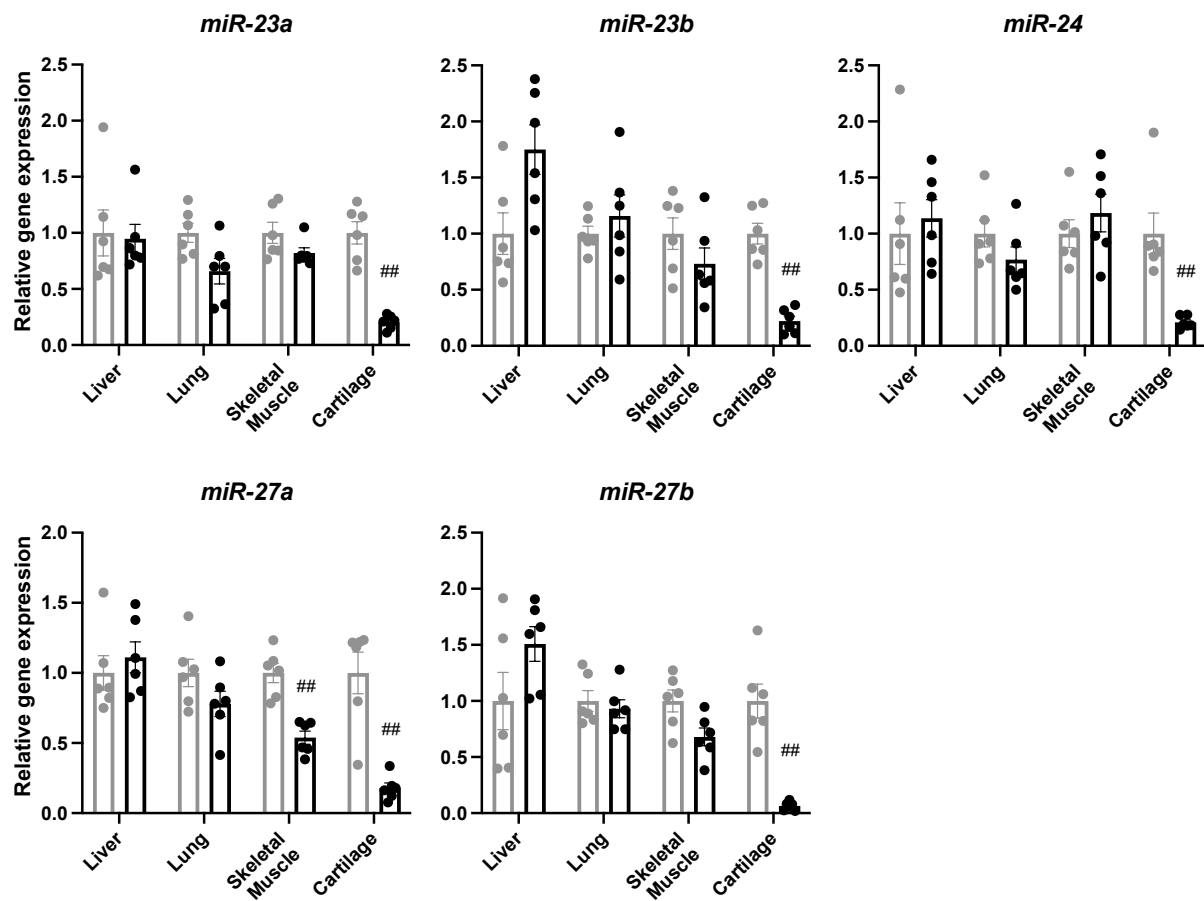


Supplementary Material

A ● Cont ● Cart-miR-23 KO



B

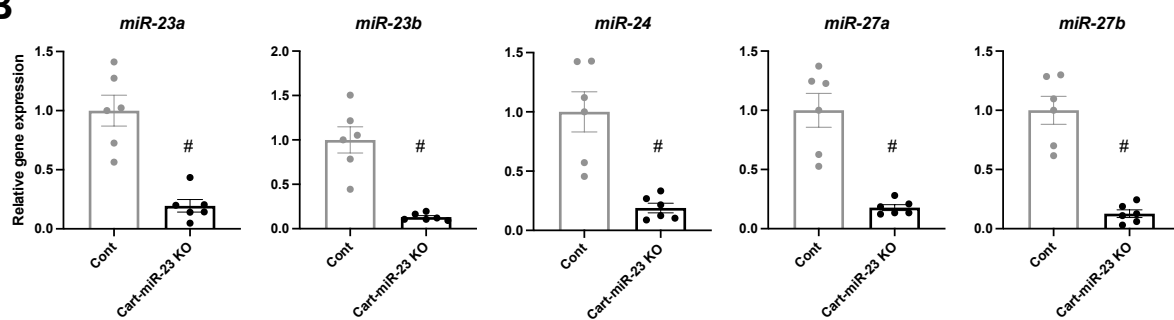
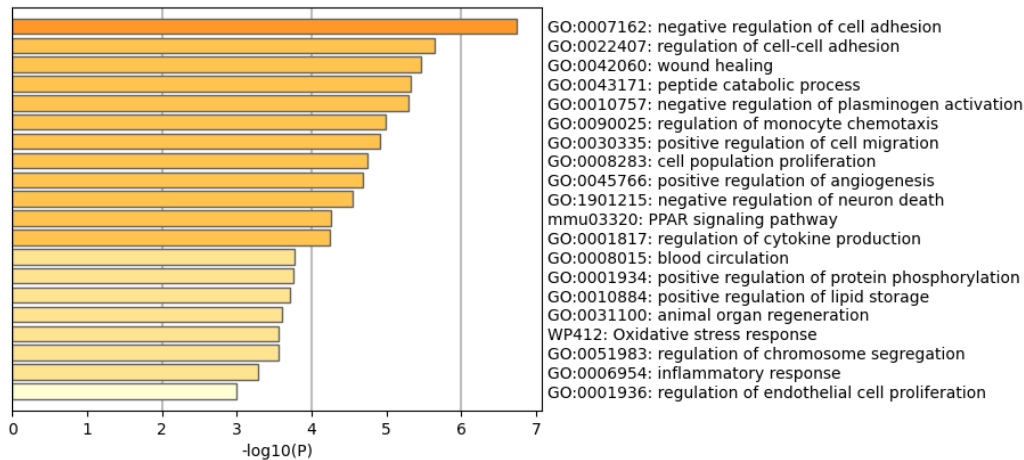


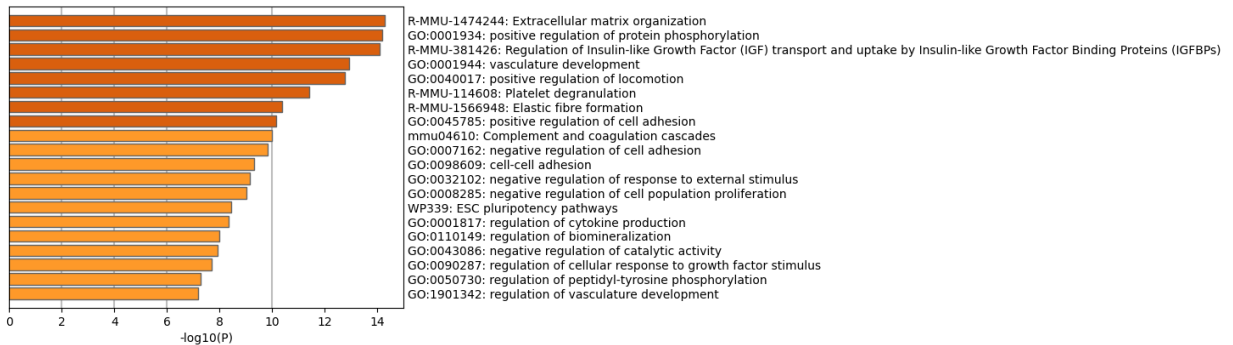
Fig. S1 Validation of cartilage-specific miR-23a/b clusters deficiency in Cart-miR-23clus KO mice.

A) Real-time analysis showed the expression of miR-23a/b clusters in liver, lung, skeletal muscle, and articular cartilage from Control and Cart-miR-23clus KO mice at 4 weeks of age. **B)** The expression of miR-23a/b clusters in articular chondrocytes from Control and Cart-miR-23clus KO mice. All data are represented as mean $\pm$ SEM. Comparisons of expression