Supplemental Materials

Endogenous TDP-43 mislocalization in a novel knock-in mouse model reveals DNA repair impairment, inflammation, and neuronal senescence

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Supplemental materials include one table and its related references, and five figures along with figure legends.

Supplemental **Table 1**: A comparison of TDP-43 pathologies, disease phenotypes and practical applications of various types of TDP-43 mouse models of ALS and FTD.

Model Type	TDP-43 variant	Disease Phenotypes	Advantages	Disadvantages	Reference
Cisgenic – Knock-in	Tdp-43ΔE2/3	Spinal motor neuron-specific Tdp-43ΔE2/3 nuclear depletion-linked ALS-like phenotypes	- Allows to study motor neuron-specific Tdp-43 pathologies and motor defects. - Tdp-43 pathology-induced astrocytosis.	- Not suitable for studying ALS/FTD-TDP-43 related RNA processing and misfolded protein clearance mechanisms. - Posses disease-irrelevant Tdp-43 mutation. - Pathological effect of Tdp-43ΔE2/3 variant in the motor cortex is unknown. - Not suitable for studying long-term age-associated ALS phenotypes.	[1]
Transgenic	rNLS (hTDP-43ΔNLS)	Insoluble phosphorylated cytoplasmic TDP-43 aggregates in the CNS, brain and muscle atrophy, leading to death.	- Allows rapid onset ALS pathology. - Presents most ALS-like symptoms upon hTDP-43ΔNLS induction. - Presents aggressive but reversible motor deficits.	- hTDP-43ΔNLS-mediated nuclear loss of wildtype murine Tdp-43. - Non-specific interaction between hTDP-43ΔNLS and murine mRNA, resulting in aberrant alternative splicing. - Aggressive model of ALS, not suitable for pre-symptomatic biomarker discovery for early detection. - The underlying mechanism of reversible motor phenotype is unclear.	[2]
Cisgenic – Knock-in	Mnx1-Tdp-43ΔNLS	Nuclear clearance and cytoplasmic-aggregation of Tdp-43ΔNLS in motor neurons and associated ALS-type motor defects and muscle weakness	- Suitable for investigating progressive motor neuron degeneration mimicking ALS. - Allows cell type-specific and conditional expression of Tdp-43ΔNLS mutant, with otherwise intact RRM domains. - Suitable for pre-symptomatic pathomechanistic discoveries and biomarker development. - Suitable for studying the gradual nuclear loss of Tdp-43ΔNLS and altered mRNA processing and expression. - Suitable for investigating Tdp-43 pathology-linked genome instability, inflammation, and neuronal senescence pathways for therapy development.	- Disease onset and manifestation are slow (10-12 months). - Not suitable for aggressive ALS pathology studies.	This study.

Transgenic	hTDP-43 WT	hTDP-43 overexpression-induced TDP-43 ubiquitination, phosphorylation and fragmentation pathologies. Moderate to aggressive ALS/FTD phenotypes.	-Allows CNS-targeted TDP-43 WT overexpression-associated disease phenotypes.	- Not suitable for examining motor neuron-specific TDP-43 proteinopathies. - Disease onset and aggressiveness vary widely from mild to severe across the transgenic subtypes. -Not reliable for ALS/FTD-TDP-43-linked RNA alternative splicing and biomarker discovery studies.	[3]
Cisgenic – knock-in	Tdp-43 Q331K	Sporadic ALS-Tdp-43 Q331K variant-induced Tdp-43 gain-of-function phenotypes.	- Suitable for studying genetic causes of male dominance in ALS/FTD. - Allows investigating Tdp-43 Q331K-related aberrant mRNA splicing. - Enables investigating Tdp-43 gain-of-function motor and behavioral phenotypes for Q331K mutant. - Allows investigating Tdp-43 pathology-induced metabolic	- Not suitable for investigating TDP-43 loss-of-function and gain-of-toxicity phenotypes. - A rare mutation model. - Lacks ALS-like limb muscle pathologies.	[4]
Transgenic	hTDP-43 A315T	TDP-43-mediated neurodegeneration in the brain and spinal cord without cytosolic aggregation of TDP-43	Allows studying - ALS-like motor and pathological phenotypes in a short span of time. - hTDP-43 overexpression-induced disease mechanism in mice.	- Non-specific TDP-43 pathology for motor neurons and motor cortex. - High levels (3-fold) of Tg-TDP-43 overexpression. - Early onset (1-2 months age) pathological symptoms resembling advanced stages of ALS/FTD. - Possibility of aberrant interactions between Tg-TDP-43 A315T and endogenous mRNA and Tdp-43. - Does not recapitulate human ALS aggregation phenotypes.	[5]

