

**Rph1 coordinates transcription of ribosomal protein gene and ribosomal RNA to control cell growth under nutrient stress conditions**

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**Supplementary Table S1. Yeast strains**

<b>Yeast strains</b>	<b>Genotype</b>	<b>Source</b>
BY4741	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Open Biosystems
W303	MATa <i>leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15</i>	Open Biosystems
R1158	MATa <i>URA3::CMV-tTA his3-1 leu2-0 met15-0 ura3-1</i>	GE Healthcare
BY4741 <i>rph1Δ</i>	<i>rph1Δ::KanMX</i>	Open Biosystems
BY4741 <i>set2Δ</i>	<i>set2Δ::KanMX</i>	Open Biosystems
HNDY001	<i>RPH1::RPH1-3xFlag-KanMX6</i>	(1)
HNDY044	as BY4741 with pRS415 [empty vector]	(1)
HNDY122	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [empty vector]	This study
HNDY123	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [endo-GFP-Rph1]	This study
HNDY124	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [endo-GFP-Rph1 H235A]	This study
HNDY125	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [endo-GFP-Rph1 JmjNΔ]	This study
HNDY126	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [endo-GFP-Rph1 ZFΔ]	This study
BY4741 <i>tor1Δ</i>	<i>tor1Δ::KanMX</i>	Open Biosystems
HNDY127	BY4741 <i>tor1Δ::KanMX</i> , <i>rph1Δ::HygB</i>	This study
HNDY128	R1158 <i>rph1Δ::HygB</i>	This study
R1158 <i>Tet-tor2</i>	<i>pTOR2::kanR-tet07-TATA</i>	GE Healthcare
HNDY129	R1158 <i>pTOR2::kanR-tet07-TATA</i> , <i>rph1Δ::HygB</i>	This study
BY4741 <i>rim15Δ</i>	<i>rim15Δ::KanMX</i>	Open Biosystems
HNDY130	BY4741 <i>rph1Δ::KanMX</i> , <i>rim15Δ::HygB</i>	This study
HNDY131	W303 <i>rph1Δ::KanMX</i>	This study
HNDY050	BY4741 with pRS415 [ <i>pMET17-GFP-Rph1</i> ]	(1)
HNDY051	BY4741 with pRS415 [ <i>pMET17-GFP-Rph1 H235A</i> ]	(1)
HNDY052	BY4741 with pRS415 [ <i>pMET17-GFP-Rph1 JmjNΔ</i> ]	(1)
HNDY053	BY4741 with pRS415 [ <i>pMET17-GFP-Rph1 ZFΔ</i> ]	(1)
HNDY054	BY4741 with pRS415 [ <i>pMET17-GFP-Gis1</i> ]	(1)
HNDY132	BY4741 with pRS415 [endo-GFP-Rph1]	This study
HNDY133	BY4741 pRS415 [endo-GFP-Rph1 S7A]	This study
HNDY134	BY4741 pRS415 [endo-GFP-Rph1 S7D]	This study
HNDY135	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [endo-GFP-Rph1 S412A]	This study
HNDY136	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [endo-GFP-Rph1 S425/S426A]	This study
HNDY137	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [endo-GFP-Rph1 S429/430A]	This study
HNDY138	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [endo-GFP-Rph1 S434A]	This study

HNDY139	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -GFP-Rph1 S557A]	This study
HNDY140	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -GFP-Rph1 S561A]	This study
HNDY141	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -GFP-Rph1 S575A]	This study
HNDY142	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -GFP-Rph1 S412D]	This study
HNDY143	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -GFP-Rph1 S425/S426D]	This study
HNDY144	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -GFP-Rph1 S429/430D]	This study
HNDY145	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -GFP-Rph1 S434D]	This study
HNDY146	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -GFP-Rph1 S557D]	This study
HNDY147	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -GFP-Rph1 S561D]	This study
HNDY148	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -GFP-Rph1 S575D]	This study
HNDY149	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -GFP-Rph1 S7A]	This study
HNDY150	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -GFP-Rph1 S7D]	This study
HNDY151	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -Rph1-3xFLAG]	This study
HNDY152	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -Rph1-3xFLAG S7A]	This study
HNDY153	BY4741 <i>rph1Δ::KanMX</i> , pRS415 [ <i>endo</i> -Rph1-3xFLAG S7D]	This study
HNDY154	BY4741 <i>RRN3::RRN3-3xFlag-HygB</i>	This study
HNDY155	BY4741 <i>rph1Δ::KanMX</i> , <i>RRN3-3xFlag-HygB</i>	This study

