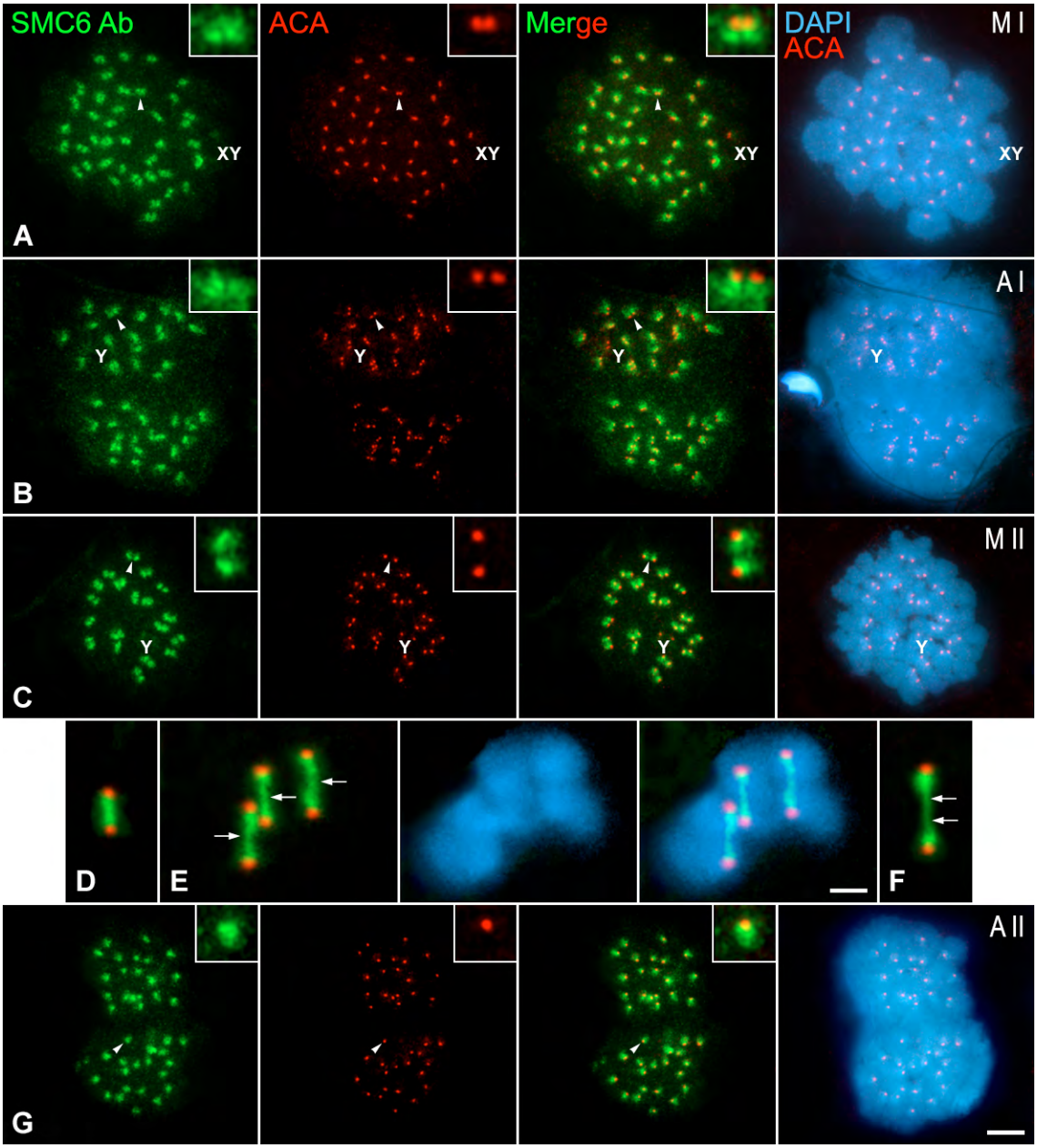

Fig. S1. SMC6 Ab labeling at centromeres is underneath the kinetochores. Double-immunolabeling of SMC6 Ab (green) and an ACA serum revealing kinetochores (red) and counterstaining of the chromatin with DAPI (blue) on spread metaphase I (**A**), anaphase I (**B**), metaphase II (**C**) and anaphase II (**G**) spermatocytes, and selected metaphase II (**D**,**E**) and early anaphase II (**F**) chromosomes from squashed spermatocytes. The sex bivalent (XY) and the Y chromosome (Y) are indicated. Arrowheads point to the centromeres that are enlarged in the insets. Arrows in (**E**,**F**) mark a strand between the Topo IIα masses below each kinetochore. Scale bars: (A-C,G) 10 µm; (D-F) 3 µm.

