SUPPLEMENTARY FIGURE LEGENDS

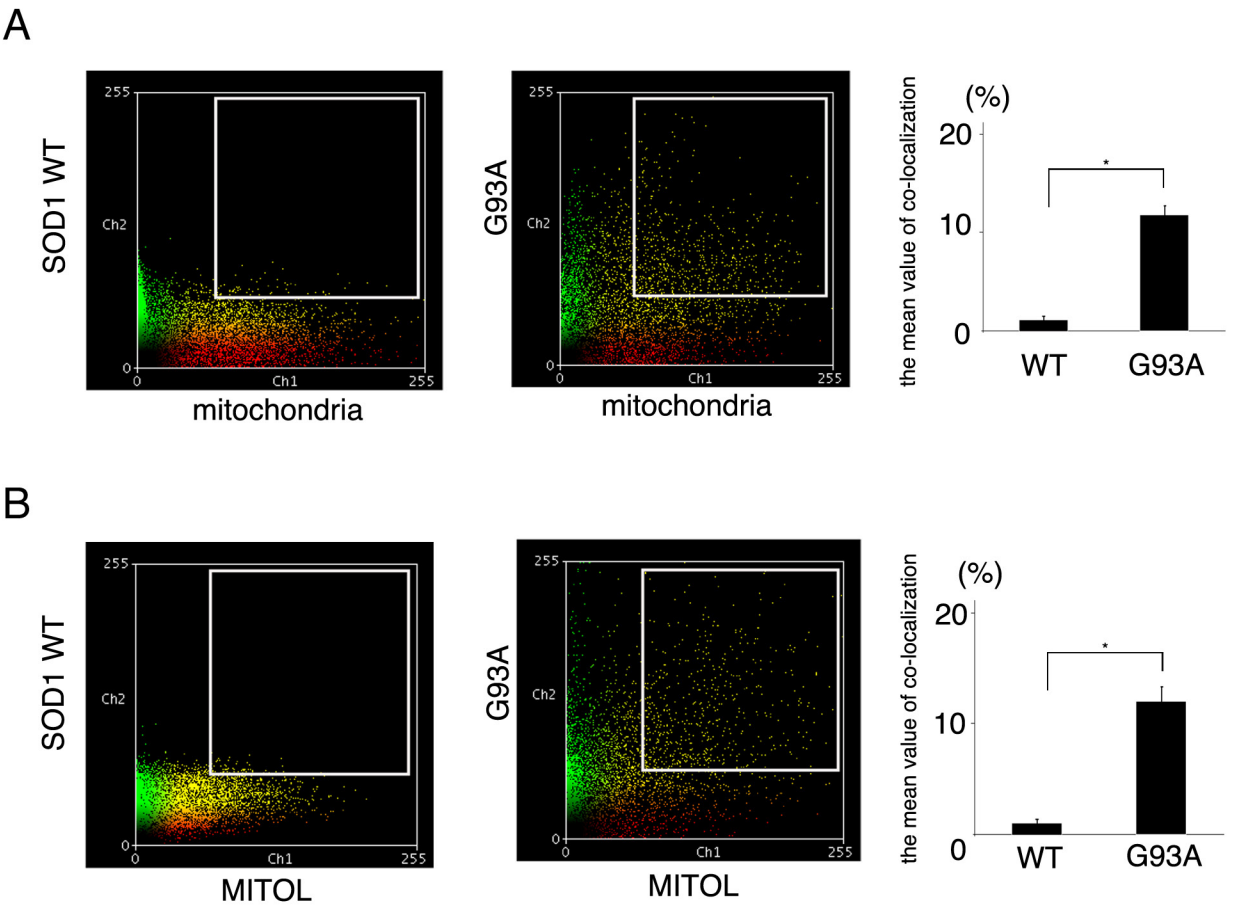
Supplementary Figure 1. Scatter plot analysis using ImageJ software indicated a higher level of co-localization of mSOD1 than that of WT SOD1 with mitochondria (A) and with MITOL (B). The regions of interest (ROI) containing high pixel intensities in x-axis (red) and y-axis (green) show the high co-localized area. The degree of co-localization within ROI was estimated in the right panels. Error bars indicated s.d (n=10). *, $p < 0.05$ (Student's *t*-test).

Supplementary Figure 2. Ubiquitination and degradation of mitochondria-targeted mSOD1 by MITOL. (A) Cycloheximide chase assay indicated the MITOL-dependent degradation of mitochondria-targeted mSOD1 but not ER-targeted mSOD1. Neuro2a cells transfected with indicated vectors were treated with cycloheximide (10 μg/ml) for indicated times and whole cell lysates were immunoblotted with indicated antibodies. (B) Enhanced MITOL-dependent ubiquitination of mitochondria-targeted mSOD1 as compared to non-tagged mSOD1. Neuro2a cells transfected with indicated vectors were immunoprecipitated with anti-FLAG antibody and immunoprecipitates were immunobloted with anti-HA or anti-FLAG antibodies. Whole lysates were immunoblotted with anti-myc antibody.

