Supplemental Information

Supplemental Methods

Supplemental Figure Legends

Supplemental Figures S1-S12

Supplemental Tables S1-S3

Supplemental References
Supplemental methods

Generation of Tetrahymena strains

To generate the C-terminal HA-tagging constructs, the coding sequences and 3’ flanking regions of the corresponding genes were PCR amplified using specific primers with 15-20 nt overlapping sequences (Supplemental Table S3). These PCR fragments, together with the neo4 cassette, were stitched together by fusion-PCR and cloned into the NotI site of pBluescript SK(-) vector (Promega) with In-Fusion cloning kit (Clontech), as previously described (1). RPB3, CBP20, PRP19, and THO2 were HA-tagged at their C-termini in their endogenous loci (2). *Tetrahymena* transformants were generated by particle bombardment (3). After phenotypic assortment in the presence of increasing concentration of paromomycin, complete replacement of the target locus in the macronucleus was confirmed by real-time PCR quantification. Complete replacement in the endogenous loci supports that the C-terminal HA-tagged proteins are functionally equivalent to their WT counterparts.

Immunofluorescence staining

The primary antibodies used were anti-RNF1 (1:500; this study), anti-NUD1 (1: 500; this study), anti-EMA1 (1: 500) (4), anti-TWI1 (1: 500) (4), anti-PDD1 (1:500; Abcam, ab5340), anti-H3K27me3 (1:500; Millipore, 07-449), anti-H3K9me3 (1:100; Millipore, 07-442). In co-staining studies, after incubation with the aforementioned antibodies (all rabbit-generated), a mouse monoclonal Alexa Fluor® 488 conjugated anti-HA antibody (Invitrogen, A21287) was used together with an Alexa Fluor® 555 conjugated goat anti-rabbit antibody (Invitrogen, A21428) in the secondary incubation. Alternatively, Alexa Fluor® 488 and Alexa Fluor® 555 labeling kits (Invitrogen) were used to separately label two primary antibodies. Digital images were collected using Olympus BX40 epi-fluorescence microscope and Zeiss LSM 510 confocal microscope.

RNA Extraction and RT-qPCR

Total RNA was extracted from WT, ΔEZL1 and ΔDCL1 cells at designated time points during conjugation using the RNeasy Protect Cell Mini Kit (Qiagen), as described (TetraFGD: http://tfgd.ihb.ac.cn/index/smphelp) (5). RNA with length less than 200 bp was enriched by mirVana™ miRNA Isolation Kit (Ambion). All RNA samples were treated with Turbo DNA™-free (Ambion) to eliminate
DNA contamination. scnRNA samples were reverse transcribed with the miScript reverse transcription kit (Qiagen). Long ncRNA samples were reverse transcribed using Superscript III® reverse transcriptase (Invitrogen) and random hexamer primers (New England Biolabs). mRNA samples were reverse transcribed with oligo(dT) primer. For the M element, quantitative PCR was performed with the M-mic-fwd primer for scnRNA, and the M-mic-fwd/rev primers for long ncRNA (Supplemental Table S3), using SYBR® green PCR master mix (Applied Biosystems).

**Histone ubiquitylation assay**

The assay was adopted from a published protocol for RNF8, a mammalian E3 ligase for ubiquitylating histones and possibly other substrates (6,7). Briefly, to the ubiquitylation buffer (50 mM Tris (pH 7.5), 5 mM MgCl₂, 2 mM NaF, 0.5 mM DTT) the following were added: 2 mM ATP, 1 µg Myc-ubiquitin, 0.1 µg E1 (human), 0.1 µg E2 (UbcH5c; human), 1 µg acid-extracted *Tetrahymena* histones, the EZL1 complex IPed from conjugating *Tetrahymena* cells, or other controls (up to 50 µl total volume). The reaction was incubated for 2h at 30°C and stopped by acidification. TCA-precipitated proteins were resolved by SDS-PAGE and ubiquitylation was detected by immunoblotting with an anti-Myc antibody. Note that endogenous ubiquitylation of *Tetrahymena* H2A and H2B does not interfere with this assay.

