OMTN, Volume *17*

Supplemental Information

miR-892b Inhibits Hypertrophy by Targeting KLF10 in the Chondrogenesis of Mesenchymal Stem Cells

Jong Min Lee, Ji-Yun Ko, Hye Young Kim, Jeong-Won Park, Farshid Guilak, and Gun-Il Im

SUPPLEMENTARY TABLES

Table S1. Primer List for Cloning of Gene Expression Plasmids

Plasmid name	Sequences (5′ to 3′)	Direction	Product	Restriction Enzyme site
pCDH-miR-892b-copGFP	GCTAGCTGGAGAGCAGTGGGATGAGCATT	Forward	miR-892b	NheI-EcoRI
	GAATTCCATCTTGAGGATGCCTGAGGTCCA	Reverse		
pECFP-hKLF10	AGATCTATGCTCAACTTCGGTGCCTC	Forward	KLF10	BglII-SalI
	GTCGACTCACTGTGTGGGAGCAGG	Reverse		
pLKO.1-shKLF10-C1	CCGGGAGTATGTATTCCTGGAACAACTCGAG TTGTTCCAGGAATACATACTCTTTTTG	Forward	KLF10 shRNA	AgeI-EcoRI
	AATTCAAAAAGAGTATGTATTCCTGGAACAA CTCGAGTTGTTCCAGGAATACATACTC	Reverse		
pLKO.1-shKLF10-C2	CCGGGAACCCTCTCAAGTGTCAAATCTCGA GATTTGACACTTGAGAGGGTTCTTTTTG	Forward	KLF10 shRNA	AgeI-EcoRI
	AATTCAAAAAGAACCCTCTCAAGTGTCAAA TCTCGAGATTTGACACTTGAGAGGGTTC	Reverse		
pMIR-KLF10-3’UTR-WT	CTAGTTTGGTCTCAGCGGGAGCCAGTGA	Forward	KLF10 3′-UTR	SpeI-HindIII
	AGCTTCACTGGCTCCCGCTGAGACCAAA	Reverse		
pMIR-KLF10-3’UTR-MUT	CTAGTTTGGTCTCAGCGGGAATTCATGA	Forward	KLF10 3′-UTR	SpeI-HindIII
	AGCTTCATGAATTCCCGCTGAGACCAAA	Reverse		
pMIR-WNT6-3’UTR-WT	CTAGTTTAGACTGGAAAAAAGCCAGTCA	Forward	WNT6 3′-UTR	SpeI-HindIII
	AGCTTGACTGGCTTTTTTCCAGTCTAAA	Reverse		
pMIR-WNT6-3’UTR-MUT	CTAGTTTAGACTGGAAAAAAATTCATCA	Forward	WNT6 3′-UTR	SpeI-HindIII
	AGCTTGATGAATTTTTTTCCAGTCTAAA	Reverse		

