Table S1. RT-PCR primer and conditions.

Primer Set	Sequences	Conditions
mPINK1	F: 5' GCTGATCGAGGAGAAGCAG 3' R: 5' GATAATCCTCCAGACGGAAGC 3'	95°C 15 min, [94°C 30 secs, 60°C 30 secs, 72°C 30 secs] 35 cycles.
hPINK1	F: 5' AGACGCTTGCAGGGCTTTC 3' R: 5' GGCAATGTAGGCATGGTGG 3'	95°C 15 min, [94°C 30 secs, 50°C 30 secs, 72°C 30 secs] 35 cycles.

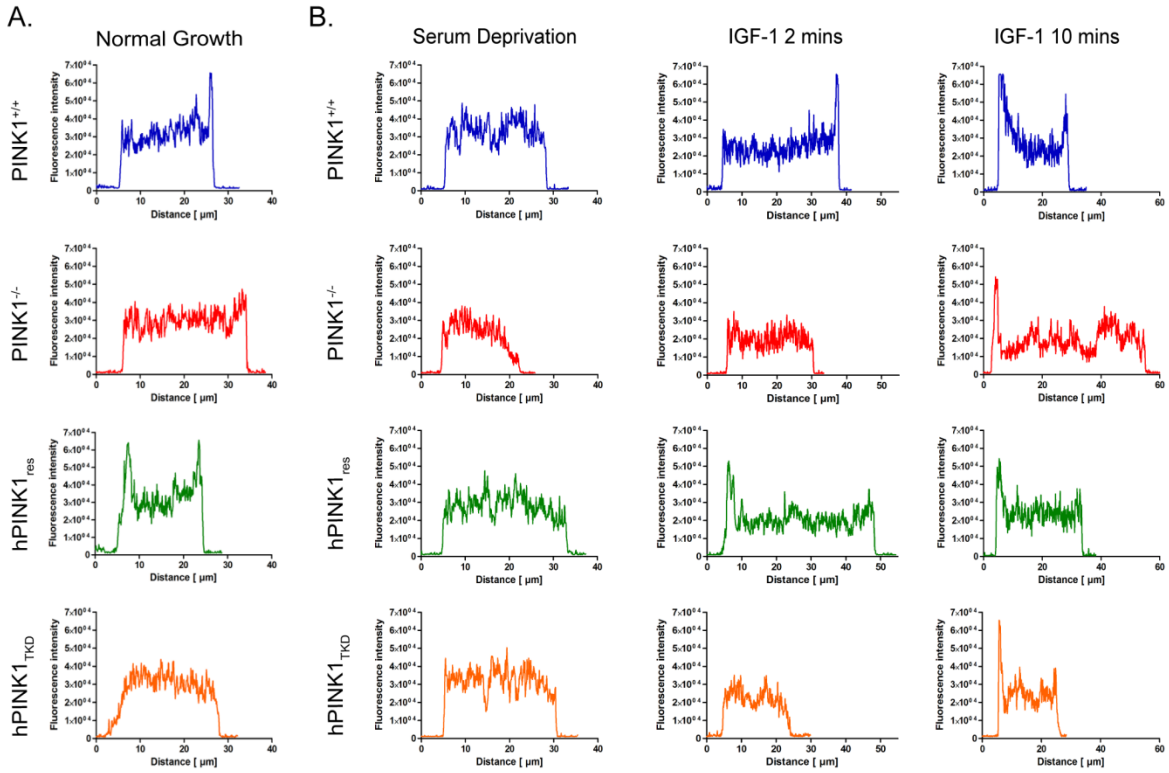

Figure S1. Increases in fluorescence intensity at the plasma membrane show that PINK1 decreases the time taken for Akt localisation to the plasma membrane in response to IGF-1 stimulation.

Representative line plots indicating the fluorescence intensity of GFP-Akt-PH along the white lines shown in Figure 2 (A) and 3 (A). Normal growth MEFs were grown in DMEM-Hi supplemented with 10% FBS. Serum-starved cells were stimulated with 10 ng/ml IGF-1 for the indicated times and subsequently stimulated with 10 ng/ml IGF-1. Scale bars 10 µm.

