Supplemental Table 1: ^{18}F-FDG uptake values measured with PET/CT imaging

Treatment Group	Baseline (max %ID/g)	Treatment Day 7 (max %ID/g)	Baseline (T:M)	Treatment Day 7 (T:M)
WT-ER Tumor Control	8.30 ± 0.35	8.05 ± 1.04	6.37 ± 0.74	5.01 ± 0.77*
WT-ER Tumor Fulvestrant	7.56 ± 0.71	4.62 ± 0.79*	6.13 ± 1.37	3.66 ± 0.57*
Y537S-ER Tumor Control	10.24 ± 1.25	9.20 ± 1.00*	7.06 ± 0.86	5.39 ± 0.63
Y537S-ER Tumor Fulvestrant	8.86 ± 1.14	6.12 ± 0.57	6.84 ± 1.30	3.89 ± 0.37
Muscle Control	1.38 ± 0.10	1.65 ± 0.09	N/A	N/A
Muscle Fulvestrant	1.36 ± 0.09	1.46 ± 0.15	N/A	N/A

*Significant change in ^{18}F-FDG uptake when compared to baseline imaging (paired t-test; N=6 tumors per treatment group).

Supplemental Table 2: ^{18}F-FFNP uptake values measured with PET/CT imaging

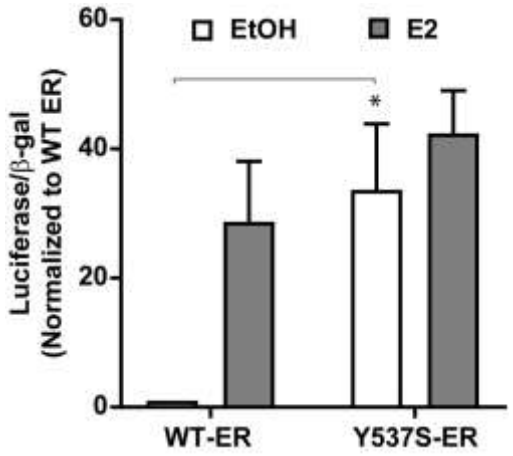
Treatment Group	Baseline (max %ID/g)	Treatment Day 7 (max %ID/g)	Baseline (T:M)	Treatment Day 7 (T:M)
WT-ER Tumor Control	3.97 ± 0.35	3.80 ± 0.31	4.95 ± 0.45	4.71 ± 0.36
WT-ER Tumor Fulvestrant	4.00 ± 0.23	2.10 ± 0.20*	5.26 ± 0.61	2.41 ± 0.18*
Y537S-ER Tumor Control	4.20 ± 0.19	3.75 ± 0.35	5.27 ± 0.54	4.61 ± 0.38
Y537S-ER Tumor Fulvestrant	4.18 ± 0.37	3.92 ± 0.50	5.41 ± 0.55	4.49 ± 0.50
Pituitary Gland Control	2.95 ± 0.07	3.17 ± 0.16	N/A	N/A
Pituitary Gland Fulvestrant	3.05 ± 0.08	2.07 ± 0.04*	N/A	N/A
Muscle Control	0.81 ± 0.04	0.84 ± 0.10	N/A	N/A
Muscle Fulvestrant	0.80 ± 0.08	0.87 ± 0.03	N/A	N/A

*Significant change in ^{18}F-FFNP uptake when compared to baseline imaging (paired t-test; N=6 tumors per treatment group).

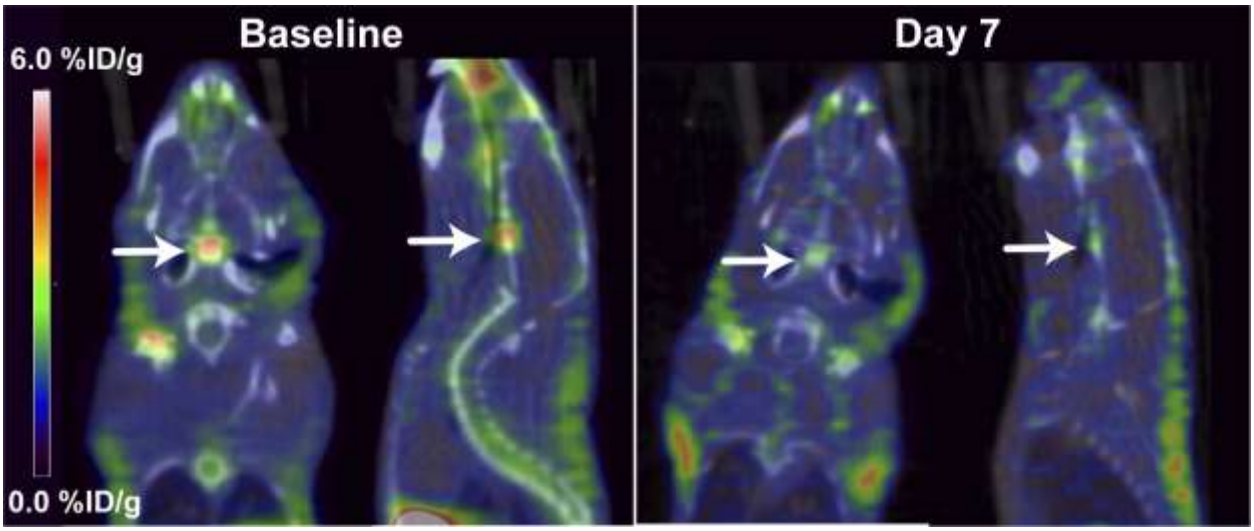
Supplemental Table 3: PR and ER immunohistochemistry of excised tumors

