

Supplementary Figure 1

A.

>ROCR transcript variant 1 (LOC102723505, ENST00000430908, AC005152.3)

AGCCTTTTAAGACACATGTTTTGGAATACAGTCAAATGCGTGCTGCTGTATGACCAGCCTATTTGAACTA
 CTTAAGCATCAACTAATTAATCTGCAAGTGAAGAATACTTCCAGCATTACTCTATAATTAGACAATCTCAG
GAGGAAACAAGAGGATTTCAAAGGGGAGTCAGATGGTTTCGCAGACCGCTACATCATTTATTTTTTCATC
 ACTCAGATTATGCCTCCAAATCCTACAGAAGTATAACAACCATAGAACAGACAAAACCAACAGCATCAA
 CAACAGAGAAACTCAGTCCAGGAGGATATGAAACCCTTTGTAATAAAGAAAGCAACATCCATGAA

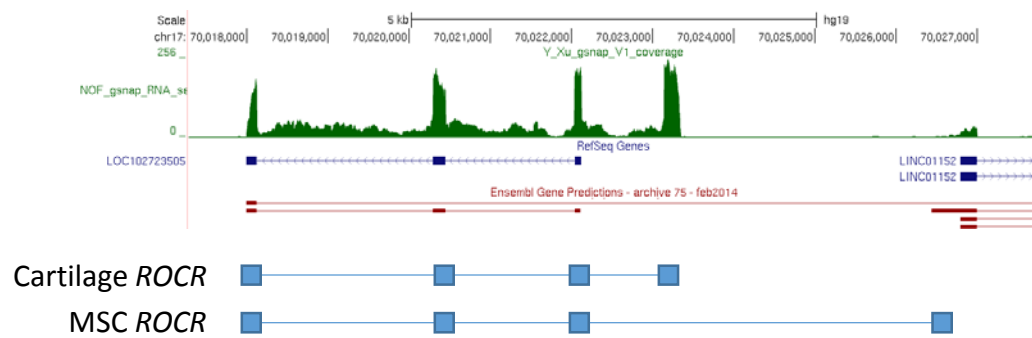
>ROCR transcript variant 2

AGTCCTTGAGGGACAAGAAAGTGGAGCCCAGGACTTTCCAGTCCCCACCTAGTGATCAACGTCTCACCC
 ACGGTCAGGGGAGCAACTGGTGGTTCTACTTTTTGAATCTGAAGGCAAAGGCAAGCTTTTTTTAGGG
 GTCCTGCTGTTGGCAGAGAAAGCTCCCAAGAGTTCTGCAGAGAAGAAGGATATAGACTCCAAAATTTTA
 CTTCCAGGGACCATTTTCAGTAGAGCCTTTTAAGACACATGTTTTGGAATACAGTCAAATGCGTGCTGCTG
TATGACCAGCCTATTTGAACTACTTAAGCATCAACTAATTAATCTGCAAGTGAAGAATACTTCCAGCATT
 CTCTATAATTAGACAATCTCAGGAGGAAACAAGAGGATTTCAAAGGGGAGTCAGATGGTTTCGCAGACC
 GCTACATCATTTATTTTTTCATCACTCAGATTATGCCTCCAAATCCTACAGAAGTATAACAACCATAGAAC
 AGACAAAACCAACAGCATCAACAACAGAGAAACTCAGTCCAGGAGGATATGAAACCCTTTGTAATAAA
 GAAAGCAACATCCATGA

>ROCR transcript variant 3

CAGCGGTTGATTGCGAGGAGCAGCGGAAGGAAGGATGTATCAAACCGAAAGAAGAAAGAACCCCTGC
 CAGTCGCCCCATGACATCCGACTCTGAACGGCAGCGATCTAGAGGCTGCGGCGTGTTCAGATTAACA
 GCAGCGAGGGCAGGAGGCAAATCAGATGCCGAGGAGCTGACTGTTTTCTTTAGCCCTGAGAGCTGAC
 TGTAGGCACAGGTTGCATATCAAAGGCAACGGGAATCAGTCTGTGGCAATATTACATCAGGGACCATTT
 CAGTAGAGCCTTTTAAGACACATGTTTTGGAATACAGTCAAATGCGTGCTGCTGTATGACCAGCCTATTT
GAACTACTTAAGCATCAACTAATTAATCTGCAAGTGAAGAATACTTCCAGCATTACTCTATAATTAGACA
TCTCAGGAGGAAACAAGAGGATTTCAAAGGGGAGTCAGATGGTTTCGCAGACCGCTACATCATTTATTT
 TTTCACTCAGATTATGCCTCCAAATCCTACAGAAGTATAACAACCATAGAACAGACAAAACCAACA
 GCATCAACAACAGAGAAACTCAGTCCAGGAGGATATGAAACCCTTTGTAATAAAGAAAGCAACATCCAT
 GA

B.

