

vMiX™, an Innovative AAV-Based RNA Interference Platform: First Applications to Treat ALS

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OBJECTIVE

To design and test a novel adeno-associated viral (AAV) vector gene silencing platform that could be applied to a wide range of genes implicated in neurodegenerative diseases, including those that cause amyotrophic lateral sclerosis (ALS).

- We sought to demonstrate proof-of-concept of the vMiX™ platform's ability to target *ATXN2* and *SOD1* to support continued development towards clinical application.

METHODS

In vitro development of vMiX™ vector (Figure 2)

- Using luciferase reporter assays to assess mRNA knockdown levels, the vMiX™ vector efficiency was compared with that of commercially-available miR-155 miRNA constructs with identical guides.
- Using protein analysis, vMiX™ vector specificity was assessed by comparing knockdown levels against wild-type and codon-optimized mRNA coding for the same protein (three mutations outside the seed sequence).

Performance of vMiX™ vector *in vitro* (Figure 3)

- A schematic of the screening strategy from *in silico* design to *in vitro* transduction is shown in Figure 3A.
- Many target sequences were selected *in silico*, converted into miRNA sequences, and included in the vMiX™ hybrid hairpin for cloning.
- The vMiX™ plasmids were transfected into mammalian cells and their activity was tested against a luciferase reporter gene.
- The most promising candidates were then tested against the target's endogenous mRNA.
- vMiX™ AAVs expressing the best candidates were transduced into mammalian cells and their activity against the target's endogenous mRNA was assessed.

Performance of vMiX™ vector *in vivo* (Figure 4)

- ATXN2* and *SOD1* mRNA knockdown was assessed 6–7 weeks after injection in two separate *in vivo* studies.
- Neonatal mice expressing the human *ATXN2* transgene with 72 polyglutamine repeats (hBACQ72) received intracerebroventricular injections of a vMiX™ AAV9-miR-*ATXN2* in a dose-range study.
- Neonatal mice expressing the human mutated *G93A SOD1* received intracerebroventricular injections of a vMiX™ AAV9-miR-*SOD1*.

RESULTS

In vitro development of vMiX™ vector (Figure 2)

- The vMiX™ vector achieved greater levels of luciferase knockdown compared with miR-155 miRNA constructs: 80% versus 30–75%, respectively (Figure 2A).
- Protein analysis revealed the vMiX™ vector achieved an 81% knockdown of a wild-type target and had no activity against the codon-optimized mRNA coding for the same protein (Figure 2B).

