Figure S1. The Cancer Genome Atlas endoplasmic reticulum to nucleus signaling 1 gene expression profiles of several cancers and corresponding normal tissue samples. Data from the Gene Expression Profiling Interactive Analysis website (http://gepia.cancer-pku.cn/index.html). TPM, transcripts per million.

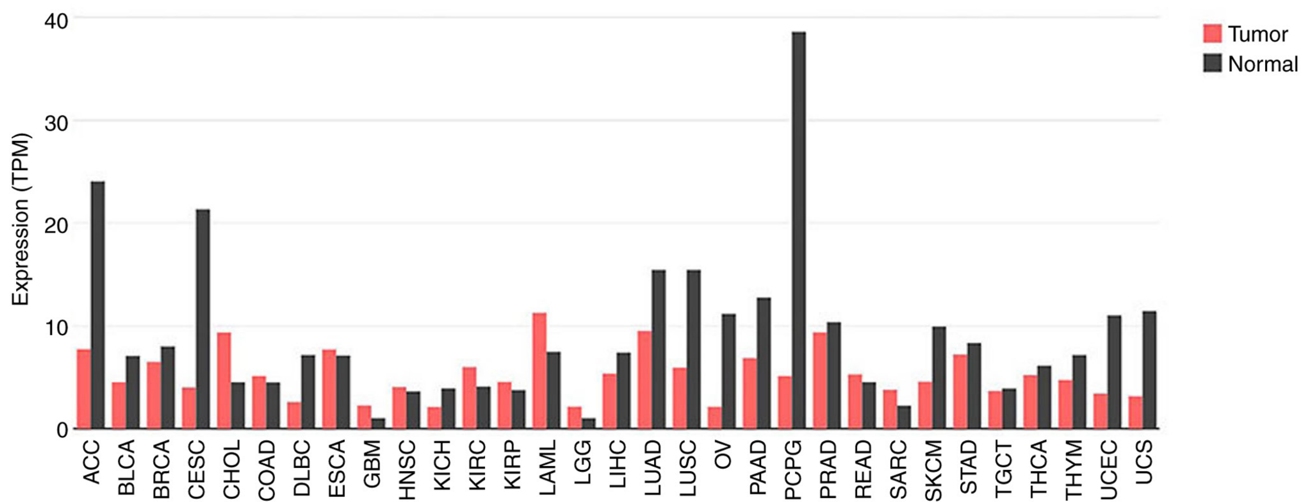

Figure S2. Dose-response of APY29-induced cytotoxicity in breast cancer cells. Cell viability of (A) MDA-MB-231, (B) MDA-MB-468, (C) 4T1 and (D) HT1080 cells. Dose-response of (E) sunitinib- and (F) KIRA6-induced cytotoxicity in MDA-MB-231 cells. Data are presented as mean ± standard error of the mean (n=6). KIRA6, inositol-requiring enzyme 1α kinase-inhibiting RNase attenuator.

