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Supplemental information

Rad53 regulates RNase H1, which promotes DNA replication through sites of transcription-replication conflict

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Figure S1

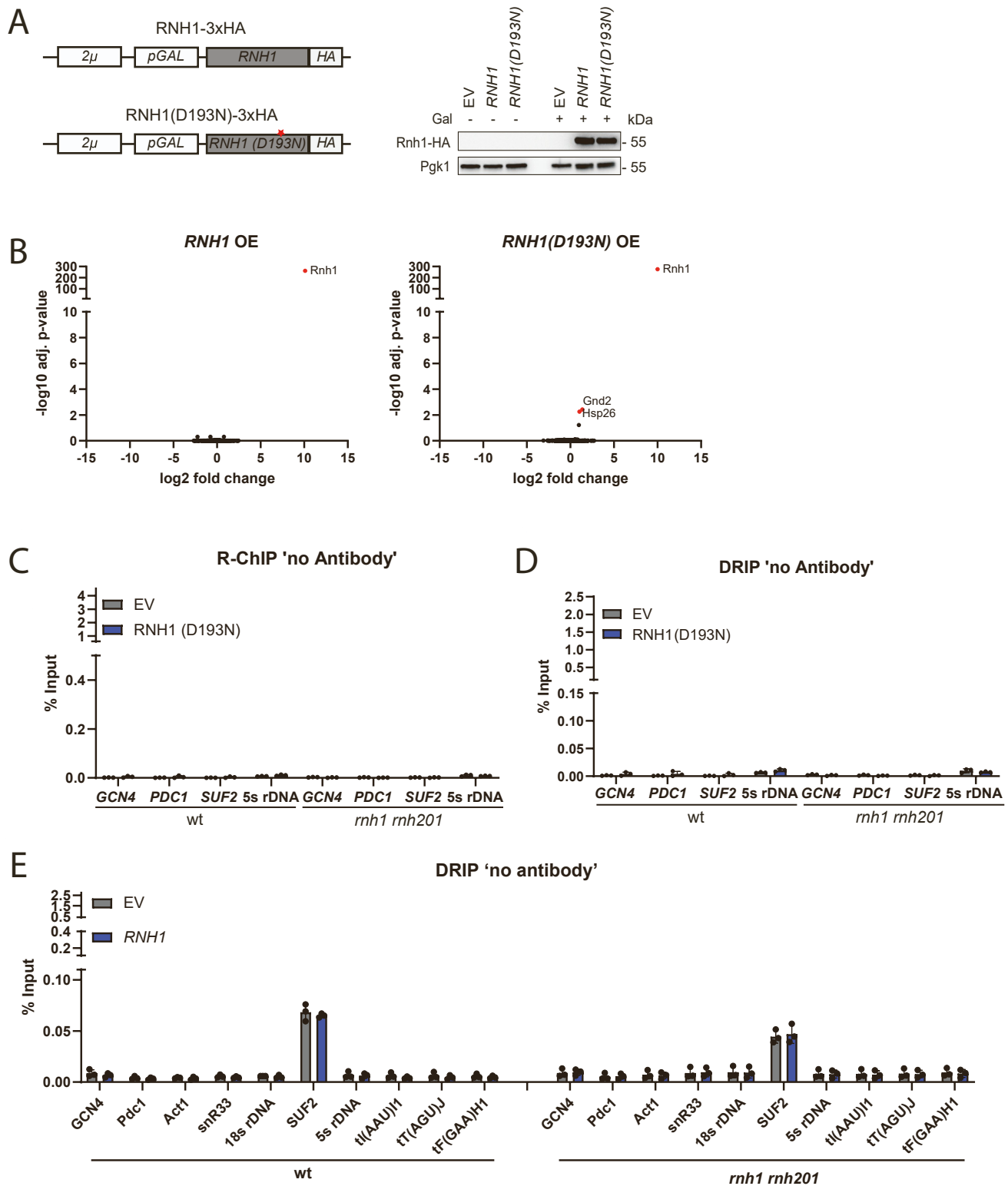


Figure S1. Overexpressed RNH1 likely acts at misregulated RNA-DNA hybrids.

(A) Left: Inducible allele for galactose induced overexpression of *RNH1*-HA and *RNH1*(D193N)-HA. Right: Confirmation of expression by Western blot analysis.

(B) Total RNA seq analysis of wild type cells overexpressing *RNH1*-HA and *RNH1*(D193N)-HA. Cells were grown to OD 0.5 in SC-Leu + 2 % raffinose prior to addition of 2 % galactose. After 2 h cells were collected for RNA extraction. Significance threshold: $p < 0.5$, $n=5$.

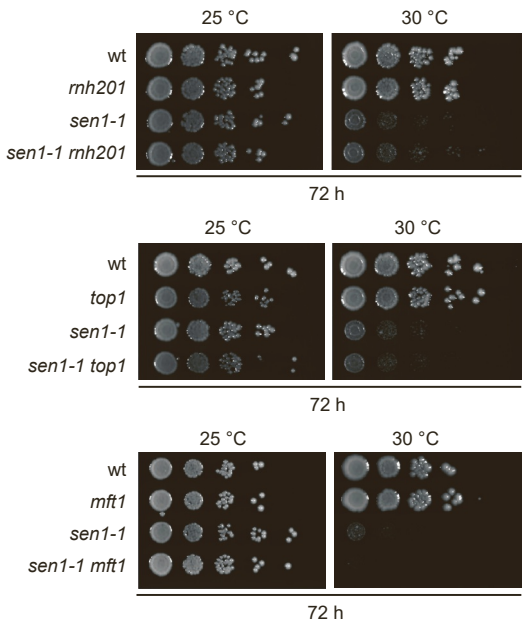
(C) No antibody control for the Rnh1(D193N) ChIP in Figure 1 C. Data are depicted as mean \pm SD, $n = 3$.

(D) No antibody control for the DRIP in Figure 1 D. Data are depicted as mean \pm SD, $n = 3$.

