Supplemental Material

Structure-function analysis and genetic interactions of the Yhc1, SmD3, SmB, and Snp1 subunits of yeast U1 snRNP and genetic interactions of SmD3 with U2 snRNP subunit Lea1

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Supplemental Figures: S1, S2, S3, S4 S5, S6, S7, S8

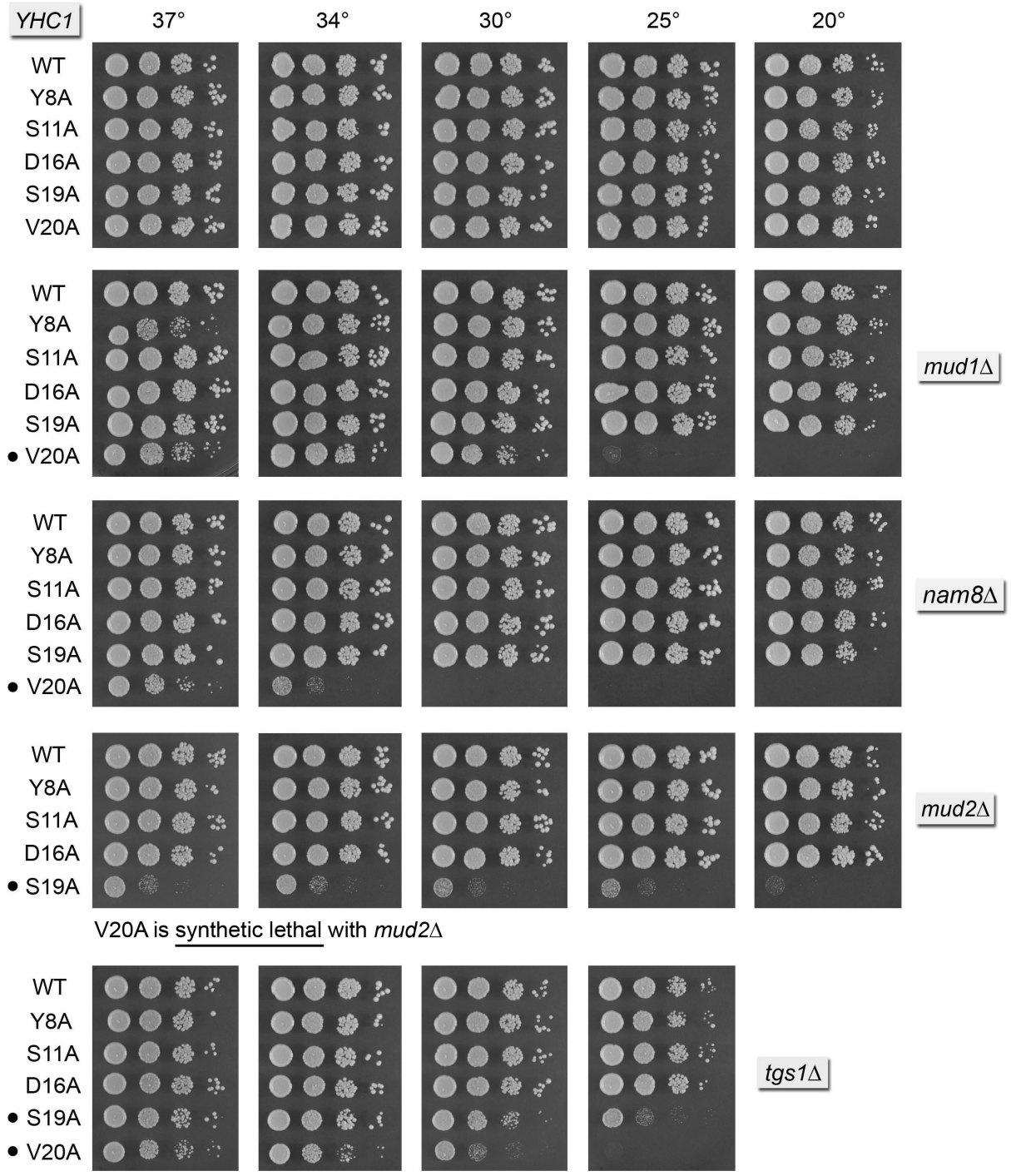


Figure S1. Genetic interactions of *YHC1-Ala* alleles. Yeast *yhc1*Δ strains bearing the indicated *YHC1-Ala* alleles on a *CEN HIS3* plasmid in an otherwise wild-type (top panel), *mud1*Δ, *nam8*Δ, *mud2*Δ, or *tgs1*Δ background as indicated were spot-tested for growth on YPD agar at the temperatures specified. Synthetic growth defects are denoted by •.

