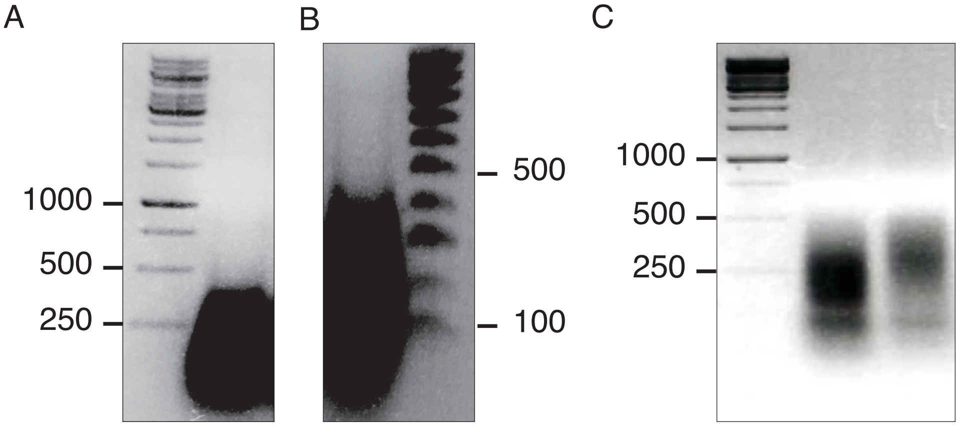
Supplementary data

Supplementary data 1: Yeast strains used in this study.

Strain	Genotype	Source
W303	*MATa ura3-1 trp1-1 leu2-3,112 his3-11,15 ade2-1 can1-100 ybp1-1 rad5-535*	R. Rothstein
KW20	W303a Pif1-Myc13*::TRP1*	(1)
MBY49	*MATα ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt13::URA3*	(2)
SG64	*MATα ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt13::URA3 prb1::*G4(ChrI)-*LEU2*	This study
KW95	W303a Mre11-Myc13*::TRP1*	This study
KW109	W303a Mms1-Myc13*::TRP1*	This study
KW110	*MATα ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt13::URA3 mms1::TRP1*	This study
KW111	*MATα ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt13::URA3 prb1::*G4(ChrI)-*LEU2 mms1::TRP1*	This study
KW136	W303a Mms1-Myc13*::TRP1 pif1-m2-HIS3*	This study
KW155	W303a *mms1::TRP1*	This study
KW166	W303a Mms1-Myc13*::TRP1 rtt101::KANMX4*	This study
KW168	W303a Mms1-Myc13*::TRP1 mms22::KANMX4*	This study
KW178	W303a Pol2-Myc13*::TRP1*	This study
KW190	W303a Pol2-Myc13*::TRP1 mms1::HIS3*	This study
KW200	*MATα ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt13::URA3 prb1::*NG(ChrVIII)-*LEU2*	This study
KW203	*MATα ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt13::URA3 prb1::*GR(ChrI)-*LEU2*	This study
KW208	*MATα ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt13::URA3 prb1::*NG(ChrVIII)-*LEU2 mms1::TRP1*	This study
KW220	*MATα ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt13::URA3 prb1::*GR(ChrI)-*LEU2 mms1::TRP1*	This study
KW231	W303a Mms1-Myc13*::TRP1* Pol2-HA*::HIS3 bar1::KANMX6*	This study
KW232	W303a Pif1-Myc13*::TRP1 mms1::HIS3*	This study
KW240	W303a Mre11-Myc13*::TRP1 mms1::HIS3*	This study
KW256	*MATα ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt13::URA3 prb1::LEU2*	This study
KW261	*MATα ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt13::URA3 prb1::LEU2 mms1::HIS3*	This study
KP808	W303a Pol2-Myc13*::TRP1 rtt1o1::URA3*	This study
KP809	W303a Mms1-Myc13*::TRP1 rtt101::KANMX4 mms22 HIS3*	This study
KP810	W303a Pol2-Myc13*::TRP1 rtt101:URA mms22::HIS3*	This study
KP811	W303a Mms1-Myc13*::TRP1, G4 Chr IV mut-LoxP*	This study
KP812	W303a Pol2-Myc13*::TRP1, G4 Chr IV mut-LoxP*	This study
KP813	W303a Pol2-Myc13*::TRP1 mms1::HIS3, G4 Chr IV mut-LoxP*	This study

Supplementary data 2: DNA fragment sizes before ChIPseq and conventional ChIP. (A,B) DNA fragment sizes after sonication of the Myc-tagged Mms1 (A, lane 2) and untagged (B, lane 1) strain used for ChIPseq analysis and DNA ladder (lane 1 and lane 2 respectively). (C) DNA fragment sizes after sonication of the Myc-tagged Mms1 (lane 2) and untagged (lane 3) strain used for conventional ChIP analysis and DNA ladder (lane 1). Each DNA was separated on a 2% agarose gel.



Supplementary data 3: Primers used in this study for qPCR.

Region	(G4$_{\text{tract3}}$) motif (3)	(G4$_{\text{tract2}}$) motif on leading strand	(G4$_{\text{tract2}}$) motif on lagging strand	Peak-call	Sequence (5´-3´)	Source
ChrI$_{\text{NC}}$ fw	+	+	-	-	TCGTATACATGCGGAGTAG	This study
ChrI$_{\text{NC}}$ rev	+	+	-	-	GTTACCACAGAATTGAACTG	This study
ChrIV$_{\text{ARO1}}$ fw					TCGTTACAAGGTGATG	(4)
ChrIV$_{\text{ARO1}}$ rev					AATAGCGGCAACAAC	(4)
ChrVI$_{\text{BR}}$ fw	+	+	+	-	TGCATAGTTCTTAGGTCTTC	This study
ChrVI$_{\text{BR}}$ rev	+	+	+	-	GTATAGCAGTGACGCGTG	This study
ChrVI$_{\text{Tel-VI-R}}$ fw					ATCATTGAGGATCTATAATC	(1)
ChrVI$_{\text{Tel-VI-R}}$ rev					CTTCACTCCATTGCG	(1)
BM VII$_{\text{BR}}$ fw	-	+	+	+	AGTCTAATCTAACTGGTCTG	This study
BM VII$_{\text{BR}}$ rev	-	+	+	+	GCCAAGAAGGCTCTAGAC	This study
ChrIX$_{\text{BR}}$ fw	+	+	+	-	AGAGTCTTTGGCACTGTTG	This study
ChrIX$_{\text{BR}}$ rev	+	+	+	-	ATTATCCCTTAATGGCCTAC	This study
ChrIX$_{\text{tRNA}}$ fw					GGAAAGATTGTACGGGAAATGG	(5)
ChrIX$_{\text{tRNA}}$ rev					GCTAATGAAACTACTAATGTCTTGC	(5)
ChrX$_{\text{BR}}$ fw	+	+	+	+	CACAAACACATAAACACATAC	This study
ChrX$_{\text{BR}}$ rev	+	+	+	+	CGGATTTCGCATAGTTGTC	This study
ChrXIa$_{\text{BR}}$ fw	-	+	+	-	GGCAACGATAGAACCAATTC	(1)
ChrXIa$_{\text{BR}}$ rev	-	+	+	-	GCAACCATTATACCATCTCC	(1)
ChrXIb$_{\text{BR}}$ fw	-	-	+	-	ACTAGGTCTCTTAGCTCTC	This study
ChrXIb$_{\text{BR}}$ rev	-	-	+	-	TTTTGAACACGTTCTACGAG	This study
ChrXIc$_{\text{BR}}$ fw	+	+	+	+	CAGTATGAAATTATCCGCTC	This study
ChrXIc$_{\text{BR}}$ rev	+	+	+	+	CACTATGGTGGACAGCTG	This study
ChrXII$_{\text{rDNA\_RFB}}$ fw					AAGATGGGTTGAAAGAGAAGGG	(1)
ChrXII$_{\text{rDNA\_RFB}}$ rev					TCATATCAAAGGCATGTCCTGT	(1)
ChrXIII$_{\text{BR}}$ fw	-	+	+	-	CCAAACCAGACCAACCATTG	(1)
ChrXIII$_{\text{BR}}$ rev	-	+	+	-	TGCTGACCACAACGAACC	(1)
ChrXIII$_{\text{NC}}$ fw	+	+	-	-	GCTTCAGCCTGGGGTAAC	This study
ChrXIII$_{\text{NC}}$ rev	+	+	-	-	GGCACCATTAGATTCACCAC	This study
ChrXIV$_{\text{NC}}$ fw	-	-	-	-	AGTGATTGTGCCGTTATAAC	This study
ChrXIV$_{\text{NC}}$ rev	-	-	-	-	CGGTTCGCACTACGATAC	This study
ChrXV$_{\text{BR}}$ fw	+	+	+	-	ATACGCAGTATGGTGATATC	This study
ChrXV$_{\text{BR}}$ rev	+	+	+	-	GTTTATTGCCGATATACCTC	This study

Supplementary data 4: Binding regions of Mms1 identified by MACS2 ‘peakcall’ command.

