RNA inhibits dMi-2/CHD4 chromatin binding and nucleosome remodelling

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Summary

The ATP-dependent nucleosome remodeller Mi-2/CHD4 broadly modulates epigenetic landscapes to repress transcription and to maintain genome integrity. Here we use individual nucleotide resolution crosslinking and immunoprecipitation (iCLIP) to show that *Drosophila* Mi-2 associates with thousands of mRNA molecules *in vivo*. Biochemical data reveal that recombinant dMi-2 preferentially binds to G-rich RNA molecules using two intrinsically disordered regions of previously undefined function. Pharmacological inhibition of transcription and RNase digestion approaches establish that RNA inhibits the association of dMi-2 with chromatin. We also show that RNA inhibits dMi-2-mediated nucleosome mobilization by competing with the nucleosome substrate. Importantly, this activity is shared by CHD4, the human homolog of dMi-2, strongly suggesting that RNA-mediated regulation of remodeller activity is an evolutionary conserved mechanism. Our data support a model in which RNA serves to protect actively transcribed regions of the genome from dMi-2/CHD4-mediated establishment of repressive chromatin structures.

Keywords

Chromatin, ATP-dependent chromatin remodelling, RNA, NuRD, iCLIP, gene regulation

Introduction

Although a growing number of chromatin regulating proteins have been demonstrated to bind RNA, the functional implications of these interactions are largely not understood (Castello et al., 2016; He et al., 2016; Hendrickson et al., 2016). In only a few cases has the functional relationship between such proteins and their cognate RNA molecules been studied in detail. One example is the Polycomb Repressive Complex 2 (PRC2), which catalyses the repressive trimethylation of lysine 27 of histone H3 (H3K27me₃). PRC2 binds RNA in a promiscuous manner while displaying a preference for G-quadruplex-forming and G-tract containing RNAs (Wang et al., 2017). Interaction with RNA can affect several PRC2 functions, sometimes with seemingly opposite outcomes. For example, RNA binding is important genome wide for association of PRC2 with PRC2-repressed targets, implicating RNA in PRC2 recruitment (Long et al., 2020). At the same time, nascent RNA evicts PRC2 from active genes (Beltran et al., 2019; Beltran et al., 2016; Long et al., 2020). Moreover, RNA binding to an allosteric regulatory site of the methyltransferase subunit Enhancer of zeste homolog 2 (EZH2) inhibits PRC2 enzymatic activity (Zhang et al., 2019). Thus, RNA binding can lead to multifaceted effects on PRC2 function.

RNA-mediated regulation of PRC2 impacts H3K27me₃ levels within PRC2-repressed regions which are essential for stable transcriptional repression of lineage-inappropriate genes and proper differentiation of ES cells (Long et al., 2020; Shan et al., 2017). H3K27 methylation is facilitated by the Nucleosome Remodelling and Deacetylation (NuRD) complex (Bracken et al., 2019; Reynolds et al., 2012b). In ES cells, NuRD removes the acetyl group from acetylated lysine 27 of histone H3 (H3K27ac), a prerequisite for PRC2 chromatin association and H3K27 methylation. Moreover, NuRD antagonizes activating SWI/SNF remodelling complexes and increases nucleosome density (Kreher et al., 2017; Liang et al., 2017; Moshkin et al., 2012). These NuRD activities are believed to further assist PRC-mediated chromatin compaction. Like PRC2, NuRD is important for ES cell differentiation (Bornelov et al., 2018; Bracken et al., 2019; Burgold et al., 2019; Ragheb et al., 2020). It is not known if NuRD function, like PRC2 function, is modulated by RNA and whether RNA can coordinately regulate both chromatin regulatory complexes.

CHD4, the ATPase subunit of NuRD, has been identified as an RNA binding protein in several large-scale screens but the consequences of RNA binding on CHD4 function have not been systematically analysed (Castello et al., 2016; He et al., 2016; Hendrickson et al., 2016). CHD4

binds PAPAS, a long non-coding RNA derived from rRNA promoters and this interaction has been suggested to aid association of NuRD with rRNA genes (Zhao et al., 2018). Our own work on the recruitment of the *Drosophila* CHD4 homologue, dMi-2, to active heat shock (HS) genes indicates that dMi-2 binding to nascent HS gene transcripts might contribute to its association with HS gene bodies (Mathieu et al., 2012; Murawska et al., 2011). However, a comprehensive view of CHD4/dMi-2 RNA binding and its functional consequences is lacking.

Here, we use iCLIP and fRIP to identify dMi-2 associated RNAs *in vivo*. We show that dMi-2 binds to thousands of mRNAs and exhibits a preference for their 3' regions. Using a combination of bioinformatic analyses, biochemical approaches and chromatin immunoprecipitation following pharmacological inhibition of RNA polymerase II, we reveal that RNA restricts dMi-2 association with chromatin. We establish that dMi-2 displays broad RNA binding activity with a preference for G-rich RNA sequences *in vitro*. We map RNA binding activity to two intrinsically disordered regions (IDRs) that are conserved between fly and human. Finally, we establish that G-rich RNA inhibits nucleosome remodelling by both *Drosophila* Mi-2 and human CHD4 and uncover the molecular mechanism of this inhibition. We propose that RNA antagonizes both dMi-2/CHD4 and PRC2 in a concerted manner to safeguard actively transcribed genes from the establishment of repressive chromatin structures.

Results

dMi-2 associates with thousands of mRNAs in vivo

To investigate the *in vivo* association of dMi-2 with RNA in an unbiased manner we used individual nucleotide resolution crosslinking and immunoprecipitation (iCLIP2). We first tested if dMi-2 can be photo-crosslinked to RNA *in vivo*. S2 cells expressing GFP-tagged dMi-2 (dMi-2-GFP) from the endogenous dMi-2 locus (Lenz et al., 2021) were irradiated with UV (254 nm) to photo-crosslink RNA and proteins. Extracts from these cells were partially digested with RNase I to shorten crosslinked RNA molecules and immunoprecipitated with GFP-Trap beads. Crosslinked RNAs were then labelled with ³²P at their 5' ends and protein/[³²P]-RNA photo-adducts were visualized by autoradiography following SDS-PAGE and transfer to a membrane (**Figure 1A**). No significant radioactive signal was detected in samples derived from UV-treated S2 control cells which did not express GFP-tagged dMi-2 (upper panel: lanes 2 to 4) or in samples derived from S2 cells expressing dMi-2-GFP that had not been exposed to UV (lanes 8 to 10). In contrast, robust signals were obtained in

samples derived from UV-crosslinked, dMi-2-GFP expressing cells treated with low or intermediate RNase concentrations (lanes 6 and 7). These signals largely disappeared when the sample was treated with the highest RNase concentration (lane 5). The radioactive signal was concentrated in the apparent molecular weight range of 250-300 kDa. This corresponds well to the apparent molecular weight of dMi-2-GFP as determined by Western blot (lower panel). These results demonstrate that dMi-2-GFP can be efficiently photo-crosslinked to RNA and, therefore, strongly imply that dMi-2 is closely associated with RNA *in vivo*.

We then repeated the above procedure with dMi-2-GFP expressing cells and control cells not expressing dMi-2-GFP. We excized membrane slices to recover protein/[³²P]-RNA photo-adducts in the 250-300 kDa range for further processing (**Supplementary Figure 1A**). Equivalent membrane regions were excized for samples derived from UV-crosslinked cells expressing dMi-2-GFP (two biological replicates) and samples derived from UV-crosslinked control cells not expressing dMi-2-GFP (control; two biological replicates). dMi-2 exists in two multi-subunit protein complexes: dNuRD and dMec (Kunert et al., 2009). Given that dMi-2 is by far the largest subunit in both complexes, restricting the analysis to photo-adducts in the 250-300 kDa range ensured that RNAs identified by iCLIP were most likely directly bound to dMi-2 as opposed to other dNuRD or dMec subunits (Lenz et al., 2021).

We processed the four membrane slices according to the iCLIP2 library preparation protocol (Buchbender et al., 2020) and DNA deep sequencing resulted in a combined total of 5.03 million reads. Of these 41.8% were derived from dMi-2-GFP replicate-1, 31.9% from dMi-2-GFP replicate-2 and less than 2% from each of the two control replicates (**Supplementary Figure 1B**). The remaining reads were missing barcode sequences and could not be assigned to any replicate. 161,569 reads (dMi-2-GFP replicate-1) and 160,021 reads (dMi-2-GFP replicate-2), respectively, uniquely mapped to RNAs encoded by the *Drosophila melanogaster* genome (*dm6*). These RNAs included tRNAs, rRNAs, snRNAs, snoRNAs and other noncoding RNAs (**Figure 1B**). However, the majority mapped to mRNAs (86.3% dMi-2-GFP replicate-1, 90.0% dMi-2-GFP replicate-2). Furthermore, the data sets of the two replicates were highly correlated (squared Pearson correlation coefficient (R^2) = 0.97; (**Supplementary Figure 1C**).

We defined dMi-2 associated RNAs as RNAs exhibiting five or more crosslinks in each replicate. 5471 RNAs fulfilled this criterion. Given dMi-2's established role in the regulation of protein coding genes, we focused our analysis on protein coding RNAs. We asked if the

propensity of dMi-2 to crosslink to a given mRNA was a function of the expression level of that mRNA. We plotted the number of iCLIP2 crosslinks to a given mRNA against the expression level of that mRNA as determined by RNA-seq (Lenz et al., 2021) (**Figure 1C**, see Methods for details). This demonstrated that there was no strict linear correlation between RNA expression level and the number of dMi-2 iCLIP2 crosslinks. We identified 580 transcripts displaying an enrichment of dMi-2 crosslinks (see Methods for details; **Figure 1C**, green dots, **Table 1**).

We performed RNA immunoprecipitation (fRIP) to corroborate our iCLIP2 data. After formaldehyde crosslinking, we used GFP-Trap beads to immunoprecipitate extracts of cells expressing dMi-2-GFP, dLint1-GFP and of cells not expressing GFP-tagged protein (control) (**Supplementary Figure 1D**). Co-precipitated RNAs were then analysed by RT-qPCR. Like dMi-2, dLint1 is a subunit of a chromatin associated repressor complex (LINT complex, (Macinkovic et al., 2019; Meier et al., 2012)). We analysed nine dMi-2-associated transcripts, seven that were preferentially crosslinked to dMi-2 and two that were not (highlighted in **Figure 1C**). None of these transcripts were significantly enriched in dLint1 precipitates compared to control precipitates (**Supplementary Figure 1E**). In contrast, significant enrichment in dMi-2 precipitates over Lint1 and control precipitates was detected for 7 transcripts. This result corroborates the iCLIP data and strengthens the hypothesis that dMi-2 associates with RNAs *in vivo*.

RNA binding is unlikely to act in the recruitment of dMi-2 to chromatin

Association of dMi-2/CHD4 with nascent RNA has previously been suggested to assist in the recruitment of dMi2/CHD4/NuRD to chromatin at heat shock and rRNA genes (Mathieu et al., 2012; Zhao et al., 2018). A widespread role for RNA binding in mediating the chromatin association of dMi-2 would predict that dMi-2 ChIP-seq and dMi-2 iCLIP2 patterns across genes are correlated. A metagene analysis of dMi-2 RNA crosslink sites revealed preferential crosslinking towards the 3' end of transcripts (**Figure 2A**; **Supplementary Figure 2A**). However, a metagene analysis of dMi-2 chromatin binding sites at these genes as determined by ChIP-seq (Lenz et al., 2021) highlights a clear preference for promoter sequences and the 5' region of genes (**Figure 2A**). In fact, dMi-2 chromatin occupancy is lowest towards the 3' end of genes. Moreover, dMi-2 target genes identified by iCLIP and ChIP-seq, respectively, show only a limited overlap (**Figure 2B**). Closer inspection of iCLIP crosslink site distribution over the intron/exon junction preceding the transcription termination site (TES; also referred to as the cleavage and polyadenylation site) reveals that crosslinking of dMi-2 to the terminal

exon is dramatically more likely than crosslinking to the terminal intron. While this observation does not rule out the possibility that dMi-2 also binds nascent RNA, it demonstrates that dMi-2 is predominantly associated with processed RNA (**Figure 2C**). Taken together, these results indicate that the binding of dMi-2 to nascent RNA is unlikely to play a dominant role in recruiting dMi-2 to chromatin.

RNA antagonizes the interaction of dMi-2 with chromatin

NuRD and PRC2 cooperate to establish repressed chromatin regions characterized by high nucleosome occupancy and nucleosomes containing H3K27me3 (Bracken et al., 2019; Reynolds et al., 2012a). RNA has recently been found to contribute to bringing PRC2 to its genomic targets but also to dissociate the complex from chromatin at actively transcribed genes (Beltran et al., 2019; Beltran et al., 2016; Long et al., 2020). Given the close cooperation between PRC2 and NuRD, we considered the possibility that RNA simultaneously removes both repressive complexes from chromatin. To test this hypothesis, we treated permeabilized S2 cells with RNase A or BSA (control). We then biochemically fractionated cells into cytoplasm, nucleoplasm and chromatin, and subjected these fractions to Western blot analysis (**Figure 2D**). We found that RNase treatment results in a decrease of the nucleoplasmic fraction of the PRC2 subunit Enhancer of zeste (E(z)) and a corresponding increase of the chromatin-bound fraction of the same protein. This observation agrees with published data that demonstrate a similar behaviour of EZH2 from mouse ES cells in this assay, suggesting that RNA-mediated chromatin displacement of PRC2 is conserved between mammals and insects (Beltran et al., 2016). The chromatin-associated fractions of dMi-2 and the dNuRD subunit dRPD3 were also increased upon RNA digestion. In contrast, the distributions of dLSD1, a histone demethylase, and of histone H3 were not significantly affected by RNase treatment. Thus, RNA-dependent displacement from chromatin is not a general property of chromatin-associated proteins in this assay.

If RNA antagonizes chromatin association of dMi-2 *in vivo*, inhibition of transcription would be expected to facilitate dMi-2 binding to chromatin. We treated S2 cells with triptolide or DMSO (control) to test this hypothesis. Triptolide induces degradation of the largest subunit of RNA polymerase II. As a consequence, transcription at active genes is strongly reduced (Beltran et al., 2016; Riising et al., 2014) (**Supplementary Figure 2B**). We used ChIP-qPCR to determine the effect of inhibition of transcription on dMi-2 chromatin binding at six established dMi-2 target genes (Kreher et al., 2017) (**Figure 2E**). Five of these showed increased dMi-2 chromatin binding in triptolide-treated cells. No such increase of dMi-2

chromatin binding was detected at an untranscribed control region (intergenic 2R). Importantly, dLint1, although bound to the same genes, displayed no increased chromatin association when transcription was inhibited. This demonstrates that increased chromatin binding upon triptolide treatment is specific for dMi-2.

Taken together, these results establish that RNA counteracts dMi-2 chromatin association *in vivo*.

dMi-2 has broad RNA binding activity in vitro

Because dMi-2 exists in two multi-subunit complexes: dNuRD and dMec (Kunert et al., 2009), dMi-2 RNA binding and the effects of RNA on chromatin association could be mediated by other subunits of these complexes. We sought to investigate the inherent RNA binding activity of dMi-2 *in vitro*. We used electrophoretic mobility shift assays (EMSA) to investigate RNA binding by purified, recombinant dMi-2 (**Supplementary Figure 3A; Table 2**). We tested dMi-2 binding to segments of two RNA molecules: the Hsp70Aa transcript, which we have previously shown to bind dMi-2 during the heat shock response (Murawska et al., 2011), and the unrelated Pgant35a transcript. The two transcripts are similar in length (Hsp70Aa: 2.4 kb, Pgant35a: 2.2 kb). For each RNA we selected four 100 nucleotide regions spanning the transcript (**Figure 3A**, regions #1, #2, #4 and #5; **Supplementary Figure 3B**). All eight RNA molecules were efficiently bound by dMi-2 (**Figure 3B**). In contrast to the apparent preference of dMi-2 for binding towards the 3' ends of transcripts *in vivo* (**Figure 2A**), we did not detect strong differences in binding to 5' and 3' regions by recombinant dMi-2 *in vitro*. These results indicate that dMi-2 has broad RNA binding activity *in vitro*.

We next asked if we could detect quantitative differences of dMi-2 binding to different RNA molecules. We performed titration experiments to derive dissociation constants (K_d) for two 100 nucleotide RNA fragments (Hsp70Aa #3 and Pgant35a #3, **Figure 3A**, **Supplementary Figure 3C**). The apparent K_d values for dMi-2 binding to Hsp70Aa and Pgant35a RNAs were 0.38 (+/- 0.06) μ M and 0.63 (+/- 0.28) μ M, respectively (**Figure 3C and 3D**). This result shows that the dMi-2 binding affinities of RNA molecules with identical length but nonidentical nucleotide sequence (and potentially different secondary structures) are overall quite similar, but can exhibit subtle differences.

