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Supplemental Material to:

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PINK1 is degraded through the N-end rule pathway

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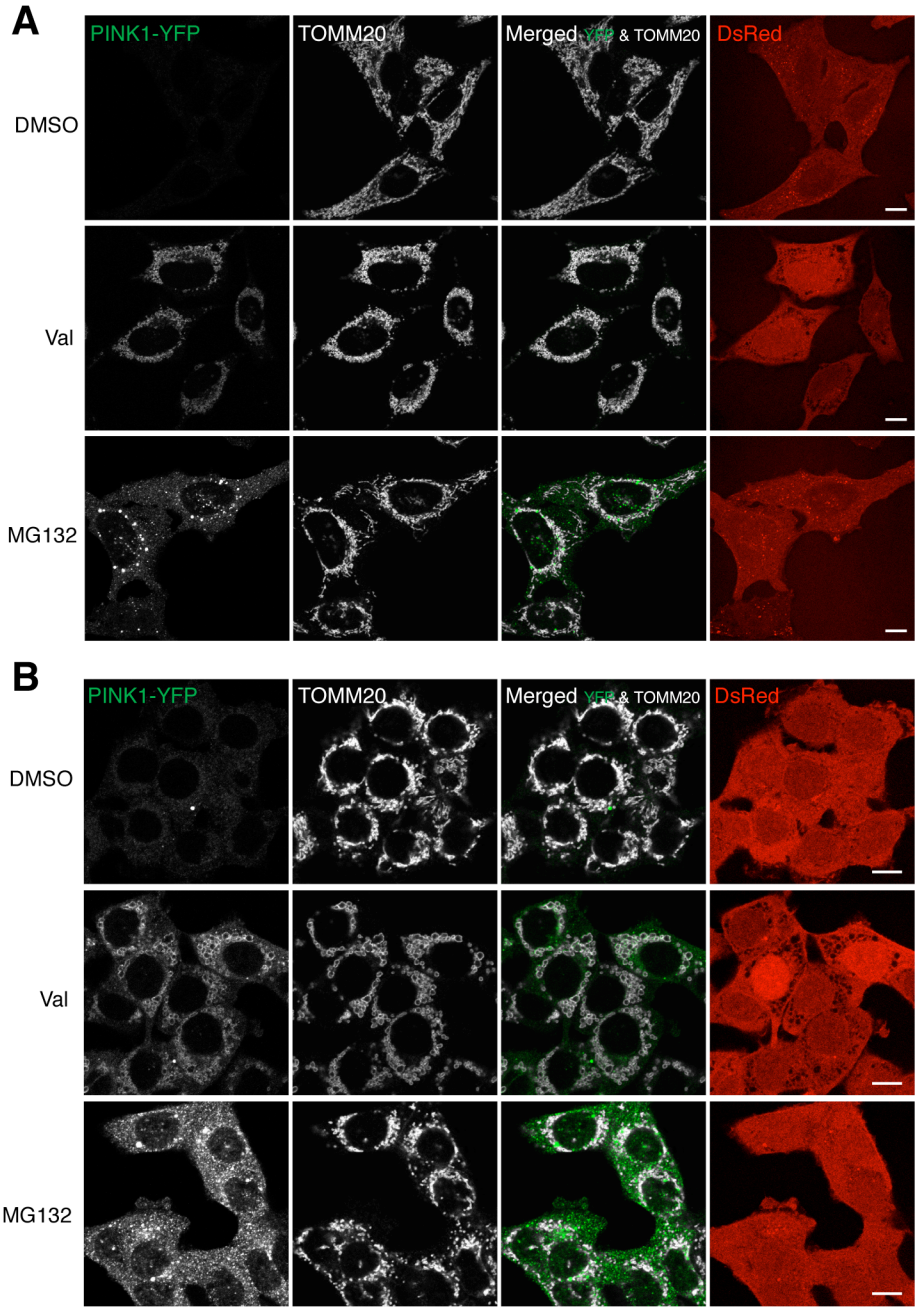

Figure S1. Cytosolic aggregates in HeLa or HCT116 cells stably expressing PINK1-YFP. HeLa (**A**) or HCT116 (**B**) cells stably expressing PINK1-YFP-IRES-DsRed treated with DMSO, valinomycin (Val), or MG132 (for 3 h for HeLa cells and for 4 h for HCT116 cells) were subjected to microscopic analysis. Cells were immunostained with anti-TOMM20. Bars, 10 μm. Of note, internal ribosome entry site (IRES) can direct coexpression of PINK1-YFP and cytosolic DsRed under a single promoter.

