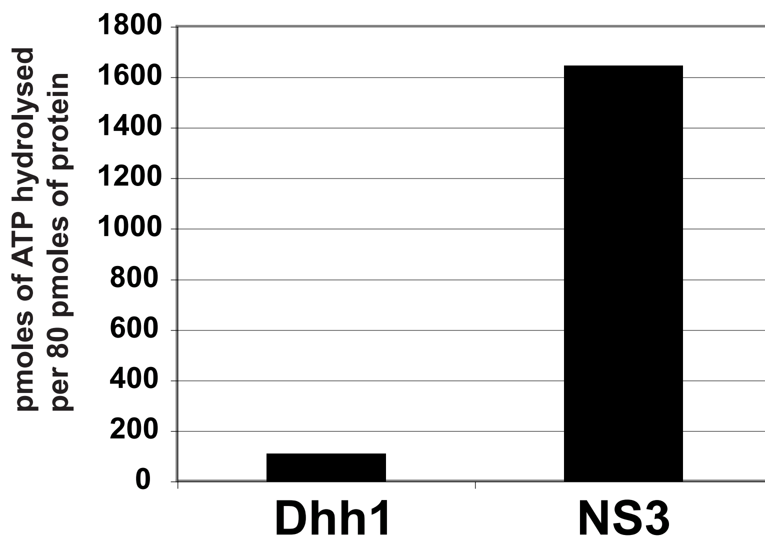
A.



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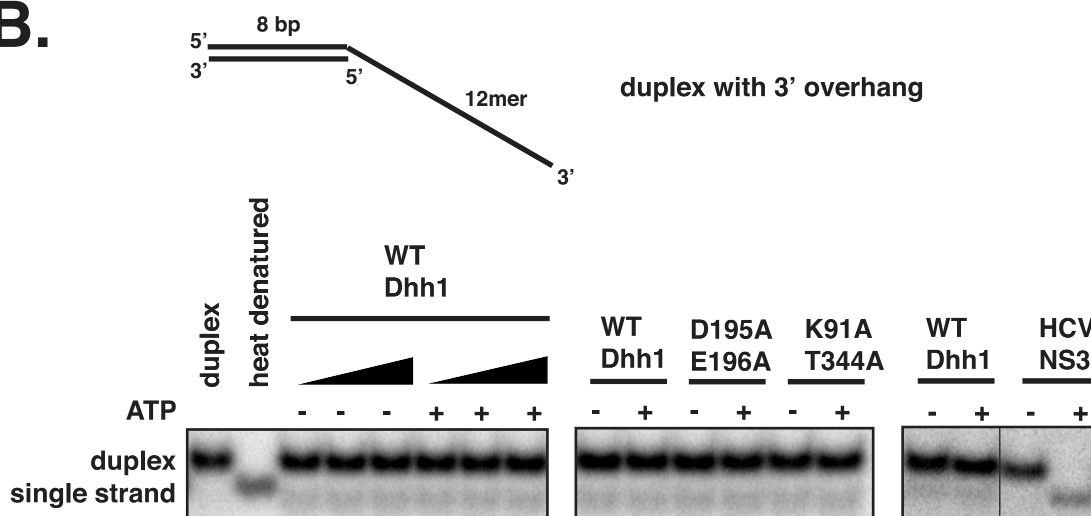
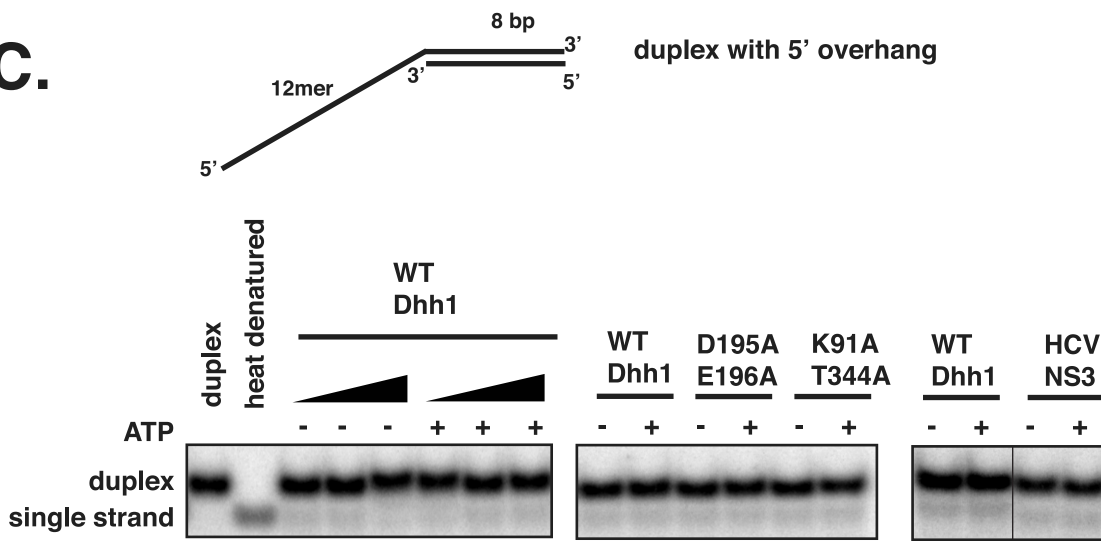
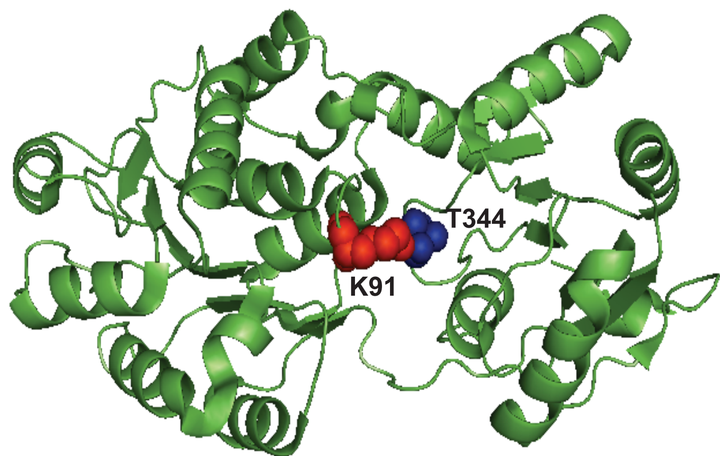


