Supplementary Figure S1 Clustal W alignment of human and *Xenopus tropicalis* MBD1 proteins. Human hMBD1 and *Xenopus* xMBD1 proteins share homology within the MBD and CxxC domains but not within the C-terminal region including the TRD. However, similar to the human MBD1, two consensus SUMO conjugation sites IKEE and VKTE (blue boxes) are present in the C-terminus of xMBD1. The human SUMO conjugation sites VKQE are indicated with red boxes.
Supplementary Figure S2  Sumoylation of MBD1 in E.coli expressing mammalian SUMO conjugation enzymes.

(A) A coomassie stained gel shows purified non-sumoylated and sumoylated GST-MBD1 proteins. Lanes 1-4 represent proteins purified from BL21 cells co-expressing the mammalian sumoylation machinery and GST-tagged wild type (WT) and lysine mutants of MBD1 K450A, K489A and 2(K-A). Sumoylated MBD1 proteins are indicated with black dots. Lanes 5-8 represent the GST-tagged MBD1 proteins purified from ordinary BL21 cells. 

(B) A Western blot of a gel as in (A) with anti-SUMO1 antibodies detects only sumoylated MBD1 proteins. 

(C) A Western blot of an identical gel as in (A) and (B) with anti-MBD1 antibodies detects non-sumoylated and sumoylated MBD1 proteins.
**Supplementary Figure S3** Mapping the region of PIAS1 required for binding to MBD1.

(A) Schematic representation of PIAS1 protein with its known domains. The regions of homology (>80%) between PIAS1 and PIAS3 are indicated. (B) pGADT7-PIAS1 deletion constructs were used to identify regions of PIAS1 that bind MBD1 in yeast two hybrid assays. The interactions were scored by growth on –Leu/-Trp/-His/-Ade quadruple drop out medium (QDO). The portion of PIAS1 required for interaction with MBD1 maps to amino acids 510-651. (C) pGADT7-PIAS1 deletion constructs (as in B) were transcribed and translated in rabbit reticulocyte lysates. 1 µl of each translation reaction was run on 12% SDS gel and the PIAS1 proteins were detected on Western blot with anti-HA antibodies. Note that anti-HA antibody recognize several unspecific bands in reticulocyte lysates. Translated PIAS1 proteins are indicated with black dots. (D) GST-MBD1 and GST-MBD1(221-480) were used to pull down *in vitro* translated PIAS1 proteins. 5 µl of each translation in 200 µl buffer were used in these assays. As in (B) the C-terminal region containing amino acids 451-651 binds to MBD1 while the rest of the protein amino acids 1-450 does not.

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Supplementary Figure S4  Mapping the region of PIAS3 required for binding to MBD1. (A) Schematic representation of PIAS3 protein with its known domains. (B) pGADT7-PIAS3 deletion constructs were used to identify regions of PIAS3 that bind MBD1 in yeast two hybrid assays. As in Figure S2 the interactions were scored by growth on −Leu/-Trp/-His/-Ade quadruple drop out medium (QDO) and quantitative β-galactosidase assays. The region of PIAS3 containing amino-acids 1-500 does not bind to MBD1 while a slightly longer protein 1-565 binds. However, the full length PIAS3 scored higher in β-galactosidase assays than the shorter 1-565 protein.
Supplementary Figure S5  PIASy does not target MBD1 for conjugation of SUMO.
(A) Western blot probed with anti-MBD1 antibody detects MBD1-SUMO accumulating in nuclear extracts of HeLa cells transfected with GFP-PIAS1 and GFP-PIAS3 but not in nuclear extracts of cells transfected with GFP-PIASy or Flag-SUMO1.
(B) GFP-PIAS1, GFP-PIAS3 and GFP-PIASy were detected in the same extracts as in (A) with anti-GFP antibody.

Figure S6  MBD1 co-immunoprecipitates with PIAS1 and PIAS3 when SETDB1 protein levels are low. PIAS1 and PIAS3 were immunoprecipitated from HeLa nuclear extracts of cells transfected with 4 µg of either control GL2 siRNA or SETDB1 siRNA. Immunoprecipitated proteins were detected on Western blots with anti-PIAS1, anti-PIAS3 and anti-MBD1 antibodies. Note that MBD1 co-immunoprecipitates efficiently with PIAS1 and PIAS3 only in extracts of cells treated with SETDB1 siRNA, and non-sumoylated MBD1 is preferentially co-immunoprecipitated. A very small amount of MBD1 can be seen co-immunoprecipitating with PIAS proteins from extracts of cells treated with GL2 siRNA. Anti-HA antibodies were used for the mock IPs.
Supplementary Figure S8  Recombinant MBD1-SUMO binds SETDB1 in vitro.
(A) Coomassie stained 10 % gel shows purified recombinant proteins (see supplemental methods for details).
(B) GST pull down with recombinant proteins shown in (A) of in vitro translated and mixed together HA-tagged CAF-1 and SETDB1. The unmodified and disumoylated GST-MBD1 proteins pull down roughly equal amounts of SETDB1 and CAF-1. Similar results were observed with the mono-sumoylated and unmodified GST-MBD1K450A and GST-MBD1K489A proteins.

