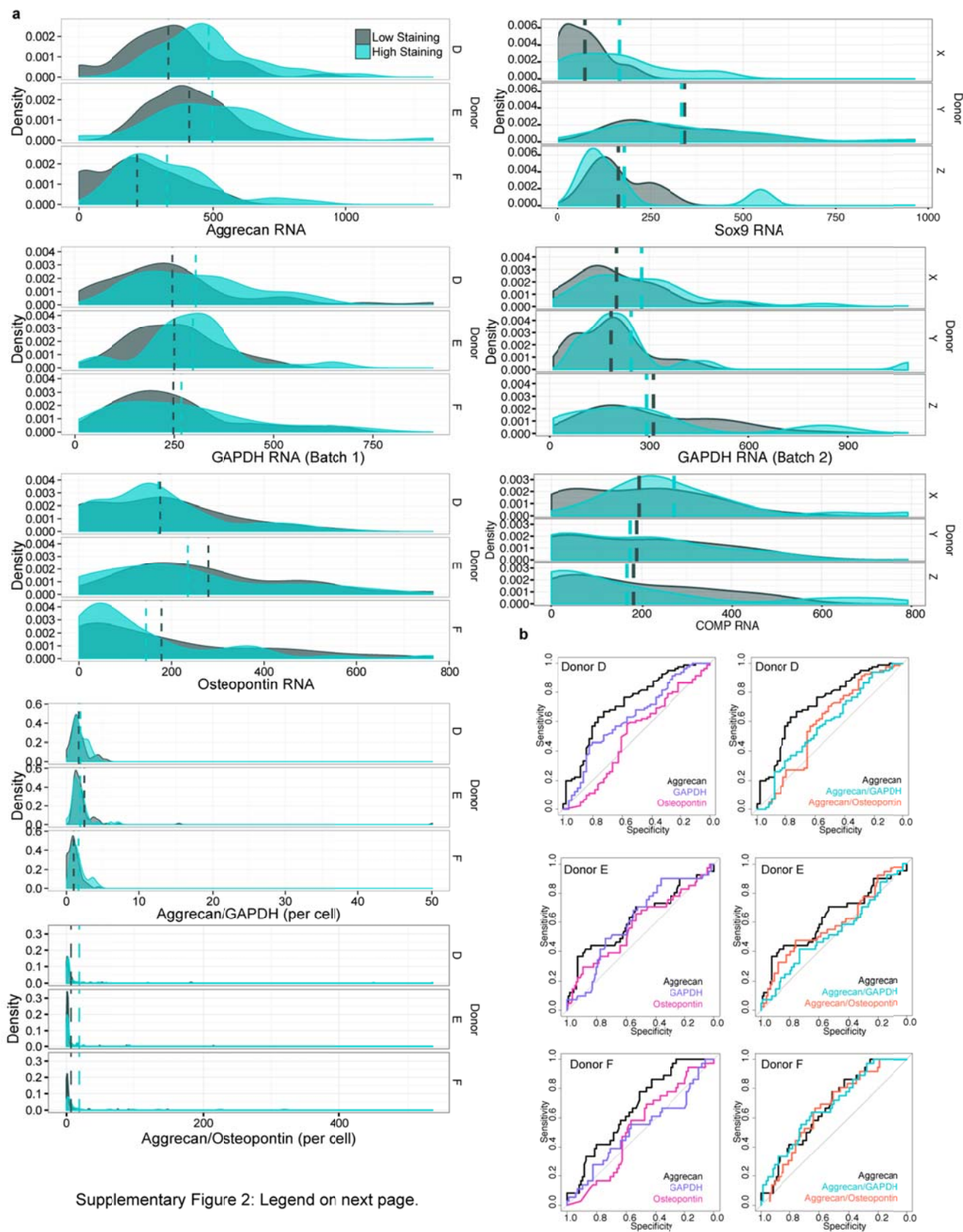


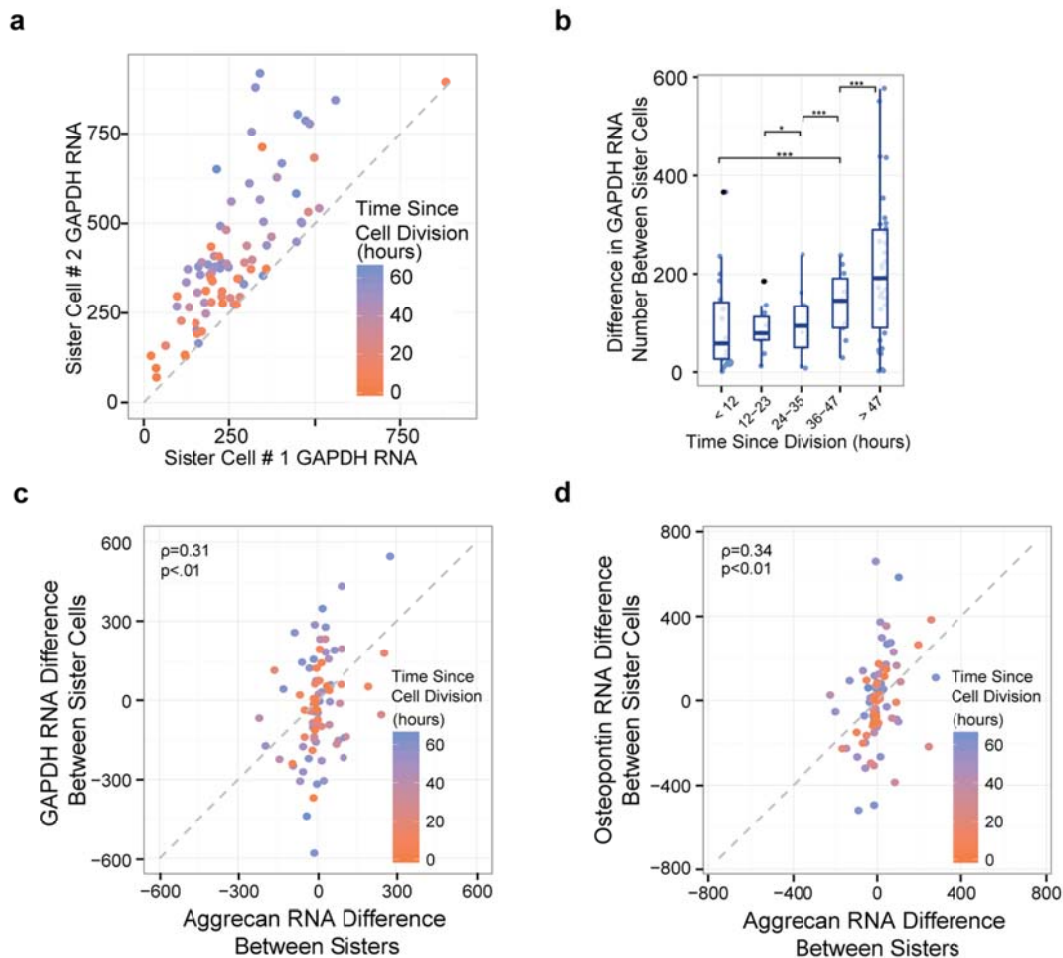
**Supplementary Figure 1: Matrix production, GAPDH copy number, and viability of MSCs in 3D culture.** a) Alcian blue staining for sulfated proteoglycans for MSCs in 3D culture, for all donors and time points, with and without TGFβ induction of chondrogenesis. Donor B is also shown in Figure 1. Scale bar = 5 mm. b) Mean GAPDH RNA counts and c) cell viability over 21 days in 3D culture. Narrow bars represent the mean within an individual donor; overlaid bars represent the mean across donors. Error bars indicate standard error (n = 24-128 cells per donor and condition). RNA count means compared by t-tests with Satterthwaite approximation and simulated adjustment for multiple comparisons. See Supplementary Table 1 for all statistical comparisons. d) Simultaneous RNA FISH and fixable dead staining established a threshold of GAPDH > 10 to differentiate live cells from dead cells for further analysis. n = 85 cells for TGFβ-, 75 cells for TGFβ+.



