Supplementary informations

Table S1

	FORWARD	REVERSE
S98A	CAATGGCTGTGACGCCCCAGATCCCG	CGGGATCTGGGGCGTCACAGCCATTG
S110A	CAGTAGGTCACGCCCCTGAGTCTGAG	CTCAGACTCAGGGGCGTGACCTACTG
S240A	GAATATACAAGCCAAAGCTCCTCCCCCTATGAATC	GATTCATAGGGGGAGGAGCTTTGGCTTGTATATTC
S388A	GTCAGAACCTGTTGCTCCTCCTAGAGACC	GGTCTCTAGGAGGAGCAACAGGTTCTGAC
Δ1-57	CGATGACGACAAGCTTGCCAGCACTGACATGG	CCATGTCAGTGCTGGCAAGCTTGTCGTCATCG
Δ1-95	CGATGACGACAAGCTTAATGGCTGTGACAGCC	GGCTGTCACAGCCATTAAGCTTGTCGTCATCG
Δ1-173	CGATGACGACAAGCTTCCGTCTCTGCAGAGG	CCTCTGCAGAGACGGAAGCTTGTCGTCATCG
Δ247-327	CCTCACCAGGCCTGCTGTTCAACACTGCCAGTG	CACTGGCAGTGTTGAACAGCAGGCCTGGTGAGG
Δ1-327	CGATGACGACAAGCTTTTCAACACTGCCAGTG	CACTGGCAGTGTTGAAAAGCTTGTCGTCATCG
Δ92-400	GAGAAAGGGCCTCTACCCACAACACACC	GGTGTGTTGTGGGTAGAGGCCCTTCTTTCT
Pin1 C113A	CTCACAGTTCAGCGACGCCAGCTCAGCCAAGGCC	GGCCTTGGCTGAGCTGGCGTCGCTGAACTGTGAG
Pin1 W34A	CATCACTAACGCCAGCCAGGCGGAGCGGCCCAGC	GCTGGGCCGCTCCGCCTGGCTGGCGTTAGTGATG

FIGURE LEGENDS

Fig. S1. Pin1 isomerase activity is required to block skeletal muscle differentiation. C2C7 myoblasts were infected with lentiviruses encoding HA-Pin1 or HA-Pin1 C113A, then induced to differentiate. (A) After 48h from serum withdrawal, cells were fixed and subjected to immunostaining using the anti-MyHC antibody (Red stain, upper panels), cell nuclei were stained by Hoechst (lower panels); bar: 50 μm. (B) The proportion of nuclei in differentiated cells is reported as ratio between the nuclei incorporated in MyHC positive cells and total nuclei. The number obtained in cells that ectopically express the dominant negative Pin1 C113A is expressed relative to the number evaluated in cells that overexpress wild type Pin1 (taken as 1). The data are presented in the histogram and represent the mean of two independent experiments. (C) Western Blot (WB) analysis performed on protein extracts of C2C7 cells infected with lentiviruses encoding HA-Pin1 or HA-Pin1 C113A with antibodies against MyHC, Pin1 and actin.

Fig. S2. The MEF2C region encompassing amino acids 92-118 is di-phosphorylated. MALDI-TOF spectra of the MEF2C 92-118 peptide before (A) and after treatment (B) with Alkaline Phosphatase. The shift of +80 and +2(80) Da (2999.17 and 3079.17 versus 2919,17 Da peptide corresponding to the unmodified peptide) upon treatment of the tryptic digest with Alkaline Phosphatase (AP)strongly suggest that peaks m/z=2999.17 and 3079.79 represent the mono- and di-phosphorylated peptide, respectively. Phosphorylated peaks are indicated with an arrow.