**Supplementary Table S2.** Primer sequences

Primer	Sequence	Purpose	Reference /Source
<i>ATG7-pro-5'</i>	CGCAAGATTTTTAACTTCCGCT	ChIP-qPCR	This study
<i>ATG7-pro-3'</i>	AGCATCGTAAAATAATGAGTTCCT	ChIP-qPCR	This study
<i>Chr V-5'</i>	GGCTGTCCAGAAATATGGGGCCGTAGTA	ChIP-qPCR	(2)
<i>Chr V-3'</i>	CACCCCGAAGCTTTCACAATAC	ChIP-qPCR	(2)
<i>rDNA A-5'</i>	CAACCGAAACCAAAACCAAC	ChIP-qPCR	(3)
<i>rDNA A-3'</i>	TGGCATGGATTTCCCTTTAG	ChIP-qPCR	(3)
<i>rDNA B-5'</i>	TTTCTCGTAAGGTGCCGAGT	ChIP-qPCR	(3)
<i>rDNA B-3'</i>	GGGGATCGAAGATGATCAGA	ChIP-qPCR	(3)
<i>rDNA C-5'</i>	TACGAGCCTCCACCAGAGTT	ChIP-qPCR	(3)
<i>rDNA C-3'</i>	AATCGACCGATCCTGATGTC	ChIP-qPCR	(3)
<i>rDNA D-5'</i>	CAATCCAACGCTTACCGAAT	ChIP-qPCR	(3)
<i>rDNA D-3'</i>	CGATGTCCGGCTCTTCCTATC	ChIP-qPCR	(3)
<i>rDNA E-5'</i>	TCCCTCCATTTCCCTCTCTT	ChIP-qPCR	(3)
<i>rDNA E-3'</i>	GGAAAGCGGGAAGGAATAAG	ChIP-qPCR	(3)
<i>5S rDNA-5</i>	GTTGCGGCCATATCTACCAG	ChIP-qPCR	This study
<i>5S rDNA-3</i>	GATTGCAGCACCTGAGTTTC	ChIP-qPCR	This study
<i>NOP1-C ter-5'</i>	CTACTGTAGACGCGGAAACC	ChIP-qPCR	This study
<i>NOP1-C ter-3'</i>	CCGCTTCTCATGTATCTACC	ChIP-qPCR	This study
<i>NOP1-pro-5'</i>	GTGTCCCTTAACCCTTTAGAGC	ChIP-qPCR	This study