ALS: amyotrophic lateral sclerosis; FTD: frontotemporal dementia; E2/3: Exon 2 and Exon 3; NLS: nuclear localization signal; CNS: central nervous system; Tg: transgenic.

Reference

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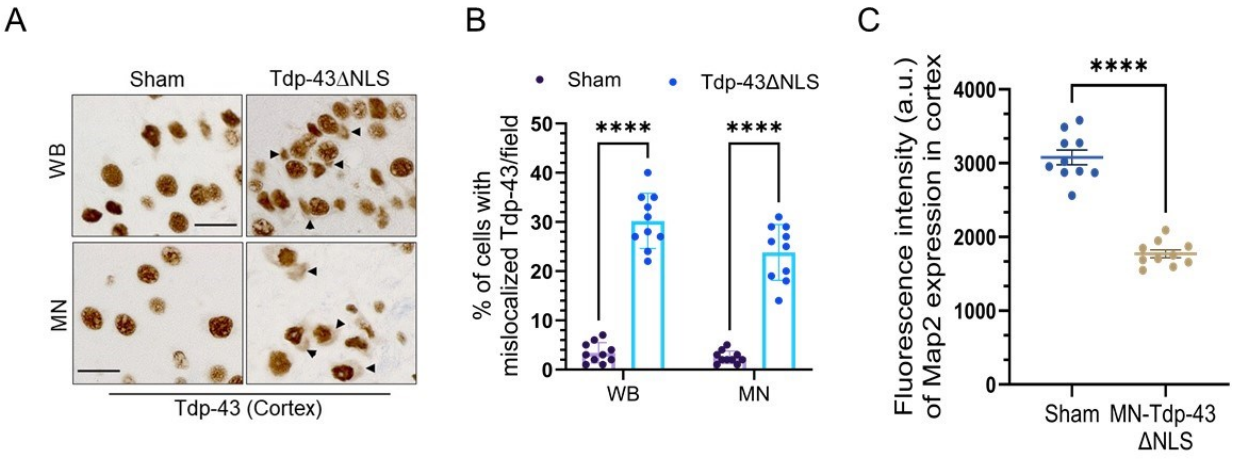
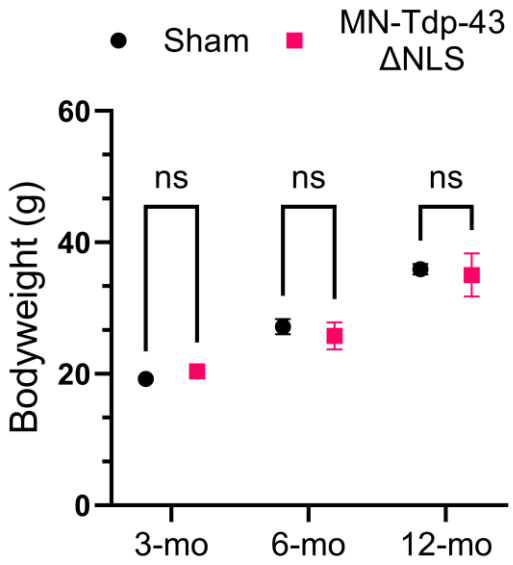
5. Wegorzewska I, Bell S, Cairns NJ, Miller TM, Baloh RH (2009) TDP-43 mutant transgenic mice develop features of ALS and frontotemporal lobar degeneration. Proc Natl Acad Sci U S A 106:18809-18814. https://10.1073/pnas.0908767106