**Supplementary Figure 2: Matrix staining intensity versus mRNA copy number and ROC curves for individual donors, markers, and marker ratios.**

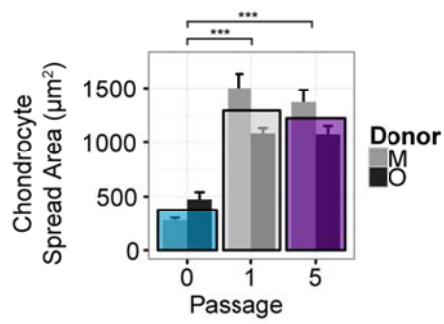
a) Distribution of aggrecan, GAPDH, osteopontin, aggrecan/GAPDH, and aggrecan/osteopontin gene expression within high- and low-performing MSC populations; separated by donor. Dashed lines represent the mean for each condition. For aggrecan/osteopontin, only cells that had at least one osteopontin (>95% of cells per donor) mRNA were included in the graph and mean statistics calculation.

b) Receiver operating characteristic curves using individual gene expression and gene expression ratios to distinguish between high- and low-performing MSCs, separated by donor. Cells/donor: D: 132, E: 153, F: 122, X: 57, Y: 42, Z: 47.



**Supplementary Figure 3: Heritability of marker copy number through cell division.**

a-b) Divergence in gene expression between sister cells as a function of time since their last division. c) Divergence in GAPDH vs aggrecan and d) osteopontin vs aggrecan between sister cells as a function of time since their last division. Box hinges denote the first and third quartiles. Whiskers extend from the hinges to the most extreme data points within (1.5 \* interquartile range) of the hinges. Means compared by t-tests with Satterthwaite approximation and simulated adjustment for multiple comparisons, \*\*\* indicates  $p<0.001$ , \*  $p<0.05$ . See Supplementary Table 6 for all statistical comparisons. n = 81 sister cell pairs.



**Supplementary Figure 4: Chondrocyte morphology with passage number.**

Chondrocyte spread area with increasing passage number during de-differentiation (n = 25-27 cells per donor per condition). Narrow bars represent the mean within an individual donor; overlaid bars represent the mean across donors. Error bars indicate standard error. Compared via one-way ANOVA with Tukey post-hoc test, \*\*\* indicates p<0.01.

Supplementary Table 1	
Comparison	Adjusted P-value
-TGFB vs +TGFB, Day 01	0.3572
-TGFB vs +TGFB, Day 04	0.0827
-TGFB vs +TGFB, Day 07	0.0361
-TGFB vs +TGFB, Day 14	0.9479
-TGFB vs +TGFB, Day 21	0.9980
+TGFB, Day 1 vs Day 4	0.0022
+TGFB, Day 1 vs Day 7	0.4637
+TGFB, Day 1 vs Day 14	<.0001
+TGFB, Day 1 vs Day 21	0.0002
+TGFB, Day 4 vs Day 7	0.0019
+TGFB, Day 4 vs Day 14	0.0002
+TGFB, Day 4 vs Day 21	0.0009
+TGFB, Day 7 vs Day 14	<.0001
+TGFB, Day 7 vs Day 21	0.0002
+TGFB, Day 14 vs Day 21	0.0016

**Supplementary Table 1: Additional statistical information related to GAPDH RNA abundance differentiating MSCs.** Companion table to Supplementary Figure 1b. P-values indicated for all planned contrasts between -TGF $\beta$  and +TGF $\beta$  groups over 21 days of culture. GAPDH RNA count means compared by t-tests with Satterthwaite approximation and simulated adjustment for multiple comparisons.

Supplementary Table 2			
Timepoint/Gene Pair	r <sup>2</sup>	Pearson's correlation coefficient	p-value
Day 1 aggrecan - osteopontin	0.24	0.49	1.0e-07
Day 21 aggrecan - osteopontin	.11	.34	.0019
Day 1 aggrecan-LPL	7.5e-5	.0087	.92
Day 21 aggrecan-LPL	.0022	.047	.67
Day 1 osteopontin-LPL	1.9e-5	.0044	.96
Day 21 osteopontin-LPL	.04	.21	.059

**Supplementary Table 2: Correlation of marker gene RNA abundance in individual differentiating MSCs.** r<sup>2</sup> and Pearson's correlation coefficient between abundance of aggrecan, osteopontin, and LPL in MSCs cultured with TGF $\beta$  in agarose for either 1 or 21 days. Test for the significance of the Pearson correlation coefficient was conducted with 103 degrees of freedom for Day 1 comparisons, and 77 degrees of freedom for Day 21 comparisons. n= 105 cells at Day 1 and 79 cells at Day 21.

