

Appendix

RNA degradation triggered by decapping is largely independent of initial deadenylation

Appendix Table S1 - page 1

Appendix Figure S1 - Robustness of the negative correlations between poly(A) tail length and half-life of RNA. - page 2

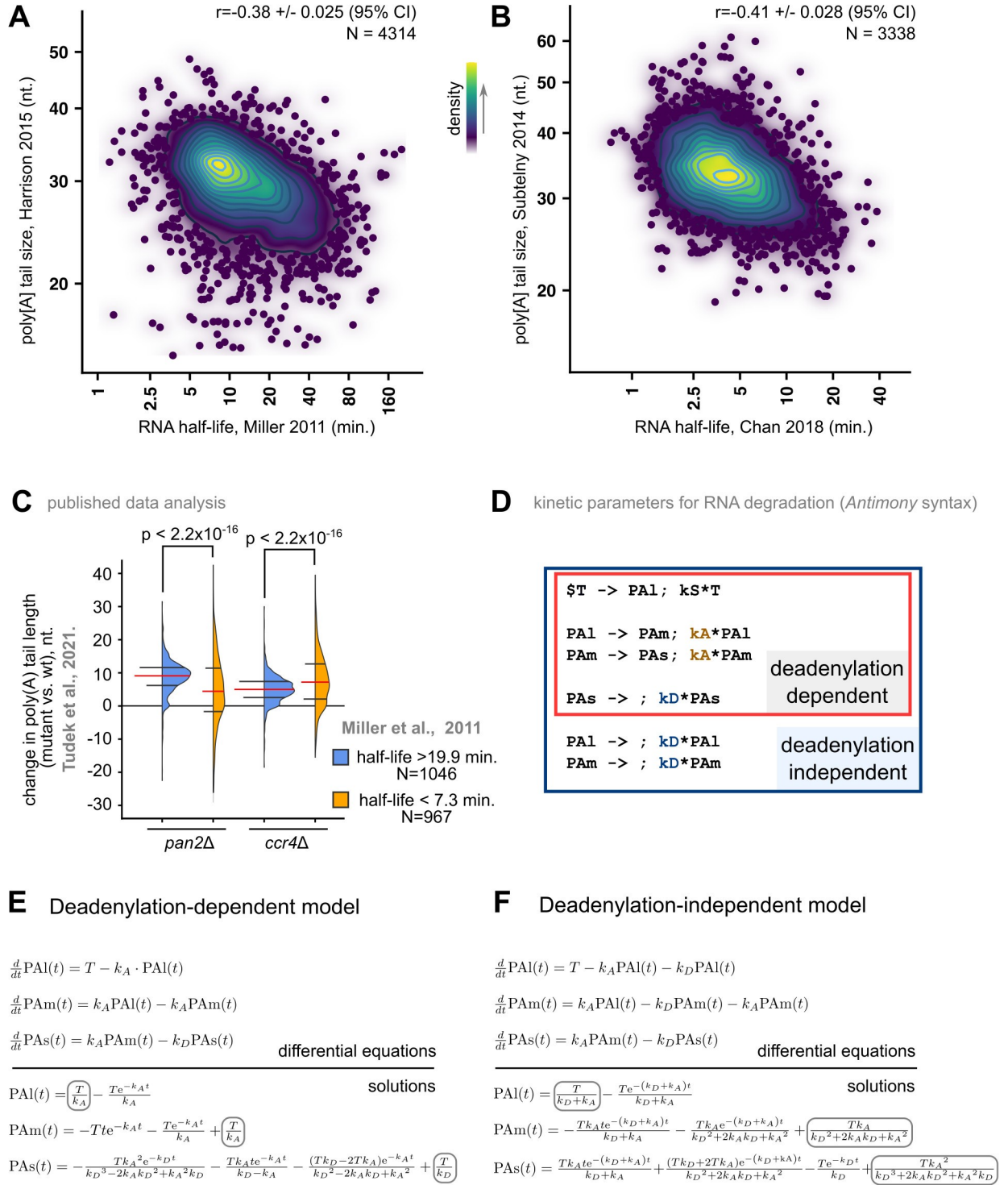
Appendix Figure S2 - Model-based scenarios for predicted effects of slowing down deadenylation and decapping. - page 4