Peaks from MACS 2.0									
Chr	Start	End	Length	Summit	pileup	LOG10 (p value)	fold_enrichment	LOG10 (p value)	name
ChrI	112580	112785	205	112690	33.00	625.095	247.853	327.508	peakcall_mms1_default_extsize180_peak_1
ChrI	130081	130284	203	130242	40.00	527.558	208.622	249.880	peakcall_mms1_default_extsize180_peak_2
ChrI	191965	192177	212	192031	40.00	809.461	266.000	481.361	peakcall_mms1_default_extsize180_peak_3
ChrII	193642	193822	180	193769	31.00	645.572	260.302	344.464	peakcall_mms1_default_extsize180_peak_4
ChrII	215788	215983	195	215843	34.00	685.749	258.984	377.494	peakcall_mms1_default_extsize180_peak_5
ChrII	463749	464101	352	464057	28.00	749.299	303.780	429.304	peakcall_mms1_default_extsize180_peak_6
ChrIII	123534	123722	188	123612	47.00	997.933	280.550	642.703	peakcall_mms1_default_extsize180_peak_7
ChrIV	427403	427648	245	427568	41.00	1.393.092	395.679	984.699	peakcall_mms1_default_extsize180_peak_8
ChrIV	461797	462449	652	462293	77.00	1.555.914	285.887	1.121.153	peakcall_mms1_default_extsize180_peak_9
ChrIV	1049062	1049304	242	1049164	27.00	488.485	232.904	219.689	peakcall_mms1_default_extsize180_peak_10
ChrIV	1239630	1239824	194	1239696	44.00	927.112	276.729	582.947	peakcall_mms1_default_extsize180_peak_11
ChrIV	1251076	1251389	313	1251202	45.00	1.238.366	336.579	853.083	peakcall_mms1_default_extsize180_peak_12
ChrV	40469	41119	650	40735	38.00	1.001.155	317.243	645.062	peakcall_mms1_default_extsize180_peak_13
ChrV	42006	42474	468	42233	42.00	1.040.526	307.755	680.290	peakcall_mms1_default_extsize180_peak_14
ChrV	43222	43945	723	43695	72.00	1.981.364	356.086	1.493.835	peakcall_mms1_default_extsize180_peak_15
ChrV	312024	312220	196	312084	44.00	625.972	219.505	328.202	peakcall_mms1_default_extsize180_peak_16
ChrV	335544	335734	190	335617	40.00	405.728	184.715	160.186	peakcall_mms1_default_extsize180_peak_17
ChrV	396417	396611	194	396505	34.00	816.523	291.130	487.064	peakcall_mms1_default_extsize180_peak_18
ChrV	442043	442465	422	442270	75.00	1.264.826	254.802	876.649	peakcall_mms1_default_extsize180_peak_19
ChrV	574897	575199	302	575071	60.00	1.073.506	258.619	708.690	peakcall_mms1_default_extsize180_peak_20

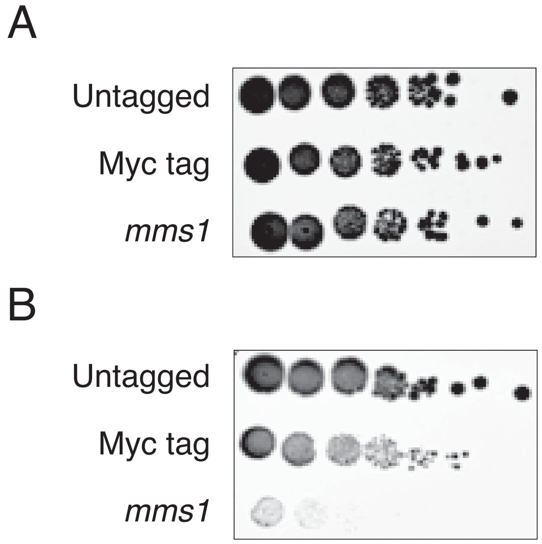
ChrVI	224946	225231	285	225020	44.00	816.422	255.176	487.064	peakcall_mms1_default_extsize180_peak_21
ChrVII	567184	567405	221	567287	38.00	718.636	253.025	403.215	peakcall_mms1_default_extsize180_peak_22
ChrVII	700602	700830	228	700704	50.00	1.771.911	424.219	1.300.422	peakcall_mms1_default_extsize180_peak_23
ChrVII	794325	794519	194	794426	38.00	1.242.680	377.673	856.834	peakcall_mms1_default_extsize180_peak_24
ChrVII	806449	806663	214	806581	36.00	1.082.030	348.574	716.266	peakcall_mms1_default_extsize180_peak_25
ChrVII	1049534	1049759	225	1049724	41.00	787.711	258.281	461.973	peakcall_mms1_default_extsize180_peak_26
ChrVII	1088958	1089156	198	1089058	56.00	883.253	238.573	545.299	peakcall_mms1_default_extsize180_peak_27
ChrVIII	189407	189652	245	189581	38.00	502.145	207.392	229.984	peakcall_mms1_default_extsize180_peak_28
ChrVIII	215840	216046	206	215971	47.00	799.402	244.240	471.951	peakcall_mms1_default_extsize180_peak_29
ChrIX	54150	54474	324	54323	38.00	657.242	239.832	353.795	peakcall_mms1_default_extsize180_peak_30
ChrIX	334127	334307	180	334294	31.00	484.923	219.695	217.488	peakcall_mms1_default_extsize180_peak_31
ChrIX	392157	392481	324	392309	26.00	537.260	250.760	257.480	peakcall_mms1_default_extsize180_peak_32
ChrX	121000	121236	236	121088	43.00	613.254	219.159	318.162	peakcall_mms1_default_extsize180_peak_33
ChrX	391940	392163	223	392038	45.00	663.878	224.383	359.497	peakcall_mms1_default_extsize180_peak_34
ChrX	400013	400260	247	400116	35.00	798.738	282.325	471.355	peakcall_mms1_default_extsize180_peak_35
ChrXI	99803	99985	182	99895	47.00	1.158.282	311.415	782.455	peakcall_mms1_default_extsize180_peak_36
ChrXI	380958	381322	364	381207	31.00	711.099	277.528	397.450	peakcall_mms1_default_extsize180_peak_37
ChrXI	519605	519853	248	519720	50.00	1.436.254	353.430	1.020.517	peakcall_mms1_default_extsize180_peak_38
ChrXII	84188	84385	197	84328	35.00	787.982	279.721	461.973	peakcall_mms1_default_extsize180_peak_39
ChrXII	369706	369916	210	369834	55.00	1.022.171	262.313	664.327	peakcall_mms1_default_extsize180_peak_40
ChrXII	451470	451833	363	451826	350.00	425.587	123.512	173.564	peakcall_mms1_default_extsize180_peak_41
ChrXII	452031	452232	201	452047	354.00	365.961	120.950	132.597	peakcall_mms1_default_extsize180_peak_42
ChrXII	455225	455521	296	455258	357.00	393.367	121.972	151.503	peakcall_mms1_

