Supplementary Information

Non-canonical CTD-kinases regulate RNA polymerase II in a gene-class specific manner

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Supplementary Tables

Supplementary Table 1. Comparison of snoRNA 3′- extension indices between *hrr25is*, *hrr25as*, T4A[1], *paf1Δ*[2], Nrd1 anchor away[3], *rrp6Δ*[4], and T4V[5]. Data missing from the chart were not included in the original manuscript.

	hrr25is	*hrr25as*	T4A	*paf1Δ*	Nrd1AA	*rrp6Δ*	T4V	*Bur1is*
SNR3	37.50	10.32	1.75	1.47	11.94	31.76	0.60	0.92
SNR71	22.76	0.83	14.76	6.28	15.19	7.18	1.40	0.80
SNR48	21.85	1.17	7.26	8.60	13.17	2.81	1.08	1.00
SNR56	20.62	1.64	2.31	2.13	18.01	0.95	0.59	0.86
SNR9	19.42	8.38	2.04	1.63		7.10	0.99	0.76
SNR45	19.17	2.01	1.18	4.31	18.55	19.03	0.67	1.10
SNR47	19.07	1.66	20.56	2.33	22.94	1.75	1.32	1.02
SNR64	18.82	1.62	7.32	3.06	12.83	1.50	1.32	1.33
SNR34	17.42	8.79	2.24	1.72	23.42	5.99	0.98	0.74
SNR82	17.41	5.83	10.11	1.73	12.87	4.60	0.89	1.17
SNR66	14.63	1.97	2.06	0.66	11.34	2.07	0.99	0.99
SNR32	14.49	8.88	1.44	2.89	17.04	5.64	0.88	0.70
SNR60	14.25	1.75	6.02	3.86	17.11	5.96	1.31	0.94
SNR5	14.19	4.88	16.24	1.90	9.74	6.81	0.84	0.80
SNR11	14.17	2.47	1.71	0.95		17.98	0.59	0.87
SNR37	12.85	1.24	0.85	0.56	8.48	38.40	0.93	0.89
SNR128	12.27	1.86	0.96	0.88	12.18	2.90	0.86	0.93
SNR10	11.75	3.72	0.92	0.75	15.59	0.88	0.83	0.78
SNR49	11.17	2.66	4.92	0.96	17.44	6.84	0.98	0.88
SNR68	9.72	1.46	4.55	2.35		2.70	0.99	1.10
SNR8	9.35	2.09	1.89	1.16	12.71	5.03	0.92	0.39
SNR46	8.72	4.14	2.03	0.86	15.42	7.65	1.07	1.16
SNR35	8.54	1.63	1.31	1.28	16.58	20.68	0.62	0.95
SNR67	8.01	1.10	0.64	0.31	7.70	2.19	0.69	0.69
SNR61	7.99	1.64	1.05	0.40	12.82	6.90	0.70	0.74
SNR13	7.79	1.15	18.57	1.12	17.26	8.20	0.65	0.78
SNR62	7.71	1.39	0.70	1.04	15.68	8.82	0.80	0.90
SNR80	7.56	4.89	0.56	0.44		3.62	0.56	0.76
SNR4	7.27	4.52	2.21	0.98	18.12	1.07	0.85	0.94
SNR39B	6.95	1.11	1.62	1.47	9.13	1.73	0.70	1.09
SNR83	6.72	1.57	1.26	1.25	12.65	3.23	0.58	0.83
SNR79	6.36	3.44	3.04	2.83	12.03	1.90	0.75	1.09
SNR31	6.34	3.45	0.85	0.46	12.38	11.95	0.62	1.19
SNR30	5.96	9.47	1.97	1.55	11.84	32.34	0.70	1.09
SNR42	5.53	1.22	4.83	2.29	10.65	14.64	0.95	1.11
SNR50	5.42	1.61	3.67	1.60	7.12	1.65	0.69	0.84
SNR58	4.84	0.93	2.15	1.56	9.97	2.68	0.71	1.23

SNR63	4.78	2.02	3.42	0.70	10.36	7.76	1.30	0.86
SNR69	4.75	1.79	4.26	1.86	11.10	3.43	0.89	0.66
SNR53	4.67	2.17	2.66	2.30	7.44	2.95	0.57	0.99
SNR33	4.60	2.53	20.55	2.18	6.64	2.27	1.03	0.68
SNR51	3.98	1.03	6.99	2.37		0.86	0.66	1.52
SNR189	3.85	9.62	5.29	1.56	6.87	4.93	0.91	0.97
SNR86	3.46	3.34	0.70	1.79		1.48	0.33	2.08
SNR40	3.38	0.99	0.78	1.47	8.48	1.20	0.60	1.04
SNR161	3.32	1.75	7.96	4.77	7.59	3.24	1.27	1.07
SNR77	3.18	0.96	0.64	0.87		1.72	0.68	1.06
SNR17A	2.98	1.34	0.79	1.64	5.44	1.29	0.55	1.30
SNR36	2.90	10.79	0.97	0.80	11.58	17.40	0.48	0.48
SNR81	2.86	5.78	10.42	3.75	5.86	5.96	0.67	0.70
SNR52	2.86	1.15	0.84	1.13		0.72	1.03	0.85
SNR54	2.78	1.02	0.77	2.12		0.86	1.15	0.90
SNR39	2.58	0.22	0.89	1.22		2.50	0.76	1.31
SNR74	2.51	1.12	0.95	0.73		1.62	0.61	0.99
SNR43	2.31	2.70	3.76	0.65	8.94	15.03	1.47	1.18
SNR72	1.99	0.88	1.78	0.83		1.53	0.59	1.64
SNR191	1.73	0.79	0.92	2.79		1.09	0.87	0.40
SNR73	1.72	1.05	0.92	0.64		2.17	0.66	0.69
SNR18	1.70	0.41	0.90	1.16	2.60	1.01	0.87	1.63
SNR38	1.60	1.12	0.98	1.54		4.17	0.68	0.54
SNR70	1.49	2.48	1.06	1.17		0.84	0.62	0.82
SNR59	1.42	0.45	1.91	1.03	7.97	9.84	0.91	0.91
SNR87	1.37	1.08	1.88	0.25	8.17	1.36	1.22	0.73
SNR17B	1.23	1.22	0.82	0.41		1.25	0.63	0.78
SNR57	1.20	0.67	0.55	0.91		1.73	0.63	0.85
SNR78	1.20	1.08	0.91	1.29		1.23	0.58	2.17
SNR84	1.18	3.04	1.04	1.63		3.48	0.67	1.52
SNR75	1.15	1.59	0.94	0.59		1.96	0.62	0.71
SNR76	1.07	0.83	0.88	0.86		2.29	0.61	0.82
SNR85	1.05	0.79	3.35	3.52	9.71		0.81	0.79
SNR44	1.01	0.82	1.24	0.55		0.50	0.66	0.69
SNR65	0.76	0.29	1.08	0.82	20.01	6.14	0.64	1.04
SNR190	0.63	1.63	0.87	1.16		0.73	0.62	0.95
SNR41	0.62	0.86	0.93	1.26		0.97	0.66	0.78
NME1	0.31	2.95	0.65	1.09			0.56	0.83
SNR24	0.21	0.52	0.97	0.66			0.63	0.77
SNR55	0.08	1.77	0.70	0.99		1.01	0.60	1.00

Supplementary Table 2. Oligonucleotides used in this study.

