Supplementary information for:

Elimination of cap structures generated by mRNA decay involves the new scavenger mRNA decapping enzyme Aph1/FHIT together with DcpS

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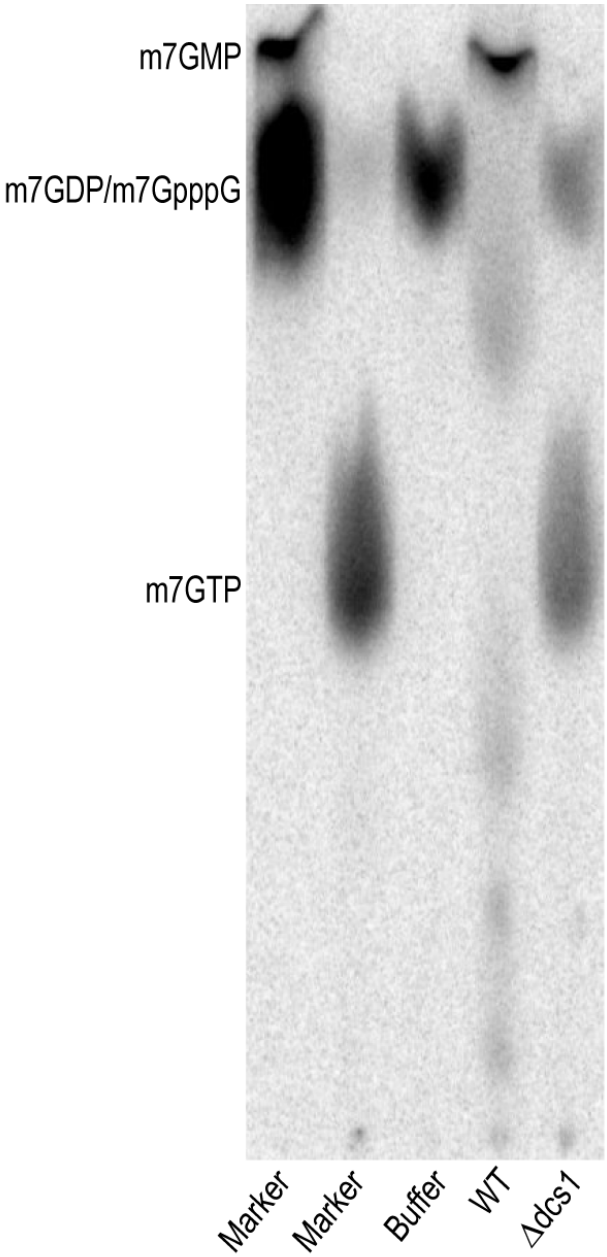
Running title: Factors mediating mRNA cap elimination

