Transcriptomopathies of pre- and post-symptomatic frontotemporal dementia-like mice with TDP-43 depletion in forebrain neurons

Lien-Szu Wu†, Wei-Cheng Cheng†, Chia-Ying Chen², Ming-Che Wu¹, Yi-Chi Wang³, Yu-Hsiang Tseng², Trees-Juen Chuang²* and C.-K. James Shen¹*

Abstract

TAR DNA-binding protein (TDP-43) is a ubiquitously expressed nuclear protein, which participates in a number of cellular processes and has been identified as the major pathological factor in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Here we constructed a conditional TDP-43 mouse with depletion of TDP-43 in the mouse forebrain and find that the mice exhibit a whole spectrum of age-dependent frontotemporal dementia-like behaviour abnormalities including perturbation of social behaviour, development of dementia-like behaviour, changes of activities of daily living, and memory loss at a later stage of life. These variations are accompanied with inflammation, neurodegeneration, and abnormal synaptic plasticity of the mouse CA1 neurons. Importantly, analysis of the cortical RNA transcripts of the conditional knockout mice at the pre- /post-symptomatic stages and the corresponding wild type mice reveals age-dependent alterations in the expression levels and RNA processing patterns of a set of genes closely associated with inflammation, social behaviour, synaptic plasticity, and neuron survival. This study not only supports the scenario that loss-of-function of TDP-43 in mice may recapitulate key behaviour features of the FTLD diseases, but also provides a list of TDP-43 target genes/transcript isoforms useful for future therapeutic research.

Keywords: Circular RNAs/ frontotemporal lobar degeneration/ loss-of-function/ Mis-processing/TDP-43

Introduction

Frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) are both incurable and rapidly progressive neurodegenerative diseases of the central nervous system, and they have overlapping spectra of pathogenic features [75]. While patients with FTLD exhibit a range of progressive changes in language dysfunction, behavioural abnormality, personality change, memory deficit, or motor neuron dysfunction [71], muscle weakness and motor neuron degeneration are the predominant symptoms of ALS [76]. The cytoplasmic ubiquitinated inclusions (UBIs) consisting of relocated nuclear TDP-43 protein is a common pathological characteristic observed in 50% of FTLD (FTLD-TDP) and 95% of ALS (ALS-TDP) [4, 18, 47, 54].

TDP-43, or TAR DNA-binding protein-43 [55], encoded by the highly conserved Tardbp gene [82] is a RNA-binding protein involved in transcriptional repression, pre-mRNA splicing, and translation [1, 49, 62, 76, 83]. TDP-43 in the diseased cells of the patients’ brains of FTLD-TDP or spinal cords of ALS-TDP is characterized with abnormal ubiquitination, hyperphosphorylation, and enhanced cleavage to generate the 25 kDa and 35 kDa C-terminal fragments (TDP-25 and TDP-35) [4, 54]. Furthermore, TDP-43 is partially or completely cleared from the nuclei of neuronal and/ or glial cells containing cytosolic TDP-43 (+) UBIs [53].
Mouse models with transgenic overexpression of TDP-43, knock-out/ knock-down of Tardbp gene expression often serve as the biological system for exploring the physiological functions of TDP-43 and its pathogenic roles in neurodegeneration [75]. Most of the transgenic TDP-43 mouse lines overexpress human TDP-43, wild type or mutants, under the control of pan-neuronal promoter, and the resulting phenotypes appear to be primarily relevant to ALS [57, 63]. Studies have engineered the mice to overexpress wild-type TDP-43 or induced depletion of TDP-43 in the forebrain region, which sufficed to cause neurodegeneration of brain [33, 43, 78]. However, the pathological features of most of these various mouse models do not follow a pattern of adult-onset diseases. Furthermore, the analysis of their behavioural deficits has been predominantly based on motor function or Alzheimer disease-related tests [75]. On the other hand, FTLD patients with behavioural abnormalities (behavioural variant frontotemporal dementia, bvFTLD) present predominantly with persistent changes in behaviour and social functioning, which manifest in disinhibition, apathy, altered food preferences and executive deficits. Also, impairment of motor function and hippocampal-dependent learning/memory are rare in early stage bvFTLD [61].

Moreover, the relative contributions of loss-of-function and gain-of-cytotoxicity to the neurodegeneration in FTLD-TDP or ALS-TDP remain to be better defined [44, 45, 47, 76, 83]. The physiological functions of TDP-43 in different mammalian tissues also await further investigation. Our previous results have shown that TDP-43 is important for early mouse embryo development [88] and that loss-of-TDP-43 function in spinal motor neurons can generate many of the ALS-TDP phenotypes [89]. To explore the normal physiological function of TDP-43 and examine whether depletion of TDP-43 expression in brain could cause the neurodegeneration in FTLD-TDP, we have utilized the Tardbp<sup>LS</sup> mouse line [88] and generated conditional knockout mice (TDP-43 cKO) with forebrain-specific deletion of Tardbp gene by crossing Tardbp<sup>loxP</sup> mice with αCaMKII-Cre mice (T29-1 line) [88], the latter of which express αCaMKII-Cre only in neurons of the adult mouse brain [79]. Mice with Tardbp<sup>loxPlox/lox</sup>Cre<sup>+</sup> alleles, referred to as TDP-43 cKO, were born at normal Mendelian ratios and appeared indistinguishable from their wild type littermate controls (Tardbp<sup>loxPlox</sup>Cre<sup>-</sup>, referred to as Ctrl) at birth. As expected, immunohistochemistry analysis of the TDP-43 cKO mice at 2 months of age confirmed the deletion of Tardbp gene and consequent depletion of TDP-43 expression in the forebrain region, in particular in the CA1 pyramidal cell layer of the hippocampus (Fig. 1a and Additional file 1: Figure S2a). Western blot analysis of TDP-43 expression in the TDP-43 cKO mice at 3 and 12 months of age also supported that depletion of TDP-43 was restricted to the cortex and hippocampus (upper panels of Fig. 1b and c), but not in cerebellum and spinal cord (lower panels of Fig. 1b). 50% of TDP-43 cKO mice died around the age of 17 months, approximately 12 months shorter than the Ctrl mice (Fig. 1d). Collectively, the results in Fig. 1 demonstrate the successful establishment of a mouse model with postnatal depletion of TDP-43 in the forebrain and the shortened life span of TDP-43 cKO mice in comparison to their wild type littermate controls.

**Perturbation of social behaviour and development of dementia-like behaviour in TDP-43 cKO mice at the early stage of behaviour variations**

TDP-43 has been identified as the major pathological protein in 50% of FTLD patients [80] and FTLD is characterized by a preponderance of abnormalities in social behaviour rather than memory, especially in the early stages of the disease [61]. Hence, we tested the social interaction behaviour of TDP-43 cKO mice by the three-chamber sociability and social novelty test [38] at 3, 6, and 12 months of age. In the test for social preference (session I), unlike theCtrls, 12-month-old TDP-43 cKO mice showed no preference for their conspecific (stranger 1) over the object by spending similar time investigating the empty cage and stranger 1 (upper panels, Fig. 2a). In the test for preference of social novelty and social recognition (session II), either 6- or 12-month-old TDP-43 cKO mice failed to demonstrate a preference for unfamiliar mouse (stranger 2) compared with the familiar one (stranger 1) (lower panels, Fig. 2a). TDP-43 cKO mice exhibited a progressive decline in their capability of social interaction as observed among the bvFTLD patients.

The light/dark box test [20] was used to assess the anxiety-like behaviour [74] of the TDP-43 cKO mice. TDP-43 cKO mice at 12-month-old showed an increased latency in moving from the brightly lit area to the dark

**Results**

**Generation of mouse lines with αCaMKII promoter-directed depletion of forebrain TDP-43**

To understand the pathophysiological role of TDP-43 in adult brain, we generated conditional knock-out mice with forebrain-specific deletion of Tardbp gene by crossing Tardbp<sup>loxP</sup> mice with αCaMKII-Cre mice (T29-1 line) [88], the latter of which express αCaMKII-Cre only in neurons of the adult mouse brain [79]. Mice with Tardbp<sup>loxPlox/lox</sup>Cre<sup>+</sup> alleles, referred to as TDP-43 cKO, were born at normal Mendelian ratios and appeared indistinguishable from their wild type littermate controls (Tardbp<sup>loxPlox</sup>Cre<sup>-</sup>, referred to as Ctrl) at birth. As expected, immunohistochemistry analysis of the TDP-43 cKO mice at 2 months of age confirmed the deletion of Tardbp gene and consequent depletion of TDP-43 expression in the forebrain region, in particular in the CA1 pyramidal cell layer of the hippocampus (Fig. 1a and Additional file 1: Figure S2a). Western blot analysis of TDP-43 expression in the TDP-43 cKO mice at 3 and 12 months of age also supported that depletion of TDP-43 was restricted to the cortex and hippocampus (upper panels of Fig. 1b and c), but not in cerebellum and spinal cord (lower panels of Fig. 1b). 50% of TDP-43 cKO mice died around the age of 17 months, approximately 12 months shorter than the Ctrl mice (Fig. 1d). Collectively, the results in Fig. 1 demonstrate the successful establishment of a mouse model with postnatal depletion of TDP-43 in the forebrain and the shortened life span of TDP-43 cKO mice in comparison to their wild type littermate controls.

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Fig. 1 (See legend on next page.)
area, an increase in time spent in the light area, and markedly decreased crossings between light and dark area in comparison to the Ctrl mice (Fig. 2b). These data suggest that depletion of TDP-43 in mice lead to develop the dementia-like behaviour [43].

Sequential impairment of learning/ memory capability and locomotor function in TDP-43 cKO mice at later stage of behaviour variations

FTLD patients usually did not display cognitive deficits until later stage of the disease [52]. We used the Morris Water Maze to examine the hippocampus-dependent learning/memory, including acquisition of spatial memory and long-term spatial memory [81] of TDP-43 cKO mice. As shown in Fig. 2c, TDP-43 cKO mice show severe learning/memory impairment at the age of 12 months (left panels of Fig. 2c). In the probe trial, only the 12-month-old TDP-43 cKO mice exhibited a significant smaller numbers of target platform crossings in comparison to the age-matched Ctrl mice (right panels of Fig. 2c).

Overall, the progressive dementia of the TDP-43 cKO mice strongly suggests that the functional requirement of TDP-43 in learning/ memory at later stage of life [43].

Defective motor coordination developed in the late stages of a proportion of patients with FTLD [11]. We thus examined the locomotor activity of TDP-43 cKO mice using the accelerated rotarod tests. While no difference in motor performance could be found between Ctrl and TDP-43 cKO mice before the age of 12 months, older TDP-43 cKO mice exhibited reduced motor performance on the 1st day of test (Fig. 2d). Interestingly, their performance would become better on the 2nd day of test, and there was no difference between the TDP-43 cKO and Ctrl mice on the 3rd day of test (Fig. 2d). This result suggested that the impairment of rotarod performance of the TDP-43 cKO mice was mainly due to their memory loss. They barely remembered how to perform on the rotarod, but could re-learn after 1st day of test. However, while the motor deficiency was also observed after 16 months of age, it could not be reversed on the 2nd or 3rd day of test (Fig. 2d). The massive degeneration of cortex might result in the reduced motor performance after 16 months of age which the TDP-43 cKO mice lose their memory and learning ability to perform rotarod. Nevertheless, the above data show that in addition to severe memory loss developed after 12 months of age, TDP-43 cKO mice also exhibit motor dysfunction after the age of 16 months. This pattern is in interesting parallel to the sequential impairment of these two neuronal functions during FTLD pathogenesis [75].

