Supplementary Material for:

Chromosome fusions triggered by noncoding RNA

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This file includes:

Oligonucleotide Sequences

Figures S1-S3

Description of Supplemental Alignments

Additional Supplemental Files

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Supplemental_Alignment_2

Supplemental_Alignment_3

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Supplemental_Alignment_5

Oligonucleotides (5' to 3')

Synthetic RNA oligonucleotides:

Contig11396-*TEBPβ* (27 nt)
/5'Phos/rUrGrGrGrUrUrArArArUrUrArCrUrCrArUrGrUrUrCrUrUrUrArUrA

Contig11396-*TEBPβ* (22 nt control)
/5'Phos/rUrArArArUrUrArCrUrCrArUrGrUrUrCrUrUrUrArUrA

Contig9.1-310.1 (27 nt)
/5'Phos/rUrCrUrUrCrArGrUrUrArUrArArUrUrArArGrArUrArArUrUrArArA

Contig11682-20527 (piRNA1) (27nt)

/5'Phos/rUrArArUrUrArUrArArUrCrUrUrCrUrUrCrArUrUrArUrUrArArUrC

Contig20527-16348 (piRNA2) (27nt)

/5'Phos/rUrUrArUrCrCrArArUrArArCrUrUrUrArUrArCrCrArCrArArArGrU

Contig7005.0 (27 nt)
/5'Phos/rUrGrUrCrUrArArUrUrGrUrGrArCrUrUrUrGrArArUrArUrArArArU

Synthetic DNA oligonucleotides used in injection:

Contig11396-*TEBPβ*
/5'Phos/TGGGTTAAATTACTCATGTTCTTTATA

Contig9.1-310.1
/5'Phos/TCTTCAGTTATAATTAAGATAATTAAA

Contig7005.0
/5'Phos/TGTCTAATTGTGACTTTGAATATAAAT

Primers used to generate the *TEBPβ/α* chimeric template:

*TEBPβ*_MDS_1F
CCCCGTTTAAGATTTATGATTTCAAGTTG

*TEBPβ*_MDS_3R
GATAACGATAACCTTGTCGTGGAGGTCG

*TEBPα*_MDS_14F
GATTACCGATGATAGAGCTGTCGAGA

*TEBPα*_MDS_18R
AACCCCAGACTAATGAATTGAGAAT

*AflII*_F (restriction site underlined)
AGCAAAGACACCTTAAGAACCCAATTCTAC

*AflII*_R (restriction site underlined)
ATTGGGTTCTTAAGGTGTCTTTGCTGGAG

Primers used to generate DNA for *in vitro* transcription of single-stranded RNA template (sense)

T7_telo_5'
GTAATACGACTCACTATAGGGCCCCAAAACCCCAAAACCCCGTTTAAGATTTATGATTTC

*TEBPα*_MDS_18R
AACCCCAGACTAATGAATTGAGAAT

Primers used to generate DNA for *in vitro* transcription of single-stranded RNA template (antisense)

T7_telo_3'
GTAATACGACTCACTATAGGGCCCCAAAACCCCAAAACCCCAAGACTAATGAATTTGAG

*TEBPβ*_MDS_1F
CCCCGTTTAAGATTTATGATTTCAAGTTG

Primers for detecting *TEBPβ/α* chimeric chromosomes (Figure 5b):

*TEBPβ*_MDS_2F
TCAAGCAACTCTACACCGAGCTATTCAAC

*TEBPα*_MDS_17R-1
GGCTCTCCCTTCCATTTGAATTCACA

***TEBPα*_MDS_17R-21**
CTAGGCTCTCCCTTCCATTTGAATTC

Primers for detecting cDNA of mRNA transcribed from *TEBPβ/α* chimeric chromosomes (Figure 5d):