<i>NOP1-pro-3'</i>	CGTGAATTTTACAGAACGGAGG	ChIP-qPCR	This study
<i>NSR1-C ter-5'</i>	AGACCATCTGGTTCTGGTGC	ChIP-qPCR	This study
<i>NSR1-C ter-3'</i>	AACCAGCGAAAGAAGCGGTA	ChIP-qPCR	This study
<i>NSR1-pro-5'</i>	AAATTTGCAAGGGCAGCTCA	ChIP-qPCR	This study
<i>NSR1-pro-3'</i>	TGCAGACAGCAACAGCTACA	ChIP-qPCR	This study
<i>PHR1-pro-5'</i>	TCACAGAACAGACAACCAGCA	ChIP-qPCR	This study
<i>PHR1-pro-3'</i>	TGATTTGCGCCTGTCCTAGT	ChIP-qPCR	This study
<i>RPL10-C ter-5'</i>	AACCAAGGACAGCAACAAGGA	ChIP-qPCR	This study
<i>RPL10-C ter-3'</i>	ACCGTCGTCCTTGACTTCAC	ChIP-qPCR	This study
<i>RPL10-pro-5'</i>	CCCTCCGAAACTAGTTAGCACA	ChIP-qPCR	This study
<i>RPL10-pro-3'</i>	AGCACTTGC GGAAAGGATGT	ChIP-qPCR	This study
<i>RPL3-C ter-5'</i>	TGGTTTCGTCCACTACGGTG	ChIP-qPCR	This study
<i>RPL3-C ter-3'</i>	TCAGCTGGGGTTTGAATCT	ChIP-qPCR	This study
<i>RPL3-pro-5'</i>	CGCACACTGGAATGAATGGC	ChIP-qPCR	This study
<i>RPL3-pro-3'</i>	AAACAGTTGTGCGTCGCTTC	ChIP-qPCR	This study
<i>RPS12-C ter-5'</i>	AGGTGAATGGGCTGGTTTGG	ChIP-qPCR	This study
<i>RPS12-C ter-3'</i>	TCAGTTTCAGCACCCCAGTT	ChIP-qPCR	This study
<i>RPS12-pro-5'</i>	ACCTTACCCGCAAGCAAACCT	ChIP-qPCR	This study
<i>RPS12-pro-3'</i>	ACTGCTCCGTGAAGAGTTTTGA	ChIP-qPCR	This study
<i>ACT1-5'</i>	GAAATGCAAACCGCTGCTCA	RT-qPCR	This study
<i>ACT1-3'</i>	TACCGGCAGATTCCAAACCC	RT-qPCR	This study
<i>ATG7-5'</i>	ATGAGCATTGTCCAGCATGTAG	RT-qPCR	This study
<i>ATG7-3'</i>	GACCTCCTGCTTTATGACTGAC	RT-qPCR	This study
<i>ETS1-5'</i>	TGGGTTGATGCGTATTGAGA	RT-qPCR	(4)
<i>ETS1-3'</i>	TCGCTGATTTGAGAGGAGGT	RT-qPCR	(4)
<i>ITS1-5'</i>	TGTTTTGGCAAGAGCATGAG	RT-qPCR	(4)
<i>ITS1-3'</i>	TCGAATGCCCAAAGAAAAAG	RT-qPCR	(4)
<i>NOP1-5'</i>	GATCTAAGTTGGCTGCCGGT	RT-qPCR	This study
<i>NOP1-3'</i>	CGGCGTAGACAACACCTTCT	RT-qPCR	This study
<i>NSR1-5'</i>	TTCCGTCCGTATCCCAACAC	RT-qPCR	This study
<i>NSR1-3'</i>	CTTCTTGGCGTCCTCCATGT	RT-qPCR	This study
<i>RPS31-5'</i>	ACAAGGTGCTGCTGAAGGT	RT-qPCR	This study
<i>RPS31-3'</i>	AGCCAAGAAAACACCAGCAC	RT-qPCR	This study
<i>RPL5-5'</i>	CTTAGCTGCTGCCTACTCCC	RT-qPCR	This study
<i>RPL5-3'</i>	TGCAAGGTTCTTCTGGCGAT	RT-qPCR	This study
<i>RPL30-5'</i>	TTGCCGCTAACACTCCAGTT	RT-qPCR	This study
<i>RPL30-3'</i>	CTTACCGACAGCAGTACCCA	RT-qPCR	This study
<i>RPL3-5'</i>	TGGTTTCGTCCACTACGGTG	RT-qPCR	This study
<i>RPL3-3'</i>	TCAGCTGGGGTTTGAATCT	RT-qPCR	This study
<i>RPS12-5'</i>	AGGTGAATGGGCTGGTTTGG	RT-qPCR	This study
<i>RPS12-3'</i>	TCAGTTTCAGCACCCCAGTT	RT-qPCR	This study
<i>RPH1-5</i>	AACTCTAAGTTTGCGCCCGA	RT-qPCR	This study
<i>RPH1-3</i>	TCTCACCAGAGTGGACGGAT	RT-qPCR	This study

18S <i>rDNA</i> -5'	GCTTGC GTTGATTACGTCCC	RT-qPCR	(4)
18S <i>rDNA</i> -3'	CACTAAGCCATTCAATCGGT	RT-qPCR	(4)
25S <i>rDNA</i> -5	CGTTCATAGCGACATTGCTT	RT-qPCR	(4)
25S <i>rDNA</i> -3	GGGTGAACAATCCAACGCTT	RT-qPCR	(4)

### Supplementary Figure legends

**Figure S1. Protein levels of Rph1 is critical for controlling cell growth under nutrient stress conditions.** The indicated strains were spotted on YPD plates with or without rapamycin (A-C). **(A)** *RPH1*-deficient cells in W303 strain were resistant to different doses of rapamycin. **(B)** Cells lack of *RPH1* in BY4741 strain was also resistant to high concentration of rapamycin. **(C)** Cells with overexpression of the indicated GFP-Rph1 constructs lead to distinct response to rapamycin (left panel). The expression levels of the indicated cells were examined by immunoblotting with various antibodies (right panel).