Progressively changes of nesting behaviour and eating habits in TDP-43 cKO mice

Beside cognitive dysfunction, patients with dementia including FTLD also exhibit decreased activities of daily living (ADL). We analyzed the changes of eating habits in TDP-43 cKO mice and found reduced food intake by the aged TDP-43 cKO mice (Additional file 1: Figure S1c) but not in younger ones (Additional file 1: Figure S1a and S1b). We also compared nest construction scores [21] between the Ctrl and TDP-43 cKO mice. There was a significant difference in the nesting behaviour between TDP-43 cKO and Ctrl mice at 12 months of age (Additional file 1: Figure S1d). Taken together, these results indicate that accompanying with the dementia phenotype, aged TDP-43 cKO mice also developed a progressive decrease of their ADL.

Brain atrophy, neuronal loss, and neuronal degeneration of the TDP-43 cKO mice

Necropsy examination showed that the 12-month-old TDP-43 cKO mice, but not 3-month-old ones, had a significant reduction of the overall brain size and its weight when compared to age-matched Ctrl mice, which appeared to be mainly due to a decrease in the size of the cortical areas (Fig. 3a). In parallel, hematoxylin and eosin staining showed aberrant cellular patterns and layering in the cortex of 12-month-old TDP-43 cKO mice (Fig. 3b and Additional file 1: Figure S2). Also, the thickness of cerebral cortex was reduced and the size of the ventricles was enlarged when compared to the Ctrl mice (Fig. 3b and c). These results are indicated that depletion of TDP-43 causes the brain atrophy in mice [43].

Massive neuronal degeneration was observed in the cortex and hippocampus of 12-month-old TDP-43 cKO mice (Additional file 1: Figure S2 b-d). In particular, Golgi staining showed that the average length of the dendrites on dendritic stems of the layer V neurons of 12-month-old TDP-43 cKO mice was shorter than that of the control.
**Fig. 2** (See legend on next page.)
group (Fig. 4a). Moreover, the numbers of neuron with the beading or shorter dendrites [48, 71, 72] were increased mainly in the layer V pyramidal neuron of TDP-43 cKO mice at the age of 12 months (Fig. 4b and c). Immunofluorescence staining showed that the number of neurofilament H marker (SMI-32)-positive neurons in layer III/V of retrosplenial cortex (RS), but not those in the somatosensory cortex, decreased in TDP-43 cKO mice (Fig. 4d). Taken together, there appears to be significant and progressive neuronal degeneration in TDP-43 cKO mice in a selective vulnerability manner, with distinct neuronal populations in different cortical layers compromised by the depletion of TDP-43.

**Chronic astrocytosis in the forebrain of TDP-43 cKO mice**

We assessed the astrocyte response during the progressive cortical degeneration describe above. As shown, GFAP-positive astrocytes in the cortical layers and hippocampus of Ctrl mice of different ages were mainly found in the corpus callosum and rarely in the neuronal layers of the cortex (upper panels, Fig. 3d). Furthermore, their slim morphology suggested a resting state of the astrocytes (upper panels, Additional file 1: Figure S3a). In stark contrast, large numbers of tufted enlarged GFAP-positive astrocytes were found in the retrosplenial cortex (RS) and in the stratum lacunosum-moleculare (SLM) region of the hippocampus of TDP-43 cKO mice (lower panels, Fig. 3d and Additional file 1: Figure S3a) and they progressively increased during aging (Additional file 1: Figure S3a and b), coinciding with the progressive thinning of the cortex exemplified in Fig. 3b. However, no microglia activation was observed at all stages of the Ctrl and TDP-43 cKO mice analyzed (Additional file 1: Figure S3c). These results indicated that progressive astrogliosis, but not microgliosis was found in TDP-43 cKO mice which reflecting the neuropathological changes. Taken together, depletion of TDP-43 in the forebrain neurons resulted in a substantial and persisting activation of the astrocytes.

**Impaired synaptic plasticity in TDP-43 cKO mice**

TDP-43 has been shown to be a modulator of synaptic plasticity in transgenic mouse models of ALS and FTLD [30]. We investigated whether depletion of TDP-43 indeed affected the synaptic plasticity by examining the Schaeffer collateral pathway of TDP-43 cKO mice for synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD). As shown in Additional file 1: Figure S4, the magnitude of long-term potentiation (LTP) and long-term depression (LTD) in the hippocampal slices from 2-month-old TDP-43 cKO mice was unaffected (Additional file 1: Figure S4a and c). At the age of 12 months, LTP in the hippocampus of TDP-43 cKO mice were significantly lower than the Ctrl mice (Additional file 1: Figure S4b and c). Alltogether, these results show that depletion of TDP-43 in the forebrain neurons affects the synaptic functions.

**Genome-wide analysis of the neocortex transcriptomes of pre- and post-symptomatic TDP-43 cKO mice in comparison to ctrl mice**

Transcripts exhibiting alternations of their expression levels or splicing patterns in TDP-43 depleted cells other than cortex have been identified before by RNA-seq analysis [5, 37, 58]. However, a transcriptome-wide analysis of the relationship between TDP-43 targeted RNAs and TDP-43 regulated behaviour/phenotypes was lacking. We used paired-end deep sequencing (see Materials and Methods) to examine the gene expression profiles in the neocortex (the region indicated in the left part of Additional file 1: Figure S2a) of TDP-43 cKO mice and their corresponding littermate controls at both the pre-symptomatic (3-month) and post-symptomatic (12-month) stages. The differential expression analysis (see Materials and Methods) revealed 52 and 121 up-/down-regulated genes at the ages of 3 months and 12 months, respectively (Fig. 5a-(i)). Interestingly, the number of the down-regulated genes was much greater in 12-month-old
Fig. 3 (See legend on next page.)
TDP-43 cKO mice than in 3-month-old cKO ones (Fig. 5a-(ii)), in correlation with the progression of the FTLD-like pathological phenotypes of the mutant mice (Fig. 2-4).

Significantly, most of the transcripts with increased abundance in TDP-43 cKO mice associated with activation of astrocytes, whereas the majority of transcripts with decreased abundance were associated with calcium signaling and synaptic transmission—likely reflecting the astrocitosis and synaptic transmission deficit that progressively occurred with aging in TDP-43 cKO cortex (Table 1). Consistent with the correlation of these up/down regulated cortex genes with the age-dependent pathogenesis of TDP-43 cKO mice, most of the gene expression differences between the TDP-43 cKO cortex and Ctrl cortex became greater magnified as the mice aged (Fig. 5a-(i) and 5a-(iv)).

**Up/down-regulated TDP-43 cKO mouse neocortex genes associated with inflammation, autophagy, and synaptic function**

Significantly, 26 genes and 10 genes were up-regulated by >2 fold in 3- and 12-month-old TDP-43 cKO mouse neocortex, respectively (Fig. 5a-(ii) and Table 1), and most of them encoded the inflammatory proteins. Three out of these up-regulated genes, i.e. Gfap, Serpina3a, and C4a/C4b, were constitutively up-regulated at both the ages of 3 months and 12 months (Additional file 1: Figure S5a), as also quantified by qRT-PCR (Additional file 1: Figure S5b). Of the three genes, increase of Gfap and Serpina3n mRNAs in reactive astrocyte was reported in brain injury and in several neurodegeneration diseases [26, 93]. We also calculated and compared the intron sizes of TDP-43-regulated genes in the Ctrl and TDP-43 cKO mouse neocortex. It was found that the total lengths of the introns of down-regulated neocortex genes in TDP-43 cKO mice (the median values being 30,061 bp and 28,604 bp at the ages of 3 months and 12 months, respectively) were larger than those of control (the median value being 11,754 bp), whereas the trend was not observed for the up-regulated neocortex genes (the median values being 9968 bp and 13,098 bp at the ages of 3 months and 12 months, respectively) (Additional file 1: Figure S5c).

To assess the empirical P values, we calculated the median values of the total intron lengths of 100 genes randomly selected from the annotated mouse protein-coding genes and the process was repeated for 10,000 times. Indeed, the down-regulated neocortex genes of the TDP-43 cKO mice at either the age of 3 months or 12 months possessed significantly longer total introns than expected, with the P values < 0.001. This result is consistent with the previous observation that down-regulated genes in striatum upon TDP-43 reduction tend to have long introns [58].

Increase of Gfap protein in the cortex and hippocampus of TDP-43 cKO mice at the ages of 3 months and 12 months was confirmed by Western blotting, respectively (Fig. 1b). Note that one additional band in GFAP immunoblotting in protein extracts of 3-month-old mice could be the isoform of GFAP protein. On the other hand, most of 20 down-regulated genes in the neocortex of 3-month-old TDP-43 cKO mice (Fig. 5a) encoded proteins involved in the functions of synapse, e.g. Dlg3, endosome, e.g. Lamp5, and autophagosome, e.g. Tecpr1 (Table 1). Furthermore, eight of these 20 down-regulated genes were constitutively repressed in the neocortex of TDP-43 cKO mice at the age of 12 month (Fig. 5a and Table 1).

We also analyzed the altered expression of several genes by Western blotting. Firstly, autophagy defect was reported in Tecpr1 gene knockout mice with increased expression of an autophagy substrate, p62 [14]. As shown in Additional file 1: Figure S5d, the level of p62 protein was increased in the cortex of TDP-43 cKO mice at all ages analyzed. Secondly, consistent with the RNA-seq data (Additional file 1: Figure S5a) and RT-qPCR analysis (Additional file 1: Figure S5b), the levels of SAP102 protein, which was encoded by the Dlg3 gene mentioned above and involved in synaptic plasticity by regulating the recycling of NMDA receptor NMDAR [13], in the cortex and synaptosome of TDP-43 cKO mice were reduced in an age-dependent manner (Additional file 1: Figure S5e). Since NMDA receptor (NMDAR)-mediated responses regulated the levels and activities of CaMKII family members [48], we also examined the levels of different synaptic proteins including the CaM kinase proteins CaMK4, NMDAR subunit NR2b, and phospho-Erk1/2. Indeed, the amounts of these proteins were all greatly reduced in the cortex.
Fig. 4 (See legend on next page.)
and/or synaptosome of 12-month-old TDP-43 cKO mice in comparison to the Ctrl mice (Additional file 1: Figure S5e), while the amount of SAP102 was decreased in the cortex and synaptosome of early stage TDP-43 cKO mice. Thus, depletion of TDP-43 in the cortex indeed would down-regulate the expression of a specific set of genes and this could contribute in part to the impairment of synaptic functions (Additional file 1: Figure S4) and behaviour deficits (Fig. 2) in an age-dependent manner.

