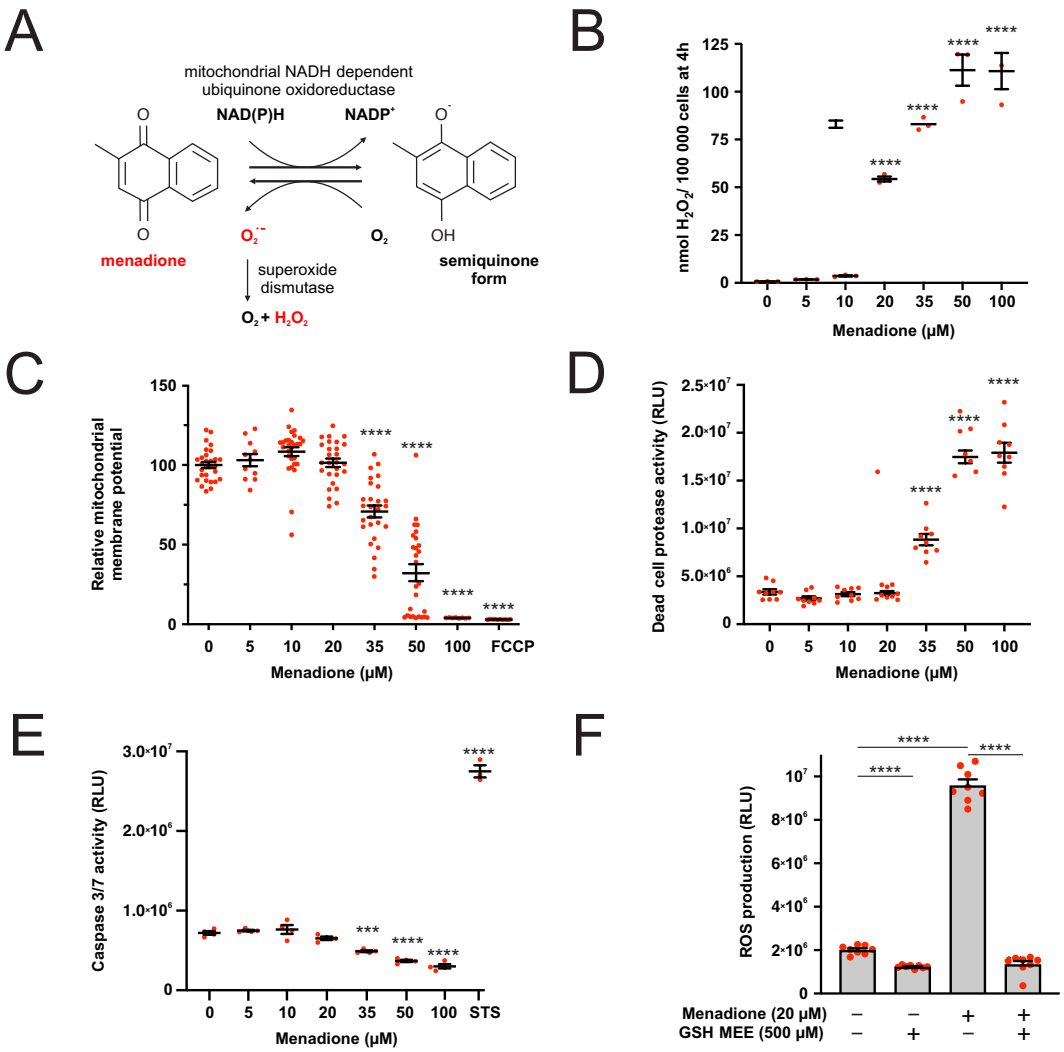

Figure S1. Menadione concentrations below 35 µM induce marked ROS production but are not toxic or apoptosis-triggering

A. Redox cycling of menadione catalyzed by mitochondrial Complex I leads to superoxide and H_2O_2 production.

B. Quantitative estimation of the dose-dependent H_2O_2 generation induced by menadione. PC6 cells were treated with menadione for 4h, and H_2O_2 released to the medium was measured using ROS-Glo™ H_2O_2 assay kit. H_2O_2 concentrations were calculated from the standard curve obtained by measuring the luminescence in the presence of different concentrations of H_2O_2. We also generated a standard curve to show exactly how much hydrogen peroxide cells produce and secrete to the media after menadione treatment ***P < 0.001 and ****P < 0.0001 compared to control group, n = 8-10 wells, Welch's ANOVA followed by Dunnett's T3 multiple comparison test.

