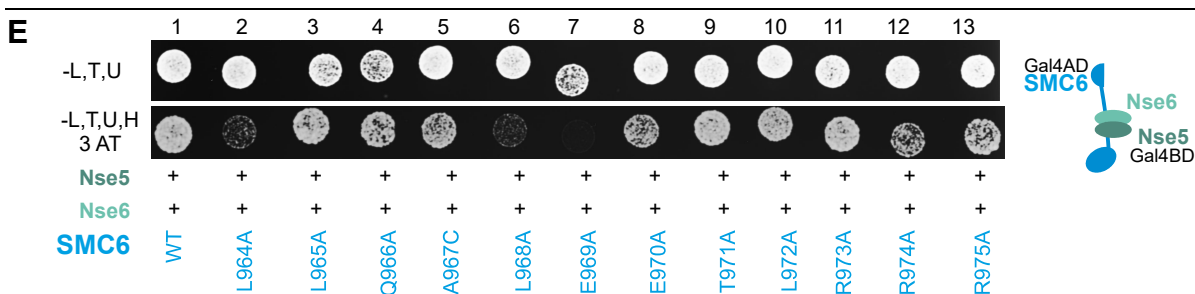
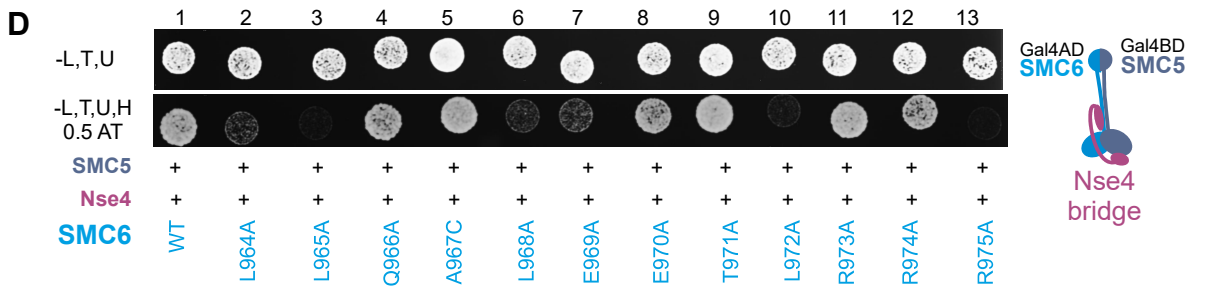
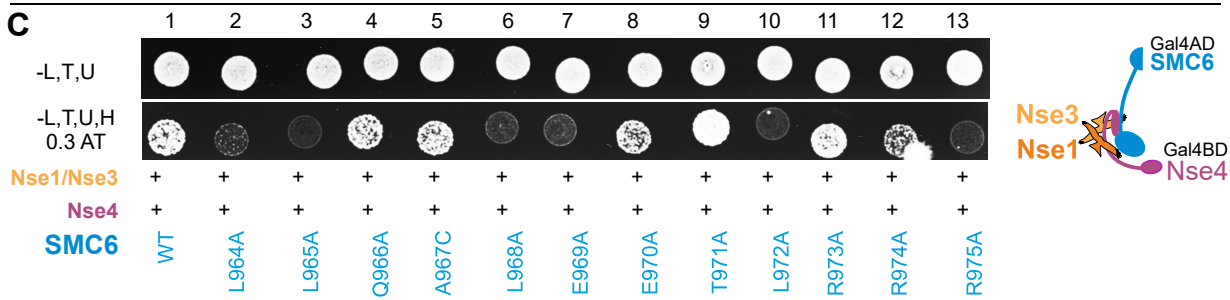


B

mutations

S.p.	955	KVLVARELTQLLQALEETLRRRNEMWTKFRKLIHLRTKELFELYLSQ	1000
A.n.	970	LKQVEEFGLLLEVLKASLNHRKERWRAFRSHISSRAKAQFTYLLSE	1015
A.c.	962	LKQIEEFRLADVLKATLKHRKHRWQIFRSHISSRAKAQFTYLLSE	1007
S.c.	940	QKKYMEIDEALNRLHNSLKARDQNYKNAEKGTQFDADMDFRASLKV	985
D.r.	911	SRQVKGLDFAFIHQLSKIMTTRHNVAEMRMYLSVRCKYNFHSMLSQ	956
X.l.	944	EGKVKHLKRFIKLLDEIMAQRYKSYQQFRRCLEFRCKIYFDSLISQ	989
O.a.	913	EGKVKNLKRFIKLLDEIMTQRYKTYQQFRRCLELRCKFYFDSLIAQ	958
L.a.	909	ENKVKTLKKFIKLLLEEIMTHRYKTYQQFRRCLELRCKLYFDNLLSQ	954
M.d.	1094	DSKVKSLKKFIKLLLEKIMAQRYSTYQQFRRCLELRCKLYFDNLLSQ	1139
D.n.	886	DNKVRTLKRFIKLLLEEIMTHRYKTYQQFRRCLELRCKLYFDNLLSQ	931
M.m.	912	DNKVRTLRRFIKLLLEEIMTHRYKTYQQFRRCLELRCKLYFDNLLSQ	957
H.s.	906	DSKVRTLKKFIKLLGEIMEHRFKTYQQFRRCLELRCKLYFDNLLSQ	951



