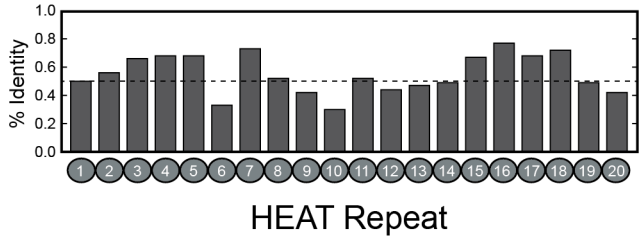
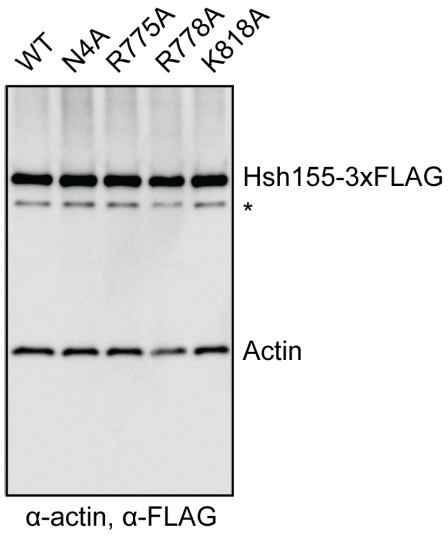


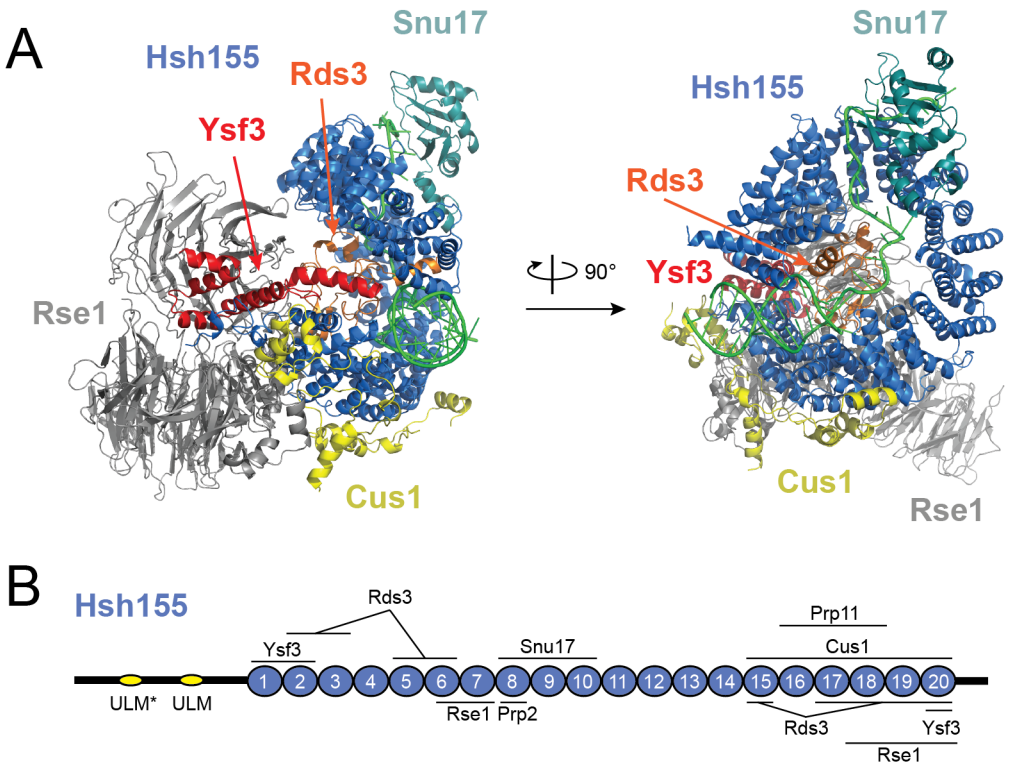
Supplemental Figure 1

Supplemental Figure 1: Amino acid sequence conservation of the SF3b1 HEAT repeats between yeast and humans. The dotted line represents 50% amino acid identity.