C. Up to 20 µM concentrations of menadione do not depolarize mitochondria. PC6 cells were treated with different concentrations of menadione for 4h, after which the relative mitochondrial membrane potential was measured using JC10 staining. Treatment with 10 µM FCCP was used as a positive control. ****P < 0.0001 compared to control group, n = 8-29 wells, Welch's ANOVA followed by Dunnett's T3 multiple comparison test.

D. Up to 20 µM concentrations of menadione do not compromise cell viability. PC6 cells were treated with menadione for 4h, after which the dead-cell protease activity was measured using CytoTox-

Glo™ Cytotoxicity Assay. ****P < 0.0001 compared to control group, n = 8-9 wells, Welch's ANOVA followed by Dunnett's T3 multiple comparison test.

E. Menadione does not provoke apoptosis under our experimental conditions. PC6 cells were treated with menadione for 4h, after which the caspase 3/7 activity was measured using Caspase-Glo® 3/7 Assay System. Ten μM staurosporin (STS) treatment was used as a positive control. ***P < 0.001 ****P < 0.0001 compared to untreated group, n = 4 wells, Ordinary one-way ANOVA followed by Dunnett's T3 multiple comparison test.

F. Menadione treatment (20 μM for 4h) induced ROS generation is abolished in the presence of 500 μM GSH-MEE in PC6 cells. ****P < 0.0001, n = 8 wells, Welch's ANOVA followed by Dunnett's T3 multiple comparison test.

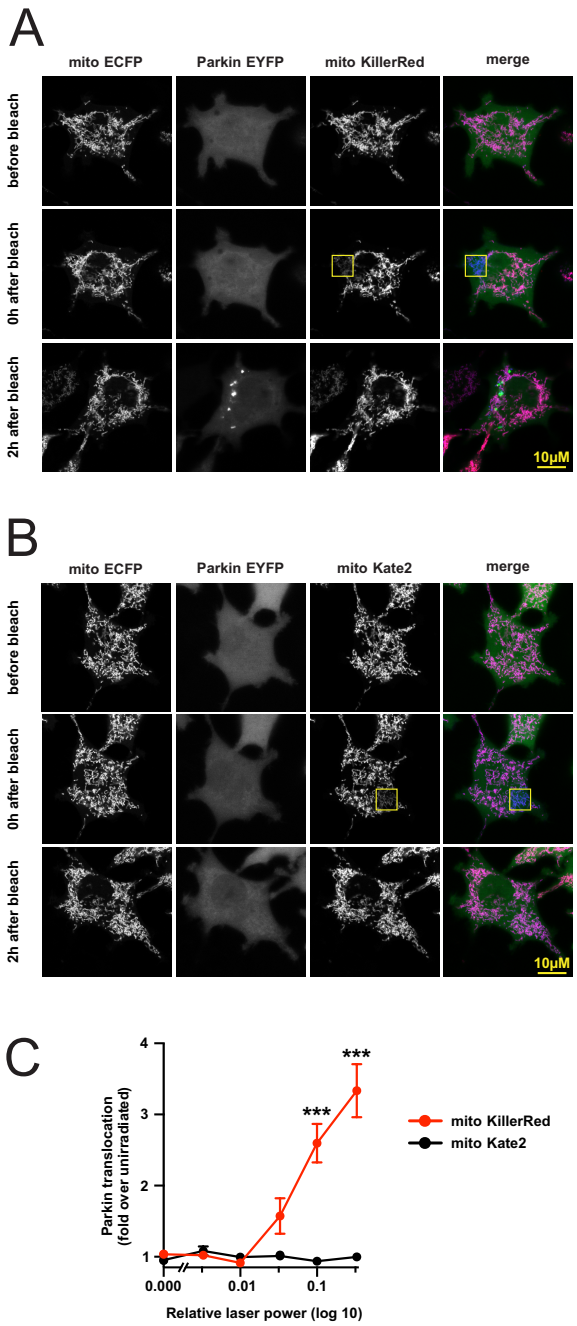

Figure S2. KillerRed generated ROS and not laser irradiation per se induces the Parkin translocation to the irradiated mitochondria

