

Supporting material

The RNA binding protein Npl3 promotes resection of DNA double-strand breaks by regulating the levels of Exo1

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

Strain	Relevant genotype	Source
JKM139	<i>MATa ho hmlΔ::ADE1 hmrΔ::ADE1 adel-100 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL-HO</i>	[1]
YLL3466	JKM139 <i>npl3Δ::NATMX</i>	This study
184/10A	JKM139 <i>mec1Δ::HIS3 sml1Δ::KANMX</i>	[2]
YLL1854	JKM139 <i>MRE11-18MYC::TRP1</i>	[3]
DMP6178/9B	JKM139 <i>MRE11-18MYC::TRP1 npl3Δ::NATMX</i>	This study
YLL3187	JKM139 <i>XRS2-3HA::URA3</i>	[4]
DMP6195/2A	JKM139 <i>XRS2-3HA::URA3 npl3Δ::NATMX</i>	This study
YLL3501	JKM139 <i>RAD50-3HA::URA3</i>	[5]
DMP6196/1B	JKM139 <i>RAD50-3HA::URA3 npl3Δ::NATMX</i>	This study
YLL3101	JKM139 <i>SAE2-3HA::TRP1</i>	This study
DMP6179/8C	JKM139 <i>SAE2-3HA::TRP1 npl3Δ::NATMX</i>	This study
DMP6030/3A	JKM139 <i>SGS1-3HA::URA3</i>	This study
DMP6182/7B	JKM139 <i>SGS1-3HA::URA3 npl3Δ::NATMX</i>	This study
DMP5923/6A	JKM139 <i>DNA2-18MYC::TRP1</i>	[4]
DMP6180/3A	JKM139 <i>DNA2-18MYC::TRP1 npl3Δ::NATMX</i>	This study
YLL1959	JKM139 <i>EXO1-18MYC::TRP1</i>	[4]
DMP6010/3B	JKM139 <i>EXO1-18MYC::TRP1 npl3Δ::NATMX</i>	This study
DMP6010/6C	JKM139 <i>EXO1-18MYC::TRP1 npl3Δ::NATMX</i>	This study
YLL1540	JKM139 <i>exo1Δ::LEU2</i>	[3]
YLL3287	JKM139 <i>rrp6Δ::NATMX</i>	[4]
DMP6293/25D	JKM139 <i>rrp6Δ::NATMX npl3Δ::NATMX</i>	This study
DMP6293/30A	JKM139 <i>rrp6Δ::NATMX npl3Δ::NATMX</i>	This study
YLL3695.1	JKM139 <i>rrp6Δ::NATMX EXO1-18MYC::TRP1</i>	This study
DMP6293/37C	JKM139 <i>rrp6Δ::NATMX EXO1-18MYC::TRP1 npl3Δ::NATMX</i>	This study
YLL3467	JKM139 <i>NPL3-3HA::TRP1</i>	This study
YLL3012	JKM139 <i>DDC2-3HA::URA3</i>	[4]
DMP6009/5A	JKM139 <i>DDC2-3HA::URA3 npl3Δ::NATMX</i>	This study
DMP5991/13A	JKM139 <i>RFA1-18MYC::TRP1</i>	This study
DMP5991/3A	JKM139 <i>RFA1-18MYC::TRP1 npl3Δ::NATMX</i>	This study
YLL3096	JKM139 <i>MEC1-9MYC::TRP1</i>	[6]
DMP6238/5C	JKM139 <i>MEC1-9MYC::TRP1 npl3Δ::NATMX</i>	This study
YLL3526	JKM139 <i>RFA3-3HA::TRP1</i>	[4]
DMP6181/4A	JKM139 <i>RFA3-3HA::TRP1 npl3Δ::NATMX</i>	This study
YLL3222	JKM139 <i>TEL1-3HA::NAT</i>	[6]
DMP6590/19C	JKM139 <i>TEL1-3HA::NAT npl3Δ::NAT</i>	This study
344-115B2	<i>MATa his3-513::TRP1::his3-537 ura3-52 trp1 leu2</i>	[7]
YLL3873	<i>MATa his3-513::TRP1::his3-537 ura3-52 trp1 leu2 npl3Δ::KAN</i>	This study