Supplementary Table 1: Oligonucleotides used in this study

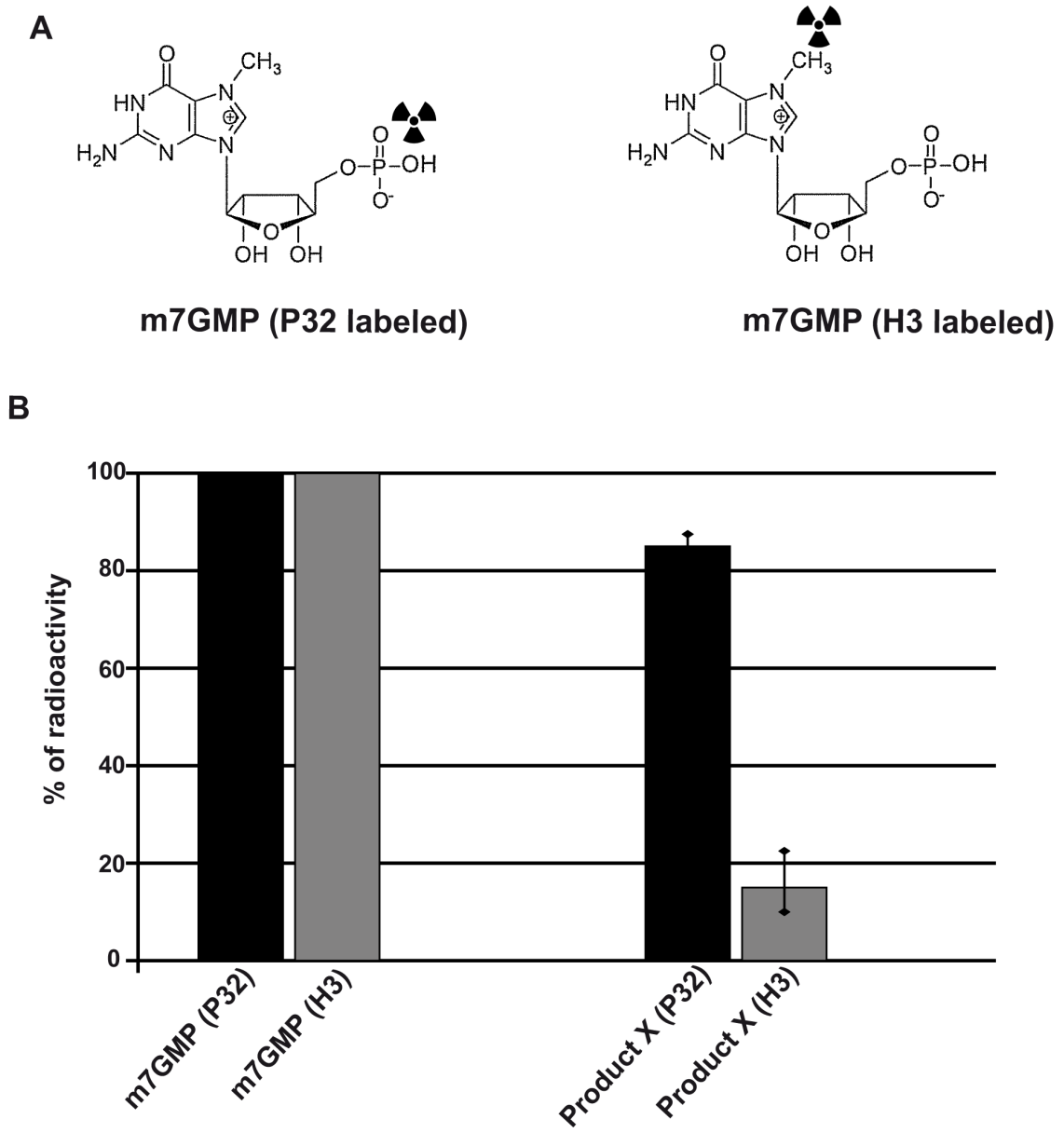
Name	Sequence
OBS6090	TCA GGT TCT TTC CGG AAA AAC ATC TCA TAA ATC ATC CCT GGA AAA CGG ATC CCC GGG TTA ATT AA
OBS6091	AGA AAA AAA AGG CGC TCA TAT GTA CCC TCC CAT GCC ATA AGT TAT GAA TTC GAG CTC GTT TAA AC
OBS6240	AGC GAA GAA AAA AAG GAA GCT CCA TTG ATC TAT CTT GGG CTC AGA CAC AGG AAA CAG CTA TGA CC
OBS6241	CCT ACT ATT TTT TAT ATC GTA AGT ATG AAT CTA TTA TTT ATT GAA GTT GTA AAA CGA CGG CCA GT
OBS6505	GGA CAG ACT GTG AAG CAC GTT AAC GTC CAT GTT CTT CCC AGG AAG
OBS6506	CTT CCT GGG AAG AAC ATG GAC GTT AAC GTG CTT CAC AGT CTG TCC
OBS6507	GGA CAG TCG GTA CCC CAC TTA AAT ACA CAC ATT ATT CCA CGT TAT
OBS6508	ATA ACG TGG AAT AAT GTG TGT ATT TAA GTG GGG TAC CGA CTG TCC
OBS6510	CCG TAC TCG AGC TAT AAC CAT TGT GTT AAA CCA G
OBS6518	GGC TAG AAT TCA TGA ATA AGC CAA TAT ATT TCA GC

