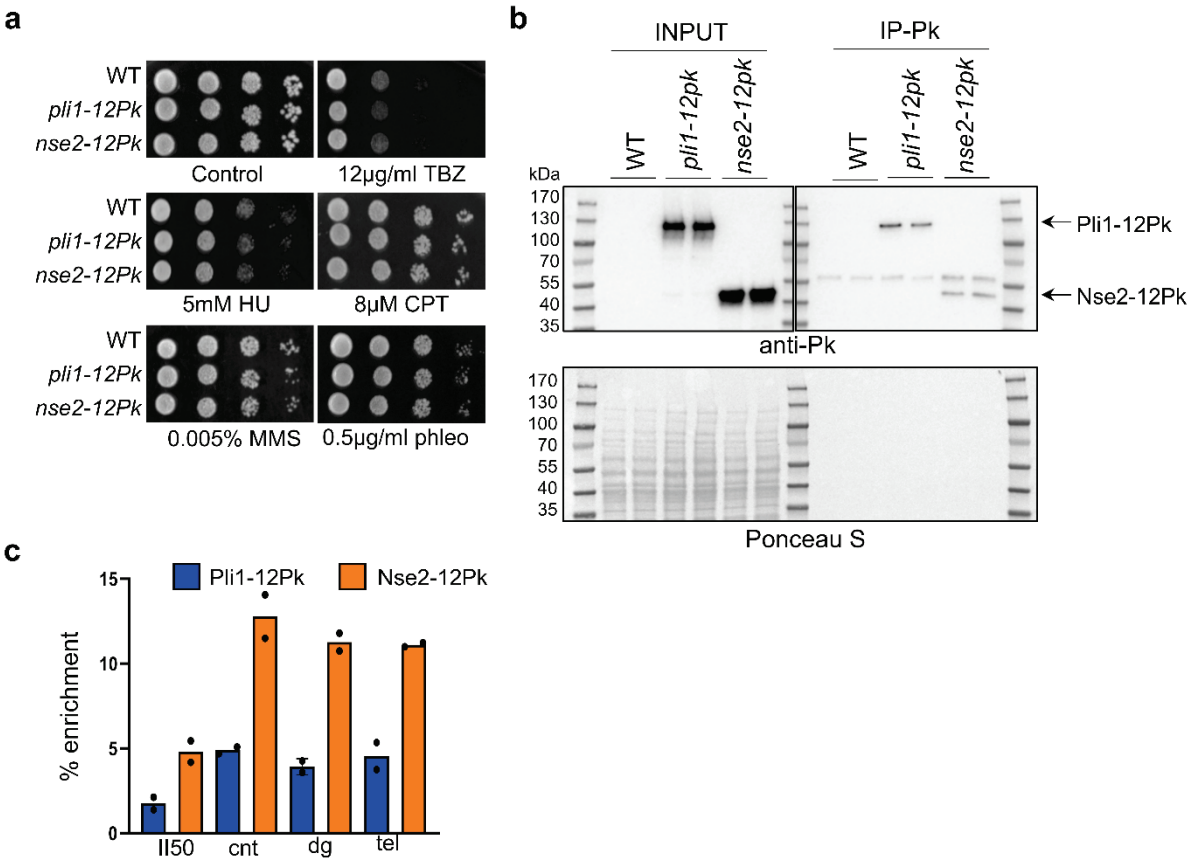
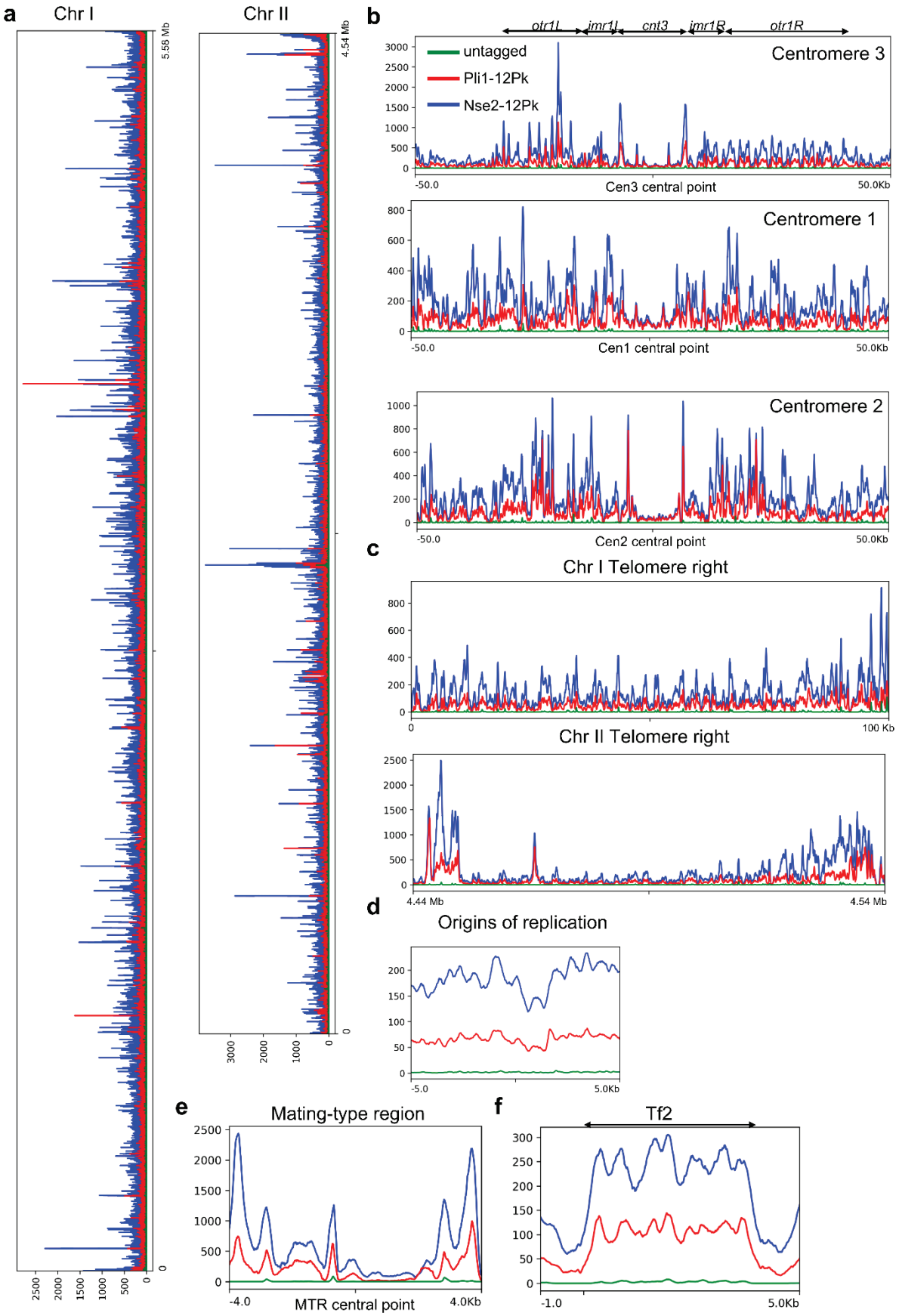
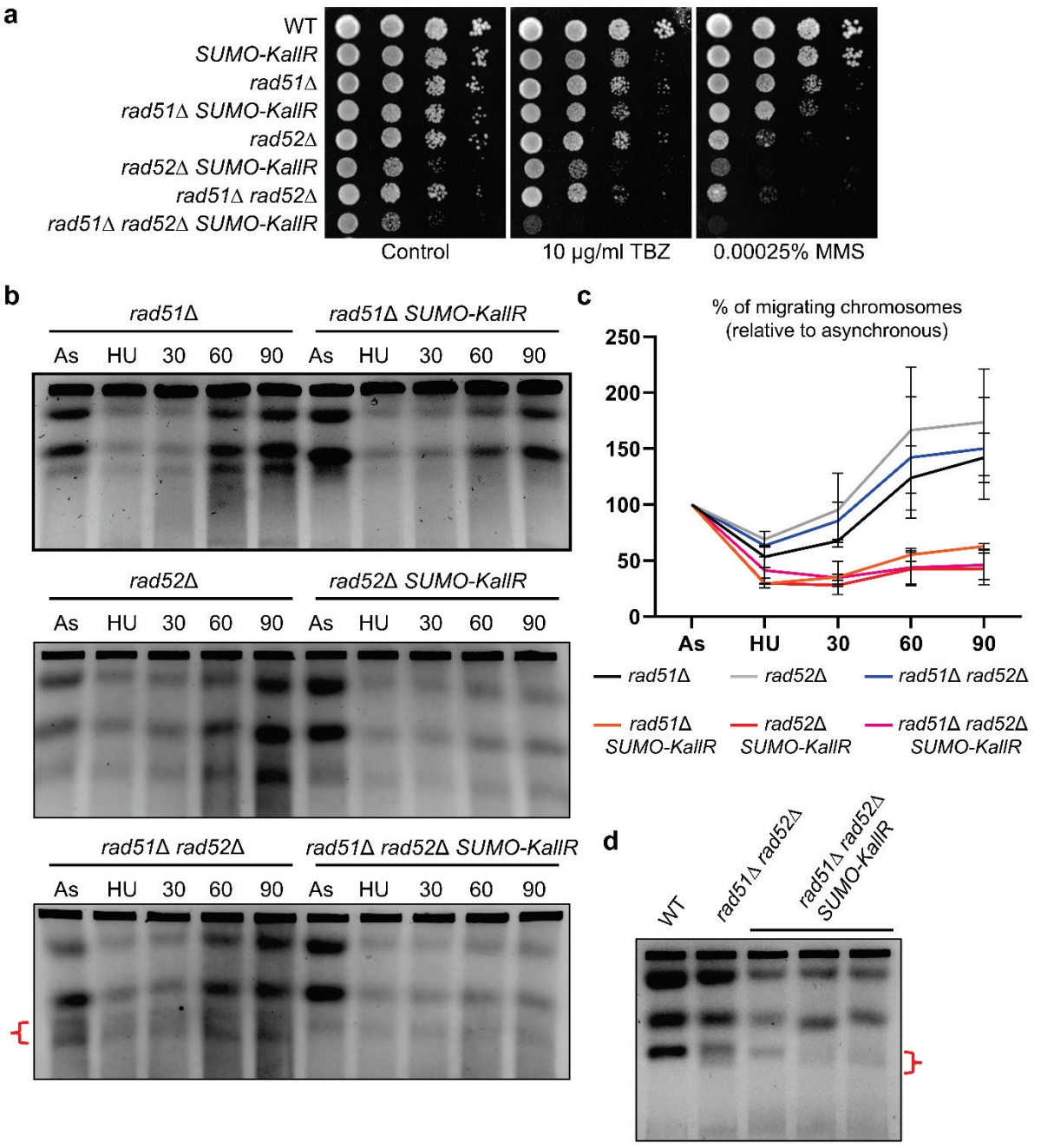


