

# TDP-43 Depletion in Microglia Promotes Amyloid Clearance but Also Induces Synapse Loss

Rosa C. Paolicelli,<sup>1,\*</sup> Ali Jawaid,<sup>2</sup> Christopher M. Henstridge,<sup>3</sup> Andrea Valeri,<sup>1</sup> Mario Merlini,<sup>4</sup> John L. Robinson,<sup>5</sup> Edward B. Lee,<sup>5</sup> Jamie Rose,<sup>6</sup> Stanley Appel,<sup>7</sup> Virginia M.-Y. Lee,<sup>5</sup> John Q. Trojanowski,<sup>5</sup> Tara Spires-Jones,<sup>3</sup> Paul E. Schulz,<sup>8</sup> and Lawrence Rajendran<sup>1,9,\*</sup>

<sup>1</sup>Systems and Cell Biology of Neurodegeneration, IREM, University of Zurich, Schlieren, Switzerland

<sup>2</sup>Brain Research Institute, University of Zurich/ETH, Zurich, Switzerland

<sup>3</sup>Center for Cognitive and Neural Systems, University of Edinburgh, Edinburgh, UK

<sup>4</sup>Center for Molecular Cardiology - Vascular Aging & Stroke, University of Zurich, Schlieren, Switzerland

<sup>5</sup>Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

<sup>6</sup>Academic Neuropathology, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK

<sup>7</sup>ALS/MDA Center, The Methodist Hospital, Houston, TX, USA

<sup>8</sup>Department of Neurology, University of Texas, Health Science Center, Houston, TX, USA

<sup>9</sup>Lead Contact

\*Correspondence: [rosachiara.paolicelli@irem.uzh.ch](mailto:rosachiara.paolicelli@irem.uzh.ch) (R.C.P.), [lawrence.rajendran@irem.uzh.ch](mailto:lawrence.rajendran@irem.uzh.ch) (L.R.)

<http://dx.doi.org/10.1016/j.neuron.2017.05.037>

## SUMMARY

Microglia coordinate various functions in the central nervous system ranging from removing synaptic connections, to maintaining brain homeostasis by monitoring neuronal function, and clearing protein aggregates across the lifespan. Here we investigated whether increased microglial phagocytic activity that clears amyloid can also cause pathological synapse loss. We identified TDP-43, a DNA-RNA binding protein encoded by the *Tardbp* gene, as a strong regulator of microglial phagocytosis. Mice lacking TDP-43 in microglia exhibit reduced amyloid load in a model of Alzheimer's disease (AD) but at the same time display drastic synapse loss, even in the absence of amyloid. Clinical examination from TDP-43 pathology cases reveal a considerably reduced prevalence of AD and decreased amyloid pathology compared to age-matched healthy controls, confirming our experimental results. Overall, our data suggest that dysfunctional microglia might play a causative role in the pathogenesis of neurodegenerative disorders, critically modulating the early stages of cognitive decline.

## INTRODUCTION

Microglia, the innate immune cells of the central nervous system (CNS), provide constant surveillance for neural functioning (Nimmerjahn et al., 2005; Davalos et al., 2005). They coordinate various critical roles throughout life, assisting early neuronal development and circuit formation and maintaining brain homeostasis (Paolicelli and Gross, 2011; Tremblay et al., 2011; Kettenmann et al., 2013). As the primary source of phagocytes in the CNS, microglia engulf cellular debris upon programmed

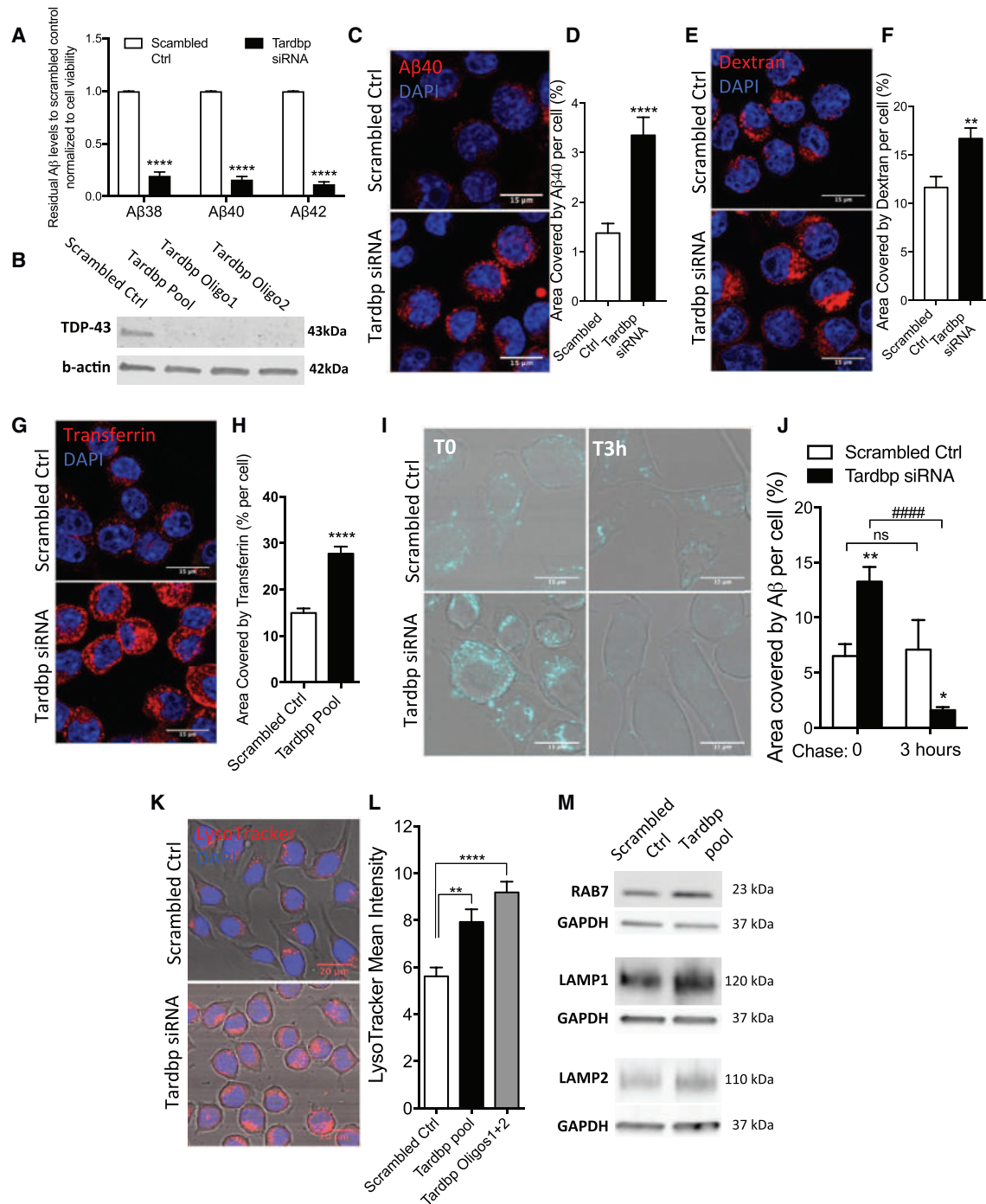
apoptosis, remove excess synapses during neural circuit maturation, and clear the brain from potentially dangerous protein aggregates (Wakselman et al., 2008; Sierra et al., 2010; Lee and Landreth, 2010; Paolicelli et al., 2011; Prinz et al., 2011). Synapse elimination is activity dependent and strictly confined to the first postnatal weeks in the rodent brain, a physiological process defined as synaptic pruning (Paolicelli et al., 2011; Schafer et al., 2012). However, recent studies indicate that this process re-activates in Alzheimer's disease (AD), in which amyloid promotes microglia-mediated removal of synapses (Hong et al., 2016). Synaptic loss, an early and highly predictive correlate of cognitive decline (Terry et al., 1991), occurs not only in AD but also in other distinct neurodegenerative disorders characterized by the presence of toxic protein aggregates. This accumulation classifies these disorders as proteinopathies.

Evidence from genome-wide association studies (GWASs) reveals that most genes associated with risk to develop such disorders are highly expressed in microglia, which implies that they could modulate immune and phagocytic functions in disease states (Derecki et al., 2014). This hypothesis suggests that they may confer susceptibility to develop the diseases by modulating microglia-mediated protein aggregates clearance rather than production. We previously demonstrated that risk genes associated with late-onset AD do not affect amyloid production, which suggests that these predisposing genetic factors contribute to disease development through different mechanisms (Bali et al., 2012). These data implicate microglia in the pathogenesis of neurodegenerative disorders. However, it remains unknown whether an intrinsic dysfunction in microglia can promote pathological synaptic pruning leading to abnormal synapse loss.

## RESULTS

### TDP-43 Regulates Microglial Phagocytosis and Clearance of A $\beta$

Microglia, the scavenger cells of the brain, play a key role as moderators of protein aggregates clearance, which occurs



**Figure 1. TDP-43 Loss Promotes Amyloid Phagocytosis and Degradation and Enhances Lysosomal Biogenesis in BV2 Microglia Cells**

(A) Residual A $\beta$ 38, A $\beta$ 40, and A $\beta$ 42 levels from HeLa swAPP-conditioned medium, after overnight incubation with BV2 cells depleted of TDP-43, normalized to scrambled control and to cell viability (means  $\pm$  SEM from three independent experiments, \*\*\*\* $p$  < 0.0001, multiple unpaired t test).

(B) Western blot confirming the knockdown efficiency of Tardbp pool and single siRNA oligos in BV2 cells compared to scrambled control.