**Figure S2. Transcriptional regulation by Rph1 under nutrient-rich medium.** **(A)** Volcano plots indicating the differentially expressed genes (DEGs) from overlapped two biological repeats in *rph1Δ* cells relative to WT. The vertical dashed gray lines in the plot represent log<sub>2</sub> normalized fold changes of ±0.5. The horizontal dashed gray line represents an adjusted *P* value of 0.05. ( $\log_2(\text{FC}) > 0.5, P < 0.05$ ). **(B)** GO analysis show the top 5 enriched upregulated gene clusters based on DEGs in A and C, respectively, by different pathways. BP: biological processes; CC: cellular components. **(C)** Boxplot showed genome-wide RPGs transcription in WT and *rph1Δ* cells. *P*-value denotes the result from two-tailed Student's *t*-test. **(D)** Expression of representative RPGs and Ribi genes was validated by RT-qPCR. Data are represented as mean ± SD from three biological replicates. *t*-test, \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001, n.s, "not significant".

**Figure S3. Overexpression of Rph1 decreased RP gene expression.** **(A)** Expression levels of Rph1 from cells expressing different *RPH1* constructs driven by the indicated promoters were examined by immunoblotting with an α-Rph1 antibody. **(B)** The indicated strains were spotted onto plates with or without rapamycin. **(C-D)** Comparison of expression levels of RP gene (C)

or rRNA (D) in different Rph1-overexpressing cells by RT-qPCR. Data are represented as mean  $\pm$  SD from three biological replicates. *t*-test, \*\**P* < 0.01; \*\*\**P* < 0.001.

**Figure S4. Genomic-wide enrichment of Rph1 in budding yeast.** (A) ChIP-seq profile of Rph1-Flag over chromosome V. (B) Genome browser view of Rph1 binding peaks on RPGs within the chromosome V of 30 kbp – 440 kbp. GSE121635 represents the ChIP-seq data obtained from NCBI. (C) Comparison of Rph1 ChIP-seq peaks between published dataset of GSE121635 and our dataset. (D) GO analysis from the published dataset of GSE121635 show the top 5 of Rph1-enriched peaks by different pathways. BP: biological processes; CC: cellular components.

**Figure S5. The enrichment of Rph1 in different genomic regions.** Enrichment of Rph1 ChIP-seq signals on RPGs (A), snoRNAs (B), rDNA loci (C), and Ribi genes (D) with or without rapamycin treatment. Data from two biological replicates. RPM: Read count Per Million mapped reads.

