Supporting Information

IRE-1-targeting caged prodrug with endoplasmic reticulum stress-inducing and XBP-1s-inhibiting activities for cancer therapy

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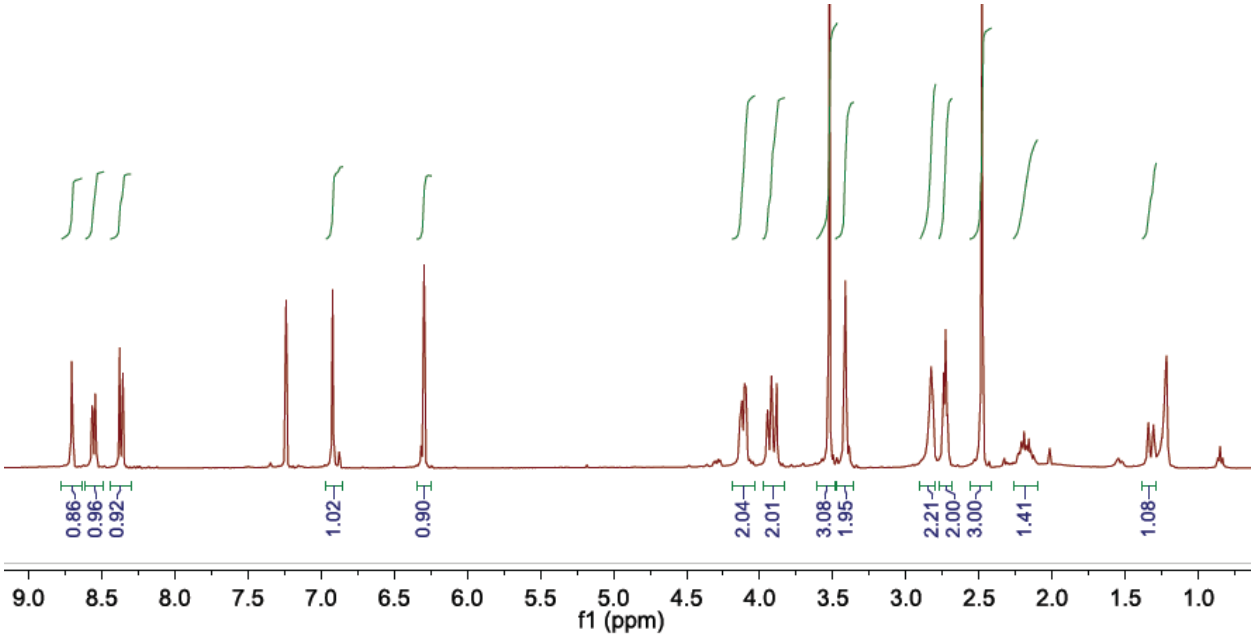
Table of Contents

