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Supplemental Data

Mammalian miRNA RISC Recruits CAF1 and PABP to Affect PABP-Dependent Deadenylation

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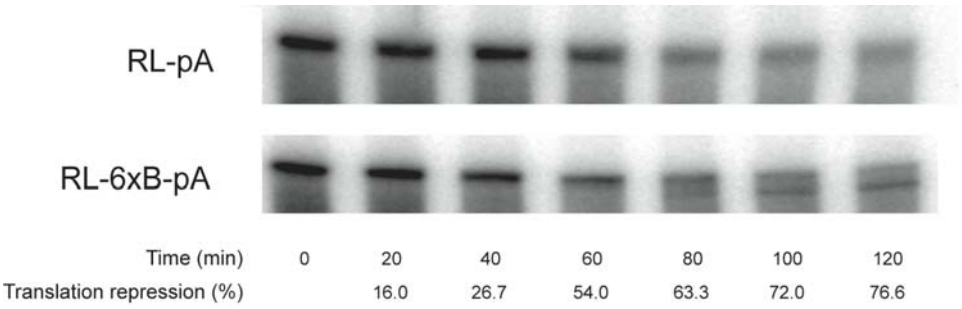

Figure S1. Time course of mRNA stability, as determined by autoradiography. Average percentage of translational repression, as determined by parallel luciferase-based assays, is labeled below each time point.

Figure S1, Fabian et al., 2009

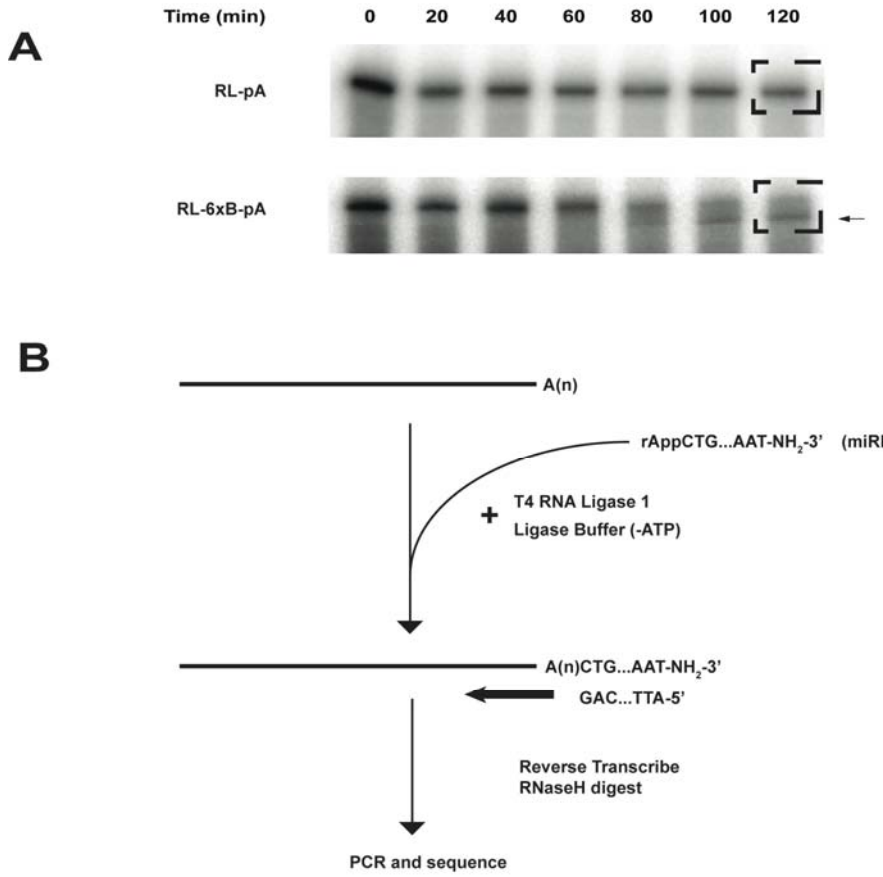

Figure S2, Fabian et al., 2009

Figure S2. Sequencing of RL-pA and RL-6xB-pA RNA. (A) Phosphorimager autoradiography of polyacrylamide/urea gels from which RNA bands were extracted and cloned. (B) Schematic of RNA cloning procedure. (C) RT-PCR products derived from extracted RNA bands and resolved on a 2% agarose gel. Sequences on the right are for RL-pA (1), full-length RL-6xB-pA (2) and deadenylated RL-6xB-pA (3).

