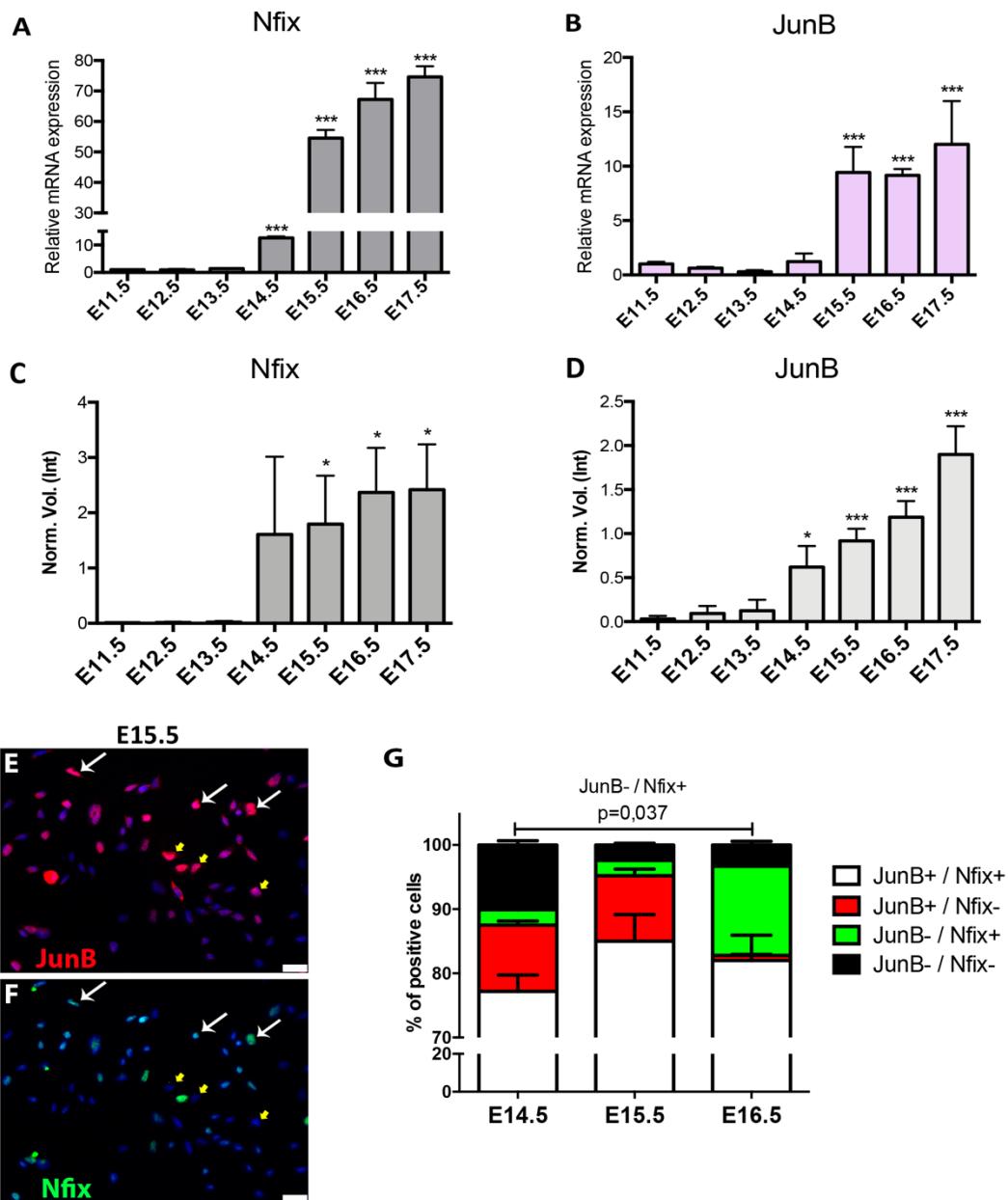


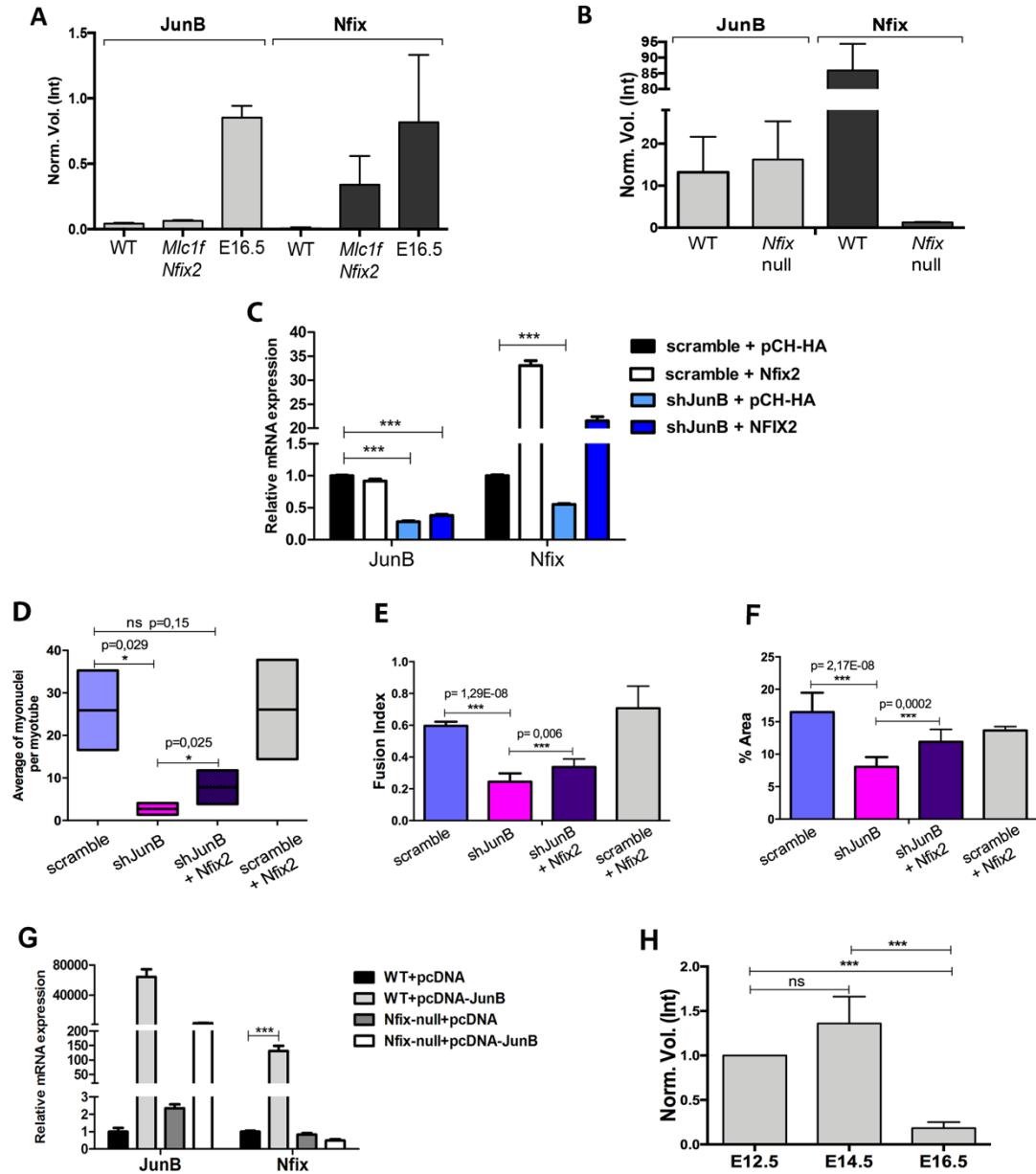
Supplementary Information



Supplementary Figure 1. JunB and Nfix are co-expressed in foetal myoblasts, with JunB earlier expressed than Nfix

