The helicase Ded1p controls use of near-cognate translation initiation codons in 5' UTRs

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The conserved and essential DEAD-box RNA helicase Ded1p from yeast and its mammalian orthologue DDX3 are critical for the initiation of translation. Mutations in DDX3 are linked to tumorigenesis2–4 and intellectual disability5, and the enzyme is targeted by a range of viruses6. How Ded1p and its orthologues engage RNAs during the initiation of translation is unknown. Here we show, by integrating transcriptome-wide analyses of translation, RNA structure and Ded1p–RNA binding, that the effects of Ded1p on the initiation of translation are connected to near-cognate initiation codons in 5' untranslated regions. Ded1p associates with the translation pre-initiation complex at the mRNA entry channel and repressing the activity of Ded1p leads to the accumulation of RNA structure in 5' untranslated regions, the initiation of translation from near-cognate start codons immediately upstream of these structures and decreased protein synthesis from the corresponding main open reading frames. The data reveal a program for the regulation of translation that links Ded1p, the activation of near-cognate start codons and mRNA structure. This program has a role in meiosis, in which a marked decrease in the levels of Ded1p is accompanied by the activation of the alternative translation initiation sites that are seen when the activity of Ded1p is repressed. Our observations indicate that Ded1p affects translation initiation by controlling the use of near-cognate initiation codons that are proximal to mRNA structure in 5' untranslated regions.

To systematically analyse how Ded1p influences translation initiation in cells, we first examined how a mutation in the enzyme altered the spectrum of ribosome footprints in cells7. We used the ded1-95 mutation (Ded1pT408I), which reduces the affinity of Ded1p for RNA, diminishes RNA unwinding and confers a temperature-sensitive growth defect to the budding yeast Saccharomyces cerevisiae8. The mutation does not affect pre-mRNA splicing or ribosome biogenesis9. We performed ribosome profiling on wild-type and ded1-95 strains before and after a temperature shift from 30 °C to 37 °C for 5 min (Extended Data Fig. 1a–h). The short time was chosen to minimize any secondary effects on ribosome footprints arising from broader translation defects.

At 30 °C, wild-type and ded1-95 strains showed virtually indistinguishable RNA expression and translation profiles (Extended Data Fig. 1i, j). After the temperature shift, translation broadly decreased in ded1-95, compared to the wild type (Extended Data Fig. 1k–n). These observations indicate that Ded1p promotes translation initiation for most mRNAs, consistent with previous findings10,11. However, translation of a subset of mRNAs coding for proteins involved in gluconeogenesis, cell wall synthesis and transcripts encoding histones were less affected by Ded1p than other mRNAs (Extended Data Fig. 2a, b).

The fraction of ribosomes on 5' untranslated regions (UTRs) markedly increased upon temperature shift in ded1-95, compared to the wild type (Fig. 1a, Extended Data Fig. 3a). The majority of mRNAs showed higher ribosome occupancy of the 5' UTR in the ded1-95 strain, which correlated with lower translation efficiency of the main open reading frame (ORF) (Fig. 1b, Extended Data Fig. 3b). To examine the link...
between increased ribosome occupancy in the 5′ UTR and diminished translation of the main ORF; we performed polyosomal fractionation with northern blot analysis of individual mRNAs. The PSA1 mRNA—the translation efficiency of which is markedly affected by Ded1p—showed a distinct shift from polysomes to monosomes in ded1-95 compared to the wild type, upon temperature shift but not at 30 °C (Fig. 1c, d, Extended Data Fig. 3c). TDH2 mRNA, which is largely unaffected by Ded1p, did not show a comparable shift (Fig. 1c, d). Collectively, these observations suggest that increased ribosome occupancy on 5′ UTRs correlates with binding of the mRNA to only a single ribosome. This notion is consistent with previous reports. Ribosome profiling on only the 80S monosome fraction upon temperature shift also showed more footprints on 5′ UTRs in the ded1-95 strain, compared to the wild type (Extended Data Fig. 3d), indicating that ribosome occupancy on 5′ UTRs broadly correlates with the binding of mRNAs to single ribosomes.

A large number of sites on 5′ UTRs with increased ribosome footprints in the ded1-95 strain were enriched with near-cognate initiation codons (Fig. 1e–g), which differ from the canonical 5′-AUG-3′ initiation codon by a single nucleotide and can create alternative translation initiation sites (ATISs). Increased ribosome occupancy on AUG codons in 5′ UTRs was also seen in the ded1-95 strain (Fig. 1f), but only a few of these sites exist in the yeast transcriptome, compared to nine different near-cognate initiation codons, which constitute roughly 14% of all codons. Ribosomes can translate from the ded1-95-activated ATISs, as demonstrated by ribosome profiling on small ORFs that start at these ATISs and finish at the respective termination codons, by the lack of ribosome accumulation at ATISs when translation was not arrested and by the periodicity of ribosome footprints starting from ATISs (Extended Data Fig. 3e–l). Collectively, the data indicate that defective Ded1p leads to ATIS activation in 5′ UTRs, which decreases polysome formation on the main ORFs and thereby over-all protein production. We conclude that Ded1p function suppresses the use of ATISs.

Although ATIS activation in ded1-95 was extensive, only a subset of all near-cognate initiation codons was used. We detected no preferred length or register of the corresponding small ORFs relative to the main ORFs. However, in the ATISs, near-cognate codons from which translation initiation is most efficient were over-represented, whereas near-cognate codons from which translation initiation is least efficient were underrepresented (Extended Data Fig. 4a). These observations show that ATIS activation is influenced by inherent codon preferences of the pre-initiation complex (PIC), although these preferences do not fully explain the ATIS activation pattern (Extended Data Fig. 4b–e).

To better understand this pattern, we examined whether remodelling of mRNA secondary structure by Ded1p is linked to ATIS activation. As an RNA helicase, Ded1p has been implicated in RNA structure remodelling, but it is not known which mRNA structures Ded1p alters in cells. To delineate the cellular mRNA structures that are remodelled by Ded1p, we used dimethyl sulfate (DMS) probing in vivo and measured changes in mRNA structure in ded1-95 and wild-type strains upon temperature shift (Extended Data Fig. 5a, b). Unwinding of mRNA structure by Ded1p was most pronounced in 5′ UTRs, compared to other mRNA regions (Fig. 2a, Extended Data Fig. 5c). Notably, ded1-95 activated ATISs were generally located 5′ of unwound RNA regions (Fig. 2b, c). Even near-cognate codons for which translation initiation is least efficient were activated, if they were located 5′ of mRNA structure (Fig. 2b). Our observations link the inability of ded1-95 to resolve mRNA structure to ATIS activation, suggesting that Ded1p suppresses ATIS activation by unwinding mRNA structure.

