

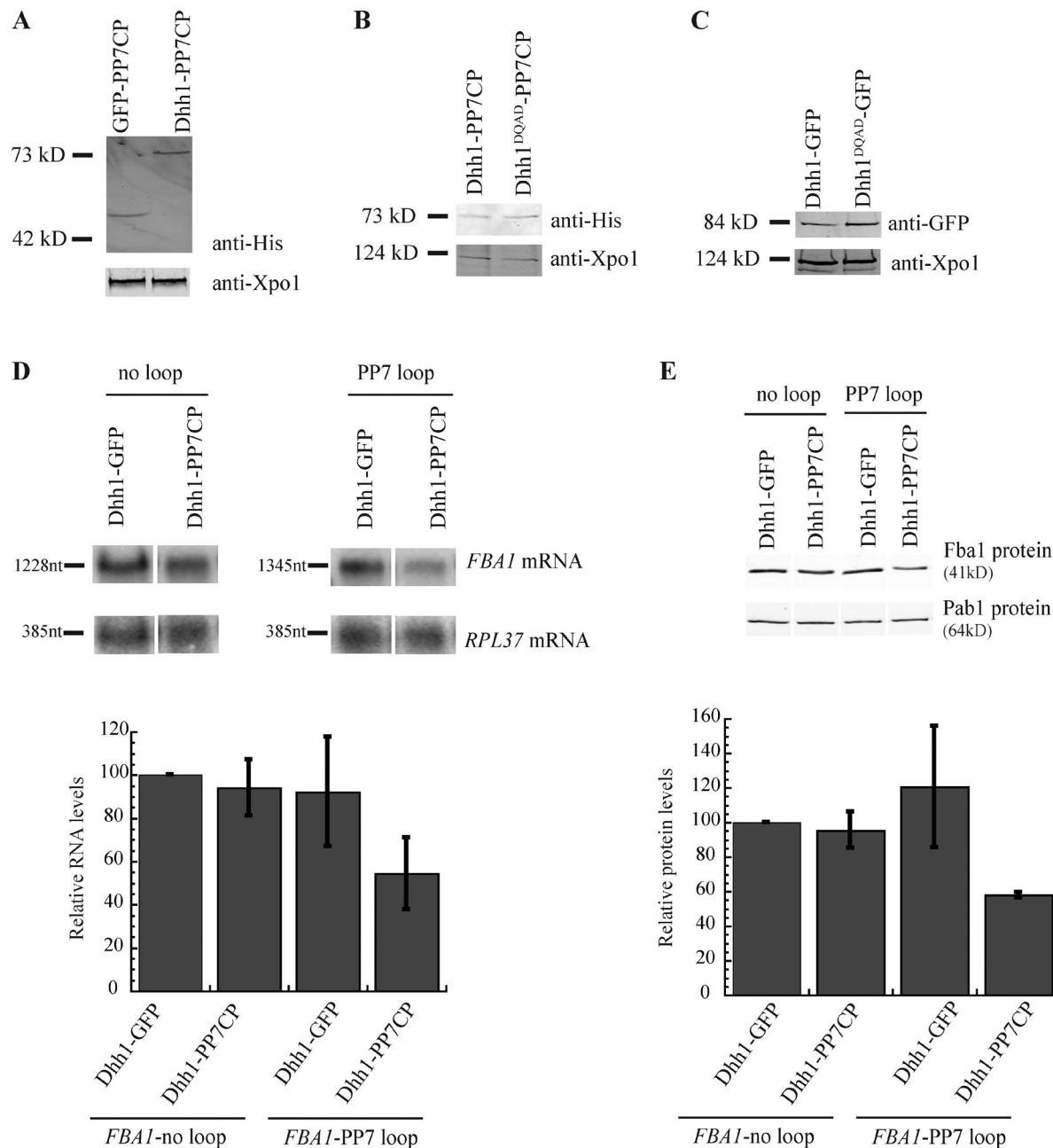
Carroll et al., <http://www.jcb.org/cgi/content/full/jcb.201007151/DC1>