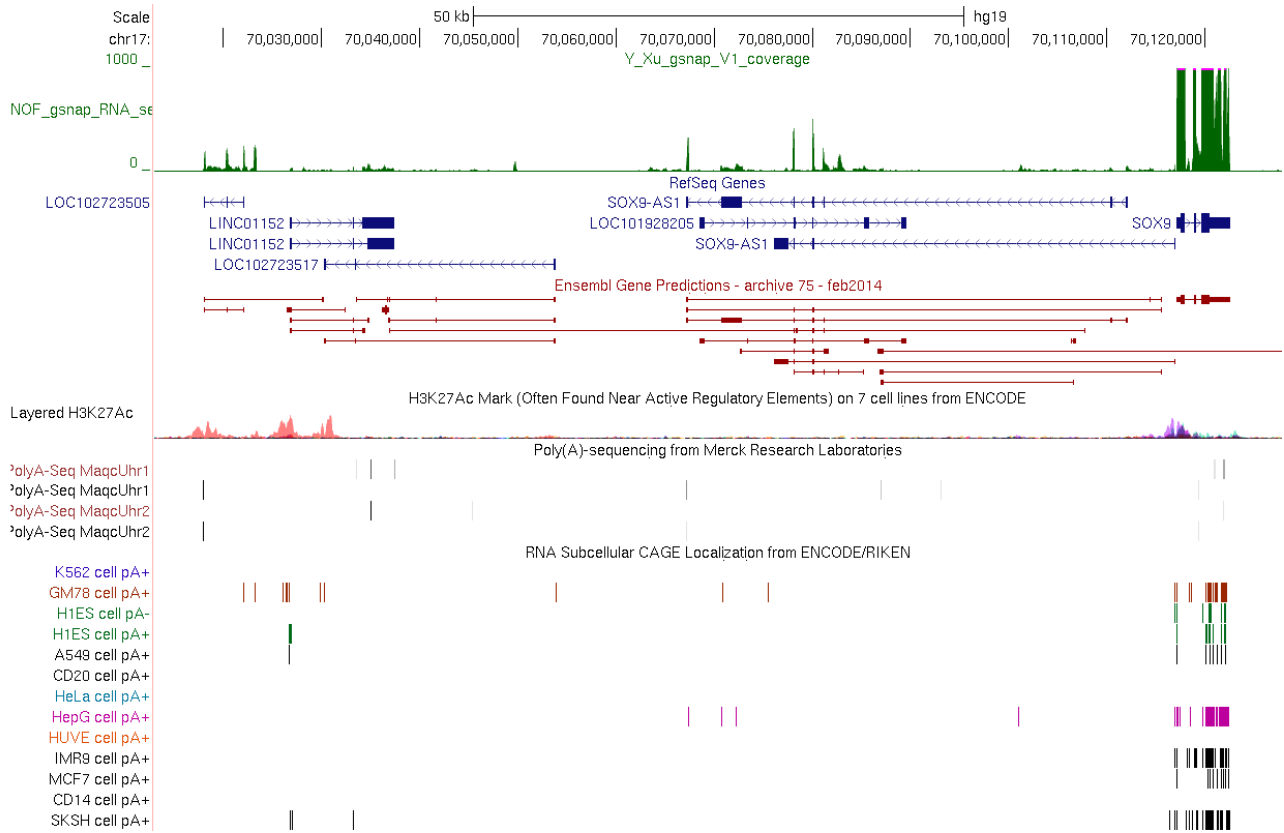


Supplementary Figure 1.

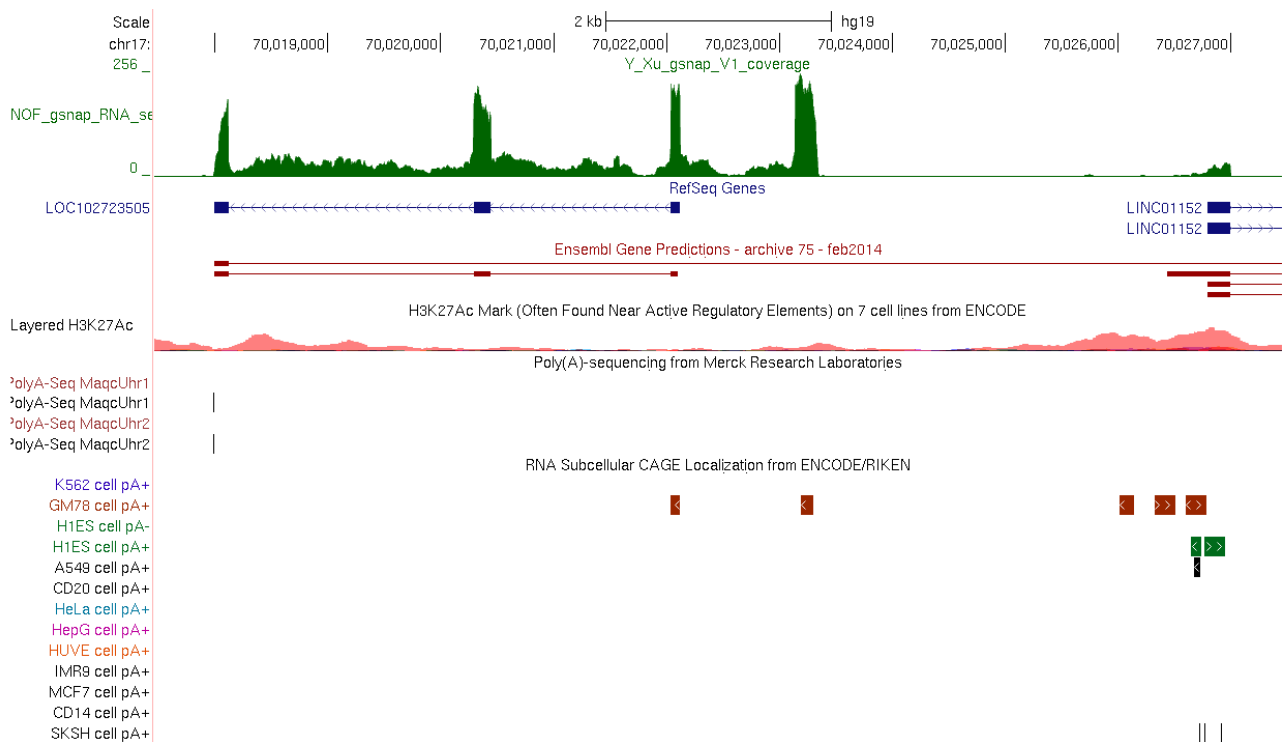
A. Transcript sequences of *ROCR* variants identified by RNA-Seq and RACE. Exons are delineated by the change between black and grey nucleotides. Primer locations are indicated by underlining (blue for exon1-2 assay, red for exon2-3 assay). B. Genome browser schematic of the two 4 exon transcript variants of *ROCR* with alternative first exons as indicated by 5'RACE from cartilage and MSC RNA.

Supplementary Figure 2

A.



B.



Supplementary Figure 2.

UCSC genome browser schematic of cartilage RNA-Seq reads aligned to the human genome with evidence of transcript start and end sites using CAGE (cap analysis gene expression) and PolyA-Seq data. A. *SOX9* and upstream locus. B. *ROCR* locus.

Supplementary Figure 3

A.

