

# Solid Lipid Curcumin Particle (SLCP) As Estrogen Replacement In TDP-43 ALS Neuropathy

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## Abstract

Despite the discovery of amyotrophic lateral sclerosis (ALS) 154 years ago in 1869, public awareness of this devastating disease increased substantially only after the 2014 Ice Bucket Challenge, which also accelerated efforts to develop effective therapies. Currently, the three approved drugs for ALS treatment—riluzole, edaravone, and AMX0035—provide only limited symptomatic relief and cannot halt or cure disease progression, highlighting the urgent need for novel therapeutic strategies. The higher incidence and more severe disease progression observed in men and postmenopausal women compared with younger women in ALS and frontotemporal lobar degeneration (FTLD) suggest that sex hormones may play critical roles in disease onset and progression. Although 17 $\beta$ -estradiol (E2) has been shown to delay neurodegeneration in both animal models and clinical studies, its therapeutic application is limited by increased risks of breast and cervical cancers. In this study, we investigated the involvement of TDP-43 in regulating the estrogen-related enzymes CYP19A1 and CYP3A4 and explored the potential of solid lipid curcumin particles (SLCP) as an alternative estrogen replacement therapy. Using PrP-TDP-43A315T mice, we examined the expression patterns of E2 and its biosynthetic and metabolic enzymes under both normal and pathological conditions. We found that E2 levels, as well as the expression of CYP19A1 and CYP3A4, exhibited sex-dependent differences between non-transgenic wild-type and PrP-TDP-43A315T mice, suggesting that toxic phosphorylated TDP-43 oligomers may impair estrogen homeostasis by suppressing estrogen biosynthesis and enhancing its degradation. Furthermore, oral administration of SLCP significantly prolonged survival in female PrP-TDP-43A315T mice and reduced the accumulation of insoluble phosphorylated TDP-43 species. SLCP also modulated estrogen biosynthesis and CYP450 enzyme activity, thereby attenuating TDP-43-mediated neurodegeneration. Collectively, our findings demonstrate for the first time that SLCP may serve as a promising alternative to conventional estrogen replacement therapy by enhancing E2 expression and reducing pathological TDP-43 accumulation, providing a potential therapeutic strategy for ALS, FTLD, and other TDP-43-related neurodegenerative diseases.

