Chen et al., Supplementary Information

Figure S1

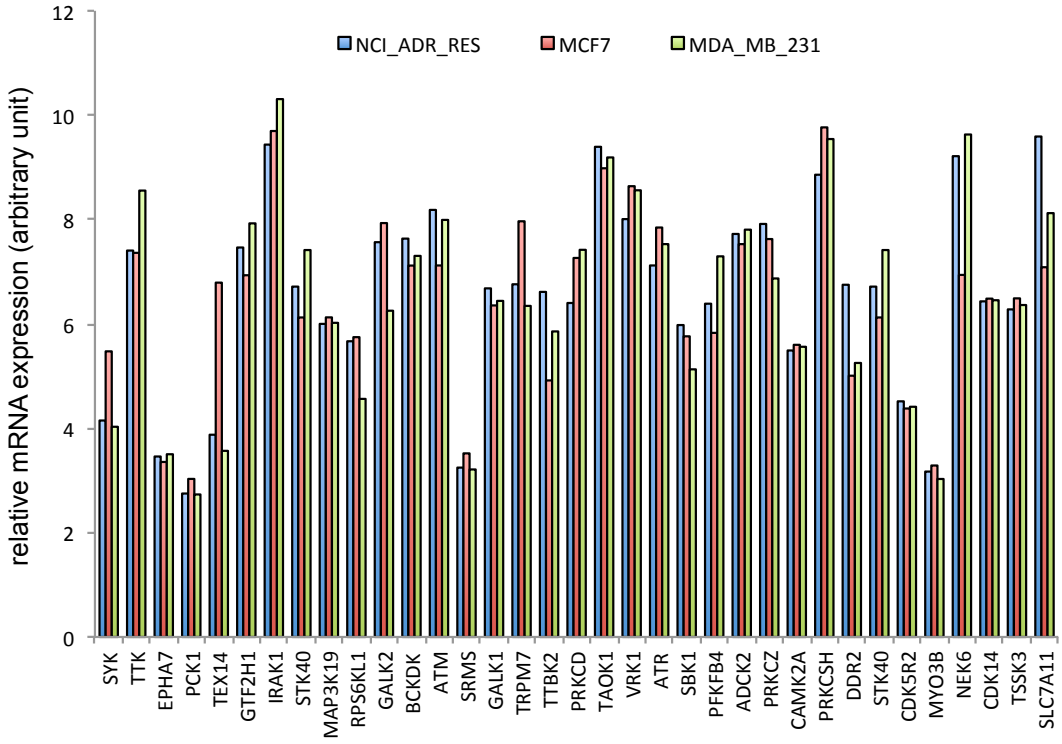

Figure S1. Relative mRNA expression of top 34 candidate kinases and *SLC7A11* in MDA-MB-231 compared with NCI-ADR-RES and MCF7 cell lines (related to Figure 1). The mRNA expressions were revealed by microarray, and the raw value of each candidate kinase probe and *SLC7A11* in RMA-normalized data was presented in the figure.