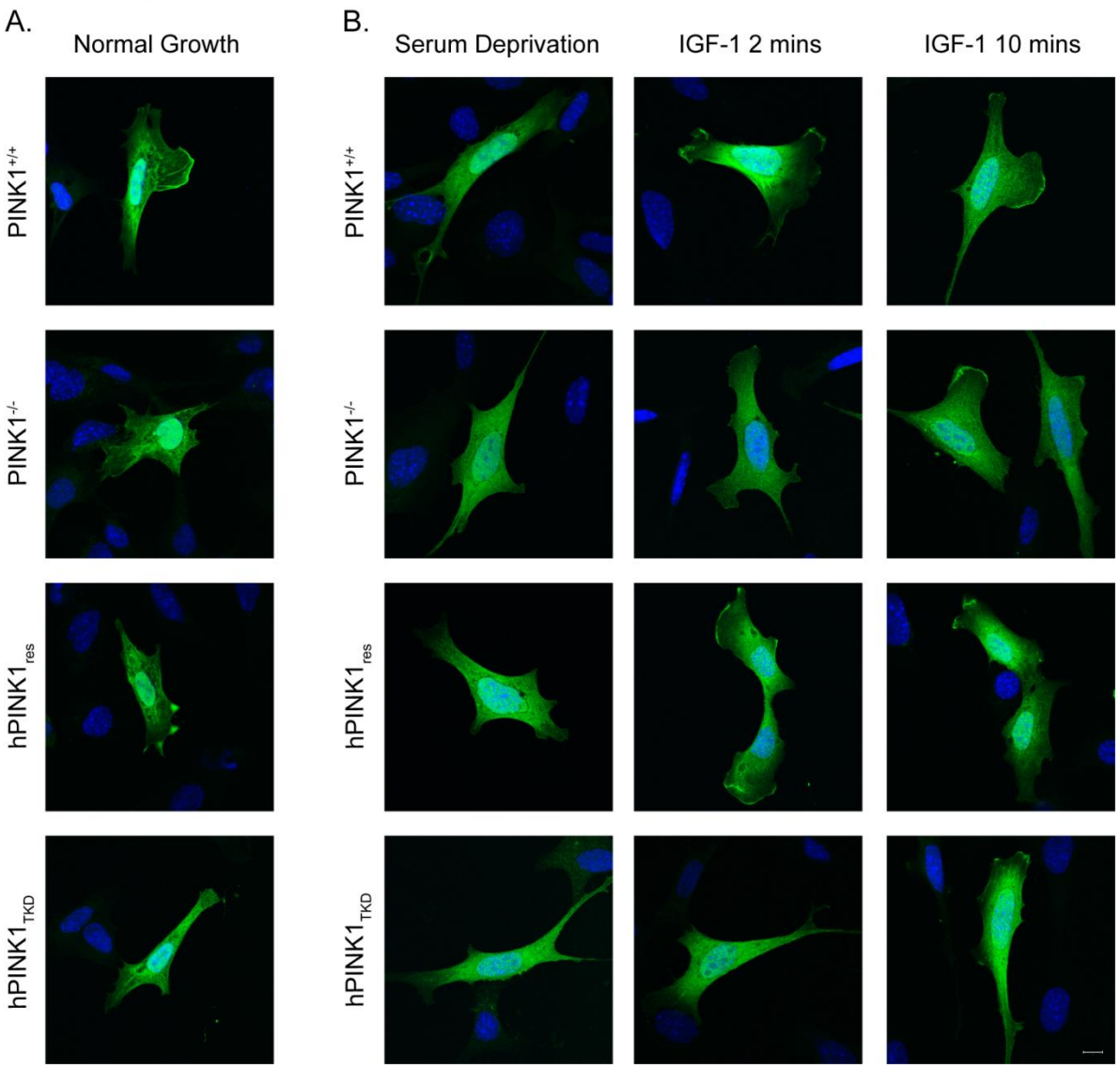

Figure S2. PINK1 regulates the localisation of Akt to the plasma membrane under normal growth conditions and decreases the time taken for Akt to get to the plasma membrane in response to IGF-1 stimulation.

A Representative confocal images showing GFP-Akt localisation in PINK1$^{+/+}$, PINK1$^{-/-}$, hPINK1$_{res}$ and hPINK1$_{TKD}$ MEFs, which were grown in DMEM-Hi supplemented with 10% FBS, transfected with GFP-Akt for 24h and stained with DAPI (blue). B Representative confocal images showing GFP-Akt localisation in PINK1$^{+/+}$, PINK1$^{-/-}$, hPINK1$_{res}$ and hPINK1$_{TKD}$ MEFs, following transfection with GFP-Akt for 24 h and stained with DAPI (blue). Serum-starved cells were stimulated with 10 ng/ml IGF-1 for the indicated times (n = 3). Scale bar 10 μm.

