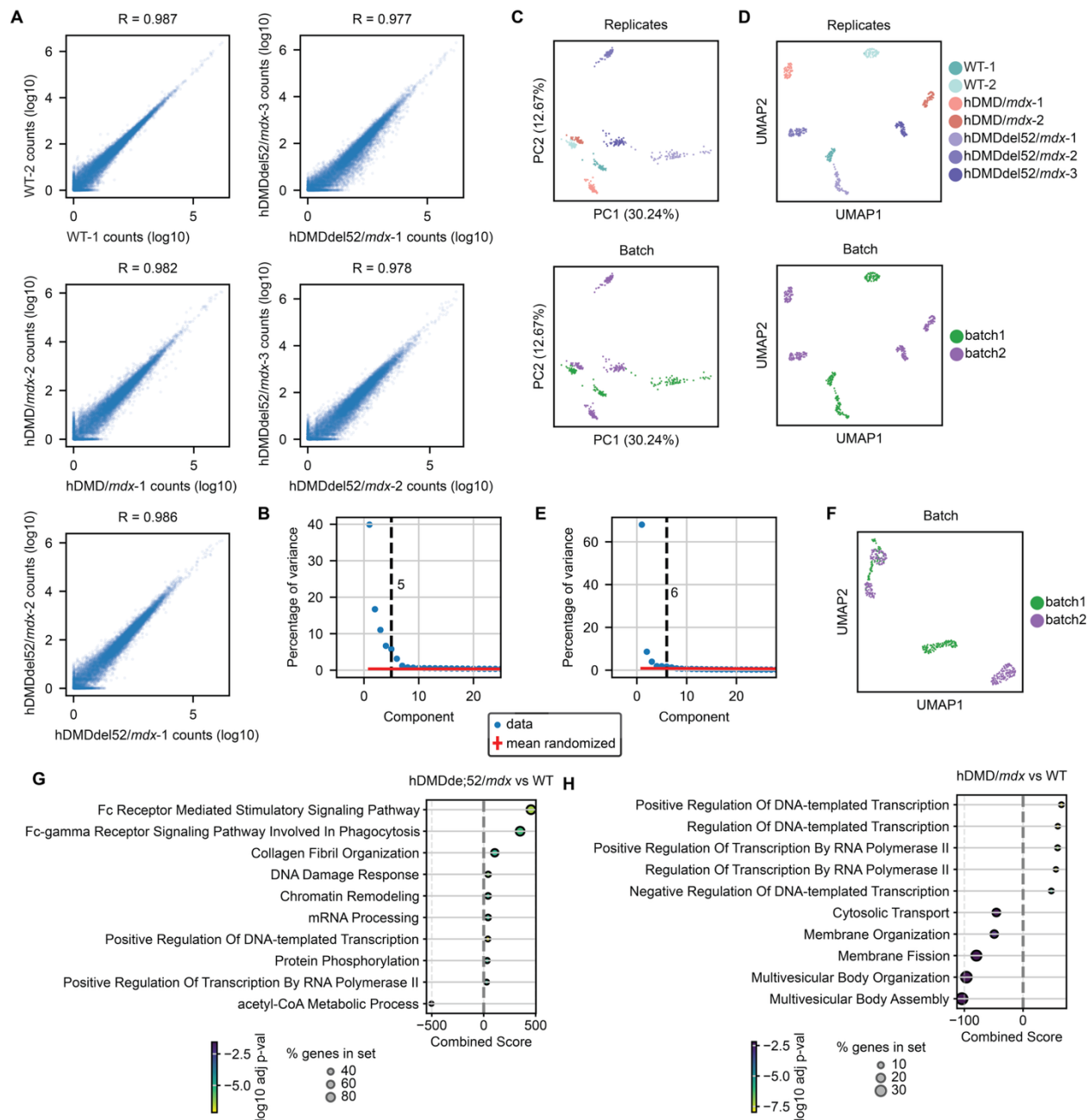


Supplementary Materials for

Compartment-specific remodeling of skeletal muscle in Duchenne muscular dystrophy

Clara Martínez Mir *et al.*

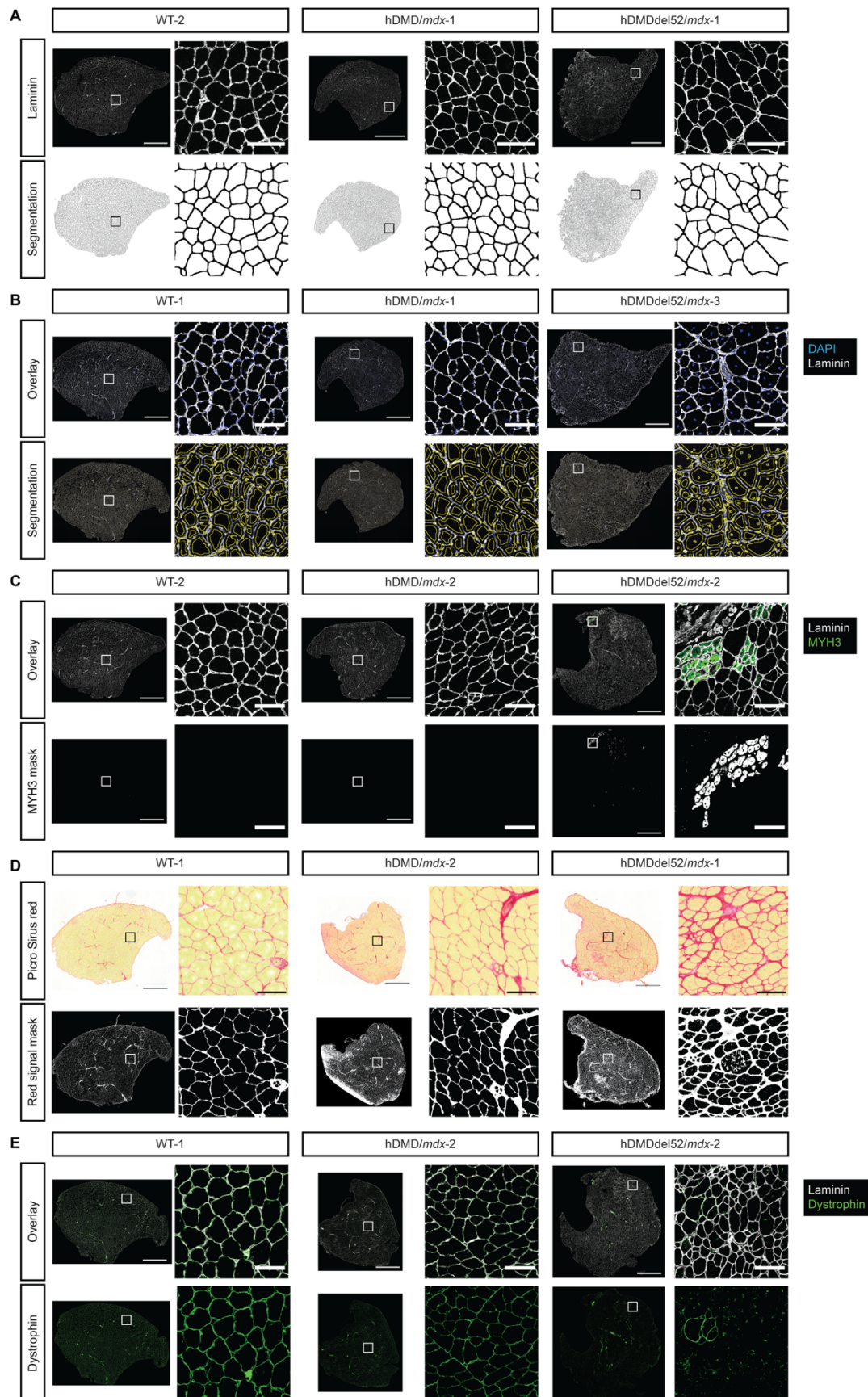
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Supplementary Fig. 1. TOMOseq on WT, hDMD/*mdx* and hDMDdel52/*mdx* TA muscles.