A

spSMC5/K57I

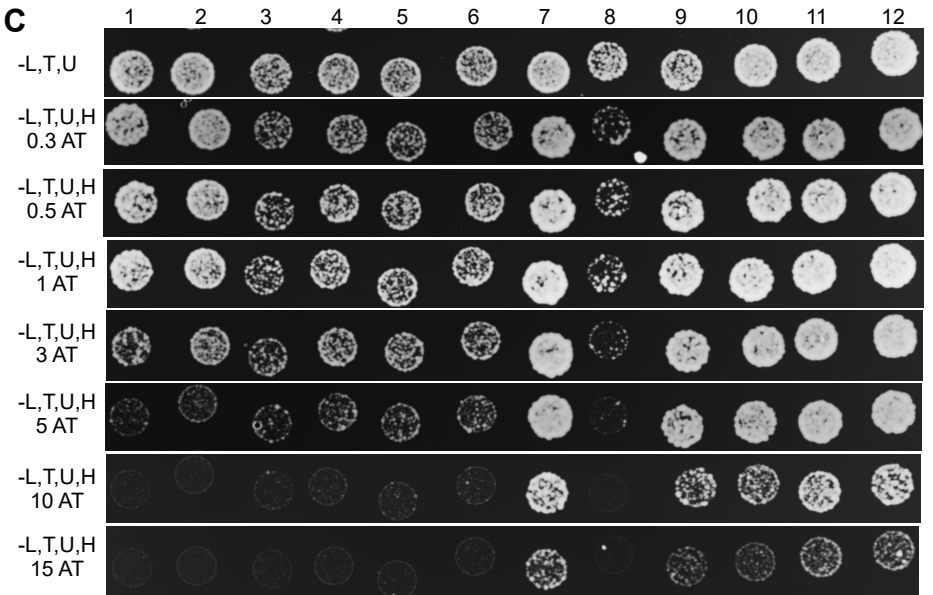
Smc	B.s.	27	TAVV	GGP	NGS	SGKS	NI	TDAI	45
Smc1	S.p.	28	TSI	IGP	NGAG	KSN	LM	DAI	46
Smc2	S.p.	28	NAIT	GLNG	SGKS	NI	LDAI	46	
Smc3	S.p.	28	NVIV	GRNG	SGKS	NF	FAAI	46	
Smc4	S.p.	151	SSI	VGP	NGS	SGKS	NV	IDAL	169
Smc5	S.p.	47	NLI	IGP	NGT	GKST	IV	SAI	65
Smc6	S.p.	120	NFVI	GHNG	SGKS	AI	LTGL	138	
Smc1	H.s.	28	TAI	IGP	NGS	KSN	LM	DAI	46
Smc2	H.s.	28	NAIT	GLNG	SGKS	NI	LDSI	46	
Smc3	H.s.	28	NVIV	GRNG	SGKS	NF	FYAI	46	
Smc4	H.s.	109	SCI	IGP	NGS	KSN	VIDSM	127	
Smc5	H.s.	76	NMIV	GANG	TGKS	SI	VCAI	94	
Smc6	H.s.	72	NFVV	GNNG	SGKS	AV	LTAL	90	

