Supporting Information

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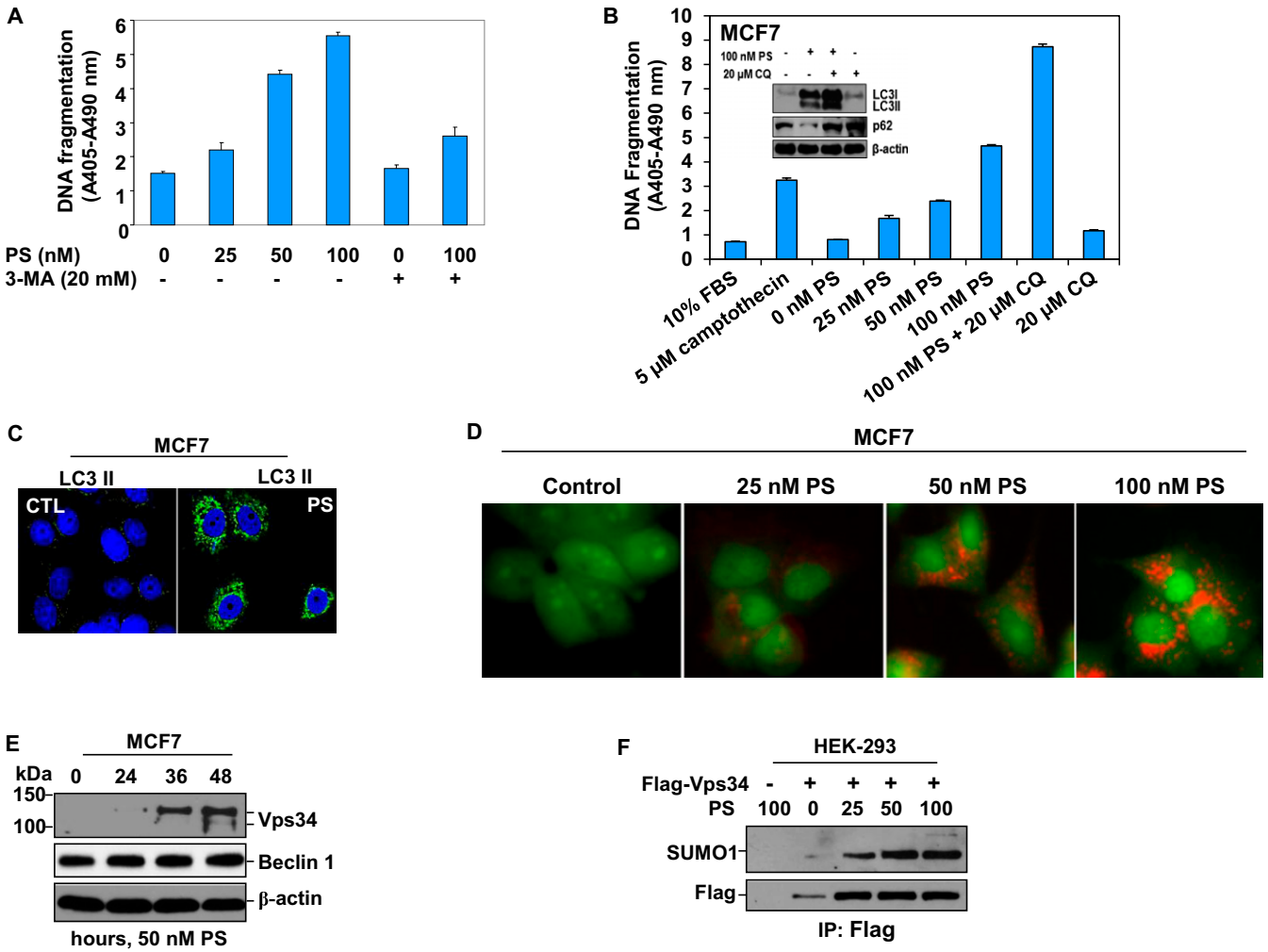

