A disease-linked IncRNA mutation in RNase MRP inhibits ribosome synthesis

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*RMRP* encodes a non-coding RNA forming the core of the RNase MRP ribonucleoprotein complex. Mutations cause Cartilage Hair Hypoplasia (CHH), characterized by skeletal abnormalities and impaired T cell activation. Yeast RNase MRP cleaves a specific site in the pre-ribosomal RNA (pre-rRNA) during ribosome synthesis. CRISPR-mediated disruption of *RMRP* in human cells lines caused growth arrest, with pre-rRNA accumulation. Here, we analyzed disease-relevant primary cells, showing that mutations in *RMRP* impair mouse T cell activation and delay pre-rRNA processing. Patient-derived human fibroblasts with CHH-linked mutations showed similar pre-rRNA processing delay. Human cells engineered with the most common CHH mutation (70AG in *RMRP*) show specifically impaired pre-rRNA processing, resulting in reduced mature rRNA and a reduced ratio of cytosolic to mitochondrial ribosomes. Moreover, the 70AG mutation caused a reduction in intact RNase MRP complexes. Together, these results indicate that CHH is a ribosomopathy.
MRP mutations are associated with a spectrum of disorders presenting with skeletal dysplasia, abnormal hair, and immune deficiency with impaired T-cell activation. The most common of these syndromes is called Cartilage Hair Hypoplasia (CHH). Patients with CHH have reduced life expectancy due to immune deficiency, but the mechanism underlying this problem is not understood. Clinically, patients often experience recurrent infections, and also have a higher incidence of autoimmunity and cancer. At a cellular level, lymphocytes from CHH patients show reduced proliferation in response to stimulation and increased activation-induced cell death. Naive T cells are small and quiescent, but on activation increase in size over 24 h before beginning to rapidly divide. This activation program is accompanied by a 10-fold increase in per-cell ribosome abundance, achieved through upregulation of both ribosomal protein (RP) and ribosomal RNA (rRNA) production.

Human rRNAs are transcribed by RNA polymerase I (RNAI) as a long precursor, 47 S pre-rRNA (Supplementary Fig. 1). This polycistronic transcript includes the 18 S rRNA, destined for the small ribosomal subunit (SSU), and the 5.8 S and 28 S rRNA components of the large subunit (LSU). The rRNA sequences are flanked by 5′ and 3′ external transcribed spacers (ETS and 3′ ETS) and separated by internal transcribed spacers (ITS1 and ITS2). A third LSU rRNA, 5 S, is transcribed separately by RNA polymerase III. During ribosome synthesis, the 47 S primary transcript undergoes a complex sequence of endonuclease cleavages and exonuclease digestion steps that remove the spacer regions. These process the 47 S RNA through a series of discrete pre-rRNA processing intermediates (Supplementary Fig. 1), to generate the mature rRNAs (Fig. 1A). Notably, human pre-rRNAs cannot be assigned to a single linear order, and there are at least two major alternative pathways. The existence of partially redundant pathways may enhance the overall efficiency and resilience of the system.

During transcription and processing, around 80 ribosomal proteins will assemble with the pre-rRNA, assisted by more than 200 protein assembly factors and over 100 small non-coding RNAs termed small nucleolar RNAs (snoRNAs) (reviewed in ref. 9). Most snoRNAs function as guides for pre-rRNA nucleotide modification (methylation, acetylation, or pseudouridine formation) or as chaperones for pre-rRNA folding (reviewed in10). All known snoRNAs fall into two large families (termed box C/D and box H/ACA snoRNAs), with the exception of RNase MRP.

The human MRP gene encodes a noncoding RNA, which associates with around 10 proteins in the RNase MRP complex. All of these proteins are shared with the RNase P complex, which processes pre-rRNAs and includes the evolutionarily related ncRNA RPPH19. RNase MRP was initially proposed to function in cleavage of an RNA primer during mouse mitochondrial DNA replication, giving its name: mitochondrial RNA processing. However, in budding yeast, RNase MRP is required for cleavage at a specific site, designated A3, in the ITS1 region of the pre-rRNA. A3 cleavage provides an entry site for the 5′ end of the major form of 5.8 S rRNA11,14. Loss of MRP activity does not fully inhibit pre-rRNA processing due to the presence of a poorly understood alternative processing pathway. Mature 5.8 S rRNA is present in two different forms in all studied animals, fungi and plants. In S. cerevisiae about 80% of 5.8 S is the short form (5.8S), generated by the MRP-dependent pathway cleavage. The MRP-independent pathway generates 5.8S, which is 5′ extended into ITS1 by 7 or 8 nucleotides. In consequence, loss of MRP activity in yeast results in a characteristic reduction in 5.8S relative to 5.8S.

Previous analyses of fibroblasts from CHH patients showed increased abundance of species with a 5′ extension into ITS1. By analogy with observations in yeast, this was predicted to reflect 5′ extension the 5.8 S rRNA. CRISPR-mediated disruption of MRP in human HEK293 cells caused pre-rRNA accumulation consistent with defective ITS1 processing at "site 2" analogous to site A3 in yeast. However, another analysis failed to observe an ITS1 processing defect at this site following MRP deletion. Several different human diseases, collectively termed ribosomopathies, are caused by mutations or haploinsufficiency in the RNAPI transcription machinery, pre-ribosome maturation factors or ribosomal proteins (reviewed in refs. 23–25). Despite their function in all cells, ribosomopathies are generally associated with quite cell-type specific diseases, most commonly hemopoietic defects (reviewed in refs. 26,27). All known ribosomopathies cause only mild deficits in the accumulation of cytoplasmic ribosomes, presumably because severe defects would be lethal in early development. In consequence, tissue specificity may be caused by the combination of a generally mild deficit in translation capacity, combined with tissue-specific exacerbating factors (perhaps high demand for protein synthesis combined with reduced activity in compensating pathways).

In this study we show that mutations in MRP impair mouse T-cell activation and delay pre-rRNA processing, a phenotype recapitulated in patient-derived human fibroblasts. In cells engineered with the most common CHH mutation, we also observed changes in RNase MRP structure and reduction in the production of cytoplasmic ribosomes, establishing the defect as a ribosomopathy.