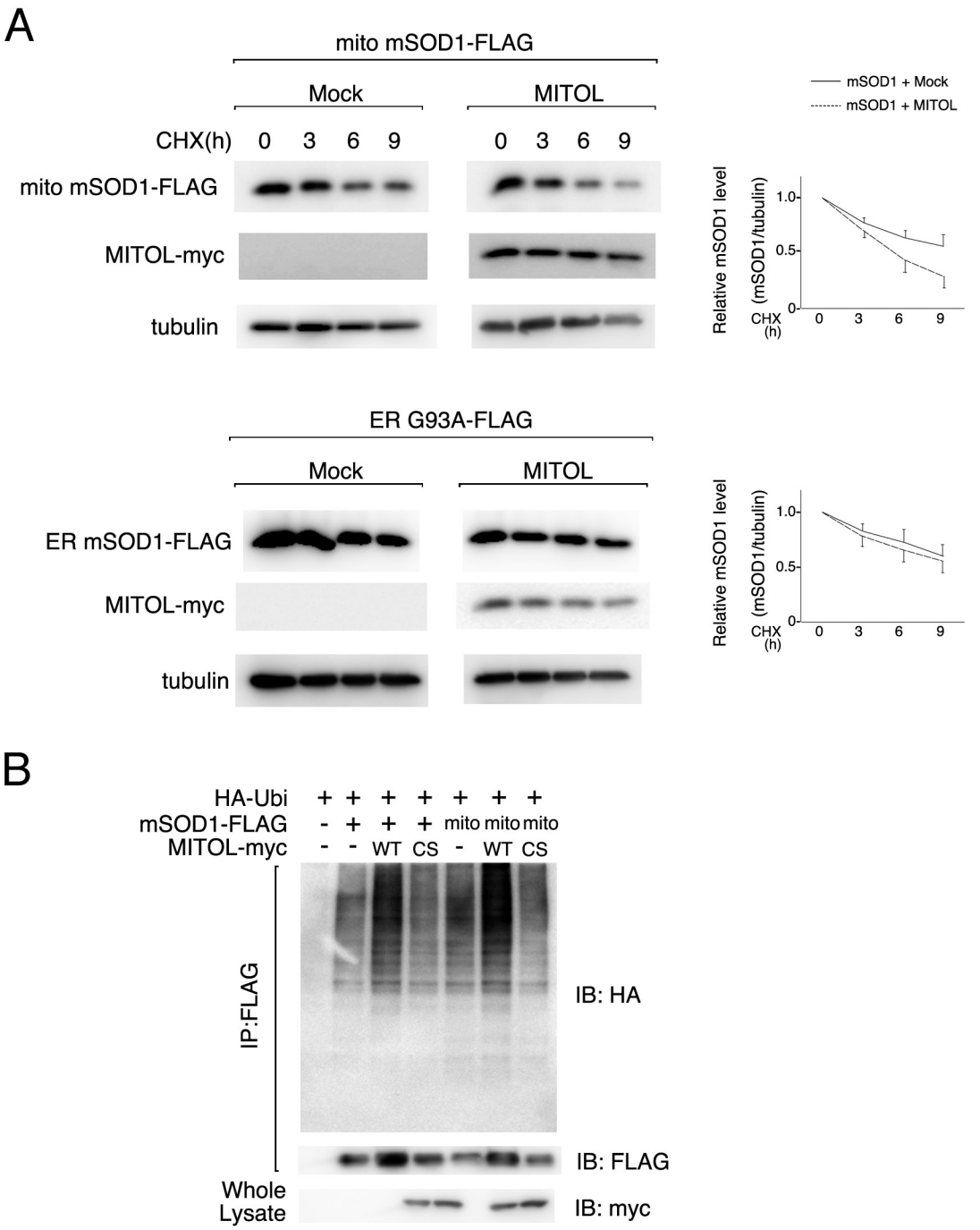
Supplementary Figure 3. Role of MITOL in quality control for short chain acyl CoA dehydrogenase (SCAD) mutant. (A) Degradation of insoluble SCAD W153R by MITOL WT but not CS mutant. SCAD mutant W153R with HA epitope tag is well known to form insoluble aggregates and induce mitochondrial stress. Co-expression of SCAD mutant with MITOL-FLAG WT, but not CS mutant, significantly reduced the accumulation of SCAD mutant in insoluble fraction. Whole lysates or soluble and insoluble fractions isolated from HeLa cells transfected with indicated vectors were immunoblotted using anti-HA or FLAG antibodies. Relative values are indicated below. (B) Degradation of SCAD W153R in MITOL WT-dependent manner. We examined whether this decrease in SCAD mutant with HA epitope tag from insoluble fraction was dependent on MITOL-FLAG expression level. MITOL reduced SCAD mutant from insoluble fraction in a dose dependent manner. Whole lysates or insoluble fraction isolated from HeLa cells trasnfected with indicated vectors were immunoblotted using anti-HA antibody. Relative values are indicated below. (C) Association of MITOL with a chaperone Hsp70.

Immunoprecipitation of MITOL-FLAG using anti-FLAG antibody from HeLa cells expressing control vector or MITOL-FLAG revealed a clear band around 70 kDa in silver stained electrophoresis gel. Mass spectrometric analysis demonstrated that this 70 kDa protein was identical to Hsp70.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3