

Supplementary Information:

Structural and functional characterization of the Spo11 core complex

Corentin Claeys Bouuaert, Sam E. Tischfield, Stephen Pu, Eleni P. Mimitou, Ernesto Arias-Palomo, James M. Berger and Scott Keeney.

Supplementary Tables.

Supplementary Table 1: XL-MS data (separate Excel file).

Supplementary Table 2: C α distances of Spo11 and Ski8 intramolecular crosslinks.

Supplementary Table 3: Oligonucleotides.

Supplementary Table 4: Plasmids.

Supplementary Table 5: Strains.

Supplementary Table 6: Sequencing statistics.

Supplementary Table 2: C α distances of Spo11 and Ski8 intramolecular crosslinks.Spo11 intramolecular crosslinks

Position 1	Position 2	Count -DNA	Count +DNA	C α distance
121	159	17	65	20.2
121	164	12	34	14
121	270	2	1	29.1
126	159	3	25	22.7
126	164	17	36	13.4
126	270	10	23	27.8
159	164	23	0	13.8
159	270	14	9	19.7
159	317	2	0	35.7
159	334	2	0	57.2
159	350	2	0	36.8
164	196	3	0	29.4
164	206	10	0	25.8
164	258	16	0	20
164	270	17	93	15.2
164	334	2	0	50.1
240	270	0	4	24.5
240	317	2	0	17.8
240	334	4	0	20
247	334	4	0	36.4
270	317	10	2	17.5
270	334	3	0	41.9
270	383	2	2	32
317	334	14	0	26.3

Ski8 intramolecular crosslinks

Position 1	Position 2	Count -DNA	C α distance
3	321	3	14.4
3	365	13	15.2
44	54	1	9.3
44	105	30	11.2
44	106	4	13.4
44	365	11	12.5
52	105	3	14.1
52	106	10	8.7
62	111	2	15.5
121	147	5	12

cb847	CATCCGTTAAAATTGTAGCTGCTGCACTGAGAATGACGTGG
cb848	CAGTTTAAAATGTACAGCTGCAACGGATGCTCAGATAAG
cb849	CTTATCTGAGCATCCGTTGCAGCTGTACATTTTAACTG
cb850	CAAACCCAGAGAGCTTGCTGCGGCTGCCAAAGGATATCCTG
cb851	CAGGATATCCTTTGGCAGCCGCAGCAAGCTCTCTGGGTTTG
cb852	GATATCCTGAAATCTATGCGCTTTCGAGTTATCAAC
cb853	GTTGATAACTCGGAAAGCGCATAGATTTTCAGGATATC
cb854	CTATTACCTTTCGAGGCATCAACAAGTCAATTTTTTTTC
cb855	GAAAAAAAATTGACAGTTGTTGATGCCTCGGAAAGGTAATAG
cb856	GAAGTGGCAACAAAGTGCAGCTCGCTCCGATATCC
cb857	GGATATCGGAGCGAGCGCACTTTGTTGCCACTTC
cb858	CAACAAAGTTCGCTCGCTGCGGCATCCTACGCCCTAACGAG
cb859	CTCGTTAGGGCGTAGGATGCCGCAGCGAGCGAACTTTGTTG
cb860	CTCCGATATCCTACGCCGCAACGAGCAATTCAGTAC
cb861	GTAAGAATTGCTCGTTGCGGCGTAGGATATCGGAG
cb862	CTACGCCCTAACGAGCGCTGCAGTACTTCTTCCAATAATG
cb863	CATTATTGGAAGAAGTACTGCAGCGCTCGTTAGGGCGTAG
cb864	CTAACGAGCAATTCAGTAGCTGCTGCAATAATGCTTAACATG
cb865	CATGTTAAGCATTATTGCAGCAGCTACTGAATTGCTCGTTAG
cb866	CAGTACTTCTTCCAATAGCGGCTAACATGATTGCCCAAG
cb867	CTTGGGCAATCATGTTAGCCGCTATTGGAAGAAGTACTG
cb868	CTTCCAATAATGCTTAACGCGGCTGCCCAAGACAAATCTTC
cb869	GAAGATTTGTCTTGGGCAGCCGCGTTAAGCATTATTGGAAG
cb870	CTTAACATGATTGCCCAAGCCGCATCTTCAACAACCGCG
cb871	CGCGGTTGTTGAAGATGCGGCTTGGGCAATCATGTTAAG
cb872	CCAAGACAAATCTTCAGCAGCCGCGTATCAAATTCTG
cb873	CAGAATTTGATACGCGGCTGCTGAAGATTTGTCTTGG
cb896	AATACCATGCTTATTACAGGTAAGGGAGCTCCAGATTTCTTGACAAGG
cb897	CCTTGTCAAGAAATCTGGAGCTCCCTTACCTGTAATAAGCATGGTATT
cb953	TAGCAATGTAATCGTCTATGACGTTAACGTCATAGACGATTACATTGCTA
cb957	TAGCAATGTAATCGTCTATGACGTTAACGTCATAGACGATTACATTGC
cb958	TAGCAATGTAATCGTCTATGACGTT-Biot
cb959	AACGTCATAGACGATTACATTGC
cb960	AA*CG*TC*AT*AG*ACGATTACATTGC
cb961	AACGTCATAGACG*AT*TA*CA*TT*GC
cb962	TCGCGCGTTTCGGTGATGA
cb963	AATTCACTGGCCGTCGTTTTAC
cb1061	TAGCAATGTAATCGTCTATGACGTTAACGTCATAGACGATTACATTGCT
cb1062	TAGCAATGTAATCGTCTATGACGTTAACGTCATAGACGATTACATTG
cb1063	TAGCAATGTAATCGTCTATGACGTTAACGTCATAGACGATTACATT
cb1064	AGCAATGTAATCGTCTATGACGTTAACGTCATAGACGATTACATTGCTA
cb1065	GCAATGTAATCGTCTATGACGTTAACGTCATAGACGATTACATTGCTA

