

YMTHE, Volume 25

Supplemental Information

Treatment with Recombinant Human MG53 Protein Increases Membrane Integrity in a Mouse Model of Limb Girdle Muscular Dystrophy 2B

Liubov V. Gushchina, Sayak Bhattacharya, Kevin E. McElhanon, Jin Hyuk Choi, Heather Manring, Eric X Beck, Jenna Alloush, and Noah Weisleder

Supplemental Information for Gushchina, et al.

Supplementary Table S1. Statistical analysis of histological features of muscular dystrophy in *Dysf*^{-/-} mice.

Skeletal Muscle	<i>P</i> value (3 vs 6 months)	<i>P</i> value (3 vs 10-12 months)	<i>P</i> value (3 vs 15-16 months)	<i>P</i> value (6 vs 10-12 months)	<i>P</i> value (6 vs 15-16 months)	<i>P</i> value (10-12 vs 15-16 months)
Histopathological area						
Gluteus	n/s	0.0019	<0.0001	0.0069	0.0002	n/s
Gastroc	n/s	n/s	0.0021	n/s	0.0027	0.0003
Central Nuclei						
Gluteus	n/s	<0.0001	<0.0001	<0.0001	<0.0001	n/s
Gastroc	n/s	n/s	<0.0001	n/s	<0.0001	<0.0001

n/s – measurements are not significantly different

P values are calculated by *Tukey-ANOVA* method using Prism software (GraphPad Prism 7.0, USA).

Supplementary Table S2. Statistical analysis of *ex vivo* studies of rhMG53 efficacy on whole FDB muscle derived from *Dysf*^{-/-} mice.

	<i>P</i> value (Dysf KO, Saline, 2 mM Ca ²⁺)	<i>P</i> value (Dysf KO, BSA, 0 mM Ca ²⁺)	<i>P</i> value (Dysf KO, BSA, 2 mM Ca ²⁺)	<i>P</i> value (Dysf KO, rhMG53, 0 mM Ca ²⁺)	<i>P</i> value (Dysf KO, rhMG53, 2 mM Ca ²⁺)	<i>P</i> value (WT 0 mM Ca ²⁺)
AUC						
Dysf KO,	<0.0001	<0.0001	<0.0001	n/s	n/s	n/s

rhMG53, 0 mM Ca ²⁺						
Dysf KO, rhMG53, 2 mM Ca ²⁺	<0.0001	<0.0001	<0.0001	n/s	n/s	n/s
WT 0 mM Ca ²⁺	0.0006	n/s	n/s	<0.0001	<0.0001	n/s
WT 2 mM Ca ²⁺	<0.0001	<0.0001	<0.0001	n/s	n/s	<0.0001
MaxFluor FM4-64						
Dysf KO, rhMG53, 0 mM Ca ²⁺	<0.0001	<0.0001	<0.0001	n/s	n/s	n/s
Dysf KO, rhMG53, 2 mM Ca ²⁺	<0.0001	<0.0001	<0.0001	n/s	n/s	n/s
WT 0 mM Ca ²⁺	n/s	n/s	n/s	<0.0001	<0.0001	n/s
WT 2 mM Ca ²⁺	<0.0001	<0.0001	<0.0001	n/s	n/s	<0.0001

n/s – measurements are not significantly different

P values are calculated by *Tukey-ANOVA* method using Prism software (GraphPad Prism 7.0, USA).

Supplementary Table S3. Effect of serum CK and LDH release in *Dysf*^{-/-} mice after treatment and downhill running exercise.

	CK level (IU/L)	LDH level (IU*10 ³ /L)
WT (C57BL/6J)	17.7 ± 4.6	55.0 ± 3.2
Dysf^{-/-} Sedentary (control)	58.5 ± 6.4	76.5 ± 3.2
Dysf^{-/-} Pre-exercise-Saline	67.6 ± 6.7	88.7 ± 5.6
Dysf^{-/-} Post-exercise-Saline	157.5 ± 14.8	148.9 ± 15.4
Dysf^{-/-} Pre-exercise-rhMG53	67.0 ± 9.8	74.5 ± 11.4
Dysf^{-/-} Post-exercise-rhMG53	123.0 ± 8.0	120.1 ± 12.1

Supplementary Table S4. Quantification analysis of IgG positive fibers from *Dysf*^{-/-} mice.

Muscle	Control*	Post-exercise-Saline*	Post-exercise-rhMG53*
Gluteus	7.9 ± 1.4	17.7 ± 1.8	13.1 ± 1.4
EDL	24.3 ± 1.1	29.8 ± 1.3	26.3 ± 1.0
Soleus	11.6 ± 1.3	18.5 ± 1.1	13.5 ± 0.8

*The quantification result was expressed as the mean of positive fibers to the total number of fibers ratio, expressed as a percentage.

Supplementary Table S5. Absorption spectroscopy analysis of EBD-injected *Dyst^{-/-}* mice after treatment and downhill running exercise.

Muscle	Control*	Post-exercise-Saline*	Post-exercise-rhMG53*
Gluteus	2.5 ± 0.13	3.2 ± 0.12	2.9 ± 0.14
Soleus	5.2 ± 0.17	6.0 ± 0.29	5.3 ± 0.21
EDL	2.5 ± 0.15	3.4 ± 0.19	2.7 ± 0.10
TA	1.7 ± 0.09	2.4 ± 0.17	2.2 ± 0.06
Gastroc	2.0 ± 0.10	2.6 ± 0.14	2.4 ± 0.05
FDB	5.5 ± 0.59	5.4 ± 0.48	5.5 ± 0.62
Diaphragm	8.2 ± 0.18	8.4 ± 0.21	8.5 ± 0.26
Kidney	11.4 ± 0.40	13.7 ± 0.48	13.5 ± 0.43

*The absorption results of EBD uptake were expressed as the mean of OD⁽⁶²⁰⁻⁸⁰⁰⁾/tissue (g).