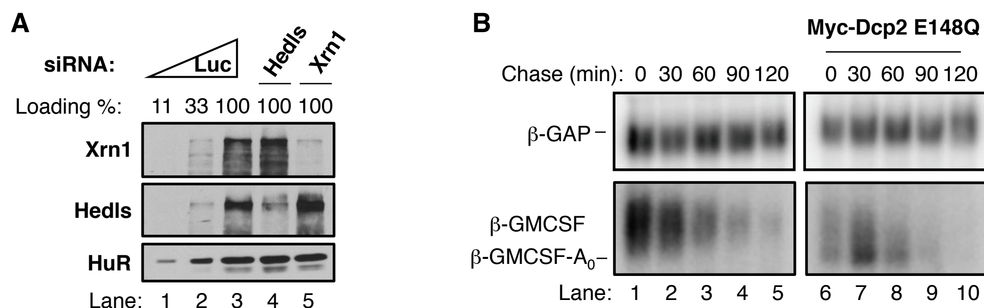


Figure S1. Supplementary information to Figure 1
(A) Western blots showing knockdown efficiencies for siRNAs targeting Hedls, Xrn1, or Luciferase as indicated.
(B) Northern blots monitoring the degradation β-globin reporter mRNA (β-GMCSF) containing an ARE from GMCSF mRNA mRNAs in tetracycline-controlled pulse-chase mRNA decay assays in HeLa Tet-off cells transfected with a control plasmid (empty vector) or a plasmid expressing catalytically inactive Dcp2 E148Q. Numbers above lanes refer to time in minutes after transcription was stopped by addition of tetracycline. β-GAP is a constitutively expressed internal control mRNA. The position of a faster migrating deadenylated species is indicated as β-GMCSF-A_0 on the left.

