SUPPLEMENTARY DATA

The helicase Pif1 functions in the template switching pathway of DNA damage bypass

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Supplementary Table 1: Yeast strains used in this study

Strain	Genotype	Source
WT (DF5)	*Mat a, his3-Δ200, leu2-3,112, lys2-801, trp1-1, ura3-52*	(1)
exo1Δ	DF5, *exo1Δ::natNT2*	This study
pif1Δ	DF5, *pif1Δ::hphNT1*	This study
exo1Δ pif1Δ	DF5, *exo1Δ::natNT2, pif1Δ::hphNT1*	This study
rev3Δ	DF5, *rev3Δ::hisG-URA3-hisG*	This study
ubc13Δ	DF5, *ubc13Δ::HIS3MX*	(2)
exo1Δ rev3Δ	DF5, *exo1Δ::natNT2, rev3Δ::hisG-URA3-hisG*	This study
pif1Δ rev3Δ	DF5, *pif1Δ::hphNT1, rev3Δ::hisG-URA3-hisG*	This study
exo1Δ pif1Δ rev3Δ	DF5, *exo1Δ::natNT2, pif1Δ::hphNT1, rev3Δ::hisG-URA3-hisG*	This study
exo1Δ ubc13Δ	DF5, *exo1Δ::natNT2, ubc13Δ::HIS3MX*	This study
pif1Δ ubc13Δ	DF5, *pif1Δ::hphNT1, ubc13Δ::HIS3MX*	This study
exo1Δ pif1Δ ubc13Δ	DF5, *exo1Δ::natNT2, pif1Δ::hphNT1, ubc13Δ::HIS3MX*	This study
pif1-m2	DF5, *pif1-m2::TRP1*	This study
exo1Δ pif1-m2	DF5, *exo1Δ::hphNT1, pif1-m2::TRP1*	This study
pif1-R3E	DF5, *pif1-R3E::TRP1*	This study
rrm3Δ	DF5, *rrm3Δ::natNT2*	This study
$PIF1^{9myc}$	DF5, *KanMX::TetO*$_7$*-RAD18, LEU2::TetR'-SSN6, TRP1::BrdU-inc,* $PIF1^{9myc}$*::hphNT1*	This study
$PIF1^{GFP}$	DF5, $PIF1^{GFP}$*::natNT2*	This study
$pif1\text{-}R3E^{GFP}$	DF5, $pif1\text{-}R3E^{GFP}$*::natNT2*	This study
$PIF1^{GFP}$ $RAD52^{mRuby2}$	DF5, $PIF1^{GFP}$*::natNT2,* $RAD52^{mRuby2}$*::HIS3MX6*	This study
pol32Δ	DF5, *pol32Δ::KanMX*	(3)
pol32Δ exo1Δ	DF5, *pol32Δ::KanMX, exo1Δ::natNT2*	This study
pol32Δ pif11Δ	DF5, *pol32Δ::KanMX, pif1Δ::natNT2*	This study
pol32Δ exo1Δ pif11Δ	DF5, *pol32Δ::KanMX, exo1Δ::natNT2 pif1Δ::hphNT1*	This study
pol32Δ ubc13Δ	DF5, *pol32Δ::KanMX, ubc13Δ::natNT2*	This study
pol32Δ pif1-m2	DF5, *pol32Δ::KanMX, pif1-m2::TRP1*	This study

