Deregulation of mitochondrial F1FO-ATP synthase via OSCP in Alzheimer’s disease

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F1FO-ATP synthase is critical for mitochondrial functions. The deregulation of this enzyme results in dampened mitochondrial oxidative phosphorylation (OXPHOS) and activated mitochondrial permeability transition (mPT), defects which accompany Alzheimer’s disease (AD). However, the molecular mechanisms that connect F1FO-ATP synthase dysfunction and AD remain unclear. Here, we observe selective loss of the oligomycin sensitivity conferring protein (OSCP) subunit of the F1FO-ATP synthase and the physical interaction of OSCP with amyloid beta (Aβ) in the brains of AD individuals and in an AD mouse model. Changes in OSCP levels are more pronounced in neuronal mitochondria. OSCP loss and its interplay with Aβ disrupt F1FO-ATP synthase, leading to reduced ATP production, elevated oxidative stress and activated mPT. The restoration of OSCP ameliorates Aβ-mediated mouse and human neuronal mitochondrial impairments and the resultant synaptic injury. Therefore, mitochondrial F1FO-ATP synthase dysfunction associated with AD progression could potentially be prevented by OSCP stabilization.
Clinical observations have suggested that mitochondrial dysfunction is among the earliest manifestations of Alzheimer's disease (AD) and constitutes a hallmark pathological feature of this neurological disorder. Previous studies have also identified impaired mitochondrial oxidative phosphorylation (OXPHOS) as a feature of mitochondrial defects in AD individuals and AD animal models. Compromised mitochondrial OXPHOS efficiency results in lowered mitochondrial bioenergetics and exaggerated production of free radicals. Indeed, ATP deficiency and oxidative damage are characteristics of brains from AD patients. Impaired mitochondrial OXPHOS efficiency is closely associated with dysfunction of mitochondrial respiratory enzymes, including mitochondrial complex I to IV, as well as the defect of F1FO-ATP synthase. Previous studies have mostly focused on the dysfunction of mitochondrial complex IV in AD. However, this concept has been recently challenged in AD individuals and AD animal models. In fact, in addition to mitochondrial respiratory enzyme defects, increasing evidence implicates the dysfunction of mitochondrial F1FO-ATP synthase in AD.

The mitochondrial F1FO-ATP synthase, which includes three components (F1, FO and the peripheral stalk), is a critical mitochondrial OXPHOS enzyme involved in the regulation of mitochondrial ATP production and in the maintenance of the mitochondrial membrane potential. The F1FO-ATP synthase can both synthesize ATP and degrade ATP when operating in reverse to generate proton backflow, increasing mitochondrial membrane potential when it is critically low. In addition to its vital function in mitochondrial OXPHOS, recent studies have shown that this enzyme contributes to the formation of the mitochondrial permeability transition pore (mPTP) through the interaction of its oligomycin sensitivity conferring protein (OSCP) subunit with cyclophilin D (CypD), the key regulator of mPTP. Extensive formation of mPTP is a severe pathological event that leads to collapsed mitochondrial membrane potential (mΔΨ), decreased mitochondrial OXPHOS capacity, elevated reactive oxygen species (ROS) generation and, eventually, cell death. Indeed, mPTP activation is thought to be a key mechanism of mitochondrial stress in AD and has been proposed to underlie its characteristic synaptic dysfunction and cognitive decline. Given its role in mitochondrial OXPHOS and mPTP formation, the deregulation of mitochondrial F1FO-ATP synthase may predispose to compromised OXPHOS efficiency and sensitized mPTP formation, which are two hallmark mitochondrial defects in AD. However, to date information on the dysfunction of F1FO-ATP synthase in AD has remained limited. Accordingly, the underlying molecular mechanisms causing the defect of this enzyme in AD remain unresolved.

In this study, we compare the levels of major F1FO-ATP synthase subunits in the brains from AD individuals, mild cognitive impairment (MCI) patients and non-AD control subjects, and find a selective decrease in the levels of OSCP during the progression of AD. We also find that, in a mouse model of AD that overexpresses the human form of amyloid beta (Aβ), the loss of OSCP is more prominent in synaptic mitochondria. In addition to OSCP loss, we also detect a direct physical interaction between OSCP and Aβ in the brains from AD cases, as well as in AD mice. Such OSCP aberrations disrupt F1FO-ATP synthase stability, leading to severe mitochondrial dysfunction and synaptic injury. Further in vivo studies show the deleterious impact of F1FO-ATP synthase dysfunction on the development of mitochondrial defects in AD mice. Importantly, the restoration of OSCP ameliorates the Aβ-mediated mitochondrial dysfunction and synaptic injury in mouse or human neurons, further supporting the role of OSCP deregulation in mitochondrial dysfunction under Aβ-rich conditions. Therefore, mitochondrial F1FO-ATP synthase dysfunction that results from OSCP aberrations may constitute a primary AD event that can be prevented by OSCP protection, suggesting OSCP as a potential new therapeutic target for AD.

Results

Loss of OSCP in AD subjects and 5xFAD mice. To examine changes of F1FO-ATP synthase in AD brains, we used immunoblotting to compare the expression levels of the major subunits of the mitochondrial F1FO-ATP synthase in protein extracts from the temporal lobe of non-AD, MCI, and AD subjects (Supplementary Table 1). Among the F1FO-ATP synthase subunits tested here, OSCP was slightly reduced in MCI brains, while this decrease was exaggerated in AD patients (Fig. 1a). Notably, the expression levels of the other major F1FO-ATP synthase subunits, including a, b, c, z, and β were not significantly changed in either MCI or AD brains (Supplementary Fig. 1a,b), implying that the OSCP loss is not a likely result of F1FO-ATP synthase reduction in AD. Next, we performed histological studies on brain sections from the temporal lobes of AD and control subjects. Our results showed a dramatic reduction of OSCP expression in neurons from the temporal lobe of AD individuals. A similar reduction of OSCP also occurred in an AD mouse model (5xFAD mice). To determine whether synaptic mitochondria are more vulnerable to OSCP loss under conditions of elevated Aβ, we separated synaptic mitochondria from 5xFAD mice at 4 and 9 months old that mimic the amyloidopathy and behavioral changes typical of the early-, and middle to late-stages of AD, respectively. The purity of isolated mitochondria was verified by means of the detection of high abundance of the mitochondrial protein voltage-dependent anion channel (VDAC) without contamination of β-actin or synaptic vesicles (Supplementary Fig. 2). Synaptic mitochondria demonstrated a significant OSCP decrease in young 5xFAD mice, and this OSCP loss was exacerbated with age (Fig. 1c). However, a significant OSCP reduction in nonsynaptic mitochondria was detected only in old 5xFAD mice (Fig. 1d), suggesting that in Aβ-rich environments synaptic mitochondria are more susceptible to OSCP loss. In sharp contrast, the expression levels of a, b and c, as well as z and β subunits remained unaltered in either synaptic or nonsynaptic mitochondria from 5xFAD mice at any tested age (Supplementary Fig. 3a–d), indicating that OSCP expression is selectively suppressed in parallel with the manifestation of AD-like symptoms in 5xFAD mice.