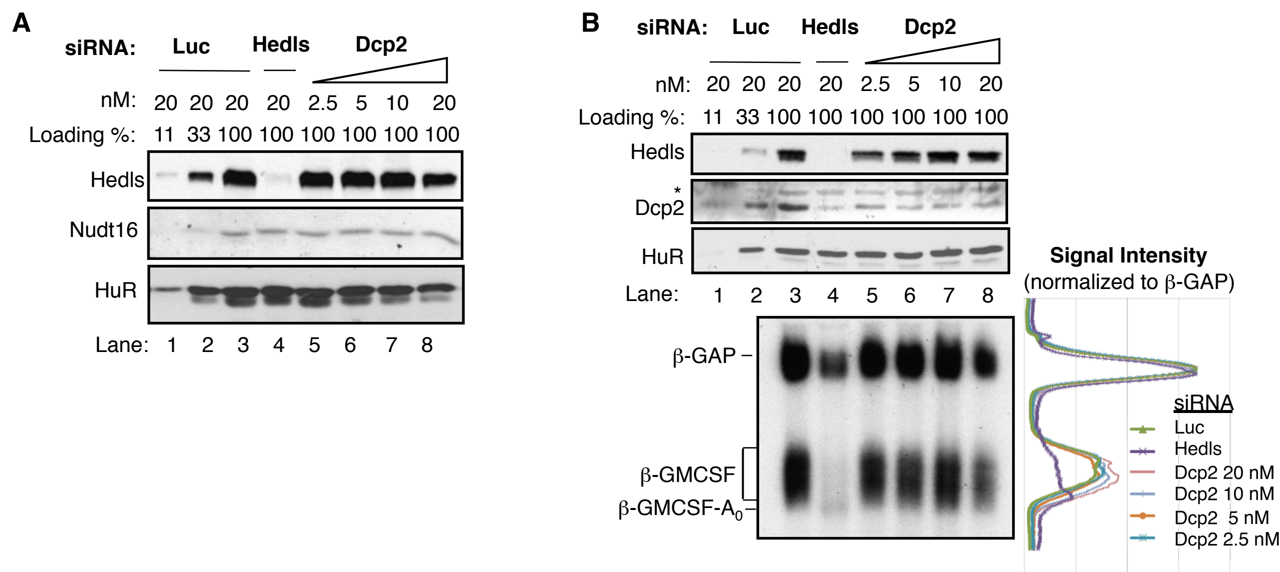


Figure S2. Effect on ARE-mRNA and Nudt16 of Hedls and Dcp2 depletion
(A) Western blots of lysates from HeLa Tet-off cells transfected with siRNAs targeting luciferase (Luc), Hedls or Dcp2. Antibodies against endogenous proteins were used to detect Hedls, Nudt16, and HuR. Numbers above lanes refer to concentration siRNA used (nM) and percent of each sample loaded (Loading %).
(B) Western (top) and Northern (bottom) blots monitoring levels of indicated proteins and mRNAs in HeLa Tet-off cells transfected with siRNAs targeting luciferase (Luc), Hedls, or Dcp2 and expressing β-GMCSF reporter and β-GAP control mRNAs. Numbers above lanes refer to concentration of siRNA used (nM) and percent of each sample loaded (Loading %). Antibodies against endogenous proteins were used to detect Hedls, Dcp2, and HuR in the top panel. A non-specific band detected by the Dcp2 antibody is indicated with an asterisk. In the Northern blots in the bottom panel, the fast migrating band appearing in lane 2 is indicated by β-GMCSF-A_0 on the left, and a graphical representation of the signal intensity across each lane, normalized for β-GAP mRNA signal, is shown on the right.

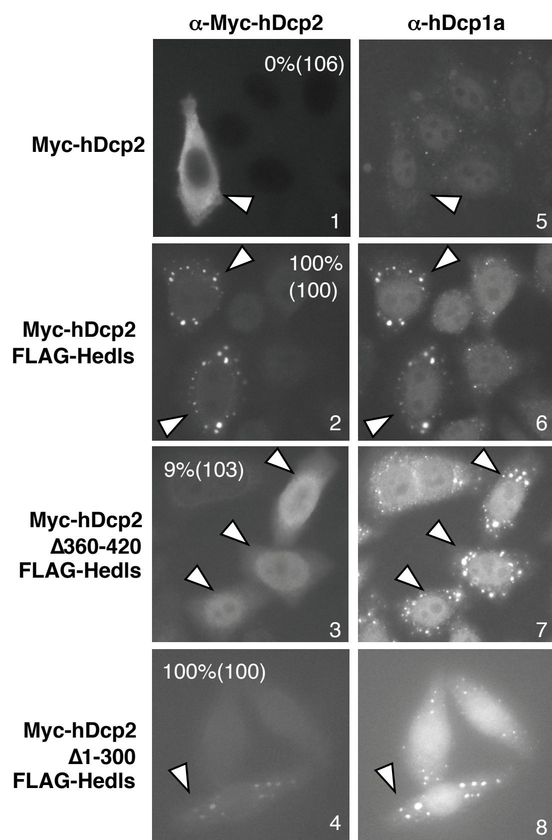


Figure S3. The C-terminus of Dcp2 is necessary and sufficient for Hedls-mediated localization to P-bodies

Immunofluorescence assays of HeLa cells co-expressing Myc-tagged wild-type or mutant Dcp2 with FLAG-Hedls. Anti-Myc antibody was used on the left panels and anti-hDcp1a was used on the right panels as a P-body marker. Arrowheads indicate transfected cells. Percentages of cells with Dcp2 in P-bodies are listed with the number of cells counted in parentheses.

