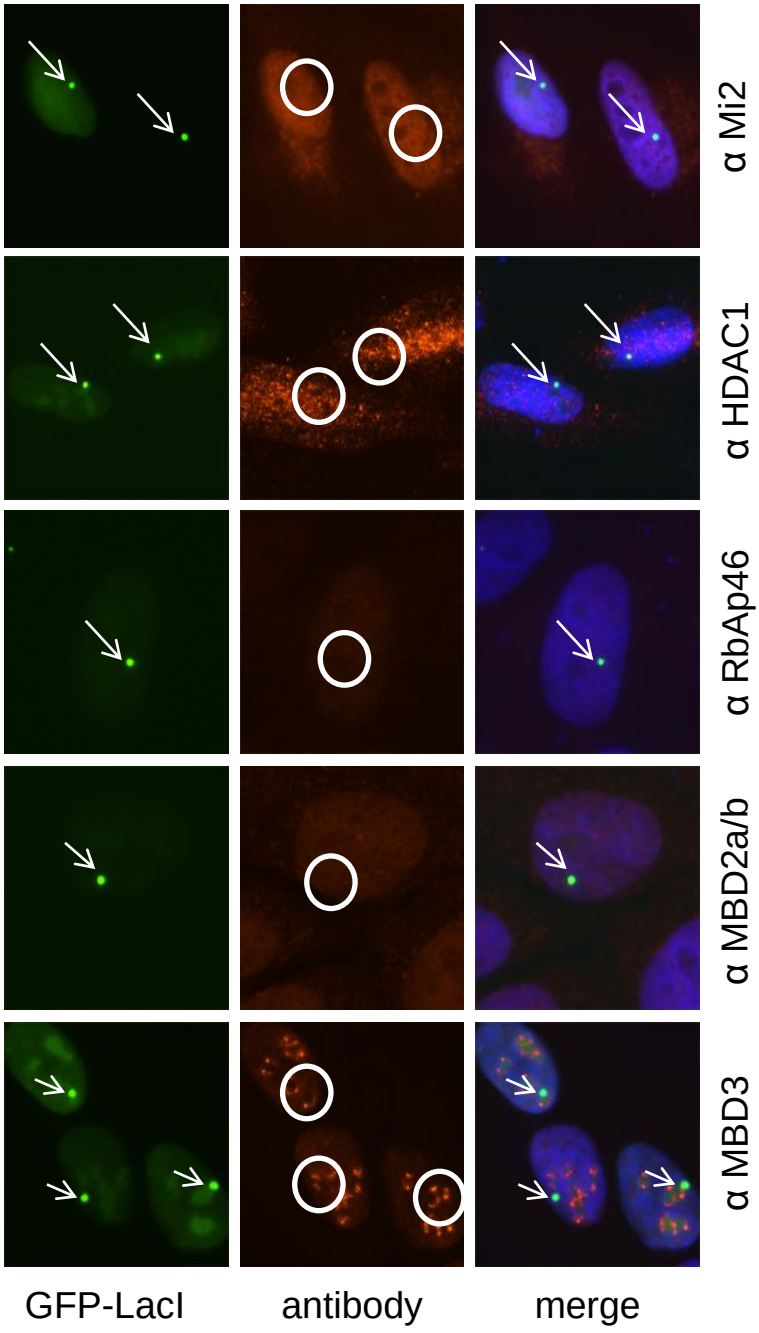
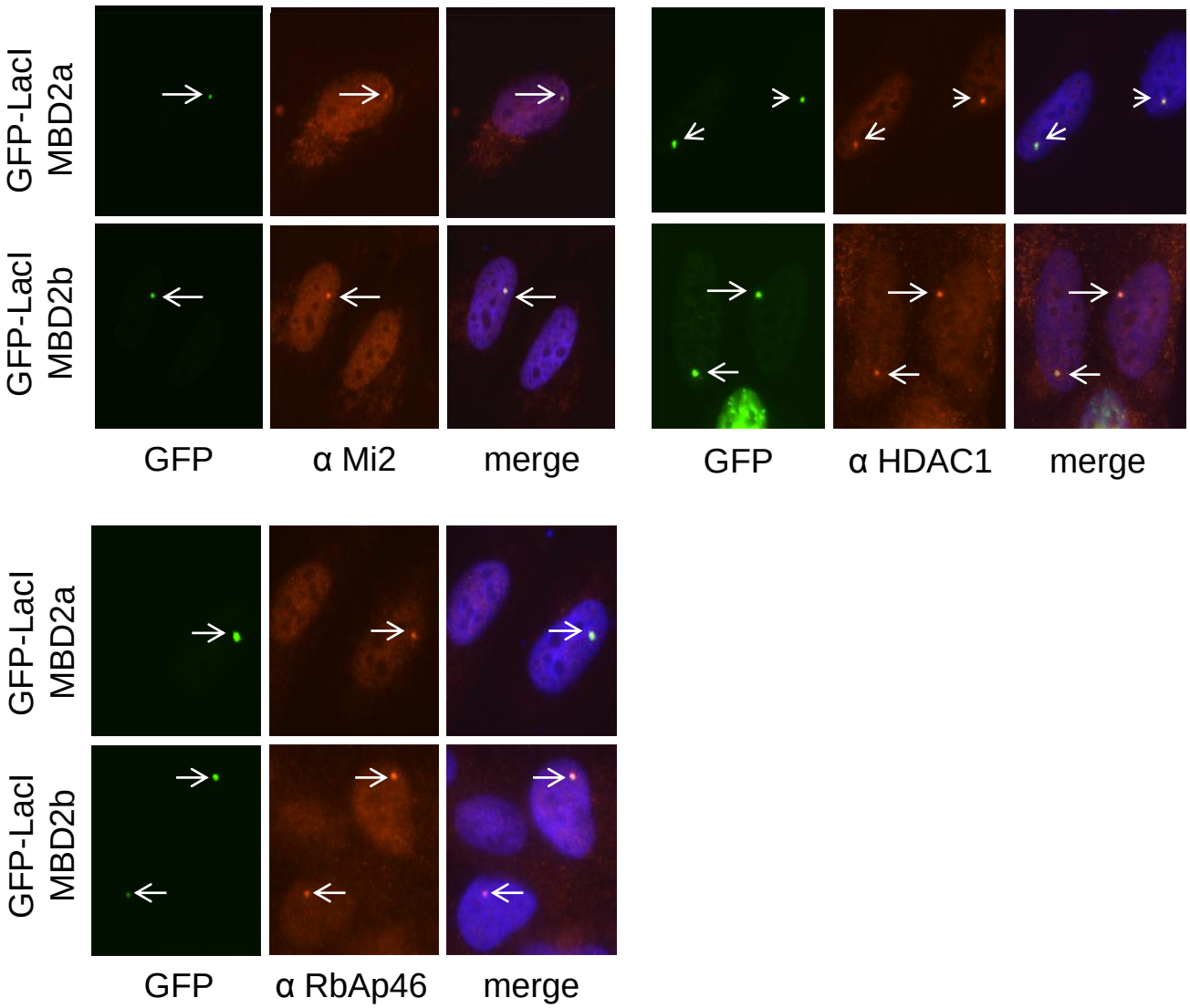
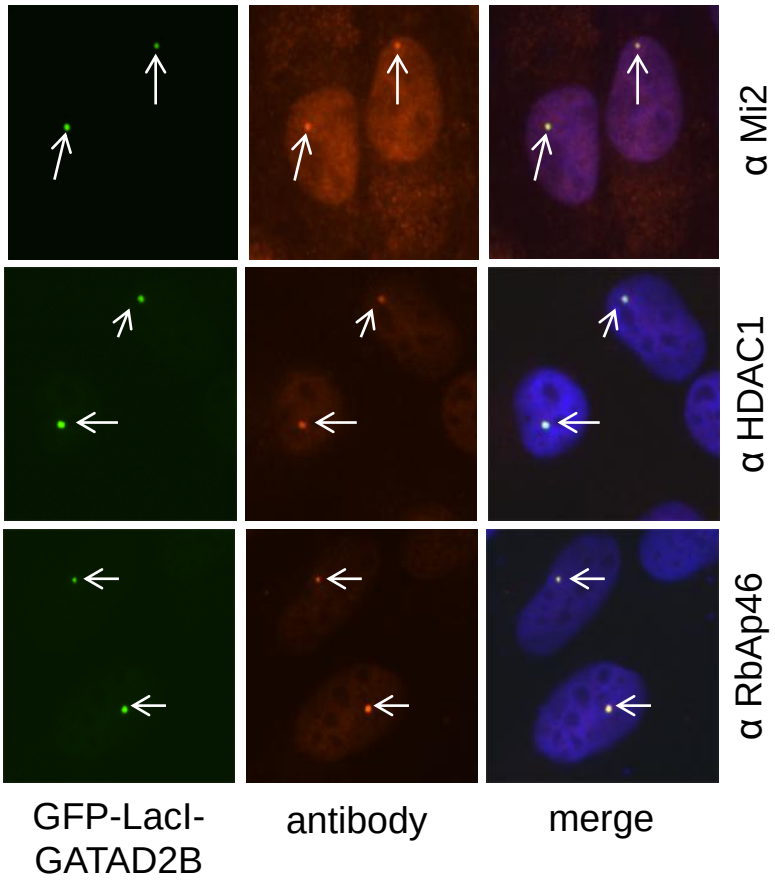