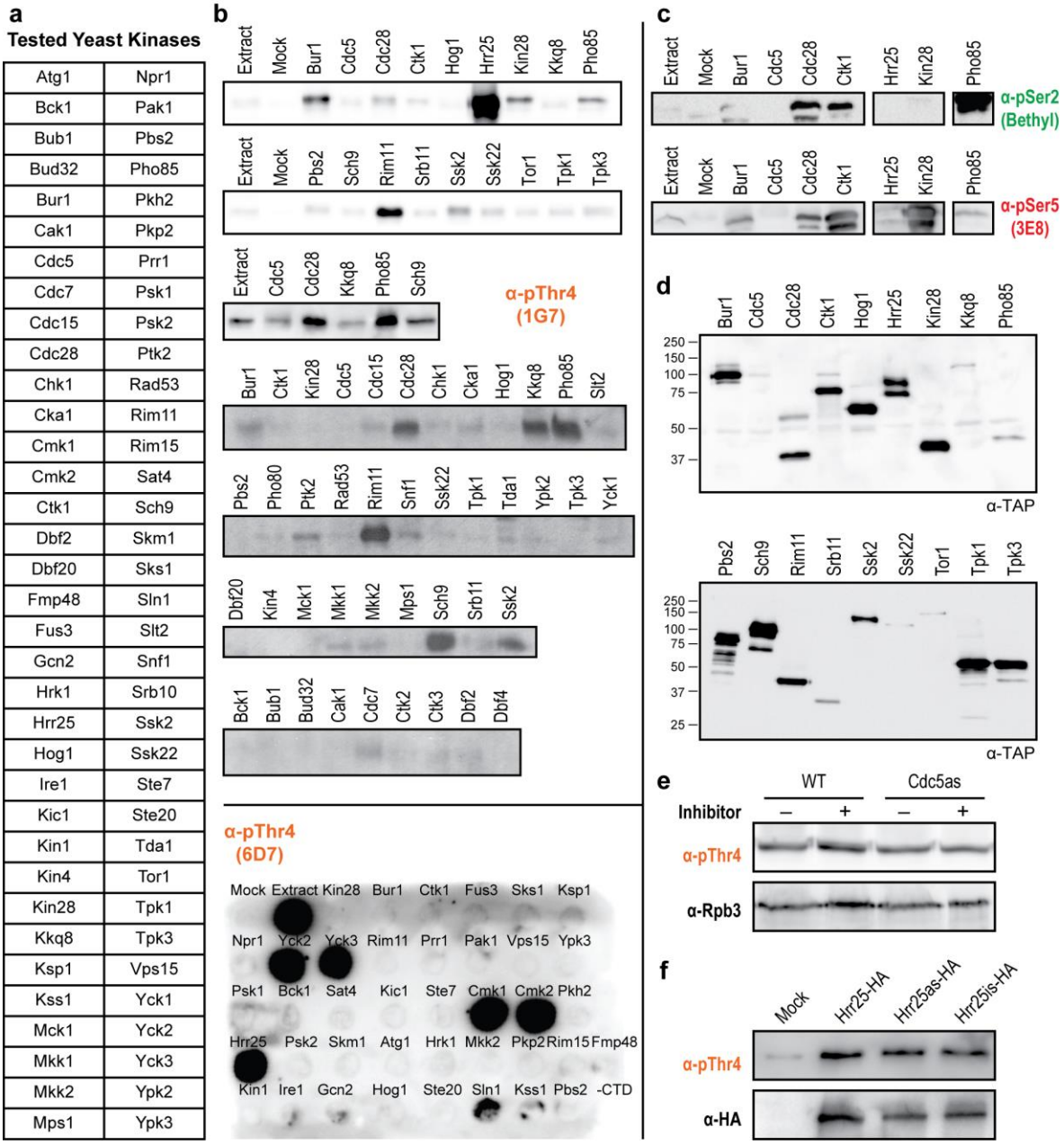
Name	Function	Sequence (5′-3′-)
Hrr25is F	SDM I23C	GTGGTTCCTTTGGTGACTGTTACCACGGCACGAAC
Hrr25is R	SDM I23C	GTTCGTGCCGTGGTAACAGTCACCAAAGGAACCAC
Hrr25 WT F	SDM WT	GGTGAATATAATGCTATGGTCATCGATCTTCTAGGCCCATCTTTG
Hrr25 WT R	SDM WT	CAAAGATGGGCCTAGAAGATCGATGACCATAGCATTATATTCACC
Hrr25 NcoI F	Hrr25-HA	TTCTTTGCCATGGCAGGGTTTG
Hrr25 NcoI R	Hrr25-HA	CAACCAAATTGACTGGCCAGC
Hrr25 pFA6a F	Hrr25-HA	GCTGGCCAGTCAATTTGGTTGcggatccccgggttaattaa
Hrr25 pFA6a R	Hrr25-HA	CTGATCGACGTCtcaTAAATCATAAGAAATTCG
Hrr25Δ F	Delete Hrr25	ATGGACTTAAGAGTAGGAAGGAAATTTCGTATTGGCAGGAAGATTGGGAGGATTGTACTGAGAGT
Hrr25Δ R	Delete Hrr25	TTACAACCAAATTGACTGGCCAGCTGGTTTATCTTGAGGCGGCTGTTGTGCTGTGCGGTATTTCA
SNR47-F	Northern Probe	TCATGTAAACGCATGGGG
SNR47-R	Northern Probe	TAATACGACTCACTATAGGAGCTACTCTGATTTACGTTACCGCC
SNR13-F	Northern Probe	TTATAAATGGCATCTCAAATCGTC
SNR13-R	Northern Probe	TAATACGACTCACTATAGGCTGTCGCTTCCGTGTCTCTTGTCCTG
SNR40-F	Northern Probe	AGTACCTTAACACATGACGAAGA
SNR40-R	Northern Probe	TAATACGACTCACTATAGGCTGATCTATTTCACGCCCAGA
SCR1-F	Northern Probe	AGGCTGTAATGGCTTTCTGGTGGGA
SCR1-R	Northern Probe	TAATACGACTCACTATAGGATATGTGCTATCCCGGCCGCCTCCA
M3F	Pma1-1 qPCR	GACGACGAAGACAGTGATAACGAT
M3R	Pma1-1 qPCR	GGACCGACGAAAAACATAACGAAC
M5F	Pma1-2 qPCR	CAACTGATGGTCGTATTGTCACTG
M5R	Pma1-2	CGAAAGTGTTGTCACCGGTAG

