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piRNAs warrant investigation in Rett Syndrome: An omics perspective

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Abstract. Mutations in the *MECP2* gene are found in a large proportion of girls with Rett Syndrome. Despite extensive research, the principal role of MeCP2 protein remains elusive. Is MeCP2 a regulator of genes, acting in concert with co-activators and co-repressors, predominantly as an activator of target genes or is it a methyl CpG binding protein acting globally to change the chromatin state and to supress transcription from repeat elements? If MeCP2 has no specific targets in the genome, what causes the differential expression of specific genes in the *Mecp2* knockout mouse brain? We discuss the discrepancies in current data and propose a hypothesis to reconcile some differences in the two viewpoints. Since transcripts from repeat elements contribute to piRNA biogenesis, we propose that piRNA levels may be higher in the absence of MeCP2 and that increased piRNA levels may contribute to the mis-regulation of some genes seen in the *Mecp2* knockout mouse brain. We provide preliminary data showing an increase in piRNAs in the *Mecp2* knockout mouse cerebellum. Our investigation suggests that global piRNA levels may be elevated in the *Mecp2* knockout mouse cerebellum and strongly supports further investigation of piRNAs in Rett syndrome.

Keywords: Rett Syndrome, MeCP2, piRNAs, LINE 1, short RNAs

Rett Syndrome (RTT), a severe neurodevelopmental disorder, leads to intellectual disability in girls. After a normal prenatal and postnatal period, patients usually present with developmental delay between 6 and 18 months of age, followed by the development of stereotypic hand movements and loss of acquired skills including voluntary hand use, language and communication. This regression is characteristic of Rett Syndrome which, in 97% of clinically diagnosed classic cases and 70% of atypical cases, is caused by mutations in the methyl CpG binding protein 2 gene, (*MECP2*) [1]. In some patients with atypical Rett Syndrome, where some but not all clinical features are seen, mutations in *CDKL5* [2] or *FOXG1* [3] are found, albeit infrequently. Diagnosis of Rett Syndrome is based on clinical criteria [4] and confirmed upon detection of a mutation in *MECP2*, *CDKL5* or *FOXG1*. However, in approximately 20% of girls clinically diagnosed with classic or atypical Rett syndrome, mutations cannot be detected in either of these genes.

MECP2 gene undergoes X chromosome inactivation (XCI) [5], which means that in any cell with two X chromosomes, RNA transcripts arise only from the *MECP2* gene on the active X chromosome. This is because the *MECP2* gene on the inactive X chromosome has been silenced. Chaumeil et al. demonstrated, through in-situ hybridization in mouse ES cells, that the *Mecp2* gene moves inside the silencing compartment of *Xist* on the 4th day after differentiation [6]. Although some genes are known to escape X inactivation in humans and mouse [7–10], Carrel et al. showed using rodent/human somatic hybrid cell lines that in humans MECP2 transcripts are not expressed from the inactive X chromosome [7]. Due to the random nature of X inactivation, part of the clinical variability in RTT is attributed to the differences in the X inactivation status of patients [11–13], however more recent data suggest X inactivation status may not adequately explain the phenotypic variations [14].

MECP2 gene is composed of 4 exons and generates two transcripts which encode two nearly identical protein isoforms [15,16]. MeCP2_e1, which commences translation from exon 1, is encoded from a transcript encompassing exons 1, 3 and 4; and MeCP2_e2, which starts translation from the end of exon 2, is generated from a transcript arising from exons 1, 2, 3 and 4, where exon 1 and most of exon 2 form the 5'UTR [15,

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16]. Although *MECP2* is expressed in all tissues, semi-quantitative PCR analysis has shown that *Mecp2_e1* may have a higher expression level over *Mecp2_e2* in the brain [16]. Mutations in exons 3 and 4 affect both protein isoforms and are frequently found in Rett patients. Mutations in exon 1 can cause Rett syndrome despite the fact that mutations in exon 1 do not affect the coding region of the MeCP2_e2 protein. Interestingly, mutations in exon 2 of the gene, which have the potential to affect the MeCP2_e2 isoform alone, have so far not been found in patients. While earlier work on an Australian patient with a recurrent deletion in exon 1 of *MECP2* gene demonstrated the absence of MeCP2_e2 protein correlated with X inactivation status, suggesting translational interference from the mutation [17], a recent publication found no evidence of loss of MeCP2_e2 protein in a Canadian patient with a similar mutation indicating that some patients may present with clinical features of Rett syndrome even in the presence of a fully functional MeCP2_e2 isoform [18]. Interestingly, this data also suggests that despite high sequence similarities there is no functional redundancy between the two protein isoforms.

Due to its property to bind methylated DNA with high affinity and its association with repressor complexes consisting of HDAC1/2 and Sin3A, MeCP2 was believed to function as a transcriptional repressor [19, 20]. The MeCP2-DNA interaction was shown to result in chromatin compaction, which is also correlated with silencing of chromatin [21]. Subsequent studies revealed that MeCP2 had binding affinity to methylated DNA as well as non-methylated DNA [22]. Absence of MeCP2 in mouse brains also results in an increase in H3Ac levels, suggesting a role for MeCP2 in chromatin modification [23,24]. Recent data suggest that MeCP2 protein may be a regulator of transcription, acting in concert with activators as well as repressors to regulate gene expression. Yasui et al. first reported that promoter occupancy by MeCP2 may not result in gene silencing [25]. Using a custom tiling array of selected chromosomal regions totalling 26.3 Mb, they performed ChIP-chip analysis on SH-SY5Y cells with antibodies against MeCP2 and RNA polymerase II. The data revealed co-occupancy of MeCP2 and RNA Polymerase II at selected promoters suggesting that MeCP2 binding may not be correlated to gene repression [25]. Using ChIP-chip assays for 24,275 promoters, they demonstrated that only 2600–4300 promoters were occupied by MeCP2, of which 1534 promoters showed strongest enrichment. Comparison with gene expression arrays in the same cell lines revealed that almost 63% of the "strongest" promoters were expressed in SH-SY5Y cells. Subsequent MeDIP-ChIP analysis revealed that just 2.2% of methylated promoters were occupied by MeCP2 [25]. These data were supported in part by Chahrour et al. who used microarrays to determine differentially expressed genes in the hypothalamus of 6 week old *Mecp2* knockout (KO) mouse and in the hypothalamus of a mouse model that overexpressed *Mecp2* under its endogenous promoter (Tg) [26]. Combining their data from the KO and Tg models, they identified 2561 genes as direct targets of MeCP2, of which ~85% were activated by MeCP2 and ~ 15% were repressed by MeCP2 [26]. Using mass spectrometry on proteins co-immunoprecipitated with an anti-MeCP2 antibody, they identified CREB1 as a co-activator associated with MeCP2 and demonstrated co-occupancy of the two proteins at an activated target *Sst [26]*. Together, these data established MeCP2 as an activator of transcription [25,26]. Thus transcriptional mis-regulation is believed to underlie the phenotype seen in patients with mutations in the *MECP2* gene. In view of the fact that FOXG1 is a member of the forkhead family of transcription regulators, it is likely that in patients carrying mutations in *FOXG1,* mis-regulation of genes may contribute to the phenotypic features. The molecular pathology leading to the clinical phenotype of Rett Syndrome in mutation negative patients remains unknown. While much has been reported on the mis-regulation of specific genes after MeCP2 knockdown (KD) or KO, such studies have not yet been reported for FOXG1. Other studies reveal subtle changes in the expression levels of specific genes after MeCP2 KD or in the *Mecp2* KO mouse brain rather than genome wide transcriptional mis-regulation [27–29].

