

Teixeira-Silva et al. Supplementary Fig1

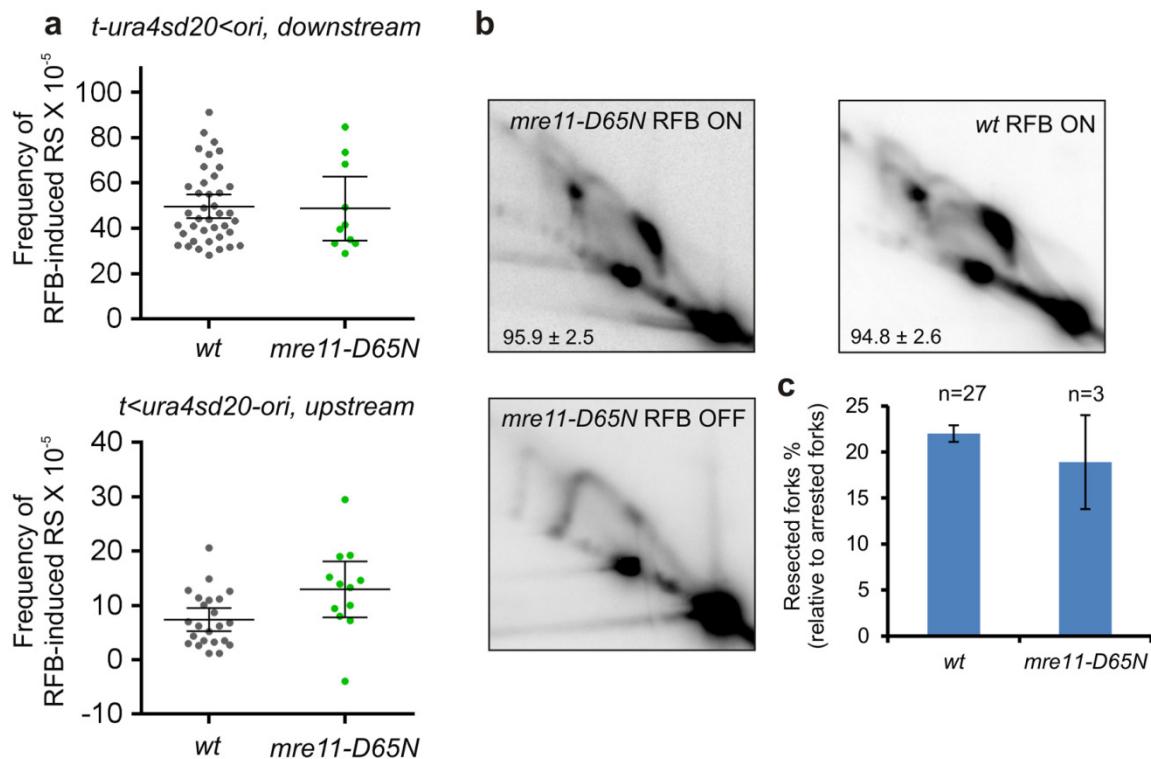
**Supplementary Figure 1: A genetic assay to monitor HR-mediated replication restart and investigate fork-resection and the RTS1-RFB.**

**(a)** Frequency of Replication Slippage (RS) in indicated strains, using indicated constructs containing the *ura-sd20* reporter gene (*t-ura4sd20-ori* in blue and *t-ura4sd20<ori* in red), in indicated conditions. Values are the mean of at least three independent experiments  $\pm$  standard errors of the mean (SEM).

P-values were calculated using the Mann and Whitney U test. The expression of Rtf1 (“-” for repressed and “+” for expressed), presence of the *RTS1*-RFB (“-” for absence and “+” for presence) and activity of the *RTS1*-RFB (“-” for its absence, “+” for active, and “L” for leaky) are indicated on the panel bellow the histogram. The red arrows indicate the values used to obtain RFB-induced RS represented on the panel B.

**(b)** Frequency of RFB-Induced RS in indicated strains. The frequency of RS monitored with the *t-ura4sd20-ori* construct was subtracted to the frequency obtained with the *t-ura4sd<ori* construct upon expression of Rtf1 to disregard genetic backgrounds effect and obtain the true induction of RS by the *RTS1*-RFB. Each dot represents one value obtained from the subtraction of each value obtained with the *t-ura4sd20<ori* construct to the mean of the values obtained with *t-ura4sd20-ori*. Bars indicate the mean value  $\pm$  95 % confidence interval (CI). P-values were calculated using the Mann and Whitney U test.

