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SPLICING MODULATION OF DMPK EXON 15 AS A THERAPEUTIC APPROACH FOR MYOTONIC DYSTROPHY TYPE 1

ENTRY DM
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INTRODUCTION

Antisense oligonucleotides (ASOs) are among the most explored therapeutic approaches for DM1. Different ASO chemistries, including phosphorothioate (PS), 2'O-methyl, 2-methoxyethyl (MOE), phosphorodiamidate morpholino oligomer (PMO) and locked nucleic acid (LNA) modifications, have been developed to improve stability, affinity and cellular uptake. Depending on their design, ASOs can either induce RNA degradation or modulate RNA processing. While several approaches have shown promising results, strategies based on RNA degradation may also affect normal DMPK expression. Therefore, more specific approaches that selectively remove the toxic CUG-expanded region while preserving the transcript are still needed.

PROJECT RATIONALE

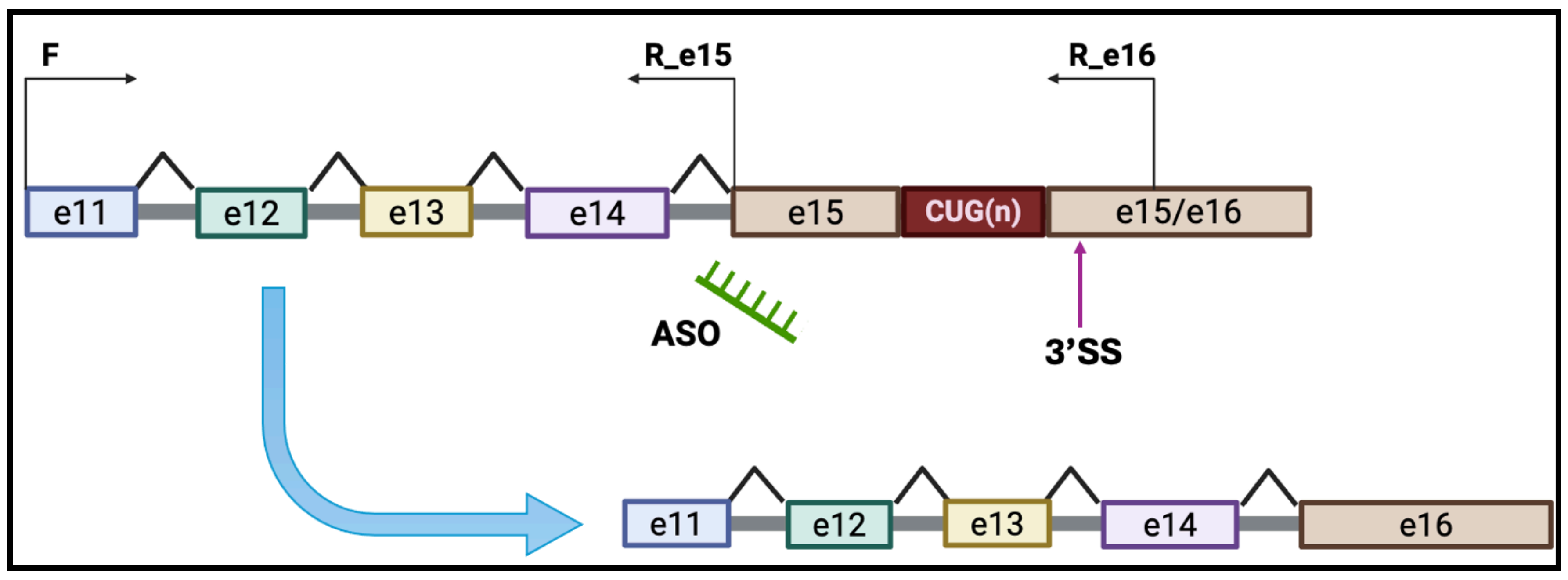


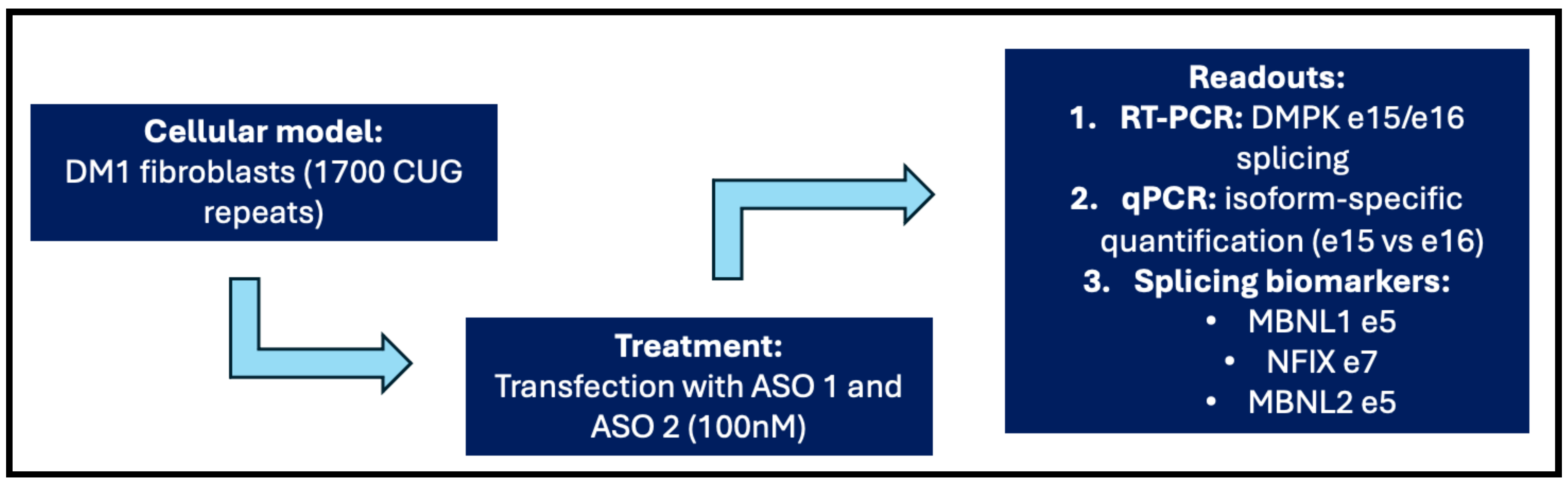
Fig. 1. ASO-mediated exon 15 skipping strategy in DMPK transcripts.