However, a recent report suggests that the absence of a functional MeCP2 may result widespread mis-regulation of repeat elements. Skene et al. investigated MeCP2 binding on selected loci in the mature mouse brain using ChIP-qPCR and demonstrated that MeCP2 was enriched all across the loci, but the enrichment was reduced over CpG islands, which are generally methylation free [24]. With bisulfite modification and sequencing of selected loci they demonstrated the recovery of predominantly methylated chromatin from the MeCP2 ChIP, re-emphasizing the role of MeCP2 as a methyl CpG binding protein [24]. Based on their investigation of the histone acetylation status by Western blotting and H3Ac ChIP-qPCR at 100 loci, they concluded that the association of MeCP2 with chromatin causes a genome-wide decrease in histone acetylation [24]. To investigate the binding sites of MeCP2

genome wide, Skene et al. performed MeCP2-ChIP-sequencing on the whole brain. Despite deep sequencing, they did not find peaks of MeCP2 occupancy, but found reads which coincided with methylated regions of the genome. Since they did not uncover specific binding targets of MeCP2 in the genome, they hypothesized that MeCP2 may act at a global level, most likely to supress transcription from the repeat regions of the genome [24]. Using qPCR, they demonstrated a 1.6 fold increase in transcripts arising from repeat sequences such as LINE-1, intra-cisternal A particles (IAPs) and major satellite DNA in the nuclear fraction of the *Mecp2* KO mouse brain. Based on their data they proposed that MeCP2 functions to repress spurious transcription of repeat elements [24] rather than to activate specific gene targets. An earlier investigation into the association between MeCP2 and LINE-1 and Alus had revealed that MeCP2 repressed LINE-1 expression and transposition, but activated Alu expression [30]. The role of MeCP2 in repressing transcription and transposition of LINE-1 elements was also corroborated by independent studies from the Gage Lab that showed that LINE-1 is over expressed in neuron progenitor cells after KD of MeCP2 and in neurons derived from Rett patients [31]. The data from Yasui et al. suggests that MeCP2 displays limited binding to methylated sites [25] and from Chahrour et al. proposes that MeCP2 acts as a transcriptional activator of specific targets [26]. In contrast, the data from Skene et al. suggests that MeCP2 binds methylated DNA, is a modulator of global chromatin state and may not have specific gene targets [24]. We note that some of these studies were conducted using microarrays or custom tiling arrays, which are generally limited to gene specific probes and exclude repeat sequences. Despite contradictory inferences on MeCP2 function, the fact remains that specific genes are mis-expressed and repeat elements are over-expressed in *Mecp2* KO mice. To reconcile the two opposing views, an alternative model would suggest that the key role of MeCP2 is to silence LINEs and similar repeat elements globally and that the observed mis-expression of genes is a downstream consequence of mis-expressed repeat elements.

Several recent reports suggest that non-coding RNAs play a role in the regulation of transcription through epigenetic modifications, [for a review see [32]]. Repeat elements, particularly LINEs, are known to participate in the silencing of genes on the X chromosome [33]. Expression of LINEs in the vicinity of genes is instrumental for their inclusion into the Xist silencing compartment [33]. It is not yet known whether transcripts from LINE elements are associated with chromatin remodelling complexes to mediate epigenetic changes and fine-tune gene transcription, but we note that the elevated repeat elements were found in the nuclear compartment of the *Mecp2* KO mouse brain cells [24] and there is emerging evidence of enrichment of LINEs in nuclear and chromatin fraction of cells [34]. A recent report using a retrotransposon capture sequencing technique (RC-seq) reveals that somatic transposition of LINE-1 (L1) in the hippocampus results in insertions, predominantly in exons and introns of protein coding genes [35]. Comparing microarray data with their RC-seq data, Baillie et al. reported that intronic L1 insertions are likely to cause overexpression of such genes in the brain, suggesting a regulatory role for L1 [35]. It is not clear if the increase in retrotransposon expression in the *Mecp2* KO brain leads to their active transposition even in post mitotic neurons. Random integration of transposons is suppressed in differentiated somatic cells by transcriptional [36] and post-transcriptional mechanisms [37]. It would be interesting to investigate if somatic retrotransposition is increased in the *Mecp2* KO brain and whether overexpressed genes identified in *Mecp2* KO mouse show novel intronic L1 insertion events.

Retrotransposons such as LINEs can be further processed into short 21–24 nucleotide double stranded siRNAs [38] or into single stranded 24–31 nucleotide long piRNAs [39,40]. Watanabe et al. described in mouse oocytes dicer dependent double stranded endogenous siRNAs mapping exclusively to retrotransposons or expressed mRNA transcripts [38]. While the presence of endogenous siRNAs has not been demonstrated in the mouse brain, given that such short RNAs are shown to regulate the expression levels of specific genes and specific retrotransposons [38,41], and that dysfunctional MeCP2 may result in the overexpression of LINE-1 [24,31], it would be interesting to investigate the presence of endogenous siRNAs in the MeCP2 KO mouse brain.

piRNAs are germ line specific short RNAs of size 24 to 31 nucleotides generated through dicer independent processing of long single strand RNA transcripts. piRNAs interact with the PIWI proteins (MILI, MIWI and MIWI2 in mouse) [39,40] and their function, though not fully understood, appears to relate to silencing of transposons especially LINE-1 [39,40,42] intracisternal A particles [39,40] and specific genes through DNA hypermethylation [43]. In mouse testis, 17% of piRNAs bound to the MIWI protein map to repeats including LINEs, SINEs and LTRs [40]. In addition, through

a unique ping-pong cycle, piRNAs are amplified from existing retrotransposon transcripts, mostly LINE-1 elements [44,45]. This amplification cycle also results in the depletion of LINE-1 in germ line cells and is believed to deplete the levels of retrotransposon transcripts after differentiation [42]. Thus piRNAs regulate expression of LINEs and Intracisternal A particles both transcriptionally and post transcriptionally. Interestingly, piRNAs have recently been shown to regulate expression of a single imprinted gene in an imprinted locus in mouse spermatogonia via DNA methylation by piRNA targeting of a non coding RNA (pitRNA) arising from the locus [43]. It is as yet not known if such specific targeting by piRNAs is a widespread phenomenon, nevertheless it highlights a mechanism through which piRNAs may regulate expression of specific genes.

