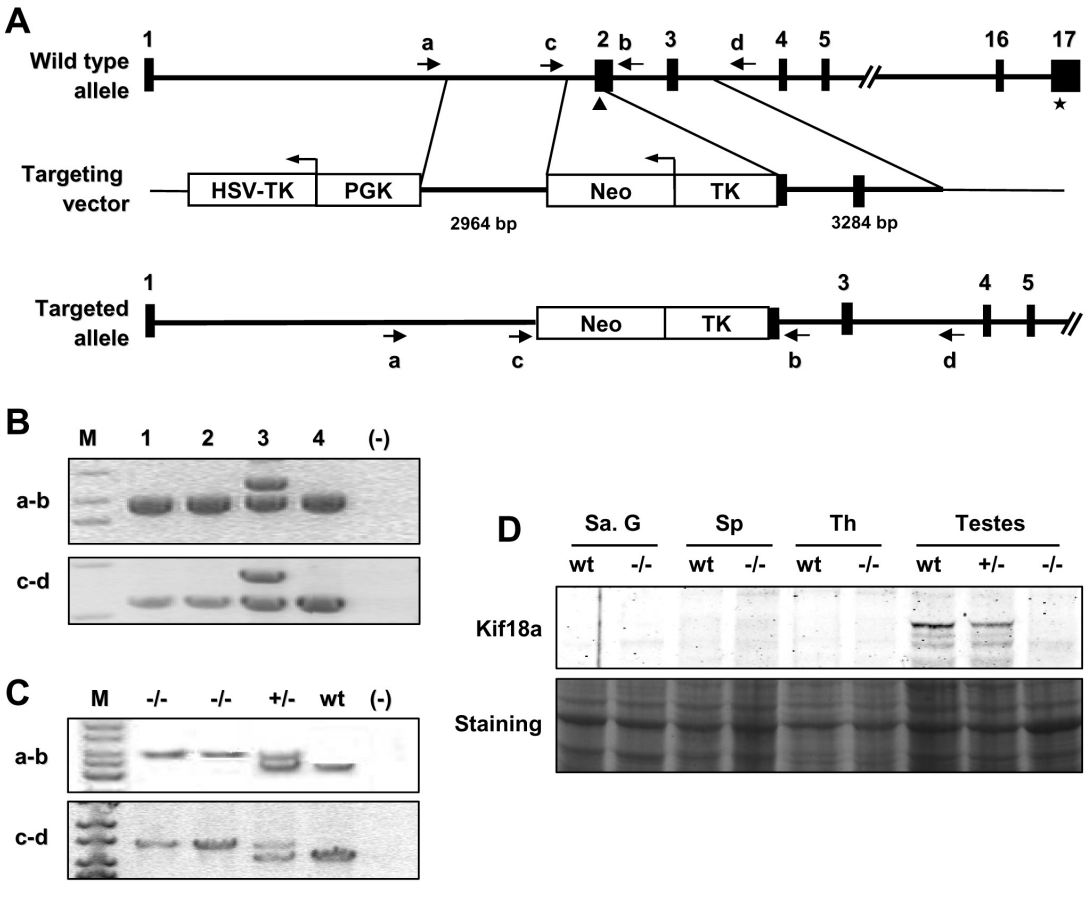


Fig. S1. Targeted disruption of *Kif18a* in mice.

A

Infertility of mutant male but not female mice.

