Figure S1. Biological validation of expression profiling data.



**A** Representative images of the morphological changes during myogenic differentiation and tadditional 3D-FISH fluorescent image data with gene locus in red and DNA counterstaining with DAPI (blue) Bar: 10  $\mu$ m. **B-C** All genes detected in the transcriptional profiling with statistical significance and at least two fold expression (up and down) were analyzed for their gene ontology using the DAVID database. Pie charts (grouped by fold changes) give the ratio of muscle related (dark grey) and proliferation related genes (light grey), respectively.

Figure S2. Morphological differences in cellular systems.



The nuclear volume of >30 nuclei from myoblasts (MB, black) and myotubes (MT, grey) were evaluated, resulting in the shown frequency distribution of volume bins. During myogenesis the nuclear volume decreases to half, which consequently reduces all distances measured. This stark morphological change together with an increased clustering of constitutive heterochromatin, compels the use of a normalization procedure.

A (diff. sys)			3D distan	ices: gene	to nuclear peri	phery		
	At	osolute 3D dist	ance [µm]		single	cell normalized	I 3D distan	ces
Gene	AVG (MT)	AVG (MB)	Δ D [µm]	GPE	AVG n (MT)	AVG n (MB)	ΔnD	GPE
Birc5	1.88 (±0.83)	1.53 (±0,66)	0.34	1.06	0.77 (±0.17)	0.76 (±0.18)	0.01	0.25
Brca1	1.67 (±0.60)	1.10 (±0.41)	0.57	0.72	0.84 (±0.14)	0.74 (±0.17)	0.10	0.22
Coro1c	1.14 (±0.63)	1.35 (±0.78)	- 0.21	1.00	0.58 (±0.20)	0.67 (±0.24)	- 0.09	0.31
Mef2c	1.23 (±0.45)	1.10 (±0.59)	0.13	0.74	0.66 (±0.15)	0.64 (±0.23)	0.02	0.28
Myom2	0.95 (±0.57)	0.74 (±0.52)	0.21	0.77	0.51 (±0.19)	0.54 (±0.30)	- 0.03	0.35
Nrde2	1.33 (±0.47)	0.96 (±0.40)	0.37	0.62	0.74 (±0.14)	0.63 (±0.18)	0.11	0.23
Obscn	1.61 (±0.69)	1.25 (±0.73)	0.36	1.00	0.72 (±0.17)	0.69 (±0.27)	0.03	0.32
Slc19a2	1.87 (±0.73)	0.91 (±0.48)	0.96	0.88	0.75 (±0.16)	0.63 (±0.23)	0.12	0.28
Трт3	1.07 (±0.45)	1.09 (±0.50)	- 0.02	0.68	0.63 (±0.18)	0.66 (±0.21)	- 0.03	0.27
Ttk	1.59 (±0.84)	1.07 (±0.78)	0.51	1.15	0.68 (±0.21)	0.58 (±0.27)	0.11	0.34
В			3D dista	ances: gen	e to chromoce	nter		
(diff. sys)	At	osolute 3D dist	ance [µm]		Single	cell normalized	I 3D distan	ces
Gene	AVG (MT)	AVG (MB)	Δ D [µm]	GPE	AVG n (MT)	AVG n (MB)	ΔnD	GPE
Birc5	1.25 (±0.55)	0.97 (±0,44)	0.29	0.70	0.66 (±0.22)	0.60 (±0.21)	0.06	0.31
Brca1	1.22 (±0.51)	1.00 (±0.56)	0.22	0.76	0.65 (±0.24)	0.67 (±0.23)	- 0.01	0.33
Coro1c	1.38 (±0.62)	1.19 (±0.59)	0.20	0.86	0.66 (±0.23)	0.63 (±0.25)	0.03	0.34
Mef2c	0.98(±0.57)	0.94 (±0.56)	0.22	0.83	0.58 (±0.23)	0.55 (±0.22)	0.04	0.31
Myom2	1.04 (±0.52)	0.97 (±0.44)	0.01	0.72	0.50 (±0.26)	0.58 (±0.21)	- 0.07	0.33
Nrde2	1.24 (±0.62)	1.38 (±0.70)	- 0.34	0.88	0.60 (±0.25)	0.72 (±0.23)	- 0.13	0.34
Obscn	1.35 (±0.62)	1.04 (±0.54)	0.20	0.82	0.61 (±0.24)	0.54 (±0.25)	0.07	0.35
Slc19a2	1.21 (±0.60)	1.23 (±0.60)	0.12	0.86	0.65 (±0.22)	0.72 (±0.22)	- 0.07	0.31
Tpm3	1.21 (±0.60)	1.08 (±0.54)	0.12	0.81	0.62 (±0.25)	0.67 (±0.22)	- 0.05	0.33
Ttk	1.01 (±0.56)	1.11 (±0.46)	- 0.11	0.72	0.51 (±0.24)	0.63 (±0.20)	- 0.11	0.32

## Table S1. 3D-distance measurements in undifferentiated myoblasts (MB) and differentiated myotubes (MT) (differentiation system).

Genes selected in the *in vitro* differentiation system were visualized by 3D-FISH using BAC probes and distances towards the nearest heterochromatin compartment indicated were measured and averaged for undifferentiated myoblasts (MB) and differentiated myotubes (MT). Average distances towards the nuclear periphery (A) or towards the nearest chromocenter (B) are given in the respective AVG column with standard deviation included in

brackets (for detailed information see also Table S3 and Figure S5). The average distance change ( $\Delta$  D) was calculated by subtracting the mean distance values of high and low expressing cells. Linear Gaussian propagation error (GPE) was calculated as the square root of the sum of squared individual standard deviations per sample. Single nucleus normalization (n) was performed as described in the methods yielding comparable distance changes unaffected by nuclear morphology differences. A negative  $\Delta$  D (red) refers to a decrease in distance or movement towards the compartment during differentiation.

