vMiX™: A novel AAV-based RNA interference platform demonstrating consistent gene silencing efficacy in mouse studies

AVIADOBIO

vMiX™

P0075

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OBJECTIVE

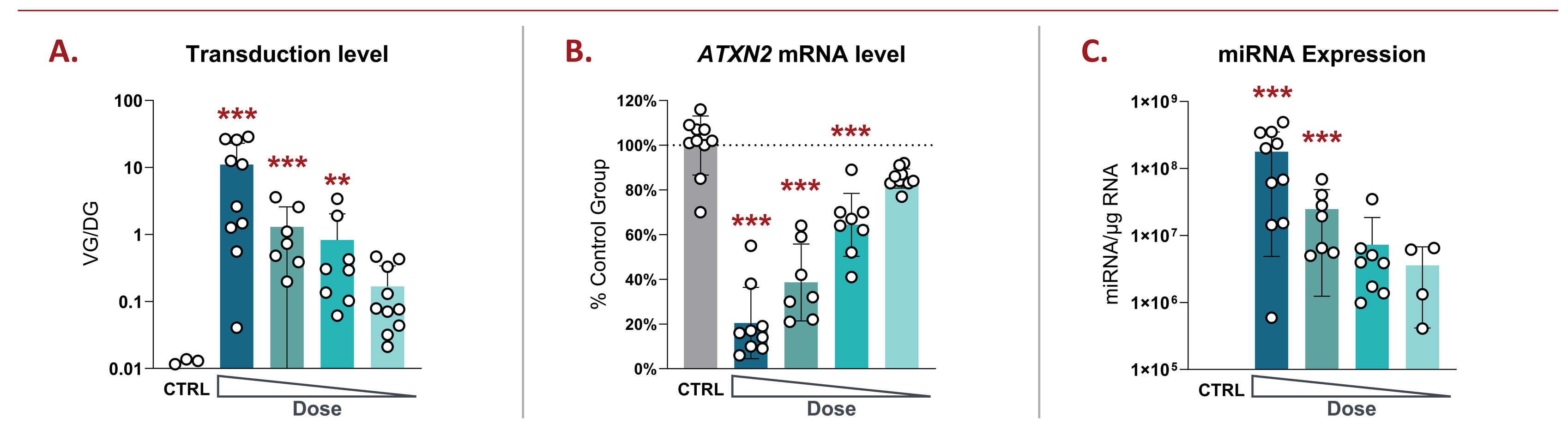
To assess the efficacy, dose-response relationships, and processing fidelity of the AAV vMiX™ platform targeting *ATXN2/Atxn2* across transgenic and wild-type mouse models relevant to ALS and FTD.

Introduction

- ALS and FTD are devastating neurodegenerative diseases characterised by progressive motor neuron loss and cognitive decline, respectively. Both conditions involve aberrant protein aggregation, with TDP-43 proteinopathy being a hallmark pathological feature. Multiple lines of evidence implicate Ataxin-2 (ATXN2) in promoting TDP-43 aggregation, making it an attractive therapeutic target.
- Reducing ATXN2 expression represents a promising therapeutic strategy that could address both the toxic gain-of-function and the pathological interaction with TDP-43.
- Current gene silencing approaches require repeated administrations, creating patient burden and compliance challenges. Using vMiX™, a novel AAV-based RNA interference platform utilising miRNA for gene silencing (see **Poster P0169**), we aimed to develop a single-administration therapeutic approach for *ATXN2* knockdown, evaluating dose-dependent efficacy and reproducibility across multiple models *in vivo*.

