Supplemental Data

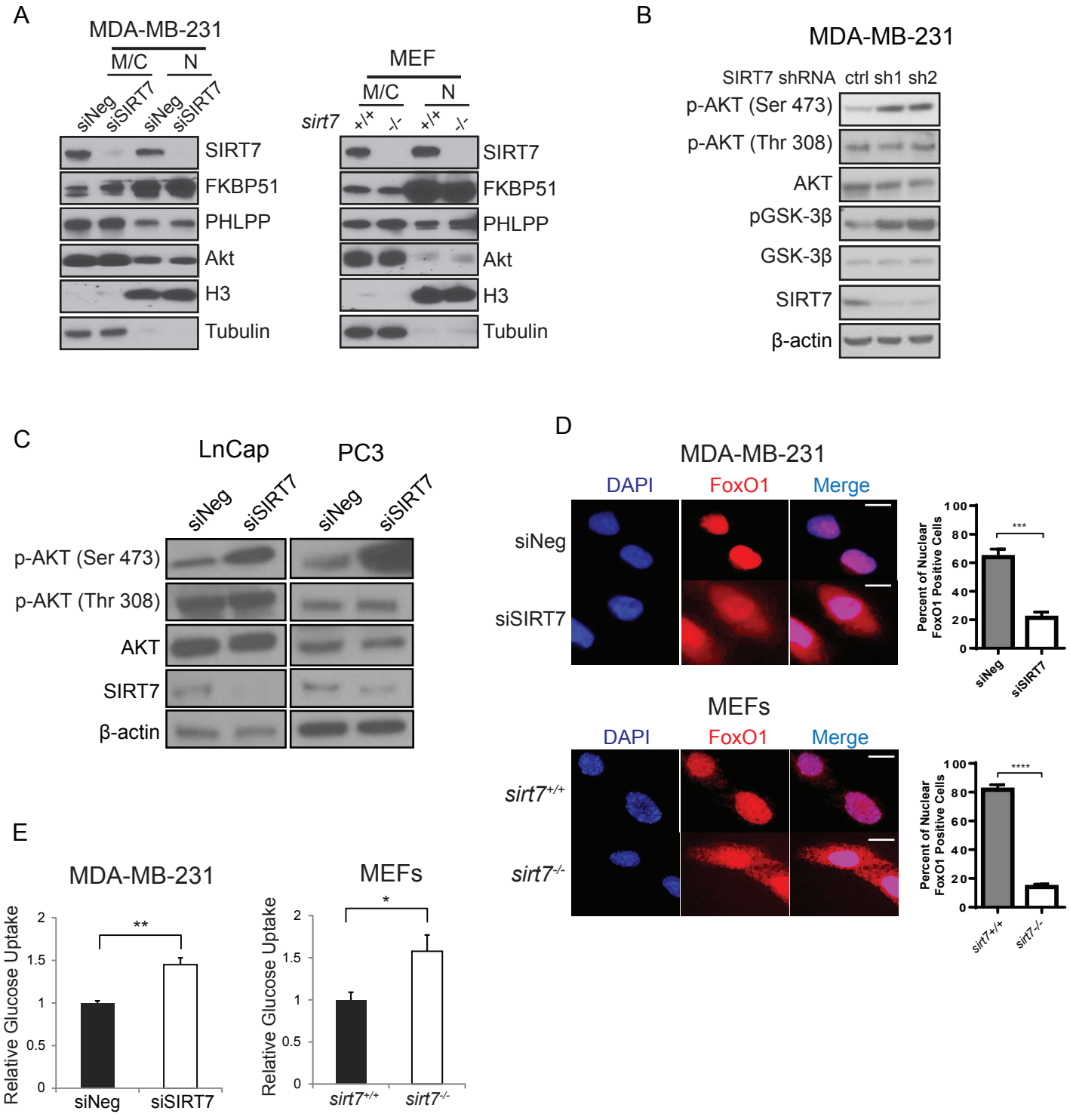

Figure S1. SIRT7 Regulates the Phosphorylation of the Akt at Ser473, Related to Figure 2

(A) MDA-MB-231 cells were transfected with SIRT7 siRNAs (siSIRT7) or control siRNA (siNeg) before fractionation. Western blot was performed to analyze SIRT7 protein levels in nuclear and membrane/cytoplasmic fractions of MDA-MB-231 and SIRT7 MEFs. Validation of antibody specificity was done by testing in SIRT7 knock-down cell or SIRT7 knock-out MEF cells.