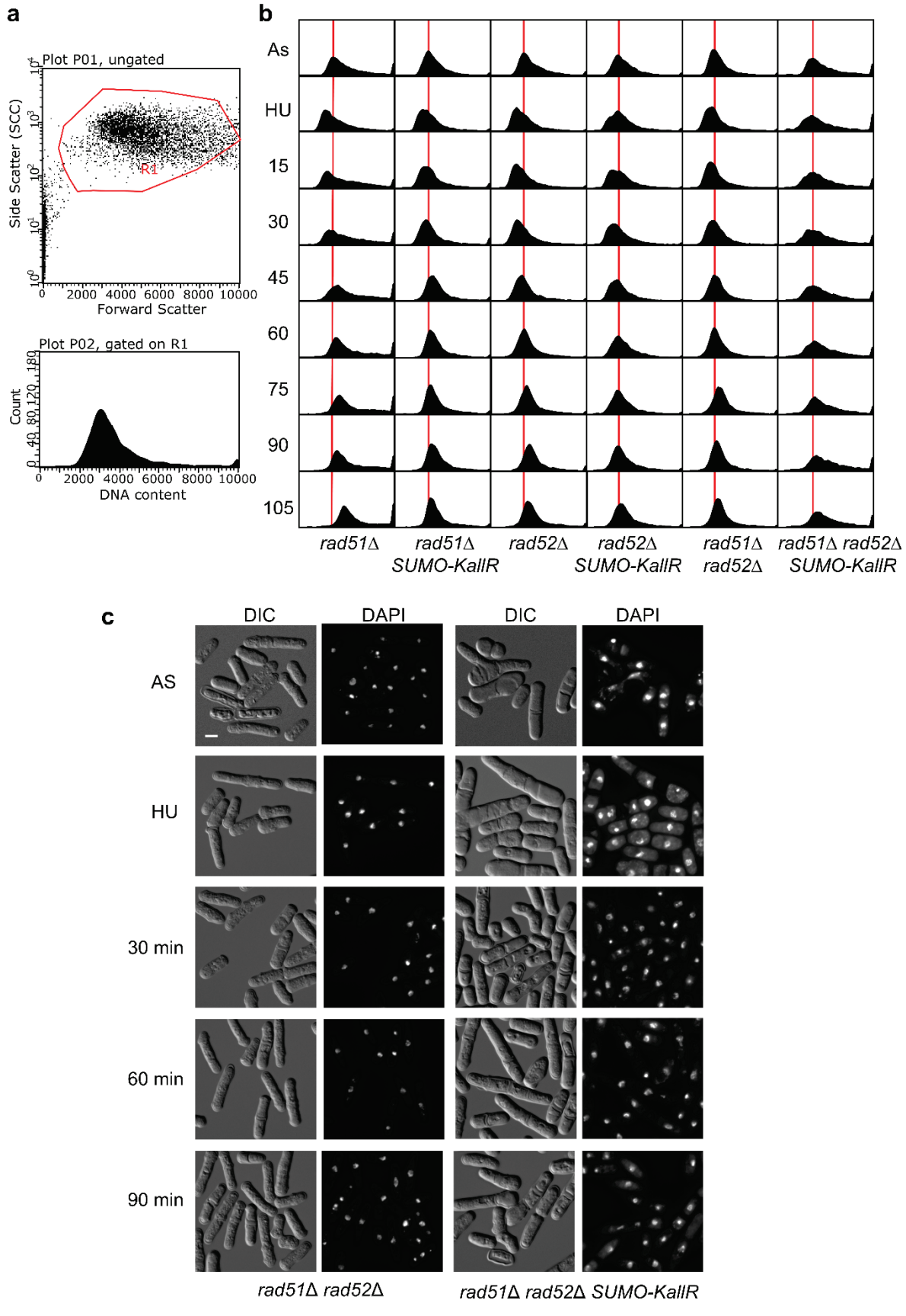
Supplementary Figure 1: Validation of Pli1-12Pk and Nse2-12Pk strains and chromatin immunoprecipitation controls. a Verification of Pli1-12Pk and Nse2-12Pk functionality. Ten-fold dilutions of indicated strains were spotted on plates containing TBZ, HU, CPT, MMS or Phleomycin (phleo) at indicated concentrations. **b** Pulldown of Pli1-12Pk and Nse2-12Pk performed using anti-Pk antibodies. Protein extracts (INPUT) and immunoprecipitated samples (IP-Pk) were analyzed by Western blot using anti-Pk antibodies. Ponceau S staining of the membrane was used as a loading control. **c** Binding of Pli1-12Pk and Nse2-12Pk to the indicated loci analyzed by ChIP-qPCR using anti-Pk antibody. Chromosome II intergenic locus (II-50) was used as internal specificity control; cnt – core of centromere, dg – outer repetitive sequences of centromere, tel – telomere. Dots represent two independent biological replicates; bars indicate the mean.


