**Supplementary Methods**

**SILAC assays**

MCF-7 cells were grown for at least three passages in stable isotope labelling by amino acids (SILAC) phenol red-free RPMI media (Sigma R1780) containing 13C615N4 arginine (heavy medium) to fully label peptides. Cells were then grown in heavy medium supplemented with 5% dialysed CSS for 24 hours before washing with PBS and switching to standard phenol red-free RPMI medium containing unlabelled arginine and AZD9496, fulvestrant, tamoxifen, estradiol or DMSO. Compounds were incubated over a 48 hour period before protein lysates were prepared in lysis buffer. Equal concentrations of sample proteins spiked with internal standards (lysate from MCF-7 cells labelled with 13C6 lysine only) were immunoprecipitated overnight at 4oC using an anti-ERα monoclonal antibody (SP1, Thermo Fisher Scientific) and then digested with 0.4 μg trypsin in 50 mmol/L ammonium bicarbonate at 37oC overnight before analysing by mass spectrometry using relative peptide quantification by selected reaction monitoring (SRM). Degradation half-life was measured using the one-phase exponential decay equation in GraphPad PRISM (Y=Span.e-K.X+Plateau) where X is time and Y is response which starts out as Span+Plateau and decreases to Plateau with a rate constant K.

**Biacore affinity measurements**

For BIAcore affinity measurements a tetra-His antibody (Qiagen) was immobilised to a biacore CM5 biosensor chip in 20 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% T20 (HBS-T) running buffer and 6His-ERα protein captured in the presence of AZD9496. The association (kass) and dissociation (kdiss) rate constants and (KD) were calculated using BIAevaluation software (v4.1, Biacore, GE Healthcare) and used to fit the ERα LBD:AZD9496 interaction to a 1:1 Langmuir binding interaction model.

**Immunoblotting**

Cells were lysed in 25 mmol/L Tris/HCL pH6.8, 3 mmol/L EDTA, 3 mmol/L EGTA, 50 mmol/L NaF, 2 mmol/L sodium orthovanadate, 270 mmol/L sucrose, 10 mmol/L -glycerophosphate, 5 mmol/L sodium pyrophosphate and 0.5% Triton X-100 supplemented with protease inhibitors (Roche) and phosphatase inhibitors (Pierce) and proteins run on 4% to 12% Tris-HCl precast gels (Bio-Rad). Membranes were probed overnight with primary antibodies (ER, Thermo Fisher Scientific SP1; PR, Dako PgR636; GAPDH, CST 2118) followed by incubation with HRP-tagged secondary antibodies (CST 7074 or 7076) and visualized on a Syngene ChemiGenius with Super-Signal West Dura Chemiluminescence Substrate (Pierce).

**RNA extraction and HTA transcript analysis**

RNA was extracted from tissue samples using the miRNeasy kit (Qiagen) and Qiacube system as per manufacturer’s instructions. RNA quantity and purity was then determined using a Nanodrop spectrophotometer and mRNA levels measured using a human transcriptome assay (HTA2.0; Affymetrix). Normalisation of HTA array data was performed using Robust Multi-array Average (RMA) method and quality control analyses were run in the Affymetrix Expression Console software.  Differential gene expression analyses were subsequently carried out for each treatment versus its control in the three groups separately.  Statistical significance in gene expression differences between the treatments and the controls was assessed using ANOVA and set at a linear fold change >1.5 and p<0.05.

**Rat Uterine and Xenograft Studies**

MCF-7 cells (5 x 106 ) were implanted subcutaneously in the hind flank of immuno-compromised (SCID) male mice the day after each mouse was surgically implanted with a 0.5 mg/21 day estrogen pellet (Innovative Research, USA). HCC1428 LTED (10 x 106 cells) were implanted transdermally into the fourth mammary fat pad of immuno-compromised (NSG) female mice 7 days after they were surgically ovariectomized. CTC-714 PDX model was derived from patient CTC cultures, consented according to the Human Biological Samples Policy and purchased from Conversant Biologics. EpCAM+CD44+ cells were suspended in phosphate buffered saline (PBS) mixed with high concentration matrigel (BD Biosciences) at 10 mg/ml and ~ 650 cells were injected into the third mammary fat pad of a [NOD/SCID (Cg-Prkdcscid Il2rgtm1Wjl/SzJ](http://jaxmice.jax.org/strain/005557.html)) mouse. In tumour transplantation study, 2 × 2 mm pieces of tumour tissue from CTC-derived tumour xenografts were implanted in the mammary fad pad of Beige Nude XID mice. Tumour growth was calculated weekly by bilateral caliper measurement (length x width) and mice randomised into vehicle or treatment groups with approximate mean start size of 0.2 to 0.4 cm3 for efficacy studies or 0.5 to 0.8 cm3 for PD studies. Mice were dosed once daily by oral gavage or subcutaneous (s.c.) injection for fulvestrant at the times and doses indicated for the duration of the treatment period. Tumour growth inhibition from start of treatment was assessed by comparison of the mean change in tumour volume for the control and treated groups. Statistical significance was evaluated using a one-tailed Student *t* test. Tumours were excised at specific time points and fragments either fixed in 10% buffered formalin or snap-frozen in liquid nitrogen and stored at -80 oC and terminal bleeds plasma PK samples collected. Measurement of estradiol levels in mouse plasma from the MCF-7 xenograft model with implanted estrogen pellets was done using a custom-made immunoassay kit from Meso scale Discovery (MSD).

**Tumour protein analysis**

Tumour fragments were added to Cell Extraction buffer (Invitrogen: FNN0011) with added Sigma Phosphatase inhibitors (No. 2 (P5726) and 3 (P0044) 1 in 100 dilution) and Roche Complete (11836145001) protease inhibitor, 1 mM DTT and homogenised, sonicated, and centrifuged before protein quantification with Bradford Reagent (Bio-Rad). Equal protein loadings were run on Bis-Tris Criterion gels (4-12% Gels) using standard methods. Detection of ER and PR protein is as described in previous immunoblotting section. Vinculin protein levels were measured as a loading control using V931 Sigma (mouse) and anti-mouse HRP-linked antibody. An unpaired two-tailed *t* test was used to examine the statistical differences between the vehicle and treated groups.

**Rat Uterine Model**

Sexually immature female Han Wistar rats (Charles River) were randomised and dosed either with vehicle, AZD9496 or tamoxifen once daily for 3 days by oral gavage or a single s.c dose of fulvestrant. At 24 hours after the final dose of each agent was given, rats were euthanised, terminal plasma samples collected and uterine tissue removed with both horns intact, blotted dry and weighed. Protein extracts were prepared for immunoblot analysis as described for xenograft studies.

**Pharmacokinetic studies**

The pharmacokinetics of AZD9496 and its active mouse metabolite was investigated using combined data from multiple studies and analysed via population PK modelling. Specific PK studies consisted of IV bolus and PO doses of the parent, and IV bolus dose of the metabolite with multiple time points per animal, 2 animals per time point and were designed to establish a parent-metabolite pharmacokinetic model. The concentration of AZD9496 and its active metabolite in plasma samples was determined within AstraZeneca Oncology DMPK. Samples were analysed for parent and metabolite using LC-MS/MS detection using analytical standards over a final concentration range of 1 nM – 10,000 nM before being analysed using Masslynx and processed using Quanlynx.