Chromatin-dependent allosteric regulation of DNMT3A activity by MeCP2

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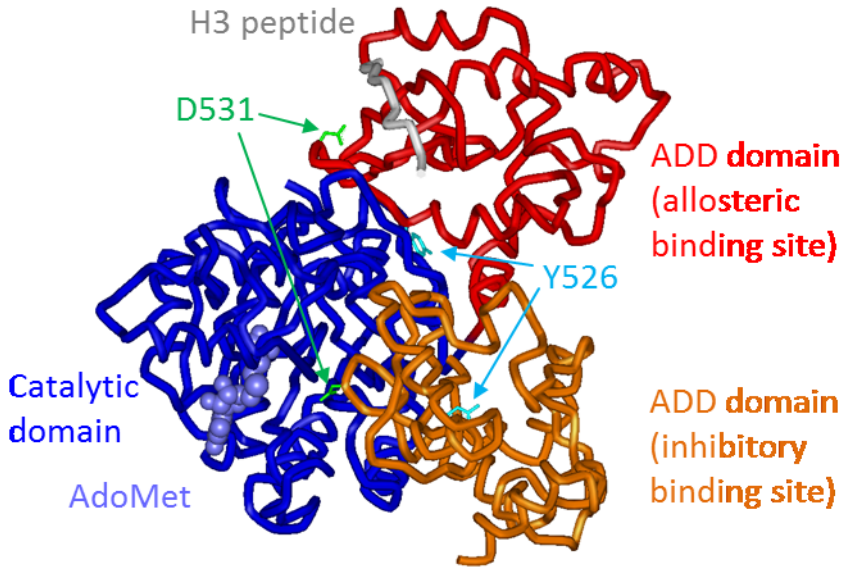
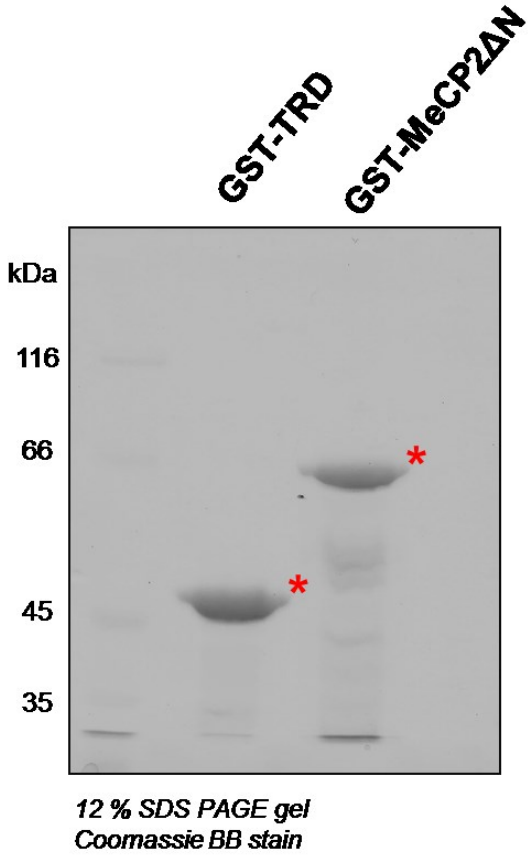
Supplemental information

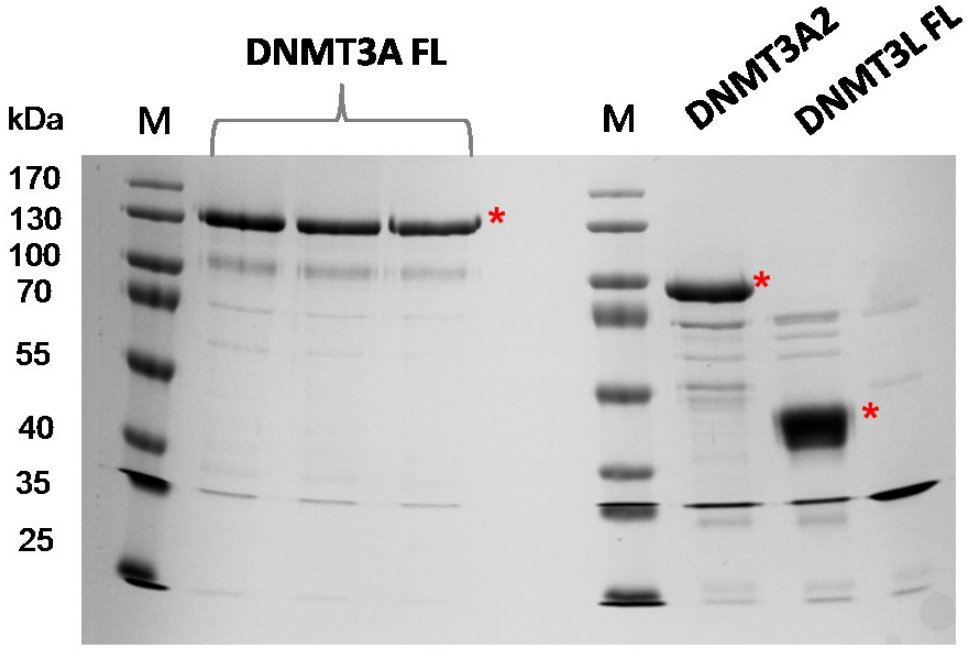
Suppl. Fig. 1: Model of the structure of a DNMT3A fragment comprising ADD (red or orange) and catalytic domain (blue). The ADD domain can bind to the catalytic domain at two interfaces, at an allosteric site (ADD domain colored in red, Y526 is at the interface), which is stabilized by binding of the H3 tail, and at an auto-inhibitory binding site (ADD domain colored in orange, D531 is at the interface). The image was generated using pdb files 4U7P and 4U7T.



