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# Analysis of orthologous groups reveals Archease and DDX1 as tRNA splicing factors

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RNA ligases play essential roles in many cellular processes in eukaryotes, archaea and bacteria including RNA repair[1,2] and stress-induced splicing of mRNA[3]. In archaea and eukaryotes, RNA ligases also feature in tRNA (transfer RNA) splicing to generate functional tRNAs required for protein synthesis[4-7]. We recently identified the human tRNA splicing ligase, a multimeric protein complex having RTCB (also known as HSPC117, C22orf28, FAAP or D10Wsu52e) as the essential subunit[8]. The functions of the additional complex components ASW, CGI-99, FAM98B and the DEAD box helicase DDX1 in the context of RNA ligation have remained unclear. Taking advantage of clusters of eukaryotic orthologous groups (KOGs), we found that Archease, a protein of unknown function, is required for full activity of the human tRNA ligase complex and, in cooperation with DDX1, facilitates the formation of an RTCB-guanylate intermediate central to mammalian RNA ligation. Our findings define a role for DDX1 in the context of the human tRNA ligase complex and suggest that the widespread co-occurrence of Archease and RtcB proteins implies evolutionary conservation of their functional interplay.

A kinetic assay using FLAG-RTCB affinity-purified from stably transfected HEK293 cells (Extended Data Figure 1a and b) and tRNA exon halves (Fig. 1a) or isolated linear intron as a minimal substrate (Fig. 1 b and c) (see materials and methods) revealed that product formation virtually ceased to occur before consumption of the substrate (Fig. 1a, b and c).

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**Author Contributions**

JP designed and carried out experiments and wrote the manuscript, JJ designed and carried out experiments and contributed to writing the manuscript, AS performed bioinformatic analysis of Archease and contributed to writing the manuscript, JM designed experiments and contributed to writing the manuscript.


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The maximum amount of ligation product formed was proportional to the initial amount of enzyme (Fig. 1c and Extended Data Figure 1c-e) suggesting that affinity-purified FLAG-RTCB requires an additional, stimulatory component for unlimited enzymatic turnover. We reasoned that the distinctive phyletic distribution of RtcB proteins in eukaryotes (*i.e.* their absence in plants and fungi[9] which rely on different ligase proteins and mechanisms[10-12], see Extended Data Figure 2a) could be used to discover proteins functionally associated with human RTCB. We therefore focused our attention on seven clusters of eukaryotic orthologous groups (KOGs) conserved in the same model organisms as RtcB (KOG3833) (Extended Data Figure 2b)[13,14]. One of these, KOG4528, encompasses Archease, a protein of unknown function predicted to act in combination with nucleic acid processing factors[15]. In *Pyrococcus abyssi* Archease improves the substrate specificity of a tRNA methyltransferase encoded downstream in a bicistronic operon[16], however, this function does not appear to be conserved in mammals[17].

We therefore added recombinant Archease (Extended Data Figure 3a) to ligase assays containing affinity-purified human FLAG-RTCB which made ligase reactions proceed to near complete consumption of substrate (Fig. 1d). Addition of recombinant Archease to stalled ligase reactions allowed the formation of product to resume (Fig. 1e, Extended Data Figure 3b and c). Two mutant versions of Archease, D39A ($Asp^{39} \rightarrow Ala^{39}$) and K144A ($Lys^{144} \rightarrow Ala^{144}$) (Extended Data Figure 4), had no effect on the ligase activity of human FLAG-RTCB (Fig. 1f).

Affinity purification of FLAG-Archease or FLAG-RTCB from HEK293 cells did not reveal a detectable association of Archease with the human tRNA ligase complex (Fig. 2a) or RNA ligase activity (Fig. 2b). However, addition of the cell permeable crosslinking reagent dithiobis[succinimidylpropionate] (DSP), to HEK293 cells prior to affinity purification, enabled us to detect a specific association of FLAG-Archease with endogenous RTCB and FAM98B (Fig. 2c and Extended Data Figure 5a and b). Failure to detect an association with FLAG- TSEN2[18], which is not stimulated by Archease (Extended Data Figure 5c and d), further attests to the specificity of the crosslink (Fig. 2d). Thus, Archease is a component of the human tRNA ligase complex. Characterization of the interaction between Archease and the tRNA ligase complex by surface plasmon resonance (SPR) yielded a dissociation constant ($K_D$) of 1.8 ± 0.9 nM (Extended Data Figure 5e-g). The inactive D39A mutant displayed similar interaction parameters as the wild type (WT) protein indicating that this residue directly mediates chemical reaction steps or conformational rearrangements required for RNA ligation rather than facilitating the interaction of Archease with RTCB.

To test whether Archease stimulates the activity of human RTCB complexes at the stage of product release, we established a gel shift assay to detect RTCB-RNA adducts forming at reaction conditions. These adducts (termed C1, C2 and C2a) appeared only transiently in presence of WT Archease (Fig 3a, lanes 1-4) but persisted upon mutagenesis of conserved residues (Fig. 3a, lanes 5-8 and Extended Figure 6a). All adducts–irrespective of whether they had formed in the presence or absence of functional Archease–exclusively contained substrate RNA species S and $P_{concat}$ (Fig. 3b, lanes 1-4 and Extended Data Figure 6b) and FLAG-RTCB (Extended Data Figure 6c). Taken together, these data argue against the release of RNA products as a rate-limiting step accelerated by Archease.

The ligase activity of RtcB from *Escherichia coli* depends on the formation of a covalent RtcB-guanylate intermediate and the subsequent transfer of GMP to the 3′-end of RNA molecules with 2′,3′- or 3′-phosphate termini[19,20]. In contrast to prokaryotic RtcB, addition of [α-$^{32}$P]GTP to RNA ligase assays did not lead to the formation of a radiolabeled human RTCB-guanylate adduct (Fig. 3c, upper panel, lane 1). Formation of a radiolabeled band corresponding to guanylated human FLAG-RTCB required the addition of WT Archease, whereas inactive Archease point mutants did not support guanylation (Fig. 3c, upper panel, lanes 2-4). In agreement with this finding, Archease stimulated the activity of the human tRNA ligase complex with high efficiency only in the presence of GTP (Fig. 3d and Extended Data Figure 7a). The co-purification of GTP or isolation of a guanylated form of RTCB during affinity purification[8,20] may account for the detection of activity corresponding to incomplete turnover cycles during the initial and apparently Archease-independent phase of the reaction (Extended Data Figure 7a). Although ATP could not serve as a nucleotidyl donor for human RTCB (Fig. 3c, lanes 5 and 6), we observed a marked stimulation of guanylation by ATP in the presence of Archease (Fig. 3e, compare lanes 1 and 2) and therefore tested whether guanylation of RTCB depends on the ATP binding DEAD box helicase DDX1 (Extended Data Figure 7b-f). Both, substitution of ATP by its non-hydrolysable ATP analogue AMPPcP and mutagenesis of two conserved motifs of DDX1 implicated in ATP binding and hydrolysis[21] (Extended Data Figure 7b, d and e) affected Archease-dependent guanylation (Fig. 3e, compare lanes 2 with lane 3 and lane 4 with lanes 5 and 6) indicating that ATP hydrolysis by DDX1 is required for maximal stimulation of the human RNA ligase by Archease. RTCB guanylation was most compromised for DDX1 K52N, the mutant displaying the lowest affinity for ATP and RNA (Fig. 3e, lane 5, Extended Data Figure 7e and f and Extended Data Figure 8a). In agreement with these results, RTCB complexes with mutagenized DDX1 exhibited diminished RNA ligase activity (Fig. 3f, Extended Data Figure 8b-e and Extended Data Figure 9) thus providing a molecular basis for the dual cofactor requirement of human RNA ligase.

Depletion of Archease by RNA interference (RNAi) impaired maturation of intron containing pre-tRNAs *in vitro*, to a comparable extent as observed upon the depletion of RTCB (Fig. 4a and Extended Data Figure 10a and b). Simultaneous depletion of Archease and RTCB suppressed pre-tRNA maturation even more (Fig. 4a). Splicing activity was restored by expressing exogenous WT but not mutant Archease (Fig. 4b and c). In agreement with the dependence of the activity of the human tRNA ligase complex on DDX1, tRNA maturation under multiple turnover conditions–*i.e.* in the presence of recombinant Archease–was recognizably impaired upon its depletion. A similar effect was observed upon the depletion of CGI-99, presumably due to the concomitant depletion of DDX1[8] (Fig. 4d compare panels 1 and 2 with panels 4 and 5 and Extended Data Figure 10c). Induction of transcription of tagged pre-tRNAs[8] revealed that the formation of mature tRNA was impaired to comparable levels in cells depleted of Archease or RTCB (Fig. 4e and Extended Data Figure 10d).

We have revealed that Archease facilitates the DDX1-dependent formation of an RTCB-guanylate intermediate central to mammalian RNA ligation and provide evidence for the role of ATP hydrolysis by the human tRNA ligase[8,22] (Extended Data Figure 7a). In human

cell lines, a significant fraction of DDX1 associates with the tRNA ligase complex, which is supported by affinity purification (Extended Data Figure 7c) and siRNA-mediated co-depletion experiments (Extended Data Figure 10c). These findings suggest that RTCB–and possibly Archease–may also be involved in the established functions of DDX1 in the context of retinoblastoma[23], cytoplasmic ribonucleoprotein particles[24], viral replication[25,26] and induction of anti-viral responses in dendritic cells[27]. Our implication of Archease in mammalian tRNA splicing has already stimulated experiments demonstrating functional cooperation of Archease and RtcB proteins in at least one archaeon[28] which may be widespread as many prokaryotic genomes harbor homologues of both proteins in operons[29,30].

## Methods

### Cloning of expression constructs for FLAG-Archease and FLAG-RTCB

The coding sequence of Archease was amplified from HeLa cDNA with the primers 12attB1_HsARCH_F (5′-AAA AAG CAG GCT CCa tgG CGC AGG AAG AGG AAG ATG TTA-3′, start codon lower case) and 12attB2_HsARCH_R (5′-AGA AAG CTG GGT CCT ACT ATT AAA TGT CAA TGA TCA CAA AAA CTT CC-3′), and cloned into pDONR201 (Invitrogen Cat. No. 11798-014) by Gateway recombination. Archease and human RTCB1 were shuttled from the pDONR201 constructs into a Gateway compatible derivative of pIM.RAG1.CMV.Neo (Cellectis Bioresearch Cat. No. IM-HS1-1) introducing an N-terminal FLAG epitope tag.

### Generation of stably transfected HEK293 cell lines

HEK293 cell lines were co-transfected with pIM.RAG1.CMV.Neo containing the FLAG-tagged gene of interest and the hsRAG1 meganuclease plasmid (Cellectis Bioresearch Cat. No. EM-HS1-1-05) according to the manufacturer's recommendations. After selection with geneticin sulphate, single cell clones were tested for expression of tagged genes by Western blot.

### Affinity purification of FLAG-Archease and FLAG-RTCB from HEK293 cells

HEK293 cells stably expressing FLAG-tagged proteins were disrupted by sonication in lysis buffer (30 mM Tris-HCl pH 7.5, 150 or 500 mM NaCl, 2 mM $MgCl_2$, 5 % [w/v] glycerol, 0.1 % [w/v] Triton® X-100, 0.1 mM PMSF, 4 mM β-mercaptoethanol, supplemented with Phosphatase Inhibitor Cocktail Set II (Merck Cat. No. C524625)). Tagged proteins were captured with FLAG-M2 agarose (Sigma Cat. No. A2220) and the affinity matrix washed five times with lysis buffer and three times with buffer P100 (30 mM Tris-HCl pH 7.5, 100 mM NaCl, 2 mM $MgCl_2$, 5 % [w/v] glycerol, 0.1 % [w/v] Triton® X-100, 4 mM β-mercaptoethanol). Bound proteins were eluted by addition of 3XFLAG peptide at a final concentration of 1 mg/mL (Sigma Cat. No. F4799).

### RNA ligase assays

Cleaved pre-tRNA transcripts or linear RNA fragment (50 nM-1 μM, typically 100 nM) were incubated with varying amounts of affinity-purified FLAG-RTCB (typically 1-5 μL of eluate per 10 μL reaction) as described previously[8]. Where indicated, recombinant,

hexahistidine tagged Archease (40 nM-10.25 μM) or a corresponding volume of buffer (10 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM DTT, 10 % [w/v] glycerol) was added to the reaction. For dose-response experiments recombinant Archease (40 nM-10.2 μM) was added to FLAG-RTCB (30 nM) pre-incubated with an excess of linear RNA fragment (300 nM) for 90 min. The rates of formation of $P_{concat}$ were plotted versus the respective concentration of Archease.

