

Supplement 1. MITOL associates with and ubiquitinates Drp1.

(A) Ubiquitination of Drp1 by MITOL. Lysates of HeLa cells transfected with indicated vectors were immunoprecipitated with anti-FLAG antibody and immunoprecipitates were immunoblotted with anti-HA or anti-FLAG antibodies. Whole lysates were immunoblotted with anti-Myc antibody.

(B) Interaction of MITOL with Drp1. Lysates of HeLa cells transfected with indicated vectors were immunoprecipitated with anti-Myc antibody and immunoprecipitates were immunoblotted with anti-FLAG or anti-Myc antibodies. Whole lysates were immunoblotted with anti-FLAG antibody to confirm the expression of FLAG-Drp1.

(C) Pulse-chase experiment showed that MITOL overexpression promoted Drp1 turnover. HeLa cells expressing FLAG-Drp1 or FLAG-Drp1/MITOL WT were labeled for 60 min with [<sup>35</sup>S]Met/Cys labeling mixture and chased in complete DMEM for indicated periods. Cells were lysed and immunoprecipitated with anti-FLAG antibody and immunoprecipitated FLAG-Drp1 were visualized by autoradiography. Relative values estimated by NIH image analysis were indicated below. Result was representative of three independent experiments.