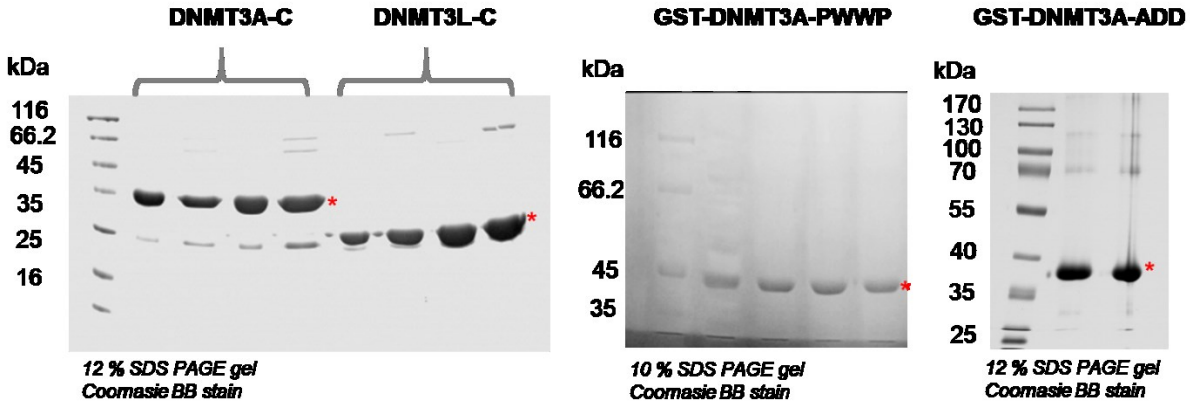
Suppl. Fig. 2: Purification of GST-tagged TRD domain of MeCP2 and of N-terminally truncated MeCP2. The image shows examples of the purified proteins separated on an SDS polyacrylamide gel and stained with Coomassie BB. The expected sizes of the proteins have been annotated with red asterisks.



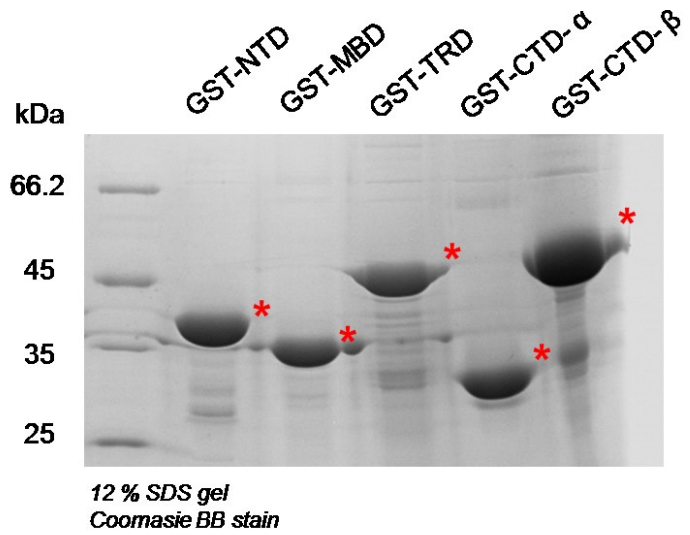
Suppl. Fig. 3: Purification of His-tagged DNMT3 proteins. The image shows examples of the purified DNMT3A full length (FL), DNMT3A2 and DNMT3L full length (FL). The proteins were separated on an SDS polyacrylamide gel and stained with Coomassie BB. The expected sizes of the proteins have been annotated with red asterisks.



