Supplemental information

Table 1. PCR primers used in this study

Gene	Gene ID	Gene description	forward primer(5'-3')	reverse primer (5'-3')	length (bp)
Actb	NM_007393	actin, beta, cytoplasmic	GGCCGGGACCTGACAGACTACCTC	GTCACGCACGATTTCCCTCTCAGC	182
Agc1	NM_007424	aggrecan 1	GTGAGGACCTGGTAGTGCGAGTGA	GAGCCTGGGCGATAGTGGAATATA	101
Col10a1	NM_009925	procollagen, type X, alpha 1	TGCCCGTGTCTGCTTTTACTGTCA	TCAAATGGGATGGGGGCACCTACT	70
Col2a1	NM_031163	procollagen, type II, alpha 1	ACGAAGCGGCTGGCAACCTCA	CCCTCGGCCCTCATCTCTACATCA	73
Dlk1	NM_010052	delta-like 1 homolog (Drosophila)	GCGTGGACCTGGAGAAAG	GGAAGTCACCCCCGATGT	276
Fn	NM_010233	fibronectin 1	CCAGGAGACAGCCGTGACCCAGACTTA	GCTCCCCGTTTGAATTGCCACCATA	50
Gtl2	NR_003633	maternally expressed 3	GGCCTGTCGCGTCTTCCTGTGC	GAGTCCTCGCGCGCTGGGCTTCCT	172
Itgb	NM_010578	integrin beta 1	GGGCGGGGCGGCTTCCTGA	GGGCCTCGGCTTCTCGGTTGGTCT	125

Supplemental figure legends

Fig. S1. Western blot analysis of Dlk1 expression in selected ATDC5 cell clones. 50 μg/well whole cell lysates from ATDC5 control cells (Vehicle) and ATDC-Dlk1 cells (Dlk1) were loaded and separated by SDS-PAGE gel. The presence of Dlk1 was checked by mouse Dlk1 antibody (Antibodies-online, #ABIN226221) at 1:500 dilution.

Fig. S2. Silver staining of the purification of FA1-Fc protein in the conditioned medium of FA1 overexpressing cells. FA1-Fc plasmid was transfected into 293F cells, 48 hours after transfection, the conditioned media was collected and purified by NAb™ Spin Kits. Lane 1. The original collected medium; Lane 2. Medium after concentrated by Amicon Ultra centrifuge filter (Millipore); Lane 3. Samples after binding with Protein A agarose columns; Lane 4-6. Three washing samples; Lane 7-9. Three eluting samples.

Fig. S3. Early changes in gene marker expression of ATDC5 during in vitro chondrogenic differentiation. Detailed presentation of the real time qPCR data obtained between day 0 and day 8 of chondrogenic differentiation of ATDC5 cells and presented in Fig. 3B and Fig. 4B. Expression of chondrognic marker genes (Col2a1, Agc1, and Col10a1) were compared to control (D0). * $P<0.05$

Fig. S4. Effects of chemical kinase inhibitors on insulin-induced chondrogenesis in ATDC5 cells. ATDC5 cells were induced to chondrogenesis by 10 μg/ml insulin for 24 days in presence of different chemical inhibitors (10 uM/each). Media was changed every other day. LY294002 and Wortmannin, target PI3K; triciribine and BML-257 target Akt; PD98059 and U0126 target MEK. Alcian blue staining (upper panel) was performed to detect the cartilage nodule formation and quantification of marker genes (Col2a1, Agc1, Col10a1) was carried out by real time qPCR at day 21 and compared to control (vehicle treated). * $P<0.05$, ** $P<0.01$.

Fig. S5. Expressions of Dlk1 and Gtl2 during chondrogenesis in mouse embryonic limb bud cells. Mouse limb buds from forelimbs and hind limbs from 8-10 litters of E11.5 mouse embryos were dissociated. Cells were re-suspended in growth media at a concentration of 2.5×10^7 cell/ml and spotted in 10-μl droplets per well of 4-well cell culture plate for micromass culturing. After cells adhered to culture dishes for 3 h at 37 ℃ in a humidified atmosphere containing 5% CO_2, DMEM/F12 media containing 10% FBS, 0.5 mM glutamine, 0.25 mM ascorbic acid (Sigma) and 1 mM β-glycerophosphate (Sigma) was added. The media were replaced every other day. Real time qPCRs was performed to check the expressions of Dlk1 and Gtl2.

Fig. S6. Temporal expression of Sox9 in ATDC5 and ATDC-Dlk1 cells during the chondrogenesis. RNA samples were collected from ATDC5 control and ATDC5 overexpressing Dlk1 (ATDC-Dlk1) cells at day 0, 4, 8, 16, 24 during chondrogenic differentiation. Real time qPCRs were performed and normalized to β-actin. Results are representative of at least three independent experiments.