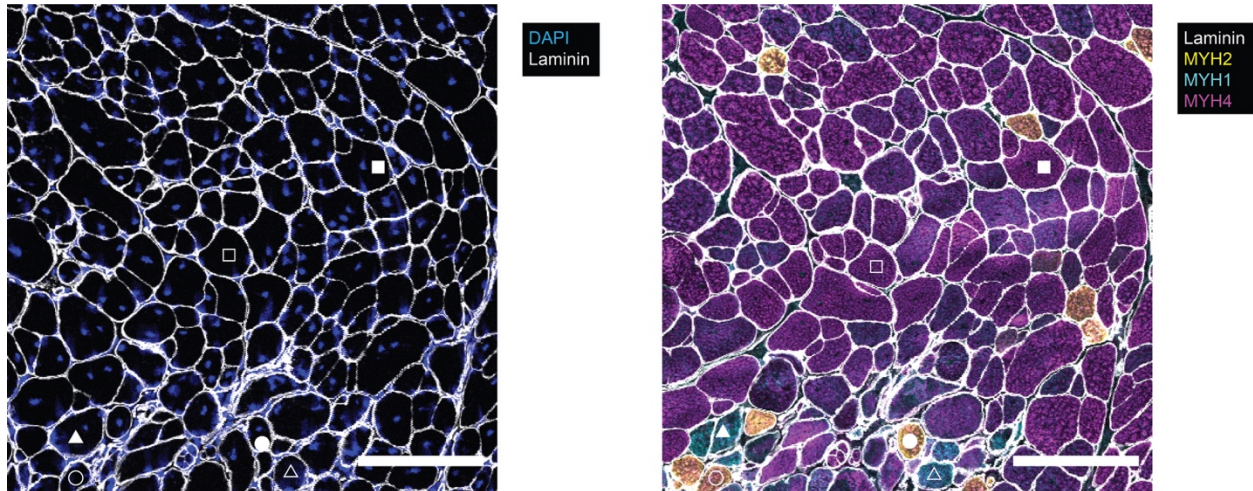
(A) Pearson correlation of total transcript counts data for each pair-wise comparison between biological replicates. (B) Scatterplots after PCA displaying n = 419 sections, where each point is a section. Colors represent biological replicate (top) or experimental batch (bottom) for each section (Supplementary Data 1). (C) Percentage of the variance explained by the principal component analysis (PCA) of the data (blue dots) and the randomized data (red crosses). Threshold (black dashed line) indicates the number of PCs that explain 1.2x more variance than the randomized data used for UMAP projection. (D) UMAP projection displaying n = 419 sections, where each point is a section. Colors represent biological replicate (top) or experimental batch (bottom) for each section (Supplementary Data 1). (E) Percentage of the variance explained by the Harmony-corrected PCs of the data (blue dots) and the randomized data (red crosses). Threshold (black dashed line) indicates the number of Harmony-corrected PCs that explain 1.2x more variance than the randomized data.

variance than the randomized data used for UMAP projection and Leiden clustering. **(F)** UMAP projection after Harmony correction displaying $n = 419$ sections, where each point is a section. Colors represent experimental batch for each section (Supplementary Data 1). **(G-H)** GO-term analysis for the DEGs between hDMDdel52/*mdx* and WT samples **(G)** or hDMD/*mdx* and WT samples **(H)**.



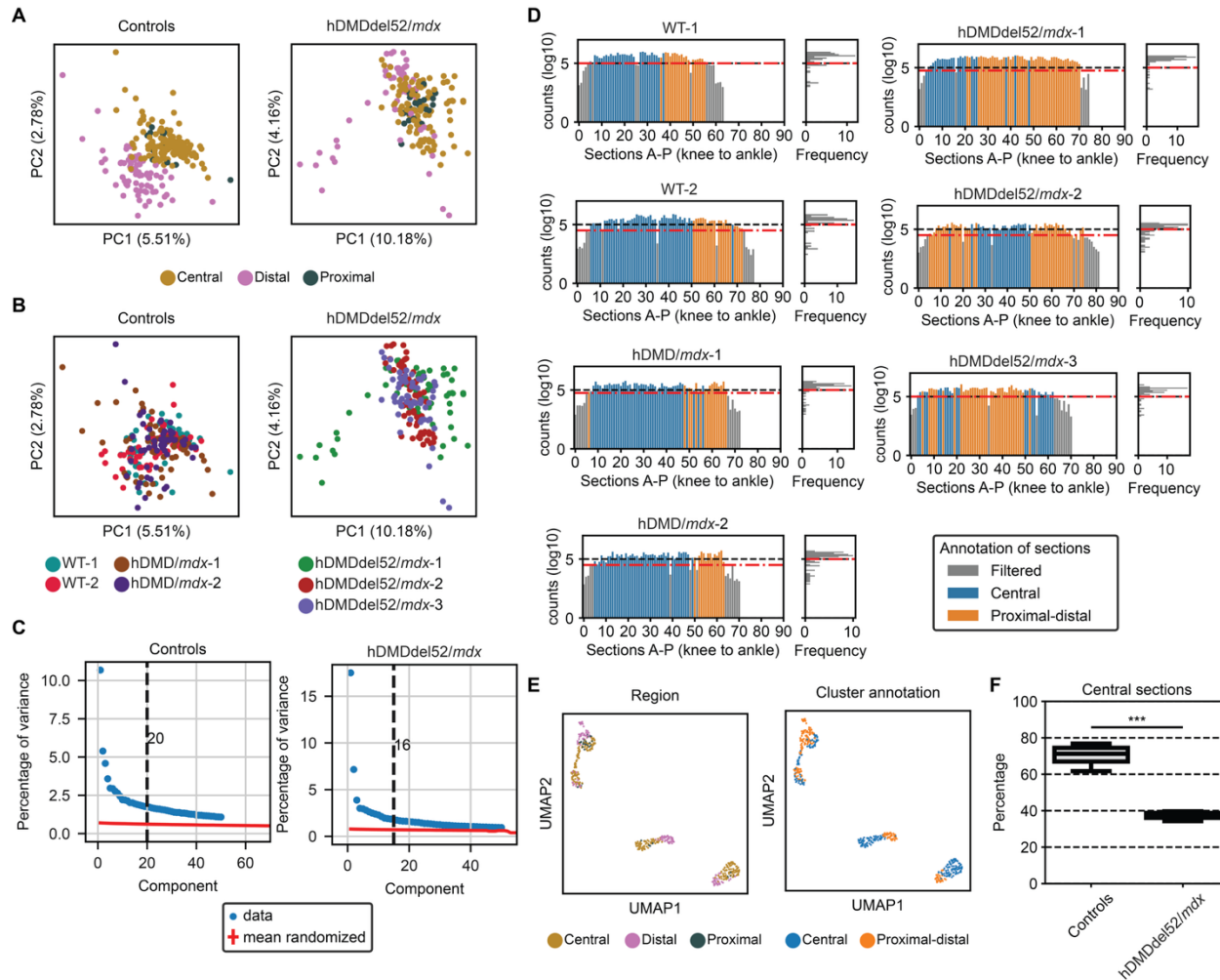
Supplementary Fig. 2. Histopathological features of TA muscles used for TOMOseq.