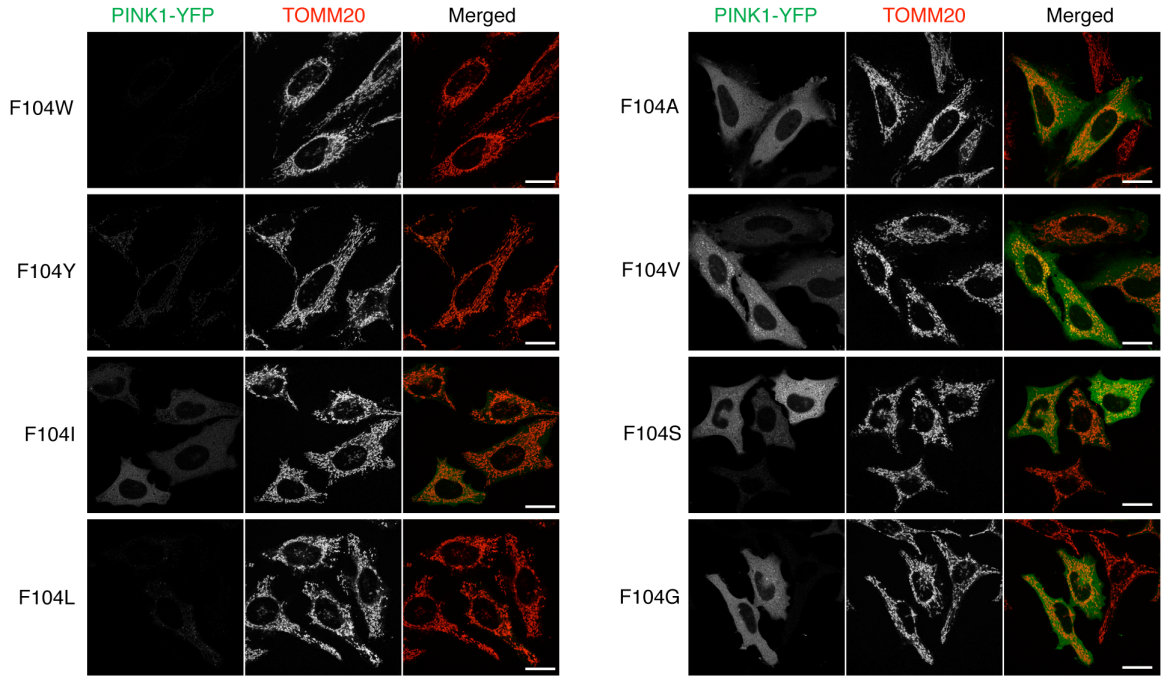

Figure S2. Expression level and localization of PINK1-YFP variants. Plasmids harboring PINK1^{F104X}-YFP (X= W, Y, I, L, A, V, S or G) were transfected into HeLa cells. Transfected cells immunostained with anti-TOMM20 and subjected to microscopic analysis. Bars, 20 μm.

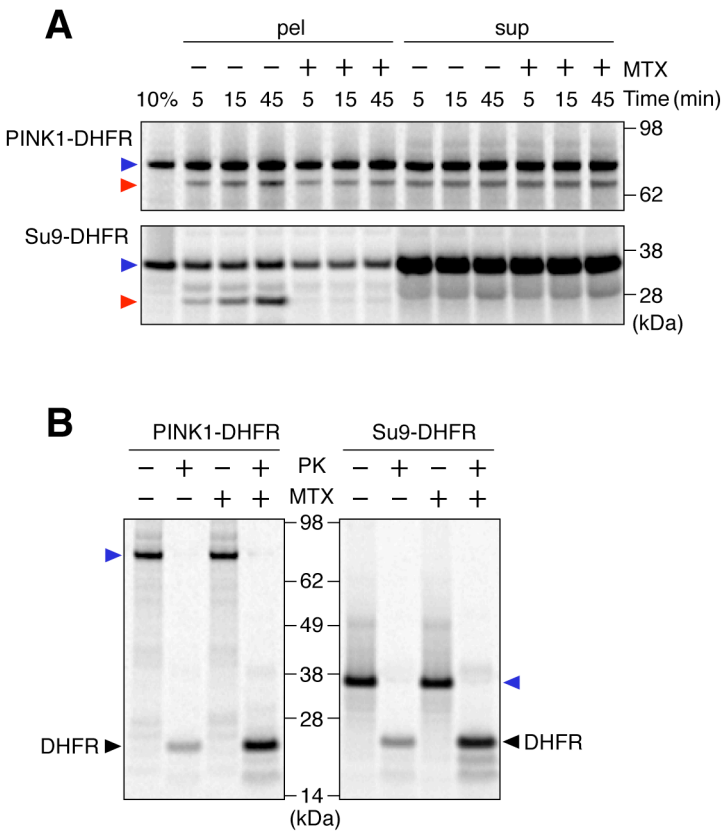

Figure S3. Retrotranslocation occurs without entire crossing of PINK1 to the outer membrane. (**A**) Radiolabelled PINK1-DHFR and Su9-DHFR were incubated with isolated mitochondria in the presence or absence of methotrexate (MTX) for the indicated times at 24°C. The mitochondrial pellet (pel) and supernatant (sup) fractions were separated by centrifugation and subjected to radioimaging. Blue and Red arrowheads represent full-length and cleaved forms. (**B**) Translation products of PINK1-DHFR or Su9-DHFR were treated with PK at 24°C in the presence or absence of MTX. The result indicates that, in the presence of MTX, the DHFR moieties become tightly folded.