a
Chr I
Chr II
5.58 Mb
4.54 Mb
b
otr1L
imr1L
cnt3
imr1R
otr1R
untagged
Pli1-12Pk
Nse2-12Pk
Centromere 3
-50.0
Cen3 central point
50.0Kb
Centromere 1
Cen1 central point
Centromere 2
Cen2 central point
c
Chr I Telomere right
100 Kb
Chr II Telomere right
4.44 Mb
4.54 Mb
d
Origins of replication
-5.0
5.0Kb
e
Mating-type region
-4.0
MTR central point
4.0Kb
f
Tf2
-1.0
5.0Kb

Supplementary Figure 2: Analysis of chromatin distribution of Nse2 and Pli1. Calibrated ChIP-seq profiles of Pli1-12Pk, Nse2-12Pk and untagged control across fission yeast chromosomes. Logarithmic cultures of indicated strains were mixed with budding yeast calibrator aliquot (exponential culture of Ku70-cMyc) and extracted DNA was used to construct DNA libraries and NGS analysis. Red line corresponds to signal obtained for Pli1-12Pk, blue line – Nse2-12Pk, green line – untagged control. **a** Binding of SUMO ligases across chromosome I and II. **b** Enrichment of Pli1 and Nse2 at centromeres of chromosomes I, II, III. **c** Enrichment of Pli1 and Nse2 at right telomeres of chromosomes I and II. **d** Analysis of Nse2 and Pli1 enrichment at active replication origins (averaged). **e** Enrichment of Pli1 and Nse2 at Mating type locus. **f** Association of Nse2 and Pli1 to Tf2 retrotransposons.