OSCP loss impacts mitochondrial and synaptic function. To investigate the impact of OSCP loss on neuronal mitochondrial function we genetically downregulated OSCP expression in primary cultured mouse neurons by using specific OSCP shRNA. Nontarget shRNA was used as control. Quantitative analysis showed substantially decreased OSCP levels in OSCP knockdown neurons (OSCP KD, Fig. 2a). The key functions of mitochondrial F1FO-ATP synthase are to maintain mΔΨ and to produce ATP. To determine the impact of OSCP loss on F1FO-ATP synthase function, we first measured mΔΨ in OSCP knockdown neurons via staining with tetramethylrhodamine methyl ester (TMRM), an indicator of mΔΨ (ref. 5). In comparison with control neurons, OSCP knockdown neurons exhibited significantly decreased intensities of TMRM (Fig. 2b), suggesting a collapsed mΔΨ in response to OSCP loss. Furthermore, in agreement with reduced mΔΨ, ATP generation was markedly reduced in OSCP knockdown neurons (Fig. 2c), suggesting deregulation of mitochondrial OXPHOS efficiency. In view of the close...
correlation between compromised OXPHOS efficiency and mitochondrial ROS production, we next compared mitochondrial ROS levels by staining with MitoSox Red, a specific fluorescent indicator of mitochondrial superoxide abundance. Our results showed significantly elevated MitoSox Red intensity in mitochondria from OSCP knockdown neurons (Fig. 2d). In addition, in comparison to the control neurons OSCP downregulated neurons also displayed significantly reduced mitochondrial population in neurites (Fig. 2e).

OSCP deregulation has also been previously linked to mPTP formation. To determine whether the loss of OSCP sensitizes mPTP opening, we subjected neurons to calcein AM-co-chloride quenching assay. When we exposed the neurons to mPTP opening, we subjected neurons to calcein AM-co-chloride quenching assay. To determine whether the loss of OSCP sensitizes mitochondrial population in neurites (Fig. 2e).

In addition, in comparison to the control neurons OSCP results showed significantly elevated MitoSox Red intensity in mitochondria from OSCP knockdown neurons (Fig. 2d). Furthermore, by downregulating OSCP by disrupting the interaction of OSCP with F1, we tried to further probe the association between OSCP loss-of-function and mPTP formation in neurons by disrupting the interaction of OSCP with F1. To this end, we downregulated the β-subunit, which is the major F1 component that interacts with OSCP in the integral F1FO-ATP synthase (Supplementary Fig. 5a). Neurons in which the β-subunit was knockeddown demonstrated significantly suppressed mΔΨ and reduced ATP generation (Supplementary Fig. 5b,c) in patterns similar to those that we observed in OSCP-deficient neurons (Fig. 2b,c). Importantly, β-subunit knockdown neurons displayed substantial mPTP activation (Supplementary Fig. 5d). These results indicated that increased mPTP formation is closely associated with F1FO complex disruption, further highlighting a key role of OSCP loss-of-function in triggering mPTP opening. These findings also support previous reports that increased F1 dissociation from the F1FO complex contributes to mPTP formation.

Mitochondria play a critical role in maintaining synaptic and neuronal function. To determine whether loss of OSCP induces synaptic dysfunction, we measured synaptic density by

**Figure 1 | Loss of OSCP in AD subjects and 5xFAD mouse brain mitochondria.** (a) Densitometric quantification of immunoreactive bands of OSCP in protein extracts from the temporal lobes of non-AD, MCI and AD patients. β-Actin was used to indicate the loading amount of proteins. The lower panel is representative of six non-AD, six MCI and four AD patients. (b) Immunohistochemical staining using anti-OSCP on brain sections from human temporal lobes showed substantial reduction of OSCP in neurons. The right panel shows representative images. Scale bar, 40 μm. (c,d) Densitometric quantification of OSCP immunoreactive bands shows the age-dependent OSCP reduction in synaptic (c) and nonsynaptic (d) mitochondria from 5xFAD mice. TOM40 was used to show the enrichment of mitochondrial fractions. Coomassie blue staining was employed to indicate the loading amount of mitochondrial proteins. Five nonTg and five 5xFAD mice at 4 months old and 5 nonTg and 6 5xFAD mice at 9 months old were used in the experiments. Error bars represent s.e.m.
staining for PSD95 and vesicular glutamate transporter 1 (vGlut1). OSCP shRNA-treated neurons showed a significant reduction in synaptic density (Fig. 3a), suggesting a deleterious influence of OSCP loss-associated mitochondrial dysfunction on synaptic function. To directly determine the influence of OSCP loss on synaptic transmission we performed whole-cell voltage-clamp recordings to examine changes in action potential-independent miniature excitatory postsynaptic currents (mEPSCs). The downregulation of OSCP significantly reduced the average mEPSC amplitude in OSCP knockdown neurons in comparison to their nontarget shRNA-treated controls (Fig. 3b,d,e), without significantly altering mEPSC frequency (Fig. 3b,c). Furthermore, direct stimulation of postsynaptic glutamate receptors by ‘puffing’ glutamate onto cultured neurons resulted in currents of significantly smaller amplitude in OSCP knockdown neuron (Fig. 3f). Taken together, these data suggest that OSCP loss impairs synaptic function, which parallels the well-documented synaptic dysfunction, and particularly a loss of postsynaptic function, in the pathology of AD. Synaptic loss and impaired synaptic transmission further characterize AD-related pathological changes. Therefore, our results suggest that reduced OSCP expression is associated with AD-like mitochondrial dysfunction and the resultant synaptic failure.

**Interaction of OSCP with Aβ results in OSCP loss-of-function.** Aβ is a key mediator of AD and previous studies have shown that Aβ deposits in AD mitochondria, targeting several mitochondrial proteins. We therefore explored whether OSCP is a binding partner of Aβ in AD brains by co-immunoprecipitating temporal lobe protein extracts using anti-OSCP antibody. We detected OSCP/Aβ interaction in AD brains (Fig. 4a), as well as in MCI brains that showed brain Aβ deposition (Supplementary Fig. 6). In contrast, non-AD brains did not exhibit the OSCP/Aβ complex (Fig. 4a). The OSCP/Aβ complex was not detected in AD brain tissue when the OSCP antibody was replaced by nonimmune IgG (Fig. 4a), validating the specificity of OSCP/Aβ binding. Similarly, the OSCP/Aβ complex was also found in 5xFAD mice by co-immunoprecipitation (Fig. 4b). Further confocal microscopy studies showed extensive colocalization of OSCP and Aβ in neocortex and hippocampus from 5xFAD mice (Fig. 4c), suggesting an interaction between OSCP and Aβ in vivo. To determine whether OSCP directly interacts with Aβ we next performed an in vitro pull-down assay using glutathione S-transferase (GST)-tagged OSCP as the bait protein. Aβ1–42,
Figure 3 | OSCP deficiency impacts synaptic function. (a) Loss of OSCP induced a decline in synaptic density. The staining of vGlut1 (blue) and PSD95 (red) were used to identify the pre- and post-synaptic components of synapses, respectively. Neuronal dendrites were identified by staining for MAP2 (green). Scale bar, 5 μm. n = 27–43 neurons from at least three independent experiments. (b–e) Loss of OSCP affected mEPSCs. (b) Representative traces of mEPSCs in control (upper panel) and OSCP KD neurons (lower panel). Scale bars represent 200 ms and 20 pA. Data were collected from 10 control neurons and 7 OSCP knockdown (OSCP KD) neurons from at least three different cultures. (c) Quantitative analysis of mEPSC frequency. (d) Representative traces of mEPSC amplitude. (e) Analysis of the cumulative fraction of mEPSC amplitude distribution. (f) Loss of OSCP reduced the amplitude of EPSCs evoked by puff-application of glutamate. Scale bars represent 500 ms and 100 pA. Data were collected from seven control neurons and eight OSCP KD neurons. The right panel shows quantitative analysis of EPSC amplitude. Error bars represent s.e.m.

but not scrambled Aβ1–42, demonstrated dose-dependent binding with OSCP (Fig. 4d), indicating direct physical interaction of OSCP with Aβ.

