Title: Bortezomib Does Not Reduce Muscular Dystrophy in the dy.sup.2J/dy.sup.2J Mouse Model of Laminin [alpha]2 Chain-Deficient Muscular Dystrophy
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Introduction

Mutations in the LAMA2 gene, encoding the laminin [alpha]2 chain of the extracellular matrix protein laminin-211, leads to congenital muscular dystrophy type 1A (MDC1A), which is a life threatening disease. Genotype-phenotype analyses have demonstrated that complete deficiency of laminin [alpha]2 chain causes a more severe phenotype whereas partial absence leads to a milder disease course. The clinical manifestations of complete laminin [alpha]2 chain-deficiency include profound hypotonia at birth, widespread muscle weakness, proximal joint contractures, scoliosis, elevated serum creatine kinase levels and delayed motor milestones. Patients may achieve unsupported sitting but very few children acquire independent ambulation. Individuals with partial laminin [alpha]2 chain deficiency often have later onset of proximal muscle weakness and delayed motor milestones but achieve independent ambulation. Histologically, MDC1A skeletal muscle displays typical dystrophic characteristics with degenerating/regenerating and atrophic fibers, early inflammation and extensive connective tissue infiltration [1-4].

Importantly, analyses of dy.sup.3K/dy.sup.3K and dy.sup.2J/dy.sup.2J mice and other MDC1A mouse models have identified several disease driving mechanisms for MDC1A. For example, we have previously shown that there is increased proteasome activity in human MDC1A myoblasts and myotubes. We have also demonstrated significantly enhanced expression of proteasome-related genes and proteins both in dy.sup.3K/dy.sup.3K as well as in dy.sup.2J/dy.sup.2J muscle, although the increase was more profound in dy.sup.3K/dy.sup.3K compared to dy.sup.2J/dy.sup.2J mice [9,10]. Moreover, administration of the proteasome inhibitors MG-132 and bortezomib, respectively, partially improved muscular dystrophy in dy.sup.3K/dy.sup.3K mice. Bortezomib improved histological hallmarks of disease, enhanced body weight, locomotion and survival and partially normalized miRNA expression and reduced the proteasome activity in human MDC1A myoblasts and myotubes [9,10]. In order to evaluate if bortezomib also has beneficial effects in the mouse model of partial laminin [alpha]2 chain-deficiency we herein explored the use of bortezomib in dy.sup.2J/dy.sup.2J mice. Quite unexpectedly, we found that bortezomib did not ameliorate any of the muscular dystrophy features in the dy.sup.2J/dy.sup.2J mouse model.

Materials and Methods

Transgenic Animals

Heterozygous dy.sup.2J/dy.sup.2J mice (B6.WK-Lama2dy-2J/J) were purchased from the Jackson Laboratory (Bar Harbor, ME) and bred and maintained in the animal facilities of the Biomedical Center at Lund University according to institutional animal care guidelines. Permission was given by the Malmö/Lund (Sweden) ethical committee for animal research (ethical permit numbers M152-14 and M180-14).

Bortezomib Treatment

Bortezomib was purchased from LC Laboratories (Woburn, MA). A stock solution was stored in -80°C (dissolved in dimethylsulfoxide) and further diluted in sterile sodium chloride before administration. Mice were either injected twice with approximately 0.4 mg/kg i.v. at 2.5 weeks of age and 0.3 mg/kg i.v. at 3.5 weeks of age, or injected altogether
six times with approximately 0.4 mg/kg i.v. at 2.5 weeks of age; 0.3 mg/kg i.v. at 3.5 weeks of age; 0.2 mg/kg i.v. at 4.5 weeks of age; 0.2 mg/kg s.c. at 5.5 and 6.5 weeks of age and finally 0.16 mg/kg s.c. at 7.5 weeks of age. Mice were subsequently analyzed at 5.5 and 8.5 weeks of age, respectively. Quadriceps and triceps muscles were processed for immunofluorescence, morphometric analysis and hydroxyproline assay. Plasma was also collected for analysis of circulating miRNAs and creatine kinase activity.

