Developmental Cell *15*

Supplemental Data

Mouse Maelstrom, a Component of Nuage, Is Essential for Spermatogenesis and Transposon Repression in Meiosis

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Supplemental Experimental Procedures

Generation of *Mael* Mutation

To generate a loss-of-function allele of *Mael*, we designed a targeting vector, which, following homologous recombination, deletes a total of 5.9 kb of genomic DNA encompassing the CpG island and first five exons of the gene (Figure 1A). The targeted region was initially replaced with a 3 kb hygromycin-thymidine kinase selection cassette flanked by lox*P* sites. Targeting was carried out in V6.5 (129SvJae x C57BL/6)F1 ES cells. Following the identification of correctly targeted ES cells by long-range genomic PCR and Southern analysis, the selection cassette was subsequently removed via transient expression of Cre recombinase. As a result of these manipulations, the mutant *Mael* allele lacks any apparent endogenous and vector-derived sequence elements that otherwise could promote expression of the 7 exons of *Mael* (with a potential to encode a 260 amino acid peptide) remaining in the mouse genome. Correctly targeted heterozygous *Mael* mutant ES cells generated in this manner were injected into wild-type CD1 blastocysts to generate male chimeras that subsequently transmitted the mutant allele to their progeny when mated to wild-type females. Genotyping is performed using primers 5'-GGC CAG GCT CTT AGG AAA GT-3' and 5'-GAT TCT TTC ACC CAA ACC TCA-3' for the wild-type allele, and 5'-GCT GGG ACT CCT GTT CAG TC and TGA TTC TTT CAC CCA AAC CTC-3' for the mutated allele.

Cot-1 In Situ Hybridization

Cot-1 in situ hybridization was performed as previously described (Turner et al., 2005). Briefly, after dissection and removal of the tunica albuginea, testes were minced with razor blades in a small quantity of cold Optimem medium. Several drops of the cell suspension were then applied to silanized slides. Slides were covered with cold CSK buffer [100 mM NaCl, 300 mM sucrose, 3 mM MgCl2, 10 mM PIPES, 0.5% Triton X-100, 1 mM EGTA, 2 mM vanadyl ribonucleoside, (pH 6.8)] for ten minutes, cold 4% paraformaldehyde for ten minutes and then rinsed in PBS. Slides were dehydrated using a graded alcohol series and then air-dried. Cot-1 probes were prepared from Cot-1 DNA (Invitrogen) using a Biotin Nick Translation Kit (Roche). For hybridization .5 μg of labeled Cot-1 DNA was used in a hybridization solution consisting of 10 μg salmon sperm DNA in 2XSSC, 10% dextran sulfate,1 mg/ml BSA and 2 mM vanadyl

ribonucleoside. Cover-slips were sealed with rubber cement, and probes were allowed to hybridize for three hours. After post-hybridization washes, slides were blocked in 4xSSC, 4 mg ml-1 bovine serum albumin and 0.001% Tween-20. Probes were detected with Texas Red anti-streptavidin (Jackson Immunoresearch) diluted 1:100 in blocking solution, followed by biotinylated anti-streptavidin at 1:100 (Jackson Immunoresearch) and rabbit anti-γ-H2AX at 1:200, and finally Texas Red anti-streptavidin (Jackson Immunoresearch) at 1:100 and Alexa 488 donkey anti-rabbit at 1:2000 (Invitrogen). Images were captured on a Leica SP5 laser scanning confocal microscope.

Southern Blot Analysis

Genomic DNA was purified by digesting tissues overnight in a lysis buffer containing Proteinase K. Lysate was PCIA extracted twice, chloroform extracted twice and then ethanol precipitated. After washing in 70% ethanol, the pellet was dried and dissolved in an appropriate volume of Tris-EDTA pH 8.0. Five to ten micrograms of genomic DNA were digested for four hours at 37º C with either MspI or HpaII restriction enzymes (NEB). Approximately one microgram of digested DNA was loaded and run on a .9% agarose gel. DNA was transferred overnight to an Immobilon-Ny+ Charged Nylon membrane (Millipore). The L1 probe corresponded to nucleotides 515–1,628 of the L1 sequence (GenBank accession no. M13002). The probe was random-primed labeled with 32P-dCTP using High Prime premixed solution (Roche). Hybridizations were carried out overnight at 65ºC.