cb1066	CAATGTAATCGTCTATGACGTTAACGTCATAGACGATTACATTGCTA
cb1067	AATGTAATCGTCTATGACGTTAACGTCATAGACGATTACATTGCTA
cb1027	ATCTTGGCAGTTAATCGAACAAGACCCGTGCAATGCTATCGACATCAAGGCCTATCGTTACGGGGTTGGA GGTCAATGGGTTCAAGGATGCAGGTGAGGAT
cb1028	CCTTGATGTCGATAGCATTGCACGGGTCTTGTTGATTAAGTCCCAAGATATCCTCACCTGCATCCTGAA CCCATTGACCTCCAACCCCGTAACGATAGG
cb1072	AACGTCATAGACGATTACATT*GC
cb1073	AACGTCATAGACGATTACA*TTGC
cb1074	AACGTCATAGACGATTA*CATTGC
cb1075	AACGTCATAGACGAT*TACATTGC
cb1076	AACGTCATAGACG*ATTACATTGC
cb1077	AACGTCATAGA*CGATTACATTGC
cb1078	AACGTCATA*GACGATTACATTGC
cb1079	AACGTCA*TAGACGATTACATTGC
cb1080	AACGT*CATAGACGATTACATTGC
cb1081	AAC*GTCATAGACGATTACATTGC
cb1082	Biot-AACGTCATAGACGATTACATTGC
cb1083	TA*GCAATGTAATCGTCTATGACGTT
cb1084	TAGC*AATGTAATCGTCTATGACGTT
cb1085	TAGCAA*TGTAATCGTCTATGACGTT
cb1086	TAGCAATG*TAATCGTCTATGACGTT
cb1087	TAGCAATGTA*ATCGTCTATGACGTT
cb1088	TAGCAATGTAAT*CGTCTATGACGTT
cb1089	TAGCAATGTAATCG*TCTATGACGTT
cb1090	TAGCAATGTAATCGTC*TATGACGTT
cb1091	TAGCAATGTAATCGTCTA*TGACGTT
cb1092	TAGCAATGTAATCGTCTATG*ACGTT
cb1093	TAGCAATGTAATCGTCTATGAC*GTT
cb1299	CATTATACCAGCTCAA _{gc} GGGTTTAGTTTATTCGC
cb1300	GCGAATAAACTAAACCCGCTTGAGCTGGTATAATG
cb1301	CAATTATTGAGTTTGAATCAG _{gc} CGACTACTCCTTAGCCAAG
cb1302	CTTGGCTAAGGAGTAGTCGGCCTGATTCAA _{gc} ACTCAATAATTG

'Biot' indicates the position of a 3'-biotin modification.

'*' indicates the position of a phosphorothioate modification.

Supplementary Table 4: Plasmids used in this study.

