SUPPLEMENTAL INFORMATION

Supplemental Figure Legends:

Figure S1.

TF binding evidence at the rs225014 locus. USCS browser hg19 screenshot. Darker bars indicate stronger evidence for binding. CTCF shows a black bar, while the other TFs show less strong evidence indicated as grey bars.

Figure S2.

The rs225014 risk allele does not influence local CTCF binding. Electromobility shift assays (EMSAs) using full-length CTCF (CTCF-FL) and a truncated protein, containing just the 11 Zinc Finger binding domain (CTCF-11ZF). We have used three different probes containing the rs225014 common allele (T, Fig. 1, lane 1-2), the minor allele (C, Fig. 1, lane 3-4) and a non-existent allele (G, Fig. 1, lane 5-6). The latter is in highest agreement with the consensus CTCF-binding sequence. As a positive control, a known CTCF site 90 base pairs upstream of rs225014 was used (ENCODE project[1]) (DIO2-CTCF2; **Fig. 1,** lane 7-8). Lanes 1, 3, 5 and 7 depict the truncated CTCF protein, containing just the CTCF DNA binding domain whereas lanes 2, 4, 6 and 8 depict the full length CTCF protein. For lane 1 to 6 no band shifts were observed, indicating that CTCF does not bind the putative CTCF binding site located at the rs225014 SNP. However, we were able to confirm the putative CTCF binding site upstream of rs225014, as for both proteins a band shift was observed (lane 7-8).

Figure S3.

***DIO2* overexpression in hBMSCs, proven by co-transduced eGFP expression. (A)** GFP expression seen in cells cultured on culture dishes **(B)** Normal light microscopy picture of a hBMSC pellet after 3 weeks of culturing. **(C)** Fluorescence picture of a non-transduced hBMSC pellet after 3 weeks of culturing. **(D)** Fluorescence picture of an hBMSC pellet transduced with the control vector containing only the sequence for eGFP after 3 weeks of culturing. **(E)** Fluorescence picture of a hBMSC pellet transduced with the *DIO2* overexpressing vector containing both the sequence for eGFP and *DIO2*. **(B, C, D,and E)** Scale bar, 200 μm.

Figure S4.

The effect of excess T3 and IOP in a human BMSC in vitro chondrogenesis model. (A-G) Alcian Blue staining of sections comparing control (top), thyroid treated (middle) and IOP treated (bottem) chondrogenic hBMSC pellets of **(A-B)** donor 54, **(C-D)** donor 55, **(E-F)** donor 56, and **(G-H)** donor 57. **(A, C, E, and G)** Scale bar, 400 μm. **(B, D, F, and H)** Scale bar, 100 μm.

Figure S5.

Calculating the relative pixel intensity; an automated histology quantification method. (A) Original image algorithm. **(B)** IsoData thresholding without rolling ball algorithm. **(C)** IsoData thresholding after roling ball algorithm. **(D)** masking-area after separation **(E)** Glycosaminoglycan histology separated from the background **(A-E)** Scale bar, 400 μm.

Supplemental Figures:

Figure S1.

