Supplementary Materials and Methods

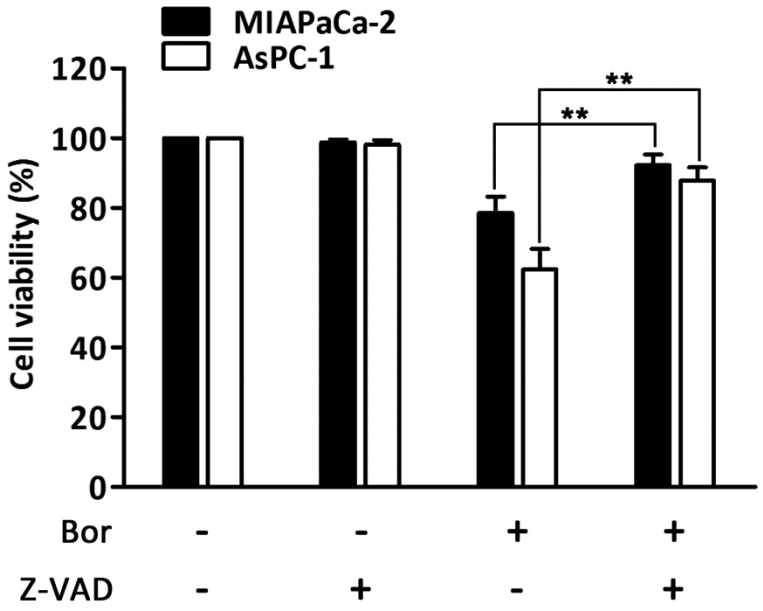
Reagents.

Anti-Beclin1, anti-Atg5, anti-Atg7 antibodies were from Cell Signaling Technology (Beverly, MA, USA).

RNA interference.

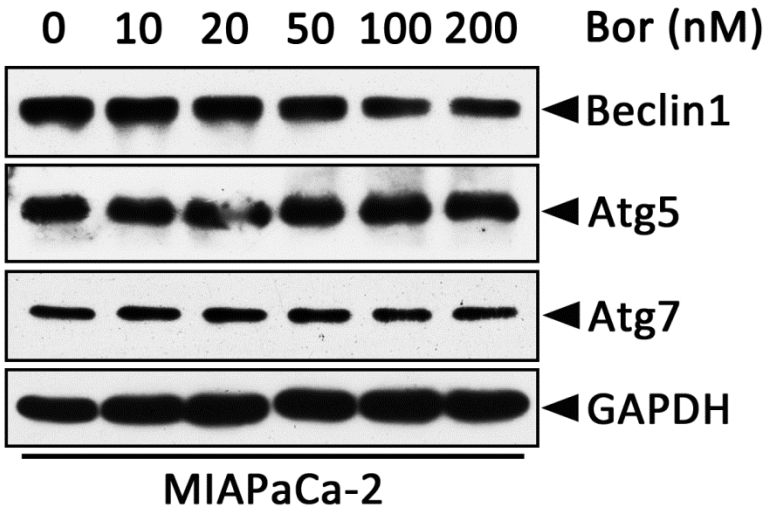
The double-stranded siRNA duplex for Beclin1 (5'-GGUCUAAGACGUCCAACAA-3'), and non-targeting scramble siRNA were synthesized chemically from Ribobio (Guangzhou, China) and resuspended in RNase-free buffer to a concentration of 20 μM. For siRNA transfection, cells were seeded into 6-well plates in antibiotic-free media and transfected at 30-50% confluency with 50 nM siRNA in Opti-MEM (Invitrogen) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Supplementary Figure legends



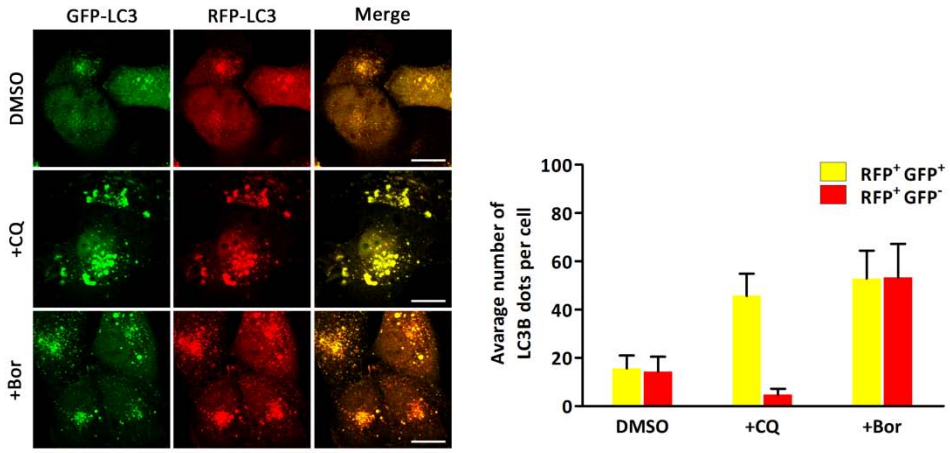
Supplementary Figure S1: Bortezomib causes PC cells apoptosis was caspase dependent.