Supplementary Table 3				
	Colony A	Colony B	Colony C	Colony D
Aggrecan CV	1.71	1.34	1.07	0.91
GAPDH CV	0.32	0.35	0.31	0.20

**Supplementary Table 3: Coefficient of variation (CV) of single cell RNA count in small MSC colonies.** GAPDH RNA abundance was less variable in small colonies than aggrecan RNA abundance. (n = 75 cells in colony A, 7 cells in colony B, 6 cells in colony C, 8 cells in colony D).

Supplementary Table 4	
Comparison	Adjusted P-value
-TGFB vs +TGFB, Day 01	0.0067
-TGFB vs +TGFB, Day 04	0.0043
-TGFB vs +TGFB, Day 07	0.0020
-TGFB vs +TGFB, Day 14	0.0017
-TGFB vs +TGFB, Day 21	0.0031
+TGFB, Day 1 vs Day 4	0.0006
+TGFB, Day 1 vs Day 7	0.0002
+TGFB, Day 1 vs Day 14	0.0047
+TGFB, Day 1 vs Day 21	0.0002
+TGFB, Day 4 vs Day 7	0.0004
+TGFB, Day 4 vs Day 14	0.0021
+TGFB, Day 4 vs Day 21	0.0002
+TGFB, Day 7 vs Day 14	0.0002
+TGFB, Day 7 vs Day 21	<.0001
+TGFB, Day 14 vs Day 21	0.0002

**Supplementary Table 4: Additional statistical information related to aggrecan RNA abundance differentiating MSCs.** Companion table to Figure 1e. P-values indicated for all planned contrasts between –TGFβ and +TGFβ groups over 21 days of culture. Aggrecan RNA count means compared by t-tests with Satterthwaite approximation and simulated adjustment for multiple comparisons.

Supplementary Table 5		
Comparison		Adjusted P-value
< 12 hrs	[12-23 hrs]	<.0001
< 12 hrs	[24-35 hrs]	<.0001
< 12 hrs	[36-47 hrs]	<.0001
< 12 hrs	> 47 hrs	<.0001
[12-23 hrs]	[24-35 hrs]	0.8759
[12-23 hrs]	[36-47 hrs]	0.7564
[12-23 hrs]	> 47 hrs	0.9997
[24-35 hrs]	[36-47 hrs]	<.0001
[24-35 hrs]	> 47 hrs	<.0001
[36-47 hrs]	> 47 hrs	<.0001

**Supplementary Table 5: Additional statistical information related to divergence in aggrecan RNA abundance between sister cells.** Companion table to Figure 4f. Means compared by t-tests with Satterthwaite approximation and simulated adjustment for multiple comparisons, n = 81 sister cell pairs.

Supplementary Table 6		
Comparison		Adjusted P-value
< 12 hrs	[12-23 hrs]	0.1649
< 12 hrs	[24-35 hrs]	0.6435
< 12 hrs	[36-47 hrs]	<.0001
< 12 hrs	> 47 hrs	<.0001
[12-23 hrs]	[24-35 hrs]	0.0173
[12-23 hrs]	[36-47 hrs]	<.0001
[12-23 hrs]	> 47 hrs	<.0001
[24-35 hrs]	[36-47 hrs]	<.0001
[24-35 hrs]	> 47 hrs	<.0001
[36-47 hrs]	> 47 hrs	<.0001

**Supplementary Table 6: Additional statistical information related to divergence in GAPDH RNA abundance between sister cells.** Companion table to Supplementary Figure 3b. Means compared by t-tests with Satterthwaite approximation and simulated adjustment for multiple comparisons, n = 81 sister cell pairs.