Figure 2: *In vitro* development of vMiX™ vector

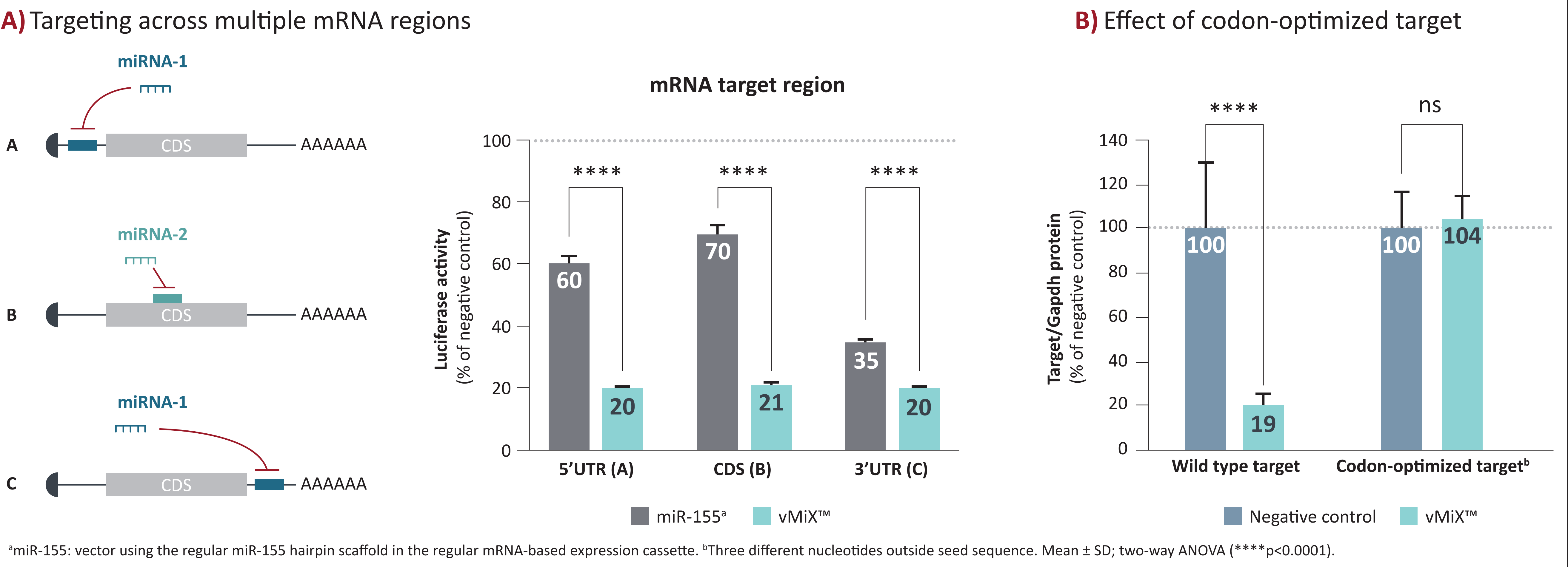
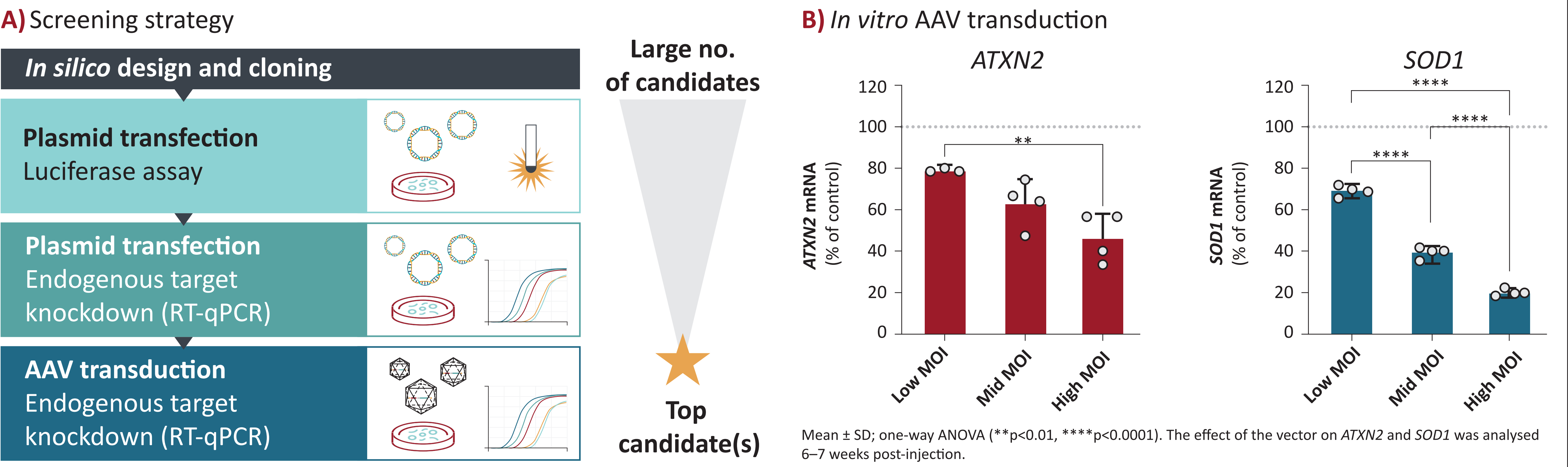


Figure 3: *In vitro* application of vMiX™ to knockdown two ALS targets, *ATXN2* and *SOD1*



