Published in final edited form as:

Neurosci Lett. 2018 June 21; 678: 8–15. doi:10.1016/j.neulet.2018.04.053.

TDP-43 interacts with mitochondrial proteins critical for mitophagy and mitochondrial dynamics

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Abstract

Transactive response DNA-binding protein of 43kDa (TDP-43) functions as a heterogeneous nuclear ribonucleoprotein and is the major pathological protein in frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis/motor neuron disease (ALS/MND). TDP-43 pathology may also be present as a comorbidity in approximately 20 to 50% of sporadic Alzheimer's disease cases. In a mouse model of MND, full-length TDP-43 increases association with the mitochondria and blocking the TDP-43/mitochondria interaction ameliorates motor dysfunction. Utilizing a proteomics screen, several mitochondrial TDP-43-interacting partners were identified, including voltage-gated anion channel 1 (VDAC1) and prohibitin 2 (PHB2), a crucial mitophagy receptor. Overexpression of TDP-43 led to an increase in PHB2 whereas TDP-43 knockdown reduced PHB2 expression in cells treated with carbonyl cyanide m-chlorophenylhydrazone (CCCP), an inducer of mitophagy. These results suggest that TDP-43 expression contributes to metabolism and mitochondrial function however we show no change in bioenergetics when TDP-43 is overexpressed and knocked down in HEK293T cells. Furthermore, the fusion protein mitofusin 2 (MFN2) interacts in complex with TDP-43 and selective expression of human TDP-43 in the hippocampus and cortex induced an age-dependent change in Mfn2 expression. Mitochondria morphology is altered in 9-month-old mice selectively expressing

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Author contributions: S.D. designed and performed most of the experiments, analyzed all data, prepared figures and tables, and contributed to writing the paper; J.S. and S.I. performed some experiments; C.K. analyzed proteomics data, generated the interactome map, and edited the manuscript; J.D. performed and analyzed proteomics and contributed to the writing; N.C. contributed to experimental design, data analysis, and writing; M.G. contributed to experimental design, data interpretation, and writing the paper. All authors read and approved the final manuscript.

Ethics approval: All experiments were approved by and performed according to the ethical guidelines provided by the Animal Care and Use Committees at Delaware State University and at the University of Delaware Animal Facilities.

Conflict of interest: The authors declare that they have no conflict of interest.

TDP-43 in an APP/PS1 background compared with APP/PS1 littermates. We further confirmed TDP-43 localization to the mitochondria using immunogold labeled TDP-43 transmission electron microscopy (TEM) and mitochondrial isolation methods. There was no increase in full-length TDP-43 localized to the mitochondria in APP/PS1 mice compared to wild-type (littermates); however, using C- and N-terminal-specific TDP-43 antibodies, the N-terminal (27kDa, N27) and C-terminal (30kDa, C30) fragments of TDP- 43 are greatly enriched in mitochondrial fractions. In addition, when the mitochondrial peptidase (PMPCA) is overexpressed there is an increase in the N-terminal fragment (N27). These results suggest that TDP-43 processing may contribute to metabolism and mitochondrial function.

Keywords

TDP-43; APP/PS1; PHB2; mitophagy; MFN2; mitochondria; PMPCA

BACKGROUND

The heterogeneous nuclear ribonucleoprotein (hnRNP) TDP-43, participates in exon skipping, RNA stability, RNA transport, splicing, translation, microRNA processing, cellular stress, and localizes to the mitochondria [6, 7, 40, 45, 48]. TDP-43 is the major pathological protein in frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis/motor neuron disease (ALS/MND) [2, 27]. In addition, TDP-43 proteinopathy may be present as a comorbidity in approximately 19-57% of cases of sporadic AD [1, 3, 18, 22, 27, 41]. The pathology of TDP-43 consists of ubiquitination, hyperphosphorylation and proteolytic cleavage of TDP-43, into 35kDa and 25kDa insoluble aggregates, the major component of the inclusion bodies [2, 31]. Full-length and C-terminal fragment (25kDa) of TDP- 43 overexpression in NSC-34 cells has been shown to increase mitophagy, but little is known about the mechanisms by which overexpression of TDP-43 induces mitophagy [19, 28, 50]. Distinct changes in mitochondrial dynamics, including mitochondrial aggregation, have been reported in some mouse models of TDP-43 [37, 39, 47, 54, 55]. Mitochondrial dynamics are orchestrated by regulators of fusion, including mitofusin 1 and 2 (MFN1 and MFN2), along with dynamin-related protein 1 (DRP1), a regulator of fission [8, 13]. Specifically, one study showed that MFN2 overexpression rescued TDP-43 induced mitochondrial dysfunction [47], while other research found that overexpression of MFN2 induced mitochondrial dysfunction and clustering [20, 54], with the latter study supporting a model of neurodegeneration involving TDP-43 overexpression [20, 54]. Additionally, a recent report showed that full-length TDP-43 localizes to the mitochondrial fraction during stress-induced conditions in autosomal dominant ALS. Interestingly, by reducing localization of TDP-43 to the mitochondria, ALS transgenic mice show improved motor function [46, 48]. However, another recent report found that ALS mutant TDP-43 did not impair mitochondrial bioenergetics [23].

