

Supplemental Figure S1 Kanki and Klionsky.

Monitoring mitophagy using Om45-GFP processing following oligomycin treatment. *A*, The wild-type strain (SEY6210) expressing Om45-GFP was grown to the logarithmic phase in YPL medium. Cells were treated with the mitochondrial complex V (ATP synthase) inhibitor oligomycin (2  $\mu$ g/ml) or ethanol as a control, for 6 h. Cell lysates equivalent to A<sub>600</sub> = 0.2 units of cells were subjected to immunoblotting with anti-GFP and anti-Pgk1 (loading control) antibody or antiserum, respectively. *B*, Cells were treated with oligomycin (2  $\mu$ g/ml) or ethanol (0  $\mu$ g/ml) as a control, for 0, 6 and 8 h. Cell lysates equivalent to A<sub>600</sub> = 0.2 units of zells were subjected to immunoblotting with anti-GFP and anti-Pgk1 (loading control) antibody or antiserum, respectively. *B*, Cells were treated with oligomycin (2  $\mu$ g/ml) or ethanol (0  $\mu$ g/ml) as a control, for 0, 6 and 8 h. Cell lysates equivalent to A<sub>600</sub> = 0.2 units of cells were subjected to immunoblotting as above. The asterisk indicates a non-specific band.



Supplemental Figure S2 Kanki and Klionsky.

Monitoring mitophagy using Om45-GFP processing during Mdm38 depletion. An *mdm38* $\Delta$  and wild-type (WT, BY4742) strain expressing Om45-GFP and a doxycycline (Dox)-regulated Mdm38-HA expression vector or the pCM189 empty vector, respectively, were cultured in SMGal medium. The cells were grown to the early logarithmic phase for 8 to 48 hours as indicated, in the presence of 5 µg/ml Dox and collected. The cell lysates equivalent to A<sub>600</sub> = 0.4 unit of cells were subjected to immunoblotting analysis. GFP processing and Mdm38-HA expression level were monitored by immunoblotting with anti-GFP, anti-HA and anti-Pgk1 (loading control) antibodies.



Supplemental Figure S3 Kanki and Klionsky.

Screening *atg* mutants for potential mitophagy defects during starvation by fluorescence microscopy. The indicated strains expressing Om45-GFP were cultured in YPL medium to mid-log growth phase, and shifted to SD-N medium for 4.5 hours. The cells were then treated with the vacuolar stain FM 4-64 (10  $\mu$ g/ml, Molecular Probes/Invitrogen) for 45 min, washed with SD-N medium and re-incubated in SD-N medium for another 45 min. After a total of 6 hours starvation, the cells were observed by fluorescence microscopy.