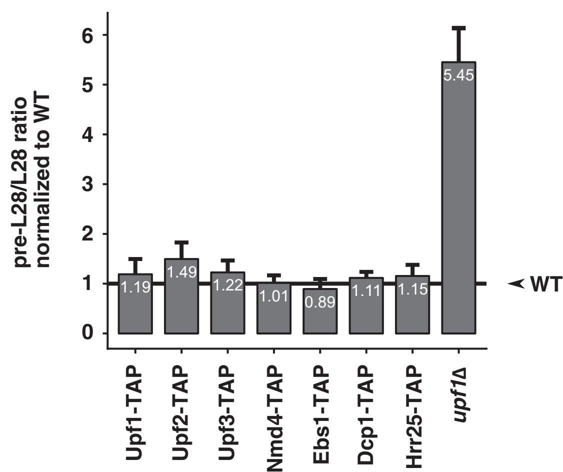
# Expanded View Figures



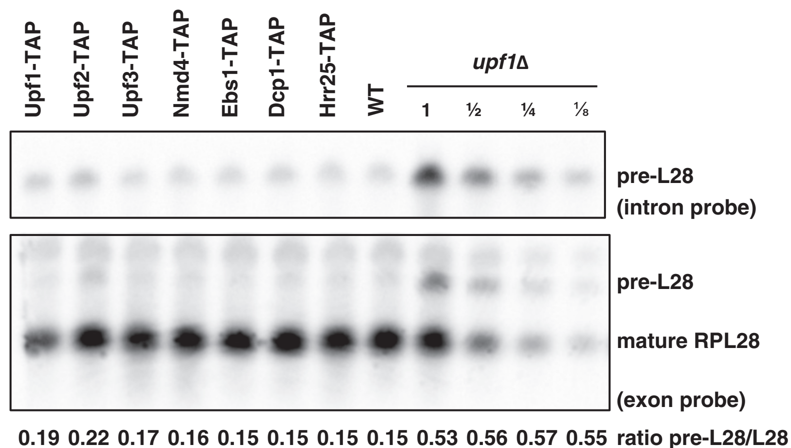




**Figure EV1. Tagged proteins are functional for NMD.**

A RNA from the indicated tagged strains and *upf1*Δ, as control, was quantified by reverse transcription followed by quantitative PCR for the unspliced precursor of RPL28 mRNA and for a normalization RNA, insensitive for NMD (RIM1). Mean values and standard deviations are depicted.

B The stabilization of the endogenous NMD target pre-RPL28 was tested in comparison with the mature form (RPL28) by northern blot using dsDNA probes and chemiluminescent detection.

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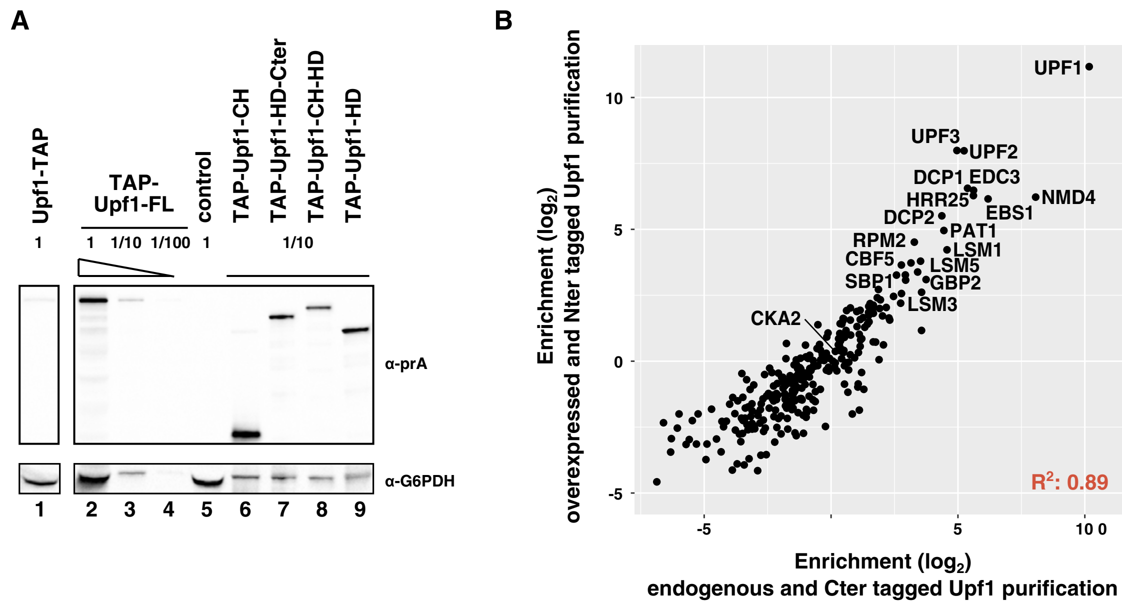


