

Evaluating U7 small nuclear RNA splice-switching therapeutics to correct Tdp-43 mediated cryptic splicing of *Stmn2* in vivo

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ABSTRACT

BACKGROUND

TDP-43 proteinopathies, including Alzheimer's Disease (AD) and Frontotemporal lobar degeneration (FTD), are characterized by the mislocalization of the normally nuclear TAR DNA binding protein (TDP-43) to the cytoplasm where it is incorporated into inclusions in affected brain regions. TDP-43 plays a crucial role in the suppression of cryptic exons in the mature RNA of many targets. One such target, Stathmin-2 (STMN2), is misspliced in the absence of functional TDP-43 leading to a reduction of STMN2 RNA and protein while the levels of cryptic splice variants are increased. STMN2 has emerged as a potential pathological driver of disease and is also promising therapeutic candidate. Cryptic exons are not conserved across species, meaning previous Tdp-43 models did not capture *Stmn2* loss of function (LOF), thus hampering our ability to study mechanisms and test therapeutics aimed at correcting Tdp-43 dependent *Stmn2* processing in vivo.

METHODS

To address this, we have depleted Tdp-43 by expressing short-hairpin RNA via adeno associated virus in the central nervous system of wild-type mice and a recently described humanized *Stmn2* (hStmn2) mouse model.

RESULTS

hStmn2 mice expressing shRNA against *Tardbp* have a reduction of *Tardbp* RNA and Tdp-43 protein, in the brain, compared to mice expressing a non-targeting control shRNA (shCTRL). Tdp-43 depletion is accompanied by cryptic splicing of endogenous mouse splicing targets, and as expected, a significant elevation of cryptic *Stmn2* RNA. We observed a significant increase in premature death in hStmn2^{+/+} mice expressing shTardbp compared to those expressing shCtrl. At 3-months of age shTardbp expressing hStmn2^{+/+} mice performed similarly to control animals on tasks assessing cognitive function, but had significant motor deficits, as judged by grip strength and rotarod. We also tested the ability of U7 small nuclear RNA (snRNA) splice-switching therapeutics to correct *Stmn2* splicing and observed a significant reduction of cryptic *Stmn2* splicing in mice treated with U7 snRNA targeting the *Stmn2* cryptic exon compared to control U7s.

CONCLUSIONS

We find that Tdp-43 dependent processing of *Stmn2* exacerbates premature death and motor deficits in conditions of Tdp-43 depletion. Further, we demonstrate target engagement of *Stmn2* U7 snRNA in this novel humanized *Stmn2* mouse model.

RESULTS

FIGURE 1: Tdp-43 depletion via shRNA in humanized (hStmn2^{+/+}) mice induces accumulation of cryptic *Stmn2* RNA.