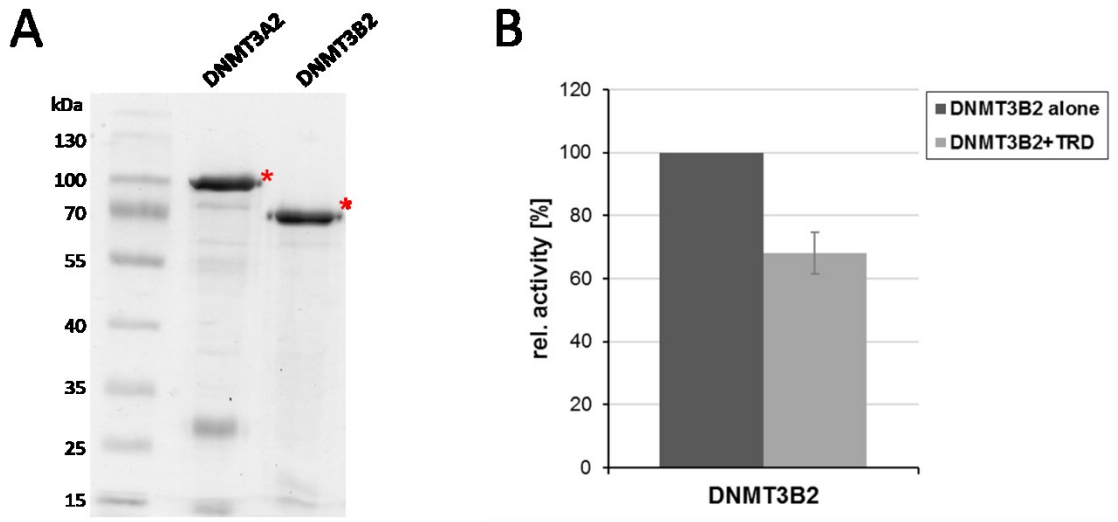
Suppl. Fig. 4: Purification of DNMT3 domains. The image shows examples of the purified His-tagged DNMT3A catalytic domain (DNMT3A-C), as well as the GST-tagged PWWP and ADD domains of DNMT3A. The proteins were separated on SDS polyacrylamide gels and stained with Coomassie BB. The expected sizes of the proteins have been annotated with red asterisks.



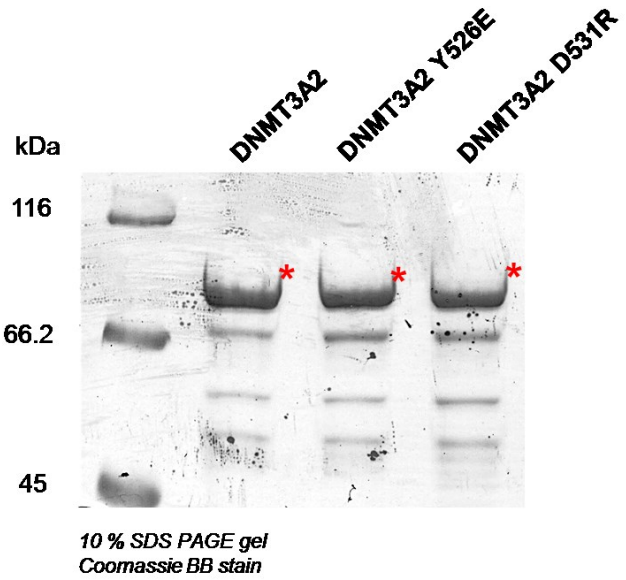
Suppl. Fig. 5: Purification of the GST-tagged MeCP2 domains. The image shows examples of the purified proteins separated on an SDS polyacrylamide gel and stained with Coomassie BB. The expected sizes of the proteins have been annotated with red asterisks.



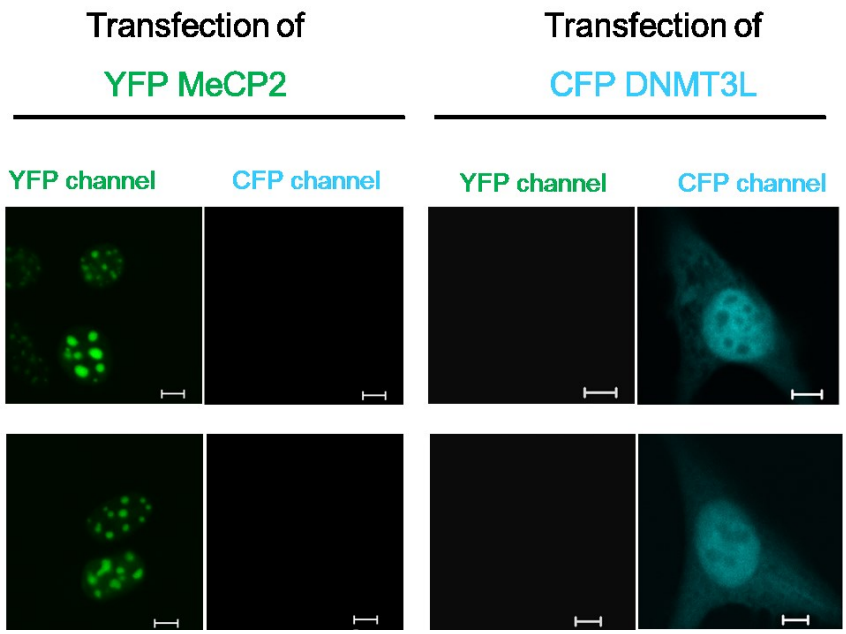
Suppl. Fig. 6: Purification of DNMT3B2 and its inhibition by MeCP2 TRD. DNMT3B2 is an N-terminally truncated form of DNMT3B cloned to correspond to DNMT3A2. A) Coomassie-stained SDS-PAGE of the purified His-tagged DNMT3A2 and DNMT3B2. The expected sizes of the proteins are indicated with red asterisks. Both protein preparations show comparable and high purity. B) Enzymatic activity assay with DNMT3B2 in the absence and presence of 3 µM TRD domain. The activity of DNMT3B2 in the absence of TRD was normalized to 100%. The error bar represents the SEM based on three independent experiments.



