

PINK1 signalling rescues amyloid pathology and mitochondrial dysfunction in Alzheimer's disease

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Mitochondrial dysfunction and synaptic damage are early pathological features of the Alzheimer's disease-affected brain. Memory impairment in Alzheimer's disease is a manifestation of brain pathologies such as accumulation of amyloid- β peptide and mitochondrial damage. The underlying pathogenic mechanisms and effective disease-modifying therapies for Alzheimer's disease remain elusive. Here, we demonstrate for the first time that decreased PTEN-induced putative kinase 1 (PINK1) expression is associated with Alzheimer's disease pathology. Restoring neuronal PINK1 function strikingly reduces amyloid- β levels, amyloid-associated pathology, oxidative stress, as well as mitochondrial and synaptic dysfunction. In contrast, PINK1-deficient mAPP mice augmented cerebral amyloid- β accumulation, mitochondrial abnormalities, impairments in learning and memory, as well as synaptic plasticity at an earlier age than mAPP mice. Notably, gene therapy-mediated PINK1 overexpression promotes the clearance of damaged mitochondria by augmenting autophagy signalling via activation of autophagy receptors (OPTN and NDP52), thereby alleviating amyloid- β -induced loss of synapses and cognitive decline in Alzheimer's disease mice. Loss of PINK1 activity or blockade of PINK1-mediated signalling (OPTN or NDP52) fails to reverse amyloid- β -induced detrimental effects. Our findings highlight a novel mechanism by which PINK1-dependent signalling promotes the rescue of amyloid pathology and amyloid- β -mediated mitochondrial and synaptic dysfunctions in a manner requiring activation of autophagy receptor OPTN or NDP52. Thus, activation of PINK1 may represent a new therapeutic avenue for combating Alzheimer's disease.

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Abbreviations: CcO = cytochrome *c* oxidase; EPR = electron paramagnetic resonance; LTP = long-term potentiation; ROS = reactive oxygen species

Introduction

Mitochondrial dysfunction and synaptic failure are prominent and early pathological features of Alzheimer's disease (Selkoe, 2002; Lin and Beal, 2006; Du *et al.*, 2010, 2012; Swerdlow, 2012; Fang *et al.*, 2015; Yu *et al.*, 2016). Perturbed bioenergetics function, aberrant mitochondrial dynamics, and increased reactive oxygen species (ROS) are observed in Alzheimer's disease-affected brains. Amyloid- β peptide has deleterious effects on mitochondrial and synaptic function (Lustbader *et al.*, 2004; Manczak *et al.*, 2006, 2011; Reddy and Beal, 2008; Takuma *et al.*, 2009; Chen and Yan, 2010; Reddy *et al.*, 2012). Damaged mitochondria are progressively accumulated in neurons, particularly in synapses over the lifetime of Alzheimer's disease-affected neurons (Du *et al.*, 2010). The accumulation of damaged mitochondria in Alzheimer's disease brains contributes to aberrant synaptic structure and function such as loss of synapses, dendritic spines, synaptic proteins, and disruption of synaptic transmission. The underlying mechanisms of amyloid- β -mediated progressive mitochondrial and synaptic degeneration remain elusive and strategies to rescue such injuries remain unavailable. However, it has become increasingly evident that defective mitochondria must be removed in order to maintain neuronal function and synaptic transmission.

PTEN-induced putative kinase 1 (PINK1) is critical to the maintenance of mitochondrial integrity and function by promoting the removal of damaged mitochondria via mitophagy (Geisler *et al.*, 2010; Batlevi and La Spada, 2011; Ashrafi *et al.*, 2014; Lazarou *et al.*, 2015; Pickrell and Youle, 2015)—a selective form of autophagy whereby defective mitochondria are specifically engulfed by autophagosomes and targeted for degradation in the lysosomes. Upon membrane depolarization, damaged mitochondria stabilize and activate PINK1 leading to the recruitment of parkin (Narendra *et al.*, 2008, 2010) from the cytosol to the mitochondria. Once recruited, parkin initiates mitophagy. However, a recent study has demonstrated that PINK1 is capable of activating mitophagy directly without parkin by recruiting the autophagy receptors optineurin (OPTN) and nuclear dot protein 52 kDa (NDP52, encoded by *CALCOCO2*) (Lazarou *et al.*, 2015). Parkin can further amplify the mitophagy signal triggered by PINK1. This new pathway further strengthens the concept that targeting PINK1 is a novel therapeutic strategy. Consistent with its protective role, PINK1 downregulation causes mitochondrial dysfunction, increased oxidative stress, and neuronal dysfunction (Beilina *et al.*, 2005; Clark *et al.*, 2006; Yang *et al.*, 2006; Gautier *et al.*, 2008; Tufi *et al.*, 2014). Several mutations of PINK1 have been found to be involved in the pathogenesis of Parkinson's disease. However, the significant role of PINK1 in Alzheimer's disease pathogenesis has yet to be elucidated.

Our present study offers new insights into PINK1-dependent amyloid pathology through autophagy signalling

and mitochondrial quality, contributing to the synaptic and cognitive dysfunction in the pathogenesis of Alzheimer's disease.

Materials and methods

Animal studies

Animal studies were carried out with the approval of the Institutional Animal Care and Use Committee of the University of Kansas Lawrence in accordance with the National Institutes of Health guidelines for animal care.

Human subjects for PINK1 expression

We obtained human brain tissues of hippocampus tissues from individuals with Alzheimer's disease and age-matched, non-Alzheimer's disease controls from the University of Arizona. Detailed information for each of the cases studied is shown in Supplementary Table 1. We obtained informed consent from all subjects.

Production of AAV2-PINK construct

Viral production and packaging using recombinant adeno-associated virus type 2 (rAAV2) encoding a protein of interest under control of the cytomegalovirus (CMV) promoter has been described in our previous study (Rappold *et al.*, 2014). Briefly, human wild-type PINK1 and mutant PINK1-L347P (Cui *et al.*, 2010) were subcloned into the pBSFBmcs shuttle vector and then the modified pFBGR plasmid backbone containing EGFP to monitor the expression of PINK1 proteins after rAAV injection. Finally, rAAV2 packaging was performed as described previously (Rappold *et al.*, 2014). Control experiments were performed with an AAV2-GFP construct.

Intrahippocampal injections with AAV2-PINK1, AAV2-mPINK1 and AAV2-GFP

Non-transgenic mice and transgenic mAPP mice that overexpress a human mutant form of amyloid precursor protein (APP) bearing both the Swedish (K670N/M671L) and the Indiana (V717F) mutations (APPSwInd, J-20 line, obtained from Jackson Lab) were used in this study. Both male and female animals were used for the described experiments. The investigators were blind to the mouse genotype in performing all experiments. For the intrahippocampal stereotaxic injections, mice were deeply anaesthetized and placed in the stereotaxic frame. Two microlitres of each AAV2 (AAV2-PINK, AAV2-mPINK or AAV2-GFP, $\sim 5 \times 10^{12}$ vg/ml) or vehicle were injected with a 10 μ l Hamilton syringe at a rate of 0.25 μ l/min by a nano-injector system. The needle was allowed to remain in the brain for an additional 5 min. Coordinates for stereotaxic injections were determined according to the Paxinos atlas of the mouse brain by using the following coordinates: ± 3.2 mm medial/lateral, -2.7 mm anterior/posterior, and -2.7 mm dorsal/ventral from the bregma. Before waking, mice were allowed to recover in a heated pad. At 2 months post-injection, mice were subjected to a behavioural test and electrophysiological analysis. Then, assessment of amyloid- β levels,

mitochondrial function and neuropathology were performed using mouse hippocampal samples.

Hippocampal neuronal cell culture, N2a-APPsw cell culture, and production of lentiviral constructs of Lenti-PINK1, Lenti-mPINK1, Lenti-triple-mPINK1 and Lenti-GFP are described in the Supplementary material.

Amyloid- β measurement, cytochrome *c* oxidase (CcO) activity assay, ATP levels, mitochondrial and the intracellular ROS, mitochondrial membrane potential measurement, immunoblotting, immunofluorescent staining and measurement of synaptic density were performed as previously described (Fang *et al.*, 2015, 2016c) and are detailed in the Supplementary material.

Behavioural test and electrophysiological studies

We performed behavioural studies to assess spatial learning and memory using the Morris water maze as previously described (Fang *et al.*, 2015) with modifications (Supplementary material).

Statistical analysis

All data were expressed as the mean \pm standard error of the mean (SEM). Data were analysed by one-way ANOVA for repeated measure analysis using commercially available software (Statview, version 5.0.1, Berkeley, CA), followed by Fisher's protected least significant difference for *post hoc* comparisons. $P < 0.05$ was considered significant.

Results

PINK1 reduces cerebral and mitochondrial amyloid- β accumulation

As PINK1 was downregulated in the brains of patients with Alzheimer's disease and transgenic Alzheimer's disease mice (Supplementary Fig. 1A–F), we sought to develop a model system in which neuronal expression of PINK1 would be restored or increased so that the consequences of PINK1-dependent signalling in an Alzheimer's disease- and amyloid- β -rich environment could be analysed. We first explored whether increasing hippocampal PINK1 expression would affect amyloid- β accumulation. To this end, we performed stereotaxic injection to deliver human PINK1 to the hippocampus via rAAV2 encoding human PINK1. As a control for PINK1 kinase activity, we also delivered the loss-of-function form of PINK1 without kinase activity (L347P, mPINK1) (Beilina *et al.*, 2005; Pridgeon *et al.*, 2007). AAV-GFP was used as a viral vector control. PINK1 expression was increased in hippocampal neurons at 2 months post AAV-PINK1 injection (Supplementary Fig. 2). AAV-PINK1 transduction significantly reduced human amyloid- β levels by 65–70% in the hippocampus of mAPP/PINK1 mice compared to mAPP mice at 8 months of age (Fig. 1A and B), a time point when progressive accumulation of cerebral amyloid- β became evident. Similarly, human amyloid- β

levels were reduced by 65–70% in the hippocampus (Supplementary Fig. 3A and B) of the 11–13-month-old mAPP mice, which were also transduced with AAV-PINK1 for 2 months. Transduction of AAV-mPINK1 did not affect cerebral amyloid- β levels in any amyloid- β measurements (Fig. 1A, B and Supplementary Fig. 3A and B). The percentage of amyloid- β immunoreactive plaque loads was also reduced in the hippocampus of mAPP/PINK1 mice receiving AAV-PINK1 but not AAV-mPINK1 (Fig. 1C and D).

Given the involvement of PINK1 in the maintenance of mitochondrial integrity and function, we evaluated the effect of PINK1 on mitochondrial amyloid- β accumulation. Consistent with our previous studies (Du *et al.*, 2008, 2010; Fang *et al.*, 2015), amyloid- β is progressively accumulated in cortical mitochondria of mAPP mice. As expected, statistically significant reduction in mitochondrial pool of amyloid- β driven by PINK1 overexpression was evident in mAPP/PINK1 mice but not in mAPP/mPINK1 mice (Supplementary Fig. 3F and G). These data indicate that PINK1 efficiently eliminates amyloid- β from mitochondria.

