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Chromatin remodelling by INO80 at promoter proximal pause sites promotes premature termination of mRNA synthesis

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29 **Abstract**

30 How co-transcriptional RNA quality control is regulated remains poorly understood. Here,
31 we report that in *S. cerevisiae* premature transcription termination of mRNAs is regulated by
32 the evolutionarily conserved ATP-dependent chromatin remodeling INO80 complex. Loss of
33 INO80 leads to an increase in promoter-proximally paused RNA Polymerase II and defective
34 progression into the gene body. We show that promoter-proximal transcriptional pausing
35 correlates with loading of RNA surveillance and transcription termination factors to mRNA
36 transcripts. Cells lacking INO80 are defective for the Nrd1-Nab3-Sen1 (NNS)-dependent
37 pathway for transcription termination at snRNA genes and promoter-proximally sites of
38 mRNA genes. We demonstrate that INO80 promotes the association of the RNA surveillance
39 and termination factor Nab2 with short promoter-proximal mRNA transcripts. We provide
40 evidence that co-transcriptional recruitment of Nab2 to chromatin is regulated by INO80,
41 which enables the interaction of Nab2 with the histone variant H2A.Z. Our work suggests a
42 chromatin mechanism for premature transcription termination at promoter-proximally
43 pausing sites, linking RNA quality control to the transcriptional process.

44 INTRODUCTION

45 Elimination of aberrant mRNAs is essential for proper gene expression. In eukaryotic cells
46 nuclear RNA surveillance and quality control mechanisms monitor mRNA biogenesis co-
47 transcriptionally and terminate transcription prematurely for degradation of the nascent RNA
48 transcript¹. Elegant genome-wide studies in the budding yeast *Saccharomyces cerevisiae*
49 have suggested that unproductive transcriptional elongation of protein coding genes is wide-
50 spread and common^{2,3}. Furthermore, promoter-proximal short mRNAs enriched in 3'-
51 oligo(A) tails have been identified⁴, indicating extensive premature termination across the
52 yeast protein-coding genome⁵. Likewise, only 10% of promoter-proximally paused
53 polymerases was shown to enter productive elongation in human cells, with the remaining
54 being prematurely terminated during abortive elongation^{6,7}.

55 The major nuclear RNA quality control pathway in yeast is mediated by the RNA
56 surveillance complex Nrd1-Nab3-Sen1 (NNS)⁸ and the Rrp6-dependent nuclear exosome
57 complex with 3' exonuclease activity⁹. The NNS-dependent nuclear RNA quality control
58 pathway acts prominently to restrict the extensive and pervasive transcription of non-coding
59 RNAs (ncRNAs)¹⁰. Nrd1 and Nab3 have also been found to be recruited at active protein-
60 coding genes with a preference near their 5' end and bind most mRNA transcripts¹¹⁻¹⁵,
61 suggesting a genome-wide role for NNS in mRNA transcription termination¹⁵ and co-
62 transcriptional control of mRNA metabolism. However, this model has been challenged by a
63 separate study, which proposed that only a few mRNA genes are subject to transcriptional
64 attenuation by the NNS complex¹⁶. Thus, although the RNA surveillance and termination
65 pathways have been characterized in molecular detail, the mechanisms regulating premature
66 transcription termination during mRNA synthesis by Pol II remain unclear.

67 In metazoans, short, promoter-associated polyadenylated mRNA transcripts¹⁷ have been
68 found to associate with Pol II pausing¹⁸, with premature termination and exosome-dependent

69 degradation coinciding with promoter-proximal pausing sites¹⁹. Experiments with chemical
70 transcriptional inhibitors indicated high rates of turnover and premature termination of
71 promoter-proximal paused Pol II^{6,20,21}, suggesting regulation of gene expression by
72 transcriptional attenuation. Even though promoter-proximal pausing plays an important
73 regulatory role in eukaryotic transcription²²⁻²⁵, how pausing is linked to premature
74 termination remains poorly understood.

75 Eukaryotic transcription is controlled by the underlying chromatin structure. ATP-dependent
76 chromatin remodelling enzymes shape the chromatin landscape and act at almost all stages of
77 RNA production controlling transcription activation and silencing, modulation of
78 transcriptional rates, transcription termination and RNA export from the nucleus²⁶⁻³⁰. In
79 mammals, premature termination of mRNA genes and promoter-proximal transcriptional
80 pausing have been associated with nucleosome organization¹⁹. However, the role of
81 chromatin remodelers in co-transcriptional RNA quality control remains elusive.

82 The multisubunit ATP-dependent chromatin remodelling complex INO80 has a well-
83 established and important role in transcription across eukaryotes³¹. INO80 is enriched at the
84 transcription start sites (TSS) of most active and poised mRNA genes in yeast and
85 mammals^{32,33}. The nucleosome remodelling activity of INO80 is important for the exchange
86 of the histone variant H2A.Z for H2A in nucleosomes and for controlling nucleosome
87 positions³¹. While it is generally proposed that the function of INO80 during transcription is
88 to control transcription initiation, INO80 physically interacts with the elongating RNA
89 Polymerase II machinery^{34,35} and binds to mRNAs^{36,37}. This indicates a -yet- undefined role
90 for INO80 in the transcriptional process.

91 Here we investigate the role of INO80 in transcription regulation in *S. cerevisiae*. Using
92 Native Elongating Transcript sequencing (NET-seq) we analyse how the process of
93 transcription is regulated by INO80 at nucleotide resolution. We show that INO80 enables

94 the progression of Pol II from the promoter-proximal region into the gene body. Our analysis
95 indicates that promoter-proximal pausing of Pol II is linked to loading of transcription
96 termination and RNA processing factors to mRNA transcripts. Mechanistically our study
97 reveals that INO80 modulates the interaction between the RNA surveillance and termination
98 factor Nab2 and H2A.Z, enabling recruitment of Nab2 to chromatin. Overall, our results
99 suggest that the chromatin remodelling function of INO80 promotes premature transcription
100 termination.

101 **RESULTS**

102 **Defective progression of Pol II from the promoter proximal region into the gene body in**
103 **the absence of INO80**

104 To illuminate the role of INO80 in transcription, we performed Native Elongating Transcript
105 NET-seq analysis in Wild-Type (WT) and *ino80Δ* yeast cells expressing a FLAG-tag version
106 of the largest subunit of the RNA Polymerase II complex, Rpb1 (Extended Data Fig. 1a, b).
107 NET-seq captures nascent RNAs bound to transcriptionally engaged Pol II and sequences the
108 3'-end of the purified RNAs, thus providing a detailed map of elongating Pol II across the
109 genome at nucleotide resolution³⁸. Therefore, and to avoid confusion with the term “nascent
110 RNAs” that is commonly used to describe newly synthesized RNAs that are not necessarily
111 associated with Pol II³⁹, we will henceforth refer to the Pol II-associated transcripts captured
112 by NET-seq as Pol II density.

113 Following removal of duplicated reads and DEseq normalization, NET-seq densities were
114 computed as normalized counts over gene length and their distribution across the gene was
115 compared between the two strains. Consistent with previous reports^{38,40}, promoter-proximal
116 region showed sense Pol II density, with a subsequent reduction further into the gene body in
117 WT cells (Fig. 1a). Interestingly, in *ino80Δ* cells, while sense Pol II density was reduced at
118 the promoter-proximal peak, it decreased further downstream the promoter-proximal region,
119 compared to WT (Fig 1a). Changes in the abundance of sense and antisense NET-seq reads at
120 mRNA genes in *ino80Δ* did not correlate (Extended Data Fig. 1c, $r=0.04$), indicating that
121 altered sense Pol II density in the absence of INO80 is independent of antisense transcription.

122 We explored whether the decrease in Pol II density downstream the promoter proximal
123 region in *ino80Δ* indicates defective progression of RNA Polymerase II into the gene body.
124 To test this possibility, we calculated Traveling Ratio (TR) values for all mRNA genes by

125 comparing the Pol II density in the gene body relative to the promoter-proximal region of the
126 first 200bp after the TSS (Fig. 1b). In WT cells, 68% of protein-coding genes exhibited
127 higher Pol II density at the promoter-proximal region compared to Pol II density in the gene
128 body for (TR<1), indicating accumulation of Pol II proximally to promoter for most of the
129 genes. Loss of INO80 led to an overall significant reduction in TR, with 809 genes exhibiting
130 a decrease in TR of at least 1.5 fold-change compared to WT (Fig. 1b). Overall, 73% of genes
131 demonstrated a TR<1 in *ino80Δ*. This indicates a genome-wide defect in progression of Pol II
132 from the promoter-proximal region into the gene body in the absence of INO80.

133 INO80 physically interacts with the elongating RNA Polymerase II complex^{34,35} and is
134 enriched within gene bodies^{33,41}. To evaluate its role during transcription elongation, we
135 asked whether the enrichment of INO80 inside gene bodies correlates with the intragenic
136 levels of Pol II. ChIP-exo analysis provides near single-nucleotide resolution of factor
137 binding to DNA⁴² and can distinguish between recruitment inside and outside the gene body
138 with great accuracy. When the Ino80⁴¹ and Rpb1 ChIP-exo densities within the gene bodies
139 were compared, a strong, positive correlation between their enrichment was observed (Fig.
140 1c, r=0.68). This indicates that the presence of INO80 inside the gene is associated with
141 transcription elongation activity.

142 Chromatin remodelling activity and functionality of the INO80 complex relies on the INO80-
143 specific subunits Arp5 and Arp8³¹. Cells lacking either *INO80* or the INO80-specific subunits
144 *ARP5* or *ARP8* grew poorly in the presence of 6-azauracil (6-AU) (Fig. 1d and Extended
145 Data Fig. 1d, e), an inhibitor of GTP biosynthesis which sensitizes transcriptional elongation
146 mutants⁴³. Therefore, these results suggest that INO80 promotes transcription elongation,
147 supporting a genome-wide, post-initiation role for INO80 in gene expression.

148 **Global downregulation of Pol II transcription in the absence of INO80 is associated**
149 **with defective mRNA degradation**

150 Increase in transcription initiation can lead to enhanced pausing of Pol II at predetermined
151 sites⁴⁴. We therefore asked whether transcription rates are increased in the absence of INO80,
152 by comparing the levels of newly synthesized mRNAs in WT and *ino80Δ* cells. Cells grown
153 in rich medium were pulse-labelled with 4-thiouracil (4tU) and mixed in a fixed ratio with
154 labelled *S.pombe* cells (spike-in) for normalization⁴⁵. In parallel, 4tU labelling was also
155 conducted in *ino80Δ* cells expressing wild-type *INO80* from a plasmid, serving as a control
156 for *ino80Δ*-mediated changes in RNA expression. Purified total (steady state) and labelled
157 (newly synthesized) RNA from all samples were subjected to sequencing.

158 After normalization to the *S. pombe* spiked-in signal and applying a cutoff of 1.5-fold change
159 and p-value < 0.05, only 3.6% of all protein coding genes showed significant increase in
160 newly synthesized mRNA levels in *ino80Δ* (n=178, Fig. 3a). Contrary, 34% of the protein-
161 coding genome demonstrated a significant reduction in mRNA synthesis in *ino80Δ* compared
162 to WT (n=1706, Fig. 3a). The downregulation of mRNA synthesis rates was INO80-
163 dependent, since in *ino80Δ* cells expressing *INO80* from an episomal plasmid, less than 4%
164 of genes had markedly decreased newly synthesized mRNA levels (n=193, Extended Data
165 Fig. 3a). Therefore, deletion of INO80 induces genome-wide downregulation of mRNA
166 synthesis rates, arguing against increased transcription initiation as the underlying cause of
167 enhanced Pol II pausing in *ino80Δ*.

168 Interestingly, despite the global decrease in newly synthesized mRNA levels in *ino80Δ*, the
169 cellular, total mRNA levels for 81% of the protein-coding genes did not significantly change,
170 while 13% were increased in the absence of INO80 (Fig. 3b). This disentanglement between
171 newly synthesized and total RNAs suggests buffering of mRNA transcript levels⁴⁶ in *ino80Δ*
172 by a compensatory mechanism. Cellular mRNA levels are determined by the rates of mRNA
173 synthesis and degradation. To understand why cellular mRNA levels remain high in *ino80Δ*,
174 we conducted comparative dynamic transcriptome analysis (cDTA)⁴⁷, which measures

175 mRNA synthesis and decay rates (SR and DR respectively) for all protein-coding genes.
176 Comparison of the SR and DR changes in *ino80Δ* relative to WT revealed that the decrease in
177 SR observed in *ino80Δ* positively correlated with a concomitant decrease in DR ($r=0.47$, p-
178 value= $2.9e^{-233}$, Fig 3c). This indicates that the defect in Pol II transcription in *ino80Δ* is
179 associated with compromised mRNA degradation, in a similar fashion to what has been
180 observed for the *rpb1-N488D* mutant⁴⁷, which reduces the processivity of Pol II⁴⁸.

181 To verify that mRNAs are stabilized in the absence of INO80, we analysed mRNA decay
182 rates following transcription inhibition with thiolutin in WT and *ino80Δ* cells. Thiolutin was
183 used at concentration below the threshold reported to affect mRNA half-life⁴⁹ and mRNA
184 values were normalized relative to the stable *scR1* RNA, which is transcribed by Pol III. The
185 relative abundance of *NRD1*, *RNAI* and *HPT1* transcripts, which showed decreased DR in the
186 cDTA analysis (Extended data Fig. 3b), was markedly higher in *ino80Δ* compared to WT
187 after transcription inhibition (Fig. 3d and Extended data Fig. 3c). Therefore, the concomitant
188 change in mRNA synthesis and degradation in *ino80Δ* suggest that the transcriptional role of
189 INO80 is linked to RNA decay by buffering steady state mRNA levels.

190 **Promoter-proximal Pol II pausing is linked to increased loading of RNA surveillance**
191 **and termination factors to mRNA transcripts**

192 The results from our NET-seq and 4tU-seq analysis prompted us to evaluate whether
193 promoter-proximal pausing is linked to nuclear RNA quality control and premature
194 transcription termination.

