



Title	Transcriptome alterations underlying metabolic dysfunction and liver disease in myotonic dystrophy type 1
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Supplemental Table 18. Correlation between splicing or gene expression alterations and demographic data.

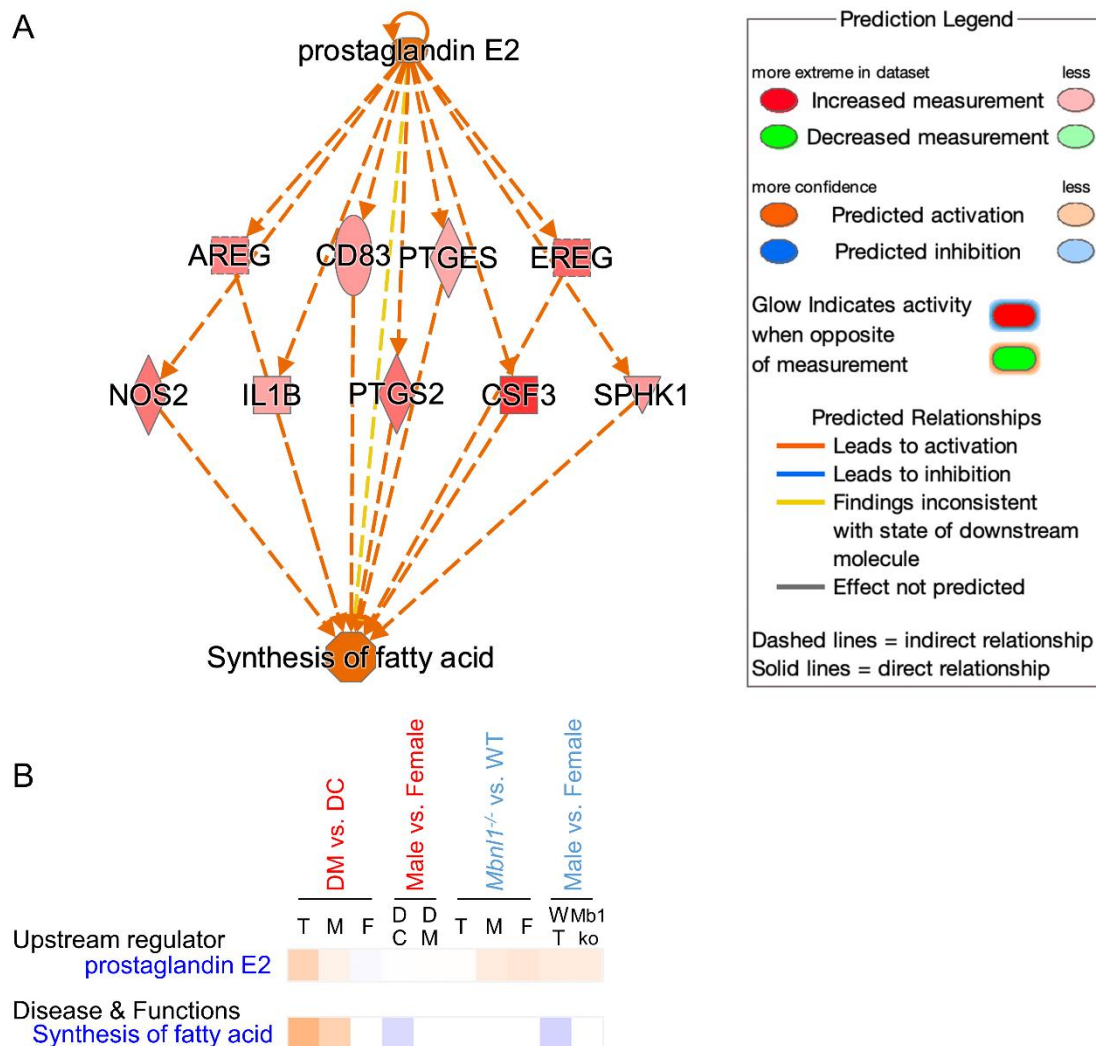
Supplemental Table 19. Correlation between splicing alterations and laboratory data.

Supplemental Table 20. Misspliced genes in the liver of DM1 patients shared with liver-specific CTG repeat-induced mouse model by Zachary Dewald et al.

Supplemental Table 21. Primer sequences used in this study.