We also compared the affinities of dMi-2 binding to double stranded RNAs and to double stranded DNAs of corresponding length and sequence (**Figure 3E and F**, **Supplementary Figure 3D and E**). dMi-2 bound to a 100 bp double stranded Hsp70Aa fragment with an

apparent K_d of 0.86 ($\pm$ 0.1) μ M. The corresponding double stranded DNA fragment of the same sequence was bound with a similar apparent K_d of 0.82 ($\pm$ 0.1) μ M. These K_d values are higher by a factor of two than the apparent K_d value for binding of dMi-2 to the corresponding single stranded RNA molecule (**Figure 3D**). This agrees with previous findings and indicates that while dMi-2 can bind to double stranded RNA and DNA it binds with higher affinity to single stranded RNA (Murawska et al., 2011). Furthermore, dMi-2 complexes with single stranded RNAs were remarkably resistant to high salt (800 mM KCl) and high detergent concentrations (8% NP40) (**Supplementary Figure 3F and 3G**), supporting the conclusion that dMi-2 has robust RNA binding activity in the absence of dMi-2-associated dNuRD or dMec subunits.

dMi-2 preferentially binds G-rich RNAs

Our analysis revealed that dMi-2 has broad RNA binding activity *in vitro*, in agreement with our finding that dMi-2 is associated with many RNAs *in vivo* (**Figure 1**). The results also indicate that dMi-2 does not bind with the same affinity to all RNA molecules, suggesting that sequence (and/or secondary structure) might modulate binding. We asked if RNA regions to which dMi-2 was crosslinked *in vivo* had a non-random sequence composition surrounding the crosslinking site that would facilitate dMi-2 binding. The relative distribution of bases surrounding dMi-2 crosslinking sites is shown in **Figure 4A**. Of note, as a consequence of UV-induced crosslinking, U is more likely to be crosslinked to proteins than other bases (Sugimoto et al., 2012). Indeed, U was identified at the crosslinking site much more often than the remaining three bases. Of these, G was the most abundant at both the crosslinking site (position 0) as well as at the neighbouring position +1. Furthermore, this analysis detected an overrepresentation of A and C at positions +4 and +5, respectively, indicating that dMi-2 preferentially associates with a GGNNAC motif *in vivo*. To further probe the effect of base composition on dMi-2 binding we used homopolymeric RNAs in EMSAs. We compared binding of dMi-2 to poly(G), poly(C), poly(U) and poly(A) 40-mers (**Figure 4B, Supplementary Figure 4A to D**). dMi-2 bound to poly(G) RNA with an apparent K_d of 0.03 ($\pm$ 0.01) μ M. The affinity for binding to the other three homopolymeric RNA fragments was too low to allow us to determine an apparent K_d value. These results indicate that dMi-2 interacts preferentially with G-rich RNA.

To confirm this finding using a different method, we compared binding of dMi-2 to (CA)₂₀ RNA with binding to (GA)₂₀ RNA in a fluorescence polarization assay. In this experiment dMi-2 bound to (GA)₂₀ RNA with an apparent K_d of 42 ($\pm$ 17) μ M (**Figure 4C**). In contrast, binding

of dMi-2 to (CA)₂₀ RNA was so weak that we were unable to calculate a K_d value. We conclude that dMi-2 has a strong preference for binding to G-rich RNA sequences *in vitro*.

Some G-rich RNA molecules can form G-quadruplex structures and some chromatin regulators such as PRC2 display preferential binding to G-quadruplex-forming RNAs (Wang et al., 2017). We confirmed that the poly(G) 40mer used in Figure 4B does indeed form a G-quadruplex structure (**Supplementary Figure 4E**). We next compared binding of dMi-2 to a G-quadruplex-forming RNA ((G₄A₄)₄ 40mer) with binding to an RNA fragment with very similar sequence composition that cannot form G-quadruplex structures ((GA)₂₀ 40mer) (**Supplementary Figure 4F and 4G**). dMi-2 bound with indistinguishable apparent affinities to both RNAs ((G₄A₄)₄: $K_d = 0.08$ (+/- 0.0) μ M; (GA)₂₀: $K_d = 0.07$ (+/- 0.01) μ M) (**Figures 4D and 4E**) indicating that dMi-2 binding to RNA is facilitated by G-rich sequences but not by G-quadruplex structures.

A novel RNA binding domain in the N-terminus of dMi-2

dMi-2/CHD4 has several domains that have previously been demonstrated to interact with nucleic acids. These include the HMG box and adjacent sequences that interact with poly(ADP-ribose) and the chromodomains and the ATPase domain, both of which interact with nucleosomal DNA during the remodelling reaction (Bouazoune et al., 2002; Kovac et al., 2018; Murawska et al., 2011; Silva et al., 2016). We used a series of dMi-2 truncation mutants to assess if these domains also contribute to RNA binding in EMSAs. **Figure 5A and 5B** show that neither of the regions with an established role in contacting nucleosomal DNA during the remodelling reaction exhibit significant RNA binding activity (compare lanes 2, 5 and 8). In contrast, fragments containing the N-terminal 376 amino acid residues, which harbour the HMG box, exhibited substantial RNA binding activity (compare lanes 2, 4 and 6). Moreover, removal of this N-terminal region resulted in a reduction of RNA binding (compare lanes 2, 3 and 7), suggesting that region 1-376 makes a major contribution to RNA binding *in vitro*.

The central HMG box in the 1-376 fragment is flanked by regions that are predicted to be disordered by PONDR (**Figure 5C**). We further truncated this region to assess the importance of the HMG box for RNA binding (**Figure 5D**). Surprisingly, the isolated HMG box failed to interact with RNA in our assay (**Figure 5E**). In contrast both disordered regions flanking the HMG box exhibited robust RNA binding activity.

Collectively, these results suggest that two N-terminal IDRs make a substantial contribution to RNA binding by dMi-2.

G-rich RNA inhibits dMi-2- and CHD4-mediated nucleosome remodelling in vitro

We next asked if RNA can interfere with dMi-2 remodelling activity *in vitro*. We selected three 40mer RNAs and verified by EMSA that all three bound to dMi-2 *in vitro* (**Supplementary Figure 6 A–D**). The (G₄A₄)₄ 40mer bound dMi-2 with an apparent K_d of 0.08 ($\pm$ 0.02) μ M and the (GA)₂₀ 40mer with an apparent K_d of 0.08 ($\pm$ 0.01) μ M. The Hsp70Aa fragment, which contains a lower percentage of G nucleotides (30%), bound more weakly with an apparent K_d of 0.31 ($\pm$ 0.1) μ M. To test remodelling activity, we used a DNA fragment with an end-positioned nucleosome. We used limiting amounts of dMi-2 to sensitize the assay for activation or inhibition. In agreement with our previous work, dMi-2 mobilized 25-30% of the histone octamer to a central position, resulting in a nucleosome that migrated more slowly during native gel electrophoresis (**Supplementary Figure 6E–G**). We then challenged the remodelling reaction by addition of increasing amounts of RNA. Addition of either of the two G-rich RNAs resulted in efficient inhibition of remodelling with a half maximal inhibitory concentration (IC₅₀) of 140.10 ($\pm$ 20.93) nM ((G₄A₄)₄) and 152.65 ($\pm$ 52.25) nM ((GA)₂₀), respectively (**Figure 6A**). In contrast, the Hsp70Aa 40mer failed to impact the remodelling reaction even when used at high concentrations (1.5 μ M). This result indicates that G-rich RNAs, which bind with high affinity to dMi-2, inhibit nucleosome remodelling. In contrast, RNAs that are not G-rich and bind dMi-2 with lower affinity are not capable of interfering with remodelling under our assay conditions.

We then turned to CHD4, the human homologue of dMi-2, and asked if RNA-mediated inhibition of remodelling was conserved between *D. melanogaster* and *H. sapiens*. In the same gel-based remodelling assay, CHD4-mediated nucleosome remodelling was robustly inhibited by addition of the G-rich (GA)₂₀ RNA (**Figure 6B**). The (CA)₂₀ RNA, on the other hand, failed to inhibit remodelling even at high concentrations (2 μ M). We also measured inhibition of CHD4-mediated remodelling by (GA)₂₀ RNA using a Real Time Fluorescence Assay (RTFA) ((Zhong et al., 2020); **Supplementary Figure 6H and 6I**). Here, we used nucleosomes carrying an AF488 fluorophore on histone H2A and a BHQ1 quencher at one end of the DNA fragment (**Supplementary Figure 6H**). Nucleosome remodelling relieves quenching of the AF488 fluorescence, allowing detection of remodelling in real time. Again, we observed an RNA concentration-dependent reduction of CHD4-mediated nucleosome remodelling (**Supplementary Figure 6I**). Together, these results establish that inhibition of nucleosome remodelling by RNA is a property that is conserved between dMi-2 and CHD4.

RNA directly inhibits binding of dMi-2/CHD4 to its nucleosomal substrate

What is the mechanism of inhibition of dMi-2/CHD4-mediated nucleosome remodelling by RNA? One possibility is that RNA engages with the active site of the enzyme or a nearby allosteric site to lower remodelling activity. Such a mechanism has been invoked to explain the inhibition of PRC2 histone methyltransferase activity by RNA (Zhang et al., 2019). However, given that we could not detect an interaction between the dMi-2 ATPase domain and RNA (**Figure 5**), we considered this possibility to be unlikely. Alternatively, the interaction of dMi-2/CHD4 with RNA might be incompatible with binding of the enzyme to the nucleosome. Consistent with this competition hypothesis, the data in Figure 6A show that RNAs that bind dMi-2/CHD4 with high affinity are more potent inhibitors of remodelling. In order to directly test if RNA can compete with CHD4 binding to the nucleosome, we used microscale thermophoresis. We measured the K_d of human CHD4 for a fluorescently labelled nucleosome to be 41 (+/-2) nM (**Figure 6C**). We then repeated the titration in the presence of (GA)₂₀ RNA and observed a significant and concentration dependent reduction in the capacity of CHD4 to bind the nucleosome: the presence of 2 μ M (GA)₂₀ RNA reduced the affinity by ~20-fold. Moreover, the same concentration of (CA)₂₀ RNA reduced the K_d by less than two-fold (**Figure 6D**), demonstrating a strong sequence dependence of the ability of RNA to inhibit nucleosome binding by CHD4.

Taken together these results suggest that preferential binding of dMi-2/CHD4 to G-rich RNAs as well as inhibition of nucleosome remodelling by G-rich RNAs is shared between fly and man.

Discussion

dMi-2 associates with a broad range of mRNAs

Our study demonstrates that mRNA inhibits the function of an ATP-dependent chromatin remodeller of the CHD family. This inhibition involves the direct interaction of RNA with two novel IDRs in dMi-2. We identify two molecular mechanisms by which RNA binding counteracts dMi-2 function: dissociation of dMi-2 from chromatin and competition with the nucleosome substrate during the remodelling reaction. As discussed below, this mRNA-mediated activity allows a coordinated and simultaneous inhibition of several repressor complexes and thereby safeguards transcriptionally active regions from the establishment of repressive chromatin structures.

We reveal that dMi-2 is associated with thousands of mRNAs *in vivo* defining it for the first time as a *bona fide* RNA binding protein (RBP). This conclusion is supported by the efficient photo-crosslinking of dMi-2 to multiple mRNAs *in vivo* identified by iCLIP2, by the co-immunoprecipitation of formaldehyde-crosslinked dMi-2/mRNA complexes and by the robust interaction of dMi-2 with numerous RNA fragments detected in *in vitro* EMSA and FP assays. We have identified a novel RNA binding region in the N-terminus of dMi-2 that comprises two predicted IDRs (residues 1-117 and 239-376) flanking an HMG box that has previously been shown to bind DNA and poly(ADP-ribose) (Silva et al., 2016). These IDRs contain regions rich in basic amino acids that might interact with the phosphate backbone of RNA. However, given that formation of dMi-2/RNA complexes is not strongly affected by high salt concentrations we expect that non-ionic interactions with RNA bases will make a substantial contribution to RNA binding.

The RNA-binding IDRs that flank the HMG box are also present in human and *C. elegans* homologs of dMi-2 (CHD4 and LET-418, respectively) indicating an RNA-binding activity that is conserved across 550 million years of evolution. These IDRs are not resolved in the recently reported cryo-EM structure of the CHD4-nucleosome complex, suggesting either that they do not adopt a well-defined conformation in presence of the nucleosome substrate or that the interactions that they form are with a higher-order (>1 nucleosome) chromatin substrate (Farnung et al., 2020). We speculate that the IDRs might adopt more ordered conformations when they encounter RNA.

Although the IDR-containing region displays potent RNA binding activity, it is important to note that dMi-2 deletion mutants lacking this region retained weak RNA binding *in vitro* (Figure 5). This result suggests that dMi-2 contains several RNA binding sites that are distributed across a wider region. These could increase binding to one RNA molecule by providing multiple contact points or allow the simultaneous binding of multiple RNA molecules.

RNA binding preference of dMi-2

dMi-2 displays a preference for binding G-rich RNA fragments *in vitro*. The basis for this selectivity is currently unclear and will require structural analyses of dMi-2/RNA complexes. dMi-2's preference for G-rich sequences is not entirely recapitulated *in vivo*. Although G residues are overrepresented at the crosslinking site and the +1 position, the surrounding region is not particularly G-rich. In fact, A and C are strongly overrepresented at the +4 and

+5 positions, respectively. It is likely that RNA binding *in vivo* is influenced by other dMi-2 complex subunits. Indeed, the *C. elegans* homolog of dMEP-1, which interacts with dMi-2 to form the dMec complex, has previously been shown to bind RNA (Belfiore et al., 2002; Kunert et al., 2009).

In addition, other RBPs might facilitate the interaction of dMi-2 with RNA via protein-protein interactions. RBPs that are preferentially associated with the 3' ends or the polyA tail of mRNAs would be of particular interest given that dMi-2 preferentially crosslinks to the 3' ends of mRNA *in vivo*. An analysis of the RNA binding properties of 24 human chromatin regulators by fRIPseq has revealed a strong relationship between CHD4- and PABP-bound RNAs (Hendrickson et al., 2016). The association of both CHD4 and PABP with mRNA was highest over the 3' ends of transcripts. Moreover, CHD4 was found to bind almost exclusively to exons. This finding mirrors our results and suggests that the observed mRNA binding preferences are fundamental features of this remodeller. It is tempting to speculate that an interaction with PABP contributes to the dMi-2/RNA interaction *in vivo*. Indeed, PABP was recovered in a screen for CHD4 interactors (Hoffmeister et al., 2017). An involvement of general RBPs in mediating dMi-2 binding would help to explain why dMi-2 associates with thousands of mRNAs that differ in sequence and secondary structure *in vivo*.

RNA inhibits nucleosome remodelling

The ability of RNAs to inhibit dMi-2 mediated nucleosome remodelling correlates with their affinity for binding to dMi-2. This observation suggests that it is RNA binding to dMi-2, rather than RNA binding to the nucleosome substrate, that directly modulates remodelling. RNA binds to PRC2 at an allosteric site close to the catalytic centre, suggesting that RNA binding results in a change in conformation (Zhang et al., 2019). In contrast the major RNA binding region of dMi-2 (aa 1-376) is far from its catalytic domain in primary sequence. Still, it is possible that the N-terminus of dMi-2 interacts with the ATPase domain in the three-dimensional structure of the enzyme even though this region could not be resolved in the structure of the CHD4-nucleosome complex (Farnung et al., 2020). Nevertheless, our finding that RNA can compete with the nucleosome substrate for binding to CHD4 suggests a different inhibition mechanism. We propose that RNA binding to dMi-2 is incompatible with the simultaneous binding to nucleosomes, at least for RNA molecules of 40 nucleotides or longer. It is conceivable that dMi-2 associated RNA interferes with the interactions of chromodomains and the ATPase domain with nucleosomal DNA; these interactions are essential for the remodelling reaction (Farnung et al., 2020; Kovac et al., 2018).

SWI/SNF nucleosome remodelling complexes have recently been demonstrated to interact with repressive, long non-coding RNAs such as Xist and Evf2 (Cajigas et al., 2015; Jegu et al., 2019). Xist binding results both in eviction of SWI/SNF complexes from the inactive X chromosome and in inhibition of remodelling and ATPase function (Jegu et al., 2019). It is remarkable that both activating SWI/SNF and repressive dMi-2/CHD4 remodelling complexes are inhibited by RNA, and yet seem to respond to different types of RNA: long non-coding RNA that function to repress transcription in the case of SWI/SNF complexes and protein-coding mRNAs that emanate from actively transcribed genes in the case of dMi-2/CHD4.

