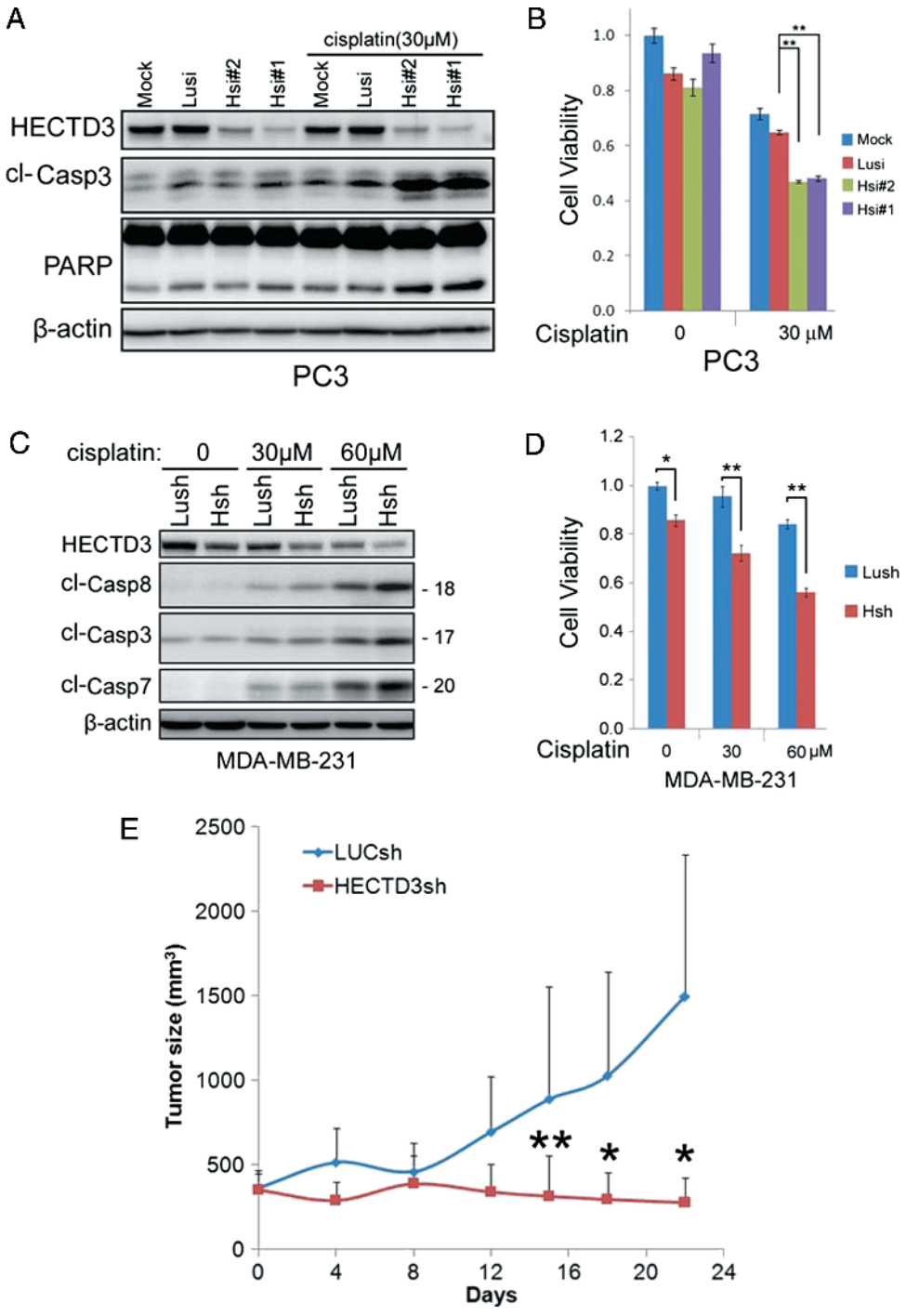

Figure W1. HECTD3 depletion increases cisplatin-induced apoptosis and tumor growth. (A) HECTD3 knockdown by two different siRNAs sensitized the PC-3 prostate cancer cells to cisplatin-induced apoptosis, as detected by PARP and Caspase-3 cleavage. Cisplatin was added 1 day after the cells were transfected with siRNA for 2 days. (B) HECTD3 knockdown by siRNA significantly sensitized PC-3 to cisplatin-induced apoptosis, as determined by the loss of cell viability detected by SRB assays. $^{**}P < .01$ (*t* test). (C) Stable knockdown of HECTD3 by shRNA sensitized the MDA-MB-231 breast cancer cells to cisplatin-induced apoptosis, as detected by the Caspase-8, Caspase-3, and Caspase-7 cleavage. The Hsi#1 siRNA target sequence was chosen for preparing the HECTD3 shRNA construct in the pSIH1-H1-Puro shRNA vector. (D) Stable knockdown of HECTD3 by shRNA decreased cell growth and sensitized the MDA-MB-231 breast cancer cells to cisplatin-induced apoptosis, as detected by the loss of cell viability and by