Fig. S1. Analysis of post mortem skeletal muscle specimens and correlation with disease severity

(A) Muscle mass of tibialis anterior (TA), gastrocnemius (GAS) and quadriceps (QUAD) muscles relative to body weight, from 5 week old wildtype and Mtm1<sup>+/−</sup> mice. Unpaired t-test with Welsh’s correction performed. (B) Correlation analysis of TA muscle mass/body weight ratio, relative to disease severity score (DSS) for 5 week old Mtm1<sup>+/−</sup> (red) and wild type mice (black). Line of best fit and 95%CI highlighted in black for all mice (r= -0.7241; p < 0.0001), line of best fit for Mtm1<sup>+/−</sup> alone (red line). Muscle mass presented alone (GAS-C; TA-E) or relative to body weight (GAS-D; TA-F), for wildtype and Mtm1<sup>+/−</sup> mice injected with 6.25, 12.5 or 25mg/kg DYN101-m targeting murine Dnm2 reduction. (G) TA/ body weight ratio represented relative to DSS for wild type (black), Mtm1<sup>+/−</sup> mice injected with 6.25 (orange), 12.5 (green), 25mg/kg (blue) DYN101-m. 5 week old Mtm1<sup>+/−</sup> mice from (B) reproduced here for comparison purposes only (red dots, shading), and are not included in statistical analysis. Data represented as a violin plots (A,C-F), individual mouse data shown. Statistical analysis: (A) Unpaired t-test with Welsh’s correction; Spearman correlation tests performed for (B), (G) Ordinary 1-way ANOVA followed by Dunnett’s multiple comparisons test performed for (C)-(F). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
Table S1. Previous disease severity scoring system (Tasfaout, Buono et al. 2017). A scoring system was set up to evaluate the clinical evolution of six centronuclear myopathy features. Difference of body weight between \( Mtm1^{-/-} \) versus WT littermate, ability to perform the hanging test, walking manner, presence or absence of ptosis and kyphosis and breathing difficulties (frequency and amplitude evaluation based on clinical observations) are recorded and a score of 0, 0.5 or 1 is given to each clinical readout. The sum represents the DSS. The higher the DSS, the more severe the phenotype, minimum 0 (healthy mouse), maximum 6 (severely affected mouse) (Tasfaout, Buono et al., 2017).

| Disease scoring category | DSS Description | Score |
|--------------------------|-----------------|-------|
| **Body weight**          | Score 0-1       | 0: 0-1g  
|                          | Difference in body weight between \( Mtm1^{-/-} \) mouse and wildtype littermate | 0.5: >1-2g  
|                          |                 | 1: >2g  |
| **Ptosis**               | Score 0 or 1    | 0 : No  
|                          | Drooping of the eyelid | 1 : Yes  |
| **Hanging test ability** | Score of 0-1, representing 0-60 seconds hanging time. | 0: 60s  
|                          |                   | 0.5: 5-60s  
|                          |                   | 1: <5  |

Adapted Yang et al. Immunology and Microbiology (2007)
| Kyphosis            | Score 0-1   | Curvature of the spine | 
|---------------------|-------------|------------------------|
|                     | Score 0: no curvature of the spine | 
|                     | Score 0.5: mild curvature of the spine | 

![Image of kyphosis scoring system](image)

Score 0: normal use of hindlimbs

Score 0.5: splayed use of hindlimbs

Score 1: loss of use of hindlimbs

Breathing difficulties: Score 0 or 1

Frequency and amplitude evaluation based on clinical observations

0: no breathing alternation

1: breathing alternation

Walking difficulties: Score 0-1

Ability to use hindlimbs

Maximum score: 6
Table S2. Husbandry and housing conditions for training and test cohorts