RNA antagonizes both dMi-2/CHD4 and PRC2

PRC2 and dMi-2/CHD4-containing NuRD collaborate to establish repressive chromatin structures. NuRD facilitates PRC2-mediated H3K27 methylation by deacetylating H3K27ac (Reynolds et al., 2012b). Moreover, NuRD increases local nucleosome density which might further contribute to PRC1-mediated chromatin compaction (Bornelov et al., 2018). The effects of RNA binding to PRC2 and NuRD are two-fold: (1) counteracting chromatin binding and (2) inhibition of enzymatic activity. Thus, two different types of enzymes - a histone methyltransferase and an ATP-dependent nucleosome remodeller - are both inhibited by RNA. This RNA-mediated mechanism provides an elegant strategy to simultaneously target the two cooperating repressor complexes - PRC2 and NuRD - and in doing so to safeguard actively transcribed genes from the establishment of repressive chromatin structures (**Figure 7**).

In summary, our work supports a general and conserved role of mRNA in the coordinated control of multiple chromatin regulating complexes. This activity constitutes a negative feedback loop in which mRNA, the ultimate product of "active chromatin", directly engages and counteracts two important complexes that generate repressive chromatin structures. We envisage that this mechanism stabilizes an active chromatin region until transcription rates are reduced by the dissociation of activating transcription factors or the binding of transcriptional repressors to enhancers and/or promoters. Reduced mRNA production would then provide a window of opportunity for PRC2 and NuRD to (re)engage with chromatin and to (re)establish repressive chromatin structures.

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Author contributions

IU, JPM, AB conceived the study; IU, OR, JPM, AB designed experiments; IU and OR performed iCLIP2; AN and TS curated sequencing data; CT and HRC analysed iCLIP2 data; JL analysed RNA-seq data; IU and MJ performed RIP-qPCR and FLAG IP; IU, MJ and OV performed FP assays; IU and YZ performed nucleosome remodelling assays; YZ performed MST and RTFA assays; IU performed ChIP-qPCR, RT-qPCR, cell fractionation, EMSA and CD assays; MG and RH synthesized RNA; IU, CT, YZ, MJ, OR, JL, HRC, JPM and AB discussed and interpreted results; IU, OR, JPM and AB wrote the manuscript.

Declaration of interests

The authors declare no competing interests.

Materials and Methods

iCLIP2: Individual nucleotide-resolution UV-crosslinking and immunoprecipitation 2 was performed as described in (Buchbender et al., 2020). Briefly, S2 cells expressing GFP-tagged dMi-2 (Lenz et al., 2021) and untagged S2 cells were washed with cold 1× PBS. Protein-RNA cross-linking was performed at 254 nm UV light (300 mJ/cm²) using a Stratagene Stratalinker. Cells were harvested by centrifugation for 5 min at 2,000 rpm at 4°C. Cell pellets were resuspended in 4 volumes (vol.) of RIPA buffer (50 mM Tris-HCl pH 7.4, 1% NP-40, 0.1% SDS, 150 mM NaCl, 5 mM EDTA), incubated for 10 min on ice and centrifuged at 13,000 rpm for 20 min at 4°C.

DNase and partial RNase digestion: To the whole cell extract, 2 vol. of RQ-1 buffer (40 mM Tris-HCl pH 8.0, 10 mM MgSO₄, 1 mM CaCl₂) was added. Subsequently, TURBO™ DNase (1:500 vol.) and RNaseOUT (1:1,000 vol.) were added to the extract and mixed gently. RNase I (Ambion, AM2294) was added to a final concentration of 10⁻³ (11.764 U/μL), 10⁻⁴ (1.307 U/μL) and 10⁻⁵ (0.145 U/μL), respectively. Samples were incubated at 37°C for 3 min with 800 rpm shaking and immediately placed on ice for 3 min. To each sample, 20 μL (1:50 vol.) of 5 M

NaCl was added and samples centrifuged at 14,800 rpm for 5 min at 4°C. The extracts were transferred to fresh tubes.

Immunoprecipitation (IP): The GFP-trap beads were washed 2× with 1 mL cold TBS-T buffer (50 mM Tris pH 7.4, 150 mM NaCl, 0.05% Tween-20). To each sample, the washed and equilibrated GFP-trap beads were added, and samples were incubated for 2 h at 4°C while rotating. After IP, the samples were centrifuged at 2,000 rpm for 5 min at 4°C and the flow-through was discarded. The beads were washed 4× with TBS1000-T buffer (50 mM Tris pH 7.4, 1000 mM NaCl, 0.05% Tween-20) and 2× with PNK buffer (70 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 0.05% NP-40). Beads were transferred to fresh Eppendorf tubes after the third wash.

On-bead phosphatase treatment: To the beads, 40 µL of 20× phosphatase mix (80 µL 10× phosphatase buffer (Fermentas), 30 µL SAP (1 U/µL, Fermentas), 10 µL RNaseOUT (40 U/µL, Invitrogen) and 680 µL DMPC treated water) was added and incubated at 37°C for 20 min. Beads were washed 2× with TBS400-T buffer (50 mM Tris pH 7.4, 400 mM NaCl, 0.05% Tween-20). Eppendorf tubes were changed after second wash and beads washed 2× with PNK buffer.

On-bead 3'-end RNA linker ligation: To the beads, 25 µL of 1× ligase mix (1.5 µL 3'-linker (100 pmol/µL), 2.5 µL of 10× T4 RNA-ligase buffer (Thermo Scientific), 0.65 µL RNA-ligase (10 U/µL, Thermo Scientific), 0.25 µL RNaseOUT (40 U/µL, Invitrogen), 2.5 µL BSA (1 mg/mL, Thermo Scientific) and 17.60 µL DMPC treated water) was added and incubated at 16°C in a thermomixer overnight. Samples were washed 2× with PNK buffer.

On-bead PNK treatment: To the beads, 20 µL of 1× PNK mix (2 µL of 10× PNK buffer (NEB), 2 µL ATP (SCP801; 12.33 pmol/µL), 0.5 µL T4 PNK Enzyme (1000 U/µL, NEB), 0.5 µL RNaseOUT (40 U/µL, Invitrogen) and 15 µL DMPC treated water) was added and incubated at 37°C for 20 min in a thermomixer. Beads were washed 1× with cold TBS-T and 1× with cold PNK buffer. All buffers were freshly supplemented with 1 mM DTT, RNase inhibitor (unless RNaseOUT is supplied) and protease inhibitor cocktail.

SDS PAGE and Blotting: The beads were mixed with 20 µL of 2× LDS loading buffer supplemented with 1mM DTT. Samples were denatured for 10 min at 70°C while shaking and loaded on 4-12% Bis-Tris gel along with a pre-stained size marker (Thermo Scientific) and electrophoresed at 200 V for 5 h. Proteins were transferred to a nitrocellulose membrane by blotting overnight at 15 V at 16°C. The protein size marker bands were drawn on a plastic

bag using a radioactive pen after placing the plastic bag on top of the membrane. The membrane was exposed, whilst in the plastic bag, to X-ray film for 16 h. The X-ray films were developed and the autoradiograms were used to detect the protein-RNA complex of interest. The corresponding protein-RNA area was marked on the nitrocellulose membrane.

RNA isolation: The selected area on the nitrocellulose membrane containing the protein-RNA complex of interest was cut along with the corresponding area in the control lane. To the nitrocellulose membrane pieces, 400 μ L of PNK buffer was added and 20 μ L of Proteinase K (20 mg/mL). Samples were incubated at 37°C for 20 min with 1,000 rpm shaking. 400 μ L of PK buffer (100 mM Tris-HCl pH 7.4, 50 mM NaCl, 10 mM EDTA and 1% SDS) and PK-Urea buffer (PK buffer containing 7 M urea) were added and samples were incubated for a further 20 min at 55°C with shaking at 1,000 rpm.

To each sample, 800 μ L of RNA-grade phenol-chloroform-isoamyl alcohol (25:24:1) was added and samples were incubated at 30°C for 5 min while shaking at 1,000 rpm. Samples were centrifuged at 13,000 rpm for 5 min at room temperature using phase-lock gel (5PRIME). The aqueous layer was transferred to a new tube without disturbing the gel. RNA was precipitated by adding 1 μ L glycoblue (Ambion, 9510), 80 μ L of 3 M sodium acetate (NaOAc) pH 5.5 and 0.7 vol. ice-cold isopropanol, mixed and incubated overnight at -80°C. Samples were centrifuged at 14,800 rpm for 20 min at 4°C, the supernatant was discarded, the RNA pellet washed with 0.9 mL of 80% ethanol, centrifuged again at 14,800 rpm for 5 min. The RNA pellet was air-dried for 3 min and dissolved in 5 μ L of RNase-free water and transferred to a PCR tube.

The RNA was reverse transcribed and iCLIP library was prepared as detailed in (Buchbender et al. 2020) and sequenced.

Western blot: Samples to be analyzed by Western blot (WB) were mixed with SDS loading buffer, denatured at 95°C for 5 min and loaded on SDS polyacrylamide gel along with a size marker and electrophoresed. The proteins were transferred to activated polyvinylidene difluoride (PVDF) membrane at 400 mA (milliamperes) for 2 h in cold conditions. The membrane was blocked with 5% milk in PBS-T (1 $\times$ PBS pH 7.4, 0.1% (v/v) Tween-20) for 30 min at room temperature while shaking and then incubated over night with primary antibody in 5% milk in PBS-T at 4°C on a shaker. The membrane was washed 3 $\times$ with PBS-T for 5 min each at room temperature on a shaker and incubated with the respective secondary antibody in 5% milk in PBS-T at room temperature for 2 h on a shaker. The membrane was

again washed 3× with PBS-T for 5 min each at room temperature on a shaker. Western blot signals were detected by chemiluminescence using HSP substrate (Milipore, WBKLS0500). The following primary antibodies were used: Rat monoclonal anti-GFP antibody (Chromotek, 80626001AB, 1:5,000), Mouse monoclonal anti-tubulin anti-body (Merck, 3272293, MAB3408, 1:10,000), Rabbit polyclonal anti-dMi-2 anti-body (custom made, 0.5 mg ml⁻¹, 1:10,000), Rabbit anti-E(z) antibody (1:3,000), anti-dLSD1, Rabbit polyclonal anti-dRPD3 (Abcam 1767, 1:10,000) and Rabbit polyclonal anti-H3 antibody (Abcam 1791, 1:10,000). The following secondary anti-bodies were used: Sheep polyclonal anti-mouse HRP (Amersham, NA931, 1:30,000), Goat polyclonal anti-Rat HRP (Invitrogen, 31470, 1:1,000).

RNA-IP-qPCR: 80 million cells were cross-linked with formaldehyde at a final concentration of 1% (v/v) and slowly shaken on a plate rocker for 10 min at room temperature. To quench this reaction, glycine was added to a final concentration of 240 mM and incubated for 5 min at room temperature while shaking. Cells were collected and spun down at 1,000 rpm for 10 min at 4°C. The supernatant was removed and the pellet was washed with 10 mL cold 1× PBS, pH 7.4. The supernatant was removed and the pellet was resuspended in 800 µl FA buffer (50 mM HEPES-KOH pH 7.6; 140 mM NaCl; 1% (v/v) Triton X-100; 0.1% Sodium deoxycholate) with freshly added protease inhibitors, 1 mM DTT and RNasin (Thermo Scientific, 100 Units (U)/mL). The cells were lysed on ice for 15 min. After the lysis, the cells were sonicated for 10 min at the high intensity setting (30 sec on/off) in a Bioruptor (Diagenode). The whole cell extract was adjusted to 25 mM MgCl₂ and 5 mM CaCl₂ and 1 µL DNase I (30 U) was added. The solution was incubated for 10 min at room temperature and stopped by adding EDTA to a final concentration of 10 mM. To remove cell debris, the extract was centrifuged at 13,000 rpm for 10 min at 4°C. 100 µL of the supernatant was removed and stored at -20°C (input sample). 500 µL of the supernatant was used for precipitation. The GFP Trap resins (ChromoTek, gta10) were equilibrated 3× with 1 mL FA buffer supplemented with protease inhibitors, 1 mM DTT and RNasin (100 U/mL). 30 µL of GFP Trap resins were used for precipitation. The IP was performed overnight at 4°C on a spinning wheel.

The resins were spun down at 2,000 rpm for 4 min at 4°C and the supernatant was removed. The resins were washed 5× with 1 mL FA buffer and 2× with 1 mL TE buffer pH 8.0 (10 mM Tris-HCl, 1 mM EDTA) with freshly added protease inhibitors, 1 mM DTT and RNasin (100 U/mL). Before the last wash with TE buffer, resins were transferred into fresh Eppendorf tubes. After the last washing step, the supernatant was removed, 100 µL of RIP elution buffer (100 mM Tris-HCl pH 8.0, 10 mM EDTA; 1% (w/v) SDS) with RNasin (40 U/mL) was added and samples incubated for 10 min at room temperature on a rotating wheel. Samples were spun down at 2,000 rpm for 4 min at room temperature and the supernatant was transferred

into new Eppendorf tubes. Again, 100 μ L of RIP elution buffer with RNAsin (40 U/mL) was added and samples incubated in a thermomixer at 1,000 rpm for 10 min at 65°C. Samples were spun down with the same settings as before and the supernatant was transferred into the same microfuge tube as the previous elution. 100 μ L of RIP elution buffer with RNAsin (40 U/mL) was added to the 'input' sample to adjust the volume to 200 μ L. Eluates and 'input' samples were adjusted to 200 mM NaCl and 20 μ g of proteinase K (10 mg/ml) was added. Samples were incubated at 42°C for 1 h and then at 65°C for 2 h for decrosslinking. The RNA was purified with the PeqGOLD Total RNA Kit (PeqLab) or RNeasy Mini Kit (Qiagen) following the manufacturer's instruction and an additional DNase I digestion was performed on the column provided in the kit. The purified RNA was then reverse transcribed into complementary DNA (cDNA) with the SensiFAST™ complementary cDNA synthesis Kit (Bioline) and used in qPCR. After cDNA synthesis, the cDNA was diluted 1:20 in nuclease-free water.

cDNA synthesis program:

25°C	->	10 min
42°C	->	15 min
85°C	->	5 min
4°C	->	∞

qPCRs were performed using SensiFast™ SYBR® Lo-ROX kit (Bioline). A master mix for each primer pair was prepared following the manufacturer's instructions. 15 μ L of master mix was used per well and 5 μ L of cDNA or nuclease-free water control were added to the appropriate wells. The 96-well plate was closed and centrifuged shortly. The RT-qPCR was performed under the following conditions-

95°C 2 min	
95°C 5 s	40 cycles
60°C 10 s	
72°C 20 s	

For the RIP experiments, the percentage of the input was calculated as-

$$\Delta Ct = Ct (input) - Ct (eluate)$$

$$\% input = 2^{\Delta Ct}$$

For plotting the values on a graph, the standard deviation was calculated as:

$$SD = \ln(2) \times \% input \times \sqrt{(SD (input))^2 + (SD (elute))^2}$$

Chromatin immunoprecipitation-qPCR: 100 million cells were seeded in 15 cm dishes and the protein-DNA contacts were cross-linked using 1% (v/v) formaldehyde (Polysciences, 04018-1) for 10 min at room temperature with shaking. The formaldehyde was quenched with 240 mM glycine (final concentration) for 10 min at room temperature with shaking. The cells were harvested and washed 2× with cold PBS pH 7.4, the supernatant was discarded. The cell pellet was lysed with 1 mL lysis buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA and 1% SDS, supplemented with protease inhibitors cocktail and 1 mM DTT) and incubated 10 min on ice. The cell lysate was sheared with Biorupter (Diagenode) with 3 cycles of 30 seconds ON/OFF for 10 min each at high power. The lysate was centrifuged at 14,800 rpm for 15 min at 4°C, the supernatant (chromatin) transferred to fresh Eppendorf tubes and the pellets discarded. Protein A beads (Invitrogen) were washed 3× with low salt buffer (20 mM Tris-HCl pH 8.0, 2 mM EDTA, 150 mM NaCl, 1% Triton X-100, 0.1% SDS) and blocked overnight in blocking buffer (2 mg/mL BSA, 2% fish skin gelatin in low salt buffer).

