Table S1. Nanopore sequencing summary

	DGRP379	DGRP732
Total Reads	878,451	1,195,332
Total Bases	7.498 Gbp	9.870 Gbp
Mean Phred Score	15.3	15.8
Mean Read Length	8,535 bp	8,257 bp
Median Read Length	4,374 bp	4,657 bp

Figure S1. Nanopore reads length distribution
The density plots show the distribution of reads lengths for the full nanopore sequence dataset used for each strain.

Figure S2. DGRP732 nanopore genome assembly before and after Hi-C scaffolding.
The top half of the figure shows the Hi-C contact matrices before and after scaffolding. Lighter shades indicate more 3D interactions between genomic regions and darker shades indicate fewer interactions. The red boxes show the location of contigs/scaffolds. The bottom half of the figure shows dotplots representing the alignment of the assembly to the *D. melanogaster iso-1* reference genome before and after scaffolding.

Figure S3. Genome assembly polishing with *Pilon*
Uninformative Illumina reads from the Hi-C data (i.e. those that do not include a ligation junction) were used as single-end data to correct sequence errors in the nanopore assemblies using the *Pilon* software package. Pilon was run for 10 iterations and the number of corrections per iteration is shown for each assembly.

Figure S4. Genomic copy number for TEs showing high strain specificity
Total copy number is shown for each TE family with >= 50% strain-specific insertions.

Figure S5. Distribution of Transposable elements, large insertions, and piRNA source loci along DGRP732 chromosome arms.
The three large chromosomes of *D. melanogaster* are shown with their missing centromeric sequence represented by grey boxes. The chromosome arms were divided into 100kb windows and the number of features per window is shown for TE insertions, other large insertion/deletion mutations, and piRNA source loci.

Figure S6. Insertion mutation sequencing coverage for euchromatin versus pericentric heterochromatin.
We categorized our insertion mutations by whether they reside in euchromatin or pericentromeric heterochromatin and then compared sequencing coverage at each insertion for the strain that carried the insertion versus the strain that lacks the insertions. For both euchromatin and heterochromatin, we find the expected pattern where the strain with the insertion has typical sequencing coverage whereas the strain lacking the insertion has low or zero coverage at that region.

Figures S7-S10. Assessment of TIDAL-fly false negatives and false positives.
We aligned paired-end Illumina sequences to locations in our assemblies that lack a TE insertion despite one being predicted by TIDAL-fly (*i.e.* potential false positives, **Figure S7**: DGRP379, **Figure S8**: DGRP732), as well as locations that contained a TE insertion missed by TIDAL-fly (*i.e.* potential false negatives, **Figure S9**: DGRP379, **Figure S10**: DGRP732). In each figure, the horizontal black lines show the alignment location of a single paired-end fragment and the pink boxes show either the window where TIDAL predicts a TE to be (but it is not present in our assemblies, **Figures S7** & **S8**) or the location of a TE that IS present in our assemblies, but was not identified by TIDAL (**Figures S9** & **S10**). All paired-end alignments were required to be concordant with at least one mate with mapping quality >= 20. The presence of multiple alignments tiled across the pink region is supportive of the assembly rather than the TIDAL prediction. The panels highlighted in red do not have tiled read alignments, leading to the possibility that TIDAL is correct, rather than the assembly.

Figure S11. *Micropia* sequence alignment
A multiple sequence alignment between consensus sequences from the novel *micropia*-like TE identified in this study and the two known *micropia* elements that it matches in RepBase: a *micropia* element has that has previously been identified in *D. melanogaster* and a *micropia* element from *D. hydei.* Excluding the divergent 5' and 3' ends, the novel element is 72% identical to the *D. hydei micropia* sequence and 68% identical to the previously described *D. melanogaster micropia* sequence.

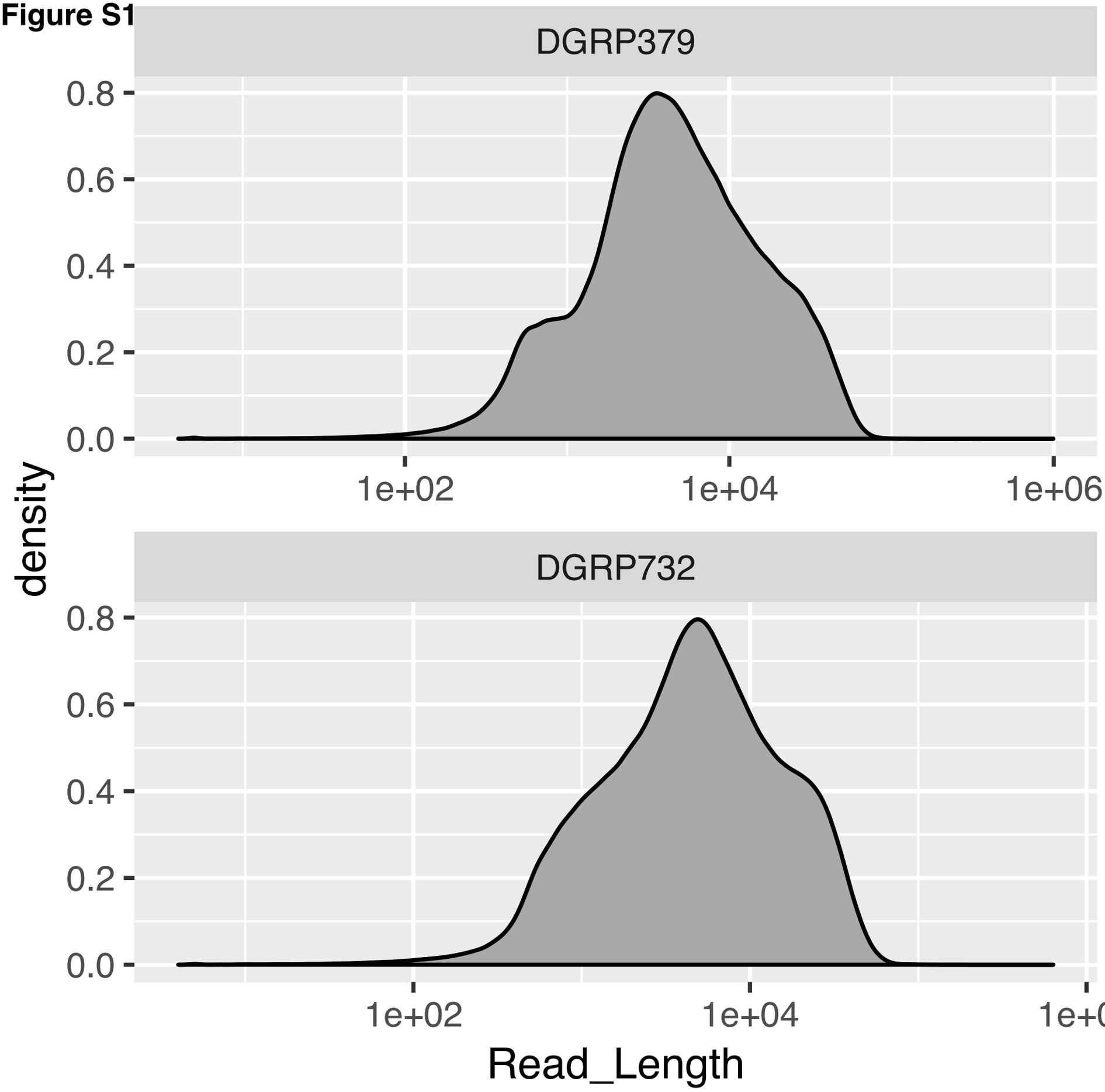
Figure S12. piRNA expression variation
Normalized expression values from piRNA producing loci were calculated for each strain. In the above scatterplot, the piRNA loci from DGRP732 were classified based on the expression of the orthologous locus in DGRP379: Up-regulated (UPREG) loci show two-fold or greater expression in DGRP732 (versus DGRP379), SIMILAR loci show less than a two-fold difference in expression, downregulated (DOWNR) loci show two-fold or greater down-regulation in DGRP732 (versus DGRP379), and INACTIVE loci were not identified as piRNA source loci in DGRP732.

