Transcriptional Repressor Domain of MBD1 is Intrinsically Disordered and Interacts with its Binding Partners in a Selective Manner

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SUPPLEMENTARY FIGURES

Figure S1. ^{15}N HSQCs overlay of MBD1-c (black) and MBD1-c-R583L/R585L mutant (orange). The two new peaks presumably from the point mutations are marked with '*'.

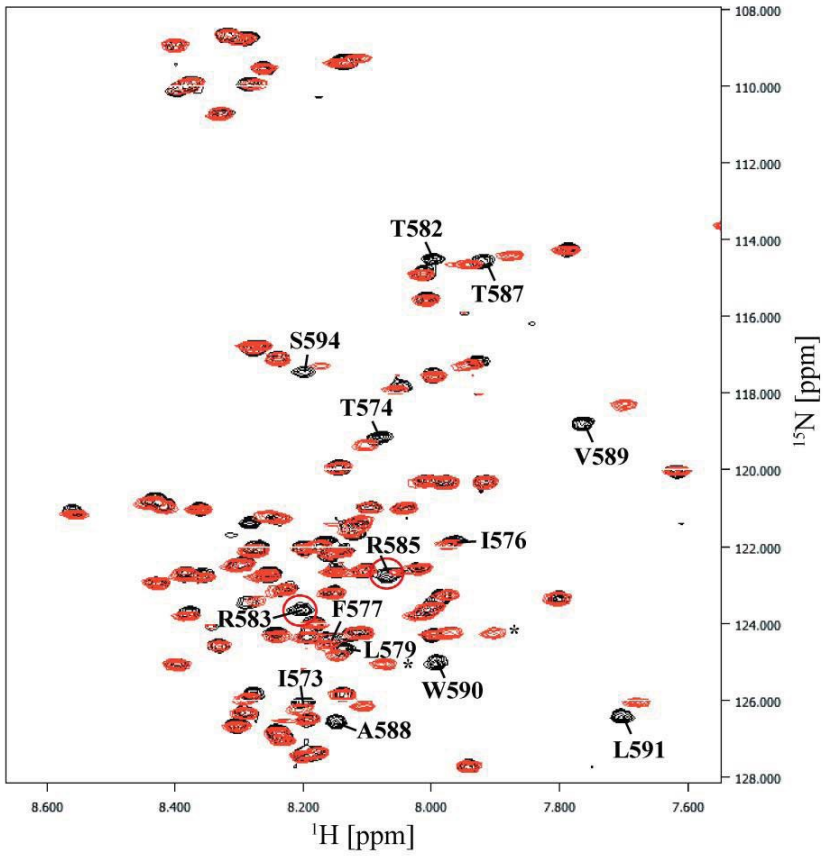


Figure S2. NOE connectivity for selected residues A588 to L591.

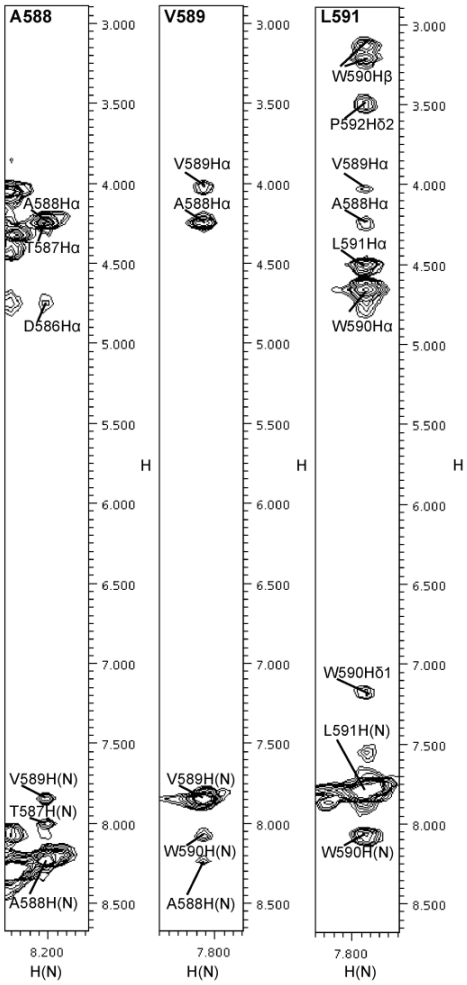


Figure S3. Relationship between average intensity retention (NMR) and isobaric heat capacity (ITC) upon MBD1-c protein interactions.

