

A

General Conditions of Patients	
Item	Non-weight bearing/weight bearing
Number of cases	15
Age	65.13± 9.32
Gender(M/F)	6/9
Outerbridge Grade	
Stage I	2
Stage II	4
Stage III	4
Stage IV	5

B

Outerbridge Grade	
Stage I	Localised softening of the cartilage with minimal or no break in the surface
Stage II	Fissuring within the softened area often longitudinal 'shark gill' type area < 1.25cm
Stage III	Breakdown of the surface and fibrillation, extending down to subchondral bone 'Crab meat' type Area > 1.25 cm
Stage IV	Early osteoarthritis; erosive changes and exposure of subchondral bone, usually involves the opposite articular surface (more often the lateral than the medial femoral surface).

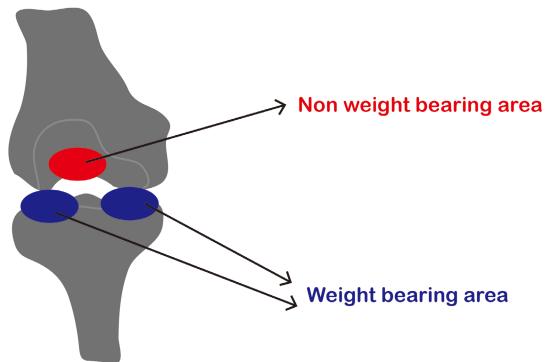
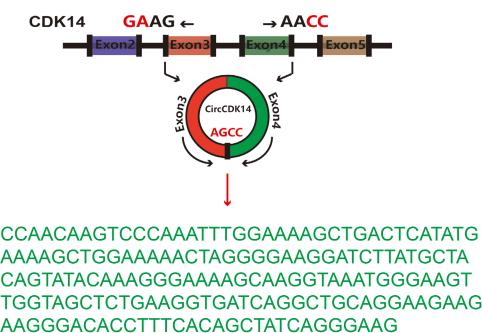
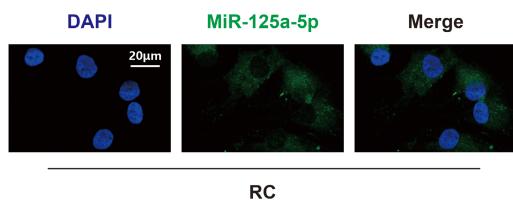
C

Figure S1 (A) General conditions of patients. (B) Standard of Outerbridge Grade. (C) Schematic diagram of the selection criteria for the knee weight-bearing area and non-weight-bearing area

A**B****C****D**

	Predicted consequential pairing of target region (top) and miRNA (bottom)
Position 977-983 of SMAD2 3' UTR	5'GCAGUAUAAUUUUUCAGGGAU...
hsa-miR-125a-5p	3' AGUGUCCAAUUUCCAG/AGUCCJ

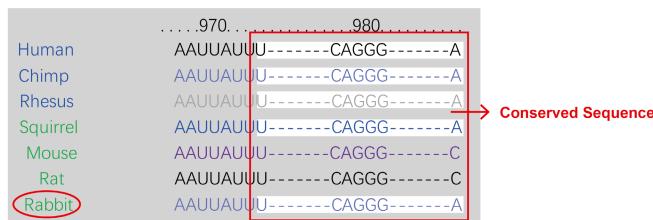
E

Figure S2 (A) The whole sequence of CircCDK14 provided by Circbase database. **(B)** The whole sequence of CircCDK14 detected by Sanger sequencing. **(C)** RNA FISH showed that the probe of human miR-125a-5p could also bind to miR-125a-5p in rabbit chondrocytes. Scale bar, 20μm **(D)** Putative miR-125a-5p binding site in the 3'UTR of Smad2 mRNA. **(E)** Sequence alignment of a putative miR-125a-5p binding site within the 3'UTR of Smad2 mRNA shows a high level of sequence conservation and complementarity with miR-125a-5p.