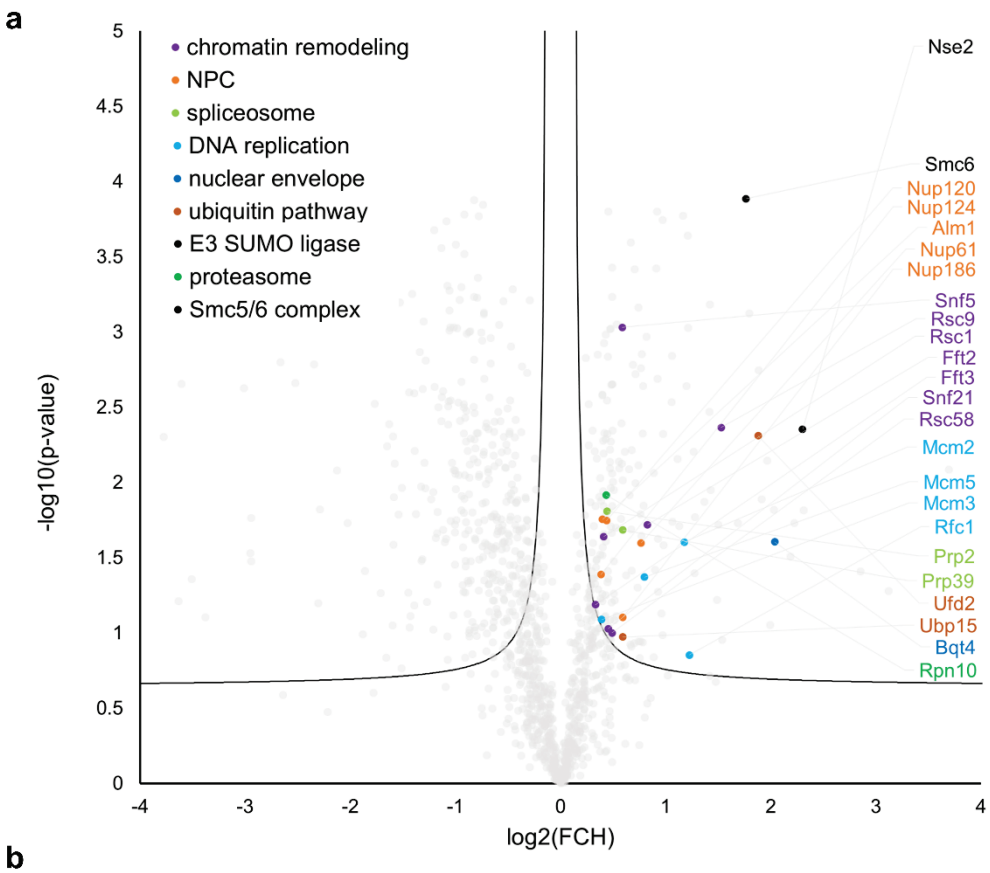


Supplementary Figure 3: SUMO chains support DNA replication independently of homologous recombination during recovery from hydroxyurea. a A drop dilution assay of indicated mutants. Ten-fold dilutions of strains were spotted on plates containing MMS or TBZ at indicated concentrations. **b** Representative images of whole chromosome migration during pulsed-field gel electrophoresis (PFGE) in indicated mutants. Logarithmically growing cells (As, asynchronous cells) were exposed to 20 mM HU for 4h (HU time point) and then released into fresh, HU-free, rich YES medium at 30^{o}C to monitor recovery of chromosomes 30, 60 and 90 min after drug removal. Red bracket – fragmentation of chromosome III. **c** % quantification of chromosomes migrating into the gel after release from HU block relative to their asynchronous profile. Values are means of 2 independent biological replicates ± SD. **d** Changes in the chromosome III length in indicated strains. DNA plugs for PFGE were prepared from the asynchronous cells of tested strains. Red bracket marks unstable chromosome III.



Supplementary Figure 4: SUMO chains enable completion of DNA replication in HR-deficient cells. a Gating strategy for conducted flow cytometry analysis; upper panel: ungated SSC-FSC plot; lower panel:

gated histogram based on SSC-FSC plot. **b** Flow cytometry analysis of DNA content in indicated strains. Logarithmically growing cells (As, asynchronous cells) were exposed to 20 mM HU for 4 h (HU time point) and then released into fresh, HU-free, rich YES medium at 30°C to monitor S-phase progression in the 15 min intervals after release. Red line marks G2 phase in asynchronous cells. **c** Representative fluorescence microscopy images of the indicated strains. Cells were treated as in (**Supplementary Fig. 3b**), then collected at 30 min intervals after release, fixed with ethanol, and stained with DAPI to visualize nuclei. Differential interference contrast (DIC) images show cell morphology. Scale bar = 5 μm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NPC
spliceosome
DNA replication
nuclear envelope
ubiquitin pathway
E3 SUMO ligase
proteasome
Smc5/6 complex
Nse2
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Nup124
Alm1
Nup61
Nup186
Snf5
Rsc9
Rsc1
Fft2
Fft3
Snf21
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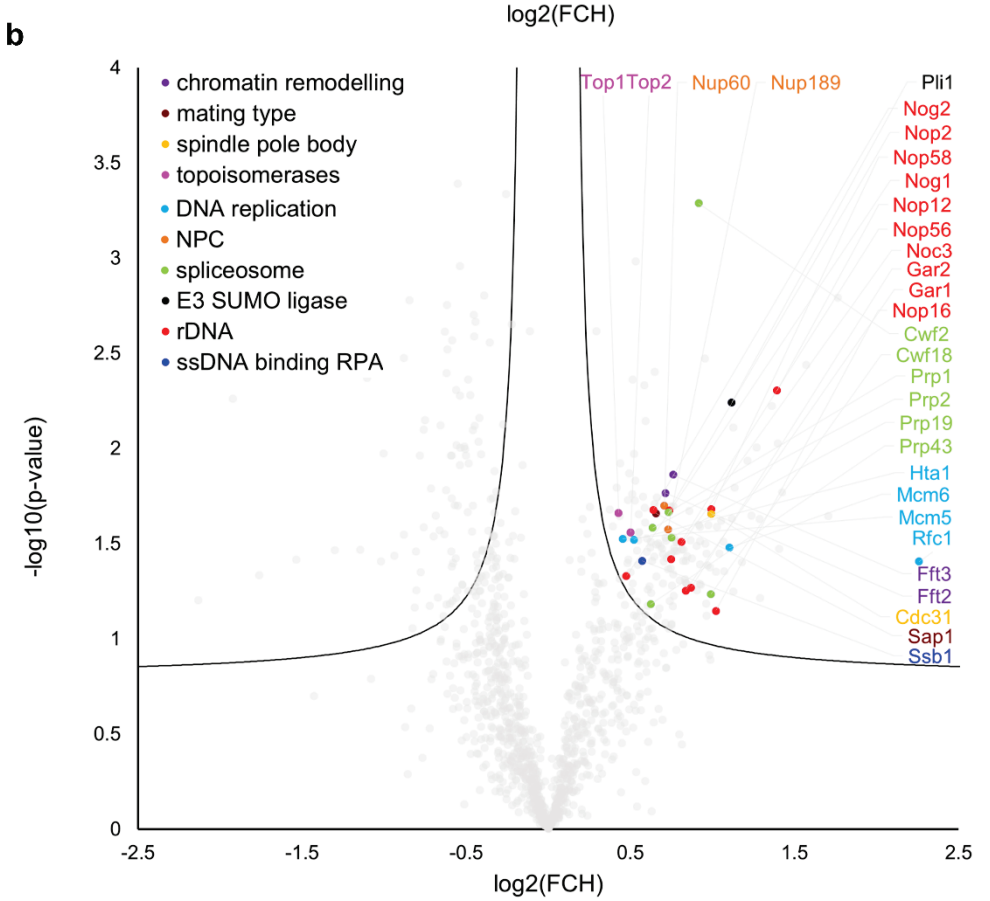
Ufd2
Ubp15
Bqt4
Rpn10
-log10(p-value)
log2(FCH)


