Supplemental Material

Loss of \( I(3)mbt \) leads to acquisition of the ping-pong cycle in \textit{Drosophila} ovarian somatic cells

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Supplemental Materials and Methods

Fly stock, cell culture and RNAi

Oregon-R was maintained at 25°C. Δmbt-OSCs and OSCs were grown at 26°C in culture medium prepared from Shields and Sang M3 Insect Medium (Sigma) supplemented with 0.6 mg/mL glutathione, 10% FBS, 10 mU/mL insulin, and 10% fly extract (Saito et al. 2009). Cell proliferation was monitored with the xCELLigence RTCA SP system (ACEA). For the RNAi of OSCs shown in Supplemental Fig. S1B, trypsinized cells (3×10^6 cells) were suspended in 100 µL of Solution V of the Cell Line Nucleofector Kit V (Amaxa Biosystems) together with 200 pmol of siRNA duplex. Transfection was conducted in electroporation cuvettes using a Nucleofector device 2b (Amaxa Biosystems). For the RNAi of Δmbt-OSCs and OSCs shown in Supplemental Fig. S1C, trypsinized cells (3×10^6 cells) were suspended in 20 µL of Solution SF of the Cell Line Nucleofector Kit SF (Amaxa Biosystems) together with 200–400 pmol of siRNA duplex. Transfection was conducted in a 96-well electroporation plate using a Nucleofector device 96-well Shuttle (Amaxa Biosystems). The transfected cells were transferred to fresh OSC medium and incubated at 26°C for 2–4 days for further experiments. S2 cells were grown at 26°C in Schneider's Insect medium (Sigma) supplemented with 10% fetal calf serum (Gibco), 50 µg/mL streptomycin, and 50 µg/mL penicillin (Sato et al. 2010). For RNAi in S2 cells, l(3)mbt cDNA was amplified by RT-PCR from OSC total RNA using gene-specific primers. It should be noted that the 5’ end of each primer contained the T7 RNA polymerase promoter sequence. PCR products were purified using the FastGene Gel/PCR Extraction Kit (NIPPON Genetics) according to the manufacturer's instructions. Purified PCR products were used to produce dsRNAs using a T7-Scribe Standard RNA IVT Kit (CELLSCRIPT). The resultant RNAs were purified according to the manufacturer’s instructions, heated for 5 min at 95°C, and then left to cool to room temperature. Then, 5×10^6 cells were suspended with 5 µg of dsRNA and incubated for 4 days at 26°C. The siRNAs and PCR primers used in this study are summarized in Supplemental Table S3.

Plasmid constructs and OSC transfection

Construction of the Myc-Aub, Myc-AGO3, and Myc-EGFP vectors used in this
study was described previously (Sato et al. 2015). To construct a Myc-Vasa expressing plasmid, a full-length vasa cDNA was amplified by RT-PCR and subcloned into pAcM under the control of the actin 5C promoter. SgRNA-expressing vectors were produced by inverse PCR using pU6-Bbsl-chiRNA (Addgene, 45946). Primers are summarized in Supplemental Table S3. OSC transfection was performed using Xfect Transfection Reagent (Clontech) as reported previously (Saito et al. 2009; Saito et al. 2010; Sato et al. 2015) or ScreenFect A (Wako) following the manufacturer’s protocol (in Fig. 4B).

**Immunoprecipitation**

Immunoprecipitation was performed as previously described (Sato et al. 2015). In brief, immunoprecipitation of Piwi and Aub complexes from Δmbt-OSCs was performed using anti-Piwi (Saito et al. 2006) and anti-Aub (Nishida et al. 2007) antibodies in NP40 buffer. Immunoprecipitation of AGO3 complexes was performed using an anti-AGO3 antibody (a kind gift from Dr. Dahua Chen, Chinese Academy of Sciences, China) in RIPA binding buffer.

