**Figure S1.** Schematic showing MeCP2 3’ UTR (top; thick black line), miR132 MRE (arrow) and nucleotide sequence conservation (vertical black lines; http://genome.ucsc.edu).
Figure S2. Transfection of cortical cultures with miR132 decreases MeCP2 protein level. Western blots are depicted in S2a and b, quantification in S2c, and graphical representation in S2d. Y-axis represents MeCP2/α-tubulin.
**Figure S3.** Western blot showing that in L6 cells (which express the short MeCP2 mRNA lacking the miR132 MRE), introduction of miR132, miR1-1, or GFP did not affect MeCP2 protein levels.
Figure S4. Treatment of rat cortical neurons with 10µM forskolin induces pre-miR132 transcript. Error bars are SEM.
Figure S5. Treatment of cortical cultures with 60mM KCl or 10µM forskolin for 6 hours decreases MeCP2 protein levels. Western blot is depicted in S5a, quantification in S5b, and graphical representation in S5c.
Figure S6. Treatment of rat cortical neurons with 10µM forskolin for 6 hours does not change the level of MeCP2 mRNA, compared with DMSO treated cells. Error bars are SEM.
Figure S7. Quantification and graphical representation of data from Fig. 1b. Introduction of either the AS 2'-O-me or AS LNA blockers increase MeCP2 protein levels under basal conditions.
Figure S8. Introduction of either the AS 2'-O-me or AS LNA blockers abolishes the forskolin-induced decrease of MeCP2 protein levels. Western blot is depicted in S8a, quantification in S8b, and graphical representation in S8c.
Figure S9. AS 2’-O-me blocker abolishes the decrease in CtBP protein levels observed after forskolin treatment. However, no increase in CtBP levels is observed after treatment with the AS LNA blocker, suggesting that the AS LNA specifically blocks the interaction between miR132 and MeCP2. Western blot is depicted in S9a, quantification in S9b, and graphical representation in S9c.
Figure S10. Introduction of AS or control LNA into rat cortical neurons does not change the level of MeCP2 mRNA compared with untransfected cells. Error bars are SEM.
Figure S11. Model for homeostatic regulation of MeCP2 mRNA by miR132.
Figure S12. Comparison of mature miR24, miR132, miR212, and miR194 levels between wildtype and Rett mice. Each of these microRNAs are predicted to target the miR132 MRE within the MeCP2 3’UTR. Error bars are SEM. Asterisks denote significant changes (p < 0.01).
Homeostatic regulation of MeCP2 Expression by a CREB-induced microRNA
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Western blots. Standard methodologies were utilized with anti-MeCP2 (Upstate), anti-CtBP1 (BD Biosciences), or anti-α-tubulin (Sigma) antibodies. Primary antibodies were used in 5% BSA/TBST at a 1:1000 concentration for MeCP2, and CtBP1, or a 1:10,000 concentration for α-tubulin. Secondary HRP-conjugated antibodies were used in 3% milk/TBST at a 1:5000 concentration. The blots were exposed using ECL plus (Amersham). Each experiment was repeated in at least three independent cortical cultures. Additional experiments and quantifications are provided as supplemental figures. Blots were quantified using the ImageJ software package (rsb.info.nih.gov/ij/).