pol32Δ exo1Δ pif1-m2	DF5, *pol32Δ::KanMX, exo1Δ::hphNT1, pif1-m2::TRP1*	This study
pol32Δ pif1-R3E	DF5, *pol32Δ::KanMX, pif1-R3E::TRP1*	This study
pol32Δ rrm3Δ	DF5, *pol32Δ::KanMX, rrm3Δ::natNT2*	This study
sgs1Δ	DF5 *KanMX::TetO$_7$-RAD18, LEU2::TetR'-SSN6, TRP1::BrdU-inc, sgs1Δ::hphNT1*	(4)
sgs1Δ exo1Δ	DF5 *KanMX::TetO$_7$-RAD18, LEU2::TetR'-SSN6, TRP1::BrdU-inc, sgs1Δ::hphNT1, exo1Δ::natNT2*	This study
sgs1Δ pif1Δ	DF5 *KanMX::TetO$_7$-RAD18, LEU2::TetR'-SSN6, TRP1::BrdU-inc, sgs1Δ::hphNT1, pif1Δ::natNT2*	This study
sgs1Δ exo1Δ pif1Δ	DF5 *KanMX::TetO$_7$-RAD18, LEU2::TetR'-SSN6, TRP1::BrdU-inc, sgs1Δ::hphNT1, exo1Δ::HIS3MX, pif1Δ::natNT2*	This study
^{His}POL30	DF5, *pol30Δ::URA3, YIp128-P30-His6-POL30::LEU2*	(5)
^{His}POL30 exo1Δ	DF5, *pol30Δ::URA3, YIp128-P30-His6-POL30::LEU2, exo1Δ::hphNT1*	This study
^{His}POL30 pif1Δ	DF5, *pol30Δ::URA3, YIp128-P30-His6-POL30::LEU2, pif1Δ::hphNT1*	This study
^{His}POL30 exo1Δ pif1Δ	DF5, *pol30Δ::URA3, YIp128-P30-His6-POL30::LEU2, exo1Δ::hphNT1, pif1Δ::natNT2*	This study
WT (W303)	*Mat a, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15, RAD5+*	R. Rothstein
W303 *exo1Δ*	W303, *exo1Δ::hphNT1*	This study
W303 *pif1Δ*	W303, *pif1Δ::hphNT1*	This study
W303 *exo1Δ pif1Δ*	W303, *pif1Δ::hphNT1, exo1Δ::natNT2*	This study
W303 *ubc13Δ*	W303, *ubc13Δ::natNT2*	This study
W303 *pif1-m1*	W303, *pif1-m1::TRP1*	This study
W303 *pif1-m2*	W303, *pif1-m2::TRP1*	This study
W303 *pif1-R3E*	W303, *pif1-R3E::TRP1*	This study
W303 *pif1-R3E^{9myc}*	W303, *pif1-R3E^{9myc}::hphNT1*	This study
smc6-56	W303, *smc6-56^{13myc}::KanMX*	(6)
smc6-56 exo1Δ	W303, *smc6-56^{13myc}::KanMX, exo1Δ::hphNT1*	This study
smc6-56 pif1Δ	W303, *smc6-56^{13myc}::KanMX, pif1Δ::natNT2*	This study
smc6-56 exo1Δ pif1Δ	W303, *smc6-56^{13myc}::KanMX, exo1Δ::hphNT1, pif1Δ::natNT2*	This study
smc6-56 ubc13Δ	W303, *smc6-56^{13myc}::KanMX, ubc13Δ::natNT2*	This study
smc6-56 ubc13Δ exo1Δ	W303, *smc6-56^{13myc}::KanMX, ubc13Δ::HIS3MX, exo1Δ::hphNT1*	This study
smc6-56 ubc13Δ pif1Δ	W303, *smc6-56^{13myc}::KanMX, ubc13Δ::HIS3MX, pif1Δ::natNT2*	This study

smc6-56 ubc13Δ exo1Δ pif1Δ	W303, *smc6-56*^{13myc}*::KanMX, ubc13Δ::HIS3MX, exo1Δ::hphNT1, pif1Δ::natNT2*	This study
smc6-56 pif1-m1	W303, *smc6-56*^{13myc}*::KanMX, pif1-m1::TRP1*	This study
smc6-56 pif1-m2	W303, *smc6-56*^{13myc}*::KanMX, pif1-m2::TRP1*	This study
smc6-56 pif1-R3E	W303, *smc6-56*^{13myc}*::KanMX, pif1-R3E::TRP1*	This study
smc6-56 pif1-R3E^{9myc}	W303, *smc6-56*^{13myc}*::KanMX, pif1-R3E*^{9myc}*::hphNT1*	This study
BrdU Inc x7	W303, *GAL, psi+, URA::GPD-TK×7, phENT1-LEU2*	(7)
BrdU Inc x7 exo1Δ	W303, *GAL, psi+, URA::GPD-TK×7, phENT1-LEU2 exo1Δ::hphNT1*	(4)
BrdU Inc x7 pif1Δ	W303, *GAL, psi+, URA::GPD-TK×7, phENT1-LEU2 pif1Δ::natNT2*	This study
BrdU Inc x7 exo1Δ pif1Δ	W303, *GAL, psi+, URA::GPD-TK×7, phENT1-LEU2 exo1Δ::hphNT1, pif1Δ::natNT2*	This study

