Fig.S1

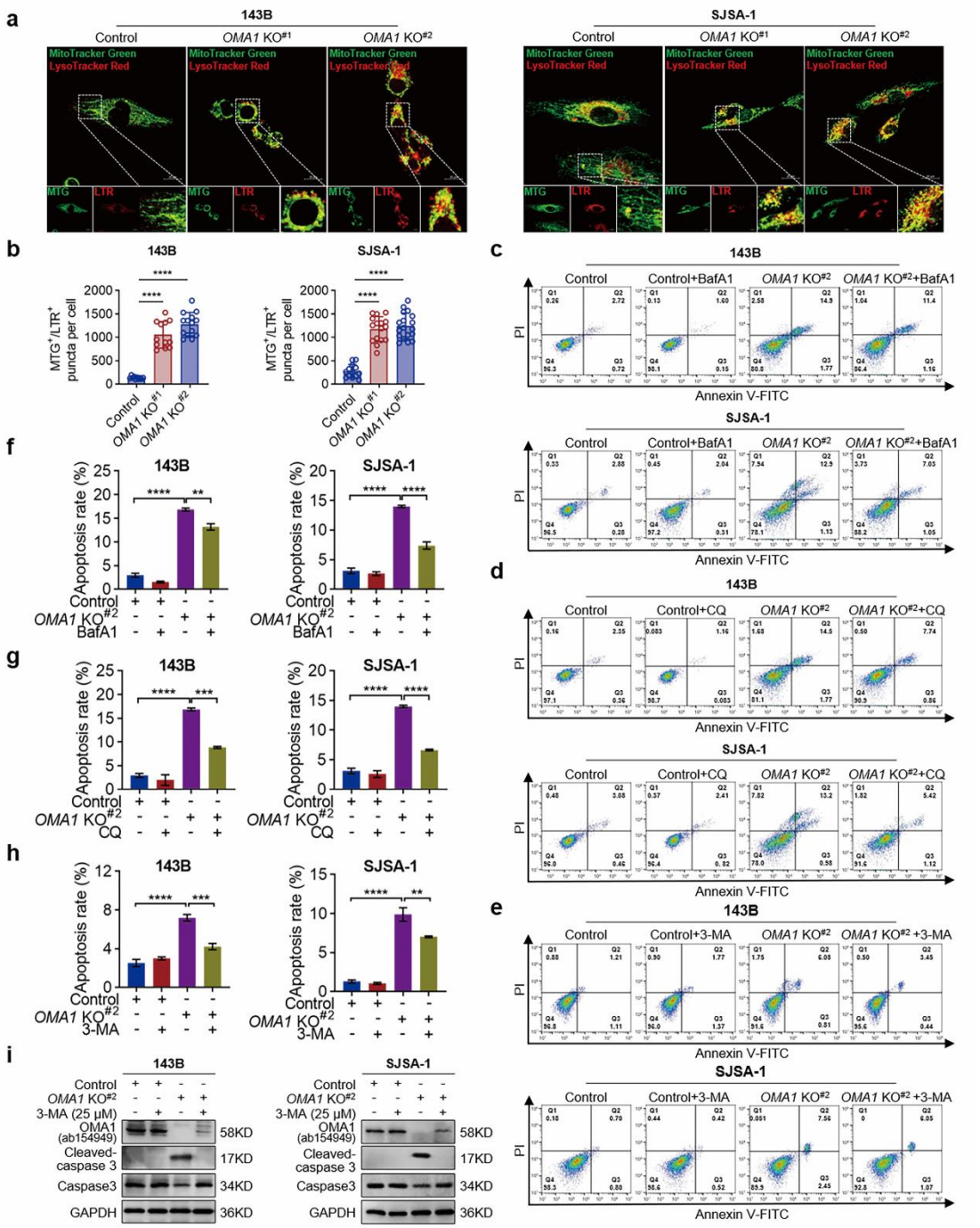

Fig.S1 OMA1 deficiency induces apoptosis of OS cells. a, b Representative confocal microscopy images of the co-localization of MitoTracker Green (MTG) and LysoTracker Red (LTR) in control and *OMA1-deficient* cells (**a**) (Scale bar, 20 μm). The MTG^{+}/LTR^{+} puncta per cell was quantified using ImageJ Plus software (**b**). Data were shown as mean ± SD and statistical significance was assessed by two-tailed unpaired Student's *t*-test (n > 3, ****$P < 0.0001$). **c-h** Apoptosis of control or *OMA1-deficient*

cells treated with BafA1 (50 nM) (**c**), CQ (100 μM) (**d**), or 3-MA (25 μM) (**e**) for 24 h and stained with annexin V-FITC/PI were analyzed using flow cytometry. The apoptosis rates were plotted followed by statistical analysis (**f-h**). Data were shown as mean ± SD, and statistical significance was assessed by two-tailed unpaired Student's *t*-test (n=3, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$). **i** Western blotting analysis of Cleaved-caspase 3 and Caspase 3 protein levels in *OMA1-deficient* cells treated with 3-MA (25 μM) for 24 h.