To investigate how Ded1p physically accomplishes this function, we determined which cellular RNAs bind to wild-type Ded1p using a high-throughput cross-linking-based approach (cross-linking-aided RNA affinity precipitation with sequencing (XL-RAP–seq)) and the individual-nucleotide resolution cross-linking and immunoprecipitation (iCLIP) technique to map Ded1p-binding sites on these RNAs (Extended Data Fig. 6a–c). Ded1p cross-linked predominantly to mRNAs and ribosomal RNA (Extended Data Fig. 6b), especially to the 40S ribosomal subunit (Fig. 3a), which is part of the PIC that scans 5′ UTRs. The most frequently cross-linked position maps to helix 16, located at the mRNA entry channel (Fig. 3a). Notable cross-linking was also observed at helix 26, which is located at the mRNA exit site, and in extension segment 6 (around nucleotide 720), which is located in the vicinity of the other cross-link sites on the solvent side of the 40S subunit (Fig. 3a). Ded1p binding to helices 16 and 26 is consistent with reported interactions between Ded1p and eIF3c and the eIF3b–eIF3g–eIF3h sub-complex, that binds near these sites (Extended Data Fig. 7a, b). Human DDX3X also binds to helix 16.

Ded1p further cross-linked to virtually all expressed mRNAs, predominantly in 5′ UTRs (Fig. 3b, c). This cross-linking pattern is consistent with the physical contact of Ded1p to the PIC. Aside from a modest preference for A and U, no sequence motifs could be identified in the mRNA cross-linking sites (Extended Data Fig. 7c). However, peaks of Ded1p cross-linking on 5′ UTRs were frequently proximal to ded1-95-activated ATISs (Fig. 3c, d), and unwound mRNA structure was located 3′ of Ded1p cross-linking sites (Fig. 3c, f).

Collectively, the data link Ded1p binding to mRNA, unwound mRNA structure, ATIS location and binding of Ded1p to the PIC. This link is illustrated by a segment of the PSA1 mRNA as an example (Fig. 4a). Ded1p binding is most pronounced 5′ of unwound RNA...
structure, indicating that Ded1p does not exclusively contact mRNA structure, but also regions that are 5' of the structure. This finding is consistent with the notion that Ded1p functions in the context of the scanning PIC. The scanning process is slowed by RNA structure20,23, and a slowed PIC conceivably permits Ded1p to survey the mRNA for structured regions that it then unwinds. Biochemical data show higher functional affinity of Ded1p for unstructured RNA, compared to structured RNA,6, rationalizing the contacts of Ded1p to unpaired mRNA that is 5' of unwound mRNA structure, as the helicase travels in a 5' to 3' direction with the PIC.

Our data collectively indicate that failure of Ded1p to resolve mRNA structure leads to ATIS activation. To directly probe the link between mRNA unwinding and ATIS activation, we generated a PSA1 mRNA with a mutation in an activated ATIS 5' of unwound RNA structure. (Fig. 4b). The mutation markedly diminished the sensitivity to Ded1p-deficiency seen with the native PSA1 mRNA (Fig. 4b, c). Alterations in the RNA structure 3' of the ATIS also decreased sensitivity to Ded1p (Extended Data Fig. 8a, b). Identical observations were made for mutations in an ATIS and the corresponding RNA structure in the ATPS mRNA (Extended Data Fig. 8c–f). These results show that the effect of Ded1p on translation initiation depends not only on RNA unwinding, but also on proximal ATISs. Without a proximal ATIS, failure of Ded1p to unwind 5' UTR structures does not abrogate scanning of the PIC and subsequent translation of the main ORF (Extended Data Fig. 9). This finding challenges the notion that cellular 5' UTR structures alone are insurmountable hindrances for the scanning PIC.

Together, our results suggest the following function for Ded1p on 5' UTNs (Fig. 4d). The enzyme associates with the PIC in the vicinity of the mRNA entry site of the small ribosomal subunit30. This site is in close proximity to eIF4G and eIF4A (Extended Data Fig. 7b), both of which bind Ded1p with high affinity and might therefore be important for recruitment and function of Ded1p on the PIC. The density of Ded1p cross-linking sites on 5' UTRs increases with distance from the 5' cap (Fig. 3b), suggesting gradual recruitment of Ded1p to the mRNA entry site during the scanning process. This notion is consistent with the reported increase of Ded1p function with greater distance from the 5' cap and with 5' UTR length.11 The mRNA binding pattern of Ded1p further suggests that Ded1p is targeted to its sites of action through association with the scanning PIC. This is an effective way to deploy the enzyme exactly at sites at which it is needed, even though these sites lack common sequence or defined structure signatures. If Ded1p is missing or defective, mRNA structure persists, the PIC stalls and either dissociates from the mRNA, continues slow scanning through the structure, or undergoes subunit joining and translation initiation if a near-cognate codon is present (Fig. 4d). Ribosomes initiating on an ATIS block subsequent scanning ribosomes from reaching the canonical initiation site, thereby decreasing translation efficiency for the main ORF (Fig. 1b). Unless an ATIS marks an N-terminal extension of the main ORF, PICs encountering 5' UTR structures without a proximal ATIS also interfere with scanning, but the kinetic pause introduced by PIC stalling, slowed scanning through the structure or a combination thereof is shorter than on an activated ATIS. Slowed PICs will eventually reach the main ORF (Extended Data Fig. 9), and therefore 5' UTR structure alone affects main ORF translation less in isolation than it does in combination with proximal ATISs. Our model for Ded1p function does not preclude additional roles of the enzyme before the PIC scanning process. However, the Ded1p function outlined above largely accounts for the observed Ded1p interactions with mRNA, and therefore, additional roles of Ded1p are probably restricted to transient Ded1p–mRNA interactions.

Finally, our data reveal a straightforward mechanism for activation of upstream ORFs. The mRNA structures in the 5' UTRs represent a large set of riboswitches that are sensitive to Ded1p. Active Ded1p turns the switches off, suppresses ATIS activation and allows efficient translation of the main ORF. Inactivation of the helicase by post-translational modifications,1 by metabolites such as AMP26, by decreased Ded1p levels or by sequestration of Ded1p in RNP granules25,27 turns the switches on, activating the ATISs and thereby inhibiting translation from the corresponding main ORFs. Certain peptides that are translated from activated ATISs might also have direct biological functions, but the regulation described here appears to be independent of functional peptides.

