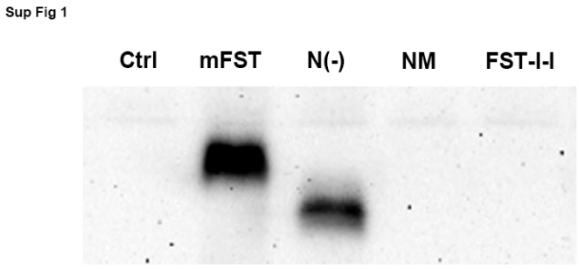
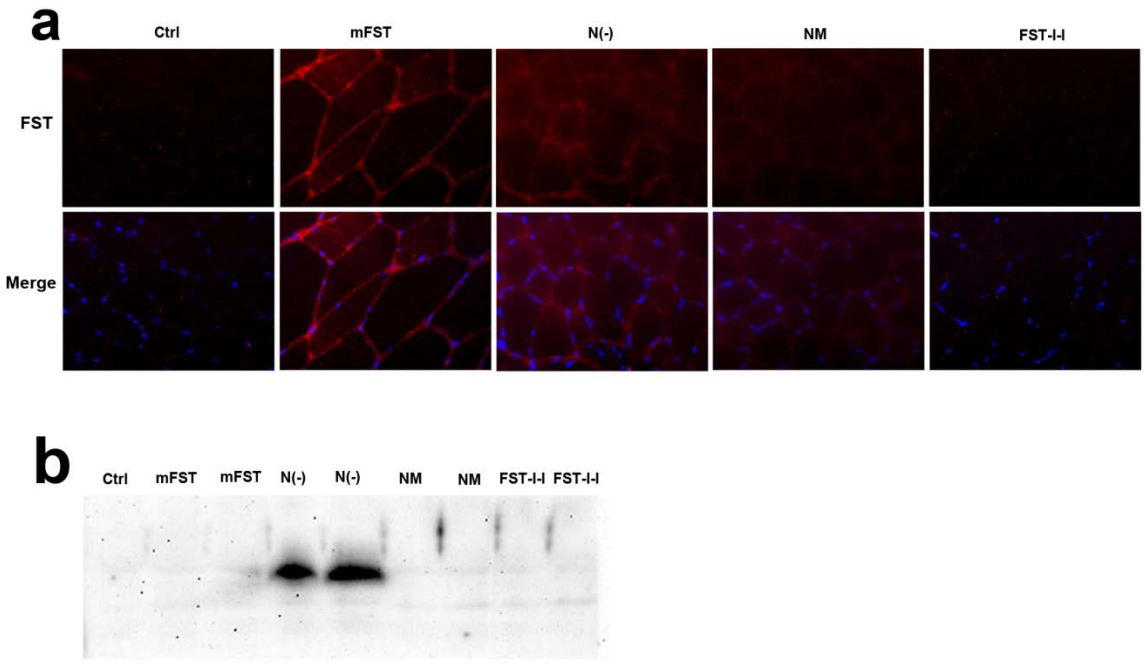


Supplementary Fig 1. Detection of FST variants expression in conditioned medium via Western blot.

The conditioned medium generated by single plasmid transfection was separated with 10% SDS/PAGE gels and then transferred to PVDF membranes. After blocking at room temperature for 1 hour with solution containing 5% non-fat milk in TBST, membranes were incubated overnight at 4°C with 1:1000 mouse monoclonal anti-follistatin (Santa Cruz Biotechnology, Inc, Cat# sc-271502), followed by 1:5000 HRP-conjugated secondary antibody incubation. Both FST and N(-) could be observed clearly. It is anticipated that the FST-I-I could not be detected because it lacks the epitope (C-terminal tail) recognized by the anti-FST antibody we utilized. We could not clearly see the N-mutant in conditioned media but detected a clear band in crude lysate (not shown).



Supplementary Fig 2. FST variants were expressed in skeletal muscles. (a) Immunofluorescent staining. TA frozen sections were fixed with pre-cold 4% paraformaldehyde for 15 minutes. After blocking with 10% horse serum in PBS, the sections were incubated with 1:75 rabbit polyclonal anti-follistatin (Santa Cruz Biotechnology, INC, Cat# sc-30194) for 3 hours followed by Cy3-conjugated secondary antibody incubation (1:500 dilution) for 45 minutes. Nuclei were stained with DAPI and 40X images were taken. The expression of FST variants can be seen around membranes in FST and NM groups, while both cytoplasm and peri-membrane area in N(-) group, compared to the control. There are no differences between FST-I-I and control, in the lack of C-terminal binding epitope recognized by the FST antibody. **(b) Western blot analysis.** Protein of the GAS muscle was extracted using lysis buffer containing 4% SDS, 125 mM Tris pH 8.8, 4% glycerol, 100 mM DTT, 0.01% BPB, and a cocktail of protease inhibitors. 30 µg of protein per lane and prestained molecular weight markers were separated with 10% SDS/PAGE gels and then transferred to PVDF membranes. After blocking at room temperature for 1 hour with solution containing 5% non-fat milk in TBST, membranes were incubated overnight at 4°C with 1:1000 rabbit polyclonal anti-follistatin, followed by 1:5000

HRP-conjugated secondary antibody incubation. Consistent with the IF results, heavy bands were detected in the N(-) group, and a weak band was detected for the FST group. This may have occurred because most of the wide type FST was secreted.