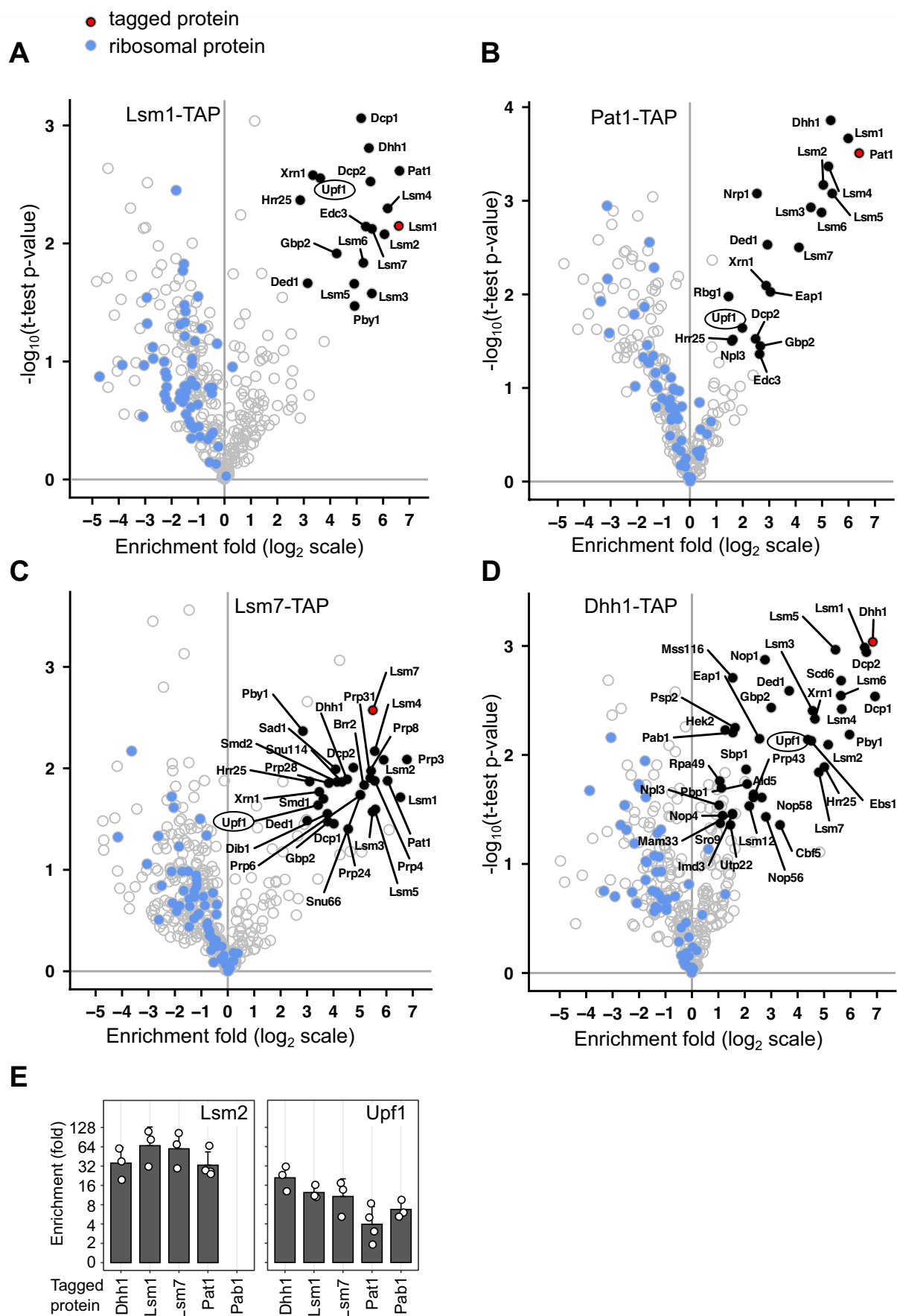


Expanded View Figures

Figure EV1. Enrichment of specific factors in purified complexes containing Upf1.

Volcano plots represent the enrichment of proteins, relative to the protein abundance in a total extract as measured by label-free mass spectrometry. Results are presented for purifications using TAP tagged versions of Lsm1 (A), Pat1 (B), Lsm7 (C), and Dhh1 (D). Ribosomal proteins are highlighted in blue. (E) Examples of enrichment values across the various purifications for Lsm2, a component of Lsm1-7 and Lsm2-8 complexes, and Upf1. Error bars represent standard deviation. *N* is 3 for Dhh1, Lsm1, Lsm7, and Pab1 purifications and 4 for Pat1.