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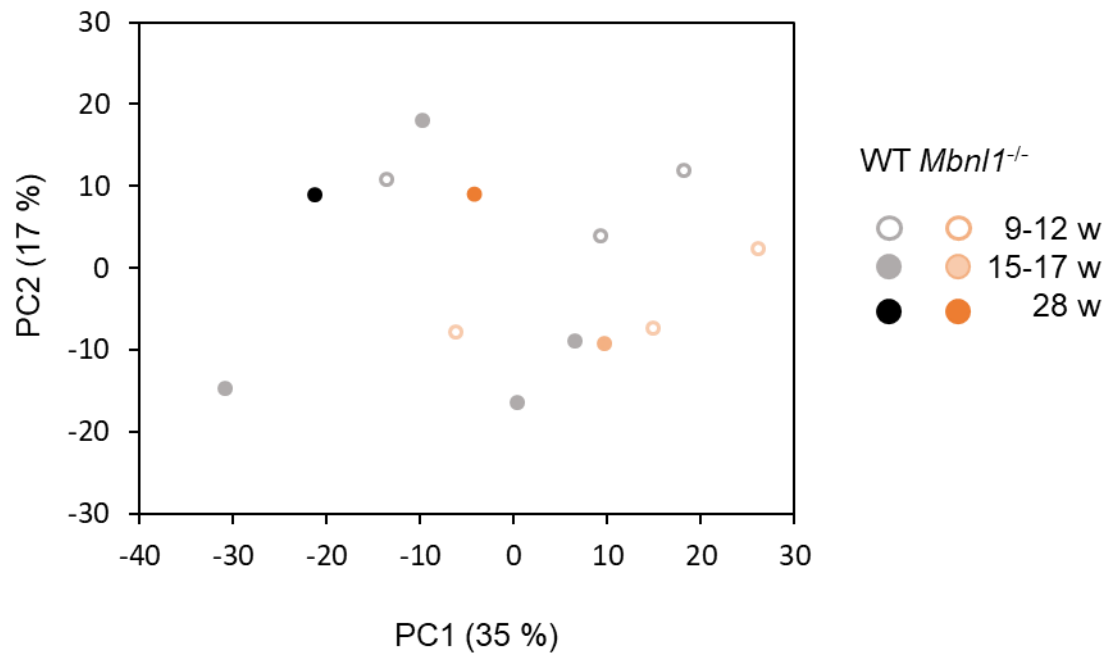
Supplemental Table 23. Antibodies used in this study.



Supplemental Figure 1. Regulator effects analysis in IPA.

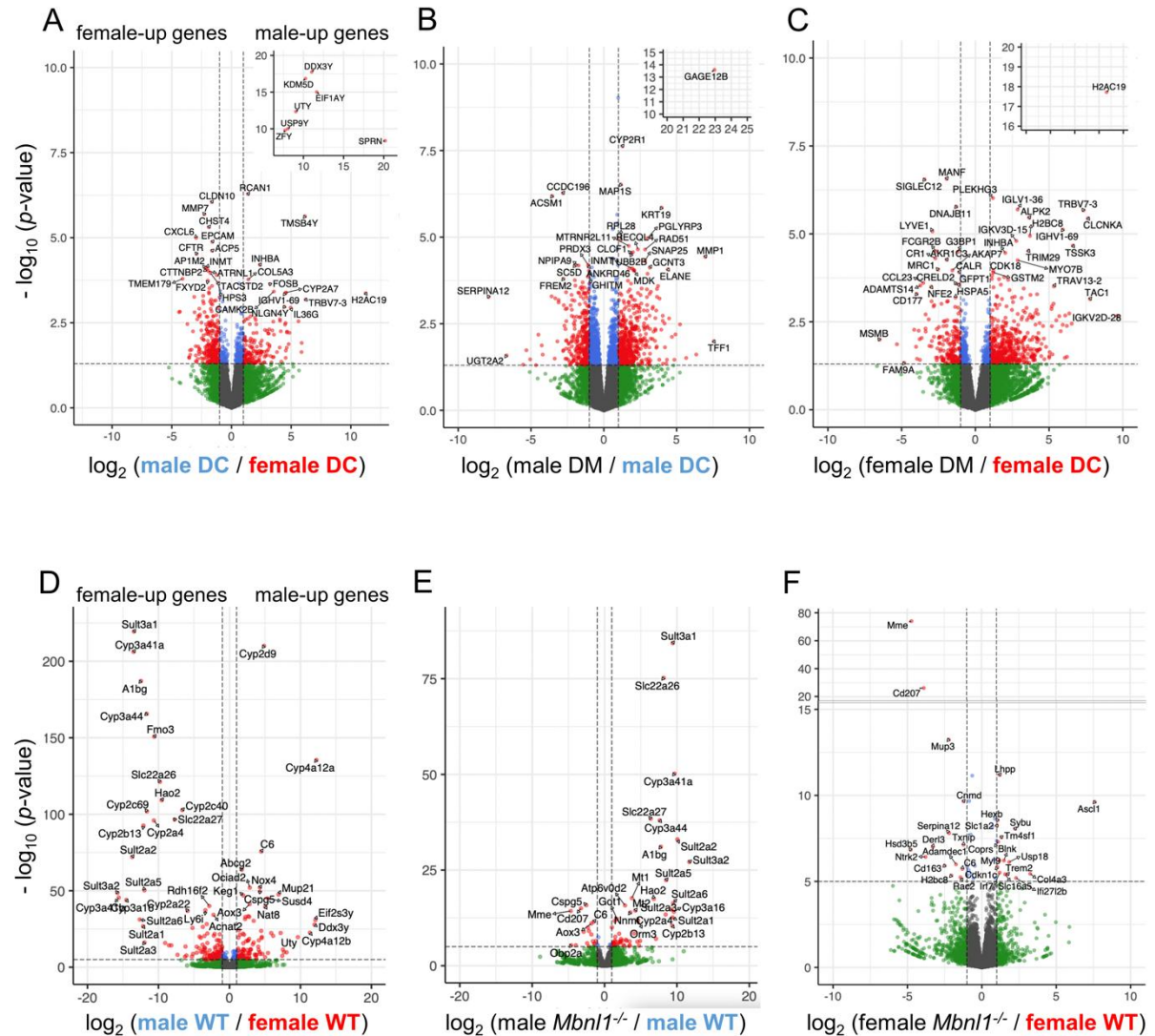
(A) A Regulator effects analysis comparing DM and DC are displayed. In the upper tier, prostaglandin E2 is predicted to be activated (orange color) as the upstream regulator. In the middle tier, the genes whose expression changes in response to the activation of upstream regulators (red = upregulation) are shown. In the lower tier, the expected phenotypic consequences of changes in gene expression are shown by considering the Ingenuity Knowledge Base. Synthesis of fatty acid is predicted to be activated (orange color). T: total (both male and female); M: male; F: female.