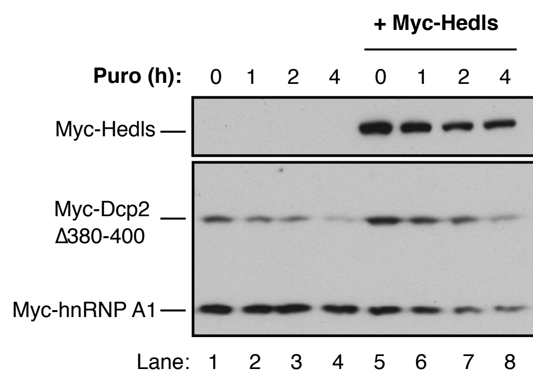
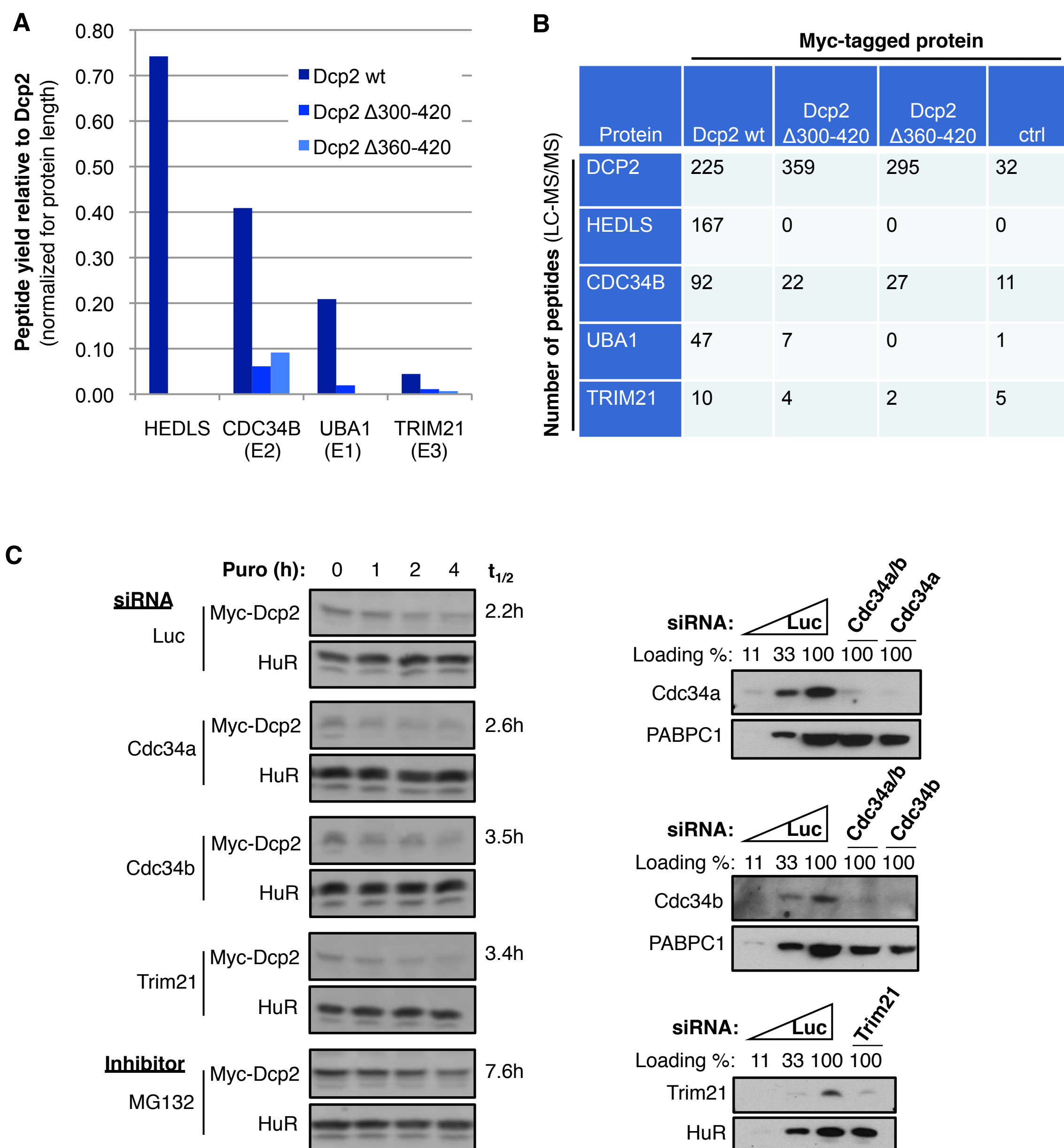


Figure S4. Dcp2 Δ380-400 is not stabilized by Hedls
Translation shut-off assays in HEK293T cells transiently expressing Myc-tagged Dcp2 Δ380-400 in the absence (lanes 1-4) or presence (lanes 5-8) of co-expressed Hedls. Myc-hnRNP A1 served as a control. Times above lanes refer to hours after translation shut-off with puromycin (Puro).



Protein	Dcp2 wt	Dcp2 Δ300-420	Dcp2 Δ360-420	ctrl
DCP2	225	359	295	32
HEDLS	167	0	0	0
CDC34B	92	22	27	11
UBA1	47	7	0	1
TRIM21	10	4	2	5

Figure S5. Ubiquitin ligases co-purify with Dcp2

(A) Graph showing relative peptide yield obtained for indicated proteins in LC-MS-MS assays of IP samples for the indicated Myc-tagged Dcp2 proteins. Numbers were calculated as number of peptides from individual proteins divided by number of peptides from Dcp2 and normalized for protein length in amino acids. The table on the right lists the actual number of peptides obtained for each protein.

(B) Table showing total number of peptides obtained from indicated Myc-Dcp2 IPs.

(C) Translation shutoff assays in HEK293 T-REx cells stably expressing tetracycline-inducible Myc-tagged Dcp2 and transfected 72 and 24 hours prior to translation shutoff with siRNAs (50 nM) targeting Luciferase (Luc), Cdc34a, Cdc34b, or Trim21. Myc-Dcp2 was induced 16 hours prior to translation shutoff by addition of 2 μg/ml tetracycline. MG132 was added 2 hours prior to translation shutoff in bottom panels. Half-lives were calculated relative to HuR and are listed on the right. Panels on the right are Western blots showing efficiency of depletion with the indicated siRNAs. Loading % refers to the fraction of samples loaded.