Figure S2

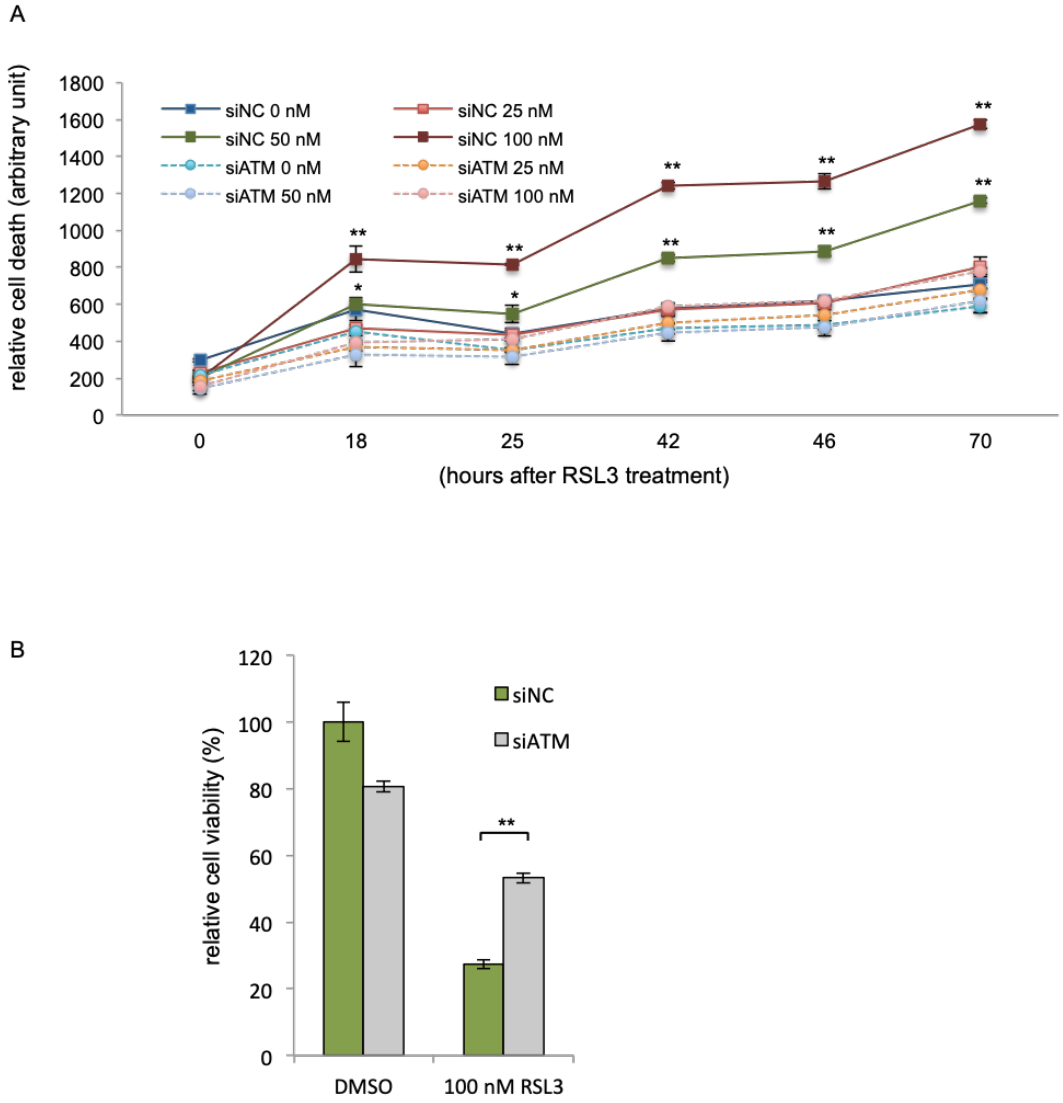

Figure S2A-B. ATM knockdown protects MDA-MB-231 cells against death triggered by RSL3 in a dose- and time-dependent manner. (A) Depletion of ATM by siRNA protects MDA-MB-231 cells against RSL3-induced death. MDA-MB-231 cells were treated with siRNA for 48 h, and then treated with indicated concentration of RSL3. The cell death was monitored from 0 to 70 h by CellTox-Green. (B) Depletion of ATM by siRNA protects MDA-MB-231 cells against RSL3-induced death. MDA-MB-231 cells were treated with siRNA for 48 h, and then treated with indicated concentration of RSL3. The cell viability was monitored by CellTiter-Glo. after 28 h of RSL3 treatment. The data are shown as mean±S.D from three biological replicates, Student's t-test; *p<0.01; **p<0.001.

Figure S2 (cont.)