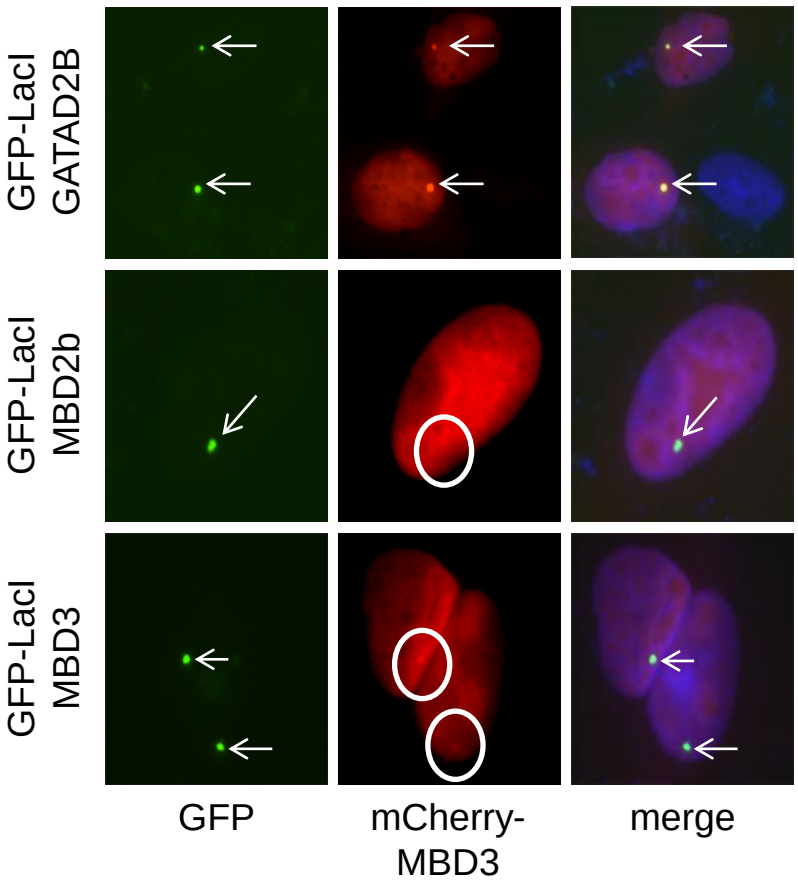
Supplementary Figure S1. GFP-LacI does not assemble MBD2-NuRD or MBD3-NuRD at the LacO-array. F42B8 cells were transfected with GFP-LacI. Transfected cells were incubated for 48h, fixed and stained with the indicated antibodies. GFP signals were detected as indicated and indirect immunofluorescence was carried out with antibodies against Mi2 (αMi2), HDAC1 (αHDAC1), RbAp46 (αRbAp46) , MBD2 (αMBD2a/b) and MBD3 (αMBD3). Merged colour images show the DAPI staining in addition. Arrows point at positive signals, whereas circles indicate lack of a signal.

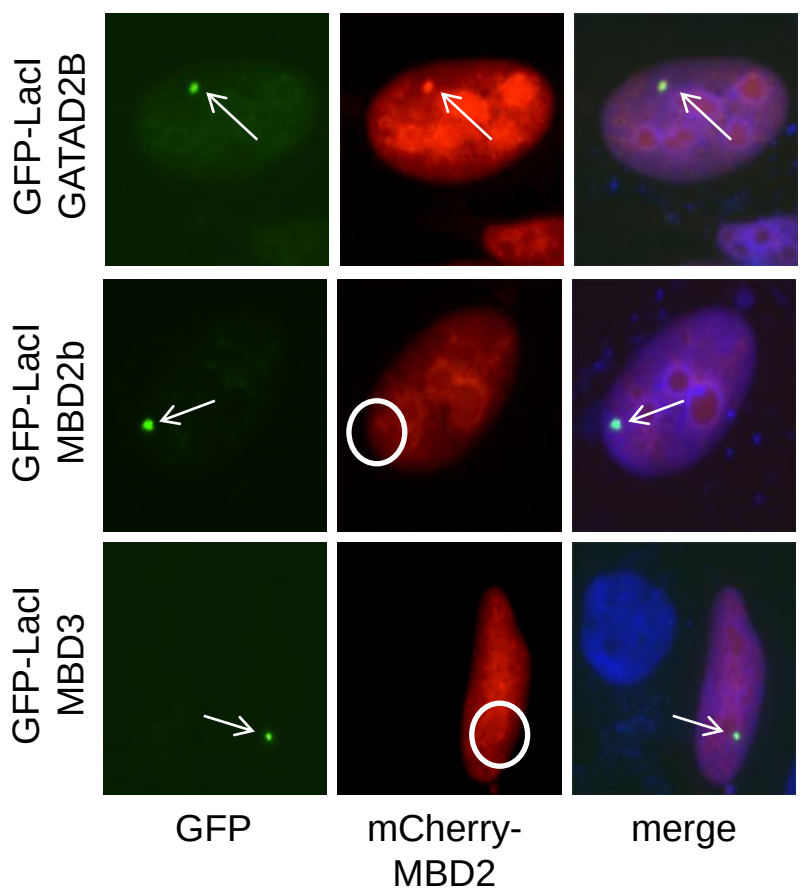


Supplementary Figure S2. GFP-LacI-MBD2a and GFP-LacI-MBD2b assemble MBD2-NuRD at the LacO-array. F42B8 cells were transfected with GFP-LacI-MBD2a or GFP-LacI-MBD2b. Transfected cells were incubated for 48h, fixed and stained with the indicated antibodies. GFP signals were detected as indicated and indirect immunofluorescence was carried out with antibodies against Mi2 (αMi2), HDAC1 (αHDAC1) and RbAp46 (αRbAp46). Merged colour images show the DAPI staining in addition.

