The meiotic TERB1-TERB2-MAJIN complex tethers telomeres to the nuclear envelope

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During meiotic prophase I, telomeres attach to and move on the nuclear envelope (NE), regulating chromosome movement to promote homologous pairing. Meiosis-specific proteins TERB1, TERB2 and MAJIN play a key role in this process. Here, we report the crystal structures of human TERB1-TERB2 and TERB2-MAJIN subcomplexes. Specific disruption of the TERB1-TERB2 or the TERB2-MAJIN interaction in the mouse Terb2 gene abolishes the telomere attachment to the NE and causes aberrant homologous pairing and disordered synapsis. In addition, depletion of SUN1 also partially disrupts the telomere-NE connection. We propose that the telomere-TRF1-TERB1-TERB2-MAJIN-NE interaction network and the telomere-LINC complex connection are likely two separate but cooperative pathways to stably recruit telomeres to the NE in meiosis prophase I. Our work provides a molecular model of the connection between telomeres and the NE and reveals the correlation between aberrant synapsis and the defective telomere attachment to the NE.
Meiosis produces reproductive cells by two consecutive rounds of nuclear division following a single round of DNA replication. The maintenance of genomic integrity during meiosis depends on the accurate chromosome segregation. Prophase I is the longest and most complex phase in meiosis and is characterized by homologous pairing and formation of synaptonemal complex (SC). Homologous pairing is essential for both genetic recombination and, more crucially, precise segregation of the homologs in the subsequent cell division step.

Vertebrate telomeres consist of an array of TTAGGG bound by the evolutionarily conserved telomere complex. SUN1 (Sad1 and UNC84 domain containing 1) and KASH5 (Klarsicht/ANC-1/Syne/homology 5) assemble into the mammalian telomere-specific LINC (linker of nucleoskeleton and cytoskeleton) complex, providing the binding sites for telomeres on the inner surface of the nuclear envelope (NE). In meiosis prophase I, cells establish the association between telomeres and the LINC complex, bridging chromosomes to the cytoskeleton for force transduction to allow chromosome movements. In most organisms, telomeres attach to and move along the NE during leptotene stage and become clustered at the side of the nucleus adjacent to the centrosome at zygote stage, resulting in a transient bouquet-like arrangement of chromosomes. The transient bouquet formation is the underlying reason for subsequent precise segregation of the homologs in the subsequent cell division step.

The TERB1–TERB2 and TERB2–MAJIN interactions. Meiotic specific proteins TERB1, TERB2, and MAJIN form a stable complex that plays a critical role in regulating the recruitment of telomeres to the NE. Our previous study revealed that TRF1 recognizes a unique motif in TERB1 via its TRF homology (TRFH) domain to regulate the telomere–NE attachment and play a vital role in assisting X–Y chromosome pairing during meiosis. In the TTM complex, TERB2 simultaneously interacts with both TERB1 and MAJIN. To gain further insight into the telomere recruitment function of the TTM complex, we first characterized the interaction between TERB1 and TERB2 using bimolecular fluorescence complementation (BiFC) assay. We found that a short fragment of TERB1 consisting of residues 587–636, which is adjacent to the TRF1-binding motif (TERB1TBM) and MAJIN2–149 of TERB1, was necessary and sufficient for binding with the N-terminal domain of TERB2 (residues 1–116) (Fig. 1a, b).

We next set out to map the interaction between TERB2 and MAJIN. MAJIN was identified as an inner nuclear membrane protein containing a putative TM domain at its C-terminus. Consistently, ectopically expressed MAJIN exhibited a clear pattern of nuclear membrane localization in U-2 OS cells, whereas TERB2 when ectopically expressed alone displayed a diffused distribution (Fig. 1c). MAJIN was able to recruit the co-expressed TERB2 to the nuclear membrane, confirming the direct interaction between TERB2 and MAJIN (Fig. 1d). Various TERB2 fragments were then evaluated for their ability to interact with MAJIN (Fig. 1d). Our data revealed that a short fragment of TERB2 at the C-terminus (residues 168–202) is sufficient for interaction with MAJIN. Consistent with previous studies, gel filtration chromatography analysis using purified proteins showed that the N-terminal fragment of MAJIN (residues 2–149) mediates the interaction with TERB2 (Fig. 1e). Collectively, these results reveal a linear interaction network within the TTM complex (Fig. 2a).

Crystal structures of TERB1–TERB2 and TERB2–MAJIN complexes. To reveal the structural basis for the interactions between TERB1 and TERB2 and between TERB2 and MAJIN, we crystallized the TERB1587–659–TERB2116 and the TERB2119–220–MAJIN2–149 complexes and determined their structures using single-wavelength anomalous dispersion (SAD) method at resolutions of 3.3 and 2.9 Å, respectively (Supplementary Tables 1 and 2). Well-defined electron density maps allowed unambiguous tracing of polypeptide chains TERB1590–649, TERB24–110, TERB2174–209, and MAJIN2–109 in the complexes (Supplementary Fig. 1a–d), whereas the rest of residues are disordered in the crystals and presumably dispensable for the interactions. Notably, the interaction domains revealed by the crystal structures are consistent with those identified by the mapping analyses (Fig. 1a–d). Hereafter, for simplicity, we will refer to TERB21–116 as TERB2NTD (N-terminal domain), TERB1587–636 as TERB1TBM (TERB2-binding motif), MAJIN1–110 as MAJINNTD, and TERB2168–202 as TERB2MMB (MAJIN-binding motif), respectively (Fig. 2a).

