

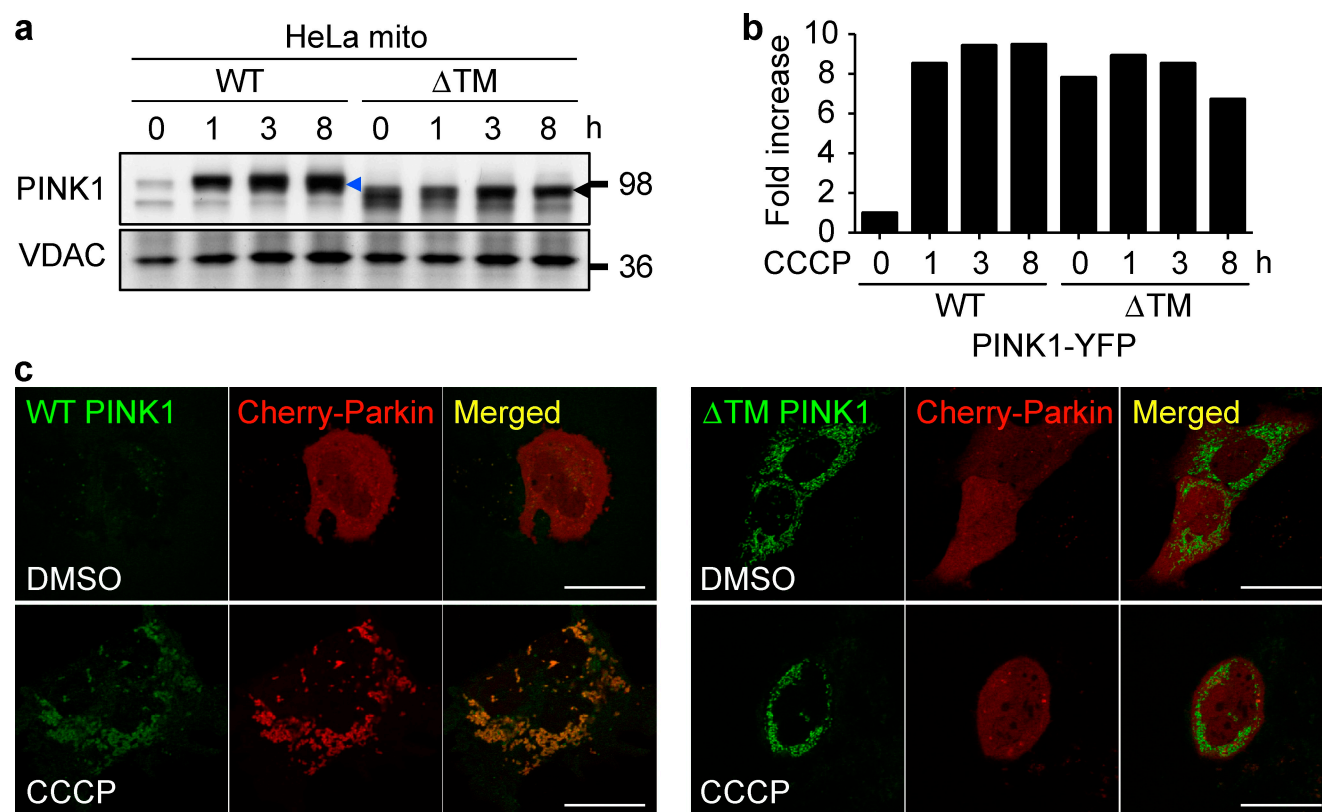
Jin et al., <http://www.jcb.org/cgi/content/full/jcb.201008084/DC1>

Figure S1. Transmembrane domain deleted-PINK1 fails to recruit Parkin after mitochondrial depolarization. (a) HeLa cells were transfected with WT (blue arrowhead) or Δ 91–117 (Δ TM)-PINK1-YFP (black arrowhead) for 18 h and treated with 10 μ M 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 Δ 91–117-PINK1-YFP. After treatment with DMSO or 10 μ M CCCP for 3 h, Parkin translocation was examined using confocal microscopy. Bars, 20 μ m.

