

Supplemental Material

Dual and Opposing Regulation of MMP1 and MMP13 by Both Arms of miR-675 in Human Articular Chondrocytes

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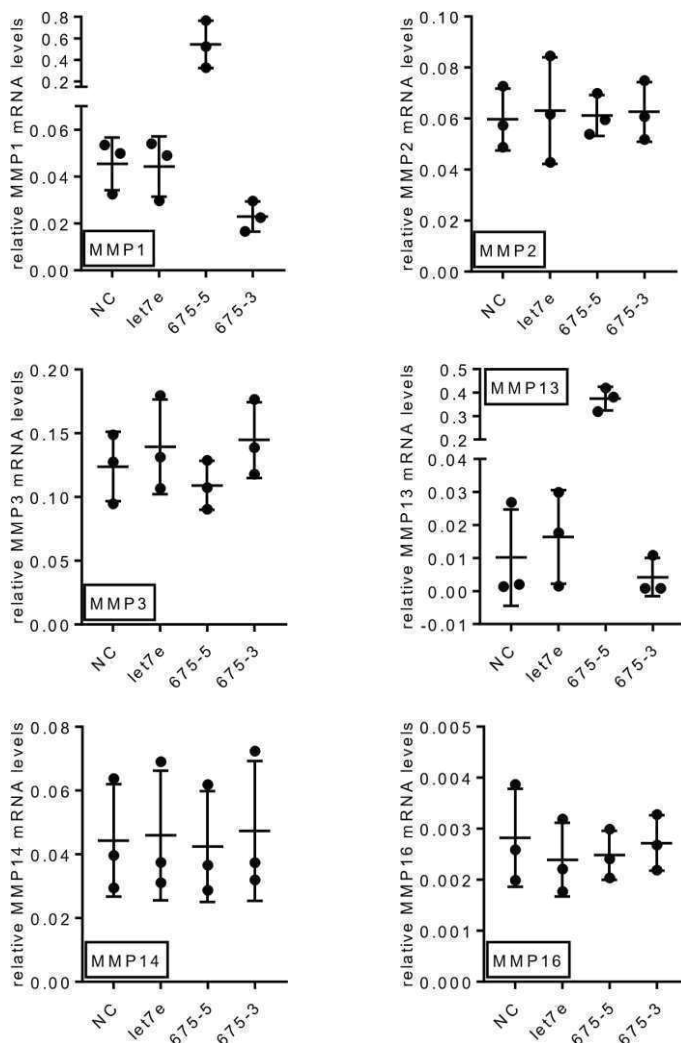
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Supplementary Table S1. Relative expression levels of MMP genes in human articular chondrocytes.

MMP	Family	Relative expression	Primer fwd	Primer rev
1	Collagenase	0.073753 ± 0.043	CCAGGTATTGGAGGGGATGC	GTCCAAGAGAATGGCCGAGT
2	Gelatinase	0.081308 ± 0.048	GGTTCATTGGCGGACTGTG	GGCCTCGTATACCGCATCAA
3	Stromelysin	0.138509 ± 0.0934	CACAGACCTGACTCGGTTCC	GAGTCAGGGGGAGGTCCATA
8	Collagenase	6.89E-05 ± 0.0001	CCATGTGCAGATTTCCAAGGC	AGACTGATACTGGTTGCTTGGT
9	Gelatinase	5.31E-05 ± 0.0002	CGACGTCTTCCAGTACCGAG	CTGGTTCAACTCACTCCGGG
10	Stromelysin	6.3E-05 ± 0.0001	ACAGCAGGGACACCGTTTTT	GGAGGAAAACCCAGGGTATGG
11	Stromelysin	0.000344 ± 0.0002	TCATGATCGACTTCGCCAGG	TCCCCTTCTCGGTGAGTCTT
12	Stromelysin	1.38E-05 ± 0.00003	ATCTGGCATTGGAGGGGATG	TGGCCAATCTCGTGAACAGC
13	Collagenase	0.061612 ± 0.047	AGACTTCCCAGGAATTGGTGA	ACGGTACTCCAGATGCTGT
14	M.B. MMP	0.047177 ± 0.0298	CCCAACATCTGTGACGGGAA	TTGGTTATTCTCACCCGCC
15	M.B. MMP	0.000282 ± 0.0002	CCTTCCTGAGCAATGACGCA	AGGCGCTCATTGTGCAATTT
16	M.B. MMP	0.002824 ± 0.0015	GCATCATTCGGGGGTGTTTT	GGTGGAAGGTAGCCGTACTT
17	M.B. MMP	4.54E-05 ± 0.00001	CCCCACCAAGTGAACAAGA	AGGGCGTAGTACATGAGTGC

