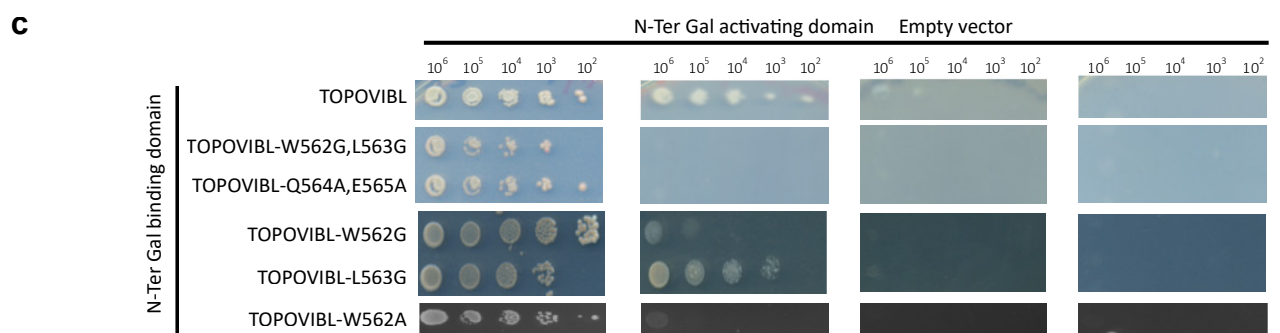
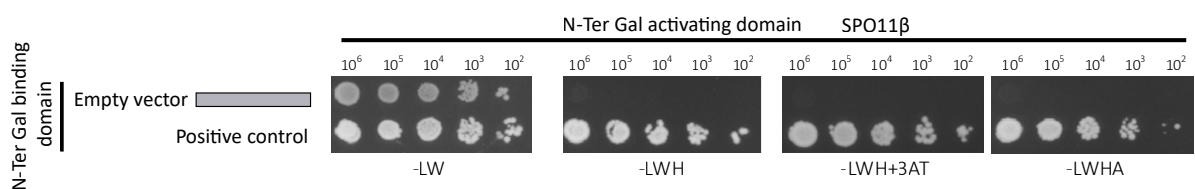
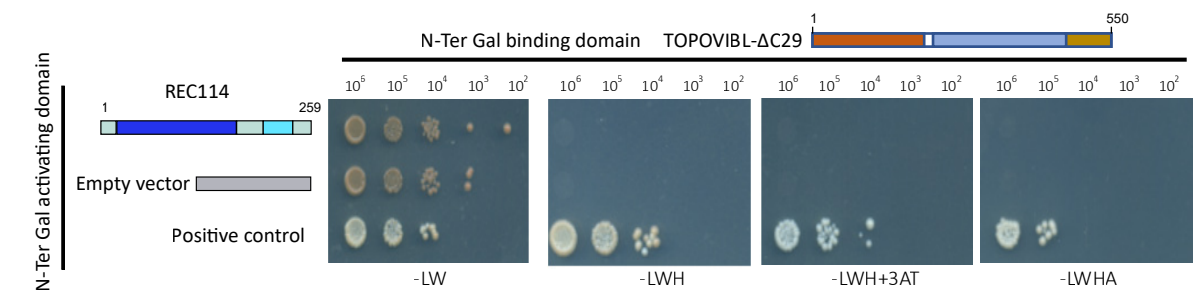
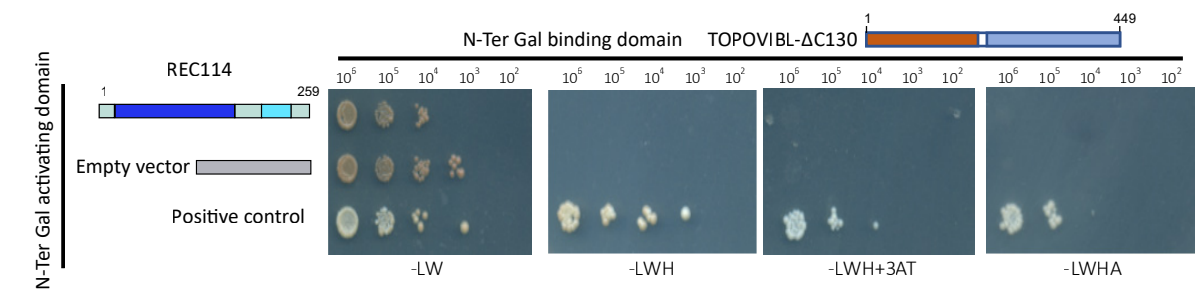
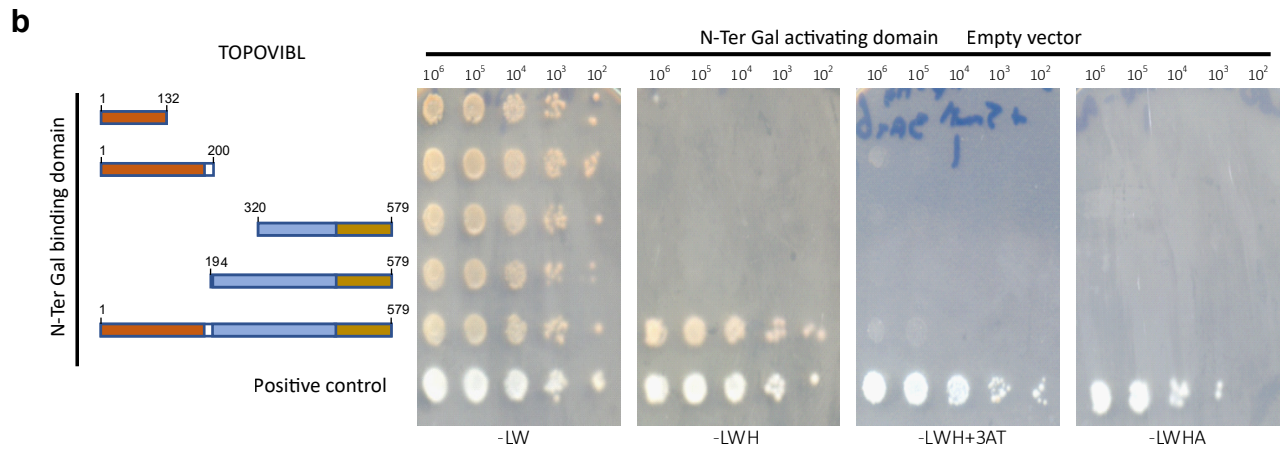
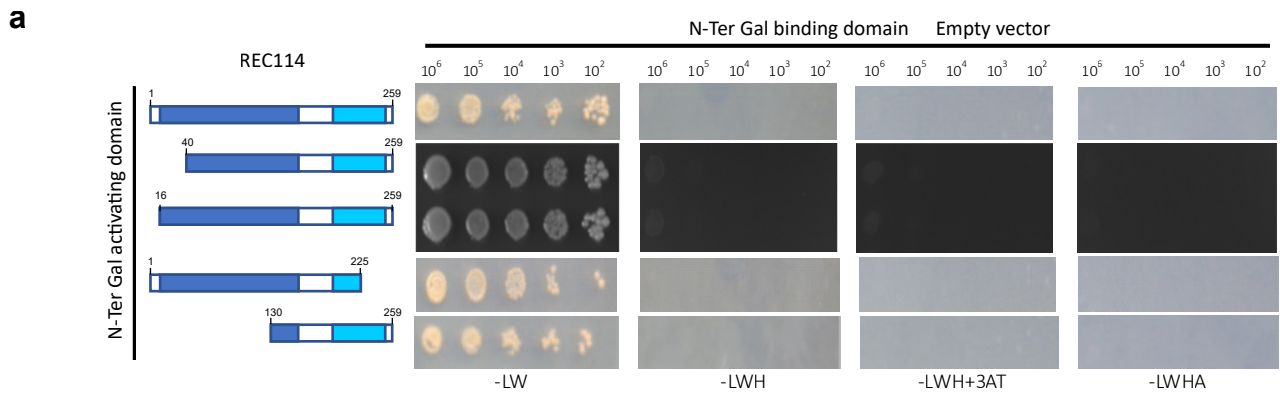


Supplementary data

Supplementary Figures 1-16

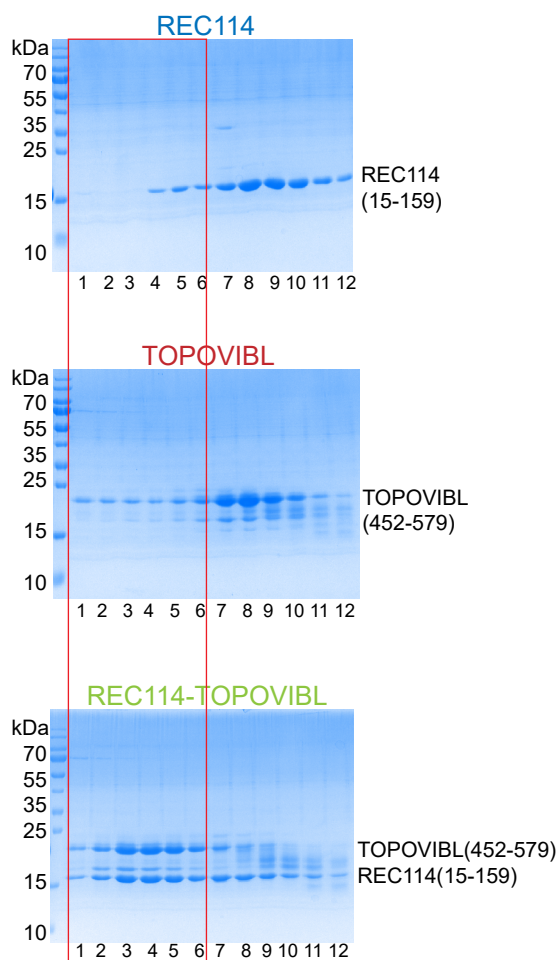
Supplementary Tables 1-4



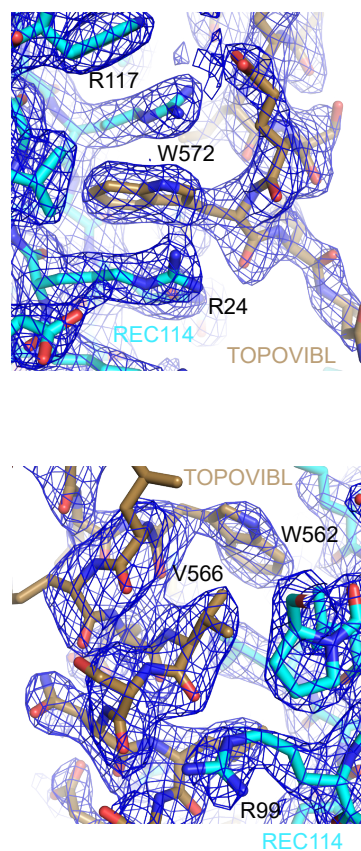
Supplementary Figure 1. Controls for Y2H

- a.** Negative controls for Y2H assays for Fig. 1b
- b.** Negative controls for Y2H assays for Fig. 1c
- c.** Negative controls for Y2H assays for Fig. 2h.

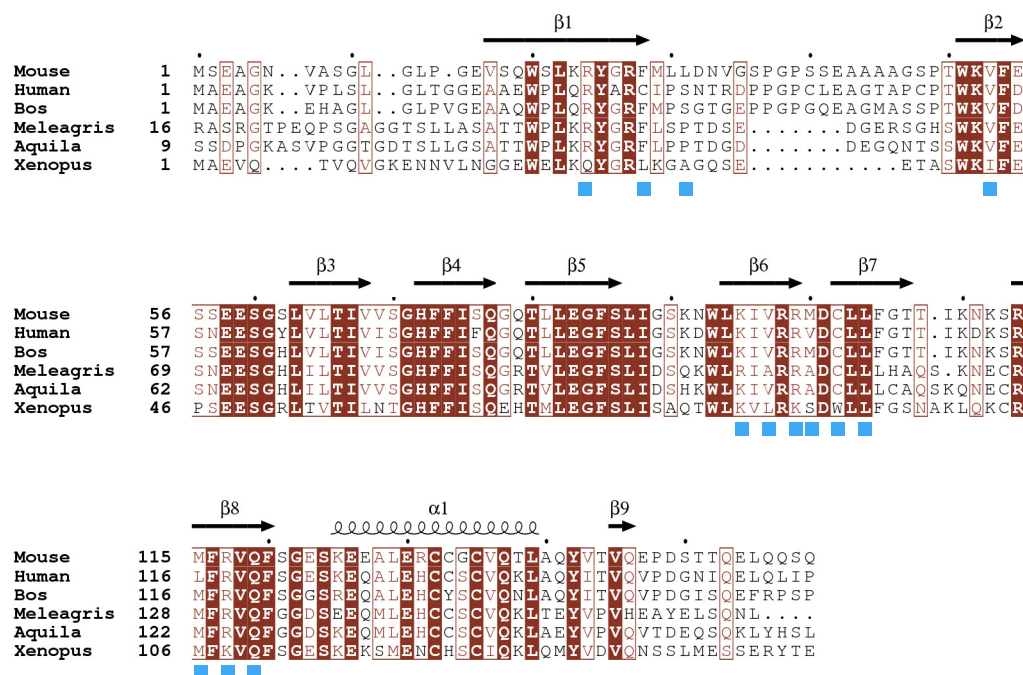
a



b



c



Supplementary Figure 2. Properties of the REC114-TOPOVIBL complex

- a. SDS-PAGE analysis of fractions 1-12 of the Superdex 200 gel filtration elution profiles of REC114¹⁵⁻¹⁵⁹, TOPOVIBL⁴⁵²⁻⁵⁷⁹ and their complex shown in Fig. 1e. The red rectangle highlights fractions containing the REC114-TOPOVIBL complex. Source data are provided as a Source Data file.
- b. Representative part of the 2Fo-Fc electron density map covering the TOPOVIBL-REC114 interface contoured at 1.0 σ .
- c. Sequence alignment of the REC114 PH domain from *M. musculus*, *H. sapiens*, *B. mutus*, *Meleagris gallopavo*, *Aquila chrisaetos canadensis* and *Xenopus laevis* used to highlight the amino-acid conservation on the structure (Fig. 2c). Brown letters: equivalent amino acids; white letters: identical amino acids. The residues interacting with TOPOVIBL are shown as blue squares.

