## Supplementary Figures

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generated in this study and the Roadmap Epigenomics project.

Figure S20 – Methylation in the six enhancer regions selected for luciferase reporter assays.

Fig. S21 – Relative SOX9 enrichment in new enhancers compared to constant enhancers



Supplementary Fig. S1 – Expression of chondrogenic markers from day 0 to day 14 of chondrogenesis. General chondrocyte markers: COMP, ACAN. Articular chondrocyte markers: COL2A1, SOX9, SIX1, PRG4, BMPR1B, ABI3BP. Hypertrophic/growth plate chondrocyte markers: ITGA4, ALPL, COL10A1, RUNX2, PTH1R



#### Frequency

Supplementary Fig. S2 – Frequency of chromatin state changes in hMSCs to chondrocytes. The y axis shows the chondrocyte chromatin state and the x axis shows what state it was prior to chondrogenesis.



## Figure S3 - GREAT GO terms for chondrocyte chromatin state 1\_TssA



## Figure S4 - GREAT GO terms for chondrocyte chromatin state 2\_TssS



Figure S5 - GREAT GO terms for chondrocyte chromatin state 3\_TssFlnk



## Figure S6 - GREAT GO terms for chondrocyte chromatin state 4\_TssFlnkU



## Figure S7 - GREAT GO terms for chondrocyte chromatin state 5\_TssFlnkD



Figure S8 - GREAT GO terms for chondrocyte chromatin state 6\_TssBiv

Job ID: 20170606-public-3.0.0-35s1sO Display name: user-provided data

### **GO Biological Process**

	-log10(Binomial p value)											
0	10	20	30	40	50	60	70	80	90	100		
ncRNA metabolic process										105.43		
DNA-dependent transcription, elongation							63.86					
transcription elongation from RNA polymerase II promoter						54.51						
tRNA metabolic process						53.45						
DNA-dependent transcription, termination					43.58							
nucleotide-excision repair				37.	23							
mRNA 3'-end processing			31	1.16								
RNA 3'-end processing			30	.41								
positive regulation of viral transcription			29	.74								
transcription elongation from RNA polymerase I promoter			29.	12								
t RNA processing			28.	97								
7-methylguanosine m RNA capping			28.	89								
7-methylguanosine RNA capping			27.8	39								
transcription initiation from RNA polymerase I promoter			27.7	70								
transcription from RNA polymerase I promoter			27.1	7								
termination of RNA polymerase I transcription			26.6	8								
branched-chain amino acid catabolic process			26.2	6								
RNA modification			25.50	)								
regulation of viral transcription			25.13	3								
protein import into mitochondrial inner membrane			25.02	2								

## Figure S9 - GREAT GO terms for chondrocyte chromatin state 7\_TxWk



## Figure S10 - GREAT GO terms for chondrocyte chromatin state 8\_TxS



Figure S11 - GREAT GO terms for chondrocyte chromatin state 9\_TxFlnk



## Figure S12 - GREAT GO terms for chondrocyte chromatin state 10\_EnhG1



# Figure S13 - GREAT GO terms for chondrocyte chromatin state 11\_EnhG1

Job ID: 20170607-public-3.0.0-46HwdS Display name: user-provided data	GO Biological Process -log10(Binomial p value)											
	0 1	0	20	30	40	50	60	70	80	90	100	
cell-matrix adhesion												105.57
muscle cell migration									72.52			
positive regulation of mesenchymal stem cell differentiation								68	.58			
positive regulation of stem cell differentiation								66.	96			
regulation of blood vessel endothelial cell migration								66.	53			
positive regulation of chondrocyte differentiation							6	1.20				
platelet-derived growth factor receptor signaling pathway							6	0.16				
regulation of mesenchymal stem cell differentiation							59	9.85				
smooth muscle cell-matrix adhesion							57	.96				
extrinsic apoptotic signaling pathway							57.	40				
cell-substrate junction assembly	_						56.	99				
regulation of alpha-beta 1 cell proliferation							55.2	20				
nucleotide-sugar metabolic process	_		_	_	_		54.2	1				
inactivation of MAPK activity	_			_	_		53.22	2				
negative regulation of blood vessel endotnellal cell migration	_				_	- 40	52.73					
positive regulation of cartilage development	-		_			4	9.99					
male germ-line sex determination	_			_	_	48	1.70					
NI S-bearing protein import into puclous	_					49	90					
EBK1 and EBK2 cascade						40	60					

