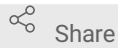




Oct 31, 2022

Kraus et al., 2022 FBX07 /Park15

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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 FBX07/PARK15 is mutated in an early-onset parkinsonian-pyramidal syndrome. Previous studies have proposed a role for FBX07 in promoting Parkin-dependent mitophagy. Here, we systematically examine the involvement of FBX07 in depolarization-dependent mitophagy in the well-established HeLa and induced-neurons cell systems. We find that FBX07^{-/-} 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 FBX07 reveals no obvious alterations in mitochondria or other organelles. These results argue against a general role for FBX07 in Parkin-dependent mitophagy and point to the need for additional studies to define how FBX07 mutations promote parkinsonian-pyramidal syndrome.

DOI

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COLLECTION CITATION

Felix Kraus 2022. Kraus et al., 2022 FBX07 /Park15 . **protocols.io**
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FUNDERS ACKNOWLEDGEMENT

NIH

Grant ID: NS083524

NIH

Grant ID: K01DK098285

ASAP

Grant ID: ASAP-000282

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CREATED

Oct 31, 2022

LAST MODIFIED

Oct 31, 2022



COLLECTION INTEGER ID




72080



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FILES



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Flow cytometry-based measurement of mitophagic flux
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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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Evaluation of mtKeima foci in induced neurons (iNeurons)
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Microscopy-based measurements of p62 recruitment in HeLa
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Microscopy-based pUb-coverage measurements of mitochondria in iNeurons
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Microscopy-based evaluation of Parkin translocation and mitophagy in FBX07-/- cell linesons)
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Immunocytochemical analysis

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