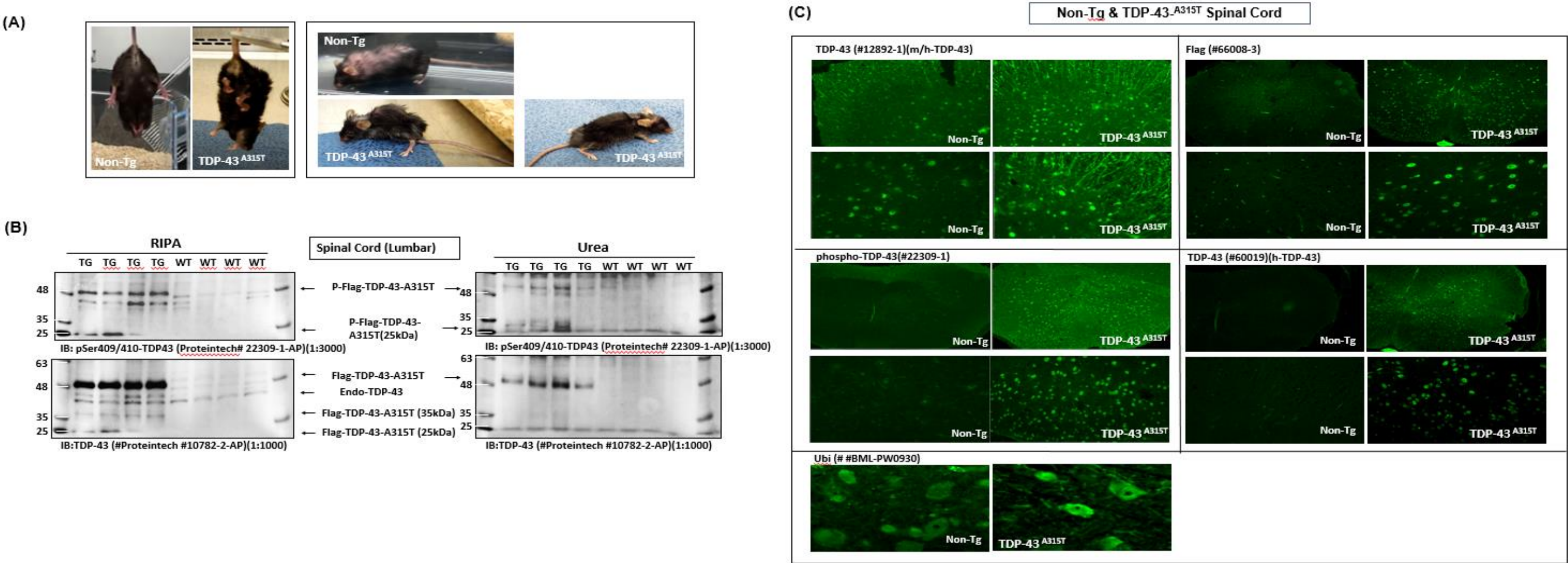


**Fig. 1. Pathological TDP-43 expressed in Prp-TDP43<sup>A315T</sup> ALS/FTLD mice.** (A) Prp-TDP43<sup>A315T</sup> mice develop motor neuron disease in both cortical and spinal motor neurons, which is reminiscent of human ALS and FTLD patients. Progressive motor phenotype including arched back, paralysis of lower limbs, gait abnormalities, hind-limb claspings, and gastrocnemius muscle atrophy is observed. (B) Post-mortem spinal cord tissue were sequentially extracted with RIPA and Urea buffers, and analyzed by Western blotting with anti-TDP-43 (#10782-2-AP)(m/h-TDP-43) and anti-phospho-TDP-43(#22309-1) antibodies. In both RIPA and Urea fraction, full length and truncated pathological phosphorylated Flag-tag human TDP-43-A315T mutant protein were only detected in TDP-43<sup>A315T</sup> ALS/FTLD mice, but not in non-Tg wild-type (WT) littermate mice. (C) The expression patterns of pathological Flag-tag human TDP-43-A315T protein in the spinal cord was further analyzed with immunofluorescent assay. The fluorescent intensity of total TDP-43 protein detected with anti-mouse/human TDP-43 (#12892-1) was significantly stronger in TDP-43<sup>A315T</sup> Tg ALS/FTLD mice spinal cord than that in non-Tg wild-type littermates mice. Phosphorylated Flag-tag human TDP-43-A315T protein expressed only in TDP-43<sup>A315T</sup> Tg ALS/FTLD mice detected by anti-Flag (#66008-3), anti-human TDP-43 (#60019)(h-TDP-43) and anti-phospho-TDP-43(#22309-1) antibodies. The ubiquitin signal in TDP-43<sup>A315T</sup> Tg ALS/FTLD mice was also stronger than that in non-Tg wild-type littermates mice.

