Jin et al., http://www.jcb.org/cgi/content/full/jcb.201008084/DC1

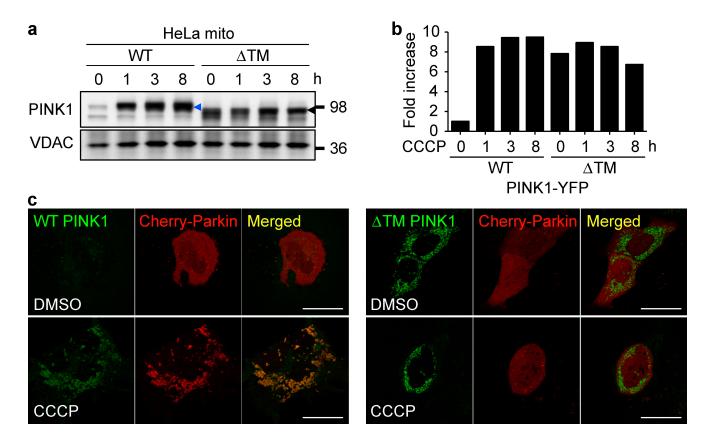


Figure S1. **Transmembrane domain deleted-PINK1 fails to recruit Parkin after mitochondrial depolarization.** (a) HeLa cells were transfected with WT (blue arrowhead) or Δ91–117 (ΔTM)-PINK1-YFP (black arrowhead) for 18 h and treated with 10 μM CCCP for different times as indicated. Cells were fractionated, and the mitochondrial fractions were immunoblotted for PINK1. VDAC was used as a mitochondrial marker. (b) The band intensity in each lane in panel a was densitometrically measured using Multi Gauge (Fujifilm). After correction for background, PINK1 band intensity in each lane was normalized to the loading control (VDAC) and calculated for fold increase. (c) PINK1 KO MEFs were transfected with mCherry-Parkin and either WT or Δ91–117-PINK1-YFP. After treatment with DMSO or 10 μM CCCP for 3 h, Parkin translocation was examined using confocal microscopy. Bars, 20 μm.

a Transmembrane domain FVVRAWGCAGPCGRAVFLAF-GLGLGLIEEKQAESRRAVSACQEIQAIFTQK-SKPGPDP 85 142 H.sapiens FVVRARGGACPCGRAVFLAF-GLGLGLIEEKQAEGRRAASACEEIQAIFTQK-NKLLPDP 88 145 B.taurus FMVRARGGAGPCGRAVFLAF-GLGLGLIEEKQAEGRRAASACQEIQAIFTQK-TKRVSDP 85 M.musculus 142 FVVRARGGAGPCGRAVFLAF-GLGLGLIEEKQAESRRAASACQEIQAIFTQK-NKQVSDP R.norvegicus 142 AFRRVIGGGS<mark>ARNRAVFLAF-GVGLGLIE</mark>QEQEEDRTSAALCQEIQAVFRKKKFQSLPKP 133 D.rerio 121 LRQRATRKLFFGDSAPFFALIGVSLASGSGVLSKEDELEGVCWEIREAASRLQNAWNHDE 180 D.melanogaster * *:*: *:.*. b Human PINK1 Drosophila PINK1 2 1 1 Hydrophobisity value Hydrophobisity value 0 0 -1 -2 -3 -2 -4 Upper cutoff -3 Lower cutoff -5 hydrophobicity 300 0 100 200 300 400 500 0 100 200 400 500 600 700 start position of window in sequence start position of window in sequence

Figure S2. Protein sequence alignment of the predicted transmembrane domain of PINK1 from various species. (a) Amino acid sequences of the predicted transmembrane domain of PINK1 from the indicated species were aligned using the ClustalW algorithm (http://www.uniprot.org). The putative transmembrane domains are indicated with a red box. "*", fully conserved; ":", strongly conserved; ".", weakly conserved residue. (b) Hydropathy plots for identifying the putative transmembrane regions were created using the Density Alignment Surface program (Cserzö et al., 1997). Sequences of FL PINK1 proteins (human and *Drosophila*) were used for the analyses.

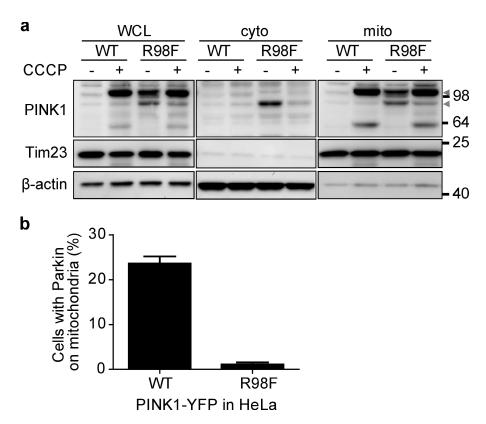


Figure S3. The R98F PINK1-YFP mutant accumulates in mitochondria without mitochondrial uncoupling but does not recruit Parkin. (a) WT or R98F mutant PINK1-YFP were transfected into HeLa cells and incubated with DMSO or 10 μM CCCP for 3 h. Cells were fractionated to mitochondria enriched and cytosolic fractions. Whole cell lysates (WCL), mitochondrial, and cytosolic fractions were analyzed for the level of expressed PINK1 with immunoblotting. (top middle) A fraction of the 52-kD form of ectopic PINK1 was found in the cytosolic fraction and might be the artifact of overexpression (see Fig. 2 a for endogenous 52-kD PINK1). Top arrowhead, FL and ΔMTS-PINK1; bottom arrowhead, 52-kD PINK1. β-Actin and Tim23 are loading controls. (b) HeLa cells were transfected with WT or R98F mutant PINK1-YFP together with mCherry-Parkin. After 1-h incubation with DMSO or 10 μM CCCP, cells (≥150/condition) were counted for mitochondrial translocation of Parkin. Counting results were represented as mean ± SEM from four replicates.

Reference

Cserzö, M., E. Wallin, I. Simon, G. von Heijne, and A. Elofsson. 1997. Prediction of transmembrane alpha-helices in prokaryotic membrane proteins: the dense alignment surface method. *Protein Eng.* 10:673–676. doi:10.1093/protein/10.6.673