SUPPLEMENTAL FIGURE 1

(A) Schematic representation of exon 32 sequence and AONs targeted sequences.

Size and frame of exon 31, 32 and 33 are presented. Exon 32 sequence is boxed. Each sequence targeted by AONs is presented in different colors, the position is indicated relative to the first nucleotide of exon 32.

(B) AON B and D skipped efficiently exon 32.

The efficiency of exon skipping using two different AON (AON B and AON D) has been assessed by RT-PCR on control cells from a non-affected individual (left panel) and from patient 1 cells (right panel). The pair of primers amplified the region comprised between exons 31 and 33 (293bp and 215bp with or without exon 32 respectively). All AONs were transfected once at day 0 of differentiation and compared to Mock transfected cells. RT- : negative control of reverse transcription. PCR- : negative control of PCR. MM (bp): molecular marker (1kb+ from Life Technologies).

(C) Efficiency of exon-skipping using AON D has been assessed by RT-PCR on cells from patient 2.

The pair of primers amplified the region comprised between exons 31 and 33 (293bp and 215bp with or without exon 32 respectively). Repetitive transfection (every 2 days) of AON starting at day 0 of differentiation (one time (AON*1), two times (AON*2) or three times (AON*3)) or Mock (3 times) has been used and the ratio of the skipped band versus non-skipped was determined in each cases. RT- : negative control of reverse transcription. PCR- : Negative control of PCR. MM: molecular marker.

(D) Exon skipping preserves the coding frame.

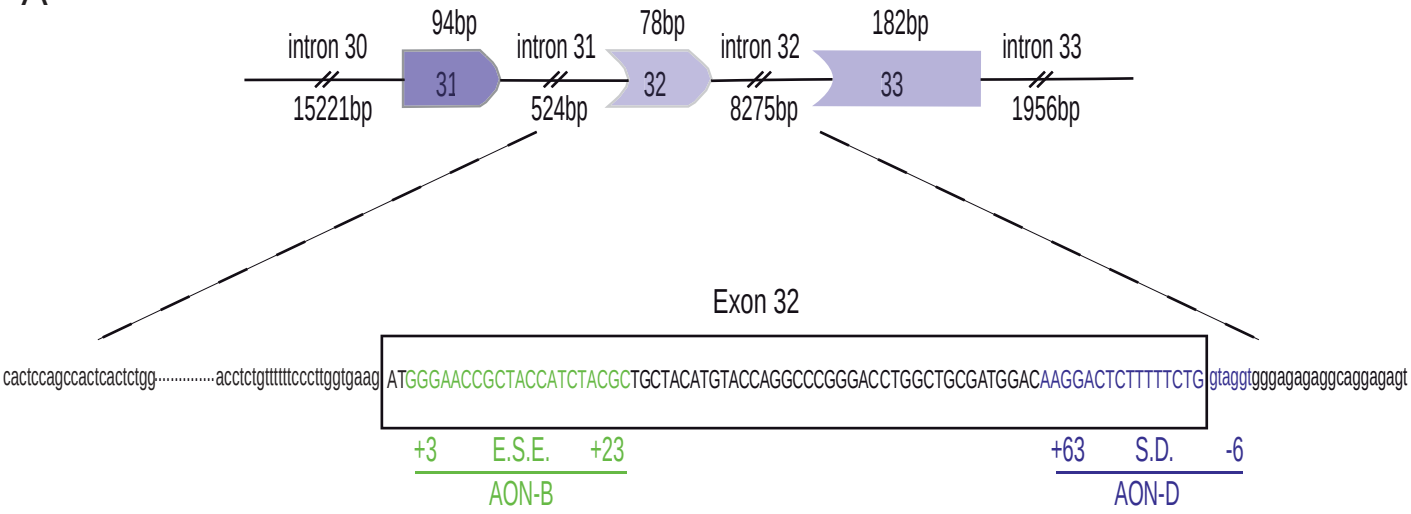
After sequencing several times products of exon-skipped mRNA in both patient cells and with both AON, we reliably found the correct fusion between exon 31 and 33. A representative Sanger sequence of this exon-exon boundary is presented.

SUPPLEMENTAL FIGURE 2

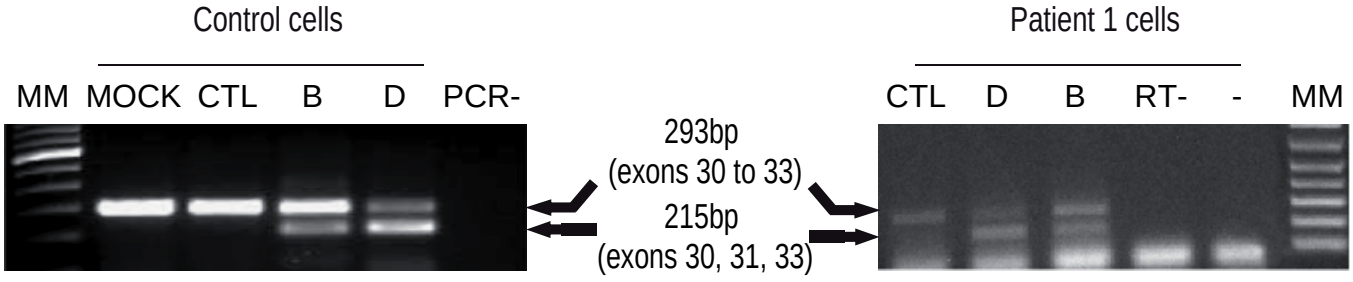
(A) Western Blot experiment performed with proteins from AON treated patient 2 cells or mock treated cells. Hamlet 2 was used to detect dysferlin and GAPDH for normalization.