Supplementary Table 2: Yeast strains used in this study

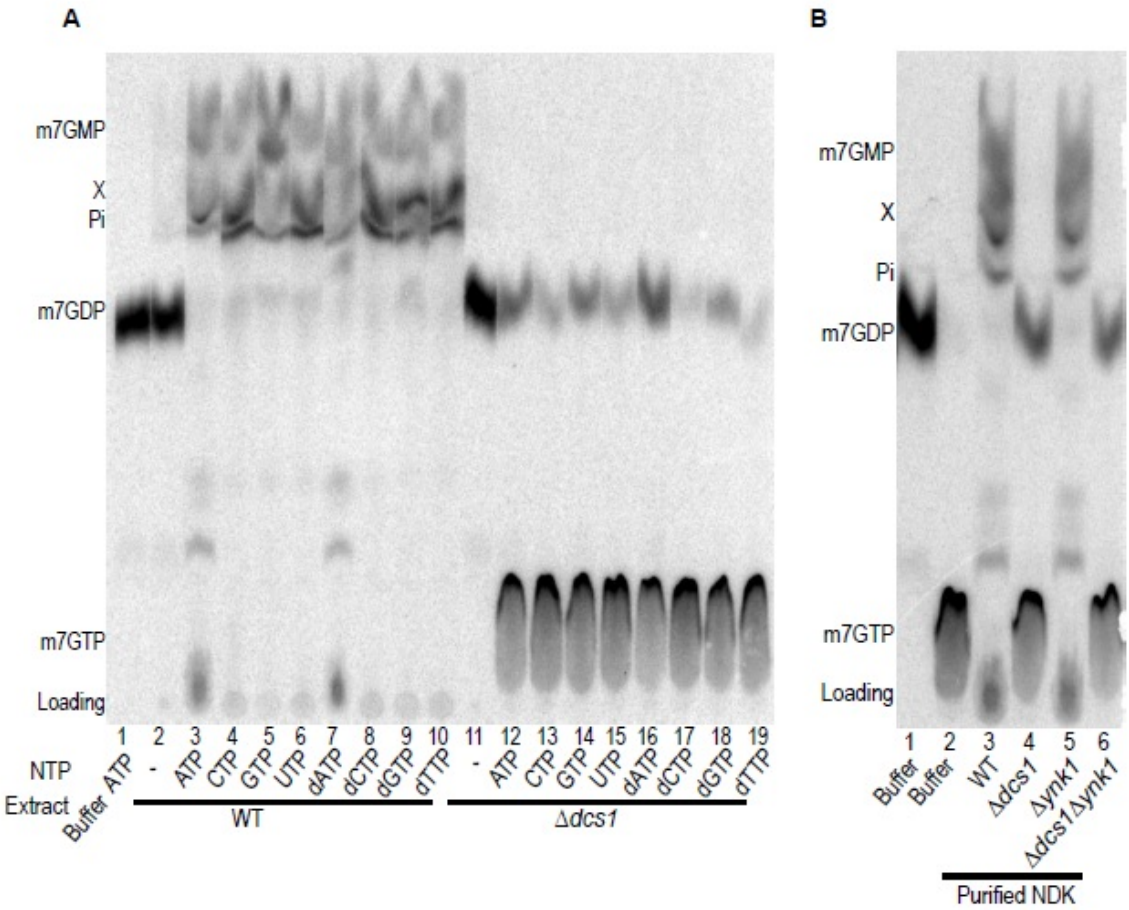
Name	Genotype	Comment (Reference)
BMA64	MAT α; ade2-1; leu2-3,112; his3-11,15; Δtrp1; ura3-1	Wild type background (1)
BSY1417	MAT a; ade2-1; leu2-3,112; his3-11,15; Δtrp1; ura3-1; Δdcs1::$_{Kl}$TRP1-TAP	(2)
BSY2810	MAT α; ade2-1; leu2-3,112; his3-11,15; Δtrp1; ura3-1; Δynk1::HIS3MX6	This work
BSY2811	MAT a; ade2-1; leu2-3,112; his3-11,15; Δtrp1; ura3-1; Δdcs1::$_{Kl}$TRP1-TAP; Δynk1::HIS3MX6	This work
BSY2967	MAT α; ade2-1; leu2-3,112; his3-11,15; Δtrp1; ura3-1; Δaph1::$_{Gg}$LEU2	This work
BSY2973	MAT a; ade2-1; leu2-3,112; his3-11,15; Δtrp1; ura3-1; Δdcs1::$_{Kl}$TRP1-TAP; Δaph1::$_{Gg}$LEU2	This work
BSY2975	MAT α; ade2-1; leu2-3,112; his3-11,15; Δtrp1; ura3-1; Δynk1::HIS3MX6; Δaph1::$_{Gg}$LEU2	This work
BSY2977	MAT α; ade2-1; leu2-3,112; his3-11,15; Δtrp1; ura3-1; Δdcs1::$_{Kl}$TRP1-TAP; Δynk1::HIS3MX6; Δaph1::$_{Gg}$LEU2	This work
YPALS	trp1-Δ1 his3Δ200 ura3-52 ade2-101 lys2-801 can1	(3)
YPALST	YPALS aph1Δ::TRP1	(3)
YPALSHU	YPALS apa1Δ::HIS3 apa2Δ::URA3	(3)
YPALSHUT	YPALS apa1Δ::HIS3 apa2Δ::URA3 aph1Δ::TRP1	(3)



