

Figure S1. Schematic diagram of the experimental design.

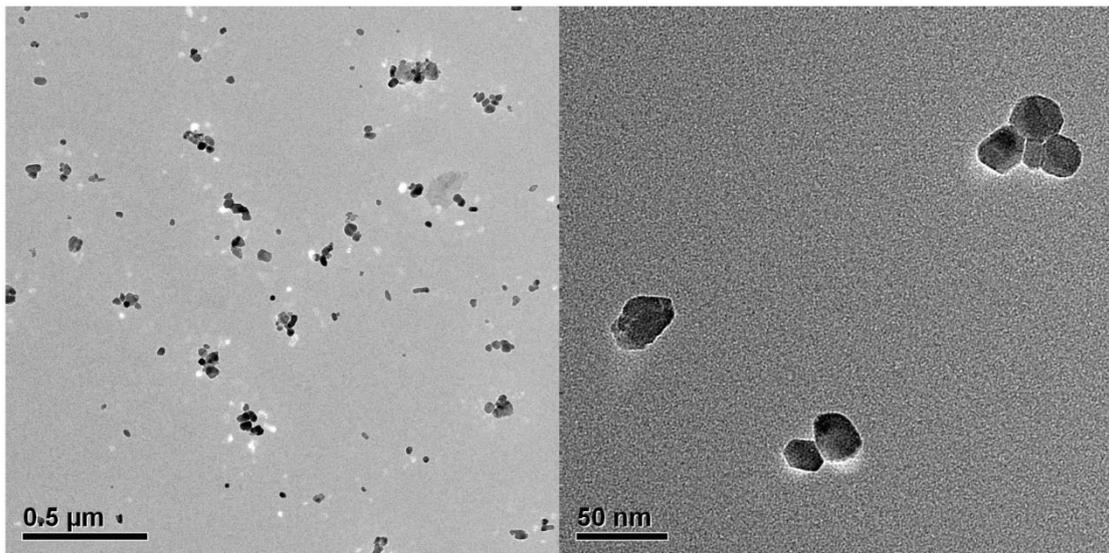


Figure S2. Transmission electron microscopy (TEM) images of ZnO NPs.

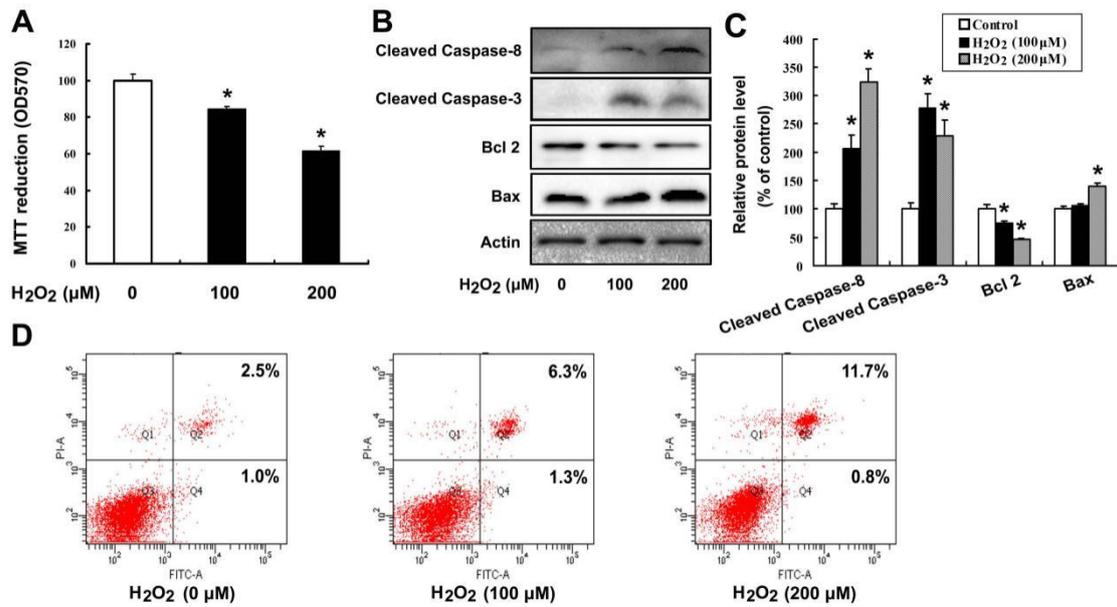


Figure S3. Oxidative stress induced apoptosis of mouse Leydig TM3 cells. Mouse Leydig TM3 cells were treated with 0–200 μM H₂O₂ for 24 h; then, cell viability (A), the protein levels of cleaved Caspase-8, cleaved Caspase-3, Bcl 2 and Bax (B) and the AnnexinV-positive staining cells (D) were tested by MTT assay, Western blot and flow cytometry, respectively. (C) The relative protein levels of cleaved Caspase-8, cleaved Caspase-3, Bcl 2 and Bax were quantified by densitometry. The experiment was done in triplicate and repeated for three times. Data were analyzed by one-way ANOVA. * $p < 0.05$.