A (ect. sys.)			3D distan	ces: gene	to nuclear peri	phery		
(,-,-,,-,,-,,-,,-,,,-,,,-,,,-,,,-,,	Ab	solute 3D dist	ance [µm]		single	cell normalized	3D distan	ces
Gene	High MeCP2 AVG	Low MeCP2 AVG	Δ D [µm]	GPE	High MeCP2 AVG n	Low MeCP2 AVG n	ΔnD	GPE
Bdnf	0.76 (±0.38)	0.74 (±0,45)	0.02	0.58	0.54 (±0.20)	0.54 (±0.23)	0.00	0.30
Birc5	1.69 (±0.65)	1.49 (±0.80)	0.20	1.04	0.79 (±0.18)	0.72 (±0.21)	0.06	0.28
Brca1	1.72 (±0.74)	1.70 (±0.64)	0.02	0.98	0.79 (±0.18)	0.81 (±0.16)	- 0.02	0.24
Cdc20	0.83 (±0.45)	0.76 (±0.45)	0.07	0.64	0.56 (±0.21)	0.53 (±0.25)	0.02	0.32
Col6a2	1.30 (±0.57)	1.04 (±0.49)	0.26	0.75	0.72 (±0.16)	0.67 (±0.20)	0.05	0.26
Myom2	1.00 (±0.69)	0.99 (±0.63)	0.00	0.93	0.57(±0.25)	0.58 (±0.63)	0.00	0.35
Prl7c1	1.38 (±0.75)	1.21 (±0.64)	0.16	0.98	0.71 (±0.24)	0.67 (±0.24)	0.04	0.34
Ttk	0.92 (±0.46)	0.82 (±0.53)	0.10	0.71	0.60 (±0.22)	0.56 (±0.25)	0.04	0.33
B (ect. Sys.)			3D dista	ances: gen	e to chromoce	nter		
	Ab	solute 3D dist	ance [µm]		Single	cell normalized	l 3D distan	ces
Gene	High MeCP2 AVG	Low MeCP2 AVG	Δ D [µm]	GPE	High MeCP2 AVG n	Low MeCP2 AVG n	ΔnD	GPE
Bdnf	1.47 (±0.79)	0.99 (±0,52)	0.48	0.94	0.72 (±0.25)	0.68 (±0.25)	0.00	0.36
Birc5	0.89 (±0.51)	0.94 (±0.49)	- 0.04	0.70	0.50(±0.22)	0.60 (±0.24)	- 0.09	0.32
Brca1	0.93 (±0.45)	0.86 (±0.51)	0.07	0.68	0.56 (±0.22)	0.54 (±0.26)	0.02	0.34
Cdc20	1.36 (±0.65)	1.04 (±0.59)	0.32	0.88	0.72 (±0.23)	0.69 (±0.21)	0.03	0.31
Col6a2	0.94 (±0.56)	0.78 (±0.47)	0.17	0.73	0.52 (±0.23)	0.59 (±0.24)	- 0.07	0.33
Myom2	0.86 (±0.48)	0.71 (±0.38)	0.15	0.62	0.57 (±0.25)	0.52 (±0.24)	0.05	0.34
Prl7c1	0.68 (±0.38)	0.69 (±0.39)	- 0.01	0.55	0.47 (±0.22)	0.50 (±0.23)	- 0.03	0.32
Ttk	1.05 (±0.61)	0.85 (±0.53)	0.20	0.81	0.58 (±0.25)	0.59 (±0.28)	- 0.01	0.37

Table S2. 3D-distance measurements in high and low expressing MeCP2 cells (ectopic heterochromatin reorganization system).

Genes selected in the ectopic MeCP2 system were visualized by 3D-FISH using BAC probes and distances towards the nearest heterochromatin compartment were measured and averaged for high and low MeCP2 expressing cells. Average distances towards the nuclear periphery (A) or towards nearest chromocenter (B) are given in the respective AVG column with standard deviation included in brackets (for detailed information see also Table S4 and Figure S6). The average distance change ( $\Delta$  D) was calculated by subtracting the mean distance values of high and low expressing cells. Linear Gaussian propagation error (GPE) was calculated as the square root of the sum of squared individual standard deviations per sample. Single nucleus normalization (n) was performed as described in the methods yielding comparable distance changes. Negative  $\Delta$  D values (red) refer to a decrease in distance or movement towards the indicated compartment in differentiation independent reorganized heterochromatin (i.e. high levels of MeCP2).



Figure S3. Average normalized 3D distances per gene and condition towards the heterochromatin compartment indicated.

Overview of normalized 3D distance measurements (see Tables S1 and S2) in the differentiation system (A) and differentiation independent ectopic MeCP2 system (B). Means are highlighted either as black circles (myotubes and high MeCP2 levels) or orange squares (myoblasts and low MeCP2 levels); whiskers indicate the 95% confidence interval.



Figure S4. Average normalized distance changes per gene and condition towards the heterochromatin compartment indicated.

Overview of normalized 3D distance changes (see Tables S1 and S2) in the differentiation system (A) and differentiation independent ectopic MeCP2 system (B).

