Figure S1

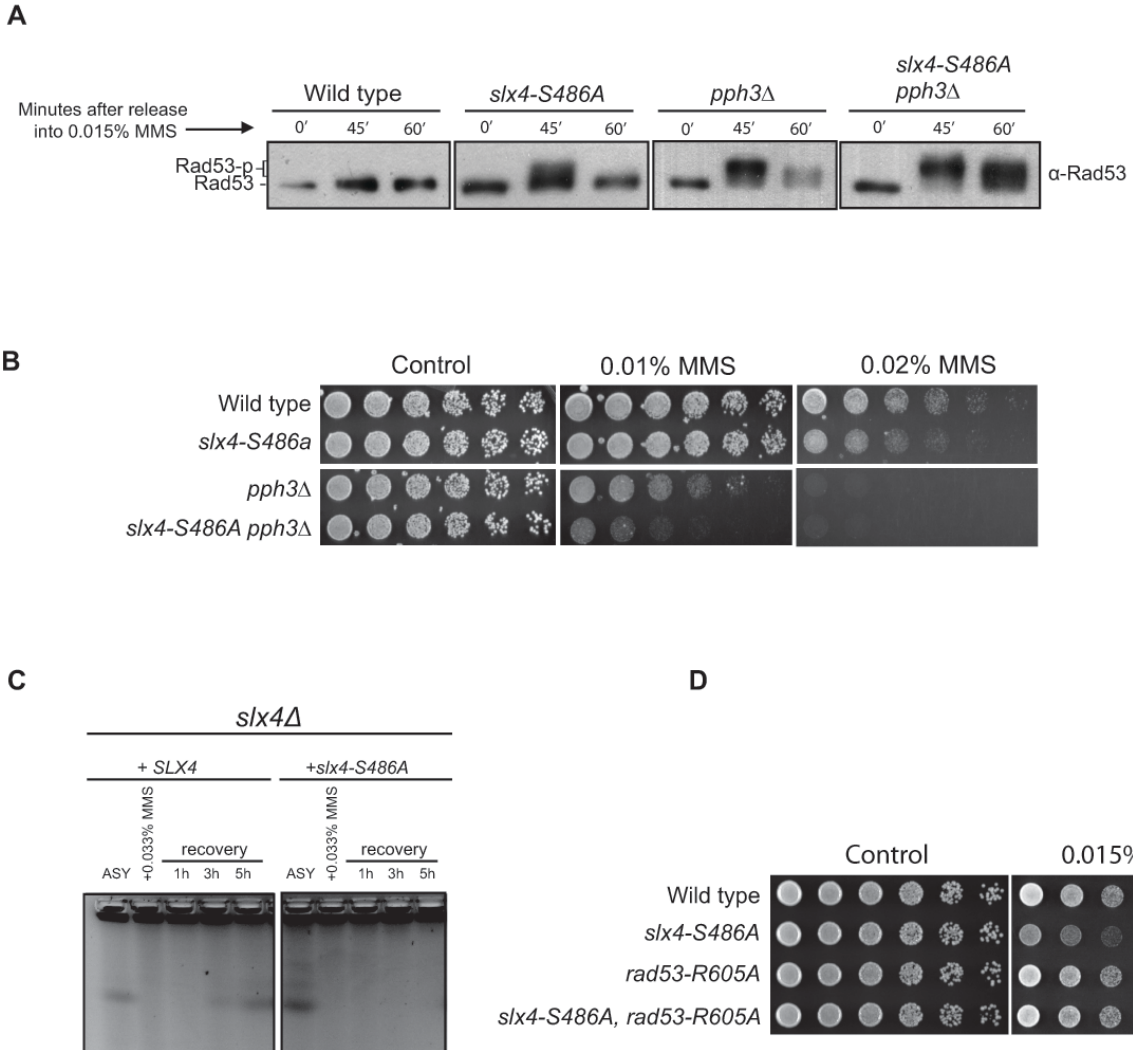
A
Minutes after release into 0.015% MMS
Wild type
slx4-S486A
pph3Δ
slx4-S486A pph3Δ
0′ 45′ 60′
Rad53-p
Rad53
α-Rad53
B
Control
0.01% MMS
0.02% MMS
Wild type
slx4-S486a
pph3Δ
slx4-S486A pph3Δ
C
slx4Δ
+ SLX4
+slx4-S486A
ASY
+0.033% MMS
recovery
1h 3h 5h
D
Control
0.015% MMS
Wild type
slx4-S486A
rad53-R605A
slx4-S486A, rad53-R605A