**Results**

Disruption of MRP impairs T cell proliferation and rRNA processing. Activating T cells require high rates of ribosome synthesis to support rapid cell division. We hypothesized that this might confer particular vulnerability to partial disruption of MRP function if its primary biological role is in pre-rRNA processing. To test this, we used T cells from OT-1 Rag-/- mice, all of which recognize the same ovalbumin-derived antigen. This system allows T cells to be activated in a relatively physiological manner, by adding Ova peptide to the culture medium. Naive primary transgenic T cells were isolated, stained with a division tracker dye (CellTrace Violet), and activated by culturing with ovalbumin peptide (Fig. 1a). After 24 h, we disrupted MRP by electroporating these cells with CRISPR components, including one of four guides targeting MRP, or a no-guide control (mock; Supplementary Fig. 2a). Cells were then left to proliferate for a further 48 h.

CRISPR RNAs guide associated CAS proteins to generate site-specific double-strand breaks in the DNA. In the absence of a repair template, these can be repaired by an error-prone nonhomologous end-joining process that generates a high frequency of nucleotide insertion and deletions (indels) at the repaired break site. Guide efficiency was therefore estimated by calculating the proportion of alleles in the population containing an indel, using the Synthego ICE tool to deconvolute Sanger sequencing traces obtained from bulk populations. This showed that guides 1 and 2 disrupted about 60% of alleles, whereas guides 3 and 4 were less efficient, mutating approximately 20% and 10%, respectively (Fig. 1b). No mutations were detected in the control. Proliferation after 48 h was assessed by flow cytometry using a division tracker dye, CellTrace Violet, which halves in fluorescent intensity each division (Fig. 1c,d). This showed that disruption of MRP markedly impaired T cell proliferation. Guide 1 again had the most profound effect, reducing the expansion of the culture to about 60% of that seen in mock-transfected cells. Guide 3 reduced expansion to around 70% of mock-treated cultures, while guides 2 and 4 showed a modest effect (about 80%). Guide efficacy in this...
**Fig. 1** Disruption of *RMRP* impairs T cell proliferation and rRNA processing. a Outline of experiment. Naïve TCR transgenic (OT-1) T cells were obtained from mouse peripheral lymph nodes and activated with N4 peptide. After 24 h, cells were electroporated with a CRISPR mix targeting Rmrp, or a mix without the guide (mock-transfected controls). After 48 h, cells were analyzed by flow cytometry for proliferation, northern blotting for pre-rRNA species, and ICE analysis to determine CRISPR guide efficiency. b CRISPR guide efficiency as determined by the Synthego Inference of CRISPR Edits (ICE) tool. A 669 bp region including *Rmrp* was amplified from DNA extracted from CRISPR-targeted cultures and sequenced with forward (Fw) and reverse (Rv) primers. The ICE tool deconvolutes Sanger sequencing traces to estimate the proportion of alleles in the population containing an indel. Graph summarizes data from three independent experiments. c T cell proliferation 48 h after CRISPR targeting of Rmrp, as measured with division tracker dye CellTrace Violet. Gated on live cells, as shown in Supplementary Fig. 2B. d Expansion of T cell cultures, calculated from data illustrated in panel c. Includes data from three independent experiments. In each replicate, expansion of CRISPR-targeted cultures was normalized to the average of mock-targeted controls in that experiment. e Pre-rRNA species detected by a probe in ITS1, in RNA from T cells 48 h after disruption of Rmrp. Cartoon on right shows species represented by each band. Pre-rRNA species indicated in green are present in both Pathway 1 and Pathway 2 (Supplementary Fig. 1). 41 S in brown is a Pathway 1 intermediate, and those in blue are Pathway 2 species. f Quantification of pre-rRNA species shown in panel e. X indicates a noncanonical precursor; analogous bands were seen in human cells with non-specifically disrupted *RMRP*21. Intensity values for each band were first normalized to the co-running 47S/45S band in that lane. For each rRNA species, the average intensity signal from the mock controls in that experiment was then subtracted. The resulting value was Log2 transformed. Panels b, d, and f show mean values from three independent experiments, with SD. Source data are provided as a Source Data file.
assay is predicted to depend on both guide cutting rates and the tolerance to small mutations of the target region in the RNA. These analyses were restricted to viable cells, as assessed by a dye for live/dead cells (Zombie Red), implying that non-lethal mutations in RMRP slow T cell division.

We next assessed whether these growth defects are associated with impaired rRNA processing. RNA was extracted from cultures 48 h after transfection and analyzed by northern hybridization, which allows each pre-rRNA species to be visualized and quantified (Fig. 1e, left). Pre-rRNA probe 170 hybridizes in ITS1 5′ to the site 2, thus detecting pre-rRNAs that have not undergone cleavage at site 2, potentially by Rnase MRP (Fig. 1e, right cartoon, and Supplementary Fig. 1)28,29. For quantitation, pre-rRNAs levels were analyzed by the Ratio Analysis of Multiple Precursor (RAMP) method28, in which the abundance of each pre-rRNA species is compared to the abundance of the 47 S primary transcript plus the initial processing product 45 S pre-rRNA. These are not resolved and are jointly designated 47 S (Fig. 1f). All cultures treated with RMRP guides showed elevated levels of 41 S pre-rRNA, the intermediate immediately downstream of 47 S, whereas later intermediates were depleted. These results indicate that removal of the 5′ external transcribed spacer (5′ ETS) was unaffected, but cleavage in ITS1 to separate the LSU and SSU precursors is delayed (Supplementary Fig. 1).

The pre-rRNA processing defect was most prominent after targeting with guide 1, the guide that had shown the greatest effect on proliferation and produced the largest proportion of mutated loci. However, the effect of other guides did not correlate well with their effect on proliferation. Guides 2 and 4 had similar effects on proliferation, but guide 4 caused more 41 S accumulation than guide 2.

Overall, these results support the model that disruption of RMRP impairs T cell proliferation by slowing pre-rRNA processing. However, disruption of different sites in the RMRP gene had different relative effects on cell division and pre-rRNA accumulation. We speculate that some guides preferentially cause lethal mutations in a smaller fraction of cells, while others induce milder defects in a larger population.