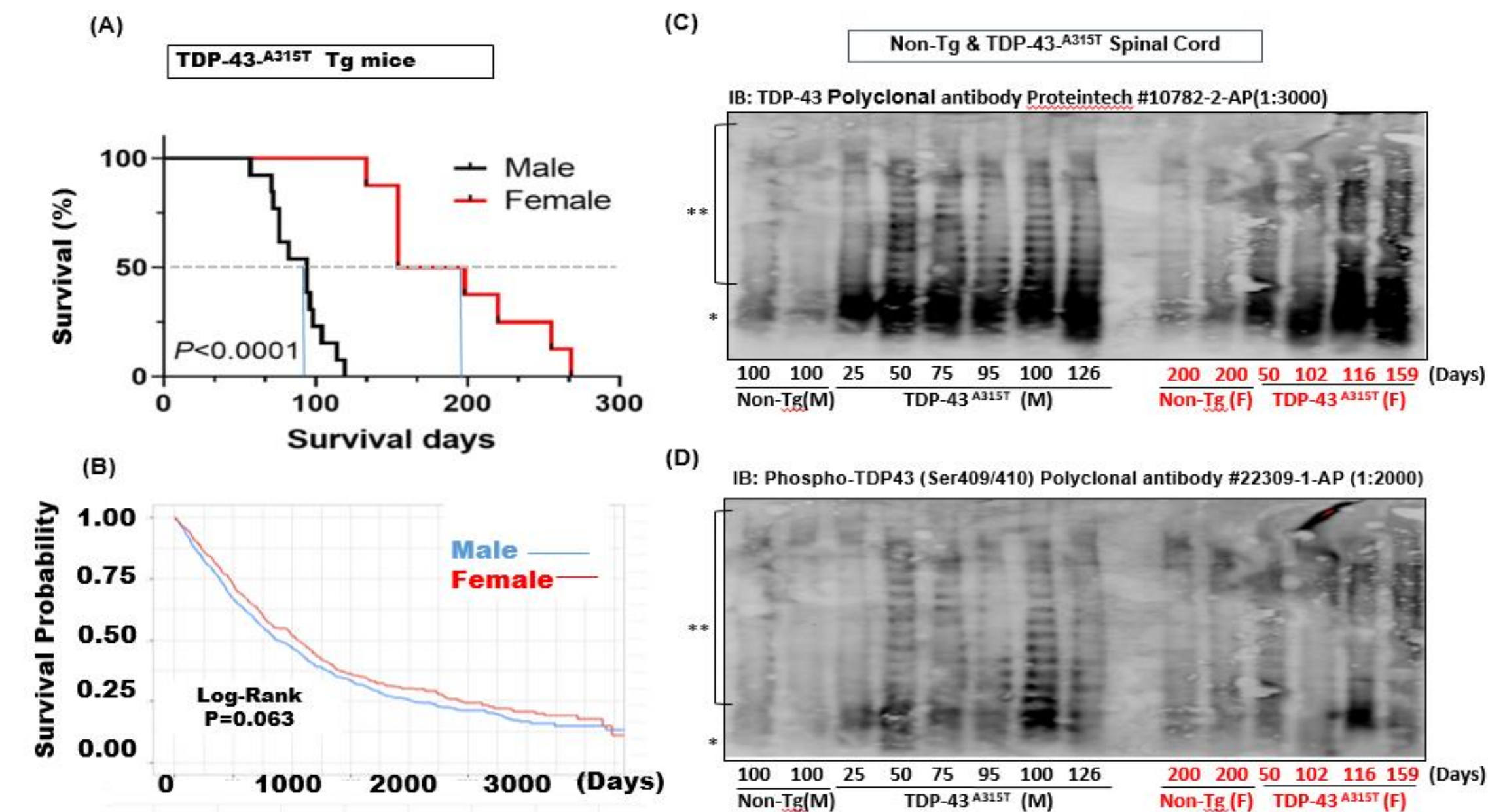


**Fig. 2. The gender difference between survival rates and pathological TDP-43 expression.** (A) Survival rates of male (black line, n = 50) and female (red line, n = 26) Prp-TDP43<sup>A315T</sup> mice was monitored over time. All Non-Tg animals survived beyond 200 days. The medium lifespan in Prp-TDP43<sup>A315T</sup> males was significantly shorter than females (96  $\pm$  18 days vs. 196  $\pm$  15 days, respectively, p < 0.001). (B) and C) SDD-AGE and immunoblotting were used to detect TDP-43 oligomerization and aggregates patterns. Spinal cord tissue lysates derived from different days of non-Tg wild-type and Prp-TDP43<sup>A315T</sup> male and female mice were analyzed by SDD-AGE. All blots were probed either with TDP-43 (#10782-1)(m/h-TDP-43) antibody or Phospho-TDP43 (Ser409/410) antibody (#22309-1-AP) (\*monomers; \*\*oligomers, polymers or aggregates).