A-B. Laser irradiation induces Parkin translocation in mitochondrial KillerRed but not in mitochondrial Kate2 expressing cells. Representative confocal images of PC6 cells transfected with mitochondrial ECFP, Parkin-EYFP and mitochondrial KillerRed (A) or mitochondrial Kate2 (B). Sub-population of mitochondria in the selected cell was irradiated in a 6.7 x 6.7 µm frame (yellow) using a 561 nm laser line (1 iteration, pixel time 25 µs, laser power 10%), and redistribution of Parkin-EYFP signal was followed over the time. Images show the cells before the irradiation, immediately after irradiation and 2h later. Note that PC6 cells tend to move or/and change their shape over time.

C. Quantification of Parkin translocation in mitochondrial KillerRed or mitochondrial Kate2 expressing cells 2h after irradiation with 0.33-33% laser power. The spatial heterogeneity of Parkin-EYFP (coefficient of variation of the intensity of individual pixels) was estimated for individual cells. ***$P < 0.001$ compared to respective Kate2 expressing group, $n = 17$ cells from 3 different dish sections, mixed-effects model (REML) analysis and by Sidak's multiple comparison test.

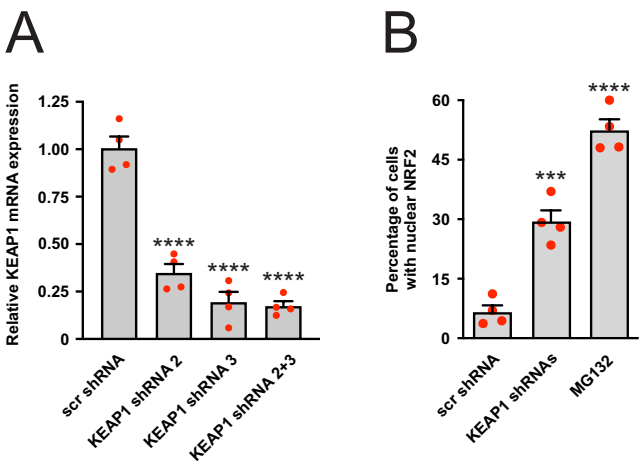

Figure S3. KEAP1 shRNAs suppress the expression of KEAP1 mRNA and induce NRF2 translocation into the nucleus

A. KEAP1 shRNAs suppress KEAP1 mRNA expression. PC6 cells were transfected with scrambled shRNA or different KEAP1 shRNAs and selected with 200 μg/ml G418 for 7 days. ****P < 0.0001 compared with scrambled shRNA group, n = 4, one-way ANOVA followed by Dunnett's multiple comparisons test.

B. Nuclear localization of NRF2 is increased in cells expressing KEAP1 shRNAs. PC6 cells were transfected with EGFP-NRF2 and scrambled shRNA or KEAP1 shRNA (combination of 2 and 3) encoding plasmids. MG132 treatment (25 μM for 4h) was used as a positive control. ***P < 0.001 and ****P < 0.0001 compared with scrambled shRNA group, n = 4 dishes, 10 fields per dish, one-way ANOVA followed by Dunnett's multiple comparisons test.