Misregulation of mitochondrial dynamics contributes to mitochondrial dysfunction in Alzheimer's disease (AD) and other neurodegenerative diseases [4, 5, 33, 35]. The *APP/PS1* transgenic mouse model co-expresses familial AD Swedish mutations ($APP^{K595N, M596L}$) and mutant human presenilin 1 (*PSEN1*-ΔE9) [21], recapitulates features of pathologic AD,

and displays alterations in metabolism that affect respiratory function, mitochondrial biogenesis, and mitophagy [32, 49]. With the different findings related to TDP-43, mitochondrial dynamics, and stress, and since we previously described an increase in cytoplasmic accumulation of endogenous TDP-43 in APP/PS1 mice, we wanted to determine if TDP-43 under stress would accumulate within the mitochondria [14]. Using several methods including proteomics, immunoprecipitation, immunogold TEM, mitochondrial fractionation, immunoblotting, and bioenergetics we studied the role that TDP-43 plays in mitophagy and mitochondrial dynamics. Our data may provide new insights into the role TDP-43 plays in mitochondrial dynamics.

METHODS

For complete methods describing mice, transmission electron microscopy, mitochondrial isolation, cell culture, transfections, siRNA, CCCP treatment, immunofluorescent staining, immunoprecipitation, Western blots, proteomics, bioenergetics analysis (Seahorse, Agilent), and statistical analysis please see supplementary material.

RESULTS

TDP-43 interacts with prohibitin 2 and increases mitophagy

Initially we investigated if a change in TDP-43 expression altered mitochondrial bioenergetics. In HEK293T cells overexpressing and knocking down TDP-43, we saw no change in normalized oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) (Seahorse, Agilent)(Fig. S1). This is supported from previous work that examined these changes in patient-derived mutant TDP-43 fibroblasts [23]. Interestingly, it has been recently reported that primary fibroblasts derived from sporadic ALS patients show no TDP-43 pathology, which may limit their use with respect to mitochondrial dysfunction [11].

To identify TDP-43 interacting partners, immunoprecipitated TDP-43 from cortex lysate in wild- type mice was evaluated through proteomic analysis. Proteomic analysis revealed several other mitochondrial proteins that interact with TDP-43 as well as non-mitochondrial proteins (Fig. S2) [14, 26, 43]. The subcellular locations, functions, and known interactions for these mitochondrial proteins are represented by the interactive map (Fig. S3). Five out of eight of the mitochondrial TDP-43 interacting proteins had known interactions with each other, including Prohibitin 2 (PHB2) and voltage-gated dependent anion channel 1 (VDAC1). We first validated these interactions with TDP-43 using co- immunoprecipitation (Fig. 1 A-B and Fig. S4). We chose to focus on the interaction between TDP-43 and PHB2 since PHB2 is a critical receptor for mitophagy [52]. Moreover, loss of PHB2 causes mitochondrial instability and leads to tau hyperphosphorylation and neurodegeneration [29].

We investigated whether TDP-43 expression regulates PHB2 protein expression levels. When TDP-43 was knocked down (siTDP-43) in HEK293T cells, PHB2 protein expression was not significantly altered under normal conditions (Fig. 1C, D & F). However, upon treatment with the mitochondria uncoupler and autophagy inducer, CCCP (10μM for 12 hours), there was a 20% significant reduction in PHB2 ($p<0.01$) when TDP-43 was knocked

down (p=0.0006) (Fig. 1C,F). When TDP-43 (v5-tagged) is overexpressed in cells treated with CCCP, (Fig. 1C,E) PHB2 levels were higher independent of CCCP treatment (Fig. 1C,G). We did not see a significant change in PHB2 levels upon CCCP treatment alone without TDP-43 overexpression (Fig. 1C,G).

PHB2 has been shown to be a crucial receptor for Parkin-mediated mitophagy and interacts with LC3-II [25]. We observed an increase in Parkin-positive punctate staining in cells treated with CCCP, which was enhanced with TDP-43 overexpression and reduced when TDP-43 was knocked down (Fig. S5). Overall, CCCP treatment led to an increase in LC3-II and a decrease in COXIV (Fig. 1C,H-J). However, there was significantly less (12%, p=0.024) LC3-II when TDP-43 expression was reduced (Fig. 1C,H). This may indicate that TDP-43 expression is required for full activation of CCCP-induced mitophagy. Interestingly, TDP-43 overexpression alone significantly increased LC3-II (75%, p=0.0012) expression to the same level induced by CCCP treatment (Fig. 3C,I). TDP-43 overexpression in cells treated with CCCP led to a marginal increase in LC3 compared to the vector control (Fig. 1C,I). Additionally, we see TDP-43 labeled mitochondria near lysosomes in mouse hippocampal sections using TEM (Fig. S6). We observed disorganized mitochondrial membranes in close proximity to TDP-43- labeled lysosomes indicating a possible link between TDP-43 and mitochondrial organization and degradation (Fig. S6).