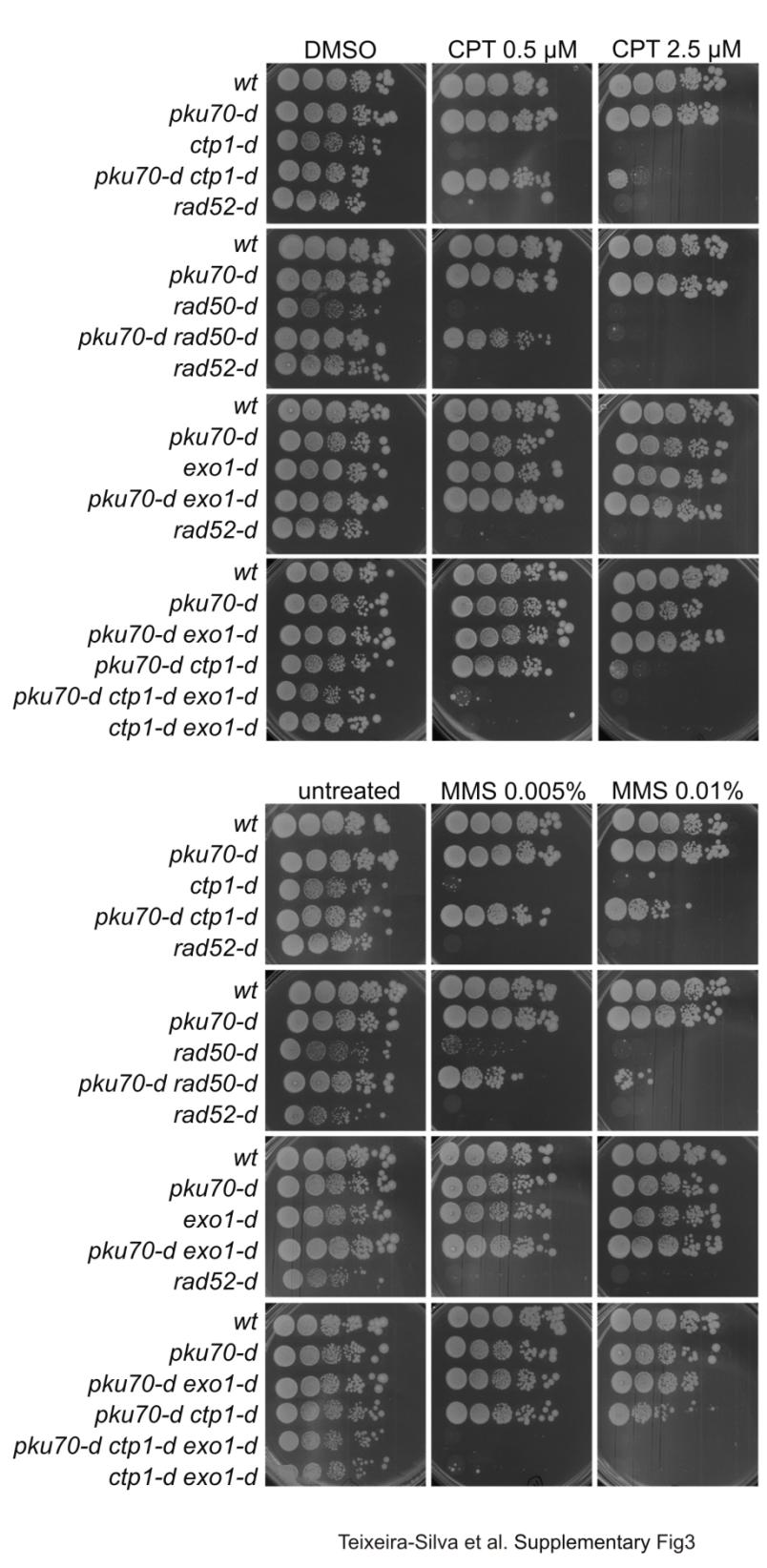
**(c) and (d)** Same representation as (A) and (B), respectively, using the constructs containing the *ura-sd20* reporter gene (*t-ura4sd20-ori* in blue and *t<ura4sd20-ori* in red), in indicated conditions.



