Supplementary data

The CNOT1 (MIF4G-DUF3819) and CNOT9 subunits stimulate deadenylation by the Ccr4-Not nuclease module

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This file contains:

- Supplementary figures Fig. S1-Fig. S5

Supplementary figures

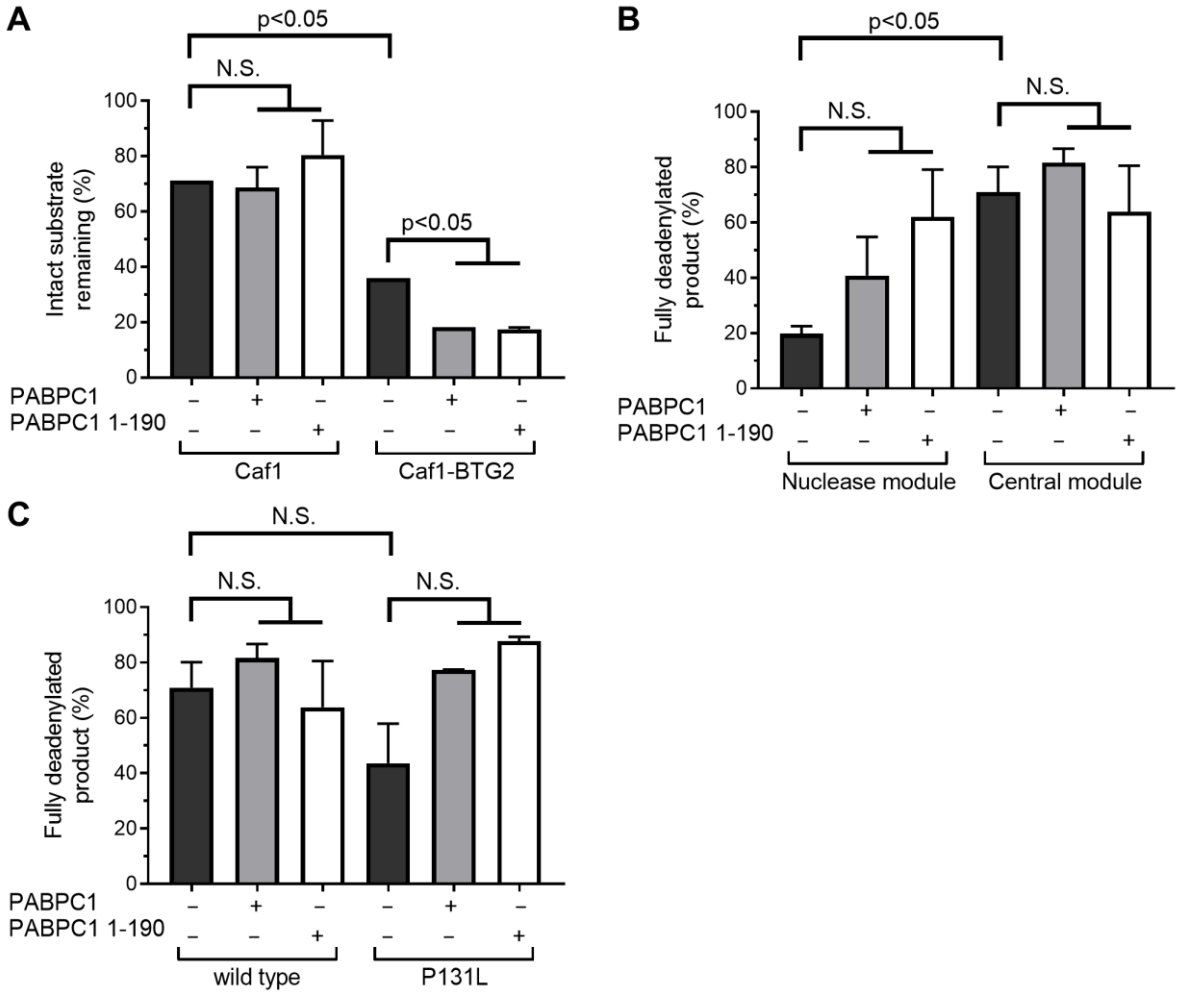

Fig. S1. Deadenylation of 5'Flc-A_{20} substrates. (A) Comparison of deadenylation by Caf1 and BTG2-Caf1. (B) Comparison of deadenylation by the trimeric nuclease module and the pentameric central module of Ccr4-Not. (C) Comparison of deadenylation by the central module of Ccr4-Not containing wild type CNOT9 or the melanoma-associated CNOT9 P131L variant. Synthetic oligonucleotides (5'Flc-$(A)_{20}$) were incubated with the indicated enzymes (100 nM) at 30°C for 60 min. Products were analysed by denaturing 50% urea/16% PAGE followed by densitometric analysis using ImageJ. Error bars show the mean and standard deviation.