Figure S13. piRNA expression variation: FPKM>=10 threshold
The same figures as in Figure 7 and Figure S12 are shown here expect a threshold of FPKM>=10 was used to identify piRNA loci, rather than FPKM>=1.

Figure S14. Smr gene model with piRNA read coverage
This figure shows an expanded view of the region in Figure 8, including the gene model of *Smr*. The intron of Smr shows much higher levels of piRNA production in DGRP379 compared to DGRP732 despite there being no major sequence differences between the two strains at this location.

Figure S15. Comparative expression of strain-specific genic piRNA loci
A substantial portion of strain-specific piRNA loci do not overlap TEs, but overlap genes instead. Comparison of expression levels of these genes between DGRP379 and 732 shows that there is no association between piRNA production from these genes and their mRNA expression level. Gene expression data is from microarrays from whole adult females (34).

Figure S1


DGRP379
DGRP732
density
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0.6
0.4
0.2
0.0
1e+02
1e+04
1e+06
Read_Length

Figure S2

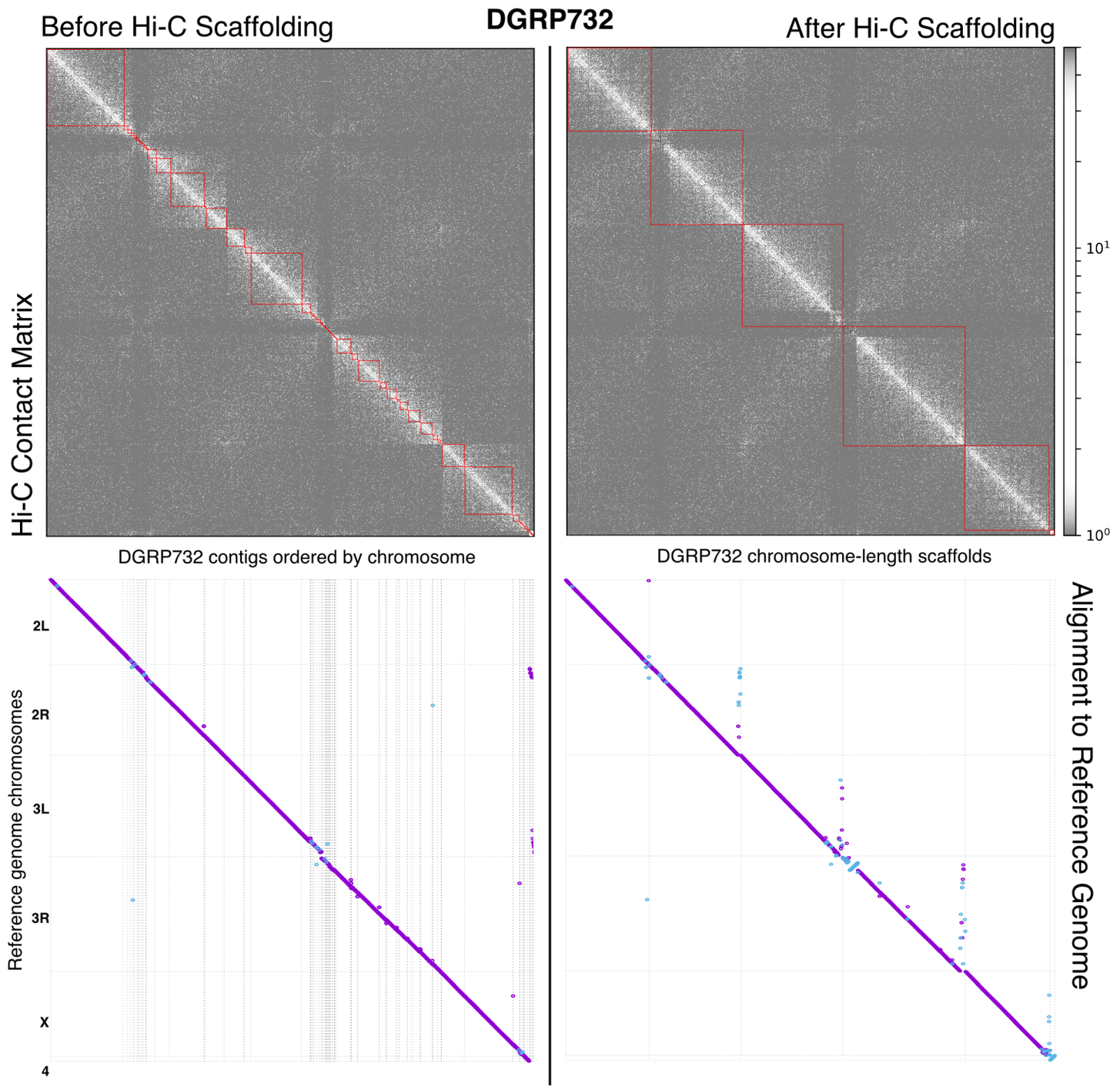

DGRP732
Before Hi-C Scaffolding
After Hi-C Scaffolding
Hi-C Contact Matrix
10^1
10^0
DGRP732 contigs ordered by chromosome
DGRP732 chromosome-length scaffolds
Reference genome chromosomes
2L
2R
3L
3R
X
4
Alignment to Reference Genome