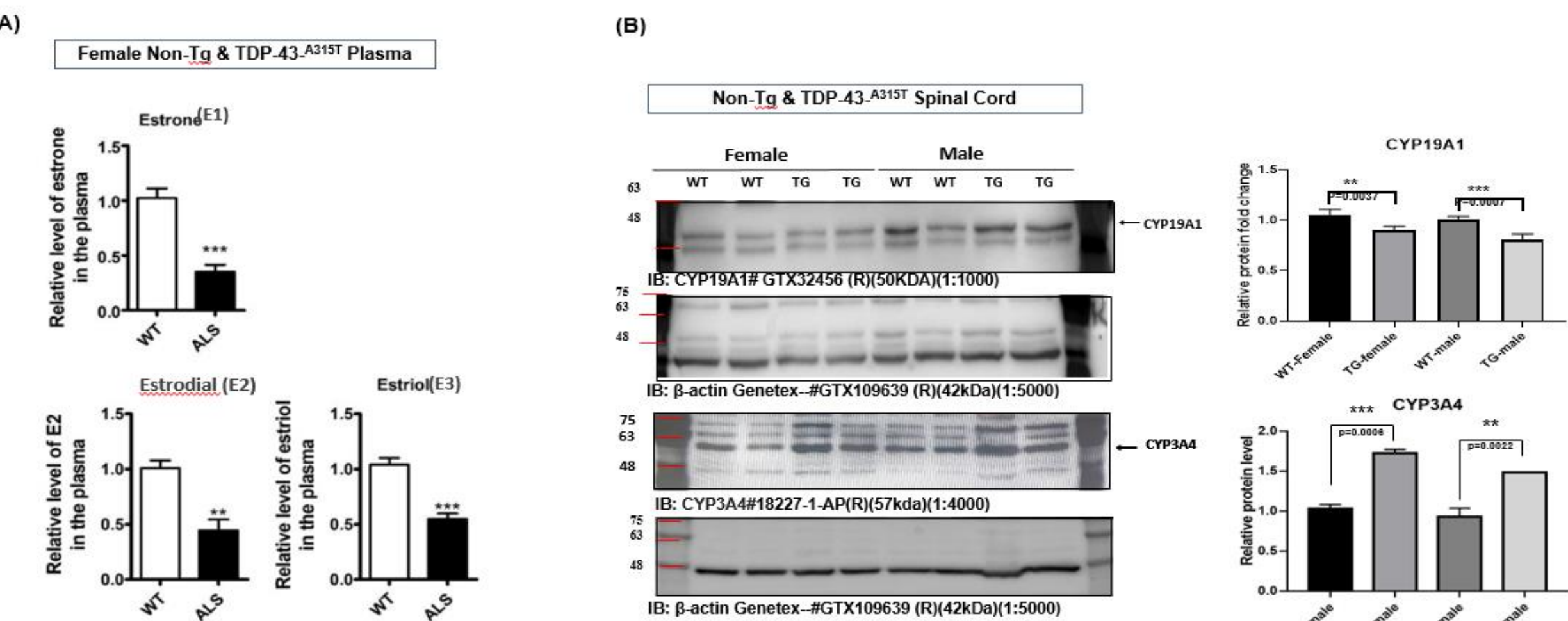


**Fig. 3. E2, CYP19A1 and CYP3A4 in Prp-TDP43<sup>A315T</sup> ALS/FTLD mice neuropathies.** (A) The plasma level of estrogens, including E1, E2 and E3 were examined in female non-Tg wild-type and prp-TDP43<sup>A315T</sup> mice, and significant decrease levels of estrogens were observed in Prp-TDP43<sup>A315T</sup> female mice comparing to those in non-transgenic female mice. (B) Post-mortem spinal cord tissue were extracted with RIPA buffers, and analyzed by Western blotting with CYP19A1 (#GTX32456), CYP3A4 (#18227-1-AP) and  $\beta$ -actin (#GTX109639) antibodies. Data are presented as the mean  $\pm$  SEM (groups were compared by a one-way ANOVA, two-tailed p value).