**Granularity analysis of IF images**

Image processing and measurement were performed by ImageJ (http://rsb.info.nih.gov/ij/). The Granulometric Filtering plugin (https://imagej.nih.gov/ij/plugins/gran-filter.html) was used to filter foci of certain sizes based on their similarity to Structuring Elements of increasing dimensions. Histograms represent granulometric size density distribution of the entire image at the corresponding channel (x-axis: foci dimension; y-axis: foci number). Note that this analysis can only process discrete foci, but not diffusive staining.
Supplemental figure legends

Supplemental figure S1. Nuclear morphology and key events during Tetrahymena conjugation.

A schematic of nuclear morphology and key events during Tetrahymena conjugation. Conjugation is induced by mixing starved cells of different mating types. Several conjugation stages are illustrated in the temporal order: meiosis (2-4h), pre-zygotic and post-zygotic divisions (4-6h), micronuclei/macronuclei differentiation (6-8h), early anlagen formation (8-12h), late anlagen development (2 mic/2 mac. 12-14h) and micronuclei re-absorption (1 mic/2 mac. >14h). Conjugating progress of DNA elimination mutants is usually arrested at the late anlagen development stage (2 mic/2 mac), without producing viable progeny. Mic: micronuclei; Mac: macronuclei; PM: parental macronuclei; AN: anlagen; OM: old macronuclei.

ncRNA transcription and scnRNA generation initiate at 2h conjugation, coincide with meiosis, and persist until late conjugation. Heterochromatin formation in anlagen, characterized by H3K27/H3K9 methylation and PDD1 binding, initiates in early anlagen and culminates in coalescence of DNA elimination bodies in late anlagen, followed by DNA elimination.

Supplemental figure S2. Epitope-tagging of EZL1 and affinity-purification of associated proteins.

(A) A schematic of the EZL1 epitope-tagging construct. The tandem FLAG-HA tag was inserted in frame before the EZL1 C-terminus by fusion PCR. A neo cassette (conferring paromomycin resistance) was inserted into the 3' UTR, ~500 bp downstream of the stop codon.

(B) Expression of epitope-tagged EZL1. Immunoblotting of whole-cell lysates from different time points (0, 3, 5, 8, 10, and 24h post-mixing) showed that EZL1 was expressed exclusively during conjugation, in a profile similar to PDD1.

(C) Functional equivalence of epitope-tagged EZL1 and wild-type (WT) EZL1. Unprocessed M element (red arrow) accumulated in conjugating ΔEZL1 cells, as a result of the DNA elimination defect (Lane 3). This defect was rescued by crossing with either WT (Lane 5 and 6: either mating type replaced with the corresponding WT strain) or epitope-tagged EZL1 cells (Lane 1 and 2: two independent strains). Conjugating WT cells was used as the positive control (Lane 4). M: DNA ladder.

(D) Affinity purification of epitope-tagged EZL1. Epitope-tagged EZL1 and WT cells were
immunoprecipitated with the anti-FLAG antibody, and immunoblotted with the anti-HA antibody. Epitope-tagged EZL1 was highly enriched in the FLAG peptide elution, while absent from the flow-through (FT).

(E) Co-fractionation of the EZL1 complex components (arrow) in gel filtration (fraction numbers indicated on top of the panel). IN (input): EZL1 and its associated proteins affinity-purified with the anti-FLAG antibody.

Supplemental figure S3. Coexistence of the EZL1 complex components.

(A) Similar expression patterns for the core components of the EZL1 complex: EZL1, ESC1, SUZ12, RNF1, RNF2, and NUD1. Microarray data (8) from *Tetrahymena* cells during growth, starvation, and conjugation are represented by the heat map to highlight the similarity between their gene expression patterns.

(B) Co-staining with an anti-HA antibody (against EZL1-FH in the tagged strain) and an RNF1-specific antibody. These two EZL1 complex components co-localized at both early and late conjugation. Green arrowhead: parental macronuclei; white arrowhead: micronuclei; green arrow: anlagen; white arrow: old macronuclei.