195 The nuclear RNA cleavage and termination factor Nab2⁵⁰ and the Trf4/Trf5-Air1/Air2-Mtr4
196 polyadenylation (TRAMP) complex are required for processing and delivering nascent
197 transcripts to the nuclear exosome for degradation⁵¹⁻⁵⁵. Nab2, Mtr4 and the Nab3 subunit of
198 the NNS complex bind most of mRNAs^{4,56}. We retrieved published genomic data for RNA

199 binding (CRAC) for Nab3, Nab2 and Mtr4^{4,56}. and corrected their binding to mRNA
200 transcripts for their respective gene transcription levels as measured by CRAC-Rpb1⁵⁶ (Fig.
201 4a). This normalisation generated an index of Transcript Instability (TI) values for all
202 protein-coding genes, allowing us to quantitatively assess the level of premature transcription
203 termination taking place within each mRNA gene.

204 We evaluated whether accumulation of Pol II at the promoter-proximal region is associated
205 with premature termination. TI values were calculated for the promoter-proximal region (TSS
206 to TSS+200) and the downstream gene body (TSS+200 to PAS). Genes were clustered in
207 four groups based on their TR values in WT conditions (Fig. 4b) and $TIs^{Mtr4/Nab3/Nab2}$ were
208 analysed for the genes of each group. Intriguingly, a reciprocal relationship between TR and
209 promoter-proximal TI was observed: genes with increased accumulation of Pol II at the
210 promoter-proximal region relative to the gene body (low TR) had high $TIs^{Mtr4/Nab3/Nab2}$, while
211 genes with little or no promoter-proximal accumulation of Pol II (high TR) had low TI (Fig.
212 4c). Contrary, no decrease in the $TIs^{Mtr4/Nab3/Nab2}$ values was observed within the gene body in
213 the four TR groups (Fig. 4c). Therefore, defective progression of Pol II from the promoter-
214 proximal region correlates with early transcription termination at the same region.

215 **Functional and physical interactions link INO80 to RNA quality control and NNS-** 216 **dependent transcription termination pathway**

217 Since in *ino80Δ* cells Pol II is stalled at genomic sites associated with increased binding of
218 RNA surveillance factors to RNA, we interrogated whether the role of INO80 in gene
219 expression is functionally linked to the RNA quality control process. Abd1 is an essential
220 m7G cap methyltransferase that prevents mRNA destabilization and degradation⁵⁷. Deletion
221 of *ARP8* in the *abd1-DAmP* strain, which expresses a hypomorphic allele of *ABD1*⁵⁸, led to
222 synthetic lethality in non-permissive conditions (Fig. 5a), suggesting that INO80 is essential
223 for cellular viability when mRNAs are destabilized. Deletion of *ARP8* in the *nrđ1-V368G*

224 (designated as *nrd1-5*) mutant strain, which is deficient in transcription termination⁸ led to
225 synthetic lethality in 6-AU (Fig. 5b). In addition, the *arp8Δ* and *nrd1-5* mutants were
226 epistatic for *INO1* expression (Fig. 5c) suggesting that the INO80 and NNS complexes work
227 in the same pathway in gene regulation. These genetic interactions link INO80 to co-
228 transcriptional RNA quality control, prompting us to test whether the INO80 complex
229 physically associates with the transcription termination machinery.

230 Protein co-immunoprecipitation analysis after nucleic acid removal demonstrated that the
231 INO80 complex physically interacts with both Nrd1 and Nab2 *in vivo* (Fig. 5d, e). Contrary,
232 we could not detect a physical interaction between the INO80 complex and the component of
233 cleavage and polyadenylation factor IA (CF IA) complex Pcf11 (Extended Data Fig. 4a),
234 which promotes transcription termination at the PAS.

235 **INO80 promotes NNS-dependent transcription termination**

236 To explore the possibility that INO80 is involved in Nrd1-dependent termination, we
237 employed the well-characterized *ACT-SNR^{TTS}-CUP* reporter system^{8,59} (Extended Data Fig.
238 5a). Briefly, cells were transformed with ACT-CUP plasmids without or with the
239 Transcription Termination Site (TTS) of the snoRNA gene *SNR13* (*SNR^{TTS}*) inserted prior to
240 the CUP1 gene, which confers resistance to copper. Termination of snoRNAs, including
241 *SNR13*, is regulated by the NNS complex^{8,60}. INO80 is co-enriched with Nrd1 at the
242 endogenous *SNR13^{TTS}* region (Extended Data Fig. 4b), making the *ACT-SNR^{TTS}-CUP*
243 reporter a suitable system to test the role of INO80 in NNS-dependent transcription
244 termination. Contrary to WT, *ino80Δ* cells transformed with the *ACT-SNR^{TTS}-CUP* plasmid
245 were able to grow in the presence of copper, suggesting read-through transcription of *CUP1*
246 (Fig. 6a). RT-qPCR analysis using primer pairs that target the regions before and after the
247 *SNR13^{TTS}* insertion site in the reporter plasmids showed a significant increase in the relative
248 transcription of *CUP1* in the *ACT-SNR^{TTS}-CUP* plasmid in *ino80Δ* and *arp8Δ* mutants by 2.8-

249 fold and 3.5-fold respectively (Fig. 6b and Extended Data Fig. 5c). Similarly, a 3-fold
250 increase was observed in *nrd1-5* cells, in accordance to previous studies⁸ (Fig. 6b and
251 Extended Data Fig. 5c). Inducible degradation of Ino80 also resulted in an acute increase in
252 relative *CUP1* levels from the *ACT-SNR^{TTS}-CUP* but not from the control *ACT-CUP* plasmid
253 lacking the transcription termination site (Extended Data Fig. 5d). Deletion of either *INO80*
254 or *ARP8* did not significantly alter gene expression of any of the known transcription
255 termination and RNA surveillance factors (Extended Data Table 2). These results support a
256 direct role for INO80 in transcription termination at the *SNR13^{TTS}* termination site.

257 To further substantiate a role for INO80 in NNS-dependent termination, we evaluated read-
258 through transcription at the termination sites of endogenous snoRNA genes. Using paired-
259 end, spiked-in RNA sequencing we mapped the sense RNA-seq reads encompassing the TTS
260 of the snoRNA genes in WT and *ino80Δ*. In the absence of INO80 read-through transcripts
261 are significantly increased for the majority of the snoRNA genes, but not for the ribosomal
262 protein coding gene *YNL096C* (Fig. 6c, d, and Extended Data Fig. 5e and Extended Data
263 Table 3). The increase in readthrough transcripts in *ino80Δ* is not due to defective
264 degradation, as termination of snoRNAs in cells deleted for the 5'-3' exoribonuclease *XRN1*
265 was normal, while deletion of the nuclear exosome subunit *RRP6* compromised termination
266 only at a small number of specific snoRNA genes, in accordance with previous studies^{61,62}
267 (Fig. 6d and Extended Data Table 2). These results suggest that INO80 promotes NNS-
268 dependent transcription termination.

269 To investigate the effect of INO80 loss on premature termination of mRNA synthesis, we
270 focused our analysis on the *NRD1* gene, which is a known target for promoter-proximal
271 transcription termination by the NNS/Rrp6 pathway^{8,61}. Ino80 is enriched at the promoter-
272 proximal region of *NRD1* (Extended Data Fig. 5f) and loss of INO80 resulted in increased
273 promoter-proximal Pol II density at *NRD1* compared to WT (Extended Data Fig. 5g). To

274 assess termination proximally to promoters and at the end of the gene we conducted
275 quantitative 3' rapid amplification of cDNA ends (3' RACE) using gene-specific primers for
276 either the promoter-proximal or the PAS region of the *NRDI* gene respectively. Deletion of
277 *RRP6* increased the relative abundance of promoter-proximally terminated *NRDI* mRNAs
278 compared to WT, while also upregulated the expression of *NRDI* transcripts terminated at the
279 end of the gene, in line with RNA-seq data (Fig. 6e and Extended Data Fig. 5h). Notably,
280 deletion of either *INO80* or *APR8* attenuated the expression of early terminated transcripts
281 compared to the PAS-terminated *NRDI* transcripts (Fig. 6e). This suggests that the INO80
282 complex promotes premature transcription termination at mRNA genes.

283 **INO80 promotes chromatin recruitment and association of Nab2 with H2A.Z**

284 To understand the mechanism underlying the function of INO80 in premature termination,
285 we hypothesized that INO80 regulates the recruitment of the RNA quality control machinery
286 to chromatin. The RNA surveillance factor Nab2 promotes transcription termination⁵⁰. Nab2
287 binds promoter-proximal mRNAs that are prematurely terminated⁴, including *NRDI*
288 (Extended Data Fig. 6a), while it is co-transcriptionally recruited to the gene body of actively
289 transcribed mRNA genes¹². We therefore tested by ChIP-qPCR whether association of Nab2
290 with chromatin depends on INO80. Co-transcriptional recruitment of Nab2 to the promoter-
291 proximal region of *NRDI* was reduced in the absence of INO80 (Fig. 7a and Extended Data
292 Fig. 6b). Likewise, Nab2 co-transcriptional recruitment at the *SNR13* gene was also
293 compromised in *ino80Δ* (Extended Data Fig. 6c). Co-immunoprecipitation for Nab2
294 confirmed that the physical interaction between Nab2 and Rpb1 was independent of INO80
295 (Extended Data Fig. 6d), excluding the possibility that the decreased recruitment of Nab2 to
296 chromatin is caused by compromised binding to the Pol II machinery. To evaluate whether
297 loss of INO80 disrupts the association of Nab2 with the promoter-proximal *NRDI* transcript,
298 we conducted quantitative RNA-IP for Nab2. In *ino80Δ* cells the binding of Nab2 to

299 promoter-proximal *NRDI* RNA was significantly reduced compared to its binding to the
300 PAS-proximal *NRDI* region (3'-*NRDI*) (Fig. 7b). These results suggest that INO80 regulates
301 recruitment of Nab2 to chromatin to promote its binding to nascent RNAs.

302 We sought to gain insight into how INO80 promotes recruitment of Nab2 to chromatin.
303 Promoter-proximal nucleosomes are enriched in the histone variant H2A.Z⁶³. H2A.Z has
304 been suggested to have a role in RNA quality control and non-PAS transcription termination
305 in *D.melanogaster* and *S.pombe*⁶⁴⁻⁶⁶. INO80 preferentially remodels nucleosomes containing
306 H2A.Z and controls the dynamic association of H2A.Z with chromatin^{33,67-69}, raising the
307 possibility that H2A.Z might be involved in NNS-dependent termination. Cells lacking
308 *HTZI*, which encodes for H2A.Z in budding yeast, grew in the presence of copper when
309 transformed with the *ACT-SNR^{TTS}-CUP* plasmid (Fig.7c and Extended Data Fig. 6f),
310 suggesting that H2A.Z suppresses read-through transcription from NNS-dependent
311 termination sites. Promoter-proximally terminated *NRDI* transcripts were decreased in *htz1Δ*
312 compared to WT (Fig. 7d), indicating that loss of H2A.Z compromises premature
313 transcription termination of mRNA genes. In line with defective early termination, co-
314 transcriptional recruitment of Nab2 at the promoter-proximal region of *NRDI* was reduced in
315 *htz1Δ* cells (Fig. 7e and Extended Data Fig. 6g). Co-immunoprecipitation assays after nucleic
316 acid removal demonstrated that H2A.Z interacts with Nab2, even after non-physiological
317 high-salt wash conditions, but not with Pcf11 (Fig. 7f and Extended Data Fig. 7h, i). Since
318 our evidence indicate that H2A.Z is involved in premature transcription termination and RNA
319 quality control at mRNA genes, we asked whether INO80 controls the interaction between
320 H2A.Z and Nab2. Remarkably, binding of Nab2 to H2A.Z was decreased in *ino80Δ* (Fig. 7f).
321 This suggests that INO80 enables the recruitment of the Nab2 RNA surveillance machinery
322 to chromatin by promoting its interaction with H2A.Z.

323 **DISCUSSION**

324 Here, we elucidate a role for INO80 in transcription. We report that INO80 enables
325 recruitment of the RNA quality control machinery to transcribed mRNA genes for early
326 transcription termination, promoting transcription elongation. Our data suggest that by
327 removal of Pol II and termination of unproductive transcription, INO80 defines a chromatin-
328 based pathway for co-transcriptional mRNA quality control. Based on our results we propose
329 that control of premature transcriptional termination by INO80-dependent chromatin
330 remodelling is a genome-wide regulatory mechanism of eukaryotic gene expression.

331 **Linking mRNA quality control to chromatin regulation**

332 What is the role of Pol II accumulation at the promoter-proximal region? Our analysis links
333 promoter-proximal accumulation of Pol II with RNA surveillance and transcription
334 termination. Binding of the RNA quality control factors Nab3, Mtr4 and Nab2 to mRNA
335 transcripts is enhanced at promoter-proximal regions. Therefore, a model emerges that
336 increased dwelling of Pol II at specific promoter-proximal sites provides the time for RNA
337 surveillance and termination factors to be loaded to nascent transcripts efficiently. Our
338 cumulative findings that in the absence of INO80 (i) Pol II accumulates at promoter proximal
339 regions, (ii) Nab2 recruitment to chromatin and binding to RNA are defective and (iii) early
340 termination is compromised, suggest that removal of Pol II molecules that are not engaged in
341 productive transcription is mediated by INO80. Given that loss of INO80 results in reduced
342 transcription rates, our results substantiate the hypothesis that chromatin promotes
343 transcription by orchestrating the co-transcriptional RNA quality control pathway. It is
344 therefore tempting to speculate that chromatin acts as a molecular switch that dictates
345 whether Pol II will either progress into productive elongation or be removed to allow for the
346 next round of transcription to take place.