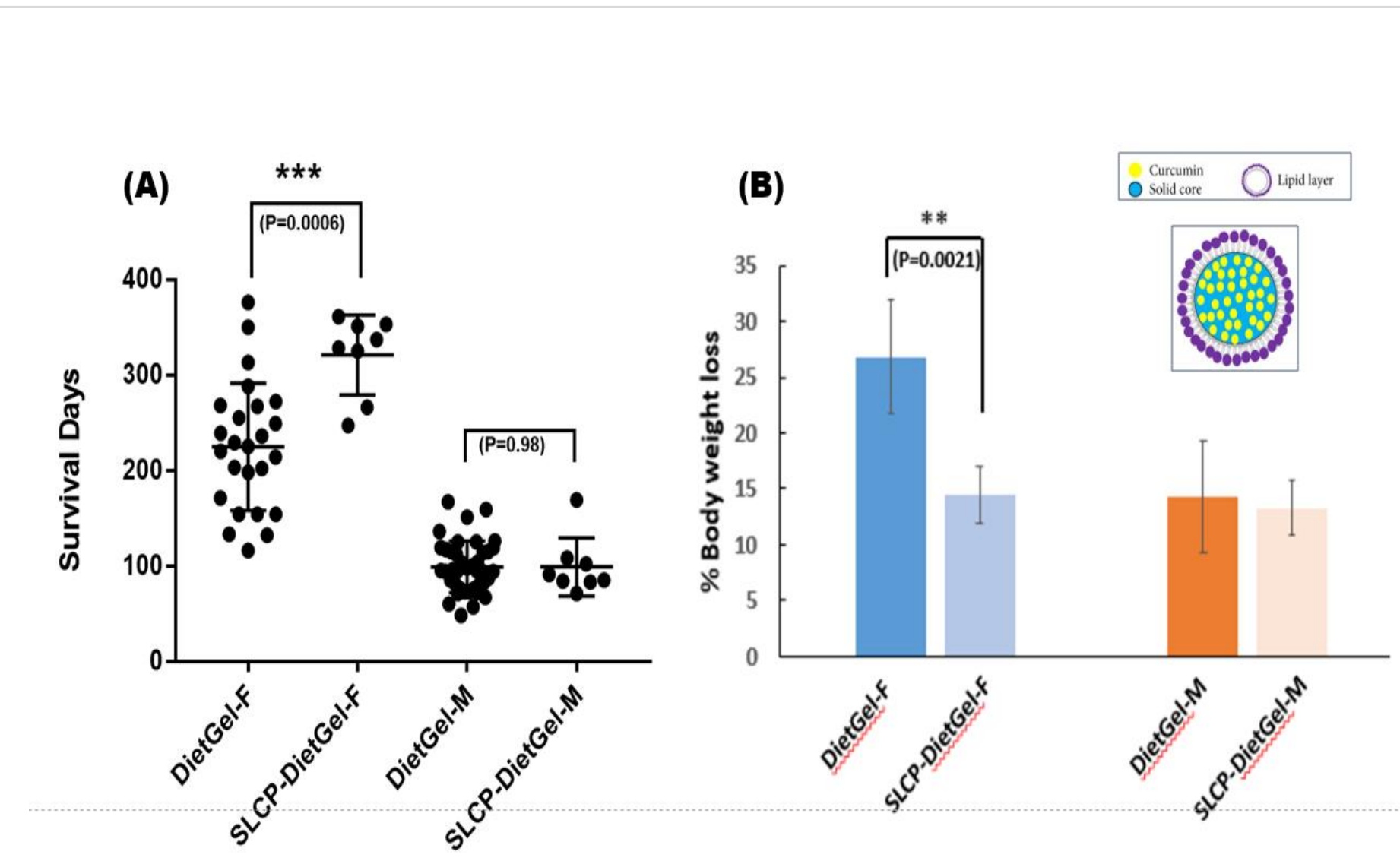


**Fig. 4. Administration of SLCP prolongs survival rates and mitigates body weight loss of female TDP-43<sup>A315T</sup> Tg ALS/FTLD mice.** (A) TDP-43<sup>A315T</sup> ALS/FTLD mice were fed with DietGel Boost containing with or without 0.5% SLCP starting at 6 weeks of age. In each graph, female and male TDP-43<sup>A315T</sup> Tg ALS/FTLD mice treated with or without SLCP are shown. The survival days of female mice treated with SLCP-DietGel was 321 $\pm$ 15 days (n=8), which is longer than the 225 $\pm$ 13 days survival days of DietGel-fed female mice (n=25). While the survival days between DietGel (n=40) and SLCP-DietGel (n=8)-treated male mice were no significant. (B) The % body weight loss in female prpTDP-43<sup>A315T</sup> mice administrated with SLCP (14.26%) was much less than those without SLCP treatment (26.83%). While there was no change in male prpTDP-43A315T mice administrated with or without SLCP.