Because increased PINK1 expression attenuated amyloid- β accumulation, we assessed the potential effect of PINK1 on amyloid- β production by detecting changes in protein expression levels of APP and amyloid- β . Strikingly decreased human APP and amyloid- β expressions were observed in hippocampal and mitochondrial fractions of mAPP/PINK1 mice, but not in mAPP/mPINK1 mice compared with mAPP mice (Fig. 1E, F and Supplementary Fig. 3), suggesting that PINK1 overexpression reduces both the cellular and mitochondrial pool of APP and amyloid- β .

PINK1 attenuates mitochondrial defects and oxidative stress in mAPP mice

Next, we determined whether PINK1-enhanced amyloid- β clearance would improve mitochondrial function. Mitochondrial membrane potential ($\Delta\Psi_m$) is critical for maintaining the physiological function of the respiratory chain to generate ATP. Consistent with our previous studies (Du *et al.*, 2008; Fang *et al.*, 2015; Yu *et al.*, 2016), mitochondrial membrane potential was reduced in mAPP hippocampal neurons as evidenced by decreased tetramethylrhodamine (TMRM) signals, whereas PINK1 rescued the loss of TMRM signals in mAPP/PINK1 hippocampal neurons (Fig. 2A and B). We then evaluated mitochondrial respiratory function by measuring CcO activity, a key enzyme in mitochondrial complex IV activity in response to amyloid- β toxicity (Caspersen *et al.*, 2005; Du *et al.*, 2008, 2011; Fang *et al.*, 2015). CcO enzyme activity was significantly decreased in mAPP/GFP hippocampus compared to non-transgenic/GFP controls, whereas CcO activity in hippocampus of mAPP/PINK1 mice was restored to the levels similar to non-transgenic/GFP mice (Fig. 2C). In parallel, the decline in ATP level in hippocampus of mAPP mice was reversed in mAPP/PINK1 mice (Fig. 2D).

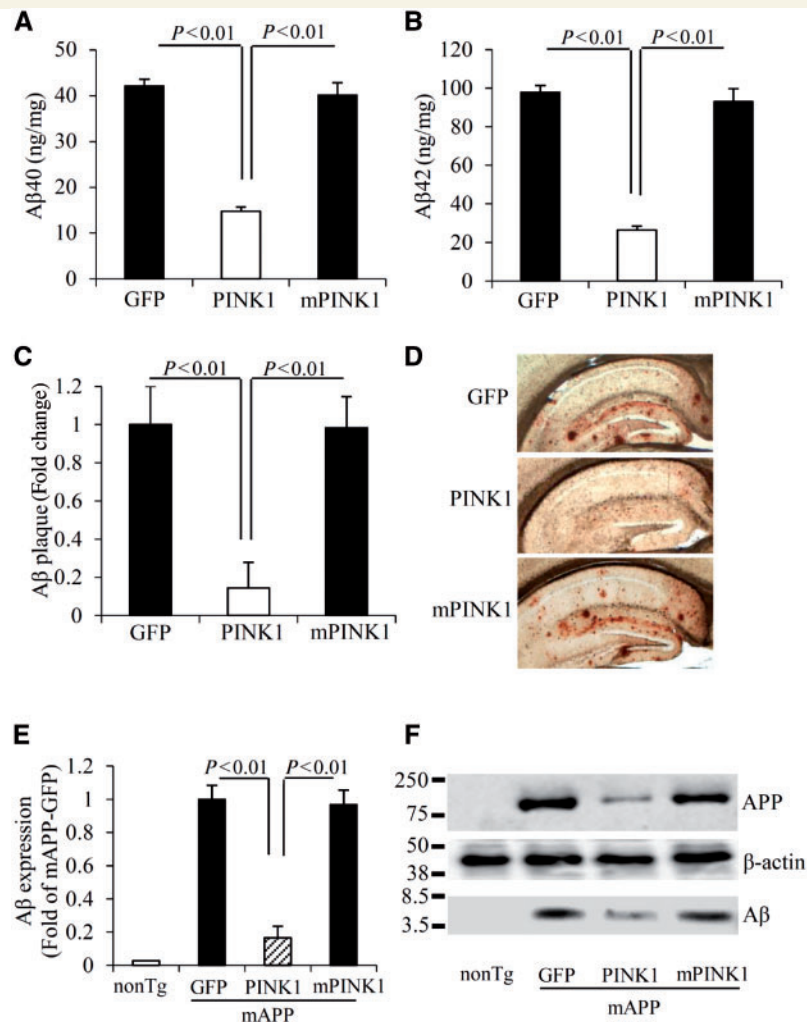


Figure 1 PINK1 reduces amyloid- β accumulation in mAPP mice. (A and B) Amyloid- β (A β) levels in the hippocampus of the indicated transgenic (Tg) mAPP mice, 2 months post-intrahippocampal injection of AAV2-PINK1 (PINK1), AAV2-mPINK1 (mPINK1), and AAV2-GFP (GFP) were measured by ELISA. $n = 9$ –13 mice per group. (C) Quantification of amyloid- β plaque was performed with the hippocampi of the indicated mice and (D) representative images showed amyloid- β deposits. $n = 6$ mice per group. (E) The bar graph presents quantification of immunoreactive bands for amyloid- β normalized to β -actin. (F) The representative immunoblots show immunoreactive bands for APP and amyloid- β proteins from indicated hippocampal homogenates, and β -actin served as a loading control. $n = 3$ mice per group.

In contrast, mAPP/mPINK1 mice did not show any protection against changes in mitochondrial membrane potential, CcO activity, and ATP levels (Fig. 2A–D).

Given that mitochondria are the main source of ROS production, we next evaluated whether increased PINK1 expression would diminish ROS overproduction in amyloid- β -affected hippocampus of 8-month-old mAPP mice. We first quantitatively measured the intracellular ROS levels in the hippocampus by highly specific electron paramagnetic resonance (EPR) spectroscopy. The intracellular ROS levels indicated by EPR peaks were significantly elevated in mAPP/GFP mice compared to non-transgenic/GFP mice. This was largely abolished in mAPP/PINK1 mice but not in mAPP/mPINK1 mice (Fig. 2E and F). Furthermore, MitoSOXTM Red signals, an indicator of mitochondria-derived superoxide was significantly increased in mAPP

hippocampi. In contrast, MitoSOXTM Red signals were significantly suppressed in mAPP/PINK1 hippocampi, but not in mAPP/mPINK1 hippocampi (Fig. 2G and H). Together, these data suggest that increased PINK1 expression/activity attenuates mitochondrial oxidative stress and mitochondrial dysfunction.

PINK1 activates autophagy signalling in amyloid- β -producing mAPP mice

Dysfunctional mitochondria are progressively accumulated in Alzheimer's disease-affected neurons (Caspersen *et al.*, 2005; Du *et al.*, 2010; Fang *et al.*, 2015). This accumulation is likely attributable to inadequate mitophagy capacity to remove defective mitochondria (Hirai *et al.*, 2001; Du

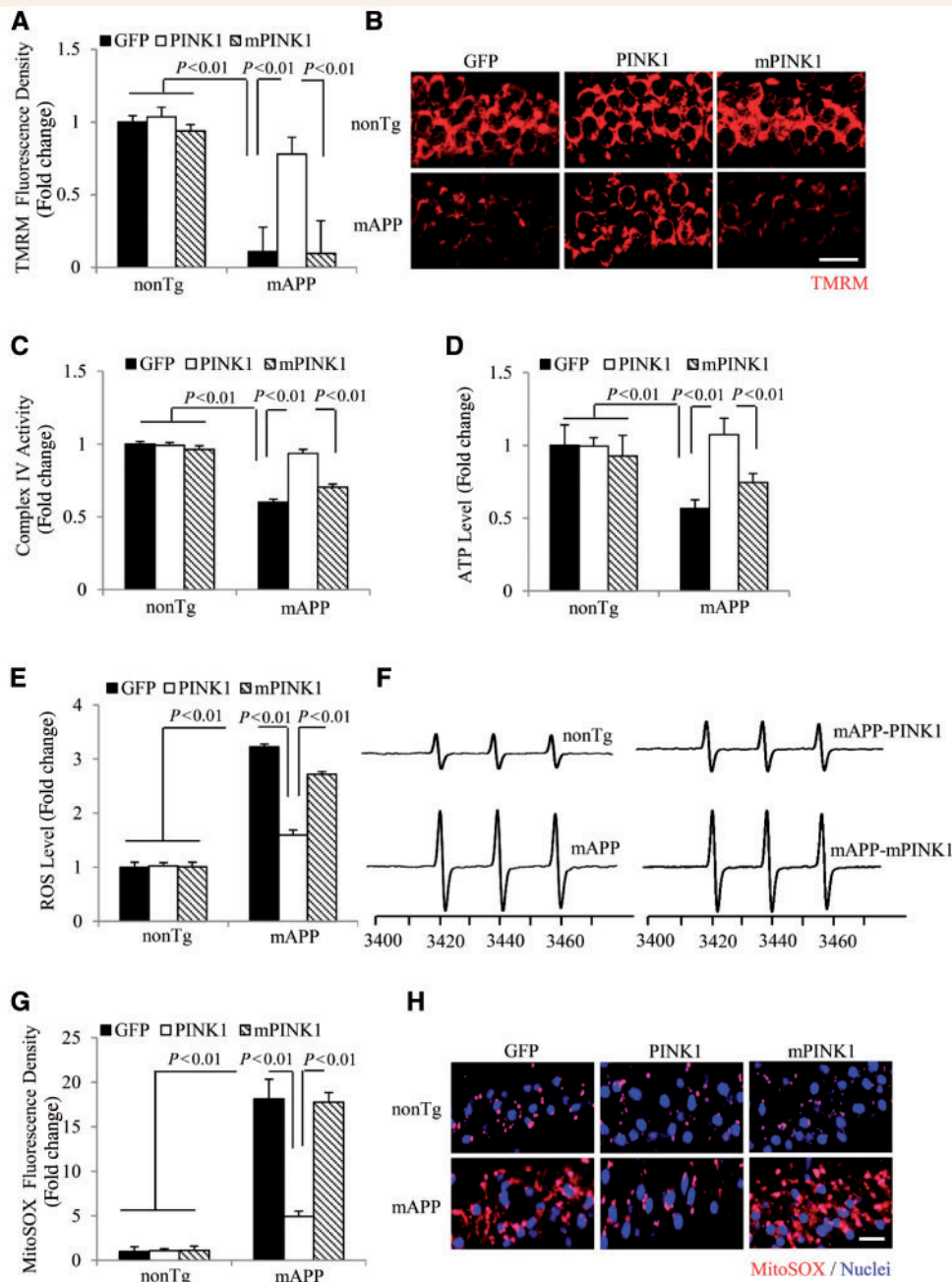


Figure 2 PINK1 rescues mitochondrial defects in mAPP mice. (A and B) Evaluation of mitochondrial membrane potential by measuring TMRM staining intensity (A) and the representative images of TMRM signal in hippocampi of the indicated mice (B). $n = 3-5$ mice per group. (C and D) CcO (complex IV, C) activity and ATP levels (D) in brain hippocampal tissues of the indicated mice. $n = 6-10$ mice per group. (E and F) Quantification of EPR spectra (E) and representative spectra of EPR (F) in the indicated mice. The peak height in the spectrum indicates levels of ROS. $n = 3-5$ mice per group. (G and H) Intensity of MitoSOX staining (G) and representative images of MitoSOXTM signals in the hippocampus of the indicated mice (H). $n = 3-5$ mice per group. Data are expressed as fold increase relative to non-transgenic mice received intrahippocampal injection of AAV2-GFP. Scale bars = 25 μm (B and H).