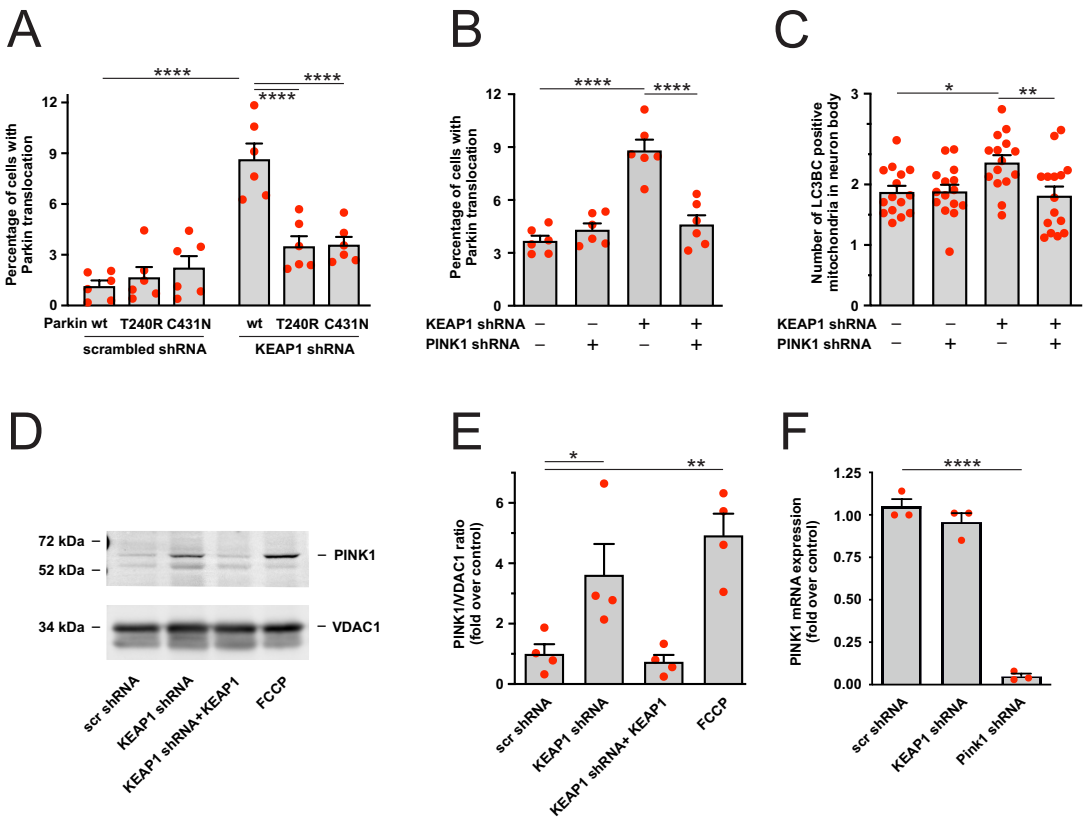

Figure S4. KEAP1 shRNAs induced Parkin translocation and mitophagy require PINK1 and Parkin ubiquitin ligase activity and induce PINK1 accumulation without affecting its mRNA expression

A. Parkin ubiquitin ligase activity is required for Parkin translocation induced by KEAP1 silencing. PC6 cells were transfected with wt Parkin-EYFP, T240R Parkin-EYFP, C431N Parkin-EYFP and scrambled shRNA or KEAP1shRNA. ****P < 0.0001, n = 6 dishes, 20 fields per dish, one-way ANOVA followed by Sidak's multiple comparisons test.

B. KEAP1 silencing does not induce Parkin translocation in the absence of PINK1. PC6 cells were transfected with Parkin-EYFP and scrambled shRNA, KEAP1 shRNA, or/and PINK1 shRNA. ****P < 0.0001, n = 6 dishes, 20 fields per dish, one-way ANOVA followed by Sidak's multiple comparisons test.

C. KEAP1 silencing does not induce mitophagy in the absence of PINK1. Neurons were transfected with EGFP-LC3B, GFP-LC3C, mitochondrial Kate2 and scrambled shRNA, KEAP1 shRNA or/and PINK1 shRNA. *P < 0.05 and **P < 0.01, n = 15 dishes, 7-10 neurons per dish, one-way ANOVA followed by Sidak's multiple comparisons test.

D-E. KEAP1 silencing leads to the accumulation of full-length PINK1. (D) depicts representative Western blot and (E) the quantitative analysis. PC6 cells were transfected with plasmids encoding scrambled shRNA, KEAP1 shRNAs with or without shRNA insensitive KEAP1 and selected with 200 µg/ml G418 for 6 days. FCCP treatment (10µM for 4 hours) was used as a positive control. *P < 0.05 and **P < 0.01, n = 4 dishes, one-way ANOVA followed by Holm-Sidak's multiple comparison test.

F. KEAP1 silencing does not augment the PINK1 mRNA expression. PC6 cells were transfected with plasmids encoding scrambled shRNA, KEAP1 shRNAs, or PINK1 shRNA and selected with 200 µg/ml G418 for 6 days followed by RT-PCR. ****P < 0.0001 compared with scrambled shRNA group, n = 3, one-way ANOVA followed by Dunnett's multiple comparisons test.

