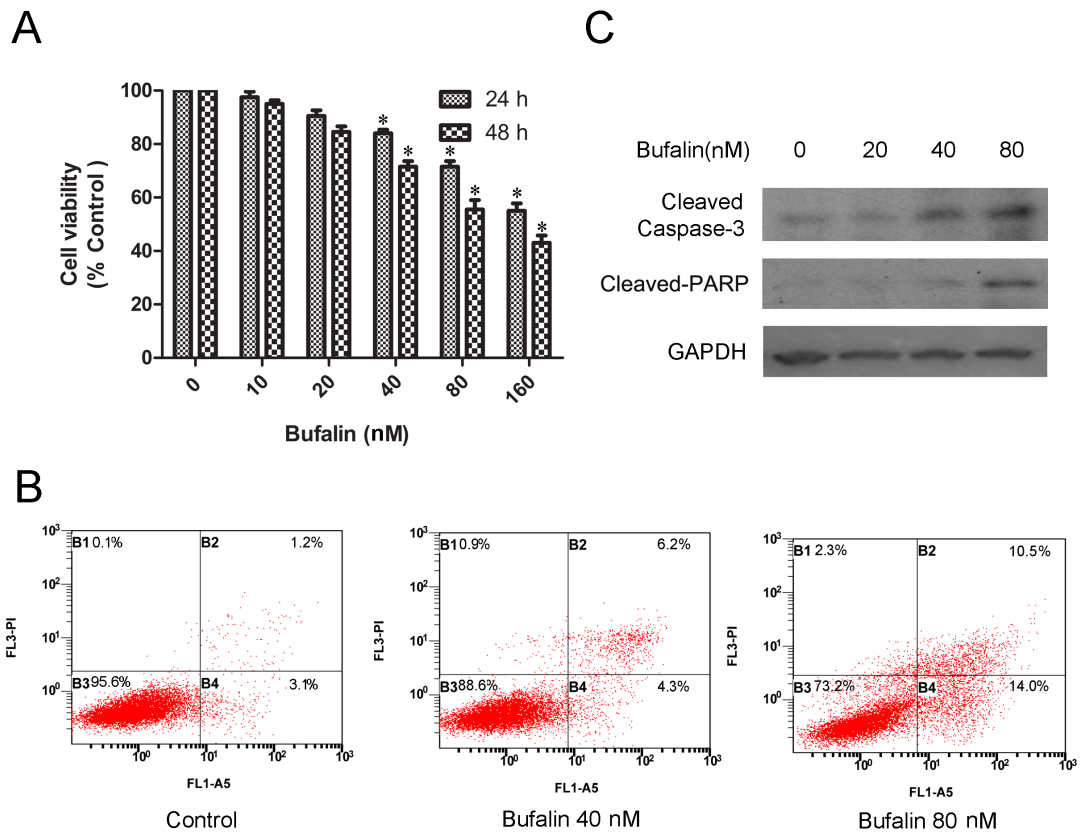

Figure S1. Bufalin inhibits LN229 cell growth and induces apoptosis. **A:** LN229 cells were treated with various concentrations of bufalin for 24 h and 48 h. Cell viability was determined by MTT assay. Data are presented as mean ± SD, n = 3. *$p < 0.05$ versus control group. **B:** LN229 cells were treated with bufalin for 24 h, stained with Annexin V and PI, and then analyzed by flow cytometry. Results are representative of three independent experiments. **C:** Western blot analysis for the expression of cleaved PARP and cleaved caspase-3.