In vivo performance of vMiX™ targeting *ATXN2/Atxn2* in four mouse models

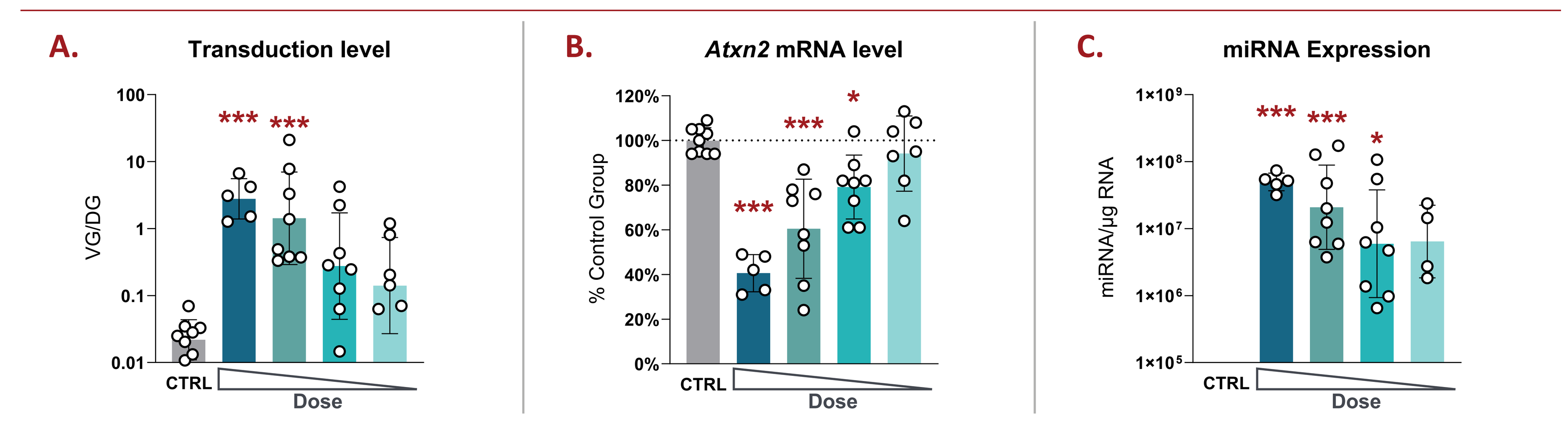
Figure 1: Human *ATXN2* knockdown in BAC-Q72 mice



Dose-dependent vector transduction up to 11.1 VG/DG **(A)** correlates with miRNA expression reaching 1.8x10⁸ copies/µg RNA **(C)**. *ATXN2* mRNA knockdown **(B)** achieves up to 79.6% reduction at highest dose (mean values), demonstrating effective dose-response relationship across all measured parameters.

Mice (N=7-10) were injected at P1 by ICV and cortices were harvested after 8 weeks. Statistical analysis: Krustal-Wallis (A, C) and 1way-ANOVA (B); ** P<0.01, *** P<0.001 vs control group).

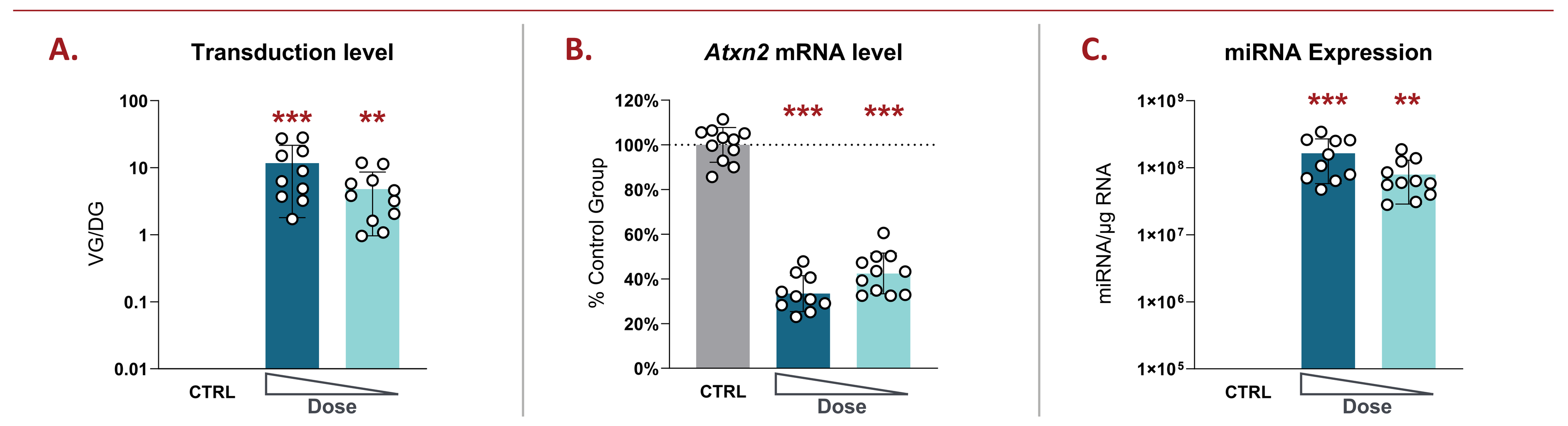
Figure 2: Mouse *Atxn2* knockdown in C57Bl/6J mice



Progressive vector transduction up to 4.4 VG/DG **(A)** drives miRNA expression levels to 5.2x10⁷ copies/µg RNA **(C)**. *Atxn2* mRNA silencing **(B)** reaches 59.4% at higher doses (mean values), confirming robust platform performance in wild-type background with clear dose-dependent efficacy.

