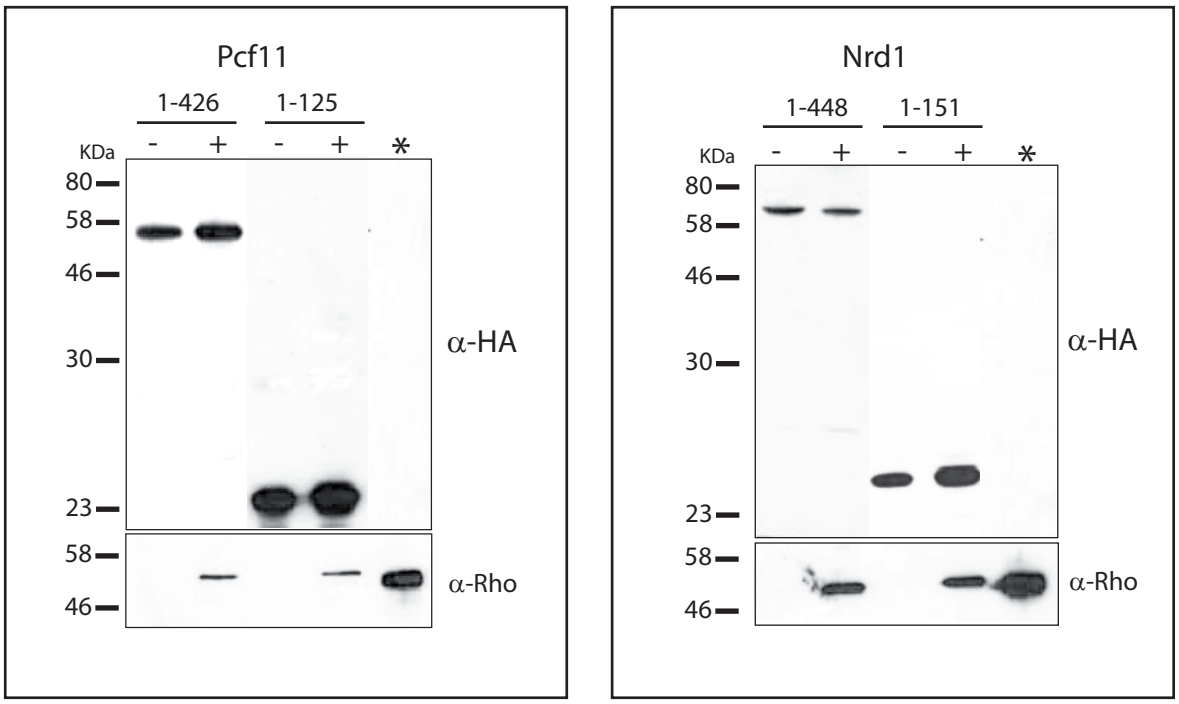

Figure S1: Western blot analyses showing the level of expression of Rho and the overproduced protein fragments. Cells harboring the Doxy-regulated Rho expression plasmid and a 2 μ construct expressing constitutively the indicated HA-tagged Pcf11 and Nrd1 protein-fragments (Pcf11: 1-426 and 1-125; Nrd1: 1-448 and 1-151) were grown under Rho-induced (+) or Rho-repressed (-) conditions. The proteins were extracted in lysis buffer, fractionated on a 10% polyacrylamide gel and analyzed by immunobloting using anti-HA antibodies (α-HA from Invitrogen) to detect the overproduced protein-fragments or custom made anti-Rho polyclonal antibodies (α-Rho from Eurogentec) to detect Rho. The proteins were visualized with a secondary antibody using a chemiluminescence detection kit from Invitrogen. Purified bacterial Rho protein (lanes indicated by asterisks) run in parallel was used as control. Protein molecular weights, according to ColorPlus prestained protein marker (New England Biolabs) run in parallel, are shown on the left of the gels.