Fig. S3. Multiple sequence alignement of MEF2 proteins. Sequences of MEF2 proteins from different species were aligned with ClustalW (www.ebi.ac.uk/Tools/clustalw). Serines 98 and 110 are conserved across MEF2 proteins from different species (MEF2A - rat gi:62078801; MEF2C - human gi:19923215; MEF2C - mouse gi:293728; MEF2C - dog gi:73952080; MEF2CB - zebrafish gi:195972877; MEF2A - human gi:5031907; MEF2A - mouse gi:76253934; MEF2A - zebrafish gi:18859003; MEF2A - chicken gi:45382367; MEF2D - mouse gi:19526812; MEF2D - human gi:5174545; MEF2D - rat gi:52138610; MEF2D - zebrafish gi:18859007).

Fig. S4. Pin1 modulates MEF2C transcriptional activity. C3H 10T1/2 cells were transfected with pGL3(desMEF2)$_3$, pTK-Renilla and the empty plasmid expression vector pcDNAI/Amp or the vectors encoding HA-Pin1 (1 μg) and MEF2C alone (100 ng) or in the indicated combinations. After 24 hours cells were lysed and luciferase reporter activity determined and normalized to Renilla activity. The data are presented as the fold activity versus that observed with the empty pcDNAI/Amp expression vector and

represent the mean ± the standard deviation of the mean for three independent experiments. (p<0,05). A fraction of cell lysates were Western Blotted for anti-MEF2C (upper panel) or anti-Pin1 (lower panel).

Fig. S5 Protein expression of cell cycle regulators upon Pin1 siRNA knockdown . C2C7 myoblasts were infected with lentiviruses encoding short hairpin RNAs, respectively a scramble control sequence (sh CTRL, lane 1) or a Pin1 silencing sequence (sh Pin1, lane 2), then cultured in growth medium for 48h. Cell lysates from siRNA transfected cells were immunoblotted for the CDK inhibitor p21, Cyclin D1, MyHC, Pin1 and total actin.

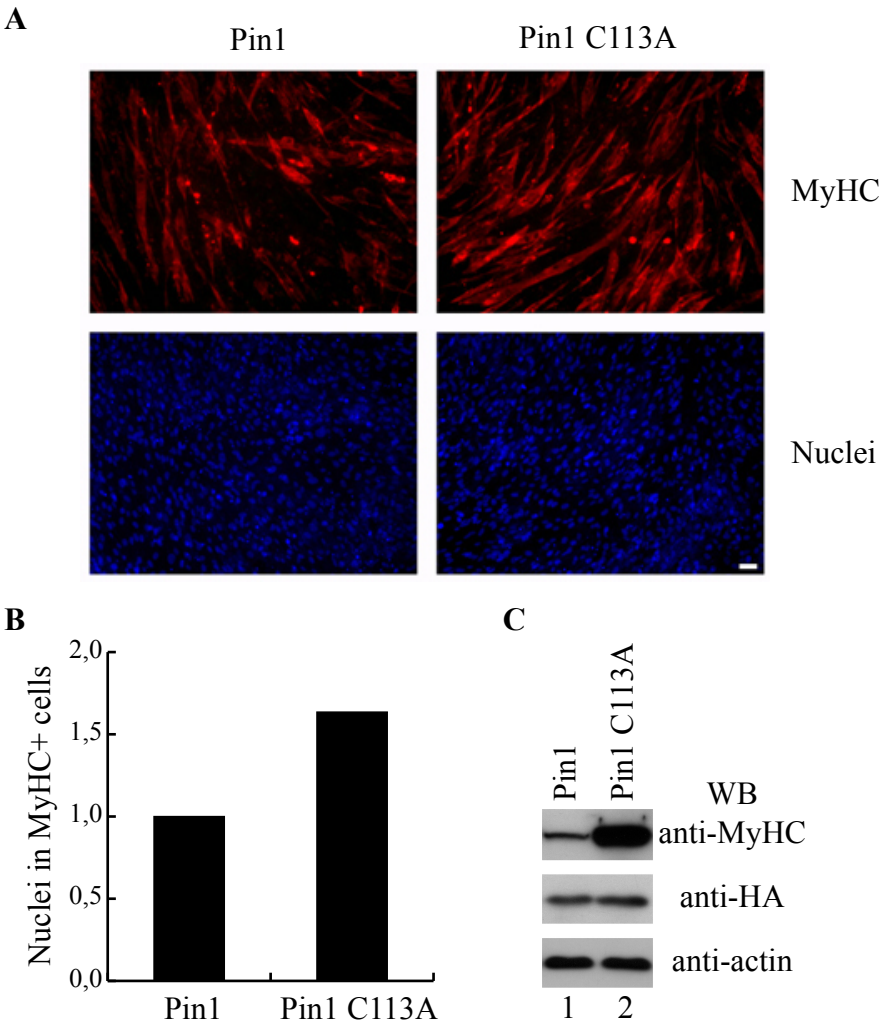
A
Pin1
Pin1 C113A
MyHC
Nuclei
B
Nuclei in MyHC+ cells
2,0
1,5
1,0
0,5
0,0
Pin1
Pin1 C113A
C
Pin1
Pin1 C113A
WB
anti-MyHC
anti-HA
anti-actin
1
2