S452

Mus_musculus/396-579

1 10 20 30 40 50 60

Mus musculus/396-579
Homo sapiens/395-577
Chelonina mydas/398-611
Rhinatrema viviparum/70-211
Danio rerio/391-562
Merluccius californicus/391-634
Aplysia californica/466-596
Patriaria miniata/30-206
Nematostella vectensis/21-315

LSVAVNSISLMSVLTGTSRSRFRKMTCLQALEADTFQFGVKLHRIFYDTTQHQFLKHSCSDT
LSVAVNSISLMTGTSRSRFRKMTCLQALEADTFQFRKTLHKVFRETTQHQFLHHKSCDT
RPFVLIGALSSVSVSSNTSQRRRECLSQVDSITDGFRLMAITAFENYTRKKRLMNGTCSIT
RPSVLTDCISISVTSADSGSRERCLSDMQVDTQGFRTVVDLSHRLNRLVLSRSTCMLN
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LPIFVLSVNSISITAGNGERTATVDMSRVSISLQSLTSRQSSQSDVGRFPTPRPKCKR
IPDIANSLLDMFIRITNTERELNVLGHSLPTENSRATMSKHKVEKLNWLLNG
VANIATEVATIVSHNDEERQCFSLMKVPNSGLSSSSLPQSHLSACRHHMGVITCAES
VPIAISALASIVSHKKNRQQRCLLITQETRVDRIEKLTNKLWCAKQKFGIS

<i>Mus_musculus/396-579</i>	79	89	99
<i>Mus musculus/396-579</i>	EQHLTPKKNISAQNTKDDH...	KNIAQFFFEES...	
<i>Homo sapiens/395-577</i>	KQQLTLEKSDAQGTEDAP...	DNSSELELLAT...	
<i>Chelonina mydas/398-611</i>	TKQVPEDASPAWLAAACDLPTAGQLKSQEASDV		
<i>Rhinatrema bivittatum/70-211</i>	RKELLEKPNVNLDTCTCTYPEARNRSQSGNASC		
<i>Danio rerio/391-562</i>	EKKVTVQVQSSPDLEEAFFENPLSSKRI		
<i>Limneria chalumnae/391-634</i>	RTSVENKQTSQSTSDSS...	DLRLMDSFETCTGSLRKHGLSLPMDSSKED	
<i>Aplysia californica/466-956</i>	CHESNADEDTDAKPLPLGNQDKDEKICVDE		WSDSQFESQERHFEDRRND
<i>Patiria miniata/30-206</i>	QVQFESKSEKAMVTSQSPASQCDLGHIPASQ		PTGADASDLDFSD.
<i>Nematostella vectensis/21-315</i>	QEASEGEQVAVPAISQASVSGSDTGKTPSTQ		TSIISSSYFDDLSD.

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Homo sapiens/395-577      . . . . . SGQAEIKRRKRRGSPFR . . . . . IEEMRALRSAR .
Chelonia mydas/398-611      . . . . . SISLINSSEPA . . . . . SLLDRVDLWGEISETKRRKTA
Rhinatrema bivittatum/70-211
Danio rerio/391-562      . . . . . IVESDTGEFFVQAEIKRRRTAAPKQGQFDSTEYIQAATGTCGRIE
Leinieria chthonica/391-634
Lepomis californicus/466-566
Patiria miniata/30-206      . . . . . FSQDLDFIDGCVGTVGNLESS . . . . . QLFREAKSAGTATSGME .
Nematostella vectensis/21-315

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Mus_musculus/396-579
129      130      140
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Homo_sapiens/395-577      . . . . . APSPSEAAAPRRP      . . . . . EATAAPLTFRGRE
Chelonina mydas/398-611      . . . . . TIENNAGEALEQEPFRGGLQERTDNLMT      . . . . . DFGSQLS      . . . . . FCLWSP
Rhinatremata bivittatum/70-211      . . . . .      . . . . . RED      . . . . . LSDNGST
Danio_zebrio/391-562      . . . . . HCDLESSSIFISKRKQTEFFPSDSPTNE      . . . . . SDFQKSP      . . . . . FSLVHL
Acanthamoeba castellanii/391-634      . . . . . EARSQGAARRR      . . . . .      . . . . . RGSDFP      . . . . . LPVCRK
Aplysia californica/466-956      . . . . . CQLSEHQNEQPPVEDPKDDSLDLVLSIQSDVQ      . . . . . SEDERLPIDSPFSPDLSKSPPCYEDTE
Patiria miniata/30-206      . . . . .      . . . . .      . . . . .      . . . . .      . . . . .
Nematostella vectensis/21-315      . . . . . CITSEPSSELGIDIPLPNNNTTQRKTQEVNSGIST      . . . . .      . . . . . LGLDKP