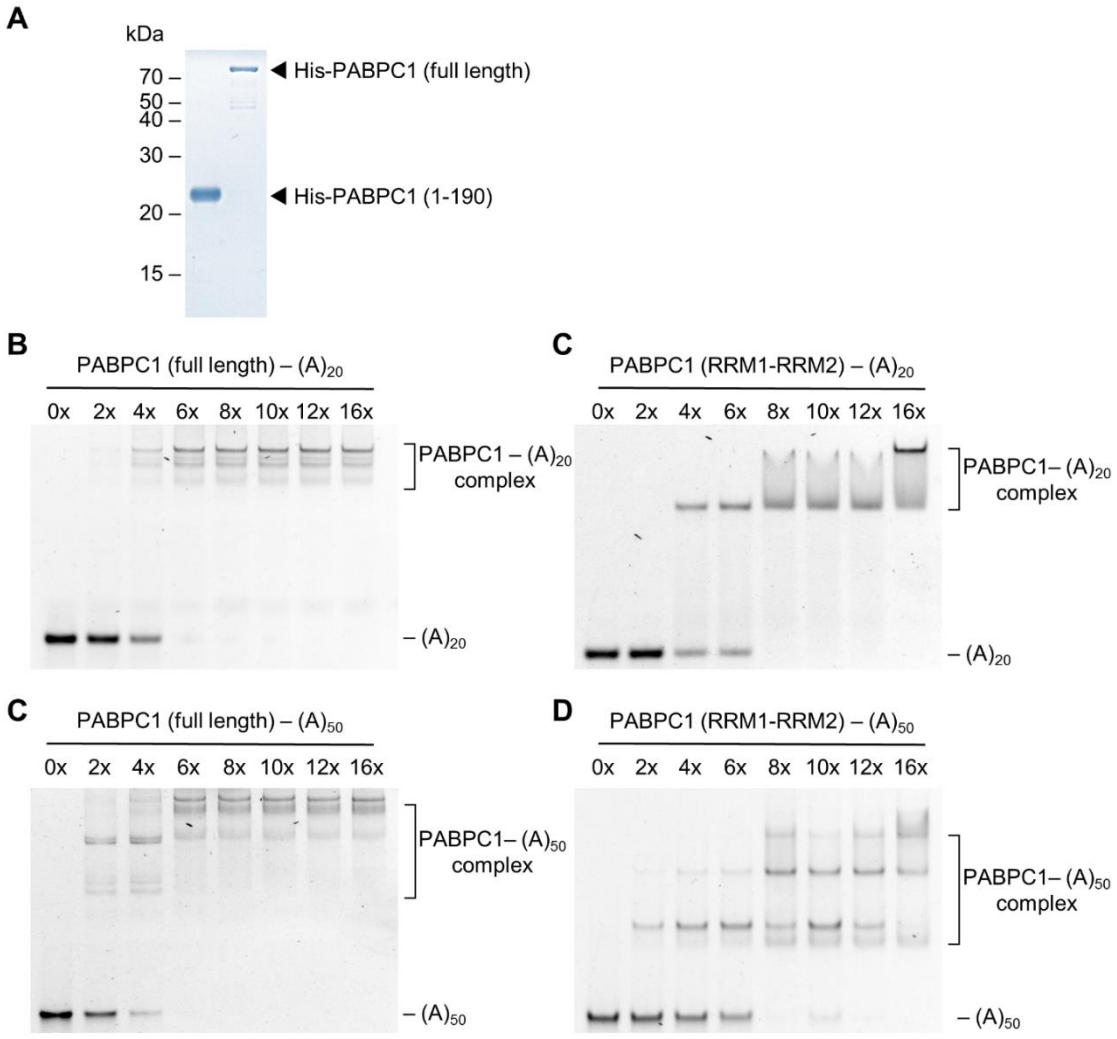

Fig. S2. Analysis of PABPC1 – poly(A) substrates by electrophoretic mobility shift assays. (A) Analysis of purified full length PABPC1 and PABPC1 (1-190). Purified proteins were analysed by 14% SDS-PAGE and visualised by staining with Coomassie Brilliant Blue. (B) Analysis of full length PABPC1 binding to poly(A)$_{20}$. (C) Analysis of PABPC1 (1-190) binding to poly(A)$_{20}$. (D) Analysis of full length PABPC1 binding to poly(A)$_{50}$. (D) Analysis of PABPC1 (1-190) binding to poly(A)$_{50}$. The molar excess of PABPC1 (0-16x) with respect to poly(A) RNA is indicated. 5' Fluorescein-labelled RNA was incubated with RNA substrate (200 nM) at room temperature for 20 min in buffer containing 20 mM Tris-HCl pH 7.9, 50 mM NaCl, 2 mM $MgCl_2$, 10% glycerol, and 1 mM β-mercaptoethanol. Then, non-denaturing loading dye (1 µl; 0.05% bromophenol blue, 0.05% xylene cyanol, 25% glycerol and 