(B) MDA-MB-231 cells were infected with the indicated shRNA lentivirus and the phosphorylation levels of Akt and GSK-3β in cell lysates were detected by the indicated antibodies.
(C) The prostate cancer cell lines LnCap and PC3 were transfected with control siRNA or SIRT7 siRNA for 72 hours and phosphorylation of Akt in cell lysates was detected by the indicated antibodies.
(D) Immunofluorescence of localization of FoxO1 in MDA-MB-231 cells transfected with SIRT7 siRNA or a control siRNA, or in *sirt7*$^{+/+}$ or *sirt7*$^{-/-}$ MEF cells. Cells are counterstained with DAPI (blue). Scale bar 10 μm. Relative quantification of nuclear only FoxO1 positive cells was normalized to control cells and data represents means ± SE. Comparison was done by 2-tailed *t-test* (***P<0.001, ****P<0.0001)
(E) Glucose uptake in both MDA-MB-231 cells and SIRT7 MEF cells were measured. Relative glucose uptake was normalized to control cells and data represents means ± SE (in 3 replicates). Data were compared with an unpaired 2-tailed *t-test* (*P<0.05, **P<0.01).

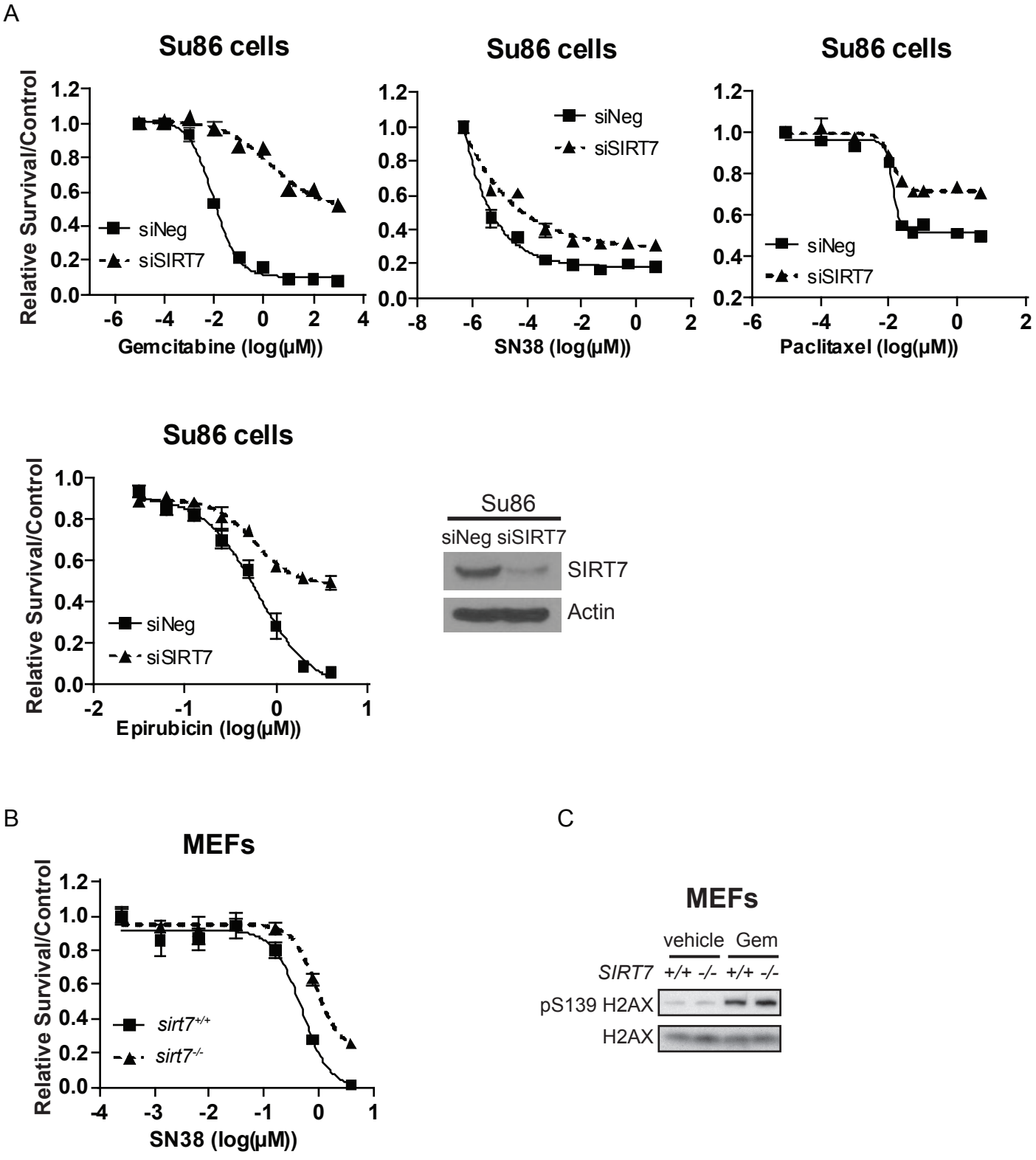