et al., 2011; Hung *et al.*, 2015; Weissmiller *et al.*, 2015). In view of the significance of autophagy in mitochondrial clearance and the involvement of PINK1 in autophagy signalling by recruiting autophagy receptors of NDP52 and OPTN to damaged mitochondria to initiate autophagy (Lazarou *et al.*, 2015), we assessed whether gain of

PINK1 function would enhance autophagy signalling. Levels of the active form of LC3-II, an autophagosome marker, were significantly increased in mAPP hippocampus compared to non-transgenic hippocampus (Fig. 3A). The percentage of LC3 positive neurons with puncta or aggregated morphology was significantly elevated in

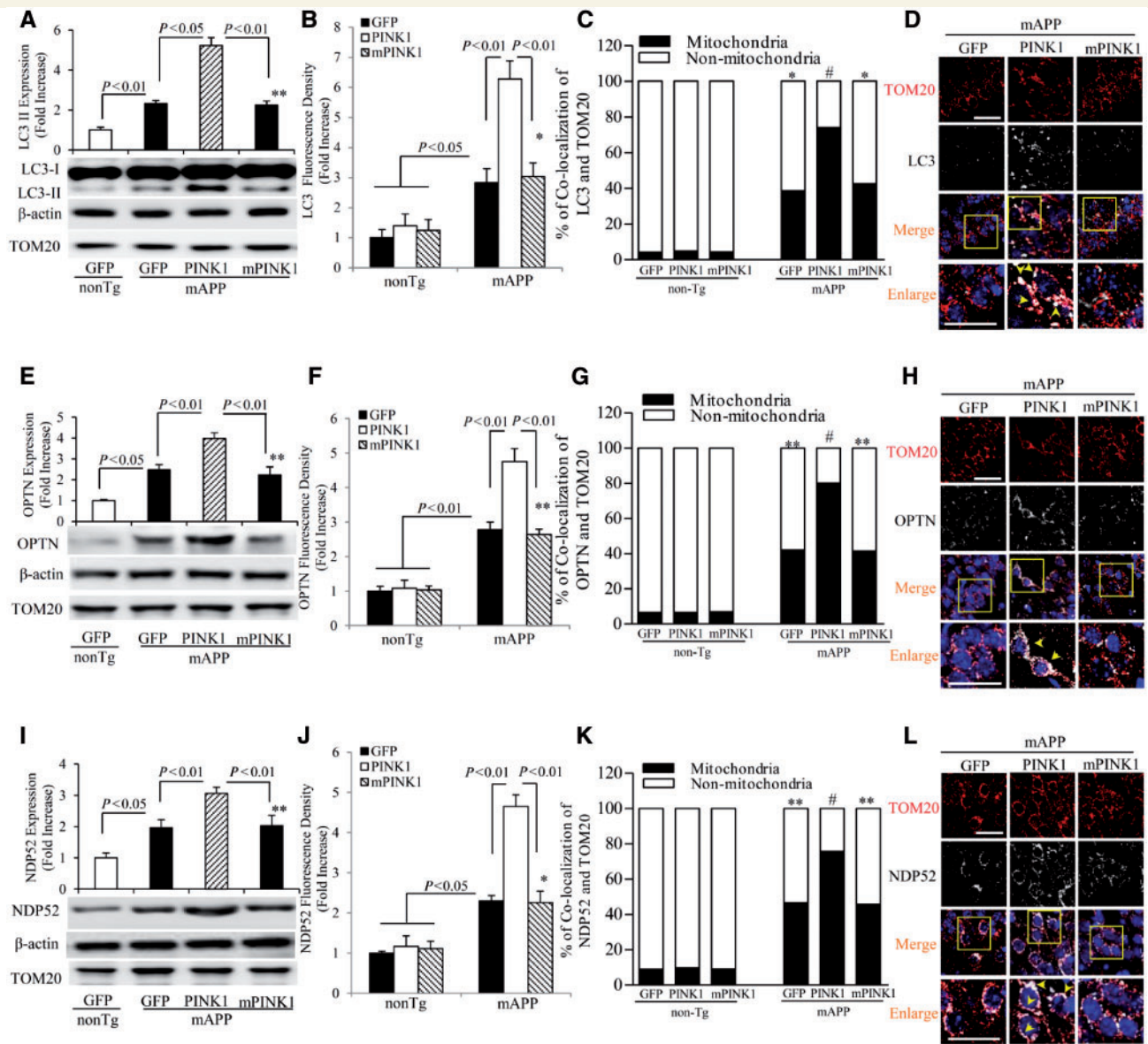


Figure 3 PINK1 activates autophagy signalling in mAPP mice. (A, E and I) Representative immunoblots show immunoreactive bands for LC3 (A), OPTN (E) and NDP52 (I) from indicated hippocampal homogenates, and β -actin served as a loading control. The bar graphs in A, E and I present quantification of immunoreactive bands for the corresponding proteins normalized to β -actin. $n = 3$ mice per group. (B, F and J) Quantification of LC3 (B), OPTN (F) and NDP52 (J) positive immunostaining intensity. Quantification of LC3, OPTN and NDP52 with TOM20 in mitochondria and non-mitochondria are shown in C, G and K, respectively. (D, H and L) Representative images of LC3 (D), OPTN (H) and NDP52 (L) in hippocampi of the indicated mice and the bottom panels show enlarged images of the interest area indicated by the box in the upper panels. $n = 3$ –5 mice and 5–8 slides per group. * $P < 0.05$, ** $P < 0.01$ versus non-transgenic (nonTg) group in A–C, E–G and I–K, # $P < 0.01$ versus mAPP/GFP or mAPP/mPINK1 mice in C, G and K. Scale bars = 25 μ m (D, H and L).

mAPP/PINK1 mice compared to mAPP/GFP mice (Fig. 3B–D). Importantly, PINK1 overexpression further elevated levels of autophagy receptor OPTN (Fig. 3E) and NDP52 (Fig. 3I) in mAPP/PINK1 mice compared to mAPP/GFP mice. Similarly, OPTN- and NDP52-positive neurons were elevated in mAPP/PINK1 hippocampi (Fig. 3F–H and J–L). Representative images show the co-localization of increased expression of autophagy proteins (LC3, OPTN, or NDP52) with mitochondrial protein TOM20

(Fig. 3D, H and L). Consistent with these observations, the mitochondrial pool of LC3, OPTN and NDP52 was elevated in mAPP/PINK1 mice (Supplementary Fig. 4A–C). The comparative graphs for the mitochondria and non-mitochondria showed increased percentage of co-localization of TOM20 with LC3II (Fig. 3C), OPTN (Fig. 3G), or NDP52 (Fig. 3K). However, mPINK1 overexpression failed to induce translocations of the above autophagy proteins into the mitochondria. In contrast, no changes in these

autophagy molecules were found in mAPP/mPINK1 mice compared to mAPP/GFP mice, either in hippocampus tissues (Fig. 3) or in mitochondria (Supplementary Fig. 4A–C). No changes in LC3, OPTN and NDP52 expression were found in non-transgenic mice transduced by PINK1 or mPINK1 (Supplementary Fig. 4D–F). These results indicate that PINK1 activates autophagy signalling in an amyloid- β -rich environment.

Lysosomes play a fundamental role in the autophagic pathway. Autophagy delivers cytoplasmic material, aggregated proteins, and organelles to lysosomes for degradation. Dysfunctional autophagy/lysosome pathway may also contribute to the accumulation of damaged organelles and proteins such as damaged mitochondria and amyloid- β (Barrachina *et al.*, 2006; Qiao *et al.*, 2008). In line with a previous observation of increased levels of lysosome-associated membrane protein 1 (LAMP1) in Alzheimer's disease brains (Barrachina *et al.*, 2006), LAMP1 expression was elevated in the hippocampi of mAPP/GFP mice. PINK1, but not mPINK1, overexpression induced more abundant LAMP1 expression in mAPP mice (Fig. 4A–C). Increased LAMP1-positive cells were co-localized with LC3 (Fig. 4D). There were no changes in LAMP1 expression or LC3 signalling in non-transgenic mice transduced with PINK1 or mPINK1 (Supplementary Fig. 4G). Cathepsin D, one of the lysosomal proteases, is important for the degradation of aggregated proteins including amyloid- β (Qiao *et al.*, 2008). Levels of cathepsin D were higher in mAPP/GFP hippocampi than non-transgenic/GFP hippocampi. PINK1-overexpressed mAPP hippocampi exhibited a robust increase in expression of cathepsin D compared with mAPP/GFP hippocampi (Fig. 4E). These results indicate the involvement of PINK1 in amyloid- β -mediated autophagy/lysosome pathways including amyloid- β degradation and clearance.

PINK1-mediated autophagy signalling contributes to the reduction in amyloid- β accumulation

Given that PINK1 overexpression significantly elevated autophagosome marker LC3-II and autophagy receptors (OPTN and NDP52) in mAPP/PINK1 brains compared to mAPP brains (Fig. 3), we evaluated whether PINK1-mediated amyloid- β clearance via activation of autophagy signals. To this end, we examined the effect of PINK1-dependent autophagy activation on amyloid- β accumulation in amyloid- β -producing neurons. Neuro2a cells stably overexpressed APPsw (N2a-APPsw) were transduced with lentivirus-PINK1 or lentivirus-vector as a control in the absence or presence of siRNA against LC3, OPTN, or NDP52, respectively. Consistent with our observations of reduced PINK1 in patients with Alzheimer's disease and mAPP mice (Supplementary Fig. 1), PINK1 level was lower in N2a-APPsw cells (Supplementary Fig. 5A and B). After PINK1 transduction, N2a-APPsw neurons revealed a significantly elevated expression of autophagy proteins, including the active form of LC3-II, OPTN and

NDP52 (Supplementary Fig. 5A, C–E and 6A–C), as well as lysosomal protein LAMP1 (Supplementary Fig. 5A and F). Next, to determine the effects of suppressing these essential autophagy proteins, cells were treated with two independent sets of siRNA against LC3, OPTN, and NDP52 at different target sites, respectively. The scramble control siRNA-treated cells were used as negative controls (Fig. 5A–C and Supplementary Fig. 6A–C). PINK1-transduced N2a-APPsw cells displayed significant lower levels of amyloid- β and APP compared to vector-transduced N2a-APPsw cells (Fig. 5D–F and Supplementary Fig. 6D and E). Conversely, knockdown of LC3, OPTN or NDP52 by siRNA in PINK1-transduced N2a-APPsw cells abolished the protective effects of PINK1 on amyloid- β accumulation and APP levels (amyloid- β ELISA results are presented in Fig. 5D, E and Supplementary Fig. 6D and E, and immunoblotting results in Fig. 5F). These data suggest that increasing PINK1 expression enhances amyloid- β clearance via activation of OPTN and NDP52 signals in addition to LC3 autophagosome.