									default_extsize180_peak_43
ChrXII	455721	456041	320	455824	358.00	528.897	127.085	251.040	peakcall_mms1_default_extsize180_peak_44
ChrXII	458652	459006	354	458788	352.00	427.775	123.523	175.395	peakcall_mms1_default_extsize180_peak_45
ChrXII	459110	459290	180	459285	355.00	374.987	121.291	138.398	peakcall_mms1_default_extsize180_peak_46
ChrXII	464853	465115	262	464975	355.00	515.768	126.738	240.564	peakcall_mms1_default_extsize180_peak_47
ChrXII	467534	468132	598	467560	354.00	373.050	121.244	137.317	peakcall_mms1_default_extsize180_peak_48
ChrXII	704511	704746	235	704580	40.00	682.404	239.637	374.708	peakcall_mms1_default_extsize180_peak_49
ChrXII	904035	904280	245	904110	32.00	472.551	214.098	208.308	peakcall_mms1_default_extsize180_peak_50
ChrXII	921240	921442	202	921330	39.00	959.489	302.822	609.679	peakcall_mms1_default_extsize180_peak_51
ChrXII	1074796	1075077	281	1074850	52.00	591.819	200.487	300.473	peakcall_mms1_default_extsize180_peak_52
ChrXIII	1731	1917	186	1804	47.00	630.240	214.612	331.825	peakcall_mms1_default_extsize180_peak_53
ChrXIII	16258	16578	320	16428	55.00	1.609.468	363.317	1.165.234	peakcall_mms1_default_extsize180_peak_54
ChrXIV	494007	494211	204	494082	30.00	498.671	225.984	227.759	peakcall_mms1_default_extsize180_peak_55
ChrXIV	547086	547297	211	547204	42.00	767.811	251.326	444.554	peakcall_mms1_default_extsize180_peak_56
ChrXIV	619048	619366	318	619157	37.00	561.935	222.102	276.947	peakcall_mms1_default_extsize180_peak_57
ChrXIV	631800	631983	183	631871	42.00	600.525	218.798	307.301	peakcall_mms1_default_extsize180_peak_58
ChrXIV	652075	652255	180	652157	34.00	669.706	255.143	363.903	peakcall_mms1_default_extsize180_peak_59
ChrXV	31003	31286	283	31151	41.00	400.039	182.258	155.643	peakcall_mms1_default_extsize180_peak_60
ChrXV	159994	160358	364	160118	63.00	943.633	234.574	596.621	peakcall_mms1_default_extsize180_peak_61
ChrXV	480108	480435	327	480246	39.00	597.875	224.877	305.507	peakcall_mms1_default_extsize180_peak_62
ChrXV	588162	588351	189	588225	31.00	600.297	248.641	307.168	peakcall_mms1_default_extsize180_peak_63
ChrXV	710139	710326	187	710244	45.00	1.570.881	411.661	1.133.412	peakcall_mms1_default_extsize180_peak_64
ChrXV	855669	855950	281	855717	20.00	418.960	239.090	169.052	peakcall_mms1_default_extsize1

									80_peak_65
ChrXV	1060686	1061023	337	1060915	40.00	1.470.885	423.391	1.049.152	peakcall_mms1_default_extsize180_peak_66
ChrXV	1091099	1091289	190	1091222	47.00	742.374	234.139	423.706	peakcall_mms1_default_extsize180_peak_67
ChrXVI	338813	339003	190	338904	54.00	752.677	222.313	432.364	peakcall_mms1_default_extsize180_peak_68
ChrXVI	536586	536770	184	536716	34.00	1.409.377	460.031	999.169	peakcall_mms1_default_extsize180_peak_69
ChrXVI	922313	922518	205	922398	30.00	675.542	272.462	368.757	peakcall_mms1_default_extsize180_peak_70
ChrXVI	945943	946241	298	945944	68.00	373.205	155.965	137.412	peakcall_mms1_default_extsize180_peak_71
ChrM	2467	2658	191	2619	40.00	1.144.746	341.038	769.971	peakcall_mms1_default_extsize180_peak_72
ChrM	6033	6275	242	6176	55.00	1.609.468	363.317	1.165.234	peakcall_mms1_default_extsize180_peak_73
ChrM	15085	15328	243	15173	20.00	590.802	302.739	299.602	peakcall_mms1_default_extsize180_peak_74
ChrM	18607	18863	256	18768	28.00	910.884	354.172	568.783	peakcall_mms1_default_extsize180_peak_75
ChrM	19247	19506	259	19354	20.00	577.220	297.502	288.352	peakcall_mms1_default_extsize180_peak_76
ChrM	24794	24974	180	24909	24.00	480.983	242.098	214.714	peakcall_mms1_default_extsize180_peak_77
ChrM	28759	29077	318	28904	17.00	982.868	522.979	630.414	peakcall_mms1_default_extsize180_peak_78
ChrM	36683	36965	282	36858	13.00	988.256	609.433	634.195	peakcall_mms1_default_extsize180_peak_79
ChrM	37939	38414	475	38264	19.00	1.601.831	831.931	1.158.768	peakcall_mms1_default_extsize180_peak_80
ChrM	40344	40537	193	40405	35.00	1.058.635	348.621	695.164	peakcall_mms1_default_extsize180_peak_81
ChrM	40814	41221	407	40936	21.00	1.318.506	620.844	921.853	peakcall_mms1_default_extsize180_peak_82
ChrM	41557	42373	816	41825	19.00	1.382.073	702.586	975.087	peakcall_mms1_default_extsize180_peak_83
ChrM	43257	43661	404	43513	26.00	841.561	346.917	508.972	peakcall_mms1_default_extsize180_peak_84
ChrM	44087	44272	185	44225	18.00	690.562	362.646	381.355	peakcall_mms1_default_extsize180_peak_85
ChrM	50659	50926	267	50781	50.00	2.070.018	493.880	1.566.621	peakcall_mms1_default_extsize180_peak_86
ChrM	53591	53813	222	53693	66.00	610.028	187.350	315.245	peakcall_mms1_default_extsize180_peak_87

ChrM	68517	68770	253	68639	30.00	775.677	300.202	451.627	peakcall_mms1_default_extsize180_peak_88
ChrM	83902	84362	460	84229	49.00	1.705.155	415.901	1.242.801	peakcall_mms1_default_extsize180_peak_89
ChrM	84630	84909	279	84761	26.00	1.287.038	515.339	894.333	peakcall_mms1_default_extsize180_peak_90
ChrM	85490	85769	279	85636	44.00	1.176.328	328.041	798.103	peakcall_mms1_default_extsize180_peak_91

Supplementary data 5: Yeast strains with Myc-tagged Mms1 grow slower than untagged tag wild type cells on MMS-containing media. Serial dilutions of no Myc tag wild type, Myc-tagged Mms1, and *mms1* cells were spotted on YPD (A) and 0.01% MMS (B) containing media. Cells were diluted at OD 0.8 six times 10-fold. n=1.



Supplementary data 6: Characteristics of G4 motifs within qPCR regions. Strand location and distance to next ARS were used to elucidate if the G4 motif is replicated on the leading or lagging strand.

Region (Chr)	Location region		Location G4		Strand location	Sequence G4 (5´-3´)	Location next ARS	
	Start	End	Start	End			Start	End
I_{NC}	61257	61473	60974	61018	W	GGGCAGCATCTCCGTTGGATTGTTGTGCATGGCCAGTGTCTTGG	70300	70469
			61437	61493	W	GGTGAACGAGTGGGGACAGTTCAATTCTGTGGTAACAAGGCCACAATTGGTGGTGG		
			61618	61662	W	GGGTTCACGATGTCTAGGTTGAATAGCGAGGGTCGCCCCGTGGG		
			61683	61712	W	GGGTGGGAACGGCGACGGAACCGCGCCGG		
			61836	61864	W	GGAGGCAGGCTGGGCTTTTTTCGACGGG		
			61703	61753	C	CCGCGCCGGTTAATAACGATCCTAACTATTGTGGGCCATGTTACGGTGCC		
VI_{BR}	255397	255624	255328	255355	W	GGTCCTAAGGTACCAAAATCCGGGGGG	256277	256431
			255498	255548	W	GGGGCACACGTGCGGGAGTTTCAAAGGGGCAGAATAGTGGGGTTCAGGGG		
			255819	255849	W	GGTAAGACCAGGTGCAAGGAGAATACTGGG		
			254976	255047	C	CCATCAATTCCTTGGGCACATCAGCCATGGAACCCTTTCTAGCCTGTGGTTTCTTTGGACCTAAATGAACC		
			255319	255349	C	CCTCTCAACGGTCCTAAGGTACCAAAATCC		
			255710	255757	C	CCGGTTTATTTCCAACCGGGAAATAAATTATTCCTAATAAAATTTCC		
VII_{BR}	806633	806696	806736	806773	W	GGTTGTTCAGTTTCTGGATGTGTTGGGATACGGACGG	834491	834734
			806484	806606	C	CCAGAACCAGATGGTCTGAAACCGCCACGGCCACCGCGAGCGCCACCACGACCACCGAATCCACGGTTACCGCCACGACCACCGCCACGACCACCAAAACCACGGCTACCGCCACGACCACC		
			807069	807109	C	CCAATGACACCACCGATGTGTTCGAATTCCTTCTTCAACC		
IX_{BR}	356233	356408	356335	356403	W	GGAGACTGATTTGGAGGGTACGGTGGGTAATAAGGGAAGGTATCGGGATTGGGGTAGGCCATTAAGGG	357160	357396
			356572	356600	C	CCGTAGCCTTTTGGTGTTCCCGTATTCC		
			356762	356806	C	CCTGTGGAGTGCCCTCGATAGATAGTTTACCCACAAGTTCATCC		
X_{BR}	391792	391919	391294	391323	W	GGTTTCGGGCCAGATTCATGGCCCTGTGG	375706	376227
			391333	391368	C	CCTACAGACAAAAAACCGTTACGTCCCGCCTCACC		
			391531	391557	C	CCGCGCAAGCCAGATCCAAGCACGCC		
			391854	391877	C	CCACTATTCAGCGCCGTCCGCCC		
			391916	391968	C	CCGGAGCAACGGGCAACCGTTTGGGGAAAGACCACACCCACGCGCGATCGCC		
			391989	392008	C	CCCCACACCCAGACCTCCC		
			392394	392448	C	CCAATTACCATGCCTAAAGAGACCCCTTCCAAAGCTGCTGCCGATGCATTGTCC		
XIa_{BR}	142007	142159	141891	141942	W	GGTAAAAAGTATCCTGGTCACCGATGGCCAAGTTACCTTCTGGGGTGATGG	153020	153135
			141974	142008	W	GGTGGAGGACCATCGAATTGGAATTGTCTGTTGG		