	qPCR	
M9F	Pma1-3 qPCR	GGGCATAGTTTTAGCTATAGGTTC
M9R	Pma1-3 qPCR	CACCAGCCAATTGCCAGGAT
Y4F	Pyk1-1 qPCR	GGTAAGCCAGTTATCTGTGCTACC
Y4R	Pyk1-1 qPCR	GCGGTTTCAGCCATAGTGGTAAC
Y5F	Pyk1-2 qPCR	AGATGCCCAAGAGCTGCTAGATT
Y5R	Pyk1-2 qPCR	CCTTGGATGGAAACGTAAGTGTC
Y6F	Pyk1-3 qPCR	CGATGAGGTGTTGCATTTTTGGAA
Y6R	Pyk1-3 qPCR	GTACCCATGTATAACCTTCCAAGT
U1P3	SNR19-1 qPCR	GGGGCCTTTCAAAAGAGAGCT
U1RP3	SNR19-1 qPCR	ATCTTCAAACTACAATCCCGACCA
U1FP4	SNR19-2 qPCR	AGAGAGCCGCAACAAGAAACGTAA
U1RP4	SNR19-2 qPCR	AGCTCACATTCTCAACTACCGT
U2FR3	SNR20-1 qPCR	CCCCCAAGTATCGGCCAAAG
U2RP3	SNR20-1 qPCR	AAGAGCTCCTTCTCCTCAATGAG
U2FP4	SNR20-2 qPCR	CACGTACTCACACATGGCCGA
U2RP4	SNR20-2 qPCR	GGAGAGAACGAGAAAGCGGAT

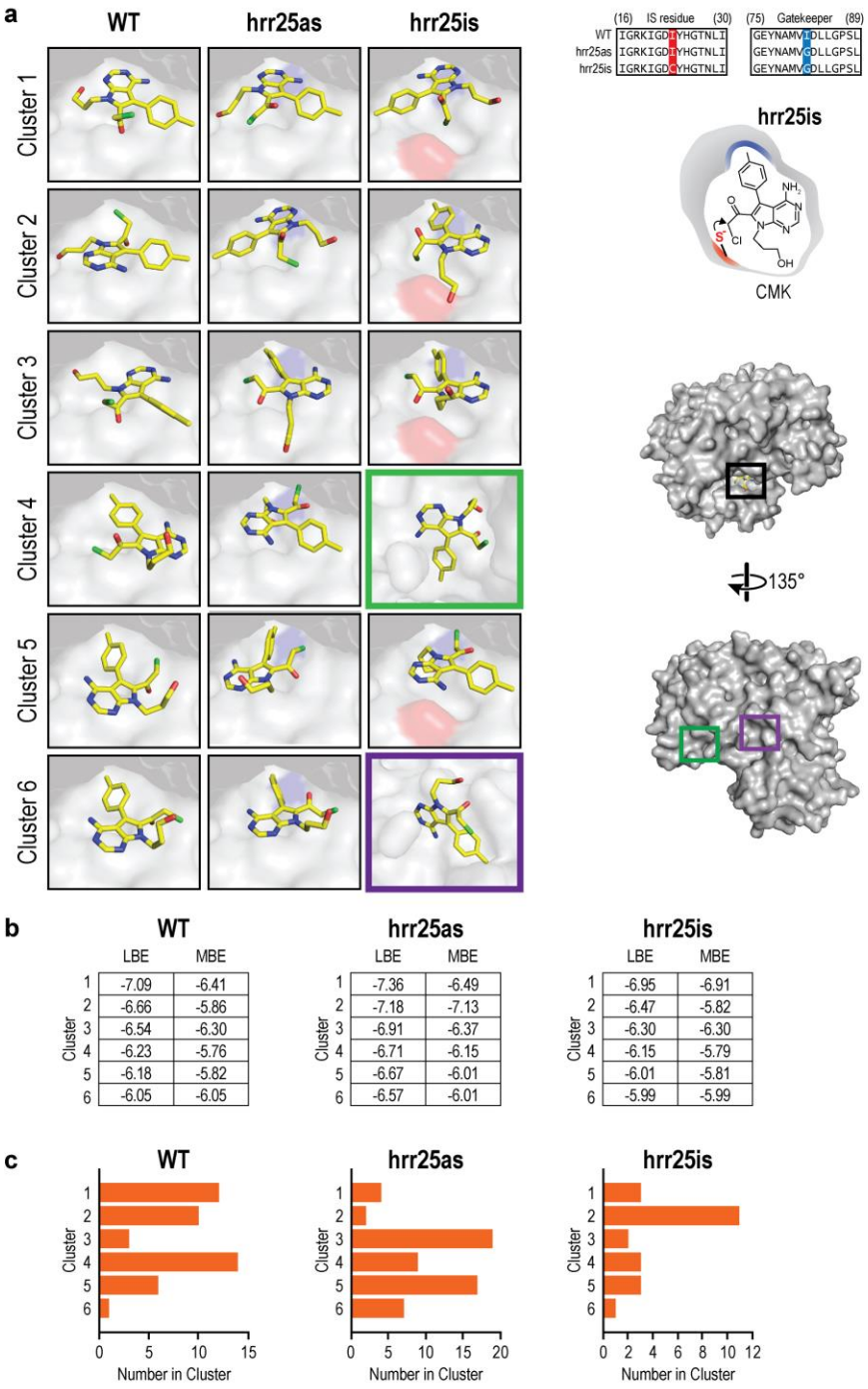
Supplementary Table 3. Strains used in this study.