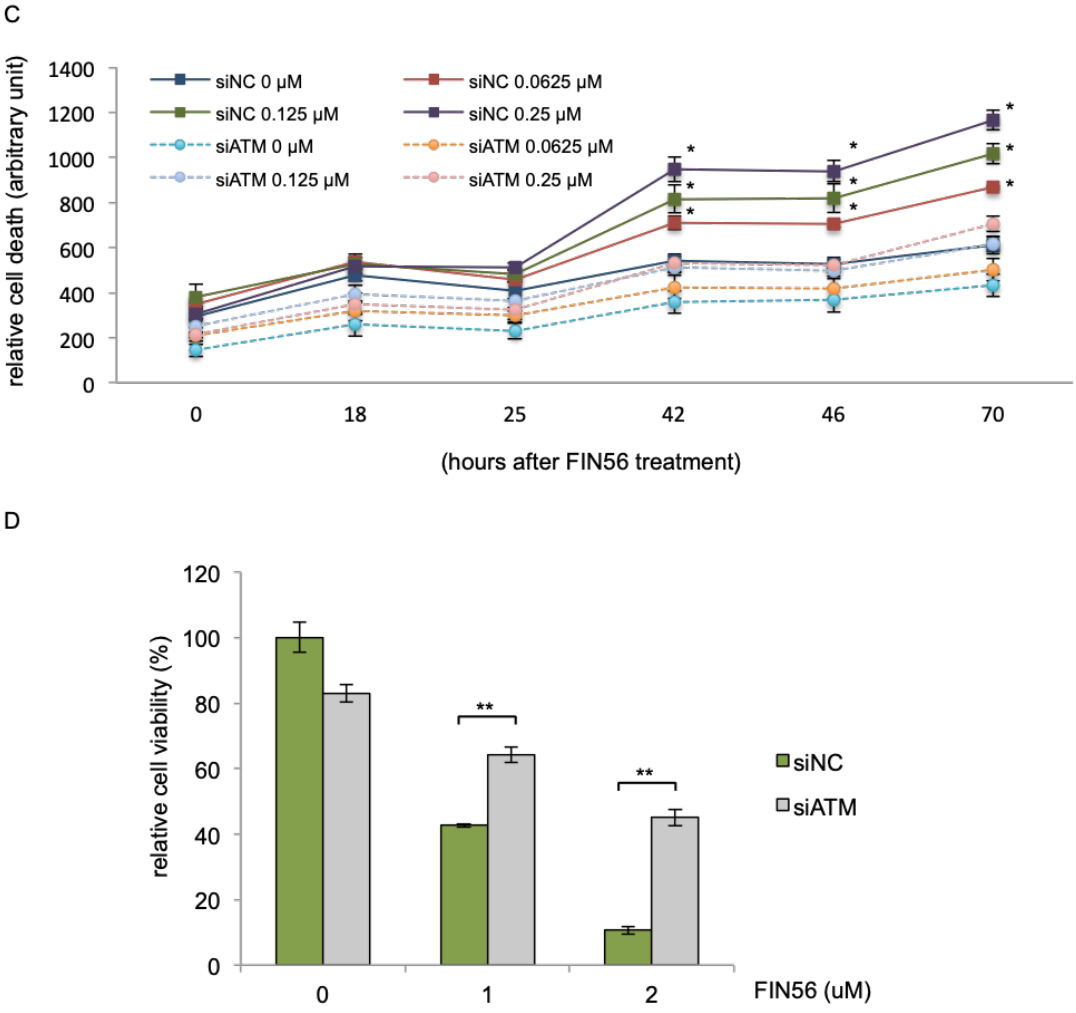

Figure S2C-D. ATM knockdown protects MDA-MB-231 cells against death triggered by FIN56 in a dose- and time-dependent manner. (C) Depletion of ATM by siRNA protects MDA-MB-231 cells against FIN56-induced death. MDA-MB-231 cells were treated with siRNA for 48 h, and then treated with indicated concentration of FIN56. The cell death was monitored from 0 to 70 h by CellTox-Green. (D) Depletion of ATM by siRNA protects MDA-MB-231 cells against FIN56-induced death. MDA-MB-231 cells were treated with siRNA for 48 h, and then treated with indicated concentration of FIN56. The cell viability was monitored by CellTiter-Glo after 28 h of FIN56 treatment. The data are shown as mean±S.D from three biological replicates, Student's t-test; *p<0.01; **p<0.001.

Figure S2 (cont.)