cDNA constructs, siRNA, oligos, and primers. The miR132 and miR1-1 hairpins were amplified from rat genomic DNA by using the following primers: miR132 forward, 5’-CTAGCCCCGCAGACACTAGC-3’; miR132 reverse, 5’-CCCCGCCTCCTCTTGTCTCTGTA-3’; miR1-1 forward, 5’-TGGCGAGAGAGTTCTAGCTTG-3; miR1-1 reverse, 5’-TGTGCACAACTTCAGCCCCATA-3’. miR132 and miR1-1 were cloned into pCAG. A dicer substrate siRNA against MeCP2 was synthesized by Integrated DNA technologies with the following sequence, 5’-CAUGGAAUCCUGUGAGCUAGUCUAC-3’. The primer sequences for real time PCR are as follows: BDNF I forward, 5’-GGCTGCGTAGAAGCAACAA-3’, reverse, 5’-CTTGTCAGGCTAGGCGGAAG-3’; BDNF III forward, 5’-CCCAGTCTCTGCCTTAGAATGG-3’, reverse, 5’-ACTCGCAGCGCTTCATGGAA-3’; GAPDH forward, 5’-ATCCAGAGCTGAACGGGAAGC-3’, reverse, 5’-TTGGGGGTAGGAACAGGAAG-3’; 18S forward, 5’-CCGCAGCTAGGAATAATGGA-3’, reverse, 5’-CCCTCTTATACATGGGCCTCA-3’; mouse miR132 forward, 5’-ATGGTGCGCCCCGCAGC-3’; reverse, 5’-CCCGCCTCCTCCTGTCTGTA-3’; mouse BDNF I forward, 5’-GGCTGCGTGCAGAAGCAACAA-3’; reverse, 5’-TCGCCAGGTAGAAGCCCATTC-3’; mouse BDNF IV forward, 5’-ACCCACCCC CGGCAGCTA-3’; reverse,
TACTCGCACGCTTCAGCGAGA-3’. The sequences of the 2’-O-methyl oligoribonucleotides (IDT) are: antisense, GGGCAACCGUGGCUUUCGAUUGUUCUGUGG; scrambled, GGGGACACCCUGGAUUCUUUGGAUCUGUGGG. The sequence of the LNA oligonucleotides (IDT) are (with modified bases underlined): antisense, 5’-TAACAGTCCTGTTATTTGACA-3’; control, 5’-TGTAGACAAATATGGCATGGCCTT-3’.

**Cell culture and stimulation.** Primary cortical cultures were prepared from P1 rats using standard protocols. Briefly, brains extracted from P1 pups were digested with papain for 1 hour, washed, and triturated by being passed through a 10mL pipette. Neurons were plated in Neurobasal A supplemented with B-27 (Invitrogen), and 10% FBS. After two hours, the plating media was replaced with Neurobasal A containing B-27. For stimulation experiments, neurons 5 DIV were treated for 6 hrs with 10 M forskolin dissolved in DMSO.

**Neuronal transfection.** P1 cortical neurons were nucleofected (Amaxa) according to the manufacturer’s protocol. Transfection efficiencies were monitored by co-transfection of a GFP reporter. After nucleofection, neurons were cultured for 5 DIV, with the exception of the miR132 experiments which were cultured for 3 DIV, due to toxicity. Transfection efficiency at 3 and 5 DIV was 60-80%. Plasmids were used at a concentration of 3.5 g per 100 1 of nucleofector solution. Oligos (2’-O-me and LNA) were used at a concentration of 10nM. siRNA was used at a concentration of 200nM.

**RT-PCR.** RNA was extracted from cultured neurons using the RNeasy kit (QIAGEN). RNA was subjected to DNase (Ambion) treatment and reverse transcribed using SuperScript II and random primers (Invitrogen). PCRs (20 l) contained 2 l of 10X PCR buffer, 2.5 mM MgCl₂, 200 M dNTP (Roche), 0.125 M primer, 1X SYBR green I (Invitrogen), and 1 unit of Platinum Taq (Invitrogen). PCR was performed on an Opticon OP346 (MJ Research) for 3 min at 94°C followed by 50 cycles at 94°C for 15s and 68° for 40s. Each reaction was normalized to 18S. Fig 2a, 2b, and 2c represent averages of six independent experiments for each condition. RT-PCR experiments for mature microRNAs
were performed using TaqMan® microRNA assays (Applied Biosystems) according to the manufacturer’s protocol.

**In vivo experiments.** 30mg pieces of cortex were isolated from three Jaenisch MeCP2 KO mice and three WT littermates. The samples were homogenized in buffer RLT (QIAGEN). After this step, RNA extraction and RT-PCR proceeded as described above. Fig 2d represents averages from three KO mice and three WT littermates.

**Statistical analysis.** Data with homogenous variances were analyzed by using the two-tailed Student *t* test. A p-value of < .01 was considered significant.