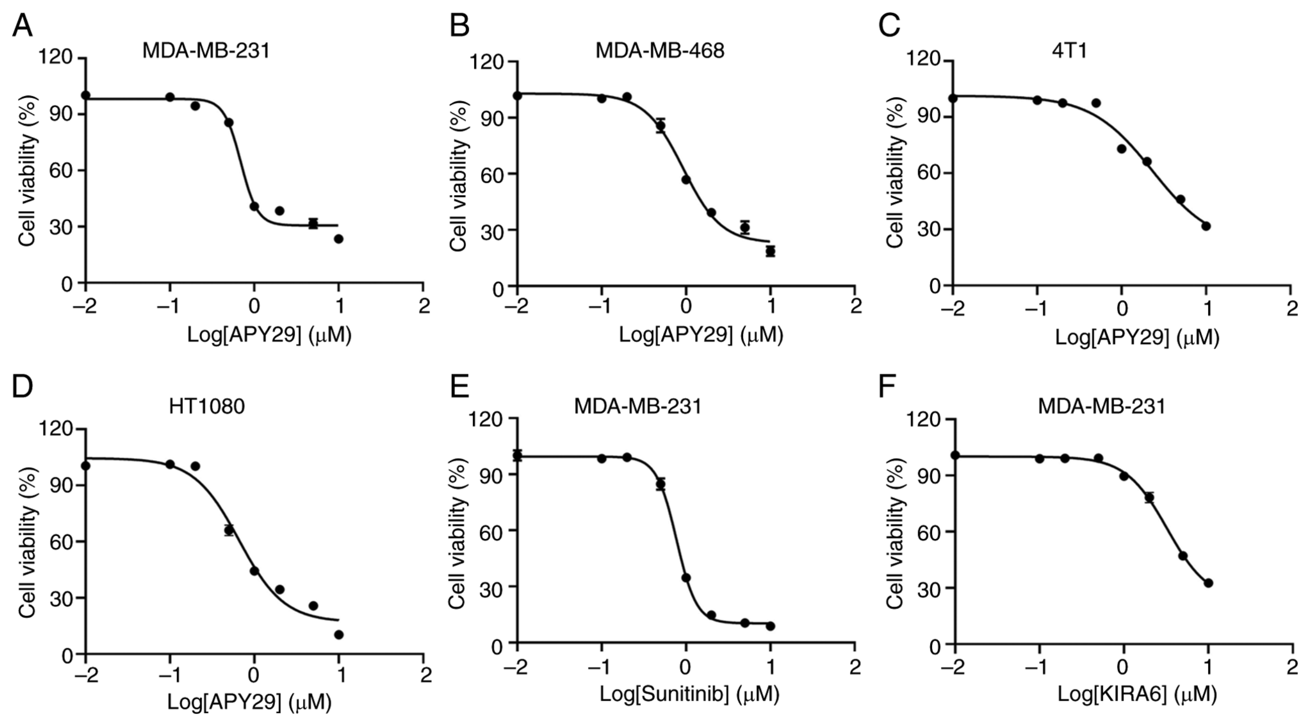

Figure S3. Cell viability in HT1080 cells treated with (A) erastin, (B) SAS, (C) glutamate or (D) cystine restriction for 24 h in the presence or absence of APY29 (0.2 μM). Cell viability in MDA-MB-231 treated with (E) RSL3 or (F) ML162 for 24 h in the presence or absence of APY29 (0.2 μM). Cell viability in HT1080 cells treated with (G) RSL3 or (H) ML162 for 24 h in the presence or absence of APY29 (0.2 μM). Data are presented as mean ± standard error of the mean (n=6). $^{****}P<0.0001$ vs. Control. RSL3, RAS-selective lethal 3; SAS, sulfasalazine.

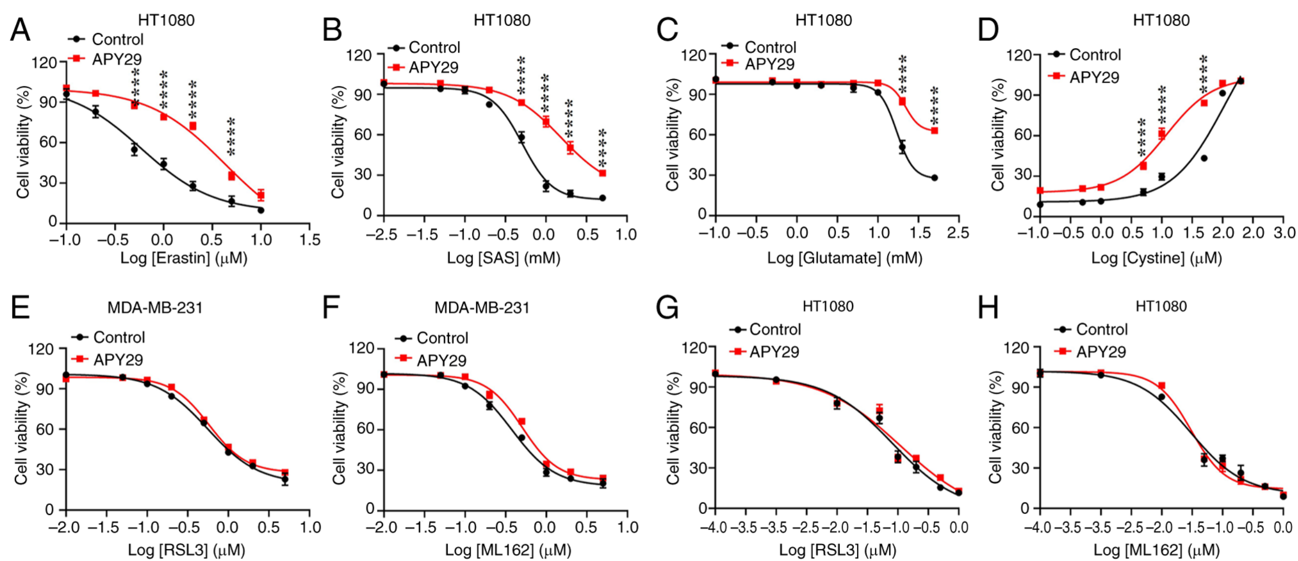

Figure S4. Western blot analysis of MDA-MB-231 cells overexpressing wild-type IRE1α and kinase-dead IRE1α mutant. Red boxes highlight sections of blots shown in the indicated figures. The target protein and corresponding β-actin were derived from the same membrane through sequential probing. IRE1α, inositol-requiring enzyme 1α; NC, negative control; WT, wildtype.