Plasmid	Description	Reference
pCCB586	Spo11 from <i>S. cerevisiae</i> (SK1 strain) in pFastBac1 (primers cb753 and cb754, digest with BamHI and XhoI)	This study
pCCB587	Ski8 from <i>S. cerevisiae</i> (SK1 strain) in pFastBac1 (primers cb755 and cb756, digest with StuI and XhoI)	This study
pCCB588	Rec102 from <i>S. cerevisiae</i> (SK1 strain) in pFastBac1 (digest with BamHI and XhoI)	This study
pCCB589	Rec104 from <i>S. cerevisiae</i> (SK1 strain) in pFastBac1 (digest with BamHI and XhoI)	This study
pCCB592	Spo11-HisFlag in pFastBac1 (inverted PCR and self-ligation using pCCB586 as a template and primers cb757 and cb758)	This study
pCCB611	Spo11-(Y135F)-HisFlag in pFastBac1 (QuikChange on pCCB592 using primers cb806 and cb807)	This study
pCCB613	Spo11-(E233A)-HisFlag in pFastBac1 (QuikChange on pCCB592 using primers cb810 and cb811)	This study
pCCB615	Ski8-HisFlag in pFastBac1 (inverted PCR and self-ligation using template pCCB587, primers cb814 and cb815)	This study
pCCB616	Rec104-HisFlag in pFastBac1 (inverted PCR and self-ligation using template pCCB589, primers cb816 and cb817)	This study
pCCB617	MBP-Rec102 in pFastBac1 (amplification of MBP from pMAL-c2x using primers cb818 and cb819)	This study
pCCB618	MBP-Ski8 in pFastBac1 (amplification of MBP from pMAL-c2x using primers cb820 and cb821)	This study
pCCB619	MBP-Rec104 in pFastBac1 (amplification of MBP from pMAL-c2x using primers cb822 and cb823)	This study
pCCB620	MBP-Spo11 in pFastBac1 (amplification of MBP from pMAL-c2x using primers cb830 and cb831)	This study
pCCB633	HisFlag-Rec102 in pFastBac1-HTB	This study
pCCB646	Spo11-(F260A)-HisFlag in pFastBac1 (QuikChange on pCCB592 using primers cb896 and cb897)	This study
pCCB814	Spo11-(K173A)-HisFlag in pFastBac1 (QuikChange on pCCB592 using primers cb1299 and cb1230)	This study
pCCB815	Spo11-(R344A)-HisFlag in pFastBac1 (QuikChange on pCCB592 using primers cb1301 and cb1302)	This study
pSK282	LexA-Rec102 Y2H vector (pCA1-Rec102)	Maleki et al., 2007
pSK305	Gal4AD-Spo11 Y2H vector	Arora et al., 2004
pSK310	Gal4AD-Rec104 Y2H vector	Arora et al., 2004
pSK276	Gal4AD empty Y2H vector	Arora et al., 2004
pSK272	LexA empty Y2H vector	Arora et al., 2004
pCCB655	pSK282 with Rec102-L26A/L27A (QuikChange primers cb834 and cb835)	This study
pCCB656	pSK282 with Rec102-W30A (cb836 and cb837)	This study
pCCB657	pSK282 with Rec102-F90A/W91A (cb838 and cb839)	This study
pCCB658	pSK282 with Rec102-E92A/E93A (cb840 and cb841)	This study
pCCB659	pSK282 with Rec102-Q94A/L95A (cb842 and cb843)	This study
pCCB660	pSK282 with Rec102-F96A/Y97A (cb844 and cb845,)	This study
pCCB661	pSK282 with Rec102-L113A/K114A/C115A (cb846 and cb847)	This study
pCCB662	pSK282 with Rec102-I117A/L118A (cb848 and cb849)	This study
pCCB663	pSK282 with Rec102-H134A/T135A/H136A (cb850 and cb851)	This study
pCCB664	pSK282 with Rec102-Y145A (cb852 and cb853)	This study

pCCB665	pSK282 with Rec102-L149A (cb854 and cb855)	This study
pCCB666	pSK282 with Rec102-S206A (cb856 and cb857)	This study
pCCB667	pSK282 with Rec102-P209A/I210A (cb858 and cb859)	This study
pCCB668	pSK282 with Rec102-L214A (cb860 and cb861,)	This study
pCCB669	pSK282 with Rec102-N217A/S218A (cb862 and cb863)	This study
pCCB670	pSK282 with Rec102-L220A/L221A/P222A (cb864 and cb865)	This study
pCCB671	pSK282 with Rec102-M224A/L225A (cb866 and cb867)	This study
pCCB672	pSK282 with Rec102-M227A/I228A (cb868 and cb869)	This study
pCCB673	pSK282 with Rec102-D231A/K232A (cb870 and cb871)	This study
pCCB674	pSK282 with Rec102-T235A/T236A (cb872 and cb873)	This study
pSK806	Spo11-HisFlag::HphMX4 in pUC19	This study
pSK809	Spo11-(F260A)-HisFlag::HphMX4 in pUC19	This study
pCCB822	Spo11-(K173A)-HisFlag::HphMX4 in pUC19	This study
pCCB823	Spo11-(R344A)-HisFlag::HphMX4 in pUC19	This study

Supplementary Table 5: Yeast strains used in this study.