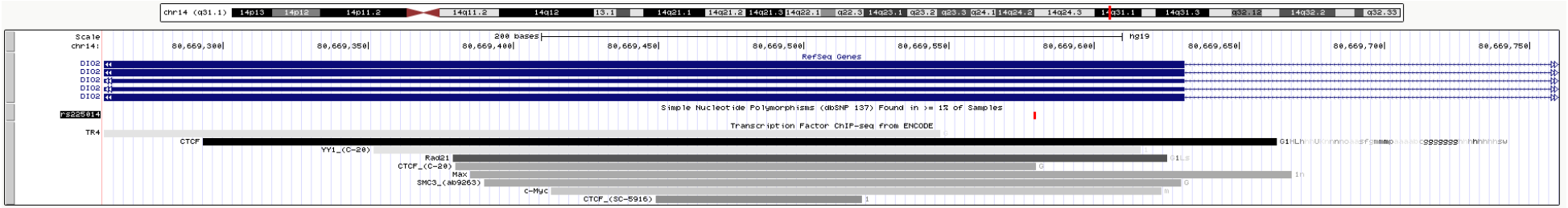
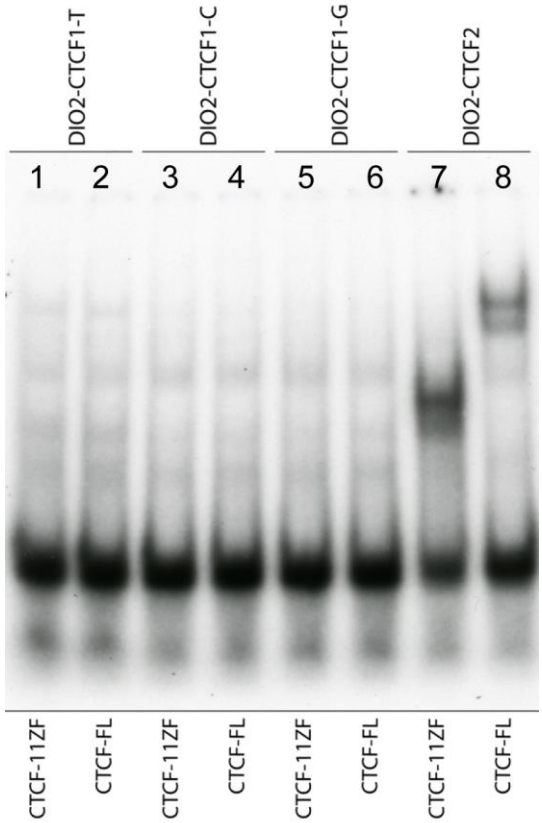


Figure S2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DIO2-CTCF1-C
DIO2-CTCF1-G
DIO2-CTCF2
1 2 3 4 5 6 7 8
CTCF-11ZF
CTCF-FL
CTCF-11ZF
CTCF-FL
CTCF-11ZF
CTCF-FL
CTCF-11ZF
CTCF-FL

Figure S3.

