Supplementary Data

SUPPLEMENTARY TABLES

Table S1. Primers used for Msx1 point mutation assays

Primer name	Primer sequence (5' to 3')
Msx1(S136A)	F: AAGGCCGAAGCCCCCGAGAA R: TTCTCGGGGGCTTCGGCCTT
Msx1(S136D)	F: AAGGCCGAAGACCCCGAGAA R: TTCTCGGGGTCTTCGGCCTT
Msx1(S152A)	F: TGGATGCAGAGTCCCCGCTTCGCCCCGCCCCCAGCCAGACGGCTG R: CAGCCGTCTGGCTGGGGGCGGGGCGAAGCGGGGACTCTGCATCCA
Msx1(S160A)	F: CCGCCCCCAGCCAGACGGCTGGCTCCCCCAGCATGCACCCTACGC R: GCGTAGGGTGCATGCTGGGGGAGCCAGCCGTCTGGCTGGGGGCGG

Table S2: List of antibodies used in this study.

Antigen	**Antibody Source (Catalog# and/or clone#)**	**Type**	**Use and dilution**	
			IF	**WB**
β-Actin	Proteintech (23660-1-AP)	Rabbit mAb		1:5000
p-Erk1/2	CST (4370S)	Rabbit mAb		1:2000
t-Erk1/2	CST (9102S)	Rabbit mAb		1:1000
Fgf9	Bioss(bs-5906R)	Rabbit pAb		1:500
Fgf18	Bioss(bs-9762R)	Rabbit pAb		1:500
Flag	Sigma (F3165, Clone M2)	Mouse mAb	1:5000	1:5000
PCNA	Abcam (ab18197)	Rabbit pAb	1:200	1:500
Ki-67	Abcam (ab16667)	Rabbit mAb	1:200	1:1000
SOX9	Abcam (ab76997)	Mouse mAb	1:200	
a-Tubulin	Proteintech (11224-1-AP)	Rabbit pAb		1:5000
Alexa Fluor 488	Invitrogen (A32723)	Goat anti-Mouse	1:500	
Alexa Fluor 488	Invitrogen (A32731)	Goat anti-Rabbit	1:500	
Alexa Fluor 546	Invitrogen (A21133)	Goat anti-Mouse	1:500	
Alexa Fluor 647	Invitrogen (A32733)	Goat anti-Rabbit	1:500	

Table S3. guideRNA information

Target gene	Sequence
Msx1	F: CGCTCTTCGCCGCGGGGCCAAGGAGAGCGTCCGTTTTAGAGCTAGAAATAGCAA R: CGCTCTTCTAACCAAGTGCTGCACCGAGCCACCCGGTGTTTCGTCCTTTCCAC

Table S4. Primers used for qRT-PCR analysis

Gene name	Primer sequence (5' to 3')
Fgf3	F: TGCGCTACCAAGTACCACC R: CACTTCCACCGCAGTAATCTC
Fgf9	F: ATGGCTCCCTTAGGTGAAGTT R: TCCGCCTGAGAATCCCCTTT
Fgf14	F: TTCTCAGGGTGTCTAAGCTGC R: GGGGATCAGTTGGGTTCTTGTT
Fgf15	F: ATGGCGAGAAAGTGGAACGG R: CTGACACAGACTGGGATTGCT
Fgf18	F: CCTGCACTTGCCTGTGTTTAC R: TGCTTCCGACTCACATCATCT
Fgfr1	F: TTTTAAAGCTAAGTAGCTGG R: AATTGGCTAGACCTTGGCAG
Fgfbp1	F: TGGCTACTCAGGCGTTCTCA R: CGTCAGAGATTTAGATGTCCTGC
Ki67	F: ACCGTGGAGTAGTTTATCTGGG R: TGTTTCCAGTCCGCTTACTTCT
Msx1	F: CTCTCGGCCATTTCTCAGTC R: TACTGCTTCTGGCGGAACTT
GAPDH	F: TGCGACTTCAACAGCAACTC R: GCCTCTCTTGCTCAGTGTCC

Table S5. Primers used for CHIP-qPCR

Gene name	Primer sequence (5' to 3')
Fgf9	F: AGCTCTGAGGTGTACGAACTTG R: ACCCATCACCCCATTGCTAAC
Fgf18-1	F: CCCTGTGTAACACCCCGATAC R: AGGCTGAAGTGGAACAGGGA
Fgf18-2	F: CCAAGACCTTCCCGAGTCTG R: AGAGTTCTCCCCATCCTGCT
Fgf18-3	F: ACTGTCGGCAATGGGACTG R: CACCAGGTCCAGCTTCGTG