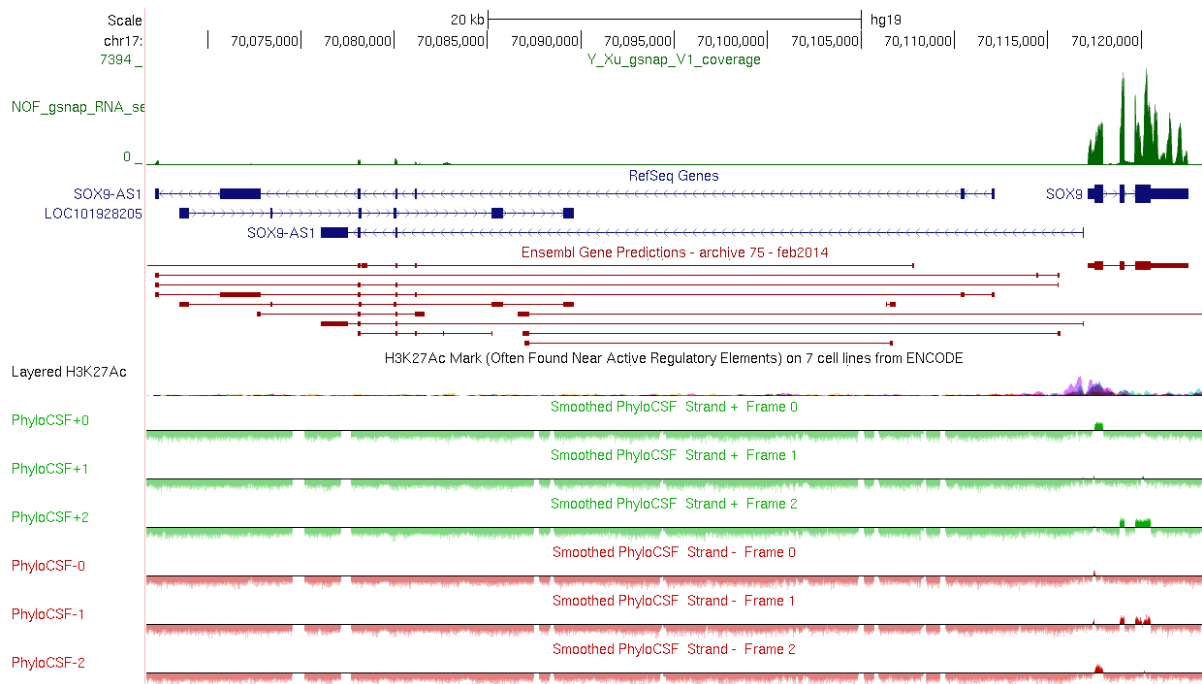
	ORFfinder		
	Start	Stop	Length (nt aa)
ENST0000430908	50	127	78 25
ROCR_HAC	279	356	78 25
ROCR_MSC	79	177	99 32
	329	406	78 25
SOX9-AS1	483	776	294 97
	2739	2930	192 63
	837	1001	165 54
	1511	1672	162 53
	125	280	156 51
	1393	1521	129 42
	76	201	126 41
	2295	2408	114 37
	2100	2204	105 34
	2137	2238	102 33
	256	351	96 31
	1908	1988	81 26
	589	666	78 25
LINC01152	2605	2757	153 50
	2571	2711	141 46
	1048	1179	132 43
	1182	1304	123 40
	1967	2086	120 39
	320	430	111 36
	286	390	105 34
	2460	2546	87 28
	861	944	84 27
	2251	2334	84 27
	1124	1201	78 25
	2803	2880	78 25
SOX9	373	1902	1530 509
	1241	1519	279 92
	3334	3564	231 76
	2668	2862	195 64
	2161	2322	162 53
	2859	2996	138 45
	2099	2227	129 42
	1927	2049	123 40
	2936	3037	102 33
	3256	3333	78 25

B.

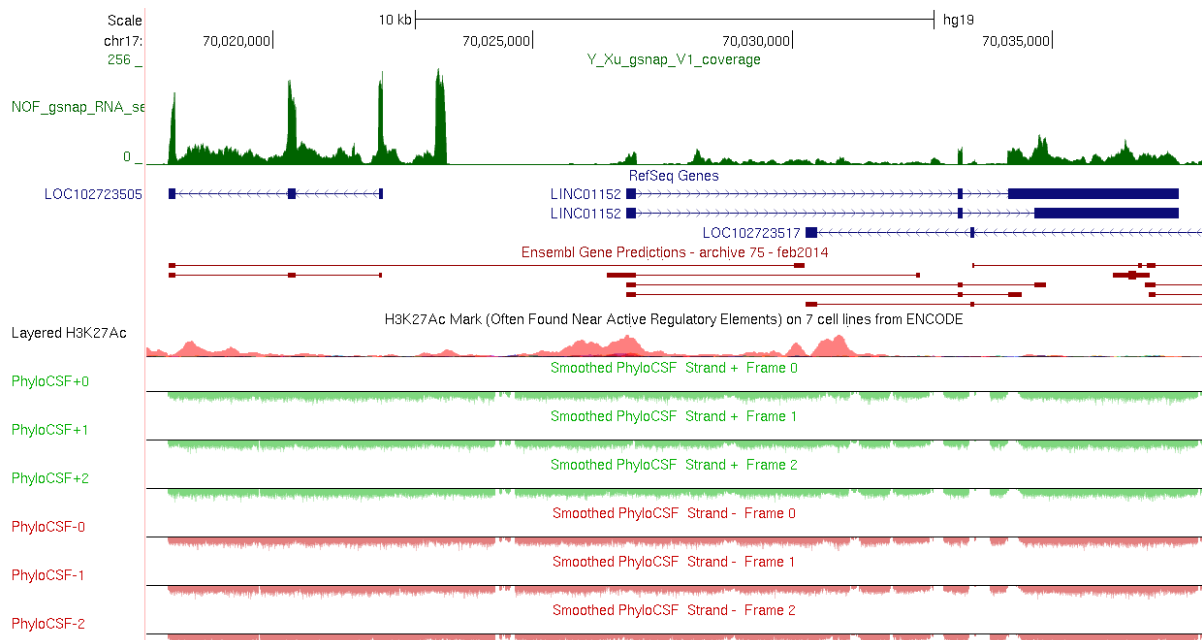
Sequence Name	RNA Size	Fickett Score	Hexamer Score	CPAT		CPC	
				Coding Probability	Coding Label	CODING POTENTIAL SCORE	C/NC
ENST00000430908	346	0.7649	-0.081	0.012	no	-1.285	noncoding noncoding
ROCR_HAC	574	0.7649	-0.081	0.012	no	-0.758	(weak)
ROCR_MSC	624	0.7649	-0.081	0.012	no	-1.332	noncoding
SOX9-AS1	3032	1.0343	-0.010	0.128	no	-1.018	noncoding
LINC01152	3578	0.9027	-0.053	0.015	no	0.364	coding (weak)
SOX9	3963	1.3018	0.647	1.000	yes	8.199	coding