The chromatin (130 µL per ChIP) was diluted 10-fold with ChIP-IP buffer (16.7 mM Tris pH 8.0, 167 mM NaCl, 1.2 mM EDTA, 0.01% (w/v) SDS, 1.1% (w/v) Triton X-100) and pre-cleared with pre-equilibrated and blocked protein A beads (80 µL slurry per ChIP) for 1 h on a rotating wheel at 4°C. Pre-cleared chromatin was centrifuged at 14,800 rpm for 10 min at 4°C. The supernatant was transferred to fresh Eppendorf tubes, 13 µL were removed and stored at -20°C (input). To the pre-cleared chromatin, 50 µL (1:1) pre-equilibrated and blocked (just as protein A beads), GFP-Trap beads (chromoTek) were added and samples incubated overnight on a rotating wheel at 4°C. The samples were centrifuged at 2,000 rpm for 5 min at 4°C, supernatant was discarded and beads were washed 3× with low salt buffer, 3× with high salt buffer (20 mM Tris-HCl pH 8.0, 2 mM EDTA, 500 mM NaCl, 1% Triton X-100, 0.1%

SDS), 1× with LiCl buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 250 mM LiCl and 0.1% NP-40) and 1× with TE buffer (10 mM Tris pH 8.0, 1 mM EDTA). For each washing step, samples were incubated on a rotating wheel at 4°C for 10 min. After the last wash, beads were resuspended in 700 µL TE buffer, transferred to fresh Eppendorf tubes and centrifuged at 2,000 rpm for 5 min at 4°C. The supernatant was discarded. 250 µL of elution buffer (100 mM NaHCO₃ and 1% (v/v) SDS) was added to each sample and incubated for 20 min on a rotating wheel at room temperature, centrifuged at 2,000 rpm for 5 min at room temperature. The supernatant was transferred to fresh Eppendorf tubes. To the beads, 250 µL of elution buffer was added followed by incubation for 20 min at room temperature on a rotating wheel and then at 95°C for 10 min. Samples were centrifuged at 14,800 rpm at room temperature for 5 min. The supernatant was pooled with the first eluate. To the input samples, 486 µL of elution buffer was added. To decrosslink, 20 µL of 5 M NaCl was added and samples were incubated at 65°C overnight with 800 rpm shaking. Protein was digested by adding 10 µL of 0.5 M EDTA, 20 µL of 1 M Tris-HCl pH 6.8 and 2 µL of Proteinase K (10 mg/mL) and samples were incubated at 45°C for 1 h with 800 rpm shaking. DNA was purified using Qiaquick PCR purification Kit (Qiagen) following the manufacturer's protocol. The DNA was eluted with elution buffer provided in the kit. The DNA was diluted 1:4 with nuclease free water and 5 µL were used for each qPCR reaction. qPCR reactions were set up and analyzed as described above (RIP-qPCR).

Triptolide treatment: S2 cells were seeded in 15 cm dishes (for ChIP) or 6-well plates (for RT-qPCR) and 10 µM triptolide (dissolved in DMSO) or an equal volume of DMSO added to the media. Cells were incubated for 3 h and were processed for ChIP-qPCR as described above or for RT-qPCR as described below.

RT-qPCR: Triptolide treated and DMSO treated cells were harvested and RNA extracted using RNeasy Total RNA isolation kit (Qiagen). RNA was reverse transcribed to cDNA using SensiFAST cDNA synthesis kit (Bioline). The cDNA was diluted 1:5 with nuclease free water and 5 µL were used per qPCR reaction. qPCR reactions were set up using SensiFAST SYBR Lo-ROX kit (Bioline). The qPCR was performed as described above for RIP-qPCR.

RNase A treatment and cell fractionation: RNase A treatment and cell fractionation was performed as explained in (Beltran et. al., 2016). Briefly, 2-4 × 10⁷ cells per condition were aliquoted into 15 mL Falcon tubes. The cells were washed with 1× PBS pH 7.4 at room temperature. Cells were permeabilized with 10 mL of Buffer P (0.05% Tween-20 in 1× PBS pH 7.4) and incubated at 4°C for 10 min with intermittent agitation. The cell suspension was

centrifuged at 1,200 rpm for 2 min at 4°C and the supernatant was discarded. The cell pellet was resuspended in 950 µL of 1× PBS pH 7.4 (room temperature). The sample was split into two vials, 450 µL each. One was labelled as ‘sample’ and another ‘mock’. The ‘sample’ vial was taken to a different room while the ‘mock’ vial was processed on an RNase-free bench. To the ‘sample’ vial, 50 µL of 10 mg/mL RNase A (Applichem) was added while to the ‘mock’ vial, 50 µL of 10 mg/mL BSA was added. Each vial was incubated on separate rotating wheels at room temperature for 30 min. Both the vials were centrifuged at 1,200 rpm for 2 min at 4°C. The supernatant was discarded. The cell pellet was washed by pipetting up and down with 10 mL cold 1× PBS.

The cell suspension was centrifuged as earlier. The pellet was resuspended in 1 mL ice-cold buffer A (10 mM HEPES pH 7.9, 10 mM KCl, 1.5 mM MgCl₂, 0.34 M sucrose, 10% glycerol and supplemented with protease inhibitor cocktail and 1 mM DTT). From this step, 2.5 µL/mL RNase inhibitor – RNaseOUT (40U/µL, Ambion), was added to each buffer for ‘mock’ sample.

Triton X-100 was added to a final concentration of 0.1% (v/v), the tubes were flicked to mix and incubated on ice for 5 min. To isolate the cytoplasmic fraction, the samples were centrifuged at 3,600 rpm for 4 min at 4°C. The supernatant was separated, cleared by centrifugation at 14,100 rpm for 15 min at 4°C and stored at -20°C. The nuclei were washed 2× with 1 mL buffer A (resuspended gently by flicking). The nuclei were lysed with 250 µL of buffer B (3 mM EDTA, 0.2 mM EGTA, supplemented with protease inhibitor cocktail and 1 mM DTT) and mixed by gently flicking the tube. For the ‘mock’ sample, the buffer B was additionally supplemented with RNaseOUT. Both samples were incubated on ice for 30 min. The nucleoplasm was separated by centrifugation at 4,100 rpm for 4 min at 4°C. The nucleoplasm was stored at -20°C. The chromatin pellet was washed twice with 1 mL of buffer B and centrifuged at 4,100 rpm for 4 min at 4°C (mixed gently by flicking the tube). The final chromatin pellet was resuspended in 200 µL of 1× Laemmli buffer supplemented with β –mercaptoethanol (β - Me). The chromatin samples were sonicated for 7 cycles (30 s ON, 30 sec OFF, at High output) in a Bioruptor (Diagenode). The samples were heated to 95°C for 5 min and stored at -20°C. The cytoplasm, nucleoplasm and chromatin fractions were loaded on 8-12% SDS-gel and electrophoresed. For the ‘mock’ sample, care was taken to clean the bench, the pipettes and all equipment with RNaseZap.

Electrophoretic mobility shift assay (EMSA): In a 10 µL standard reaction, DNA/RNA was incubated with the protein of interest in EMSA buffer (40 mM KCl, 20 mM Tris pH 7.6, 1.5

mM MgCl₂, 0.5 mM EGTA, 10% glycerol, 200 ng/uL BSA). EMSA buffer was freshly supplemented with protease inhibitor cocktail, 1 mM DTT and 40U/mL of RiboLock RNase inhibitor (40U/μL, Thermo Scientific), for RNA EMSA only, for 15 min at room temperature. The samples were loaded on a pre-run 5% native polyacrylamide gel and electrophoresed at 100 V for 1 h. The gel was stained with 1× SYBR gold stain (Thermo Scientific) and imaged with Bio-Rad Chemi Doc Touch system. The bands were quantified by Bio-Rad Image Lab software and after subtracting the background intensity, the bound fractions were calculated as-

Fraction bound

$$= \frac{\text{Intensity of shifted band}}{\text{Intensity of unshifted band} + \text{Intensity of shifted band}}$$

Percent bound was calculated as-

$$\text{Percent bound} = \text{Fraction bound} \times 100$$

The apparent dissociation constant (app. K_d) was calculated using the hill slope equation as

$$Y = B_{max} \times X^h \div (Kd^h + X^h)$$

Where,

B_{max} = the maximum specific binding in the same unit as Y, K_d = ligand concentration needed to achieve a half-maximal binding at equilibrium in the same unit as X and h = hill slope.

FLAG immunopurification: The Sf9 cells expressing the FLAG-tagged protein of interest were harvested by centrifugation at 3,200 rpm for 25 min at 4°C. The supernatant was discarded and the cell pellet washed with 30 mL of cold 1× PBS (Phosphate buffer saline), pH 7.4. The centrifugation was repeated as earlier and the supernatant was discarded. The cellular pellet was resuspended and lysed with buffer BC-250 (0.4 mM EDTA pH 8.0, 20 mM HEPES pH 7.9, 10% (v/v) glycerol, 250 mM NaCl and freshly supplemented with protease inhibitors and 1 mM DTT), transferred to 50 mL Falcon tubes and incubated on ice for 15 min. The lysate was snap-frozen in liquid nitrogen and thawed. The freeze-thaw cycle was repeated. The lysate was then cleared by centrifugation at 10,400 rpm for 75 min at 4°C. The supernatant was transferred to a pre-chilled 50 mL Falcon tube and the cell debris was discarded. An aliquot from the whole cell extract was removed as 'input', mixed with 5× SDS

loading buffer and stored at -20°C. Anti-FLAG M2 agarose affinity gel (Sigma, A2220) were equilibrated by washing 3× with buffer BC-250 (10× bead vol.). To the extract, pre-equilibrated anti-FLAG M2 agarose affinity gel was added and samples were incubated at 4°C for 4 h on a rotator. After immunoprecipitation, the solution was transferred to Bio-Rad Econo-pac gravity-flow columns (Bio-Rad, #732-1010), the flow-through was collected. The beads were washed with 40× bead volume of BC-250, BC-500 (BC buffer with 500 mM NaCl), BC-1000 (BC buffer with 1,000 mM NaCl), BC-500, BC-250 and BC-100 (BC buffer with 100 mM NaCl) buffer in that order. Each buffer was freshly supplemented with protease inhibitor cocktail and 1 mM DTT. The purified protein of interest was eluted with 2× the volume of beads with elution buffer (FLAG peptide (5 mg/mL) diluted 1:20 in BC-100 buffer). The elution was repeated twice more. The elutes were pooled and concentrated in pre-chilled Centricons (Thermo Scientific).

The concentrated elute was dialyzed with buffer BC-50 (BC buffer with 50 mM NaCl and 5% glycerol) and loaded on a 5 mL HiTrap Q Fast Flow anion exchange chromatography column (Sigma) using an ÄKTA™ Pure FPLC (GE Healthcare). The flow through was collected. The bound fraction was washed with BC-50 buffer (20 column volumes) and gradually eluted with BC-2M buffer (BC buffer with 2 M NaCl and 5% glycerol). The flow through and elute sample fractions were loaded on denaturing polyacrylamide (Coomassie stain) and 5% native polyacrylamide (SYBR Gold stain) gels and electrophoresed to verify the purity of the fractions. The respective purified protein fractions were pooled, dialyzed to BC-100 buffer, aliquoted, snap frozen and stored at -80°C.

Native Polyacrylamide Gel Electrophoresis: EMSA reactions were loaded on native polyacrylamide gels. A 100 mL solution of 5% native polyacrylamide gel was prepared by mixing 12.5 mL of Rotiphorese Gel 40 (19:1, acrylamide:bisacrylamide) (Roth) with 5 mL of 10× Tris-Boric acid-EDTA (TBE) buffer (1 M Tris, 0.9 M boric acid and 0.01 mM EDTA). 400 µL of ammonium persulfate (APS) (Roth) and 80 µL tetramethylethylenediamine (TEMED) (Roth) were added and the solution poured in a glass chamber. The gel was allowed to polymerize at room temperature for 30 min, then pre-run for 1 h at 100 V. After the EMSAs were loaded, the gel was electrophoresed for 1 h at 100 V at RT in 0.5× TBE buffer. The gel was incubated in a 1× SYBR gold (Thermo Scientific) staining solution for 5 min at room temperature with shaking. The gel was washed 2× with deionized water and imaged with a Bio-Rad ChemiDoc Touch system.

Denaturing PAGE for RNAs: RNA samples were loaded on denaturing polyacrylamide gels to verify the quality of the RNAs. The gels were poured in the same manner as the native gels described above, but were additionally supplemented with 8% urea. The RNA samples were mixed with RNA loading buffer (Thermo Scientific), heated to 65°C for 5 min, instantly cooled for 1 min on ice and loaded on a pre-run denaturing gel and electrophoresed at 100 V for 30 min in 0.5× TBE buffer. The gels were processed as described above.

Fluorescence Polarization Assay: For FP assays, a protein dilution series was performed, leading to a protein concentration ranging from 0.5 μM to 0.0001 μM in 50 μL BC-100 buffer (0.4 mM EDTA pH 8.0; 20 mM HEPES pH 7.9; 100 mM NaCl; freshly added protease inhibitors and 1 mM DTT). The RNA working stock was diluted with BC-100 buffer to a concentration of 10 nM. 50 μL of the 10 nM RNA working stock was added into each well, leading to a final concentration of 5 nM in a 100 μL reaction. The plate was shortly spun down and fluorescence polarization measured at time point 180 min at 26°C. “Excitation” was set to “Filter” for measuring using a Spark® 20M multimode reader (Tecan). The following formulae were used for calculating the app. K_d and the EC50 values:

→ Formula for calculating the K_d :

$$y = T0 + (TE - T0) \times [(A0 + X + Kd) \div 2 - \sqrt{((A0+X+Kd) \div 2)^2 - A0 \times X}) \div A0]$$

→ Formula for calculating the EC50: (formula non-linear curve fit -> log(agonist) vs. response (three parameters)

$$y = lowest\ value + (highest\ value - lowest\ value) \div (1 + 10^{\log EC50 - X})$$

Circular dichroism (CD) spectroscopy: RNA (10 μM) in 170 μL volume was allowed to fold as described in (Wang et al. 2017). CD spectra were measured in a 0.1 cm path-length quartz cell. The measurements were taken from 220 nm to 350 nm with a data pitch of 0.1 nm and scanning speed of 50 nm/min and a band width of 1 nm. The cell holder temperature was regulated and the cuvette chamber was flushed with dry Nitrogen (N₂) gas. Spectra were measured at temperatures indicated in the figures. Three replicates were taken for each measurement and the mean data were analyzed by using CAPITO CD analysis and plotting software (Wiedemann et al. 2013). The data were smoothened with Savitzky-Golay filter and mean residue ellipticity (θ in grad cm² dmol⁻¹) was plotted.

Nucleosome remodelling assay: A typical nucleosome remodelling reaction was set up in a volume of 10 μ L. 50 nM end-positioned mono-nucleosomes with 80 bp flanking DNA were mixed with different concentrations of dMi-2 in BC-100 buffer (0.4 mM EDTA pH 8.0, 20 mM HEPES pH 7.9, 10% (v/v) glycerol, 100 mM NaCl and freshly supplemented with protease inhibitors and 1 mM DTT). Reactions were incubated for 5 min at 26°C, then, 2 mM ATP (Sigma) and 6 mM $MgCl_2$ were added and reactions were incubated at 26°C for 45 min. Reactions were stopped by adding competitor plasmid DNA (5 μ g/reaction) and samples were incubated on ice for 10 min and loaded on a 5% native polyacrylamide gel and electrophoresed at 100 V for 3 h in 0.5 $\times$ TBE buffer. Gels were stained with 1 $\times$ SYBR gold staining solution, imaged with Bio-Rad ChemiDoc Touch system and bands were quantified with Bio-Rad Image Lab software. For effects of RNA on remodelling activity of dMi-2, RNA was titrated in increasing concentrations before the addition of ATP and $MgCl_2$. The IC50 was calculated in GraphPad Prism software using the following equation-

$$Y = Bottom + (Top - Bottom) \div (1 + (X \div IC50))$$

Where IC50 is the concentration of agonist that gives a response half way between Bottom and Top. Top and Bottom are plateaus in the units of the Y axis.

Remodelling reactions with hCHD4 were performed in the following buffer: 50 mM Tris-HCl pH 7.5, 50 mM NaCl, 3 mM $MgCl_2$ and 1 mM ATP. Samples were incubated at 37°C for 1 h, stopped with competitor DNA and loaded onto the gel and processed as described above.

Microscale thermophoresis (MST) Assay: CHD4 was concentrated to ~2 μ M and buffer exchanged into the dilution buffer (50 mM HEPES pH 7.5, 50 mM NaCl, 3 mM $MgCl_2$) using Amicon Ultra-0.5 mL Centrifugal Filters (100 K MWCO, Merck Millipore). For each MST assay, a set of nine titration points was prepared by 1:2 serial dilution using the same dilution buffer. The diluted CHD4 were then mixed with 50 mM HEPES pH 7.5, 100 ng/ μ L BSA, 0.5% (v/v) glycerol and 25 nM 46^{Cy5}w60 nucleosome. Following 10 min incubation on ice, the samples were loaded into premium coated capillary tubes (Nanotemper) before undergoing MST in a Monolith™ NT.115 instrument. Thermophoresis was conducted at 80% LED power, and 20% MST power. Thermophoresis data were then analyzed with MO. Affinity Analysis software v2.3 and plotted using GraphPad Prism software.

Real time fluorescence assay (RTFA): Nucleosomes assembled from 0^{BHQ1}w60 DNA and AF488-labelled histone octamer were used for RTFAs. The assay was performed at 37°C and

the fluorescence intensity was monitored at 526 nm. The reaction mixtures (typically 50 μ L) containing 60 nM nucleosome, 10 nM CHD4 in 50 mM Tris-HCl pH 7.5, 50 mM NaCl and 3 mM $MgCl_2$ were loaded into the wells of a Corning® black nonbinding surface half-area 96-well plate, which was then subsequently put into a Tecan® Infinite M1000 Pro plate reader pre-warmed to 37°C. Reactions were monitored for 5 min prior to the addition of 1 mM ATP by the injector unit of the plate reader, and the fluorescence changes were continued to be monitored for another 25 min, with data points recorded every 30 s. The data were normalized with a nucleosome-only control.