Figure S1

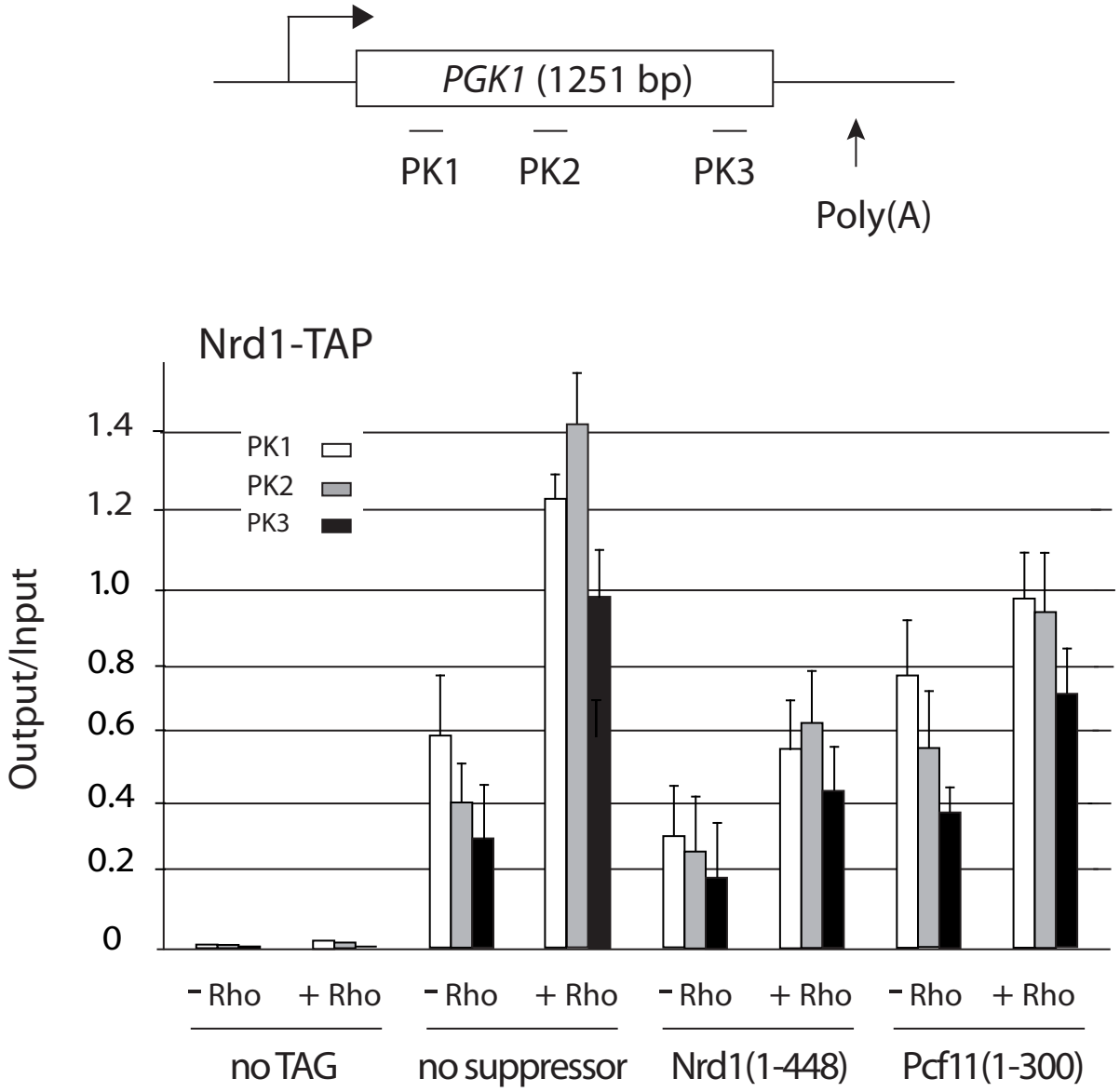

Figure S2: ChIP analyses of Nrd1 recruitment across the *PGK1* locus

(Upper panel) Schematic representation of the *PGK1* gene with the horizontal bars denoting the positions of the PCR products used in the ChIP analyses. **(Lower panel)** Quantifications of the fold enrichment for *PGK1* DNA in Nrd1-TAP immunoprecipitates from cells expressing Rho (+Rho) or not (- Rho) and co-transformed with either the empty 2µ vector vector for the assays without suppressor or the plasmids over-expressing the suppressors Nrd1 (1-448) or Pcf11 (1-300). The « no TAG » control strain is W303 transformed with the empty 2µ vector and either the (- Rho) or the (+Rho) plasmids. Immunoprecipitated samples (output) were normalized to input. The average of three independent experiments is shown with error bars representing standard deviations.