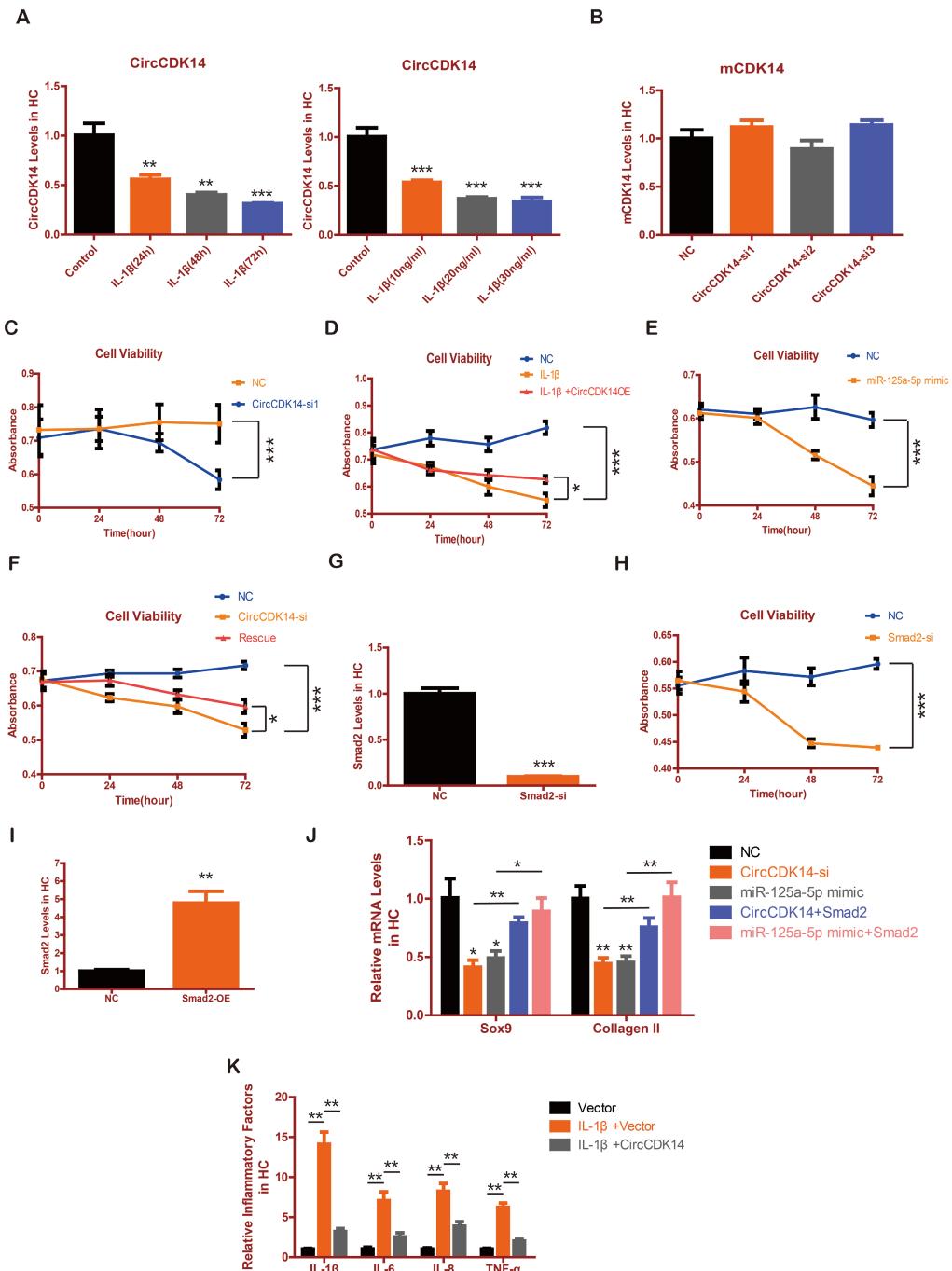


Figure S3 (A) Expression of CircCDK14 after IL-1 β (10ng/mL) treatment with different time gradient. Expression of CircCDK14 after 24 h IL-1 β treatment with different concentration gradient. (n=3) **p<0.01, ***p<0.001. **(B)** Three siRNAs specific against CircCDK14 have no effect on CDK14 mRNA. **(C)** CCK8 assay demonstrated that siRNA-mediated CircCDK14 knockdown suppressed HC cell proliferation. Data represents the mean \pm SD (n = 5). ***p<0.001. **(D)** CCK8 assay demonstrated that the treatment of IL-1 β suppressed HC cells proliferation, while the overexpression of CircCDK14 could antagonize this effect. Data represents the mean \pm SD (n = 5). *p<0.05, ***p<0.001. **(E)** CCK8 assay demonstrated that the overexpression of miR-125a-5p suppressed HC cell proliferation. Data represents the mean \pm SD (n = 5). ***p<0.001. **(F)** CCK8 assay showed the effect of siRNA-mediated CircCDK14 knocking down on HC cell proliferation could be rescued by inhibition of miR-125a-5p. *p<0.05, ***p<0.001. **(G)** The knock down efficiency of Smad2 in HC was detected by qRT-PCR. (n=3) ***p<0.001.

(H) CCK8 assay demonstrated that siRNA-mediated Smad2 knockdown suppressed HC cell proliferation. Data represents the mean \pm SD ($n = 5$). *** $p < 0.001$. **(I)** The overexpression efficiency of Smad2 in HC detected by qRT-PCR. ($n=3$) ** $p < 0.01$. **(J)** qRT-PCR showed that overexpression of Smad2 could antagonize the effects of CircCDK14-si and miR-125a-5p mimic on Sox9 and Collagen II. ($n=3$) * $p < 0.05$, ** $p < 0.01$. **(K)** qRT-PCR showed that the overexpression of CircCDK14 inhibited IL-1 β -induced inflammatory response in HC. ($n=3$) ** $p < 0.01$.