*TEBPβ*_MDS_2F
TCAAGCAACTCTACACCGAGCTATTCAAC

*TEBPα*_MDS_16R
CGAGCTTCTTTCTGGCATCA

*TEBPα*_MDS_17R-2
CTAGGCTCTCCCTTCCATTTGAATTCACA

Primers for detecting *contig11396-TEBPβ* fusion chromosome (Figure 1c, 1d):

contig11396-F-inner
GCCAGCTAATGCACATGGAATGTGC

TEBPβ -R-inner
GCCATCAAGCAGAAAGGAGACCCC

Primers for sequencing complete *contig11396-TEBPβ* fusion chromosome (supplemental sequencing alignment 1):

contig11396-F-outer
CACCACTTGGCACTCCTAATTC

TEBPβ -R-outer
CAATTTGCAAGATATAGACCAATAATTTTAAG

Primers for detecting *contig9.1-contig310.1* fusion chromosome (Figure 2c):

contig9.1-F
CACTCATGCTTTAGGAAACTATGATGTATGGG

contig310.1-R
CATGTGGGAGTGCCTTGAGTGAG

Primers for detecting triple-chromosome fusion chromosome (Figure 3d and S1b,c):

11682mds6_newLinkF

CTACAGCATCACTGAATTCTTCAGTC

16348F_linkR

GTTAGTTTACAAGTGCCATTTCTG

Primers for detecting *contig7005* fusion chromosome (Figure 4b):

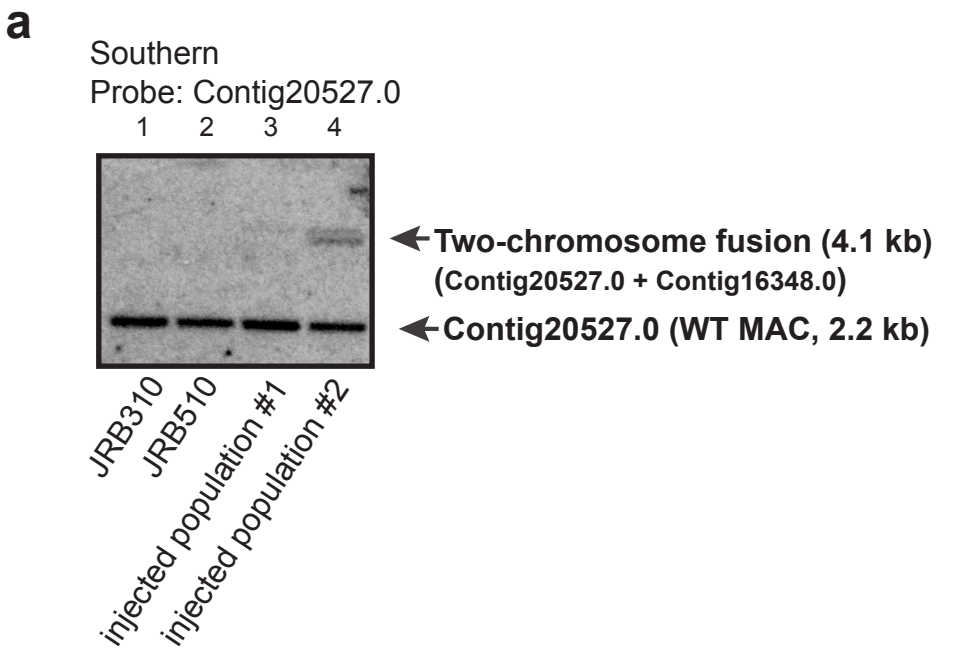
contig7005-F
CGACCCTATAATCCAAGATTTCAAATCC

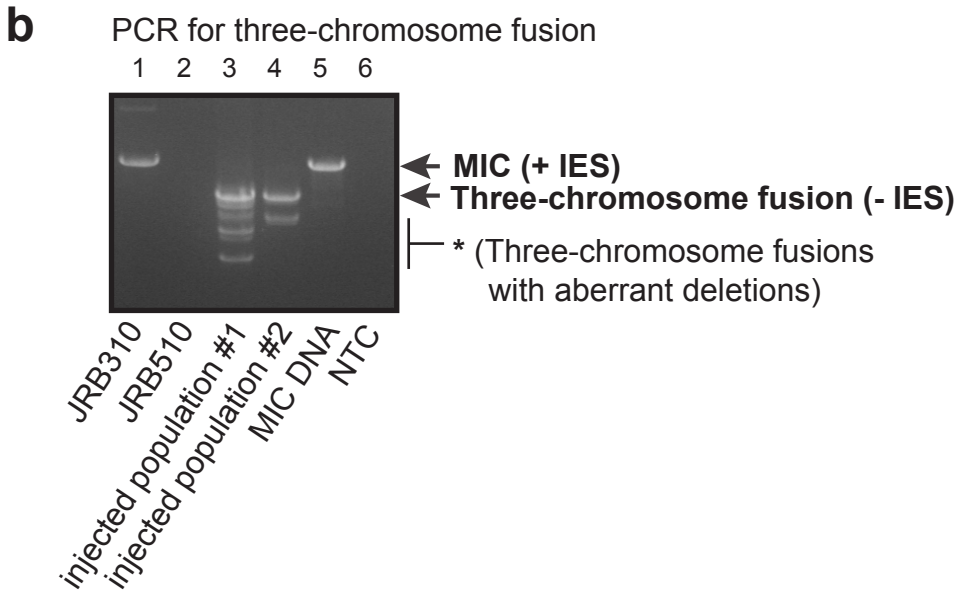
contig7005-R
GGGTTTTGCTTCAAAGTAAGGTTTGAGAGGG