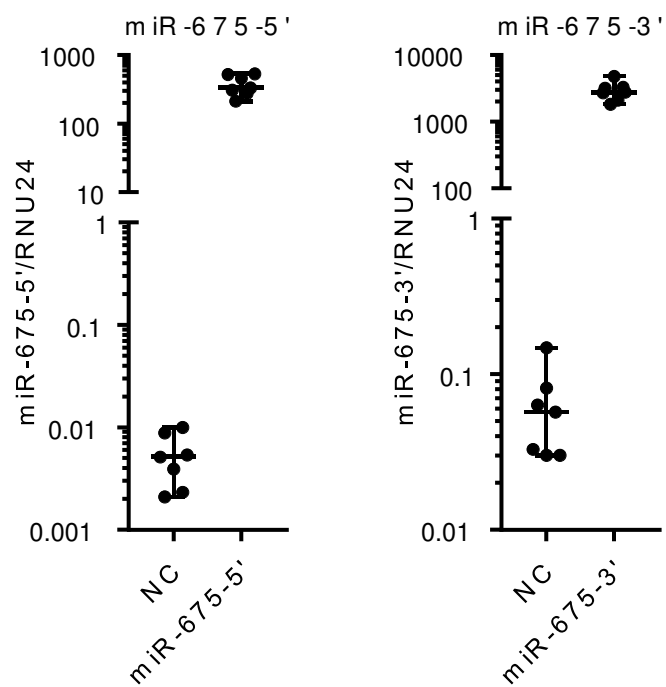
Supplementary Table S1. Relative expression levels of several members of the MMP family, representing the collagenase, stromelysin, gelatinase and Membrane-bound MMP categories. Levels are shown in human articular chondrocytes at P2 isolated from three different donors after SYBR qPCR relative to the housekeeping gene RPLP0 using the ΔC_t method and displayed ± stdev. Levels > 0.0005 were considered significant in expression and are shaded in grey. M.B., membrane-bound. Following primers were used: RPLP0-FWD, CCATTGAAATCCTGAGTGATGTG; RPLP0-REV, CTTGCTGGCTCCCACTTT; MMP primer sequences are listed in the two right most columns.

Supplementary Figure S1



	let7e	miR-675-5'	miR-675-3'			miR-675-5'	miR-675-3'
NC	0.7249	0.0176	0.0182	MMP1	let7e	0.0175	0.0092
	0.571	0.6555	0.45	MMP2		0.5195	0.7985
	0.6987	0.3821	0.567	MMP3		0.4462	0.3486
	0.3454	0.004	0.3782	MMP13		0.0061	0.1777
	0.5334	0.7506	0.6882	MMP14		0.5211	0.9892
	0.0882	0.3526	0.7284	MMP16		0.6033	0.1116

Supplementary Figure S2



Legend to Supplementary Figures S1 and S2.

Supplementary Figure S1. Expression levels of six MMPs after overexpression of mimic miR-675-5' and mimic miR-675-3' in human articular chondrocytes from three donors. Transfection was carried out as described in Materials and Methods and expression levels were analysed relative to RPLP0 using the Δ Ct method. The table lists p values of paired t-tests relative to either the negative control mimic or to the unrelated miR let-7e. NC, negative control miR mimic.

Supplementary Figure S2. Levels of miR-675-5' and miR-675-3' using mimics in human articular chondrocytes from seven donors at passage 2 48 hrs after overexpression. Data are shown after TaqMan qPCR relative to the small housekeeping RNA RNU24 and evaluated using the Δ Ct method. NC, negative control mimic.