Piwi induces piRNA-guided transcriptional silencing and establishment of a repressive chromatin state

Supplementary Materials

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1 Supplementary Methods

1.1 High throughput data analysis

Except for where specifically specified otherwise, all data processing was carried out using custom-written python scripts. The dm3/BDGP assembly, release 5 version of the Drosophila melanogaster genome was used.

1.2 ChIP-seq and ChIP-seq data processing

Sequencing libraries were sequenced on the Illumina HiSeq 2000 (50bp reads). The resulting sequencing reads were trimmed down to 36bp and mapped against the genome using Bowtie 0.12.7 (Langemad et al., 2009) with the following settings: ''-v 2 --best --strata'', i.e. no mismatches and an unlimited number of locations to which a read can map to. Read mapping statistics for these libraries are presented in Supplementary Table 4.

1.3 Gene expression quantification using RNA-seq

RNA-seq libraries were built from polyA-selected RNA from fly ovaries following standard protocols (Mortazavi & Williams et al., 2008) and sequenced on the HiSeq 2000 (50bp reads). For the purposes of expression quantification, reads were mapped as 50mers, using TopHat 1.4.1 (Trapnell et al., 2009) and splice junctions from the ENSEMBL62 dm3 annotation with otherwise default settings. Gene expression was quantified in RPKMs/FPKMs (Reads/Fragments Per Kilobase per Million mapped reads/fragments) for the refSeq annotation (downloaded from the UCSC browser) with Cufflinks 2.0.2 (Trapnell et al., 2010, Trapnell et al., 2012). Read mapping statistics for these libraries are presented in Supplementary Table 4.

1.4 Repeat analysis

The usual practice when mapping ChIP-seq data is to retain only unique alignments as the ambiguity of the allocation of multimapper seriously confounds most analyses. In this study it was necessary to examine repeats but not absolutely necessary to properly allocate multimappers to each individual repeat. We therefore adopted the following two strategies for processing our ChIP-seq and RNA-seq data and examining ChIP enrichment over the expression of repeat elements:

1.4.1 Repeat analysis on RepeatMasker-annotated repeat elements

Both ChIP-seq and RNA-seq reads were trimmed down to the same length (36bp) and again aligned with Bowtie 0.12.7 against the dm3 genome but this time with the following options: "-v 0 -a --best --strata -q", i.e. no mismatches and an unlimited number of locations to which a read can map to. Read mapping statistics for these alignments are presented in Supplementary Table 5. For each read r, an integer multiplicity score NHr was defined (corresponding to the number of positions in the genome the read maps to) and for each individual instance of a repeated element RE (as defined in the RepeatMasker repeat element annotation downloaded from UCSC) an RPM score was calculated as follows:
\[
RPM_{RE} = \sum_{r \in RE} \frac{1}{NH_r}
\]

A combined repeat RPM score was calculated as the sum of the RPMs for each individual instance of that repeat:

\[
TotalRPM_{RE} = \sum_{RE} RPM_{RE}
\]

For RNA-seq data, repeat expression change was assessed as the RPM ratio between the shPiwi and shWhite libraries. For Pol II ChIP-seq data, an additional confounding factor exists as the differences in signal between two regions is the result of the combination of the actual change in occupancy and the difference in ChIP strength between the two experiments. We therefore used the total Pol II RPMs over transcription start sites in order to assess the difference in ChIP strength and derive a normalization factor to be used for rescaling of the repeat RPMs of one libraries so that they are comparable to those in the other (here, this factor turned out to be very close to 1).