Teixeira-Silva et al. Supplementary Fig2

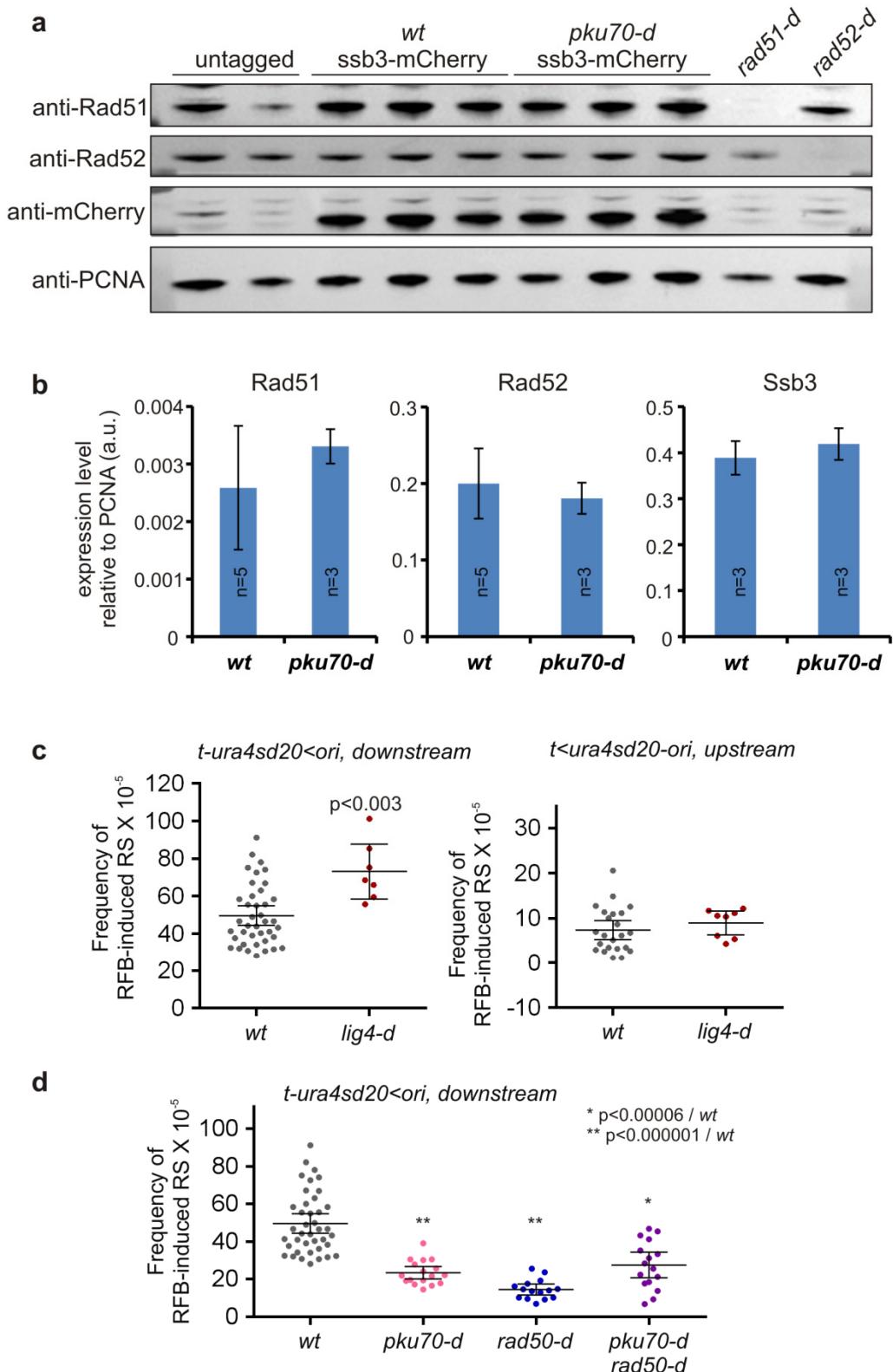
**Supplementary Figure 2: The nuclease activity of Mre11 is dispensable for fork-resection and restart.**