Physical binding of Aβ could result in functional changes of its target proteins2. Therefore, it is important to determine whether the Aβ interaction influences OSCP function by tethering F1 via binding with α- and β-subunits41. To address this question, GST-tagged OSCP was pre-incubated with or without Aβ1–42. After washing off unbound Aβ1–42, we conducted in vitro pull-down assays and found that the binding of OSCP to α- or β-subunits was significantly reduced by Aβ1–42, suggesting that the interplay between OSCP and Aβ1–42 disrupts the ability of OSCP to bind to the F1 entity (Fig. 4f). To further evaluate the association between Aβ and impaired OSCP function, we generated deleted forms of OSCP based on the predicted Aβ binding sites on OSCP (Supplementary Fig. 7a–c). We found that the ability of OSCPΔ107–121 to interact with Aβ was significantly reduced (Fig. 4e). Furthermore, OSCPΔ107–121 showed extensive binding with α- or β-subunits regardless of the presence or absence of Aβ1–42 (Fig. 4f). These results further indicate a direct and specific influence of Aβ on OSCP, leading to reduced mitochondrial bioenergetics and activated mPTP formation.

To examine the impact of Aβ on OSCP binding to the FIFO-ATP synthase within mitochondrial membranes we exposed brain mitochondria to Aβ1–42. As predicted, co-immunoprecipitation showed OSCP/Aβ complexes in Aβ-exposed mitochondria, indicating that Aβ1–42 can enter mitochondria and bind to OSCP within the FIFO-ATP synthase (Supplementary Fig. 8a). Next, we examined FIFO-ATP synthase stability by conducting blue-native PAGE (BN-PAGE)42,43. Mitochondria were exposed to vehicle-, Aβ1–42- or Ca²⁺-treatment. Ca²⁺ is thought to disrupt the FIFO complex through OSCP52. Aβ induced a marked increase in F1 dissociation from the FIFO complex, similar to Ca²⁺-treatment, while little OSCP was detected in the free F1 (Supplementary Fig. 8b,c). The identification of FIFO dimer, monomer and F1 by β subunit blots was further validated by using an antibody against F1 (Supplementary Fig. 8d). Together these data suggest that the Aβ-induced FIFO complex instability is at least partly due to a reduction in OSCP’s ability to hold F1 and FO together. As expected, Aβ1–42-treated mitochondria displayed mitochondrial OXPHOS inhibition as evident from the significantly suppressed ATP synthesis (Supplementary Fig. 8e). To dissect the influence of Aβ on mitochondrial OXPHOS enzymes, we measured the enzymatic activities of mitochondrial complexes I through IV and
mitochondrial complex IV, whose deactivation is thought to be
F1FO-ATP synthase catalytic activity (Fig. 5a–c). In contrast,
mitochondrial respiratory control ratio, ATP synthesis and
from 5xFAD mice exhibited an early and marked decrease in the
specific effect (Supplementary Fig. 10a–c), synaptic mitochondria
from 5xFAD mice also demonstrated an age- and genotype-
reduction, as well as a
OSCP aberrations and 5xFAD mouse mitochondrial dysfunction.
We hypothesized that if the above effects of OSCP loss and
OSCP/Aβ interactions impact OSCP function in vivo and in vitro. (a) Co-immunoprecipitation of OSCP and Aβ in AD patient temporal lobes.
Results shown are representatives from three non-AD and three AD patients. Aβ1–42 peptide and AD brain extracts were used as positive controls for Aβ
immunoreactive bands. (b) Co-immunoprecipitation of OSCP and Aβ in 5xFAD mouse neocortex. Results shown are representatives from three mice in
each group. Aβ1–42 peptide was used as a positive control for Aβ immunoreactive bands. (c) Colocalization of OSCP (green) and Aβ (red) in neocortex
and hippocampus from 5xFAD mice. Neurons were identified by staining for NISSL (blue). Scale bar, 10 μm. (d) OSCP and Aβ interaction determined by an
in vitro pull-down assay. (e) Amino-acid residue numbers are given for mature OSCP protein (with known mitochondrial signalling peptide removed).
SP is the mitochondrial signalling peptide. Wild-type OSCP and OSCP107–121 were used for pull-down assay. OSCP107–121 displayed lowered capacity
to interact with Aβ compared to wild-type OSCP. (f) In vitro pull-down assay showed that Aβ1–42 suppresses the ability of OSCP, but not OSCP107–121,
to bind α- and β-subunits as demonstrated by decreased α- and β-immunoreactive bands. n = 6–10 samples per group. Error bars represent s.e.m.
of the F1FO-ATP synthase. F1FO-ATP synthase activity was
significantly reduced in exogenous Aβ-treated mitochondria
(Supplementary Fig. 8f); while the activities of mitochondrial
complexes I to IV remained unaltered (Supplementary Fig. 8g–j),
suggesting that F1FO-ATP synthase dysfunction confers Aβ
toxicity to mitochondrial OXPHOS under the experimental
conditions tested.

OSCP aberrations and 5xFAD mouse mitochondrial dysfunction.
We hypothesized that if the above effects of OSCP loss and
OSCP/Aβ interplay on F1FO-ATP synthase function could be
extrapolated to an in vivo setting, 5xFAD mice would display
F1FO-ATP synthase dysfunction, particularly in synaptic
mitochondria, which demonstrated early and extensive OSCP
reduction, as well as Aβ deposition (Supplementary Fig. 9a,b). To
address this, we first measured mitochondrial OXPHOS function
in 5xFAD mitochondria. Although nonsynaptic mitochondria
from 5xFAD mice also demonstrated an age- and genotype-
specific effect (Supplementary Fig. 10a–c), synaptic mitochondria
from 5xFAD mice exhibited an early and marked decrease in the
mitochondrial respiratory control ratio, ATP synthesis and
F1FO-ATP synthase catalytic activity (Fig. 5a–c). In contrast,
mitochondrial complex IV, whose deactivation is thought to be
the major OXPHOS defect in AD8–10, exhibited only a relatively
mild decrease (Supplementary Fig. 10g,h), again suggesting that
F1FO-ATP synthase deregulation contributes to the extensive
OXPHOS suppression in 5xFAD mice. Next, we examined F1FO
complex proton-flow coupling, which reflects F1FO complex
integrity44,45. Synaptic mitochondria from 5xFAD mice demonstrated a significant increase in oligomycin-insensitive
respiration (that is, uncoupled electron transport, Fig. 5d). This
was further supported by a F1FO-ATP synthase coupling assay
which showed that 5xFAD synaptic mitochondria had markedly
blunted sensitivity to oligomycin-A inhibition (Fig. 5e,f). Such
changes were also seen in nonsynaptic mitochondria, but the
effect size was considerably smaller (Supplementary Fig. 10d–f).
These results imply that F1FO complex destabilization occurs in
5xFAD brain mitochondria. Direct evidence of F1FO complex
instability in 5xFAD mitochondria was collected using
BN-PAGE. Free F1 was identified by immunoblotting using
specific antibodies recognizing the β subunit as previously
described42. We found an age-dependent increase in F1
dissociation (Fig. 5g,h), confirming the destabilization of the
F1FO complex in 5xFAD mitochondria. Lastly, because mPTP
formation is thought to be a consequence of F1FO complex
uncoupling via OSCP deregulation, we measured mPTP
Figure 5 | F1FO-ATP synthase deregulation in 5xFAD mouse synaptic mitochondrial. (a) Synaptic mitochondria from 5xFAD mice demonstrated an age-dependent decrease in their respiratory control ratio (RCR). Six nonTg and 5 5xFAD mice at 4 months old and 6 nonTg and 5 5xFAD mice at 9 months old were used. (b) Synaptic mitochondria from 5xFAD mice had an age-dependent decrease in ATP synthesis. Six nonTg and 6 5xFAD mice at 4 months old and those from six nonTg and seven 5xFAD mice at 9 months old were used in the experiments. (c) Synaptic mitochondria from 5xFAD mice demonstrated an early decrease in the F1FO-ATP synthase catalytic activity at 4 months old which was exacerbated at 9 months old. Five mice of each group at 4 months old and seven nonTg and nine 5xFAD mice at 9 months old were used. (d) The left panel is the representative of images collected from seven nonTg and six 5xFAD mice at 4 months old, and six nonTg and five 5xFAD mice at 9 months old. F1 was determined by probing with anti-β-subunit antibody and the molecular weight of the bands. The same amount of samples was used for SDS-PAGE and Tom40 and β-subunit were detected to show the loading amount of mitochondrial proteins. The right panel is the coomassie blue staining before immunoblotting to indicate the loading amount of samples. Error bars represent s.e.m.

