

SUPPLEMENTARY MATERIAL

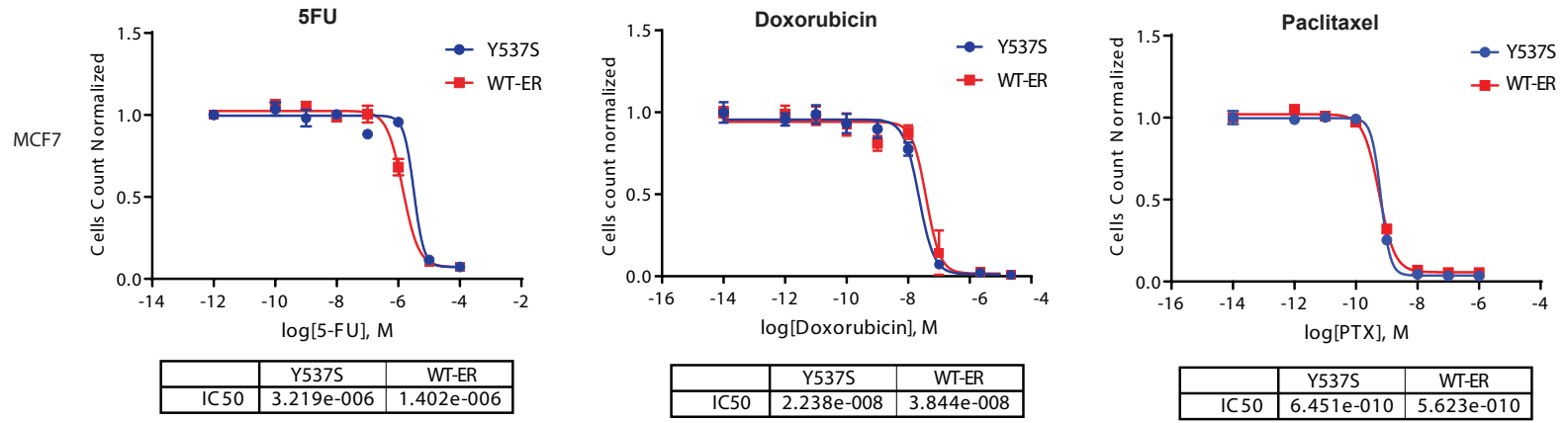
Pure Estrogen Receptor Antagonists Potentiate Capecitabine Activity in *ESR1*-mutant Breast Cancer