Fig. S1. Treatment with panobinostat (PS) induces apoptosis and autophagic vacuole formation in breast cancer cells. (*A* and *B*) DNA fragmentation (apoptosis) in MCF7 cells following treatment with the indicated concentration of PS and/or 3-methyladenine (3-MA) (*A*) or PS and chloroquine (CQ) (*B*) for 48 h. Treatment with 5 μM camptothecin was used as a positive control for DNA fragmentation. (*Inset*) The effects of cotreatment on the expression levels of p62 and LC3 in the MCF7 cells. (*C* and *D*) Treatment with PS induces autophagic vacuoles in breast cancer cells. (*C*) Confocal microscopic evaluation of LC3B in MCF7 cells treated with 100 nM PS for 24 h. Original magnification is 40×. (*D*) Confocal microscopic evaluation of acridine orange-staining in MCF7 cells treated with PS as indicated, for 24 h. Original magnification is 40×. (*E*) Treatment with PS induces Class III PI 3-kinase Vps34 and Beclin 1 in MCF7 cells. Anti-Vps34 antibody detected two bands, a major upper and minor lower band with a size difference of ∼20 kDa. (*F*) PS induces Flag–Vps34 and its SUMOylation by small ubiquitin-related modifier (SUMO) 1 in HEK-293 cells.