Supplementary Figure 3

C.



D.

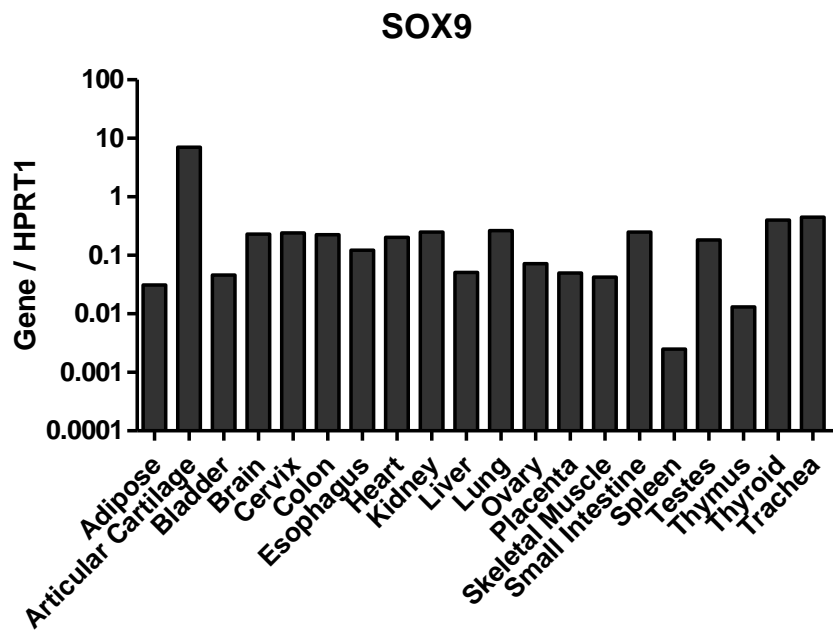


Supplementary Figure 3.

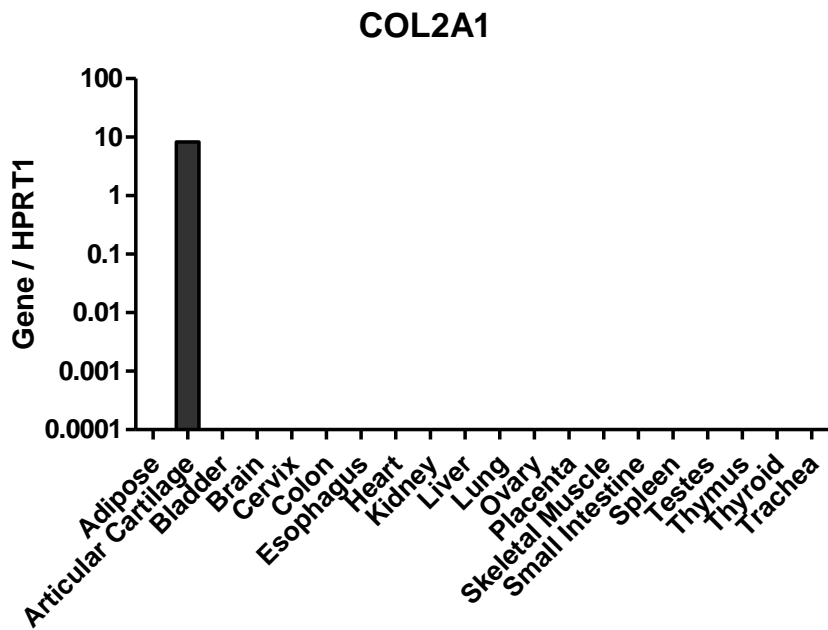
In silico analysis indicates a lack of coding potential for both *SOX9-ASI* and *ROCR*, with the existence of A. only very short open reading frames (ORF Finder) and B-D. codon substitution rates indicative of noncoding transcripts. B. CPAT and CPC. C. PhyloCSF on *SOX9* and *SOX9-ASI* locus. D. PhyloCSF on *ROCR* locus.

Supplementary Figure 4

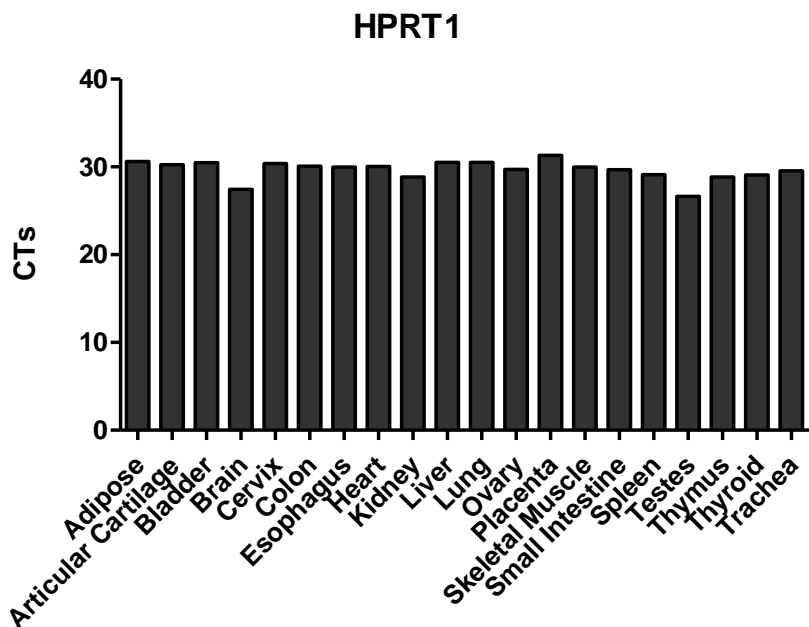
A.