Table S2. Primers and Probes List

Experiment	Gene or Probe	Sequences (5′ to 3′)	Direction	Accession No.
Rq-PCR (Human)	*Col2a1*	AACCAGATTGAGAGCATCCG	Forward	NM_033150
		ACCTTCATGGCGTCCAAG	Reverse	
	Sox9	ACTTGCACAACGCCGAG	Forward	NM_000346
		CTGGTACTTGTAATCCGGGTG	Reverse	
	Col1a1	CCCCTGGAAAGAATGGAGATG	Forward	NM_000088
		TCCAAACCACTGAAACCTCTG	Reverse	
	Col10a1	ACGATACCAAATGCCCACAG	Forward	NM_000493
		GTACCTTGCTCTCCTCTTACTG	Reverse	
	Klf10	AAAGTTCCCATCTGAAGGCC	Forward	NM_001032282
		TCACAACCTTTCCAGCTACAG	Reverse	
	Ihh	ATGAAGGCAAGATCGCTCG	Forward	NM_002181
		GATAGCCAGCGAGTTCAGG	Reverse	
	Ptch1	TCTTGGTGTTGGTGTGGATG	Forward	NM_000264
		ATTGCTGATGGACGTGAGG	Reverse	
	Ptch2	TGCTCTTTCTGGGACTGTTG	Forward	NM_003738
		AGCTTCTCCTTGGTGTAATGC	Reverse	
	Smo	ACAGCTACATCGCGGCCTTC	Forward	NM_005631
		AACAGCAGGGTAGCGATTCGAG	Reverse	
	Gli-2	AGCAGCAGCAACTGTCTGAGTGA	Forward	NM_005270
		GACCTTGCTGCGCTTGTGAA	Reverse	
	Wnt3a	ATCAAGATTGGCATCCAGGAG	Forward	NM_033131
		CAATGGCGTGGACAAAGG	Reverse	
	Wnt7a	GGGACTATGAACCGGAAAGC	Forward	NM_004625
		GGCCTGGGATCTTGTTACAG	Reverse	
	Wnt6	GAGAGTGCCAGTTCCAGTTC	Forward	NM_006522
		TGATGGCGAACACGAAGG	Reverse	
	Wnt9b	AGTGCCAGTTTCAGTTCCG	Forward	NM_003396
		GGAAAGCTGTCTCTTTGAAGC	Reverse	
	Ctnnb1	GTTCAGTTGCTTGTTCGTGC	Forward	NM_001098209
		GTTGTGAACATCCCGAGCTAG	Reverse	
	Alpl	GACAAGAAGCCCTTCACTGC	Forward	NM_000478
		AGACTGCGCCTGGTAGTTGT	Reverse	
	Gapdh	ACATCGCTCAGACACCATG	Forward	NM_002046
		TGTAGTTGAGGTCAATGAAGGG	Reverse	
Northern blot	hsa-miR-892b	Bio-TCTACCCAGAAAGGAGCCAGTG	Anti-sense	MI0005538
EMSA	IHPro-753-P1	AGGTTTGCGCCTGGCGGGGCACCCCAGAGCC	Top	
		GGCTCTGGGGTGCCCCGCCAGGCGCAAACCT	Bottom	
	IHpro-710-P2	AGGCGAGGCCAGGGCGGGGTGGGGCGCGTCC	Top	
		GGACGCGCCCCACCCCGCCCTGGCCTCGCCT	Bottom	
	IHpro-689-P3	GGGGCGCGTCCAGGCGGGGAGGGCAAACTCG	Top	
		CGAGTTTGCCCTCCCCGCCTGGACGCGCCCC	Bottom	
ChIP	IHpro-345	TGGGTTGCGGTCTCCGTG	Forward	
		GGAAATGGAAGAGATCCGGGC	Reverse	
Genotyping	mKLF10-5-15	CCT TCCTGCCAACAACTCTC	Forward	
	mKLF10-3-25	TCTGAGGAGTGACCCTTGCT	Reverse	
	Neo-5-1	TCGCCTTCTTGACGAGTTCT	Forward	