(A) Representative images of adjacent central sections (left) and zoom in on region of interest (right) of TA muscles used for TOMOseq to quantify CSA, (B) centralized nuclei, (C) MYH3 expression, (D) fibrosis with Picro Sirius red staining, and (E) dystrophin expression. One central tissue section was analyzed per muscle. Scale bar full sections, 1000 μm ; scale bar zoom in, 100 μm .



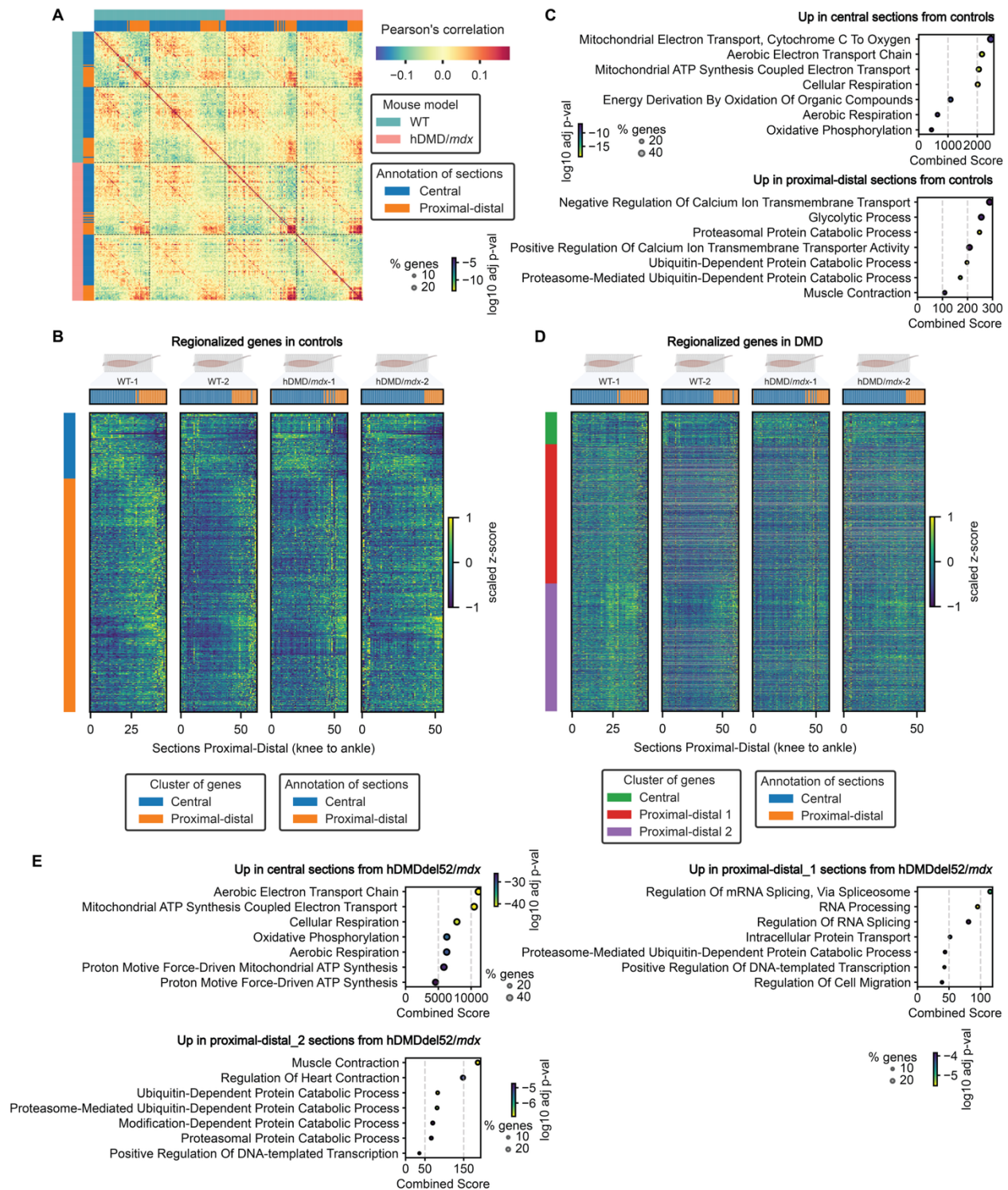
Supplementary Fig. 3. Analysis of centralized myonuclei across fiber types in hDMDdel52/*mdx* TA muscles used for TOMOseq.

Representative consecutive cross-sections of hDMDdel52/*mdx* TA muscles used for spatial transcriptomic analysis. Adjacent serial sections were stained with laminin and DAPI (left panel) or with laminin and fiber type markers MYH1, MYH2, and MYH4 (right panel) to assess the presence of centralized myonuclei across different myofiber populations. Symbols indicate representative centrally nucleated fibers corresponding to MYH1-positive (triangles), MYH2-positive (circles), and MYH4-positive (squares) fibers identified across serial sections. Scale bar, 150 μm .



Supplementary Fig. 4. Regionalization of gene expression in controls and hDMDdel52/mdx tissues.