### Histology and Immunofluorescence

Quadriceps and triceps muscle from *dy^2J/dy^2J*, bortezomib-treated *dy^2J/dy^2J*, wild-type (WT) and bortezomib-treated WT mice were dissected after euthanasia and frozen in optimal cutting temperature compound (Tissue-Tek OCT; Sakura Finetek, Torrance, CA) in liquid nitrogen. Transverse cryosections of 7 μm were stained with hematoxylin and eosin (H&E), Masson's trichrome (using an HT15 commercial kit; Sigma-Aldrich, St. Louis, MO) or biotinylated wheat germ agglutinin (WGA), which was detected with fluorescein avidin D (Vector Laboratories, Burlingame, CA). Sections were also processed for immunofluorescence analyses according to standard procedures with rat monoclonal anti-tenascin-C (MTn15) [11] and rat monoclonal anti-CD11b (M1/70, BD Pharmingen, San Diego, California). Anti-tenascin-C, anti-CD11b and WGA stained sections were analyzed and images captured with a Zeiss Axioplan fluorescence microscope (Carl Zeiss Microscopy, Jena, Germany) using an ORCA 1394 ER digital camera (Hamamatsu Photonics, Hamamatsu City, Japan) and Openlab software version 3 (Improvement, Coventry, UK).

### Morphometric Analysis

Quantifications were performed on cross sections of entire quadriceps and triceps muscle. H&E and Masson's trichrome stained sections were scanned using an Aperio ScanScope CS2 scanner with ScanScope console version 8.2.0.1263 (Aperio, Vista, CA). For quantification of tenascin-C and CD11b labeling, we used multiple Tiff-format images at x 10 magnification covering the whole muscle. The area within muscle corresponding to Masson's trichrome-positive area and to tenascin-C and CD11b labeling was quantified relative to the entire area of the quadriceps and triceps cross section. Images were converted to 8-bit-mode images and the measurements were set to a threshold that was manually adjusted for every individual image (the total muscle area versus stained area, measured in square pixels). The images were analyzed using ImageJ software version 1.43u (NIH, Bethesda, MD). Central nucleation was also quantified using ImageJ. The fiber area of biotinylated WGA stained muscle fibers was measured and quantified using Adobe Photoshop CS5 extended version (Adobe Systems, San Jose, CA).

### Hydroxyproline Assay

OCT blocks with quadriceps and triceps muscle were thawed and washed in PBS. The muscles were weighed and incubated overnight in 200 [μl] concentrated HCl (12 M) at 95°C. Twenty five [μl] of hydrolyzate was neutralized with 25 [μl] NaOH (6 M) and incubated with 450 [μl] chloramine-T reagent (0.056 M) at room temperature for 25 min. A volume of 500 [μl] freshly prepared Ehrlich's reagent [1 M 4-(dimethylamino)benzaldehyde] was added to each sample and incubated at 65°C for 1 h. After cooling on ice, 100 [μl] in duplicates was transferred to a 96-well plate and absorbance was read at 560 nm. Standards from 4-hydroxyproline at concentrations ([μg/ml; 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0] were treated the same way as the samples. Absorbance (A_560) of standards was plotted against amount of hydroxyproline ([μg]) and a linear regression was performed to determine slope and intercept. All absorbance values were subtracted with blank (0[μg/ml hydroxyproline]). Content of hydroxyproline in samples was calculated by equation: [see PDF for formula] Collagen conversion factor = 13.5 [12,13]. Values are presented as relative amount of collagen.

### Exploratory Locomotion Test

Exploratory locomotion was evaluated in an open-field test. In each experiment, a mouse was placed into a new cage and allowed to explore the cage for 5 minutes. The time that the mouse spent moving around was measured, as well as number of stand-ups (on hindlimbs).

### Grip Strength

Forelimb grip strength was measured on a grip-strength meter (Columbus Instruments, Columbus, OH) as previously described [14]. In short, the mouse was held by the base of the tail and allowed to grasp the flat wire mesh of the pull bar with its forepaws. When the mouse got a good grip it was slowly pulled away by its tail until it released the pull bar. Each mouse was allowed to pull the pull bar five times. The two lowest values were rejected and the mean of the three remaining values was counted. Animals were not subjected to any training prior to the experiment.
RNA Isolation from Plasma, cDNA Synthesis and qPCR for miRNA Detection