|                  | TRAINING COHORT                                                                 | TEST COHORT                                                                 |
|------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Training cohort  | Training cohort data was generated from historical data of Mtm1<sup>−/−</sup> mice located at the IGBMC animal facility, Illkirch, France (Koch, Buono et al. 2020). N=38 mice | Test cohort data: In vitro fertilization (IVF) was performed (Janvier Laboratories, Rennes, France) with samples taken from Mtm1<sup>−/−</sup> mice from the colony used to generate the training cohort. Following IVF and colony generation, mice were transferred to Chronobiotron animal facility (Strasbourg, France), for colony amplification and phenotyping (test cohort). N=20 mice |
| Husbandry        | Light cycle: 12hr/12hr                                                         | Janvier Laboratories: Light cycle: 12hr/12hr                                |
| conditions       | Temperature: 19-22°C                                                          | Temperature: ±21°C ± 2°C                                                   |
|                  | Enrichment: nests to help nesting.                                             | Enrichment: all cages contain sticks to gnaw (Aspenbrick). Breeding cages were systematically enriched with nests. |
| Housing          | Light cycle: 12hr/12hr                                                         | Chronobiotron animal facility: Light cycle: 12hr/12hr                      |
| conditions       | Temperature: 19-22°C                                                          | Temperature: ±21°C ± 2°C                                                   |
|                  | Enrichment: nests                                                              | Enrichment: nests and cardboard tunnel                                      |
| Weaning age      | 3 weeks of age                                                                 | 3 weeks of age                                                             |
| Description of   | Cage type 2: Individually ventilated cages: no Floor surface: 370 cm<sup>2</sup> | Cage type 2: Individually ventilated cages: no Brand: Tecniplast (#1264C Eurostandard) or Erhet |
| cage             | For housing during experimental procedure, cage type 3 was rarely used because of the larger size and increased distance to access food | Floor surface: 370 cm<sup>2</sup> Dimensions: L x W x H : 268 x 215 x 141 mm |
|                  | Access to food and water: Gel diet was added inside the cage to facilitate access. Food pellets were added at multiple places inside the cages. | Access to food and water: Gel diet was added inside the cage to facilitate access. Food pellets were added at multiple places inside the cages. Longer nozzles on water bottles were used to reduce the distance between floor and bottle nozzle. |
| Animal density   | 4 animals per cage: type II cage 5 animal per cages: type III cage             | 4 animals per cage: type II cage 5 animal per cages: type III cage          |
| Genotype ratio   | Typically 1 or 2 wildtype mice were housed in each cage with Mtm1<sup>−/−</sup> mice from the same litter, whenever feasible. |                                                                                           |
| Change frequency | Once per week                                                                   |                                                                                           |
| Diet             | Food composition: SAFE® gel diet breeding                                       | SAFE® gel diet water                                                         |
|                  | Cereals 17.3%                                                                  | Cereals 17.3%                                                               |
|                  | Proteins 6.6%                                                                  | Proteins 6.6%                                                               |
|                  | Vitamins, minerals 1.2%                                                        | Vitamins, minerals 1.2%                                                     |
|                  | Fibers 1.4%                                                                     | Fibers 1.4%                                                                |
|                  | Water 72.9%                                                                    | Water 98.2%                                                                |
Supplementary Materials and Methods

Joint Models for Disease Severity Scores. Joint models for disease severity scores are presented in the associated manuscript. The mice where followed over a period $[0; \tau]$. For each subject $i$, we observe the following:

- Longitudinal measures: $\{y_{ij}; j = 1, ..., n_i\}$ at times $\{t_{ij}; j = 1, ..., n_i\}$
- Survival time and indicator:
  $T = \min(ST, C)$ and $\delta = I(ST \leq C) = \begin{cases} 1 & \text{if uncensored observation} \\ 0 & \text{if censored observation} \end{cases}$

The longitudinal measurements and time to event were jointly measured via a latent bivariate process, which was realized independently in each subject $W_i = \{W_{1i}, W_{2i}\}$

The longitudinal sub-model is defined as: The observed response $y_{ij}$ scaled between 0 and 1 ($i = 1, ..., M$ and $j = 1, ..., n_i$), is defined as $y_{ij} \sim \text{Beta}(a_{ij}, b_{ij})$; where the beta distribution is defined by the parameters $a_{ij}$ and $b_{ij}$, which are defined, respectively, by the mean $\mu_{ij}$ and “the sample size” $\nu$ of the distribution as follows:

$a_{ij} = \mu_{ij} \ast \nu$

$b_{ij} = (1 - \mu_{ij}) \ast \nu$

The parameter $\nu$ is estimated from the data, and the mean $\mu_{ij}$ is defined as a mixed model with logit-link function, where $T$ is a constant to center time:

$\chi_{ij} = \alpha_i + \beta \ast (T + t_{ij})$

$\mu_{ij} = \frac{1}{1 + \exp(-\chi_{ij})}$

The equation of the mean contains a random effect on the intercept $W_{1i} = \alpha_i \sim N(0, \sigma_a^2)$. In turn, the survival sub-model is then defined via a Weibull survival model:

$S(t_{ij}) = \exp \left(-\bigl(\zeta_i \ast t_{ij}\bigr)^k\right)$

Where:

$\zeta_i = \exp(-W_{2i})$

$W_{2i} = \gamma \ast \alpha_i$

Where $\gamma$ expresses the induced association. A negative value of gamma implies that the larger value of the DSS Score the smaller the probability to stay in the study. A positive value would however imply the opposite. For simplicity in estimation, $k = 1$ in all estimations of the model indicating a constant failure rate. The model for the evolution of weight over time is similar to the one proposed here over.