### RNA mobility shift assays

Reactions (5 μL FLAG-RTCB eluate, 1.5 μM linear RNA substrate, 20 μM recombinant Archease, 30 μL total volume) were incubated at 37 °C and aliquots of 5 μL quenched by transfer to ice. Samples were resolved by 6 % PAGE (170 mm × 240 mm × 1 mm) in 0.5 × TBE run at 5 W, 4 °C for 3 h. For analysis of the RNA content of shifted complexes, gels were visualized by phosphorimaging, bands excised and passively eluted in 200 mM Tris-HCl pH 7.5, 25 mM EDTA pH 8.0, 300 mM NaCl, 2 % SDS. RNA was recovered by ethanol precipitation and analyzed on 12.5 % denaturing PAGE gels.

### Preparation of the linear RNA fragment used for ligation assays

Hybrid pre-tRNA was transcribed as described previously[8] using the AmpliScribe® T7-Flash® kit (Epicentre Cat. No. ASF3507) as recommended by the manufacturer. The recovered transcripts were cleaved with recombinant tRNA endonuclease from *Methanocaldococcus jannaschii* as previously described[8] and the cleavage products resolved on preparative denaturing polyacrylamide gels. The linear intron was recovered by passive elution and dissolved at an appropriate concentration in 10 mM Tris-HCl pH 7.5, 100 mM KOAc, 6 mM $Mg(OAc)_2$ and 150 μM spermine-HCl pH 7.5.

### Quantitative evaluation of RNA ligation assays

The intensities of bands corresponding to substrate (S), concatemerized ($P_{concat}$), circularized concatemer ($P_{concat,circ}$) and circularized ($P_{circ}$) product were determined by phosphorimaging and the evaluation software ImageQuant (GE Lifesciences). All intensities were corrected by subtraction of appropriate background values. Concentrations of $P_{concat}$ were calculated by dividing the sum of counts corresponding to $P_{concat}$ and $P_{concat,circ}$ by the total signal in the respective lane and multiplication of the resulting fraction with the appropriate substrate concentration.

### Preparation of recombinant hexahistidine tagged human Archease

pET28a-Archease was created by amplification of the coding sequence of Archease from HeLa cDNA using the primers NdeI_ARCH_F (5′-AAG CGT CAT ATG ATG AAG GGC GGA AGT AG-3′) and XhoI_ARCH_R (5′-ATT CGA CTC GAG TTA TTA AAT GTC AAT GAT CAC-3′) and ligation into pET28a using the restriction enzymes *Nde*I and *Xho*I. Hexahistidine tagged Archease was expressed in *E. coli* BL21-CodonPlus® (DE3)-RIL and purified by Ni-NTA affinity chromatography. After a subsequent gel filtration in 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM DTT, 10 % [w/v] glycerol, protein fractions were flash-frozen in liquid nitrogen and kept at −80 °C for long-term storage.

### Bioinformatic analysis of Archease

For the alignment of Archease proteins the family was collected with an NCBI-BLASTp search[31] within the NCBI non-redundant protein database applying default parameters and highly significant expectation values ($< 10^{-3}$). The proteins were aligned using MAFFT (version 6, L-INS-I method)[32] and graphically processed with ClustalX[33]. The NMR structure of Archease from *Methanobacterium thermoautotrophicum*[34] was colored by the degree of conservation using Pymol (http://www.pymol.org). The normalized conservation score was calculated with al2co, with an independent-count based sequencing weighting scheme, the sum-of-pairs measure conservation calculation method and the BLOSUM62 scoring matrix[35] and plotted using a continuous color scale with yellow indicating the highest- and blue indicating the lowest degree of conservation.

### Cloning of mutant versions of human Archease

Aspartate (D) 39, Threonine (T) 147 and Lysine (K) 144 were converted into Alanine residues by overlap extension PCR and sub-cloned into the vectors pDONR201, gcDNA3.1_myc and pET28a.

### Surface plasmon resonance (SPR)

SPR measurements were performed on a Biacore 3000 instrument (GE Healthcare Europe). Hexahistidine tagged Archease and the Archease mutant D39A were covalently immobilized on the surface of a CM5 optical sensor chip at densities of 1239 and 1679 RU, respectively, using the Biacore amine coupling protocol. Amine activated flow cell 1 was used as a reference to allow generation of background-subtracted binding sensorgrams. FLAG-RTCB complexes were passed over the flow cells at 30 μL/min in HBS-P buffer (10 mM HEPES-KOH pH 7.4, 150 mM NaCl, 6 mM $MgCl_2$, 0.005 % v/v Surfactant P2) supplemented with 0.5 mM ATP or AMPPcP and 0.5 mM GTP. Sensorgrams were generated with 193 s association and 500 s dissociation phases and are shown as subtractive curves against an amine-activated reference surface. After each run the chip was regenerated using 10 mM glycine-HCl pH 1.5. For determination of binding kinetics, sensorgrams were analyzed by mathematical curve fitting aimed at $Chi^2 < 1$, based on a Langmuir 1:1 interaction model using the BiaEvaluation 4.1 software.

### *In vitro* nucleotidylation of FLAG-RTCB complexes

2.5 μL FLAG-RTCB were mixed with 0.5 μL hexahistidine tagged WT or mutant Archease (10 μM final concentration), 3.25 μL nucleotidylation buffer (20 mM Tris-HCl pH 7.5, 200 mM KOAc, 12 mM $Mg(OAc)_2$, 300 μM spermine-HCl pH 7.5, 1 mM DTT, 1 μM ATP, 1 μM GTP, 1 % (w/v) Triton® X-100, 30 % (w/v) glycerol) and 0.25 μL (92.5 kBq) [α-$^{32}$P]adenosine-5′-triphosphate (111 TBq/mmol, Perkin Elmer) or [α-$^{32}$P]guanosine-5′-triphosphate (111 TBq/mmol, Perkin Elmer). Reactions were incubated at 37 °C and analyzed by SDS-PAGE.

### ATPase assays

Reaction mixes (10 μL) containing 5 μL of WT, K52N or E371Q mutant FLAG-DDX1, 50 mM Tris-HCl pH 8.0, 2.5 mM MgCl2, 37 kBq [γ-$^{32}$P]ATP (111 TBq/mmol, Perkin Elmer)

and 1 μg poly($A^+$) RNA were incubated for 60 min at 37 °C and stopped by adding EDTA to a final concentration of 25 mM. 1 μL aliquots were spotted onto TLC plates (PEI Cellulose F, Merck) and developed in 0.375 M $K_2HPO_4$ pH 3.5. Intensities of non-hydrolyzed ATP and $P_i$ were determined by phosphorimaging using the evaluation software ImageQuant.

### ATP cross-linking assays

Reaction mixes containing 5 uL of WT, K52N or E371Q mutant FLAG-DDX1, 50 mM Tris-HCL pH 8.0, 2.5 mM $MgCl_2$, and 74 kBq [α-$^{32}$P]ATP (111 TBq/mmol, Perkin Elmer) were incubated at 37 °C, UV-crosslinked on ice for 15 min at 254 nm using a Herolab UVT-14S Transilluminator and analyzed by SDS-PAGE. After electrophoresis the gels were stained with Coomassie Blue, dried and visualized by phosphorimaging.

### RNA binding assays

[5′-$^{32}$P]cytidine-3′,5′-bisphosphate labeling of RNA has been described previously[8]. 2.5 μL of WT, mutant (K52N, E371Q) FLAG-DDX1 were incubated with 2 μL of binding buffer (250 μM EDTA pH 8.0, 100 mM KCl, 3 mM $MgCl_2$, 12.5 mM DTT, 7.5 mM ATP, 0.5 mM GTP, 10 U/mL RNasin® (Promega Cat. No. N2611), 65 % (w/v) glycerol) and 1 μL labeled RNA oligonucleotide (5′-UCG AAG UAU UCC GCG UAC GU-3′) (200 nM) on ice for 20 min. Membranes were pre-soaked in 20 mM HEPES pH 7.3 and assembled from top to bottom as follows in a slot-blot apparatus: nitrocellulose (Schleicher & Schuell) to trap soluble protein-RNA complexes, and Hybond-N nylon (Amersham Biosciences) to bind free RNA molecules. After assembly, reaction mixtures were applied to each slot and filtered through the membranes. Each slot was washed twice with 0.3 mL of 20 mM HEPES pH 7.3. Membranes were air dried and visualized by phsphorimaging. Intensities of bound and unbound RNAs were determined using the evaluation software ImageQuant.

### Cell culture and transfection of siRNAs

HeLa and HEK 293 cells were cultured and propagated using standard procedures. siRNAs and DNA plasmids were transfected using Lipofectamine 2000® reagent (Invitrogen Cat. No. 11668) according to the manufacturer's instructions. Archease (Dharmacon Cat. No. L-017915-01) and human RTCB (Dharmacon Cat. No. L-017647-00) were depleted by transfection of ON-TARGETplus® siRNA reagents. In addition, a custom RNA duplex (5′-TGA CAT TTA AGA CAC CAA A[dT][dT]-3′ annealed to 5′-TTT GGT GTC TTA AAT GTC A[dT][dT]-3′, deoxythimidine at the 3′ end is indicated by [dT]) targeting Archease in its 3′ untranslated region was designed using the program RNAxs 4 and obtained commercially from Dharmacon.

### Pre-tRNA maturation and cleavage assays

Pre-tRNA maturation and cleavage assays were carried out as previously described[8].

### Construction of pSUPER.retro.neo+GFP-ScPhe

The sequence coding for *Saccharomyces cerevisiae* pre-tRNA3-PheGAA, Chr. 131,5 was fused to a Tet-inducible H1 promoter[36] and introduced into pSUPER.retro.neo+GFP

(OligoEngine Cat. No. VEC-PRT-0005/0006) using the restriction enzymes *Eco*RI, *Hin*dIII and *Bgl*II. The resulting construct was introduced into HeLa T-Rex® cells (Invitrogen Cat. No. R714-07) by retroviral infection following standard procedures. GFP-positive single cells were seeded into 96-well plates, expanded and tested for inducible expression of the reporter tRNA by Northern blot analysis. Expression of the reporter tRNA transcripts was induced by addition of 1 μg/mL of doxycycline hyclate (Sigma Cat. No. D9891) to cell culture media for the indicated time.

### Northern blotting

Northern blot analysis of RNA was carried out essentially as previously described[8]. Blots were hybridized with 10 pmol of a [5′-$^{32}$P]-labeled LNA probe detecting the 3′-exon of ScPhe-tRNA (probe 5.6, 5′-TG[G] TG[G] GA[A] TT[C] TG[T] GG[A] TC[G]-3′, LNA nucleotides are symbolized as [N]). Equal loading was confirmed by hybridizing the blots with a [5′-$^{32}$P]-labeled DNA probe detecting U6 snRNA (5′-GCA GGG GCC ATG CTA ATC TTC TCT GTA TCG-3′).

### Western blotting

Proteins were transferred onto Immobilon® P PVDF membranes (Millipore Cat. No. IPVH00010) and blots were developed using ECL reagent (GE Lifesciences Cat. No. RPN2109) as recommended by the manufacturer. The polyclonal antibody against RTCB used in this study has been described before[8]. Antibodies recognizing β-actin (Abcam Cat. No. ab8227), FAM98B (Sigma Cat. No. HPA008320), DDX1 (Bethyl Cat. No. A300-521A) and the c-myc (Sigma Cat. No. M4439) and FLAG (Sigma Cat. No. F3165) epitope tags were obtained from the mentioned commercial sources.

### Quantitative reverse transcriptase PCR

RNA was prepared using Trizol® reagent (Invitrogen Cat. No. 15596) and reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems Cat. No. 4368813) according to the manufacturer's instructions. Quantitative PCR was performed using GoTaq® qPCR Master Mix (Promega Cat. No. A6002). Relative expression levels were normalized relative to human cyclophylin B using the primers HsCYCB_F (5′-GTA ATC AAG GAC TTC ATG ATC CAG GG-3′) and HsCYCB_R (5′-AAC TTT GCC AAA CAC CAC ATG CTT GC-3′). Archease was detected using the primers ARCH_QPCR_F (5′-GCA TGG GGA GAT ACT CTG GA-3′) and ARCH_QPCR_R (5′-CTT CCC GGG GTA TGA AGA AT-3′). The primers used for detection of RTCB have been described previously[8]. All primers were designed using the Primer3 software (v.0.4.0).