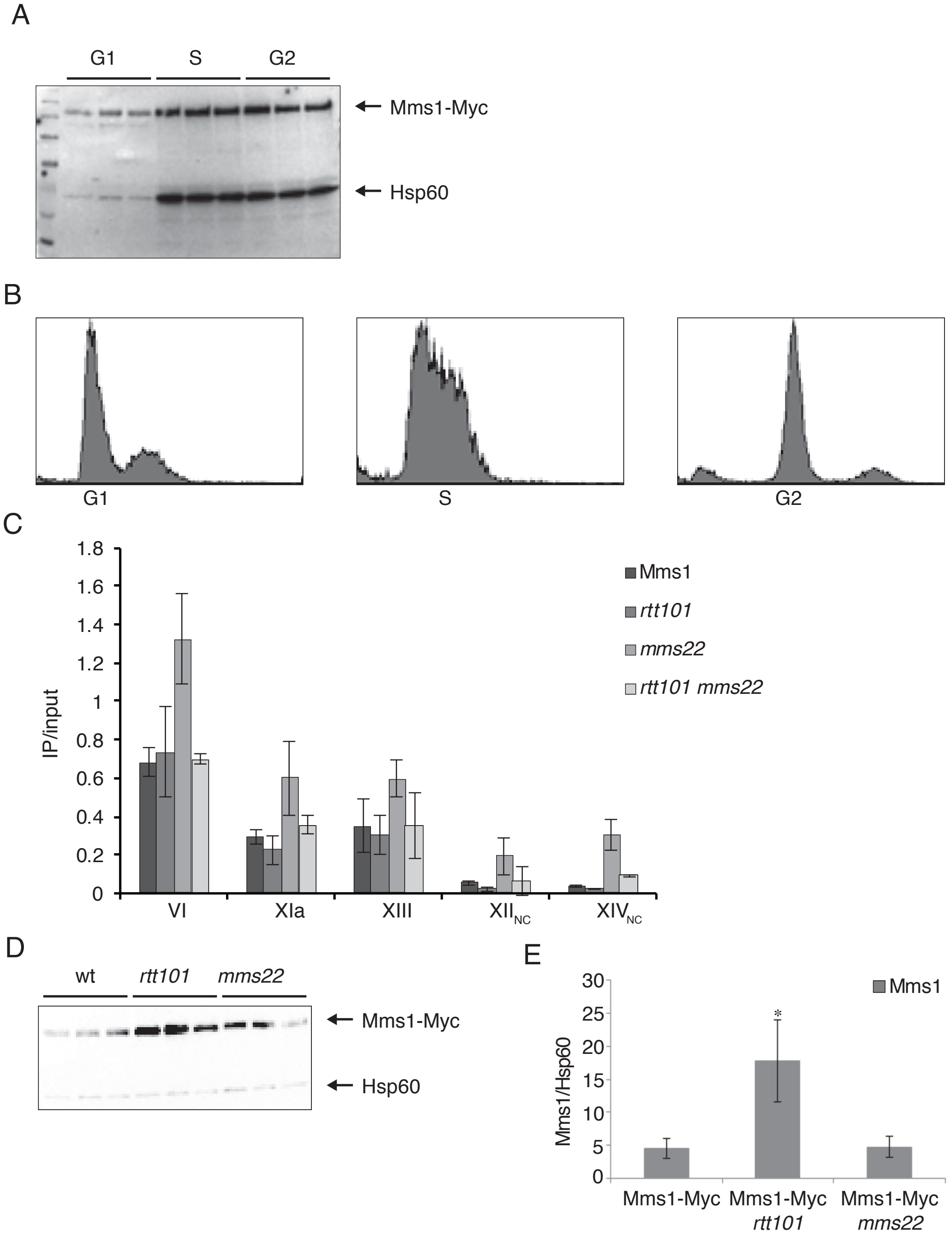
			142127	142155	W	GGAGCTGGAGCTGGAGATGGTATAATGG		
			142174	142238	W	GGCTTGGATTTGGCCATCAGTGATTTGGGAGACTGGAGCAACAGTAGTATTGGTGGTGGCTTGG		
			142428	142487	W	GGGAGACGGCTGCAGCGGTAGTCTTAGCTGAGGTAGTCTTGGTGGTGGCTTGGATTTGG		
			142576	142595	W	GGTTTTGGTAGTGGCTTGG		
			142627	142666	W	GGCTCTCTTAGCCTTGGAGGAAGCAGTAGTGGCAATTGG		
			141903	141949	C	CCTGGTCACCGATGGCCAAGTTACCTTCTGGGGTGATGGACCAACC		
			142019	142055	C	CCAATTCTACCTTTACCGTCAGTCAGGATACCACCC		
XIb$_{BR}$	503681	503933	503309	503359	W	GGAGAATCGAATCCTGGCATTAGTGGGATAAGAATAAAGGCTTTCCAAGG	517017	517265
			503654	503686	W	GGCGGGGTTAGTAATGGAGTTAACCTACTAGG		
			503529	503560	C	CCGTTTCCTTTAGCCCAAGAGACCAGTTCCC		
			504100	504138	C	CCAAAGACTATCTGTCCCAACAATATTCCCCACATTCC		
			504279	504335	C	CCTTTGCCATTAAGAGGGCCTCGCTTAACGACCAAAAATAATCACGCCCATCTACC		
XIc$_{BR}$	519523	519599	519028	519058	W	GGTGCCTGGATCTCGAGGCCGCGGCACTGG	517017	517265
			519142	519185	W	GGGCAAGTAGGTCTTTCTGCACGGCCCGGCCCGGGTCGTGCGG		
			519699	519719	W	GGCGGCTGCTGATGGAAAGG		
			519889	519957	W	GGACAAGGTGCTGTCTCCTTGGACTGGTTAGGTCTAGGCGGCTGGGCTTCCATCATGGACATGAACGG		
			519018	519099	C	CCTAGTTCCTGGTGCCTGGATCTCGAGGCCGCGGCACTGGAAAAGCCCTTTCTTTTCCAGATCGGGAAACCTAATGAGTCC		
			519264	519304	C	CCATCTAATGTGTTTCCTTCTCGAGACCTCGGCGTCTCCC		
			519557	519637	C	CCTCCACCGCCGTCTTGGCCGCTCCAGCTGTCCACCATAGTGACAACCACCACCACAACGACAAGCGTGCCGTTGTCACC		
			519676	519697	C	CCAGCTGCCACCACCGCTACC		
			519737	519832	C	CCACCACTACTTTGTCCTCGACTGCCGCCGCCGCTACTACCTCTGCCGCCGCCTCTTCTTCCTCCTCTTCCTCTTCCTCCTCTTCCTCTTCTTCC		
			519863	519889	C	CCATTTCCTGTTCTGATTTCCCATCC		
			520009	520063	C	CCAGGTTACGCTAAGACCCAATGGCCTTCTGAACAACCTTCCGATGGTAGATCC		
XIII$_{NC}$	250596	250670	250258	250297	W	GGTTAATAGACGTGGTAGAGTTCCACAGGCCAGAATGGG	263063	263296
			250327	250417	W	GGCCACTGGCGGTATTGCAGCAGGTGCTGCGGCTACCTCTTCTGGTCTTAGCGGTGGTATGACACCAGGATGGAGCTCCTTCGATGGTGG		
			250442	250732	W	GGAGGCTCAGGTGGTGGCGGTGTCTCCTCATGGGGTGGTGCTTCCACTTGGGGTGGCCAAGGTAATGGAGGTGCATCCGCTTGGGGCGGTGCTGGCGGCGGTGCCTCAGCTTGGGGCGGCCAAGGTACTGGTGCTACTTCTACTTGGGGTGGTGCTTCAGCCTGGGGTAACAAATCAAGTTGGGGCGGTGCATCCACTTGGGCGTCGGGTGGTGAATCTAATGGTGCCATGTCTACTTGGGGTGGTACCG		