SUPPLEMENTARY REFERENCES

1. Finley,D., Ozkaynak,E. and Varshavsky,A. (1987) The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation, and other stresses. *Cell*, **48**, 1035-1046.
2. Ulrich,H.D. and Jentsch,S. (2000) Two RING finger proteins mediate cooperation between ubiquitin-conjugating enzymes in DNA repair. *EMBO J.*, **19**, 3388-3397.
3. Saugar,I., Parker,J.L., Zhao,S. and Ulrich,H.D. (2012) The genome maintenance factor Mgs1 is targeted to sites of replication stress by ubiquitylated PCNA. *Nucleic Acids Res.*, **40**, 245-257.
4. Garcia-Rodriguez,N., Morawska,M., Wong,R.P., Daigaku,Y. and Ulrich,H.D. (2018) Spatial separation between replisome- and template-induced replication stress signaling. *EMBO J.*, **37**, e98369.
5. Stelter,P. and Ulrich,H.D. (2003) Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. *Nature*, **425**, 188-191.
6. Choi,K., Szakal,B., Chen,Y.H., Branzei,D. and Zhao,X. (2010) The Smc5/6 complex and Esc2 influence multiple replication-associated recombination processes in Saccharomyces cerevisiae. *Mol. Biol. Cell*, **21**, 2306-2314.
7. Bianco,J.N., Poli,J., Saksouk,J., Bacal,J., Silva,M.J., Yoshida,K., Lin,Y.L., Tourriere,H., Lengronne,A. and Pasero,P. (2012) Analysis of DNA replication profiles in budding yeast and mammalian cells using DNA combing. *Methods*, **57**, 149-157.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Nuclear, but not mitochondrial Pif1 participates in TS, and both Exo1 and Pif1 function in the same pathway as Ubc13. A. Disruption of the nuclear form of Pif1 (*pif1-m2*) but not the mitochondrial form (*pif1-m1*) suppresses the MMS sensitivity of *smc6-56*. Serial dilutions of relevant strains were spotted onto plates containing the indicated concentrations of MMS. **B.** Exo1 and Pif1 are epistatic to Ubc13 with respect to the suppression of the MMS sensitivity of *smc6-56.* Serial dilutions of relevant strains were spotted onto plates containing the indicated concentrations of MMS. **C.** The double mutant *exo1Δ pif1-m2* rescues the cold sensitivity of *pol32Δ* more efficiently than either single mutant. Serial dilutions of relevant strains were spotted onto YPD plates and incubated at the indicated temperatures.

Figure S2: Deletion of *PIF1* reduces the amount of X-shaped molecules in early S phase. 2D gel analysis of a 6 kbp region upstream the ARS305 was performed with the indicated strains, treated as in Figure 2D. The 3.4 kbp HindIII-HindIII fragment analysed by 2D gel electrophoresis is shown on the left. Quantification of X-shaped molecules, relative to *sgs1Δ* and normalized to the n-spot is shown at the bottom.

Figure S3: Deletion of Pif1 reduces the overall length of replication tracts in the presence of DNA damage. Quantification of replication tract length on DNA fibres from Figure 4. Number of EdU tracts analysed: *WT* = 177; *exo1Δ* = 120; *pif1Δ* = 81; *exo1Δ pif1Δ* = 150. Significance was calculated by the Mann-Whitney test (ns: not significant; ****: $p < 0.0001$). Black bar = mean.

Figure S4: Mutant *pif1-R3E* is expressed at near wild-type levels and only compromises the nuclear function of the protein. A. Western blots of total cell extracts, showing protein levels of Pif1^{9myc} and *pif1-R3E*^{9myc} (two independent clones each) in *WT* and *smc6-56* background. Pgk1 served as loading control. **B.** The *pif1-R3E* mutation does not increase tolerance to zinc. Serial dilutions of relevant strains were spotted onto plates containing the indicated concentrations of $ZnCl_2$.

Figure S5: Pif1 co-localizes with Rad52 upon MMS treatment. Representative images of *WT* cells synchronized in G1 with α-factor and released in media containing 0.033% MMS for 90 min. A magnified section of the images are shown at the bottom. Scale bar = 5 µm.

Figure S6: Deletion of *RRM3* aggravates the cold sensitivity of *pol32Δ*. Serial dilutions of relevant strains were spotted onto YPD plates and incubated at the indicated temperatures.

Figure S1 (García-Rodríguez et al.)