Supplementary Figures: 5

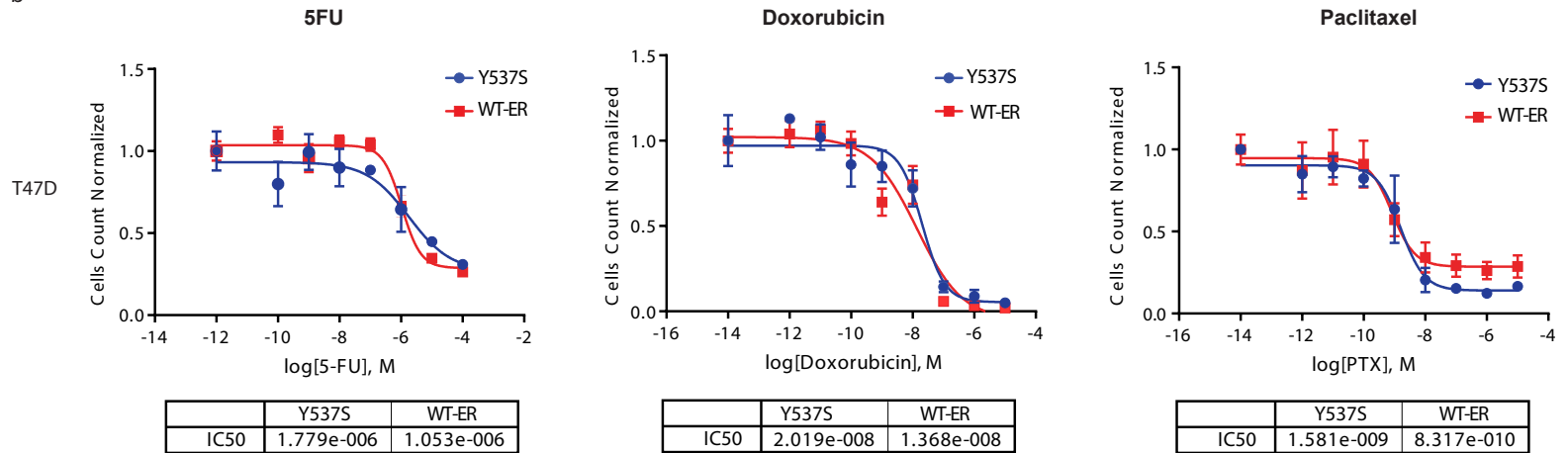
Supplementary Data Files: 2

Supplementary Figure 1. IC50 studies performed on **(a)** MCF7 cells expressing either WT ER or the doxycycline induced Y537S ER mutation, or **(b)** *TP53* mutant T47D cells expressing WT ER or the doxycycline induced Y537S mutation, treated with 5FU, doxorubicin or paclitaxel (PTX). **(c)** Quantification of the colony confluency of control or p53 knock-out MCF7 cells expressing WT ER or the doxycycline inducible Y537S mutation, following treatment with vehicle, 5FU or fulvestrant as single agents or fulvestrant in combination with 5FU. Data are shown as mean $\pm$ SE. *** $P < 0.001$. KO: knock out

