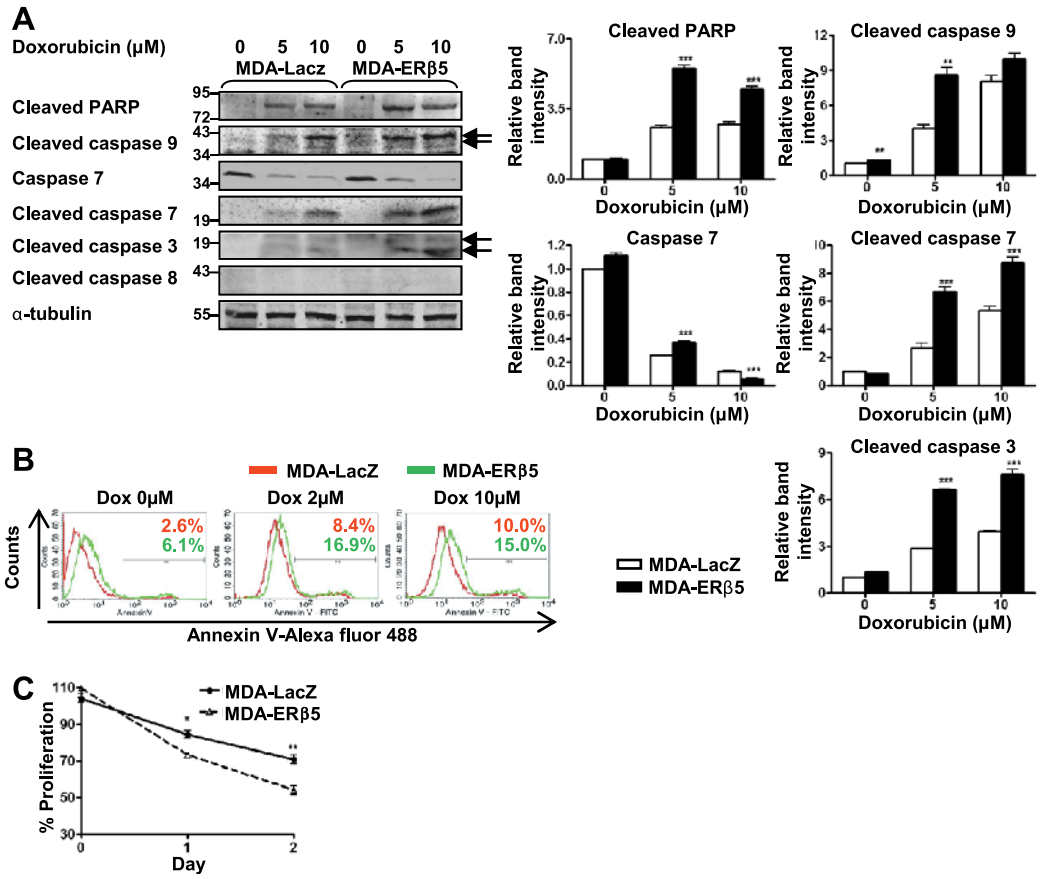

Figure W1. Ectopic ERβ5 sensitizes MDA-MB-231 to doxorubicin-induced apoptosis. (A) ERβ5 and LacZ stably expressed MDA-MB-231 (MDA-ERβ5 and MDA-LacZ) were treated with different concentrations of doxorubicin (0, 5, and 10 μM) for 24 hours. Whole-cell lysates were extracted for Western blot analysis with antibodies as indicated. Band intensities were measured and normalized to the intensity of α-tubulin. The relative band intensities were compared with that of untreated LacZ stably expressed cells. Results are the average of three measurements in a representative experiment. Data are presented as means ± SD. Similar results were obtained in cells incubated with doxorubicin for 12 hours. The statistical significance of the difference in densitometric analysis between MDA-ERβ5 and MDA-LacZ at the same concentration of doxorubicin is shown as $*P < .05$, $**P < .01$, and $***P < .001$. (B) Annexin V/7-AAD staining assays were performed in ERβ5 (green) and LacZ (red) stably expressed MDA-MB-231 cells. Cells were incubated with different concentrations of doxorubicin (0, 5, and 10 μM) for 18 hours. The percentage of annexin V–positive (apoptotic) cells was determined by FACS. Three independent experiments were performed. The results shown are from a representative experiment. (C) The cell viability of MDA-MB-231 stably expressed cell lines was measured by MTS assay. Equal numbers of cells (3×10^3) were seeded on 96-well plates. Cells were incubated with 5 μM doxorubicin after 24 hours. The data were recorded on the first and second days after drug treatment and represented as the percentage of cell viability relative to that of untreated cells. The results are the average of three independent experiments; each was performed in triplicate. Data are represented as means ± SD. The statistical significance of the difference in cell viability between ERβ5 and LacZ stably expressed cells is shown as $*P < .05$.