**Mis-regulation of RNA processing**

Aberrant RNA processing was increasingly recognized as a potential contributor to the development/ pathogenesis of neurological diseases [68]. Among the different RNA processing events, alternative splicing (AS) is one major mechanism for the enhancement of transcriptome diversity. A growing number of human diseases were correlated with RNA mis-splicing [3]. There are several different types of alternative splicing events including inclusion/exclusion of conserved (consistently present in wild type RNAs) or non-conserved (cryptic) exon as well as the alternative splicing site selection leading to extension of conserved exons. To investigate the regulatory role of AS in the forebrain neurons of TDP-43 cKO mice, we used the Cufflink [77] and MISO (Mixture of Isoforms) [40] programs to examine usage changes of alternatively spliced exons (ASEs) and poly(A) sites. In comparison to the Ctrl mice, 55 and 57 transcript processing events exhibited remarkably usage changes of ASEs or poly(A) sites. In contrast, 44 and 54 transcript processing events with significant usage changes were found only in 3- and 12-month-old TDP-43 cKO mice, respectively (Additional file 1: Figure S6 and Table 2). Notably, some of these transcript events with significant usage changes were found only in 3-month-old TDP-43 cKO mice (Additional file 1: Figure S7 and Table 2).

We then examined some of the differential RNA processing events by RT-qPCR and/or semi-quantitative RT-PCR (Additional file 1: Figure S8 and Figure S9). For instance, Sortilin 1 (Sort1), a member of a family of cellular vacuolar protein sorting 10 (VSP10)-domain receptors, was primarily expressed in neurons and a key player in regulating the neuronal viability and function [87]. It was proposed that TDP-43 regulates the splicing of Sort1 mRNA in mouse striatum and cell lines [58, 60]. We confirmed Sort1(wt) as the main mRNA isoform encoding sortilin in mouse cortex by RT-PCR and Western blotting (Additional file 1: Figure S9a and b). However, depletion of TDP-43 expression in the neocortex of TDP-43 cKO mice led to the accumulation at all stages of the higher molecular weight RNA isoform Sort1(e17b) encoding a non-functional progranulin receptor Sortilin 1(e17b) [60] (Additional file 1: Figure S8a, S9a and b). Quantification analysis of Sort1 mRNA levels by qRT-PCR in different mouse brain areas confirmed this observation in both 3- and 12-month-old TDP-43 cKO mice (Additional file 1: Figure S8a). Other events of conserved and cryptic exon inclusions induced by depletion of TDP-43 were also confirmed by qRT-PCR, as exemplified for Dnajc5,CaMK1g,and Adnp2, respectively (Additional file 1: Figure S8). Notably, the expression levels of the wild type isoforms of Sort1 and Dnajc5 in the cortex of TDP-43 cKO mice were unaltered (Additional file 1: Figure S9c), while those of CaMK1g and Adnp2 were unchanged in the cortex of 3-month-old TDP-43 cKO mice (upper panels, Additional file 1: Figure S9d) but moderately increased at the age of 12 months (lower panels, Additional file 1: Figure S9d).

**Mis-regulation of circular RNA processing in TDP-43 cKO mice**

Circular RNAs (circRNAs) are RNA molecules in which a covalent linkage termed a “backsplice” has formed between a downstream 3’ splice site and an upstream 5’ splice site in a linear pre-messenger RNA [73]. Previous
### a (i)

|                  | Genes without changes (FPKM>0.2) | Up-regulated genes (FPKM>0.2, q<0.05) | Down-regulated genes (FPKM>0.2, q<0.05) | Splicing-changed genes (q<0.05) |
|------------------|----------------------------------|----------------------------------------|----------------------------------------|-------------------------------|
| 3 Months         | 13,981                           | 32                                     | 20                                     | 55                            |
| 12 Months        | 12,736                           | 22 (4)                                 | 99 (8)                                 | 57 (37)                       |

### (ii)

**3 Months**
- Up-regulated (q<0.05): 32
- >2 fold change: 28
- Down-regulated (q<0.05): 20
- >2 fold change: 6

**12 Months**
- Up-regulated (q<0.05): 22
- >2 fold change: 10
- Down-regulated (q<0.05): 99
- >2 fold change: 9

### (iii)

**3 Months**
- Log2 (FPKM_{control}/FPKM_{3 months})

**12 Months**
- Log2 (FPKM_{control}/FPKM_{12 months})

- Genes altered at 3 Months
- Genes altered at 12 Months
- Genes altered at both 3 and 12 Months

### b

**3 Months**
- Total=55 events
- Cryptic exon inclusion: 5%
- Exon inclusion: 11%
- Exon exclusion: 16%
- Exon extension: 33%
- Poly(A) extension: 35%

**12 Months**
- Total=57 events
- Cryptic exon inclusion: 6%
- Exon inclusion: 12%
- Exon exclusion: 12%
- Exon extension: 39%
- Poly(A) extension: 32%

Fig. 5 (See legend on next page)
studies have lead to the identification of thousands of circRNAs in diverse species [15, 31, 36, 67, 84] that are enriched in neuronal tissues and may play specific roles in neuronal processes [29, 65, 92]. Analysis of our RNA-seq data by the NCLscan pipeline [19] revealed that the expression levels of 182 circRNAs in the neocortex were significantly different between the TDP-43 cKO and Ctrl mice (Fig. 6 and Additional file 2: Table S1). Among them, the expression levels of 22 circRNAs were significantly altered in the neocortex of 3- as well as 12-month-old TDP-43 cKO mice when compared to the Ctrl mice. The levels of 39 circRNAs were changed only at the age of 3 months and 121 circRNAs were altered only at the age of 12 months (Fig. 6a and Additional file 2: Table S1). Notably, a considerable percentage of the circRNAs and their corresponding co-linear mRNA isoforms exhibited different changes of their expression levels in TDK-43 cKO in comparison to the Ctrl mice (Fig. 6b). This result reflects the previous observation that circRNAs and their co-linear counterparts could compete with each other for biogenesis during splicing [6, 16]. The biological significance of the alterations of expression levels of the circRNAs in the TDP-43 cKO mouse neocortex await to be examined.

Discussion
TDP-43 proteinopathy is associated with more than 95% of ALS (ALS-TDP) and more than 50% of FTLD (FTLD-TDP) [47]. A gain-of-toxicity mechanism for early pathogenesis of FTLD-TDP or ALS-TDP has been suggested in view of the aberrant RNA metabolism and/or perturbed autoregulation of TDP-43 caused by mutant TDP-43 in different mouse models [5, 24, 58, 59, 86, 89]. One the other hand, a common characteristic of TDP-43 pathology at the later stage of FTLD-TDP or ALS-TDP is the loss of nuclear TDP-43 with concomitant cytoplasmic TDP-43 accumulation in neurons and glia [54]. This nuclear clearing provides a disease mechanism that is at least partially driven by the loss of normal TDP-43 function in the nucleus, as supported by studies of different mouse models with knockout or knockdown of TDP-43 expression [89, 91]. The presence of the cytoplasmic TDP-43(+) inclusions would also cause gain of one or more cytotoxic properties [44].

As summarized in Fig. 7, this study shows that CaMKII-directed conditional depletion of TDP-43 expression in the forebrain neurons has adverse effects on the mice, leading to shorter life span and a range of age-dependent phenotypes on the behavioural, cellular, as well as molecular levels that mimic FTLD, especially bvFTLD [61, 75]. Specifically, depletion of TDP-43 in aCaMKII-expressing neurons in the mouse forebrain (Fig. 1) results in progressive perturbation of social behaviour (Fig. 2a), development of dementia-like behaviour (Fig. 2b), and impairment of learning/memory (Fig. 2c). The behaviour deficits are accompanied with brain atrophy and neurodegeneration in the cortical hippocampal region and massive astrocytosis (Fig. 3-4). These findings together with analysis of the transcriptomes/gene expression profiles of mouse neocortex at the pre-symptomatic and post-symptomatic stages (Fig. 5, Fig. 6 and Additional file 1: Figures S5-S10) demonstrate the function of TDP-43 in cognition, and synaptic function in the adult brain. Notably, the large neurons in cortical layer V of TDP-43 cKO mice are more vulnerable to TDP-43 depletion (Fig. 4). This data is consistent with the finding by Yang et al. [91], in which 10% reduction of TDP-43 protein level in the forebrain region of a TDP-43 knockdown mouse model could causes ~25% loss of large neurons in cortical layer V. Thus, there appears to be a selective vulnerability of the forebrain neurons, with distinct neuronal populations in different cortical layers compromised by the depletion of TDP-43. Notably, depletion of TDP-43 did not affect the sensory neurons of above mouse model [91]. Overall, this study demonstrates that loss-of-function of TDP-43 in the
Table 1: The genes with altered mRNA levels in the neocortex of 3-month and 12-month-old TDP-43 cKO mice in comparison to the Ctrl s are listed.

| Gene symbol | Name | RefSeq ID | Log2(TDP-43 cKO/ Ctrl) 3 Months | Log2(TDP-43 cKO/ Ctrl) 12 Months | Significant (q<0.05) | FTLD patient | White et al. [86] | Other references |
|-------------|------|-----------|---------------------------------|---------------------------------|---------------------|---------------|----------------|----------------|
| Rabggtb     | Rab geranylgeranyltransferase beta subunit | NM_011231 | 6.21                           | -0.14                          | ●                   |               |                |                |
| Clec7a      | C-type lectin domain containing 7A | NM_020008 | 6.06                           | 2.09                           | ●                   |               | (Chen-Plotkin et al. [17]) |
| Itgad       | Integrin alpha-D | NM_001028872 | 2.99                           | 1.45                           | ●                   |               |                |                |
| Brd3        | bromodomain containing 3 | NM_023336 | 2.88                           | 0.11                           | ●                   |               |                |                |
| Atf3        | activating transcription factor 3 | NM_007498 | 2.47                           | 1.90                           | ●                   |               |                |                |
| Slc6a3      | solute carrier family 6 member 3 | NM_0100020 | 2.19                           | 0.80                           | ●                   |               |                |                |
| Cc19        | chemokine (C-C motif) ligand 6 | NM_011338 | 2.15                           | 0.15                           | ●                   |               |                |                |
| Cc16        | chemokine (C-C motif) ligand 9 | NM_009139 | 2.09                           | 0.18                           | ●                   |               |                |                |
| Cep112      | centrosomal protein 112 | NM_029586 | 1.76                           | 0.04                           | ●                   |               |                |                |
| Pafah2      | platelet-activating factor acetylhydrolase 2 | NM_001285872 | 1.69                           | 1.50                           | ●                   |               |                |                |
| Itgad       | Integrin alpha-D | NM_001028872 | 1.48                           | 0.86                           | ●                   |               | (Chen-Plotkin et al. [17]) |
| Lyz1, Lyz2 | lysozyme 1 | NM_017372 | 1.48                           | 0.86                           | ●                   |               |                |                |
| Ddhd1       | DDHD domain containing 1 | NM_176845 | 1.44                           | 0.13                           | ●                   |               |                |                |
| Myo1f       | myosin IF | NM_053214 | 1.39                           | 0.60                           | ●                   |               | (Polymenidou et al. [58]) |
| Trem2       | triggering receptor expressed on myeloid cells 2 | NM_001272078 | 1.16                           | 0.25                           | ●                   |               |                | (Polymenidou et al. [58]) |
| Ptpn6       | protein tyrosine phosphatase, non-receptor type 6 | NM_001077705 | 1.15                           | 0.63                           | ●                   |               | (Polymenidou et al. [58]) |
| Ifit3b      | interferon-induced protein with tetratricopeptide repeats 3B | NM_001005858 | 1.11                           | 0.31                           | ●                   |               | (Polymenidou et al. [58]) |
| Clqa        | complement component 1, q subcomponent, alpha polypeptide | NM_007572 | 1.02                           | 0.42                           | ●                   |               | (Polymenidou et al. [58]) |
| Tyrobp      | Tyrp protein tyrosine kinase binding protein | NM_011662 | 1.01                           | 0.19                           | ●                   |               | (Polymenidou et al. [58]) |
| Lgals3bp    | lectin, galactoside-binding, soluble, 3 binding protein | NM_011150 | 0.99                           | 0.38                           | ●                   |               | (Chen-Plotkin et al. [17]) |
| Clqb        | complement component 1, q subcomponent, beta polypeptide | NM_009777 | 0.96                           | 0.41                           | ●                   |               | (Polymenidou et al. [58]) |
Table 1 The genes with altered mRNA levels in the neocortex of 3-month and 12-month-old TDP-43 cKO mice in comparison to the Ctrls are listed (Continued)