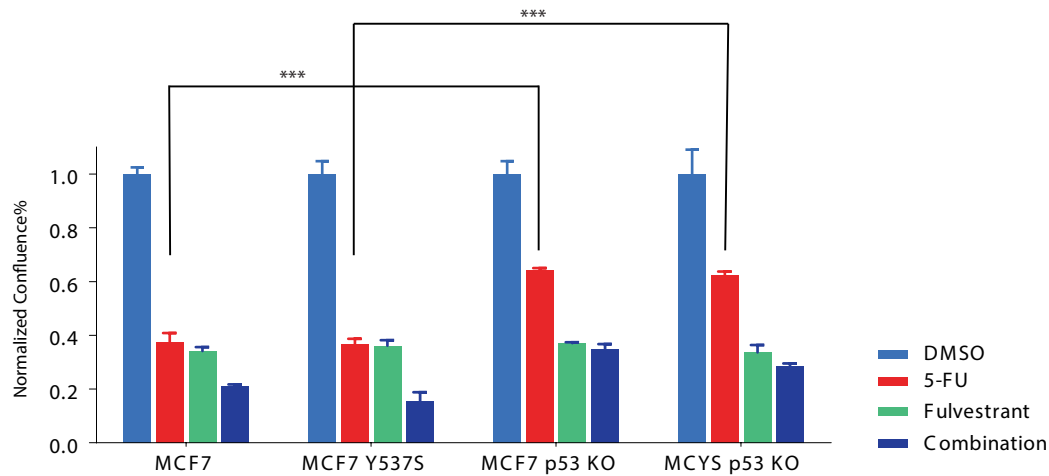
a



b



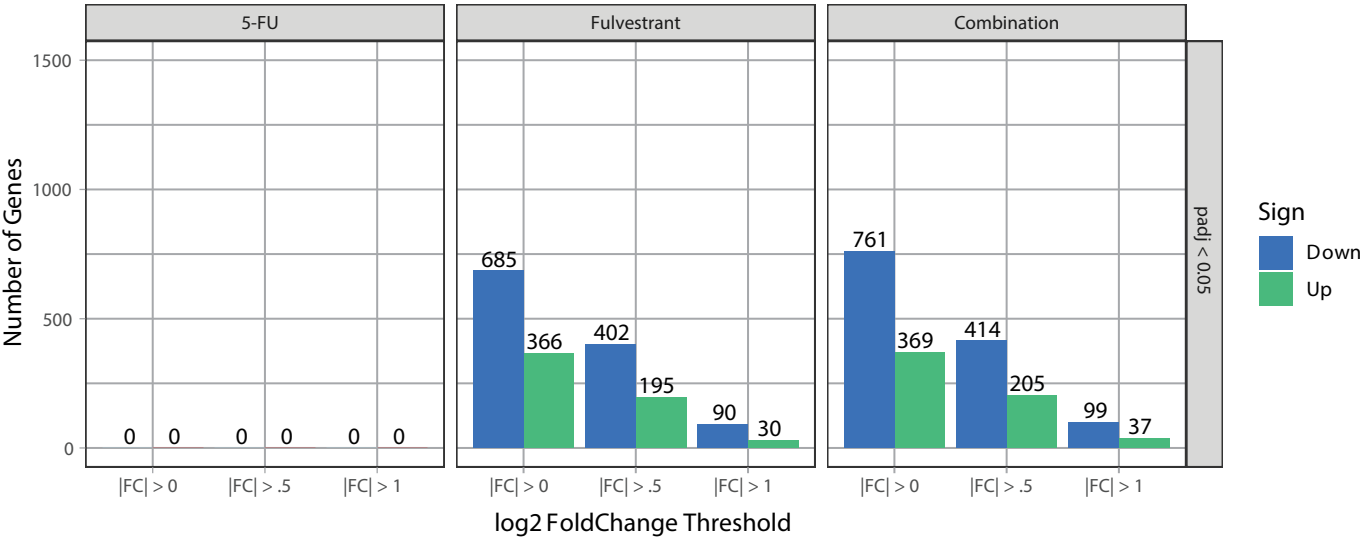
c



Supplementary Figure 2. (a-b) Number of downregulated (blue bars) and upregulated (green bars) genes in MCF7 cells without **(a)** or with **(b)** the expression of the Y537S mutation treated with 5FU, fulvestrant, or 5FU in combination with fulvestrant compared to vehicle treatment at different absolute log₂FC ($|FC| > 0$, $|FC| > 0.5$ or $|FC| > 1$) and FDR<0.05 thresholds. ER: estrogen receptor; WT: wild type; FC: fold change; FDR: false discovery rate.