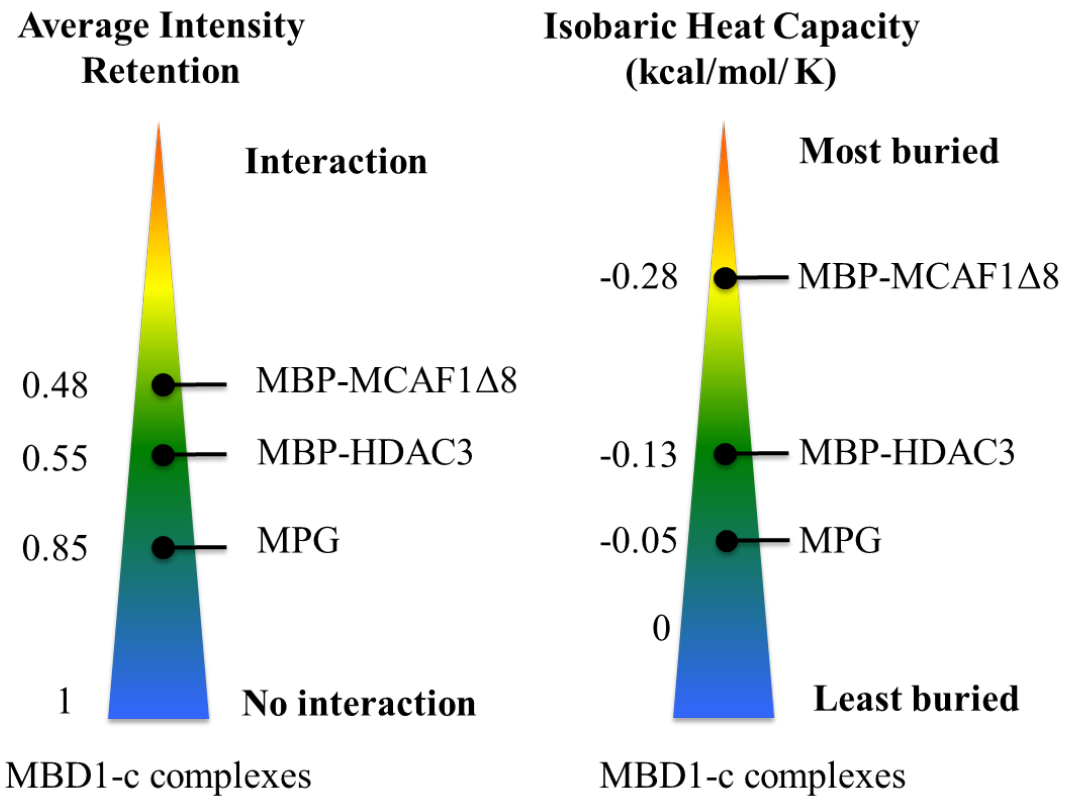
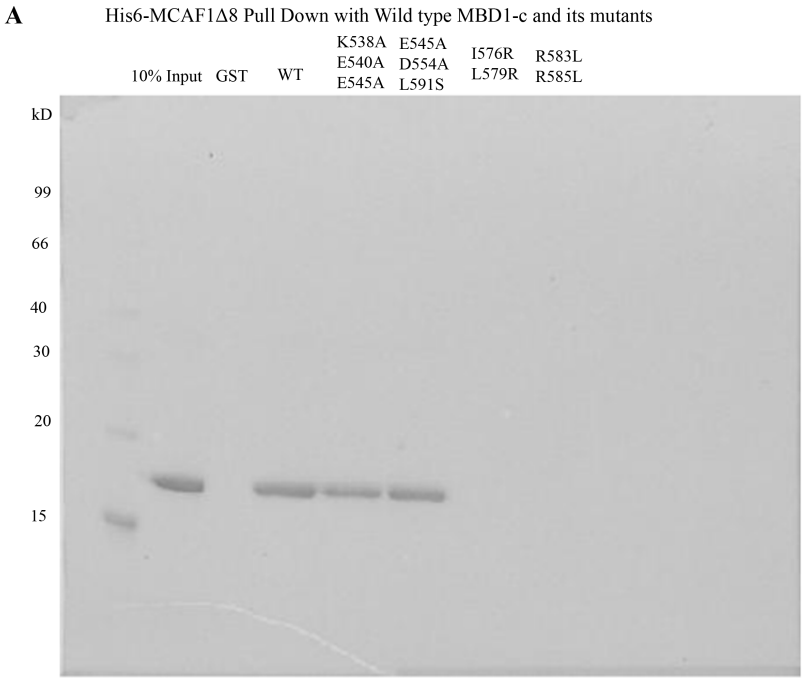