Strain	Genotype	Plasmid	Reference
TAP library	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0		Open Biosystems, currently GE Healthcare
WT HRR25	his3Δ leu2Δ ura3Δ Hrr25Δ::kanMX4	pRS316-HRR25	E. Hurt Lab
hrr25as	his3Δ leu2Δ ura3Δ Hrr25Δ::kanMX4	pRS315-hrr25-I82G	E. Hurt Lab
hrr25is	his3Δ leu2Δ ura3Δ Hrr25Δ::kanMX4	pRS315-hrr25-I82G,I23C	This study
WT HRR25-HA	his3Δ leu2Δ ura3Δ Hrr25Δ::kanMX4	pRS315-hrr25-HA	This study
hrr25is-HA	his3Δ leu2Δ ura3Δ Hrr25Δ::kanMX4	pRS315-hrr25-HA (I82G, I23C)	This study
pho85as	MATa pho85as1::HPH his3Δ1 leu2Δ0 ura3Δ0 met15Δ0		B. Andrews Lab
cdc28as	MATa cdc28as1::NAT his3Δ1 leu2Δ0 ura3Δ0 met15Δ0		B. Andrews Lab
bur1as	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bur1 L149G		S. Hahn Lab
ctk1as	MATα his3Δ200 leu2-3,112 ura3-52 rpb1Δ187::HIS3 ctk1-F259G-HA3::kan	RPB1-XTPH LEU2	A. Greenleaf Lab
kin28as	MATα ade2Δ::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 kin28 L83G		S. Hahn Lab
LL20 (HRR25 WT)	MATα leu2-3,112 his3-11,15		M. Stark Lab
ARB97 (*hrr25-3*)	MATα leu2-3,112 his3-11,15 hrr25-3/kti4-1		M. Stark Lab
WT RTT103-TAP	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Rtt103-TAP::His3 Hrr25Δ::URA3	pRS315-hrr25-HA	This study
hrr25is RTT103-TAP	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Rtt103-TAP::His3 Hrr25Δ::URA3	pRS315-hrr25-HA (I82G, I23C)	This study

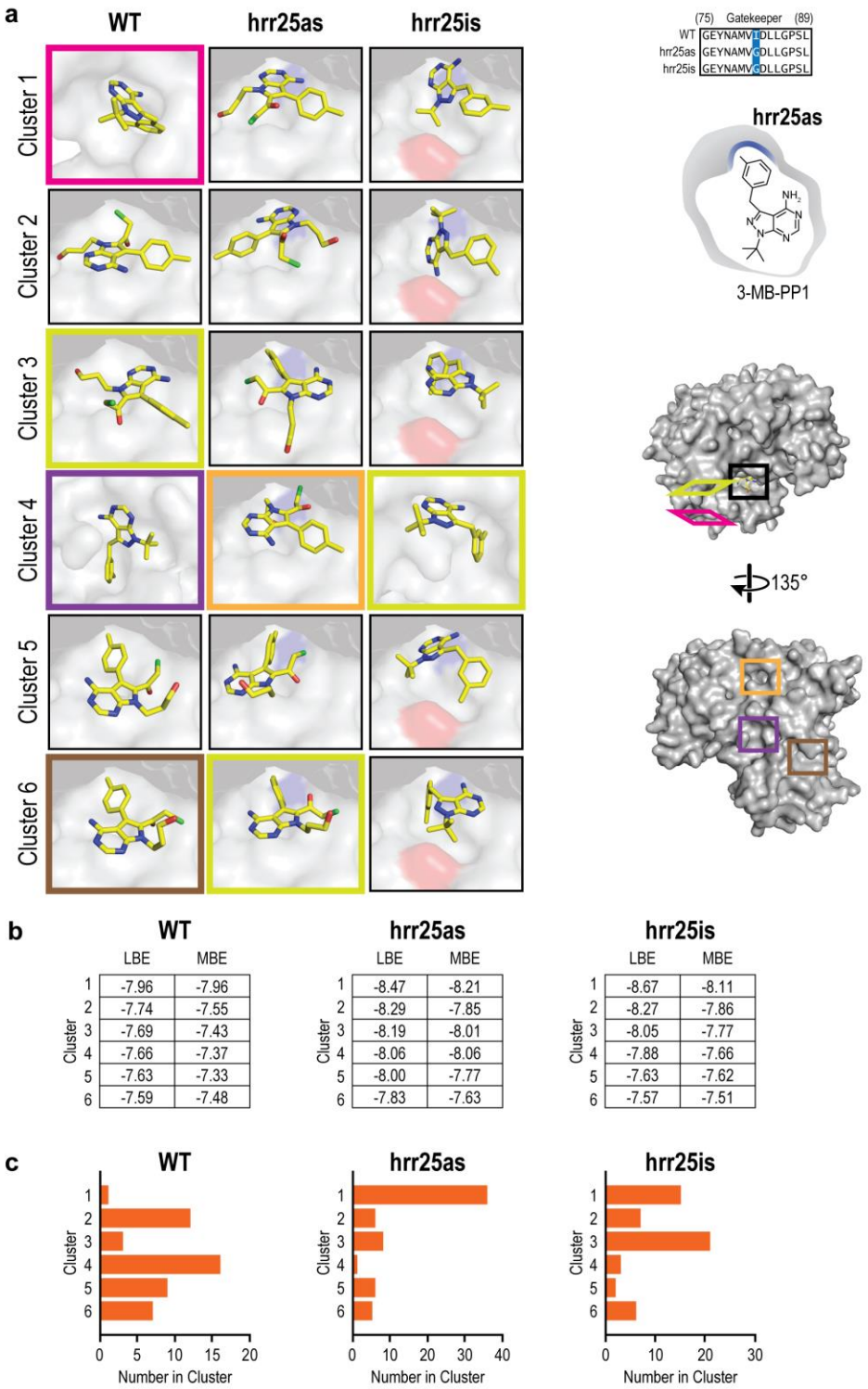
Supplementary Figures



Supplementary Figure 1 | Additional kinase data. (**a**) List of 70 yeast kinases tested. (**b**) Representative in vitro kinase assays (Western blots or dot blots) probing for pThr4 with the indicated antibodies. (each kinase was tested from at least two independent purifications). Full length blots are shown in Supplementary Fig. 12. (**c**) Representative in vitro kinase assays probing pSer2 or pSer5, and (**d**) evidence that kinases have been purified. (**e**) In vivo inhibition of WT or Cdc5as cells probed with antibodies targeting pThr4 or Rpb3. (n = 2 independent experiments). (**f**) WT Hrr25, Hrr25as, and Hrr25is were purified via HA tag. An in vitro kinase assay showing the activity of each kinase (α-pThr4) with respect to total amount of kinase (α-HA).