Selective expression of TDP-43 in the hippocampus and cortex leads to an age-dependent change in fusion and fission

Since TDP-43 has been shown to localize to mitochondria, we were interested in whether TDP-43 overexpression affects mitochondrial fusion/fission dynamics. Mitofusin2 (MFN2) is a critical regulator of mitochondrial fusion. Knocking down TDP-43 in HEK293T cells led to a 37 ± 0.016% (p=0.0006) reduction in MFN2 protein whereas TDP-43 overexpression marginally increased MFN2 levels (Fig. S7A-C). We determined that TDP-43 interacts in complex with MFN2 by immunoprecipitation using cortical human brain tissue (Fig. S7D). Mitochondrial morphology was altered in 9-month old APP/PS1 mice expressing hTDP-43 in the cortex and hippocampus (Camk2a-tTA/tetO-hTDP-43/APP/PS1) that correlates with the increase in MFN2 expression (Fig. 1A,E-G). Furthermore, we utilized mice with selective expression of human TDP-43 in cortex and hippocampus (Camk2a-tTA/tetO-hTDP-43) and examined changes in MFN2 as well as dynamin-related protein 1 (DRP1), which is reported to play a key role in fission, at 4 months and 9 months of age. The dynamin family GTPase fusion protein, optic atrophy 1 (OPA1) showed no change in expression (Fig. 2B, E) [10]. In the cortex and hippocampus of 4-month old mice, there was no difference in MFN2 expression but there was a two-fold increase in MFN2 in Camk2a/hTDP-43 age (1.94 ± 0.24, p=0.012) and CAmk2a/hTDP-43/APP/PS1 (1.82 ± 0.22 p=0.025) mice at 9 months of (Fig. 2B-C,E-F). There was a 3.24-fold ± 0.55, (p=0.018) increase in phospho- DRP1(Ser637) implicated in fission inhibition in Camk2a/hTDP-43 mice compared to wild-type littermates in 4-month-old mice. Though there was an increase trend in expression of pDRP1(Ser637) in APP/PS1 and Camk2a/hTDP-43 it was not significantly increased at 9 months of age(WT to APP/PS1 (p=0.44); WT to Camk2a/hTDP-43 (p=0.10) (Fig. 2B,D,E,G)[8, 13]. Taken together with the morphological changes

in mitochondria observed these results indicate that TDP-43 may play a role in age-dependent changes in mitochondrial dynamics through regulation of MFN2.

Ultrastructure of endogenous TDP-43 proximal to mitochondria

TDP-43 has been shown to mislocalize from the nucleus to the cytoplasm in response to beta- amyloid toxicity. Likewise, in both FTLD and ALS abnormally aggregated TDP-43, in the form of inclusion bodies, is found in the cytoplasm. Interestingly, abnormal TDP-43 inclusion bodies are also frequently seen in late-onset AD and other age-related neurodegenerative diseases [17]. Recently, it was shown that TDP-43 with ALS-associated mutations localized to mitochondria whereas very little wild-type TDP- 43 was detected in mitochondria [47]. In APP/PS1 mice there is an increase in cytoplasmic endogenous TDP-43 compared to wild-type mice [14]. Immunogold TEM revealed both a nuclear and cytoplasmic distribution of endogenous TDP-43 in 8-month old wild-type and APP/PS1 mice (Fig. S8). TDP-43 distribution throughout hippocampal cells was similar between APP/PS1 and wild-type mice (Fig. S8). TDP-43 was also found in close proximity to mitochondria to a similar degree in both the APP/PS1 and wild-type mice (Fig. 3A and B, Fig. S8), which may indicate that TDP-43 does no play a role in the mitochondrial dysfunction seen in APP/PS1 mice. In the APP/PS1 mice, there appeared to be no distinct aggregation of TDP-43 (Fig. S8). There were clusters of TDP-43 speckles in the cytoplasm that may be indicative of sites of active translation or stress granules, similar to the ultrastructure of TIA1, an RNA binding protein that promotes stress granule formation and has been shown previously to localize to TDP- 43 (Fig. S8) [15]. This is similar to what has been observed in sporadic ALS [30, 34]. With TDP-43 localized to mitochondria and recent work showing TDP-43 present in the mitochondrial fraction, we next wanted to determine if TDP-43 increased in the mitochondrial fraction in APP/PS1 mice [46, 48].