SRB assays. $^{*}P < .05$; $^{**}P < .01$ (*t* test). (E) Stable knockdown of HECTD3 by shRNA sensitized MDA-MB-231 xenografts to cisplatin in SCID mice. In total, twenty-four 4- to 5-week-old SCID mice were purchased from Harlan (Indianapolis, IN) and randomly distributed into two even groups (Lucsh and HECTD3sh). Tumor cells (2.0×10^6) were subcutaneously injected into the fat pad of both sides of each mouse. Tumor volumes were measured twice a week and calculated as $1/2 \times \text{length} \times \text{width}^2$. When the tumor volume reached 300 ± 50 mm^3, the mouse was injected with cisplatin (20 mg/kg) twice a week. All mice were sacrificed after 3 weeks of cisplatin treatment. $^{*}P < .05$; $^{**}P < .01$ (*t* test).

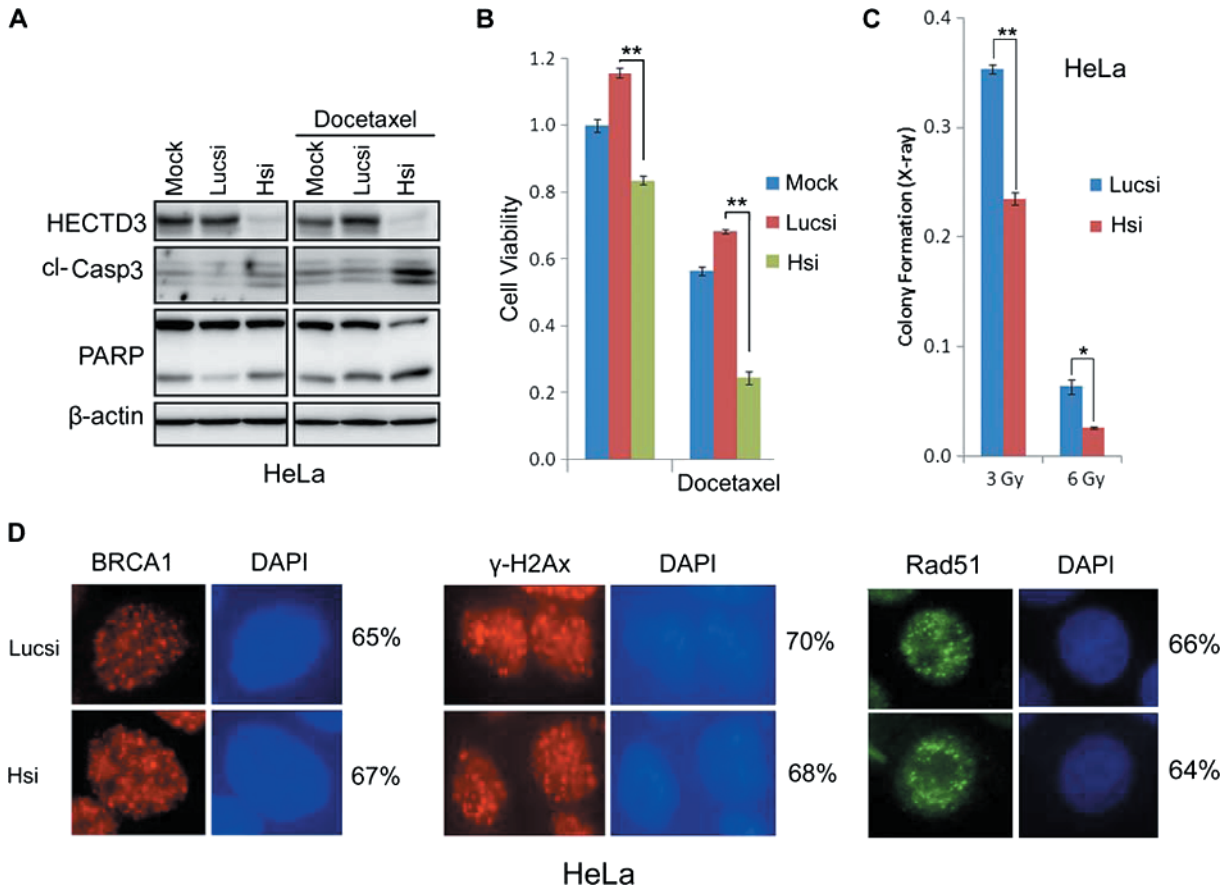

Figure W2. HECTD3 depletion increases docetaxel and γ irradiation–induced apoptosis but does not affect DNA repair in HeLa. (A) HECTD3 knockdown by siRNA sensitized HeLa to docetaxel (10 nM)–induced apoptosis, as detected by PARP and Caspase-3 cleavage. (B) HECTD3 knockdown by siRNA significantly decreased cell growth and sensitized HeLa to docetaxel (10 nM)–induced apoptosis, as determined by loss of cell viability detected by SRB assays. $^{**}P < .01$ (*t* test). (C) HECTD3 knockdown by siRNA significantly sensitized HeLa to γ irradiation–induced apoptosis, as determined by the colony formation assays. $^{*}P < .05$; $^{**}P < .01$ (*t* test). (D) Percentage of cells with positive BRCA1, γ-H2Ax, or Rad51 foci following exposure to irradiation (8 Gy, 6 hours) is similar in HeLa cells with or without HECTD3 depletion. The representative microphotographs of irradiation-induced foci in HeLa cells transfected with the Lucsi control or HECTD3 siRNA are shown. Cells were treated with 8-Gy irradiation and then fixed 6 hours later with 4% paraformaldehyde, blocked, and stained with anti-BRCA1, γ-H2Ax, or Rad51 antibodies. 4′,6-Diamidino-2-phenylindole (DAPI) was used to stain cell nuclei. The percentage of cells with positive foci is shown on the right. The cells with more than 10 foci were considered as positive.