| Gene symbol | Name                                                    | RefSeq ID | Log2(TDP-43 cKO/Ctrl) | Significant (q<0.05) | FTLD patient | White et al. [86] | Other references |
|-------------|---------------------------------------------------------|-----------|------------------------|-----------------------|--------------|-------------------|-----------------|
|             |                                                         | 3 Months  | 12 months              |                       | 3 Months      | 12 months        |                 |
|             |                                                         | Common MB- | Common MB-              |                       | frontal cortex| frontal cortex    |                 |
| C1qc        | complement component 1, q subcomponent, C chain        | NM_007574 | 0.90                  | 0.39                  | ●             | ●                |                 |
| Fcrls       | Fc receptor-like 5, scavenger receptor                 | NM_030707 | 0.91                  | -0.11                 | ●             | ●                |                 |
| Fcgr3       | Fc receptor, IgG, low affinity III                     | NM_010188 | 0.83                  | 0.53                  | ●             | ●                | (Polymenidou et al. [58]) |
| Cts3        | cathepsin S                                            | NM_001267695 | 0.79              | 0.28                  | ●             | ●                | (Polymenidou et al. [58]) |
| Ripor1      | Rho family interacting cell polarization regulator 1    | NM_001081241 | -1.48            | -0.28                 | ●             | ●                |                 |
| Plod3       | procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3     | NM_011962  | -1.23                 | 0.14                  | ●             | ●                |                 |
| Hist1h4m    | histone cluster 1, H4m                                  | NM_175654  | -1.18                 | -0.30                 | ●             | ●                |                 |
| Zfp101      | zinc finger protein 101                                 | NM_009542  | -1.02                 | -0.02                 | ●             | ●                |                 |
| Dlg3        | discs large MAGUK scaffold protein 3                    | NM_007863  | -0.87                 | 0.00                  | ●             | ●                | (Chen-Plotkin et al. [17]) |
| Otof        | otoferlin                                               | NM_001100395 | -0.86            | -0.34                 | ●             | ●                | (LaClair et al. [43]) |
| Dock4       | dedicator of cytokinesis 4                              | NM_172803  | -0.79                 | -0.52                 | ●             | ●                | (Polymenidou et al. [58]) |
| Abr         | active BCR-related gene                                 | NM_001291186 | -0.79            | -0.41                 | ●             | ●                | (LaClair et al. [43]) |
| Syt17       | synaptotagmin XVII                                      | NM_138649  | -0.70                 | -0.48                 | ●             | ●                | (Polymenidou et al. [58]) |
| Unc13a      | unc-13 homolog A (C. elegans)                           | NP_001025044 | -0.77              | -0.48                 | ●             | ●                | (Polymenidou et al. [58]) |
| Rab11fip5   | RAB11 family interacting protein 5 (class I)            | NM_001003955 | -0.69            | -0.58                 | ●             | ●                | (Polymenidou et al. [58]) |
| Fgf21       | fibroblast growth factor 21                             | NM_020013  | 0.00                  | 0.00                  | ●             | ●                | (Polymenidou et al. [58]) |
| Gfap        | glial fibrillary acidic protein                         | NM_001131020 | 1.74              | 1.43                  | ●             | ●                | (Chen-Plotkin et al. [17]) |
| Serpina3n   | serine (or cysteine) peptidase inhibitor, clade A, member 3N | NP_035588  | 1.27                  | 1.34                  | ●             | ●                | (Chen-Plotkin et al. [17]) |
| C4a,C4b     | complement component 4a/b                               | NM_011413  | 1.64                  | 1.46                  | ●             | ●                | (Chen-Plotkin et al. [17]) |
| A2m         | alpha-2-macroglobulin                                   | NM_175628  | 1.37                  | 2.09                  | ●             | ●                |                 |
Table 1: The genes with altered mRNA levels in the neocortex of 3-month and 12-month-old TDP-43 cKO mice in comparison to the Ctrls are listed (Continued)

| Gene symbol | Name                                                                 | RefSeq ID | Log2(TDP-43 cKO/ Ctrl) | Significant (q<0.05) | FTLD patient | Other references |
|-------------|----------------------------------------------------------------------|-----------|-------------------------|----------------------|--------------|------------------|
|             |                                                                     |           | 3 Months     | 12 months            | 3 Months     | 12 months       | 5 Months frontal cortex | 20 Months frontal cortex |
|              |                                                                      |           | 3 Months     | 12 months            | 5 Months frontal cortex | 20 Months frontal cortex |
| Tecpr1      | tectonin beta-propeller repeat containing 1                         | NP_081686 | -1.06        | -0.93                | ● ●          |                 | ● ●              |                     |
| Tarsl2      | threonyl-tRNA synthetase-like 2                                      | NM_172310 | -1.00        | -0.90                | ● ●          |                 | ● ●              |                     |
| Pitpmn3     | PITPM family member 3                                               | NM_001024927 | -0.97      | -0.96                | ● ●          | ● (up)          | ● (up)            |                     |
| Epop        | elongin Bc and polycomb repressive complex 2 associated protein     | NM_175332 | -0.80        | -0.90                | ● ●          |                 | ● ●              |                     |
| Arc         | activity regulated cytoskeletal-associated protein                  | NM_001276684 | -0.84      | -0.80                | ● ●          |                 |                 |                     |
| Cry2        | cryptochrome 2 (photolyase-like)                                     | NM_009963 | -0.78        | -0.58                | ● ●          |                 |                 |                     |
| Lamp5       | lysosomal-associated membrane protein family, member 5              | NM_029530 | -0.71        | -0.96                | ● ●          |                 | ● ●              |                     |
| Fam171a2    | family with sequence similarity 171, member A2                       | NM_199200 | -0.70        | -0.65                | ● ●          |                 | ● ●              |                     |
| Oxt         | oxytocin/neurophysin I prepropeptide                                | NM_011025 | 1.11         | 2.95                 | ● ●          |                 | ● ●              |                     |
| Plin4       | perilipin 4                                                          | NM_020568 | 0.61         | 1.85                 | ● ●          |                 | ● ●              |                     |
| Irs4        | insulin receptor substrate 4                                        | NP_034702 | 0.22         | 1.79                 | ● ●          |                 | ● ●              |                     |
| Itih3       | inter-alpha trypsin inhibitor, heavy chain 3                        | NM_008407 | 0.69         | 1.27                 | ● ●          |                 | ● ●              |                     |
| Etnppl      | ethanolamine phosphate phosphorylase                                 | NM_00163587 | 0.63      | 1.07                 | ● ●          |                 | ● ●              |                     |
| AW551984    | expressed sequence AW551984                                        | NM_001199556 | 0.08      | 1.04                 | ● ●          |                 | ● ●              |                     |
| Agt         | angiotensinogen (serpin peptidase inhibitor, clade A, member 8)      | NM_007428 | 0.31         | 0.97                 | ● ●          |                 | ● ●              |                     |
| Chd11       | chromodomain helicase DNA binding protein 1-like                    | NM_002639 | 0.06         | 0.96                 | ● ●          |                 | ● ●              |                     |
| Ugp2       | UDP-glucose glycoprotein glucosyltransferase 2                      | NM_001081252 | 0.26      | 0.87                 | ● ●          |                 | ● ●              |                     |
| Vip         | vasoactive intestinal polypeptide                                    | NM_00131969 | 0.57      | 0.79                 | ● ●          |                 | ● ●              |                     |
| Sparc       | secreted acidic cysteine rich glycoprotein                          | NM_001290817 | 0.48      | 0.77                 | ● ●          |                 | ● ●              |                     |
| Bialp3      | BAI1-associated protein 3                                            | NM_001163270 | 0.24      | 0.72                 | ● ●          |                 | ● ●              |                     |
| Nnat        | neuronatin                                                           | NM_001291128 | 0.37      | 0.70                 | ● ●          |                 | ● ●              |                     |
| Fam107a     | family with sequence similarity 107, member A                        | NM_183187 | 0.38         | 0.69                 | ● ●          |                 | ● ●              |                     |
| Sdc4        | syndecan 4                                                           | NM_011521 | 0.33         | 0.68                 | ● ●          |                 | ● ●              |                     |

(LaClair et al. [43]) (Chen-Plotkin et al. [17]) (Polymenidou et al. [58])
Table 1: The genes with altered mRNA levels in the neocortex of 3-month and 12-month-old TDP-43 cKO mice in comparison to the Ctrls are listed (Continued)

