

Supplementary information

Targeted Demethylation of the TGF β 1 mRNA Promotes Myoblast Proliferation via Activating the SMAD2 Signaling Pathway

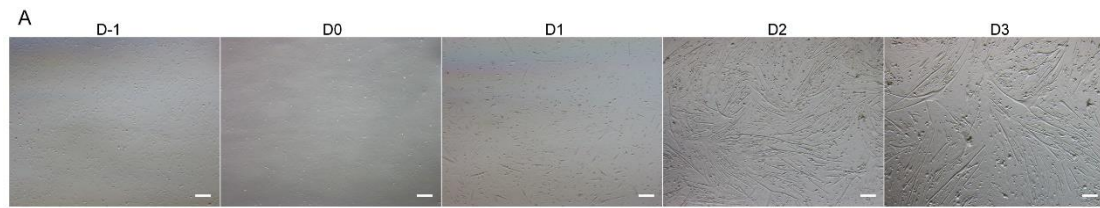
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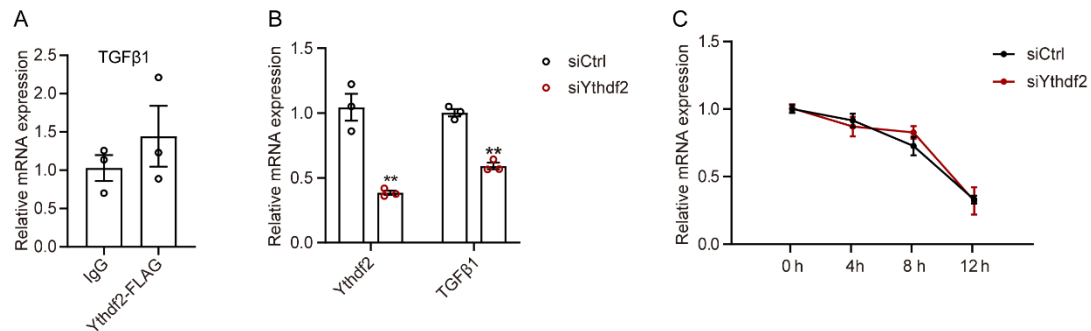
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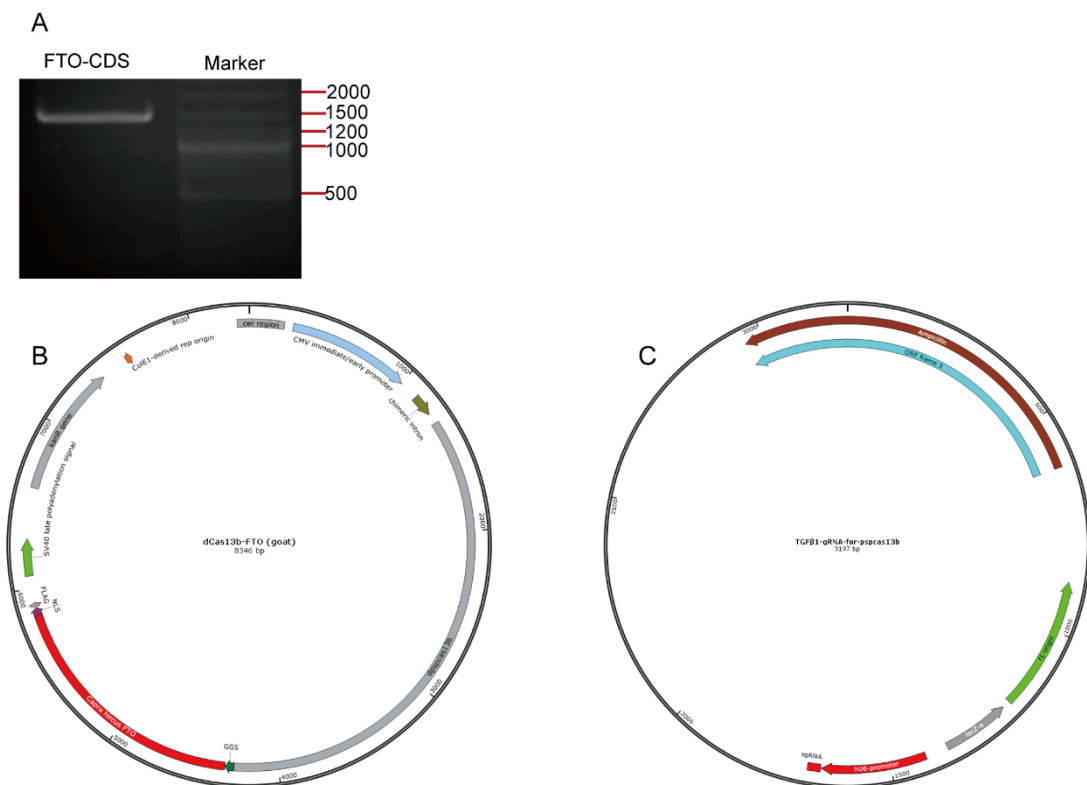
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Supplementary Figure S1. Phase contrast microscopy images of GPMs at days -1 (D-1), 0 (confluence [D0]), 1 (D1), 2 (D2), and 3 (D3) of differentiation. Scale bars, 100 μ m.