Appendix Figure S3 - Correlation of SLAM-Seq results with previous mRNA half-life estimates. - page 5

Appendix Table S1. HIS3 coding sequence for reporters (non-OPT/OPT)

non-OPT-HIS3 coding region HIS3 sequence
ATGGACTACAAGGACGACGACGACAAGACAGAGCAGAAAAGCACTAGTAAAGCGGATAACAAATGAGACAAAGATTCAGATAGCGATAA GTTTAAAAGGAGGTCCCCTAGCGATAGAGCATTCGATATTTCCCGAGAAAGAGGCAGAAGCAGTAGCAGAGCAGGCAACACAGTCGCA AGTGATAAATGTGCATACAGGAATAGGGTTTCTGGATCATATGATACATGCGCTGGCAAAACATTTCGGGCTGGTCGCTAATAGTGGAG TGCATAGGAGATTTACATATAGATGATCATCACACAACAGAGGATTGCGGGATAGCTCTCGGTCAAGCATTAAAGAGGCGCTAGGGG CAGTGCGTGGAGTAAAAAGGTTTGGATCAGGATTTGCGCCTTTGGATGAGGCACCTTCCAGAGCGGTGGTAGATCTTTCGAACAGGCC GTATGCAGTTGTGGAGCTTGGCCTCCAGAGGGAGAAAGTAGGAGATCTCAGTTGCGAGATGATACCGCATTTTCTTGAGAGCTTTGCA GAGCGAGCAGGATAACCCTCCATGTGGATTGCCGTGCGAGGCAAAAATGATCATCATCGGAGTGAGAGTGCGTTTTAAAGCTCTTGCGG TAGCAATACGCGAGGCCACATCGCCCAATGGCACCAATGATGTTCCCTCGACAAAAGGGGTACTTATGTAG
OPT-HIS3 coding region HIS3 sequence
ATGGACTACAAGGACGACGACGACAAGACTGAACAAAAGGCCTTGGTTAAGCGTATTACTAACGAAACCAAGATTCAAATTGCCATCT CTTTGAAGGGTGGTCCATTGGCCATTGAACACTCCATCTTCCCAGAAAAGGAAGCTGAAGCTGTTGCTGAACAAGCCACTCAATCCCA AGTCATTAACGTCCACACTGGTATTGGTTTTCTTGGACCACATGATTCACGCTTTGGCCAAGCACTCCGTTGGTCCTTGATCGTTGAA TGTATTGGTGACTTGCACATTGACGACCACCACCACTGAAGACTGTGGTATTGCTTTGGGTCAAGCTTTCAAGGAAGCCTTGGGTG CCGTCCGTGGAGTAAAAAGGTTTGGATCAGGTTTCGCCCATTTGGACGAAGCTTTGTCCAGAGCCGTCGTTGACTTGTCCAACAGACC ATACGCTGTTGTGAATTGGGTTTGCAAAGAGAAAAGGTTGGTGACTTGCTTTGTGAAATGATCCACACTTCTTGGAATCCTTCGCT GAAGCTTCCAGAATTACCTTGCACGTTGACTGTTTGCCTGGTAAGAACGACCACCACCGTTCTGAATCTGCCTTCAAGGCTTTGGCCG TTGCCATTAGAGAAGCCACCTCCCCAAACGGTACCAACGACGTTCCATCCACCAAGGGTGTTTTGATGTAG