The TERB1TBM–TERB2NTD complex adopts a compact globular fold, resembling a single folding unit (Fig. 2b). TERB2NTD consists of a three-stranded β sheet (β1–β2–β3) at one end and six α helices that flank the β sheet and scatter at the periphery of the structure (Fig. 2b). The TERB1TBM is a
helix-turn-helix (HtH) motif with a long tail that folds back to the middle turn between the two helices (Fig. 2b). Notably, Cys592 and Cys595 at the N-terminus of the tail together with Cys618 and His621 in the middle of the HtH motif chelate a Zn\(^{2+}\) ion, playing a key role in defining the overall shape of TERB1\_TBM (Fig. 2c). TERB1\_TBM adopts an extended conformation and packs against the concaved surface of TERB2\_NTD, burying \(\sim 1400 \, \text{Å}^2\) of surface area at the interface (Fig. 2d). The
Fig. 1 Characterization of the interactions among TERB1, TERB2, and MAJIN. a Illustration of different fragments of TERB1 (upper panel). Bimolecular fluorescence complementation (BiFC) data with full-length TERB2 and TERB1 fragments (lower panel). Cells expressing TERB2-VC only (blue, as a negative control) or TERB2-VC with -VN vector (green, as a negative control) or TERB1-VC with different TERB2-VN fragments (red) were subjected to fluorescence-activated cell sorting (FACS) analysis. b Illustration of various fragments of TERB2 (upper panel). BiFC data with full-length TERB1 and TERB2 fragments (lower panel). Cells expressing TERB1-VC only (blue, as a negative control) or TERB1-VC with -VN vector (green, as a negative control) or TERB1-VC with different TERB2-VN truncations (red) were subjected to FACS analysis. c Equator images of U-2 OS cells expressing SUN1-GFP (green) together with mCherry-MAJIN (red) (up) or Flag-TERB2 (magenta) (down). SUN1-GFP was used to indicate the nuclear envelope localization. Cells were stained with 4,6-diamidino-2-phenylindole (DAPI; blue) to show the nuclear position. Scale bars, 10 μm. d Schematics of various TERB2 fragments (upper panel). Equator images of U-2 OS cells expressing mCherry-MAJIN (red) and Flag-TERB2 truncations. Cells were stained with Flag antibody (magenta) and DAPI (blue). Scale bars, 10 μm. e Gel filtration chromatography profile of the TERB2MBM-MAJINNTD complex. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis image of the peak in the gel filtration chromatography profile is shown.

Mutational analysis of TERB1–TERB2 and TERB2–MAJIN interfaces. To corroborate our structural analysis, we examined whether missense mutations of the interface residues of TERB2 could weaken or disrupt the TERB1–TERB2 and TERB2–MAJIN interactions. We examined the interaction of mutant proteins transiently expressed in human embryonic kidney 293T cells. For the TERB1–TERB2 interaction, we focused on a hydrogen bond between Tyr56TERB2 and Glu62TERB1 in the core of the binary interface (Fig. 4a). A substitution of Tyr56TERB2 with a negatively charged glutamate residue would generate an electrostatically unfavorable clash that could severely interfere with the interaction. Consistent with this structural information, co-immunoprecipitation (Co-IP) experiments revealed that, while wild-type (WT) TERB1 and TERB2 showed the expected interaction, the TERB2 Y56E mutation completely abolished the interaction with TERB1 in 293T cells (Fig. 4b). For the TERB2–MAJIN interaction, we focused on the hydrophobic interface between the C-terminal tail of TERB2MBM and the hydrophobic wedge formed by strands S1 in TERB2MBM and β1, β2, and β4 in MAJINNTD, which are critical for stabilization of the interaction (Fig. 4c). In particular, located at the center of this interface, the side chain of Phe192 of TERB2 is buried in a pocket formed by a group of hydrophobic residues of both MAJIN and TERB2 (Fig. 4c). Again consistent with the crystal structure, substitution of Phe192TERB2 with a negatively charged bulk arginine residue completely abolished the TERB2–MAJIN interaction in 293T cells (Fig. 4d). These results indicated that single point mutations at the TERB1–TERB2 and TERB2–MAJIN interfaces are sufficient to disrupt the ability of TERB2 to bind TERB1 and MAJIN, respectively. Notably, the Y56E mutation of TERB2 only disrupted the TERB2 interaction with TERB1 but not the one with MAJIN (Fig. 4b, d). And vice versa the F192R mutation only affected the interaction with MAJIN but not with TERB1 (Fig. 4b, d). Therefore, our Co-IP data strongly suggested that these mutations only disrupt the desired interactions of TERB2 in cells. Both Tyr56 and Phe192 are highly conserved in mice. To elucidate the meiotic role of the TTM complex, we generated knock-in mice with the TERB1-binding-deficient mutation Y56E and the MAJIN-binding-deficient mutation F188R in the Terb2 gene using the CRISPR–Cas9 method (Supplementary Fig. 2a–d). The heterozygous mice were healthy and fertile and were intercrossed to produce homozygous mice. Heterozygous and homozygous mutant progeny were identified.

Fig. 3 Characterization of the TERB2MBM–MAJINNTD complex. a Illustration of different fragments of TERB2MBM and MAJINNTD complexes in one asymmetric unit in the crystal structure, two protomer complexes apparently form a heterotetramer with an extensive hydrophobic dimeric interface between two MAJINNTD molecules (Fig. 3e and Supplementary Fig. 1e and 1f). Interestingly, the third protomer form a second heterotetramer with another complex from an adjacent unit cell with the identical hydrophobic interface (Supplementary Fig. 1g). Consistent with a recent study, this observation suggests that TERB2 and MAJIN could form a heterotetramer with a 2:2 stoichiometry. Notably, TRF1 itself forms a stable dimer and binds two TERB1 molecules through its TRFH domain. Therefore, TRF1, TERB1, TERB2, and MAJIN can potentially form a large complex with two sets of each protein with multiple dimeric interfaces (Fig. 3f). Together with previous data, the crystal structures of the TERB1–TERB2 and the TERB2MBM–MAJINNTD complexes provide a molecular model of the telomere–NE connection through the TRF1–TERB1–TERB2–MAJIN interaction network (Fig. 3f).

In the TERB2MBM–MAJINNTD complex, MAJINNTD adopts a compact fold with a curved four-stranded β sheet (-β1–β2–β3–β5) holding helix a1 in the center (Fig. 3a). The TERB2MBM polypeptide forms an extended structure that wraps around the surface of MAJINNTD (Fig. 3a). The driving force for the binding of TERB2MBM to MAJINNTD is van der Waals interactions (Fig. 3b). The highly hydrophobic TERB2MBM polypeptide meanders along a hydrophobic groove on the surface of MAJINNTD and buries a total of ~1300 Å² surface area at the interface (Fig. 3b, c). The C-terminus of TERB2MBM and the loop between strands β3 and β5 of MAJINNTD contact each other and fold into an intermolecular anti-parallel β sheet that packs onto the central β sheet of MAJINNTD to further strengthen the interaction (Fig. 3d). It is worthy of noticing that, although there are three TERB2MBM–MAJINNTD complexes in one asymmetric unit in the crystal structure, two protomer complexes apparently form a heterotetramer with an extensive hydrophobic dimeric interface between two MAJINNTD molecules (Fig. 3e and Supplementary Fig. 1e and 1f). Interestingly, the third protomer form a second heterotetramer with another complex from an adjacent unit cell with the identical hydrophobic interface (Supplementary Fig. 1g). Consistent with a recent study, this observation suggests that TERB2 and MAJIN could form a heterotetramer with a 2:2 stoichiometry. Notably, TRF1 itself forms a stable dimer and binds two TERB1 molecules through its TRFH domain. Therefore, TRF1, TERB1, TERB2, and MAJIN can potentially form a large complex with two sets of each protein with multiple dimeric interfaces (Fig. 3f). Together with previous data, the crystal structures of the TERB1–TERB2 and the TERB2MBM–MAJINNTD complexes provide a molecular model of the telomere–NE connection through the TRF1–TERB1–TERB2–MAJIN interaction network (Fig. 3f).
by polymerase chain reaction (PCR) and DNA sequencing analyses (Supplementary Fig. 2b and 2d). Pups were born close to the expected Mendelian ratio (Supplementary Table 3), suggesting that the Terb2 mutations did not impair normal embryonic development. Reverse transcription PCR and DNA sequencing were performed to confirm that mRNA transcripts of the mutant Terb2 genes carried the desired mutations in homozygous testes (Supplementary Fig. 2b and 2d). Quantitative PCR and western blotting analyses of 2-week-old mouse testes revealed that the Terb2 mutations do not alter the in vivo mRNA and protein expression levels of TERB2 (Supplementary Fig. 2e–g). The mRNA levels of TERB1 and MAJIN were also not affected by the Terb2 mutations (Supplementary Fig. 2e, f).