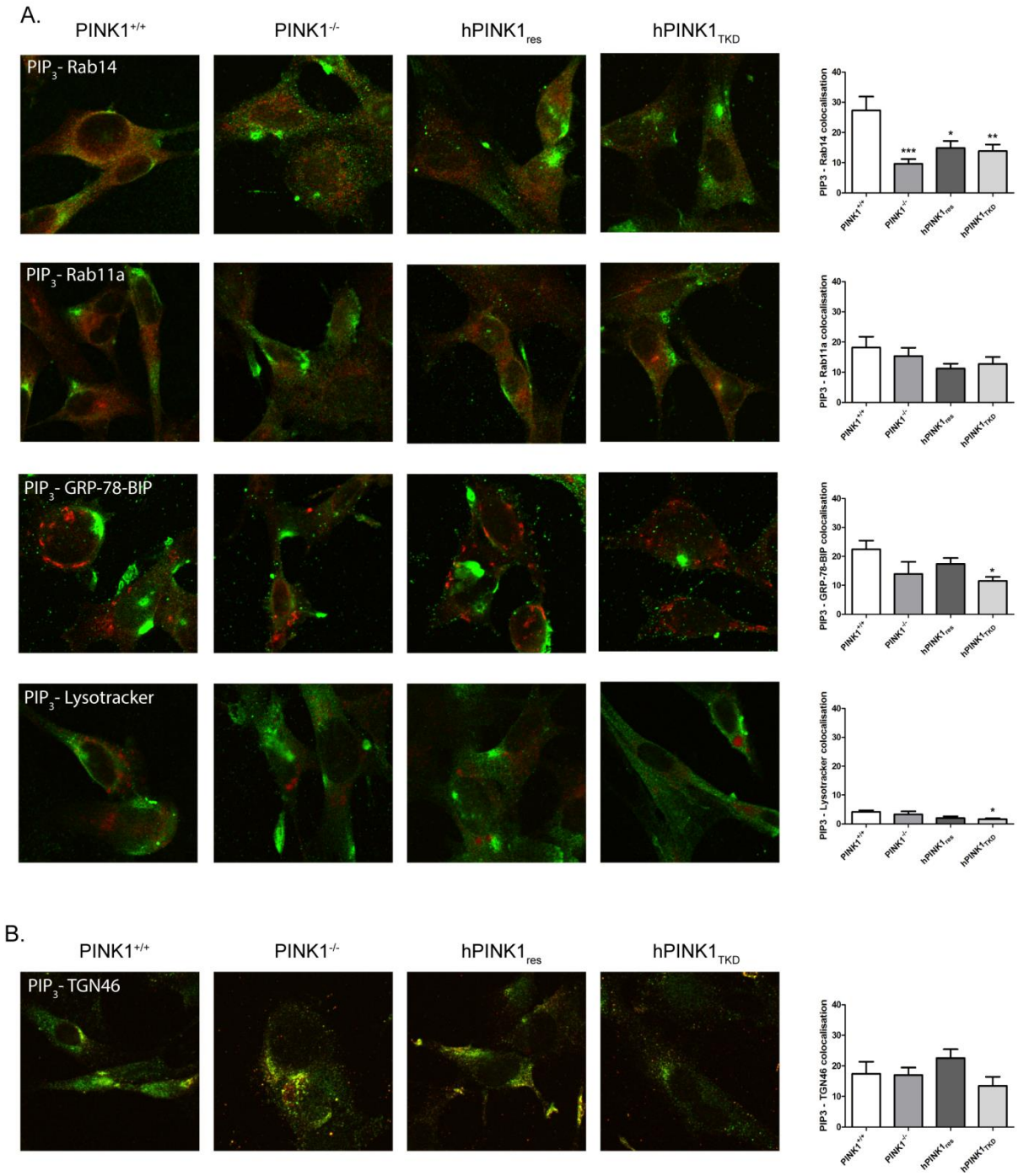

Figure S3. Colocalisation analysis using endomembrane markers Rab14, Rab11a, GRP-78-BIP and Lysotracker reveals selective localisation of PIP_3 to the medial-Golgi.

Representative confocal images showing PIP_3 colocalisation with endomembrane markers in $PINK1^{+/+}$, $PINK1^{-/-}$, $hPINK1_{res}$ and $hPINK1_{TKD}$ MEFs, which were grown in DMEM-Hi supplemented with 10% FBS. A, B Cells were immunostained for PIP_3 (green) and Rab 14, Rab 11a, GRP-78-BIP, Lysotracker and TGN46 (red). Scale bar 10 µm. Data information: In A, B, data are presented in corresponding graphs as mean ± SEM (n=3 for each). *=$p < 0.05$, **=$p < 0.01$, and ***=$p < 0.0001$ with respect to $PINK1^{+/+}$ MEFs (One-way ANOVA).