	Gene	Bir	rc5	Bro	ca1	Cor	ro1c	Ме	f2c	Мус	om2	Nrc	de2	Ob	scn	Slc1	9a2	Тр	<i>m</i> 3	Т	tk
A	Datase t	MT	MB	MT	MB	MT	MB	MT	MB	MT	MB	MT	MB	МТ	MB	MT	MB	МТ	MB	МТ	MB
	Sampl e size	77	80	74	76	188	51	100	126	86	92	61	77	129	61	111	116	113	84	100	83
ter	AVG	1.25	0.97	1.22	1.00	1.38	1.19	1.16	0.94	0.98	0.97	1.04	1.38	1.24	1.04	1.35	1.23	1.21	1.08	1.01	1.11
ocen	STD	0.55	0.44	0.51	0.56	0.62	0.59	0.61	0.56	0.57	0.44	0.52	0.70	0.62	0.54	0.62	0.60	0.60	0.54	0.56	0.46
nom	Q0	0.18	0.25	0.08	0.00	0.00	0.16	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.19	0.18	0.00	0.00	0.11	0.16	0.00
to cł	Q1	0.91	0.65	0.89	0.58	0.99	0.75	0.79	0.61	0.52	0.67	0.74	0.88	0.82	0.62	0.94	0.82	0.78	0.71	0.59	0.80
ene	Q2	1.18	0.92	1.19	1.00	1.35	1.18	1.07	0.82	0.89	0.87	1.03	1.28	1.18	1.03	1.23	1.17	1.20	1.03	0.92	1.09
m];g	Q3	1.63	1.24	1.53	1.20	1.71	1.67	1.46	1.15	1.38	1.17	1.39	1.88	1.56	1.38	1.74	1.62	1.58	1.38	1.34	1.39
n] s	Q4	2.99	2.22	2.52	3.15	3.31	2.40	3.84	3.46	2.36	2.28	2.21	3.32	3.17	2.37	3.71	3.21	3.04	2.49	3.05	2.14
abs. distances [µm];	SEM	0.06	0.05	0.06	0.06	0.05	0.30	0.06	0.19	0.06	0.22	0.07	0.08	0.05	0.07	0.06	0.26	0.06	0.23	0.06	0.05
s. dist	ΔD	0.2	29	0.	22	0.2	20	0.3	22	0.0	01	-0.	34	0.3	20	0.	12	0.	12	-0	.11
ab	GPE	0.1	70	0.	76	0.8	86	0.	83	0.	72	0.8	88	0.8	82	0.8	86	0.	81	0.	72
	AVG	0.66	0.60	0.65	0.67	0.66	0.63	0.58	0.55	0.50	0.58	0.60	0.72	0.61	0.54	0.65	0.72	0.62	0.67	0.51	0.63
ces	STD	0.22	0.21	0.24	0.23	0.23	0.25	0.23	0.22	0.26	0.21	0.25	0.23	0.24	0.25	0.22	0.22	0.25	0.22	0.24	0.20
stan	Q0	0.16	0.17	0.08	0.09	0.07	0.13	0.05	0.08	0.07	0.13	0.07	0.10	0.07	0.13	0.13	0.07	0.07	0.16	0.10	0.07
ed di	Q1	0.53	0.44	0.49	0.54	0.51	0.46	0.41	0.41	0.28	0.45	0.45	0.59	0.44	0.33	0.51	0.60	0.41	0.51	0.29	0.45
aliz(	Q2	0.68	0.60	0.68	0.71	0.69	0.62	0.58	0.54	0.46	0.55	0.64	0.78	0.64	0.56	0.67	0.79	0.65	0.70	0.50	0.65
norm	Q3	0.87	0.78	0.86	0.86	0.86	0.88	0.78	0.72	0.75	0.69	0.81	0.90	0.80	0.77	0.85	0.90	0.81	0.85	0.71	0.80
ı snə	Q4	1.00	0.97	1.00	0.98	0.99	0.97	1.00	1.00	0.97	0.99	0.98	1.00	1.00	0.95	0.99	1.00	0.99	0.98	0.99	0.97
ncle	SEM	0.03	0.02	0.03	0.03	0.02	0.13	0.02	0.08	0.03	0.10	0.03	0.03	0.02	0.03	0.02	0.09	0.02	0.09	0.02	0.02
single r	ΔD	0.06		-0.01		0.03		0.04		-0.07		-0.13		0.07		-0.07		-0.05		-0.11	
3	GPE	0.31		0.33		0.34		0.31		0.33		0.34		0.35		0.31		0.33		0.32	

## Table S3. A) Statistics summary table of 3D distance measurements (differentiation system).

	Gene	Birc5		Brca	1	Coro	01c	Mef2	?c	Myo	m2	Nrde	2	Obso	cn	Slc1	9a2	Трт	3	Ttk	
В	Datase t	MT	MB	MT	MB	MT	MB	MT	MB	MT	MB	MT	MB	MT	MB	MT	MB	MT	MB	MT	MB
	Sample size	77	80	74	76	188	51	100	126	86	92	61	77	129	61	111	116	113	84	100	83
ery	AVG	1.88	1.53	1.67	1.10	1.14	1.35	1.23	1.10	0.95	0.74	1.33	0.96	1.61	1.25	1.87	0.91	1.07	1.09	1.59	1.07
eriph	STD	0.83	0.66	0.60	0.41	0.63	0.78	0.45	0.59	0.57	0.52	0.47	0.40	0.69	0.73	0.73	0.48	0.45	0.50	0.84	0.78
d .or	Q0	0.30	0.35	0.45	0.29	0.08	0.00	0.21	0.00	0.20	0.00	0.50	0.30	0.32	0.00	0.43	0.00	0.16	0.00	0.16	0.00
to ni	Q1	1.31	1.16	1.27	0.79	0.62	0.78	0.89	0.62	0.54	0.21	0.98	0.60	1.09	0.65	1.26	0.58	0.82	0.76	1.01	0.43
ene	Q2	1.73	1.51	1.64	1.04	1.06	1.24	1.23	1.08	0.76	0.76	1.32	0.97	1.57	1.18	1.81	0.79	0.99	1.07	1.38	0.97
ח]; g	Q3	2.38	1.93	2.12	1.37	1.53	1.81	1.57	1.51	1.37	1.13	1.63	1.25	2.06	1.94	2.37	1.17	1.33	1.43	2.09	1.46
un] s	Q4	4.08	3.69	3.06	2.19	3.35	3.54	2.15	2.56	3.01	2.14	2.43	2.10	3.50	2.57	3.69	2.57	2.19	2.54	3.75	4.13
nces	SEM	0.09	0.07	0.07	0.05	0.05	0.36	0.05	0.20	0.06	0.16	0.06	0.05	0.06	0.09	0.07	0.18	0.04	0.22	0.08	0.02
s. dista	ΔD	0.3	34	0.	57	-0.	21	0.	13	0.3	21	0.3	37	0.3	36	0.9	96	-0.	02	0.	51
abs	GPE	1.(	06	0.	72	1.(	00	0.1	74	0.1	77	0.0	62	1.0	00	0.8	88	0.0	68	1.	15
	AVG	0.77	0.76	0.84	0.74	0.58	0.67	0.66	0.64	0.51	0.54	0.74	0.63	0.72	0.69	0.75	0.63	0.63	0.66	0.68	0.58
ces	STD	0.17	0.18	0.14	0.17	0.20	0.24	0.15	0.23	0.19	0.30	0.14	0.18	0.17	0.27	0.16	0.23	0.18	0.21	0.21	0.27
stan	Q0	0.28	0.34	0.43	0.32	0.15	0.11	0.22	0.05	0.20	0.03	0.45	0.26	0.28	0.05	0.31	0.06	0.17	0.04	0.18	0.05
ip pi	Q1	0.67	0.64	0.75	0.61	0.42	0.52	0.55	0.47	0.37	0.23	0.63	0.48	0.60	0.51	0.62	0.50	0.55	0.54	0.54	0.36
alize	Q2	0.81	0.80	0.86	0.76	0.60	0.73	0.68	0.68	0.46	0.62	0.73	0.61	0.73	0.74	0.78	0.64	0.63	0.66	0.70	0.60
lorm	Q3	0.91	0.91	0.95	0.87	0.74	0.87	0.78	0.83	0.66	0.81	0.85	0.79	0.87	0.94	0.89	0.83	0.77	0.82	0.86	0.80
u sna	Q4	1.00	1.00	1.00	1.00	0.99	1.00	0.89	0.99	0.97	1.00	0.98	1.00	1.00	1.00	0.99	1.00	0.95	1.00	1.00	1.00
iucle	SEM	0.02	0.02	0.02	0.02	0.01	0.14	0.02	0.09	0.02	0.10	0.03	0.02	0.02	0.03	0.02	0.09	0.02	0.10	0.02	0.03
single n	ΔD	0.0	01	0.	10	-0.	09	0.0	02	-0.	03	0.	11	0.0	03	0.	12	-0.	03	0.	11
0	GPE	0.2	25	0.	22	0.3	31	0.:	28	0.3	35	0.:	23	0.3	32	0.:	28	0.:	27	0.	34