**Western blotting**

Western blotting was performed in principle as described previously (Miyoshi et al. 2005). Anti-Piwi, anti-Aub, anti-AGO3, anti-Krimp, anti-Spn-E, anti-Tud, anti-Vret, anti-Mael, anti-Armi, anti-GTSF1, anti-Yb, and anti-sDMA-AGO3 antibodies have been described previously (Saito et al. 2006; Nishida et al. 2007; Gunawardane et al. 2007; Nishida et al. 2009; Saito et al. 2010; Sato et al. 2011; Nagao et al. 2011; Ohtani et al. 2014; Sato et al. 2015). The anti-Qin antibody was kindly gifted by Dr. T. Kai. The mouse monoclonal anti-Vasa antibody was raised specifically against the full-length protein, essentially as described previously (Ishizuka et al. 2002) and was purified from the culture supernatant of hybridoma cells under standard procedures using Thiophilic-Superflow Resin (BD Biosciences). The anti-βTub antibody was obtained from the Developmental Studies Hybridoma Bank and used at a dilution of 1:1,000. Anti-mouse IgG, HRP-linked antibody (MP Biomedicals, 55558), and anti-rabbit IgG, HRP-linked antibody (CST) were used at dilutions of 1:5,000 and 1:1,000, respectively.
**Immunofluorescence**

Immunostaining of Δmbt-OSCs and OSCs was performed as previously described (Sato et al. 2015). The anti-Myc (Sigma C3956) antibody was used at 1:500. Anti-Piwi, anti-Aub, and anti-Krimp, anti-AGO3, and anti-Yb antibodies have been described previously (Saito et al. 2010; Sato et al. 2015; Nishida et al. 2007; Gunawardane et al. 2007). The anti-Vasa antibody was used at 1:250. Alexa Fluor488-conjugated anti-mouse IgG, Alexa Fluor546 conjugated anti-mouse IgG1, and Alexa Fluor488-conjugated anti-mouse IgG2a (Molecular Probes) were used as secondary antibodies. Cells were mounted in VECTASHIELD with DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) (Vector). All images were collected using a Zeiss LSM510 laser scanning microscope (Carl Zeiss). Image processing and annotation were performed using Adobe Photoshop (Adobe), ZEN (Carl Zeiss), and ImageJ software (National Institute of Health).

**qRT-PCR**

qRT-PCR was performed as previously described (Sato et al. 2015). In brief, total RNAs were isolated using ISOGEN (Nippon Gene) according to the manufacturer’s instructions. Total RNAs were treated with DNase to eliminate DNA contamination. Total RNA (1 µg) was annealed with an oligo-dT primer and reverse transcribed using a Transcriptor First strand cDNA Synthesis Kit (Roche) according to the manufacturer’s instructions. The resulting cDNAs were amplified with StepOnePlus (Applied Biosystems) using SYBR Premix Ex Taq (TaKaRa). The primer sets used are shown in Supplemental Table S1. The amplification efficiency of a qPCR reaction was calculated based on the slope of the standard curve. After confirming the amplification efficiency values (between 95% and 105%), relative steady-state RNA levels were determined from the threshold cycle for amplification.

**Visualization of small RNAs**

Visualization of small RNAs was performed as previously described (Sato et al. 2015). In brief, for RNA labeling with radioisotopes, total RNAs and
immunopurified RNAs were dephosphorylated with CIP (NEB), and then labeled with $\gamma^{32}$P-ATP using T4 polynucleotide kinase (TaKaRa). The radiolabeled RNAs were separated on a denaturing polyacrylamide gel. The signal was detected using a Typhoon FLA 9500 (GE Healthcare).

**Preparation of total RNA libraries and bioinformatic analysis**
rRNAs were removed from isolated total RNAs using a Ribo-Zero rRNA Removal Kit (Human/Mouse/Rat) (Illumina). Total mRNA libraries were prepared using a TruSeq Stranded mRNA HT Sample Prep Kit (Illumina) according to the manufacturer’s instructions and sequenced using a HiSeq 2500 (Illumina). Paired-end sequence reads were split into forward and reverse reads and mapped separately on the *Drosophila* genome (Dm3, BDGP Release 5), and then annotated to *Drosophila* genes defined in the UCSC Genome Browser (FlyBase) using Cuffcompare. Differential expression analysis of mRNA-seq expression profile was performed using edgeR package in R. The lists of differentially expressed genes ($p < 0.05$) were applied for functional annotation (gene ontology) analysis using DAVID. Paired-end sequence reads were also mapped to the transposon consensus sequences (Senti et al. 2015). To compare mRNA abundance between two libraries, FPKM (fragments per kilobases of exons per million mapped reads) normalization was performed.