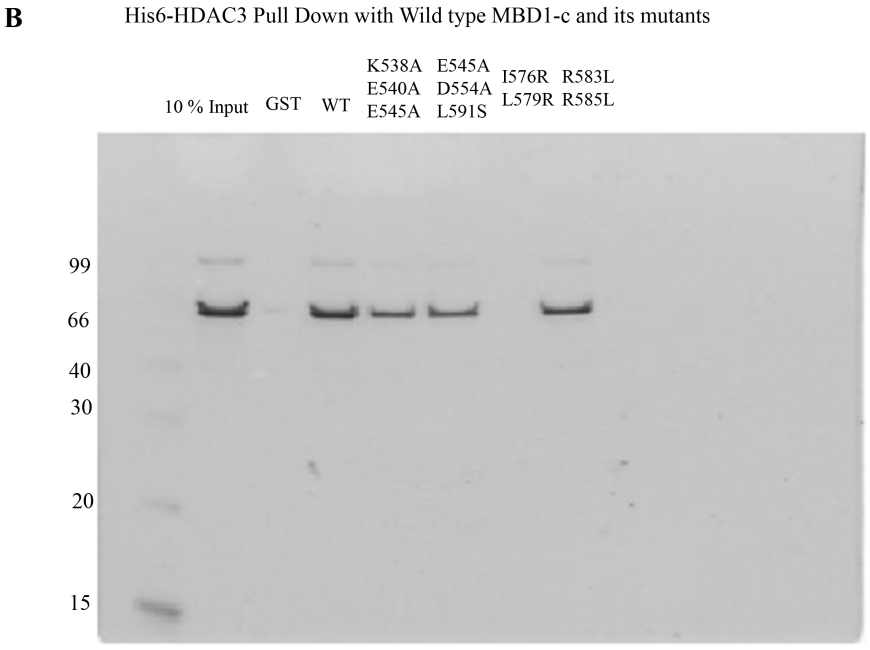
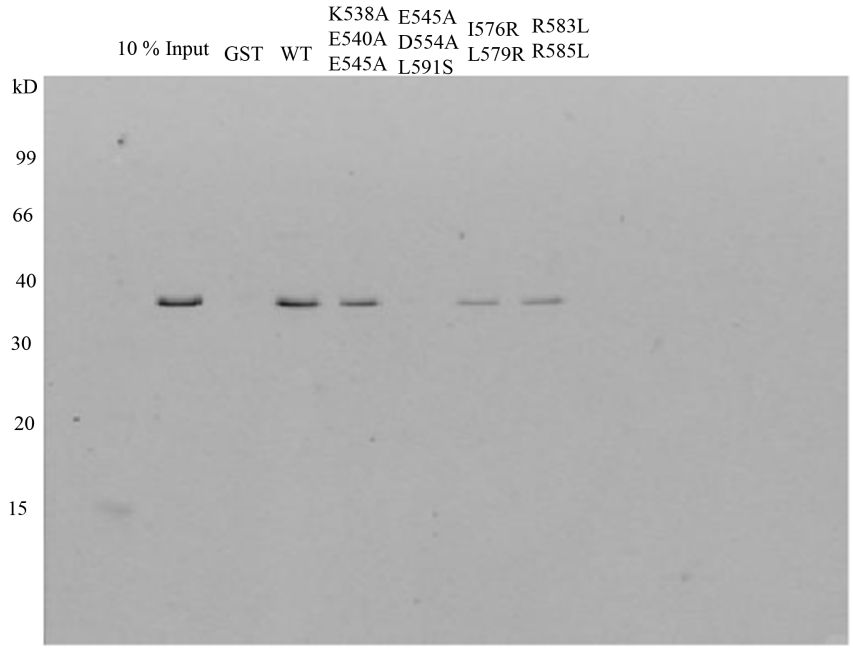


