Figure S1. Bortezomib induces cell apoptosis in HL-60 cells. (A) Cells were treated with bortezomib (20 nM) for 0, 24 and 48 h, and then cells were stained using Annexin V and PI. The percentage of apoptotic cells was assessed using FACS analysis. (B) Bar graph showing the percentage of cells negative for Annexin V and PI staining. Data are presented as the mean ± standard deviation of three independent repeats. **P<0.01 and ***P<0.001 vs. control (0 h). $^{\&\&\&}$P<0.001 24 vs. 48 h. (C) Cells were treated with bortezomib (20 nM) for 0, 24 and 48 h, and the expression levels of cleaved PARP, cleaved caspase-3, Bcl-2 and Bax were assessed by western blotting. PI, propidium iodide; PARP, poly(ADP-ribose) polymerase.

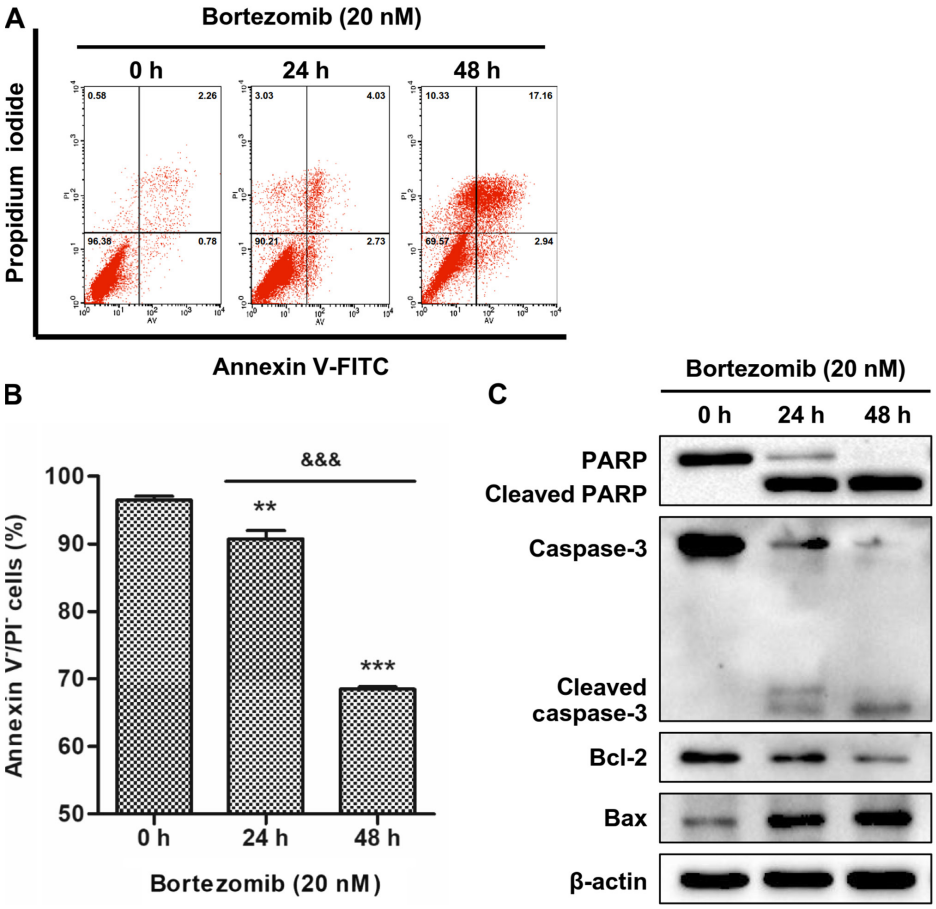

Figure S2. Bortezomib induces autophagy in HL-60 cells. (A) Cells were treated with bortezomib (20 nM) for 0 or 24 h. Transmission electron microscopy was performed to detect autophagosomes. Arrows indicate the autophagosomes. Scale bar, 500 nm. (B) Cells were treated with bortezomib (20 nM) for 0, 24 and 48 h. The expression levels of p62 and LC3 were assessed by western blotting. (C) Treated cells were stained with MDC, and staining was assessed using fluorescence microscopy to identify autophagic vacuoles. Scale bar, 50 μm. (D) Percentages of punctate dots were quantified by counting the number of cells exhibiting the punctate pattern of MDC staining among 100 cells. Data are presented as the mean ± standard deviation of three independent repeats. **P<0.01 vs. control (0 h). M, mitochondrial structures; N, nucleus; C, cytoplasm; MDC, monodansylcadaverine.

