

B

168 unique peptides, 207 unique spectra, 2087 total spectra, 684/968 amino acids (71% coverage)

MRFEFESDA NEEMHVNSHD DVDSIPETLR AWLVTMTTSP RIHDIRASIR VVDFLDRFSL TNERVARRCE KFVYQSEENN CIPSNVAVIEF QIPDELLYKR LHEQLNAYTQ LVCYSSLLKVL SDDDLKLTME LVKGLKETAD	SHLNEVLTEL YAFPQEFFFLK QVNRSSQKSL WMLVRHTATT IVCVTALYDC RIVEICRYDI AKTATSLSK FYDADFELV SSIYLHYFAT EFHKKDFSKLY RKKIKKKQL FLLLLSDMRD EEMIEQIETF HLSPLGIKSH	ESVLELVVEY LLDIDMPLFO SQAATDMLYT IQCDIMRCLC COLLPTYLIN DSVRSVALKT LDRREKIPFL DMNNHRQQL HISKRSKKPG KEILDIFNST NGNDENTLV RNCITFNIFL KAWSMKFKK EKRRWKKNFI	EGPSLONVSI LSQHYFEDYK SNSKAKTVR LIVNKLSEKS VSSFEFLKHM EESMHSIHTN KRLLEDEVNVO TVNFFMLICS LALNKFQIFA KAAKVAIOKN SSGNPSYFFK AVSAANRFKK	CASIPSEKKRK SNSADFMLETF IRFQLFLVER NOTAEILVLR LSDAESQIRK NGKGINIVSS FESWFRDSSD QOAMLRNPL RFFHRLAVNK KEMVOLRDVN GNSFSLSKVY PEDTEEKELW ISSSGALDYD	WGTQDEFSLT INFALVASGC LSKVYVEDAF DLTSRFIDMI ISLKIIDLVS CIFDGGKPNR FESWFRDSSD AKLLLYDRDR LLOKYRESGV VVQEDILFAI GELIOSLEWGP TITMTTLFLG NNLFLWDPYN ISRPTTAS	ENEENNEIET QPFFVTNFDVQ LGDSTFMEITK HDIGDVLSTK SHEPEKDDQDF ISTMRILVAH HIFSRVKDDDD AKDTNSVLVPL SSYFFIKQVE DNVYNDLAES GELIOSLEWGP SKANNWDINF RARDPPTLSH
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30 unique peptides, 32 unique spectra, 72 total spectra, 304/561 amino acids (54% coverage)

MFYNQDVLTK RVWAHQYSEF VDLHVDMSTP DIOELTKGTI RALKRRKVKQK LKPWIEKLP RLLDTKVRKR NFYEAKTAI	EKGGMGVIWL HSQVSTLHLR TOPKENPNIS NSDPSLOAAS LLEPDENIEL SNNTPEIIDD LPHSPSPSP YENNGRITFS	AATLGSKHS VRKELDHFTS VLETDPDSTV OHSNLGSVQR STRTLSSQWRK VLRNIDTSEV VEFLPALES SLLPNDLKR	RKLHKKDIMS RPFKNIDION YLINTSQNP EYNSEEQESR NYVERMIALE EVGRDVGQEL YVQAQFSLH	VDIDEACDFV EQTNPKQLLL LRYMVSFPVY IHMFEIDEDV ATKYVRRRGA GLNIPWNTSS SLDSDDFVL SLATKSAFLV	AFSPEPLALR AEDPAFIPEV EDSRVAFSTE LPLVPLOSK SSAKKELNQV RSNSAINSKS YKNTQEENAH KODKPYSEIS	LSNLMLIGVT SLYDAFNLP PLDQCFDEN MDEHNENEP FFDWEFHP HOTGSEHST RMLSMKECA VSLNLKSTDA
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63 unique peptides, 67 unique spectra, 137 total spectra, 627/1233 amino acids (51% coverage)