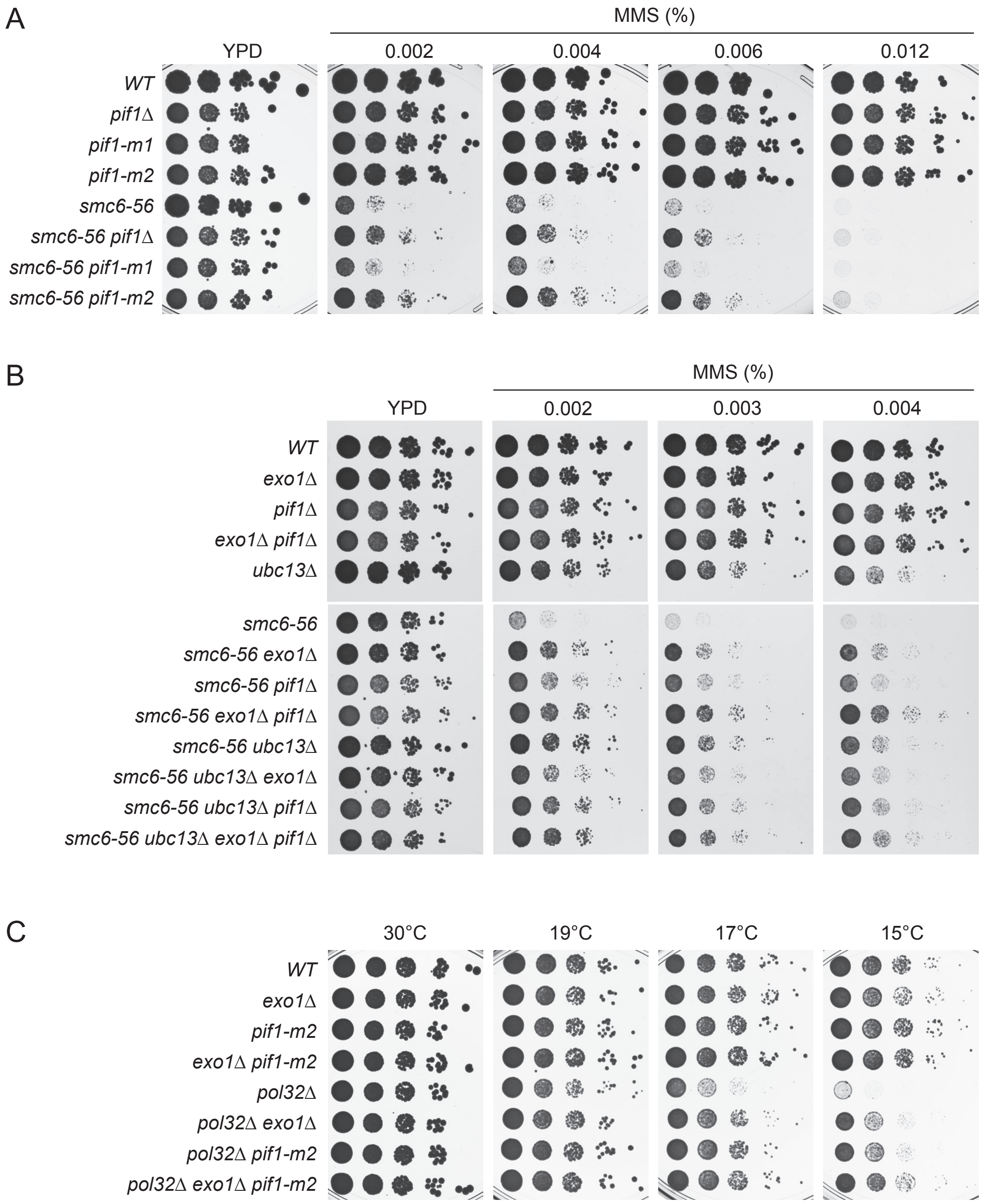

Figure S2 (García-Rodríguez et al.)