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Mus_musculus/396-579      . . . . . TQAAHG.
Homo_sapiens/395-577      . . . . . HREAHG.
Chelonia_mydas/398-611    . . . . . MFLSEREEGF
Rhinatrema bivittatum/70-211 . . . . . KKGTDAS . . . . . MFLSEREEGF
Danio_xerio/391-562       . . . . . PQIQQAR . . . . . SNSIMHSQI
Ictalurus_noveboracensis/391-634 . . . . . HRDREA . . . . . PQIQQAR
Aplysia_californica/466-956 . . . . . LCDDPLIELSVVESNSHVHMNRNSSPAETDAGLELEFPMLPHETADACKALLSLFDNSLTQ
Patiria miniata/30-206    . . . . .
Nematostella vectensis/21-315 . . . . . LRNN . . . . . IPQNTQNTCFSS
```

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Mus_musculus/396-579
Mus_musculus/396-579
Homo_sapiens/395-577
Chelonia_mydas/398-611
Rhinatrema bivittatum/70-211
Danio rerio/391-562
Latimeria chalumnae/391-634
Haplochromis caeruleus/466-956
Patiria miniata/30-206
Nematostella vectensis/21-315

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Mus_musculus/396-579
Mus_musculus/396-579
Homo_sapiens/395-577
Chelonia_mydas/398-611
Rhinatrema bivittatum/70-211
Danio_xerio/391-562
Latimeria chalumnae/391-634
Aplysia californica/466-956
Patiria miniata/30-206
Nematostella vectensis/21-315

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Mus_musculus/396-579                               .RAQAAEAASAPG                      GLEDL
Homo_sapiens/395-577                              .RALAPGRASIGS                    RLEDVL
Chelonia_mydas/398-611                             .TAVSEDGARSS                     NLWQGHL
Rhinatrema_bivittatum/70-211                       .YDTFKDGSLLQGO                   QEDKL
Danio_xerio/31-562                                  .TEDPKPSVKRLNE                  QVSEQ
Latimeria_chalumnae/391-634                        .CTDWCAAGGGSRHGS                VMGEDW
Lepidosteusosteichthys/466-566                    .DITDWMSCSVETSTSCICDAKFRPRGKSPDVLMVDG    VSGSLNSF
Patiria_miniatra/30-206                            .SVKSFVEVSQSKS                 MLEDE
Nematostella_vectensis/21-315 ADP     .TGLEFHTEKDEE                 NLLKE DLE TKDALH
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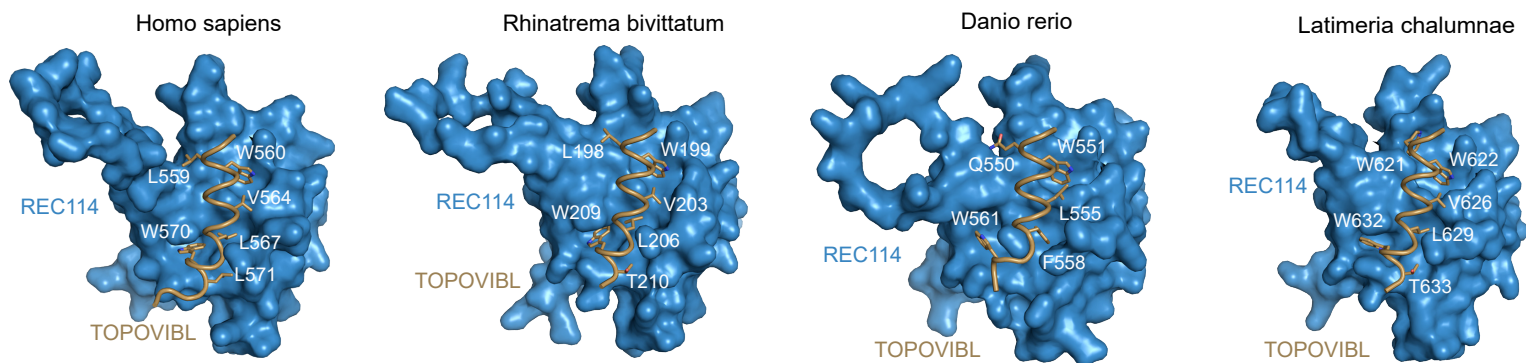
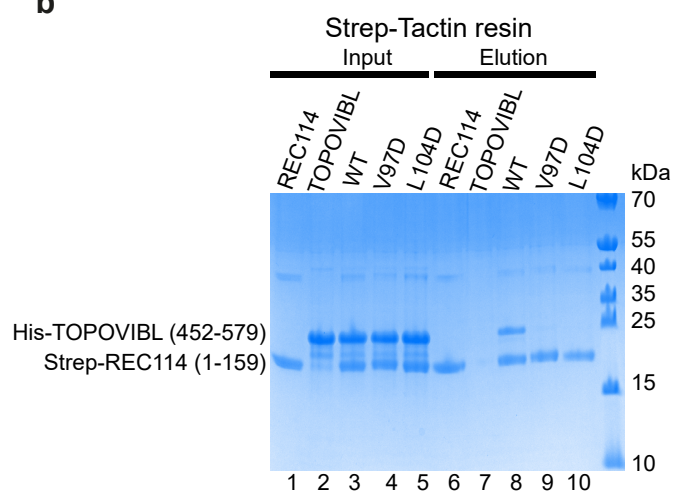
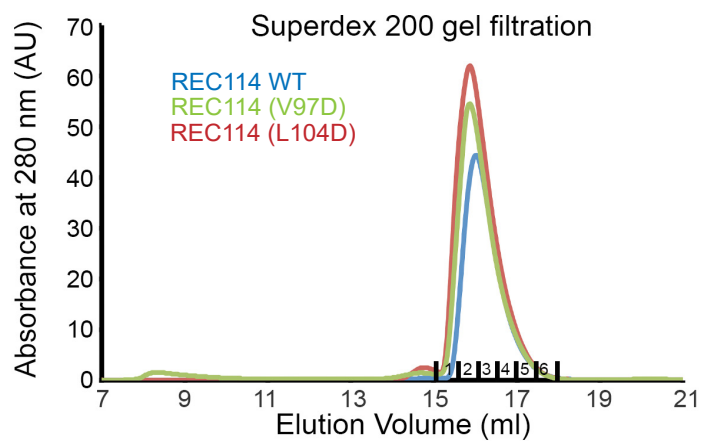
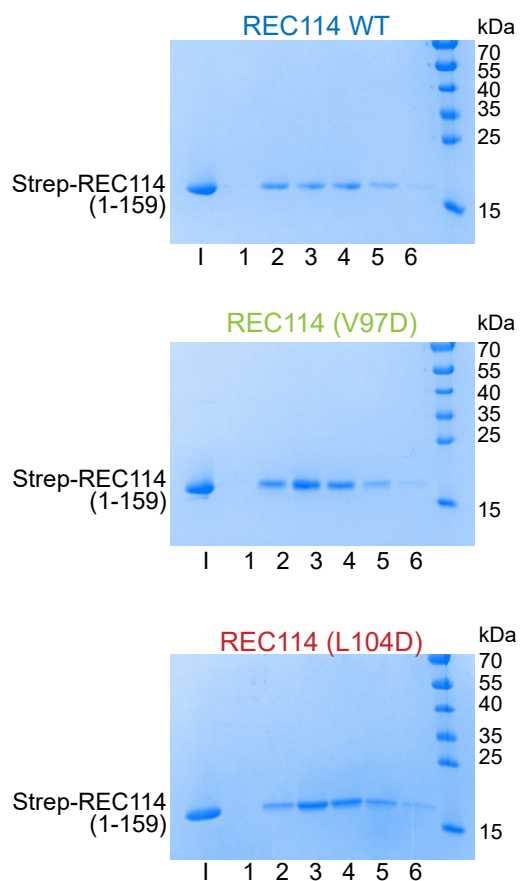
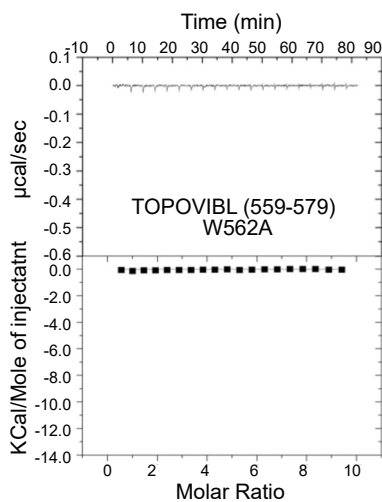
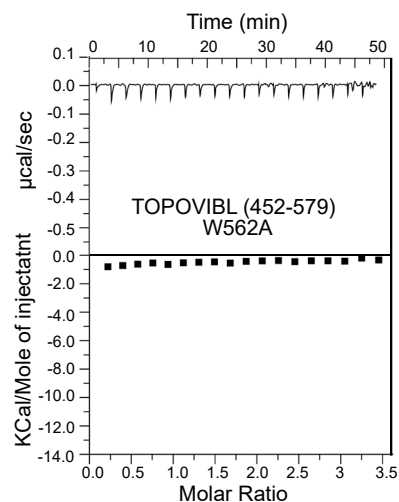
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                                170      180
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Chelonia_mydas/398-611    . . . . . WLDVSNLSEWNTS.
Rhinatrema_bivittatum/70-211 . . . . . WLDVSKLDWTC.
Danio_zebr/391-562        . . . . . WLDVSNLSEWLN.
Latimeria_chalumna/391-634 . . . . . WLDVSNLSEWLN.
Aplysia_californica/466-596 . . . . . WLDVSNLSEWLN.
Patiria_mininata/30-206   . . . . . WLDVLDSEFENE.
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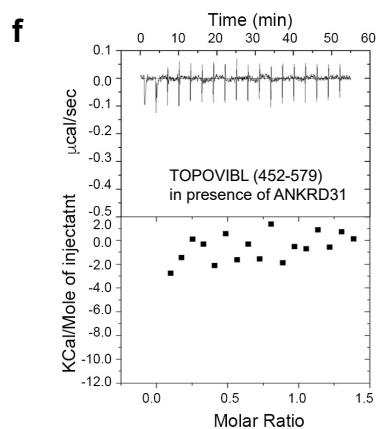
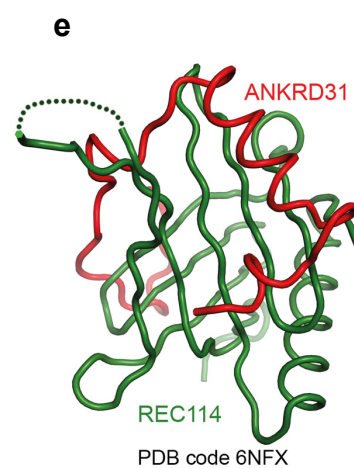
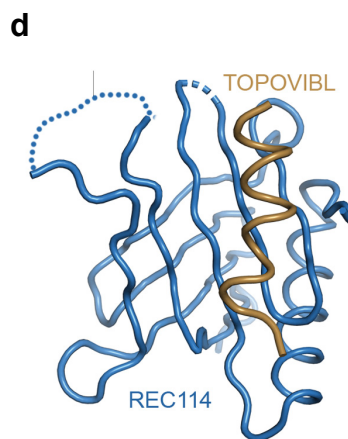
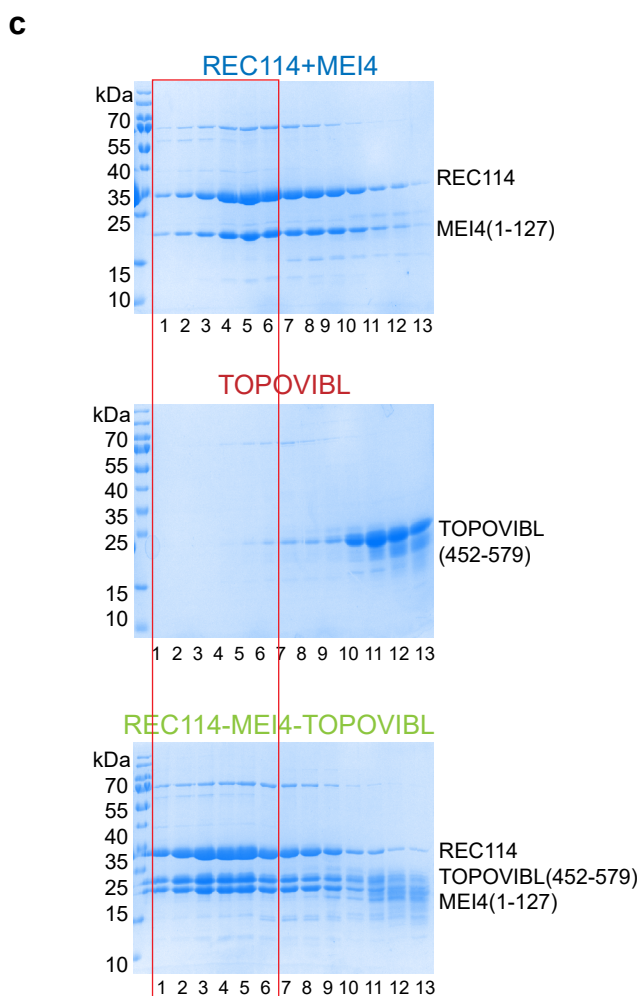
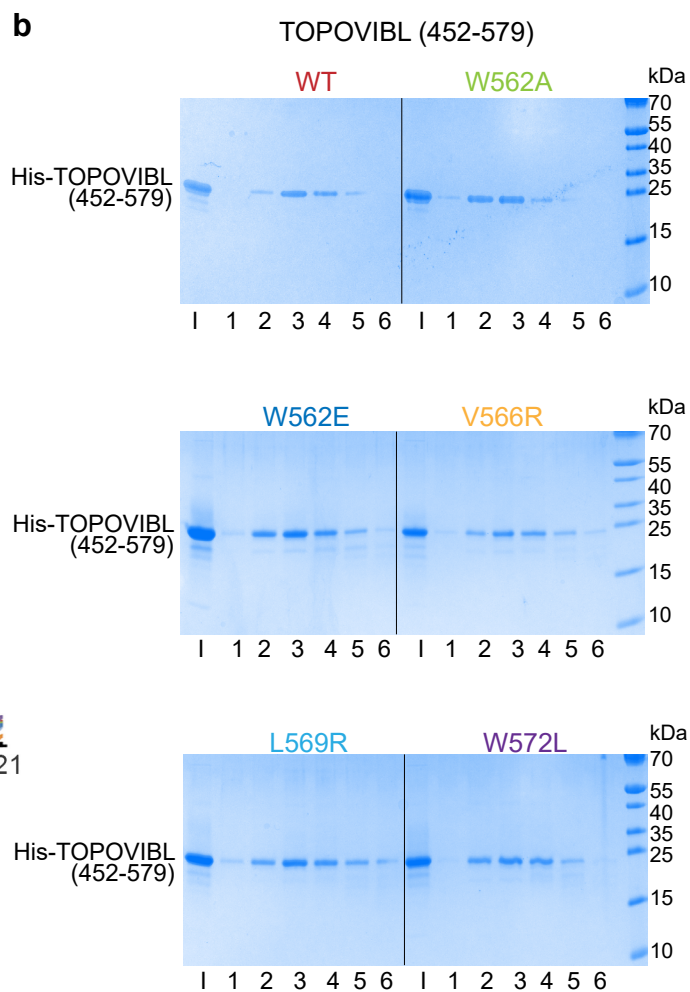
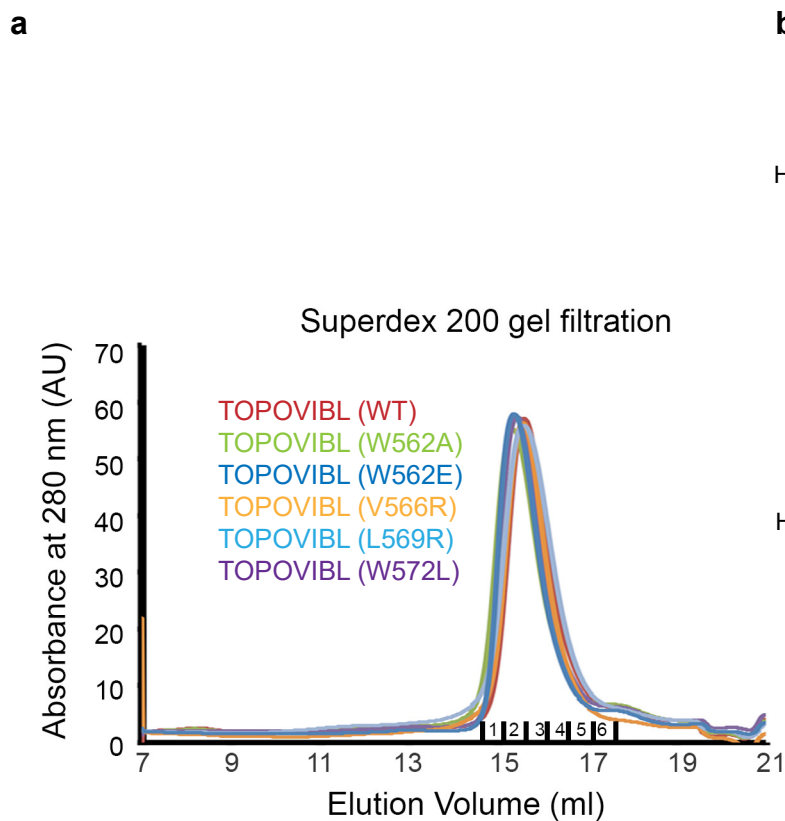
Supplementary Figure 3. Conservation of TOPOVIBL C-terminal region

Alignment of TOPOVIBL C-terminal region from nine metazoan species (as in Fig. 1f). The region aligned starts from the three conserved helices of the transducer domain ($\alpha 5$, $\alpha 6$ and $\alpha 7$) up to the C-terminal end. The position of S452 in *M. musculus* TOPOVIBL which defines the N-terminal boarder of the protein used in biochemical assays (452-579) is shown by an arrow. Brown letters: equivalent amino acids; white letters: identical amino acids.

a**b****c****d****e****f**

Supplementary Figure 4. Properties of the TOPOVIBL-REC114 complex and mutants

- a.** The TOPOVIBL-REC114 complex models predicted by AlphaFold2²⁹. The structure of the REC114 PH domain is shown as surface in blue. Key interacting residues of TOPOVIBL are shown as sticks. Most of the interactions observed in the mouse crystal structure are conserved in the predicted complexes of other species. In *D. rerio*, the residue corresponding to mouse Val566 is Leu555. This mutation seems to be compensated by the valine to glutamine mutation at position 92 in REC114.
- b.** Pull-down experiments of Strep-tagged REC114¹⁻¹⁵⁹ mutants indicated above the lanes with TOPOVIBL⁴⁵²⁻⁵⁷⁹. All proteins were first purified by affinity chromatography and gel filtration. A total of 1.2% of the input (lanes 1–5) and 1.2% of the eluates (lanes 6–10) were analyzed on 12% SDS-PAGE gels stained with coomassie brilliant blue. Control lanes 1-2 and 6-7 show inputs and elutions of REC114 and TOPOVIBL alone, respectively.
- c.** Superdex 200 gel filtration elution profiles of WT and mutant Strep-REC114¹⁻¹⁵⁹. The elution profiles are similar, indicating that the REC114 mutations do not significantly affect the overall structure of this REC114 fragment.