MGRLLRLLEVE LQRDNTDFTD EQKTEAERYQ GSIRRNLLAF TSLSAAEKFR LVQDLESKIR KILEASADRN KECIDYIKEQ VARDLSYKRN CVITSDTYWK QIQVEKVEEK ERMHKFIEKD TKLSGNI TLL KFWVEVDYDE AAKNAKERFN PPMKRFRFMD ISLKNQFLSK	NFKSYRQHOI SSNPTTAYVK SQKEKRDSDA DRKVRKQEK LKDMDKEQEL SLHSEVSD ESKQDQAKRE RIGIMTFFPM LNAKTIVTLEG LHSLESEIISL IFSGFCRRIQ QESIDNYEQN ESEIDRYVSE LDEEDYTNMS AVKOKRLOK SEALSGEKTMA QALVGIYRD	IGPFEDFTSI LMYELDNQEG LSKLVQOISG LVYLLWKLPH IASKRPELIS KQLRLLPDK TERADLAKI ALYALKRIYP DTIAASPVNO TVIHKTGLIT LKDKYTVVSR ISDIHTYDEI REALESEVAT WHAILRQKRL ESMASVLOEK QAAFHISEQ IDPIYKELTK QALALFAIS QOENSSRTL	IGPNGAOKSN REYKRAITPS SLEYKSEYDK LEKSISSNA IAEKALESKS BEYEGLRSEA NEKLELELE EVKGRIDLE KFRGTHKGAR GGSSNRSKAK SVEDKKKETO HRTFTQSTO AEAELELLTE EDIDVPLREG LREYSELDQ IDPIYKELTK YQPPFFVLD INVRDYFDL	LMDAISFVLG GATEYKIDEE SKDEQDKAVN EVTRLKADS NLRKIQKAA DKLNSNLLFK KHDDQKRLT TPTQKKYESA LAIDVLFNFS HWDDHDFDL HYEELIKEQ KQLEFTKQKS DFASENSKTE SLTSIPIDDV MSPNLAIFER SPAFFLGGTA EIDAALDQTN LKY	VKSSHLRSTN IVTFSEYCGS LSAHSFNKR QLIERRDENT ETIEKEDYDQA LQTLNRNIKV WSELFHKTQE LLENRAISFEK KILLAASEKK SNSGDITMGE LETVETRLAK YLTLLDDLEP VTKIANYIRO	VKELIYRGGI LQKENILVRA GINAELROYQ KEIEKLKNE STLQVLENQK TISQKDSLTS LNEELQSSLO ATVSVETQAVA GNTLICDSMT IGELIYQKSS RNFVKSRDEL QRVSDTRLRL LVGKRLVSEL EPSEVINFE LDEEFAARK YFLGGIKFHAM HASSGFQFVV
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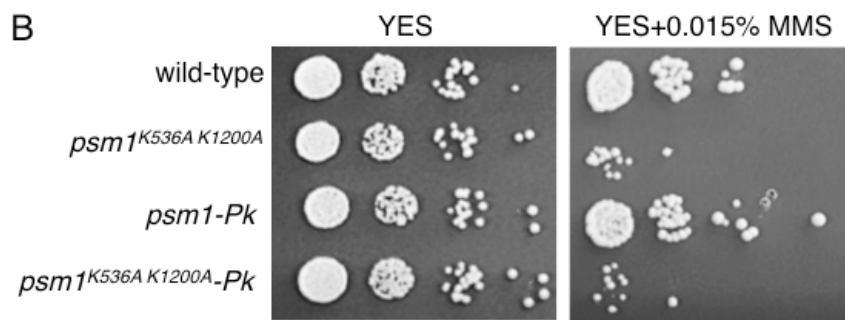
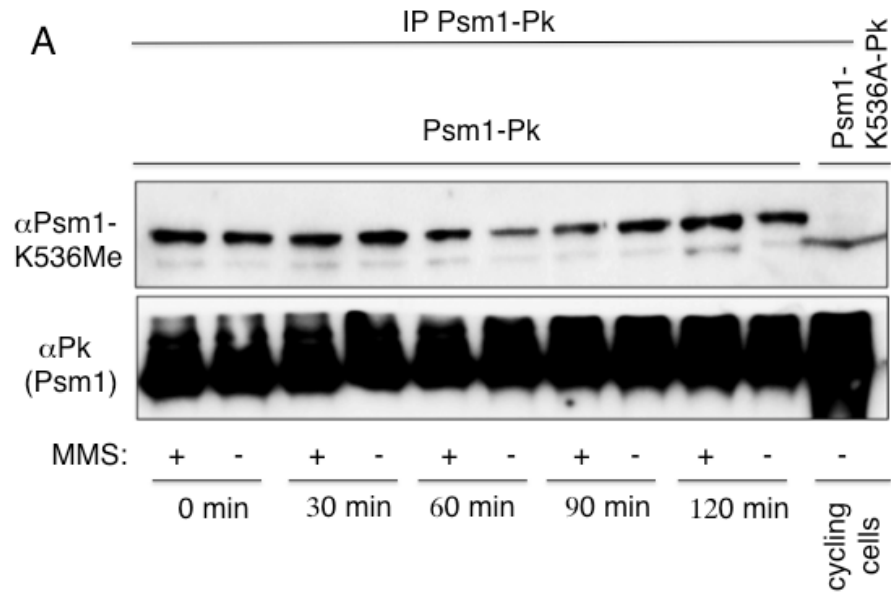
57 unique peptides, 62 unique spectra, 123 total spectra, 612/1194 amino acids (51% coverage)