1.4.2 Repeat analysis on consensus repeat sequences

An orthogonal strategy for the analysis of repeat occupancy and expression change that we employed was to map reads against consensus repeat sequences (obtained from FlyBase version FB2012.05 (McQuilton et al., 2012)). Reads were mapped with the following settings: "-v 3 -a --best --strata -q" (allowing for up to 3 mismatches and unlimited number of multimappers). Read mapping statistics for these alignments can be found in Supplementary Table 6. Read counts for each repeat were calculated (normalizing for multimapper multiplicity as described above) and normalized for sequencing depth against the total number of reads mappable to the genome (derived from the alignment without limits to read multiplicity discussed in the previous section) and finally, normalized for the length of the consensus sequences (RPKMs).

Results from both analyses were very similar and so only plots for RepeatMasker repetitive elements are shown.

1.5 Differential expression and occupancy analysis

In order to identify differentially expressed genes and transposons we used a combination of eXpress quantification (Roberts & Pachter, 2013) and DESeq (Anders & Huber, 2010) differential read count analysis. For each replicate, RNA-seq reads were aligned against the transcriptome and the quantification values for all transcripts belonging to the same gene were summed to derive gene-level quantifications. The “effective counts” values were used for downstream analysis. As only a minority of reads align to transposons, differential expression analysis only on transposons is not reliable. For this reason, we combined raw read counts for transposons (derived for the RepeatMasker annotation as described above or for the consensus sequences) with the eXpress quantifications on genes and ran DESeq to evaluate the statistical significance of the observed expression changes over the two shWhite and shPiwi replicates.

Differential occupancy of H3K9me3 was estimated as follows. First, the genome was divided into 1000bp bins and the H3K9me3 read count was estimated for each using the alignments generated with unlimited number of locations a read can map (dividing each alignment by the read multiplicity as discussed above). Next, DESeq was run on the H3K9me3 replicates to identify regions enriched or depleted upon Piwi knock down (p-value of 0.05 threshold was applied). Neighboring depleted regions were merged into contiguous clusters.

Pol II occupancy change over transposons was estimated from the combined RPM values for RepeatMasker transposons and from RPKM values for consensus transposons after taking into account that the difference in ChIP signal between two regions is the result of the combination of the actual change in occupancy and the difference in ChIP strength between the two experiments. We therefore used the total Pol II RPMs over TSSs in order to assess the difference in ChIP strength and derive a normalization factor to be used for rescaling of the repeat RPMs of libraries so that they are comparable to those in the other (this factor turned out to be close to 1 for both sets of replicates).
2 Supplementary References

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### 3 Supplementary Tables

**Supplementary Table 1:** List of genes significantly upregulated upon Piwi knockdown. Shown are the DESeq $log_2(Fold\text{Change})$ and p-values as calculated from two biological replica.