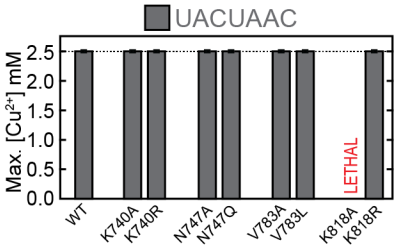
Supplemental Figure 2

Supplemental Figure 2: Western blot confirming expression of lethal Hsh155 point mutants (R775A, R778A, and K818A). Epitope-tagged Hsh155 WT and N4A (T178A, R181A, R182A, R186A) are viable and included as controls. Actin was used as a loading control, and a protein present in yeast that is cross-reactive with the FLAG-tag antibody is marked (*).



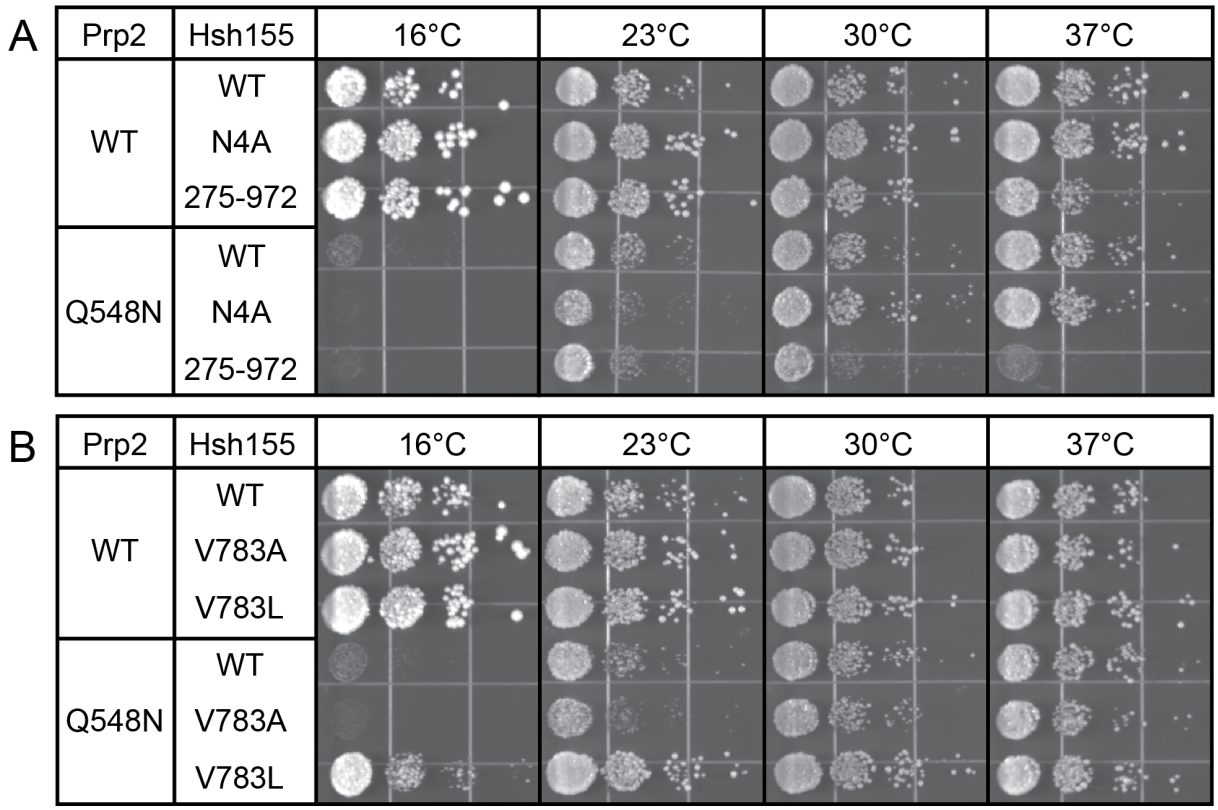
Supplemental Figure 3

Supplemental Figure 3: Structure of yeast Hsh155 and contacts to spliceosomal proteins and RNAs within the cryo-EM structure of the activated spliceosome (pdb 5GM6) (Yan et al. 2016). (**A**) Ribbon diagram showing protein and RNAs in contact with Hsh155 (blue). Figure was generated using PyMol. (**B**) Schematic of the interactions shown in (A).