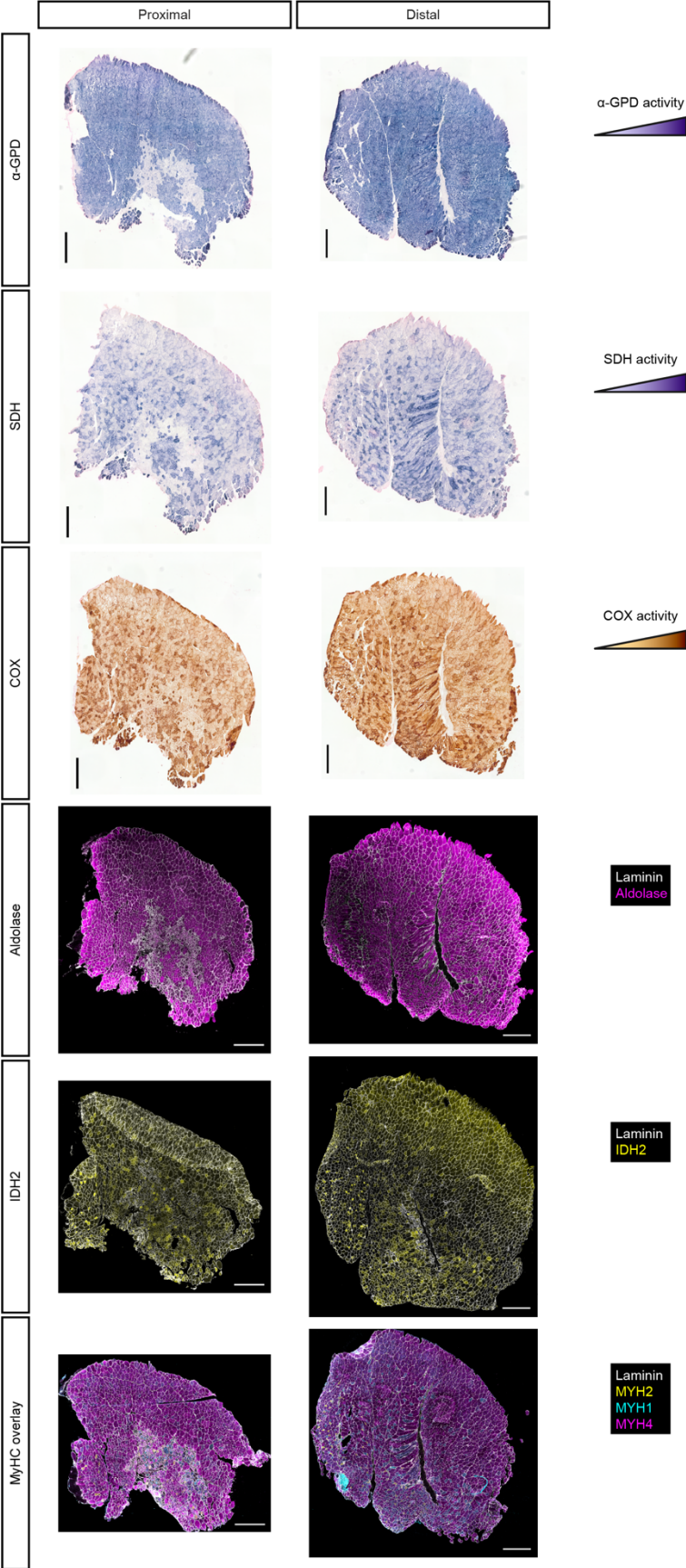
(A) Scatterplots after PCA displaying $n = 228$ (controls, left) and $n = 191$ (hDMDdel52/mdx, right) sections, where each point is a section. Colors represent manual region annotation based on anatomical features of controls (left) or hDMDdel52/mdx samples (right) for each section (Supplementary Data 2,3). (B) Scatterplots after PCA displaying $n = 228$ (controls, left) and $n = 191$ (hDMDdel52/mdx, right) sections, where each point is a section. Colors represent biological replicate of controls (left) or hDMDdel52/mdx samples (right) for each section (Supplementary Data 2,3). (C) Percentage of the variance explained by the PCA of the data (blue dots) and the randomized data (red crosses) on controls (left) and hDMDdel52/mdx samples (right). Threshold (black dashed line) indicates the number of PCs that explain 1.2x more variance than the randomized data used for Leiden clusters. (D) UMAP projection displaying $n = 419$ sections after batch correction with Harmony, where each point is a section. Colors represent manual region annotation based on anatomical features (left) or Leiden cluster annotation (right) for each section (Supplementary Data 1,2,3). (E) Distribution of the percentage of sections annotated as central in controls or hDMDdel52/mdx. Statistical significance was assessed using a two-sided t-test. Significance levels: *** < 0.001. Box plot characteristics: center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; circles, outliers.



Supplementary Fig. 5. Spatial transcriptomics in TAs of hDMDdel52/*mdx* and control mouse models.

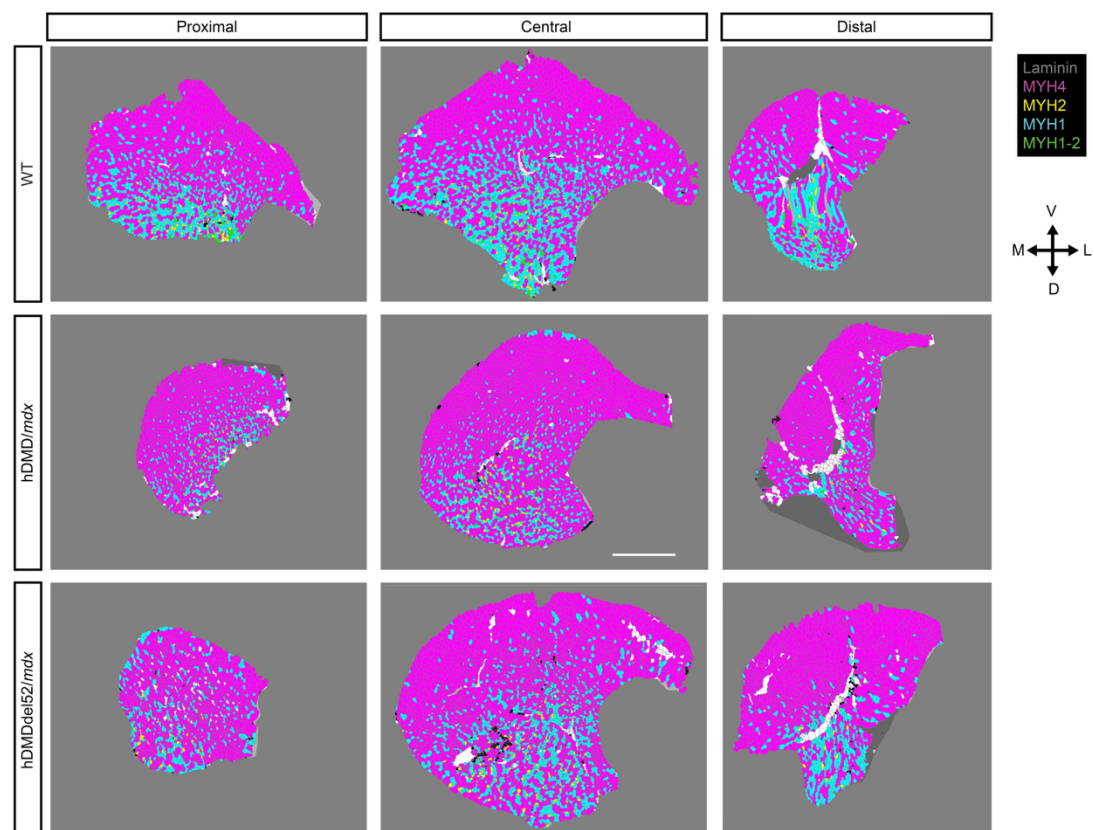
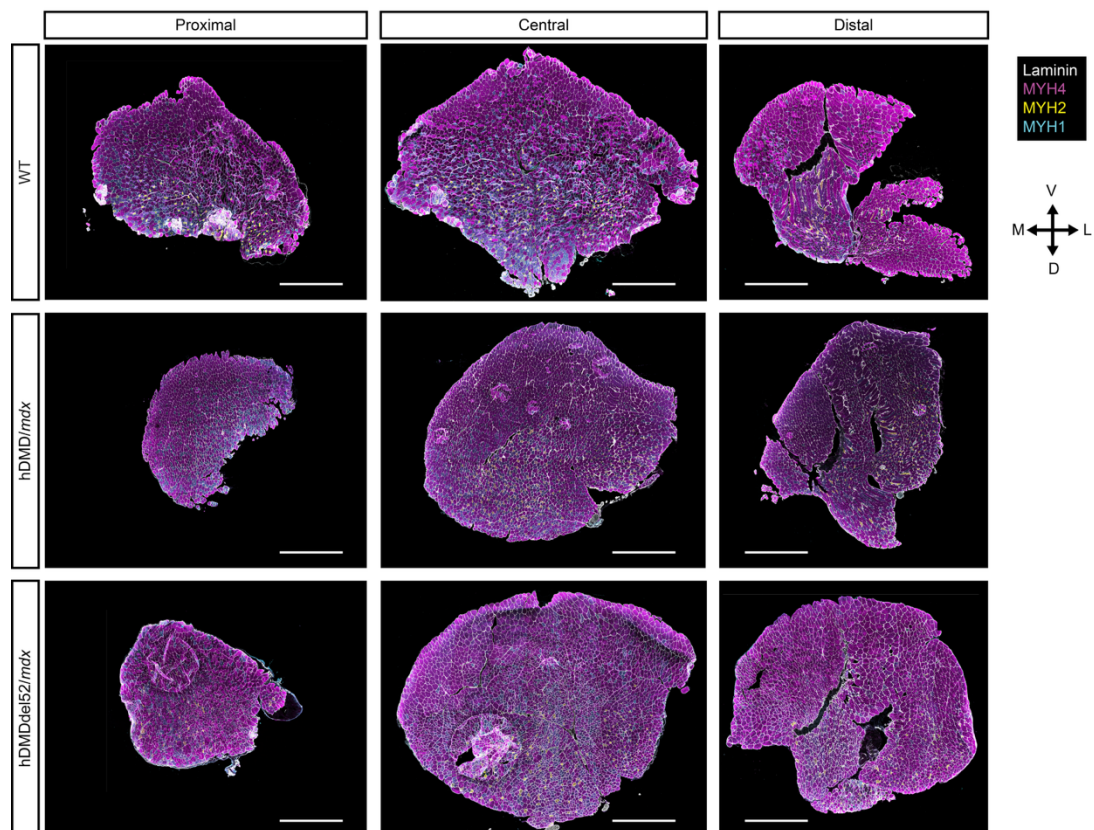
(A) Pairwise correlation heatmap of individual sections among all genes detected at more than four transcripts in at least three consecutive sections in all control samples. Sections are colored by Leiden cluster annotation and mouse model. (B,D) Heatmaps display the average proximal-distal expression patterns of the 733 DEG detected in control regionalization (B) and 1601 DEG detected in hDMDdel52/*mdx*