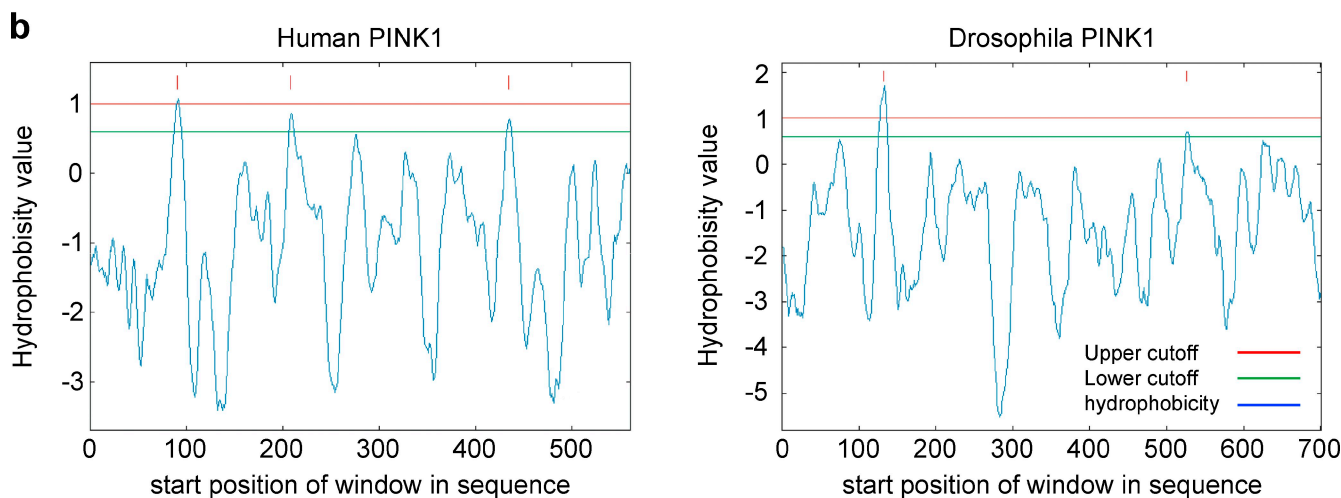


Figure S2. **Protein sequence alignment of the predicted transmembrane domain of PINK1 from various species.** (a) Amino acid sequences of the predicted transmembrane domain of PINK1 from the indicated species were aligned using the ClustalW algorithm (<http://www.uniprot.org>). The putative transmembrane 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 (Cserző et al., 1997). Sequences of FL PINK1 proteins (human and *Drosophila*) were used for the analyses.

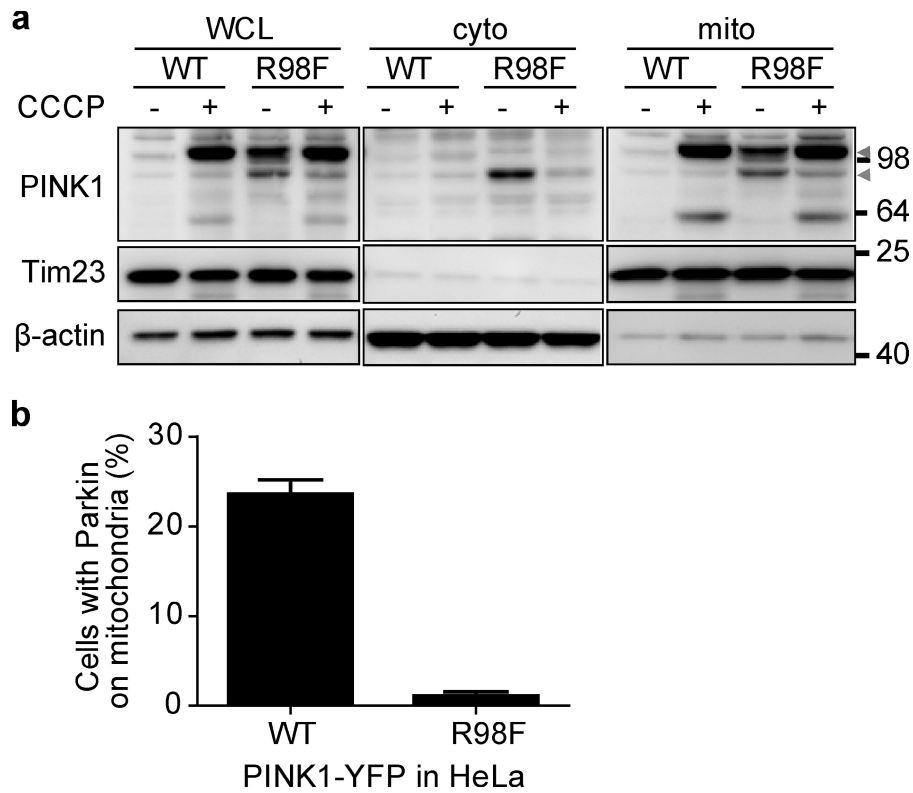


Figure S3. **The R98F PINK1-YFP mutant accumulates in mitochondria without mitochondrial uncoupling but does not recruit Parkin.** (a) WT or R98F mutant PINK1-YFP were transfected into HeLa cells and incubated with DMSO or 10 μ M CCCP for 3 h. Cells were fractionated to mitochondria enriched and cytosolic 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 Δ MTS-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 μ M CCCP, cells (≥ 150 /condition) were counted for mitochondrial translocation of Parkin. Counting results were represented as mean \pm 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