Suppl. Fig. 7: Purification of DNMT3A2 allosteric mutants. The image shows examples of the purified His-tagged proteins separated on an SDS polyacrylamide gel and stained with Coomassie BB. The expected sizes of the proteins have been annotated with red asterisks.



Suppl. Fig. 8: Absence of crosstalk between the YFP and CFP channels in the fluorescence microscopy. YFP-tagged MeCP2 and CFP-tagged DNMT3L were individually transfected into NIH3T3 cells and images were captures in the YFP and CFP channels.



Suppl. Fig. 9: Sequence of the 585mer DNA methylation substrate (left part) and HpaII digestion of unmethylated and pre-methylated 585mer. Samples were separated on an agarose gel and stained with Ethidium bromide. The absence of digestion of the methylated sample indicates complete methylation.

585mer Sequence

GGTAAAGGAAGCAGTAAGGGGCATACCCCGCGCGAAG
CGAAGGACAACCTGAAGTCCACGCAGTTGCTGAGTGT
GATCGATGCCATCAGCGAAGGGCCGATTGAAGGTCCG
GTGGATGGCTTAAAAAGCGTGCTGCTGAACAGTACGC
CGGTGCTGGACACTGAGGGGAATACCAACATATCCGG
TGTCACGGTGGTGTTCCGGGCTGGTGAGCAGGAGCAG
ACTCCGCCGGAGGGATTTGAATCCTCCGGCTCCGAGA
CGGTGCTGGGTACGGAAGTGAAATATGACACGCCGAT
CACCCGCACCATTACGTCTGCAAACATCGACCGTCTG
CGCTTTACCTTCGGTGTACAGGCACTGGTGGAAACCA
CCTCAAAGGGTGACAGGAATCCGTCGGAAGTCCGCCT
GCTGGTTCAGATACAACGTAACGGTGGCTGGGTGACG
GAAAAAGACATCACCATTAAGGGCAAAACCACCTCGC
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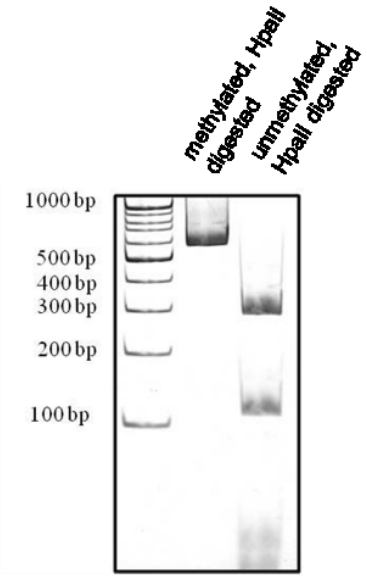
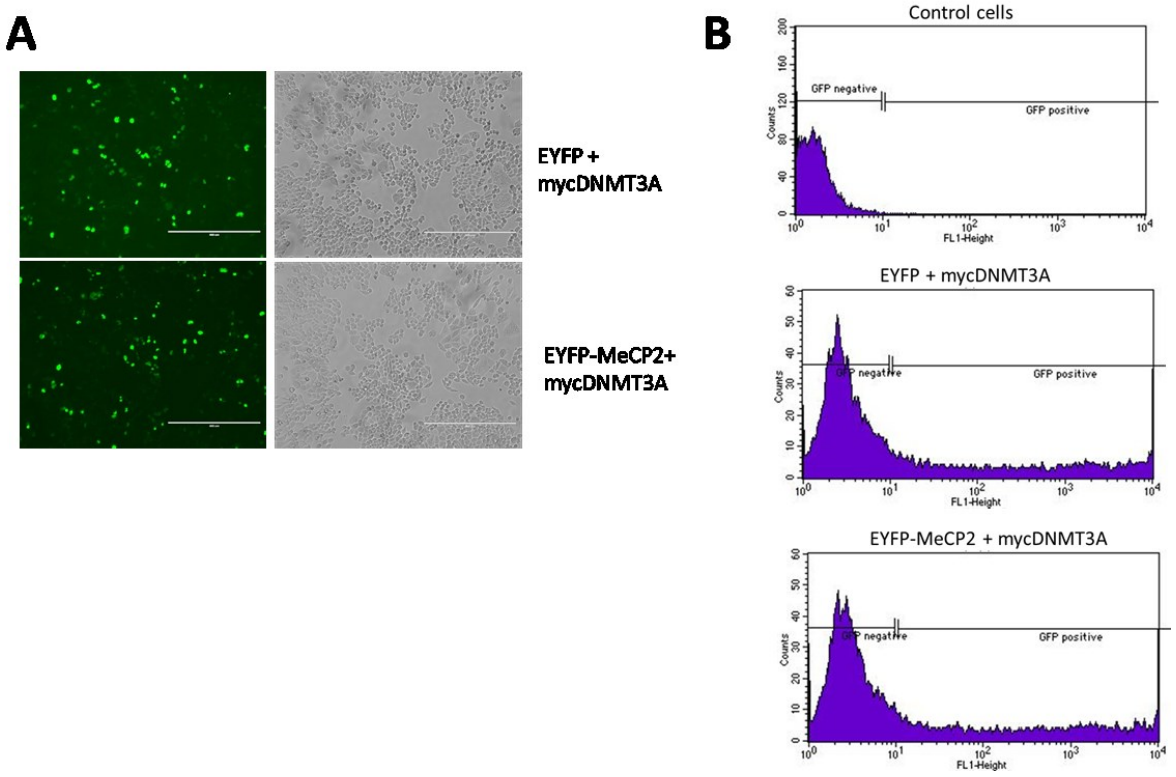
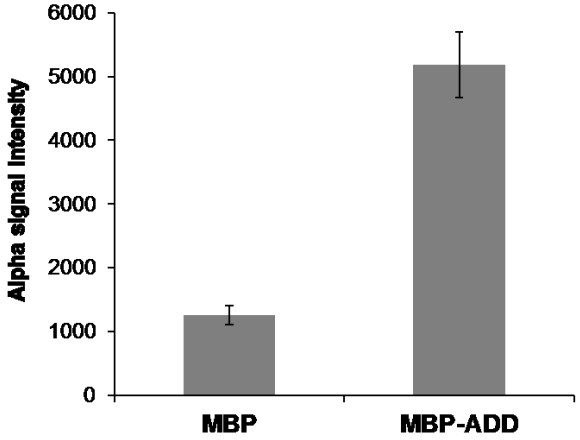
GCGCCCGTTTAATATCCGGATGCGCAGGATGACGCCG
GACAGCACCACAGACCAGCTGCAGAACAAA