						GTGATAGGTCAGCCTACGGCGGGGCTTCCACCTGGGGAGG		
			250760	250822	W	GGCGGAGCTTCTGCATGGGGTAACCAAGACGATGGAAATAGGTCTGCTTGGAACAACCAAGG		
			250838	250861	W	GGTGGTAACAGTACATGGGGAGG		
			250280	250331	C	CCACAGGCCAGAATGGGCCCAAGTTACGTCAGTGCCCCAAGAAACATGGCC		
			250689	250724	C	CCGGTGATAGGTCAGCCTACGGCGGGGCTTCCACC		
XIII_{BR}	672879	673048	673216	673259	W	GGTGATGGGGTGTCTGGAGTGGATTCCAAAACGGCATTAACGG	649309	649552
			673509	673530	W	GGAAGTGGCTGGCTTCTCTGG		
			672461	672483	C	CCAAATAGCCTGAGTTACCACC		
			672802	672848	C	CCGATTTCAGAACCACCACGGGATTGCCAACCCAACATATCCTTCC		
			672878	672894	C	CCAAACCAGACCAACC		
XIV_{NC}	88913	89103	88727	88775	W	GGGTTTTAATGTGGTAAACAAGATGGCCCTTACGGGGCTCTTAGTGGG	89531	89804
			88907	88950	C	CCCTAAGTGATTGTGCCGTTATAACTTCCATTCGGGTGATACC		
XV_{BR}	318817	318975	318436	318471	W	GGCACAAGCTCAGGCTCAGGCACAGGCACAGGTGG	309359	309925
			319281	319318	W	GGGTTGGACATCCTGTATGGGCTTCCGAAGATTCGGG		
			318523	318565	C	CCATCCTTCTAACCGAGGTATTCCACAGCAAAACTTGCCTCC		
			318581	318614	C	CCTCCAACAAACGGTTCGACCGTACATGAAGCC		
			318638	318661	C	CCACCCCACTTCATGCCCTTACC		
			318863	318902	C	CCTAGCATTGTGCCTGTGGTCCCTGAACCCACTGAGCCC		

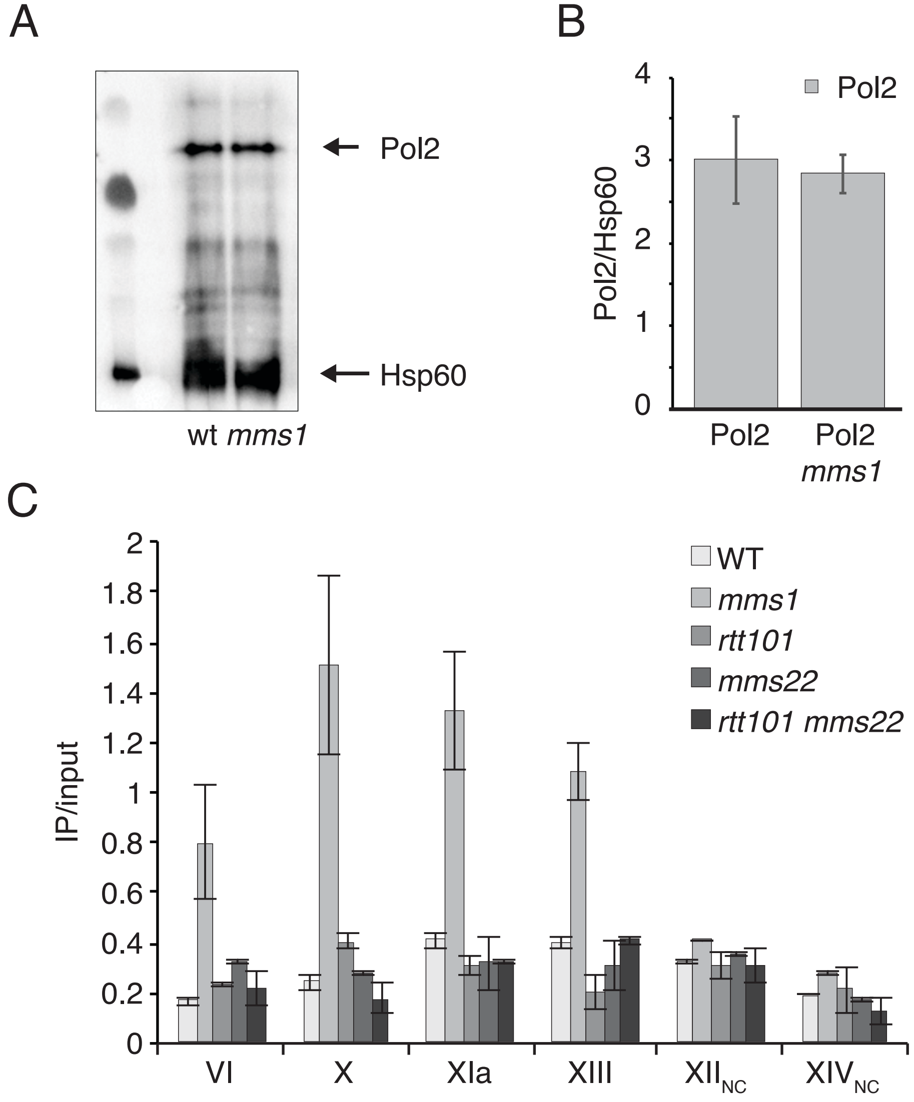
Supplementary data 7: Sequences of oligodeoxynucleotides used for folding and CD.

Name	Feature	Region	Sequence (5´-3´)
KW336	MEME motif lagging strand	$\mathrm{ChrVII}_{\mathrm{BR}}$	GGTCGTGGCGGTAGCCGTGG
KW341	G4 motif lagging strand	$\mathrm{ChrXI}_{\mathrm{BR}}$	GGTGGTATCCTGACTGACGGTAAAGG
KW342	no G4 motif lagging strand	$\mathrm{ChrXIII}_{\mathrm{NC}}$	GGTGGAAGCCCCGCCGTAGGCTGACCTATCACCGG
KW399	no G4 motif lagging strand	$\mathrm{ChrI}_{\mathrm{NC}}$	GGCACCGTAACATGGCCCACAATAGTTAGGATCGTTATTAACCGGCGCGG
KW400	G4 motif lagging strand	$\mathrm{ChrXV}_{\mathrm{BR}}$	GGCACAAGCTCAGGCTCAGGCACAGGCACAGGTGG

