Supporting Information

Enhancing tumor chemotherapy and overcoming drug resistance through autophagy mediated intracellular dissolution of zinc oxide nanoparticles †

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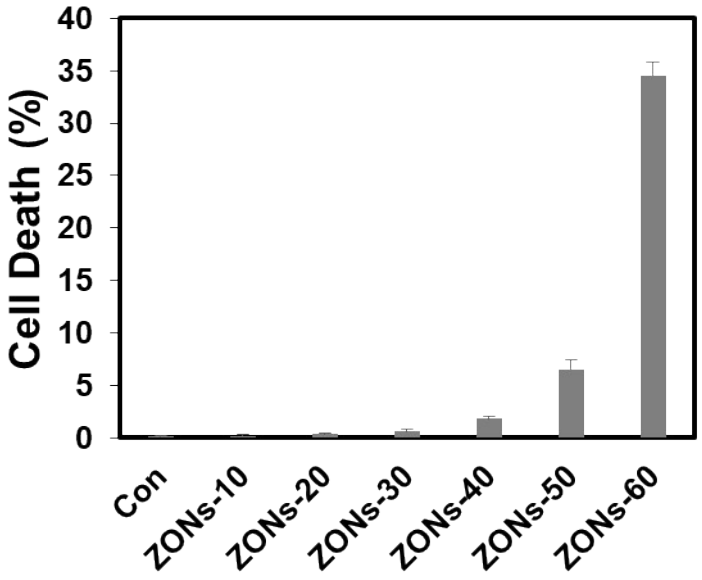

Figure S1. Hoechst 33342/PI staining assay reveals the dose-dependent cell death of HeLa cells when cells treated with different concentrations of ZONs (0, 10, 20, 30, 40, 50, 60 μg/mL) for 24h. (Mean ± s.e.m., n = 3)

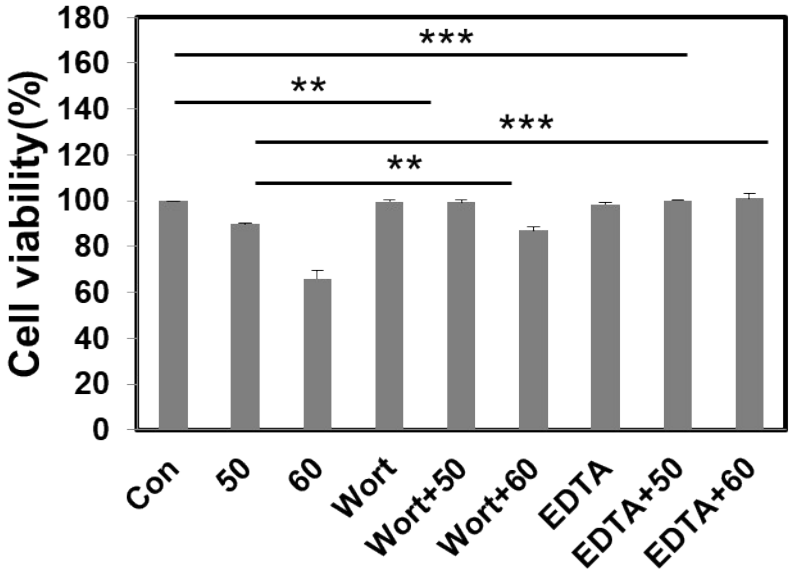

Figure S2. MTT assay indicated that autophagy inhibition by wortmannin (Wort, 500 nM) or abrogating zinc release by EDTA (1 mM) could abrogate the inhibitory effect on HeLa cells induced by ZONs (50, 60 μg/mL, 24 h). (Mean ± s.e.m., n = 3, **p < 0.01, ***p < 0.001)