This mechanism for activation of upstream ORFs is probably used in biological processes. This notion is supported by several lines of evidence. First, there is a marked increase in sequence conservation in the RNA regions around activated ATIS (Extended Data Fig. 10a). This finding challenges the notion that cellular 5' UTR structures alone are insurmountable hindrances for the scanning PIC.

Fig. 3 | Ded1p cross-linking to the 40S ribosomal subunit and to mRNAs. a, Left, fraction of iCLIP reverse transcription stops on 18S rRNA. Moving average of ±2 nt, values represent the average from two independent experiments. Numbers denote predominant cross-linking sites. Right, the position of the three predominant Ded1p cross-linking sites (red) in the crystal structure of the 40S ribosomal subunit30, RNA, grey; ribosomal proteins, cyan; Ded1p cross-link sites, red. ES6, extension segment 6. b, Metagene profile of Ded1p association to mRNAs, calculated from two independent iCLIP experiments (moving average of ±1 nt). Stop, translation stop site; PAS, polyadenylation site. c, Ded1p cross-linking to the 5' UTR of the PSA1 mRNA. Top, fraction of reverse transcription stops per nucleotide, normalized to transcript length. For comparison, differential DMS-MapSeq (middle) and ribosome profiling (bottom) tracks of the 5' UTR of PSA1 mRNA for wild-type DED1 and ded1-95 are shown (5 min, 37 °C). Similar results were obtained in two independent experiments. d, Enrichment of Ded1p cross-linking within 20 nt of ATISs (n = 274) normalized to the background distribution of Ded1p binding (moving average of ±1 nt, reverse transcription stops normalized for each mRNA). The dashed line marks the ATIS position. The shaded area marks a significant difference in Ded1p binding between the regions in the vicinity of an ATIS and in the vicinity of a random position within the same 5' UTR (P = 0.013, two-tailed t-test). e, Enrichment of differential DMS-Mapseq counts (Fig. 2c) within 40 nt of Ded1p binding sites (n = 178, high-stringency ATIS) on 5' UTRs. The shaded area marks a significant difference in RNA structure between the regions downstream of a Ded1p binding site and downstream of a random position within the same 5' UTR (P = 0.008, two-tailed t-test).
The activation of ATISs proximal to 5' UTR structures during meiosis suggests a role for the levels of Ded1p in this process. Collectively, our observations show that the regulatory program linking Ded1p to mRNA structure and ATIS activation is used in a physiological cellular process. The results indicate that intricate translation control and activation of upstream ORFs can be based on simple, ubiquitous elements: a helicase, mRNA structure and near-cognate initiation codons.

Online content

Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0258-0.

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Yeast strains, plasmids and oligonucleotides. Yeast strains used in this study are listed in Supplementary Table 1. Strains were grown at 30 °C unless stated otherwise. Primers, northern blot probes and other DNA oligonucleotides are listed in Supplementary Table 2. RNA oligonucleotides were prepared as described.31. DNA plasmids are listed in Supplementary Table 4.

Generation of a yeast strain expressing Ded1p–HTBHI. Construction of plasmid pEJ21 containing the N-terminally haemagglutinin (HA)-tagged Ded1p has previously been described,32 pEJ21 was then used to generate the plasmid pEJ5. The HA tag was replaced by a sequence containing a HpaI and a SpHl site (amplification with primers X1 and X2), generating pEJ1. The HpaI–TEV–Biotin–His6 (HTBHI) tag was amplified from pFA6-HTBHI-kamMX6 plasmid (gift from P. Kaiser) with primers X3 and X4. The resulting PCR product was cloned into pEJ1 via its HpaI and SpHl sites yielding pEJ2. A second HpaI tag was introduced by site-directed mutagenesis with primers X5 and X6 generating pEJ3. The C-terminal His6–TEV–Biotin–His6 (HTBHI) tag was introduced into pEJ4 by amplification of pEJ3 with primers X8 and X9 and subcloning with PlMlI and SpHl into pEJ4, yielding pEJ5. pEJ5 was linearized and used to transform BY4741 by standard lithium acetate transformation yielding yeast strain yEBP71.

Generation of a yeast strain expressing Ded1p–His6–FLAG2. Yeast strain yDPB740, containing a C-terminal His6–FLAG2 tag on the endogenous DED1 allele, was generated from BY4742 using standard methods. In brief, a homologous recombination template was designed comprising the 40 nucleotides upstream and downstream of the DED1 stop codon flanking the His6–FLAG2 tag (with stop codon) and kanMX6 drug resistance cassette. This template was generated by amplifying from pFA6-6–His3–FLAG–kanMX6 plasmid with primers DW1 and DW2. PCR product was used to transform BY4742 by standard lithium acetate transformation yielding yeast strain yDPB740.

Generation of a yeast strain expressing wild-type, ΔATIS and secondary structure mutants of PSA1 and ATP5 mRNAs. FLAG-tagged PSA1 and ATP5 strains were generated from the respective cDNAs using standard methods as described above (pEJ14, pEJ15 and pEJ18, pEJ19, respectively). The FLAG-tag was appended at the 3′ terminus of the ORF, respectively. For the calculation of log2 fold change (FC) of an mRNA or a gene expression, the following equation was used:

\[ \log_{2} \left( \frac{\text{expression in mutant}}{\text{expression in control}} \right) = \log_{2} \left( \frac{\text{expression in mutant}}{\text{expression in control}} \right) \]

where expression in mutant is the expression of the mutant strain, and expression in control is the expression of the wild-type strain. The log2 FC indicates the fold change in expression between the mutant and control strains. The log2 FC is calculated using the AND software (www.yeastgenome.org). All other parameters were kept at default settings.34. The abundance of mRNAs in ribosome or monosome-protected fragments as well as in the fragmented RNA control libraries were determined using Cufflinks software.

Sizing, concentration and quality of each DNA library was assessed with the High Sensitivity DNA kit on an Agilent2100 Bioanalyzer system. Up to eight DNA libraries were pooled before performing 50 bp single end read sequencing on an Illumina HiSeq2500 V2 in rapid run mode.

