Supplemental Methods

**Smad4 immunoblot**

12 week old S4 WT or Heterozygous mice with WT Sgcg were sacrificed and quadriceps femoris muscle was harvested. The muscles were snap frozen, powdered by mortar and pestle under liquid nitrogen, and resuspended in cell lysis buffer (10mM Tris pH 7.0, 1mM EDTA pH 8.0, 150mM NaCl, 1% TritonX100) with HALT protease and phosphatase inhibitor with EDTA (Pierce Scientific, Rockford, IL). 50µg protein was separated on a 10% denaturing gel, transferred to PVDF membrane, blocked with 1% BSA in TBS-T for 1 hour, blotted overnight with a 1:1000 dilution of rabbit anti-SMAD4 (Santa Cruz, H-552) at 4°C, washed 3 times with TBS-T, incubated with a 1:2500 dilution of goat anti-rabbit IgG-HRP conjugate for 1 hour at room temperature, again washed, and imaged using ECL prime (Amersham GE, Piscataway, NJ).

**Luciferase activity**

The gastrocnemius and soleus muscles were harvested and processed as described (18). Luciferase activity was measured using a Promega Glomax 20/20 luminometer. Twenty µl of sample was combined with 100µl of assay reagent, and luminescence was measured for 10 seconds after a 2 second delay. Each set of lysates was measured 3 times and averaged. Protein concentration was determined using the Bio-Rad Protein Assay and a bovine serum albumin (BSA) standard. Data presented are calculated by dividing luciferase counts by the protein present in the sample and multiplying by 1000.
Supplemental Figure 1. Cardiotoxin injury induces expression from a SMAD reporter allele. To measure SMAD signaling, mice carrying an SBE-luciferase transgene were used (17). In this transgene, an artificial sequence that binds SMAD2/3 acts as an enhancer for firefly luciferase. The gastrocnemius/soleus muscle group was injected with cardiotoxin, leaving the contralateral muscle as uninjured control. Injured muscle showed greater SMAD signaling than uninjured muscle (p <0.01 by Mann-Whitney test, n=5 mice) indicating that acute injury of normal muscle is sufficient to induce TGFβ signaling.
Supplemental Figure 2. *Smad4 (S4)* mice encode an amino-terminally deleted SMAD4. The structure of the SMAD4 mRNA is shown in the upper portion of the figure. Boxes represent exons, gaps represent introns. The first and second in-frame ATG sites are noted, as is the stop codon (TGA). Mice with a targeted deletion of exon 2 in SMAD4, indicated by the black box, were previously generated (19). SMAD4 protein has two MAD homology (MH1 and MH2) domains. With deletion of the first ATG, the next available ATG would delete MH1. Skeletal muscle lysates from WT and S4/+ mice were blotting with anti-SMAD4 antibody. In WT mice, SMAD4 migrated at its predicted size of 60 kDa. In SMAD4 heterozygous skeletal muscle, full length SMAD4 and a unique band at ~47 kDa, the predicted size of SMAD4ΔN, were detected.