Figure S3

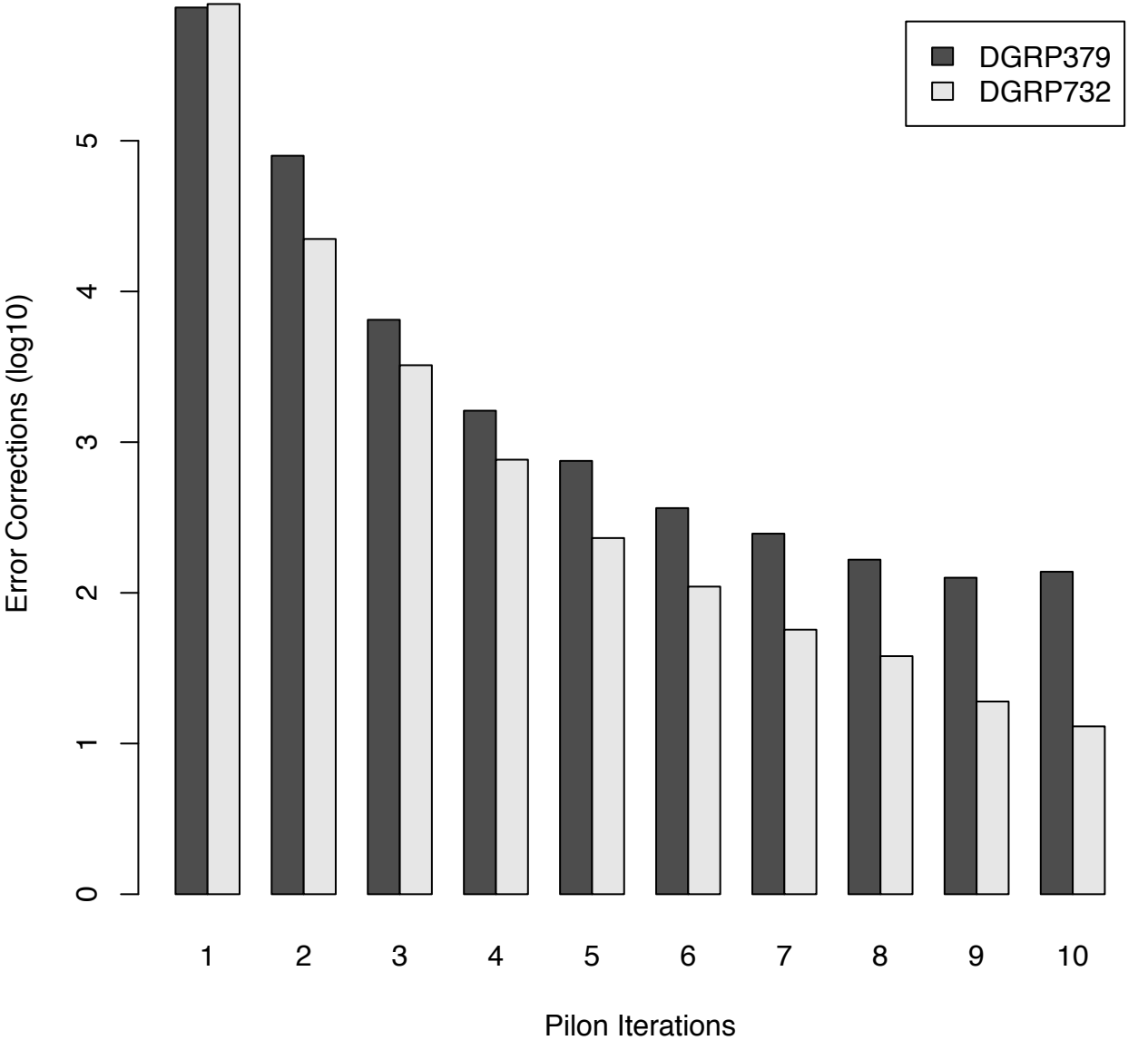

DGRP379
DGRP732
Error Corrections (log10)
0
1
2
3
4
5
1
2
3
4
5
6
7
8
9
10
Pilon Iterations

