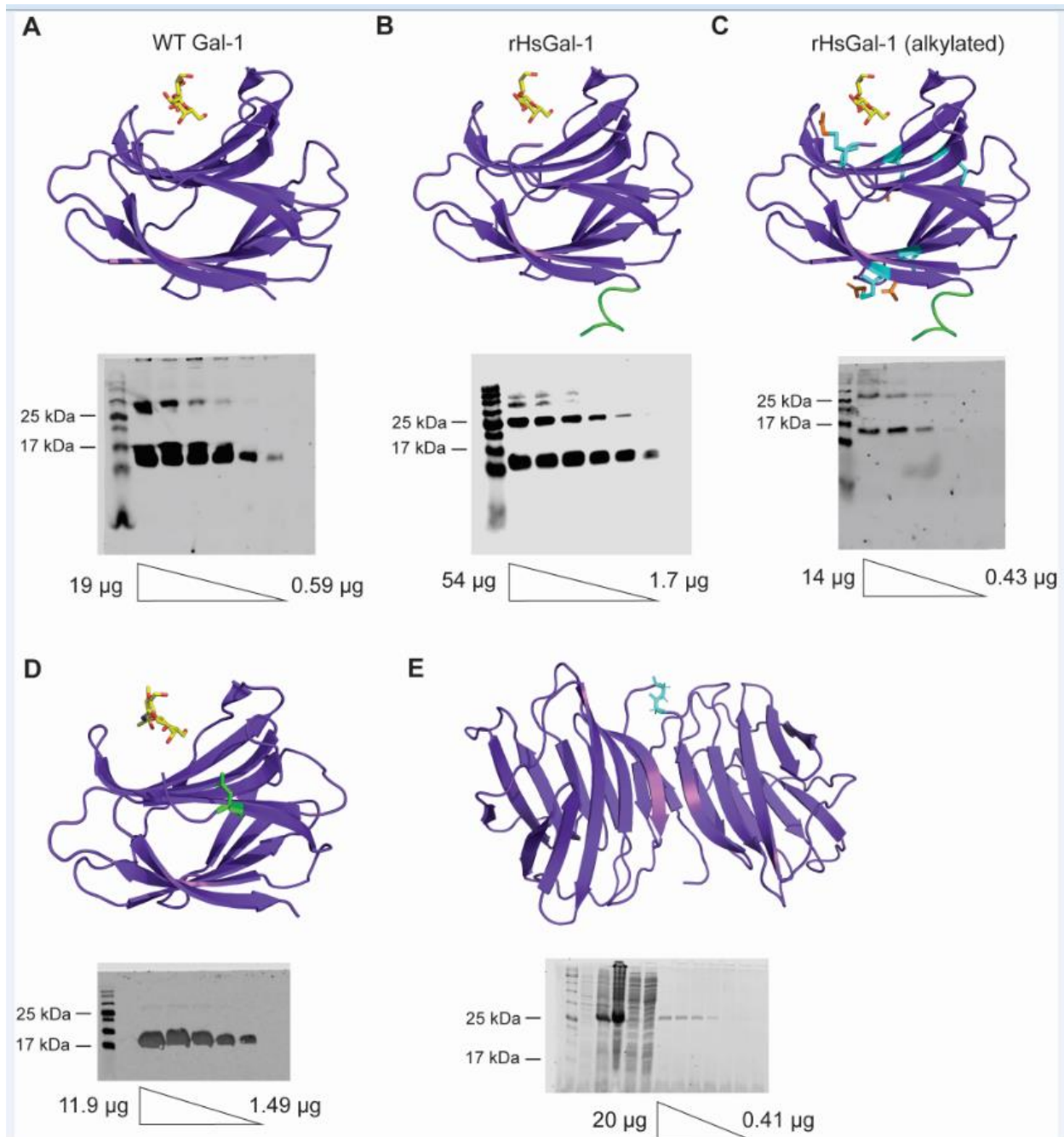
