Appendix

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Supplementary Figures

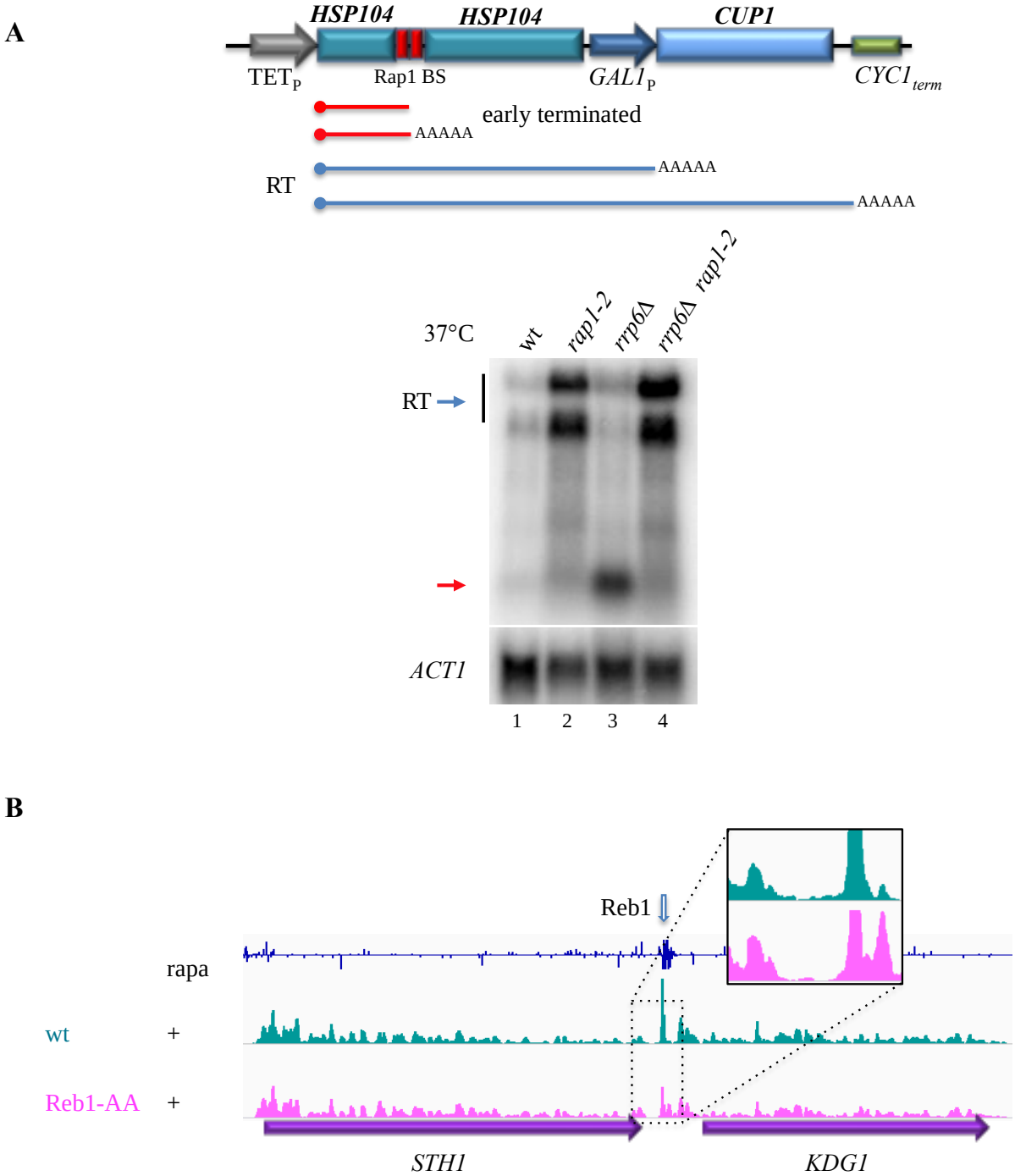

Figure S1. A. Northern blot analysis of transcripts derived from a construction containing the two Rap1 sites upstream of the *HYP2* gene in the context of the TET$_P$-*HSP104-GAL1*$_p$-*CUP1* reporter (scheme shown on top). Strains have been grown at permissive temperature (25°C) and shifted to 37°C for 4 hour to inactivate the *rap1-2* thermosensitive mutant. The transcript derived from termination at the Rap1 sites is indicated by a red arrow. Because of its instability, this transcript is best detected in *rrp6*Δ cells (lane 2). **B.** Snapshot showing the RNAPII CRAC signal at a site of Reb1-dependent transcriptional roadblock. The roadblock peak decreases significantly upon nuclear depletion of Reb1 by the addition of rapamycin for 1 hour to the Reb1-AA strain. As a control, the parental strain (containing untagged Reb1) was also treated with rapamycin for the same time. The inset contains a magnification of the region of the roadblock illustrating the appearance of a readthrough signal upon Reb1 depletion.