level were performed by Mann-Whitney tests in each tissue. Holm-Sidak corrections were applied to correct for multiple comparisons. [#]P<0.05, ^{##}P<0.01. n=6 per group.

A Proteins and transcripts: Cart-miR-23a/b KO >2 folds



B Proteins: Cart-miR-23a/b KO >2 folds



C Transcripts: Cart-miR-23a/b KO >2 folds

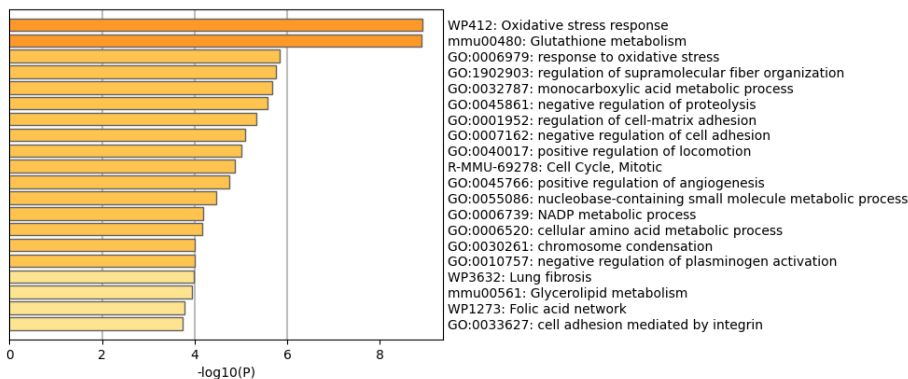


Fig. S2 Top 20 gene ontology enrichment analysis terms for the genes which **A)** proteins and transcripts are both have been upregulated (>2 folds) **B)** proteins have been upregulated (>2 folds) **C)** transcripts have been upregulated (>2 folds) in articular chondrocytes from Cart-miR-23a/b KO mouse compared to Control mouse.