(A-B) qRT-PCR analysis on purified Myf5^{GFP-P/+} myoblasts dissected from E11.5 up to E17.5 muscles, showing the expression profile of Nfix (A) and JunB (B) at different developmental stages. The statistical analyses are compared to E11.5 sample (**p<0.005; n=5). (C, D) Densitometry analysis of Nfix (C) and JunB (D), related to

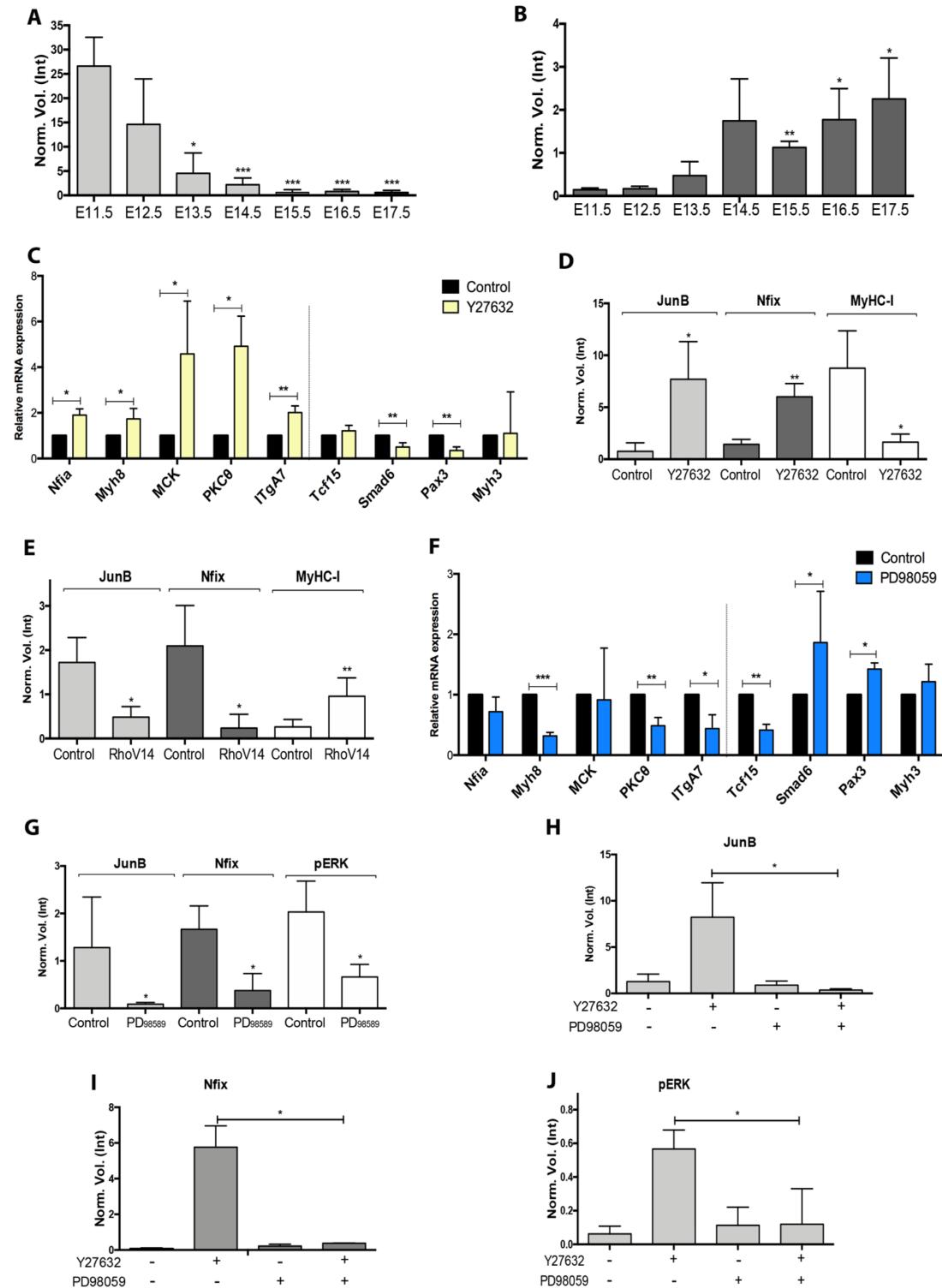
Western blot showed in Fig. 1A. The statistical analyses were performed comparing all the results to E11.5 (* $p<0.05$; n=5; *** $p<0.005$; n=5). **(E-F)** Immunofluorescence for JunB (red) and Nfix (green) on freshly isolated Myf5^{GFP-P/+}-purified myoblasts at E15.5. Nuclei were counterstained with Hoechst. White arrows indicate myoblasts coexpressing JunB and Nfix, while yellow arrows indicate myoblasts expressing JunB but not Nfix. Scale bars: 25 μ m. **(G)** Graph showing the percentage of JunB+/Nfix+ (white bar), JunB+/Nfix- (red), JunB-/Nfix+ (green) and Junb-/Nfix- (black). Percentages are calculated on the total number of nuclei (* $p<0.05$; n=5).