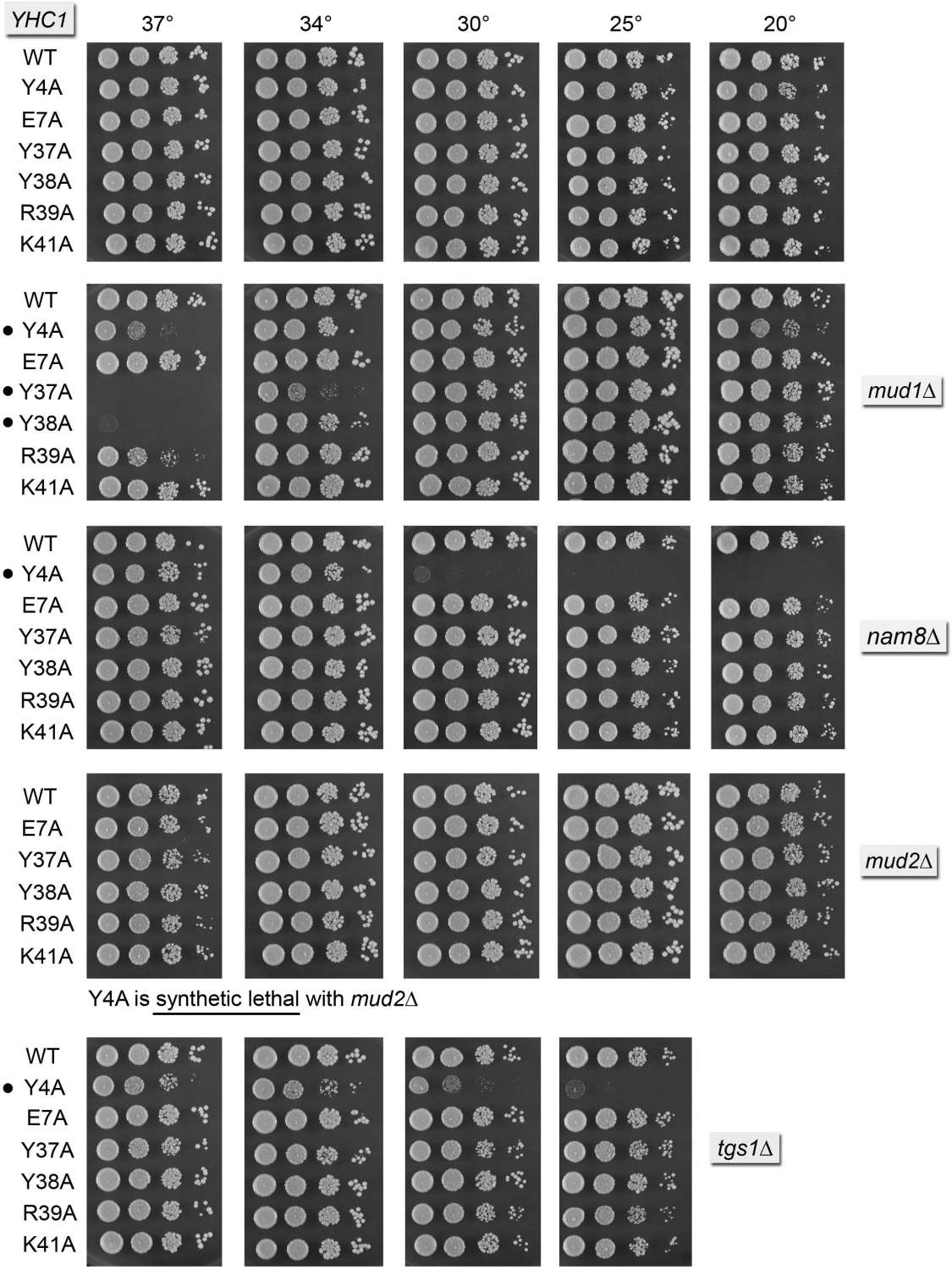


Figure S2. Genetic interactions of *YHC1-Ala* alleles. Yeast *yhc1*Δ strains bearing the indicated *YHC1-Ala* alleles on a *CEN HIS3* plasmid in an otherwise wild-type (top panel), *mud1*Δ, *nam8*Δ, *mud2*Δ, or *tgs1*Δ background as indicated were spot-tested for growth on YPD agar at the temperatures specified. Synthetic growth defects are denoted by •.

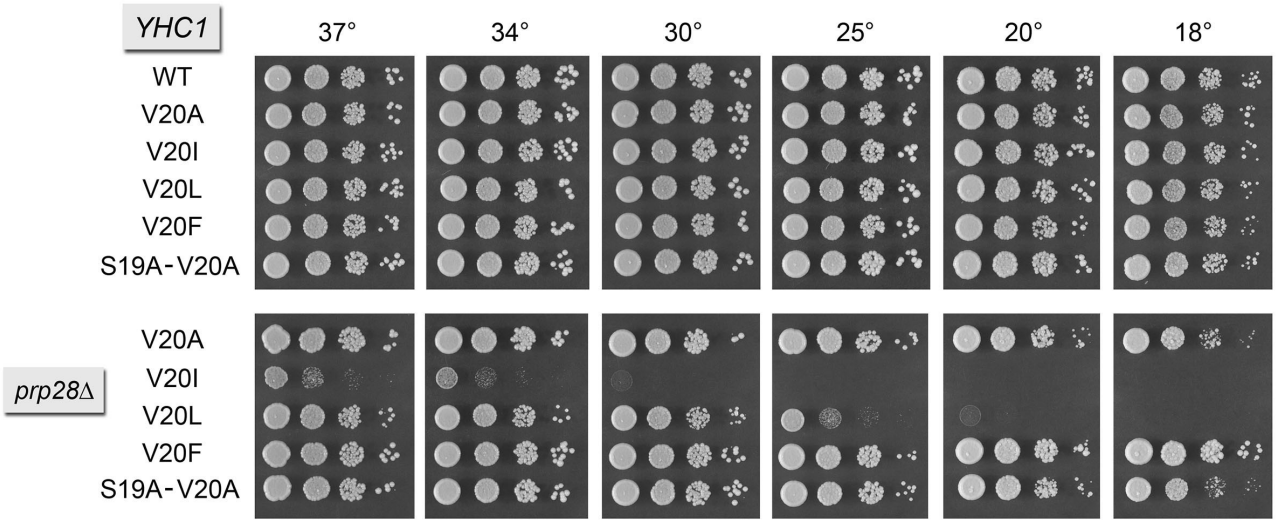


Figure S3. Val20 mutations bypass Prp28. Complementation of *yhc1*Δ (*top panel).* Yeast *yhc1*Δ strains bearing the indicated *YHC1-Ala* alleles were spot-tested for growth on YPD agar at the temperatures specified. Prp28 bypass (*bottom panel*). Yeast *prp28*Δ *yhc1*Δ cells bearing the indicated *YHC1* allele on a *CEN LEU2* plasmid were spot-tested for growth on YPD agar at the temperatures specified.