Protection of OSCP restoration on Aβ-exposed mouse neurons. To further address the role of OSCP deregulation for the induction of mitochondrial dysfunction in an Aβ-rich environment we examined whether the Aβ-mediated mitochondrial dysfunction can be attenuated by OSCP restoration. Therefore, we overexpressed OSCP in mouse neurons, aiming to restore OSCP levels and to dilute the OSCP/Aβ interaction which would
be expected to protect OSCP function against Aβ toxicity. The control and OSCP overexpressing (OSCP OE) neurons were exposed to a treatment with oligomeric Aβ1–42. By immunoblotting and immunostaining (Fig. 6a,b), we found that Aβ-mediated OSCP reduction in control neurons was significantly ameliorated by OSCP upregulation. Further mitochondrial functional assays showed that OSCP overexpression substantially attenuated the decreased mΔΨ, lowered ATP production and decreased neuritic mitochondrial population as well as sensitized mPTP formation in Aβ-treated neurons (Fig. 6c–f). Notably, OSCP overexpression by itself did not significantly alter the expression level of CypD (Supplementary Fig. 11). To investigate the specific protection of OSCP restoration against Aβ toxicity on neuronal mitochondria we overexpressed the F1FO-ATP synthase β-subunit, which forms the catalytic core of the F1FO-ATP synthase. We then treated the control and β-subunit-overexpressing (β-subunit OE) neurons with Aβ. Aβ treatment did not induce detectable changes in the expression levels of β-subunit in either control or β-subunit OE neurons when compared with their vehicle-treated counterparts (Supplementary Fig. 12a), which supports our findings of the unaltered β-subunit levels in AD subjects (Supplementary Fig. 1a,b) as well as in 5xFAD mice (Supplementary Fig. 3a,c). However, further experiments showed that the overexpression of β-subunit did not demonstrate significant protection against Aβ-induced mΔΨ collapse, ATP reduction or decrease in neuritic mitochondrial population, as well as mPTP activation (Supplementary Fig. 12b–e), further supporting a specific protective effect of OSCP restoration in AD-related conditions.

We used exogenous Aβ treatment to mimic the high level of Aβ over-production in AD subjects; however, it is not clear whether OSCP restoration has similar protective effects in neurons that generate endogenous Aβ. To address this question we used primary neuron cultures from 5xFAD mice. In comparison to nonTg neurons, cultured 5xFAD neurons demonstrated a significant loss of OSCP expression, which was prevented by OSCP overexpression (Supplementary Fig. 13a). Importantly, OSCP overexpression in 5xFAD neurons exhibited protective effects on mΔΨ, ATP production and neuritic mitochondrial population, as well as mPTP formation.

Figure 6 | OSCP overexpression ameliorates Aβ-induced mitochondrial dysfunction in mouse neurons. Mouse cortical neurons were exposed to 500 nM oligomeric Aβ1–42 for 24 h. (a) Attenuated oligomeric Aβ1–42-induced OSCP reduction by OSCP overexpression. Western blot images show OSCP expression level in mouse primary neurons which is representative of seven samples in each group. (b) Shows immunofluorescent staining of OSCP (green) in primary cultured neurons. COXIV (red) was used to determine the localization of OSCP in mitochondria. *P < 0.01 versus other groups. Scale bar, 20 μm. (c) OSCP overexpression protected mitochondrial membrane potential against oligomeric Aβ1–42 toxicity. The upper panel of representative images shows the TMRM staining and the lower panel shows phase contrast images. Scale bar, 20 μm. *P < 0.01 versus other groups. n = 40–64 neurons from at least three independent experiments. (d) OSCP overexpression protected neuron ATP reduction against oligomeric Aβ1–42 toxicity. *P < 0.01 versus other groups. n = 12 samples of each group from at least three independent experiments. (e) Attenuated Aβ-induced reduction in neuritic mitochondrial population by OSCP overexpression. *P < 0.01 versus other groups. n = 22–27 neurons from at least three independent experiments. The upper panel of representative images shows the merged staining of MAP2 (green, dendrite) and Mito-Dsred (red, mitochondria). Scale bar, 20 μm. The middle panel shows Mito-Dsred staining and the lowest panel shows enlarged images from the indicated areas. (f) Ameliorated Aβ-sensitized mPTP formation by OSCP overexpression. Ionophore (2 μM) was used to trigger mPTP formation. *P < 0.05 versus vehicle-treated control and OSCP OE neurons. #P < 0.05 versus Aβ-treated OSCP OE neurons. n = 4–6 independent experiment. Control refers to neurons infected with lentivirus carrying backbone vector. OSCP OE refers to neurons infected with lentivirus carrying OSCP cDNA. Error bars represent s.e.m.
(Supplementary Fig. 13b–e) against endogenous Aβ toxicity in similar patterns as we observed in neurons treated with exogenous Aβ, confirming the protective effects of OSCP restoration against Aβ toxicity.

Given the crucial role of mitochondria in sustaining synaptic transmission and plasticity, and the attenuation of mitochondrial defects by OSCP overexpression, we next examined whether OSCP restoration also protects synaptic function against Aβ toxicity. Control and OSCP OE neurons were exposed to vehicle- or oligomeric Aβ1–42-treatment before the synaptic density was analysed using immunofluorescent staining of PSD95 and vGlut1. Oligomeric Aβ1–42-treated control neurons displayed a significant reduction in synaptic density in comparison to control neurons receiving vehicle treatment (Fig. 7a). In sharp contrast, the Aβ-induced synaptic loss was significantly ameliorated by OSCP overexpression (Fig. 7a). OSCP overexpression by itself did not affect baseline levels of synaptic density (Fig. 7a). In addition, OSCP overexpression protected against the reduction in mEPSC amplitude induced by Aβ toxicity (Fig. 7b,d,e). The relatively preserved mEPSC frequency of Aβ-treated neurons (Fig. 7b,c) may reflect distinct time-dependent changes in pre- and post-synaptic function during acute Aβ treatment.