(B) A heatmap showing the Z-score obtained using the comparative Ingenuity Pathway Analysis. It predicted prostaglandin E2 as upstream regulator and synthesis of fatty acid as disease & functions.



Supplemental Figure 2. Gene expression alteration in *Mbnl1*^{-/-} mice in relation to age of mice.

PCA plot in Fig 2B based on the gene count data for 5 *Mbnl1*^{-/-} and 8 WT mice samples was re-presented with different symbols according to mouse age group.



Supplemental Figure 3. Volcano plots indicating sex-dependent gene expression alterations.

(A-C) Volcano plots indicating differentially expressed genes (DEGs) in the liver of patients with DM1. DEGs, with $|\log_2\text{FC}| > 1$ and $p\text{-value} < 0.05$, are shown in red. In the comparison of DC samples between male and female (A), 463 DEGs (171 upregulated and 292 downregulated) were identified. In male DM (B), 873 DEGs (545 upregulated and 328 downregulated) were identified. In female DM (C), 655 DEGs (472 upregulated and 183 downregulated) were identified.

(D-F) Volcano plots indicating DEGs in the liver of *Mbnl1*^{-/-} mice. DEGs, with $|\log_2\text{FC}| > 1$ and $p\text{-value} < 0.05$ are shown in red. In the comparison of WT samples between male and female (D), 594 DEGs (272 upregulated and 322 downregulated) were identified. In male *Mbnl1*^{-/-} (E), 434 DEGs (271 upregulated and 163 downregulated) were identified. In female *Mbnl1*^{-/-} (F), 326 DEGs (118 upregulated and 208 downregulated) were identified.