B.



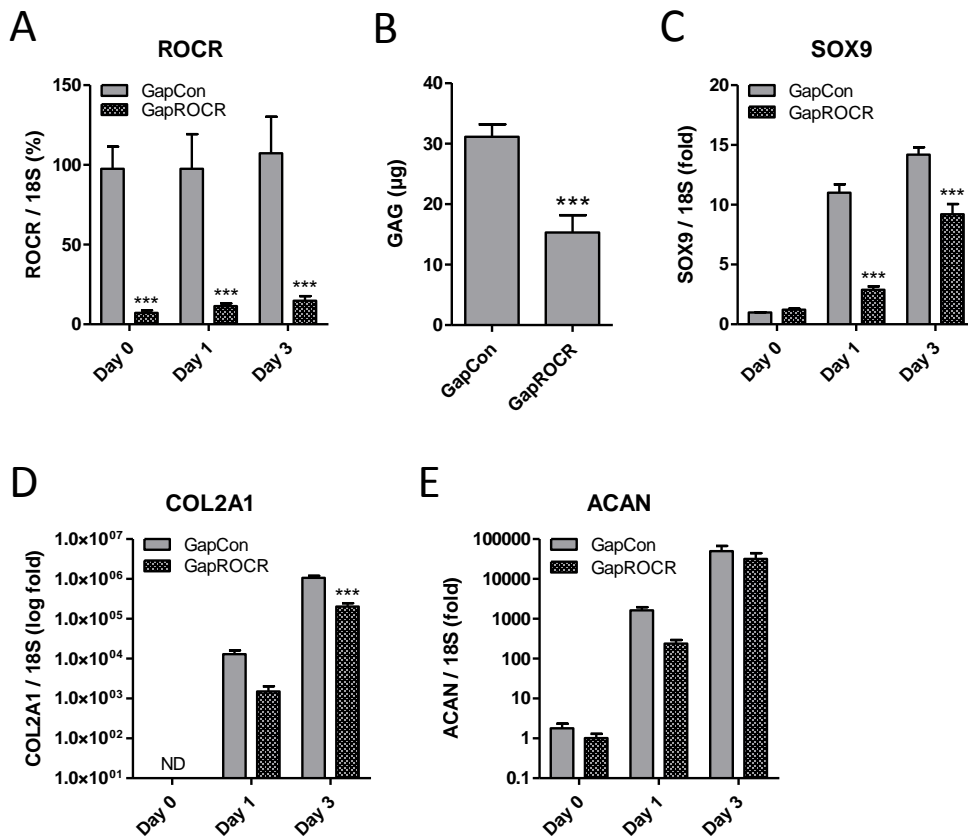
C.



Supplementary Figure 4.

Expression of A. *SOX9*, B. *COL2A1* and C. *HPRT1* in an RNA tissue panel measured by real-time RT-PCR. Values are the technical mean of data pooled from 3 donors per tissue.