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B

			spSMC6/S1045R		spSMC5/E995Q																																
Smc	B.s.	1087	LL	SGGER	ALTA	IALL	FS	ILK	VRPV	PF	CVL	DE	VEA	ALDE	1125																						
Smc1	S.p.	1130	QL	SGG	EKTMA	ALAL	LF	AI	HSY	QPS	PF	FVL	DE	IDA	ALDQ	1168																					
Smc2	S.p.	1084	EL	SGG	QRS	LVAL	LIMS	LL	KYK	PAP	MY	IL	DE	IDA	ALDL	1122																					
Smc3	S.p.	1095	QL	SGG	QKSL	CALT	LI	F	AI	QR	CD	PAP	F	NI	LDE	CDANLDA	1133																				
Smc4	S.p.	1228	NL	SGG	EKTL	S	SL	AL	V	F	AL	H	NY	K	P	T	PL	Y	V	M	D	E	I	D	A	A	L	D	F	1266							
Smc5	S.p.	964	RQ	SGG	ERS	V	ST	I	M	Y	L	L	S	L	Q	G	L	A	I	A	P	F	R	I	V	D	E	I	N	Q	G	M	D	P	1002		
Smc6	S.p.	1042	GL	SGG	EKS	F	A	T	I	C	M	L	L	S	I	W	E	A	M	S	C	P	L	R	C	L	D	E	F	D	V	F	M	D	A	1080	
Smc1	H.s.	1126	NL	SGG	EKT	V	A	A	L	A	L	I	F	A	I	H	S	Y	K	P	A	P	P	F	F	V	L	D	E	I	D	A	A	L	D	N	1164
Smc2	H.s.	1083	EL	SGG	QRS	L	V	A	L	S	L	I	L	S	M	L	L	F	K	P	A	P	I	Y	I	L	D	E	V	D	A	A	L	D	L	1121	
Smc3	H.s.	1113	QL	SGG	QKSL	V	A	L	A	L	I	F	A	I	Q	K	C	D	P	A	P	F	Y	L	F	D	E	I	D	Q	A	L	D	A	1151		
Smc4	H.s.	1189	NL	SGG	EKTL	S	SL	AL	V	F	AL	H	H	Y	K	P	T	PL	Y	F	M	D	E	I	D	A	A	L	D	F	1227						
Smc5	H.s.	989	HQ	SGG	ERS	V	ST	M	L	Y	L	M	A	L	Q	E	L	N	R	C	P	F	R	V	D	E	I	N	Q	G	M	D	P	1027			
Smc6	H.s.	985	AL	SGG	ERS	F	S	T	V	C	F	I	L	S	L	W	S	I	A	E	S	P	F	R	C	L	D	E	F	D	V	Y	M	D	M	1023	

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SMC6	WT	WT	SR	SR	WT	WT	WT	WT	SR	SR	WT	WT
SMC5	WT	EQ	WT	EQ	KI	KI+EQ	WT	EQ	WT	EQ	KI	KI+EQ
Nse1	-	-	-	-	-	-	+	+	+	+	+	+
Nse3	-	-	-	-	-	-	+	+	+	+	+	+
Nse4	+	+	+	+	+	+	+	+	+	+	+	+



Supplementary Table 1: SMC6(aa875-1024) peptide library

aa	Smc6 peptide sequence
875-899	TNILREKEAKKVQCAQVVADYTAKA
880-904	EKEAKKVQCAQVVADYTAKANTRCE
885-909	KVQCAQVVADYTAKANTRCERVPVQ
890-914	QVVADYTAKANTRCERVPVQLSPAEL
895-919	YTAKANTRCERVPVQLSPAELDNEI
900-924	NTRCERVPVQLSPAELDNEIERLQM
905-929	RVPVQLSPAELDNEIERLQMQIAEW
910-934	LSPAELDNEIERLQMQIAEWRNRTG
915-939	LDNEIERLQMQIAEWRNRTGVSVEQ
920-944	ERLQMQIAEWRNRTGVSVEQAAEDY
925-949	QIAEWRNRTGVSVEQAAEDYLNAKE
930-954	RNRTGVSVEQAAEDYLNAKEKHDQA
935-959	VSVEQAAEDYLNAKEKHDQAKVLVA
940-964	AAEDYLNAKEKHDQAKVLVARLTQL
945-969	LNAKEKHDQAKVLVARLTQLLQALE
950-974	KHDQAKVLVARLTQLLQALEETLRR
955-979	KVLVARLTQLLQALEETLRRRNEMW
960-984	RLTQLLQALEETLRRRNEMWTKFRK
965-989	LQALEETLRRRNEMWTKFRKLITLR
970-994	ETLRRRNEMWTKFRKLITLRTKELF
975-999	RNEMWTKFRKLITLRTKELFELYLS
980-1004	TKFRKLITLRTKELFELYLSQRNFT
985-1009	LITLRTKELFELYLSQRNFTGKLVI
990-1014	TKELFELYLSQRNFTGKLVIKHQEE
995-1019	ELYLSQRNFTGKLVIKHQEEFLEPR
1000-1024	QRNFTGKLVIKHQEEFLEPRVYPAN