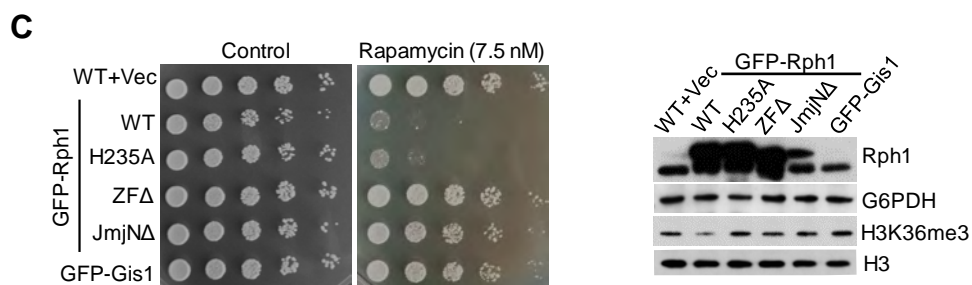
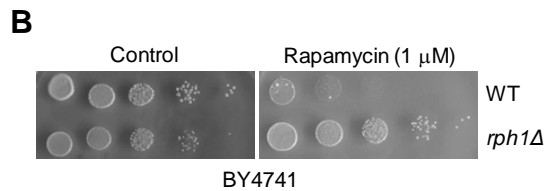
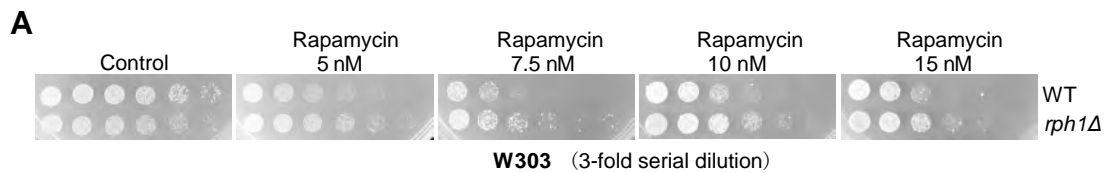
**Figure S6. Deletion of Rph1 did not affect Rrn3 association of 35S rDNA loci and processivity of RNAPII transcription under untreated or treated rapamycin conditions.** (A) ChIP-qPCR analysis were performed to examine RNAPII (Rpa190-Flag) enrichment over different rDNA loci in WT or *rph1* $\Delta$  cells without rapamycin treatment. IgG ChIP served as a negative control. (B) Western blot showed protein levels of the integrated 3xFlag tagged Rrn3 from each colony in either WT or *rph1* $\Delta$  cells using an anti-Flag antibody. (C-D) Relative enrichment of Rrn3 with the indicated rDNA loci in WT or *rph1* $\Delta$  cells was normalized to chromosome V locus by ChIP-qPCR analysis without (C) or with (D) rapamycin treatment. (E) Processivity assay of RNAPII transcription were performed in the *rph1* $\Delta$  cells relative to WT. The IP/input value for the 3' end (the D locus) was divided by the value for the 5' end (the B locus), and the ratio ("processivity") for *rph1* $\Delta$  was normalized to that for WT. Data are represented as mean  $\pm$  SD from three biological replicates. *t*-test, n.s, represents "not significant"

**Figure S7. Rim15-dependent Rph1 phosphorylation regulates cell growth under rapamycin stress conditions.** (A) The phosphorylation states of Rph1-Flag at different time points upon rapamycin stress were examined using a phos-tag gel. (B) Phosphorylation sites on Rph1 protein identified in previous studies as indicated in the Saccharomyces Genome Database (SGD) were marked in red. (C-D) Rapamycin sensitivity of various Rph1 mutants were monitored by a spot assay (top panel), and protein levels of various GFP-Rph1 mutants expressed in *rph1Δ* strain were examined by immunoblotting (bottom panel). G6PDH served as a loading control. (E) Cells with overexpression of WT, S7A or S7D Rph1 constructs were spotted on YPD plates with or without different doses of rapamycin.

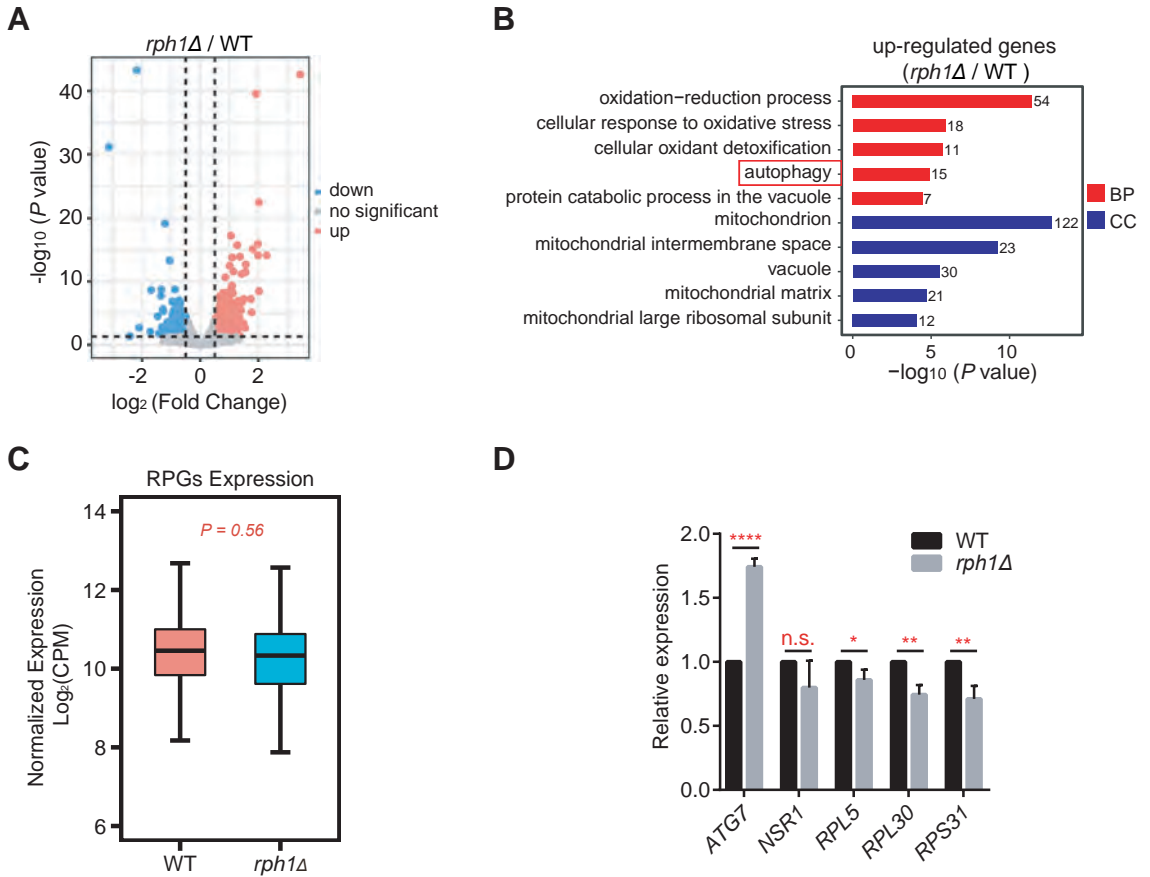
### Reference:

1. Li, F., Zheng, L.D., Chen, X., Zhao, X., Briggs, S.D. and Du, H.N. (2017) Gcn5-mediated Rph1 acetylation regulates its autophagic degradation under DNA damage stress. *Nucleic Acids Res.*, **45**, 5183-5197.
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4. Laribee, R.N., Hosni-Ahmed, A., Workman, J.J. and Chen, H. (2015) Ccr4-not regulates RNA polymerase I transcription and couples nutrient signaling to the control of ribosomal RNA biogenesis. *PLoS Genet.*, **11**, e1005113.

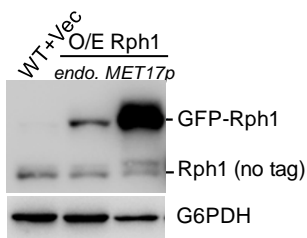
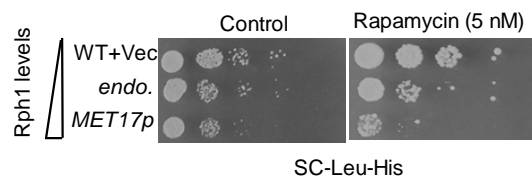
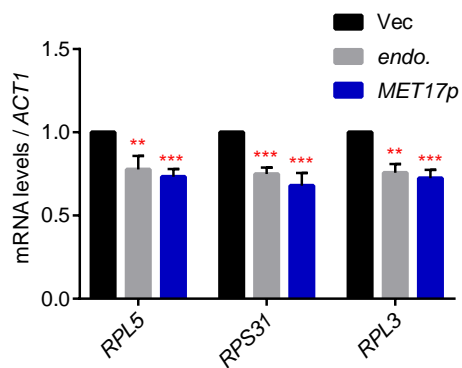
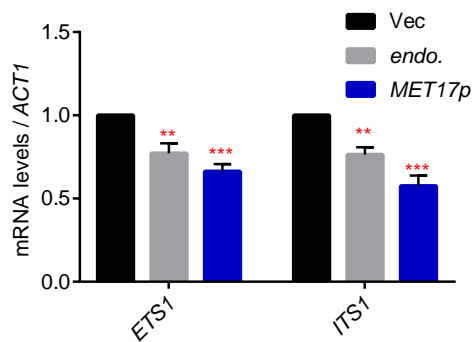
**Figure S1**