PINK1 improves synaptic function and learning memory in mAPP mice and alleviates synaptic loss

To investigate whether PINK1-mediated mitochondrial function links to synaptic network, we first assessed synaptic transmission under basal conditions and during long-term potentiation (LTP), a form of synaptic plasticity that is widely studied as a cellular model for learning and memory. LTP recorded in CA1 neurons of hippocampal slices was significantly declined in mAPP/GFP mice compared to non-transgenic/GFP or non-transgenic/PINK1 mice, whereas LTP was significantly restored in hippocampal slices from mAPP/PINK1 mice compared with those from mAPP/GFP mice (Fig. 6A and B). PINK1 expression also enhanced basal synaptic transmission [field-excitatory post-synaptic potential (fEPSPs), Supplementary Fig. 7A] in CA1 neurons of non-transgenic mice. These data demonstrate that PINK1 activation significantly alleviates amyloid-induced synaptic injury.

To determine whether the protective effect of PINK1 on synaptic function reflects improvement in cognitive function, we evaluated spatial reference memory and target searching strategy using a Morris water maze (Fig. 6C–F). Compared to mAPP/GFP mice and mAPP/mPINK1 mice, mAPP/PINK1 mice showed a shorter latency for locating the hidden platform during the training session (Fig. 6C). In the Probe test, mAPP/PINK1 mice increased both the number of times crossing the target and the time in the target quadrant (Fig. 6D–F). These results indicate that mAPP/PINK1 mice retained a better search strategy. There were no significant differences in latency, number of times crossing the target, and time in the target quadrant between non-transgenic/GFP and non-transgenic/PINK1 mice, indicating that PINK1 overexpression has no effect on baseline spatial reference memory. Mice among tested groups had

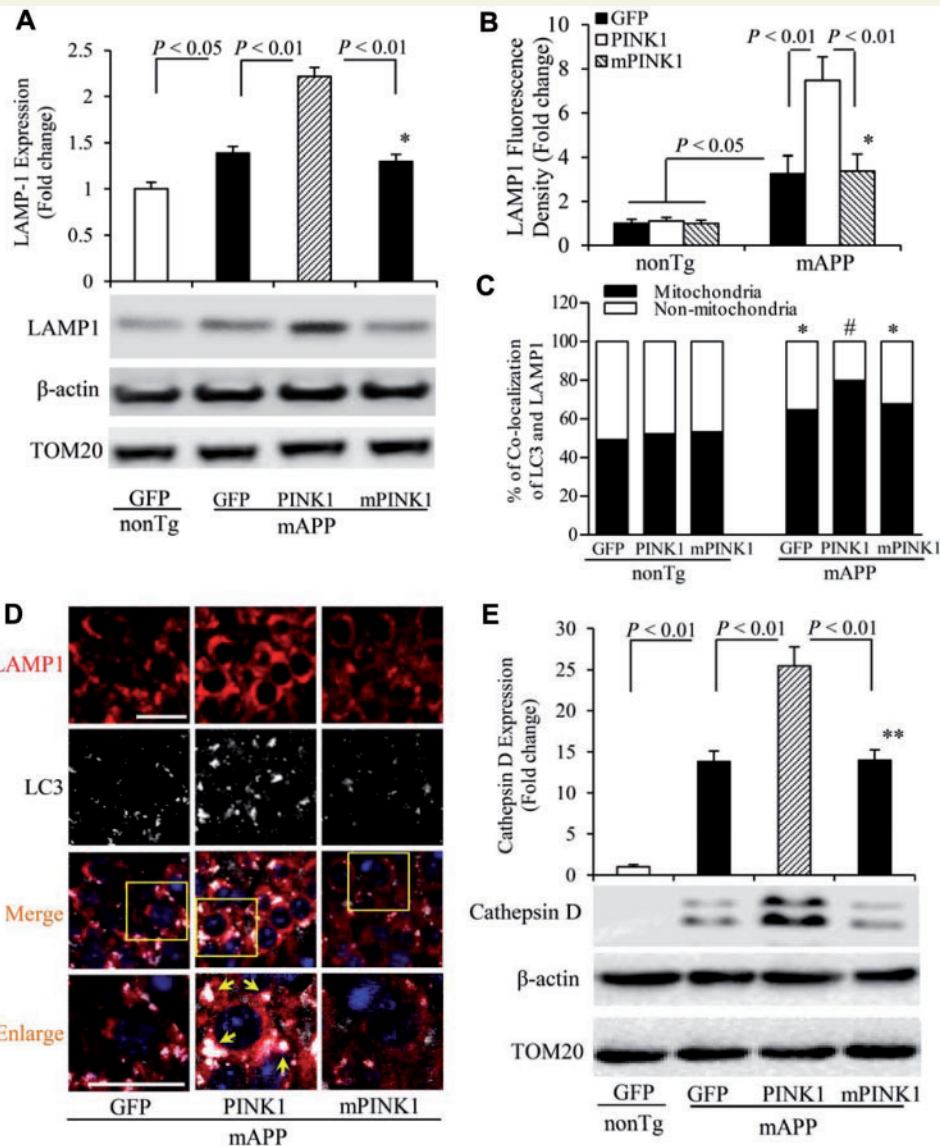


Figure 4 PINK1 promotes lysosomal recruitment in mAPP mice. (A) Representative immunoblots show immunoreactive bands for LAMP1 protein from hippocampal homogenates from indicated mice, and β -actin served as a loading control. The bar graph (A) presents quantification of immunoreactive bands for LAMP1 protein normalized to β -actin. $n = 3$ mice per group. (B–D) Quantification of LAMP1-positive immunostaining intensity is shown in B, and quantifications of LAMP1 in mitochondria and non-mitochondria are shown in C. Representative images of LAMP1 in hippocampi of indicated mice are shown in D, and the bottom panels show enlarged images of the field of interest indicated by the box in the top panels. (E) Representative immunoblots show immunoreactive bands for cathepsin D from hippocampal homogenates of indicated mice, and β -actin served as a loading control. The bar graph (E) presents quantification of immunoreactive bands for cathepsin D protein relative to β -actin. $n = 3$ –5 mice and 5–8 slides per group. * $P < 0.05$, ** $P < 0.01$ versus non Tg group in A–C and E. # $P < 0.01$ versus mAPP/GFP or mAPP/mPINK1 mice in C. Scale bars = 25 μ m in D.

similar swimming speed as measured by the visual swimming speed test (Supplementary Fig. 7B). Thus, the observed difference in spatial learning and memory is due to defects in cognition rather than differential motility or motivation. These data indicate that increased neuronal PINK1 expression and activity improve learning and memory in mAPP mice.

Given that synaptic loss is an early feature of Alzheimer's disease, we assessed the morphological integrity of synapses

in cultured Alzheimer's disease neurons carrying mutant human APP (mAPP neurons) and neurons exposed to amyloid- β . Introduction of lentivirus encoding human PINK1 into mAPP neurons resulted in an increase in the length of dendrites, compared to mAPP neurons transduced by lentivirus control vector encoding GFP (Fig. 6G). Synaptic density was quantified by measuring synaptophysin (pre-synaptic marker)-positive clusters attaching to dendrites. PINK1-transduced mAPP neurons displayed increased

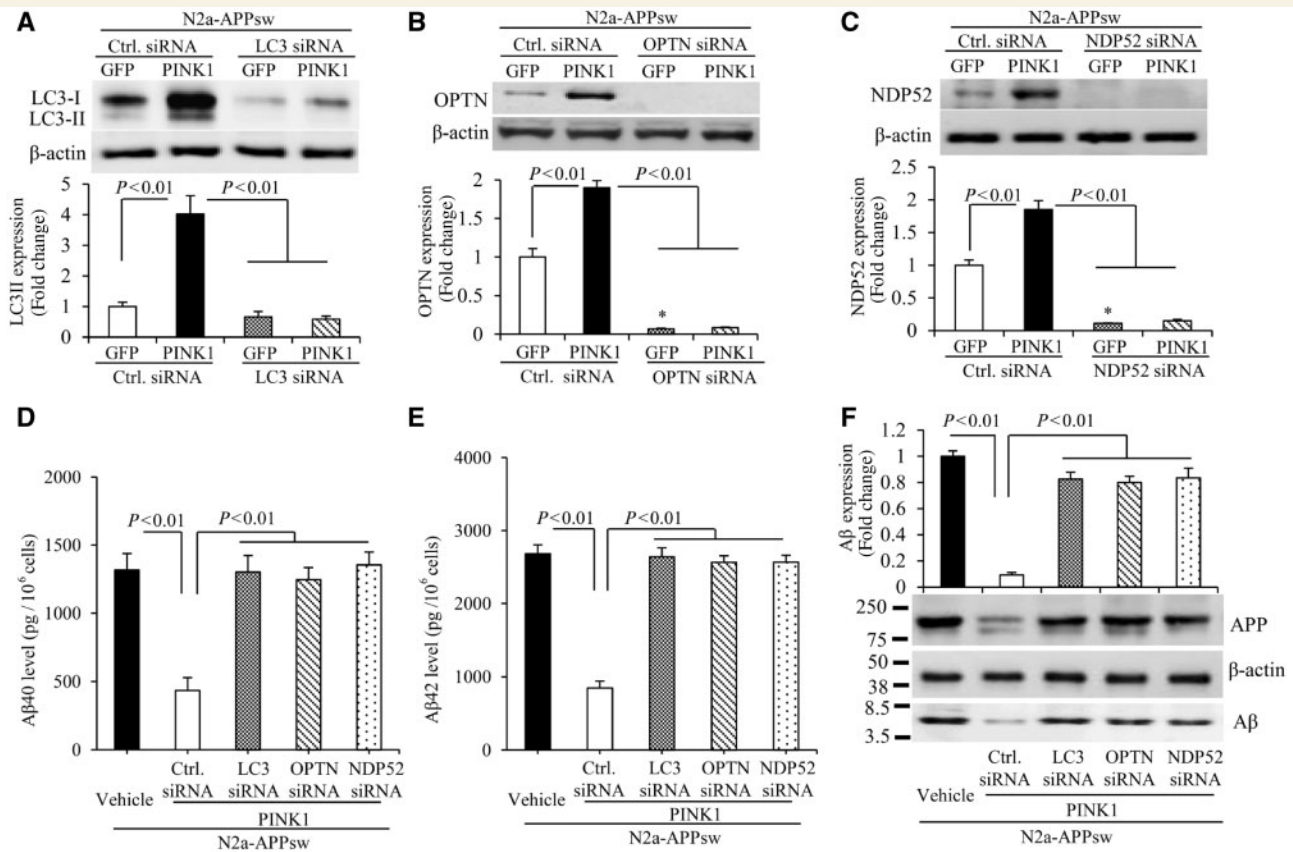


Figure 5 PINK1 decreases amyloid- β accumulation via activation of autophagy signalling in N2a-APPsw cells. (A–C)