MYITIKAVIQG ATVMSAYVEV OGRVTSLTNA AVYHKNDNER LELLRVEKQD YNAIVSEADD DEMENELKAK SRSEKALGTT ATQILDVIFIK SQYARSHQLN LITACLDLLOK LSSEMDLDP RIDSEELNDR BRNAKDRSLL FTKQRDSLLA LSQSIQEDDI FNILLDECDAN FVEG	FKSYKDYTVI TFANADNRP KDSERLELLK RDELYAIVSR NDEDYTNIMK LNKRIMLLKN LSRKKEIEIS MDRNTSNGIR ENAGRVFMP GITLSGDRSD AQLSLKQFER KDVEALKSL RDSLLKYENK LARKKCEKNEK RREELRRSOE MDIDTPSQRS LDAQYRSIA AMVKEMSKTS	EPLSPHHNV TGKSEVLLRR EVAGTQIYEN EHDEINSVLD SKVVALSOS QKQSLLDKOS LESQGDMSQ AVKQIAERLK ENKLRPKAVT KKGALTACYR DHIPLKDQFER GQIENLDEL LQTIKSSS IKSLQVLPPE SISELTTVLA SIDNYTGSI AMVKEMSKTS	VGRNGSGKSN TIGLKKDEYS RRAESNKIMD ALEQDRIFAAL QLSRQIEFSK RTSOFTTKKE LLANITSINE LEGYVGPLCE YPDASSALPL DYRNSRLDAI TITGETTDLQ DAITKERAHI SEQOMRHEI AFIKVYVTS QRKDEAIERT RVSFNCKDDE QFICTTFRPE	FFAAIRFVLS LDKKTIVSKTE ETIQKSEKID ERNDDDSGAF KDESSKLNIL RDEWIRNQLL RKENLTDKRR LRFKVDNRFKV HGYLEDPK KNVKTYQIKF EMMHHKSRML EARKTALYE EISDKRNELE NAIVKKLHKI FKQVAKSFSE QLNINQLSGG MVKVADNFYQ	DAYTHLSREE VINLLESAGF ELLQYIEERL IQREERTERL SLESKIEK QINRRINSTK SLWREEAKLK AVEATAGNSL DAAIKVFFSK SDLOESLEK ELVLELHLL LNTNLYLRRN SLEELQHEVA NEALKDQYGSV IFVKLVPAQR QKSLCALTLI VMFNHKKSTV	ROALLHEGPG SRSNPPYIVP RELEEEKNDL HAEITELNHS TRIEQALPK ENSNDYLKTEY SSIEENKDDL PHIVVDNDET PLKAEIGSON TRIEQALPK REIESIEFDQK EQOANDLKSE PLKAEIGSON TRIEQALPK NKKAYEOPFN GELVMNRRSE FAIQRCDEPAP EISIKCEAMA
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Figure S1 Progression of *pat1*-induced meiosis and mass-spectrometry analysis of cohesin subunits.