(C) Destabilization of NUD1 in the absence of any of the EZL1 complex component. Granularity analysis of the NUD1 foci in the images was provided in Supplemental Fig. S11D.

Supplemental figure S4. Components of Polycomb repressive complexes in *Tetrahymena* and *Drosophila*.

(A) The EZL1 complex components in *Tetrahymena*. PDD1 is included here for comparison, even though it is only loosely associated with the EZL1 complex core components. Positions for conserved domains were marked. The same color scheme is used for both *Tetrahymena* and *Drosophila*.

(B) Core components of the Polycomb repressive complexes in *Drosophila*.

Supplemental figure S5. Interaction between PDD1 and the EZL1 complex.

(A) Co-IP of PDD1 with the EZL1 complex. PDD1 was pulled down with HA-tagged EZL1 (EZL1-HA), with
or without PFA crosslinking. NUD1 was probed as the positive control.

(B) Influence of salt concentration on the interaction between PDD1 and the EZL1 complex. The EZL1 associated proteins were pulled down with anti-HA agarose beads, washed by buffers with different NaCl concentrations (0, 50, 100, and 150mM), and immunoblotted for PDD1 as well as NUD1 (positive and loading control). Note that decreasing amounts of PDD1 were associated with the EZL1 complex in the presence of increasing salt concentrations.

(C) Co-IP of the EZL1 complex with PDD1. NUD1 was pulled down with HA-tagged PDD1 (PDD1-HA). The interaction was not affected by the mutation disabling methyl-lysine binding (Y49A). Benzonase was included in the IP buffer to eliminate interference from nucleic acids.

(D) Co-localization of PDD1 and the EZL1 complex. Late conjugating cells with HA-tagged RNF1 (RNF1-HA) were co-stained with the anti-PDD1 and anti-HA antibodies. Note the overlapping staining in developing MAC (anlagen). Green arrowhead: parental macronuclei; white arrowhead: micronuclei; green arrow: anlagen; white arrow: old macronuclei.

(E) Transient localization of the EZL1 complex at the conjusome. IF staining of HA-tagged RNF1 revealed its presence in the conjusome (yellow arrows), a membraneless structure (most likely formed by phase separation) known to contain PDD1 (9).

**Supplemental figure S6.** Additional analyses of NUD1.

(A) Conserved domains in *Tetrahymena* NUD1. Three conserved glutamate residues (E) in the NUDIX box are indicated.

(B) Sequence alignment of the NUD1 NUDIX box and the C-terminal domain with other NUDIX domain-containing proteins.

(C) IF staining by the anti-NUD1 antibody. Specific staining in conjugating WT cells, but not ΔNUD1 cells. Green arrowhead: parental macronuclei; white arrowhead: micronuclei.

(D) Immunoblotting by the anti-NUD1 antibody. A band of the size predicted for NUD1 was detected in nuclear extract from WT cells; a band of slightly increased size was detected in cells with epitope-tagged NUD1 (NUD1-FH); no band was detected in ΔNUD1 cells.
Supplemental figure S7. *Tetrahymena* RBBP4 not associated with the EZL1 complex.

RNF1 was not co-immunoprecipitated with RBBP4 (RBBP4-FH, anti-FLAG), the *Tetrahymena* orthologue of *Drosophila* NURF55 and mammalian RBBP4/RBBP7 (auxiliary components in metazoan PRC2). IP with EZL1-FH was used as the positive control.

Supplemental figure S8. Additional analyses of RNF1 and RNF2.

(A) Presence of RING finger domains in *Tetrahymena* RNF1 and RNF2, as well as *Drosophila* dRing.

(B) Sequence alignment of ring-finger domains from *Tetrahymena* RNF1 and RNF2, as well as representative RING finger-containing PcG proteins in other species.

(C) Phylogenetic analysis of selected PcG proteins with RING-finger domains.