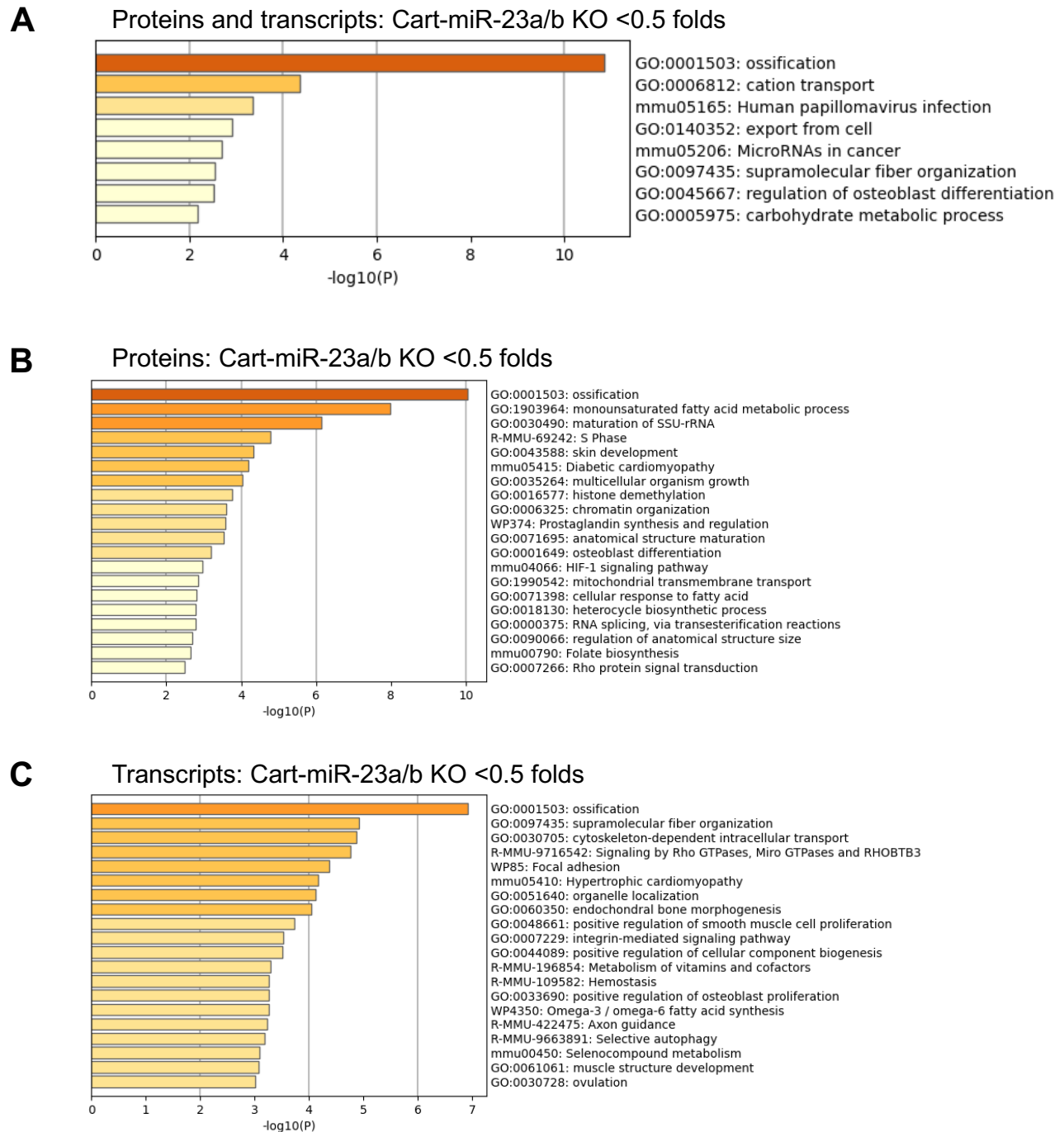


Fig. S3 Top gene ontology enrichment analysis terms for the genes which **A)** proteins and transcripts are both have been downregulated (<0.5 folds) **B)** proteins have been downregulated (<0.5 folds) **C)** transcripts have been downregulated (<0.5 folds) in articular chondrocytes from Cart-miR-23a/b KO mouse compared to Control mouse.