Intact TTM complex is required for fertility. Both male and female mutant mice developed normally into adults with no obvious somatic defects. However, repeated breeding attempts of

![Fig. 2 Crystal structure of the TERB1T2BM–TERB2NTD complex.](image-url)
homozygous Terb2Y56E and Terb2F188R male mice with WT female animals did not yield any offspring (Supplementary Table 4). Although mutant mice exhibited similar body weight compared to their WT littermates, anatomical analysis revealed that the adult testes of homozygous mutant mice were much smaller than those of their WT siblings (Fig. 5a, b). Histological examination showed that adult mutant seminiferous tubules were narrower and devoid of both elongated spermatids and mature sperms (Fig. 5c). Indeed, the epithelium of the majority of the seminiferous tubules was abnormal and contained only a single layer of Sertoli cells and very few spermatogonial cells at the tubule periphery (Fig. 5d). Consequently, there was no mature sperm in either the caput or cauda epididymal lumen of the homozygous Terb2Y56E and Terb2F188R mice (Supplementary Fig. 3a). Fluorescence-activated cell sorting (FACS) analysis showed that spermatogenesis process in both Terb2Y56E and Terb2F188R mice was disrupted at the tetraploid stage of primary spermatocytes (Supplementary Fig. 3b). Furthermore, terminal dexynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay revealed obvious positive signals in mutant seminiferous tubules, suggestive of massive apoptotic events that likely account for the absence of post-meiotic germ cells in Terb2Y56E and Terb2F188R testes (Fig. 5e, f). To further characterize the meiotic defect in Terb2Y56E and Terb2F188R mice, we examined the meiotic prophase I progression by immunofluorescence (IF) staining of the SC proteins SYCP3 and SYCP1.

Fig. 3 Crystal structure of the TERB2MBM–MAJIN_{NTD} complex. a Ribbon diagrams of two orthogonal views of the TERB2MBM–MAJIN_{NTD} complex. TERB2MBM is colored in magenta and MAJIN_{NTD} in blue. b Electrostatic surface potential of TERB2MBM-binding site of MAJIN_{NTD}. Positive potential, blue; negative potential, red. c, d Detailed interactions at the TERB2MBM–MAJIN_{NTD} interface. The color scheme is the same as in a. Residues important for the interaction are shown in stick models. Salt bridges and hydrogen-bonding interactions are shown as magenta dashed lines. e Ribbon diagram shows the potential heterotetramer mode of the TERB2MBM–MAJIN_{NTD} complex revealed in the crystal structure. f Structural model of the connection between telomeres and the NE through the TRF1–TERB1–TERB2–MAJIN interaction network.
Fig. 4 Mutational analyses of the TERB1–TERB2 and TERB2–MAJIN interactions. a, c Detailed interactions around Tyr56^{TERB2} in the TERB1_{T2BM}–TERB2_{NTD} complex (a) and around Phe192^{TERB2} in the TERB2_{MBM}–MAJIN_{NTD} complex (c). b, d Co-immunoprecipitation (Co-IP) of human TERB1 (b) or MAJIN (d) with wild-type (WT) or mutant Flag-tagged human TERB2 in 293T cells. The levels of each protein in the input and IP samples were analyzed by immunoblotting with the indicated antibodies. Input contains 5% of the input whole-cell lysate used for IPs. e Multiple sequence alignment of the N-terminal (top) and C-terminal (bottom) regions of TERB2 required for its association with TERB1 and MAJIN, respectively. Sequence positions are shown based on the human TERB2. The residues of Tyr56 (Try56 in mouse) and Phe192 (Phe188 in mouse) are indicated by blue arrowheads. f, g Co-IP of mouse TERB1 (f) or mouse MAJIN (g) with WT or mutant Flag-tagged mouse TERB2 in 293T cells. The levels of each protein in the input and IP samples were analyzed by immunoblotting with the indicated antibodies. Input contains 5% of the input whole-cell lysate used for IPs. Source data are provided as a Source Data file.
In both Terb2\textsuperscript{Y56E} and Terb2\textsuperscript{F188R} testes, although the presence of leptotene and zygote spermatocytes was evident, meiotic progression was apparently arrested at a pachytene-like stage where autosomal SC formation was never completed but with some partially synapsed lateral elements as shown by the positive staining of SYCP1 (Fig. 5g, h). The prophase I arrest was further confirmed by the Hoechst 33342 staining profile of the Terb2\textsuperscript{Y56E} and Terb2\textsuperscript{F188R} spermatocytes (Supplementary Fig. 3c). We never observed any Terb2\textsuperscript{Y56E} and Terb2\textsuperscript{F188R} spermatocytes that reached the mid-pachytene stage with typical WT SYCP3 and SYCP1 staining pattern along the entire autosomes (Fig. 5g, h).