Appendix Supplementary Figures

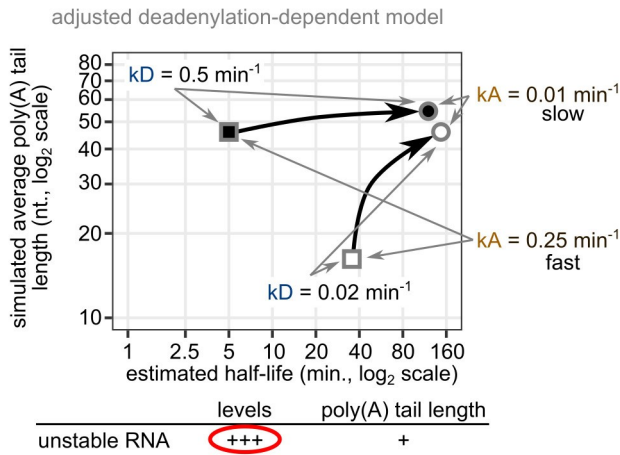


Appendix Fig. S1. Robustness of the negative correlations between poly(A) tail length and half-life of RNA. (A), (B) Negative correlations between published results, similar to the ones shown in Fig. 2A and 2B, including results for poly(A) tail size (Harrison *et al*, 2015; Subtelny *et al*, 2014) and

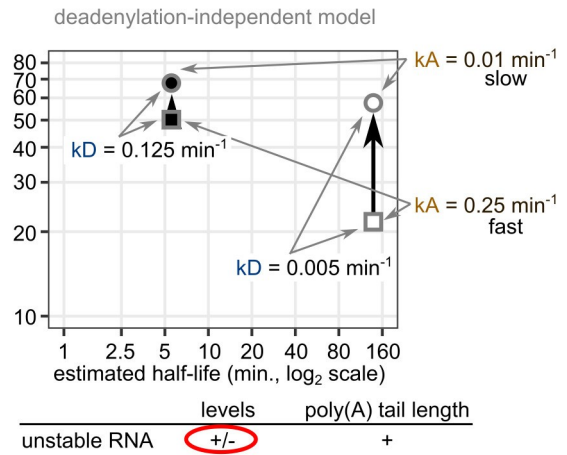
estimates of RNA half-life (Chan *et al*, 2018; Miller *et al*, 2011). **(C)** RNAs for which half life is short, in the first quartile, half life inferior to 7.3 min, or long, in the third quartile, over 19.9 min (Miller *et al*, 2011), were selected to evaluate how their poly(A) size changed in deadenylation mutants, as measured by Nanopore sequencing (Tudek *et al*, 2021). The quartiles of the distributions are indicated, median in red, as well as the p-value of a Wilcoxon rank sum test with continuity correction for the difference between the two populations. **(D)** Parameters used to simulate RNA degradation measurements that include poly(A) tail information and kinetic equations using the *Antimony* syntax (Smith *et al*, 2009). "T" represents all the processes leading to mRNA formation. PA_i , PA_m and PA_s represent concentrations of different poly(A) tail RNA species. **(E)** The set of differential equations used to estimate time changes for the three modeled poly(A) species in a deadenylation-dependent model (upper region) and the solutions (lower region). The terms that correspond to steady-state are circled in gray. **(F)** Similar to (E), for the deadenylation-independent model.