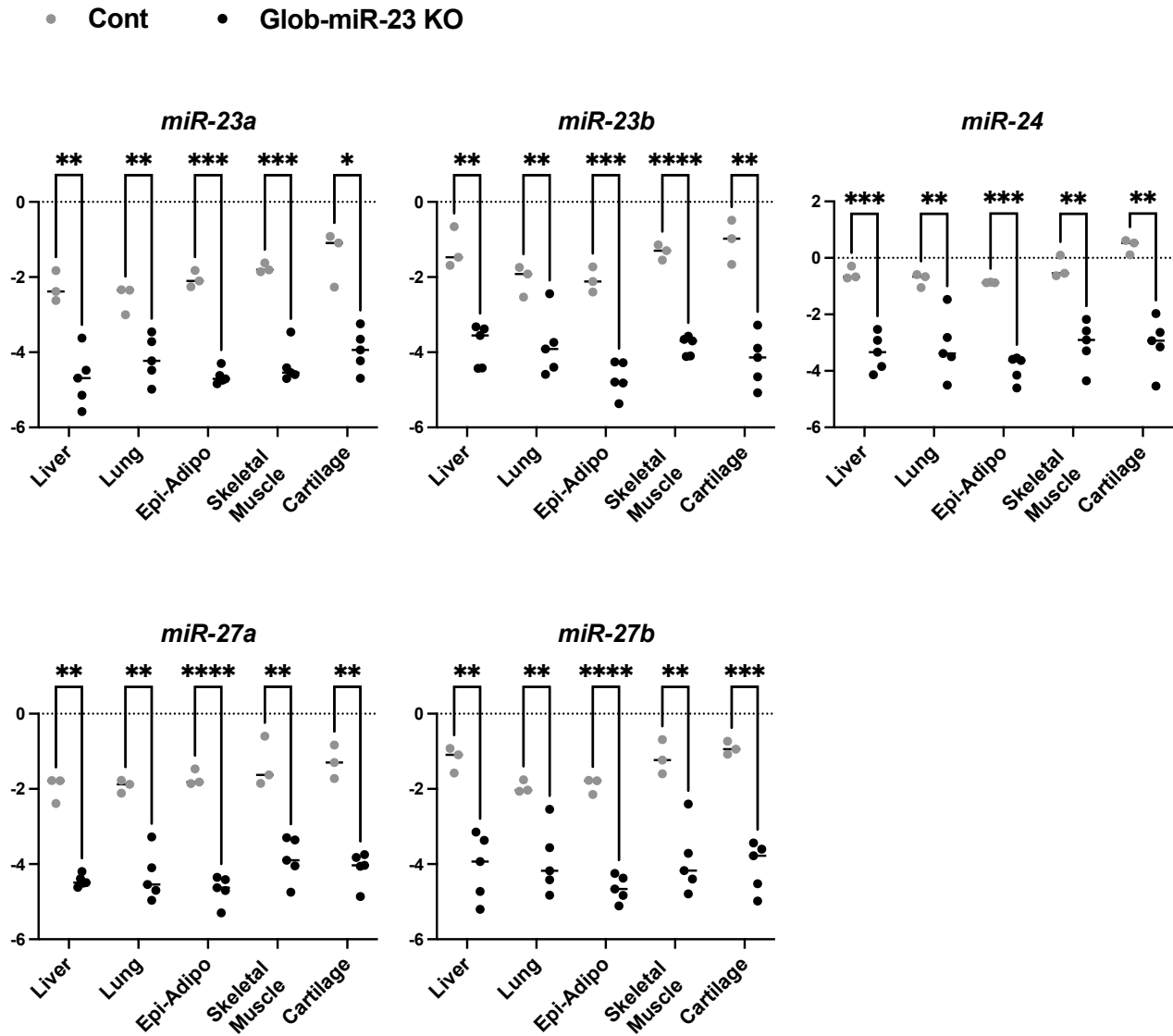


Fig. S4 Validation of miR-23a/b clusters deficiency in various tissues of Glob-miR-23clus KO mice.

Real-time PCR analysis showed the expression of miR-23a/b clusters in liver, lung, epididymal adipose tissue, skeletal muscle, and articular cartilage from Control (n=3) and Glob-miR-23clus KO mice (n=5) at 4 weeks of age. All data are represented as mean $\pm$ SEM. Comparisons of expression level were performed by multiple welch test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Table. S1

	Primer sequence
	F: GCTCCAACCTTCCTACGGATCGATGC
<i>miR-23a cluster*</i>	R-1: CCTGCCTCTACCTCTGGAGTCTAGGA
	R-2: GTGGTGCAGCTGGTATTCCCAAATC
	F: TGCCCCCTGAGTGAGCAAATCC
<i>miR-23b cluster*</i>	R-1: TGGCTTGCCTGTGACCAAGCAT
	R-2: GGTGTCCTTCATTGAATGACTGCC
	F: GCATTACCGGTCGATGCAACGAGTGATGAG
<i>Cre recombinase</i>	R: GAGTGAACGAACCTGGTCGAAATCAGTGCG

*For the genotyping of the miR-23a or miR-23b cluster, primer-F and primer-R-1 were used for the Cart:miR-23clusKO mice and the combinations of primer-F with primer-R-1 and primer-F with primer-R-2 were used for the Glob-miR-23clusKO mice.

