

Checkpoint-dependent RNR induction promotes fork restart after replicative stress

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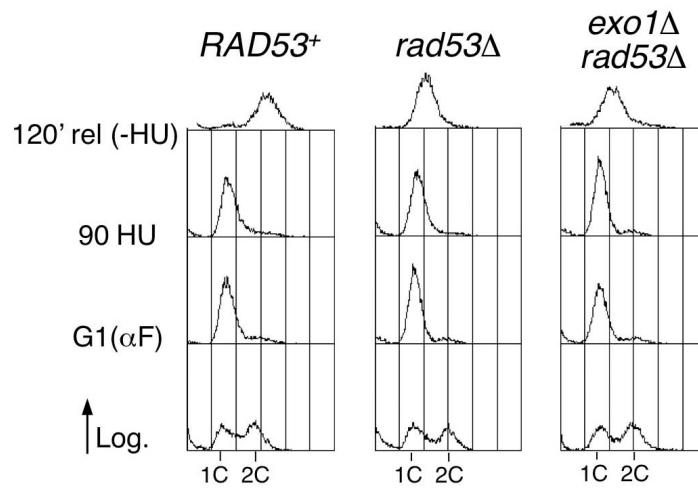
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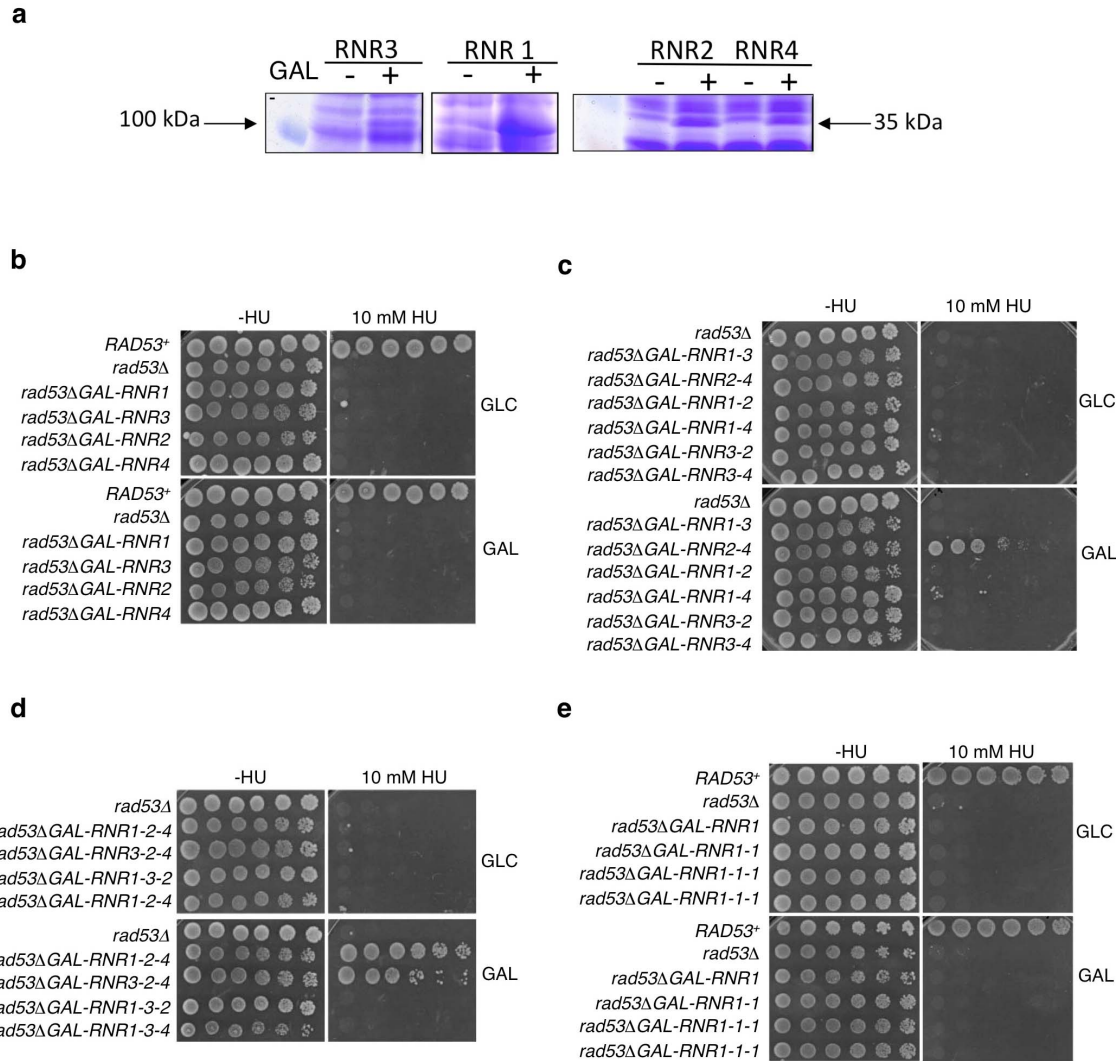
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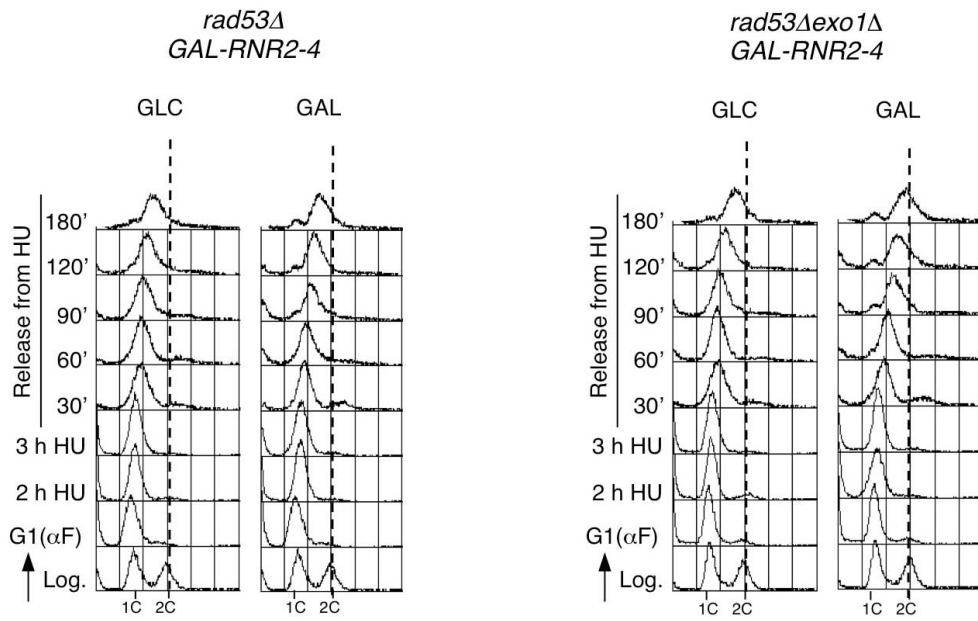