| Gene symbol | Name                                              | RefSeq ID     | Log2(TDP-43 cKO/ Ctrl) | Significant (q<0.05) | FTLD patient | White et al. [86] | Other references |
|-------------|---------------------------------------------------|---------------|-------------------------|----------------------|--------------|-------------------|------------------|
| Ptgsds      | prostaglandin D2 synthase (brain)                 | NM_008963     | 0.27                    | 0.65                 | ●            | (Chen-Plotkin et al. [17]) |
| Scg2        | secretogranin II                                  | NM_001310680  | 0.21                    | 0.58                 | ●            | (Chen-Plotkin et al. [17]) |
| Apod        | apolipoprotein D                                  | NM_001301353  | 0.38                    | 0.54                 | ●            | (Chen-Plotkin et al. [17]) |
| Tnn1c1      | tropomyosin C1, slow skeletal and cardiac type    | NP_033419     | -1.20                   | -2.38                | ●            | (Chen-Plotkin et al. [17]) |
| Gm10635     | predicted gene 10635                              | NR_045336     | -1.10                   | -2.42                | ●            | (Polymenidou et al. [58]) |
| Mylk3       | myosin light chain kinase 3                       | NM_175441     | -1.04                   | -2.28                | ●            | (Chen-Plotkin et al. [17]) |
| Mecom       | MDS1 and EVI1 complex locus                       | NM_007963     | -1.42                   | -2.42                | ●            | (Chen-Plotkin et al. [17]) |
| Rsps1       | R-spondin 1                                       | NP_619624     | -0.80                   | -1.46                | ●            | (Polymenidou et al. [58]) |
| Myh4        | myosin, light polypeptide 4                        | NM_010858     | -0.29                   | -1.36                | ●            | (Polymenidou et al. [58]) |
| Ddit4l      | DNA-damage-inducible transcript 4-like            | NM_030143     | -0.65                   | -1.13                | ●            | (Polymenidou et al. [58]) |
| Gmr2        | glutamate receptor, metabotropic 2                | NM_001160353  | -0.52                   | -1.12                | ●            | (Polymenidou et al. [58]) |
| Cpne9       | copine family member IX                           | NM_170673     | -0.70                   | -1.04                | ●            | (Polymenidou et al. [58]) |
| Ryr1        | ryanodine receptor 1                              | NP_033135     | -0.58                   | -0.98                | ●            | (Polymenidou et al. [58]) |
| Rassf3      | Ras association domain family member 3           | NP_620406     | -0.26                   | -0.91                | ●            | (Polymenidou et al. [58]) |
| Egr1        | early growth response 1                           | NM_007913     | -0.42                   | -0.90                | ●            | (Chen-Plotkin et al. [17]) |
| Kcn12       | potassium channel, subfamily T, member 2          | NM_001081027  | -0.54                   | -0.89                | ●            | (Chen-Plotkin et al. [17]) |
| Rims3       | regulating synaptic membrane exocytosis 3         | NM_182929     | -0.38                   | -0.84                | ●            | (Polymenidou et al. [58]) |
| Pisd-ps3    | phosphatidylserine decarboxylase, pseudogene 3    | NR_003518     | 0.10                    | -0.86                | ●            | (Polymenidou et al. [58]) |
| Kcnab3      | potassium voltage-gated channel, shaker-related subfamily, beta member 3 | NM_010599 | -0.11 | -0.86 | ● | (Polymenidou et al. [58]) |
| Fos         | FBJ osteosarcoma oncogene                         | NM_010234     | -0.77                   | -0.84                | ●            | (Polymenidou et al. [58]) |
| Scube1      | signal peptide, CUB domain, EGF-like 1           | NM_001271472  | -0.43                   | -0.83                | ●            | (Polymenidou et al. [58]) |
| Kcnh5       | potassium voltage-gated channel, subfamily H (eag-related), member 5 | NM_172805 | -0.45 | -0.83 | ● | (Polymenidou et al. [58]) |
Table 1: The genes with altered mRNA levels in the neocortex of 3-month and 12-month-old TDP-43 cKO mice in comparison to the Ctrls are listed (Continued)

| Gene symbol | Name                                                                 | RefSeq ID       | Log2(TDP-43 cKO/ Ctrl) | Significant (q<0.05) | FTLD patient | White et al. [86] | Other references                | 5 Months frontal cortex | 20 Months frontal cortex |
|-------------|----------------------------------------------------------------------|-----------------|------------------------|----------------------|---------------|--------------------|----------------------------------|-------------------------|--------------------------|
| Inf2        | inverted formin, FH2 and WH2 domain containing                       | NM_198411       | -0.35                  | -0.83                |               |                   |                                  | Common MB-              | Common MB-               |
| Acvr1c      | activin A receptor, type IC                                          | NM_001111030    | -0.13                  | -0.82                |               |                   |                                  |                         |                          |
| Hipl4       | homeodomain interacting protein kinase 4                            | NP_001028487    | -0.51                  | -0.81                |               |                   |                                  |                         |                          |
| Scrt1       | scratch family zinc finger 1                                         | NM_130893       | -0.26                  | -0.77                |               |                   |                                  |                         |                          |
| HaplN4      | hyaluronan and proteoglycan link protein 4                           | NM_177900       | -0.47                  | -0.75                |               |                   |                                  |                         |                          |
| Kif5a       | kinesin family member 5A                                             | NM_001039000    | -0.52                  | -0.76                |               |                   |                                  |                         |                          |
| Arhgap10    | Rho GTPase activating protein 10                                      | NP_001074833    | 0.00                   | -0.75                |               |                   |                                  |                         |                          |
| Camk2n1     | calcium/calmodulin-dependent protein kinase II inhibitor 1           | NM_025451       | -0.40                  | -0.74                |               |                   |                                  |                         |                          |
| Car10       | carbonic anhydrase 10                                                | NP_082572       | -0.33                  | -0.73                |               |                   |                                  |                         |                          |
| Camk4       | calcium/calmodulin-dependent protein kinase IV                       | NM_009793       | -0.24                  | -0.73                |               |                   |                                  |                         |                          |
| Rims1       | regulating synaptic membrane exocytosis 1                           | NM_001012623    | -0.42                  | -0.73                |               |                   |                                  |                         |                          |
| Eipr1       | EARP complex and GARP complex interacting protein 1                  | NM_201357       | -0.68                  | -0.73                |               |                   |                                  |                         |                          |
| Rgs4        | regulator of G-protein signaling 4                                  | NM_009062       | -0.52                  | -0.73                |               |                   |                                  |                         |                          |
| Rapgef4     | Rap guanine nucleotide exchange factor (GEF) 4                       | NM_001204165    | -0.23                  | -0.70                |               |                   |                                  |                         |                          |
| Dagla       | diacylglycerol lipase, alpha                                          | NM_198114       | -0.43                  | -0.69                |               |                   |                                  |                         |                          |
| Diras2      | DIRAS family, GTP-binding RAS-like 2                                | NM_001024474    | -0.52                  | -0.69                |               |                   |                                  |                         |                          |
| Gp1bbb      | glycoprotein lb, beta polypeptide                                     | NM_001001999    | -0.65                  | -0.68                |               |                   |                                  |                         |                          |
| Ano3        | anoctamin 3                                                          | NM_001128103    | -0.33                  | -0.67                |               |                   |                                  |                         |                          |
| Gm3         | glutamate receptor, metabotropic 3                                   | NM_181850       | -0.26                  | -0.67                |               |                   |                                  |                         |                          |
| Nefm        | neurofilament, medium polypeptide                                    | NM_008691       | -0.53                  | -0.66                |               |                   |                                  |                         |                          |
| Gabra3      | gamma-aminobutyric acid (GABA) A receptor, subunit alpha 3           | NM_008067       | -0.57                  | -0.65                |               |                   |                                  |                         |                          |
Table 1: The genes with altered mRNA levels in the neocortex of 3-month and 12-month-old TDP-43 cKO mice in comparison to the Ctrls are listed (Continued)

| Gene symbol | Name                                               | RefSeq ID   | Log2(TDP-43 cKO/ Ctrl) | Significant (q<0.05) | FTLD patient | White et al. [86] | Other references |
|-------------|----------------------------------------------------|-------------|------------------------|----------------------|--------------|-------------------|------------------|
|             |                                                    | 3 Months    | 12 months              |                      |              |                   |                  |
| Rph3a       | rabphilin 3A                                       | NM_001302344| -0.39 -0.65            | ●                    | (Chen-Plotkin et al. [17]) |
| Tbr1        | T-box brain gene 1                                 | NM_009322   | -0.51 -0.63            | ●                    |              | (Polymenidou et al. [58]) |
| Sath1       | special AT-rich sequence binding protein 1         | NM_001163630| -0.39 -0.64            | ●                    |              |                  |
| Sh3gl2      | SH3-domain GRB2-like 2                             | NM_019535   | -0.22 -0.63            | ●                    | (Chen-Plotkin et al. [17]) |
| Ephb6       | Eph receptor 86                                     | NM_001146351| -0.37 -0.63            | ●                    | (Chen-Plotkin et al. [17]) |
| Kcnh3       | potassium voltage-gated channel, subfamily H (leag-related), member 3 | NM_010601 | -0.43 -0.62 | ●                      |                  |
| Ngn         | neurogranin                                        | NM_022029   | -0.35 -0.62            | ●                    | (Chen-Plotkin et al. [17]) |
| Cnmd2       | cell adhesion molecule 2                           | NM_001145977| -0.17 -0.61            | ●                    | (Chen-Plotkin et al. [17]) |
| Camk2k      | calcium/calmodulin-dependent protein kinase kinase 2, beta | NM_001199676| -0.44 -0.61            | ●                    | (Chen-Plotkin et al. [17]) |
| Robb        | RAR-related orphan receptor beta                    | NM_001043354| -0.34 -0.60            | ●                    |              |                  |
| Scn4b       | sodium channel, type IV, beta                      | NP_001013408| 0.05 -0.60             | ●                    |              |                  |
| Camk2a      | calcium/calmodulin-dependent protein kinase II alpha| NM_001286809| -0.50 -0.60            | ●                    | (Chen-Plotkin et al. [17]) |
| Nnr1        | neuritin 1                                         | NM_153529   | -0.24 -0.60            | ●                    | (Chen-Plotkin et al. [17]) |
| Cdk5r2      | cyclin-dependent kinase 5, regulatory subunit 2 (p39) | NM_009872   | -0.42 -0.60            | ●                    |              |                  |
| Rbfox3      | RNA binding protein, fox-1 homolog (C. elegans) 3  | NM_001024931| -0.34 -0.60            | ●                    |              |                  |
| Gca         | grancalcin                                         | NP_663498   | 0.08 -0.59             | ●                    |              |                  |
| Oxbpl1a     | oxysterol binding protein-like 1A                  | NM_001252489| -0.39 -0.59            | ●                    |              |                  |
| Vsnl1       | visinin-like 1                                     | NM_012038   | -0.32 -0.59            | ●                    | (Chen-Plotkin et al. [17]) |
| Adcy1       | adenylate cyclase 1                                | NM_009622   | -0.25 -0.59            | ●                    | (Chen-Plotkin et al. [17]) |
| Neurod6     | neurogenic differentiation 6                       | NM_009717   | -0.41 -0.59            | ●                    |              | (Polymenidou et al. [58]) |