MIAPaCa-2 and AsPC-1 cells were treated with bortezomib (100 nM) for 24 h in the presence or absence of Z-VAD (50 μM). Cell viability was measured by CCK-8 assay. Each bar represents means ± SD of three separate experiments. **p < 0.01



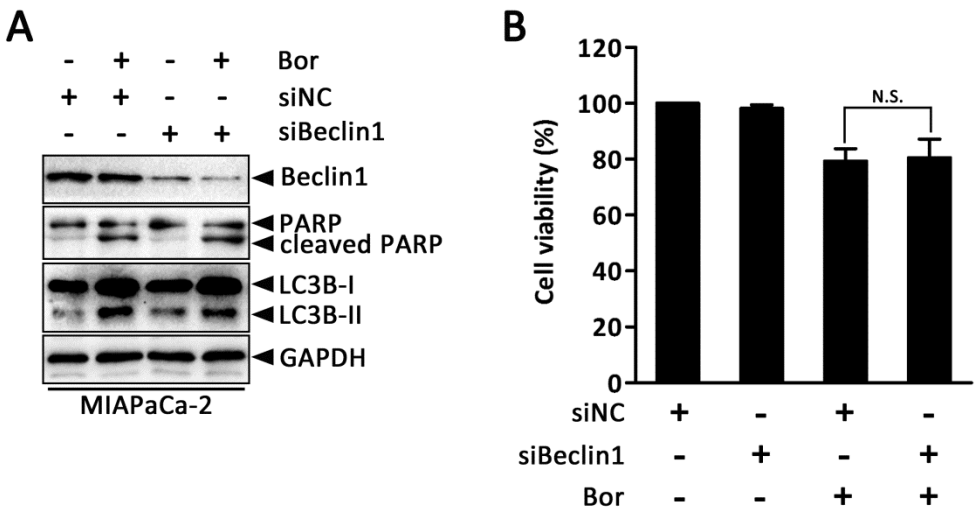
Supplementary Figure S2: Effects of bortezomib on the expression of several autophagy-related proteins in PC cells.

Western blot analysis of Beclin1, Atg5, and Atg7 protein levels after MIAPaCa-2 cells were treated with the indicated concentrations of bortezomib for 24 h.



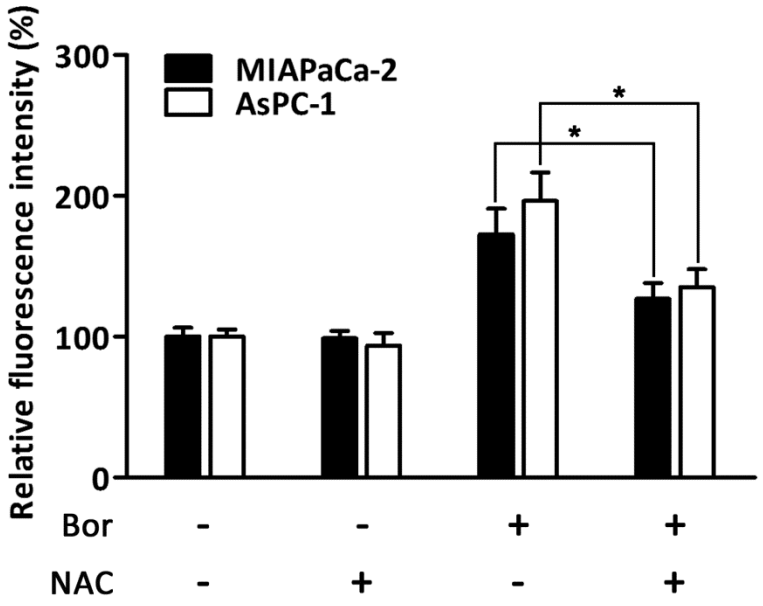
Supplementary Figure S3: Bortezomib induces autophagic flux in PC cells.

AsPC-1 cells transfected with GFP-mRFP-LC3B were treated with DMSO (<0.1%), CQ (10 μM), or bortezomib (100 nM) for 24 h, and then observed for the change of both green and red fluorescence using a confocal microscope. Scale bar: 20 μm. The numbers of acidified autophagosomes (GFP^-/RFP^+) versus neutral autophagosomes (GFP^+/RFP^+) per cell in each condition were quantified. Data are presented as mean ± SD from three separate experiments.