IF analysis showed that TERB1, TERB2, and MAJIN were all localized at the ends of chromosomal axes in WT pachytene spermatocytes (Supplementary Fig. 4a–f)\textsuperscript{12,13}. In contrast, TERB1
Fig. 5 Disruption of the TERB1-TERB2-MAJIN complex results in defective spermatogenesis in mice. a Testes from 6-week-old wild-type, Terb2Y56E, and Terb2F188R mice. Scale bars, 5 mm. b Body weight (top) and testis weight (bottom) of 6-week-old male mice of the indicated genotypes. c Hematoxylin and eosin-stained histological cross-sections of testes. Arrowheads indicate abnormal spermatocyte-like cells in mutant seminiferous tubules. Asterisks show vacuolated seminiferous lacking spermatozoa and spermatids. Scale bars, 100 μm. d Immunofluorescence (IF) staining for SOX9 (green) of testis sections from adult male mice. DNA was stained by 4,6-diamidino-2-phenylindole (DAPI; blue). Asterisks show seminiferous containing a single layer of Sertoli cells and very few spermatogonial cells at the tubule periphery. Scale bars, 100 μm. e TUNEL staining of testis sections from 6-week-old mice. DNA was stained with DAPI (blue) and apoptosis was detected by IF terminal deoxinucleotidyl transferase-mediated dUTP-fluorescein nick end labeling (TUNEL) (green). Many TUNEL-positive cells were observed in both Terb2F188R and Terb2F188R tests. Scale bars, 1 mm. f Population of TUNEL-positive tubules shown as mean and s.d. n = 3 mice of each indicated genotype. Statistical significances (*P < 0.05, **P < 0.01, ***P < 0.001) were assessed by two-tailed t tests. g Representative spermatocyte nuclei from 6-week-old mice at different stages stained with DAPI and antibodies of SYCP3 and SYCP1. Pac-like Pachytene-like. h Quantification of the frequencies of meiotic stages shown in g. Data are from three independent experiments of different mice and error bars indicate s.d. More than 100 nuclei from each mouse were counted. Lep LtUpetone, Zyg Zygote, Pac-like Pachytene-like, Pac Pachytene, Dip Diploolte, Dia Diakinesis. Source data are provided as a Source Data file.

and TERB2 but not MAJIN were present at chromosomal ends in Terb2F188R pachytene-like spermatocytes, and only TERB1 was retained at chromosomal ends in Terb2Y56E spermatocytes (Supplementary Fig. 4a–f). This result was in agreement with the in vitro Co-IP analysis in 293T cells (Fig. 4f, g), suggesting that the TERB2 mutations Y56E and F188R, respectively, disrupted the TERB1-TERB2 and the TERB2-MAJIN interactions in mutant spermatocytes in an expected manner.

Like their male siblings, homozygous Terb2Y56E and Terb2F188R female mice were also infertile (Supplementary Table 4). Although the mutant female mice exhibited similar body weight as their WT littermates, ovaries from mutant adults were greatly reduced in size as compared with those of WT mice (Supplementary Fig. 5a, b). Histological examination failed to identify any growing follicles in Terb2Y56E and Terb2F188R ovaries (Supplementary Fig. 5c). Moreover, both Terb2Y56E and Terb2F188R fetal oocytes displayed a complete meiotic arrest at pachytene-like stage similar to the mutant spermatocytes (Supplementary Fig. 5d, e). The most advanced fetal oocytes observed in the mutant mice exhibited abnormal pattern of SC formation with only partially synapsed lateral elements (Supplementary Fig. 5d, e). Taken together, we concluded that the intact TTM complex is required for gametogenesis and targeted disruption of the complex results in complete meiotic arrest in prophase I in both sexes.

Disruption of the TTM complex induces disordered synapsis. The presence of only partially synapsed lateral elements in the arrested mutant nuclei indicated a severe defect in synapsis (Fig. 5g, h). Moreover, the mutant spermatocytes exhibited a large increase of TRF1 foci and SYCP3 filaments compared to WT pachytene spermatocytes (Fig. 6a, b), suggestive of synapsis defects in both chromosome bodies and telomeric ends. To investigate the underlying mechanism for the synapsis defects in mutant mice, we performed fluorescence in situ hybridization (FISH) chromosome painting to examine the homologous association in spermatocytes. Majority of the arrested mutant spermatocytes exhibited separated chromosome signals (Supplementary Fig. 6a–d), consistent with the presence of large number of SYCP3 filaments (Fig. 6a). FISH experiments with probes to chromosomes 5 and 8 revealed similar results (Supplementary Fig. 6a–d), suggesting that defect of homologous association is not specific to particular chromosomes.

The partial synapsis in arrested mutant spermatocytes revealed by SYCP1 staining suggests a possibility of non-homologous synapsis formation (Fig. 5g). When only chromosome 5 was counted, most of the detected axial structures were asynapsed with no SYCP1 signals (~60%), some displayed self-pairing (~20%), some exhibited non-homologous pairing (~5%), and <20% showed likely homologous pairing (Fig. 6d). The most convincing evidence for the presence of heterologous chromosome synapsis in Terb2-mutant spermatocytes was the synapsis formation on a chromosome 5 that was clearly separated from its homolog revealed by chromosome painting (Fig. 6c). Notably, some chromosome 5 even homologously associated displayed partial synapsis (Fig. 6c). The aberrant synapsis in the mutant spermatocytes was confirmed by the stimulated emission depletion (STED) super-resolution microscopy. The mutant spermatocytes exhibited a lot of self-alignment, including self-synapsis of chromosomal termini (Fig. 6e). STED also revealed misaligned telomeres in some apparently fully synapsed axial structures (Fig. 6e, the zoom-in view). All these misalignments at chromosome ends explained the large number of telomere foci observed in Terb2-mutant spermatocytes (Fig. 6a, b). Taken together, our results indicated that disruption of the TTM complex causes failure of homologous association and disordered synapsis and thus leads to prophase I arrest.

Disruption of the TTM complex impairs meiotic double-strand break (DSB) repair. Meiotic DNA recombination that is initiated via programmed DNA DSBs is coupled with SC formation. To further investigate the synapsis defects observed in Terb2Y56E and Terb2F188R male mice, we examined the effects of Terb2 mutations on homologous recombination-directed DSB repair. In both Terb2Y56E and Terb2F188R male spermatocytes, DMC1 exhibits positive γ-H2AX staining from leptotene through zygotene in a similar fashion as the WT spermatocytes (Fig. 7a). This result suggested that the initiation of the programmed DSBs is not affected in Terb2 mutant spermatocytes. It is noteworthy that, although reduced, γ-H2AX signals were still obvious in mutant pachytene-like spermatocytes, whereas the signal can only be observed in the X-Y body in WT pachytene nuclei (Fig. 7a), indicating that the programmed DSB repair process is severely impaired and no Terb2Y56E and Terb2F188R spermatocytes reach mid-pachytene stage (Fig. 7a, b). Next, we analyzed the location of DMC1, a protein involved in early repair of DSBs. In WT zygote spermatocytes, DMC1 assembles on the axial elements of chromosomes and gradually disappear towards pachynema (Fig. 7c, d). However, the Terb2Y56E and Terb2F188R spermatocytes exhibited persistent DMC1 foci (Fig. 7c, d), suggesting that the loading of DMC1 was not affected but subsequent process was arrested in mutant spermatocytes. To further examine the defect of DSB repair in mutant mice, we analyzed the distributions of MSH4, a marker of intermediate steps in recombination that forms discrete foci along aligned chromosomes, and of MLH1, a key player in the last step of recombination leading to the formation of crossovers. We found that majority of SYCP3 filaments lacked MSH4 foci in the mutant pachytene-like pairings.
spermatocytes, suggestive of a great reduction of DNA–DNA interaction between homologous chromosomes (Fig. 7e, f). This is consistent with the result of chromosome painting assays that most chromosomes are spatially separated from their homologs (Supplementary Fig. 6a–d). As expected, IF staining revealed no MLH1 foci in the mutant spermatocytes (Fig. 7g, h), suggesting a complete absence of crossover formation between homologous chromosomes. Collectively, our data demonstrated that disruption of the TTM complex causes severe impairment of homologous recombination and persistent DSBs.

