Winbanks et al., http://www.jcb.org/cgi/content/full/jcb.201211134/DC1

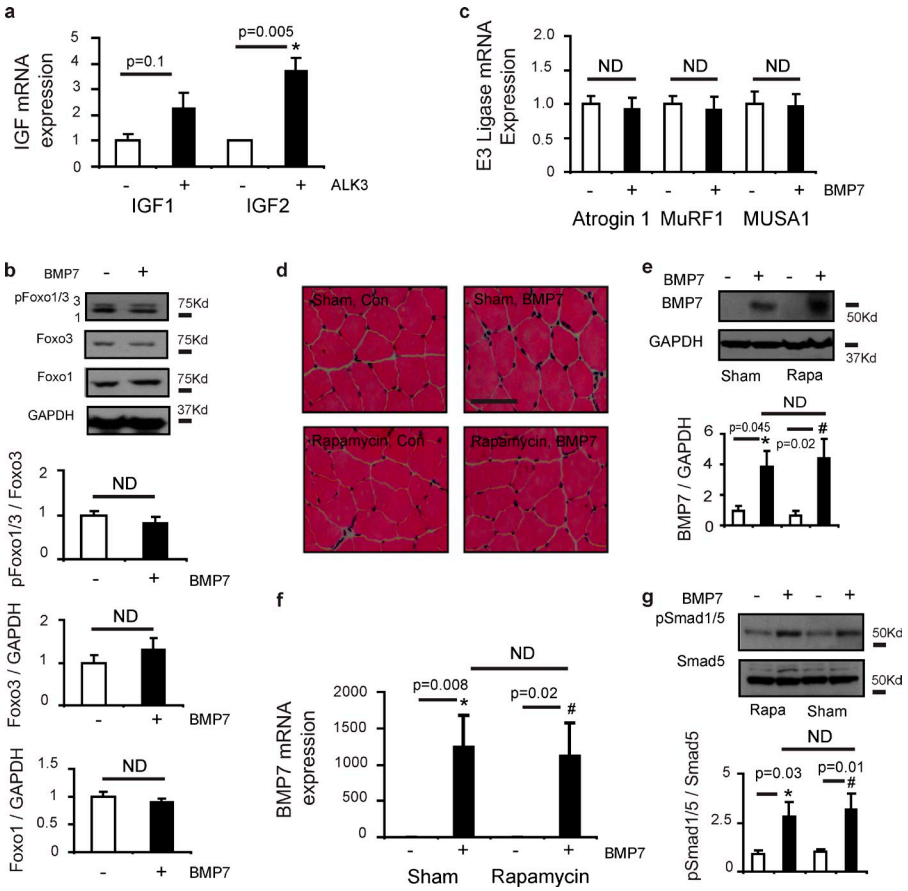

Figure S1. **Rapamycin prevents hypertrophy induced by rAAV6:BMP7.** (a) IGF1 and IGF2 transcription was measured in TA muscles examined 28 d after administration of rAAV6:ALK3 (*, P = 0.005 vs. control; *n* = 4 per treatment). (b) FoxO1^{T24}/FoxO3a^{T32} phosphorylation and total abundance was measured 28 d after rAAV6:BMP7 administration by Western blotting (P = ND). (c) Transcription of the E3 ubiquitin ligases atrogin1, MuRF1, and Musa1/Fbxo30 was measured by RT-PCR in muscle examined 28 d after administration of rAAV6:BMP7. P = ND. (d) Representative H&E images demonstrate rapamycin prevents hypertrophy of myofibers otherwise caused by administration of rAAV6:BMP7. Bar, 100 µm. (e and f) BMP7 mRNA (*n* = 7–8 per treatment) and protein expression (*n* = 3 per treatment) were measured 28 d after rAAV6:BMP7 administration in the presence of rapamycin or vehicle. RT-PCR data are presented according to the ΔΔCT method of analysis and normalized to a value of 1 (*, P = 0.008 vs. control; #, P = 0.02 vs. control). (g) The administration of rapamycin to mice did not prevent the phosphorylation of Smad1/5 in muscles administered rAAV6:BMP7 (*n* = 4 per treatment; *, P = 0.03 vs. control; #, P = 0.01 vs. control). Data are presented as means ± SEM.

