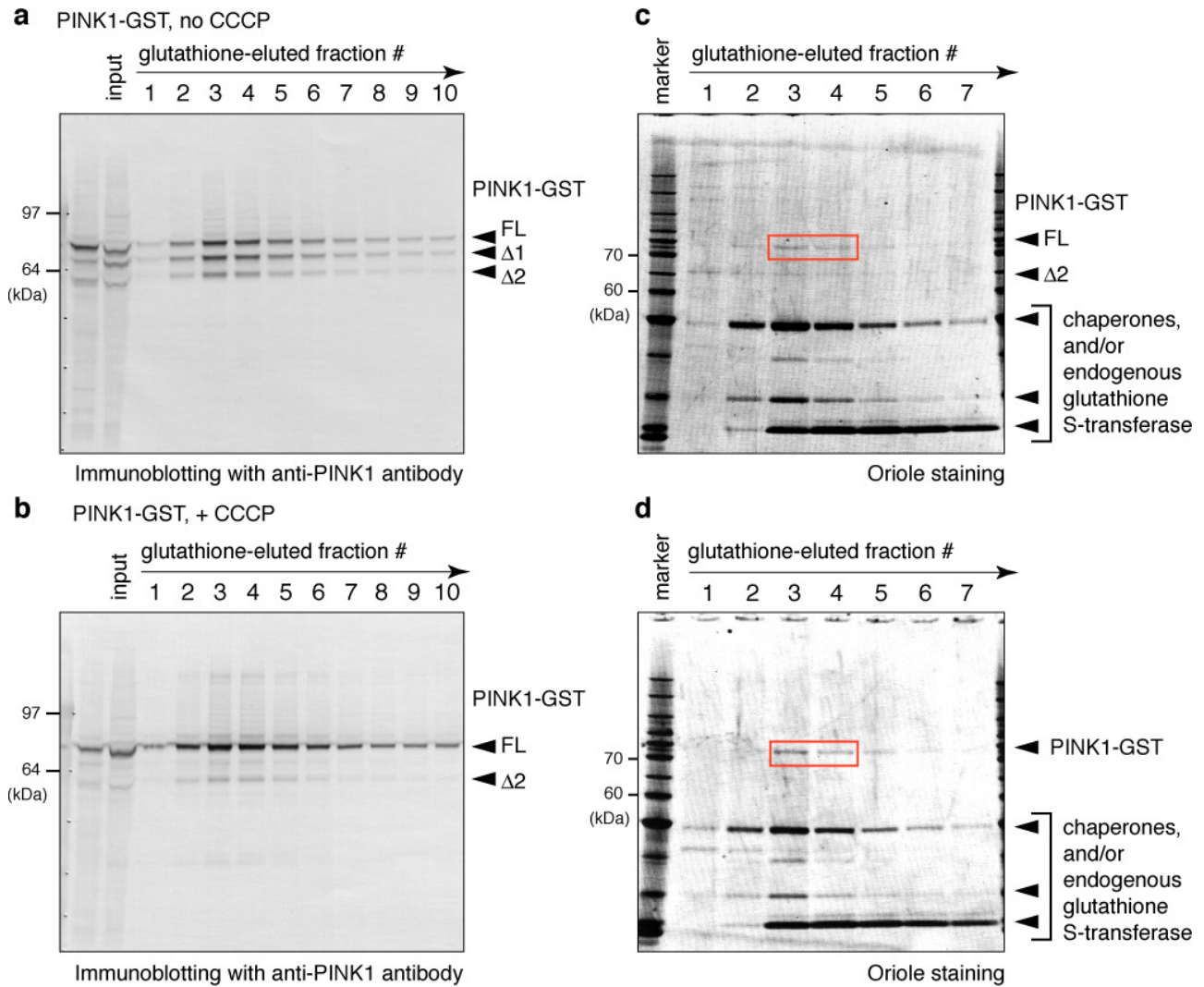


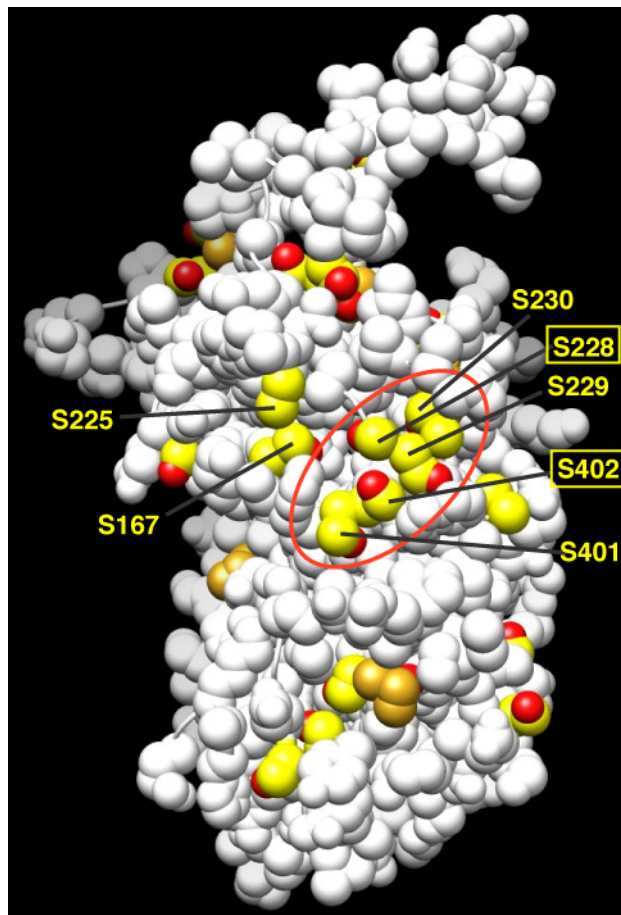
Supplementary Figure S1. PINK1 with various pathogenic mutations localized on mitochondria.

PINK1^{-/-} MEFs expressing various pathogenic PINK1 mutants were treated with 10 μM CCCP for 1 h and then subjected to immunocytochemistry with anti-PINK1 antibody. *PINK1*^{-/-} MEFs were used to avoid the signal derived from endogenous PINK1. Bars, 10 μm.



Supplementary Figure S2. Sample collection for MS analysis.

(a), (b) PINK1-GST was purified from HEK293 cells stably expressing PINK1-GST in the absence (a) or presence (b) of CCCP by standard purification methods. Eluates from glutathione sepharose were subjected to immunoblotting with an anti-PINK1 antibody. (c), (d) Eluates from glutathione sepharose were subjected to Oriole-staining in the absence (c) or presence (d) of CCCP. Although the yields are not optimal, the portion of the gels corresponding to PINK1-GST, which were used for MS analysis, are shown in red.



Supplementary Figure S3. Ser residues comprise a small patch on the surface of PINK1 structural model.

Ser228, Ser229, Ser230, Ser401 and Ser402 are spatially very close and localize to the surface of PINK1 and cluster together in a small patch. Ser and Thr residues are shown in yellow and gold, respectively, with their respective hydroxyl groups highlighted in red.

Supplementary Table S1. List of plasmids used.