Mutations associated with Cartilage Hair Hypoplasia impair pre-rRNA processing. Fibroblasts from CHH patients have reduced growth rates and cell cycle defects31. We tested whether these problems are associated with impaired pre-rRNA processing, by measuring pre-rRNA species in patient-derived, CHH fibroblasts (Fig. 2a). Cells from healthy volunteers were used as controls. Being slow growing, these cells contained substantially less pre-rRNA than activated T cells, so obtaining high-quality RNA quantitation was challenging. Unlike RMRP-disrupted mouse T cells, the 41 S pre-rRNA in patient fibroblasts was not clearly increased relative to 47 S (Fig. 2b). 41 S pre-rRNA is generated in processing Pathway 1, by initial removal of both the 5′ ETS and 3′ ETS from 47 S, leaving the SSU and LSU rRNA precursors joined (Supplementary Fig. 1)29. In Pathway 2, initial cleavage at site 2 in ITS1 separates the 30 S (precursor for the SSU) and 32 S pre-rRNA (precursor for the LSU). Relative to 41 S, the 30 S pre-rRNA was significantly less abundant in CHH fibroblasts than controls. We propose that slowed site 2 cleavage, caused by reduced Rnase MRP activity, leads to preferential utilization of Pathway 1 over Pathway 2 in CHH patient cells (Fig. 2b).

To overcome the challenges of working with pre-rRNA from fibroblasts, we generated a cell line homozygous for the most common CHH-associated mutation, an A to G transition at position 71 on the current reference sequence (NBCI sequence NR_003051.3; Supplementary Fig. 3a)31. Prior literature refers to this mutation as 70AG based on previous reference sequences, and we use 70AG for consistency. K562 cells, a suspension line derived from chronic myelogenous leukemia, were selected as the parental line32.

The 70AG mutation was introduced by combining CRISPR/CAS cleavage with a single-stranded repair templates including the A -> G mutation flanked by homology arms. Position 70A overlaps a PAM site compatible with CRISPR-Cas12a (previously Cpf1), allowing the 70AG mutation to be introduced without creating extraneous mutations in the ncRNA33. Four independent, homozygous CRISPR-derived clones were obtained (Supplementary Fig. 3b). Levels of the RMRP ncRNA were not significantly reduced in these cells compared to wild-type levels, when assessed by qPCR (Supplementary Fig. 3c). For unknown reasons, one clone (clone C) showed slightly reduced levels of the ncRNA RPPH1, which forms the related Rnase P complex (Supplementary Fig. 3c)33. All tested 70AG clones grew on average more slowly than parental cells: over 48 h, mutant cell cultures expanded 25% less than wild-type controls (Supplementary Fig. 3d).

The cell lines gave good yields of high-quality RNA for pre-rRNA northern hybridization (Fig. 2c). RAMP analyses with an ITS1 probe (Probe 119; Fig. 2c left) showed that the 41 S:47 S ratio was consistently increased in 70AG mutants, with an average band intensity 0.08 (Log2) above wildtype. In contrast, the ratios of 30 S:47 S and 26 S:47 S were reduced, with an average change of −0.05 and −0.06, respectively (Fig. 2d). An ITS2 probe (Probe 123; Fig. 2c right), showed a visible reduction in 32 S relative to the 47 S band. Quantifying this showed that 32.4 S:7 S was decreased by an average of −0.15 fold. To further validate these results, we used qPCR to measure the ratio of pre-rRNA ITS amplicons to 5′ETS amplicons, both normalized to wildtype levels (Supplementary Fig. 3e, f). This showed that ITS-containing species were increased relative to the 5′ETS amplicons, consistent with an accumulation of 41 S. Overall, we conclude that more 47 S is processed in Pathway 1 (via 41 S) in 70AG mutants, with a decrease in Pathway 2 species (notably 30 S and 26 S), as found in CHH patient cells.

In S.cerevisiae, loss of MRP activity in yeast favors accumulation of the longer 5.8S SL relative to the 6–8 nucleotide shorter form, 5.8S4,14,15,17. It was previously reported that fibroblasts from CHH patients have perturbations in the 5.8 S rRNA population, with an increased abundance of species with a 5′ extension into ITS1, as measured by qPCR20. We assessed the ratio of 5.8S5 to 5.8S6 in the 70AG K562 cells, using polycrylamide gels for RNA separation to give nucleotide-level resolution (Fig. 2e). In northern hybridization, 5.8S5 and 5.8S6 were clearly resolved, with 5.8S5 being more abundant. However, the 5.8S5 to 5.8S6 ratio was the same in parental and 70AG cells, at approximately 1.25 (Fig. 2f). It may be that the reported, extended 5.8 S rRNA qPCR products were generated by accumulated pre-rRNAs, particularly 41 S, which includes the ITS1-5.8 S region (Supplementary Fig. 1)20.

The 70AG mutation in RMRP reduces cytosolic ribosome abundance. We next assessed if the processing delay in 70AG cells resulted in a lower abundance of mature rRNA species. This is difficult to quantify using northern blotting because rRNA comprises the majority of cellular RNA as changes in rRNA abundance are masked when a set quantity of total RNA is loaded on the gel. Instead, we used flow cytometry to measure per-cell 18 S and 28 S RNA signals, using fluorescently-labeled oligonucleotide probes, a technique called FlowFISH (Flow-cytometry based fluorescently labelled in-situ hybridization)6,39. Probes with
scrambled nucleotide sequences were used as negative controls and gave low background (Fig. 3a). All 70AG clones tested had significantly reduced rRNA signals compared to the parental lines (Fig. 3b), but there was some variability. Clone C showed the greatest reduction, with an rRNA signal at about 60% of parental cells. Clone D showed approximately 80% of wildtype signal, with clone F at 70%.

The ratio between 18S and 28S did not vary significantly between wild-type and mutant cells in the FlowFISH data. To support the conclusion that 18S and 28S rRNA were equally affected, RNA samples were run on a BioAnalyzer to quantify the relative fluorescence between mature rRNA species (Fig. 3c)\textsuperscript{36}. As expected, the ratio of 28S to 18S fluorescence was about 2 in all samples tested (reflecting the greater length of 28S rRNA).
with no significant differences between the mutants and parental cells.

As per-cell rRNA was reduced in 70AG mutants, we next tested whether they also had a reduction in mature ribosomes using Total RNA-Associated Protein Purification (TRAPP)37. This method quantifies the RNA-bound proteome, with proteins recovered in proportion to their interaction with RNA. To do this, in vivo RNA-protein complexes are stabilized by UV irradiation in living cells (Fig. 4a). Cells are then lysed in denaturing conditions, and RNA-associated proteins captured by binding the RNA portion of the complex to a silica column. Unbound proteins are washed away and the remaining silica-bound RNA-associated proteins digested with trypsin. Released peptides are eluted for analysis by mass spectrometry (LC-MS-MS). Wildtype and 70AG cells were directly compared by growing each in media with isotopically-labeled amino acids, respectively, and mixing samples 1:1 (total of 4 WT and 9 mutant samples). Nonsignificant differences between the mutants and parental cells.