**Preparation of small RNA libraries and bioinformatic analysis**
Cloning and analysis of small RNA libraries were performed essentially as previously described (Sato et al. 2015). In brief, 20–30 nt small RNA libraries were prepared manually based on the manufacturer’s instructions of a TruSeq Small RNA Sample Prep Kit (Illumina), and then sequenced using MiSeq (Illumina). Reads perfectly mapped on the *Drosophila* genome (Dm3, BDGP Release 5) were used. Alignments overlapping with rRNAs, tRNAs and snoRNAs were removed, and then small RNAs in the range of 24–30 nt were selected. The mapped reads for immunoprecipitation libraries do not reflect actual cellular abundance and, therefore, we performed a relative comparison of how piRNA sequences are loaded on to each PIWI protein. For transposon analysis, we mapped all 24–30 nt small RNAs to *Drosophila* transposable
element sequences obtained from the UCSC Genome Browser (RepeatMasker). Sense/antisense strand bias, sequence logo, and ping-pong signature on each transposon or all transposons were analyzed as previously described (Sato et al. 2015). To calculate ping-pong $Z_{10}$ scores, overlaps at position 1–9 and 11–25 nt were used as background. Phasing analysis was performed as previously described (Han et al. 2015). The score for a 3'-to-5' distance was calculated by $\Sigma_{\text{minimal}} (M_i, N_{i+x})$; $M_i$ is the number of upstream reads whose 3' ends are located at position $i$, and $N_{i+x}$ is the number of downstream reads whose 5' ends are located at position $i+x$. To calculate the ping-pong $Z_1$ score, overlaps at position 0 and 2–50 nt were used as background.

Deep sequencing datasets have been deposited in the DDBJ Sequence Read Archive under the accession number DRA004265.
Figure legends for Supplemental Figures and Tables

Supplemental Figure S1. Ectopic expression of piRNA factors in \textit{l(3)mbt}-depleted OSCs. (A) Genomic structure of the \textit{l(3)mbt} gene and siRNA target sites. (B) qRT-PCR shows that \textit{vasa} and \textit{ago3} were up-regulated in OSCs upon \textit{l(3)mbt} RNAi treatment. In contrast, the \textit{piwi} mRNA level was unchanged by \textit{l(3)mbt} depletion. \textit{rp49} was used as an internal control. \textit{N = 3}; error bars indicate SEM. (C) Western blotting confirms up-regulation of Aub, AGO3, and Vasa by \textit{l(3)mbt} depletion in OSCs. \textit{β} Tubulin (\textit{β}Tub) was employed as a loading control.

Supplemental Figure S2. Expression of piRNA factors and MBTS/non-MBTS genes in \textit{Δmbt}-OSC. (A) Transcript abundance of small RNA biogenesis factors (germ specific, germ + soma, and soma specific) (Handler et al. 2013) in OSCs and \textit{Δmbt}-OSC measured by mRNA-seq. FPKM: Fragments per kilobase of exon per million mapped sequence reads. (B) Left: Scatter plot comparing transcript abundance (mRNA-seq) of MBTS and non-MBTS genes in OSCs and \textit{Δmbt}-OSC. Classification of MBTS and non-MBTS genes was carried out with reference to Janic et al. (Janic et al. 2010). Right: Transcript abundance of MBTS and non-MBTS genes in OSCs and \textit{Δmbt}-OSC measured by mRNA-seq. (C) Real-time monitoring of cell proliferation. Cell-sensor impedance was expressed as an arbitrary unit called the Cell index. The Cell index values were plotted against time.

Supplemental Figure S3. Characterization of piRNAs in \textit{Δmbt}-OSC. (A) Pie charts summarizing the annotation of small RNA populations in total small RNAs, and Piwi/Aub/Ago3-bound piRNAs. (B) Small RNA size profiles for total small RNAs, and Piwi/Aub/Ago3-bound piRNAs. Sequences corresponding to rRNA, tRNA, snRNA, and snoRNA were omitted before analysis. (C) Mapping of Piwi/Aub/Ago3-bound piRNAs in \textit{Δmbt}-OSC and Piwi-bound piRNAs in parental OSCs (Ishizu et al. 2015) to \textit{flam}, \textit{tj}, and 42AB. (D) Mapping of Piwi/Aub/Ago3-bound piRNAs in \textit{Δmbt}-OSC to \textit{DM412}, \textit{DM297}, and \textit{mdg1} transposons. Mapping of Piwi-bound piRNAs in OSCs was also shown as a
Supplemental Figure S4. Characterization of phased piRNAs in Δmbt-OSCs. (A) Analyses of phased piRNAs in total small RNAs, and Piwi/Aub/AGO3-bound piRNAs in Δmbt-OSCs. The distance between the 3' end of the upstream piRNA and the 5' end of downstream piRNAs on the same genomic strand was analyzed. (B) Comparison of phased piRNAs in Piwi-bound piRNAs in OSCs (Ishizu et al. 2015) and Δmbt-OSCs. (C) Analyses of phased piRNAs in AGO3-bound piRNAs in Δmbt-OSCs and Myc-AGO3-bound piRNAs in OSCs (Sato et al. 2015).

