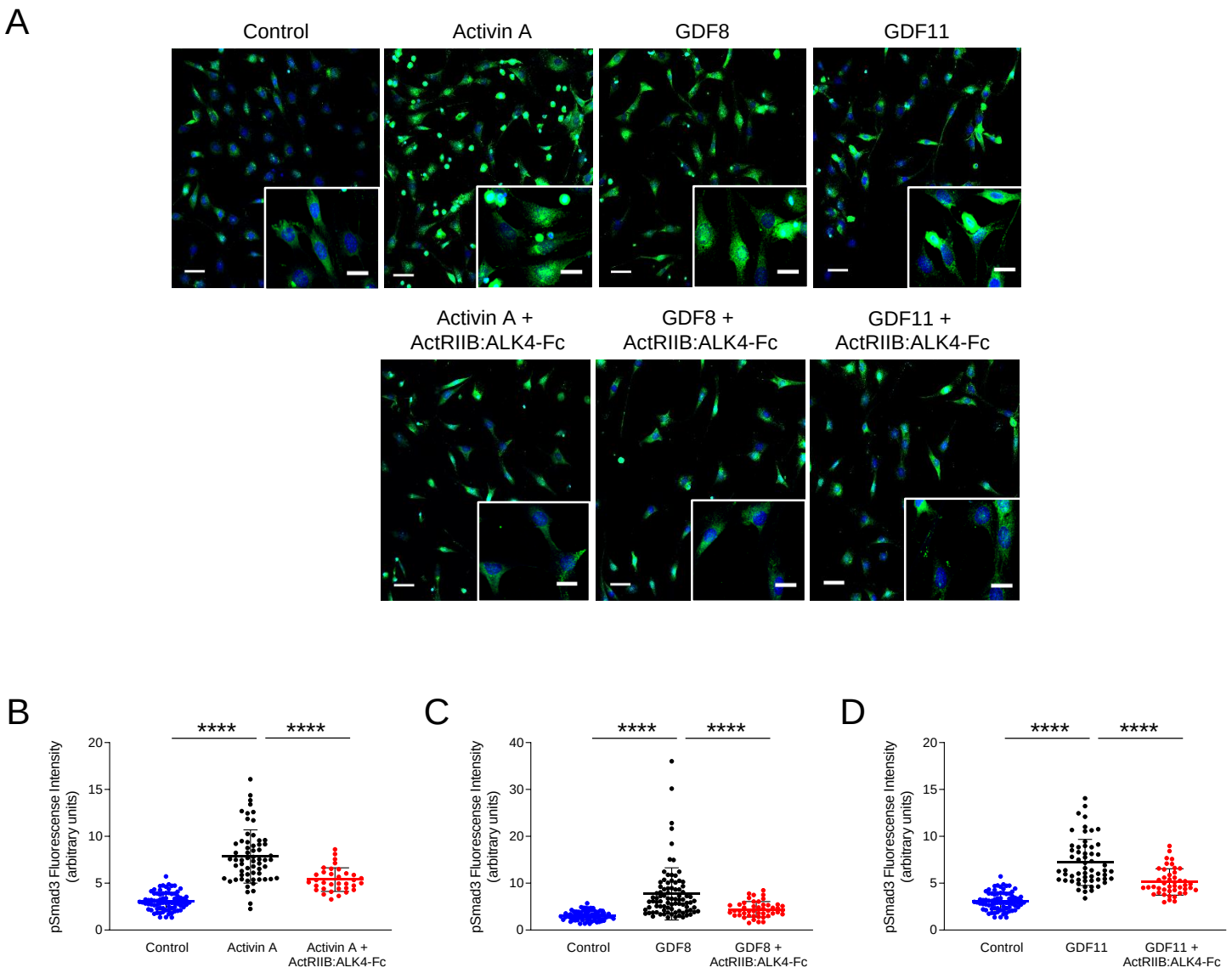
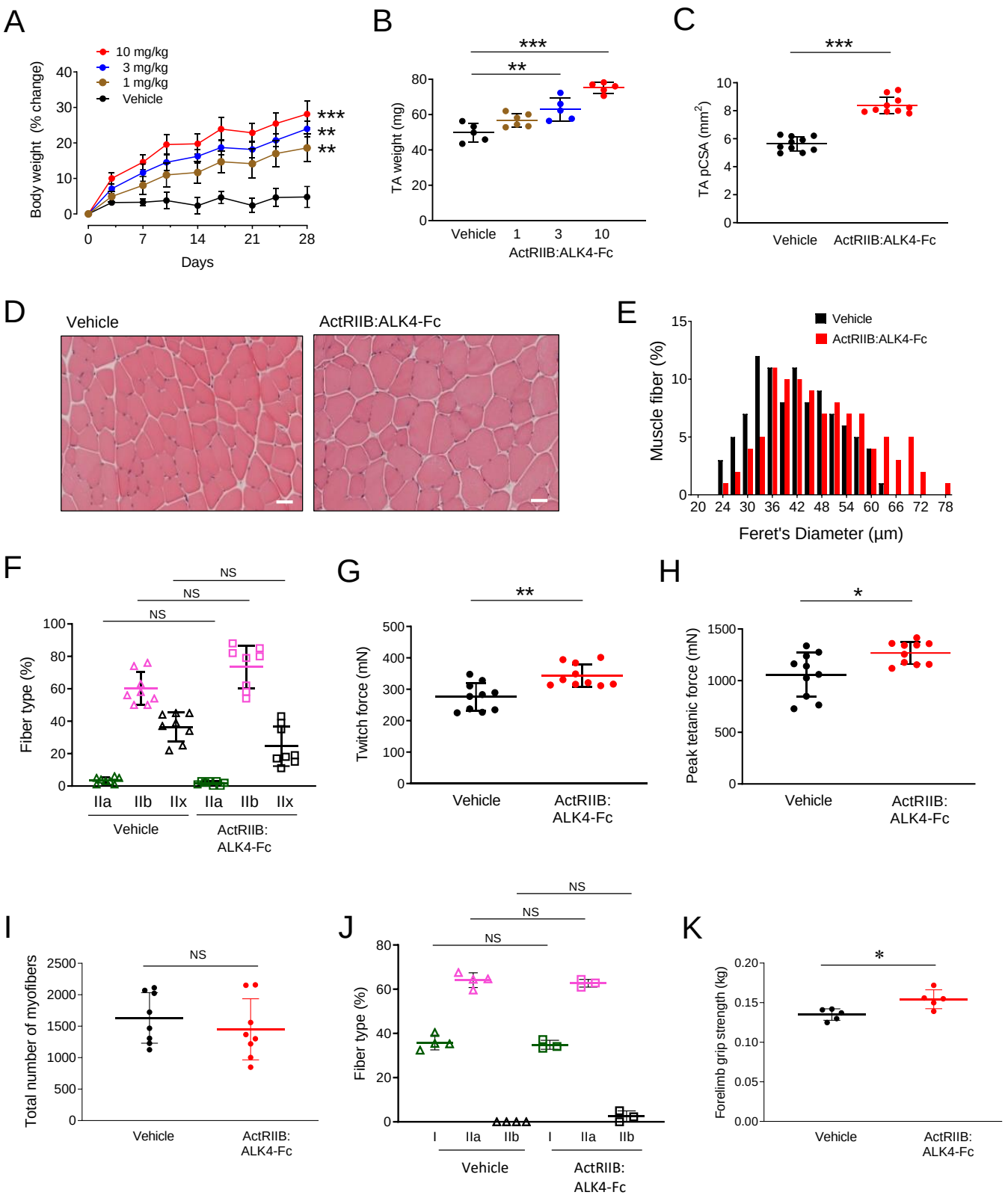
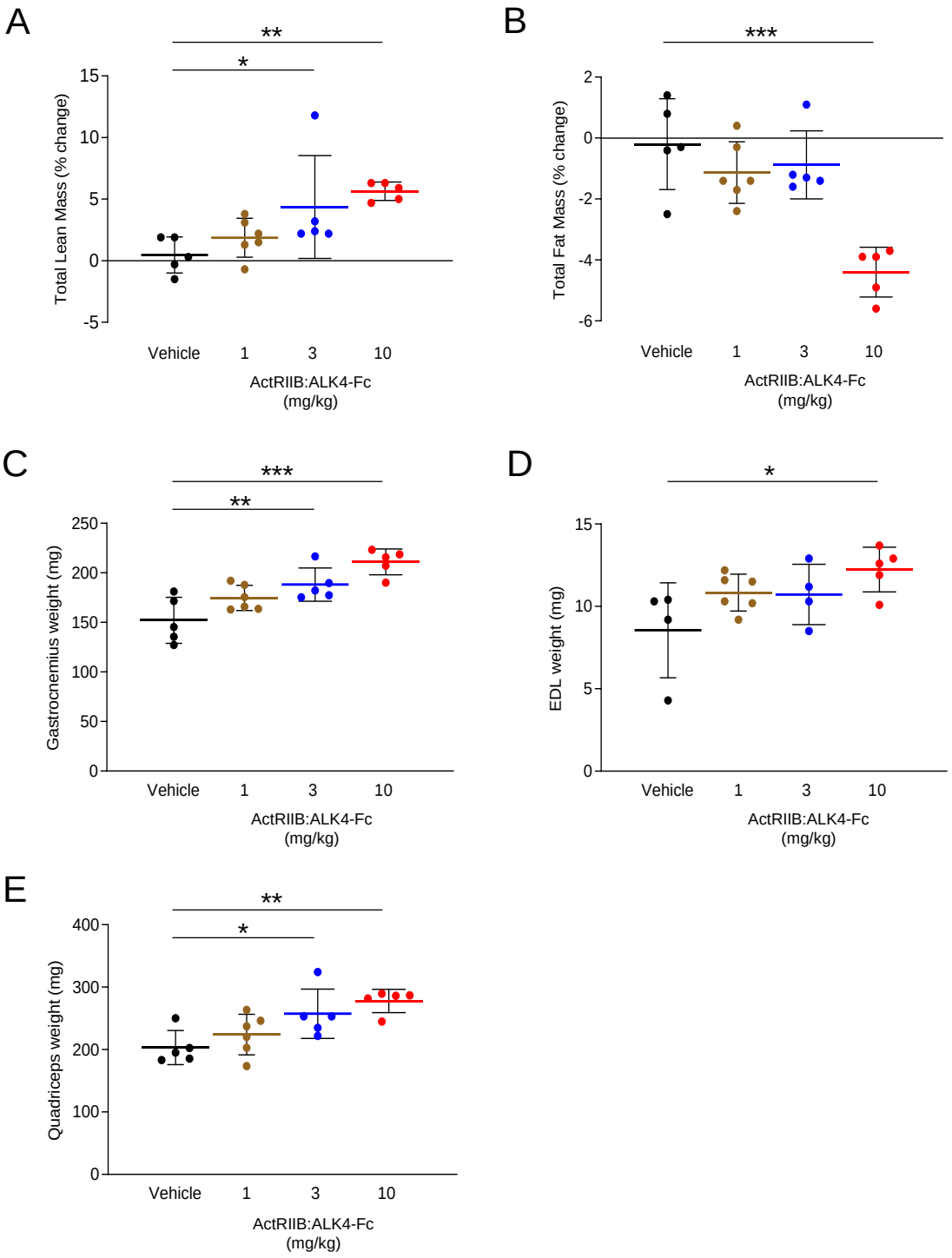


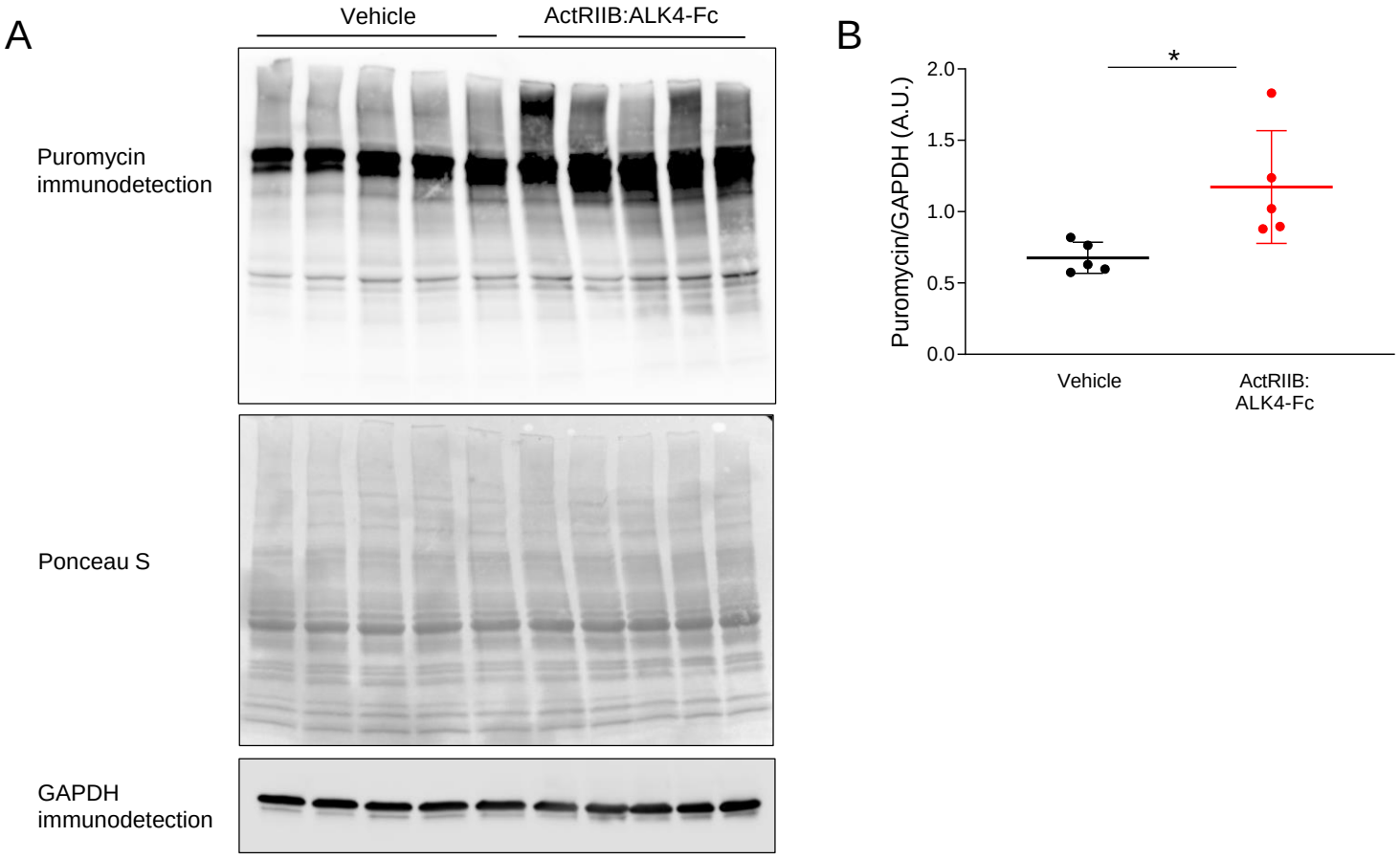
Supplemental Figure 1. ActRIIB:ALK4-Fc inhibits Smad3 signaling *in vitro*. Immunofluorescence microscopic images of C2C12 cells treated with activin A, GDF8, or GDF11 – with or without ActRIIB:ALK4-Fc – for 30 minutes, then stained to detect phospho-Smad3 (pSmad3, green) and nuclei (DAPI, blue). (**A**) Scale bars = 50 μm or 20 μm (insets). (**B-D**) Quantitative analysis of fluorescence intensity for phospho-Smad3. Data are means ± SEM; n = 34-88 cells per group. Group differences were assessed by one-way ANOVA with Tukey's adjustment. ****$P < 0.0001$.