Table S2. Primers used in this study

Primer name	Primer sequence
qRT-PCR on <i>EXO1</i> RNA	
PP1 For	5'- GACAAGCGGAAGACAAACTGAC - 3'
PP1 Rev	5'- TCCACAACAGTCATGGAGGG - 3'
PP2 For	5'- TTATTGGTGCCAGGTCACAG - 3'
PP2 Rev	5'- CGATGTCCCTTTTCTTACTTTCC - 3'
PP3 For	5'- GTCGAAATGCCTCAAGACAAG - 3'
PP3 Rev	5'- CAATGGCTTTTTCCCAAAGTAG - 3'
PP4 For	5'- GCGGTCTACATTCGCTATCAAC - 3'
PP4 Rev	5'- GTTCTGTGAGCCCGTTTGTC - 3'
PP5 For	5'- TGCCTTCTTATGCCGCTGTA - 3'
PP5 Rev	5'- AGTGGGATTGTGTCTACGCT - 3'
PP6 For	5'- TTTTCCAAGAGCAGCAG - 3'
PP6 Rev	5'- GCCAAGGCGAGTAGTAGAA - 3'
5' RACE on <i>EXO1</i> RNA	
QT	5'- CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCTTTTTTTTTT TTTTTTT - 3'
QO	5'- CCAGTGAGCAGAGTGACG - 3'
QI	5'- GAGGACTCGAGCTCAAGC - 3'
EXO1NEST1	5'- CGGTAATCAGGCCAACCT - 3'
EXO1NEST2	5'- GGAATGGCATCACCATCG - 3'
ChIP analysis at DSB	
DSB1 For	5'- CATGCGGTTACATGACTTT - 3'
DSB1 Rev	5'- CACCCAAGAAGGCGAATAAG - 3'
DSB3 For	5'- CGAGGAAAATGGTGGGATAA - 3'
DSB3 Rev	5'- GGACGACTTTAAGATGGAAGGA - 3'
ARO For	5'- TGAGTCGTTACAAGGTGATGCC - 3'
ARO Rev	5'- ACCTACAGGAGGACCCGAAA - 3'
HO-cut efficiency at <i>MAT</i> locus	
HOCUT For	5'- GTGGCATTACTCCACTTCAA - 3'
HOCUT Rev	5'- TCACCACGTACTTCAGCATA - 3'
TRP3 For	5'- CATCCTGCTTGAAGGTTACT - 3'
TRP3 Rev	5'- ATCATCAGATGACCTTCCTC - 3'