Northern Blot Analysis

Small RNAs were isolated from testes using the mirVana miRNA Isolation Kit (Ambion). Approximately 1.5 µg of small RNAs were loaded on a 15% TBE-Urea gel with radioactive size markers. Gels were run until the bromophenol blue loading dye neared the bottom of the gel—about 70 minutes at 180V. RNA was transferred to Immobilon-Ny+ Charged Nylon membrane (Millipore) for one hour at 300 mA using a semi-dry electroblotter. Northern blot probes were synthetic DNA oligonucleotides end-labeled with 32P using T4 polynucleotide kinase. Pachytene and pre-pachytene piRNA probes were as previously reported (Aravin et al., 2007b) Probes were hybridized overnight at 37ºC in ULTRAhyb-Oligo hybridization buffer (Ambion). After washing blots were exposed to film at -80ºC using amplification screens for 3-7 days.

RT-PCR and qPCR

Total testis RNA was isolated using TRIZOL (Invitrogen) reagent as per the manufacturers recommendations. RNA preparations were treated with TURBO DNA-free (Ambion) using the "rigorous" treatment protocol. Five micrograms of RNA were used as a template for cDNA synthesis with SuperScript III Reverse Transcriptase (Invitrogen). For qPCR reactions, random hexamer primers were used, otherwise a poly-dT primer was used. Resulting cDNA was diluted 1:10 with water for downstream PCR reactions. Quantitative PCR was carried out using SYBR Green for detection in an MJ Research DNA Engine Opticon. A standard curve was generated using a TOPO-cloned L1 5' UTR amplicon, and actin was used for normalization. Primers for qPCR were used as described (Carmell et al., 2007). Long-range high-fidelity PCR for amplification of piRNA precursors was performed as in (Ro et al., 2007).