Figure S1

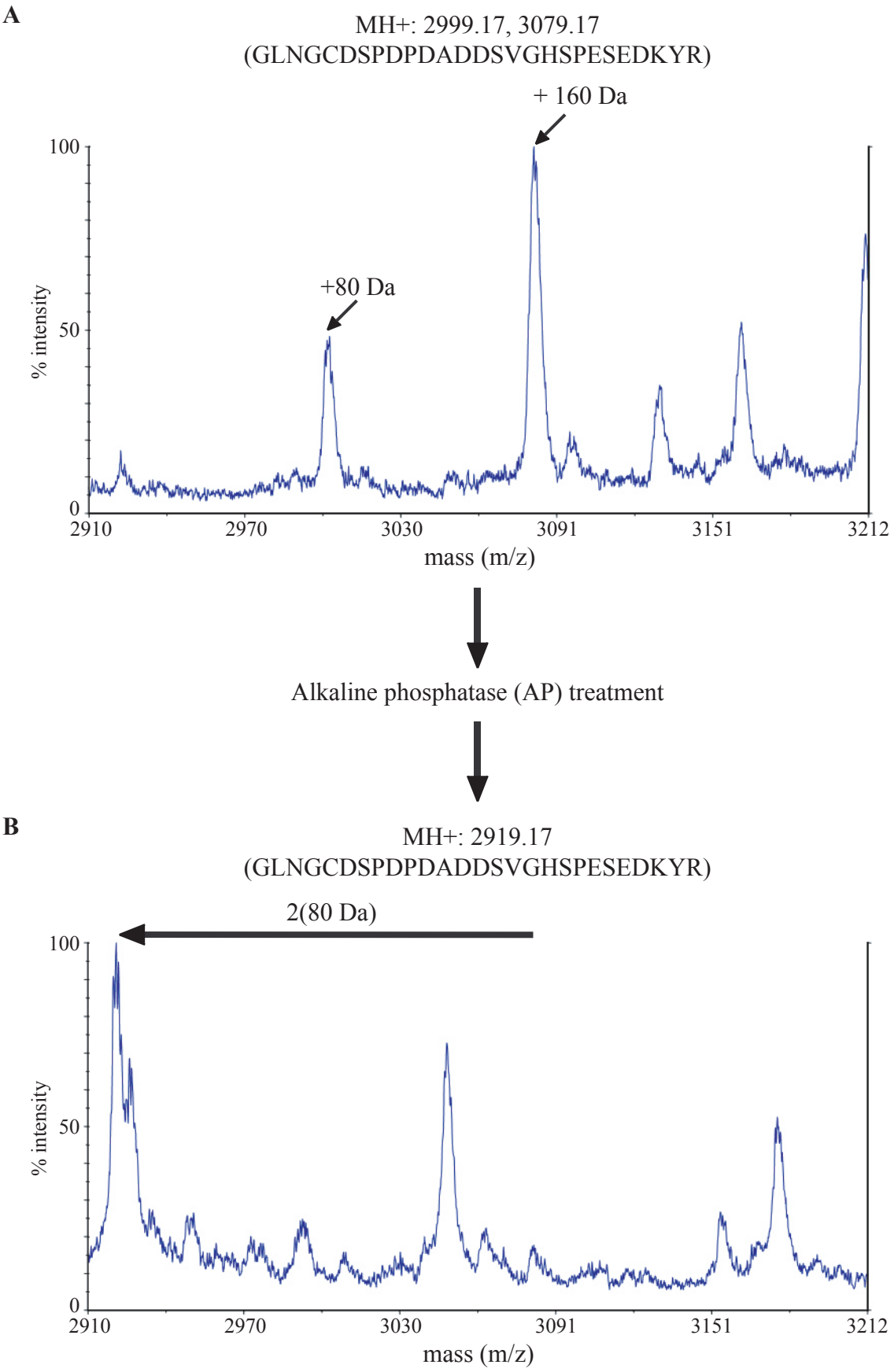
A
MH+: 2999.17, 3079.17
(GLNGCDSPDPDADDSVGHSPESEDKYR)
+ 160 Da
+80 Da
100
50
0
% intensity
2910
2970
3030
3091
3151
3212
mass (m/z)
Alkaline phosphatase (AP) treatment
B
MH+: 2919.17
(GLNGCDSPDPDADDSVGHSPESEDKYR)
2(80 Da)
100
50
0
% intensity
2910
2970
3030
3091
3151
3212
mass (m/z)