COPIES OF ^{1}H NMR SPECTRA

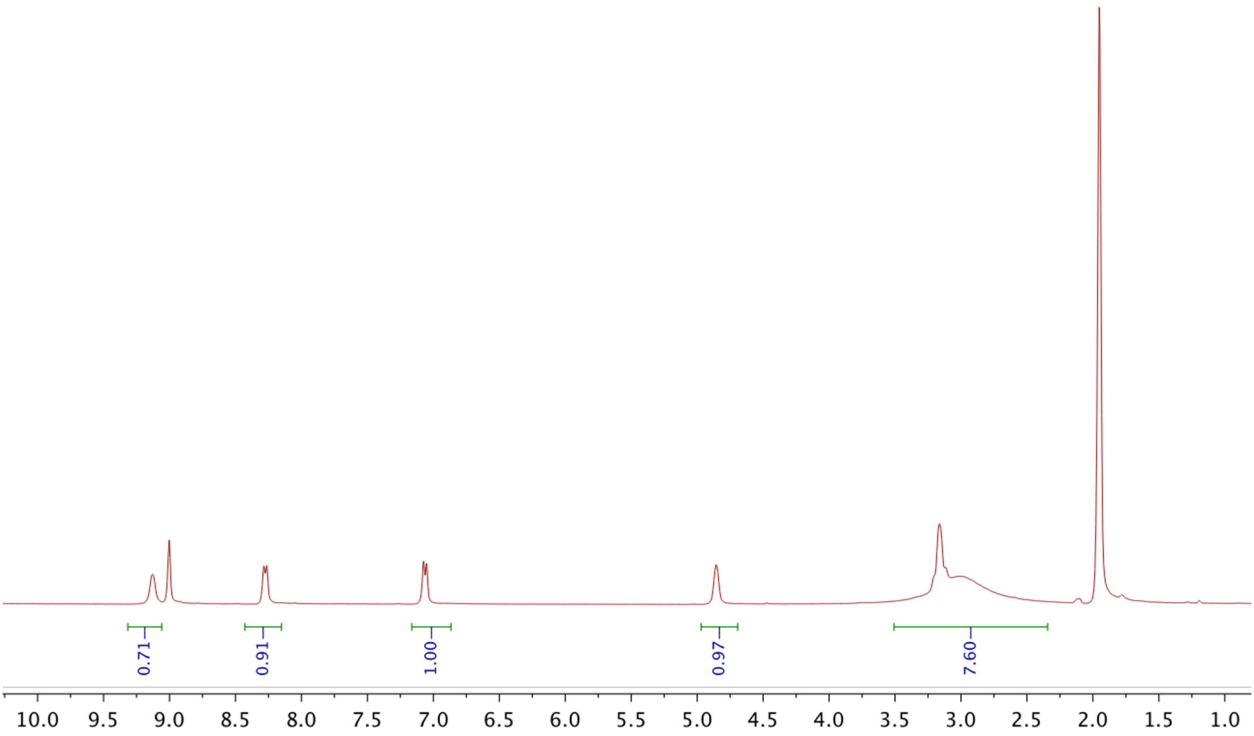
TC-D-F07

400 MHz ^{1}H NMR ($CDCl_3$):



E-H01

400 MHz ^{1}H NMR (CD_3CN):



Supplementary Figures and Legends

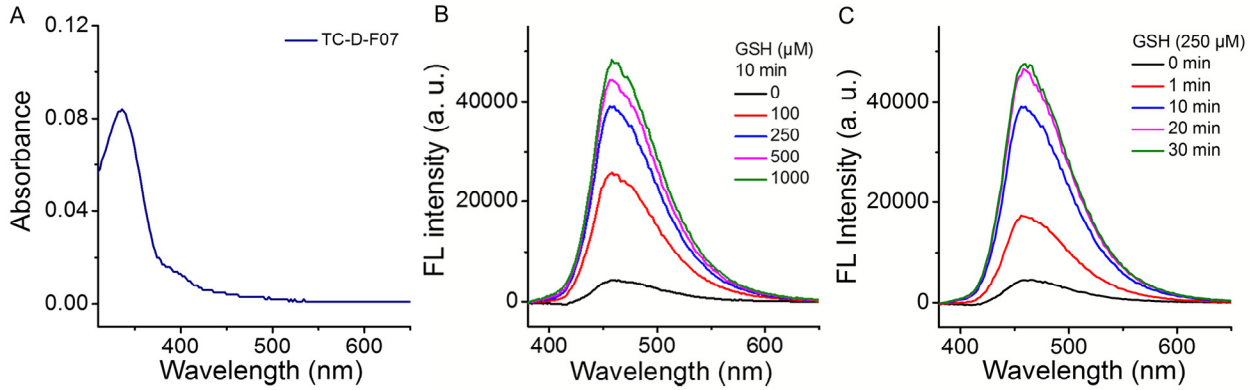


Figure S1. (**A**) Absorbance curves of TC-D-F07 (10 μM) in the DMSO/PBS (v/v = 1:99) solution. (**B**) The fluorescence from liberated D-F07 increases upon incubation of TC-D-F07 (2.5 μM in DMSO/PBS solution (v/v = 1:99), E_x = 360 nm) with 0 to 1000 μM GSH at room temperature for 10 min. (**C**) The fluorescence from liberated D-F07 increases upon incubation of TC-D-F07 (2.5 μM in DMSO/PBS solution (v/v = 1:99), E_x = 360 nm) with 250 μM GSH at room temperature for 0-30 min.