(D) No detectable ubiquitin E3 ligase activity associated with the EZL1 complex. Ubiquitylation levels increased with more E2 ligase (Ubc5), even in the absence of E3. An E2 conjugase amount (with low background was determined by titration (top panel). Ubiquitylation assay was performed with HA-ubiquitin and acid-extracted *Tetrahymena* histones as the substrates. Ubiquitylation assay samples were resolved by SDS-PAGE and analyzed by immunoblotting with the anti-HA antibody. When increasing amount of the EZL1 complex was introduced, no obvious change in the banding pattern was observed, compared to no input (-) and wild-type mock IP input (WT IP). We argue that the EZL1 complex lacks ubiquitin E3 ligase activity, though we cannot rule out that the result is due to the incompatibility between the mammalian E2 employed in this assay and the *Tetrahymena* E3.

(E) RIN1, but not RNF1 or RNF2, is required for the bulk of H2A ubiquitylation in *Tetrahymena* cells. Histones were acid extracted from WT, Δ*RIN1*, Δ*RNF1*, and Δ*RNF2* conjugating cells (5h post-mixing). Immunoblots were probed with anti-H2A and anti-H2B antibodies. The minor band on top of the major H2A band, representing mono-ubiquitylated H2A (H2Aub1), was abolished in the Δ*RIN1* lane, but not much affected in lanes corresponding to Δ*RNF1*, Δ*RNF2* as well as WT cells. H2B mono-ubiquitylation (H2Bub1) was examined as a negative control. The result is consistent with RIN1’s role reported previously (10).

Supplemental figure S9. Additional evidence supporting the EZL1 complex’s role in processing IESs and
CBSs.

(A) A schematic for generating alternative DNA elimination products (M long or M short) of the M element (M mic), a well-characterized IES (11,12). Box: IES; line: MDS; arrow: PCR primers.

(B) Single-cell PCR assay of DNA elimination at the M element. Note that the two processed forms, M long and M short, were detected in WT conjugation progeny, while only the unprocessed form, M mic, was detected in ΔRNF1.

(C) Time course analysis of DNA elimination at the M element. In WT cells (CU427 and CU428) and the null mutants of the EZL1-associated proteins (ΔESC1, ΔSUZ12, ΔRNF1, ΔRNF2, and ΔNUD1 cells, all derived from CU427 and CU428), only M long was found in their parental MAC. The appearance of M short in conjugant progeny is therefore indicative of successful processing of the M element in the developing MAC. Note that M short was only detected in WT conjugation progeny, while the unprocessed form, M mic, was detected in all the mutants.

(D) A schematic for CBS processing. After breakage at CBS, chromosomal ends are healed by de novo telomere addition. Box: CBS; line: MDS; circle: telomeres; arrow: PCR primers.

(E) Single-cell PCR assay of CBS processing. Note that only the unprocessed form—a long PCR product amplified with locus-specific primers spanning the CBS—was detected in ΔRNF1, while the processed forms—short PCR products anchored at one end by the telomere-specific primer—were only detected in WT conjugation progeny.

Supplemental figure S10. Accumulation of scnRNA and ncRNA in the EZL1 complex mutants.

(A) Accumulation of scnRNA in ΔRNF1 as well as WT cells during conjugation. Total RNA from WT and the mutant at starvation and different conjugation time points (hours post-mixing) were resolved on 12% sequencing gel and visualized by ethidium bromide staining. The position of conjugation-specific scnRNA is marked by arrowheads.

(B) Accumulation of M-related scnRNA during conjugation. RNA samples from WT and designated RNAi and Polycomb repression mutants (ΔDCL1, ΔEZL1, ΔESC1, ΔSUZ12, ΔRNF1, ΔRNF2, ΔNUD1, and ΔPDD1) at different conjugation time points (0, 2, 4, 6, 8, 10, 12, and 24h post-mixing) were reverse transcribed and analyzed by quantitative PCR for the levels of M-specific scnRNAs. Expression levels
were normalized against total RNA input (OD260) and plotted relative to the levels before conjugation
initiation.

(C) Accumulation of the M element transcripts during conjugation. RNA samples from WT and the mutants
(same as above) were reverse transcribed and analyzed by quantitative PCR for the levels of transcripts
from the M element.

