

SUPPLEMENTARY INFORMATION

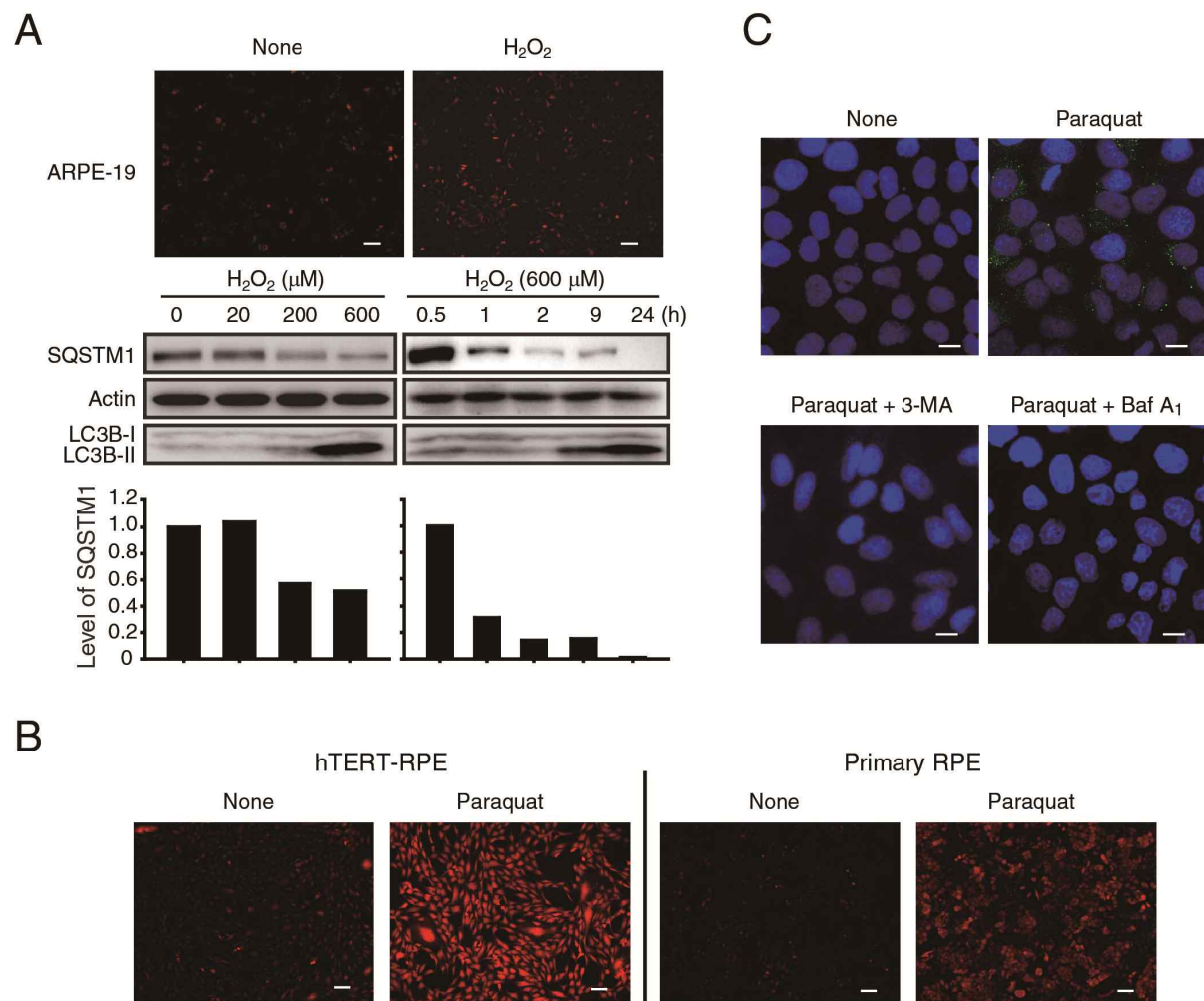


Figure S1. Reactive oxygen species induce autophagy in RPE cells. **(A)** Fluorescence microscopy images and western blot analysis of ARPE-19 cells exposed to H₂O₂. Fluorescence microscopy images of DCFDA-stained ARPE-19 cells exposed to H₂O₂ (600 μM) for 24 h (top panel). Western blot analysis of autophagy markers such as SQSTM1 and LC3-I, -II in ARPE-19 cells (bottom panel). Cells were exposed to various concentrations of H₂O₂ for 24 h or fixed concentration of H₂O₂ for various times. **(B)** Fluorescence microscopy images of DCFDA-stained hTERT-RPE and human primary RPE cells exposed to paraquat (400 μM) for 24 h. **(C)** ARPE-19 cells were treated with 400 μM paraquat in the absence or presence of either 10 mM 3-MA or 50 nM Baf A₁ for 24 h. Fluorescence microscopy images show autophagic vacuoles formed in ARPE-19 cells, which are stained as green puncta. Nuclei are represented with blue fluorescence (TOPRO-3 staining).