(A) *pat1-114/pat1-114 mat-Pc* cells expressing Rec11-TAP were arrested by nitrogen starvation and released into meiosis at 34°C by inactivation of Pat1 (as described in Fig. 2A). Cells were harvested at the indicated time points (hours), fixed with ethanol and analysed by flow-cytometry. In parallel, fixed cells were stained with DAPI and nuclei were counted in 100 cells per time point. Shown are the fractions of cells that contained one nucleus (1n), two nuclei (2n) or more than two nuclei (3n or more) at the indicated time points.

(B) Cohesin subunits associated with Rec11-TAP were isolated and analysed by mass-spectrometry as described in Figure 1. Sequence coverage is indicated in yellow.



C

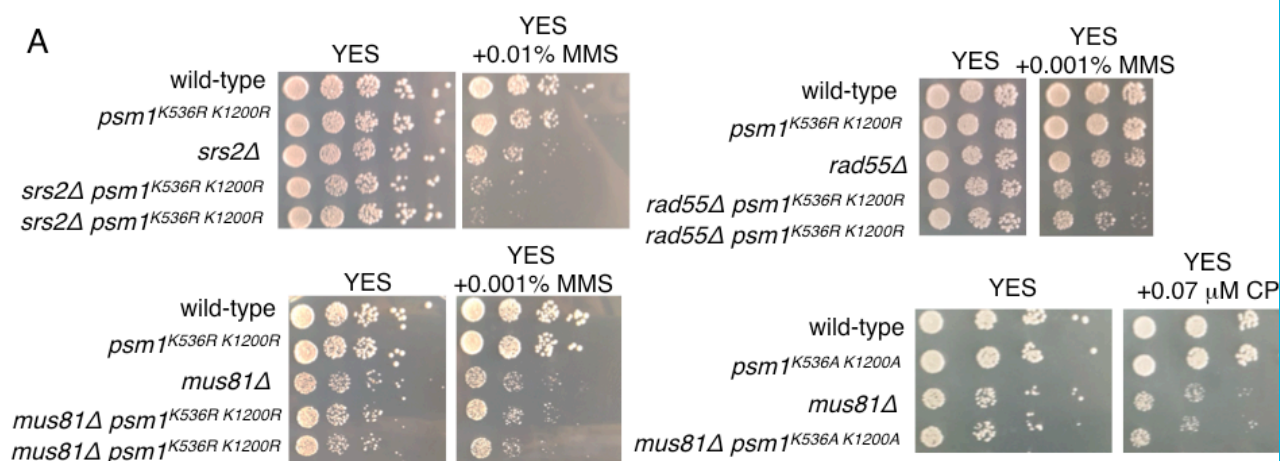
		anaphase		metaphase		
		sister chromatids segregated	missegregation of sister chromatids	cohesed <i>cen2</i> -GFP	split <i>cen2</i> -GFP	
		wild-type	100%	0	43%	57%
		<i>psm1^{K536A K1200A}</i>	100%	0	45%	55%
+0.02% MMS		wild-type	100%	0	40%	60%
		<i>psm1^{K536A K1200A}</i>	100%	0	45%	55%

Figure S2. The levels of Psm1-K536me do not change in response to MMS treatment, Psm1-Pk is functional and chromosome segregation during mitosis is normal in *psm1*^{K536A K1200A} mutant cells.

(A) Psm1-Pk protein was immunoprecipitated (IP) from the indicated time points after adding 0.01% MMS (10 mg of total proteins were applied to 50 µg of packed beads). Samples were analysed by Western blotting using anti-Psm1-K536me and anti-Pk antibodies. Cycling cells expressing Psm1-K536A-Pk were used as a negative control.

(B) Sensitivity of cells expressing Psm1-Pk and Psm1^{K536A K1200A}-Pk to methyl methanesulfonate (MMS). Serial dilutions of wild-type, *psm1*^{K536A K1200A}, *psm1-Pk* and *psm1*^{K536A K1200A}-*Pk* strains were spotted on YES containing or lacking the MMS and grown for 2 days at 32°C. Psm1-Pk is functional as assayed by this MMS-sensitivity test.