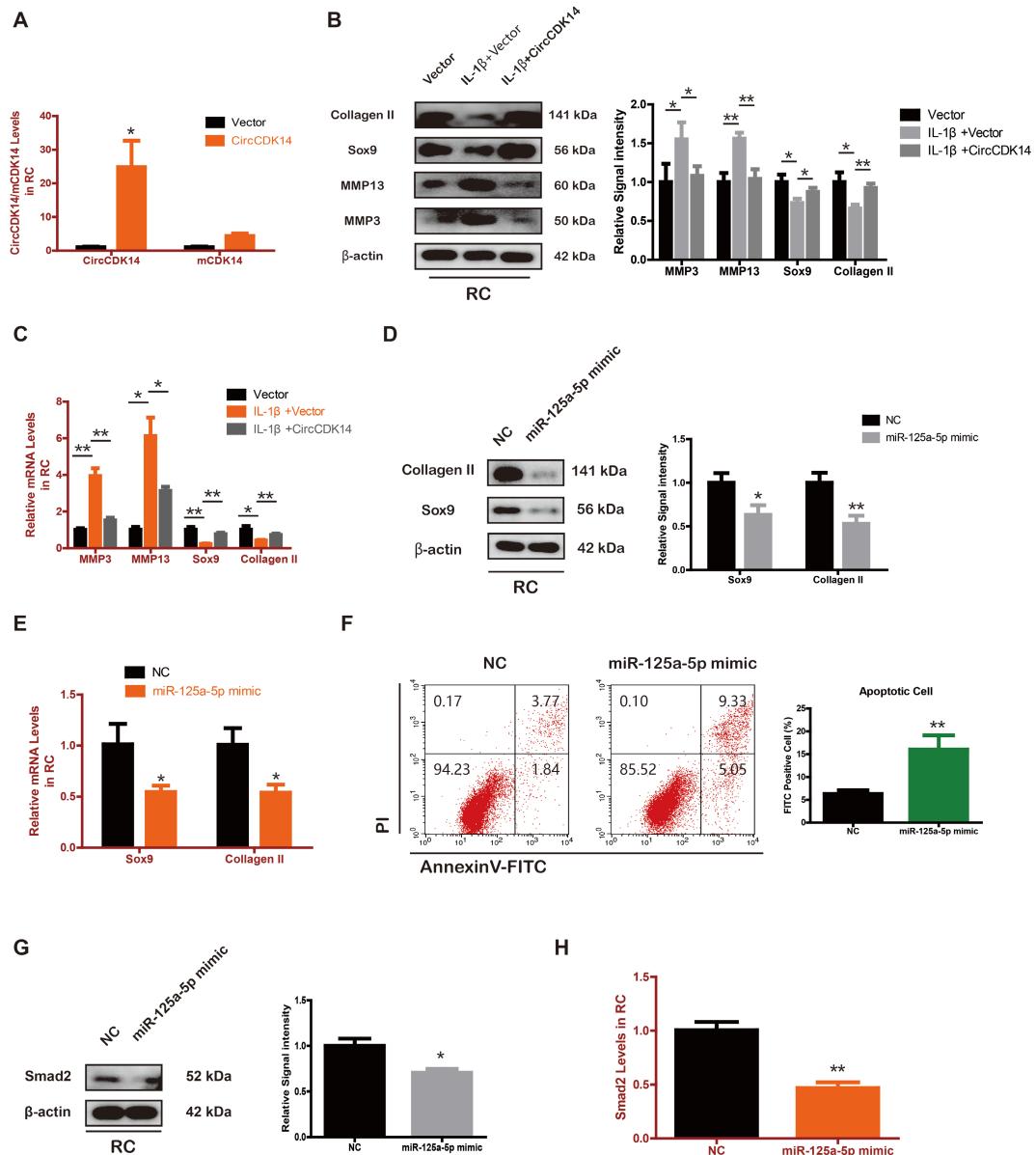


Figure S4 (A) The overexpression efficiency of CircCDK14 in RCs detected by qRT-PCR. ($n=3$) * $p < 0.05$.

(B) Western blot analysis of MMP3, MMP13, Sox9 and Collagen II in RCs after treating with IL-1 β (10ng/mL) for 24 h and the saving effects of overexpressed CircCDK14 on IL-1 β . The optical density analysis was performed from the results of three independent experiments of Western blot samples. * $p < 0.05$, ** $p < 0.01$.

(C) The expression levels of MMP3, MMP13, Sox9 and Collagen II in RCs was detected by qRT-PCR after treating with IL-1 β (10ng/mL) for 24 h and the saving effects of overexpressed CircCDK14 on IL-1 β . ($n=3$) * $p < 0.05$, ** $p < 0.01$.

(D) Western blot analysis of Sox9 and Collagen II in RCs when miR-125a-5p was upregulated. The optical density analysis was performed

from the results of three independent experiments of Western blot samples. *p<0.05, **p<0.01. (E) The expression levels of Sox9 and Collagen II in RCs were detected by qRT-PCR when miR-125a-5p was upregulated. (n=3) *p<0.05. (F) RCs were transfected with miR-125a-5p mimic, followed by Flow cytometry assay. The percentage of apoptotic cells was shown. (n=3) **p<0.01. (G) Western blot analysis of Smad2 in RCs when treated with miR-125a-5p mimic. The optical density analysis was performed from the results of three independent experiments of Western blot samples. *p<0.05. (H) The expression level of Smad2 in RCs was detected by qRT-PCR when miR-125a-5p was upregulated. (n=3) **p<0.01.

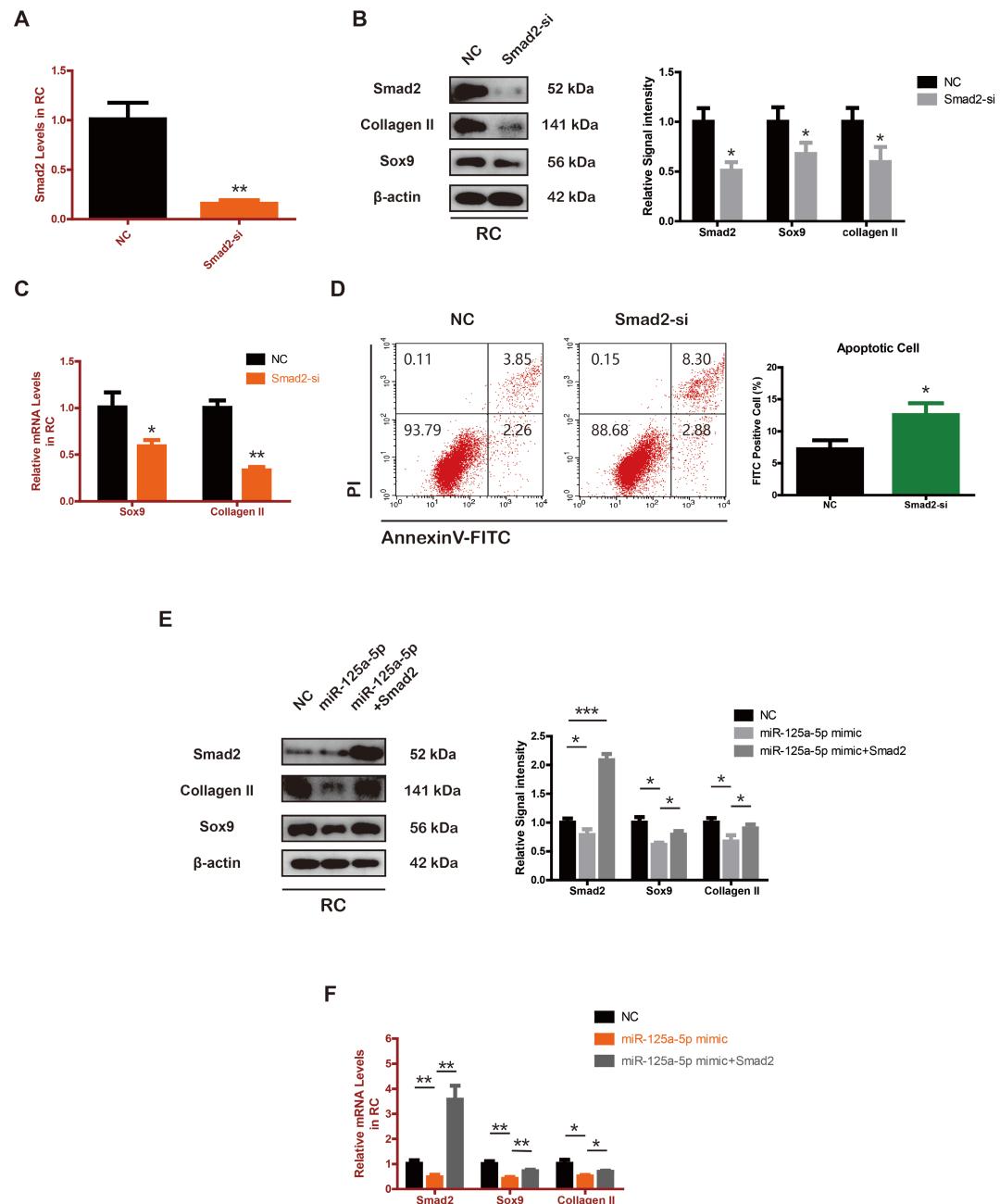


Figure S5 (A) The knock down efficiency of Smad2 in RCs was detected by qRT-PCR. (n=3) **p<0.01. **(B)** Western blot analysis of Smad2, Sox9 and Collagen II when Smad2 was downregulated in RCs. The optical density analysis was performed from the results of three independent experiments of Western blot samples. *p<0.05. **(C)** The expression level of Sox9 and Collagen II in RCs was detected by qRT-PCR