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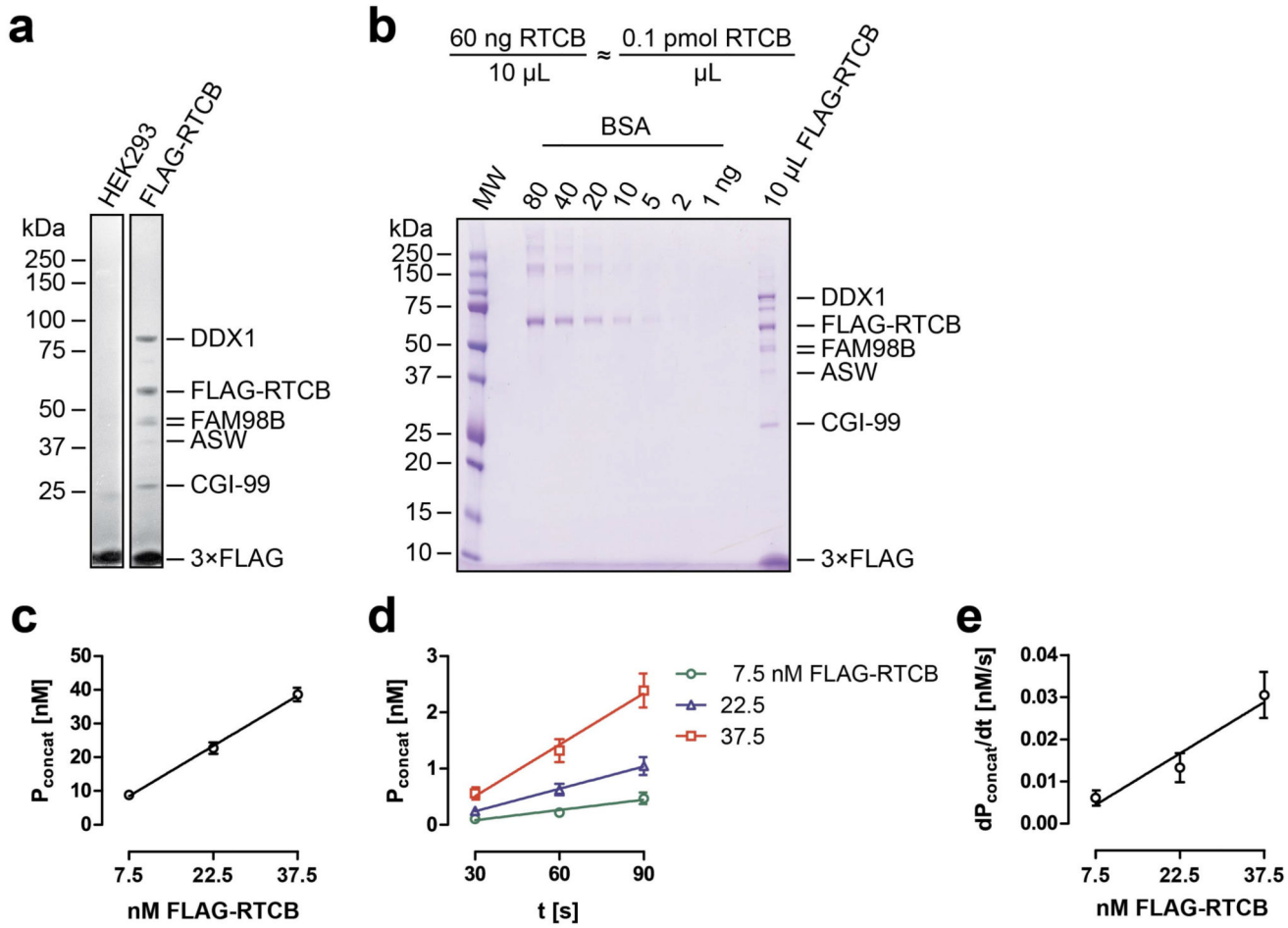
## Extended Data




**Extended Data Figure 1. Kinetic characterization of FLAG-RTCB**

**a**, SDS-PAGE (Coomassie blue) of FLAG-RTCB affinity-purified from HEK293 cells. **b**, SDS-PAGE of a 10 µL aliquot of FLAG-RTCB and comparison with a dilution series of bovine serum albumin (BSA). FLAG-RTCB concentrations were between 40 and 80 ng per 10 µL corresponding to an approximate concentration of 100 nM. **c**, Fit of time course data depicted in Fig. 1c to an exponential one phase association model restricting the bottom value to zero using the software GraphPad®. Obtained plateau values were plotted against the concentration of FLAG-RTCB. The linear relation of plateau product concentrations with the amount of FLAG-RTCB suggests that human RNA ligase catalyses a limited number of substrate turnovers. (mean ± standard error of the mean, SEM, $N = 3$) **d**, Estimation of initial rates of ligation from time course data in Fig. 1c. (mean ± SD, $N = 3$) **e**, Reaction rates derived by linear regression from the data depicted Fig. 1c were plotted against the concentration of FLAG-RTCB. (mean ± SD, $N = 3$)

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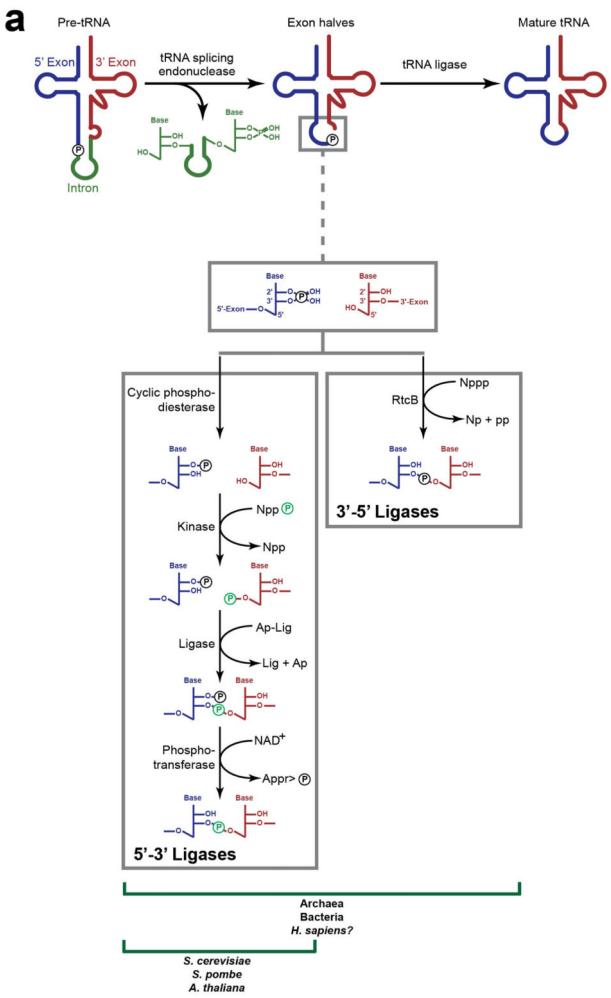


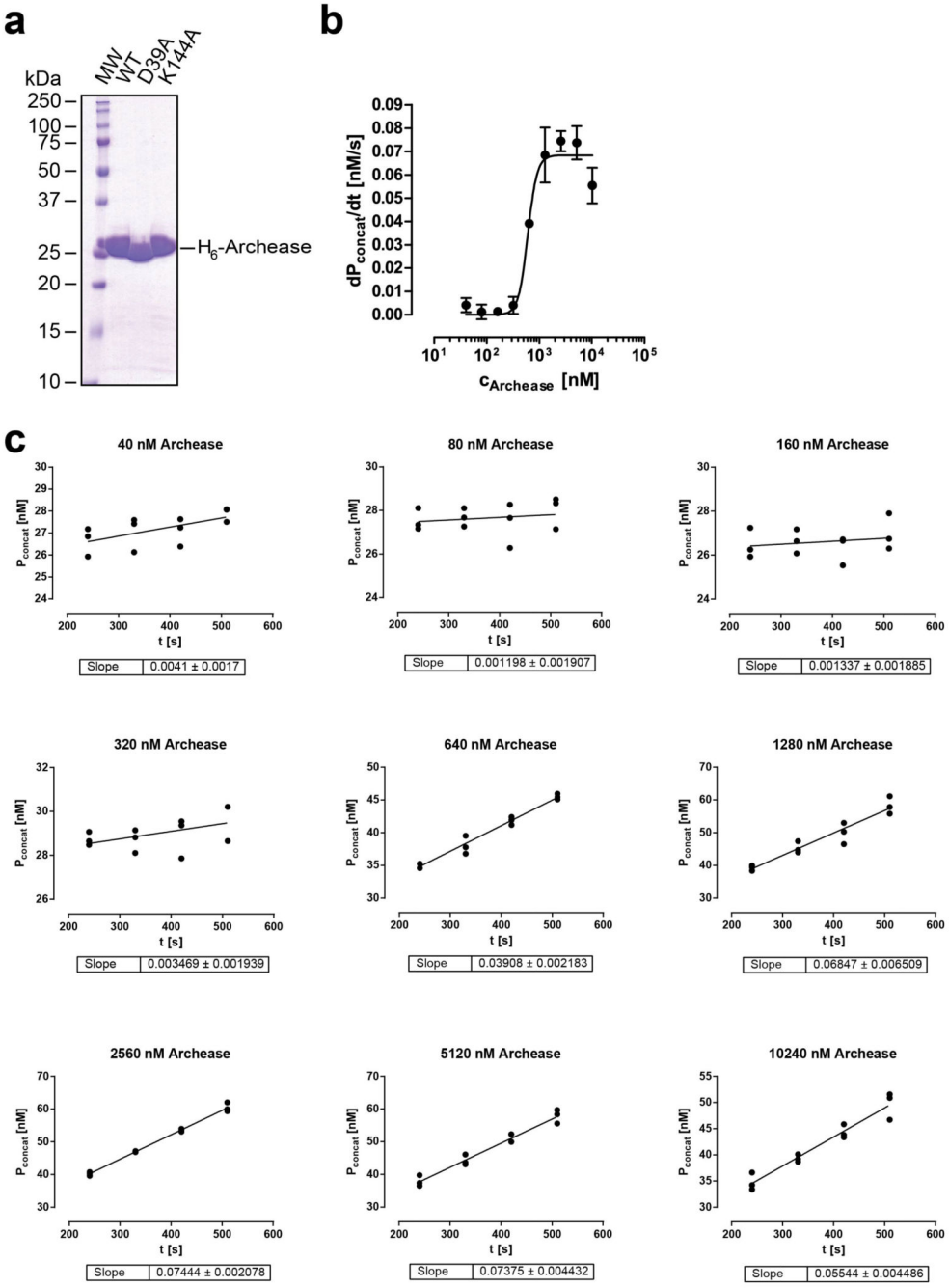

b

| A. thaliana | C. elegans | D. melanogaster | H. sapiens | S. cerevisiae | S. pombe | E. cuniculi | KOG No. | Annotation |
|---|---|---|---|---|---|---|---|---|
| − | + | + | + | − | − | + | KOG0612 | Rho-associated, coiled-coil containing protein kinase |
| − | + | + | + | − | − | + | KOG1146 | Homeobox protein |
| − | + | + | + | − | − | + | KOG1675 | Predicted cyclin |
| − | + | + | + | − | − | + | KOG1734 | Predicted RING-containing E3 ubiquitin ligase |
| − | + | + | + | − | − | + | KOG3668 | Phosphatidylinositol transfer protein |
| − | + | + | + | − | − | + | KOG3788 | Predicted divalent cation transporter |
| − | + | + | + | − | − | + | KOG3833 | Uncharacterized conserved protein, contains RtcB domain |
| − | + | + | + | − | − | + | KOG4528 | Uncharacterized conserved protein, Archease |

**Extended Data Figure 2. Archease and RTCB proteins share their phyletic distribution in eukaryotic model genomes**

**a**, Whereas most organisms appear to rely on both 5′-3′- and 3′-5′ RNA ligase mechanisms, the genomes of the vast majority of plants and fungi (as exemplified by the model organisms *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Arabidopsis thaliana*) do not encode for RtcB proteins known to catalyze 3′-5′ RNA ligation. **b**, The table lists protein families exhibiting the same phyletic pattern as RtcB proteins (ftp://ftp.ncbi.nih.gov/pub/COG/KOG/kog). Archease is the only protein of unknown function

represented in this group of KOGs. + indicates presence of a protein assigned to a KOG in a given model organism, whereas - indicates its apparent absence.