Quantification of rRNA signals obtained in FlowFISH experiments. In each experiment, intensity values for each 70AG clone were normalized to the signal from wild-type cells. The gating strategy is shown in Supplementary Fig. 2c. The plot shows mean of data from three independent experiments, with SD. About 1,300 SILAC ratios were recovered per mix (Fig. 4b). The mean ratio was 1 (log transformed to 0), confirming that there was no systematic bias between samples (Fig. 4b). As expected, mutant cells showed a small decrease in average abundance of cytoplasmic RPs, implying reduced interaction between RPs and rRNA (Fig. 4c). Human cells have two separate populations of ribosomes, cytoplasmic and mitochondrial, which differ in both rRNA and protein composition39. The processing of mitochondrial ribosomes is independent of RMRP. Strikingly, recovery of cytoplasmic RPs from 70AG cells was significantly lower than recovery of mitochondrial RPs, relative to wild-type cells (RPMs; Fig. 4c). This difference was statistically significant (p < 0.0001). The same result was obtained in a repeat experiment where SILAC labelling was swapped such that mutant cells were “heavy” and wild-type cells “light”, confirming that it was not a technical artifact caused by SILAC labelling.

There are two possible explanations for this result. First, 70AG cells might genuinely have more mitochondrial ribosomes. RNase MRP was originally identified as cleaving an RNA primer required for mouse mitochondrial DNA replication. The 70AG mutation could potentially increase the efficiency of this process and increase mitochondrial copy number12. Alternatively, the result could be caused by normalization to total RNA. In TRAPP, equal amounts of RNA from wildtype and mutant cells are mixed in order to purify RNA-bound proteins37. If mutant cells have less total RNA per cell, due to reduced rRNA abundance, more cell-equivalents will be included in the mix relative to the wild-type. This will cause an apparent reduction in ribosomal proteins in the TRAPP-purified proteome compared to other abundant RNA-interacting proteins, such as mitochondrial RPs.

Cytoplasmic RPs are also abundant relative to the total proteome so a similar effect would be expected for analyses of...
total protein. Indeed, the same trend was seen in SILAC total proteome data (Supplementary Fig. 4a, b).

70AG mutation in RMRP reduces the abundance of intact RNase MRP complexes. RNase MRP is a ribonucleoprotein complex comprised of the RMRP ncRNA and about 10 proteins. All of these are reported to be shared with an evolutionarily related ribonucleoprotein complex, RNase P, which cleaves the 5′ leader from pre-tRNAs. RNase P includes the ncRNA RPPH1 in place of RMRP and has a similar abundance to MRP40,41. Mutations in the shared POP1 protein cause diseases that overlap with skeletal phenotypes of RMRP mutations42–44. In recent cryo-EM structures of yeast RNase MRP45,46, Pop1 was shown to be the main structural protein in the complex. Its C-terminus interacts with the C-domain of the yeast RMRP ncRNA (called NME1), while the N terminal is in contact with the S-domain. No high-resolution structure of human RNase MRP has yet been reported.

In TRAPP data, POP1 and another RNase MRP/P complex protein, RPP38, showed consistently reduced RNA-association in 70AG mutants compared to controls (Fig. 5a). In analyses of total protein, POP1 was slightly less abundant in mutants than parental cells, but this was not statistically significant (SD crossing 1; Fig. 5b). The results raised the question of which RNAs are most associated with POP1 in vivo, as these interactions must be reduced in 70AG cells to cause the reduced recovery of POP1 in TRAPP. To address this, we used the technique of crosslinking and analysis of cDNAs (CRAC; shown schematically in Supplementary Fig. 5) to map RNAs associated with individual MRP/P complex proteins.47. In addition to POP1, we performed this analysis on POP4, as the yeast structures suggested this protein to be in close proximity to the known pre-rRNA substrate.46. A CRISPR-based approach was used to insert a FLAG-HIS7 tag onto the N or C termini of these proteins in K562 cells. Clones with homozygous tags were selected for analysis and RNA:protein complexes were stabilized by UV crosslinking in vivo. The bait protein was then tandem purified in stringent, denaturing conditions, and co-purifying RNAs sequenced47. Notably, RMRP comprised the majority of RNAs recovered with POP1, making up 77–82% of sequencing reads (Fig. 5c).

RPPH1 was significantly less recovered with POP1, accounting for 17–23% of reads. As the abundance of RMRP was not significantly reduced in 70AG mutants (Supplementary Fig. 3c), this result indicates that the reduced total RNA interactions of POP1 in 70AG cells likely reflects impaired POP1:MRP interactions. POP4 CRAC recovered the opposite ratio from POP1, with 79–86% of reads representing RPPH1 (Fig. 5c). Sites of RNA:protein crosslinks in CRAC are often revealed by single-base deletions in the sequencing data.47. Mapping such crosslinks...
**Fig. 5 70AG mutation in RMRP reduces the abundance of intact RNase MRP complexes.**

**a** SILAC ratios (70AG/wildtype cells) obtained for MRP/P complex proteins in TRAPP (mean and SD). Each dot represents data from an independent SILAC mix (total of 4 mixes, processed in 2 independent experiments). **b** SILAC ratios (70AG/wildtype cells) obtained for POP1 protein in proteome. Includes data from two SILAC mixes, showing mean value. **c** RNA species recovered from MRP/P complex proteins in CRAC. Graphs show relative proportion of mapped reads representing each indicated RNA or RNA biotype, in two independent experiments. **d** Pileup of reads containing single base deletions (indicating RNA:protein crosslink site) in RMRP ncRNA, in POP1 or negative control CRAC. Representative of results from two independent experiments. **e** Possible secondary structure of RMRP, indicating sites of crosslinking with two RNase MRP complex proteins (POP1 and POP4). Nucleotides with reproducibly increased SHAPE reactivity scores in RMRP 70AG cells are indicated. The line designated P4 indicates a predicted interaction that generates a conserved pseudoknot structure. Source data are provided as a Source Data file (panels **a**, **b** and **c**), and as processed data files (panel **d**).
across RMRP showed a clear, single site of interaction with POP1, centered on nucleotide 201 (Fig. 5d), whereas crosslinks on RPPH1 were more diffuse with several smaller peaks (Supplementary Fig. 6a, b). Conversely, POP4 CRAC showed two clear peaks in RPPH1 (at positions 122 and 186), but multiple smaller peaks for RMRP (Supplementary Fig. 6c, d). There was some overlap in the recovered crosslinking sites for POP1 and POP4. This has previously been seen for other RNPs (see for example48). Our interpretation is that in vivo RNPs can show flexibility, perhaps on different substrates or at different steps during their function.