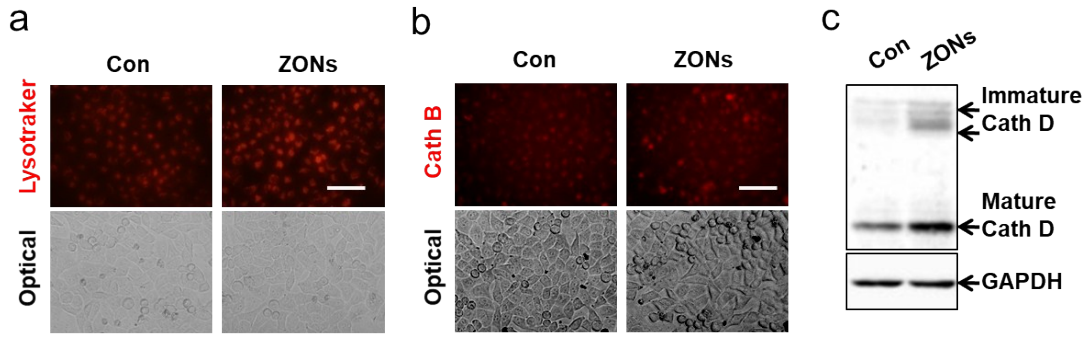

Figure S3. The lysosomal acidity and activity are enhanced by ZONs treatment. Fluorescent microscopy images of HeLa cells stained with 75 nM LysoTracker Red (a) or Magic Red CTSB Substrate (b) when treated with ZONs (50 μg/mL, 24 h). Scale bar, 100 μm. (c) Western blot analysis of mature cathepsin D formation in HeLa cells treated with ZONs (50 μg/mL, 24 h).

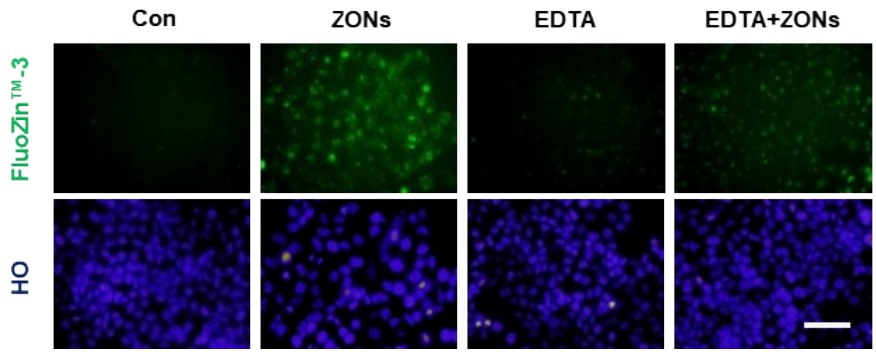

Figure S4. Fluorescent microscope images reveal that EDTA (1 mM) decrease the level of the intracellular dissolved zinc ions in HeLa cells incubated with ZONs (50 μg/mL, 24 h). In this section, FluoZin™-3 (5 μM, 30 min) was used as probe. Scale bar, 100 μm.

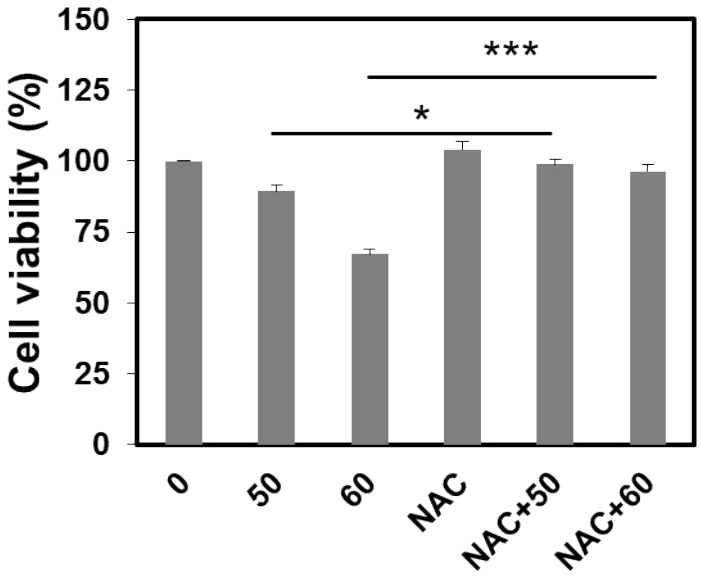

Figure S5. MTT assay shows that the ROS scavenger NAC (10 mM) could abrogate ZONs (50, 60 μg/mL, 24 h) induced the inhibitory effect on cell proliferation, indicating that ROS generation contributes to the inhibition of cell proliferation in HeLa cells induced by ZONs. (Mean ± s.e.m., n = 3, *$p < 0.05$, ***$p < 0.001$)