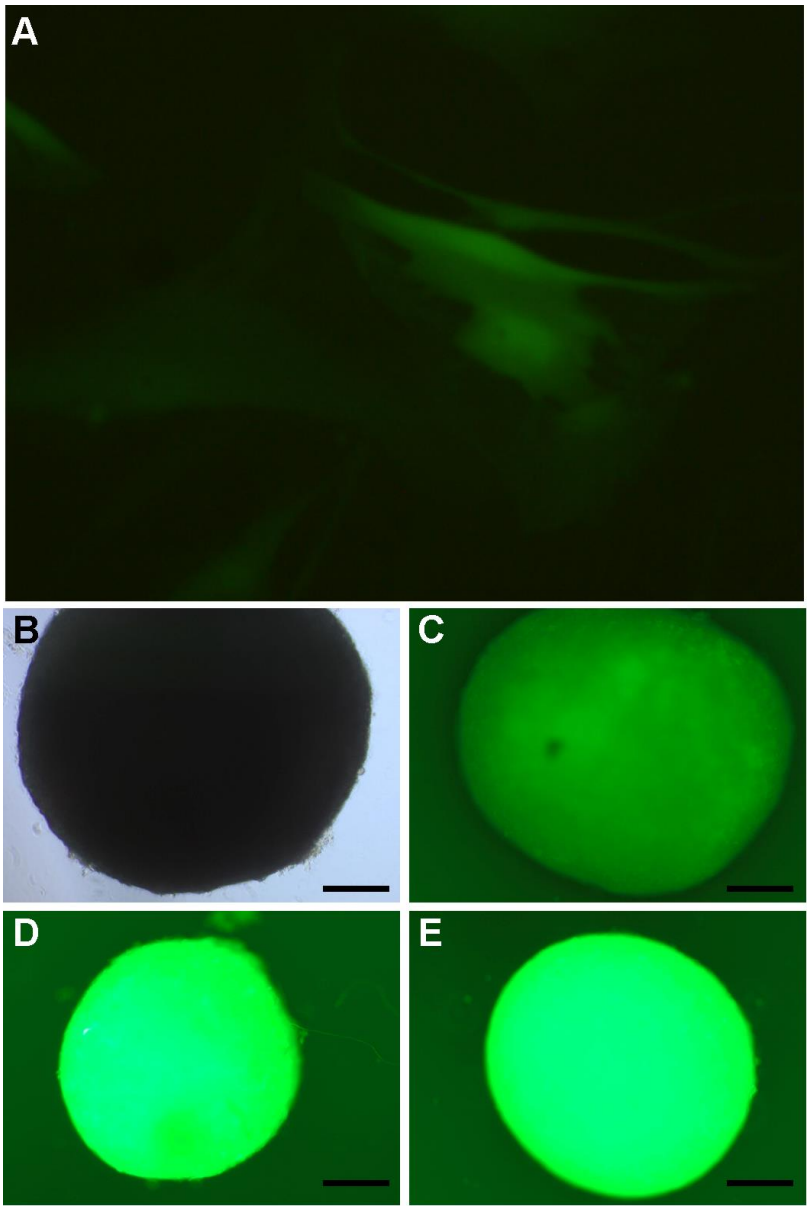


Figure S4.