**Supplemental figure S11.** Inter-dependency of TWI1, EMA1 and the EZL1 complex localization.

(A) WT, ΔTWI1, ΔEMA1, and ΔNUD1 cells at late conjugation (10h post-mixing) were IF stained by the
anti-TWI1, anti-EMA1, and anti-NUD1 antibodies, respectively. Cells were permeabilized with
detergent before fixation to reduce aggregation artifacts. Note the absence of staining for an antibody
in the corresponding KO cells.

(B) Dynamic distributions of TWI1, EMA1, and the EZL1 complex can be attributed to two opposing
forces for dispersing and coalescing, mediated by interactions between them.

(C) Granularity analysis of NUD1 signals in Fig. 5A (left) and PDD1 signals in Fig. 6A (right). Only the top
panels were analyzed, as they did not have diffusive staining that cannot be analyzed by this method.

(D) Granularity analysis of PDD1 signals in Supplemental Fig. S3C.

**Supplemental figure S12.** PDD1 is not absolutely required for EZL1-dependent H3K27 and H3K9
methylation.

(A) PDD1 is not absolutely required for H3K27me3 and H3K9me3 in the developing MAC. WT and ΔPDD1
(homozygous homokaryon) cells at late conjugation (10h post-mixing) were IF stained by anti-
H3K27me3 and anti-H3K9me3 antibodies, respectively. H3K9me3 levels were reduced—but not
eliminated—in ΔPDD1, contradicting a previous report (13). Note the lack of signal aggregation in the
mutant.

(B) EZL1-dependent H3K27me3 in the parental MAC is not affected in the methyl-binding deficient PDD1
mutant. WT, EZL1 H721A (methyltransferase dead), and PDD1 Y49A (methyl-binding deficient) cells at
early conjugation (6h post-mixing) were IF stained by the anti-H3K27me3 antibody. Note H3K27me3
staining in the parental MAC of WT and PDD1 Y49A cells, but not in the parental MAC of EZL1 H721A
cells. H3K27me3 in the MIC is not EZL1-dependent, serving as a positive control here.
**Supplemental figure S1.** Nuclear morphology and key events during *Tetrahymena* conjugation.
Supplemental figure S2. Epitope-tagging of EZL1 and affinity-purification of associated proteins.
Supplemental figure S3. Coexistence of the EZL1 complex components.
Supplemental figure S4. Components of Polycomb repressive complexes in *Tetrahymena* and *Drosophila*.
Supplemental figure S5. Interaction between PDD1 and the EZL1 complex.
Supplemental figure S6. Additional analyses of NUD1.
Supplemental figure S7. *Tetrahymena* RBBP4 not associated with the EZL1 complex.
Supplemental figure S8. Additional analyses of RNF1 and RNF2.
Supplemental figure S9. Additional evidence supporting the EZL1 complex’s role in IES and CBS processing.
Supplemental figure S10. Accumulation of scnRNA and ncRNA in the EZL1 complex mutants.
Supplemental figure S11. Inter-dependency of TWI1, EMA1 and the EZL1 complex localization.
Supplemental figure S12. PDD1 is not required for EZL1-dependent H3K27 and H3K9 methylation.
Supplemental Tables

**Supplemental table S1.** Summary of mass spectrometry results.

**Supplemental table S2.** *Tetrahymena* strains used in this study.

**Supplemental table S3.** PCR primers used in this study.
### EZL1 IP

| Protein | # unique peptides |
|---------|------------------|
| RNF1    | 20               |
| RNF2    | 18               |
| **EZL1** | **16**           |
| NUD1    | 12               |
| ESC1    | 10               |
| SUZ12   | 2                |

### EZL1 X-link IP

| Protein | # unique peptides |
|---------|------------------|
| RNF1    | 4                |
| RNF2    | 6                |
| **EZL1** | **15**          |
| NUD1    | 4                |
| ESC1    | 7                |
| SUZ12   | 2                |
| **EMA1** | **4**            |

### RNF1 IP

| Protein | # unique peptides |
|---------|------------------|
| RNF1    | 7                |
| RNF2    | 4                |
| **EZL1** | **7**           |
| NUD1    | 2                |
| ESC1    | 3                |
| **PDD1** | **6**           |