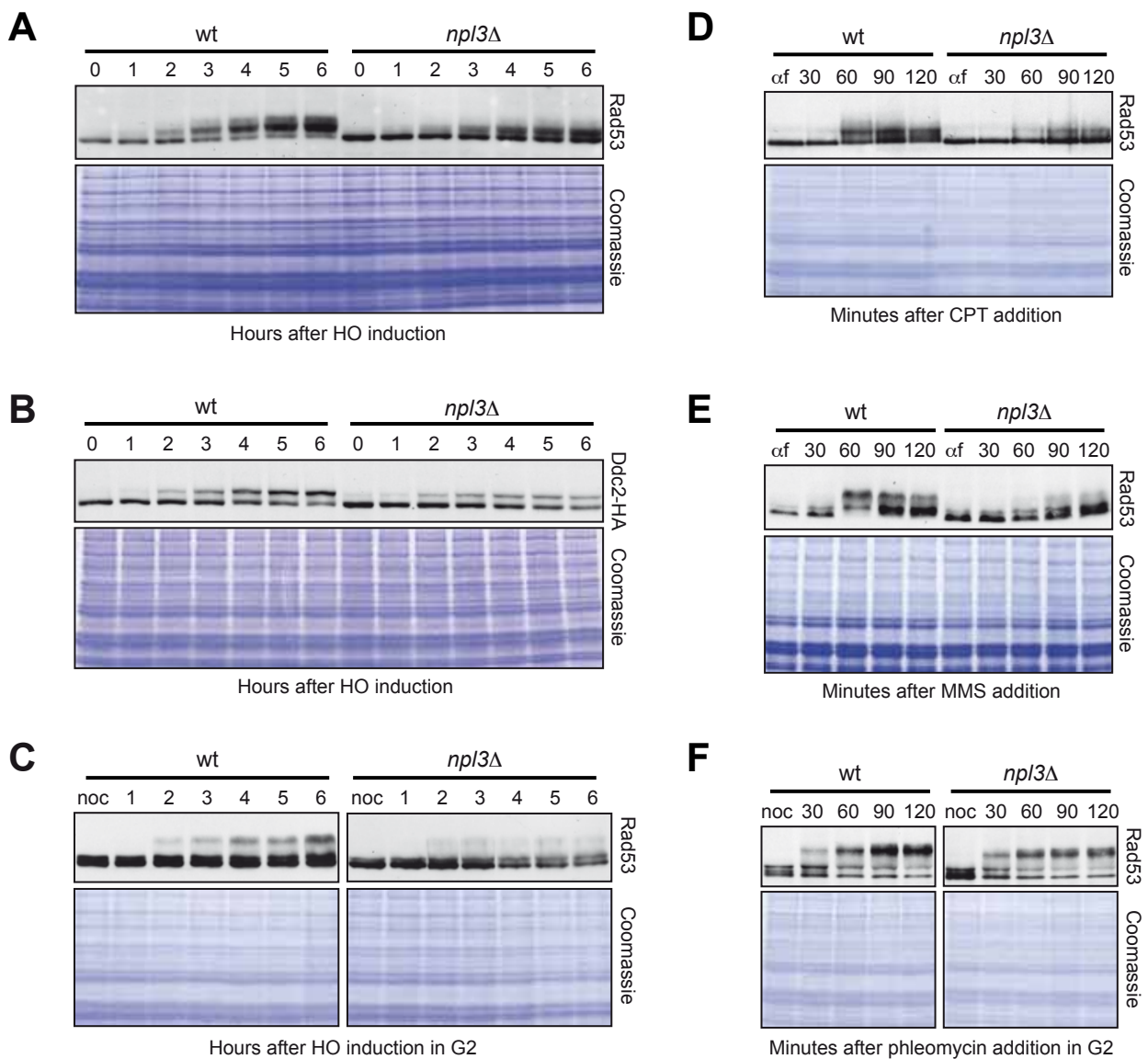


Figure S1. Loading control of western blot in Figure 1. (A-C) Galactose was added to exponentially growing (A,B) or G2-arrested (C) YEPY cell cultures to induce HO. Protein extracts were subjected to western blot with anti-Rad53 (A,C) or anti-HA (B) antibodies or stained with Coomassie as a loading control. (D-E) YEPY G1-arrested cell cultures (α f) were released in fresh medium containing CPT (50 μ M) (D) or MMS (0,02%) (E). Protein extracts were subjected to western blot with anti-Rad53 antibodies or stained with Coomassie. (F) Phleomycin (15 μ g/ml) was added to YEPY G2-arrested cell cultures in the presence of nocodazole to maintain the G2 arrest. Protein extracts were analyzed by western blot with anti-Rad53 antibodies or stained with Coomassie.