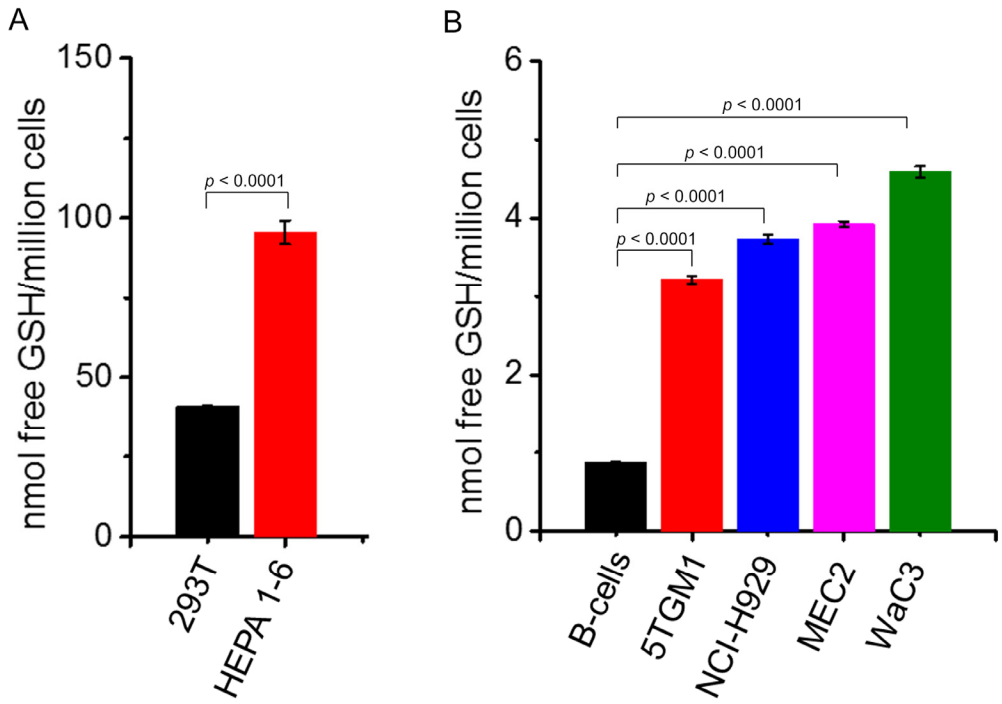


Figure S2. (**A**) Free GSH concentration was assayed in 293T and HEPA 1-6 cells and plotted as means ± SD. (**B**) Free GSH concentration was assayed in primary B cells, 5TGM1, NCI-H929, MEC2, and WaC3 cells and plotted as means ± SD.