Mitochondrial isolation reveals N-terminal and C-terminal TDP-43 fragments

In order to examine if there was an increase in mitochondrial TDP-43, we used wild-type and APP/PS1 mouse cortical and hippocampal tissue and performed Percoll density gradient fractionation to isolate mitochondria [38]. Furthermore, we utilized two different TDP-43 antibodies to specifically detect N-terminal and C-terminal TDP-43 fragments [14]. We detected distinctly different TDP-43 fragment sizes enriched in mitochondria fractions: a 27kDa N-terminal fragment (N27) and a 30kDa C-terminal fragment (C30) (Fig. 3C). Although we expected to see an increase in full-length TDP-43 in APP/PS1 mice, we detected very little full-length TDP-43, which was not expressed significantly more highly than non- transgenic mice (Fig. 3C-D). However, there was a marginal increase in N-terminal TDP-43 (27kDa) (not significant) and a significant decrease in C-terminal TDP-43 (30kDa) (WT 1.00 ± 0.110, APP/PS1 0.552 ± 0.116, $p=0.049$) (Fig. 3C, E-F). There was also a significant increase in the previously characterized 35kDa and 25kDa species in the total cell lysates of APP/PS1 mice compared to wild-type, which were not found enriched in mitochondria (Fig. 3C, F-G). In total cell lysates, the N-terminal TDP-43 antibody differentially detected expression of the 35kDa TDP-43 whereas the C-terminal antibody detected 25kDa TDP-43, which are both significantly increased in APP/PS1 mice (Fig. 3C, G-H). Although histone H3 was used as a marker for possible nuclear protein contamination in mitochondria fractions, we noted a significant decrease in histone H3 expression in

APP/PS1 (0.457 ± 0.0304, p=0.006) compared to wild- type (1.00 ± 0.0962), which may indicate a loss of soluble histone expression that needs further investigation (Fig. 3C, I).

Due to the presence of the 27kDa and 30kDa TDP-43 fragments in the mitochondrial fractions, we hypothesized that TDP-43 was processed by mitochondrial peptidases in order to facilitate entry into mitochondria. We overexpressed several mitochondrial peptidases and screened for 27kDa and 30kDa TDP-43 by Western blotting. We chose to focus on mitochondrial processing peptidase (MPP), presenilin- associated rhomboid-like protease (PARL), and ATPase family gene 3-like 2 (AFG3L2) because they were shown to affect degradation of phosphatase and tensin homologue-induced kinase 1 (PINK1), which recruits Parkin to mitochondrial membranes during Parkin-mediated mitophagy [16]. There was no increase in TDP-43 processing of the C30 fragment however there was significant increase of 2.37 ± 0.11 (p=0.004) of the N27 fragment in total lysate as a result of overexpression of peptidase mitochondrial processing alpha subunit (PMPCA) (Fig. S9). This mechanism of TDP-43 processing by PMPCA needs further investigation.

DISCUSSION

Mitochondria are involved in multiple processes in the cell including: oxidative phosphorylation to produce ATP, lipid metabolism, calcium homoeostasis, apoptosis, and other functions [12, 53]. With the high-energy demand of neurons mitochondria are vital for synaptic homeostasis [42]. Mitochondrial dysfunction is evident in age-related neurodegenerative diseases including, but not limited to, Parkinson disease, ALS, AD, and Huntington disease [4, 5, 33, 35]. Full-length TDP-43 localizes to the mitochondria during stress in familial ALS and both wild-type and mutant TDP-43 bind to mitochondria-transcribed mRNAs for mitochondria-encoded NADH:ubiquinone oxidoreductase core subunit 3 (ND3) and subunit 6 (ND6) which interfere with their expression causing complex I disassembly [48]. However, one report described low levels of TDP-43 associated with the mitochondria and no change in mitochondrial bioenergetics in ALS patient-derived fibroblasts and an ALS mouse model [23]. It has been recently shown that no TDP-43 pathology was observed in primary fibroblasts derived from sporadic ALS patients [11]. Pathogenic mechanism in ALS in part show increases in oxidative stress and mitochondrial dysfunction (reviewed in [9]). With no phosphorylated TDP-43 pathology present in patient derived fibroblasts and only ubiquitin pathology seen in the TARDBP A315T mouse model used for both these studies could limit recapitulation of the mitochondrial dysfunction observed in ALS patients [11, 51].

A proteomic screen of TDP-43 interacting proteins identified the recently discovered mitophagy receptor PHB2 as interacting with TDP-43 [52]. We hypothesize that TDP-43, through its interaction with PHB2, may be involved in tethering mitochondria for degradation. When TDP-43 is knocked down, less activation of CCCP-induced mitophagy occurs. The complex interaction between TDP-43 and PHB2 may also be mediated through MFN2 and/or VDAC1 that was also pulled down with TDP-43. It has been previously reported that muscle-specific Mfn2 knockout mice reduces mitophagy and alters metabolic homeostasis in an age-dependent manner [36]. A recent study in *Drosophila melanogaster* showed that increasing mitofusin/marf ameliorated neurodegeneration in flies

overexpressing human TDP-43 [24]. In mice overexpressing TDP-43, mitochondria aggregation was associated with an increase in fission proteins [54, 55]. Through selective expression of TDP-43 in the hippocampus and cortex at 4 months of age there is an increase in pDRP1(Ser637) shown to be inhibitory of fission and an age-dependent increase in the fusion protein, MFN2, which also interacts in complex with TDP-43. This may relate to the age-dependent changes associated with fusion/fission dynamics observed in our model of TDP-43 overexpression. We further show in human cells that knocking down TDP-43 also decrease MFN2, which suggests that TDP-43 may have a pivotal role in mitochondrial dynamics.