The main difference lies in the equation for the mean. Indeed, the equation is assumed to be a linear gaussian model that evolves a the square root of time rather than a beta model.

The disease severity data from individual mice are included only until the point of death. The death of the mouse is then factored into the joint longitudinal survival model for disease severity scores. The above model used here allows the expected range to be presented following the correction for limited sample size, variability, and survival differences. The output is presented as the line of best fit (black line) and prediction intervals (shaded zone). A correct model fit occurs when 95% of the observed individual data is contained, for each time point, in the intervals depicted. Individual data from all living mice is shown as an overlay of the model to confirm this point on each graph, and thus the validity of the model.
Standard Operating Procedure for Disease Severity Score

This SOP describes the disease severity scoring (DSS) system, based on the original (DSS) published in Tasfaout, Buono et al 2017, and subsequently optimized in Buono et al (associated manuscript).

Objective

The goal of this SOP is to detail the procedure of allocating a disease severity score (DSS) in mice. The DSS is a parameter which is used for the evaluation of the disease severity of the myopathic phenotype in Mtm1<sup>−/−</sup> mice. The calculation is based on 4 parameters: difference of body weight, hanging test ability, kyphosis and walking difficulties. The minimal score is 0 (no myopathic phenotype) and the maximal score is 5 (characterizing a very severe myopathic phenotype).

1. Abbreviations and Definitions

| Abbreviation | Definition                  |
|--------------|-----------------------------|
| DSS          | Disease Severity Score      |
| NHS          | Natural History Study       |
| SOP          | Standard Operating Protocol |
| NA           | Not applicable              |

2. Risk Assessment / Related documents

3. Ethics

Mice should be handled according to national legislation on animal care and experimentation. This SOP is in alignment with the French and European legislation. Procedures and Protocols should be approved by the institutional Ethics Committee.

4. Materials and Equipment

- Type 3 cage: 425 x 265 x 150 mm
- Metal Grid: 410 x 270 mm
- Timer
- Scale
- Lid of transport box (optional)

5. Reagents

NA
6. Protocol

1) Body weight

Measure body weight, to 2 decimal places. The score for this parameter (score between 0 and 1) is the difference of a Mtm1<sup>-/-</sup> mouse body weight from week n to n+1:

\[ X(g) = (\text{body weight week } n+1(g)) - (\text{body weight week } n(g)) \]

| Body weight | SCORE |
|-------------|-------|
| \( X \geq 0.25g \) | 0     |
| -0.25g > X > 0.25g | 0.5   |
| \( X \leq -0.25g \) | 1     |

Table 1: Body weight to score conversion

2) Hanging test ability

This test must be done one mouse at a time:

a. Take one type 3 cage and one grid.

b. Place mouse in the middle of the grid

c. Turn the grid upside down. The suspending animal should hold on to the grid in order to avoid falling.
   - Test must be set up at a certain height, around 40 cm, for mouse to not being influenced to jump.

d. Prevent the mouse from turning over to the other side of the grid or at pellets food and bottle place by barring with the hand (without touching the mouse) or by using the lid of a transport box.

e. The latency to fall will be measured three times (60 seconds each) for each mouse, with a minimum interval of 10 minutes between trials.
   - The latency time measurements begin when the mouse is hanging free on the wire and end with the animal falling to the cage underneath the wire or grid.
   - If performing the whole body hanging test for the first time, mouse can fall as soon as grip is turned upside down. If this is the case, the first assay can be considered as familiarization of the mouse with the testing conditions and will not be considered as one of the three assays of the test. Only time (seconds) for the three next trials will be reported in the dedicated table (Table 2).
   - If a mouse falls for any other reason that muscle strength default (eg if a mouse is not willing to do the test, voluntary jump from the grip, or falls because of your hands...), this will not be considered as a trial. This must be recorded in the note section in the dedicated table (Table 2).
   - A mouse should normally explore the grid. If a mouse stays in place and does not explore the grip. This must be recorded in the note section in the dedicated table (Table 2).