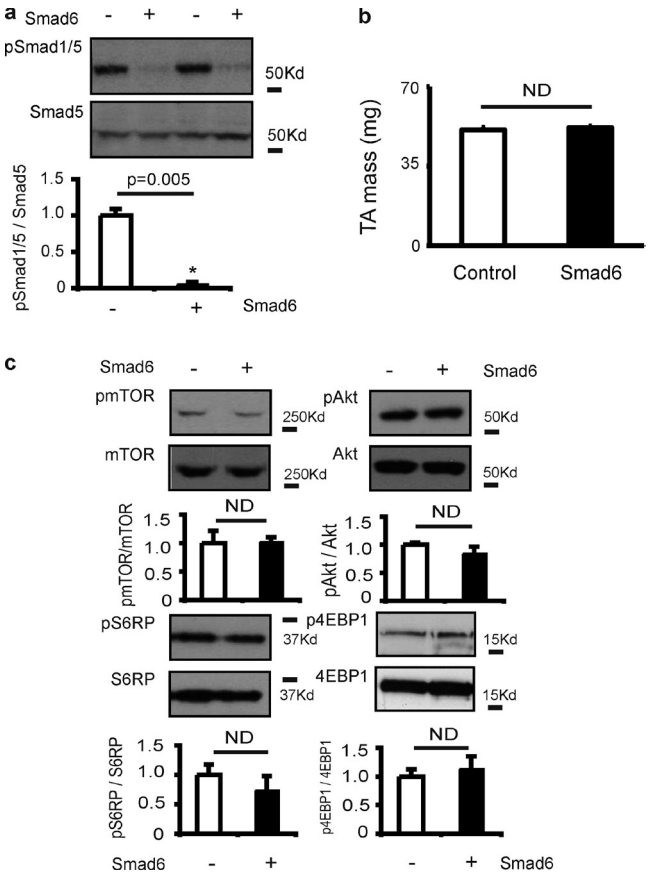

Figure S2. **Increased expression of Smad6 does not alter basal skeletal muscle mass.** (a) Administration of rAAV6:Smad6 to mouse muscles 28 d before examination potently suppressed Smad1/5$^{S463/465}$ phosphorylation, as determined by Western blot (n = 3 per treatment; *, P = 0.005 vs. control). (b) TA muscle mass as recorded 28 d after administration of rAAV6:Smad6. n = 3 per treatment. ND, not different. (c) The administration of rAAV6:Smad6 to mouse limb muscles did not alter the phosphorylation of Akt^{S473}, mTOR^{S2448}, S6RP$^{S235/236}$, or 4EBP1$^{T37/46}$ as determined by Western blot (ND, not different). n = 3–7 per treatment. Data are presented as means ± SEM.