b
chromatin remodelling
mating type
spindle pole body
topoisomerases
DNA replication
NPC
spliceosome
E3 SUMO ligase
rDNA
ssDNA binding RPA
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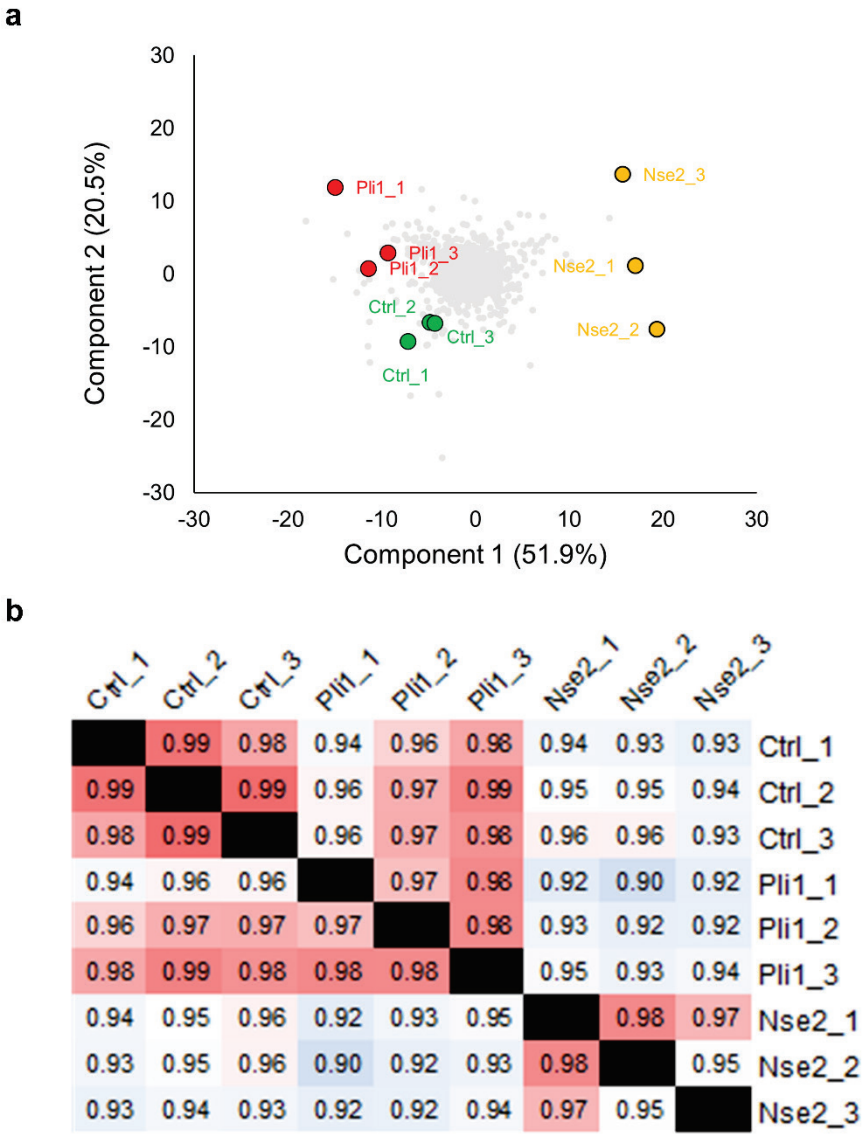
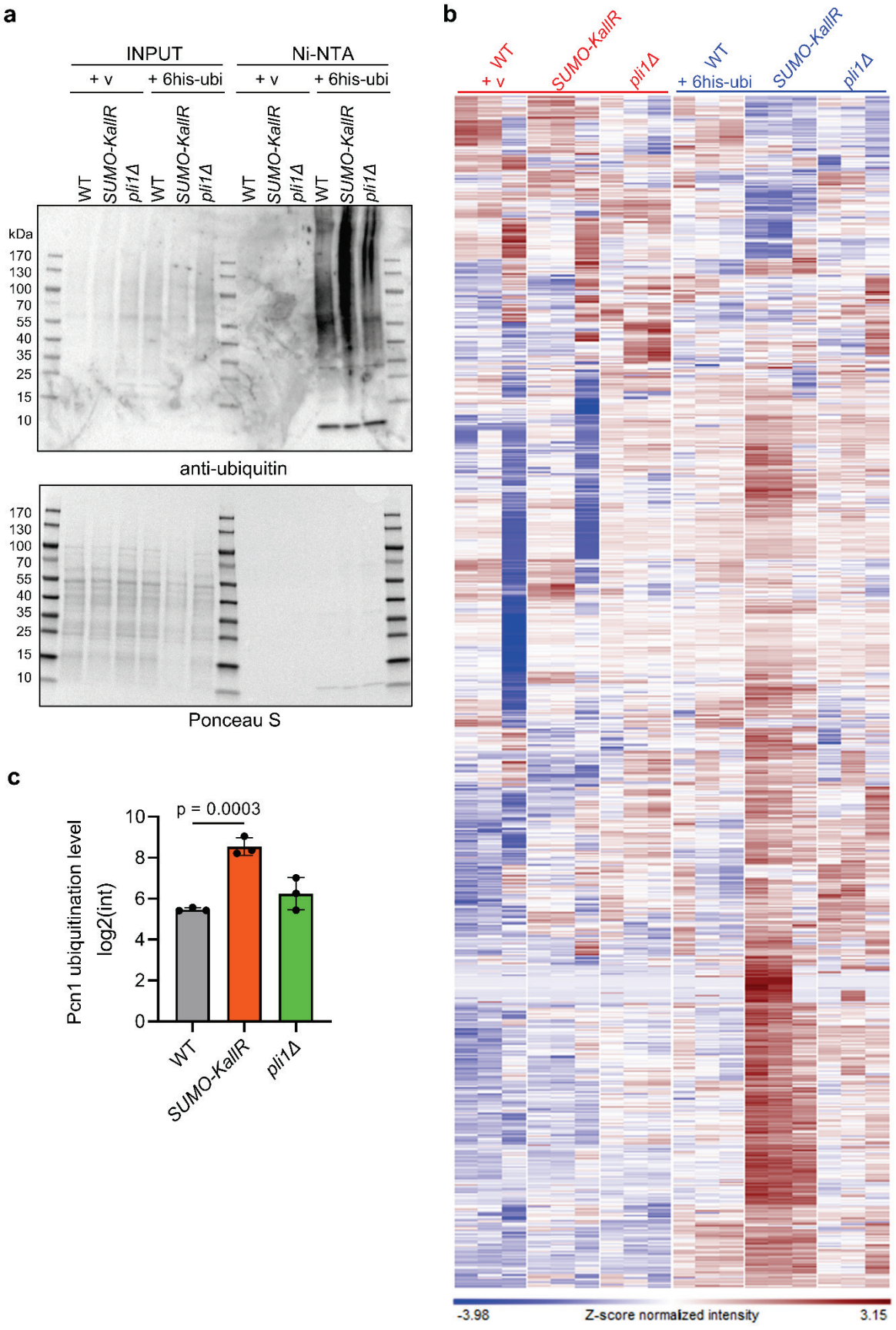
Nup60
Nup189
Pli1
Nog2
Nop2
Nop58
Nog1
Nop12
Nop56
Noc3
Gar2
Gar1
Nop16
Cwf2
Cwf18
Prp1
Prp2
Prp19
Prp43
Hta1
Mcm6
Mcm5
Rfc1
Fft3
Fft2
Cdc31
Sap1
Ssb1
-log10(p-value)
log2(FCH)