Figure S4

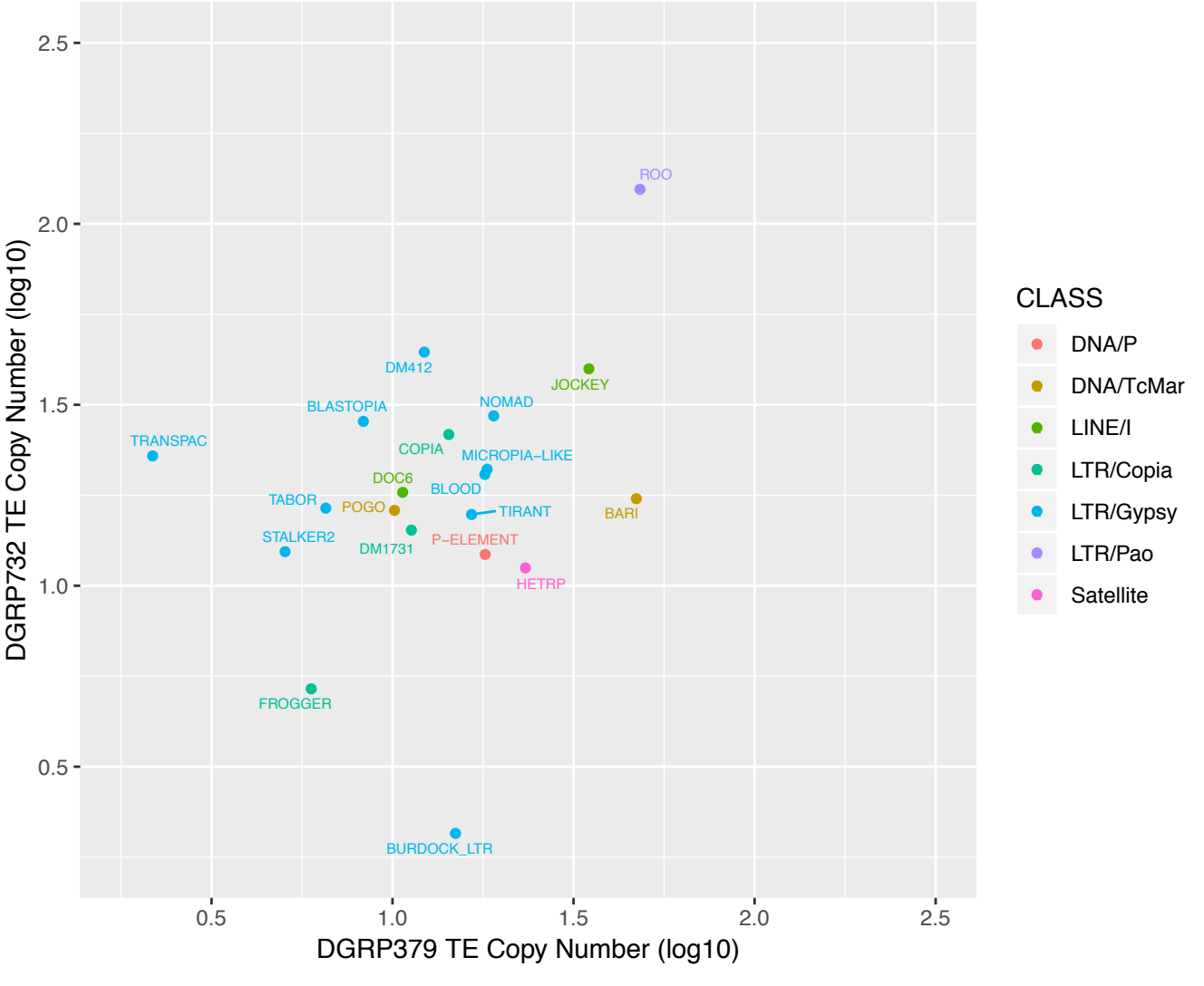
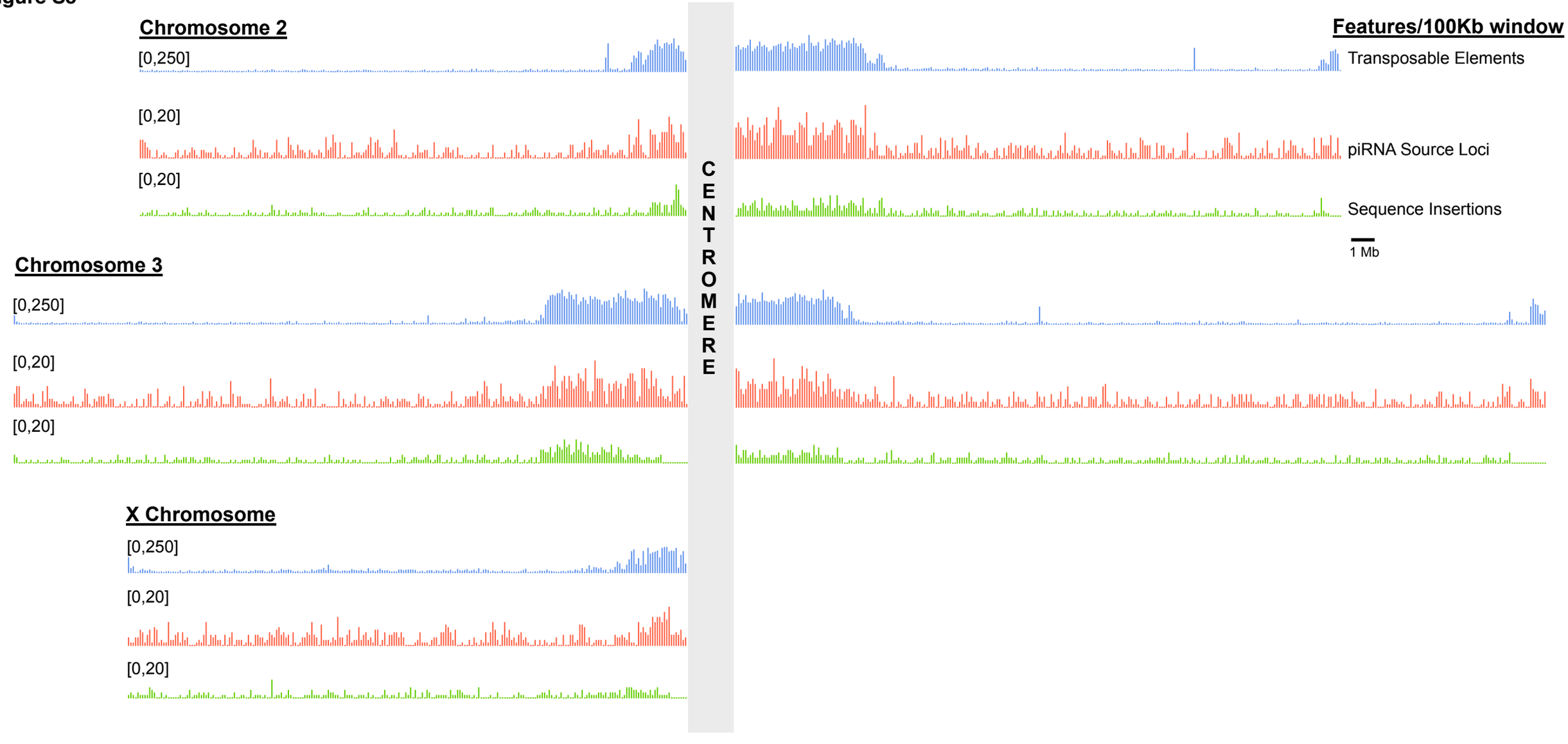

DGRP732 TE Copy Number (log10)
DGRP379 TE Copy Number (log10)
2.5
2.0
1.5
1.0
0.5
0.5
1.0
1.5
2.0
2.5
ROO
DM412
JOCKEY
NOMAD
BLASTOPIA
TRANSPAC
COPIA
MICROPIA-LIKE
DOC6
BLOOD
TABOR
POGO
TIRANT
BARI
STALKER2
DM1731
P-ELEMENT
HETRP
FROGGER
BURDOCK_LTR
CLASS
DNA/P
DNA/TcMar
LINE/I
LTR/Copia
LTR/Gypsy
LTR/Pao
Satellite

