Expanded View Figures

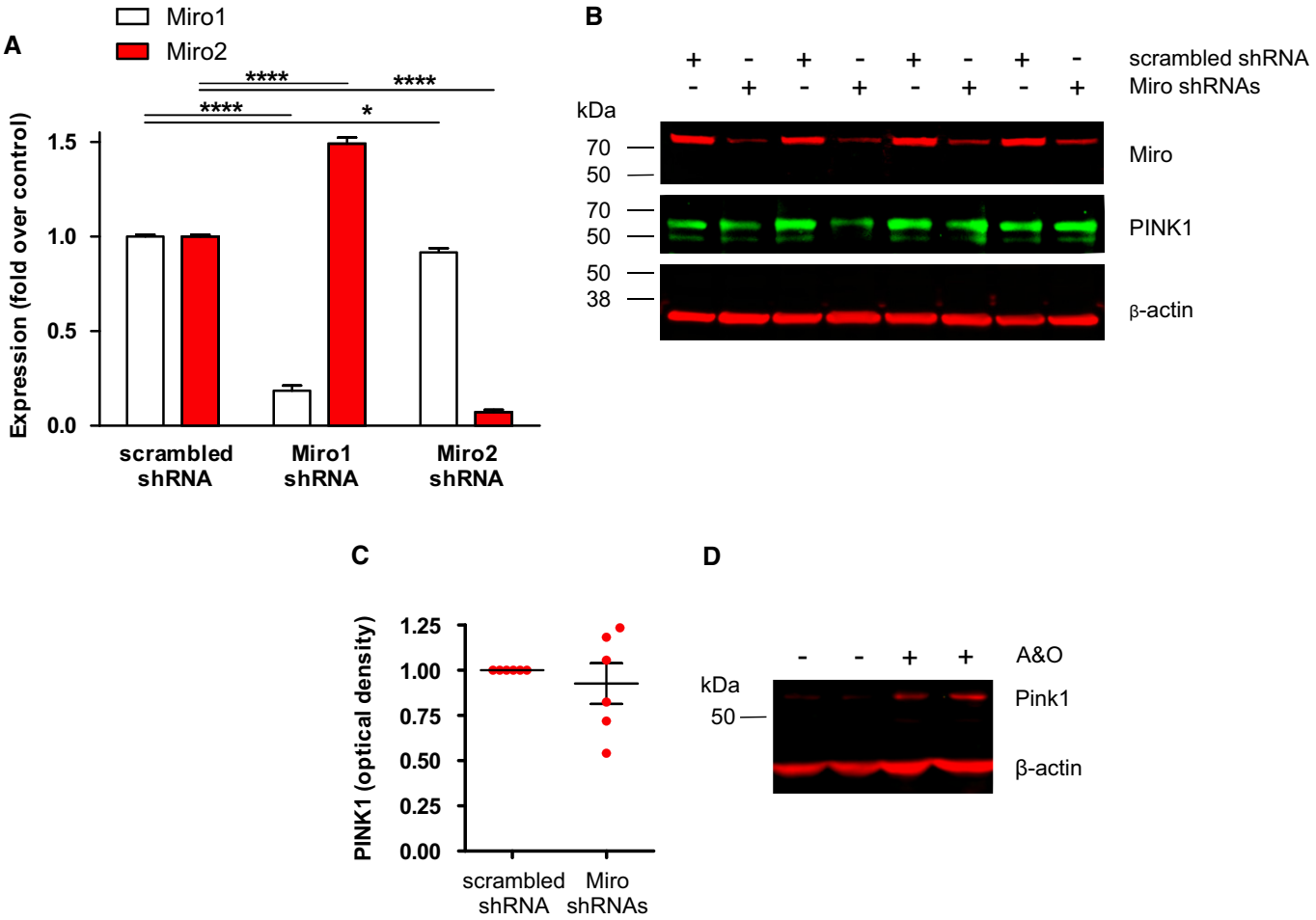

Figure EV1. Miro shRNAs specifically suppress the expression of Miro1 and Miro2 and do not affect the expression level of overexpressed PINK1.

A PC6 cells were transfected with either scrambled shRNA, Miro1 shRNA or Miro2 shRNA and selected for 7 days with 200 μg/ml G418. Total RNA was extracted using RNAeasy mini kit (Qiagen), and first-strand synthesis was performed using 5 μg of total RNA with Maxima First Strand cDNA Synthesis Kit (Thermo). cDNAs were subjected to qPCR using specific primers for CYC (housekeeping gene), Miro1 and Miro2 using a QuantStudio 12K Flex from Applied Biosystems by Life technologies. Acquired data were analysed using the delta Ct method and normalised to transfection efficiency, which was estimated separately. *$P < 0.05$ and ****$P < 0.0001$ compared with respective scrambled shRNA group, $n = 3$ samples, one-way ANOVA.

