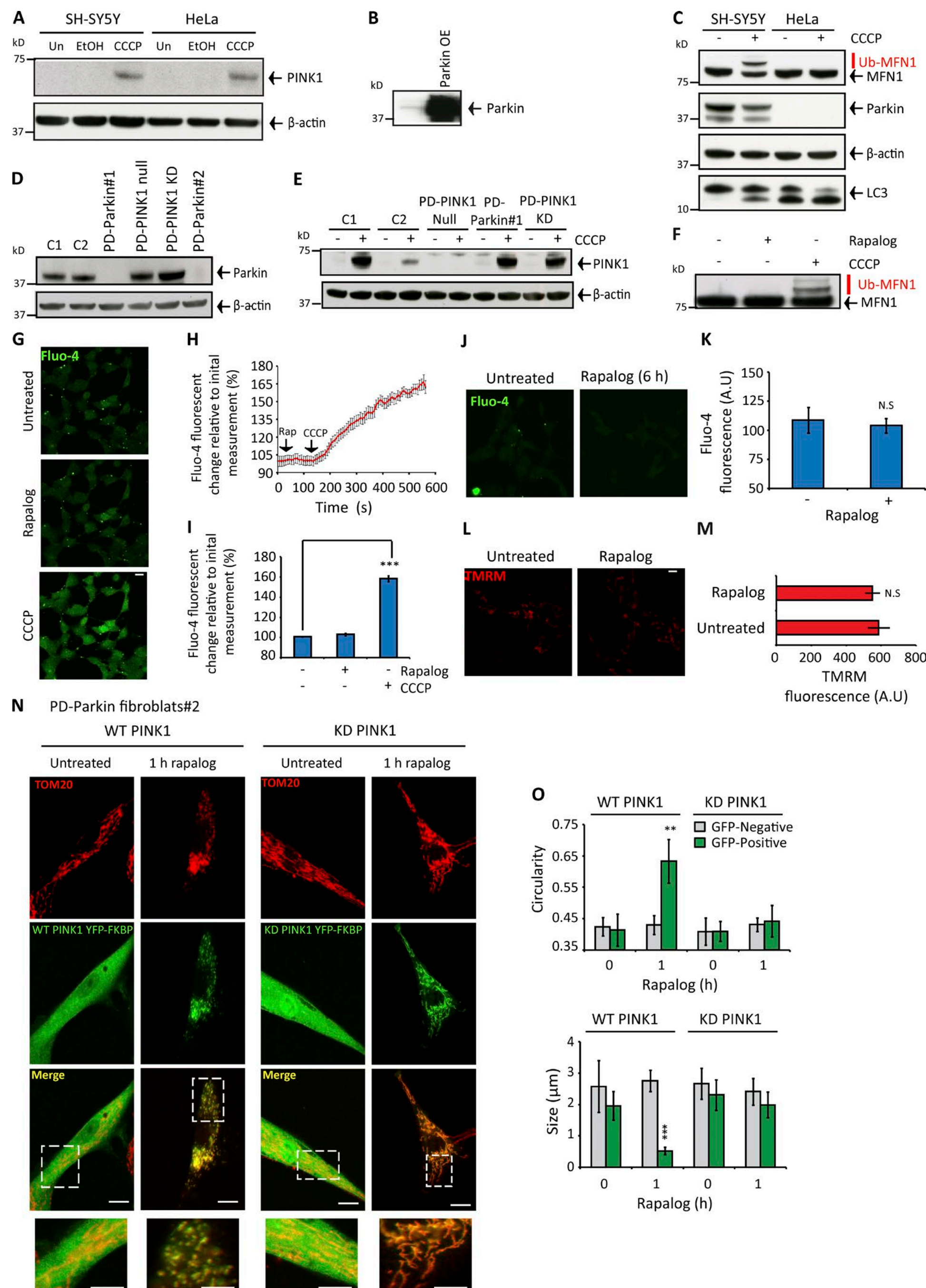

Figure S1. **Parkin and PINK1 expression in SH-SY5Y, HeLa and primary human fibroblasts, the effect of rapalog on cytosolic calcium levels and mitochondrial membrane potential, and targeting PINK1 to the OMM of Parkin-deficient fibroblasts induces robust mitochondrial fission.** (A) PINK1 protein in untreated (Un), ethanol (EtOH), and 18-h CCCP-treated SH-SY5Y and HeLa cells. (B) Parkin stable overexpression in SH-SY5Y cells. (C) MFN1 ubiquitylation and endogenous Parkin expression in untreated and 2-h CCCP-treated SH-SY5Y and HeLa cells. Formation of LC3-II is shown to confirm CCCP efficacy. (D and E) Parkin and PINK1 protein expression in fibroblast cultures from two control (C1 and C2), PD-PINK1 null, PD-PINK1 KD, and two PD-Parkin (#1 and #2). Indicated fibroblasts were 18-h CCCP treated to determine PINK1 expression. (F) Effect of 6-h rapalog treatment and 1-h CCCP treatment on MFN1 in SH-SY5Y cells. (G–K) Cytosolic calcium level (Fluo-4 fluorescence) in representative fields of SH-SY5Y cells after indicated rapalog or CCCP treatment and quantified as the mean maximum fluorescent change after treatment as percentage change from initial measurement, or in arbitrary fluorescence units. (L and M) Mitochondrial membrane potential in representative fields of SH-SY5Y cells determined by TMRM fluorescence intensity in arbitrary units, ±2-h rapalog treatment. (N and O) Mitochondrial morphology and quantification in PD-Parkin#2 fibroblasts transfected with WT or KD PINK1Δ110-YFP-FKBP/FRB-Fis1, ± 1-h rapalog treatment. All experiments were repeated at least three times, and error bars indicate SDM. **, $P < 0.01$; ***, $P < 0.001$. Bars: (G and L) 60 μM; (N) 20 μM; (insets) 10 μM. A.U., arbitrary units.