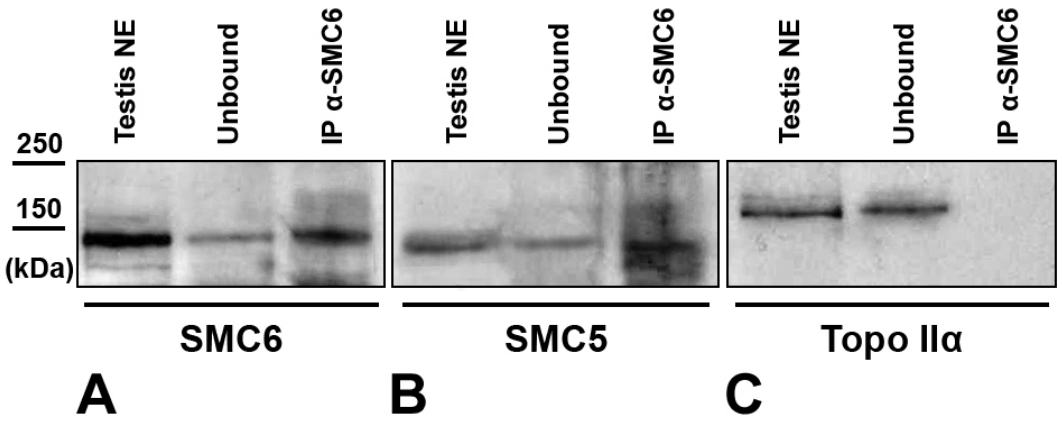

Fig. S2. Immunoprecipitation assay for SMC6, SMC5 and Topo IIα. Western blotting using antibodies against SMC6 (**A**), SMC5 (**B**) and Topo IIα (**C**) on testis nuclear extracts (NE), supernatant of non-immunoprecipitated proteins (Unbound), and SMC6 co-immunoprecipitated complexes (IP α-SMC6). Bands of approximately 145 kDa are detected with anti-SMC6 and anti-SMC5 antibodies in NE, unbound and IP α-SMC6 lanes. A band of approximately 170 kDa is detected with the anti-Topo IIα antibody only in NE and unbound lanes. No band of anti-Topo IIα is detected in IP α-SMC6.

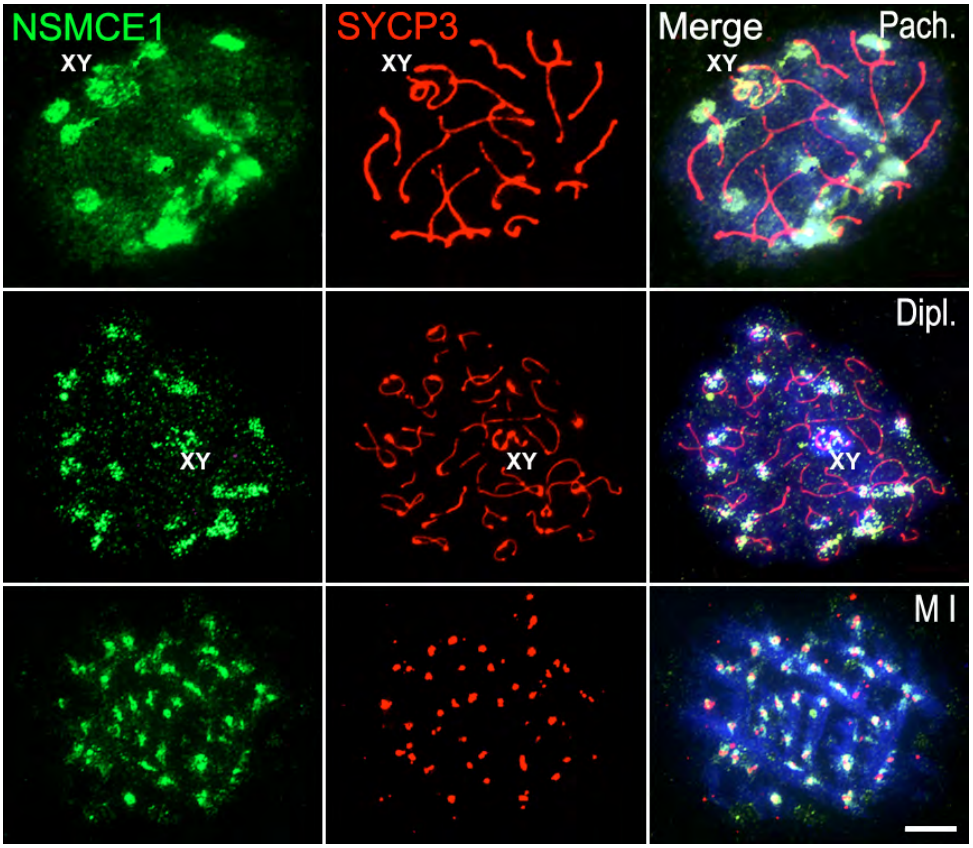

Fig. S3. NSMCE1 localizes to chromocenters and the sex body in prophase I and at metaphase I centromeres. Double-immunolabeling of NSMCE1 (green) and SYCP3 (red) and counterstaining of the chromatin with DAPI (blue) on spread pachytene (Pach.), diplotene (Dipl.) and metaphase I (M I) spermatocytes. Scale bar: 10 µm.