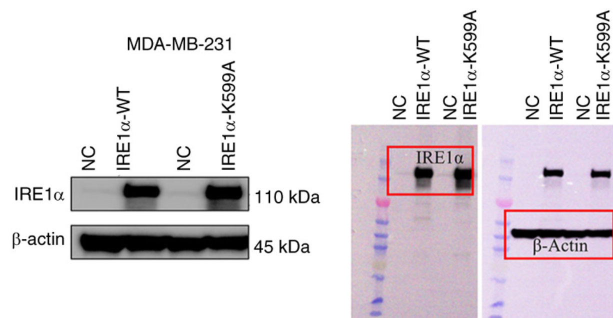

Figure S5. Reverse transcription-quantitative PCR results. mRNA levels of xCT were analyzed using reverse transcription-quantitative PCR in (A) MDA-MB-231, (B) MDA-MB-468 and (C) 4T1 cells following 24-h treatment with APY29 (0.2 μM). Data are presented as mean ± standard error of the mean (n=5).

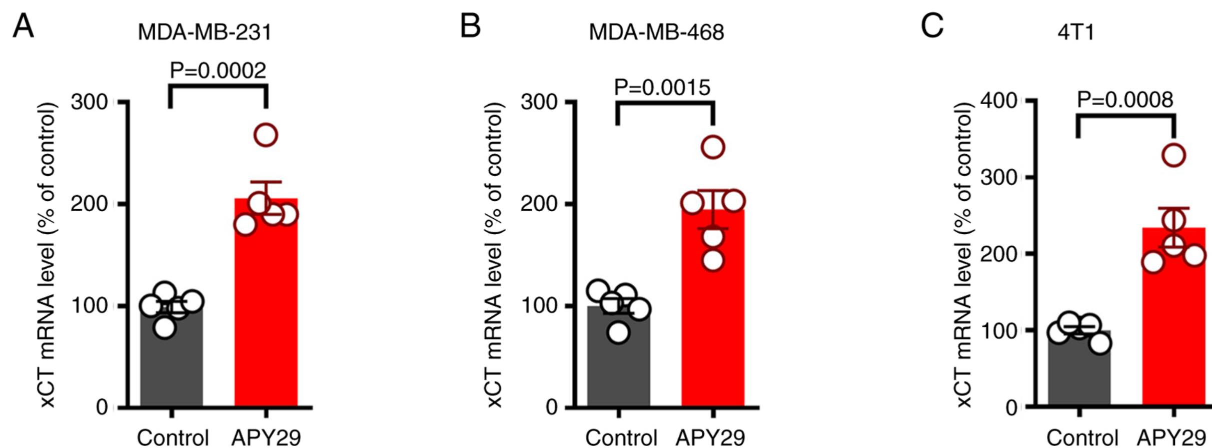

Figure S6. Western blotting results. Protein expression of xCT was analyzed using western blotting in (A) MDA-MB-468 and (B) 4T1 cells following 24-h treatment with APY29 (0.2 μM). GSH levels were measured in MDA-MB-468 cells following 12-h treatment with (C) erastin (5 μM) or (D) SAS (1 mM), in the presence or absence of APY29 (0.2 μM). GSH levels were measured in 4T1 cells following 12-h treatment with (E) erastin (5 μM) or (F) SAS (1 mM), in the presence or absence of APY29 (0.2 μM). Data are presented as mean ± standard error of the mean (n=3). xCT, solute carrier family 7 member 11; GSH, glutathione; SAS, sulfasalazine.

