Characterization of Second-Hit Mouse Models for Amyotrophic Lateral Sclerosis and Parkinson's Disease Using Plasma Neurofilament-Light Chain as a Biomarker

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Introduction

Neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD) are characterized by progressive neuronal loss and overlapping pathological mechanisms. To enhance translational relevance and phenotypic robustness of transgenic animal models for these diseases, we employed second-hit paradigms in established respective transgenic mouse lines: heterozygous TAR6 for ALS, and M83 for PD. Each model was subjected to a disease-relevant secondary insult to potentiate pathology: M83 mice were injected with α-synuclein pre-formed fibrils (PFFs) to model Parkinson's, TAR6 mice were administered AAV-TDP-43 intrathecally (IT) to better model ALS.

Methods

The primary objective was to evaluate the impact of these "second-hits" on disease progression and biomarker level, with a focus on neurofilament-light chain (NF-L) levels in plasma and cerebrospinal fluid (CSF). NF-L, a sensitive marker of axonal damage, was quantified longitudinally to assess its utility in differentiating disease burden across models and interventions.

PD M83 α-synuclein (Asyn) Seeding Model: This work was performed as a collaboration project with Amyl Therapeutics. Male M83 transgenic animals received a unilateral injection into the dorsal neostriatum and the overlying somatosensory cortex of either PBS (sham) or ASyn A53T mutant pre-formed fibrils (PFFs). One additional group of sham injected wild type (WT) animals served as control.

NF-L was assessed with a commercial immunosorbent assay from Uman Diagnostics, in terminal CSF and plasma taken 12 weeks after injection. Quantitative immunoflourescent analysis of phosphorylated ASyn (pSer129-Asyn) and pFTAA, a marker for disease-associated protein aggregates was performed in fixed brains focusing on caudate putamen (CPu) and substantia nigra (SN).

ALS AAV-TDP43 Model: Heterozygous TDP43 mice (mixed sex) as well as, sex-matched non-transgenic littermates at an age of 10 weeks were used. AAV9-hTDP-43, AAV9-empty or sterile PBS as control were intrathecally injected into lumbar region at the L5-L6 intervertebral space. 6 or 12 weeks after injection, blood, CSF and tissues were collected. NF-L was assessed with a commercial immunosorbent assay from Uman Diagnostics in body fluids. Brain tissue was subjected to mRNA expression analysis assessing TDP43 via qPCR.

To gain additional insight into behavioral data of this new ALS model please visit Roland Rabl's poster "Characterization of an Inducible Intrathecal TDP43-AAV Amyotrophic Lateral Sclerosis Model" on Monday, Nov. 17, 1:00 PM – 5:00 PM (poster #212).

Conclusion

Our findings demonstrate that second-hit paradigms significantly amplify neurodegenerative phenotypes. In both presented models, the addition of the second hit led to highly increased NF-L level in both plasma and CSF, indicating severe axonal degeneration in addition to the basic phenotype.

In the M83 α-synuclein seeding model we also observed that major PD hallmarks, including pSer129-Asyn, as well as intracellular aggregates shown as pFTAA staining, were significantly enhanced upon PFF seeding. This comparative approach underscores the value of combinatorial modelling in preclinical research and highlights NF-L as a cross-disease biomarker for neurodegeneration.