regionalization (**D**) in all control samples. Colors represent clusters of genes and annotation of sections. (**C,E**) GO-term analysis of the up-regulated genes in central and proximal-distal sections in control samples (**C**) or in central, proximal-distal 1 and proximal-distal 2 sections in hDMDdel52/*mdx* samples (**E**). Muscle diagrams in **B** and **D** were created with BioRender.com.



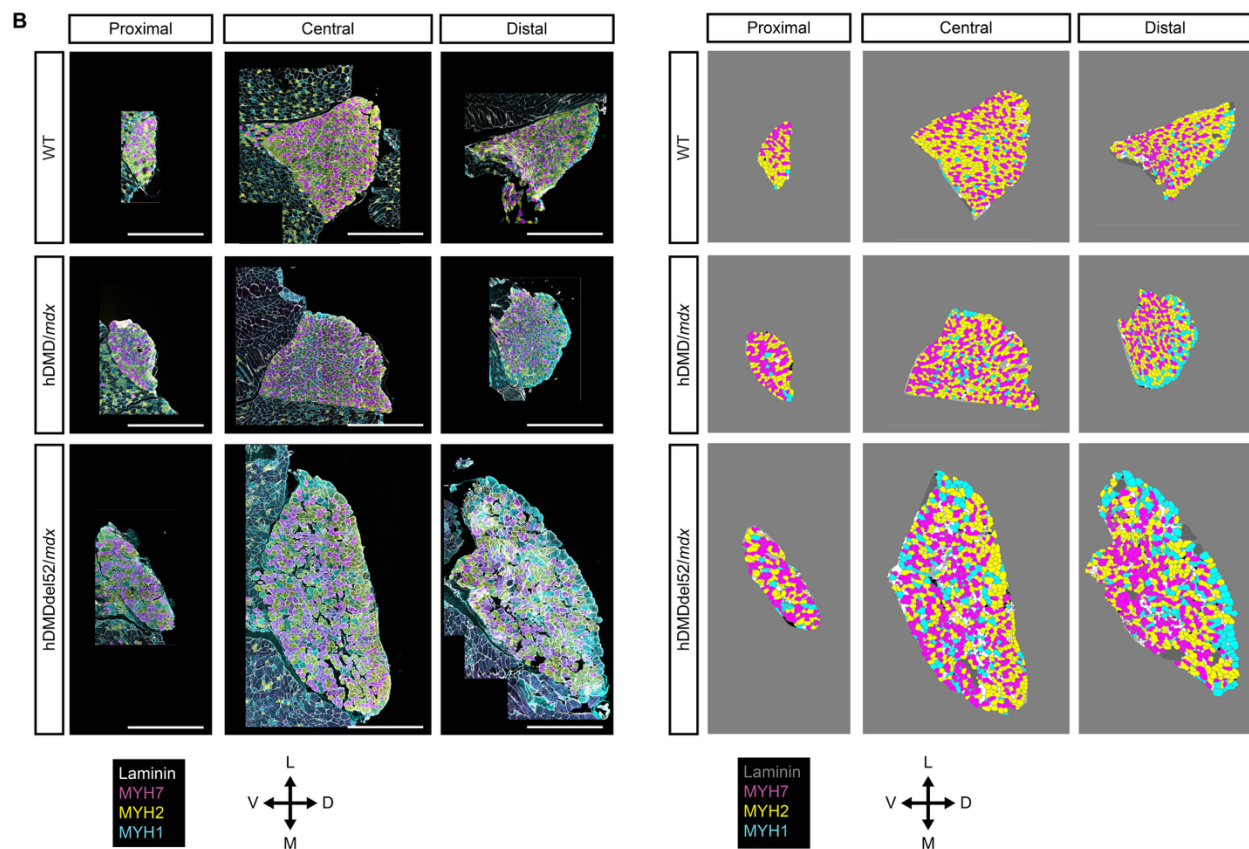
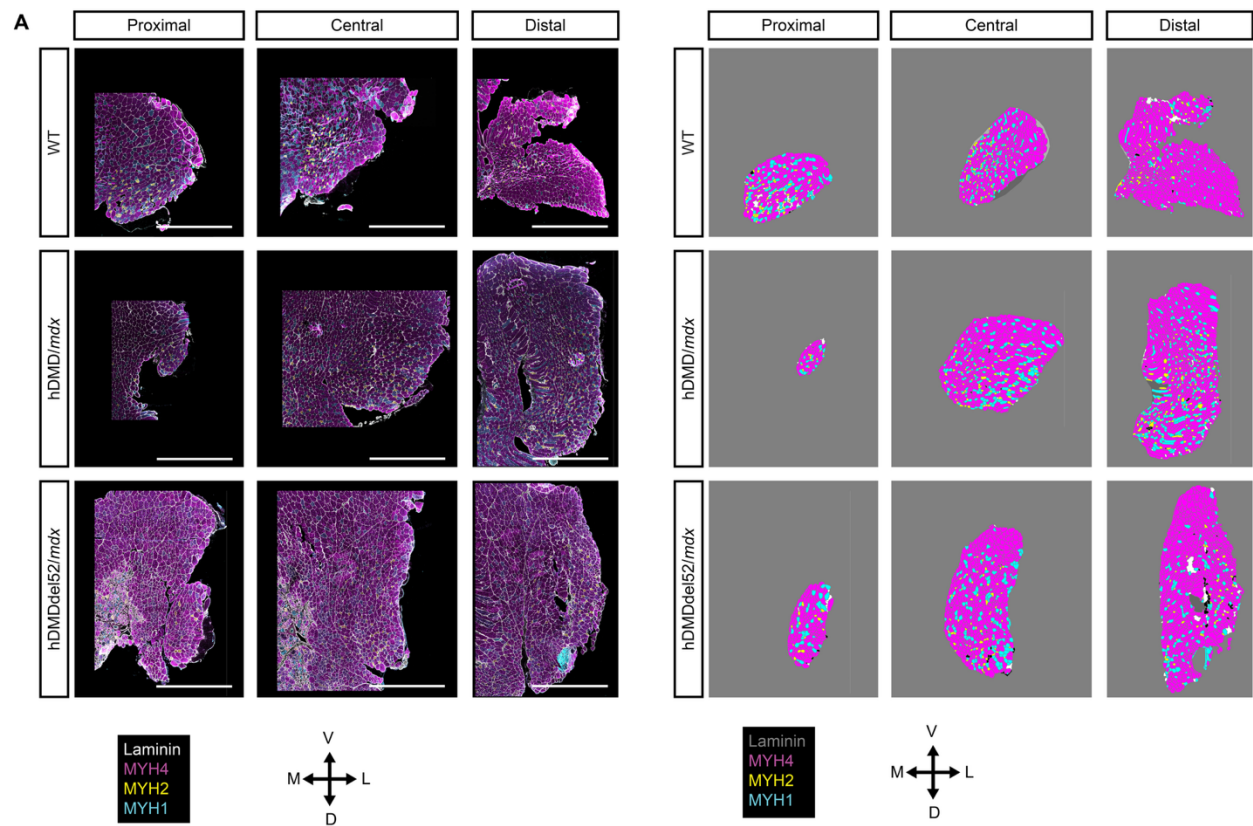
Supplementary Fig. 6. Metabolic changes in proximal and distal regions of hDMDdel52/*mdx* TA muscles.