(B) Single AON treatment is efficient to improve myotube formation Box plots represent the number of nuclei per myotubes in control (n = 70), Mock treated patient cells (n = 26), AON treated patient 2 cells (n = 29). Boxes extend from the 25th to the 75th percentile values. Horizontal bars indicate the median value. * : $p<0.01$ compared to mock treated patient cells. AON treatment increased the number of nuclei per cells.

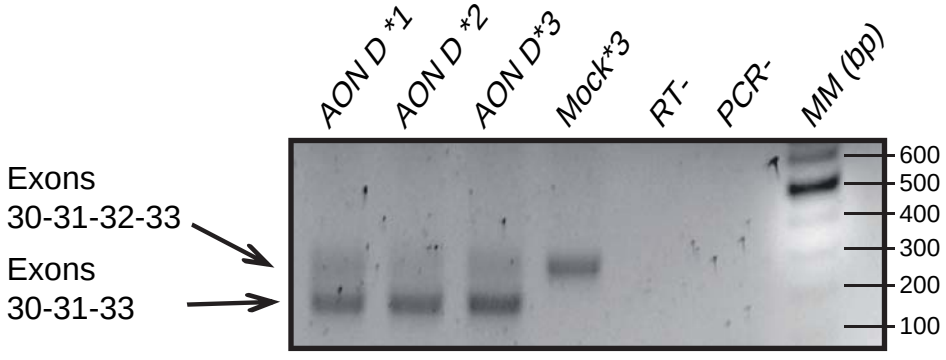
A


intron 30
15221bp
94bp
31
intron 31
524bp
78bp
32
intron 32
8275bp
182bp
33
intron 33
1956bp
Exon 32
cactccagccactcactctgg.....acctctgtttttccctggtgaag ATGGGAACCGCTACCATCTACGCTGCTACATGTACCAGGCCCGGGACCTGGCTGCGATGGACAAGGACTCTTTTTCTG gtaggtgggagagaggcaggagagt
+3 E.S.E. +23
AON-B
+63 S.D. -6
AON-D

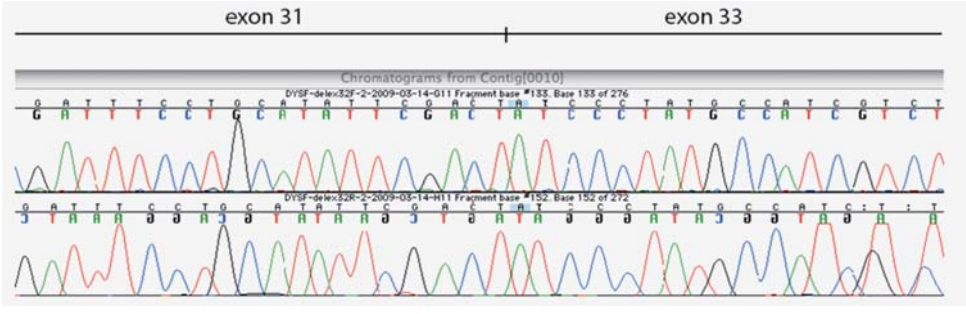
B


Control cells
MM MOCK CTL B D PCR-
293bp
(exons 30 to 33)
215bp
(exons 30, 31, 33)
Patient 1 cells
CTL D B RT- - MM