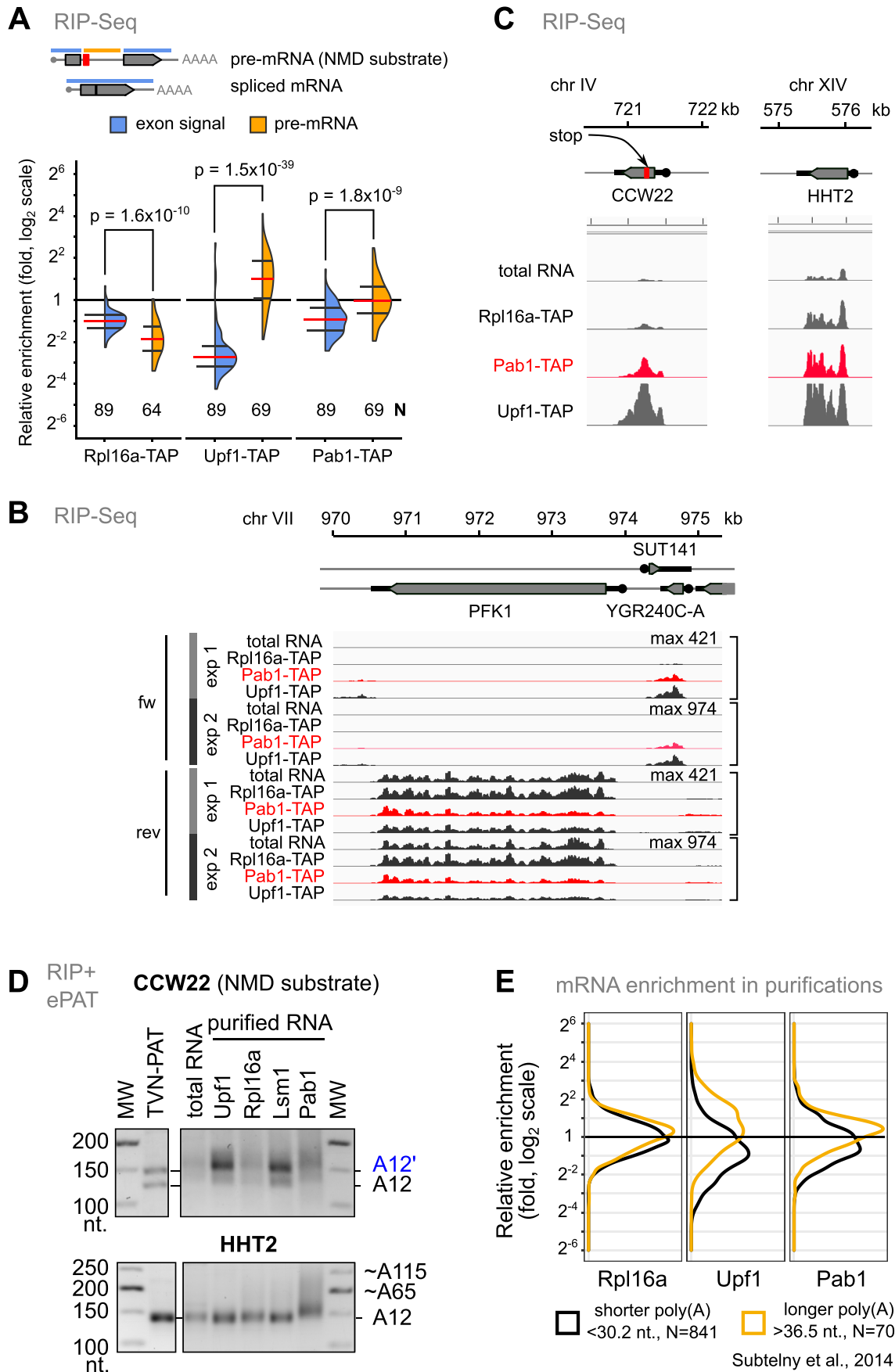
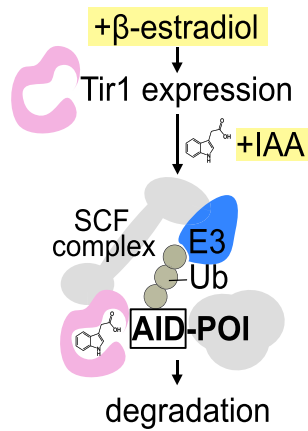
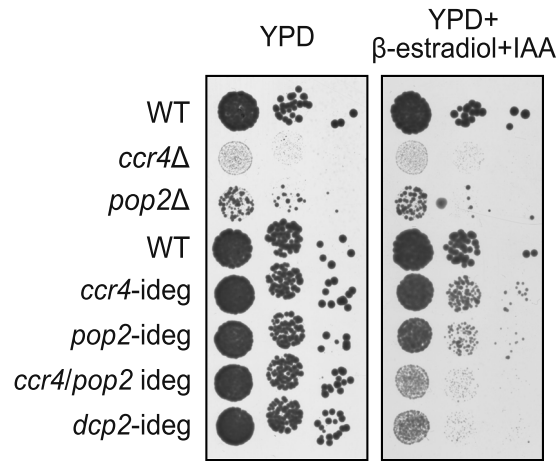
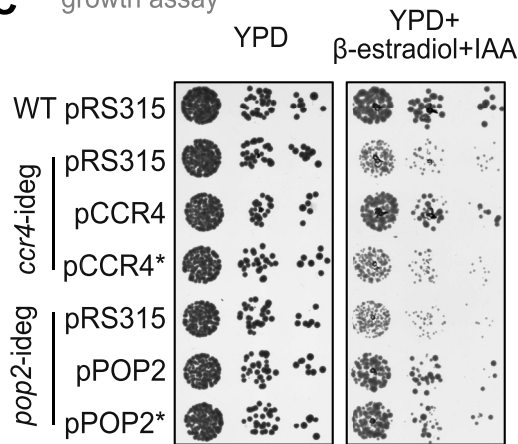
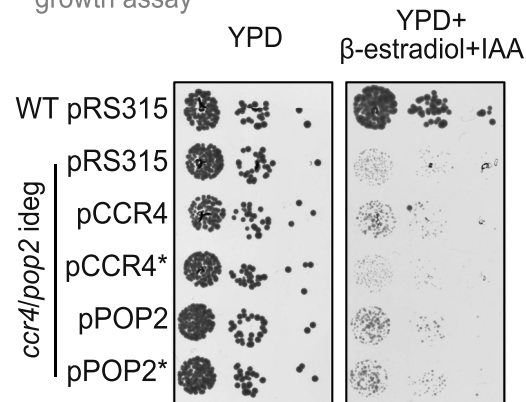