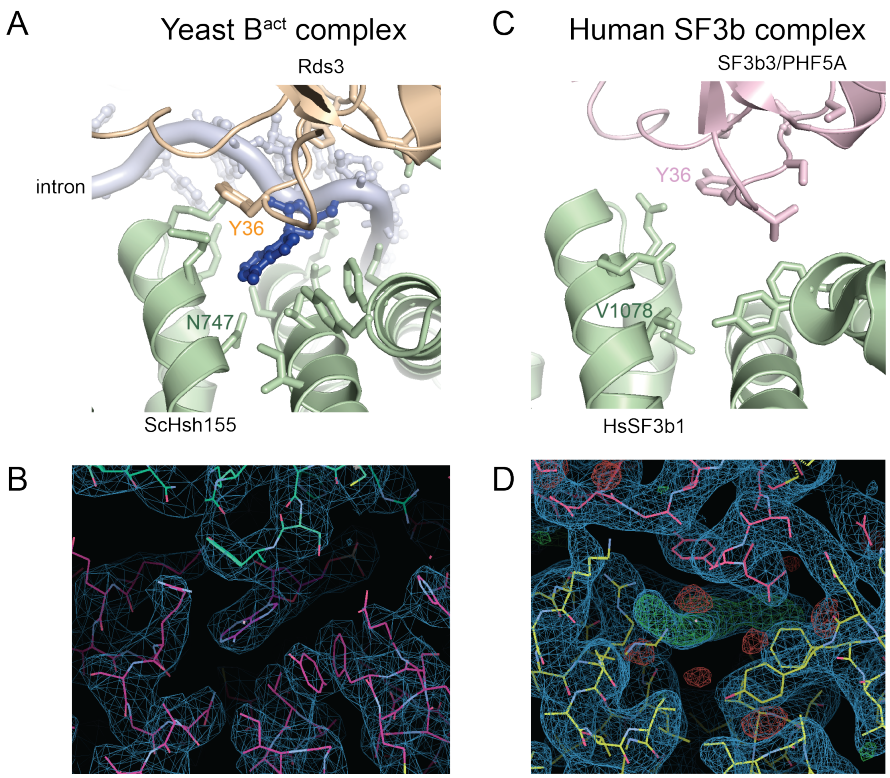
Supplemental Figure 4

Supplemental Figure 4: Results from a Cu^{2+} growth assay of strains carrying mutations to the BP-A pocket and containing a consensus BS substitution reporter plasmid. Each bar represents the average of three independent experiments, and error bars represent the standard deviation.