Supplementary Figure 5: Analysis of Nse2 and Pli1 interaction networks. Results of three independent proteomic analyses of the Pk pulldown of respective SUMO ligase. **a** Significantly enriched proteins (top right-hand side) are identified as possible Nse2-12Pk interactors. **b** Significantly enriched proteins (top right-hand side) are identified as possible Pli1-12Pk interactors. All hits were grouped according to their cellular function based on the PantherDB GO Terms. Functions of interest are shown on the plots.



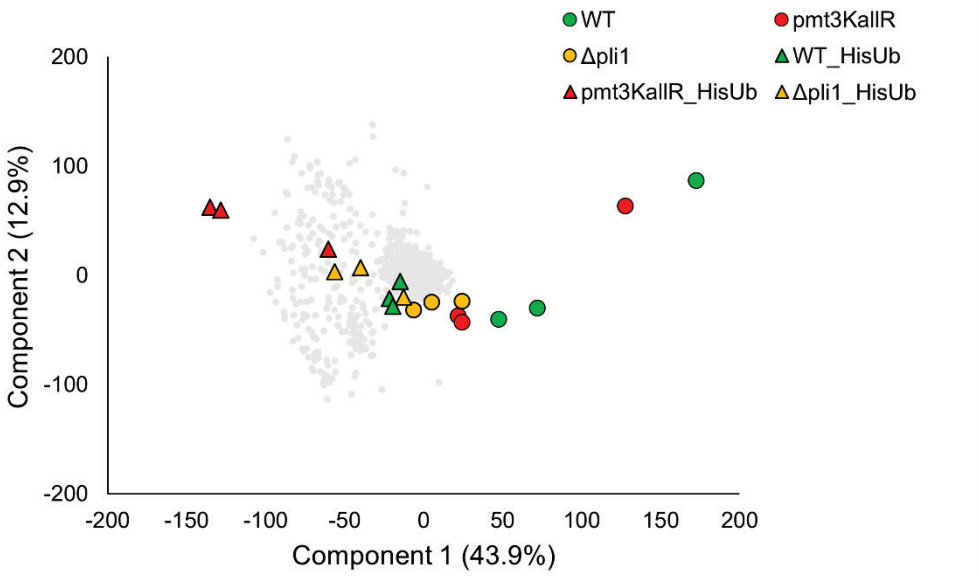
Supplementary Figure 6: Reproducibility analyses related to Supplementary Fig. 5 (pulldown of Nse2-12Pk and Pli1-12Pk). a Principal component analysis (PCA) of protein log2 intensities for independent biological replicates from proteomic analyses of the indicated strains: untagged (Ctrl), Pli1-12Pk and Nse2-12Pk. Small grey points indicate each protein's resultant cluster, while large colored points with black outlines indicate each sample's resultant cluster. **b** Protein log2 intensity Pearson correlation coefficient matrix for independent biological replicates from proteomic analyses of the indicated strains: untagged (Ctrl), Pli1-12Pk and Nse2-12Pk.