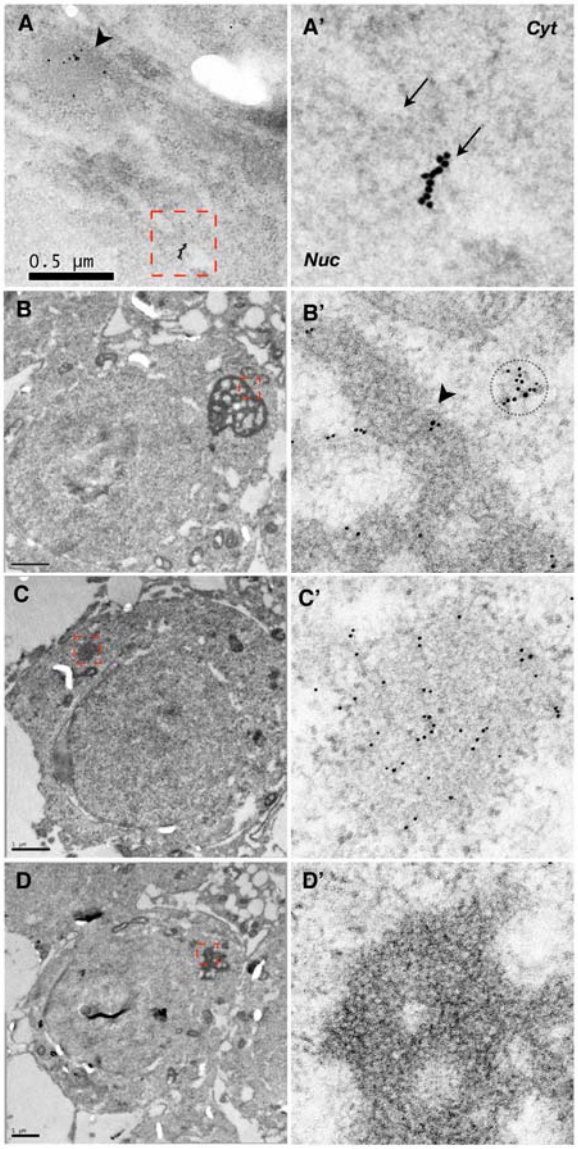

Figure S1. Immunoelectron Microscopy Localization of MAEL
(A–C) IEM of wild-type male germ cells using anti-MAEL antibody (21st Century).
(A) MAEL localization to perinuclear nuage (arrow) and a nuclear pore (red dashed square) (bar – 0.5 µm).
(A') A close-up of the nuclear pore-associated MAEL cluster. Arrows indicate the opening of the nuclear pore.
(B) MAEL localization to the chromatoid body of round spermatid (bar – 1 µm).
(B') A close-up of a part of the chromatoid body and an associated cytoplasmic cluster from (B) (red dashed square).
(C) MAEL localization to a second nuage in round spermatids (bar – 1 µm).
(C') A close-up of the small nuage from (C) (red dashed square).
(D) Control IEM of wild type round spermatid using normal rabbit serum instead of anti-MAEL antibody.
(D') A close up of the chromatoid body shown in (D).

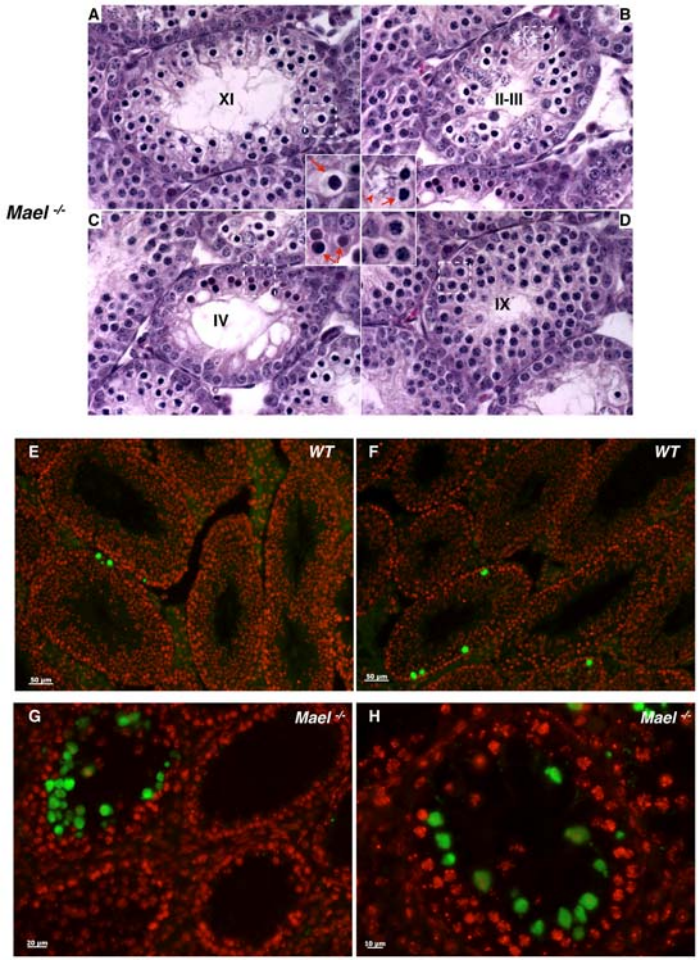

Figure S2. Histological and TUNEL Analyses of *Mael*$^{-/-}$ Testes
(A) Epithelial stage XI tubule containing atypical zygotene *Mael*$^{-/-}$ spermatocytes with densely stained chromatin (inset, arrow).
(B) Epithelial stage II-III tubule with two types of atypical *Mael*$^{-/-}$ spermatocytes (inset, arrow and arrowhead).
(C) Epithelial stage IV tubule containing *Mael*$^{-/-}$ apoptotic cells (inset, arrows).
(D) Epithelial stage IX tubule containing leptotene *Mael*$^{-/-}$ spermatocytes with normal appearance.
(E and F) Low level of apoptosis in the wild-type testes. Bar – 50 μm.
(G and H) High level of apoptosis in selected tubules of a *Mael*$^{-/-}$ testis. Bar – 20 μm (G), 10 μm (H).

