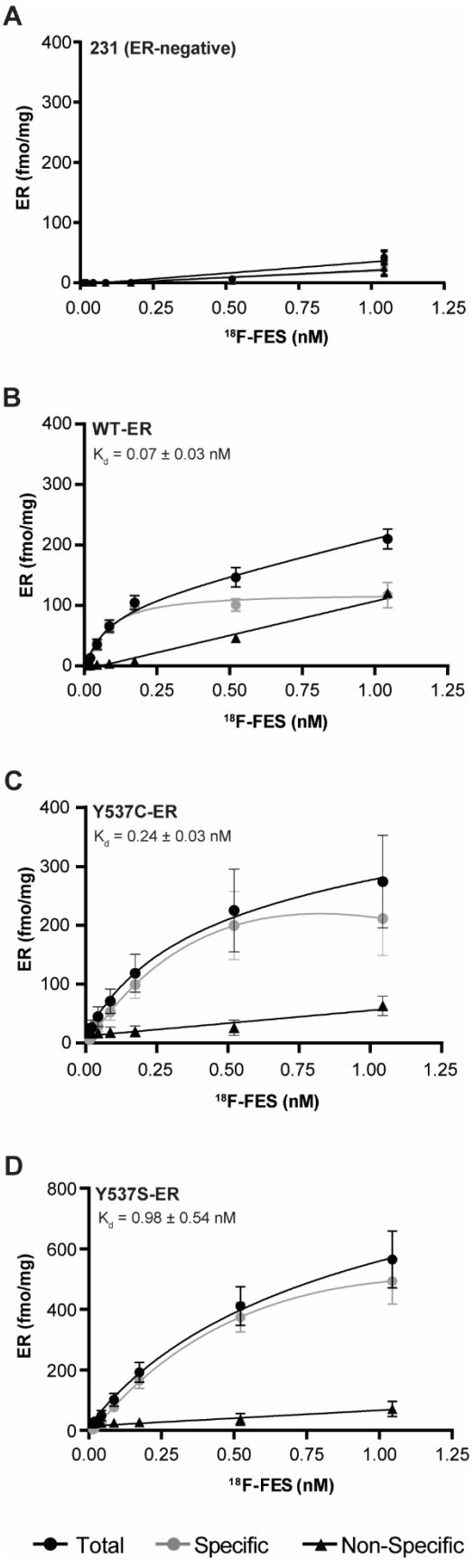
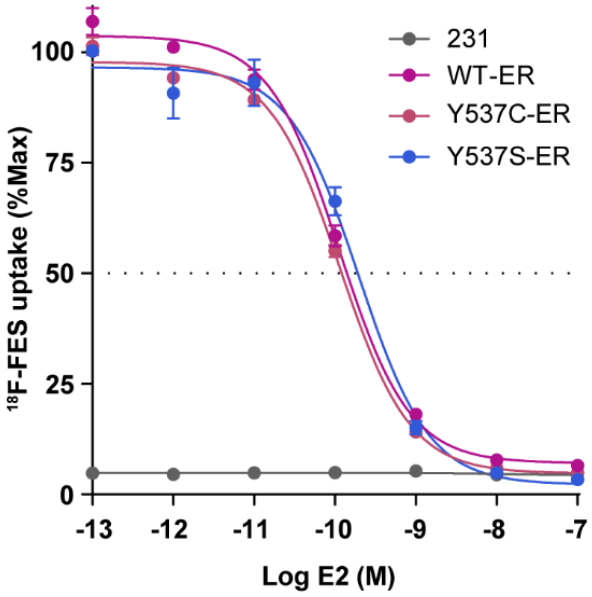


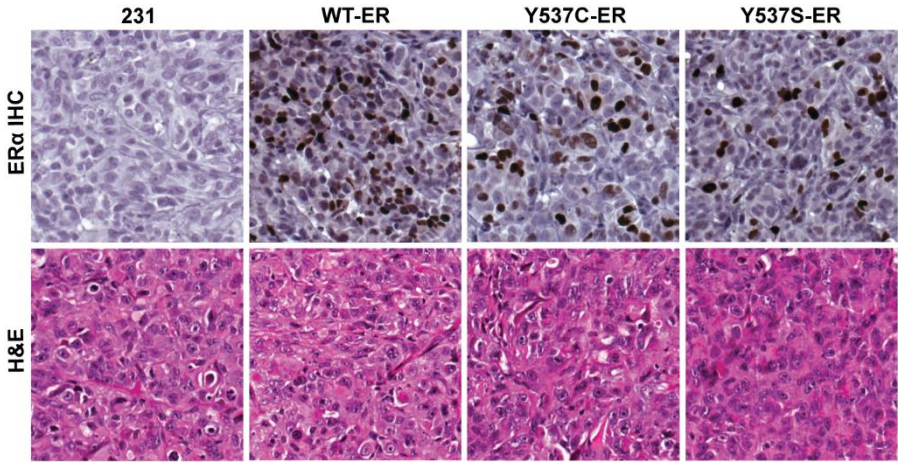
Supplemental Figure 1: Saturation binding of ^{18}F-FES in Y537C-ER and Y537S-ER cells compared with WT-ER

Parental ER-negative MDA-MB-231 (A), WT-ER (B), Y537C-ER (C), and Y537S-ER cells (D) were seeded in 24-well plates then incubated in estrogen-deprived media with 5 µg/ml doxycycline for 24 h. Plates were treated with 0.002-0.222 MBq (0.06-6 µCi) ^{18}F-FES and were incubated for 1 h at 37 ^{o}C. Total, non-specific, and specific binding was determined from nonlinear regression. Values represent the mean±SEM of 3 independent experiments performed in triplicate.