Figure S5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[0,20]
[0,20]
Chromosome 3
[0,250]
[0,20]
[0,20]
X Chromosome
[0,250]
[0,20]
[0,20]
CENTROMERE
Features/100Kb window
Transposable Elements
piRNA Source Loci
Sequence Insertions
1 Mb

Figure S6

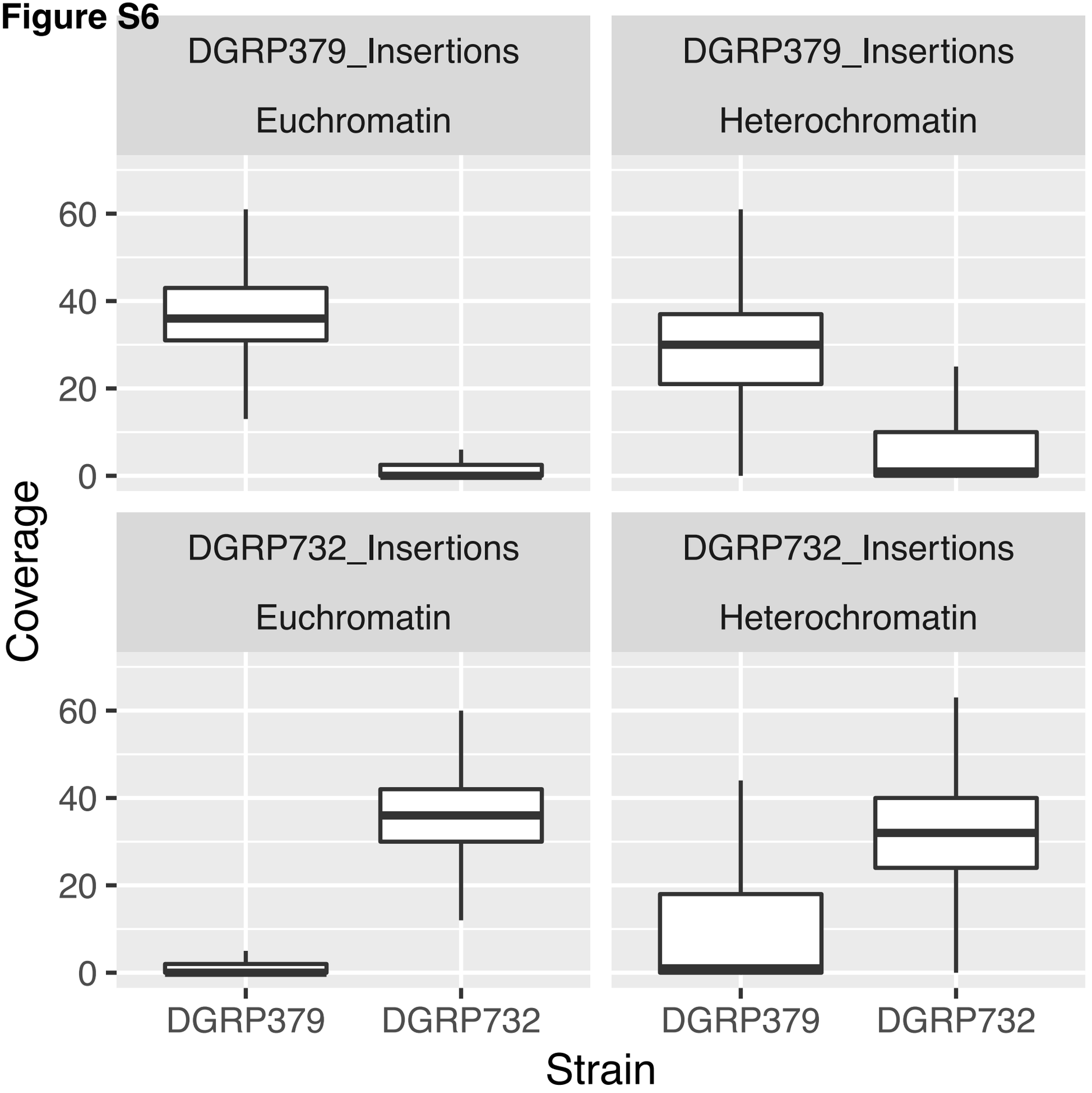
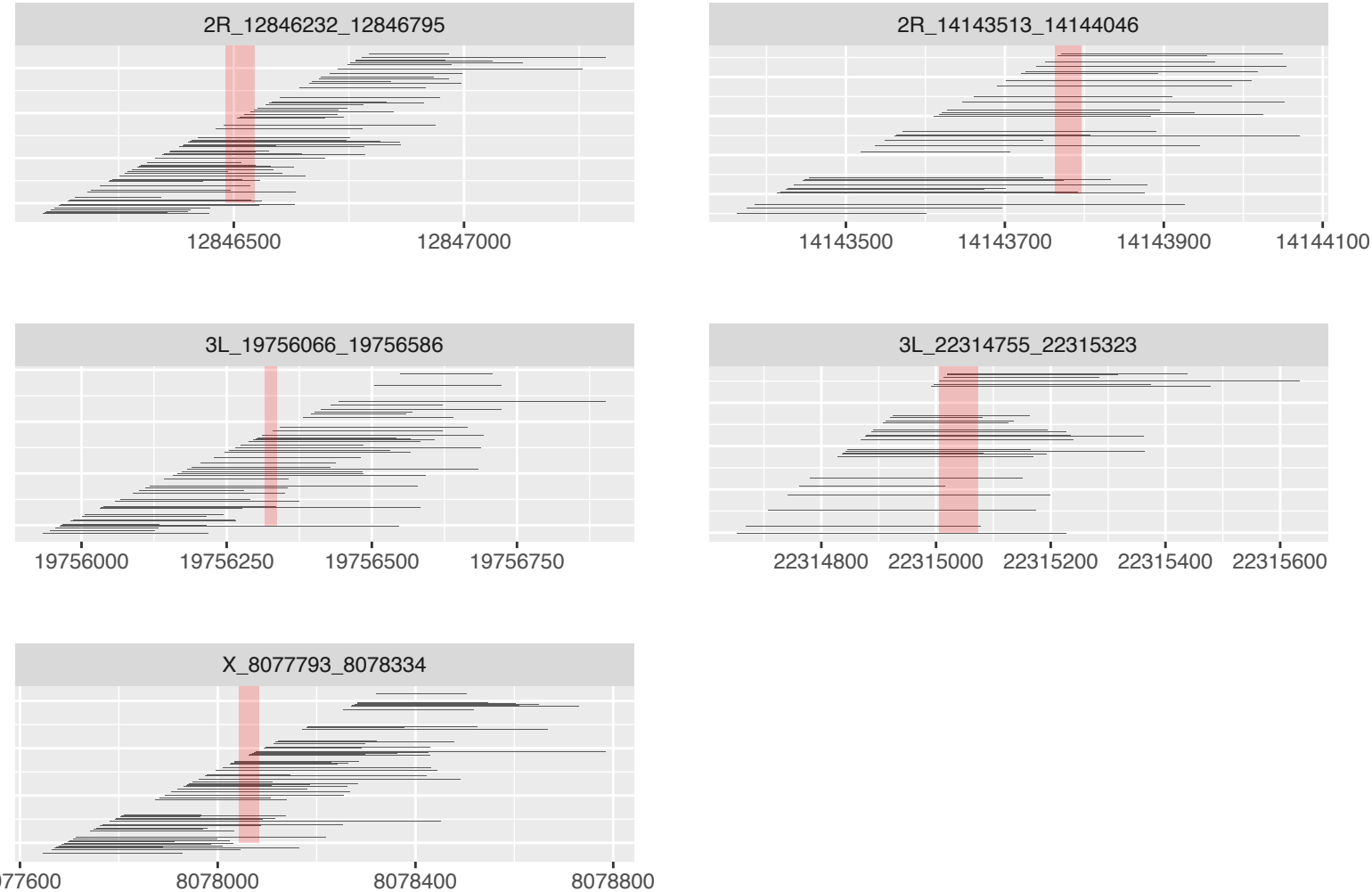