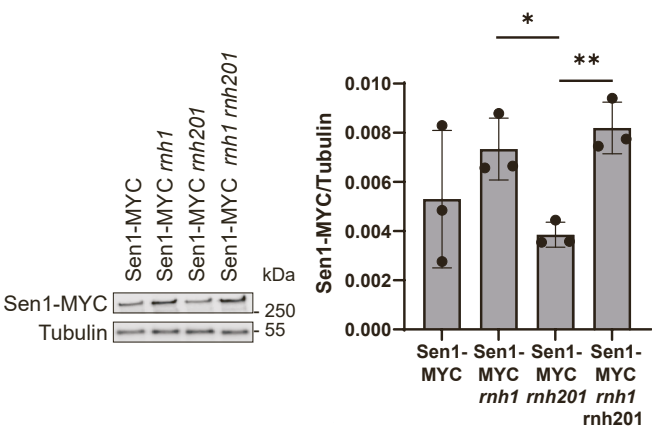
(E) No antibody control for the DRIP in Figure 1 E. Data are depicted as mean \pm SD, $n = 3$.

Figure S2

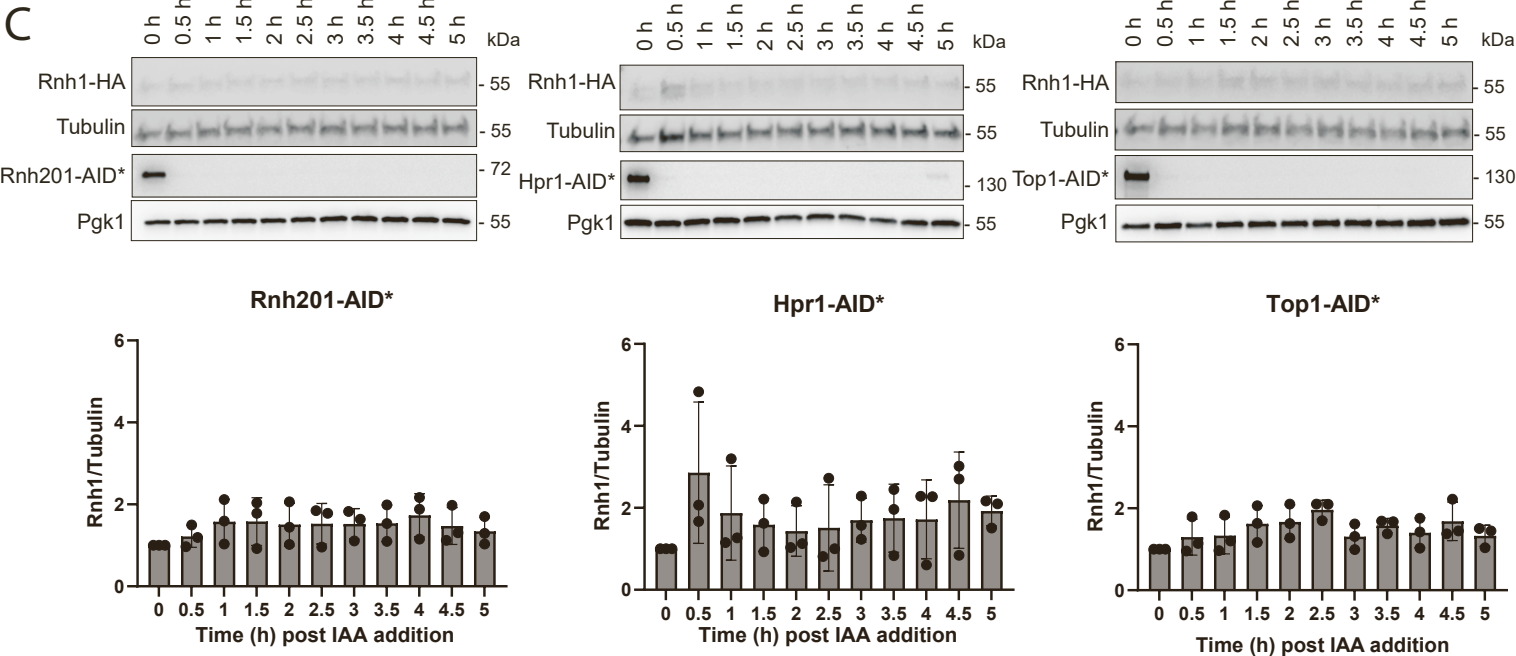
A



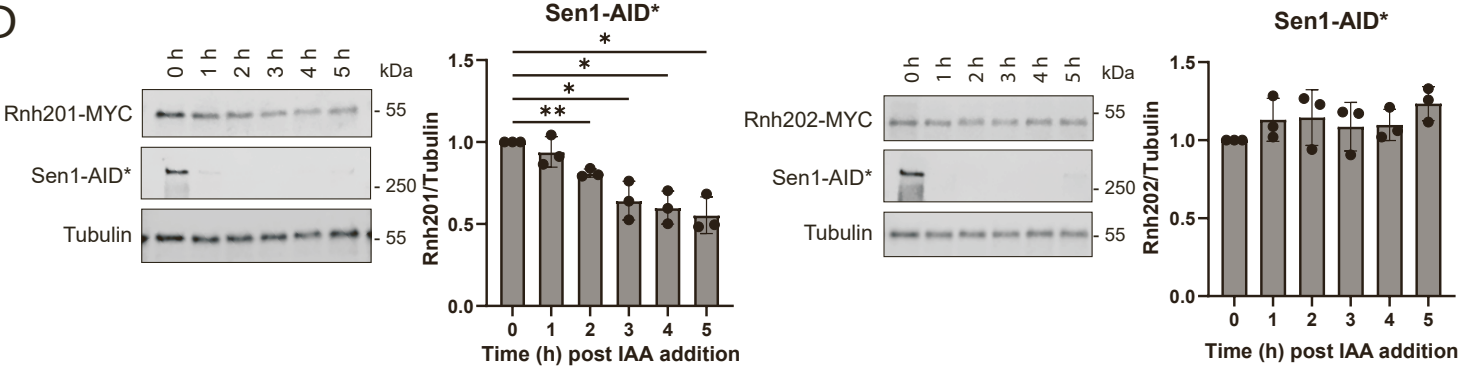
B



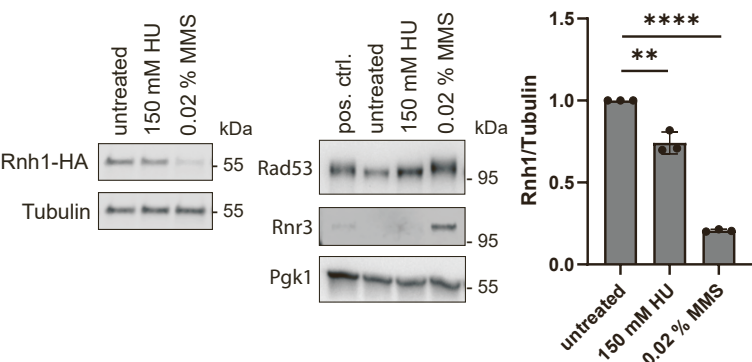
C



D



E



F

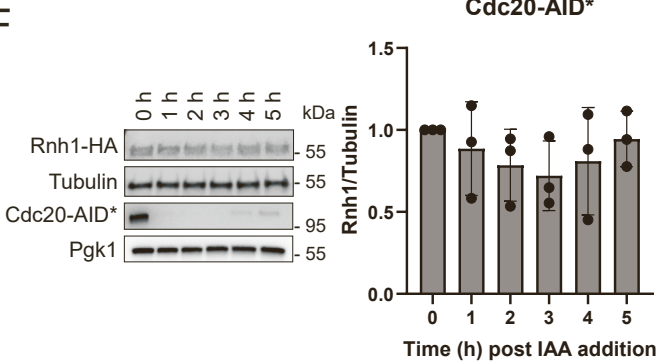


Figure S2. Increase of Rnh1 levels is specific to Sen1 loss.

- (A) Tenfold serial dilutions of the indicated strains were spotted on YPD plates and incubated at 25°C and 30 °C for 3 days.
- (B) Western blot analysis of endogenous Sen1-MYC levels in wild type and cells carrying the deletion of *RNH1*. Data are depicted as mean \pm SD, n = 3. Unpaired t-test (*: p<0.05, **: p<0.01).
- (C) Western blot analysis of endogenous Rnh1-HA levels over a time course of 5 h after depleting Rnh201-AID*, Hpr1-AID* or Top1-AID*. Values for quantification were normalized to 0 h time point. Data are depicted as mean \pm SD, n = 3.
- (D) Western blot analysis of endogenous Rnh201-MYC and Rnh202-MYC levels over a time course of 5 h after depleting Sen1-AID*. Values were normalized to the 0 h time point. Data are depicted as mean \pm SD, n = 3. Paired t-test (*: p<0.05, **: p<0.01).
- (E) Western blot analysis of endogenous Rnh1-HA levels in cells grown in the presence of 150 mM HU or 0.02 % MMS for 5 h. The positive control is a wild type sample grown in 0.02% MMS for 2 h. Values were normalized to the untreated samples. Data are depicted as mean \pm SD, n = 3. Paired t-test (**: p<0.01, ****: p<0.0001).
- (F) Western blot analysis of endogenous Rnh1-HA levels over a time course of 5 h after depleting Cdc20-AID*. Values were normalized to the 0 h time point. Data are depicted as mean \pm SD, n = 3.