| Gene         | $log_2(fold\text{change})$ | p-value     |
|--------------|-----------------------------|-------------|
| CG14628      | Inf                         | 6.77E-10    |
| CG15056      | Inf                         | 8.05E-03    |
| CG18823      | Inf                         | 2.77E-02    |
| CG31054      | Inf                         | 3.28E-12    |
| CG4984       | Inf                         | 2.59E-02    |
| Sdic1        | Inf                         | 3.80E-02    |
| yellow-c     | Inf                         | 9.49E-03    |
| blanks       | 9.97                        | 7.11E-04    |
| Rpt6R        | 9.5                         | 3.17E-05    |
| CG32259      | 7.65                        | 3.91E-02    |
| Rpt3R        | 6.42                        | 1.73E-07    |
| Oseg5        | 6.32                        | 8.24E-07    |
| Shawl        | 6.1                         | 2.02E-09    |
| CG18193      | 5.63                        | 1.56E-02    |
| CG15201      | 5.37                        | 4.01E-02    |
| CG12493      | 5.24                        | 1.87E-02    |
| TrxT         | 5.19                        | 5.90E-03    |
| Salt         | 5.14                        | 4.61E-02    |
| CG4650       | 4.74                        | 2.73E-02    |
| CR18854      | 4.59                        | 3.91E-11    |
| Rbp4         | 4.48                        | 1.69E-03    |
| PebIII       | 4.42                        | 5.82E-03    |
| CG5791       | 4.33                        | 1.52E-02    |
| CG13321      | 4.26                        | 2.81E-09    |
| CG3884       | 3.79                        | 1.47E-02    |
| CG12655      | 3.68                        | 3.73E-03    |
| CG10151      | 3.45                        | 6.51E-05    |
| CG5281       | 3.32                        | 9.62E-05    |
| GstD2        | 3.32                        | 3.00E-02    |
| CG30108      | 3.3                         | 3.83E-06    |
| IM1          | 3.3                         | 1.59E-02    |
| CG10440      | 3.23                        | 2.18E-02    |
| CG34291      | 3.2                         | 3.13E-02    |
| CG16758      | 3.14                        | 1.34E-02    |
| CG6776       | 3.1                         | 3.14E-05    |
| Cyp12d1-p    | 3.03                        | 1.37E-03    |
| CG18186      | 2.94                        | 1.18E-05    |
| Obp99b       | 2.86                        | 5.56E-04    |
| CG1600       | 2.82                        | 2.48E-04    |
| CG13936      | 2.79                        | 4.55E-02    |
| Hsp70Ab      | 2.77                        | 7.62E-03    |
| CG7470       | 2.7                         | 2.51E-04    |
| Gfat1        | 2.65                        | 4.23E-03    |
| CG9960       | 2.6                         | 2.87E-03    |
| Ptp52F       | 2.58                        | 1.58E-03    |
| GstD10       | 2.58                        | 4.32E-02    |
| GstD5        | 2.57                        | 2.29E-02    |
| Mdr49        | 2.57                        | 1.13E-02    |