Until recently, piRNAs and their associated proteins were presumed to be germ line specific in mouse, but a report published last year confirmed the presence of MIWI and its associated piRNAs in the mouse hippocampus through sequencing, RIP-qPCR, northern blots, western blots and in situ hybridization studies [46]. Through bioinformatics analysis, Lee at al. identified specific piRNAs expressed in the brain and showed through piRNA inhibition studies, that one piRNA in the brain, DQ541777, may play a role in regulating the size of dendritic spines [46]. It appears plausible that in the absence of MeCP2, over expression of repeat elements, particularly LINE-1 may result in an increase in piRNA amplification from transposons. It would be interesting to investigate whether akin to germ line cells, in brain also, the increase in piRNAs result in depletion of retrotransposon transcript levels through transcriptional and post transcriptional silencing. The mechanism of transcriptional silencing by piRNAs through DNA methylation may require recruitment of repressor complexes by proteins that bind methylated DNA, including MeCP2, thus highlighting a feedback loop for MeCP2 requirement.

To investigate our hypothesis that piRNAs may be overexpressed in the *Mecp2* KO mouse brain, we analysed a short RNA library made from mouse cerebellum [47]. To identify miRNAs differentially expressed in the cerebellum, Wu et al. performed short RNA sequencing of pooled 6 week old pre-symptomatic wildtype and *Mecp2* KO cerebellum ($n = 4$ in each pool) using the SOLiD version 2 sequencer [47]. We downloaded the pooled libraries from the DDBJ database (DDBJ accession number SRP005132). ncRNAs were downloaded from NONCODE version 3 [48] and a total of 75,814 mouse piRNAs were extracted from this database. As the SOLiD reads correspond to the 5' ends of small RNAs, we directly mapped the respective reads from the pooled WT and KO libraries, using SHRiMP version 2.2.2 [49] with the default parameters, to the mouse piRNAs. After mapping we corrected tag numbers for reads multi-mapping to more than one piRNA, so that if a read mapped equally well to 2 or more individual piRNA sequences, the tag numbers were divided by the number of times it multi mapped, followed by equal assignment to all the piRNAs. For expression analysis we did not take into consideration multi mapped reads that mapped to 5 or more piRNAs and also filtered out reads that had less than 5 tags in the KO samples. The tag numbers were normalized by tags per million before comparison between wildtype and KO samples. Our very preliminary analysis of piRNAs in the cerebellum reveals 357 piRNAs in the cerebellum libraries (Supplementary Table 1). While 81% (287) of the individual piRNAs found in the cerebellum have a higher expression in KO, 59% (208) piRNAs show an expression change of over 1.5 fold in the KO cerebellum compared with the wildtype (Fig. 1B and supplementary Table 1). Overall, we found a striking 1.9 fold increase in the total piRNAs in the KO cerebellum in comparison with the wildtype cerebellum (Table 1 and Fig. 1A) suggesting a global increase in piRNAs in the *Mecp2* KO sample. We next investigated whether the 20 most abundant piRNAs identified by Lee et al. in the mouse hippocampus were represented in the mouse cerebellum [46]. We found 19 out of the 20 piRNAs reported by Lee et al. in the cerebellum libraries (Table 2) including DQ 541777 (the piRNA implicated in regulating the size of dendritic spines) and of these, 12 piRNAs (60%) revealed a fold change of over 1.5 in the KO cerebellum (Table 2). Interestingly, DQ541777 is the 5^{th} most abundant piRNA in the cerebellum libraries, the two most highly abundant piRNAs in the cerebellum libraries map to rRNA loci, which were incidentally excluded in the hippocampal analysis [46].

Based on our preliminary findings, we suggest a model for Rett Syndrome where, in the absence of a functional MeCP2, the over-expressed repeat elements lead to an increase in the total piRNAs. The over-represented piRNAs may function, not only to deplete the load of repeat transcripts in cells, but also to fine-tune the expression level of specific genes. Thus piRNA mis-regulation may contribute to some of the differences in gene expression seen in the *Mecp2* KO mouse brain.

While our analyses provide preliminary evidence of genome wide piRNA over-expression in the *Mecp2*

Table 1
piRNA analysis in the short RNA libraries made from 6 week old pooled cerebellum from the wildtype mouse and the *Mecp2* knockout mouse [38] (DDBJ accession number SRP005132)

	WT cerebellum	KO cerebellum
Read ID	SRR089647	SRR089648
Total sRNA reads	3660124	2789136
Total reads mapped to piRNAs (no filter)	362089	522238
Reads mapped to piRNAs (filter out < 5 reads in KO)	356283	518589
piRNA mapped tags normalized by TPM	97341.74762	185931.8441
Fold change (tpm normalized piRNAs over WT)	1	1.910093548

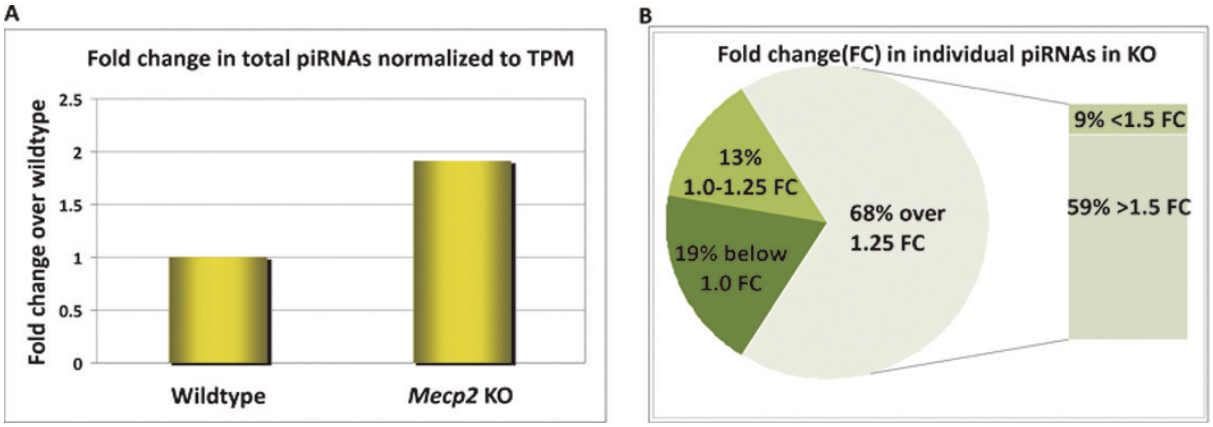

Fig. 1. piRNA levels are elevated in *Mecp2* KO cerebellum. Read numbers for individual piRNAs found in the wildtype (WT) and *Mecp2* knockout (KO) samples were normalized to tags per million as described in the text. After filtering out the piRNAs with less than 5 reads in the KO sample, the total piRNA reads were summed up. The histogram in panel A shows that the total numbers of piRNAs are almost doubled (1.9 fold) in the KO sample suggesting a global rise in piRNAs. The fold change relative to WT was calculated for each individual piRNA in KO. The pie chart in panel B reveals that 81% of piRNAs show a higher expression level in the KO sample. Of these, 59% have a fold change of over 1.5 in the KO sample (see supplementary Table 1). (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-2012-0932)

KO cerebellum, this data was generated from libraries without replicates. Thus additional detailed investigations are warranted to affirm the over-representation of piRNAs and gain insights into the extent of their contribution to the gene mis-regulation seen in the Rett mouse model. We did not venture into the identification of gene targets of mis-regulated piRNAs and their intersection with the known mis-regulated genes or repeats. Such data may provide insights into the mis-regulation of some genes and the biogenesis of the over-represented piRNAs. Notably, in humans and mouse, an absence of MeCP2 results in fewer dendritic spines when compared with wildtype neurons [50,51]. While inhibition of DQ541777 was reported to cause a decrease in spine density, whether the overexpression of piRNAs, including DQ541777, can cause such morphological changes in the brain is not yet known. Further, recent reports have demonstrated that unlike mRNAs, miRNAs are stable in extracellular environments including blood serum [52,53]. Although such analyses have not been conducted for piRNAs, if piRNAs are found to be stable in extracellular fluids, differentially expressed piRNAs may potentially represent clinical biomarkers for the diagnosis and prognosis of Rett Syndrome.