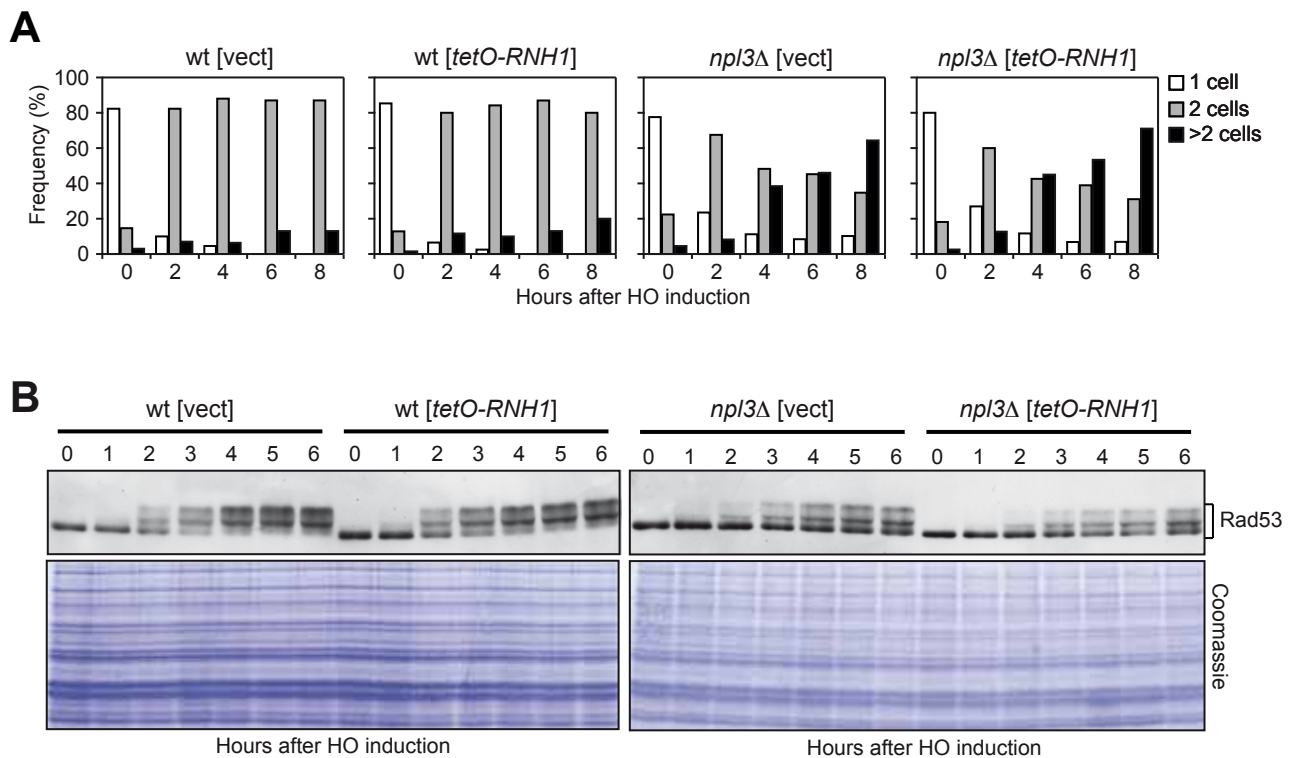


Figure S2. High levels of RNase H1 do not suppress the checkpoint defect of cells lacking Npl3. (A) Exponentially growing cell cultures of wild type JKM139 and an otherwise isogenic *npl3*Δ strain, both carrying a centromeric plasmid either expressing the *RNH1* gene from the *tetO* promoter or empty (vect), were arrested in G1 with α -factor and plated on galactose-containing plates (time zero) to follow microcolonies formation. (B) YEPR exponentially growing cell cultures were transferred in YEPRG to monitor Rad53 phosphorylation by western blot. The same amounts of protein extracts were separated on SDS-PAGE and stained with Coomassie as a loading control.

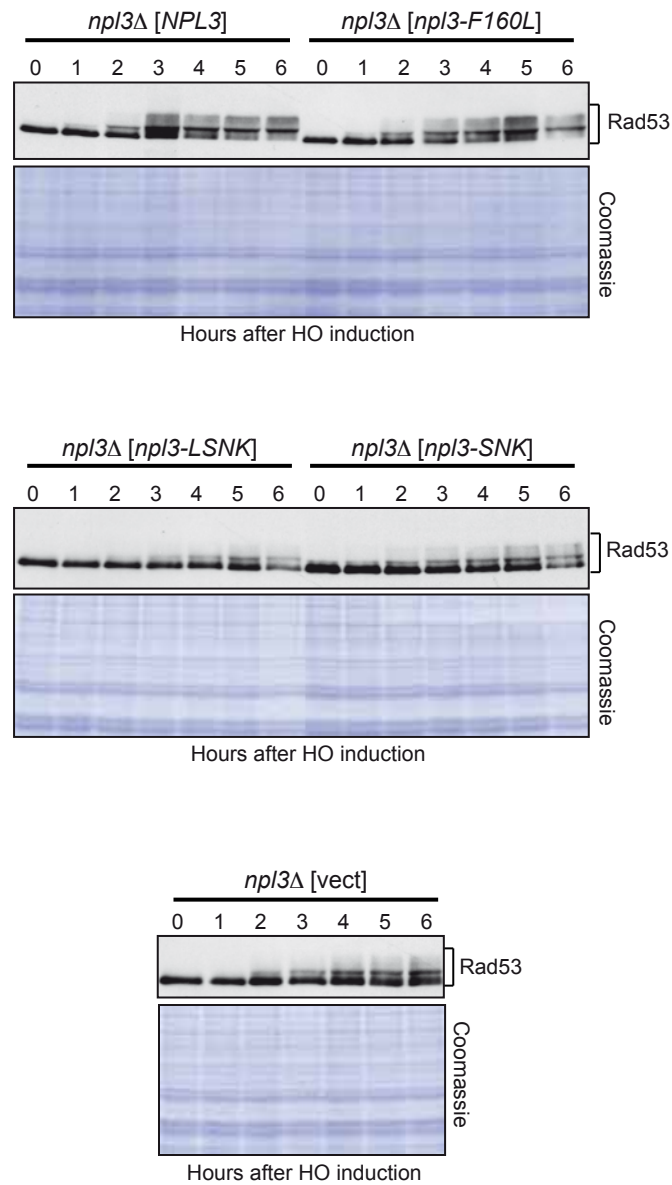


Figure S3. Loading control of western blot in Figure 3. Exponentially growing YEPR cell cultures were transferred to YEPRG at time zero. Protein extracts were subjected to western blot analysis with anti-Rad53 antibodies or stained with Coomassie as a loading control.

