p62/SQSTM1 is required for the protection against endoplasmic reticulum stress-induced apoptotic cell death

Jeong Su Park[a,#], Sue Young Oh[d,#], Da Hyun Lee[a,b], Yu Seol Lee[a], Su Haeng Sung[a], Hye Won Ji[a], Moon Joo Lee[a], Yong-ho Lee[c], Sue Goo Rhee[a] and Soo Han Bae[a,*]

[a]Severance Biomedical Science Institute, Yonsei Biomedical Research Institute, Yonsei University College of Medicine, 50-1, Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea;

[b]Brain Korea 21 PLUS Project for Medical Science, Yonsei University;

[c]Division of Endocrinology and Metabolism, Department of Internal Medicine, Yonsei University College of Medicine, 50-1, Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea;

[d]Department of Oral Biology, Yonsei University College of Dentistry, 50-1, Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea

*Corresponding author. Address: [1]Severance Biomedical Science Institute, Yonsei Biomedical Research Institute, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea; Tel.: +82-2-2228-0756, Fax: +82-2-2227-8129; E-mail address: soohanbae@yuhs.ac (S. H. Bae)

#These authors contributed equally this work.

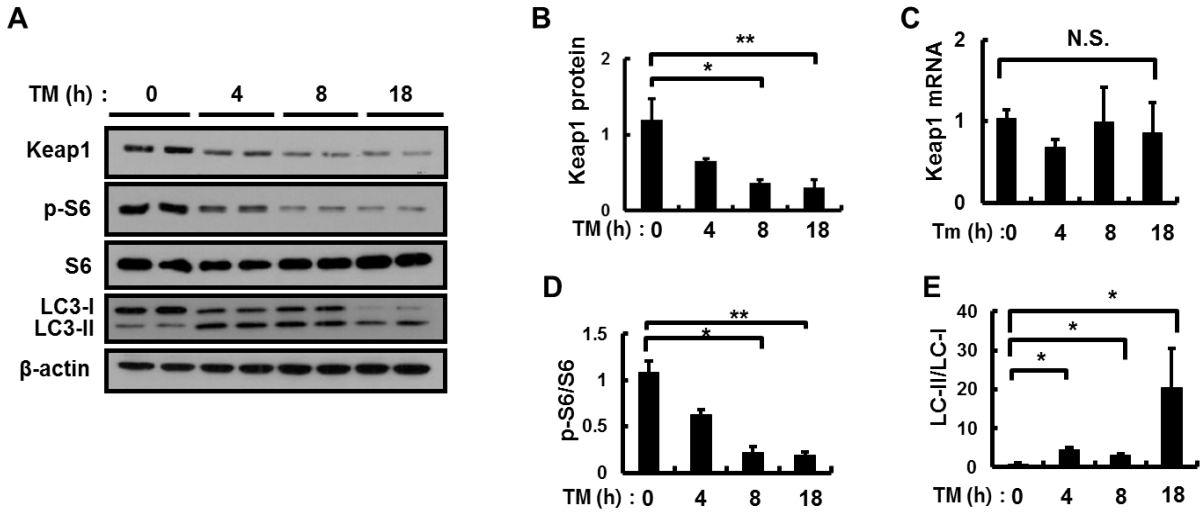
Supporting Experimental Procedures

Cell culture and reagents

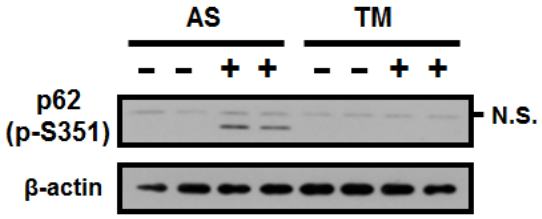
Green fluorescent protein (GFP)-conjugated LC3 (GFP-LC3) expressing HeLa cells (GFPLC3/HeLa) cells were maintained under 5% CO_2 at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin, and streptomycin. The following primary antibodies were used: anti-p-p62 (gift from Drs. Rhee and Komatsu) and anti-β-actin (Abclon). Tunicamycin (TM) and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich.

