Landes Bioscience www.landesbioscience.com

LANDES BIOSCIENCE

Supplemental Material to:

Xiaolei Xie, Li Le, Yanxin Fan, Lin Lv and Junjie Zhang

Autophagy is induced through the ROS-TP53-DRAM1 pathway in response to mitochondrial protein synthesis inhibition

Autophagy 2012; 8(7)
http://dx.doi.org/10.4161/auto.20250

www.landesbioscience.com/journals/autophagy/article/20250

Supplemental Material

Autophagy is induced through the ROS-TP53-DRAM1 pathway in response to mitochondrial protein synthesis inhibition

Xiaolei Xie, Li Le, Yanxin Fan, Lin Lv and Junjie Zhang

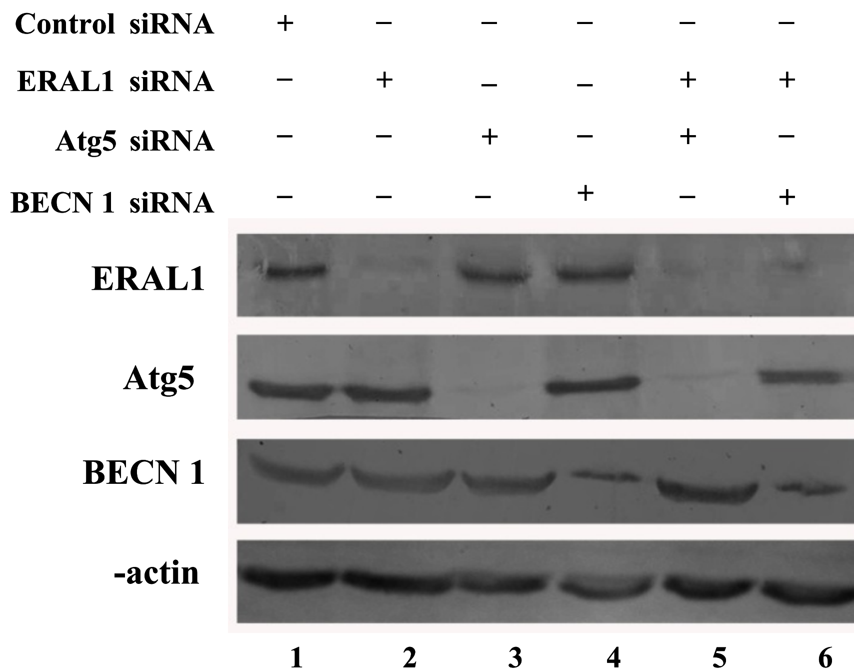

Figure S1. The down-regulation effects of siRNAs. Hela cells were transfected with the indicated siRNA (s) against ERAL1, Atg5 or BECN1. Cells were harvested at 72 h post-siRNA transfection and subjected to Western blotting to detect the indicated proteins. Lane 1, control siRNA (100 nM); lane 2, *ERAL1* siRNA (50 nM) and control siRNA (50 nM); lane 3, *Atg5* siRNA (50 nM) and control siRNA (50 nM); lane 4, *BECN1* siRNA (50 nM) and control siRNA (50 nM); lane 5, *ERAL1* siRNA (50 nM) and *Atg5* siRNA (50 nM); lane 6, *ERAL1* siRNA (50 nM) and *BECN1* siRNA (50 nM).

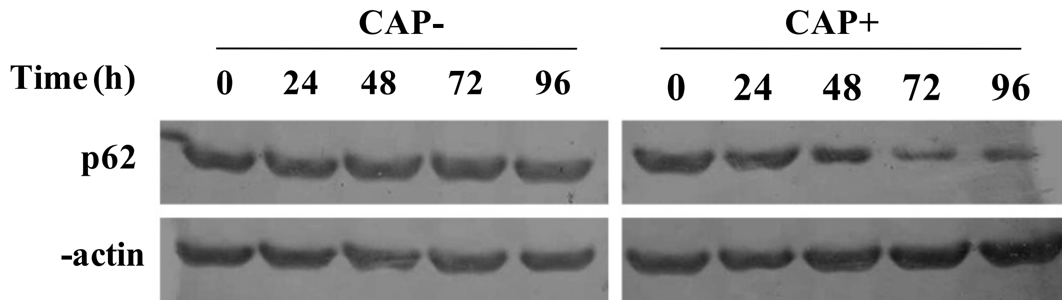

Figure S2. SQSTM1/p62 protein levels are decreased in CAP-treated HCT116 *TP53*$^{+/+}$ cells. HCT116 *TP53*$^{+/+}$ cells were treated with or without CAP (50 μg/ml). At indicated time point post-CAP treatment, SQSTM1/p62 protein levels were detected by western blot.