- (a) Frequency of RFB-induced RS downstream (top panel, *t-ura4sd20<ori*) and upstream (bottom panel, *t<ura4sd20-ori*) in indicated strains. Each dot represents one sample. Bars indicate the mean value  $\pm$  95 % CI. Statistics were calculated using the non-parametric Mann and Whitney U test.
- (b) Representative RI analysis by 2DGE in indicated strains upon activation (RFB ON) or not (RFB OFF) of the *RTS1*-RFB. Numbers indicate the % of forks blocked at the *RTS1*-RFB  $\pm$  SD.
- (c) Quantification of forks undergoing resection (“tail signal”), relative to the intensity of terminally-arrested forks. Values are the mean of at least 3 independent experiments  $\pm$  95% CI.



**Supplementary Figure 3: The deletion of *pku70* partially rescues the sensitivity of *ctp1-d* and *rad50-d* cells to CPT and MMS, in an Exo1-dependent manner.**

Tenfold serial dilution of indicated strains on plates containing the indicated doses of CPT and MMS.



Teixeira-Silva et al. Supplementary Fig4

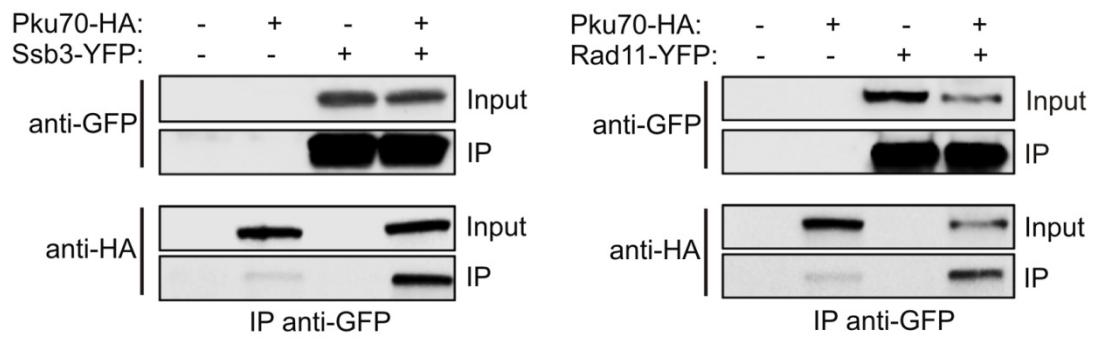
**Supplementary Figure 4: Ku promotes efficient replication restart, independently of NHEJ.**

**(A)** Frequency of RFB-induced RS downstream (left panel, *t-ura4sd20<ori*) and upstream (right panel, *t<ura4sd20-ori*) in indicated strains. Each dot represents one sample. Bars indicate the mean value ± 95 % CI. Statistics were calculated using the non-parametric Mann and Whitney U test.

**(B)** Expression of Rad51, Rad52 and Ssb3-mCherry in *wt* and *pku70-d* strains. Untagged, *rad51-d* and *rad52-d* strains were included as controls for antibodies specificity. PCNA was used as loading control.

**(C)** Quantification of protein expression relative to PCNA. Values indicate the mean (a.u.) of at least 3 independent experiments ± SD.

**(D)** Frequency of upstream RFB-induced RS in indicated strains. Each dot represents one sample. Bars indicate the mean value ± 95 % CI. Statistics were calculated using the non-parametric Mann and Whitney U test.