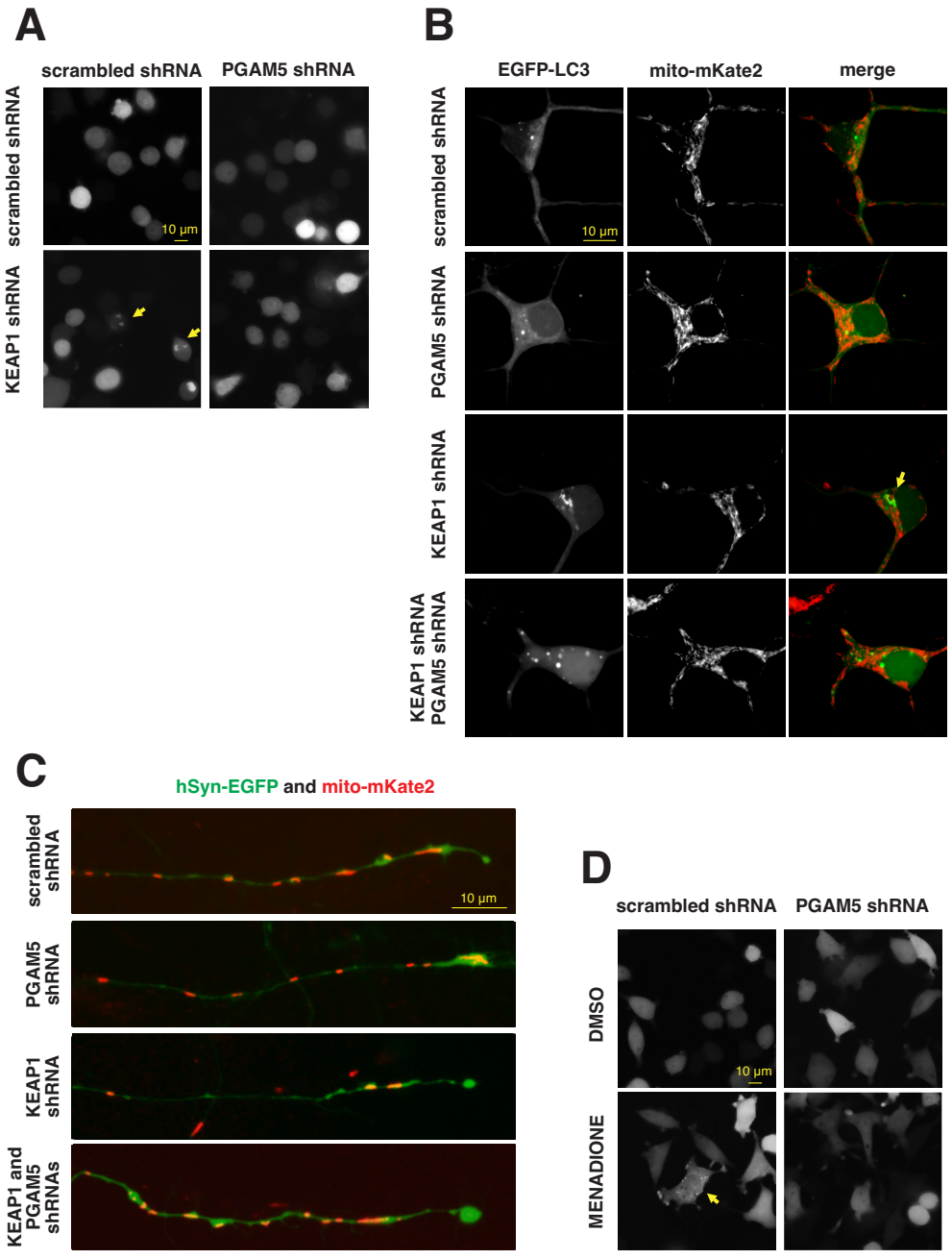

Figure S5. Representative images of Parkin translocation, mitophagy and mitochondrial density in PGAM5 silenced cells.

A. Parkin translocation in KEAP1 silenced PC6 cells in the absence of PGAM5. PC6 cells were transfected with Parkin-EYFP and scrambled shRNA, KEAP1 shRNA, or/and PGAM5 shRNA expressing plasmids.

B. Mitophagy in KEAP1 silenced neurons in the absence of PGAM5. Primary cortical neurons were transfected with a mix of EGFP-LC3B and GFP-LC3C, mitochondrial Kate2 and scrambled shRNA, KEAP1 shRNA or/and PGAM5 shRNA expressing plasmids.