Representative images of proximal and distal TA muscle sections from hDMDdel52/*mdx* mice showing staining for metabolic enzymes: SDH, COX, IDH2 (oxidative markers), α -GPD, and aldolase (glycolytic markers), alongside fiber type markers MYH1, MYH2, and MYH4. Scale bar, 500 μ m.



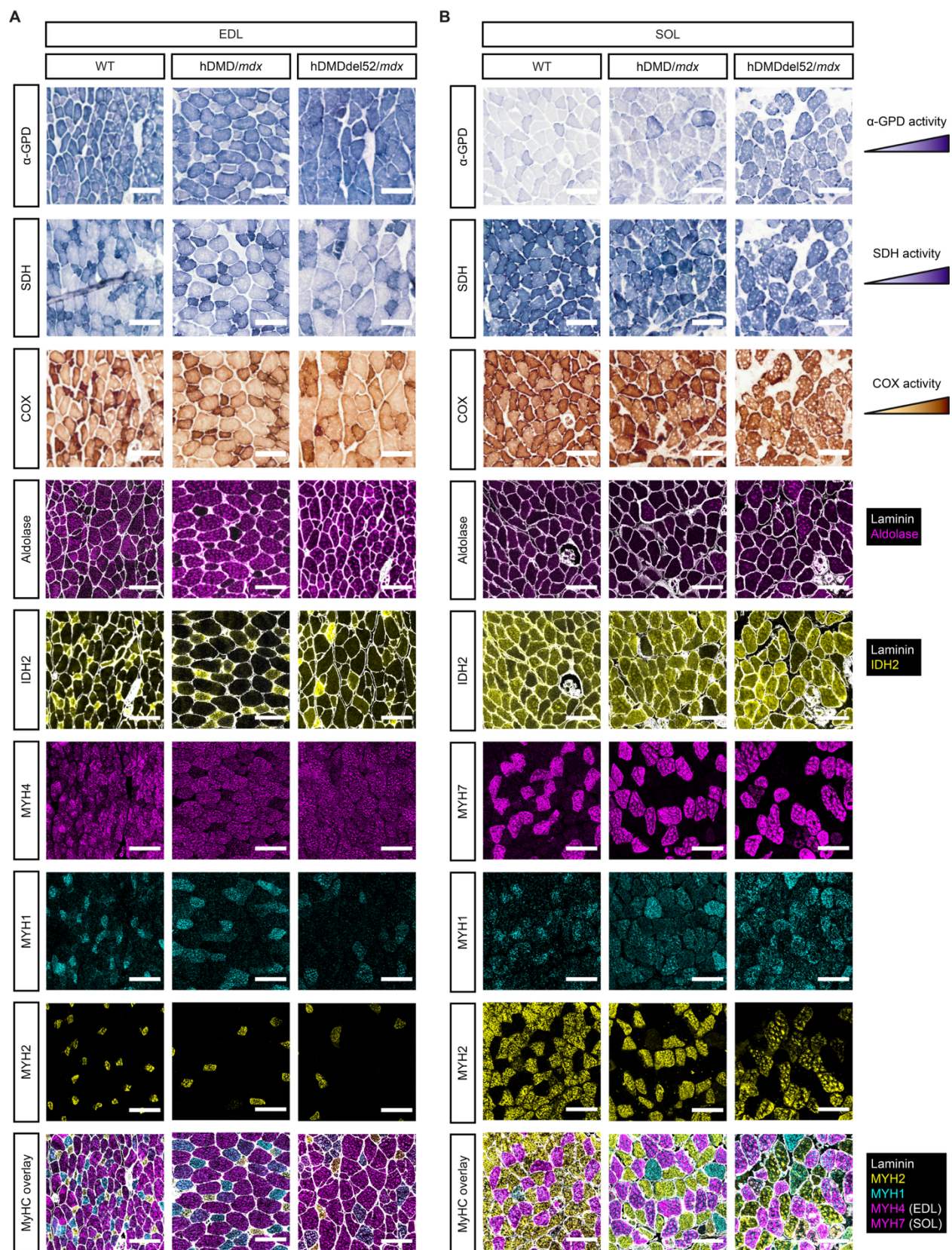
Supplementary Fig. 7. MyHC distribution in TA muscles.