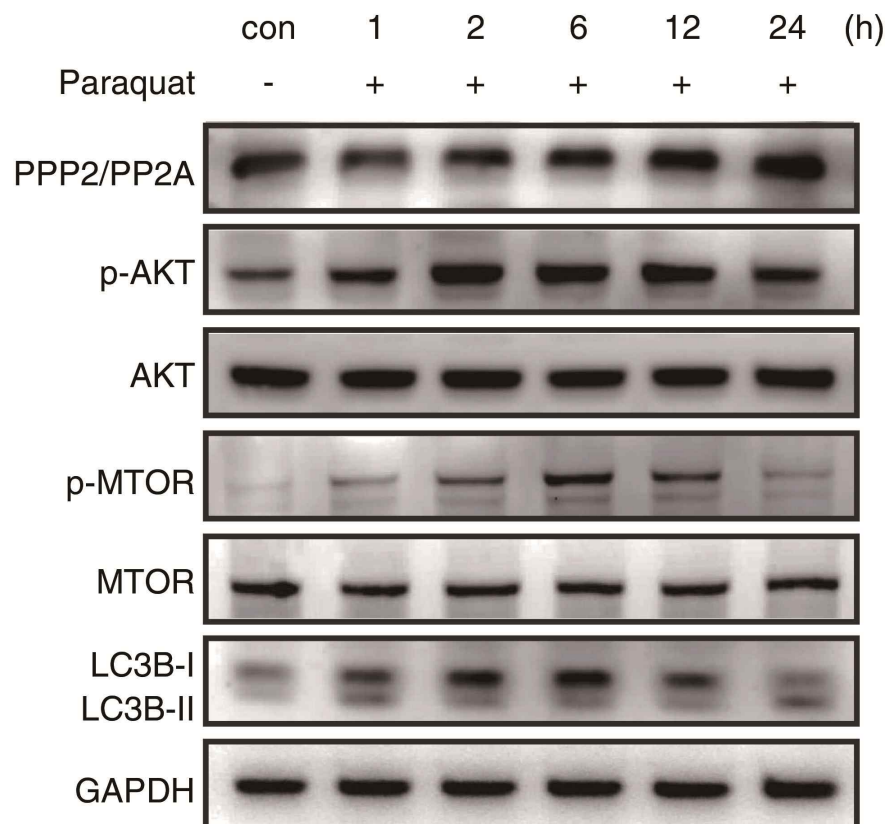


Figure S2. Oxidative stress-induced autophagy is mediated by the AKT-MTOR pathway in RPE cells. Western blot analysis of **PPP2/PP2A**, AKT and its phosphorylated form (p-AKT), MTOR and its phosphorylated form (p-MTOR), and LC3-I, -II in ARPE-19 cells. Cells were exposed to 400 μ M paraquat for various times (1 to 24 h).