1× TBE) was added to an aliquot (5 µl) and samples were analysed by 8% PAGE cast and run in 1× TBE. Polyacrylamide gels (acrylamide:bisacrylamide=19:1; 10x10x0.1 cm) were run at 120 V (Protein XCell; ThermoFisher) for 50-60 min at room temperature. RNA was visualised using a FujiFilm LAS-4000 imaging system equipped with an epi-Blue LED illuminator.

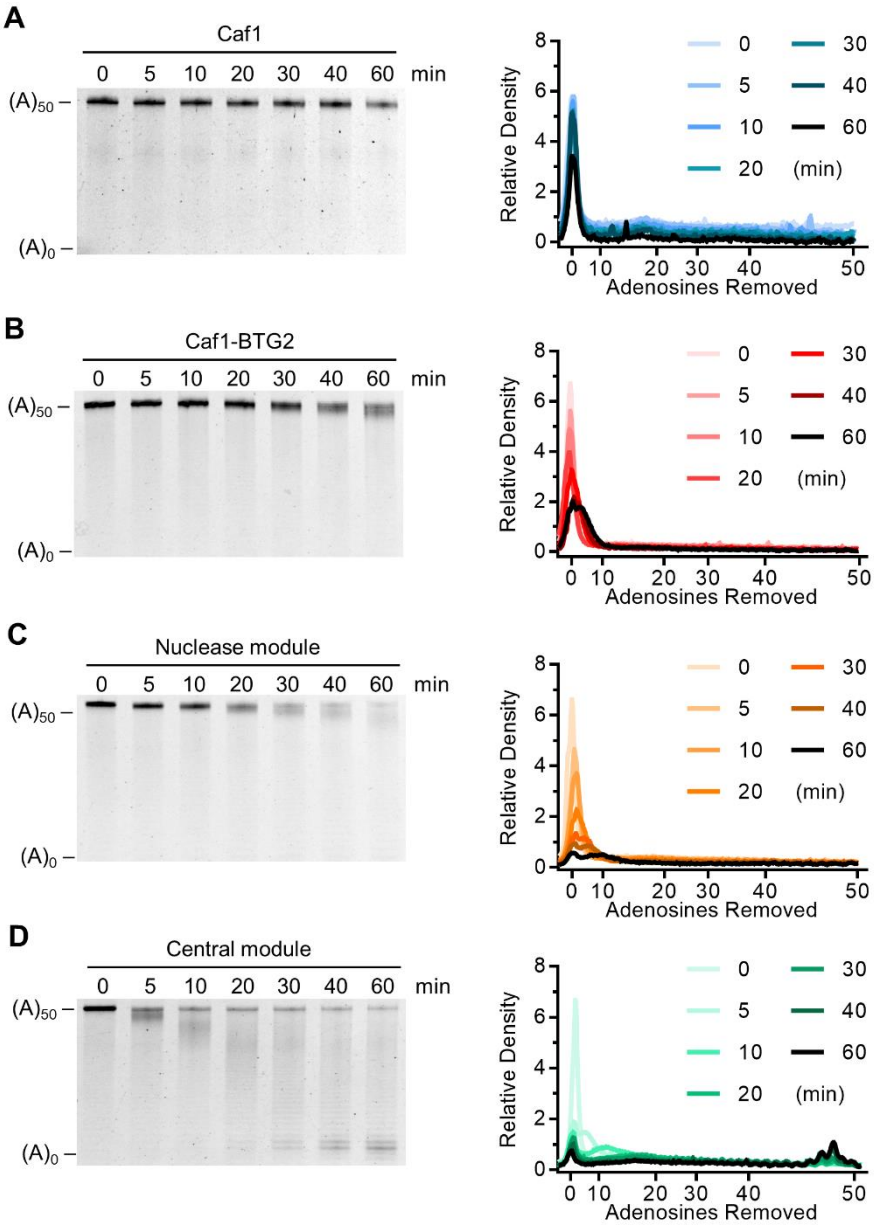

Fig. S3. Deadenylation of $(A)_{50}$ substrates by Caf1 and Caf1-containing complexes. Deadenylation of $(A)_{50}$ by (A) Caf1; (B) the Caf1-BTG2 dimer; (C) a trimeric BTG2-Caf1-Ccr4 nuclease module; and (D) a pentameric BTG2-Caf1-Ccr4-CNOT1-CNOT9 core complex. Synthetic oligonucleotides (5'Flc-CCUUUCC$(A)_{50}$) were incubated with the indicated enzymes (100 nM) at 30°C and stopped at the indicated time points. Products were analysed by denaturing 50% urea/16% PAGE (*left*). Densitometric analysis was carried out using ImageJ (*right*).