As previously described [12], blood was collected from heart puncture and transferred to anticoagulant tubes (EDTA), which were centrifuged at 1100 x g for 10 min in 4°C. Total RNA from blood plasma was extracted using the manufacturer's (miRNeasy Serum/Plasma kit; Qiagen, Valencia, CA) instructions. Briefly, the samples were thawed on ice and then centrifuged at 3000 x g for 5 min in 4°C. Fifty [μl] plasma was transferred to a new microcentrifuge tube containing 190 [μl] of QIAzol mixture containing 0.8 [μg]/[μl] MS2 bacteriophage RNA (Roche Applied Science, Penzberg, Germany) and incubated for 5 min. Fifty [μl] of chloroform was added to each tube and incubated for 2 min followed by centrifugation at 12000 x g for 15 min in 4°C. The supernatant was transferred to a new microcentrifuge tube and 435 [μl] ethanol was added to each sample. The sample was transferred to a spin column, then a rinse step was performed with 1 x 700 [μl] RWT buffer, 1 x 500 [μl] RPE buffer and 1 x 500 [μl] 80% ethanol. Total RNA was eluted by adding 14 [μl] RNase-free water to the membrane followed by centrifugation at 12000 g for 1 min. The RNA was stored at -80°C. Two [μl] of eluted blood plasma RNA was reverse transcribe in a 15 [μl] reaction using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA). Two [μl] of cDNA was assayed in 20 [μl] PCR reaction according to the protocol for the TaqMan Fast Advanced Master Mix. The amplification was performed in 96-well plates in a LightCycler 480 qPCR system (Roche Diagnostics, Basel, Switzerland). The determination of C_T (by the second-derivative method) was done using the manufacturer's LightCycler software. MiRNA levels were calculated relative to miR-122. Primers/Probes for miR-1, miR-133a and miR-122 were designed by Applied Biosystems (assay ID 002246, 002222 respectively 002245).

Creatine kinase assay

Blood was collected from heart puncture and transferred to anti-coagulant tubes (EDTA) and centrifuged at 1100 x g for 10 min at 4°C. Plasma was analyzed at Clinical Chemistry Laboratory at Skåne University Hospital. The CK_P_S Cobas method was used to quantify enzyme activity.

Statistical Analysis

Data were analyzed using the Kruskal-Wallis test with a Dunn’s multiple comparison test to determine differences between groups followed Mann-Whitney U-test to determine the differences between two respective groups. Statistical significance was accepted for \( P < 0.05 \).

Results and Discussion

Two bortezomib injections do not reduce muscular dystrophy in \( dy^{2J} /dy^{2J} \) mice

In our previously published study, we demonstrated that \( dy^{3K} /dy^{3K} \) mice injected twice with bortezomib had higher body weight, were more active in an exploratory locomotion test, survived longer and displayed improved muscle morphology compared to non-treated \( dy^{3K} /dy^{3K} \) mice [10]. In order to analyze whether bortezomib has beneficial effects in \( dy^{2J} /dy^{2J} \) mice as well, we injected \( dy^{2J} /dy^{2J} \) mice with bortezomib using a similar administration schedule. Thus, wild-type and \( dy^{2J} /dy^{2J} \) mice were i.v. injected (0.3-0.4 mg/kg) at 2.5 and 3.5 weeks of age and analyzed at 5.5 weeks of age. The \( dy^{2J} /dy^{2J} \) quadriceps muscle displayed an increased number of small muscle fibers compared with wild-type muscle (S1 Fig). Bortezomib administration in wild-type mice significantly increased the proportion of small muscle fibers (cross-sectional area 1-1000 [μm]^2 ) and reduced the proportion of large fibers (cross-sectional area 1500-2500 [μm]^2 ) (S1 Fig). However, bortezomib administration did not alter fiber size distribution in \( dy^{2J} /dy^{2J} \) quadriceps muscle (S1 Fig). A significantly increased number of muscle fibers with centrally located nuclei was noticed in 5.5-week-old \( dy^{2J} /dy^{2J} \) quadriceps muscle compared to wild-type muscle, but central nucleation was not affected by bortezomib treatment (regardless of genotype) (S1 Fig). Laminin [alpha]2 chain-deficiency is also characterized by pathological fibrosis and \( dy^{2J} /dy^{2J} \) quadriceps muscle at 5.5 weeks of age displayed increased fibrosis compared with wild-type (roughly 2.5-fold) as shown by increased tenascin-C deposition and Masson's trichrome staining. Yet, two injections of bortezomib administration did not reduce fibrosis in \( dy^{2J} /dy^{2J} \) quadriceps muscle (S1 Fig). Finally, we analyzed the body weight. It was only mildly reduced in 5.5-week-old \( dy^{2J} /dy^{2J} \) mice compared to wild-type animals (reduction was not statistically significant) and bortezomib administration had no significant impact on body weight, regardless of genotype (S1 Fig). In summary, two bortezomib injections in \( dy^{2J} /dy^{2J} \) mice at 2.5 and 3.5 weeks of age did not have any beneficial effects on muscle morphology.
Six bortezomib injections do not reduce muscular dystrophy in dy^{2J}/dy^{2J} mice