Mice (N=5-9) were injected at P1 by ICV and cortices were harvested after 8 weeks. Statistical analysis: Krustal-Wallis (A, C) and 1way-ANOVA (B); * P<0.05, ** P<0.01, *** P<0.001 vs control group).

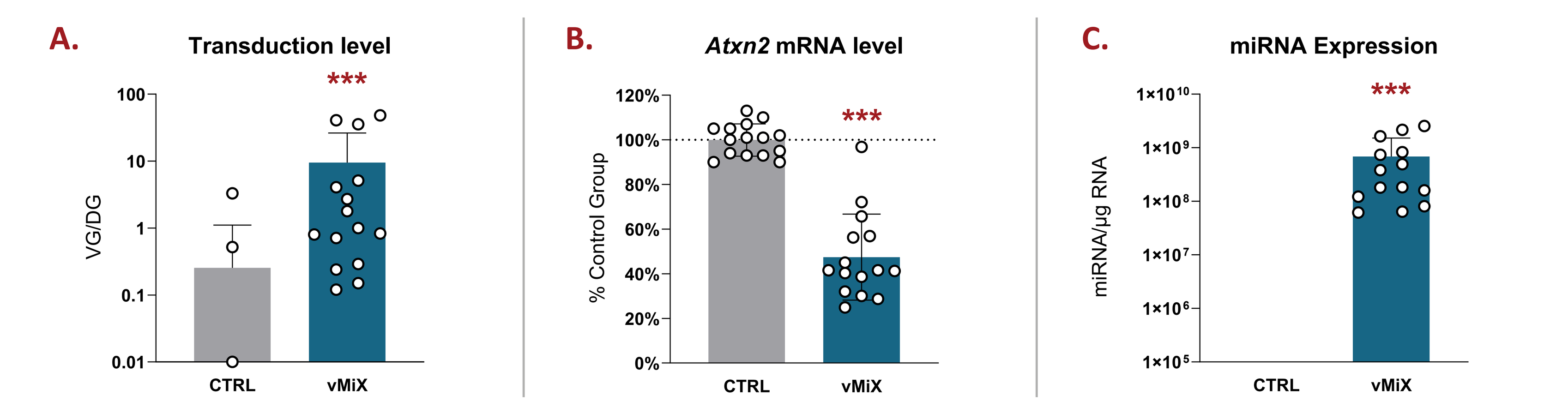
Figure 3: Mouse *Atxn2* knockdown in TDP-43 rNLS8 mice



High dose achieves 11.7 VG/DG transduction **(A)** with miRNA expression 1.6x10⁸ copies/µg RNA **(C)**. *Atxn2* mRNA knockdown **(B)** reaches 66.6% for both doses after 11 weeks (mean values), demonstrating sustained platform efficacy in this ALS/FTD model.

Mice (N=10-11) were injected at P1 by ICV and cortices were harvested after 11 weeks. Statistical analysis: Krustal-Wallis (A, C) and 1way-ANOVA (B); ** P<0.01, *** P<0.001 vs control group).

Figure 4: Mouse *Atxn2* knockdown in $TDP43^{WTxQ331K}$ mice

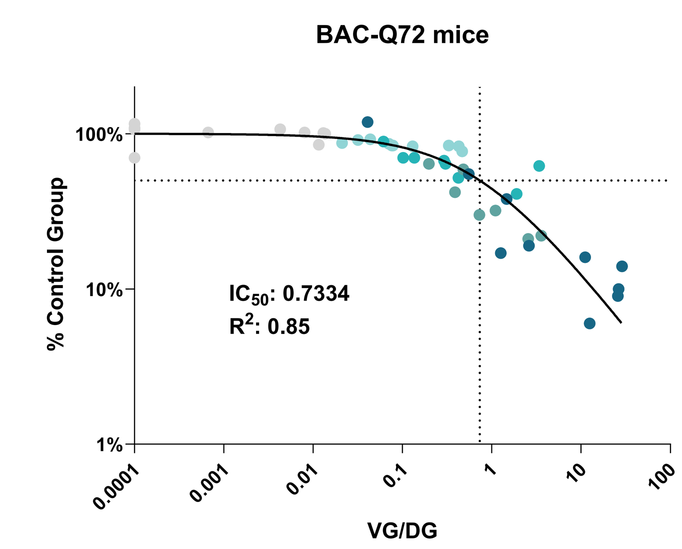


Single-dose treatment produces 9.5 VG/DG transduction **(A)** and 6.9x10⁸ miRNA copies/µg RNA expression **(C)**. *Atxn2* mRNA levels **(B)** show 52.6% reduction after 18 weeks (mean values), confirming therapeutic potential with maintained long-term efficacy in this ALS/FTD model.