Protection of OSCP restoration on Aβ-exposed human neurons. Because AD is a human disease, it is of considerable interest to know whether OSCP restoration confers similar protection to mitochondria in Aβ-exposed human neurons. Therefore we derived human neurons from human neural stem cells (Supplementary Fig. 14) and treated them with oligomeric Aβ1–42. Immunoblotting for OSCP levels and co-immunoprecipitation of OSCP and Aβ revealed that the OSCP loss and the formation of the OSCP/Aβ complex (Fig. 8a,b) observed in AD individuals were mirrored in Aβ-treated human OE neurons. Next, OSCP was overexpressed in human neurons (Fig. 8c). Control and OSCP OE human neurons were then exposed to oligomeric Aβ1–42 at 500 nM and then processed for our assays of mitochondrial function.

**Figure 7 | OSCP overexpression protects mouse neurons against Aβ induced synaptic dysfunction.** (a) Attenuated Aβ-induced synaptic density reduction in OSCP OE neurons. *P<0.01 versus other groups. n = 26–43 neurons collected from at least three independent experiments. Synapses were visualized by the staining for vGlut1 (Blue) and PSD95 (red) to identify the pre- and postsynaptic components of synapses, respectively. Neuronal dendrites were determined by MAP2 (green). Scale bar, 5 μm. (b–e) OSCP overexpression protected mEPSCs from Aβ toxicity. (b) Representative traces of mEPSC recordings from control and OSCP OE neurons in the presence or absence of Aβ. Scale bars represent 200 ms and 20 pA. The data were collected from 10 vehicle-treated control neurons, 8 Aβ-treated control neurons, 7 vehicle-treated OSCP OE neurons and 8 Aβ-treated OSCP OE neurons. (c) Quantitative analysis of mEPSC frequency. (d) Quantitative analysis of mEPSC amplitude. *P<0.05 versus other groups. (e) The cumulative fraction of mEPSC amplitude distribution. (f) Overexpression of OSCP prevented the reduction in amplitude of glutamate-evoked EPSCs that results from Aβ toxicity in control neurons. Scale bars represent 500 ms and 100 pA. Data were collected from seven vehicle-treated control neurons, nine Aβ-treated control neurons, five vehicle-treated OSCP OE neurons and seven Aβ-treated OSCP OE neurons. Error bars represent s.e.m.

NATURE COMMUNICATIONS | DOI: 10.1038/ncomms11483 | www.nature.com/naturecommunications

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function. Our results showed that Aβ-induced mitochondrial dysfunctions in ATP production, mΔΨ and neuritic mitochondrial population, as well as mPTP formation (Fig. 8d–g) were substantially reduced by OSCP overexpression in human neurons. Moreover, the overexpression of OSCP in human neurons did not have a detectable influence on baseline levels of the assayed parameters in comparison to control neurons (Fig. 8d–g), which further supports our observations in OSCP OE mouse neurons.

Discussion

In this study, we find that OSCP loss and OSCP/Aβ interaction constitute the major OSCP alterations in the brains from AD patients and 5xFAD mice. Among the major subunits of F1FO-ATP synthase, OSCP is selectively decreased in the brains of AD subjects and 5xFAD mice. Moreover, the early decrease of OSCP expression in synaptic mitochondria from 5xFAD mice indicates that synaptic mitochondria are more vulnerable to Aβ-induced mitochondrial alterations. This reveals a novel form of synaptic mitochondrial stress in AD and supports a causative role of synaptic mitochondrial dysfunction in the development of early synaptic dysfunction in the disease. Another critical finding of this study is the interaction of OSCP and Aβ. In recent years, the accumulation of Aβ in mitochondria, and particularly in synaptic mitochondria, has received considerable attention. Aβ is probably transported via a translocase in the outer mitochondrial membrane and impacts mitochondrial function via multiple pathways, including the interaction of Aβ with several mitochondrial proteins such as CypD. Amyloid beta-binding alcohol dehydrogenase and dynamin-like protein 1 (ref. 40). Our finding of significant interplay between OSCP and Aβ further supports our understanding of the intracellular influence of Aβ toxicity in AD. Indeed, given the extensive Aβ accumulation in mitochondria, we cannot exclude the possibility that mitochondrial Aβ may bind to other proteins embedded in the inner mitochondrial membrane or to other subunits of mitochondrial F1FO-ATP synthase, which could also be involved in compromised F1FO-ATP synthase function in AD. In view of

Figure 8 | OSCP overexpression ameliorates Aβ-induced mitochondrial dysfunction in human neurons. (a) Human neurons were exposed to 500 nM oligomeric Aβ1–42 for 4 days, then subjected to immunoblotting detection of OSCP levels. Aβ induced a significant reduction in OSCP expression level. The lower panel shows representative immunoblotting images. Tom40 was used as the loading control. n = 4–5 samples of each group. (b) Co-immunoprecipitation of OSCP and Aβ in Aβ-treated human neurons. (c) Significantly increased OSCP expression in the OSCP overexpressing neurons. The lower panel shows representative immunoblotting images. Tom40 was used as the loading control. n = 4 samples of each group. (d) Preserved ATP production by OSCP overexpression. *P < 0.01 versus other groups. n = 6–10 samples of each group. (e) Preserved mitochondrial membrane potential by OSCP overexpression. *P < 0.01 versus other groups. n = 17–23 neurons from at least 3 time independent experiments. Right panels are representative images of TMRM staining (upper row) and phase contrast (lower row). Scale bar, 20 µm. (f) Preserved neuritic mitochondrial population by OSCP OE. *P < 0.05 versus other groups. Right panels are representative images. Dendrites were determined by the staining of MAP2 (green); and neuritic mitochondrial TOM-dsred (red). Scale bar, 20 µm. (g) Attenuated Aβ-sensitized mPTP formation by OSCP overexpression. Ionophore (2 µM) was used to trigger mPTP formation. *P < 0.05 versus vehicle-treated control and OSCOE OE neurons. #P < 0.05 versus Aβ-treated OSCOE OE neurons. n = 4–7 independent experiments. Error bars represent s.e.m.
the critical role that the β-subunit plays for the catalytic functions of F1FO-ATP synthase, we examined whether the β-subunit is a binding partner of Aβ. Our data (not shown) suggest that the β-subunit is not a likely binding partner of Aβ. Future investigation will explore whether Aβ may affect other mitochondrial proteins that contribute to mitochondrial F1FO-ATP synthase dysfunction, as well as mPTP activation in AD.

Our findings of reduced OSCP levels and the interaction of Aβ with OSCP, which impair mitochondrial function in AD-related pathological settings, implicate that Aβ toxicity induces OSCP aberrations, leading to mitochondrial F1FO-ATP synthase dysfunction in AD-sensitive brain areas. However, increasing evidence suggests that AD is a systemic disease. In addition to AD-affected brain areas, Aβ is detected in circulating blood51 and platelets52,53, as well as in other tissues such as intestine and kidney54. The systemic distribution of Aβ raises the possibility of Aβ interaction with OSCP in cells outside the nervous system, which may potentially cause mitochondrial dysfunction. Moreover, in view of the complexity of mitochondrial abnormalities in AD our findings of OSCP aberrations may only provide one mechanism for systemic mitochondrial dysfunction. Therefore, it will be important for future studies to evaluate the impact of OSCP alterations in AD on a systems level.