Disruption of the TTM complex abolishes telomere–NE linkage. Next, we analyzed telomere distribution patterns in structurally preserved spermatocytes by staining of SYCP3, the NE marker Lamin B, and the telomeric DNA probe. IF-FISH assays revealed that majority of telomere foci in WT nuclei are localized to the nuclear periphery (Fig. 8a). In contrast, both the Terb2Y56E and Terb2F188R spermatocytes exhibited scattered telomeric signals within the nuclei, suggesting that most of telomeres in Terb2 mutant spermatocytes did not attach to the NE (Fig. 8a, b). Similar conclusion was obtained when testis sections were used...
for IF-FISH analysis (Supplementary Fig. 7). To test whether some synapsed chromosomes in Terb2 mutant spermatocytes may properly attach to the NE, we prepared testis tissue for in situ electron microscopic analysis, by which stretches of SC and sites of telomere–NE attachment can easily be detected. As anticipated, synapsed stretches of SC attached to the NE were clearly evident in WT spermatocytes but not in Terb2Y56E or Terb2F188R nuclei (Fig. 8c, d), further confirming that disruption of the TTM complex abolishes the telomere–NE attachment. SUN1 resides on the NE and is required for the telomere-LINC complex connection. Notably, no SUN1 foci were detected at chromosomal ends on Terb2Y56E or Terb2F188R spermatocyte spreads (Fig. 8e, f). This result suggested that the telomere–NE attachment mediated by the TTM complex is necessary for the telomere-LINC complex connection. On the other hand, although there were still telomere foci at the nuclear periphery, we observed an increased number of internal telomere signals in Sun1+/− spermatocytes compared to WT (Fig. 8a, b), suggesting that, despite SUN1 is not a prerequisite for the telomere–NE association, it plays an important role in the stable attachment of telomeres to the NE. This is consistent with the reduced frequency of telomere–NE junction observed by electron microscopic analysis in Sun1+/− spermatocytes (Fig. 8c, d). Together, we conclude that both the TTM complex and SUN1 (the LINC complex) contribute to the stable telomere–NE association.

Disruption of TTM complex causes Speedy A congregation at NE. CDK2 was previously shown to localize to telomeres, late recombination nodels, and the sex body in prophase I nuclei; playing an essential role in meiotic prophase I progression, a noncanonical activator of CDK2, was recently identified as a key regulator for CDK2 recruitment to meiotic telomeres. Knockout studies showed that both CDK2 and Speedy A contribute to efficient NE attachment of telomeres. This prompted us to examine the distribution of Speedy A and CDK2 in the absence of the telomere–NE attachment in Terb2Y56E and Terb2F188R spermatocytes. Consistent with previous studies, WT zygotene and pachytene spermatocytes displayed clear staining at both Speedy A and CDK2 at telomeres (Fig. 9a, c). In sharp contrast, telomere-bound Speedy A and CDK2 were greatly reduced in Terb2Y56E and Terb2F188R zygotene spermatocytes (Fig. 9a–d), suggesting that the telomere–NE attachment is important for the stable association of Speedy A and CDK2 with telomeres. Strikingly, however, many Terb2 mutant spermatocytes exhibited sustained congregation of Speedy A at the NE, suggesting that a fraction of Speedy A associates with the NE independent of the telomere–NE attachment (Fig. 10a, b). Moreover, this congregation of Speedy A at the NE is not detectable in Sun1+/− spermatocytes, indicating that Speedy A congregation at the NE is SUN1 dependent (Fig. 10a, b). Collectively, our data revealed two pools of Speedy A in the absence of telomere–NE attachment, one on the NE and the other at telomeres. The substantial reduction of telomere-bound Speedy A and CDK2 in Terb2 mutant spermatocytes suggested that the telomere–NE attachment promotes the association of Speedy A and CDK2 to the telomeres.

To investigate the mechanism of Speedy A congregation at the NE when the TTM complex is disrupted, we next analyzed SUN1 distribution in Terb2Y56E and Terb2F188R spermatocytes. In WT spermatocytes, teratomas, led by the LINC complex, cluster together to form the bouquet during a short time window in late zygotene stage and disperse along the NE after early pachytene. Our IF-FISH data of testis sections revealed ~4% of WT nuclei showing congregated SUN1 staining (Fig. 10c, d), consistent with the conclusion that telomere bouquet formation is a transient event. Notably, congregation of SUN1 at the NE was also observed in both Terb2Y56E and Terb2F188R nuclei, suggesting that SUN1 moving and clustering on the NE in early prophase I is independent of the telomere–NE connection (Fig. 10c). Strikingly, similar to the sustained congregation of Speedy A (~38%) at the NE, the population of nuclei displaying congregation of SUN1 in mutant mice was also much higher (~35%) compared to that in WT spermatocytes, suggesting that the dispersion of SUN1 and Speedy A is severely delayed or arrested in mutant nuclei (Fig. 10a–d). Together, we concluded that, in Terb2 mutant spermatocytes, a pool of Speedy A that is not associated with telomeres is recruited to and congregated at the NE in a SUN1-dependent manner.