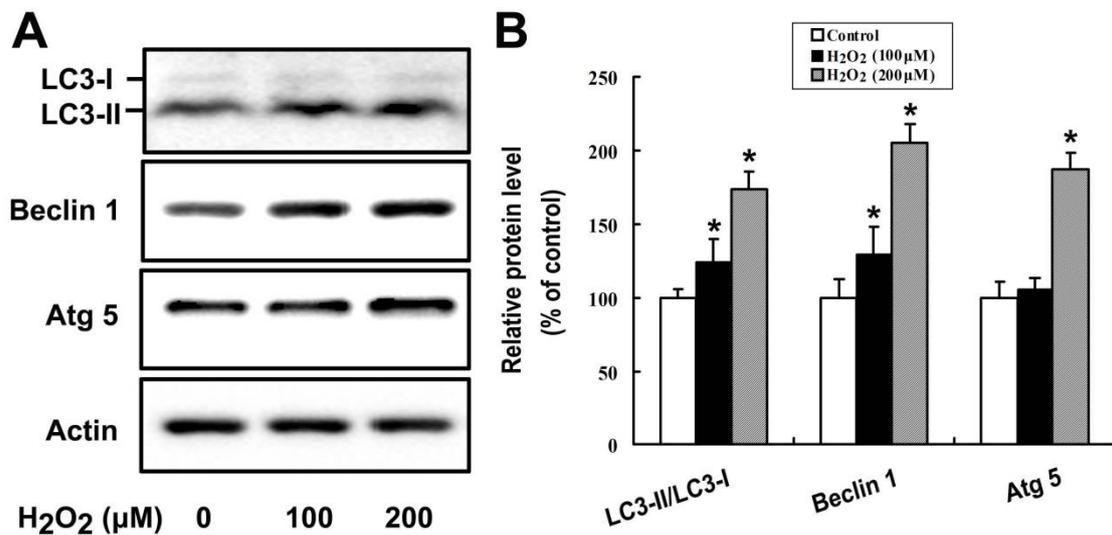


Figure S4. Oxidative stress induced autophagy of mouse Leydig TM3 cells. (A) Mouse Leydig TM3 cells were treated with 0–200 μM H₂O₂ for 24 h; then, the protein levels of LC3, Atg5 and Beclin1 were quantified by Western blot; Actin was used as an internal control. (B) The relative protein levels of LC3, Atg5 and Beclin1 were quantified by densitometry. The experiment was done in triplicate and repeated for three times. Data were analyzed by one-way ANOVA. * $p < 0.05$.

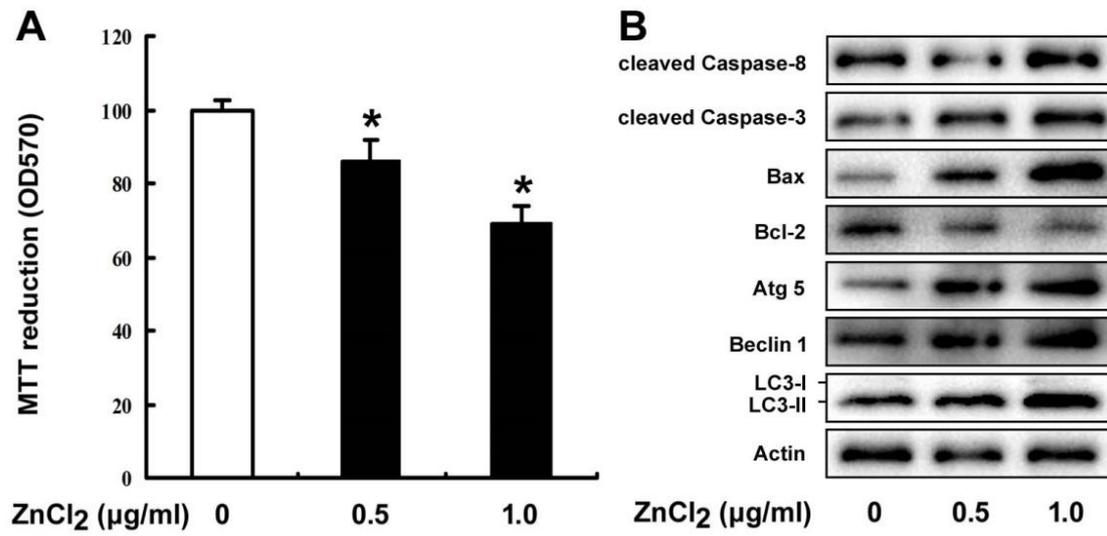


Figure S5. ZnCl₂ induced apoptosis and autophagy of mouse Leydig TM3 cells. Mouse Leydig TM3 cells were treated with 0–1 µg/mL ZnCl₂ for 24 h; then cell viability (A) and the levels of apoptosis and autophagy related proteins (B) were determined by MTT assay and Western blot, respectively. The experiment was done in triplicate and repeated three times. Data were analyzed by one-way ANOVA. * $p < 0.05$.