Suppressed mitochondrial OXPHOS is a hallmark of mitochondrial defects in AD5,3. However, the detailed mechanisms of this mitochondrial deficit in AD are not fully understood. It is well accepted that AD is accompanied by pronounced changes in the function of mitochondrial complex IV (refs 8–10). Indeed, we have found a significant decrease of complex IV activity in 5xFAD neuronal mitochondria when comparing it to the corresponding complex IV activity in nonTg mice. However, the contribution of complex IV dysfunction to ATP deficiency in AD is still controversial11–13. Given the redundant capacity of complex IV in mitochondria, it has been argued that the slight decrease in complex IV activity does not explain severe ATP deprivation in AD11, suggesting that other mitochondrial OXPHOS enzymes also become dysfunctional. F1FO-ATP synthase is a critical OXPHOS enzyme synthesizing ATP18,19. To date, our knowledge on the functional state of mitochondrial F1FO-ATP synthase in AD is extremely limited. Earlier studies suggested that F1FO-ATP synthase is not involved in AD because the F1FO-ATP synthase enzyme activity in the brains or platelets of AD patients25 appeared to be unchanged, and increased enzymatic activity was observed in platelets from probable AD subjects26. However, this concept has been challenged in recent years by evidence that showed alterations of this enzyme in AD-sensitive brain regions, and particularly in neurons14–17.

Indeed, as we show in this study, synaptic mitochondria are more vulnerable to Aβ-induced F1FO-ATP synthase dysfunction in 5xFAD mice, whereas nonsynaptic mitochondria are relatively preserved. Further, we show that F1FO complex uncoupling is a prominent defect in 5xFAD mouse brain mitochondria. The coupling state of this enzyme in AD, however, was largely overlooked in previous studies, which failed to fully evaluate F1FO-ATP synthase deregulation in AD. Therefore, our results showing that F1FO-ATP synthase deregulation is associated with suppressed neuronal mitochondrial OXPHOS efficacy provide a novel mitochondrial mechanism of neuronal stress in AD and impact our current understanding of mitochondrial OXPHOS deficits in this disease.

The molecular identity of mPTP has long been a critical scientific issue23. Recently, uncoupled mitochondrial F1FO-ATP synthase was identified as the molecular basis of mPTP23. Specifically, the prevalent model proposes that OSCP is the binding target of CypD, which is a non-pore forming regulator of mPTP opening. The interaction of CypD with OSCP triggers the instability of the F1FO complex, thus resulting in the dissociation of F1 and eventually the formation of a nonselective leak channel within c rings22,24. These findings have suggested a key role of OSCP deregulation in the induction of mPTP. However, whether this model applies to neuronal mitochondria has not been comprehensively investigated. By downregulating OSCP in neuronal mitochondria, we found substantially sensitized mPTP formation. Importantly, this OSCP loss-associated mPTP activation is indispensable of the function of CypD, thus serving as strong evidence of the role of OSCP deregulation for inducing mPTP formation in neurons. Our in vivo results further link mPTP over-activation to OSCP aberrations in AD-relevant pathological settings. Sensitized mPTP formation is well documented as a key mitochondrial dysfunction in AD. Our previous studies have shown that mPTP blockade by CypD depletion protects mitochondrial and neuronal stress in a mouse model of AD, suggesting that mPTP regulation could be a therapeutic strategy for AD treatment5,26. The physiological function of mPTP has been noticed in recent years57–59. Molkentin and colleagues57 found increased susceptibility of heart failure in CypD-deficient mice outlining the risks associated with inhibiting the physiological functions of mPTP and CypD. Therefore, it would be preferable to develop a strategy that can reduce mPTP over-activation under pathological states like AD while preserving mPTP function under normal physiological conditions. In this study, we found that OSCP restoration reduces Aβ-induced mPTP over-activation without having a detectable influence on baseline mPTP formation. Therefore, protecting OSCP seems to be a promising avenue for the regulation of mPTP for AD treatment.

Lastly, mitochondrial dysfunction has been identified as a causative factor of synaptic failure in AD. Therefore, supporting mitochondrial function seems to be a viable strategy to protect synaptic strength and plasticity to delay the cognitive decline in...
AD patients. We found that loss of OSCP induces synaptic dysfunction, which was paralleled by reduced mitochondrial function. Notably, the restoration of OSCP mitigated Aβ-mediated mitochondrial dysfunction and further preserved synaptic function as evidenced by our measures of synaptic density and synaptic transmission. Therefore, our results confirm the deleterious impact of mitochondrial injury on synaptic function and further highlight the therapeutic role of OSCP restoration for the protection of synaptic strength to ameliorate cognitive impairments in AD.

In summary, we have uncovered a novel mechanism of mitochondrial dysfunction mediated via OSCP disruption in an AD-relevant pathological setting. Moreover, our results indicate a role of mitochondrial F1FO-ATP synthase deregulation in the development of AD mitochondrial defects that has long been overlooked. However, other factors may also contribute to mitochondrial F1FO-ATP synthase dysfunction in AD. For example, a previous study has shown that the mitochondrial α-subunit interacts with extracellular domain of amyloid beta precursor protein (APP) and Aβ on neuronal surface ATPases.

METHODS

Mice. Animal studies were approved by the University of Texas at Dallas Institutional Animal Care and Use Committee (IACUC) and were performed in accordance with the National Institutes of Health guidelines for animal care. 5xFAD mice overexpress a human form of mAPP-bearing mutations (SwFlLon) and PSEN1 mutations (M146L and L286V) linked to familial AD. CypD-deficient mice (B6;129-(SwFlLon) and PSEN1 mutations (M146L and L286V) linked to familial AD). 5xFAD mice were used in the experiments. Day 0–1 nonTg, 5xFAD and CypD-deficient (B6SJL-Tg(APPswFlLon, PSEN1*M146L*L286V) 6799Vas/Mmjax, mixed gender) and 5xFAD mice (B6;129-Pten+/+ with 5σ-mt, mixed gender) were used in the experiments. Frozen brain samples and paraffin-embedded brain slices were requested from UT Southwestern Medical Center ADC Neuropathology Core, and were used in the experiments.

Mitochondria preparation. Synaptic and nonsynaptic mitochondria were isolated from tissue as previously described, brain tissues were homogenized in ice cold isolation buffer (225 mM mannitol, 75 mM sucrose, 2 mM K2PO4, 0.1% BSA, 5 mM Hepes, 20 mM K2HPO4, 50 mM KCl, 5 mM MgCl2, 50 mM KCl, 0.2 mM EDTA, 0.2 mM NADH and 1 mM phosphoenolpyruvate) in the presence of oligomycin-A at 0.4, 0.1 and 1 μg oligomycin-A per mg mitochondrial protein for 15 min at room temperature. After the incubation, ATP synthase activity was measured spectrophotometrically using NADH-linked, ATP-regenerating system.

ATP synthase catalytic activity assay. ATP synthase activity was measured spectrophotometrically using NADH-linked, ATP-regenerating system. Briefly, mitochondria of appropriate amount were placed in ATP synthase assay buffer (100 mM Tris-HCl (pH 7.4), 2 mM MgCl2, 50 mM KCl, 2 mM NaCl, 0.25 mM NADH and 1 mM phosphoenolpyruvate) in the presence of oligomycin-A at 0.4, 0.1 and 1 μg oligomycin-A per mg mitochondrial protein for 15 min at room temperature. After the incubation, ATP synthase activity was measured spectrophotometrically using NADH-linked, ATP-regenerating system.

ATP synthase coupling assay. Mitochondrial fractions (15 μg) were placed in ATP synthase assay buffer (225 mM mannitol, 75 mM sucrose, 2 mM K2PO4, 0.1% BSA, 5 mM HEPES, and 20 mM K2HPO4, 50 mM KCl, 5 mM MgCl2, 50 mM KCl, 0.2 mM EDTA, 0.2 mM NADH and 1 mM phosphoenolpyruvate) in the presence of oligomycin-A at 0.4, 0.1 and 1 μg oligomycin-A per mg mitochondrial protein for 15 min at room temperature. After the incubation, ATP synthase activity was measured spectrophotometrically using NADH-linked, ATP-regenerating system.