Supplemental Figure S5. Piwi maintains its transposon silencing functions in Δmbt-OSCs. (A) Immunofluorescence reveals that Piwi is localized in the nucleus of Δmbt-OSCs. Scale bars indicate 10 µM. (B) Scatter plot comparing transcript abundance (mRNA-seq) of transposons in OSCs and Δmbt-OSCs.

Supplemental Table S1. List of Gene Ontology terms of the biological process category associated with differentially expressed genes in OSCs and Δmbt-OSCs.

Supplemental Table S2. List of differentially expressed genes in OSCs and Δmbt-OSCs.

Supplemental Table S3. List of siRNAs and primers. RNA is shown in italics.
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Supplemental Fig. S1

A

B

C

Supplemental Fig. S1

A

B

C
Supplemental Fig. S3

A

B

Supplemental Fig. S3

A

B

Supplemental Fig. S3

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Supplemental Fig. S3

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Supplemental Fig. S3

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Supplemental Fig. S3

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Supplemental Fig. S3

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Supplemental Fig. S3

A

B

Supplemental Fig. S3

A

B

Supplemental Fig. S3

A

B

Supplemental Fig. S3

A

B
Supplemental Fig. S3 (continued)

C

flamenco cluster

Position on Chr. X [Mbp]

0 1000 2000 3000 4000 5000

0 1000 2000 3000 4000 5000

0 1000 2000 3000 4000 5000

0 1000 2000 3000 4000 5000

traffic jam

Chr. 2L: 19464129 - 19468157

ORF

0 20 40

0 20 40

0 20 40

0 20 40

42AB cluster

Position on Chr. 2R [Mbp]

0 20 40

0 20 40

0 20 40

0 20 40

D

LTR-Gypsy-DM412

Piwi

AGO3

OSC Piwi

LTR-Gypsy-DM297

Piwi

AGO3

OSC Piwi

LTR-Gypsy-MDG1

Piwi

AGO3

OSC Piwi
Supplemental Fig. S3 (continued)

E

| OSC | Myc-EGFP | Myc-AGO3 | Oregon-R Ovary |
|-----|----------|----------|----------------|
|     | AGO3     |          |                |
|     | sDMA-AGO3|          |                |
Supplemental Fig. S4

A

\[ \Delta \text{mbt-OSC} \]

\[ \text{Total} \quad Z_1 = 13.8 \]

\[ \text{Piwi} \quad Z_1 = 22.3 \]

\[ \text{Aub} \quad Z_1 = 11.5 \]

\[ \text{AGO3} \quad Z_1 = 2.9 \]

B

\[ \Delta \text{mbt-OSC} \]

\[ \text{Piwi} \quad Z_1 = 22.3 \]

\[ \text{OSC} \quad Z_1 = 16.8 \]

C

\[ \Delta \text{mbt-OSC} \]

\[ \text{AGO3} \quad Z_1 = 2.9 \]

\[ \text{OSC} \]

\[ \text{Myc-AGO3} \quad Z_1 = 12.9 \]
Supplemental Fig. S5

A

B

Δmbt-OSC

\[ R^2 = 0.988 \]

Δmbt-OSC FPKM

OSC FPKM

copia
gypsy
blood
mdg1
DM297
stalker2
DM412
Transpac
gtwin
idefix
t-element
micropia
Tabor
circe
gypsy6
gypsy12

A

Piwi

Piwi DAPI

Supplemental Fig. S5
**Supplemental Table S1.** List of Gene Ontology terms of the biological process category associated with differentially expressed genes in OSCs and Δmbt-OSCs.