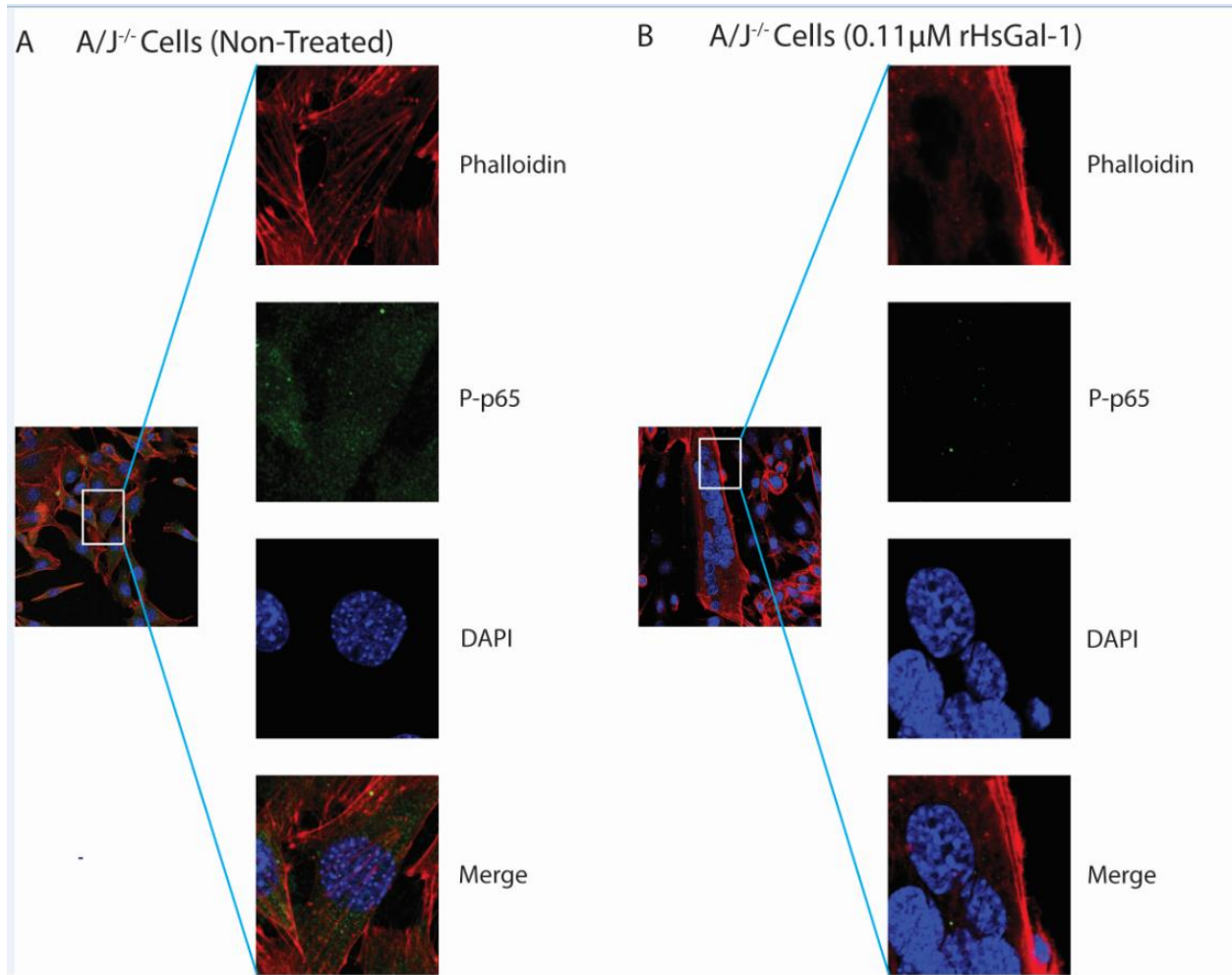