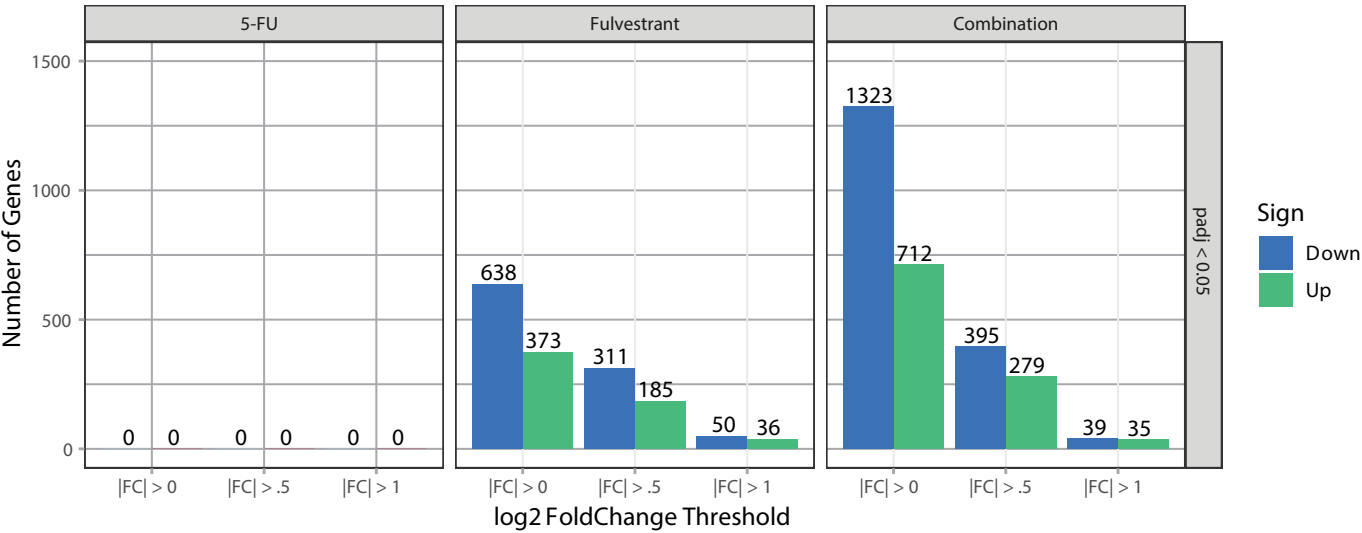
a

Differential Expression Summary
WT-ER Veh_vs_Treatment

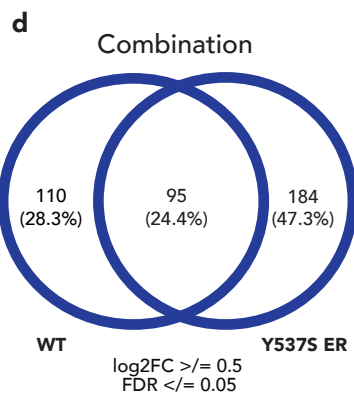
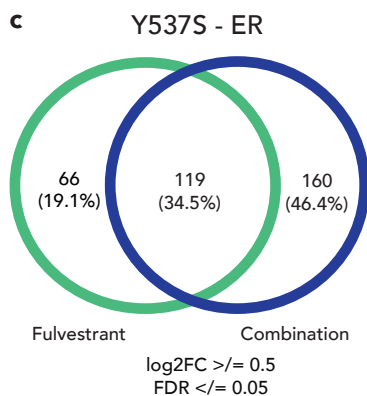
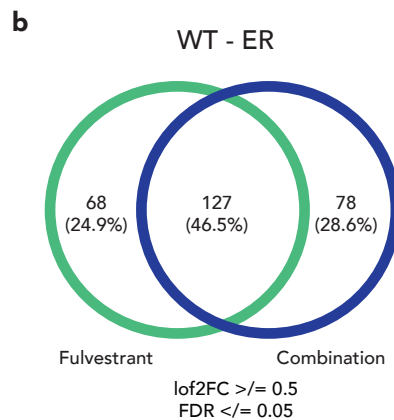
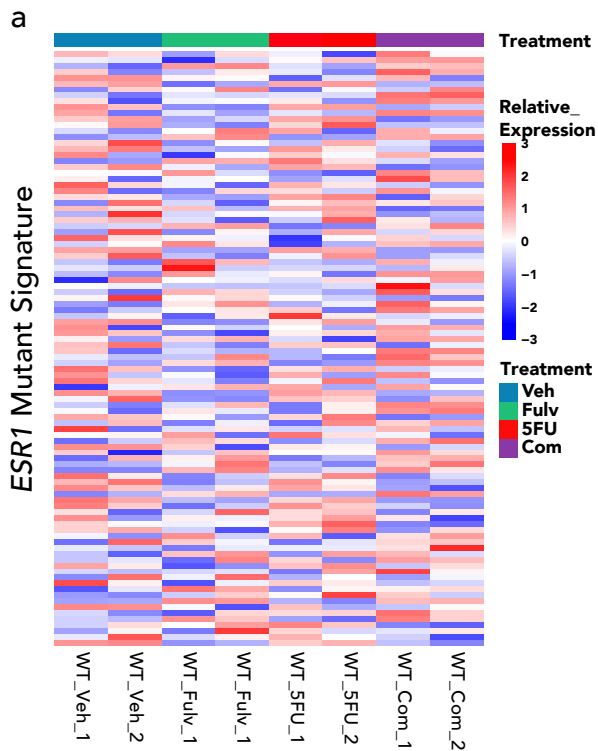


b

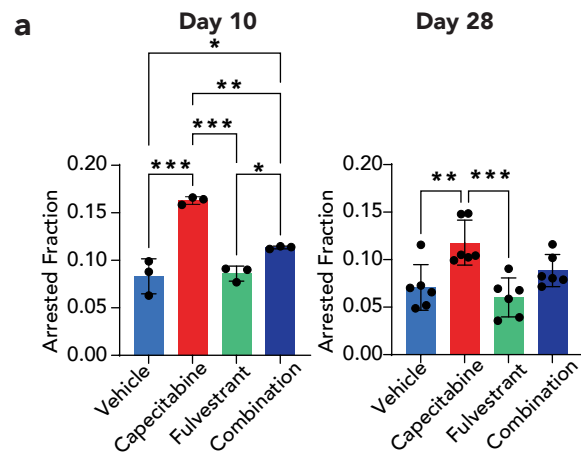
Differential Expression Summary
Y537S-ER Veh_vs_Treatment