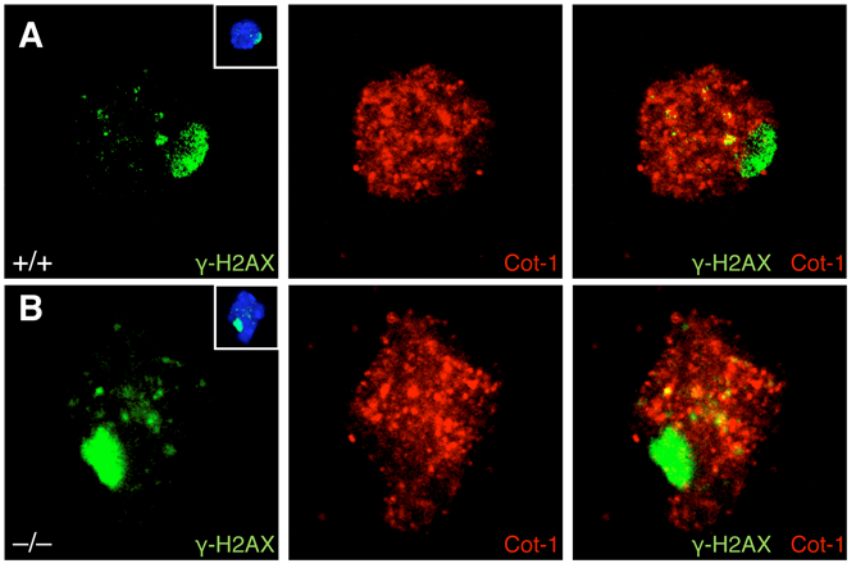

Figure S3. MAEL Is Not Required for MSCI
Cot-1 in situ hybridization is used to identify nascent, un-spliced transcripts. Both wild-type (A) and mutant (B) spermatocytes have equally low levels of Cot-1 hybridization signal.