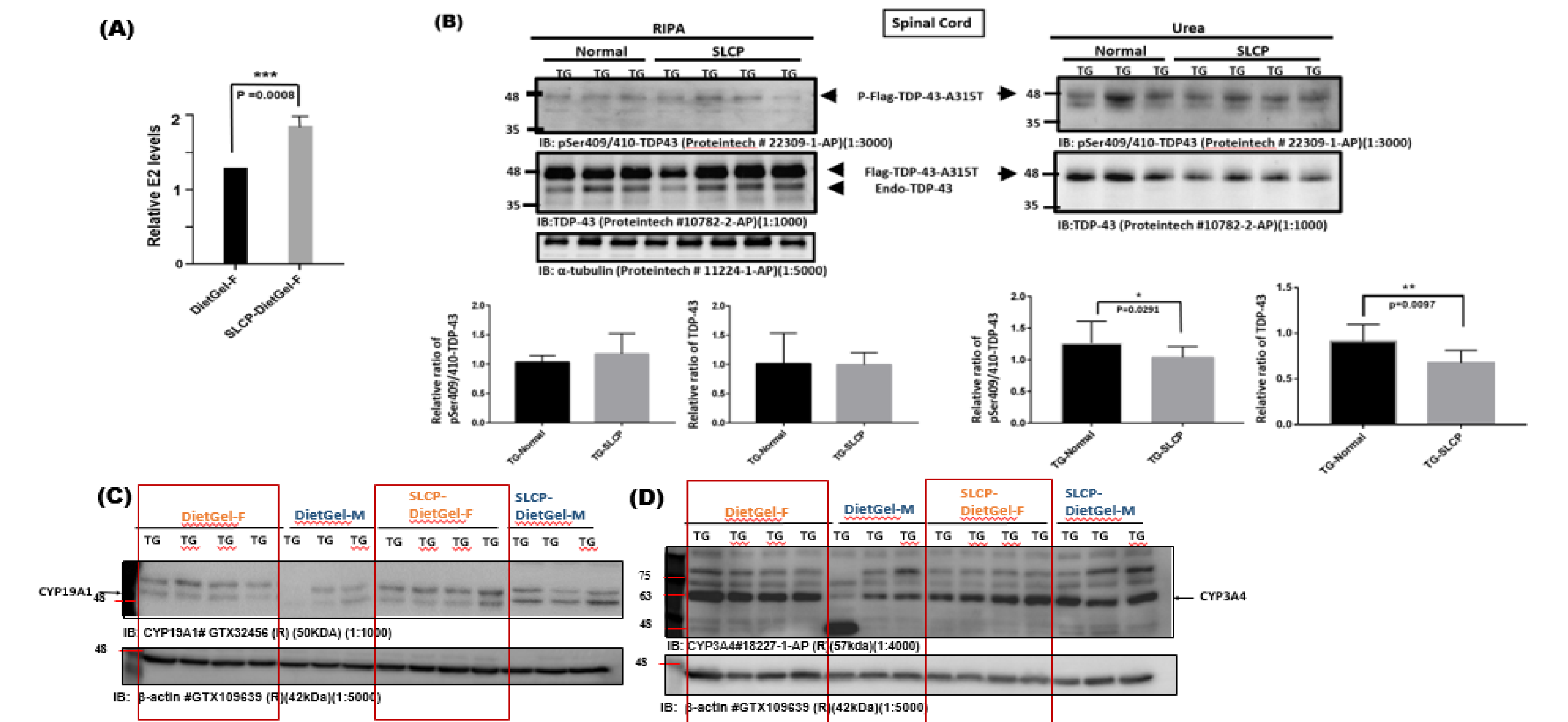