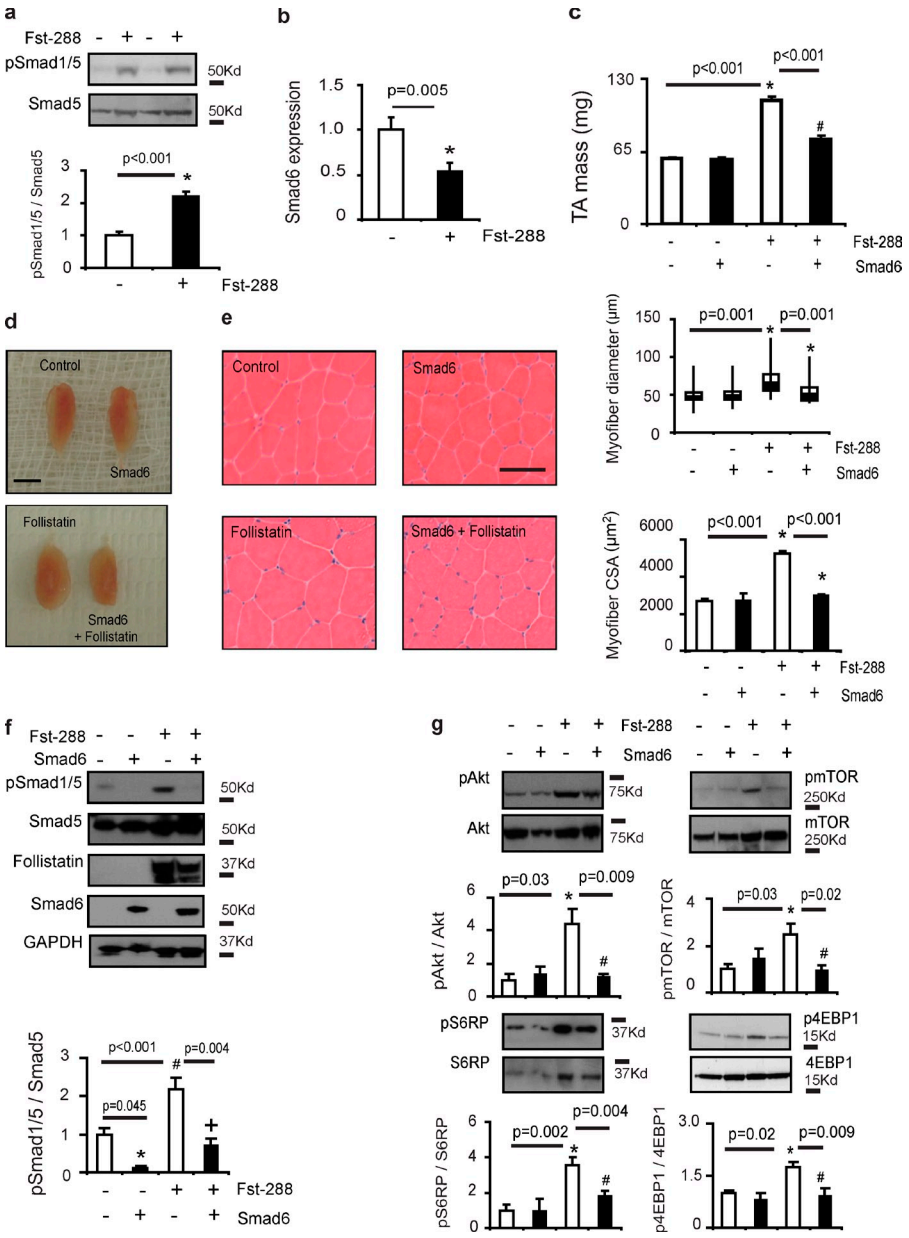

Figure S3. **Hypertrophy of skeletal muscle alters the phosphorylation of BMP-regulated Smads.** (a) Smad1/5$^{S463/465}$ phosphorylation was assessed in mouse TA muscles injected 28 d earlier with rAAV6:Fst-288 (n = 7–8 per treatment; *, P < 0.001 vs. control). (b) Smad6 transcription was measured by RT-PCR in TA muscles examined 28 d after administration of rAAV6:Fst (n = 5 per treatment; *, P = 0.005 vs. control). (c) TA muscles were administered rAAV6:Fst-288 alone or rAAV6:Fst-288 and rAAV6:Smad6 28 d before analysis (n = 4–7 per treatment; *, P < 0.001 vs. control; #, P < 0.001 vs. Fst-288 treated muscles). (d) Representative images demonstrate the repressive effect of Smad6 on follistatin-288–induced TA muscle hypertrophy. Bar, 5 mm. (e) Representative H&E images and quantification demonstrate that Smad6 attenuates follistatin-mediated increases in myofiber diameter (*, P < 0.001 vs. control; *, P < 0.001 vs. Fst-288) and area (*, P < 0.001 vs. control; *, P < 0.001 vs. Fst-288). Myofiber diameter is presented as box and whisker plots comprising minimum, lower quartile, median, upper quartile, and maximum values for myofiber diameter. Bar, 100 μm. (f) Protein expression of follistatin and Smad6 as determined by Western blot. rAAV6:Smad6 reduced Smad1/5$^{S463/465}$ phosphorylation (n = 8–14 per treatment; *, P = 0.045 vs. control; #, P < 0.001 vs. control; +, P = 0.004 vs. Fst-288). (g) The phosphorylation of Akt^{S473} (*, P = 0.03 vs. control; #, P = 0.009 vs. Fst-288), mTOR^{S2448} (*, P = 0.03 vs. control; #, P = 0.02 vs. Fst-288), S6RP$^{S235/236}$ (*, P = 0.002 vs. control; #, P = 0.004 vs. Fst-288), and 4EBP1$^{T37/46}$ (*, P = 0.02 vs. control; #, P = 0.009 vs. Fst-288) was assessed in response to the administration of rAAV6:Smad6 and rAAV6:Fst-288 by Western blot. n = 4–9 per treatment. Data are presented as means ± SEM.