PD: M83 α-Synuclein Seeding Model

Neurofilament-Light Chain

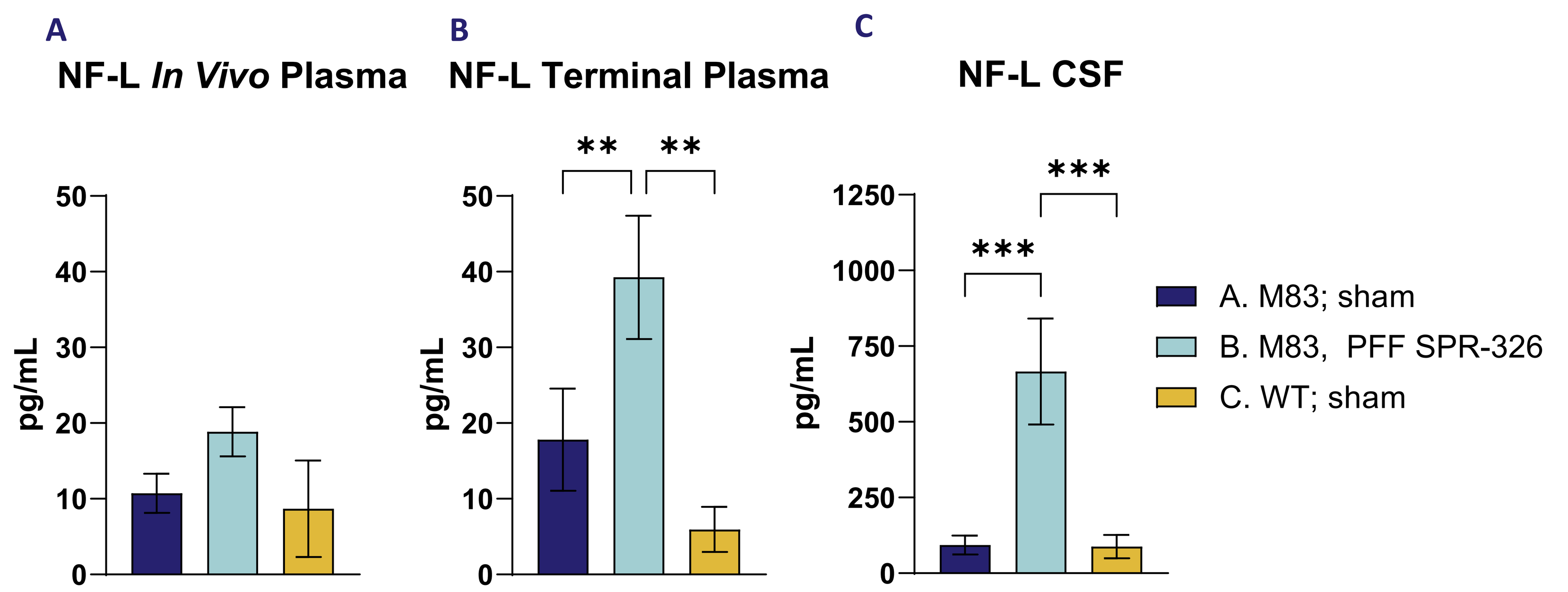

Figure 1: Analysis of neurofilament-light chain (NF-L) levels in plasma and terminal CSF samples of PFF or sham injected M83 mice and wild type (WT) control. NF-L levels were quantified by ELISA in *in vivo* plasma collected 6 weeks post-injection (**A**) as well as in terminal plasma (**B**) and CSF samples collected at 12 weeks post injection (**C**). Mean ± SEM. One-way ANOVA followed by Bonferroni's *post hoc* test. n = 6-16 per group and time point for plasma analysis, n = 4 for group A and C and n = 10 for group B. **p <0.01, ***p <0.001.