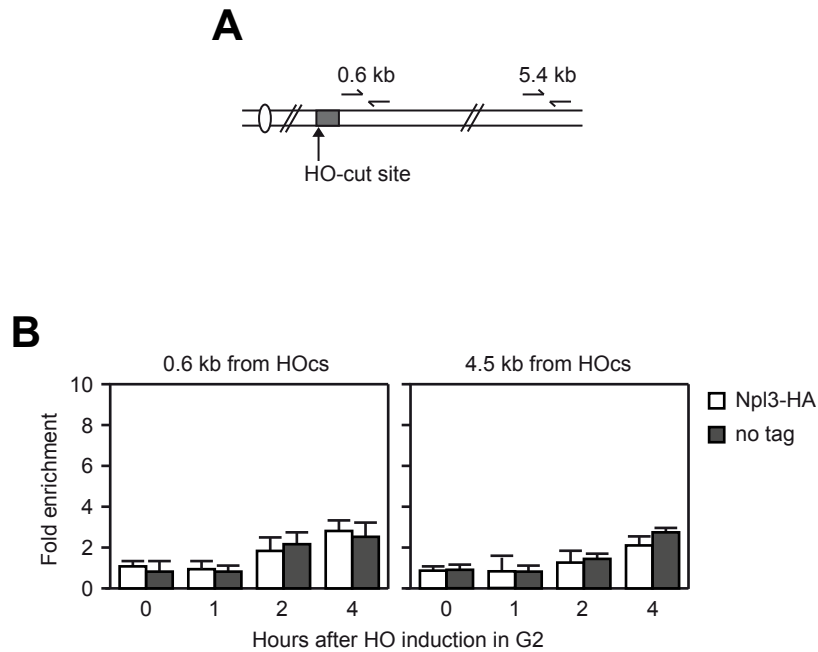


Figure S4. Npl3 is not enriched at an HO-induced DSB. (A) Schematic representation of *MAT* locus on chromosome III. The relative positions of the HO cleavage site and of primer pairs used for chromatin immunoprecipitation (ChIP) are shown. (B) G2-arrested YEPR wild type (no tag) and *NPL3-HA* cell cultures were transferred to YEPRG in the presence of nocodazole to maintain the G2 block and subjected to ChIP analysis with anti-HA antibodies and subsequent qPCR. Relative fold enrichment of the Npl3-HA fusion protein at the indicated distances from the HO cleavage site was determined. Plotted values are the mean values +SD (n=3).