**Extended Data Figure 3. Dose-response curve of stimulation of FLAG-RTCB by recombinant Archease**

**a**, SDS-PAGE analysis of purified recombinant WT and mutant (D39A, K144A) hexahistidine tagged human Archease. **b**, Dose-response curve of stimulation of the ligase activity (225 nM substrate) of FLAG-RTCB (22.5 nM) by Archease (40 nM to 10.2 μM). The rates of formation of $P_{concat}$ (mean ± SD, $N = 3$) were plotted versus the respective

concentration of Archease. Affinity-purified FLAG-RTCB required relatively high concentrations of recombinant Archease (approximately 1 μM) for half maximal activation of its RNA ligase activity. **c,** A master reaction was assembled as described in the methods section (30 nM FLAG-RTCB, 300 nM substrate), incubated at 37 °C for 90 min (to reach the plateau phase) and transferred to ice. 4.5 μL aliquots of this pre-incubation were mixed with 1.5 μL of recombinant Archease solution to final concentrations of 40 nM - 10.25 μM and equilibrated for 4 min at 37 °C. The concentrations of all cofactors present in the reaction mixture were adjusted accordingly. Aliquots of the reaction were quenched at 4, 5.5, 7 and 8.5 min and analyzed by denaturing electrophoresis. Pilot experiments were carried out before to ensure near linear behavior of the reactions at the chosen conditions. Reaction rates were determined by linear regression and are stated as slopes [nM/s] ± standard error of the mean ($N$ = 3).



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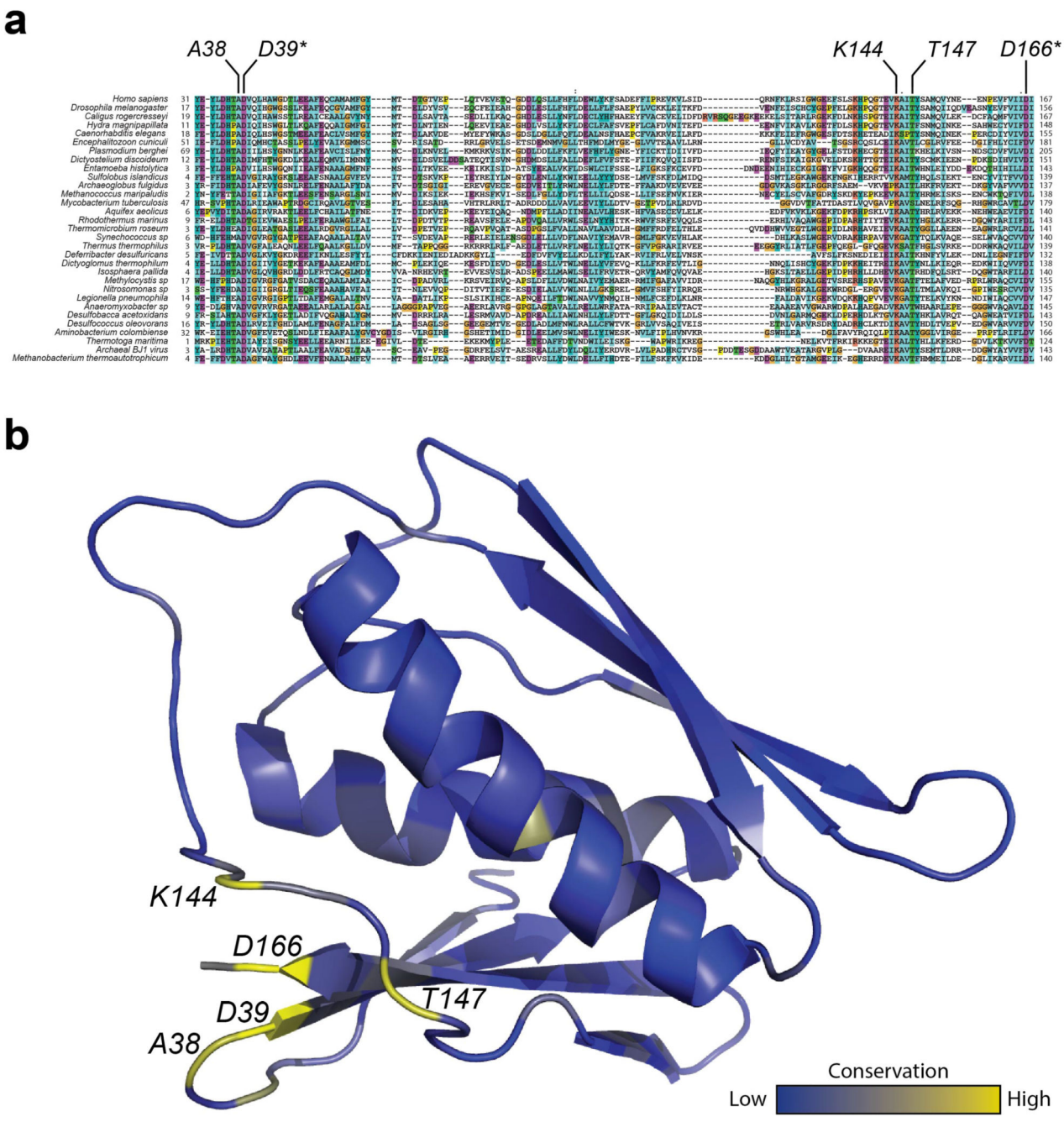
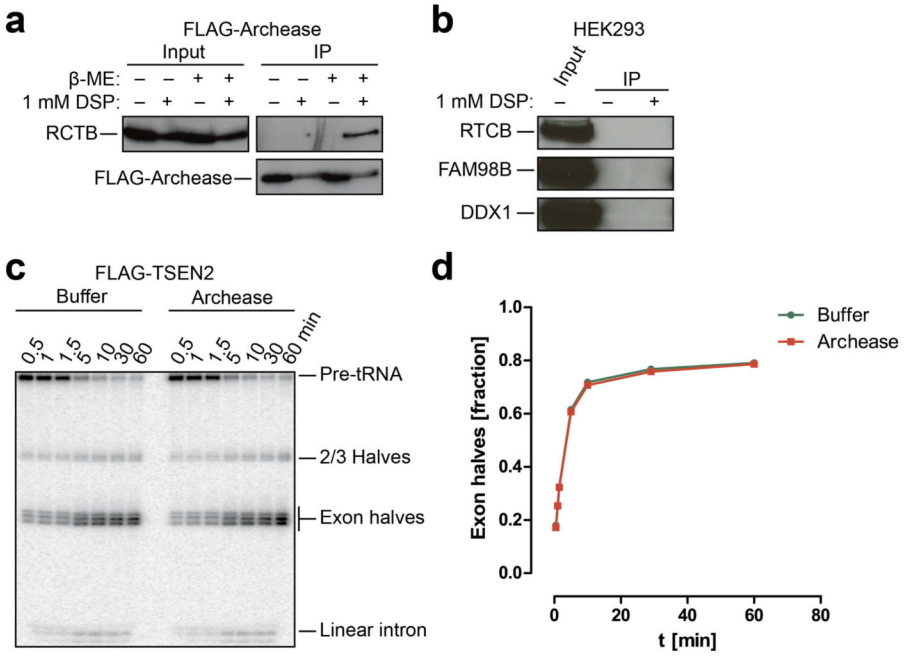



**Extended Data Figure 4. Archease is highly conserved from bacteria and archaea to metazoa**

**a,** Alignment of Archease proteins from various prokaryotic and eukaryotic organisms. Positions of residues examined in this study are indicated above. Residues marked by an asterisk have recently been implicated in metal binding in a structure of Archease from *Pyrococcus horikoshii*[28]. **b**, Archease residues examined by mutagenesis (D39, K144 and T147) are highly conserved and positioned in close proximity on the surface of the protein as suggested by an NMR structure of Archease from *Methanobacterium*

*thermoautotrophicum*. Colors indicate conservation (yellow: highest-, blue: lowest degree of conservation).




**g**

| (His)$_6$-Archease | GTP | ATP | AMPPcP | $k_a$ $10^5$ M$^{-1}$ s$^{-1}$ | $k_d$ $10^{-4}$ s$^{-1}$ | $K_D$ nM |
|---|---|---|---|---|---|---|
| wild type | – | – | – | 7.3 ± 2.7 | 7.7 ± 2.1 | 1.2 ± 0.6 |
| | + | + | – | 5.4 ± 3.9 | 7.6 ± 0.5 | 1.8 ± 0.9 |
| | + | – | + | 6.1 ± 4.0 | 6.5 ± 1.9 | 1.3 ± 0.5 |
| D39A | – | – | – | 7.2 ± 2.9 | 8.4 ± 2.8 | 1.3 ± 0.7 |
| | + | + | – | 5.9 ± 3.9 | 7.7 ± 2.1 | 1.5 ± 0.5 |
| | + | – | + | 6.8 ± 4.5 | 6.6 ± 1.1 | 1.2 ± 0.6 |

**Extended Data Figure 5. Affinity-purified FLAG-Archease associates with RTCB**

**a,** Reducing (2 M β-mercaptoethanol) and non-reducing Western blot of FLAG-Archease purified from HEK293 cells in presence or absence of DSP. **b,** Western blot of FLAG control eluates prepared from non-transfected HEK293 cells treated with DSP or DMSO. **c**, tRNA cleavage assay (100 nM substrate) of FLAG-TSEN2 (~10 nM) affinity-purified from

HEK293 cells in presence or absence of Archease (7.5 μM). **d,** Quantification of data shown in c by phosphorimaging. **e**, SPR sensorgram overlay demonstrating concentration dependent binding of Archease to RTCB. **f**, Mathematical sensorgram fitting to a Langmuir 1:1 interaction model (using FLAG-RTCB as analyte) at the indicated concentrations. Data are displayed as subtractive curves against an amine-activated reference surface. **g**, Kinetic constants ($k_a$, $k_d$, $K_D$) for Archease and FLAG-RTCB complexes of hexahistidine tagged WT and mutant (D39A) Archease in the absence of nucleotide triphosphate cofactors, the presence of GTP and ATP or GTP and the non-hydrolysable ATP analogue AMPPcP. Neither inactivating mutagenesis of Archease nor substitution or omission of cofactors affected the obtained binding constants to a significant extent. Unchanged affinity for Archease in presence of non-hydrolysable AMPPcP indicates that ATP hydrolysis is not required for recruitment of Archease to the ligase complex.