C. Mitochondrial density in the axons of KEAP1 silenced neurons in the absence of PGAM5. The neurons were transfected with neuronal marker hSyn-EGFP, mitochondrial DsRed2 and KEAP1 shRNA or/and PGAM5 shRNA and mitochondrial density was quantified at the end of the axon.

D. Menadione-induced Parkin translocation in PGAM5 silenced PC6 cells. PC6 cells expressing Parkin-EYFP and scrambled or PGAM5 shRNA were treated with DMSO or 20 µM menadione for 4h.

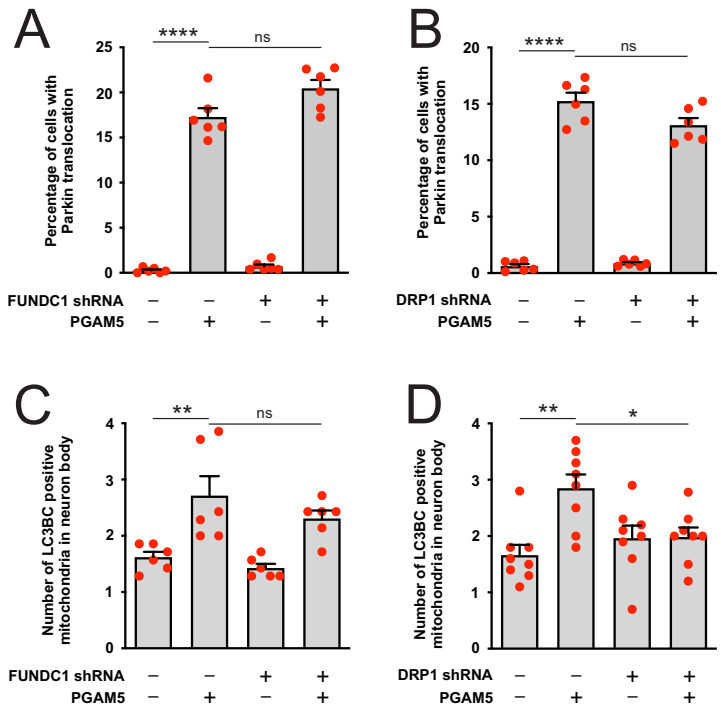

Figure S6. FUNDC1 and DRP1 are not involved in PGAM5 induced Parkin translocation

A. FUNDC1 silencing does not inhibit PGAM5-induced Parkin translocation. PC6 cells were transfected with Parkin-EYFP with or without PGAM5 and scrambled or FUNDC1 shRNA. ****P < 0.0001, n = 6 dishes, 20 fields per dish, Welch's ANOVA followed by Dunnett's T3 multiple comparisons test.

B. DRP1 silencing does not inhibit PGAM5-induced Parkin translocation. PC6 cells were transfected with Parkin-EYFP with or without PGAM5 and scrambled or DRP1 shRNA. ****P < 0.0001, n = 6 dishes, 20 fields per dish, Welch's ANOVA followed by Dunnett's T3 multiple comparisons test.

C. FUNDC1 silencing does not inhibit PGAM5-induced mitophagy. Primary cortical neurons were transfected with EGFP-LC3B, GFP-LC3C, Kate2 targeted to the mitochondria with or without PGAM5 and scrambled or FUNDC1 shRNAs. **P < 0.01, n = 6 dishes, 7 cells per dish, one-way ANOVA followed by Sidak's multiple comparison test.

D. DRP1 silencing inhibits PGAM5-induced mitophagy. Primary cortical neurons were transfected with EGFP-LC3B, GFP-LC3C, Kate2 targeted to the mitochondria with or without PGAM5 and scrambled or DRP1 shRNA. *P < 0.05 and **P < 0.01, n = 8 dishes, 10 cells per dish, one-way ANOVA followed by Sidak's multiple comparison test.