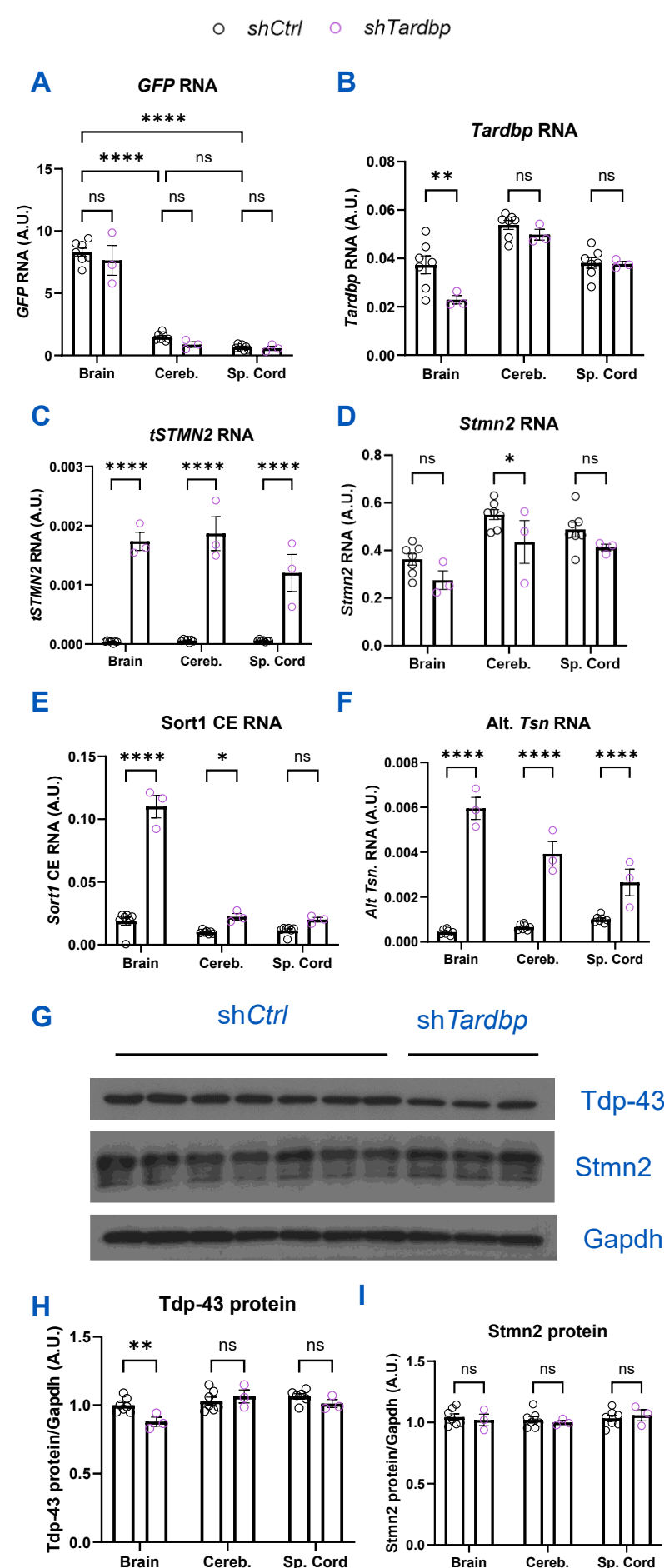


Fig 1. A-I) Data from 1-month-old hStmn2^{+/+} mice ICV injected with shRNA targeting *Tardbp* (shTardbp, n=3) or non-targeting control (shCtrl, n=7). **A-F)** RT-qPCR analysis from injected mice. Representative western blot **(G)** of the forebrain and quantification of Tdp-43 **(H)**, and Stmn2 protein **(I)**. Data are presented as mean ± SEM. *p ≤ 0.05, ** p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, and non-significant (ns), as analyzed by 2-way ANOVA.

FIGURE 2: Tdp-43 depletion via shRNA in hStmn2^{+/+} mice exacerbates premature lethality and motor deficits