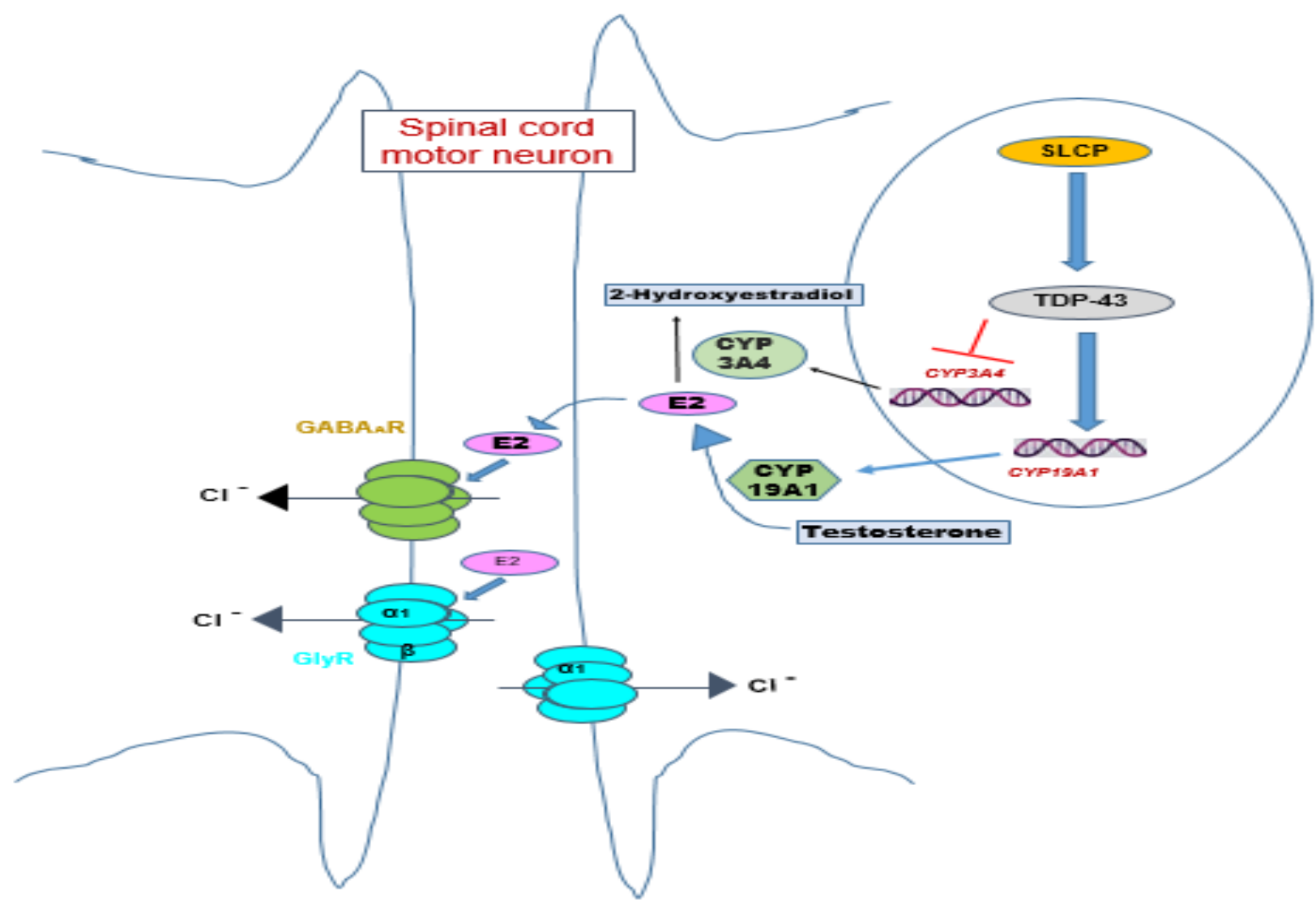