(C) Segregation of chromosome 2 was scored in a wild-type and *psm1*^{K536A K1200A} strains carrying chromosome 2 marked by *cen2*-GFP. Strains were grown at 32°C for 3 hours in YES medium with or without 0.02% MMS. Cells were fixed and immunostained for tubulin and GFP. DNA was visualized by DAPI staining. At least 100 anaphase or metaphase cells were examined under the fluorescence microscope.



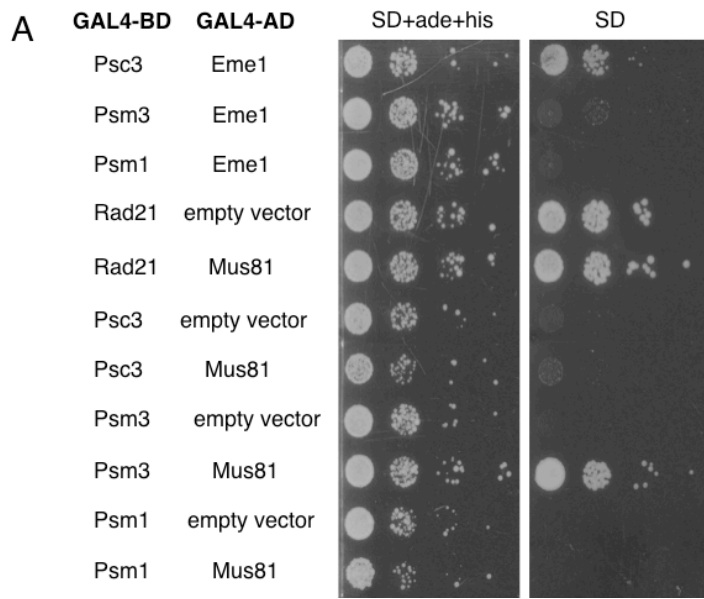
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	% plating efficiency (± standard deviation)			
	YES	YES +0.001% MMS	YES +0.002% MMS	YES +0.004% MMS
wild-type	62.9 ± 9.7	62.6 ± 4.1	60.8 ± 5.6	61.8 ± 5.0
<i>psm1^{K536A K1200A}</i>	80.6 ± 2.7	78.1 ± 4.0	83.7 ± 4.4	78.7 ± 4.4
<i>psm1^{K536R K1200R}</i>	72.5 ± 3.2	72.7 ± 3.1	71.1 ± 6.6	71.6 ± 4.1
<i>mus81Δ</i>	40.4 ± 2.6	13.5 ± 2.6	0.6 ± 0.2	-
<i>mus81Δ psm1^{K536A K1200A}</i>	35.3 ± 2.0	12.3 ± 0.9	0.5 ± 0.3	-
<i>mus81Δ psm1^{K536R K1200R}</i>	40.3 ± 3.6	12.1 ± 2.2	0.3 ± 0.2	-
<i>sfr1Δ</i>	68.4 ± 9.2	-	67.2 ± 5.2	65.1 ± 6.4
<i>sfr1Δ psm1^{K536A K1200A}</i>	75.1 ± 3.2	-	72.6 ± 8.7	2.6 ± 1.3