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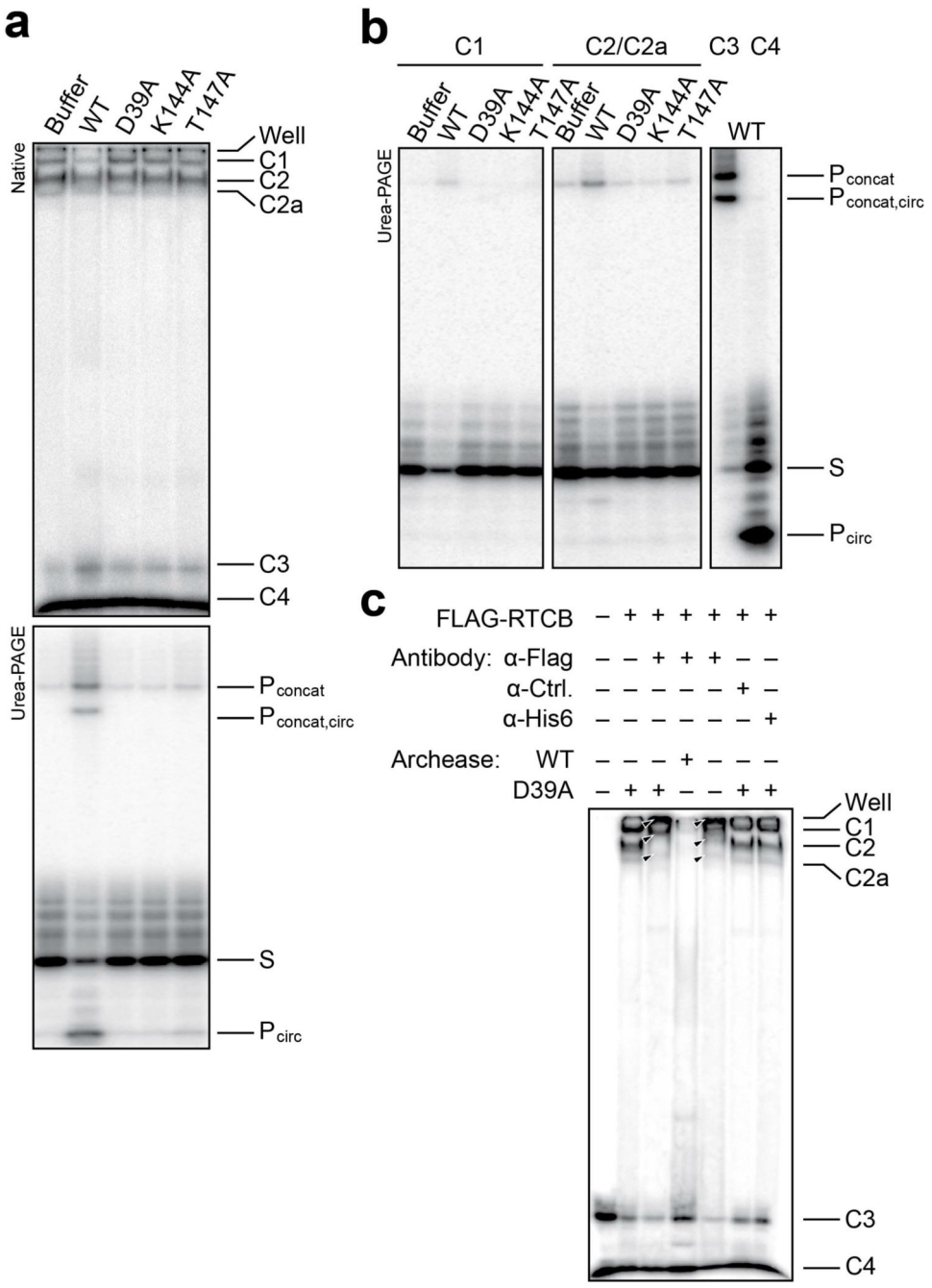
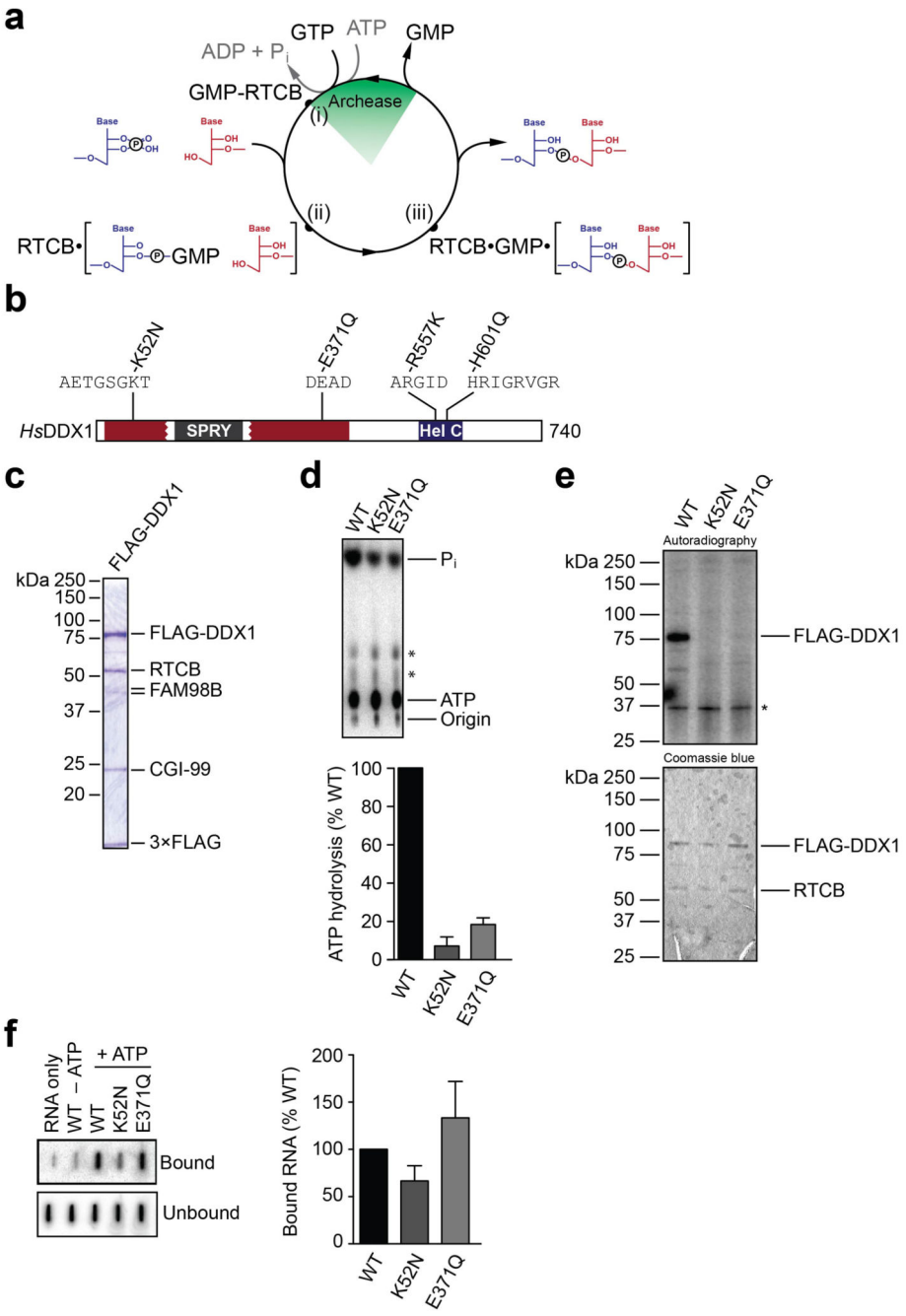



**Extended Data Figure 6. Mechanistic aspects of the stimulation of RTCB by Archease**

**a,** Native PAGE of FLAG-RTCB-RNA adducts formed in presence of WT or mutant (D39A, K144A, T147A) Archease at 5 min (upper panel). Denaturing PAGE of the same reactions (lower panel). **b,** Denaturing PAGE of FLAG-RTCB-RNA adducts isolated from the gel shown in a. (Note that part of Extended Data Figure 6b is identical to Fig 3b where the results for the two further mutants were not shown for clarity). **c**, Antibody supershift assays (α-FLAG retards FLAG-RTCB, α-His6 retards recombinant Archease, α-Ctrl.) of FLAG-RTCB-RNA adducts assembled in presence of mutant (D39A) Archease (30 min).

Incubation with WT Archease demonstrates that antibody incubation does not inhibit ligation. Arrowheads indicate the position of supershifted complexes. A marked supershift in the presence of anti-FLAG antibodies indicates that the visualized complexes contain FLAG-RTCB.




**Extended Data Figure 7. Mechanistic aspects of RTCB-catalyzed RNA ligation and characterization of mutant versions of DDX1**

**a**, Tentative model illustrating the mode of action of human Archease (indicated by the green shaded area) in the RTCB reaction cycle. Prior to activating RNA 2′,3′ cyclic

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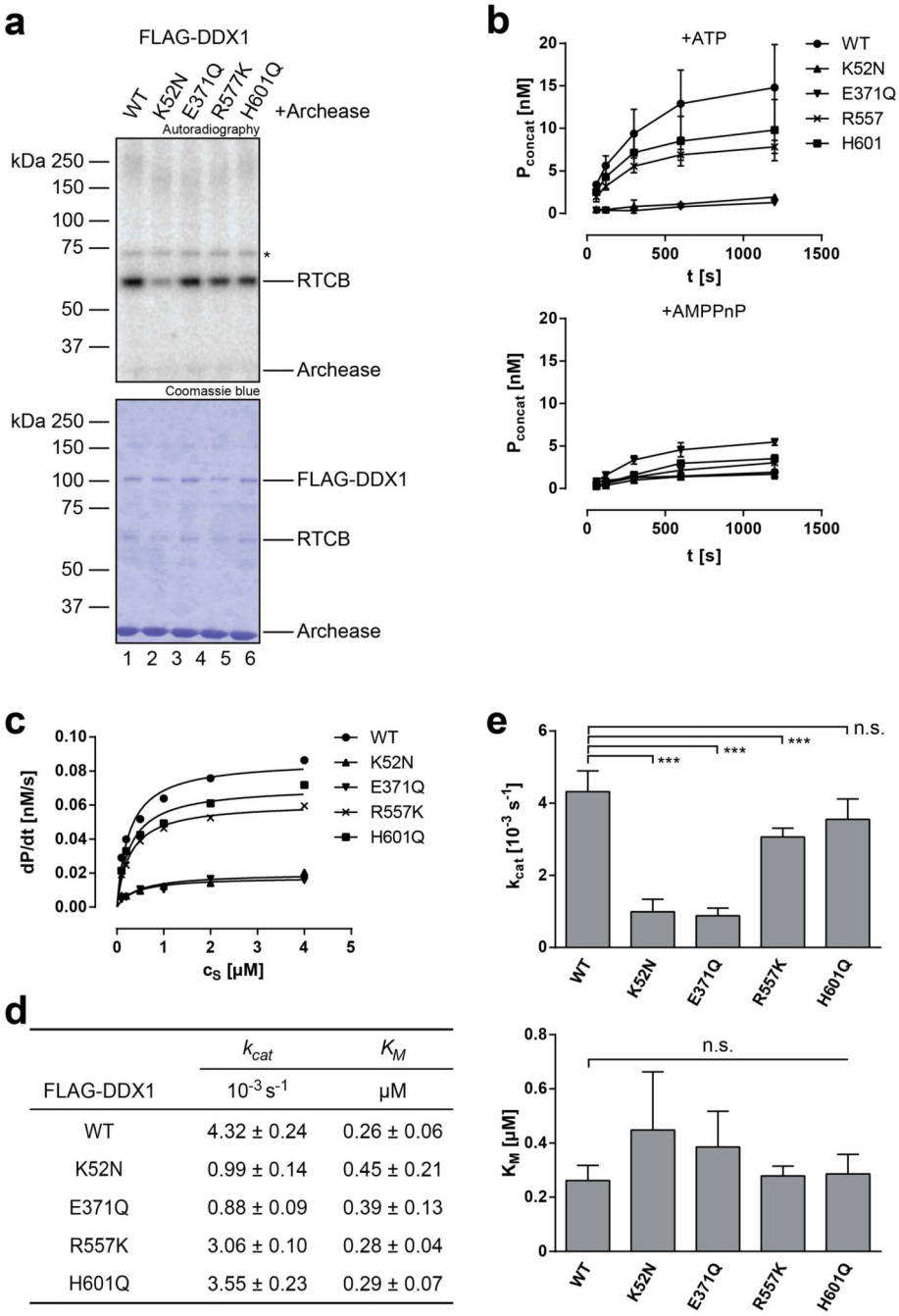
phosphate termini for ligation, RTCB undergoes guanylation resulting in a guanylated form of the enzyme (i). GMP is then transferred to the 3′ end of the RNA substrate (ii). After ligation the active site of RTCB is occupied by the products of the reaction (iii). Another round of catalysis requires guanylation of the RTCB active site which depends on Archease and is extensively stimulated by ATP hydrolysis by DDX1. **b**, Domain structure of DDX1 (DEAD helicase domain in dark red, SPRY domain in grey and c-terminal helicase domain in blue). DDX1 was mutagenized in the ATPase A motif (K52N, predicted to interfere with ATP binding), in the ATPase B motif (E371Q, predicted to interfere with ATP hydrolysis), or in the C-terminal helicase domain (R557K and H601Q, predicted to interfere with helicase activity)[21]. **c**, SDS-PAGE- of a 10 μL aliquot of FLAG-DDX1 purified from HEK293 cells. **d,** ATPase assay of WT and mutant (K52N, E371Q) FLAG-DDX1 (upper panel), quantified signals are displayed in the lower panel (mean ± SEM, $N = 4$). **e**, SDS-PAGE analysis of FLAG-DDX1 (WT, K52N and E371Q) crosslinked to [α-$^{32}$P]ATP. The upper panel (autoradiography) reveals affected ATP crosslinking for the two mutants K52N and E371Q while the lower panel (Coomassie blue) confirms equal loading of the purified complexes. **f**, RNA filter binding assay of WT and mutant (K52N, E371Q) FLAG-DDX1 (left panel). Quantified signals obtained for WT FLAG-DDX1 in the presence of ATP were set to 100 % (right panel, mean ± SEM, $N = 9$).