Mice (N=15) were injected at P1 by ICV and cortices were harvested after 18 weeks. Statistical analysis: Mann-Whitney (A, C) and unpaired T-test (B); *** P<0.001 vs control group).

Dose Response: IC_{50} curves for *ATXN2/Atxn2* achieved with vMiX™

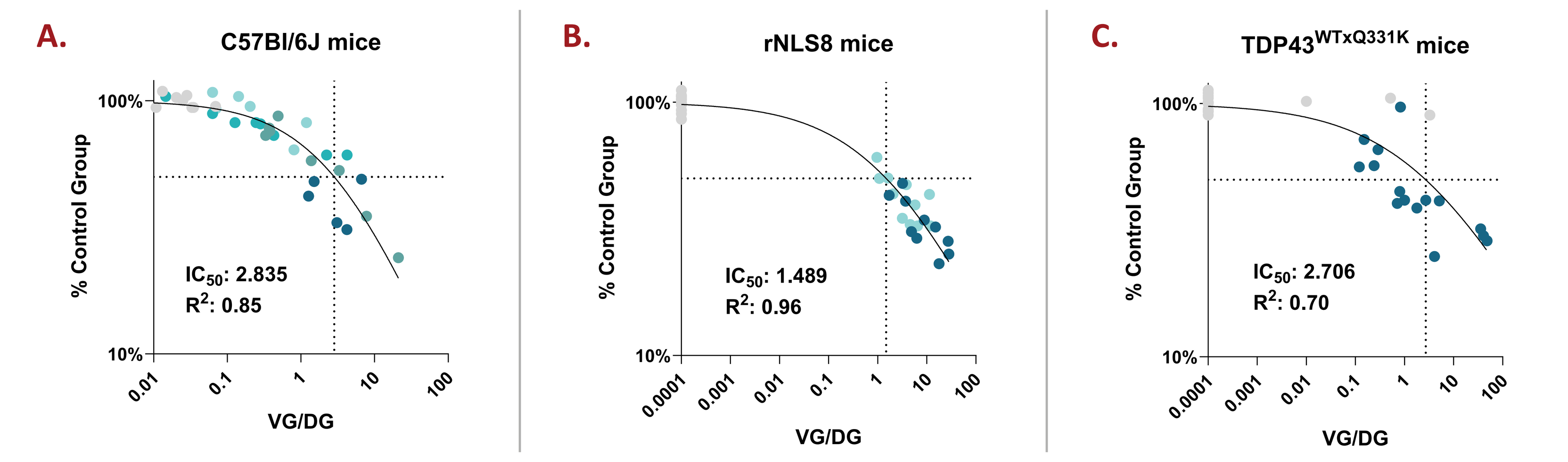
Figure 5: Human *ATXN2* dose-response relationship in BAC-Q72 mice



- IC_{50} of 0.73 VG/DG establishes the minimal vector dose required for 50% *ATXN2* knockdown in BAC-Q72 mice, providing a critical benchmark for therapeutic dosing strategies.
- Strong correlation (R^2 = 0.85) across four orders of magnitude transduction levels demonstrates highly predictable dose-response relationship and robust platform performance.

Mice were injected at P1 by ICV and samples harvested after 8 weeks (Figure 1).