- d.** SDS-PAGE analysis of fractions 1-6 of the Superdex 200 gel filtration elution profiles shown in c. I indicates input samples loaded onto the column.
- e.** ITC measurement of the interaction affinity between REC114¹⁵⁻¹⁵⁹ and the TOPOVIBL⁵⁵⁹⁻⁵⁷⁶ peptide harbouring the W562A mutation (the wild-type control is in Fig. 1g).
- f.** ITC measurement of the interaction affinity between REC114¹⁻¹⁵⁹ and TOPOVIBL⁴⁵²⁻⁵⁷⁹ (W562A) (the wild-type control is in Fig. 1g). Source data are provided as a Source Data file.



Supplementary Figure 5. Properties of the TOPOVIBL-REC114-MEI4 complex and mutants

- a.** Superdex 200 gel filtration elution profiles of WT and mutant TOPOVIBL⁴⁵²⁻⁵⁷⁹. The elution profiles are similar, indicating that the TOPOVIBL mutations do not significantly affect the overall structure of this TOPOVIBL fragment.
- b.** SDS-PAGE analysis of fractions 1-6 of the Superdex 200 gel filtration elution profiles shown in **a**. I indicates input samples loaded onto the column.
- c.** SDS-PAGE analysis of fractions 1-13 of the Superose 6 gel filtration elution profiles shown in Fig. 2i. The red rectangle highlights fractions of containing the TOPOVIBL-REC114-MEI4 complex.
- d. and e.** Comparison of the REC114-TOPOVIBL (d) and the REC114-ANKRD31 (e) structures.
- f.** ITC measurement of the interaction between REC114¹⁵⁻¹⁵⁹ and TOPOVIBL⁴⁵²⁻⁵⁷⁹ shows the absence of binding when REC114 is pre-saturated with ANKRD31¹⁸⁰⁸⁻¹⁸⁵⁷. Source data are provided as a Source Data file.

a

CTGGAGGACTTGTGGCTACAGGAGGTATCCAATCTGTCCGAGTGGCTGAATCCGGGACACAGGTCC TAA

HR, allele *Top6b*^{W562A}

4 bp deletion,
allele *Top6b*^{Δ17Ct}

CTGGAGGACTTGTgCgCTgCAGGAGGTATCCAATCTGTCCGAGTGGCTGAATCCGGGACACAGGTCC TAA

CTGGAGGACTTGTGGCTTACAGGAGGTATCCAATCTGTCCGAGTGGCTGAATCCGGGACACAGGTCC TAA

b

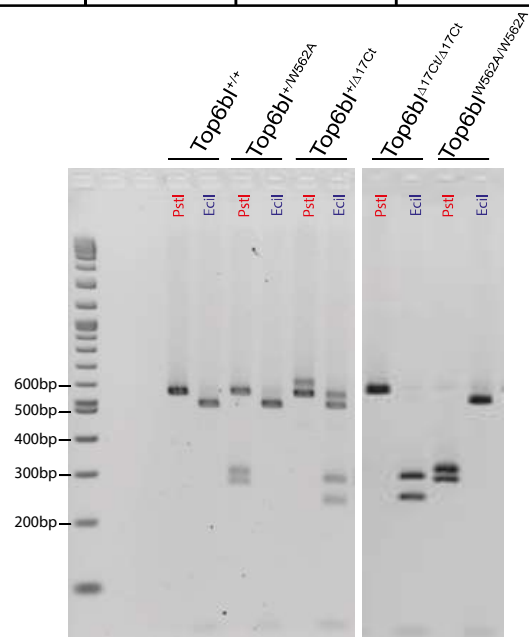
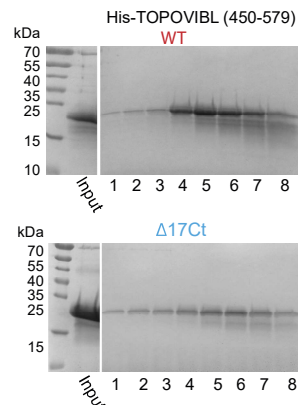
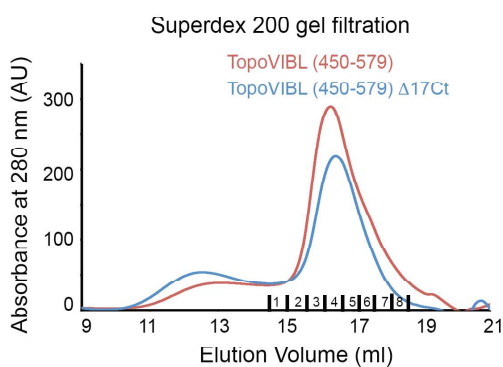
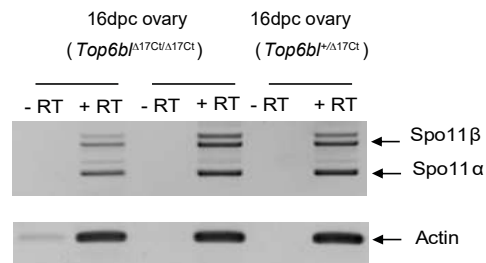
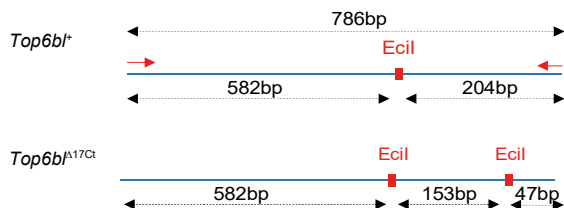
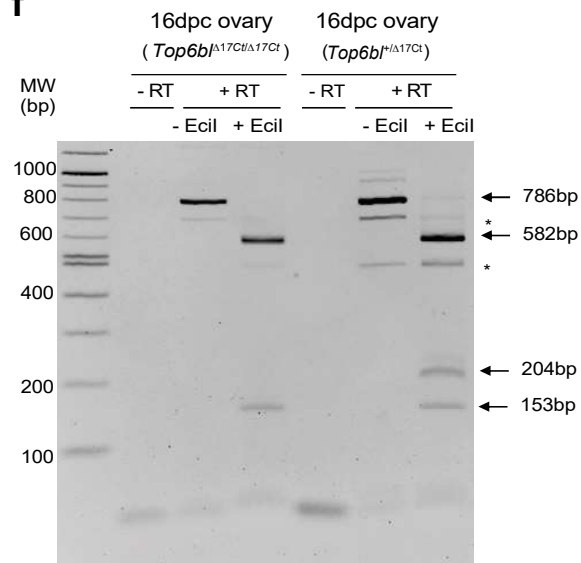
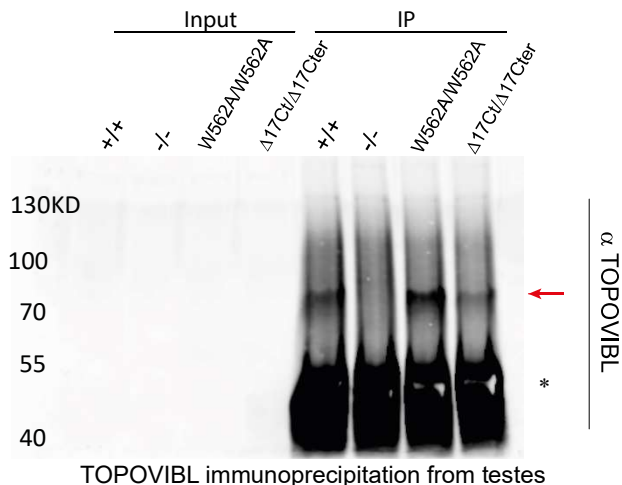
TOPOVIBL ...LEDLWLQEVSNLSEWLNPGHRS*

TOPOVIBL-W562A ...LEDLALQEVSNLSEWLNPGHRS*

TOPOVIBL-Δ17Ct ...LEDLWRRYPICPSG*

c

	<i>Top6b</i> ^l	<i>Top6b</i> ^{W562A}	<i>Top6b</i> ^{Δ17Ct}
PstI	569 bp	294 + 275 bp	569 bp
EciI	517 + 52 bp	517 + 52 bp	280 + 233 + 52 bp

**d****f****e**

Supplementary Figure 6. Generation and characterization of mice carrying *Top6bl*^{W562A} and *Top6bl*^{Δ17Ct} alleles.

a. *Top6bl*, *Top6bl*^{W562A} and *Top6bl*^{Δ17Ct} cDNA sequences. The top sequence corresponds to the 65 last nucleotides of the *Top6bl* open reading frame. Orange and blue letters mark exons 15 and 16, respectively. The TGG codon in green corresponds to the W562 residue in the protein sequence. Red arrows mark the position of the predicted CRISPR-Cas9 cleavage sites, corresponding to the three guide RNAs used. TAA in black: original stop codon.

The middle sequence corresponds to the repair product by homologous recombination (HR) following CRISPR-Cas9-mediated DSB formation. The TGG codon is replaced by the GCG codon (red letters). The G residue in purple corresponds to a silent substitution to create a *Pst*I restriction site for genotyping. TAA in black: original stop codon.

The bottom sequence corresponds to the product of the deletion of four nucleotides in the sequence following DSB formation and repair (grey crossed letters). TGA in black: new stop codon due to frameshift.

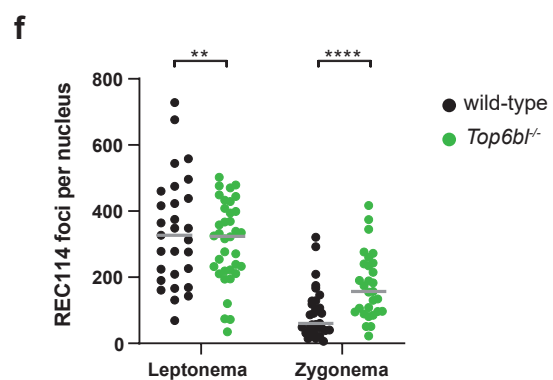
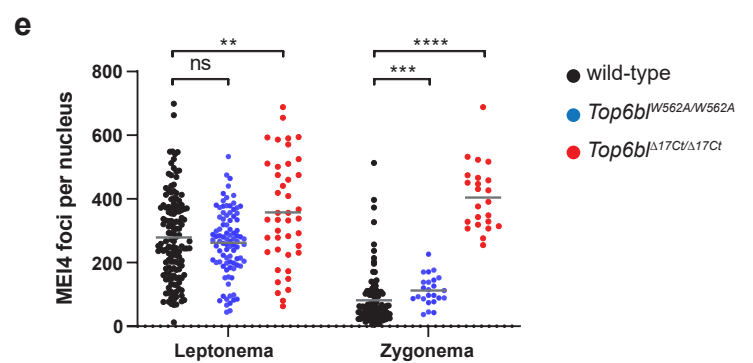
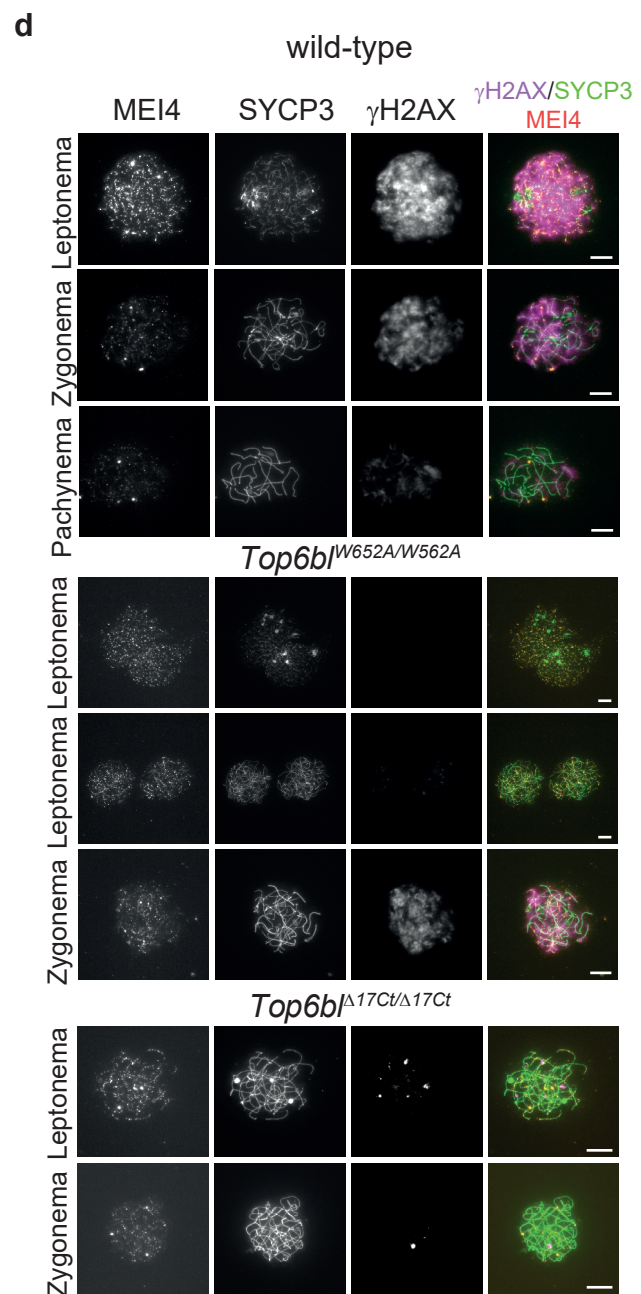
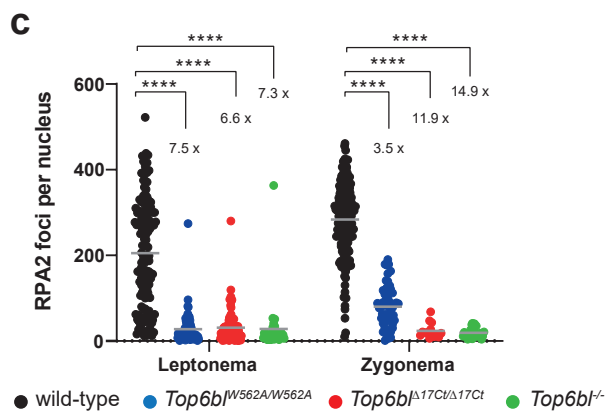
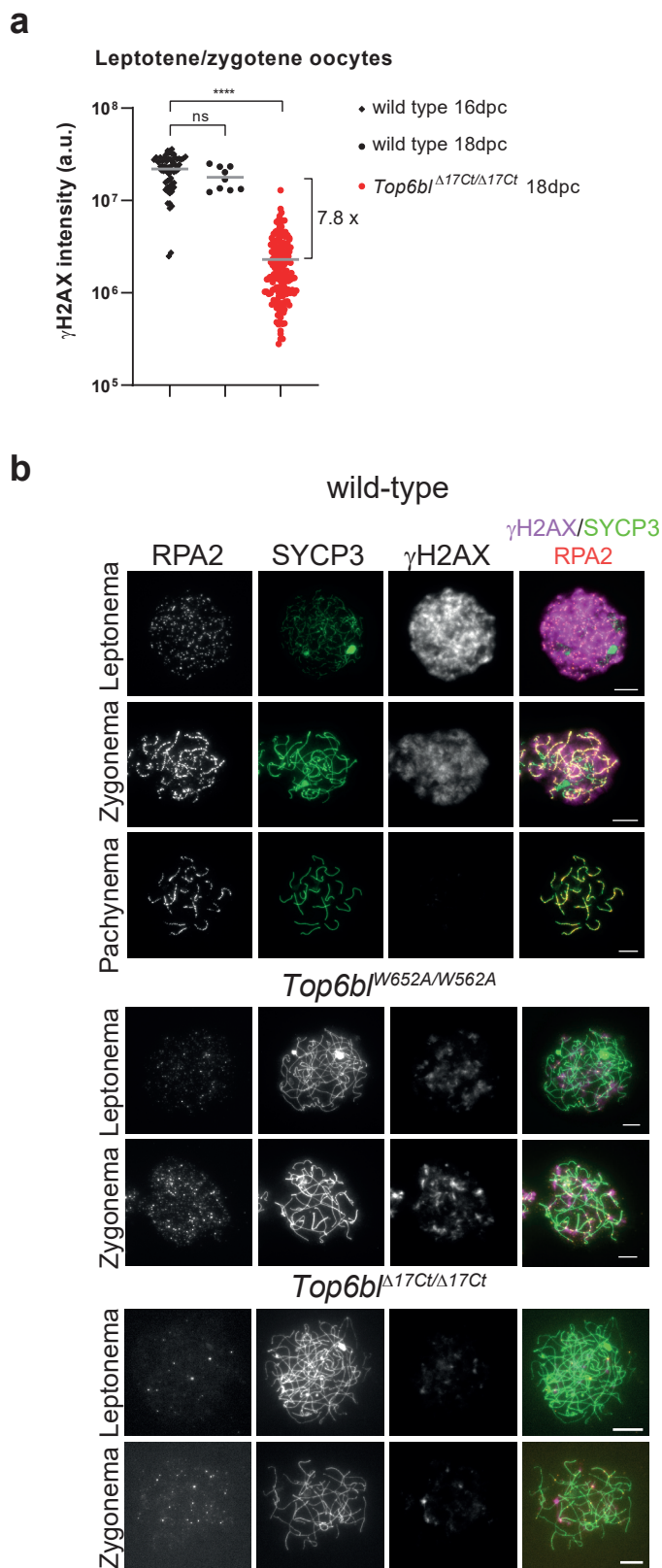
b. Protein sequences of the C-terminal part of TOPOVIBL, TOPOVIBL-W562A and TOPOVIBL-Δ17Ct. Blue letters correspond to the original residues, bold red A to the mutant substitution of W562 in the TOPOVIBL-W562A sequence, and red letters to the nine-residue substitution in the TOPOVIBL-Δ17Ct sequence. *: Stop.

c. Genotyping strategy. Following DNA extraction from tails of *Top6bl*^{+/+}, *Top6bl*^{+/W562A}, *Top6bl*^{+/Δ17Ct}, *Top6bl*^{W562A/W562A} and *Top6bl*^{Δ17Ct/Δ17Ct} mice, PCR amplification with the primer pair specific for a 569 bp region containing the mutations was performed. PCR products were digested with *Pst*I or *Eci*I. The top table presents the expected size following digestion of the different alleles (red correspond to *Pst*I digestion, and dark blue to *Eci*I digestion). Bottom panel: DNA fragments separated on agarose gels.

d. Left panel: Overlay of the Superdex 200 gel filtration elution profiles of wild-type TOPOVIBL⁴⁵²⁻⁵⁷⁹ and TOPOVIBL⁵²⁻⁵⁷⁹ Δ17Ct. Both profiles are similar, indicating that the Δ17Ct mutation does not significantly affect the overall structure of this TOPOVIBL fragment. Right panel: SDS-PAGE analysis of the input and fractions 1-8 of the Superdex 200 gel filtration elution profiles. Gels were stained with coomassie brilliant blue.