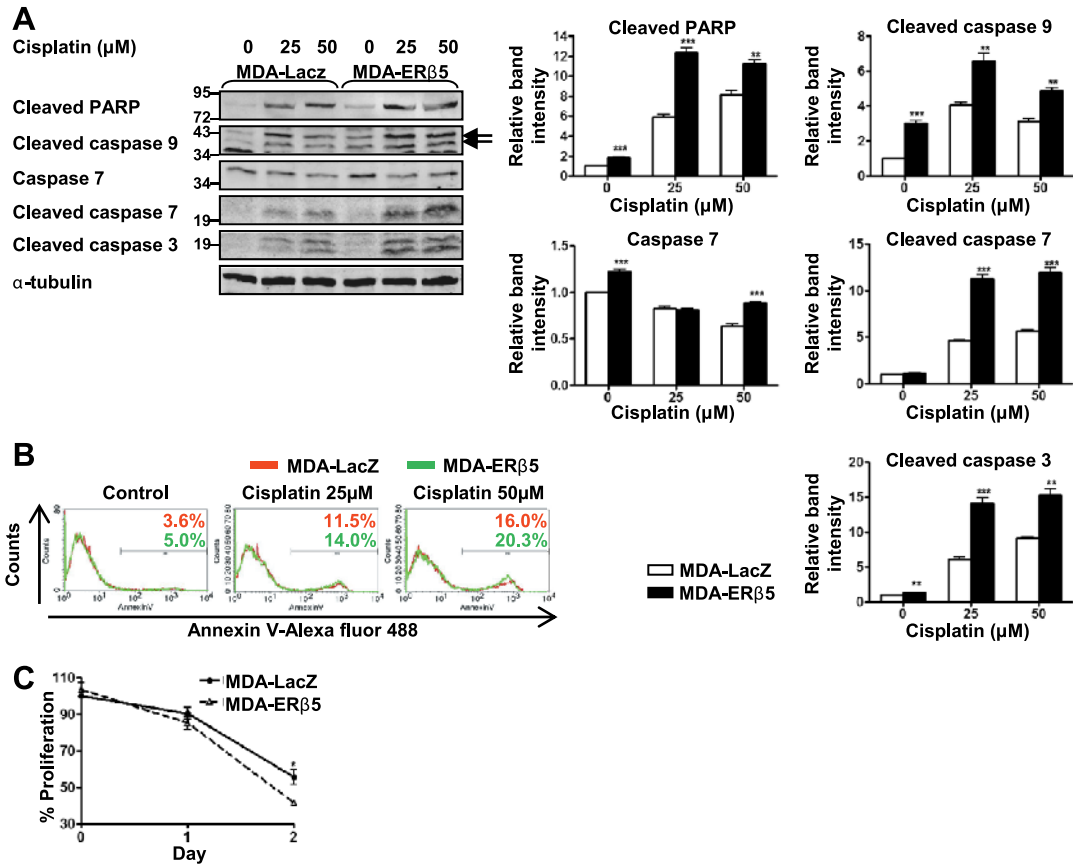

Figure W2. Ectopic ERβ5 sensitizes MDA-MB-231 to cisplatin-induced apoptosis. (A) ERβ5 and LacZ stably expressed MDA-MB-231 were treated with different concentrations of cisplatin (0, 25, and 50 μM) for 24 hours. Whole-cell lysates were extracted for Western blot analysis with antibodies as indicated. The measurement of relative band intensities and analysis of data were similar to those in Figure W1*A*. The statistical significance of the difference in densitometric analysis between MDA-ERβ5 and MDA-LacZ at the same concentration of cisplatin is shown as $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. (B) Annexin V/7-AAD staining assays were performed in ERβ5 (green) and LacZ (red) stably expressed MDA-MB-231 cells. Cells were incubated with different concentrations of cisplatin (0, 25, and 50 μM) for 18 hours. Other experimental details and analysis of data were similar to those in Figure W1*B*. (C) The cell viability of MDA-MB-231 stably expressed cell lines was measured by MTS assay. Cells were incubated with 50 μM cisplatin. The procedures of experiment and analysis of data were similar to those described in Figure W1*C*. The statistical significance of the difference in cell viability between ERβ5 and LacZ stably expressed cells is shown as $^{**}P < .01$.