## Supplementary Figures and Video

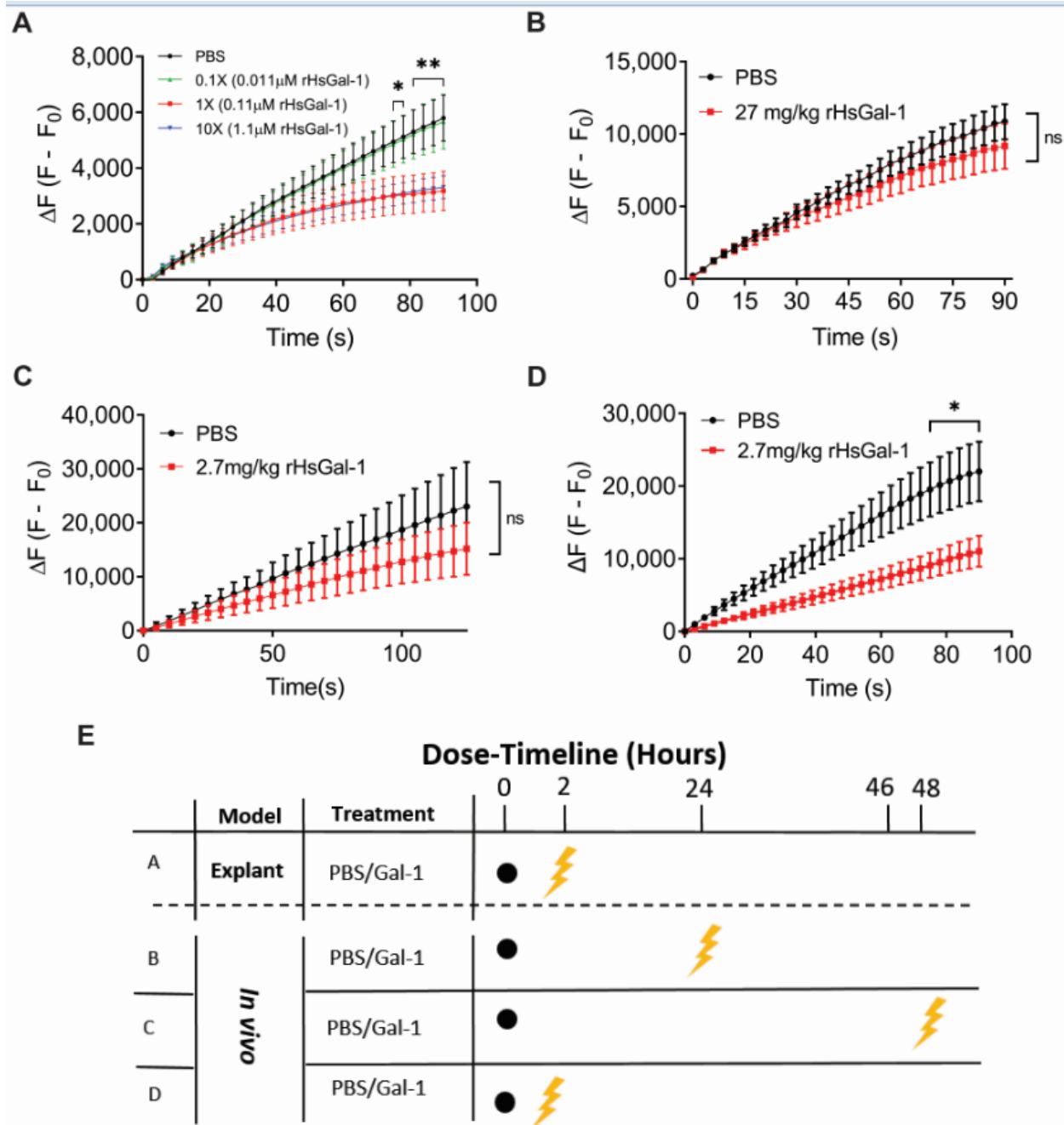


**Figure S1.** Structure and Purification of different types of Gal-1. **(A)** Crystal structure of wild-type galectin-1 (WT Gal-1) bound to generic  $\beta$ -galactoside ligand (PDB 1GZW) and western blot image of WT Gal-1 at decreased dosage. **(B).** Model of recombinant human galectin-1 (rHsGal-1) bound to generic  $\beta$ -galactoside ligand (modeled from PDB 1GZW) and western blot image of rHsGal-1 at decreased dosage. **(C)** Model of alkylated rHsGal-1 bound to generic  $\beta$ -galactoside ligand (modeled from PDB 1GZW) and western blot image of alkylated rHsGal-1 at decreased dosage. **(D)** Representative structure of the fixed monomeric form of Gal-1 and western blot image

with serial dilution for verification of proper construction. **(E)** Representative structure of the fixed dimeric form of Gal-1 and western blot image with serial dilution for verification of proper construction.