The pathological increase in TDP-43 in the mitochondria may contribute to the changes associated with ALS [48]. In APP/PS1 mice we observed no increase in full-length TDP-43 in the mitochondria fraction; however, N- and C- fragments of TDP-43 were enhanced in the mitochondrial fraction. There was also a marginal decrease in the TDP-43 C30 fragment in APP/PS1 mitochondria, which may indicate that a balance between the N- and C-terminal fragments are required for normal homeostasis of TDP-43 in the mitochondria. When the mitochondrial peptidase PMPCA is overexpressed there was an increase in the N27 fragment. It has been previously reported that PMPCA interacts in complex with ubiquinol-cytochrome c reductase core protein 1 (UQCRC1) [44]. Interestingly, UQCRC1 was one of the mitochondrial proteins that co-immunoprecipitated with TDP-43. Although the relationship between PMPCA and TDP-43 processing and the role of TDP-43 plays in mitophagy needs further investigation, these data collectively help to further characterize the relationship between TDP-43 and the mitochondria.

CONCLUSION

Our findings do not show an increase in full-length TDP-43 localized to the mitochondrial fraction in APP/PS1 mice. Interestingly, the TDP-43 C- and N-terminal fragments are enriched in the mitochondrial fraction and when the mitochondrial peptidase PMPCA is overexpressed we observe an increase in the N27 fragment. The role of these fragments needs further study. Investigating proteins that interact with TDP-43 may elucidate the normal function and delicate balance TDP-43 plays in neurodegeneration. These observations expand our understanding of mitophagy and fusion/fission dynamics and provide novel insights into the possible role TDP-43 plays in mitochondrial dysfunction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Randall Massey at the University of Wisconsin-Madison Electron Microscope Facility and Jean Ross at the Delaware Biotechnology Institute, University of Delaware.

Funding: This work was funded by the Alzheimer's Association New Investigator Research Grant: NIRG-12-241456, the National Institute on Aging: 1K01AG042500, Delaware IDeA Network of Biomedical Research Excellence (INBRE) Pilot Award: NIH-NIGMS: 5P20GM103446, NIH-NIGMS Centers of Biomedical Research Excellence (COBRE): 5P20GM103653 and a Delaware Economic Development Office Grant from the State of Delaware.

Abbreviations

APP	amyloid precursor protein
VDAC1	voltage dependent anion channel 1
TDP-43	TAR DNA binding protein of 43 kDa
PSEN1/PS1	presenilin 1
AD	Alzheimer disease
hnRNP	heterogeneous nuclear ribonucleoprotein
ALS	amyotrophic lateral sclerosis
MND	motor neuron disease
CCCP	carbonyl cyanide m-chlorophenyl hydrazine
PHB2	prohibitin 2, MFN2, mitofusin 2
DRP1	dynamin-related protein 1
N27	N-terminal TDP-43 fragment
C30	C-terminal TDP-43 fragment
ND3	mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 3
ND6	mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 6
PMPCA	peptidase mitochondrial processing alpha subunit

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Highlights

- Mitochondrial dysfunction is evident in age-related neurodegenerative diseases (Parkinson's disease, ALS, AD, and Huntington disease).
- TDP-43 is a major pathological protein in FTD, ALS, and AD and interacts with several mitochondrial proteins.
- TDP-43 interacts with and regulates MFN2 and PHB2, thus affects mitochondrial dynamics and mitophagy.
- We observed no increase in full-length TDP-43 in mitochondria in APP/PSEN1 mice, however there was a decrease in a 30kDa C-terminal TDP-43 fragment.
- Overexpression of the mitochondrial processing peptidase alpha subunit, PMPCA, increased 27kDa N-terminal TDP-43 expression.
- These observations expand our understanding of mitochondrial dynamics and may provide novel insights into the role TDP-43 plays in mitochondrial dysfunction.