*Continued on next page*
| Gene       | log₂(FoldChange) | p-value  |
|-----------|-----------------|----------|
| Lsd-1     | 2.48            | 7.31E-04 |
| scpr-A    | 2.47            | 3.65E-03 |
| GstE5     | 2.45            | 3.60E-02 |
| Cyp28d1   | 2.34            | 1.05E-02 |
| CG7408    | 2.34            | 4.42E-02 |
| CG9380    | 2.3             | 1.04E-02 |
| CG15347   | 2.28            | 2.26E-02 |
| CG14629   | 2.27            | 1.03E-02 |
| CG32572   | 2.26            | 7.74E-03 |
| CG5399    | 2.24            | 4.98E-03 |
| Jheh3     | 2.2             | 8.99E-03 |
| CG5171    | 2.19            | 3.17E-02 |
| CG9743    | 2.17            | 4.68E-02 |
| Hsp23     | 2.13            | 8.02E-04 |
| RpS19b    | 2.1             | 4.61E-02 |
| Lip4      | 2.07            | 6.69E-03 |
| Hsp70Aa   | 2.06            | 8.74E-05 |
| IM2       | 2.05            | 3.62E-02 |
| Pompr     | 2               | 8.31E-04 |
| pncr008   | 1.99            | 4.42E-03 |
| CG5853    | 1.96            | 1.08E-02 |
| CG9360    | 1.93            | 2.94E-02 |
| CG30104   | 1.93            | 5.42E-03 |
| CG12290   | 1.92            | 2.58E-02 |
| ref(2)P   | 1.92            | 1.26E-03 |
| Pro alpha5| 1.92            | 1.56E-03 |
| CR42871   | 1.91            | 3.78E-02 |
| Pros28.1  | 1.86            | 1.72E-03 |
| Pros35    | 1.86            | 5.95E-03 |
| CG6299    | 1.85            | 5.75E-03 |
| Pro beta7 | 1.8             | 3.75E-03 |
| CG15445   | 1.79            | 5.28E-03 |
| qsm       | 1.78            | 1.13E-02 |
| CG11378   | 1.78            | 2.50E-02 |
| DnaJ-H    | 1.76            | 2.53E-03 |
| CG17331   | 1.74            | 4.46E-03 |
| Jheh1     | 1.73            | 8.66E-03 |
| dgo       | 1.7             | 2.67E-02 |
| IM3       | 1.69            | 3.05E-02 |
| CG3348    | 1.69            | 4.28E-02 |
| Pro beta5 | 1.68            | 8.07E-03 |
| CG3958    | 1.67            | 1.50E-02 |
| Pro beta1 | 1.65            | 6.22E-03 |
| Hmu       | 1.65            | 1.08E-02 |
| msd1      | 1.64            | 7.74E-03 |
| CG4199    | 1.64            | 1.08E-02 |
| cathD     | 1.63            | 9.09E-03 |
| CG10208   | 1.62            | 1.45E-02 |
| Gel       | 1.61            | 1.41E-02 |
| GstE3     | 1.61            | 1.75E-02 |
| Pro beta2 | 1.6             | 6.70E-03 |
| sev       | 1.58            | 2.74E-02 |
| Gene       | log₂(FoldChange) | p-value     |
|------------|-----------------|-------------|
| Prosalpha7 | 1.58            | 7.14E-03    |
| CG5167     | 1.57            | 2.87E-02    |
| Lsm10      | 1.57            | 1.72E-02    |
| Rpn9       | 1.57            | 9.83E-03    |
| Rpn6       | 1.56            | 1.13E-02    |
| Rpt1       | 1.55            | 8.58E-03    |
| CG2046     | 1.55            | 6.56E-03    |
| CG5384     | 1.55            | 1.59E-02    |
| CG12795    | 1.54            | 7.79E-03    |
| Pros29     | 1.53            | 1.19E-02    |
| Roc1a      | 1.53            | 1.11E-02    |
| Rpn12      | 1.52            | 2.12E-02    |
| CG13779    | 1.51            | 8.89E-03    |
| Cyp9f2     | 1.51            | 7.47E-03    |
| Pros54     | 1.51            | 3.31E-02    |
| Pros26     | 1.49            | 1.46E-02    |
| Tsf1       | 1.49            | 3.31E-03    |
| Pros25     | 1.47            | 1.99E-02    |
| CG33099    | 1.46            | 3.51E-02    |
| Pros45     | 1.46            | 1.90E-02    |
| Cyp12d1-d  | 1.41            | 3.26E-02    |
| CG11885    | 1.41            | 3.85E-02    |
| p47        | 1.4             | 1.86E-02    |
| Rpt4       | 1.39            | 4.25E-02    |
| Uch-L3     | 1.39            | 2.00E-02    |
| CG6218     | 1.36            | 2.05E-02    |
| Sirt4      | 1.36            | 3.52E-02    |
| PHGPx      | 1.36            | 1.86E-02    |
| Rpn11      | 1.36            | 2.56E-02    |
| Mov34      | 1.36            | 2.08E-02    |
| CG12398    | 1.36            | 3.46E-02    |
| CalpB      | 1.35            | 3.57E-02    |
| Jheh2      | 1.32            | 3.59E-02    |
| Clc        | 1.31            | 2.97E-02    |
| Ube3a      | 1.31            | 3.51E-02    |
| borr       | 1.28            | 4.07E-02    |
| Irc        | 1.28            | 3.78E-02    |
| Txl        | 1.27            | 2.78E-02    |
| Rpn3       | 1.27            | 2.72E-02    |
| CG42488    | 1.23            | 2.32E-02    |
| TER94      | 1.21            | 3.78E-02    |
| Ice        | 1.19            | 4.30E-02    |
| CG4572     | 1.18            | 3.84E-02    |
| Cyt-b5     | 1.17            | 3.81E-02    |
| Prosbeta3  | 1.16            | 4.38E-02    |
| CG4673     | 1.16            | 4.35E-02    |
| CG13349    | 1.15            | 4.32E-02    |
| CG9436     | 1.12            | 4.70E-02    |
| SelG       | 1.11            | 4.04E-02    |
Supplementary Table 2: PCR primers