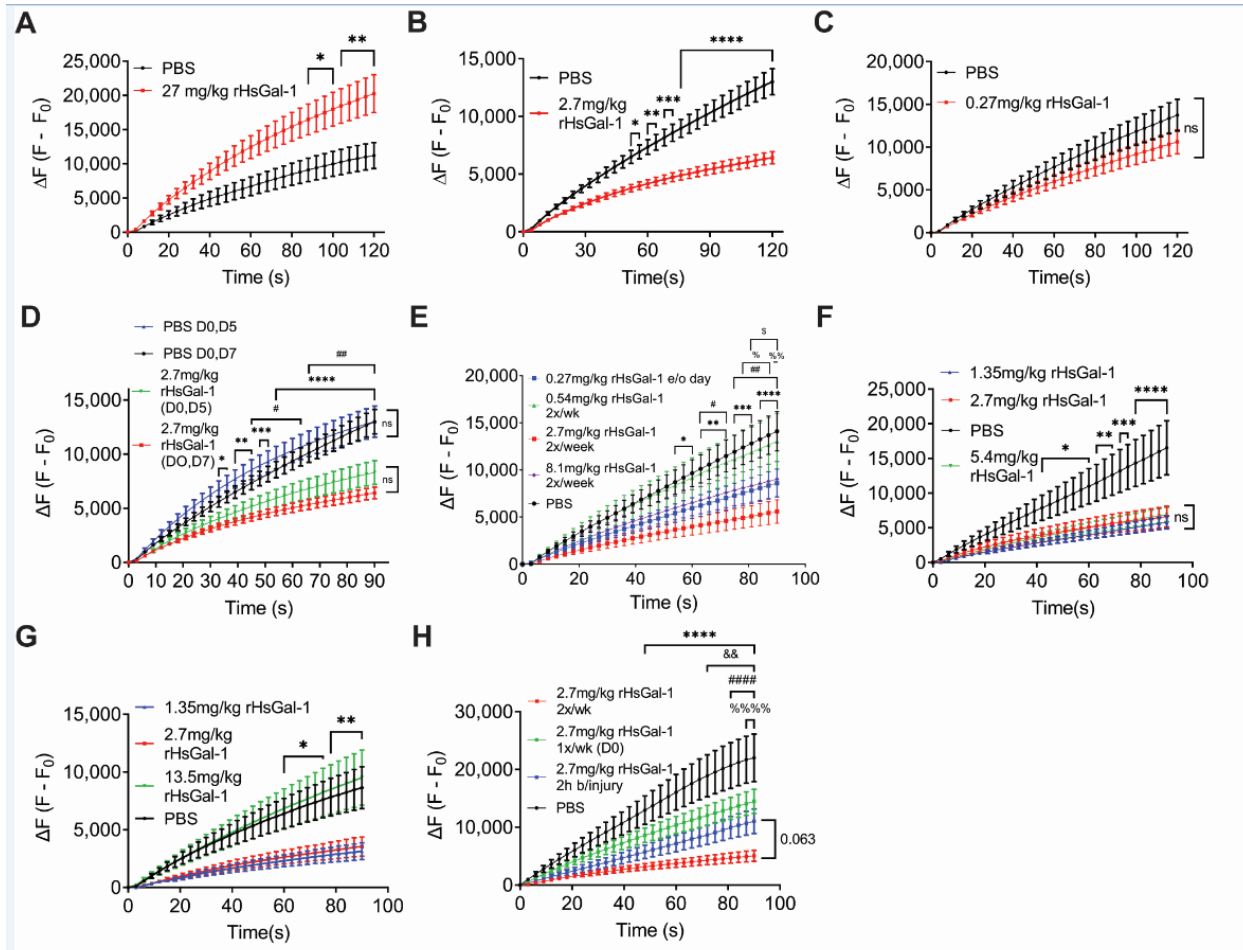


**Figure S2.** In vitro treatment with rHsGal-1 treatment decreases expression of the phosphorylated p65. Representative images of NT (A) or 48 h treated A/J<sup>-/-</sup> myotubes with 0.11 μM rHsGal-1 in (B). A/J<sup>-/-</sup> NT or 0.11 μM rHsGal-1 treated myotubes cultured and immunostained with Phospho-p65 (green), Phalloidin (red), and DAPI (blue).