Immunoblotting of N2a-APPsw cell lysates for LC3 (A), OPTN (B) and NDP52 (C) in the indicated groups of cells. N2a-APPsw cells were transduced with lentivirus encoding GFP or PINK1 and co-transfected with siRNA against LC3 (A), OPTN (B), NDP52 (C) and control siRNA. The expression levels of LC3-II, OPTN, and NDP52 were eliminated in cells treated with corresponding siRNA compared to control siRNA. Representative immunoblots show the immunoreactive bands for LC3-II (A), OPTN (B), and NDP52 (C), and β -actin served as a loading control. The bar graphs present quantification of immunoreactive bands for LC3-II (A), OPTN (B), and NDP52 (C) normalized to β -actin. * $P < 0.01$ versus control siRNA treatment group in B and C. $n = 3$ independent experiments of each group. (D–F) The levels of amyloid- β_{40} (A β_{40}) (D) and amyloid- β_{42} (A β_{42}) (E) in the N2a-APPsw cells transduced with lentivirus encoding GFP or PINK1 and co-transfected with siRNA against LC3, OPTN, NDP52 or control siRNA were measured by amyloid- β ELISA ($n = 4$). (F) Representative immunoblots show immunoreactive bands for APP and amyloid- β proteins in N2a-APPsw cell lysates with above treatment, and β -actin served as a loading control. The bar graph (F) presents quantification of immunoreactive bands for amyloid- β relative to β -actin. $n = 3$ independent experiments of each group.

synaptophysin density compared to vector-transduced mAPP neurons (Fig. 6H and I). Accordingly, loss of synaptophysin protein was completely restored in mAPP/PINK1 mice but not in mAPP/mPINK1 mice compared to mAPP/GFP mice (Fig. 6J).

PINK1-mediated autophagy signalling contributes to amyloid- β -induced mitochondrial and synaptic abnormalities

We then determined whether PINK1-mediated autophagy activity depends on OPTN or NDP52 autophagy signalling. Consistent with our observation obtained in mAPP/PINK1 mice (Fig. 3), PINK1-transduced primary neurons significantly elevated expression of autophagy proteins, including the active form of LC3-II, OPTN, and NDP52,

compared to vector-induced neurons (Fig. 7A–C and Supplementary Fig. 8A). These effects were significantly diminished by siRNA knockdown of LC3-II, OPTN or NDP52 when the PINK1-transduced neurons were exposed to amyloid- β stimuli, compared to scramble control siRNA-treated neurons (Fig. 7A–C and Supplementary Fig. 8A). In addition, confirmation of such suppressive effects on activation of LC3, OPTN, or NDP52 proteins was achieved by the second set of siRNA knockdown of these genes (Fig. 7A–C and Supplementary Fig. 8A). Accordingly, loss of LC3, OPTN, or NDP52 in PINK1-expressed neurons failed to protect against amyloid- β -induced alterations in mitochondrial and synaptic function as evidenced by increased ROS levels (Fig. 7D–F, Supplementary Fig. 8B and C, and representative images of MitoSOXTM Red staining in Supplementary Fig. 9A), reduced mitochondrial membrane potential (Fig. 7G, and representative images of TMRM red

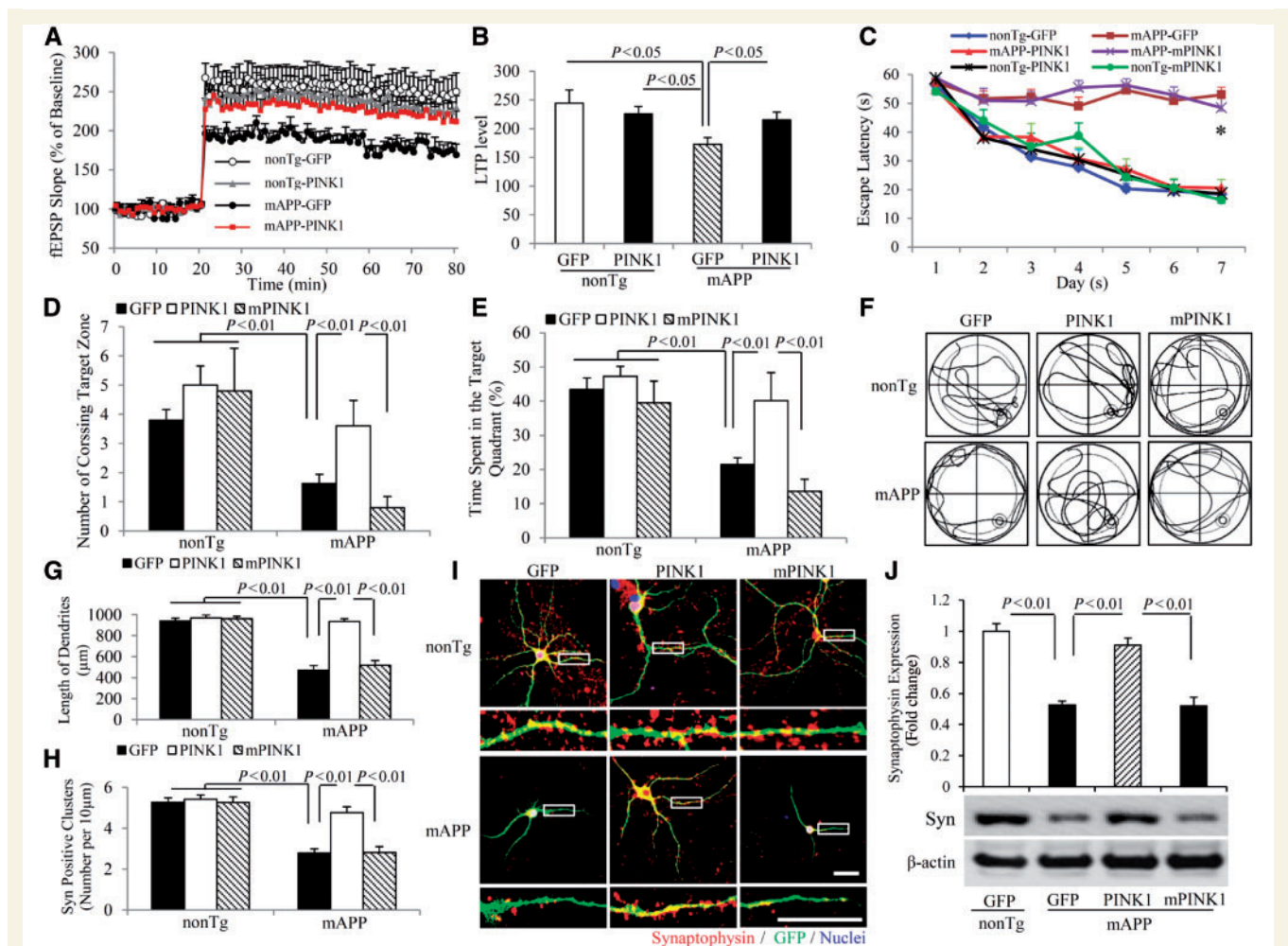


Figure 6 PINK1 reduces LTP and learning impairments. (A and B) LTP was recorded in hippocampal CA1 neurons of the indicated mice at 12 months of age. Data are presented as mean \pm standard error (SE). $n = 3$ –5 mice per group. (C–F) Effect of PINK1 on learning and memory in mAPP mice. (C) Mean escape latency to the hidden platform during each day of the training session. (D) Mean number of crossings of the target during probe trials. (E) Time spent in the quadrant with the hidden platform in probe trials. (F) Pattern of representative searching traces for the indicated transgenic (Tg) mice in search of the target. $n = 5$ –10 mice per group. * $P < 0.05$ compared to non-transgenic (nonTg)/GFP, non-transgenic/PINK1, non-transgenic mPINK1 and mAPP/PINK1 mice. (G–J) Effect of PINK1 on synaptic density. Hippocampal neurons cultured from non-transgenic and mAPP mice *in vitro* Day 5 (DIV 5) were transduced with lentivirus encoding PINK1, mPINK1 or GFP vector, respectively. Neurons at *in vitro* Day 14 were stained with Syn (red). Quantifications of the total length of dendrites (G) and the number of Syn-positive clusters (H) per micrometre of dendrite length are counted in the indicated groups of neurons. $n = 16$ –21 neurons per group. (I) Representative images of immunofluorescence staining for Syn (red) and GFP (green) in the indicated groups of cells. The lower panels show enlarged images of the interest area indicated by the box in the upper panels. (J) The bar graph presents quantification of immunoreactive Syn bands normalized to β -actin from the hippocampal homogenates of the indicated transgenic mice. The representative immunoblots show the immunoreactive bands for Syn protein, and β -actin served as a loading control. $n = 3$ independent experiments of each group.

staining in Supplementary Fig. 9B), decreased mitochondrial density (Fig. 7H, and representative images of SODII staining in Supplementary Fig. 9A), complex IV activity (Fig. 7I and Supplementary Fig. 8D), ATP levels (Fig. 7J and Supplementary Fig. 8E), neuronal process length, and synaptic density (Fig. 7K–M and Supplementary Fig. 8F–H). In parallel, knockdown by the second set of siRNA against LC3, OPTN, or NDP52 resulted in similar suppression of PINK1-induced protective effect on amyloid- β -triggered neuronal, mitochondrial and synaptic alterations (Supplementary Fig. 8). These data highlight

the role of PINK1-mediated autophagy signalling via OPTN and NDP52 in protection against amyloid- β -insulted mitochondrial damage and synaptic injury.

Interestingly, upon a higher concentration of amyloid- β (1 μ M) treatment, some mitochondria appeared as a doughnut-like shape in the cultured hippocampal non-transgenic neurons (Supplementary Fig. 10), whereas increasing PINK1 reduced the numbers and percentage of donut-shaped mitochondria (Supplementary Fig. 10). This result further validates that PINK1 rescues mitochondrial defects in amyloid- β -induced neurotoxicity.