Histological Analysis

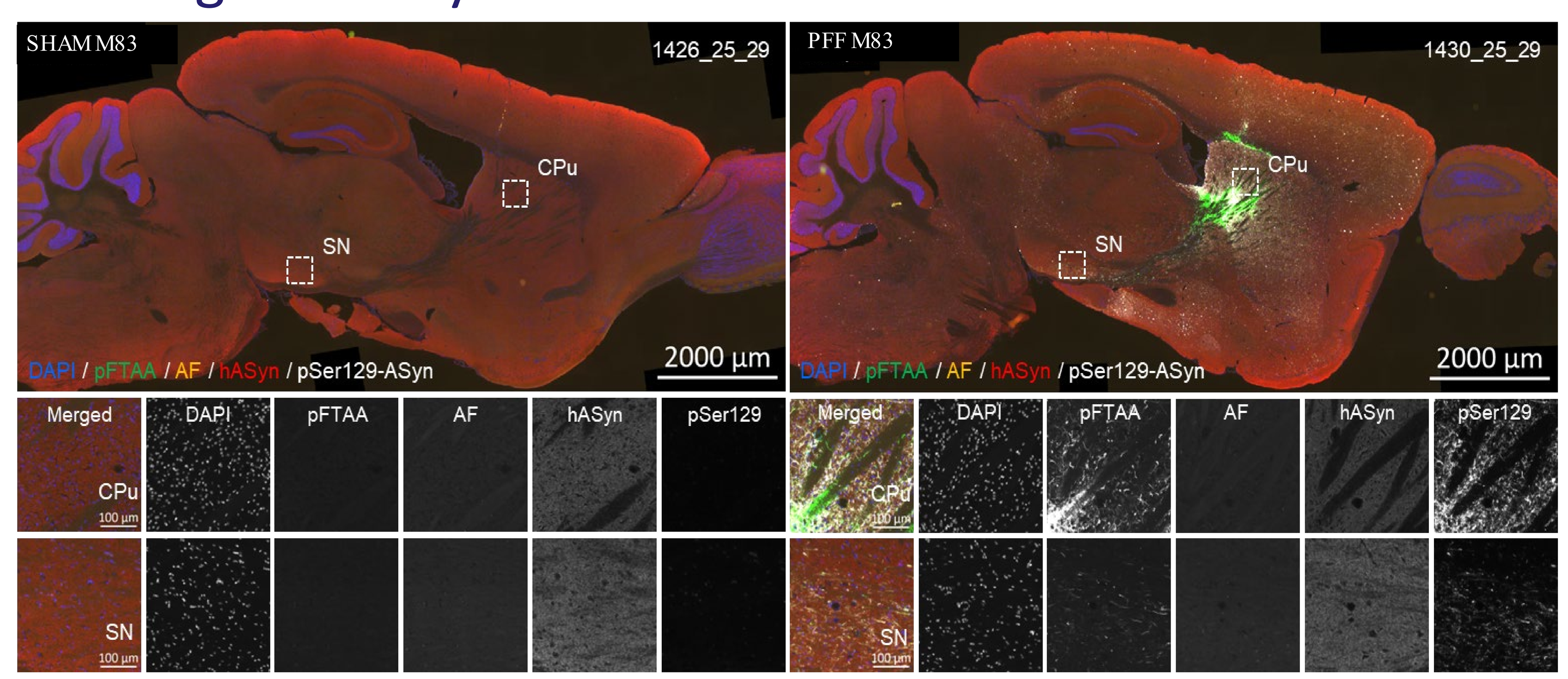

Figure 2: Examples of immunofluorescent labeling of hASyn, pSer192-ASyn and pFTAA. Images show representative labeling on sagittal sections; nuclei are labeled with DAPI. Single channel magnifications show labeling in the caudate putamen (CPu) and substantia nigra (SN); images were taken at the position indicated by the rectangle.