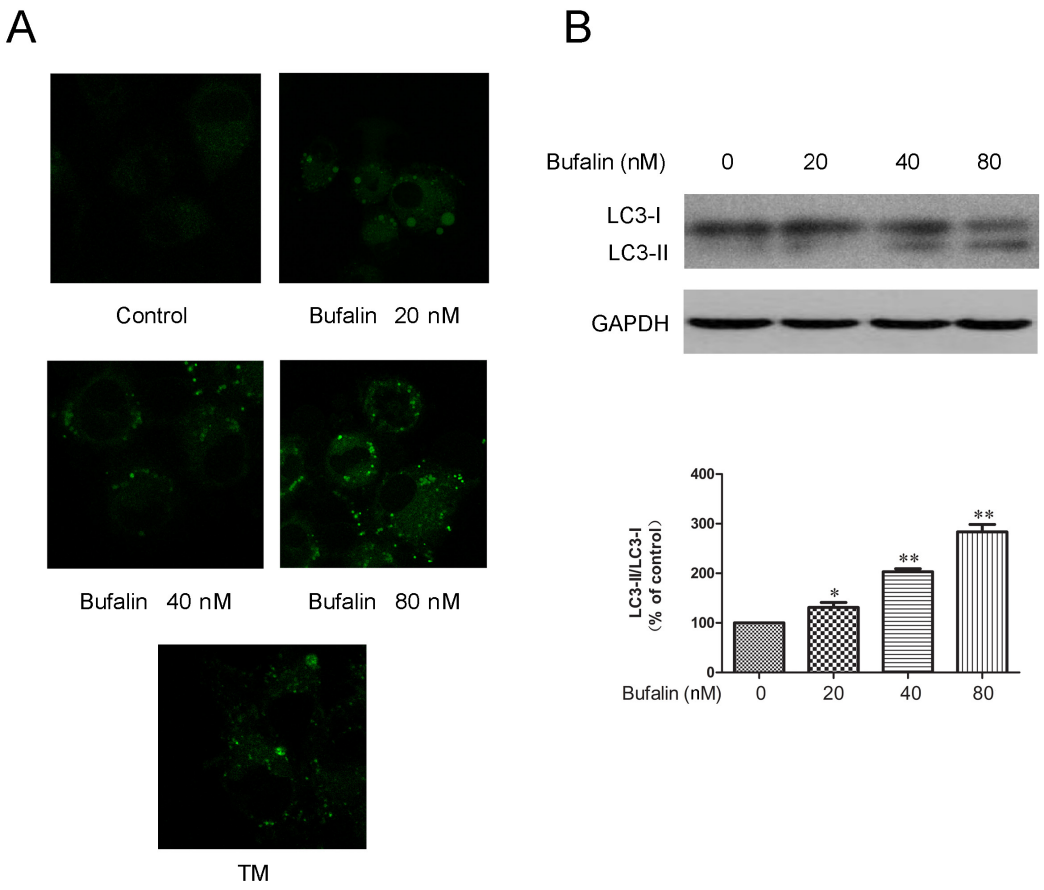

Figure S2. Bufalin induces autophagy in LN229 cells. **A:** LN229 cells were treated with 20-80 nM bufalin for 24 h and stained by MDC. MDC staining of cells treated with temozolomide (TM) was presented as a positive control. **B:** Total cell extracts were assayed by western blotting for expression of LC3-I and LC3-II. Relative levels of LC3-II to LC3-I ratio are indicated in the graphs. Data were quantified using ImageJ software (mean ± SD, n=3). **p*<0.05, ***p*< 0.01 vs control.