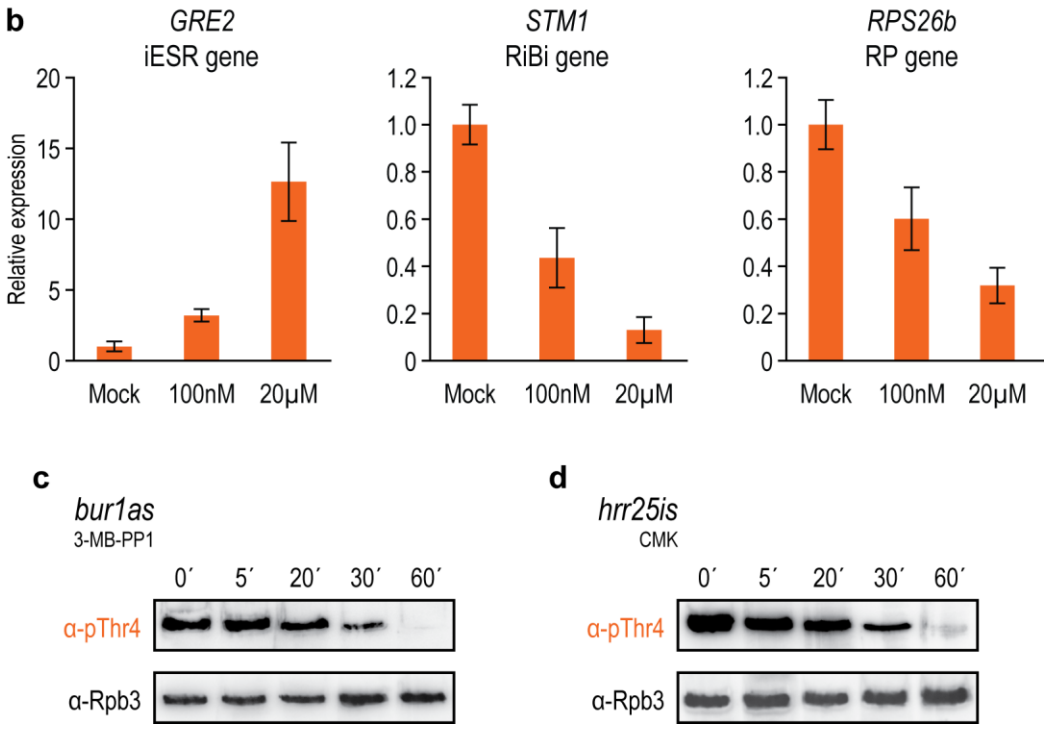
Supplementary Figure 2 | Docking of CMK into Hrr25. (**a**) Substitutions to create hrr25is and a schematic are shown (right). Six clusters of CMK docked into WT, hrr25as, and hrr25is are shown. Structures were clustered if RMSD < 2.0 Å. CMK docked into the active site unless surrounded by a colored box. The location of the docking is shown to the right. (**b**) Lowest binding energy (LBE) for each cluster and mean binding energy (MBE) for each cluster are shown. (**c**) The number of structures in each cluster are shown as a histogram. For each structure, 100 models of the inhibitor in the active site were created. Structures were clustered if the RMSD ≤ 2 Å.



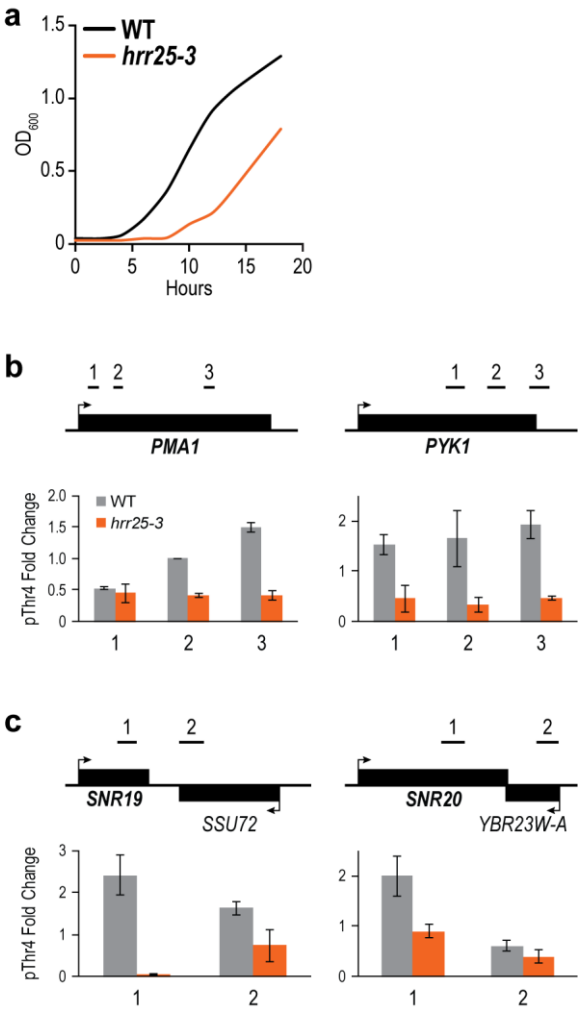
Supplementary Figure 3 | Docking of 3-MB-PP1 into Hrr25. (**a**) Substitutions to create hrr25as and a schematic are shown (right). Six clusters of 3-MB-PP1 docked into WT, hrr25as, and hrr25is are shown. Structures were clustered if RMSD < 2.0 Å. 3-MB-PP1 docked into the active site unless surrounded by a colored box. The location of the docking is shown to the right. (**b**) Lowest binding energy (LBE) for each cluster and mean binding energy (MBE) for each cluster are shown. (**c**) The number of structures in each cluster are shown as a histogram.

a

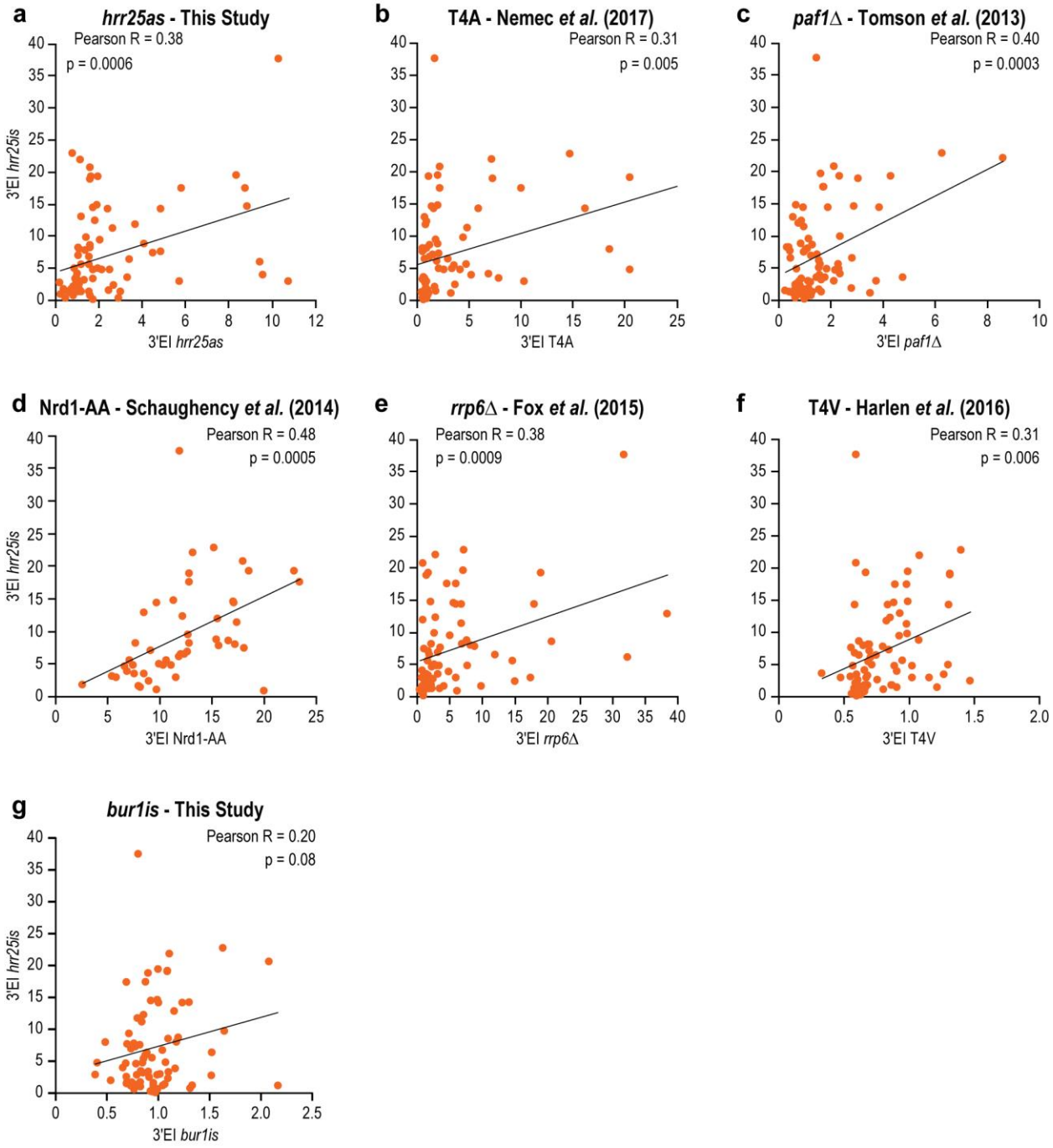
	WT	Hrr25as	Hrr25is
CMK	> 200 μM	> 200 μM	4.8 μM
3-MB-PP1	88 μM	94 nM	82 nM