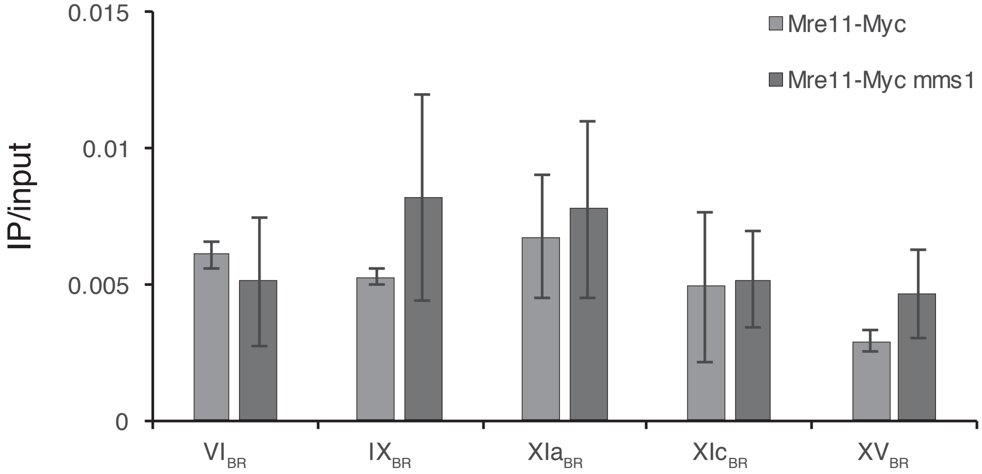
Supplementary data 8: Mms1 binds independent of Rtt101 or Mms22 to G-rich regions. (A) Western blot analysis of Myc-tagged Mms1 protein levels in G1, S and G2 phase. Hsp60 serves as a loading control. N=3 biological replicates. Mms1 protein is present in all cell cycle phases but peaks in G1 phase (quantifications see Figure 2B). (B) FACS analysis of yeast cells arrested in G1, S and G2 phase used for conventional ChIP analysis. (C) ChIP and qPCR analysis of Mms1-Myc in *rtt101*, *mms22*, and *rtt101 mms22* cells. Plotted are the IP/input values as means ± SD. N≥3 biological replicates. (D) Western blot analysis of Myc-tagged Mms1 protein levels in wild type (wt), *rtt101* and *mms22*2 cells. Hsp60 serves as a loading control. N=3 biological replicates. (E) Quantified Myc-tagged Mms1 protein levels in wild type (wt), *rtt101* and *mms22* cells after western blot analysis. Hsp60 was used as a reference protein. Shown are mean Myc-tagged Mms1 levels normalized to Hsp60 ± SD. N=3 biological replicates.



Supplementary data 9: DNA Pol2 levels and occupancy. (A) Western blot analysis of Myc-tagged DNA Pol2 protein levels in wild type (wt) and *mms1* cells. Hsp60 serves as a loading control. N=3 biological replicates. (B) Quantified Myc-tagged DNA Pol2 protein levels in wild type (wt) and *mms1* cells after western blot analysis. Hsp60 was used as a reference protein. Shown are mean Myc-tagged DNA Pol2 levels normalized to Hsp60 ± SD. N=3 biological replicates. (C) ChIP analysis of DNA Pol2-Myc in wild type, *rtt101*, *mms22* and *rtt101 mms22* cells. qPCR analysis of DNA Pol2-Myc association at four Mms1 BR and two NC regions. Plotted are the IP/input values as mean value ± SD. N≥3 biological replicates.



Supplementary data 10: Mms1 does not recruit Mre11. Conventional ChIP was performed with Myc-tagged Mre11 in wild type and *mms1* cells. The association of Myc-tagged Mre11 was analyzed by qPCR using primer pairs for the shown regions. Plotted are the IP/input values ± SD. N≥3 biological replicates.

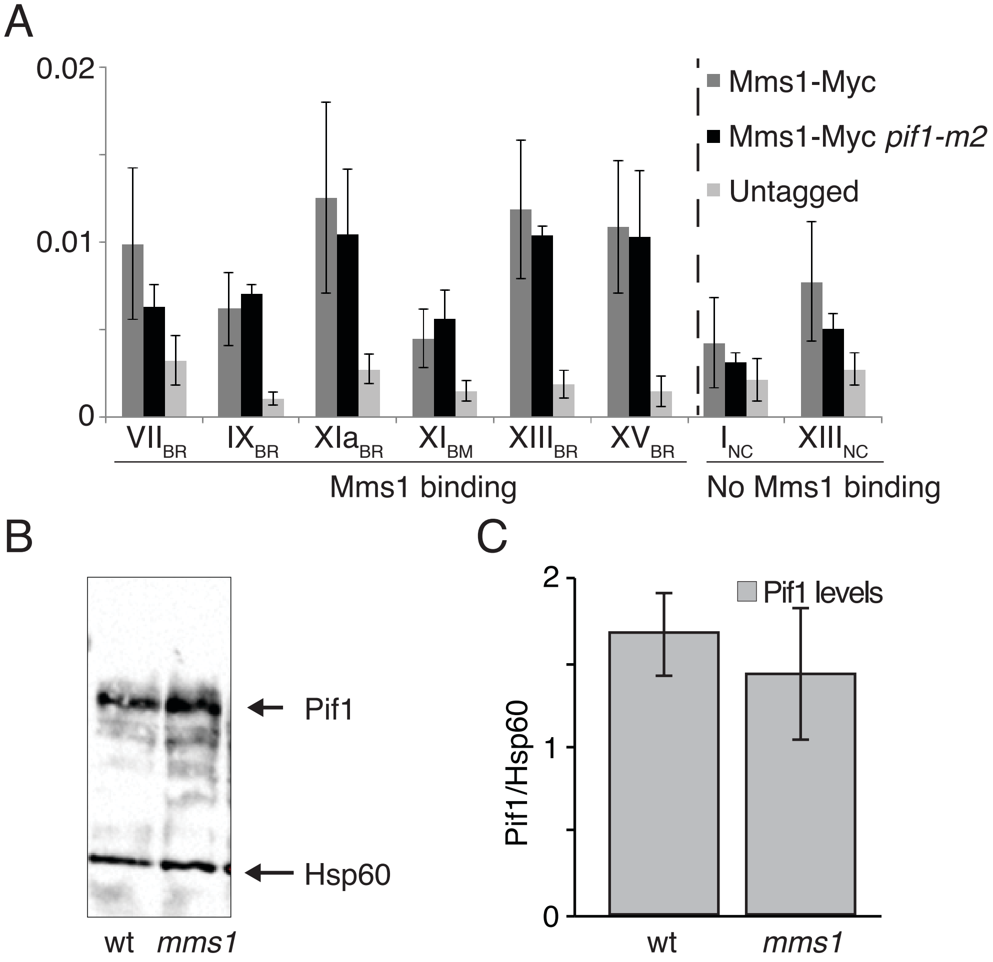


Supplementary data 11: Mms1 binding regions that overlap Pif1 (1) binding regions.

mms1_peak_Chr	mms1_peak_start	mms1_end	pif1_peak_Chr	pif1_peak_start_coord	pif1_peak_end_coord
ChrI	112580	112785	ChrI	111548	114298
ChrI	130081	130284	ChrI	129548	131298
ChrI	191965	192177	ChrI	189298	193548
ChrII	463749	464101	ChrII	463082	464332
ChrIV	461797	462449	ChrIV	461450	461700
ChrIV	1239630	1239824	ChrIV	1239450	1240200
ChrIV	1251076	1251389	ChrIV	1250700	1251700
ChrV	40469	41119	ChrV	40562	41562
ChrV	42006	42474	ChrV	40562	41562
ChrV	42006	42474	ChrV	42812	43562
ChrV	43222	43945	ChrV	42812	43562
ChrV	335544	335734	ChrV	333062	336812
ChrVI	224946	225231	ChrVI	224415	225415
ChrVII	567184	567405	ChrVII	567541	569041
ChrVII	700602	700830	ChrVII	700916	701166
ChrVII	806449	806663	ChrVII	806666	806916
ChrVIII	189407	189652	ChrVIII	188763	190263
ChrIX	54150	54474	ChrIX	51865	55115
ChrIX	334127	334307	ChrIX	333615	334365
ChrIX	392157	392481	ChrIX	389365	391365
ChrIX	392157	392481	ChrIX	392365	393115
ChrX	121000	121236	ChrX	120811	121311
ChrX	400013	400260	ChrX	399561	401061
ChrXI	380958	381322	ChrXI	380319	382069
ChrXI	519605	519853	ChrXI	519444	519694
ChrXI	519605	519853	ChrXI	520319	521319
ChrXII	84188	84385	ChrXII	84818	86318
ChrXII	458652	459006	ChrXII	459318	460818
ChrXII	459110	459290	ChrXII	459318	460818
ChrXII	904035	904280	ChrXII	902568	903068
ChrXII	904035	904280	ChrXII	903568	903818
ChrXII	921240	921442	ChrXII	920943	921193
ChrXIV	494007	494211	ChrXIV	493616	494366
ChrXIV	547086	547297	ChrXIV	547241	547491
ChrXIV	652075	652255	ChrXIV	650366	651366
ChrXV	31003	31286	ChrXV	28945	31445
ChrXV	159994	160358	ChrXV	159945	160695
ChrXV	480108	480435	ChrXV	479695	480445