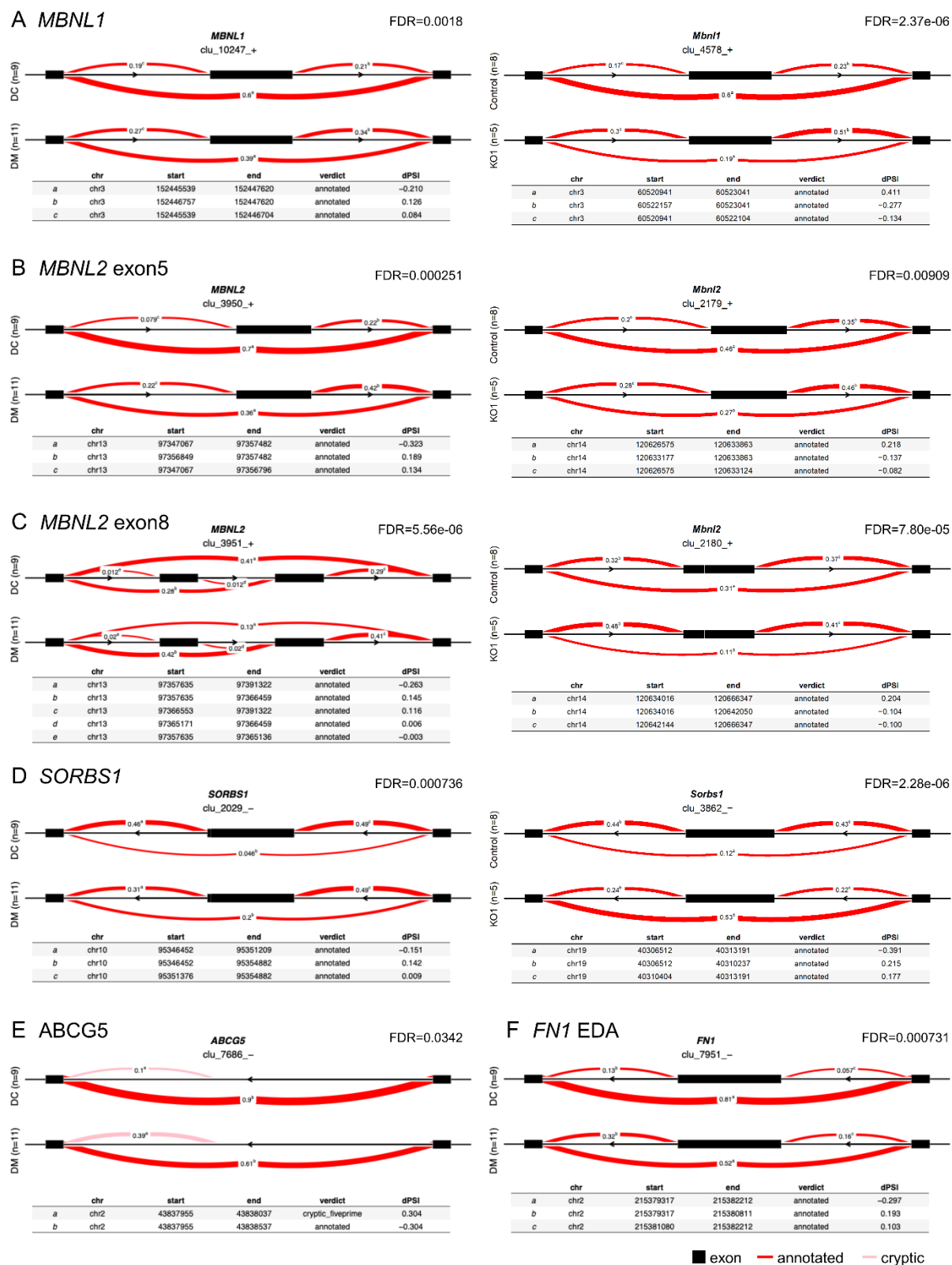


Figure S5. Sashimi plots of aberrantly mis-spliced genes.

(A-D) Sashimi plots of significant intron clusters known mis-spliced events regulated by MBNL proteins in both DM (left) and *Mbnl1*^{-/-} (right) livers.

(E, F) Sashimi plots of significant intron clusters with high Δ PSI values in DM.