Figure S2

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

Strain name	Genotype	Source
BMA41	*MAT*α *ade2-1 ura3-1 leu2-3,112 his3-11,15 trp1*Δ *can1-100*	Baudin-Baillieu et al.,1997*
W303	*MAT*α *ade2-1 ura3-1 leu2-3,112 his3-11,15 trp1-1 can1-100*	Thomas and Rothstein, 1989**
Δ*rrp6*	as BMA41 with *rrp6*Δ::*KAN*	Mosrin-Huaman et al., 2009
DLY1144	as W303 with *nrd1*Δ::*KAN [pRS415-NRD1-HA, LEU2, CEN/ARS]*	D. Libri laboratory
DLY1146	as W303 with *nrd1*Δ::*KAN [pRS415-nrd1-102-HA, LEU2, CEN/ARS]*	D. Libri laboratory
NHY001	as W303 with *nrd1*Δ::*KAN [pAG415-NRD1, LEU2, CEN/ARS]*	This study
YNH026	as W303 with *nrd1*Δ::*KAN [pAG415-nrd1*Δ*1-151, LEU2, CEN/ARS]*	This study
YPN102	as W303 with *nab3-11*	P. Thuriauχ laboratory
DLY913	as W303 with *pcf11*Δ::*TRP1 [pNOPL-PCF11, LEU2, CEN/ARS]*	D. Libri laboratory
DLY915	as W303 with *pcf11*Δ::*TRP1 [pNOPL-pcf11-13, LEU2, CEN/ARS]*	D. Libri laboratory
DLY914	as W303 with *pcf11*Δ::*TRP1 [pNOPL-pcf11-2, LEU2, CEN/ARS]*	D. Libri laboratory
rpo21-Δ*104*	as W303 with *rpo21-*Δ*104*	P. Thuriauχ laboratory
DLY1124	as W303 with *NRD1-TAP*::*HIS3*	D. Libri laboratory
DLY388	as W303 with *RRP41-TAP*::*KAN*	D. Libri laboratory
DLY391	as W303 with *RRP4-TAP*::*HIS3*	D. Libri laboratory
DLY269	as W303 with *RRP6-TAP*::*HIS3*	D. Libri laboratory

*Baudin-Baillieu, A., Guillemet, E., Cullin, C., and Lacroute, F. (1997). Construction of a yeast strain deleted for the TRP1 promoter and coding region that enhances the efficiency of the polymerase chain reaction-disruption method. Yeast *13*, 353-356.

**Thomas, B.J., and Rothstein, R. (1989). The genetic control of direct-repeat recombination in Saccharomyces: the effect of rad52 and rad1 on mitotic recombination at GAL10, a transcriptionally regulated gene. Genetics *123*, 725-738.