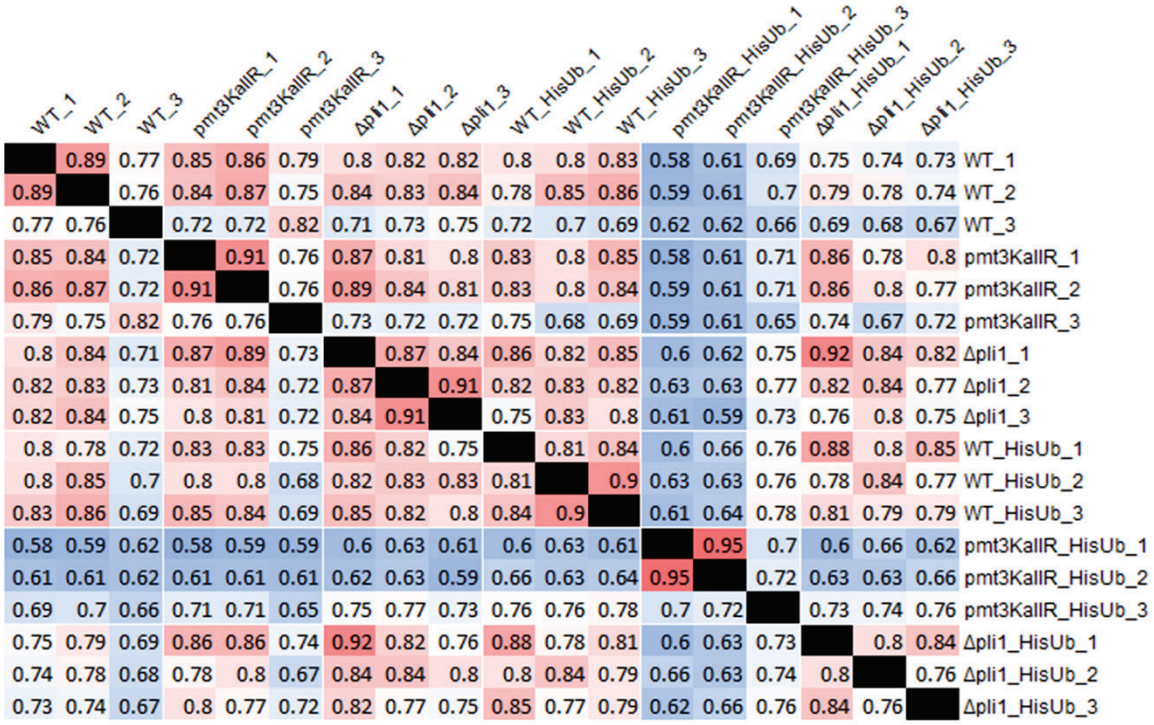
Supplementary Figure 7: Changes in protein ubiquitination upon SUMO chain depletion and *pli1+* deletion (related to Figure 4). a Ubiquitination pattern from indicated strains transformed with empty vector (marked as '+v') or pREP1-6His-ubiquitin (marked as '+6his-ubi'). Cells were disrupted in

denaturing conditions and subjected to Ni-NTA pulldown. Ubiquitination status of precipitated proteins was examined with anti-ubiquitin antibody. Ponceau S was included for both Western blots to show the amount of protein loaded onto the gels. Representative blots from n=3 biological replicates used for mass spectrometry analysis. **b** Heat map presenting the effects of SUMO chain depletion and Pli1 deletion on ubiquitination of proteins. Ubiquitination pattern from indicated strains transformed with empty vector (marked as '+v', in red font) or pREP1-6His-ubiquitin (marked as '+6his-ubi', in blue font). Only statistically significant changes are presented. **c** Ubiquitination pattern of endogenous Pcn1 (spPCNA) in background devoid of SUMO chains or *pli1+* deletion. Single values represent 3 independent biological replicates for each sample.

a

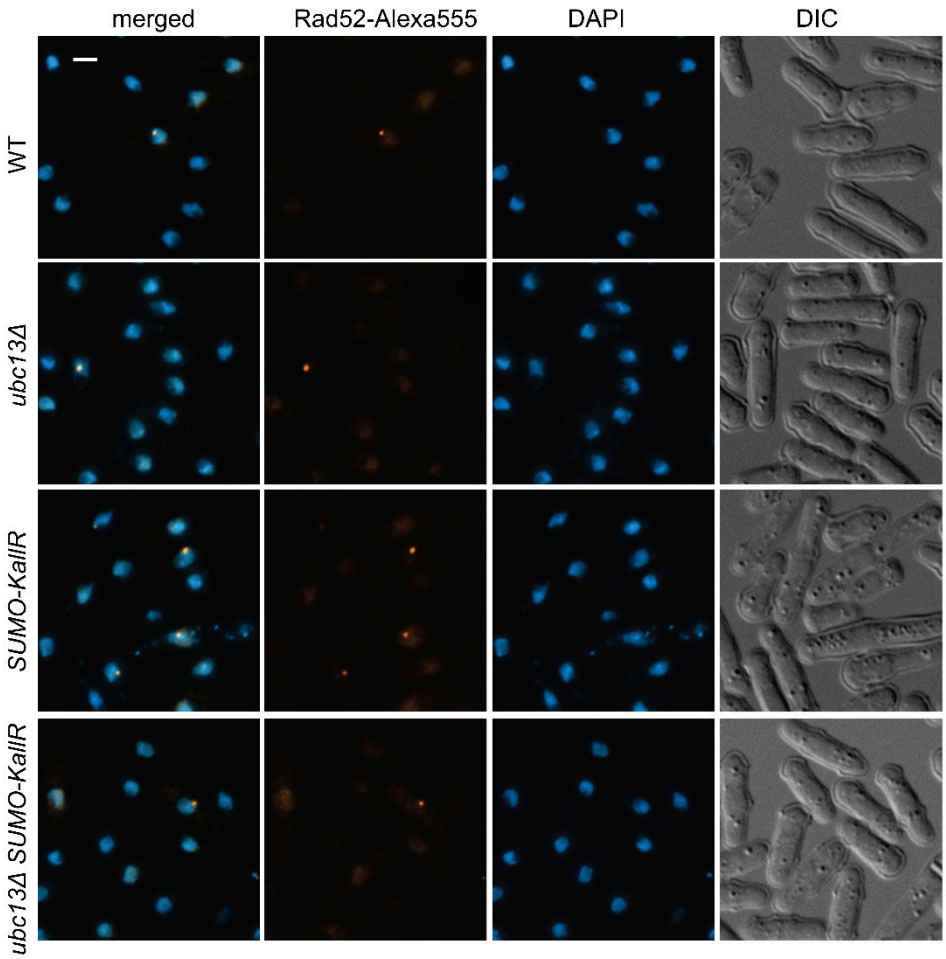


b



Supplementary Figure 8: Reproducibility analyses (related to Figure 4 and Supplementary Figure 7). a Principal component analysis (PCA) of protein log2 intensities for independent biological replicates from proteomic analyses of the indicated strains: WT, *pli1Δ* and *SUMO-KallR* (pmt3KallR) transformed with empty vector or pREP1-6His-ubiquitin (marked as _HisUb). Small grey points indicate each protein's resultant cluster, while large colored points with black outlines indicate each sample's resultant cluster. **b** Protein log2 intensity Pearson correlation coefficient matrix for independent biological replicates from

proteomic analyses of the indicated strains: WT, *pli1Δ* and *SUMO-KallR* (pmt3KallR) transformed with empty vector or pREP1-6His-ubiquitin (marked as _HisUb).