Schematic representation of the ASO strategy designed to promote exon 15 skipping and exon 16 inclusion, thereby bypassing the CUG repeat-containing region. The alternative 3' splice site (3'SS) associated with exon 16 is indicated. Primer positions used for transcript analysis are shown: a common forward primer (F), a reverse primer detecting exon 15-containing transcripts (R_e15), and a reverse primer specific for exon 16-containing transcripts (R_e16). The F/R_e16 primer pair was additionally used for conventional RT-PCR analysis of DMPK transcript isoforms.

- The CTG expansion in DMPK is located within exon 15, generating toxic CUG-containing transcripts
- This toxic RNA drives disease pathology through sequestration of MBNL proteins
- An alternative splicing event allowing usage of exon 16 can bypass the repeat-containing region
- ASO-mediated modulation of exon 15/16 splicing offers a targeted strategy to reduce toxic RNA species while preserving transcript expression

OBJECTIVES

- To assess whether ASOs can modulate DMPK exon 15/16 alternative splicing in cellular models
- To evaluate the potential of this strategy to reduce toxic CUG-containing transcript species in DM1

METHODS



Cellular model:
DM1 fibroblasts (1700 CUG repeats)

Treatment:
Transfection with ASO 1 and ASO 2 (100nM)

Readouts:
1. **RT-PCR:** DMPK e15/e16 splicing
2. **qPCR:** isoform-specific quantification (e15 vs e16)
3. **Splicing biomarkers:**
   - MBNL1 e5
   - NFIX e7
   - MBNL2 e5