347 **Chromatin regulation of premature transcription termination by INO80**

348 How does INO80 promote premature termination? The physical interactions of the INO80
349 complex with the transcription termination and RNA surveillance factors Nrd1 and Nab2, and
350 the enrichment of INO80 at NNS-dependent termination regions suggest a direct involvement
351 for INO80 in the co-transcriptional RNA quality control pathway. Nab2 associates with the
352 RNA cleavage factor Hrp1⁷⁰ and both factors are involved in mRNA quality control and
353 NNS-dependent termination^{4,71,72}. The INO80-dependent recruitment of Nab2 to chromatin
354 could therefore promote co-transcriptional cleavage of the nascent RNA, leading to
355 destabilization of the transcription elongation complex and subsequent release of Pol II from
356 chromatin. As INO80 interacts with and facilitates release of poly-ubiquitinated Rpb1 from
357 chromatin³⁵, an intriguing possibility is that Rpb1 ubiquitination is involved in the process,
358 providing specificity for selective eviction of Pol II molecules by INO80.

359 In agreement with a role for H2A.Z in RNA quality control and non-poly(A) transcription
360 termination⁶⁴⁻⁶⁶, our results suggest that INO80 promotes recruitment of Nab2 to chromatin
361 by regulating its interaction with H2A.Z (Figure 7). This implies that remodelling of H2A.Z-
362 containing nucleosomes by INO80⁶⁷⁻⁶⁹ is important for premature termination. It is therefore
363 likely that intermediate steps in the H2A.Z exchange reaction by INO80, for example
364 disposing of DNA from the surface of H2A.Z-H2B dimers⁶⁹, might be involved in the
365 process. How H2A.Z promotes recruitment of the RNA quality control machinery to
366 chromatin is not clear. It would not be surprising if regulation of premature termination
367 entails posttranslational modifications on H2A.Z, which would command its role in the RNA
368 quality control pathway.

369 Promoter-proximal transcriptional pausing has recently been linked to premature termination
370 in metazoans^{6,19-21}. Uncontrolled transcription elongation and dysregulated Pol II pausing
371 have been implicated in human disease^{73,74}. Mutations in the RNA surveillance machinery
372 have been associated with increased transcriptional stress and genomic instability⁷⁵, both

373 hallmarks of cancer⁷⁶. Dysregulated expression of INO80 leads to uncontrolled gene
374 expression and is associated with disease^{31,77,78}. We therefore anticipated that our reported
375 chromatin mechanism of co-transcriptional RNA quality control has general relevance for
376 regulation of eukaryotic gene expression and in disease.

377 **Competing interests**

378 The authors declare that they have no competing interests. BFP has a financial interest in
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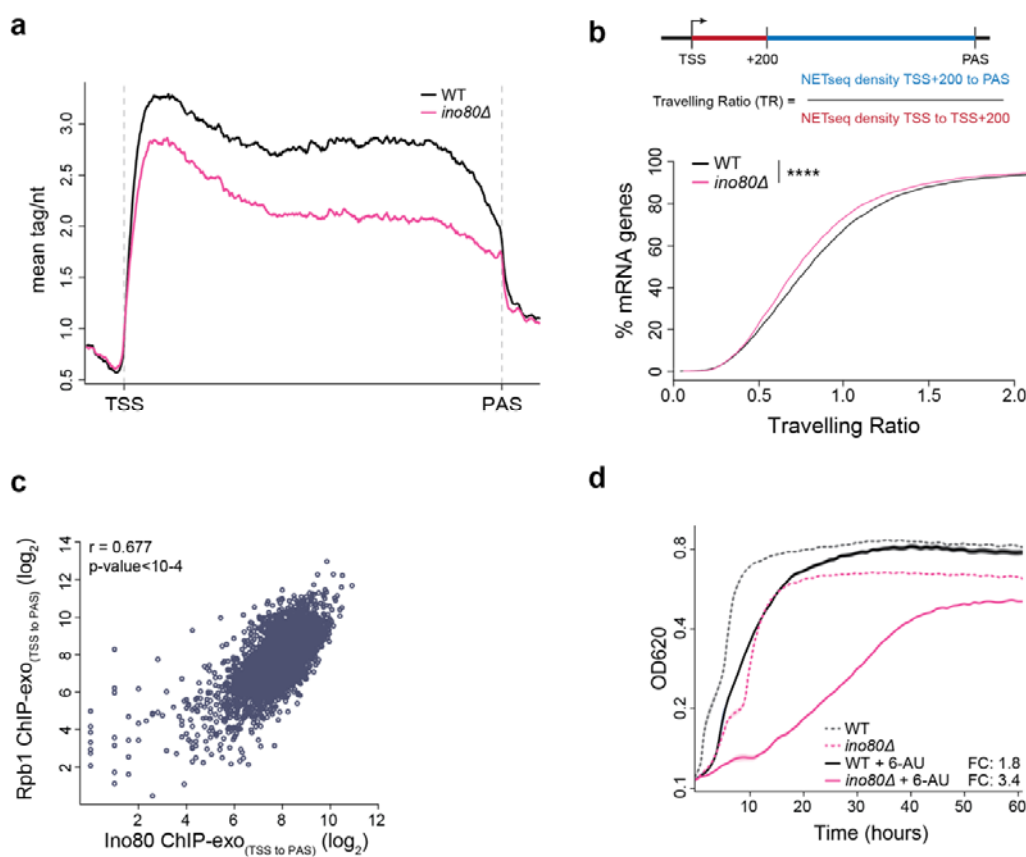
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Figure 1



597

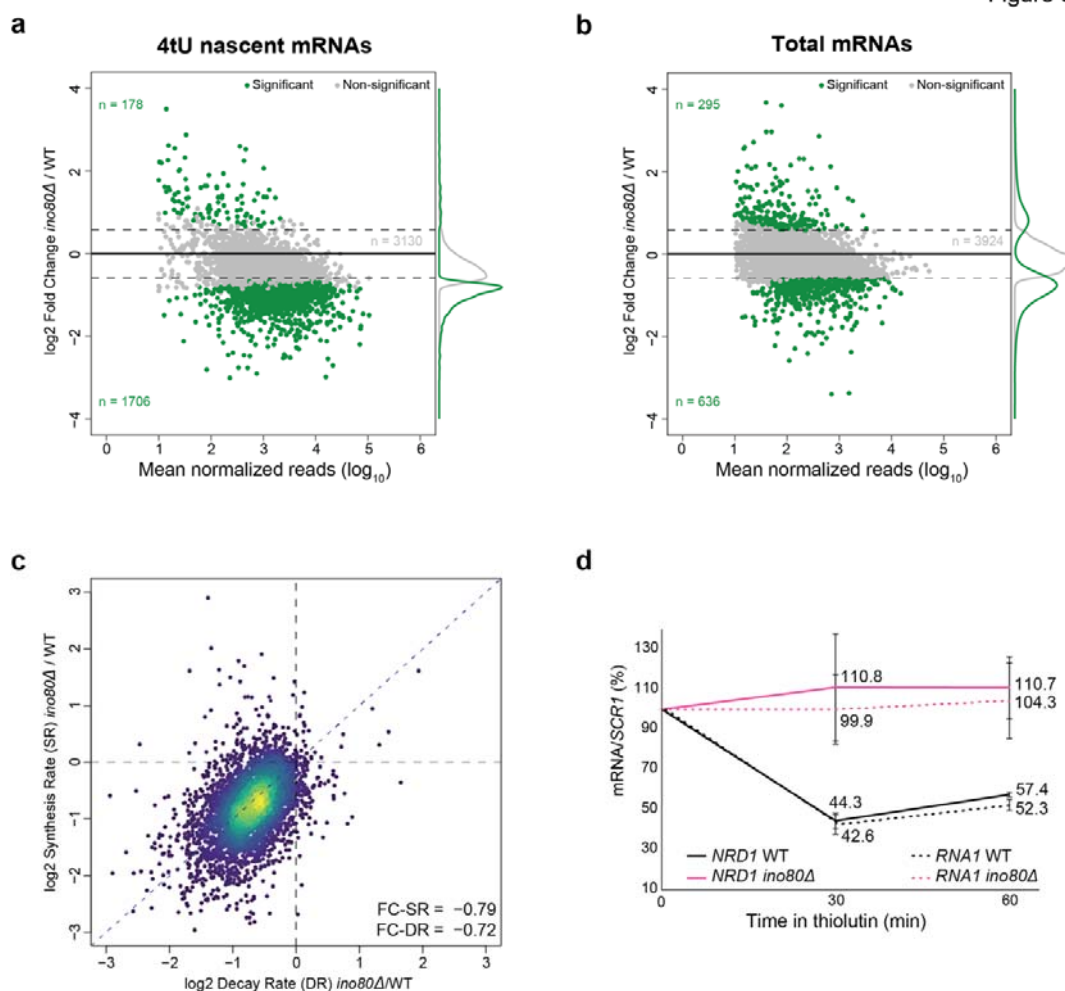
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599 **Fig. 1. Accumulation of nascent mRNA transcripts on chromatin proximally to**
 600 **promoters and defective transcriptional elongation in the absence of INO80.**

601 **a**, Metagenesis analysis for NET-seq density profiles averaged across scaled mRNA genes (n=
 602 5432) in WT and *ino80Δ*. Tags were aligned to both Transcription Start Site (TSS) and
 603 Polyadenylation Site (PAS) for each gene. n=2. **b**, Top: Schematic representation and
 604 formula for calculating the Traveling Ratio (TR) from NET-seq mRNA densities. Bottom:
 605 Cumulative distribution plot of Traveling Ratio for mRNA genes from a (n=5432). Genes
 606 with TR>2 (WT: n=354, *ino80Δ*: n=314) are not shown. ****p < 0.0XXX with K-S test. **c**,
 607 Scatterplot analysis comparing Ino80³³ and Rpb1 ChIP-exo densities between the
 608 transcription start site and polyadenylation site (TSS to PAS) in mRNA yeast genes
 609 (n=5798). Pearson's correlation coefficient (r) and p-value are indicated. **d**, Cell proliferation

610 analysis for WT and *ino80Δ* strains grown exponentially in SC-ura and in SC-ura containing
611 50 μg/ml 6-azauracil (6-AU) liquid media for the indicated time. Cell density was measured
612 at OD⁶²⁰ and plotted on a log₂ scale. Doubling time was calculated from OD⁶²⁰: 0.2 to OD⁶²⁰:
613 0.4 for all strains in both conditions. Fold Change ratio (FC) of doubling time in 6-AU over
614 SC-ura for each strain is shown. n=4.

Figure 3



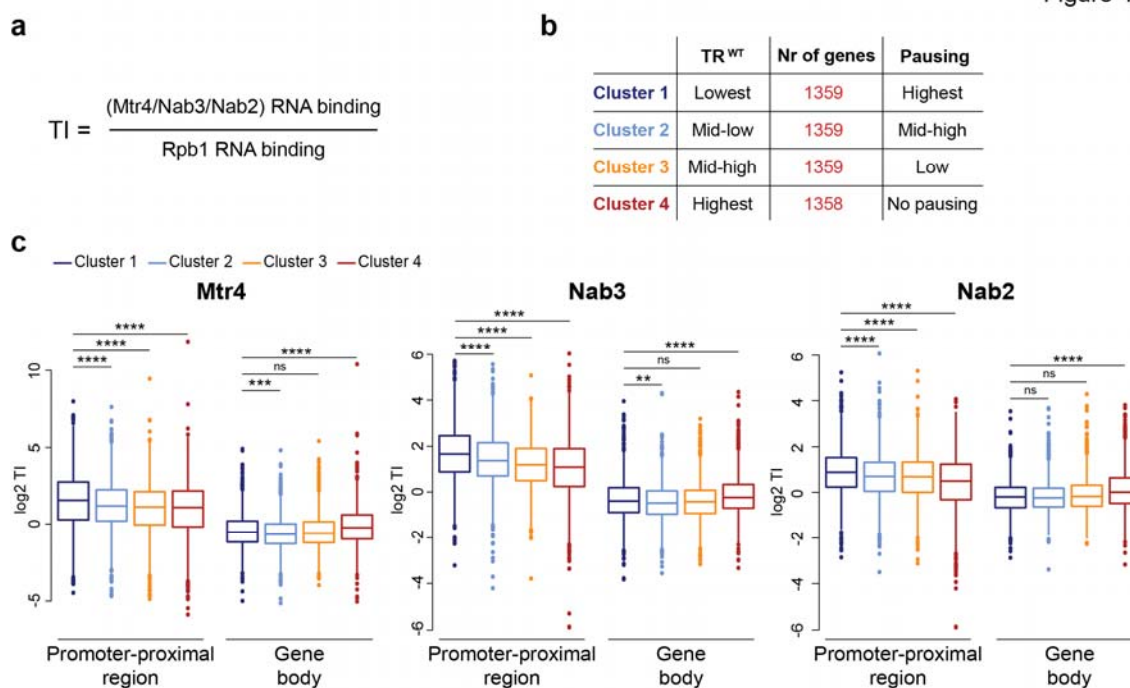
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616 **Fig. 3. mRNA synthesis and decay rates are reduced in the absence of INO80.**

617 **a, b**, Scatterplot analysis for fold changes between WT and *ino80Δ* in newly synthesized
618 mRNA (a) and steady-state mRNA (b) levels (n=5012 genes) plotted on a log₂ scale.
619 Thresholds of 1.5-fold change and 0.05 p values were considered for significant change
620 (green dots). n=3. **c**, cDTA profiles for *ino80Δ*. For all analysed genes, changes in synthesis
621 rates (SR) were plotted against the changes in mRNA decay rates (DR). Fold-change in SR
622 and DR were calculated between *ino80Δ* and WT and plotted on a log₂ scale. 90% of genes
623 are contained within the outer contour. Yellow and red dots correspond to 60% of genes. **d**,
624 RT-qPCR analysis for *NRD1* and *RNA1* RNAs was conducted in WT and *ino80Δ* before (T₀)
625 and after addition of thiolutin in YPD. RNA abundance at each time-point was normalized

626 over the respective abundance of the *SCR1* RNA. RNA before thiolutin treatment was set at
 627 100% in both strains. Remaining RNA was calculated as the amount of normalized RNA in
 628 the indicated time-points relative to the normalized RNA at T_0 . Values represent the average
 629 from at least three independent experiments. Bars, standard errors.