when treated with Smad2-si. (n=3) *p<0.05, **p<0.01. (D) Flow cytometry experiment indicated that siRNA-mediated Smad2 knockdown increased RCs apoptosis rate. (n=3) *p<0.05. (E) Western blot analysis of Smad2, Sox9 and Collagen II when Smad2 was upregulated in RCs. The optical density analysis was performed from the results of three independent experiments of Western blot samples. *p<0.05. ***p<0.001. (F) The expression level of Smad2, Sox9 and Collagen II in RCs was detected by qRT-PCR when Smad2 was upregulated in RCs. (n=3) *p<0.05, **p<0.01.

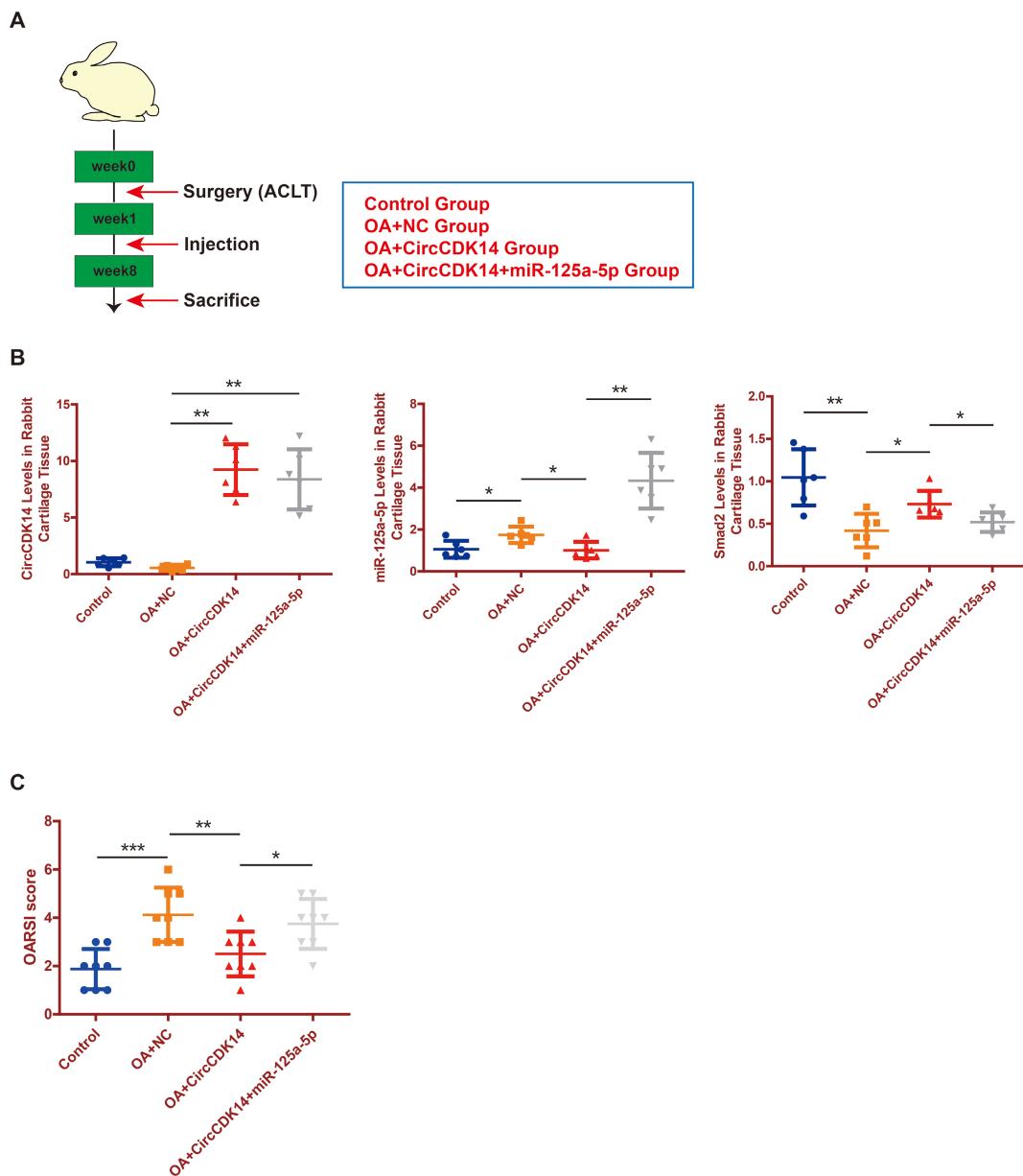


Figure S6 (A) Establishment of a rescue model for rabbit OA targeting CircCDK14. **(B)** The expression levels of CircCDK14, miR-125a-5p and Smad2 in rabbit joint tissues were detected by qRT-PCR (n=6). *p<0.05. **p<0.01. **(C)** OARSI scoring was performed according to Safranin-O/fast green staining results. (n=8) *p<0.05, **p<0.01.