**Figure EV2. N-terminal and C-terminal tagged Upf1 enrich similar sets of specific proteins.**

A Estimation of the levels of overexpression for N-terminal tagged Upf1 fragments, in comparison with chromosomally C-terminal tagged protein. G6PDH was used as a loading control. Serial dilutions were used to test the ability of the immunoblot signal to estimate protein levels.

B Average enrichment values for purifications done with chromosomally C-terminal tagged Upf1 (*x*-axis) and N-terminally TAP-tagged Upf1.

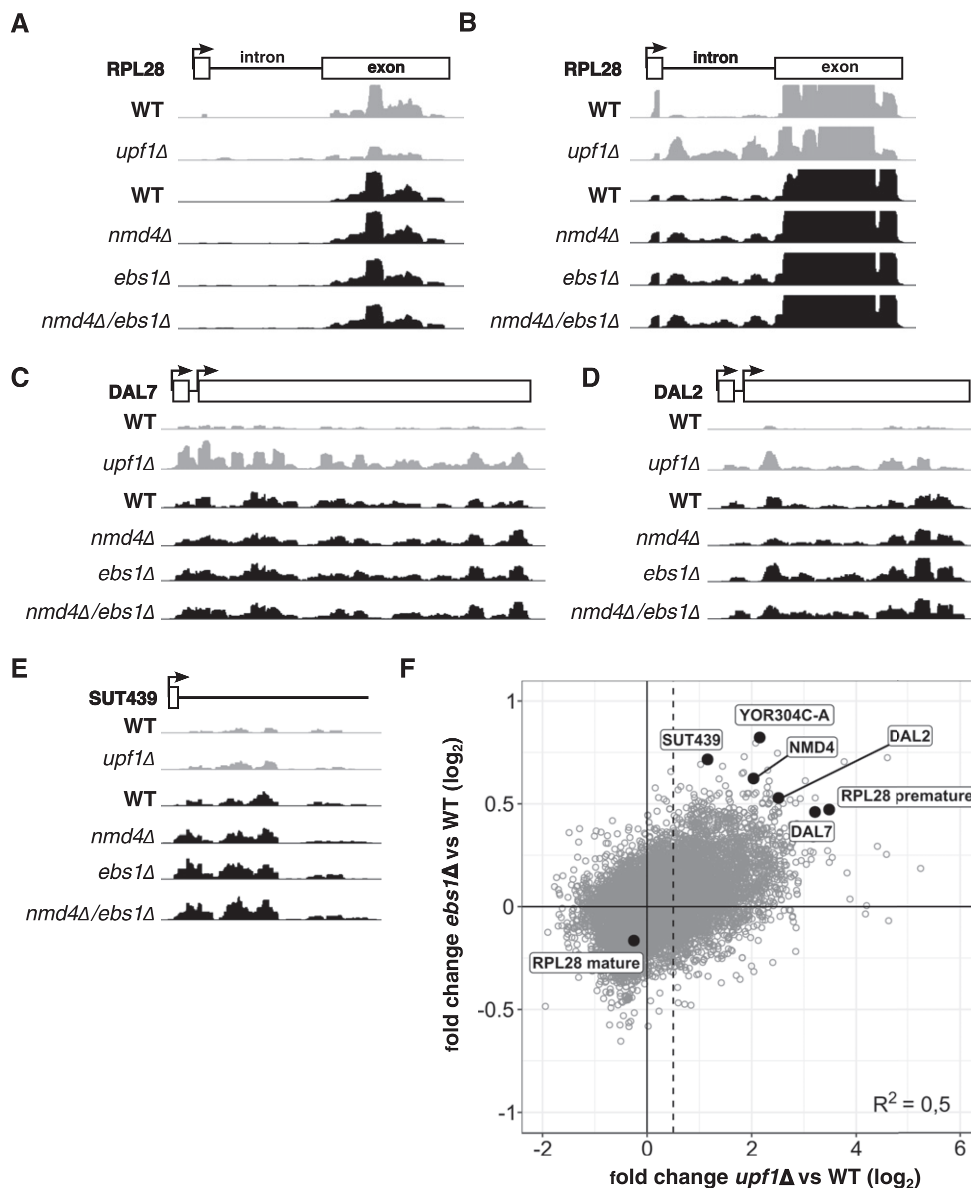


**Figure EV3. Deletion of *NMD4* and *EBS1* stabilize a set of transcripts that is also stabilized in the absence of *UPF1*.**

A–E Examples of NMD substrates sequencing profiles in WT, *upf1*Δ, *nmd4*Δ, *ebs1*Δ and *nmd4*Δ/*ebs1*Δ experiments. NMD substrates belong to different classes, intron containing (RPL28; A and B, different scaling to show intron signal), uORF (DAL7, DAL2; C and D) and non-coding RNA (SUT439; E). Profiles were normalized using the samples median counts.

F Scatter plot of transcript $\log_2$ fold change in *upf1*Δ against *ebs1*Δ. The dashed line represents the limit over which RNA was considered as stabilized, with a $\log_2$ value of 0.5 (1.4 fold change).