**Fig. 5. Expression pattern of pathological TDP-43-A315T mutant proteins, CYP19A1 and CYP3A4 in TDP-43<sup>A315T</sup> Tg ALS mice administrated with or without SLCP.** (A) The plasma level of E2 was examined in Prp-TDP43<sup>A315T</sup> mice treated with or without SLCP. (B) Post-mortem spinal cord tissue were sequentially extracted with RIPA and Urea buffers, and analyzed by Western blotting with anti-TDP-43 (#10782-2-AP)(m/h-TDP-43) and anti-phospho-TDP-43(#22309-1) antibodies. In RIPA fraction, pathological phosphorylated and Flag-tag human TDP-43-A315T mutant protein have no significant change between control and SLCP groups, while the insoluble phosphorylated and Flag-tag human TDP-43-A315T mutant protein were significantly decreased in SLCP-treated mice comparing to that with normal saline-treatment. Data are presented as the mean  $\pm$  SEM (groups were compared by a one-way ANOVA, two-tailed p value). (C and D) Post-mortem spinal cord tissue were extracted with RIPA buffers, and analyzed by Western blotting with CYP19A1 (#GTX32456), CYP3A4 (#18227-1-AP) and  $\beta$ -actin (#GTX109639) antibodies. In female Prp-TDP43<sup>A315T</sup> mice with SLCP treatment, comparing to normal saline-treated group, the expression levels of the aromatase CYP19A1 has no significant change, whereas CYP3A4 levels were significantly declined, indicating SLCP slows down the degradation rates of estrogen through attenuating CYP3A4 expression in female Prp-TDP43<sup>A315T</sup> mice. While in male Prp-TDP43<sup>A315T</sup> mice treated with SLCP, comparing to normal saline-treated group, both of the aromatase CYP19A1 and CYP3A4 levels were significantly increased, leading to unchanged levels of estrogen in these male mice. Data are presented as the mean  $\pm$  SEM (groups were compared by a one-way ANOVA, two-tailed p value).



## Summary

The higher incidence and more severe disease progressing observed in men and postmenopausal women than young women in ALS and FTLD rise up an important role of sexual hormones, especially 17 $\beta$  estradiol (E2), in the disease onset and pathology progression in ALS and FTLD. Nowadays, there are still no promising medications and treatments to completely cure ALS and FTLD diseases, thus developing a drug for those patients is imminent. Numerous studies have proved the beneficial effect of E2 as a therapeutic strategy in numerous neurodegenerative diseases, however, its unwanted adverse side effects overshadow its bright side. Thus exploring the alternative replacement therapy for E2 provide potential therapeutic strategies for ameliorating the disease progression in FTLD and ALS diseases. Unrevealing these underlying mechanism is thus essential to provide more comprehensive understanding about TDP-43 neuropathies.

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