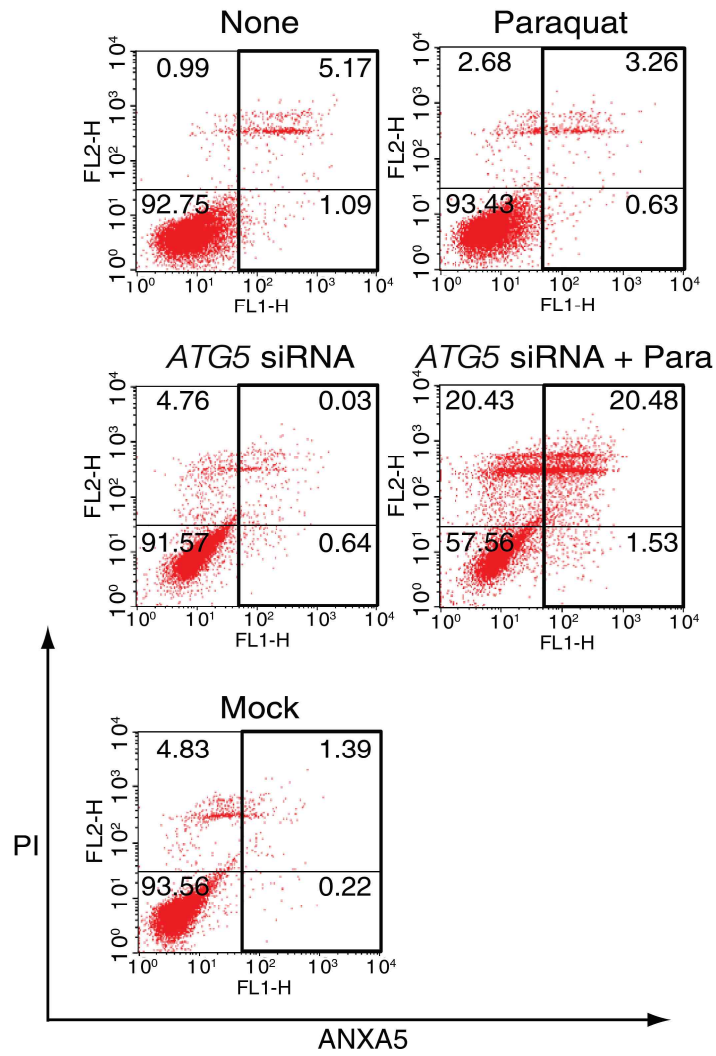


Figure S3. Inhibition of autophagy increases apoptotic cell death in RPE cells under oxidative stress. ARPE-19 cells were transfected with *ATG5* siRNA in the absence or presence of paraquat (400 μ M) for 24 h. Cells were stained with FITC conjugated ANXA5 and propidium iodide (PI) and analyzed by FACS.

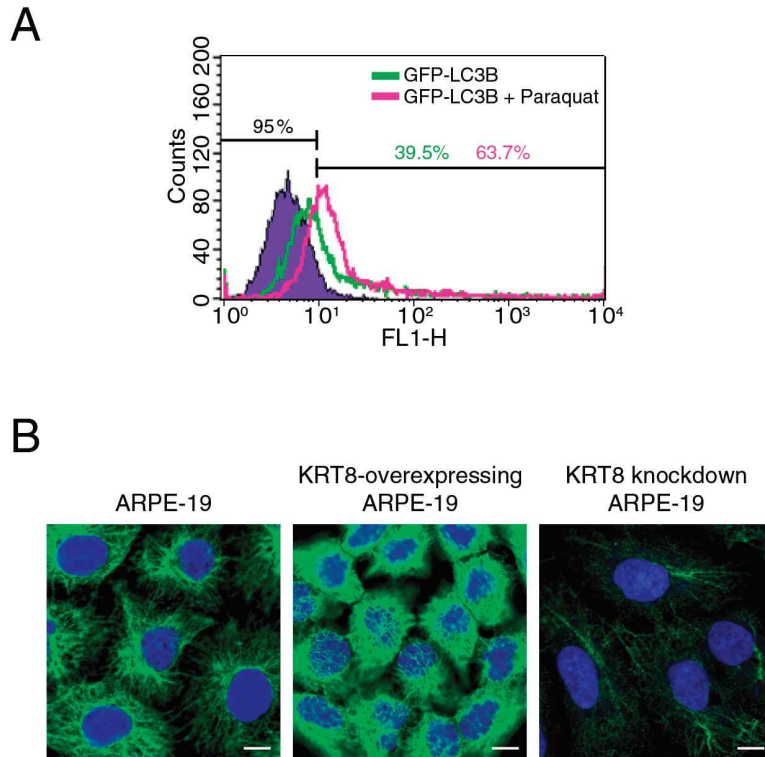
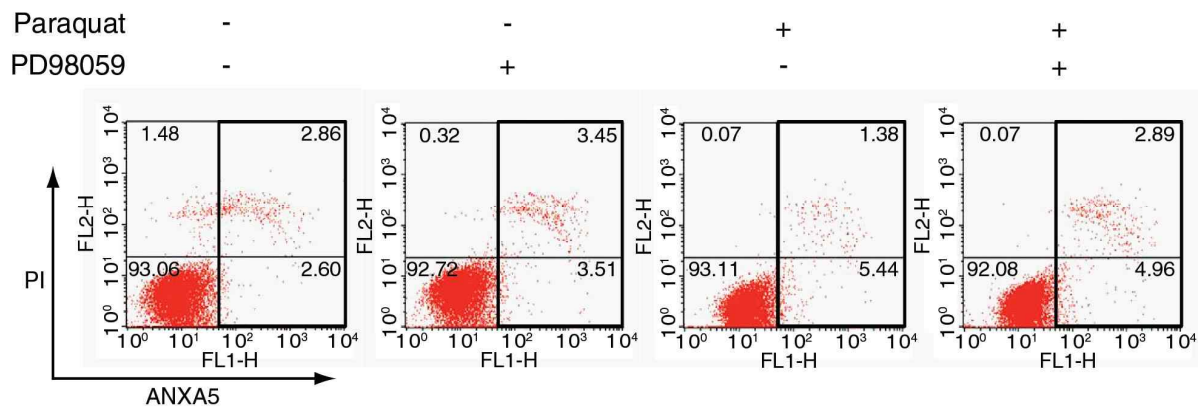