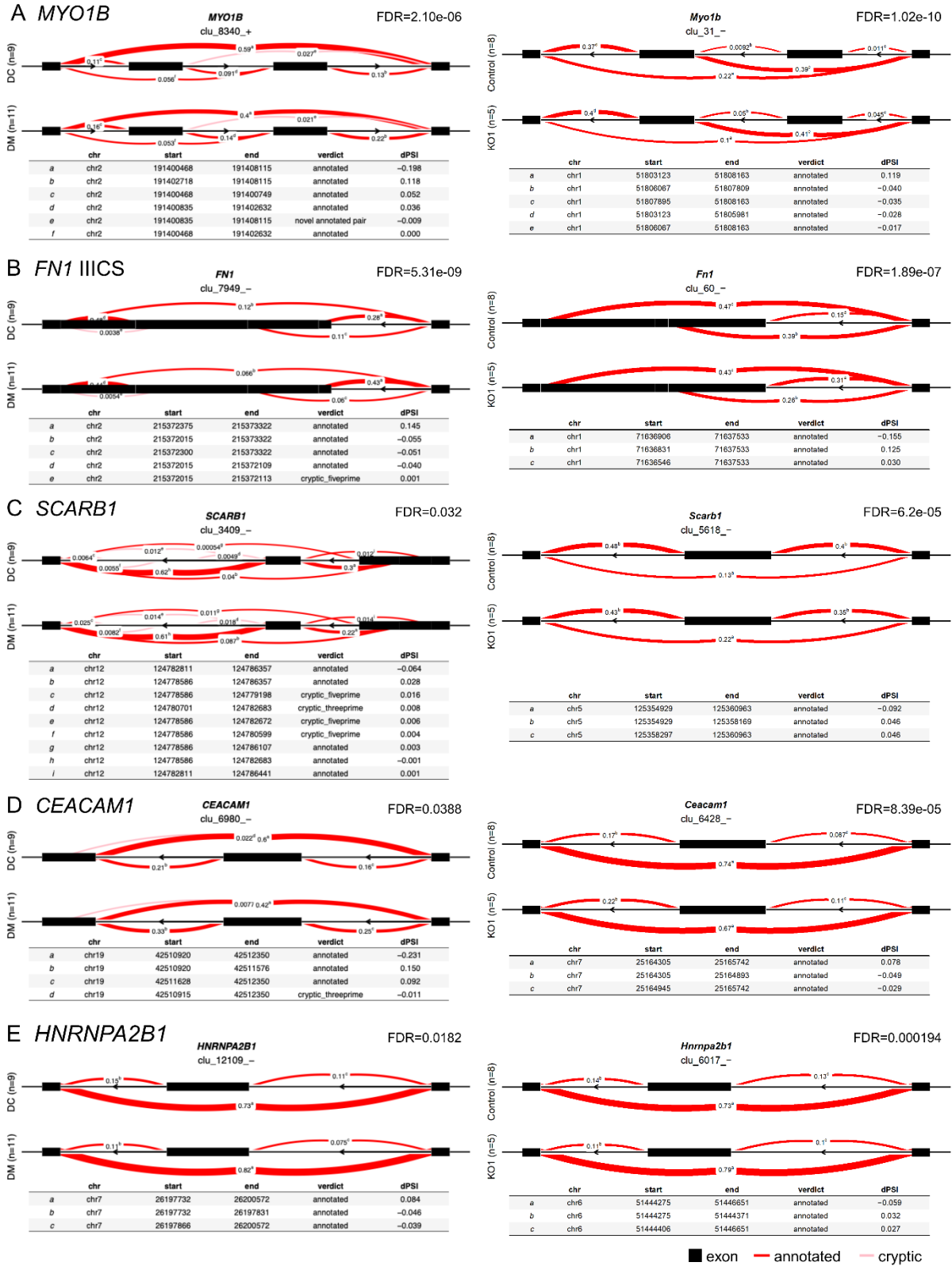


Figure S6. Sashimi plots of aberrantly mis-spliced genes.

(A-E) Sashimi plots of significant intron clusters in genes associated with liver dysfunction and metabolic disorders in both DM (left) and *Mbn1*^{-/-} (right).

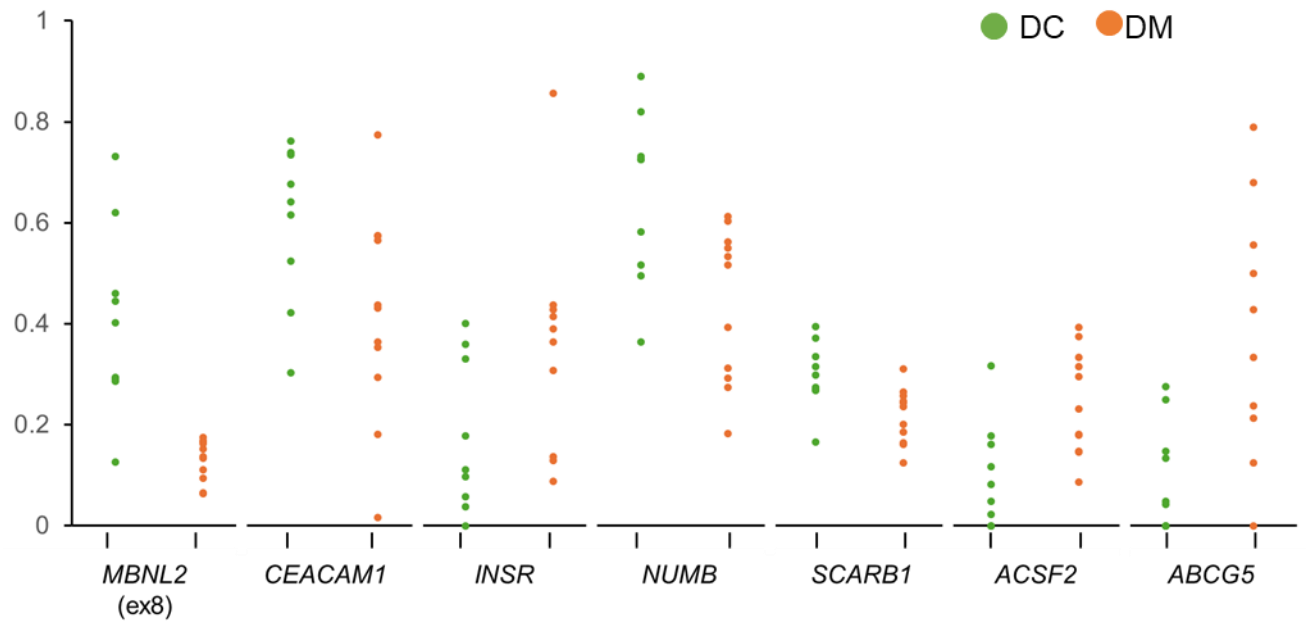


Figure S7 Representative misspliced events identified by RNA-seq analysis using LeafCutter in the liver of patients with DM1

The scatter plots show RNA-seq data from individual patient samples for the same splicing abnormalities presented in Fig. 3G by RT-PCR. It should be noted that the output from LeafCutter represents the excision level within specific intron clusters and is therefore not identical to the inclusion ratios measured by RT-PCR.