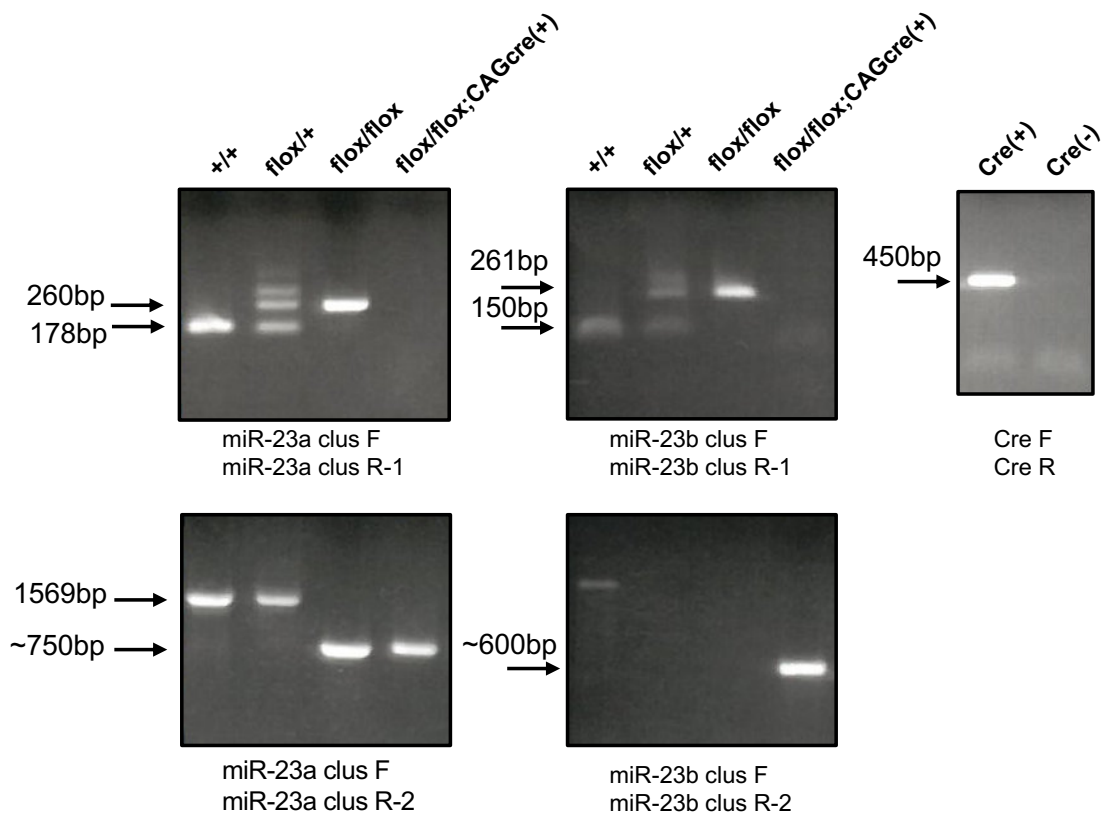


Table. S2

Gene Name	Assay ID
<i>Col2a1</i>	Mm01309565m1
<i>Acan</i>	Mm00545807m1
<i>Mmp13</i>	Mm00439491m1
<i>Adamts5</i>	Mm00478620m1
<i>Il6</i>	Mm00446190m1
<i>Runx2</i>	Mm00446190m1
<i>Gapdh</i>	Mm99999915g1
<i>miR-23a-3p</i>	RT/TM000399
<i>miR-23b-3p</i>	RT/TM000400
<i>miR-24-3p</i>	RT/TM000402
<i>miR-27a-3p</i>	RT/TM000408
<i>miR-27b-3p</i>	RT/TM000409
<i>miR-140-5p</i>	RT/TM000462
<i>miR-455-3p</i>	RT/TM002244
<i>miR-455-5p</i>	RT/TM001280
<i>U6 snRNA</i>	RT/TM001973

Real time PCR was performed with the TaqMan Gene Expression Assay probes

Table. S3

Antibody	Catalog Number	Manufacturer
COX2 (mouse, monoclonal)	sc-376861	Santa Cruz Biotechnology
iNOS (rabbit, polyclonal)	ab15323	Abcam
MMP13 (mouse, monoclonal)	MA5-14238	Thermo Fisher Scientific
SMAD3 (rabbit, monoclonal)	ab40854	Abcam
GAPDH (mouse, monoclonal)	015-25473	FUJIFILM Wako