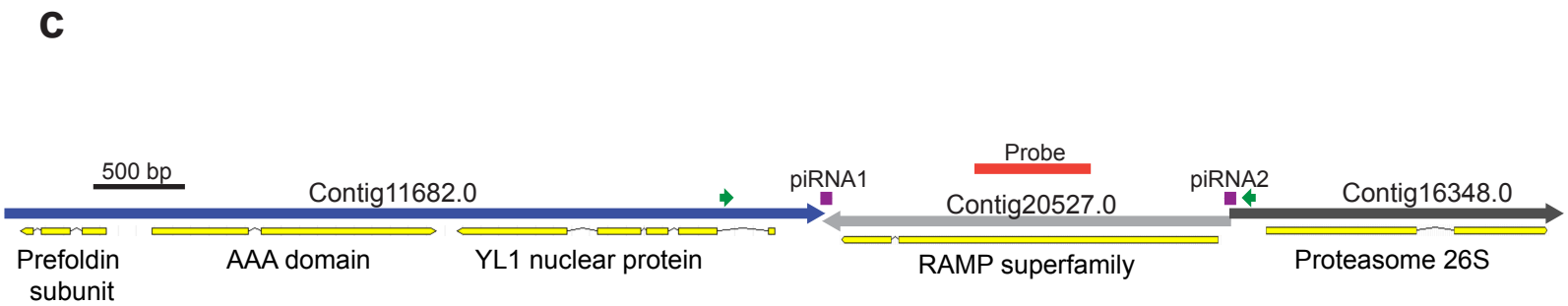
Primers for detecting *contig7005* fusion chromosome (Figure S2):

contig7005-F2
CCCAAACCCTCCACAGATGCTTG

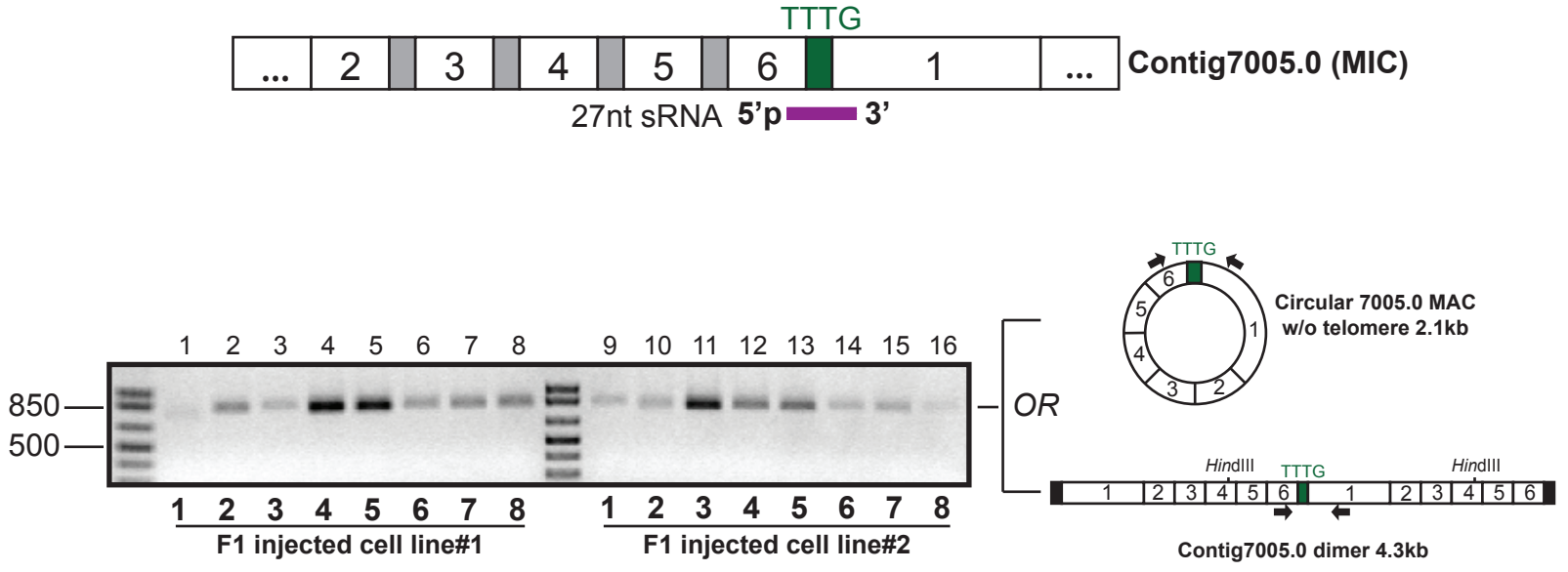
contig7005-R
GGGTTTTGCTTCAAAGTAAGGTTTGAGAGGG