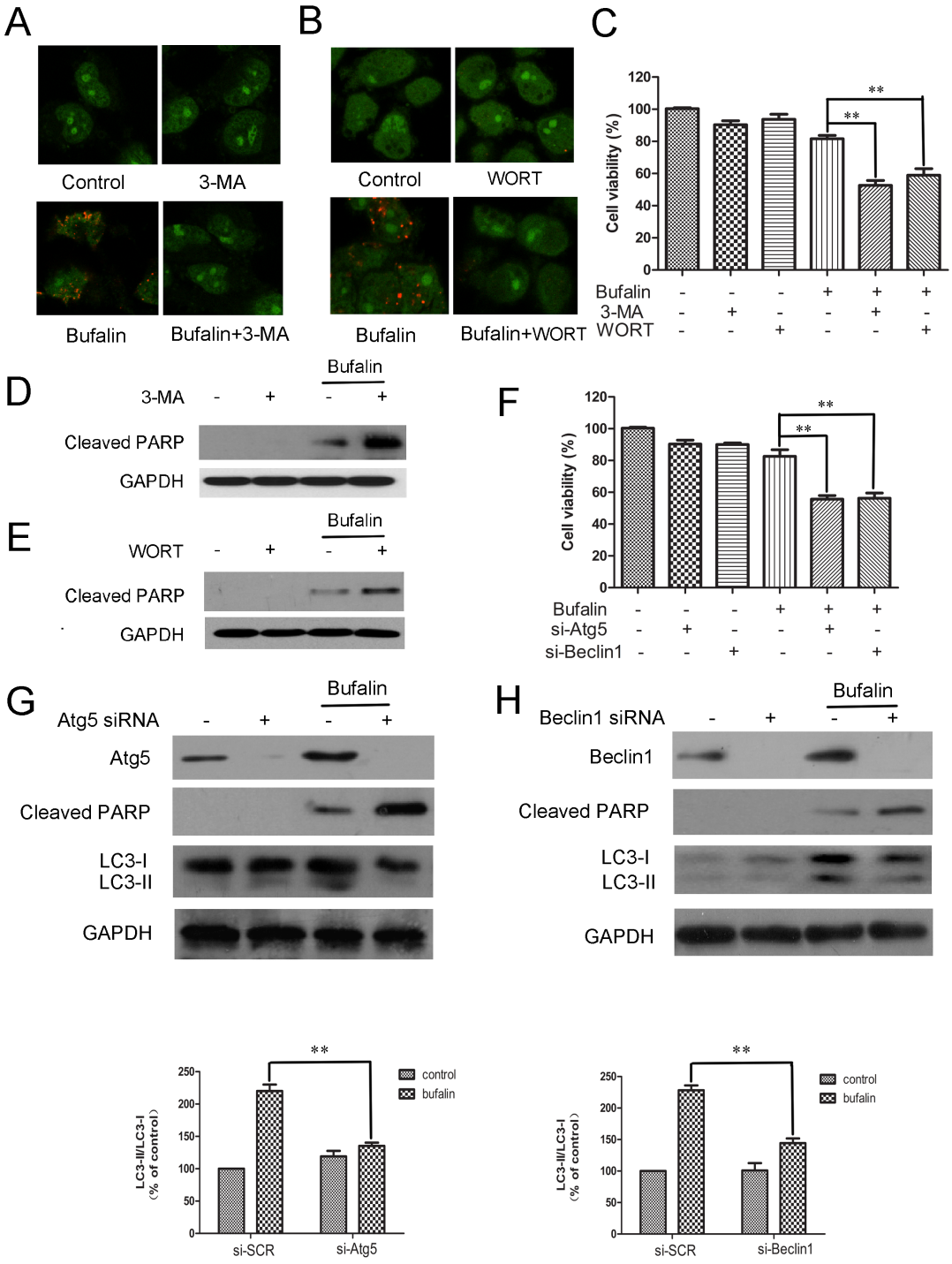

Figure S4. The inhibition of autophagy enhances bufalin-induced apoptosis. **A-E:** LN229 cells were pretreated or not with 3 mM 3-MA (**A**, **C** and **D**) or with 1 μM WORT (**B**, **C** and **E**) for 1 h and further treated with 40 nM bufalin for 24 h. **A-B:** Acridine orange labeling of acidic vesicles in LN229 cells. **C:** MTT assay was employed to detect the cell viability. Data are presented as mean ±SD of three separate experiments. **p<0.01 versus bufalin treatment alone. **D-E:** Total cell

extracts were assayed for immunoblot analysis of cleaved PARP in LN229 cells. **F:** The viability of LN229 cells was measured by MTT assay. The cells were transfected with Atg5 siRNA or Beclin1 siRNA as described in the text, and then exposed to 40 nM bufalin for 24 h. Data are presented as mean ±SD of three separate experiments. $^{**}p<0.01$ versus bufalin treatment alone. **G-H:** Effects of Atg5 (**G**) and Beclin1 (**H**) knockdown on UA-induced LC3-I to LC3-II conversion and PARP cleavage. Treatment was as described for **F**. Western blot was performed to assess the expression of indicated proteins. Relative levels of LC3-II to LC3-I ratio are indicated in the graphs. Data were quantified using ImageJ software (mean ± SD, n =3). $^{**}p<0.01$ versus bufalin treatment alone.

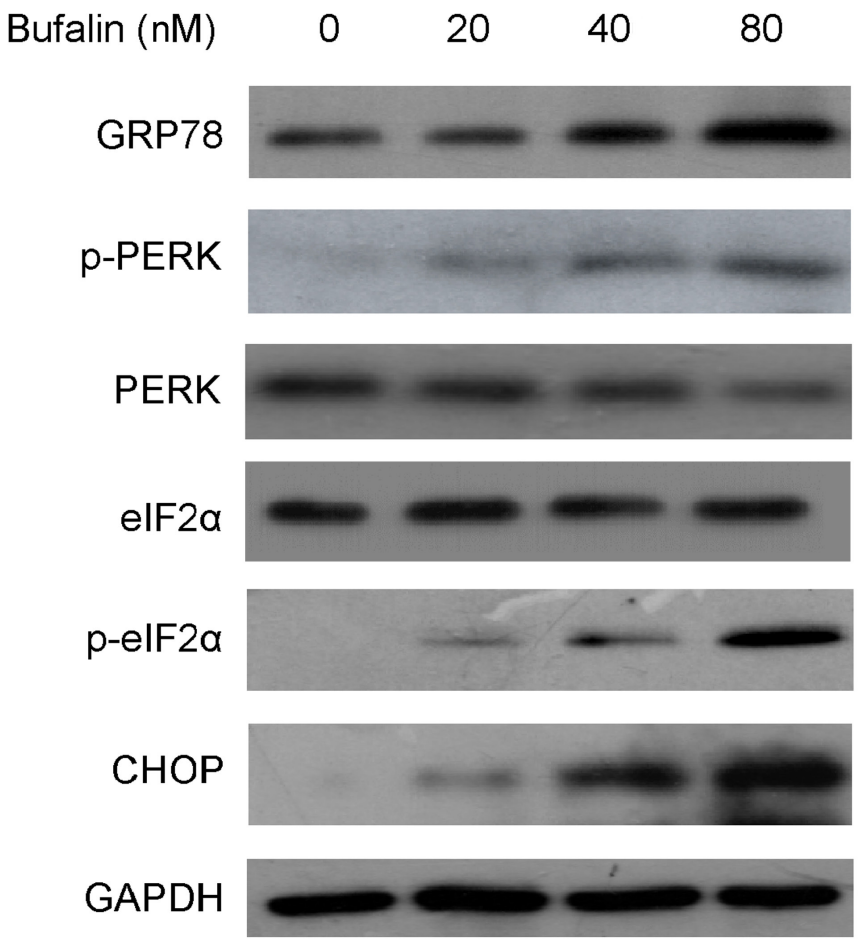

Figure S5. Bufalin induced ER stress in LN229 cells. LN229 cells were treated with various concentrations of bufalin for 24 h. Total cell extracts were assayed by western blotting for expression of GRP78, PERK, p-PERK, eIF2α, p-eIF2α and CHOP.