Figure EV2. Unstable mRNAs are associated with Pab1.

(A) Relative enrichment of pre-mRNA for ribosomal protein genes (intron signal, orange) in comparison with the spliced mRNA for the same category (exon signal, blue). The red horizontal line indicates the median of the enrichment values, with the first and third quartiles indicated. The p-values correspond to a Welch two sample t-test, N is the number of values in each category. (B) Example of signal intensity for RNAs associated with Pab1-TAP, Rpl16a-TAP, or Upf1-TAP for a region of the yeast genome that corresponds to SUT141, an unstable RNA, in comparison with the PFK1 stable mRNA (transcribed in proximity but from the opposite strand). The vertical scale is the same for the samples of the same replicated experiment. Pab1 associated RNA signal is depicted in red. The image was obtained with the Integrated Genome Viewer, IGV. (C) Similar to (B) for the regions of transcripts CCW22, and NMD substrate (left), and HHT2 (right). (D) Estimation of poly(A) tail distribution for CCW22 and HHT2 in the RNA fractions enriched with Upf1, Rpl16a, Lsm1, and Pab1. The TVN-PAT lane corresponds to a control experiment with an anchored 3' primer. For CCW22, two 3' ends were detected (noted A12, and A12'). (E) The distribution of the enrichment values for two extreme categories of mRNA, with long (orange) or short average poly(A) tails (black) was depicted for the purified samples in association with Rpl16a-TAP, Upf1-TAP, and Pab1-TAP. A Wilcoxon ranks sum test with continuity correction comparing the distribution of relative enrichment values for long poly(A) tails mRNA versus short poly(A) tails, led to the following p values (for a minimum 25% increase): 0.9997 for Rpl16a, less than 2×10^{-16} for Upf1 and 1.9×10^{-8} for Pab1.