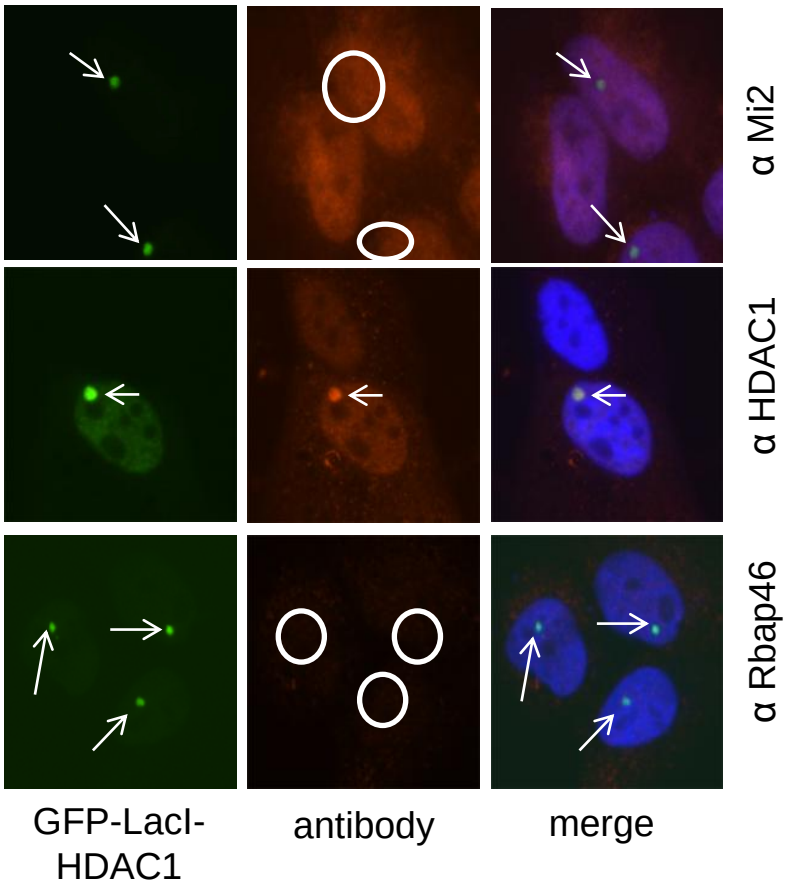


Supplementary Figure S3. GFP-LacI-GATAD2B assembles NuRD at the LacO-array. F42B8 cells were transfected with GFP-LacI-GATAD2B. Transfected cells were incubated for 48h, fixed and stained with the indicated antibodies. GFP signals were detected as indicated and indirect immunofluorescence was carried out with antibodies against Mi2(αMi2), HDAC1 (αHDAC1) and RbAp46 (αRbAp46). Merged colour images show the DAPI staining in addition.

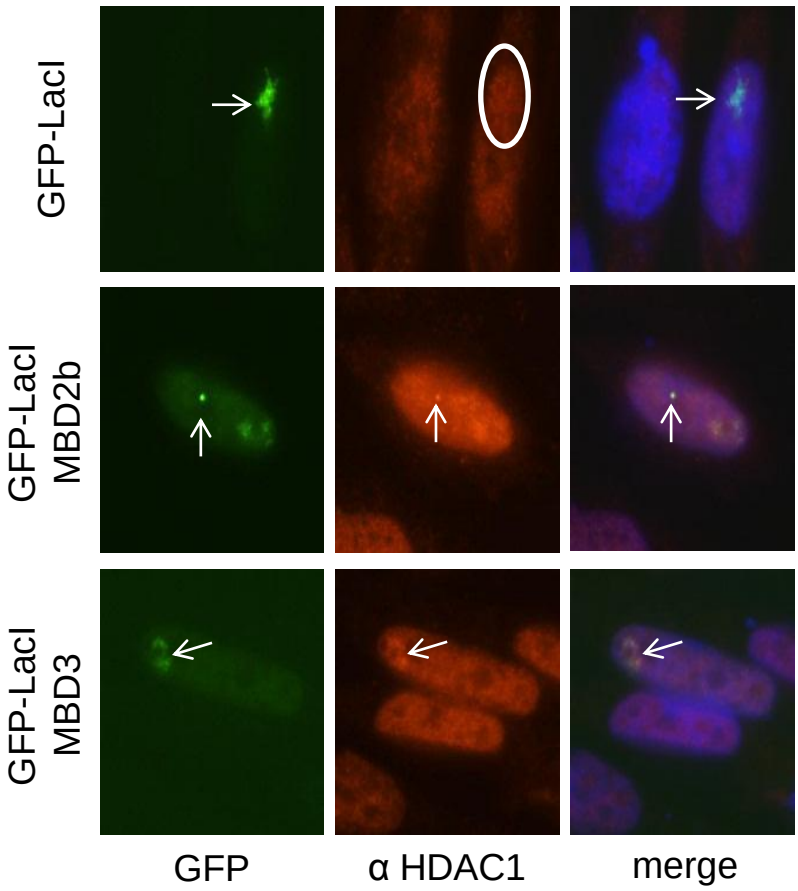


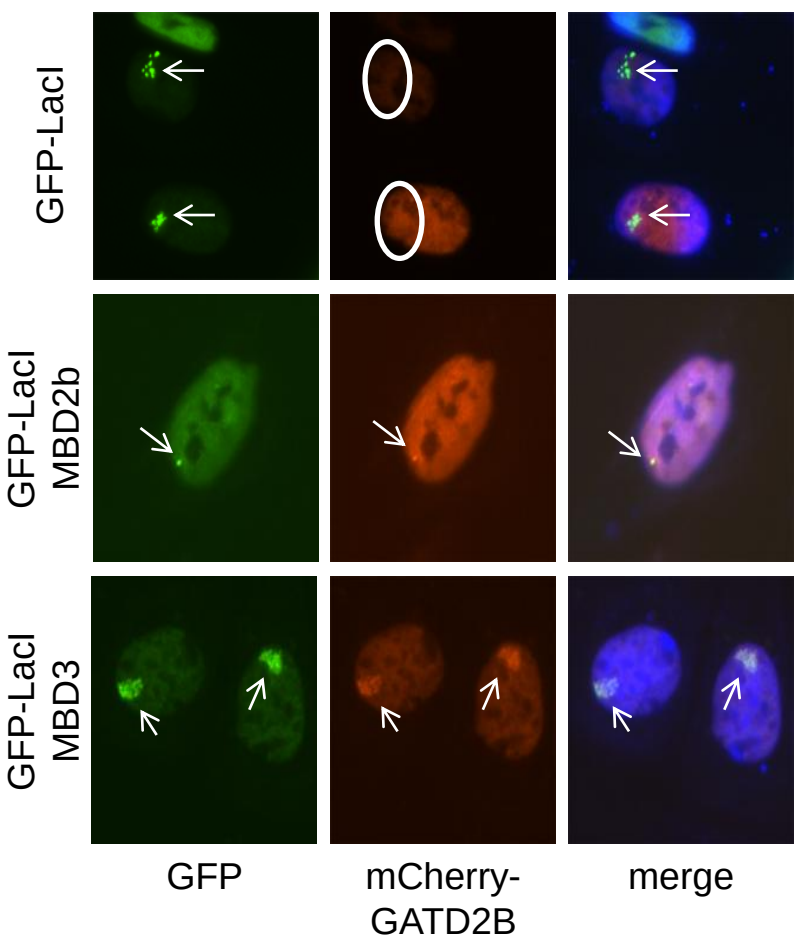


Supplementary Figure S4. GATAD2B recruits both MBD factors to the LacO-array, whereas neither GFP-LacI-MBD2 nor GFP-LacI-MBD3 recruit the paralogous factor. F42B8 cells were co-transfected with GFP-LacI-GATAD2B, GFP-LacI-MBD2b or GFP-LacI-MBD3 and mCherry-MBD3 or mCherry-MBD2. Transfected cells were incubated for 48h, fixed and stained with the indicated antibodies. GFP and mCherry signals were detected as indicated. Merged colour images show the DAPI staining in addition. Arrows point at positive signals, whereas circles indicate lack of a signal.