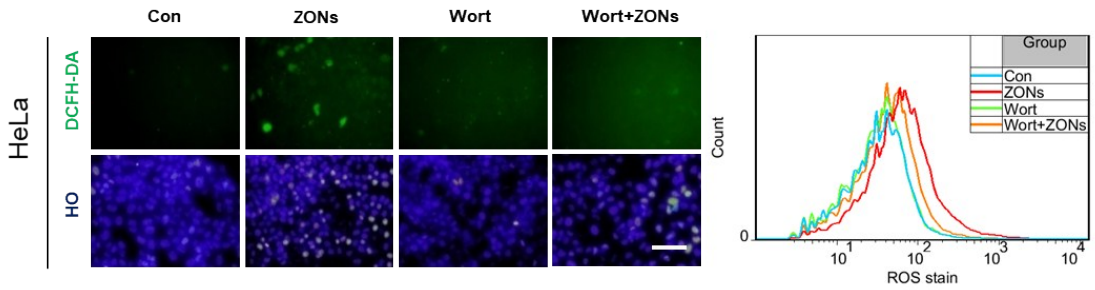

Figure S6. Wortmannin rescues the ROS generation induced by ZONs in HeLa cells. Fluorescent microscope pictures and flow cytometric analysis reveal that wortmannin (500 nM) decrease the level of the intracellular ROS in the HeLa cells incubated with ZONs (50 μg/mL, 24 h). In this section, DCFH-DA (10 μM, 30 min) was used as probe. Scale bar, 100 μm.