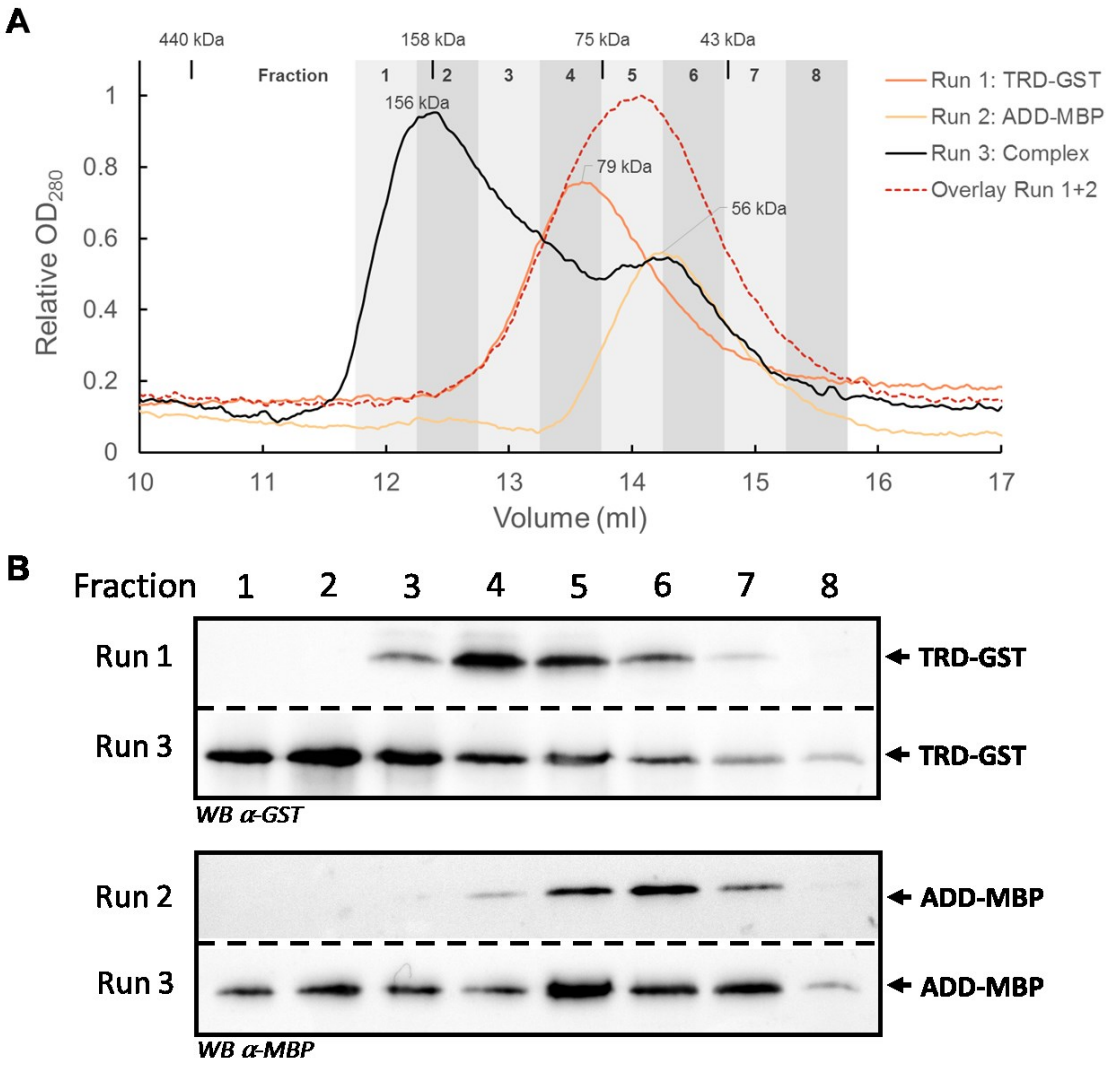
Suppl. Fig. 10: Co-expression of myc-tagged DNMT3A with EYFP-MeCP2 or EYFP in HEK293 cells. A) Fluorescence microscopy images captured 48 hours post transfection. The scale bar corresponds to 400 µm. B) FACS profiles of untransfected control cells and double transfected cells. Transfected cells were analyzed by FACS analysis using a Calibur system (BD Bioscience) equipped with 488 nm laser and CellQuest Pro software. The data confirm roughly equal expression of EYFP-MeCP2 and EYFP.



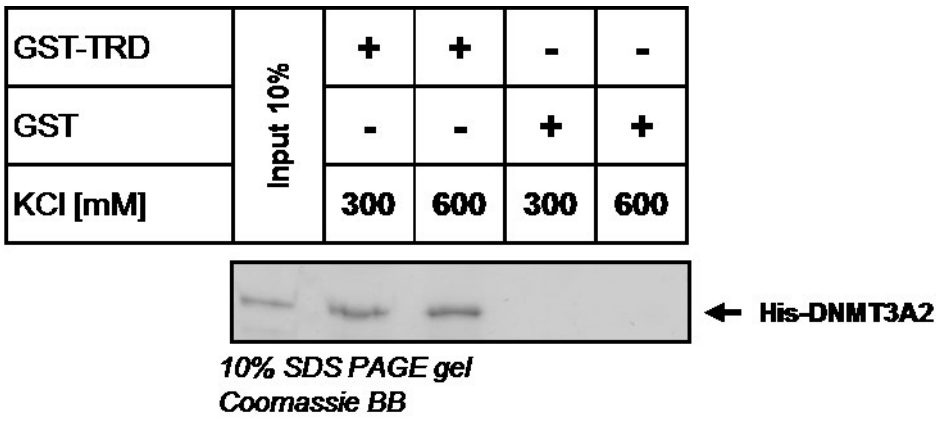
Suppl. Fig. 11: Complex formation of the GST-TRD and MBP-ADD domains detected by Alpha-assay. Alpha glutathione donor beads (PerkinElmer) were used for GST-TRD and anti-MBP AlphaLISA acceptor beads (PerkinElmer) for MBP-tagged proteins. MBP was used as negative control. Each protein (2 nM GST-TRD, 1 nM MBP-ADD or MBP) was separately incubated for 20 min at RT with 20 µg/ml of the corresponding beads to allow for binding. Afterwards, the corresponding samples were mixed and Alpha-signal measurements were taken after 20 min using an EnSpire Alpha Plate Reader (PerkinElmer).