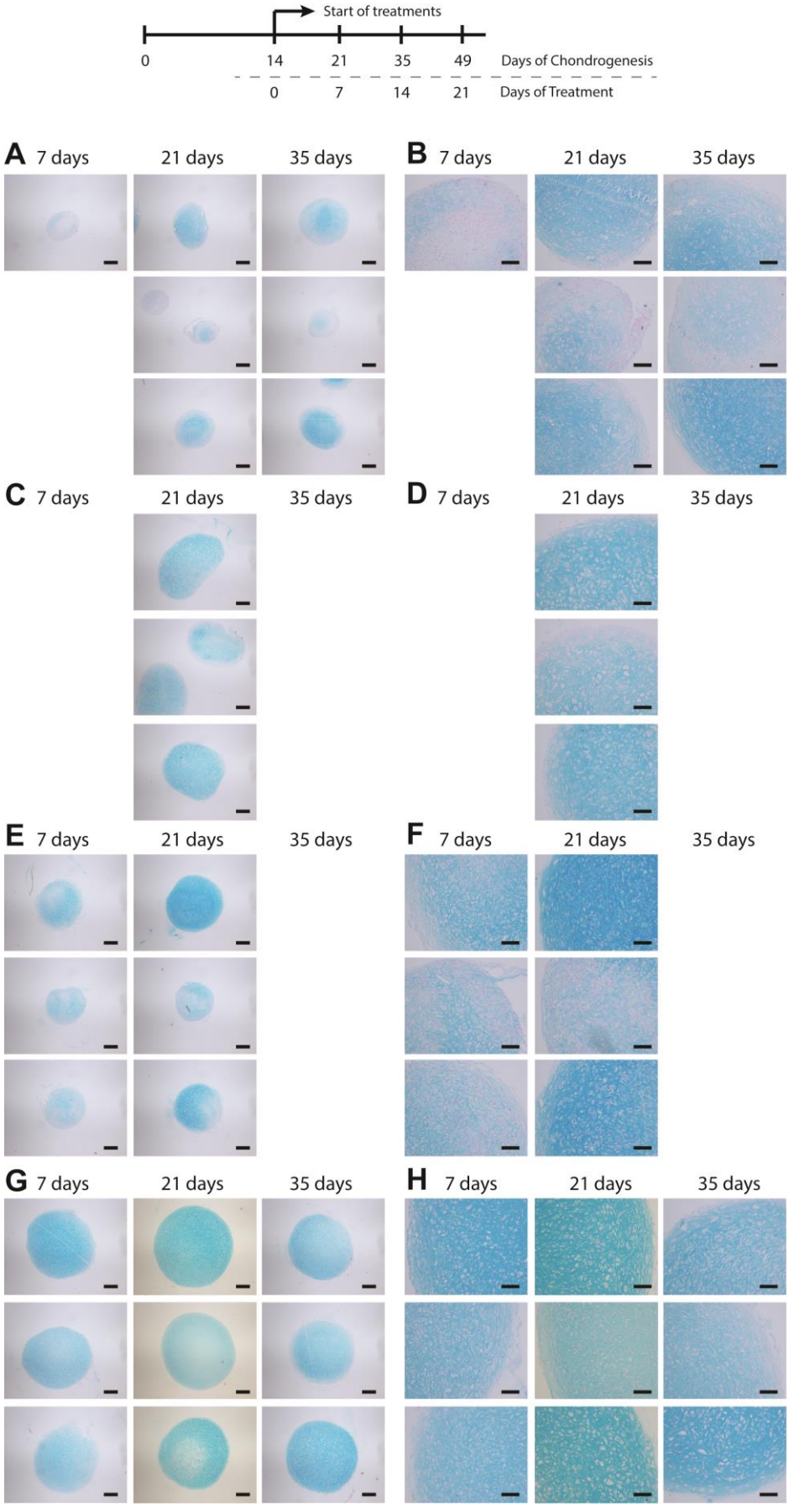
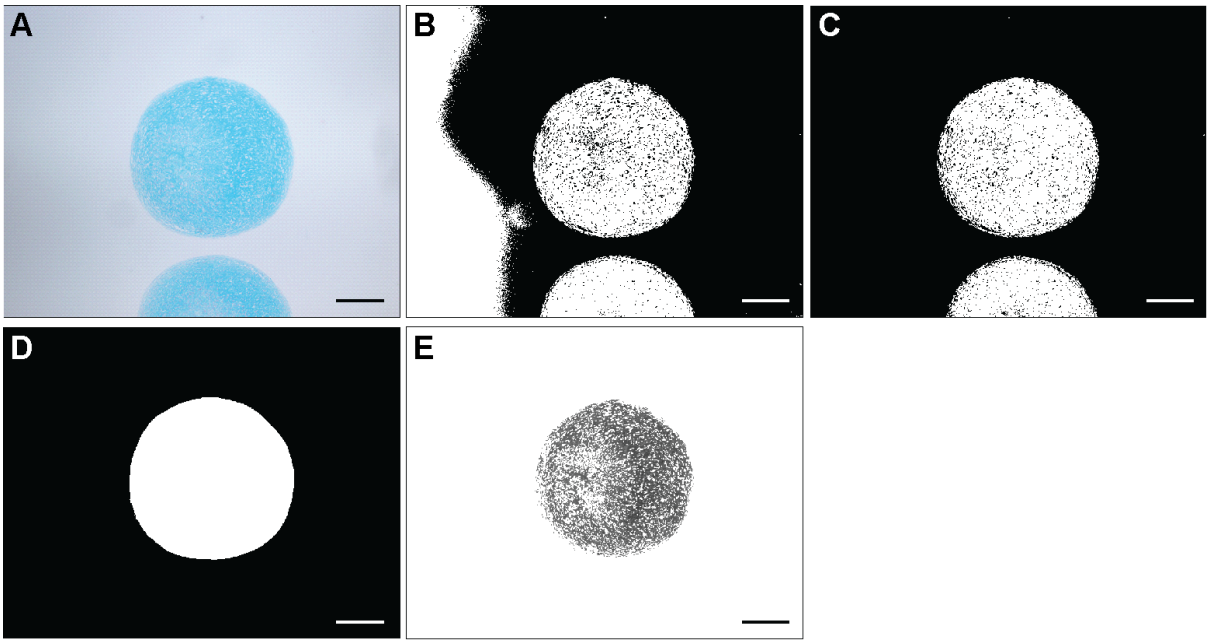

Start of treatments
0
14
21
35
49
Days of Chondrogenesis
0
7
14
21
Days of Treatment
A 7 days
21 days
35 days
B 7 days
21 days
35 days
C 7 days
21 days
35 days
D 7 days
21 days
35 days
E 7 days
21 days
35 days
F 7 days
21 days
35 days
G 7 days
21 days
35 days
H 7 days
21 days
35 days

Figure S5.