(C–H) Representative confocal micrograph (C) and relative quantification of BV2 cells uptaking fluorescently labeled A $\beta$ 40 (D) (scrambled control,  $n$  = 171 and Tardbp siRNA  $n$  = 147 BV2 cells); (E and F) dextran (control  $n$  = 47, Tardbp siRNA  $n$  = 47 cells) and (G and H) transferrin (control  $n$  = 68, Tardbp siRNA  $n$  = 68 cells); \*\* $p$  < 0.005, \*\*\*\* $p$  < 0.0001 using two-tailed unpaired t test.

(I and J) Representative confocal images of scrambled control and Tardbp knockdown BV2 cells (I), with relative quantification soon after (T0, control  $n$  = 23; Tardbp siRNA  $n$  = 35 cells) and 3 hr (T3 hr, control  $n$  = 13; Tardbp  $n$  = 27 cells) after 60 min incubation with 1  $\mu$ M A $\beta$ 40 (J). Values are shown as mean  $\pm$  SEM,

(legend continued on next page)

intracellularly through enzymatic degradation following phagocytosis or extracellularly through degradation by secreted enzymes (Vekrellis et al., 2000; Nakanishi, 2003; Ries and Sastre, 2016; Solé-Domènech et al., 2016). We selected 18 top-ranked genes associated with neurodegenerative diseases and, using a loss-of-function approach, we screened them for their role to modulate microglial clearance of beta amyloid (A $\beta$ ), a well-established target for microglial phagocytosis and degradation (Paresce et al., 1996; Frenkel et al., 2013). Among the candidates we tested, TDP-43 exhibited the strongest A $\beta$  clearance in BV2 cells, i.e., residual A $\beta$  peptide levels measured from medium containing endogenous murine A $\beta$  showed a significant reduction after exposure to cells in which TDP-43 gene was knocked down (Figures S1A–S1D).

TDP-43 is a 43 kDa DNA-RNA binding protein encoded by the *Tardbp* gene and is a known transcriptional repressor, mRNA binding protein, and splicing factor (Buratti and Baralle, 2001; Lagier-Tourenne et al., 2010; Polymenidou et al., 2011; Baralle et al., 2013). Ubiquitinated TDP-43 aggregates represent the predominant constituent of cytoplasmic inclusions in glia and neurons in frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) patients, who show severe neuronal loss in frontal or motor cortex, respectively (Neumann et al., 2006). In the last years, the number of neurodegenerative disorders associated with TDP-43 pathology has considerably increased (Cook et al., 2008; Buratti and Baralle, 2009). The accompanying cell death in these disorders may arise from a combination of a toxic gain of function and a loss of nuclear TDP-43, both of which are associated with the presence of cytoplasmic aggregates (Cohen et al., 2011; Gendron and Petrucelli, 2011). Although a gain of toxicity induced by cytoplasmic inclusions can significantly contribute to the pathology (Xu et al., 2011; Medina et al., 2014; Walker et al., 2015), TDP-43 loss of function in neurons has been shown to be sufficient for inducing neuronal loss, accompanied by neuropathological alterations (Kraemer et al., 2010; Wu et al., 2012; Iguchi et al., 2013; Vanden Broeck et al., 2013). However, no evidence so far existed to support a role for loss of TDP-43 in microglia in the pathogenesis of the disease.

We wanted to confirm whether the enhanced clearance observed upon *Tardbp* knockdown could be also replicated with human A $\beta$ . To this end, TDP-43-depleted BV2 cells were incubated overnight with conditioned medium derived from HeLa cells overexpressing the Swedish mutation of the human Amyloid Precursor Protein (sweAPP). This assay ensured that BV2 cells were exposed to medium containing high levels of human A $\beta$ . Consistent with our findings from the murine A $\beta$  screen, TDP-43 depletion resulted in a higher clearance capacity of all the A $\beta$  species measured, compared to a scrambled control (Figure 1A). We achieved efficient TDP-43 depletion using either siRNA pools or single oligos (Figure 1B), with consistent results on amyloid clearance (Figure S1E).

To determine whether enhanced phagocytosis was the mechanism that mediated this enhanced clearance, we measured the internalization of fluorescently labeled A $\beta$  peptide, a cargo previously reported to be phagocytosed by microglia (Paresce et al., 1996). In a validation experiment, the internalization of the A $\beta$  peptide was followed in time lapse with the pH-dependent LysoTracker dye, to ensure that the cargo was trafficked to intracellular acidic compartments (Movie S1; Figure S2A and S2B). TDP-43 depletion significantly enhanced intracellular levels of fluorescent A $\beta$  (Figures 1C and 1D). Consistent with the enhanced uptake, we found a similar effect using fluorescently labeled dextran (Figures 1E and 1F) and transferrin (Figures 1G and 1H), which target uptake-mediated cargo. These results indicate that TDP-43 depletion in microglia increases the overall phagocytic activity.

Next, we determined whether the increased uptake was functionally followed by enhanced intracellular degradation. For that, we quantified the fluorescent signal of internalized A $\beta$ 40 3 hr after the uptake (T = 3 hr) and found a significant reduction in intracellular fluorescence, despite a higher uptake as measured by the initial amount (T = 0 hr) (Figures 1I and 1J). Since TDP-43 depletion increased intracellular degradation and amyloid is sorted to the lysosomal compartment for degradation in microglia (Cole et al., 1992), we examined whether increased lysosomal function occurs after TDP-43 depletion. We found higher levels of acidic late endosomal/lysosomal structures indicated by the pH-sensitive LysoTracker staining (Figures 1K and 1L). In addition, increased levels of lysosomal markers, such as LAMP1 and LAMP2, also accompanied the increased changes in acidic organelles in both BV2 cells (Figure 1M) and primary microglia cultures depleted of TDP-43 (Figures S3A–S3C). TDP-43 loss was recently shown to promote the nuclear translocation of TFEB, a transcription factor regulating lysosomal biogenesis (Xia et al., 2016). To investigate whether this was the case in microglia cells depleted of TDP-43, we assessed a subset of CLEAR (coordinated lysosomal expression and regulation) genes transcripts, downstream of TFEB, by RT-PCR. We found that the expression of *Lamp1*, *CtsD*, *CtsB*, *Clcn7*, *vATP6v1h*, *Psap*, and *Psen2* in TDP-43-depleted cells was higher than in scrambled control (Figure S3D). Overall, these data identify and validate TDP-43 as a regulator of microglial phagocytosis and clearance of A $\beta$ .

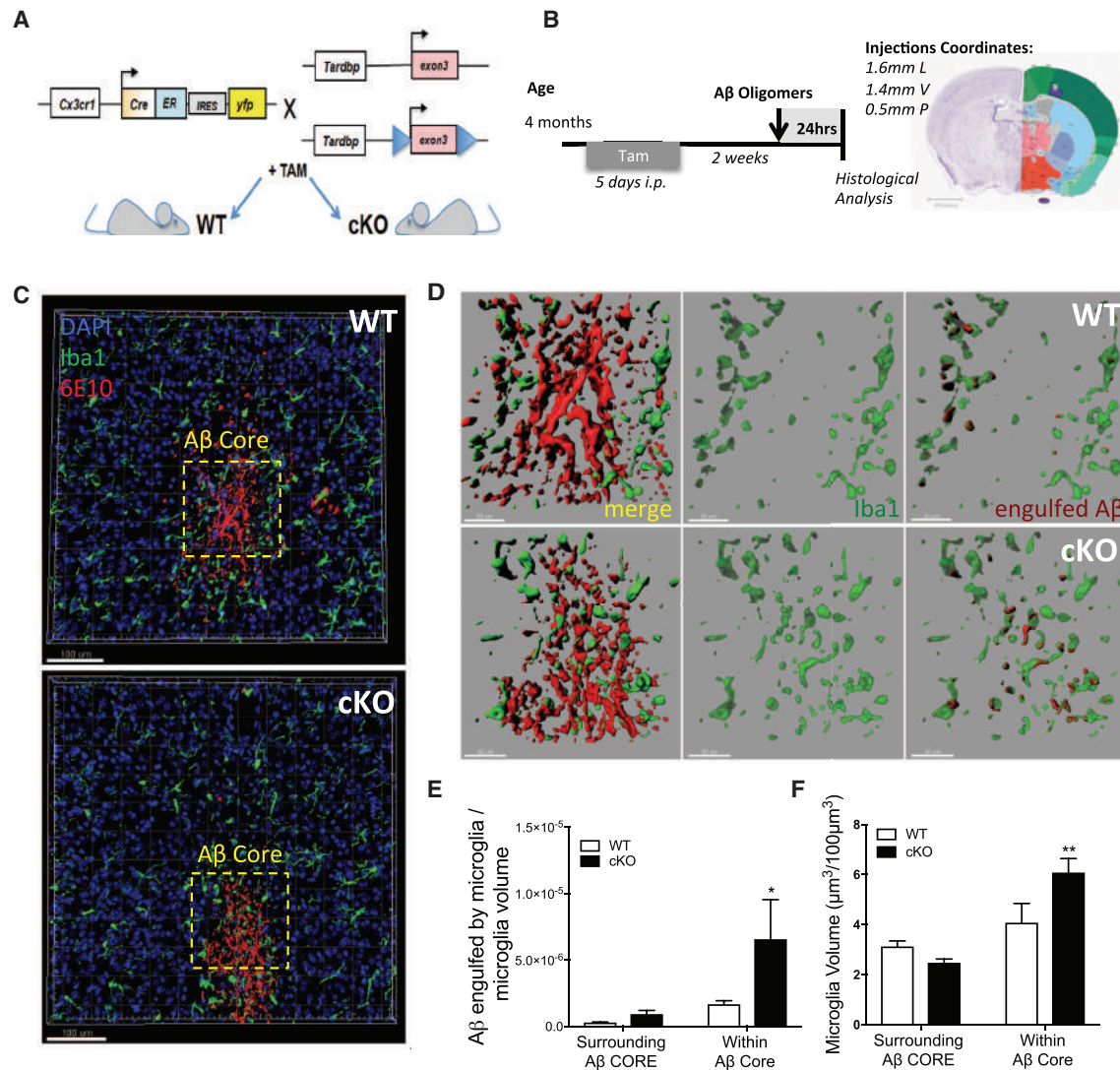
### Conditional Microglial TDP-43 Depletion *In Vivo* Promotes Phagocytosis of Stereotactically Injected A $\beta$

We then determined the physiological relevance of these findings *in vivo* by generating a microglial-specific inducible conditional TDP-43 knockout mouse line (cKO). We crossed mice expressing tamoxifen-inducible CRE recombinase (CreER) under the control of the endogenous *Cx3cr1* microglia-specific promoter (*Cx3cr1*<sup>creER-YFP</sup>; Parkhurst et al., 2013) with

\*p < 0.05, \*\*p < 0.01 versus scrambled control; ####p < 0.0001 *Tardbp* siRNA-T3 hr versus *Tardbp* siRNA-T0, using two-way ANOVA, followed by Bonferroni multiple comparison test.