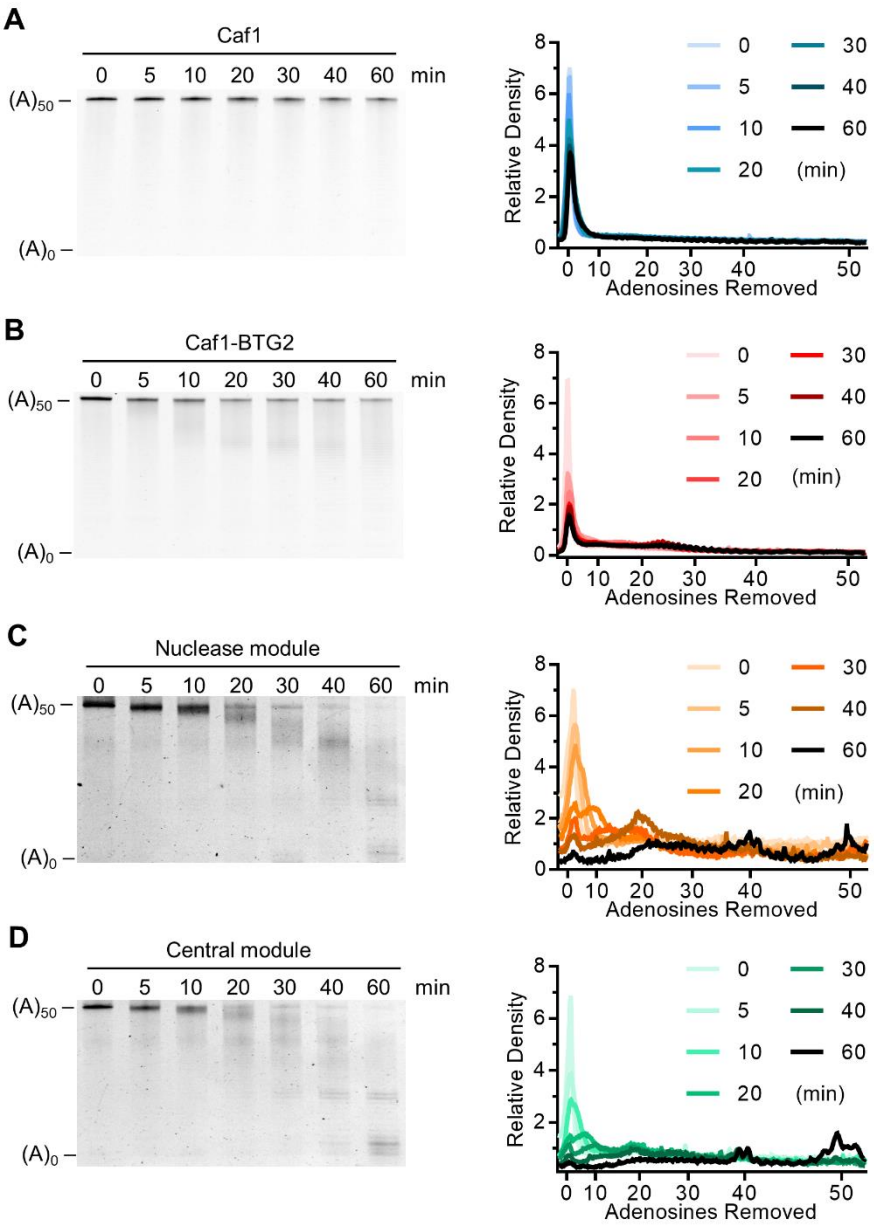

Fig. S4. Deadenylation of full length PABPC1-(A)$_{50}$ substrates by Caf1 and Caf1-containing complexes. Deadenylation of full length PABPC1-(A)$_{50}$ by (A) Caf1; (B) the Caf1-BTG2 dimer; (C) a trimeric BTG2-Caf1-Ccr4 nuclease module; and (D) a pentameric BTG2-Caf1-Ccr4-CNOT1-CNOT9 core complex. Synthetic oligonucleotides (5'Flc-CCUUUCC(A)$_{50}$) were incubated with the indicated enzymes (100 nM) at 30°C and stopped at the indicated time points. Products were analysed by denaturing 50% urea/16% PAGE (*left*). Densitometric analysis was carried out using ImageJ (*right*).