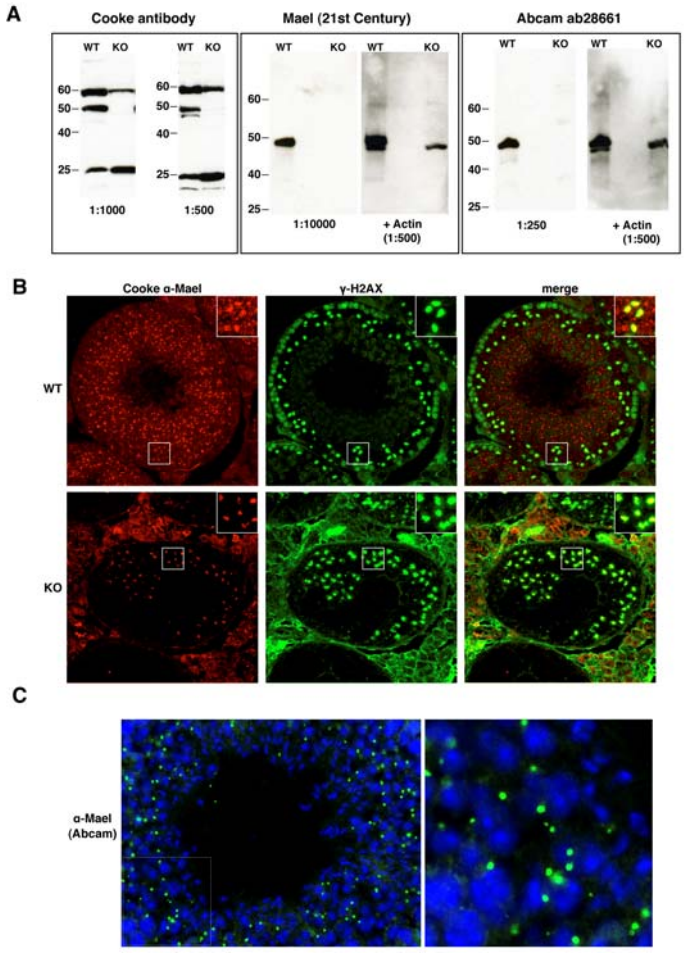

Figure S4. Validation of Anti-MAEL Antibodies

(A) Western blot analysis of wild-type (WT) and *Mael*$^{-/-}$ mutant (KO) testicular lysates probed with three different rabbit polyclonal anti-MAEL antibodies of indicated origin. The membranes, initially probed with anti-MAEL antibodies generated at 21st Century and Abcam, were re-probed with anti-actin antibody (Santa-Cruz) to show equal amounts of protein lysates in the WT and KO lanes.
(B) The Cooke lab rabbit anti-Mael antibody strongly stains what appears to be the chromatoid body, and more weakly stains the sex body of spermatocytes, co-localizing with γ-H2AX staining (inset). In testis lacking MAEL (KO), sex body staining with the Cooke lab antibody is intact.
(C) Abcam anti-MAEL antibody ab28661 labels cytoplasmic spheres in the wild-type spermatocytes and chromatoid bodies in round spermatids but does not recognize the sex body.

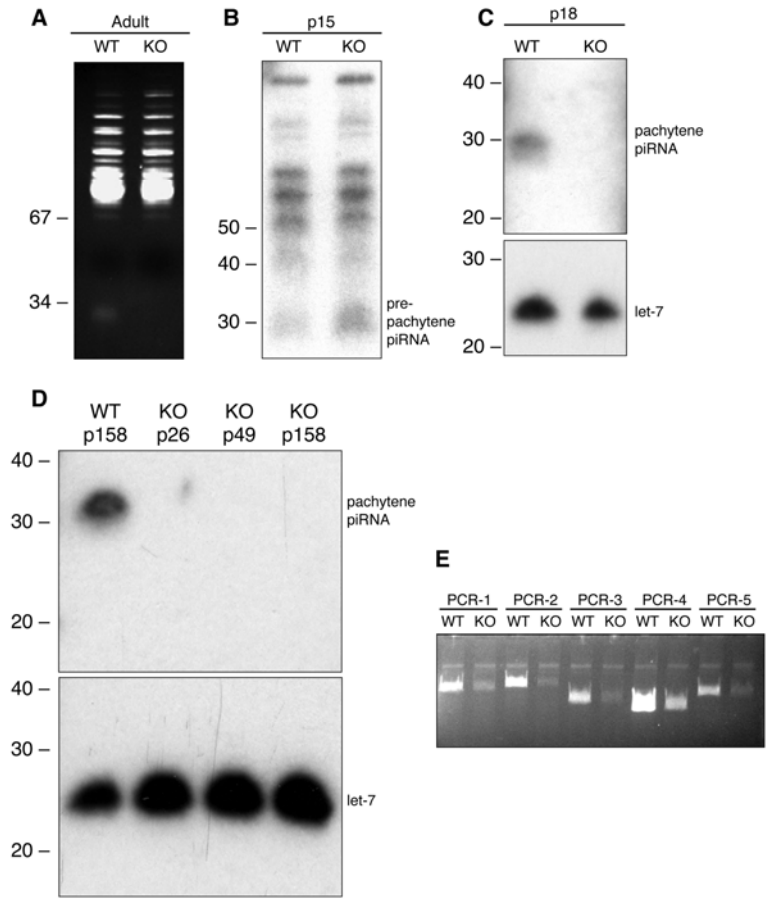

Figure S5. Biogenesis of piRNAs In the Absence of MAEL
(A) EtBr-stained small RNAs from wild-type and *Mael* mutant testes. A characteristic cloud of ~ 30 – 32 nt piRNAs is present in the wild-type sample only.
(B) Northern blot analysis of expression of SINE-element pre-pachytene piRNA in p15 wild-type and *Mael* mutant samples. Control Northern blot was probed with a probe to let-7 miRNA to demonstrate the presence of the small RNA component in the *Mael* mutant sample.
(C - D) Northern blot analysis of pachytene piRNAs in wild type and *Mael* mutant testes of indicated age. Control blot as in (B).
(E) Six RT-PCR amplicons (PCR-1 through PCR-6) reveal expression of a long piRNA precursor transcript from Chromosome 6.