Table S2 - Plasmids used in this study

Plasmid name	Yeast sequences	Rho expression	Backbone vector	Source
pCM185	*CEN TRP1*			Euroscarf
pCM189	*CEN URA3*			Euroscarf
pCM185-Rho	*CEN TRP1*	*pTetO$_7$::Rho-NLS*	pCM185	Mosrin-Huaman et al., 2009
pCM189-Rho	*CEN URA3*	*pTetO$_7$::Rho-NLS*	pCM189	This study
pCM189-Rho-KE	*CEN URA3*	*pTetO$_7$::Rho-KE-NLS*	pCM189	This study
pRS415-*NRD1*-HA	*CEN LEU2 NRD1*		pRS415	D.Libri laboratory
pRS415-*nrd1-102*-HA	*CEN LEU2 nrd1-102*		pRS415	D.Libri laboratory
pNOPL*-*PCF11*	*CEN LEU2 PCF11*		pRS315-2XProtA-TEV	D.Libri laboratory
pNOPL*-*pcf11-2*	*CEN LEU2 pcf11-2*		pRS315-2XProtA-TEV	D.Libri laboratory
pNOPL*-*pcf11-13*	*CEN LEU2 pcf11-13*		pRS315-2XProtA-TEV	D.Libri laboratory
pAG425GPD	*2µ LEU2*		pAG425GPD-ccdB	Addgene
FL (*PCF11*)	*2µ LEU2 PCF11*		pAG425GPD-ccdB	Mosrin-Huaman et al., 2009
1-426	*2µ LEU2 pcf11* 1-426		pAG425GPD-ccdB	This study
1-300	*2µ LEU2 pcf11* 1-300		pAG425GPD-ccdB	This study
1-145	*2µ LEU2 pcf11* 1-145		pAG425GPD-ccdB	This study
1-125	*2µ LEU2 pcf11* 1-125		pAG425GPD-ccdB	This study
ΔCID (*PCF11*)	*2µ LEU2 pcf11Δ1-125*		pAG425GPD-ccdB	This study
M1	*2µ LEU2 pcf11* 1-145 M1 (A66D)		1-145	This study
M2	*2µ LEU2 pcf11* 1-145 M2 (D68A, S69A)		1-145	This study
M3	*2µ LEU2 pcf11* 1-145 M3 (D68A, S69A, I70A)		1-145	This study
FL (*NRD1*)	*2µ LEU2 NRD1*		pAG425GPD-ccdB	This study
1-151	*2µ LEU2 nrd1* 1-151		pAG425GPD-ccdB	This study
1-178	*2µ LEU2 nrd1* 1-178		pAG425GPD-ccdB	This study
1-369	*2µ LEU2 nrd1* 1-369		pAG425GPD-ccdB	This study
1-448	*2µ LEU2 nrd1* 1-448		pAG425GPD-ccdB	This study
ΔRRM	*2µ LEU2 nrd1Δ340-410*		pAG425GPD-ccdB	This study
NRD1	*CEN LEU2 NRD1*		pAG415GPD-ccdB	This study
ΔCID (*NRD1*)	*CEN LEU2 nrd1Δ1-151*		pAG415GPD-ccdB	This study

*Senger, B., Simos, G., Bischoff, F.R., Podtelejnikov, A., Mann M., and Hurt, E. (1998). Mtr10p functions as a nuclear import receptor for the mRNA-binding protein Npl3p. EMBO J *17*, 2196-2207.

Table S3 – Primers used in this study for the construction of overexpression plasmids (full length, deleted, and mutated ORFs)