(K and L) Representative confocal images of LysoTracker staining (K) and relative mean intensity quantification in control (n = 33) and TDP-43-depleted BV2 cells using siRNA *Tardbp* pool oligos, n = 36 or siRNA best 2 oligos, n = 36 (L). Data are shown as mean  $\pm$  SEM, \*\*p < 0.01, \*\*\*\*p < 0.0001, using one-way ANOVA followed by Dunnett's post hoc test.

(M) Representative blots for late endosomal/lysosomal markers in control and *Tardbp* knockdown BV2 cells.



**Figure 2. Inducible-Conditional Depletion of TDP-43 from Microglia Induces Enhanced Phagocytosis of Aβ42 Oligomers Administered by Stereotaxic Injections**

(A) Schematic representation of mouse breeding strategy for microglia-specific inducible conditional line, to obtain  $Cx3cr1^{CreER};Tardbp^{+/+}$  (WT) and  $Cx3cr1^{CreER};Tardbp^{floxed/floxed}$  (cKO) experimental subjects.

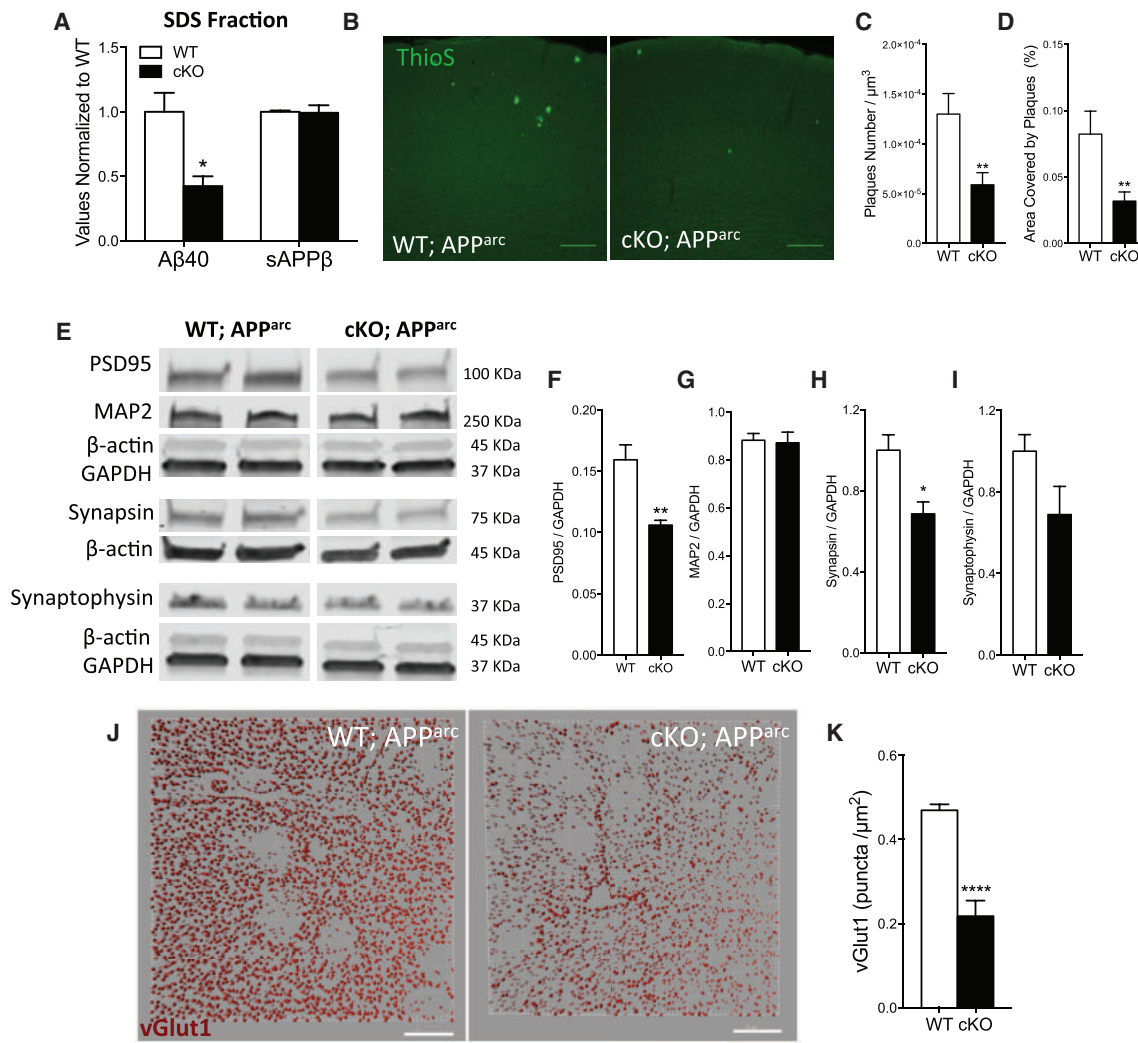
(B) Timeline for stereotaxic injections of Aβ oligomers upon tamoxifen treatment in WT and cKO mice and relative coordinates of injections.

(C and D) 3D reconstruction of confocal stack acquisition in the somatosensory cortex of WT and cKO mice, 24 hr after the injection of 100 μM Aβ42 oligomers (C). Dashed-yellow frames enclosing Aβ core are zoomed in (D), showing a representative reconstruction of Iba1-positive microglia processes in green, surrounding (WT) or infiltrating (cKO) the 6E10-positive amyloid core in red. Increased engulfment of amyloid is appreciable in cKO microglia cells compared to WT controls.

(E and F) Quantification of microglia processes and Aβ engulfment surrounding or within amyloid core injection. Data are shown as mean ± SEM from WT, n = 8, and cKO, n = 7, stacks, acquired from n = 3 animals per genotype, \*p < 0.05, \*\*p < 0.01, using two-way ANOVA followed by uncorrected Fisher's LSD test.

$Tardbp^{floxed}$  mice (Chiang et al., 2010) (Figure 2A). We confirmed that  $Tardbp$  transcript levels were significantly downregulated specifically in microglia isolated from cKO mice upon tamoxifen administration compared to WT controls, whereas overall cortical levels remained unchanged (Figures S4A and S4B). In addition, nuclear TDP-43 depletion was also confirmed at the protein level, in CRE-treated microglial primary cultures prepared from  $Tardbp^{floxed}$  mice (Figures S4C and S4D).

We then confirmed enhanced phagocytic uptake upon TDP-43 depletion *in vivo* by injecting Aβ42 oligomers in the cortex (100 μM, as prepared in Fa et al., 2010) and quantifying amyloid uptake 24 hr later (Figures 2B–2E). Interestingly, we observed a significant increase of microglia cells in close proximity to the amyloid core in cKO mice compared to WT littermates, whereas no differences occurred in the area surrounding the Aβ core (Figures 2D and 2F).



**Figure 3. Depletion of TDP-43 from Microglia Enhances Amyloid Clearance but Exacerbates Synaptic Loss in a Mouse Model of AD**

(A) Multiplexed electrochemiluminescent assay measurements of A $\beta$ 40 and sAPP $\beta$  levels in the SDS-soluble fraction of cortex homogenates from 7-month-old APP<sup>arc</sup> mice lacking TDP-43 in microglia. Mean  $\pm$  SEM, n = 4 mice per genotype, \*\*p < 0.01, using two-way ANOVA, followed by Sidak's post hoc test. (B) Representative max-projections of confocal stacks from cortex of WT;APP<sup>arc</sup> or cKO;APP<sup>arc</sup> mice stained with Thioflavin S. (C and D) Quantification of ThioS plaque density (C) and area covered by plaques from the cortex of 7-month-old APP<sup>arc</sup>, WT (n = 36) and cKO (n = 36) with acquisitions from 4 animals per genotype (D). Mean  $\pm$  SEM, \*\*p < 0.01, using two-tailed unpaired t test. (E–I) Representative blots for synaptic markers, from the cortex of 7-month-old APP<sup>arc</sup> mice, WT or KO for microglial TDP-43 (E). Quantification of western blots for PSD95 (F), MAP2 (G), synapsin (H), and synaptophysin (I) normalized for GAPDH reference gene. Mean  $\pm$  SEM, n = 4–5 mice per genotype, \*p < 0.05, \*\*p < 0.01, using two-tailed unpaired t test. (J and K), Representative 3D reconstruction from confocal acquisitions of vGlut1 immunoreactivity in the cortex of WT and cKO mice (J) and relative quantification (K) (WT n = 25, cKO n = 17, acquisitions from 4 mice per genotype; \*\*\*\*p < 0.0001, two-tailed t test).