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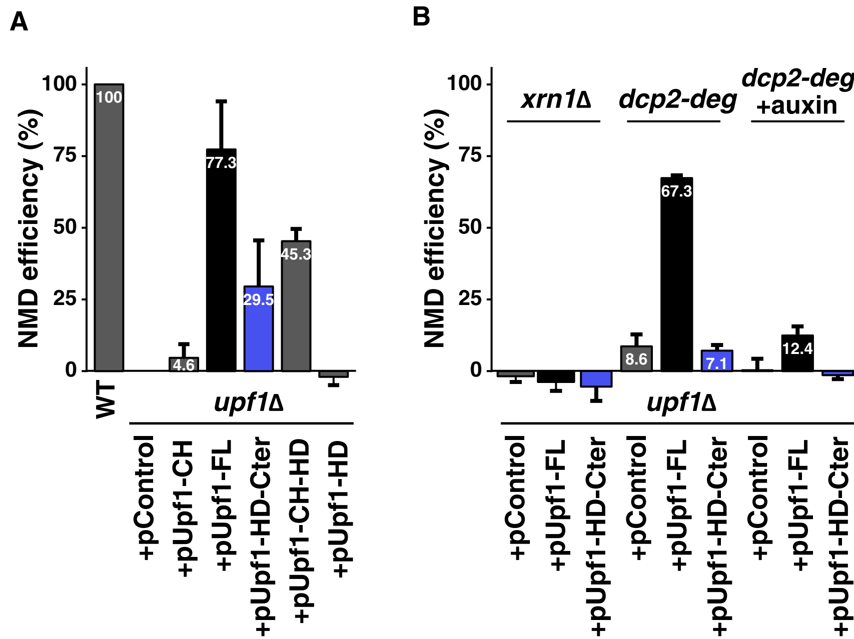


**Figure EV4. The helicase domain of Upf1 alone can destabilize RPL28 pre-mRNA, an NMD substrate.**

A Total RNA from wild-type or a *upf1Δ* strain transformed with an empty plasmid (pControl) or plasmids expressing various Upf1 fragments (see Fig 3A) was tested by reverse transcription and quantitative PCR. The levels of RPL28 pre-mRNA were normalized using an NMD-insensitive transcript (RIM1), and an NMD efficiency score was calculated based on the difference between a wild-type (100%) and the *upf1Δ* (0% NMD) strain.

B Complementation of *UPF1* deletion was tested in combination with the deletion of *XRN1*, and in strains with a degron-regulated Dcp2 protein, when protein degradation was not induced (dcp2-deg) or was induced by addition of auxin (dcp2-deg + auxin) and incubation for 1 h. Values represent average and standard deviation for at least three independent experiments.

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