Figure S1. Effects of mbnl mutations on mbnl mRNA levels.
A-C. qRT-PCR showing the levels of (A) mbnl1, (B) mbnl2, and (C) mbnl3 mRNAs in WT and mbnl mutant 5 days post fertilization whole larval zebrafish.
D-F. qRT-PCR showing relative levels of (D) mbnl1 mRNA, (E) mbnl2 mRNA, and (F) mbnl3 mRNA in WT 5 dpf whole larvae, adult skeletal muscle, heart, brain, cornea, and intestine.
G-J. RNA-Seq data showing the normalized counts of WT mbnl1, mbnl2, and mbnl3 RNAs in unfertilized eggs (from Mehjabin et al., 2019), and (H) mbnl1, (I) mbnl2, and (J) mbnl3 mRNAs in WT and mbnl mutant adult zebrafish skeletal muscle.

Data information: In (A-J) data are presented as mean ± SEM. Each dot represents RNA from a pool of 5 larvae or one adult fish. Data were analyzed by ordinary one-way ANOVA with Tukey’s multiple comparisons test. Data bars that do not share a letter above them are significantly different from one another. Raw data and statistical analysis details are in Table S5.
Figure S2. Single mbnl mutant zebrafish were not decreased in size.

A-C. Standard length of (A) 83 dpf clutchmates from an incross of 1+/B fish, (B) 83 dpf clutchmates from an incross of 2+/A fish, and (C) 78 dpf clutchmates from an incross of 3+/C fish. Example images of fish of each genotype are next to each graph. Scale bar = 10 mm.

D. Standard length of 7 dpf larval fish.

Data information: In (A-D) mbnl1 mutant alleles are denoted as 1A and 1B, mbnl2 alleles as 2A, and mbnl3 alleles as 3A, 3B, and 3C. Data are presented as mean ± standard deviation. Each dot represents one fish. Data were analyzed by ordinary one-way ANOVA with Tukey's multiple comparisons test. Data bars that do not share a letter above them are significantly different from one another. Raw data and statistical analysis details are in Table S5.
Figure S3. Adult zebrafish WT and mbnl mutant muscle fibers were structurally similar.
A. Representative hematoxylin and eosin stained transverse sections of epaxial muscle fibers from the tails of adult zebrafish. Scale bar = 100 µm. B. Distribution of muscle fiber cross-sectional areas from transverse sections of epaxial muscle fibers from the tails of adult zebrafish. Individual muscle fibers were measured from 5-6 sections each of 5 fish from each genotype. WT n=2928, 1B/B;2A/A n=3819, 1B/B;2A/A;3C/C n=2846. Data were analyzed using the Kruskal-Wallis test with Dunn's multiple comparisons test. Curves with different letters are significantly different from one another.
**Figure S4.** The introns surrounding zebrafish *mbnl1* exon 5 contain potential Mbnl protein binding sites.

Zebrafish *mbnl1* exon 5 (bold upper case letters) and its surrounding intronic sequences (lower case letters) were aligned with orthologous human sequences using the EMBOSS Water program. Potential YGCY (Y=pyrimidine) Mbnl protein binding sites are indicated in red.
**Figure S5. Zebrafish mbnl2 exon 5 and its surrounding introns contain potential Mbnl protein binding sites.**

Zebrafish mbnl2 exon 5 (bold upper case letters) and its surrounding intronic sequences (lower case letters) were aligned with orthologous human sequences using the EMBOSS Water program. Potential YGCY (Y=pyrimidine) Mbnl protein binding sites are indicated in red.
Figure S6. Alternative splicing of *mbnl2* exon 5 was misregulated across tissues in zebrafish *mbnl* mutants.

A-F. RT-PCR analysis showing percent spliced in (PSI) of *mbnl2* exon 5 in WT and *mbnl* mutant (A) whole 5 dpf larvae, and in adult (B) skeletal muscle, (C) heart, (D) brain, (E) cornea, and (F) intestine. *MBNL2* exon 5 inclusion is increased in human DM1 patients (Tabs 5A and S7A of Table S5).

Data information: In (A-F) *mbnl1* mutant alleles are denoted as 1A and 1B, *mbnl2* alleles as 2A, and *mbnl3* alleles as 3A, 3B, and 3C. Representative RT-PCR gels are shown above each graph with band sizes in bp shown on the left. White boxes represent constitutive exons and gray boxes represent alternative exons. Dividing lines indicate samples run on separate gels. Each dot represents RNA from one adult fish or a pool of five larvae. Data are presented as mean ± SEM. Data were analyzed by ordinary one-way ANOVA with Tukey's multiple comparisons test. Data bars that do not share a letter above them are significantly different from one another. Raw data and statistical analysis details are in Table S5.
Figure S7. Misregulation of many DM-associated alternative splicing events was conserved in zebrafish mbnl mutants.

A. Change in percent spliced in values (ΔPSI) between mutant and WT are shown for orthologous exons in zebrafish 1B/B;2A/A;3C/C skeletal muscle and in human DM1 patient-derived myotubes.