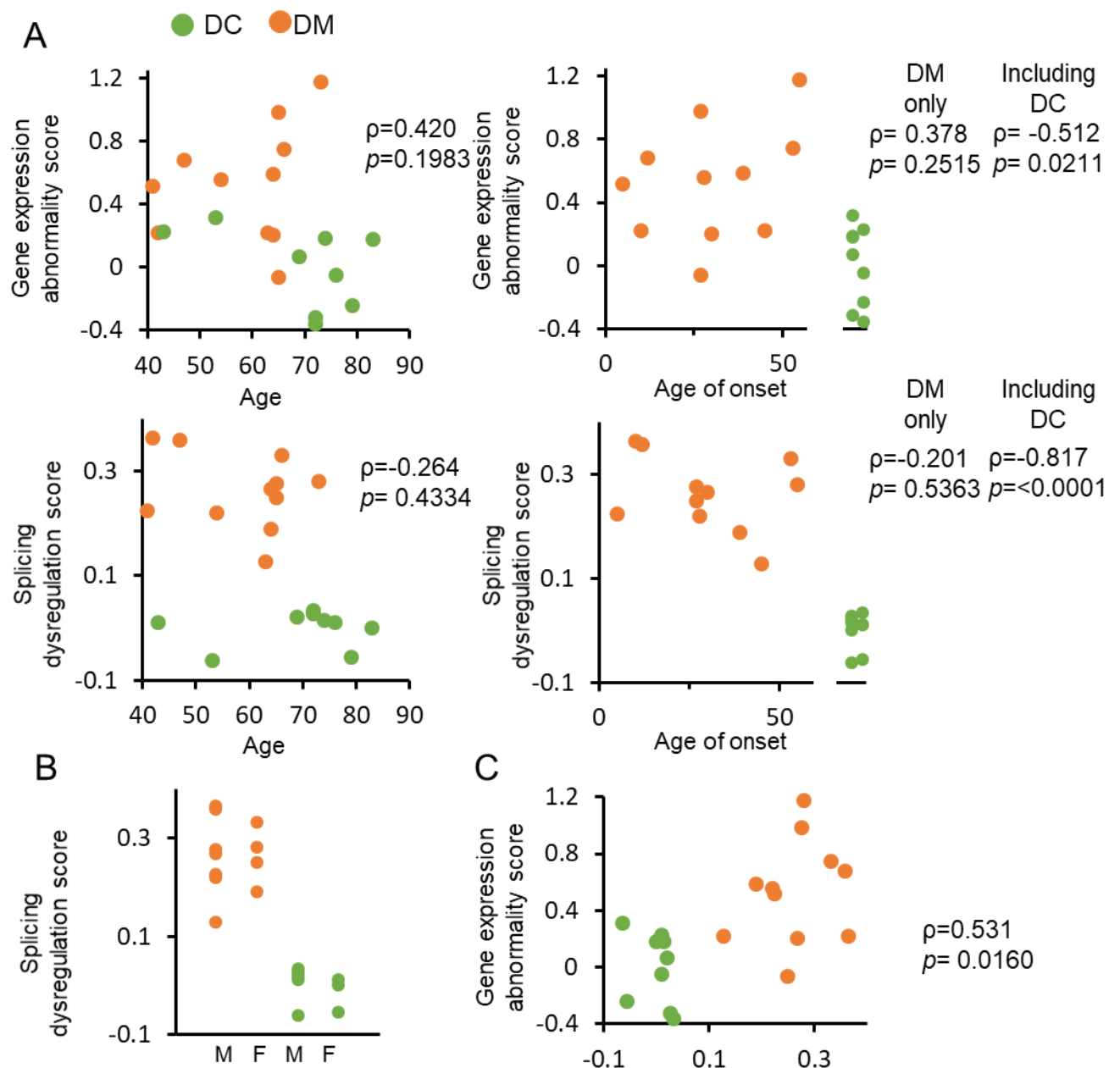


Figure S8. Gene expression and splicing dysregulation in patients with DM in relation to age, age of onset and sex.

(A) Scatterplots showing the relationship between the gene expression abnormality scores and the splicing dysregulation scores with age and age of onset. When disease controls (DC) were included, both scores — particularly the splicing dysregulation score — correlated with age of onset.

(B) Scatterplot of splicing dysregulation scores by sex.

(C) Scatterplot of gene expression abnormality scores versus splicing dysregulation scores. A correlation was observed between the two scores. ρ : Spearman's rank correlation coefficient, p -value: $\text{Prob}>|\rho|$