Figure S2. SIRT7 Depletion Sensitizes Su86 Cells to Different Genotoxic Agents, Related to Figure 3
(A) Su86 cells were transfected with control siRNA or SIRT7 siRNA and were then treated with the indicated drugs for 72 hours. Cell survival was determined by MTS assay.
(B) *sirt7*$^{+/+}$ or *sirt7*$^{-/-}$ cells were treated with the indicated drugs. Cell survival was determined by MTS assay.
(C) Cells treated with vehicle or 1μM gemcitabine for 24 hours and cells were subsequently subjected to western blot. Each point indicates the mean value for three independent experiments. Error bars represent standard error of the mean (n=3).

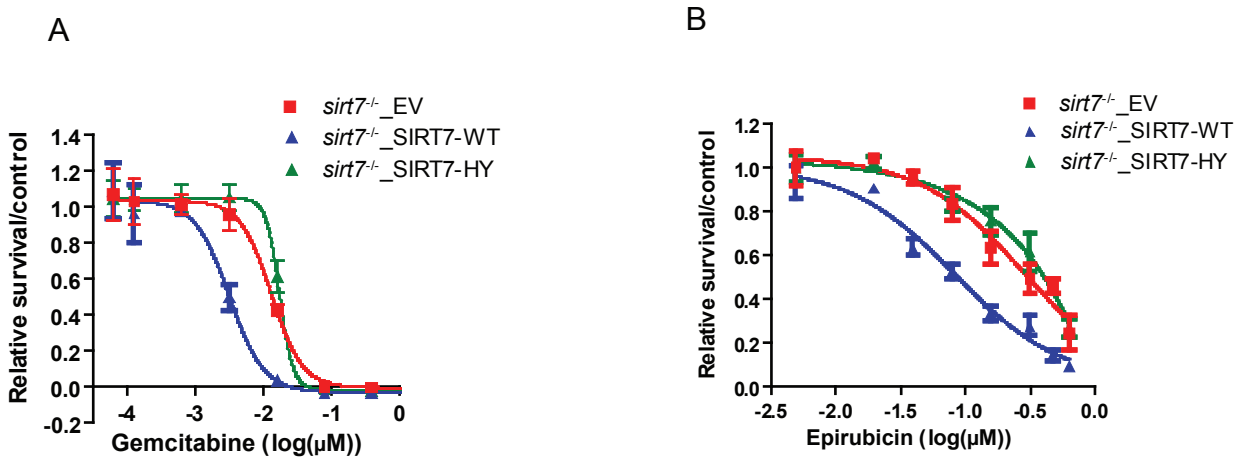

Figure S3. SIRT7 Regulates Cell Sensitivity to Different Genotoxic Agents, Related to Figure 3

sirt7$^{-/-}$ MEF cells transfected with empty vector (EV), SIRT7 wild type (WT) and SIRT7 H187Y mutants were treated with gemcitabine (A) or epirubicin (B). Cell survival was determined by MTS assay. Each point indicates the mean value for three independent experiments. Error bars represent standard error of the mean (n=3).