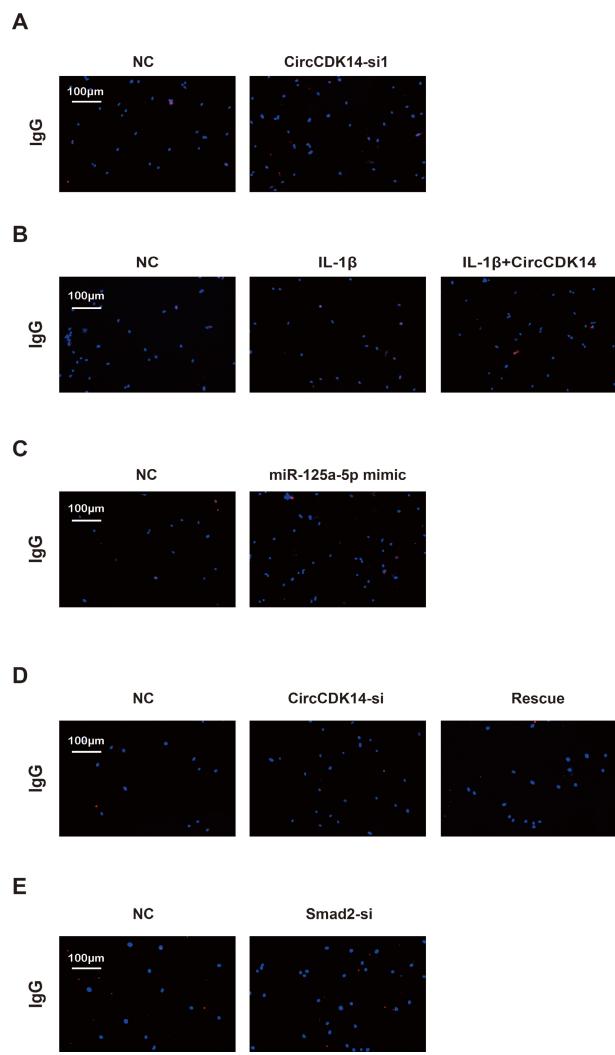


Figure S7 (A) Negative control (IgG) of IF in NC and CircCDK14-si1 groups. scale bar, 100μm. **(B)** Negative control (IgG) of IF in NC, IL-1 β and IL-1 β +CircCDK14 groups. scale bar, 100μm. **(C)** Negative control (IgG) of IF in NC and miR-125a-5p groups. scale bar, 100μm. **(D)** Negative control (IgG) of IF in NC, CircCDK14-si and rescue groups. scale bar, 100μm. **(E)** Negative control (IgG) of IF in NC and Smad2-si groups. scale bar, 100μm.

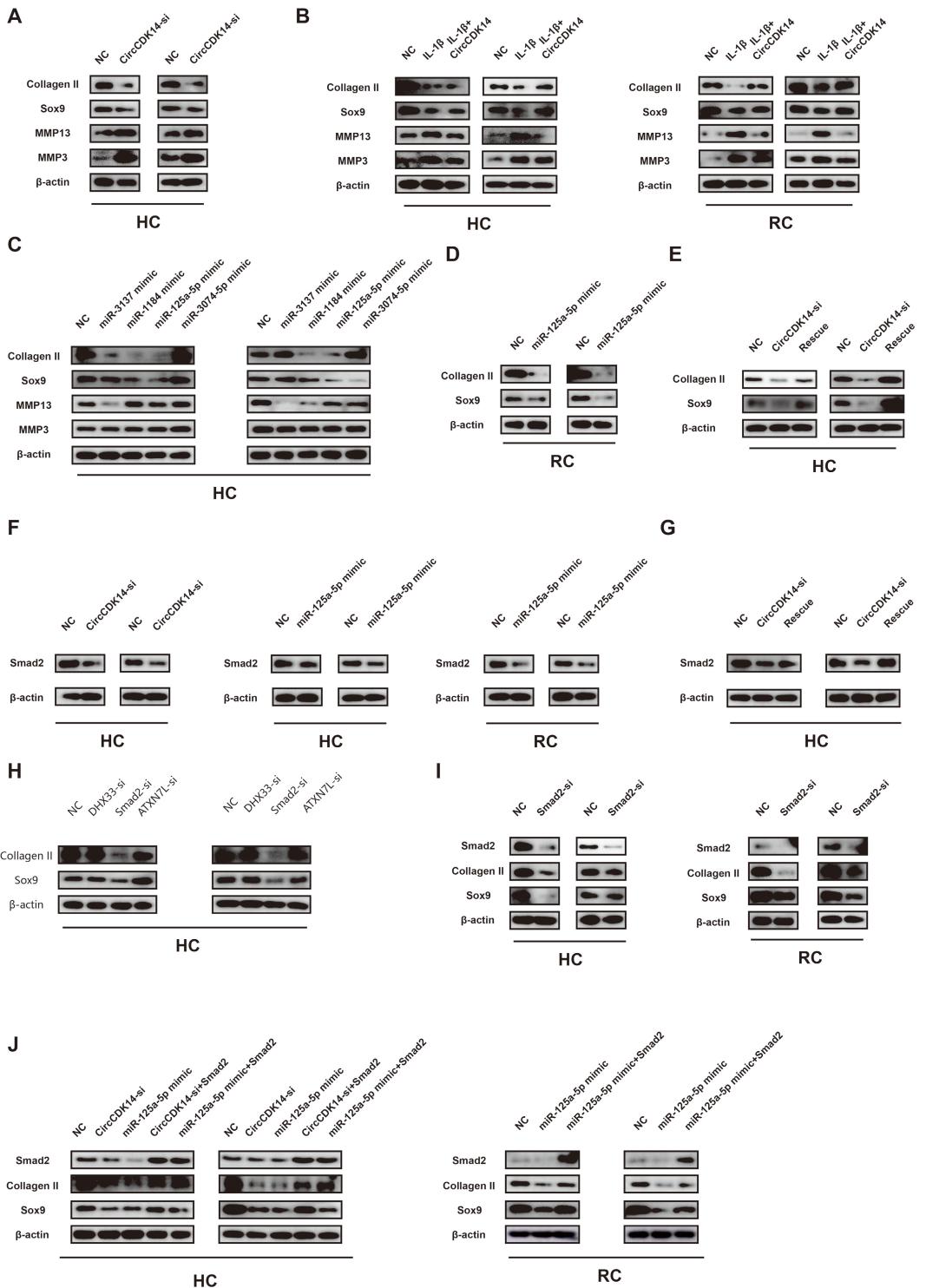


Figure S8 **(A)** Western blots of MMP3, MMP13, Sox9 and Collagen II when CircCDK14 was downregulated in HCs. **(B)** Western blots of MMP3, MMP13, Sox9 and Collagen II in HCs and RCs after treating with IL-1 β (10ng/mL) for 24 h and the saving effects of overexpressed CircCDK14 on IL-1 β . **(C)** Western blots of MMP3, MMP13, Sox9 and Collagen II in HCs when the selected 4 candidate miRNAs were overexpressed respectively. **(D)** Western blots of Sox9 and Collagen II in RCs when miR-125a-5p was upregulated. **(E)** Western blots showed that the downregulation of miR-125a-5p antagonized the effect of CircCDK14-si on Sox9 and Collagen II in HCs. **(F)** Western blots of Smad2 when treated with CircCDK14-si or miR-125a-5p mimic in HCs and RCs. **(G)** Western blots showed that the