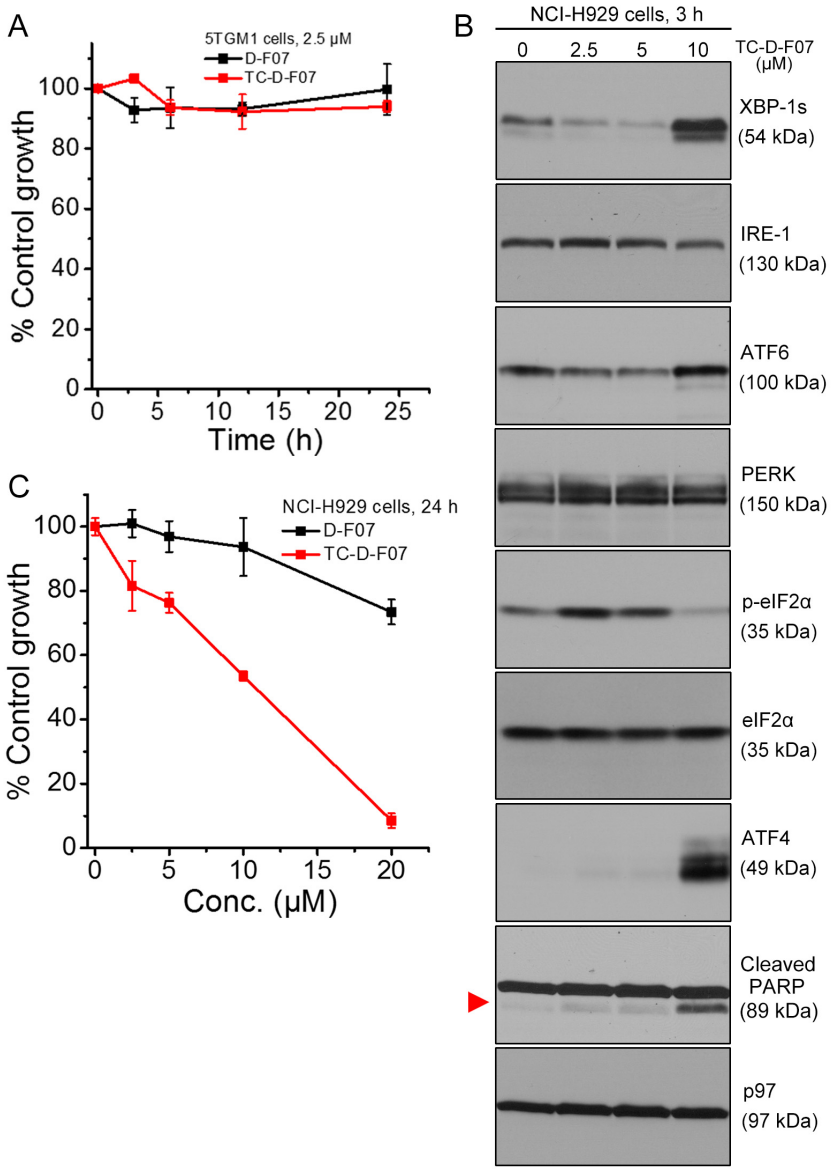


Figure S3. (**A**) 5TGM1 cells were treated with DMSO, D-F07, or TC-D-F07 at 2.5 μM for 3, 6, 12, or 24 h, and subjected to XTT assays. Percentages of growth were determined by comparing treated groups with control groups (DMSO). Each data point derived from four independent groups receiving the same treatment was plotted as means ± SD. Data were representative of three independent experiments. (**B**) NCI-H929 cells were treated with TC-D-F07 at indicated concentrations for 3 h, lysed, and analyzed for the expression of indicated proteins by immunoblots. Cleaved PARP is indicated by a red arrowhead. (**C**) NCI-H929 cells were treated with DMSO, D-F07, or TC-D-F07 at indicated concentrations for 24 h and subjected to XTT assays. Percentages of growth were determined by comparing treated groups with control groups (DMSO). Each data point derived from four independent groups receiving the same treatment was plotted as means ± SD. Results are representative of three independent experiments.