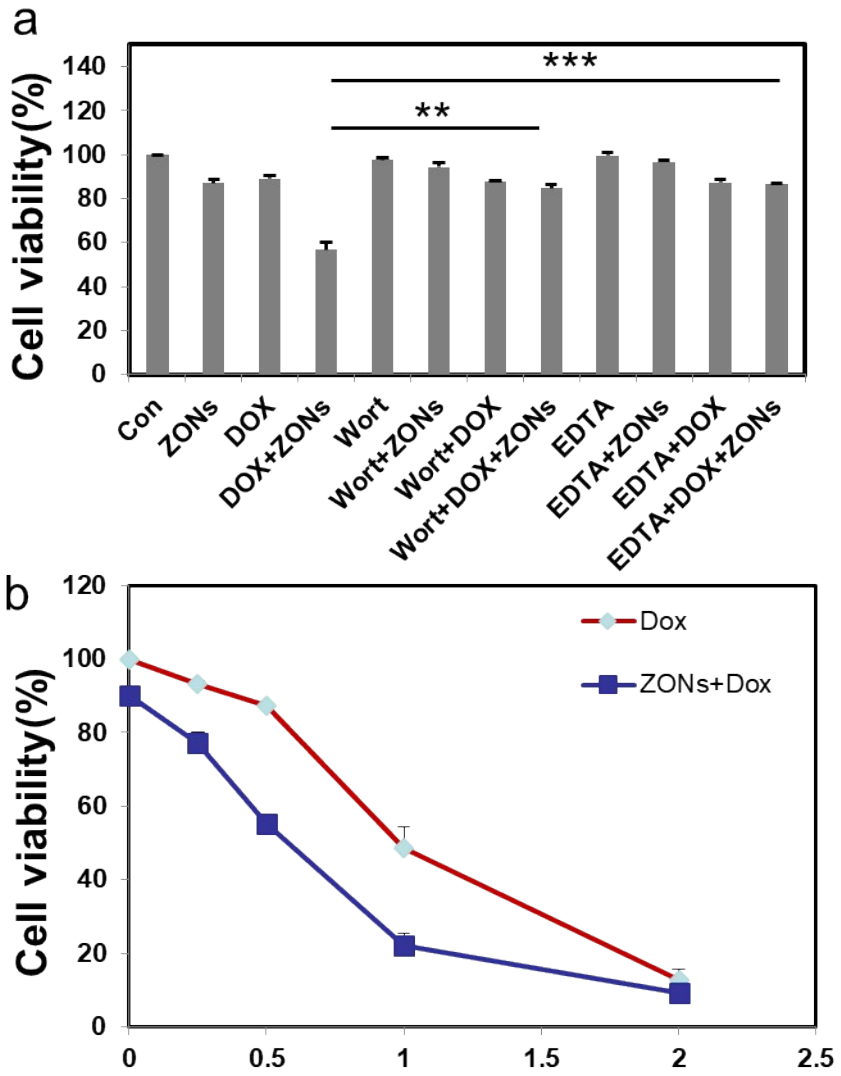

Figure S7. (a) The cell viability of HeLa cells separately treated with vehicle control, ZONs (50 μg/mL), doxorubicin (0.5 μg/mL) and ZONs (50 μg/mL) plus doxorubicin (0.5 μg/mL) for 48h in the presence or absence of wortmannin (500 nM) or EDTA (1 mM), are assessed by MTT assay. (Mean ± s.e.m., n = 3, **p < 0.01, ***p < 0.001.) (b) The MTT assay results showed that ZONs (50 μg/mL) plus different concentrations of doxorubicin (0, 0.25, 0.5, 1, 2 μg/mL) induced more inhibitory effect than doxorubicin alone in HeLa cells.

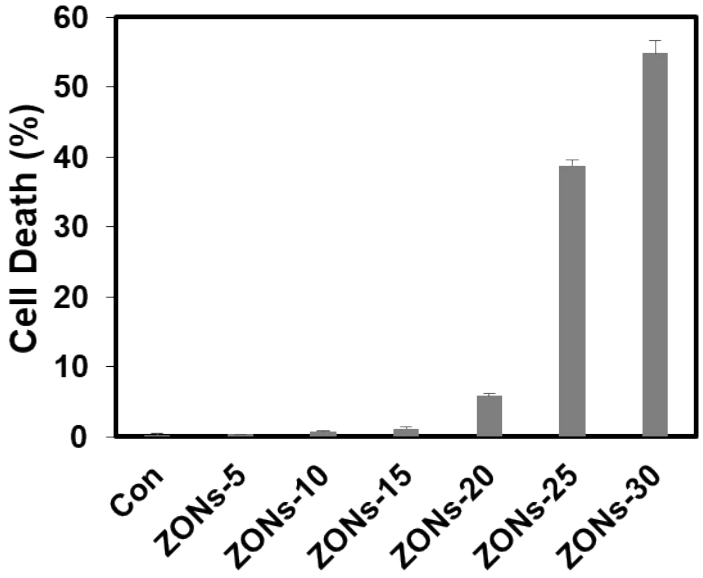

Figure S8. Hoechst 33342/PI staining assay reveals the dose-dependent cell death of MCF-7/ADR cells when cells treated with different concentrations of ZONs (0, 5, 10, 15, 20, 25, 30 μg/mL) for 24h. (Mean ± s.e.m., n = 3)

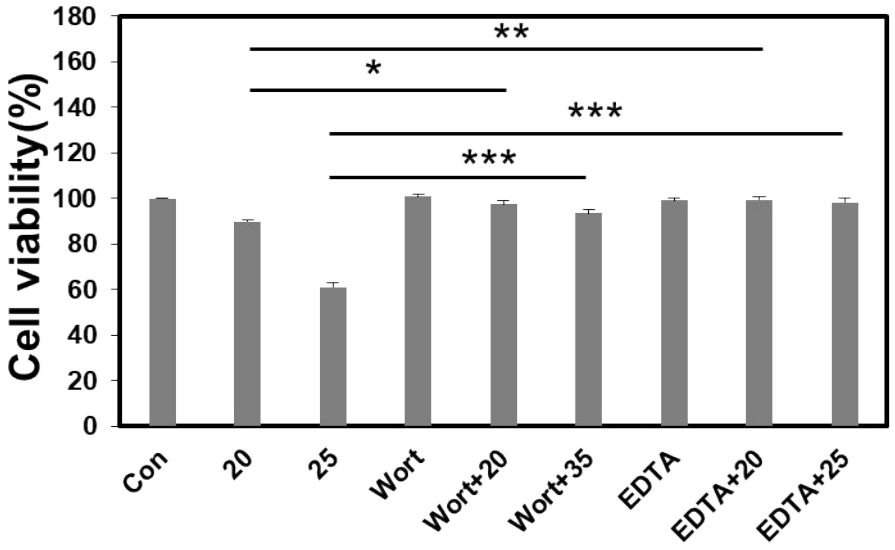

Figure S9. MTT assay indicated that autophagy inhibition by wortmannin (Wort, 500nM) or abrogating zinc release by EDTA (1 mM) could abrogate ZONs (20, 25 μg/mL, 24 h) induced the inhibitory effect on MCF-7/ADR cells. (Mean ± s.e.m., n = 3, *p < 0.05, **p < 0.01, ***p < 0.001)