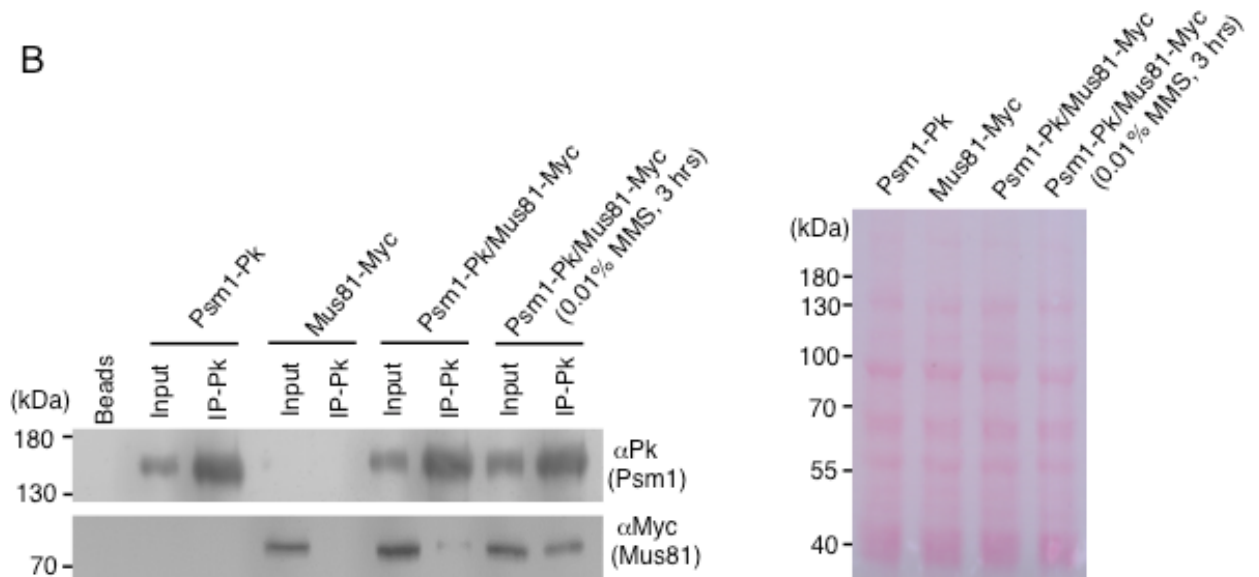
Figure S3. Genetic interactions between *psm1*^{K536RA K1200A}, *psm1*^{K536R K1200R} and mutations in DNA repair-related genes.

(A) Cells were grown on YES medium for one day, diluted in 5-fold steps, spotted onto YES plates containing the indicated amounts of methyl methanesulfonate (MMS) or camptothecin (CPT) and incubated for 3 days at 30°C.

(B) The plating efficiency indicates the percentage of cells plated onto a plate capable of forming colonies. To determine the plating efficiency, the number of cells plated on the freshly prepared YES plates containing the indicated concentrations of MMS was divided by number of colonies formed after 4 days of incubation. Data are means ± standard deviation of at least four independent experiments.



B



C

Psm1-K536		
Dm_SMC1	DRMINMCQPTHKRYNVAVTKVLGKFMEAIIVDTEKTARHCIQILKEQMLEVETFLPLDYL	598
Hs_SMC1A	GRLIDLCQPTQKKYQIAVTKVLGKNMDAIIVDSEKTGRDCIQYIKEQRGEPETFLPLDYL	576
Xl_SMC1A	GRLIDLCQPTQKKYQIAVTKVLGKNMDAIIVDSEKTGRDCIQYIKEQRGEPETFLPLDYL	576
Hs_SMC1B	GRLFDLCHPIHKKYQLAVTKVFRGITAIIVVASEKVAKDCIRFLKEERAEPETFLALDYL	576
Mm_SMC1B	GRLDLCHPIHKKYQLAVTKLFGRYMVAIVVASEKIAKDCIRFLKAERAEPETFLALDYL	576
Sp_Psm1	GRIIDLCTPTQKKYESAIAAALGKNFDAIVVETQAVAKECIDYIKEQRIGIMTFFPMDTI	583
Sc_SMC1	GLVHDLCHPKKKEYGLAVSTILGKNFDSVIVENLTVAQECIAFLKKQRAGTASFIPLDTI	590
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Psm1-K1200		
Dm_SMC1	APFFVLDEIDAALDNTNIGKVASYIRDHT-TNLQTIVISLKEEFYGHADALVGITPGEED	1221
Hs_SMC1A	APFFVLDEIDAALDNTNIGKVANYIKEQSTCNFQAIIVISLKEEFYTKAESLIGVYPEQGD	1209
Xl_SMC1A	SPFFVLDEIDAALDNTNIGKVANYIKEQSMSNFQAIIVISLKEEFYTKAESLIGVYPEQGD	1209
Hs_SMC1B	-----VSSYIKEQTQDQFQMIIVISLKEEFYSRADALIGIYPEYDD	1131
Mm_SMC1B	APFFVLDEVDAALDNTNIGKVSYSYIKEQSQEQFQMIIVISLKEEFYSKADALIGVYPEHNE	1204
Sp_Psm1	SPFFVLDEIDAALDQTNVTKIANYIRQHASSGFQFVVISLKNQLFSKSEALVGIYRDQQE	1213
Sc_SMC1	SPFFVLDEVDAALDITNVQRIAAAYIRRHNPDLQFIVISLKNMFMFKSDALVGVYRQQQE	1210
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Figure S4. Physical interactions between subunits of cohesin and Mus81-Eme1 complexes and conservation of Psm1 lysine residues 536 and 1200 in other organisms.

