

Supplementary Material

Structure-function analysis of the Yhc1 subunit of yeast U1 snRNP and genetic interactions of Yhc1 with Mud2, Nam8, Mud1, Tgs1, U1 snRNA, SmD3 and Prp28

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Figures S1, S2, S3, S4, S5

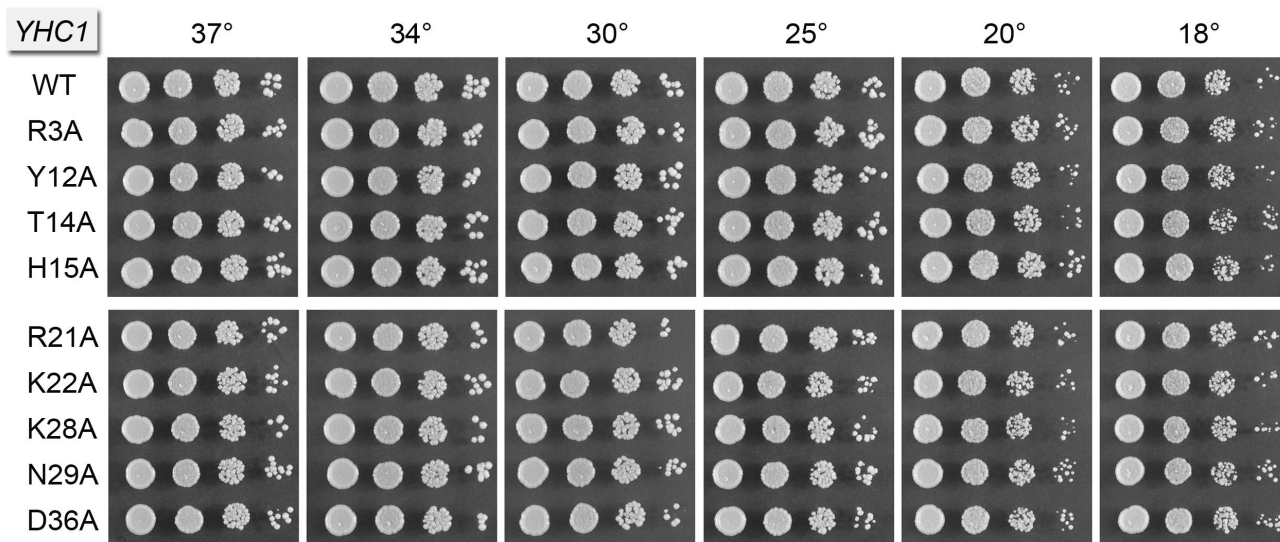


Figure S1. **Effects of alanine mutations on Yhc1 function *in vivo*.** The *yhcl* Δ p[*CEN HIS3 YHC1*] strains obtained after plasmid shuffle, bearing the *YHC1* alleles indicated at left, were spot tested for growth on YPD agar at the temperatures specified.