Table S3. Antibody List

Name	Experiments	Type	Manufacturer (Cat No.)	Dilution Ratio	React. Buffer/ Ag. retrieval
COL2A1	Western blotting	Ms Monoclonal	EMD Millipore (MAB8887)	1/1,000	PBS-T
SOX9	Western blotting	Rb Polyclonal	Abcam (ab59265)	1/1,000	PBS-T
COL1A1	Western blotting	Ms Monoclonal	Abcam (ab90395)	1/500	PBS-T
COL10A1	Western blotting	Rb Polyclonal	Abcam (ab58632)	1/500	TBS-T
ALP	Western blotting	Ms Monoclonal	Abcam (ab54778)	1/1,000	PBS-T
KLF10	Western blotting	Rb Polyclonal	Abcam (ab73537)	1/1,000	PBS-T
	IHC			1/100	Citrate Bfr. /20 min at 95°C
IHH	Western blotting	Rb Monoclonal	Abcam (ab52919)	1/2,000	PBS-T
	IHC			1/200	0.2% Triton X-100 /20 min at RT
PTCH1	Western blotting	Rb Polyclonal	Abcam (ab53715)	1/1,000	PBS-T
PTCH2	Western blotting	Rb Polyclonal	Thermo Scientific (PA1-46223)	1/1,000	PBS-T
SMO	Western blotting	Rb Polyclonal	Abcam (ab38686)	1/1,000	PBS-T
GLI-2	Western blotting	Rb Polyclonal	Abcam (ab26056)	1/1,000	PBS-T
WNT3A	Western blotting	Rb Polyclonal	Abcam (ab28472)	1/1,000	PBS-T
WNT6	Western blotting	Rb Polyclonal	Biovision (3570-100)	1/500	PBS-T
WNT7A	Western blotting	Rb Polyclonal	Abcam (ab100792)	1/500	PBS-T
WNT9B	Western blotting	Rb Polyclonal	Abcam (ab69287)	1/500	PBS-T
β-catenin	Western blotting	Ms Monoclonal	Santa Cruz (sc-59737)	1/1,000	PBS-T
GAPDH	Western blotting	Ms Monoclonal	Santa Cruz (sc-47724)	1/1,000	TBS-T
KLF10	ChIP	Rb Polyclonal	GeneTex (GTX108661)	1/500	50 mM HEPES, 140mM NaCl, 1mM EDTA
Osteocalcin	IHC	Ms Monoclonal	Abcam (ab13418)	1/80	Pepsin Enzymatic /20 min at RT

SUPPLEMENTARY FIGURES

Figure S1

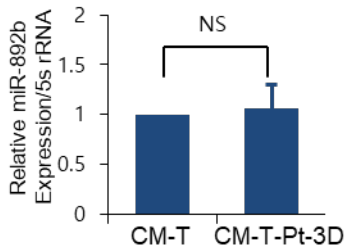

Figure S1. Expression of miR-892b induced by PTHrP at the early stage of chondrogenic differentiation.

PTHrP was treated only for the first 3 days of chondrogenic culture. The expression of miR-892b was analyzed after 3 weeks of differentiation, and compared with the group not treated with PTHrP. The data are shown as the mean ± SD, NS = not significant (P = 0.56), 2-tailed Student's t-test, n = 3 donors.

Figure S2

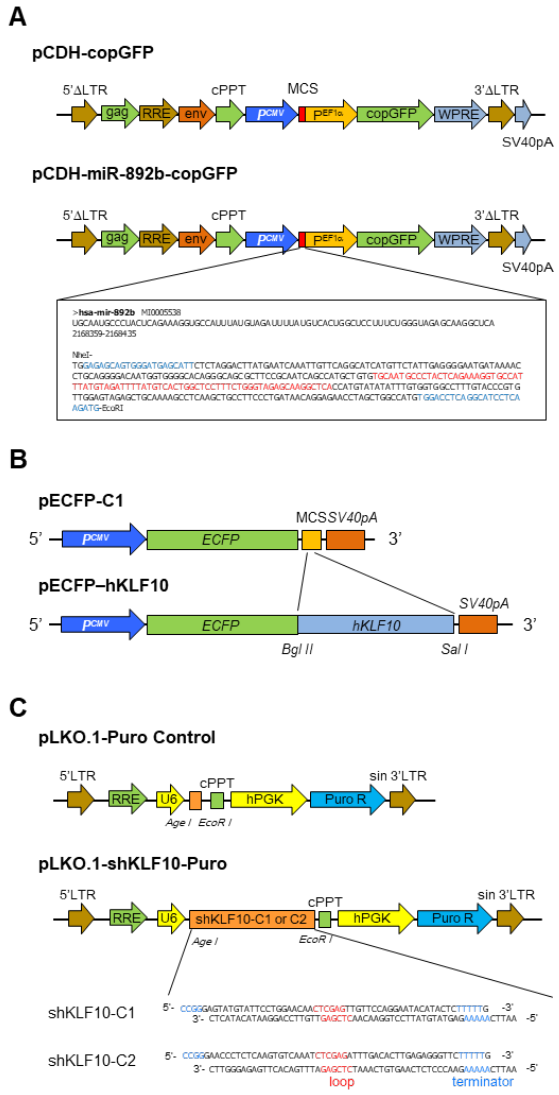

Figure S2. Construction of gene expression plasmids.