## Table S3. B) Statistics summary table of 3D distance measurements (differentiation system).

Detailed statistical overview of 3D distance measurements within the differentiation system. (A) Addresses distances and distance changes  $\Delta$  D towards chromocenters (B) towards the nuclear periphery. "Data set" refers to the respective chromatin state (i.e., either myoblasts [MB] or myotubes [MT]). The total number of measurements is indicated as sample size. For each data set absolute and normalized values are given as: mean distances (AVG), standard deviation (STD), min. (Q0), max. (Q4), median (Q2), standard error of the mean (SEM), and mean distance changes ( $\Delta$  D) (see also Figure S5).  $\Delta$  D was calculated as the difference of mean MT and mean MB distances. The linear Gaussian propagation error (GPE) was calculated as the square root of the sum of squared individual standard deviations per sample.

		Gene	Bdnf		Birc	5	Brca	1	Cdc	20	Col6	a2	Муо	m2	Prl2o	c2	Ttk	
	4	MeCP2 level	high	low	high	low	high	low										
		Sample size	54	77	47	59	62	72	61	77	68	74	72	65	56	77	78	61
		AVG	1.47	0.99	0.89	0.94	0.93	0.86	1.36	1.04	0.94	0.78	0.86	0.71	0.68	0.69	1.05	0.85
		STD	0.79	0.52	0.51	0.49	0.45	0.51	0.65	0.59	0.56	0.47	0.48	0.38	0.38	0.39	0.61	0.53
Ē	ter	Q0	0.00	0.25	0.00	0.08	0.00	0.00	0.08	0.00	0.08	0.00	0.08	0.00	0.00	0.00	0.00	0.00
es [I	cen	Q1	0.94	0.57	0.54	0.56	0.66	0.47	0.82	0.69	0.56	0.48	0.47	0.41	0.42	0.43	0.61	0.43
tanc	omc	Q2	1.45	0.94	0.80	0.90	0.89	0.77	1.20	0.88	0.80	0.69	0.77	0.67	0.61	0.66	0.92	0.88
e dis	chr	Q3	2.01	1.28	1.11	1.19	1.17	1.26	1.91	1.27	1.21	1.01	1.22	0.92	0.98	0.94	1.40	1.24
olute	ne tc	Q4	3.09	2.53	2.44	2.08	2.01	2.20	2.46	4.03	2.88	2.62	2.57	1.89	1.64	1.70	2.77	2.36
abso	ger	SEM	0.11	0.06	0.07	0.06	0.06	0.06	0.08	0.07	0.07	0.06	0.06	0.05	0.05	0.04	0.07	0.07
		ΔD	0.	48	-0	.04	0.	07	0.	32	0.	17	0.	15	-0.	.01	0.	20
		GPE	0.	94	0	.70	0.	68	0.	88	0.	73	0.	62	0.	55	0.	81
		AVG	0.72	0.68	0.50	0.60	0.56	0.54	0.72	0.69	0.52	0.59	0.57	0.52	0.47	0.50	0.58	0.59
		STD	0.26	0.25	0.22	0.24	0.22	0.26	0.23	0.21	0.23	0.24	0.25	0.24	0.22	0.23	0.25	0.28
ed		Q0	0.09	0.23	0.10	0.13	0.09	0.08	0.11	0.09	0.13	0.09	0.12	0.08	0.07	0.05	0.09	0.07
naliz		Q1	0.54	0.43	0.34	0.43	0.41	0.32	0.55	0.55	0.35	0.41	0.33	0.32	0.31	0.33	0.37	0.37
norn	ses	Q2	0.80	0.75	0.45	0.58	0.57	0.48	0.77	0.69	0.49	0.61	0.57	0.53	0.47	0.49	0.60	0.66
eus	tanc	Q3	0.94	0.90	0.71	0.80	0.71	0.76	0.94	0.89	0.69	0.77	0.79	0.70	0.62	0.69	0.78	0.84
nucl	dis	Q4	1.00	0.99	0.97	0.97	0.96	0.95	0.99	1.00	0.99	1.00	0.98	0.95	0.91	0.92	1.00	0.98
gle I		SEM	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04
sin		ΔD	0.	03	-0	.09	0.	02	0.	03	-0.	.07	0.	05	-0.	.03	-0	.01
		GPE	0.	36	0	.32	0.	34	0.	31	0.	33	0.	34	0.	32	0.	37

Table S4. A) Statistics summary table of 3D distance measurements (ectopic MeCP2 system).