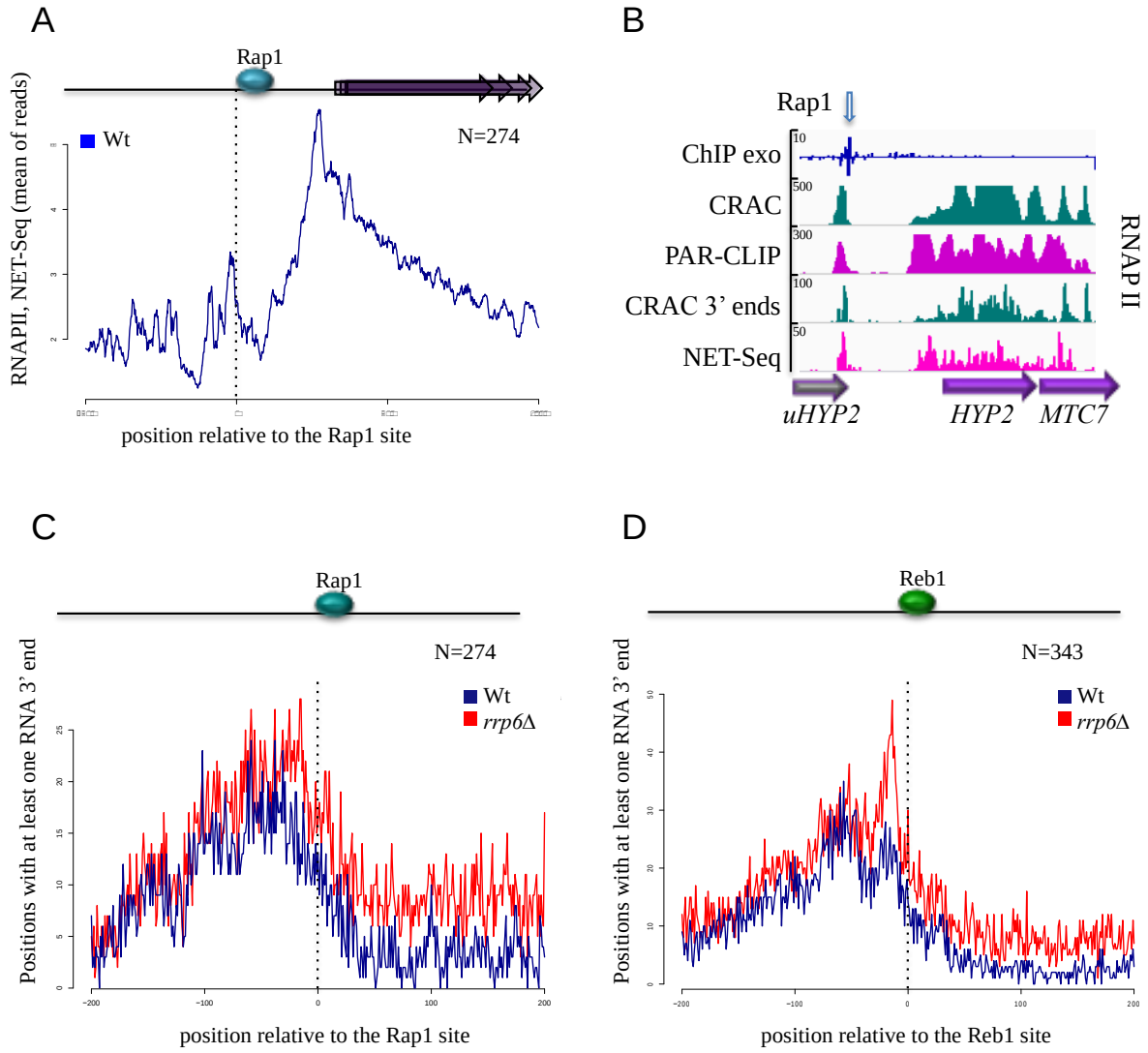

Figure S2. A. Metasite analysis analogous to the one shown in Figure 3A, but using NET-Seq, instead of CRAC data. **B.** Comparison of the CRAC, NET-Seq and PAR-CLIP signals at a site of Rap1 RB. For better comparison between CRAC and NET-Seq only the read 3' ends have been plotted in the last to tracks. Note the prominent presence of an RNAPII pausing peak revealed with all techniques. **C-D.** Aggregate distribution of RNA 3'-ends in wt or *rrp6*Δ cells as indicated upstream of Rap1 and Reb1 binding sites. The presence of these RNAs testifies to the occurrence of termination events at sites of roadblock.

