

Supplementary figures

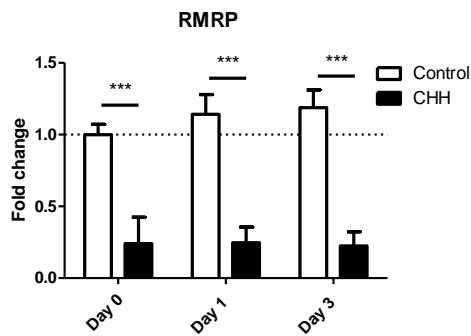


Figure S1: Expression levels of *RMRP* were significantly lower in CHH cells throughout the experiment. Data (FPMK values) are represented as mean \pm standard deviation, represented as FC to control Day 0, *** indicates statistically significant difference $FDR \leq 0.001$.

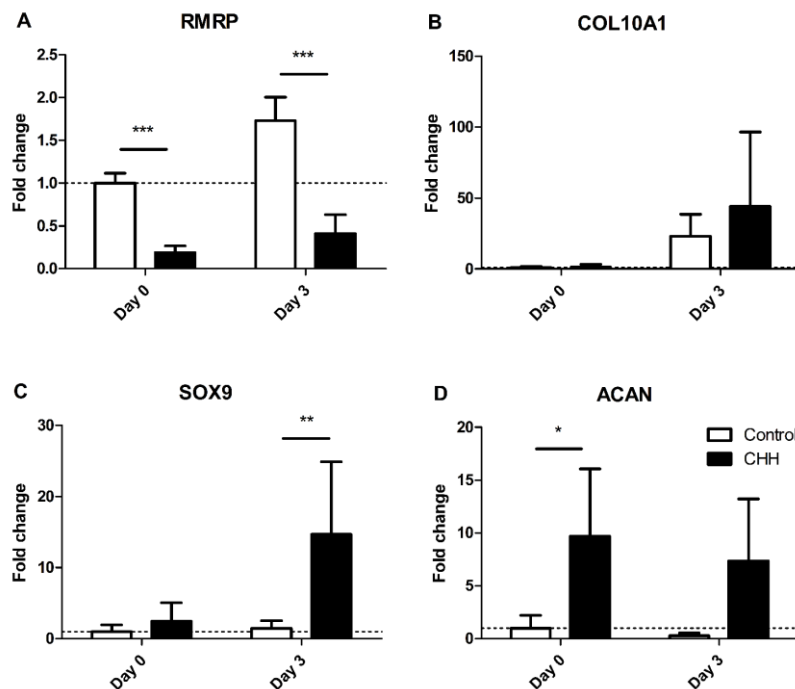


Figure S2: RT-qPCR validation of RNA sequencing data – expression levels of *RMRP*, *COL10A1*, *SOX9*, and *ACAN*. Data were normalized to GAPDH expression and are shown as fold change compared to Day 0 control condition, mean \pm SD. Statistical significance was assessed by two-way ANOVA, Bonferroni post-tests, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Supplementary tables

Gene	Forward primer	Reverse primer
<i>GAPDH</i>	5'-ACTTTGTGAAGCTCATTTCTGGTA-3'	5'-GTGGTTTGAGGGCTCTTACTCCTT-3'
<i>SOX9</i>	5'-AGTACCCGCACCTGCACAAC-3'	5'-CGCTTCTCGCTCTCGTTCAG-3'
<i>ACAN</i>	5'-GCAGCTGGGCGTTGTCA-3'	5'-TGAGTACAGGAGGCTTGAGGAC-3'
<i>COL10A1</i>	5'-ATGATGAATACACCAAAGGCTACCT-3'	5'-ACGCACACCTGGTCATTTTCTG-3'

Table S1: RT-qPCR primer sequences used in this study.

	Controls						CHH vs controls					
	Day 1 / Day 0		Day 3 / Day 0		Day 3 / Day 1		Day 0		Day 1		Day 3	
	FC	FDR	FC	FDR	FC	FDR	FC	FDR	FC	FDR	FC	FDR
Collagens												
COL3A1	.	.	3.8	2.6E-02
COL5A2	0.2	3.2E-08
COL5A3	7.1	2.2E-06	.	.	0.3	4.4E-02	.	.	0.3	3.6E-03	.	.
COL6A3	5.8	1.1E-05	3.6	2.8E-03	0.3	2.9E-03	.	.
COL7A1	20.2	6.1E-12	20.1	1.3E-11	0.3	1.7E-02	.	.
COL8A2	.	.	3.0	2.2E-02	6.8	1.6E-04
COL9A2	4.8	1.5E-02	6.2	5.6E-03	11.6	1.1E-04
COL9A3	23.3	8.5E-03
COL10A1	96.9	5.5E-08	321.6	1.9E-10
COL14A1	.	.	5.1	2.6E-02	.	.	5.6	3.2E-02	7.8	4.1E-03	4.4	3.6E-02
COL21A1	8.8	2.7E-03	6.9	6.3E-03	.	.
COL22A1	29.7	9.5E-03
COL23A1	.	.	11.3	2.1E-02
COL24A1	8.4	5.9E-03	18.8	2.4E-04	0.1	3.7E-03	.	.
COL27A1	8.5	7.5E-16	6.0	3.0E-11
Proteoglycans												
ASPN	12.8	2.98E-03	0.12	1.2E-02
BGN	.	.	3.3	1.51E-02
GPC2	3.3	3.47E-02	3.4	4.04E-02
HAPLN3	0.4	4.13E-02	0.4	2.62E-02	.	.	0.01	7.9E-03	0.0	1.2E-02	0.02	2.4E-02
HAS1	13.9	9.10E-04	17.9	4.52E-04
HAS2	3.8	2.81E-03	6.6	2.80E-05	0.3	9.5E-03	0.25	2.6E-03
LUM	.	.	9.9	1.27E-02	11.7	2.71E-02
MATN3	3.7	4.91E-02
OGN	.	.	30.4	2.97E-04	44.8	5.15E-04