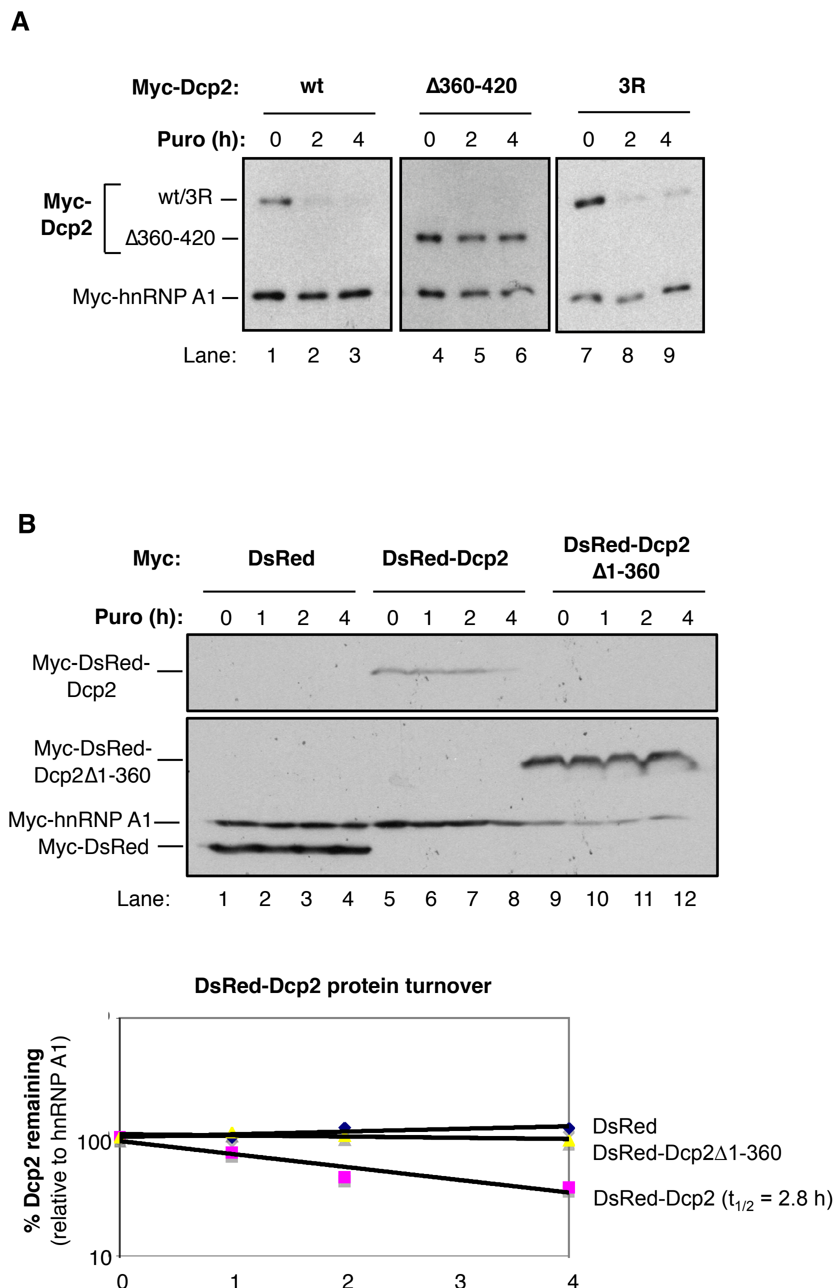


Figure S6. The Dcp2 C-terminus is not sufficient to trigger protein degradation
(A) Translation shut-off assays in HEK293T cells transiently expressing Myc-tagged Dcp2 wild-type (wt), Δ360-420, or mutant Dcp2 with all three lysines within the C-terminal 60 amino acids mutated to arginines (3R). Times above lanes refer to hours after translation shut-off with puromycin (Puro).
(B) Same as *A*, but using HEK293T cells transiently expressing indicated DsRed fusion proteins. Co-transfected Myc-hnRNPA1 served as a stable control. Times above lanes refer to hours after translation shut-off with puromycin (Puro). DsRed fusion protein levels were quantified relative to Myc-hnRNP A1 and are graphed below.