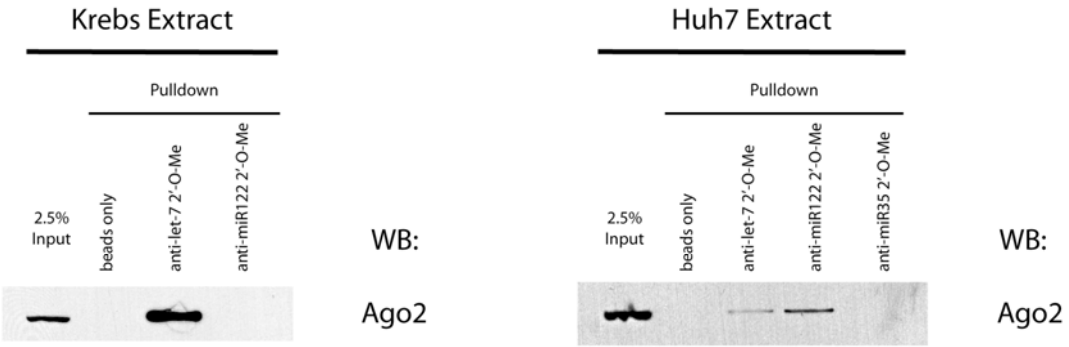

Figure S3. Pulldown of miRNA-loaded Ago2 from Krebs and Huh7 extracts. Ago2 was pulled down from micrococcal nuclease-treated extracts using biotin-conjugated anti-let-7 or anti-miR122 2'-*O*-Me oligonucleotides and streptavidin Dynabeads. Isolated complexes were subjected to SDS-PAGE and probed with anti-Ago2 antibody.

Figure S3, Fabian et al., 2009

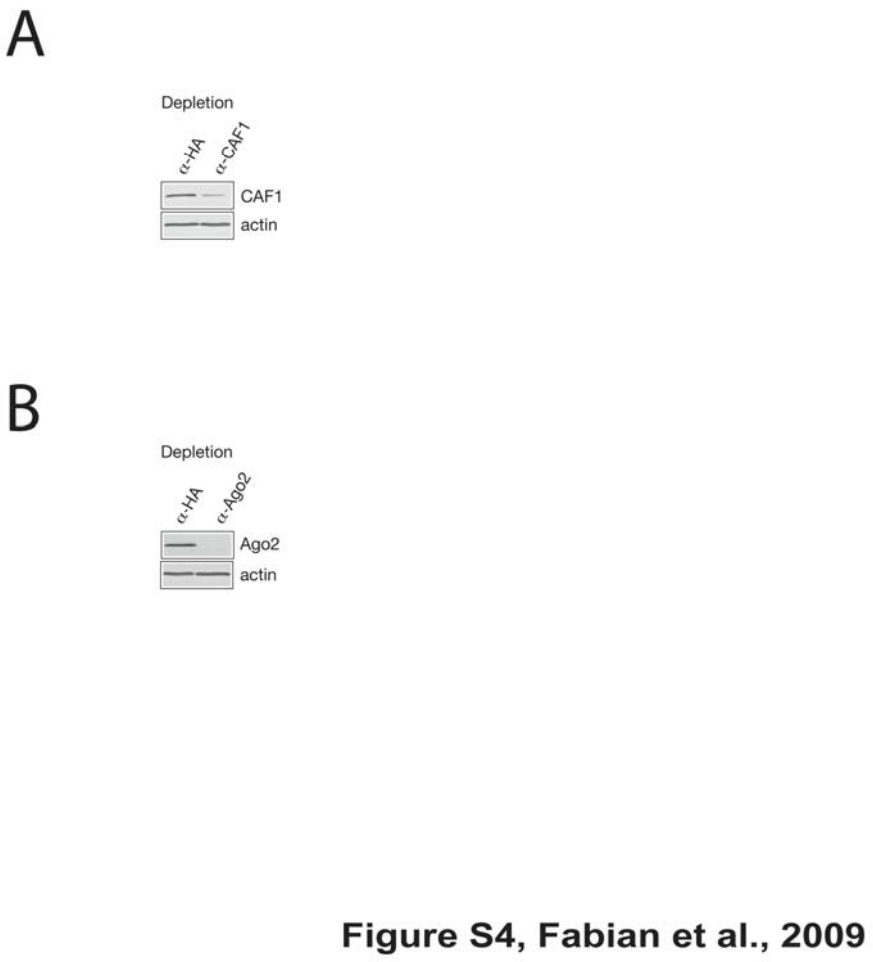

Figure S4. Western blot analysis of Ago2- and CAF1-depleted Krebs-2 extracts. Krebs extracts were depleted with either mouse or rabbit anti-HA (control) or anti-CAF1 or anti-Ago2 antibodies and probed with anti-CAF1, anti-Ago2 and anti-ß-actin antibodies.