downregulation of miR-125a-5p antagonized the effect of CircCDK14-si on Smad2 in HCs. **(H)** Western blots of Sox9 and Collagen II when the selected 3 candidate mRNAs were downregulated respectively in HCs. **(I)** Western blots of Smad2, Sox9 and Collagen II when Smad2 was downregulated in HCs and RCs. **(J)** Western blots of Smad2, Sox9 and Collagen II when Smad2 was upregulated in HCs and RCs.

Tables

Table S1 Primer sequences used in this study

Primer set	Gene ID	Primers	Sequences
CircCDK14 (human, Divergent)		Foward Reverse	TGGGAAGTTGGTAGCTCTGAAG AGATCCTTCCCTAGTTTTC
mCDK14 (human, Convergent)		Foward Reverse	GCTGGAAAAACTAGGGGAAGG CTTCCTGCAGCCTGATCAC
β-actin (human)	60	Foward Reverse	AGAGCTACGAGCTGCCTGAC AGCACTGTGTTGGCGTACAG
GAPDH (human)	2597	Foward Reverse	AGCCACATCGCTCAGACAC GCCAATACGACCAAATCC
MMP3 (human)	4314	Foward Reverse	CCTACAAGGAGGCAGGCAAG CCCGTCACCTCCAATCCAAG
MMP13 (human)	4322	Foward Reverse	TCGGCCACTCCTTAGGTCTT AAGTGGCTTTGCCGGTGTA
Sox9 (human)	6662	Foward Reverse	GCTCTGGAGACTCTGAACGA CCGTTCTTCACCGACTTCCT
Collagen II (human)	1280	Foward Reverse	CCAGATGACCTTCCTACGCC TTCAGGGCAGTGTACGTGAAC
Smad2 (human)	4087	Foward Reverse	GGCGGAGAAAGCAGCTG ACTGGAGGCAAAACTGGTGT
IL-6 (human)	3569	Foward Reverse	ACTCACCTCTCAGAACGAATTG CCATCTTGGAAAGGTTAGGTTG
IL-8 (human)	3576	Foward Reverse	TTTGCCAAGGAGTGCTAAAGA AACCTCTGCACCCAGTTTC
TNF-α (human)	7124	Foward Reverse	CCTCTCTTAATCAGCCCTTG GAGGACCTGGGAGTAGATGAG
IL-1β (human)	3553	Foward Reverse	ATGATGGCTTATTACAGTGGCAA GTCGGAGATTCTGTAGCTGGA
KLF13 (human)	51621	Foward Reverse	CGGCCTCAGACAAAGGGTC TTCCCGTAAACTTCTCGCAG
MAP2K7 (human)	5609	Foward Reverse	CCACGTCATTGCCGTTAAC GCACGATGTAGGGCAGTC
LIN28B (human)	389421	Foward Reverse	CATCTCCATGATAAACCGAGAGG GTTACCCGTATTGACTCAAGGC
ARID3A (human)	1820	Foward Reverse	ACCACGGCGACTGGACTTA CACAGGTGTCCCCTCGCITC
DHX33 (human)	56919	Foward Reverse	GATGAAGCTCACGAACGGACT CCACATCCATCGTAGCTGACA
ZSWIM6 (human)	57688	Foward Reverse	AAGCGGCTGCGTAGACAAC GGCTCCGATTGTATTGCAGGT
HK2	3099	Foward	GAGCCACCACTCACCCACT

(human)		Reverse	CCAGGCATTGGCAATGTG
MAP3K1 (human)	4214	Foward Reverse	CATCAGGTCGCACAGTGAAAT TCAGGGCTATATGGTGAGAAGC
LIFR (human)	3977	Foward Reverse	TGGAACGACAGGGTTCACT GAGTTGTGTTGGGTCACTAA
ZDHHC9 (human)	51114	Foward Reverse	CCCAGGCAGGAACACCTTT CCGAGGAATCACTCCAGGG
ATXN7L3 (human)	56970	Foward Reverse	ACAGCAAACTAGAGGCCATCG CCTTCATGCTATCAGGGTCCG
GAPDH (Rabbit)	100009074	Foward Reverse	GCTTCTCTCGTGCAGTGCTA GATGCCCTCCCGTGTGATGA
MMP3 (Rabbit)	100009111	Foward Reverse	CAATCCCTCATGGACCGGC CAGCAGCCTGTTGAACACC
MMP13 (Rabbit)	100008685	Foward Reverse	CCAGTCTCTATGGTCCAGG TGCTTCCGCATAGCACTGAA
Sox9 (Rabbit)	100008944	Foward Reverse	GGGGTGATGAGCTTGCTGA AAAACAGGATCTGTCGCCGT
Collagen II (Rabbit)	100009005	Foward Reverse	GGATAGACCCAACCAAGGC GGGTCCCTGGCTGTTAGA
Smad2 (Rabbit)	100359137	Foward Reverse	GCTCTTTCGAGTGGTC CACCTGAAGACGACCGTCAA
U6 (human)	26827	Foward Reverse	CTCGCTTCGGCAGCACA AACGCTCACGAATTIGCGT
hsa-miR-211-5p	406993	Foward Reverse	GTTCCCTTGTATCCTCGCCT Universal Reverse Primer (CW BIO)
hsa-miR-5088-3p	100847074	Foward Reverse	TCCCTTCTCCCTGGGCCCTCA Universal Reverse Primer (CW BIO)
hsa-miR-6875-3p	102466755	Foward Reverse	CCATTCTCCTGCCCTGGCTCCAT Universal Reverse Primer (CW BIO)
hsa-miR-3137	100422926	Foward Reverse	TATTCTGTAGCCTGGGAGCAATGGG Universal Reverse Primer (CW BIO)
hsa-miR-4778-3p	100616464	Foward Reverse	CGCGCTTCTTCCTTGCAGAGTTGA Universal Reverse Primer (CW BIO)
hsa-miR-204-5p	406987	Foward Reverse	CGTCCCTTGTATGCCCTGGCT Universal Reverse Primer (CW BIO)
hsa-miR-1184	100302111	Foward Reverse	CCTGCAGCGACTTGATGGC Universal Reverse Primer (CW BIO)
hsa-miR-937-5p	100126338	Foward Reverse	GTGAGTCAGGGTGGGCT Universal Reverse Primer (CW BIO)
hsa-miR-125a-5p	406910	Foward Reverse	TCCCTGAGACCCCTTAACCTGTGA Universal Reverse Primer (CW BIO)
hsa-miR-3667-3p	100500882	Foward Reverse	AGACCTTCCTCCATGGGTCTTT Universal Reverse Primer (CW BIO)