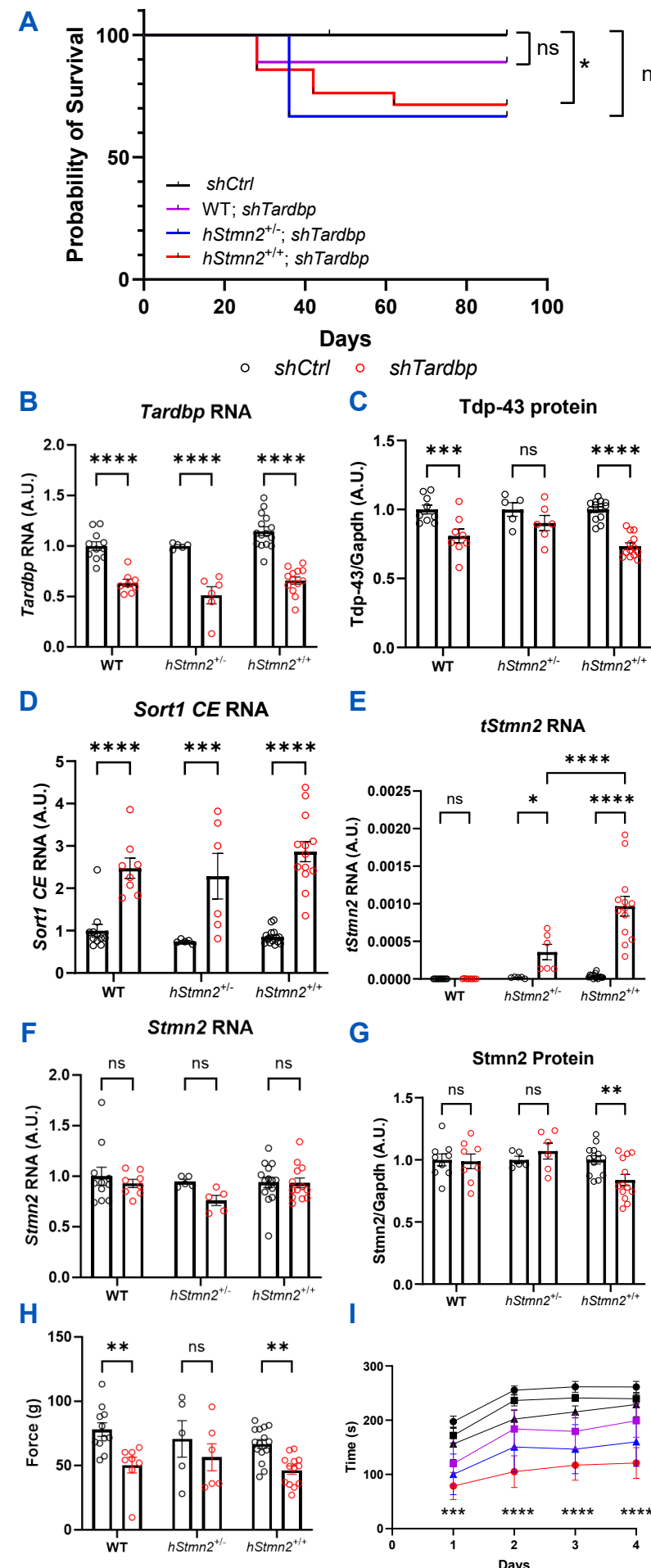


Fig 2. A) Survival curve of WT (n=8-11), hStmn2^{+/+} (n=5-6), and hStmn2^{+/+} (n=13-15) injected with shCtrl or shTardbp aged to 3-months. Levels of *Tardbp* RNA **(B)** and Tdp-43 protein **(C)** from injected mice. **D-F)** RT-qPCR analysis from injected mice. Results of grip strength **(G)** rotarod day 4 **(H)** from all groups. Data are presented as mean ± SEM. *p ≤ 0.05, ** p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, and non-significant (ns), as analyze by Mantel-Cox survival analysis **(A)** or 2-way ANOVA **(B-I)**.

FIGURE 3: Stmn2 U7 small RNA rescues premature lethality and restores STMN2 splicing

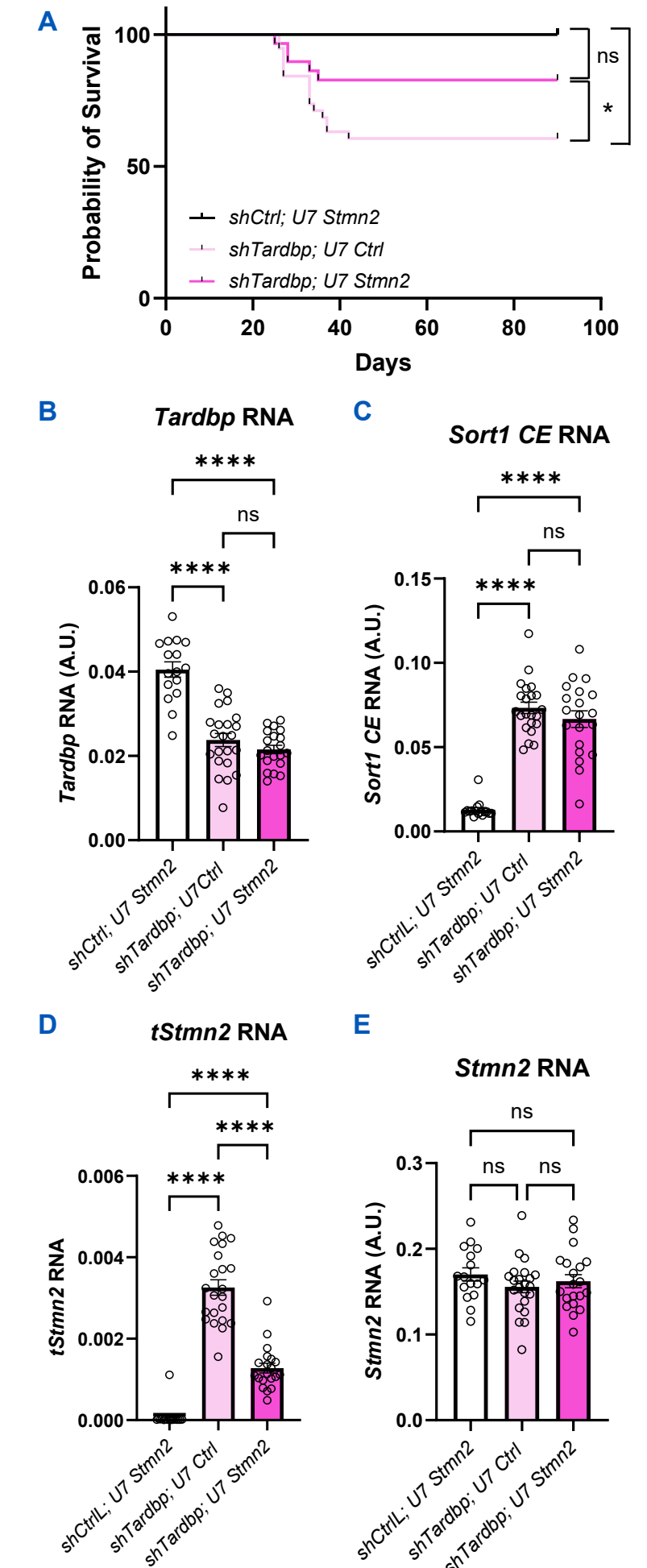


Fig 3. A) Survival curve of hStmn2^{+/+} injected with shCtrl; U7 Stmn2 (n=19), shTardbp; U7 Ctrl (n=23), and shTardbp; U7 Ctrl (n=23) aged to 3-months old. **B-E)** RT-qPCR analysis from injected mice. Data are presented as mean ± SEM. *p ≤ 0.05, ** p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, and non-significant (ns), as analyze by Mantel-Cox survival analysis **(A)**, or 2-way ANOVA **(B-E)**.