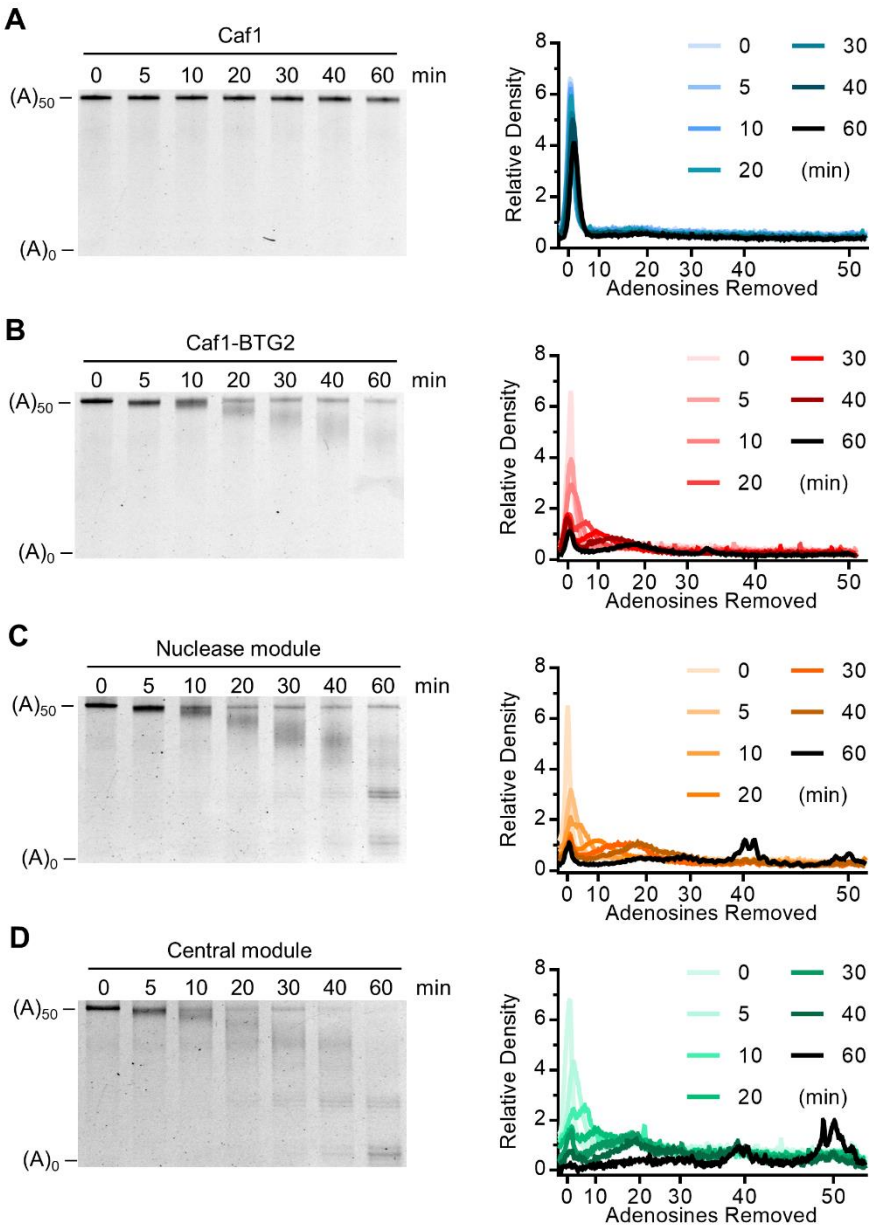

Fig. S5. Deadenylation of PABPC1 (RRM1-RRM2)-$(A)_{50}$ substrates by Caf1 and Caf1-containing complexes. Deadenylation of PABPC1 (RRM1-RRM2)-$(A)_{50}$ by (A) Caf1; (B) the Caf1-BTG2 dimer; (C) a trimeric BTG2-Caf1-Ccr4 nuclease module; and (D) a pentameric BTG2-Caf1-Ccr4-CNOT1-CNOT9 core complex. Synthetic oligonucleotides (5'Flc-CCUUUCC$(A)_{50}$) were incubated with the indicated enzymes (100 nM) at 30°C and stopped at the indicated time points. Products were analysed by denaturing 50% urea/16% PAGE (*left*). Densitometric analysis was carried out using ImageJ (*right*).