There is overwhelming complexity in unravelling the molecular pathogenesis of the phenotype seen in Rett syndrome. Although the miRNA repertoire of *Mecp2* KO cerebellum has been investigated using next generation sequencing approaches, the field would benefit in re-identifying mis-regulated transcripts through deep, long and short (to identify sRNAs other than miRNAs), RNA sequencing of specific brain regions. Additionally, RC-seq conducted to identify somatic integration events would reveal whether the overexpressed genes are correlated with intronic LINE-1 insertion events. And while it has been established for the *Mecp2* KO mouse model, that investigating a specific brain region is more fruitful than investigating the whole brain [26], analysis of neuronal subtypes would be even more insightful. Until now, the isolation of neuronal subtypes from adult mouse brain using high throughput techniques such as FACS sorting was challenging, yielding few nuclei and poor quality RNA. A recently pub-

Table 2
Comparison of the top twenty piRNAs reported in the hippocampus [38] with the piRNAs found in the cerebellum

Top 20 piRNAs in hippocampus	WT hippocampus tag numbers	WT cerebellum tag numbers	KOcerebellum tag numbers	WT cerebellum TPM	KO cerebellum TPM	FC KO/WT
DQ541777	16130	1995.50	2411.00	545.20	864.43	1.59
DQ705026	6257	154.00	377.00	42.08	135.17	3.21
DQ555094	3439	202.00	140.00	55.19	50.19	0.91
DQ719597	2459	168.00	306.00	45.90	109.71	2.39
DQ689086	1514	65.00	78.00	17.76	27.97	1.57
DQ540285	1433	457.40	548.23	124.97	196.56	1.57
DQ540981	1360	126.50	124.00	34.56	44.46	1.29
DQ720186	849	336.00	251.00	91.80	89.99	0.98
DQ555093	775	189.50	129.50	51.77	46.43	0.90
DQ540862	639	21.50	33.50	5.87	12.01	2.04
DQ540284	635	456.90	548.23	124.83	196.56	1.57
DQ541506	580	523.90	627.40	143.14	224.94	1.57
DQ539915	304	35.50	28.00	9.70	10.04	1.04
DQ540861	252	20.50	31.50	5.60	11.29	2.02
DQ715526	207	20.00	20.00	5.46	7.17	1.31
DQ543676	182	438.90	518.70	119.91	185.97	1.55
DQ722288	175	2.00	13.00	0.55	4.66	8.53
DQ551351	168	Not found	Not found	Not found	Not found	Not found
DQ550765	118	10.75	9.50	2.94	3.41	1.16
DQ708131	115	3.00	6.00	0.82	2.15	2.62

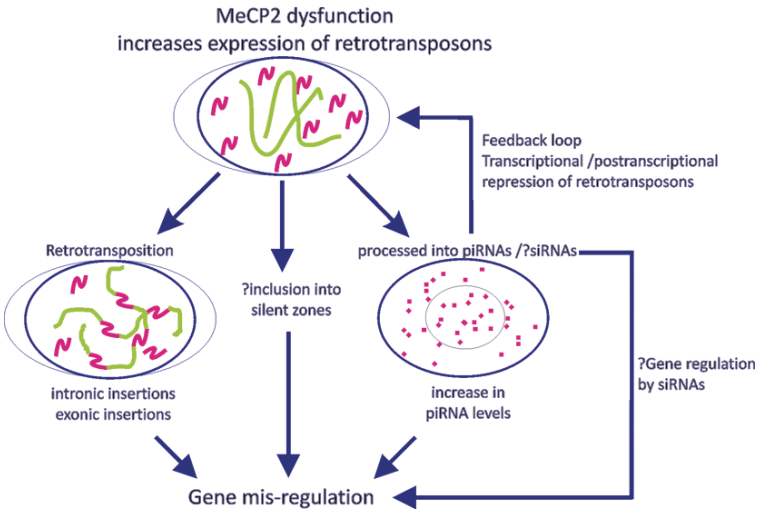

Fig. 2. Schematic of the proposed model showing that changes in the expression level of some genes may be a consequence of the increase in expression of retrotransposons. DNA is depicted in green, retrotransposon transcripts in pink and piRNAs as pink dots. See text for details. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-2012-0932)

lished trehalose enhanced technique for FACS sorting individual neuronal subtypes could help isolate high quality RNA from *Mecp2* null neuronal subtypes for transcriptome sequencing [54].

In conclusion, we propose that overexpression of LINE-1 may contribute to the mis-regulation of some genes in Rett syndrome, mediated through insertional events or by an increase in piRNAs (Fig. 2). Our preliminary data suggests that piRNA expression levels may be altered globally in the absence of MeCP2. Application of next generation sequencing technologies may resolve some key questions regarding MeCP2 function and the downstream consequences of a dysfunctional MeCP2.

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Supplemental material

Supplementary Table 1

List of piRNAs, found in the cerebellum samples in wildtype and Mecp2 KO samples with their tag numbers and normalized tags per million (Tpm) values. This list was generated after filtering out piRNAs with less than 5 tags in the Mecp2 KO sample. Top 20 piRNAs reported to be present in the hippocampus [38] are highlighted in **bold**. Tpm was calculated by dividing the tag numbers by the total number of reads in the library (see Table 1, 3660124 for wildtype (WT) and 2789136 for Mecp2 KO). Fold change was calculated by dividing the Tpm normalized tag numbers for Mecp2 KO with WT