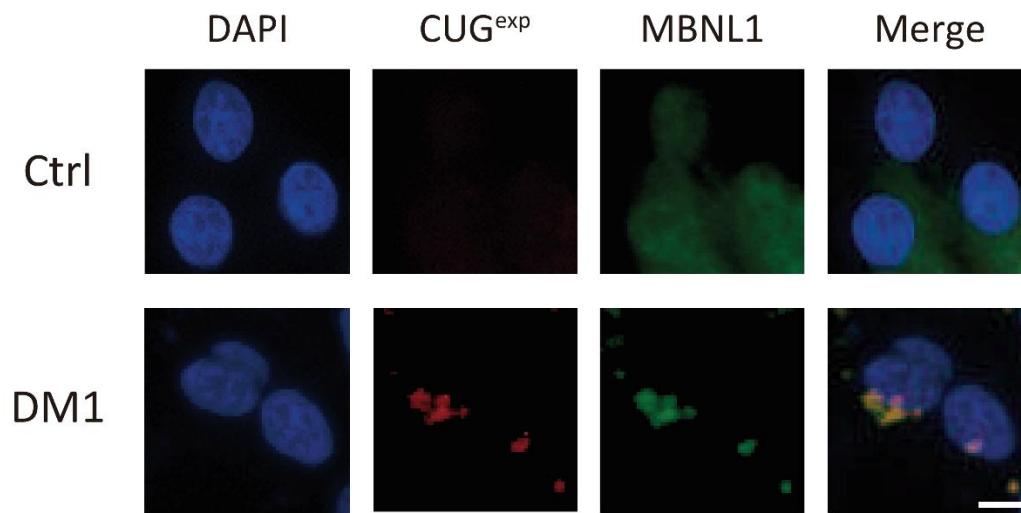
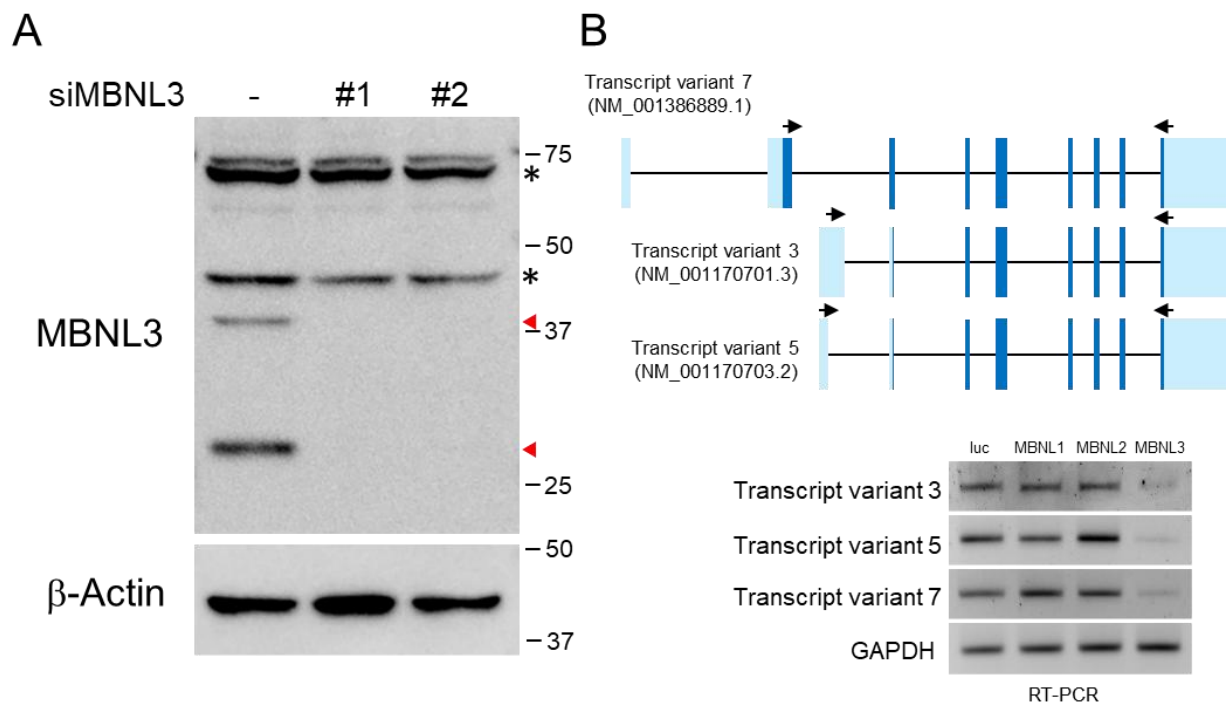


Figure S9. Fluorescent in situ hybridization (FISH) and immunofluorescence (IF).