Sex	n	Plugs	Litters	Offspring
M (mt) x F (wt)	10	22	0	0
F (mt) x M (wt)	14	13	9	56
M (wt) x F (wt)	14	14	12	79

B

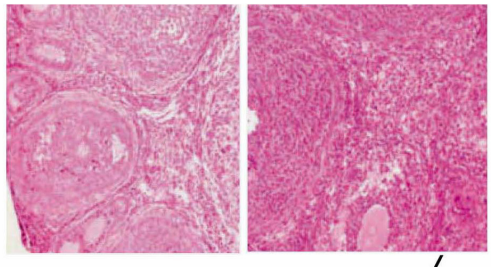
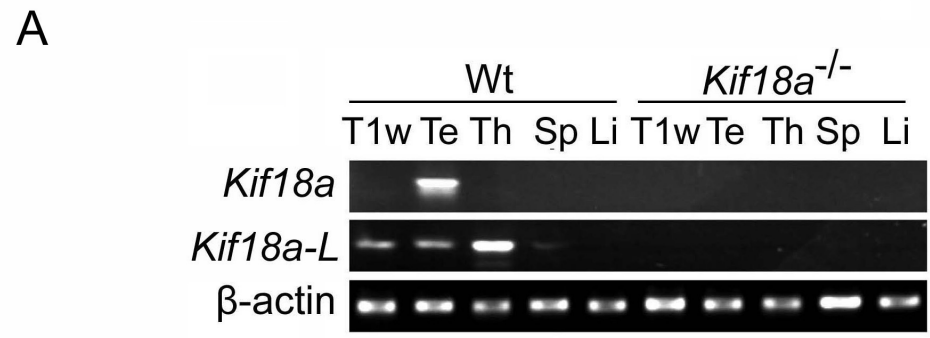
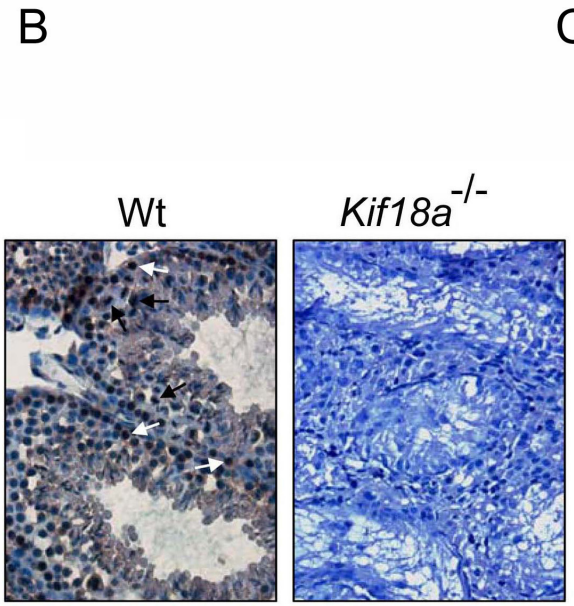


Fig. S2. Kif18a deletion causes infertility in male but not female mice.