**Figure S2**

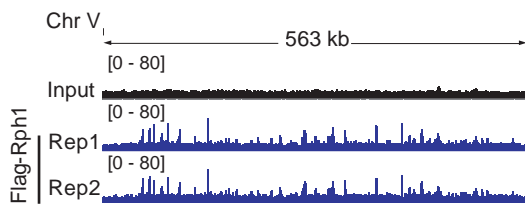




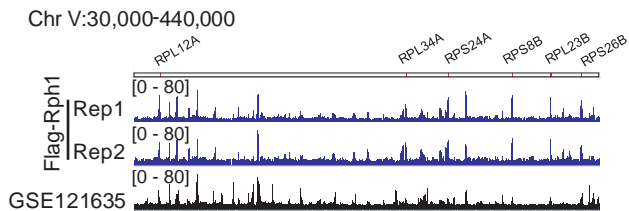
**Figure S3****A****B****C****D**

**Figure S4**

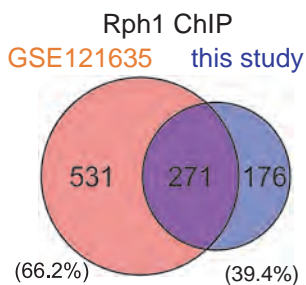
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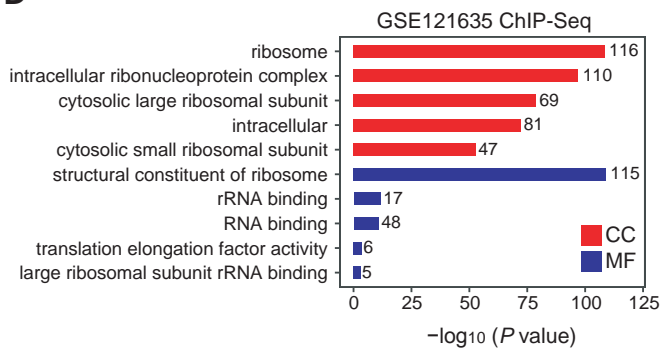
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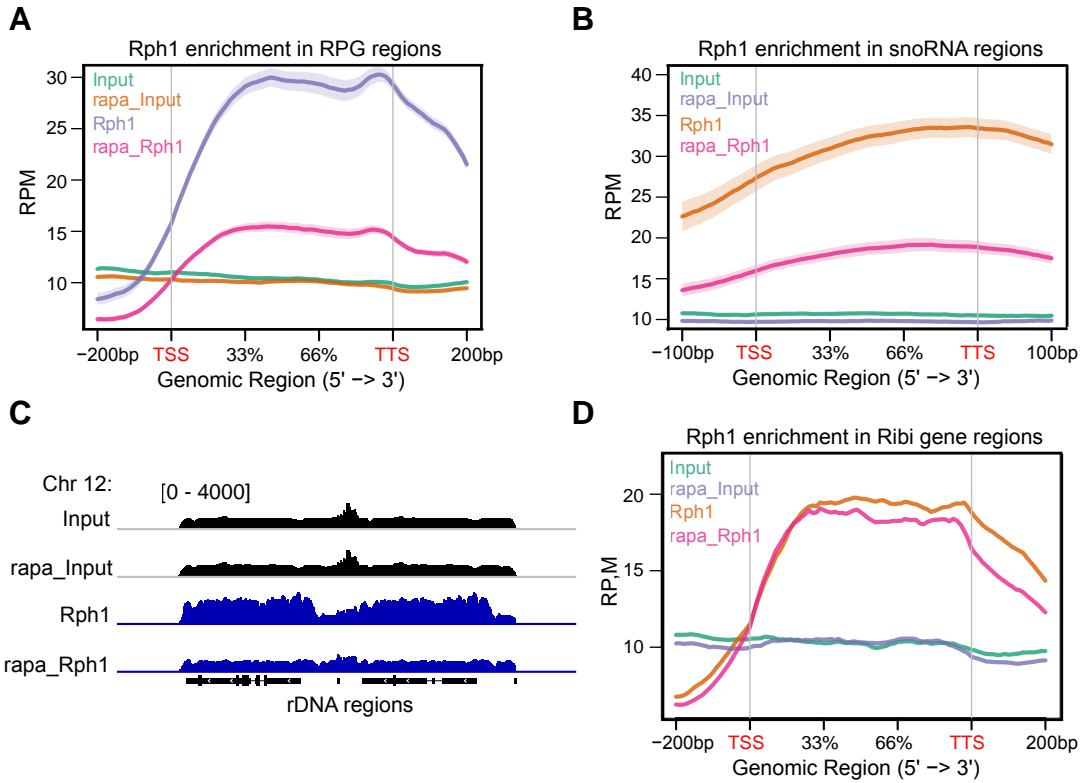