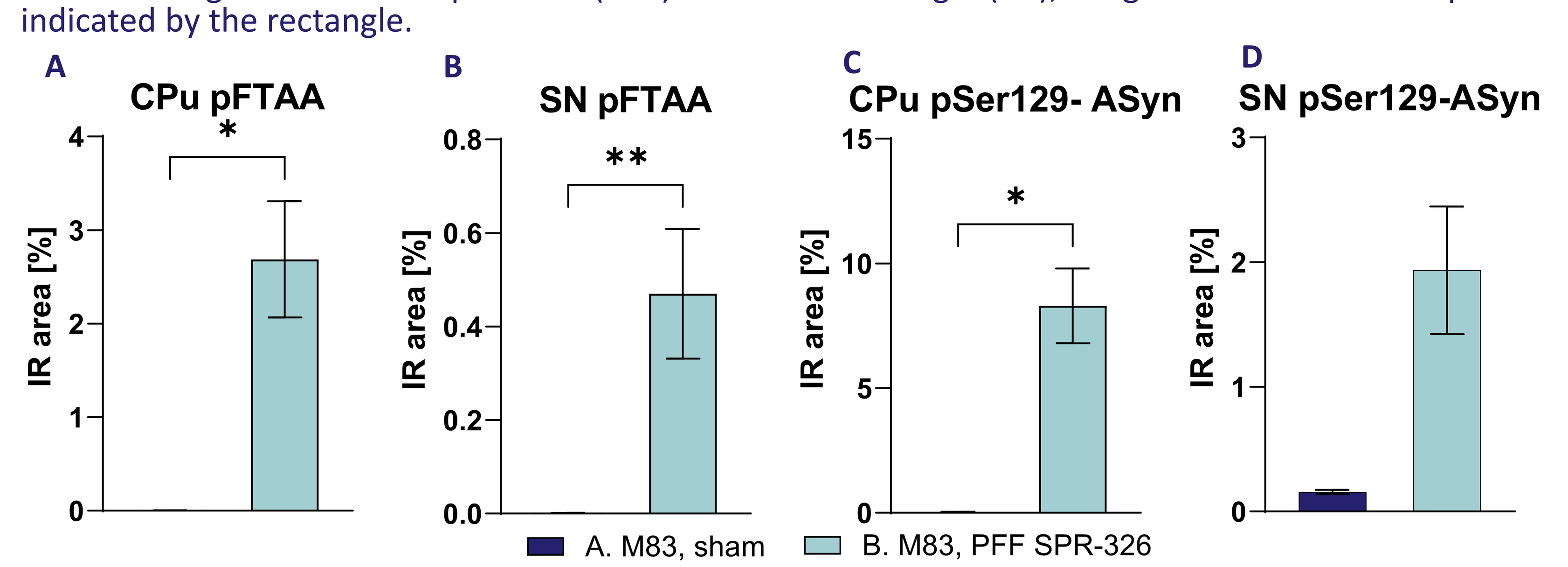

Figure 3: Quantitative immunofluorescent analysis. 12 weeks post-injection brains were fixed, ipsilateral hemispheres were cut sagittally, and sections were subjected to immunofluorescent labeling. Fluorescent area of pFTAA staining (**A, B**), as well as ASyn (pSer129-Asyn) (**C, D**), immunofluorescent area in caudate putamen (CPu) (**A, C**) and substantia nigra (SN) (**B, D**). Graphs present the means of immunofluorescent signal on five brain sections per mouse (A: n = 3; B: n = 9). Mean ± SEM; Unpaired two-tailed T-Test. *p <0.05, **p <0.01.