**Conditional Depletion of Microglial TDP-43 Enhances Amyloid Clearance but Also Exacerbates Synaptic Loss in a Mouse Model of AD**

In the light of all our findings indicating that TDP-43 depletion in microglia enhances the phagocytic uptake of A $\beta$ , we hypothesized that this would promote clearance to reduce the total amyloid burden in a mouse model of AD. To this end, we crossed our Cx3cr1<sup>CreER</sup>;Tardbp<sup>floxed</sup> mice with mice overexpressing human APP carrying the Arctic and Swedish mutations (APP<sup>arc</sup>, Knobloch et al., 2007). Again, in this mouse model of

AD, A $\beta$  levels measurement revealed a significant reduction in cKO;APP<sup>arc</sup> compared to WT;APP<sup>arc</sup> littermates (Figure 3A) in the SDS fraction of brain homogenates and showed a similar trend in the TBS fraction (Figures S5A and S5B), confirming our *in vitro* results that TDP-43 depletion in microglia enhanced A $\beta$  clearance. We found no differences in sAPP $\beta$  levels, a soluble intermediate product in the generation of A $\beta$ , indicating that the amyloidogenic processing of APP was not affected, as also suggested by comparable levels of the full-length APP (Figure 3A; Figures S5A and S5C). These results definitely show

that TDP-43 depletion in microglia promotes A $\beta$  clearance, rather than affecting production.

To investigate whether the enhanced A $\beta$  clearance had any bearing on the amyloid load, we performed ThioS staining and observed a significant reduction in the cortex of cKO;APP<sup>arc</sup> mice compared to WT (Figures 3B–3D), with no change in plaque size (Figure S5D). Levels of Iba1 and CD45 markers in microglia surrounding the plaques were comparable in cKO and WT controls (Figures S5E–S5G).

Since amyloid oligomers and plaques are considered the primary cause of synaptotoxicity in AD patients, we hypothesized that enhancing microglial-mediated amyloid clearance should preserve synapses. To our surprise, despite the reduction in amyloid load, we found a significant decrease in cortical synaptic markers in these mice as assayed by western blot (Figure 3E). Specifically, PSD95, a scaffold protein located in dendritic spines, was significantly reduced (Figure 3F), while levels of MAP2, a dendritic structural protein, were comparable between WT and cKO mice. These results suggest a specific reduction in dendritic spines rather than a general decrease in neuronal branches (Figure 3G). Consistently, levels of synapsin and synaptophysin were also reduced (Figures 3H and 3I). In addition, quantification of immunoreactive puncta for the synaptic marker vGlut1 also confirmed a drastic reduction in glutamatergic terminals (Figures 3J and 3K). These data show that microglia lacking TDP-43 can mediate enhanced removal and clearance of amyloid in an AD mouse model, but also in parallel, induce significant synapse loss. Overall these findings suggest that abnormally phagocytic microglia remove not only amyloid but also synapses.

### **In Vivo Depletion of TDP-43 from Microglia Results in Enhanced Synapse Loss Even in the Absence of Amyloid**

Microglia are shown to re-activate synaptic pruning in the presence of A $\beta$  oligomers (Hong et al., 2016). Since we observed synapse loss in mice depleted of microglial TDP-43 in APP transgenic model, we next asked whether amyloid is required for the synapse loss to occur.

To answer this question, we quantified the levels of synaptic markers in the cortex of WT and cKO mice where no human APP gene was overexpressed and thus no amyloid load was present. Here again, we found a significant decrease in vGlut1 and PSD95 (Figures 4A–4C). Since demyelination can occur in many neurodegenerative disorders, we also assayed levels of myelin-binding protein (MBP) isoforms and found a significant decrease (Figures 4A and 4D). The decrease in PSD95 was significant despite no changes in MAP2 levels, indicative of a selective synapse loss rather than general neuronal death (Figures 4A and 4E). Consistent with these findings, we observed a significant decrease in cortical dendritic spine density in cKO mice (Figures 4F and 4G). vGlut1 immunohistochemistry also revealed a significant decrease in mice depleted of microglial TDP-43 compared to controls (Figures 4H and 4I). These results conclusively show that synapse loss occurs due to microglial TDP-43 depletion in mice, independent of amyloid load.

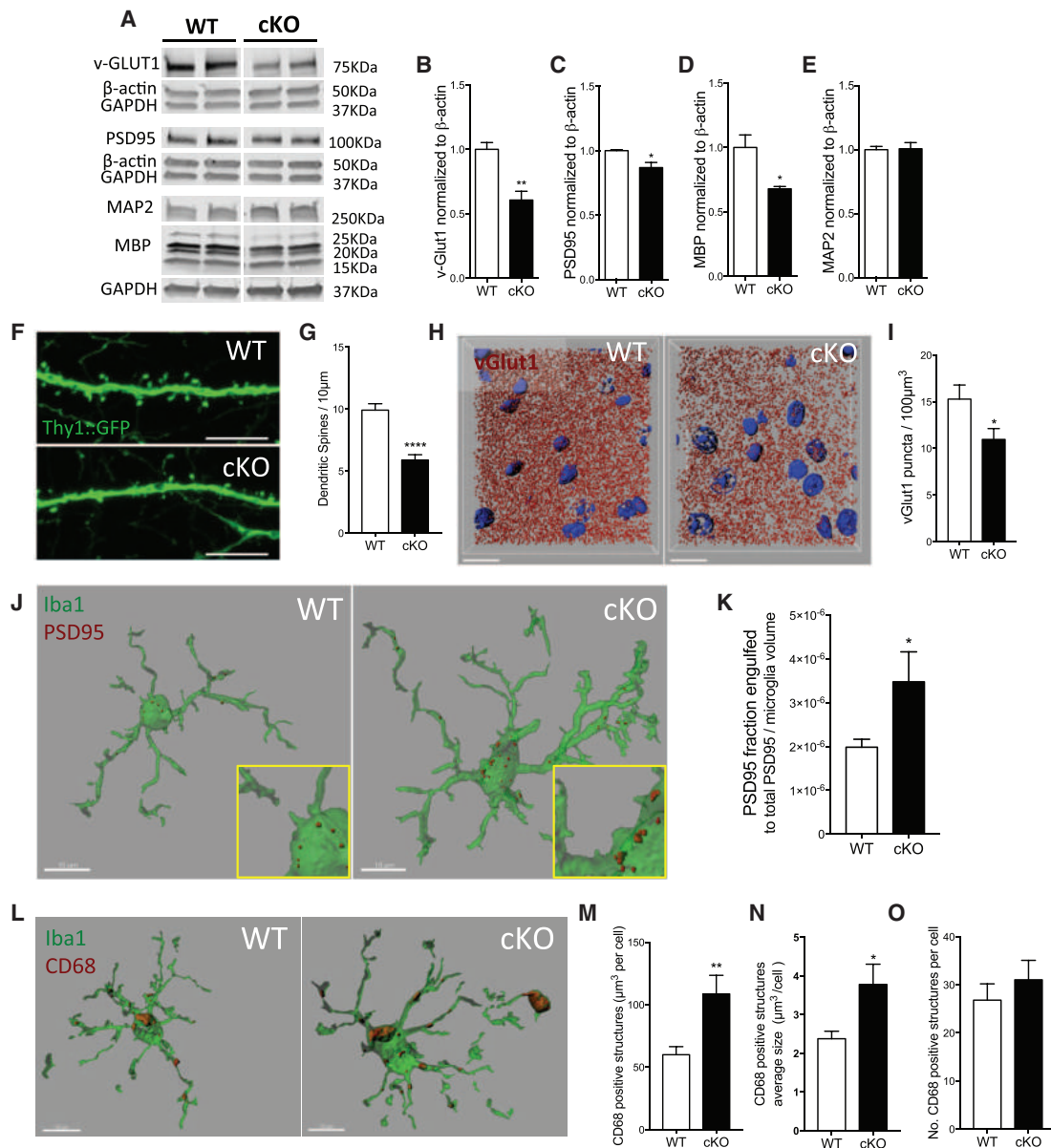
To directly assess the role of microglia in synapse elimination in these mice, we quantified synapse engulfment through 3D reconstruction of confocal acquisitions. Since we observed syn-

aptic immunoreactive puncta within CD68-positive phagocytic structures inside microglia cells (Figure S6), our signal co-localization was specific. We then quantified PSD95 immunoreactive puncta within and surrounding microglia cells. There was a significant increase in the fraction of synaptic marker engulfed by TDP-43 depleted microglia compared to WT controls (Figures 4J and 4K). We also observed a significant increase in the phagocytic marker CD68 (Figure 4L). The cells had increased size and total volume of CD68-positive structures, despite no change in number of structures (Figures 4M–4O). Overall, these data show that abnormal microglial phagocytosis induced by TDP-43 depletion mediates synapse loss, regardless of the presence of amyloid.