Supplementary Figure 5

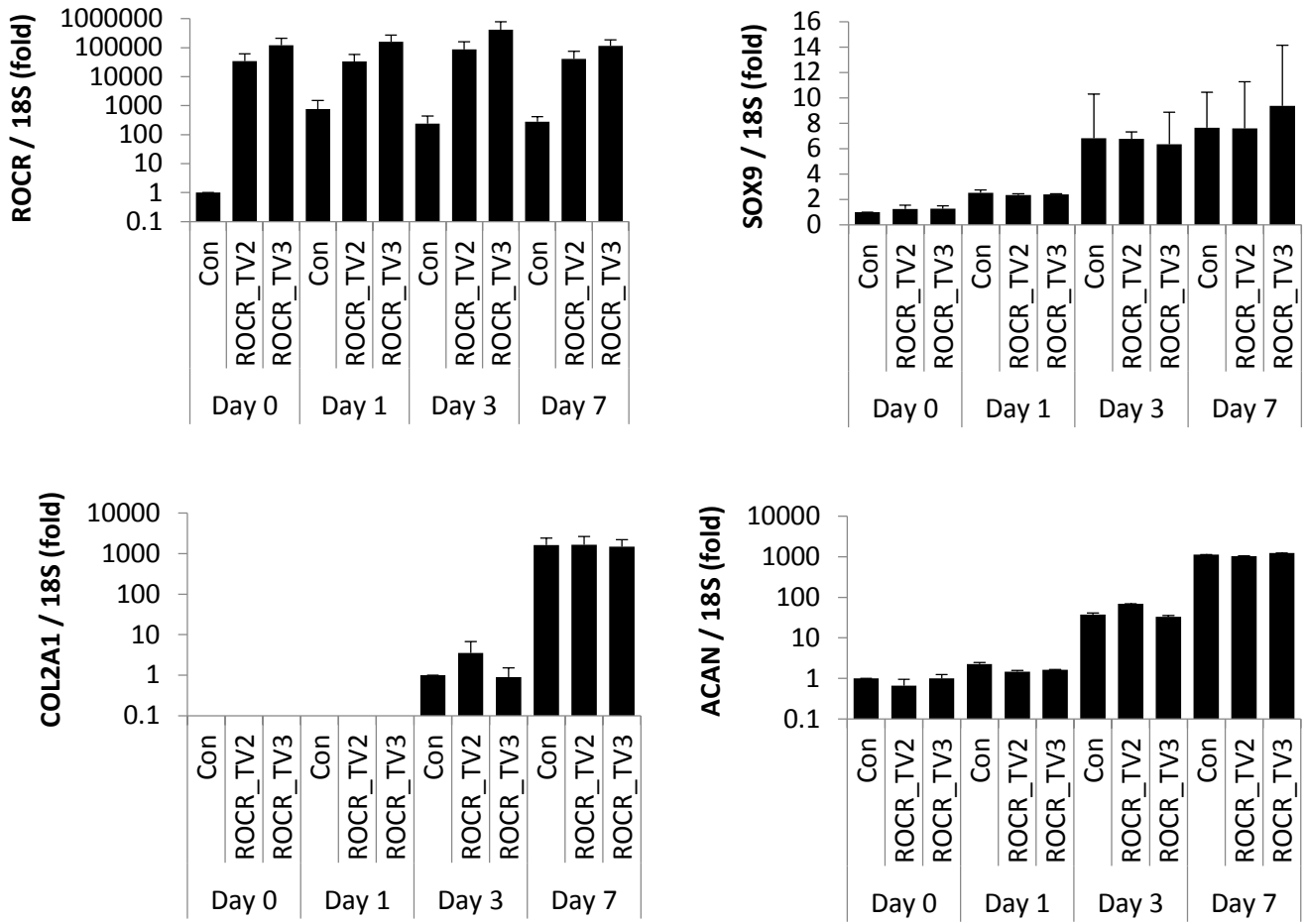


Supplementary Figure 5.

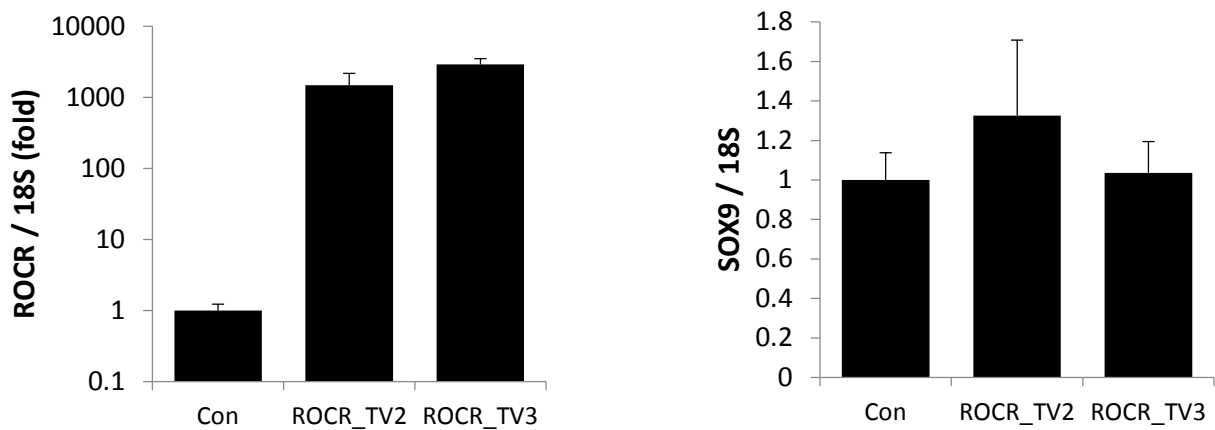
MSCs were transfected for 1 day with *ROCR*-targetting or non-targetting control GapmeR prior to chondrogenic differentiation in a V-bottom 96 well plate. A. *ROCR* expression in RNA extracted from Day 0, 1 and 3 chondrogenic pellets. Expression is presented as a percentage of non-targetting control levels. B. GAG levels assayed by DMB assay in Day 7 chondrogenic pellets. C-E. Expression of (C) *SOX9*, (D) *COL2A1*, (E) *ACAN* in RNA extracted from Day 0, 1 and 3 chondrogenic pellets. Values are the mean \pm SEM of data pooled from 4 MSC donors. *** = $P < 0.001$ for *ROCR* GapmeR versus non-targetting GapmeR.

Supplementary Figure 6

A



B

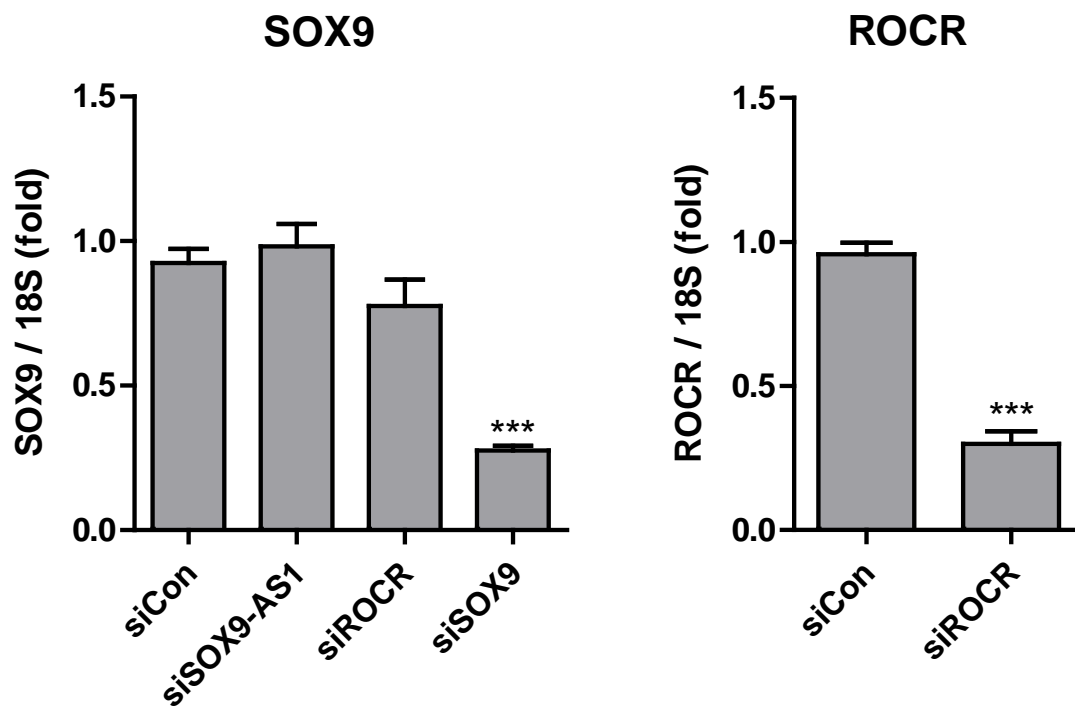


Supplementary Figure 6.