piRNA accession	Length	Number of tags in wildtype cerebellum	Number of tags in Mecp2 KO cerebellum	WT_Tpm	Mecp2 KO_Tpm	Fold_change_ KO/WT	Reported as top 20 piRNAs in brain
DQ546606	29	147865.67	229912.17	40399.09	82431.32	2.04	No
DQ540966	30	146001.83	228493.17	39889.86	81922.56	2.05	No
DQ540188	25	29252.92	24557.00	7992.33	8804.52	1.10	No
DQ558990	30	6166.50	11048.00	1684.78	3961.08	2.35	No
DQ541777	**30**	**1995.50**	**2411.00**	**545.20**	**864.43**	**1.59**	**Yes**
DQ703900	32	4252.00	1621.50	1161.71	581.36	0.50	No
DQ540229	31	1495.50	1033.33	408.59	370.49	0.91	No
DQ719271	21	1355.00	1009.83	370.21	362.06	0.98	No
DQ540053	29	810.00	822.00	221.30	294.71	1.33	No
DQ708952	22	694.00	720.00	189.61	258.14	1.36	No
DQ541506	**28**	**523.90**	**627.40**	**143.14**	**224.94**	**1.57**	**Yes**
DQ542358	31	446.00	549.50	121.85	197.01	1.62	No
DQ540285	**32**	**457.40**	**548.23**	**124.97**	**196.56**	**1.57**	**Yes**
DQ540284	**31**	**456.90**	**548.23**	**124.83**	**196.56**	**1.57**	**Yes**
DQ540283	31	456.90	548.23	124.83	196.56	1.57	No
DQ543676	**31**	**438.90**	**518.70**	**119.91**	**185.97**	**1.55**	**Yes**
DQ701563	26	384.00	489.50	104.91	175.50	1.67	No
DQ541630	25	680.50	464.00	185.92	166.36	0.89	No
DQ705026	**29**	**154.00**	**377.00**	**42.08**	**135.17**	**3.21**	**Yes**
DQ551624	28	196.00	349.33	53.55	125.25	2.34	No
DQ551625	29	196.00	349.33	53.55	125.25	2.34	No
DQ701020	19	281.00	339.00	76.77	121.54	1.58	No
DQ715971	25	461.17	327.00	126.00	117.24	0.93	No
DQ710909	23	428.17	312.00	116.98	111.86	0.96	No
DQ719597	**28**	**168.00**	**306.00**	**45.90**	**109.71**	**2.39**	**Yes**
n200793	31	463.83	302.50	126.73	108.46	0.86	No
DQ711996	22	313.83	295.00	85.74	105.77	1.23	No
DQ724236	18	264.83	277.00	72.36	99.31	1.37	No
DQ541689	30	401.30	273.15	109.64	97.93	0.89	No
DQ720186	**23**	**336.00**	**251.00**	**91.80**	**89.99**	**0.98**	**Yes**
DQ714752	31	115.30	242.15	31.50	86.82	2.76	No
DQ559312	26	380.00	224.00	103.82	80.31	0.77	No
n202644	19	173.00	222.50	47.27	79.77	1.69	No
DQ725273	26	335.80	221.70	91.75	79.49	0.87	No
n204765	21	145.00	210.17	39.62	75.35	1.90	No
DQ558144	25	376.00	209.00	102.73	74.93	0.73	No
DQ696996	22	206.50	192.00	56.42	68.84	1.22	No
DQ707524	20	191.50	191.00	52.32	68.48	1.31	No
DQ553318	26	181.00	163.00	49.45	58.44	1.18	No
DQ714439	28	178.47	147.18	48.76	52.77	1.08	No
DQ555094	**32**	**202.00**	**140.00**	**55.19**	**50.19**	**0.91**	**Yes**
DQ716505	21	80.25	138.50	21.93	49.66	2.26	No
DQ719430	23	79.25	137.00	21.65	49.12	2.27	No
DQ706273	22	79.25	137.00	21.65	49.12	2.27	No
DQ712837	23	53.00	135.00	14.48	48.40	3.34	No
DQ549760	29	191.00	133.00	52.18	47.69	0.91	No
DQ555093	**29**	**189.50**	**129.50**	**51.77**	**46.43**	**0.90**	**Yes**
DQ550329	28	126.00	127.00	34.43	45.53	1.32	No
DQ540952	28	91.70	124.27	25.05	44.55	1.78	No
DQ540981	**30**	**126.50**	**124.00**	**34.56**	**44.46**	**1.29**	**Yes**
DQ709462	30	90.67	108.33	24.77	38.84	1.57	No

Supplementary Table 1, continued

piRNA accession	Length	Number of tags in wildtype cerebellum	Number of tags in Mecp2 KO cerebellum	WT_Tpm	Mecp2 KO_Tpm	Fold_change_ KO/WT	Reported as top 20 piRNAs in brain
DQ540984	30	62.93	103.50	17.19	37.11	2.16	No
DQ713872	22	81.00	97.00	22.13	34.78	1.57	No
DQ552696	30	120.00	91.00	32.79	32.63	1.00	No
DQ719488	24	248.00	90.00	67.76	32.27	0.48	No
DQ541470	27	127.72	89.05	34.89	31.93	0.91	No
DQ551913	27	57.33	80.25	15.66	28.77	1.84	No
DQ689086	**27**	**65.00**	**78.00**	**17.76**	**27.97**	**1.57**	**Yes**
DQ717385	22	89.50	64.33	24.45	23.07	0.94	No
DQ548183	28	65.50	60.00	17.90	21.51	1.20	No
DQ540859	30	77.50	58.50	21.17	20.97	0.99	No
DQ556354	30	20.00	58.50	5.46	20.97	3.84	No
DQ696831	31	33.00	54.00	9.02	19.36	2.15	No
DQ540872	32	107.50	54.00	29.37	19.36	0.66	No
DQ540944	26	47.68	53.08	13.03	19.03	1.46	No
DQ542796	28	49.00	53.00	13.39	19.00	1.42	No
DQ546708	30	43.00	52.00	11.75	18.64	1.59	No
DQ541352	25	44.93	51.08	12.28	18.32	1.49	No
DQ540974	29	66.50	49.50	18.17	17.75	0.98	No
DQ540526	28	32.00	49.00	8.74	17.57	2.01	No
DQ551739	28	22.67	47.22	6.19	16.93	2.73	No
DQ540403	30	24.00	46.00	6.56	16.49	2.52	No
DQ540915	30	54.00	46.00	14.75	16.49	1.12	No
DQ539904	28	45.00	45.50	12.29	16.31	1.33	No
DQ699095	24	20.00	44.50	5.46	15.95	2.92	No
DQ723924	20	20.00	44.00	5.46	15.78	2.89	No
DQ541614	28	18.38	43.58	5.02	15.63	3.11	No
DQ564913	30	29.33	41.92	8.01	15.03	1.88	No
DQ553409	27	45.50	41.50	12.43	14.88	1.20	No
DQ547181	28	18.00	41.00	4.92	14.70	2.99	No
DQ545450	28	32.00	41.00	8.74	14.70	1.68	No
DQ687520	26	41.93	39.08	11.46	14.01	1.22	No
DQ689768	30	26.00	39.00	7.10	13.98	1.97	No
DQ540964	25	42.50	39.00	11.61	13.98	1.20	No
DQ701846	29	46.00	38.00	12.57	13.62	1.08	No
DQ540058	27	17.00	37.00	4.64	13.27	2.86	No
DQ706530	31	34.00	36.17	9.29	12.97	1.40	No
DQ710928	30	54.00	36.00	14.75	12.91	0.87	No
DQ706818	28	52.00	36.00	14.21	12.91	0.91	No
DQ546549	25	33.83	36.00	9.24	12.91	1.40	No
DQ540988	28	23.17	35.50	6.33	12.73	2.01	No
DQ540862	**30**	**21.50**	**33.50**	**5.87**	**12.01**	**2.04**	**Yes**
DQ696491	29	41.72	32.92	11.40	11.80	1.04	No
DQ540861	**27**	**20.50**	**31.50**	**5.60**	**11.29**	**2.02**	**Yes**
DQ563946	30	5.00	31.00	1.37	11.11	8.14	No
DQ562907	29	9.73	30.70	2.66	11.01	4.14	No
DQ702901	29	13.00	30.67	3.55	11.00	3.10	No
DQ707624	26	14.93	30.07	4.08	10.78	2.64	No
DQ540780	26	16.33	30.00	4.46	10.76	2.41	No
DQ540412	27	37.00	29.00	10.11	10.40	1.03	No
DQ539915	**32**	**35.50**	**28.00**	**9.70**	**10.04**	**1.04**	**Yes**
DQ565590	31	1.00	28.00	0.27	10.04	36.74	No
DQ698557	30	10.93	27.65	2.99	9.91	3.32	No
DQ689686	22	28.75	27.50	7.85	9.86	1.26	No
DQ541113	31	15.50	27.50	4.23	9.86	2.33	No
DQ555883	30	59.75	27.30	16.32	9.79	0.60	No
DQ711586	30	27.00	27.00	7.38	9.68	1.31	No
DQ564866	27	14.83	27.00	4.05	9.68	2.39	No
DQ562906	27	8.73	26.70	2.39	9.57	4.01	No