Supplemental Fig. 1



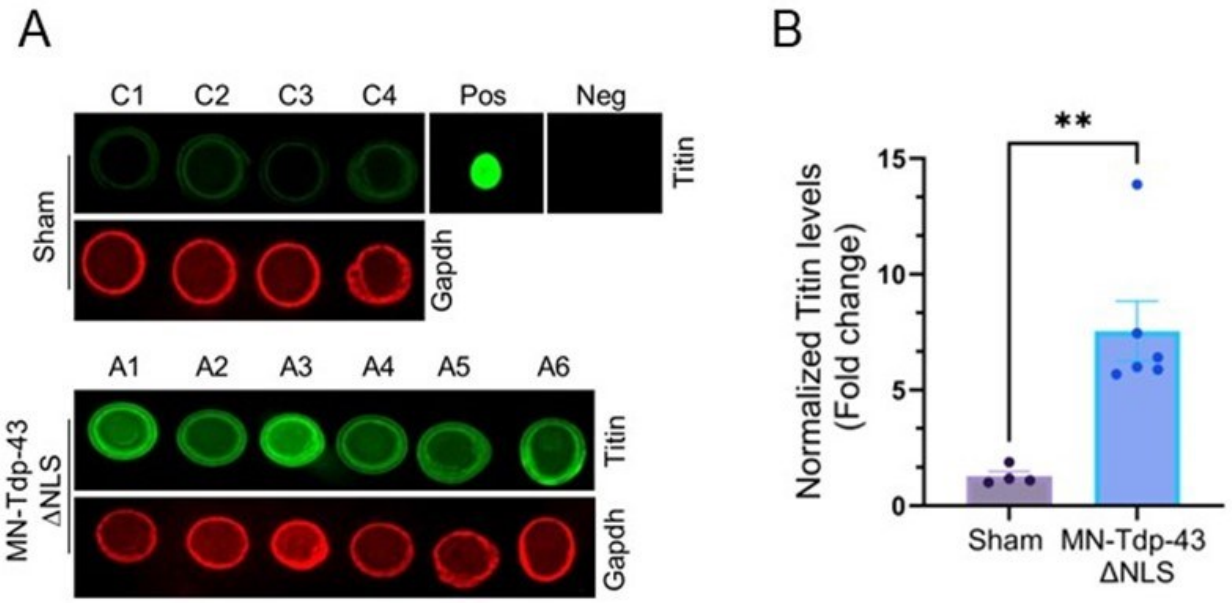
Supplemental Fig. 1. (Related to **Fig. 3**) **Immunohistochemical analysis of Tdp-43 expression phenotypes in different Tdp-43ΔNLS mice models**. (**A-B**) Immunohistochemistry (IHC) with anti-TDP-43 antibody in the motor cortex of ALS mice Ubc-Cre::Tdp-43ΔNLS [whole body (WB)-Tdp-43ΔNLS hence after; *upper panel*] and Mnx1-Cre::Tdp-43ΔNLS [motor neuron (MN)-Tdp-43ΔNLS hence after; *lower panel*]. Scale bar = 20 μm (**A**). (**B**) Quantitation of cells with Tdp-43 mislocalization phenotype in sham versus WB- or MN-Tdp-43ΔNLS mice cortical tissues (N = 6 mice/group) by two-way ANOVA. ****, $P < 0.0001$. (**C**) Quantitation of fluorescence intensity (arbitrary unit, a.u.) of Map2 levels in Layers III-IV of the cortex of sham and MN-Tdp-43ΔNLS mice. Data are expressed as mean ± SEM and analyzed by t-test. ****, $P < 0.0001$.