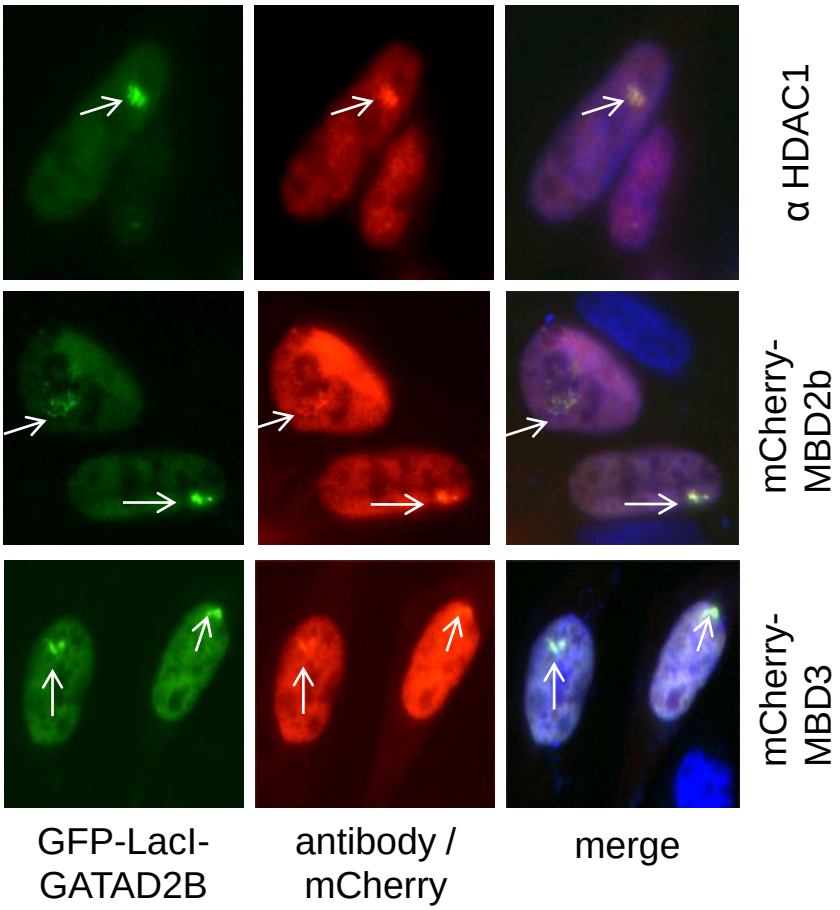


Supplementary Figure S5. GFP-LacI-HDAC1 does not assemble NuRD at the LacO-array. F42B8 cells were transfected with GFP-LacI-HDAC1. Transfected cells were incubated for 48h, fixed and stained with the indicated antibodies. GFP signals were detected as indicated and indirect immunofluorescence was carried out with antibodies against Mi2(αMi2), HDAC1 (αHDAC1) and RbAp46 (αRbAp46). Merged colour images show the DAPI staining in addition.