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Table 1 The genes with altered mRNA levels in the neocortex of 3-month and 12-month-old TDP-43 cKO mice in comparison to the Ctrls are listed (Continued)

| Gene symbol | Name | RefSeq ID | Log2(TDP-43 cKO/ Ctrl) | Significant (q<0.05) | FTLD patient | White et al. [86] | Other references |
|-------------|------|-----------|------------------------|----------------------|--------------|------------------|-----------------|
| AI593442    | expressed sequence AI593442 | NM_001286641 | -0.19 -0.58 | ● | (Chen-Plotkin et al. [17]) |
| Stx1a       | syntaxin 1A (brain) | NM_016801 | -0.28 -0.57 | ● | (Polymenidou et al. [58]) |
| Nat8l       | N-acetyltransferase 8-like | NP_001001985 | -0.34 -0.57 | ● | (Chen-Plotkin et al. [17]) |
| 3110035E14Rik | RIKEN CDNA 3110035E14 gene | NM_178399 | -0.28 -0.57 | ● | (Chen-Plotkin et al. [17]) |
| Zbtb18      | zinc finger and BTB domain containing 18 | NM_001012330 | -0.22 -0.57 | ● | (Polymenidou et al. [58]) |
| Hlf         | hepatic leukemia factor | NM_172563 | -0.39 -0.56 | ● | (Polymenidou et al. [58]) |
| Lynx1       | Ly6/neurotoxin 1 | NM_011838 | -0.28 -0.56 | ● | (Polymenidou et al. [58]) |
| Cadps2      | Ca2+-dependent activator protein for secretion 2 | NM_001252105 | -0.53 -0.56 | ● | (Chen-Plotkin et al. [17]) |
| Psd         | pleckstrin and Sec7 domain containing | NM_133694 | -0.47 -0.56 | ● | (Chen-Plotkin et al. [17]) |
| Dhcr24      | 24-dehydrocholesterol reductase | NM_053272 | -0.49 -0.54 | ● | (Chen-Plotkin et al. [17]) |
| 2900011O08Rik | RIKEN CDNA 2900011O08 gene | NM_144518 | -0.53 -0.53 | ● | (Chen-Plotkin et al. [17]) |
| Dlgap3      | discs, large (Drosophila) homolog-associated protein 3 | NM_001302081 | -0.43 -0.52 | ● | (Chen-Plotkin et al. [17]) |
| Blhhe40     | basic helix-loop-helix family, member e40 | NM_011498 | -0.35 -0.53 | ● | (Chen-Plotkin et al. [17]) |
| Pcp4        | Purkinje cell protein 4 | NM_008791 | -0.04 -0.52 | ● | (Chen-Plotkin et al. [17]) |
| Ctnnap1     | contactin associated protein-like 1 | NM_016782 | -0.38 -0.52 | ● | (Chen-Plotkin et al. [17]) |
| Cep170b     | centrosomal protein 1708 | NM_001024602 | -0.42 -0.51 | ● | (Chen-Plotkin et al. [17]) |
| Plcx2d2     | phosphatidylinositol-specific phospholipase C, X domain containing 2 | NM_001134480 | -0.16 -0.50 | ● | (Chen-Plotkin et al. [17]) |
| Cplx2       | complexin 2 | NM_009946 | -0.26 -0.50 | ● | (Chen-Plotkin et al. [17]) |
| Phyhip      | phytanoyl-CoA hydroxylase interacting protein | NM_145981 | -0.38 -0.50 | ● | (Chen-Plotkin et al. [17]) |
| Snap25      | synaptosomal-associated protein 25 | NM_001291056 | -0.43 -0.50 | ● | (Chen-Plotkin et al. [17]) |
| Scl8a2      | solute carrier family 8 (sodium/calcium exchanger), member 2 | NM_001347561 | -0.42 -0.50 | ● | (Chen-Plotkin et al. [17]) |
| Gene symbol | Name | RefSeq ID | Log2(TDP-43 cKO/Ctrl) | Significant (q<0.05) | FTLD patient | Other references |
|-------------|------|-----------|-----------------------|----------------------|--------------|------------------|
| D430041D05Rk | RIKEN cDNA D430041D05 gene | NM_001033347 | -0.47 | -0.49 | ● | |
| Tubb4a | tubulin, beta 4A class IVA | NM_009451 | -0.38 | -0.49 | ● | |
| Ppp3r1 | protein phosphatase 3, regulatory subunit B, alpha isoform (calcineurin B, type I) | NM_024459 | -0.25 | -0.48 | ● | (Chen-Plotkin et al. [17]) |
| Epdrl | ependymin related protein 1 (zebrafish) | NM_134065 | -0.35 | -0.48 | ● | ● |
| Scn1b | sodium channel, voltage-gated, type I, beta | NM_011322 | -0.20 | -0.47 | ● | ● |
| Jph3 | junctophilin 3 | NM_020605 | -0.39 | -0.47 | ● | ● |
| Syt13 | synaptotagmin XIII | NM_030725 | -0.33 | -0.45 | ● | ● (Polymenidou et al. [58]) |
| Snap91 | synaptosomal-associated protein 91 | NM_001277982 | -0.53 | -0.45 | ● | (Chen-Plotkin et al. [17]) |
| Zfp365 | zinc finger protein 365 | NM_178679 | -0.24 | -0.44 | ● | |
| Atp1a1 | ATPase, Na+/K+ transporting, alpha 1 polypeptide | NM_144900 | -0.33 | -0.44 | ● | |

The down-regulated genes are indicated by the “-” sign in the columns of Log2 (TDP-43 cKO/Ctrl). The genes that have been reported to have altered mRNA levels in the FTLD patients and in the striatum or hippocampus upon TDP-43 depletion are indicated in the far right column.
Table 2 Genes with altered mRNA processing patterns in the neocortex of TDP-43 cKO mice

| Gene          | Location               | Strand | Significant | Δmiso<ψ | References |
|---------------|------------------------|--------|-------------|---------|------------|
| Cryptic Cdh22 | Ch2:165183239-165183371 | -      | •           | 0.61    | 0.54       | White et al. [86] |
| Cryptic Camk1g| Ch1:193368267-193368952 | -      | •           | 0.46    | 0.59       | Jeong et al. [37] |
| Cryptic Slc45a1| Ch4:150630400-150630454 | -      | •           | 0.32    | 0.13       | Jeong et al. [37] |
| Cryptic Synj2bp | Ch12:81509828-81510051 | -      | •           | 0.39    | 0.30       | Jeong et al. [37] |
| Cryptic Hgsnat| Ch8:25945949-25945996  | -      | •           | 0.22    | 0.19       | Jeong et al. [37] |
| Cryptic Adnp2  | Ch18:80138153-80138304 | -      | •           | 0.45    | 0.49       | Jeong et al. [37] |
| Cryptic Abca8b | Ch11:09975240-109975477 | -      | •           | 0.18    | 0.18       | Jeong et al. [37] |
| Cryptic Upf3a  | Ch8:13789928-13789967  | +      | •           | 0.17    | 0.07       | Jeong et al. [37] |
| Cryptic Letm1  | Ch5:33779574-33779604  | -      | •           | 0.13    | 0.04       | Jeong et al. [37] |
| Inclusion Sort1 | Ch3:108355472-108355570 | +      | •           | 0.35    | 0.40       | Polymenidou et al. [58] |
| Inclusion Islr2-02 | Ch9:58200272-58200461  | -      | •           | 0.36    | 0.37       | Polymenidou et al. [58] |
| Inclusion Islr2-01 | Ch9:58200272-58200443  | -      | •           | 0.30    | 0.29       | Polymenidou et al. [58] |
| Inclusion Bsg   | Ch10:80136663-80136743 | -      | •           | 0.25    | 0.13       | Polymenidou et al. [58] |
| Inclusion Vps13d | Ch4:145099352-145099463 | -      | •           | 0.23    | 0.18       | Polymenidou et al. [58] |
| Inclusion Smg5  | Ch3:88340469-88340763  | +      | •           | 0.20    | 0.10       | Polymenidou et al. [58] |
| Inclusion Smarca4| Ch9:21677953-21678051  | +      | •           | 0.20    | 0.24       | Polymenidou et al. [58] |
| Inclusion Uggt2 | Ch14:119043908-119044028| -      | •           | 0.19    | 0.08       | Polymenidou et al. [58] |
| Inclusion Elac2 | Ch11:65005454-65005505 | -      | •           | 0.15    | 0.12       | Polymenidou et al. [58] |
| Inclusion Kcnmb4| Ch10:116443772-116443912| -      | •           | 0.11    | 0.08       | Polymenidou et al. [58] |
| Inclusion Dnajc5 | Ch2:181548926-181549000 | +      | •           | 0.10    | 0.13       | Polymenidou et al. [58] |
| Inclusion Sun1  | Ch5:139230773-139230838 | +      | •           | -0.14   | 0.32       | Polymenidou et al. [58] |
| Inclusion Pdp1-01 | Ch4:11965614-11965648  | -      | •           | 0.40    | 0.24       | Polymenidou et al. [58] |
| Inclusion Tmem2  | Ch19:21780171-21780252 | +      | •           | 0.40    | 0.19       | Polymenidou et al. [58] |
| Inclusion Zfp30  | Ch7:29788049-29788175  | +      | •           | 0.32    | -0.04      | Polymenidou et al. [58] |
| Inclusion Zkscan16| Ch4:58943943-58944160  | +      | •           | 0.29    | 0.18       | Polymenidou et al. [58] |
| Inclusion Nfia-02 | Ch4:98081725-98081816  | +      | •           | 0.12    | 0.02       | Polymenidou et al. [58] |
Table 2 Genes with altered mRNA processing patterns in the neocortex of TDP-43 cKO mice (Continued)

| Gene     | Location          | Strand | Significant | Δmisoψ | White et al. [86] | References |
|----------|-------------------|--------|-------------|--------|-------------------|------------|
| Inclusion | Lrrk2             | Ch15:91785371-91785527 | +      | ●     | 0.06  | -0.01 |
| Inclusion | Atxn1             | Ch13:45849519-45849588 | -      | ●     | 0.10  | 0.09  |
| Inclusion | Ranbp17           | Ch11:33283908-33283990 | -      | ●     | -0.17 | 0.28  |
| Inclusion | Srr               | Ch11:74919437-74919662 | -      | ●     | 0.11  | 0.22  |
| Inclusion | Atad2b            | Ch12:4970406-4970468   | +      | ●     | -0.03 | 0.15  |
| Inclusion | Kctd10            | Ch11:114376771-114376866 | -      | ●     | 0.05  | 0.06  |
| Inclusion | Pdp1-02           | Ch4:11965614-11965648 | -      | ●     | #REF! | 0.05  |
| Inclusion | Mettl22           | Ch16:4821257-4821667  | +      | ●     | 0.00  | 0.03  |
| Exclusion | Cobl              | Ch11:12306958-12307128 | -      | ●     | -0.34 | -0.41 |
| Exclusion | Scamp1            | Ch13:94210577-94210678 | -      | ●     | -0.31 | -0.26 |
| Exclusion | Ddx50             | Ch10:62627521-62627682 | -      | ●     | -0.27 | -0.37 |
| Exclusion | Kcnip2-02         | Ch19:45797091-45797186 | -      | ●     | -0.23 | -0.29 |
| Exclusion | Dtwd1             | Ch2:126158410-126158553 | +      | ●     | -0.27 | -0.19 |
| Exclusion | Nlgn3             | ChX:101307075-101307134 | +      | ●     | -0.22 | -0.15 |
| Exclusion | Lzts1             | Ch8:69182213-69182331  | -      | ●     | -0.12 | -0.15 |
| Exclusion | Nxxn1             | Ch17:90701988-90702011 | -      | ●     | -0.09 | -0.16 |
| Exclusion | Shisa4            | Ch1:135373152-135373285 | -      | ●     | -0.07 | -0.14 |
| Exclusion | Rdh13             | Ch7:4444978-4445122   | -      | ●     | 0.08  | -0.26 |
| Exclusion | Atp11b            | Ch3:35843571-35843696  | +      | ●     | -0.44 | -0.28 |
| Exclusion | Gpatch1           | Ch7:35281332-35281480  | -      | ●     | -0.36 | 0.00  |
| Exclusion | Kcnip2-01         | Ch19:45796279-45796332 | -      | ●     | -0.21 | -0.18 |
| Exclusion | Nlfa-01           | Ch4:98041551-98041679  | +      | ●     | -0.15 | 0.00  |
| Exclusion | Dzip3             | Ch16:48951543-48952160 | -      | ●     | -0.13 | 0.01  |
| Exclusion | Cacna1b-01        | Ch2:24618255-24618362  | -      | ●     | -0.04 | 0.01  |
| Exclusion | Tmcc2             | Ch1:132380657-132381172 | -      | ●     | -0.05 | 0.01  |
| Exclusion | Pcm1-01           | Ch8:41313302-41313460  | +      | ●     | -0.08 | -0.01 |
Table 2: Genes with altered mRNA processing patterns in the neocortex of TDP-43 cKO mice (Continued)