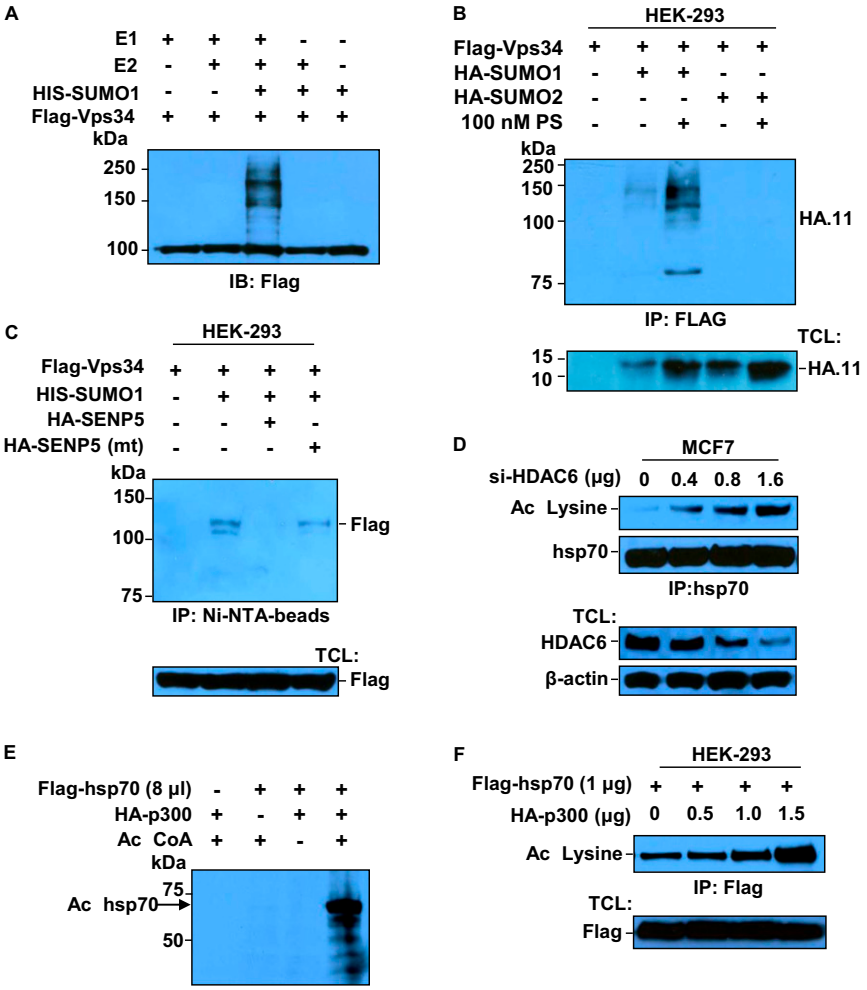

Fig. S2. SUMOylation at lysine-840 enhances lipid kinase activity of Vps34. (*A*) Immunoblot analysis of SUMO1-mediated in vitro SUMOylation of Flag–Vps34. In vitro SUMOylation of in vitro translated Vps34 was performed with a kit [containing SUMO1 activating enzyme subunit1 (E1) and Ubiquitin-like protein activating enzyme (E2)] from LAE Biotech International and detected by anti-Flag antibody. (*B*) Vps34 has high affinity for SUMO1 but not SUMO2. Immunoblot analysis of Flag-immunoprecipitated Vps34 from HEK-293 cells cotransfected with Flag–Vps34 and HA–SUMO1 or HA–SUMO2 for 24 h, then treated with 100 nM PS for 24 h, as indicated. (*C*) SENP5 is the SUMO-specific protease for Vps34. Immunoblot analysis of Vps34 in His-tag immunoprecipitates from HEK-293 cells cotransfected with Flag–Vps34, His–SUMO1, and wild-type or mutant HA–SENP5 for 48 h, as indicated. The expression of Flag–Vps34 in the total cell lysates served as the loading control. (*D*) Histone deacetylase (HDAC) 6 depletion by siRNA induces acetylation of heat shock protein (hsp) 70. Immunoblot analyses of acetylated lysine in hsp70 immunoprecipitates from MCF7 cells transfected with the indicated amounts of HDAC6 siRNA vector for 48 h. Immunoblot analyses were also performed for expression levels of HDAC6 and β-actin in the cell lysates. (*E* and *F*) p300 is an acetyltransferase for hsp70. (*E*) Recombinant p300 can acetylate hsp70 in vitro. Immunoblot analysis of p300-mediated in vitro acetylated hsp70. In vitro translated Flag–hsp70 was combined with recombinant p300 and acetyl CoA and subjected to in vitro acetylation followed by SDS/PAGE and immunoblot analyses. (*F*) Immunoblot analyses of acetylated lysine in Flag immunoprecipitates from HEK-293 cells transfected with Flag–hsp70 and the indicated amounts of HA–p300 cDNA. Immunoblot analysis was also performed for expression levels of Flag–hsp70 in the total cell lysates.