(A) Strains expressing Mus81 or Eme1 fused to the GAL4 transcription activation domain and Psm1, Psm3, Psc3 or Rad21 fused to the GAL4 DNA-binding domain were spotted at 10-fold serial dilutions on SD plates or SD plates supplemented with adenine and histidine (SD+ade+his). The empty vector pGADT7 containing GAL4 transcription activation domain was used as a negative control. Growth of the strain expressing Psm3 fused to the GAL4 DNA-binding domain and Mus81 fused to the GAL4 transcription activation domain on the plate without histidine and adenine (SD) indicates interaction between Psm3 and Mus81. A weak interaction between Psc3 and Eme1 was also detected. The interaction between Rad21 and Mus81 is a false positive because the control strain carrying Rad21 fused to the GAL4 DNA-binding domain and an empty vector containing GAL4 transcription activation domain was also able to grow on the plate without histidine and adenine (SD).

(B) Protein extracts were prepared from cycling cells expressing Psm1-Pk, Mus81-Myc or both Psm1-Pk and Mus81-Myc grown in YES or YES+0.01% MMS, as indicated. Proteins bound to anti-V5 agarose beads, which bind the Pk tag on Psm1, were analyzed for Mus81 by Western blotting with anti-Myc antibody. Ponceau staining of the membrane after protein transfer shows protein amounts loaded in inputs.

(C) ClustalW generated alignment of Smc1 protein sequences from *Drosophila melanogaster* (Dm_SMC1), human (Hs_SMC1A, Hs_SMC1B), *Xenopus laevis* (Xl_SMC1A), mouse (Mm_SMC1B), *S. pombe* (Sp_Psm1) and *S. cerevisiae* (Sc_SMC1). Positions of Psm1 lysines K536 and K1200 are indicated.