Supplementary Figure S7  Expression of RFP-MBD1 plasmids in HeLa cells.
HeLa cells were transfected with plasmids expressing the indicated proteins. The cells were collected 48 hours after the transfection and one half of the cells were used to prepare nuclear extracts (-NEM) and the other half for extraction of total RNA. RFP-MBD1 proteins were detected on Western blots probed with anti-MBD1 antibody. The RNA was used for RT-PCRs shown in Figure 6.

Transfections:

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Supplementary methods:

**Sumoylation of MBD1 in E.coli**
The plasmids pBADE12, encoding His-tagged Aos1, Uba2 and Ubc9 (chloramphenicol resistance) and pKR-SUMO1 were described previously (Mencia and de Lorenzo, 2004). Full length human MBD1 was cloned into pGEX-4T1 (GE Healthcare) in frame with the GST tag. Single and double lysine-to-alanine substitutions at K450 and K489 of GST-MBD1 were generated by mutagenic PCR. The three expression plasmids pBADE12, pKR-SUMO1 and pGEX-MBD1 were transformed into BL21 (DE3) cells as described (Mencia and de Lorenzo, 2004). Bacterial colonies grown on plates with triple ampicillin/kanamycin/chloramphenicol selection were picked up, grown in liquid culture, induced with IPTG and the recombinant proteins were purified by affinity chromatography according to standard procedures.

**Yeast two-hybrid assays**
PIAS1 and PIAS3 deletion fragments were cloned into pGAD-T7rec vector and introduced into a haploid Y187 (MATα) yeast strain. The resulting colonies were analysed for expression by Western blots and mated with AH 109 strain (MATα) yeast expressing full length MBD1 expressed from a pGBK-T7 plasmid. After mating the yeast were plated on plates of minimal SD-double drop out (DDO) medium (-Trp/-Leu) or SD-quadruple drop out medium (QDO: -His/-Ade/-Trp/-Leu) and scored for growth. Colonies from each mating grown on DDO were also analysed by liquid β-galactosidase assays according to standard protocols.

**Purification of recombinant MBD1 and MBD1-SUMO proteins.**
ER2566 E.coli cells (NEB) were transformed simultaneously with pBADE12 (encoding Aos1, Uba2 and Ubc9), pRSFduet-SUMO1 (expressing 6His-SUMO1) and pGEX-MBD1 (wild type or mutants). Bacterial colonies grown on plates with triple ampicillin/kanamycin/chloramphenicol selection were picked up, grown in 1L of liquid culture, induced with IPTG at OD600 = 0.5 and the recombinant GST-MBD1-SUMO1 was purified by affinity chromatography (first on Ni-chelating sepharose column followed by glutathione sepharose) according to standard procedures. Unmodified GST-MBD1 was derived from GST-MBD1-SUMO1. 300 µl beads carrying GST-MBD1-SUMO1 were incubated with 5 units of yeast SUMO-isopeptidase ULP1 (Invitrogen) for 1 hour at 4C. The beads were washed with PBS to remove ULP1 and the sumoylated and desumoylated proteins were analysed in Polyacrilamide SDS gels (Supplemental Figure 8A). Subsequently the sumoylated and desumoylated GST-MBD1 proteins were used for *in vitro* pull down assays. pGEX-GFP plasmid was a gift from Ken Sawin. GST-GFP protein was purified according to standard procedures.

**In vitro pull downs**
pGADT7-SETDB1 and pGADT7-CAF-1 plasmids containing the N-terminus of SETDB1 and the C-terminus of CAF-1 were described previously (Sarraf and Stancheva, 2004). Typically 1 µg of each pGAD plasmid was transcribed and translated in 50 µl rabbit reticulocyte lysate (TnT T7 kit, Promega). For GST pull downs 100 ng of glutathione sepharose-bound recombinant proteins were incubated with 100 µl of NE1 buffer (20mM HEPES pH=8; 10 mM KCl; 1 mM MgCl; 0.1% Triton X-100, 0.5 mM DTT) supplemented with protease inhibitors cocktail (Sigma) and 1 µl of each (SETDB1 and CAF-1) translation reaction. The mixtures were incubated on rotating wheel for 1 hour at 4C, the beads were washed 3-4 times with NE2 buffer (20M HEPES pH=8; 10 mM KCl; 1 mM MgCl; 400 mM NaCl, 0.1% Triton X-100 and 0.5 mM DTT). The bound proteins were resolved in 10% SDS gels and detected on Western blots with monoclonal anti-HA antibodies (provided by CRUK).

**References:**