### **TDP-43 Pathology Is Associated with Lower Prevalence of AD and Higher Microglial Phagocytic Markers in Post-mortem Human Brains**

In line with our findings, we predicted that enhanced microglia-mediated clearance would affect cognitive decline by targeting synapses yet simultaneously reducing amyloid plaque load. This dual function could complicate the diagnosis of AD, which has been a topic of discussion for a very long time—whether amyloid load correlates with the cognitive decline (Braak and Braak, 1998; Serrano-Pozo et al., 2011; Nelson et al., 2012). We evaluated the prevalence of AD in a large cohort (n = 698) of ALS patients that typically exhibit TDP-43 pathology (Table S1). We selected an age cutoff of 65 years or older, as individuals over the age of 65 are at increased risk of sporadic AD. The prevalence of AD in ALS patients aged 65 to 74 years was comparable to what is expected in the normal population (reference to Hebert et al., 2013); however, the AD prevalence was considerably lower in ALS patients aged 75 years and above (Figure 5A). Notably, cognitive evaluation revealed a subtle cognitive dysfunction in non-AD ALS patients older than 75 years, despite excluding patients with over-lapping FTL (Figure 5B). These findings suggest that TDP-43 pathology is associated with reduced amyloid burden and may underlie subtle cognitive deficits in non-AD ALS patients. These clinical data support our overall hypothesis that dysfunctional microglia (as due to TDP-43 pathology) can mediate both enhanced amyloid clearance and synapse loss. This suggests that TDP-43 pathology might promote neurodegeneration through synapse loss on one hand, but on the other might also reduce the risk for enhancing the amyloid load and thus decrease the prevalence of AD.

To verify that the observed decreased prevalence of AD in ALS patients is secondary to decreased amyloid burden, we quantified amyloid pathology in an independent brain autopsy cohort, composed of healthy controls, AD cases, and TDP-43 cases (ALS and FTL-TDP-43). The quantification of A $\beta$  was performed using Thal A $\beta$  phase (TAP) scoring system. TAP relies on immunohistochemistry and evaluates presence or absence of all A $\beta$  plaques spatially across several neocortical, limbic, and subcortical regions of the brain. TAP staging is superior to other methods of A $\beta$  quantification in its sensitivity for A $\beta$ , as well as prediction of dementia symptoms (Boluda et al., 2014). Using TAP scoring, we observed A $\beta$  plaque burden to be comparable to age-matched controls in 65- to 74-year-old ALS/FTL-TDP patients. However, similar to how AD prevalence was lower in



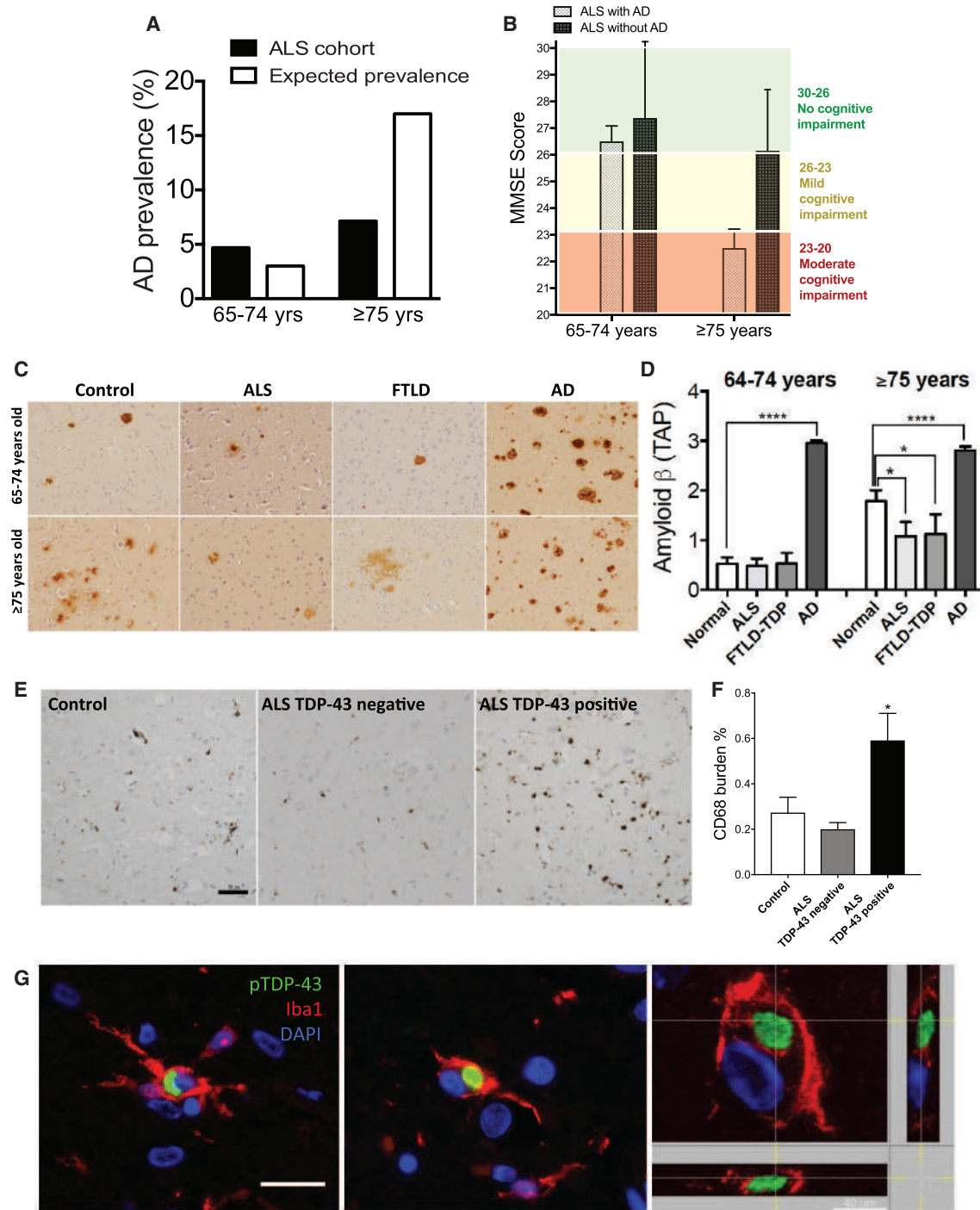
**Figure 4. Selective Depletion of TDP-43 from Microglia Results in Enhanced Synaptic Loss in Mice Even in the Absence of Amyloid**

(A–E) Representative blots of synaptic markers in the motor/somatosensory cortex of WT and cKO 8-month-old mice (A) and relative quantification for vGlut-1 (B), PSD95 (C), MBP (D), and MAP2 (E) normalized to β-actin reference gene. Mean ± SEM, n = 3–4 mice per genotype, \*p < 0.05, \*\*p < 0.01, unpaired two-tailed t test. (F and G) Representative confocal micrograph of dendritic spines from motor/somatosensory cortex of WT and cKO mice (F) (scale bar: 10 μm), and relative quantification (G) (WT n = 53, cKO n = 47 segments, from 4 animals per genotype). (H and I) Representative 3D reconstruction of vGlut1 immunoreactive puncta in the somatosensory cortex of WT and cKO mice (H) (scale bar: 15 μm), and relative quantification (I) (WT n = 8, cKO n = 10 acquisitions from 3 animals per genotype; \*p < 0.05, using two-tailed t test). (J and K) Representative 3D reconstruction of single microglia cells engulfing PSD95 (J) and quantified as fraction of engulfed PSD95 normalized to microglia volume (K) (means ± SEM, WT n = 12 and cKO n = 12 cells from 3 animals per genotype; \*p < 0.05, using two-tailed t test). (L–O) Representative 3D reconstructions showing CD68-positive structures within Iba1-microglia cells (L) (scale bar: 10 μm). Quantification of CD68 structures total volume per cell (M), average size per CD68-structure (N), and number of CD68-positive structures per cell (O) (WT n = 16 and cKO n = 20, from 3 animals per genotype).

ALS patients who were 75 years or older, Aβ pathology was significantly reduced in the brains collected from ALS/FTLD-TDP patients 75 years or older, compared to the age-matched controls (Figures 5C and 5D). These findings suggest that

TDP-43 pathology might promote enhanced amyloid clearance and hence prevent against AD.

To further validate the increase in the microglial phagocytic marker CD68 observed in our mouse model, we examined an



**Figure 5. Prevalence of AD in ALS/FTLD Patients Is Significantly Lower than in Healthy Controls**

(A) Prevalence of AD in ALS patients compared to expected prevalence in a normal age-matched population. ALS cohort was divided into two sub-groups according to their age at the time of AD screening: 65–74 and ≥75 years. The expected prevalence of AD in these age groups is 3% and 17%, respectively. AD prevalence is 4.7% in ALS patients aged 65–74 years but increases to only 7.1% in those ≥75 years.

(B) MiniMental State Examination (MMSE) scores reported from ALS patients with or without AD, indicate a mild cognitive impairment in ALS patients above 75, despite no AD.

(C) Representative images of beta-amyloid load in Control, ALS, FTLD, and AD biopsies in 65–74 and ≥75 years cases.

(D) Amyloid burden quantified according to the Thal A $\beta$  phase (TAP) scoring system, in 65–74 and ≥75 years controls, ALS, FTLD, and AD cases indicates decreased A $\beta$  in ALS and FTLD cases ≥75 years (mean  $\pm$  SEM, Control  $n = 40$ , ALS  $n = 35$ , FTLD  $n = 25$ , AD  $n = 62$ , \* $p < 0.05$ , \*\*\*\* $p < 0.0001$ , by one-way ANOVA of unpaired t test, followed by Holm-Sidak's multiple comparisons test).