Figure S1 The *slx4-S486A* mutant phenocopies cells lacking *SLX4* in the response to MMS-induced replication stress. (A) Anti-Rad53 immunoblots of WT, *slx4-S486A*, *pph3Δ* and *pph3Δ slx4-S486A* strains showing Rad53 phosphorylation status after MMS treatment. Experiment was performed as described in Figure 1B. (B) Serial dilution assays showing the effect of MMS treatment upon the sensitivity of wild type, *slx4-S486A*, *pph3Δ* and *pph3Δ slx4-S486A* strains. Four-fold serial dilutions were spotted on YPD plates and grown for 2–3 days at 30°C. (C) Analysis of fully replicated chromosomes measured by PFGE in wild type and *slx4-S486A* strains. Asynchronous (ASY) cells were treated with 0.033% MMS for 3 hours and then released in MMS-free media at different time points. (D) Serial dilution assay showing the effect of a hypomorphic *RAD53* allele (*rad53-R605A*) on MMS sensitivity of wild type and *slx4-S486A* strains.

Figure S2

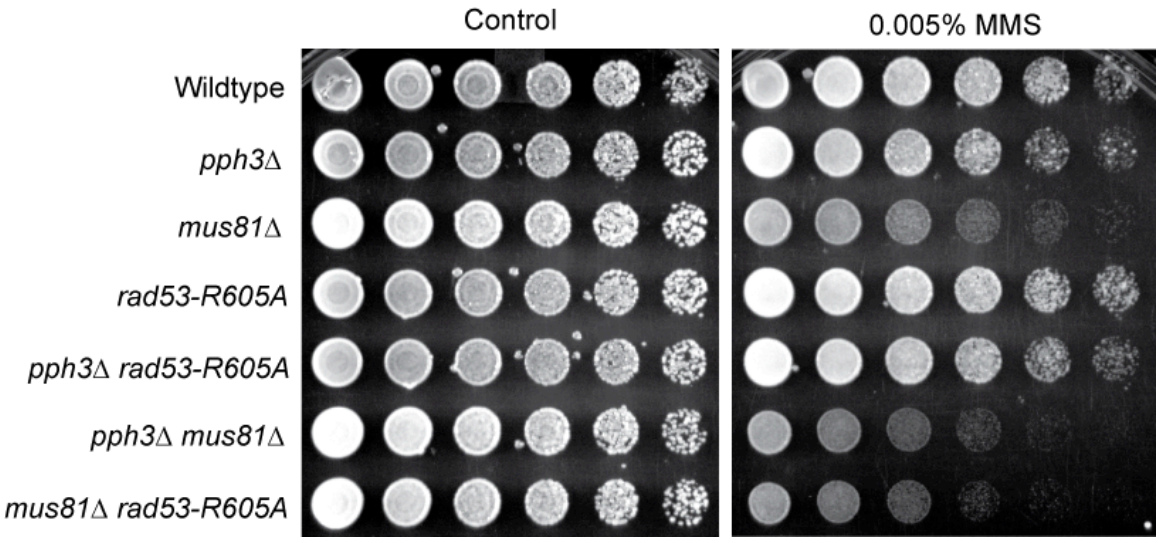
Control
0.005% MMS
Wildtype
pph3Δ
mus81Δ
rad53-R605A
pph3Δ rad53-R605A
pph3Δ mus81Δ
mus81Δ rad53-R605A

Figure S2 Effect of the presence of the *rad53-R605A* allele on the MMS sensitivity of indicated strains lacking. Four-fold serial dilutions were spotted on YPD plates and grown for 2–3 days at 30°C.

Tables S1-S2

Available for download as Excel files at www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.181479/-/DC1

Table S1. *S. cerevisiae* strains used in this study.

Table S2. Plasmids used in this study.