Fig. S1

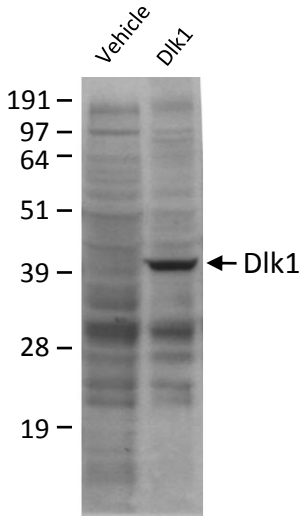


Fig. S2

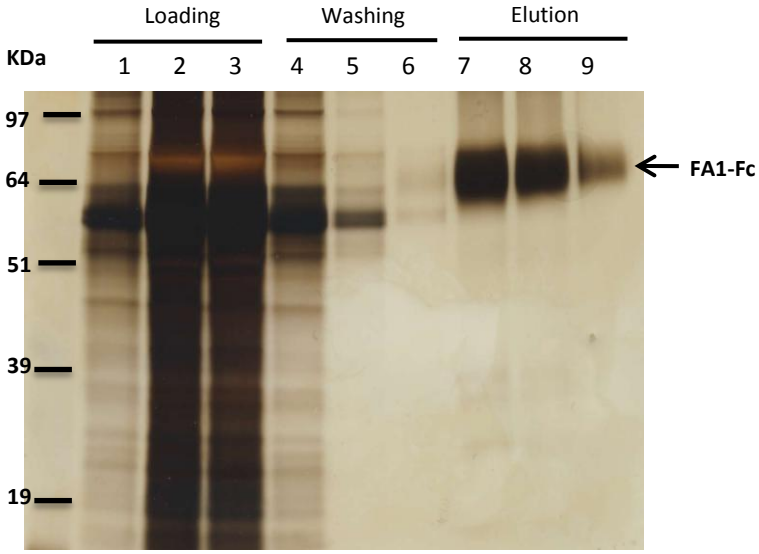


Fig. S3

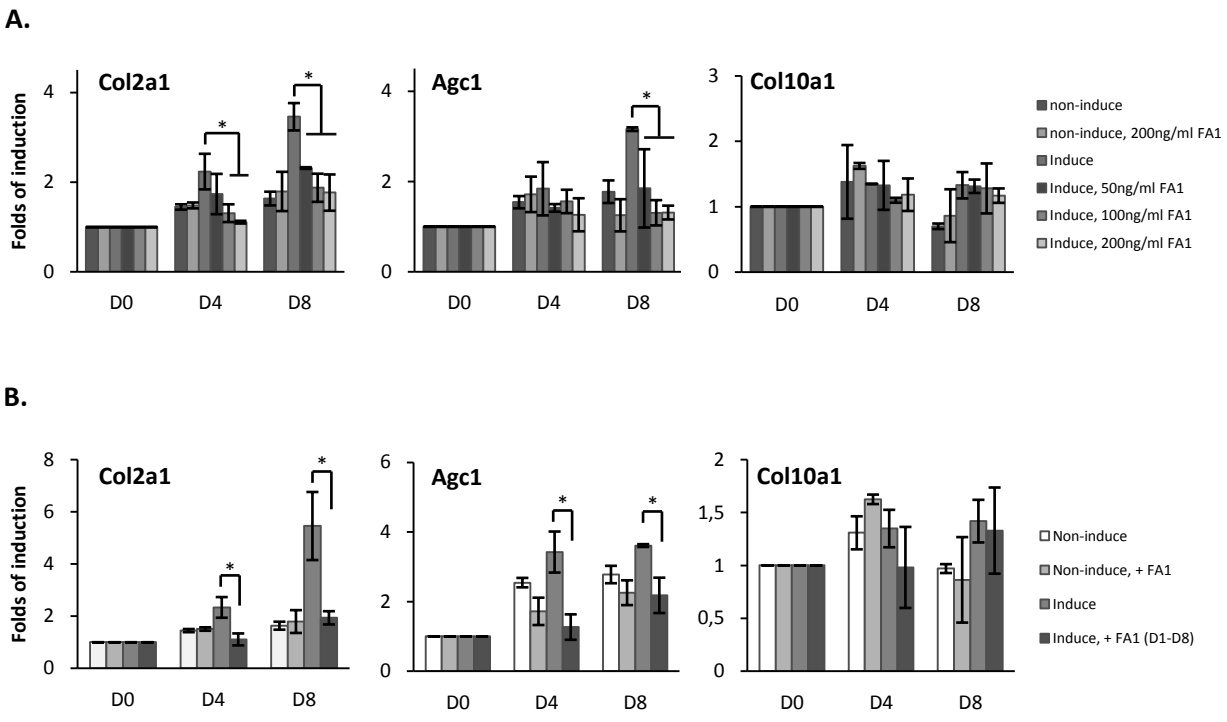

A.
Col2a1
Agc1
Col10a1
Folds of induction
D0
D4
D8
non-induce
non-induce, 200ng/ml FA1
Induce
Induce, 50ng/ml FA1
Induce, 100ng/ml FA1
Induce, 200ng/ml FA1
B.
Col2a1
Agc1
Col10a1
Folds of induction
D0
D4
D8
Non-induce
Non-induce, + FA1
Induce
Induce, + FA1 (D1-D8)

Fig. S4

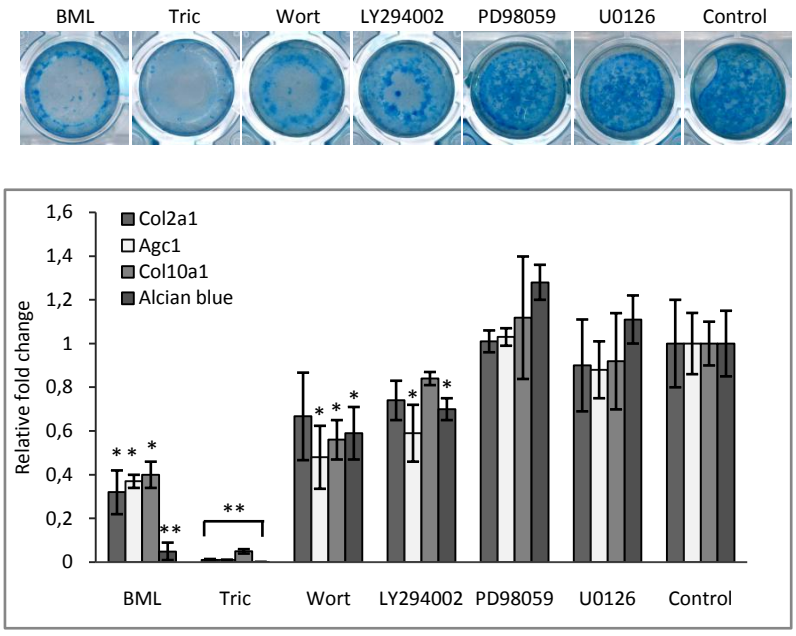

BML