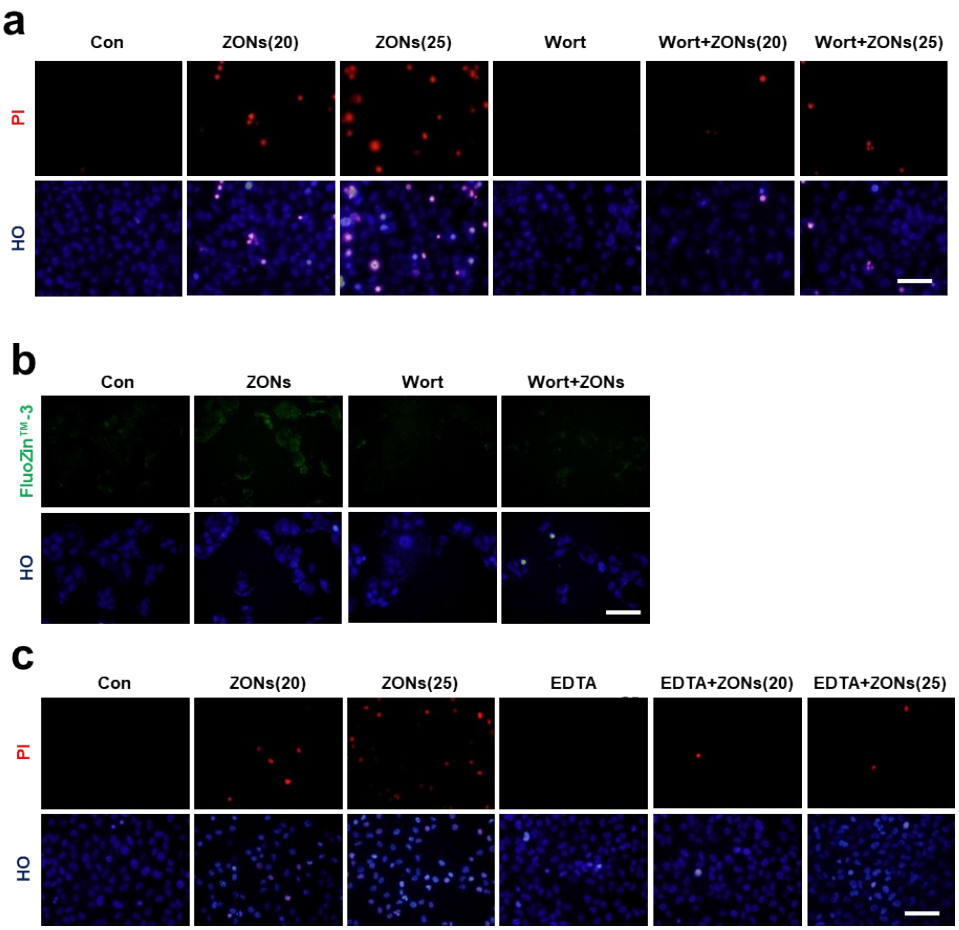

Figure S10. (a) Hoechst 33342/PI staining analysis evinces that wortmannin (Wort, 500 nM) could abrogate ZONs (20, 25 μg/mL, 24 h) induced cell death in MCF-7/ADR cells. (b) Fluorescent microscope pictures reveal that wortmannin (500 nM) obviously decrease the relase of zinc ions in MCF-7/ADR cells incubated with ZONs (20 μg/mL, 24 h). In this section, FluoZin™-3 (5 μM, 30 min) was used as probe. (c) Hoechst 33342/PI staining analysis visions that EDTA (1 mM) could abrogate ZONs (20, 25 μg/mL, 24 h) induced cell death in MCF-7/ADR cells. Scale bar, 100 μm.