A inducible degron schematics**B** growth assay**C** growth assay**D** growth assay**E** ePAT assay (HHT2)

CCR4* = CCR4(E556A)
POP2* = POP2(D310A)

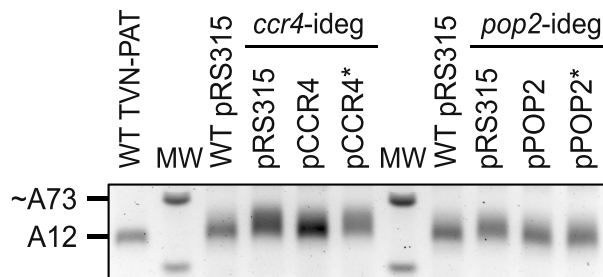
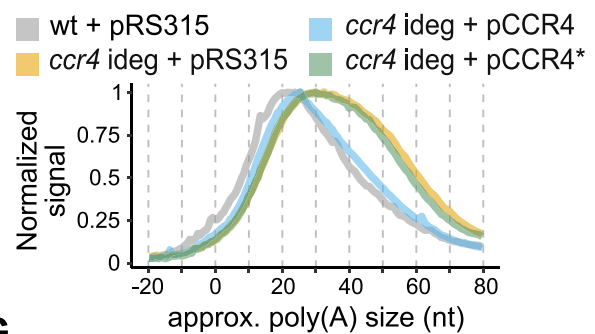
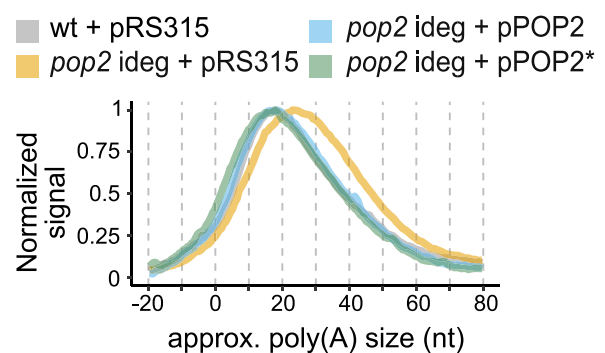
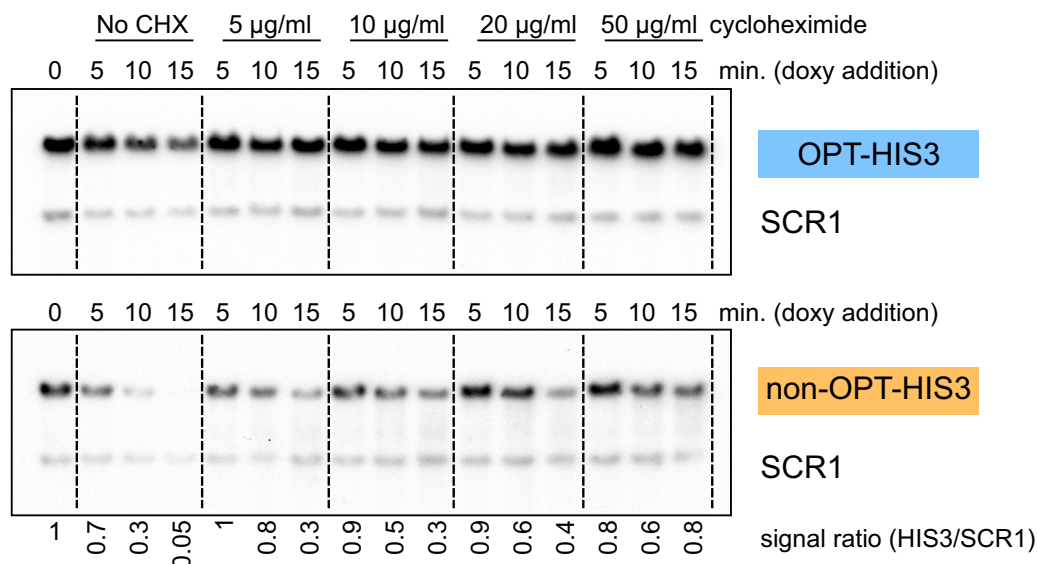
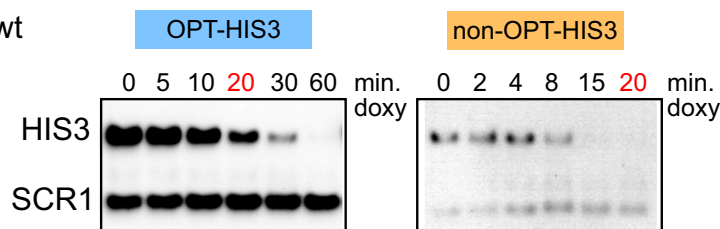
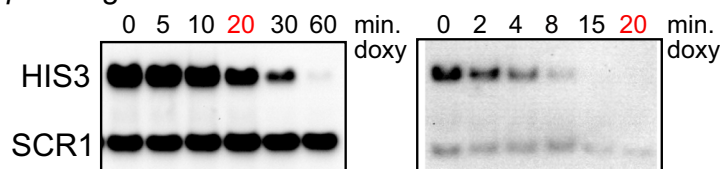
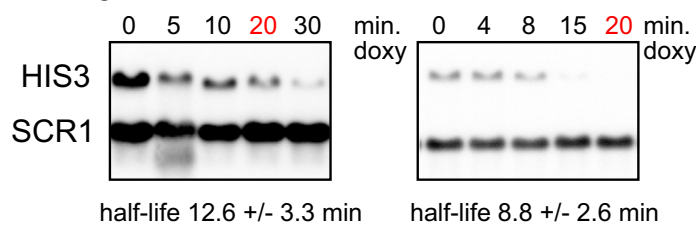
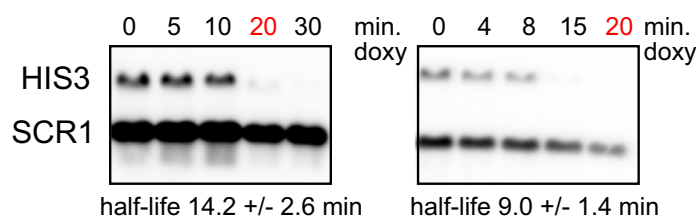
**F****G**