Figure 6: Mouse *Atxn2* dose-response comparison across models

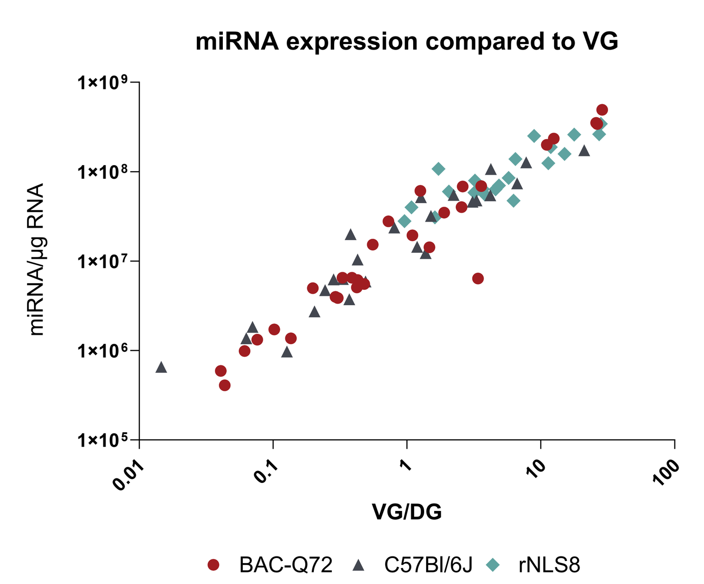


Consistent IC_{50} values across mouse models (1.49-2.84 VG/DG) demonstrate platform robustness. The correlations (R^2 > 0.70) confirm reproducible dose-response relationships independent of genetic background or disease state. Notably, targeting the endogenous mouse *Atxn2* gene requires slightly higher doses compared to human *ATXN2* knockdown (Figure 5), indicating species-specific dosing considerations.

Mice were injected at P1 by ICV and samples harvested after 8, 11, and 18 weeks (Figures 2, 3, 4).

Vector transduction and miRNA expression relationship

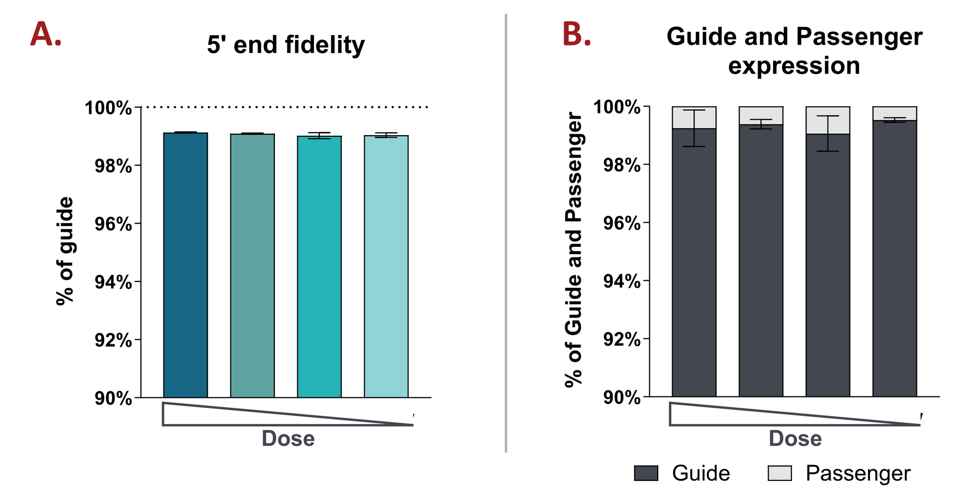
Figure 7: Correlation between vector transduction and miRNA expression levels



- Strong linear correlation between vector transduction levels (0.01 to 30 VG/DG) and miRNA expression ($5.0x10^5$ to $5.0x10^8$ copies/µg RNA) from multi-dose studies (Figures 1-3).
- Consistent relationship across BAC-Q72, C57Bl/6J, and rNLS8 models demonstrates reliable vector processing independent of genetic background.
- Data confirms efficient miRNA processing from delivered vectors, with no evidence of saturation effects across the tested dose range.

vMiX™ miRNA processing quality and guide strand fidelity

Figure 8: vMiX™ miRNA processing fidelity and guide strand selection



- vMiX™ miRNAs maintain >99% 5' end fidelity across all doses (Figure 1), ensuring precise target recognition. This high fidelity is critical for therapeutic specificity and minimizing off-target effects.
- Guide strand selection exceeds 99% efficiency with minimal passenger strand retention. This optimal processing ensures maximal therapeutic activity while reducing potential off-target effects from passenger strand loading.

CONCLUSIONS

- **vMiX™ achieved consistent *ATXN2/Atxn2* knockdown across four mouse models with well-characterized dose-response relationships, validating platform efficacy in both disease-relevant and wild-type backgrounds.**
- **High miRNA processing quality (>99% 5' end fidelity and guide selection) combined with predictable vector-to-expression correlations demonstrate the platform's therapeutic precision and reliability.**
- **These findings establish vMiX™ as a promising gene silencing platform with quantifiable performance metrics suitable for clinical translation and broader neurotherapeutic applications.**

ABBREVIATIONS: AAV: adeno-associated virus; **ALS:** amyotrophic lateral sclerosis; **ANOVA:** analysis of variance; **CTRL:** control group; **FTD:** Frontotemporal dementia; **ICV:** Intracerebroventricular Injection; **mRNA:** messenger RNA; **miRNA:** microRNA; **VG/DG:** viral genome per diploid genome.

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