DGRP379_Insertions
Euchromatin
DGRP379_Insertions
Heterochromatin
DGRP732_Insertions
Euchromatin
DGRP732_Insertions
Heterochromatin
Coverage
60
40
20
0
DGRP379
DGRP732
Strain

Figure S7


2R_12846232_12846795
12846500
12847000
2R_14143513_14144046
14143500
14143700
14143900
14144100
3L_19756066_19756586
19756000
19756250
19756500
19756750
3L_22314755_22315323
22314800
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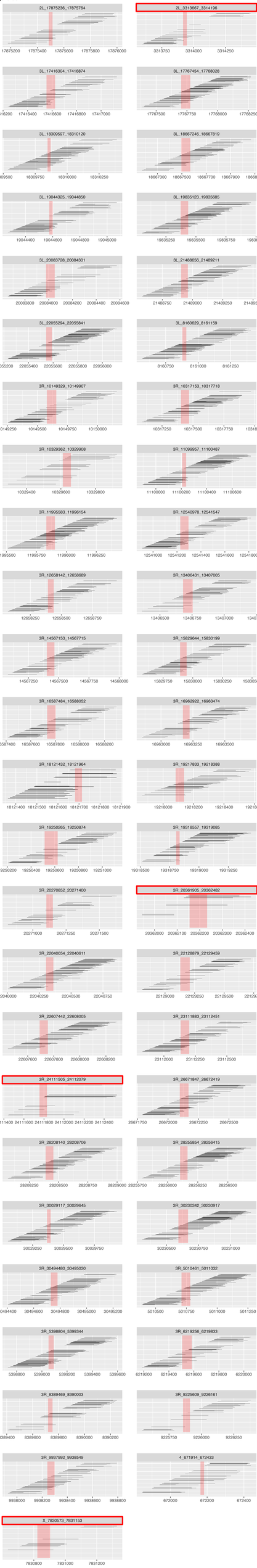
22315400
22315600
X_8077793_8078334
8077600
8078000
8078400
8078800

Figure S8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17875200 17875400 17875600 17875800 17876000
2L_3313667_3314196
3313750 3314000 3314250
3L_17416304_17416874
17416200 17416400 17416600 17416800 17417000
3L_17767454_17768028
17767500 17767750 17768000 17768250
3L_18309597_18310120
18309500 18309750 18310000 18310250
3L_18667246_18667819
18667300 18667500 18667700 18667900 18668100
3L_19044325_19044850
19044400 19044600 19044800 19045000
3L_19835123_19835685
19835250 19835500 19835750 19836000
3L_20083728_20084301
20083800 20084000 20084200 20084400 20084600
3L_21488656_21489211
21488750 21489000 21489250 21489500
3L_22055294_22055841
22055200 22055400 22055600 22055800 22056000
3L_8160629_8161159
8160750 8161000 8161250
3R_10149329_10149907
10149250 10149500 10149750 10150000
3R_10317153_10317718
10317250 10317500 10317750 10318000
3R_10329362_10329908
10329400 10329600 10329800
3R_11099957_11100487
11100000 11100200 11100400 11100600
3R_11995583_11996154
11995500 11995750 11996000 11996250
3R_12540978_12541547
12541000 12541200 12541400 12541600 12541800
3R_12658142_12658689
12658250 12658500 12658750
3R_13406431_13407005
13406500 13406750 13407000 13407250
3R_14567153_14567715
14567250 14567500 14567750 14568000
3R_15829644_15830199
15829750 15830000 15830250 15830500
3R_16587484_16588052
16587400 16587600 16587800 16588000 16588200
3R_16962922_16963474
16963000 16963250 16963500
3R_18121432_18121964
18121400 18121500 18121600 18121700 18121800 18121900
3R_19217833_19218388
19218000 19218200 19218400 19218600
3R_19250265_19250874
19250200 19250400 19250600 19250800 19251000
3R_19318557_19319085
19318500 19318750 19319000 19319250
3R_20270852_20271400
20271000 20271250 20271500
3R_20361905_20362482
20362000 20362100 20362200 20362300 20362400
3R_22040054_22040611
22040000 22040250 22040500 22040750
3R_22128879_22129459
22129000 22129250 22129500 22129750
3R_22607442_22608005
22607600 22607800 22608000 22608200
3R_23111883_23112451
23112000 23112250 23112500
3R_24111505_24112079
24111400 24111600 24111800 24112000 24112200 24112400
3R_26671847_26672419
26671750 26672000 26672250 26672500
3R_28208140_28208706
28208250 28208500 28208750 28209000
3R_28255854_28256415
28255750 28256000 28256250 28256500
3R_30029117_30029645
30029250 30029500 30029750
3R_30230342_30230917
30230500 30230750 30231000
3R_30494480_30495030
30494400 30494600 30494800 30495000 30495200
3R_5010461_5011032
5010500 5010750 5011000 5011250
3R_5398804_5399344
5398800 5399000 5399200 5399400 5399600
3R_6219256_6219833
6219200 6219400 6219600 6219800 6220000
3R_8389469_8390003
8389400 8389600 8389800 8390000 8390200
3R_9225609_9226161
9225750 9226000 9226250
3R_9937992_9938549
9938000 9938200 9938400 9938600 9938800
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7830800 7831000 7831200