Teixeira-Silva et al. Supplementary Fig5

**Supplementary Figure 5:** Higher exposure of the blot showed on the figure 5

**Supplementary Table 1:** Strains used in this study (related to all figures).

Strain	Genotype	Reference
SL350	<i>h-smt0 t-ura4<sup>+</sup>&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Lambert et al. 2005
YC6	<i>h-smt0 t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Iraqui et al. 2012
YC21	<i>h-smt0 t&gt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Iraqui et al. 2012
YC13	<i>h-smt0 t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Iraqui et al. 2012
YC76	<i>h-smt0 rad51::KAN t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Iraqui et al. 2012
YC82	<i>h+ rad51::KAN t&gt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
YC80	<i>h-smt0 rad51::KAN t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Iraqui et al. 2012
YC86	<i>h+ rad52::NAT t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Iraqui et al. 2012
YC91	<i>h-smt0 rad52::NAT t&gt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
YC90	<i>h-smt0 rad52::NAT t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Iraqui et al. 2012
II264	<i>h- exo1::NAT t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Tsang et al. 2014
II324	<i>h- smt0 exo1::NAT t&lt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Tsang et al. 2014
II268	<i>h- exo1::NAT t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Iraqui et al. 2012
II383	<i>h- rqh1::KAN t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II376	<i>h- rqh1::KAN t&lt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II381	<i>h- rqh1::KAN t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II487	<i>h- rqh1::KAN exo1::NAT t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II492	<i>h- rqh1::KAN exo1::NAT t&lt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II490	<i>h- rqh1::KAN exo1::NAT t&lt;ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II445	<i>h- smt0 ctp1::NAT t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II440	<i>h- smt0 ctp1::NAT t&gt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II437	<i>h- smt0 ctp1::NAT t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II518	<i>h- ctp1::HYGRO t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II527	<i>h+ ctp1::HYGRO t&gt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32 his<sup>+</sup></i>	This study
II525	<i>h- ctp1::HYGRO t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32 his<sup>+</sup></i>	This study
YC65	<i>h- smt0 rad50::KAN t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Iraqui et al. 2012
YC70	<i>rad50::KAN t&gt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
YC67	<i>h- smt0 rad50::KAN t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Iraqui et al. 2012
II541	<i>h- ctp1::HYGRO rad50::KAN t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II547	<i>h- ctp1::HYGRO rad50::KAN t&gt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II544	<i>h- ctp1::HYGRO rad50::KAN t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II483	<i>h+ ctp1::HYGRO exo1::NAT t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704</i>	This study
II486	<i>h- ctp1::HYGRO exo1::NAT t&gt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704</i>	This study
II484	<i>h- ctp1::HYGRO exo1::NAT t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704</i>	This study
II532	<i>h- rad50::KAN exo1::NAT t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II535	<i>h- rad50::KAN exo1::NAT t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study
II538	<i>h- rad50::KAN exo1::NAT t&gt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	This study

YC259	h-	<i>mre11-D65N t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
YC264	h-	<i>mre11-D65N t&gt;ura4SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
YC262	h-	<i>mre11-D65N t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
II594	h+	<i>pku70::leu2 t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
II603	h-	<i>pku70::leu2 t&gt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
II598	h+	<i>pku70::leu2 t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS18	h+	<i>pku70::leu2 ctp1::HYGRO t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS30	h-	<i>pku70::leu2 ctp1::HYGRO t&gt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS23	h-	<i>pku70::leu2 ctp1::HYGRO t-ura4S-D20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS1	h-	<i>pku70::leu2 rad50::KAN t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS6	h-	<i>pku70::leu2 rad50::KAN t&gt;ura4SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS12	h+	<i>pku70::leu2 rad50::KAN t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS52	h-	<i>pku70::leu2 exo1 ::NAT t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS56	h+	<i>pku70::leu2 exo1 ::NAT t&gt;ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS178	h+	<i>pku70::leu2 exo1 ::NAT t-13xter-ura4-SD20-ura5&lt;ori sup35:nmt41:rtf1<sup>+</sup> ura5::HYGRO ade6-704 leu1-32</i>	<i>This study</i>
AS63	h+	<i>pku70::leu2 exo1 ::NAT ctp1::HYGRO t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS67	h-	<i>pku70::leu2 exo1 ::NAT ctp1::HYGRO t&gt;ura4sd20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS70	h-	<i>pku70::leu2 exo1 ::NAT ctp1::HYGRO t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS120	h+	<i>lig4::KAN t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS116	h+	<i>lig4::KAN t&gt;ura4sd20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS111	h+	<i>lig4::KAN t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32 his3-DL</i>	<i>This study</i>
YC266	h-	<i>t-13xter-ura4-SD20-ura5&lt;ori sup35:nmt41:rtf1<sup>+</sup> ura5::HYGRO ade6-704 leu1-32</i>	Ait-Saada et al. 2017
AS128	h-	<i>pKu70::leu2 t-13xter-ura4-SD20-ura5&lt;ori sup35:nmt41:rtf1<sup>+</sup> ura5 ::HYGRO ade6-704 leu1-32</i>	<i>This study</i>
II606	h-	<i>ctp1::NAT t-13xter-ura4-SD20-ura5&lt;ori sup35:nmt41:rtf1<sup>+</sup> ura5::HYGRO ade6-704 leu1-32</i>	<i>This study</i>
AS182	h+	<i>pku70 :: leu1 ctp1::NAT t-13xter-ura4-SD20-ura5&lt;ori sup35:nmt41:rtf1<sup>+</sup> ura5::HYGRO ade6-704 leu1-32</i>	<i>This study</i>
II561	h-	<i>rad50::KAN t-13xter-ura4-SD20-ura5&lt;ori sup35:nmt41:rtf1<sup>+</sup> ura5::HYGRO ade6-704 leu1-32</i>	<i>This study</i>
AS254	h+	<i>pku70::leu2 rad50 ::KAN t-13xter-ura4-SD20-ura5&lt;ori sup35:nmt41:rtf1<sup>+</sup> ura5 ::HYGRO ade6-704 leu1-32</i>	<i>This study</i>
AS97	h90	<i>pku70-3HA:ura4<sup>+</sup> t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS132	h+	<i>rad50::KAN pku70-3HA:ura4<sup>+</sup> t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AS39	h+	<i>arg3::psv40-GFP-LacI** ssb3-mCherry:KAN lacO 7,9Kb:KAN t-ura4<sup>+</sup>&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	Ait-Saada et al. 2017
AS206	h+	<i>pku70::leu2 arg3::psv40-GFP-LacI** ssb3-mCherry:KAN lacO 7,9Kb:KAN t-ura4<sup>+</sup>&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AC147	h+	<i>ssb3-YFP:NAT t-ura4<sup>+</sup>&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
SL1507	h+	<i>pku70-3HA:ura4<sup>+</sup> ssb3-YFP:NAT t-ura4-SD20-ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
AC61	h-smt0	<i>rad11-YFP:NAT t-ura4<sup>+</sup>&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>
SL1498	h+	<i>pku70-3HA:ura4<sup>+</sup> rad11-YFP:NAT t-ura4-SD20&lt;ori sup35:nmt41:rtf1<sup>+</sup> ade6-704 leu1-32</i>	<i>This study</i>