e. TOPOVIBL immunoprecipitation. Following extraction of total protein from 14 dpp mouse testes obtained from *Top6bl*^{+/+}, *Top6bl*^{W562A/W562A}, *Top6bl*^{Δ17Ct/Δ17Ct} and *Top6bl*^{-/-} mice, TOPOVIBL was immunoprecipitated using the homemade anti-TOPOVIBL antibody. Six testes of each genotype were used for one immunoprecipitation. The immunoblot was revealed using the homemade anti-TOPOVIBL antibody. The red arrow shows the TOPOVIBL expected band. *corresponds to a non-specific band (immunoglobulin heavy chain).

f. Expression of *Top6bl* assessed by RT-PCR. RNA extracted from ovaries of 16 dpc *Top6bl*^{Δ17Ct/Δ17Ct} or *Top6bl*^{+/Δ17Ct} mice was incubated with or without reverse transcriptase (RT), amplified by PCR and incubated with or without *EciI* restriction enzyme. The size of the fragments after *EciI* digestion are shown for the *Top6bl*⁺ and *Top6bl*^{Δ17Ct} alleles. Stars indicate non-specific amplification products. Control PCR assays for monitoring the expression of the two *Spo11* splice variants (*Spo11α* and *β*) and of actin are shown. Source data are provided as a Source Data file.



Supplementary Figure 7. Immunocytochemistry on oocytes

a. Quantification of γ H2AX signal intensity in leptotene (L)/zygotene (Z) oocytes from 16 and 18 dpc wild-type and 18dpc *Top6bl* ^{Δ 17Ct/ Δ 17Ct} mice. Number of nuclei: 64, 9, and 167, respectively. Grey bars show the mean values. P values were determined using the two-tailed unpaired Mann-Whitney test. Progression into prophase at 18dpc was: L 46%, Z 54%, Pachynema (P) 0% in *Top6bl* ^{Δ 17Ct/ Δ 17Ct}, and L 2%, Z 8%, P 90% in wild-type oocytes.

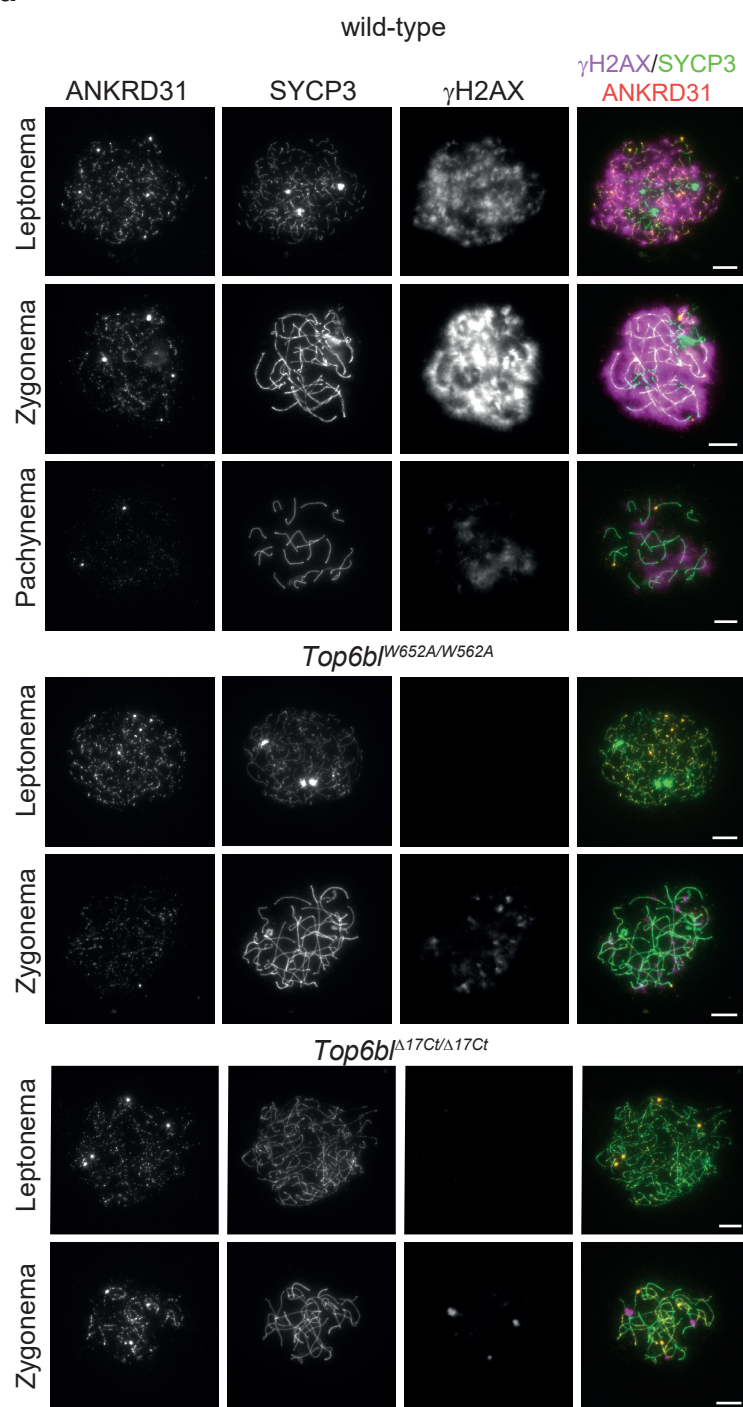
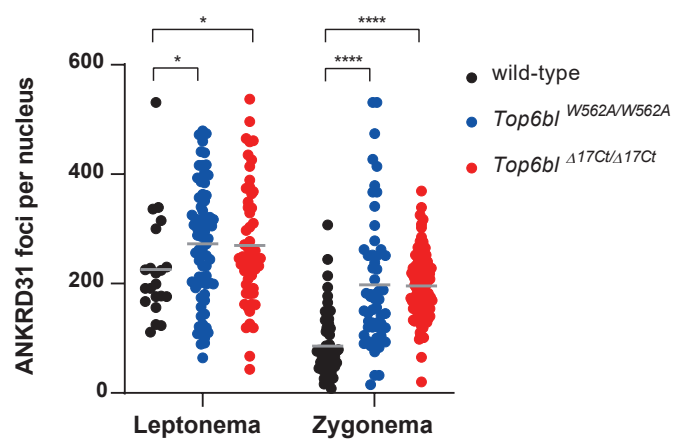
b. Immunostaining of RPA2, SYCP3 and γ H2AX in oocytes from 16 dpc wild-type (+/+), *Top6bl*^{W562A/W562A}, and *Top6bl* ^{Δ 17Ct/ Δ 17Ct} ovaries. Scale bar, 10 μ m.

c. Quantification of RPA foci per nucleus in leptotene and zygotene oocytes from 16 dpc wild-type (+/+ or *Top6bl*^{+/ Δ 17Ct}), *Top6bl*^{W562A/W562A}, *Top6bl* ^{Δ 17Ct/ Δ 17Ct}, and *Top6bl*^{-/-} mice (n=4 wild-type and n=2 mice per mutant genotype). Number of nuclei per genotype at leptotene (157, 85, 14, and 29) and at zygonema (165, 55, 105, and 28). Grey bars show the mean value. P values were determined using the two-tailed unpaired Mann-Whitney test. Ratios of the wild-type and mutant mean values are shown.

d. Immunostaining of MEI4, SYCP3 and γ H2AX in oocytes from 16 dpc wild-type (+/+), *Top6bl*^{W562A/W562A}, and *Top6bl* ^{Δ 17Ct/ Δ 17Ct} ovaries. Scale bar, 10 μ m.

e. Quantification of axis-associated MEI4 foci in leptotene and zygotene oocytes from 15 and 16 dpc wild-type (+/+ or *Top6bl*^{+/ Δ 17Ct}), *Top6bl*^{W562A/W562A}, and *Top6bl* ^{Δ 17Ct/ Δ 17Ct} mice (n=3 wild-type, n=1 *Top6bl*^{W562A/W562A}, and n=2 *Top6bl* ^{Δ 17Ct/ Δ 17Ct} mice). Number of nuclei per genotype at leptotene (139, 91, and 42) and at zygonema (106, 24, and 23). Grey bars show the mean values. P values were determined using the two-tailed unpaired Mann-Whitney test.

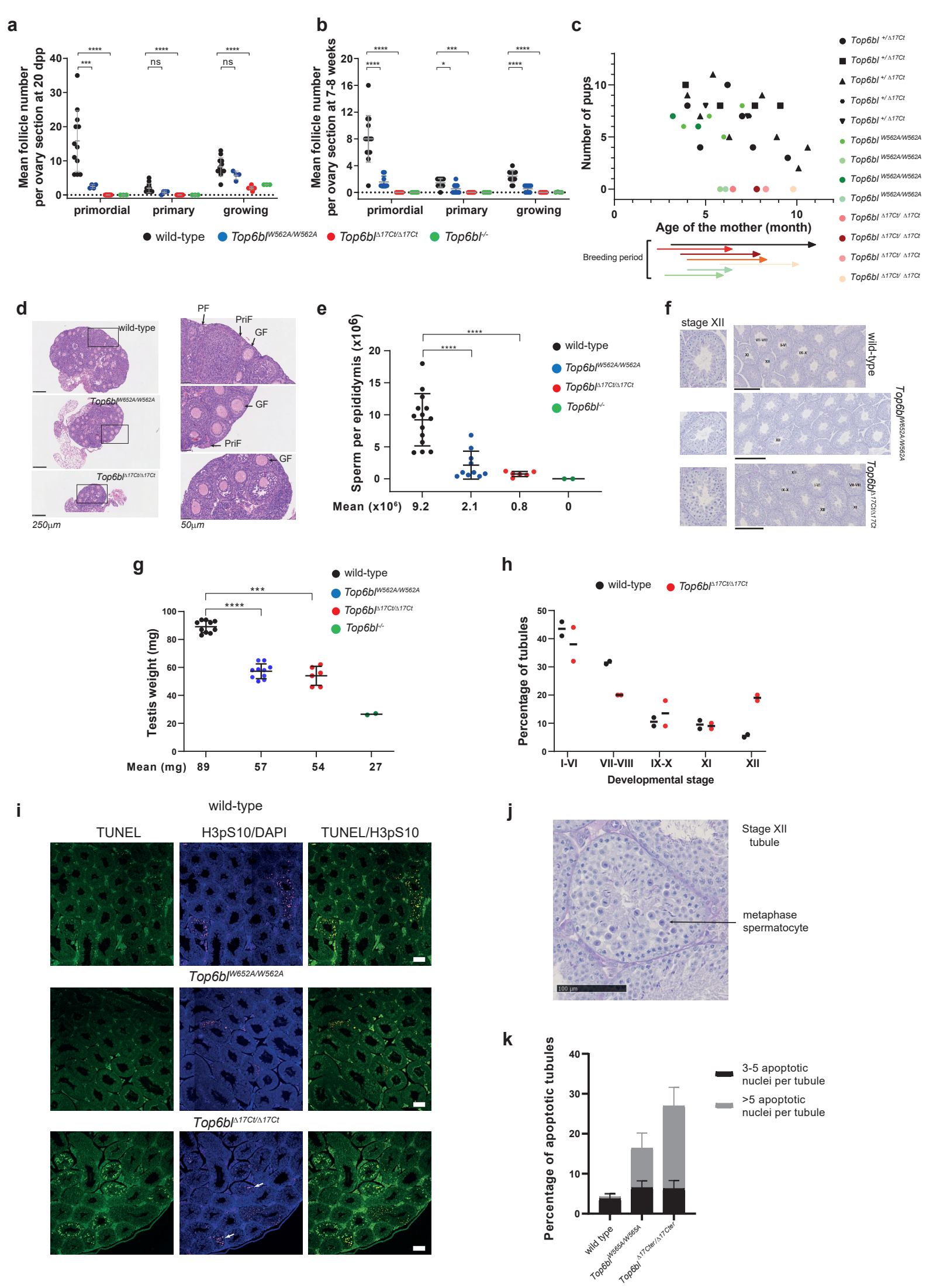
f. Quantification of axis-associated REC114 foci in leptotene and zygotene oocytes from 16 dpc wild-type (+/+) and *Top6bl*^{-/-} mice (n=1 mouse/genotype). Number of nuclei: 29 and 36 L, and 31 and 29 Z, respectively. Grey bars show the mean values. P values were determined using the two-tailed unpaired Mann-Whitney test. Source data are provided as a Source Data file.

a**b**

Supplementary Figure 8. Immunocytochemistry of ANKRD31 on oocytes

a. Immunostaining of ANKRD31, SYCP3 and γ H2AX in oocytes from 16 dpc wild-type (+/+), *Top6bl*^{W562A/W562A}, and *Top6bl* ^{Δ 17Ct/ Δ 17Ct} ovaries. Scale bar, 10 μ m.

b. Quantification of axis-associated ANKRD31 foci in leptotene and zygotene oocytes from 16 and 18 dpc wild-type (+/+ or *Top6bl*^{+/ Δ 17Ct}), *Top6bl*^{W562A/W562A}, and *Top6bl* ^{Δ 17Ct/ Δ 17Ct} mice (n=3 wild-type, n=1 *Top6bl*^{W562A/W562A}, and n=2 *Top6bl* ^{Δ 17Ct/ Δ 17Ct} mice). Number of nuclei per genotype at leptotema (21, 74, and 56) and at zygonema (56, 57, and 108). Grey bars show the mean values. P values were determined using the two-tailed unpaired Mann-Whitney test. Source data are provided as a Source Data file.



Supplementary Figure 9. Histology on ovaries and testis

a. Quantification of primordial, primary, and growing follicles in ovaries from 20 dpp wild-type homozygous or heterozygous (+/+, *Top6bl*^{+/Δ17Ct}, *Top6bl*^{+/W562A}, +/ *Top6bl*⁻), *Top6bl*^{W562A/W562A}, *Top6bl*^{Δ17Ct/Δ17Ct}, and *Top6bl*^{-/-} mice. Each data point is the mean ± SD of at least 10 sections from one ovary. Number of mice: 6, 2, 4, and 2, respectively. P values were determined using the two-tailed unpaired Mann-Whitney test.