To determine whether the preference for POP1 crosslinking to the RNA component of MRP over P was conserved in yeast, we tested yeast Pop1 in CRAC. This showed that, indeed, the bias was even more pronounced: 89–94% of reads were from the RNase MRP RNA (NME1 in yeast) and 3–7% from RNase P (RPR1; Supplementary Fig. 7a). However, unlike human POP1, the yeast protein showed three distinct crosslink sites across the MRP RNA (Supplementary Fig. 7b), suggesting that the structure of human and yeast RNase MRP complexes may not be identical in vivo.

POP1 apparently shows a conserved, preferential association with RMRP ncRNA in vivo. Levels of POP1 and RMRP are maintained in 70AG cells, so the reduced POP1 recovery in TRAPP indicates that the mutation likely destabilizes the POP1:RMRP interaction. Potentially this could be associated with altered folding of the RMRP RNA in vivo. This hypothesis is supported by the finding from yeast structural data that, although human disease-causing mutations in RMRP cluster near the catalytic center of RMRP, these sites do not appear to bind the known substrate of yeast MRP (the A3 fragment of ITS1 in pre-rRNA), implying that they may act by impairing RNA folding or complex stability.

We assessed this possibility using selective 2′-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP49–51. In this method, an electrophilic chemical probe (1M7 in this case) is added to cultured cells and modifies RNA nucleotides in proportion to their flexibility. Sites of modification are detected as single-base mutations in sequencing data after gene-specific reverse transcription and cDNA sequencing. Overall, the obtained RMRP SHAPE reactivity profiles were similar between wildtype and 70AG cells (Supplementary Fig. 8), indicating no large-scale refolding in the mutants. Visual inspection revealed few changes, however, the deltaSHAPE tool50 performs statistical comparisons based on average reactivity at each nucleotide (Supplementary Fig. 9). This identified two areas showed small, but apparently reproducible, increases in flexibility in 70AG cells (indicated in green in Fig. 5e and boxed in Supplementary Fig. 9), one just downstream of the mutation (nucleotides 73 and 74) and one further 3′ (106 and 107).

We conclude that the disease-associated mutation likely results in subtle structural destabilization of the RNase MRP complex.

Discussion
Ribosomopathies are a diverse group of human disorders in which ribosome production or function is defective23–25. Clinical features of these disorders are variable; bone marrow dysfunction leading to anemia or cytopenia is common, as are skeletal anomalies and an increased risk of cancer. Like CHH, many ribosomal disorders have surprisingly tissue-specific phenotypes, despite ribosomes being present in almost all cells26,27. Two broad hypotheses have been proposed to explain this. First, the phenotype may reflect decreased ribosome number or function in specific cell types that crucially depend on poorly translated mRNAs, vulnerable to reduced translation. In Diamond Blackfan anemia (DBA), in which mutations in ribosomal proteins cause defective erythropoiesis, ribosome numbers are reduced without altered composition28. This results in reduced translation of transcripts with unstructured 5′ untranslated regions (UTRs), including a specific reduction in the translation of GATA2, a key hematopoietic transcription factor. Alternatively, and not mutually exclusively, some cell types may rely on specialized ribosomes, the assembly of which may be affected by particular mutations25.

Here, we present evidence that CHH is a ribosomopathy caused by a defect in pre-rRNA processing. Previous work reported increased levels of ITS1-containing RNA precursors in CHH patient cells29,30, but downstream effects on ribosome abundance have not previously been demonstrated. In this study, patient fibroblasts and a cell line with a disease-linked mutation (70AG in RMRP) showed delayed rRNA processing in a pattern consistent with decreased cleavage at the presumed RNase MRPR target (site 2 in ITS1). These cells also have reduced rRNA per cell, and reduced intact cytosolic ribosomes relative to the mitochondrial ribosome pool. Moreover, the 70AG mutation reduces the abundance of intact RNase MRP complexes, probably by destabilizing the interaction between RMRP and POP1. The overall effects on ribosome synthesis are modest, but this is expected from a disease-related mutation in an essential gene, since carriers must develop almost normally in order to be classed as patients.

A high-resolution structure of the human RNase MRP complex is not currently available, and the POP1/Pop1 crosslinking sites suggest that there may be appreciable differences from the yeast structure. Based on evolutionary comparisons41,54, the predicted secondary structure of RMRP is shown in Fig. 5c. The line designated P4 (Fig. 5e) indicates a predicted interaction that generates a conserved pseudoknot structure. This will fold the core of the RNA into a compact 3D structure postulated to play a role in catalyzing substrate RNA cleavage. Within this structure, all indicated RNA sites will likely be in close proximity. The large POP1 protein (115 kDa) crosslinked at a single site on RMRP (Fig. 5e). In contrast, the smaller POP4 protein (25 kDa) crosslinked at multiple sites. Notably, one of these POP4 binding sites overlapped with one of the POP1 crosslinking sites, suggesting conformational changes within the complex, perhaps during different steps during pre-ribosome association and function. Chemical probing of structural flexibility in vivo using SHAPE-MaP49–51 did not reveal substantial changes in the mutant, although modest changes in the structure are indicated (green in Fig. 5e) at sites in the vicinity of the mutation. We speculate that changes in the overall structure of the MRP core impact negatively on the stability of protein interactions and catalytic activity in vivo.

These results advance our understanding of CHH, but also raise some intriguing questions. Most notably, why are the phenotypes of DBA and CHH different, if reduced ribosome number is a common pathological mechanism? Some aspects of the two disorders do overlap; for example, some CHH patients have bone marrow dysfunction similar to that seen in DBA35. However, other aspects are unique, notably the T cell dysfunction which is life-limiting in CHH but not a feature of DBA1.

In conclusion, the results in this study point to CHH being a disorder of ribosome synthesis and suggest experimental approaches to further explore this complex disease.

Methods
Human cell culture. K562 cells were obtained from ATCC (cat. CCL-243), and grown in RPMI 1640 Medium with GlutaMAX Supplement (Gibco; cat. 61870036), further supplemented with 1x final concentration Antibiotic-Antimycotic (Gibco; cat. 15240096) and 10% fetal calf serum (Sigma; cat. F2442). Cells were grown to a density of 0.5–1×10⁶ cells/mL, then diluted or used for experiments.