Figure S2

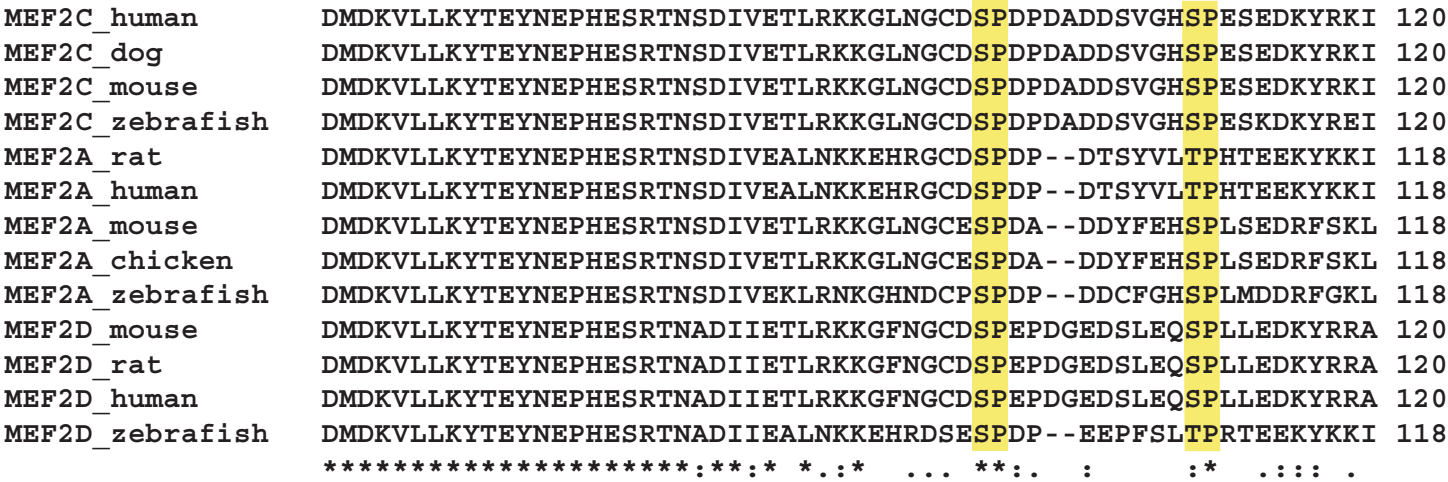
MEF2C_human DMDKVLLKYTEYNEPHESRTNSDIVETLRKKGLNGCDSPDPDADDSVGHSPESEDKYRKI 120
MEF2C_dog DMDKVLLKYTEYNEPHESRTNSDIVETLRKKGLNGCDSPDPDADDSVGHSPESEDKYRKI 120
MEF2C_mouse DMDKVLLKYTEYNEPHESRTNSDIVETLRKKGLNGCDSPDPDADDSVGHSPESEDKYRKI 120
MEF2C_zebrafish DMDKVLLKYTEYNEPHESRTNSDIVETLRKKGLNGCDSPDPDADDSVGHSPESKDKYREI 120
MEF2A_rat DMDKVLLKYTEYNEPHESRTNSDIVEALNKKEHRGCDSPDP--DTSYVLTPHTEEKYKKI 118
MEF2A_human DMDKVLLKYTEYNEPHESRTNSDIVEALNKKEHRGCDSPDP--DTSYVLTPHTEEKYKKI 118
MEF2A_mouse DMDKVLLKYTEYNEPHESRTNSDIVETLRKKGLNGCESPDA--DDYFEHSPLSEDRFSKL 118
MEF2A_chicken DMDKVLLKYTEYNEPHESRTNSDIVETLRKKGLNGCESPDA--DDYFEHSPLSEDRFSKL 118
MEF2A_zebrafish DMDKVLLKYTEYNEPHESRTNSDIVEKLRNKGHNDCPSPDP--DDCFGHSPLMDDRFGKL 118
MEF2D_mouse DMDKVLLKYTEYNEPHESRTNADIIETLRKKGFNGCDSPEPDGEDSLEQSPLLEDKYRRA 120
MEF2D_rat DMDKVLLKYTEYNEPHESRTNADIIETLRKKGFNGCDSPEPDGEDSLEQSPLLEDKYRRA 120
MEF2D_human DMDKVLLKYTEYNEPHESRTNADIIETLRKKGFNGCDSPEPDGEDSLEQSPLLEDKYRRA 120
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Figure S3