b. Quantification of primordial, primary, and growing follicles in ovaries from 7-8-week-old wild-type (+/+ or *Top6bl*^{+/W562A}), *Top6bl*^{W562A/W562A}, *Top6bl*^{Δ17Ct/Δ17Ct}, and *Top6bl*^{-/-} mice. Each data point is the mean ± SD of 20 sections from one ovary. P values were determined using the two-tailed unpaired Mann-Whitney tests. Number of mice: 7, 5, 3, and 2 respectively.

c. Fertility tests. *Top6bl*^{+/Δ17Ct}, *Top6bl*^{W562A/W562A} and *Top6bl*^{Δ17Ct/Δ17Ct} females (n= 5, 4 and 4 respectively), from 2.3 to 10.6 months of age, were mated with heterozygous males (*Top6bl*^{+/Δ17Ct} or *Top6bl*^{+/W562A}). Each symbol refers to a female. Only two of the four *Top6bl*^{W562A/W562A} females were fertile (large circle, dark green, and small circle), and after the last progeny, they were not mated any longer. Breeding periods of non-productive females and for *Top6bl*^{+/Δ17Ct} females are shown.

d. Haematoxylin and eosin-stained sections of ovaries from 20 dpp wild-type (+/+), *Top6bl*^{W562A/W562A} and *Top6bl*^{Δ17Ct/Δ17Ct} mice (samples from panel a). Scale bars, 250 and 50 μm in the left and right panel, respectively. Primordial (PriF), primary (PF), and growing (GF) follicles are indicated.

e. Sperm count in *Top6bl* mutants. Sperm in epididymis was counted in 2-month-old mice. Each data point is one epididymis (number of mice= 7, 5, 3 and 1 for wild-type (+/+ or +/-), *Top6bl*^{W562A/W562A}, *Top6bl*^{Δ17Ct/Δ17Ct}, and *Top6bl*^{-/-}, respectively). The mean ± SD is shown. P values were determined using the two-tailed unpaired Mann-Whitney test.

f. Periodic acid-Schiff staining of testis sections from 2 month-old wild-type (+/+), *Top6bl*^{W562A/W562A}, and *Top6bl*^{Δ17Ct/Δ17Ct} mice. Scale bar, 250μm. Zoomed images of stage XII tubules are shown on the right.

g. Testis weight in *Top6bl* mutants. Testis weight was measured in 2 month-old mice. Each data point is one testis (number of mice= 5, 5, 3, and 1 for wild-type (+/+), *Top6bl*^{W562A/W562A}, *Top6bl*^{Δ17Ct/Δ17Ct}, and

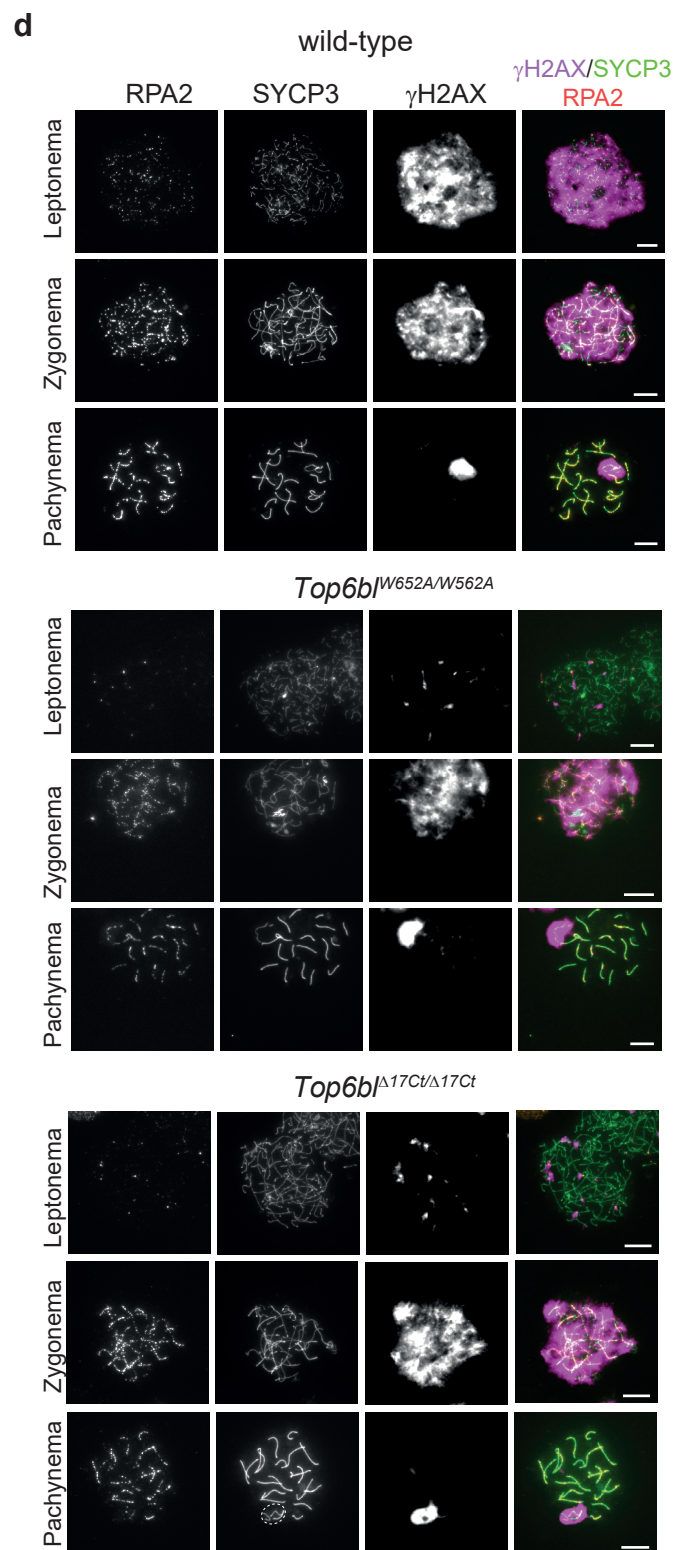
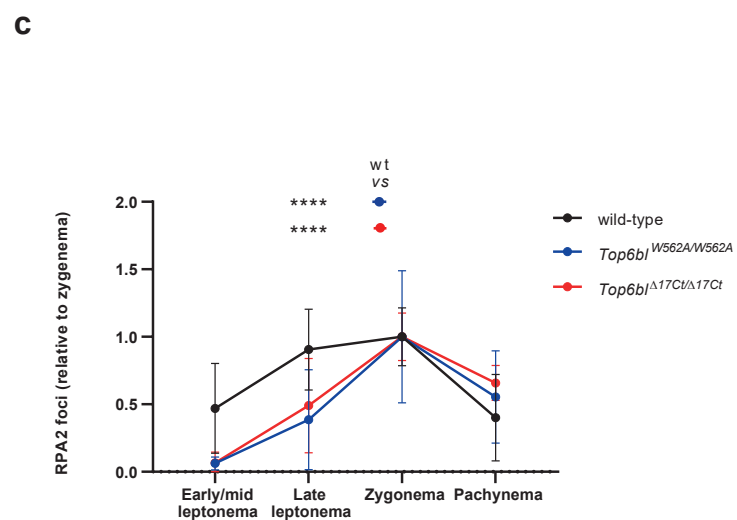
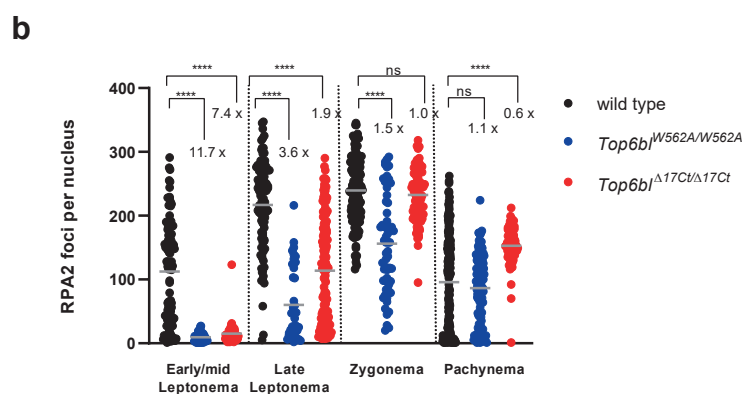
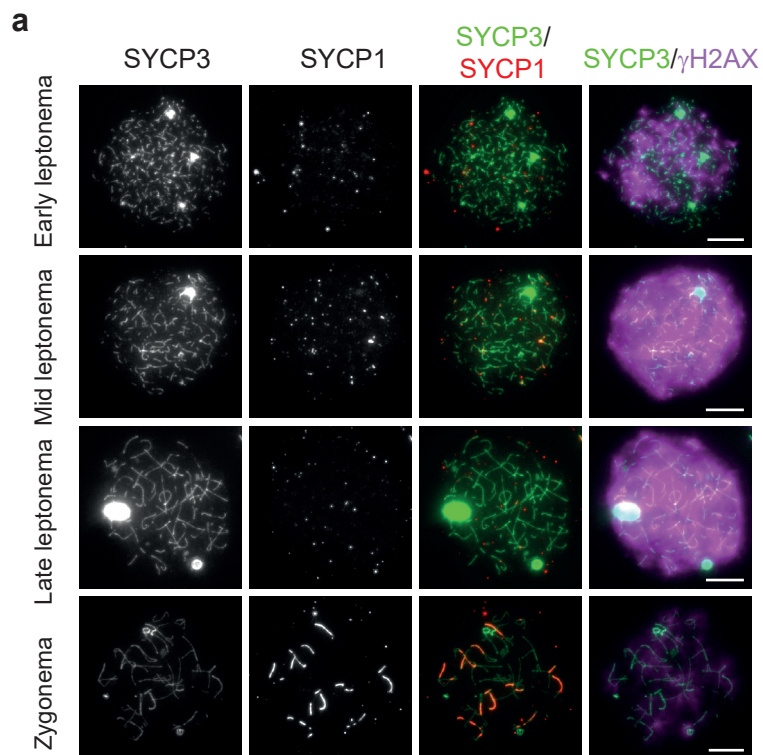
Top6bl^{-/-}, respectively). The mean value $\pm$ SD is shown. P values were determined using the two-tailed unpaired Mann-Whitney test.

h. Staging of tubules in testis sections from 2-month-old wild-type and *Top6bl* ^{$\Delta 17Ct / \Delta 17Ct$} mice (n=2 mice/genotype). Tubules were divided in five groups based on several criteria. I-VI: round spermatid with acrosomes, 0 to ~25% of the perimeter of the spermatid, elongated spermatids on the lumen; VII-VIII: round spermatids with acrosomes, ~50% of the perimeter; IX-X: elongated spermatids with acrosomes not hook-shaped; XI: elongated spermatids with a hook; XII: presence of metaphases. Number of staged tubules: 310 in wild-type and 366 in *Top6bl* ^{$\Delta 17Ct / \Delta 17Ct$} mice. The horizontal bars show the mean value. The distributions are statistically different (Pearson's Chi-Square: 36.028).

i. Detection of apoptosis and metaphase in testis sections. Sections were labelled with TUNEL (green) and H3pS10 (red), and stained with DAPI (blue). Scale bar, 100 μ m. White arrowheads indicate tubules positive for H3pS10 and TUNEL.

j. A stage XII tubule with metaphase spermatocytes (from *Top6bl* ^{$\Delta 17Ct / \Delta 17Ct$} mice). Scale bar, 100 μ m. Several metaphases with condensed chromosomes are seen, and one (arrow) shows relatively more intense nuclear staining, suggesting apoptosis.

k. Detection of apoptosis by TUNEL assay. Tubules were classified in three categories: <3, 3 to 5, and >5 apoptotic nuclei. The mean values $\pm$ SD of the 3 to 5 and >5 categories are shown. Number of 2 month-old mice: 4 wild-type (+/+ and *Top6bl* ^{$+ / \Delta 17Ct$}) and 2 mice per mutant. The percentage of tubules with <3, 3 to 5, and >5 apoptotic nuclei was significantly different between wild-type and *Top6bl*^{W562A/W562A} and *Top6bl* ^{$\Delta 17Ct / \Delta 17Ct$} mice (Chi-square Pearson: 354.03 and 811.63, respectively). Source data are provided as a Source Data file.



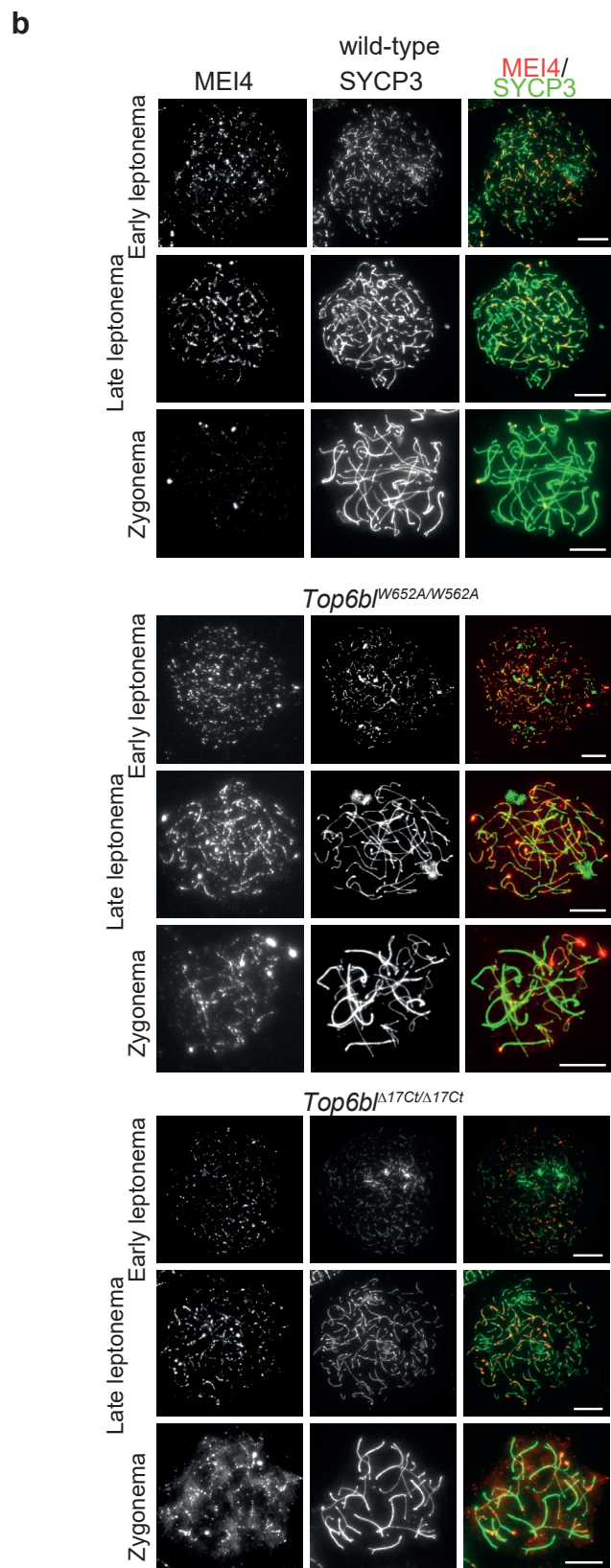
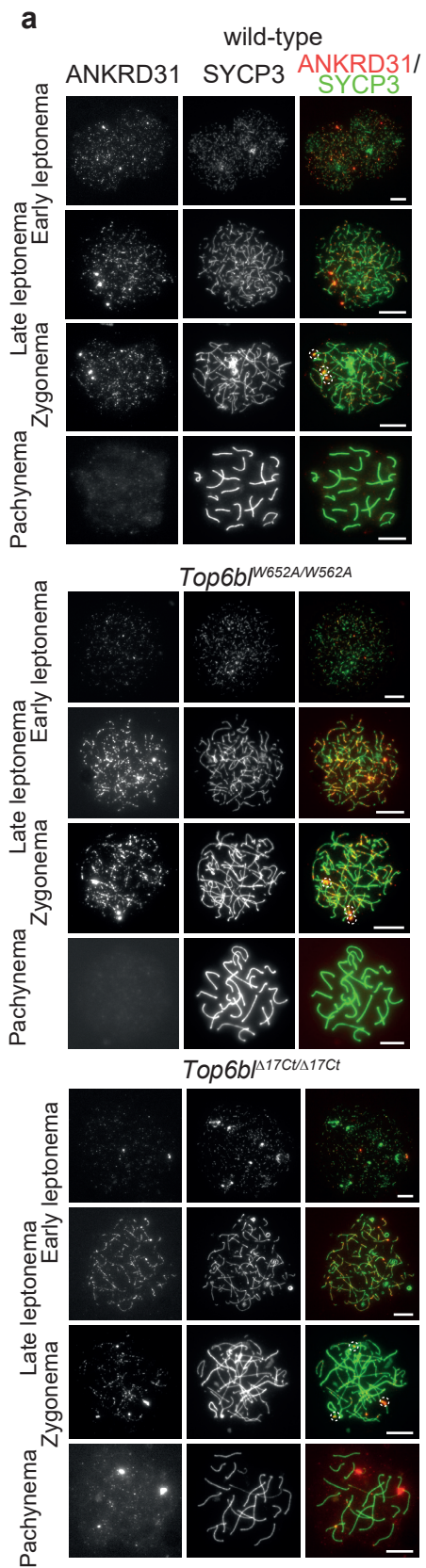
Supplementary Figure 10. Staging and immunocytochemistry on spermatocytes

a. Identification of spermatocytes at early, mid, late leptotene and zygonema. Examples of wild-type nuclei at the different stages by staining with SYCP3 (green), SYCP1 (red) and γ H2AX (magenta). Scale bar, 10 μ m.

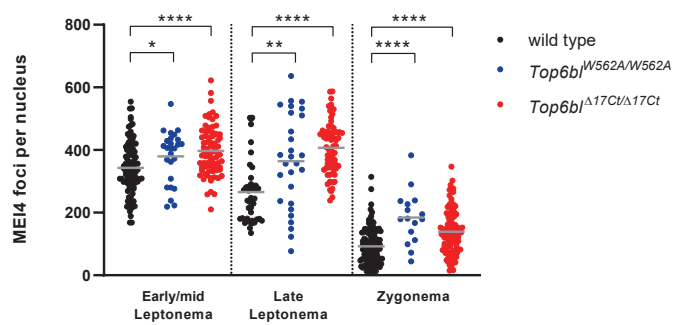
