Supplemental Information

Supplemental Figure1. The kinase activity of PINK1 is not required for BNIP3-PINK1 interaction

HEK293 cells expressing myc-BNIP3 and PINK1-flag variants, including wild-type and two kinase dead mutants (G309D and D384N) in various combination (indicated on the top of panels) followed by 20 μM CCCP treatment for 2 hours. Right panels: Lysates were immunoprecipitated with an anti-flag tag antibody (to precipitate PINK1) followed by immunoblotted with antibodies against BNIP3 or flag-tag (to detect PINK1). Left panels: Cell lysates were immunoblotted with an anti-BNIP3 antibody (BNIP3) or anti-PINK1 (PINK1) are shown as the input controls. β-tubulin is shown as loading controls.

Supplemental Figure 2. BNIP3 promotes mitochondria localization of PINK1

HeLa cells co-expressing PINK1-flag and DsRed-mito were transfected with either myc-tagged BNIP3 WT or ΔTM followed by immunostaining with antibodies against myc-tag (to detect BNIP3 WT or ΔTM, blue) and flag-tag (to detect PINK1, green). Mitochondria were labeled by expressing DsRed-mito (red). The cell nuclei were stained with DAPI (gray). Colocalization of BNIP3 variants, mitochondria and PINK1 is also shown (Merge). Cells treated with 20 μm CCCP for 2 hours was used as a positive control (top panel). Bar = 10 μm. Note that colocalization of mitochondria and PINK1 is remarkably enhanced in cells transfected with BNIP3 WT comparing to that in cells either transfected with control plasmid or expressing BNIP3 ΔTM.

Supplemental Figure 3. *BNIP3* prevents mitochondrial clustering in DA neurons caused by loss of *PINK1*

Whole-mount immunostaining of dorsolateral protocerebral posterior DA neurons in 5-day-old wild-type (WT) or $PINK1^{B9}$ (*PINK1* null) null adult flies expressing either

th-gal4 and mito-GFP (*TH*) or *th-gal4*, mito-GFP and BNIP3 (*TH>BINP3*) were immunotained with either an anti-GFP antibody (to detect mitochondria, mito-GFP, green) or an anti-TH antibody (to label DA neurons, red). Colocalization of mitochondria and DA neurons are shown in merged pictures (Merge). An amplified mitochondrial view is shown (right panels, Zoom). Bar=10 μm. Note that mitochondrial fragmentation is observed in neurons expressing BNIP3.

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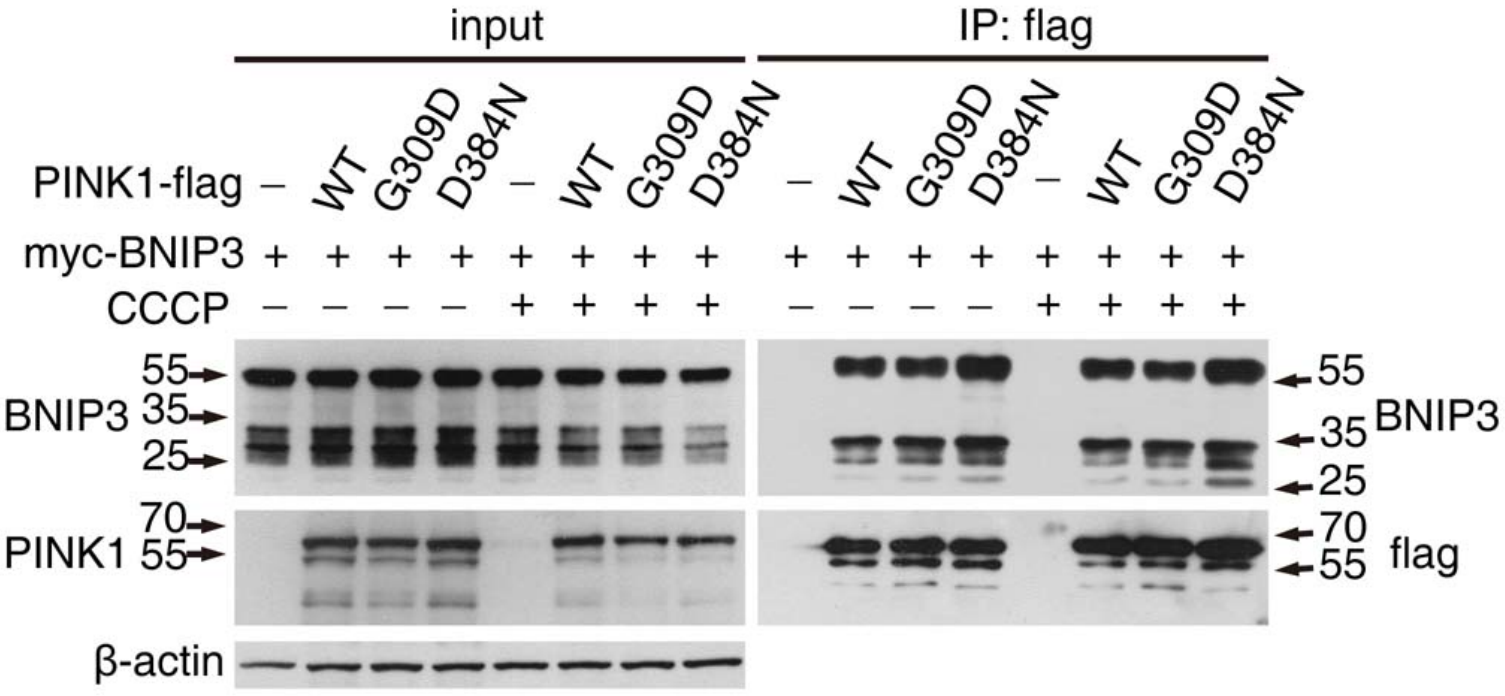