B Representative Western blot image of untagged PINK1 expression in PC6 cells expressing either scrambled shRNA or a combination of Miro1 and Miro2 shRNA. Note that the level of endogenous Miro proteins detected with Miro antibody recognising both isoforms was clearly decreased in the Miro shRNA-treated samples.

C Quantification of PINK1 band intensity from six independent samples shows that there is no statistical significance between the groups (*t*-test with Welch's correction).

D Treatment with A&O (both 15 μM) for 3 h led to clearly visible endogenous PINK1 accumulation.

Data information: Data are presented as means ± SEM.
Source data are available online for this figure.

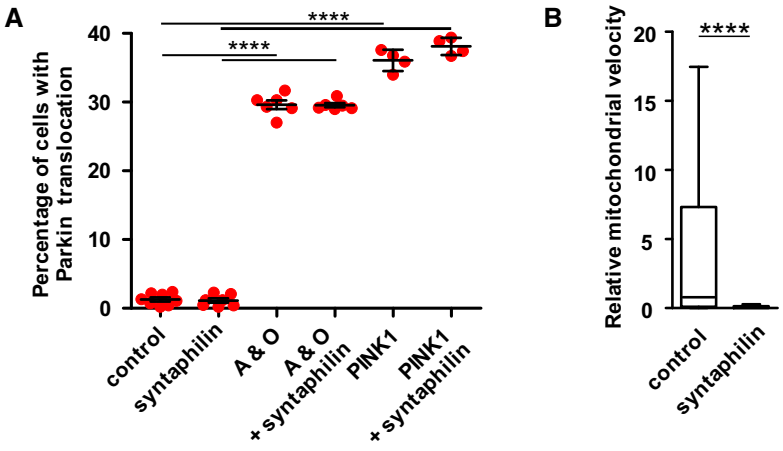

Figure EV2. Syntaphilin overexpression does not affect Parkin translocation.

A Syntaphilin overexpression has no effect on A&O- or Pink1-induced EYFP-Parkin translocation in PC6 cells (****$P < 0.0001$, $n = 4$–8 dishes, 20 fields per dish, one-way ANOVA).

B Syntaphilin overexpression led to an almost complete inhibition of mitochondrial motility in primary cortical neurons. Data are presented as a Tukey boxplot (****$P < 0.0001$, $n = 305$–339 individual mitochondria from 12 axons per group pooled from 3 individual dishes, Mann–Whitney test).

Data information: Data are presented as means ± SEM or as a Tukey plot (median ± 1.5 times interquartile range).
Source data are available online for this figure.