In order to analyze whether additional injections could be advantageous, we tested the following bortezomib injection regimen: three i.v. injections at 2.5, 3.5 and 4.5 weeks of age and three s.c. injections at 5.5, 6.5 and 7.5 weeks of age and final analysis at 8.5 weeks of age. We started with 0.4 mg/kg and then gradually decreased the dose to 0.16 mg/kg in order to avoid serious adverse effects of bortezomib. Still, it has been demonstrated that bortezomib significantly attenuates the severity of collagen-induced arthritis (another musculoskeletal disorder) in mice within this dose range [15]. Also, in several previous studies it has been demonstrated that bortezomib is taken up in skeletal muscle after intravenous injections in mice and rats [16-18].

One hindlimb (quadriceps femoris) and one forelimb (triceps brachii) muscle were chosen for histological analyses. We found that the muscle morphology of dy^{2J}/dy^{2J} muscle was not further improved by additional injections of bortezomib. Dystrophic changes such as fiber size variability, central nucleation and connective tissue infiltration were evident to a similar degree in quadriceps and triceps muscle of untreated and bortezomib-treated dy^{2J}/dy^{2J} mice (Fig 1). In 8.5-week-old dy^{2J}/dy^{2J} animals, the quadriceps and triceps muscle fiber-size distribution was shifted to smaller diameters compared to wild-type muscles. However, bortezomib administration did not affect fiber size distribution in either dy^{2J}/dy^{2J} quadriceps or triceps muscle (Figs 2A and 3A). An increased number of muscle fibers with centrally located nuclei was also noticed in 8.5-week-old dy^{2J}/dy^{2J} quadriceps and triceps muscle compared with wild-type muscles but again, central nucleation was not affected by bortezomib treatment (Figs 2B and 3B). We evaluated fibrosis with two independent methods. We found significantly increased tenascin-C expression in dy^{2J}/dy^{2J} quadriceps muscle compared with wild-type muscle and a trend for enhanced tenascin-C expression in dy^{2J}/dy^{2J} triceps muscle. Nonetheless, there was no reduction of tenascin-C expression in quadriceps and triceps muscle of bortezomib-treated dy^{2J}/dy^{2J} mice (Figs 2C and 3C). We also used a biochemical collagen quantification assay, which revealed significantly increased collagen content (approximately 2-3-fold) in dy^{2J}/dy^{2J} in quadriceps and triceps muscle. This increase did not change upon administration of bortezomib to dy^{2J}/dy^{2J} animals (Figs 2D and 3D). Since inflammation is a feature of MDC1A, we assessed the inflammatory response in treated and non-treated muscle. We observed a significant upregulation of CD11b-positive cells (monocytes/macrophages) in dy^{2J}/dy^{2J} quadriceps and triceps muscle. Bortezomib did not reduce the number of CD11b-positive immune cells in quadriceps muscle, but there was a slight but significant reduction in triceps muscle (Figs 2E and 3E).

**Fig 1. Bortezomib treatment does not improve the muscle morphology of dy^{2J}/dy^{2J} quadriceps and triceps muscle.** Hematoxylin and eosin staining of cross-sections of quadriceps and triceps muscle from wild-type (WT), bortezomib-treated WT, dy^{2J}/dy^{2J} and bortezomib-treated dy^{2J}/dy^{2J} mice (8.5-week-old) revealed myopathic changes (muscle degeneration/regeneration, fiber size variability and connective tissue infiltration) in both dy^{2J}/dy^{2J} and bortezomib-treated dy^{2J}/dy^{2J} mice. Magnification x 8.6. [see PDF for image]