Supplementary Table 2: Primers used for PCR

SMC5 In-Fusion pGBKT7	oLV511	fw	CTGCATATGGCCATGGATGGCCTTAGGCCT
	oLV486	rev	GCCGCTGCAGGTCGACTTATGACGAAGAAATGAGTGC
Nse6 In-Fusion p416ADH1	EB77	fw	CCGCTCTAGAAGTAGTATGAATGCGTCTAATAACATTTCAA
	EB78	rev	ATGACTCGAGGTCGACTTATCTTTTGTACGCTTGCC
Nse4 In-Fusion pGADT7	oLV575	fw	CAGATTACGCTCATATGTCCTCCATTGATAAACG
	oLV576	rev	CGAGCTCGATGGATCCTCAGCCATACCAAGTATTACTGT
insertion into p416ADH1	KB353	fw	CTTTAATTTGCGGCCGGGAGCTCGCCGGGATC
	KB354	rev	CTATAGGGCGAATTGG
(G ₄ S) ₆ linker insertion	oLV579	fw	CATTACTACCGTCGAaGGTGGAGGAGGCTCT
	oLV580	rev	TATTTTCTTGGTCGACTGACCCTCCGCCT
3'end of Nse4	JP414	fw	CTCGAGATAACGCTTCGTAATTAAGAT
	JP415	rev	GTCGACCTTGCATAAATACTTAGTCC
Nse4 In-Fusion pGEM	oLV680	fw	TAGAACTAGTGGATCCATGTCCTCCATTGATAAACG
	oLV681	rev	GCTTGATATCGAATTCTCAGCCATACCAAGTATTACT

Supplementary Table 3: Primers used for site-directed mutagenesis

SalI mutation in MCS	oLV522	fw	TCAAGCTTATCGATACCGTgGACCTCGAGTCATGTAATTAGT
	oLV523	rev	ACTAATTACATGACTCGAGGTCcACGGTATCGATAAGCTTGA
SalI insertion into Nse4	oLV520	fw	CTGAATGAACGTAACATTACTACCgtcgacCAAGAAAATAACACCACTAAAAATG
	oLV521	rev	CATTTTTAGTGGTGTATTTTTCTTGtgcgacGGTAGTAATGTTACGTTTCATTTCAG
Nse1 Q18A M21A	JP891	fw	GACAAGCATAAATTCATTCTTgcATATATAgcGTGTCGCACAGCTGGTGTGG
	JP892	rev	CAACACCAGCTGTGCGACACgcTATATATgcAAGAATGAATTTATGCTTGTC
Nse4 L62C	oLV291	fw	GAAGCAACCTTAGATGCTTTACTGtgTACTAAAACGGTTGATCTGGCTTC
	oLV292	rev	GAAGCCAGATCAACCGTTTTAGTAcacAGTAAAGCATCTAAGGTTGCTTC
Nse4 T63R	oLV612	fw	AACCTTAGATGCTTTACTGCTTAgaAAAACGGTTGATCTGGCT
	oLV613	rev	AGCCAGATCAACCGTTTTtcTAAGCAGTAAAGCATCTAAGGTT
Nse4 K64C	oLV295	fw	GCAACCTTAGATGCTTTACTGCTTACTtgcACGGTTGATCTGGCTTCCA
	oLV296	rev	TGGAAGCCAGATCAACCGTgcaAGTAAGCAGTAAAGCATCTAAGGTTGC
Nse4 T65R	oLV614	fw	AGATGCTTTACTGCTTACTAAAAGGGTTGATCTGGCTTCCA
	oLV615	rev	TGGAAGCCAGATCAACCCTTTTAGTAAGCAGTAAAGCATCT
Nse4 V66C	oLV417	fw	GATGCTTTACTGCTTACTAAAACGtgTGATCTGGCTTCCATTAAAGC
	oLV418	rev	GCTTTAATGGAAGCCAGATCAcAcCGTTTTAGTAAGCAGTAAAGCATC
Nse4 D67C	oLV297	fw	ATGCTTTACTGCTTACTAAAACGGTTtgTCTGGCTTCCATTAAAGCTAGG
	oLV298	rev	CCTAGCTTTAATGGAAGCCAGAcAaAACCCTTTTAGTAAGCAGTAAAGCAT
Nse4 L68C	oLV377	fw	ACTGCTTACTAAAACGGTTGATtgcGCTTCCATTAAAGCTAGGCA
	oLV378	rev	TGCCTAGCTTTAATGGAAGCgcaATCAACCGTTTTAGTAAGCAGT
Nse4 L62C T65R	oLV676	fw	AGCAACCTTAGATGCTTTACTGtgTACTAAAAGGGTTGATCTGGCTTCCA
	oLV677	rev	TGGAAGCCAGATCAACCCTTTTAGTAcacAGTAAAGCATCTAAGGTTGCT
Nse4 del187-91	oLV654	fw	TTGGAAGGCCCAAGTTTAATATTGAAATTAAGCAATTCCTCAACTATCC
	oLV655	rev	GGATAGTTGAGGAATTGCTTAATTTCAATATTAAACTTGGGCCTTCCAA
Smc5 E995Q	oLV494	fw	GCTCCGTTTCGAATAGTTGATcAAATAAATCAAGGAATGGATCCTC
	oLV495	rev	GAGGATCCATTCCCTTGATTTATTTgATCAACTATTCGAAACGGAGC
Smc5 K57I	oLV674	fw	TTTGATTATCGGTCCAAATGGGACAGGTAtaAGCACAATTGTTTCAG
	oLV675	rev	CTGAAACAATTGTGCTtaTACCTGTCCATTTGGACCGATAATCAAA