Processing of the ribosome profiling data was performed as described.35. In brief, adaptor sequences and ribosomal reads were removed. Remaining reads were mapped to the sacCer3 genome with the TopHat software (parameters set as: --no-novel-juncs -N 2 –read-edit-dist 2 –max-insertion-length 3 –max-deletion-length 3 -g 2 https://www.yeastgenome.org). All other parameters were kept at default settings.36. The abundance of mRNAs in ribosome or monosome-protected fragments as well as in the fragmented RNA control libraries were determined using Cufflinks software.37. These values were used to calculate transcriptional efficiency as described.38. For the calculation of log2ATE values we also included a constant factor reflecting the change in the overall size of the mRNA pool, derived from the spacer (5′ UTRs) CCA (Saccharomyces cerevisiae C). All other parameters were kept at default settings.29. The abundance of mRNAs in ribosome or monosome-protected fragments as well as in the fragmented RNA control libraries were determined using Cufflinks software.33. These values were used to calculate transcriptional efficiency as described.38. The shift in the CRD (ΔCRD) was calculated as described.41. The shift in the CRD was defined relative to the entire length of the mRNA according to:

\[ \Delta \text{CRD} = ((\text{CRD}_{\text{ded1-95}} – 95) – (\text{CRD}_{\text{wild-type DEDI}})) / \text{mRNA length} \]

A negative ΔCRD value marks increased ribosome accumulation in the 5′ UTR in ded1-95.

ATIS were identified according to a previous described algorithm.22. In brief, a position is considered an ATIS, (i) if minimal ribosome count value (>1 nt of the nucleotide under consideration) is greater than nine (high-stringency ATIS) or four (medium-stringency ATIS) in all replicates; (ii) if the ratio of ribosome occupancy between two neighbouring nucleotides 5′ to 3′ (position “n”/“n+1”position) is greater than or equal to three (high-stringency ATIS) in all replicates, or greater or equal of three in one and greater than or equal to 1.75 in the other replicates (medium-stringency ATIS); and (iii) if the normalized ribosome count in ded1-95 cells 5′ after temperature shift to 37 °C is 1.5-fold higher that in wild-type DEDI in all replicates. This algorithm identified high-stringency ATIS and 2,126 medium-stringency ATIS. Near-cognate codons were identified in 239 high-stringency ATIS (65%) and 1,382 medium-stringency ATIS (65%) within a moving window of ±1 nt. Canonical AUG initiation codons were found in 4% high-stringency ATIS, 16% medium-stringency ATIS and 9% low-stringency ATIS. Near-cognate codons on 5′ UTRs of mRNA genes with a 5′ UTR length between 20 and 500 nt. After removal of near-cognate codons in medium-stringency ATIS, ATIS identified 60,666 near-cognate codons.

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XL-RAP–seq. Yeast cells containing HTBHM-tagged DED1 were grown in rich medium to an OD600 of 1.0–1.5, collected by brief centrifugation at 4000g, re-suspended in ice-cold water or remaining YPD medium, transferred to a Petri dish, and subjected to UV-light in a Stratalinker (600 cm2 × 254 nm) on ice. Cells were washed in ice-cold water, sedimented by centrifugation for 5 min at 9000 × g, frozen on dry ice and stored at −80°C.

Frozen cells were lysed in QIA-1M buffer (100 mM NaH2PO4 pH 8, 10 mM Tris, 1 mM Urea, 8 μM imidazole, 0.5% (v/v) IGEPA, 2.5 mM β-mercaptoethanol, 1 mM PMSF protease inhibitor cocktail) with glass beads six times for 30 s in a Beadbeater system (Biospec products). Glass beads were removed, and lysates were centrifuged at 5250 × g for 30 min. Cleared lysates were incubated with Ni2+-agarose (40 μl slurry per g dry pellet weight, pre-equilibrated in buffer QIA-1M; Qiagen) overnight at 4°C. Ni2+-beads were washed in 25 ml of wash buffer 1 (0.3 M NaCl, 10 mM Tris, 100 mM NaH2PO4, 8 μM Urea, 10 mM imidazole) and sample was eluted with 10 μl elution buffer 1 (0.3 M NaCl, 100 mM Tris, 50 mM NaH2PO4, 8 μM Urea, 500 mM imidazole, 10% (v/v) glycerol). Eluates were then incubated with 12.5 μl equilibrated streptavidin-conjugated agarose resin (Pierce Technologies) per g pellet dry weight overnight at 4°C. Streptavidin beads were washed with 12.5 ml wash buffer 2 (0.3 M NaCl, 100 mM Tris, 8 μM Urea, 0.5 mM EDTA, 1 mM DTT) and twice with 1.5 ml 1× 1.5 ml TEV-salt buffer (50 mM Tris pH 7.5, 300 mM NaOAc, 1 mM EDTA, 0.25% (v/v) SDS) overnight at 4°C with subsequent ethanol precipitation.

The RNA was suspended in 15 μl RNase-free water and circu-larized with CircLigase I (Epipentre) according to the manufacturer’s instructions. The circularized cDNA was used for amplification with Phusion polymerase (NEB) and primers X98 and X99 (Supplementary Information 2). PCR settings were: 30 s at 98°C and then 24 PCR cycles (10 s at 98°C, 30 s at 58°C, 30 s at 72°C). PCR products were applied to 10% non-denaturing PAGE and visualized by SYBR Gold. Products with 75–90bp were extracted from gel slices, and ethanol precipitated as described above. PCR products were amplified with Phusion polymerase and primers X100 and X101 for five cycles using the same PCR settings as above. PCR products were purified and subjected to UV-light in a Stratalinker (245 mm × 254 mm square Petri dish; Sigma) at room temperature. Cells were collected by centrifugation for 5 min at 2000g, washed twice in ice-cold PBS, frozen in liquid nitrogen, and stored at −80°C.