Supplemental Fig. 2



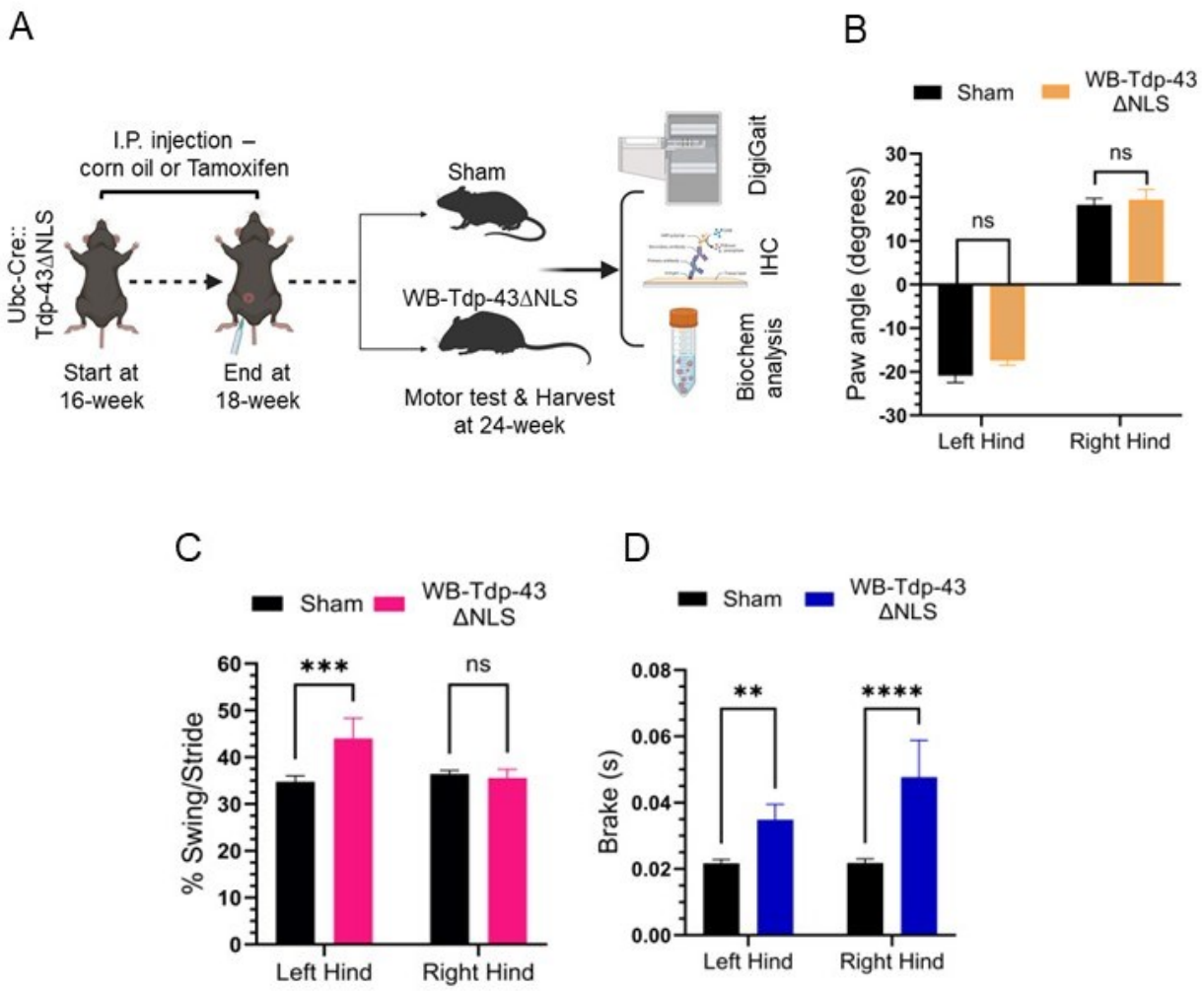
Supplemental Fig. 2. Assessment of bodyweight at different developmental stages. Quantitation of bodyweight of Mnx1-Cre::Tdp-43ΔNLS (MN-Tdp-43ΔNLS) mice**.** Data are expressed as mean ± SEM and analyzed by two-way ANOVA. N = 6 per group. ns = non-significant.

