Supporting Information

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Fig. S1. Sensitivity to DNA damage caused by mph1 mutations in WT, rad51 Δ , and rad52 Δ backgrounds. Experiments were carried out as described in Fig. 2A.

Α	YPD	MMS (0.01%)	HU (10 mM)	С				~	
WT 🖸) 🛞 🍀 🏅	• # •		smc	6-P4		A		180 120
mms21-11				3///0		1			60'
mph1-E210Q) 🕲 👍 🤅	• 💿 🕲 🐳 🔹	💿 🕲 🍰 強						Log
mms21-11 mph1-E210Q) 🛞 🍇 🤞	🔘 🎯 🖅 🤞	💿 🛞 😁 😬		1	1	1		100
mph1-Q603D) 🛞 🛬 👻	• 💮 🛞 🐳 🤸	🔘 🌒 🍰 🏭	smcl	6-P4	.23	21		120
mms21-11 mph1-Q603D) 💮 🔅 🖗 (: 💿 🐵 🚓 🦛	⊙ ⊗ ☆ <	mpn1-Qe	503D				60' Noc
					2				Log
В	YPD	MMS (0.001%)	HU (10 mM)	UV (40 J/m ²)	1	1.	1		180
WT 💽) 💿 🤀 🤃	. 💿 💿 🎄 🦕	🔍 🔘 🔅 🔘 🔘	🕽 🔵 🚳 👋 🧰 mms2	21-11		1	Enti	120 [°] 60'
smc6-P4) 🚳 🌾 🧯	• 🕑 🔅		٠					Noc
mph1-E210Q) 🔿 😤 .	💿 🍘 🗱 💀	● 樽 条 🕴	🔊 🌒 🖓 📑					Log
smc6-P4 mph1-E210Q) 🌑 🤤 in	· 💿 🛞 🔆 🤫	💿 🌚 🔅 .) (. : 🗧 🛞 🖲	1	1	1. 1		180
mph1-Q603D	ى 🐨 🕲 (💿 🚳 🎄 🤮	💿 🏶 🕸 🕚	mms2	21-11 503D		12	m i	120
smc6-P4 mph1-Q603D	6	• 🙆 🙆 18 · · .	💿 🚳 🚓 🕂 (10.0				Noc

Fig. S2. Helicase mutations of *mph1* suppress the sensitivity to DNA damage and the accumulation of recombination intermediates in *mms21–11* and *smc6-P4* cells. (A and B) *mph1-E210Q* and -*Q603D* suppress the sensitivity to DNA damage of *mms21–11* cells (A) and *smc6-P4* cells (B). Experiments were carried out as described in Fig. 2.A. (C) *mph1-Q603D* suppresses the accumulation of X-shaped recombination intermediates in *mms21–11* and *smc6-P4* cells. Experiments were carried out as described in Fig. 4.

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Table S1. The levels of Mph1, Rad52, and Pol30 foci in WT cells

% Cells containing foci of these proteins	Normal growth	MMS treatment*	
Mph1	6% (50/789)**	13% (72/578)**	
Rad52	33% (264/789)	44% (254/578)	
Pol30	19% (152/789)	32% (183/578)	
% Mph1 foci co-localized with			
Rad52 foci	76% (38/50)	81% (58/72)	
Pol30 foci	30% (15/50)	32% (23/72)	
Both Rad52 and Pol30 foci	22% (11/50)	21% (15/72)	

*Cells were treated with 0.03% MMS for 2 h.

** $P \le 0.025\%$.

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Table S2. Strains and plasmids used in this study

