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# Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.

For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

## Experimental design

1. Sample size

   Describe how sample size was determined.

   Sample size was determined by standard methods (Rosner B. Fundamentals of Biostatistics. 7th ed. Boston, MA: Brooks/Cole; 2011) after first estimating the effect size relative to standard deviation for the most sensitive measurement previously used in comparing dystrophic to wild type animals (Song, et al, J Appl Physiol 122: 593–602, 2017). Experiments were designed with the input of our collaborating biostatistician to insure that appropriate formulas were applied for sample size calculation a priori.

2. Data exclusions

   Describe any data exclusions.

   No data was excluded from the analyses.

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   A very clear and detailed protocol was written out before performing any experiment in order to ensure the same method was used every time. The same person would be assigned to conduct an assay in order to minimize any human variability. All attempts at replicating the experimental findings were successful. When feasible, every experiment was repeated at least twice. Actual animal experiments were conducted once solely due to their limited availability.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   All animals within a group were randomized and assigned a code.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   All animals within a group were assigned a code. The code was held by an independent member of the lab that was not involved with data acquisition or analysis. All of the studies were performed while the experimenter remained blinded. Only after all the data for a group was collected and analyzed were the codes broken.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|---|---|
| ☐ | ☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| ☐ | ☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ A statement indicating how many times each experiment was replicated |
| ☐ | ☒ The statistical test(s) used and whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☐ | ☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ☐ | ☒ Test values indicating whether an effect is present<br>*Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.* |
| ☐ | ☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
| ☐ | ☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation) |

*See the web collection on statistics for biologists for further resources and guidance.*

## Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

GraphPad Prism 7 software for Mac OSX was used to analyze the data in this study. Dynamic Muscle Control v.5.3 software and Aurora Mouse 1200A System was used to measure the force during the Ex-vivo Eccentric Contraction protocol. ClustalW and MacVector version 13.5.1was used to for aligning protein sequences. DNA sequences for species listed in figure legends and manuscript text were queried by tBLASTn. FGENESH+ (Softberry) was used with organism specific gene-finding parameters and Hidden Markov Model plus similar protein-based gene prediction to identify putative coding sequences from assembled contigs.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

## Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

The N-19 antibody was only available for sale by a third party and has been discontinued by the Santa Cruz supplier.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

N-19 utrophin is a polyclonal antibody (supplier name: Santa Cruz biotech, catalog number: sc-74060, lot number:G3010). According to the manufacturer's website, the N-19 utrophin antibody is recommended for detection of utrophin of human origin by WB, IP, IF and ELISA; also reactive with additional species, including canine. See relevant citation, Cerletti M, et al. 2003. Dystrophic phenotype of canine X-linked muscular dystrophy is mitigated by adenovirus-mediated utrophin gene transfer. Gene Ther. 10(9): 750-757. PMID: # 12704413. See manufacturers website for further details: https://www.scbt.com/scbt/product/utrophin-antibody-n-19. Its important to note that the N-19 antibody has been discontinued by the supplier.

MANCHO7 utrophin is a monoclonal antibody (supplier name: Santa Cruz, catalog number: sc-81557, clone name: MANCHO7, lot number:A1614). According to the manufacturer's website, the MANCHO7 antibody is recommended for detection of utrophin of mouse, rat and human origin by WB, IP and IF. See relevant citation, van Putten, M. et al. 2012. Comparison of skeletal muscle pathology and motor function of dystrophin and utrophin deficient mouse strains. Neuromuscul. Disord.. 22: 406-417. PMID: # 22284942. See manufacturers website for further details: https://www.scbt.com/scbt/product/utrophin-antibody-mancho7

Gamma-sarcoglycan is a polyclonal antibody (supplier name: Novus Biologicals, catalog number: NBP1-59744, lot number:N/A). According to the manufacturer's website, this gamma-sarcoglycan antibody is recommended for detection in mouse, rat and human origin by WB, IP and IF. See manufacturers website for further details: https://www.novusbio.com/products/gamma-sarcoglycan-antibody_nbp1-59744

MANEX1011B(1C7) is a mouse monoclonal IgG2a raised against exons10/11 of human dystrophin. MANEX1011B(1C7) was deposited to the DSHB by Morris, G.E. (Supplier name:

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

We used HEK293 cells as made available by the University of Pennsylvania cell center. The original reference for this widely used human cell line is as follows: J Gen Virol. 1977 Jul;36(1):59-74.
Characteristics of a human cell line transformed by DNA from human adenovirus type 5.

Graham FL, Smiley J, Russell WC, Nairn R.

b. Describe the method of cell line authentication used.

These cells were authenticated by the cell center, but not reauthenticated by our lab.

c. Report whether the cell lines were tested for mycoplasma contamination.

These cells were tested by the cell center and confirmed that there was no mycoplasma contamination, but not retested by our lab.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

## Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Wild type (c57BL/10J) and mdx mice (c57BL/10ScSn-Dmdmdx/J) were purchased from the Jackson Laboratory (Bar Harbor, ME) and then bred in house. Both males and females were used for these studies. Therapeutic agent was administered in all selected mice between 9 ± 2 days days of age. Creatine Kinase, in-vivo functional assays, western blot, and immunohistochemistry analyses were all done at 8 weeks of age. Sustained expression of recombinant µUtrophin was also assessed at 16 weeks of age.

For our larger mammalian studies, two distinct groups of the Golden Retriever Muscular Dystrophic (GRMD) and and one group of German Shorthaired Pointer (GSHPMD) canine model were used . All dogs were bred and genotyped at Texas A&M University. The first GRMD group included five affected male, one male wild type and three carrier females. Pups were injected at 6-10 days of age and euthanized at 7 weeks of age. The second GRMD group included two male GRMD dogs that were injected at 7.5 weeks and euthanized at 14.5 weeks of age. The GSHPMD received an intramuscular injection at 7 years of age, while muscle biopsies were collected 4 weeks post injection.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.