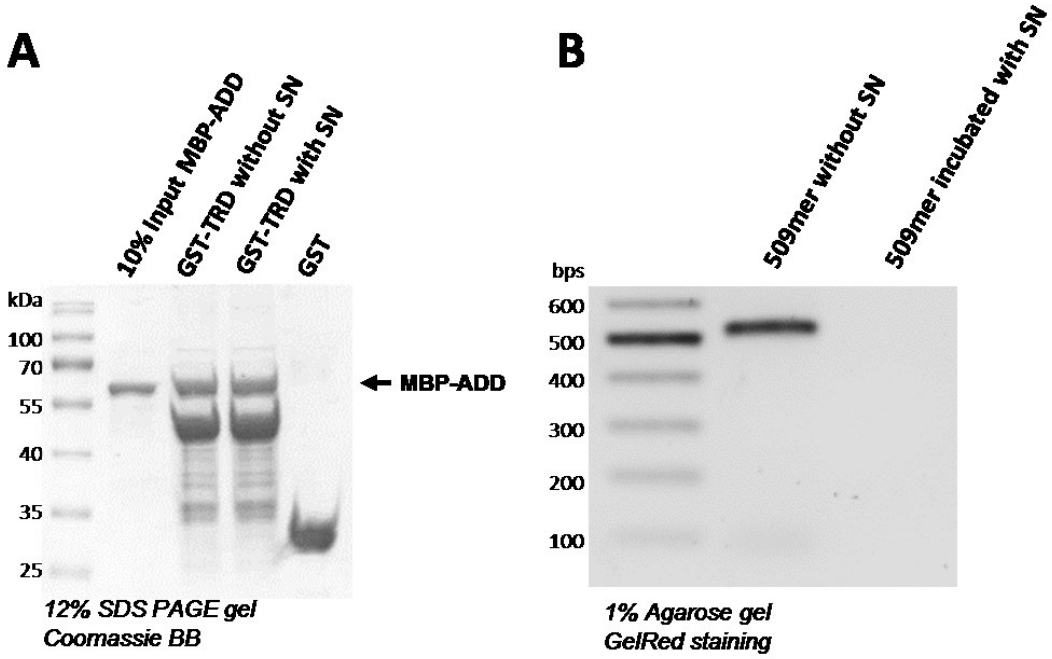
Suppl. Fig. 12: Detection of the interaction of ADD and TRD by size exclusion chromatography using a Superdex™ 200 10/300 GL column (24 ml column volume, GE Healthcare) and a BIORAD NGC Quest™ 10 system operated at a constant flow rate of 0.7 mL/min. The column was equilibrated with two column volumes (CV) of running buffer (20 mM Hepes pH 8.0, 200 mM KCl, 2 mM DTT and 10% Glycerol). For size calibration, the Gel filtration calibration kit HMW (GE Healthcare) was used. For the single domains, the protein solutions (7.6 µM GST-TRD and 2.6 µM for MBP-ADD) were prepared in running buffer and 100 µl injected using a sample loop. For the protein complex analysis, the same amounts of the proteins were pre-incubated in running buffer for 1 h at 22°C and injected afterwards. The resulting chromatograms were analyzed in Chromlab 5.0. A) Chromatograms: The isolated MBP-ADD domain (yellow curve) elutes at 56 kDa fitting to a monomer (theoretical weight 58 kDa). The isolated GST-TRD domain (orange curve) elutes at 79 kDa roughly fitting to a dimer (theoretical weight of the monomer 43 kDa). This result is in agreement with the known tendency of GST to dimerize. The sum of both signals is shown by the red, dashed line. The elution profile of both pre-incubated proteins is shown in black, showing an additional peak at 156 kDa, roughly corresponding to a mixture of 1:2 (56 kDa + 79 kDa = 135 kDa) and 2:2 complexes (2x56 kDa + 79 kDa = 191 kDa). B) Protein analysis in fractions (500 µl) collected as annotated. 15 µl of each fraction were separated on a 12% SDS gel and analyzed by western blot using anti-GST (2745770, GE Healthcare Bio-Sciences, USA) and anti MBP (E8032S, New England BioLabs, USA) antibodies.


A
440 kDa
158 kDa
75 kDa
43 kDa
Fraction
1
2
3
4
5
6
7
8
156 kDa
79 kDa
56 kDa
Run 1: TRD-GST
Run 2: ADD-MBP
Run 3: Complex
Overlay Run 1+2
Relative OD280
Volume (ml)
B
Fraction 1 2 3 4 5 6 7 8
Run 1
Run 3
TRD-GST
TRD-GST
WB α-GST
Run 2
Run 3
ADD-MBP
ADD-MBP
WB α-MBP