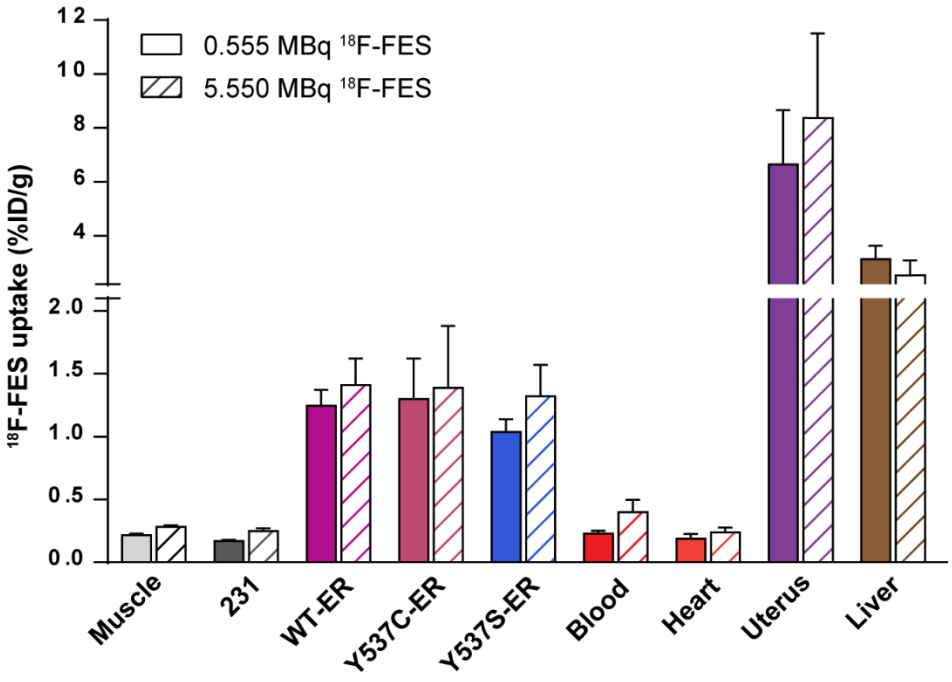
Supplemental Figure 2: Competition binding of ^{18}F-FES with E2 in Y537C-ER and Y537S-ER cells compared with WT-ER

Parental ER-negative MDA-MB-231, WT-ER, Y537C-ER, and Y537S-ER cells were seeded in 24-well plates with estrogen-deprived media and were treated with 5 µg/mL doxycycline for 24 h. Increasing amount of cold E2 ($1x10^{-13}$ to $1x10^{-7}$ M) were added with 0.037 MBq (1 µCi) ^{18}F-FES for 1 h and incubated at 37 ^{o}C. Decay-corrrected counts per minute were normalized to wells containing ^{18}F-FES without E2 to calculate percentage maximum uptake values. MDA-MB-231 cell values were expressed relative to WT-ER to demonstrate binding specificity. Values represent the mean±SEM of 3 independent experiments performed in triplicate.



Supplemental Figure 3: ER immunohistochemistry of excised tumors post ^{18}F-FES biodistribution experiment

Representative 20x magnification images of ER immunohistochemistry (upper panel) and hematoxylin-eosin (H&E) staining (lower panel) of excised tumors.



Supplemental Figure 4: ^{18}F-FES tissue biodistribution panel

Biodistribution of ^{18}F-FES at 0.555 MBq (15 µCi, 10 mice) and 5.55 MBq (150 µCi, 9 mice) injected doses in muscle, xenograft tumors, blood, heart, uterus and liver 1 h after tail vein injection. Data are expressed as %ID/g (mean±SEM). $p \geq 0.05$ for ^{18}F-FES uptake at 0.55 MBq compared with 5.55 MBq.

Supplemental Table 1: Primers for site-directed mutagenesis PCR using human ERα

Mutation	Sense Primer	Antisense Primer
Y537C	G TGC AAG AAC GTG GT**A** CCC CTC T**G**T GAC CTG CTG CTG G	C CAG CAG CAG GTC A**C**A GAG GGG **T**AC CAC GTT CTT GCA C
Y537S	G TGC AAG AAC GTG GT**A** CCC CTC T**C**T GAC CTG CTG CTG G	C CAG CAG CAG GTC A**G**A GAG GGG **T**AC CAC GTT CTT GCA C

Supplemental Table 2: Sanger sequencing primers

Primers	pBluescript Vector	pUHD 10-3 Vector
Forward	TGTAAAACGACGGCCAGT	TCGAGTAGGCGTCTACGGT
Internal	GCTGCAAGGCCTTCTTCAAG	GCTGCAAGGCCTTCTTCAAG
Reverse	CAGGAAACAGCTATGAC	ATAAAGCAATAGCATCAC

Supplemental Table 3: Quantitative PCR primers

Target	Forward Primer (5'-3')	Reverse Primer (5'-3')
Progesterone Receptor (PGR)	TGACACCTCCAGTTCTTTGC	AACACCATTAAGCTCATCCAAG
Trefoil factor-1 (TFF1)	CGCCTTTGGAGCAGAGAG	ACCACAATTCTGTCTTTCACG
Ribosomal protein 36B4	GACAATGGCAGCATCTACAAC	GCAGACAGACACTGGCAAC