Figure S9

Figure S10

Figure S11

Figure S12

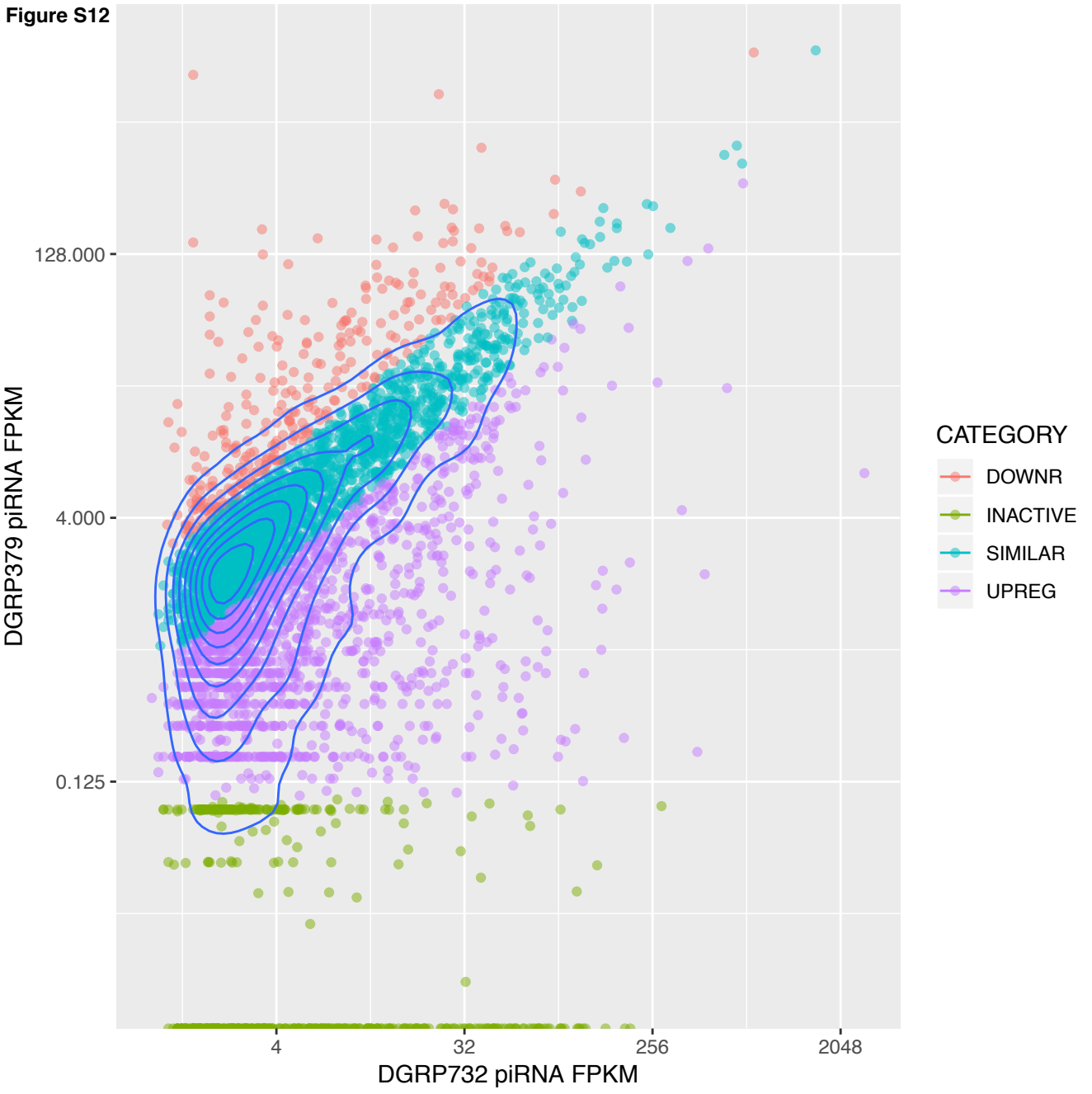

128.000
4.000
0.125
DGRP379 piRNA FPKM
4
32
256
2048
DGRP732 piRNA FPKM
CATEGORY
DOWNR
INACTIVE
SIMILAR
UPREG

Figure S13

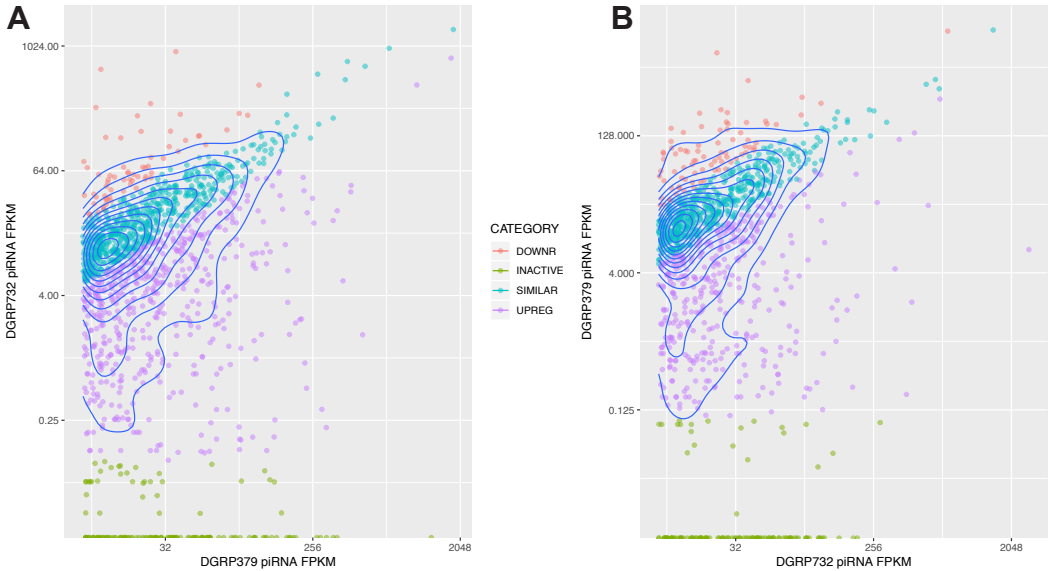
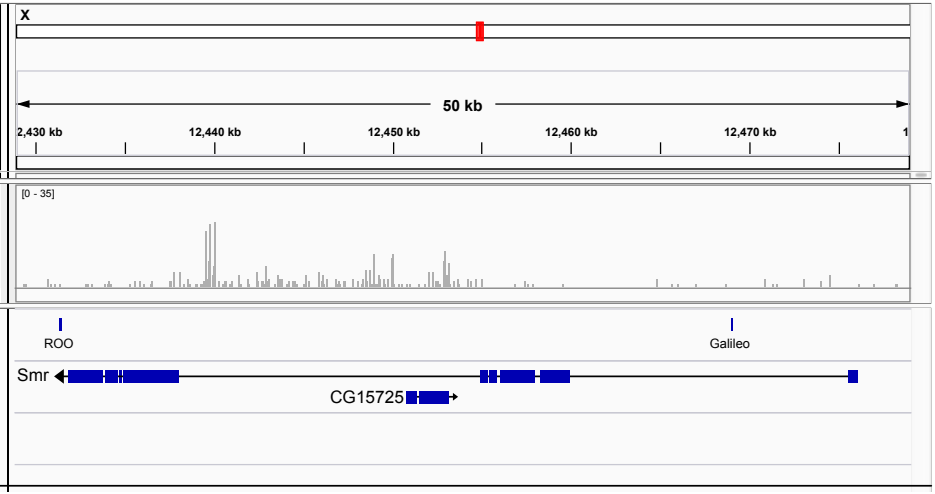

A
1024.00
64.00
4.00
0.25
DGRP732 piRNA FPKM
32
256
2048
DGRP379 piRNA FPKM