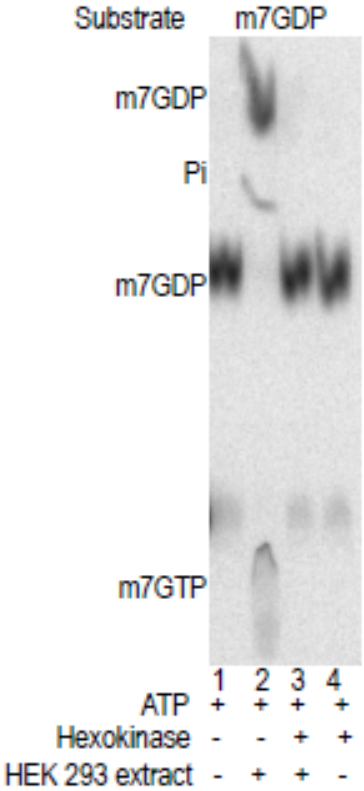
Supplementary Figure 1 m7GDP conversion in yeast extracts. Purified radiolabeled m7GDP was incubated with the reaction buffer, or whole extracts from wild type or Δ*dcs1* strains. The reaction products were separate by TLC using $(NH_4)SO_3$ as resolving buffer that allow better separation of the lower migrating products. Marker lanes present the migration patterns of purified m7GMP, m7GDP, and m7GpppG (the latter two co-migrating) in one lane or m7GTP in another lane.



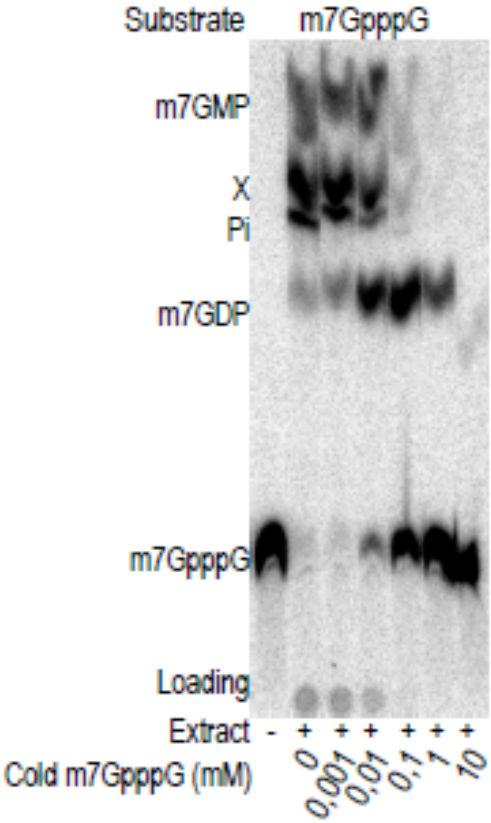
Supplementary Figure 2 Product X contains the phosphate but not the m7 methyl group of m7GMP. (A) Structure of the two substrates used indicating the locations of the radiolabels in P32 labeled m7GMP (left) and H3 labeled m7GMP. (B) Fraction of radioactivity present in product X generated by incubation in yeast extract relative to the m7GMP substrate (100%) for both the P32 labeled and H3 labeled compounds.