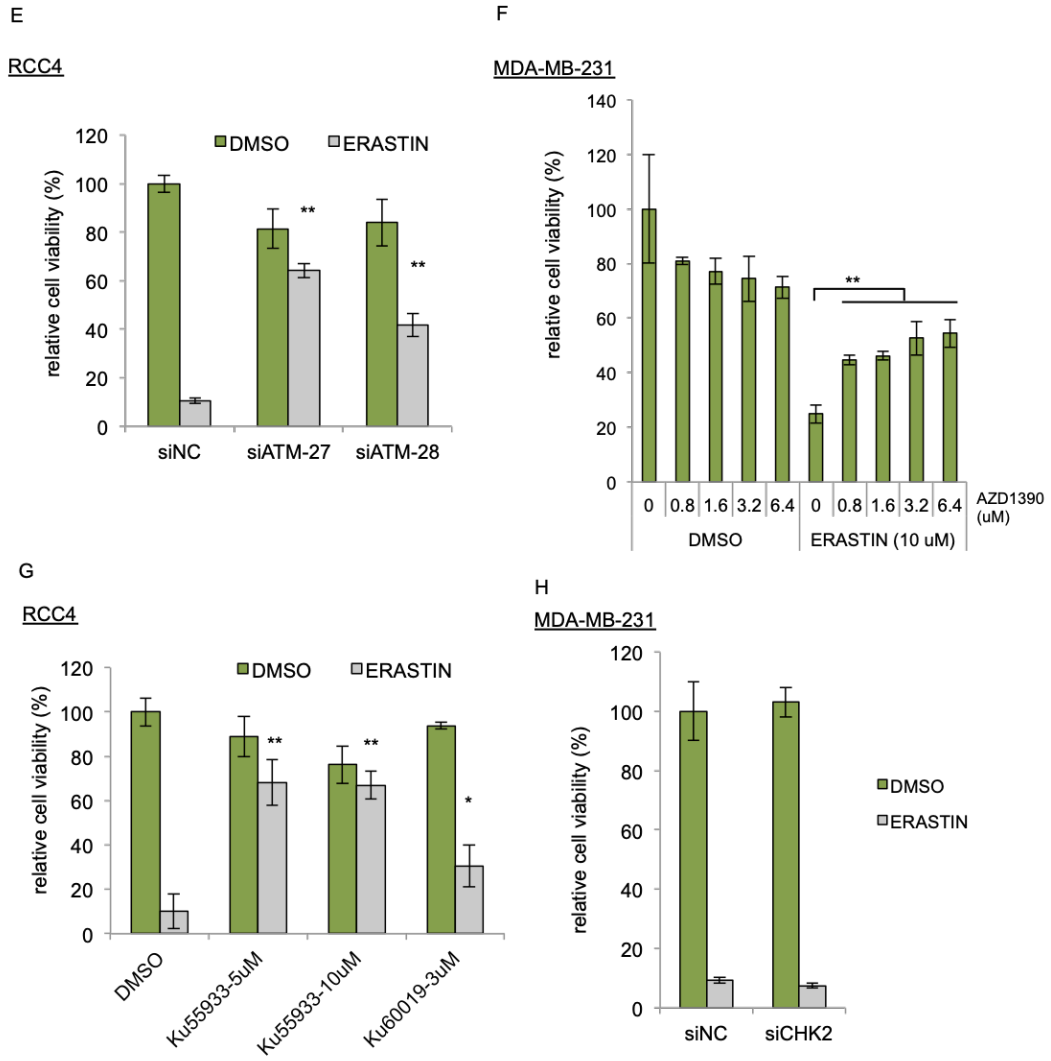

Figure S2E-H. Inhibition of ATM protects RCC4 and MDA-MB-231 cells against erastin-induced death. (E) Depletion of ATM by two individual siRNAs (siATM-27 and siATM-28) recued RCC4 cells from erastin-induced death. RCC4 cells were transfected with indicated siRNA for 72 h and treated with erastin (10 μM) for 18 h before viability determination by CellTiter-Glo. (F) Inhibition of ATM by AZD1390 protects MDA-MB-231 cells against erastin-induced death. MDA-MB-231 cells were pretreated the indicated inhibitor for 48 h, and then treated with DMSO or erastin (10 μM) for 28 h before viability determination by CellTiter-Glo. (G) ATM inhibitor Ku-55933 and Ku-60019 rescued cells from erastin-induced death. RCC4 cells were treated the indicated inhibitor for 29 h, and then treated with DMSO or erastin (10 μM) for 18 h before viability determination by CellTiter-Glo. (H) CHK2 depletion dose not rescue MDA-MB-231 cells from erastin-mediated cell death. MDA-MB-231 cells transfected with indicated siRNA for 72 h and treated with

erastin (10 μM) for 18 h before viability determination by CellTiter-Glo. The data are shown as mean±S.D from three biological replicates, Student's t-test; *p<0.05; **p<0.01.

Figure S2 (cont.)