Table. S4

microRNA	Chondrocyte		Cho-EVs	
	Rank	Normalized Counts	Rank	Normalized Counts
hsa-let-7a-5p	1	47048.64	5	12162.12
hsa-miR-125b-5p	2	35194.21	2	35106.55
hsa-miR-4454	3	26155.59	1	49397.53
hsa-let-7b-5p	4	21205.61	12	4336.57
hsa-miR-21-5p	5	20252.24	3	17950.79
hsa-miR-23a-3p	6	13823.68	4	15808.79
hsa-miR-29b-3p	7	13301.61	14	3962.05
hsa-let-7g-5p	8	7865.05	15	3193.30
hsa-miR-100-5p	9	7533.65	8	5939.79
hsa-miR-99a-5p	10	5992.56	12	4336.57
hsa-miR-720	11	5370.90	6	7096.21
hsa-miR-221-3p	12	5224.52	24	2293.13
hsa-miR-199a-3p+hsa-miR-199b-3p	13	4641.19	9	5210.46
hsa-miR-222-3p	14	4160.89	11	4737.38
hsa-miR-27b-3p	15	4019.85	18	2904.19
hsa-miR-15b-5p	16	3885.41	20	2509.96
hsa-miR-23b-3p	17	3869.70	16	3134.16
hsa-miR-15a-5p	18	3536.10	22	2345.69
hsa-miR-22-3p	19	3050.78	21	2385.12
hsa-miR-125a-5p	20	2810.47	32	1373.25
hsa-miR-191-5p	21	2556.97	19	2825.34
hsa-miR-374a-5p	22	2547.24	26	1951.46
hsa-miR-26a-5p	23	2530.90	42	762.19
hsa-miR-148a-3p	24	2329.55	23	2339.12
hsa-miR-199a-5p	25	2218.98	27	1866.04
hsa-miR-376a-3p	26	1856.48	17	3055.31
hsa-miR-29a-3p	27	1789.57	29	1662.35
hsa-miR-16-5p	28	1717.63	28	1714.92
hsa-miR-337-5p	29	1683.71	31	1438.95
hsa-miR-140-5p	30	1445.29	36	1136.71
hsa-let-7i-5p	31	1313.67	46	670.20
hsa-let-7c	32	1133.99	58	473.08
hsa-miR-34a-5p	33	1117.03	51	525.65
hsa-miR-376c	34	1084.04	33	1327.25
hsa-miR-361-5p	35	1059.54	40	834.46
hsa-miR-199b-5p	36	1042.58	52	519.07
hsa-let-7e-5p	37	1042.27	70	341.67
hsa-miR-377-3p	38	882.69	30	1550.65
hsa-let-7f-5p	39	838.71	94	243.11
hsa-miR-130a-3p	40	809.50	37	1005.30
hsa-miR-382-5p	41	718.40	56	479.65
hsa-miR-155-5p	42	712.12	778	6.57
hsa-miR-106a-5p+hsa-miR-17-5p	43	681.96	71	335.10
hsa-miR-127-3p	44	626.99	43	729.33
hsa-miR-24-3p	45	626.36	47	663.63
...	...	...	...	...
hsa-miR-27a-3p	98	167.74	126	164.26