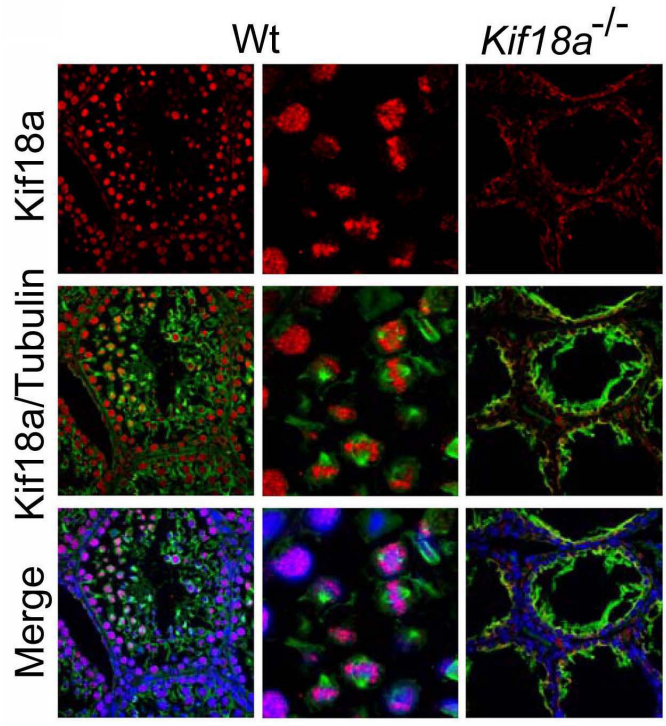
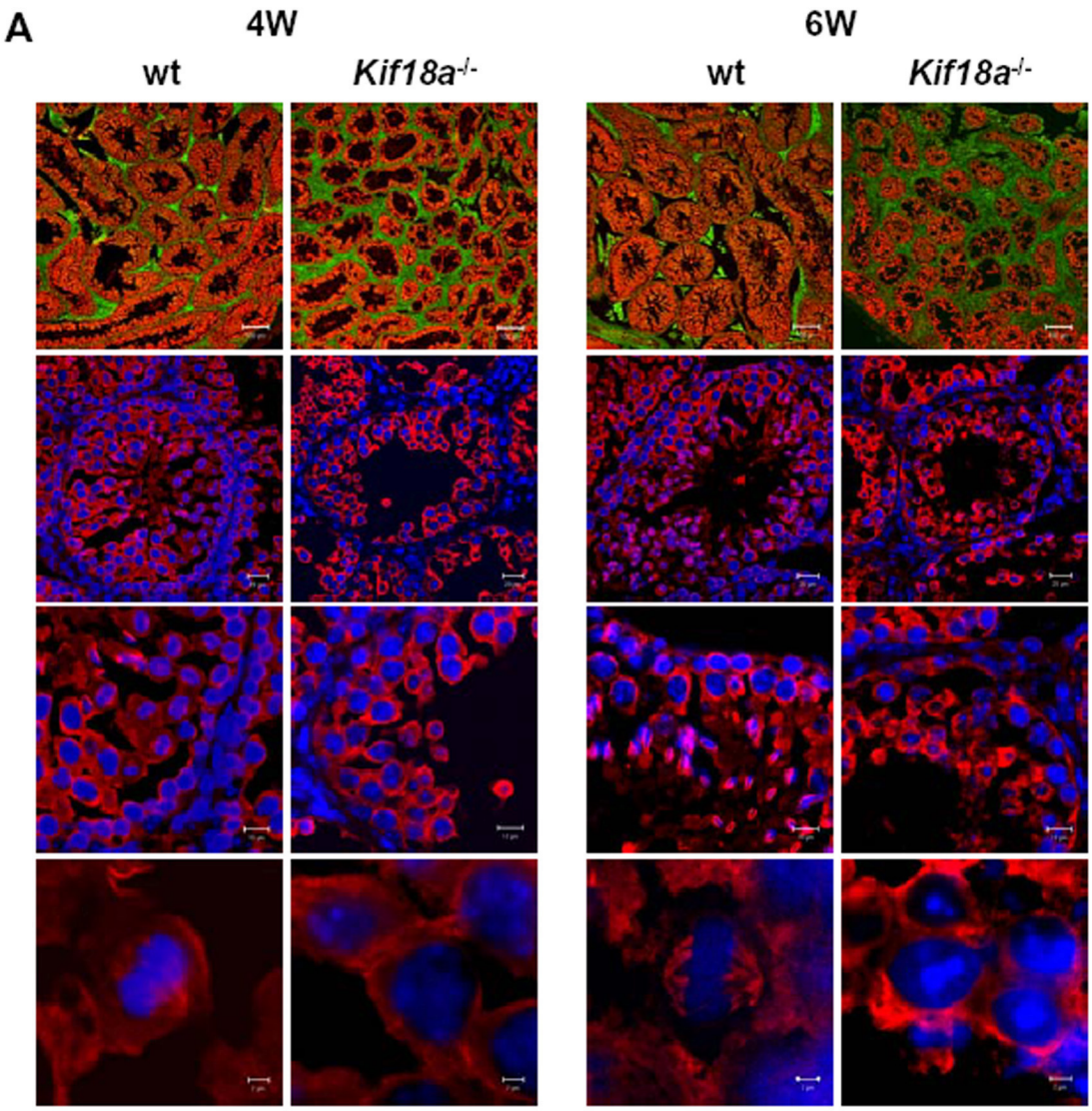


