Supplementary Information

SI Materials and Methods

Construction of *TPB2* mutants

The oligo DNAs *TPB2*-T1 and *TPB2*-T2 (SI Appendix, Table S1), which target "T1" and "T2", respectively (Figure. 3B), in the first exon of *TPB2* were introduced into the BbsI-digested pC9T vector (1) using the Gibson Assembly system (NEB). The resulting pC9T-T1 and T2 were digested with XhoI and introduced to the MAC *BTU1* locus of B2086 and CU428 cells using a biolistic gun. Six transformants of each were assorted until they grew in 10 mg/mL paromomycin without cadmium. Then, Cas9 expression was induced in 1 μg/mL $CdCl_2$ for 6 h, and single cells were isolated in drops and cultured for 1-2 days. Eight clones from each transformant line were chosen, and their *TPB2* MIC and MAC loci were amplified by PCR using the primer pair *TPB2*-a and *TPB2*-c or *TPB2*-b and *TPB2*-c, respectively (SI Appendix, Table S1). Clones #38 and #41 from B2086, which harbor the frameshift mutation *tpb2-1fs* in the MIC, and clones #6 and #44 from CU428, which harbor the frameshift mutations *tpb2-2fs* and *tpb2-3fs*, respectively in the MIC (see Figure 3B), were used in this study.

Western blotting

Whole-cell proteins from $5x10^4$ (for Tpb2p detection) or $5x10^3$ (for α-tubulin detection) cells were separated by SDS-PAGE and transferred to Immobilon-P membranes (Millipore). The blots were incubated with a 1:3,000-diluted rabbit anti-Tpb2 antibody (2) or a 1:10,000-diluted mouse anti-alpha-tubulin antibody (12G10, Developmental Studies Hybridoma Bank) in blocking solution (1% BSA, 1% skim milk, 0.1% Tween20 in PBS). Thereafter, the blots were visualized by incubation with a 1:10,000-diluted an HRP-conjugated anti-rabbit or mouse IgG antibody (Jackson Immuno Research) in blocking solution, followed by a chemiluminescent reaction. To reduce nonspecific signals, the anti-Tpb2 antibody was pre-absorbed with fixed starved *Tetrahymena* cells.

Immunofluorescent staining. Cells were fixed as described previously (3). The fixed cells were incubated with a 1:1,000-diluted anti-H3K9me3 (Abcam, Ab8898) or anti-H3K27me3 (Merck Millipore, 07-449) in blocking solution (3% BSA, 10% Goat Serum,

0.1% Tween 20 in PBS) followed by incubation with a 1:1000-diluted Alexa 488-conjugated anti-rabbit IgG. The samples were counter-stained with 10 ng/ml DAPI.

References for SI Materials and Methods

1. Suhren JH, et al. (2017) Negative Regulators of an RNAi-Heterochromatin Positive Feedback Loop Safeguard Somatic Genome Integrity in Tetrahymena. *Cell Rep* 18(10):2494–2507.
2. Vogt A, Mochizuki K (2013) A domesticated PiggyBac transposase interacts with heterochromatin and catalyzes reproducible DNA elimination in Tetrahymena. *PLoS Genet* 9(12):e1004032.
3. Loidl J, Scherthan H (2004) Organization and pairing of meiotic chromosomes in the ciliate Tetrahymena thermophila. *J Cell Sci* 117(Pt 24):5791–5801.