**d**

| FLAG-DDX1 | $k_{cat}$ $10^{-3}$ s$^{-1}$ | $K_M$ μM |
|---|---|---|
| WT | 4.32 ± 0.24 | 0.26 ± 0.06 |
| K52N | 0.99 ± 0.14 | 0.45 ± 0.21 |
| E371Q | 0.88 ± 0.09 | 0.39 ± 0.13 |
| R557K | 3.06 ± 0.10 | 0.28 ± 0.04 |
| H601Q | 3.55 ± 0.23 | 0.29 ± 0.07 |

**Extended Data Figure 8. Effect of mutagenesis of DDX1 on the activity of the human RNA ligase complex**

**a**, SDS-PAGE of WT or mutant (K52N, E271Q, R577K, H601Q) FLAG-DDX1 incubated with Archease and [α-$^{32}$P]GTP. **b**, Ligase assays (100 nM substrate) of 20 nM WT or mutant (K52N, E271Q, R577K, H601Q) FLAG-DDX1 in the presence of Archease (10 μM) and 0.5 mM ATP (upper panel) or AMPPnP (lower panel). **c**, Michaelis Menten kinetics of WT or mutant (K52N, E271Q, R577K, H601Q) DDX1 (20 nM RNA ligase complex, 10 μM Archease). Reaction rates were estimated as indicated in Extended Data Figure 9. **d**,

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Michaelis Menten parameters (mean ± SEM) were obtained by nonlinear regression using GraphPad. $k_{cat}$ values were obtained by multiplication of $V_{max}$ values by the concentration of ligase complex. **e**, Michaelis Menten parameters of WT and mutant FLAG-DDX1 (mean ± SEM). Comparisons between WT and mutant FLAG-DDX1 were performed by z-test. The z-statistics were computed as (<WT> - <mutant>)/(sqrt(SEM(<WT>)^2 + SEM(<mutant>)^2). From these z-values we obtained Bonferoni-corrected two sided p-values (n.s. $p > 0.05$, *** $p < 0.0004$). The mutants K52N and E371Q exhibit a marked reduction of $k_{cat}$ with only insignificant changes of $K_M$ indicating that ATP binding and hydrolysis contribute to catalytic steps (such as guanyl transfer) of human RNA ligase rather than substrate binding.



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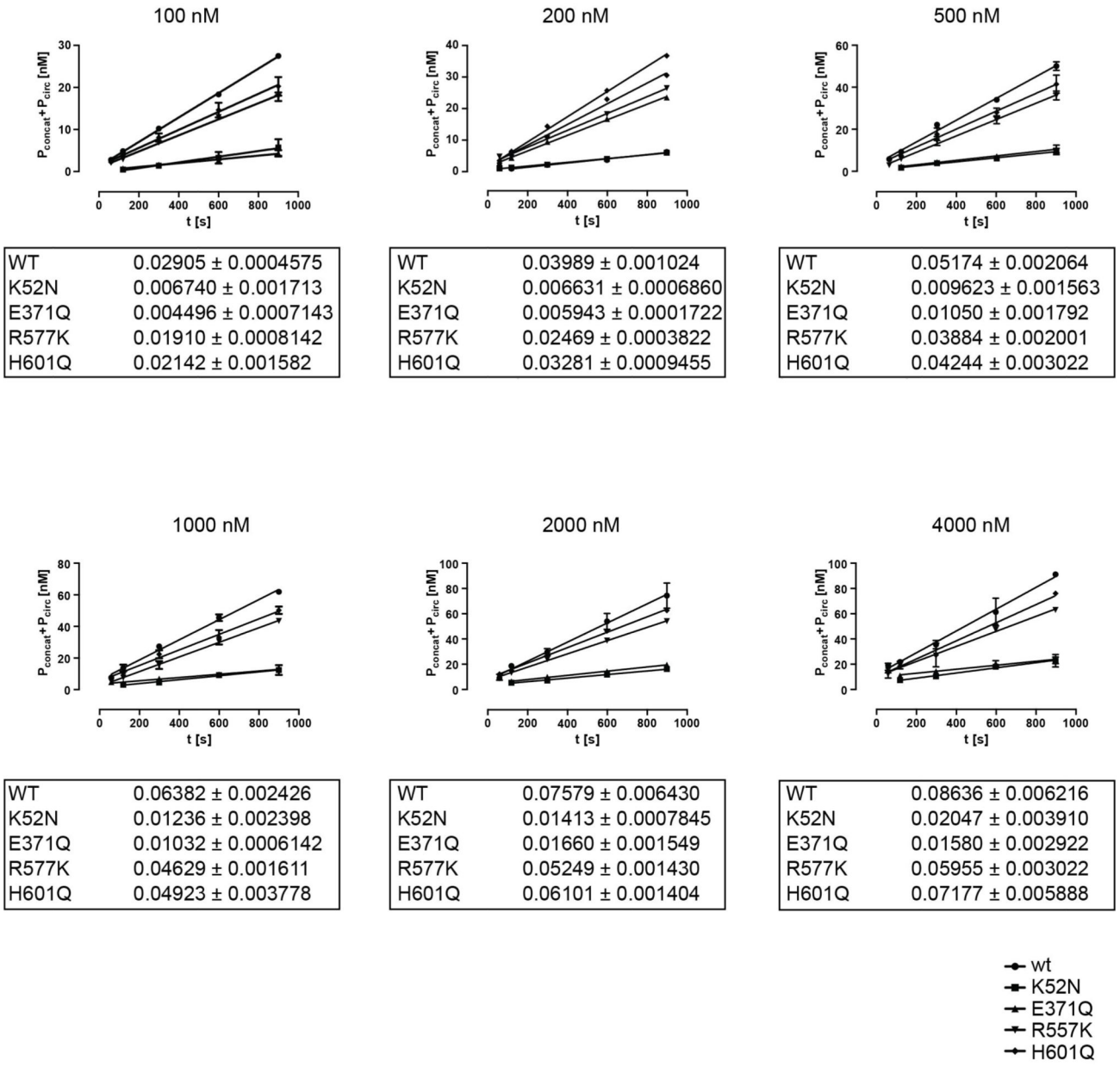



| | |
|---|---|
| WT | 0.02905 ± 0.0004575 |
| K52N | 0.006740 ± 0.001713 |
| E371Q | 0.004496 ± 0.0007143 |
| R577K | 0.01910 ± 0.0008142 |
| H601Q | 0.02142 ± 0.001582 |

| | |
|---|---|
| WT | 0.03989 ± 0.001024 |
| K52N | 0.006631 ± 0.0006860 |
| E371Q | 0.005943 ± 0.0001722 |
| R577K | 0.02469 ± 0.0003822 |
| H601Q | 0.03281 ± 0.0009455 |

| | |
|---|---|
| WT | 0.05174 ± 0.002064 |
| K52N | 0.009623 ± 0.001563 |
| E371Q | 0.01050 ± 0.001792 |
| R577K | 0.03884 ± 0.002001 |
| H601Q | 0.04244 ± 0.003022 |

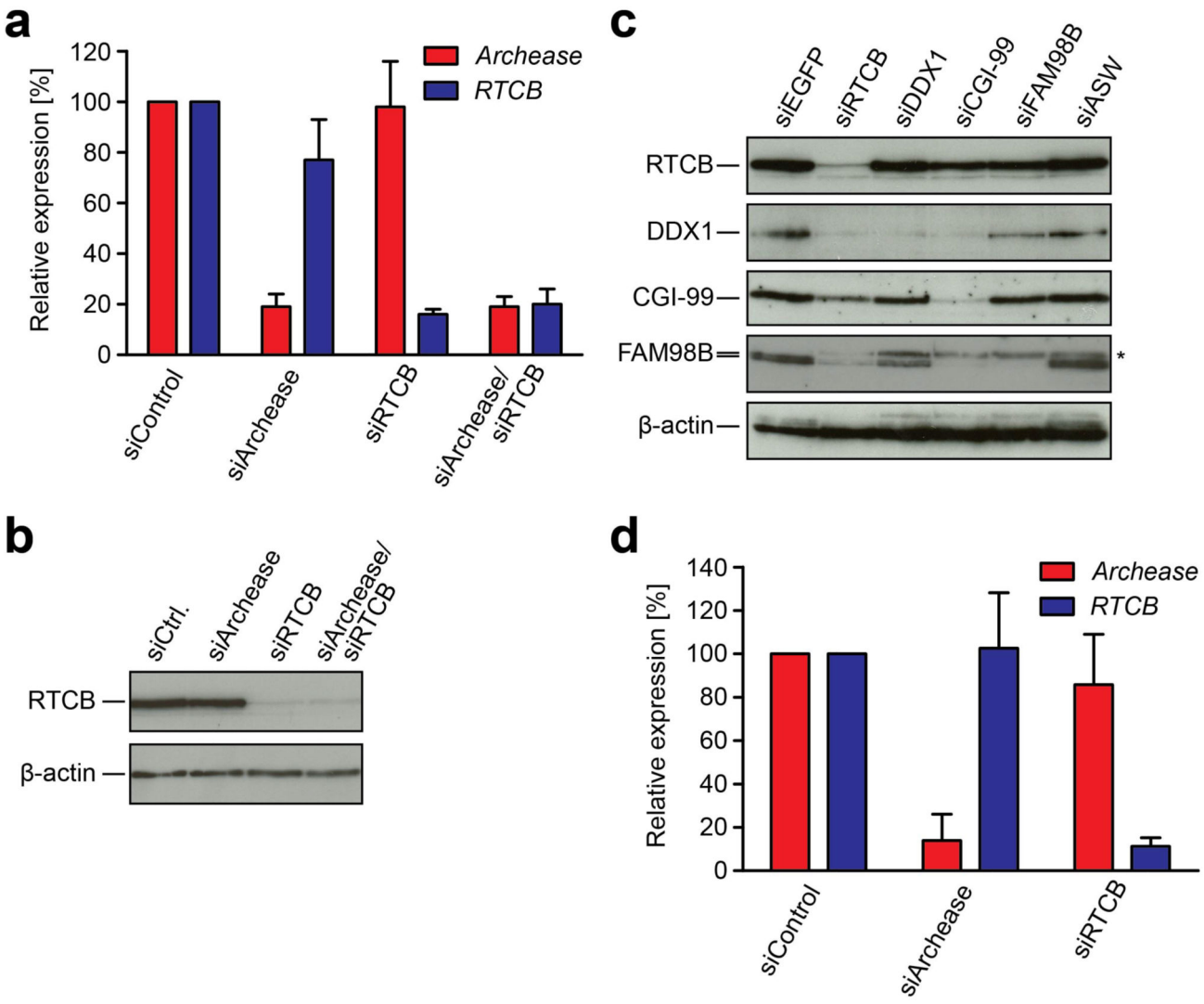
| | |
|---|---|
| WT | 0.06382 ± 0.002426 |
| K52N | 0.01236 ± 0.002398 |
| E371Q | 0.01032 ± 0.0006142 |
| R577K | 0.04629 ± 0.001611 |
| H601Q | 0.04923 ± 0.003778 |

| | |
|---|---|
| WT | 0.07579 ± 0.006430 |
| K52N | 0.01413 ± 0.0007845 |
| E371Q | 0.01660 ± 0.001549 |
| R577K | 0.05249 ± 0.001430 |
| H601Q | 0.06101 ± 0.001404 |

| | |
|---|---|
| WT | 0.08636 ± 0.006216 |
| K52N | 0.02047 ± 0.003910 |
| E371Q | 0.01580 ± 0.002922 |
| R577K | 0.05955 ± 0.003022 |
| H601Q | 0.07177 ± 0.005888 |

**Extended Data Figure 9. Determination of RNA ligase reaction rates for estimation of Michaelis Menten parameters of FLAG-DDX1 complexes**

The progress of reactions (20 nM RNA ligase complex, 100 nM – 4 μM substrate, 10 μM Archease) was recorded for 15 min assuming near-linear behaviour of the reaction in this time frame (mean ± SEM, $N = 2$). Reaction rates were determined by linear regression, reaction rates (considering both concatemerized and circular product species) for each preparation (WT, K52N, E371Q, R577K, H601Q) are stated below each graph ([nM/s], mean ± SEM).




**Extended Data Figure 10. Depletion of Archease does not indirectly impair tRNA processing by concomitant depletion of RTCB**

**a,** Quantitative reverse transcriptase PCR (qRT-PCR) of *RTCB* and *Archease* messenger RNAs in the cells used to prepare the extracts for the experiments shown in Fig. 4a. Expression levels are plotted as relative amounts of transcripts with respect to control-treated cells (mean ± SD, *N* = 3 technical replicates). **b,** Western blot of RTCB in extracts used for the experiments shown in Fig. 4a. **c**, Western blot of tRNA ligase complex members in extracts used for the experiments shown in Fig. 4d. **d,** qRT-PCR of *RTCB* and *Archease* messenger RNA in cells analyzed in Fig. 4b. Expression levels are plotted as relative amounts of transcripts with respect to control treated cells (mean ± SD, *N* = 3 technical replicates).