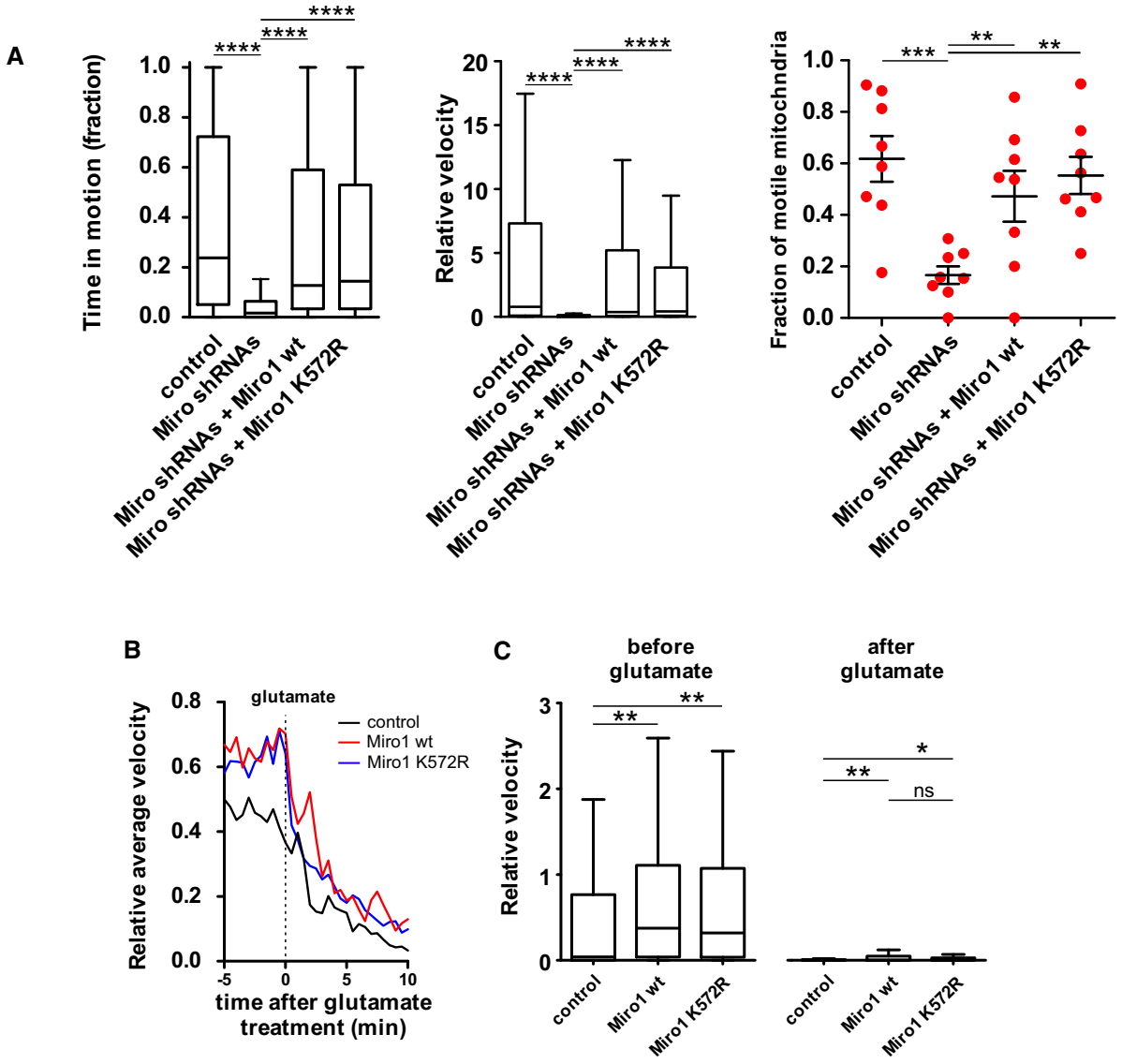

Figure EV3. Miro1 K572R mutation does not affect Miro1 function in mitochondrial motility in neurons.

A Suppression of Miro1 and Miro2 with shRNAs led to an almost complete inhibition of mitochondrial motility; co-expression of shRNAs with shRNA-resistant WT Miro1 or K572R mutant both efficiently rescued mitochondrial motility (left panel: fraction of time in motion, middle panel: relative mitochondrial velocity). Data are presented as a Tukey boxplot. ****$P < 0.0001$, $n = 110$–133 individual mitochondria from 8 axons per group pooled from 4 individual dishes, Kruskal–Wallis test. Co-expression of shRNA-resistant Miro1 K572R also restored the fraction of motile mitochondria (right panel), **$P < 0.01$ and ***$P < 0.001$, $n = 8$ axons, one-way ANOVA.

B, C Glutamate-induced (30 μM glutamate/1 μM glycine in HBSS) arrest of mitochondrial motility was similar between Miro1 K572R-overexpressing neurons and Miro1 WT-overexpressing neurons (B). (C) shows motility parameters before and after glutamate treatment. Data are represented as a Tukey boxplot. *$P < 0.05$, and **$P < 0.01$, $n = 143$–160 mitochondria from 10 to 13 axons (one axon per dish), Kruskal–Wallis test.

Data information: Data are presented as means ± SEM or as a Tukey plot (median ± 1.5 times interquartile range).
Source data are available online for this figure.