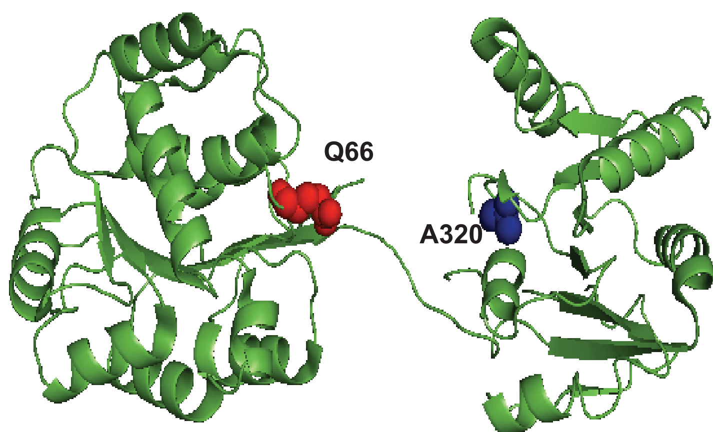
C.



Dhh1



eIF4A



mjDEAD

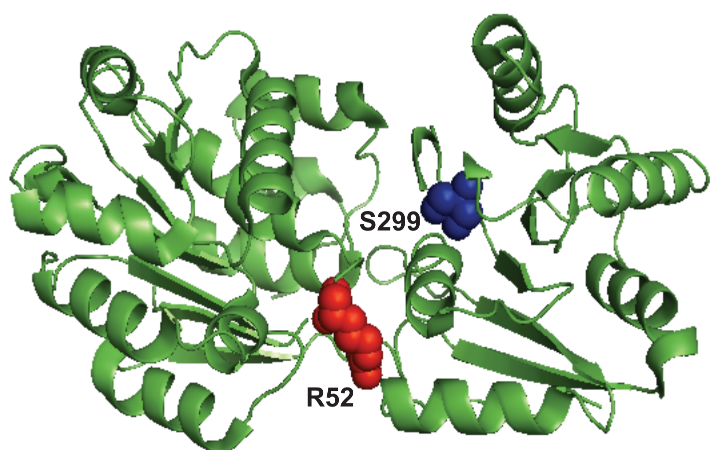


Fig S3

N' domain

C' domain

F66
Q73
R322
S340
T344
K96
D195
E196
K91
R370
G346
R345
R89

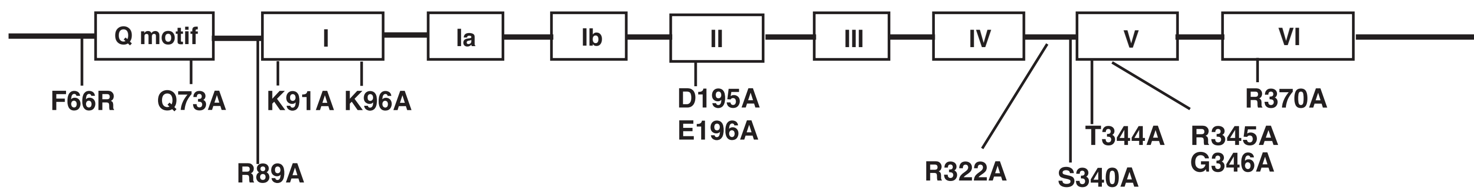
ATP hydrolysis

ATP binding

inter-domain interaction

RNA binding

other residues mutated



Strain	Description	Genotype
PH499	WT	*MAT a ade2-101; his3-Δ200; leu2-Δ1; ura3-52; trp1-Δ63; lys2-801*
YJR148	DHH1 deletion	PH499 with *Δdhh1::HIS3*
JR1429	Integrated *DHH1*	Isogenic to YJR148; *DHH1*::TRP1
JR1430	Integrated F66R Q73A	Isogenic to YJR148; *dhh1* F66R/Q73A::*TRP1*
JR1431	Integrated D195A E196A	Isogenic to YJR148; *dhh1* D195A/E196A::*TRP1*
JR1432	Integrated K91A T344A	Isogenic to YJR148; *dhh1* K91A/T344A::*TRP1*
JR1433	Integrated R322A S340A	Isogenic to YJR148; *dhh1* R322A/S340A::*TRP1*
JR1450	Integrated *DHH1-GFP*	Isogenic to YJR148; *DHH1-GFP*::*KanMx*
JR1451	Integrated F66R Q73A-*GFP*	Isogenic to YJR148; *dhh1* F66R/Q73A-*GFP*::*KanMx*
JR1452	Integrated D195A E196A-*GFP*	Isogenic to YJR148; *dhh1* D195A/ E196A-*GFP*::*KanMx*
JR1453	Integrated K91A T344A-*GFP*	Isogenic to YJR148; *dhh1* K91A/T344A-*GFP*::*KanMx*
JR1453	Integrated R322A S340A-*GFP*	Isogenic to YJR148; *dhh1* R322A/S340A-*GFP*::*KanMx*

Supplemental Table 1.

Supplemental figure legends

Supplementary Figure 1. Comparison of the biochemical activities of Dhh1 and NS3 helicase. (A) ATPase activity. ATPase assay was performed at 30 °C for 60 minutes using 4uMof Dhh1 and HCV NS3 helicase and 20 ug poly (U) RNA. The activity was plotted as pmol of ATP hydrolyzed per 80 pmol of protein. Details are found in the methods section of the m,anuscript. (B) Helicase assays were carried out using 2 nM [32P]-labeled 3' and 5' overhang substrates (B and C). Dhh1 was titrated into the