### OSC < Δmbt-OSC

| Term                                                              | PValue   |
|-------------------------------------------------------------------|----------|
| GO:0055114~oxidation reduction                                    | 1.41E-04 |
| GO:0007155~cell adhesion                                         | 0.004    |
| GO:0045727~positive regulation of translation                    | 0.006    |
| GO:0006026~aminoglycan catabolic process                          | 0.008    |
| GO:0022610~biological adhesion                                    | 0.009    |
| GO:0046012~positive regulation of oskar mRNA translation          | 0.009    |
| GO:0000272~polysaccharide catabolic process                       | 0.010    |
| GO:0006928~cell motion                                           | 0.011    |
| GO:0042461~photoreceptor cell development                         | 0.013    |
| GO:0060429~epithelium development                                 | 0.014    |
| GO:0009792~embryonic development ending in birth or egg hatching  | 0.019    |
| GO:0007398~ectoderm development                                   | 0.019    |
| GO:0042462~eye photoreceptor cell development                     | 0.021    |
| GO:0048729~tissue morphogenesis                                   | 0.022    |
| GO:00010648~negative regulation of cell communication             | 0.023    |
| GO:0007422~peripheral nervous system development                  | 0.025    |
| GO:0001700~embryonic development via the syncytial blastoderm    | 0.026    |
| GO:0007354~zygotic determination of anterior/posterior axis, embryo| 0.028    |
| GO:0034329~cell junction assembly                                 | 0.031    |
| GO:0046530~photoreceptor cell differentiation                     | 0.034    |
| GO:0007127~meiosis I                                             | 0.034    |
| GO:0006032~chitin catabolic process                               | 0.034    |
| GO:0032270~positive regulation of cellular protein metabolic process | 0.034  |
| GO:0051247~positive regulation of protein metabolic process       | 0.034    |
| GO:0042067~establishment of ommatidial polarity                   | 0.037    |
| GO:0007164~establishment of tissue polarity                       | 0.038    |
| GO:0002009~morphogenesis of an epithelium                        | 0.038    |
| GO:0007561~imaginal disc eversion                                | 0.039    |
| GO:0048598~embryonic morphogenesis                               | 0.039    |
| GO:0009952~anterior/posterior pattern formation                   | 0.042    |
| GO:0008544~epidermis development                                 | 0.043    |
| GO:0001754~eye photoreceptor cell differentiation                | 0.044    |
| GO:0030178~negative regulation of Wnt receptor signaling pathway  | 0.045    |
| GO:0001745~compound eye morphogenesis                            | 0.045    |
| GO:0009880~embryonic pattern specification                       | 0.046    |
| GO:0048610~reproductive cellular process                          | 0.047    |
| GO:0007411~axon guidance                                         | 0.048    |
| GO:0016477~cell migration                                        | 0.050    |
| GO:0048134~germ-line cyst formation                              | 0.050    |

### OSC > Δmbt-OSC

| Term                                                              | PValue   |
|-------------------------------------------------------------------|----------|
| GO:0000022~mitotic spindle elongation                             | 0.010    |
| GO:0051231~ spindle elongation                                     | 0.011    |
| GO:0006412~translation                                            | 0.015    |
| GO:0007052~mitotic spindle organization                           | 0.017    |
| GO:0007051~ spindle organization                                  | 0.038    |
| GO:0000278~mitotic cell cycle                                     | 0.041    |
| GO:0006350~transcription                                          | 0.048    |
| GO:0009303~rRNA transcription                                     | 0.050    |
| GO:0008272~sulfate transport                                      | 0.050    |
List of differentially expressed genes in OSCs and Δmbt-OSCs.