ATP synthesis assay. Aliquots of mitochondria were analysed by using the ATP Luminescent assay kit (Abcam). Briefly, mitochondria were placed in isolation buffer (225 mM mannitol, 75 mM sucrose, 2 mM K2PO4, 0.1% BSA, 5 mM HEPES, and 20 mM K2HPO4, 50 mM KCl, 5 mM MgCl2, 50 mM KCl, 0.2 mM EDTA, 0.2 mM NADH and 1 mM phosphoenolpyruvate) in the presence of oligomycin-A at 0.4, 0.1 and 1 μg oligomycin-A per mg mitochondrial protein for 15 min at room temperature. After the incubation, ATP synthase activity was measured spectrophotometrically using NADH-linked, ATP-regenerating system.

Mitochondrial respiration assays. Purified mitochondria were energized by glutamate (5 mM) and malate (5 mM) and subjected to respiration assays on a Clark electrode. Oxygen consumption was triggered by the addition of ADP.

Mitochondrial swelling assay. Mitochondria were suspended in 0.5 ml of swelling assay buffer (50 μg of mitochondrial protein, 150 mM KCl, 5 mM HEPES, and 20 mM K2HPO4, 5 mM glutamate (pH 7.3)). Mitochondrial swelling was triggered by the addition of calcium (500 mM mg 1 of protein). Swelling was observed by immediately and continuously recording changes at OD540 nm by using a spectrophotometer (Ultrospec 2100, Amersham Biosciences) for a total of 600 s at 10 s intervals.

Immunoblotting analysis. Samples were prepared in sample loading buffer (50 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 1% β-mercaptoethanol, 12.5 mM EDTA and 0.02% bromophenol blue) and proteins were separated by SDS–PAGE (10 or 12% Bis-Tris gels, Life Technologies), and then transferred to an Immobilon-P membrane for blotting (Bio-Rad Laboratories). After blocking in TBS buffer (20 mM Tris-HCl, 150 mM sodium chloride) containing 5% (wt/vol) nonfat dry milk for 1 h at room temperature, the membrane was then incubated and gently shaken overnight at 4 °C with primary antibodies. This was followed by incubation with the corresponding secondary antibody for 1 h at room temperature. The following antibodies were used: mouse monoclonal anti ATP5A (Life Technologies, #459240, 1:5,000), rabbit polyclonal anti ATP5B (Santa Cruz, sc-33618,1:2,000) and mouse monoclonal anti-OSCP (Santa Cruz, sc-365162,1:5,000), goat polyclonal anti subunit c (Santa Cruz, #sc-132636,1:1,000), goat polyclonal anti subunit f1 (Santa Cruz, #sc-132637,1:1,000), rabbit polyclonal anti ATP5A (Santa Cruz, sc-365162,1:5,000), rabbit polyclonal anti TOM40 (Santa Cruz, sc-11414,1:1,000), rabbit polyclonal anti Aβ (CST, #8243, 1:5,000), mouse anti-β-actin (Sigma-Aldrich, #4541, 1:1,000), mouse anti-Cyclin D (Millipore, #AP1035, 1:3,000), rabbit anti-VDAC (CST, #4661, 1:5,000), Goat anti-mouse IgG HRP conjugated and goat anti-rabbit IgG HRP conjugated (Life Technologies, #625320 and 656120, 1:2,000-8,000). Images were collected on Bio-Rad Chemidoc Imaging System. Image J software (National Institutes of Health) was used to analyse the scanned blots and to quantify protein signal intensity.

Co-immunoprecipitation. Mitochondria from cerebral cortices of transgenic mice or human subjects were suspended in buffer (500 μg ml−1, 50 mM Tris, 150 mM NaCl, 1 mM EDTA, protease inhibitors (Calbiochem, set V, EDTA free), 0.1% NP-40, pH 7.5) and then subjected to five freeze-thaw cycles, followed by centrifugation by centrifugation at 12,000g for 10 min at 4 °C. We immunoprecipitated the resulting supernatant with mouse antibody to OSCP (Santa Cruz, sc-365162, 0.5 μg IgG per
100 μg protein) at 4°C overnight, followed by an incubation with protein A/G agarose (Pierce) for 2 h at room temperature. Nonimmune IgG (0.5 μg IgG per 100 μg protein) was used as a negative control. We subjected the resultant immunoprecipitant to immunoblotting with antibody to β-actin (CST, #8243, 1:4,000).

**Immunostaining analysis.** For brain slices, slices were deparaffinized and rehydrated before they were subjected to antigen retrieval by boiling in citric acid buffer for 15 min. For cultured neurons, neurons were fixed in 4% paraformaldehyde for 30 min at 37°C. After blocking (5% Goat serum, 0.3% Triton X-100, PBS), the slices were incubated with antibodies against Aβ (CST, #8243, 1:200), COXIV (CST, #48484, 1:300), MAP2 (Sigma-Aldrich, #M4403, 1:300), OSCP (Santa Cruz, #sc-365162, 1:200) and with Nissl staining (Life Technologies, #N21479, 1:200) in combination or separately. After washing in PBS, the slices were probed with appropriate secondary antibodies including Alexa Fluor® 488 and Alexa Fluor® 594 (Life Technologies, 1:500). Images were collected on a Nikon confocal microscope.

**GST–OSCP pulldowns.** GST pulldown was performed according to manufacturer’s protocol (Pierce). Briefly, the human OSCP cDNA (Gene Name: ATP5O, NCBI Gene ID: 539) or deleted form of OSCP cDNA was transformed into BL21 (DE3) pLysS plSyS Escherichia coli (Promega) using the pGEX-4T-1 plasmid (GE Healthcare). After transformation and selection single colony was chosen for PCR to verify positive transformation. After overnight growth and induction by IPTG (Sigma-Aldrich), bacteria were pelleted and then were lysed by sonication in 1× PBS containing 0.2 mM PMSF and 100 μg ml⁻¹ lysozyme. After sonication bacterial debris was removed by centrifugation at 12,000 g for 15 min at 4°C. Supernatant was collected and incubated with glutathione agarose high-capacity, high-performance resin (Pierce) for 2 h. Glutathione beads were then washed and incubated overnight at 4°C with mitochondria lysates or Aβ peptide. After washing, protein was eluted from the beads and separated by SDS-PAGE. Immunoblotting or coomassie staining was performed to visualize results.

**Protein–protein predictions.** HADDOCK (high ambiguity-driven protein–protein docking)²⁴ was used to predict protein–protein interaction between ATP5O (PDB# 2WSS; s chain) and amyloid β (PDB# 1Z0Q). The modelling was performed in ambiguous interaction restraints with CPRTS active and passive constraints. Models with z-scores below ~1.4 were selected and amino acids on OSCP within 4 Å of amyloid beta were determined using PyMOL.⁵⁴

**Deletion constructs.** Regions of interest were determined by both HADDOCK modelling and based on literature analysis.⁶⁵,⁶⁶ Four OSCP deletions constructs were made; OSCPA1–18, OSCPA16–190, OSCPA1–18, 168–190 and OSCPA107–121. These constructs were generated via PCR and inserted into a GST-bearing vector (pGEX-4T-1). The vector was subsequently transformed into BL21(DE3) plSyS E. coli (Promega). After transformation and selection a single colony was chosen for PCR to verify positive transformation. Protein products were obtained via IPTG induction and purification with glutathione agarose high-capacity, high-performance resin (Pierce).