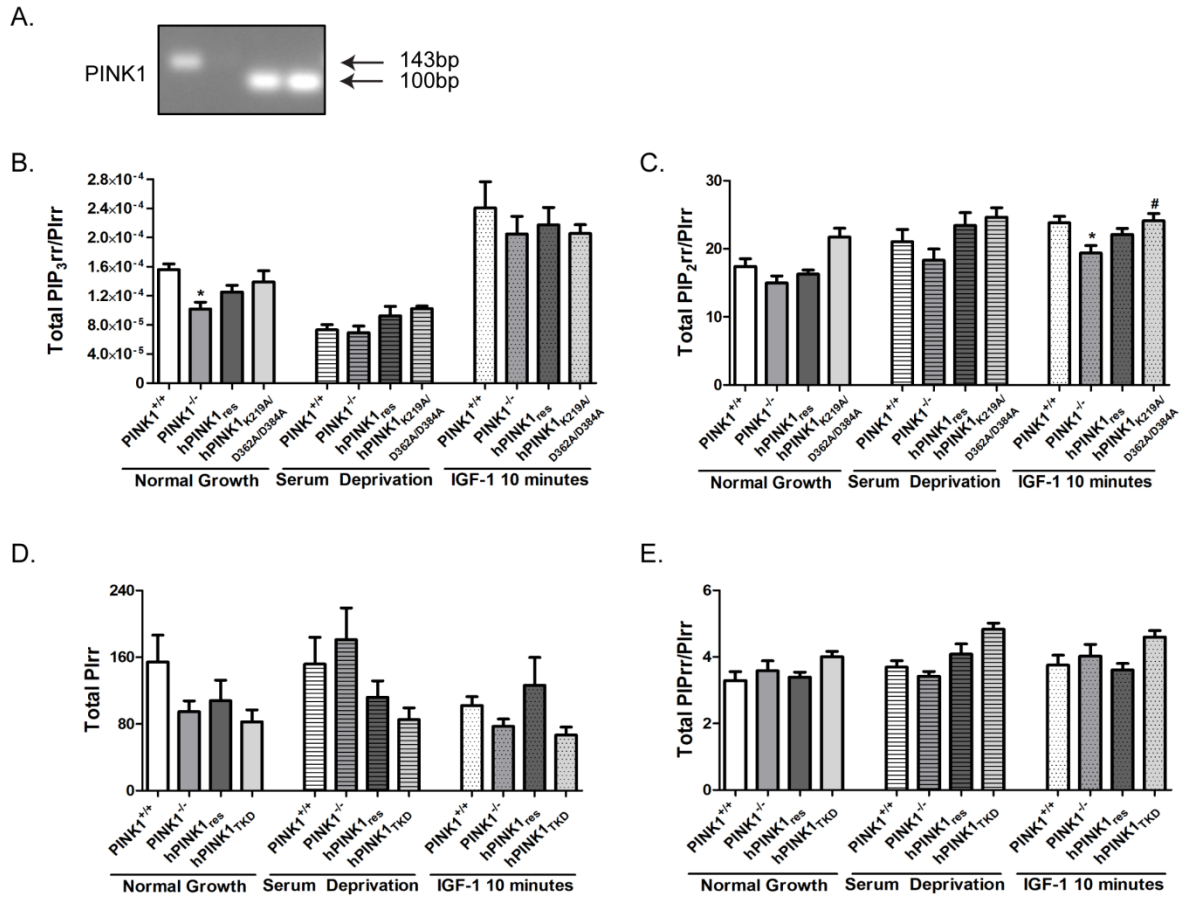

Figure S4. PINK1 modification in MEFs, PINK1 modulates PIP_3 and PIP_2 levels.

A. Agarose Gel showing PINK1 deletion and re-expression as confirmed by RNA extraction and RT-PCR analysis. Total PIP_3 (B), PIP_2 (C), PI (D), and PIP (E) levels as measured by mass spectrometry in $PINK1^{+/+}$, $PINK1^{-/-}$, $hPINK1_{res}$ and $hPINK1_{TKD}$ MEFs grown in DMEM-Hi supplemented with 10% FBS, serum deprived for 24h or stimulated with 10 ng/ml IGF-1 for 10 min (n = 3). Data information: In B-E, data are presented in corresponding graphs as mean ± SEM. *=$p < 0.05$ with respect to $PINK1^{+/+}$ MEFs, #=$p < 0.05$ with respect to $PINK1^{-/-}$ MEFs (Two-way ANOVA).