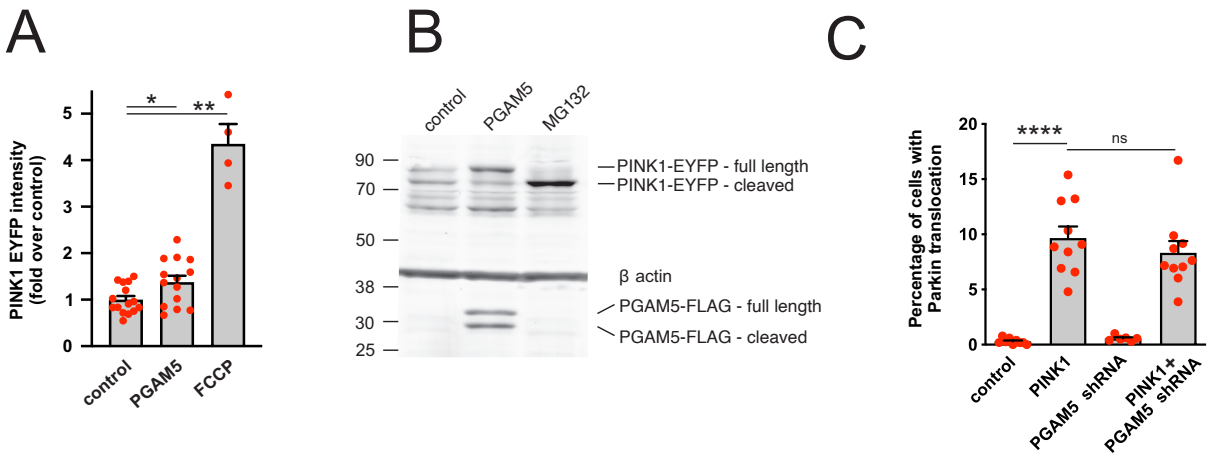

Figure S7. Overexpression of PGAM5 leads to accumulation of full length PINK1

A. PGAM5 overexpression induces PINK1 accumulation. PC6 cells were transfected with PINK1-EYFP and PGAM5 wt or treated with 10 µM FCCP for 3h, and fluorescence intensity of PINK1-EYFP was measured in individual cells. *P < 0.05 and **P < 0.01, n = 14-15 dishes (n=4 in FCCP group), 6 fields per dish, 5 cells per field. Welch's ANOVA followed by Dunnett's T3 multiple comparisons test.

B. PGAM5 overexpression leads to the accumulation of overexpressed full-length PINK1. HeLa cells were transfected with PINK1-EYFP with or without PGAM5 and treated with DMSO or 25 µM MG132 for 4h. Total cell lysates were resolved on SDS-PAGE and immunoblotted for indicated proteins.

C. PGAM5 silencing does not block the PINK1 overexpression-induced Parkin translocation. PC6 cells were transfected with Parkin-EYFP, PINK1 and scrambled shRNA or PGAM5 shRNA. ****P < 0.0001, n = 6-10 dishes, 20 fields per dish, Welch's ANOVA followed by Dunnett's T3 multiple comparisons test.

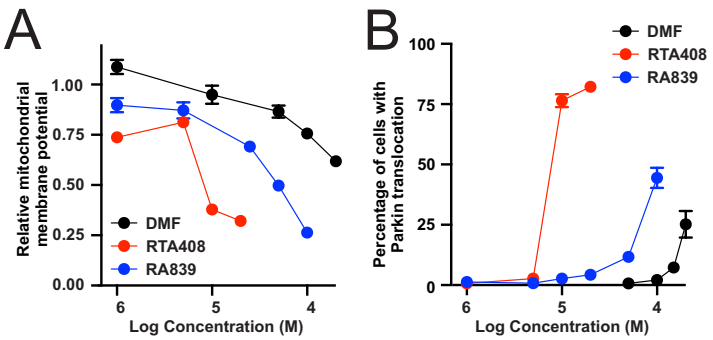

Figure S8. Electrophilic KEAP1 inhibitors depolarise mitochondria and induce robust Parkin translocation to mitochondria

A. Electrophilic KEAP1 inhibitors decrease the mitochondrial membrane potential. PC6 cells were treated with KEAP1 inhibitors for 24 h, after which the cells were stained with ratiometric mitochondrial membrane potential sensor JC10 (n=8-14 wells per data point).

B. Electrophilic KEAP1 inhibitors induce Parkin translocation to mitochondria. PC6 cells expressing Parkin-EYFP were treated with KEAP1 inhibitors for 24 h (n=3 dishes per data point, 20 fields per dish).