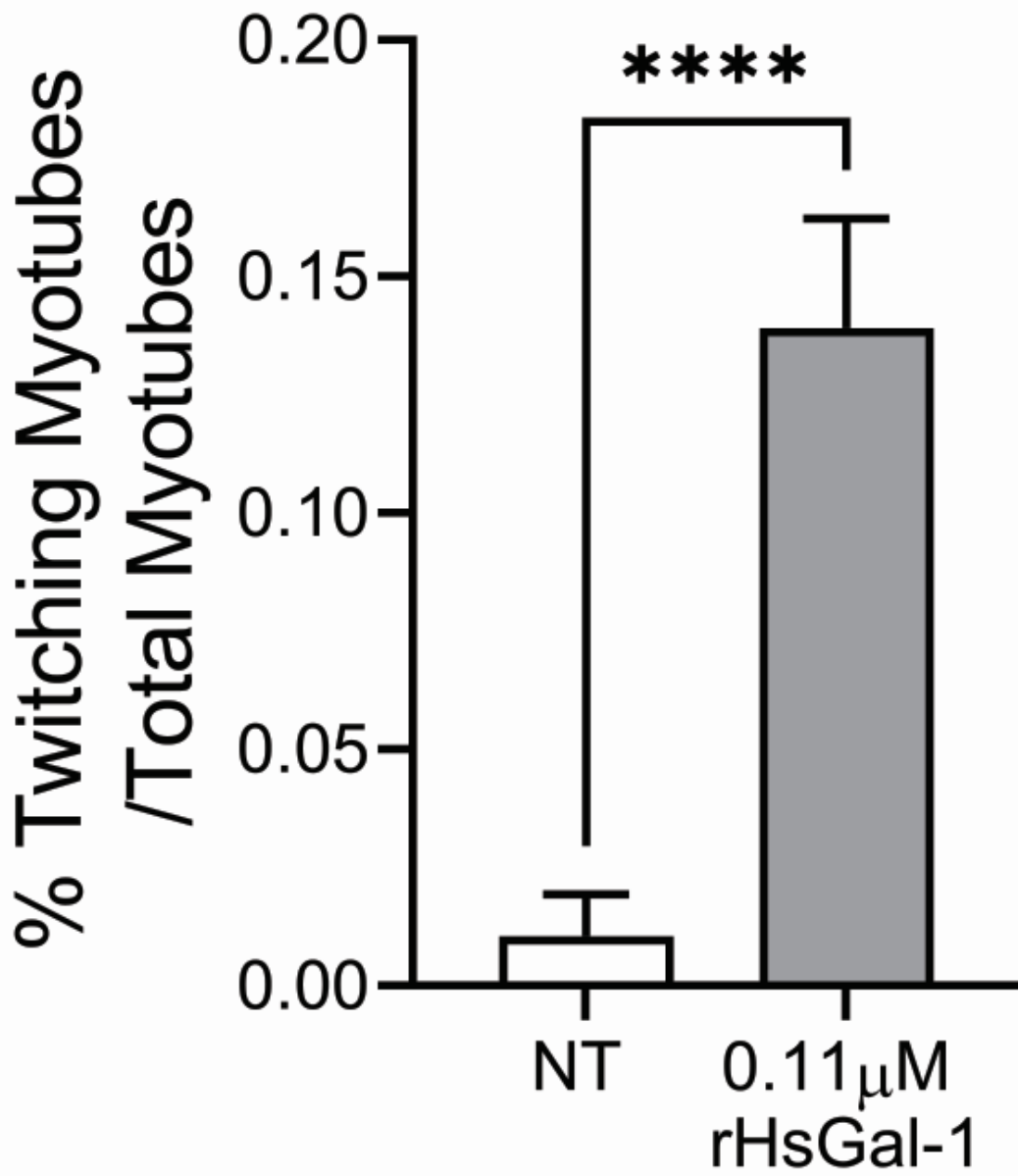
**Figure S3.** rHsGal-1 Dose optimization in *explant* and in vivo BLA/J myofibers. **(A)** Quantified laser injury assay on explant mouse muscles from BLA/J mice treated with 0.011  $\mu$ M rHsGal-1 (0.1X), 0.11  $\mu$ M rHsGal-1 (1X), 1.1  $\mu$ M rHsGal-1 (10X) compared to PBS treated control. **(B)** Quantified laser injury assay on mouse muscles from BLA/J mice treated in vivo 27 mg/kg rHsGal-1 (10X) compared to PBS treated control. Muscles were taken and injured 24 h after treatment. **(C)** Quantified laser injury assay on mouse muscles from BLA/J mice treated in vivo 2.7 mg/kg rHsGal-1 (1X) compared to PBS treated control. Muscles were taken and injured 48 h after treatment. **(D)** Quantified laser injury assay on mouse muscles from BLA/J mice treated in vivo 2.7 mg/kg rHsGal-1 (1X) compared to PBS treated control. Muscles were taken and injured 2 h

after treatment. **(E)** Timeline for experiments shown in A-E. Black dot = treatment. Yellow bolt = injury. Values were measured by Tukey's multiple comparison test and indicated by: \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , and \* $p < 0.05$  between control and rHsGal-1 treated mice.



