Supplemental Figure Legends.

Supplemental Figure 1. (A) Confocal microscopy images of rat cortical neurons exhibiting co-transfection of mtDsRed2 and either Empty-Vector control plasmid or hu-Parkin expressing plasmid with staining for Parkin expression (PRK8 antibody) at 2wk post-transfection. (B) Co-expression of hu-Parkin and PINK1-GFP in Rat cortical neurons. (i) Panels exhibiting expression of DsRed2 with Parkin staining 24hrs after co-transfection of hu-Parkin and PINK1-GFP expressing plasmids. (ii) Panels exhibiting expression of DsRed2 with PINK1-GFP staining 24hrs after co-transfection of hu-Parkin and PINK1-GFP expressing plasmids. To enhance the GFP signal, cells were probed using an anti-GFP antibody, with AlexaFluor647 secondary. The presented images are of the AlexaFluor647 staining of GFP. (iii) GFP staining in neurons co-transfected with mtDsRed2 and Empty-Vector control plasmid, demonstrating no detection of GFP without the PINK1-GFP plasmid present. (C) Examples of parkin localization in neurons 24hr following transfection with hu-Parkin and PINK1-GFP plasmids. Cells were exposed to DMSO vehicle control for 1-6 hr. Cells represent (i) no parkin-mitochondria localization, (iI) moderate co-localization and (iii) excessive co-localization.

Supplemental Figure 2. Tetramethyl Rhodamine Methyl Ester (TMRM) analysis was used to determine that CCCP was sufficiently depolarizing cells using previously described methods (Ward, 2010) with minor modifications., DIV10 rat primarily cortical neurons and HeLa cells plated on glass coverslips were pre-incubated in 25nM or 20nM TMRM in media, respectively, for 10-30 min. Cells were then transferred to a glass-bottomed culture dish for imaging (MatTek). Cells were placed in an incubation media (MEM pH 7.4, supplemented with 2% glutamax, 20 mM HEPES, 33 mM glucose (or galactose, for galactose-cultured HeLa cells), 1mM Na-pyruvate) containing an equal concentration of TMRM. Living cells were imagined on an Olympus Fluoview FV1000, with TMRM visualized using a 568nm wavelength detection setting, 10μs/pixel. A baseline fluorescence was obtained, and then 1-fold media added containing a 2x concentration of either CCCP (10uM final concentration) or DMSO vehicle control. Live imaging was used to capture fluorescence loss, indicative of mitochondrial depolarization, over time.

1A and 1B represent sequential images of live-imaging TMRM fluorescence with either DMSO vehicle control (A) or 10uM CCCP(B) in neurons over the course of 20min. 1C and 1D

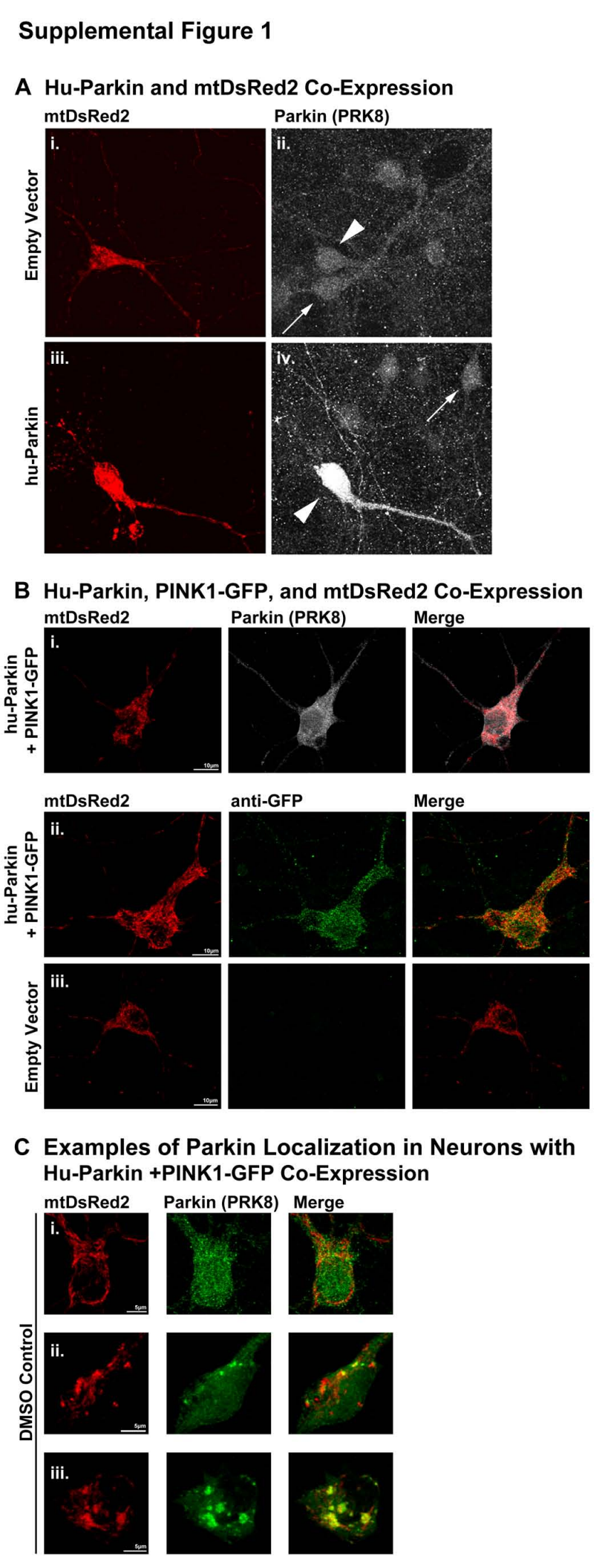
represent sequential images of live-imaging TMRM fluorescence with either DMSO vehicle control (A) or 10uM CCCP(B) in HeLa cells cultured in glucose-DMEM, imaged over 1hr. 1E and 1F represent sequential images of live-imaging TMRM fluorescence with either DMSO vehicle control (A) or 10uM CCCP(B) in HeLa cells cultured in glucose-DMEM, imaged over 2hr.

Supplemental Movie 1 – Time-lapse movie (30 frames/sec) of 20min live-cell TMRM fluorescence in DIV10 rat cortical neurons exposed to DMSO Vehicle Control. Images were taken every 10 sec for 20 min without adjustment of laser settings.

Supplemental Movie 2 – Time-lapse movie of 20min live-cell TMRM fluorescence in DIV10 rat cortical neurons exposed to 10μM CCCP. Images were taken every 10sec for 20min without adjustment of laser settings. Note the gradual loss of fluorescence over time, indicating CCCP-induced depolarization of the mitochondria.

Ward, M.W. (2010). Quantitative Analysis of Membrane Potentials. In *Live Cell Imaging*, Methods in Molecular Biology 591, D.B. Papkovsky, ed. (Humana Press), pp. 335-351.

Supplemental Figure 1



Supplemental Figure 2