vector	description	source
Plasmids for retrovirus-mediated expression		
pMXs-puro-PINK1 WT-3×Flag	For expression of WT PINK1	Ref. 6
pMXs-puro-PINK1 KD-3×Flag	For expression of KD PINK1	Ref. 6
pMXs-puro-PINK1-GST	For expression of PINK1-GST	This study
Plasmids for transient expression of pathogenic mutants of PINK1 (mainly used in Figs. 1 and 2)		
pCMV14-PINK1 WT-3×Flag	For expression of WT PINK1	Ref. 57
pCMV14-PINK1 KD-3×Flag	For expression of KD PINK1	Ref. 46
pCMV14-PINK1 C92F-3×Flag	For expression of A168P PINK1	This study
pCMV14-PINK1 A168P-3×Flag	For expression of A168P PINK1	This study
pCMV14-PINK1 E240K-3×Flag	For expression of E240K PINK1	This study
pCMV14-PINK1 H271Q-3×Flag	For expression of H271Q PINK1	Ref. 57
pCMV14-PINK1 G309D-3×Flag	For expression of G309D PINK1	Ref. 57
pCMV14-PINK1 L347P-3×Flag	For expression of L347P PINK1	Ref. 46
pCMV14-PINK1 G386A -3×Flag	For expression of G386A PINK1	This study
pCMV14-PINK1 G409V-3×Flag	For expression of G409V PINK1	Ref. 46
pCMV14-PINK1 E417G-3×Flag	For expression of E417G PINK1	Ref. 57
pCMV14-PINK1 534insQ-3×Flag	For expression of 534insQ PINK1	This study
Plasmids for transient expression of various PINK1-GFPs (used in Fig. 2)		
pEGFP-N1-PINK1 WT-GFP	For expression of WT PINK1-GFP	This study
pEGFP-N1-PINK1 KD-GFP	For expression of KD PINK1-GFP	This study
Plasmids to determine the autophosphorylation site of PINK1 (mainly used in Figs. 3 and 4)		
pcDNA3.1-non tagged PINK1 WT	For transient expression of non-tagged WT PINK1	Ref. 58
pcDNA3.1-non tagged PINK1 S393A	For expression of S393A PINK1	This study
pcDNA3.1-non tagged PINK1 S401A	For expression of S401A PINK1	This study
pcDNA3.1-non tagged PINK1 S402A	For expression of S402A PINK1	This study
pcDNA3.1-non tagged PINK1 S(393, 401)A	For expression of S393A S401A PINK1	This study
pcDNA3.1-non tagged PINK1 S(393, 402)A	For expression of S393A S402A PINK1	This study
pcDNA3.1-non tagged PINK1 S(401, 402)A	For expression of S401A S402A PINK1	This study
pcDNA3.1-non tagged PINK1 S(393,401,402)A	For expression of S393A, S401A, S402A PINK1	This study
pcDNA3.1-non tagged PINK1 S167A	For expression of S167A PINK1	This study
pcDNA3.1-non tagged PINK1 S225A	For expression of S225A PINK1	This study
pcDNA3.1-non tagged PINK1 S228A	For expression of S228A PINK1	This study
pcDNA3.1-non tagged PINK1 S229A	For expression of S229A PINK1	This study

pcDNA3.1-non tagged PINK1 S230A	For expression of S230A PINK1	This study
pcDNA3.1-non tagged PINK1 S(225,402)A	For expression of S225A S402A PINK1	This study
pcDNA3.1-non tagged PINK1 S(228,402)A	For expression of S228A S402A PINK1	This study
pcDNA3.1-non tagged PINK1 S(229,402)A	For expression of S229A S402A PINK1	This study
pcDNA3.1-non tagged PINK1 S(230,402)A	For expression of S230A S402A PINK1	This study
Plasmids to check the significance of PINK1 autophosphorylation (used in Fig. 5)		
pCMVTNT-PINK1 WT-3HA	For strong expression of WT PINK1	This study
pCMV(d1)TNT-PINK1 WT-3HA	For weak expression of WT PINK1	This study
pCMV(d1)TNT-PINK1 S(228,402)A-3HA	For weak expression of S228A S402A PINK1	This study
pCMV(d1)TNT-PINK1 S(228,402)D-3HA	For weak expression of S228D S402D PINK1	This study
Plasmids to express GFP-Parkin		
pEGFP-C1-GFP-Parkin	For transient expression of GFP-Parkin	Ref. 6
pMXs-puro-GFP-Parkin	For retrovirus-mediated expression of GFP-Parkin	Ref. 6

WT and KD mean wild-type and kinase dead, respectively.

Supplementary References

57 Shiba, K. *et al.* Parkin stabilizes PINK1 through direct interaction. *Biochem Biophys Res Commun.* 383, 331-335, <http://dx.doi.org/10.1016/j.bbrc.2009.04.006> (2009).

58 Takatori, S. *et al.* Cytoplasmic localization and proteasomal degradation of N-terminally cleaved form of PINK1. *Neurosci Lett.* 430, 13-17, <http://dx.doi.org/10.1016/j.neulet.2007.10.019> (2008).