Supplementary Material

Table S1. Primary and secondary antibodies used in this study.

PRIMARY ANTIBODIES				DILUTION	
Antibody	**Host**	**Source**	**Cat. number or reference**	**IF**	**WB**
hSMC6 Le (6B1)	Rabbit	Alan R. Lehmann	Taylor et al., 2001	1:10	1:1,000
hSMC6 Ab	Rabbit	Abcam	ab-18039	1:10	1:2,000-1:10,000
hSMC6 Sc	Rabbit	Santa Cruz	sc-134543	1:10	--
hSMC5 Le (5A1)	Rabbit	Alan R. Lehmann	Taylor et al., 2001	1:10	--
hSMC5 Ge	Rabbit	GeneTex	GTX115669	1:100	1:1,000
hSMC5	Rabbit	Abcam	ab-18038	--	1:2,000
hNSMCE1	Rabbit	Abcam	ab-66956	1:100	1:1,000
hNSMCE4A	Rabbit	Abgent	AP-9909a	--	1:250
hNSMCE4B	Rabbit	GeneTex	GTX119734	--	1:1,000
mSYCP3	Mouse	Santa Cruz	sc-74569	1:100	--
mSYCP3	Rat	Mary A. Handel	Eaker et al., 2001	1:500	--
mTEX12	Guinea-pig	Christer Höög	Hamer et al., 2006	1:500	--
mSYCP1	Mouse	José L. Barbero	--	1:10	--
SYCP1	Rabbit	Novus Biologicals	NB300-229	--	1:10,000
Kinetochores (ACA)	Human	Antibodies Incorporated	15-235	1:50	--
hTopo IIα (4E12)	Mouse	Akihiko Kikuchi	Cobb et al., 1999	1:10	1:50
Aurora-B	Mouse	BD Transduction Laboratories	611082	1:20	--
γH2AX	Mouse	Millipore	05-636	1:500	--
H3S10ph	Rabbit	Millipore	06-570	--	1:20,000
α-Tubulin	Rabbit	Sigma	T9026	--	1:5,000
SECONDARY ANTIBODIES				**DILUTION**	
Antibody	**Host**	**Source**	**Cat. number or reference**	**IF**	**WB**
Alexa 488 α-rabbit IgG	Donkey	Molecular Probes		1:500	--
Alexa 594 α-mouse IgG	Donkey	Molecular Probes		1:500	--
Alexa 594	Donkey	Molecular		1:500	--

α-rat IgG		Probes			
Alexa 594 α-human IgG	Donkey	Molecular Probes		1:500	--
Alexa 647 α-Guinea-pig IgG	Donkey	Molecular Probes		1:500	--
Horseradish peroxidase α-Rabbit IgG	Goat	Invitrogen	A10533	--	1:20,000

Supplementary Material

Table S1. Primers used in this study.

Gene	Forward primers (5'-.....-3')	Reverse primers (5'-.....-3')	Amplicon size (bp)
Smc5	*TGGCGACTCCGAGCGGGAAG*	*GCCTGCGAGTCCGAGGCAAA*	296
Smc6	*CATGCGTGCTTTGTCCGGCG*	*AGGTCGGAAGGGCAGTGTGGT*	295
Nsmce1	*CCGGCGTGATCTCGCTGGTTTG*	*GCAGACGCCGAACCTCCCAC*	144
Nsmce2	*TGCCTTGGACCTTGTGGAGACT*	*TCAGCTCCCTCAGCTGTTGCT*	278
Nsmce3	*TTCCAGGTTGGTTCGCGGGC*	*GGATGCGGAGATGAGGGGCG*	283
Nsmce4a	*GCGCTCTGACCTGAGCTCGT*	*ATGCGAGGCTGTCTTGGCCG*	270
Nsmce4b	*AGCTGGTCCGGAGTCTGGCA*	*GCCTGCGGATTTTGCGGCAC*	207
Sycp1	*CATCAGCGAAGATTGCTTTG*	*ATTGATGAGGAAAGCCGAGA*	253
Actb	*TAAAGACCTCTATGCCAACACAGT*	*CACGATGGAGGGGCCGGACTCATC*	241