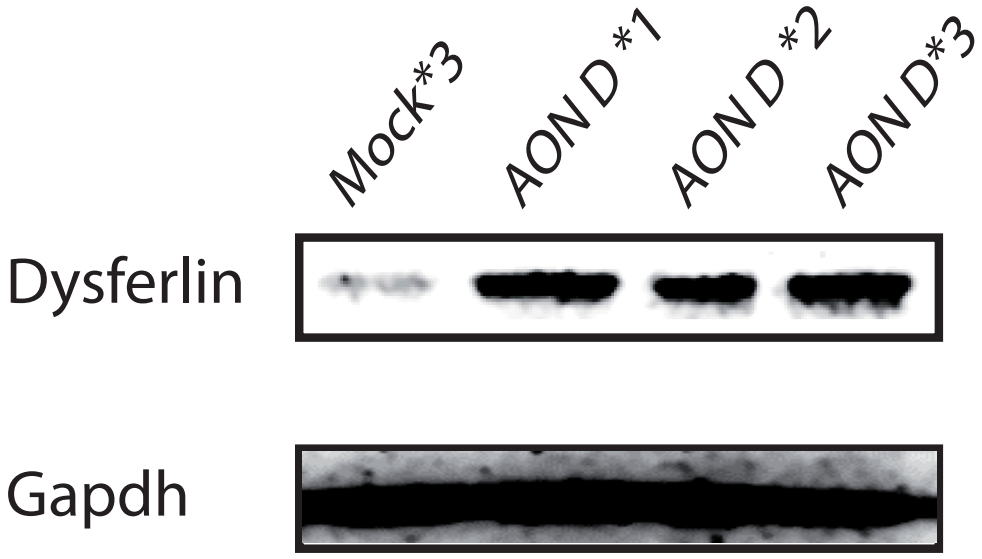
C


AON D *1
AON D *2
AON D*3
Mock*3
RT-
PCR-
MM (bp)
Exons
30-31-32-33
Exons
30-31-33
600
500
400
300
200
100

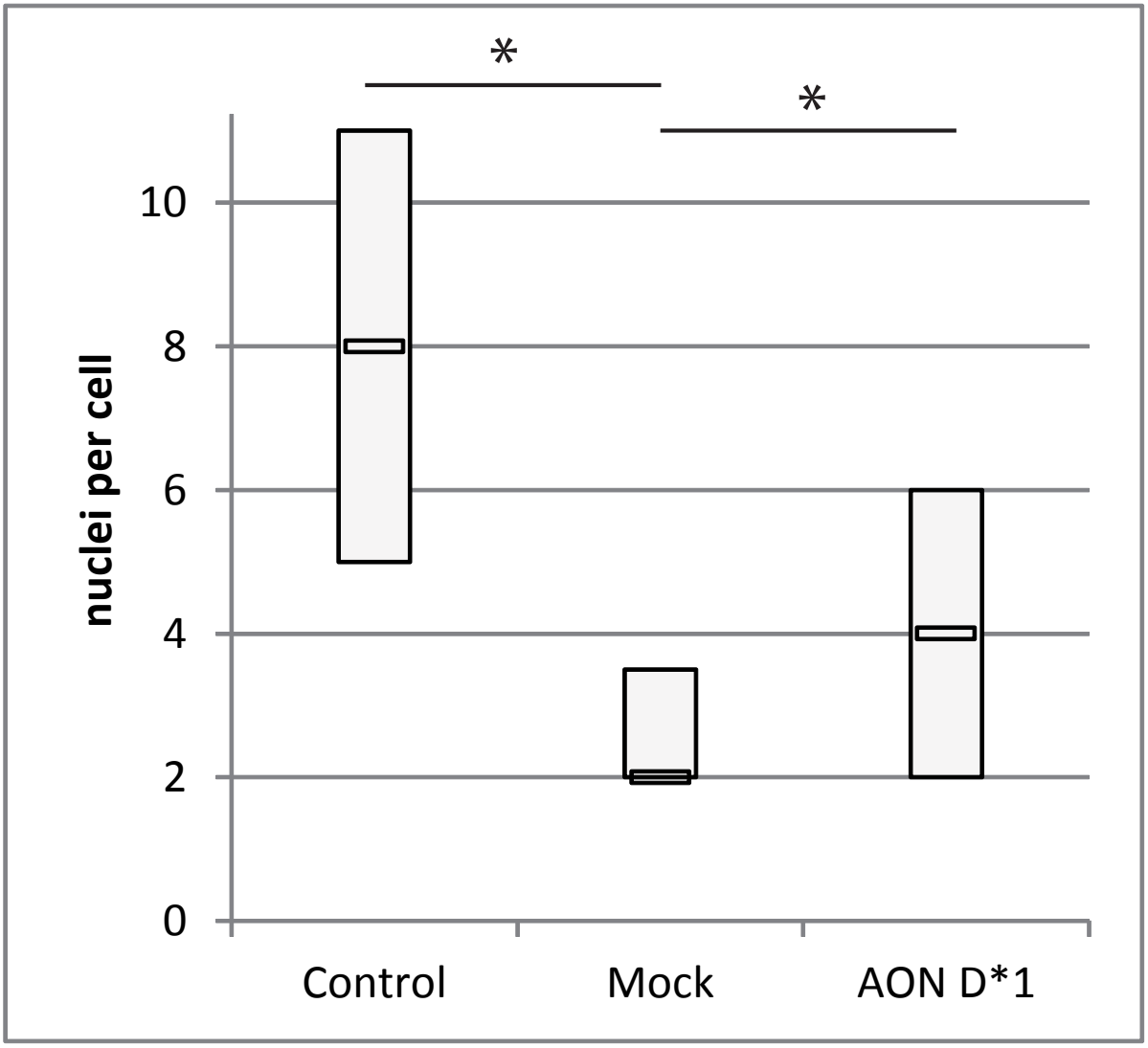
D


exon 31
exon 33
Chromatograms from Contig[0010]

A


Mock*3
AON D *1
AON D *2
AON D*3
Dysferlin
Gapdh

B


*
*
nuclei per cell
10
8
6
4
2
0
Control
Mock
AON D*1