assay from 0 to 1 μM (left side). Wild type Dhh1 and mutants were analyzed at 1 μM (middle). The NS3 protein, a helicase from Hepatitis C virus was analyzed as control (0.5 μM). Reactions were performed at 30°C. The trapping strand was included in the reactions (8-mer RNA that is complementary to the 8 bp displaced strand). Reactions were quenched after 15 minutes by the addition of EDTA to 100 mM and SDS to 0.33%. Products were resolved on native polyacrylamide gels.

Supplementary Figure 2: Comparison of crystal structures of Dhh1 with that of eIF4A and mjDEAD. The crystal structures of Dhh1 (PDB id. 1S2M), *S. cerevisae* eIF4A (PDB id. 1FUU) and *M. janaschii* mjDEAD (PDB id. 1HV8) were analyzed using PyMOL. The positions of K91 and T344 are highlighted in the crystal structure of Dhh1. The position of orthologous residues in eIF4A and mjDEAD are highlighted in the respective crystal structures. K91 in Dhh1 is replaced by Q66 in eIF4A and R52 in mjDEAD, whereas T344 in the C' domain of Dhh1 is replaced by A320 in eIF4A and S299 in mjDEAD. Unlike Dhh1, eIF4A and mjDEAD lack interdomain interactions.

Supplementary Figure 3: Location of the mutants on the crystal structure of Dhh1. The crystal structure of Dhh1 was analyzed using PyMOL software (PDB id. 1S2M). The positions of amino acid residues that were mutated to alanines are color coded as follows: residues involved in ATP hydrolysis (K96, D195, E196 and R373) are highlighted in red, (F66 and Q73) involved in ATP binding are highlighted in orange, residues involved in interdomain interactions (K91 and T344) are highlighted in blue, residues that affect RNA binding (R322, S340 and R370), are shown in green and other residues (R89, R345 and G346) are shown in black. A schematic of the helicase domain structure of Dhh1 is shown below.