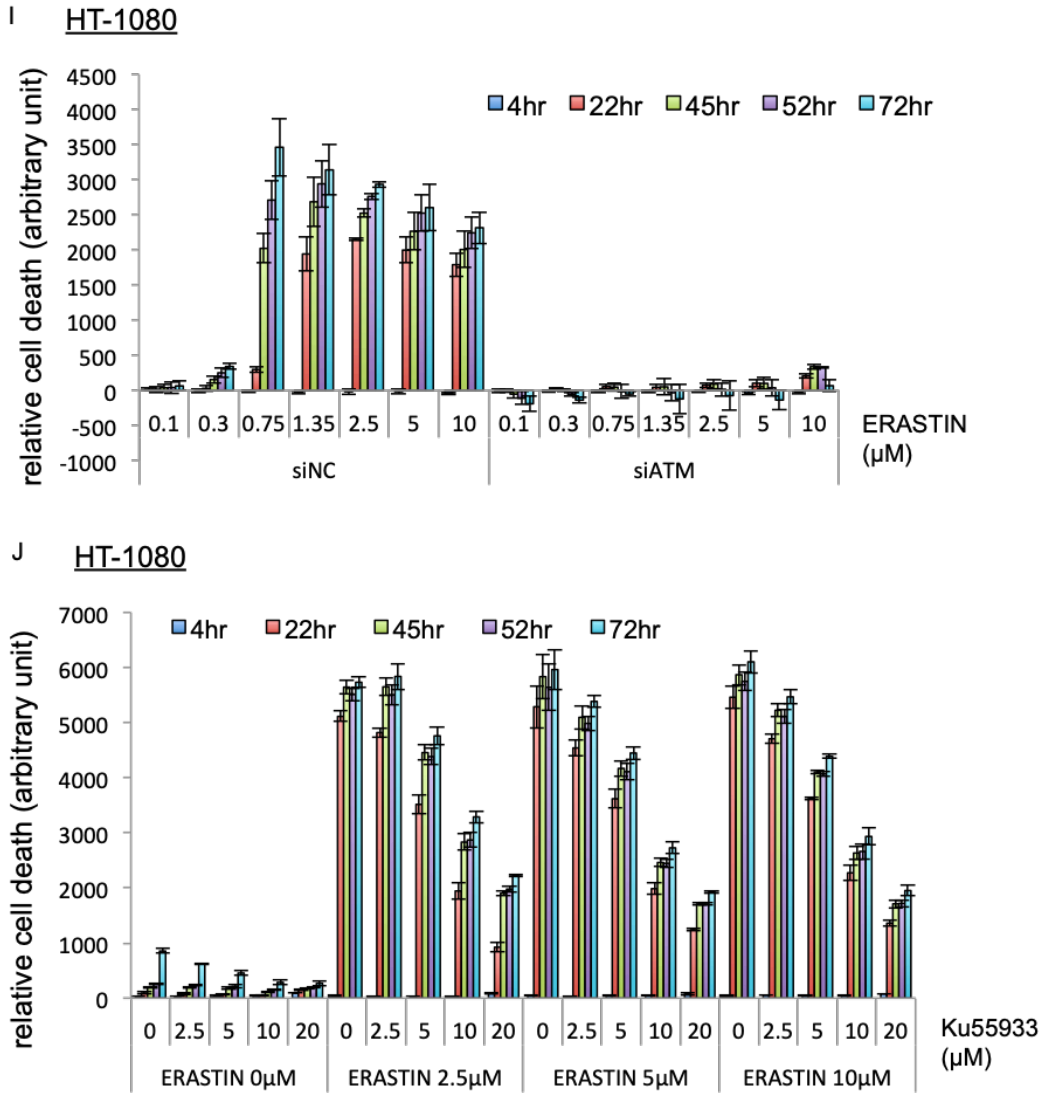

Figure S2I-J. Inhibition of ATM by siRNA or chemical inhibitors protects HT-1080 against erastin-induced death in a time- and does-dependent manner. (I) Depletion of ATM by siRNA protects HT-1080 cells against erastin-induced death. HT-1080 cells were treated with siRNA for 48 h, and then treated with indicated concentration of erastin. The cell death was monitored from 0 to 72 h by CellTox-Green. All the data were normalized to DMSO control in each group. (J) Inhibition of ATM by Ku-55933 protects HT-1080 cells against erastin-induced death. HT-1080 cells were treated with Ku-55933 for 48 h, and then treated with indicated

concentration of erastin. The cell death was monitored from 0 to 72 h by CellTox-Green. The data are shown as mean±S.D from three biological replicates.