Cho-EVs: Chondrocyte-derived extracellular vehicles

Table. S5

Gene name	Mass Spectrometry (Identified Peptide Count)			RNA-seq (TPM)		
	Cont	Cart-miR-23 KO	Fold Change	Cont	Cart-miR-23 KO	Fold Change
Hmox1	447277000	1479628000	3.31	53.07	153.81	2.90
Serpine2	394245800	792757500	2.01	370.31	954.20	2.58
Serpinb1a	235063600	512885100	2.18	17.47	45.60	2.61
Gpnmb	184437900	429767000	2.33	125.39	343.42	2.74
Maoa	170023800	492563200	2.90	4.59	29.34	6.39
Plin4	148102900	420612600	2.84	12.81	45.97	3.59
Tpm2	136333500	339893200	2.49	61.33	124.83	2.04
Tagln	125071800	769259900	6.15	1.80	24.45	13.58
Ass1	73640130	196621700	2.67	2.82	12.43	4.40
Erap1	70216530	141026100	2.01	2.88	7.47	2.59
Aldh1a1	68154590	239258900	3.51	99.69	277.79	2.79
Fabp4	66201750	245077400	3.70	60.89	262.29	4.31
Mttp	48886180	106763900	2.18	2.42	5.58	2.31
Anpep	46278680	154543800	3.34	3.03	8.88	2.94
Lox	45187450	346526700	7.67	244.49	981.62	4.01
Emb	41188020	82608200	2.01	402.69	977.26	2.43
Icam1	26620030	474050000	17.81	11.73	79.41	6.77
Pdlim4	25021980	76676650	3.06	12.15	41.01	3.38
C3	22629220	49426810	2.18	0.56	3.28	5.87
Enpep	22315770	59819150	2.68	0.46	2.27	4.95
Gfer	21487370	44308620	2.06	57.62	142.16	2.47
Serpine1	20536510	67098690	3.27	40.05	85.40	2.13
Synn	20516480	42151550	2.05	1.35	6.74	5.00
Il1rn	20042700	60765570	3.03	8.90	40.04	4.50
Plpp3	15472890	66761900	4.31	21.82	52.04	2.39
P4ha3	14752360	43063280	2.92	38.11	81.68	2.14
Rufy3	10616410	29066600	2.74	4.39	8.82	2.01
Rrm2	8757544	37681400	4.30	13.09	37.48	2.86
Lgals9	7630653	17574580	2.30	4.41	11.09	2.52
Top2a	5817056	12842100	2.21	8.98	19.58	2.18
Ttyh3	5485562	35086060	6.40	10.46	21.88	2.09
Timp1	5446187	13657780	2.51	621.13	1797.41	2.89
Mt2	5337880	11282930	2.11	1470.42	7069.73	4.81
Sorl1	5273122	15998130	3.03	5.00	10.23	2.04
Vcam1	5265081	33679840	6.40	7.73	24.00	3.11
Xdh	5139618	17784530	3.46	0.54	4.17	7.71
Atp1b1	4760890	20713530	4.35	0.37	6.54	17.62
Cyp1b1	4467622	24652690	5.52	32.66	109.35	3.35
Tinagl1	4294986	8662477	2.02	15.15	49.53	3.27
Nid1	4017366	9807333	2.44	2.83	6.57	2.32
Eng	3920848	19044830	4.86	3.49	7.33	2.10
Cxcl12	3743041	82994030	22.17	389.90	2831.06	7.26
Clu	3581137	24472920	6.83	122.16	321.40	2.63
Prss23	3410412	23391200	6.86	33.51	71.82	2.14
Ppif	3340422	16465260	4.93	3.68	13.91	3.77
Myoc	3077883	19585150	6.36	17.54	86.38	4.93
Hs6st2	3016240	28088240	9.31	42.12	119.35	2.83
Tjp2	2679283	6709965	2.50	1.44	4.74	3.30

(Continue...)

Table. S5

Gene name	Mass Spectrometry (Identified Peptide Count)			RNA-seq (TPM)		
	Cont	Cart-miR-23 KO	Fold Change	Cont	Cart-miR-23 KO	Fold Change
Crlf1	2508715	18624450	7.42	8.73	43.59	4.99
Anln	2236792	5140888	2.30	2.29	4.81	2.10
Lpl	2222832	15952490	7.18	78.62	158.78	2.02
Ccn3	2150312	14033260	6.53	9.01	56.57	6.28
Ephb6	2130838	6526919	3.06	6.36	17.09	2.69
Tdrp	2066204	4313554	2.09	0.78	3.42	4.40
Pm20d1	1981226	7933452	4.00	0.28	0.76	2.75
Il3ra	1862561	4298940	2.31	1.19	2.61	2.20
Mgst1	1576546	4471263	2.84	4.10	10.84	2.64
Fabp7	1540417	4058362	2.63	1.19	3.93	3.30
Gba2	1414731	4404064	3.11	1.08	2.68	2.48
Nes	1177270	10239480	8.70	0.73	2.41	3.30
Postn	1162173	4845063	4.17	3.07	8.10	2.64
Lpin1	1133115	2668860	2.36	9.38	22.18	2.37
Ccdc126	1089689	2845145	2.61	2.44	5.38	2.20
Me3	1084754	2434075	2.24	0.65	1.66	2.57
Prg4	1061632	3226544	3.04	65.32	140.99	2.16
Dlk1	990338	9978799	10.08	25.79	347.08	13.46
Ggt5	948152	5120349	5.40	2.33	6.60	2.83
Kif1a	939535	6737272	7.17	0.93	2.18	2.34
Mustn1	868357	20732120	23.88	3.87	34.11	8.81
Gbp2	867185	5713468	6.59	0.38	2.51	6.61
Mki67	846723	1722916	2.03	0.95	2.09	2.20
Hmmr	796488	2638066	3.31	2.46	8.66	3.52
Spc24	726270	1658242	2.28	4.90	12.34	2.52
Ltbp2	687049	5748239	8.37	0.85	11.37	13.40
Zwint	639869	4229712	6.61	30.27	156.87	5.18
Nek6	585576	2256154	3.85	4.72	12.84	2.72
Parvb	440050	1648774	3.75	0.76	1.67	2.20
Coq5	412104	1030297	2.50	6.11	14.49	2.37
Grem1	397151	3486111	8.78	1.85	6.35	3.44
Tpx2	341218	2428677	7.12	5.50	14.22	2.59
Fhit	336059	736413	2.19	3.34	7.35	2.20
Ube2c	327086	804840	2.46	29.70	82.83	2.79
Sneg	282876	1942897	6.87	1.12	13.61	12.11
Rab3b	275670	989514	3.59	0.58	1.58	2.75
Kif20a	229068	1139925	4.98	1.83	10.05	5.51
Wdfy1	224811	10656230	47.40	2.10	19.22	9.14
Pyroxd2	203128	1065998	5.25	1.53	4.37	2.86
Igfbp2	168292	1435285	8.53	5.86	17.74	3.03
Tmod1	139475	1196372	8.58	0.91	3.00	3.30
Prc1	125860	636219	5.05	12.02	32.39	2.69
ErbB2	83480	183644	2.20	1.34	3.15	2.36
Plau	65157	1603249	24.61	0.82	2.25	2.75
Ttyh2	25759	181938	7.06	7.69	15.72	2.04
Msr1	24735	60913	2.46	6.72	17.02	2.53