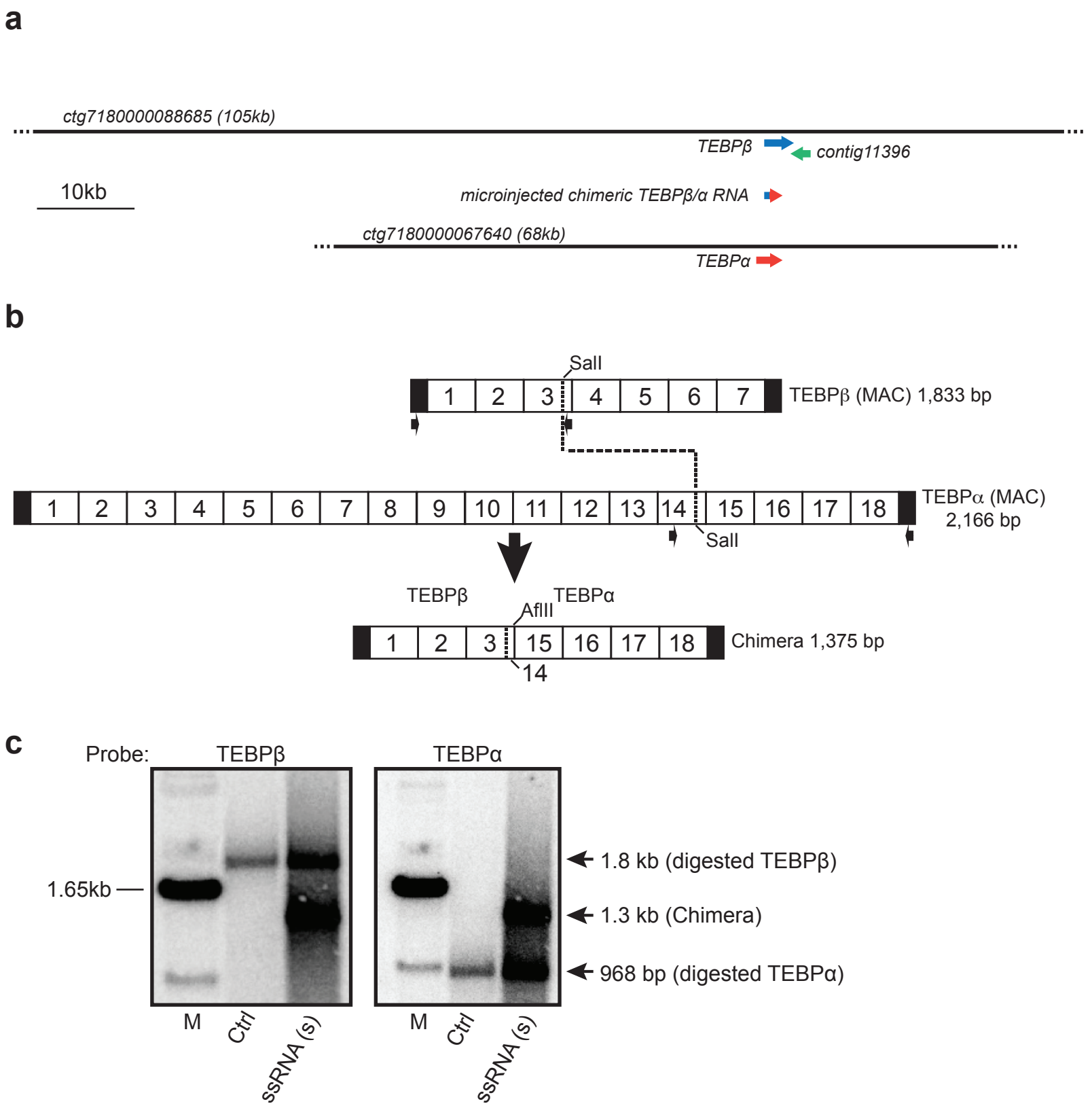




Supplemental Figure 1: Evidence that co-injection of two piRNAs leads to detectable levels of fusion of two and three chromosomes. piRNA1 and piRNA2 (small purple bars; Supplementary Data Alignment 4) were co-injected into populations of *Oxytricha* cells in two separate experiments (marked #1 and #2). JRB310 and JRB510 are two WT strains. **a**, Southern analysis of DNA isolated from these cells detected only the fusion of Contigs *20527.0* and *16348.0* (indicated by upper arrow), at higher levels in experiment #2. A probe for *Contig11682.0* failed to hybridize to any fusion band (data not shown), indicating that the more abundant fusion contained just *Contig20527.0* and *Contig16348.0*. **b**, PCR analysis revealed the presence of a triple chromosome fusion in both injected populations, along with aberrant deletion-containing products indicated by an asterisk (*). NTC, no template control. **c**, Map of predicted triple-fusion chromosome structure containing 5 genes. Protein coding exons are in yellow, oriented according to reading frame. PCR primers are green arrows; the three somatic chromosomes (*Contig11682.0*, *Contig20527.0*, and *Contig16348.0*) are annotated in blue, and light and dark grey, respectively. The probe sequence for the Southern shown in panel a is indicated as a red bar. Nine independent PCR products from injected population #1 (panel b lane 3) were completely sequenced and are shown in Figure 3b and Supplementary Data Alignment 4.



