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online data

SUPPLEMENTARY ONLINE DATA

Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser^{65}

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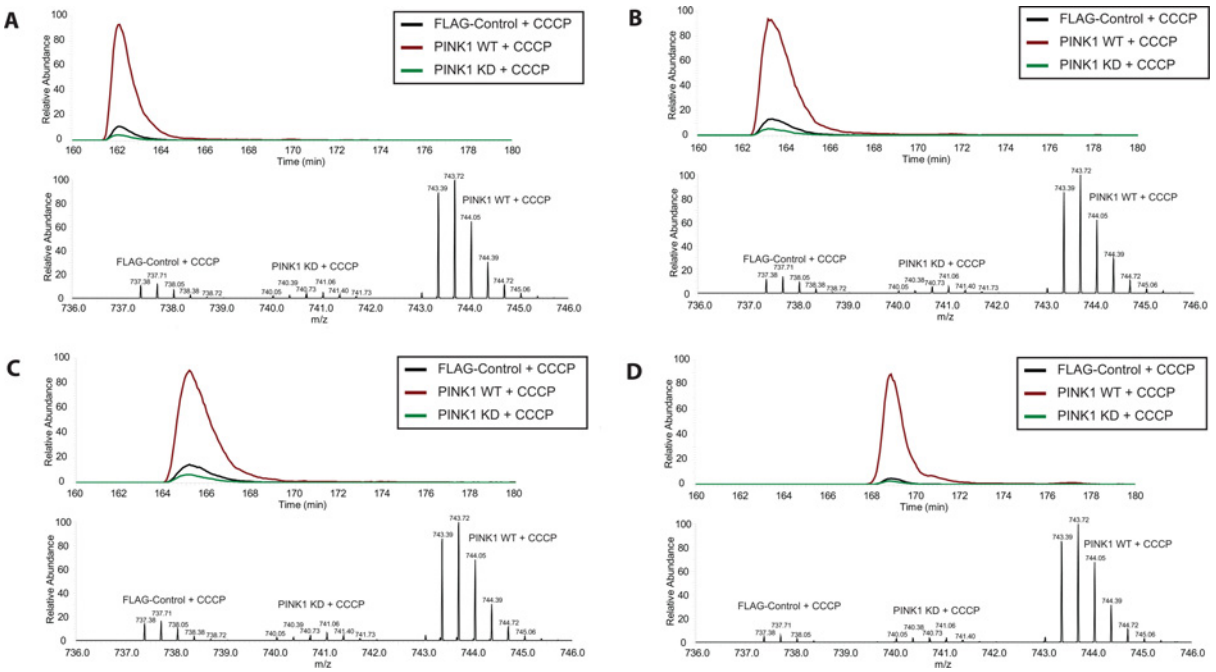

Figure S1 Reproducible phosphorylation of ubiquitin upon PINK1 stimulation by CCCP *in vivo*

Flp-In T-Rex HEK-293 cells stably expressing FLAG-empty, wild-type PINK1–FLAG or kinase-inactive PINK1–FLAG were grown in light, heavy and medium SILAC media respectively. Cells in each condition were stimulated with 10 μM CCCP for 3 h. Subsequently, membrane fractions were enriched by ultracentrifugation and solubilized in 1 % RapiGest. Lysates from each of the three conditions were mixed at 1:1:1 and digested with trypsin before phosphopeptide enrichment by HILIC and TiO_2, and analysis by MS. Data analysis was performed using MaxQuant. (**A**–**D**) Extracted ion chromatograms representing the ubiquitin Ser^{65} phosphopeptide TLSDYNIQKEpSTLHLVLR in the three SILAC-labelled conditions over all four biological replicates. There is a ~14-fold increase in abundance in cells transfected in the wild-type PINK1–FLAG compared with the kinase-inactive PINK1–FLAG.

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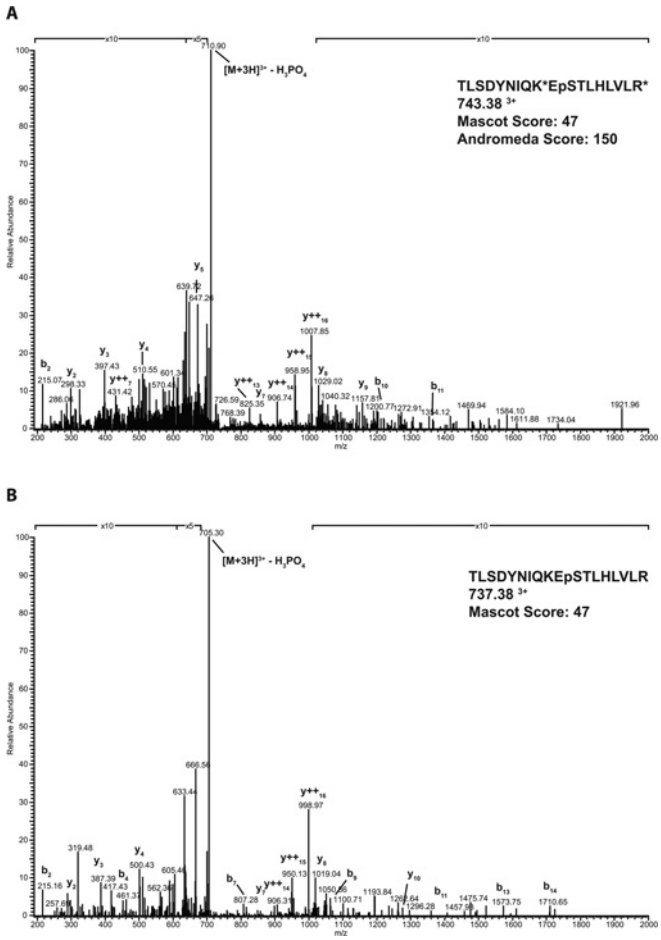

Figure S2 MS/MS fragmentation spectra of the phosphorylated ubiquitin peptide TLSDYNIQKEpSTLHLVLR

(**A**) MS/MS spectrum of the heavy (R10K8)-labelled peptide TLSDYNIQKEpSTLHLVLR identified in wild-type PINK1–FLAG-transfected cells. (**B**) Virtually identical MS/MS spectrum of TLSDYNIQKEpSTLHLVLR from a digest of ubiquitin *in vitro* phosphorylated by TcPINK1. Note that the doubly charged ion $[1105.5650]^{2+}$ of the same peptide was also positively identified in both experiments with Andromeda and Mascot Scores of 202 and 54 respectively.

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