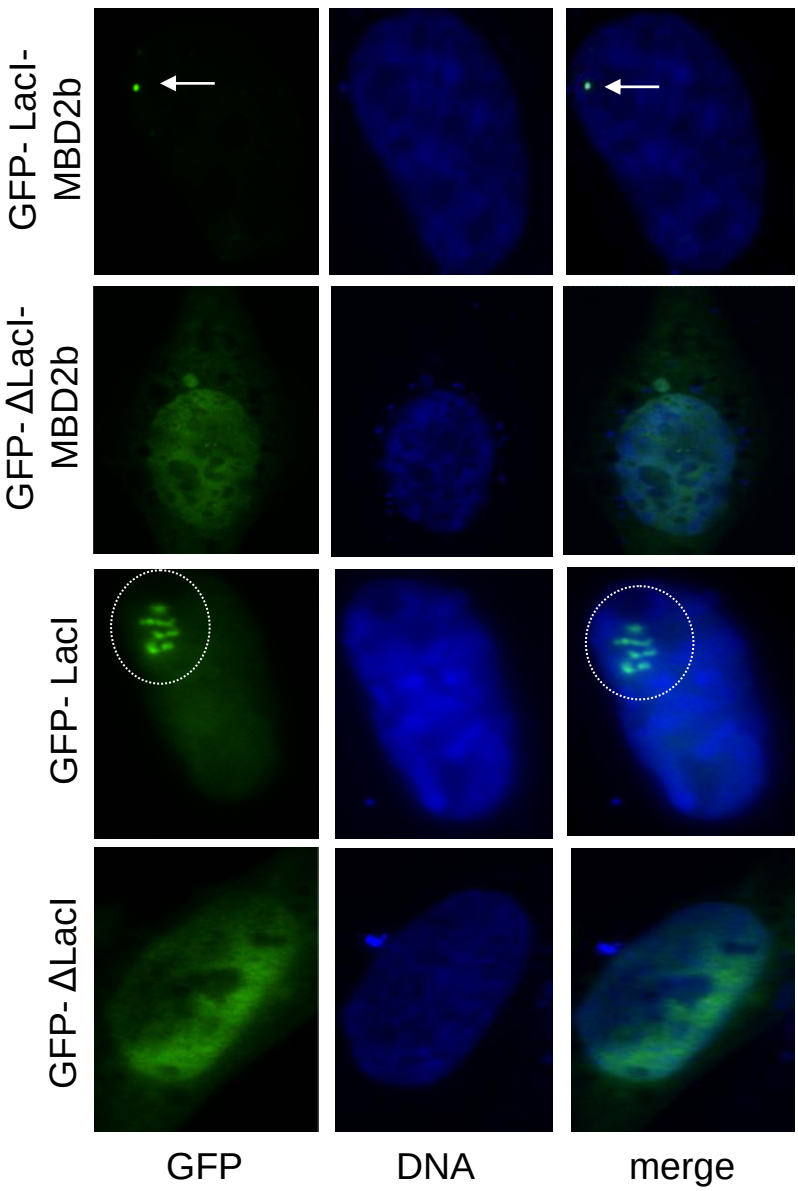




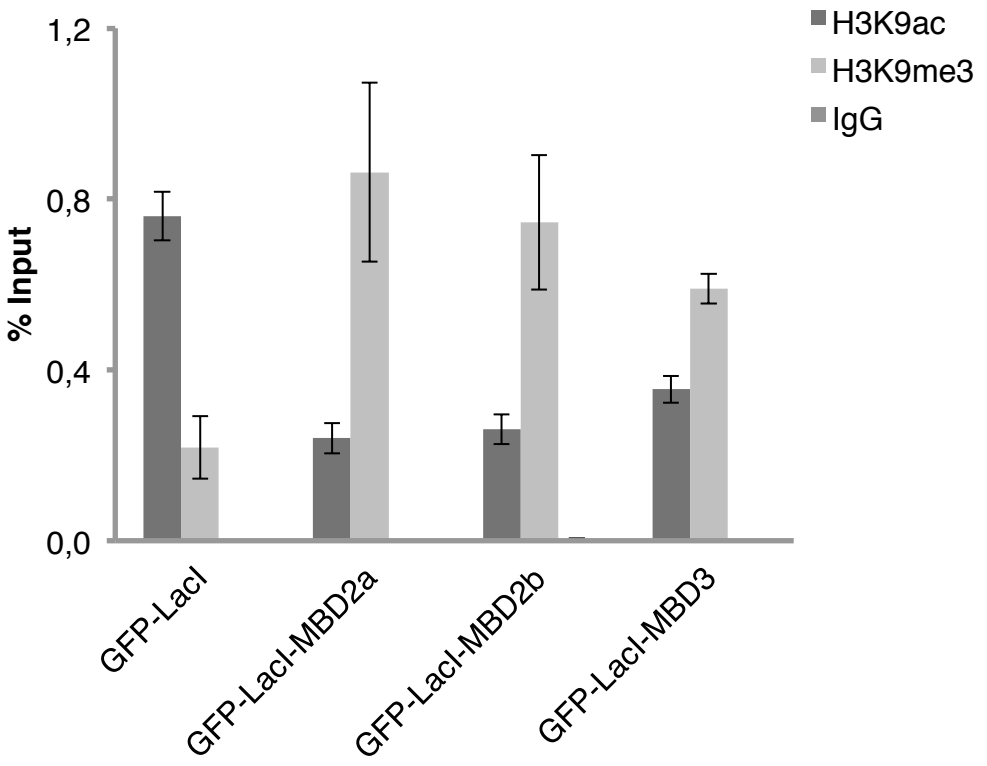
Supplementary Figure S6A. GFP-LacI-MBD2b and GFP-LacI-MBD3 assemble the NuRD-complex at the euchromatic LacO-array. RREB1 cells were transfected with GFP-LacI, GFP-LacI-MBD2b or GFP-LacI-MBD3 or co-transfected in addition with mCherry-GATAD2B. Transfected cells were incubated for 48h, fixed and stained with the indicated antibody. GFP and mCherry signals were detected as indicated and indirect immunofluorescence was carried out with antibody against HDAC1 (αHDAC1). Merged colour images show the DAPI staining in addition. Arrows point at positive signals, whereas circles indicate lack of a signal.



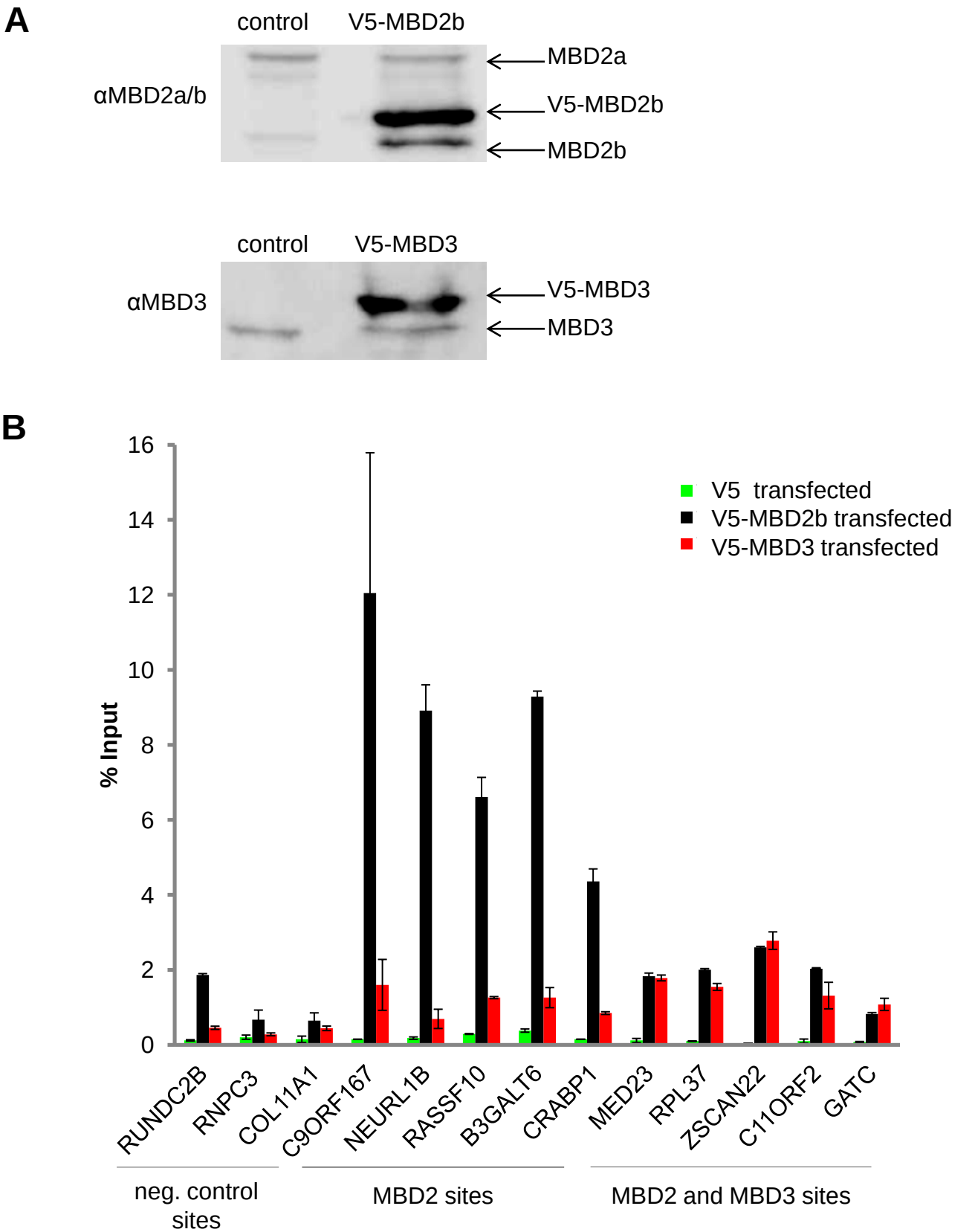
Supplementary Figure S6B. GFP-LacI-GATAD2B recruits GFP-LacI-MBD2b and GFP-LacI-MBD3 to the euchromatic LacO-array. RREB1 cells were transfected with GFP-LacI-GATAD2B or co-transfected in addition with mCherry-MBD2b or mCherry MBD3. Transfected cells were incubated for 48h, fixed and stained with the indicated antibody. GFP and mCherry signals were detected as indicated and indirect immunofluorescence was carried out with antibody against HDAC1 (αHDAC1). Merged colour images show the DAPI staining in addition.