Fig. S3. ***Kif18a*** **is specifically expressed in adult mouse testes, restricted to spermatogenic cells during mitosis and meiosis.**



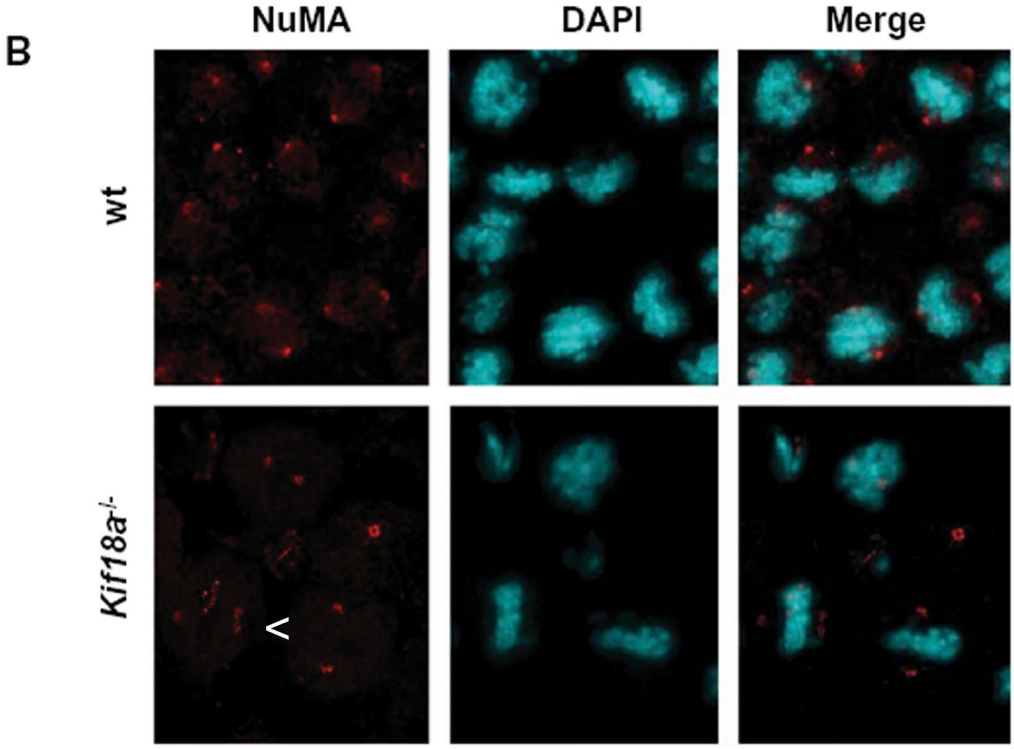


Fig.S4. Spindle pole and chromosome congression defects in *Kif18a*$^{-/-}$ meiotic cells.