Strain	Genotype
SKY661	<i>MATa</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>leu2::hisG</i> , <i>trp1::hisG</i> , <i>ndt80::kanMX</i> , <i>LexA(op)-LacZ::URA3</i>
SKY662	<i>MATα</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>leu2::hisG</i> , <i>trp1::hisG</i> , <i>ndt80::kanMX</i> , <i>LexA(op)-LacZ::URA3</i>
SKY1311	<i>MATα</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>trp1::hisG</i> , <i>arg4-nsp</i> , <i>rec102Δ::URA3</i>
SKY1312	<i>MATa</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>trp1::hisG</i> , <i>arg4-bgl</i> , <i>rec102Δ::URA3</i>
SKY5788	<i>MATa</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>arg4-Nsp</i> , <i>nuc1Δ::LEU2</i> , <i>SPO11-His6-flag3-HphMX4</i>
SKY5789	<i>MATα</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>arg4-Nsp</i> , <i>nuc1Δ::LEU2</i> , <i>SPO11-His6-flag3-HphMX4</i>
SKY5797	<i>MATa</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>arg4-Nsp</i> , <i>nuc1Δ::LEU2</i> , <i>SPO11(F260A)-His6-flag3-HphMX4</i>
SKY5798	<i>MATα</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>arg4-Nsp</i> , <i>nuc1Δ::LEU2</i> , <i>SPO11(F260A)-His6-flag3-HphMX4</i>
SKY5806	<i>MATa</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>arg4-Nsp</i> , <i>nuc1Δ::LEU2</i> , <i>SPO11-His6-flag3-HphMX4</i> , <i>sae2Δ::KanMX6</i>
SKY5807	<i>MATα</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>arg4-Nsp</i> , <i>nuc1Δ::LEU2</i> , <i>SPO11-His6-flag3-HphMX4</i> , <i>sae2Δ::KanMX6</i>
SKY6621	<i>MATa</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>arg4-Nsp</i> , <i>nuc1Δ::LEU2</i> , <i>SPO11(F260A)-His6-flag3-HphMX4</i> , <i>sae2::KanMX6</i>
SKY6622	<i>MATα</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>arg4-Nsp</i> , <i>nuc1Δ::LEU2</i> , <i>SPO11(F260A)-His6-flag3-HphMX4</i> , <i>sae2::KanMX6</i>
SKY6615	<i>MATa</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>SPO11(K173A)-His6-flag3-HphMX4</i>
SKY6616	<i>MATα</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>SPO11(K173A)-His6-flag3-HphMX4</i>
SKY6617	<i>MATa</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>SPO11(R344A)-His6-flag3-HphMX4</i>
SKY6618	<i>MATα</i> , <i>ho::LYS2</i> , <i>lys2</i> , <i>ura3</i> , <i>leu2::hisG</i> , <i>SPO11(R344A)-His6-flag3-HphMX4</i>

All strains are from the SK1 background.

Supplementary Table 6: Sequencing statistics.

S1-seq				
Dataset	Total Sequenced	Total Mapped^a		Uniquely mapped to sacCer2^b
Spo11 sae2 sample 1	13,883,720	12,589,142 (90.7%)		8,735,385 (69.4%)
Spo11 sae2 sample 2	10,602,809	9,622,551 (90.8%)		6,748,120 (70.1%)
Spo11-F260A sae2 sample 1	7,814,010	7,351,934 (94.1%)		4,498,753 (61.2%)
Spo11-F260A sae2 sample 2	29,127,904	27,114,286 (93.1%)		16,477,694 (60.8%)
Spo11-oligos				
Dataset	Total Sequenced	Total Mapped^c	Total Filtered^d	Uniquely mapped to sacCer2^e
Spo11-F260A sample 1	16,534,430	15,909,570 (96.2%)	14,160,043 (85.6%)	13,247,190 (80.1%)
Spo11-F260A sample 2	13,176,831	12,813,774 (97.2%)	11,453,354 (86.9%)	11,175,114 (84.8%)
Spo11 wild type sample 1	9,599,502	9,428,776 (98.2%)	8,236,687 (85.8%)	8,043,287 (83.8%)
Spo11 wild type sample 2	9,968,154	9,312,052 (93.4%)	8,274,465 (83%)	7,794,920 (78.2%)

^a Percent of sequences mapped relative to total number of reads.

^b Percent of sequences mapped uniquely relative to total number of mapped reads.

^c The fraction of sequences that could not be mapped likely reflect sequencing errors, adapter dimers, PCR dimers and bona fide Spo11-oligos derived from genomic regions that are unique to SK1 (i.e., not found in the S288C reference genome assembly).

^d Total number of reads that were filtered to remove reads with poor alignment and/or adapter clipping.

^e Total number of reads that mapped to only one position in the genome.