Table S6. The sequences of RNAs oligo

siRNA	siRNA sequence (5’ to 3’)
Fgf9	Sense: GCACCAGAAAUUUACACAUTT Antisense: AUGUGUAAAUUUCUGGUGCTT
Fgf18	Sense: GUGGGAAGCACAUUCAAGUTT Antisense: ACUUGAAUGUGCUUCCCACTT
Negative control	Sense: UUCUCCGAACGUGUCACGUTT Antisense: ACGUGACACGUUCGGAGAATT

Table S7. Kinase of phosphorylation sites of Msx1 predicted online

Location	Phosphorylated sites	HMM Bit score	E-value	Catalytic kinase
136	VKAE**S**PEKL	-3.1	21	CDK1
152	SPRF**S**PPPA	-2.4	16	CDK1
152	SPRF**S**PPPA	-3.8	19	ATM
160	ARRL**S**PPAC	5.5	0.24	PKA
160	ARRL**S**PPAC	-1.5	12	IKK

SUPPLEMENTARY FIGURES

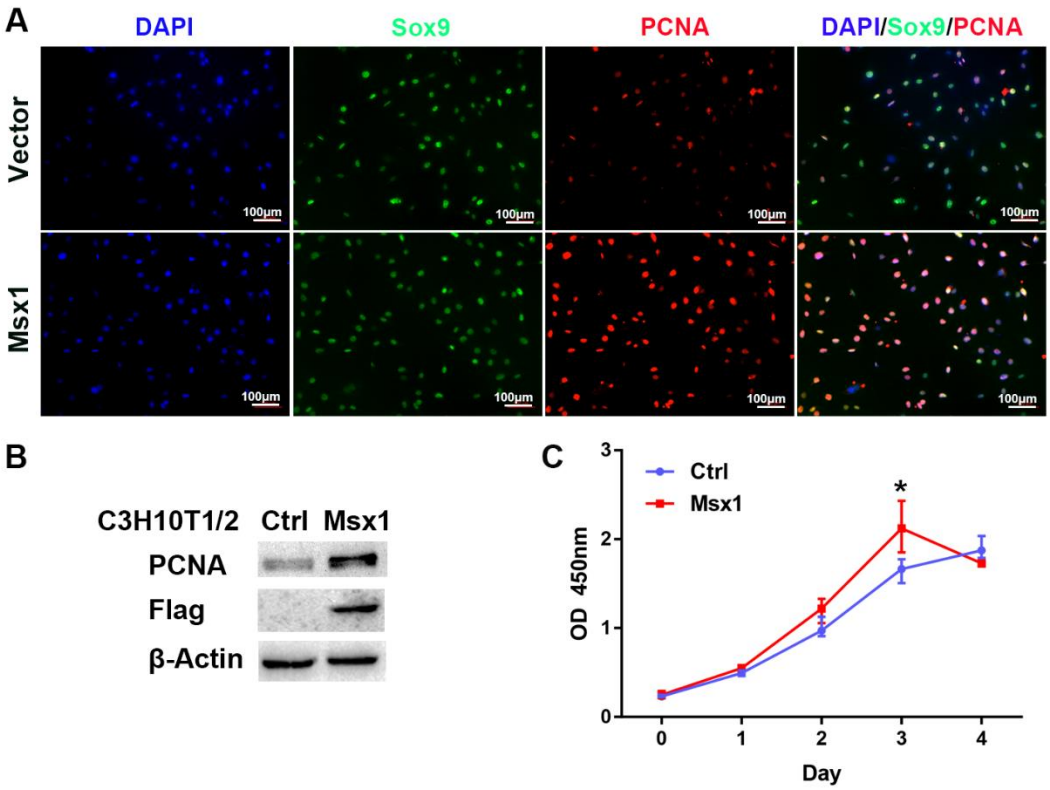

Figure S1. Overexpression of Msx1 promotes C3H10T1/2 cell proliferation. (A) IF assays to detect PCNA expression in C3H10T1/2 cells overexpressing Msx1 and the control. DAPI was used to counterstain the nuclei. Scale bar = 100 μm. (B) Western blotting to detect PCNA expression in C3H10T1/2 cells overexpressing Msx1 and the control. (C) CCK-8 assays to assess the proliferation rate of C3H10T1/2 cells

overexpressing Msx1 and the control.