Supplementary Table 1, continued

piRNA accession	Length	Number of tags in wildtype cerebellum	Number of tags in Mecp2 KO cerebellum	WT_Tpm	Mecp2 KO_Tpm	Fold_change_ KO/WT	Reported as top 20 piRNAs in brain
DQ564777	27	11.00	26.70	3.01	9.57	3.19	No
DQ562139	27	29.00	26.00	7.92	9.32	1.18	No
DQ725665	32	27.67	26.00	7.56	9.32	1.23	No
n205237	30	28.17	25.50	7.70	9.14	1.19	No
DQ702517	30	76.58	25.33	20.92	9.08	0.43	No
DQ725422	27	22.50	25.00	6.15	8.96	1.46	No
DQ540976	27	41.50	24.30	11.34	8.71	0.77	No
DQ564776	26	8.00	24.20	2.19	8.68	3.97	No
DQ709768	28	7.00	24.00	1.91	8.60	4.50	No
DQ540175	32	42.00	24.00	11.48	8.60	0.75	No
DQ540963	29	21.32	23.83	5.82	8.55	1.47	No
DQ691499	30	41.00	23.50	11.20	8.43	0.75	No
DQ701440	28	14.00	23.00	3.83	8.25	2.16	No
DQ539926	27	17.00	22.50	4.64	8.07	1.74	No
DQ724091	30	17.00	22.50	4.64	8.07	1.74	No
DQ715990	30	18.33	22.17	5.01	7.95	1.59	No
DQ719178	29	21.50	22.00	5.87	7.89	1.34	No
DQ687463	26	17.00	22.00	4.64	7.89	1.70	No
DQ552936	30	57.00	22.00	15.57	7.89	0.51	No
DQ703911	30	14.00	22.00	3.83	7.89	2.06	No
DQ555802	28	19.00	22.00	5.19	7.89	1.52	No
DQ540860	30	35.00	22.00	9.56	7.89	0.82	No
DQ707092	26	7.93	21.65	2.17	7.76	3.58	No
DQ555882	29	53.75	21.30	14.69	7.64	0.52	No
DQ721541	28	16.33	21.17	4.46	7.59	1.70	No
DQ715697	31	16.33	21.17	4.46	7.59	1.70	No
n199527	26	17.33	21.00	4.74	7.53	1.59	No
DQ709916	25	11.00	21.00	3.01	7.53	2.51	No
DQ565303	30	11.30	20.82	3.09	7.46	2.42	No
DQ718173	30	11.30	20.82	3.09	7.46	2.42	No
DQ692434	29	11.30	20.82	3.09	7.46	2.42	No
DQ691288	24	20.92	20.50	5.71	7.35	1.29	No
n199120	16	38.00	20.50	10.38	7.35	0.71	No
DQ715526	**28**	**20.00**	**20.00**	**5.46**	**7.17**	**1.31**	**Yes**
DQ567738	29	8.00	20.00	2.19	7.17	3.28	No
DQ720914	31	15.00	20.00	4.10	7.17	1.75	No
DQ540280	30	14.50	20.00	3.96	7.17	1.81	No
DQ540869	29	16.52	19.93	4.51	7.15	1.58	No
DQ540975	26	27.50	19.50	7.51	6.99	0.93	No
DQ541100	28	15.50	19.00	4.23	6.81	1.61	No
DQ712821	24	21.00	19.00	5.74	6.81	1.19	No
DQ545972	30	19.00	19.00	5.19	6.81	1.31	No
DQ702126	29	18.15	18.25	4.96	6.54	1.32	No
DQ703459	30	13.00	18.17	3.55	6.51	1.83	No
DQ699219	30	21.00	18.00	5.74	6.45	1.12	No
DQ540965	32	15.17	18.00	4.14	6.45	1.56	No
DQ707037	28	8.55	17.60	2.34	6.31	2.70	No
DQ541147	28	10.50	17.50	2.87	6.27	2.19	No
DQ555881	28	43.25	17.30	11.82	6.20	0.52	No
DQ568996	31	7.00	17.00	1.91	6.10	3.19	No
n202030	32	11.67	17.00	3.19	6.10	1.91	No
DQ703779	25	7.00	17.00	1.91	6.10	3.19	No
DQ545225	30	5.00	17.00	1.37	6.10	4.46	No
DQ541000	28	13.18	16.93	3.60	6.07	1.69	No
n197343	38	13.00	16.50	3.55	5.92	1.67	No
DQ540868	26	13.18	16.10	3.60	5.77	1.60	No
DQ541218	26	79.00	16.00	21.58	5.74	0.27	No
DQ718197	27	10.50	16.00	2.87	5.74	2.00	No