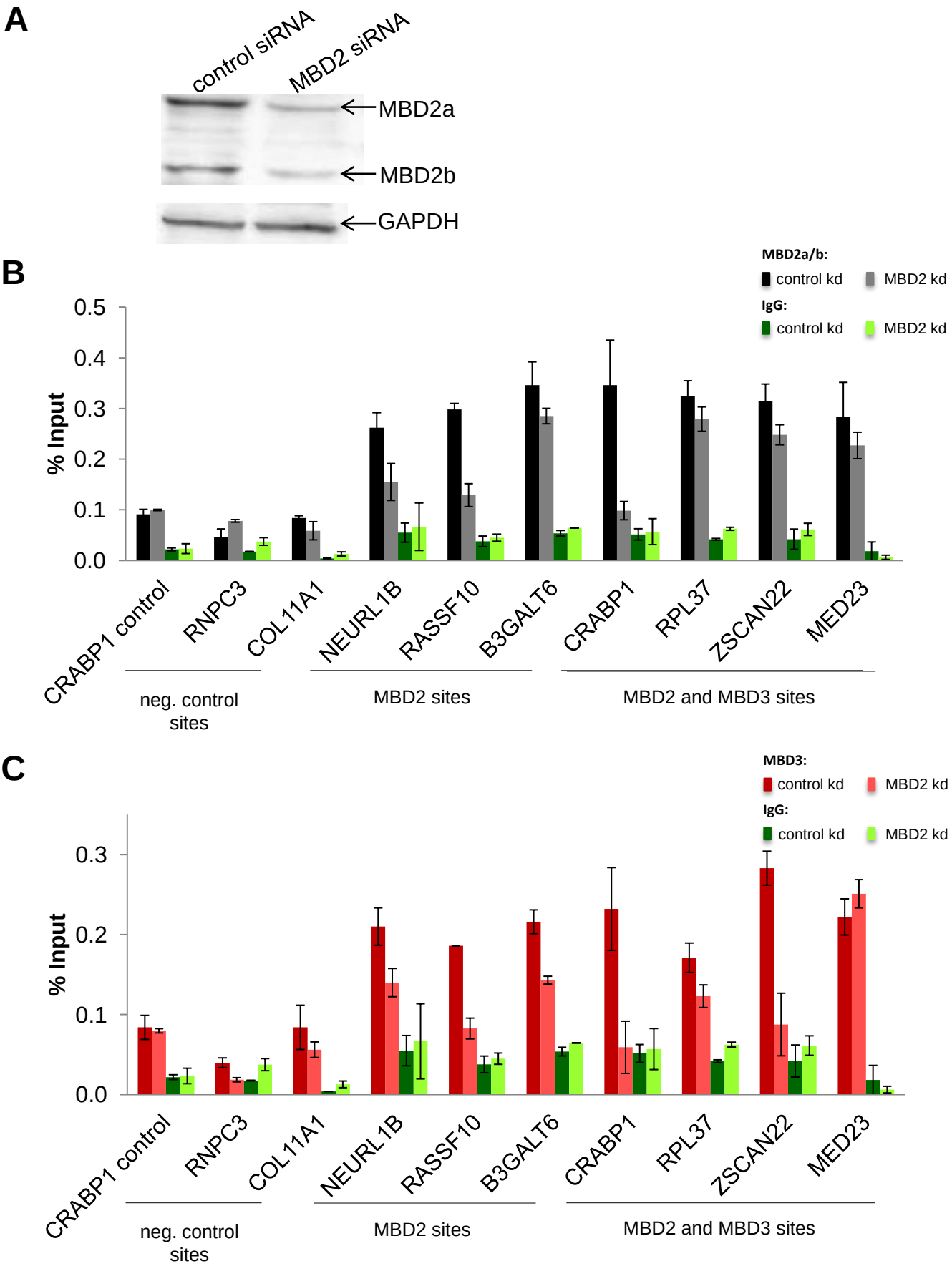
Supplementary Figure S7. Compaction of the euchromatic LacO-array in RREB1 cells is caused by binding of MBD2-NuRD to the array. RREB1 cells were transfected with GFP-LacI, GFP- ΔLacI, GFP-LacI-MBD2b or GFP- ΔLacI-MBD2b. Transfected cells were incubated for 48h, fixed and GFP (green) and DAPI(blue) signals were detected as indicated. The arrows point to the compact array, the expanded array is indicated by the circle.



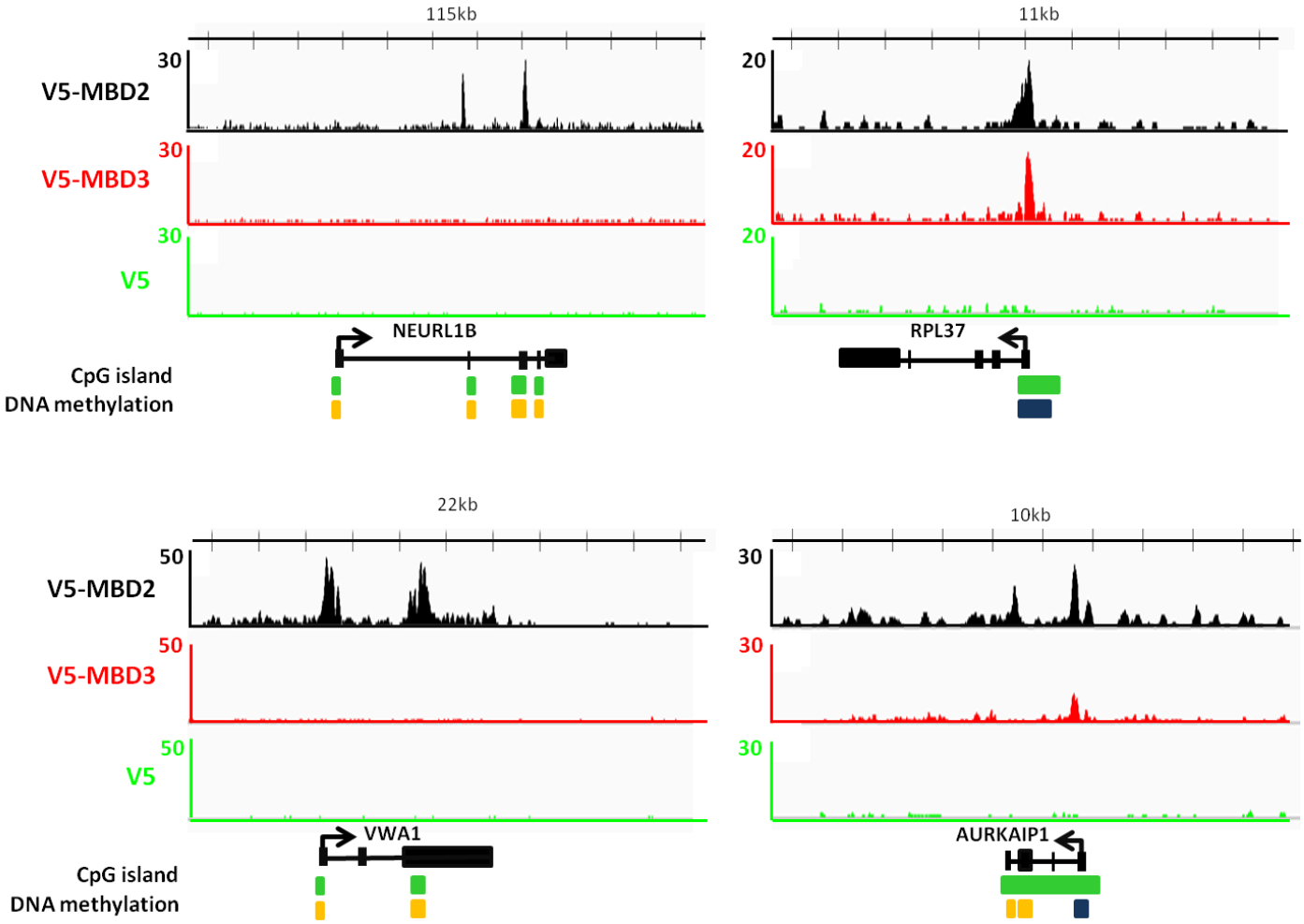
Supplementary Figure S8. GFP-LacI-MBD2a/b convert euchromatin to heterochromatin. RREB1 cells containing the integrated euchromatic LacO-array were transiently transfected with GFP-LacI, -MBD2a, -MBD2b or –MBD3. Chromatin modification change was analysed by chromatin immunoprecipitation using antibodies against H3K9ac or H3K9me3.