**Fig 2. Bortezomib treatment does not normalize fiber size distribution or reduce fibrosis and inflammation in dy^{2J}/dy^{2J} quadriceps muscle.** (A) In dy^{2J}/dy^{2J} mice, a higher proportion of muscle fibers had small diameters. The fiber-size distribution was not changed toward the values of wild-type quadriceps muscle in the bortezomib-injected dy^{2J}/dy^{2J} mice. (B) Bortezomib did not affect the number of centrally nucleated fibers in quadriceps muscle of dy^{2J}/dy^{2J} mice. (C) Bortezomib did not decrease the tenascin-C positive area in quadriceps muscle of dy^{2J}/dy^{2J} animals. (D) The relative collagen content in quadriceps muscle was not changed in bortezomib-treated dy^{2J}/dy^{2J} mice. (E) Bortezomib did not significantly reduce the amount of CD11b-positive immune cells in dy^{2J}/dy^{2J} quadriceps muscle. Data are expressed as means ± SEM. Number of mice analyzed is indicated in data bars. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001. [see PDF for image]

**Fig 3. Bortezomib treatment does not normalize fiber size distribution or reduce fibrosis in dy^{2J}/dy^{2J} triceps muscle.** (A) In dy^{2J}/dy^{2J} mice, a higher proportion of muscle fibers had small diameters. The fiber-size distribution was not changed toward the values of wild-type triceps muscle in the bortezomib-injected dy^{2J}/dy^{2J} mice. (B) Bortezomib did not affect the number of centrally nucleated fibers in triceps muscle of dy^{2J}/dy^{2J} mice. (C) Bortezomib did not decrease the tenascin-C positive area in triceps muscle of dy^{2J}/dy^{2J} animals. (D) The relative collagen content in triceps muscle was not significantly altered in bortezomib-treated dy^{2J}/dy^{2J} mice. (E) Bortezomib slightly reduced the amount of CD11b-positive immune cells in dy^{2J}/dy^{2J} triceps muscle. Data are
expressed as means ± SEM. Number of mice analyzed is indicated in data bars. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001. [see PDF for image]

Next, we assessed the overall health status of bortezomib-treated dy2J/dy2J animals by investigating if bortezomib injections contributed to increased body weight, improved locomotive behavior and enhanced muscle strength. The body weight was significantly reduced in 8.5-week-old dy2J/dy2J male mice compared to wild-type male mice and bortezomib did not enhance the body weight of male dy2J/dy2J mice. Instead, multiple injections of bortezomib decreased the body weight (approximately 10%) of male wild-type mice compared with untreated wild-type male mice (Fig 4A). In female wild-type mice, on the other hand, multiple injections of bortezomib did not cause any weight loss (data not shown). We have previously demonstrated that exploratory locomotion of dy3K/dy3K mice in an open field test is significantly reduced compared to wild-type animals [9,10]. Similarly, dy2J/dy2J mice at the age of 8.5 weeks were also significantly less active (roughly 1.1-fold) compared to wild-type mice. Bortezomib did, however, not enhance the exploratory locomotion of dy2J/dy2J mice (Fig 4B). We also analyzed the average number of stand-ups over a 5-minute period and observed that 8.5-week-old dy2J/dy2J mice displayed a significant reduction (around 3-fold) in stand-up activity compared to wild-type mice. No increase in stand-up activity was noted upon bortezomib treatment (Fig 4C). Subsequently, we measured the muscle strength of dy2J/dy2J and wild-type forelimbs using a grip-strength meter. Dy2J/dy2J mice were significantly weaker (approximately 2.3-fold) compared to control mice and bortezomib did not increase the grip strength (Fig 4D).

Fig 4. Bortezomib treatment does not increase weight, locomotion, muscle strength or reduce plasma levels of CK and miRNA. (A) The body weight was significantly reduced in dy2J/dy2J male mice and bortezomib-treated wild-type and dy2J/dy2J male mice compared to wild-type mice. (B) Administration of bortezomib did not improve the exploratory locomotion of dy2J/dy2J animals in an open-field test. (C) Bortezomib did not increase the number of stand-ups in dy2J/dy2J mice. (D) Grips strength testing revealed no increase in fore-limb muscle strength in bortezomib-treated dy2J/dy2J mice. (E) Analysis of CK levels in plasma revealed a significant increase in CK levels in dy2J/dy2J mice, which was not significantly reduced by bortezomib. (F) RT-qPCR analysis showed enriched plasma levels of miR-1 and miR-133a in dy2J/dy2J animals, which were not decreased by bortezomib. Blue bar represents wild-type mice; green bortezomib-treated wild-type; red dy2J/dy2J; orange bortezomib-treated dy2J/dy2J. Data are expressed as means ± SEM. Number of mice analyzed is indicated in data bars, except in panel F, in which n = 4. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001. [see PDF for image]