Figure S3

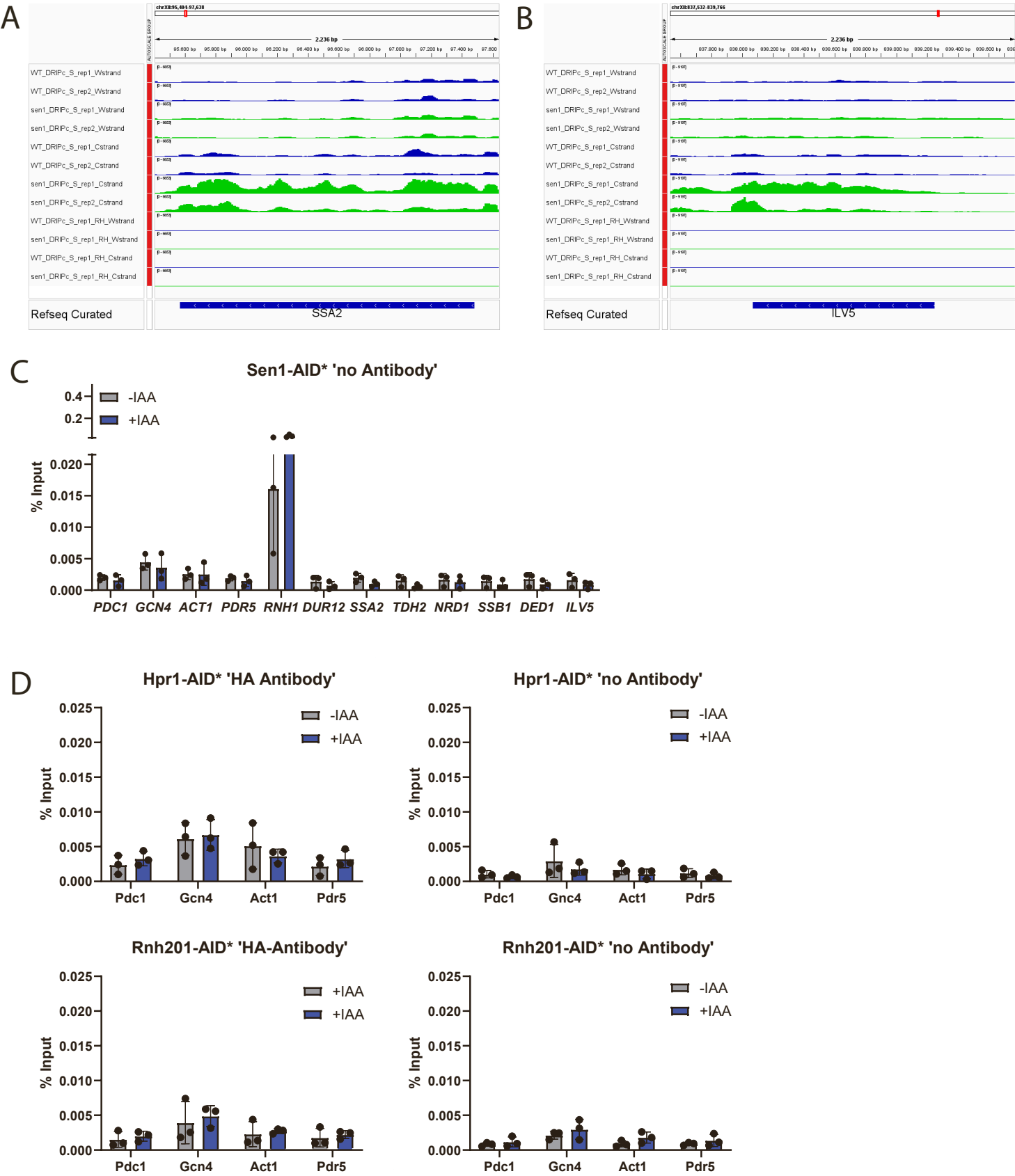
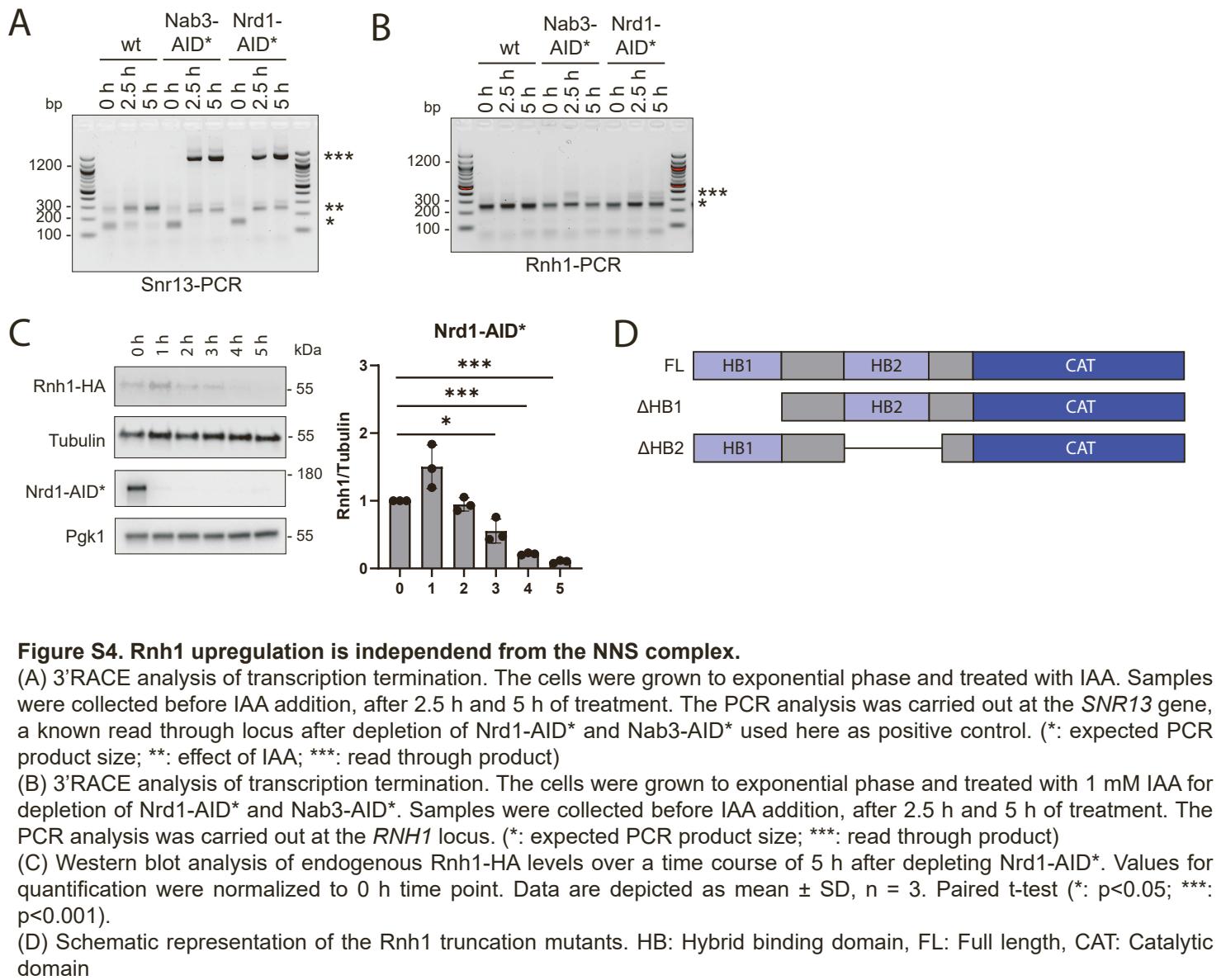


Figure S3. Rnh1 is recruited to Sen1-specific loci upon Sen1 loss.
(A) and (B) Genome Browser view of the example loci *SSA2* and *ILV5* from the DRIPc-seq experiment published in San Martin-Alonso et al (2021).
(C) No antibody control for Figure 2 F. Data are depicted as mean \pm SD, n = 3.
(D) Chromatin immunoprecipitation of endogenous catalytically inactive Rnh1-HA in a Hpr1-AID* or Rnh201-AID* background after 5 h of IAA treatment or without treatment. Left: Immunoprecipitation with HA-antibody, right: control without antibody. Data are depicted as mean \pm SD, n = 3.

Figure S4**Figure S4. Rnh1 upregulation is independent from the NNS complex.**

(A) 3'RACE analysis of transcription termination. The cells were grown to exponential phase and treated with IAA. Samples were collected before IAA addition, after 2.5 h and 5 h of treatment. The PCR analysis was carried out at the *SNR13* gene, a known read through locus after depletion of Nrd1-AID* and Nab3-AID* used here as positive control. (*: expected PCR product size; **: effect of IAA; ***: read through product)

(B) 3'RACE analysis of transcription termination. The cells were grown to exponential phase and treated with 1 mM IAA for depletion of Nrd1-AID* and Nab3-AID*. Samples were collected before IAA addition, after 2.5 h and 5 h of treatment. The PCR analysis was carried out at the *RNH1* locus. (*: expected PCR product size; ***: read through product)

(C) Western blot analysis of endogenous Rnh1-HA levels over a time course of 5 h after depleting Nrd1-AID*. Values for quantification were normalized to 0 h time point. Data are depicted as mean \pm SD, n = 3. Paired t-test (*: p<0.05; ***: p<0.001).