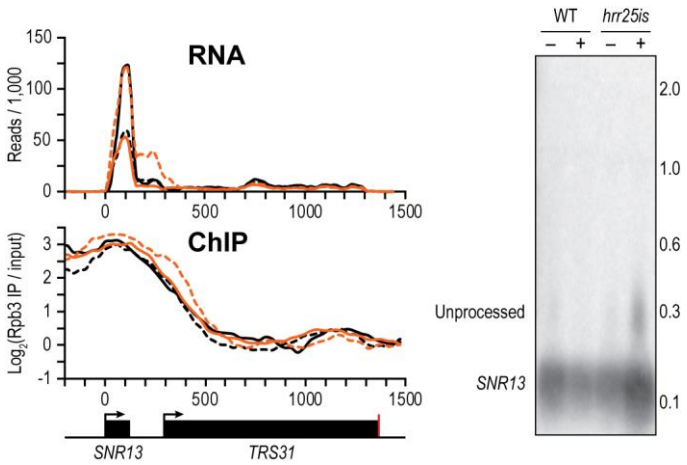
Supplementary Figure 4 | Additional inhibition data. (**a**) WT, Hrr2as, and Hrr25is cells were treated with either CMK or 3-MB-PP1 at concentrations ranging from 20 pM to 200 μM. For each concentration, the OD_{600} after 16 hours of inhibition is plotted in Fig 3d. IC50 values were calculated with GraphPad Prism. (**b**) *hrr25as* was treated with 3-MB-PP1 at the indicated concentrations. The relative expression via qPCR of an induced ESR gene (*GRE2*), ribosome biogenesis gene (*STM1*), and a ribosome protein gene (*RPS26b*) are shown. Error bars are standard error of the mean (n=3 independent experiments). Even when *hrr25as* was treated with 3-MB-PP1 at its IC50, signatures of the environmental stress response were evident. (**c**) *bur1as* was inhibited with 3-MB-PP1 and (**d**) *hrr25is* was inhibited with CMK for the indicated times, lysed, and analyzed via western blot probing pThr4 (1G7) or Rpb3 as a loading control. (n = 3 independent experiments). Full length blots are shown in Supplementary Fig. 12.



Supplementary Figure 5 | Defects in *hrr25-3* cells mirror Hrr25 inhibition. (**a**) Growth curves of WT (black) or *hrr25-3* (orange) cells (left). (**b,c**) ChIP qPCR of pThr4 marks at representative (**b**) protein coding genes or (**c**) noncoding genes. Locations of amplicons are shown and numbered. ChIP in WT (grey) or *hrr25-3* (orange) cells are shown. Error bars are standard deviation (n=3).

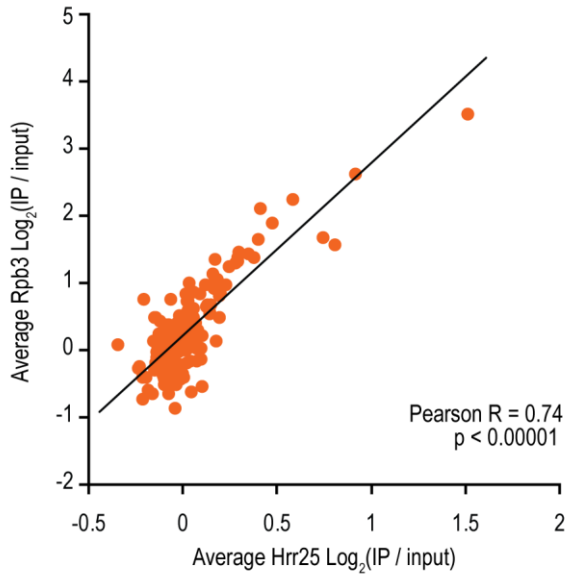


Supplementary Figure 6 | Comparison of 3′-extension indices to other datasets. Comparison of readthrough in *hrr25is* to (**a**) *hrr25as* (**b**) T4A[1], (**c**) *paf1Δ*[2], (**d**) Nrd1 anchor away[3], (**e**) *rrp6Δ*[4], (**f**) T4V[5], or (**g**) *bur1is.*



Supplementary Figure 7 | Additional evidence of readthrough in *hrr25is*. RNA-seq from WT (black) or *hrr25is* (orange) cells treated with either DMSO (solid) or CMK (dotted) are shown at *SNR13* (top). Rpb3 ChIP (bottom) in the same strains is shown at the same locus. A Northern blot probing for *SNR13* is shown (right). (n = 2 independent experiments). Full length blots are shown in Supplementary Fig. 12.