**Supplementary Table 7**

Passage Comparison		Aggrecan/GAPDH (Pooled Population) Adjusted P-value	Aggrecan per Cell Adjusted P-value	GAPDH per Cell Adjusted P-value
0	1	0.0079	0.2108	0.0006
0	3	0.0004	0.9342	0.0006
0	5	0.0002	0.9999	0.0008
0	7	0.0002	1.0000	0.0008
0	9	0.0011	0.9984	0.0025
1	3	0.7036	0.6621	1.0000
1	5	0.5040	0.2891	0.9945
1	7	0.4593	0.1658	0.9929
1	9	0.9377	0.3623	0.7026
3	5	0.9993	0.9783	0.9981
3	7	0.9982	0.8850	0.9972
3	9	0.9944	0.9934	0.7784
5	7	1.0000	0.9988	1.0000
5	9	0.9510	0.9999	0.9343
7	9	0.9309	0.9941	0.9431

**Supplementary Table 7: Additional statistical information related to RNA abundance in chondrocytes during passage-induced de-differentiation.** Companion table to Figure 5b-d. P-values indicated for all planned contrasts between chondrocytes at different passages (passages 0-9). Pooled aggrecan/GAPDH levels compared via one-way ANOVA with Tukey's post-hoc test. Single cell RNA count means compared by t-tests with Satterthwaite approximation and simulated adjustment for multiple comparisons.

**Supplementary Table 8**

Comparison	Aggrecan per Cell Adjusted P-value	GAPDH per Cell Adjusted P-value	Aggrecan/GAPDH per Cell Adjusted P-value
Day 1, P0 vs P5	0.0016	0.0406	0.9983
P0, Day 1 vs 14	0.0897	0.0006	0.0176
Day 14, P0 vs P5	0.0002	0.6714	0.0588
P5, Day 1 vs 14	<.0001	<.0001	0.1982

**Supplementary Table 8: Additional statistical information related to RNA abundance in chondrocytes during re-differentiation in agarose.** Companion table to Figure 5h-j. P-values indicated for all planned contrasts between chondrocytes at different passages (passages 0 and 5) and culture time points (days 1 and 14). Pooled aggrecan/GAPDH levels compared via one-way ANOVA with Tukey's post-hoc test. Single cell RNA count means compared by t-tests with Satterthwaite approximation and simulated adjustment for multiple comparisons.