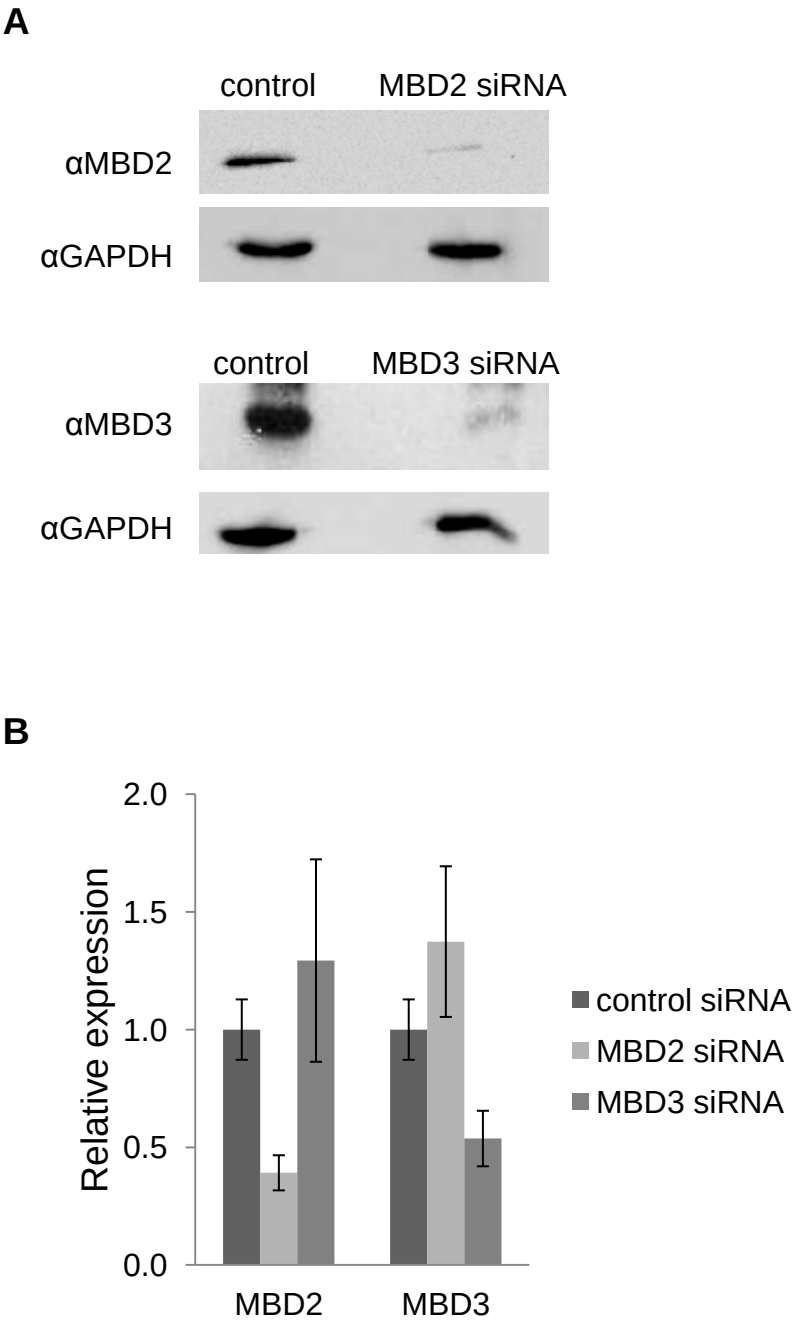
Supplementary Figure S9. Verification of V5-MBD2 or V5-MBD3 binding. (A) Detection of expressed V5-MBD2b and V5 MBD3. Western Blot analysis of endogeneous MBD2a, MBD2b, MBD3 and of expressed V5-MBD2b and V5-MBD3 in HeLa cells. HeLa cells were transfected with V5-MBD2b or V5-MBD3. Subsequent western blot was done with the indicated MBD2 (αMBD2a/b) and MBD3(αMBD3) antibodies. Untransfected HeLa cells were used as control. (B) Recruitment of V5-MBD2b or V5-MBD3 to endogenous binding sites. Binding analysis by ChIP-qPCR was performed 48h after transfection using V5 antibody and calculated relative to input.



Supplementary Figure S10. Verification of endogenous MBD2 and MBD3 binding. (A) Western Blot of the MBD2a/b knock down. (B) MBD2 binding is reduced after MBD2 knock down (kd). Binding analysis by ChIP-qPCR was performed in HeLa cells after control kd or MBD2 kd using MBD2a/b antibody as well as control IgG. (C) ChIP shows crossreactivity of the MBD3 anti body to MBD2. ChIP-qPCR was performed in HeLa cells after control kd or MBD2 kd using MBD3 antibody as well as control IgG.

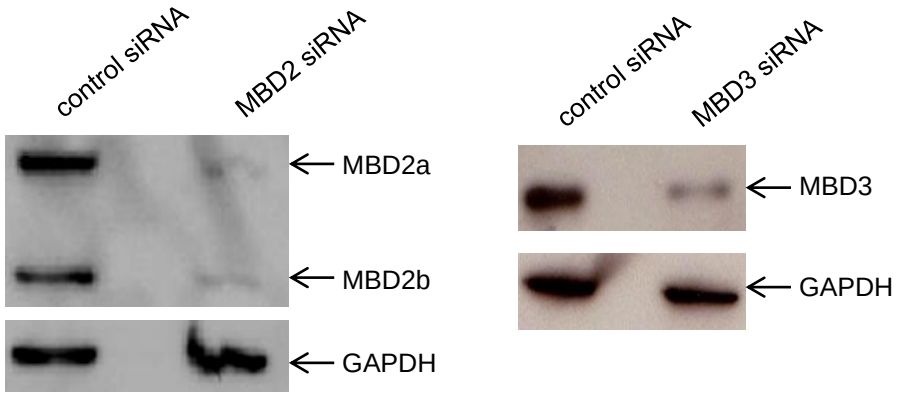


Supplementary Figure S11. Selected snapshots of the genome browser view at the NEURL1B, RPL37, VWA1 and AURKAIP1 loci**.** ChIPseq results are shown after V5-MBD2, V5-MBD3 or V5 expression in HeLa cells. CpG islands (green bars), methylation (blue) versus non-methylation (yellow) is taken from (45) and public data downloaded from UCSC (Methyl 450K Bead Arrays from ENCODE/HAIB.



Supplementary Figure S12. MBD2 and MBD3 expression analysis after knock down.
(A) Western Blot analysis of endogeneous MBD2 and MBD3 expression in HeLa cells. HeLa cells were transfected with MBD2 siRNA, MBD3 siRNA or control siRNA. Subsequent western blot was done with the indicated MBD2 (αMBD2) and MBD3 (αMBD3) antibodies. GAPDH was used as control. (B) Analysis of MBD2 and MBD3 expression on RNA level. RNA was isolated form HeLa cells six days after treatment with either MBD2-, MBD3- or control siRNA. Subsequent qRT-PCR was done.

