isolation of endogenous ULK1, and blotted with an anti-ULK1 antibody. (**H**) GFP-MUL1 and ULK1-MYC were cotransfected with Flag-ATG13 or Flag vector, and immunoprecipitation was performed with an anti-Flag antibody. Coimmunoprecipitated ULK1 and MUL1 were detected through western blotting with anti-GFP and anti-MYC antibodies respectively. (**I**) Truncated forms of ULK1-MYC were constructed based on its functional domains. GFP-MUL1 was cotransfected with full-length or truncated forms of ULK1-MYC, and immunoprecipitation was performed with an anti-MYC antibody. Coimmunoprecipitated MUL1 was detected using an anti-GFP antibody. (**J**) *Ulk1-/-* MEFs were transfected with exogenous wild-type ULK1 or truncation ULK1Δ CTD, followed by treatment with selenite for 12 h before western blotting analysis of the indicated proteins.

**Figure 4.** Overexpression of MUL1 and treatment with selenite promotes ULK1 degradation through proteasome pathway. (**A**) HeLa cells were treated with CHX (10 μM, 12 h) and selenite (5 μM) for the indicated time, with or without MG132, and subjected to western blotting analysis of the ULK1. (**B**) Quantification of ULK1 protein levels in (**A**) (mean ±SEM, from 3 independent experiments). (**C**) HeLa cells were treated with the indicated agents (FCCP 5 μM; hypoxia with 1% O2; selenite 5 μM), and then subjected to western blotting analysis of ULK1 (The intensity of indicated bands was measured with ImageJ software). (**D**) After transfection with plasmids as indicated, HeLa cells were treated with BAF (10 nM, 6 h) or MG132 (5 μM, 6 h) prior to harvesting, followed by western blotting analysis of the GFP-ULK1 level. (**E**) After transfection with MUL1-MYC as indicated, HeLa cells were treated with BAF (10 nM, 6 h) or MG132 (5 μM, 6 h), followed by western blotting analysis of the ULK1 protein level. (**F**) HeLa cells were transfected with MUL1-MYC or MUL1ΔR-MYC (MUL1 with absence of the RING finger domain) for 24 h, and subjected to western blotting analysis of the indicated protein levels (The intensity of indicated bands was measured with ImageJ software). (**G**) HeLa cells transfected with MUL1-MYC for 24 h, followed by treatment with CHX (10 μM) for the indicated time, with or without MG132, and subjected to western blotting analysis of the ULK1 protein level. (**H**) Quantification of ULK1 protein levels in (**G**). Mean ±SEM, from 3 independent experiments.

**Figure 5.** MUL1 promotes ubiquitination of ULK1. (**A**) HEK 293T cells were transfected with MUL1-MYC, MUL1ΔR-MYC or the empty MYC-vector together with GFP-ULK1 and HA-Ub. Ubiquitination assays were performed as described in Materials and Methods. The ubiquitination level of GFP-ULK1 was detected using an anti-HA antibody. (**B**) *In vitro* ubiquitination assays were performed as described in Materials and Methods. The ubiquitinated form of ULK1 was immunoblotted using an anti-ULK1 antibody. (**C**) MUL1 knockdown cells transfected with GFP-ULK1 were subjected to ubiquitination assays for analysis with an anti-Ub antibody. (**D**) Quantitative analysis of ubiquitinated GFP-ULK1 level as described in (**C**) (The intensity of bands was measured with Image J software. mean±SEM; from 3 independent experiments). (**E**) HEK 293T cells were transfected with the indicated plasmids for 24 h, and subsequently a ubiquitination assay was performed for analysis with an anti-Ub antibody. (**F**) HeLa cells transfected with GFP-ULK1 were treated with 5 μM of selenite for the indicated time, followed by ubiquitination assays for analysis with an anti-Ub antibody. (**G**) HeLa cells were treated with 5 μM of selenite for the indicated time, followed by ubiquitination assays for analysis with an anti-Ub antibody. (**H**) Scrambled RNA- or si*MUL1*-transfected cells were treated with or without selenite for 12 h, followed by ubiquitination assays for analysis with an anti-Ub antibody. (**I**) NIH-3T3 cells were treated with 5 μM of selenite for the indicated time, followed by ubiquitination assays for analysis with an anti-Ub antibody. (**J**) NIH-3T3 cells were treated with selenite for the indicated time, with or without MG132, and subjected to western blotting analysis of the ULK1 protein level.

**Figure 6.** MUL1 response to selenite-induced ROS stress depending on conserved cysteines 62 and 87. (**A**)HeLa cells treated with selenite as indicated were stained with MitoSox Red and subjected to flow cytometry. (**B**) HeLa cells were pretreated with NAC (10 mM) or GSH-EE (10 mM) for 30 min, followed by treatment with 5 μM of selenite for 12 h. Cell lysates were subjected to western blotting analysis of the indicated proteins. (**C**) HeLa cells transfected with GFP-ULK1 were pretreated with NAC (10 mM) or GSH-EE (10 mM) for 30 mins, followed by treatment of selenite (12 h). The ubiquitination assays were performed as described in Materials and Methods. (**D**) Alignment of the MUL1 amino acids in difference species. (**E**) HeLa cells with MUL1 knockdown were transfected with exogenous MYC-tagged wild-type MUL1 or MUL1 with cysteine mutations (C62S, C87S), followed by treatment with selenite (5 μM, 12 h) before western blotting analysis of the indicated proteins (The intensity of the indicated bands was measured with ImageJ software.).