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## References

1. Chan CM, Zhou C, Huang RH. Reconstituting bacterial RNA repair and modification in vitro. Science. 2009; 326:247. [PubMed: 19815768]
2. Nandakumar J, Schwer B, Schaffrath R, Shuman S. RNA repair: an antidote to cytotoxic eukaryal RNA damage. Molecular cell. 2008; 31:278–286. [PubMed: 18657509]
3. Sidrauski C, Cox JS, Walter P. tRNA ligase is required for regulated mRNA splicing in the unfolded protein response. Cell. 1996; 87:405–413. [PubMed: 8898194]
4. Filipowicz W, Shatkin AJ. Origin of splice junction phosphate in tRNAs processed by HeLa cell extract. Cell. 1983; 32:547–557. [PubMed: 6186399]
5. Konarska M, Filipowicz W, Gross HJ. RNA ligation via 2′-phosphomonoester, 3′5′-phosphodiester linkage: requirement of 2′,3′-cyclic phosphate termini and involvement of a 5′-hydroxyl polynucleotide kinase. Proceedings of the National Academy of Sciences of the United States of America. 1982; 79:1474–1478. [PubMed: 6280184]
6. Laski FA, Fire AZ, RajBhandary UL, Sharp PA. Characterization of tRNA precursor splicing in mammalian extracts. The Journal of biological chemistry. 1983; 258:11974–11980. [PubMed: 6413507]
7. Zofallova L, Guo Y, Gupta R. Junction phosphate is derived from the precursor in the tRNA spliced by the archaeon Haloferax volcanii cell extract. RNA. 2000; 6:1019–1030. (New York, N.Y). [PubMed: 10917597]
8. Popow J, et al. HSPC117 is the essential subunit of a human tRNA splicing ligase complex. Science. 2011; 331:760–764. [PubMed: 21311021]
9. Popow J, Schleiffer A, Martinez J. Diversity and roles of (t)RNA ligases. Cell Mol Life Sci. 2012 doi:10.1007/s00018-012-0944-2.
10. Englert M, Beier H. Plant tRNA ligases are multifunctional enzymes that have diverged in sequence and substrate specificity from RNA ligases of other phylogenetic origins. Nucleic acids research. 2005; 33:388–399. [PubMed: 15653639]
11. Konarska M, Filipowicz W, Domdey H, Gross HJ. Formation of a 2′-phosphomonoester, 3′,5′-phosphodiester linkage by a novel RNA ligase in wheat germ. Nature. 1981; 293:112–116. [PubMed: 7266664]
12. Phizicky EM, Consaul SA, Nehrke KW, Abelson J. Yeast tRNA ligase mutants are nonviable and accumulate tRNA splicing intermediates. The Journal of biological chemistry. 1992; 267:4577–4582. [PubMed: 1537841]
13. Koonin EV, et al. A comprehensive evolutionary classification of proteins encoded in complete eukaryotic genomes. Genome biology. 2004; 5:R7. [PubMed: 14759257]
14. Tatusov RL, et al. The COG database: an updated version includes eukaryotes. BMC bioinformatics. 2003; 4:41. [PubMed: 12969510]
15. Canaves JM. Predicted role for the archease protein family based on structural and sequence analysis of TM1083 and MTH1598, two proteins structurally characterized through structural genomics efforts. Proteins. 2004; 56:19–27. doi:10.1002/prot.20141. [PubMed: 15162483]
16. Auxilien S, El Khadali F, Rasmussen A, Douthwaite S, Grosjean H. Archease from Pyrococcus abyssi improves substrate specificity and solubility of a tRNA m5C methyltransferase. The Journal of biological chemistry. 2007; 282:18711–18721. doi:M607459200 [pii] 10.1074/jbc.M607459200. [PubMed: 17470432]
17. Brzezicha B, et al. Identification of human tRNA:m5C methyltransferase catalysing intron-dependent m5C formation in the first position of the anticodon of the pre-tRNA Leu (CAA). Nucleic acids research. 34:6034–6043. doi:gkl765 [pii] 10.1093/nar/gkl765 (2006). [PubMed: 17071714]

18. Paushkin SV, Patel M, Furia BS, Peltz SW, Trotta CR. Identification of a human endonuclease complex reveals a link between tRNA splicing and pre-mRNA 3′ end formation. Cell. 2004; 117:311–321. [PubMed: 15109492]
19. Chakravarty AK, Subbotin R, Chait BT, Shuman S. RNA ligase RtcB splices 3′-phosphate and 5′-OH ends via covalent RtcB-(histidinyl)-GMP and polynucleotide-(3′)pp(5′)G intermediates. Proceedings of the National Academy of Sciences of the United States of America. 109:6072–6077. doi:1201207109 [pii] 10.1073/pnas.1201207109 (2012). [PubMed: 22474365]
20. Tanaka N, Chakravarty AK, Maughan B, Shuman S. A novel mechanism of RNA repair by RtcB via sequential 2′,3′-cyclic phosphodiesterase and 3′-phosphate/5′-hydroxyl ligation reactions. The Journal of biological chemistry. 2011; 286:43134–43143. [PubMed: 22045815]
21. Pause A, Sonenberg N. Mutational analysis of a DEAD box RNA helicase: the mammalian translation initiation factor eIF-4A. The EMBO journal. 1992; 11:2643–2654. [PubMed: 1378397]
22. Perkins KK, Furneaux H, Hurwitz J. Isolation and characterization of an RNA ligase from HeLa cells. Proceedings of the National Academy of Sciences of the United States of America. 1985; 82:684–688. [PubMed: 3856222]
23. Godbout R, Packer M, Bie W. Overexpression of a DEAD box protein (DDX1) in neuroblastoma and retinoblastoma cell lines. The Journal of biological chemistry. 1998; 273:21161–21168. [PubMed: 9694872]
24. Kanai Y, Dohmae N, Hirokawa N. Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. Neuron. 2004; 43:513–525. [PubMed: 15312650]
25. Cao D, Haussecker D, Huang Y, Kay MA. Combined proteomic-RNAi screen for host factors involved in human hepatitis delta virus replication. RNA. 2009; 15:1971–1979. (New York, N.Y.). [PubMed: 19776158]
26. Edgcomb SP, et al. DDX1 Is an RNA-Dependent ATPase Involved in HIV-1 Rev Function and Virus Replication. Journal of molecular biology. 2012; 415:61–74. [PubMed: 22051512]
27. Zhang Z, et al. DDX1, DDX21, and DHX36 helicases form a complex with the adaptor molecule TRIF to sense dsRNA in dendritic cells. Immunity. 2011; 34:866–878. [PubMed: 21703541]
28. Desai KK, Cheng CL, Bingman CA, Phillips GN Jr. Raines RT. A tRNA splicing operon: Archease endows RtcB with dual GTP/ATP cofactor specificity and accelerates RNA ligation. Nucleic acids research. 2014 doi:10.1093/nar/gkt1375.
29. Dandekar T, Snel B, Huynen M, Bork P. Conservation of gene order: a fingerprint of proteins that physically interact. Trends in biochemical sciences. 1998; 23:324–328. doi:S0968-0004(98)01274-2 [pii]. [PubMed: 9787636]
30. Ciria R, Abreu-Goodger C, Morett E, Merino E. GeConT: gene context analysis. Bioinformatics. 2004; 20:2307–2308. doi:10.1093/bioinformatics/bth216 bth216 [pii]. [PubMed: 15073003]

## Methods References

31. Altschul SF, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research. 1997; 25:3389–3402. [PubMed: 9254694]
32. Katoh K, Toh H. Recent developments in the MAFFT multiple sequence alignment program. Briefings in bioinformatics. 2008; 9:286–298. [PubMed: 18372315]
33. Thompson JD, Gibson TJ, Higgins DG. Multiple sequence alignment using ClustalW and ClustalX. Current protocols in bioinformatics / editoral board, Andreas D. Baxevanis … [et al. 2002 Chapter 2, Unit 2 3. [PubMed: 18792934]
34. Yee A, et al. An NMR approach to structural proteomics. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:1825–1830. doi:10.1073/pnas.042684599 99/4/1825 [pii]. [PubMed: 11854485]
35. Pei J, Grishin NV. AL2CO: calculation of positional conservation in a protein sequence alignment. Bioinformatics. 2001; 17:700–712. [PubMed: 11524371]
36. van de Wetering M, et al. Specific inhibition of gene expression using a stably integrated, inducible small-interfering-RNA vector. EMBO reports. 2003; 4:609–615. [PubMed: 12776180]

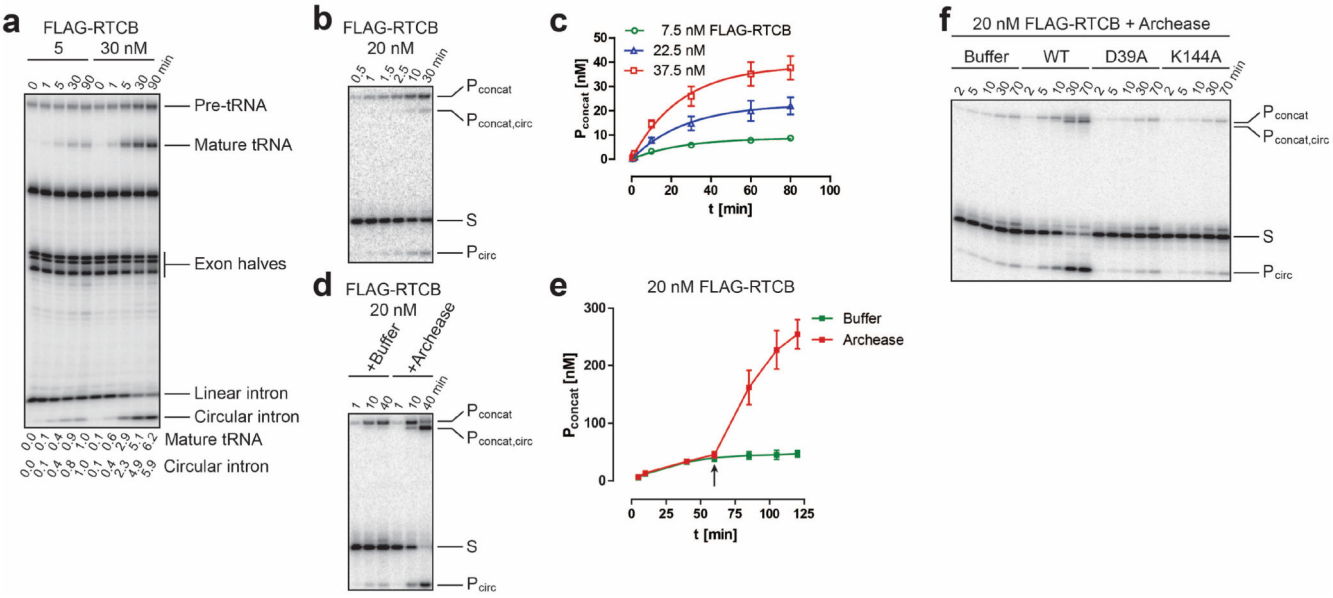


**Figure 1. Archease facilitates multiple enzymatic turnover of RTCB**

**a**, FLAG-RTCB (5 or 30 nM) was incubated with cleaved pre-tRNA transcripts. Numbers below lanes indicate amounts of mature tRNA and intron (arbitrary numbers relative to 5 nM FLAG-RTCB at 90 min, set to 1.0). **b**, FLAG-RTCB converts linear RNA fragments (S, 100 nM) into concatemerized- ($P_{concat}$), circularized concatemer- ($P_{concat,circ}$) and circularized ($P_{circ}$) products. **c**, The endpoint of ligation reactions (1 μM linear RNA substrate) depends on FLAG-RTCB concentration. Values represent mean and standard deviation (SD) of technical replicates ($N$ = 3). **d**, RNA ligase assays (100 nM substrate) in presence or absence of recombinant Archease (10 μM). **e**, Addition of Archease (10 μM, arrow) re-starts stalled ligase reactions (500 nM substrate). (mean ± SD, $N$ = 3) **f**, Ligase reactions in presence of buffer, recombinant WT, D39A or K144A mutant Archease (10 μM) with linear substrate (1 μM).