Figure S14 - GREAT GO terms for chondrocyte chromatin state 12\_EnhA

#### **GO Biological Process**

	-log10(Binomial p value)								
a	) 5	5 1	0	15	20	25	30	35	
forebrain neuroblast division									38.53
negative regulation of stem cell differentiation									38.06
positive regulation of transforming growth factor beta production							30	).82	
anatomical structure regression							30	.32	
blood vessel endothelial cell differentiation							29.	95	
forebrain radial glial cell differentiation							28.56	;	
arterial endothelial cell differentiation						2	26.71		
negative regulation of lipoprotein oxidation						2	26.62		
positive regulation of GTP catabolic process						2	6.52		
negative regulation of lipoprotein lipid oxidation						2	6.31		
negative regulation of cytokine production involved in inflammatory response						2	6.31		
negative regulation of T cell migration						2	6.31		
positive regulation of cell proliferation in bone marrow						2	6.29		
Notch signaling involved in heart development						25	.60		
renal interstitial cell differentiation						25	.18		
odontoblast differentiation						24.	93		
positive regulation of peptidyl-tyrosine autophosphorylation						24.	71		
negative regulation of smooth muscle cell-matrix adhesion						24.5	58		
smoothened signaling pathway involved in ventral spinal cord interneuron specification						24.3	8		
moothened signaling pathway involved in spinal cord motor neuron cell fate specification						24 3	18		

## Figure S15 - GREAT GO terms for chondrocyte chromatin state 14\_EnhP



## Figure S16 - GREAT GO terms for chondrocyte chromatin state 15\_Repr



Figure S17 - GREAT GO terms for chondrocyte chromatin state 16\_Quies



Figure S18 - ChromHMM emission parameters for Roadmap's 18 state model (A) and (B) chondrogenesis 16 state model. Roadmap's 1\_TssA state and the chondrogenesis 2\_TssS state comprises equal probabilities of H3K4me3 and H3K27ac histone marks. Likewise, Roadmap's 9\_EnhA1 and chondrogenesis 13\_EnhS have similar levels of H3K4me1 and H3K27ac.Other states considered comparable were Roadmap's 5\_Tx with chondrogenesis 8\_TxS, Roadmap's 6\_TxWk with chondrogenesis 7\_TxWk, Roadmap's 11\_EnhWk with chondrogenesis 14\_EnhP, Roadmap's 16\_ReprPC with chondrogenesis 15\_Repr and Roadmap's 18 Quies with chondrogenesis 16 Quies



*Figure S19 – Heatmaps of Jaccard similarity of cell types between equivalent chromatin states generated in this study and the Roadmap Epigenomics project. The Jaccard similarity metric ranges from 1 (identical overlap) to 0 (no overlap).* 



Figure S20 – Methylation in the six enhancer regions selected for luciferase reporter assays. Methylation beta values are shown on the y-axis and the CpG location within the enhancer is shown on the x-axis.

Logo	Database ?	ID ?	Alt ID ?	p-value ?	E-value ?	TP Thresh	TP (%) ?	FP (%) ?
<sup>₽</sup> <mark>₩<sub>≂</sub>ҿ₀, ₅<sub>₹</sub>ҳҬҬҀҬ<sub>ҫҫ</sub></mark>	motifs	3	AADRVNNBYWTTGTKY	3.26e-30	3.58e-29	1.07	6504 (10.6%)	8405 (8.7%)
	motifs	11	TCAAAG	6.19e-17	6.81e-16	5.73	4838 (7.9%)	6369 (6.6%)
<b><sup>®</sup>C<sub>E</sub>ATIGIT</b> E	motifs	10	CYATTGTTY	7.75e-17	8.52e-16	1.06	11681 (19.0%)	16498 (17.2%)
AATCAAT <sub>333</sub> AGTGAT <sub>4</sub>	motifs	7	AATCAATKKYAGTGATW	4.35e-4	4.78e-3	3.21	600 (1.0%)	711 (0.7%)
<sup>⋬</sup> <mark>௲Ç<sub>₹</sub>₳₶₶₢₶₶</mark> ₽	motifs	9	YCYATTGTTYT	1.18e-3	1.30e-2	1.08	3607 (5.9%)	5063 (5.3%)
	motifs	6	RAACAATTKCAGTGTTT	4.07e-1	4.48e0	23.80	171 (0.3%)	189 (0.2%)
	motifs	2	YSWTTGKYY	5.73e-1	6.31e0	3.28	7952 (13.0%)	11794 (12.3%)
AATGAATT <sub>3</sub> CAGTCATT	motifs	8	AATGAATTKCAGTCATT	6.88e-1	7.56e0	33.18	137 (0.2%)	149 (0.2%)
<b>ATGAAT</b> AT <b>JCAGTCAT</b>	motifs	5	ATGAATRTKCAGTCAT	9.89e-1	1.09e1	3.78	556 (0.9%)	747 (0.8%)
<b>ATCAAT<sub>e</sub>T<sub>a</sub>CAGTGAT</b>	motifs	4	ATCAATRTKCAGTGAT	1.00e0	1.10e1	3.78	433 (0.7%)	643 (0.7%)
AACAAT	motifs	1	AACAATRTGCAGTGTT	1.00e0	1.10e1	14.43	224 (0.4%)	308 (0.3%)

*Figure S21 – Relative enrichment of SOX9 binding motifs in new enhancers to constant enhancers*