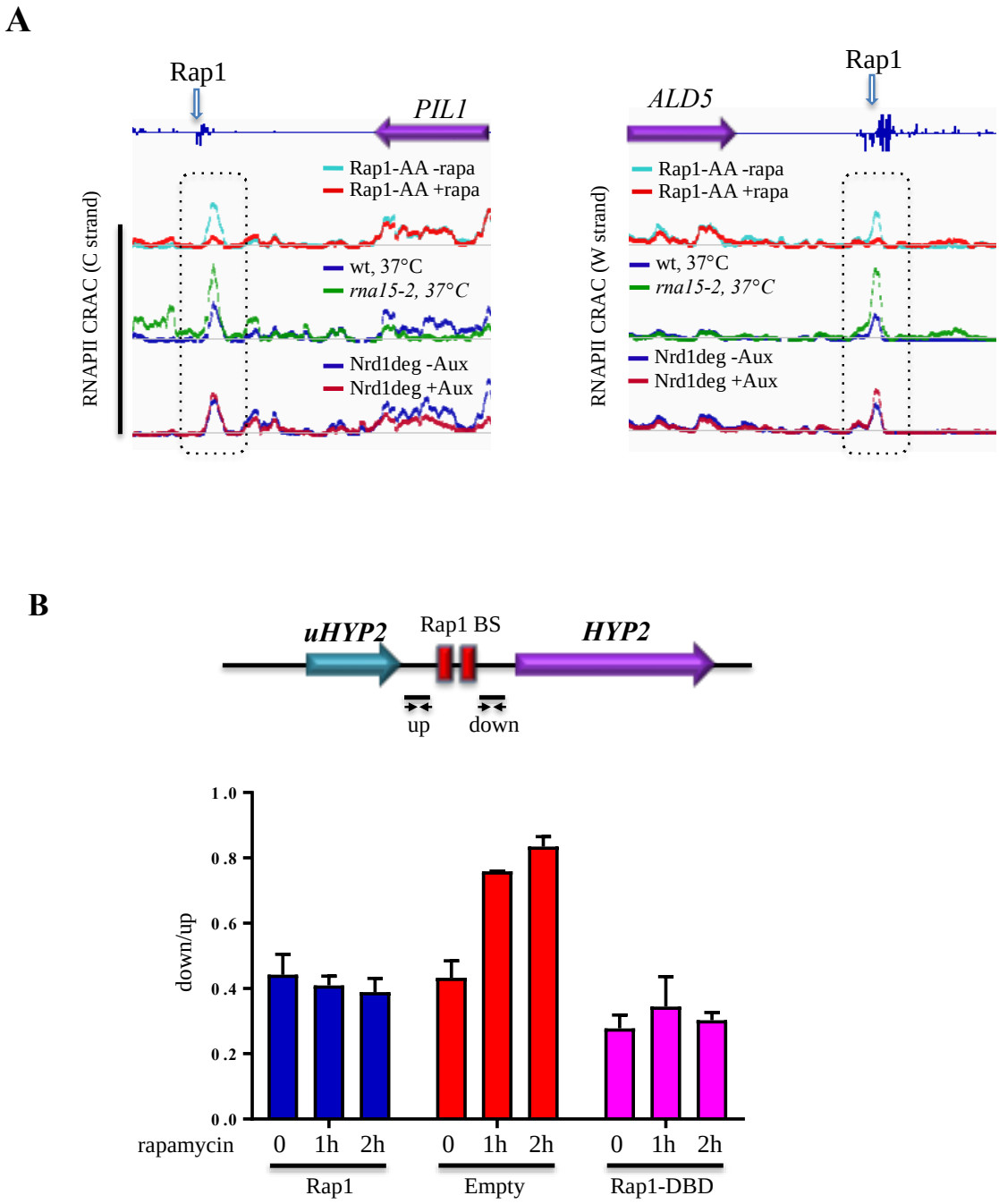

Figure S3. A. Representative snapshots illustrating the impact of the NNS complex on roadblock termination. The RNAPII CRAC signal at sites of roadblock termination downstream of *PIL1* and *ALD5* is shown (dotted rectangle). In both cases, the roadblock peak is insensitive to depletion of Nrd1 (bottom track, compare blue and red lines). As a control, the RB peak is strongly diminished by depletion of Rap1 (top track) and strongly increased upon mutation of the CPF pathway (middle track) because polymerases that fail to terminate at the CPF terminator accumulate at the RB site. **B.** Expression of the Rap1 DNA binding domain alone induces roadblock termination upstream of the *HYP2* locus. RT-qPCR analyses of transcripts around the Rap1 sites (n= 2, error bars indicate the half difference between the replicates). The ratio between the RT-qPCR signal detected before (up) and after (down) the roadblock (down/up) is used as a measure of roadblock efficiency upon depletion of Rap1 in the presence of ectopic Rap1, the Rap1 DNA binding domain (Rap1-DBD) or an empty vector as indicated. Data have been corrected for the different efficiency of amplification of the two amplimers. A schematic representation of the *HYP2* locus is shown on the top. The amplimers used for the RT-qPCR analysis are located 46nt before the first Rap1 site ("up") and immediately after the second Rap1 site ("down").

Table S1. Strains used in this study

Library #	Name	Genotype	Reference