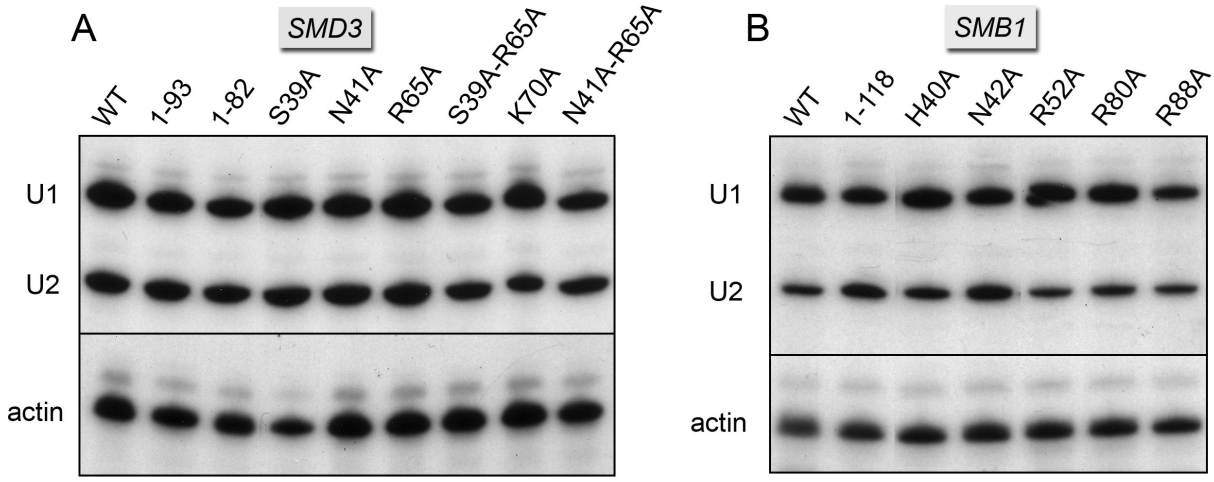


Figure S4. Primer extension analysis of U1 and U2 snRNA levels. Primer extension reactions with a ^{32}P-labeled primers complementary to U1 snRNA, U2 snRNA, and actin mRNA were performed as described previously (Schwer et al. 2013) using as template total cellular RNA isolated from the indicated *SMD3* and *SMB1* strains. The reaction products were analyzed