Immunofluorescence staining and confocal microscopy

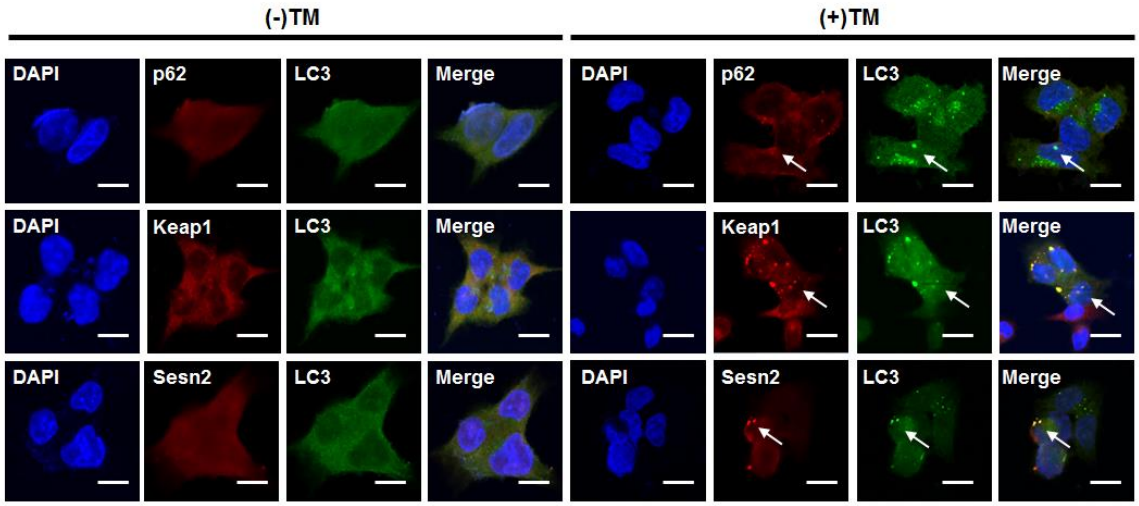
Green fluorescent protein (GFP)-conjugated LC3 (GFP-LC3) expressing HeLa cells (GFPLC3/HeLa) cells seeded on glass coverslips were treated with DMSO or tunicamycin (2 μg/mL) for 24 h, and then fixed and permeabilized with 0.1% Triton X-100 in PBS for 15 min at room temperature. After PBS washes, cells were blocked with 1% BSA for 1 h at room temperature. The cells were then incubated for overnight with primary antibodies at 4°C. After PBS washes, cells were incubated with secondary antibodies for 1h at room temperature. Finally, cells were washed with PBS 3 times and mounted onto slides with mounting medium and observed on an LSM700 confocal microscope (Carl Zeiss, Jena, Germany) at 800x magnification. The following primary antibodies were used: Sesn2 (1:500; abclon by custom antibody service), p62 (1:500; Abnova, H00008878-M01), Keap1 (1:500; Proteintech, 10503-2-AP). Secondary antibodies used are from Invitrogen: Donkey anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 568 and Donkey anti-Mouse IgG Secondary Antibody, Alexa Fluor 568. The nuclei were counterstained with DAPI signals were visualized on a confocal microscope at 800x magnification.



Supplementary Figure 1. Tunicamycin induces autophagic Keap1 degradation in MEF. **(**A) MEF cells were incubated with TM (2 μg/mL) for indicated periods. Lysates of MEF cells were subjected to immunoblot analysis with antibodies against Keap1, p-S6, S6, LC3, and β-actin (loading control). (B) Densitometric analysis of Keap1 immunoblots obtained as described in (A). Total RNA isolated from cells treated as described in (A) was subjected to qRT-PCR analysis of Keap1 mRNA expression (C). (D) Densitometric analysis of p-S6/S6 immunoblots obtained as described in (A). (E) Densitometric analysis of p-S6/S6 immunoblots obtained as described in (A). Data are presented as mean ± SD from three independent experiments. *$p < 0.05$ and **$p < 0.005$.



Supplementary Figure 2. Tunicamycin did not affect the level of p62 phosphorylation (p-S351). Hepa1c1c7 cells were incubated with DMSO, sodium arsenite (AS, as positive control, 10 μM), or Tunicamycin (TM, 2 μg/mL) for 24h. The cells were lysed and subjected to immunoblot analysis with antibodies against p62 (p-S351) and β-actin (loading control).



Supplementary Figure 3. Co-localization of Keap1, p62, Sesn2, and LC3 proteins under ER stressed condition induced by tunicamycin. Confocal microscopy analysis of colocalization of Keap1, p62, Sesn2, and LC3 proteins. Green fluorescent protein (GFP)-conjugated LC3 (GFP-LC3) expressing HeLa cells (GFPLC3/HeLa) cells were incubated with TM (2 μg/mL) for 24h, Nuclei were also stained with DAPI, and representative single optical sections and overlay (merge) images are shown. Scale bars, 10 μm. Representative puncta are marked by arrows.