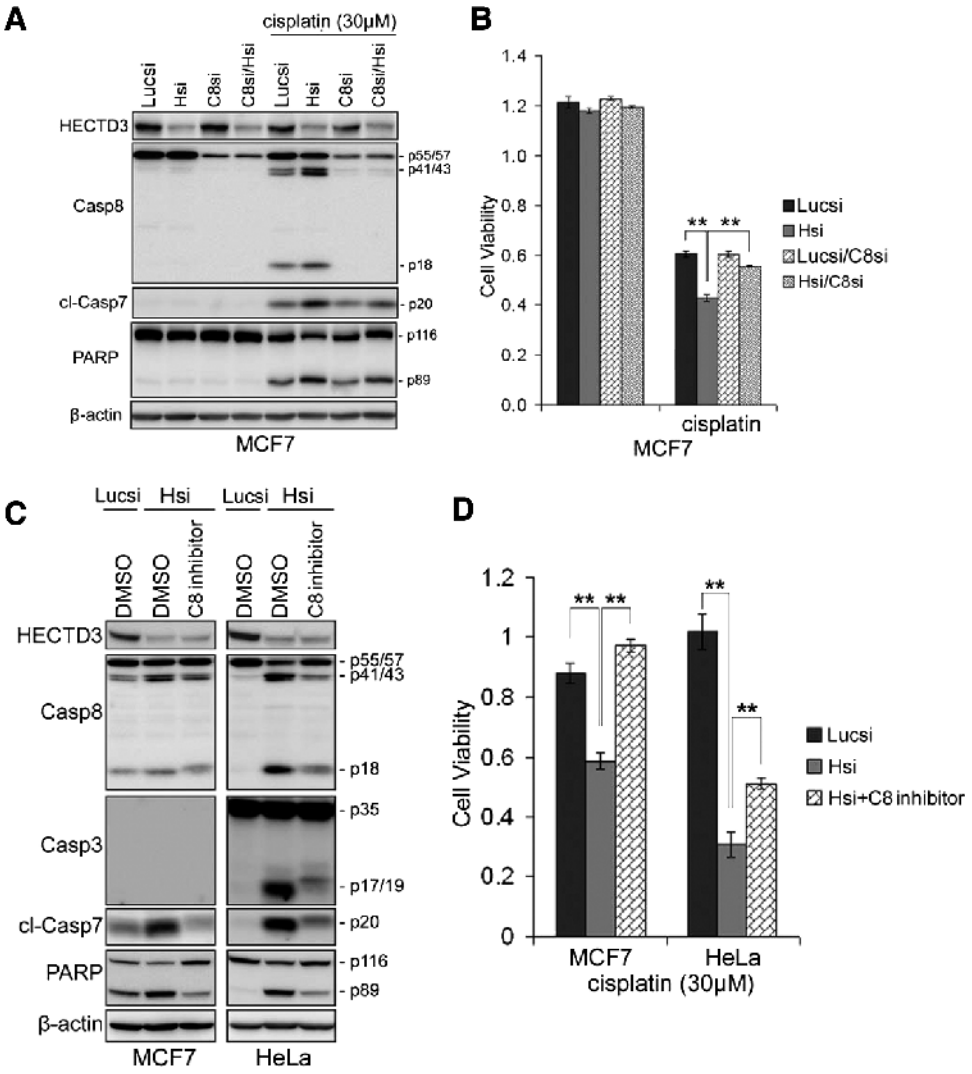

Figure W3. Depletion or inhibition of Caspase-8 at least partially rescues the HECTD3 siRNA proapoptotic function in the presence of cisplatin. (A) MCF7 cells were transfected with siRNAs for 48 hours and treated with cisplatin. Apoptosis was measured by Caspase-8, Caspase-7, and PARP cleavage. (B) Caspase-8 depletion in MCF7 partially rescued the HECTD3 siRNA and cisplatin-induced cell viability decrease as measured by SRB assays. $^{**}P < .01$ (*t* test). (C) The Caspase-8 inhibitor at least partially rescued the HECTD3 siRNA and cisplatin-induced apoptosis. MCF7 and HeLa cells were transfected with siRNAs for 1 day. InSolution Caspase-8 inhibitor II (Calbiochem, Philadelphia, PA, Cat. No. 218840) was added to the cells for 4 hours. Following that, cisplatin (30 μM) was added for 24 hours. Apoptosis was measured by Caspase-8, Caspase-3, Caspase-7, and PARP cleavage. (D) The Caspase-8 inhibitor at least partially rescues the HECTD3 siRNA and cisplatin-induced apoptosis in MCF7 and HeLa. Cell viability was measured by SRB assays. $^{**}P < .01$ (*t* test).