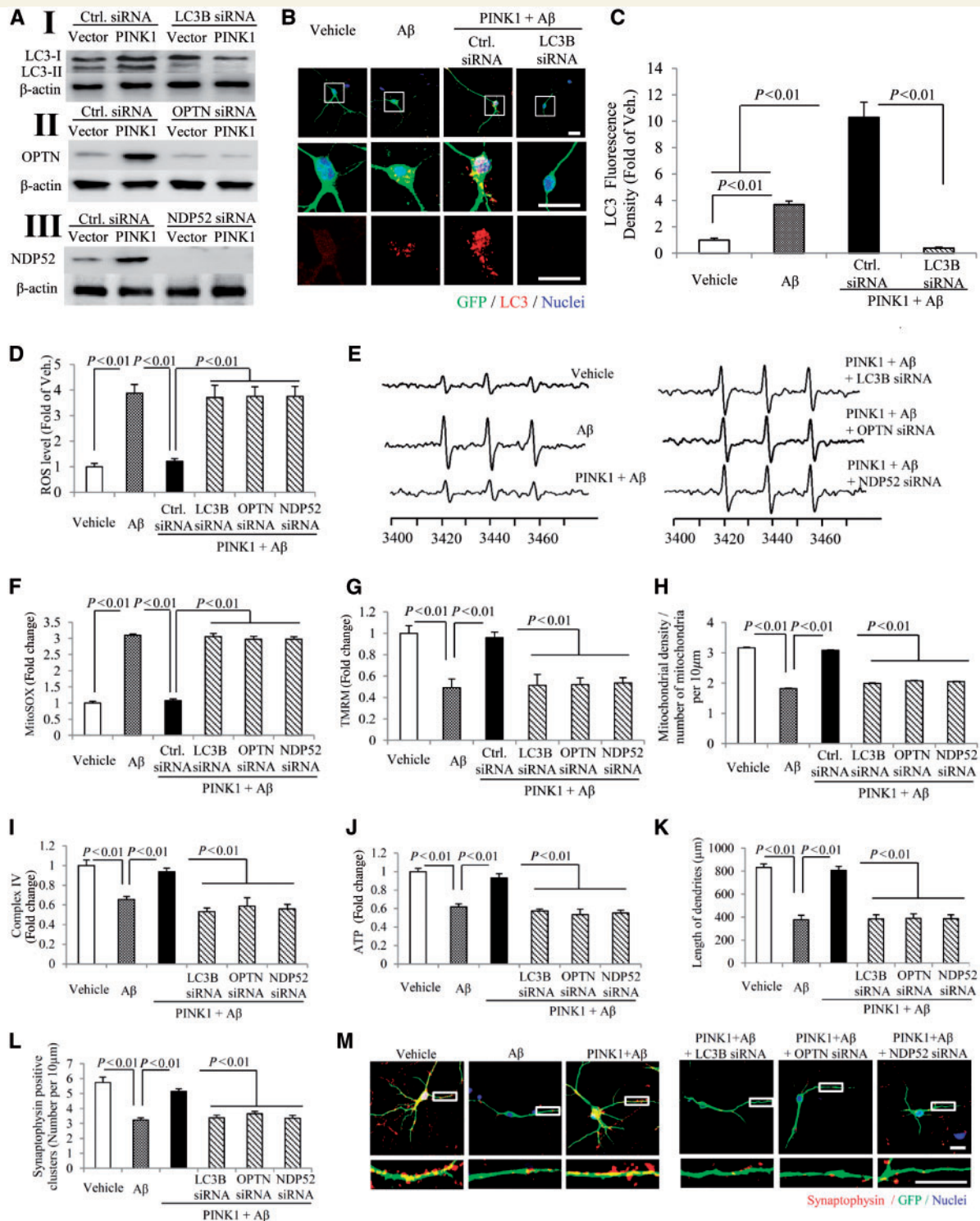


Figure 7 Effect of autophagy signalling on PINK1/amyloid- β -induced mitochondrial and synaptic alterations. (A) Immunoblotting of cell lysates for LC3 (I), OPTN (II) and NDP52 (III) in the indicated groups of cells. Hippocampal neurons at *in vitro* Day 10 (DIV 10) were transfected with PINK1 and co-transfected with siRNA against LC3, OPTN, NDP52 and control siRNA. The expression levels of LC3-II, OPTN, and NDP52 were eliminated in neurons treated with corresponding siRNA compared to control siRNA (I–III). $n = 3$ independent experiments. (B and C) Representative images for PINK1/GFP (green), LC3 (red) and nuclei (blue) in the indicated groups of neurons (B) and quantification of LC3-positive puncta (C). (D and E) Quantification of EPR spectra (D) and representative spectra of EPR (E) in the indicated neurons. (F–J) Quantifications of MitoSOX staining (F), TMRM staining (G), mitochondrial density (H), CcO activity (I), and ATP level (J) in the indicated neurons. (K–M) Quantifications of length of dendrites (K), synaptophysin-positive clusters (L), and representative images (M) for synaptophysin (red), GFP (green), and nuclei (blue). The lower panels show enlarged images of field of interest indicated by the box in the upper panels (M). $n = 10$ neurons for LC3-positive puncta counting, MitoSOX[™] and TMRM staining quantifications, $n = 10$ for EPR, $n = 3$ for CcO activity and ATP level, and $n = 20$ neurons for quantifications of length of dendrites and synaptophysin-positive clusters. Scale bars = 25 μm in B.

Effects of PINK1 deficiency on amyloid- β accumulation, mitochondrial function, LTP and learning memory in mAPP mice

To validate the role of PINK1 in amyloid pathology and neuronal function further, PINK1 knockout (*Pink1*^{-/-}) mice were crossed with mAPP mice to generate PINK1-deficient mAPP mice (mAPP/*Pink1*^{-/-}). Next we evaluated the effects of PINK1 deficiency on amyloid- β level, LTP and learning impairments and mitochondrial defects in mAPP mice at 5–6 months of age, an early stage of amyloid pathology prior to mitochondrial defect. As shown in Fig. 8A, PINK1 protein was absent in the hippocampi from both *Pink1*^{-/-} and mAPP/*Pink1*^{-/-} mice. Notably, amyloid- β ₄₀ and amyloid- β ₄₂ levels were significantly elevated by 2-fold in mAPP/*Pink1*^{-/-} hippocampi compared to mAPP mice at 5–6 months of age (Fig. 8B and C). The percentage of amyloid- β immunoreactive plaque loads was also 2-fold higher in mAPP/*Pink1*^{-/-} hippocampi (Supplementary Fig. 11A and B). Similarly, amyloid- β levels and amyloid- β plaque load were further elevated in mAPP/*Pink1*^{-/-} mice at 9 months of age (Supplementary Fig. 11C–F). These results demonstrated that lack of PINK1 promotes amyloid- β accumulation and amyloid pathology in mAPP mice. To validate the effect of PINK1 on amyloid- β accumulation further, we measured mouse endogenous amyloid- β in PINK1 null mice. Interestingly, amyloid- β ₄₂, a pathological toxic form of amyloid- β , was significantly elevated by 1.2-fold when compared to age-matched non-transgenic mice (Supplementary Fig. 11G and H) and implies the involvement of PINK1 in amyloid- β accumulation.

Furthermore, mAPP/*Pink1*^{-/-} mice displayed early deficit in synaptic function as shown by the reduction of LTP in CA1 neurons of hippocampus compared to mAPP mice and non-transgenic mice (Fig. 8D and E). There were no effects of PINK1 deficiency on the basal synaptic transmission (fEPSPs, Supplementary Fig. 12A) either in *Pink1*^{-/-} or mAPP/*Pink1*^{-/-} mice compared to non-transgenic mice. These data demonstrate that loss of PINK1 exacerbates/promotes synaptic injury prior to cerebral amyloid pathology/deposits.

Similarly, mAPP/*Pink1*^{-/-} mice displayed a much longer latency for locating the hidden platform during the training session (Fig. 8F) compared with *Pink1*^{-/-}, mAPP mice and non-transgenic littermates at 5–5.5 months of age (Fig. 8F–I). In the Probe test, mAPP/*Pink1*^{-/-} mice revealed the reduction in both the number of times crossing the target (Fig. 8G and I) and time in the target quadrant (Fig. 8H and I). These results demonstrate that mAPP/*Pink1*^{-/-} mice retained a poorer search strategy. Mice among tested groups had similar swimming speed as measured by the visual swimming speed test (Supplementary Fig. 12B), indicating that the observed difference in spatial learning and memory is due to defects in cognition. These results suggest an early perturbation of

learning and memory, consequent to loss of PINK1 in mAPP mice.

We evaluated the effect of PINK1 deficiency on mitochondrial function and oxidative stress further. At the early stage of 5–6 months of age, no significant changes in CcO activity, a key complex IV enzyme, and ATP level were observed in mAPP or *Pink1*^{-/-} mice compared to non-transgenic controls, while complex IV activity and ATP levels were significantly reduced only in mAPP/*Pink1*^{-/-} mice (Fig. 8J and K). The ROS levels were significantly increased in mAPP/*Pink1*^{-/-} mice compared to mAPP mice (Fig. 8L and M). Thus, lack of PINK1 exacerbates mitochondrial perturbation and oxidative stress relevant to amyloid- β pathogenesis.

PINK1 kinase activity is essential for reducing amyloid- β accumulation and rescuing amyloid- β -induced mitochondrial defects and synaptic abnormalities

Finally, to validate the role of PINK1 kinase activity on amyloid- β accumulation and synaptic and mitochondrial alterations, we examined the effects of a triple kinase dead mutants of PINK1 (K219A, D362A and D384A, triple-mPINK1) as a classical kinase dead mutant form (Beilina *et al.*, 2005; Pridgeon *et al.*, 2007) in mAPP neurons. Compared to wild-type PINK1 transduced-mAPP neurons, triple-mPINK1 transduced mAPP neurons failed to attenuate amyloid- β accumulation and had no protective effect on synaptic loss (Supplementary Fig. 13D–G) and mitochondrial dysfunction (Supplementary Fig. 13H–I). These results were similar to those from the mutant PINK1-L347P used in this study. Therefore, PINK1 kinase activity is required for the protection from amyloid pathology and amyloid- β -mediated mitochondrial and synaptic perturbation.

Discussion

Alzheimer's disease is a neurodegenerative disorder clinically characterized by progressive dementia and pathologically characterized by early mitochondrial and synaptic degeneration associated with cerebral and mitochondrial amyloid- β accumulation and intra-neuronal fibrillary tangles with hyperphosphorylated tau (Selkoe, 2002; Lin and Beal, 2006; Du *et al.*, 2010, 2012; Yu *et al.*, 2016). The pathogenesis of Alzheimer's disease involves multiple pathways, which may explain the limited clinical benefits of simply removing amyloid- β from patients. For example, early synaptic mitochondrial damage may cause damage to the brain for many years before an Alzheimer's patient experiences memory loss (Du *et al.*, 2010, 2012). In the present study, we have provided substantial evidence of multiple PINK1-dependent pathways relevant to amyloid- β clearance,