(A) After genomic DNA PCR, an intron fragment containing pre-miR-892b gene was cloned into NheI-EcoRI sites of pCDH-copGFP lentiviral plasmid. The blue letters represent PCR priming sites, and the red letters represent a sequence correspond to pri-miR-892b. (B) Human KLF10 gene amplified from cDNA of hMSCs was cloned into BglII-SalI sites of pECFP-C1 nonviral plasmid. The hKLF10 gene is fused to ECFP gene, enhanced cyan fluorescent protein. (C) pLKO.1 lentiviral plasmid was used to construct a lentiviral plasmid expressing KLF10 shRNAs. KLF10 shRNAs were designed by BLOCK-iT^{TM} RNAi Designer (Thermo Scientific). The top and bottom single strands correspond to hKLF10 shRNAs was annealed, respectively, and then ligated to AgeI-EcoRI sites of pLKO.1 lentiviral plasmids.

Figure S3

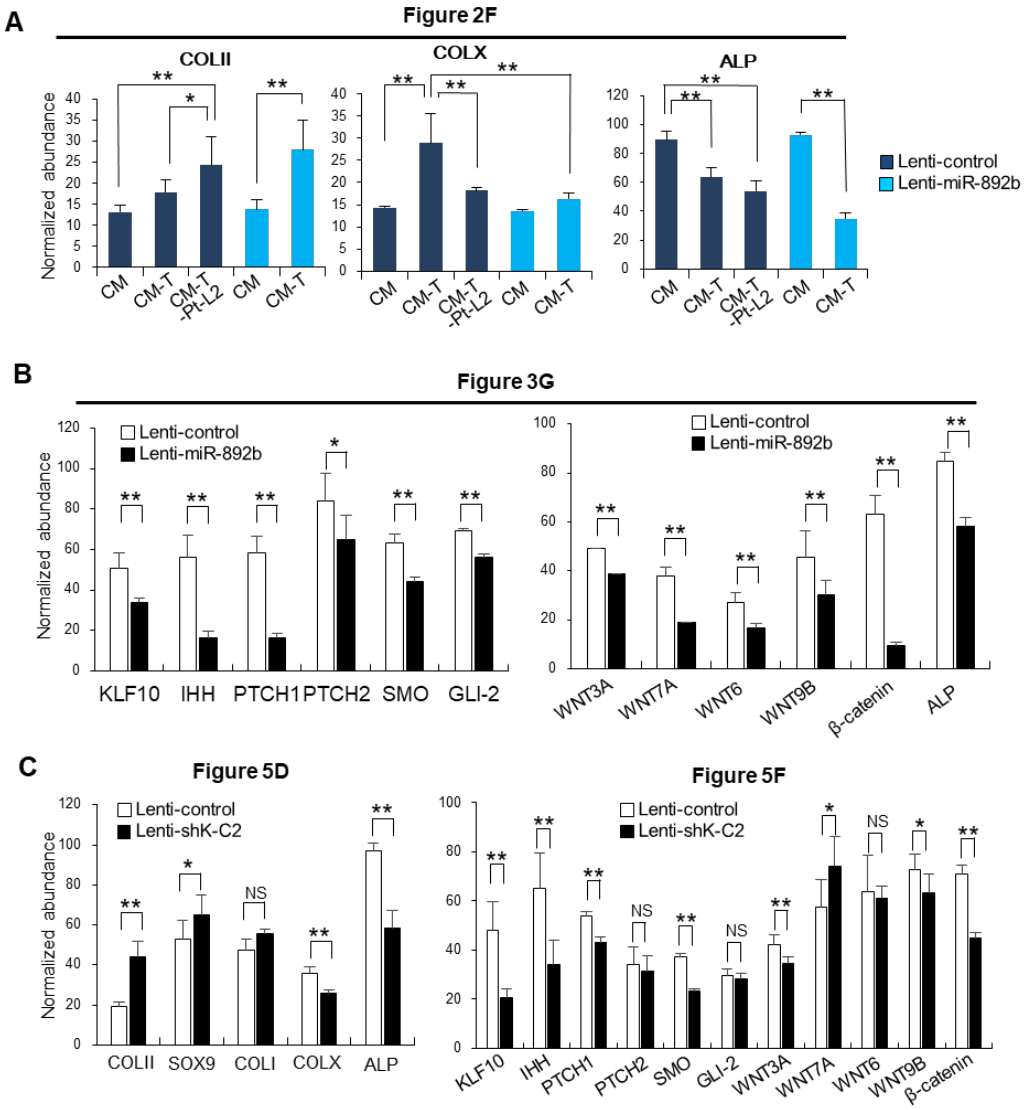

Figure S3. Statistical quantification of western blot image (by ImageJ).