Supplementary Fig. S1. Analysis of replication resumption in *RAD53+*, *rad53Δ* and *rad53Δexo1Δ* strains after HU treatment. The experiment was performed as described in Fig. 1c. DNA samples of the specified strains were taken at the indicated time points and DNA content was determined by flow cytometry.

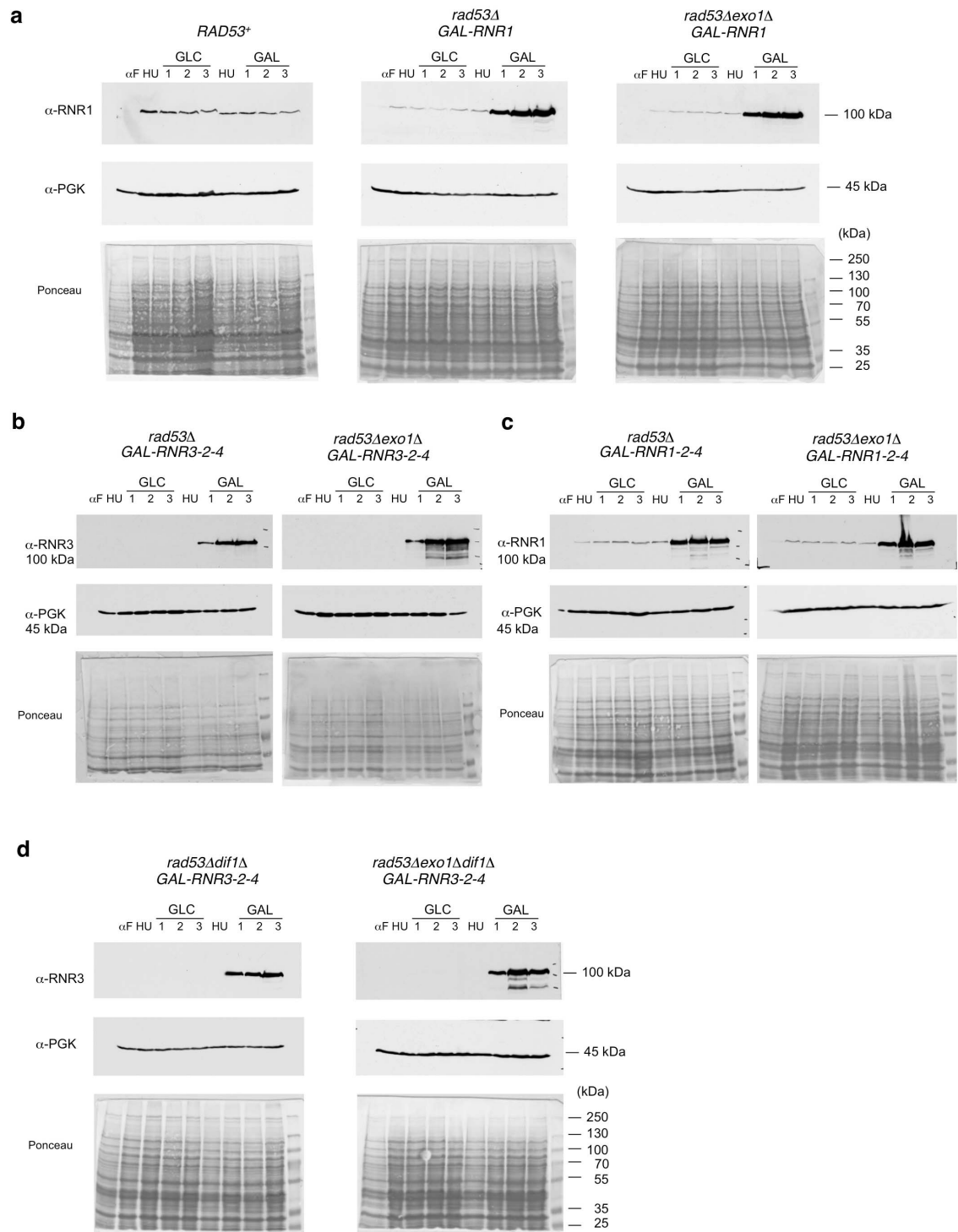


Supplementary Fig. S2. Effect of *RNR* induction on viability in the presence of HU.

(a) Expression of the different RNR subunits was examined upon induction/repression of the GAL1 promoter. Protein extracts from cultures grown overnight in YP supplemented with glucose or galactose were separated by 7.5% and 12% SDS-PAGE and stained with Coomassie Blue. (b) 1:5 serial dilutions of exponential cultures of the indicated strains were tested for sensitivity to HU in YPD plates (top) or YPGAL plates (bottom) by using a drop assay on plates. (c) Drop assays of the indicated strains as described in (b). (d) HU-sensitivity of the indicated strains was tested as described in (b). (e) Drop assays of the indicated strains as described in (b).



Supplementary Fig. S3. Effect of *RNR2-4* induction and *EXO1* deletion on replication restart in *rad53Δexo1Δ* mutants after fork stalling. Cultures of *rad53ΔGAL-RNR2-4* and *rad53Δexo1Δ GAL-RNR2-4* strains were grown in YPRAF medium, arrested in G1 with α -factor, and released into fresh medium in the presence of 0.2 M HU for 3 hours. After that, cultures were released into fresh medium in the presence of Glc (left panel) or Gal (right panel). DNA content was measured by flow cytometry at the indicated times.



Supplementary Fig. S4. Full-length images of western blots. (a), (b), (c) Full-length western blots in Fig. 3a, Fig. 3b and Fig. 3c respectively are shown. (d) Full-length western blots in Fig. 3e are shown. All gels have been run under the same experimental conditions.

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

All the strains used in this work are derived from W303 (*ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*)