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Name	Genotype	Sources
W1588-4C	MATa ade2–1 can1–100 his3–11,15 leu2–3,112 trp1–1 ura3-1 RAD5+	R. Rothstein
X2240-8C	MATa smc6–56–13myc::HIS3 rad51 Δ ::LEU2	This study
T294–11B	MATa SMC5-13Myc:HIS3	Lab collection
X1996–12B	MATα SMC5-13Myc::HIS3 MPH1-3Flag::HIS3	This study
X1546–12B	MATa MPH1–13myc::HIS3	This study
T538–2	MATa MPH1–3Flag::HIS3	This study
T497–1	MATa MPH1-YFP	This study
1483-2Ca	MATa mph1\L::KAN	H. Klein
W3111-1C	MATa rad51A::LEU2	R. Rothstein
X1958–1C	$MAT\alpha$ mph1 Δ ::KAN rad51 Δ ::LEU2	This study
T80–14	ΜΑΤα mms21–11::URA3	(1)
X2007–3D	MATa mph1\L::KAN mms21–11::URA3	This study
T382-P4	MATa smc6-P4–13myc::HIS3	This study
X1787–4A	MATa mph1\L::Kan smc6-P4–13myc::HIS3	This study
X2020-10B	MATα smc6-P4-13myc::HIS3 rad51Δ::LEU2	This study
X2123–2A	MATa smc6–56–13myc::HIS3	This study
X2123–3C	MATa mph1\L::KAN smc6-56-13myc::HIS3	This study
W1958-4D	$MAT\alpha sqs1\Delta$::HIS3	R. Rothstein
X1907–2D	$MAT\alpha mph1\Delta$::KAN sqs1 Δ :HIS3	This study
X2489–9C	MATa MPH1-YFP::HIS3 RAD52-RFP CFP-POL30	This study
X1991–1A	MATa lys2_his3-11::pCUP1-GFP12-lacl12::HIS trp1-1::LacO::TRP1 MCD1:3XHA:URA3:3XHA	This study
X2359–7A	MATa lys2\Lambda his3-11::pCUP1-GFP12-lacl12::HIS trp1-1::LacO::TRP1 mph1\L::KAN	This study
X2006-31C	MATa lys2_his3-11::pCUP1-GFP12-lacl12::HIS smc6-56-13mvc-HIS3MX6 trp1-1::LacO::TRP1	This study
X2358–14A	MATa lys2\(\Lambda\) his3-11::pCUP1-GFP12-lacl12::HIS3 smc6-56-13mvc-HIS3MX6 trp1-1::LacO::TRP1 mph1\(\Lambda\):KAN	This study
pXZ 216	pOAD-MPH1	This study
pXZ 194	, pOBD-SMC5	This study
pXZ 198	POBD-NSE1	This study
pXZ 55	POBD-MMS21	This study
pXZ 191	POBD-NSE3	This study
pXZ 196	pOBD-NSE4	This study
pXZ 199	, POBD-NSE5	This study
pXZ 193	, POBD-NSE6	This study
pXZ 195	pOBD-SMC6	This study
P134	, GAL-MPH1	OpenBiosystem
X700-10D	, MATa SMC6-YFP::KAN	This study
T597–1	MATα mph1-0603D-YFP::HIS5	This study
T617	MATa mph1-E2100-YFP::HIS5	This study
X2183–1B	MATa mms21-11::URA3 mph1-Q603D-YFP::HIS5	This study
X2170-4A	MATα mms21–11::URA3 mph1-E210Q-YFP::HIS5	This study
X2176–1C	MATa smc6-P4-13mvc::KAN mph1-O603D-YFP::HIS5	This study
X2171–14B	MATa smc6-P4-13mvc::KAN mph1-E2100-YFP::HIS5	This study
X2217–14C	MATa smc6-56-13mvc::KAN mph1-0603D-YFP::HIS5	This study
X2212-12A	MATa smc6-56-13mvc::KAN mph1-F2100-YFP: HIS5	This study
X2233–15A	MATa SMC5-13mvc::HIS3 mph1-O603D-YFP::HIS5	This study
T295-3C	MATa SMC6-13mycriHIS	This study
X2452–7B	MATα SMC6-13myc::HIS3 MPH1-3Flag::HIS3	This study
T130–4B	MATα NSE6–13mvc::KAN	This study
X2453–16A	MATα NSE6-13mvc::KAN MPH1-3Elag::HIS3	This study
X707–8B	MATa srs2A::H/S3	This study
X1804-20D	MATα srs2A::HIS3 mph1A::KAN	This study
X795–4B	$MAT\alpha$ srs2 Δ ::TRP1 mms21-11-3HA::KAN	This study
X801	$MATa/w sost \Lambda^{+}HS3/+ ms21-11HA^{+}KAN/+ rad51\Lambda^{+}IEU2/+$	This study
nX7115	pFT28a-Smc5	(1)
Mph10	pGEX4T3-Mph1	This study
X532	MATAIA mms21-11-11/IRA31+ SMC6-YFPL+ ade3A	This study
T733	MATala mms21/\KAN/+ mb1/\/IRA3/+	This study
T732-2	$M\Delta T_{alg} sim (5KA)/+ mph (1IRA)/+$	This study
		This study

Strains in this study are derivatives of W1588–4C, a *RAD5* derivative of W303 (*MATa* ade2–1 can1–100 ura3–1 his3–11,15 leu2–3,112 trp1–1 rad5–535) (2). When applicable, a single representative of each genotype is listed. Strains containing WT proteins fused with various tags exhibit normal cell growth or resistance to DNA damaging agents, suggesting that the fusion constructs are fully functional.

1. Zhao X, Blobel G (2005) A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. *Proc Natl Acad Sci USA* 102:4777–4782. 2. Thomas BJ, Rothstein R (1989) Elevated recombination rates in transcriptionally active DNA. *Cell* 56:619–630.