Description of Supplemental Alignments

All alignments were produced with Geneious software package (version 5.6.3, Biomatters.com; www.geneious.com) using the Geneious alignment algorithm. The first line shows a reference sequence(s) to which the Sanger sequencing reads or assembled clones were aligned, with accompanying annotation. Macronuclear Destined Sequences (MDSs) are indicated by gray annotation bars. Purple annotations indicate injected piRNAs and light blue indicates pointer sequences (regions of microhomology at MDS recombination junctions). Green arrows indicate oligonucleotide primer locations.

Supplemental_Alignment_1: *Contig11396-TEBPβ* fusion Sanger alignment. PCR amplified molecules were produced using red primers in <link rid="fig1">Figure 1</link>a (annotated in the alignment with dark green arrows), and 11 independent full-length clone sequences were obtained. The source DNA template for PCR was derived from the cell population shown in <link rid="fig1">Figure 1</link>b, lanes 1, 3, and 5. Reference alleles for the somatic *contig11396* gene structure from wild-type strains JRB310 (homozygous) and JRB510 (2 alleles) are shown in the first part of the alignment. Also shown are the germline gene structures for strain JRB310 (annotated "MIC_11396_TEBPb"), with gray or black boxes indicating the MDSs for the 2 chromosomes, and IESs represented as gaps in the alignment (shown as dashes). Start and stop codons for each gene are indicated as red arrows. The sequence corresponding to the microinjected 27nt RNA is annotated with a purple arrow. Other annotations as above. The aberrant deletions described in the text are shown on page 3 of the alignment, where 3 clones have the 71 bp deletion flanked by a "GAA" cryptic pointers (marked "cp1" with light blue arrows) and 7 clones deleted 27 bp flanked by cryptic "ATG" pointers (labeled cp2 with light blue arrows). A single intron in *contig11396* is annotated with light green triangles at the GT…AG splicing signals.

Supplemental_Alignment_2: *Contig9.1-310.1* fusion Sanger alignment. Three cloned PCR products from <link rid="fig2">Figure 2</link>c, lane 2 (primers in <link rid="fig2">Fig. 2</link>a), showing proper unscrambling of multiple alleles, in the sequenced region of the fusion chromosome. Because of the complex scrambled germline locus,

3 reference sequences are provided: representative somatic alleles of *contig9.1*, and *contig310.1*, as well as a portion of the germline contig from which the 2 somatic sequences derive. All annotations as above.

Supplemental_Alignment_3: *Contig7005* fusion Sanger alignment, beginning with the pointer between MDS segments 1 and 2. Partial sequence of the cloned inverse PCR product from <link rid="fig4">Figure 4</link>b, lane 2, confirms that the molecule has the programmed fused junction and contains all MDSs with IESs (colored) correctly spliced at labeled pointers. The shorter sequences (3 clones derived from Fig. S2, lane 11) confirm the fusion from MDS 5 to MDS 1. All annotations are as above.

Supplemental_Alignment_4: *Contig11682-Contig20527-Contig16348* triple-chromosome fusion Sanger alignment. All annotations as above, except that MDSs are indicated in blue (*Contig11682*), light gray (*Contig20527*), or dark gray (*Contig16348*) to distinguish the different chromosomes. Open reading frames are indicated in yellow—there are 3 genes on *Contig11682* (a Prefoldin subunit, AAA domain protein, and YL1 nuclear protein) and one gene each on *Contig20527* (RAMP superfamily protein) and *Contig16348* (Proteasome 26S). Thus the triple fusion results in the formation of a chromosome with 5 genes. A TBE1 transposable element comprising IES2 of *Contig11682* is indicated in gold. Clones 52 and 56 are the predicted fusion products; aberrant deletions are present in clones 49, 55, 60, 62, 63, 69, and 71 and are always flanked by crpytic pointers: an "ATTTA," marked cp1, flanks the aberrant deletion proximal to piRNA1. On the piRNA2 side, aberrant deletions are flanked by "ATA," marked cp2 at the left, paired with 3 alternative right-side "ATA" cryptic pointers marked "a" (clones 55, 62, 63, and 69), "b" (clone 60), or "c" (clone 49).

Supplemental_Alignment_5: *TEBPβ-TEBPα* chimeric Sanger alignment. Sequences obtained from PCR reactions shown in <link rid="fig5">Figure 5</link>b (labeled Chimeric0#_s or _as, from lanes 2 and 3, respectively) and <link rid="fig5">Figure 5</link>d (labeled cDNA0#_s, from lane 9). The reference sequences are an alignment consensus and reference alleles for TEBPbeta and TEBPalpha from strain JRB310, as well as the Injected Template (the microinjected long RNA to guide rearrangement). A C-to-A nucleotide substitution, introduced into the Injected_Template, is indicated as a purple single-nucleotide substitution in the annotation. It is far enough from any recombination junction such that we

observed no substitution transfer, as in Nowacki *et al.*<link rid="bib18">[18]</link> This allows use of this site to distinguish endogenous from injected molecules. The sequences differ from the injected template at many segregating sites (gold), which confirm endogenous rearrangement. A *Sal*I site used to construct the fusion is indicated in yellow. Additional cDNA sequences were recovered but not shown.