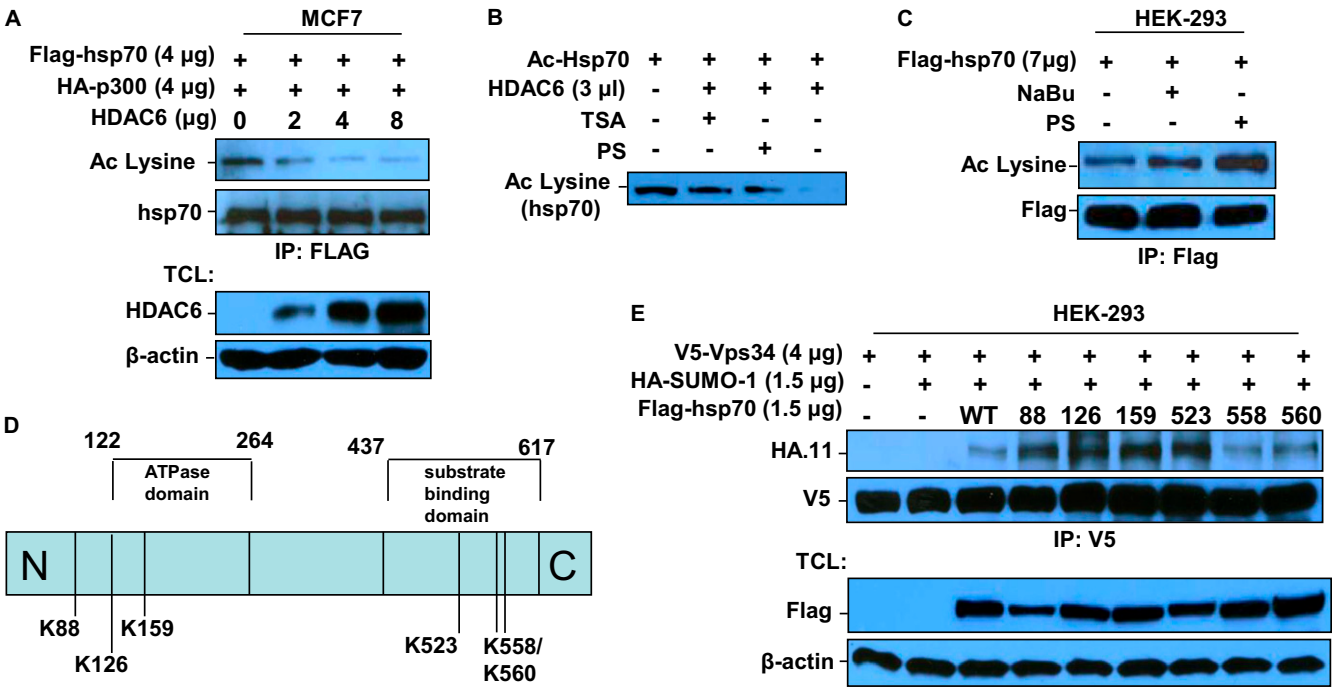

Fig. S3. Effects of hsp70 acetylation on the SUMOylation of Vps34 in breast cancer cells. (*A*) HDAC6 deacetylates hsp70 in breast cancer cells. Immunoblot of acetylated lysine in hsp70 immunoprecipitates from MCF7 cells transfected with Flag–hsp70, HA–p300, and HDAC6 cDNA, as indicated. Immunoblot analyses were also performed for expression levels of HDAC6 and β-actin in the total cell lysates. (*B*) HDAC inhibitors attenuate HDAC6-mediated deacetylation of hsp70. Acetylated Flag–hsp70 (purified from PS-treated HEK-293 cells expressing Flag–hsp70) was combined with in vitro translated HDAC6 in the presence or absence of 400 nM TSA or 100 nM PS. The attenuation of hsp70 acetylation was detected with anti-acetylated lysine antibody. (*C*) HDAC inhibitors induce varying amounts of hsp70 acetylation. Immunoblot analyses of acetylated lysine in Flag immunoprecipitates from HEK-293 cells transfected with Flag–hsp70 and treated with 1.0 mM sodium butyrate (NaBu) or 50 nM PS for 24 h. (*D*) Schematic representation of acetylation sites on hsp70 identified by mass spectroscopy following treatment of Flag–hsp70 expressing HEK-293 cells with PS for 16 h. (*E*) Acetylation mimicking hsp70 mutations (K to Q) enhance Vps34 SUMOylation. Immunoblot analysis of SUMO1 in Vps34 immunoprecipitates from HEK-293 cells cotransfected with V5–Vps34, HA–SUMO1, and Flag–hsp70 wild type or K-to-Q mutants, as indicated. Immunoblot analyses were also performed for expression levels of Flag–hsp70 and β-actin in the total cell lysates.