Figure S3

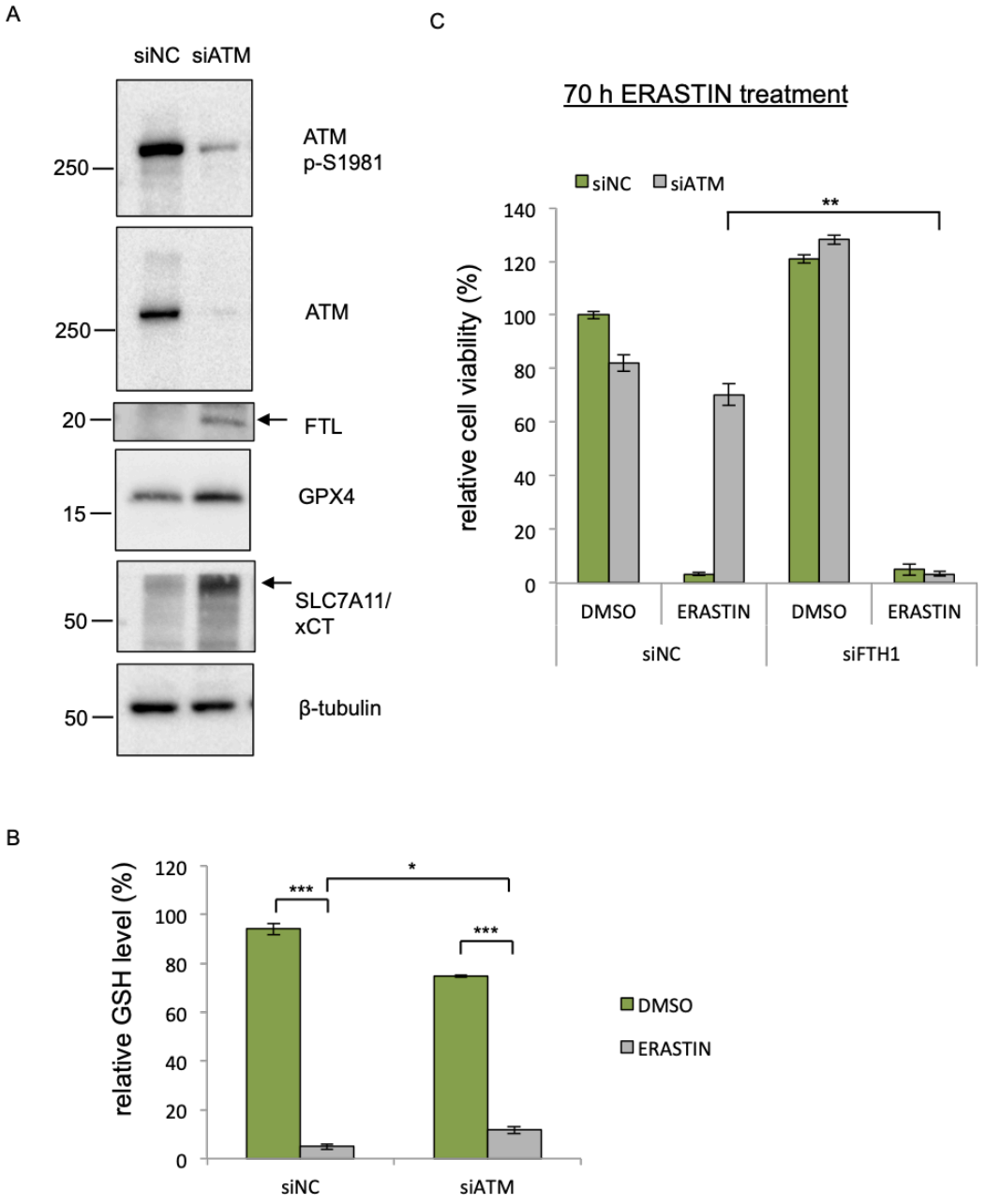

Figure S3. The induction of iron regulatory genes by ATM inhibition is essential for protection against ferroptosis (related to Figure 3). (A) The protein expression of FTL, xCT/SLC7A11, and GPX4 in ATM knockdown cells. Cell lysates from ATM depleted (siRNA 70 h) and siNC (control) MDA-MB-231 cells were analyzed by Western blots with indicated antibodies. The expected bands corresponding to FTL and xCT proteins are indicated by arrows. (B) Reduced glutathione (GSH) is depleted in both siNC and siATM cells in the presence of erastin. MDA-MB-231 cells were transfected with siNC or siATM for 48 h and treated with erastin (2.5 μM) for additional 18 h. The amount of GSH in each indicated condition was measured by

GSH/GSSG-Glo assay (Promega, #V6611). The relative amount of GSH is presented after normalized to the cell viability (CellTiter-Glo) at each condition. (C) FTH1 is essential for prolonged ferroptosis protection by ATM depletion. MDA-MB-231 cells were transfected with indicated siRNA for 72 h, and incubated with DMSO or erastin (10 μM) for 70 h, and the viability was determined by CellTiter-Glo. The data are shown as mean±S.D from three biological replicates, Student's t-test; *$p<0.01$; **$p<0.001$; ***$p<0.0001$