| Name               | sequence               |
|--------------------|------------------------|
| RP49-f(14)         | CCGCTTCAAGGGACAGTATCTG |
| RP49-r(14)         | ATCTCGCCGAGTAAACGC    |
| lacZpromoter-f     | ATCGCCCTTCCCAACAGTTGC |
| lacZpromoter-r     | TTCTGTTGCCCAGAACCAGG |
| lacZreporter-f     | TGCACATTTCGGAGTACGTCG |
| lacZreporter-r     | GATTTCGCGCCTGCTACC    |

Supplementary Table 3: ChIP-seq datasets read mapping statistics

| Library                      | Read Length | Uniquely mapped reads |
|------------------------------|-------------|-----------------------|
| Ovary shPiwi Rep1 H3K9me3   | 36          | 11,093,401            |
| Ovary shPiwi Rep1 Input      | 36          | 23,783,156            |
| Ovary shPiwi Rep1 Pol II     | 36          | 21,233,655            |
| Ovary shWhite Rep1 H3K9me3  | 36          | 17,745,203            |
| Ovary shWhite Rep1 Input     | 36          | 22,091,234            |
| Ovary shWhite Rep1 Pol II    | 36          | 18,377,757            |
| Ovary shPiwi Rep2 H3K9me3    | 36          | 22,467,219            |
| Ovary shPiwi Rep2 H3K9me3 Input | 36      | 14,843,946            |
| Ovary shPiwi Rep2 Pol II     | 36          | 9,627,221             |
| Ovary shPiwi Rep2 Pol II Input | 36      | 2,985,999             |
| Ovary shWhite Rep2 H3K9me3  | 36          | 21,135,950            |
| Ovary shWhite Rep2 H3K9me3 Input | 36      | 16,619,035            |
| Ovary shWhite Rep2 Pol II    | 36          | 5,731,448             |
| Ovary shWhite Rep2 Pol II Input | 36      | 1,629,660             |

Supplementary Table 4: RNA-seq datasets read mapping statistics (TopHat 1.4.1 mappings)

| Library            | Read Length | Unique   | Multi    | Unique splices | Multi splices |
|--------------------|-------------|----------|----------|----------------|---------------|
| Ovary              | 50          | 19,868,793 | 3,249,894 | 2,021,378      | 31,552        |
| Ovary shWhite Rep1 | 50          | 4,266,297  | 868,256  | 389,035        | 5,895         |
| Ovary shPiwi Rep1  | 50          | 5,886,236  | 906,534  | 606,030        | 8,962         |
| Ovary shWhite Rep2 | 50          | 10,345,357 | 1,186,659 | 607,786        | 18,881        |
| Ovary shPiwi Rep2  | 50          | 12,764,829 | 1,393,823 | 1,177,886      | 25,302        |
### Supplementary Table 5: Repeat analysis mapping statistics (whole genome with unlimited multimappers, zero mismatches)