ORF	Plasmid name	Forward	Reverse
PCF11	1-426	5'*GGGG***ACAAGTTTGTACAAAAAAGCAGGCT***CCACC***ATGGATCACGACACAGAAGTTATAGT 3'	5'*GGGG***ACCACTTTGTACAAGAAAGCTGGGT***C*CTATCATTACTA**CTTACCACACACGCTACATTTATTTG 3'
	1-300		5'*GGGG***ACCACTTTGTACAAGAAAGCTGGGT***C*CTATCATTACTA**TTCAACGCCAGAAATATTACCAAAAAGAG 3'
	1-145		5'*GGGG***ACCACTTTGTACAAGAAAGCTGGGT***C*CTATCATTA**GTTTTTCTGGTGGAGACGA 3'
	1-125		5'*GGGG***ACCACTTTGTACAAGAAAGCTGGGT***C*CTATCATTACTA**GCCTTCAAACAGAGG 3'
	∆ CID (*PCF11*)	5'*GGGG***ACAAGTTTGTACAAAAAAGCAGGCT***CCACC***ATGTCAGCACTGGAGAAAATTG 3'	5'*GGGG***ACCACTTTGTACAAGAAAGCTGGGT***C*CTATCACTATTA**TTTTGTGACCAATTTC 3'
	M1	5'AAGCTATACGCTTTTTATGACTTGGACTCCATTTGTAAG 3'	5'CTTACAAATGGAGTCCAAGTCATAAAAAGCGTATAGCTT3'
	M2	5'CGCTTTTTATGCCTTGGCCGCCATTTGTAAGAATGTTGG 3'	5'CCAACATTCTTACAAATGGCGGCCAAGGCATAAAAAGCG 3'
	M3	5'CGCTTTTTATGCCTTGGCCGCCGCTTGTAAGAATGTTGG 3'	5'CCAACATTCTTACAAGCGGCGGCCAAGGCATAAAAAGCG 3'
NRD1	NRD1	5'*GGGG***ACAAGTTTGTACAAAAAAGCAGGCT***CCACC***ATGCAGCAGGACGACGATTTTC 3'	5'*GGGG***ACCACTTTGTACAAGAAAGCTGGGT***C*CTAGCAAATAAAGGGTGGAGTAAAG 3'
	∆CID (*NRD1*)	5'*GGGG***ACAAGTTTGTACAAAAAAGCAGGCT***CCACC***ATGGACATATCGAATAACACC 3'	
	1-151	As NRD1 plasmid	5'*GGGG***ACCACTTTGTACAAGAAAGCTGGGT***C*CTATTATCA**AGCAAAGCATTTTGACCTGATGGCG 3'
	1-178		5'*GGGG***ACCACTTTGTACAAGAAAGCTGGGT***C*CTATCATTA**CAAGATTTGTTTCGACCTCTGCTTGGG 3'
	1-369		5'*GGGG***ACCACTTTGTACAAGAAAGCTGGGT***C*CTATCATTA**AATGACGCTTTGTACTTCAGCAAAAGG 3'
	1-448		5'*GGGG***ACCACTTTGTACAAGAAAGCTGGGT***C*CTATCATTA**ACCAGAAGTGCCACCCCATTGCGC 3'
	∆RRM	5'-GGTCCAAGAGATTGTTGTGACTATCAACACGGTTACAGTATTATTCCTATGCACAGGC 3'	5'GATAGTCACAACAATCTCTTGGACCACGGCTGTAGACTTTAATATATCTGGTGG 3'

Bold sequences correspond to the Gateway attB-attachment sites and the stop codons whenever necessary. Italics and underlined sequences represent the linker nucleotides and the modified codons, respectively.

Table S4 - Oligonucleotides and amplicons used in the Northern blot analyses

Targets	Amplicons, bp	Oligonucleotide sequence	
PGK1	NA	5’ CCTTACCGAAGTACTTCAATTCC 3’	
18S	NA	5’ GGTTAAGGTCTCGTT 3’	
5.8S	NA	5’ TTTCGCTGCGTTCTTCATC 3’	
		Forward	**Reverse**
PMA1	631	5’ GATGGTCGTATTGTCACTGAAGAC 3’	5’ CAATAGCATCCAAACCCTTCTTC 3’

NA, not applicable

Table S5 – PMA1 and PGK1 primers used in the ChIP experiments

Alias	Name	Sequence
PM1	F-151	5' CAATCTAATCACGGTGTCGACGACGAAGAC 3'
	R-390	5' GGCTTCCATAACGAATTGAATTGGACCGAC 3'
PM2	F-1010	5’ GTTTGCCAGCTGTCGTTACCACCAC 3’
	R-1235	5’ GCAGCCAAACAAGCAGTCAACATCAAG 3’
PM3	F-2251	5' AACCTACCAAGATTATGGGGTATGTC 3’
	R-2532	5' CCAACCGAATAAGGTAAACATGGTAGCGATG 3’
PK1	F-455	5’ GGCTGATGTTTACATCAACGATGCCT 3’
	R-650	5’ TCAGCAACCTTGGCACCACCTAAG 3’
PK2	F-751	5’ ATCGGTGACTCCATCTTCGACAAGGC 3’
	R-927	5’ CCAGCCAGCTGGAATACCTTCCTTGTC 3’
PK3	F-1053	5’ GGCTTTGTTAGACGAAGTTGTCAAGAGC 3’
	R-1229	5’ GCAACACCTGGCAATTCCTTACCTTCC 3’