Supplementary Table 9						
Target:	Aggrecan	GAPDH	Osteopontin	Lipoprotein Lipase	COMP	Sox9
# probes:	32 probes	20 probes	24 probes	32 probes	32 probes	7 probes
Probe 1	gtccttgtctccatagcaac	agaattaaagcagccctgg	cccaagaggcagaagcaaat	ggagtcctgagagcaaattt	gtaatctccttgacctgctg	aaggggtccaggagattcat
Probe 2	acacgtcataggtttcgttg	tcattgatggcgacgatgctc	gtttaactggaagggcggag	caggaatgaggtggcaagtg	catcaccgtgttttcagga	ctgctcgtcggtcatcttca
Probe 3	cttgaagtggaacttctcc	agaccatgtagtgaaggtca	tacagcatctgggtattgtt	tgctgtggttgaagtacag	gttgctgtctcggtagaag	cttggggaactgttctctc
Probe 4	tttctgggatgtccacaaag	ttgactgtgccgttgaactt	aagtctgctctgagatggg	caactctacacattcctgt	ctggtattgatgcagcgaac	gacggcctcggatgcaca
Probe 5	cccttacttcagggacaaac	ctttcattgatgacgagct	gacacagaattctgtgtgctc	gacactggataatgctgctg	cgtacaaacctgcttgg	cgtagcccttgagcactgg
Probe 6	actgatgtcctactaccag	gatctcgtcctggaagatg	gggtatttgttgtgtca	ataaactggccacatcctg	cggtctcacactgtaaatg	ggtagggccaccagctcca
Probe 7	gcagagatttctgaccttc	tctcatggtagtagagacc	ggcttctcattggacttactt	agtaaattcatccgccatc	attgacgcatacagagtgg	ttgttctgtctgagccgtt
Probe 8	actgatgtcctactaccag	agagatgatgacctcttgg	gtcatctagatcgtctgtt	atccaagagatgcacattg	gaaagcctctaagtctgtg	
Probe 9	aaggtcctactccagaag	ttgtatacttctctgtgtt	cgctggagtcattagattg	ttcttattggtcagactcc	cagttgtccttctgactg	
Probe 10	agaaggaagtcactaaggt	gaggcattgctgacaactt	aatggtcagggtcatcagtg	catactcgaagttaggtcca	gcagttgtccttctcattga	
Probe 11	agtctgctgagatcctctac	ccaaagtgtgcatggatgac	tcagaatggtgagactcgtc	caggagaaaggcacttgg	cttctgccatccgtatttc	
Probe 12	tctactccagaagcagagac	aagcaggatgatattctgg	gctgattgtggaatcagtg	ccctgggtgaatgtgtgtaa	cattctgctcgtcttctc	
Probe 13	actgatgtcctactaccag	ttgagctcaggatgacctt	ccgtagggataaacggagtg	ttctggattccgatactcg	ctttctgatctgagttggc	
Probe 14	caccagaaggaagaactctg	gtcagatccacaacagacac	tcttactcagctcctaag	cgtagggtaaatgtccaca	ttgtcacaggcatctctac	
Probe 15	actgatgtcctactaccag	catactggcaggtttctcc	ttagatcggcggaacttctt	caatgttacatcctggttgg	gtctgctgttctctctgag	
Probe 16	actgatgtcctactaccag	gtcctcagtgtagcctagaa	tgtggcatctggactctgaa	ctctgcaatcacacggagag	gtcttggctgctgcacaa	
Probe 17	cctccagaaggaagtctact	tgagtgctgcttgaagtc	ctgactcgtctcttagtg	taggccttactggatttctc	cagttgtcctctgagctctg	
Probe 18	agaaggaagtcactaaggt	aatgagcttgacaaagtgtt	tggcgtgagttcttggaaa	ggcagagaccttctcaaaag	ccatcattgtcgtcatcgtc	
Probe 19	gaaggtattctgccaaggtc	ttgctgtagccaattcatt	gaatgctgttcttacctt	tgacctgttgatctcgtag	ctagacggcagttgtcccta	
Probe 20	gtccactgaaatcaggaagg	tactcctggaggccatgtg	ttcctgactcctaatcaga	tacatcttctgcttctttt	acctgtccgcatacaagtc	
Probe 21	caaagagtcagagggttctc		aattctggctgagtttgg	gcatctgagaacgagctctc	ggacacacatcagcttctc	
Probe 22	ccactaatgtcaggaacc		atgatcaggtctagctgtg	cctggttgggtatgtatta	accagttagggtctatctg	
Probe 23	gtcacccagatatttctcc		ttcaggtgttctctctt	cagtgccatacagagagatc	atctccataacctggttag	
Probe 24	gatttctggtgtccagagg		attgacctcagaagaggcac	aaaagggatgttctcgtct	gtcgtgttcatcgtctgta	
Probe 25	caattgtcctcagaagacgt			ttgtgaaactcaggcagg	tggaactgtccttcaaatc	
Probe 26	aagttcagaatgctgaag			aatatccacctcctgtaaa	ggtagccaaagatgaacca	
Probe 27	ccaagatcccaaatggtct			ggaccagctgaagtagaat	catgaccacatagaagctgg	
Probe 28	accactggattcaaaaagct			caccttttttgagctctc	cagtactgtctcctcatctg	
Probe 29	tgggactgatgaccttcta			gataagacatttctcccgg	taggatgtctgtccttcca	
Probe 30	cagatcagcttcatggaagg			tatcacaggtgacttctct	cctcgtagaatgcactcta	
Probe 31	ggattcagttctagacgctg			actgtcatggcatttcaca	catggtcgtatccagatca	
Probe 32	attgatctcgtatcgtctc			cagccagacttctattcag	atgatgttctcctgggagaa	

**Supplementary Table 9: RNA-FISH probe sequences.**

Oligonucleotide probe sequences used to assay aggrecan, GAPDH, osteopontin, LPL, COMP and Sox9 RNA abundance.