Bioinformatic Pipeline: The raw iCLIP2 sequencing reads were assigned to the different libraries by the specific barcode part of the unique molecule identifier (UMI) and deduplicated based on total sequence identity with an in-house script. UMIs were removed from the read sequences and added to the read identifier. Additionally, poly(A) tails with at least eight consecutive A's were removed from reads and trimmed reads longer than 19 nucleotides were aligned to the *Drosophila melanogaster* genome (dm6) using bwa-mem (0.7.17-r1188, [arXiv:1303.3997v2](https://arxiv.org/abs/1303.3997v2)) with minimum score output (-T) set to 19. Alignments were deduplicated based on UMIs and mapping position using UMI-tools (1.0.1, doi:10.1101/gr.209601.116) dedup functionality with default parameters. Only reads mapping uniquely to the genome were considered for further analysis while the rest of the reads were discarded. The uniquely mapped reads were attributed to transcript annotations based on their genomic coordinates using featureCounts from the SubRead (v1.6.4, doi:10.1093/bioinformatics/btt656) software suite for non-overlapping features (-O) and only counting strand specific alignments (-s 1).

Sample Correlation- The read counts from the two biological replicates were checked for correlation by calculating the squared Pearson Correlation Coefficient for paired samples. For visualization a linear model was fitted using the same metric. (there is no analysis at all. There is a simple formula for the Pearson Correlation Coefficient for paired samples and you square that to get R^2).

Metagene Analysis- A metagene representation was generated based on read starts from both libraries using bamCoverage, calculateMatrix and plotProfile from the deepTools suite (3.4.3, doi:10.1093/nar/gkw257) with options “-bs 1 --ignoreDuplicates --minMappingQuality 1 --exactScaling --Offset 1”. Additionally, a detail graphic for the TTS of all transcripts with iCLIP reads was generated by manually plotting cross link positions derived from alignment start position minus one within +/-500 nucleotides of the TTS. Transcripts were sorted by

mapped number of reads and their proximity to TTS. Relative density of cross links was calculated for the respective region.

Intron-Exon coverage- The cumulative coverage across the last intron-exon junction of all transcripts with at least two exons was determined using a custom script and plotted for +/- 10 nucleotides around the junction.

DE-Seq analysis- We compared iCLIP crosslinking results and mRNA expression level, as determined by RNA-Seq (Lenz *et al.*, 2021) to identify mRNAs that preferentially associate with dMi-2. RNA-seq reads were mapped in the same manner as iCLIP reads but without prior trimming. Uniquely mapping reads were then counted using featureCounts as described above. In contrast to iCLIP, RNA-Seq read counts were normalized to FPKM (fragments per kilobase in transcript per million reads in library) to account for the difference in protocols (there is one read per cross-linked transcript molecule in iCLIP, while RNA-Seq can produce multiple reads per molecule especially for longer transcripts). DESeq2 (doi:10.1186/s13059-014-0550-8) was used to identify mRNAs more frequently captured in iCLIP experiments than expected from their proportional abundance in RNA-Seq data. We chose a threshold for adjusted p-value of < 0.05 and putative log fold difference of iCLIP vs. RNA-Seq reads of > 4 to identify mRNAs that we regard as significantly enriched.

Position relative to cross-link site- Genomic sequences +/-200 nucleotides around all identified cross-linking sites were extracted and absolute as well as relative base content per relative position to the cross-linking site calculated to identify base specific biases at the cross-linking site. Relative base content per position was converted to percent of the median content for each base within the 400-nucleotide window. Additionally, a sequence logo was generated with genomic sequences of +/-20 nucleotides around the crosslinking site using kpLogo (v1.1, doi:10.1093/nar/gkx323) and parameters “-gapped -startPos 21”.

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Figure Legends

Figure 1: dMi-2 associates with thousands of mRNAs *in vivo*

(A) dMi-2 binds RNA *in vivo*. Autoradiograph (upper panel) and dMi-2 Western blot (WB) (lower panel). S2 cells expressing dMi-2-GFP (dMi-2-GFP) or control S2 cells (untagged-dMi-2) were UV-irradiated (+UV) or left untreated (-UV) as indicated. Samples were treated with decreasing concentrations of RNase I as shown on top. Red closing bracket indicates dMi-2-RNA complexes. Protein molecular weights are indicated on the left. **(B)** The distribution of different types of RNAs in replicates-1 and -2 are represented as pie charts, the total number of reads (n) in each replicate is shown below. **(C)** Correlation of the number of crosslinks per gene (iCLIP2 counts, y-axis) with RNA-Seq abundances (RNA-seq, FPKM, x-axis). We used DESeq2 to determine robustly and significantly enriched mRNAs (green dots) across the two biological iCLIP replicates and three RNA-Seq replicates (fold difference > 4 and $p < 0.05$). RNAs chosen for validation by fRIP-qPCR are indicated.

Figure 2: RNA binding does not play a major role in recruiting dMi-2 to chromatin

(A) Distribution of dMi-2 iCLIP2 crosslinking sites (green), dMi-2 ChIPseq (blue) and ChIPseq input (grey) across a metagene. The transcription start site (TSS) and transcription end site (TES) are indicated. Left Y-axis shows ChIP-seq signal strength (RPKM), right Y-axis shows iCLIP2 signal strength (number of crosslinks). **(B)** Venn diagram showing the overlap between dMi-2 bound RNAs and genes as determined by iCLIP2 and ChIP-seq, respectively. **(C)** Accumulated iCLIP2 read coverage across the last intron-exon junction. All annotated genes with at least two exons were aligned at the last junction between intron and exon. The first base of the exon is at position zero. **(D)** S2 Cells were permeabilized and treated with RNase A (+) or BSA (-). Cells were fractionated into cytoplasmic, nuclear and chromatin and analysed by Western Blot as indicated. Histone H3 was used as loading control. The western blot signals were quantified using ImageJ software. The BSA treated control signals were set to 1 and the fold change in the RNase A treated samples was calculated and is shown below each panel. **(E)** Control (blue and red), dMi-2-GFP (green and purple), and dLINT1-GFP (orange and black) expressing S2 cells were treated with DMSO or 10 μ M Triptolide, respectively, for 3 hours, as indicated on the right. Chromatin was prepared and subjected to ChIP-qPCR analysis. Individual data points of two independent biological replicates were plotted as percent of input. Error bars indicate the standard deviation between two biological replicates.

Figure 3: dMi-2 has broad RNA binding activity in vitro

(A) Schematics representing the regions of Hsp70Aa (black) and Pgant35a (blue) genes from which corresponding RNAs were generated. The RNAs #1, #2, #3, #4 and #5 are all 100 nucleotides in length. RNA #6 is 40 nucleotides in length. The location of 100 bp double stranded RNA (dsRNA) and DNA fragments from the Hsp70Aa gene, used in Figure 3E are indicated (dsRNA, DNA). Scale is indicated on the right. **(B)** dMi-2 binding to RNA fragments derived from Hsp70Aa and Pgant35a genes. For EMSAs, 220 nM of each RNA and 112 nM of recombinantly purified dMi-2 was used per reaction. RNA without protein was loaded in lanes 1, 3, 5, 7, 9, 11, 13 and 15. Gels were stained with SYBR Gold. **(C)** EMSAs with Hsp70Aa #3 RNA (lanes 1-10) and Pgant35a #3 RNA (lanes 11-20). 250 nM RNA was used in each reaction and recombinantly purified dMi-2 was titrated from 0.1 to 1.5 μ M. RNA without protein was loaded in lanes 1 and 11, respectively. Representative image of three independent biological replicates is shown. **(D)** Quantification of the EMSA shown in (C). The fraction of the RNA bound by dMi-2 is plotted against the concentration of dMi-2. Data points from three independent biological replicates are shown. The apparent dissociation constant (app. K_d) indicated in (D) and (F), were derived with Hill slope equation using GraphPad Prism software. **(E)** EMSA of corresponding 100 bp double stranded RNA (dsRNA) and double stranded DNA (dsDNA) fragments derived from Hsp70Aa gene. 250 nM dsRNA or dsDNA was used for each reaction and dMi-2 was titrated from 0.1 to 1.5 μ M. dsRNA and dsDNA controls without protein were loaded in lanes 1 and 11, respectively. Representative image of two independent biological replicates. **(F)** Quantification of the EMSA shown in (E). Data points from two independent biological replicates are shown. The gels were stained with SYBR Gold.

Figure 4: dMi-2 preferentially binds G-rich RNAs

(A) KpLogo sequence plot indicating enrichment of G-nucleotides at the crosslinking site and possible preferential binding to 5'- GGNNAC-3' sequence. Nine nucleotides up- and downstream (-9, +9) of the crosslinking site (0) are plotted. The size of nucleotide letters represents the enrichment as (p-value) above expected frequency sampled from random mRNA sequences. Crosslinking to uridine nucleotides is favoured due to use of UV-C irradiation. Therefore, the higher frequency of U-nucleotides is disregarded. The second most frequently crosslinked nucleotide is guanine. **(B)** Quantification of EMSAs of homopolymer RNAs Poly (A) (blue), Poly (U) (green), Poly (C) (red) and Poly (G) (purple). Each RNA is 40 nucleotides in length. The apparent dissociation constant (app. K_d) was calculated using Hill Slope equation

with GraphPad Prism. Individual data points of three biological replicates are plotted. K_d values for Poly (C), Poly (A) and Poly (U) RNA could not be defined (n.d.). The K_d for Poly (G) is indicated. **(C)** Fluorescence polarization assays to determine the binding efficiency of recombinantly purified dMi-2 with FAM-labelled (GA)₂₀ RNA (blue) and (CA)₂₀ RNA (red). 5 nM RNA was used in each reaction and dMi-2 titrated from 0.0001 μ M to 0.5 μ M. Fluorescence polarization was measured in triplicates. Triplicate data points from two independent biological replicates are plotted. The apparent K_d was calculated using Hill Slope equation with GraphPad Prism. **(D)** EMSAs showing the binding of dMi-2 to G-quadruplex forming (G₄A₄)₄ RNA (left panel) and to non-G-quadruplex forming (GA)₂₀ RNA (right panel). 250 nM RNA was used for each reaction and dMi-2 was titrated from 0.01 μ M (lanes 2) to 0.64 μ M (lanes 8). RNA without protein was loaded in lanes 1. Each EMSA is a representative figure of at least two independent biological replicates. The gels were stained with SYBR gold. **(F)** Quantification of EMSAs in (D). (G₄A₄)₄ RNA: blue and (GA)₂₀ RNA: red. App. K_d was determined by Hill Slope equation using GraphPad Prism. Individual data points of two independent biological replicates are plotted.

Figure 5: A novel RNA binding domain in the N-terminus of dMi-2

(A) Scheme representing wild type (WT) and truncation mutants of dMi-2. High mobility group box (HMG) (green), Plant homeodomains (PHD) (orange), chromodomains (CD) (black) and ATPase domain (blue) of dMi-2 are marked. Plus (+) and minus (-) signs on the right indicate the RNA binding properties of full length and truncation mutants with (+++) representing the best RNA binding. **(B)** EMSA of dMi-2 WT and truncation mutants. 250 nM of Hsp70Aa #6 RNA (see Figure 3A) was used for each reaction against 500 nM recombinantly purified proteins. RNA without protein was loaded as control in lane 1. The dMi-2-RNA complexes are shown by arrows on the left. **(C)** Prediction of intrinsically disordered regions (IDRs) in the N-terminal part of dMi-2. PONDR was used with optimal settings to predict IDRs. PONDR Score 1.0 (y-axis) is the highest score for the prediction of IDR while a PONDR Score of 0.0 indicates a well folded structure. **(D)** Scheme representing the truncation mutants of the dMi-2 N-terminal region. HMG-box domain is denoted in green. Plus (+) and minus (-) signs on the right indicate the RNA binding properties of the truncation mutants of dMi-2 with (+++) being the highest RNA binding. **(E)** EMSA of dMi-2 (1-117) (lane 2), (118-238) (lane 3) and (239-376) (lane 4). 250 nM Hsp70Aa #6 RNA (see Figure 3A) was used for each reaction with 1.5 μ M proteins. RNA without protein was loaded as control in lane 1. Arrows on the left show the dMi-2-RNA complexes.

Figure 6: G-rich RNA inhibits dMi-2- and CHD4-mediated nucleosome remodelling *in vitro*

(A) Remodelling inhibition of dMi-2 by RNA. Remodelling reactions were set up using 15 nM of the end-positioned mononucleosomes with 80 bp of flanking DNA in each experiment as denoted by plus sign, except in controls (minus signs on the top). End-positioned mononucleosomes, lanes 1 are represented on the left of each panel. 50 nM dMi-2 was added in each reaction (plus sign), except in controls (minus sign on the top). RNAs (G₄A₄)₄, (GA)₂₀ and (Hsp70Aa #6) were titrated from 0.1 to 1.2 μ M, as indicated. Remodelled nucleosome, represented on the left, can be observed in lanes 2-6 of (G₄A₄)₄ and (GA)₂₀ and lanes 2-10 in (Hsp70Aa #6). Reactions were loaded on native polyacrylamide gels, stained with SYBR gold stain. Representative images of at least three independent biological experiments. Bottom panel, quantification of remodelling assays performed in presence of increasing amounts of (G₄A₄)₄ RNA (blue), (GA)₂₀ RNA (red) and Hsp70Aa #6 RNA (green). The IC₅₀ values were calculated with non-linear regression using GraphPad Prism. Individual data points of two independent biological replicates are plotted. **(B)** Remodelling reactions of end-positioned mononucleosomes with 60 bp flanking DNA (0W60) with human CHD4 performed in presence of increasing amounts of (CA)₂₀ RNA (lanes 3-7) and (GA)₂₀ RNA (lanes 8-12). The un-remodelled and remodelled nucleosome positions are indicated on the left. Free DNA is indicated on left. For each reaction 100 nM nucleosomes and 10 nM CHD4, respectively, were used. RNA was titrated from 0.5 to 2 μ M. The nucleosomal DNA carried a 5'-Cy3 label, and the gel was imaged with a Typhoon FLA 9000 laser scanner. **(C) and (D)** Microscale thermophoresis (MST) assays showing competition between RNA and nucleosomes for binding to human CHD4. CHD4 was titrated against 25 nM Cy5-labelled nucleosome. Following 10 min incubation on ice, the samples were loaded into Monolith NT.115 instrument for MST measurement. Each binding curve is generated via GraphPad Prism and is a fit to at least three technical replicates (shown as individual dots).

Figure 7: RNA antagonizes NuRD and PRC2 to protect transcriptionally active chromatin environments.

Upper panel: In the absence of active transcription, NuRD and PRC2 associate with chromatin by binding to DNA bound repressors (R) or by direct interaction with nucleosomes. NuRD remodels nucleosomes to increase local nucleosome density and deacetylates nucleosomes (e.g. K27ac) to facilitate PRC2 mediated nucleosome methylation. PRC2 generates K27me3 marked nucleosomes and together with PRC1 establishes repressive

chromatin structures. Lower panel: During active transcription, NuRD and PRC2 are removed from chromatin by RNA. Nucleosome density and K27me3 can no longer be maintained. Activators (A) recruit histone acetyltransferases and demethylates (not shown) to remove repressive and add active histone modifications. As long as the region is actively transcribed NuRD and PRC2 cannot access chromatin. In addition, their remodelling and histone methylation activities are directly inhibited by RNA.

Supplementary Figure Legends

Supplementary Figure 1: dMi-2 associates with thousands of mRNAs *in vivo*

(A) iCLIP2 autoradiographs, replicate-1 (left panel) and replicate-2 (right panel). Same cell lines and treatment conditions as mentioned in Figure 1A. The red boxes indicate the regions of the membranes that were cut out for iCLIP2 sequencing library preparation. Molecular weight is indicated on the left in lane 1 of each replicate. **(B)** Distribution of iCLIP2 reads. dMi-2-GFP replicates-1 and -2 are indicated in orange and yellow, respectively. The control untagged-dMi-2 replicates-1 and -2 are indicated in green and violet, respectively. 23.5% of the reads, indicated in cyan, had no barcode and were not included in our downstream analysis. **(C)** Squared Pearson correlation coefficient (R^2) of the iCLIP2 data sets from replicate-1, on x-axis, and replicate-2, on y-axis. The $R^2 = 0.968$ indicates high correlation between the two replicates. **(D)** Western blot analysis of untagged control and GFP tagged cells lines. Input (IN) from each cell line was loaded in lanes 1, 4 and 7, respectively. Flow through (FT) was loaded in lanes 2, 5 and 8, respectively. GFP-beads (B) were loaded after GFP-immunoprecipitation in lanes 3, 6 and 9, respectively. Western blot membranes were probed with anti-GFP antibody. Anti -Tubulin antibody was used as a loading control. Molecular weights are indicated on the left. **(E)** fRIP-qPCR validation of dMi-2 associated RNAs indicated in Figure 1C. RIP-qPCRs were performed with extracts from control (blue), dLINT1-GFP- (black) and dMi-2-GFP- expressing (purple) cell lines. Percent of input is plotted. Individual data points of three independent biological replicates are shown.