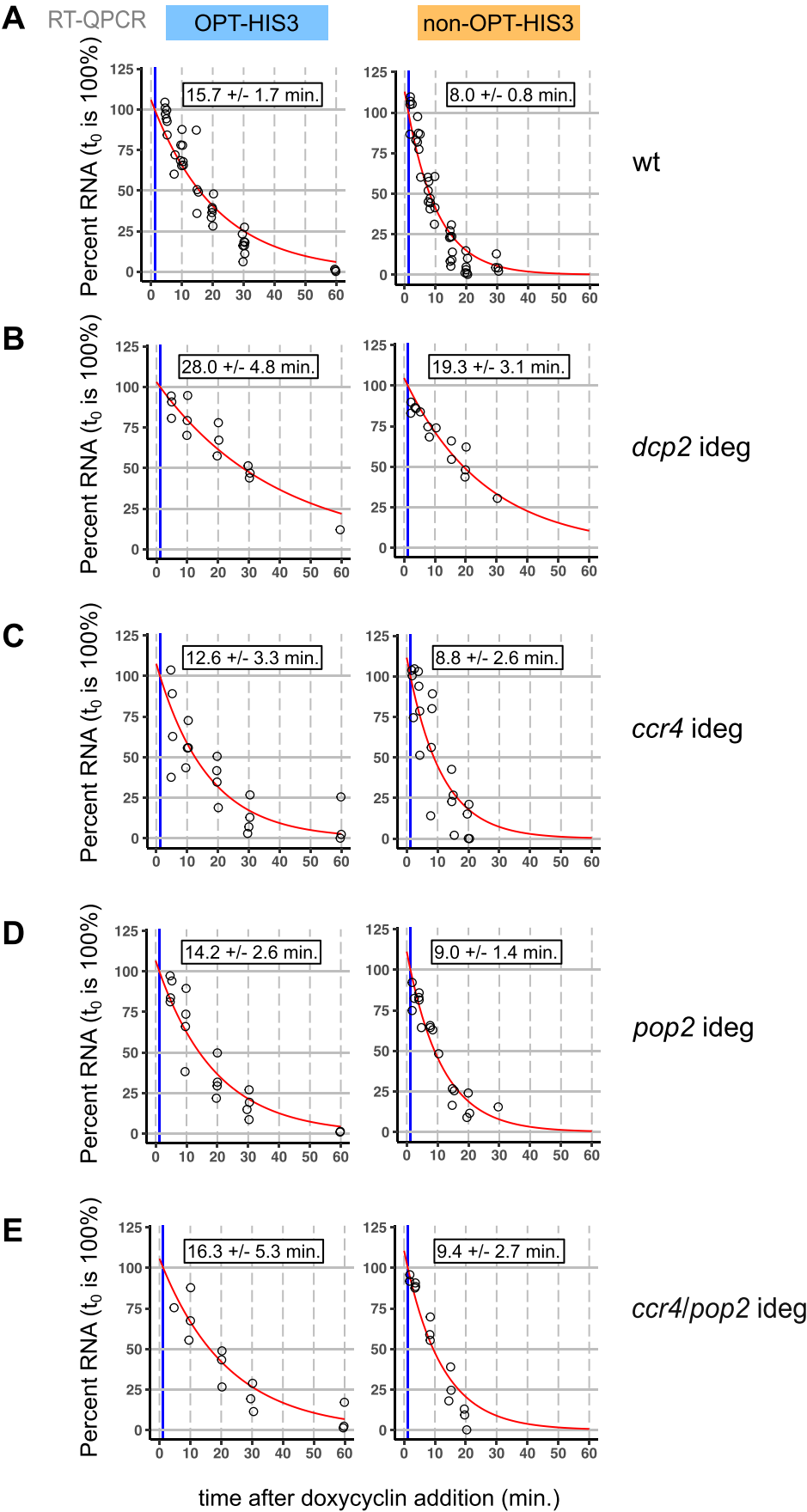

Figure EV3. An inducible degron system for deadenylases.

(A) Schematics of the developed inducible degron system in which *O. sativa* Tir1 expression is under the control of an estrogen sensitive promoter. In the presence of the plant auxin hormone indole-3-acetic acid (IAA), Tir1 targets a E3 ubiquitin ligase to the AID domain fused to the protein of interest (POI) for its rapid proteasome degradation. (B) Serial dilution plate growth assay for deletion and inducible degron (ideg) strains affecting deadenylases of the CCR4/NOT complex and the decapping enzyme Dcp2. Growth on rich medium (YPD, left) was compared with growth on rich medium containing β -estradiol and IAA (right). (C) Similar to (B) for the strains depleted for Ccr4 or Pop2 that were transformed with a control plasmid (pRS315), pRS315-CCR4 or pRS315-POP2. The asterisks indicates mutated versions of Ccr4, E556A, and Pop2, D310A. (D) Similar with (C), but for the concomitant depletion of Ccr4 and Pop2. (E) Changes in the poly(A) tail size of HHT2 measured using ePAT in the strains presented in panel (C). The TVN-PAT lane indicates the relative position of the A12 RNA. (F) Poly(A) size profile for HHT2 under Ccr4 depletion conditions was extracted from panel (E) and normalized to the signal in each lane. (G) Similar to (F), but for the depletion of Pop2.

A Northern blot**B** wt**C** *upf1-ideg***D** *ccr4-ideg***E** *pop2-ideg*