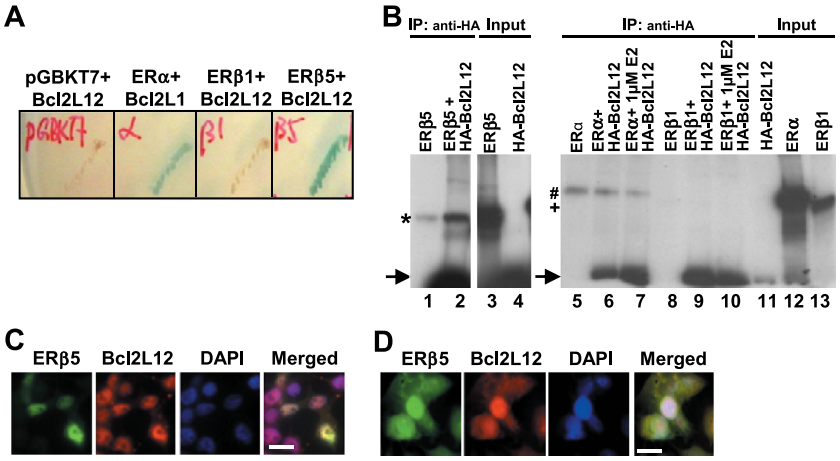

Figure W3. Bcl2L12 specifically interacts with ERβ5 but not ERβ1 or ERα. (A) Full-length ERβ5 and ERα, but not ERβ1, interact with Bcl2L12 in yeast. ERβ1, ERβ5, ERα, or empty vector (pGBKT7) was transformed with Bcl2L12 into yeast cells. Transformed cells were seeded on QDO containing X-α-galactosidase until growth of blue colonies. (B) Bcl2L12 interacts with ERβ5 but not ERβ1 or ERα *in vitro*. ERβ5, ERβ1, ERα, and HA-tagged Bcl2L12, respectively, were translated *in vitro* and labeled with [^{35}S] methionine. Lysates were mixed and incubated in the absence or presence of E2 as indicated, followed by immunoprecipitation with anti-HA affinity gel. The immunoprecipitates were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and analyzed by X-ray autoradiography. The asterisk (*), number sign (#), and plus sign (+) respectively indicate the positions of ERβ5, ERα, and ERβ1. (C and D) ERβ5 colocalized with Bcl2L12 in 293T and MCF-7–ERβ5 cells. The two cell lines were grown in full-serum medium. The 293T cells were transfected with ERβ5 and Bcl2L12. Antibodies to ERβ and Bcl2L12 were used for immunostaining. DAPI was used as a nuclear marker. Images in C and D were captured by fluorescence microscopy. Bar = 20 μm.

