Supplementary Data

PINK1 Stimulates Interleukin-1β-mediated Inflammatory Signaling via the Positive Regulation of TRAF6 and TAK1

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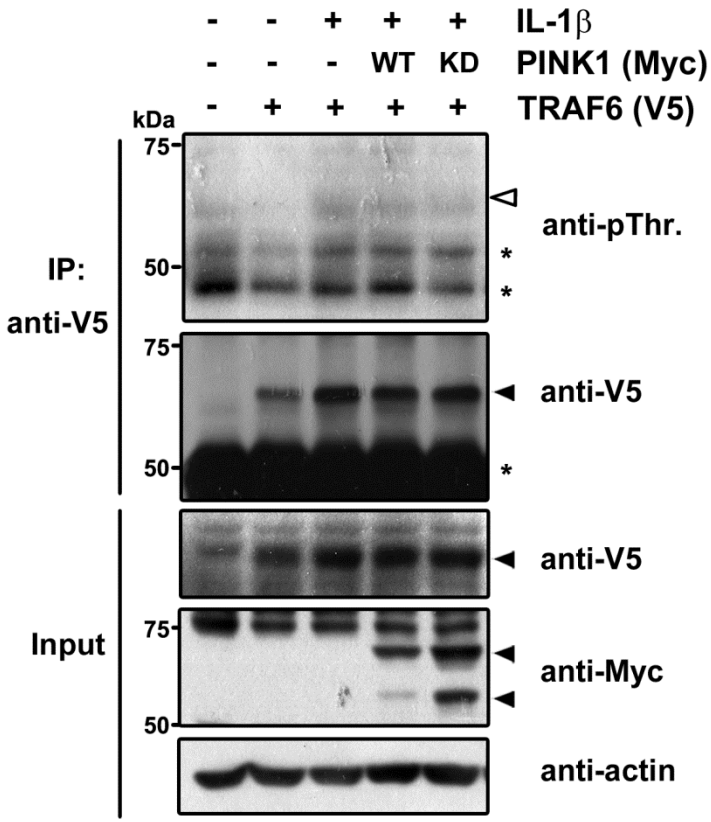
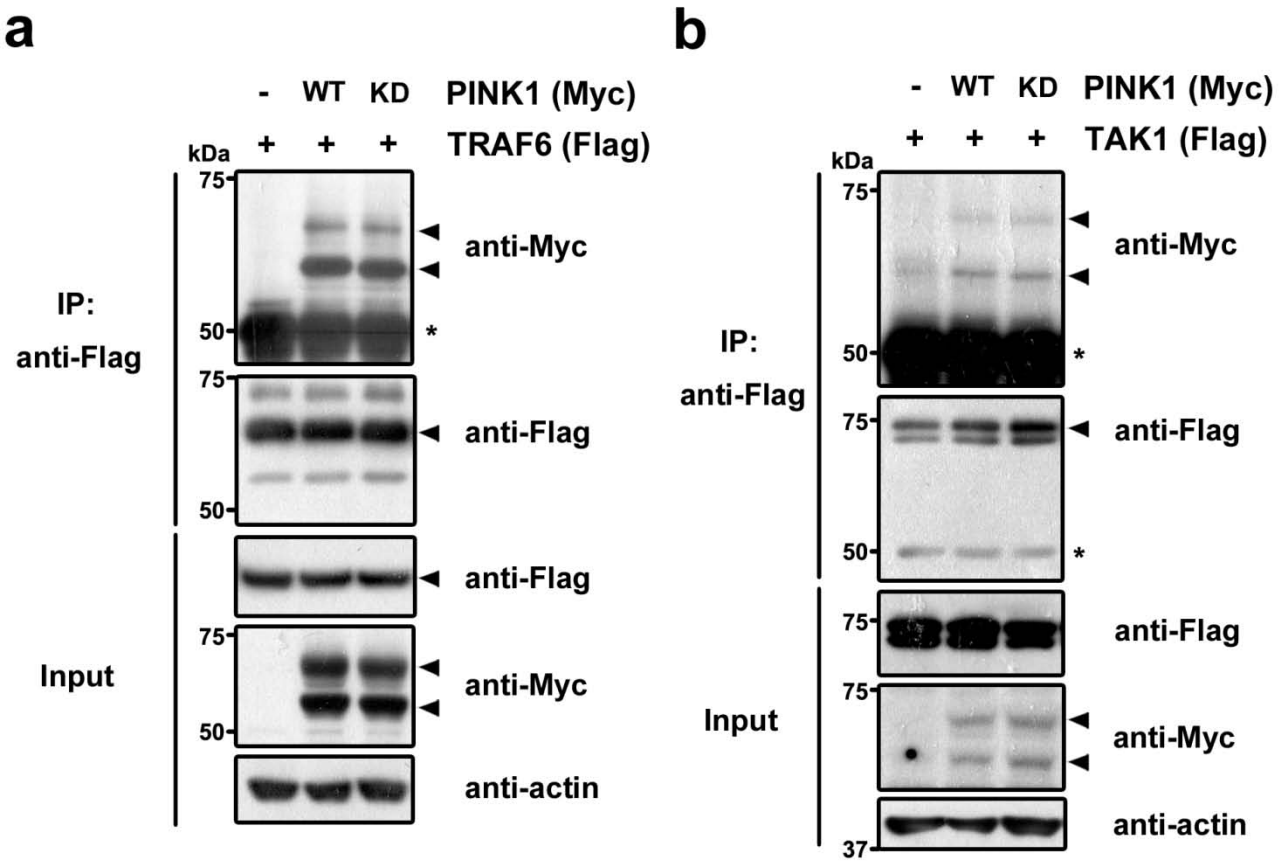
Supplementary Figures and Legends



Supple. S1: PINK1 has no effect on TRAF6 phosphorylation. Where specified, 293 IL-1RI cells were mock-transfected or transfected with V5-TRAF6 alone or together with either Myc-hPINK1-WT (WT) or Myc-hPINK1-KD (KD) for 42 h. After cells were then left untreated or treated with 10 ng/ml IL-1β for 15 min, cell lysates were immunoprecipitated with anti-V5 antiserum, followed by immunoblotting with anti-phospho-threonine antibody. The proper expression of transfected proteins in each cell lysate was confirmed by immunoblotting with the indicated antibodies. Asterisk and open arrow indicate IgG heavy chains and non-specific band, respectively, Actin served as a loading control.



Suppl. S2 Kinase-deficient PINK1 mutant still binds to TRAF6 and TAK1. a and b. Where indicated, 293 IL-1RI cells were mock-transfected or transfected with Flag-TRAF6 (a), -TAK1 (b) alone or together with Myc-hPINK1-WT or Myc-hPINK1-KD for 42 h and treated with 10 ng/ml IL-1β for 15 min. Cell lysates were immunoprecipitated with anti-Flag antiserum (a and b), followed by immunoblotting with anti-Myc antibody. The proper expression of transfected proteins in each cell lysate was confirmed by immunoblotting with the indicated antibodies. Asterisk indicates IgG heavy chains and actin served as a loading control.