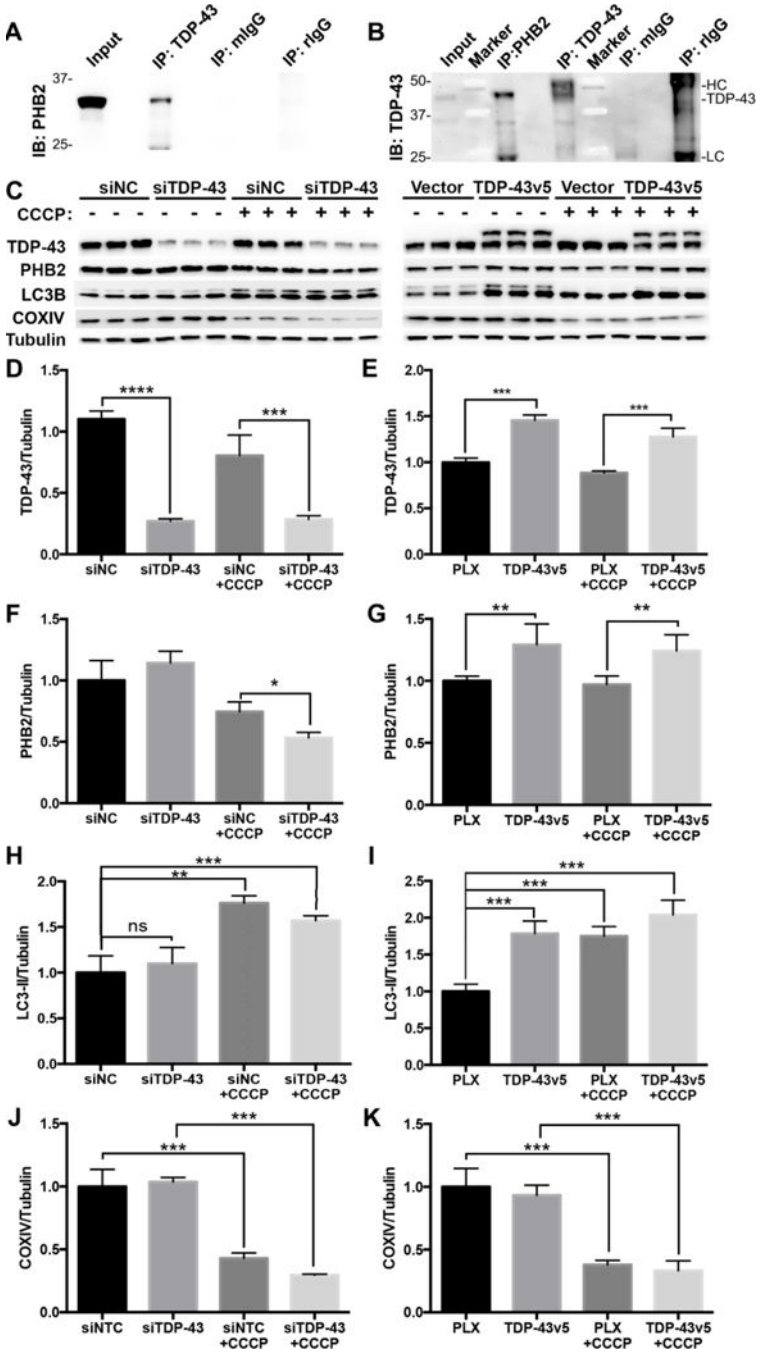

Figure 1. TDP-43 expression alters PHB2 expression and induces LC3-II

A) Immunoprecipitation of TDP-43 and immunoblot (IB) with anti-PHB2. mIgG indicates mouse IgG control, rIgG indicates rabbit IgG control. **B)** Immunoprecipitation of PHB2 and TDP-43 and immunoblot for TDP-43. **C)** Western blot for TDP-43, PHB2, LC3B, COXIV and Tubulin from lysed HEK293T cells transfected with siNC (negative control), siTDP-43, vector (PLX), or v5 tagged TDP-43 (TDP-43v5) and treated with either 10μM CCCP (indicated by + above lane) or DMSO as a control (indicated by -) for 12 hours. **D-E)** Quantitation of TDP- 43/Tubulin from Western blot above. **F-G)** Quantitation of PHB2/

Tubulin from Western blot above. **H-I)** Quantitation of LC3-II/tubulin from Western blot above. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. ANOVA with Tukey correction for Type I error. Error bars represent standard deviation.