Supplementary Table 1, continued

piRNA accession	Length	Number of tags in wildtype cerebellum	Number of tags in Mecp2 KO cerebellum	WT_Tpm	Mecp2 KO_Tpm	Fold_change_ KO/WT	Reported as top 20 piRNAs in brain
DQ714526	30	18.07	15.67	4.94	5.62	1.14	No
DQ540689	29	29.50	15.50	8.06	5.56	0.69	No
DQ540059	26	11.00	15.50	3.01	5.56	1.85	No
DQ724251	22	11.75	15.40	3.21	5.52	1.72	No
DQ714788	30	26.50	15.33	7.24	5.50	0.76	No
DQ540867	25	11.93	15.10	3.26	5.41	1.66	No
DQ693545	30	4.67	15.00	1.28	5.38	4.22	No
DQ717257	29	17.00	14.50	4.64	5.20	1.12	No
DQ544489	29	8.92	14.37	2.44	5.15	2.11	No
DQ551953	28	12.23	14.25	3.34	5.11	1.53	No
DQ541776	29	3.65	14.23	1.00	5.10	5.12	No
DQ541806	27	19.00	14.00	5.19	5.02	0.97	No
DQ540253	31	27.00	14.00	7.38	5.02	0.68	No
DQ686298	21	7.00	14.00	1.91	5.02	2.62	No
DQ706110	30	23.50	14.00	6.42	5.02	0.78	No
DQ545604	27	17.00	14.00	4.64	5.02	1.08	No
n202750	30	12.00	14.00	3.28	5.02	1.53	No
DQ696259	29	6.00	14.00	1.64	5.02	3.06	No
DQ723396	30	6.00	13.50	1.64	4.84	2.95	No
DQ555880	27	24.25	13.30	6.63	4.77	0.72	No
DQ716469	31	5.40	13.08	1.48	4.69	3.18	No
DQ722288	**28**	**2.00**	**13.00**	**0.55**	**4.66**	**8.53**	**Yes**
DQ558886	26	4.00	13.00	1.09	4.66	4.26	No
DQ548138	27	6.00	13.00	1.64	4.66	2.84	No
DQ566603	30	9.00	13.00	2.46	4.66	1.90	No
DQ694433	25	19.00	13.00	5.19	4.66	0.90	No
DQ559729	29	16.00	13.00	4.37	4.66	1.07	No
DQ541629	26	10.18	12.93	2.78	4.64	1.67	No
DQ540202	31	16.50	12.50	4.51	4.48	0.99	No
DQ710188	27	2.00	12.00	0.55	4.30	7.87	No
DQ541627	28	11.00	12.00	3.01	4.30	1.43	No
DQ725115	29	46.00	12.00	12.57	4.30	0.34	No
DQ715208	24	0.50	12.00	0.14	4.30	31.49	No
DQ718174	22	5.50	12.00	1.50	4.30	2.86	No
DQ690565	31	9.00	12.00	2.46	4.30	1.75	No
DQ721627	30	36.00	12.00	9.84	4.30	0.44	No
DQ563182	31	4.00	12.00	1.09	4.30	3.94	No
DQ725966	19	12.33	11.67	3.37	4.18	1.24	No
DQ717747	32	14.00	11.33	3.83	4.06	1.06	No
DQ698641	27	1.00	11.00	0.27	3.94	14.44	No
DQ705481	22	7.00	11.00	1.91	3.94	2.06	No
DQ702236	29	7.00	11.00	1.91	3.94	2.06	No
DQ697536	31	9.10	10.92	2.49	3.91	1.57	No
DQ693633	30	17.67	10.50	4.83	3.76	0.78	No
DQ692951	30	9.07	10.50	2.48	3.76	1.52	No
DQ558403	26	8.33	10.42	2.28	3.73	1.64	No
DQ716691	28	11.87	10.33	3.24	3.70	1.14	No
DQ548430	30	11.17	10.08	3.05	3.62	1.18	No
DQ709071	29	8.17	10.00	2.23	3.59	1.61	No
DQ568824	30	4.00	10.00	1.09	3.59	3.28	No
DQ709273	31	11.00	10.00	3.01	3.59	1.19	No
DQ719680	26	5.00	10.00	1.37	3.59	2.62	No
DQ719784	21	12.00	10.00	3.28	3.59	1.09	No
DQ691624	22	12.60	10.00	3.44	3.59	1.04	No
DQ719096	29	6.10	9.98	1.67	3.58	2.15	No
DQ693813	30	13.17	9.98	3.60	3.58	1.00	No
DQ709946	26	4.83	9.83	1.32	3.53	2.67	No
DQ540134	28	3.00	9.83	0.82	3.53	4.30	No

Supplementary Table 1, continued

piRNA accession	Length	Number of tags in wildtype cerebellum	Number of tags in Mecp2 KO cerebellum	WT_Tpm	Mecp2 KO_Tpm	Fold_change_ KO/WT	Reported as top 20 piRNAs in brain
DQ551740	30	4.80	9.75	1.31	3.50	2.67	No
DQ551741	32	5.00	9.75	1.37	3.50	2.56	No
DQ708438	31	0.50	9.50	0.14	3.41	24.93	No
n204129	26	10.50	9.50	2.87	3.41	1.19	No
DQ698521	27	11.50	9.50	3.14	3.41	1.08	No
DQ550765	**31**	**10.75**	**9.50**	**2.94**	**3.41**	**1.16**	**Yes**
DQ707509	31	22.50	9.25	6.15	3.32	0.54	No
DQ712474	28	22.50	9.25	6.15	3.32	0.54	No
DQ712486	30	22.50	9.25	6.15	3.32	0.54	No
DQ724038	21	7.23	9.17	1.98	3.29	1.66	No
DQ717846	29	7.83	9.08	2.14	3.26	1.52	No
DQ727278	29	3.00	9.00	0.82	3.23	3.94	No
DQ703937	31	3.17	9.00	0.87	3.23	3.73	No
DQ553641	29	5.00	9.00	1.37	3.23	2.36	No
DQ698382	31	7.00	9.00	1.91	3.23	1.69	No
DQ547060	30	5.83	8.92	1.59	3.20	2.01	No
DQ554102	30	4.83	8.57	1.32	3.07	2.33	No
DQ541631	30	5.75	8.50	1.57	3.05	1.94	No
DQ694480	28	4.42	8.33	1.21	2.99	2.48	No
DQ562379	25	8.83	8.17	2.41	2.93	1.21	No
DQ703493	26	7.05	8.13	1.93	2.92	1.51	No
DQ699015	30	8.00	8.00	2.19	2.87	1.31	No
DQ565695	31	4.00	8.00	1.09	2.87	2.62	No
DQ687262	30	16.00	8.00	4.37	2.87	0.66	No
DQ540853	26	1.00	8.00	0.27	2.87	10.50	No
DQ541719	29	4.00	8.00	1.09	2.87	2.62	No
DQ721809	29	5.00	8.00	1.37	2.87	2.10	No
DQ686705	26	3.00	8.00	0.82	2.87	3.50	No
DQ719574	27	9.50	8.00	2.60	2.87	1.11	No
DQ698794	29	11.00	8.00	3.01	2.87	0.95	No
DQ569658	30	13.67	7.67	3.73	2.75	0.74	No
DQ684777	30	16.33	7.67	4.46	2.75	0.62	No
DQ548429	28	8.67	7.58	2.37	2.72	1.15	No
DQ569911	31	3.83	7.50	1.05	2.69	2.57	No
DQ714299	28	2.25	7.50	0.61	2.69	4.37	No
DQ705429	28	2.02	7.48	0.55	2.68	4.87	No
DQ700644	30	10.00	7.33	2.73	2.63	0.96	No
DQ542520	32	8.40	7.27	2.30	2.61	1.14	No
DQ725358	22	7.88	7.22	2.15	2.59	1.20	No
DQ715253	28	4.57	7.08	1.25	2.54	2.04	No
DQ540081	28	5.00	7.00	1.37	2.51	1.84	No
DQ559964	27	1.00	7.00	0.27	2.51	9.19	No
DQ555047	31	13.00	7.00	3.55	2.51	0.71	No
DQ546953	32	7.00	7.00	1.91	2.51	1.31	No
DQ697785	18	18.00	7.00	4.92	2.51	0.51	No
DQ712498	27	14.00	7.00	3.83	2.51	0.66	No
DQ703255	29	2.00	7.00	0.55	2.51	4.59	No
DQ703079	27	5.00	7.00	1.37	2.51	1.84	No
DQ559781	28	5.00	7.00	1.37	2.51	1.84	No
DQ726397	22	1.00	7.00	0.27	2.51	9.19	No
DQ540951	28	106.00	7.00	28.96	2.51	0.09	No
DQ707442	32	1.17	7.00	0.32	2.51	7.87	No
DQ695733	31	1.17	7.00	0.32	2.51	7.87	No
DQ550956	30	2.00	6.67	0.55	2.39	4.37	No
DQ704045	30	2.00	6.67	0.55	2.39	4.37	No
DQ698371	26	4.50	6.50	1.23	2.33	1.90	No
DQ540939	26	4.50	6.50	1.23	2.33	1.90	No
DQ686264	26	5.33	6.33	1.46	2.27	1.56	No