a

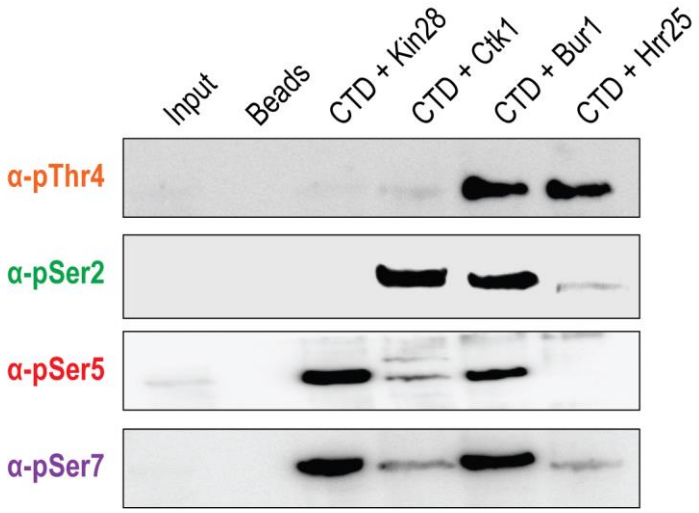


b

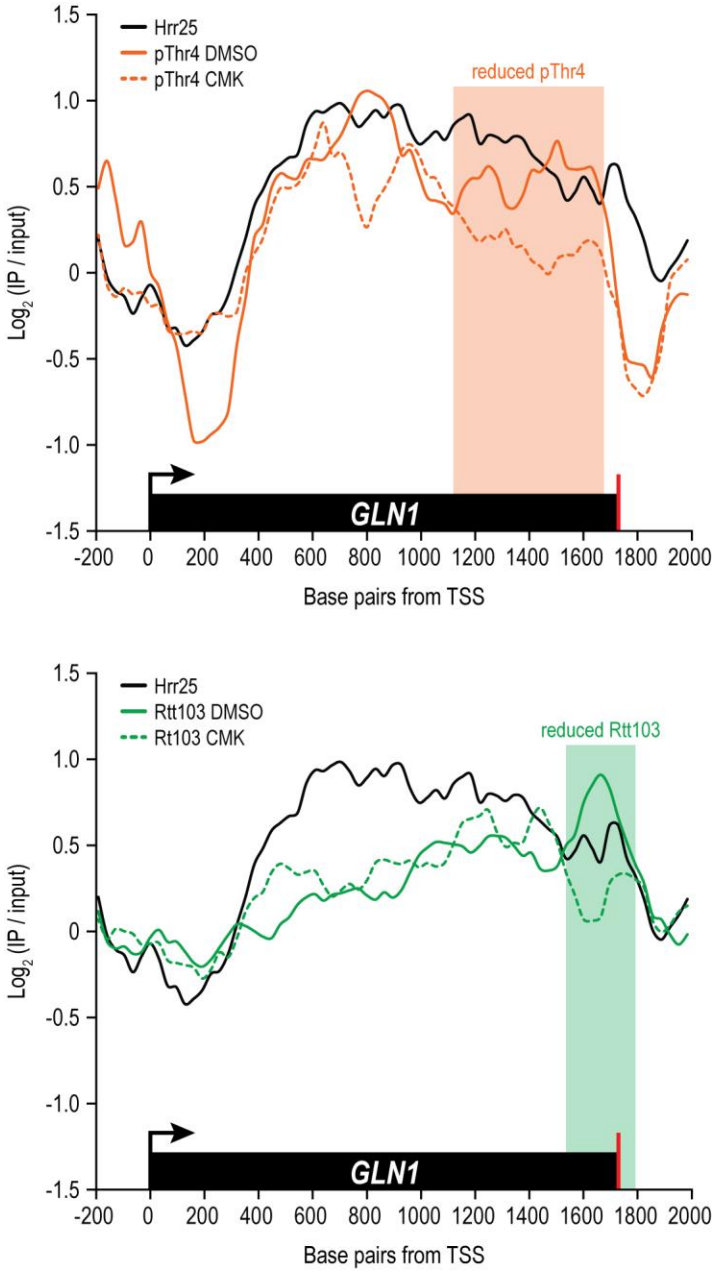
GO Biological Process

Cluster 1	Transcription, DNA-dependent	p=3.3e-5
Cluster 2	DNA repair Response to DNA damage stimulus	p=1.2e-5 p=5.8e-5
Cluster 3	Protein transport	p=4.2e-5
Cluster 4	Protein folding Ribosome biogenesis SRP-dependent cotranslational protein target to membrane	p=1.8e-5 p=6.7e-5 p=7.2e-5
Cluster 5	Translation Ribosome biogenesis Maturation of SSU-rRNA from tricistronic rRNA transcript rRNA procesing rRNA export from nucleus Translational elongation	p<1e-14 p=4.1e-12 p=2.6e-11 p=3.3e-9 p=4.7e-8 p=6.2e-8

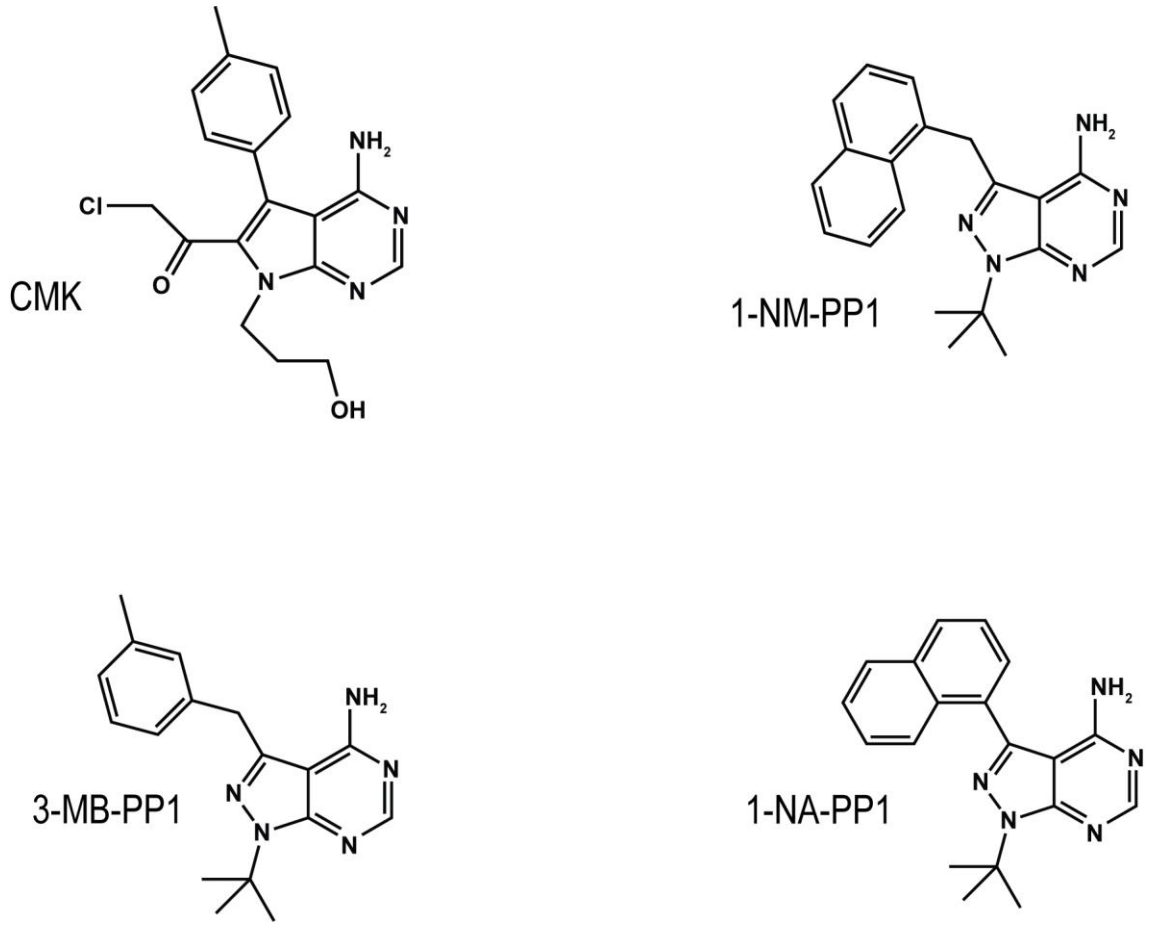
Supplementary Figure 8 | Hrr25 profiles correlate with Pol II at protein coding genes. (**a**) Scatterplot of average Rpb3 (y-axis) or Hrr25 (x-axis) ChIP signal across each protein coding gene. Pearson R and p-value are shown. (**b**) GO annotations ChIP clusters from Fig. 5a.