Frozen cells were lysed in CLIP lysis buffer (50 mM Tris-HCl pH 7.8, 300 mM NaCl, 1% Triton X-100, 1 mM PMSE, protease inhibitor cocktail (Roche)) with glass beads six times for 1 min in a Disruptor Genie System (Scientific Industries). Lysates were centrifuged after removal of the glass beads at 10,000g, twice for 5 min. Cleared lysates (~26.5 A260 units) were incubated with anti-FLAG M2 Magnetic Beads (20 μl slurry pre-equilibrated in CLIP Lysis Buffer; Sigma) in a total volume of 1 ml at 4°C overnight at 4°C. Beads were washed twice in 1 ml FLAG Wash Buffer (50 mM Tris-HCl pH 7.8, 1 mM NaCl, 0.1% NP-40) and twice in 1 ml FLAG Elution Buffer (50 mM Tris-HCl pH 7.8, 150 mM NaCl, 0.1% NP-40). Proteins were eluted twice in 95 μl FLAG Elution Buffer containing 150 μg ml−1 3–FLAG tag peptide (Sigma). Eluates were further analyzed with 10 μl of 1× 1.5 ml RNase I (Ambion), diluted 1:5000 in FLAG elution buffer for 15 min at room temperature. Reactions were quenched with 960 μl 8 M guanidine–HCl, 90 μl Dilution Buffer (600 mM Tris-HCl pH 7.8, 3.93 M NaCl), 6.4 μl 2 M imidazole, 10.8 μl 10% NP-40 and 12.8 μl 500 mM β-mercaptoethanol. RNase-treated eluates were incubated further with Ni-NTA magnetic agarose beads (50 μl slurry pre-equilibrated in Ni-NTA binding buffer (50Mm Tris-HCl pH 7.8, 300 mM NaCl, 10 mM imidazole, 6 M guanidine-HCl, 0.1% NP-40, 5 mM β-mercaptoethanol; Qiagen) overnight at 4°C. Ni2+-beads were washed twice in 1 ml CLIP wash buffer I (50 mM Tris-HCl pH 7.8, 500 mM NaCl, 10 mM imidazole, 6 M guanidine-HCl, 0.1% NP-40, 5 mM β-mercaptoethanol) and three times in 1 ml CLIP wash buffer II (50 mM Tris-HCl pH 7.8, 10 mM MgCl2, 0.5% NP-40, 10 mM β-mercaptoethanol). Beads were then incubated with 30 μl dephosphorylation mix (50 mM Tris-HCl pH 7.8, 10 mM MgCl2, 10 mM β-mercaptoethanol, 3 M BU TSAP (Promega), 30 U Superscript-Asp-In (Ambion)) for 30 min at 37°C in a thermomixer at 1000 rpm. Reactions were terminated by adding 1 ml CLIP wash buffer I, and beads were washed three times in 1 ml 1× PNK buffer, re-suspended in 30 μl ligation mix (50 mM Tris-HCl pH 7.8, 10 mM MgCl2, 10 mM β-mercaptoethanol, 10% PEG8000 (NEB), 10% DMSO, 2 μl 3′ adenylated adaptor X103, 30 U T4 RNA Ligase 1 (NEB), 30 U SUPERSplice-In (Ambion)) and incubated for 5 h at 22°C in a thermomixer at 1000 rpm. Ligation reactions were monitored by adding 1 ml CLIP wash buffer II and beads were washed three times in 1 ml 1× PNK buffer, re-suspended in 30 μl ligation mix (50 mM Tris-HCl pH 7.8, 10 mM MgCl2, 10 mM β-mercaptoethanol, 10% PEG8000 (NEB), 10% DMSO, 2 μl 3′ adenylated adaptor X103, 30 U T4 RNA Ligase 1 (NEB), 30 U SUPERSplice-In (Ambion)) and incubated for 30 min at 37°C in a thermomixer (1000 rpm). Reactions were terminated by adding 1 ml CLIP Wash Buffer I. Beads were washed three times in 1 ml CLIP Wash Buffer I, and three times in 1 ml CLIP wash buffer II. Beads were suspended in 30 μl Tris-HCl pH 7.8, 50 mM NaCl, 10 mM imidazole, 0.1% NP-40, 5 mM β-mercaptoethanol). Beads were washed twice in 1 ml CLIP Wash Buffer I, and three times in 1 ml CLIP Wash Buffer II. Beads were suspended in 30 μl Tris-HCl pH 7.8, 50 mM NaCl, 10 mM imidazole, 0.1% NP-40, 5 mM β-mercaptoethanol). Beads were washed twice in 1 ml CLIP Wash Buffer I, and three times in 1 ml CLIP Wash Buffer II. Beads were suspended in 30 μl Tris-HCl pH 7.8, 50 mM NaCl, 10 mM imidazole, 0.1% NP-40, 5 mM β-mercaptoethanol). Beads were washed twice in 1 ml CLIP Wash Buffer I, and three times in 1 ml CLIP Wash Buffer II.
Sequences were prepared as previously described. In brief, after the trimming of adaptor sequences, reads were mapped to sacCer3 with Bowtie2 or TopHat software with similar settings used for the ribosome profiling data, outlined above. Identical reads were subsequently collapsed and duplications removed.

The cross-link position of Ded1p to RNA was defined as 1 nt of the 5′ mapped nucleotide of a sequencing read.

**DMS-MaPseq.** Yeast strains (wild-type Ded1 and ded1-95) were grown in YPD at 30°C. Overnight cultures were diluted to OD600nm = 0.09 and grown to an OD600nm = 0.6. An equal volume of 44°CYPD medium was added to achieve an immediate temperature shift to 37°C, as outlined for the ribosome profiling experiments. Cultures were incubated in a 37°C water bath for 3 min. At this time, DMS (Sigma) was added to a 5% (v/v) final concentration and incubation was continued with stirring for 3 min. DMS was quenched by adding 30 ml of ice-cold stop solution (30% 3-mercaptoethanol, 50% (v/v) isomyl alcohol). Cells were quickly transferred to ice, collected by centrifugation at 3,500 x g at 4°C for 4 min, and washed with 10 ml 30% 3-mercaptoethanol solution. Cells were re-suspended in 0.6 ml RNA lysis buffer (6 mM EDTA, 45 mM NaOAc, pH 5.5). Total RNA was purified with hot acid phenol (Ambion) and ethanol precipitation. Sequencing libraries were prepared as previously described.

Fastq files were stripped of linker sequences and filtered for overall quality using the FASTX-Toolkit Clipper and Quality Filter functions (http://hannonlab.cshl.edu/fastx_toolkit/), respectively, requiring that 80% of sequenced bases have a quality score >25. Reads were aligned against the yeast genome (sacCer3) using Tophat v2.1.0 with Bowtie2 with the following settings for a 50 bp sequencing run: --no-novel-juncs -N 5 --read-gap-length 7 --read-edit-dist 7 --max-insertion-length 5 --max-deletion-length 5 -g 3. All non-uniquely aligned reads were then removed. Owing to empirically determined mutation enrichment from non-template regions, the 5′ end of each read was trimmed from the 3′ end of each read. Mismatches located within 3 nt of an indel were also discarded for future analysis.