Treatment Group	PR+ (%)	PR Intensity	ER+ (%)	ER Intensity
WT-ER Tumor Control	94.00 ± 6.00	Strong (N=5/5)	41.0 ± 4.58	Moderate (N=2/5) Weak (N=3/5)
WT-ER Tumor Fulvestrant	56.25 ± 23.00	Moderate (N=4/4)	0	None (N=4/4)
Y537S-ER Tumor Control	80.00 ± 13.78	Strong (N=5/5)	46.4 ± 12.1	Moderate (N=1/5) Weak (N=4/5)
Y537S-ER Tumor Fulvestrant	80.00 ± 18.77	Strong (N=5/5)	0*	None* (N=5/5)

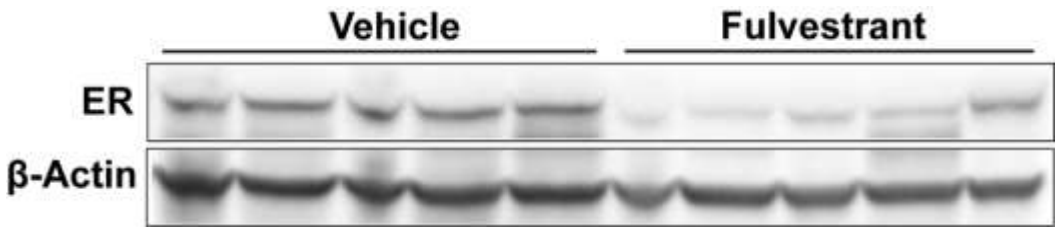
*Residual ER protein detectable by Western blot analysis (Supplemental Figure 3)



Supplemental Figure 1: Estrogen-dependent and estrogen-independent ER transcriptional activity of CRISPR-edited T47D breast cancer cells expressing WT-ER and Y537S-ER. Cells were grown in steroid hormone-depleted media for 3 days and were seeded in a 6-well plate (500,000 cells/well). On day 5, cells were co-transfected with estrogen receptor response element (ERE)-luciferase (0.75 μg) and cytomegalovirus-β-galactosidase (0.25 μg) reporter plasmids using Lipofectamine 3000 (Life Technologies). The following day, cells were treated with ethanol (EtOH) vehicle or 10 nM 17β-estradiol (E2) for 24 hours. Luciferase reporter gene activity (Promega) and β-galactosidase activity (Tropix) were measured according to the manufacturers' protocol. ERE-luciferase reporter gene activity was normalized to β-galactosidase activity to control for transfection efficiency. Data was normalized to WT-ER in the absence of E2 (N=3 independent experiments). *$P < 0.05$ compared to WT-ER.



Supplemental Figure 2: Representative horizontal/coronal and sagittal fused ^{18}F-FFNP PET/CT images demonstrating pituitary gland uptake (arrows) at baseline and 7 days after starting fulvestrant treatment.



Supplemental Figure 3: Western blot for ER and β-actin protein in the excised Y537S-ER tumors from the ^{18}F-FFNP biodistribution assay on day 7 post treatment with either ethanol vehicle control or fulvestrant. Tumor lysates were prepared from flash-frozen tumors excised on day 7 post treatment from the ^{18}F-FFNP biodistribution assay. Crushed flash-frozen tumors were lysed using radioimmunoprecipitation assay buffer (Sigma) with 2 mM sodium orthovanadate, protease (1:500) and phosphatase (1:100) inhibitor cocktails (Sigma). Protein concentration was determined with Bradford assay (Bio-Rad). Equal amounts of protein were run on 10% sodium-dodecyl sulfate-polyacrylamide gel electrophoresis (Bio-Rad) and transferred to a polyvinylidene difluoride membrane (Millipore). Saturating amounts of antibodies were used for ER (1:1,000 clone 6F11; Leica Biosystems), β-actin (1:20,000 clone AC-15; Sigma) as a loading control, and horseradish peroxidase-conjugated anti-mouse IgG (1:3,000; GE Healthcare).