Supplementary Figure S2. Ythdf2 was not involved in m⁶A regulated TGF β 1 expression: (A) Ythdf2 RIP-qPCR analysis of TGF β 1 mRNA level in GPMs transfected with YTHDF2-FLAG vector; (B) GPMs were treated with Ythdf2 siRNA (siYthdf2), and the mRNA expression of Ythdf2 and TGF β 1 were measured at 36 h; (C) After transfection with siCtrl or siYthdf2 for 36 h, cells were treated with actinomycin D for the indicated times, and the relative expression level of TGF β 1 was checked by qRT-PCR.



Supplementary Figure S3 the supplement to Figure 6: (A) The represented FTO amplification product using skeletal muscle cDNA of goat; The plasmid profiles of reconstructed CRIPSR/dCas13b-FTO plasmid (B) and plasmids containing gRNA targeted TGFβ1 (C).

Supplementary Table S2. Details for siRNA sequence.

Target	Sense (5' to 3')	Anti-sense (5' to 3')
Ctrl	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
TGFβ1	GUGGAGCUGUACCAGAAAUTT	AUUUCUGGUACAGCUCCACTT
FTO	ACAAGCAUGGCUGUUUAUUTT	AAUAAACAGCCAUGCUUGUGC
Mettl3	GGAGAUAGAGAGCCUUCUUTT	AAGAAGGCUCUCUAUCUCCTT

Supplementary Table S3 Details of primer sequences used for quantitative real-time PCR

Items	Primer sequence (5'-3')	Fragment size (bp)
TGFβ1	F: CTCCGTCTGCCTCCTCCTGTC	147
	R: TAGGGTCCAAGGTGCTCAGTCAAG	
Ythdf2	F: CAGGCATCAGTAGGGCAACA	228
	R: GGACCGCAACTTCTCCAAGA	
SMAD2	F: TGGACCACCGCTTTTGGTAA	272
	R: CCATCTAGAGACCTTGGTATGGT	
SMAD3	F: GCCCACAGGTTCTCAAGGAA	285
	R: TTCAACATCGGGCTGTGAGT	
FTO	F: AACGAGAGCGCGAAGCTAAG	274
	R: CCGATGAGGATGCGAGAGAC	
Mettl3	F: GGTCTGGCCTCTTCCACATC	229
	R: CTTGAACCTGAGCCCGACCT	
	R: GAAAGTCGTTGTGGGCGGTT	
MyHC	F: CTGTCCAAGTTCCGCAAGGT	182
	R: GAGCTTCGTTGCACCCTCAA	
	R: TTCATGGTGGCTGCATCAGT	
18S rRNA	F: GTGATGGGGATCGGGGATTG	116

	R: GTAGCGACGGGCGGTGTGTA	
GAPDH	R: CCGTTCGACAGATAGCCGTAA	296
	F: AGGATCTCGCTCCTGGAAGAT	
qPCR- SELECT	R: ATGCAGCGACTCAGCCTCTG	
	F: TAGCCAGTACCGTAGTGCGTG	

Supplementary Table S4. Primers used for SELECT qPCR in the present study

SELECT	UP (5' to 3')	DOWN (5' to 3')
TGFβ1-A358	tagccagtaccgtagtgctgtgctgtctggggtcc tcaag	5phos /cctgcctcgcgaggcaacgccagaggctgagt cgctgcat

Supplementary Table S5. Details of antibodies

Antibodies	Cat No.	Source	Dilution of WB
MyHC	Ab11083	Abcam	1:3000
TGFβ1	Ab215715	Abcam	1:1000
PCNA	ab18197	Abcam	1:1000
BAX	50599-2-Ig	Proteintech	1:5000
BCL2	12789-1-AP	Proteintech	1:1000
FTO	bs-7056R	Bioss	1:1000
METTL3	15073-1-AP	Proteintech	1:1000
Smad2	AF6497	Affinity	1:1000
Phospho-Smad2 (Ser467)	AF3449	Affinity	1:1000
FLAG	T0003	Affinity	1:5000
TUBA	11224-1-AP	Proteintech	1:5000
GADPH	60004-1-IG	Proteintech	1:8000
Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP	31460	Invitrogen	1:10000
HRP-conjugated Affinipure Goat Anti-Mouse IgG(H+L)	SA00001-1	Proteintech	1:8000