| Library                     | Read Length | Unique   | Multi    |
|-----------------------------|-------------|----------|----------|
| Ovary shPiwi Rep1 H3K9me3  | 36          | 9,469,110| 4,511,259|
| Ovary shPiwi Rep1 Input     | 36          | 20,029,978| 2,042,023|
| Ovary shPiwi Rep1 Pol II    | 36          | 17,994,285| 1,994,455|
| Ovary shWhite Rep1 H3K9me3 | 36          | 15,101,194| 5,076,952|
| Ovary shWhite Rep1 Input    | 36          | 18,568,175| 1,435,948|
| Ovary shWhite Rep1 Pol II   | 36          | 15,589,380| 1,675,468|
| Ovary shWhite Rep1 RNA-seq  | 36          | 3,682,085 | 6,376,989|
| Ovary shPiwi Rep1 RNA-seq   | 36          | 5,119,512 | 5,808,312|
| Ovary shWhite Rep2 RNA-seq  | 36          | 8,658,005 | 4,005,709|
| Ovary shPiwi Rep2 RNA-seq   | 36          | 10,573,906| 3,641,282|
| Ovary shPiwi Rep2 H3K9me3   | 36          | 13,315,195| 3,808,164|
| Ovary shPiwi Rep2 H3K9me3 Input | 36    | 13,489,170| 3,501,374|
| Ovary shPiwi Rep2 Pol2      | 36          | 8,137,867 | 1,183,428|
| Ovary shPiwi Rep2 Pol2 Input| 36          | 2,424,728 | 698,521  |
| Ovary shWhite Rep2 H3K9me3  | 36          | 19,021,830| 9,010,645|
| Ovary shWhite Rep2 H3K9me3 Input | 36      | 12,018,516| 5,698,668|
| Ovary shWhite Rep2 Pol2     | 36          | 4,858,338 | 824,157  |
| Ovary shWhite Rep2 Pol2 Input| 36         | 1,303,208 | 873,869  |