**Supplementary Table 2:** List of primers used in this study (related to Fig. 1g, 2d, 3d and 4d).

Name	Distance (bp) from RTS1 / position	Sequence (5'-3')	Experiment
L3F	110	TTTAAATCAAATCTCCATGCG	ssDNA qPCR
L3R		TGTACCCATGAGCAAAGTC	ssDNA qPCR
L400F	450	ATCTGACATGGCATTCTCA	ssDNA qPCR
L400R		GATGCCAGACCGTAATGACA	ssDNA qPCR
L1800F	1800	GGCAAAGTAGATCCGACAGC	ssDNA qPCR
L1800R		TGAATACGCCGTTACTCCTAAAG	ssDNA qPCR
L2200F	2200	AAGGCAAGAACGCTGAGAC	ssDNA qPCR
L2200R		GGCATGCATACTACCCGATAA	ssDNA qPCR
II50F	Locus control (ChrII)	CACCGCAGTTCTACGTATCCT	ssDNA qPCR
II50R		CGATGTAACGGTATGCGGT	ssDNA qPCR
II150F	Locus control (ChrII)	ATCGTCAATCCATTCCGTCT	ssDNA qPCR
II150R		AACCATCTAACATACGATATGAATCCT	ssDNA qPCR
L5F	-153	AGGGCATTAAAGGCTTATTACAGA	ChIP Ku70
L5R		TCACGTTAACCAACATCCA	ChIP Ku70
L3F	110	TTTAAATCAAATCTCCATGCG	ChIP Ku70
L3R		TGTACCCATGAGCAAAGTC	ChIP Ku70
L400F	450	ATCTGACATGGCATTCTCA	ChIP Ku70
L400R		GATGCCAGACCGTAATGACA	ChIP Ku70
Ade6-23	Chromosome III	GGCTGCCTTACCATCATTC	ChIP Ku70
Ade6-25		TTAAGCTGAGCTGCCAAGGT	ChIP Ku70

**Supplementary Excel file 1:** Statistics on RFB-induced RS (related to Fig 1c, 2a, 3a, 4a, 5a).