		Gene	Bdnf		Bircs	5	Brca	1	Cdc2	20	Col6	a2	Муо	m2	Prl2c	:2	Ttk	
	В	MeCP2 level	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low
		Sample size	54	77	47	59	62	72	61	77	68	74	72	65	56	77	78	61
		AVG	0.76	0.74	1.69	1.49	1.72	1.70	0.83	0.76	1.30	1.04	1.00	0.99	1.38	1.21	0.92	0.82
		STD	0.38	0.45	0.65	0.80	0.74	0.64	0.45	0.45	0.57	0.49	0.69	0.63	0.75	0.64	0.46	0.53
Ē	Jery	Q0	0.16	0.00	0.08	0.19	0.08	0.16	0.21	0.00	0.20	0.20	0.00	0.00	0.11	0.08	0.00	0.00
es [h	eripł	Q1	0.41	0.40	1.22	0.97	1.17	1.27	0.45	0.39	0.94	0.64	0.48	0.47	0.78	0.63	0.56	0.43
ance	ar p	Q2	0.78	0.64	1.71	1.36	1.72	1.60	0.78	0.79	1.18	0.99	0.80	0.81	1.48	1.37	0.87	0.67
dist	ucle	Q3	1.03	1.04	2.21	2.04	2.23	2.14	1.08	1.17	1.63	1.39	1.36	1.38	1.80	1.76	1.35	1.23
olute	to n	Q4	1.48	1.99	2.92	3.53	3.34	3.31	2.40	1.77	3.30	2.15	2.99	2.73	3.35	2.43	1.78	2.32
absc	ene	SEM	0.05	0.05	0.10	0.10	0.09	0.08	0.06	0.05	0.07	0.06	0.08	0.08	0.10	0.07	0.05	0.07
	g	ΔD	0.	02	0.	.20	0.0	02	0.0	07	0.3	26	0.	00	0.	16	0.	10
		GPE	0.	58	1.	.04	0.9	98	0.0	64	0.1	75	0.	93	0.	98	0.	71
		AVG	0.54	0.54	0.79	0.72	0.79	0.81	0.56	0.53	0.72	0.67	0.57	0.58	0.71	0.67	0.60	0.56
		STD	0.20	0.23	0.18	0.21	0.18	0.16	0.21	0.25	0.16	0.20	0.25	0.24	0.24	0.24	0.22	0.25
ed		Q0	0.20	0.04	0.13	0.18	0.12	0.18	0.24	0.04	0.21	0.23	0.02	0.03	0.15	0.14	0.03	0.04
naliz		Q1	0.41	0.39	0.69	0.62	0.72	0.75	0.35	0.31	0.61	0.50	0.37	0.35	0.54	0.50	0.44	0.36
norn	ses	Q2	0.52	0.51	0.84	0.77	0.83	0.83	0.56	0.58	0.72	0.68	0.53	0.55	0.77	0.76	0.62	0.53
sna	tanc	Q3	0.72	0.75	0.92	0.90	0.92	0.94	0.72	0.75	0.84	0.84	0.83	0.76	0.89	0.86	0.77	0.79
Jucle	dis	Q4	0.90	0.98	1.00	1.00	1.00	1.00	0.98	0.98	0.98	1.00	1.00	0.99	0.99	1.00	0.98	0.99
gle I		SEM	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.03
sin		ΔD	0.	00	0.	.06	-0.	.02	0.0	02	0.0	05	0.	00	0.	04	0.	04
		GPE	0	30	0	28	0	24	0	32	0.	26	0	35	0.	34	0.	33

Table S4. B) Statistics summary table of 3D distance measurements (ectopic MeCP2 system).

Detailed statistical overview of 3D distance measurements within the ectopic MeCP2 expression system. (A) Addresses distances and distance changes  $\Delta$  D towards chromocenters (B) towards the nuclear periphery. "Data set" refers to the respective chromatin state (i.e. either high or low MeCP2 expression). The total number of measurements is indicated as sample size. For each data set are given absolute and normalized values as: mean distances (AVG), standard deviation (STD), min. (Q0), max. (Q4), median (Q2), standard error of the mean (SEM), and mean distance changes ( $\Delta$  D) (see Figure S6).  $\Delta$  D was calculated as the difference of mean "high MeCP2" and mean "low MeCP2" distances. The linear Gaussian propagation error (GPE) was calculated as the square root of the sum of squared individual standard deviations per sample.



Figure S5. Tukey box plots of absolute and normalized 3D distances in the differentiation system.

For each observed gene the nuclear position was visualized by 3D-FISH using BAC probes in myoblasts (MB) and myotubes (MT). 3D distance measurements were performed as previously described in methods. The distribution of absolute distances (in µm) towards the nuclear periphery (left) and the nearest chromocenter (right) are shown in light grey (upper x-axis). The distribution of measurements normalized against individually simulated background distributions per nucleus (single cell normalized distances) is shown in dark grey (lower x-axis). Outliers are indicated as dots or triangles; sample means are indicated by crosses and median values are indicated by vertical lines within boxes.



Figure S6. Tukey box plots of absolute and normalized 3D distances in the ectopic MeCP2 system.

For each observed gene the nuclear position was visualized by 3D-FISH using BAC probes in high and low MeCP2 expressing cells. 3D distance measurements were performed as previously described in methods. The distribution of absolute distances (in µm) towards the nuclear periphery (left) and the nearest chromocenter (right) are shown in light grey (upper x-axis). The distribution of measurements normalized against individually simulated background distributions per nucleus (single cell normalized distances) is shown in dark grey (lower x-axis). Outliers are indicated as dots; sample means are indicated by crosses and median values are indicated by vertical lines within boxes.



Figure S7. Graphical summary of BAC clone composition, its genomic region and maximum gene expression fold changes.

BAC IDs and mouse chromosome numbers are shown for each gene selected. The Y-axis indicates the expression fold change (-log 10) for each system (i.e., ectopic system: high level versus low level MeCP2; differentiation system: myotubes versus myoblasts). The X-axis gives the respective genomic location in powers of 10. Shaded boxes highlight the respective BAC region. Within BAC regions, genes are drawn to scale as blue arrows (orientation indicated by arrowheads) and the genes of interest are highlighted in red. Data sets boxed in pink highlight genes selected in the ectopic MeCP2 system; black boxes highlight data sets selected for the differentiation system. Genes selected in both systems are grouped in the middle with their individual expression fold changes for each system.