Supplementary Table Legends:

Table S1.

Characteristics of samples used in methylation and expression assessment. ([a]) Genotype for the rs225014 SNP

Table S2

Overview of quantified CpG dinucleotides across the *DIO2* locus, as depicted in Figure 2. ([a]) Location of the respective CpG dinucleotides in relation to the *DIO2* TSS. **([b])** Difference in methylation between preserved and matched OA affected samples. **([c])** Crude *P*-value of differential methylation. ([d]) Bonferroni adjusted *P*-value of differential methylation. **([e])** Number of samples used in the analyses for quantification of methylation **([f])** β-estimate of the methylation variable in the regression analysis with *DIO2* expression as outcome. **([g])** Crude *P*-value of the methylation variable in the regression analysis. **([h])** Bonferroni adjusted *P*-value of the methylation variable in the regression analysis. **([i])** Number of samples used in the regression analysis.

Table S3

Multivariate analysis the individual effects. Effects of CpG -2031 methylation, joint site (hip or knee) and rs225014 alleles on *DIO2* expression in articular cartilage

Table S4

Characteristics of hBMSC donors of the RAAK study. ([a]) Genotype for the rs225014 SNP.

Table S5

Used EMSA probes. The CTCF consensus motif is underlined and the nucleotide changes between the probes are indicated in bold.

Table S6

Sequenom amplicons primer sequences.

Table S7

ChIP primer sequences.

Table S8

Real time quantitative PCR primers.

Supplemental Tables:

Table S1

Donor	Gender	Age	Joint	rs225014[a]
1	Female	78	Hip	TC
2	Female	79	Hip	TT
3	Male	67	Hip	TT
4	Male	69	Knee	TC
5	Female	77	Hip	TC
6	Female	74	Hip	TT
7	Male	72	Hip	TC
8	Female	75	Hip	TT
9	Male	54	Knee	TT
10	Male	64	Knee	TC
11	Female	65	Knee	TT
12	Female	68	Hip	TT
13	Male	61	Hip	TC
14	Female	75	Hip	TT
15	Male	61	Knee	TT
16	Female	80	Hip	TT
17	Female	78	Knee	TT
18	Male	44	Hip	TC
19	Female	79	Hip	TC
20	NA	NA	Knee	CC
21	NA	NA	Hip	TC
22	NA	NA	Hip	TC
23	Male	63	Hip	TC
24	Female	69	Hip	TC
25	Female	46	Knee	TT
26	Female	68	Knee	TT
27	NA	69	Knee	TT
28	Male	64	Hip	TC
29	Female	58	Knee	TC
30	Female	57	Knee	TC
31	Female	62	Hip	TC
32	Female	67	Knee	TC
33	Male	68	Hip	TC
34	Male	67	Hip	TC

35	Female	53	Hip	TT
36	Male	82	Knee	TT
37	Female	70	Knee	TC
38	NA	NA	Knee	TT
39	Female	60	Knee	TT
40	NA	NA	Knee	TC
41	Male	69	Knee	TT
42	Male	77	Hip	TC
43	Female	66	Knee	TT
44	NA	NA	Knee	TC
45	Female	80	Knee	CC
46	Female	80	Knee	TT
47	NA	NA	Knee	CC
48	NA	NA	Knee	TC
49	Male	69	Knee	TT
50	Male	NA	Knee	TC
51	Female	62	Knee	TC
52	Male	NA	Knee	TC