Tric
Wort
LY294002
PD98059
U0126
Control
Col2a1
Agc1
Col10a1
Alcian blue
Relative fold change
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
BML
Tric
Wort
LY294002
PD98059
U0126
Control

Fig. S5

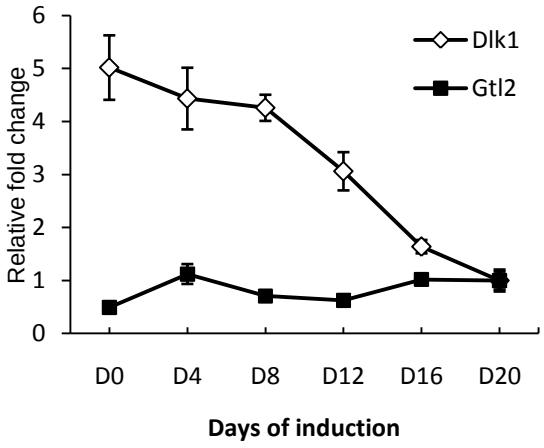
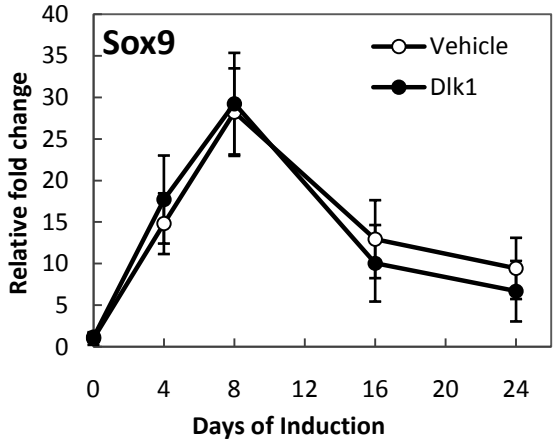

Dlk1
Gtl2
Relative fold change
0
1
2
3
4
5
6
D0
D4
D8
D12
D16
D20
Days of induction

Fig. S6


Sox9
Vehicle
Dlk1
Relative fold change
40
35
30
25
20
15
10
5
0
0
4
8
12
16
20
24
Days of Induction