Ge	ene	Mef2c	Tpm3	Myom2	Obscn	Slc19a2	Nrde2	Brca1	Coro1c	Ttk	Birc5
Ma ex	aximum gene pression value	109.9 5	27.91	19.12	14.44	1.22	-1.25	-4.13	-5.44	-6.26	-9.48
	Gene activity	2.35	1.05	2.87	1.29	-1.13	-1.17	1.11	1,00	-1.25	-1.14
νo	CpG island	10	80	18	66	23	30	67	38	20	54
vinc	%GC content	37.81	45.85	44.08	46.64	41.60	45.19	48.06	50.55	40.10	51.18
h dq	Gene density	9	39	7	63	14	13	50	25	7	23
2-M	SINE	3.22	15.88	3.54	11.45	6.35	10.27	21.70	12.04	5.19	11.87
	LINE	37.77	15.23	13.31	8.62	15.04	10.47	2.45	2.09	21.10	2.06
	Gene activity	-1.01	1	1.34	1.11	-1.06	1.06	1.01	1.05	-1.17	-1.01
Jow	CpG island	24	147	58	117	63	63	127	99	35	162
vinc	%GC content	38.34	44.13	44.15	46.35	41.84	43.72	47.08	49.80	39.05	50.7
dq	Gene density	32	186	47	157	82	60	221	105	29	153
5-M	SINE	3.38	13.38	4.97	12.46	7.14	8.86	17.57	12.27	4.45	14.78
	LINE	31.27	19.02	11.16	8.95	15.86	16.85	4.82	3.21	25.54	1.88

Table S5. Genes analyzed during differentiation and their genomic context.

Table S6. Genes analyzed in MeCP2 expressing cells and their genomic context.

Gene	е	Bdnf	Prl7c1	Myom2	Cdc20	Brca1	Birc5	Ttk	Col6a2
Maxi expr	mum gene ession value	2.13	-1.16	1.32	-1.16	-1.14	-1.13	-1.13	-1.52
	Gene activity	1.51	1.34	1.05	1.06	1.04	-1.07	-1.42	-1.13
2	CpG island	12	25	18	49	67	54	20	36
юрг	%GC content	38.98	42.02	44.08	47.15	48.06	51.18	40.10	47.42
o wir	Gene density	9	24	7	34	50	23	7	21
Mbp	SINE	5.13	9.02	3.54	14.82	21.70	11.87	5.19	9.93
2-1	LINE	25.65	21.75	13.31	8.07	2.45	2.06	21.10	8.53
	Gene activity	1.51	1.28	-1.05	1.12	1.04	-1.01	1,00	-1.01
2	CpG islands	27	38	58	128	127	162	35	76
lop	%GC content	38.09	39.16	44.15	46.14	47.08	50.7	39.05	45.73
wir	Gene density	77	57	47	143	221	153	29	131
Mbp	SINE	3.52	5.12	4.97	16.78	17.57	14.78	4.45	8.62
2-1	LINE	32.35	28.27	11.16	7.40	4.82	1.88	25.54	14.70

The gene activity of genomic regions was calculated as the average of all Affymetrix probe sets overlapping with the corresponding genomic regions. The number of genes (gene density) and the number of CpG islands was retrieved from the Ref-genes and CpG entries respectively in the genome browser (m38 assembly) overlapping with the corresponding genomic coordinates. GC content (fraction of GC within sequence), LINE and SINE density (percentage of covered sequences) was calculated using the corresponding genomic regions submitted to RepeatMasker (http://www.repeatmasker.org, version open-4.0).

		Chrom	ocenter			Perip	hery	
Gene	MB	MT	Low MeCP2	High MeCP2	MB	MT	Low MeCP2	High MeCP2
Mef2c	2.4x10 <sup>-05</sup>	3.9x10 <sup>-04</sup>	n/a	n/a	6.7x10 <sup>-09</sup>	7.2x10 <sup>-13</sup>	n/a	n/a
Трт3	1.3x10 <sup>-07</sup>	1.3x10 <sup>-03</sup>	n/a	n/a	9.4x10 <sup>-09</sup>	4.4x10 <sup>-12</sup>	n/a	n/a
Myom2	2.2x10 <sup>-06</sup>	6.2x10 <sup>-01</sup>	3.5x10 <sup>-02</sup>	1.2x10 <sup>-03</sup>	1.1x10 <sup>-01</sup>	2.4x10 <sup>-14</sup>	2.6x10 <sup>-05</sup>	3.3x10 <sup>-03</sup>
Obscn	3.8x10 <sup>-01</sup>	5.2x10 <sup>-04</sup>	n/a	n/a	5.0x10 <sup>-08</sup>	2.6x10 <sup>-13</sup>	n/a	n/a
Slc19a2	4.2x10 <sup>-13</sup>	1.6x10 <sup>-06</sup>	n/a	n/a	3.9x10 <sup>-05</sup>	5.1x10 <sup>-18</sup>	n/a	n/a
Nrde2	1.3x10 <sup>-12</sup>	8.4x10 <sup>-04</sup>	n/a	n/a	1.5x10 <sup>-13</sup>	1.8x10 <sup>-16</sup>	n/a	n/a
Brca1	8.0x10 <sup>-09</sup>	2.8x10 <sup>-05</sup>	6.8x10 <sup>-03</sup>	1.7x10 <sup>-03</sup>	4.3x10 <sup>-14</sup>	3.7x10 <sup>-31</sup>	1.1x10 <sup>-28</sup>	3.0x10 <sup>-22</sup>
Coro1c	1.6x10 <sup>-04</sup>	3.8x10 <sup>-07</sup>	n/a	n/a	6.1x10 <sup>-10</sup>	2.7x10 <sup>-06</sup>	n/a	n/a
Ttk	7.8x10 <sup>-07</sup>	2.6x10 <sup>-02</sup>	3.7x10 <sup>-02</sup>	2.0x10 <sup>-03</sup>	1.7x10 <sup>-01</sup>	4.2x10 <sup>-08</sup>	1.4x10 <sup>-02</sup>	5.1x10 <sup>-04</sup>
Birc5	6.5x10 <sup>-05</sup>	1.5x10 <sup>-08</sup>	1.0x10 <sup>-03</sup>	4.4x10 <sup>-07</sup>	8.4x10 <sup>-18</sup>	7.6x10 <sup>-17</sup>	5.4x10 <sup>-12</sup>	6.1x10 <sup>-22</sup>
Bdnf	n/a	n/a	4.5x10 <sup>-09</sup>	8.8x10 <sup>-11</sup>	n/a	n/a	7.0x10 <sup>-02</sup>	6.3x10 <sup>-05</sup>
Prl7c1	n/a	n/a	6.0x10 <sup>-03</sup>	1.1x10 <sup>-04</sup>	n/a	n/a	1.0x10 <sup>-08</sup>	2.5x10 <sup>-10</sup>
Cdc20	n/a	n/a	4.0x10 <sup>-10</sup>	1.7x10 <sup>-11</sup>	n/a	n/a	6.4x10 <sup>-01</sup>	1.1x10 <sup>-07</sup>
Col6a2	n/a	n/a	5.5x10 <sup>-03</sup>	4.0x10 <sup>-05</sup>	n/a	n/a	4.2x10 <sup>-08</sup>	5.3x10 <sup>-16</sup>

Table S7. Chi square result for gene distributions<sup>1</sup>.