Supplementary Figure 2: RNA binding does not play a major role in recruiting dMi-2 to chromatin

(A) Genes with iCLIP2 reads are aligned at the transcription end site (TES = 0). Crosslinking sites are indicated in blue. The cumulative crosslinking density for all genes is plotted on top. The grey shaded area from position -19 to -1 indicates a part of the transcript where reads are extremely unlikely to be mapped due to the minimum length of trimmed reads of 18 nucleotides. Crosslinks in this zone are mostly due to partial alignments containing a poly(A) signal, very rarely read-through events or false positive mappings. The colour code on the right indicates the number of supporting reads for each crosslinking site from 1 to ≥ 5 . **(B)** S2 cells were treated with 10 μ M triptolide (red) or an equal volume of DMSO (blue) for 3 hours. RNA was isolated from each sample and cDNA synthesized. qPCRs were performed using primers for the genes indicated. The expression of each gene was measured relative to DMSO control which was set to 1. Individual data points of three independent biological replicates are plotted as fold change. Error bars indicate standard deviation between three biological replicates.

Supplementary Figure 3: dMi-2 has broad RNA binding activity *in vitro*

(A) Immunopurification of FLAG tagged dMi-2 protein. 15 μ l of eluted dMi-2 (lane 4) was subjected to SDS PAGE along with 0.1% input (14.4 μ g) and 14.4 μ g of flow through (lanes 2 and 3, respectively). The gel was stained with Coomassie Brilliant Blue. Molecular weight marker was loaded in lane 1. **(B)** 150 ng of RNAs derived from Hsp70Aa and Pgant35a genes (Figure 3A) were separated on urea denaturing polyacrylamide gels (lanes 1-4 and 5-8, respectively) and visualized with SYBR gold staining. **(C)** #3 RNAs (100-mer ssRNAs) from Hsp70Aa and Pgant35a along with ssRNA size marker are loaded on urea denaturing polyacrylamide gels. **(D)** 100 bp dsRNA and DNA generated from Hsp70Aa gene (lanes 1 and 2, respectively) were loaded on a native polyacrylamide gel and electrophoresed. **(E)** To verify the double stranded nature of dsRNA, it was treated with RNase III (lane 4) which specifically cuts dsRNA. Hsp70Aa #3 RNA, a ssRNA of equal length, was used as a control (lane 3). Untreated controls were loaded in lanes 1 and 2. The specific degradation of dsRNA upon RNase III treatment (lane 4), confirms the double stranded nature of dsRNA. **(F)** EMSA showing binding of dMi-2 to RNA in increasing salt conditions. 250 nM of Hsp70Aa #6 RNA (Figure 3A) and 500 nM of recombinantly purified dMi-2 was used for each reaction. RNA without protein was loaded in lane 1 as control. dMi-2-RNA complex is indicated on the left. **(G)** EMSA showing binding of dMi-2 to RNA in increasing NP40 concentrations. 50 nM of Hsp70Aa #3 RNA and 173 nM of dMi-2 was used for each reaction. No NP40 was added in

lanes 1 and 2. Lane 3 was left empty. EMSA samples were loaded on native polyacrylamide gels and stained with SYBR gold.

Supplementary Figure 4: dMi-2 preferentially binds G-rich RNAs

(A-D) EMSAs of Poly (A), (C), (U) and (G) RNAs, each 40-mer in length. 250 nM RNA was used in each reaction and dMi-2 was titrated from 0.01 to 0.9 μ M. Samples were loaded on native polyacrylamide gels. The gels were stained with SYBR gold. **(E-G)** Circular dichroism (CD) spectroscopy of Poly (G) RNA, (G₄A₄)₄ and (GA)₂₀ RNA, each 40-mer in length. CD spectra were measured at temperatures as indicated and between 220 to 300 nm. The CD values were analysed and plotted by CAPITO CD analysis and plotting software and are shown as mean residue ellipticity (θ in grad cm² dmol⁻¹). Poly (G) and (G₄A₄)₄ RNAs form characteristic G-quadruplex absorbance pattern with negative peaks between 237-241 nm and positive peaks between 260-265 nm. At least 3 measurements were taken at each temperature and mean values plotted. The red lines in (F) and (G) denote the zero (0) value on Y-axis.

Supplementary Figure 5: A novel RNA binding domain in the N-terminus of dMi-2

FLAG-tagged dMi-2 Wild type (WT) and its truncation mutants were purified by FLAG immunopurification. The protein samples were loaded on SDS gels in **(A)**, **(C)**, **(E)** and **(G)**. Molecular weight markers were loaded in lane 1 for each gel. The gels were stained with Coomassie Brilliant Blue. Additional EMSAs of dMi-2 WT and its truncation mutants in **(B)**, **(D)**, **(F)** and **(H)**. In **(B)**, 50 ng of Hsp70Aa #3 RNA (Figure 3A) was used for each reaction and 173 ng each of dMi-2 WT (lane 2), dMi-2 Δ N (lane 4), dMi-2 Δ C (lane 6) and dMi-2 (691-1271) (lane 8). In **(D)**, 250 nM Hsp70Aa #6 RNA was used for each reaction with titrations of dMi-2 WT or dMi-2 Δ 376, as indicated. In **(F)**, 250 nM Hsp70Aa #6 RNA was used in each reaction and dMi-2 (1-376), lanes 2-4 or dMi-2 (377-690), lanes 5-7, were titrated from 0.5 to 2 μ M. In **(H)**, 250 nM Hsp70Aa #6 RNA was used in each reaction and 1.5 μ M protein each from dMi-2 (1-117) lane 2, (118-238) lane 3 and (239-376) lane 4. RNA without protein was loaded for each EMSA in lane 1. The gels were stained with SYBR gold.

Supplementary Figure 6: G-rich RNA inhibits dMi-2- and CHD4-mediated nucleosome remodelling *in vitro*

EMSA of **(A)** $(G_4A_4)_4$ RNA, **(B)** $(GA)_{20}$ RNA and **(C)** Hsp70Aa #6 RNA. 250 nM RNA was used in each reaction and dMi-2 titrated from 10 to 640 nM, lanes 2-8, each. The gels were stained with SYBR gold. RNA without protein was loaded in lanes 1 and denoted on the left. Representative images of at least two independent biological experiments. **(D)** Quantification of the EMSAs shown in (A-C). Apparent dissociation constant (app. K_d) was calculated using Hill Slope equation (Blue: $(G_4A_4)_4$ RNA, Red: $(GA)_{20}$ RNA and Green: Hsp70Aa #6 RNA). Individual data points of at least two independent biological replicates are plotted. Nucleosome remodelling assays with titrations of different RNAs **(E)** $(G_4A_4)_4$ RNA, **(F)** $(GA)_{20}$ RNA and **(G)** Hsp70Aa #6 RNA. 15 nM of the end-positioned mononucleosomes with 80 bp of flanking DNA were used in each experiment as denoted by plus sign, except in controls (minus signs on the top). End-positioned mononucleosomes, lanes 1, represented on the left. 50 nM dMi-2 was added in each reaction (plus sign), except in controls (minus sign on the top). Remodelled nucleosome (represented on the left, can be observed in lanes 2-6 of (E) and (F) and lanes 2-10 in (G)). Free RNA in each gel can be observed at the bottom. Reactions were loaded on native polyacrylamide gels, stained with SYBR gold stain. Red arrows point out the remodelled nucleosome band and red asterisk marks in lanes 7-10 of (E) and (F) point out the inhibition of remodelling. Blue asterisk mark in lane 10 of (G) points out remodelled nucleosome even at the highest concentration of Hsp70Aa #6 RNA. Representative images of at least three independent biological experiments. **(H)** Schematic representation of the end-positioned mononucleosomes (0w60) used in Real Time Fluorescence Assay (RTFA). The histone octamer is labelled with a fluorophore AF488 (in green). The nucleosomal DNA is end-labelled with a fluorescence quencher BHQ1 (in black). When the nucleosome is end-positioned, the quencher remains in close proximity to the fluorophore and no FRET signal is detected (left panel). When CHD4, in presence of ATP, remodels the nucleosome, the octamer is moved away from the quencher and the fluorescence is detected (right panel). **(I)** RTFA, as detailed in (H), was used to measure the inhibition of remodelling function of CHD4 in presence of different amounts of $(GA)_{20}$ RNA, 0.5 μ M (green), 1.0 μ M (purple) and 2.0 μ M (orange). RTFA signal in nucleosome only (blue) and in the absence of RNA (red) was measured as controls. RTFA signals were measured at 37 °C for 30 minutes (x-axis), with ATP being injected at 5 min time point. Averaged data from three experiments was plotted.

Table 1: dMi-2-GFP iCLIP-seq list of enriched mRNAs with Fold change > 4 and ($p < 0.05$)

FlyBase Gene Report	name	log2FC
FBgn0053868	<i>His2B:CG33868</i>	10.8002796376212
FBgn0052580	<i>Muc14A</i>	9.45703266969185
FBgn0052320	<i>CG32320</i>	7.98431559774432
FBgn0037811	<i>CG12592</i>	7.34205351830939
FBgn0261836	<i>Msp300</i>	6.90512108738892
FBgn0033141	<i>CG12831</i>	6.83376678488231
FBgn0259110	<i>mmd</i>	6.48009829238807
FBgn0034126	<i>jtb</i>	6.31564321070758
FBgn0003016	<i>osp</i>	6.15032241570444
FBgn0267033	<i>mamo</i>	6.13947123092771
FBgn0013275	<i>Hsp70Aa</i>	6.01423597289303
FBgn0042131	<i>CG18808</i>	5.82723197292279
FBgn0285926	<i>Imp</i>	5.51261175360771
FBgn0030914	<i>CG6106</i>	5.35333678436727
FBgn0042627	<i>FASN2</i>	5.34698447517476
FBgn0032683	<i>kon</i>	5.33454638168472
FBgn0026206	<i>mei-P26</i>	5.28106560202889
FBgn0016977	<i>spen</i>	5.12465549706164
FBgn0265487	<i>mbl</i>	5.09354674179842
FBgn0284408	<i>trol</i>	5.02973784581865
FBgn0016976	<i>stnA</i>	5.02202858797867
FBgn0016975	<i>stnB</i>	5.02202858797867
FBgn0034140	<i>Lst</i>	5.01429266653495
FBgn0264490	<i>Eip93F</i>	5.01429266653495
FBgn0010113	<i>hdc</i>	4.82922645855303
FBgn0038826	<i>Syp</i>	4.80554742361066
FBgn0004652	<i>fru</i>	4.73165515675901
FBgn0040765	<i>luna</i>	4.70726440615755
FBgn0053554	<i>Nipped-A</i>	4.62241953495861
FBgn0003165	<i>pum</i>	4.58493032464889
FBgn0266696	<i>Svil</i>	4.56984780051999
FBgn0261552	<i>ps</i>	4.54412038731827

FBgn0086758	<i>chinmo</i>	4.50048111975893
FBgn0052113	<i>Vps13D</i>	4.4066220711289
FBgn0013733	<i>shot</i>	4.3883913027958
FBgn0027339	<i>jim</i>	4.38457515666963
FBgn0004656	<i>fs(1)h</i>	4.3649173105306
FBgn0032136	<i>Apoltp</i>	4.36486760035777
FBgn0283451	<i>br</i>	4.33824319934438
FBgn0033661	<i>CG13185</i>	4.32920287048232
FBgn0011230	<i>poe</i>	4.28356352372469
FBgn0261574	<i>kug</i>	4.2827128208743
FBgn0262656	<i>Myc</i>	4.27141752469343
FBgn0260400	<i>elav</i>	4.23457122505278
FBgn0000479	<i>dnc</i>	4.20062776178223
FBgn0265523	<i>Smr</i>	4.18250497919764
FBgn0036851	<i>CG14082</i>	4.17569410266339
FBgn0052296	<i>Mrtf</i>	4.16936958085499
FBgn0025741	<i>PlexA</i>	4.12430657847216
FBgn0010300	<i>brat</i>	4.07380164125748
FBgn0036398	<i>upSET</i>	4.06134713863072
FBgn0261793	<i>Trf2</i>	4.0403012026309
FBgn0261797	<i>Dhc64C</i>	4.03134331769893
FBgn0265045	<i>Strn-Mlck</i>	3.96752078846758
FBgn0039959	<i>CG17514</i>	3.95073446887442
FBgn0264975	<i>Nrg</i>	3.94381527946911
FBgn0030674	<i>CG8184</i>	3.90684931717943
FBgn0283521	<i>lola</i>	3.88370229293706
FBgn0035142	<i>Hipk</i>	3.88267307312318
FBgn0085430	<i>CG34401</i>	3.88106441668734
FBgn0261617	<i>nej</i>	3.87889444131911
FBgn0052767	<i>CG32767</i>	3.877811390303
FBgn0011666	<i>msi</i>	3.85600196686996
FBgn0260003	<i>Dys</i>	3.8502939063812
FBgn0283499	<i>InR</i>	3.84300467611203
FBgn0263396	<i>sqd</i>	3.84026621842002
FBgn0259170	<i>alpha-Man-Ia</i>	3.8302661807562

FBgn0259789	<i>zld</i>	3.82192351680268
FBgn0266557	<i>kis</i>	3.82151639555282
FBgn0031107	<i>HERC2</i>	3.81224333164984
FBgn0002526	<i>LanA</i>	3.805099444757
FBgn0035424	<i>Larp4B</i>	3.8021684823626
FBgn0085407	<i>Pvf3</i>	3.78230261448468
FBgn0026869	<i>Thd1</i>	3.76329605650254
FBgn0011224	<i>heph</i>	3.75833576673696
FBgn0085446	<i>CG34417</i>	3.74416715758126
FBgn0004435	<i>Galphaq</i>	3.73700919148138
FBgn0259176	<i>bun</i>	3.7325444173771
FBgn0052479	<i>Usp10</i>	3.7106283740648
FBgn0029979	<i>mahe</i>	3.70083745707653
FBgn0052529	<i>Hers</i>	3.69450431101918
FBgn0028397	<i>Tob</i>	3.68980501695715
FBgn0016694	<i>Pdpl</i>	3.68476402351277
FBgn0285917	<i>sbb</i>	3.67453804230069
FBgn0261802	<i>CG42748</i>	3.67074461112003
FBgn0043884	<i>mask</i>	3.66411181002783
FBgn0000546	<i>EcR</i>	3.66042646641071
FBgn0050069	<i>CG30069</i>	3.64908467905196
FBgn0040297	<i>Nhe2</i>	3.64055360918131
FBgn0010905	<i>Spn</i>	3.6394433747121
FBgn0263352	<i>Unr</i>	3.63765257248846
FBgn0000568	<i>Eip75B</i>	3.63389715711136
FBgn0040752	<i>Prosap</i>	3.63019268796045
FBgn0002945	<i>nkd</i>	3.62847814774335
FBgn0267912	<i>CanA-14F</i>	3.62012387182123
FBgn0053100	<i>eIF4EHP</i>	3.61374655497384
FBgn0085478	<i>CG34449</i>	3.59778213391869
FBgn0000567	<i>Eip74EF</i>	3.58569829686856
FBgn0032600	<i>BuGZ</i>	3.58098124511512
FBgn0004607	<i>zfh2</i>	3.5682779897614
FBgn0003862	<i>trx</i>	3.56813599914016
FBgn0263289	<i>scrib</i>	3.56332797796981