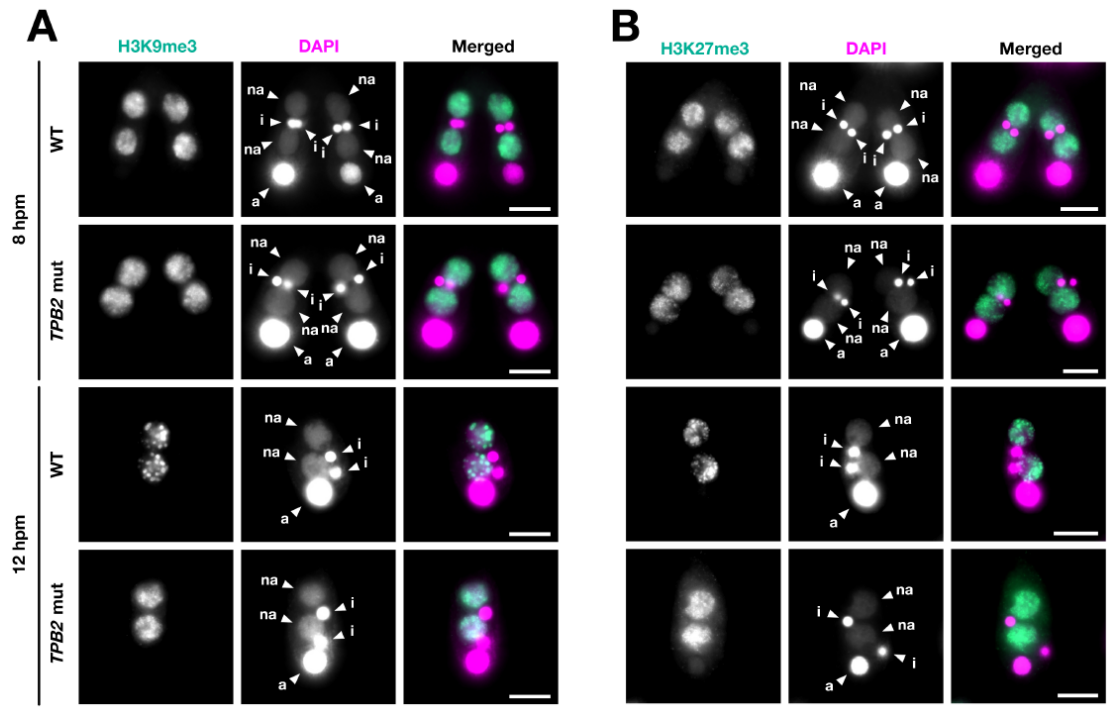

Figure S1. Accumulation of heterochromatic histone modifications

The accumulation of tri-methylation at histone H3 lysine 9 (H3K9 me3) (A) and lysine 27 (H3K27 me3) (B) in wild-type (WT) cells and *TPB2* mutants (*TPB2* mut, mating of #6 and #38) at 8 and 12 hr post-mixing (hpm) was analyzed by immunofluorescent staining using anti-H3K9 me3 and anti-H3K27 me3 antibodies (green). DNA was counterstained with DAPI (magenda). The parental MACs (a), the MICs (i) and the new MACs (na) are marked with arrowheads. Scale bar = 10 μm.

