**Supplementary Figure Legends**

**Figure S1.** ERα positive breast cancer cells are rescued from tamoxifen induced growth arrest by USP9X knockdown, but ERα negative breast cancer cells are not.

Knockdown of *USP9X* rescues tamoxifen-induced growth arrest in T47D cells. T47D cells were infected with the shRNA against *USP9X* recovered from the initial screen, pRS-USP9X II or pRS-GFP as control. Cells were cultured for 4-6 weeks in the presence of 1μM 4OH-tamoxifen. When colonies appeared cells were fixed and subsequently stained. Plate confluency was determined by calculating the number of cell-covered pixels related to the total surface area.

**Figure S2.** QPCR validation of ChIP-seq data at 45 minutes ligand treatment.

(A) Genome browser snapshots of ERα chromatin binding events at the RARA and NRIP1 loci. Cells were ligand treated for 48 hours, prior to fixation. sh*GFP* (blue) and sh*USP9X* (red) conditions were tested. Tag count and genomic coordinates are shown.

(B) ERα ChIP-QPCR validation of the genomic locations, indicated in A. Cells were ligand treated during 45 minutes prior to fixation. sh*GFP* (white) and sh*USP9X* (black) conditions were tested. Enrichment over negative control region was assessed. Error bar shows SD values from triplicate measurements.

**Figure S3.** Heatmap and quantifications for subsets of ChIP-seq data.

(A) Heatmap visualization of ERα chromatin binding events in sh*GFP* cells, separately analyzing binding sites shared or unique for vehicle, E2 or 4-OHT conditions, as indicated in Figure 4C. All peaks were vertically aligned and centered on the top of the peak, with a window of 5 kb. Number of binding sites for each subgroup are shown.

(B) Quantifications of read count at ERα chromatin binding sites for different subgroups of chromatin as shown in A, with a 2.5 kb window. Y-axis shows average tag count.

(C) Heatmap visualization of ERα chromatin binding events in sh*USP9X* cells, separately analyzing binding sites shared or unique for vehicle, E2 or 4-OHT conditions, as indicated in Figure 4C. All peaks were vertically aligned and centered on the top of the peak, with a window of 5 kb.

(D) Quantifications of read count at ERα chromatin binding sites for different subgroups of chromatin as shown in C, with a 2.5 kb window. Y-axis shows average tag count.

**Figure S4.** Ingenuity Pathway enrichment and GO analysis of 1874 sh*USP9X*-affected genes not associated with estrogen induction.

(A) Top canonical pathways

(B) GO analyses for top enriched functions (top) and processes (bottom). FDR and p values are indicated.

**Figure S5.** Survival analysis for 1874 sh*USP9X*-affected genes not associated with estrogen induction.

(A) The 1874 sh*USP9X*-affected genes, not associated with E2-treatment (see Fig. 5A), were tested as a classifier in two cohorts of ERα positive breast cancer patients who received adjuvant tamoxifen treatment (Loi: n=250; Buffa: n=134) ([35](#_ENREF_35), [36](#_ENREF_36)). Kaplan-Meier survival curves for distant metastasis free survival (DMFS) were generated, with p values indicated.

(B) Hazard rates with 95% confidence intervals, separately analyzing the Loi cohort (left) and Buffa cohort (right). The USP9X knockdown tamoxifen classifier was directly compared with the 1874 genes that were differentially expressed after sh*USP9X*, but not affected by E2 stimulation (see Figure 5A).

**Figure S6.** The USP9X knockdown tamoxifen classifier does not predict outcome after adjuvant chemotherapy in ERα positive disease.

The USP9X knockdown tamoxifen classifier was tested in a cohort of breast cancer patients (a subset from the 295 patients in the van de Vijver cohort (39)) with ERα positive breast tumors treated with adjuvant chemotherapy only. Kaplan-Meier survival curves for distant metastasis free survival (DMFS) were generated, with p values indicated.