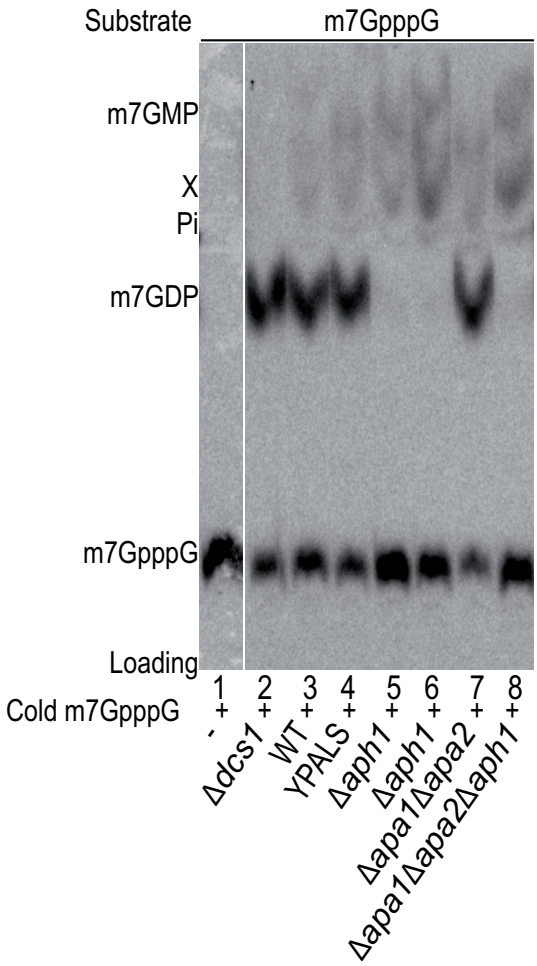
Supplementary Figure 3 m7GDP conversion in yeast extracts. (A) Purified radiolabeled m7GDP was incubated with the reaction buffer (lane 2), wild type (lanes 3-10) or Δ*dcs1* (lanes 12-19) yeast whole extracts. The indicated (deoxy-)nucleoside triphosphate were added at a final concentration of 2 mM. (B) Radiolabeled m7GDP was incubated with buffer (lanes 1), or whole extracts from wild type yeast (lane 2), Δ*dcs1* mutant (lane 3), Δ*ynk1* mutant (lane 4), or Δ*dcs1*Δ*ynk1* mutant (lane 5). All reactions contained ATP and were supplemented with purified NDK from yeast (Sigma-Aldrich).



Supplementary Figure 4 m7GDP conversion in HEK293 extract. m7GDP was incubated with the reaction buffer (lanes 1 and 4) and HEK293 total extract (lanes 2 and 3). An ATP depleting system (hexokinase and glucose) was added to reactions (lanes 3 and 4).



Supplementary Figure 5 DcpS inhibition. m7GpppG was incubated with the reaction buffer or yeast wild type extract in presence of an increasing concentration of cold m7GpppG used as DcpS inhibitor.



Supplementary Figure 6 Testing the effects of inactivation of various HIT family members on cap degradation in yeast extracts. A TLC analysis of m7GpppG conversion products is presented. Products resulting from incubation with buffer (lane 1), or with whole cell extracts from Δdcs1 (BSY1417, lane 2), wild type WT (BMA64, lane 3), YPALS (wild type isogenic with deletion mutants, lane 4), Δ*aph1* (YPALST, lanes 5 and 6), Δ*apa1*Δ*apa2* (YPALSHU, lane 7), or Δ*apa1*Δ*apa2*Δ*aph1* (YPALSHUT, lane 8) yeast strains are shown. Reactions were incubated with cold m7GpppG at a final concentration of 10 µM to inhibit DcpS.

Supplementary references

1. Baudin-Baillieu, A., Guillemet, E., Cullin, C. and Lacroute, F. (1997) Construction of a yeast strain deleted for the TRP1 promoter and coding region that enhances the efficiency of the polymerase chain reaction-disruption method. *Yeast*, **13**, 353-356.
2. van Dijk, E., Le Hir, H. and Seraphin, B. (2003) DcpS can act in the 5'-3' mRNA decay pathway in addition to the 3'-5' pathway. *Proc Natl Acad Sci U S A*, **100**, 12081-12086.
3. Chen, J., Brevet, A., Blanquet, S. and Plateau, P. (1998) Control of 5',5'-dinucleoside triphosphate catabolism by APH1, a Saccharomyces cerevisiae analog of human FHIT. *J Bacteriol*, **180**, 2345-2349.