(D) Schematic representation of the Rnh1 truncation mutants. HB: Hybrid binding domain, FL: Full length, CAT: Catalytic domain

Figure S5

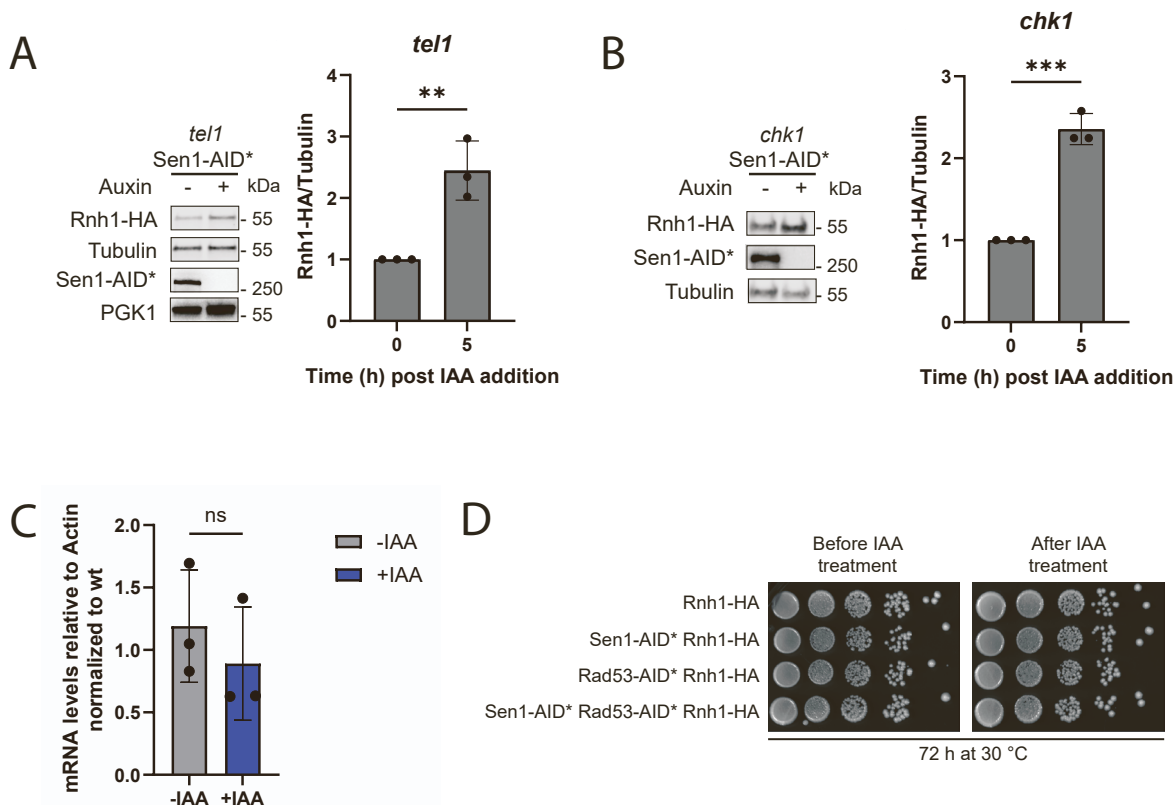


Figure S5. The replication checkpoint is required for Rnh1 accumulation

(A) Western blot analysis of endogenous Rnh1-HA levels in a *TEL1* deletion in the presence or absence of Sen1-AID* (IAA treatment for 5 h). Values were normalized to the -IAA sample. Data are depicted as mean \pm SD, $n = 3$. Paired t-test (**: $p < 0.01$).

(B) Western blot analysis of endogenous Rnh1-HA levels in a *CHK1* deletion in the presence or absence of Sen1-AID* (IAA treatment for 5 h). Values were normalized to the -IAA sample. Data are depicted as mean \pm SD, $n = 3$. Paired t-test (*: $p < 0.05$).

(C) Transcript levels of Rnh1 after depletion of Rad53-AID*. Cells were grown to exponential phase and treated with or without 1 mM IAA for 5 h. mRNA levels were first normalized to Actin before calculating the ratio Rad53-AID*/wild type. Data are depicted as mean \pm SD, $n = 3$.

(D) Impact of Rad53-AID* Sen1-AID* depletion on cell viability. Cells were grown to exponential phase and treated with or without 1 mM IAA for 5 h. The cells were washed thrice in sterile ddH₂O. A tenfold serial dilution of these samples was spotted on YPD plates and incubated at 30 °C for 3 days.

Figure S6

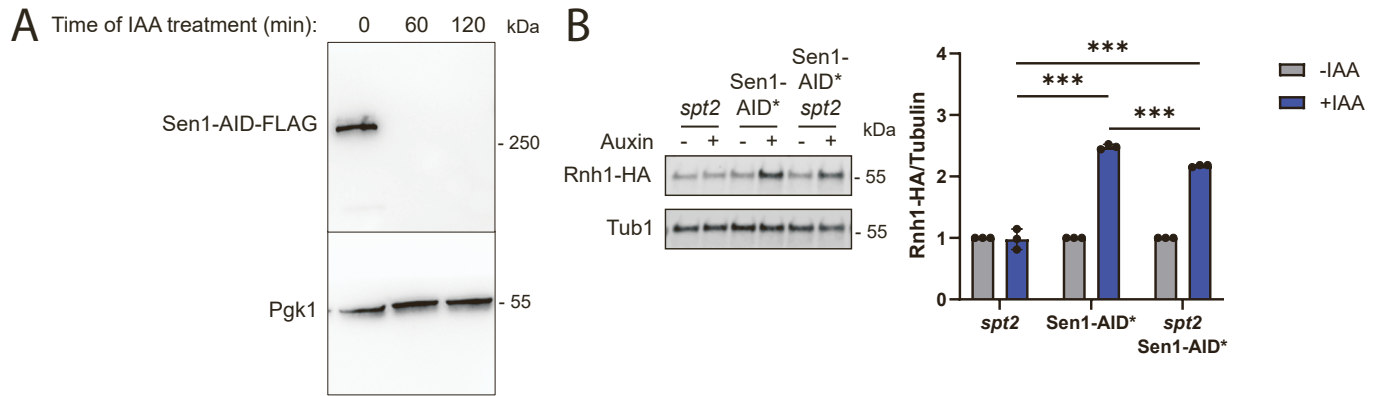


Figure S6. Rnh1 assists with timely replication completion through TRCs.