Supplementary Table 1, continued

piRNA accession	Length	Number of tags in wildtype cerebellum	Number of tags in Mecp2 KO cerebellum	WT_Tpm	Mecp2 KO_Tpm	Fold_change_ KO/WT	Reported as top 20 piRNAs in brain
DQ550027	30	1.20	6.33	0.33	2.27	6.93	No
DQ543701	30	10.17	6.33	2.78	2.27	0.82	No
DQ554152	31	3.85	6.18	1.05	2.22	2.11	No
DQ550614	31	7.42	6.13	2.03	2.20	1.09	No
DQ557476	29	5.08	6.08	1.39	2.18	1.57	No
DQ723756	31	0.83	6.08	0.23	2.18	9.58	No
DQ694583	30	2.00	6.00	0.55	2.15	3.94	No
DQ541882	32	2.25	6.00	0.61	2.15	3.50	No
DQ563242	30	1.00	6.00	0.27	2.15	7.87	No
DQ714655	21	2.50	6.00	0.68	2.15	3.15	No
DQ708131	**27**	**3.00**	**6.00**	**0.82**	**2.15**	**2.62**	**Yes**
DQ565679	30	2.50	6.00	0.68	2.15	3.15	No
DQ695473	27	3.50	6.00	0.96	2.15	2.25	No
DQ551797	29	5.00	6.00	1.37	2.15	1.57	No
n202533	30	3.00	6.00	0.82	2.15	2.62	No
DQ719363	29	3.50	6.00	0.96	2.15	2.25	No
DQ564810	31	3.00	6.00	0.82	2.15	2.62	No
DQ542432	29	3.50	6.00	0.96	2.15	2.25	No
DQ687025	30	2.00	6.00	0.55	2.15	3.94	No
DQ705141	29	3.00	6.00	0.82	2.15	2.62	No
DQ550009	32	2.50	6.00	0.68	2.15	3.15	No
DQ712132	27	3.92	5.92	1.07	2.12	1.98	No
DQ710634	26	3.92	5.92	1.07	2.12	1.98	No
DQ688047	31	0.90	5.87	0.25	2.10	8.55	No
DQ540133	27	3.00	5.83	0.82	2.09	2.55	No
DQ724045	31	5.17	5.83	1.41	2.09	1.48	No
DQ717785	29	3.67	5.67	1.00	2.03	2.03	No
DQ697835	30	0.90	5.62	0.25	2.01	8.19	No
DQ550424	30	6.95	5.62	1.90	2.01	1.06	No
DQ699107	32	5.08	5.55	1.39	1.99	1.43	No
DQ692222	31	4.08	5.55	1.12	1.99	1.78	No
DQ559632	29	5.03	5.52	1.38	1.98	1.44	No
DQ539909	31	3.50	5.50	0.96	1.97	2.06	No
DQ699690	29	3.00	5.50	0.82	1.97	2.41	No
DQ694268	30	6.17	5.50	1.68	1.97	1.17	No
DQ704413	30	3.50	5.50	0.96	1.97	2.06	No
DQ540217	30	2.50	5.50	0.68	1.97	2.89	No
DQ705397	27	3.00	5.50	0.82	1.97	2.41	No
DQ541518	32	2.83	5.42	0.77	1.94	2.51	No
DQ561657	31	2.87	5.30	0.78	1.90	2.43	No
DQ713688	20	6.47	5.27	1.77	1.89	1.07	No
DQ695662	27	0.20	5.25	0.05	1.88	34.45	No
DQ554873	32	1.92	5.03	0.52	1.80	3.45	No
DQ718455	31	1.92	5.03	0.52	1.80	3.45	No
DQ569362	29	2.00	5.00	0.55	1.79	3.28	No
DQ711635	30	2.00	5.00	0.55	1.79	3.28	No
DQ541249	31	5.00	5.00	1.37	1.79	1.31	No
DQ718385	28	6.00	5.00	1.64	1.79	1.09	No
DQ701776	29	38.00	5.00	10.38	1.79	0.17	No
DQ561111	29	2.00	5.00	0.55	1.79	3.28	No
DQ697860	31	8.00	5.00	2.19	1.79	0.82	No
DQ541101	27	5.50	5.00	1.50	1.79	1.19	No
DQ691503	28	0.00	5.00	0.00	1.79	5.00	No
DQ701204	27	6.00	5.00	1.64	1.79	1.09	No
DQ695609	26	8.50	5.00	2.32	1.79	0.77	No
DQ545504	27	10.00	5.00	2.73	1.79	0.66	No
DQ696494	26	0.00	5.00	0.00	1.79	5.00	No
DQ726803	30	1.00	5.00	0.27	1.79	6.56	No

Supplementary Table 1, continued

piRNA accession	Length	Number of tags in wildtype cerebellum	Number of tags in Mecp2 KO cerebellum	WT_Tpm	Mecp2 KO_Tpm	Fold_change_ KO/WT	Reported as top 20 piRNAs in brain
DQ695702	32	0.00	5.00	0.00	1.79	5.00	No
DQ563823	30	1.00	5.00	0.27	1.79	6.56	No
DQ551895	27	5.00	5.00	1.37	1.79	1.31	No
DQ567700	26	5.50	5.00	1.50	1.79	1.19	No
DQ688232	31	6.50	5.00	1.78	1.79	1.01	No
DQ700848	28	3.00	5.00	0.82	1.79	2.19	No
DQ710935	30	1.00	5.00	0.27	1.79	6.56	No
DQ563772	27	23.00	5.00	6.28	1.79	0.29	No
DQ691599	28	6.00	5.00	1.64	1.79	1.09	No
DQ721750	21	2.00	5.00	0.55	1.79	3.28	No
DQ722234	31	0.50	5.00	0.14	1.79	13.12	No
DQ709374	31	5.25	5.00	1.43	1.79	1.25	No
DQ709264	32	8.00	5.00	2.19	1.79	0.82	No
DQ554082	30	3.50	5.00	0.96	1.79	1.87	No
DQ541574	29	4.50	5.00	1.23	1.79	1.46	No
DQ552422	31	2.50	5.00	0.68	1.79	2.62	No
		356282.87	518589.20	97341.75	185931.84	1.91	19