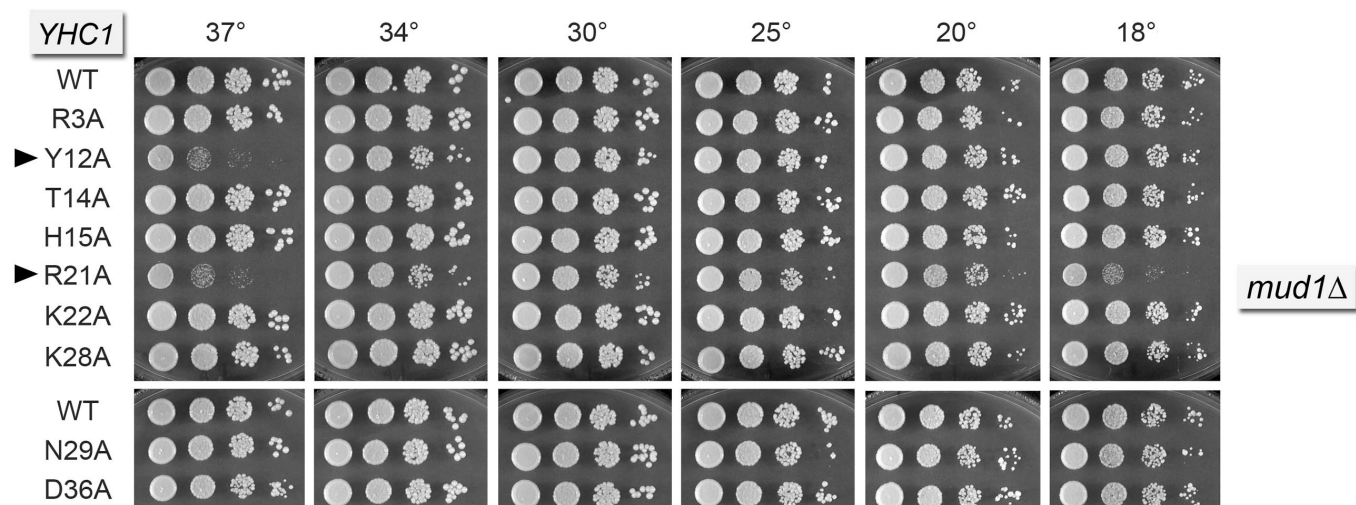


Figure S2. **Synthetic interactions of Yhc1-Ala mutants with Mud1.** Yeast *mud1Δ yhc1Δ* strains bearing the indicated *YHC1* alleles were spot-tested for growth at the temperatures specified. The Y12A and R21A alleles that displayed synthetic sickness at one or both extremes of temperature are indicated by ► at left.

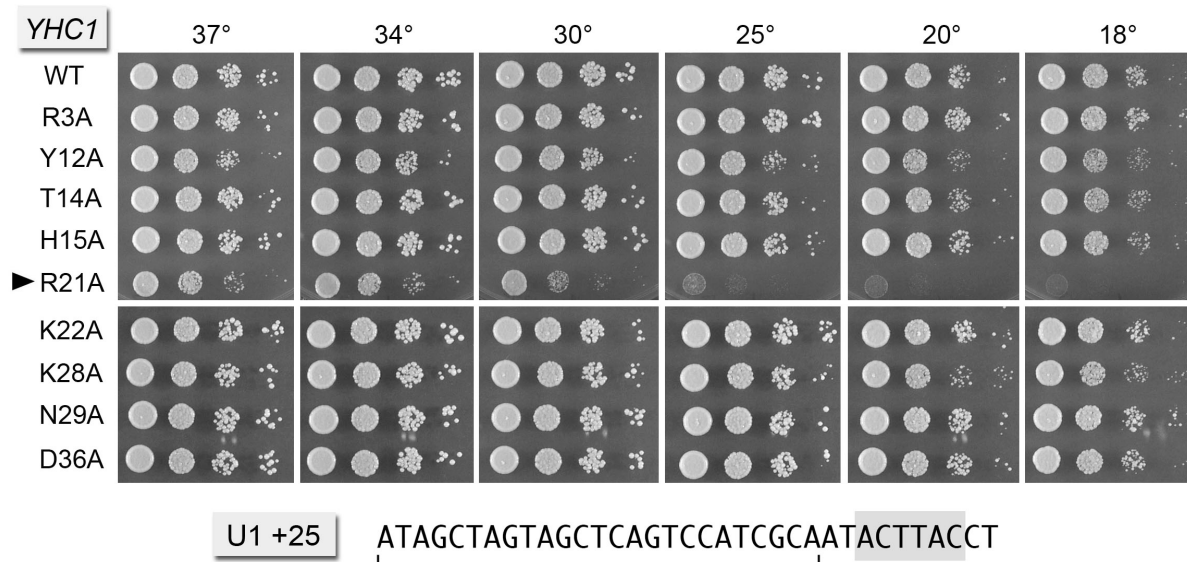


Figure S3. **Synthetic interaction of *YHC1-R21A* with U1 snRNA 5'-extension *U1+25*.** The DNA sequence encoding the 5' end of the U1+25 sRNA is shown at *bottom*. The extra 25-nucleotide spacer segment between the 5'-TMG cap and native U1 sequence is demarcated by the bracket. The segment encoding the ³ACUUAC⁸ motif that pairs with the intron 5'SS is highlighted in grey. The *U1+25 yhc1Δ* strains bearing the indicated *YHC1* alleles were spot-tested for growth at the temperatures specified. The synthetic growth defect of the *R21A* allele is denoted by ▶ at *left*.