**Bioinformatic analyses.** Yeast genomic sequence conservation scores were obtained from S. cerevisiae genome database (https://www.yeastgenome.org). Positional coordinates of mRNAs including transcription start sites and polyadenylation sites are based on sacCer3 and reported measurement. Genome-wide datasets were visualized by IGV software. Structural models of the small and large ribosomal subunits including initiation factors were generated with the Chimera software. Analyses of Gene Ontology term enrichment were carried out with GOrilla software using a single ranked list of genes.

RNA structure prediction was carried out with sequences 0–99 nt of the first nucleotide of an alternative start codon using the RNAfold web server. Constraint settings were derived from DMS-MaPseq data as follows: a nucleotide was set as ‘unpaired’ if the DMS-MaPseq counts of a given nucleotide exceeded the value of 0.49 relative to the third highest count number in the range of 100 nt downstream of an ATIS.

Statistical significance of enrichment or depletion in certain regions (for example, Figs. 2c, 3d, e, Extended Data Fig. 10a) was determined by comparing weighted data vectors of the observed variable to the background value. To this end, we calculated t-values with the wtd.t.test function in R. The algorithm is based on the mean and 1/(standard errors) as an estimate of the means accuracy. The given P values correspond to a two-tailed t-test.

Further bioinformatic analyses and multiple linear regressions were performed with R with customized scripts using RStudio software (https://www.rstudio.com/). Code is available upon request. Normalization of the datasets including ribosome protected fragments, monosome-protected fragments, Ded1p CILIP-seq and DMS-MaPseq counts were performed relative to the total number of counts of the entire mRNA.

To compute Ded1p binding density, DMS-MaPseq ratios, or sequence conservation values in the vicinity of ATIS, it was important to normalize for inherent positional trends within the exact region in the respective iCLIP, DMS-MaPseq and sequence conservation datasets. For example, values for DMS-MaPseq ratios (counts ded1-95 counts wild type), and iCLIP reads show an upward trend with increasing distance from the 5′ cap in 5′ UTRs. To normalize for inherent positional trends, we calculated a background distribution for the vicinity of each ATIS. We randomly choose a position in the respective section of a given mRNA, and determined the signal distribution in the vicinity of this position (for example, position: −5 relative to the 3′ 5′ of an ATIS). This process was repeated four times. The background value reflects the average of these five calculated values. Reported enrichment values represent the ratio of the measured signal over the background value at each indicated position. Values are given in all plots as log10(measured signal/background signal).

Statistical significance of enrichment or depletion was determined by calculating the t-value of the observed variable on the basis of the mean and s.d. of the background value.

Metagenic profiles were calculated by averaging normalized Ded1p iCLIP counts and DMS-MaPseq counts after binning transcript coordinates from 5′ UTRs, ORFs and 3′ UTRs in bins reflecting 2% of each section of mRNA. Ded1p binding sites and the midpoint of RNA secondary structures were determined by Piranha peak calling software (http://smithlabresearch.org).

Calling parameters were optimized on the basis of visual inspection. To call peak sites of RNA secondary structures, a genome-wide dataset of log(counts of DMS-MaPseq wild type/counts ded1-95) was used as input file.
Extended Data Fig. 1 | mRNA expression, and translation profiles in wild-type DED1 and ded1-95. a, Correlation of ribosome footprint counts between two biological replicates in wild-type DED1 at 30 °C (n = 5,523). b, Correlation of mRNA expression levels between two biological replicates in wild-type DED1 at 30 °C (n = 5,372). c, Correlation of mRNA expression levels in wild-type DED1, 5 min after temperature shift to 37 °C (n = 5,523). d, Correlation of mRNA expression levels between two biological replicates in ded1-95 at 30 °C (n = 5,523). e, Correlation of ribosome footprint counts between two biological replicates in wild-type DED1, 5 min after temperature shift to 37 °C (n = 5,372). f, Correlation of mRNA expression levels in wild-type DED1, 5 min after temperature shift to 37 °C (n = 5,523). g, Correlation of ribosome footprint counts between two biological replicates in ded1-95, 5 min after temperature shift to 37 °C (n = 5,372). h, Correlation of mRNA expression levels in wild-type DED1, 5 min after temperature shift to 37 °C (n = 5,523). i, Correlation of mRNA expression levels between wild-type DED1 and ded1-95 at 30 °C (n = 2,976). Each data point represents the average of at least two replicates. j, Correlation of translational efficiencies between wild-type DED1 and ded1-95 at 30 °C (n = 2,976). Each data point represents the average of at least two replicates. k, Representative polysome profiles of wild-type DED1 and ded1-95 strains at 30 °C and 5 min after shift to 37 °C. Similar results were obtained in three independent experiments. l, Changes in translational efficiencies (∆TE) for mRNAs in ded1-95, compared to wild-type DED1, 5 min after temperature shift (mean of two biological replicates). The dotted line indicates no change. m, Fraction of 18S rRNA in polysome fractions, compared to the entire sample, at 30 °C and 5 min after temperature shift to 37 °C. Each bar represents the average of three independent experiments. Empty circles represent each replicate. n, Cumulative distribution of translational efficiencies of wild-type DED1 and ded1-95, 5 min after temperature shift to 37 °C (n = 2,976). Each data point represents the average of at least two replicates.
Extended Data Fig. 2 | A subset of mRNAs is largely insensitive to Ded1p. a, mRNA groups defined by Gene Ontology term with translation that is strongly affected (green) or largely unaffected by Ded1p (blue). Box plots (group median) of change in translational efficiencies. Box boundaries, upper and lower quartiles; error bars, 1.5 × interquartile range. The black box plot marks changes in translational efficiencies for all mRNAs (Fig. 1b). mRNAs for each Gene Ontology term were extracted from the Saccharomyces Genome Database (https://www.yeastgenome.org). The false discovery rate q value indicates the enrichment P value according to a hypergeometric model after correction for multiple testing using the Benjamini and Hochberg method46. b, Box plots (as in a) of 5′UTR lengths and median of the shift in the normalized centre of ribosome density (Fig. 1b) for Gene Ontology term defined mRNA groups, colour-coded as in a.
Extended Data Fig. 3 | Activation of ATIS in ded1-95 upon temperature shift.