Supplementary Figure 3. (a). *ESR1* mutant gene signature for MCF7 cells expressing WT ER treated with vehicle (Veh), 5FU, fulvestrant (Fulv), or 5FU in combination with fulvestrant (Com) showing relative expression values across samples (row centered and scaled $\log_2(\text{FPKM}+1)$ expressions) with rows and columns unclustered. *ESR1* mutant gene signature consists of the top 100 genes by $\log_2$ fold change up in Y537S mutant vehicle treated cells compared to WT vehicle treated cells. **(b-c)** Venn diagrams showing the number of genes upregulated in MCF7 cells without **(b)** or with **(c)** the expression of Y537S mutation treated with fulvestrant or 5FU in combination with fulvestrant compared to vehicle treated cells for $\log_2\text{FC} \geq 0.5$ and $\text{FDR} \leq 0.05$ filtering thresholds. **(d)** Venn diagram showing the number of genes upregulated in MCF7 cells treated with 5FU in combination with fulvestrant compared to vehicle treated cells between cells without (WT) or with (Y537S ER) expression of the Y537S mutant for $\log_2\text{FC} \geq 0.5$ and $\text{FDR} \leq 0.05$ filtering thresholds. ER: estrogen receptor; WT: wild-type; FC: fold change; FDR: false discovery rate.



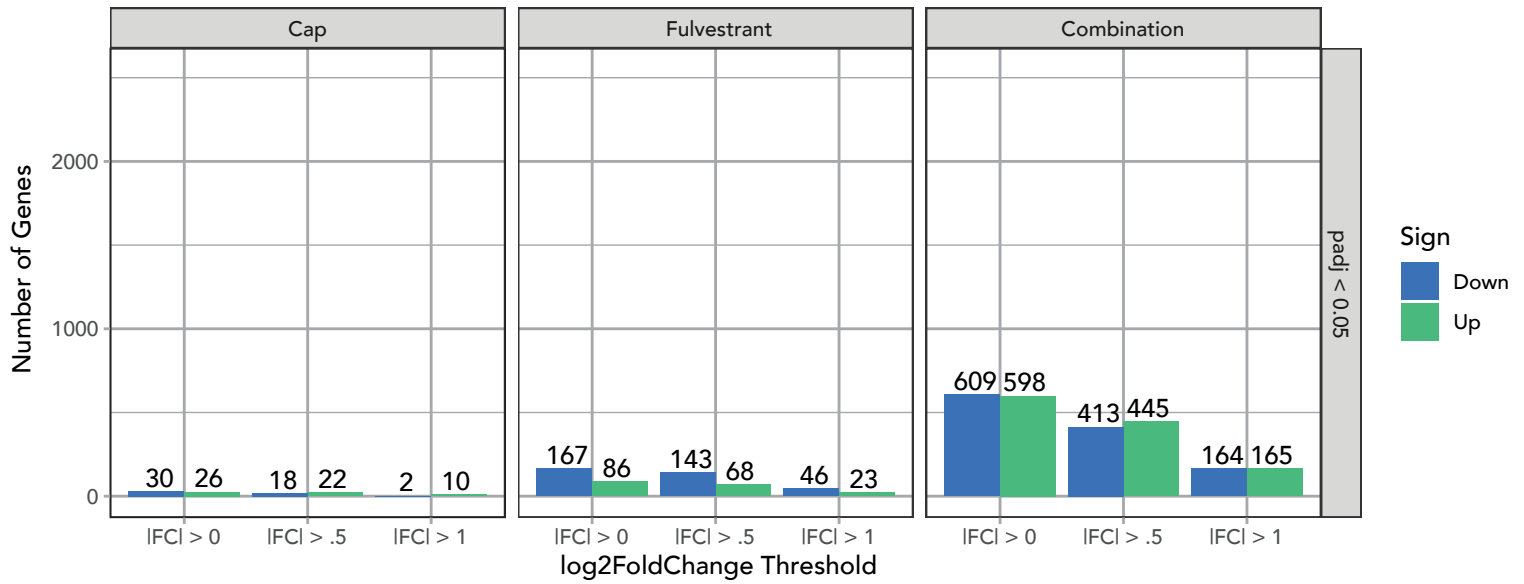
Supplementary Figure 4. (a) The arrested fraction of cells at day 10 and day 28 in the Y537S ER mutant (1526) patient derived xenograft model. **(b-c)** Number of downregulated (blue bars) and upregulated (green bars) genes in PDX tumors expressing the Y537S mutation treated with 10 days **(b)** and 28 days **(c)** of capecitabine (Cap), fulvestrant, or capecitabine in combination with fulvestrant compared to vehicle treatment at different absolute log2 fold change ($|FC| > 0$, $|FC| > 0.5$ or $|FC| > 1$) and $FDR < 0.05$ thresholds. PDX: patient-derived xenograft; ER: estrogen receptor; WT: wild-type; FC: fold change; FDR: false discovery rate.