Discussion
During meiotic prophase I, telomeres attach to and move on the NE, leading chromosome movements, a dynamic process essential for meiosis. SUN1 and MAJIN are two membrane proteins that provide binding sites on the inner surface of the NE for telomere attachment. However, it has been unclear how these two proteins and their binding partners cooperate together to establish the stable attachment of telomeres to the NE. In this study, we determined the crystal structures of the human TERB1NTD–TERB2NTD and TERB2MBM–MAJINSTD complexes and helped reveal at molecular level how these proteins establish the telomere–NE connection. We generated knock-in mice with TERB2 mutations that specifically disrupt its interaction with TERB1 (Terb2Y56E) or MAJIN (Terb2F188R). Both mutations resulted in complete disruption of the telomere–NE attachment in mouse spermatocytes and suggested that the TTM complex is essential for telomere–NE connection. In addition, we also examined the meiosis progress in Sun1−/− mice. Consistent with previous data that SUN1 plays an important role in telomere–NE association, we found that the telomere–NE attachment was partially disrupted by the deletion of SUN1 (Fig. 8a, b). A previous study revealed a direct connection between the TTM complex and the LINC complex through an
interaction between SUN1 and TERB1[15]. However, disruption of the interactions within the TTM complex results in complete abolishment of the telomere–NE attachment and depletion of SUN1 from telomeres (Fig. 8a–f), indicating that the SUN1–TERB1 interaction might be weak and the stable telomere–LINC complex connection requires additional interaction(s) between telomeres and SUN1. Taken together, we propose that the telomere–TRF1–TERB1–TERB2–MAJIN–NE interaction network and the telomere–LINC complex connection are likely two separate but cooperative pathways to stably recruit telomeres to the NE in meiosis prophase I. To fully understand the telomere–NE connection, further study is needed to reveal the molecular mechanism of the telomere–LINC complex connection.

The telomere–LINC connection is suggested to be a prerequisite for meiotic chromosome movements and bouquet formation that is required for efficient progression of prophase I[10].
Telomere localization of Speedy A and its potential role in regulating telomere–NE attachment in meiotic prophase I prompted us to examine its distribution when telomeres are depleted from the NE. We observed two pools of Speedy A, one with telomeres in the nucleoplasm and the other on the NE, when telomeres are depleted from the NE by mutations of TERB2 (Figs. 7a and 10a). The presence of telomere-associated Speedy A is consistent with a recent study, which identified a direct interaction between Speedy A and TRF1 (Fig. 9a, b)32. However, a substantial reduction of Speedy A at telomeres in both Terb2587 and Terb2116 spermatocytes compared to WT indicated that the telomere–NE attachment promotes the recruitment of Speedy A to telomeres (Fig. 9a, b). On the other hand, the NE-associated Speedy A that appears as congregations without telomere signals is dependent on the presence of SUN1 (Fig. 10a, b), suggesting that this pool of Speedy A moves and congregates in a LINC complex-dependent manner. Taken together, we speculate that Speedy A is likely capable of interacting with both telomeres and the LINC complex and thus might function as the missing linkage between telomeres and the LINC complex during prophase I, stabilizing the telomere–NE connection. It will be of great interest to test this hypothesis to fully understand the mechanisms of stable telomere–NE connection and telomere movement along the NE driven by the LINC complex.

The knock-in mice with specific and accurate disruption of the TERB1–TERB2 or the TERB2–MAJIN interactions enable us to systematically assess the consequent meiosis defects caused by the disruption of the telomere–NE attachment. During WT early prophase I, the conserved programmed DSB repair triggers a genome-wide search for DNA sequence homology22. In both Terb2587 and Terb2116 spermatocytes, the γ-H2AX signal was detected at leptotene and zygotene stages similar to that in WT spermatocytes (Fig. 7a, b), indicating the normal initiation of the programmed DSBs. Consistently, loading of DMC1 on the chromosomes suggests that DNA–RAD51 complexes were formed in mutant spermatocytes (Fig. 7c, d). However, several lines of evidence suggest that the subsequent DSB repair process was severely impaired. First, the DMC1 signal is persistently remained on the chromosomes in mutant pachytene-like spermatocytes (Fig. 7c, d). Second, majority of homologous chromosomes are not well aligned to each other (Fig. 6a, b) or not even associated to each other as revealed by the chromosome painting assays (Supplementary Fig. 6a–d). Third, in mutant spermatocytes, no MLH1 foci were observed on the chromosomes, suggestive of no crossover formation (Fig. 7g, h). Collectively, we conclude that disruption of the telomere–NE connection does not interfere the initiation of the programmed DSBs, but it interrupts the subsequent homologous recombination-directed repair process. Strikingly, besides the asynapsed chromosomes, we observed a substantial level of self-pairing and nonhomologous pairing of chromosomes (Fig. 6c, d), suggesting that disruption of telomere–NE attachment results in disorderly synapsis. It has been suggested that telomere-led meiotic chromosome movements facilitate chromosome pairing10,14 and that the telomere–LINC connection is a prerequisite for these chromosome movements10. Disruption of the telomere–NE attachment prevents telomere–LINC complex connection and therefore disables meiotic chromosome movements. We speculate that lack of chromosome movements may induce incorrect heterologous pairing, resulting in the observed heterologous synapsis in Terb2 mutant spermatocytes. Future studies will be needed to fully understand how telomere–chromosome movements influence homologous pairing in meiosis.

Methods

Protein expression and purification. Human TERB1(587–659) and TERB2(220–659) were co-expressed in Escherichia coli BL21 CodonPlus (DE3) cells (Strategene) using a modified pRSETDuet vector with a 6×His tag fused at the N-terminus of TERB2. The primers used for cloning are as follows. TERB1587–659-F: AAT TGG ATC ATG CTG ACC TAC CGT TGC, TERB1587–659-R: TTT CTC GAG CTA TTT CCT CTT CAA TTT GTT. The presence of meiotic stages shown in Fig. 6a, b or not even associated to each other as revealed by the Immuno fluorescence staining of spermatocyte chromosome spreads for SYCP3 (green) and MSH4 (red). White arrows indicated chromosomes with MSH4 foci in the mutant nuclei. f) Quantification of the number of MSH4 foci on the SYCP3 filaments per nucleus shown in e, g) Immuno fluorescence staining of spermatocyte chromosome spreads for SYCP3 (green) and MLH1 (red). h) Quantification of the number of MLH1 foci on the SYCP3 filaments per nucleus shown in g. DNA were stained with 4,6-diamidino-2-phenylindole (a, c, e, g). Source data are provided as a Source Data file.
**Fig. 8** Intact TERB1–TERB2–MAJIN complex is required for telomere–nuclear envelope (NE) attachment.  

**a** Equator images of structurally preserved zygote spermatocytes from wild-type (WT), Terb2Y56E, Terb2F188R, and Sun1−/− immunostained for SYCP3 and Lamin B and subjected to fluorescence in situ hybridization of telomeric DNA probe (TelC). DNA was stained by 4,6-diamidino-2-phenylindole (DAPI). Scale bars, 5 μm.  