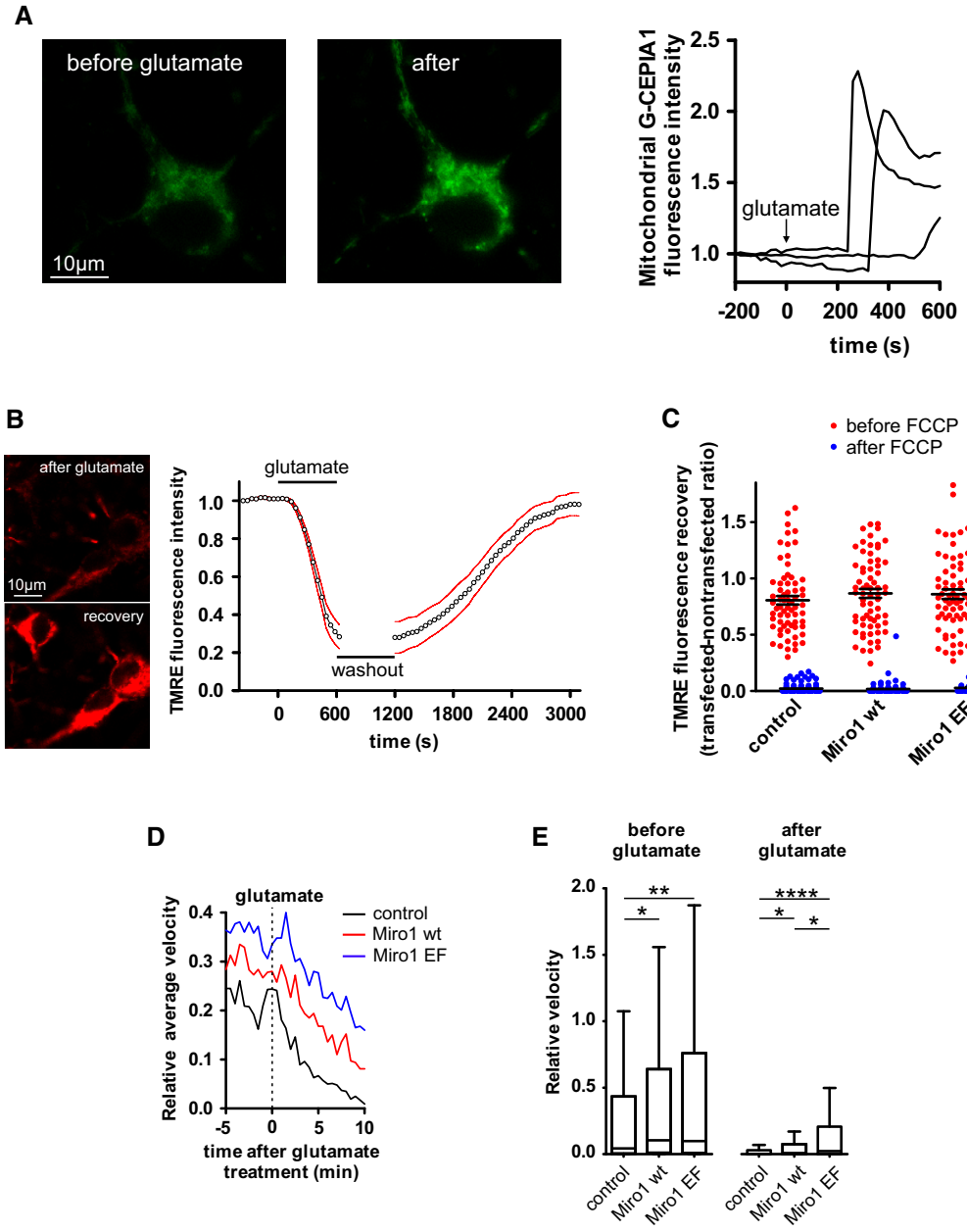

Figure EV4. Glutamate treatment leads to increased mitochondrial Ca^{2+}, mitochondrial depolarisation and inhibition of mitochondrial motility.

A Treatment of neurons for 10 min with 100 µM glutamate/10 µM glycine led to increased mitochondrial Ca^{2+} uptake, visualised by increased mitochondrial G-CEPIA1 fluorescence.

B Glutamate treatment led to mitochondrial depolarisation, visualised by a decline in TMRE fluorescence intensity. The mitochondrial membrane potential was almost completely restored by 30-40 min after glutamate washout (dots show mean, red lines the SEM).

C Further analysis demonstrated no difference in mitochondrial membrane potential recovery between control, Miro1 WT- and Miro1-EF-transfected neurons after glutamate washout (n = 65–192 neurons; ns, Kruskal–Wallis test). Note that FCCP treatment (3 µM for 15 min) almost completely abolished TMRE fluorescence.

D, E Mean mitochondrial velocity decreased after glutamate treatment (D). Panel (E) shows relative velocity before and after glutamate treatment. Note that the decrease in mitochondrial velocity was reduced in Miro1-EF-expressing neurons. Data are represented as Tukey boxplot (*P < 0.05, **P < 0.01 and ****P < 0.0001, n = 260–366 mitochondria, Kruskal–Wallis test).

Data information: Data are presented as means ± SEM or as a Tukey plot (median ± 1.5 times interquartile range).
Source data are available online for this figure.