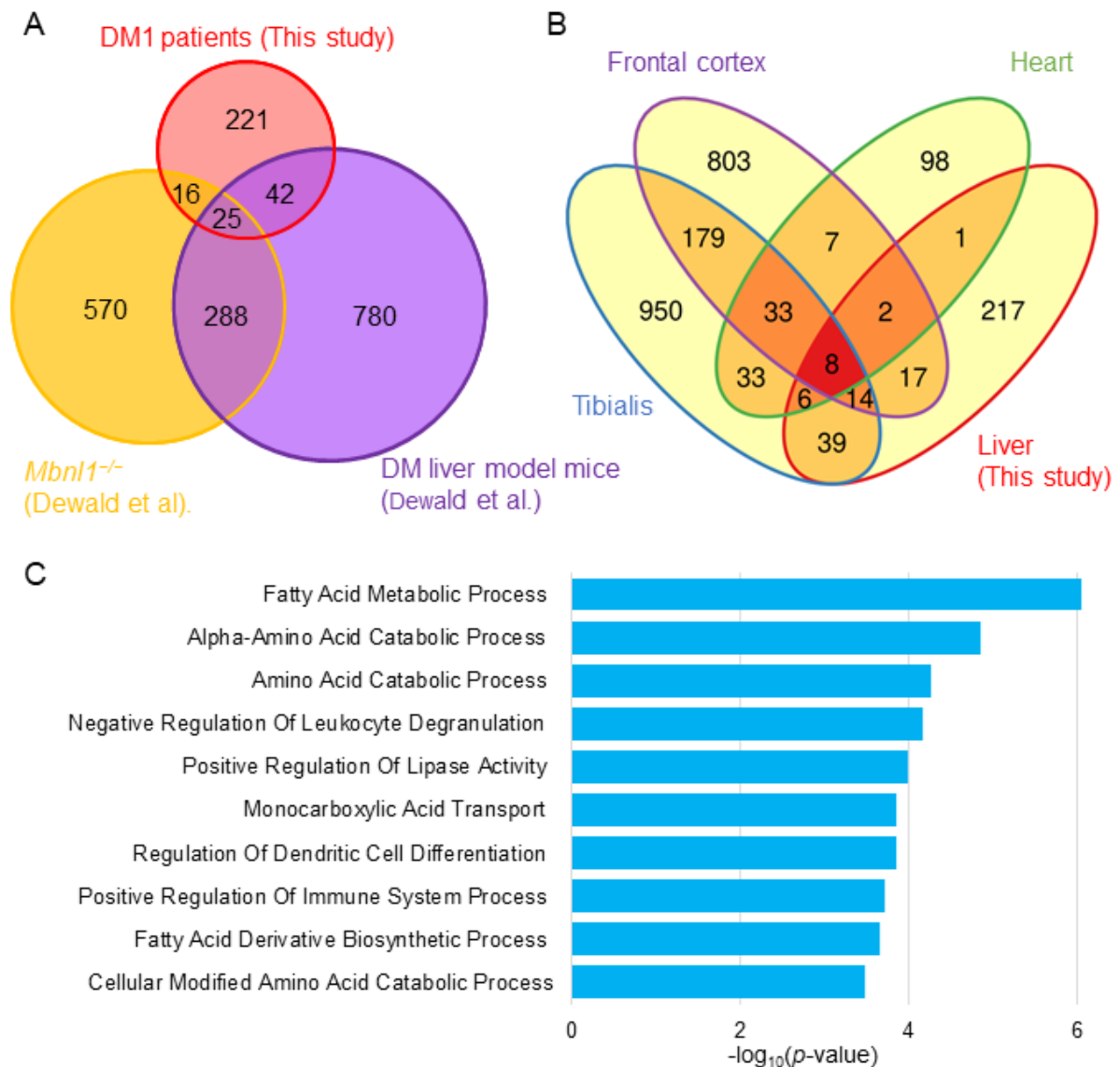
Representative fluorescent in situ hybridization combined with immunofluorescence (FISH-IF) images of liver cryosections from a disease control (upper panels) and a patient with DM1 (lower panels), showing CUG RNA foci (red) and MBNL1 protein localization (green). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, blue). Scale bar, 5 μ m.



Supplementary Figure 10. Detection of MBNL3 isoforms expressed in HepG2 cells.

(A) Western blot analysis of HepG2 cells transfected with siMBNL3 #1 and #2 targeting two distinct sequences in MBNL3. Red arrowheads indicated MBNL3 isoforms. Asterisks indicate non-specific bands that were not decreased by siMBNL3 treatment.

(B) Upper panel shows schematic diagram of MBNL3 transcript variants. Black arrows indicate the position of the primers to detect each transcript variant. A common reverse primer was used for amplifying the three transcript variants. Variants 5 and 7 encode an identical protein isoform. RT-PCR analysis of siRNA-treated HepG2 cell cDNA are showed in the lower panel. GAPDH was used as a reference for PCR amplification.



Supplemental Figure 11. Overlaps of previous studies.

(A) Venn diagram showing the overlap of mis-spliced genes identified in the livers of patients with DM1 in this study (red) with those identified in the livers of liver-specific DM1 mouse model (violet) and *Mbnl1*-knockout mice (yellow) in the study by Zachary Dewald *et al.*

(B) Venn diagram showing the overlap of mis-spliced genes identified in the livers of patients with DM1 in this study (red) with those identified in the tibialis (blue) and hearts (green) of patients with in the study by Eric Wang *et al.*, and in the frontal cortex (violet) of patients with DM1 in the study by Brittney Otero *et al.*

(C) Results of GO biological process analysis of the 217 mis-spliced genes uniquely identified in the livers of patients with DM1, as shown in the liver-only segment from (B).