(A) Western blot confirming the depletion of Sen1-AID-FLAG in the microscopy assay performed in Figure 5D.

(B) Western blot analysis of endogenous Rnh1-HA levels in a *SPT2* deletion in the presence or absence of Sen1-AID* (IAA treatment for 5 h). Values were normalized to the -IAA samples. Data are depicted as mean \pm SD, n = 3. Unpaired t-test (***: p < 0.001).

Table S1. Yeast Strains used in this study

Strain name	Genotype	Source
yAL637	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sen1-1::KAN rnh201::HYG	This study
yCW3, 4 and 5	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0	This study
yCW13, 19, 25	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 + pBL211	This study
yCW15, 21, 27	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 + pBL352	This study
yCW17, 23, 28	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 + pBL633	This study
yCW70, 71, 244 and 250	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sen1-1::kan + pBL211	This study
yCW72, 73, 246 and 252	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sen1-1::kan + pBL352	This study
yCW74, 75, 248 and 254	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sen1-1::kan + pBL633	This study
yCW78, 79 and 80	MATalpha his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 rad52::kan + pBL211	This study
yCW340, 341 and 342	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2 rnh1::kan	This study
yCW347, 348 and 349	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2 rnh201::hyg	This study
yCW354, 355 and 356	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2 rnh1::kan rnh201::hyg	This study
yCW372, 373	his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 rnh1::kan rnh201::hyg + pBL211	This study
yCW374, 375	his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 rnh1::kan rnh201::hyg + pBL352	This study
yCW376, 377	his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 rnh1::kan rnh201::hyg + pBL633	This study
yCW540, 541 and 542	MATa his3Δ1 LEU2::AFB2-LEU2 met15Δ0 ura3Δ0 RNH1-6xHA::KAN Rnh201-AID*-9Myc-His	This study
yCW545, 546 and 547	MATa his3Δ1 LEU2::AFB2-LEU2 met15Δ0 ura3Δ0 RNH1-6xHA::KAN Sen1-AID*-9Myc-His	This study
yCW550, 551 and 552	MATa his3Δ1 LEU2::AFB2-LEU2 ura3Δ0 RNH1-6xHA::KAN Top1-AID*-9Myc-His	This study
yCW694 and 695	MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Hpr1-AID*-9Myc-His Trp1::GDP-OsTIR1(F74G)-Nat RNH1-6xHA::KAN	This study
yCW696	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Hpr1-AID*-9Myc-His Trp1::GDP-OsTIR1(F74G)-Nat RNH1-6xHA::KAN	This study
yCW730, 731 and 732	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 met15Δ0 LYS2 top1::HYG + pBL211	This study
yCW733, 734 and 735	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 met15Δ0 LYS2 top1::HYG + pBL352	This study
yCW736, 737 and 738	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 met15Δ0 LYS2 top1::HYG + pBL633	This study
yCW828	MATalpha his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 Can1 lyp1Δ::GPD-OsTIR1(F74G)-NAT Nab3-AID*-9xMYC::Hyg	This study
yCW829	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 Can1 lyp1Δ::GPD-OsTIR1(F74G)-NAT Nab3-AID*-9xMYC::Hyg	This study
yCW831, 832 and 833	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 Can1 lyp1Δ::GPD-OsTIR1(F74G)-NAT Nab3-AID*-9xMYC::Hyg Rnh1-HA (Kan)	This study
yCW834	MATalpha his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 Can1 lyp1Δ::GPD-OsTIR1(F74G)-NAT Nrd1-AID*-9xMYC::Hyg	This study
yCW835	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 Can1 lyp1Δ::GPD-OsTIR1(F74G)-NAT Nrd1-AID*-9xMYC::Hyg	This study
yCW837 and 838	MATalpha his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 Can1 lyp1Δ::GPD-OsTIR1(F74G)-NAT Nrd1-AID*-9xMYC::Hyg Rnh1-HA (Kan)	This study
yCW839	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 Can1 lyp1Δ::GPD-OsTIR1(F74G)-NAT Nrd1-AID*-9xMYC::Hyg Rnh1-HA (Kan)	This study
yCW919, 920 and 921	MATa his3Δ1 leu2Δ0 ura3Δ0 rnh1::hyg	This study
yCW922, 923 and 924	MATa his3Δ1 leu2Δ0 ura3Δ0 sen1-1::Kan	This study
yCW925, 926 and 927	MATa his3Δ1 leu2Δ0 ura3Δ0 rnh1::Hyg sen1-1::Kan	This study