B-F. (B,C,E) RNA-Seq and (D,F) RT-PCR analyses showing PSI of (B) mbnl2 exon 5, (C-D) aplp2 exon 7, and (E-F) atp6v1h exon 6 in WT and mbnl mutant adult zebrafish skeletal muscle. Orthologous human exon inclusion in DM patient tissues is increased for MBNL2 and decreased for APLP2 and ATP6V1H (Tabs 5A and S7A of Table S5).

Data information: In (A) ρ is the Spearman’s rank correlation coefficient. In (A-F) homozygous mbnl1 mutant alleles are denoted as 1A and 1B, mbnl2 alleles as 2A, and mbnl3 alleles as 3A, 3B, and 3C. Representative RT-PCR gels are shown above each graph with band sizes in bp shown on the left. White boxes represent constitutive exons and gray boxes represent alternative exons. Each dot represents RNA from one fish. Data are presented as mean ± s.e.m. Raw data and statistical analysis details are in Table S5.
**Figure S8. Zebrafish aplp2 exon 7 and its surrounding introns contain potential Mbnl protein binding sites.**

Zebrafish aplp2 exon 7 (bold upper case letters) and its surrounding intronic sequences (lower case letters) were aligned with orthologous human sequences using the EMBOSS Water program. Potential YGCY (Y=pyrimidine) Mbnl protein binding sites are indicated in red.
Figure S9. Zebrafish *atp6v1h* exon 6 and its surrounding introns contain potential Mbnl protein binding sites.

Zebrafish *atp6v1h* exon 6 (bold upper case letters) and its surrounding intronic sequences (lower case letters) were aligned with orthologous human sequences using the EMBOSS Water program. Potential YGCY (Y=pyrimidine) Mbnl protein binding sites are indicated in red.
**Figure S10.** The introns surrounding zebrafish atp2a1l exon 23 contain potential Mbnl protein binding sites.

Zebrafish atp2a1l exon 23 (bold upper case letters) and its surrounding intronic sequences (lower case letters) were aligned with orthologous human sequences using the EMBOSS Needle program. Potential YGCY (Y=pyrimidine) Mbnl protein binding sites are indicated in red.
| Zebrafish | Human |
|-----------|-------|
| actccattttgt......tcagacaattgctgacc............tcat | actgcccttctcaggctctcagagaas...cgaccccccccccagccaa |
| gtcctgttgaggac.....agacactttttcgcttcaca.ggggttc... | ggcctggaaatgcccagctagagaat............acagtgggggtg |
| tatagg.........gctg....ttgatgtttttatatatatattt | ggcagagggagttggggtgctgaacttggag............ |
| atttatgtctctg....ctctctgagggtgtttttataaaaagagctgtg..gtt | cttctctg.tggcttcgctgtgctagtggggcttgagGCGGGAG |
|atatatttcgcatggcgcagaatgtggtttt....tcatcaaactccca.. | tgggcctggcttctctgcggggct..ggggtaaccctttgctt |
| ctaatacacacaaaagtccaa..........accctcattcactgcagtc | atcatacacacaaaagtccaa..........accctcattcactgcagtc |
| atgtgccagccccccacccatcctctctctcccctt | acacctcttcagccataagctt |

**Figure S11. The introns surrounding zebrafish ryr1b exon 72 contain potential Mbnl protein binding sites.**

Zebrafish ryr1b exon 72 (bold upper case letters) and its surrounding intronic sequences (lower case letters) were aligned with orthologous human sequences using the EMBOSS Water program. Potential YGCY (Y=pyrimidine) Mbnl protein binding sites are indicated in red.
Zebrafish $ank^3b$ exon 36 / Human ANK3 exon 35

|   | Zebrafish       | Human       |
|---|-----------------|-------------|
| 1 | ggtgcacag.gttgtgcttttttttcagagctctggttgcggcttt | gatgtacagttcttgatatttttt...........tttctaatcagct.gcatt |
| 51 | ttcttttcatttgc.ttgcccttttatgtctct...tca.cttgtggcttt | tt..tttaaaattttgtt.tgc.................tttctaatcagct.gcatt |
| 101 | tgcgt..aatcacaatcgcctttgtcatctgtatgtttctttccc | tgcggt..aacacaatcgcctttgtcatctgtatgtttctttccc |
| 151 | cttgtccttttactcagtttatcccccctctgtttcttcaatttgtcatctgtatgtttctttccc | cttgtccttttactcagtttatcccccctctgtttcttcaatttgtcatctgtatgtttctttccc |
| 201 | ccataaaagGATGTTGATGGATTCAGATCCCGAGGAAGAG | ccataaaagGAGACAGAGTCAGATCAAGATGTAAAGGAGtaataagtgtggtccc |
| 251 | ct..ctattttcatttccatgtttggtgttgctagttgctttagctct.........g | .taaacacttc.........tgcttttattctataagtag |
| 301 | gattg..attg..tttgt...........tttaagggcttttaacatagag | aatagaattgaatctgtgatgcagagttgcagaggg..taaagtgt |
| 351 | taccatacatc..aacacatagctgtgctgcaattttact..ca | ..........ggaaacatataaattat........atttttcaggtctta |
| 401 | agttg.................atctctttt.........tttactcatcctatag | agttgagtttaaggtatatatatttttttaatgttcttc.ttttcaacttc.tcttc |
| 451 | cgacaatattgtgtgcttgagcagtctcgag.actaaaa.ata | cacaagct.............acttaagagtacttaatata |