a, Fraction of ribosome footprints on 5′ UTRs in wild-type DED1 and ded1-95, (5 min, 37 °C, n = 3,273). The red line indicates the mean. Statistical significance for the difference between ded1-95 and wild-type DED1: P = 1.2 × 10⁻¹¹⁹ (two tailed t-test). A similar result was obtained in an independent replicate (P = 5.4 × 10⁻¹⁹²).
b, Changes in the fraction of ribosomes on 5′ UTRs for all mRNAs (n = 2,660) in wild-type DED1 compared to ded1-95, 5 min after temperature shift to 37 °C. The values on the x-axis represent the ratio (log₂) of the fraction of ribosomes on each 5′ UTR in the wild type, divided by the fraction of ribosomes on the same 5′ UTR in ded1-95. Each value represents the mean of two independent biological replicates.
c, Representative northern blots of PSA1 after sucrose gradient centrifugation for wild-type DED1 and ded1-95, at 30 °C. A similar result was obtained in an independent biological replicate.
d, Fraction of ribosome footprints on 5′ UTRs in wild-type DED1 and ded1-95, (5 min, 37 °C), measured only in 80S monosomes (n = 973, reads from two independent experiments combined). Statistical significance for the difference between ded1-95 and wild-type DED1: P = 1.2 × 10⁻⁵⁰ (two tailed t-test).
e, Mean ribosome occupancy within 10 nt 3′ and 5′ of the high-confidence ATIS in 5′ UTRs (moving average ± 1 nt, 5 min, 37 °C), measured without cycloheximide.
f, Mean ribosome occupancy within 10 nt 5′ and 3′ of the high-confidence ATIS on 5′ UTRs (n = 274) for ded1-95, 5 min after temperature shift. The dashed line indicates the first nucleotide.
Extended Data Fig. 4 | Characteristics of small open reading frames associated with activated ATISs. 

**a**, Enrichment or depletion of each near-cognate codon in ATISs over the background distribution of the codon. P values determined using a two-tailed t-test. 

**b**, Mean translation initiation site score (positions −6 to +6, excluding +1 to +3), calculated according to previously published methods, for high-stringency ATIS (n = 274, red), and TIS of main ORFs (n = 4,972, grey). A TIS score exceeding 0.01 is considered a potential translational initiation site. 

**c**, Changes in translational efficiencies (ΔTE) for mRNAs in ded1-95, compared to wild-type DED1, 5 min after temperature shift for all mRNAs (Fig. 1b) and ATIS-containing mRNAs. 

**d**, Length of the small open reading frames (smORFs) associated with ded1-95-activated ATIS. smORFs encoding N-terminal extensions were excluded from the analysis. 

**e**, Type of smORFs associated with ded1-95-activated ATIS. The bar graphs show the fraction of smORFs that falls into each category. The distribution of changes in translation efficiency (ΔTE) for RNAs with each type of smORF did not differ significantly.
Extended Data Fig. 5 | mRNA structure unwinding by Ded1p in cells using DMS MaPSeq. a, Schematic for DMS-MaPseq approach to monitor RNA structure unwinding by Ded1p. All DMS-MaPSeq experiments were performed 5 min after temperature shift. b, Representative DMS MaPSeq tracks in the *PSA1* 5′ UTR 5 min after temperature shift for wild-type *DED1* (grey) and *ded1-95* (red). Bars show normalized reverse transcription stops. A similar result was obtained in an independent replicate. The average Pearson's correlation coefficient of DMS-MaPseq counts per 5′ UTR between two replicates (5 min after temperature shift to 37 °C) were $R = 0.57$ ($n = 864$) for the wild type and $R = 0.63$ ($n = 692$) for *ded1-95*. The ribosome occupancy track for *ded1-95* is shown for reference. c, Unwinding of mRNA structure by Ded1p for different mRNA regions. Similar results were obtained in two independent experiments.
Extended Data Fig. 6 | XL-RAP–seq and iCLIP. a, Correlation of sequence reads (fragments per kilobase of exon per million fragments mapped, FPKM) per mRNA for two independent biological XL-RAP–seq replicates (n = 2,992). 
b, Fraction of mRNA (40%) and rRNA (44%) cross-linked to wild-type Ded1p as a fraction of all sequencing reads (mean of two independent experiments). n = 4,280 mRNAs exceed a minimal read count of FPKM ≥ 10. c, Correlation of the number of reverse transcription stops (FPKM) per mRNA for the two independent iCLIP approaches. Replicate 1, FLAG-tagged Ded1p; replicate 2, HTBH-tagged Ded1p; n = 4,007.
Extended Data Fig. 7 | Ded1p binding sites on 18S RNA and mRNAs. 

a, Ded1p binding sites on helix 720 (exit) and helix 16 (entry) (red) are in close proximity to the binding sites of eIF3c (purple) and eIF3b (green) on the 40S ribosomal subunit42 (rRNA, grey; ribosomal proteins, cyan). 
b, Localization of Ded1p (apricot) on helix 16 of the PIC. Schematic model of the yeast PIC with eIF3 (http://www.bangroup.ethz.ch/research/eukaryotic_translation_initiation.html and references therein); the positioning of eIF4G 572-853 and eIF4A is derived from previously published work48. The position of the eIF4G C terminus is hypothetical. The helicase core of Ded1p was modelled in analogy to the DDX3 core structure49, with the RNA binding site in contact with helix 16 at the main iCLIP cross-link sites. The position of the low-complexity N terminus of Ded1p is hypothetical. 
c, Sequence logo of Ded1p binding sites on mRNAs. Sets of 104 binding sites were randomly sampled from all Ded1p cross-linking sites and used as input to create a sequence logo (http://weblogo.berkeley.edu). All subsets yielded essentially the same sequence logo as shown here. Position zero denotes the reverse transcription stop.
Extended Data Fig. 8 | Representative northern blots of PSA1 (Δ2°) and ATP5 (ΔATIS, Δ2°) for wild-type DED1 and ded1-95.