ALS: Inducible Intrathecal TDP43-AAV Model

Neurofilament-Light Chain

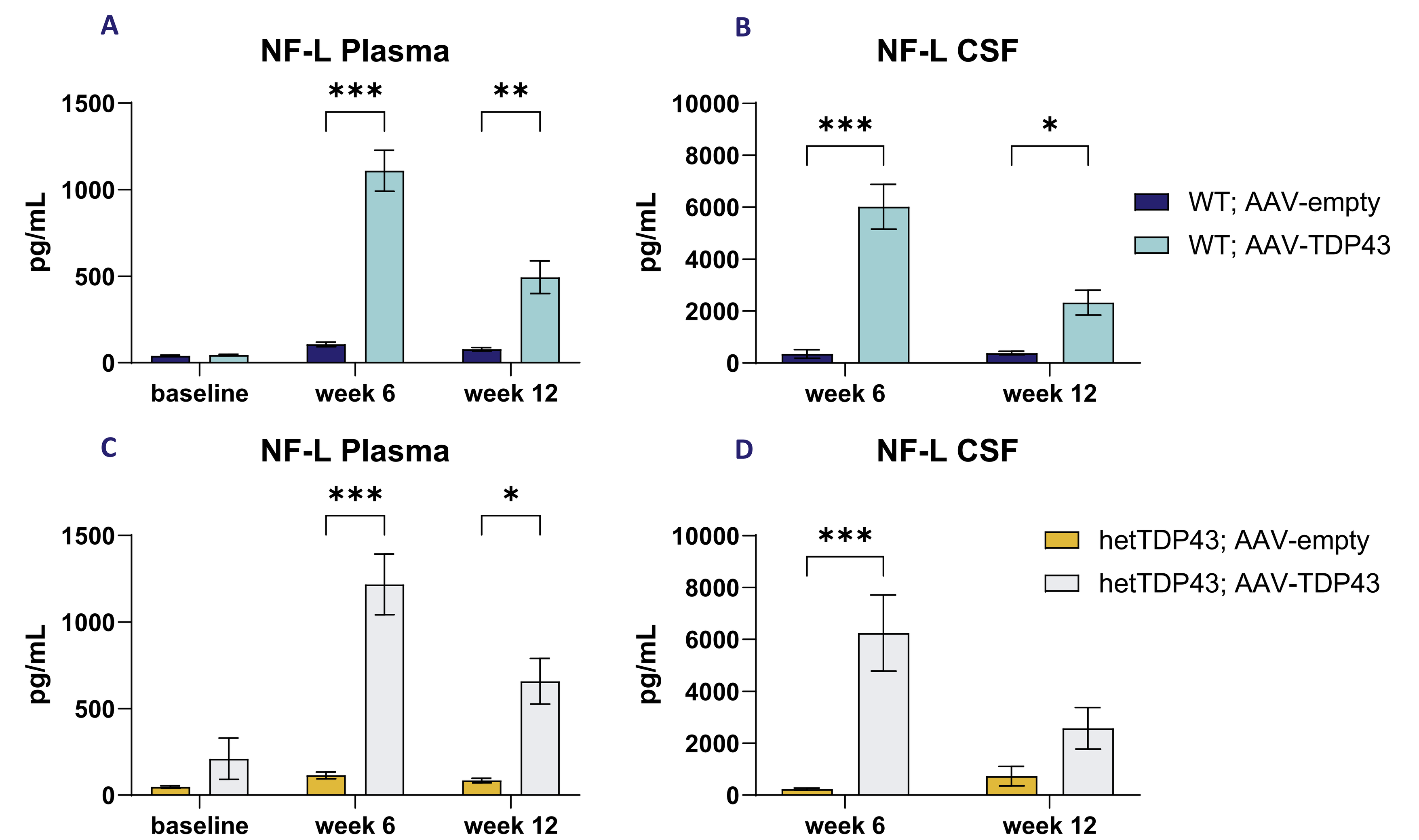

Figure 4: Analysis of neurofilament-light chain (NF-L) levels in plasma and terminal CSF samples in IT AAV9-hTDP43 injected mice. NF-L levels were quantified by ELISA in *in vivo* plasma collected at baseline, 6 and 12 weeks post-injection (**A, C**) as well as in terminal CSF samples collected at 6 and 12 weeks post-injection (**B, D**). Mean ± SEM. Two-way ANOVA followed by Bonferroni's *post-hoc* test. n = 6-16 per group and time point for plasma analysis, n = 6-8 per group for CSF analysis. *p <0.05, **p <0.01, ***p <0.001.

mRNA Expression Analysis

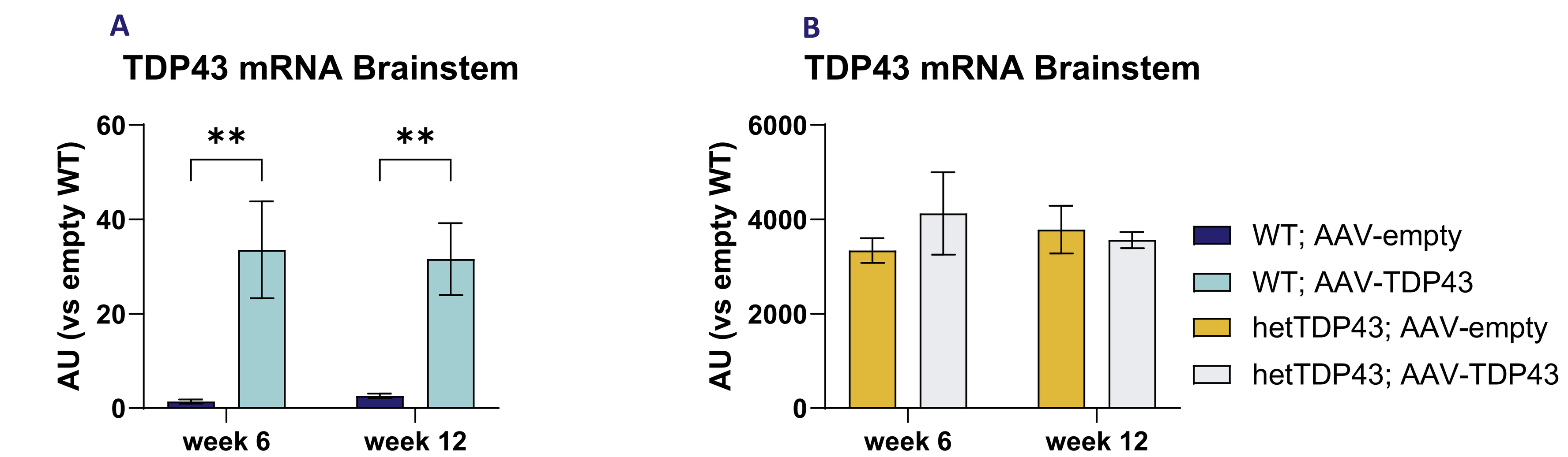

Figure 5: Analysis of hTDP43 mRNA expression level in brainstem of IT AAV9-hTDP43 injected mice. mRNA expression, given as relative expression versus empty vector injected wild type (WT) mice, was analyzed in brainstem of wild type (**A**) and heterozygous hTDP43 mice (**B**), collected at 6 and 12 weeks post-injection. Mean ± SEM. Two-way ANOVA followed by Bonferroni's *post hoc* test. n = 6 per group. **p <0.01.

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For more information about the models please visit: www.scantox.com

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