b. Quantification of axis-associated RPA2 foci in early/mid and late leptotene, zygotene and pachytene spermatocytes from 12-14dpp wild-type (+/+ or *Top6bl*^{+/Δ17Ct}; n=3), *Top6bl*^{W562A/W562A} (n=2), and *Top6bl*^{Δ17Ct/Δ17Ct} (n=2) mice. Number of nuclei per genotype at early/mid leptotene (105, 36, and 41), late leptotene (91, 38, and 131), zygotene (135, 62, and 85), and pachytene (178, 119, and 80). Grey bars show the mean values. P values were determined using the two-tailed unpaired Mann-Whitney test.

c. RPA2 focus variation during prophase. For each genotype (wild-type, *Top6bl*^{W562A/W562A}, and *Top6bl*^{Δ17Ct/Δ17Ct}), the number of RPA2 foci at the different stages (dataset as in panel b) was normalized to the mean number at zygonema (set to 1). The mean value $\pm$ SD is shown. Statistical significance between wild-type and each mutant (*Top6bl*^{W562A/W562A}; *Top6bl*^{Δ17Ct/Δ17Ct}) was tested at late leptotene using the two-tailed unpaired Mann-Whitney test.

d. Immunostaining of RPA2, SYCP3 and γ H2AX in spermatocytes from 12-14dpp wild-type (+/+), *Top6bl*^{W562A/W562A}, and *Top6bl*^{Δ17Ct/Δ17Ct} spermatocytes. A white dotted circle highlights the unsynapsed X and Y chromosomes at pachynema (SYCP3 staining) in *Top6bl*^{Δ17Ct/Δ17Ct} mice. Scale bar, 10 μ m. Source data are provided as a Source Data file.

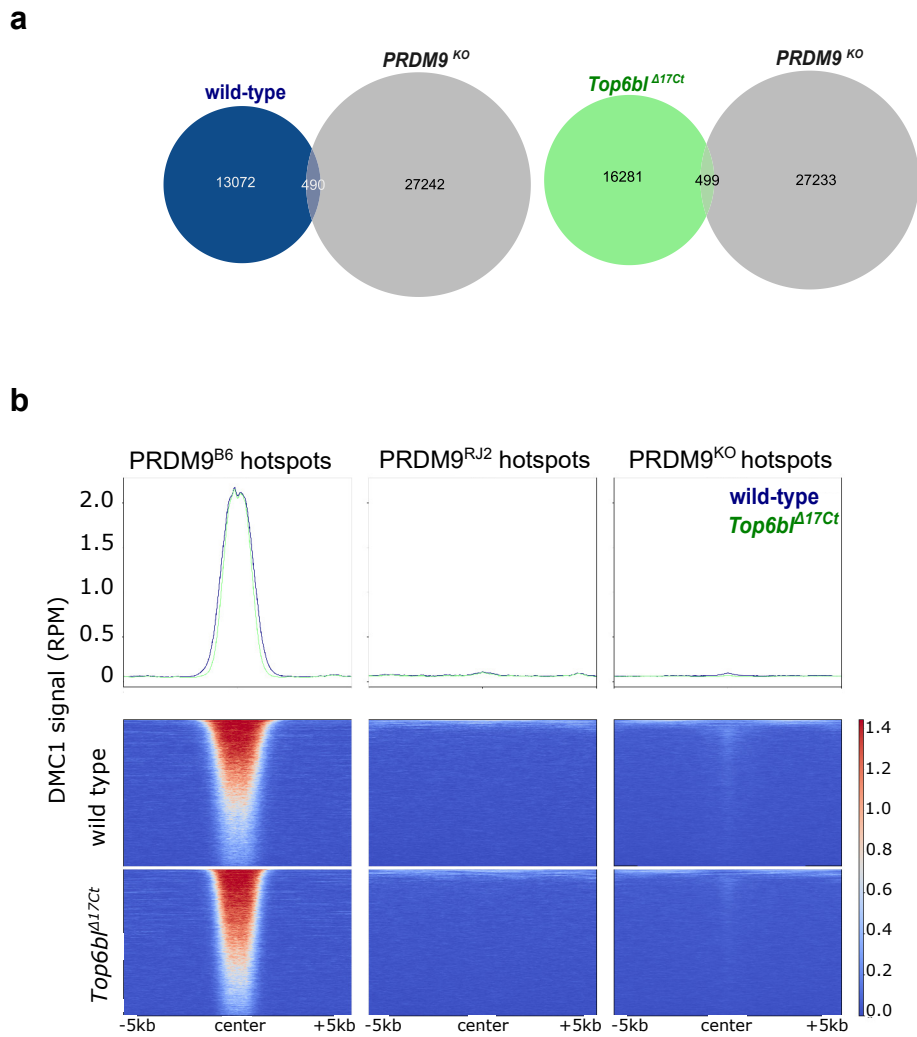


c



Supplementary Figure 11. Immunocytochemistry on spermatocytes

- a.** Immunostaining of ANKRD31 and SYCP3 in spermatocytes from 12-14dpp wild-type (+/+), *Top6bl*^{W562A/W562A}, and *Top6bl*^{Δ17Ct/Δ17Ct} mice. At zygonema, dotted white circles (merge panels) highlight ANKRD31 aggregates at the ends of some chromosome axes. Scale bar, 10μm.
- b.** Immunostaining of MEI4 and SYCP3 in spermatocytes from 12-14 dpp wild-type (+/+), *Top6bl*^{W562A/W562A}, and *Top6bl*^{Δ17Ct/Δ17Ct} mice. Scale bar, 10μm.
- c.** Quantification of axis-associated MEI4 foci in early/mid, late leptotene and zygotene spermatocytes from 12-14 dpp wild-type (+/+ or *Top6bl*^{+ /Δ17Ct}; n=2), *Top6bl*^{W562A/W562A} (n=1), and *Top6bl*^{Δ17Ct /Δ17Ct} (n=2) mice. Number of nuclei: 97 early/mid L, 43 late L, 114 Z in wild-type; 26 early/mid L, 28 late L and 16 Z in *Top6bl*^{W562A/W562A}; 77 early/mid L, 68 late L and 103 Z in *Top6bl*^{Δ17Ct /Δ17Ct}. Grey bars show the mean values. P values were determined using the two-tailed unpaired Mann-Whitney test. Source data are provided as a Source Data file.



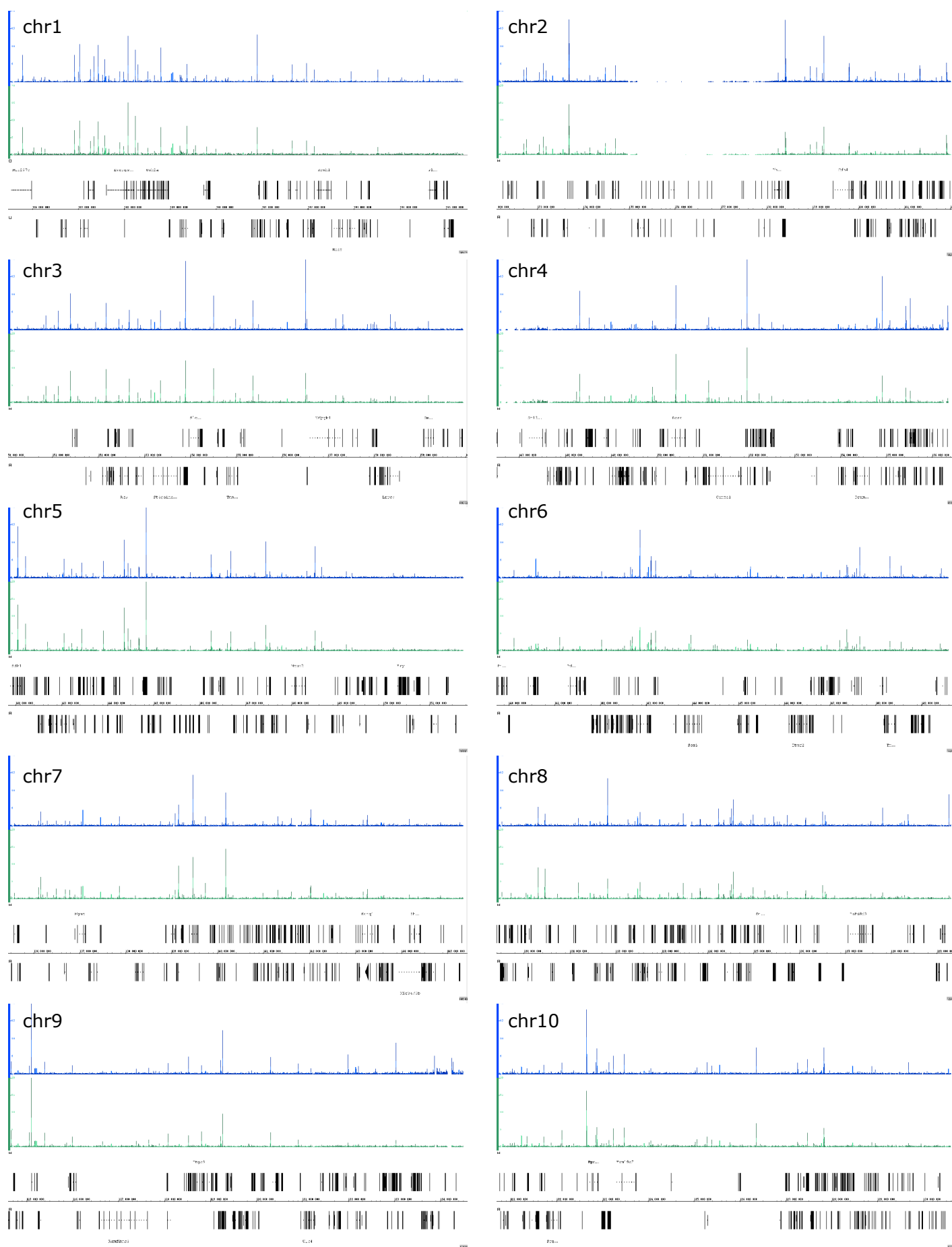
Supplementary Figure 12. Hotspot overlap analysis

a. Overlap between wild-type and *Top6bl*^{Δ17Ct/Δ17Ct} hotspots and the default hotspots used in the *PRDM9*^{KO} mouse strain. The overlaps were restricted to the central 400bp around the estimated hotspot centre.

b. DMC1-SSDS signal (heatmap and average plots) from wild-type and *Top6bl*^{Δ17Ct/Δ17Ct} mice at *PRDM9*^{B6}-, *PRDM9*^{RJ2}- and *PRDM9*^{KO}-defined hotspots.

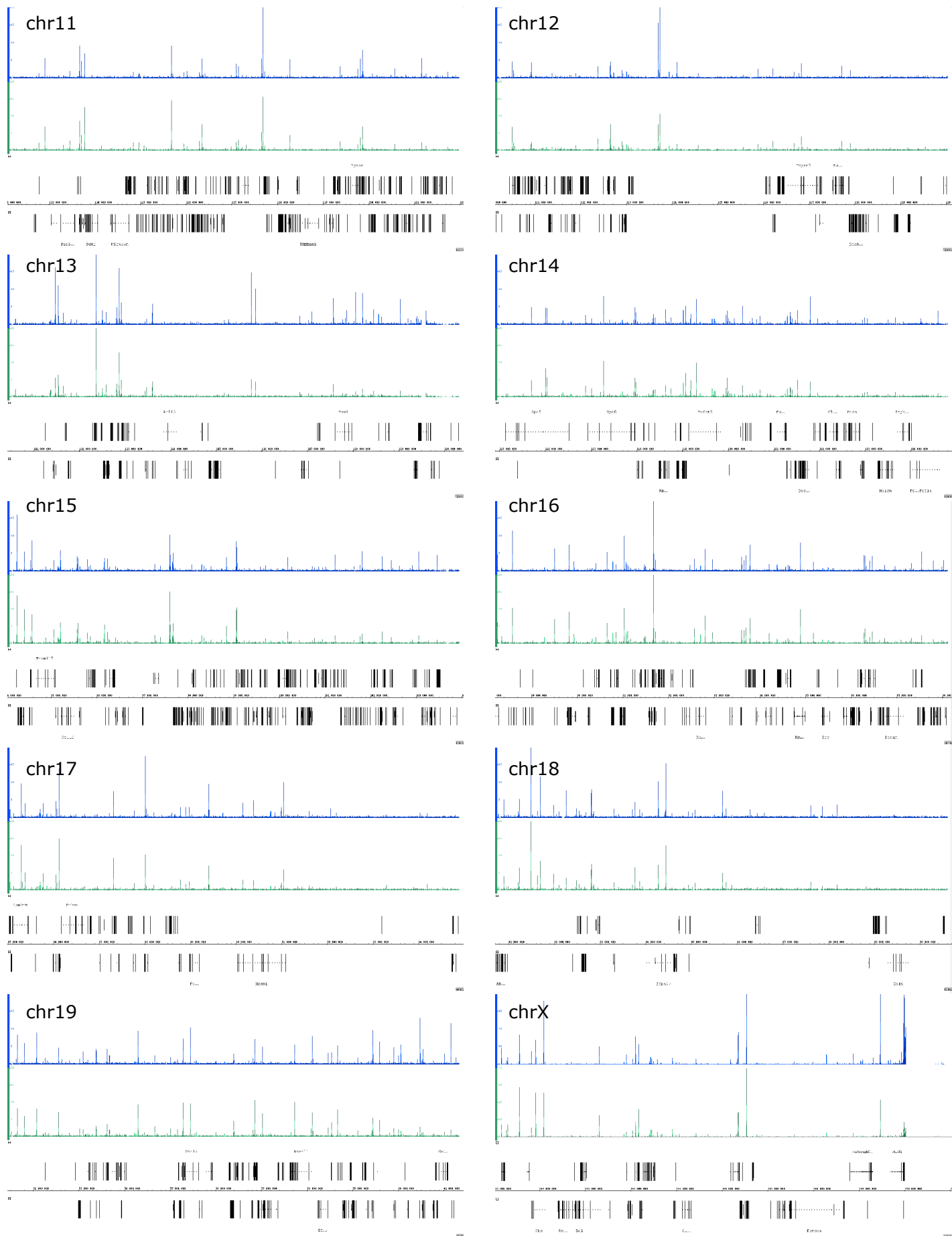
Supplementary Figure 13. Hotspots signal quantification analysis

- a.** DMC1-SSDS signal from *Top6bl* ^{$\Delta 17Ct/\Delta 17Ct$} and wild-type mice at wild-type hotspots (13562) and at hotspots specifically activated in *Top6bl* ^{$\Delta 17Ct/\Delta 17Ct$} (3519), and H3K4me3 signal (heatmap and average plots) from B6 and RJ2 mice. Peaks are ranked as a function of the DMC1 or H3K4me3 signal intensity.
- b.** DMC1-SSDS signal in *Top6bl* ^{$\Delta 17Ct/\Delta 17Ct$} , *Ankrd31*^{-/-} and their respective wild-type control mice. Signal is represented as box plots (as defined in Methods) computed for hotspots (n= 13562) ranked according to increased DMC1-SSDS signal intensity and grouped into 10 bins (1 bin = 10% of the total hotspot number; bin 1 = weakest signal, bin 10= strongest signal).
- c.** Control for the sub-telomeric effect. Quantification was done as in Fig. 6f, but with ratios calculated between two random pools of 10 non-sub-telomeric consecutive hotspots (box plot as defined in Methods).
- d.** Sub-telomeric/Non-sub-telomeric hotspot density ratios for unchanged, decreased, and increased hotspots, as defined by DESeq2 analysis, for sub-telomeric regions defined using various distances from the telomeric annotated ends (1-2-3-4-5-7-10Mb). The log2 ratio was plotted and showed excess (positive values) or lack (negative values) of hotspots at sub-telomeric regions compared with non-sub-telomeric regions.
- e.** Distribution of hotspots within 3 Mb from annotated telomeric ends and according to their DESeq2 category (unchanged, decreased, or low signal). Note the absence of increased hotspots within this sub-telomeric window size. The normalized wild-type DMC1-SSDS signal is shown on the y-axis.



Supplementary Figure 14. Browser windows of DMC1-SSDs

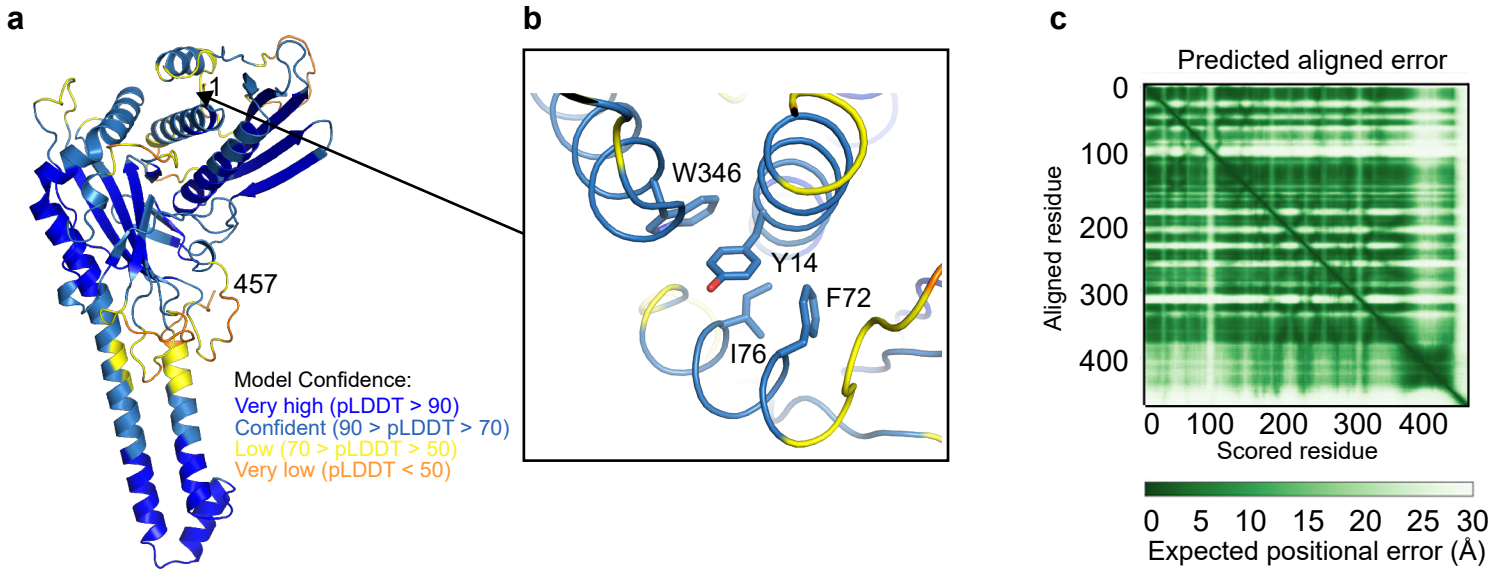
Browser windows of DMC1-SSDs in wild-type (blue) and *Top6bl*^{Δ17Ct/Δ17Ct} (green) mice within the 10Mb q-arm telomere-proximal regions of chromosomes 1 to 10.