Figure 4



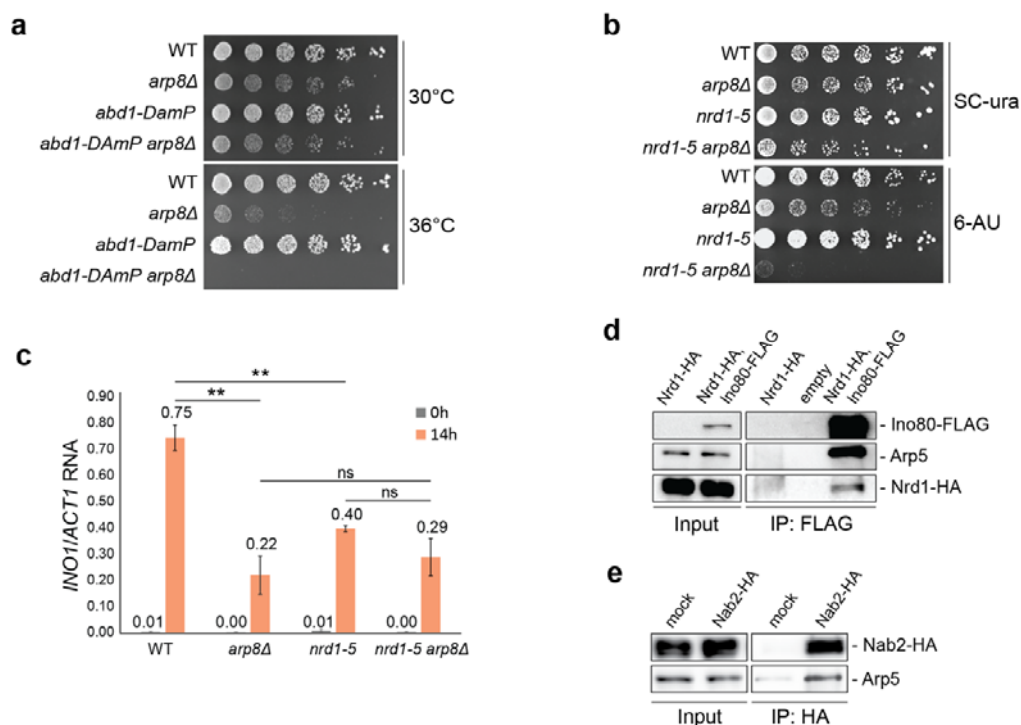
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631

632 **Fig. 4. Loading of the RNA surveillance and termination factors Mtr4, Nab3 and Nab2**
 633 **on promoter-proximal mRNA transcripts.**

634 **a**, Formula for calculating Transcript Instability (TI) as the ratio of the averaged CRAC
 635 densities of Nab3⁵⁶, Mtr4 or Nab2⁴ to CRAC densities of Rpb1⁵⁶ for mRNA genes. **b**,
 636 Schematic table for clustering mRNA genes according to the relative TR in WT (TR^{WT}). The
 637 number of genes and the level of promoter-proximal pausing are indicated for each cluster. **c**,
 638 Boxplot analysis comparing TIs calculated for the promoter-proximal and gene body regions
 639 of mRNA genes in the four clusters defined by their relative TR^{WT}. p-values were calculated
 640 by Wilcoxon rank-sum test. ****, p<0.0001. ***, p<0.001. **, p<0.01. ns, non-significant.

Figure 5



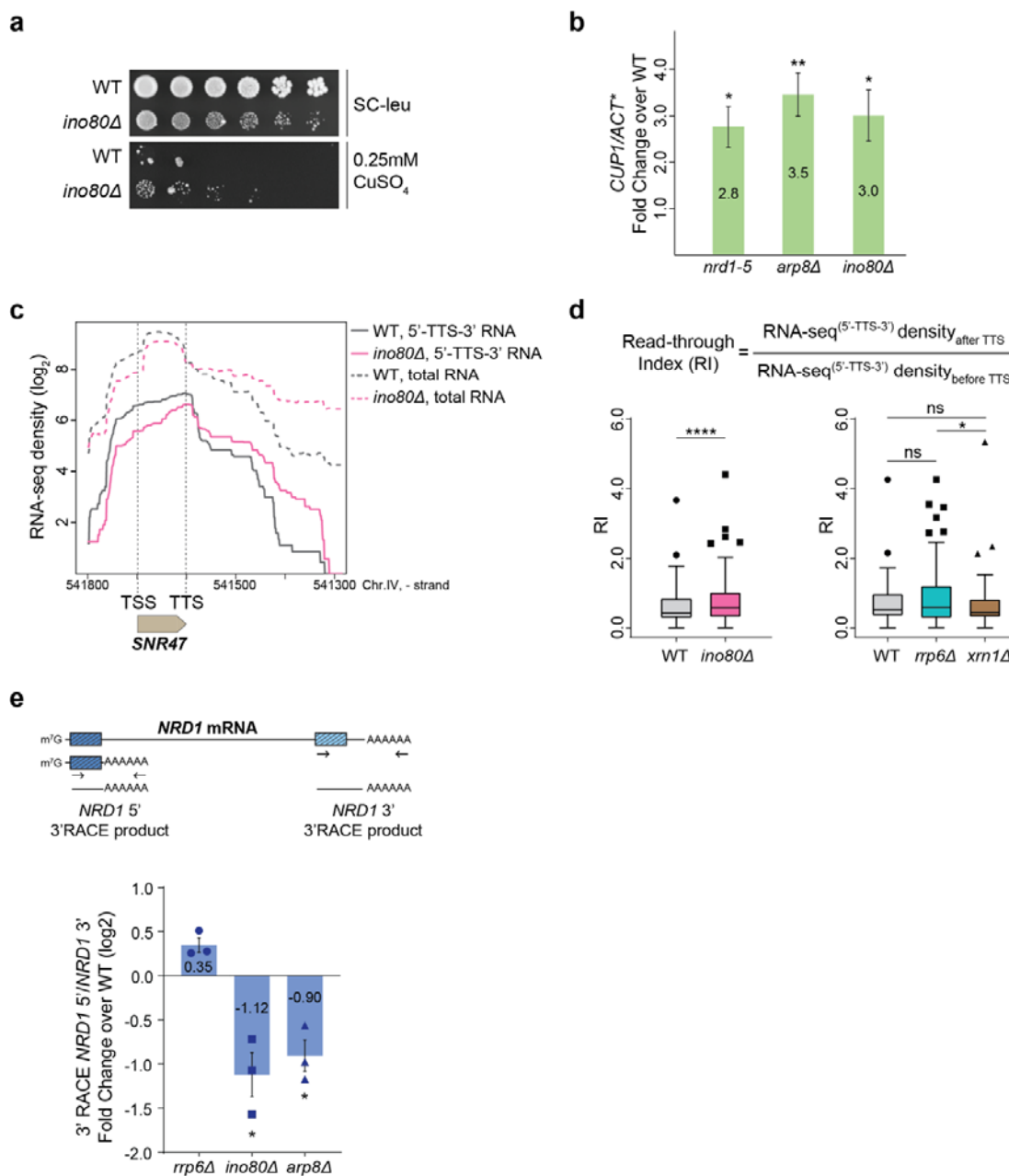
641

642 **Fig. 5. Genetic and physical interactions of INO80 with the RNA quality control**
 643 **pathway.**

644 **a**, 5-fold serial dilution of exponentially grown cells from the indicated strains were plated
 645 onto YPD and incubated at 30°C (permissive temperature) or 37°C (non-permissive
 646 temperature) for 4-5 days. **b**, 5-fold serial dilution of exponentially grown cells from the
 647 indicated strains were plated onto both SC-ura and SC-ura + 50μg/ml 6-Azaauracil (6-AU)
 648 and incubated at 30°C for 4-5 days. **c**, RT-qPCR analysis for *INO1* RNA was conducted in
 649 the indicated strains grown exponentially in SC media (0h) or SC without inositol for 14h.
 650 *INO1* RNA was normalized over *ACT1* RNA. Bars, standard errors (n=3). Asterisks indicate
 651 statistical significance of the indicated change as calculated by Tukey's multiple comparisons
 652 test. ns: non-significant. **d**, Nucleic acid-free lysates from cells co-expressing Nrd1-HA with
 653 Ino80 either untagged or tagged with FLAG epitope were subjected to FLAG-IP. Inputs and
 654 IP samples were immunoblotted for FLAG, Arp5, HA. Immunoblot against Pgk1 served as a
 655 control. **E**, Nucleic acid-free lysates from cells expressing Nab2-HA were subjected to either

656 HA-IP (Nab2-HA) or IgG-IP (mock). Inputs and IP samples were immunoblotted for Arp5
657 and HA.

Figure 6



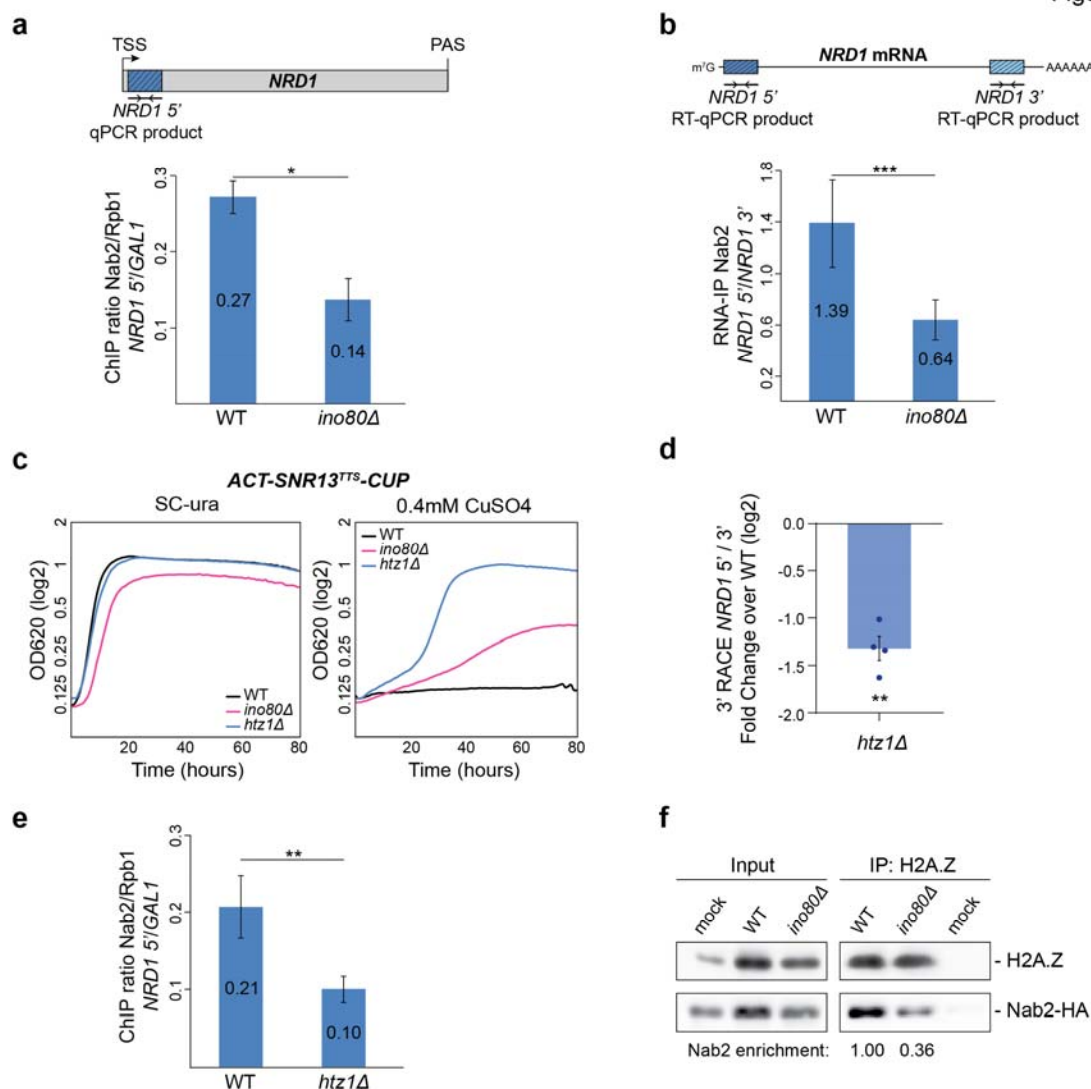
658

659 **Fig. 6. NNS-dependent transcriptional termination is defective in *ino80Δ*.**

660 **a**, 5-fold serial dilution of cells from the indicated strains were plated onto both SC-leu and
 661 SC-leu + 0.25mM CuSO₄ and incubated at 30°C for 3 (SC-leu) and 7 days (CuSO₄). **b**, RT-
 662 qPCR analysis was conducted in WT, *nrd1-5*, *arp8Δ* and *ino80Δ* cells transformed with
 663 either *ACT-CUP* or *ACT-SNR^{TTS}-CUP* plasmids. The *CUP1/ACT** ratio from the *ACT-*
 664 *SNR^{TTS}-CUP* plasmid was normalized over the same ratio from the control *ACT-CUP*

665 plasmid (Extended Fig. 4c) to correct for differential transcription across all strains. Read-
666 through at the *SNR13*^{TTS} for each indicated strain is shown as Fold Change Ratio over WT.
667 Bars, standard errors from three independent experiments. p-values were calculated by t-test
668 after testing for normal distribution by Shapiro-Wilk test. *, p<0.05. **, p<0.01. **c**, Snapshot
669 of RNA-seq densities in WT and *ino80Δ* across the *SNR47* snoRNA gene. Solid lines, density
670 of reads encompassing the transcription termination site (5'-TTS-3') of the *SNR47* gene.
671 Broken lines, total RNA-seq density. Reads encompassing the TTS were selected from the
672 total RNA-seq reads as containing 5' upstream the *SNR47* TTS and 3' at or after the *SNR47*
673 TTS. Genomic coordinates of Chromosome IV around the *SNR47* gene (coordinates: 541700
674 to 541602) are shown. The closest downstream gene is *YDR042C* (TSS: Chromosome IV,
675 541203). n=4. **d**, Upper panel, formula for calculating the Read-through Index (RI) from
676 RNA-seq densities across snoRNA genes that encompass transcription termination sites (5'-
677 TTS-3'). RNA-seq reads with 5' upstream the TTS and 3' at or after the TTS were selected
678 from the total RNA-seq read pool and the ratio of the RNA-seq read density after TTS to the
679 RNA-seq read density before TTS in the selected reads was calculated. Lower panel, boxplot
680 analysis comparing RI from the indicated strains across snoRNA genes. p-values were
681 calculated by Wilcoxon matched-pairs rank test after testing for normal distribution. *****,
682 p<0.0001. *, p<0.05. ns, non-significant. **e**, Top: Scheme illustrating nested 3'RACE for
683 termination events at the promoter-proximal (5') and PAS (3') regions of the *NRD1* gene
684 (*NRD1* 5', dark blue). Bottom: 3'RACE analysis for *NRD1* termination events was conducted
685 in WT and the indicated mutant strains. The relative enrichment of polyadenylated promoter-
686 proximal to PAS-terminated transcripts (5'/3') was calculated for all strains and the fold
687 change in each mutant compared to WT was plotted on a log₂ scale. n=3. p-values were
688 calculated by t-test after testing for normal distribution by Shapiro-Wilk test. *, p<0.05.