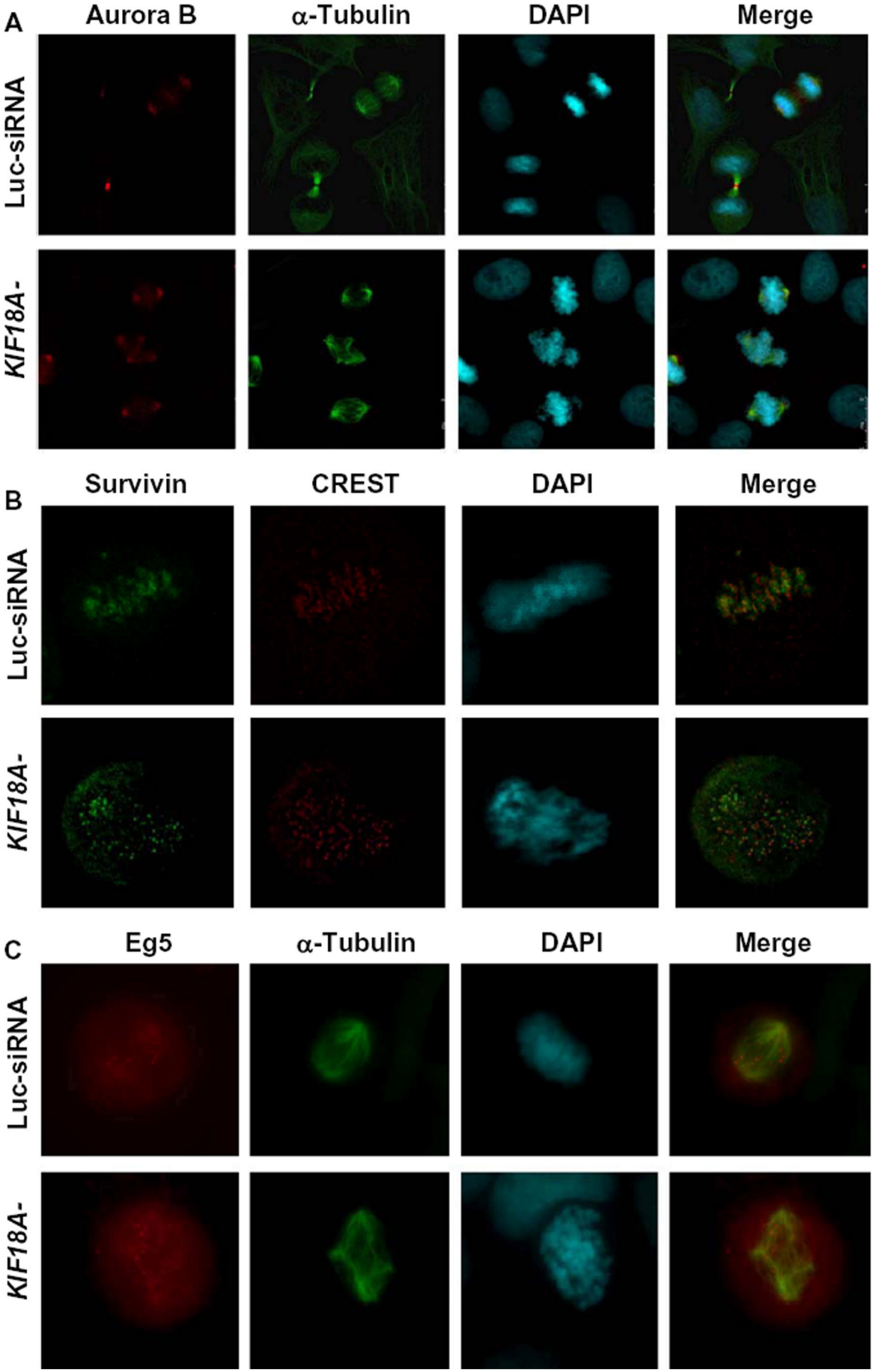


Fig.S5. Expression and cellular distribution of Aurora B, survivin and Eg5 in KIF18A-depleted mitotic HeLa cells