<sup>1</sup> Grey boxes identify random gene distribution.



Figure S8. Exemplary analysis of neighborhood gene activity.

The average gene activity of genes in the neighborhood was calculated using the gene expression profiling data. The expression data however do not cover all genes in the region. Therefore, the gene activity may not necessarily reflect the gene activity of the complete neighborhood. An example on how such an activity profile looks is given. From the 29 genes in the 5-Mbp neighborhood (the x-axis shows 2.5 Mbp up and downstream from the gene center) of the *Ttk* protein kinase locus (red square), 15 were not identified by our myogenesis expression profile (grey squares) and 13 were identified (black squares). In the distribution of gene activities below one can see that an equaly strong upregulated gene lies right next to the gene of interest which could directly influence positional changes of *Ttk*.

Jost et al. Additional File

File***	Date	Chip	Alias	Outlier*	RawQ	SF	TGT	NF	Bckgrnd	Noise	No_P
CAR_01_MB1_190805_430_2.CHP	11:44AM 08/23/2005	Mouse430_2	MB1	36	2.19	0.775	200	1	61.9	4.6	47.00%
CAR_02_MB2_190805_430_2.CHP	11:44AM 08/23/2005	Mouse430_2	MB2	54	2.35	0.805	200	1	70.31	4.39	46.30%
CAR_03_MB3_190805_430_2.CHP	11:44AM 08/23/2005	Mouse430_2	MB3	44	2.22	0.907	200	1	67.71	4.2	45.50%
CAR_04_MB4_190805_430_2.CHP	11:44AM 08/23/2005	Mouse430_2	MB4	39	2.31	0.852	200	1	69.13	4.13	46.40%
CAR_05_MB6_190805_430_2.CHP	11:44AM 08/23/2005	Mouse430_2	MB6	53	2.31	0.752	200	1	69.29	4.58	46.70%
CAR_06_MT1_190805_430_2.CHP	11:44AM 08/23/2005	Mouse430_2	MT1	56	2.34	0.884	200	1	68.61	4.35	48.00%
CAR_07_MT_190805_430_2.CHP	11:44AM 08/23/2005	Mouse430_2	MT0	34	2.3	0.775	200	1	69.04	4.68	47.90%
CAR_08_MT3_190805_430_2.CHP	11:44AM 08/23/2005	Mouse430_2	MT3	52	2.1	0.94	200	1	65.56	4.02	46.80%
CAR_09_MT4_190805_430_2.CHP	11:44AM 08/23/2005	Mouse430_2	MT4	40	2.34	0.72	200	1	65.25	4.59	49.30%
CAR_10_MT2_190805_430_2.CHP	11:44AM 08/23/2005	Mouse430_2	MT2	109	2.31	0.881	200	1	66.71	4.95	46.90%
CAR_11_R5_1_240805_430_2.CHP	01:28PM 08/25/2005	Mouse430_2	R5_1	29**	3.85	0.802	200	1	128.29	12	39.40%
CAR_12_R5_3_240805_430_2.CHP	01:30PM 08/25/2005	Mouse430_2	R5_3	104**	4.36	0.903	200	1	157.98	13.15	37.90%
CAR_13_R5_5_240805_430_2.CHP	01:30PM 08/25/2005	Mouse430_2	R5_5	32	2.46	0.891	200	1	74.9	4.72	42.90%
CAR_14_R5_1807_240805_430_2.CHP	01:31PM 08/25/2005	Mouse430_2	R5_1807	15	2.67	1.065	200	1	85.87	5.87	41.80%
CAR_15_R5_10_240805_430_2.CHP	01:32PM 08/25/2005	Mouse430_2	R5_10	32	2.34	0.994	200	1	73.42	4.78	42.70%
CAR_16_R4p1_240805_430_2.CHP	01:33PM 08/25/2005	Mouse430_2	R4_1	35	2.41	1.01	200	1	74.51	4.83	42.70%
CAR_17_R4p2_240805_430_2.CHP	01:34PM 08/25/2005	Mouse430_2	R4_2	114	2.24	0.936	200	1	69.63	4.55	45.30%
CAR_18_R4p3_240805_430_2.CHP	01:36PM 08/25/2005	Mouse430_2	R4_3	35	2.24	1.039	200	1	69.85	4.28	42.60%
CAR_19_R4p4_240805_430_2.CHP	01:36PM 08/25/2005	Mouse430_2	R4_4	53	2.28	1.052	200	1	70.69	4.1	42.70%
CAR_20_R4p1807_240805_430_2.CHP	01:38PM 08/25/2005	Mouse430_2	R4_1807	46	2.38	1.1	200	1	73.25	4.73	43.40%
Minimum				15	2.1	0.72			61.9		0.379
Maximum				114	4.36	1.1			157.98		0.493

## Table S8. Transcription profiling parameters and output.

\*Number of outlier in the Nalimov test at p < 0.001

\*\*No arrays have been rejected from the statistical analysis in respect to the fact that the quality of the sub-optimal arrays (red marked) is not clearly