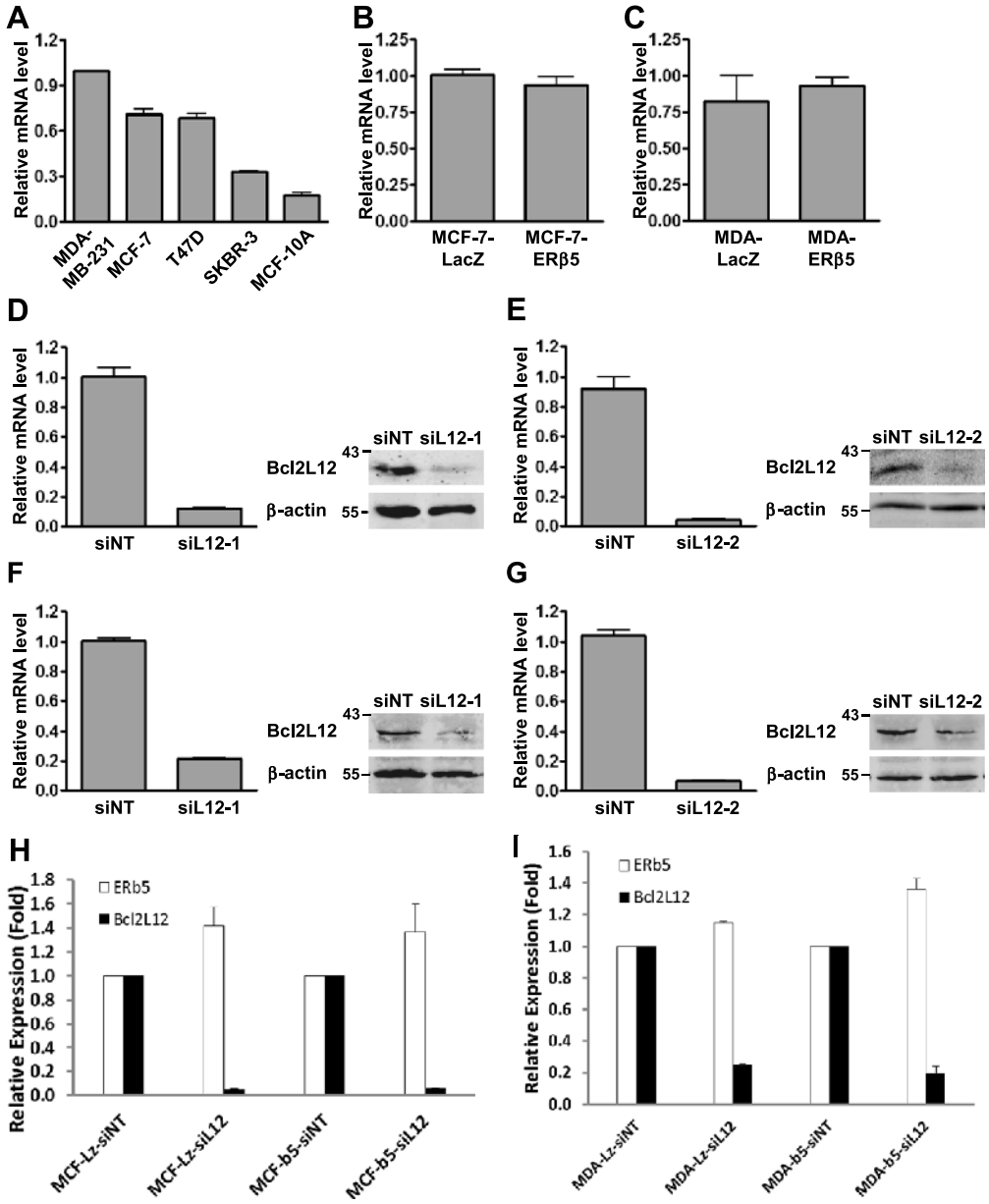

Figure W4. Expression of Bcl2L12 was significantly reduced by gene-specific siRNAs in MCF-7 and MDA-MB-231. (A) Expression level of Bcl2L12 in different mammary cell lines was determined. The cDNAs of BCa cell lines (MDA-MB-231, MCF-7, T47D, and SKBR-3) and mammary epithelial cells (MCF-10A) were used in quantitative RT-PCR. The results are the average of two independent experiments. (B and C) Expression of Bcl2L12 is not altered by the ectopic expression of ERβ5 in BCa cells. The cDNAs of MCF-7–ERβ5/−LacZ (B) and MDA-ERβ5/−LacZ (C) were used in quantitative RT-PCR. The results are the average of two independent experiments. (D–G) Bcl2L12 siRNAs efficiently reduced its expression in MCF-7 and MDA-MB-231. Two Bcl2L12-specific siRNAs (siL12-1 and siL12-2) were transfected into MCF-7 (D and E) and MDA-MB-231 (F and G). After 24-hour transfection, cells were transfected for the second time. siNT was used as negative control. The relative expression of Bcl2L12 was measured by quantitative RT-PCR and Western blot analysis. β-Actin was used as loading control in Western blot analysis. The results of quantitative RT-PCR are the average of three independent experiments. All data are represented as means ± SD. (H and I) ERβ5 and Bcl2L12 expression in MCF-7–ERβ5/−LacZ and MDA-ERβ5/−LacZ cells with or without Bcl2L12 siRNA knockdown.