Supplementary Figure 9: Examples of immunofluorescent images of Rad52 in tested strains (related to Figure 5). Examples of immunofluorescence images of logarithmic strains probed with anti-Rad52 (primary) and anti-Rabbit Alexa Fluor 555 (secondary) antibody. Chromatin was stained with DAPI fluorescent dye. Scale bar = 5 µm.

Supplementary Table 1: Strains and plasmids used in this study

Strain number	Genotype	Source
IDN1	*ade6-704 leu1-32 ura4-D18, h+*	S. Lambert
IDN365	*ade6-704 leu1-32 ura4-D18 pmt3-KallR, h+*	Markowska et al., 2025
IDN375	*leu1-32 ura4-D18 gar2-mCherry:Kan rad52-YFP:Kan, h?*	this study
IDN378	*leu1-32 ura4-D18 gar2-mCherry:Kan rad52-YFP:Kan pmt3-KallR, h?*	this study
IDN372	*ade6-704 leu1-32 ura4-D18 rad52-YFP:Kan, h?*	S. Lambert
IDN373	*ade6-704 leu1-32 ura4-D18 rad52-YFP:Kan pmt3-KallR, h?*	Markowska et al., 2025
IDN470	*ade6-704 leu1-32 ura4-D18 pcn1-K164R h?*	S. Lambert
IDN486	*ade6-704 leu1-32 ura4-D18 pcn1-K164R pmt3-KallR h?*	Markowska et al., 2025
IDN180	*ade6-704 leu1-32 ura4-D18 rad51::Kan, h?*	S. Lambert
IDN318	*ade6-704 leu1-32 ura4-D18 rad51::Kan pmt3-KallR, h?*	this study
IDN179	*ade6-704 leu1-32 rad52::Kan, h?*	S. Lambert
IDN341	*ade6-704 leu1-32 ura4-D18 rad52::Kan pmt3-KallR, h?*	this study
IDN421	*ade6-704 leu1-32 ura4-D18 rad52::Kan rad51::Kan, h?*	this study
IDN423	*ade6-704 leu1-32 rad52::Kan rad51::Kan pmt3-KallR, h?*	this study
IDN640	*ade6-704 leu1-32 ura4-D18 rad52-6his-3flag:Kan, h-*	Markowska et al., 2025
IDN642	*leu1-32 ura4-D18 rad52-6his-3flag:Kan pmt3-KallR h+*	Markowska et al., 2025
IDN520	*ade6-704 leu1-32 ura4-D18 pli1::Hph, h+*	Markowska et al., 2025
IDN254	*ade6-704 leu1-32 ura4-D18 nse2-12Pk:Kan, h+*	this study
IDN244	*ade6-704 leu1-32 ura4-D18 pli1-12Pk:Kan, h+*	this study
IDN591	***ade6-704 leu1-32 ura4-D18 rad52-4xpmt3-6his-3flag:Hph, h?***	Markowska et al., 2025
IDN593	***ade6-704 leu1-32 ura4-D18 rad52-4xpmt3-6his-3flag:Hph pmt3-KallR, h?***	Markowska et al., 2025
IDN819	***ade6-D/ade6-704 leu1-32 ura4-D18 ubc13::Kan, h+***	NBRP
IDN822	***ade6-D/ade6-704 leu1-32 ura4-D18 ubc13::Kan pmt3_KallR***	this study
IDN824	***ade6-D leu1-32 ura4-D18 rad52-6his-3flag:Kan ubc13::Kan***	this study
IDN825	***ade6-D leu1-32 ura4-D18 rad52-6his-3flag:Kan ubc13::Kan pmt3_KallR***	this study
AN008	*MATa Ku70-13cMyc ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 (S. cerevisiae)*	laboratory collection

IDN_P13	*pREP41-MSC+*	Addgene 52690
IDN_P107	*pREP1-6his-Ubi*	D. Dziadkowiec

Supplementary Table 2: Primers used in this study

Primer name	Sequence (5'-3')	Experiment
II50_F	CACCGCAGTTCTACGTATCCT	ChIP (Control locus on ChrII)
II50_R	CGATGTAACGGTATGCGGTA	ChIP (Control locus on ChrII)
rdna_F	GGACGGTGGCCATGGAA	Rad52-YFP
rdna_R	CATTCGGCCGGTGAG	Rad52-YFP
cnt_F	CAACCGTTGCAACTTACATCAGCA	Pli1-12Pk, Nse2-12Pk
cnt_R	CCGGTCGCCAAATAGCAATGAGAT	Pli1-12Pk, Nse2-12Pk
dg_F	TACCGTGATTAGCCTTACTCCGCA	Pli1-12Pk, Nse2-12Pk
dg_R	ACCGCAAGATAGAGTAGGATGGGT	Pli1-12Pk, Nse2-12Pk
tel_F	TCAAAGTTGGCGACGTTGCTGATG	Pli1-12Pk, Nse2-12Pk
tel_R	AAGCAATGTGTGGAGCAACAGTGG	Pli1-12Pk, Nse2-12Pk
KK146	GAATTCGGTAAGCGTTGGATTG	southern rDNA 2DGE
KK147	GAATTCTTCTTTCACATC	southern rDNA 2DGE
KK161	CCTGGGTGAGATCAGTAGTC	southern 28S rDNA
KK162	TTCACCATCTTTCGGGTCCC	southern 28S rDNA