	Controls						CHH vs controls					
	Day 1 / Day 0		Day 3 / Day 0		Day 3 / Day 1		Day 0		Day 1		Day 3	
	FC	FDR	FC	FDR	FC	FDR	FC	FDR	FC	FDR	FC	FDR
PODNL1	2.9	2.08E-02
PRG4	101.6	5.61E-08	19.2	5.58E-04	6.81	1.4E-02
SDC1	2.4	9.58E-03	2.7	2.47E-03
SDC3	0.2	6.83E-11	0.5	2.06E-02	2.8	1.77E-03	.	.	3.3	3.5E-05	.	.
TSKU	3.2	7.36E-08	3.2	2.44E-07
ECM degradation enzymes												
ADAM12	.	.	2.5	4.6E-02
ADAM19	4.5	4.3E-02	6.1	1.8E-02	0.2	2.1E-02	0.2	1.3E-02
ADAM21	9.3	5.4E-03	.	.
ADAM28	5.5	3.0E-02
ADAMTS1	0.1	7.4E-05	0.1	2.8E-06	4.1	6.4E-03
ADAMTS4	6.6	4.7E-09	2.7	5.7E-03	0.4	3.8E-02
ADAMTS5	6.1	1.1E-03
ADAMTS6	2.2	1.1E-04
ADAMTS7	.	.	0.2	2.8E-03	2.9	3.1E-02
ADAMTS8	10.7	9.4E-03
ADAMTS10	2.2	3.7E-02	3.3	1.3E-03
ADAMTS12	.	.	2.9	1.1E-03
ADAMTS14	30.7	3.8E-22	19.6	2.5E-17	.	.	3.3	2.8E-03	0.5	4.0E-02	.	.
ADAMTS15	0.1	5.3E-03
ADAMTS16	11.7	3.0E-03	0.2	2.8E-02	.	.
ADAMTSL1	0.3	3.9E-05	0.5	1.5E-02
ADAMTSL4	0.2	4.3E-07	0.1	2.9E-10	.	.	2.6	8.6E-03	6.8	6.7E-09	5.3	8.0E-07
ADAMTSL5	0.3	5.6E-06	0.5	7.6E-03	2.4	3.4E-03	.	.
CASP1	0.1	3.6E-03
CTSB	.	.	0.2	3.7E-03	4.1	2.4E-02	10.7	1.6E-04
CTSK	.	.	2.9	2.4E-04	3.2	6.3E-04	2.2	2.4E-02	2.9	5.1E-04	.	.

	Controls						CHH vs controls					
	Day 1 / Day 0		Day 3 / Day 0		Day 3 / Day 1		Day 0		Day 1		Day 3	
	FC	FDR	FC	FDR	FC	FDR	FC	FDR	FC	FDR	FC	FDR
MMP1	14.1	3.9E-04	11.4	1.7E-03
MMP2	.	.	5.0	4.0E-03
MMP3	.	.	2.1	4.7E-02	7.0	2.9E-02
MMP9	4.6	4.8E-02
MMP10	137.6	7.4E-08			0.0	4.0E-04	20.0	6.1E-04
MMP11	8.0	2.4E-04	32.1	6.6E-09
MMP12	8.8	2.5E-02
MMP13	.	.	94.8	2.4E-06	27.1	1.5E-03	0.1	2.8E-03
MMP14	3.7	2.6E-08	2.2	2.7E-03	2.0	7.5E-03
MMP24	0.3	2.0E-02	0.3	1.2E-02	3.8	1.1E-02	.	.

Table S2: List of significantly DE collagens, proteoglycans, and ECM-degrading enzymes in control cells over time and in CHH vs control cells. Changes in expression were calculated as fold change (FC) to control Day 0 and genes were considered significantly upregulated when $FC \geq 2.0$ and $FDR \leq 0.05$, while downregulated when $FC \leq 0.5$ and $FDR \leq 0.05$.