(legend continued on next page)



independent brain autopsy cohort. To this end, we tested the levels of cortical CD68 in the context of ALS pathology. Importantly, we found that CD68 burden was significantly higher in ALS patients with TDP-43 pathology as compared to ALS patients without TDP-43 inclusions, or to healthy controls (Figures 5E and 5F; Table S2), supporting a critical role for TDP-43 in regulating microglial function. Finally, to assess the clinical relevance of the described microglial phenotype, we investigated whether TDP-43 aggregates could be found in the microglia of TDP-43 pathology cases. To this aim, we analyzed eight motor neurodegenerative (MND) cases with TDP-43 pathology and four healthy controls. In the MND cases, but not in the controls, we could observe examples of cytoplasmic inclusions positively stained for phospho-TDP-43 (pTDP-43) within microglial cells positive for Iba1 (Figure 5G), indicating that microglial TDP-43 can contribute to TDP-43 pathology. Importantly, though rare, pTDP-43 inclusions in microglia were found in all of the MND cases examined. Overall these findings corroborate the clinical relevance for a dysfunctional, abnormally phagocytic microglial phenotype in TDP-43 pathology.

## DISCUSSION

Here we show that loss of TDP-43 in microglia enhances phagocytosis and amyloid clearance following acute A $\beta$  oligomers injection and in a mouse model of AD. The abnormal phagocytosis induced by loss of TDP-43 concurrently resulted in an excessive loss of synapses independent of amyloid deposition. Our data demonstrate enhanced lysosomal function following TDP-43 depletion. However, further studies are now required to identify the exact mechanism through which TDP-43 regulates lysosome biogenesis and also enhanced phagocytosis. One possibility is that TDP-43 negatively controls lysosomal biogenesis genes such as the CLEAR genes that are regulated by the transcription factor TFEB. In this study, we found that TDP-43 depletion results in the increase transcription of genes related to lysosomal biogenesis; however, we focused only on a selected set of CLEAR genes. Whether TDP-43 regulates lysosome biogenesis through the entire CLEAR gene network needs to be further elucidated. Alternatively TDP-43 could also regulate mTOR activity, which in turn regulates lysosome biogenesis via the Raptor-TSC2-TFEB-lysosome/autophagosome biogenesis. Recent study performed in HeLa cells showed that TDP-43 loss promotes autophagosomal biogenesis as a direct consequence of decreased Raptor mRNA stability and promotes nuclear translocation of TFEB, a transcription factor master regulator of lysosomal genes (Ying et al., 2016). Our findings suggest that TDP-43 depletion positively regulates lysosomal genes downstream of TFEB. However, it remains to be tested whether TDP-43 loss leads to decreased Raptor mRNA and regulation of mTOR activity in microglia cells.

TDP-43 cytoplasmic inclusions can occur in multiple neurodegenerative diseases, which are collectively defined as TDP-43 proteinopathies. This classification highlights a key role for TDP-43 in the disease pathogenesis (Cohen et al., 2011). Previous studies indicate a causal role for TDP-43 neuronal pathology in the pathogenesis of neurodegeneration and synaptic loss using animal models of disease. These studies suggest, in fact, a cell-autonomous TDP-43 neurotoxicity (Igaz et al., 2011; Xu et al., 2011; Diaper et al., 2013; Yang et al., 2014; Medina et al., 2014; Handley et al., 2016).

Recent studies revealed additional non-autonomous functions for TDP-43 pathology in *Drosophila* glia, which result in defective synapses and axonal wrapping of motor neurons (Romano et al., 2015). However, neither a direct demonstration of microglial TDP-43 function nor its direct contribution to the pathological phenotype has been yet proposed. Here we provide evidence that phosphorylated TDP-43 aggregates can be found in the microglia of human post-mortem brains with TDP-43 pathology, thus opening the possibility that microglial TDP-43 might contribute to the pathogenesis of the disease.

In our model, a microglial-specific dysfunction induced by a loss of TDP-43 mediates a non-cell-autonomous neurotoxic effect, which could sum to other cellular phenotypes and critically contribute to the disorder. However, we cannot exclude the involvement of other Cx3cr1-positive non-parenchymal myeloid cells, considering that Cx3cr1-driven recombination also occurs in perivascular and meningeal macrophages (Goldmann et al., 2016).

Our data show that abnormal phagocytosis and clearance elicited in microglia following TDP-43 loss is ultimately paradoxical. These processes are not entirely beneficial in the context of a complex organism, since microglial phagocytic activity might not only enable clearance of protein aggregates but also synaptic connection loss. These mixed responses may underlie the failure of many AD drug treatment clinical trials to improve cognitive function, despite the progressive reductions in amyloid burden.

Synaptic pruning by microglia can re-activate in the presence of A $\beta$  oligomers, since complement molecules upregulate in the disease state to ultimately mediate synapse removal (Hong et al., 2016). Here we show that an intrinsic dysregulation of microglia induced by TDP-43 depletion is sufficient to trigger abnormal synapse loss, even in the absence of A $\beta$  oligomers. Our results further suggest that microglial dysfunction underlies the pathogenesis of many disparate and distinct neurodegenerative disorders. In line with our results, a recent study reported that selective neuronal depletion of TDP-43 was sufficient to reduce amyloid burden and to exacerbate cognitive deficits in an AD mouse model, suggesting that common mechanisms induced by loss of TDP-43 may partially explain the enhanced amyloid clearance (LaClair et al., 2016).

(E and F) Representative micrographs for immunostaining against CD68 in cortical section of healthy control, and ALS cases negative and positive for TDP-43 pathology, respectively (E). Relative quantification of CD68 burden in ALS patients with and without TDP-43 pathology indicates increased burden in ALS patients with TDP-43 pathology (F) (mean  $\pm$  SEM, Control  $n = 6$ ; \* $p < 0.05$ , ALS TDP-43 negative ( $n = 11$ ) versus ALS TDP-43 positive ( $n = 16$ ), by using two-tailed unpaired  $t$  test); scale bar: 50  $\mu$ m.

(G) Representative confocal  $z$  stack and orthogonal projections of microglial cells positive for Iba1 and pTDP-43 markers, in human post-mortem cortical sections of MND cases; scale bar: 20  $\mu$ m; scale bar for the orthogonal projection: 40  $\mu$ m.

Consistent with our experimental findings, we show that the prevalence of AD was considerably reduced in a cohort of ALS patients when compared to the expected AD prevalence in the normal population. Nevertheless, these ALS patients without AD still exhibited a subtle decline in cognitive function, which suggests an underlying loss of synapses. Importantly, neuropathological examination of post-mortem human brains showed a significant reduction of amyloid burden in ALS/FTLD-TDP-43 cases, as compared to age-matched healthy controls. These findings suggest that TDP-43 pathology might lead to enhanced A $\beta$  clearance and hence delay AD. However, in the same dataset, a comparison of A $\beta$  levels within AD cases with or without TDP-43 pathology did not reveal any major difference. This observation could suggest that the enhanced clearance through TDP-43 pathology might modulate the initial stages of amyloid deposition, but have no effects once the amyloid burden has established. Further studies are required to better elucidate the correlation between AD and TDP-43 pathology.

Indeed, a recent study from a population-based sample revealed a strong association of TDP-43 inclusions with late-onset dementia, but not with AD markers of amyloid and tau (Keage et al., 2014). Similarly, another study reported that the prevalence of amyloid positivity on PET in FTLN patients was lower than healthy age-matched controls, further corroborating our findings (Ossenkoppele et al., 2015). Furthermore, reduced amyloid pathology with concomitant exaggerated microglial CD68 levels and activation in TDP-43-positive ALS cases strongly supports our hypothesis (as also observed in Brettschneider et al., 2012).

Emerging evidence suggests that clearance mechanisms play a crucial role in neurodegenerative disorders, particularly in sporadic cases where no mutations may cause the disease. In addition, genome-wide association studies (GWASs) have identified a growing number of risk factors associated with microglial function. Certainly, identifying the pathways that underlie microglia-induced pathological synaptic pruning is of foremost importance. These pathways could elucidate cellular mechanisms common to the early stages of many distinct neurodegenerative disorders and may also reveal new powerful and efficient therapeutic targets. We believe these interventions could selectively target the paradoxical effects we discovered to prevent synapse loss and cognitive decline.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **CONTACT FOR REAGENT AND RESOURCE SHARING**
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Animal studies
  - Human studies
- **METHODS DETAILS**
  - Cell Culture
  - siRNAs
  - Acute Isolation of Adult Microglia
  - A $\beta$  Clearance Assay and Cell Proliferation Assay

- A $\beta$  measurement
- Phagocytosis and degradation of fluorescently labeled A $\beta$
- Western Blot and Brain Tissue protein extracts
- Stereotactic injections of A $\beta$
- Immunohistochemistry and Microscopy
- Human Sample Processing For A $\beta$ / TDP-43 detection
- Human samples Immunofluorescence Protocol
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

## SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures, two tables, and one movie and can be found with this article online at <http://dx.doi.org/10.1016/j.neuron.2017.05.037>.

## AUTHOR CONTRIBUTIONS

R.C.P., M.M., and A.V. performed *in vitro* and animal experiments. A.J., S.A., and P.E.S. designed and conducted the clinical studies. J.L.R., E.B.L., V.M.-Y.L., and J.Q.T. designed and conducted the post-mortem examination relative to the amyloid burden. C.M.H., J.R., and T.S.-J. conducted the post-mortem examination relative to CD68 and pTDP-43-microglia co-localization in ALS cases. R.C.P. and L.R. conceived the project, designed the experiments, and wrote the manuscript. All authors contributed to the edits of the paper.