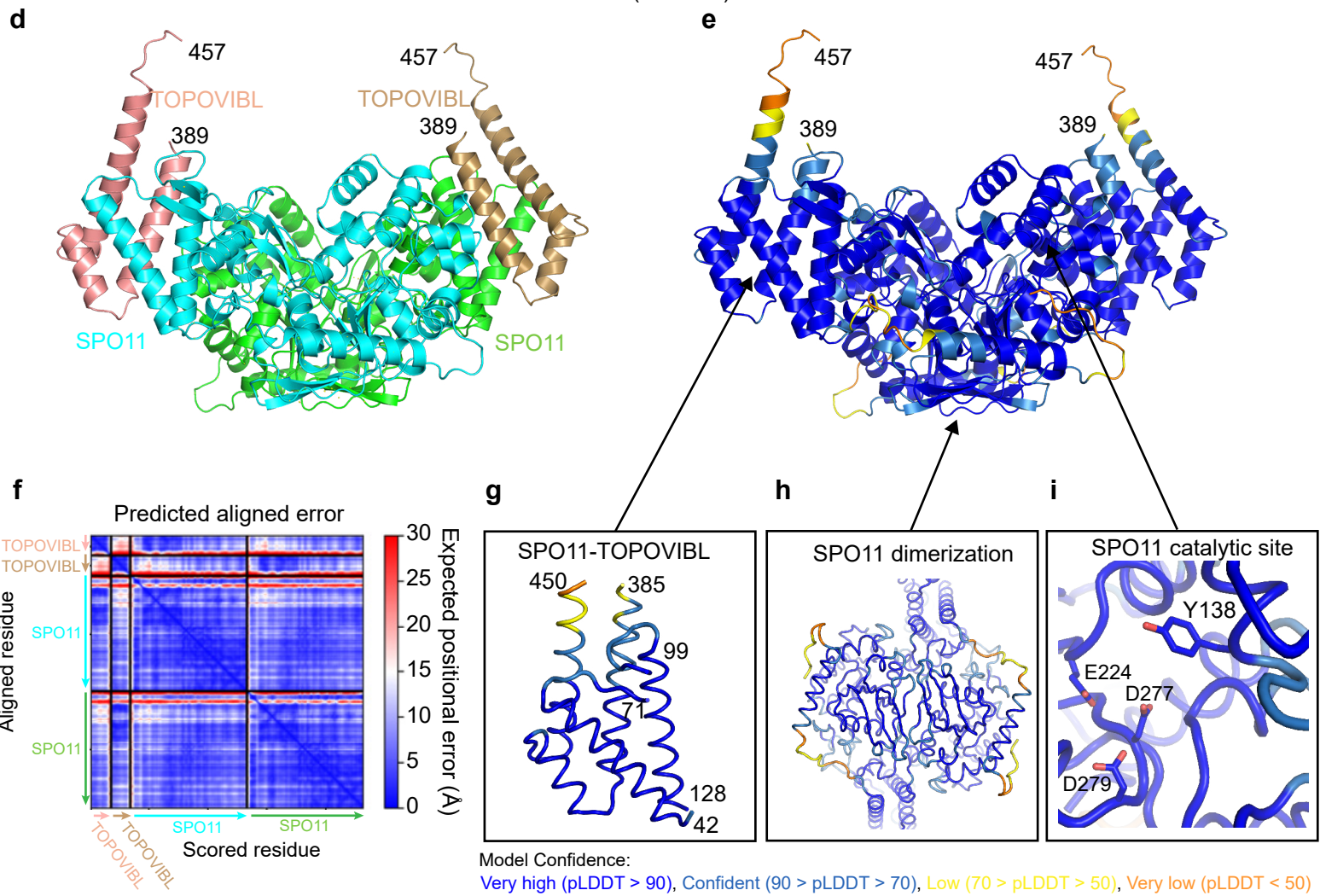
Supplementary Figure 15. Browser windows of DMC1-SSDS

Browser windows of DMC1-SSDS in wild-type (blue) and *Top6bl*^{Δ17Ct/Δ17Ct} (green) mice within the 10Mb q-arm telomere-proximal regions of chromosomes 11 to X.

TOPOVIBL (1-457) AF model -AF-J3QMY9



SPO11-TOPOVIBL (389-457) AF model



Supplementary Figure 16. Quantitative evaluations of AlphaFold2 models

- a.** AlphaFold2 model of the mouse TOPOVIBL¹⁻⁴⁵⁷, including the GHKL-like, linker and transducer domains coloured according to the AlphaFold2 per-residue estimate of confidence (pLDDT).
- b.** Detail of the TOPOVIBL GHKL-like domain shown in Fig. 8b and coloured according to pLDDT.
- c.** Predicted aligned error plot for the model shown in **a**.
- d.** AlphaFold2 model of the SPO11-TOPOVIBL³⁸⁹⁻⁴⁵⁷ complex coloured by protein chain.
- e.** AlphaFold2 model of the SPO11-TOPOVIBL³⁸⁹⁻⁴⁵⁷ complex coloured according to pLDDT.
- f.** Predicted aligned error plot for the model shown in **d**.
- g-i** Details of the SPO11-TOPOVIBL complex shown in Fig. 8c-e coloured according to pLDDT.

	TOPVIBL-REC114
Data collection	
Space group	<i>P</i> 6 ₁ 22
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	108.7, 108.7, 83
α β γ (°)	90, 90, 120
Resolution (Å)	62.3-2.26 (2.35-2.26) ^a
<i>R</i> _{merge} (%)	6.7(169)
I / σ I	23.9 (1.4)
<i>CC</i> _{1/2} (%)	99.9 (57.8)
Completeness (%)	84.4 (38.9)
Redundancy	18 (12.2)
Refinement	
Resolution (Å)	94.03-2.26
No. reflections	11285
<i>R</i> _{work} / <i>R</i> _{free}	0.226/0.249
<i>B</i> -factors	70
R.m.s. deviations	
Bond lengths (Å)	0.006
Bond angles (°)	1.412

^a Values in parentheses are for highest resolution shell.

Supplementary Table 1

Data collection and refinement statistics.

	Unchanged-Decreased-Increased									
	Subtel 1M	Subtel 2M	Subtel 3M	Subtel 4M	Subtel 5M	Subtel 1M	Subtel 2M	Subtel 3M	Subtel 4M	Subtel 5M
chr1	0.19688	1	1	1	1	0.337954	0.707152	0.95226	1	0.30121
chr2	0.19688	1	0.9538	1	1	0.118562	0.728138	0.170249	0.26614	0.26614
chr3	NA	0.09331	0.00059	0	0.00062	NA	0.209354	0.000178	1.00E-06	8.70E-05
chr4	2.00E-05	0	0	0	0	1.20E-05	0	0	0	0
chr5	NA	0.09472	1	1	1	NA	0.214951	0.530377	1	1
chr6	0.1378	0.00765	1	1	1	0.275433	0.00955	0.263484	0.426418	0.848232
chr7	NA	1	1	1	1	NA	0.867484	1	1	0.92624
chr8	0.00204	9.00E-05	0.00548	0.2626	1	0.005766	5.50E-05	0.001712	0.039004	0.37051
chr9	2.00E-05	0	0	0	0	5.00E-06	0	0	0	0
chr10	0.07514	0.00201	0.06632	0.68638	1	0.213463	0.003835	0.016055	0.106033	0.211257
chr11	0.00041	0.00046	0.13714	0.58528	1	0.000331	0.000331	0.022116	0.06639	0.301525
chr12	NA	1	1	1	1	NA	1	1	0.877914	0.708196
chr13	NA	0.00056	0	0	0	NA	0.000478	0	0	0
chr14	0.00156	0.20581	0.25	1	1	0.004174	0.094238	0.094238	0.886754	0.784146
chr15	0.00041	0.58244	0.25	1	1	0.001482	0.189291	0.0572	0.170896	0.329774
chr16	0.85779	1	1	1	1	0.596137	1	1	0.932596	1
chr17	NA	NA	NA	1	0.02142	NA	NA	NA	0.622188	0.003701
chr18	NA	NA	1	1	1	NA	NA	1	0.644324	0.875619
chr19	0	0	0.00162	0.05963	0.26777	0	0	0.00019	0.004521	0.020866
chrX	NA	0	0	0	0	NA	0	0	0	0

Supplementary Table 2.

Statistics to test the biased distribution of hotspot densities at sub-telomeric vs. non-sub-telomeric regions in *Top6bl* ^{$\Delta 17Ct$} samples: Pearson's Chi square adjusted p-value for tests considering two (unchanged/decreased) or three (unchanged/decreased/increased) hotspot categories for different sizes of sub-telomeric regions. NA: not applicable due to the absence of hotspot with quantifiable signal.

Guides for Cirspr	6509_Top6bl_sgRNA1	CCCGCAGGACTTGTGGCTAC
	6510_Top6bl_sgRNA2	GCAGGACTTGTGGCTACAGG
	6511_Top6bl_sgRNA3	TCCTGTAGCCACAAGTCCTG
Donor for CrispR	6512_Top6bl_donor1	TTCTTGGCTTGCTGGGCGTCTCAGAGAGAATGCTTAGGAC CTGTGTCCCGGATTTCAGCCACTCGGACAGATTGGATACCTC CTGCAGCGCCAAGTCCTGCGGGAGCAGAGGAGGCGGTGGT GAGGA
	6513_Top6bl_donor2	GCGGCCTGGAGGTACCGCAGCGAGAGCAGGGCGGGTGCG GGCCTGAGCTACCACCGCCGGGTCCTCACCACCGCCTCCTC TGCTCCCGCAGGACTTGCGCTGCAGGAGGTATCCAATCT GTCCGAG
Genotyping	oliAN 320	CAACCATGGCAGGGAAGAATC
	oliAN 3201	TACCGACAGTAAACGCAGCC
RT-PCR <i>Top6bl</i>	Oli63	GGACCAGCTCTGCTACTTTT
	Oli70	CAGAGAGAATGCTTAGGACC
RT-PCR <i>Spo11</i>	Spo11:116U22	CTGTTGGCCATGGTGAAGAGAG
	Spo11:655L22	TGTGCCGACTAACATTCAAGGA

Supplementary Table 3

Oligonucleotides

Genotype	Pachytene with full autosomal synapsis (%)	Nuclei (n)
+/+	76.6	77
+/ <i>Top6bl</i> ^{W562A}	86.7	30
wild-type	83.2	107
<i>Top6bl</i> ^{W562A} / <i>Top6bl</i> ^{W562A}	86.4	81
<i>Top6bl</i> ^{W562A} / <i>Top6bl</i> ^{W562A}	96.3	27
<i>Top6bl</i> ^{W562A} mutant	88.9	108
+/+	91.0	79
+/ <i>Top6bl</i> ^{Δ17Ct}	72.2	54
wild-type	88	133
<i>Top6bl</i> ^{Δ17Ct} / <i>Top6bl</i> ^{Δ17Ct}	84.4	96
<i>Top6bl</i> ^{Δ17Ct} / <i>Top6bl</i> ^{Δ17Ct}	85.2	108
<i>Top6bl</i> ^{Δ17Ct} mutant	85	204

Supplementary Table 4

Percentage of pachytene spermatocytes with full synapsis