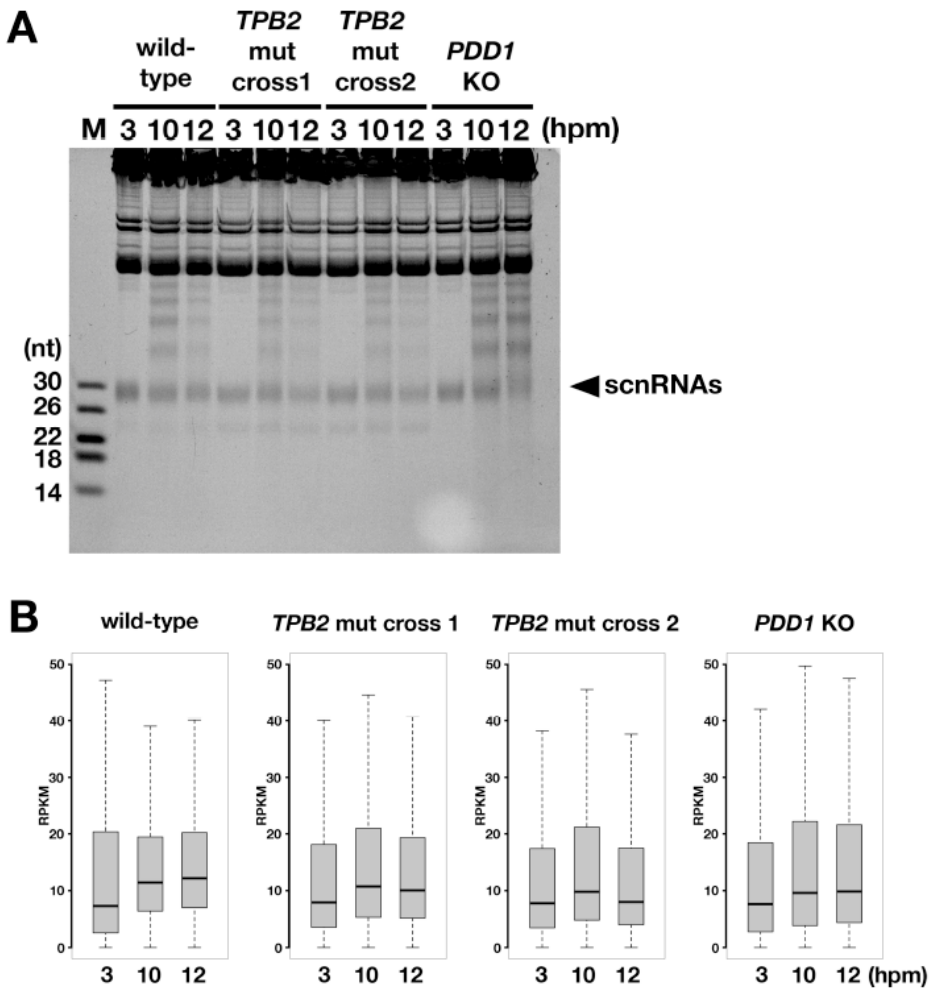

Figure S2. Accumulation of total and Type-A IES-mapping scnRNAs

Wild-type, *TPB2* mutant (cross 1 = #6 x #38, cross 2 = #41 x #44) and *PDD1* KO strains were mated and their small RNAs at 3, 10 and 12 hr post-mixing (hpm) were analyzed. (A) Total RNA was separated in a denaturing gel and stained by GelRed. The position of scnRNAs marked by an arrowhead. (B) Small RNAs were sequenced and normalized numbers (RPKM [reads per kilobase of unique sequences per million]) of 26- to 32-nt small RNAs that uniquely matched the 4,695 Type-A IESs are shown as box plots. The horizontal bar in the box indicates the median value. The bars on the top and bottom of the box indicate the minimum and maximum values, respectively, within 1.5x IQR.

Table S1: Oligo DNAs

TPB2_T1	GCTTTATAAGATTTCAAAAACTTTAATAGTTGCCTGTTGTAATGTATGACGAGTTTTAGAGCTAGAAATAGCAAG
TPB2_T2	GCTTTATAAGATTTCAAAAACTTTAATAGTTGGACAAAAAAGAACTTATGAGTTTTAGAGCTAGAAATAGCAAG
IES5_cir_FW	TTGAACTCACTATACTTCAGGC
IES5_cir_RV	AATAGCTTGTTCACTTTTGTGG
IES737_cir_FW	CTCTAATTTAAGTGTAATTGTTTTGAG
IES737_cir_RV	AATAATAAAATTTAAGTGGGAGGG
IES1147_cir_FW	TCGTATTCTATGATAAAATCATGGG
IES1147_cir_RV	GTATGTAGCCTTGAAAAAGGC
IES1988_cir_FW	ATTTGGTCAACCTAAATTAAAGG
IES1988_cir_RV	AGTCCTTACATAAAAGGCTTAG
IES4092_cir_FW	GAAATATTGGCAGTAGCATGTTTGAC
IES4092_cir_RV	TTCATCGTGATTGGAGATGTGG
IES4874_cir_FW	AAATATTGCTGCTGGACAGAGAG
IES4874_cir_RV	AATAGATGTTGAAAAGGGGGTG
RegionG_FW	ATTGCTTCGTATATCAAAAAAC
RegionG_RV	CTCAGCCAGAAACTGAAAATAGGG
RegionN_FW	CAATGCAGAAAGTGATTCTCAAC
RegionN_RV	CGTCTAAAAGTTAGTAACCAATC
RegionO_FW	GTCAATAGATGTGGGATTGAG
RegionO_RV	CTAGACAGTTATCAACTAAGATAAG
TPB2_a	TAAACTTAAAGAGAATCAGAAATCAACC
TPB2_b	ATGTTATTAAAAAAAAATCTGAGCTACCTC
TPB2_c	TAGAGAATTCAGTCCATCTCTTTTCCAC