**Oligomeric Aβ preparation.** Aβ1–42 peptide (GenicBio) was diluted in 1.1,1,3,3,3,3-hexafluoro-2-propanol to 1 μM using a glass-tight Hamilton syringes with a Teflon plunger. The clear solution was then aliquoted in microcentrifuge tubes, and it was dried by evaporation in the fume hood. Peptide film was diluted in DMSO to 5 mM and sonicated for 10 min in bath sonicator. The peptide solution was resuspended in cold HAM’S F-12 to 100 μM and immediately vortexed for 30 s. The solution was then incubated at 4°C for 24 h.

**ELISA assay for mitochondrial Aβ measurement.** Aβ level in mitochondrial fractions was measured by using human Aβ1–40 and Aβ1–42 ELISA kits (Life Technologies) following the manufacturer’s instructions.

**Mouse neuron culture.** Cortices or hippocampi were dissected from day 0–1 pups in cold Hank’s buffer (without Ca2+ and Mg2+), dissociated with 0.05% trypsin at 37°C for 15 min followed by 10 times trituration in ice cold neurobasal A medium. Cells were then passed through 40 μm mesh cell strainer (Corning) and centrifuged for 2 min at 200g. The pellet was gently resuspended in neuron culture medium (neurobasal A with 2% B27 supplement, 0.5 mM l-glutamine, 50 μM l-menthol, and 50 μg ml⁻¹ streptomycin) and plated on poly-l-lysine (Sigma-Aldrich) coated culture plates (Corning) or Lab-Tek chamber slides (Nunc, #177445) with an appropriate density.

**Human neural stem cell culture and neural cell differentiation.** Human neural stem cells (StemPro Neural Stem Cells, #A10509-01, Life Technologies) were cultured and differentiated into neurons as manufacturer’s instruction. Differentiated neurons were determined by the morphology as well as the staining of neuronal-specific marker βIII tubulin using an antibody against βIII tubulin (CST, #D17G9, 1:300) followed by a secondary antibody of Alexa Fluor® 594 (Life Technology, #A-11037, 1:500).

**OSCP knockdown in mouse primary neurons.** Lentivirus-expressing shRNA targeted to mouse OSCP was packaged with lentivirus shRNA construct (clone TRCN0000076166: 5'-CCGGGCTGCTGAGTCCAAACAACTGCGATTGGTTTGACACTGAGAAGTTTG-3', Sigma-Aldrich), packaging vector psiPAX2 (Addgene) and envelope vector pMD2.G (Addgene). Lentivirus-expressing nontarget shRNA control (SHC002, Sigma-Aldrich) was used as a control. Mouse primary neurons were cultured for 3 days before infection with lentivirus at an m.o.i. of 5. The virus containing medium was removed after 2 h and fresh culture medium was replaced to continue culturing. Neurons were treated and collected for experiments after a further 7 days in culture.

**β Subunit knockdown in mouse primary neurons.** Lentivirus-expressing shRNA targeted to mouse β-subunit was packaged with lentivirus shRNA construct (clone TRCN0000076228: 5'-CCCGGTGCAACTGTCCTCCATACTCGAGATATGGAAGACAGTCTGAGATTG-3', Sigma-Aldrich), packaging vector psiPAX2 (Addgene) and envelope vector pMD2.G (Addgene). Lentivirus-expressing nontarget shRNA control (SHC002, Sigma-Aldrich) was used as a control. Mouse primary neurons were cultured for 3 days before infection with lentivirus at an m.o.i. of 5. The virus containing medium was removed to replace with fresh culture medium was replaced to continue culturing. Neurons were treated and collected for experiments after a further 7 days in culture.

**OSCP overexpression in neurons.** Human OSCP cDNA (Gene Name: ATP5O, NCBI Gene ID: 539) were inserted in to lentivirus vector with human polyubiquitin promoter-C (Addgene). Lentiviruses were packaged and applied on primary neurons similar as shRNA lentiviral vector. Oligomeric Aβ (500 nM) was used on neurons for 24 h before cell collection.

**Preparation and treatment of OSCP OE human neurons.** Neural stem cells were plated on poly-ornithine (Sigma-Aldrich) and laminin (Life Technologies) coated culture plates in complete StemPro neural stem cell SFM (Life Technologies) for neuron differentiation. 3 days after differentiation, 10 μM fluoro deoxy uridine (Sigma-Aldrich) and 10 μM uridine (Sigma-Aldrich) were added to remove mitotic cells. After 2 days differentiation cells were infected with expressing lentivirus at an m.o.i. of 5. Then were treated with 500 nM Aβ for 4 days after 7 days differentiation.

**Mitochondrial membrane potential assay.** Neurons were incubated with 200 nM TMRE (Sigma-Aldrich) which is cell-permeable red colour fluorescent dye and a specific indicator of mitochondrial membrane potential. After the incubation of TMRE for 30 min in an incubator, the dye was washed by using pre-warmed neurobasal A medium and the TMRE staining on neuronal mitochondria was imaged on an inverted fluorescent microscope with on-stage incubator (Nikon). The mitochondrial membrane potential in neurites was analysed by using Nikon NIS Advanced Research software.

**Mitochondrial superoxide assay.** Mitochondrial superoxide was determined by using Mitosox Red (Life Technologies)²³. Neurons were incubated with 2 μM Mitosox Red for 30 min in an incubator followed by washing using pre-warmed neurobasal A medium. The images of Mitosox Red staining were collected on a Nikon inverted confocal microscope with on-stage incubator. The intensity was subsequently analysed by using Nikon NIS Advanced Research software.

**Calcine AM-cobalt chloride quenching assay.** Neurons were subjected to the labelling of 1 μM calcine (Life Technologies) and then incubated with 1 μM cobalt chloride (Sigma-Aldrich) to remove cytosolic calcine staining. The changes of mitochondrial calcine were recorded in the absence or presence of ionophore, A23187 (Sigma-Aldrich) which induces mitochondrial calcium overload on a Nikon inverted fluorescent microscope with on-stage incubator (37°C, 5% CO₂) for 30 min. The results were subsequently analysed by using Nikon NIS Advanced Research software.

**Neuritic mitochondrial population measurement.** Primary neurons were infected by lentivirus-expressing mitochondrial targeted DiRed at 2 days in vitro (DIV). At 9–10 DIV the neurons were fixed and stained with anti-MAP2.
(Sigma-Aldrich, #M4403, 1:400) followed by goat anti-mouse IgG conjugated with Alexa 488 (Invitrogen, #A11029, 1:500). Images were collected under a Nikon confocal microscope and analyzed using NIH Image J software. The distribution and variance were normal and similar in all groups. P < 0.05 was considered significant. All data were expressed as the mean ± s.e.m.

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Acknowledgements
We thank Dr Mark Richter for advices on the experiments on F1FO-ATP synthase and the critical reading of the manuscript. We thank Dr Gail Breen for the insightful discussion. We thank Ms. Janani Ramachandra and Britney Campbell for mouse genotyping. We thank the UT Southwestern Medical Center ADC Neuropathology Core supported by ADC grant (AG12300) to provide human brain tissues. This study is supported by research funding from NIH (R00AG037716, NSFC (31271145, 81200847) and SDNSF (QJ201318)). LG was supported by Alzheimer’s Association (NIRG-12-242803). IMP was supported by NS077015.

Author contributions
H.D. and L.G. initiated the study. H.D., L.G., S.K. and J.M.P. designed the study. S.B., L.G., A.P., J.T., L.W., N.T., E.G., L.L., S.K. and H.D. performed experiments. S.B., L.G., A.P., S.K. and H.D. analysed the data. H.D. prepared the manuscript.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.