Table S2

Location[a]	Mean Preserved	Mean OA	Differential methylation Preserved versus OA cartilage				Correlation between methylation and *DIO2* expression			
			Beta[b]	*P*-value[c]	Adjusted *P*-value[d]	N[e]	Beta[f]	*P*-value[g]	Adjusted *P*-value[h]	N[i]
CpG +15180	0.377	0.391	0.015	0.12293	1	102	-0.581	0.57794	1	85
CpG +15148	0.897	0.902	0.005	0.42454	1	103	-1.960	0.23275	1	86
CpG +15015	0.846	0.842	-0.004	0.39223	1	103	-3.284	0.10764	1	86
CpG +8802	0.678	0.681	0.001	0.92605	1	76	-2.745	0.04668	1	64
CpG +8771	0.852	0.846	-0.007	0.03448	0.793	98	-0.303	0.90671	1	82
CpG +8758	0.906	0.903	-0.003	0.36266	1	101	-1.245	0.69968	1	84
CpG +8742	0.944	0.942	-0.002	0.42346	1	99	-0.126	0.97346	1	82
CpG +8731	0.972	0.974	0.001	0.58860	1	103	-8.680	0.12942	1	86
CpG +8635	0.716	0.724	0.009	0.12923	1	102	0.422	0.80058	1	85
CpG +8547	0.872	0.866	-0.005	0.13632	1	97	2.832	0.35395	1	80
CpG +8527	0.758	0.779	0.017	0.01689	0.389	100	-0.904	0.49324	1	83
CpG +8481	0.167	0.182	0.015	0.00913	0.210	103	-0.201	0.91184	1	86
CpG -219	0.011	0.012	0.000	0.92106	1	94	7.420	0.19666	1	79
CpG -322	0.024	0.026	0.002	0.60637	1	98	-2.373	0.59233	1	83
CpG -473	0.015	0.016	0.001	0.15828	1	102	-7.684	0.64305	1	85
CpG -571	0.031	0.032	0.002	0.28463	1	97	2.355	0.77655	1	80
CpG -642	0.013	0.015	0.003	0.08636	1	98	5.473	0.41953	1	81
CpG -1754	0.874	0.908	0.032	0.00449	0.103	63	1.089	0.42829	1	54
CpG -1761	0.947	0.935	-0.012	0.00011	0.002 **	98	-2.094	0.48780	1	82
CpG -1838	0.763	0.732	-0.031	0.00129	0.030 *	102	-2.468	0.01598	0.368	86
CpG -1942	0.940	0.934	-0.006	0.02555	0.588	103	-1.530	0.67832	1	86
CpG -2009	0.950	0.942	-0.008	0.01004	0.231	103	-2.142	0.54294	1	86
CpG -2031	0.153	0.182	0.028	0.00005	0.001 **	103	4.959	0.00010	0.002 **	87

Table S3

	Beta	Standard Error	Statistic	*P*-value
Intercept	-0.905	0.808	-1.120	0.262
CpG -2031	4.008	1.708	2.347	0.019
Joint	-0.247	0.168	-1.471	0.141
rs225014	0.557	0.154	3.629	0.0003
Gender	0.202	0.146	1.385	0.166
BMI	-0.017	0.013	-1.260	0.208
Age	-0.007	0.007	-0.987	0.323

Table S4

Donor	Sex	Age	rs225014[a]
53	female	66	TC
54	female	72	TT
55	female	81	CC
56	female	59	TC
57	male	80	TC

Table S5

Probe/Primer	Sequence
EMSA DIO2-CTCF1-T F	TGGGTACCATTGCCACTGTTGTCACCTCCTTCTGTACTGGAGACATGCACCACACT
EMSA DIO2-CTCF1-T R	AGTGTGGTGCATGTCTCCAGTACAGAAGGAGGTGACAACAGTGGCAATGGTACCCA
EMSA DIO2-CTCF1-C F	TGGGTACCATTGCCACTGTTGTCACCTCCTTCTGCACTGGAGACATGCACCACACT
EMSA DIO2-CTCF1-C R	AGTGTGGTGCATGTCTCCAGTGCAGAAGGAGGTGACAACAGTGGCAATGGTACCCA
EMSA DIO2-CTCF1-G F	TGGGTACCATTGCCACTGTTGTCACCTCCTTCTGGACTGGAGACATGCACCACACT
EMSA DIO2-CTCF1-G R	AGTGTGGTGCATGTCTCCAGTCCAGAAGGAGGTGACAACAGTGGCAATGGTACCCA
EMSA DIO2-CTCF2 F	GTCAAGTGGCTGAGCCAAAGTTGACCACTAGTGGGCGCTCAGGGCTGGCAAAGTCAAGA
EMSA DIO2-CTCF2 R	TCTTGACTTTGCCAGCCCTGAGCGCCCACTAGTGGTCAACTTTGGCTCAGCCACTTGAC