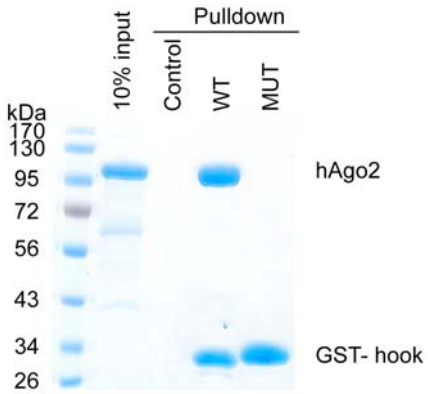

Figure S5. GST-pulldown of recombinant Ago2 with either wild-type or mutant GST-tagged Argonaute hook peptides. Pulldown efficiency was analyzed by SDS-PAGE followed by Coomassie staining.

Figure S5, Fabian et al., 2009

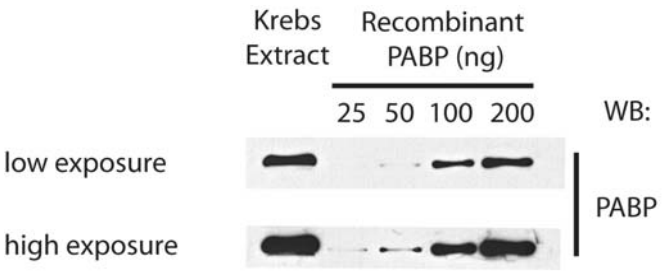

Figure S6. Quantification of endogenous PABP levels in Krebs extract. One in vitro reaction volume of Krebs extract was separated next to 25, 50, 100 (1.33 picomoles) and 200 ng of recombinant PABP by SDS-PAGE and analyzed by Western blotting using anti-PABP antibody.

Figure S6, Fabian et al., 2009

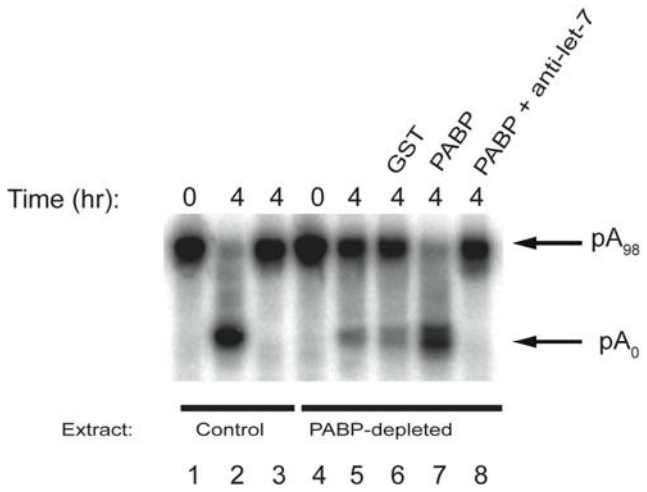

Figure S7, Fabian et al., 2009

Figure S7. Effects of anti-let-7 2'-*O*-Me oligonucleotide on miRNA-mediated deadenylation. A-capped 6xB-3'UTR RNA incubated in either mock-depleted (lanes 1-3) or PABP-depleted extract (lanes 4-8). PABP-depleted extract was supplemented with recombinant GST or GST-PABP (100 ng, which is the equivalent of roughly 50% of endogenous PABP present in an *in vitro* reaction) in the presence or absence of anti-let-7 2'-*O*-Me oligonucleotide, and RNA stability was monitored by autoradiography. Polyadenylated and deadenylated mRNAs are marked on the right of the panel.