| Gene ID   | Osc Expression | Δmbt-OSC Expression | Log2 Fold Change | P-value |
|-----------|----------------|---------------------|-----------------|---------|
| CG10674   | 155.07         | 31.83               | 4.94            | 1.43E-02|
| CG17667   | 51.62          | 20.96               | 2.46            | 3.50E-02|
| RpL35A    | 16.5           | 4.59                | 3.52            | 1.57E-02|
| gdl-ORF39 | 114.29         | 647.21              | 5.70            | 4.22E-02|
| Gyk       | 22.34          | 22.79               | 0.39            | 4.09E-02|
| xmas-1    | 26.28          | 11.55               | 2.29            | 3.74E-02|
| CG14882   | 42.84          | 62.77               | 1.48            | 3.80E-02|
| CG7694    | 83.95          | 1.97                | 43.66           | 3.22E-02|
| CG12279   | 22.34          | 7.37                | 3.03            | 3.55E-02|
| CG3999    | 22.34          | 3.77                | 6.13            | 3.53E-02|
| CG30022   | 42.84          | 1.44                | 29.72           | 3.42E-02|
| CG6842    | 1.43           | 1.50                | 0.07            | 7.30E-03|
| CG17970   | 1.43           | 1.53                | 0.09            | 6.59E-03|
| CG31957   | 1.43           | 1.53                | 0.09            | 5.40E-03|
| OSC       | Δmbt-OSC       |                     |                 |         |
**Supplemental Table S3.** List of siRNAs and primers. RNA is shown in italics.

| experiment                                                                 | Name      | sense                        | antisense                   |
|---------------------------------------------------------------------------|-----------|------------------------------|-----------------------------|
| RNAi in OSC and Δmbt-OSC                                                  | siEGFP    | GGCAAGCUUGACCUUGAGGTT        | ACUUCAGGGUGACCUUGGCTT       |
|                                                                            | siLM(3)mbt-1 | CUCUCCUGAGGAGUCGUAATT      | UUACGAGACAUUCGAGAGGTT       |
|                                                                            | siLM(3)mbt-2 | CCGCCUGGUAGUCAAGGAAATT     | UUUCUGUAGACCGCGUGGTT        |
|                                                                            | siLuc     | CGUAUGGGGAAUUCUCUAGTT      | UCAGAGAUAUCGGCGUACGTT       |
|                                                                            | siVasa    | GCAGAGCAUAUACGAGGUUTT       | AACUUGCUGAUUGUCUGCCTT       |

| experiment                                                                 | Name      | forward primer (5' > 3')    | reverse primer (5' > 3')   |
|---------------------------------------------------------------------------|-----------|-------------------------------|-----------------------------|
| RNAi in S2                                                                | dsRNA     | TAATACGACTCTATAGGGAGAGGTTGAG  | TAATACGACTCTATAGGGAGAGGTTGAG |
|                                                                            | l(3)mbt   | CGCCGGAAGATATCAGACG       | AGGAGACGTGTCCTGG          |

| experiment                                                                 | Name      | forward primer (5' > 3')    | reverse primer (5' > 3')   |
|---------------------------------------------------------------------------|-----------|-------------------------------|-----------------------------|
| qRT-PCR                                                                   | vasa      | AGCGTCCAACGATGCAA              | AACATCGCTGCGGTCGA           |
|                                                                            | ago3      | CTGCCATTGTCGCCCTCATA          | GGGAGTTGTCCTCCTTT           |
|                                                                            | piwi      | CAAGGGCGGATAATGGACA           | CCATCGCTGGAGTGTAAG          |
|                                                                            | l(3)mbt   | GCCCCGGGAATATCAGACG       | AGGAGACGTGTCCTGG          |

| experiment                                                                 | Name      | forward primer (5' > 3')    | reverse primer (5' > 3')   |
|---------------------------------------------------------------------------|-----------|-------------------------------|-----------------------------|
| Genomic PCR                                                               | l(3)mbt   | TACGACTTCTGGGTGAACGC          | GACATCCCTCCACATCGCAA        |

| experiment                                                                 | Name      | Primer sequence               | plasmid                    |
|---------------------------------------------------------------------------|-----------|-------------------------------|-----------------------------|
| plasmid construction                                                      | VasRA-KpnI-F | TTTTGCTACCATGCTGAGAAGTGATG    | pAcM-Vasa                  |
|                                                                            | VasRA-XhoI-R | TTTTACATGCGATCCATTGCTCTTCTCTC | pAcM-Vasa                  |
|                                                                            | CRISPR-lmbt-E2-F1 | TACGCTGGAGAAGTTAAGAATAGC    | sgRNA E2                   |
|                                                                            | CRISPR-lmbt-E2-R2 | AAGCGGTAACGTATGGG         | sgRNA E2                   |
|                                                                            | CRISPR-lmbt-GM76-F1 | AGCCGCTATTGTTTGAAGCTAGAATAGC | sgRNA GM76                 |
|                                                                            | CRISPR-lmbt-GM76-R2 | CAACTGAGACGCTATGGG       | sgRNA GM76                 |