Fig. S2

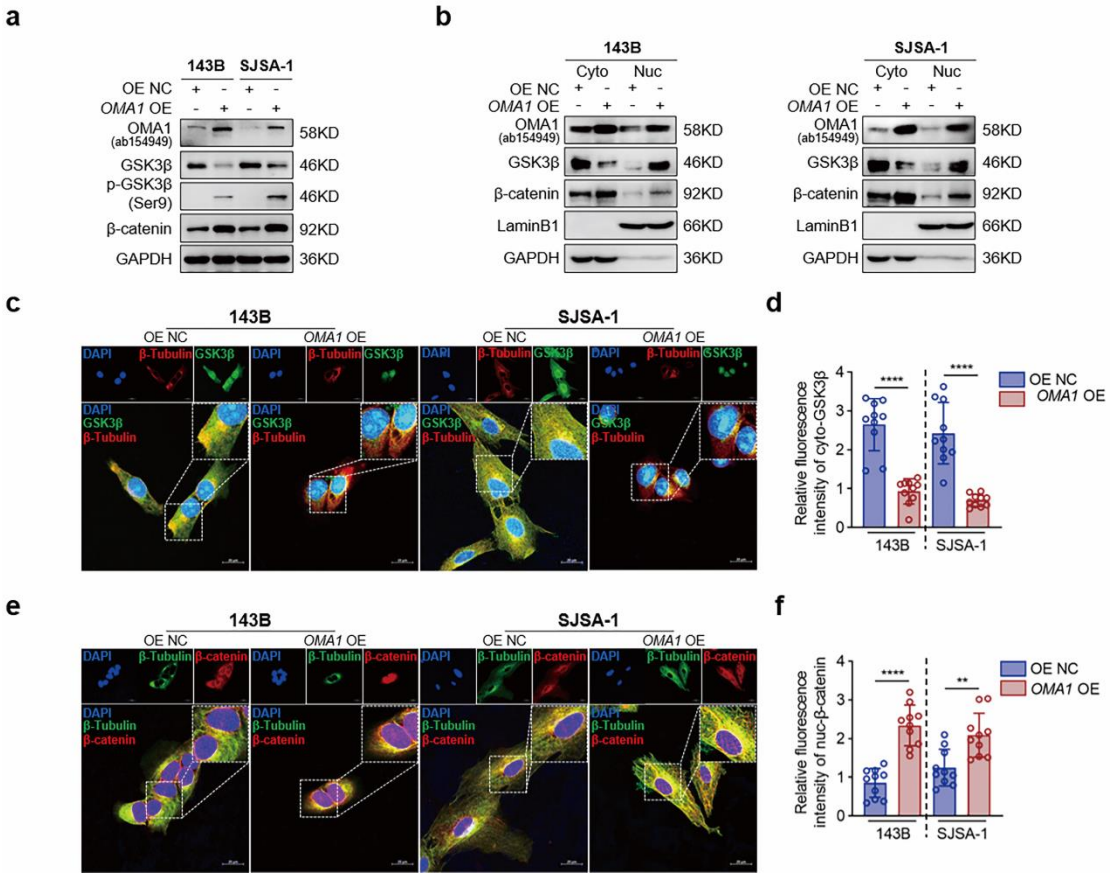

Fig. S2 OMA1 overexpression decreased cytosolic GSK3β and increased β-catenin nuclear transportation. a The whole cell lysates of OMA1 overexpression cells were determined by Western blotting with antibodies against GSK3β, p-GSK3β, β-catenin, or p53. **b** The protein levels of GSK3β, and β-catenin in the cytoplasm (Cyto) and nucleus (Nuc) of *OMA1-overexpressing* OS cells were analyzed by nuclear/cytosolic fractionation and Western blotting. **c-f** Representative confocal

microscopy images of the co-localization of β-tubulin and cytosolic GSK3β (**c**) or nuclear β-catenin (**e**) in *OMA1-deficient* cells (Scale bar, 20 μm). The relative fluorescence intensity of cytosolic GSK3β (**d**) or nuclear β-catenin (**f**) was quantified using ImageJ Plus software. Data were shown as mean ± SD and statistical significance was assessed by two-tailed unpaired Student's *t*-test (n > 3, $^{**}P < 0.01$, $^{****}P < 0.0001$).