A
10 mg/kg
3 mg/kg
1 mg/kg
Vehicle
Body weight (% change)
Days
***
**
**
B
TA weight (mg)
***
**
Vehicle
1
3
10
ActRIIB:ALK4-Fc
C
TA pCSA (mm2)
***
Vehicle
ActRIIB:ALK4-Fc
D
Vehicle
ActRIIB:ALK4-Fc
E
Vehicle
ActRIIB:ALK4-Fc
Muscle fiber (%)
Feret's Diameter (μm)
F
Fiber type (%)
NS
NS
NS
IIa
IIb
IIx
IIa
IIb
IIx
Vehicle
ActRIIB:
ALK4-Fc
G
Twitch force (mN)
**
Vehicle
ActRIIB:
ALK4-Fc
H
Peak tetanic force (mN)
*
Vehicle
ActRIIB:
ALK4-Fc
I
Total number of myofibers
NS
Vehicle
ActRIIB:
ALK4-Fc
J
Fiber type (%)
NS
NS
NS
I
IIa
IIb
I
IIa
IIb
Vehicle
ActRIIB:
ALK4-Fc
K
Forelimb grip strength (kg)
*
Vehicle
ActRIIB:
ALK4-Fc

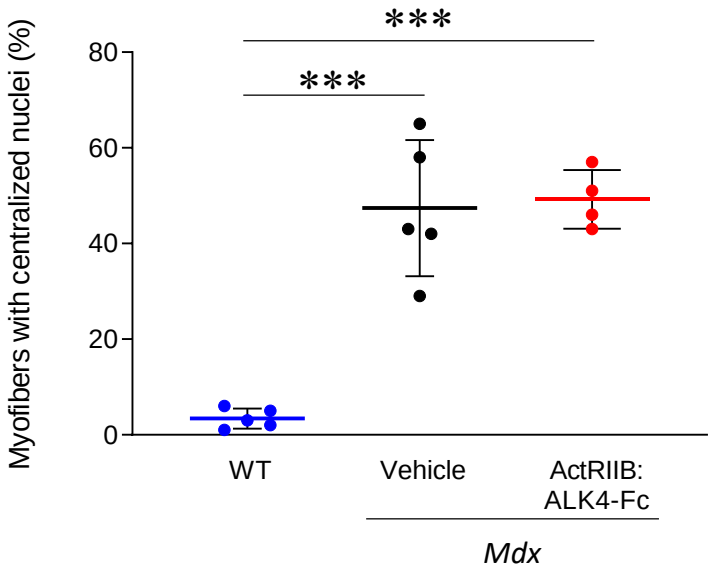
Supplemental Figure 2. ActRIIB:ALK4-Fc induces muscle hypertrophy and improves muscle function in wild-type mice. Eight or twelve-week-old wild-type C57BL/6 mice were injected s.c. with vehicle or 1, 3 or 10 mg/kg ActRIIB:ALK4-Fc twice weekly for 28 days. (**A**) Percent change in body weight from baseline over time. Data are means ± SEM (n = 8). Group differences were assessed by repeated measure one-way ANOVA with Dunnett's adjustment. ** $P < 0.01$, *** $P < 0.001$ vs. vehicle. (**B**) TA muscle weight. Data are means ± SEM (n = 5-6). Group differences were determined by one-way ANOVA with Dunnett's adjustment. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS = not significant. In single-dose experiments, C57BL/6 mice were injected s.c. with ActRIIB:ALK4-Fc (10 mg/kg) or vehicle (PBS) twice weekly for 28 days. (**C**) pCSA of the TA muscle (n = 10). (**D**) Representative TA muscle sections stained with hematoxylin and eosin. Scale bar = 50 μm. (**E**) Histograms of muscle fiber diameter with 100 fibers analyzed per treatment group. (**F**) Proportion of muscle fiber subtypes IIa, IIb, and IIx in TA muscle sections as a function of treatment (n = 8 mice). (**G**) Twitch force (n = 10). (**H**) Peak tetanic force (n = 10). (**I**) Total number of myofibers were counted in the whole TA sections (n = 8 per group). (**J**) Proportion of muscle fiber subtypes I, IIa, and IIb in soleus muscle sections as a function of treatment. Data are means ± SEM (n = 4-5 per group). (**K**) Forelimb grip strength. Data are means ± SEM (n = 5 per group). Group differences were assessed by either unpaired Student's t-test or one-way ANOVA with Tukey's adjustment. NS = not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS = not significant.