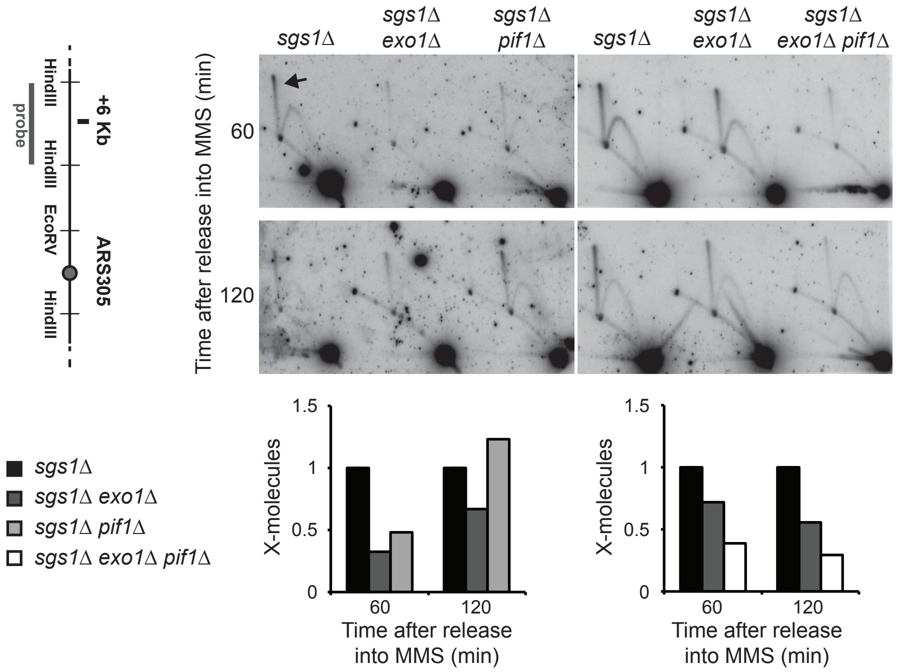

Figure S3 (García-Rodríguez et al.)

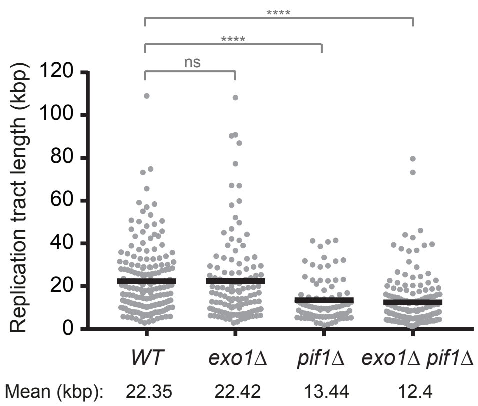

Figure S4 (García-Rodríguez et al.)

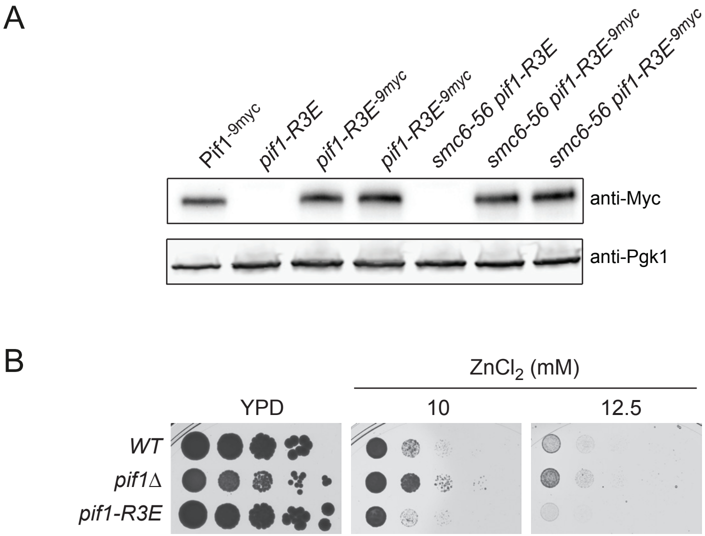

Figure S5 (García-Rodríguez et al.)

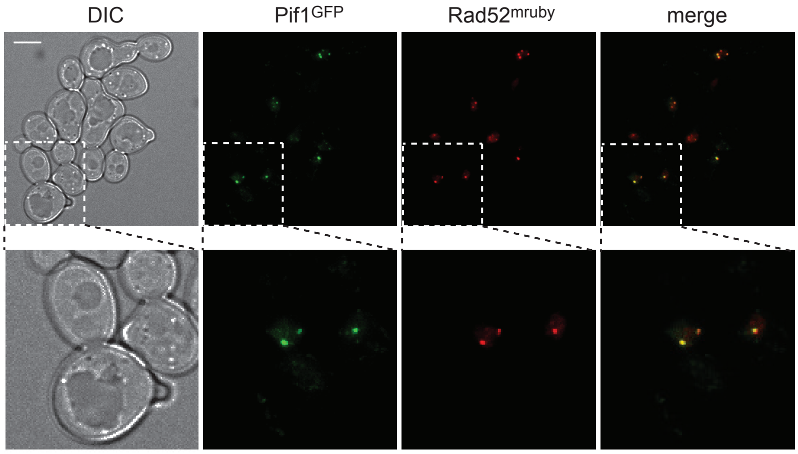

Figure S6 (García-Rodríguez et al.)