by denaturing PAGE and visualized by autoradiography. All lanes in the U1+U2 or actin image panels are from the same gel and autoradiographic exposure, from which intervening lanes were cropped (e.g. between 1-82 and S39A in A; between 1-118 and H40A and between N42 and R52A in B) and the flanking lanes moved laterally.

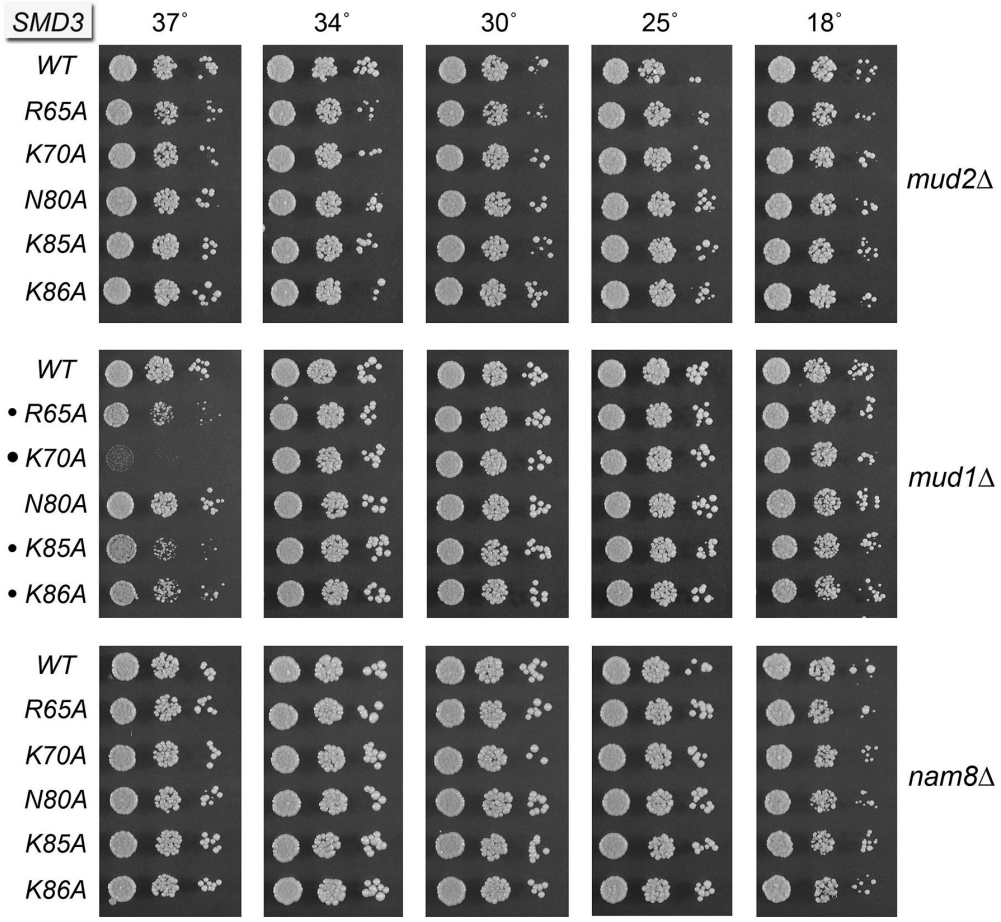
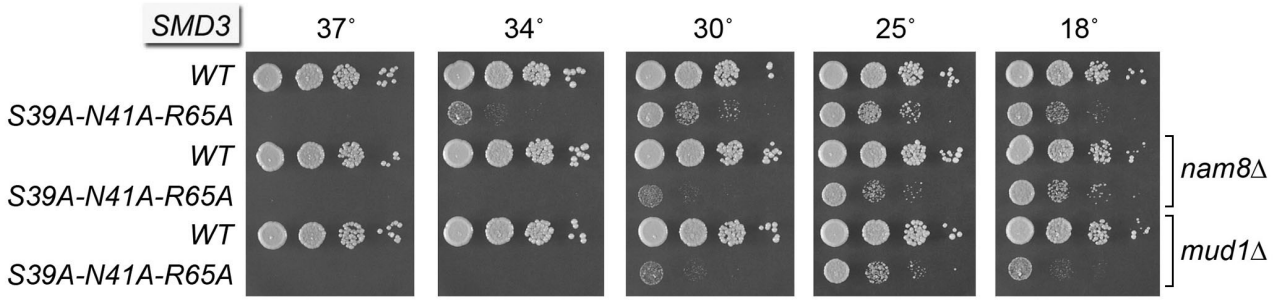