Red  | Bait  
Black | Prey

**Supplemental table S1.** Summary of mass spectrometry results.
| Strain     | Description                                | Origin/Reference |
|-----------|--------------------------------------------|-----------------|
| CU427     | WT                                         | Tetrahymena Stock Center |
| CU428     | WT                                         | Tetrahymena Stock Center |
| ΔDCL1     | Germline knockout                          | (14)            |
| ΔTWI      | Germline knockout                          | (15)            |
| ΔEMAI     | Somatic knockout                           | (4)             |
| ΔPDD1     | Germline knockout                          | (16)            |
| ΔEZL1     | Somatic knockout                           | (12)            |
| ΔESC1     | Somatic knockout                           | This study       |
| ΔSUZ12    | Somatic knockout                           | This study       |
| ΔRNFI     | Somatic knockout                           | This study       |
| ΔRNF2     | Somatic knockout                           | This study       |
| ΔNUD1     | Somatic knockout                           | This study       |
| EZL1-HA   | C-terminal HA tagging, endogenous locus, somatic | This study       |
| EZL1 H721A-HA | C-terminal HA tagging, endogenous locus, somatic | This study       |
| ESC1-HA   | C-terminal HA tagging, endogenous locus, somatic | This study       |
| SUZ12-HA  | C-terminal HA tagging, endogenous locus, somatic | This study       |
| RNFI-HA   | C-terminal HA tagging, endogenous locus, somatic | This study       |
| RNF2-HA   | C-terminal HA tagging, endogenous locus, somatic | This study       |
| NUD1-HA   | C-terminal HA tagging, endogenous locus, somatic | This study       |
| PDD1-HA   | C-terminal HA tagging, endogenous locus, somatic | This study       |
| PDD1 Y49A-HA | C-terminal HA tagging, endogenous locus, somatic | This study       |
| RPB3-HA   | C-terminal HA tagging, endogenous locus, somatic | (2)             |
| CBP20-HA  | C-terminal HA tagging, endogenous locus, somatic | (2)             |
| PRP19-HA  | C-terminal HA tagging, endogenous locus, somatic | (2)             |
| THO2-HA   | C-terminal HA tagging, endogenous locus, somatic | (2)             |

**Supplemental table S2.** *Tetrahymena* strains used in this study.
### Primmers for inserting HA tag (red sequences corresponding to the tag)

| Oligo name          | Sequence (5'-3')                                                                 |
|---------------------|---------------------------------------------------------------------------------|
| ESC1-Nfwd-HA        | GATGACAAGGGATACCCCTAAGGCTCCCGACTACGCC AGCTTTATAGAAAAAATAGAATAT GAA            |
| ESC1-Nrev-HA        | GTCTAGGGGATATCCTTCGTCTGTGAGCTCC TTATCTGCTGTTTAAATGCTTAAT                           |
| RNF1-Cfwd-HA        | GATGACAAGGGATACCCCTAAGGCTCCCGACTACGCC TTATCTGCTGTTTAAATGCTTAAT                           |
| RNF1-Crev-HA        | GATGACAAGGGATACCCCTAAGGCTCCCGACTACGCC AGCTTTATAGAAAAAATAGAATAT GAA            |
| RNF2-Cfwd-HA        | GATGACAAGGGATACCCCTAAGGCTCCCGACTACGCC AGCTTTATAGAAAAAATAGAATAT GAA            |
| RNF2-Crev-HA        | GATGACAAGGGATACCCCTAAGGCTCCCGACTACGCC AGCTTTATAGAAAAAATAGAATAT GAA            |
| NUD1-Cfwd-HA        | GATGACAAGGGATACCCCTAAGGCTCCCGACTACGCC AGCTTTATAGAAAAAATAGAATAT GAA            |
| NUD1-Cr2547-HA      | GATGACAAGGGATACCCCTAAGGCTCCCGACTACGCC AGCTTTATAGAAAAAATAGAATAT GAA            |
| SUZ12-Cf4661-HA     | GATGACAAGGGATACCCCTAAGGCTCCCGACTACGCC AGCTTTATAGAAAAAATAGAATAT GAA            |
| SUZ12-Cr4660-HA     | GATGACAAGGGATACCCCTAAGGCTCCCGACTACGCC AGCTTTATAGAAAAAATAGAATAT GAA            |