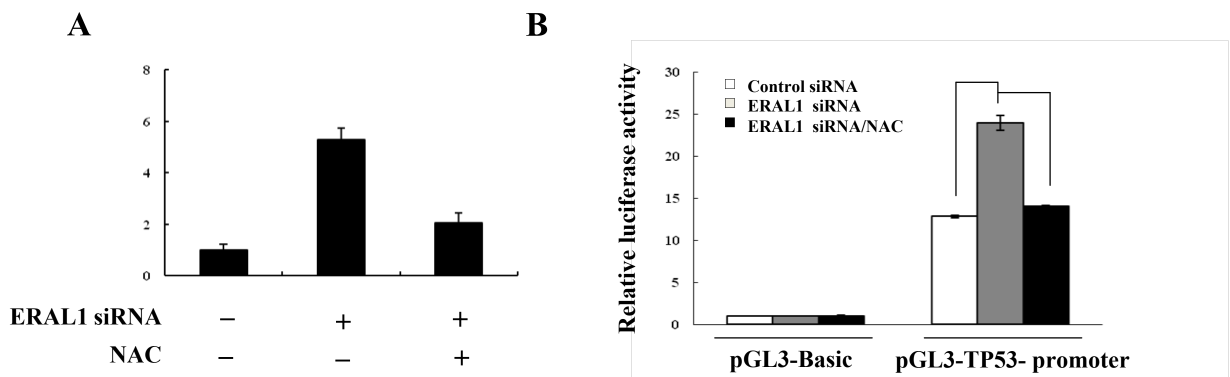

Figure S3. ROS increase *TP53* (Tumor Protein 53) expression at transcriptional level by enhancing *TP53* promoter activity in *ERAL1* siRNA-treated cells. (**A**) Hela cells were transfected with control siRNA or *ERAL1* siRNA in the presence or absence of NAC as indicated. *TP53* mRNA levels were analyzed by Real-Time PCR at 72 h post-siRNA transfection. (**B**) Hela cells were transfected with pGL3-Basic vector or pGL3-TP53-promoter together with β-gal expressing plasmid, and then transfected with control siRNA or *ERAL1* siRNA in the presence or absence of NAC as indicated. Luciferase activity was measured at 72 h post-siRNA transfection and normalized to plasmid transfection efficiency with β-galactosidase activity as internal control. Representative data were from three independent experiments. The p value derived from Student's t test is (**) $p<0.001$.