Figure S4. GST pull-down of MCAF1Δ8, HDAC3 and MPG with MBD-c and its mutants

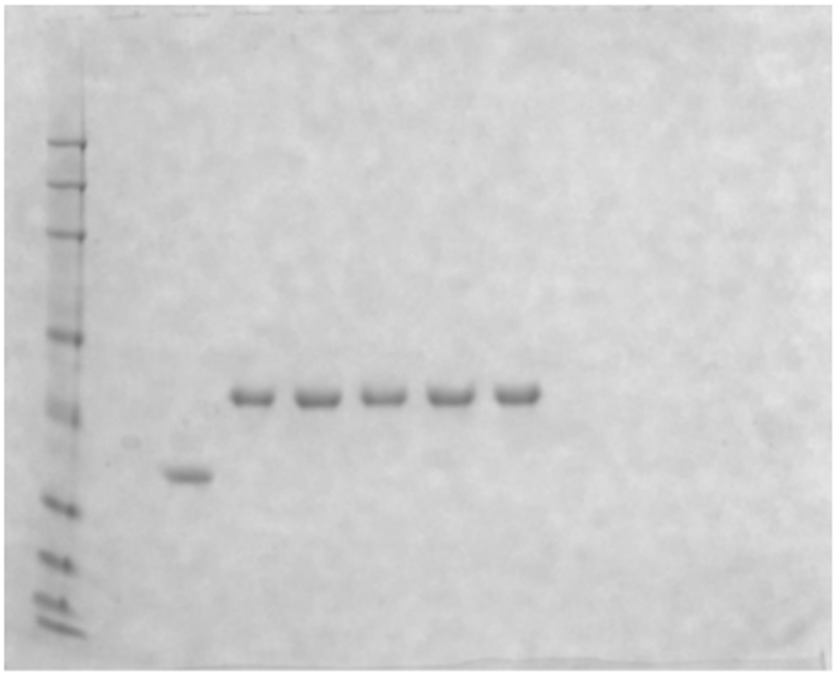




C His6-MPG Pull Down with Wild type MBD1-c and its mutants



D GST and GST-MBD1-c Input



SUPPLEMENTARY TEXT

Subcloning

The gene fragment encoding residues 507-605 of MBD1, including the transcription repressor domain (TRD, residues 529-592) was PCR amplified using the Pfu DNA polymerase (Fermentas) with the forward primer ATTGAATTCGACGAGTGGACACCAG and reverse primer TATCTCGAGCTACTGCTTTCTAGCTC (1st Base), containing the EcoRI and XhoI restriction sites (underlined), respectively. The gene fragment was subcloned into the pETM (pET32a modified) vector with an N-terminal His_6-tag and pGEX6P-1 vector with an N-terminus GST tag for protein expression and purification. In the case of MPG, the full length gene of MPG was amplified with a forward primer containing an EcoRI site (ATTGAATTCATGCCCGCGCGCAGC) and a reverse primer with an XhoI site (TTACTCGAGTCAGGCCTGTGTGTCCTGC) and then subcloned into the pETM vector.

The gene encoding for the MCAF1 residues 1154-1270 that interacts with TRD of MBD1, hereafter named as MCAF1Δ8, was amplified with the forward primer with an EcoRI site (ATTGAATTCGAAGCTGCCAGCACATCTCTGCCTC) and reverse primer with a NotI site (ATTGCGGCCGCTTAACTGCTCTGGGTAGAAGAG) and then subcloned into the multiple cloning site 1 (MCS1) of the pETduet-1 vector with a N-terminal MBP tag and the disulphide bond chaperone (DsbC) at the second multiple cloning site, and the same restriction sites were used for cloning into pETM vector with an N-terminal His_6-tag for protein expression. Similarly, full length HDAC3 was amplified using forward primer with an EcoRI site (ATTGAATTCATGGCCAAGACCGTGGCCTATTTC) and reverse primer with a NotI site (ATTGCGGCCGCTTAAATCTCCACATCGCTTTCCTTGTCATT) and cloned into MCS1 of the pETduet-1 vector with a N-terminal MBP tag and the disulphide bond chaperone (DsbC) at the second multiple cloning site.

Protein expression and purification of MBD1Δ, MPG, MBP-MCAF1Δ8 and MBP- HDAC3

Expression of pGEX-6P-1:MBD1-c followed almost the same above conditions but during

purification, the lysate was allowed to bind to glutathione sepharose beads (GE Healthcare), equilibrated with buffer A for 3 h at 4 °C, then washed with buffer A for 3 times. The GST tag was removed from MBD1-c using 1unit of Prescission protease per mg of GST- MBD1Δ at 4 °C. The cleaved MBD1-c protein was loaded onto a Superderx 75 column (GE Healthcare), equilibrated with the buffer (20 mM sodium phosphate buffer (pH 6.5), 50 mM NaCl and 3 mM DTT) is then eluted and concentrated..