◀ Figure EV4. Degradation of OPT-HIS3 and non-OPT-HIS3 reporter mRNAs depends on translation and decapping.

(A) Dose-dependent effect of the translation inhibitor cycloheximide (from 0 to 50 µg/ml) was tested by Northern blotting for optimal (upper panel) and non-optimal (lower panel) HIS3 reporters. SCR1 was used as a loading control. Degradation rates for optimized HIS3 (left panel) and non-optimal HIS3 (right panel) were visualized by Northern blot (HIS3 reporter signal) in comparison with an SCR1 control. (B) Similar to (A), in a wild type strain. (C) Similar to (A) after depletion of Upf1. (D) Similar to (A) after depletion of Ccr4. (E) Similar to (A) after depletion of Pop2. For each situation, the estimated half-life and the 95% confidence interval obtained from RT-qPCR and the results are indicated.



**Figure EV5. Half-life of reporter RNAs as measured by RT-qPCR.**

(A) The half-life of HIS3 reporter, codon-optimized (left panel) or non-optimized (right panel), was measured by RT-qPCR following transcription block with doxycyclin. For each decay experiment, the values were normalized at time 0, considered to represent 100%. Indicated estimates correspond to half-life in minutes with a 95% confidence interval. All the experiments were performed independently at least three times. (B) Similar to (A), but after depletion of Dcp2. (C) Similar to (A), but after depletion of Ccr4. (D) Similar to (A), but after depletion of Pop2. (E) Similar to (A) but after concomitant depletion of Ccr4 and Pop2.