**b** Quantification of internal telomere foci number shown in **a**.  

**c** Electron micrographs showing telomeres (magenta arrowheads) and nuclear membrane (red arrows) in WT pachytene and mutant pachytene-like spermatocytes. Scale bars, 500 nm.  

**d** Populations of nuclei from spermatocyte sections showing at least one telomere–NE attachment. Only nuclei with stretches of synaptonemal complex (SC) observed were counted for the quantification. Data are from three independent experiments with different mice and error bars indicate s.d. About 100 nuclei containing SC stretches from each mouse were counted for quantification. n is the total number of nuclei with SC stretches used for counting.  

**e** Chromosome spreads from adult spermatocytes were immunostained with anti-SYCP3 (green) and anti-SUN1 (red). DNA was stained by DAPI. The zoom-in views show one chromosome end from each spread. Scale bars, 10 μm.  

**f** Dot blot showing population of chromosome ends with SUN1 foci in one nucleus. Source data are provided as a Source Data file.
BL19U1. A 2.9-Å Se-SAD dataset of the TERB2119–220–MAJIN2–149 complex was collected at the Se peak wavelength (0.97776 Å) and was processed by HKL300033. Three selenium atoms were located and refined, and the single-wavelength anomalous diffraction data phases were calculated using Phenix34. The initial SAD map was substantially improved by solvent flattening. An initial model was automatically built into the modified experimental electron density map. The model was then refined using Phenix34, together with manual building in Coot35. All the structural figures were generated using PyMOL36.

Fig. 9 Telomere localization of Speedy A and CDK2 is reduced in mutant nuclei. a Immunofluorescence-fluorescence in situ hybridization (IF-FISH) images of chromosome spreads from adult spermatocytes of the indicated genotypes. Chromosome spreads were immunostained for SYCP3 (green) and Speedy A (red) and subjected to telomere FISH (TelC-FISH) (magenta). DNA was stained by 4,6-diamidino-2-phenylindole (DAPI; blue). Zyg Zygotene, Pac Pachytene, En showing enhanced signals (red) of Speedy A with a lower cutoff. White arrowheads indicate sex chromosomes. Scale bars, 10 μm.

b Quantification of the relative intensity of Speedy A foci at telomeres in wild-type (WT) or Terb2-mutant spermatocytes in a. c IF-FISH images of chromosome spreads from adult spermatocytes of the indicated genotypes. Chromosome spreads were immunostained for SYCP3 (green) and CDK2 (red) and subjected to telomere FISH (TelC-FISH) (magenta). DNA was stained by DAPI (blue). Zyg Zygotene, Pac Pachytene, En showing enhanced signals (red) of CDK2 with a lower cutoff. White arrowheads indicate sex chromosomes. Scale bars, 10 μm. d Quantification of the relative intensity of CDK2 foci at telomeres in WT or Terb2-mutant spermatocytes in c. Source data are provided as a Source Data file.
Cell culture. HEK293T and U-2 OS cells were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences. Cells were cultured at 37 °C under 5% CO2 in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Hyclone). No mycoplasma contaminations were detected in these cells.

BiFC assay. cDNA fragments of human TERB1 and TERB2 were constructed into pLVX-IRES-Puro vectors (Clontech) and fused at their C termini with a Venus protein N-terminus (1–154) (-VN) or C-terminus (155–238) (-VC). Plasmids were transfected into HEK293T cells with the help plasmids pCMV-dR8.9 and pCMV-
**Fig. 10** Speedy A and SUN1 exhibit congregation in Terb2 mutant spermatocytes. a, Immunofluorescence-fluorescein in situ hybridization (FISH) showing distribution of Speedy A (a) and SUN1 (c) in spermatocytes. Sections of 2-week-old testes of different genotypes were as indicated for SYCP3 and Speedy A (a) or SYCP3 and SUN1 (c) and subjected to telomere FISH (TelC). DNA was stained by 4,6-diamidino-2-phenylindole. White arrowheads indicate congregation of Speedy A (a) or SUN1 (c). White arrows in a indicate Speedy A signals co-localized with telomeres. Scale bars, 5 μm. b Population of nuclei that display Speedy A congregation in a. Data are from three independent experiments with different mice and error bars indicate s.d. c Population of nuclei that display SUN1 congregation in c. Data are from three independent experiments with different mice and error bars indicate s.d. For each experiment, >100 spermatocytes from each indicated genotype were counted for the frequencies of nuclei showing congregation of Speedy A (b) or SUN1 (d). Source data are provided as a Source Data file.

SVS-G with X-tremeGENE HE DNA Transfection Reagent (Roche) according to the manual. The lentiviral supernatants were harvested 36 h later and filtered by 0.22-μm filter (Millipore). Lentiviruses for -VN and -VC protein expression were used for co-infection of HEK293T cells. After 36 h, infected HEK293T cells were subjected to FACS analysis for Venus signals.

**Animals.** Mice were housed under controlled environmental conditions with free access to water and food. Experimental protocols were approved by the regional ethical committee of the National Center for Protein Science Shanghai (approval #SIBCB-8342-1510-043). Every effort was made to minimize and refine the experiments to avoid animal suffering.

**Generation of Terb2-mutant mice and SUN1+/− mice.** The mutant mice were generated by CRISPR/Cas9-mediated genome engineering. Briefly, a single guide RNA (sgRNA: CAT TAT CTT CTA TGT ACT CG for Terb2g5286,GCC AAA TAT CCT GAG CTA CTG CC for Terb2a1888, and AGA CCA AAG TGG ACC ACT TC for Sun1−/−) was cloned into a sgRNA vector and was then used as a template to synthesize sgRNAs using the MEGAshortscript T7 Kit (Life Technologies). Cas9 mRNA was in vitro transcribed from plasmid MLM613 (Addgene #42251) using the mMESSAGE mMACHINE T7 Ultra Kit (Life Technologies). For microinjection, Cas9 mRNA (100 ng/μL) was mixed with sgRNA (50 ng/μL) and 100 ng/μL donor oligos (CAT TCT TGT TTA CAT AAC CAG TAT CCT TCT TCT TAC AAT ATA TCA GGT CAG AGA CAT GAT AGA TAA TAG TAC TGC TTT CCA TCA TCT TGG GCA AAT AAC TAC TGA AA for Terb2g5286), CAT ACG CAT TAT AAT TGA TGC CTA ACG GAA ATT CTT CAG AGA AGT ACA CAG GAG CAC GCC TCC ATG CTC AGG ATC TTT GGA GTC TAT ACA TCA TCG AGA AAT TAA ATG GTT TGC TAT for Terb2a1888, and no donor for Sun1−/−) and microinjected into the cytoplasm of fertilized eggs collected from C57BL/6 mice for passing the desired mutations through the germline. For Sun1−/+−, heterozygous mouse containing a frameshift at Hs149 were bred with C57BL/6 mice for passing the desired mutations and homozygous Sun1−/− mice were confirmed by western blotting.