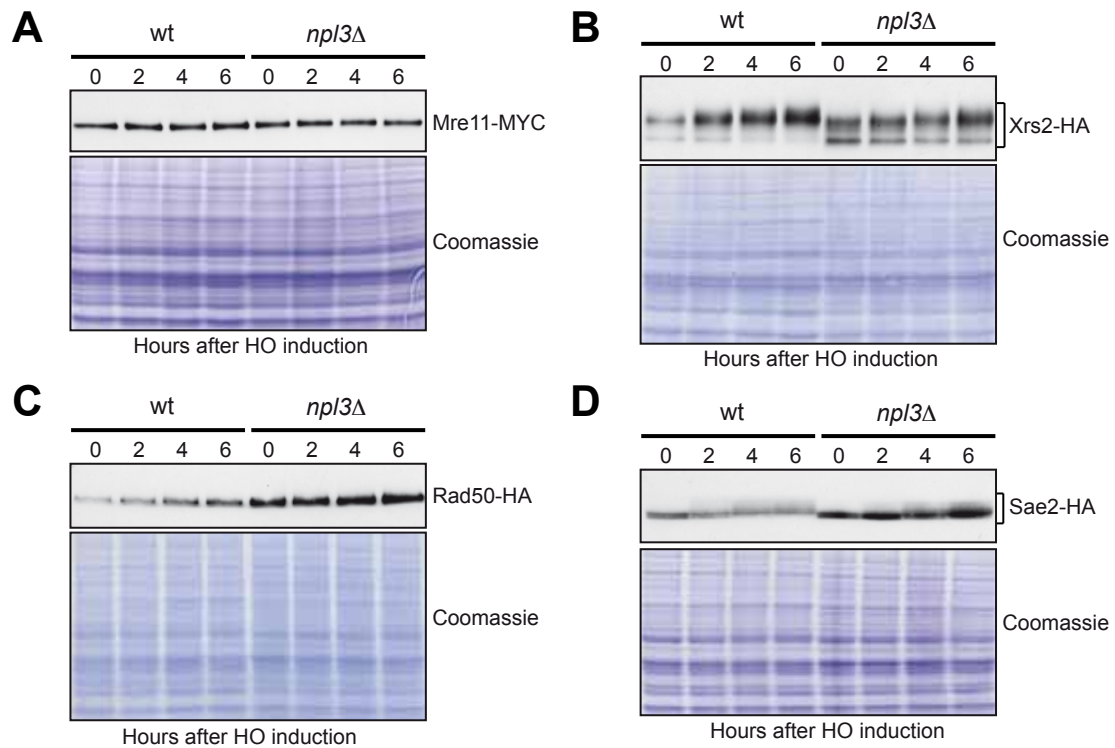


Figure S5. Levels of resection proteins in the absence of Npl3. Exponentially growing YEPR cell cultures of JKM139 derivative strains expressing the indicated tagged proteins were transferred in YEPRG at time zero. Protein extracts were subjected to western blot with antibodies specific for the indicated tags. The same amounts of protein extracts were separated on SDS-PAGE and stained with Coomassie as a loading control.

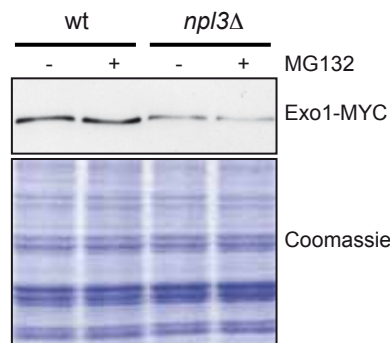


Figure S6. Exo1 is not degraded by proteasome in the absence of Npl3. Protein extracts prepared from exponentially growing YEPD cell cultures treated with the proteasome inhibitor MG132 (75 μM) for 3 hours (+) or untreated (-) were subjected to western blot analysis with anti-MYC antibodies. The same amounts of protein extracts were separated on SDS-PAGE and stained with Coomassie as a loading control.

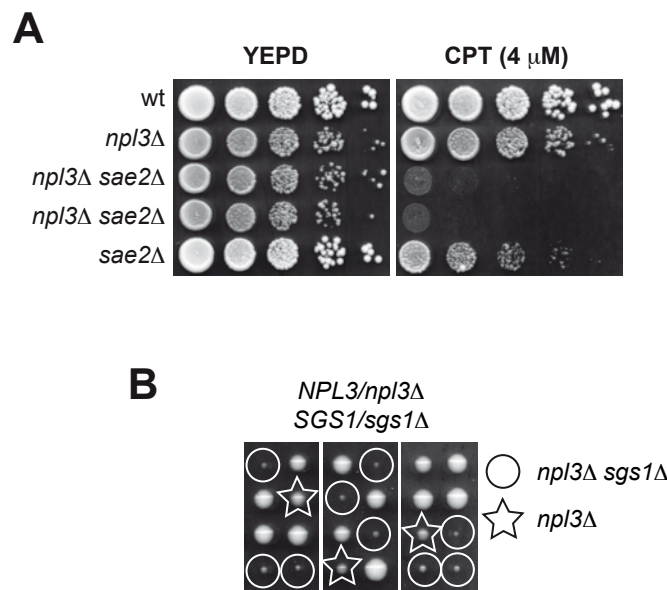


Figure S7. The lack of Npl3 increases the defects of cells lacking Sae2 or Sgs1. (A) Exponentially growing cell cultures of JKM139 derivative strains were serially diluted (1:10) before being spotted out onto YEPD plates with or without CPT. (B) Meiotic tetrads from diploid cells with the indicated genotype were dissected on YEPD plates that were incubated at 30°C for 3 days, followed by spore genotyping.