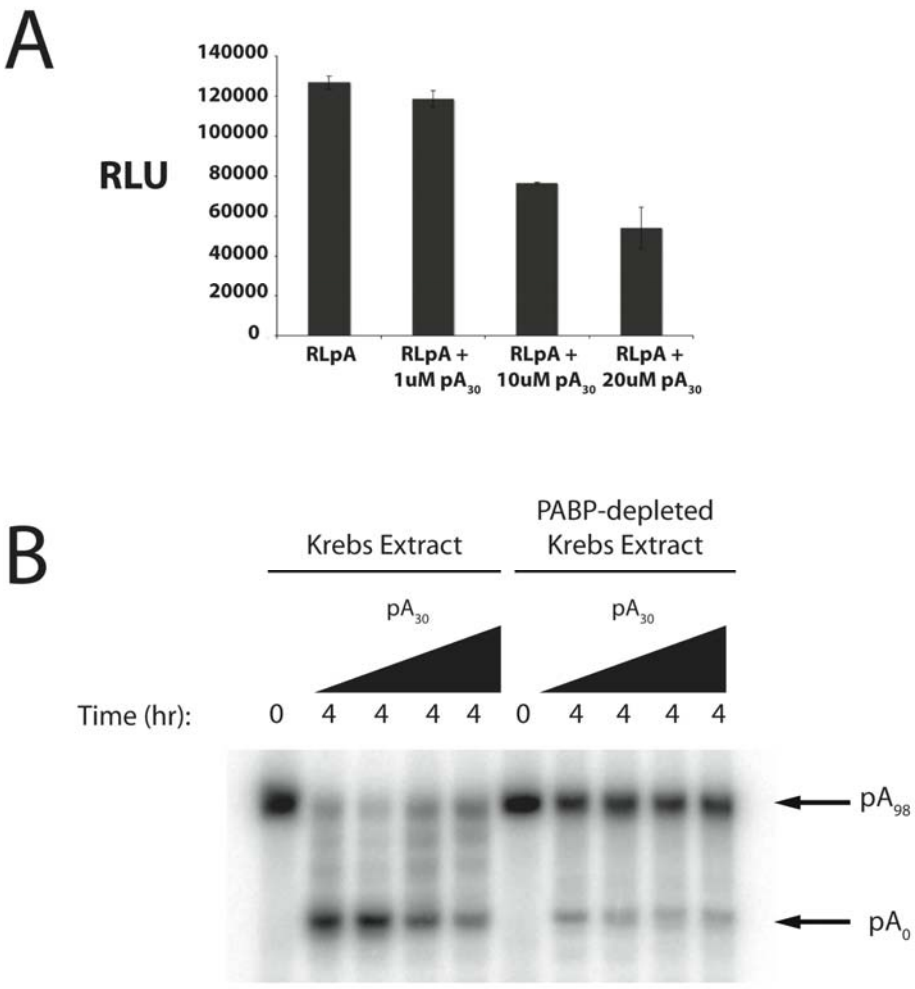

Figure S8, Fabian et al., 2009

Figure S8. Effects of free poly(A) on general translation and miRNA-mediated deadenylation. (A) Free poly(A) oligonucleotide (pA_{30}) was added to Krebs extract at increasing concentrations in the presence of RLpA mRNA, and translation was assayed

after a 1 hour incubation at 30^{o}C. Error bars represent the standard deviation of three independent experiments. (B) pA_{30} was added to Krebs extract either containing or depleted of PABP in the presence of radiolabeled 6xB-3'UTR RNA, and deadenylation was monitored by autoradiography.

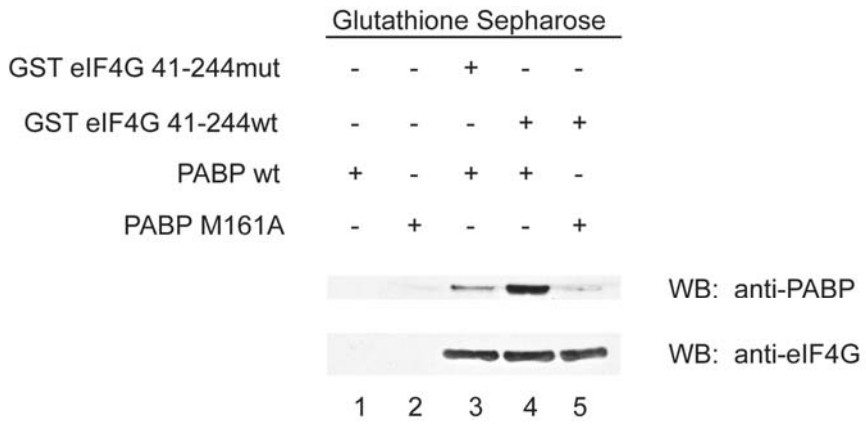

Figure S9. Interaction of PABP with eIF4G. Wild type PABP was incubated with glutathione-Sepharose beads on its own (lane 1), or with beads coupled to GST eIF4G 41-244wt or mut (lanes 3, 4). M161A PABP was incubated with glutathione-Sepharose beads on its own (lanes 2), or with beads coupled to GST eIF4G 41-244wt (lane 5). Beads were washed with binding buffer and bound proteins were analyzed by SDS-PAGE and Western blotting.

Figure S9, Fabian et al., 2009

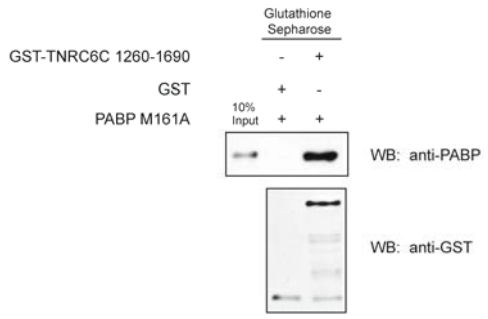

Figure S10, Fabian et al., 2009

Figure S10. The TNRC6C C-terminus can interact with PABP M161A. Wild type PABP was incubated with glutathione-Sepharose beads coupled to GST or GST-TNRC6C (1260-1690). Beads were washed with binding buffer and bound proteins were analyzed by SDS-PAGE and Western blotting.