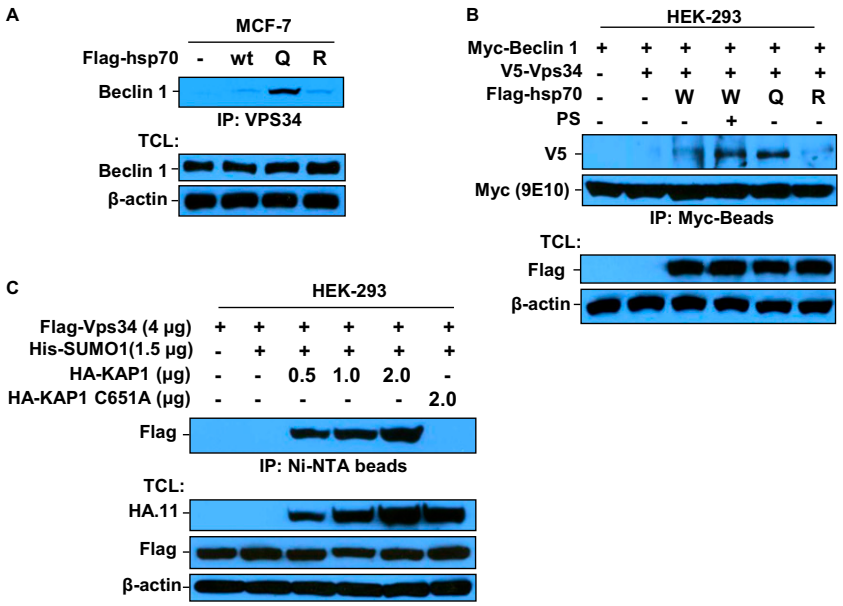

Fig. S4. Acetylation of hsp70 enhances Vps34 SUMOylation and Beclin-1–Vps34 complex formation in breast cancer cells. (*A*) K159Q mutant hsp70 increases endogenous Beclin-1–Vps34 complex formation in MCF7 cells. Immunoblot analysis of Beclin 1 in Vps34 immunoprecipitates from MCF7 cells transfected with wild-type, K159Q, or K159R hsp70 cDNA. Immunoblot analyses were also performed for expression levels of Beclin 1 and β-actin in the cell lysates. (*B*) K159Q mutant hsp70 increases Beclin-1–Vps34 complex formation. Immunoblot analyses of Vps34 in Myc–Beclin-1 immunoprecipitates from HEK-293 cells cotransfected with Myc–Beclin-1, V5–Vps34, and Flag–hsp70 wt or K159Q/K159R mutants, as indicated. Wild-type hsp70-transfected cells were treated with 50 nM PS for 24 h. Immunoblot analyses were also performed for expression levels of Flag (hsp70) and β-actin in the cell lysates. (*C*) KRAB–ZFP-associated protein 1 (KAP1)-mediated SUMOylation of Vps34 is dose-dependent. Immunoblot analysis of Flag–Vps34 in His-SUMO immunoprecipitates from HEK-293 cells transfected with Flag–Vps34, His-SUMO1, and HA–KAP1 or HA–KAP1 C651A mutant, as indicated. Immunoblot analyses were also performed for expression levels of HA–KAP1, Flag–Vps34, and β-actin in the cell lysates.

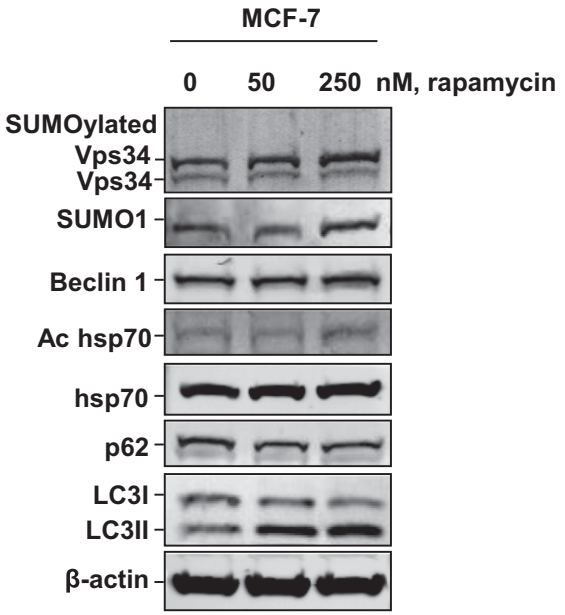

Fig. S5. Treatment with rapamycin causes acetylation of hsp70, SUMOylation of Vps34, and induction of autophagy in human breast cancer cells. MCF7 cells were treated with the indicated concentrations of rapamycin for 8 h. At the end of treatment, cell lysates were prepared and immunoblot analyses were performed for the expression levels of Vps34, SUMOylated Vps34, Beclin 1, hsp70, p62, LC3, and β-actin in the cell lysates.

Table S1. Constructs created and used in this study

Construct	Vector backbone	Cloning site
HA–SUMO2	pCDNA3–HA	BamHI/XbaI
HA–SENP5	pCDNA3–HA	BamHI/XbaI
Myc–Beclin 1	pCDNA3.1/Myc–HisA	BamHI/EcoRI
V5–Vps34	pCDNA4/V5–HisB	NotI/XbaI
Flag–Vps34	pCDNA3–FLAG	NotI/XbaI
Flag–hsp70	pCDNA3–FLAG	BamHI/HindIII