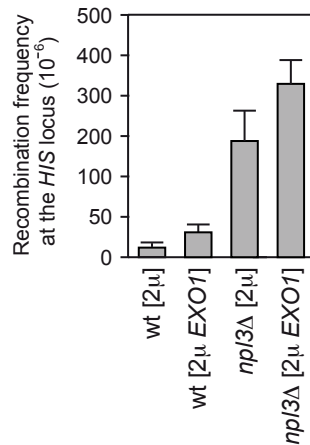


Figure S8. Exo1 high levels do not reduce the elevated frequency of mitotic recombination caused by Npl3 lack. Wild type and *npl3* Δ strains carrying the *his3-513::TRP1::his3-537* heteroallelic duplication on chromosome XV were transformed with either a *EXO1* 2 μ plasmid or an empty vector (2 μ). 10 independent clones for each strain were plated on complete medium plates to evaluate their viability and on plates lacking histidine to select the His⁺ recombinants generated by mitotic recombination at the *HIS3* locus. The number of the His⁺ colonies was evaluated and normalized to the viability of the strain to determine the recombination frequency.

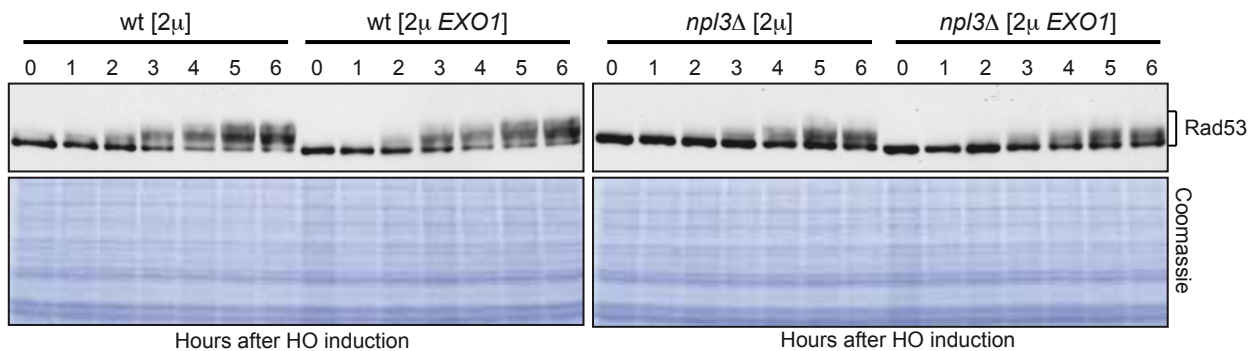


Figure S9. Loading control of western blot in Figure 5. Exponentially growing YEPR cell cultures were transferred in YEPRG at time zero. Protein extracts were subjected to western blot with anti-Rad53 antibodies or stained with Coomassie as a loading control.