RESULTS

A. Transcript outcome: qPCR quantification

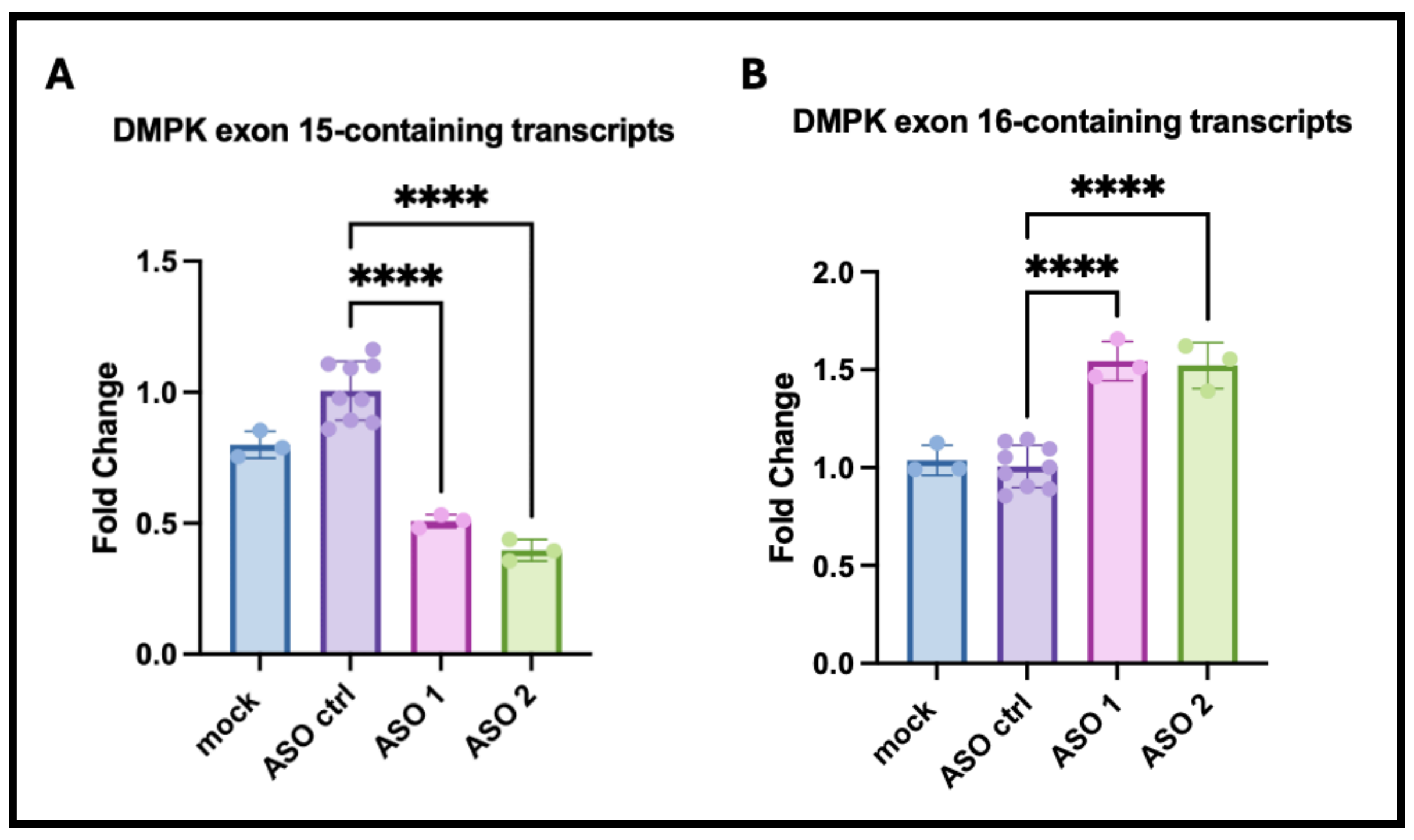


Fig 2. qPCR analysis of DMPK exon 15 and exon 16-containing transcripts following ASO treatment in DM1 fibroblasts.
*Relative expression of exon 15-containing (Panel A) and exon 16-containing (Panel B) DMPK isoforms in untreated DM1 fibroblasts (mock), ASO control-treated cells, and cells treated with ASO1 or ASO2. Data are shown as fold change normalized to ASO control. ASO1 and ASO2 induce a significant reduction in exon 15-containing transcripts and an increase in exon 16-containing isoforms, consistent with a splicing switch at the DMPK terminal exon. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test versus ASO control (**** $p < 0.0001$).*