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Patient and control fibroblasts were obtained from Prof. Sophie Hambli (Newcastle University). They were grown in DMEM with GlutaMax (Gibco; cat. 10566-064) supplemented with 10% fetal calf serum. Cells were grown to a confluency of 70–90% then split or used for experiments. To split, cells were washed once with sterile PBS, then incubated with 0.25% Trypsin-EDTA (Gibco; cat. 25200-056; 0.2x volume of culture media removed) until detached. Trypsin was inactivated with 5 volumes of media, and cells pelleted at 100 g for 5 minutes before resuspension in culture media. All mammalian cell cultures were maintained at 37 °C with 5% CO2.

Flow cytometry. Samples were prepared as described for individual methods, below. Samples were acquired on a BD LSRFortessa flow cytometer, using BD FACSDiva software, version 8.0.1. Initial data analysis was done with FlowJo version 10.6, with subsequent analysis as indicated in figure legends.

CRISPR targeting of Rmrp in primary mouse T cells. Ragl KO, C57BL/6J mice were bred at the University of Edinburgh. All experimental procedures were approved by a current project license under the authority of the Animals (Scientific Procedures) Act 1986, and additionally followed the University of Edinburgh’s ethical guidance as overseen by its AWERB committee.

Peripheral lymph nodes were dissected from Rag1 knockout mice homozygous for the OTI allele. Lymph nodes were massaged through a 70 µm cell strainer. Cells were washed once with IMDM (Gibco; cat. 12440053) supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 U/mL streptomycin, and 50 µM 2-mercaptoethanol, counted, and resuspended at 10566016 cells/mL in IMDM supplemented with 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin, 100 U/mL streptomycin, and 50 µM 2-mercaptoethanol. Cells were washed once with sterile PBS, then incubated with 0.25% Trypsin-EDTA (Gibco; cat. A36496) was added, and the combined mix heated to 37 °C for 10 minutes. A 1:10 dilution of 0.1% BSA in PBS was then prepared by mixing loading dye, 36% formaldehyde, and 1 mg/mL ethidium bromide in the ratio 14:1:1. RNA samples were mixed with an equal volume of pre-mix, and heated at 70 °C for 10 min, followed by cooling on ice for 5 min before loading. The gel was run at 140 V for 4.5 h. After visualization, RNA was transferred to BrightStar-Plus Positively Charged Nylon Membrane by overnight downward capillary transfer in 10x SSC. Membranes were then crosslinked, as above.

Radioactive oligonucleotide probing of northern membranes. 100x Denhardt’s solution was prepared by dissolving 1 g of BSA fraction V, 1 g of Ficoll 400, and 1 g of PVP in 50 mL of MQ. Hybridization solution was prepared by combining 25 mL of 0.5x SSC, 5 mL of 100x Denhardt’s solution, and bringing to 100 mL with MQ. Oligo labelling reaction mix was prepared by mixing 1 µL of 10 µM oligo with 13 µL of MQ, 2 µL of 10x T4 PNK Reaction Buffer (NEB; cat. B0201S), 1 µL of 100 mM DTT, 1 µL of T4 PNK (NEB; cat. M0201L) and 3 µL of 12P-ATP (100 Ci/µL; Hartmann Analytic). The labelling reaction was performed at 37 °C for 1 h. In the meantime, the membrane was pre-hybridized in hybridization solution at 50 °C with shaking. Following labelling, unincorporated ATP was removed from the reaction mix using a mini-Q Spin Oligo Column (Roche; cat. 1814397001), following provided instructions. The recovered reaction mix was added to the membrane in 100 µL of hybridization solution for overnight hybridization.

The following morning, the membrane was washed four times with hybridization wash solution (2x SSC, 0.1% SDS). Two washes were done at room temperature, followed by a wash at 50 °C before a final room temperature wash. Membranes were then exposed to a phosphoimager screen. Before re-hybridization with a different probe, membranes were stripped by incubating twice for 10 min with boiling stripping solution (0.1x SSC, 0.1% SDS). Sequences of oligonucleotide probes used are shown in Supplementary Table 2. Acquired images were analyzed with ImageJ version 1.51.3.

Gels were run for 1660 V hours (for example, 80 V for 20 h), and samples transferred onto BrightStar Plus Positively Charged Nylon Membrane (Invitrogen; cat. AM10100) at 0.5x TRIS for 3 h at 40 V. The membrane was then crosslinked at 254 nM in a Stratallinker cross-linking device (Stratagene).

Agarose gel for long RNA species. 50x TRIS/TRI buffer was prepared by mixing 10 µL of triethanolamine and 13.5 g tricine, and bringing the volume to 50 mL with MQ. Agarose gel mix was prepared by combining 285 mL of MQ, 3 g of agarose and 6 mL of 50x TRIS/TRI. Agarose was melted in a microwave, allowed to cool to just above room temperature, at which point 10.5 mL of 36% formaldehyde was added and the gel poured.

Gel loading dye was prepared by mixing 84 µL of 50x TRI/TRI, 4 µL of 0.5 M EDTA, 10 µL of 1% Bromophenol Blue, and 1.84 mL of MQ. Pre was then prepared by mixing loading dye, 36% formaldehyde, and 1 mg/mL ethidium bromide in the ratio 14:1:1. RNA samples were mixed with an equal volume of pre-mix, and heated at 70 °C for 10 min, followed by cooling on ice for 5 min before loading. The gel was run at 140 V for 4.5 h. After visualization, RNA was transferred to BrightStar-Plus Positively Charged Nylon Membrane by overnight downward capillary transfer in 10x SSC. Membranes were then crosslinked, as above.

CRISPR-Cpf1 mediated knock-in of RMRP 70AG mutation. A single-stranded repair template was designed including the mutation with homology arms. For POP1, a 2xFLAG-6xHIS tag was inserted at the N terminus. For POP4, a C terminal 6xHIS 1xFLAG tag was used, with the HIS and FLAG moieties separated by a 4x Ala linker.