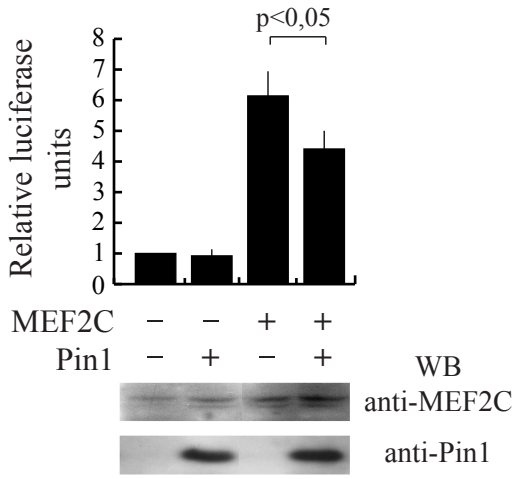
p<0,05
Relative luciferase units
8
7
6
5
4
3
2
1
0
MEF2C − − + +
Pin1 − + − +
WB
anti-MEF2C
anti-Pin1

Figure S4

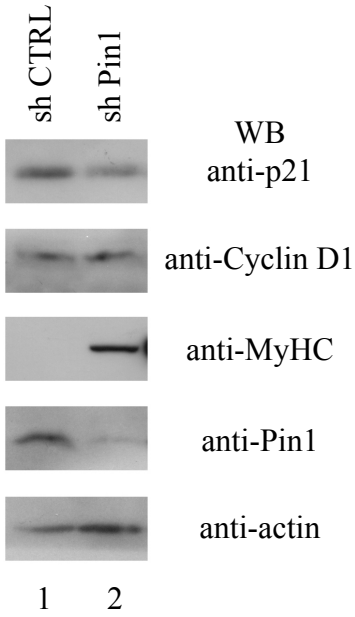
sh CTRL
sh Pin1
WB
anti-p21
anti-Cyclin D1
anti-MyHC
anti-Pin1
anti-actin
1
2

Figure S5