Figure S1. PP7CP fusion proteins are expressed to similar levels, and Dhh1-PP7CP-induced reduction of *FBA1* mRNA and protein levels is specific to tethered Dhh1. (A–C) Western blot analysis of PP7CP fusion proteins. For each Western blot, protein extracts were prepared from mid-log phase yeast cells, and blots were probed with an anti-his antibody. (A) GFP-His-PP7CP and Dhh1-His-PP7CP fusion protein expression in the wild-type strain used for the tethering assay (KWY1571). (B) Dhh1-His-PP7CP and Dhh1^{DQAD}-His-PP7CP fusion proteins in *dhh1Δ* yeast used for tethering assay (KWY2532). (C) Dhh1-GFP and Dhh1^{DQAD}-GFP fusion proteins in *dhh1Δ* yeast used for microscopy. (D) Dhh1-GFP or Dhh1-PP7CP proteins were expressed in strains with either *FBA1* tagged with a PP7-binding loop (right two lanes) or *FBA1* with no loop (left two lanes). (D) *FBA1* RNA levels were analyzed by Northern blotting and normalized to *RPL37* mRNA. (E) Western blot analysis of Fba1 protein levels in tethered and nontethered strains compared with the loading control Pab1. Graphs depict mean protein levels \pm SD from four independent experiments.

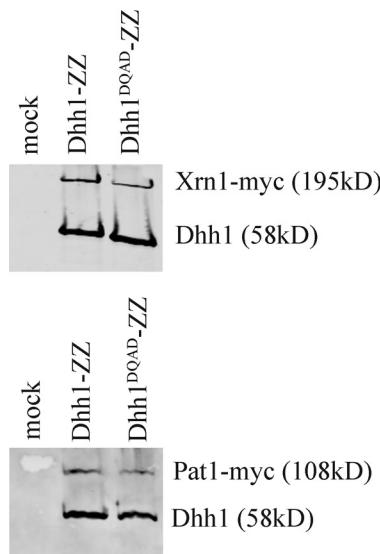


Figure S2. Equal amounts of mRNA decay factors copurify with wild-type and ATPase mutant Dhh1. Xrn1 (top) or Pat1 (bottom) proteins were tagged with a 13x myc tag in *dhh1Δ* cells carrying an empty vector or a vector containing Dhh1-ZZ or Dhh1^{DQAD}-ZZ. ZZ-tagged proteins were affinity purified, and co-purifying proteins were analyzed by Western blotting.

Table S1. Plasmids used in this study

Plasmids	Description	Source
pKW2304	pRS316 PDhh1-PP7CP	This study
pKW2306	pRS316 PDhh1-GFP	This study
pKW2312	pRS316 PDhh1-Dhh1-GFP	This study
pKW2313	pRS316 PDhh1-Dhh1 ^{DQAD} -GFP	This study
pKW2321	pRS316 PDhh1-Dhh1-6xHis-PP7CP	This study
pKW2322	pRS316 PDhh1-Dhh1 ^{DQAD} -6xHis-PP7CP	This study
pKW2420	pRS316 PDhh1-GFP-6xHis-PP7CP	This study
pKW2421	pRS316 PDhh1-Dhh1-CBP-TEV-ZZ	This study
pKW2422	pRS316 PDhh1-Dhh1 ^{DQAD} -CBP-TEV-ZZ	This study
pKW1008	pFA6a-Flag-KAN	This study
pKW1606	pFA6a-Flag-PP7-KAN	This study
pKW2090	pFA6a- γ EGFP-HIS5MX	Sheff and Thorn, 2004
pKW1809	pFA6a-mCherry-KAN	Westfall et al., 2008
pKW1061	pFA6a-13myc-KAN	Bähler et al., 1998