## ACKNOWLEDGMENTS

This work was supported by Swiss National Science Foundation (SNF), Synapsis Foundation Alzheimer Research Switzerland ARS, Velux Stiftung, Cure Alzheimer Fund, Sinergia grant, and Interdisciplinary Core grant of the SNF. R.C.P. was funded by Forschungskredit University of Zurich. T.S.-J. and C.M.H. were funded by Alzheimer's Research UK, European Research Council and MND Scotland. We wish to thank all of our patient donors and their families for their valuable contributions to this work. We greatly acknowledge the work of Prof. Colin Smith and Chris-Anne MacKenzie from the Edinburgh Brain Bank for the provision of human tissue. The Edinburgh Brain Bank is a Medical Research Council funded facility with research ethics committee (REC) approval (11/ES/0022). We are grateful to the MND Register, hosted by the Euan Macdonald Centre for MND Research and funded by MND Scotland. In addition, thanks to Motor Neurone Disease Scotland for supporting the work of C.M.H. T.S.-J. is supported by the European Research Council (ALZSYN), Alzheimer's Research UK and the Scottish Government Chief Scientist Office, a Wellcome Trust-University of Edinburgh Institutional Strategic Support Fund, Alzheimer's Society, and would like to acknowledge the FENS Kavli Network of Excellence.

Received: September 12, 2016

Revised: January 28, 2017

Accepted: May 26, 2017

Published: June 29, 2017

## REFERENCES

- Arnold, S.E., Toledo, J.B., Appleby, D.H., Xie, S.X., Wang, L.S., Baek, Y., Wolk, D.A., Lee, E.B., Miller, B.L., Lee, V.M., and Trojanowski, J.Q. (2013). Comparative survey of the topographical distribution of signature molecular lesions in major neurodegenerative diseases. *J. Comp. Neurol.* 521, 4339–4355.
- Bali, J., Gheini, A.H., Zurbruggen, S., and Rajendran, L. (2012). Role of genes linked to sporadic Alzheimer's disease risk in the production of  $\beta$ -amyloid peptides. *Proc. Natl. Acad. Sci. USA* 109, 15307–15311.
- Baralle, M., Buratti, E., and Baralle, F.E. (2013). The role of TDP-43 in the pathogenesis of ALS and FTLN. *Biochem. Soc. Trans.* 41, 1536–1540.

- Boluda, S., Toledo, J.B., Irwin, D.J., Raible, K.M., Byrne, M.D., Lee, E.B., Lee, V.M., and Trojanowski, J.Q. (2014). A comparison of A $\beta$  amyloid pathology staging systems and correlation with clinical diagnosis. *Acta Neuropathol.* **128**, 543–550.
- Braak, H., and Braak, E. (1998). Evolution of neuronal changes in the course of Alzheimer's disease. *J. Neural Transm. Suppl.* **53**, 127–140.
- Brettschneider, J., Toledo, J.B., Van Deerlin, V.M., Elman, L., McCluskey, L., Lee, V.M., and Trojanowski, J.Q. (2012). Microglial activation correlates with disease progression and upper motor neuron clinical symptoms in amyotrophic lateral sclerosis. *PLoS ONE* **7**, e39216.
- Brooks, B.R. (1994). El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial "Clinical limits of amyotrophic lateral sclerosis" workshop contributors. *J. Neurol. Sci.* **124** (Suppl), 96–107.
- Buratti, E., and Baralle, F.E. (2001). Characterization and functional implications of the RNA binding properties of nuclear factor TDP-43, a novel splicing regulator of CFTR exon 9. *J. Biol. Chem.* **276**, 36337–36343.
- Buratti, E., and Baralle, F.E. (2009). The molecular links between TDP-43 dysfunction and neurodegeneration. *Adv. Genet.* **66**, 1–34.
- Chiang, P.M., Ling, J., Jeong, Y.H., Price, D.L., Aja, S.M., and Wong, P.C. (2010). Deletion of TDP-43 down-regulates Tbc1d1, a gene linked to obesity, and alters body fat metabolism. *Proc. Natl. Acad. Sci. USA* **107**, 16320–16324.
- Cohen, T.J., Lee, V.M., and Trojanowski, J.Q. (2011). TDP-43 functions and pathogenic mechanisms implicated in TDP-43 proteinopathies. *Trends Mol. Med.* **17**, 659–667.
- Cole, G.M., Bell, L., Truong, Q.B., and Saitoh, T. (1992). An endosomal-lysosomal pathway for degradation of amyloid precursor protein. *Ann. N Y Acad. Sci.* **674**, 103–117.
- Cook, C., Zhang, Y.J., Xu, Y.F., Dickson, D.W., and Petrucelli, L. (2008). TDP-43 in neurodegenerative disorders. *Expert Opin. Biol. Ther.* **8**, 969–978.
- Davalos, D., Grutzendler, J., Yang, G., Kim, J.V., Zuo, Y., Jung, S., Littman, D.R., Dustin, M.L., and Gan, W.B. (2005). ATP mediates rapid microglial response to local brain injury in vivo. *Nat. Neurosci.* **8**, 752–758.
- Deierborg, T. (2013). Preparation of primary microglia cultures from postnatal mouse and rat brains. *Methods Mol. Biol.* **1041**, 25–31.
- Derecki, N.C., Katzmarski, N., Kipnis, J., and Meyer-Luehmann, M. (2014). Microglia as a critical player in both developmental and late-life CNS pathologies. *Acta Neuropathol.* **128**, 333–345.
- Diaper, D.C., Adachi, Y., Sutcliffe, B., Humphrey, D.M., Elliott, C.J., Stepto, A., Ludlow, Z.N., Vanden Broeck, L., Callaerts, P., Dermaut, B., et al. (2013). Loss and gain of Drosophila TDP-43 impair synaptic efficacy and motor control leading to age-related neurodegeneration by loss-of-function phenotypes. *Hum. Mol. Genet.* **22**, 1539–1557.
- Fa, M., Orozco, I.J., Francis, Y.I., Saeed, F., Gong, Y., and Arancio, O. (2010). Preparation of oligomeric beta-amyloid 1-42 and induction of synaptic plasticity impairment on hippocampal slices. *J. Vis. Exp.* (41), 1884.
- Feng, G., Mellor, R.H., Bernstein, M., Keller-Peck, C., Nguyen, Q.T., Wallace, M., Nerbonne, J.M., Lichtman, J.W., and Sanes, J.R. (2000). Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. *Neuron* **28**, 41–51.
- Frenkel, D., Wilkinson, K., Zhao, L., Hickman, S.E., Means, T.K., Puckett, L., Farfara, D., Kingery, N.D., Weiner, H.L., and El Khoury, J. (2013). Scara1 deficiency impairs clearance of soluble amyloid- $\beta$  by mononuclear phagocytes and accelerates Alzheimer's-like disease progression. *Nat. Commun.* **4**, 2030.
- Gendron, T.F., and Petrucelli, L. (2011). Rodent models of TDP-43 proteinopathy: investigating the mechanisms of TDP-43-mediated neurodegeneration. *J. Mol. Neurosci.* **45**, 486–499.
- Goldmann, T., Wieghofer, P., Jordão, M.J., Prutek, F., Hagemeyer, N., Frenzel, K., Amann, L., Staszewski, O., Kierdorf, K., Krueger, M., et al. (2016). Origin, fate and dynamics of macrophages at central nervous system interfaces. *Nat. Immunol.* **17**, 797–805.
- Handley, E.E., Pitman, K.A., Dawkins, E., Young, K.M., Clark, R.M., Jiang, T.C., Turner, B.J., Dickson, T.C., and Blizzard, C.A. (2016). Synapse Dysfunction of Layer V Pyramidal Neurons Precedes Neurodegeneration in a Mouse Model of TDP-43 Proteinopathies. *Cereb. Cortex*. Published online August 5, 2016. <http://dx.doi.org/10.1093/cercor/bhw185>.
- Hebert, L.E., Weuve, J., Scherr, P.A., and Evans, D.A. (2013). Alzheimer disease in the United States (2010–2050) estimated using the 2010 census. *Neurology* **80**, 1778–1783.
- Hong, S., Beja-Glasser, V.F., Nfonoyim, B.M., Frouin, A., Li, S., Ramakrishnan, S., Merry, K.M., Shi, Q., Rosenthal, A., Barres, B.A., et al. (2016). Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* **352**, 712–716.
- Igaz, L.M., Kwong, L.K., Lee, E.B., Chen-Plotkin, A., Swanson, E., Unger, T., Malunda, J., Xu, Y., Winton, M.J., Trojanowski, J.Q., and Lee, V.M. (2011). Dysregulation of the ALS-associated gene TDP-43 leads to neuronal death and degeneration in mice. *J. Clin. Invest.* **121**, 726–738.
- Iguchi, Y., Katsuno, M., Niwa, J., Takagi, S., Ishigaki, S., Ikenaka, K., Kawai, K., Watanabe, H., Yamanaka, K., Takahashi, R., et al. (2013). Loss of TDP-43 causes age-dependent progressive motor neuron degeneration. *Brain* **136**, 1371–1382.
- Jawaid, A., Murthy, S.B., Wilson, A.M., Qureshi, S.U., Amro, M.J., Wheaton, M., Simpson, E., Harati, Y., Strutt, A.M., York, M.K., and Schulz, P.E. (2010). A decrease in body mass index is associated with faster progression of motor symptoms and shorter survival in ALS. *Amyotroph. Lateral Scler.* **11**, 542–548.
- Keage, H.A., Hunter, S., Matthews, F.E., Ince, P.G., Hodges, J., Hokkanen, S.R., Highley, J.R., Denning, T., and Brayne, C. (2014). TDP-43 pathology in the population: prevalence and associations with dementia and age. *J. Alzheimers Dis.* **42**, 641–650.
- Kettenmann, H., Kirchhoff, F., and Verkhratsky, A. (2013). Microglia: new roles for the synaptic stripper. *Neuron* **77**, 10–18.
- Knobloch, M., Konietzko, U., Krebs, D.C., and Nitsch, R.M. (2007). Intracellular Abeta and cognitive deficits precede beta-amyloid deposition in transgenic arcAbeta mice. *Neurobiol. Aging* **28**, 1297–1306.
- Kraemer, B.C., Schuck, T., Wheeler, J.M., Robinson, L.C., Trojanowski, J.Q., Lee, V.M., and Schellenberg, G.D. (2010). Loss of murine TDP-43 disrupts motor function and plays an essential role in embryogenesis. *Acta Neuropathol.* **119**, 409–419.
- LaClair, K.D., Donde, A., Ling, J.P., Jeong, Y.H., Chhabra, R., Martin, L.J., and Wong, P.C. (2016). Depletion of TDP-43 decreases fibril and plaque  $\beta$ -amyloid and exacerbates neurodegeneration in an Alzheimer's mouse model. *Acta Neuropathol.* **132**, 859–873.
- Lagier-Tourenne, C., Polymenidou, M., and Cleveland, D.W. (2010). TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration. *Hum. Mol. Genet.* **19** (R1), R46–R64.
- Lee, C.Y., and Landreth, G.E. (2010). The role of microglia in amyloid clearance from the AD brain. *J. Neural Transm. (Vienna)* **117**, 949–960.
- Lee, E.B., Leng, L.Z., Zhang, B., Kwong, L., Trojanowski, J.Q., Abel, T., and Lee, V.M. (2006). Targeting amyloid-beta peptide (Abeta) oligomers by passive immunization with a conformation-selective monoclonal antibody improves learning and memory in Abeta precursor protein (APP) transgenic mice. *J. Biol. Chem.* **281**, 4292–4299.
- McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., and Stadlan, E.M. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* **34**, 939–944.
- Medina, D.X., Orr, M.E., and Oddo, S. (2014). Accumulation of C-terminal fragments of transactive response DNA-binding protein 43 leads to synaptic loss and cognitive deficits in human TDP-43 transgenic mice. *Neurobiol. Aging* **35**, 79–87.
- Nakanishi, H. (2003). Microglial functions and proteases. *Mol. Neurobiol.* **27**, 163–176.
- Neary, D., Snowden, J.S., Gustafson, L., Passant, U., Stuss, D., Black, S., Freedman, M., Kertesz, A., Robert, P.H., Albert, M., et al. (1998). Frontotemporal

- lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology* 51, 1546–1554.
- Nelson, P.T., Alafuzoff, I., Bigio, E.H., Bouras, C., Braak, H., Cairns, N.J., Castellani, R.J., Crain, B.J., Davies, P., Del Tredici, K., et al. (2012). Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. *J. Neuropathol. Exp. Neurol.* 71, 362–381.
- Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M., et al. (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314, 130–133.
- Neumann, M., Kwong, L.K., Lee, E.B., Kremmer, E., Flatley, A., Xu, Y., Forman, M.S., Troost, D., Kretschmar, H.A., Trojanowski, J.Q., and Lee, V.M. (2009). Phosphorylation of S409/410 of TDP-43 is a consistent feature in all sporadic and familial forms of TDP-43 proteinopathies. *Acta Neuropathol.* 117, 137–149.
- Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 308, 1314–1318.
- Ossenkoppele, R., Jansen, W.J., Rabinovici, G.D., Knol, D.L., van der Flier, W.M., van Berckel, B.N., Scheltens, P., Visser, P.J., Verfaillie, S.C., Zwan, M.D., et al.; Amyloid PET Study Group (2015). Prevalence of amyloid PET positivity in dementia syndromes: a meta-analysis. *JAMA* 313, 1939–1949.
- Paolicelli, R.C., and Gross, C.T. (2011). Microglia in development: linking brain wiring to brain environment. *Neuron Glia Biol.* 7, 77–83.
- Paolicelli, R.C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., Giustetto, M., Ferreira, T.A., Guiducci, E., Dumas, L., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458.
- Paresce, D.M., Ghosh, R.N., and Maxfield, F.R. (1996). Microglial cells internalize aggregates of the Alzheimer's disease amyloid beta-protein via a scavenger receptor. *Neuron* 17, 553–565.
- Parkhurst, C.N., Yang, G., Ninan, I., Savas, J.N., Yates, J.R., 3rd, Lafaille, J.J., Hempstead, B.L., Littman, D.R., and Gan, W.B. (2013). Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell* 155, 1596–1609.
- Polymenidou, M., Lagier-Tourenne, C., Hutt, K.R., Huelga, S.C., Moran, J., Liang, T.Y., Ling, S.C., Sun, E., Wancewicz, E., Mazur, C., et al. (2011). Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. *Nat. Neurosci.* 14, 459–468.
- Prinz, M., Priller, J., Sisodia, S.S., and Ransohoff, R.M. (2011). Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat. Neurosci.* 14, 1227–1235.
- Ries, M., and Sastre, M. (2016). Mechanisms of A $\beta$  Clearance and Degradation by Glial Cells. *Front. Aging Neurosci.* 8, 160.
- Romano, G., Appocher, C., Scorzeto, M., Klima, R., Baralle, F.E., Megighian, A., and Feiguin, F. (2015). Glial TDP-43 regulates axon wrapping, GluRIIA clustering and fly motility by autonomous and non-autonomous mechanisms. *Hum. Mol. Genet.* 24, 6134–6145.
- Schafer, D.P., Lehrman, E.K., Kautzman, A.G., Koyama, R., Mardinly, A.R., Yamasaki, R., Ransohoff, R.M., Greenberg, M.E., Barres, B.A., and Stevens, B. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74, 691–705.
- Serrano-Pozo, A., Frosch, M.P., Masliah, E., and Hyman, B.T. (2011). Neuropathological alterations in Alzheimer disease. *Cold Spring Harb. Perspect. Med.* 1, a006189.
- Sierra, A., Encinas, J.M., Deudero, J.J., Chancey, J.H., Enikolopov, G., Overstreet-Wadiche, L.S., Tsirka, S.E., and Maletic-Savatic, M. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* 7, 483–495.
- Solé-Domènech, S., Cruz, D.L., Capetillo-Zarate, E., and Maxfield, F.R. (2016). The endocytic pathway in microglia during health, aging and Alzheimer's disease. *Ageing Res. Rev.* 32, 89–103.
- Terry, R.D., Masliah, E., Salmon, D.P., Butters, N., DeTeresa, R., Hill, R., Hansen, L.A., and Katzman, R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann. Neurol.* 30, 572–580.
- Toledo, J.B., Van Deerlin, V.M., Lee, E.B., Suh, E., Baek, Y., Robinson, J.L., Xie, S.X., McBride, J., Wood, E.M., Schuck, T., et al. (2014). A platform for discovery: The University of Pennsylvania Integrated Neurodegenerative Disease Biobank. *Alzheimers Dement.* 10, 477–484.
- Tremblay, M.E., Stevens, B., Sierra, A., Wake, H., Bessis, A., and Nimmerjahn, A. (2011). The role of microglia in the healthy brain. *J. Neurosci.* 31, 16064–16069.
- Vanden Broeck, L., Naval-Sánchez, M., Adachi, Y., Diaper, D., Dourlen, P., Chapus, J., Kleinberger, G., Gistelincq, M., Van Broeckhoven, C., Lambert, J.C., et al. (2013). TDP-43 loss-of-function causes neuronal loss due to defective steroid receptor-mediated gene program switching in *Drosophila*. *Cell Rep.* 3, 160–172.
- Vekrellis, K., Ye, Z., Qiu, W.Q., Walsh, D., Hartley, D., Chesneau, V., Rosner, M.R., and Selkoe, D.J. (2000). Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. *J. Neurosci.* 20, 1657–1665.
- Wakselman, S., Béchade, C., Roumier, A., Bernard, D., Triller, A., and Bessis, A. (2008). Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor. *J. Neurosci.* 28, 8138–8143.
- Walker, A.K., Tripathy, K., Restrepo, C.R., Ge, G., Xu, Y., Kwong, L.K., Trojanowski, J.Q., and Lee, V.M. (2015). An insoluble frontotemporal lobar degeneration-associated TDP-43 C-terminal fragment causes neurodegeneration and hippocampus pathology in transgenic mice. *Hum. Mol. Genet.* 24, 7241–7254.
- Wu, L.S., Cheng, W.C., and Shen, C.K. (2012). Targeted depletion of TDP-43 expression in the spinal cord motor neurons leads to the development of amyotrophic lateral sclerosis-like phenotypes in mice. *J. Biol. Chem.* 287, 27335–27344.
- Xia, Q., Wang, H., Hao, Z., Fu, C., Hu, Q., Gao, F., Ren, H., Chen, D., Han, J., Ying, Z., and Wang, G. (2016). TDP-43 loss of function increases TFEB activity and blocks autophagosome-lysosome fusion. *EMBO J.* 35, 121–142.
- Xu, Y.F., Zhang, Y.J., Lin, W.L., Cao, X., Stetler, C., Dickson, D.W., Lewis, J., and Petrucelli, L. (2011). Expression of mutant TDP-43 induces neuronal dysfunction in transgenic mice. *Mol. Neurodegener.* 6, 73.
- Yang, C., Wang, H., Qiao, T., Yang, B., Aliaga, L., Qiu, L., Tan, W., Salameh, J., McKenna-Yasek, D.M., Smith, T., et al. (2014). Partial loss of TDP-43 function causes phenotypes of amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. USA* 111, E1121–E1129.
- Ying, Z., Xia, Q., Hao, Z., Xu, D., Wang, M., Wang, H., and Wang, G. (2016). TARDBP/TDP-43 regulates autophagy in both MTORC1-dependent and MTORC1-independent manners. *Autophagy* 12, 707–708.