**Figure S1.** Selenite induced mitophagy mediated by MUL1 in HeLa cells. (**A**) HeLa cells transfected with GFP-LC3 were treated with 5 μM of selenite for the indicated times, followed by staining with TOMM20 (Red) for mitochondria and the immunofluorescence microscopic analysis of GFP-LC3 puncta. (**B**) Quantification of GFP-LC3 punctate structures associated with mitochondria (TOMM20) described in (**A**). Mean ±SEM; n=50 cells from 3 independent experiments, one-way ANOVA, *\*P<0.05, \*\*P<0.01*. (**C**) HeLa cells were treated with 5 μM of selenite for the indicated time and subjected to western blotting analysis of the indicated proteins. (**D**) mRNA levels of *MUL1*, *ULK1* and *LC3B* were detected through qRT-PCR (mean ±SEM, 3 independent experiments, two-way ANOVA, *\*\*\*P<0.001*). (**E**) Quantification of autophagosomes containing mitochondria described in Fig. 1I (n=15 cells from 3 independent experiments, Student t test, *\*\*\*P<0.001*).

**Figure S2.** Low concentrations of selenite did not cause HeLa cell apoptosis. HeLa cells were treated with selenite for 12 h and subjected to flow cytometry. Cell apoptosis was detected through ANXA5/annexin V and PI double staining. [Insert a space before units.]

**Figure S3.** Measurement of the effects of the siRNA-mediated knockdown of mitochondrial E3 ligases. (**A**) HeLa cells with SMURF1 knockdown and *park2-/-* MEFs were treated with selenite for 12 h and subjected to western blotting analysis of the indicated proteins. (**B**) Specific siRNAs targeted to *RNF185*, *AMFR*, *MARCH5*, *SMURF1* and *MUL1* were transfected into HeLa cells for the indicated time. The mRNA levels of the E3 ligases were detected through qRT-PCR (mean±SEM; from 3 independent experiments, two-way ANOVA, *\*\*\*P<0.001*).

**Figure S4.** Ectopic expression of MUL1 induces mitophagy depending on ULK1 and ATG5, but not PARK2. (**A**) *WT*, *Ulk1-/-* and *Atg5-/-* MEFs were transfected with MUL1-MYC, and LC3 puncta were analyzed using an anti-LC3B antibody. (**B**) *park2-/-* MEFs were cotransfected with MUL1-MYC and GFP-LC3, and GFP-LC3 puncta were analyzed. (**C**) *WT* and *Ulk1-/-* MEFs were transfected with MUL1-MYC for 24 h, followed by western blotting to detect the indicated protein levels.

**Figure S5.** MUL1 interacts with ULK1. (**A**) HEK 293T cells were transfected with GFP vector or GFP-MUL1 together with ULK1-MYC, and immunoprecipitation was performed using an anti-GFP antibody. Coimmunoprecipitated ULK1 was detected through western blotting with an anti-MYC antibody. (**B**) HEK 293T cells were transfected with MYC vector or ULK1-MYC together with GFP-MUL1, and immunoprecipitation was performed using an anti-MYC antibody. Coimmunoprecipitated MUL1 was detected through western blotting with an anti-GFP antibody.

**Figure S6.** ULK1 degrade through proteasomes specifically upon treatment with selenite. HeLa cells were treated as indicated (EBSS 6 h; FCCP 5 μM; hypoxia with 1% O2, selenite 5 μM), and ULK1 protein level was detected with western blotting.

**Figure S7.** MUL1 induced mitophagy was not inhibited by expression of exogenous MFN1 or MFN2. HeLa cells expressing Cherry-LC3 were transfected with GFP-MFN1 or GFP-MFN2 together with MUL1-MYC, followed by immunofluorescence microscopy to detect and Cherry-LC3 puncta.

**Figure S8.** FUNDC1 was not involved in MUL1- or selenite-induced mitophagy. (**A**) HeLa cells with or without FUNDC1 knockdown were transfected with the indicated plasmids and subjected to western blotting analysis. (**B**) HeLa cells with or without FUNDC1 knockdown were treated with selenite as indicated and subjected to western blotting analysis.

**Figure S9.** MUL1 is involved in ROS-induced mitophagy. HeLa cells transfected with scrambled RNA or si*MUL1* were treated with the indicated ROS inducers, and then subjected to western blotting analysis indicates protein levels.

**Figure S10.** Localization of wild type MUL1 and its mutants on mitochondria. HeLa cells were transfected with the wild type MUL1 and its mutants (C62S, C87S) for 24 h, and then subjected to immunofluorescence microscopy analysis.