Smc6 L964A	oLV179	fw	GCTAGACTCACGCAAgcATTGCAAGCTTTAGAAGA
	oLV180	rev	TCTTCTAAAGCTTGCAATgcTTGCGTGAGTCTAGC
Smc6 L965A	oLV550	fw	TGCTAGACTCACGCAACTAgcGCAAGCTTTAGAAGAGACGT
	oLV551	rev	ACGTCTCTTCTAAAGCTTGCgcTAGTTGCGTGAGTCTAGCA
Smc6 Q966A	oLV552	fw	CTAGACTCACGCAACTATTGgcAGCTTTAGAAGAGACGTTAC
	oLV553	rev	GTAACGTCTCTTCTAAAGCTgcCAATAGTTGCGTGAGTCTAG
Smc6 A967C	oLV315	fw	AGACTCACGCAACTATTGCAAtgTTTAGAAGAGACGTTACGAAGGC
	oLV316	rev	GCCTTCGTAACGTCTCTTCTAAAcATTGCAATAGTTGCGTGAGTCT
Smc6 L968A	oLV183	fw	GCAACTATTGCAAGCTgcAGAAGAGACGTTACGAAG
	oLV184	rev	CTTCGTAACGTCTCTTCTgcAGCTTGCAATAGTTGC
Smc6 E969A	oLV554	fw	ACGCAACTATTGCAAGCTTTAgcAGAGACGTTACGAAGGCGT
	oLV555	rev	ACGCCTTCGTAACGTCTCTgcTAAAGCTTGCAATAGTTGCGT
Smc6 E970A	oLV187	fw	CTATTGCAAGCTTTAGAAGcGACGTTACGAAGGCGT
	oLV188	rev	ACGCCTTCGTAACGTCgCTTCTAAAGCTTGCAATAG
Smc6 T971A	oLV556	fw	ACTATTGCAAGCTTTAGAAGAgCGTTACGAAGGCGTAATG
	oLV557	rev	CATTACGCCTTCGTAACGcCTCTTCTAAAGCTTGCAATAGT
Smc6 L972A	oLV558	fw	TGCAAGCTTTAGAAGAGACGgcACGAAGGCGTAATGAAATGT
	oLV559	rev	ACATTTTCATTACGCCTTCGTgcCGTCTCTTCTAAAGCTTGCA
Smc6 R973A	oLV189	fw	GCTTTAGAAGAGACGTTAgcAAGGCGTAATGAAATGTG
	oLV190	rev	CACATTTTCATTACGCCTTgcTAACGTCTCTTCTAAAGC
Smc6 R974A	oLV191	fw	CTTTAGAAGAGACGTTACGAgcGCGTAATGAAATGTGGAC
	oLV192	rev	GTCCACATTTTCATTACGCgcTCGTAACGTCTCTTCTAAAG
Smc6 R975A	oLV193	fw	GAAGAGACGTTACGAAGGgcTAATGAAATGTGGACCAAATTC
	oLV194	rev	GAAATTTGGTCCACATTTTCATTAgcCCTTCGTAACGTCTCTTC
Smc6 S1045R	oLV498	fw	AAGTCAGCGTTCAAGGATTAcgAGGGGGTGAAAAATCTTTTG
	oLV499	rev	CAAAAGATTTTTTACCCCCTcgTAATCCTTGAACGCTGACTT