To introduce the tags, the 0.6 µL of 200 µm guide RNA was mixed with 0.6 µL of 200 µm tracrRNA (Alt-R CRISPR-Cas9 tracrRNA (IDT; cat. 1072532), heated to 95 °C for 5 min then allowed to cool. Then, 2.1 µL of PBS and 1.7 µL of 61 µM Cas9 enzyme was added (Alt-R S.p. Cas9 Nuclease V3; IDT; cat. 1081058) and the mixture incubated at room temperature for 20 minutes. Meanwhile, 1 x 106 cells per transfection were pelleted, washed with PBS, and resuspended in 100 µL of Nucleofector Solution from the Cell Line Nucleofector Kit V (Lonza; cat. VCA-1003). The transfection mixture was added with 5 µL of Cas9gRNA mix (made above), 1 µL of 100 µM electroporation enhancer (IDT; cat. 10779519) and 3 µL of 10 µM sODN. Cells and transfection mixture were combined and electroporated with a Lonza Nucleofector 2B Device, using supplied settings for K562 cells. Cells were then gently transferred to 1.5 mL of K562 media supplemented with 25 µM hDR Enhancer (IDT; cat. 10810702), centrifuged to 1 mL, and transferred to 100 µL of MQ. Cells were mixed in a 96 well plate. Clones were screened by PCR and sequencing after about 2 weeks, using check primers shown in Supplementary Table 3.

CRISPR-Cpf1 mediated knock-in of RMRP 70AG mutation. A single-stranded repair template was designed including the mutation with homology arms. 1.6 µL of 100 µM Cpf1 crRNA was mixed with 1.4 µL of PBS and 2 µL of Cpf1 Nuclease (Alt-R CRISPR-Cpf1; discontinued) and incubated for 20 min at RT. Thereafter the procedure was the same as for CRISPR-Cas9 mediated protein tagging, except the electroporation enhancer was Cpf1-specific (IDT; cat. 1076300).

For protein tagging experiments, guide RNAs, repair templates, and check primers were ordered from IDT in the sequences from Supplementary Table 3.

Protein tagging in human cell lines. For protein tagging experiments, single-stranded templates were designed including the tag with homology arms. For POP1, a 2xFLAG-6xHIS tag was inserted at the N terminus. For POP4, a C terminal 6xHIS 1xFLAG tag was used, with the HIS and FLAG moieties separated by a 4x Ala linker.

To introduce the tags, the 0.6 µL of 200 µm guide RNA was mixed with 0.6 µL of 200 µm tracrRNA (Alt-R CRISPR-Cas9 tracrRNA (IDT; cat. 1072532), heated to 95 °C for 5 min then allowed to cool. Then, 2.1 µL of PBS and 1.7 µL of 61 µM Cas9 enzyme was added (Alt-R S.p. Cas9 Nuclease V3; IDT; cat. 1081058) and the mixture incubated at room temperature for 20 minutes. Meanwhile, 1 x 106 cells per transfection were pelleted, washed with PBS, and resuspended in 100 µL of Nucleofector Solution from the Cell Line Nucleofector Kit V (Lonza; cat. VCA-1003).

The transfection mix was added with 5 µL of Cas9gRNA mix (made above), 1 µL of 100 µM electroporation enhancer (IDT; cat. 10779519) and 3 µL of 10 µM sODN. Cells and transfection mixture were combined and electroporated with a Lonza Nucleofector 2B Device, using supplied settings for K562 cells. Cells were then gently transferred to 1.5 mL of K562 media supplemented with 25 µM hDR Enhancer (IDT; cat. 10810702), centrifuged to 1 mL, and transferred to 100 µL of MQ. Cells were mixed in a 96 well plate. Clones were screened by PCR and sequencing after about 2 weeks, using check primers shown in Supplementary Table 3.
The mixture was heated to 65 °C for 5 min, then cooled on ice. In-cell SHAPE-MaP 2 washes with 1 mL FFBW, and 2 washes with 1 mL FACS buffer. being pelleted. Cells were then resuspended in staining mix for 3 h at 37 °C, before (Promega; cat. N211A). Once FFSA was cool, FFSA and FFSB were combined 1:1 2.5 µL of FISH probes diluted to 50 ng/µL; and 8.75 µL of MQ. FFSA was then formaldehyde added. Cells were left to nutation, then stained with Imperial protein stain (Thermo Scientiﬁc). Samples were acquired and analyzed as described for TRAPP. Proteins were digested on the column with 0.25 µg of Trypsin/Lys-C protease mix (Promega; cat. V5071), and peptides eluted for mass spectrometry. Samples were acquired by the Proteomics Facility at the Wellcome Centre for Cell Biology, University of Edinburgh, on an Orbitrap Fusion Lumos Tribib Mass Spectrometer (Thermo Fisher Scientiﬁc, UK). Raw data were processed by the MaxQuant software platform, version 1.6.1, searching against the UniProt reference proteome set. Further analysis used custom scripts. A paper describing this modiﬁed TRAPP protocol is in preparation.

The SHAPE-MaP protocol described here is adapted from work published by the Weeks lab 49–51,59. Wildtype or MRMP 70ΔG K562 cells were grown to low phase, then washed with PBS and 0.8 × 10^6 cells/resuspended in 900 µL media. The SHAPE reagent 1M7 (Sigma; cat. 908401) was added to a final concentration of 10 mM by adding 100 µL of 100 mM reagent resuspended in DMSO. For unmodiﬁed samples, the same volume of DMSO was added. The in-cell acylation reaction was left to proceed for 15 min at 37 °C. Then cells were pelleted, washed with PBS, and lysed in Trizol (Invitrogen; cat. 15996026). RNA was extracted using the manufacturer’s phase separation protocol, and 580 ng RNA diluted in 500 µL MQ. Reverse transcription mix was made by adding 4 µL of 2.5 mM dNTP and 50 µL of RMRP RT primer (5’-ACAGCCGGGCGTCAGA-3’) at 2 U/mL. The mixture was heated to 65 °C for 5 min, then cooled on ice.

Next, the mixture was supplemented with 4 µL of 5x FirstStrand buffer (provided with SuperScript II; Invitrogen; cat. 18060-414), 4 µL of freshly prepared 30 mM MnCl2, 2 µL of 100 mM DTT, and 1 µL of RNasin Ribonuclease Inhibitor (Promega; cat. N2511), and incubated at 23 °C for 2 min. 1 µL of the SuperScript II enzyme was then added. Reverse transcription was then done in a thermocycler following the same conditions as for TRAPP. The reaction was performed using the following program: 40 cycles of 10 s at 94 °C, 10 s at 60 °C and 15 s at 72 °C.

For some experiments, further analyses were undertaken, as indicated in Figure legends.