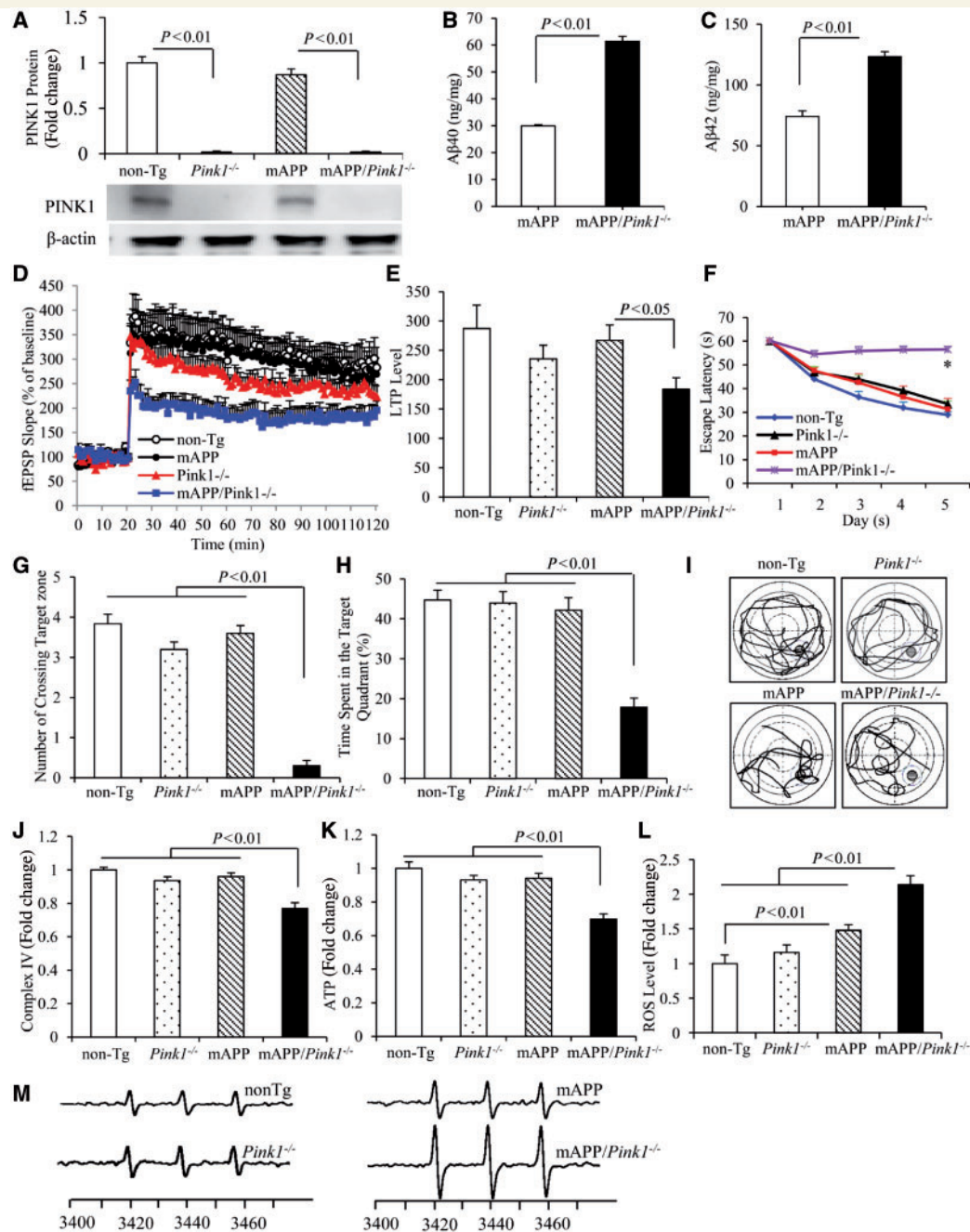


Figure 8 PINK1 deficiency accelerates amyloid- β accumulation and exaggerates abnormalities in LTP, learning and memory, and mitochondrial function in mAPP mice. (A) Representative immunoblots show immunoreactive bands for PINK1 protein from hippocampal homogenates of non-transgenic (non-Tg) *PINK1* knockout (*Pink1*^{-/-}), mAPP, and mAPP/*Pink1*^{-/-} mice at 5–6 months of age, and β -actin served as a loading control. The bar graph (A) presents quantification of immunoreactive bands for PINK1 protein normalized to β -actin. $n = 3$ mice per group. (B and C) Amyloid- β levels in hippocampi of mAPP and mAPP/*Pink1*^{-/-} mice (5–6 months old) were measured by amyloid- β ELISA. $n = 6$ mice per group. (D and E) LTP was recorded in hippocampal CA1 neurons of the indicated mice. Data are presented as mean \pm SE. $n = 3$ –5 mice per group. (F–I) Effect of *PINK1* knockout on learning and memory in mAPP mice. Learning and memory were tested using a Morris water maze (MWM) in the indicated groups of mice. (F) Mean escape latency to the hidden platform during each day of the training session. * $P < 0.01$ compared to non-transgenic, *Pink1*^{-/-} and mAPP mice. (G) Mean number of crossings of the target during probe trials. (H) Time spent in the quadrant with the hidden platform in probe trials. (I) Pattern of representative searching traces for the indicated transgenic (Tg) mice in search of the target. $n = 5$ –10 mice per group. (J and K) CcO (complex IV, J) activity and ATP levels (K) in brain hippocampal tissues of the indicated mice. $n = 6$ mice per group. Quantification of EPR spectra (L) in the indicated transgenic mice. Data are expressed as fold increase relative to non-transgenic mice. (M) Representative spectra of EPR. The peak height in the spectrum indicates levels of ROS. $n = 5$ –6 mice per group.

Alzheimer's disease-specific mitochondrial and synaptic properties, and cognitive function in the Alzheimer's disease mouse model *in vivo* and *in vitro*. Importantly, we also demonstrate that PINK1 kinase activity is crucially involved in amyloid pathology and Alzheimer's disease- and amyloid- β -mediated mitochondrial and synaptic perturbation. Finally, lack of PINK1 promotes and exacerbates amyloid accumulation accompanied by abnormalities in synaptic and cognitive function as well as mitochondrial function, before such alterations were observed in mAPP mice.

First, we observed that expression of PINK1 is associated with amyloid pathology. PINK1 expression is significantly decreased in Alzheimer's disease-affected human brains and Alzheimer's disease mice overexpressing human amyloid- β . Given that amyloid pathology and oxidative stress are age-related, we examined the direct effect of amyloid- β or oxidative stress on PINK1 expression. PINK1 expression level was significantly increased in neurons treated with amyloid- β for 12 h or with H₂O₂ for 1.5–6 h, and then declined after 24 h of amyloid- β treatment or 9–12 h of H₂O₂ treatment. These data suggest that prolonged stimulation with amyloid- β or oxidative stress results in a significant reduction of PINK1 expression (Supplementary Figure. S14A and B). Consistent with these results *in vitro*, PINK1 expression levels correlate with amyloid pathology as shown by age-associated changes in PINK1 levels (Supplementary Fig. 14C). Compared to 3-month-old mAPP mice, PINK1 was first elevated in 6-month-old and then declined in 12–20-month-old mAPP mice with severe amyloid pathology and mitochondrial defects. These data suggest that amyloid- β and oxidative stress activate the induction of PINK1 at the early stage of these toxic stimuli to protect cells from stress-induced perturbation. However, sustained toxic stimuli eventually suppress PINK1 expression, which may explain the reduction in PINK1 in Alzheimer's disease brain and amyloid- β -rich mAPP mice. Compromised PINK1 function may cause the observed amyloid- β - and Alzheimer's disease-related impairment in mitochondrial function and ability of clearance and degradation of amyloid- β . Indeed, restoring/increasing PINK1 function in amyloid- β -enriched brains improves mitochondrial and synaptic function and eliminates amyloid pathology in the Alzheimer's disease mouse model *in vivo* and *in vitro*. Conversely, genetic deletion or knockdown of PINK1 exacerbates amyloid- β -mediated detrimental effects. Previous studies reported that PINK1 accumulates on depolarized mitochondria in cells cultured under acute induction of pathological condition, such as depolarized mitochondria by exposing cells to CCCP (Narendra *et al.*, 2010; Meissner *et al.*, 2011). These results are consistent with our observations in *in vitro* cultured neurons exposed to amyloid- β and oxidative stress insults (Supplementary Fig. 14A and B), and *in vivo* mAPP mice at early stages of amyloid pathology (Supplementary Fig. 14C).

Second, we have provided substantial evidence of the effects of PINK1 on amyloid pathology from *in vitro* and *in vivo* not only in PINK1 overexpression and but also in PINK1 deficiency of mAPP mice. There are several

potential mechanisms underlying PINK1-involved amyloid- β accumulation and amyloid pathology through amyloid- β production and clearance/degradation. We have demonstrated that PINK1 overexpression or depletion suppressed or enhanced ROS production in mAPP mice (Fig. 2E–H), together with improvement in early deficits in mitochondrial function (Fig. 2A–D). These results suggest that PINK1-mediated mitochondrial ROS production links to amyloid pathology in both PINK1/mAPP (Figs 1 and 2) and PINK1KO/mAPP mice (Fig. 8J–M and Supplementary Fig. 11). Indeed, scavenging mitochondria-derived ROS by mito-TEMPO not only blunt ROS production but also largely reduced cellular amyloid- β and APP levels in human amyloid- β -producing N2a-APP cells carrying human mutant APP gene in a dose-dependent manner (data not shown). Furthermore, mito-TEMPO robustly inhibited amyloid- β -induced activation of NF- κ B by suppressing phosphorylation of NF- κ B subunits p50 and p65 together with reduced amyloid- β and APP levels (data not shown). Oxidative stress decreases the activity of alpha-secretase while promoting the expression and activation of β - and γ -secretase, enzymes critical to the generation of amyloid- β from APP (Tamagno *et al.*, 2002, 2008; Chen *et al.*, 2008; Shen *et al.*, 2008; Quiroz-Baez *et al.*, 2009; Oda *et al.*, 2010; Yoo *et al.*, 2010). Oxidative stress mediated the activation of transcription factors including the redox-sensitive activation protein (AP1) and NF- κ B enhances β -site APP cleaving enzyme 1 (BACE1) (Sambamurti *et al.*, 2004; Chami *et al.*, 2012; Chen *et al.*, 2012). In fact, the promoter and 5' untranslated region of the BACE gene contain binding sites for multiple transcription factors, including NF- κ B, thereby activation of NF- κ B by oxidative stress may in turn promote BACE expression (Sambamurti *et al.*, 2004). PINK1 has been shown to protect against oxidative stress by targeting mitochondrial chaperone (Pridgeon *et al.*, 2007). Thus, the PINK1/ROS/NF- κ B pathway could contribute to alter APP/amyloid- β levels, possibly through APP transcription or α -, β -, or γ -secretases. Additional evidence of the impact of PINK1 signalling on NF- κ B activation is demonstrated by the activation of the autophagy receptor OPTN. It has been reported that OPTN knockdown results in NF- κ B activation (Akizuki *et al.*, 2013). Therefore, activation of NF- κ B due to a loss of PINK1/OPTN function could also be responsible for increased APP and amyloid- β . We demonstrated that knockdown of OPTN resulted in increased APP and amyloid- β in PINK1-expressing mAPP/amyloid- β -producing neurons (Fig. 5).

Furthermore, PINK1-mediated autophagy signalling to enhance mitochondrial clearance and lysosome activity may also contribute to amyloid- β degradation and clearance. Activation of PINK1 upregulates expression levels of LC3 and autophagy receptors (OPTN and NDP52) in amyloid- β -enriched mAPP brain. Blockade of these autophagy signals abolishes the protective effects of PINK1 on amyloid- β accumulation, suggesting that PINK1-activated autophagy pathways via OPTN and NDP52 are responsible for reducing

amyloid pathology. We also observed that augmenting PINK1 expression activates autophagy/lysosome pathway by upregulation of lysosome-related protein such as LAMP1 and cathepsin D. The PINK1-involved autophagosome–lysosome pathway (Nguyen *et al.*, 2016) could be another possible mechanism underlying amyloid- β metabolism and clearance in mAPP mice.