**Figure S12. The introns surrounding zebrafish $ank^3b$ exon 36 contain potential Mbnl protein binding sites.**

Zebrafish $ank^3b$ exon 36 (bold upper case letters) and its surrounding intronic sequences (lower case letters) were aligned with orthologous human sequences using the EMBOSS Water program. Potential YGCY (Y=pyrimidine) Mbnl protein binding sites are indicated in red.
**Figure S13. Differentially expressed genes in zebrafish *mbnl* mutants and human DM1 myotubes belong to overlapping gene ontology pathways.**

Significantly enriched gene ontology (GO) pathways for genes that were differentially expressed in 1<sup>B/B;</sup> 2<sup>A/A;</sup> 3<sup>C/C</sup> vs. WT adult zebrafish skeletal muscle. Red asterisks indicate those GO pathways that were also enriched in differentially expressed genes between human DM1 vs. WT myotubes. See Table S6 for complete lists of differentially expressed genes and enriched GO pathways in zebrafish and human. Data information: p. adjust indicates adjusted p-value. Count indicates number of differentially expressed genes belonging to each GO pathway. Raw data are in Table S6.
Figure S14. Zebrafish triple homozygous mbnl mutants swim at decreased speed.

This figure contains additional analysis of data from the five minute swim test in Fig. 2.

A-C. Speed in cm/s of fastest five second interval within the five-minute swim test for (A) single, (B) double, and (C) triple homozygous mbnl mutant fish.

D-F. Speed in cm/s of fastest three second interval within the five-minute swim test for (D) single, (E) double, and (F) triple homozygous mbnl mutant fish.

G-H. Average speed in cm/s of 30 fastest one second intervals within the five-minute swim test for (G) single, (H) double, and (I) triple homozygous mbnl mutant fish.

Data information: mbnl1 mutant alleles are denoted as 1A and 1B, mbnl2 alleles as 2A, and mbnl3 alleles as 3A, 3B, and 3C. In (A-I) each dot represents one fish and data are presented as mean ± s.e.m. In (A,B,D,E,G,H) data were analyzed by ordinary one-way ANOVA with Tukey’s multiple comparisons test and in (C,F,I) data were analyzed by an unpaired Student’s t-test. Data bars that do not share the same letter above them are significantly different from one another. Raw data and statistical analysis details are in Table S5.
**Table S1. Primers used in this study.**

Click here to download Table S1

**Table S2. Zebrafish mbnl mutant sequences.**

For each zebrafish mbnl mutant used in this study, this table lists the University of Oregon allele number, exon that was mutated within the target gene, guide RNA (gRNA) used to generate the mutant, forward primers, reverse primers, and restriction sites used to genotype the mutants by restriction fragment length polymorphism, ZFIN Gene ID for the targeted gene, GRCz11 genome coordinates and coding sequence of the targeted exon, and predicted protein coding sequence. Sequences that are changed compared to WT are indicated in bold. Sequences of primers from this table are listed in Table S1.

Click here to download Table S2

**Table S3. Zebrafish single, double, and triple homozygous mbnl mutants are viable to adulthood.**

For each zebrafish single, double, and triple homozygous mutant mbnl genotype, this table lists the cross that was used to generate the genotype, the percent of adult offspring expected of the genotype according to Mendelian genetics, and the actual percent and number of adult offspring observed with the genotype.

Click here to download Table S3

**Table S4. Significantly misregulated alternative splicing events identified by RNA-Seq in zebrafish mbnl mutants.**

Each tab contains a list of alternative splicing events (cassette exon, mutually exclusive exon, retained intron, alternative 5’ splice site, or alternative 3’ splice site) that were identified by RNA-Seq analysis as being significantly misregulated in adult zebrafish mbnl mutant skeletal muscle compared to WT. All genome coordinates are from the GRCz11 genome assembly, except for WT vs. 1B/B;2A/A;3C/C, for which a list of significantly misregulated cassette exons identified from a preliminary analysis of the GRCz10 genome assembly is also shown. Complete RNA-Seq data are available from NCBI GEO #GSE145270.

Click here to download Table S4
Table S5. Source data and statistical analyses for figures.
Each tab contains the source data and details of statistical analysis for the indicated figure.

Click here to download Table S5

Table S6. Differential gene expression and gene ontology (GO) analysis of zebrafish adult skeletal muscle WT vs. 1B/B;2A/A;3C/C and unaffected vs. DM1 human myotubes

The first two tables contain lists of genes that were significantly differentially expressed in WT vs. 1B/B;2A/A;3F/F adult zebrafish skeletal muscle or in unaffected vs. DM1 human myotubes. The second two tables contain lists of significantly enriched GO terms for the differentially expressed zebrafish or human genes.

Click here to download Table S6