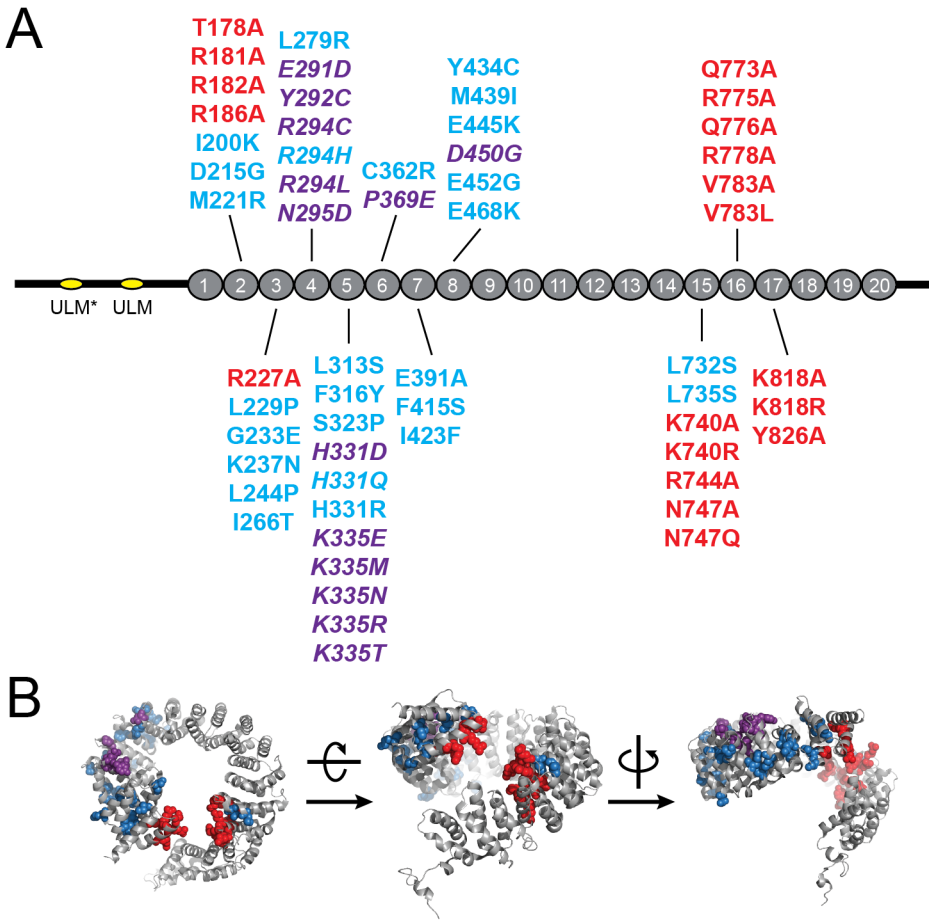
Supplemental Figure 5

Supplemental Figure 5: Representative temperature sensitivity assays of mutant Hsh155 and Prp2 strains. (**A**) N-terminal mutants of Hsh155 were combined with Prp2 Q548N. Both Hsh155 mutants fail to suppress Prp2 growth defects at 16ºC. (**B**) BP-A pocket mutations can suppress Prp2 Q548N growth defects at 16ºC. Yeast expressing the Hsh155 V783L mutation grow significantly better than WT Hsh155 when combined with Prp2 Q548N at 16 or 23ºC.



Supplemental Figure 6

Supplemental Figure 6. Structural comparison of the BP-A pocket found in the activated yeast spliceosome (pdb 5GM6; Yan et al. 2016) with the homologous region in human SF3 (pdb 5IFE; Cretu et al. 2016). (**A**) Ribbon diagram of yeast SF3b1/Hsh155 (green) in complex with the U2/BS duplex (grey). The BP-A is shown in blue and Hsh155 amino acids forming the BP-A pocket are shown in stick representation. Rds3 (beige) forms part of the BP-A pocket and the conserved Y36 is shown. (**B**) Modeled density from the structure shown in (A). (**C**) Ribbon diagram of human SF3b1 (green) as part of the SF3 complex. The Rds3 homolog, SF3b3/PHF5A (pink) forms part of the BP-A pocket and the conserved tyrosine 36 is shown (Cretu et al. 2016). (**D**) Electron density of the structure shown in (C). Note that the SF3 complex co-purified with an unidentified ligand which occupies the BP-A pocket of SF3b1. Density from the yeast spliceosome was obtained from the PDB while that from the human SF3 complex was obtained from the Uppsala Electron-Density server (http://eds.bmc.uu.se/). The figure was prepared using PyMOL, and Coot (https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/



Supplemental Figure 7

Supplemental Figure 7: Schematic of previously studied Hsh155 mutations. (**A**) Diagram showing the location of Hsh155 point mutations. Blue residues were identified in Tang et al. 2016, purple residues were characterized in both Tang et al. 2016 and Carrocci et al. 2017, and red residues were studied in this manuscript. Italicized residues are implicated in MDS (Jenkins and Kielkopf 2017). (**B**) Pymol rendering of SF3b1 showing the location of residues indicated in panel A (pdb 5GM6; Yan et al. 2016).