LEGENDS FOR SUPPLEMENTAL FIGURES

Figure S1. Disruption of the murine *Kif18a* gene. To construct the targeting vector, a ~8-kb genomic fragment of the mouse *Kif18a* gene was obtained and cloned into the vector pPNT (Tybulewicz et al., 1991). The PGK-neo cassette replaced a 2.0-kb *Kif18a* genomic fragment containing part of exon 2 (Fig. S1A). After linearization, the targeting vector was electroporated into ES cells derived from 129/Sv. ES cell clones were selected with G418 and GANC, then screened for homologous recombination using PCR. (Fig. S1B). Putative positive clones were further confirmed by southern blotting. The primer sequences used for PCR analyses are as follows: (5' arm primers: 5'-CAGTTTGTCCTTACTTGGTG-3' and 5'-ATCTGCTGAAATCTCAAAACTGCT-3'; 3' arm primers: 5'-GTGTTAACACAATGTATCTCTGAC-3' AND 5'-AATTGTGGAGCGACTAAGACCC-3'). Chimeric mice were generated by standard microinjection techniques with C57BL/6 blastocysts and germ line transmission was monitored by coat color marker. Genomic DNA extracted from tails of mouse offspring was used for Southern blotting (Fig. S1C). Proteins from several mouse tissues (salivary gland, spleen, thymus, and testes) of various genotypes were collected. Equal amounts of lysates were blotted for Kif18a. Mice containing Kif18a mutation were maintained in C57BL/6J background and maintained in the 129vJ background at Shanghai Research Center for Model Organisms. Genotyping was performed with primers specific to wild-type or to the *Kif18a* mutant allele using genomic DNA extracted from mouse tails.

Figure S2. *Kif18a* deletion causes infertility in male but not in female mice. (A) Summary of breeding of *Kif18a* mutant mice during the first year. **(B)** Sections of wt and *Kif18a*$^{-/-}$ ovaries at week 4 were stained with H&E and representative images were shown.

Figure S3. Kif18a is expressed in mouse testes. (A) RT-PCR coupled with DNA sequencing analysis revealed that Kif18a-L was composed of 12 exons (exon 1 to 12; accession No. AK036722) and that Kif18a contained all 17 exons (Accession No. NM-139303). The apparent discrepancy between the number of exons and the size of Kif18a-L mRNA was due to a unique sequence (1676 bp long) at the 3'UTR. RNA isolated either from week 1 (T1w) and week 3 testes or from week 3 thymus, spleen and liver were subjected to PCR analysis using isoform-specific primers. Representative data were shown. (B) Sections of wt and *Kif18a*$^{-/-}$ seminiferous tubules were analyzed for *Kif18a*$^{-/-}$ expression using immunohistochemistry. Arrows indicate Kif18a-positive spermatogonial cells and spermatocytes. (C) Sections of wt and *Kif18a*$^{-/-}$ seminiferous tubules were stained with antibodies to Kif18a (red) and α-tubulin (green). DNA was stained with DAPI (blue). Specific signals were detected by fluorescence microscopy.

Figure S4. Spindle pole and chromosome congression defects in *Kif18a*$^{-/-}$ meiotic cells. (A) Sections of wild-type and *Kif18a*$^{-/-}$ testes of 4 and 6 weeks old were stained with the α-tubulin antibody conjugated with Texas Red and the antibody conjugated with FITC. The DNA was stained with DAPI. Seminiferous tubule shrinkage and hyperplasia in stroma were visible in *Kif18a*-deficient testes. Metaphase plate and bipolar meiotic spindles were easily observed in wt seminiferous tubules whereas they were largely absent from *Kif18a* mutant ones. (B) Sections of wild-type and *Kif18a*$^{-/-}$ testes of 4 weeks' old were stained with the NuMA antibody. DNA was stained with DAPI. Each wt meiotic cell contained two spindle poles. On the other hand, additional NuMA foci was observed in *Kif18a*$^{-/-}$ meiotic cells.

Figure S5. Expression and distribution of Aurora B, survivin and Eg5 in KIF18A-depleted mitotic HeLa cells. (A) HeLa cells transfected with KIF18A or luciferase siRNA for 24 h were

stained with antibodies to Aurora B (red) and α-tubulin (green). DNA was stained with DAPI (blue). (B) HeLa cells transfected with *KIF18A* or luciferase siRNA for 24 h were stained with antibodies to survivin (red) and α-tubulin (green). DNA was stained with DAPI (blue). (C) HeLa cells transfected with *KIF18A* or luciferase siRNA for 24 h were stained with antibodies to Eg5 (red) and α-tubulin (green). DNA was stained with DAPI (blue). Please note that the amount of Aurora B, survivin, and Eg5 was not significantly affected after *KIF18A* depletion.