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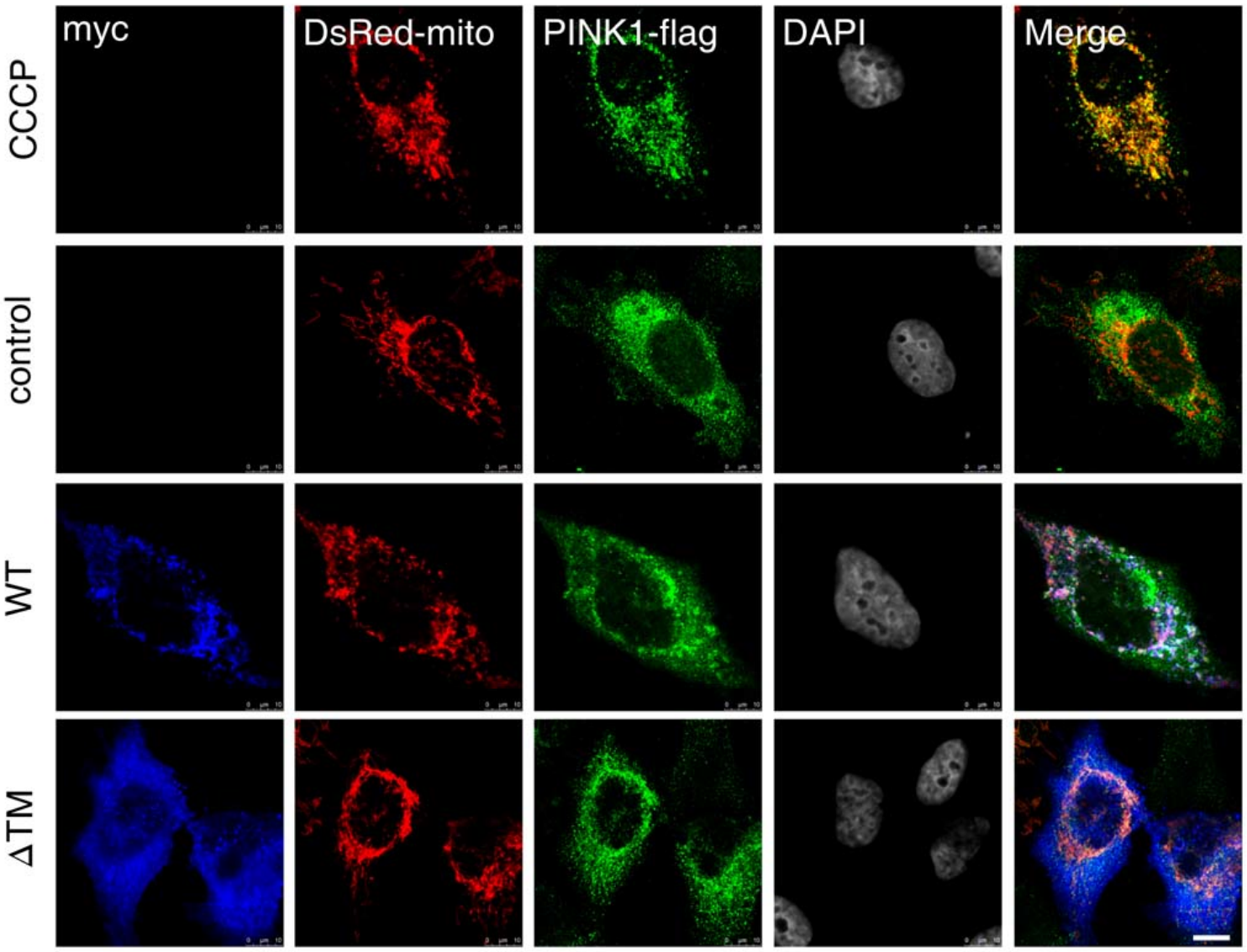

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