Supporting Information

Parkin recruitment to impaired mitochondria for nonselective ubiquitylation is facilitated by MITOL

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Running title: MITOL assists Parkin in mitochondrial localization
Keywords: Parkin, Parkinson's disease, mitophagy, ubiquitin, ubiquitin ligase
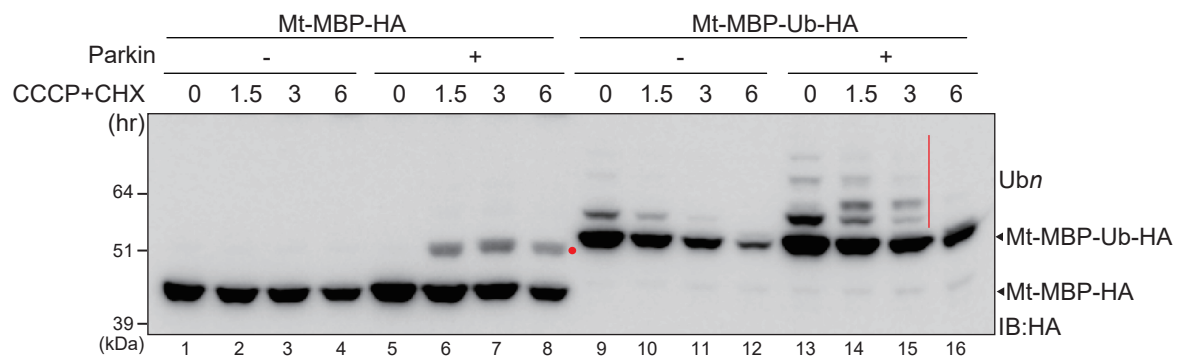

Table of content

Figure S1.

Intact HeLa cells or HeLa cells stably expressing GFP-Parkin were transfected with Mt-MBP-HA or Mt-MBP-Ub-HA. They were then treated with 15 µM CCCP + 50 µg/ml cycloheximide (CHX) for the indicated times (hr), and then immunoblotted with an anti-HA antibody. The red dot and red bar indicate ubiquitylation of Mt-MBP-HA and Mt-MBP-Ub-HA, respectively.
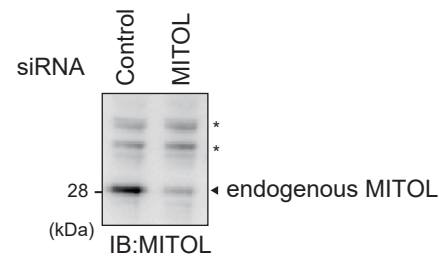
Figure S2.

HeLa cell lysates treated with control or *MITOL* siRNA were immunoblotted with an anti-MITOL antibody.

Endogenous MITOL was reduced in *MITOL*-knock down cells. The asterisks indicate cross-reacting bands.
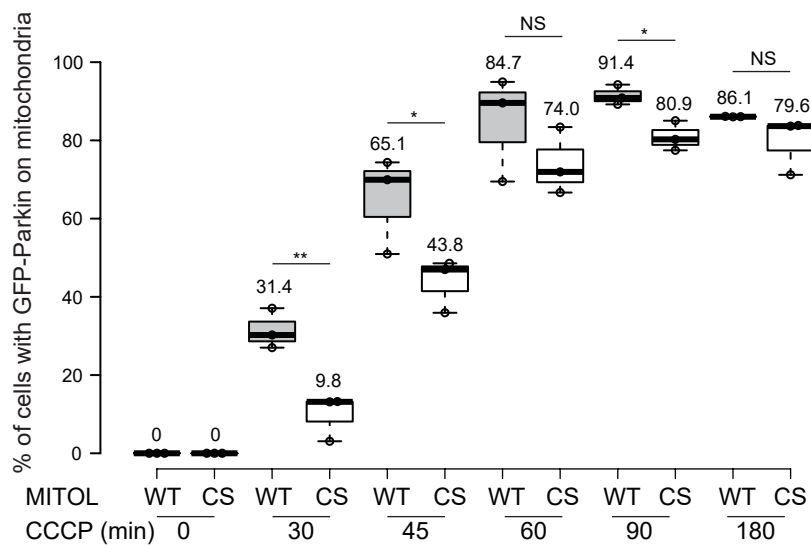
Figure S3.

Statistical analysis of the subcellular localization of GFP-Parkin in HeLa cells stably expressing 3xFlag-MITOL WT or an E3-inactive C65S/C68S (CS) mutant following 15 µM CCCP treatment for the indicated times (min).

The percentages of cells with Parkin-positive mitochondria were calculated using > 100 cells.

Numbers in the box-and-whisker plot are mean values across three independent experiments.

Statistical significance was calculated using a one-tailed Welch's *t*-test. *, *P*<0.05; **, *P*<0.01. NS, Not Significant.