SUPPLEMENTAL MATERIALS:

S1. Expression vector and stable transfection.

The pCMX-RORα1 plasmind was digested with the restriction enzymes *Kpn*I and *Bam*HI to obtain the full-length RORα1 cDNA which as then cloned into the *Kpn*I-*Bam*HI sites of the pcDNA3.1/Zeo (+) expression vector to generate the expression construct pcDNA3.1-RORα1 that was stably transfected into MCF-7 cells. The pcDNA3.1/Zeo (+) (pcDNA3.1-vt) vector was used to transfect MCF-7 cells, which were then used as controls in our studies for RORα1 transfected cells. MCF-7 cells were plated on 100 mm cell culture dishes at a density of 1.5×10^6 cells per dish in RPMI-1640 medium supplemented with 10% FBS. Cells were transfected with 6 μg of pcDNA3.1-RORα1 or pcDNA3.1-vt, using lipofectamine following the manufacturer's instructions. Following transfection, cells were allowed to recover in RPMI-1640 medium supplemented with 10% FBS for 48 h. Cells were split 1:5 onto 100 mm cell culture dishes in a selective medium (RPMI-1640 medium containing 10% FBS and 100 μg/ml zeocin) and to grow for 14 days. During this period, the cells were re-fed with fresh selective medium every 3 days. Individual zeocin-resistant cells were isolated and cloned using cloning cylinders.

S2. Transient transfection and luciferase transcriptional reporter assays.

Parental MCF-7 cells were plated onto 35 mm 6-well plates at a density of 2×10^5 cells/well in RPMI-1640 medium supplemented with 10% FBS. After 24 h of serum-starvation, cells were transfected in serum-free RPMI-1640 medium for 6 h with 2 μg of the RORE$\alpha 2_3$-tk-LUC reporter construct or 1 μg of pCMVβ control plasmid per well, using 6 μl of lipofectamine (Life Technologies, Inc.). The RORE$\alpha 2_3$-tk-LUC reporter construct contains three copies of the ROREα2 upstream of the thymidine kinase promoter and luciferase gene. Following transient transfection, cells were rinsed twice and re-fed with 2 ml RPMI-1640 medium supplemented with 10% FBS. Cells were then harvested with 200 μl cell lysis buffer. Total cellular protein concentration was measured by Bio-Rad Bradford protein assay and β-galactasidase activity was measured by the *o*-nitrophenyl β-D-galactopyranoside (ONPG) assay. Luciferase was measured using a Model 2010 luminometer (Analytical Luminescence) and luciferase activity was normalized to both the protein concentration and the β-galactosidase activity.

S3. cDNA microarray analysis.

RNA from the MCF-vt, MCF-ROR#3, and MCF-ROR#8 cell clones was isolated by RNeasy Mini kits (Qiagen, Valencia, CA) following the manufacturer's instructions. The cDNA microarray analysis was performed by the Gene Therapy Center (Tulane University, employing Affymetrix Gene Chip microarray (Affymetrix, Santa Clara, CA). In brief, eight micrograms of total RNA was used to synthesize double-stranded DNA (Superscript Choice System/GIBCO/BRL Life Technologies, Rockville, MD). The DNA was purified and In vitro transcription was conducted to produce biotin-labeled cRNA using a BioArray HighYield RNA Transcription Labeling Kit (Enzo Diagnostics, Farmingdale, NY). Twenty-five micrograms of biotinylated cRNA was fragmented to 50 to 200 nucleotides and hybridized for 16 h at 45° C to Affymetrix HG-U133A array, which contains 22,215 oligonucleotides representing 15,003 human genes. After washing, the array was stained with streptavidin-phycoerythrin (Molecular Probes, Eugene,

OR). Staining signal was amplified by biotinylated anti-streptavidin (Vector Laboratories, Burlingame, CA) and by a second staining with streptavidin-phycoerythrin, and then scanned on a Hewlett-Packard Gene Array Scanner. The expression data were analyzed using Microarray Suite 5.0 (MAS5.0).

S4. Quantitative real time PCR (qPCR).

qPCR was performed using an iCycler iQ apparatus (Bio-Rad) associated with the iCycler Optical System Interface software (version 2.3; Bio-Rad) as we described previously [x] The sequences of primers used are shown in below. For the analysis of AKR1C1 and AKR1c3 mRNA expession, PCR analysis was performed in triplicate in a volume of 20 μL, using 96-well optical-grade PCR plates and an optical sealing tape (Bio-Rad). The differences in the expression of all the gene transcripts were normalized with respect to GAPDH expression. The thermal cycling conditions used an initial DNA denaturation step at 95 °C for 8 min followed by 35 cycles of denaturation at 95 °C for 15 s, annealing and extension at 60 °C for 1 min. The relative level of expression was calculated with the formula $2^{-\Delta ct}$.

S5. Table 1. q PCR primers.

AKR1C1
forward 5'-GTAAAGCTTTAGAGGCCAC-3'
reverse 5'-ATAAGGTAGAGG TCAACATAA-3'

AKR1C3
forward 5'-GAAGTAAAGCTTTGGAGGTCA-3'
reverse 5'-GTCAACATAGTCCAATTGAGC-3',

GAPDH
forward 5'-CAACTACATGGTCTACATG-3'
reverse 5'- CTCGCTCCTGGAAGATG-3.