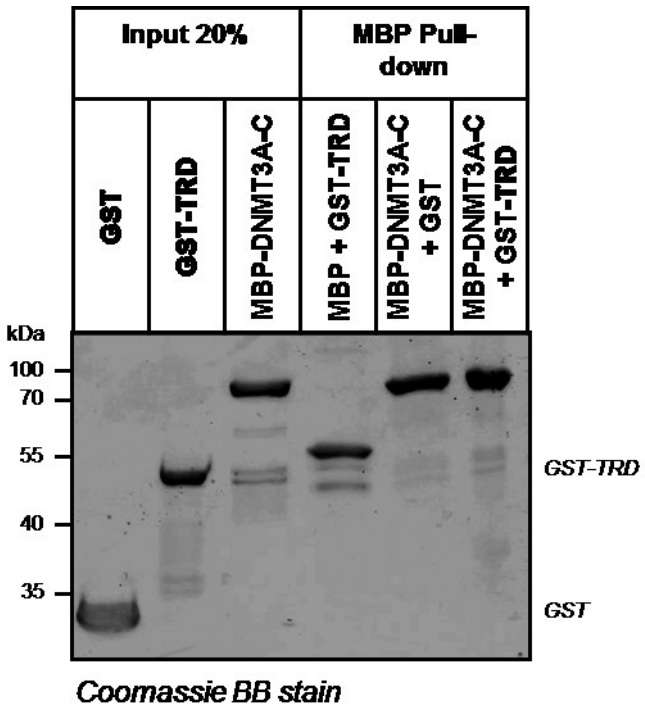
Suppl. Fig. 13: The interaction between DNMT3A2 and GST-TRD is resistant to high salt washes. Coomassie BB staining of the pull-down of His-tagged DNMT3A2 with GST-TRD conducted in buffer containing 300 and 600 mM KCl.



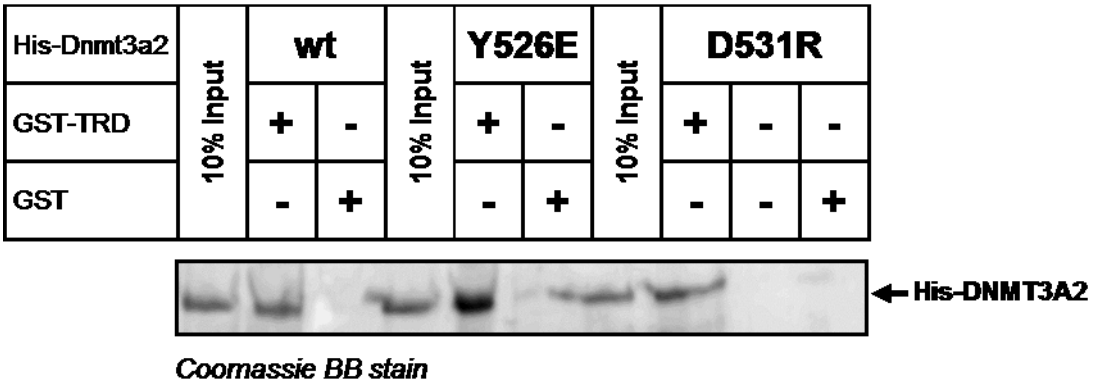
Suppl. Fig. 14: The interaction of the DNMT3A-ADD and MeCP2-TRD domains is nuclease resistant. A) GST pull-down experiments of MBP-ADD and GST-TRD were conducted in the presence and absence of non-specific Serratia nuclease (30 nM, incubated for 1 hour at 4 °C with shaking). Proteins were detected by Coomassie BB staining. The equal pulldown in absence and presence of Serratia nuclease (SN) shows that the interaction is not mediated by nucleic acids. B) Confirmation of the nuclease activity of Serratia nuclease. 1 µg of 509 bp long double stranded DNA PCR product was incubated for 1 hour at 4°C with and without 30 nM Serratia nuclease in 100 µL interaction buffer. Afterwards, 20 µL of the reaction mixtures were analyzed by agarose gel electrophoresis.



Suppl. Fig. 15: DNMT3A-C and MeCP2-TRD do not interact. Coomassie BB staining of the pull-down of MBP-DNMT3A-C and GST-TRD showing that there is no detectable interaction between the two domains.



Suppl. Fig. 16: Interaction of DNMT3A2 Y526E and D531R allosteric mutants with TRD. The Coomassie BB stained image of a pull-down of His-DNMT3A2 wild type and allosteric mutants by GST-tagged TRD shows that the DNMT3A2 Y526E and D531R mutants still interact with TRD.



Suppl. Fig. 17: Loss of TRD mediated inhibition of DNMT3A2 in the presence of higher concentrations of the H3 peptide. DNA methylation activities of DNMT3A2 and DNMT3A2 bound to H3 peptide (amino acid sequence 1-19, 25 µM) were determined in the absence (dark grey) or presence (light grey) of TRD (3 µM). Error bars indicate the SEM based on three independent experiments.