| Gene          | Location           | Strand | Significant | Δmisoψ | White et al. [86] | References          |
|---------------|--------------------|--------|-------------|--------|-------------------|---------------------|
| Exclusion     | Cdk19-02           | +      | ●           | -0.02  | -0.04             |                     |
| Exclusion     | Max                | -      | ●           | -0.08  | -0.05             |                     |
| Exclusion     | Phactr2            | -      | ●           | -0.16  | -0.08             |                     |
| Exclusion     | Cdk19-01           | +      | ●           | 0.02   | -0.05             |                     |
| Exclusion     | Rimbp2             | -      | ●           | -0.01  | -0.09             |                     |
| Exclusion     | Clasp1-01          | +      | ●           | 0.02   | -0.12             |                     |
| Exclusion     | Clasp1-03          | +      | ●           | 0.07   | -0.12             |                     |
| Exclusion     | Cnthn-01           | +      | ●           | -0.02  | -0.20             |                     |
| Exclusion     | Agfg1              | +      | ●           | -0.16  | -0.26             |                     |
| Exclusion     | Repin1             | +      | ●           | -0.07  | -0.24             |                     |
| Exclusion     | Ppp3ca             | +      | ●           | -0.09  | -0.11             | ●(inclusion) ●(inclusion) |
| Exclusion     | Hspa13             | -      | ●           | 0.01   | -0.08             |                     |
| Extension     | Wbscr22            | -      | ●           | 0.17   | 0.19              | Jeong et al. [37]   |
| Extension     | Chga               | +      | ●           | 0.08   | 0.06              | Jeong et al. [37]   |
| Extension     | Bptf               | -      | ●           | -0.10  | -0.11             |                     |
| PolyA extension | Rapgef1          | -      | ●           | 0.17   | 0.21              | Jeong et al. [37]   |
| PolyA extension | Kctd2            | +      | ●           | 0.14   | 0.11              |                     |
| PolyA extension | Kcnj4            | -      | ●           | 0.09   | 0.06              |                     |
| PolyA extension | Ergic1           | +      | ●           | 0.05   | 0.09              |                     |
| PolyA extension | Syt17            | -      | ●           | 0.06   | 0.16              |                     |
| PolyA extension | Polr1b           | +      | ●           | 0.14   | 0.13              |                     |
| PolyA extension | Elk1             | -      | ●           | 0.19   | 0.22              |                     |
| PolyA extension | Ppp3cc           | -      | ●           | 0.08   | 0.09              |                     |

The ψ (PSI, percentage of spliced in) score was defined as the percentage of transcripts containing the alternative splicing events and/or alternative poly(A) site usage. The mRNAs with increase of splicing events, i.e. conserved exon inclusion/ exclusion, cryptic exon inclusion, and exon extension, as well as change of poly(A) site usage are indicated by Δψ > 0, mRNA with decrease of the processing events are indicated by Δψ < 0. Unpaired t test was used to calculate the significance from data of 3 independent samples. Note that changes of the pre-mRNA processing events of several genes including Cob1 in the TDP-43 cKO mice are opposite to those observed in the TDP-43(Q331K) knock-in mice (White et al. [86]).
### Fig. 6

(See legend on next page.)
forebrain neurons would lead to a range of pathological changes of the mice on the phenotypic, molecular, and cellular levels that mimic those in FTLD-TDP.

Since one-fifth of the genes the expression of which are altered in TDP-43 cKO mice are also affected in FTLD patients [17] (Table 1), mis-regulation of TDP-43 RNA targets through the loss of TDP-43 function could at least in part contribute to the impairment of synaptic functions and disease pathogenesis of FTLD-TDP.

Comparison of our RNA-seq data (Table 1) to the Mouse Genome Informatics database (MGI) [8] has revealed that the mRNA levels of genes associated with anxiety-related behaviour and/or social behaviours are significantly altered in the neocortex of TDP-43 cKO mice (Additional file 3: Table S2). Notably, Slc6a3, a gene encoding a sodium-dependent dopamine transporter and associated with anxiety disorder in autism spectrum disorder [56], is upregulated (by 4 fold) in the neocortex of TDP-43 cKO mice but only at the age of 3 months (Additional file 3: Table S2) [7, 9, 23, 28, 32, 39, 41, 50, 51, 66, 69, 90]. On the other hand, 16 of the rest 17 anxiety- and/or social behaviour-related genes listed in Additional file 3: Table S2 are up- or down-regulated in the neocortex TDP-43 cKO mice mainly at the age of 12 months when the behaviour abnormality shows up (Fig. 2). Eg1 was also demostrated in a TDP-43Q331K knockin mice at the age of 20 months (Table 1) [86].

Similarly, the cognition deficiencies of the TDP-43 cKO mice are also associated with altered expression levels of specific genes, particularly those involved in synaptic transmission, as revealed by the transcriptome analysis (Table 1 and Additional file 1: Figure S10) and electro-physiology measurement (Additional file 1: Figure S4). Besides those illustrated in Additional file 1: Figure S5, the expression level of dlg3, which encodes a major excitatory postsynaptic density protein SAP102 important in NMDARs recycling [95], is significantly down-regulated in 3-month-old TDP-43 cKO mice as compared with Ctrl mice (Table 1). In
addition, several other synaptic function-associated genes, e.g. **Dlga3p**, **snap25**, are down-regulated in the cortex of 12-month-old TDP-43 cKO mice (Table 1). Among the proteins encoded by these genes, Dlga3p (PSD95-associated protein 3) is an excitory postsynaptic protein implicated in the pathogenesis of obsessive-compulsive behaviours [85], and Snap-25 is a component of the SNARE protein complex and a promising cerebrospinal fluid biomarker for synapse degeneration in Alzheimer’s disease [10]. With respect to the abnormal LTP and LTD (Additional file 1: Figure S4), the protein levels of CaMKII and p-Erk (Additional file 1: Figure S5c and d) are decreased in the cortex of TDP-43 cKO mice. Altogether, it appears that combined deficiencies of the expression of a set of forebrain genes contributed to the age-dependent cognition impairment of TDP-43 cKO mice. However, down-regulation of synaptic genes could also be the results from the degeneration of the according brain region with neuron loss in TDP-43 cKO mice.

Patients with dementia (e.g., FTLD) often exhibit activation of inflammatory response [27]. In TDP-43 cKO mice, the progressive increase of astrocytosis in SLM region of hippocampus and RS region of the cortex (Additional file 1: Figure S3) is associated with upregulation of a range of inflammatory genes, including **Gfap**, **C4a/C4b**, **Serpina3n**, and **A2m**, at both 3- and 12 months of age (Fig. 7 and Table 1). Particularly, **Serpina3n** is a marker of persistent reactive gliosis response induced in inflammation [93] and in ALS [25]. Several other genes induced in microglia activation, e.g., **Cst7** and **Clec7a**, are also upregulated in TDP-43 cKO mice (Fig. 5d and Table 1). Taken together, the transcriptome analysis indicates that chronic neuroinflammation in TDP-43 cKO mice and by implication in FTLD-TDP patients’ results in part from mis-regulation of these genes.

Depletion of TDP-43 in the mouse forebrain also results in aberrant splicing of ~50 RNA transcripts in the cortex of 3- and/ or 12-month-old TDP-43 cKO mice (Fig. 5 and Table 2). Importantly, among these transcripts, **Sort1**, **Adnp2**, and **Cdh22**, the mutations or functional variants of which are associated with aging or neurodegenerative disorders [35, 42]. Furthermore, aberrant splicing of several genes including **Dnajc5**, **Sort1**, **Pdp1**, and **Kcnip2**, which also occur in TDP-43 knockdown cells of the striatum [58], does not affect the levels of their wild type mRNA isoforms (Additional file 1: Figure S9c). Consistently, the amounts of **Dnajc5** protein in the cortex of TDP-43 cKO and Ctrl mice are similar (data not shown). On the other hand, the truncated sortilin protein accumulates in the cortex of TDP-43 cKO mice at different ages (Additional file 1: Figure S9b). Interestingly, decreasing the **Sort1 e17b** inclusion was reported by TDP-43-Q331K knock-in mice with a mild FTD phenotype [86] (Table 1). Since sortilin is a major neuronal APOE receptor [12], the truncated **Sort1** (e17b) could act as a decoy receptor [60] competing with the wild type sortilin in the cortex of TDP-43 cKO mice thus affecting the neuronal viability and causing neurodegeneration.

Finally, cryptic exons are present in transcripts from a set of genes, including **Camk1g**, **Hgsnat**, **Synj2bp**, **Adnp2**, and **Abca8b**, in the neocortex of 3- and/ or 12-month-old TDP-43 cKO mice (Table 2). Intriguingly, inclusion of the cryptic exon in **CaMK1g** would result in the loss-of-function of CaMK1y that has been implicated in aging and ALS [22]. Besides **CaMK1g** and other gene transcripts reported previously [37, 46], we identified some novel cryptic exon inclusion events in several transcripts upon TDP-43 depletion (Table 2). Notably, cryptic exon inclusions often introduce premature termination codons (PTCs) and thereby result in nonsense-mediated decay (NMD) [34] of the inserted RNA transcripts. It has also been reported that TDP-43 could autoregulate itself through NMD [58]. Thus, we speculate that the accumulation of a portion of the cryptic exon-containing transcripts in the cortex of TDP-43 cKO mice might result from loss of TDP-43 function in NMD of these transcripts.

**Conclusions**

In summary, we have generated a conditional mouse model (TDP-43 cKO) with depletion of TDP-43 in the neurons of cortex and hippocampus. The TDP-43 cKO mice exhibit a spectrum of age-dependent social behaviour change, dementia-like behaviour, impairment of the cognition functions, and decline of ADL. The development of neurodegenerative pathology in TDP-43 cKO mice is closely associated with their behaviour and cognition changes, both of which are well correlated with the age-dependent alterations of the cortex transcriptomes. Notably, the transcriptomopathies of the TDP-43 cKO mouse cortex consist of changes of mRNA levels as well as pre-mRNA splicing patterns of many genes. Related to the latter changes, alternative splicing is known to play an essential role in brain function and mutations in factors involved in splicing regulation cause a range of neurological diseases [68]. Transcriptome analyse of autopsied brains from patients with ALS/FTLD reported have identified thousands of alternative splicing changes in part regulated by hnRNP [59] including TDP-43.

Overall, this study not only supports that loss-of-function of TDP-43 could be a major cause for FTLD-TDP pathogenesis, but also suggests a list of potential TDP-43 target genes that may be useful for future therapeutic development of FTLD-TDP.

**Materials and methods**

**Generation of TDP-43 conditional knockout mouse**

The **Tardbp** allele was knocked out specifically in the postmitotic pyramidal neurons in the forebrain by crossing
mice carrying the Tardbp conditional allele (Tardbp<sup>lox/lox</sup>) with mice carrying a Cre-recombinase transgene driven by the CaMKIIα-promoter. The viability and weight of the mice were monitored regularly. Genotyping of the mice was performed by PCR of genomic DNAs from the tail biopsies.