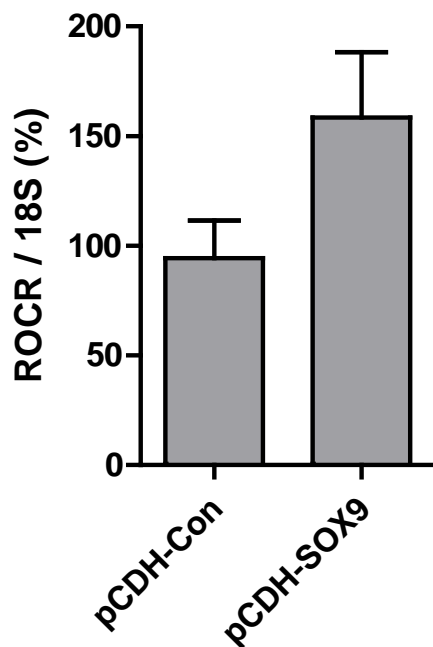
A. MSC were transduced for 2 days with *ROCR*-expressing lentivirus particles (transcript variant 2 and 3) prior to chondrogenic differentiation for up to 7 days. Expression of *ROCR*, *SOX9*, *COL2A1* and *ACAN* in RNA extracted from Day 0, 1, 3 and 7 chondrogenic pellets. Values are the mean \pm SEM of data pooled from 2 MSC donors. B. *ROCR* and *SOX9* expression in HAC transduced with *ROCR*-expressing lentivirus particles (transcript variant 2 and 3) for 3 days. Values are the mean \pm SEM of data pooled from 2 OA HAC donors.

Supplementary Figure 7

A.



B.



Supplementary Figure 7.

A. *SOX9* and *ROCR* expression in HAC transfected for 2 days with the indicated siRNAs. Values are the mean \pm SEM of data pooled from 4 OA HAC donors. *** = $P < 0.001$. B. *ROCR* expression in MSCs transduced with *SOX9* or control lentivirus (pCDH) for 2 days. Values are the mean \pm SEM of data pooled from 4 MSC donors.

Supplementary Figure 8

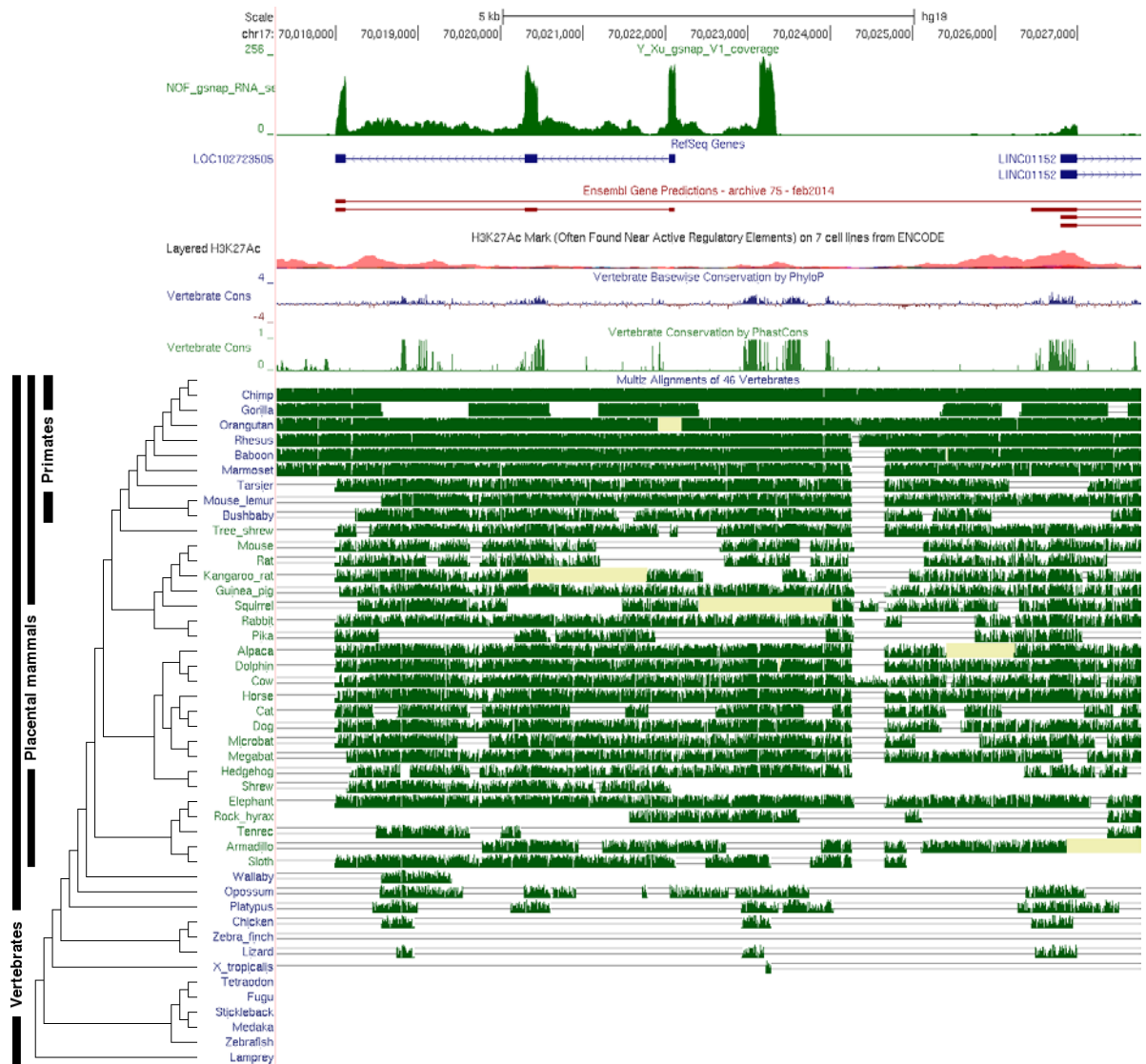
A.