b

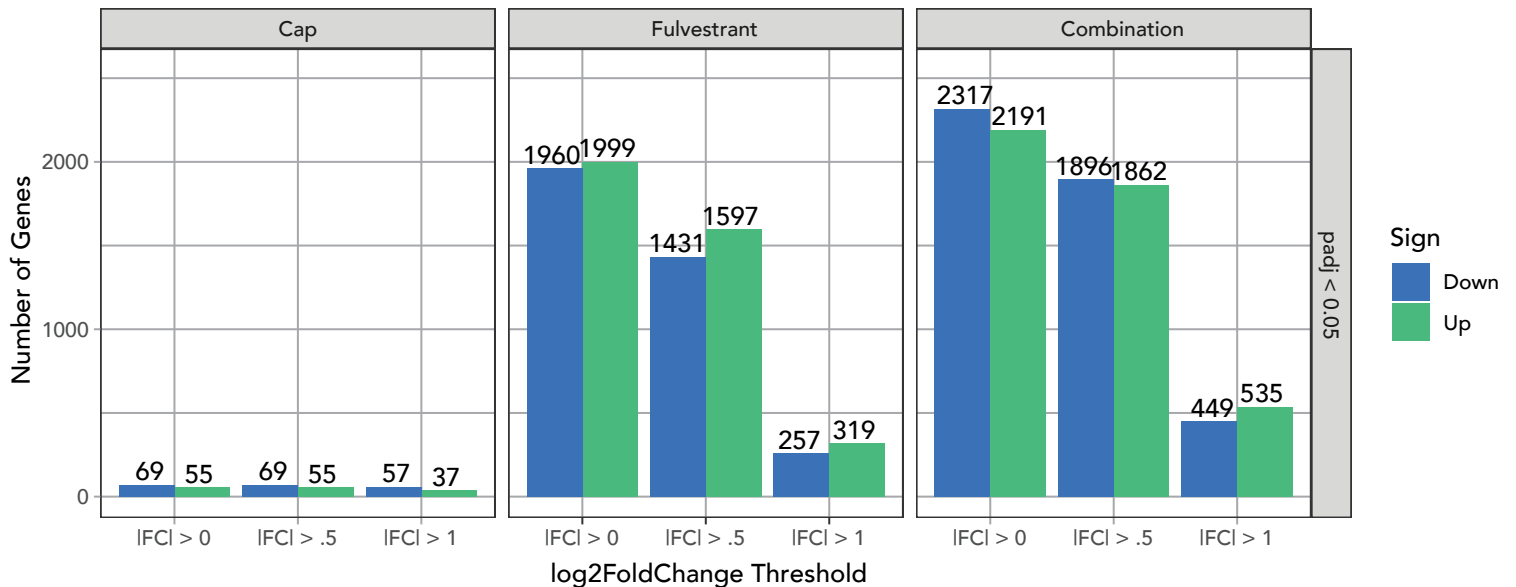
PDX Differential Expression Summary

10d Veh_vs_Treatment

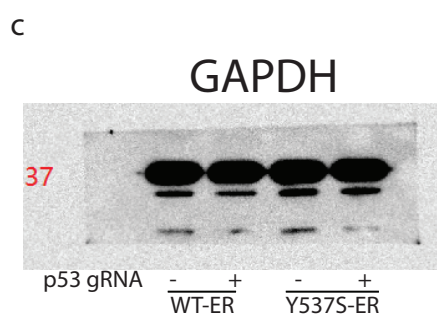
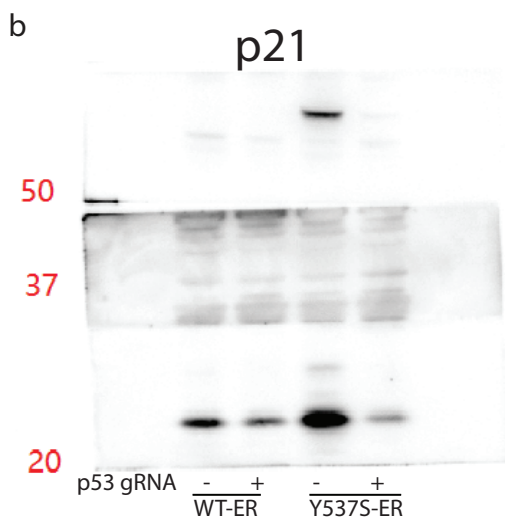
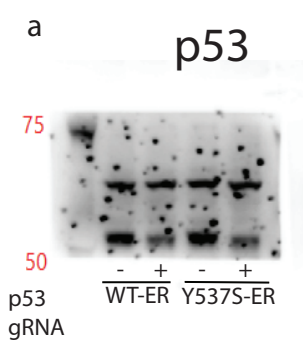