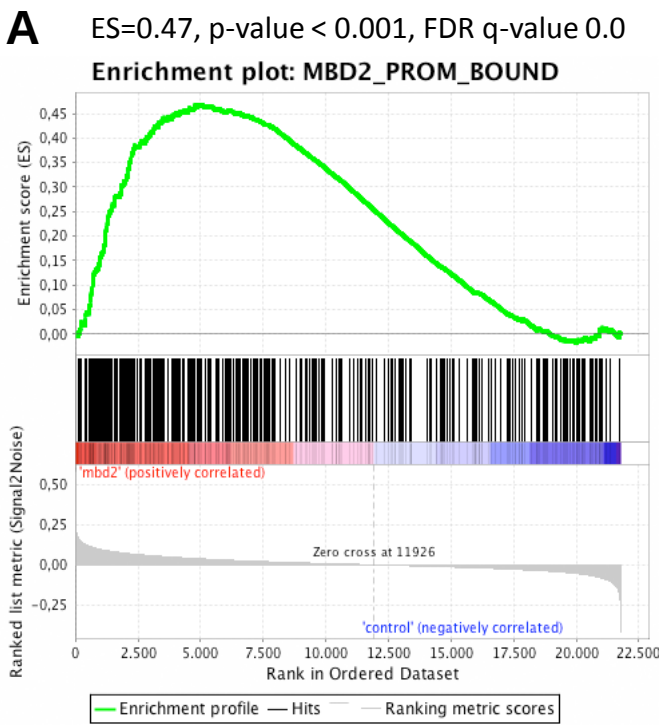
A

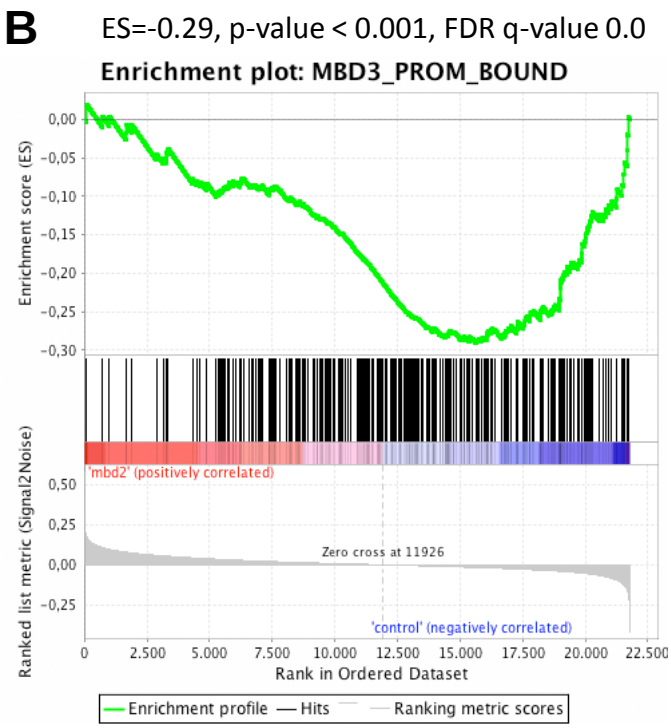


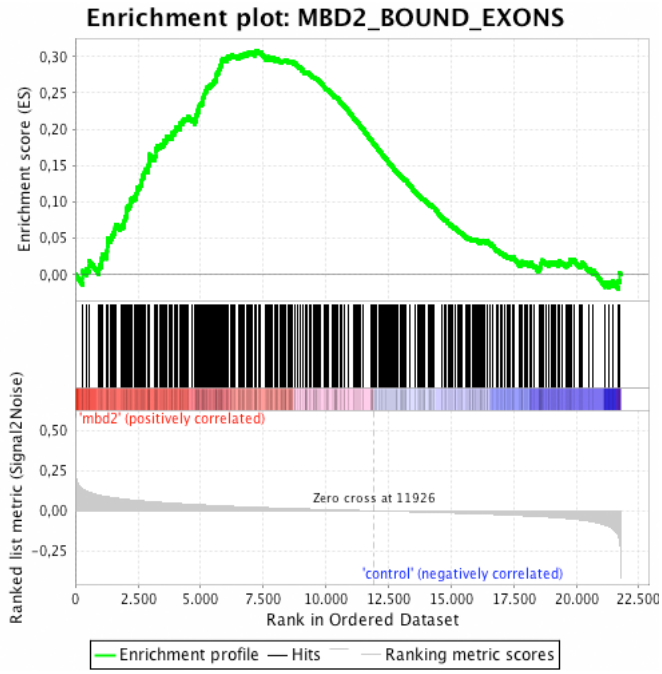
B

gene	exp. value	**microarray**	SD	**RT**	SD	MBD2/3 binding
		MBD2kd		MBD2kd		
		fold change		fold change		
BGN	7.2	1.6	0.44	9.2	5.04	
SERPINF1	5.7	2.1	0.62	7.9	0.40	
PTGS1	7.1	1.5	0.51	2.9	1.55	
CACNA1H	7.7	1.5	0.30	2.7	0.86	2
PTK7	7.0	1.3	0.07	1.4	0.16	2
RASSF5	6.2	1.3	0.15	1.3	0.08	2
PRKCZ	8.3	1.0	0.21	1.1	0.30	2
NUF2	9.1	-1.2	0.13	-2.3	0.01	2;3
CDH13	9.9	-2.2	0.07	-2.2	0.10	
CUL4A	10.2	-1.2	0.13	-1.5	0.23	2;3
SERPINE2	9.6	-1.6	0.38	-1.3	0.36	
NIF3L1	6.0	-1.3	0.21	-1.2	0.17	2;3
PLEKHM3	8.1	-1.2	0.11	-1.2	0.12	2;3
MUC13	8.7	-1.8	0.45	-1.1	0.10	
GAPDH	13.6	1.0	0.02	1.1	0.14	
gene	exp. value	**microarray**	SD	**RT**	SD	MBD2/3 binding
		MBD3kd		MBD3kd		
		fold change		fold change		
BGN	7.2	1.4	0.56	3.3	1.42	
SERPINF1	5.7	2.3	1.03	3.9	2.23	
PTGS1	7.1	1.5	0.33	1.0	1.00	
CACNA1H	7.7	1.3	0.16	1.3	0.01	2
PTK7	7.0	1.1	0.10	1.2	0.19	2
RASSF5	6.2	1.0	0.06	1.0	0.03	2
PRKCZ	8.3	1.1	0.20	1.4	0.20	2
NUF2	9.1	-1.2	0.09	-2.9	0.10	2;3
CDH13	9.9	-2.0	0.13	-2.0	0.41	
PIGC	6.4	-1.8	0.23	-1.2	0.17	2;3
SERPINE2	9.6	-1.3	0.29	-1.2	0.07	
NIF3L1	6.0	-1.2	0.15	-1.5	0.34	2;3
PLEKHM3	8.1	-1.2	0.05	-1.1	0.12	2;3
MUC13	8.7	-1.7	0.47	-1.4	0.17	
GAPDH	13.6	1.0	0.03	-1.1	0.02	

Supplementary Figure S13. Validation of endogenous MBD2- and MBD3-target gene expression after MBD2- and MBD3-kd. (A) Western Blot of the MBD2- and MBD3-kd. (B) qRT-PCR was performed after MBD2kd or MBD3kd. Results are shown in comparison to the microarray data (calculated from two to three biological replicates, SD=standard deviation).







Supplementary Figure S14. MBD2 binding to promoters is associated with gene repression and combined MBD2/MBD3 binding with gene activation.
Gene set enrichment analysis (GSEA) was performed with 3 different gene sets derived from our MBD2/3 binding analysis. All gene sets were cut down to the top 300 binding events/genes with a) MBD2 binding in promoters, b) MBD3 binding in promoters and c) MBD2 binding over exons (no binding to promoters was allowed). At the bottom of the plot the "Signal-to-Noise" ratio (SNR) statistic is depicted ranking all genes according to their correlation with either the MBD2 RNAi phenotype (red, i.e. genes induced after MBD2 RNAi) or the control RNAi phenotype (blue, genes repressed after MBD2 RNAi) from left to right. Above, black bars represent the positions of genes from individual gene sets according to the SNR statistic. The green line at the top plot represents the running sum statistic of the enrichment score for the respective gene set under analysis. On top of the individual plots the nominal p value for the respective gene set as well as the false discovery rate are shown. (A) Genes with MBD2-bound promoters clearly tend to be associated with genes up-regulated after MBD2-RNAi whereas (B) genes with MBD3-bound promoters clearly tend to be associated with genes down-regulated after MBD2-RNAi. (C) Genes with MBD2-binding across at least one exon show a tendency similar to (A).