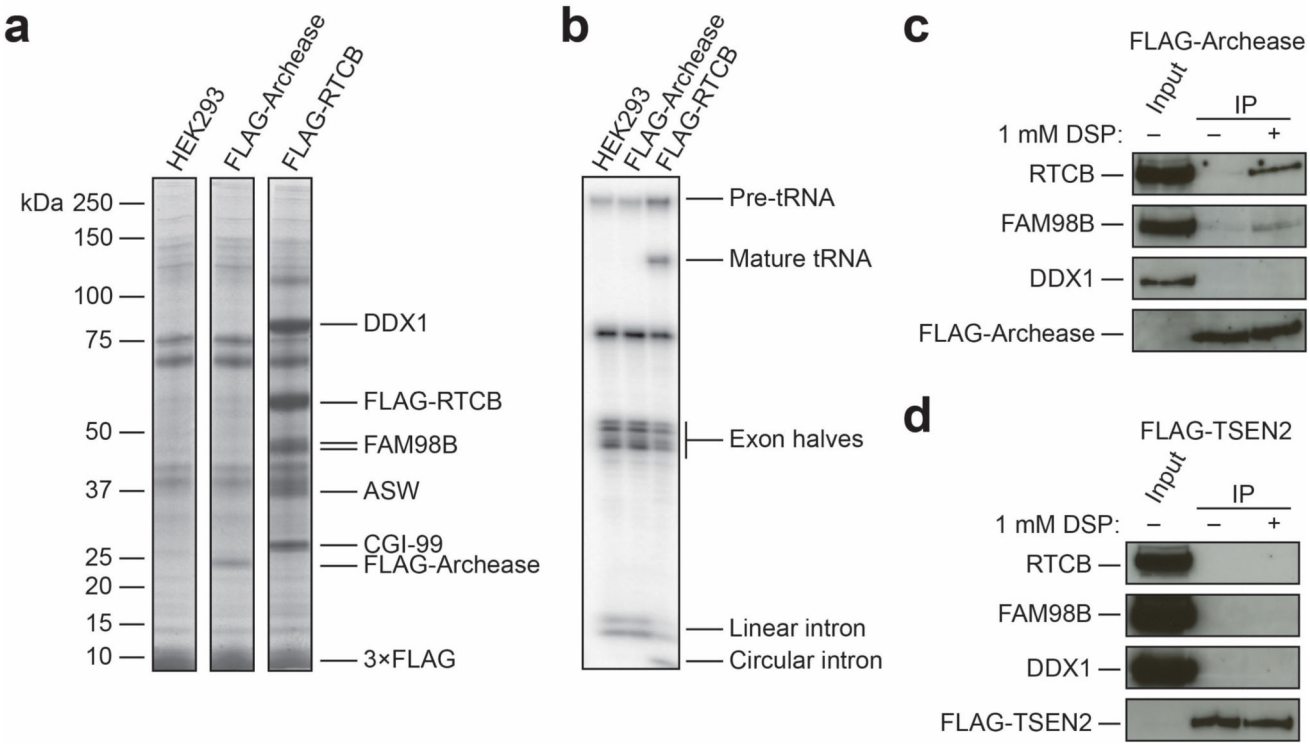


**Figure 2. Archease co-purifies with human RTCB upon *in vivo* crosslink**

**a**, SDS-PAGE analysis (Coomassie blue) of FLAG-Archease and FLAG-RTCB affinity-purified from HEK293 cells (150 mM NaCl). **b**, RNA ligase assay (cleaved pre-tRNA transcripts) of FLAG-Archease and FLAG-RTCB preparations analyzed in **a**. **c**, Western blot of FLAG-Archease affinity-purified from HEK293 cells treated with 1 mM DSP or DMSO. **d**, Western blot of FLAG-TSEN2 affinity-purified from HEK293 cells treated with 1 mM DSP or DMSO.




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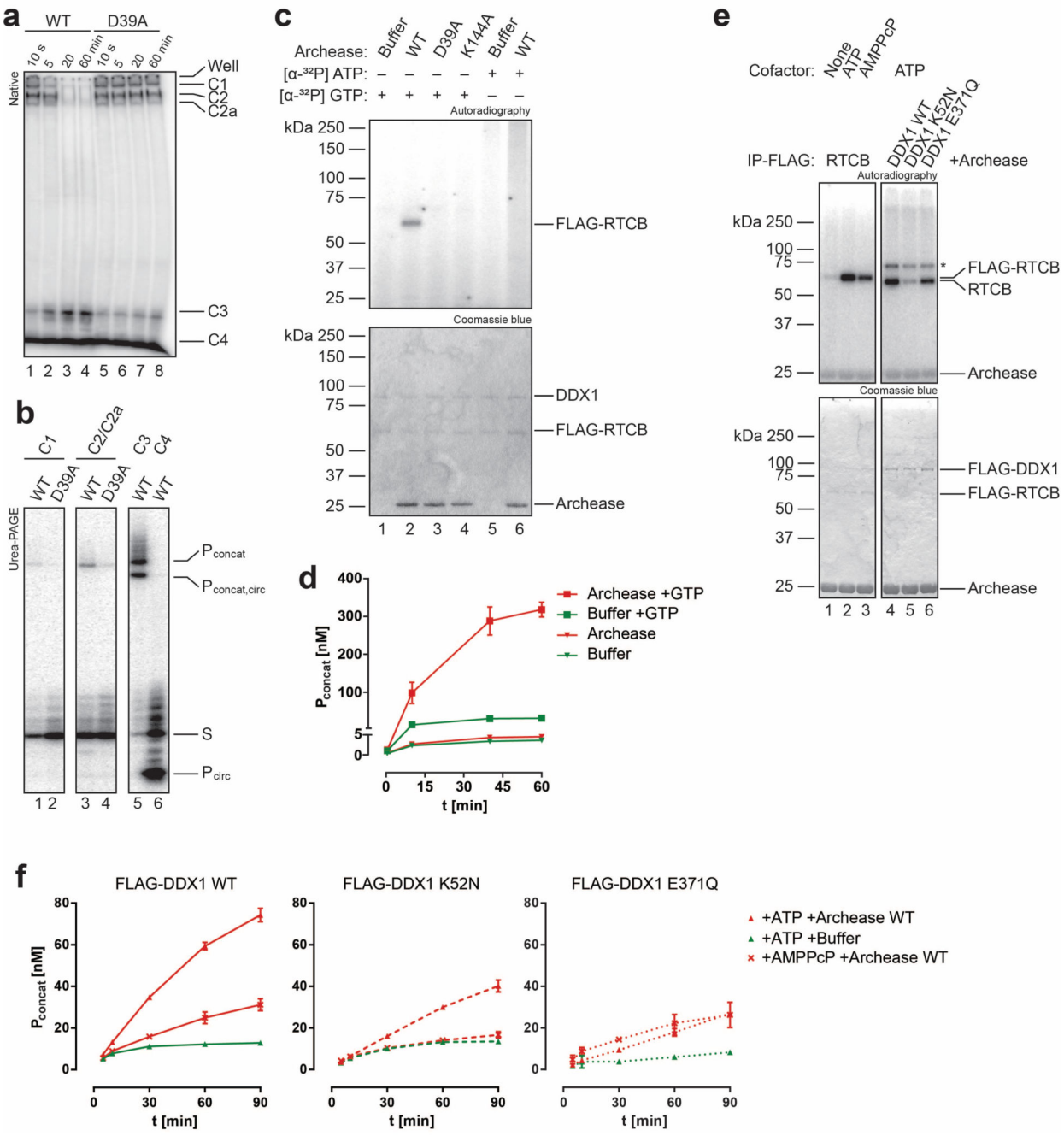


**Figure 3. Archease co-operates with DDX1 to guanylate RTCB *in vitro***

**a**, Time-course native PAGE of FLAG-RTCB-RNA adducts in presence of WT or D39A mutant Archease. **b**, Denaturing PAGE of the RNA content of FLAG-RTCB-RNA adducts at 5 min in presence of WT or mutant Archease. **c**, SDS-PAGE of FLAG-RTCB incubated with buffer, WT or mutant (D39A, K144A) Archease and [α-$^{32}$P]GTP or [α-$^{32}$P]ATP. **d**, Ligation reactions (20 nM FLAG-RTCB, 500 nM substrate) in presence of buffer or Archease (10 μM) omitting (triangles) or including GTP (0.5 mM, squares). (mean ± SD, *N* = 3) **e**, SDS-PAGE of FLAG-RTCB, WT or mutant FLAG-DDX1 (K52N, E371Q)




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incubated with 0.1 mM EDTA, ATP or AMPPcP, [α-$^{32}$P]GTP and Archease. The asterisk indicates an unrelated band.**f**, Ligase assays (1 μM substrate) of 20 nM WT or mutant (K52N, E371Q) FLAG-DDX1 in the presence (red triangles and crosses) or absence (green triangles) of Archease (10 μM) and 0.5 mM ATP (red and green triangles) or AMPPcP (red crosses). (mean ± SD, *N* = 3)



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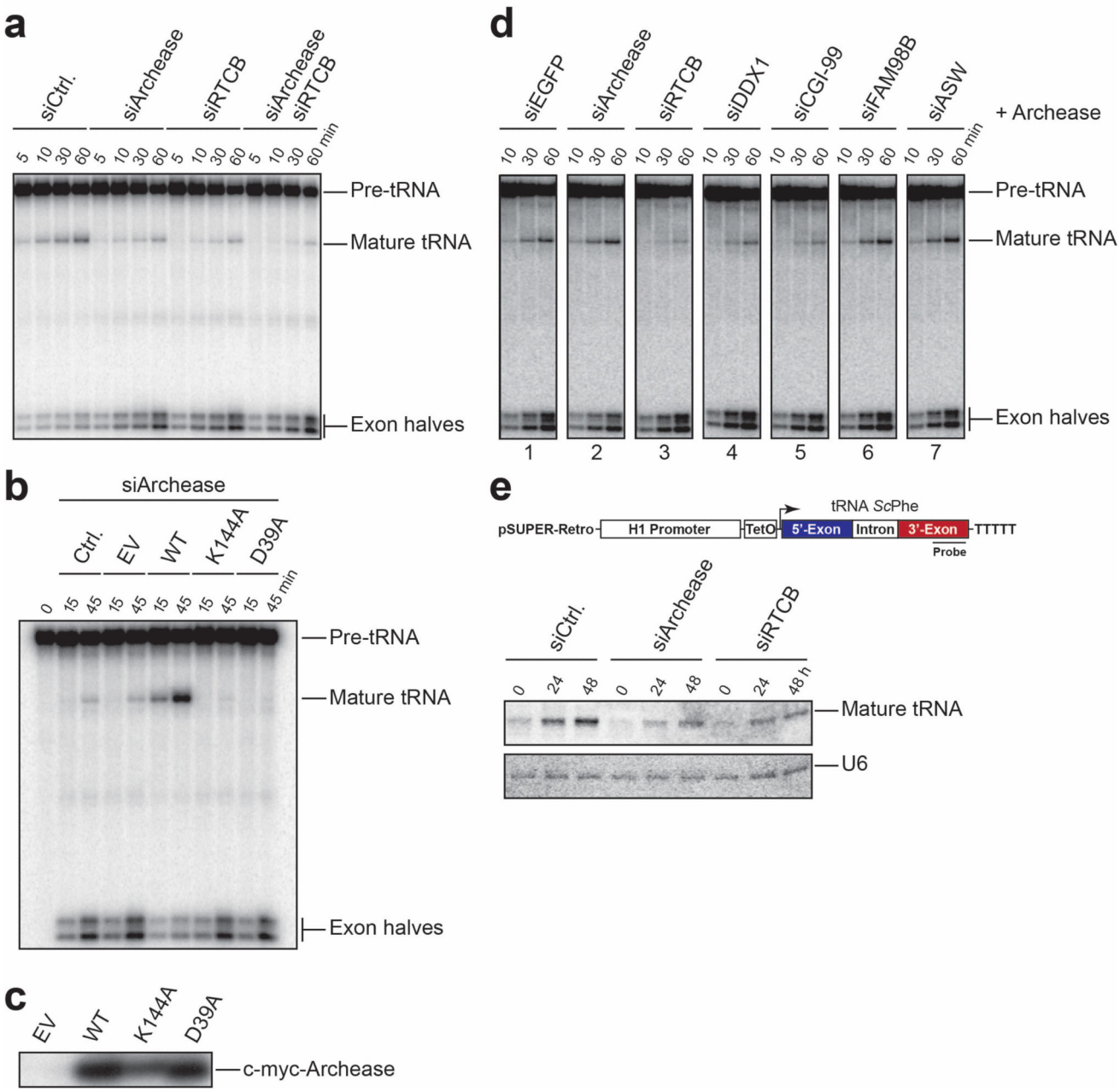


**Figure 4. tRNA maturation relies on RTCB, Archease and DDX1 *in vitro* and in living cells**

**a**, *In vitro* tRNA maturation assay of HeLa extracts RNAi-depleted of Archease, RTCB or both. **b**, *In vitro* tRNA maturation assay of HeLa extracts RNAi-depleted of Archease and simultaneously transfected with vector (EV), c-myc–tagged WT or mutant (D39A, K144A) Archease. **c**, Western blot of extracts assayed in **b**. **d**, *In vitro* tRNA maturation assay of HeLa extracts RNAi-depleted of the indicated tRNA ligase complex subunits supplemented with Archease (10 μM). **e**, Northern blot of RNA isolated from HeLa cells RNAi-depleted of Archease or RTCB. Samples were isolated at the indicated time points after induction of tagged pre-tRNA.




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