### Primmers for generating knockout constructs for the EZL1 complex components (red sequences are required for neo4-cassette insertion)

| Oligo name          | Sequence (5'-3')                                                                 |
|---------------------|---------------------------------------------------------------------------------|
| ESC1_5fwd1          | GCAAGTTTGGGCTTAAATTAAATCAG                                                                 |
| ESC1_3rev1          | CCATCCAAATTTCTACGTCAAGATTG                                                                 |
| ESC1_3rev1_neoPL    | CTCTCGAGTGGCGCGCCGCGGTGG CATCTCAATATCTATAAATAAQGCTTAATAGAAAAAATAGAATAT GAA            |
| RNF1_5fwd1_neoSma   | TTCAAAATTTTTACTGGAACCCGGGAAG GTGATTTTTCTACATAAAAACTCA                                           |
| RNF1_3rev1_neoPL    | GAAAAGTTTGGGCTTAAATTAAATCAG                                                                 |
| RNF1_3rev1_neoPL    | CTCTCGAGTGGCGCGCCGCGGTGG CATCTCAATATCTATAAATAAQGCTTAATAGAAAAAATAGAATAT GAA            |
| RNF1_Cfwd1_neoSma   | TTCAAAATTTTTACTGGAACCCGGGAAG GTGATTTTTCTACATAAAAACTCA                                           |
| RNF2_5fwd1_neoPL    | CTCTCGAGTGGCGCGCCGCGGTGG CATCTCAATATCTATAAATAAQGCTTAATAGAAAAAATAGAATAT GAA            |
| RNF2_5fwd1_neoPL    | CTCTCGAGTGGCGCGCCGCGGTGG CATCTCAATATCTATAAATAAQGCTTAATAGAAAAAATAGAATAT GAA            |
| RNF2_3rev1_neoPL    | CTCTCGAGTGGCGCGCCGCGGTGG CATCTCAATATCTATAAATAAQGCTTAATAGAAAAAATAGAATAT GAA            |
| RNF2_5fwd1_neoPL    | CTCTCGAGTGGCGCGCCGCGGTGG CATCTCAATATCTATAAATAAQGCTTAATAGAAAAAATAGAATAT GAA            |
| NUD1_5fwd1           | CTCTCGAGTGGCGCGCCGCGGTGG CATCTCAATATCTATAAATAAQGCTTAATAGAAAAAATAGAATAT GAA            |
| NUD1_5fwd1           | CTCTCGAGTGGCGCGCCGCGGTGG CATCTCAATATCTATAAATAAQGCTTAATAGAAAAAATAGAATAT GAA            |
| NUD1_5fwd1           | CTCTCGAGTGGCGCGCCGCGGTGG CATCTCAATATCTATAAATAAQGCTTAATAGAAAAAATAGAATAT GAA            |
| SUZ12_5f3556_Nodf    | CTCTCGAGTGGCGCGCCGCGGTGG CATCTCAATATCTATAAATAAQGCTTAATAGAAAAAATAGAATAT GAA            |
| SUZ12_35209_nf       | CTCTCGAGTGGCGCGCCGCGGTGG CATCTCAATATCTATAAATAAQGCTTAATAGAAAAAATAGAATAT GAA            |
| SUZ12_35210_ntr      | CTCTCGAGTGGCGCGCCGCGGTGG CATCTCAATATCTATAAATAAQGCTTAATAGAAAAAATAGAATAT GAA            |

### Primmers for detecting NMC-associated CBS processing

| Oligo name          | Sequence (5'-3')                                                                 |
|---------------------|---------------------------------------------------------------------------------|
| NMC3_r356           | GATGTTAGATAATTTAAAATTCTC                                                                 |
| NMC1_55600          | GATGTTAGATAATTTAAAATTCTC                                                                 |
| NMC1_r55732          | GATGTTAGATAATTTAAAATTCTC                                                                 |