hsa-miR-7156-5p	102466995	Foward Reverse	CTGCAGCCACTTGGGAACTGGT Universal Reverse Primer (CWBIO)
hsa-miR-3074-5p	100422842	Foward Reverse	AGTCCTGCTGAAGTGAGCCAG Universal Reverse Primer (CWBIO)

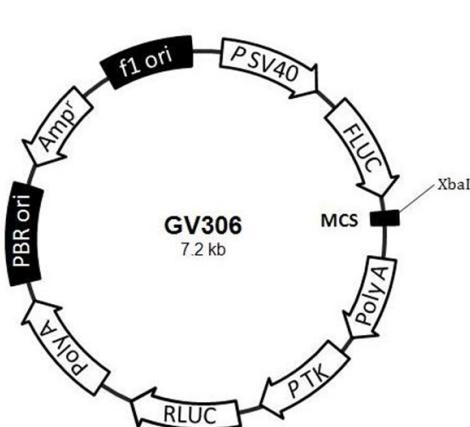
Table S2 Details about type of OARSI scoring (mean/max/sum)

	Crotrol	OA+NC	OA+CircCDK14	OA+CircCDK14+miR-125a-5p
Mean	1.88	4.13	2.50	3.75
Max	3	6	4	5
Min	1	2	1	2
Sum	15	33	20	30
Stdev	0.78	1.05	0.87	0.97

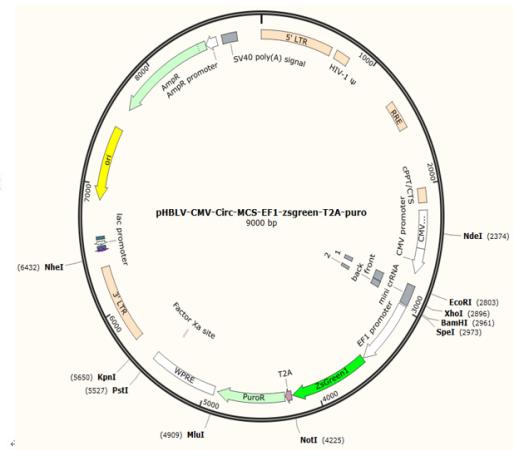
Table S3 Sequences of siRNAs, miRNA mimic/inhibitor and probes

CircCDK14 siRNA#1	GAAGCCAACAAGTCCCAA
CircCDK14 siRNA#2	TATCAGGGAAAGCCAACAAG
CircCDK14 siRNA#3	CAGGGAAGCCAACAAGTCC
Smad2 siRNA	GAATTGAGCCACAGAGTAA
DHX33 siRNA	GTGTGATGCTTCAGCTTCT
ATXN7L3 siRNA	GGCCTAGGTTCCAACAAGA
miR-125a-5p mimic	Ribobio
miR-125a-5p inhibitor	Ribobio
miR-3137 mimic	Ribobio
miR-1184 mimic	Ribobio
miR-3074-5p mimic	Ribobio
Probes for FISH	
CircCDK14	5'-CY3-GGACTTGGCTTCCCTGATAGCTG-3'
MiR-125a-5p	5'-FAM-TCACAGGTTAAAGGGTCTCAGGGA-3'
Probes for Pull-down assay	
CircCDK14	CTTGGCTTCCCTGATAG

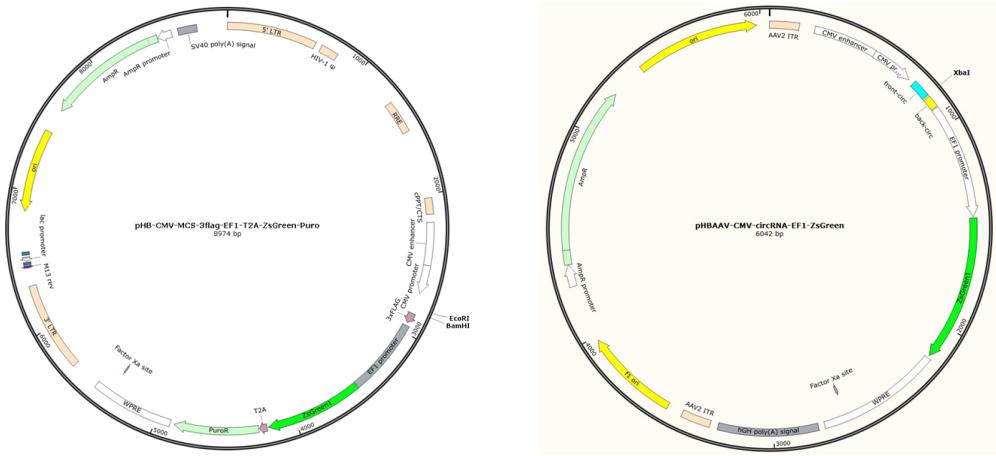
Table S4 Maps of vectors used in this study