The Western band was quantified using the ImageJ program, normalized to GAPDH band and statistically processed. (A) Statistical quantification of Figure 2F. The data are shown as the mean ± SD, *p < 0.05, **p < 0.01, one-way ANOVA followed by the Tukey test. (B) Statistical quantification of Figure 3G. The data are shown as the mean ± SD, *p < 0.05, **p < 0.01 by paired, 2-tailed Student's t-test. (C) Statistical quantification of Figure 5D and 5F. The data are shown as the mean ± SD, *p < 0.05, **p < 0.01, NS = not significant, by paired, 2-tailed Student's t-test.

Figure S4

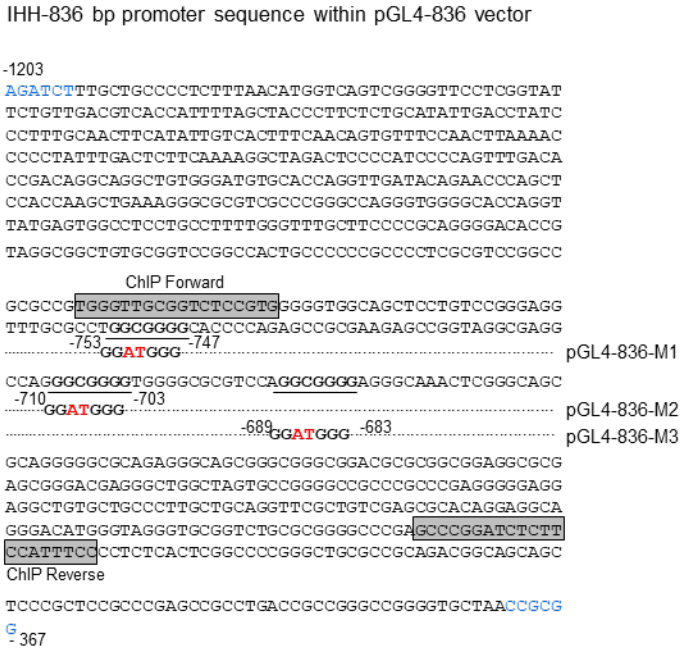

Figure S4. Point mutation of GC-rich regions within Indian hedgehog proximal promoter.

Nucleotide sequence represent an 836-bp fragment containing three predicted SP1/KLF10 binding sites. The GGCGGGG binding sites were substituted to GGATGGG by gene synthesis. The mutated 836-bp fragments were respectively synthesized and subcloned to pGL4 vector.