Supplemental Figure 2: Transmission of fused chromosome of *Contig7005.0* after 40 generations of vegetative growth. Actively growing *Oxytricha* cultures (Figure 4, lines #1 and #2), undergoing daily division were maintained for 40 days under vegetative growth conditions (see Materials and Methods). At the end of that time, eight cells from each culture were isolated and subjected to single-cell PCR to test for the presence of the fused chromosome, using the PCR primers shown as small arrows. WT (uninjected) and no-template PCR controls recovered no product and are not shown in the figure.



Supplemental Figure 3: Design and confirmation of *TEBPα* and *β* fusion. **a**, Germline map of *TEBPα* (red) and *TEBPβ* (blue)-encoding contigs drawn to scale. (Neighboring *contig11396*, used in piRNA-mediated fusion experiments described elsewhere in this manuscript, is indicated in green.) The injected chimeric template RNA is indicated between the two contig sequences. Thick black lines indicate micronuclear sequence, with 10kb scalebar shown. Arrowheads indicate gene orientation on MDS sequences, but the scale of the figure precludes annotating each MDS separately. *TEBPβ* is located on a 105 kb MIC genome contig, while *TEBPα* is located on a 68 kb MIC contig (Chen *et al.* 2014). The total micronuclear distance separating the two genes is at least 49kb. **b**, The fusion template was constructed by ligating two *Sal*I digested PCR products as shown. Introduction of an *Afl*II site permits discrimination of endogenously produced fusion products from injected sequences. **c**, Southern analysis of *Afl*II digested genomic DNA confirms the abundant presence of *TEBPβ/α* chimeric DNA molecules in the progeny of injected cells. M, marker.