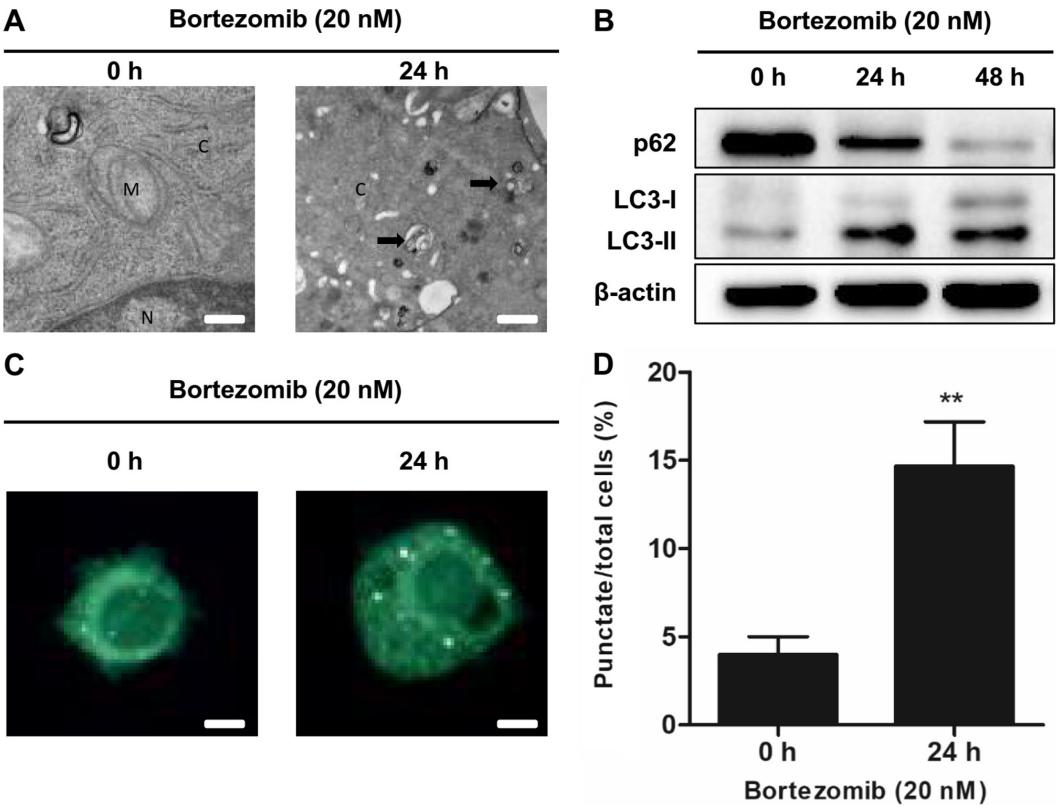

Figure S3. Downregulation of Beclin-1 enhances apoptosis induced by bortezomib in HL-60 cells. Cells were infected with lentiviruses expressing shRNAs (non-targeting control or Beclin-1). Puromycin-resistant cells were pooled after each infection. Cells transfected with the control shRNA and shBeclin-1 were treated with or without bortezomib (20 nM) for 24 h, and the expression levels of cleaved caspase-3, cleaved PARP, Bcl-2, p62 and Beclin-1, and LC3-I to LC3-II conversion were determined by western blotting. CTRL, control; PARP, poly(ADP-ribose) polymerase; shRNA/sh, short hairpin.