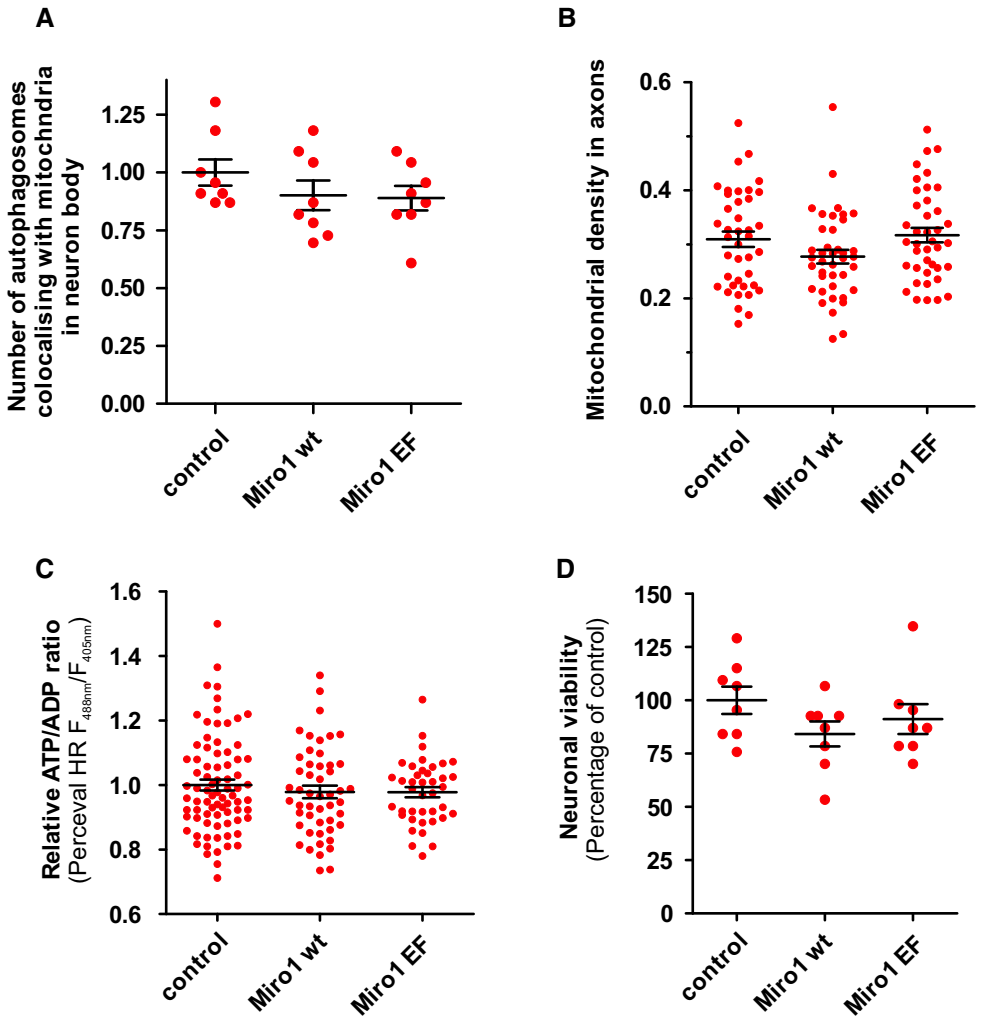

Figure EV5. Mitophagy, mitochondrial density, ATP level and neuronal survival were not affected in Miro-1 WT and Miro1 EF-expressing neurons.

A Mitochondrial co-localisation with the autophagosome marker EGFP-LC3B in neuronal soma measured at 5–6 h after HBSS treatment was not different in Miro1 WT or Miro1 EF-transfected neurons when compared with control neurons (n = 8 dishes, one-way ANOVA).

B Mitochondrial density in axons was not significantly different in HBSS-treated Miro1 WT- or Miro1 EF-expressing neurons when compared with control neurons (n = 40 axons from 4 dishes, Kruskal–Wallis test).

C Cytosolic ATP measured using the ATP/ADP sensor Perceval HR was not affected in Miro1 WT- or Miro1 EF-expressing neurons when compared with control neurons (measured 6–8 h after HBSS treatment, n = 40–70 neurons from 4 to 7 dishes, one-way ANOVA).

D Neuronal survival was not affected in Miro1 WT- or Miro1 EF-expressing neurons (estimated 24 h after HBSS treatment, n = 8 dishes, repeated-measures ANOVA).

Data information: Data are presented as means ± SEM.
Source data are available online for this figure.