Representative images of immunofluorescence staining of fiber types (top) and assigned fiber type based on clustering analysis (bottom) in proximal, central and distal areas of WT, hDMD/*mdx* and hDMDdel52/*mdx* TA muscles. Yellow, type 2a myofiber (MYH2); cyan, type 2x myofiber (MYH1); magenta, type 2b myofiber (MYH4); grey, laminin subunit α -1. Scale bar, 1000 μ m. Arrows display lateral-medial and dorsal-ventral orientation of the tissue sections. L, lateral; D, dorsal; M, medial; V, ventral.



Supplementary Fig. 8. MyHC distribution in EDL and SOL muscles.

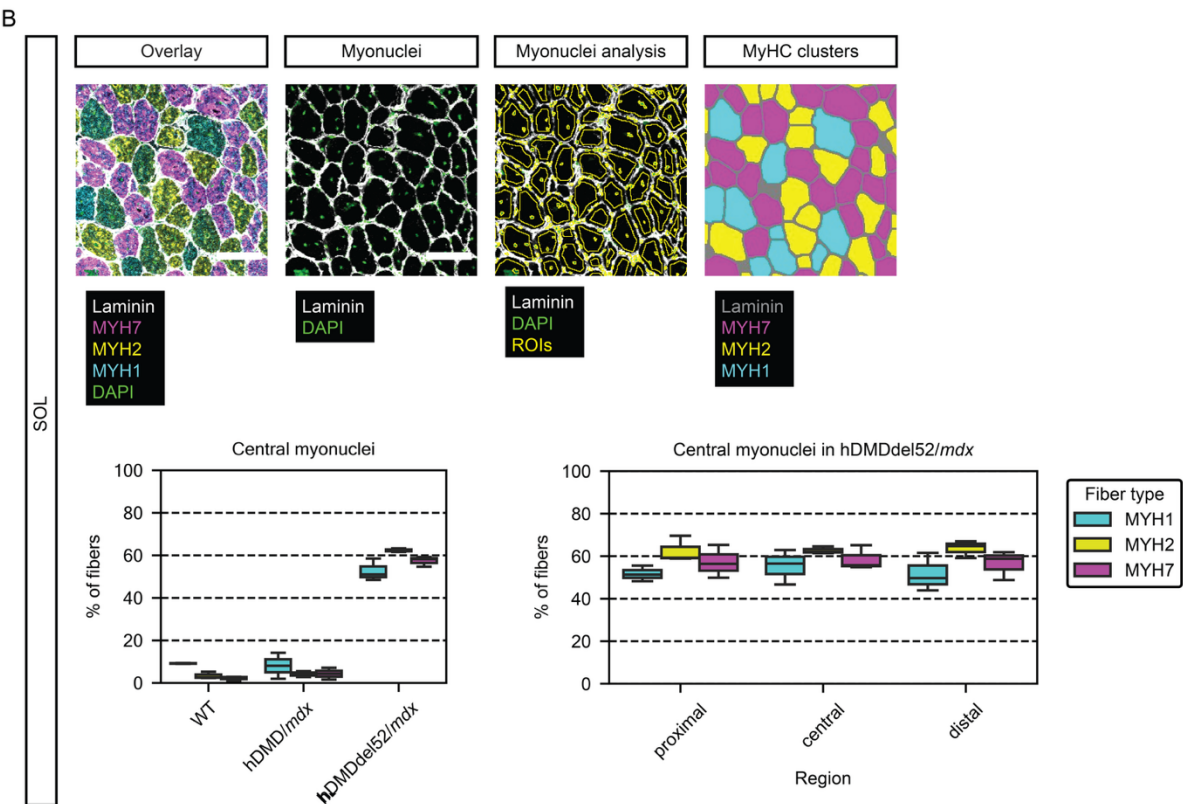
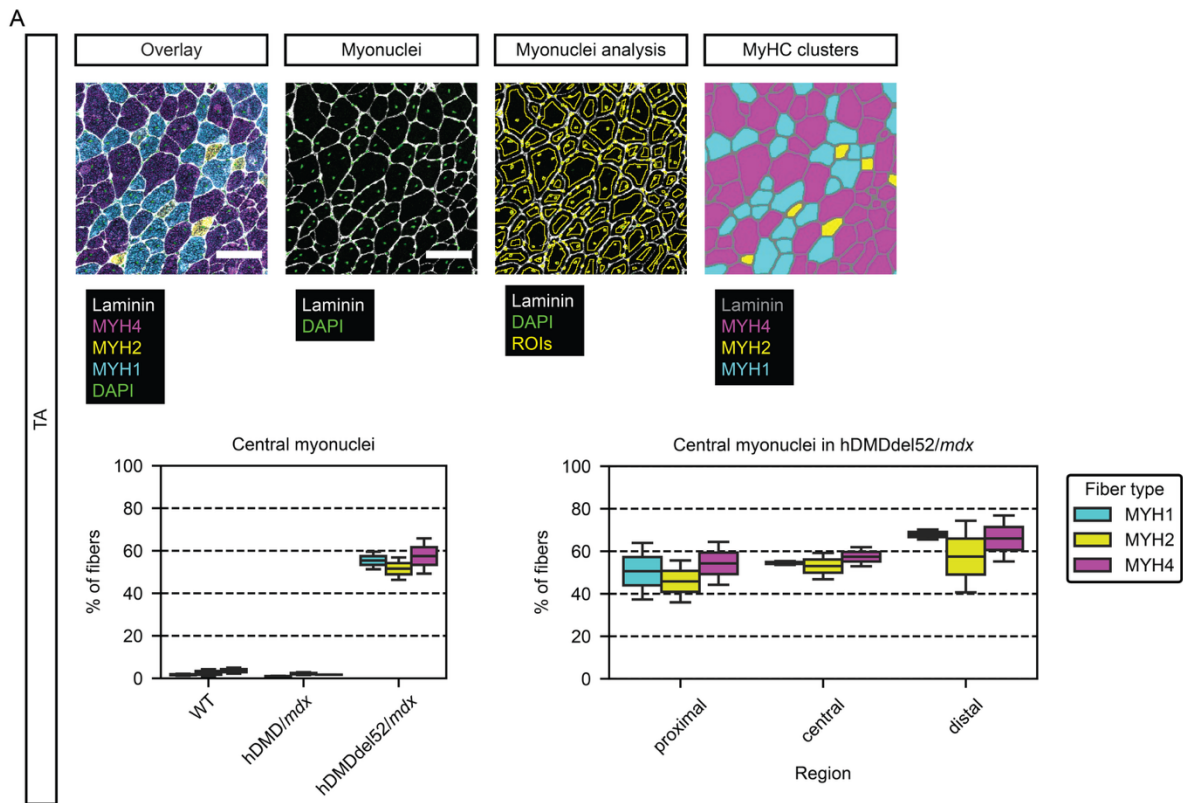
(A,B) Representative images of immunofluorescence staining of fiber types (left) and assigned fiber type based on clustering analysis (right) in proximal, central and distal areas of WT, hDMD/*mdx* and hDMDdel52/*mdx* EDL (**A**, top) and SOL (**B**, bottom) muscles. Yellow, type 2a myofiber (MYH2); cyan, type 2x myofiber (MYH1); magenta, type 2b myofiber (MYH4) or type 1 myofibers (MYH7); grey, laminin subunit α -1. Scale bar, 1000 μ m. Arrows display lateral-medial and dorsal-ventral orientation of the tissue sections. L, lateral; D, dorsal; M, medial; V, ventral.