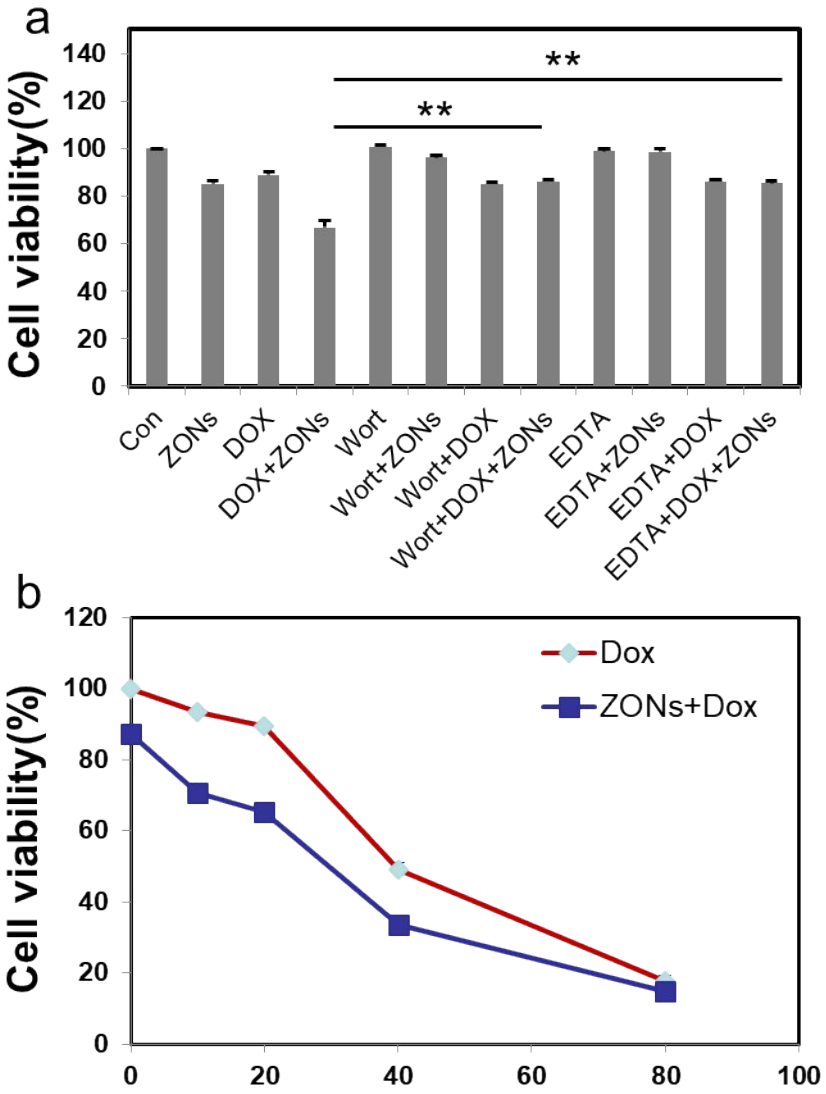

Figure S11. (a) The cell viability of MCF-7/ADR cells separately treated with vehicle control, ZONs (20 μg/mL), doxorubicin (20 μg/mL) and ZONs (20 μg/mL) plus doxorubicin (20 μg/mL) for 48h in the presence or absence of wortmannin (500 nM) or EDTA (1 mM), are assessed by MTT assay. (Mean ± s.e.m., n = 3, **p < 0.01.) (b) The MTT assay results showed that ZONs (20 μg/mL) plus doxorubicin induced more inhibitory effect than doxorubicin in MCF-7/ADR cells.

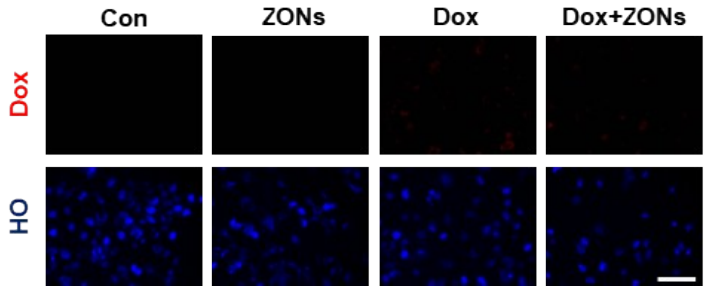

Figure S12. Fluorescent microscope pictures reveal that ZONs had no effect on endocytosis of Dox in MCF-7/ADR cells. Scale bar, 100 μm.