Supplemental Fig. 3



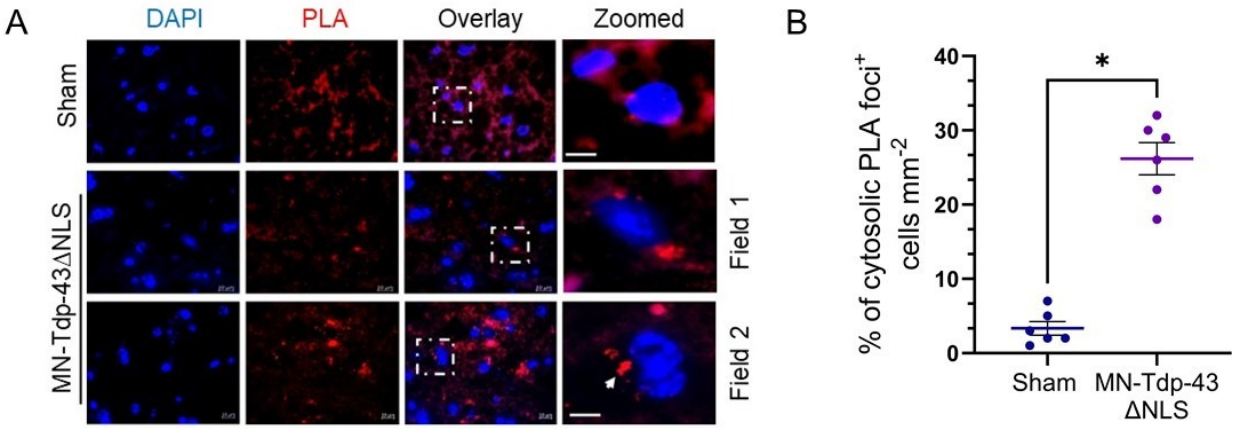
Supplemental Fig. 3 (Related to **Fig. 5**). **Motor neuron (MN)-specific Tdp-43ΔNLS expression causes muscle atrophy and gait deficits in Tdp-43 mutant mice.** (**A-B**) Dot blot analysis of Titin (N-terminal fragment) in soleus muscle tissues of MN-Tdp-43ΔNLS (N = 6; A1 – A6) and sham (N = 4; C1 – C4) mice. Gapdh levels were used as the loading controls. Anti-Titin antibody was used as the Positive (Pos) control and lysis buffer as the Negative control (Neg) (**A**). (**B**) Quantitation of normalized mouse Titin levels in two groups. Data are expressed as mean ± SD and analyzed by multiple t-tests. **, $P < 0.01$.

Supplemental Fig. 4



Supplemental Fig. 4 (Related to **Fig. 5**). **Motor function analysis in whole body (WB) Tdp-43ΔNLS expressing mice**. (**A-D**) Schematic illustration of vehicle (corn oil) in sham or tamoxifen treatment in WB-Tdp-43ΔNLS male mice by intraperitoneal (i.p.) administration at 16-week age at a dose of 75 mg/kg every other day for two consecutive weeks (18 weeks), and analysis of motor function at 24-week and harvest for histopathological and biochemical analyses (**A**). DigiGait analysis of paw angle (degrees) (**B**), percent (%) swing/stride (**C**), and brake duration (sec) (**D**). Data are expressed as mean ± SD and analyzed by two-way ANOVA. N = 6 per group. ns = non-significant; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

Supplemental Fig. 5



Supplemental Fig. 5 (Related to **Fig. 7**). **MN-Tdp-43ΔNLS mice present the cytosolic sequestration of DNA ligase 4 by Tdp-43ΔNLS.** (**A-B**) Proximity ligation assay (PLA) exhibiting pathological trapping of murine endogenous DNA ligase 4 by the cytosolic Tdp-43ΔNLS in the spinal cord tissue specimens (Fields 1 & 2), as shown by white arrowheads, compared to sham controls. Nuclei were counterstained with DAPI (**A**). Scale bars = 10 μm. (**B**) Quantitation of percent of cells with cytosolic PLA foci positivity per mm^2 of microscopic area. Data are expressed as mean ± SEM and analyzed by Wilcoxon's t-test. *, $P < 0.05$.