Supplementary Fig. 9. Metabolic changes in EDL and TA muscles of hDMDdel52/*mdx* and controls mouse models.

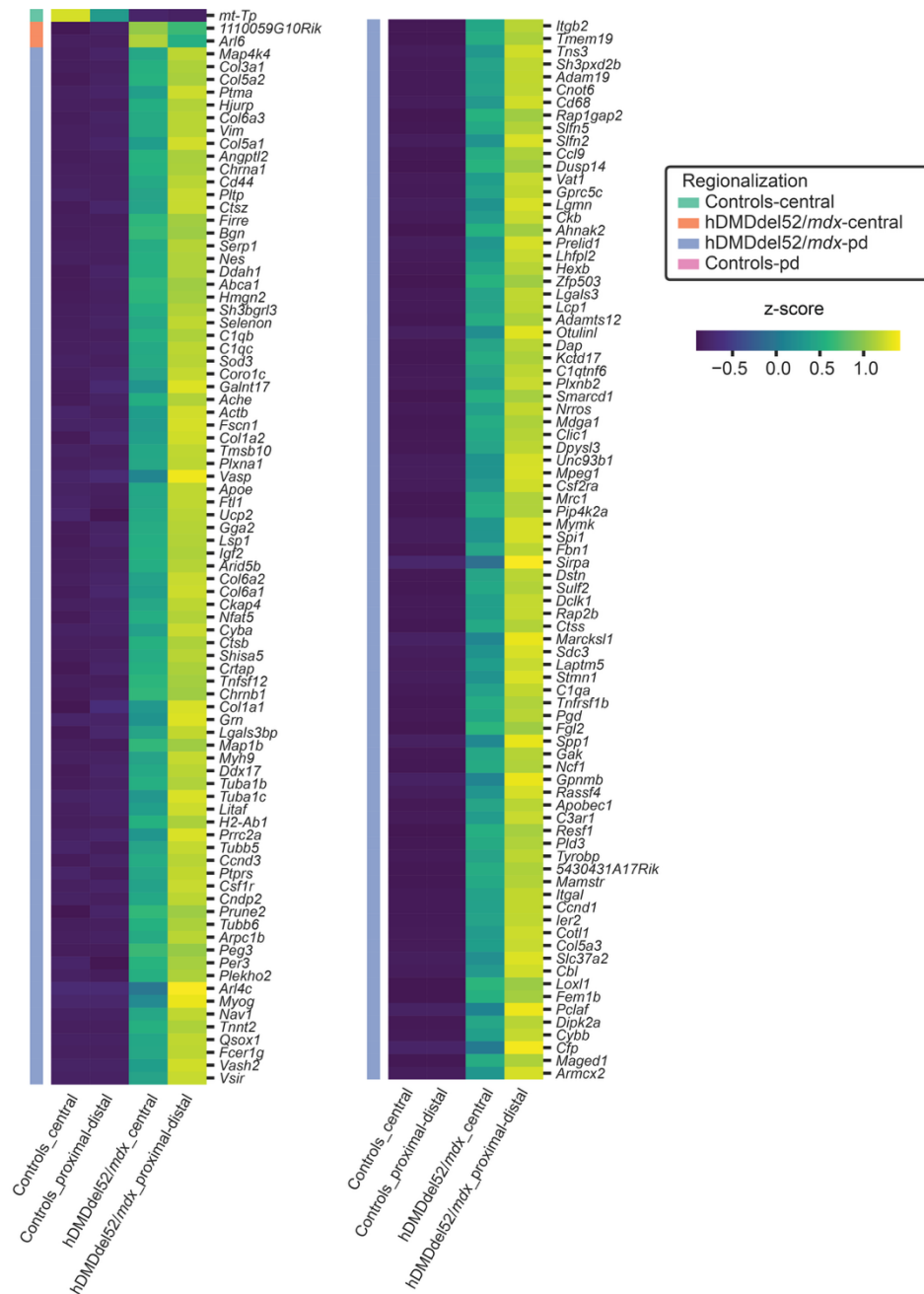
(A,B) Representative images of EDL (A) and SOL (B) muscle sections from WT, hDMD/*mdx* and hDMDdel52/*mdx* mice showing staining for metabolic enzymes: SDH, COX, IDH2 (oxidative markers), α -GPD, and aldolase (glycolytic markers), alongside fiber type markers MYH1, MYH2, and MYH4. Scale bar, 100 μ m.

100 um scale bar!



Supplementary Fig. 10. Quantification of centralized myonuclei across fiber types in TA and SOL muscles.

(A,B) Representative consecutive cross-sections of hDMDdel52/*mdx* TA (A) or SOL (B) muscles stained with laminin, DAPI and fiber type markers MYH1, MYH2, and MYH4 (TA) or MYH7 (SOL) to assess the presence of centralized myonuclei across different myofiber populations. Quantification of myofiber containing centralized myonuclei (any fiber containing one or more myonuclei in their center were accounted for) in WT (n=6), hDMD/*mdx* (n=5), and hDMDdel52/*mdx* (n=6) in TA sections (A, left plot) and in WT (n=9), hDMD/*mdx* (n=6), and hDMDdel52/*mdx* (n=9) in SOL sections (B, left plot). Quantification of myofiber containing centralized myonuclei (any fiber containing one or more myonuclei in their center were accounted for) by region in hDMDdel52/*mdx* TA sections (proximal, n=2; central, n=2; distal, n=2) (A, right plot) and SOL sections (proximal, n=3; central, n=3; distal, n=3) (B, right plot). Scale bar, 100 μ m.



Supplementary Fig. 11. Previously identified differentially expressed genes exhibiting region-dependent disease effects in hMDMdel52/*mdx* muscle.

Heatmaps showing the average expression patterns of 167 genes that were identified as globally differentially expressed between control and hMDMdel52/*mdx* muscles and additionally exhibited significant region-disease interaction effects. Genes are grouped according to stronger enrichment in controls or hMDMdel52/*mdx* sections. Additional color bars indicate whether expression was enriched in proximal-distal (pink for controls, blue for hMDMdel52/*mdx*) or central (green for controls, orange for hMDMdel52/*mdx*) compartments. Expression values represent z-score transformed group means across control and hMDMdel52/*mdx* proximal-distal and central sections. Color bars indicate whether genes showed enhanced disease-associated dysregulation in proximal-distal or central compartments.

Supplementary Table 1. Characterization of disease progression makers in hDMDdel52/*mdx* TA muscles used for TOMOseq.

Centralized myonuclei, mean number of centralized myonuclei per myofiber; Myofibers with centralized myonuclei (%), percentage of myofibers containing centralized myonuclei; MYH3 signal (%), percentage of MYH3-positive area; Fibrotic deposition (%), percentage of fibrotic deposition determined by Picro Sirius red staining.

	Centralized myonuclei	Myofiber with centralized myonuclei (%)	MYH3 signal (%)	Fibrotic deposition (%)
hDMDdel52/<i>mdx</i>-M1	0.920913	63.69427	0.4	33.5
hDMDdel52/<i>mdx</i>-M2	0.690436	49.67478	0.5	65.8
hDMDdel52/<i>mdx</i>-M3	1.083414	68.18182	0.2	36.1