DLY671	BMA64	*MAT**a** ura3-1; ade2-1; his3-11,15; leu2-3,112; can1-100; trp1*Δ	F. Lacroute
DLY678	*trf4*Δ *rrp6*Δ	As BMA, *MAT**a**; trf4::KAN; rrp6::URA*	Libri Lab
DLY815	*rrp6*Δ	As BMA, *MAT**a**; rrp6::KAN*	Libri Lab
DLY2241	W303	*ade2-1; ura3-1; his3-11,15; trp1-1; leu2-3,112; can1-100*	(Thomas & Rothstein, 1989)9)
DLY2207	*rap1-2*	As W303 *MAT**a**; rap1-2*	(Kurtz & Shore, 1991)1)
DLY2242	*rsp5-1*	As W303 *rsp5-1::HIS*	(Harreman *et al*, 2009)
DLY2547	HHY212 Anchor away loxed	As W303, *MAT**a** ; tor1-1; fpr1::loxP*; *RPL13A-2xFKBP12::loxP*; *ura3-1*	(Haruki *et al*, 2008)
DLY2568	Reb1-AA	as DLY2547, *REB1-FRB::KAN*	this study
DLY2570	Rap1-AA	as DLY2547, *RAP1-FRB::KAN*	this study
DLY2571	Rpb1-HTP	As BMA, *MAT**a**; RPB1-HTP::TRP1Kl*	this study
DLY2736	Reb1-AA Rpb1-HTP	As DLY2568, *MAT**a**; RPB1-HTP::TRP1Kl*	this study
DLY2754	Rpb1-HTP *rna15-2*	As DLY2571, *MAT rna15-2*	this study
DLY2838	Rpb1-HTP Anchor away	As W303; *MAT RPB1-HTP::URAKl, tor1-1; fpr1::NAT; RPL13A-2xFKBP12::loxP-TRP1-loxP*	this study
DLY2840	Rap1-AA Rpb1-HTP	As DLY2838, *MAT RAP1-FRB-RAP1::LEU*	this study
DLY2859	WT AA Rpb1-HTP *rrp6*Δ	As DLY2838, *MAT rrp6::HIS5Sp*	this study
DLY2860	Reb1AA Rpb1-HTP *rrp6*Δ	As DLY2736, *MAT**a** rrp6::HIS5Sp*	this study
DLY2861	Rap1 AA Rpb1-HTP *rrp6*Δ	As DLY2840, *MAT* ; *rrp6::HIS5Sp*	this study
DLY2867	Rpb1-HTP Nrd1-AID	*As 2571, MAT**a**, RPB1-HTP::TRP1kl NRD1-3Flag-mAID, KAN::OsTIR1*	this study
DLY3080	*rap1-2 rrp6::KAN*	As DLY2207, *rrp6::KAN*	this study