Table. S6

No.	Gene Symbol	Ensembl ID	Predicted target* of			
			miR-23-3p	miR-27-3p	miR-24-3p	miR-140-5p
1	RAP1B	ENST00000250559.9	✓	✓	✓	✓
2	NCOA1	ENST00000405141.1	✓	✓	✓	✓
3	STRN	ENST00000263918.4	✓	✓	✓	✓
4	DTNA	ENST00000283365.9	✓	✓	✓	✓
5	CELF2	ENST00000379261.4	✓	✓	✓	✓
6	NLK	ENST00000407008.3	✓	✓	✓	✓
7	CTCF	ENST00000264010.4	✓	✓		✓
8	YES1	ENST00000577961.1	✓	✓		✓
9	RAP2B	ENST00000323534.2	✓	✓		✓
10	ABCA1	ENST00000374736.3	✓	✓		✓
11	PPP1R12A	ENST00000261207.5	✓	✓		✓
12	PAX9	ENST00000361487.6	✓	✓		✓
13	WDR37	ENST00000358220.1	✓	✓		✓
14	ADAMTS5	ENST00000284987.5	✓	✓		✓
15	FOXP2	ENST00000408937.3	✓	✓		✓
16	LPHN2	ENST00000370715.1	✓	✓		✓
17	PARD6B	ENST00000371610.2	✓	✓		✓
18	BAZ2B	ENST00000392782.1	✓	✓		✓
19	BMP2K	ENST00000335016.5	✓	✓		✓
20	MFHAS1	ENST00000276282.6	✓	✓		✓
21	ZHX1	ENST00000395571.3	✓	✓		✓
22	SATB2	ENST00000417098.1	✓	✓		✓
23	ANKFY1	ENST00000341657.4	✓	✓		✓
24	JHDM1D	ENST00000397560.2	✓	✓		✓
25	CELF1	ENST00000395290.2	✓	✓		✓
26	TEAD1	ENST00000361905.4	✓	✓		✓
27	PRR14L	ENST00000434485.1	✓	✓		✓
28	WEE1	ENST00000299613.6	✓	✓		✓
29	STOX2	ENST00000308497.4	✓	✓		✓
30	MARK1	ENST00000366918.4	✓	✓		✓
31	TRIM44	ENST00000299413.5	✓	✓		✓
32	APBP2	ENST00000083182.3	✓	✓		✓
33	VCPIP1	ENST00000310421.4	✓		✓	✓
34	NDST1	ENST00000261797.6	✓		✓	✓
35	B3GNT1	ENST00000311181.4	✓		✓	✓
36	YOD1	ENST00000315927.4	✓		✓	✓
37	SNX27	ENST00000368843.3	✓		✓	✓
38	SYS1	ENST00000243918.5	✓		✓	✓
39	LHFPL2	ENST00000515007.2	✓		✓	✓
40	FAM175B	ENST00000298492.5	✓		✓	✓
41	PURB	ENST00000395699.2	✓		✓	✓
42	FAM46A	ENST00000369754.3	✓		✓	✓
43	ANKRD52	ENST00000267116.7	✓		✓	✓
44	CCDC85C	ENST00000380243.4		✓	✓	✓
45	HEG1	ENST00000311127.4		✓	✓	✓
46	TSC22D2	ENST00000361875.3		✓	✓	✓
47	PDGFRA	ENST00000257290.5		✓	✓	✓
48	ROR1	ENST00000371079.1		✓	✓	✓
49	ZBTB20	ENST00000462705.1		✓	✓	✓
50	MED13	ENST00000397786.2		✓	✓	✓
51	RAD54L2	ENST00000409535.2		✓	✓	✓
52	RC3H1	ENST00000367696.2		✓	✓	✓
53	ERC2	ENST00000288221.6		✓	✓	✓
54	NFAT5	ENST00000354436.2		✓	✓	✓
55	AP2B1	ENST00000262325.7		✓	✓	✓

*Source: TargetScan 8.0 https://www.targetscan.org/vert_80/