Figure S4

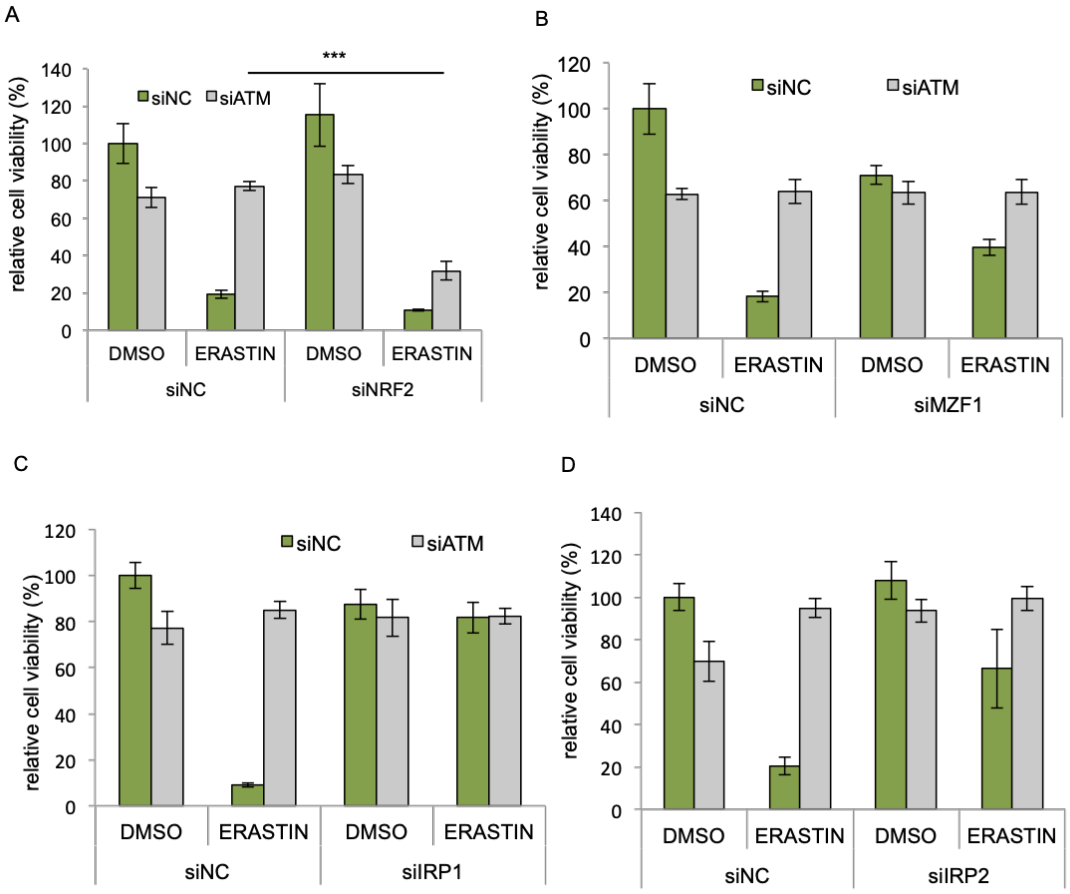

Figure S4. Depletion of MZF1, IRP1, or IRP2 does not affect ATM-silencing mediated ferroptosis protection in MDA-MB-231 cells while NRF2 depletion mitigates ATM-mediated ferroptosis protection.

(A to D) MDA-MB-231 cells transfected with indicated siRNAs for 72 h, treated with DMSO or erastin (10 μM) for 18 to 30 h and the viability was determined by CellTiter-Glo. The data are shown as mean±S.D from three biological replicates.