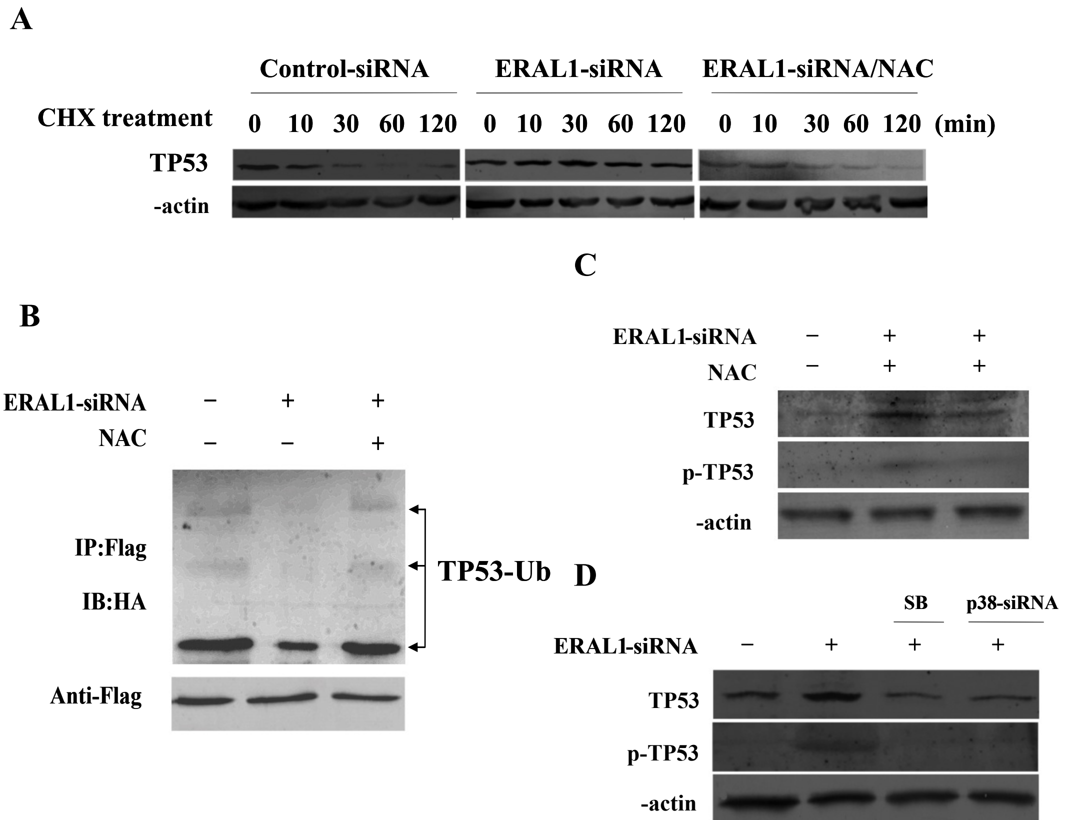

Figure S4. TP53 is activated at post-translational level through the ROS-MAPK14/p38 pathway in *ERAL1* siRNA-treated cells. (**A**) TP53 stability in Hela cells with ERAL1 knockdown. Hela cells were transfected with *ERAL1* siRNA in the absence or presence of NAC. At 72 h post-siRNA transfection, cells were treated with CHX (20 μM). TP53 protein levels were detected by western blotting at indicated time points after the addition of CHX. (**B**) TP53 ubiquitination in Hela cells with ERAL1 knockdown. Hela cells cotransfected with HA-ubiquitin and TP53-Flag plasmid were transfected with *ERAL1* siRNA h in the absence or presence of NAC. At 72 h post-siRNA transfection, cells were treated with MG132 for 2 h, and then subjected to immunoprecipitation with anti-Flag agarose beads, followed by western blot analysis with anti-HA antibody to detect ubiquitinated proteins (top panel), and with anti-Flag antibody to determine the TP53-Flag levels (bottom panel). (**C**) TP53 phosphorylation in Hela cells with *ERAL1* knockdown. Hela cells were transfected with ERAL1 siRNA in the absence or presence of NAC. At 72 h post-siRNA transfection, cells were subjected to western blotting to detect the levels of TP53 and

p-TP53 (Ser15) respectively. (**D**) The role of MAPK14/p38 in TP53 phosphorylation in Hela cells with ERAL1 knockdown. Hela cells were treated with ERAL1 siRNA for 72 h together with MAPK14 inhibitor SB202190 (SB) or *MAPK14*-specific siRNA, and then subjected to western blotting to detect the levels of TP53 and p-TP53 (Ser15) respectively.

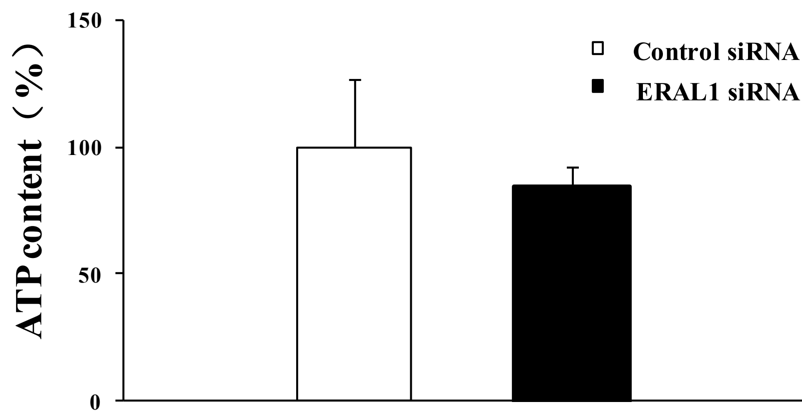

Figure S5. Intracellular ATP levels in *ERAL1* siRNA or control siRNA-treated Hela cells. Hela cells were cultured in normal DMEM medium and transfected with *ERAL1* siRNA or control siRNA respectively. Intracellular ATP levels were measured 72 h post-siRNA transfection with the ATP-Lite Assay Kit (TOO7) according to the manufacturer's direction (Vigorous Biotechnology, China).