FBgn0264707	<i>RhoGEF3</i>	3.55815203137006
FBgn0283657	<i>Tlk</i>	3.55695406810382
FBgn0267336	<i>Glut4EF</i>	3.53084643846658
FBgn0023479	<i>teq</i>	3.52818050338941
FBgn0036165	<i>chrb</i>	3.52163484398033
FBgn0260634	<i>eIF4G2</i>	3.52128170385548
FBgn0286778	<i>CG46385</i>	3.5142655680346
FBgn0259745	<i>wech</i>	3.49010643440631
FBgn0029114	<i>Tollo</i>	3.48130830860426
FBgn0086674	<i>Tpst</i>	3.47650915420401
FBgn0004198	<i>ct</i>	3.47225869320717
FBgn0051998	<i>CG31998</i>	3.46989323416501
FBgn0053087	<i>LRP1</i>	3.45453633687723
FBgn0262160	<i>CG9932</i>	3.45092768873571
FBgn0015269	<i>Nfl</i>	3.44876936407616
FBgn0001122	<i>Galphao</i>	3.44845291492252
FBgn0266717	<i>Bruce</i>	3.44492005125665
FBgn0250754	<i>CG42232</i>	3.44320665275096
FBgn0052423	<i>shep</i>	3.44319538672698
FBgn0033159	<i>Dscaml</i>	3.44183985016598
FBgn0261934	<i>dikar</i>	3.43707054163435
FBgn0052133	<i>Ptip</i>	3.42728351637155
FBgn0039920	<i>CG11360</i>	3.42007160617459
FBgn0039928	<i>Cals</i>	3.4145470485376
FBgn0000635	<i>Fas2</i>	3.40403417781517
FBgn0266801	<i>CG45263</i>	3.39219887597165
FBgn0002643	<i>mam</i>	3.38968029893869
FBgn0026086	<i>Adar</i>	3.38883409311273
FBgn0036004	<i>Jarid2</i>	3.38308793052713
FBgn0004395	<i>unk</i>	3.38295557036386
FBgn0261671	<i>tweek</i>	3.38226224328963
FBgn0085434	<i>NaCP60E</i>	3.38199769231437
FBgn0261549	<i>rdgA</i>	3.38199769231437
FBgn0003415	<i>skd</i>	3.37900372704959
FBgn0041092	<i>tai</i>	3.37010573765372

FBgn0033010	<i>Atf6</i>	3.35706130141735
FBgn0014388	<i>sty</i>	3.35458588850229
FBgn0286516	<i>aqz</i>	3.35292354750985
FBgn0004167	<i>kst</i>	3.35050831229279
FBgn0004449	<i>Ten-m</i>	3.34606086706945
FBgn0261822	<i>Bsg</i>	3.34348060293133
FBgn0260794	<i>ctrip</i>	3.34281364883831
FBgn0025740	<i>PlexB</i>	3.33426992284789
FBgn0264953	<i>Piezo</i>	3.33052226811085
FBgn0262737	<i>mub</i>	3.32572560063108
FBgn0003391	<i>shg</i>	3.32448490211995
FBgn0029504	<i>CHES-1-like</i>	3.30768140409356
FBgn0263490	<i>mld</i>	3.30414734457373
FBgn0002673	<i>twe</i>	3.30305909078457
FBgn0031698	<i>Ncoa6</i>	3.29128342704834
FBgn0021764	<i>sdk</i>	3.28901098269
FBgn0086686	<i>l(3)L1231</i>	3.2646294718284
FBgn0001078	<i>ftz-fl</i>	3.26417477578184
FBgn0259735	<i>mtgo</i>	3.2561152947444
FBgn0039633	<i>CG11873</i>	3.25499268303909
FBgn0052062	<i>Rbfox1</i>	3.24925612910232
FBgn0262739	<i>AGO1</i>	3.24587077721261
FBgn0000163	<i>baz</i>	3.24020373967179
FBgn0262743	<i>Fs(2)Ket</i>	3.23950100186323
FBgn0040388	<i>boi</i>	3.23656496660836
FBgn0014163	<i>fax</i>	3.23627305400339
FBgn0039209	<i>REPTOR</i>	3.22224426178399
FBgn0004396	<i>CrebA</i>	3.22144748849293
FBgn0033636	<i>tou</i>	3.21037135373029
FBgn0039831	<i>CG12054</i>	3.20807220954901
FBgn0263929	<i>jvl</i>	3.20234815023948
FBgn0029676	<i>HIP-R</i>	3.19393183019868
FBgn0262975	<i>cnc</i>	3.18485371316302
FBgn0265623	<i>Su(z)2</i>	3.1733913193136
FBgn0039727	<i>Vps13B</i>	3.16963220685496

FBgn0259168	<i>mnb</i>	3.16503976795187
FBgn0085436	<i>Not1</i>	3.1527052537297
FBgn0000541	<i>E(bx)</i>	3.13447764610696
FBgn0261244	<i>inaE</i>	3.13102301524477
FBgn0261811	<i>pico</i>	3.12093344475092
FBgn0032633	<i>Lrch</i>	3.11701059560866
FBgn0003301	<i>rut</i>	3.10769586102493
FBgn0263706	<i>CG43658</i>	3.10471562946762
FBgn0030243	<i>CG2186</i>	3.10095665296189
FBgn0003525	<i>stg</i>	3.09940269412615
FBgn0260965	<i>CG42588</i>	3.09869818486504
FBgn0262582	<i>cic</i>	3.09322351281376
FBgn0020306	<i>dom</i>	3.09115766849551
FBgn0030266	<i>CG11122</i>	3.08189061616957
FBgn0039590	<i>CG10011</i>	3.08029928355784
FBgn0085423	<i>GramD1B</i>	3.07473982893751
FBgn0003137	<i>Ppn</i>	3.0723859502579
FBgn0264307	<i>orb2</i>	3.06586608908757
FBgn0263346	<i>smash</i>	3.06506306903183
FBgn0025936	<i>Eph</i>	3.04927954974251
FBgn0004227	<i>nonA</i>	3.03510370990698
FBgn0260486	<i>Ziz</i>	3.03464784352583
FBgn0264324	<i>spg</i>	3.03091887610756
FBgn0003048	<i>pcx</i>	3.03042550216857
FBgn0266580	<i>Gp210</i>	3.0175721334852
FBgn0026083	<i>tyf</i>	3.01615631438175
FBgn0052000	<i>anne</i>	3.01385864470053
FBgn0011817	<i>nmo</i>	3.0126332727389
FBgn0036735	<i>Edc3</i>	3.00039518386434
FBgn0262614	<i>pyd</i>	2.99965147976344
FBgn0003870	<i>ttk</i>	2.99758069839968
FBgn0036374	<i>Spt20</i>	2.99248434088424
FBgn0011656	<i>Mef2</i>	2.98779671394399
FBgn0002645	<i>Map205</i>	2.98167777396056
FBgn0259214	<i>PMCA</i>	2.9809277190444

FBgn0003371	<i>sgg</i>	2.97996051170067
FBgn0011837	<i>Tis11</i>	2.97487211307862
FBgn0026427	<i>Su(var)2-HP2</i>	2.97432374037429
FBgn0031738	<i>CG9171</i>	2.97369016149345
FBgn0261854	<i>aPKC</i>	2.96540913913289
FBgn0032817	<i>CG10631</i>	2.95539765239398
FBgn0031118	<i>RhoGAP19D</i>	2.95527648174142
FBgn0004876	<i>cdi</i>	2.94939203997566
FBgn0039466	<i>CG5521</i>	2.94410455065222
FBgn0000382	<i>csw</i>	2.93670978762071
FBgn0036446	<i>CG9384</i>	2.93605771520376
FBgn0260934	<i>par-1</i>	2.93306625401769
FBgn0030766	<i>mthl1</i>	2.93061247024479
FBgn0263987	<i>spoon</i>	2.92717972704771
FBgn0020224	<i>Cbl</i>	2.91662054381818
FBgn0036746	<i>Crtc</i>	2.90537352750203
FBgn0035106	<i>rno</i>	2.90460279384508
FBgn0010051	<i>Itp-r83A</i>	2.90273704447419
FBgn0086655	<i>jing</i>	2.90201761594318
FBgn0022764	<i>Sin3A</i>	2.8861920193383
FBgn0261444	<i>CG3638</i>	2.8827488548919
FBgn0261285	<i>Ppcs</i>	2.87971687010065
FBgn0262730	<i>dtn</i>	2.87812818239281
FBgn0001297	<i>kay</i>	2.86859479846172
FBgn0262617	<i>Nuak1</i>	2.86490291641935
FBgn0263006	<i>SERCA</i>	2.86423609190985
FBgn0051635	<i>CG31635</i>	2.86403681268674
FBgn0259212	<i>cno</i>	2.86154470129852
FBgn0005624	<i>Psc</i>	2.85883324097677
FBgn0039214	<i>puf</i>	2.8502109345035
FBgn0053208	<i>Mical</i>	2.84891808140212
FBgn0003028	<i>ovo</i>	2.83065947875137
FBgn0025726	<i>unc-13</i>	2.82077350836129
FBgn0002781	<i>mod(mdg4)</i>	2.81813406152301
FBgn0003345	<i>sd</i>	2.81601978946513

FBgn0029903	<i>pod1</i>	2.81547257223379
FBgn0085370	<i>Pde11</i>	2.81536571581785
FBgn0085412	<i>CG34383</i>	2.80792030918454
FBgn0015589	<i>Apc</i>	2.80451313895737
FBgn0259171	<i>Pde9</i>	2.79648308869151
FBgn0265998	<i>Doa</i>	2.79605604571792
FBgn0030512	<i>Nadsyn</i>	2.78841435848043
FBgn0034570	<i>CG10543</i>	2.78162934907266
FBgn0260442	<i>rhea</i>	2.77761946673531
FBgn0005631	<i>robo1</i>	2.7769636097824
FBgn0264493	<i>rdx</i>	2.77626319561565
FBgn0264495	<i>gpp</i>	2.77403839959632
FBgn0036518	<i>RhoGAP71E</i>	2.76869941727135
FBgn0051224	<i>CG31224</i>	2.76448256044201
FBgn0000581	<i>E(Pc)</i>	2.76283691839791
FBgn0034240	<i>MESR4</i>	2.76240177983413
FBgn0263993	<i>CG43736</i>	2.75994931351646
FBgn0001169	<i>H</i>	2.75265183573361
FBgn0263392	<i>Tet</i>	2.74889286482095
FBgn0036844	<i>Mkp3</i>	2.74178206088994
FBgn0262579	<i>Ect4</i>	2.74164315594264
FBgn0036576	<i>CG5151</i>	2.74139315638568
FBgn0262103	<i>Sik3</i>	2.73460943545281
FBgn0024921	<i>Tnpo</i>	2.73401091114471
FBgn0261397	<i>didum</i>	2.73136070132917
FBgn0010382	<i>CycE</i>	2.72974163868659
FBgn0003507	<i>srp</i>	2.72093330075867
FBgn0286831	<i>sov</i>	2.71996880851096
FBgn0024734	<i>PRL-1</i>	2.71857630468388
FBgn0004370	<i>Ptp10D</i>	2.71685556126891
FBgn0004838	<i>Hrb27C</i>	2.71502169320622
FBgn0037705	<i>mura</i>	2.71442075186685
FBgn0003502	<i>Btk29A</i>	2.70728325553527
FBgn0259984	<i>kuz</i>	2.70218573454958
FBgn0037902	<i>CG5281</i>	2.6960386883981

FBgn0265988	<i>mv</i>	2.69549738552988
FBgn0020412	<i>JIL-1</i>	2.693800416969
FBgn0285955	<i>cv-c</i>	2.6917436091765
FBgn0036734	<i>CG7564</i>	2.69087996488417
FBgn0040324	<i>Ephrin</i>	2.6896366929364
FBgn0023458	<i>Rbcn-3A</i>	2.6869344999041
FBgn0266019	<i>rudhira</i>	2.6860473218105
FBgn0267861	<i>Maf1</i>	2.68523612828502
FBgn0016070	<i>smg</i>	2.68350823790166
FBgn0000119	<i>arr</i>	2.68297840408335
FBgn0061200	<i>Nup153</i>	2.6738236003526
FBgn0000578	<i>ena</i>	2.6667129227645
FBgn0260970	<i>Ubr3</i>	2.66594800603171
FBgn0266084	<i>Fhos</i>	2.66511672835558
FBgn0020251	<i>sfl</i>	2.66403695852226
FBgn0263102	<i>psq</i>	2.6584051364151
FBgn0011818	<i>oaf</i>	2.65667401747727
FBgn0024555	<i>flfl</i>	2.65545777199525
FBgn0031150	<i>bves</i>	2.652465254001
FBgn0086690	<i>Plp</i>	2.65177408603601
FBgn0034734	<i>CG4554</i>	2.64743996853497
FBgn0259927	<i>CG42450</i>	2.64512369157988
FBgn0000247	<i>ca</i>	2.63746346130591
FBgn0023407	<i>B4</i>	2.63696519188326
FBgn0003483	<i>spn-E</i>	2.63650998004523
FBgn0265974	<i>ttv</i>	2.63536274112336
FBgn0026179	<i>siz</i>	2.6305123740467
FBgn0263131	<i>CG43373</i>	2.62989293314375
FBgn0266410	<i>CG45050</i>	2.62362727460374
FBgn0278608	<i>Dsp1</i>	2.62117296723614
FBgn0052654	<i>Sec16</i>	2.61928641494117
FBgn0030049	<i>Trf4-1</i>	2.6155556000095
FBgn0003416	<i>sl</i>	2.61534764521833
FBgn0000464	<i>Lar</i>	2.61092808278158
FBgn0003117	<i>pnr</i>	2.60859696767451

FBgn0004648	<i>svr</i>	2.60239052390867
FBgn0261710	<i>nocte</i>	2.59794022696811
FBgn0023213	<i>eIF4G1</i>	2.59232114877232
FBgn0085450	<i>Snoo</i>	2.59080276135639
FBgn0262733	<i>Src64B</i>	2.59059455650457
FBgn0041585	<i>olf186-F</i>	2.59048197961029
FBgn0031981	<i>Megf8</i>	2.58720184872033
FBgn0039419	<i>CG12290</i>	2.58600056303399
FBgn0010825	<i>Gug</i>	2.58300976952829
FBgn0265630	<i>sno</i>	2.58110539895081
FBgn0038197	<i>foxo</i>	2.58101111516715
FBgn0002905	<i>mus308</i>	2.57910465099039
FBgn0051320	<i>HEATR2</i>	2.57883017429885
FBgn0015778	<i>rin</i>	2.56931396354219
FBgn0005640	<i>Eip63E</i>	2.5585194868648
FBgn0285892	<i>tea</i>	2.55783249557159
FBgn0015396	<i>jumu</i>	2.55534492596839
FBgn0001624	<i>dlg1</i>	2.55505073146627
FBgn0013343	<i>Syx1A</i>	2.5546444663924
FBgn0028863	<i>CG4587</i>	2.55362847357971
FBgn0004509	<i>Furl</i>	2.55274572070552
FBgn0000017	<i>Abl</i>	2.54819289960723
FBgn0000719	<i>fog</i>	2.5471645343893
FBgn0003256	<i>rl</i>	2.54650205299112
FBgn0027492	<i>wdb</i>	2.54479447769425
FBgn0026376	<i>Rgl</i>	2.5408249384042
FBgn0023097	<i>bon</i>	2.53974131454693
FBgn0030505	<i>NFAT</i>	2.53535444246016
FBgn0020309	<i>crol</i>	2.53285460792943
FBgn0004875	<i>enc</i>	2.53037984556701
FBgn0011826	<i>Pp2B-14D</i>	2.52994717269263
FBgn0004369	<i>Ptp99A</i>	2.52718960839937
FBgn0011274	<i>Dif</i>	2.52622928276393
FBgn0086779	<i>step</i>	2.52336323716752
FBgn0039923	<i>MED26</i>	2.50990106139535

FBgn0039904	<i>Hcf</i>	2.50706206838391
FBgn0026401	<i>Nipped-B</i>	2.50278175970482
FBgn0000097	<i>aop</i>	2.50208135258897
FBgn0024277	<i>trio</i>	2.49594705243386
FBgn0087008	<i>e(y)3</i>	2.49484920682123
FBgn0013263	<i>Trl</i>	2.47943508634996
FBgn0260941	<i>app</i>	2.47812516625264
FBgn0032821	<i>CdGAPr</i>	2.47595732733249
FBgn0261556	<i>CG42674</i>	2.47264191444187
FBgn0026575	<i>hang</i>	2.46899307225253
FBgn0031126	<i>Cyp6v1</i>	2.46406990187567
FBgn0262473	<i>Tl</i>	2.46093927376994
FBgn0033194	<i>Vps13</i>	2.45921579032316
FBgn0015772	<i>Nak</i>	2.45919160179705
FBgn0263395	<i>hppy</i>	2.45772707196989
FBgn0032395	<i>Wdr81</i>	2.45759753557472
FBgn0261545	<i>CG42663</i>	2.45542236011173
FBgn0001105	<i>Gbeta13F</i>	2.4517895390429
FBgn0035400	<i>CG11537</i>	2.44217333855291
FBgn0036451	<i>CG9425</i>	2.44206538210106
FBgn0036522	<i>CG7372</i>	2.44041972376882
FBgn0023510	<i>Rbcn-3B</i>	2.44020782129427
FBgn0037021	<i>CG11399</i>	2.43649622489732
FBgn0037248	<i>srl</i>	2.43635760122446
FBgn0036621	<i>roq</i>	2.42955082149738
FBgn0004860	<i>ph-d</i>	2.42903756534114
FBgn0052350	<i>Vps11</i>	2.42896249587558
FBgn0041094	<i>scyl</i>	2.42661298409011
FBgn0011725	<i>twin</i>	2.42563498489733
FBgn0025802	<i>Sbf</i>	2.42397895015361
FBgn0265082	<i>Cdep</i>	2.41861685099219
FBgn0261873	<i>sdt</i>	2.41407758789775
FBgn0003447	<i>sn</i>	2.41249859207593
FBgn0010316	<i>dap</i>	2.41134446229863
FBgn0263112	<i>Mitf</i>	2.40780166769646