a, Representative RNA blots (5 min, 37 °C) for the PSA1 mRNA with altered secondary structure, 3′ of the ATIS (Δ2°). Similar results were obtained in three independent biological replicates. b, Quantification of RNA blots for accumulation of the PSA1 Δ2° mRNA in monosomes in ded1-95, compared to wild-type DED1. The line indicates the average. The P value for the difference in monosome accumulation was determined using a one-tailed t-test. c, Representative ribosome profiling tracks for the 5′ UTR of ATP5 in wild-type DED1 and ded1-95 (at 30 °C and after temperature shift). The near-cognate initiation codon is highlighted by a star. For comparison, Ded1p cross-linking (iCLIP) and unwinding (DMS-MapSeq) for each nucleotide are indicated. The ratio of normalized DMS-MapSeq counts of wild type/ded1-95 in two categories: yellow triangles, 0.6 – 1.0 (moderately unwound) and red triangles, >1.0 (strongly unwound). d, DMS-MapSeq-constrained secondary-structure model of a fragment of the ATP5 mRNA 5′ UTR. The ATIS is marked by a line. Ded1p cross-linking (iCLIP) and unwinding (DMS-MapSeq) for each nucleotide are indicated. e, Representative RNA blots (5 min, 37 °C) for wild-type ATP5 mRNA and the same mRNA with mutations in the ATIS (ΔATIS) or with altered secondary structure 3′ of the ATIS (Δ2°) for wild-type DED1 and ded1-95. Similar results were obtained in two independent experiments. f, Quantification of RNA blots for accumulation of the ATP5 ΔATIS and Δ2° mRNA in monosomes in ded1-95, compared to wild-type DED1. Lines indicate averages and P values were determined using a one-tailed t-test.
Extended Data Fig. 9 | Ded1p binding and mRNA remodelling can occur without decreased translation efficiency if no near-cognate initiation codon is present. Ded1p iCLIP track, differential DMS-MaPseq track (5 min, 37 °C) and ribosome occupancy tracks (5 min, 37 °C) of wild-type DED1 and ded1-95 for ADH3 mRNA, the translation of which is largely unaffected by Ded1p (ΔTE = −0.1). 5′ UTR and ORF are marked. iCLIP and DMS-MaPseq tracks show Ded1p binding and remodelling of the 5′ UTR, ribosome profiling tracks indicate no significant accumulation of ribosomes in the 5′ UTR. Similar results were obtained in two independent experiments.
Extended Data Fig. 10 | ATIS conservation across fungi and Ded1p-mediated activation of upstream ORFs starting from near-cognate initiation codons. 

**a**, Sequence conservation in fungi around high-confidence ATIS (moving average of ±1 nt). Positive values indicate higher sequence conservation than the average of five randomly chosen positions on the same 5′ UTR for each ATIS, negative values indicate less sequence conservation (conservation scores were obtained from the sacCer3 phastCons7way dataset, on the basis of sequence homology between the following species: *S. cerevisiae*, *S. paradoxus*, *S. mikatae*, *S. kudriavzevii*, *S. bayanus*, *Naumoovozyma castellii* and *Lachancea kluyveri*)

**b**, Ribosome occupancy tracks (30 °C and 5 min, 37 °C) of wild-type *DED1* and ded1-95 for ALA1 mRNA. 5′ UTR and ORF are indicated. The ACG initiation codon (−25, marked) has been previously shown to function as an ATIS for the mitochondrial isoform of Ala1p. Similar results were obtained in two independent biological replicates for each experiment.

**c**, Ribosome occupancy tracks (5 min, 37 °C) of ded1-95 and wild-type *DED1* (vegetative control and anaphase II) for PSA1 mRNA. ATISs are marked by dashed lines. Similar results were obtained in two (vegetative control) and four (anaphase II) independent experiments.

**d**, Ribosome-protected fragments mapping to *DED1* in vegetative cells and cells in anaphase II. Data are the mean of two (vegetative) and four (anaphase II) independent experiments, circles represent each replicate.
# Reporting Summary

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## Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| n/a | Confirmed |
|-----|-----------|
| ☐  | ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐  | ☒ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐  | ☐ The statistical test(s) used AND whether they are one- or two-sided  |
| ☐  | ☐ Only common tests should be described solely by name; describe more complex techniques in the Methods section. |
| ☐  | ☒ A description of all covariates tested |
| ☐  | ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐  | ☒ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted  |
| ☐  | ☒ Give P values as exact values whenever suitable. |
| ☐  | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☐  | ☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐  | ☒ Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated |
| ☐  | ☒ Clearly defined error bars |
| ☐  | ☒ State explicitly what error bars represent (e.g. SD, SE, CI) |

Our web collection on statistics for biologists may be useful.

## Software and code

| Policy information about availability of computer code |
|-------------------------------------------------------|
| Image Quant5.2 (Molecular Dynamics) |

| Data collection | FASTX-Toolkit (NGS read processing), TopHat2.1.0 (read mapping for ribosome profiling), Cufflinks (NGS read counting for ribosome profiling), EXCEL (Data arrangement and manipulation), Custom Code (R, Python; aTIS identification and further data analysis), Bowtie2 (xRAP, NGS read mapping), TopHat 2.1.0, Bowtie2 (NGS read mapping for iCLIP and DMS-MapSeq), IGV genome browser (NGS data visualization), Chimera (Structure visualization), GOrilla (GO-term analysis), RNAsnold web server (RNA secondary structure prediction), Piranha (NGS iCLIP and DMS-MapSeq, peak calling software), Weblogo (NGS iCLIP, sequence logo), Pymol (protein structure visualization), Kaleidagraph (Plots) |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

NGS data are publicly available (GSE93959)

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☑ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Samples sizes were determined by NGS data depth. The number of data points was more than sufficient to arrive at the drawn conclusions in a statistically significant manner (evidenced by p values). For molecular biology experiments, three or more independent replicates were measured, allowing the drawing of statistically significant conclusions (evidenced by p values).

Data exclusions
Data points representing low sequencing read numbers were excluded from analyses (customary for sequencing experiments). Cutoffs, where applicable, are noted for each experiment in Materials and Methods Section.

Replication
All experiments were performed in two or more biological replicates (described for each experiment in Materials and Methods Section).

Randomization
Randomization was not relevant to our study, since we did not deal with human or animal subjects.

Blinding
Randomization was not relevant to our study, since we did not deal with human or animal subjects.

Reporting for specific materials, systems and methods

Materials & experimental systems
n/a
☑ Involved in the study
☐ Unique biological materials
☑ Antibodies
☐ Eukaryotic cell lines
☐ Palaeontology
☐ Animals and other organisms
☐ Human research participants

Methods
n/a
☑ Involved in the study
☐ ChIP-seq
☐ Flow cytometry
☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials
All unique materials are freely available from the authors.

Antibodies

Antibodies used
anti-Ded1p, anti-Hxk1p

Validation
anti-Ded1p is a polyclonal antibody raised against full length recombinant Ded1p. The Ab has been validated extensively against recombinant Ded1p and for specificity (e.g. refs.24,25). anti-Hxk1 antibody is a commercial antibody (U.S. Biological)