Supplementary Figure 2. Silencing of JunB impairs the differentiation of foetal myoblasts

(A) Graphs for densitometric quantification of JunB and Nfix expression normalized against housekeeping protein Vinculin, respectively related to Fig. 2B (A) and Fig. 2D (B). **(C)** qRT-PCR for JunB and Nfix on foetal myoblasts (E16.5) following the transduction with scramble or shJunB vector and with control or Nfix2-over-expressing vector. *** $p<0.005$; n=5. **(D)** Average of myonuclei per myotubes of foetal myoblasts,

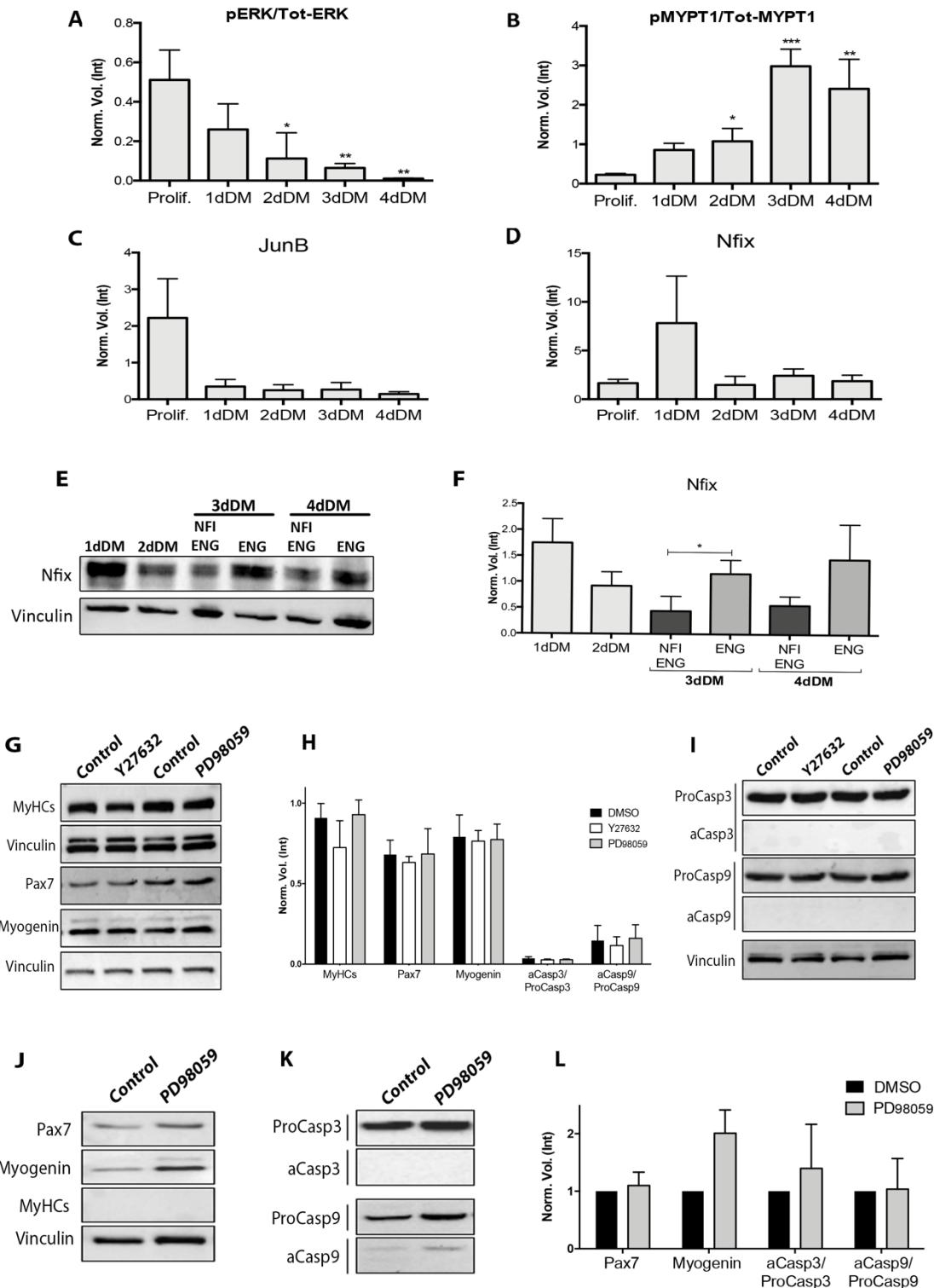
transduced with scramble or shJunB vector and with control or *Nfix2*-over-expressing vector, and induced to the terminal differentiation (*p<0.05; n=5). **(E)** Calculation of the fusion index as the ratio of the number of nuclei inside myotubes to the number of total nuclei of control (**p<0.005; n=5). **(F)** Quantification of MyHCs positive area (MF20) using image analysis software (**p<0.005; n=5). **(G)** qRT-PCR on wild-type (WT) and *Nfix*-null embryonic myoblasts (E12.5), transfected with JunB-overexpressing vector or with a control vector (pcDNA). ***p<0.005; n=5. **(H)** Quantitative densitometry of active Rho GTPases normalised according to the ratio between total Rho GTPases and β-tubulin. (***, p <0.001; n =5).



Supplementary Figure 3. Analysis of embryonic and foetal specific genes and protein levels upon ROCK and ERK kinase modulation.

(A) Densitometry quantification of the ratio of pMYPT1 on Tot-MYPT1, linked to Fig.4B. The statistical analyses are compared to E11.5 (*p<0.05; n=5; ***p<0.005;