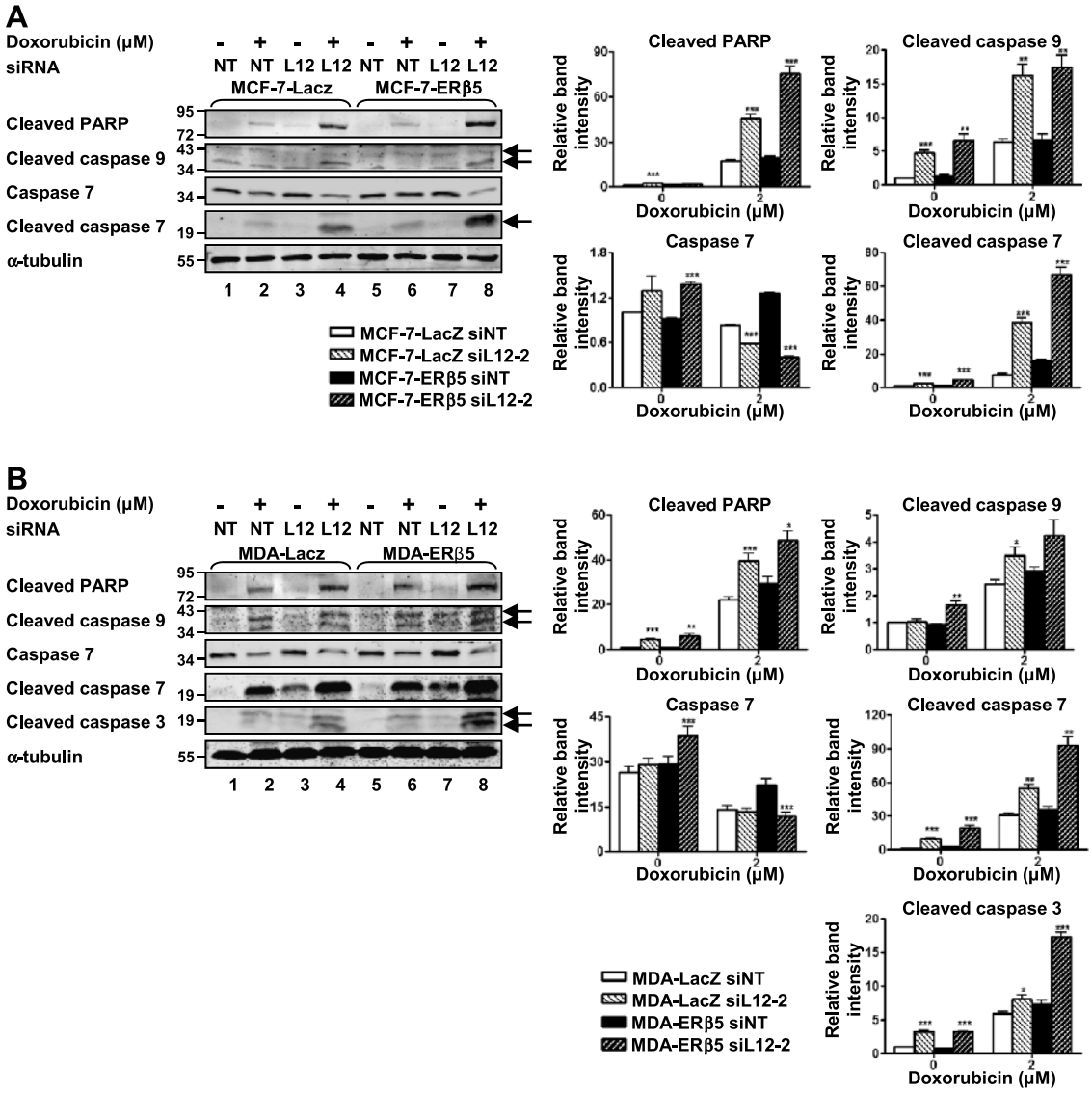

Figure W5. Knockdown of Bcl2L12 by siL12-2 sensitizes doxorubicin-induced apoptosis in MCF-7 and MDA-MB-231. (A and B) siNT or siL12-2 was transfected twice into MCF-7–ERβ5/−LacZ (A) and MDA-ERβ5/−LacZ (B). MCF-7 cells were incubated with 2 μM doxorubicin, and MDA-MB-231 cells were incubated with 5 μM doxorubicin. Whole-cell lysates were extracted for Western blot analysis with antibodies as indicated. The measurement of relative band intensities and analysis of data were similar to those in Figure W1*A*. The statistical significance of the difference in densitometric analysis between the cells treated with siNT and siL12-1 at the same concentration of doxorubicin is shown as $*P < .05$, $**P < .01$, and $***P < .001$.