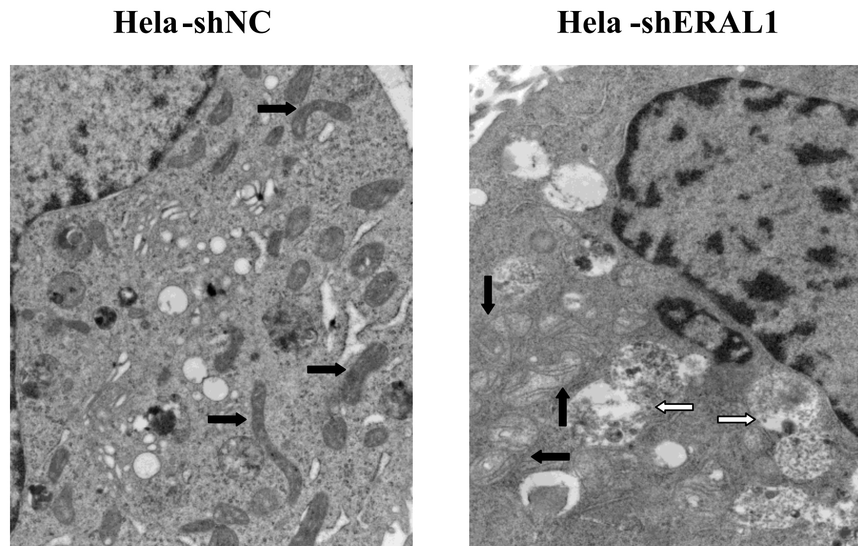

Figure S6. Electron-microscope pictures of Hela cells with stably expression of ERAL1-shRNA (Hela-shERAL1) or scramble shRNA (Hela-shNC). Solid arrows represent mitochondria, and empty arrows represent autophagic vacuoles.

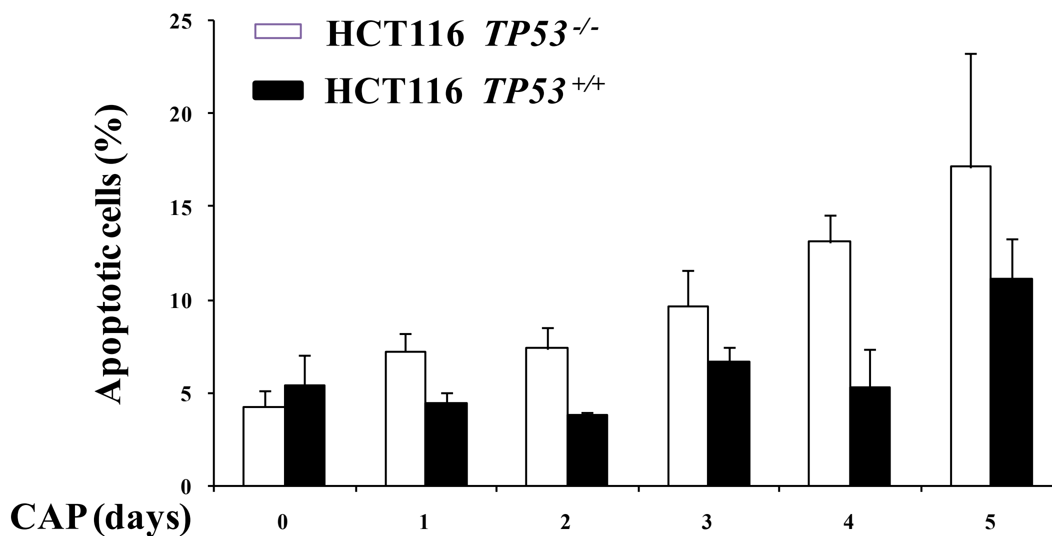

Figure S7. Analysis of apoptosis in CAP-treated HCT116 *TP53*$^{+/+}$ and HCT116 *TP53*$^{-/-}$ cells. HCT116 *TP53*$^{+/+}$ and HCT116 *TP53*$^{-/-}$ cells were treated with CAP (50 μg/ml) for the indicated time, and then fixed overnight in 70% ethanol. The cells were washed twice with PBS and incubated in PBS containing RNase A (10 μg/ml) for 30 min at 37°C. The cells were then stained with propidium iodide (0.5 mg/ml) for 30 min at 4°C. The percentage of apoptotic cells were identified by the sub-G1 DNA content measured with flow cytometry.