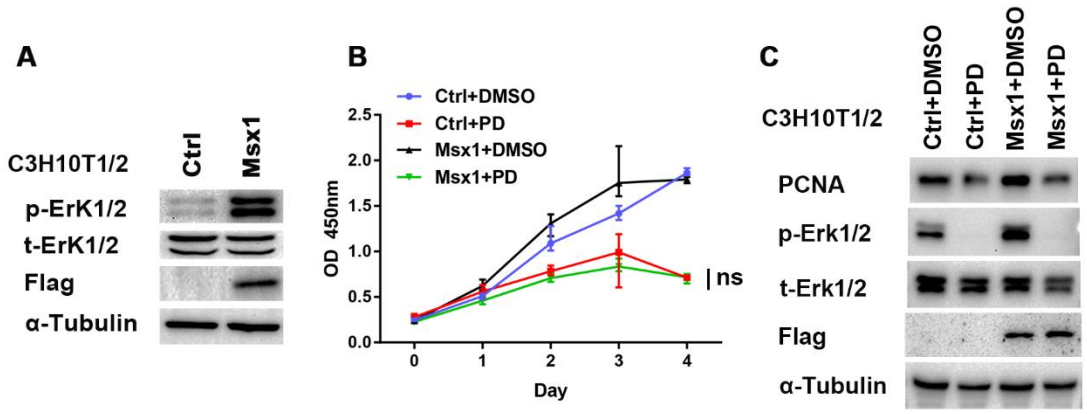

Figure S2. Msx1 promotes C3H10T1/2 cell proliferation by activating the MAPK signaling pathway. (A) Western blotting assays to detect the level of p-Erk1/2 in Msx1-overexpressing C3H10T1/2 cells and the control. (B) CCK-8 assays to assess the impact of PD0325901 on C3H10T1/2 cell proliferation promoted by Msx1. C3H10T1/2 cells overexpressing Msx1 or the control were treated with 1uM PD0325901 or DMSO, respectively. (C) Western blotting assays to detect the impact of PD0325901 on the expression of proliferation marker promoted by Msx1 in C3H10T1/2 cells.

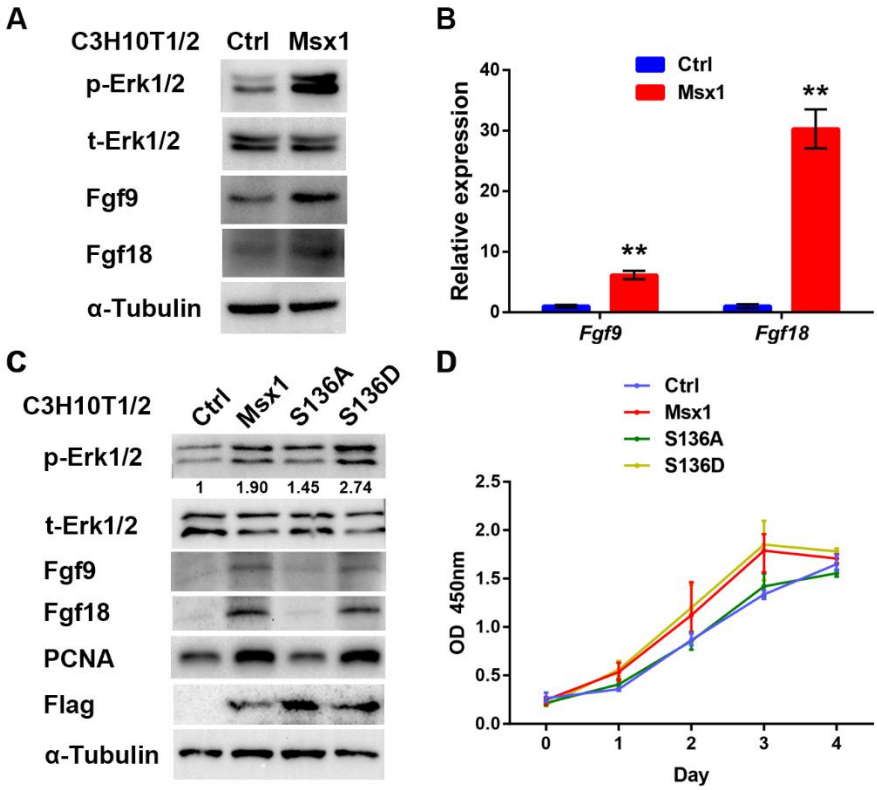

Figure S3. Msx1 up-regulated Fgf9 and Fgf18 expression by the phosphorylation of 136S in C3H10T1/2 cells. (A) Western blotting to confirm upregulation of Fgf9 and

Fgf18 by Msx1 in C3H10T1/2 cells. (B) qRT-PCR assays to determine the mRNA level of *Fgf9* and *Fgf18* in C3H10T1/2 cells overexpressing Msx1 and the control. Values are the means ± SD. $^{**}P < 0.001$. (C) Western blotting to assess the effect of Msx1 phosphorylation site Ser136 on the levels of p-Erk1/2, PCNA, Fgf9 and Fgf18 in C3H10T1/2 cells. Numbers under western blot bands represent relative quantifications over t-Erk1/2. (D) CCK-8 assays to examine the effect of Msx1 Ser136 phosphorylation on the proliferation of C3H10T1/2 cells.