Lastly, we measured plasma creatine kinase (CK) levels and the expression of two muscle-specific miRNAs in plasma from 8.5-week-old animals in order to examine the sarcolemmal integrity of skeletal muscle fibers. A significant increase in CK levels was observed in dy2J/dy2J mice but bortezomib did not significantly lower the CK levels in the blood (Fig 4E). We have recently demonstrated that the expression of miR-1 and miR-133a is significantly increased in plasma from both dy3K/dy3K and dy2J/dy2J mice. More importantly, administration of bortezomib resulted in a partial normalization of plasma levels of miR-1 and miR-133a in dy3K/dy3K mice [12]. However, bortezomib failed to reduce miR-1 and miR-133a plasma levels in dy2J/dy2J mice (Fig 4F).

In this study we demonstrated that bortezomib treatment has no significant beneficial effects in dy2J/dy2J mice. Bortezomib failed to improve the muscle phenotype as measured by histological and functional assays. These data are in contrast to our previous reports in which we found that proteasome inhibitors MG-132 and bortezomib partially improved the phenotype of dy3K/dy3K mice, which display a more severe phenotype compared to dy2J/dy2J animals [8-10]. The lack of beneficial effects was somewhat surprising considering that proteasome activity appears augmented in dy2J/dy2J muscle, but it should be noted that while the expression of proteasome-related genes (e.g. MuRF-1, atrogin-1 and ubiquitin) is increased more than 10-fold in dy3K/dy3K muscle it is only increased 1.5-2-fold in dy2J/dy2J muscle. Also, the general muscle wasting is not extensive in dy2J/dy2J mice as the body weight is only slightly decreased at 5.5 and 8.5 weeks of age. This is in sharp contrast to dy3K/dy3K mice that are severely emaciated at 3-4 weeks of age [8-10,19].

Another possibility for the absence of beneficial effects in dy2J/dy2J animals is that dy2J/dy2J and dy3K/dy3K mice may require different dosing of bortezomib. We previously treated dy3K/dy3K mice with 0.4 mg/kg bortezomib as 0.8 mg/kg (previously administered to mdx mice [18]) did not improve lifespan of dy3K/dy3K mice [10]. Similarly, we first administrated 0.8 mg/kg in two dy2J/dy2J mice but these mice died shortly after injection (note that dy2J
mice have a life span of several months) (data not shown) and therefore we subsequently injected 0.4 mg/kg, followed by lower doses. Still it is possible, but not very likely, that 0.5-0.7 mg/kg could have been advantageous in dy^{2J}/dy^{2J} mice.

In conclusion, these findings do not support a putative role for bortezomib in the treatment of MDC1A with partial laminin [alpha]2 chain-deficiency. However, we would like to stress that proteasome inhibition (two injections of bortezomib) diminished the severity of muscle dysfunction in dy^{3K}/dy^{3K} mice [9,10] but we have not yet evaluated the effects of multiple injections in dy^{3K}/dy^{3K} mice. Nevertheless, it is plausible that proteasome inhibition could be a useful supportive therapy in patients with complete deficiency of laminin [alpha]2 chain.

Supporting Information

S1 Fig Two bortezomib injections do not improve muscle morphology, reduce fibrosis or increase body weight in dy^{2J}/dy^{2J} mice.

(A) The shift in fiber-size distribution was not affected by administration of bortezomib to dy^{2J}/dy^{2J} mice. (B) Bortezomib did not affect the number of centrally nucleated fibers in quadriceps muscle of dy^{2J}/dy^{2J} mice. (C) Bortezomib did not decrease the tenasin-C positive area in quadriceps muscle dy^{2J}/dy^{2J} animals. (D) Masson's trichrome staining of transverse cryosections of dy^{2J}/dy^{2J} quadriceps muscle did not reveal reduced collagen content upon bortezomib treatment. (E) The body weight did not change between genotypes. Data are expressed as means ± SEM. Number of mice analyzed is indicated in data bars. * P < 0.05; ** P < 0.01; *** P < 0.001.

(TIF)

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Author Contributions

Conceived and designed the experiments: ZK MD. Performed the experiments: ZK. Analyzed the data: ZK MD. Wrote the paper: ZK MD.

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