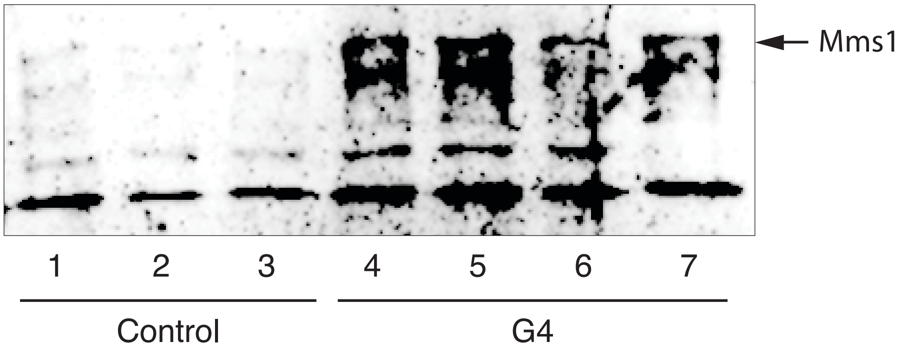
ChrXV	480108	480435	ChrXV	481070	481320
ChrXV	710139	710326	ChrXV	709570	709820
ChrXV	1060686	1061023	ChrXV	1060570	1060820
ChrXVI	536586	536770	ChrXVI	536543	536793
ChrXVI	922313	922518	ChrXVI	921918	922918

Supplementary data 12: Mms1 is independent of Pif1. (A) Conventional ChIP was performed with Myc-tagged Mms1 in wild type and *pif1-m2* cells. The association of Myc-tagged Mms1 was analyzed by qPCR using primer pairs for the shown regions. Plotted are the IP/input values ± SD. N≥3 biological replicates. (B) Western blot analysis of Myc-tagged Pif1 protein levels in wild type (wt) and *mms1* cells. Hsp60 serves as a loading control. N=3 biological replicates. (C) Quantified Myc-tagged Pif1 protein levels in wild type (wt) and *mms1* cells after western blot analysis. Hsp60 was used as a reference protein. Shown are mean Myc-tagged Pif1 levels normalized to Hsp60 ± SD. N=3 biological replicates.



Supplementary data 13: Affinity purification of MMS1. Yeast proteins were isolated from the Myc-tagged Mms1 *pif1-m2* strain using the following lysis buffer (0.1 M HEPES pH7.5, 0.01 M potassium acetate, 10% glycerin, 0.5% Nonidet P-40, 1 mM EDTA pH 8.0, 1 mM dithiothreitol, 1 μg/ml leupeptin, 1 μg/ml aprotinin and 0.5 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride). To identify Mms1-Myc interacting to G4 we modified a previous published affinity purification protocol (6). Briefly, dynabeads M-270 streptavidin (Invitrogen) were washed with BS/THES buffer (22 mM Tris-HCl pH 7.5, 10 mM HEPES pH 7.5, 8,9% saccharose, 62 mM NaCl, 5 mM $CaCl_2$, 50 mM KCl, 1 mM EDTA pH 8.0, 12% glycerin, 1 mM DTT, 1 μg/ml leupeptin (LP), 1 μg/ml aprotinin (AP) and 0.5 mM AEBSF). The lysate was cleared by pre-incubation first with avidin and then with the washed beads for 60 min at 4°C. Oligodeoxynucleotides (G4 and mutated G4) were biotinylated (SIGMA) and G4 folding protocols were performed. Dynabeads M-280 streptavidin (Invitrogen) were washed with BW buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA pH 8.0, 2 M NaCl, 1% 0.1 M phenylmethylsulfonyl fluoride (PMSF)). Beads were mixed with biotinylated DNA (7000 pmol) and incubated for 60 min at room temperature (RT). The immobilized DNA was washed three times with TE. The beads were then blocked with 0.1% (w/v) BSA in 2xBW buffer for 15 min at 4°C. Beads were washed twice with BS/THES buffer and once with 3750 μl BS/THES buffer containing 5 μg DNA (random oligodeoxynucleotides). Beads were resuspended in BS/THES buffer and incubated with cleared protein lysate, 50 mM potassium acetate and 100 fold excess DNA (compared to bound bait DNA, random oligodeoxynucleotides) for 12 h at 4°C. Beads were washed twice with BS/THES buffer with 5 μg PCR product containing G4 motifs followed by five washing steps with BS/THES buffer. The samples were mixed with 6x SDS loading buffer, boiled and loaded onto a 10% SDS PAGE gel. Western blot analysis was performed against Myc-tagged Mms1.

Mms1 co-purifies with G4 structures. Biotinylated control DNA (lane 1-3) as well as biotinylated G4 structures (lane 4-7) were incubated with total yeast cell lysate in which Mms1 was endogenous Myc-tagged and Pif1 was mutated (*pif1-m2*). Western blot analysis, directed against Mms1-Myc, showed that Mms1 co-purifies with G4 structures (lane 4: Chr VI-$G4_{\text{tract3}}$, lane 5: Chr IX-$G4_{\text{tract3}}$, lane 6: Chr XV-$G4_{\text{tract2}}$, lane 7: Chr XIII-$G4_{\text{tract2}}$) and not with control DNA (lane 1: G-rich DNA, lane 2: Chr VI-$G4_{\text{tract3mut}}$, lane 3: Chr XV-$G4_{\text{tract2mut}}$). Arrow indicates Mms1.

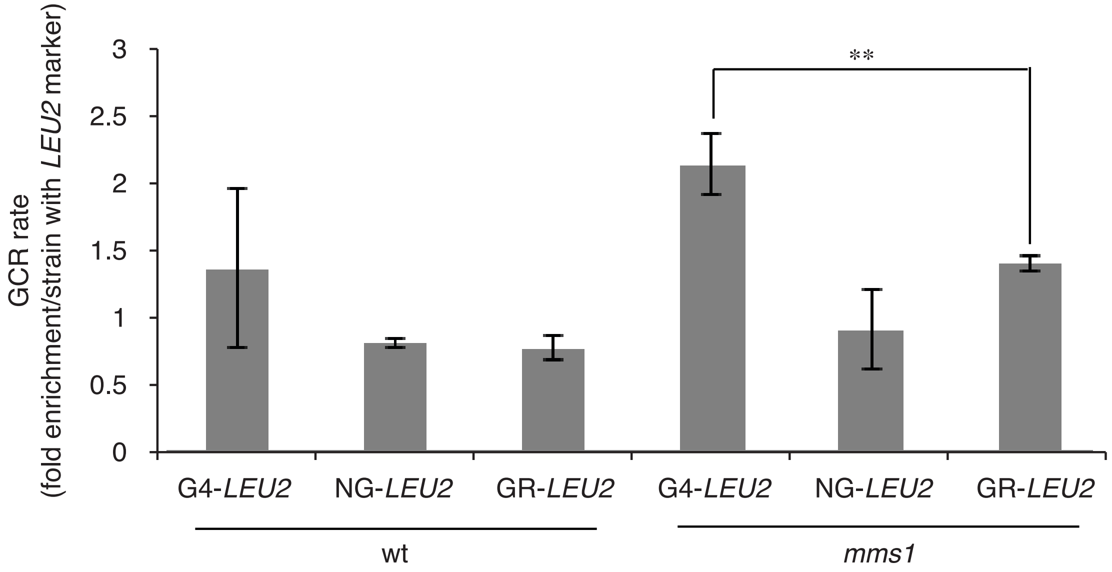


Supplementary data 14: GCR assay. (A) Inserts used in the GCR assay. (B) The GCR rate was determined for wild type (wt) and *mms1* cells with an inserted sequence. Shown are mean values ± SD as fold enrichment over strain with inserted Leu marker. n=7 biological replicates, N≥3. Statistical significance is noted in the figure. This was determined by student's T-test. **:p<0.01.