**Co-IP and western blot.** cDNA fragments were constructed into pEGFP-N1 (Clontech) and transfected into HEK293T cells. After 36 h, cells were collected and lysed in lysis buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% Glycerol, 0.5% TritonX-100, 1 mM EDTA) supplemented with EDTA-free complete protease inhibitor (SDS-PAGE) separation. To detect WT and mutant TER2 proteins in spermatocytes, testis samples from mouse littersmates were homogenized in phosphate-buffered saline (PBS) buffer. Cells were pelleted and lysed by sonication in lysis buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% Glycerol, 0.5% TritonX-100, 1 mM EDTA). Cell lysates were centrifuged and the supernatants were subjected to SDS-PAGE separation. After SDS-PAGE, proteins were blotted onto PVDF membranes (Millipore). The blots were incubated in blocking buffer (3% fat-free milk in PBS buffer supplemented with 0.05% Tween-20) at room temperature (RT) for 1 h and incubated with primary antibodies in blocking buffer at 4 °C overnight. Blots were washed with TBST and incubated in the horseradish peroxidase (HRP)-labeled secondary antibodies at RT for 1 h. After being washed, blots were developed with ECL Prime Western Blotting System (GE Healthcare, RP22322).

**Histological analysis.** The testes and ovaries were dissected and fixed in 4% paraformaldehyde (PFA) in PBS at 4 °C for 8 h. Tissues were dehydrated by treatment with an ethanol series (50%, 75%, 95%, 100%), cleared twice in xylene, and embedded in paraffin. Tissue sections (5 μm) were prepared with a Leica RM2235 rotary microtome. Sections were stained with hematoxylin and eosin according to the standard protocol (BBI, E607318). TUNEL assay was carried out with the TUNEL ApoGreen Detection Kit (Yeasen, 40307ES20).

**Squash and spread preparations.** Squash and surface-spread spermatocytes were prepared as described previously with minor modifications14,15. Briefly, for squashed spermatocytes, freshly extracted 14-dpp (days past parturition) testes were minced into small pieces with tweezers in 2% PFA and 0.05% Triton X-100 and 0.5% Tween 20. The resulting fragments were then washed twice in 1% PFA and 0.5% Triton X-100 before being collected.
fixed for 10 min. Several small pieces of tubules were then put on a slide previously rinsed with ethanol and chloroform mixture (1:1), covered with a cover slip, and gently pressed to distribute the material. The slides were immersed in a 1% PFA solution, 0.5 mM dithiothreitol, and protease inhibitor cocktail (Roche) for 30 min. Tubules were then moved to 100 mM sucrose solution (pH 8.2) and spermatoocytes were detached by pipetting. Drops of cell suspension were then placed on the upper right corner of cover slips (Thermo, 12-545-84) soaked in 1% PFA solution (Sigma, P6148), pH 9.2, supplemented with 0.15% Triton X-100, and placed on the upper right corner of cover slips (Thermo, T_7011254584) and

Electron microscopy. Testes were dissected and incubated in a fixative consisting of 2.5% glutaraldehyde in PBS for 2 h at 4 °C. Samples were then washed three times with PBS and fixed in 1% Osmotic in PBS for 2 h at 4 °C and then washed three times with PBS and two times with ddH₂O. Pre-embedding staining was performed overnight at 4 °C in the dark using 0.5% uranyl acetate. Samples were washed with ddH₂O and then dehydrated in an ethanol series (25%, 50%, 75%, 95%, and 100%). For plastic embedding, samples were incubated in 3:1 ethanol:1:1 ethanol: resin, 1:1:1 ethanol: resin, and 1:1 ethanol: resin, for 40 min for each, then kept overnight in 100% resin. Samples were put into fresh resin and further permeated for 3 h at RT. Resin polymerization was done at 60 °C for at least 48 h. Resin blocks were cut with an ultra-microtome (Leica) and serial sections of 100-nm thickness each section were collected on formvar copper grid (KYKT) and visualized under an electron microscope (FEI, Talos L202C) with a voltage acceleration of 120 kV.

IF staining and FISH. For IF staining of cultured cells, cells were grown on cover slips (Thermo, T_011254854) and fixed by 4% PFA, permeabilized with 0.15% Triton X-100, blocked with 5% bovine serum albumin (BSA) at RT for 1 h, and incubated with primary antibodies at 4 °C for overnight. For IF staining of spermatocyte spreads, spreads were washed with PBS buffer supplemented with 0.15% Triton X-100, blocked with 5% BSA at RT for 1 h, and incubated with primary antibodies at 4 °C for overnight. For IF staining of paraffin-embedded tissue sections, sections on cover slips were dewaxed twice in fresh xylene for 15 min each and rehydrated in 100%, 95%, 75%, 50% ethanol and PBS for 10 min each. Epitope retrieval was carried out by boiling sections in TUF TARGET UNMASKING BUFFER (Roche)) for 30 min. Tubules were then moved to 100 mM sucrose solution (pH 6.82, 50 mM sucrose, 17 mM sodium citrate, 5 mM EDTA, 0.5 mM dithiothreitol, and protease inhibitor cocktail (Roche)) for 30 min. Tubules were then moved to 100 mM sucrose solution (pH 8.2) and spermatoocytes were detached by pipetting. Drops of cell suspension were then placed on the upper right corner of cover slips (Thermo, 12-545-84) soaked in 1% PFA solution (Sigma, P6148), pH 9.2, supplemented with 0.15% Triton X-100, and placed on the upper right corner of cover slips (Thermo, T_7011254584) and

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Author contributions

Y.W., Y.C., C.H. and M.L. designed the study. Y.W., Y.C., and C.H. carried out biochemical assays. Y.W., L.N. and L.W. crystallized the TERB1–TERB2 and TERB2–MAJIN complex. J.W., Y.W. and L.W. collected X-ray data and determined the crystal structure. Y.C. and J.C. participated in the generation of the knock-in mice. Y.W., Y.C., J.L., H.C. and C.H. performed the experiments to characterize the meiotic phenotype. Y.W., Y.C., C.H. and M.L. analyzed data. Y.W., C.H., J.W. and M.L. drafted the manuscript. All authors reviewed and edited the manuscript.

Additional information

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