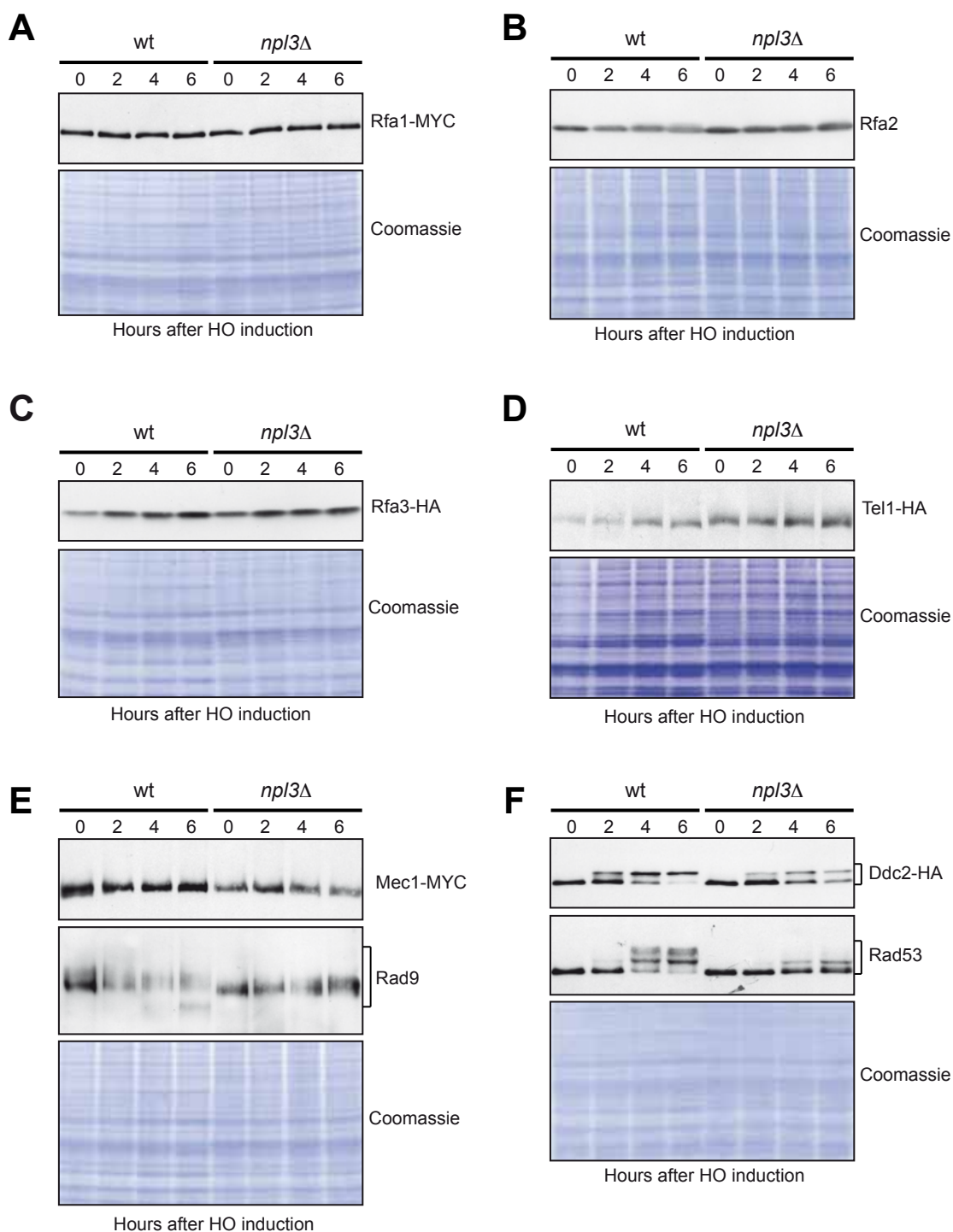


Figure S10. Levels of checkpoint proteins in the absence of Npl3. Exponentially growing YEPR cell cultures of JKM139 derivative strains expressing the indicated tagged proteins were transferred in YEPRG at time zero. Protein extracts prepared at different time points after HO induction were subjected to western blot analysis with antibodies specific for the indicated proteins or tags. The same amounts of protein extracts were separated on SDS-PAGE and stained with Coomassie as a loading control.

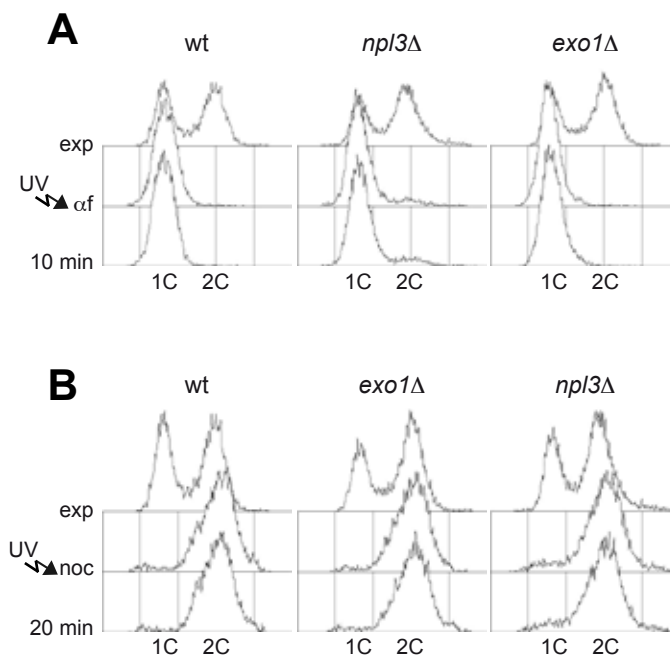


Figure S11. Cell cycle arrests of cells treated with UV. (A) Exponentially growing YEPD cell cultures of wild type JKM139 and otherwise isogenic *npl3Δ* and *exo1Δ* strains (exp) were arrested in G1 with α -factor (αf), UV irradiated (75 J/m^2), and held in G1 in the presence of α -factor. FACS analyses of DNA content to verify the cell cycle arrest in G1. (B) Exponentially growing YEPD cell cultures of the strains in (A) (exp) were arrested in G2 with nocodazole (noc), UV irradiated (75 J/m^2), and held in G2 in the presence of nocodazole. FACS analyses of DNA content to verify the cell cycle arrest in G2.

References

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