**FlowFISH.** The FlowFISH method 85 was adapted. Probe sequences shown in Supplementary Table 6 are published 86,87. Probes conjugated to ﬂuorophores were ordered from IDT.
CRAC protocol for yeast Pop1, CRAC was performed following a published protocol\(^6\). For human CRAC, technical modifications were made to this protocol. The full human CRAC protocol is described below.

Sequencing and analysis of CRAC data. CRAC libraries were sequenced either on a MiSeq or HiSeq, both with 150 base reads. Yeast CRAC data were processed using custom scripts calling utilities from the PyCRAC collection\(^6\). First, raw FASTQ files were demultiplexed using pyBarcodeFilter. Then, adapters and low-quality sequences were removed with Flexbar (version 3.4.0\(^6\)). Next, PCR duplicates were collapsed with pyFastDuplicateRemover. Reads were then aligned to the *Saccharomyces cerevisiae* genome sequence from Ensembl release EF4.74 by Novoalign version 2.07.00. Read counts were produced using pyReadCounters, and pileups (Roche; cat. 11873580001; 1 tablet per 50 mL lysis buffer), and left to lyse for 2 h. Using a magnetic rack, beads were then washed three times with 1 mL buffer LB, supplemented with 150 μg/mL of RNase inhibitor (Promega; cat. N2511), in 1x NuPAGE MOPS SDS Running Buffer (Invitrogen; cat. NP0005), followed by a rinse with 100% ethanol and 3 M NaCl, and combining.

RNA dephosphorylation. Beads on the column were washed twice with 0.75 mL of buffer WB1, spinning at 1000 RCF (4 °C for 20 min). The supernatant was removed and pellets washed with 70% ethanol, before being allowed to dry. The pellet was resuspended in 11 μL of MQ, and left to rehydrate for 10 min at RT. 4 μL of NuPage LDS sample buffer (Invitrogen; cat. NP0007) was added, together with 2-Mercaptoethanol to a final concentration of 2%. Samples were then denatured at 65 °C for 10 min, and loaded onto a NuPage 4-12% polyacrylamide gel (Invitrogen; cat. NP0321). The gels ran at 120 V in 1xNuPAGE MOPS SDS Running Buffer (Invitrogen; cat. NP0005), then transferred onto Hybond-CExtra membrane (Fischer Scientific; cat. 10564755) in 1x NuPAGE transfer buffer (Invitrogen; cat. NP0006) supplemented with 10% methanol, for 2 h at 100 V.

Library preparation. DNA libraries were amplified using the primers P5 Forward (AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCCTTGCGGATGACTGTGATCTGAAGTCATCTCCAGGAGATGTGACAAAACTATATGC) and P6 Reverse (CAAGCAGAAGACGGTCACGAGATCTACACTCTTTCCCTACACGACGCTTTCGCAGTCAT and PE miRcat Reverse (CAGAGCAGAAGACGGTCACGAGATCTACACTCTTTCCCTACACGACGCTTTCGCAGTCAT and PE miRcat Reverse (CAGAGCAGAAGACGGTCACGAGATCTACACTCTTTCCCTACACGACGCTTTCGCAGTCAT)), and P7 Forward (GGCACCGAGATCTACACTCTTTCCCTACACGACGCTTTTGTACAAAAACAGCAGAAGGAAGCTCTT CATCGAGATCTACACTCTTTCCCTACACGACGCTTTCGCAGTCAT and PE miRcat Reverse (CAGAGCAGAAGACGGTCACGAGATCTACACTCTTTCCCTACACGACGCTTTCGCAGTCAT and PE miRcat Reverse (CAGAGCAGAAGACGGTCACGAGATCTACACTCTTTCCCTACACGACGCTTTCGCAGTCAT)). PCR reactions were assembled in 50 μL and contained 0.3 μL of Phusion High-Fidelity DNA Polymerase (NEB; cat. M0530S), 10 μL of provided 5x Phusion buffer, 5 μL of 2.5 μM dNTP mix, 1 μL of each primer, and 2 μL of cDNA. cDNA was amplified using the following programme: initial denaturation at 98 °C for 30 s, then 18 to 22 cycles of 98 °C for 10 s, 65 °C for 30 s and 72 °C for 30 s. The final extension was at 72 °C for 5 min. Three reactions were completed for each sample. Once complete, the PCR reactions were pooled for each sample and purified using AMPure XP beads (Beckman Coulter; cat. A6880). Per reaction mix, 2 volumes of AMPure buffer and 0.2 volumes of AMPure XP beads were added. Samples were mixed and incubated for 5 min at RT. The supernatant was then removed on a magnetic rack, and beads washed twice with 200 μL of 80% ethanol. After the last wash, ethanol was removed and the beads left to dry.

Purified DNA was then eluted by resuspending the beads in 10 μL of MQ, and 2 μL of 4x DNA gel loading dye added (Thermo Scientific; cat. R0811). A 3 M MgCl\(_2\) solution (Lonza; cat. 500750) was prepared in 1x TBE. The samples were loaded and run at 80 V until dye reached the bottom of the gel. The smear corresponding to the DNA library was extracted by a Zymoclean Gel DNA Recovery Kit (Zymoc; cat. D4007), and fluorometrically quantified. Libraries were diluted to approximately 5 nM and pooled, and submitted for sequencing on an Illumina HiSeq2500 platform at the Wellcome Trust Clinical Research Facility, University of Edinburgh.

Statistics. All statistics were calculated using Prism 9.0.0, with tests as indicated in figure legends. In general, plots show mean values +/− standard deviations.
Data availability

The data that support this study are available from the corresponding author upon reasonable request. The GEO accession number for all sequence data reported in this paper is GSE171021. The proteomics data are available through the ProteomeXchange partner repository with the dataset identifier PXD025029. Human reference sequences used in this paper were taken from GRCh38.p13 [https://www.ncbi.nlm.nih.gov/assembly/GCF_00001405.39]. Yeast sequences are from the Saccharomyces cerevisiae genome (Ensembl release EF47.4 [ftp://ftpensembl.org/pub/release-74/gtf/ saccharomyces_cerevisiae]). Mouse sequences are from GRCm39 [https://www.ncbi.nlm.nih.gov/assembly/GCF_00001635.27]. Source data are provided with this paper.

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Author contributions

N.R., R.Z., and D.T. conceived the project and wrote the manuscript. N.R., V.S., D.W., T.T., and C.S. performed experiments. N.R., V.S., D.W., T.T., A.H., R.Z., and D.T. designed experiments. N.R., V.S., C.S., and T.T. analyzed data. All authors edited and reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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