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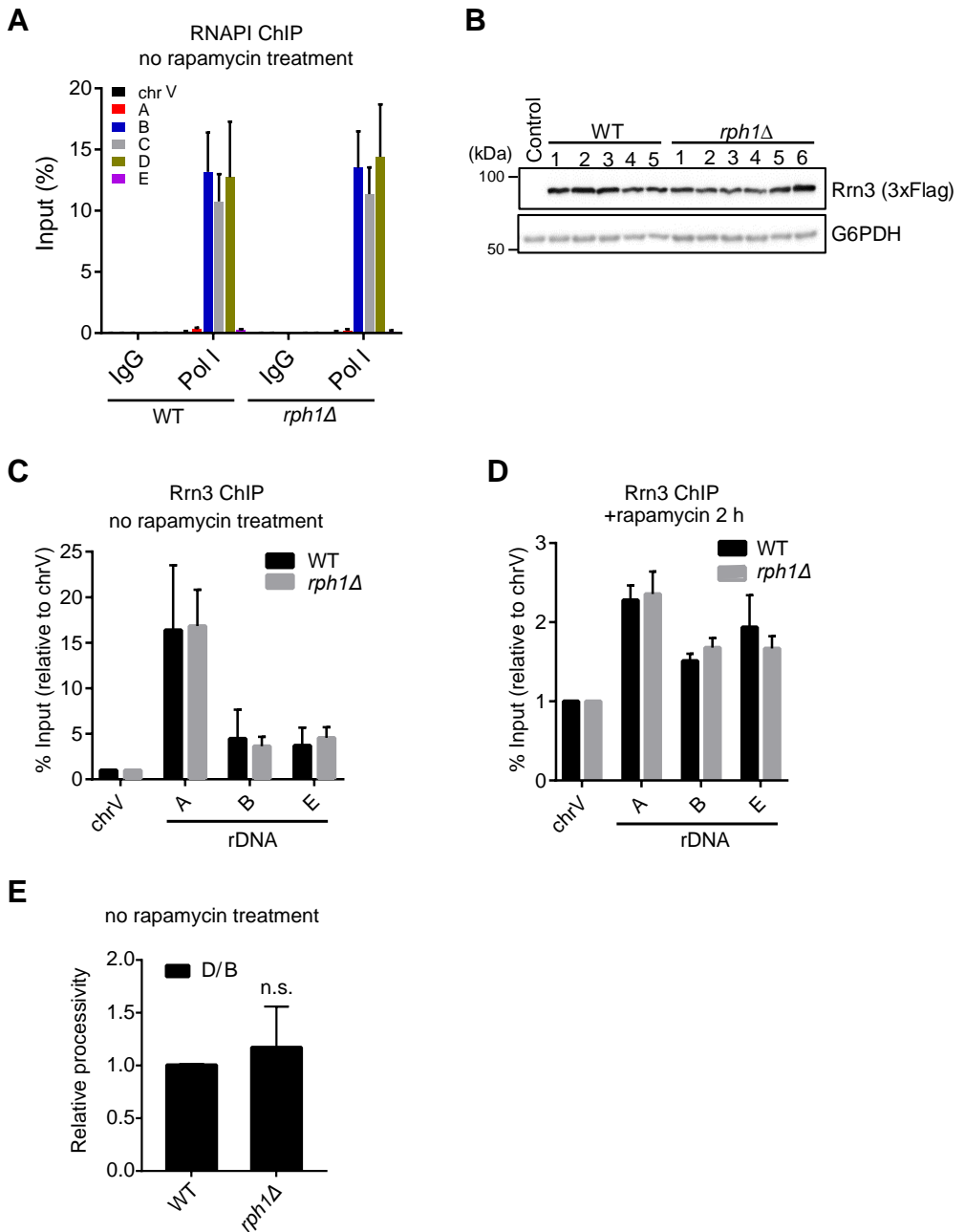


**D**



**Figure S5**

**Figure S6**



**Figure S7**