Predicted effects of slowing down deadenylation

A

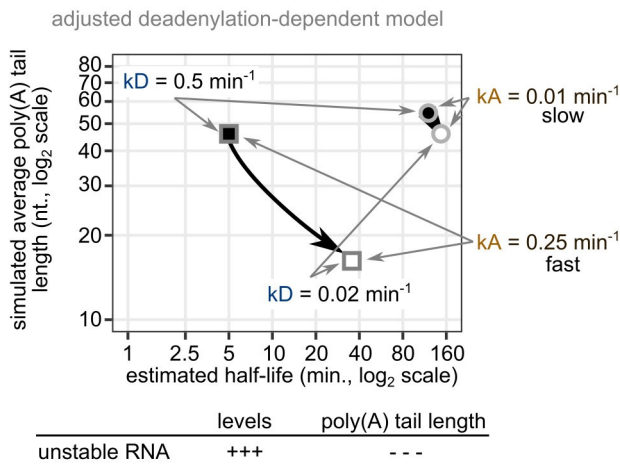


B

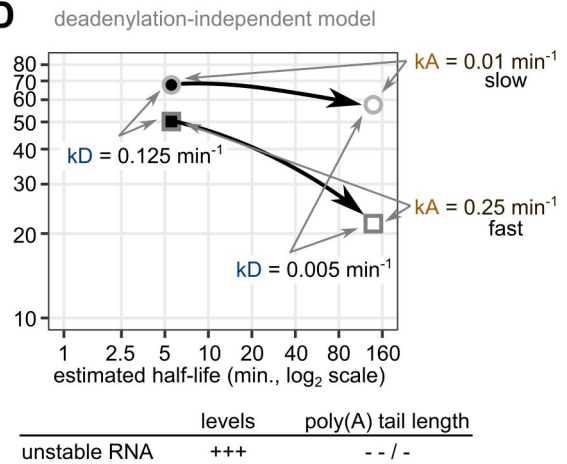


Predicted effects of slowing down decapping

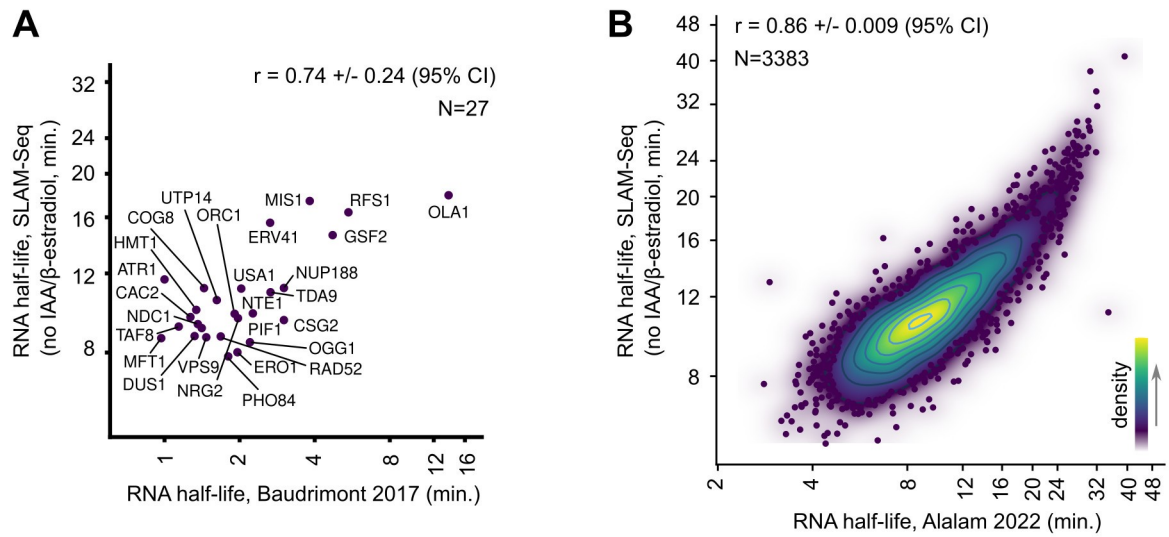
C



D



Appendix Fig. S2. Model-based scenarios for predicted effects of slowing down deadenylation (A, B) and decapping (C, D). For each situation, the expected shift in poly(A) tail length and stability for a stable and an unstable RNA are indicated by black arrows. The experimental conditions can only estimate changes in poly(A) tails and levels for relatively unstable mRNAs (situation depicted under each graph).



Appendix Fig. S3. Correlation of SLAM-Seq results with previous mRNA half-life estimates. (A) Comparison with individual results from Baudrimont et al. 2017. **(B)** Comparison with SLAM-Seq results of Alam et al., 2022. In both panels, Pearson's product moment correlation coefficients and their 95% confidence intervals are indicated.