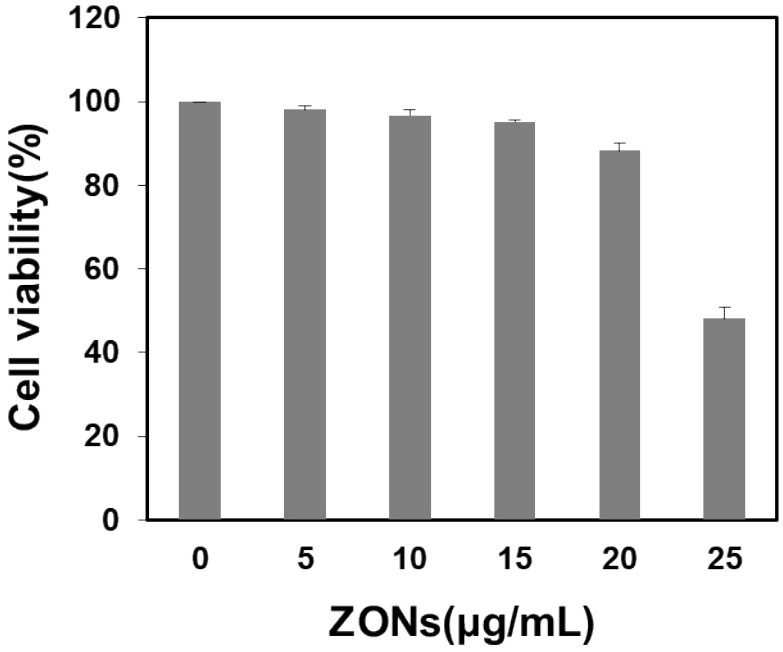

Figure S13. MTT assay reveals the dose-dependent inhibitory effect on 4T1 cells when cells treated with different concentrations of ZONs (0, 5, 10, 15, 20, 25 μg/mL) for 24h. (Mean ± s.e.m., n = 3)

