Supplemental Figure 1: Pre-activated and catalytic spliceosome purifications. A. Acrylamide Denaturing gel of radio labeled pre-mRNA during *in vitro* assembly (lanes 1-3) and the final elution (FE) after following size exclusion and amylose affinity chromatography. B. CPM percent total vs fraction of fractionated 10-30% glycerol gradient centrifugation of *in vitro* assembled pre-activated spliceosomes. Fraction 1 is the top of the gradient. Right peak corresponds to pre-activated spliceosomes. C. Native agarose gel of *in vitro* assembled pre-activated spliceosomes with and without the addition of the RNA oligo containing a 5′ splice site consensus. D. Denaturing acrylamide gel of glycerol gradient peaks as seen in B. stained for total RNA to visualize snRNA components.

Supplemental Figure 2: Mass spectrometry results for Prp8 peptides containing acetylated lysine residues 595, 597, 1300 and 1306 for catalytic and pre-activation spliceosomes.

Supplemental Figure 3: Structural organization of *S. cerevisiae* Prp8 lysines 670, 672, 1372 and 1378 and human Prp8 lysines 595, 597, 1300 and 1306. A. *S. cerevisiae* pre-activated spliceosome assembled *in vitro* with 2 uM ATP (Plaschka et al. 2017). B. *S. cerevisiae* catalytic spliceosome assembled *in vitro* with 2 mM ATP (Galej et al. 2016). C. Human pre-activated spliceosome (side chains un-modeled due to resolution limitations) assembled *in vitro* with 2 mM ATP (Bertram et al. 2017).

Supplemental Figure 4: A. Growth of serially diluted *Prp8-K670/672A* yeast relative to WT Prp8 yeast. B. ACT1-CUP1 splicing reporter assays of *Prp8-K670/672A* yeast containing various branch and 3′ splice site consensus mutations. BrC and BrG are mutations of the pre-mRNA branch point adenosine. Mutations at 301-304 are mutations to the pre-mRNA 3′ splice site consensus. C. RT-qPCR analysis of G5A reporter pre-mRNA and spliced mRNA was performed as described in the main text, with the following clarifications. Four different primer sets were used. The 5'Exon-Intron primer set (F: tttttcttcccaagatcgaaa; R: ttaaatgggatggtgcaagc) amplicon spans the 5'exon-intron boundary and reports on the level of pre-mRNA. The Intron-3'exon primer set (F: gcttcattctttttgttgctatattatatgtttagaggttg; R: cattggcactcatgaccttc) amplicon spans the intron-3'exon boundary and reports on the level of pre-mRNA and lariat intermediate. The junction primer set (F: gaattaacaatggattctggttgc; R: cattggcactcatgaccttc) amplicon spans the exon-exon mRNA junction, and reports on the abundance of fully spliced mRNA. The 3'exon primer set (F: ggtgtaacagcgacgacaaa; R: tttcccagagcagcatgatt) amplicon is contained within the 3' exon and reports on total reporter RNA abundance, as the 3'exon is present in pre-mRNA, lariat intermediate, and spliced mRNA product. Total RNA prepared from 3 separate biological replicates was analyzed in technical triplicate. The mean relative abundance of each amplicon from each primer set was normalized to that of wild type yeast expressing a wild type ACT-CUP reporter and plotted on a log scale. Error bars are SEM.

Supplemental Figure 5: Structural organization of Prp8, Isy1 and Snu114 in *S. cerevisiae* catalytic spliceosome (Galej et al. 2016). A. Structure of Prp8 (grey), Isy1 (green), Snu114 (yellow) and U5 snRNA (Blue). B. 45 degree rotation of panel A. C. 45 degree rotation of panel B.