Table S2. Oligonucleotides used in this study

Library #	Sequence (5'-3')	Use
DL190	TTGAGCCAACGTCAAAATCGTTAGAGCCCTTTCTGTAAATTGCGTTTGGTCGTTCAT	Northern blot probe, against HSP104
DL2627	ATTCAAAAGCGAACACCGAATTGACCATGAGGAGACGGTCTGGTTTAT	Northern blot probe, U4
DL3248	AGCGTCCAGCTACAGCGT	RT-Q-PCR (uHYP2, up)
DL3249	AACGGGAACGGCGACTTG	RT-Q-PCR (uHYP2, up)
DL3198	TGTCGCCTCACACGGACC	RT-Q-PCR (uHYP2, down)
DL3199	CCTCGATGTATTCCGTAG	RT-Q-PCR (uHYP2,down
L3-6N-GA	/5rApp/GCTtcNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT/3ddC/	3'-adapter
L3-6N-GU	/5rApp/GCTacNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT/3ddC/	3'-adapter
L3-6N-AC	/5rApp/GCTgtNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT/3ddC/	3'-adapter
L3-6N-UC	/5rApp/GCTgaNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT/3ddC/	3'-adapter
L5miRCat	5-/5InvddT/CTTGrGrCrArCrCrCrGrArGrArArUrUrCrCrA-3	5'-adapter
RT L3-2	5-ACACTCTTTCCCTACACGACGCTCTTCCG-3	RT primer
P5_3prime	5-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3	PCR
miRCat_PCR2	5-CAAGCAGAAGACGGCATACGAgatcCTTGGCACCCGAGAAT-3	PCR

Table S3. Plasmids used in this study

Library	Name	Description	Reference
pDL431	pCM190(TRP)-TET-HSP104-X3-HSP104-GAL1-LACZ	Reporter containing a Reb1-dependent terminator	(Colin *et al*, 2014)
DL435	pCM190(TRP)-TET-HSP104-X118-HSP104-GAL1-LACZ	Reporter containing a Rap1-dependent terminator	This study
DL468	pCM190(URA)-TET-HSP104-X118-HSP104-GAL1-CUP1	Reporter containing a Rap1-dependent terminator	This study
DL436	pCM190(TRP)-TET-HSP104-X118-ΔRap1 BS-HSP104-GAL1-LACZ	Same as DL435, containing a precise deletion of the Rap1 binding site	This study
DL878	pcM185(HIS)-P_{RAP1} *RAP1*	Plasmid expressing full length Rap1 under control of the *RAP1* promoter	This study
DL879	pcM185(HIS)- P_{RAP1} -*RAP1-DBD*$_{358-601}$	Plasmid expressing the DNA binding domain of Rap1 (aa. 358-601) under control of the *RAP1* promoter	This study

SUPPLEMENTAL REFERENCES

Colin J, Candelli T, Porrua O, Boulay J, Zhu C, Lacroute F, Steinmetz LM & Libri D (2014) Roadblock termination by reb1p restricts cryptic and readthrough transcription. *Mol. Cell* **56** 667–680

Harreman M, Taschner M, Sigurdsson S, Anindya R, Reid J, Somesh B, Kong SE, Banks CAS, Conaway RC, Conaway JW & Svejstrup JQ (2009) Distinct ubiquitin ligases act sequentially for RNA polymerase II polyubiquitylation. *Proc. Natl. Acad. Sci. U. S. A.* **106** 20705–20710

Haruki H, Nishikawa J & Laemmli UK (2008) The anchor-away technique: rapid, conditional establishment of yeast mutant phenotypes. *Mol. Cell* **31** 925–932

Kurtz S & Shore D (1991) RAP1 protein activates and silences transcription of mating-type genes in yeast. *Genes Dev.* **5** 616–628

Malabat C, Feuerbach F, Ma L, Saveanu C & Jacquier A (2015) Quality control of transcription start site selection by nonsense-mediated-mRNA decay. *eLife* **4**

Thomas BJ & Rothstein R (1989) Elevated recombination rates in transcriptionally active DNA. *Cell* **56** 619–630