B. Target effect: DMPK splicing (RT-PCR)

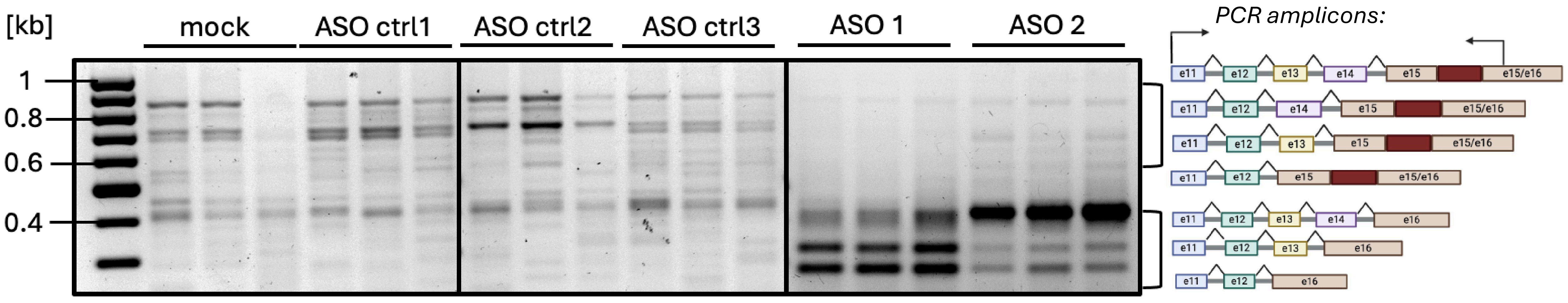


Fig 3. ASO-mediated modulation of DMPK alternative splicing in DM1 fibroblasts.
RT-PCR analysis of DMPK transcripts in untreated DM1 (1700 CUG) fibroblasts (mock) and DM1 fibroblasts treated with ASO1 or ASO2. ASO treatment promotes a shift toward exon 16 usage and reduced exon 15-containing transcripts.