f. Time (seconds) when mouse falls should be reported in the dedicated table (Table 2).
   - 60 seconds is the maximum time allowed
Table 2: Data collection table for hanging test

| Mice ID number | Mice genotype | Age (weeks) | Test 1 | Test 2 | Test 3 | Note |
|----------------|---------------|-------------|--------|--------|--------|------|

Note: Mouse genotype should be blinded until experiment is complete

The score for this parameter (score between 0 and 2) represents the mean hanging time according to the formula: \( \text{Time (s)} > \frac{(60-\text{Time (s)})}{60s} \times 2 = \text{value (0-2)} \)

Examples:
- 1s > (60-1)/60*2 = 1.97
- 15s > (60-15)/60*2 = 1.5
- 30s > (60-30)/60*2 = 1
- 60s > (60-60)/60*2 = 0

3) Kyphosis

This parameter reflects the curvature of the spine. The score is noted as:

Score 0: no curvature of the spine
Score 0.5: mild curvature of the spine
Score 1: severe curvature of the spine

Adapted from Gabellini et al. Nature (2006)
4) Walking difficulties

This parameter reflects the ability to use hindlimbs and the ability of mice for walking. The score is noted as:

Score 0: normal use of hindlimbs
Score 0.5: splayed use of hindlimbs
Score 1: loss of use of hindlimbs

**Note:** if score 1:
- indicate if the hindlimbs are paralyzed or not
- indicate if the mouse can still move

7. Calculation of DSS

Disease Severity Score (DSS) is the sum of the scores of the 4 parameters: body weight, hanging test ability, kyphosis and walking difficulties. The maximal DSS is 5.

**Note:** always refer to ethics application for humane endpoints.

8. Data Collection

All the data concerning the DSS will be reported in the dedicated table (table 3).

**Table 3: Data collection table for Disease Severity Score**

| Mice ID | Mice genotype | Age (weeks) | Body weight | Score body weight | Score hanging | Score walking | Score kyphosis | TOTAL DSS |
|---------|---------------|-------------|-------------|-------------------|---------------|---------------|----------------|----------|

**Note:** Mouse genotype should be blinded until experiment is complete

9. Data Analysis

The data will be expressed as a sum of the 4 different parameters: body weight, hanging test ability, kyphosis and walking difficulties
Table 4. Disease severity score (DSS). Tabular summary of DSS procedure, based on natural history study data analysis and modeling from training and test cohorts of *Mtm1*⁻/⁻ mice (Buono et al, associated manuscript).

| Disease severity score | DSS Description | Score |
|------------------------|-----------------|-------|
| **Body weight**        | Score 0-1       |       |
|                        | Difference in body weight between *Mtm1*⁻/⁻ mouse from week n to n+1. |       |
|                        | Score 0: x ≥ 0.25g |       |
|                        | Score 0.5: -0.25 > x > 0.25 |       |
|                        | Score 1: x ≤ -0.25g |       |
|                        | With x being the weight difference |       |
| **Hanging time**       | Score of 0-2, representing 0-60 seconds hanging time. |       |
|                        | Time (secs) > [(60-Time (s))/60s]*2 = value (0-2) |       |
|                        | Examples: |       |
|                        | 1s > (60-1)/60*2=1.967 |       |
|                        | 15s > (60-15)/60*2=1.5 |       |
|                        | 30s > (60-30)/60*2=1 |       |
|                        | 60s > (60-60)/60*2=0 |       |
| **Kyphosis**           | Score 0-1       |       |
|                        | Curvature of the spine |       |
|                        | Score 0: no curvature of the spine |       |
|                        | Score 0.5: mild curvature of the spine |       |
|                        | Score 1: severe curvature of the spine |       |
| ![Kyphosis](image)     | Adapted from Gabellini et al. Nature (2006) |       |
| **Walking difficulties** | Score 0-1 |       |
|                        | Ability to use hindlimbs |       |
|                        | Score 0: normal use of hindlimbs |       |
|                        | Score 0.5: splayed use of hindlimbs |       |
|                        | Score 1: loss of use of hindlimbs |       |
| ![Walking & gait](image) |       |       |
| **Maximum score**      | 5               |       |
References

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