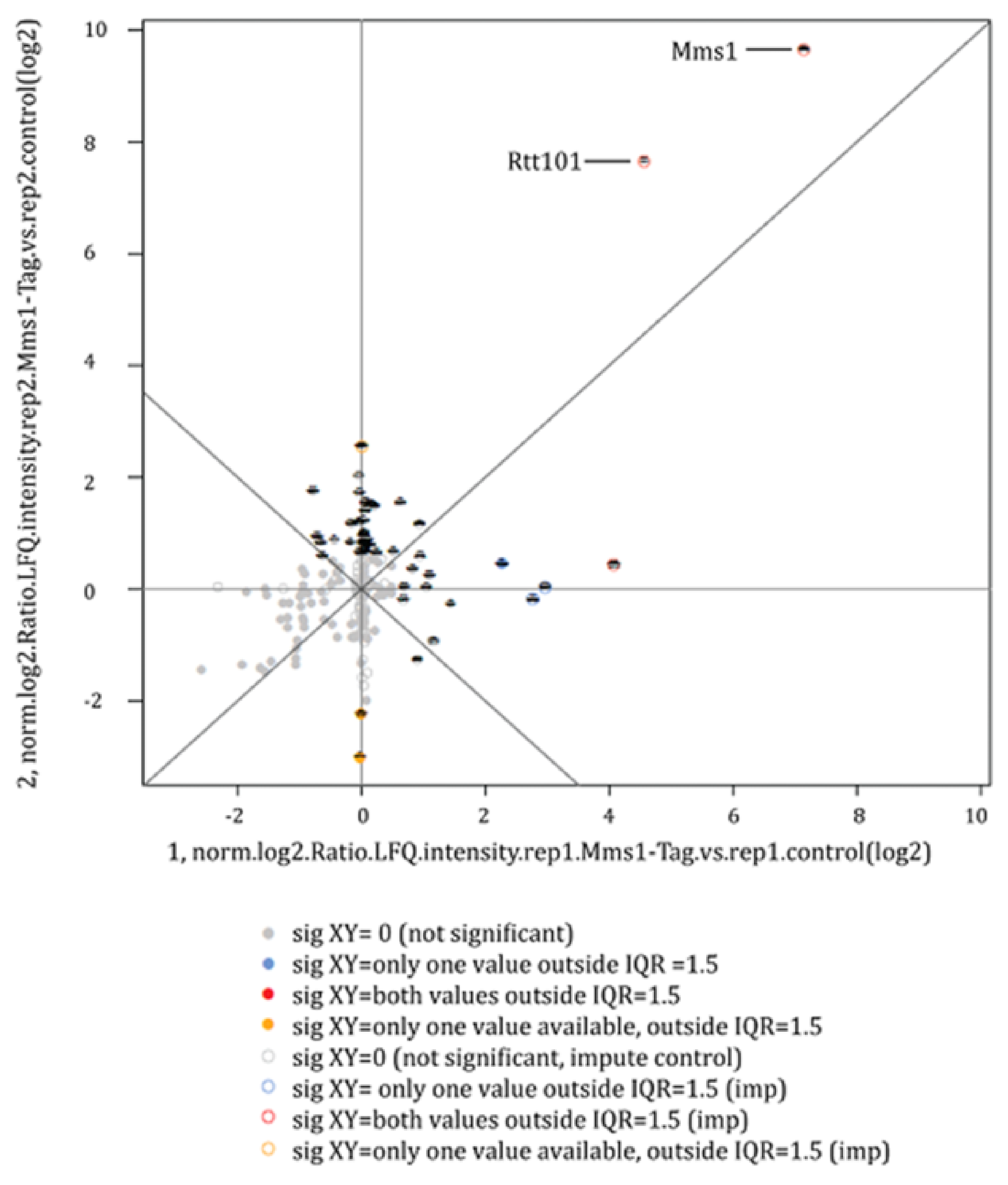
A

G4 motif (ChrI) (G4-LEU2)	lagging strand	CCCAACAATTATCTCAAAATTCCCCCAATTCTCATCAGTAACACCCCACCCC
Non-G-rich (ChrVII) (NG-LEU2)	lagging strand	CTAATCTTTCAGCGTTGTAAATGTTGGTACCCAAACCCAATTGTCTACAAGTTTCCTTAGC
G-rich (ChrI) (GR-LEU2)	lagging strand	ATGGTGGTCATCTCAGTAGATGTAGAGGTGAAAGTACCGGTCCATGGCTCGGT

B



Supplementary data 15: Immunoprecipitation and mass spectrometry (MS) to identify binding partners of Mms1. Protein lysate was prepared from Mms1-Myc and untagged cells according to ChIP analysis (see main script), except another lysis buffer was used (0.1 M HEPES pH7.5, 0.01 M potassium acetate, 10% glycerin, 0.5% Nonidet P-40, 1 mM EDTA pH 8.0, 1 mM dithiothreitol, 1 μg/ml leupeptin, 1 μg/ml aprotinin and 0.5 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride). The samples were treated with DNase and RNase. Incubation with antibody and beads was performed as described for ChIP. After four times wash with PBS, proteins were denatured and submitted to MS analysis. For MS analysis, proteins were reduced in 50 mM DTT for 10 min at 90 °C, and alkylated with 120 mM iodoactamide for 20 min at RT in the dark. Protein precipitation was performed according to Wessel and Fluegge with chloroform/methanol (7). Precipitated proteins were dissolved in 0.5 % sodium deoxycholate in 100mM ammonium bicarbonate. Digests were performed with trypsin (trypsin-to-protein ratio: 1:100) overnight at 37 °C. SDC was removed by extraction with ethylacetate (8). Peptides were dried in a vacuum concentrator to remove remaining ethylaceate. Peptides were desalted using C18 stage tips (9). Each Stage Tip was prepared with three disks of C18 Empore SPE Disks (3M) in a 200 μl pipet tip. Peptides were eluted with 60 % acetonitrile in 0.1 % formic acid, dried in a vacuum concentrator, and stored at -20 °C. Peptides were dissolved in 2 % acetonitrile/0.1 % formic acid prior to nanoLC-MS/MS analysis. NanoLC-MS/MS analyses were performed on an Orbitrap Fusion (Thermo Scientific) equipped with an EASY-Spray Ion Source and coupled to an EASY-nLC 1000 (Thermo Scientific). Peptides were loaded on a trapping column (2 cm x 75 μm ID, PepMap C18, 3 μm particles, 100 Å pore size) and separated on an EASY-Spray column (50 cm x 75 μm ID, PepMap C18, 2 μm particles, 100 Å pore size) with a 180-minute linear gradient from 3 % to 40 % acetonitrile and 0.1 % formic acid. Both MS and MS/MS scans were acquired in the Orbitrap analyzer with a resolution of 60,000 for MS scans and 15,000 for MS/MS scans. HCD fragmentation with 35 % normalized collision energy was applied. A Top Speed data-dependent MS/MS method with a fixed cycle time of three seconds was used. Dynamic exclusion was applied with a repeat count of 1 and exclusion duration of 120 seconds; singly charged precursors were excluded from selection. Minimum signal threshold for precursor selection was set to 50,000. Predictive AGC was used with an AGC target value of 5e5 for MS scans and 5e4 for MS/MS scans. EASY-IC was used for internal calibration. Raw MS data files were analyzed with MaxQuant version 1.5.3.12 (10). Database search was performed with Andromeda, which is integrated in the utilized version of MaxQuant. The search was performed against the UniProt Saccharomyces cerevisiae (strain S288c) reference proteome database. Additionally, a database containing common contaminants was used. The search was performed with tryptic cleavage specificity with 2 allowed miscleavages. Protein identification was under control of the false-discovery rate (<1% FDR on protein and peptide level). In addition to MaxQuant default settings (e.g. at least 1 razor/unique peptide for identification, 2 allowed miscleavages), the search was performed against following variable modifications: Protein N-terminal acetylation, Gln to pyro-Glu formation (N-term. Q) and oxidation (on Met). For protein quantitation, the LFQ intensities were used (11). Proteins with less than two identified razor/unique peptides were dismissed. Missing LFQ intensities in the control samples were imputed with values close to the baseline if intensities in the corresponding IP samples were present. Data imputation was performed with values from a standard normal distribution with a mean of the 5% quantile of the combined LFQ intensities and a standard deviation of 0.1. Shown are the results of a Mms1 co-immunoprecipitation. Log2-ratios of protein intensities (label-free quantitation, LFQ, MaxQuant) from two replicates are plotted against each other. N=2 biological replicates. Missing values in one or both control experiments have been imputed with baseline intensities to allow ratio calculations (open circles). Significance calculations are based on boxplot statistics (grey: not significant (n.s.); blue: one replicate >1.5x interquartile range (IQR), the other n.s.; orange: >1.5xIQR, only data available from one replicate; red: at least >1.5xIQR in two or >3xIQR in one replicate.



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