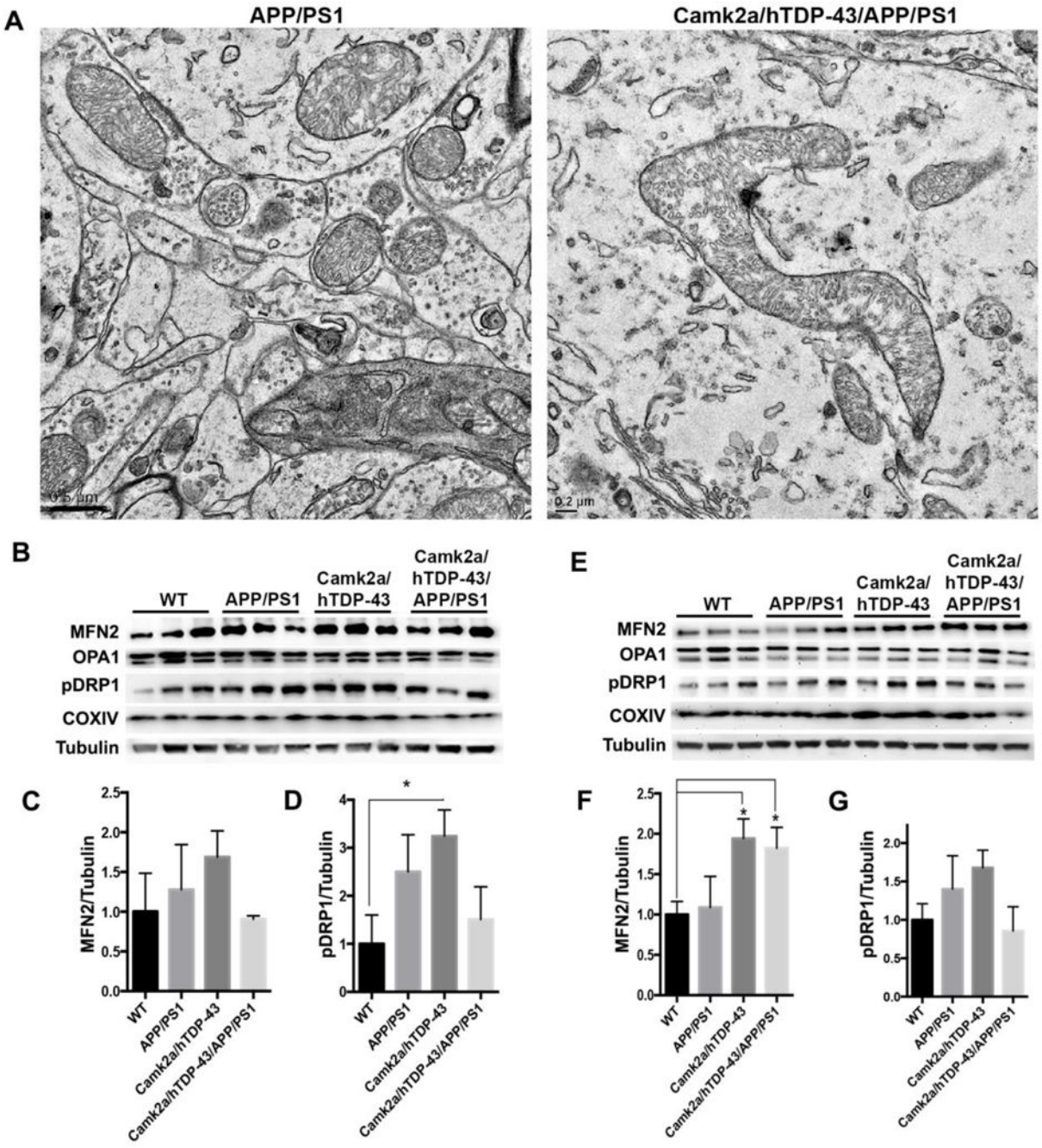

Figure 2. Selective expression of TDP-43 induces an age-dependent increase in Mitofusin-2

A) Transmission electron microscopy of hippocampal neurons in 9-month old APP/PS1 and Camk2a/hTDP- 43/APP/PS1 at 20,000×. B) Western blot for MFN2, OPA1, phospho-DRP1 (pDRP1) (Ser637), COXIV and Tubulin from lysate of cortex extracted from 4-month-old non-transgenic (WT), APP/PS1, Camk2a/hTDP-43, and Camk2a/hTDP-43/APP/PS1 mice. **C** & **D)** Quantitation of 4-month-old MFN2 and pDRP1 expression over Tubulin normalized to wild-type. **E)** Western blot for MFN2, OPA1, pDRP1(Ser637), and Tubulin from lysate of cortex extracted from 9-month-old non-transgenic (WT), APP/PS1, and Camk2a/hTDP-43

mice. **F & G)** Quantitation of 9-month-old MFN2 and pDRP1 expression over Tubulin normalized to wild-type.