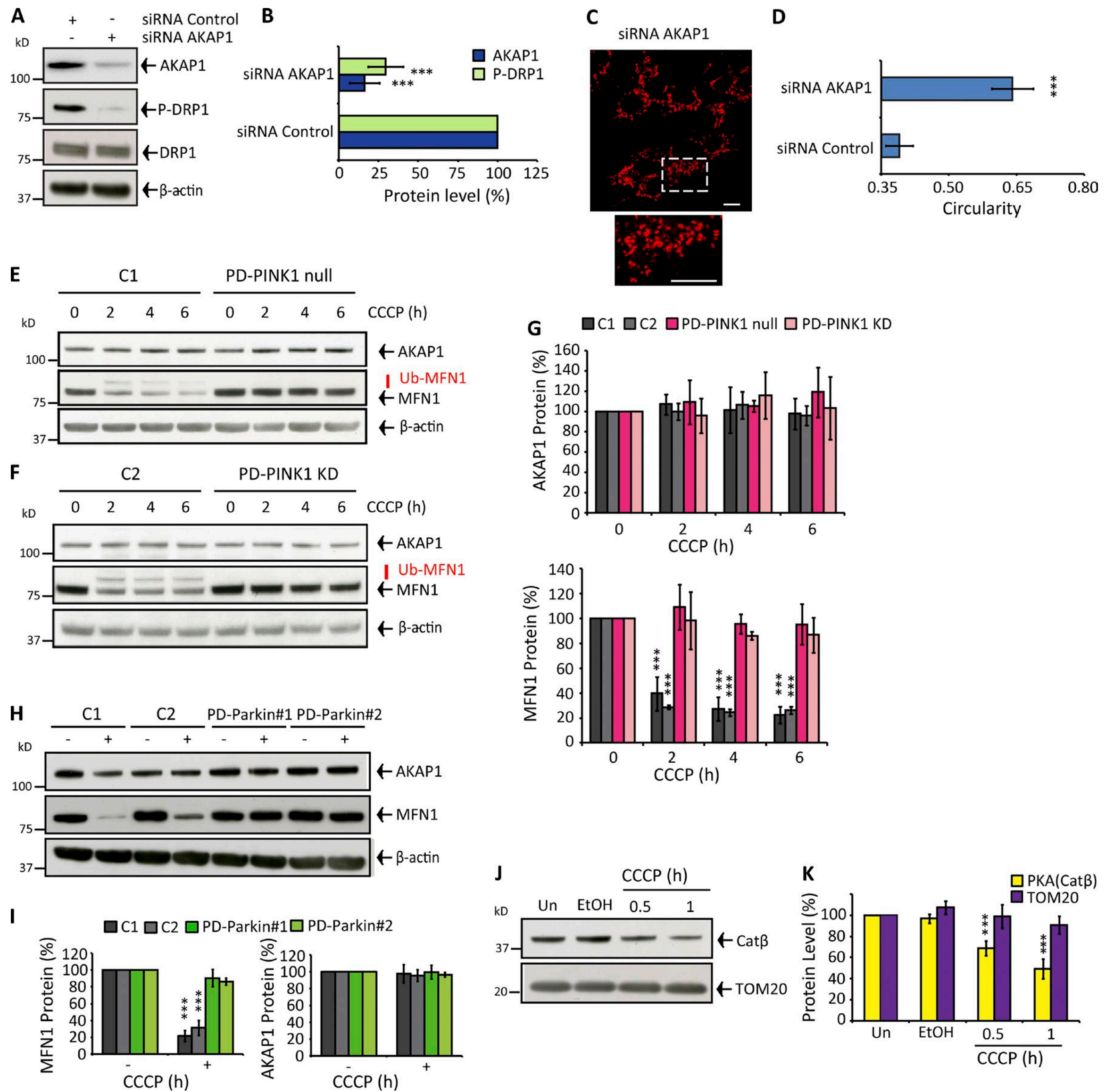

Figure S2. **Effect of AKAP1 silencing on mitochondrial fission and DRP1 phosphorylation, AKAP1 stability in depolarized cells, and retention in depolarized mitochondria.** (A–D) AKAP1 silencing in SH-SY5Y cells. DRP1 phospho–serine 637 abundance adjusted for DRP1. Mitochondrial morphology in AKAP1-silenced cells. (E–I) AKAP1 and MFN1 protein levels (corrected for β-actin) during CCCP treatments in C1, C2, PD-PINK1 null, PD-PINK1 KD, and PD-Parkin#1/#2 fibroblast cultures. (J and K) Catβ and TOM20 protein levels in mitochondria isolated from CCCP-treated SH-SY5Y cells. All experiments were repeated at least three times, and error bars indicate SDM. Bars: 20 µM; (insets) 10 µM. ***, $P < 0.001$.