Figure 7



689

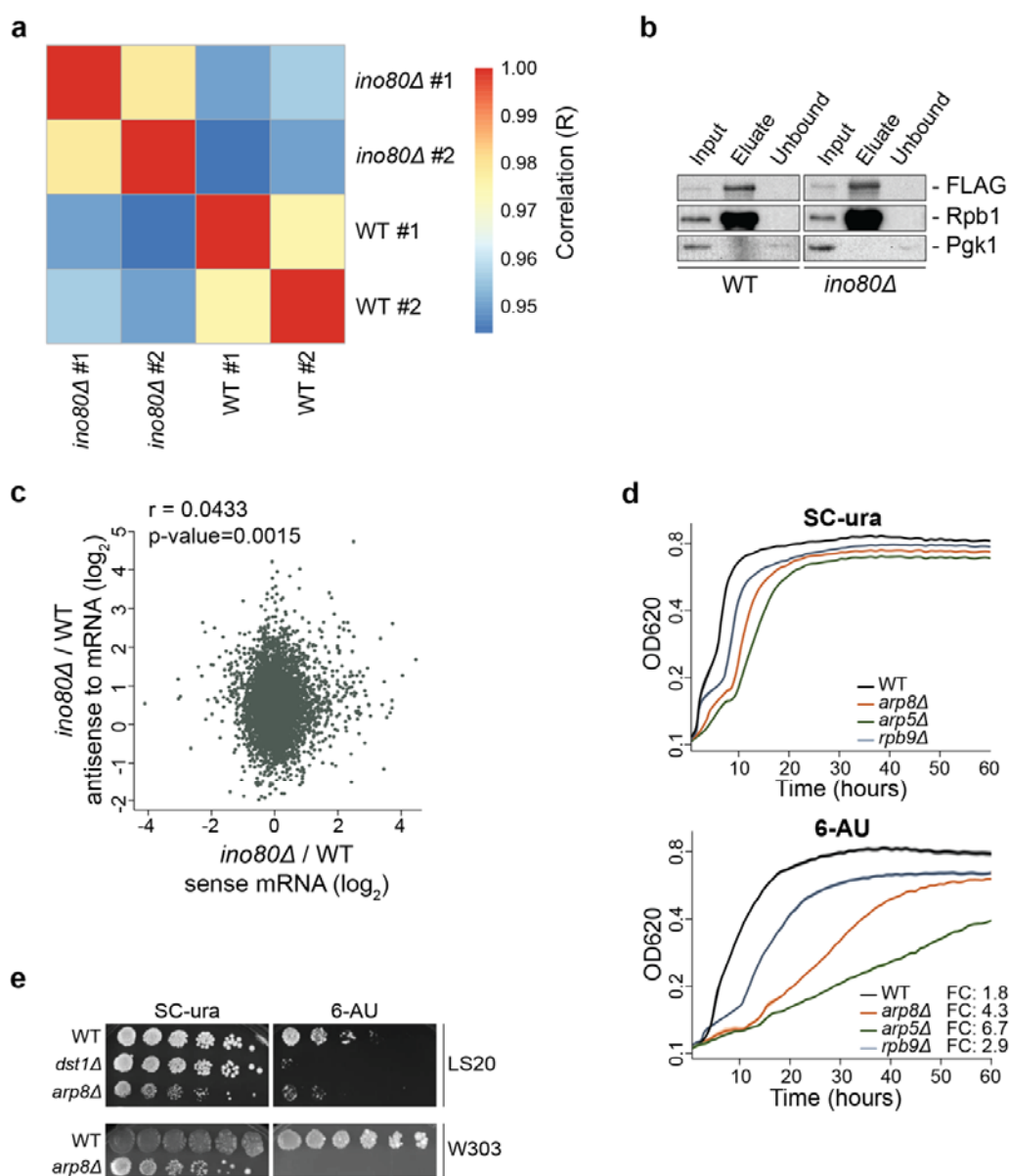
690

691 **Fig. 7. INO80 promotes the co-transcriptional recruitment of Nab2 to chromatin and its**
 692 **association with H2A.Z.**

693 **a**, Chromatin immunoprecipitation for Nab2-HA and Rpb1 in WT and *ino80Δ* cells. Fold
 694 enrichment of each protein at the promoter-proximal region of *NRD1* gene (*NRD1* 5') (upper
 695 panel, shaded in dark blue) was calculated relative to the *GAL1* promoter after correction for
 696 input DNA levels. ChIP ratio at the promoter-proximal region of *NRD1* gene (*NRD1* 5') was
 697 calculated as Fold enrichment of Nab2-HA over Fold enrichment of Rpb1. Bars, standard

698 errors from four independent experiments. p-values were calculated by t-test after testing for
699 normal distribution by Shapiro-Wilk test. *, $p < 0.05$. **b**, RNA immunoprecipitation for Nab2-
700 HA in WT and *ino80Δ* cells. Ratio of binding of Nab2 to the promoter-proximal *NRDI* RNA
701 (upper panel, *NRDI* 5', dark blue) over binding to the 3' region of *NRDI* RNA (upper panel,
702 *NRDI* 3', light blue) after correction for input (total) RNA levels in the indicated strains.
703 Bars, standard errors from four independent experiments. p-values were calculated by t-test
704 after testing for normal distribution by Shapiro-Wilk test. ***, $p < 0.001$. **c**, Cell proliferation
705 analysis for the indicated strains transformed with the *ACT-SNR^{TTS}-CUP* plasmid grown
706 exponentially in SC-ura (left panel) and in SC-ura liquid media containing 0.4mM CuSO₄ (6-
707 AU) (right panel) for the indicated time. Cell density was measured at OD⁶²⁰ and is shown on
708 a log₂ scale. **d**, Nested 3'RACE analysis for termination events at the *NRDI* gene was
709 conducted in WT and *htz1Δ*. Analysis was conducted as in Fig. 6e. n=4. p-values were
710 calculated by t-test after testing for normal distribution by Shapiro-Wilk test. **, $p < 0.01$. **e**,
711 Chromatin immunoprecipitation for Nab2-HA and Rpb1 in WT and *htz1Δ* cells. Analysis was
712 conducted as in (a). Bars, standard errors from three independent experiments. p-values were
713 calculated by t-test after testing for normal distribution by Shapiro-Wilk test. **, $p < 0.01$. **f**,
714 Representative H2A.Z immunoprecipitation experiment. Nucleic acid-free lysates from WT
715 and *ino80Δ* cells expressing Nab2-HA were subjected to H2A.Z-IP. Inputs and IP samples
716 were immunoblotted for H2A.Z and HA. Nab2-HA enrichment in H2A.Z pulldowns is
717 calculated as HA/H2A.Z IP ratio, corrected for the relative Nab2-HA abundance in the input
718 samples. Nab2-HA enrichment in WT is set arbitrarily to 1.0. All analysis was conducted
719 from the same SDS-page gel.

Extended Data Fig. 1



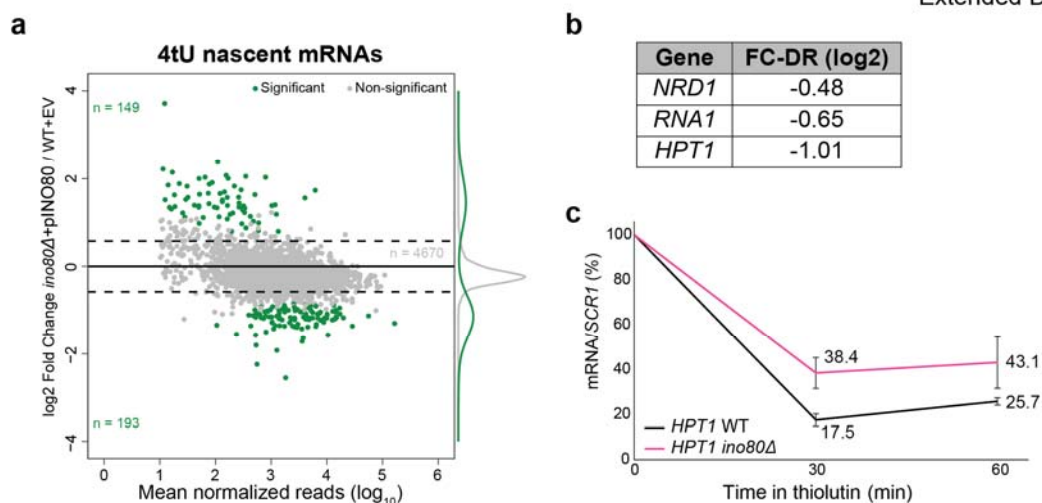
720

721 **Extended Data Fig. 1. NET-seq and 6-AU cell growth analysis in the absence of INO80.**

722 **a**, Heatmap of Pearson's correlation coefficients (R) for all pairwise combinations of NET-
 723 seq reads in independent biological replicates. **b**, Rpb1-FLAG is efficiently
 724 immunoprecipitated from yeast cells during NET-seq analysis. Lysates from WT and *ino80Δ*
 725 cells expressing *RPB1-FLAG* were subjected to FLAG immunoprecipitation using FLAG
 726 beads. Rpb1-FLAG was subsequently eluted from the beads with FLAG peptide. Input,

727 eluate and unbound samples were immunoblotted against FLAG epitope and total Rpb1.
728 Immunoblotting against Pgk1 serves as loading control. All samples were resolved in the
729 same SDS-page gel. **c**, Scatterplot analysis comparing the changes in NET-seq densities
730 between WT and *ino80Δ* for sense transcripts and their relative antisense transcripts over
731 mRNA gene regions, plotted on a log₂ scale. Pearson's correlation coefficient (r) is indicated.
732 **d**, Cell proliferation analysis for the indicated strains grown exponentially in SC-ura (top
733 panel) and in SC-ura containing 50μg/ml 6-azauracil (6-AU) (bottom panel) liquid media for
734 the indicated time. Cell density was measured at OD⁶²⁰ and shown on a log₂ scale. Shading
735 surrounding each line denotes standard error from four independent experiments. The mutant
736 strain deleted for the RNA Polymerase II subunit Rpb9 (*rpb9Δ*) has reported transcriptional
737 elongation defects in 6-AU⁷⁹ and was used as control. Doubling time from OD⁶²⁰ 0.2 to
738 OD⁶²⁰ 0.4 was calculated for all strains in both conditions. Fold Change ratio (FC) of
739 doubling time in 6-AU over SC-ura is shown (bottom panel). **e**, 5-fold serial dilution of cells
740 from the indicated strains were plated onto both SC-ura and SC-ura + 50μg/ml 6-Azaauracil
741 (6-AU) and incubated at 30°C for 4-5 days. Genetic backgrounds (LS20, W303) are
742 indicated. The mutant strain deleted for the Dst1/TFIIS transcription elongation factor (*dst1Δ*)
743 was used as control⁸⁰.

Extended Data Fig.3

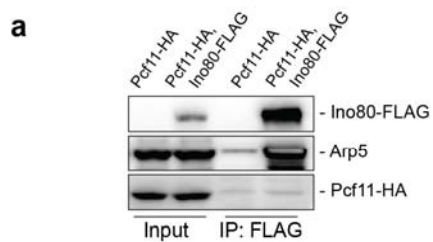


744

745

746 **Extended Data Fig. 3. Ectopic expression of *INO80* rescues the nascent mRNA synthesis**
747 **defect of *ino80*Δ cells.**

748 **a**, Scatterplot analysis for fold changes in newly synthesized mRNA between *ino80*Δ cells
749 expressing a WT allele of *INO80* from the pRS416 plasmid (*pINO80*) and WT cells
750 transformed with the empty vector pRS416 (EV) plotted on a log₂ scale. Analysis as in
751 Fig.3a. **b**, Fold changes in mRNA decay rates between *ino80*Δ and WT for the indicated
752 genes as calculated by cDTA analysis. **c**, RT-qPCR analysis for *HPT1* RNA was conducted
753 in WT and *ino80*Δ before (T₀) and after addition of thiolutin in YPD as in Fig. 3d.