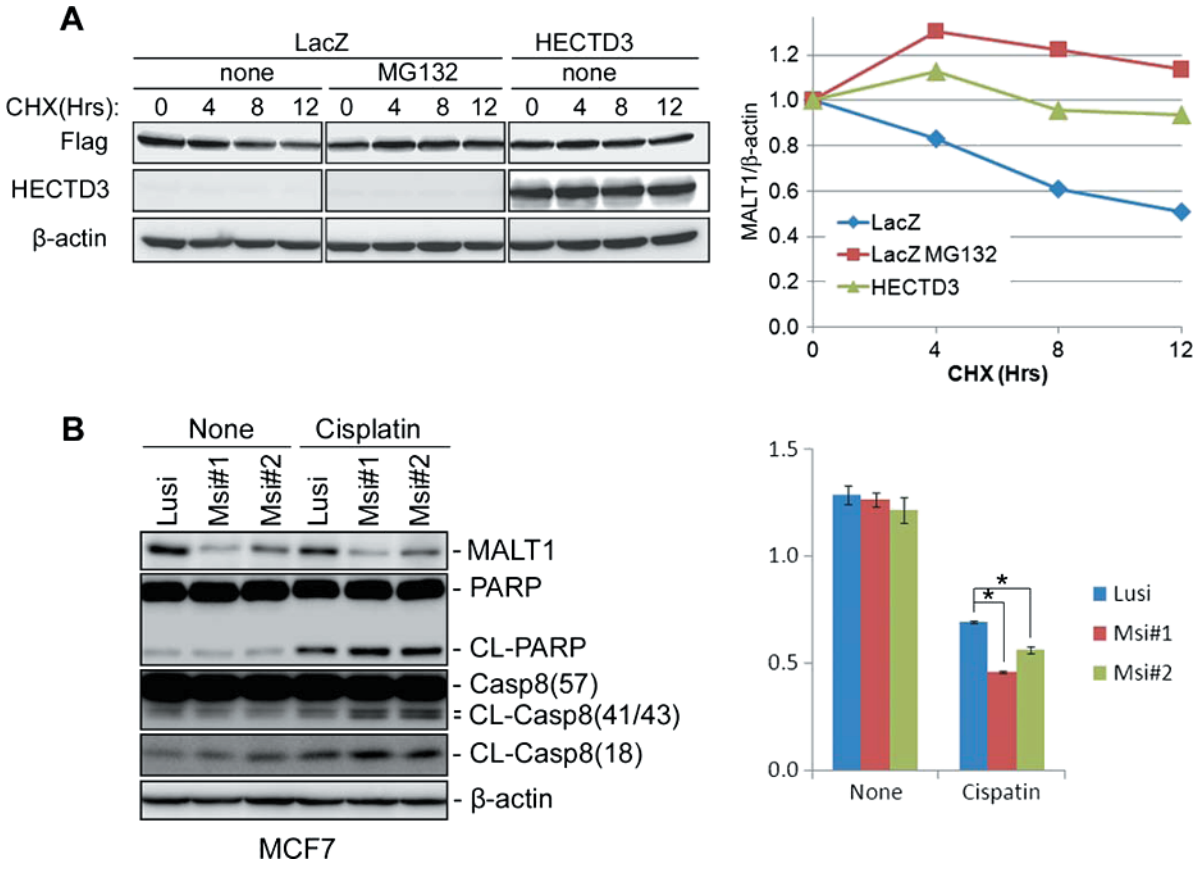

Figure W4. HECTD3 increases MALT1 protein stability and promotes MCF7 survival from cisplatin. (A) HECTD3 overexpression decreased the exogenous MALT1 protein degradation in HEK293FT cells, as detected by the CHX chase assay. MG132 blocked the degradation of MALT1. The cells were incubated with 50 μg/ml CHX for different times (4, 8, and 12 hours) and were collected for Western blot. β-actin was used as the control. The exposure times for each panel have been adjusted to better compare protein half-lives. Quantitative data were shown on the right. HECTD3 overexpression or MG132 delayed the degradation of MALT1. (B) MALT1 knockdown by two different siRNAs sensitized MCF7 cells to cisplatin-induced apoptosis, as detected by the PARP and Caspase-8 cleavage (left) and loss of cell viability detected by SRB assays (right). *$P < .05$ (*t* test).