Supplementary Methods for:
Treatment of sporadic inclusion body myositis with bimagrumab

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Cell culture. Approximately $5x10^5$ Human Skeletal Muscle Cells (HuSkMC) (#CC-2561, Lonza, Walkersville, MD) were cultured on three 100mm x 20mm plates (#430167, Corning, Corning, NY) in filtered growth media containing 20% Fetal Bovine Serum (FBS) (#S11550, Atlanta Biologicals, Lawrenceville, GA) and 1.2% P/S/L glutamine (#25200056, GIBCO/Invitrogen, Carlsbad, CA) in Dulbecco's Modified Eagle Medium (DMEM) with high glucose, P/S/L glutamine and sodium pyruvate (#11995-073, Invitrogen). Once cells were 90% confluent (Day 3), media were changed to DMEM without FBS and cell plates were treated with recombinant GDF8 (#788-G8/CF, R&D Systems, Minneapolis, MN) to final concentrations of 0 ng/mL, 10 ng/mL and 100 ng/mL. After 24 hours of incubation, HuSkMC were trypsinized, counted, washed with 1X PBS and stored in -80°C.

Muscle lysates. Whole muscle lysates (WML) were prepared using 10-20 mg of cryostat sectioned muscle sonicated on ice in a 10:1 v/w dilution of lysis buffer (containing 20 mMTris pH 7.6, 2% SDS, 5 mM DTT, 1X protease and phosphatase inhibitors). Protease (#11 836 170 001, Roche, Indianapolis, IN) and phosphatase (#04 906 845 001, Roche) inhibitor tablets were dissolved in ultrapure distilled water (#10977, GIBCO) to a 7X concentration then diluted to a 1X concentration in lysis buffer. Lysates were centrifuged at 10,000 g for 10 min at 4°C and the supernatant removed and studied. HuSkMC cells were lysed in the same fashion and the micro BCA assay (#23235, Pierce, Rockford,IL) was used to determine protein concentration.

Western Blots. SDS-PAGE and gel transfer were performed as previously described (Salajegheh et al Ann Neurol 2010;67:53-63) with 60 μg of muscle tissue from each sample. Pre-determined highly reactive WML and HuSkMC lysates at 30 μg were used as positive controls in multiple blots. After 1 hr blocking in 5% fat free milk, blots were incubated with rabbit monoclonal anti-pSMAD2 antibody (# 3108, Cell Signaling Technology, Danvers, MA) at a dilution of 1:250 in 5%milk/PBST0.1% for 17 hours at 4°C then 3 hours at room temperature (RT) with shaking. After an initial 5 min rinse, blots were more thoroughly washed 4 times for 10 min each in PBST0.1%. The blots were then incubated with pre-absorbed secondary HRP-tagged goat anti-rabbit IgG antibody (# Star124P, AbDSerotec, Kidlington, UK) at the dilution of 1:5000 in 5%milk/PBST0.1% for 1 hour at RT, with shaking, and washed as above.

To determine reactivity against SMAD, immunoblotting was repeated as above using rabbit polyclonal anti-SMAD2/3 primary antibody (# 3102, Cell Signaling Technology, Danvers, MA) at the dilution of 1:1000 for 17 hours at 4°C and secondary HRP-tagged goat anti-rabbit IgG antibody (#Star124P, AbDSerotec) at the dilution of 1:5000 for 1 hour at RT. Reactivity against actin was determined by immunoblotting using rabbit polyclonal anti-actin primary antibody (#sc1616, Santa Cruz Biotechnology, Santa Cruz, CA) at the dilution of 1:10,000 for 1 hour at RT and secondary HRP-

tagged goat anti-rabbit IgG antibody (#Star124P, AbDSerotec) at the dilution of 1:10,000 for 1 hour at RT. After each antibody incubation, blots were washed for 5 min, 5 times.

SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford IL) and High Resolution Kodak films were used for visualization of the bands. The blots were stripped using Restore Western Blot Stripping Buffer (#21062, Pierce) for 20 minutes at 37°C, washed twice for 10 minutes with PBST 0.1% and blocked for 1 hr in 5% milk/PBST 0.1%.

Western blot quantitation was performed on inverted images using ImageJ (NIH). Band intensity (area x average pixel intensity) was calculated for pSMAD2/3, SMAD2/3, and actin bands, normalized for blot image background intensity, and the ratios of pSMAD2/3 to actin calculated.