Firefly_Luciferase-Renilla_Luciferase vector



HBLV-CMV-Circ-MCS-EF1-zsgreen-t2a-puro vector



pHB-CMV-MCS-3flag-EF1-t2a-puro

pHBAAV-CMV-mcrRNA-EF1-ZsGreen vector

Table S5 RNAseq Analyses

id	Circbase id	log2FC(OA/Control)	pval	Fdr	regulate	significant
17:27765535 277665881	hsa_circ_0106434	-7.2095933	2.94E-10	1.78E-06	down	yes
1:102979382 102989571	hsa_circ_0110251	-7.0146886	9.33E-11	1.13E-06	down	yes
19:6702127 6702579	hsa_circ_0002130	-5.7256104	3.17E-08	0.000127 66	down	yes
8:17558295 17562110	hsa_circ_0005805	-5.830035	7.60E-07	0.001834 71	down	yes
5:59180595 59215968	hsa_circ_0072568	-5.2760784	1.18E-07	0.000355 93	down	yes
5:65170617 65215487	hsa_circ_0008667	-3.9400185	6.71E-05	0.061309 68	down	yes
4:88475841 88479507	hsa_circ_0070421	-4.5431287	4.12E-06	0.007102 84	down	yes
7:90747681 90790652	hsa_circ_0001722	-4.4750758	3.16E-06	0.006361 29	down	yes
4:150467673 150491035	hsa_circ_0006867	-4.2555494	1.27E-05	0.015102 05	down	yes
17:27778690 27779056	None	-4.2259783	1.16E-05	0.015102 05	down	yes
2:223912253 223918010	hsa_circ_0008365	-4.0531467	0.000134 32	0.087850 3	down	yes
20:54157169 54171670	hsa_circ_0060927	-4.2436025	8.27E-05	0.061309 68	down	yes
9:6880012 6893232	hsa_circ_0001839	4.487324826	0.333264 016	1	down	yes
5:65170617 65215487	hsa_circ_0008667	-3.9400185	6.71E-05	0.061309 68	down	yes

1:46077515 46080750	hsa_circ_0003161	-3.9505609	8.63E-05	0.061309 68	down	yes
8:17543318 17543715	hsa_circ_0083429	-4.576294	1.42E-05	0.015102 05	down	yes
15:83923904 83970637	hsa_circ_0104766	-3.7749837	0.000138 2	0.087850 3	down	yes
19:6697344 6697794	None	-3.5244413	0.000307 33	0.176758 27	down	yes
20:45815541 45815913	hsa_circ_0060551	-3.6184204	0.000380 76	0.199947 9	down	yes
1:27668226 27669346	hsa_circ_0002470	-3.85047	0.001064 67	0.389668 52	down	yes
7:84110470 84134951	hsa_circ_0134888	-3.4826538	0.002962 86	0.732848 44	down	yes
12:100204940 10020574 4	hsa_circ_0027857	-3.4815857	0.000969 6	0.373593 12	down	yes
19:48845370 48852326	hsa_circ_0051782	-3.44724	0.000596 45	0.270529 9	down	yes
21:41432050 41437152	None	-3.5076824	0.000844 07	0.364096 36	down	yes
4:102304317 102315830	hsa_circ_0002782	-3.8014826	0.003360 46	0.738059 44	down	yes
1:93305388 93325895	None	-3.4219483	0.000989 81	0.373593 12	down	yes
5:72861808 72865729	hsa_circ_0002692	0.599662703	0.482010 652	1	down	yes
12:100204940 10020575 7	hsa_circ_0096948	-3.3785056	0.001597 88	0.477396 29	down	yes
3:29868858 29899755	hsa_circ_0064644	1.782047111	0.157236 939	1	down	yes
5:138424075 138425582	hsa_circ_0004763	-3.355593	0.001421 97	0.451961 5	down	yes
19:41248514 41248820	hsa_circ_0002882	-1.376539581	0.205755 968	1	down	yes
13:75713182 75727098	hsa_circ_0030441	4.06243391	4.95E-06	0.007480 29	up	yes
10:115120185 11512953 5	hsa_circ_0020093	3.52956202	1.50E-05	0.015102 05	up	yes
8:41661430 41661941	hsa_circ_0001792	3.31749642	7.68E-05	0.061309 68	up	yes
1:247159006 247159813	hsa_circ_0017348	5.42175065	8.25E-05	0.061309 68	up	yes
1:246591512 246591941	hsa_circ_0017310	3.25903817	0.000436 72	0.210987 67	up	yes

11:78119182 78121174	hsa_circ_0008019	3.15142761	0.003156 68	0.738059 44	up	yes
11:63895159 63895633	hsa_circ_0022614	2.51804111	0.002213 61	0.581217 93	up	yes
2:72674532 72733118	None	4.56218595	0.000986 11	0.373593 12	up	yes
8:30474779 30479377	hsa_circ_0007409	-0.955555813	0.652030 171	1	up	yes
9:84702159 84710791	hsa_circ_0139142	2.86567306	0.003360 93	0.738059 44	up	yes
7:157366502 157367483	hsa_circ_0002451	2.1840816	0.012959 83	1	up	yes
10:32805015 32824604	hsa_circ_0093573	2.47034385	0.007794 52	1	up	yes
9:120536372 120539164	None	2.2484411	0.009920 09	1	up	yes
9:87698656 87700237	hsa_circ_0087421	-2.095067331	0.068295 109	1	up	yes
20:38040435 38066256	hsa_circ_0008006	2.91024396	0.009293 94	1	up	yes
11:92352096 92355404	hsa_circ_0000348	4.16268873	0.003033 82	0.732848 44	up	yes
21:36338779 36344707	None	4.11644082	0.001996 54	0.548049 15	up	yes
20:25496663 25498346	hsa_circ_0004812	2.04618052	0.003007 82	0.732848 44	up	yes
16:58560212 58560362	None	3.91773309	0.006797 91	1	up	yes
5:32724698 32739030	hsa_circ_0072107	2.31448473	0.016287 71	1	up	yes
6:168543625 168608239	hsa_circ_0078696	4.56983277	0.001708 75	0.479961 2	up	yes
2:241030284 241030571	hsa_circ_0004526	2.3421537	0.009547 36	1	up	yes
20:17956933 17961326	hsa_circ_0009173	-2.010872082	0.096621 893	1	up	yes
3:111913319 111920419	hsa_circ_0001326	-1.042522917	0.326337 728	1	up	yes
1:23071225 23072197	hsa_circ_0010835	3.52357915	0.001114 72	0.395989 24	up	yes
20:64022757 64026058	hsa_circ_0115583	2.15899955	0.015696 04	1	up	yes
5:72877228 72883232	None	2.0345252	0.010784 39	1	up	yes

4:105691976 105695668	hsa_circ_0007668	2.60053265	0.022674 81	1	up	yes
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