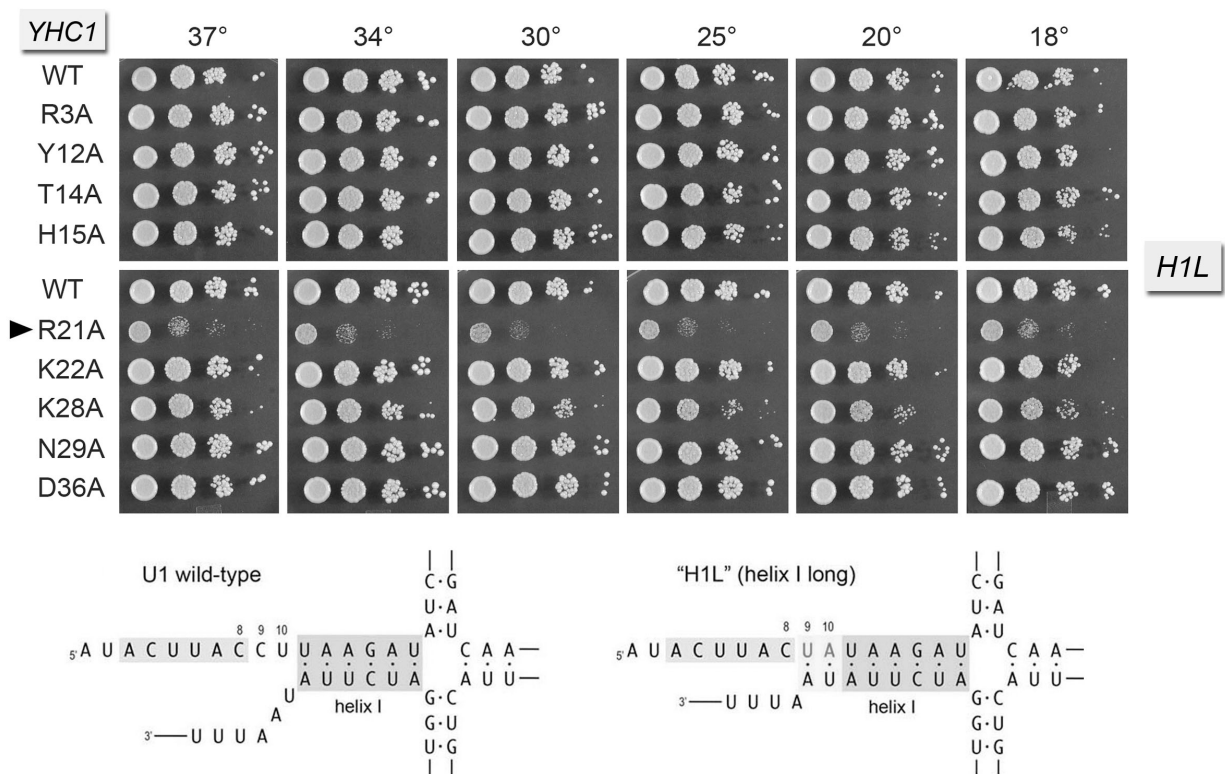


Figure S4. **Synthetic interaction of Yhc1-R21A with U1 snRNA “helix 1 long” mutant.** The primary and predicted secondary structures at the 5' end of wild-type U1 RNA and the H1L (“helix 1 long”) mutant are shown at *bottom*. The ³ACUUAC⁸ segment that pairs with the intron 5'SS is highlighted in light grey. Helix 1 is shaded dark grey. The mutation of ⁹CU¹⁰ to ⁹UA¹⁰ extends helix 1 by two base pairs. The *U1-H1L yhc1Δ* strains bearing the indicated *YHC1* alleles were spot-tested for growth at the temperatures specified. The strong synthetic growth defect of the *R21A* allele is denoted by ► at *left*.

	<i>R21A</i>	<i>K28A</i>	<i>Y12A</i>	<i>T14A</i>	<i>K22A</i>	<i>H15A</i>	<i>N29A</i>	<i>D36A</i>
<i>mud2Δ</i>	lethal	very sick	very sick	sick				
<i>nam8Δ</i>	very sick							
<i>tgs1Δ</i>	very sick	sick	very sick	sick				
<i>mud1Δ</i>	sick		sick					
<i>prp28Δ</i>	bypass	bypass						
<i>U1-U^{5C}</i>	very sick		sick					
<i>U1+25</i>	very sick							
<i>U1-H1L</i>	very sick							
<i>U1-[+1]</i>	very sick	very sick		sick	very sick	sick		
<i>U1-ΔU¹⁰</i>	lethal	lethal	very sick	lethal	very sick	very sick	very sick	↑↑

Figure S5. **Summary of mutational synergies of Yhc1-Ala mutants.** Synthetically lethal pairs of alleles are highlighted in red boxes. Other negative pairwise interactions are classified as very sick (yellow boxes) or mildly sick (light green boxes). Allelic pairs that resulted in gain-of-function genetic effects are highlighted in blue boxes, including bypass of the lethality of *prp28Δ* by *R21A* and *K28A* and suppression of the growth defect of *U1-ΔU¹⁰* by *D36A*.