**Figure S4.** 2.7 mg/kg is the best dose of rHsGal-1 improving membrane repair in explant and in vivo Bla/J myofibers. **(A)** Quantified laser injury assay on BLA/J mouse muscles treated in vivo with 27 mg/kg rHsGal-1 compared to PBS treated control. Mice were treated three times in 7 days, and muscles were taken on the seventh day to be injured and imaged. **(B)** Quantified laser injury assay on mouse muscles from BLA/J mice treated in vivo 2.7 mg/kg rHsGal-1 compared to PBS treated control. Mice were treated on day 0 and day 7. Muscles were taken and injured 7 days after first treatment. **(C)** Quantified laser injury assay on mouse muscles from BLA/J mice treated in vivo 0.27 mg/kg rHsGal-1 compared to PBS treated control. Mice were treated on day 0 and day 7. Muscles were taken and injured 7 days after first treatment. **(D)** Quantified laser injury assay on mouse muscles from BLA/J mice treated in vivo 2.7 mg/kg rHsGal-1 (1X) compared to PBS treated control. One rHsGal-1 and one PBS-treated mouse were treated on days 0 and 7, while the other rHsGal-1 and PBS-treated mice were treated on days 0 and 5. Muscles were taken and injured 7 days after first treatment. \* = PBS vs. 2.7 mg/kg rHsGal-1 (D0, D7); # = PBS vs. 2.7 mg/kg rHsGal-1 (D0, D5). **(E)** Quantified laser injury assay on mouse muscles from BLA/J mice treated in vivo with 0.27 mg/kg rHsGal-1 every other day, 0.54 mg/kg rHsGal-1 on days 0 and 7, 2.7 mg/kg rHsGal-1 on days 0 and 7, and 8.1 mg/kg rHsGal-1 on days 0 and 7 compared to PBS treated control. All muscles were taken and injured 7 days after first treatment. # = 0.54 mg/kg vs.

2.7mg/kg; \* = PBS vs. 2.7 mg/kg; % = PBS vs. 0.27 mg/kg; \$ = PBS vs. 8.1 mg/kg. **(F)** Quantified laser injury assay on mouse muscles from BLA/J mice treated in vivo with 1.35 mg/kg rHsGal-1, 2.7 mg/kg rHsGal-1, and 5.4 mg/kg rHsGal-1 compared to PBS treated control. Mice were treated on days 0 and 7. Muscles were taken 7 days after first treatment. **(G)** Quantified laser injury assay on mouse muscles from BLA/J mice treated in vivo with 1.35 mg/kg rHsGal-1, 2.7 mg/kg rHsGal-1, and 13.5 mg/kg rHsGal-1. Mice were treated on days 0 and 7. Muscles were taken 7 days after first treatment. **(H)** Quantified laser injury assay on mouse muscles from BLA/J mice treated in vivo with 2.7 mg/kg rHsGal-1 or PBS control. Mice were treated on days 0 and 7, day 0 only, or day 7 only. \* = PBS vs. rHsGal-1 D0,7; # = PBS vs. rHsGal-1 D7 only; %= rHsGal-1 D0,7 vs. rHsGal-1 D0 only; and = PBS vs. rHsGal-1 D0 only. Values for all graphs were measured by Tukey's multiple comparison test and indicated except where noted by: \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , and \* $p < 0.05$  between control and rHsGal-1 treated mice.



**Figure S5.** Quantification of the percentage of twitching myotubes. \*\*\*\* $p < 0.0001$  between NT and rHsGal-1 treated myotubes.

**Supplemental Video 1.** Effect of rHsGal-1 on twitching ability of A/J<sup>-/-</sup> myotubes. Video taken 15 days post-treatment, comparing T and NT myotubes.