### Supplementary Table 6: Repeat analysis mapping statistics (consensus repeats)

| Library                     | Read Length | Unique   | Multi    |
|-----------------------------|-------------|----------|----------|
| Ovary shWhite Rep1 RNA-seq  | 36          | 14,016   | 4,615    |
| Ovary shPiwi Rep1 RNA-seq   | 36          | 39,413   | 9,692    |
| Ovary shWhite Rep2 RNA-seq  | 36          | 15,309   | 7,910    |
| Ovary shPiwi Rep2 RNA-seq   | 36          | 27,691   | 10,559   |
| Ovary shPiwi Rep1 H3K9me3   | 36          | 2,720,971| 283,437  |
| Ovary shPiwi Rep1 Input     | 36          | 1,123,614| 133,470  |
| Ovary shPiwi Rep1 Pol II    | 36          | 515,368  | 109,711  |
| Ovary shWhite Rep1 H3K9me3  | 36          | 3,208,049| 318,559  |
| Ovary shWhite Rep1 Input    | 36          | 739,854  | 83,425   |
| Ovary shWhite Rep1 Pol II   | 36          | 346,044  | 74,633   |
| Ovary shPiwi Rep2 H3K9me3   | 36          | 5,487,961| 409,778  |
| Ovary shPiwi Rep2 H3K9me3 Input | 36      | 2,819,017| 340,708  |
| Ovary shPiwi Rep2 Pol II    | 36          | 380,988  | 79,937   |
| Ovary shPiwi Rep2 Pol II Input| 36         | 318,557  | 38,475   |
| Ovary shWhite Rep2 H3K9me3  | 36          | 5,556,191| 463,857  |
| Ovary shWhite Rep2 H3K9me3 Input | 36      | 1,718,729| 205,205  |
| Ovary shWhite Rep2 Pol II   | 36          | 220,634  | 52,925   |
| Ovary shWhite Rep2 Pol II Input| 36         | 174,554  | 25,171   |
Supplementary Figure 1: Fluorescence Loss in Photobleaching (FLIP) experiments indicate fast redistribution of most of nuclear Piwi and slower movement of the Piwi-YK mutant. Amount of fluorescence decrease after 110 bleaching iterations for H2A-RFP, GFP-Piwi and GFP-Piwi-YK mutant and GFP in a nurse cell nucleus is shown. In each case significant fluorescence loss (red pixels) is observed along the bleach axis. Both GFP and WT GFP-Piwi has extensive loss of fluorescence (≥75%) across much of the nucleus, except for specific loci. GFP-Piwi-YK mutant exhibits far less change (≤40%) in regions far from the site of bleaching. H2A-RFP control undergoes very little change in intensity away from the bleach region. Note that the apparent slower redistribution of free GFP is likely due to simultaneous nuclear import from the unbleached cytoplasmic pool. Bars = 5µm. Arrowheads indicate position of bleach stripe across the nucleus.
| Gene            | RNA log2(Fold Change) | Chip log2(Fold Change) |
|-----------------|-----------------------|------------------------|
| TC1DM           | 0.192 ± 0.04          | 0.192 ± 0.04           |
| FROGGER_LTR     | 0.192 ± 0.04          | 0.192 ± 0.04           |
| BAR1            | 0.179 ± 0.06          | 0.179 ± 0.06           |
| BEL1            | 0.172 ± 0.06          | 0.172 ± 0.06           |
| Jockey2         | 0.158 ± 0.14          | 0.158 ± 0.14           |
| QUASIMODO_1     | 0.155 ± 0.03          | 0.155 ± 0.03           |
| MINOS           | 0.155 ± 0.03          | 0.155 ± 0.03           |
| HOSO3           | 0.154 ± 0.03          | 0.154 ± 0.03           |
| DM1731_LTR      | 0.146 ± 0.06          | 0.146 ± 0.06           |
| DM1761_LTR      | 0.144 ± 0.04          | 0.144 ± 0.04           |
| DIVER           | 0.14 ± 0.02           | 0.14 ± 0.02            |
| DM297_LTR       | 0.138 ± 0.02          | 0.138 ± 0.02           |
| Gypsy7_LTR      | 0.132 ± 0.04          | 0.132 ± 0.04           |
| ACCORD2_LTR     | 0.123 ± 0.04          | 0.123 ± 0.04           |
| DNAREP1_LTR     | 0.123 ± 0.04          | 0.123 ± 0.04           |
| HETRP_LTR       | 0.122 ± 0.04          | 0.122 ± 0.04           |
| NAMAD_LTR       | 0.12 ± 0.02           | 0.12 ± 0.02            |
| IDEFIX          | 0.11 ± 0.01           | 0.11 ± 0.01            |
| PROTOS          | 0.104 ± 0.01          | 0.104 ± 0.01           |
| Gypsy1          | 0.104 ± 0.01          | 0.104 ± 0.01           |
| PROTOS_A        | 0.108 ± 0.01          | 0.108 ± 0.01           |
| Invasor2_LTR    | 0.092 ± 0.01          | 0.092 ± 0.01           |
| TRANSB4         | 0.086 ± 0.01          | 0.086 ± 0.01           |
| DOC3_LTR        | 0.076 ± 0.01          | 0.076 ± 0.01           |
| MARINA          | 0.076 ± 0.01          | 0.076 ± 0.01           |
| LOOPER          | 0.076 ± 0.01          | 0.076 ± 0.01           |
| TV1             | 0.057 ± 0.01          | 0.057 ± 0.01           |