c PDX Differential Expression Summary

28d Veh_vs_Treatment

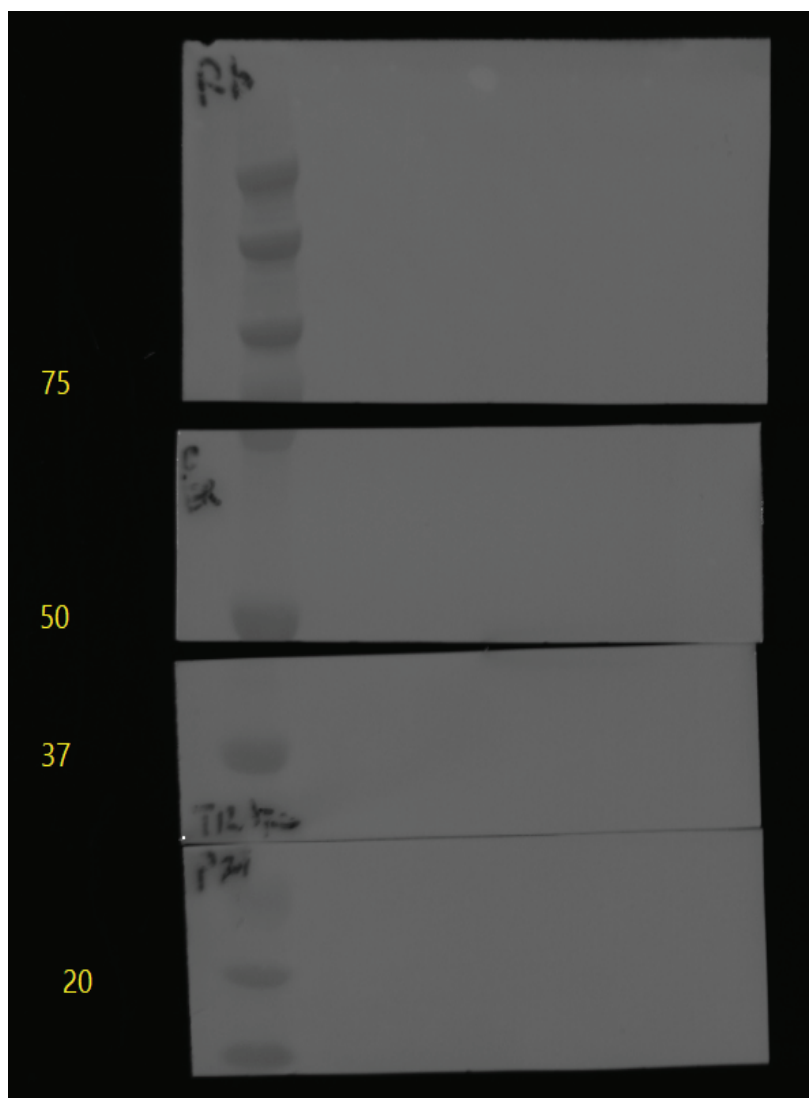


Supplementary Figure 5. Western blotting of p53 **(a)** and p21 **(b)** after p53 silencing in MCF7 cell with wild-type ER or Y537S ER mutation. **(c)** GAPDH as house-keeping protein. **(d)** molecular weight markers. WT: wild-type; ER: estrogen receptor.



d

Ladder



Supplementary Data 1. List of upregulated genes for fulvestrant and capecitabine treated.

See excel file.

Supplementary Data 2. List of antibodies used for Cyclic immunofluorescence.

See excel file.