n=5). **(B)** Densitometric quantification of the ratio pERK/Tot-ERK, related to Fig. 4C. The statistical analyses are compared to E11.5 (*, p <0.1; **, p <0.05; n=5). **(C)** qRT-PCR analysis on embryonic differentiated myotubes (E12.5) treated with vehicle (control) or with Y27632 of embryonic genes: *Tcf15*, *Smad6*, *Pax3* and *Myh3* (right part). The levels of expression of foetal specific genes (*Nfia*, *Myh8*, *MCK*, *PKCθ* and *ITgA7*) were represented on the left side of the graph (*, p <0.1; **, p <0.05; n=5). **(D)** Densitometric quantification of Western blots of JunB, Nfix and MyHC-I of embryonic myoblast treated with Y27632 (related to Fig. 5B) (*, p <0.1; **, p <0.05; n=5). **(E)** Quantification of the densitometry data from Western blot showed in Fig. 5D on fetal myoblasts expressing RhoV14 or a control vector (*, p <0.1; **, p <0.05; n=5). **(F)** qRT-PCR analysis on foetal myoblasts (E16.5) treated with vehicle (control) or with PD98059 of embryonic (*Tcf15*, *Smad6*, *Pax3* and *Myh3* - right part) and foetal (*Nfia*, *Myh8*, *MCK*, *PKCθ* and *ITgA7* - left part) genes (*, p <0.1; **, p <0.05; ***, p <0.01; n=5). **(G)** Densitometric quantification of Western blots, related to Fig. 5K, for JunB, Nfix and pERK, expressed as a ratio of pERK on Tot-ERK, upon PD98059 treatment of fetal myoblasts (*, p <0.1; n=5). **(H-J)** Densitometric quantification of Western blot in Fig. 5L of JunB (H), Nfix (I) and pERK (J), as ration of pERK on Tot-ERK upon Y27632 and/or PD98059 treatment of embryonic myoblasts (*, p <0.1; n=5).

**Supplementary Figure 4. Protein expression analysis on juvenile MuSCs.**

(A-D) Densitometric quantification of Western blot in Fig.7A for pERK/Tot-ERK (A), pmypt1/Tot-MYPT1 (B), JunB (C) and Nfix (D) on juvenile MuSCs in proliferating

conditions (Prolif.) or during differentiation (day in differentiation medium; dDM). Each sample is compared to E11.5 for the statistical analysis (*, p <0.1; **, p <0.05; ***, p <0.01; n=5). **(E-F)** Western blot following transfection with dominant-negative NFI-*engrailed* (NFI-ENG) or *engrailed* domain (ENG), Nfix during differentiation (3, 4dDM) (E), and its related densitometric quantification (F). Vinculin is used as housekeeping control (*, p <0.1; n=5). **(G-H)** Western blot on juvenile MuSCs treated with PD98059 or Y27632 until the differentiation, showing Pax7, Myogenin, sarcomeric Myosins (MyHCs) (G), and their relative densitometric analysis (H). Vinculin is used as housekeeping control. **(I)** Western blot on juvenile MuSCs treated with PD98059 or Y27632, showing Caspase3 (total Caspase3 or ProCasp3; active Caspase3 or aCasp3) and Caspase9 (total Caspase9 or ProCasp9; active Caspase9 or aCasp9) until the differentiation. Vinculin was used to normalise the amount of protein. **(J-K)** Western blot assay on proliferating juvenile MuSCs, treated over-night with PD98059, for Pax7, Myogenin (J) and active Caspase3 and 9 (aCasp3 and aCasp9) (K). **(L)** Densitometric quantification of Western blots on proliferative satellite cells treated with vehicle or PD98059, showing Pax7, Myogenin, the ratio of aCasp3 on total Caspase3 (ProCasp3) and the ratio of aCasp9 on ProCasp9.

