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ABSTRACT

The protein kinase PINK1 and ubiquitin ligase Parkin promote removal of damaged mitochondria via a feed-forward mechanism involving ubiquitin (Ub) phosphorylation, Parkin activation, and ubiquitylation of mitochondrial outer membrane proteins to support recruitment of mitophagy receptors. The ubiquitin ligase substrate receptor FBXO7/PARK15 is mutated in an early-onset parkinsonian-pyramidal syndrome. Previous studies have proposed a role for FBXO7 in promoting Parkin-dependent mitophagy. Here, we systematically examine the involvement of FBXO7 in depolarization-dependent mitophagy in the well-established HeLa and induced-neurons cell systems. We find that FBXO7$^{-/-}$ cells have no demonstrable defect in: 1) kinetics of pUb accumulation, 2) pUb puncta on mitochondria by super-resolution imaging, 3) recruitment of Parkin and autophagy machinery to damaged mitochondria, 4) mitophagic flux, and 5) mitochondrial clearance as quantified by global proteomics. Moreover, global proteomics of neurogenesis in the absence of FBXO7 reveals no obvious alterations in mitochondria or other organelles. These results argue against a general role for FBXO7 in Parkin-dependent mitophagy and point to the need for additional studies to define how FBXO7 mutations promote parkinsonian-pyramidal syndrome.

FILES

Protocol

NAME

Flow cytometry-based measurement of mitophagic flux

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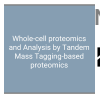

NAME

Whole-cell proteomics and Analysis by Tandem Mass Tagging-based proteomics

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Evaluation of pUb kinetics using 3D-SIM

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Microscopy-based evaluation of mtKeima flux in hESC-derived Ctrl and FBXO7-/- iNeurons

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