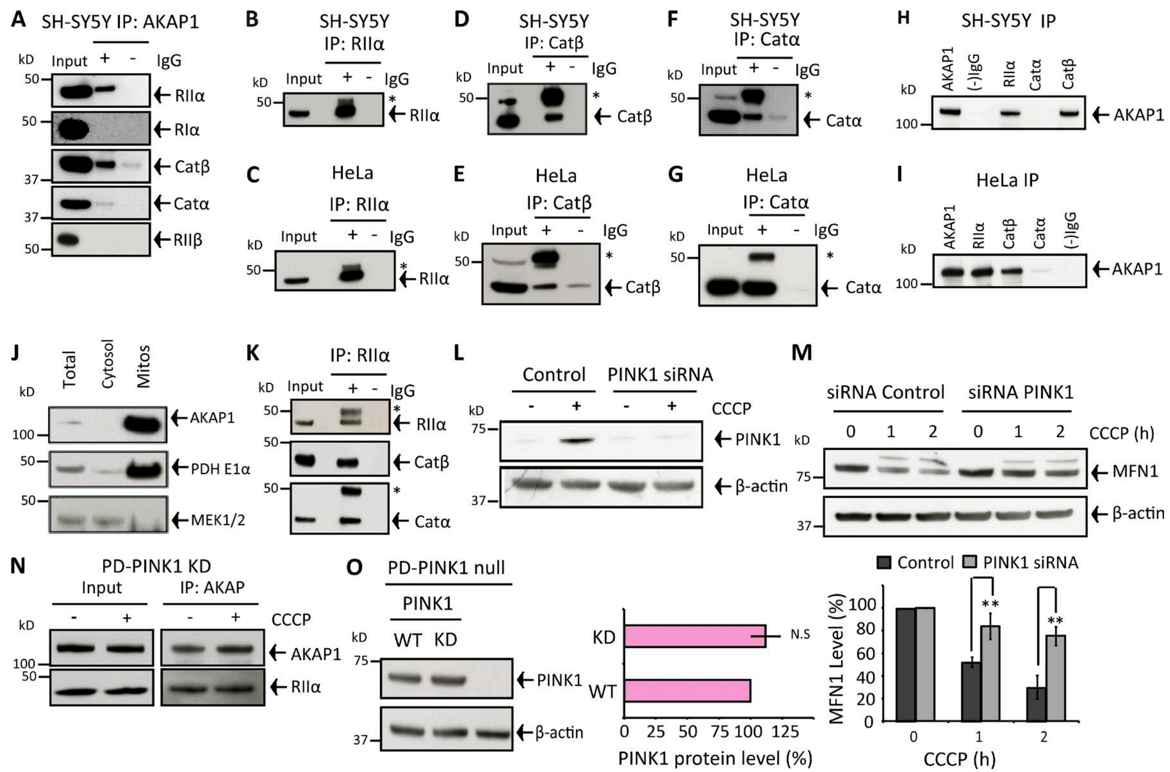

Figure S3. **AKAP1–PKA reverse precipitation in SH-SY5Y and HeLa cells, RIIα precipitation from cytosolic fractions, the efficacy of *pink1*-silencing in SH-SY5Y cells, and preservation of the AKAP1–PKA axis in CCCP-treated PINK1 KD fibroblasts.** (A) AKAP1 IP from untreated SH-SY5Y cells and immunoblots for RIα, RIIα, Catβ, Catα, or RIIβ. (B–I) PKA IPs from untreated SH-SY5Y or HeLa cells, and indicated PKA/AKAP1 immunoblots. IgG-negative controls are shown. *, IgG band. (J and K) RIIα IP from HeLa cell cytosolic fractions and indicated PKA immunoblots. Cytosolic fractionation demonstrated by AKAP1, PDH E1α, and MEK 1/2 immunoblots on samples from total, cytosolic, and mitochondrial (Mitos) fractions. (L and M) PINK1 protein in 18-h CCCP-treated SH-SY5Y cells ± 48-h control or *pink1* silencing. Non-ubiquitylated MFN1 levels were corrected for β-actin with or without 1- and 2-h CCCP treatment. (N) AKAP1-RIIα CoIP from PD-PINK1 KD fibroblasts ± 2-h CCCP treatment. RIIα levels were adjusted for AKAP1 and normalized to untreated samples. (O) PINK1 protein level (adjusted for β-actin) in WT or KD (K219M) PINK1 transfected PD-PINK1 null fibroblasts. All experiments were repeated at least three times, and error bars indicate SDM. **, $P < 0.01$.