**RNA-seq analysis**

For RNA-seq, the rRNA-depleted cortical RNAs from three biological replicates of sex-matched TDP-43 cKO as well as littermate Ctrl mice were converted to cDNAs and sequenced them in a strand-specific manner at National Center of Genomic Medicine (NCGM). The RNAs were extracted from intact mouse cortical tissues and their concentrations determined using NanoDrop 8000 (Thermo Scientific). The RNA integrity was determined by Fragment Analyzer (Advanced Analytical Technologies). cDNAs from 5 μg of total RNA was used as an input material for library preparation using TruSeq RNA Sample Preparation Kit v2 (Illumina). Insert sizes of the libraries were confirmed using Fragment Analyzer (Advanced Analytical Technologies). The libraries were multiplexed and then sequenced on Illumina HiSeq2000 (Illumina) to generate 50 M of pair end 100 base pair reads per library. Data were processed using the TopHat, Cufflinks [77] and MISO [40].

**RNA-seq normalization**

The number of mapped fragments per kilobase of exon, per million mapped reads (FPKM) for each annotated protein-coding gene was determined to establish a metric of normalized gene expression. Approximately 80–85% of annotated protein-coding genes in mouse satisfied at least 1 FPKM in either condition. To judge the significance of differences between TDP-43 cKO and Ctrl mice, false discovery rates (q < 0.05) were used as the criteria for different replicates. The q value is an adjusted P value taking in to account the false discovery rate (FDR). We calculated the q value because the expression levels of thousands of genes from a small sample set (3 individual mice) were measured. The expression levels of transcripts in the neocortex of TDP-43 cKO mice relative to Ctrl mice were represented by log2 transformed values.

**Analysis of alternative splicing**

The ψ (PSI, percentage of spliced in) score was defined as the percentage of transcripts containing the alternative splicing events and/ or alternative poly(A) site usage. The mRNAs with increase of splicing events, i.e. conserved exon inclusion/ exclusion, cryptic exon inclusion, and exon extension, as well as change of poly(A) site usage are indicated by Δψ > 0. mRNAs with decrease of the processing events are indicated by Δψ < 0. Multiple t test was used to calculate the significance from data of 3 independent samples and the Holm-Sidak method was used to correct for multiple t test.

**Clustering analysis of protein-coding and noncoding transcripts**

For the clustering, transcripts with an estimated expression of a minimum of 0.2 FPKM in both TDP-43 cKO and Ctrl mice were selected for analysis. The list of protein-coding transcripts was compiled based on the RefSeq/Enterz/Vega definitions.

**Identification and analysis of circRNAs**

circRNAs were identified by NCLscan (version 1.6; https://github.com/TreesLab/NCLscan/TreesLab/NCLscan) [20], which was reported to outperform other publicly-available tools in terms of precision and to be robust to background noise [19, 94], on the basis of the mouse reference genome (GRCm38) and the GENCODE annotation (version M10). The differential expression analysis was performed by DEseq2 [2] and edgeR [64], in which the circRNA supporting reads were normalized by Relative Log Expression (RLE) and Trimmed Mean of M-values (TMM), respectively. The P values were evaluated by the Wald test (DEseq2) and the Fisher’s exact test (edgeR), and then adjusted by the Benjamini-Hochberg procedure. In this study, the significantly differential expression of circRNAs between different stages should satisfy DEseq adjusted P < 0.05 and edgeR adjusted P < 0.05 simultaneously.

**Quantitative reverse transcription PCR (qRT-PCR)**

Total RNAs were extracted from the cortex, hippocampus, and cerebellum of 3-and 12-month-old TDP-43 cKO mice and their Ctrl littermates (N = 6 for each genotype) using TRIzol reagents (Thermo Fisher Scientific). Synthesis of cDNA followed the manufacturer’s protocol (Invitrogen). qRT-PCR was performed using Roche qPCR FastMix (Roche). Primers were designed using a primer design software (LightCycler Probe Design Software 2.0 from Roche). The expression levels were normalized to gapdh, and data are represented as fold change relative to the Ctrl mRNA levels. Significant differences were determined using un-paired t tests.

**Nesting behaviour**

Single-housed mice were transferred into a new cage with nest-building material, a 5 × 5 cm square of white compressed cotton pads (Nestlets TM; Ancare, Bellmore, NY) in a random corner. After 6, 24, and 48 h, nest building was scored on a scale of 0–5, as previously
described [21]. All data are shown means ± SEM and analyzed using un-paired t tests.

**Social interaction test**

This test has been successfully employed to study social affiliation and interest in social novelty or social discrimination (social memory). The test was performed as described previously [70]. Briefly, in the first 10-min session, a test mouse was placed in the center of the three-chamber unit, where two empty wire cages were located in the left and right chambers to habituate the test mouse. The mouse was allowed to freely explore each chamber. In the second 10-min session, an age- and gender-matched C57BL/6j mouse (S1) that had never been exposed to the test mouse, was placed in one of the two wire cages. The wire cage on the other side remained empty (E). Then, the test mouse was placed in the center, and allowed to freely explore the chamber for 10 min. The test mouse was removed and in the last 10-min session, a second age- and gender-matched C57BL/6j stranger mouse (S2) that had never been exposed to the test mouse, was placed in one wire cage, which previously served as an empty cage. Thus, the test mouse would now have the choice between a mouse that was already familiar (S1) and a new stranger mouse (S2). The test mouse was placed in the center, and allowed to freely explore the chamber for 10 min. The movement of the mouse was recorded by a camera. The recorded video file was further analyzed by off-line video tracking software (EthoVision XT 7.0). Time spent in each chamber, and time spent within a 5 cm radius proximal to each wire cage were measured. All data shown are means ± SEM and analyzed using two-way ANOVA with Bonferroni's post hoc analysis.

**Light/ dark box test**

The light–dark box was custom made (45 × 20 × 20 cm) and divided into two parts: 1/3 was painted black, covered by the lid, and separated from the white compartment by the wall containing an opening (13 × 5 cm) at the floor level. The light side was illuminated by two 40 W light bulbs 50 cm above the floor. Each mouse was released in the center of the light compartment (facing away from the opening) and allowed to explore the area for 10 min. Video tracking equipment and software (EthoVision XT 8, Noldus) were used for following the animal's position and movement. In addition, rearings and attempts (stretched attend postures at the opening) to enter either the light or the dark compartment were recorded manually.

**Rotarod rod test**

Mice were trained at the age of 2 months and then subjected to rotarod test monthly. The latencies before falling from the rod was recorded for 3 consecutive days.

**Immunohistochemistry and histochemistry staining**

Mice were anesthetized and perfused with 4% paraformaldehyde. The hemispheres were embedded in paraffin, sliced into 10-μm sections, and mounted on slides. For immunostaining, the sections were washed with 0.1 M PBS buffer, quenched by 1% H2O2, blocked in serum with 0.05% Triton X-100 for 30 min, and incubated in rabbit anti-TDP-43 (Genetex, 1:1000), mouse anti-angi fibrillar acidic protein (GFAP) (1:1000), mouse anti-SMI-32 (1:1000) and mouse anti-NeuN (Millipore, 1:200) at 4 °C overnight. After rinsing in PBS, the sections were incubated with biotinylated goat antibody (Vector) for 60 min, followed by incubation with the avidin–biotin complex (Vector) for 45 min. The reaction products were developed by 3,3′-diaminobenzidine (Sigma, St Louis, MO).

To identify the cellular localization of TDP-43, slices were combined with hematoxylin. The hematoxylin and eosin (H&E) stain was used to visualize the overall morphology of the mouse brain. Nissl staining was applied to characterize the hippocampus size, cortical layer thickness, and neuron number. All data are presented as the mean with standard error, and statistical significance was tested by paired t-test.

**Electrophysiology**

Standard protocol was followed as described previously, with some modifications. In short, the mice were decapitated and their brains rapidly removed and placed in ice-cold artificial CSF (ACSF). 450 μm thick sections of isolated mouse hippocampus were transferred into an interface-type holding chamber in oxygenated ACSF (95% O2 and 5% CO2) at room temperature to allow recovery for at least 90 min before the recording. The field excitatory postsynaptic potential (fEPSP) at Schaffer collateral branches in CA1 region of the hippocampal slices was recorded. Three trains of stimuli at 100 Hz separated by 60 s were applied for LTP induction, and low frequency stimulation (1 Hz for 15 min) were applied for LTD induction. The slopes of the fEPSP were measured and the synaptic responses were normalized to the average of the baseline. All data are presented as the mean with mean standard deviation. Statistical significance was tested by paired t-test.

**Statistical analyses**

GraphPad Prism software was used for most statistical analysis. The statistical significance between means of control and TDP-43 cKO mouse groups was calculated by the two-tailed Student's t-test. For comparisons involving more than two groups, one-way or two way ANOVA were used. Data are presented as measures for group means, and P < 0.05 was considered significant.
Additional files

**Additional file 1:** Figure S1. Altered activity of daily living (ADL) of TDP-43 cko mice. Figure S2. Immunohistochemistry staining of brain slices. Figure S3. Persisting reactive astrogliosis in TDP-43 cko mouse forebrain. Figure S4. Electrophysiology measurements. Figure S5. Mis-regulated genes in TDP-43 cko mice. Figure S6. Alternations of the processing events of cortical RNAs in TDP-43 cko mice. Figure S7. The alternative uses of poly(A) sites in the cortical RNAs of TDP-43 cko mice. Figure S8. qRT-PCR validation of RNA splicing events altered in 3- and 12-month-old TDP-43 cko mice. Figure S9. Validation of altered splicing events in TDP-43 cko mice. Figure S10. Calcium signaling and synaptic long term potentiation pathway analysis using Ingenuity Pathway Analysis (IPA). (PDF 2990 kb)

**Additional file 2:** Table S1. List of the chromosome numbers, donor positions, acceptor positions, gene names of the transcripts from which the individual circularRNAs change in the neocortex of 3- and 12-month-old TDP-43 cko mice, but not Ctrl mice are listed. (PDF 141 kb)

**Additional file 3:** Table S2. Changes of the mRNA levels of social behaviour-related genes in the neocortex of TDP-43 cko mice relative to Ctrl mice. (PDF 72 kb)

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Authors’ contributions

L.S.W and W.C.C. designed, performed, and interpreted this study with the expertise of Hsing Tsung Wu in bioinformatic analysis is greatly appreciated. The expertise of Sue-Ping Lee and Shu-Mei Huang in the Microscopy Core at IMB and all members of Bioinformatics Core at IMB are greatly appreciated: We sincerely convey our gratitude to IMB and Academia Sinica. This work was supported by the Frontier of Science Award from the National Science Council and a Senior Investigator Award from the Academia Sinica, Taipei, Taiwan (R.O.C.).

Competing interests

The authors declare that they have no competing interests.

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Author details

1Institute of Molecular Biology, Academia Sinica, Nankang, Taipei 115, Taiwan, Republic of China. 2Genomics Research Center, Academia Sinica, Taipei, Taiwan. 3Research Center for Environmental Changes, Academia Sinica, Taipei, Taiwan, Republic of China.

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