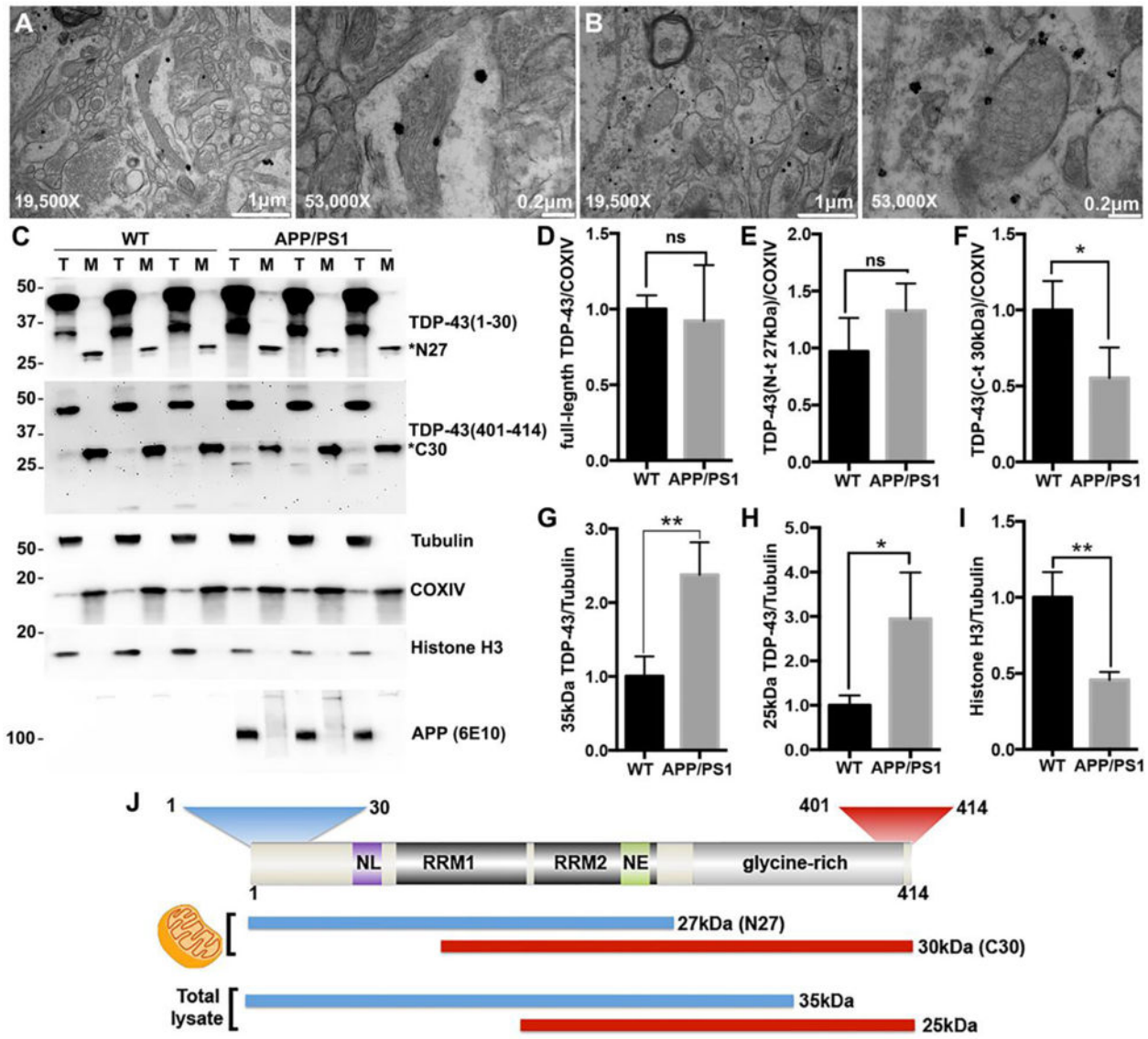

Figure 3. Mitochondrial fraction enhanced TDP-43 N- and C- terminal fragments

Transmission electron micrograph of immunogold labeled TDP-43 in the hippocampus of 8-month-old **A)** wild-type and **B)** APP/PS1 mice. TDP-43 localizes around the mitochondria in wild-type and APP/PS1 mice; 19,500× (left) and 53,000× (right) magnification. **C)** Mitochondrial fraction (Percoll gradient) from the cortex/hippocampus of 9-month-old wild-type and APP/PS1 mice, immunoblotted for TDP-43 N-terminal (N-t), C-terminal (C-t), tubulin, COXIV, Histone H3, and APP (6E10) **D)** Quantification of mitochondrial full-length TDP-43 normalized to COXIV. **E)** Quantification of N-terminal TDP-43 (27kDa) normalized to COXIV in mitochondrial fractions (band indicated by N27 in **C**). **F)** Quantification of C-terminal TDP-43 (30kDa) normalized to COXIV in mitochondrial fractions (band indicated by C30 in **C**). **G)** Quantification of 35kDa TDP-43 (N-terminal) from whole cell lysate normalized to Tubulin. **H)** Quantification of 25kDa TDP- 43 (C-terminal) from whole cell lysate normalized to Tubulin. **I)** Quantification of histone H3 normalized to Tubulin from whole cell lysates. T, total lysate; M, mitochondrial fraction;

*p<0.05, **p<0.01. Unpaired t test, two-tailed. Error bars represent standard deviation. **J)** Domain structure of TDP-43 displaying antibody epitopes for TDP-43 **N-terminal (1-30)** and **C-terminal (401-414)** and the various forms of TDP- 43 detected by each from cortex/ hippocampus mitochondrial fractions and total lysates.