| Gypsy3_LTR      | 0.059 ± 0.00          | 0.059 ± 0.00           |
| FB4_DM          | 0.059 ± 0.00          | 0.059 ± 0.00           |
| DMC1R1A         | 0.050 ± 0.00          | 0.050 ± 0.00           |
| PROTOP          | 0.049 ± 0.01          | 0.049 ± 0.01           |
| Invasor1_LTR    | 0.041 ± 0.00          | 0.041 ± 0.00           |
| Invader5_LTR    | 0.034 ± 0.00          | 0.034 ± 0.00           |
| STARKER         | 0.025 ± 0.00          | 0.025 ± 0.00           |
| HMBSEAGLE       | 0.025 ± 0.00          | 0.025 ± 0.00           |
| TRANSPAC_LTR    | 0.024 ± 0.00          | 0.024 ± 0.00           |
| Gypsy6_LTR      | 0.024 ± 0.00          | 0.024 ± 0.00           |
| OSVALDO_LTR     | 0.024 ± 0.00          | 0.024 ± 0.00           |
| TDL2            | 0.024 ± 0.00          | 0.024 ± 0.00           |
| UHU             | 0.024 ± 0.00          | 0.024 ± 0.00           |
| GDM3_LTR        | 0.024 ± 0.00          | 0.024 ± 0.00           |
| TABOR_LTR       | 0.024 ± 0.00          | 0.024 ± 0.00           |
| DMRPR           | 0.024 ± 0.00          | 0.024 ± 0.00           |
| TIRANT_LTR      | 0.023 ± 0.00          | 0.023 ± 0.00           |
| DIVER_LTR       | 0.023 ± 0.00          | 0.023 ± 0.00           |
| NOMAD3          | 0.023 ± 0.00          | 0.023 ± 0.00           |
| TIRANT          | 0.022 ± 0.00          | 0.022 ± 0.00           |
| HELENA_LTR      | 0.022 ± 0.00          | 0.022 ± 0.00           |
| RT1_LTR         | 0.022 ± 0.00          | 0.022 ± 0.00           |
| DMSAT6          | 0.022 ± 0.00          | 0.022 ± 0.00           |
| LINE1_LTR       | 0.021 ± 0.00          | 0.021 ± 0.00           |
| Gypsy11_LTR     | 0.021 ± 0.00          | 0.021 ± 0.00           |
| GTWIN_LTR       | 0.021 ± 0.00          | 0.021 ± 0.00           |
| XDMAR           | 0.021 ± 0.00          | 0.021 ± 0.00           |
| DIVER2_LTR      | 0.021 ± 0.00          | 0.021 ± 0.00           |
| ROO_LTR         | 0.018 ± 0.00          | 0.018 ± 0.00           |
| Gypsy9_LTR      | 0.017 ± 0.00          | 0.017 ± 0.00           |
| BS3_LTR         | 0.016 ± 0.00          | 0.016 ± 0.00           |
| SAR_DM          | 0.015 ± 0.00          | 0.015 ± 0.00           |
| TRANSB2         | 0.014 ± 0.00          | 0.014 ± 0.00           |
| MDG1_LTR        | 0.013 ± 0.00          | 0.013 ± 0.00           |
| ROOA_LTR        | 0.013 ± 0.00          | 0.013 ± 0.00           |
| FDW_LTR         | 0.012 ± 0.00          | 0.012 ± 0.00           |
| G2_LTR          | 0.012 ± 0.00          | 0.012 ± 0.00           |
| ARS406_D1_LTR   | 0.012 ± 0.00          | 0.012 ± 0.00           |
Supplementary Figure 2 (preceding page): Piwi regulates transposon levels through transcriptional repression. The change in the levels of transposable element transcripts and RNA Polymerase II occupancy upon Piwi knockdown is shown. RNA-seq and ChIP-seq experiments were carried out in shWhite and shPiwi ovaries in two replicates. Differential expression was assessed using DESeq (see methods). The first column shows the statistical significance of the observed expression change (in log_{10}(p-value)); upregulated and downregulated genes are sorted separately in order of decreasing significance. The second column shows the average change in RNA levels as defined by DESeq. The third column shows the average change in Pol II occupancy between the two replicate experiments.

Supplementary Figure 3: Piwi depletion increases RNA Pol II association with promoters of transposable elements. RNA Polymerase II ChIP-seq signal over the consensus sequences of selected transposable elements in the control (shWhite) and Piwi-depleted (shPiwi) ovaries. Pol II occupancy increases in the promoter regions (LTRs) of transposons upon germline knockdown of Piwi. Transposons expressed in somatic follicular cells such as ZAM are not affected.
Supplementary Figure 4: Piwi depletion does not alter H3K9me3 occupancy over differentially expressed genes. Scatter plot indicating average H3K9me3 mark levels upon Piwi depletion (shPiwi) and control (shWhite) over genes that were previously identified in the RNA-seq experiments to be differentially expressed upon Piwi knockdown. (red: upregulated genes, green: downregulated genes). The average signal of two biological replicates was taken after subtraction of the corresponding input signals.