Table S2. Yeast strains used in this study

Yeast strains	Genotype	Source
W303	MAT α /a ade2-1 ura3-1 his3-11,15 trp1-1 leu203, 112 can1-100	
KWY1570	W303a FBA1- <i>flag</i> ::KANMX	This study
KWY1571	W303a FBA1- <i>flag</i> -PP7::KANMX	This study
KWY2580	W303a/a RPL25- <i>flag</i> -PP7::KANMX	This study
KWY2548	W303a ccr4::KANMX FBA1- <i>flag</i> -PP7::NAT	This study
KWY2257	W303a xrn1::KANMX FBA1- <i>flag</i> -PP7::NAT	This study
KWY2269	W303a dcp1::KANMX FBA1- <i>flag</i> -PP7::NAT	This study
KWY2526	W303a dhh1::NAT FBA1- <i>flag</i> -PP7::KANMX DCP2-GFP::HIS	This study
KWY2530	W303a dhh1::NAT FBA1- <i>flag</i> ::KANMX DCP2-GFP::HIS	This study
KWY2459	W303a xrn1::NAT FBA1- <i>flag</i> ::KANMX DCP2-GFP::HIS	This study
KWY2461	W303a xrn1::NAT FBA1- <i>flag</i> -PP7::KANMX DCP2-GFP::HIS	This study
KWY293	W303a dhh1::KANMX	Fischer and Weis, 2002
KWY2532	W303a dhh1::NAT FBA1- <i>flag</i> -PP7::KANMX	This study
KWY2193	W303a dhh1::KANMX, DCP2-mCherry::NAT	This study
KWY2907	W303a dhh1::NAT XRN1-13myc::KAN	This study
KWY2908	W303a dhh1::NAT PAT1-13myc::KAN	This study

Table S3. Oligonucleotides used in this study

Oligonucleotides	Sequence	Description
UC1949	5'-TGTTCATTATTATCATCATGGACCAAGGCCATAAAATGTTATCTCGT-3'	QuikChange for Dhh1 ^{DQAD}
UC1950	5'-ACGAGATAACATTATCGGCTTGGCCATGATGAATAATGAACA-3'	QuikChange for Dhh1 ^{DQAD}
UC587	5'-CGGCCCTATTATCGTCATCGTCTTATAGTCGTTAAT-3'	Antisense to FLAG tag for Northern analysis
UC1347	5'-TGTAAAGATCTGGCTTAGCAGCTGGATAACACCACAGGAGGAAC- AGGTCTCTTGAACATGGAAAGAACG-3'	Antisense to Rpl37A for Northern analysis
UC3501	5'-TACCTTGAGGAATGTGAGCGGCTGAACAGCGTCAGCGGT-3'	Antisense to Adh1 for Northern analysis
UC3187	5'-CCAGACAGAGAGACGGATTCTCACGCCCTGCCAACG-3'	Antisense to Scr1 for Northern analysis
UC3919	5'-TATGCTCCAAGGCTGTTCC-3'	Sense to RPL25 for qPCR
UC3920	5'-GCGGTTTCAGAAGTGTGG-3'	Antisense to RPL25 for qPCR
UC3761	5'-TTGCTTCTCATCAGTCACC-3'	Sense to CRH1 for qPCR
UC3762	5'-GGAAGCCACTGTTCTGG-3'	Antisense to CRH1 for qPCR
UC3788	5'-CCTTGGGCAAGGGATAGTT-3'	Sense to SCR1 for qPCR
UC3789	5'-CTGCCAGGACAAATTACG-3'	Antisense to SCR1 for qPCR
UC3315	5'-AGACCGGTGTCATCGTGGTAAGATGTCACACAACATTATTAC- TTACGCTCCCTATAGTGAGTCGTAAAATT-3'	Sense to Fba1 with T7 promoter for in vitro transcription
UC3316	5'-TCCAGCTTACGGTATCCCAGTTGCTTACACTCTGACCACTGTG- CCAAGACCCCTATAGTGAGTCGTAAAATT-3'	Sense to Fba1 with T7 promoter for in vitro transcription
UC3317	5'-GTCTCCACGGTGGTCCGGTACTGTCCAAGAA TTCCACACTGGTATCCCTATAGTGAGTCGTAAAATT-3'	Sense to Fba1 with T7 promoter for in vitro transcription
UC3318	5'-TCCCCGGGTTAATTAACGACTATAAAGACGATGACCGATAATAG- GGCGGCCCTATAGTGAGTCGTAAAATT-3'	Sense to FLAG with T7 promoter for in vitro transcription

qPCR, quantitative PCR.

References

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