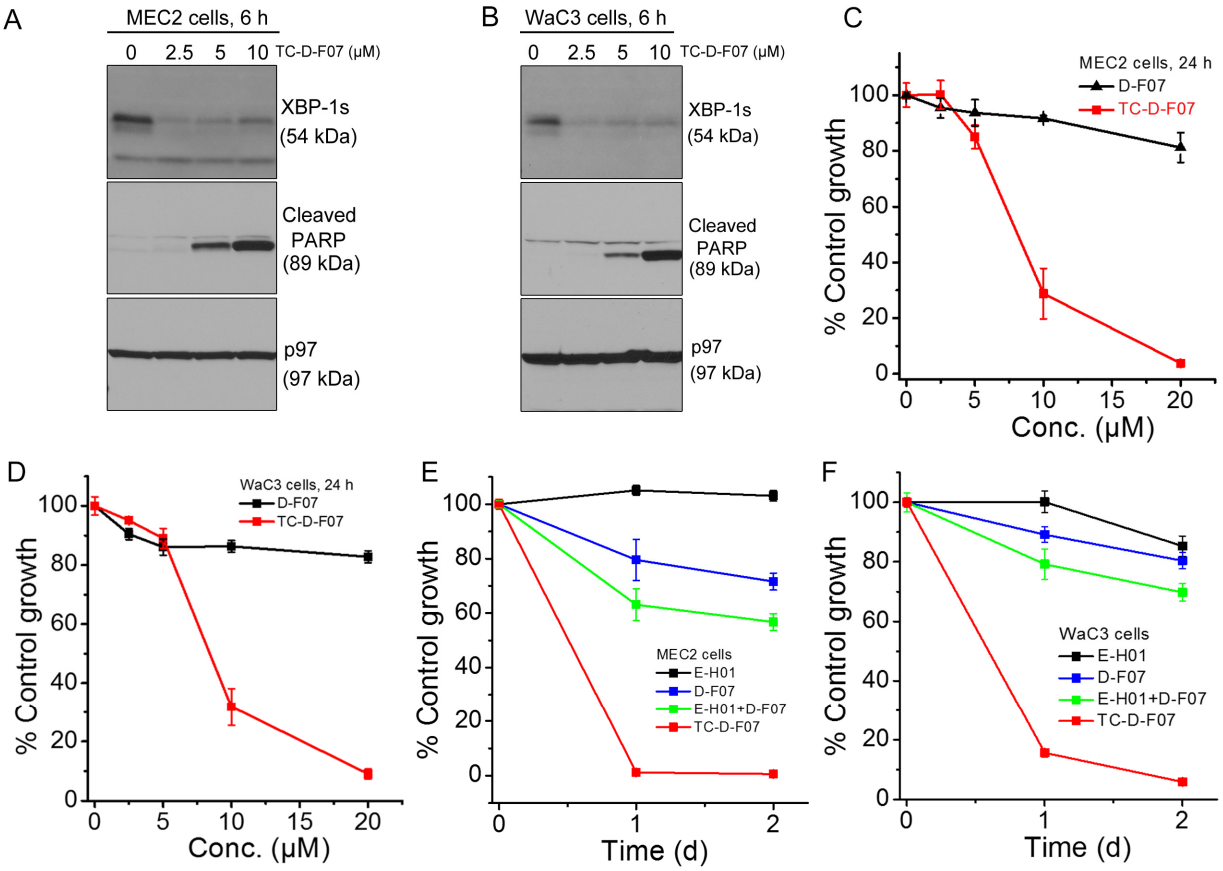


Figure S4. (**A-B**) MEC2 (A) and WaC3 (B) cells were treated with TC-D-F07 at indicated concentrations for 6 h, lysed, and analyzed for the expression of indicated proteins by immunoblots. (**C-D**) MEC2 (C) and WaC3 (D) cells were treated with DMSO, D-F07, or TC-D-F07 at indicated concentrations for 24 h and subjected to XTT assays. Each data point derived from four independent groups receiving the same treatment was plotted as means ± SD. Results are representative of three independent experiments. (**E-F**) MEC2 (E) and WaC3 (F) cells were treated with DMSO, E-H01, D-F07, E-H01 plus D-F07, or TC-D-F07 at 20 μM for 2 days, subjected to XTT assays, and similarly analyzed as in panel C or D. Results are representative of three independent experiments.