Table S6

Probe/Primer	Sequence (5'-3')
Amplicon 1 F	GAAAGTTTTTTGTTGTGTGTTAGAA
Amplicon 1 R	CTCCCTTCTTAAATAAATTATTACCATTAT
Amplicon 2 F	GAAAGTTTTTTGTTGTGTGTTAGAA
Amplicon 2 R	AAAACATCACTTCATACCATAATTTAAATA
Amplicon 3 F	GATTAGGTTATGAGGGTTTTTTTT
Amplicon 3 R	AACAATAAAAATTTATTTAATTCACATTTC
Amplicon 4 F	TTAAGTAGTAGGTGTAAGTTTGTGGTTAG
Amplicon 4 R	ACCCTATTCATTCATTCATTCAAAAC
Amplicon 5 F	TTTTTATGTGGTTAAAATTTTTATGATTAT
Amplicon 5 R	ACCCTATTCATTCATTCATTCAAAAC
Amplicon 6 F	TAAAGTTTGGAAGTATTTTTTGAAG
Amplicon 6 R	TTCTTTCTTAAATTACCAAAATTTTT
Amplicon 7 F	GTTTGTGGTGTAAAGTGTTTTTTTT
Amplicon 7 R	TTTTACTTTTCTATTCACTACAATCCTAAC
Amplicon 8 F	ATAGATAGATAGTAAGAAGGGAAAGATAGA
Amplicon 8 R	AATCCAATTACCTCTATCAAAATCC
Amplicon 9 F	GTTGTAGGAGAAGGGGTTTTTTT
Amplicon 9 R	CACCTTCTTAACTTTACCAACCCTA

Table S8

GAPDH	Forward	5'-TGCCATGTAGACCCCTTGAAG-3'
	Reverse	5'-ATGGTACATGACAAGGTGCGG-3'
ACAN	Forward	5'-AGAGACTCACACAGTCGAAACAGC-3'
	Reverse	5'-CTATGTTACAGTGCTCGCCAGTG-3'
COL2A1	Forward	5'-CTACCCCAATCCAGCAAACGT-3'
	Reverse	5'-AGGTGATGTTCTGGGAGCCTT-3'
COL10A1	Forward	5'-GGCAACAGCATTATGACCCA-3'
	Reverse	5'-TGAGATCGATGATGGCACTCC-3'
ADAMTS5	Forward	5'-GTGGTGAAGGTGGTGGTGCT-3'
	Reverse	5'-CTCATGGTCATCTCCCAGCTG-3'
MMP13	Forward	5'-TTGAGCTGGACTCATTGTCG-3'
	Reverse	5'-GGAGCCTCTCAGTCATGGAG-3'
ALPL	Forward	5'-CAAAGGCTTCTTCTTGCTGGTG-3'
	Reverse	5'-CCTGCTTGGCTTTTCCTTCA-3'
RUNX2	Forward	5'-CTGTGGTTACTGTCATGGCG-3'
	Reverse	5'-AGGTAGCTACTTGGGGAGGA-3'
EPAS1	Forward	5'-ACAGGTGGAGCTAACAGGAC-3'
	Reverse	5'-CCGTGCACTTCATCCTCATG-3'
DIO2	Forward	5'-TTCCAGTGTGGTGCATGTCTC-3'
	Reverse	5'-AGTCAAGAAGGTGGCATGTGG-3'
COL1A1	Forward	5'-GTGCTAAAGGTGCCAATGGT-3'
	Reverse	5'-ACCAGGTTCACCGCTGTTAC-3'

References

Reference List

1 Euskirchen GM, Rozowsky JS, Wei CL, Lee WH, Zhang ZD, Hartman S, *et al.* Mapping of transcription factor binding regions in mammalian cells by ChIP: comparison of array- and sequencing-based technologies. *Genome Res* 2007;17:898-909.