Extended Data Fig.4

754

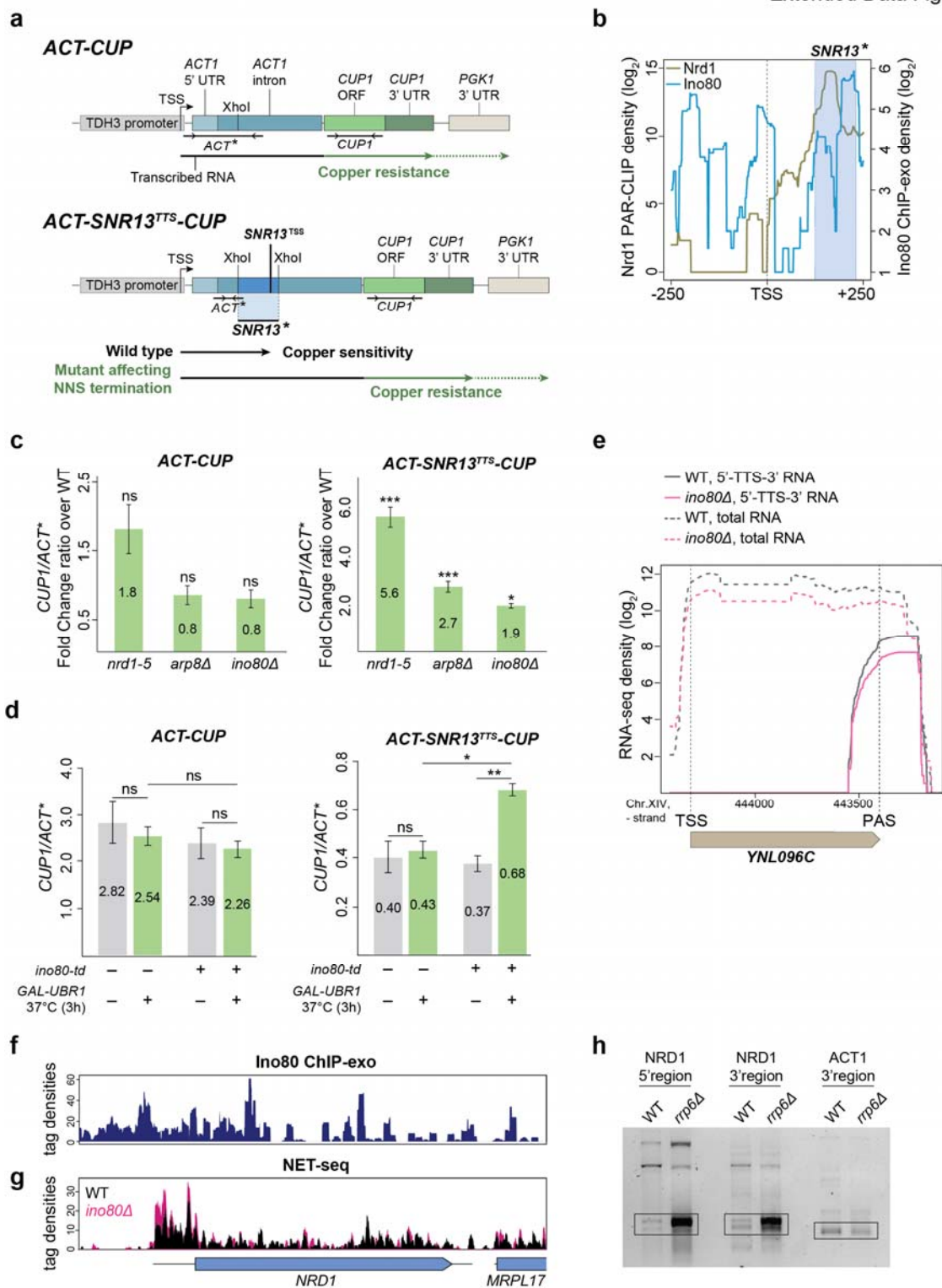
755 **Extended Data Fig. 4. INO80 does not interact with Pcf11.**

756 **a**, Nucleic acid-free lysates from cells co-expressing Pcf11-HA with Ino80 either untagged or

757 tagged with FLAG epitope were subjected to FLAG-IP. Inputs and IP samples were

758 immunoblotted for FLAG, Arp5 and HA. Samples were resolved in the same SDS-page gel.

Extended Data Fig.5



759

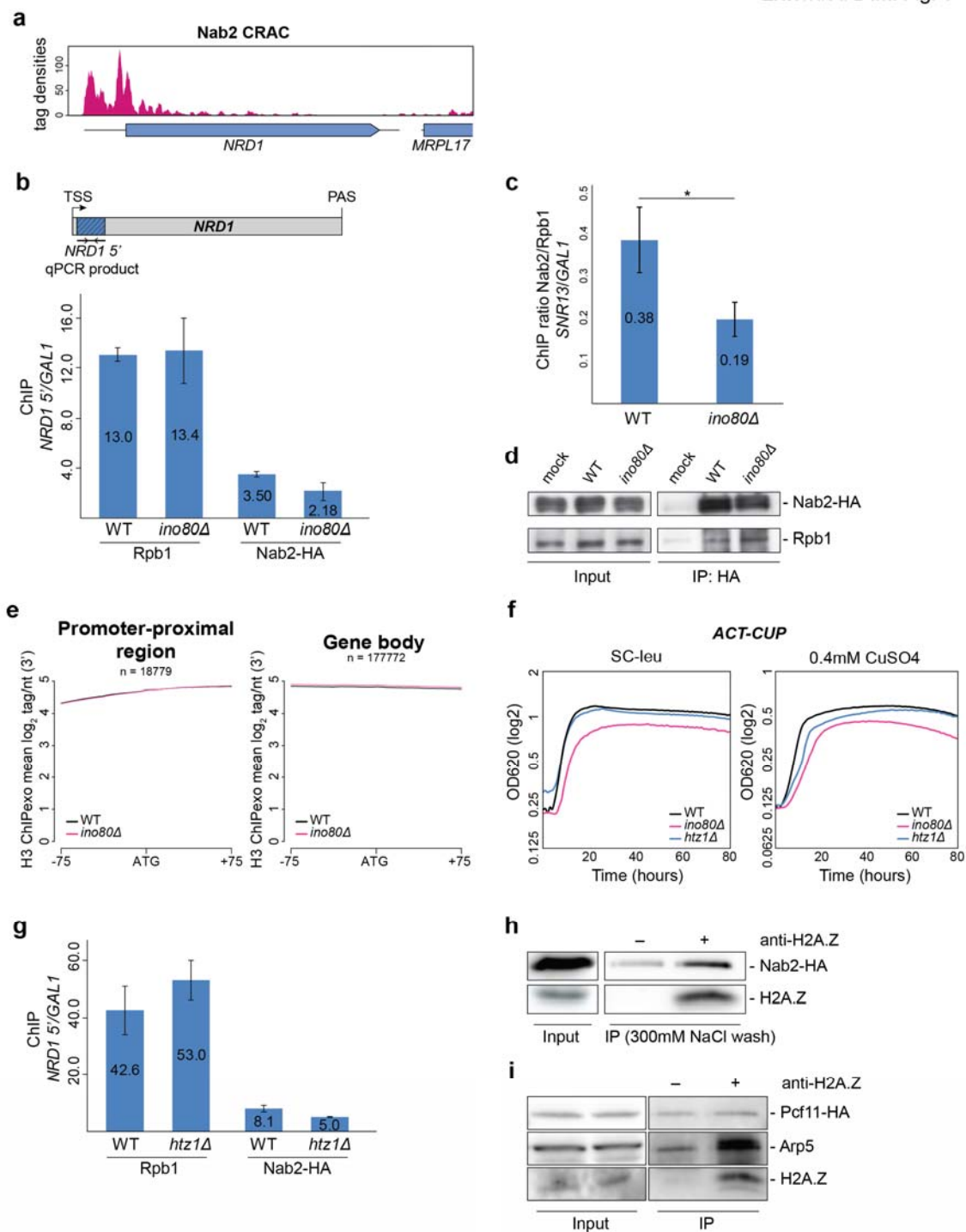
760

761 **Extended Data Fig. 5. Defective NNS-dependent transcription termination in *ino80Δ***
762 **cells.**

763 **a**, Schematic representation of the *ACT-CUP* (pGAC24) and *ACT-SNR^{TTS}-CUP* (pGAC24-
764 *SNR13^{TTS}*) reporter systems for transcription termination reported in⁸. The regions amplified
765 by RT-qPCR for *ACT** and *CUP1* are underlined. The region of the *SNR13* gene between
766 +125 to +232bp from TSS that was cloned in the *ACT-SNR13^{TTS}-CUP* plasmid⁸ is shaded in
767 blue (*SNR13**). The expected phenotype of cells transformed with either the *ACT-CUP* or the
768 *ACT-SNR13^{TTS}-CUP* plasmid is shown. **b**, Snapshot of Nrd1 PAR-CLIP density profile
769 from⁸¹ and Ino80 ChIP-exo density profile from³³ across the *SNR13* snoRNA gene. Tags
770 were aligned relative to TSS and plotted on a log₂ scale. The region between +125 to +232bp
771 from TSS cloned in the *ACT-SNR13^{TTS}-CUP* plasmid described in (a)⁸ is highlighted in blue
772 (*SNR13**). **c**, RT-qPCR analysis for the *CUP1/ACT** ratio was conducted in the indicated
773 strains transformed with either the *ACT-CUP* (left panel) or *ACT-SNR^{TTS}-CUP* (right panel)
774 plasmids. Bars, standard errors from three independent experiments. Asterisks indicate
775 statistical significance of the change in the respective mutant compared to the WT as
776 calculated by t-test. ns: non-significant. *, p<0.05. ***, p<0.001. **d**, RT-qPCR analysis was
777 conducted in both WT and the *ino80-td* degenon-inducible strain (*ino80-td*)⁸² transformed
778 with the *ACT-CUP* control plasmid described in a. Galactose-induced activation for 3 hours
779 at 37°C (+) and glucose-induced suppression (–) of the *UBR1* N-recognin in the two strains is
780 indicated. Bars, standard errors from four independent experiments. p-values were calculated
781 by t-test after testing for normal distribution by Shapiro-Wilk test. ns, non-significant. **e**,
782 Snapshot representing RNA-seq densities in WT and *ino80Δ* across the ribosomal protein
783 coding gene YNL096C. Solid lines, density of reads encompassing the transcription
784 termination site (5'-TTS-3') as described in Fig. 6c. Analysis was performed around the
785 polyadenylation site (PAS). Broken lines, total RNA-seq density. Genomic coordinates of

786 Chromosome XVI around the *YNL096C* gene are shown. The closest gene downstream of
787 *YNL096C* is the *PHO23* mRNA gene (TSS: Chromosome XIV, 442509). **f**, Snapshot
788 representing ChIP-exo densities of Ino80 from ⁴¹ across the *NRDI* gene. **g**, Snapshot
789 representing NET-seq densities in WT and *ino80Δ* across the *NRDI* gene. **h**, Representative
790 image of nested 3'RACE analysis of termination events at the *NRDI* 5' and 3' regions
791 according to the scheme in Figure 6e and at the 3' of *ACT1* gene in WT and *rrp6Δ* on an
792 agarose gel. Boxes indicate the most frequent termination sites. Ladder is 100bp. *ACT1*
793 serves as loading control.

Extended Data Fig. 6



794

795 **Extended Data Fig. 6. Co-transcriptional recruitment of Nab2 to chromatin depends on**
 796 **INO80 and H2A.Z.**

797 **a**, Snapshot representing CRAC densities for Nab2 from ⁴ across the *NRD1* gene. **b**,

798 Chromatin immunoprecipitation for Rpb1 and Nab2-HA in WT and *ino80Δ* cells. Fold

799 enrichment analysis at the promoter-proximal region of *NRD1* gene (*NRD1* 5') (upper panel,
800 shaded in dark blue) was conducted as Fig. 7a. **c**, Chromatin immunoprecipitation for Nab2-
801 HA and Rpb1 in WT and *ino80Δ* cells. Fold enrichment of each protein at the TTS region of
802 *SNR13* gene was calculated relative to the *GALI* promoter after correction for input DNA
803 levels. ChIP ratio at the TTS region of *SNR13* gene was calculated as Fold enrichment of
804 Nab2-HA over Fold enrichment of Rpb1. Bars, standard errors from four independent
805 experiments. p-values were calculated by t-test after testing for normal distribution by
806 Shapiro-Wilk test. *, p<0.05. **d**, Nucleic acid-free lysates from WT and *ino80Δ* cells both
807 expressing Nab2-HA were subjected to HA-IP. Inputs and IP samples were immunoblotted
808 for Rpb1 and HA. Mock, WT protein lysate subjected to immunoprecipitation with a control
809 IgG antibody. **e**, Metagene analysis for ChIP-exo density profiles of histone H3 in WT and
810 *ino80Δ* aligned at the A nucleotide of the ATG sites in the TSS to TSS+200bp (Promoter-
811 proximal) and the TSS+200 to PAS (Gene body) regions. n=Number of ATG sites in each
812 region. **f**, Cell proliferation analysis for the indicated strains transformed with the *ACT-CUP*
813 plasmid grown exponentially in SC-ura (left panel) and in SC-ura liquid media containing
814 0.4mM CuSO₄ (6-AU) (right panel) for the indicated time. Cell density was measured at
815 OD⁶²⁰ and is shown on a log₂ scale. **g**, Chromatin immunoprecipitation for Rpb1 and Nab2-
816 HA in WT and *htz1Δ* cells. Fold enrichment analysis at the promoter-proximal region of
817 *NRD1* gene (*NRD1* 5') was conducted as Fig. 7a. **h**, Nucleic acid-free lysates from WT cells
818 expressing Nab2-HA were subjected to immunoprecipitation with either an anti-H2A.Z
819 antibody (+) or a non-specific IgG antibody as a control (-), then washed in the presence of
820 300mM NaCl. Inputs and IP samples were immunoblotted for HA and H2A.Z. All analysis
821 was conducted from the same SDS-page gel. **i**, Nucleic acid-free lysates from cells
822 expressing Pcf11-HA were subjected to immunoprecipitation with either an anti-H2A.Z
823 antibody (+) or a non-specific IgG antibody as a control (-). Inputs and IP samples were

824 immunoblotted for HA, Arp5 and H2A.Z. The western for Pcf11-HA was over-exposed in
825 order to detect the background levels of Pcf11 binding to the beads. All analysis was
826 conducted from the same SDS-page gel.