MPG was over-expressed with an N-terminal His_6-tag in the pET-M vector. In short, the construct was transformed into E. coli BL21 (DE3) competent cells and the protein was overexpressed with the induction of 0.5 mM IPTG at Abs_{600nm} of 0.6, grown at 25 ºC for 6 hours and affinity purified using Ni-NTA resin. The protein was next purified using a cation exchange Hi-Trap SP-HP (GE Healthcare) column (MPG is highly basic with pI of 9.65) with buffer A: 20 mM sodium phosphate (pH 8.0) and buffer B: 20 mM sodium phosphate (pH 8.0) and 1 M NaCl. MPG was further purified using a Superdex 75 column (GE Healthcare) equilibrated with 20 mM sodium phosphate buffer (pH 6.5), 50 mM NaCl and 3 mM DTT. The protein eluted as a single homogenous peak, at its theoretical 35 kD. The purified MPG protein was concentrated using ultra filtration membrane (Merck Millipore) with 10 kD Molecular Weight Cut Off (MWCO).

MBP-MCAF1Δ8 and MBP-HDAC3 were over-expressed using the pETduet-1 vector as an N-terminal MBP-fusion protein. Seed cultures of transformed E. coli BL21 (DE3) competent cells were added to large scale LB medium containing 100 µg/ml ampicillin. The cell density was monitored until Abs_{600nm} was 0.6 to 0.7, before adding 0.1 mM IPTG for protein over-expression for another 16 h at 18 °C. Cells were harvested and cell pellet was stored at -80 °C until needed. The cell pellet was resuspended in binding buffer A: 50 mM Tris-HCl buffer (pH 8.0), 150 mM NaCl, 2 mM DTT, 5 % Glycerol and 0.1 % Triton X-100. The cell suspension was lysed by sonication on ice and the supernatant was loaded onto an amylose (NEB) column and subsequently allowed to bind for 2 h at 4 °C. The weakly bound proteins were removed by extensive washing using binding buffer A for 3 to 4 times. The MBP:MCAF1Δ8 and HDAC3

fusion protein was eluted with 20 mM maltose in buffer A. Thus purified MBP-MCAF1Δ8 and MBP-HDAC3 was passed through a Superderx 200 column (GE Healthcare) equilibrated with 20 mM sodium phosphate (pH 6.5), 50 mM NaCl, and 3 mM DTT. MBP-MCAF1Δ8 and MBP-HDAC3 eluted as a single homogenous peak. The MBP-tag was kept to stabilize MCAF1Δ8 and HDAC3 proteins. The purified protein was concentrated using ultra filtration membrane (Merck Millipore) with 10 kD MWCO.

Supplementary Table1. List of genes, primers and vectors used in the study.

S.	Gene	Fwd Primer	Rev Primer	Vector
1	MBD1-c	5'ATTGAATTCGACGAGTG GACACCAG3'	5'TATCTCGAGCTACTGC TTTCTAGCTC3'	pET-M
2	MBD1-c	5'ATTGAATTCGACGAGTG GACACCAG3'	5'TATCTCGAGCTACTGC TTTCTAGCTC3'	pGEX 6P-1
3	MCAF1Δ8	5'ATTGAATTCGAAGCTGCC AGCACATCTCTGCCTC3'	5'ATTGCGGCCGCTTAAC TGCTCTGGGTAGAAGAG	pET-M
4	MCAF1Δ8	5'ATTGAATTCGAAGCTGCC AGCACATCTCTGCCTC3'	5'ATTGCGGCCGCTTAAC TGCTCTGGGTAGAAGAG	pET duet-1
5	HDAC3	5'ATTGAATTCATGGCCAA GACCGTGGCCTATTTC3'	5'ATTGCGGCCGCTTAAA TCTCCACATCGCTTTCCT	pET duet-1
6	MPG	5'ATTGAATTCATGCCCGC	5'TTACTCGAGTCAGGCC	pET-M

Supplementary Table2. Table of thermodynamic parameters for MBD1-c binding with its protein partners.

Protein	Temperature (K)	ΔH (kcal/mol)	ΔS (kcal/mol/K)	ΔG (kcal/mol)
MBP-MCAF1Δ8	288	-1.72 ± 0.6	0.0185 ± 0.005	-7.05 ± 1.22
	298	-3.92 ± 1.2	0.0105 ± 0.019	-7.06 ± 1.82
	308	-7.37 ± 2.31	-0.0006 ± 0.0002	-7.20 ± 1.13
MBP-HDAC3	288	-0.8 ± 0.32	0.0233 ± 0.009	-7.5 ± 0.97
	298	-1.75 ± 0.78	0.0196 ± 0.002	-7.61 ± 1.09
	308	-3.3 ± 1.2	0.0144 ± 0.06	-7.74 ± 1.3
MPG	288	-0.39 ± 0.15	0.0236 ± 0.007	-7.18 ± 0.42
	298	-0.87 ± 0.09	0.0214 ± 0.002	-7.24 ± 0.76
	308	-1.32 ± 0.37	0.0195 ± 0.003	-7.33 ± 0.79