Table S1. Primers used in this study

Primer Name	Primer sequence	Usage	Reference
<i>Nfix</i> (Fw)	CACTGGGGCGACTTGTAGAG	qRT-PCR	Mourikis et al., 2012
<i>Nfix</i> (Rev)	AGGCTGACAAGGTGTGGC		
<i>JunB</i> (Fw)	CCTGTGTCCCCCATCAACAT	qRT-PCR	
<i>JunB</i> (Rev)	CAGCCTTGAGTGTCTCACCT		
<i>MyHC-I</i> (Fw)	AGGGCGACCTCAACGAGAT	qRT-PCR	Mathew et al., 2011
<i>MyHC-I</i> (Rev)	CAGCAGACTCTGGAGGCTCTT		
<i>MyHC-emb</i> (Fw)	GCAAAGACCCGTGACTTCACCTCTAG	qRT-PCR	Mathew et al., 2011
<i>MyHC-emb</i> (Rev)	GCATGTGGAAAAGTGTACAGTGG		
<i>Eno3</i> (Fw)	TTCTACCGCAACGGCAAGTA	qRT-PCR	
<i>Eno3</i> (Rev)	GACCTTCAGGAGCAGGCAAT		
<i>Myogenin</i> (Fw)	CTGGGGACCCCTGAGCATTG	qRT-PCR	
<i>Myogenin</i> (Rev)	ATCGCGCTCCTCCTGGTTGA		
<i>RhoA</i> (Fw)	AGCTTGTTGAAAGACATGCTTG	qRT-PCR	
<i>RhoA</i> (Rev)	GTGTCCCATAAACGCCACTCTAC		
<i>Gapdh</i> (Fw)	GGCATGGACTGTGGTCATGA	qRT-PCR	
<i>Gapdh</i> (Rev)	TTCACCACCATGGAGAAGGC		
<i>Tcf15</i> (Fw)	GCAGCTGCTTGAAGATGAG	qRT-PCR	
<i>Tcf15</i> (Rev)	CGGTCCCTTACACAACGCAGG		
<i>Pax3</i> (Fw)	GGGAACCTGGAGGCATGTTA	qRT-PCR	
<i>Pax3</i> (Rev)	GTTCCTCGTCCCAGCAATTA		
<i>Smad6</i> (Fw)	ATCACCTCCTGCCCTGT	qRT-PCR	
<i>Smad6</i> (Rev)	CTGGGGTGGTGTCTCTGG		
<i>PKCθ or Prkcq</i> (Fw)	ATGGACAACCCCTCTACCC	qRT-PCR	
<i>PKCθ or Prkcq</i> (Rev)	GCGGATGTCTCCTCTCACTC		
<i>ITGA7</i> (Fw)	GGCTGGGCTGTTAGTCCTG	qRT-PCR	
<i>ITGA7</i> (Rev)	ATGGGCTCGGTGATAGTTGGT		
<i>MCK or Ckm</i> (Fw)	TTCCTTGTGTGGGTGAACGA	qRT-PCR	
<i>MCK or Ckm</i> (Rev)	TTTCAGCTTCTCTCCATC		
<i>NfiA</i> (Fw)	TGGCATACTTGTACATGCAGC	qRT-PCR	
<i>NfiA</i> (Rev)	ACCTGATGTGACAAAGCTGTCC		
<i>Myh8</i> (Fw)	GTCACGCAATGCAGAAGAGA	qRT-PCR	
<i>Myh8</i> (Rev)	CAGGTCCCTCACCGTCTGTT		
<i>MyHC-2b promoter</i> (Fw)	GAGGTCCGTAGTCAGTCTCTTT	ChIP qRT-PCR	Raffaello et al., 2010
<i>MyHC-2b promoter</i> (Rev)	TACCCCAAGTGTAGGCTCA		
<i>Nfix promoter -1400bp</i> (Fw)	ACACTAGGATTGAGGAAGACTTAGA	ChIP qRT-PCR	
<i>Nfix promoter -1400bp</i> (Rev)	CAAGGCCTTCTGGGGCTC		
<i>Nfix promoter -200bp</i> (Fw)	TTGAGAATCCACCCAAGCCC	ChIP qRT-PCR	
<i>Nfix promoter -200bp</i> (Rev)	AAGCCAACGCCTGATTCTGA		
<i>Nfix promoter -1000bp</i> (Fw)	CAAAGAGGCATCCACTTGCAG	ChIP qRT-PCR	
<i>Nfix promoter -1000bp</i> (Rev)	GTTCAGGAGTCTCAGCATCG		
<i>Intergenic</i> (Fw)	TCGGACCGGAGTGTAGGAA	ChIP qRT-PCR	An et al., 2011
<i>Intergenic</i> (Rev)	ACCCTGGAGTCTCAGCATCG		