827

828 **Extended Data Table 1 - Yeast strains used in this study**

Name	Genotype	Source
YM50	LS20 [<i>MatΔ ade2 can1r cyh2r lys S URA3-52 trp1Δ his3-Δ200GAL:HO leu 2Δ</i>]	Sandell & Zakian, <i>Cell</i> 1993
YM85	LS20 <i>ino80Δ::KANMX</i>	Papamichos-Chronakis & Peterson, <i>Nat Struct Mol Biol</i> 2008
YM133	W303 <i>ubr1Δ::HIS3-GAL-Myc-UBR1</i>	Jónsson <i>et al.</i> , <i>Mol Cell</i> 2004
YM135	W303 <i>ubr1Δ::HIS3-GAL-Myc-UBR1 ino80Δ::URA3-HA-ino80-td</i>	Jónsson <i>et al.</i> , <i>Mol Cell</i> 2004
YM143	LS20 <i>htz1Δ::hisG-ura3Δ</i>	Papamichos-Chronakis <i>et al.</i> , <i>Cell</i> 2011
YM181	LS20 <i>arp8Δ::KANMX</i>	Papamichos-Chronakis <i>et al.</i> , <i>Cell</i> 2011
YM198	LS20 <i>hda1Δ::HPHMX</i>	This study
YM199	LS20 <i>arp8Δ::KANMX hda1Δ::HPHMX</i>	This study
YM311	W303 [<i>MATa ade2-1 can1-100 his3-11 leu2,3,112 trp1-1 ura3-1</i>]	Verma <i>et al.</i> , <i>Mol Cell</i> 2011
YM334	W303 <i>arp8Δ::KANMX</i>	Lafon <i>et al.</i> , <i>Mol Cell</i> 2015
YM467	LS20 <i>dst1Δ::KANMX</i>	This study
YM468	LS20 <i>rpb9Δ::KANMX</i>	This study
YM498	LS20 <i>rco1Δ::HPHMX</i>	This study
YM499	LS20 <i>arp8Δ::KANMX rco1Δ::HPHMX</i>	This study
YM753	BY4741 [<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>] <i>NRD1-3XHA::HIS3</i>	This study
YM801	46a [<i>MATa ura3 his3 trp1 lys2 ade2 leu2 cup1Δ</i>]	Steinmetz & Brow, <i>Mol Cell Biol</i> 1996
YM802	46a <i>nrd1-5</i>	Steinmetz & Brow, <i>Mol Cell Biol</i> 1996
YM838	LS20 <i>arp5Δ::KANMX</i>	This study
YM850	BY4741 <i>PCF11-3XHA::HIS3</i>	This study
YM885	LS20 <i>RPB1-3XFLAG::KANMX</i>	This study

YM900	BY4741 <i>PCF11-3XHA::HIS3</i> <i>INO80-5XFLAG::NATMX</i>	This study
YM901	BY4741 <i>NRD1-3XHA::HIS3 INO80-5XFLAG::NATMX</i>	This study
YM903	LS20 <i>RPB1-3XFLAG::KANMX ino80Δ::HPHMX</i>	This study
YM1002	46a <i>arp8Δ::HPHMX</i>	This study
YM1036	46a <i>nrd1-5 arp8Δ::HPHMX</i>	This study
YM1085	LS20 <i>xrn1Δ::KANMX</i>	This study
YM1139	LS20 <i>rrp6Δ::KANMX</i>	This study
YM1141	46a <i>rco1Δ::KANMX</i>	This study
YM1147	46a <i>nrd1-5 arp8Δ::HPHMX rco1Δ::KANMX</i>	This study
YM1197	BY4741 <i>abd1-DAmP::KANMX</i>	Breslow <i>et al</i> , <i>Nat Methods</i> 2008
YM1325	LS20 <i>NAB2-3XHA::KANMX</i>	This study
YM1331	LS20 <i>NAB2-3XHA::KANMX ino80Δ::HPHMX</i>	This study
YM1332	LS20 <i>NAB2-3XHA::KANMX ino80Δ::HPHMX</i>	This study
YM1452	LS20 <i>NAB2-3XHA::KANMX htz1Δ::HPHMX</i>	This study

830 **Extended Data Table 2 – Expression of genes encoding for RNA surveillance proteins is**
 831 **similar in WT, *ino80Δ* and *arp8Δ* cells.** RNA abundance for the listed genes was measured
 832 by spiked-in total RNA-seq analysis in the indicated strains. Values represent average from
 833 two biological replicates for each experiment. The RNA-seq experiment for WT⁽¹⁾ and
 834 *ino80Δ* was conducted independently from WT⁽²⁾ and *arp8Δ*. Values represent average from
 835 two biological replicates for each experiment.

Pathway/complex	Gene	WT⁽¹⁾	<i>ino80Δ</i>	Fold Change	WT⁽²⁾	<i>arp8Δ</i>	Fold Change
<i>NNS</i>	<i>NRD1</i>	0.61	0.67	1.1	0.43	0.37	0.8
	<i>NAB3</i>	0.54	0.44	0.8	0.30	0.30	1.0
	<i>SEN1</i>	0.46	0.58	1.3	0.29	0.25	0.8
<i>Exosome</i>	<i>RRP6</i>	0.68	0.54	0.8	0.46	0.46	1.0
	<i>DIS3/RRP44</i>	0.82	0.73	0.9	0.64	0.62	1.0
<i>TRAMP</i>	<i>MTR4</i>	0.97	0.74	0.8	0.87	0.89	1.0
	<i>TRF4</i>	0.45	0.48	1.1	0.28	0.32	1.1
	<i>AIR1</i>	0.38	0.30	0.8	0.29	0.29	1.0
	<i>AIR2</i>	0.21	0.22	1.1	0.11	0.15	1.4
<i>Cleavage and Polyadenylation</i>	<i>HRP1</i>	1.21	1.01	0.8	1.04	0.83	0.8
	<i>PCF11</i>	0.26	0.24	0.9	0.19	0.18	1.0
	<i>NAB2</i>	0.70	0.59	0.8	0.51	0.54	1.1
	<i>PAB1</i>	8.95	6.85	0.8	4.53	3.89	0.9
<i>Capping</i>	<i>CEG1</i>	0.42	0.44	1.1	0.19	0.22	1.1
	<i>CET1</i>	0.27	0.26	1.0	0.17	0.19	1.1
<i>Early mRNA processing</i>	<i>CBC1</i>	0.96	0.74	0.8	0.58	0.68	1.2
	<i>GBP2</i>	1.43	1.09	0.8	0.83	0.74	0.9
	<i>THO2</i>	0.48	0.51	1.0	0.27	0.31	1.2
<i>Export</i>	<i>MEX67</i>	0.52	0.50	1.0	0.32	0.34	1.1
<i>Cytoplasmic decay</i>	<i>XRN1</i>	1.87	1.76	0.9	0.91	0.91	1.0
	<i>SKI2</i>	0.40	0.40	1.0	0.28	0.31	1.1

836

837 **Extended Data Table 3 – Readthrough index (RI) of budding yeast *SNR* snoRNA genes**
 838 **as measured by total RNA-seq from the indicated strains.** The RNA-seq experiment for
 839 WT⁽¹⁾ and *ino80Δ* was conducted independently from WT⁽²⁾, *xrn1Δ*, *rrp6Δ*. Values represent
 840 average from two biological replicates for each experiment.

	WT ⁽¹⁾	<i>ino80Δ</i>	WT ⁽²⁾	<i>xrn1Δ</i>	<i>rrp6Δ</i>
<i>NME1</i>	0.05	0.10	0.09	0.12	0.06
<i>SNR11</i>	0.18	0.17	0.26	0.23	0.24
<i>SNR128</i>	0.29	0.36	0.36	0.38	0.29
<i>SNR13</i>	0.15	0.28	0.44	0.36	0.25
<i>SNR161</i>	0.20	0.21	0.30	0.20	0.15
<i>SNR189</i>	0.22	0.23	0.47	0.39	0.33
<i>SNR190</i>	0.01	0.01	0.01	0.01	0.01
<i>SNR3</i>	0.90	1.05	0.91	0.87	0.68
<i>SNR30</i>	0.11	0.16	0.25	0.24	0.51
<i>SNR31</i>	0.18	0.15	0.17	0.17	0.13
<i>SNR32</i>	0.64	0.63	0.73	0.83	0.67
<i>SNR33</i>	0.41	0.39	0.47	0.37	0.51
<i>SNR34</i>	0.49	0.55	0.55	0.41	0.29
<i>SNR35</i>	0.17	0.20	0.27	0.17	0.17
<i>SNR36</i>	0.36	0.38	0.33	0.29	0.25
<i>SNR37</i>	0.32	0.37	0.46	0.42	0.72
<i>SNR39B</i>	0.41	0.57	0.50	0.41	0.93
<i>SNR4</i>	0.17	0.18	0.18	0.15	0.34
<i>SNR40</i>	0.61	0.70	0.62	0.70	0.53
<i>SNR41</i>	0.23	0.22	0.22	0.31	0.34
<i>SNR42</i>	0.42	0.37	0.98	0.59	0.95
<i>SNR43</i>	0.23	0.24	0.35	0.40	0.73
<i>SNR45</i>	1.15	1.04	0.99	0.84	1.57
<i>SNR46</i>	0.37	0.58	0.52	1.06	0.20
<i>SNR47</i>	0.56	1.21	0.52	0.56	1.61
<i>SNR48</i>	0.59	0.71	0.75	0.76	0.93
<i>SNR49</i>	0.53	0.62	0.43	0.41	0.21
<i>SNR5</i>	0.42	0.44	0.53	0.45	0.57
<i>SNR50</i>	1.30	1.08	1.00	0.97	1.76
<i>SNR51</i>	0.79	1.30	1.03	0.76	1.45
<i>SNR52</i>	0.47	0.51	0.52	0.42	0.60
<i>SNR53</i>	0.54	0.79	0.52	0.65	0.74
<i>SNR55</i>	0.39	0.57	0.42	0.55	0.28
<i>SNR56</i>	0.40	0.68	0.46	0.52	2.72
<i>SNR57</i>	0.66	0.64	0.60	0.62	0.71
<i>SNR58</i>	1.01	0.87	1.14	0.69	1.07

SNR61	1.57	2.83	1.19	0.85	2.76
SNR62	1.78	2.03	1.66	1.51	3.55
SNR63	0.40	0.52	0.66	0.43	0.55
SNR64	0.33	0.49	0.22	0.23	0.49
SNR65	0.74	0.95	1.09	0.30	3.17
SNR66	1.23	2.42	1.07	1.33	1.74
SNR67	1.07	1.01	1.08	1.14	1.44
SNR68	0.36	0.75	0.82	0.60	1.09
SNR69	0.68	0.88	0.75	0.80	0.70
SNR70	1.56	1.72	2.16	2.34	1.28
SNR71	1.38	2.61	1.41	1.39	3.46
SNR72	0.39	0.62	0.33	0.37	0.42
SNR74	1.10	1.29	1.05	1.53	0.47
SNR75	2.10	2.46	1.72	2.14	1.37
SNR76	0.57	0.52	0.46	0.44	0.30
SNR77	1.07	1.27	1.07	1.13	0.62
SNR78	3.67	4.40	4.26	5.35	4.26
SNR79	1.73	1.63	1.65	1.23	2.45
SNR8	0.27	0.27	0.21	0.22	0.27
SNR80	0.06	0.17	0.14	0.13	0.23
SNR81	0.15	0.28	0.23	0.19	0.12
SNR82	0.66	0.77	0.92	0.89	0.94
SNR83	0.35	0.46	0.46	0.30	1.03
SNR84	0.58	0.77	0.60	0.81	0.26
SNR85	0.02	0.02	0.03	0.02	0.08
SNR86	0.41	0.40	0.25	0.26	0.17
SNR87	0.93	1.16	1.04	0.90	1.38
SNR9	0.28	0.32	0.38	0.36	0.27

842 **METHODS**

843 **Bioinformatics Tools and genome annotations**

844 Unless otherwise indicated, version of the tools used for all bioinformatics analyses are as
845 follows:

846 Samtools version 1.10

847 Bedtools version 2.29.2

848 Bowtie2 version 2.4.1

849 Hisat2 version 2.2.0

850 R version 3.6.2

851 BamCoverage version 3.5.0

852 Fastqc version 0.11.9

853 Cutadapt version 2.10

854 Trim_galore 0.6.4

855 MarkDuplicates version 2.23.1

856 FeatureCounts version 2.0.0

857 The *Saccharomyces cerevisiae* reference genome (S288C version R64-2-1) was downloaded
858 from Saccharomyces Genome Database (<https://www.yeastgenome.org/>). The annotation
859 used in the analysis for mRNA genes was produced by Antonin Morillon's lab, and based on
860 CAGE-seq. This also includes the following annotations: XUT genes from ⁸³, SUT and CUT
861 genes from XUT et al (2009) and NUT genes from ¹⁶. Annotations for other transcript types
862 (snRNA, snoRNA, etc) were downloaded from Saccharomyces Genome Database.

863 The *Schizosaccharomyces pombe* reference genome and annotation (ASM294v2.47) were
864 downloaded from pombase (www.pombase.org). Only protein coding genes were used in this
865 analysis.