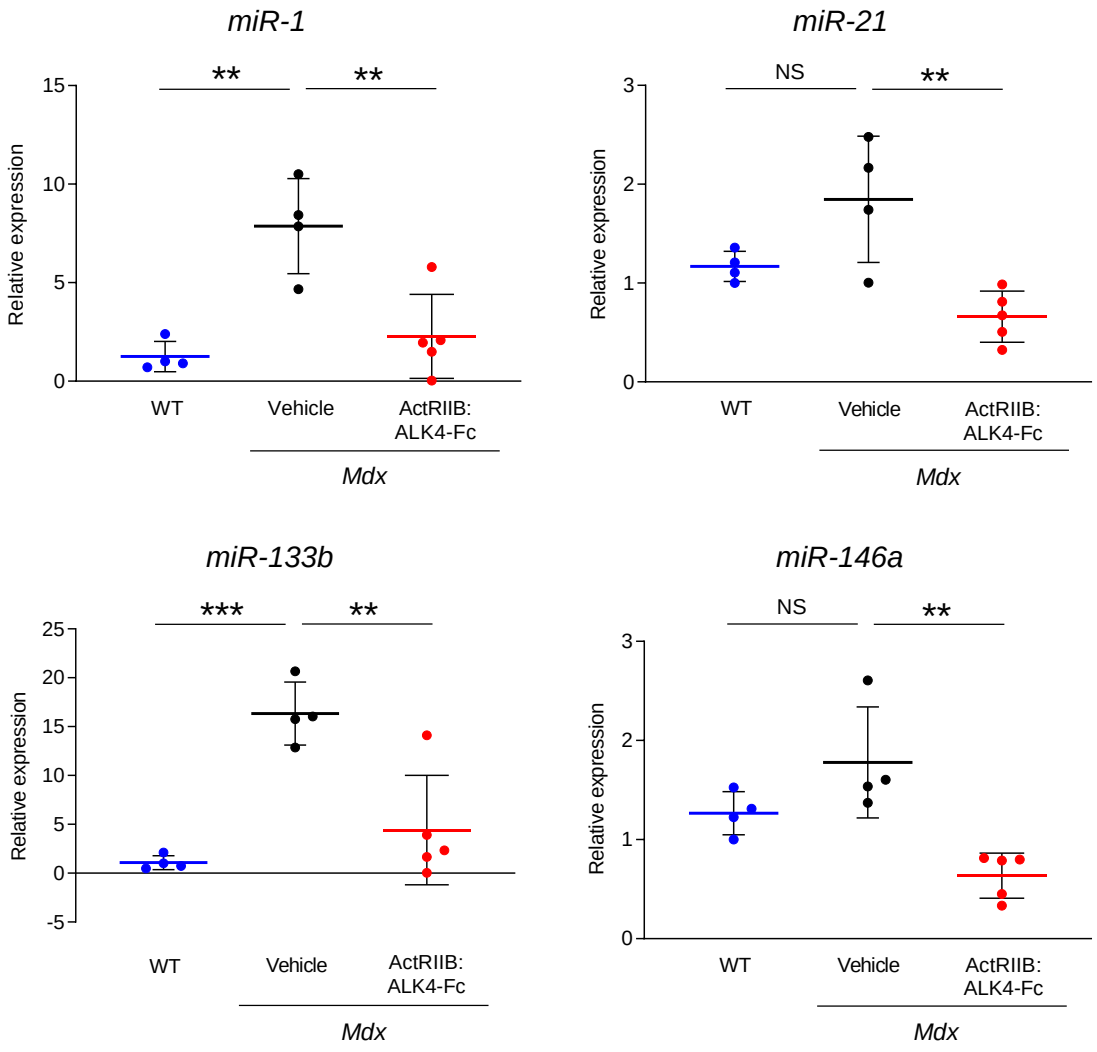
Supplemental Figure 3. ActRIIB:ALK4-Fc exerts dose-dependent effects on lean body mass, fat mass, and muscle mass in wild-type mice. Wild-type C57BL/6 mice were injected s.c. with vehicle or 1, 3, or 10 mg/kg of ActRIIB:ALK4-Fc twice weekly for 28 days. Percent change in (**A**) total lean mass and (**B**) total fat mass determined by whole-body NMR scan (n = 5-6). Weights of **(C)** gastrocnemius (n = 5-6), (**D**) extensor digitorum longus (EDL) (n = 4-6), and (**E**) quadriceps (n = 5-6). Data are means ± SEM. Group differences were assessed by one-way ANOVA with Dunnett's adjustment. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.