Strain	Genotype	Source
YJT72	<i>MATa sml1Δ::URA3</i>	Tercero and Diffley, 2001
YJT75	<i>MATa sml1Δ::URA3 rad53Δ::LEU2</i>	Tercero and Diffley, 2001
YMS666	<i>MATa sml1Δ::URA3 rad53Δ::LEU2 exo1Δ::HIS3</i>	This work
YEC514	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 ura3::pRS306-GAL-RNR1-URA3</i>	This work
YEC397	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 his3::pRS303-GAL-RNR3-HIS3</i>	This work
YEC398	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 trp1::pRS304-GAL-RNR2-TRP1</i>	This work
YEC393	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 leu2::pRS305-GAL-RNR4-LEU2</i>	This work
YEC520	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 ura3::pRS306-GAL-RNR1-URA3</i> <i>his3::pRS303-GAL-RNR3-HIS3</i>	This work
YEC535	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 trp1::pRS304-GAL-RNR2-TRP1</i> <i>leu2::pRS305-GAL-RNR4-LEU2</i>	This work
YEC515	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 ura3::pRS306-GAL-RNR1-URA3</i> <i>trp1::pRS304-GAL-RNR2-TRP1</i>	This work
YEC518	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 ura3::pRS306-GAL-RNR1-URA3</i> <i>leu2::pRS305-GAL-RNR4-LEU2</i>	This work
YEC405	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 trp1::pRS304-GAL-RNR2-TRP1</i> <i>his3::pRS303-GAL-RNR3-HIS3</i>	This work
YEC414	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 his3::pRS303-GAL-RNR3-HIS3</i> <i>leu2::pRS305-GAL-RNR4-LEU2</i>	This work
YEC553	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 ura3::pRS306-GAL-RNR1-URA3</i> <i>trp1::pRS304-GAL-RNR2-TRP1 leu2::pRS305-GAL-RNR4-LEU2</i>	This work
YMS252	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 trp1::pRS304-GAL-RNR2-TRP1</i> <i>his3::pRS303-GAL-RNR3-HIS3 leu2::pRS305-GAL-RNR4-LEU2</i>	This work
YEC513	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 ura3::pRS306-GAL-RNR1-URA3</i> <i>trp1::pRS304-GAL-RNR2-TRP1 his3::pRS303-GAL-RNR3-HIS3</i>	This work
YEC519	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 ura3::pRS306-GAL-RNR1-URA3</i> <i>his3::pRS303-GAL-RNR3-HIS3 leu2::pRS305-GAL-RNR4-LEU2</i>	This work
YEC685	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 trp1::pRS304-GAL-RNR1-TRP1</i> <i>his3::pRS303-GAL-RNR1-HIS3</i>	This work
YEC688	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 ura3::pRS306-GAL-RNR1-URA3</i> <i>his3::pRS303-GAL-RNR1-HIS3</i>	This work
YEC691	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 ura3::pRS306-GAL-RNR1-URA3</i> <i>trp1::pRS304-GAL-RNR1-TRP1 his3::pRS303-GAL-RNR1-HIS3</i>	This work
YEC532	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 ura3::pRS306-GAL-RNR1-URA3</i> <i>exo1Δ:: hghMX</i>	This work
YEC536	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 trp1::pRS304-GAL-RNR2-TRP1</i> <i>leu2::pRS305-GAL-RNR4-LEU2 exo1Δ:: hghMX</i>	This work
YMS349	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 trp1::pRS304-GAL-RNR2-TRP1</i> <i>his3::pRS303-GAL-RNR3-HIS3 leu2::pRS305-GAL-RNR4-LEU2 exo1Δ::</i> <i>hghMX</i>	This work
YEC554	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 ura3::pRS306-GAL-RNR1-URA3</i> <i>trp1::pRS304-GAL-RNR2-TRP1 leu2::pRS305-GAL-RNR4-LEU2</i> <i>exo1Δ:: hghMX</i>	This work
YMS260	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 trp1::pRS304-GAL-RNR2-TRP1</i> <i>his3::pRS303-GAL-RNR3-HIS3 leu2::pRS305-GAL-RNR4-LEU2</i> <i>exo1Δ:: hghMX dif1Δ::URA3</i>	This work
YEC561	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 ura3::pRS306-GAL-RNR1-URA3</i> <i>trp1::pRS304-GAL-RNR2-TRP1 leu2::pRS305-GAL-RNR4-LEU2</i> <i>exo1Δ:: hghMX dif1Δ::URA3</i>	This work
YMS346	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 trp1::pRS304-GAL-RNR2-TRP1</i> <i>his3::pRS303-GAL-RNR3-HIS3 leu2::pRS305-GAL-RNR4-LEU2</i> <i>dif1Δ::URA3</i>	This work
YMS269	<i>MATa sml1Δ::kanMX rad53Δ::natNT2 exo1Δ::hghMX</i> <i>his3::pRS303-GAL-RNR3-HIS3 leu2::pRS305-GAL-RNR4-LEU2</i> <i>trp1::pRS304-GAL-RNR2-TRP1 ARS608Δ::URA3 ARS609Δ::ADE2</i>	This work