yCW929, 930 and 931	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 mft1::Kan + pBL211	This study
yCW932, 933 and 934	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 mft1::Kan + pBL352	This study
yCW935, 936 and 937	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 mft1::Kan + pBL633	This study
yCW967, 968 and 969	MATa his3Δ1 LEU2::AFB2-LEU2 ura3Δ0 RNH201-6MYC-URA sen1::AID-HIS	This study
yCW971, 972 and 973	MATa his3Δ1 LEU2::AFB2-LEU2 ura3Δ0 RNH202-3MYC-URA sen1::AID-HIS	This study
yCW977, 978 and 979	MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Trp1::GDP-OsTIR1(F74G)-Nat RNH1-6xHA::KAN Sen1-AID*-9Myc-His + pBL211	This study
yCW980, 981 and 982	MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Trp1::GDP-OsTIR1(F74G)-Nat RNH1-6xHA::KAN Sen1-AID*-9Myc-His + pBL1117	This study
yCW1013, 1014, 1015	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 top1::HYG	This study
yCW1019, 1020, 1021	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 top1::HYG sen1-1::Kan	This study
yCW1049, 1050 and 1051	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 mft1::Hyg rnh1::Kan	This study
yCW1052, 1053 and 1054	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 mft1::Hyg	This study
yCW1061, 1062 and 1063	MATa his3Δ1 GPD-AFB2 (Leu) ura3Δ0 RNH1-6xHA::KAN Sen1-AID*-9Myc-His bar1::Nat	This study
yCW1069, 1070 and 1071	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 rnh1::kan top1::HYG	This study
yCW1380, 1381 and 1382	MATa his3Δ1 GPD-AFB2 (Leu) ura3Δ0 Rnh1(D913N)-6xHA::KAN Sen1::AID-HIS	This study
yCW1384, 1385 and 1386	MATa his3Δ1 GPD-AFB2 (Leu) ura3Δ0 Rnh1(D913N)-6xHA::KAN Rnh201::AID-HIS	This study
yCW1429, 1430 and 1431	MATa his3Δ1 leu2Δ ura3Δ0 Rnh1(D913N)-6xHA::KAN Hpr1-AID*-9Myc-His Trp1::GDP-OsTIR1(F74G)-Nat	This study
yCW1433, 1434 and 1435	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2 sen1-1::Hyg mft1::Kan	This study
yCW1506	MATa his3Δ1 leu2Δ0 ura3Δ0 Sen1-AID-Myc (His) pGpd-AFB2 (Leu)	This study
yCW1509	MATa his3Δ1 leu2Δ0 ura3Δ0 Sen1-AID-Myc (His) pGpd-AFB2 (Leu) rnh1::KAN	This study
yCW1603, 1604 and 1605	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2 + pBL211 (EV)	This study
yCW1609, 1610 and 1611	MATalpha his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 rnh1::HIS + pBL211 (EV)	This study
yCW1615, 1616 and 1617	MATa his3Δ1 leu2Δ0 ura3Δ0 Trp1::GDP-OsTIR1(F74G)-Nat Sen1-AID*-9MYC (HIS) rnh1::Kan + pBL211 (EV)	This study
yCW1618, 1619 and 1620	MATa his3Δ1 leu2Δ0 ura3Δ0 Trp1::GDP-OsTIR1(F74G)-Nat Sen1-AID*-9MYC (HIS) rnh1::Kan + pBL352 (RNH1 OE)	This study
yCW1621, 1622 and 1623	MATa his3Δ1 leu2Δ0 ura3Δ0 Trp1::GDP-OsTIR1(F74G)-Nat Sen1-AID*-9MYC (HIS) + pBL211 (EV)	This study
yFB130	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 lys2Δ0	This study
yFB405 and 406	MATa his3Δ1 leu2Δ0 ura3Δ0 sen1-1::KAN rnh201::NAT	This study
yFB1175	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rnh1::KAN rnh201::HYG + pBL906	This study
yFB1355 and 1356	MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rnh1::KAN rnh201::HYG + pBL797	This study
yFB1364	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rnh1::KAN rnh201::HYG + pBL804	This study
yFB1367	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rnh1::KAN rnh201::HYG + pBL808	This study
yFB1424 and 1425	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 + pBL906	This study
yFB1426 and 1427	MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 + pBL797	This study
yFB1428 and 1429	MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 + pBL837	This study

yFB1492 and 1493	MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rnh1::KAN rnh201::HYG + pBL837	This study
yFB1541 and 1542	MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rnh1::KAN rnh201::HYG + pBL906	This study
yFB2426	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 RNH1-6xHA::KAN Sen1::AID-HIS GPD-AFB2::LEU	This study
yFB2427	MATalpha his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 RNH1-6xHA::KAN Sen1::AID-HIS GPD-AFB2::LEU	This study
yFB2433	MATalphahis3Δ1 leu2Δ0 ura3Δ0 met15Δ0 RNH1-ΔHB1-6xHA::KAN Sen1::AID-HIS GPD-AFB2::LEU	This study
yFB2434	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 RNH1-ΔHB1-6xHA::KAN Sen1::AID-HIS GPD-AFB2::LEU	This study
yFB2450	MATahis3Δ1 leu2Δ0 ura3Δ0 met15Δ0 RNH1-ΔHB2-6xHA::KAN Sen1::AID-HIS GPD-AFB2::LEU	This study
yFB2451	MATalphahis3Δ1 leu2Δ0 ura3Δ0 met15Δ0 RNH1-ΔHB2-6xHA::KAN Sen1::AID-HIS GPD-AFB2::LEU	This study
yML69, 70, 71	MATa his3Δ1 leu2Δ ura3Δ0 RNH1-6xHA::KAN	This study
yML73, 74, 75	MATa his3Δ1 leu2Δ0 ura3Δ0 SEN1-AID-MYC-HIS GPD-AFB2 (LEU) RNH1-6xHA::KAN	This study
yML101, 102, 103	MATa his3Δ1 leu2Δ0 ura3Δ0 SEN1-AID-MYC-HIS GPD-AFB2 (LEU) RNH1-6xHA::KAN chk1::HYG	This study
yML113, 114, 115	MATa his3Δ1 leu2Δ0 ura3Δ RNH1-6xHA::KAN RAD53-AID*-9MYC-HYG GDP AFB2 (LEU)	This study
yML117, 118, 119	MATa his3Δ1 leu2Δ0 ura3Δ RNH1-6xHA::KAN RAD53-AID*-9MYC-HYG GDP AFB2 (LEU) sen1::AID-MYC-HIS	This study
yML77, 78, 79	MATa his3Δ1 leu2Δ0 ura3Δ0 SEN1-AID-MYC-HIS GPD-AFB2 (LEU) RNH1-6xHA::KAN rad9::NAT	This study
yML93, 94, 95	MATa his3Δ1 leu2Δ0 ura3Δ0 SEN1-AID-MYC-HIS GPD-AFB2 (LEU) RNH1-6xHA::KAN mrc1::HYG	This study
yML169, 107, 171	MATa his3Δ1 leu2Δ0 ura3Δ0 SEN1-AID-MYC-HIS GPD-AFB2 (LEU) RNH1-6xHA::KAN rad9::NAT Mrc1-AID*::URA	This study
yML85, 86, 87	MATa his3Δ1 leu2Δ0 ura3Δ0 SEN1-AID-MYC-HIS GPD-AFB2 (LEU) RNH1-6xHA::KAN tel1::HYG	This study
yML199,201	MATalpha his3Δ1leu2Δ0 ura3Δ0 Sen1-MYC-His	This study
yML203,205	MATalpha his3Δ1leu2Δ0 ura3Δ0 rnh1::KAN Sen1-MYC::His	This study
yOV91	MATs his3Δ1 LEU2::AFB2-LEU2 met15Δ0 ura3Δ0 Rad53-AID-Myc (Hyg)	This study
ySGS20, 21	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LYS2 RNH1-6xHA (KAN) sen1-3-9xMyc (His) rnh201::NAT	This study
ySGS22	MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LYS2 RNH1-6xHA (KAN) sen1-3-9xMyc (His) rnh201::NAT	This study
ySGS23, 24	MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LYS2 RNH1-6xHA (KAN) sen1-3-9xMyc (His)	This study
ySGS25	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LYS2 RNH1-6xHA (KAN) sen1-3-9xMyc (His)	This study
ySGS26, 27	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LYS2 RNH1-6xHA (KAN) rnh201::NAT	This study
ySGS28	MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LYS2 RNH1-6xHA (KAN) rnh201::NAT	This study
ySGS29	MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LYS2 RNH1-6xHA (KAN)	This study
ySGS30, 31	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LYS2 RNH1-6xHA (KAN)	This study