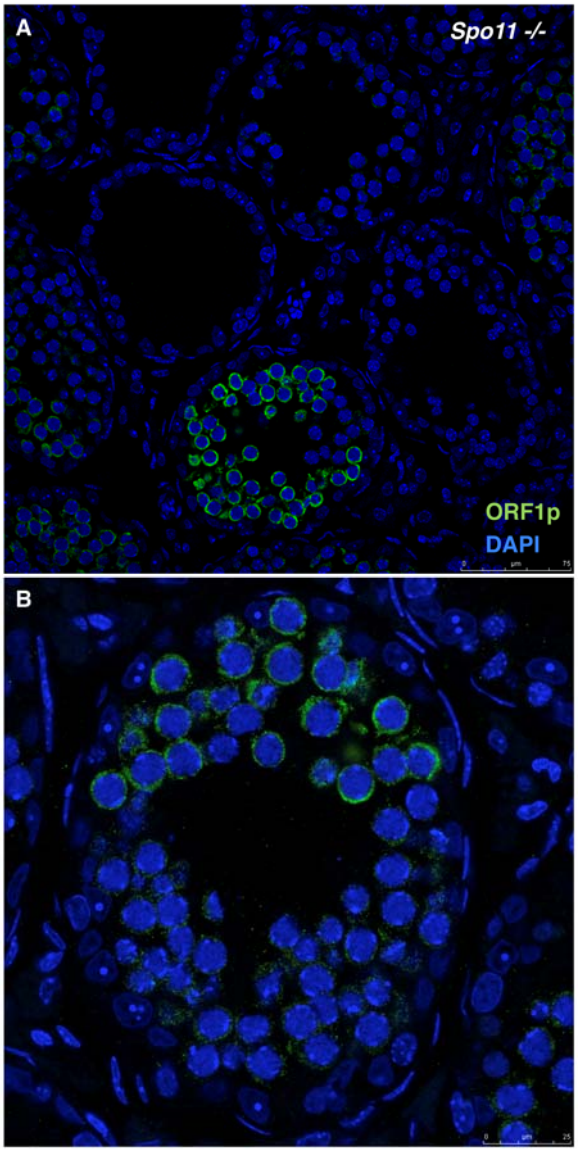

Figure S6. L1 ORF1p Expression Is Normal in a *Spo11* Meiotic Mutant
(A) Low power image of immunofluorescence detection of L1 ORF1p expression in *Spo11* homozygous mutant testes. Like in a wild-type testis, *Spo11*-deficient tubules contain low to moderate levels of cytoplasmic ORF1p.
(B) A close-up image of a *Spo11*$^{-/-}$ tubule containing arrested spermatocytes with low cytoplasmic ORF1p levels (lower half of the tubule) and moderate cytoplasmic ORF1 levels (upper half of the section).