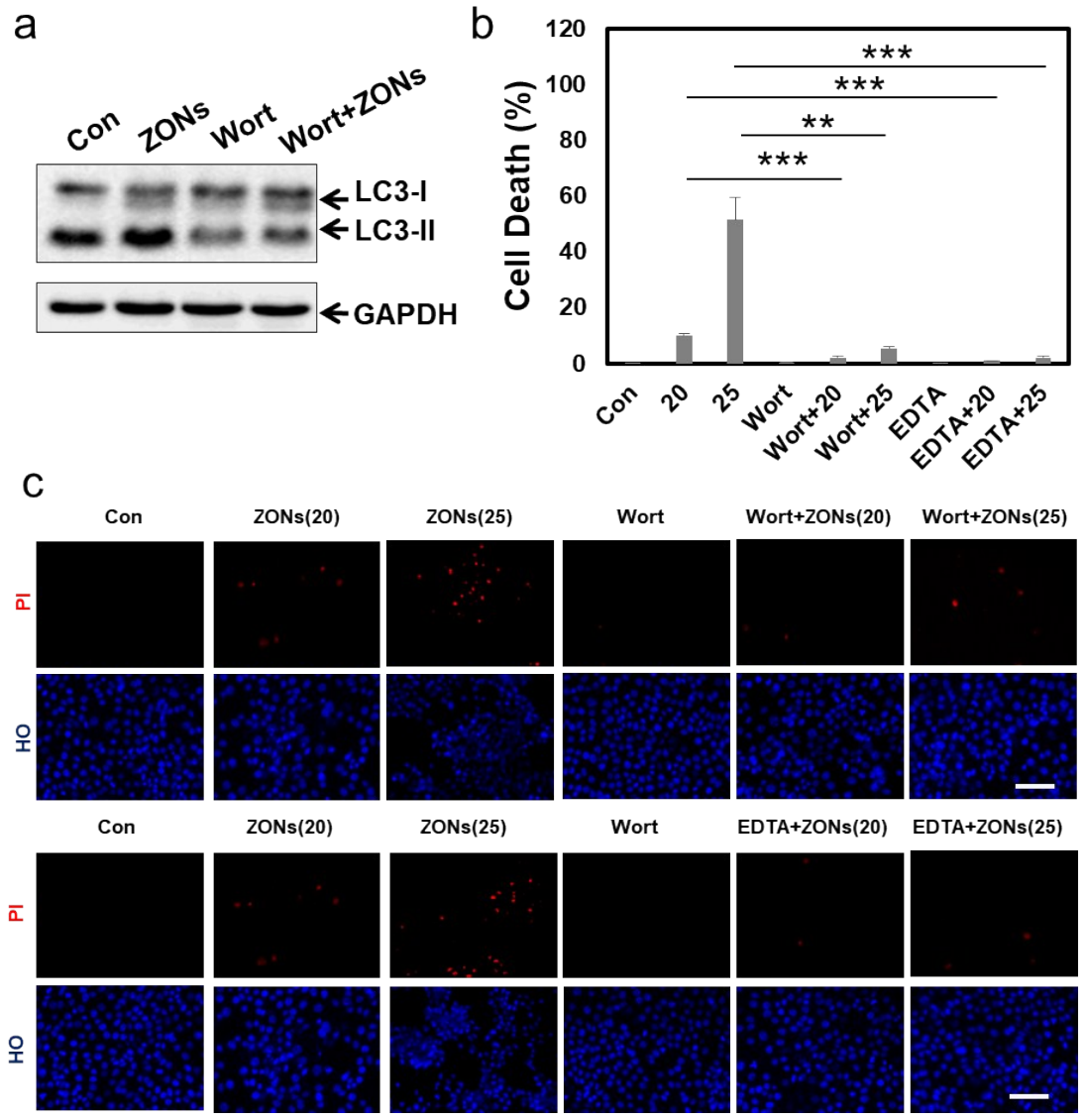

Figure S14. (a) Western blot analysis exhibits that the conversion of LC3-I to LC3-II induced by ZONs (20 μg/mL, 24h) is abrogated by autophagy inhibitor wortmannin (Wort, 500 nM) in 4T1 cells suggesting the induction of autophagy by the ZONs. (b-c) Hoechst 33342/PI staining analysis evinces that wortmannin (Wort, 500 nM) or EDTA (1 mM) could abrogate ZONs (20, 25 μg/mL, 24 h) induced cell death in 4T1 cells. Scale bar, 100 μm. Cell death are assessed by Hoechst 33342/PI staining and expressed as the percentage of PI-stained cells. (Mean ± s.e.m., n = 3, **$p < 0.01$, ***$p < 0.001$.)

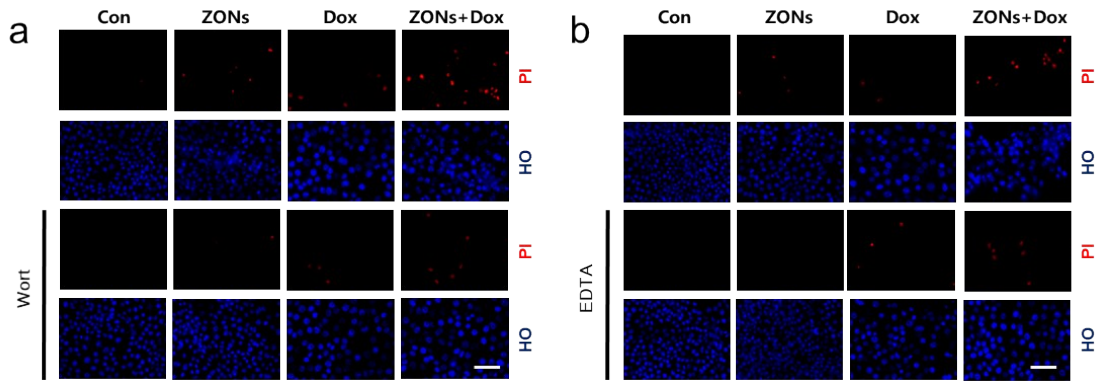

Figure S15. (a) 4T1 cells are separately treated with vehicle control, ZONs (20 μg/mL), doxorubicin (0.25 μg/mL) and ZONs (20 μg/mL) plus doxorubicin (0.25 μg/mL) for 48 h in the presence or absence of wortmannin (500 nM). Scale bar, 100 μm (b) Hoechst 33342/PI staining display the death of 4T1 cells separately treated with vehicle control, ZONs, doxorubicin and ZONs plus doxorubicin for 48 h in the presence or absence of EDTA (1 mM). Scale bar, 100 μm.