## Methods and Materials

### Generation of *Pnldc1* mutant mice by CRISPR/Cas9

To generate *Pnldc1* mutant mice, Cas9 mRNA was produced by in vitro transcription and purified using a RNeasy Mini Kit (Qiagen). sgRNA was produced using the MEGAshortscript Kit (Ambion) and purified using the MEGAclear Kit (Ambion). Cas9 mRNA and sgRNA injection to mouse zygotes obtained by mating of wild-type C57BL/6 males with C57BL/6 superovulated females.

### Quantitative and semi-quantitative RT-PCR Assay

Total RNA was extracted from the samples using TRIzol reagent and 1 μg of total RNA was reverse transcribed using a PrimeScript™ RT Master Mix. For semiquantitative RT-PCR, 500 nM of forward and reverse primer and 1 μl diluted cDNA, with a final reaction volume of 20 μl. The products were taken after 28-30 cycles and analyzed by gel electrophoresis. The primer sequences were listed as follows: *Line1*, F: GAGAACATCGGCACAACAATC, R: TTTATTGGCGAGTTGA GACCA; *IAP*, F: CAGACTGGGAGGAAGAAGCA, R: ATTGTTCCCTCACTGGCAAA; pre-piR1, F: GTTAGCGAAGGACATTATTCTAACC, R: TGACATGAACACAGGTGCTCAGAT; pre-piR2, F:CTATGCTTATGATGGCATTGGAGAG, R:TTCCAGTTCAACAGGGACACGGGAC; pre-piR3,F:GTTCTCACTTTATCAGCTCTCAAG, R: TGAGAGTGGCATCTAAATGTTTAG; pre-piLR, F: GTGAAGCTAAGGATGCTGGGATAG, R: ACAGGAT GTCCCCTGAAATCAGTC; Pre-pachytene cluster 10, F: GGCCATAGGTTAACTTTCAGA AGTC; pri-let7g, F: GTACGGTGTGGACCTCATCA, R: TCTTGCTGTGTCCAGGAAAG; *Sycp2*, F: TCACCAATGGGAAGCTGTCA, R: TTGCCTAGTTCTAGAGGGTT; *Actb*, F: A GAAGAGCTATGAGCTGCCT, R: TCATCGTACTCCTGCTTGCT.

### Immunoblotting

Tissues were rinsed with PBS and lysed in cold RIPA buffer supplemented with protease inhibitor cocktail tablets (Roche). Protein concentration was determined by Bicinchoninic Acid (BCA) assay according to manufacturer instructions (Vazyme Biotech). Primary Antibodies used: anti-MILI (Abcam), anti-MIWI (Abcam), anti-TDRD1[1], anti-TDRKH

(Proteintech), anti-LINE1 ORF1p (gifts from Prof. Ramesh Pillai, University of Geneva, Switzerland), β-Actin (Sigma).

**Immunofluorescence, TUNEL assay and Chromosome spread**

For cryosections, testes were fixed in 4% paraformaldehyde (PFA) at 4℃, dehydrated in sucrose and embedded in OCT. Primary antibodies used for immunofluorescence were as follows: anti-MILI, anti-TDRD6, anti-MIWI and anti-LINE1 ORF1p (gifts from Prof. Ramesh Pillai, University of Geneva, Switzerland), anti-γH2AX (Millipore), anti-TDRKH (Proteintech), anti-MT-CO1 (Abcam). TUNEL staining was performed on 5 μm frozen sections with the ApopTag Fluorescein in Situ Apoptosis Detection kit (Milipore) according to the manufacturer's instructions. Chromosome spreads of prophase I spermatocytes were performed as previously described [2, 3]. Primary antibodies were as follows: anti-SYCP1 (Abcam), anti-SYCP3 (Abcam), anti-γH2AX (Millipore). All the staining of testis sections and spread confocal images were visualized on a confocal microscope (Carl Zeiss, LSM700).

**Histological Analysis and Electron Microscopy (EM) Analyses**

For histology, testes were fixed in Bouin’s solution (Sigma) overnight, dehydrated in ethanol, embedded in paraffin, sectioned, and stained with hematoxylin (Sigma) and eosin (Sigma). EM followed a standard protocol used at the Biomedical Imaging Core facility at Nanjing Medical University.

**Immunoprecipitation of MILI/MIWI-piRNA Complex**

Mouse testes were homogenized and tissue lysates were immunoprecipitated with anti-MILI and MIWI antibody as previously described[1]. In brief, the mouse testes were homogenized in a lysis buffer and incubate at 4 ºC for 1 hr. And then the extract was centrifuged at 15,000 rpm for 40 minutes. Precleared samples was incubated with anti-MILI/MIWI antibody for 12 h at 4 ℃. After binding with protein A Sepharose beads for 3 hr, wash three times with wash buffer, the retained proteins were eluted 3 times by 150 μl 0.1 M glycine (pH 2.5), neutralized by adding 150 μl 1 M Tris pH 8.

**Small RNA sequencing and bioinformatic analyses**

15-45 nt total small RNAs from P10 and P18 *Pnldc1*$^{+/+}$ and *Pnldc1*$^{-/-}$ testes and Mili/Miwi-associated small RNAs were used for preparation of libraries for deep sequencing by Illumina Solexa technology. The sequences reads that mapped to the genome (mm9) were considered for analysis. We loaded small RNA sequencing data to NCBI database, and the sequencing reads are available from the National Center for Biotechnology Information's SRA database under the accession number PRJNA398115.

**Statistical Analysis**

All data are reported as mean ± SD unless otherwise noted in the figure legends. Significance was tested by using the 2-tailed unpaired Student's t test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) using Prism 7.0 (GraphPad Software, La Jolla, CA, USA).

**References**

1 Reuter M, Chuma S, Tanaka T, Franz T, Stark A, Pillai RS. Loss of the Mili-interacting Tudor domain-containing protein-1 activates transposons and alters the Mili-associated small RNA profile. *Nature structural & molecular biology* 2009; **16**:639-646.

2 Peters AH, Plug AW, van Vugt MJ, de Boer P. A drying-down technique for the spreading of mammalian meiocytes from the male and female germline. *Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology* 1997; **5**:66-68.

3 Kolas NK, Marcon E, Crackower MA *et al.* Mutant meiotic chromosome core components in mice can cause apparent sexual dimorphic endpoints at prophase or X-Y defective male-specific sterility. *Chromosoma* 2005; **114**:92-102.