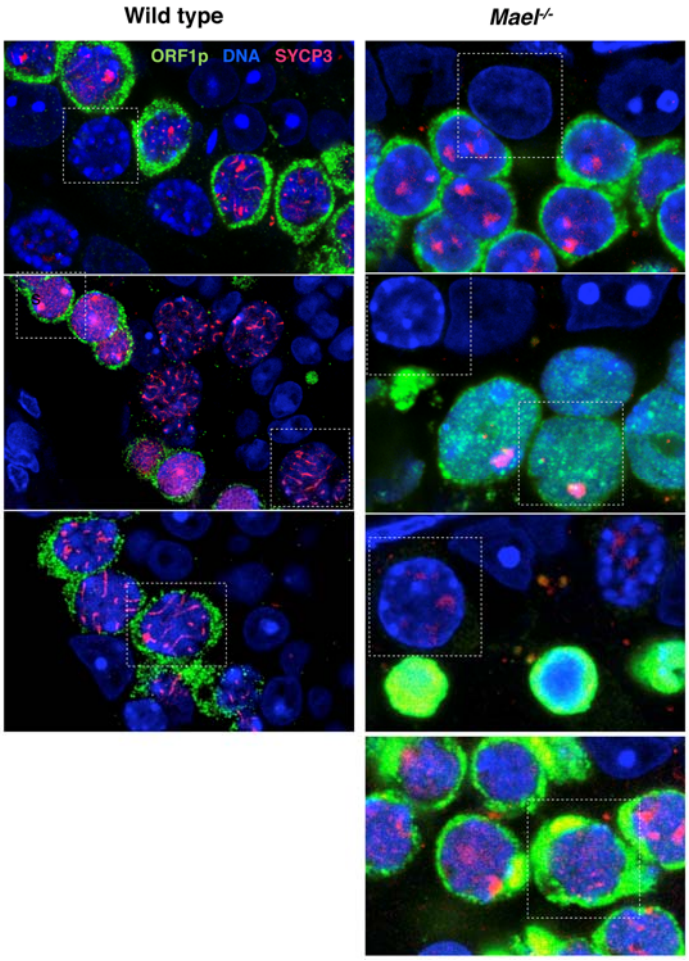

Figure S7. **Patterns of ORF1p Localization In the Wild-Type and *Mael* Mutant Germ Cells**
Cells used as cut-outs in the Figure 6D are indicated.

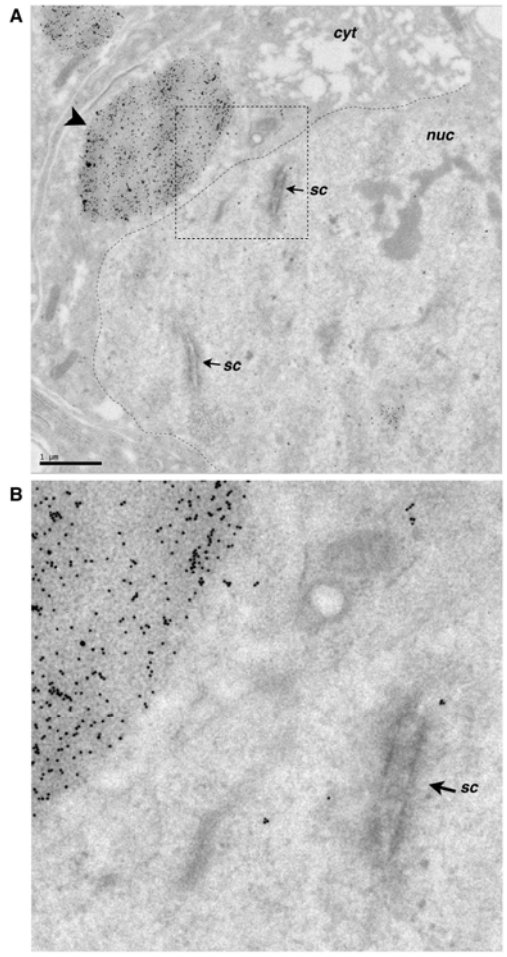

Figure S8. Immunoelectron Microscopy of L1 ORF1p In a *Mael*$^{-/-}$ Spermatocyte
(A and B) Massive amounts of L1 RNPs are present in the cytoplasmic enclave (arrowhead) of a *Mael*-deficient spermatocyte. Individual gold particles can be also observed throughout the nucleus. Synaptonemal complexes are indicated with arrows. Nuclear envelop is outlined with a dashed line. Boxed area is shown in (B) at a higher magnification. Bar – 1 µm.