File	No_A	No_M	Avg_P	Avg_A	Avg_M	Avg_All	GAPDH	18S	β_ΑCTIN	BIOB	BIOC
CAR_01_MB1_190805_430_2.CHP	51.30%	1.60%	590.5	28	76.2	293.4	0.86	0.32	1.16	0.83	1.11
CAR_02_MB2_190805_430_2.CHP	51.90%	1.70%	619.3	25.9	73.4	301.7	0.84	0.28	1.16	0.71	1.03
CAR_03_MB3_190805_430_2.CHP	53.00%	1.60%	636.3	27.7	82.7	305.4	0.82	0.33	1.15	0.92	1.1
CAR_04_MB4_190805_430_2.CHP	51.90%	1.70%	618.9	26.8	77.4	302.3	0.81	0.38	1.14	0.75	1.05
CAR_05_MB6_190805_430_2.CHP	51.70%	1.60%	604.1	24.5	67.5	295.8	0.82	0.42	1.13	0.76	1.08
CAR_06_MT1_190805_430_2.CHP	50.30%	1.70%	574.6	28.5	73.5	291.1	0.81	0.53	1.17	0.8	0.96
CAR_07_MT_190805_430_2.CHP	50.30%	1.70%	577.1	25.6	68.7	290.8	0.81	0.58	1.17	0.78	1.01
CAR_08_MT3_190805_430_2.CHP	51.60%	1.60%	604.1	26.5	70.7	297.6	0.8	0.52	1.19	0.76	1.03
CAR_09_MT4_190805_430_2.CHP	48.90%	1.80%	537.8	28.8	89.9	280.7	0.79	0.62	1.13	1.18	1.02
CAR_10_MT2_190805_430_2.CHP	51.30%	1.70%	579.9	30.3	83.1	289.1	0.79	0.62	1.26	0.79	1.07
CAR_11_R5_1_240805_430_2.CHP	58.90%	1.70%	676.5	38.3	121.7	291.3	1.82	0.35	1.61	0.81	0.75
CAR_12_R5_3_240805_430_2.CHP	60.40%	1.70%	696.8	44.9	130.4	293.7	1.95	0.27	1.91	0.69	0.8
CAR_13_R5_5_240805_430_2.CHP	55.50%	1.70%	678.6	26.6	82.4	307.2	1.92	0.3	1.61	0.93	0.87
CAR_14_R5_1807_240805_430_2.CHP	56.60%	1.60%	694.5	31.2	87.8	309.4	2.06	0.23	1.66	0.94	0.88
CAR_15_R5_10_240805_430_2.CHP	55.60%	1.70%	680.8	27.2	85.8	307.4	1.71	0.22	1.67	0.75	1.15
CAR_16_R4p1_240805_430_2.CHP	55.60%	1.70%	687.7	26.8	82.4	310	1.77	0.27	1.83	0.81	1.25
CAR_17_R4p2_240805_430_2.CHP	53.10%	1.60%	661.1	22.1	62.7	312.3	1.93	0.3	1.9	0.96	1.16
CAR_18_R4p3_240805_430_2.CHP	55.80%	1.60%	693.8	26.4	76.1	311.5	1.96	0.25	1.71	0.75	1.04
CAR_19_R4p4_240805_430_2.CHP	55.70%	1.60%	691.5	25.8	90.2	310.9	2.25	0.26	1.76	0.91	1.14
CAR_20_R4p1807_240805_430_2.CHP	55.00%	1.70%	687.8	25.1	73.9	313.2	2.15	0.25	1.6	1.07	1.16
Minimum			537.8				0.79		1.13		
Maximum			696.8				2.25		1.91		

File	BIOD	CRE	DAP	LYS	PHE	THR	TRP	Alpha1	Alpha2	Tau
CAR_01_MB1_190805_430_2.CHP	2.28	1.32	0.25	1.9	30.66	2.23	1.95	0.05	0.065	0.015
CAR 02 MB2 190805 430 2.CHP	2.05	1.31	0.6	0.5	11.01	0.2	0.67	0.05	0.065	0.015
CAR_03_MB3_190805_430_2.CHP	2.57	1.39	0.26	2	6.46	0.9	0.79	0.05	0.065	0.015
CAR_04_MB4_190805_430_2.CHP	2.4	1.3	2.31	1.04	10.33	1.58	0.09	0.05	0.065	0.015
CAR_05_MB6_190805_430_2.CHP	2.11	1.32	0.63	0.61	27.86	1.38	0.18	0.05	0.065	0.015
CAR_06_MT1_190805_430_2.CHP	2.29	1.23	0.35	2.46	33.86	0.49	1.64	0.05	0.065	0.015
CAR_07_MT_190805_430_2.CHP	1.95	1.16	1.06	0.3	19.17	0.56	0.49	0.05	0.065	0.015
CAR_08_MT3_190805_430_2.CHP	2.34	1.25	2.3	11.72	25.17	1.15	0.39	0.05	0.065	0.015
CAR_09_MT4_190805_430_2.CHP	1.89	1.28	0.13	0.84	18.21	0.78	0.56	0.05	0.065	0.015
CAR_10_MT2_190805_430_2.CHP	2.37	1.18	1.17	9.14	30.6	0.51	1.05	0.05	0.065	0.015
CAR_11_R5_1_240805_430_2.CHP	2.38	1.16	16.68	14	6.93	7.19	0.19	0.05	0.065	0.015
CAR_12_R5_3_240805_430_2.CHP	2.37	1.21	14.75	49.1	15.92	17.66	0.31	0.05	0.065	0.015
CAR_13_R5_5_240805_430_2.CHP	2.42	1.24	21.04	13.27	13.45	7.87	0.67	0.05	0.065	0.015
CAR_14_R5_1807_240805_430_2.CHP	2.78	1.29	24.49	9.26	9.92	6.76	1.55	0.05	0.065	0.015
CAR_15_R5_10_240805_430_2.CHP	2.87	1.18	19.31	12.92	7.44	5.85	0.1	0.05	0.065	0.015
CAR 16 R4p1 240805 430 2.CHP	2.82	1.21	22.2	10.09	6.73	18.36	0.79	0.05	0.065	0.015
CAR_17_R4p2_240805_430_2.CHP	2.63	1.15	18.8	6.34	8.56	3.89	0.06	0.05	0.065	0.015
CAR_18_R4p3_240805_430_2.CHP	2.75	1.21	25.14	14.2	6.87	10.17	0.07	0.05	0.065	0.015
CAR_19_R4p4_240805_430_2.CHP	2.57	1.25	32.03	30.61	6.81	7.43	0.12	0.05	0.065	0.015
CAR_20_R4p1807_240805_430_2.CHP	2.68	1.28	26.04	12.09	12.94	8.28	0.13	0.05	0.065	0.015
Minimum										
Maximum										