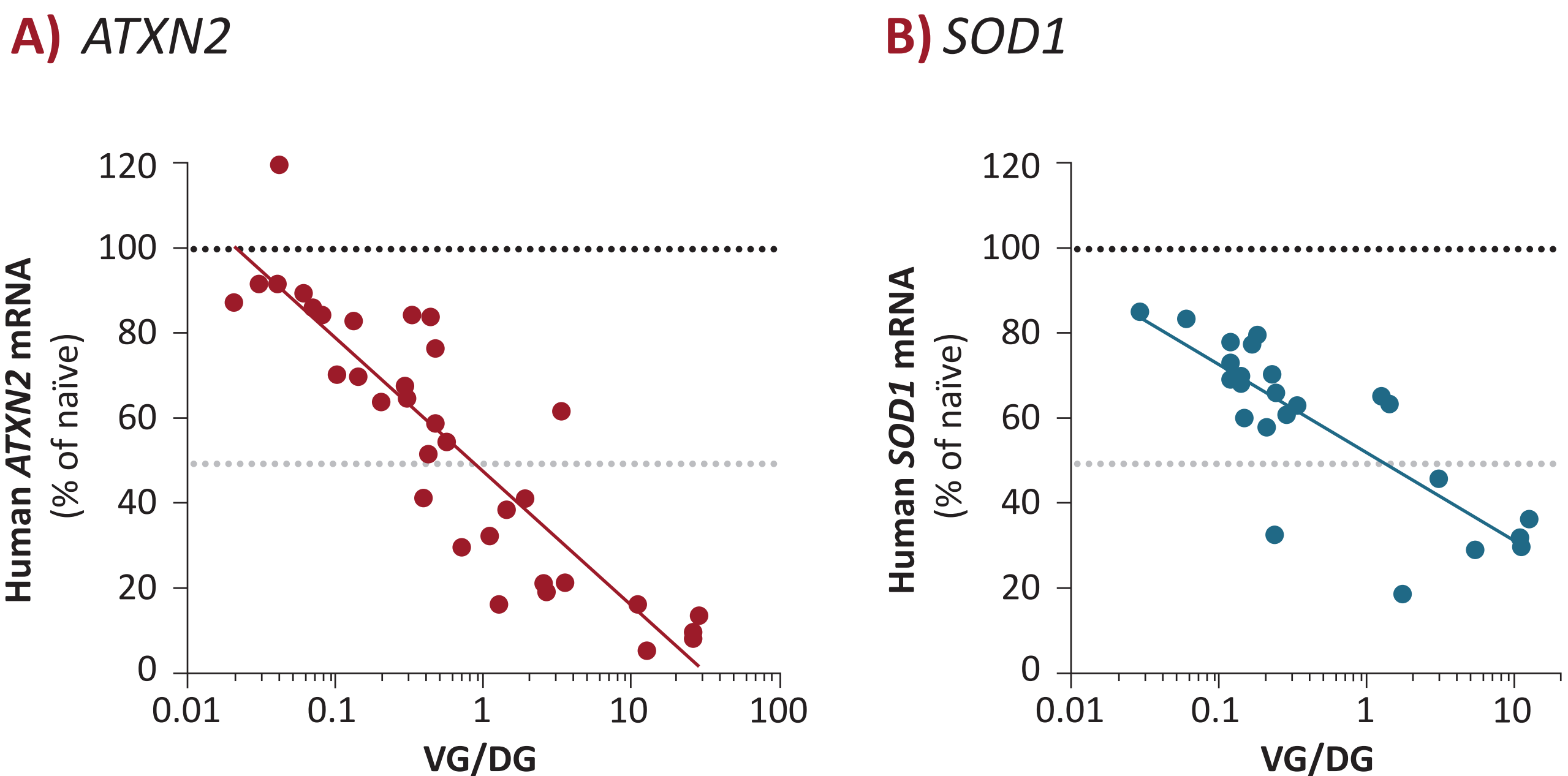
Performance of vMiX™ vector *in vitro* (Figure 3)

- The best performing candidate yielded dose-dependent knockdown of *ATXN2* and *SOD1* *in vitro*, with a maximum knockdown of 54% and 79%, respectively (Figure 3B).

Performance of vMiX™ vector *in vivo* (Figure 4)

- AAV9-miR-*ATXN2* demonstrated a dose-dependent response in human *ATXN2* mRNA knockdown mice (Figure 4A).
 - A maximum knockdown of 89.1% was reached with 10–30 viral genome/diploid genome (VG/DG).
- AAV9-miR-*SOD1* demonstrated a dose-dependent response in human *SOD1* mRNA knockdown mice, with the decrease dependent on the level of AAV transduction across the cortex and spinal cord (Figure 4B).
- The two vMiX™ vectors performed the same way with an EC50 of approx. 1 VG/DG.

Figure 4: *In vivo* confirmation of vMiX™ knockdown activity with *ATXN2* and *SOD1*



CONCLUSIONS

- For the first time, we show an RNA of interest (a miRNA here) can be expressed outside the canonical promoter-transgene-termination-signal expression cassette.
- The design of the vMiX™ vector allowed early decoupling of mRNA and miRNA sequences to avoid competition between the two pathways.
- The hybrid hairpin improved the essential step of miRNA processing, allowing a high fidelity of the predicted guide sequence and a good guide-to-passenger ratio (see Poster 1222 for more information).
- The vMiX™ vector's activity was confirmed *in vitro* and *in vivo* against two different targets (*ATXN2* and *SOD1*) involved in ALS, and showed potential for treating ALS.

REFERENCES: ¹Meijboom KE. and Brown RH. Neurotherapeutics. 2022;19:1159–79.
ABBREVIATIONS: AAV: adeno-associated viral; ALS: amyotrophic lateral sclerosis;
ANOVA: analysis of variance; *ATXN2*: Ataxin-2; CDS: coding sequence; mRNA: messenger

RNA; miRNA: microRNA; MOI: multiplicity of infection; NS: non-significant; RT-qPCR: reverse transcription-quantitative polymerase chain reaction; SD: standard deviation; *SOD1*: superoxide dismutase 1; UTR: untranslated region; VG/DG: viral genome/diploid genome.

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