Figure S5

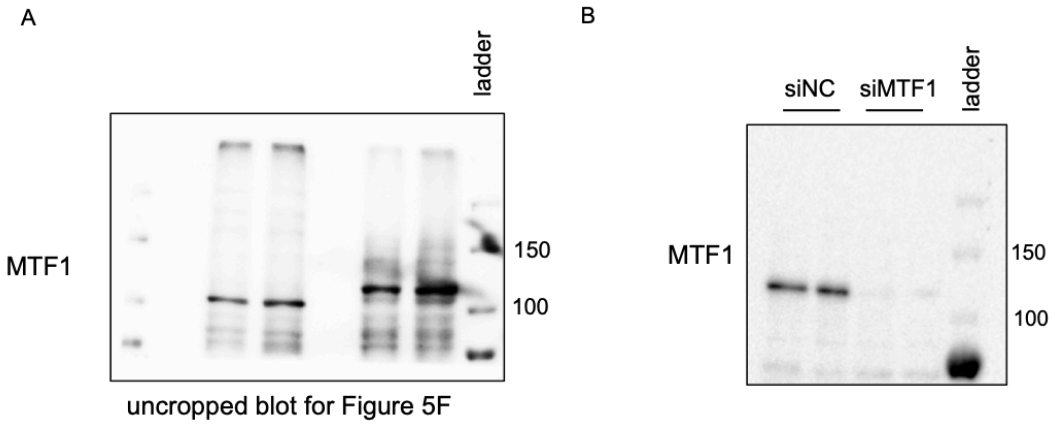

Figure S5. Validation of MTF1 antibody and siRNA in MDA-MD-231 cells. (A) Uncropped blot for Figure 5F. (B) MDA-MB-231 cells were transfected with siNC or siMTF1 for 48 h, and the whole cell lysates were collected for Western blots using the indicated antibody. The endogenous MTF1 protein migrats between 100-150 kDa.