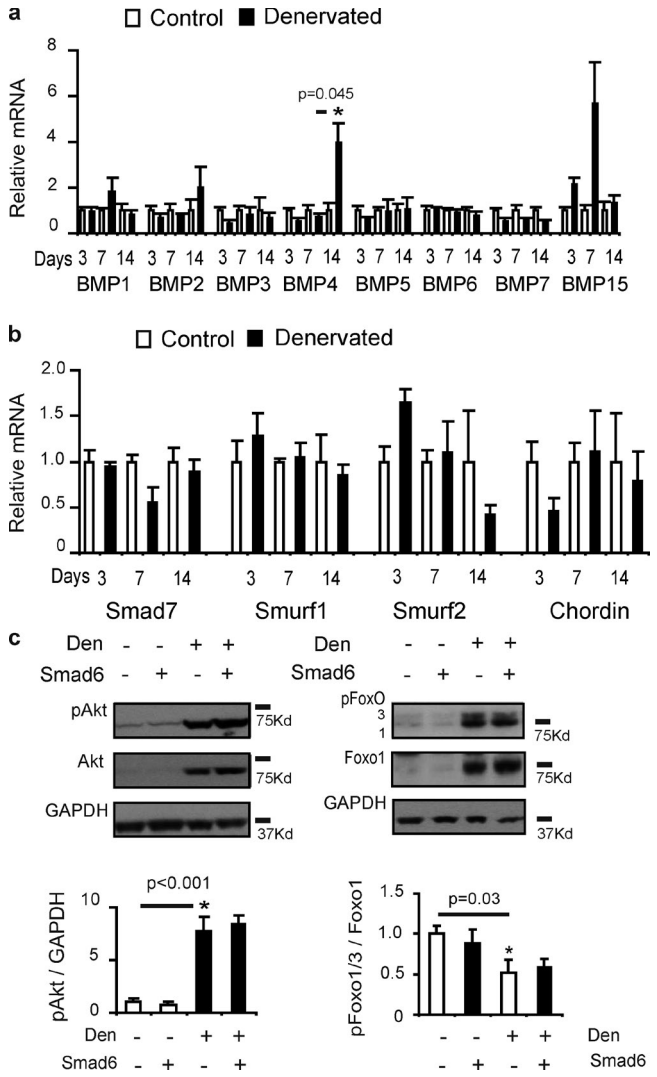

Figure S4. **Transcription of BMP ligands and BMP pathway inhibitors in denervated muscles.** (a) Expression of BMP ligands 1–7 and BMP 15 was determined by RT-PCR at 3 d (n = 6 per treatment), 7 d (n = 5–6 per treatment), and 14 d (n = 3–6 per treatment) after denervation in TA muscles. (b) Expression of BMP negative regulators Smad7, Smurf1, Smurf2, and Chordin was determined at 3 d (n = 6 per treatment), 7 d (n = 5–6 per treatment), and 14 d (n = 3–6 per treatment) after denervation by RT-PCR. Data are presented as means ± SEM. (c) TA muscles were homogenized 14 d after denervation and administration of rAAV6:Smad6 or sham vector. Lysates were analyzed by Western blotting for Akt^{S473} (n = 4–5 per treatment; *, P < 0.001 vs. control) and $FoxO1^{T24}$/$FoxO3a^{T32}$ phosphorylation (n = 6–8 per treatment; *, P = 0.03 vs. control). Data are presented as means ± SEM.

