cells treated with vehicle vs. 48 h E, or 24 h E plus 4-hydroxytamoxifen (TOT) vs. E (GSE848), and for MCF7 cells vehicle vs. E for 24 h, and MCF7 tumors E treated vs. 48 h EWD (GSE3834). To generate a broad list of *in vitro* estrogen- regulated genes, a union of genes regulated by estrogen $p<0.05$, >1.5 fold was generated for MCF7 cells from both data sets, which yielded 793 up- and 1272 down- regulated genes. Venn diagrams were used to generate lists of genes that were cross- regulated by the broad MCF7 data set and PE4 and PT12 tumors, filtering on annotated genes only. Individual lists of E+TOT vs. E

(GSE848) and EWD vs. E (GSE3834) were used to compare to endocrine therapy regulated genes in PT12. For comparison with endocrine therapies, Venn comparisions were made between PT12 (E+Tam vs. E; EWD vs. E) and MCF7 (E+TOT 24 h vs. E) and MCF7 (EWD 48 h vs. E).

Ingenuity pathways analysis. Genelists ($p<0.05$) were imported into the Ingenuity® software program. These represented placebo vs. E from PE4 and PT12, and E+Tam vs. E and EWD vs. E for PT12. For MCF7 cells, filtered lists ($p<0.05$, >1.5 fold change) from either GSE848 or GSE3834 were imported and analyzed. Estrogen signaling was among the top pathways significantly changed in both PE4 and PT12, and these diagrams were exported for presentation in Figure 5D. The top (greatest fold) pathway significantly changed between placebo and estrogen treated tumors PE4 and PT12 were exported for presentation in Figure S6. A diagram of the top changed genes/networks in Tam vs. E and EWD vs. E treated PT12 were exported for presentation in Figure 6D.