### Primmers for detecting NMC-associated IES processing

| Oligo name          | Sequence (5'-3')                                                                 |
|---------------------|---------------------------------------------------------------------------------|
| NMC1_r55732          | GATGTTAGATAATTTAAAATTCTC                                                                 |

### Primmers for MDS control

| Oligo name          | Sequence (5'-3')                                                                 |
|---------------------|---------------------------------------------------------------------------------|
| JMJ1_f2071          | GATGTTAGATAATTTAAAATTCTC                                                                 |
| JMJ1_f2236          | GATGTTAGATAATTTAAAATTCTC                                                                 |

### Primmers for detecting mobilization of the Tc1/mariner element (2)

| Oligo name          | Sequence (5'-3')                                                                 |
|---------------------|---------------------------------------------------------------------------------|
| Tc1_f3169           | GATGTTAGATAATTTAAAATTCTC                                                                 |
| Tc1_f3201           | GATGTTAGATAATTTAAAATTCTC                                                                 |
| Tc1_f8878           | GATGTTAGATAATTTAAAATTCTC                                                                 |
| Tc1_f9835           | GATGTTAGATAATTTAAAATTCTC                                                                 |

### Primmers for detecting DNA elimination at the M element

| Oligo name          | Sequence (5'-3')                                                                 |
|---------------------|---------------------------------------------------------------------------------|
| M-5fwd-1            | GATGTTAGATAATTTAAAATTCTC                                                                 |
| M-3rev-1            | GATGTTAGATAATTTAAAATTCTC                                                                 |

### Primmers for detecting CBS processing at 819-mic

| Oligo name          | Sequence (5'-3')                                                                 |
|---------------------|---------------------------------------------------------------------------------|
| 819-F1              | GATGTTAGATAATTTAAAATTCTC                                                                 |
| 819-R1              | GATGTTAGATAATTTAAAATTCTC                                                                 |
| 819-F2              | GATGTTAGATAATTTAAAATTCTC                                                                 |
| 819-R2              | GATGTTAGATAATTTAAAATTCTC                                                                 |

### Primmers for ChiP and RIP

| Oligo name          | Sequence (5'-3')                                                                 |
|---------------------|---------------------------------------------------------------------------------|
| M_f108(M1)          | GATGTTAGATAATTTAAAATTCTC                                                                 |
| M_r230(M1)          | GATGTTAGATAATTTAAAATTCTC                                                                 |
| M_f359(M2)          | GATGTTAGATAATTTAAAATTCTC                                                                 |
| M_r757(M2)          | GATGTTAGATAATTTAAAATTCTC                                                                 |
| M_f813(M3)          | GATGTTAGATAATTTAAAATTCTC                                                                 |
| M_r988(M3)          | GATGTTAGATAATTTAAAATTCTC                                                                 |
| JMJ1_f2071          | GATGTTAGATAATTTAAAATTCTC                                                                 |
**Primers for RT-PCR analysis of TE expression**

| Primer   | Sequence                        |
|----------|---------------------------------|
| hAT f727 | TGCTGCACAAATCTTATTC             |
| hAT r904 | TCCATCTAGTCAGTTAGTC             |
| Tcl f4018| TTGAAAAAGATAAAAAAGAGAGCACG     |
| Tcl r4215| CAAGTTTGGCGTTAGTAAAGC          |
| ngoA f2090| CAAGTTGAAAGTCAGACTGTC         |
| ngoA r2234| CACACATCTTTTAGCAAAGTAG        |
| TPB2 f4412| ACACAAGCAAGATCATCTTATGG       |
| TPB2 r4577| TCTTTGTTTAGTAGATAGCTGCG       |

**Primers for quantitative PCR analysis of scnRNAs and precursor ncRNAs associated with the M element**

| Primer   | Sequence                        |
|----------|---------------------------------|
| M-mic-fwd| GGTGTTTCTAGTGTAGTTGAGTAG       |
| M-mic-rev| TTGAAAGCTAAAGTGTAGCTTTCGTC     |

**Supplemental table S3.** PCR primers used in this study.
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