It has been shown that amyloid- β localizes in the extracellular and intracellular compartments, including cell surfaces, endosome, lysosome, and outer mitochondrial membrane (Choy *et al.*, 2012; Sannerud *et al.*, 2016), which plays a role in cellular amyloid- β accumulation and neuronal perturbation. Emerging evidence has highlighted the role of mitochondrial amyloid- β in Alzheimer's disease pathogenesis. Amyloid- β progressively accumulates in the mitochondria of the Alzheimer's disease brain and several transgenic Alzheimer's disease mouse models overexpressing amyloid- β (Hirai *et al.*, 2001; Fernandez-Vizarra *et al.*, 2004; Lustbader *et al.*, 2004; Caspersen *et al.*, 2005; Crouch *et al.*, 2005; Devi *et al.*, 2006; Manczak *et al.*, 2006; Gillardon *et al.*, 2007; Du *et al.*, 2008, 2012; Hansson Petersen *et al.*, 2008; Yao *et al.*, 2009; Abramowski *et al.*, 2012), including transgenic Alzheimer's disease mice expressing human APP_{swe}. Increased degradation of clearance of mitochondrial pool of amyloid- β by activating presequence protease (PreP), a novel mitochondrial amyloid- β degrading enzyme, not only reduced mitochondrial amyloid- β accumulation but also significantly affects total cerebral amyloid- β levels (Fang *et al.*, 2015), suggesting that mitochondrial amyloid- β is not just a 'spilling over' from cellular aggregation and that PreP/amyloid- β in mitochondria also has an important regulating effect on total brain amyloid- β levels (Fang *et al.*, 2015). Given that mitochondria function as guardians in the cytosol pathway (Ruan *et al.*, 2017), and that exogenous or intracellular amyloid- β is capable of direct transport into mitochondria via mitochondrial channel proteins such as TOMM40 (Hansson Petersen *et al.*, 2008), endoplasmic reticulum/mitochondria contact (Caspersen *et al.*, 2005; Hedskog *et al.*, 2013), the receptor for advanced glycation end product (RAGE), endoplasmic reticulum/mitochondrial contact (Caspersen *et al.*, 2005; Hedskog *et al.*, 2013), or by an unknown mechanism, the mitochondrial pool of amyloid- β may undergo dynamic changes in different intracellular compartments, contributing to the balance of intracellular/extracellular amyloid- β accumulation.

In summary, possible mechanisms underlying PINK1-altered amyloid- β accumulation are: (i) activation of β APP transcription via NF- κ B/oxidative stress induced by amyloid- β and mitochondrial damages; (ii) modulating APP processing by altering α -, β -, or γ -secretase activities via NF- κ B/oxidative; and (iii) activation of PINK1-mediated autophagy signal promoting amyloid- β degradation and clearance, which could have an impact on crosstalk of mitochondria with cerebral and mitochondrial amyloid- β metabolism. Detailed mechanisms of how PINK1 affects APP/amyloid- β metabolism and production or processing require further investigations.

Third, PINK1 is an important regulator of mitochondrial quality control, which is essential for maintaining proper mitochondrial function and integrity by regulating the balance between the elimination of damaged mitochondria through autophagy, and augmentation of mitochondrial bioenergy (Lazarou *et al.*, 2015). Alzheimer's disease-affected brains reveal a decrease in healthy mitochondria and an increase in dysfunctional and damaged mitochondria, leading to disruption of mitochondrial homeostasis (Du *et al.*, 2010; Gan *et al.*, 2014a; Fang *et al.*, 2015; Yu *et al.*, 2016). We demonstrate that restoring PINK1 expression/activity protects against amyloid- β -induced aberrant mitochondrial function as shown by increased membrane potential, key respiratory enzyme activity and ATP levels. Increased PINK1 function significantly suppresses mitochondrial oxidative stress and ROS generation. Blockade of autophagosome LC3-II activation and autophagy receptor (OPTN or NPD52)-activated signalling failed to rescue amyloid- β -mediated aberrant mitochondrial and synaptic function through PINK1, suggesting that PINK1-dependent autophagy signalling is responsible for suppression of amyloid- β -induced perturbation. Lysosomes play a fundamental role in the autophagic pathway by degrading damaged organelles and aggregated protein such as damaged mitochondria and amyloid- β (Saido and Leissring, 2012; Orr and Oddo, 2013). Elevated levels of both LAMP1 and cathepsin D in mAPP/PINK1 mice suggest that PINK1 enhances autophagy-lysosome activity, promoting clearance and degradation of damaged mitochondria and amyloid- β .

Finally, PINK1-mediated mitochondrial function has been linked to synaptic plasticity, as we observed that PINK1 overexpression significantly improved synaptic and cognitive function in a mouse model of Alzheimer's disease. Our previous studies showed that the addition of ROS-scavenging enzymes alleviated amyloid- β -induced decline in LTP (Du *et al.*, 2008; Ma *et al.*, 2011). Given that PINK1 protects against ROS accumulation and generation, the loss of antioxidant effects of PINK1 may also be a mechanism underlying the impairments in synaptic plasticity and memory in Alzheimer's disease (Liu *et al.*, 2003; Serrano and Klann, 2004; Esposito *et al.*, 2006; Du *et al.*, 2008). Mitochondria produce most of the ATP and control the local Ca^{2+} homeostasis required for maintaining synaptic function, phosphorylation, and synaptic plasticity (Rizzuto *et al.*, 2012). The active concentration of mitochondria in specific neuronal regions, such as growth cones and synapses (Li *et al.*, 2004; Chang *et al.*, 2006; Fang *et al.*, 2016a, b), is important for neuronal function. Mitochondria become dysfunctional through the permeability transition induced by the synergistic effects of oxidative stress and dysregulation of cytosolic free Ca^{2+} (Xie, 2004). Indeed, PINK1 overexpression suppressed cyclophilin D expression in mAPP/PINK1 brains (data not shown); therefore, PINK1 may be involved in other mechanisms such as impaired mitochondrial transition pore (Du *et al.*, 2008, 2011), Ca^{2+} -regulated signalling pathways, oxidative stress-mediated kinase systems and activation of transcription factors (Vitolo *et al.*, 2002; Xie, 2004; Origlia *et al.*, 2008; Du *et al.*, 2014).

Notably, to address whether PINK1 kinase activity is required for amyloid pathology and amyloid- β -induced defects, we examined the effect of inactive mutant forms of PINK1, mPINK1 (L347P, defined as a loss-of-function form of PINK1) or triple-mPINK1 (PINK1 kinase dead mutant, K219A, D362A and D384A) as a classical kinase dead mutant form that is stable but lacking PINK1 kinase activity (Beilina *et al.*, 2005; Pridgeon *et al.*, 2007) on Alzheimer's disease pathology, mitochondrial function, synaptic density and learning and memory abilities. PINK1 overexpression significantly reduced amyloid- β accumulation, alleviated mitochondrial and synaptic alterations, and improved learning and memory. The mutant PINK1 without kinase activity had no protective effects on these parameters. Thus, PINK1 activity is essential for the maintenance of mitochondrial and cognitive function as well as amyloid- β levels reflecting the balance between rates of production and clearance.

Using genetically manipulated PINK1 expression (gain and loss of PINK1 function) and inactive mutant PINK1 without kinase activity as a control to validate the role of PINK1 kinase activity, we comprehensively evaluated the effects of PINK1 on mitochondrial function. Our results clearly demonstrated the mitochondrial improvements dependent on PINK1 kinase activity. Furthermore, mitochondrial improvements disappeared by genetically deletion or knockdown of *PINK1*.

Mitochondrial dynamics are important for maintaining mitochondrial morphology, distribution and function. Amyloid- β or Alzheimer's disease-derived mitochondria contributes importantly to the impaired mitochondrial dynamics by increased mitochondrial fission protein Drp1 or fusion MFN2 (Wang *et al.*, 2008, 2009a, b; Gan *et al.*, 2014a, b). Blocking excessive mitochondrial fission Drp1 or recruiting fusion MFN2 improves mitochondrial function (Gan *et al.*, 2014a, b; Huang *et al.*, 2015). This suggests that modulating mitochondrial dynamics genes that control mitochondrial fission or fusion to maintain normal balance of mitochondrial dynamics have protective effects on mitochondrial defects relevant to amyloid- β toxicity. In view of the involvement of PINK1 in mitochondrial dynamics showing overexpression of PINK1 reduces Drp1-induced mitochondrial fission (Cui *et al.*, 2010), PINK1 could be directly or indirectly involved in amyloid- β -mediated imbalance between mitochondrial fission and fusion rates. The direct link of PINK1 to amyloid- β -induced abnormal mitochondrial dynamics requires further investigation.

Parkin serves as a downstream substrate for PINK1. Damaged mitochondria are removed by autophagy after activation of PINK1 kinase and the E3 ubiquitin ligase parkin (Narendra *et al.*, 2008, 2010). Parkin amplifies the mitophagy signal triggered by PINK1. It is possible to have the similar protective effect on mitochondrial function by enhancing parkin-involved mitochondrial clearance. To clarify, the present study is to elucidate PINK1 signalling independent on parkin in amyloid- β -induced mitochondrial

perturbation by recruiting the autophagy receptors OPTN and NDP52 (Lazarou *et al.*, 2015).

Taken together, we have provided substantial evidence showing PINK1-dependent amyloid- β pathology and mitochondrial alterations from *in vitro* neuronal and *in vivo* transgenic mouse model. Our studies have clearly demonstrated that downregulation of PINK1 is associated with Alzheimer's disease pathology and oxidative stress. Restoring the expression and activity of neuronal PINK1 significantly reduced amyloid- β accumulation and improved mitochondrial function and synaptic plasticity. As a result, cognitive function decline in Alzheimer's disease mice was substantially reversed by PINK1 overexpression. Importantly, non-functional mutant human PINK1 lacking kinase activity has no such protective effects against Alzheimer's disease-related mitochondrial defects or behaviour changes in both *in vitro* and *in vivo* settings. Lack of PINK1 accelerates amyloid- β accumulation and exaggerates mitochondrial and synaptic damages. Notably, our findings highlight a novel mechanism by which PINK1 kinase activity-dependent signalling promotes rescue of amyloid pathology and amyloid- β -mediated mitochondrial and synaptic dysfunction in a manner requiring activation of autophagy receptor OPTN or NDP52. We propose that compromised PINK1 function induced by ROS or amyloid- β in the Alzheimer's disease milieu perturbs mitochondrial clearance, leading to imbalanced mitochondrial homeostasis via increased accumulation of damaged mitochondria and impaired degradation of proteins including amyloid- β , which contributes importantly to amyloid and mitochondrial pathology and synaptic degeneration in Alzheimer's disease. Thus, activation of PINK1 may present a new therapeutic approach for halting Alzheimer's disease progression at the early stage through mitochondrial quality control combined with eliminating amyloid pathology.

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Supplementary material

Supplementary material is available at *Brain* online.

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