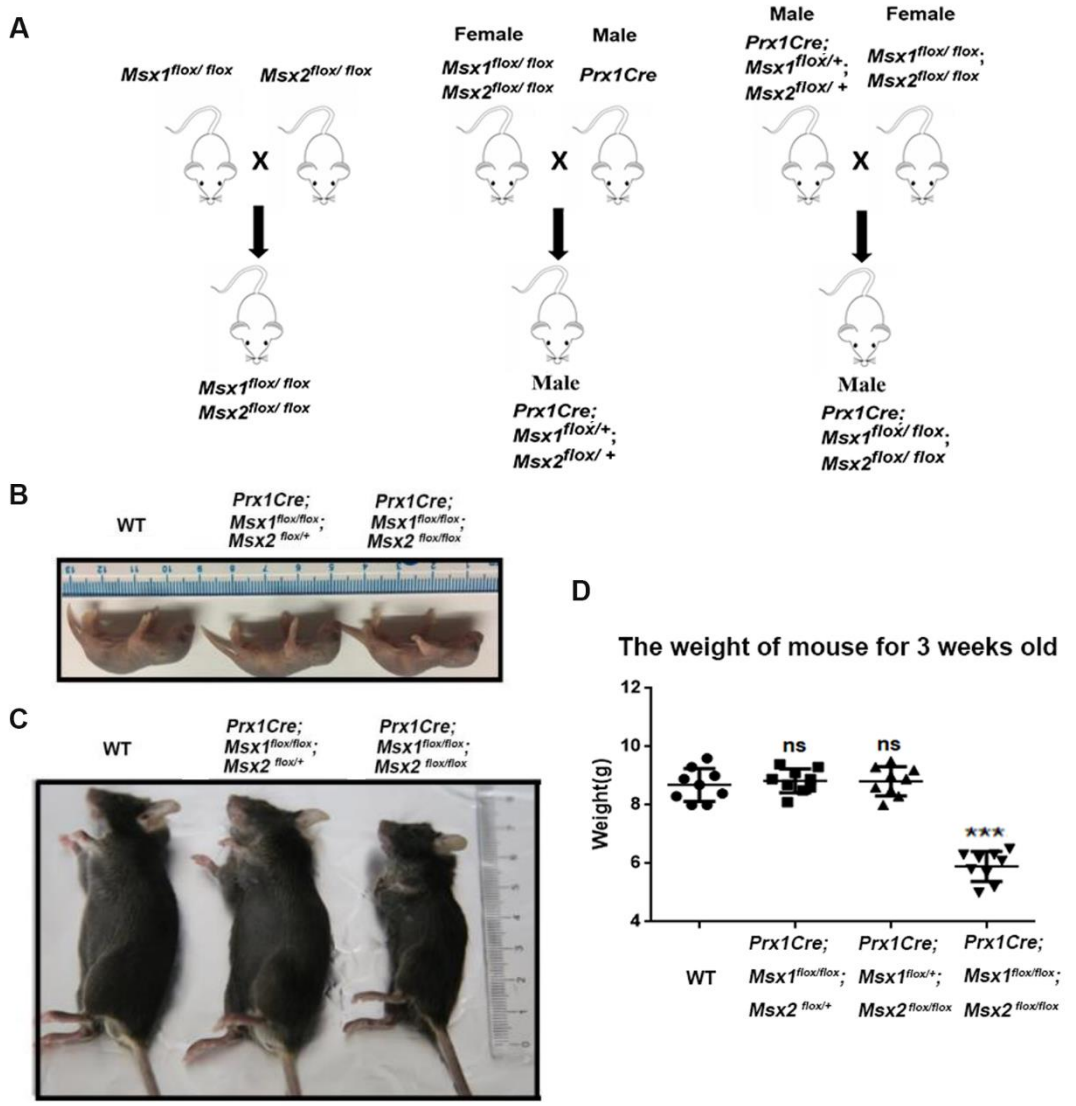

Figure S4. General appearance of mice with *Msx* inactivated in the lateral plate mesoderm. (A) Strategies for obtaining different genotype mice by crossing $Msx^{flox/flox}$ mice with *Prx1-Cre* mice. (B and C) Representative view of male control (wild-type), *Prx1-Cre*; $Msx1^{flox/flox}$; $Msx2^{flox/+}$, *Prx1-Cre*; $Msx1^{flox/flox}$; $Msx2^{flox/flox}$ mice at 2 days (B) or 6 weeks (C) after birth. (D) Body weight statistical analysis of male control (wild-type), *Prx1-Cre*; $Msx1^{flox/flox}$; $Msx2^{flox/+}$, *Prx1-Cre*; $Msx1^{flox/+}$; $Msx2^{flox/flox}$, *Prx1-Cre*; $Msx1^{flox/flox}$; $Msx2^{flox/flox}$ mice measured at 3 weeks old (n=9). Values are the means ± SD. $^{***}P < 0.0001$.