Table S1. *S. pombe* strains

Strain	Genotype	Used in Figure
JG16384	<i>h⁻/h⁻ ade6-M210/ade6-M216 pat1-114/pat1-114 Rec11-TAP::kanMX6/Rec11-TAP::kanMX6 mat-Pc::lys1</i>	1, S1
JG17622	<i>h⁻ ade6-210 psm1::psm1-pk9::kanMX6</i>	2B, S2A, S2B
JG17624	<i>h⁻ ade6-210 psm1::psm1-K536A K1200A-pk9::kanMX6</i>	2B, S2A, S2B
JG17680	<i>h⁺/h⁻ ade6-M210/ade6-M216 psm1+/psm1::psm1-K536A K1200A-hphMX4</i>	3A
JG16543	<i>h⁺/h⁻ ade6-M210/ade6-M216 psm1+/psm1::natMX4 lys1-37/lys1+</i>	3A
JG17331	<i>h⁺ ade6-M216 lys1-37</i>	2C, 3, 4, S3
JG17184	<i>h⁺ psm1::psm1-K536A::hphMX4</i>	3B
JG17187	<i>h⁻ psm1::psm1-K1200A::hphMX4</i>	3B
JG17189	<i>h⁻ psm1::psm1-K536A K1200A::hphMX4</i>	2C, 3, 4, S3, S2B
JG17179	<i>h⁺ psm1::psm1⁺::hphMX4</i>	3B
JG17543	<i>h⁹⁰ rqh1::kanMX4</i>	4A
JG17879	<i>psm1::psm1-K536A K1200A::hphMX4 rqh1::kanMX4</i>	4A
JG17792	<i>h⁹⁰ srs2::kanMX4</i>	4A, S3
JG17875	<i>psm1::psm1-K536A K1200A::hphMX4 srs2::kanMX</i>	4A
JG17827	<i>h⁹⁰ eme1::kanMX4</i>	4A
JG17869	<i>psm1::psm1-K536A K1200A::hphMX4 eme1::kanMX4</i>	4A
JG17884	<i>h⁹⁰ mus81::kanMX4</i>	4A, S3
JG17924	<i>h⁻ psm1::psm1-K536A K1200A::hphMX4 mus81::kanMX4</i>	4A, S3
JG17551	<i>h⁺ ade6-52 slx1::kanMX6</i>	4A
JG17877	<i>psm1::psm1-K536A K1200A::hphMX4 slx1::kanMX6</i>	4A
JG17468	<i>h⁺ ura4-D18 leu1-32 rad57::kanMX4</i>	4A
JG17863	<i>h⁻ psm1::psm1-K536A K1200A::hphMX4 rad57::kanMX4</i>	4A
JG17469	<i>h⁺ ura4-D18 leu1-32 rad55::kanMX4</i>	4A, S3
JG17861	<i>psm1::psm1-K536A K1200A::hphMX4 rad55::kanMX4 clone 1</i>	4A
JG17862	<i>psm1::psm1-K536A K1200A::hphMX4 rad55::kanMX4 clone 2</i>	4A
JG17823	<i>h⁺ ura4-D18 leu1-32 rad52::kanMX4</i>	4A
JG17865	<i>h⁻ psm1::psm1-K536A K1200A::hphMX4 rad52::kanMX4</i>	4A
JG17746	<i>h⁹⁰ sfr1::natMX4</i>	4A, S3
JG17871	<i>psm1::psm1-K536A K1200A::hphMX4 sfr1::natMX4</i>	4A, S3
JG17680	<i>h⁻/h⁺ ade6-M210/ade6-M216 psm1+/psm1::psm1-K536A K1200A::hphMX4</i>	3A
JG16543	<i>h⁻/h⁺ ade6-M210/ade6-M216 psm1+/psm1::natMX4</i>	3A
JG16539	<i>h⁻/h⁺ ade6-M210/ade6-M216</i>	3A
JG18082	<i>psm1::psm1-K536R K1200R::hphMX4 mus81::kanMX4 clone1</i>	S3
JG18083	<i>psm1::psm1-K536R K1200R::hphMX4 mus81::kanMX4 clone2</i>	S3
JG18092	<i>psm1::psm1-K536R K1200R::hphMX4 srs2::kanMX4 clone1</i>	S3
JG18093	<i>psm1::psm1-K536R K1200R::hphMX4 srs2::kanMX4 clone2</i>	S3
JG18096	<i>psm1::psm1-K536R K1200R::hphMX4 rad55::kanMX4 clone1</i>	S3
JG18097	<i>psm1::psm1-K536R K1200R::hphMX4 rad55::kanMX4 clone2</i>	S3
JG18168	<i>h⁻ psm1::psm1-K536R K1200R::hphMX4</i>	S3
JG17214	<i>h⁹⁰ ade6-216 ura4-D18 cen2(D107)[:: kanr-ura4+-lacOp] his7+::lacI-GFP psm1::phygUra4Tpsm1+</i>	S2C
JG17254	<i>h⁺ cen2(D107):Kan-ura4+-lacO his7+::lacI-GFP psm1::pHygUra4T psm1K536A K1200A</i>	S2C
JG11363	<i>h⁺ rad21-K1-ura4+ leu1-32 ura4D18 ade6-210</i>	4B
JG18180	<i>h⁻ rad21-K1-ura4+ leu1-32 ura4D18 ade6-210</i>	4B
JG18178	<i>rad21-K1 mus81::kanMX4</i>	4B
JG18182	<i>rad21-K1 eme1::kanMX4</i>	4B
JG18183	<i>rad21-K1 rad55::kanMX4</i>	4B
JG18184	<i>rad21-K1 rqh1::kanMX4</i>	4B
JG18140	<i>h⁺ ade6-M210 psm1-pk9::kanMX4</i>	S4B
JG18186	<i>h⁻ leu1 ura4 mus81-myc::kanMX6</i>	S4B
JG18196	<i>psm1-pk9::kanMX4 mus81-myc::kanMX6</i>	S4B

(other auxotrophic markers have not been scored)