Figure S5. Tests of mutation synergy of *SMD3-Ala* alleles. Yeast *smd3*Δ strains bearing the indicated *SMD3-Ala* alleles on a *CEN LEU2* plasmid in *mud2*Δ, *mud1*Δ, or *nam8*Δ backgrounds as indicated were spot-tested for growth on YPD agar at the temperatures specified. Synthetic growth defects are denoted by •.



S39A-N41A-R65A is synthetic lethal with mud2Δ and tgs1Δ

Figure S6. Phenotype and mutational synergies of *SMD3-S39A-N41A-R65A*. Yeast *smd3*Δ strains bearing *SMD3 (WT)* or *SMD3-S39A-N41A-R65A* alleles on a *CEN LEU2* plasmid in an otherwise wild-type (top two rows), *nam8*Δ, or *mud1*Δ background, as indicated, were spot-tested for growth on YPD agar at the temperatures specified. *SMD3-S39A-N41A-R65A* was lethal in the *mud2*Δ and *tgs1*Δ strains.

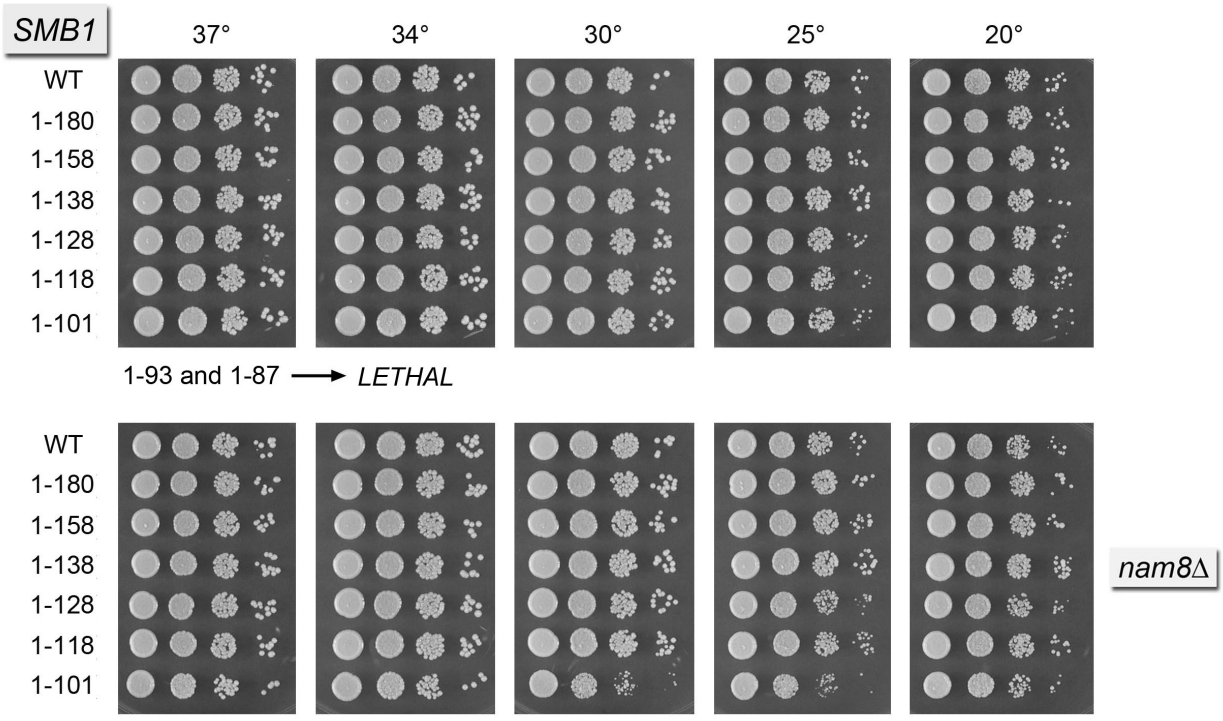


Figure S7. C-terminal truncations demarcate a minimized functional SmB. Wild-type *SMB1* and the series of C-terminally truncated *SMB1* alleles on *CEN HIS3* plasmids were tested for activity by plasmid shuffle in *smb1*Δ (top panel) and *smb1*Δ *nam8*Δ strains (bottom panel). *SMB1-(1-93)* and *SMB1-(1-87)* failed to complement *smb1*Δ in the plasmid shuffle assay and were deemed lethal. The growth phenotypes of the indicated viable FOA-resistant strains were spot tested for growth on YPD agar at the temperatures specified.

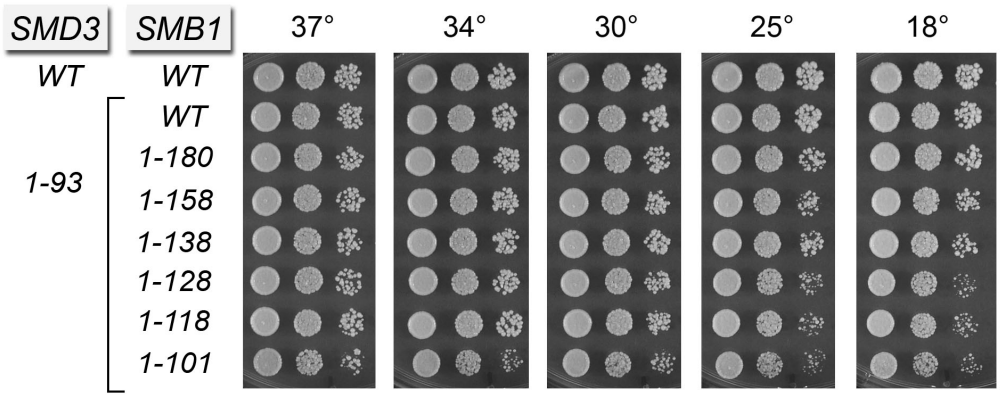


Figure S8. Lack of strong mutational synergies between SmD3 and SmB C-terminal truncations. *SMD3 (WT)* and *SMD3-(1-93)* alleles on *CEN LEU2* plasmids were co-transformed with *SMB1 (WT)* and *SMB1-ΔC* alleles on *CEN HIS3* plasmids into a yeast *smd3*Δ *smb1*Δ p(*CEN URA3 SMD3 SMB1*) strain. The growth phenotypes of the indicated viable FOA-resistant strains recovered after plasmid shuffle were spot tested for growth on YPD agar at the temperatures specified.