Fig.S3

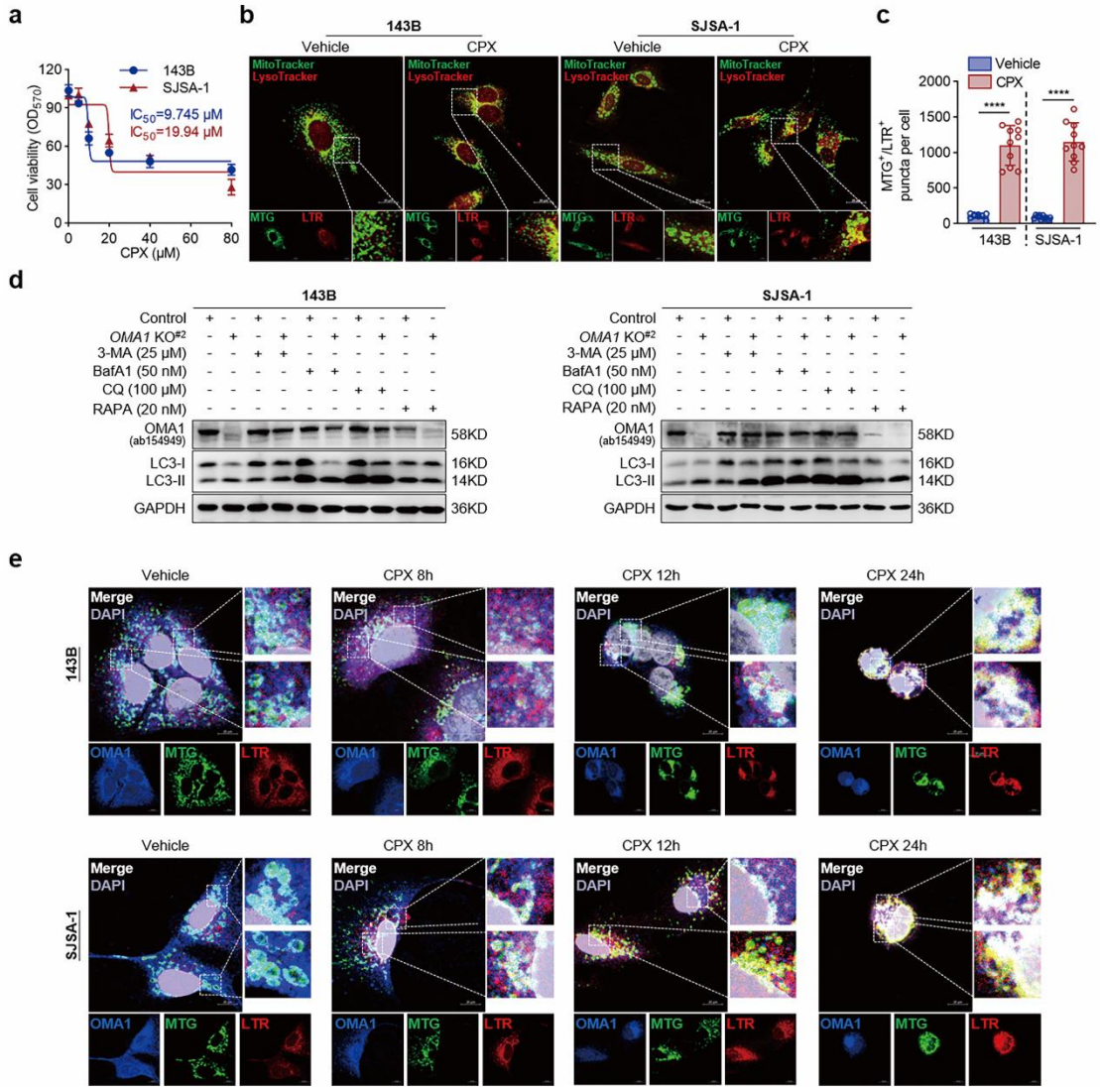

Fig.S3 CPX promotes OMA1 degradation via the ubiquitination and lysosome pathways in OS cells. a The IC_{50} value of OS cells treated with a serial dose of CPX for 24 h was evaluated using an MTT cellular proliferation and cytotoxicity assay kit. Data were shown as mean ± SD (n=6). **b, c**

Representative confocal microscopy images of the co-localization of MitoTracker Green (MTG) and LysoTracker Red (LTR) in OS cells treated with CPX (10 μM) for 24 h (**b**) (Scale bar, 20 μm). The MTG^+/LTR^+ puncta per cell was quantified using ImageJ Plus software (**c**). Data were shown as mean ± SD and statistical significance was assessed by two-tailed unpaired Student's *t*-test (n > 3, ****$P <$ 0.0001). **d** Western blotting analysis of OMA1 protein levels in control and *OMA1-deficient* cells treated with an indicated concentration of three autophagy inhibitors or activator RAPA for 24 h. **e** Representative images of OS cells treated with 10 μM CPX before immunostaining with MitoTracker Green (MTG), LysoTracker Red (LTR), and endogenous OMA1 (Scale bar, 10 μm).