Figure S4. Transfection efficiency of GFP-LC3B plasmid DNA into ARPE-19 cells and different KRT8 expression in ARPE-19 cells by either plasmid DNA or anti-KRT8 siRNA transfection. **(A)** ARPE-19 cells were transfected with GFP-LC3B-encoding plasmid DNA. Transfection efficiency was measured by measurement of GFP fluorescence using flow cytometer in the absence or presence of paraquat (400 μ M). **(B)** Fluorescence microscopy images of ARPE-19 cells for KRT8 expression, which were differently prepared by either KRT8 plasmid DNA or anti-KRT8 siRNA transfection. Cells were immunostained for KRT8 (green fluorescence) and nuclei (blue fluorescence). Scale bar: 5 μ m.

A



B

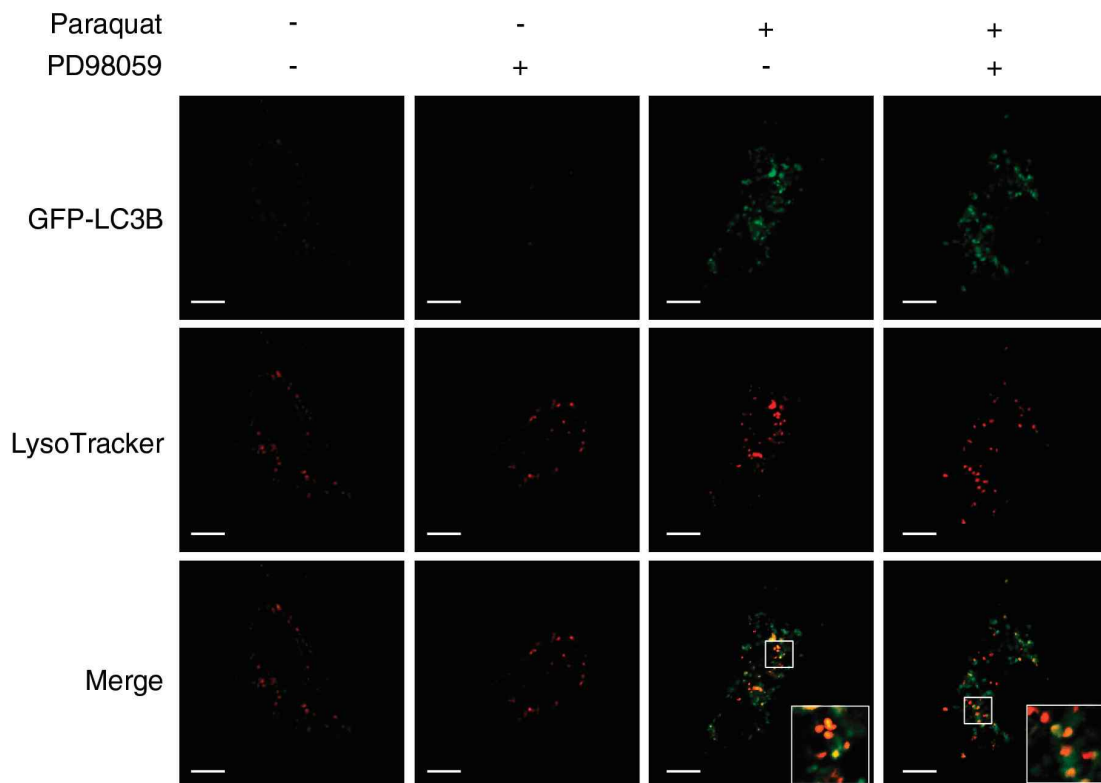


Figure S5. Apoptotic cell death and inhibition of autophagy are not affected by PD98059 in RPE cells under oxidative stress. **(A)** FACS analysis of ARPE-19 cells exposed to paraquat (400 μ M) for 24 h in the absence or presence of PD98059 (20 μ M). **(B)** ARPE-19 cells transfected with GFP-LC3B were treated with paraquat (400 μ M) in the absence or presence of PD98059 (20 μ M) for 24 h. Fluorescence microscopy images of the cells were obtained by monitoring GFP-LC3B puncta (green fluorescence) and the lysosomes were stained with LysoTracker Red DND-99 (red fluorescence). Scale bar: 10 μ m. Enlarged and merged fluorescence images show the detection of GFP-LC3B puncta and lysosomes.