C. Functional readout: MBNL-dependent splicing biomarkers

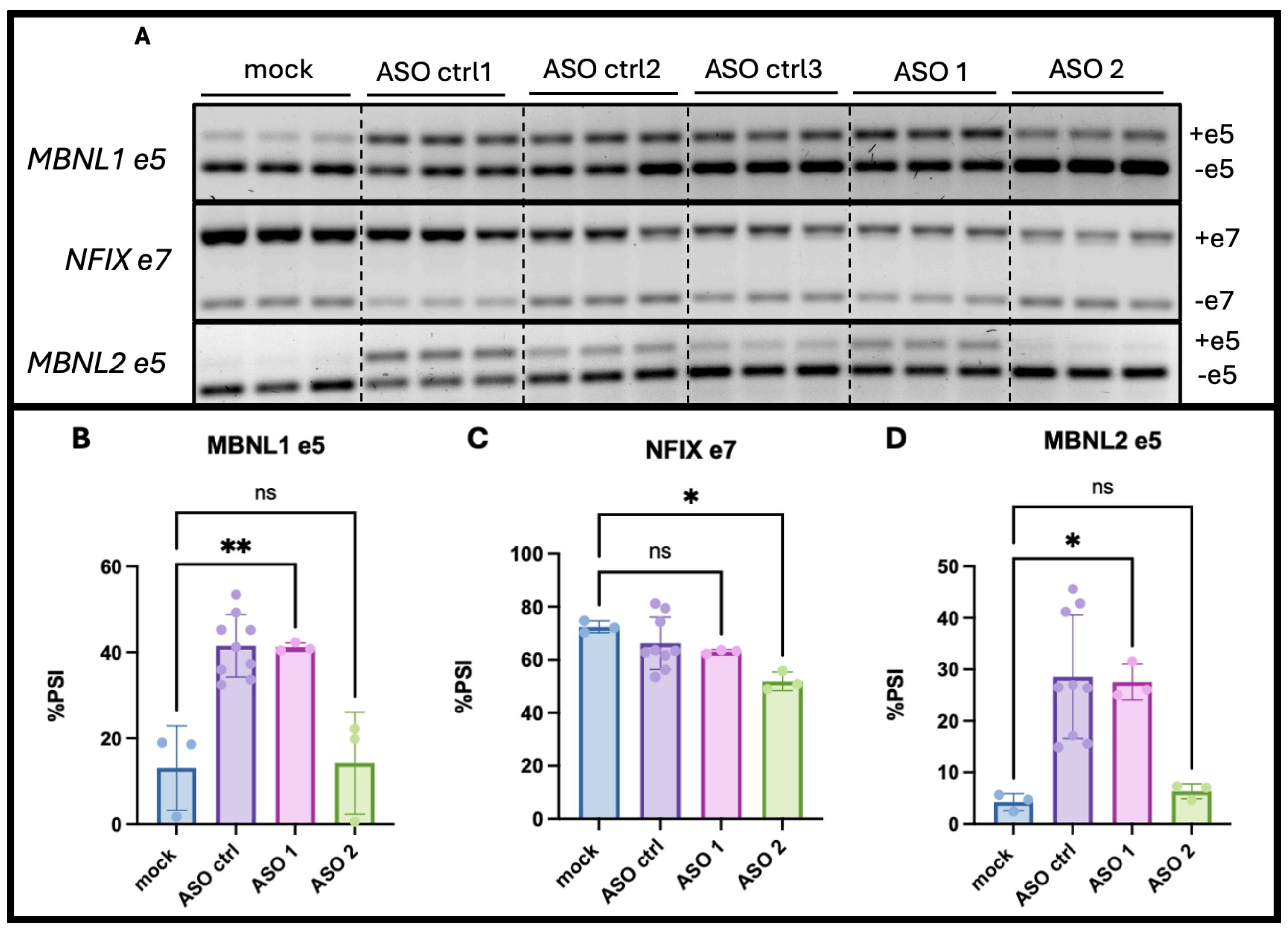


Fig. 4. Functional readout of MBNL-dependent alternative splicing in DM1 fibroblasts treated with ASOs.
(A) Representative RT-PCR gels showing alternative splicing patterns of MBNL1 exon 5, NFIX exon 7 and MBNL2 exon 5 in DM1 fibroblasts transfected with mock, control ASOs (ASO ctrl1–3) or candidate ASOs (ASO 1 and ASO 2). Inclusion (+) and skipping (−) isoforms are indicated.
(B–D) Quantification of percent splicing inclusion (PSI) for MBNL1 exon 5 (B), NFIX exon 7 (C) and MBNL2 exon 5 (D). Individual biological replicates are shown as dots and mean ± SD is indicated. Statistical analysis was performed using one-way ANOVA followed by multiple comparisons testing. Selected pairwise comparisons are indicated in the graphs. P values are represented as follows: $p < 0.05$ (*) and $p < 0.01$ (**).

CONCLUSIONS

- **Efficiency in DMPK modulation:** It was robustly demonstrated that ASO 1 and ASO 2 treatment successfully directs DMPK splicing in DM1 fibroblasts, significantly decreasing transcripts containing the toxic exon 15 and promoting the inclusion of the functional exon 16.
- **Limited effect on the cellular phenotype:** Despite the reduction of exon 15 in DMPK, inclusion levels of two analyzed splicing biomarkers (MBNL1 e5 and MBNL2 e5) remained similar to untreated cells (mock).

FUTURE PERSPECTIVES

- **Technical optimization:** Refine both qPCR and RT-PCR protocols to improve consistency and detection sensitivity.
- **ASO screening:** Evaluate a broader library of ASOs to identify sequences with higher efficacy and functional rescue.
- **Complex cellular models:** Transition from fibroblasts to myoblasts/myocytes, hiPSC-derived muscle and neuronal cells to assess tissue-specific responses.
- **Mechanistic & global analysis:** Perform nuclear foci analysis (FISH) and RNA-seq to determine the global transcriptomic impact of the selected ASOs.

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