Supplemental Figure 4. ActRIIB:ALK4-Fc increases protein synthesis *in vivo*. C57BL/6 mice were treated s.c. with ActRIIB:ALK4-Fc or vehicle twice weekly for one week, and puromycin was administered 30 minutes prior to muscle collection. (**A**) TA muscle lysates were analyzed by immunoblot to detect puromycin-labeled peptides (upper panel). Also shown are Ponceau S staining (center panel) and GAPDH immunodetection (lower panel). (**B**) Quantitative analysis of western blotting. Data are means ± SEM. Group differences were assessed by unpaired Student's t-test. **P* < 0.05.

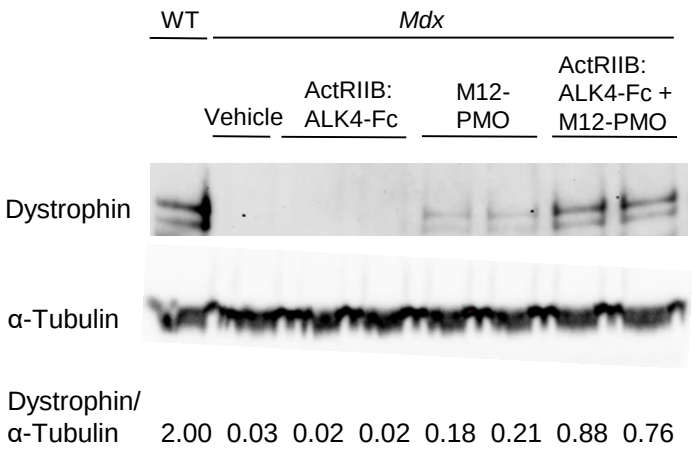


Supplemental Figure 5. ActRIIB:ALK4-Fc did not alter the distribution of nuclei within TA myofibers in a mouse model of DMD. Twelve-month-old C57BL/6 wild-type and BL10.*mdx* mice were treated with ActRIIB:ALK4-Fc (10 mg/kg) or vehicle (PBS) twice weekly for 8 weeks. Percentage of TA myofibers with centralized nuclei based on analysis of all myofibers in the cross-sectional area per mouse. Data are means ± SEM (n = 4-5 per group). Group differences were assessed by one-way ANOVA with Tukey's adjustment. ***$P < 0.001$.



Supplemental Figure 6. ActRIIB:ALK4-Fc reduces elevated levels of circulating miRNA muscle biomarkers in aged *mdx* mice. Fourteen-month-old C57BL/6 wild-type and B10.*mdx* mice were injected s.c. with ActRIIB:ALK4-Fc (10 mg/kg) or vehicle (PBS) twice weekly for 8 weeks. Serum levels of miR-1, miR-21, miR-133b, and miR-146a were determined by PCR. Data are means ± SEM (n = 4-5 per group). Group differences were assessed by one-way ANOVA with Tukey's adjustment. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant.

A



Supplemental Figure 7. Combined treatment with ActRIIB:ALK4-Fc and M12-PMO increases dystrophin expression in aged *mdx* mice. Thirteen-month-old BL10.*mdx* mice were injected with ActRIIB:ALK4-Fc (10 mg/kg) or vehicle (PBS) s.c. twice weekly, M12-PMO (25 mg/kg) i.p. once weekly, or a combination of ActRIIB:ALK4-Fc and M12-PMO for 4 weeks. (**A**) TA muscle lysates were analyzed by immunoblot to detect dystrophin (upper panel) and α-tubulin (lower panel), which served as a control for protein loading.