Mus musculus cDNA sequence BC006965 (BC006965), long non-coding RNA
 Sequence ID: [NR_024085.1](#) Length: 1952 Number of Matches: 1

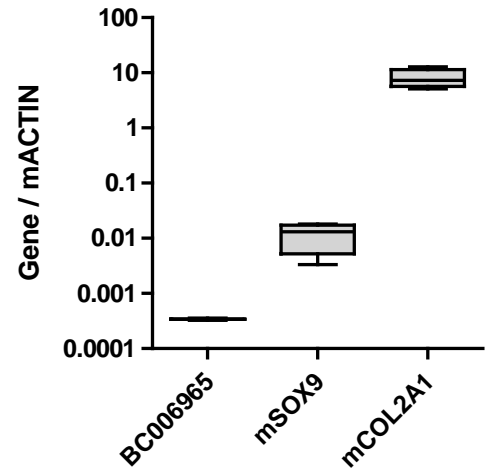
Score	Expect	Identities	Gaps	Strand
50.0 bits(54)	9e-04	66/92(72%)	0/92(0%)	Plus/Plus

Query	Score	Expect	Identities	Gaps	Strand
65	GAACTACTTAAGCATCAAC	124	TAATTAATCTGCAAGTGAAGAA	TACTCTCCAGCATTACTCTA	
Sbjct	644	GAAGTGCCTCAGCATCCAC	703	TAATTAATCCATCAGCAAAGAA	TGCTGCCTCCATCTCTCCA
125	TAATTAGACAATCTCAGG	156	GAGGAAACAAGAGGA		
Sbjct	704	TCATTAGCCAGTCTTCAA	735	AGAAGCAAGAGGA	

B.



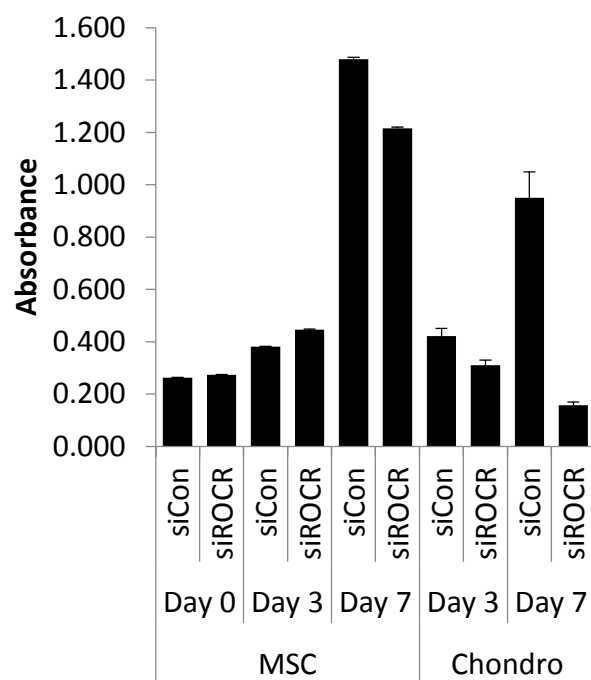
C.



Supplementary Figure 8.

A. Blastn result of homology search for *ROCR* sequence in mouse. B. UCSC genome browser schematic of the *ROCR* locus showing vertebrate conservation. C. Expression of *Bc006965*, *Sox9* and *Col2a1* in C57BL/6 mouse xiphoid cartilage RNA.

Supplementary Figure 9



Supplementary Figure 9.

MSCs were transfected for 2 days with *ROCR*-targetting or non-targetting control siRNA prior to chondrogenic differentiation in a V-bottom 96 well plate or maintenance in MSC medium for up to 7 days. Media were collected immediately prior to the induction of chondrogenesis (Day 0) and after 3 and 7 days. Cytotoxicity was measured with the use of the Promega CytoTox 96 kit.

Supplementary Table 1.

Primer and siRNA/GapmeR sequences.

Primer	Sequence	Probe
SOX9-AS1 exon4-5 F	cctgagcatttctcgtctg	65
SOX9-AS1 exon4-5 R	caggacaaggtggatgaaca	
SOX9-AS1 exon5-6 F	ctggcaagaggcaggaaa	38
SOX9-AS1 exon5-6 R	gctctcatccaaggtgaacag	
ROCR exon2-3 F	tgctgtatgaccagcctattg	11
ROCR exon2-3 R	tcctctgtttcctcctgaga	
ROCR exon1-2 F	ctgaaggcaaaggcaagc	18
ROCR exon1-2 R	caaacatgtgtcttaaaggctct	
LINC01152 F	cctcacttcctcctcttctg	19
LINC01152 R	ccccatctcatctgttttga	
RUNX2 F	gtgcctaggcgcatttca	29
RUNX2 R	gctcttctactgagagtgaagg	
ALPL F	agaaccccaaaggcttcttc	31
ALPL R	cttgcttttccttcatggt	
ADIPOQ F	ggtgagaagggtgagaaagga	85
ADIPOQ R	ttcaccgatgtctcccttag	
FABP4 F	ccaccataaagagaaaacgagag	31
FABP4 R	gtggaagtgacgcctttcat	
L-SOX5a F	ttacctcaggagttgaaagga	38
L-SOX5a R	gcttgtcaccatggctacct	
SOX6 F	gcttctggactcagcccttta	67
SOX6 R	ggcccttagcctttggta	
ROCR RACE GSP1	TTTGAAATCCTCTTGTTTC	
ROCR RACE GSP2	CAAAACATGTGTCTTAAAAGGCTCT	
siRNA	Target sequence	
siSOX9-AS1	CAAAGGAGCTAAAGAAGAA	
siROCR-1	GGAGGAAACAAGAGGATTT	
siROCR-2	CAACCATAGAACAGACAAA	
GapmeR	Target sequence	
GapROCR	ACTTAAGCATCAACTA	

Supplementary Table 2.

Cartilage RNA-Seq lncRNA expression and *SOX9* expression.

[Click here to Download Table S2](#)

Supplementary Table 3.

Expression of *ROCR* and *SOX9-AS1* in cell types from Human Protein Atlas and Illumina BodyMap RNA-Seq data.

[Click here to Download Table S3](#)