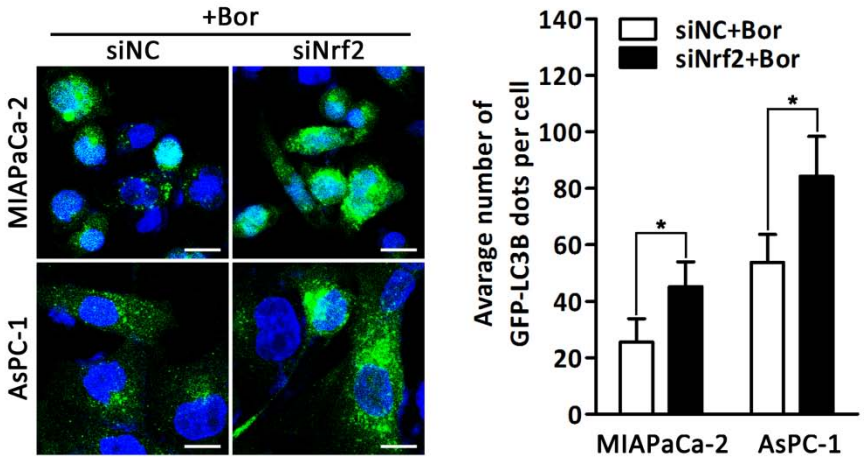
Supplementary Figure S4: Bortezomib induces autophagy in PC cells via a Beclin1-independent pathway.

MIAPaCa-2 cells were transfected with Beclin1 siRNAs for 48 h and then cells were treated with bortezomib (100 nM) for an additional 24 h. The indicated protein levels (A) and the cell viability (B) were determined by western blot and CCK-8 assay, respectively. Each bar represents means ± SD of three separate experiments. N.S, not significant



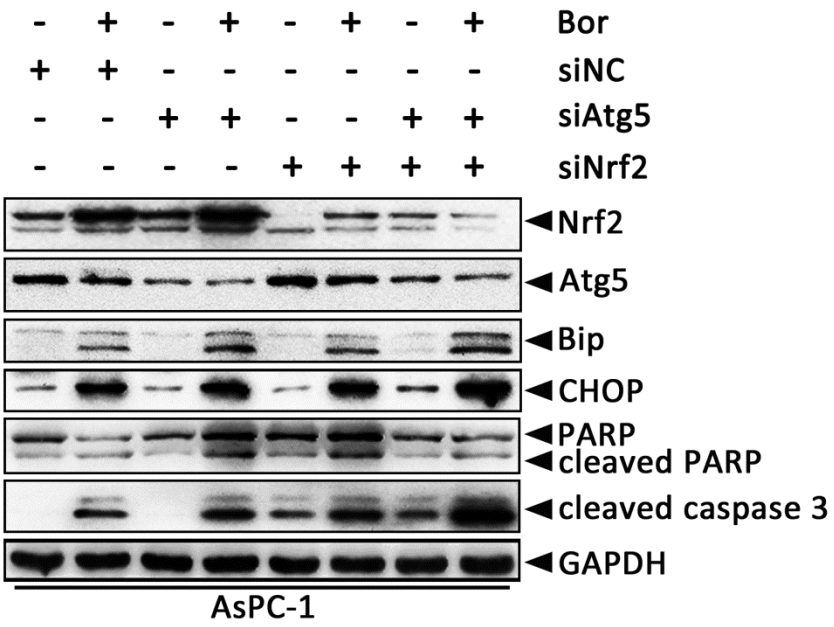
Supplementary Figure S5: ROS plays a critical role in bortezomib-induced upregulation of the intracellular calcium level.

MIAPaCa-2 and AsPC-1 cells were pretreated with NAC (5 mM) for 30 min, and then exposed to bortezomib (100 nM) for 24 h. The cytosolic calcium levels was determined by Fluo-3/AM staining and analyzed by flow cytometry. Each bar represents means ± SD of three separate experiments. *$p < 0.05$



Supplementary Figure S6: Knockdown of Nrf2 increased bortezomib-induced the accumulation of GFP-LC3B puncta in PC cells.

MIAPaCa-2 and AsPC-1 cells transfected with GFP-LC3B were transfected with Nrf2 siRNAs for 48 h and then cells were treated with bortezomib (100 nM) for an additional 24 h. The number of GFP-LC3B dots in each cell was quantified, and at least 50 cells were included for each group. Scale bar: 20 μm. Each bar represents means ± SD of three separate experiments. *$p < 0.05$.



Supplementary Figure S7: Combined inhibition of autophagy and Nrf2 pathway augments bortezomib-induced ER stress and apoptosis in PC cells.

AsPC-1 cells were transfected with Atg5 or Nrf2 siRNA, or combinations thereof for 48 h, and then cells were treated with bortezomib (100 nM) for an additional 24 h. The indicated protein levels were analyzed by western blot.