Table S2: Oligonucleotides used in this study

Name	Sequence	Target gene	Direction	Application	Source
oAL8	GGACACGATGGTGATCCA GG	Rnh1	Forward	Confirmation of C-terminal AID tag, 3'RACE	
oAL31	ATGGACAGCAGGAAGAA CG	Rnh201	Forward	3'RACE	
oAL76	ccttgatacgagcgtaacctca	Pdc1	Forward	qPCR	
oAL78	gaaggtatgagatgggctggtaa	Pdc1	Reverse	qPCR	
oBL292	CCCAGGTATTGCCGAAAG AATGC	Actin	Forward	ddPCR; qPCR	
oBL293	TTTGTTGGAAGGTAGTCA AAGAAGCC	Actin	Reverse	ddPCR; qPCR	
oCW68	TACGCGGTTAGAAAGGGC AG	Rnh1	Forward	ddPCR	
oCW69	CCAGCATGCGTTGAACTT CC	Rnh1	Reverse	ddPCR	
oCW118	AGTTCCCACCGACGAGAA AC	Rnh201	Forward	ddPCR	
oCW119	ATGCAGTAGAGCGAGTCT GC	Rnh201	Reverse	ddPCR	
oCW120	CACTTTTACACTGCCGCCA C	Rnh202	Forward	ddPCR	
oCW121	GGCCCTTGCCGAATTGA AA	Rnh202	Reverse	ddPCR	
oCW122	GCCATCCTCTCGGGAAAC AC	Rnh203	Forward	ddPCR	
oCW123	CGCCAATTCGTTGCGATC AG	Rnh203	Reverse	ddPCR	
oCW128	TTCGTATACCCGGGTACC AATTTTTTTTTTTTTTTTTT	Poly-A	Reverse	3'RACE RT	Mischo et al., 2011
oCW129	TTGGTACCCGGGTATACG AA	3'RACE adaptor	Reverse	3'RACE adaptor primer	Mischo et al., 2012
oCW130	GTGGTGACACTGCCACTG TC	Pgk1	Forward	3'RACE	
oCW137	GTGCCATGAATGTAGGCC TAAATTC	Rnh202	Forward	3'RACE	
oCW139	CGAATTGGCGCGATTGCA AG	Rnh203	Forward	3'RACE	
oCW140	GATGAATATGAGTGCATT TGGCTCG	Snr13	Forward	3'RACE	
oCW157	GATGGACGTAGTGAGTGC ATTTGTAGACC	Sen1	Forward	sen1-3 HR template for CRISPR, confirmation of sen1-3	

oCW179	TTTGGTCTCACGCATAAA ATTCTGGGAATCATGTGT TTTAGAGCTAGAAATAGC AAGTTA	pBL1158	Forward	Golden Gate assembly (Bsal) of pBL1168	
oCW180	TTTGGTCTCAGGATTGCG CAAGCCGGAATCG	pBL1158	Reverse	Golden Gate assembly (Bsal) of pBL1168	
oCW181	aaacaactgtgtgaccttgag	pBL1168	Forward	confirmation of pBL1168	
oCW182	acacatgtatctcagatatctcatt atatc	pBL1168	Reverse	confirmation of pBL1168	
oCW183	CACCAAAGAGCGACTTAG ATCTAAAG	Sen1	Reverse	sen1-3 HR template for CRISPR, confirmation of sen1-3	
oCW184	GTCAGAGGCTATATTTCA CTGGAGAA	Pdr5	Forward	qPCR	(San Martin-Alonso <i>et al.</i> , 2021)
oCW185	TACGTCTTGTTTCGGCCTT AATC	Pdr5	Reverse	qPCR	(San Martin-Alonso <i>et al.</i> , 2021)
oFB346	AGCTGATGATTGGAACGT ACC	Sen1	Reverse	confirmation of sen1-3	
oFB349	CTGGACAGAGATTGTGGT ATCTC	Sen1	Forward	confirmation of sen1-3	
oLP178	TTGTGCCCGAATCC AGTGA	Gcn4	Forward	qPCR	(Santos-Pereira <i>et al.</i> , 2013; San Martin-Alonso <i>et al.</i> , 2021)
oLP179	TGGCGGCTTCAGTG TTTCTA	Gcn4	Reverse	qPCR	(Santos-Pereira <i>et al.</i> , 2013; San Martin-Alonso <i>et al.</i> , 2021)

Table S3: Plasmids used in this study

Plasmid identifier	Plasmid name	Source
pBL211	pRS425 pGal	Gift from M. Peter
pBL352	pRS425 pGal-RNH1-HA	Graf et al., 2017
pBL633	pRS425 pGal-RNH1(D193N)-HA	Wagner and Luke, 2022
pBL797	pRS416 pGal-RNH1-HA	This study
pBL804	pGal-RNH1(D193N)-HA delta52	This study
pBL808	pGal-RNH1(D193N)-HA delta53-168	This study
pBL837	pRS416 pGal-RNH1(D193N)-HA	Misino et al., 2022
pBL906	pRS416 pGal-3HA	Misino et al., 2022
pBL959	pRS426-Gal-EV-3HA	This study
pBL967	pRS426-Gal-RNH1(D193N)-3HA	This study
pBL1117	pRS425 pGal-RNH1-3Myc	This study
pBL1158	pMYT095 CRISPR/Cas9-gRNA empty backbone	Addgene
pBL1168	CRISPR/Cas9-Sen1-gRNA	This study