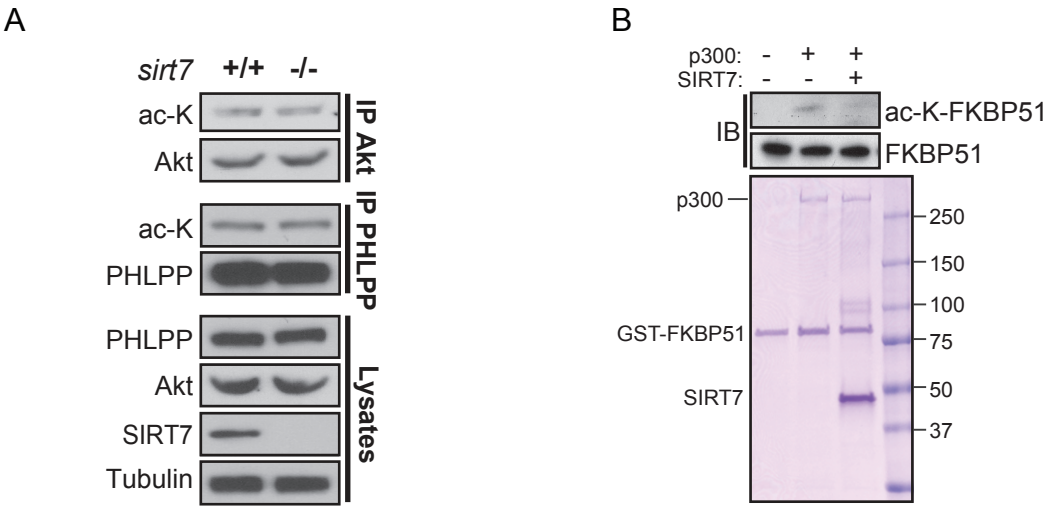

Figure S4. SIRT7 does not Regulate Acetylation of Akt or PHLPP, Related to Figure 5

(A) *sirt7*$^{+/+}$ and *sirt7*$^{-/-}$ MEF cells were subjected to IP using anti-Akt or PHLPP antibodies followed by immunoblotting with anti-acetylation antibody.

(B) *In vitro* deacetylation assay demonstrating that p300 acetylates FKBP51 and SIRT7 deacetylates FKBP51.

A

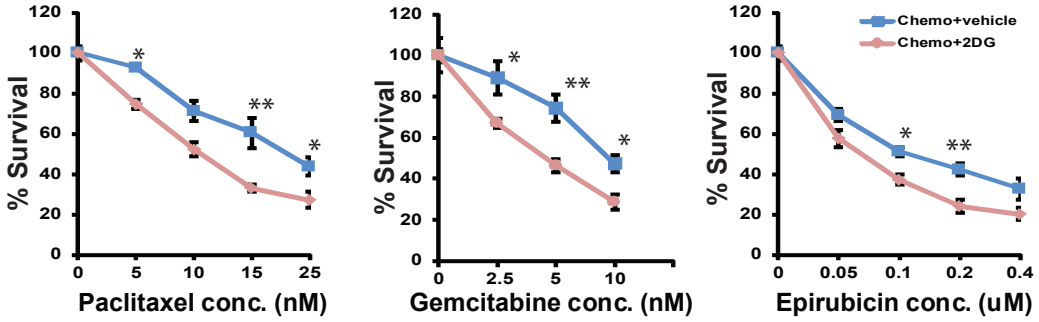

Figure S5. 2DG Sensitized Breast Cancer Cells to Genotoxic Agents, Related to Figure 7

(A) MDA-MB-231 cells were treated with the indicated different doses of drugs alone or combined with 5mM 2DG. After 14 days, colony formation assay was performed and cell survival was calculated. Data represents mean ± SD from triplicate experiments. P-value was calculated by Student's t-test. *P<0.05. **P<0.01.

Supplemental Experimental Procedures

Lentiviral infection

The sequences for SIRT7 shRNA were 5'-TAGCCATTTGTCCTTGAGGAA-3' and 5'-CACCTTTCTGTGAGAACGGAA-3' (Barber et al., 2012). For lentiviral packaging, HEK293T cells (75% confluency) were co-transfected with either SIRT7 knockdown or pLKO.1 control constructs together with packaging and envelope plasmids using Lipofectamine 2000 (Life Technologies Invitrogen) following the manufacturer's instructions. ShRNA lentiviral particles were collected and indicated cells were transduced in the presence of 8mg/ml polybrene. After 48 hours, infected cells were selected with puromycin for 72 hours.