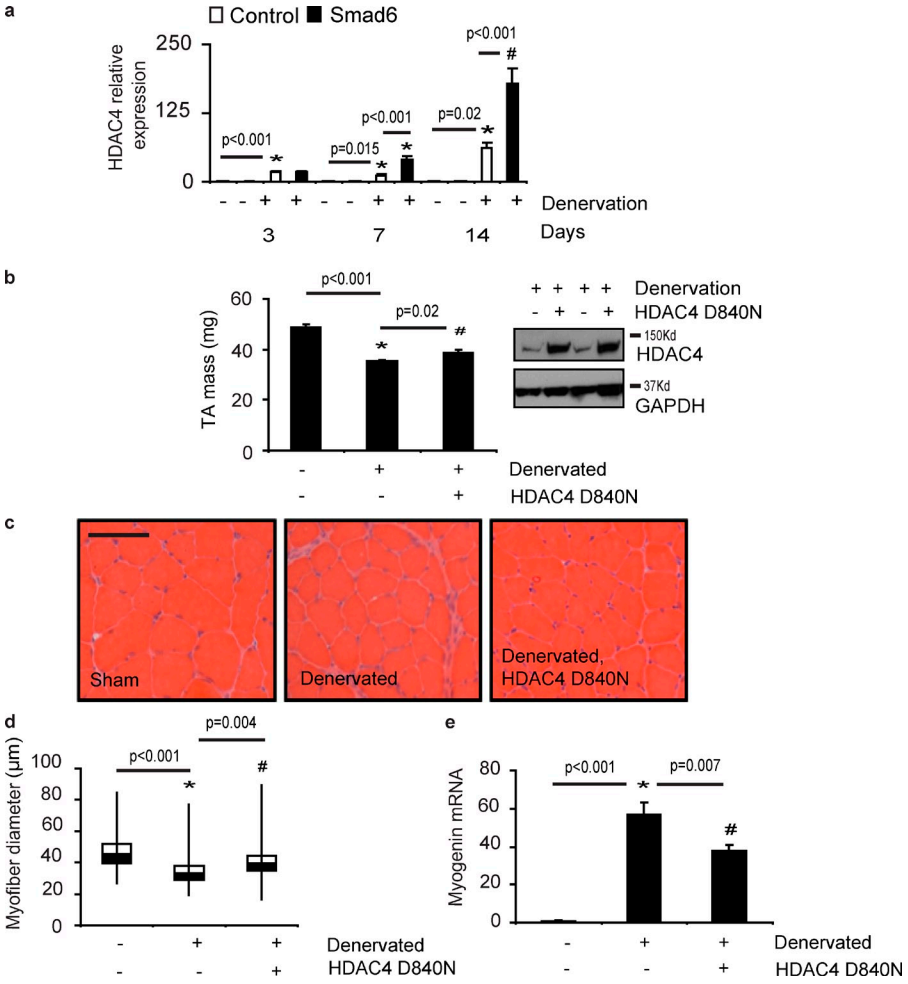

Figure S5. **Effect of dominant-negative HDAC4 in denervation-induced muscle wasting.** (a) HDAC4 transcription was measured at 3 d (*, P < 0.001 vs. control; *n* = 6 per treatment), 7 d (*n* = 5–6 per treatment; *, P = 0.015 vs. control; #, P < 0.001 vs. denervation), and 14 d (*n* = 3–6 per treatment; *, P = 0.02 vs. control; #, P < 0.001 vs. denervation) after denervation via RT-PCR. (b–d) TA muscles were denervated and treated with rAAV6:HDAC4^{D840N} 8 d before analysis, at which time, muscle mass (*, P < 0.001 vs. control; #, P = 0.02 vs. denervation) and myofiber diameter (*, P < 0.001 vs. control; #, P = 0.004 vs. denervation) was assessed. *n* = 4–5 per treatment. Expression of the dominant-negative HDAC4 mutant in denervated muscle was readily detectable by Western blot. Myofiber diameter is presented as box and whisker plots comprising minimum, lower quartile, median, upper quartile, and maximum values. Bar, 100 μm. (e) Myogenin transcription was measured by RT-PCR in muscles examined 8 d after denervation while transduced with rAAV6:HDAC4^{D840N} or sham vector (*, P < 0.001 vs. control; #, P = 0.007 vs. den; *n* = 3–5 per treatment).