FBgn0039302	<i>Nup358</i>	2.40606703947924
FBgn0267964	<i>CG46244</i>	2.40404964375311
FBgn0002431	<i>hyd</i>	2.40281841637812
FBgn0015520	<i>nonA-1</i>	2.40191424018986
FBgn0037344	<i>CG2926</i>	2.39992487571929
FBgn0004655	<i>wapl</i>	2.39989089784983
FBgn0027603	<i>Ulp1</i>	2.39710915992238
FBgn0032223	<i>GATAd</i>	2.39634451649019
FBgn0016081	<i>fry</i>	2.39344457225599
FBgn0259243	<i>Pka-R1</i>	2.39232550308458
FBgn0000307	<i>chif</i>	2.39231308871872
FBgn0264491	<i>how</i>	2.39118665169054
FBgn0026375	<i>RhoGAPp190</i>	2.39102094244172
FBgn0001324	<i>kto</i>	2.38596539533345
FBgn0265042	<i>Irk1</i>	2.38297263091889
FBgn0000273	<i>Pka-C1</i>	2.37696218390045
FBgn0004390	<i>RasGAP1</i>	2.37486462758511
FBgn0030680	<i>CG8944</i>	2.37340714143728
FBgn0020386	<i>Pdk1</i>	2.3727985103154
FBgn0050183	<i>CG30183</i>	2.37121829783637
FBgn0003392	<i>shi</i>	2.36263125229868
FBgn0035397	<i>CG11486</i>	2.36177342549171
FBgn0036043	<i>CG8177</i>	2.35854757997247
FBgn0035959	<i>CG4911</i>	2.35836490466317
FBgn0262124	<i>uex</i>	2.35817837980896
FBgn0267795	<i>Frl</i>	2.3576825855114
FBgn0002873	<i>mud</i>	2.35656922771361
FBgn0003209	<i>raw</i>	2.35007959980049
FBgn0267348	<i>LanB2</i>	2.34985035375732
FBgn0004606	<i>zfh1</i>	2.34806397191747
FBgn0030809	<i>Ubr1</i>	2.34724074399706
FBgn0032796	<i>CG10188</i>	2.34437811370054
FBgn0284225	<i>CG46309</i>	2.34290978191281
FBgn0261954	<i>east</i>	2.33596010857812
FBgn0039431	<i>plum</i>	2.33094658374251

FBgn0283427	<i>FASN1</i>	2.33004623210842
FBgn0033504	<i>CAP</i>	2.32523646705443
FBgn0014141	<i>cher</i>	2.3219777816587
FBgn0264895	<i>RapGAP1</i>	2.31861998857016
FBgn0262734	<i>eIF4H1</i>	2.31825158632215
FBgn0036032	<i>CG16711</i>	2.31492141777987
FBgn0021796	<i>Tor</i>	2.31471774911897
FBgn0011481	<i>Ssdp</i>	2.31366884885179
FBgn0033155	<i>Br140</i>	2.30583949606892
FBgn0026147	<i>TTLL4A</i>	2.29747302803618
FBgn0031969	<i>pes</i>	2.29319594340789
FBgn0086451	<i>l(2)k09022</i>	2.28979262552197
FBgn0263232	<i>Nxf3</i>	2.28526622496391
FBgn0051158	<i>Efa6</i>	2.28422412767222
FBgn0032341	<i>Reps</i>	2.28127980299909
FBgn0243516	<i>Vrp1</i>	2.28127817852858
FBgn0036741	<i>anchor</i>	2.280969446207
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FBgn0053217	<i>CG33217</i>	2.27395155547263
FBgn0031759	<i>lid</i>	2.27321662528559
FBgn0032456	<i>MRP</i>	2.27044580631546
FBgn0266111	<i>ana3</i>	2.26939379175172
FBgn0031885	<i>Mnn1</i>	2.26813095649748
FBgn0030400	<i>Pits</i>	2.26460689042668
FBgn0003118	<i>pnt</i>	2.26258012116944
FBgn0037760	<i>FBXO11</i>	2.26117870867679
FBgn0052756	<i>CG32756</i>	2.26078840174657
FBgn0035842	<i>CG7504</i>	2.26078840174657
FBgn0052103	<i>SCaMC</i>	2.25863951748893
FBgn0026432	<i>Grip163</i>	2.25641233546752
FBgn0033802	<i>CG17724</i>	2.25459669574268
FBgn0284223	<i>CG46307</i>	2.24686457234522
FBgn0050015	<i>CG30015</i>	2.24442575141235
FBgn0042185	<i>MCU</i>	2.2427688595078
FBgn0029123	<i>SoxN</i>	2.23954047974951

FBgn0261383	<i>IntS6</i>	2.23657110238323
FBgn0031990	<i>PAPLA1</i>	2.23542383772161
FBgn0262740	<i>Evi5</i>	2.23438410598615
FBgn0030412	<i>Tomosyn</i>	2.23401869685696
FBgn0034087	<i>clu</i>	2.23187014960679
FBgn0264270	<i>Sxl</i>	2.22632554379118
FBgn0031187	<i>Usp2</i>	2.22394931577402
FBgn0259224	<i>CG42324</i>	2.22133349648103
FBgn0020257	<i>Ppa</i>	2.22077693249726
FBgn0028476	<i>Usp1</i>	2.21974854291646
FBgn0026189	<i>prominin-like</i>	2.2192287460897
FBgn0262872	<i>milt</i>	2.21691085967949
FBgn0034975	<i>enok</i>	2.21460531066863
FBgn0039944	<i>CG17162</i>	2.21287549675706
FBgn0015278	<i>Pi3K68D</i>	2.21213047307464
FBgn0038968	<i>CG12499</i>	2.21086051514842
FBgn0003463	<i>sog</i>	2.20832887634621
FBgn0000721	<i>for</i>	2.20778344343556
FBgn0031006	<i>rictor</i>	2.20585106788442
FBgn0003721	<i>Tm1</i>	2.20206271537516
FBgn0005632	<i>faf</i>	2.20135566073757
FBgn0042693	<i>wrd</i>	2.20078729456201
FBgn0034158	<i>CG5522</i>	2.19639281222816
FBgn0039141	<i>spas</i>	2.19538872246171
FBgn0034083	<i>lbk</i>	2.19295808676032
FBgn0015513	<i>mbc</i>	2.18909815396437
FBgn0036534	<i>DCP2</i>	2.18898764101792
FBgn0032938	<i>CG8671</i>	2.18733289497642
FBgn0025455	<i>CycT</i>	2.18493006626074
FBgn0004889	<i>twis</i>	2.18311973418013
FBgn0029687	<i>Vap33</i>	2.18231528793915
FBgn0031896	<i>CG4502</i>	2.17920385113533
FBgn0263391	<i>hts</i>	2.17870935704716
FBgn0000317	<i>ck</i>	2.17396869743185
FBgn0058002	<i>ND-AGGG</i>	2.17318212077496

FBgn0000524	<i>dx</i>	2.17129668624419
FBgn0003396	<i>shn</i>	2.17086724359754
FBgn0259228	<i>C3G</i>	2.17003674627658
FBgn0031952	<i>cdc14</i>	2.16993074893325
FBgn0005386	<i>ash1</i>	2.16962247455508
FBgn0035237	<i>CG13917</i>	2.16835868148038
FBgn0260938	<i>tay</i>	2.1658575456593
FBgn0026181	<i>Rok</i>	2.16565195875127
FBgn0015371	<i>chn</i>	2.16524386372645
FBgn0261885	<i>osa</i>	2.16491991225475
FBgn0004391	<i>shtd</i>	2.16353536538267
FBgn0283741	<i>prage</i>	2.1627287689669
FBgn0261239	<i>Hr39</i>	2.16198891111363
FBgn0029825	<i>CG12728</i>	2.16133510381566
FBgn0041111	<i>lilli</i>	2.16106057603673
FBgn0051992	<i>gw</i>	2.15474998721364
FBgn0038504	<i>Sur-8</i>	2.14635996182577
FBgn0058263	<i>MFS17</i>	2.14419470102632
FBgn0046692	<i>Stlk</i>	2.14380167504649
FBgn0032120	<i>CG33298</i>	2.13264937043225
FBgn0039528	<i>dsd</i>	2.13166722662859
FBgn0264607	<i>CaMKII</i>	2.13110556965004
FBgn0261570	<i>CG42684</i>	2.13109214969947
FBgn0050011	<i>gem</i>	2.13053274233609
FBgn0030812	<i>wcy</i>	2.13052392827129
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FBgn0011747	<i>Ank</i>	2.12513324330182
FBgn0027548	<i>nito</i>	2.1242793674139
FBgn0003892	<i>ptc</i>	2.12054722297233
FBgn0003277	<i>RplI215</i>	2.11968763870511
FBgn0035023	<i>ITP</i>	2.1190042754919
FBgn0085443	<i>spri</i>	2.11854207792077
FBgn0027948	<i>msps</i>	2.1181843969848
FBgn0039945	<i>CG17159</i>	2.1175895390335
FBgn0025866	<i>CalpB</i>	2.11711652863622

FBgn0032006	<i>Pvr</i>	2.11254030945481
FBgn0040230	<i>dbo</i>	2.11158906049993
FBgn0261618	<i>larp</i>	2.11058275262565
FBgn0284235	<i>CG46319</i>	2.11045849283883
FBgn0040010	<i>CG17493</i>	2.10794201754853
FBgn0026577	<i>CG8677</i>	2.1033120362932
FBgn0063485	<i>Lasp</i>	2.10208243781143
FBgn0264605	<i>CG43954</i>	2.10125365441681
FBgn0015286	<i>Rala</i>	2.096522405419
FBgn0003041	<i>pbl</i>	2.09056590123978
FBgn0265297	<i>pAbp</i>	2.08901635476039
FBgn0260789	<i>mxc</i>	2.08825587776901
FBgn0001168	<i>h</i>	2.0865058772932
FBgn0039907	<i>lgs</i>	2.08587200697008
FBgn0266487	<i>CG45086</i>	2.08542670851974
FBgn0035688	<i>fnt</i>	2.08494827868727
FBgn0032363	<i>Dlg5</i>	2.08342146918111
FBgn0031681	<i>Pgant5</i>	2.08308887874727
FBgn0265778	<i>PDZ-GEF</i>	2.07757110483131
FBgn0031030	<i>Tao</i>	2.0757835738441
FBgn0000183	<i>BicD</i>	2.07492374540185
FBgn0029881	<i>pigs</i>	2.07441618807715
FBgn0030344	<i>Nrd1</i>	2.0719067755965
FBgn0266450	<i>Kr-h1</i>	2.0716438763146
FBgn0033913	<i>CG8468</i>	2.07128296365492
FBgn0004101	<i>bs</i>	2.06937035205546
FBgn0022768	<i>Pp2C1</i>	2.06844328886748
FBgn0052850	<i>CG32850</i>	2.06370671723186
FBgn0014075	<i>Uggt</i>	2.06201824934049
FBgn0033569	<i>CG12942</i>	2.05910660703589
FBgn0261823	<i>Asx</i>	2.05775802305879
FBgn0013756	<i>Mtor</i>	2.05608880140724
FBgn0034046	<i>tun</i>	2.05606508771281
FBgn0263108	<i>BtbVII</i>	2.05567475858338
FBgn0001316	<i>klar</i>	2.05378415309378

FBgn0037240	<i>Cont</i>	2.05319027054982
FBgn0037720	<i>CG8312</i>	2.04760291043297
FBgn0002921	<i>Atpalpha</i>	2.04654126515635
FBgn0025633	<i>CG13366</i>	2.04524519260379
FBgn0032123	<i>Oatp30B</i>	2.04459278854709
FBgn0005617	<i>msl-1</i>	2.03751144023282
FBgn0016917	<i>Stat92E</i>	2.03560644896501
FBgn0037770	<i>Art4</i>	2.03212653592837
FBgn0003410	<i>sina</i>	2.02953402706872
FBgn0030466	<i>CG15744</i>	2.02715697745512
FBgn0264560	<i>garz</i>	2.02588702575747
FBgn0010309	<i>pigeon</i>	2.02031322334521
FBgn0034657	<i>LBR</i>	2.01628140677471
FBgn0011766	<i>E2f1</i>	2.01459943305984
FBgn0052676	<i>stx</i>	2.01354758724351
FBgn0002716	<i>mei-W68</i>	2.01141652315342
FBgn0037831	<i>Cap-H2</i>	2.00752263397315
FBgn0021760	<i>chb</i>	2.00709774689602
FBgn0033246	<i>ACC</i>	2.00592253890408
FBgn0026620	<i>tacc</i>	2.00397501700747
FBgn0014007	<i>Ptp69D</i>	2.00168898842332
FBgn0015477	<i>Rme-8</i>	2.00021075832301

Table 2: RNAs used in EMSAs

RNA	Sequence
Poly (A)	AAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAA
Poly (U)	UUUUUUUUUUUUUUUUUUUU UUUUUUUUUUUUUUUUUUUU
Poly (C)	CCCCCCCCCCCCCCCCCCCC CCCCCCCCCCCCCCCCCCCC
Poly (G)	GGGGGGGGGGGGGGGGGGGG GGGGGGGGGGGGGGGGGGGG
(G ₄ A ₄) ₄	AAAAAGGGGAAAAGGGGAAAAGGGGA AAAGGGGAAAAAA
(GA) ₂₀	GAGAGAGAGAGAGAGAGAGA GAGAGAGAGAGAGAGAGAGA
Hsp70Aa #1	CUCACACACAAUGCCUGCUAUUGGAAU CGAUCUGGGCACCACCUACUCCUGCGU GGGUGUCUACCAACAUGGCAAGGUGGA GAUUAUCGCCAACGACCAG
Hsp70Aa #2	GAGGACAUGAAGCACUGGCCUUUCAAG GUUGUAAGCGACGGCGGAAAGCCCAAG AUCGGGGUGGAGUAUAAGGGUGAGUC CAAGAGAUUUGCCCCCGAGG
Hsp70Aa #3	CCUACGGACUGGACAAGAACCUCAAGG GUGAGCGCAAUGUGCUUAUCUUCGACU UGGGCGGCGGCACCUUCGAUGUCUCC AUCCUGACCAUCGACGAGGG

Hsp70Aa #4	GCUGAUCGAGCGCAACUGCCGCAUUCC GUGCAAGCAGACUAAGACGUUCUCCAC AUACGCGGACAACCAGCCCGGAGUCUC CAUUCAGGUGUAUGAGGGC
Hsp70Aa #5	GCAGGCCGAGAUUGAUCGCAUGGUGAA CGAGGCUGAAAAGUACGCCGACGAGGA CGAGAAGCAUCGCCAGCGAAUAACCUC UAGAAAUGCCCUGGAGAGC
Hsp70Aa #6	CCUACGGACUGGACAAGAACCUCAAGG GUGAGCGCAAUGU
Hsp70Aa dsRNA	CCUACGGACUGGACAAGAACCUCAAGG GUGAGCGCAAUGUGCUUAUCUUCGACU UGGGCGGCGGCACCUUCGAUGUCUCC AUCCUGACCAUCGACGAGGG
Pgant35a #1	UCGGCAGGAUGGAGGAUCGACGAAGG GAACGCAACGCCACGGGCCGAGCUGAG CUACCAGGCCAGGGUCACCGUCGGAUG CACUCCAAAUGCCAGCAUUA
Pgant35a #2	UCUUCGGGGCGAUCCCAGACACUCGGC ACAAGGUGUGCGAUCGCCAGGAGACCA CGGAAGCGGAAAAUCUGCCCCAGGCCU CCAUAGUCAUGUGCUUCUA
Pgant35a #3	GUCAUUGGUGCACGCGAGGCCGUGGG CGAUGUGCUCGUCUCCUCGACUCGCA UAUCGAGGUCAAUCAGCAGUGGCUGGA GCCGCUGCUGCGCCUCAUCA

Pgant35a #4	CUGCAUGUGCCGGGCGAGGAGUCCAA GAAGUCGGCGGCAGCGCCCAUCUUUCA GCCUUGGCACUCGCGAAAACGUAACUA UGUCGACACAUUCCAGCUGC
Pgant35a #5	AAGUGCCACGAGAUGCUCGGCGAUCAG CAGUGGCGGCACACCCGCAACGCCAAC AGUCCCGUCUACAACAUGGCCAAAGGC ACCUGCCUCCGGGCAGCUG

Figure 1

Supplementary Figure 1

Figure 2

Supplementary Figure 2

Figure 3

Figure 3 continued

C

D

E

F

Supplementary Figure 3

Figure 4

Supplementary Figure 4

Figure 5

Supplementary Figure 5

Figure 6

Supplementary Figure 6

Supplementary Figure 6 continued

Supplementary Figure 6 continued

Figure 7