Nuclear and Cytoplasmic Fraction Extraction

Nuclear and cytoplasmic fractions were extracted using a commercial nuclear extract kit (Active Motif, California, USA) following the manufacturer's instructions. Briefly, approximately 10^6 cells were used for the fractionation. Cells were washed three times with buffer A containing phosphatase inhibitors and centrifuged for 5 min at 500 rpm at 4°C. Cell pellets were resuspended in 500 µl Hypotonic buffer B and incubated for 15 min on ice. Then, 25 µl detergents were added before centrifugation for 30 sec at 13000 rpm at 4°C. Supernatant (cytoplasmic fraction) was boiled with SDS and subjected to SDS-PAGE. Pellets were resuspended in 50 µl lysis buffer C and incubated on ice for 30 min, followed by centrifugation at 13000 rpm for 10 min at 4 °C. Supernatant (nuclear fraction) was boiled with SDS and subjected to SDS-PAGE.

Immunofluorescence Staining

Cells were seeded on coverslips and cultured for 24 hours. After washing with PBS, cells were fixed with 4% paraformaldehyde for 10 min and permeabilized in 0.5% Triton X-100 solution for 5 minutes at room temperature. Cells were then blocked with 5% goat serum and then incubated with indicated primary antibodies for 1 hr at 37 °C. After washing with PBS, cells were incubated with secondary antibody for 30 min at 37°C. DAPI staining was performed to visualize nuclear DNA and coverslips were mounted onto glass slides with anti-fade solution. A Nikon ECLIPSE E800 fluorescence microscope was used to visualize the imaging.

Glucose Uptake Assays

MDA-MB-231 cells were reverse transfected with control siRNA (siNeg) or siRNA targeting SIRT7 (siSIRT7) using lipofectamine RNAiMAX per manufacturer's instructions. *sirt7*$^{+/+}$ and *sirt7*$^{-/-}$ MEFs, control or SIRT7 depleted MDA-MB-231 cells were seeded into a 96-well plate at a density of 1×10^4 – 5×10^4 cells/well. Glucose uptake assay kit (Cayman Chemical, USA) was used to perform the assays. Cells treated with 100 µl glucose-free medium containing 150 µg/ml 2-NBDG were incubated at 37 °C in the 5% CO_2 incubator for 6 hours. Plates were then centrifuged for 5 min at 400 rpm at room temperature. Supernatant was removed and 100 µl cell-based assay buffer was added to each well. Uptake of 2-NBDG was detected using an excitation wavelength of 485 nm and an emission wavelength of 535 nm (Infinite M1000PRO, Tecan AG, Switzerland).

Drug treatment

Assays with different drug dilutions for cancer cells were as follows: for paclitaxel: 500, 100, 25, 5, 1, 0.2, 0.04, 0.008, 0 µM; gemcitabine: 10-fold dilutions: 1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001 µM; epirubicin: 2-fold dilutions: 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0 µM; drug dilutions for MEF cells were as follows: for paclitaxel: 100, 25, 6.25, 1.5625, 0.39, 0.098, 0.024, 0.006, 0 µM; gemcitabine: 10-fold dilutions: 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001 µM; epirubicin: 2-fold dilutions: 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078, 0.0039, 0.002, 0 µM;

Antibodies

Antibodies against Akt, phospho-Akt (Ser473), phospho-Akt (Thr308), Foxo1, phospho-Foxo1 (Thr 24), Gsk3β, and phosphor-Gsk3β were purchased from Cell Signaling. Mouse monoclonal SIRT7 antibody was purchased from Santa Cruz (sc-365344). PHLPP antibody was obtained from Bethyl Laboratories. Anti-Flag (M2), anti-HA, and anti-β-actin antibodies were purchased from Sigma. Anti-acetylated lysine antibodies were purchased from Rockland and Millipore. Agarose beads immobilized with acetylated lysine antibody were purchased from ImmuneChem.

Colony Formation Assay

Cells were treated with indicated drugs for 10-14 days at 37°C, 5% CO2 to allow colony formation. Colonies were then stained with 5% GIEMSA and counted. Results were generated from three independent experiments and normalized for plating efficiencies.

Supplemental References

Barber, M.F., Michishita-Kioi, E., Xi, Y., Tasselli, L., Kioi, M., Moqtaderi, Z., Tennen, R.I., Paredes, S., Young, N.L., Chen, K., *et al.* (2012). SIRT7 links H3K18 deacetylation to maintenance of oncogenic transformation. Nature *487*, 114-118.