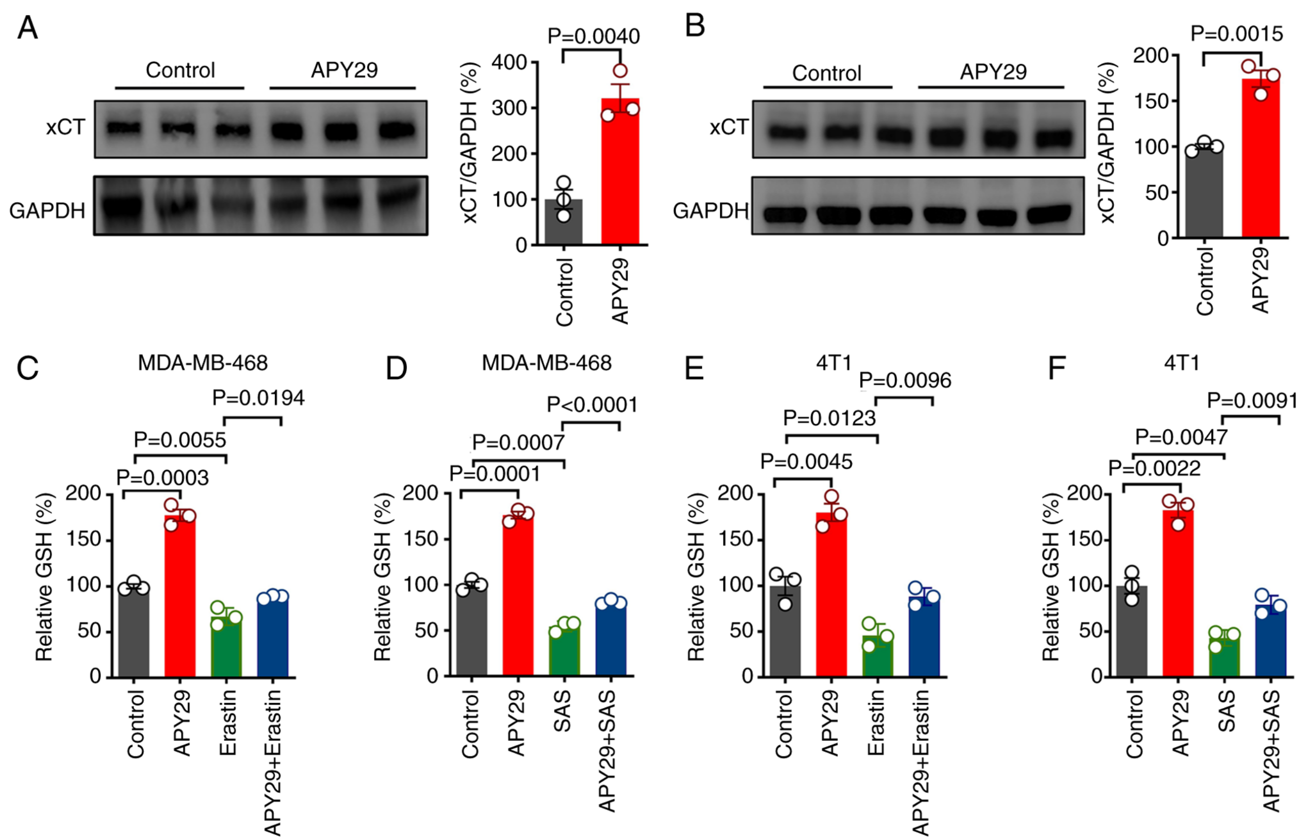

Figure S7. Scans of uncropped blots. Red boxes highlight sections of blots shown in the indicated figures. The target protein and corresponding loading control (β-actin or GAPDH) for each set were derived from the same membrane through sequential probing. xCT, solute carrier family 7 member 11; ACSL4, acyl-CoA synthetase long chain family member 4; GPX4, glutathione peroxidase 4.

Fig. 7A

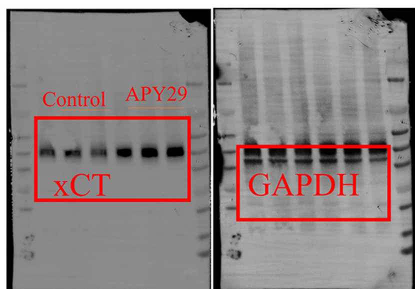

Fig. 7B

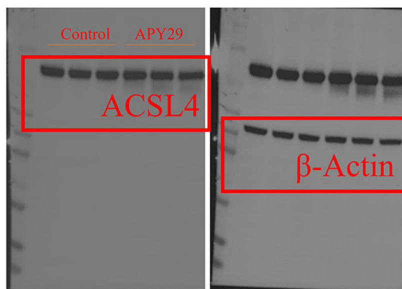

Fig. 7C

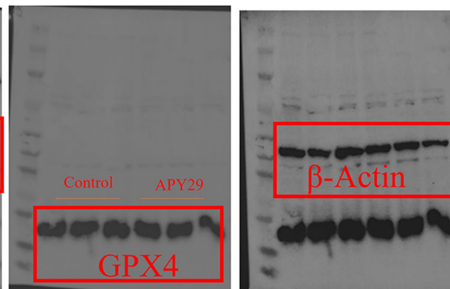

Fig. S6A

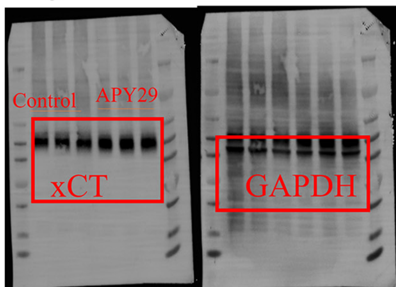

Fig. S6B