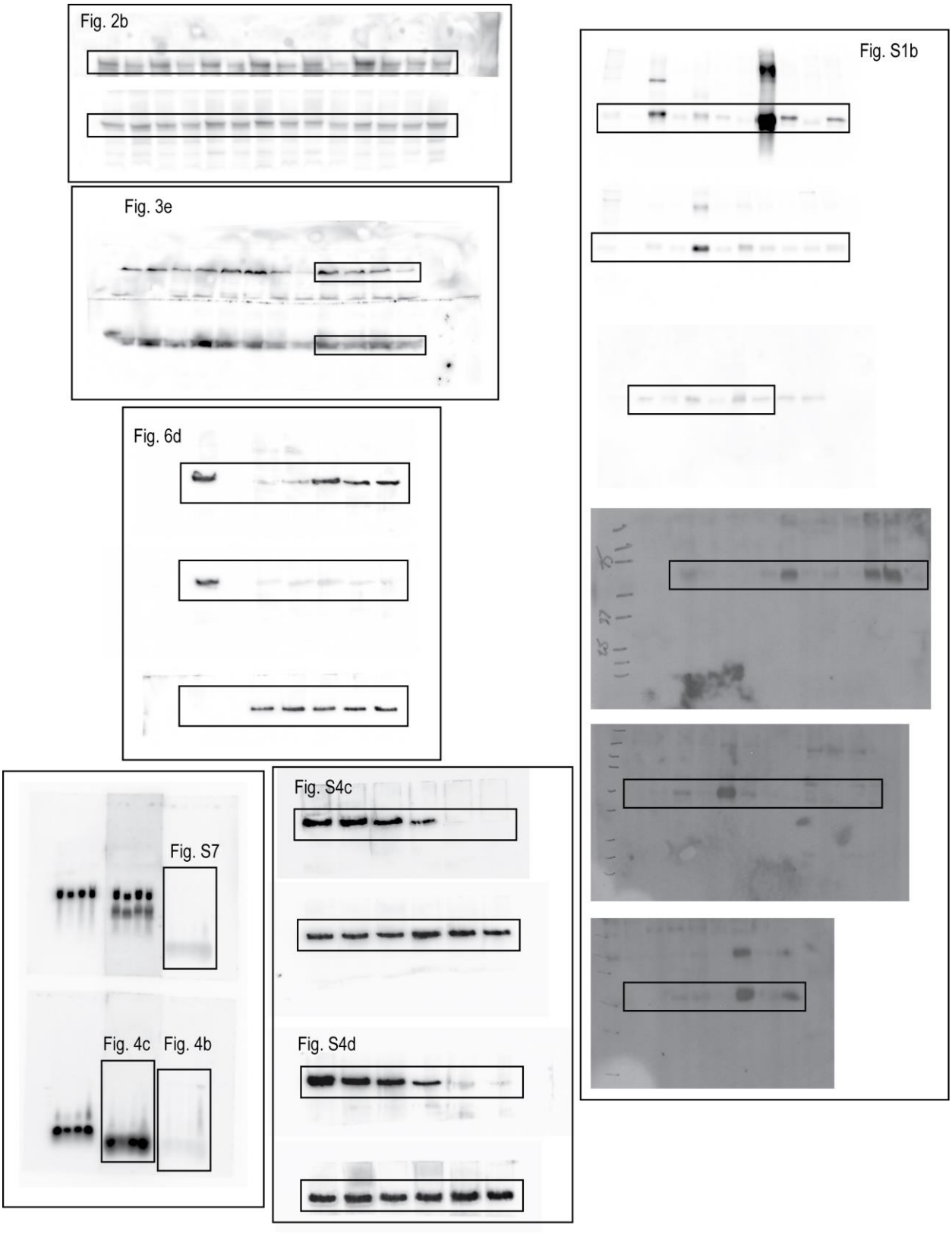
Supplementary Figure 9 | Confirmation of CTD phosphorylation. Bead-bound GST-CTD was incubated with Kin28, Ctk1, Bur1, or Hrr25 to phosphorylate the CTD at various positions. Blots were probed with antibodies targeting pThr4 (1G7), pSer2 (Bethyl), pSer5 (3E8), or pSer7 (4E12). "Beads" refers to sepharose beads lacking bound CTD (negative control). Input refers to Rtt103, loaded at 10% the abundance of Rtt103 incubated with CTD as in Fig. 6. (n = 2 independent experiments).



Supplementary Figure 10 | Dissection of pThr4 deposition and Rtt103 recruitment. Chromatin immunoprecipitation was performed with antibodies targeting Hrr25 (HA), pThr4 (1G7), or Rtt103 (TAP) in *hrr25is* strains treated with either DMSO (solid) or CMK (dotted). Shown are representative levels at the protein coding gene Gln1 (arrow is TSS and red bar is CPS). Hrr25 (black) occupancy is generally uniform across the gene. pThr4 (orange) levels begin to decrease ~600bp before the CPS upon hrr25is inhibition. Rtt103 (green) is subsequently depleted about 100bp from the CPS.



Supplementary Figure 11 | Chemical structures of compounds used.



Supplementary Figure 12 | Raw, uncropped blots.

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