**TGF-β requires the activation of canonical and non-canonical signalling pathways to induce skeletal muscle atrophy**

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**Supplementary Figure legends**

**Figure S1: TGF-β1 activated the Smad pathway in C2C12 myotubes.**

Skeletal muscle cells were differentiated into myotubes for five days and incubated with 10 ng/mL of TGFβ1 during the times indicated in the figure. The Smad2 and Smad3 proteins (phosphorylated and totals) were evaluated by Western blot analysis. Molecular weights are shown in kDa **(A, C)**. A densitometry analysis was conducted. The levels of phosphorylated proteins were normalised to total proteins respectively and expressed as fold of induction relative to control without TGFβ1 (time 0) **(B, D)**. The results shown correspond to the mean ± SD from three independent experiments. Control: condition without TGFβ1 and inhibitor. (\*, P < 0.05 versus control).

**Figure S2: SiS3 inhibits the Smad3 phosphorylation induced by TGF-β1 in C2C12 myotubes.**

Myoblasts C2C12 differentiated into myotubes for five days were pre-incubated for 1 h with SiS3 inhibitor of Smad3 and then were incubated with 10 ng/mL of TGFβ1. **(A)** Myotubes pre-incubated in absence or presence of 1or 3M of SiS3 and then for 1 hour with TGFβ1. The levels of phosphorylated and total Smad3 were evaluated by Western blot. **(B)** The densitometric analysis of the bands was performed and the results were expressed and plotted as fold of induction relative to control. **(C)** C2C12 myotubes were pre-incubated with phosphorylation inhibitors: SiS3, UO126, SP600125 (3 M, 5 M and 5 M respectively) and then were incubated with TGFβ1. The protein levels phosphorylated and totals were evaluated by Western blot for Smad3 then of 1 h of incubation with TGFβ1. **(D)** Quantitative analysis is shown in the graph. The results were expressed as fold of induction relative to control. For A and C, the molecular weights are shown in kDa. For B and D, the quantitative analysis was performed normalising the levels of phosphorylated proteins to total proteins respectively. The results shown correspond to the mean ± SD from three independent experiments. Control: condition without TGF-β1 and inhibitor. (\*, P < 0.05 versus control; #, P < 0.05 versus TGF-without inhibitors).

**Figure S3: TGF-β1 induce the phosphorylation of ERK 1/2 and JNK 1/2 but not p38 MAPK in C2C12 myotubes.**

C2C12 myoblasts were differentiated into myotubes (5 days) and incubated with TGFβ1 (10 ng/mL) for the times indicated in the figure. The ERK 1/2, JNK 1/2 and p38 MAPK proteins levels (phosphorylated and totals) were determined by Western blot analysis. Molecular weights are shown in kDa **(A, C, E)**. A quantitative analysis was conducted for each protein. The levels of phosphorylated proteins were normalised to total proteins respectively, and expressed as fold of induction relative to control. Control: condition without TGFβ1 and inhibitor (time 0) **(B, D, F)**. The results are expressed as mean ± SD from three independent experiments (\*, P < 0.05 versus control).

**Figure S4: Inhibition of ERK1/2 and JNK1/2 phosphorylation induced by TGF-β1 in C2C12 myotubes.**

Myoblasts C2C12 differentiated into myotubes for five days were pre-incubated for 1 h with inhibitors of ERK1/2 and JNK1/2 phosphorylation and then were incubated with 10 ng/mL of TGFβ1. **(A)** Myotubes were pre-incubated in absence or presence of 1 or 5M of UO126, and then for 15 min with TGFβ1. The ERK1/2 levels (total and phosphorylated) were analysed by Western blot. **(B)** Quantitative analysis is shown in the graph. The results were expressed as fold of induction relative to control. **(C)** Myotubes were pre-incubated in absence or presence of 5 or 10M of SP600125. Then, the incubation with TGFβ1 was performed for 15 min. The JNK1/2 levels (total and phosphorylated) were evaluated by Western blot. **(D)** The quantitative analysis of the bands is shown in the graph. For A, C and E, the molecular weights are shown in kDa. For B and D, the quantitative analysis was performed normalising the levels of phosphorylated proteins to total proteins respectively. The results shown correspond to the mean ± SD from three independent experiments. Control: condition without TGF-β1 and inhibitor. (\*, P < 0.05 versus control; #, P < 0.05 versus TGF-without inhibitors).

**Figure S5: UO126 and SP600125 compounds were specifics for the inhibition respective of ERK1/2 and JNK1/2 phosphorylation induced by TGF-β1 in C2C12 myotubes.**

C2C12 myotubes were pre-incubated with phosphorylation inhibitors: UO126, SP600125 (5 M and 5 M respectively) and then were incubated with TGFβ1. The protein levels phosphorylated and totals were evaluated by Western blot for ERK1/2 **(A)** and JNK1/2 **(C)** then of 15 min of incubation with TGFβ1. The molecular weights are shown in kDa. Quantitative analysis of the bands was performed and normalised respect to the total protein correspondent in each case **(B, D)**. The results were plotted as fold of induction relative to the vehicle control, and correspond to the mean ± SD from three independent experiments. Control: condition without TGFβ1 and inhibitor. (\*, P < 0.05 versus control; #, P < 0.05 versus TGF-without inhibitors).