866 **NET-seq analyses**

867 NET-seq was performed from yeast strains containing Rpb1-FLAG (3XFLAG), alone or
868 along with deletion of *INO80*. All strains appeared fully functional, with identical Ser2 and
869 Ser5 phosphorylation levels on Rpb1 C-terminal domain and identical growth rates to the
870 respective untagged strains (data not shown). NET-Seq libraries were constructed from
871 biological duplicates of YM903 (*ino80Δ*, *RPB1-FLAG*) and YM885 (WT, *RPB1-FLAG*) cells
872 and sequenced as previously described⁸⁴. Reads shorter than 10 nt were filtered out. Reads
873 were mapped with Bowtie2 using default parameters. Duplicates reads were marked using
874 Picard MarkDuplicates. Coverage files (bigWig) were produced by custom script. Reads
875 locations from the alignment files (bam) were turned into bed files using bedtools bamtobed
876 (with « -split » option), ignoring duplicates reads and reads with mapping quality below 30,
877 piled up along the genome and turned into bigWig files using a python script. FeatureCounts
878 was used for quantification with options « -O --fraction s 2 --ignoreDup ». Normalization
879 factors were computed using DESeq2 method, using the whole count matrix. Travelling
880 Ratio was computed as: (read density on gene body) / (read density on TSS), with gene body
881 the region between TSS+200 nt to TTS and TSS the region from TSS to TSS + 200 nt. Read
882 density was computed as : (# reads on region / region length) *1000.

883 **4tU labeling and purification of newly synthesized RNAs**

884 RNA labeling and purification were performed as previously described with some minor
885 adjustments^{45,85}. Briefly, 20 mL of wildtype and mutant *S. cerevisiae* cells were grown at
886 30°C to an OD600 ≈0.8 in YPD. Newly synthesized (ns) RNAs were labelled for 6 min with
887 4-thiouracil (4tU, Sigma-Aldrich) added to the yeast cultures to a final concentration of 5
888 mM. Similarly, *S. pombe* RNAs were labeled for 6 min in YES medium at 32°C. Cells were
889 pelleted directly after labeling and flash frozen in liquid nitrogen. All experiments were
890 performed in biological triplicates. Prior to RNA extraction *S. cerevisiae* and *S. pombe* cells
891 were mixed in a ratio of 3:1 and total RNA was extracted with Ribopure Yeast Kit (Ambion,

892 Life Technologies) according to the manufacturer's guidelines. 150 μ g of total RNA were
893 biotinylated for 3 hours at room temperature using 200 μ L of 1 mg/mL EZ-link HPDP-
894 Biotin (Pierce) with 100 μ L of biotinylation buffer (100 mM Tris- HCl pH 7.5, 10 mM
895 EDTA) and 100 μ L of DMSO adjusted to a final volume of 1 mL with DEPC-treated RNase-
896 free water (Sigma-Aldrich). After biotinylation, excess biotin was removed by mixing 1 mL
897 of chloroform to the reaction and the liquid phases were separated by centrifugation for 5 min
898 at 4°C and max speed. The aqueous phase was recovered and precipitated with isopropanol
899 (1/10 volumes 5M NaCl and 2.5 volumes isopropanol). The recovered RNA was resuspended
900 in 100 μ L DEPC-treated RNase-free water and incubated for 10 min at 65°C followed by 5
901 min on ice. nsRNAs were bound to 100 μ L of μ MACS streptavidin microbeads (Miltenyi
902 Biotec) for 90 min at room temperature and purification was performed using the μ MACS
903 streptavidin starting kit (Miltenyi Biotec). Columns were first equilibrated with 1 mL of
904 washing buffer (100 mM Tris-HCl at pH 7.5, 10 mM EDTA, 1M NaCl, 0.1% Tween20).
905 Samples were loaded and the flow-through was reapplied 2 times to the columns. The
906 columns were washed five times with increasing volumes of washing buffer (600, 700, 800,
907 900, and 1000 μ L). Ultimately, labeled RNAs were eluted twice with 200 μ L of 100mM
908 DTT. nsRNAs were precipitated overnight in 1/10 volume of 3 M NaOAc, 3 volumes of
909 100% ethanol and 20 μ g of RNA-grade glycogen. The RNAs were recovered by
910 centrifugation and RNA pellets were wash with ice-cold 70% ethanol and resuspended in 20
911 μ L of DEPC-treated RNase-free water (Sigma-Aldrich). Samples were stored at -80°C until
912 further use. Differential gene expression analysis was performed using DESeq2 1.16.1
913 Bioconductor R package on *Saccharomyces cerevisiae* counts normalized with size factors
914 computed by the median-of-ratios method proposed by Anders and Huber on
915 *Schizosaccharomyces pombe* counts (Anders and Huber 2010). cDTA analysis was
916 performed as described (Schwalb et al. 2012).

917

918 **Total RNA-seq**

919 Yeast strains were processed in duplicate. ERCC RNA Spike-in control mixes (Ambion,
920 catalogue #4456740) were added to 1µg of total RNA according to manufacturer. Ribosomal
921 RNAs were depleted from total RNA using the RiboMinus™ Eukaryote v2 Kit (Life
922 Technologies). Depletion efficiency and quality control of rRNA-depleted RNA was assessed
923 by analysis in RNA Pico 6000 chip for 2100 Bioanalyzer (Agilent). Total RNA-seq libraries
924 were prepared from 50ng of rRNA-depleted RNA using the TruSeq® Stranded Total RNA
925 Sample Preparation Kit (Illumina). Paired-end sequencing (2x50 nt) was performed on a
926 HiSeq 2500 sequencer giving between 65 and 84 million reads. Reads were mapped using
927 version 2.0.6 of TopHat, with a tolerance of 3 mismatches and a maximum size for introns
928 2Kb. Tags densities were normalized on spike-in controls for all subsequent analyses.

929 **ChIP-exo**

930 The ChIP-exo assay has been previously described (Rhee HS, Pugh BF. ChIP-exo method for
931 identifying genomic location of DNA-binding proteins with near-single-nucleotide accuracy.
932 Curr Protoc Mol Biol. 2012;Chapter 21:Unit 21). In brief, yeast strains were grown to
933 exponential phase in yeast extract peptone (YP) + 2% dextrose (30°C to OD600 nm = 0.8),
934 then subjected to 1% formaldehyde crosslinking for 15 min at 25°C. After quenched with
935 125mM final concentration of glycine for 5 min, cells were harvested and washed. Sonicated
936 chromatin was prepared by standard methods. Standard ChIP methods were used for histone
937 H3 (antibody Ab1791, Abcam) followed by lambda exonuclease treatment and library
938 construction. Libraries were sequenced on an Illumina 2000 sequencer. Reads were uniquely
939 mapped to the reference genome (sacCer3 build) using version 2.1.0 of Bowtie2 with a
940 tolerance of 1 mismatch in seed alignment. Data were normalized on the total number of
941 uniquely mapped reads, for each sample.

942 **Cell proliferation analysis in liquid media**

943 Liquid growth rate analysis was conducted using the CG-12 robot (S&P Robotics Inc.). Yeast
944 cells transformed with a URA3-containing plasmid (pRS416) and grown exponentially in
945 SC-ura at 30°C were diluted to OD₆₂₀ 0.1 in either SC-ura or SC-ura + 50µg/ml 6-AU and
946 four 150µl samples from each strain were plated onto 96-well transparent plates covered with
947 breathable films. Plates were incubated at 30°C for several days and OD₆₂₀ was measured in
948 the CG-12 robot (S&P Robotics Inc.) every hour with a shaking of 1000rpm for 1 minute
949 before each read. Liquid growth rate analysis was not possible in the *nrđ1-5* and associated
950 strains due to flocculation. All cell proliferation experiments were repeated at least twice.

951 **Growth rates analysis on agar plates**

952 Growth of yeast strains was compared by plating 5-fold serial dilutions on SC / SC-inositol,
953 SC-ura / SC-ura + 50µg/ml 6-azauracil (6-AU, Sigma), SC-leu / SC-leu + 0.4mM CuSO₄
954 (Sigma) or SC-leu / SC-leu + 0.25 CuSO₄ and incubating the plates at 30°C for several days.
955 All plating experiments were performed at least three times.

956 **RT-qPCR**

957 Total RNA was extracted either with RNeasy Mini Kit (Qiagen) or by classical acid phenol-
958 chloroform extraction. RNAs abundance was measured with SuperScript® III Platinum®
959 SYBR® Green One-Step qRT-PCR Kit (Invitrogen) using specific primers for the targets of
960 interest in a 10µl reaction volume, on Applied Biosystem Step-One Plus machines. RNAs
961 abundance of each sample was compared with standard curves analysis. Abundance of *CUP1*
962 from transcriptional read-through analyses using the *ACT-CUP* reporter system was
963 compared to *ACT** RNA specifically transcribed from the same plasmid (Extended Data
964 Fig.5a). Abundance of *NRĐ1* promoter-proximal RNA (*NRĐ1* 5') was compared to levels of
965 *NRĐ1* promoter-distal RNA (*NRĐ1* 3').

966 **Co-immunoprecipitation**

967 Benzonase-treated yeast cell extracts were incubated with 20 μ l Anti-FLAG Affinity Gel
968 (Sigma Aldrich) in 10mM Tris-Cl pH 7.5, 150mM NaCl, 0.05mM EDTA, 0.5% NP40, 1X
969 Protease Inhibitor Cocktail (Roche) and 0.01mM PMSF for 3h. Beads were washed twice in
970 10mM Tris-Cl pH 7.5, 150mM NaCl, 0.05mM EDTA, 1X Protease Inhibitor Cocktail
971 (Roche) and 0.01mM PMSF and resuspended in SDS sample buffer. Western blot analyses
972 were performed using Monoclonal anti-FLAG M2 antibody (Sigma F3165), Anti-HA tag
973 antibody (Abcam, ab9110) and Anti-Arp5 antibody (Abcam, 12099). Images were acquired
974 using Syngene G:BOX. The experiment was reproduced three times. Immunoprecipitations
975 for H2A.Z and HA-tag were performed similarly on benzonase-treated yeast cell extract
976 using 36 μ l Protein-A sepharose beads coupled with 6 μ l of appropriate antibody (Anti-
977 H2A.Z, Active Motif 39647; Anti-HA tag, Abcam ab9110). Control immunoprecipitations
978 (mock) were performed with a non-specific antibody (Anti-Rabbit IgG, Promega). Western
979 blot analyses were performed using the following antibodies: Anti-HA, Anti-Arp5, Anti-
980 H2A.Z and Anti-Rpb1 y-80 (Santa Cruz Biotechnology, sc-25758). After the
981 immunoprecipitation for H2A.Z shown in Figure S6G, beads were washed twice in 10mM
982 Tris-Cl pH 7.5, 150mM NaCl, 0.05mM EDTA, 1X Protease Inhibitor Cocktail (Roche) and
983 0.01mM PMSF, then washed a third time in the same buffer containing 300mM NaCl and
984 finally resuspended in SDS sample buffer All experiments were reproduced three times.

985 **Ino80 inducible degradation**

986 The assay for inducible degradation of Ino80⁸² was performed as previously described^{35,86}
987 Briefly, a degron tag was fused to INO80 (Ino80-td) in cells carrying the UBR1 gene, that
988 encodes for the N-recognin Ubr1, under the control of the GAL1 promoter, and cells were
989 transformed with the *ACT-CUP* plasmid reporter system (Extended Data Fig. 5a). Cells were
990 grown in SC-leu media containing raffinose as carbon source at 25°C until OD600~0.6-0.7,
991 then shifted to SC-leu media containing galactose to induce *UBR1* expression. Subsequently,

992 complete Ino80-td protein degradation by the Ubr1 pathway was induced by 3 hours of heat
993 shock at 37°C. Cells were then immediately harvested by centrifugation at 4°C, 3500rpm for
994 3 minutes, then washed twice with ice-cold water and snap-frozen in liquid nitrogen for RNA
995 analysis. Yeast cells carrying the GAL1-driven UBR1 gene along with an unmodified INO80
996 genes were use as control. Furthermore, both (GAL1-UBR1) and (GAL1-UBR1 Ino80-td)
997 cells were grown in SC-leu media containing glucose as a negative control for UBR1
998 expression (Extended Data Fig. 5d, grey bars). RNA analysis was conducted by RT-qPCR as
999 described above.

1000 **ChIP-qPCR**

1001 Chromatin immunoprecipitation was performed as previously described in (Papamichos-
1002 Chronakis M, Watanabe S, Rando OJ, Peterson CL. Global regulation of H2A.Z localization
1003 by the INO80 chromatin-remodeling enzyme is essential for genome integrity. Cell.
1004 2011;144(2):200-13.). Sonication was performed on a Bioruptor® Pico sonication device
1005 (Diagenode) with ten 30-sec pulses with a 30-sec break between pulses. Immunoprecipitation
1006 was carried out with Anti-HA tag antibody (Abcam ab9110) and Anti-Rpb1 y-80 antibody
1007 (Santa Cruz Biotechnology, sc-25758) in parallel. The recovered DNA was subjected to
1008 quantitative real-time PCR using Platinum® SYBR® Green qPCR SuperMix-UDG Kit
1009 (Invitrogen) with specific primers for the targets of interest in 10µl reaction volume, on
1010 Applied Biosystem Step-One Plus machines.

1011 **RNA-IP**

1012 RNA immunoprecipitation was performed as described in (Selth LA, Gilbert C, Svejstrup JQ.
1013 RNA immunoprecipitation to determine RNA-protein associations in vivo. Cold Spring Harb
1014 Protoc. 2009;2009(6):pdb prot5234.). Sonication was performed on a Bioruptor® Plus
1015 sonication device (Diagenode) with two 15-sec pulses at 50% amplitude with a 1-min break

1016 between pulses. Immunoprecipitation was carried out with Anti-HA tag antibody (Abcam
1017 ab9110). The recovered RNA was analysed by RT-qPCR as described above.

1018 **De novo analysis of public ChIP-exo and CRAC data**

1019 NAB3 and POLII CRAC data were taken from GEO dataset GSE70191 and processed as
1020 described in ⁵⁶. NAB2 and MTR CRAC data were taken from GEO dataset GSE46742 and
1021 processed as described in ⁴. ChIP-exo H3 data were downloaded from NCBI SRA dataset
1022 SRA059355 and processed as described in ⁸⁷.