CATEGORY
DOWNR
INACTIVE
SIMILAR
UPREG
B
128.000
4.000
0.125
DGRP379 piRNA FPKM
32
256
2048
DGRP732 piRNA FPKM

Figure S14

DGRP379



DGRP732

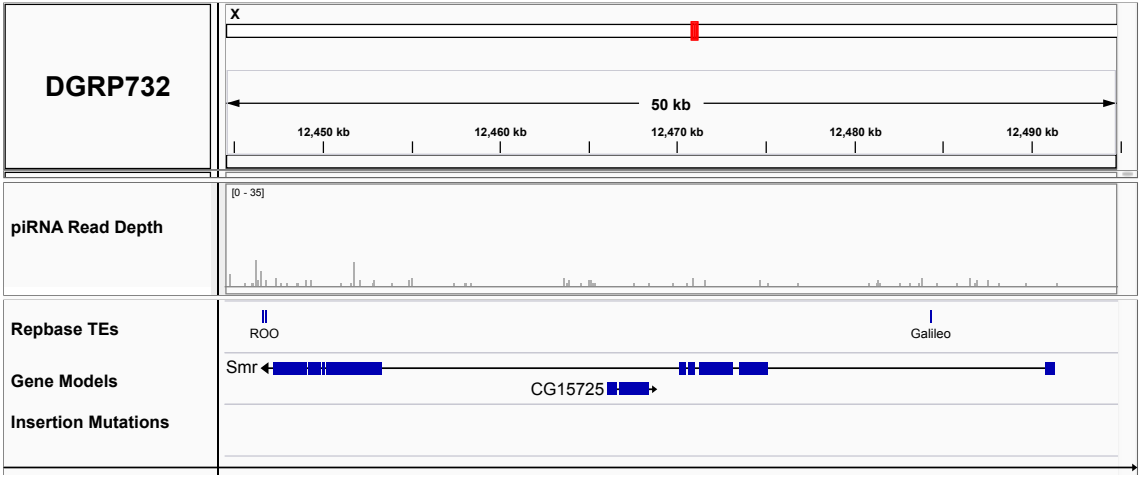
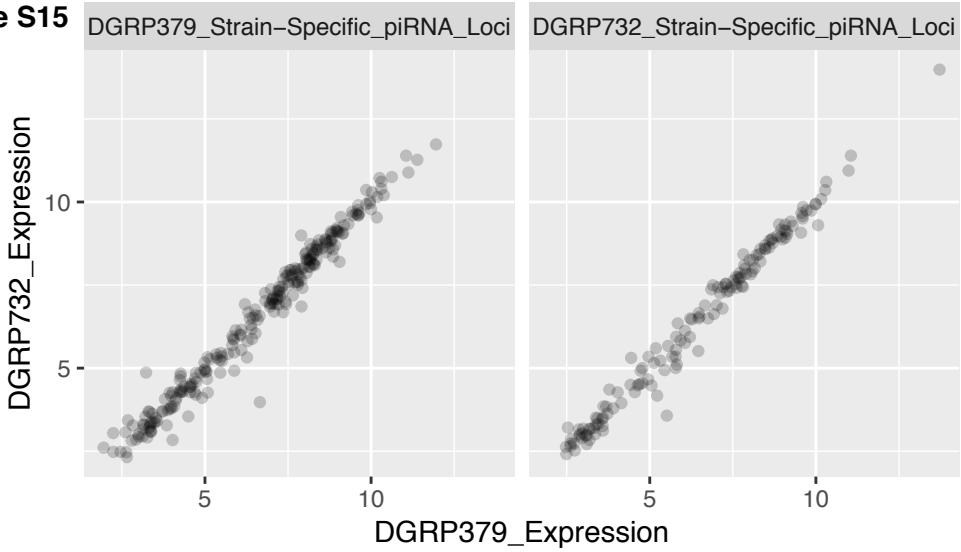


Figure S15


DGRP379_Strain−Specific_piRNA_Loci
DGRP732_Strain−Specific_piRNA_Loci
DGRP732_Expression
10
5
5
10
5
10
DGRP379_Expression