Supplementary Information for

Saccharomyces cerevisiae Ecm2 Modulates the Catalytic Steps of pre-mRNA Splicing

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RUNNING TITLE: Multiple Functions of Ecm2 During Splicing

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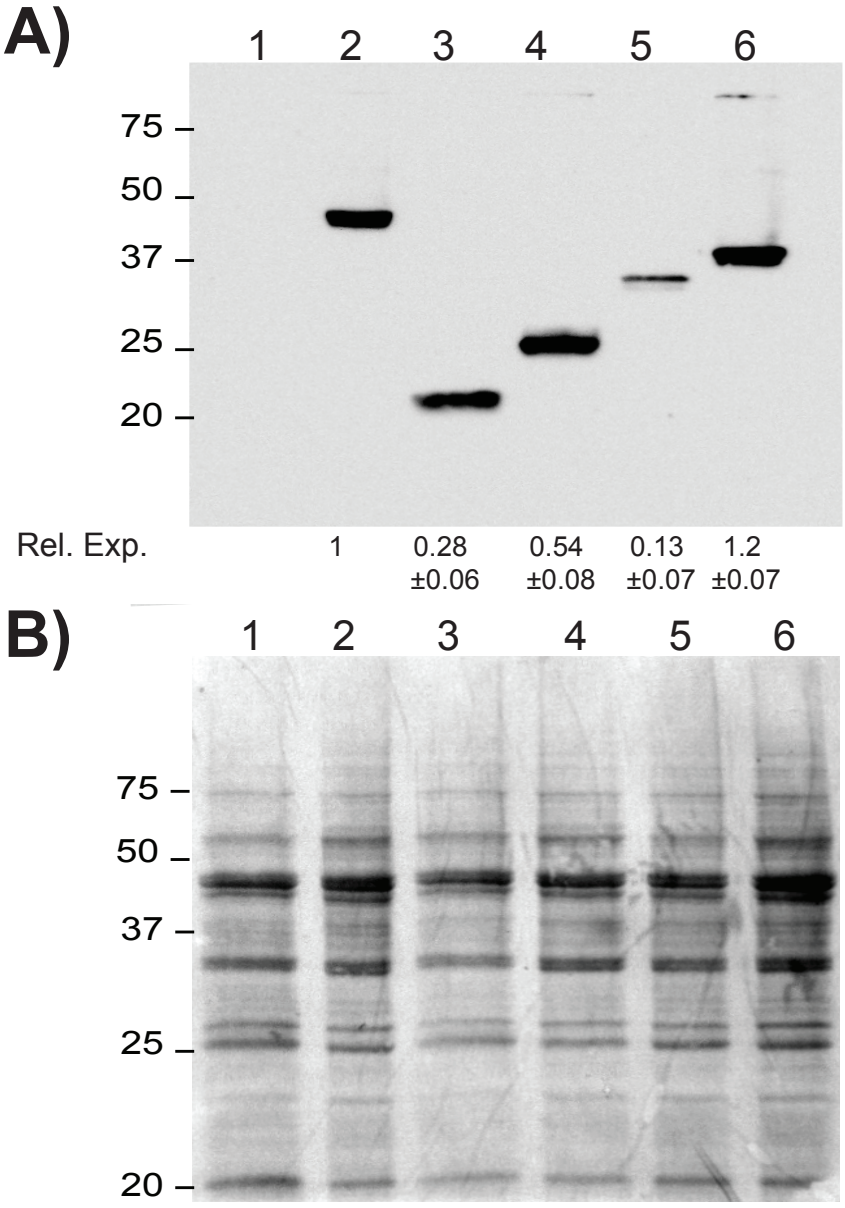
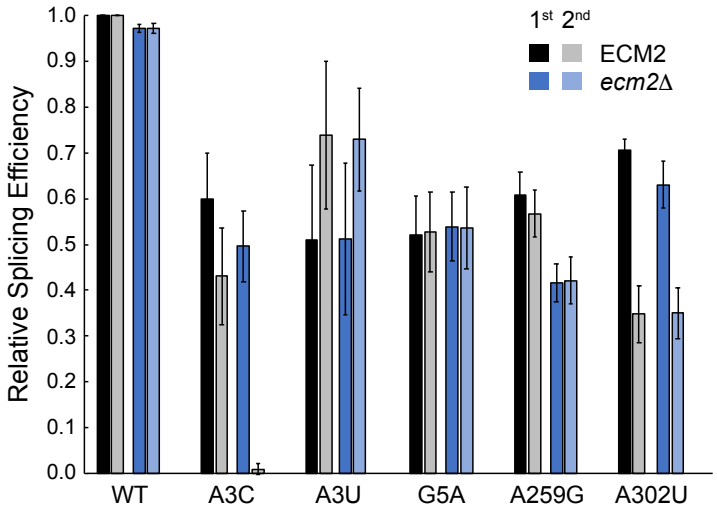
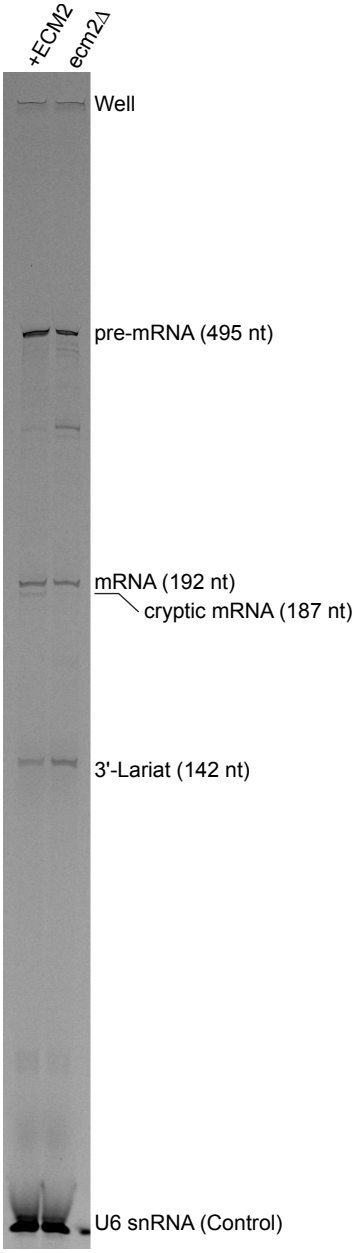
Contents:



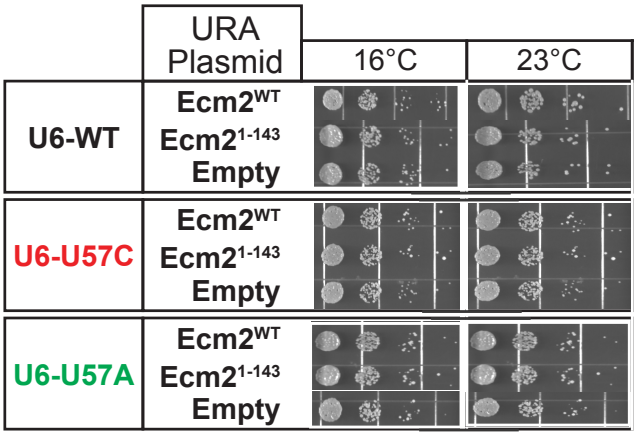
Supplemental Figure S1. Western blot analysis of Ecm2 truncation mutant expression. (A) Western blot results (B) Ponceau stain of the blot shown in (A). For both panels, lane 1 is lysate from a yeast strain expressing $Ecm2^{WT}$ (no Flag epitope tag); lane 2 is $Ecm2^{WT}$-3xFLAG; lane 3 is $Ecm2^{1-144}$-3xFLAG; lane 4 is $Ecm2^{1-198}$-3xFLAG; lane 5 is $Ecm2^{1-266}$-3xFLAG, and lane 6 is $Ecm2^{1-326}$-3xFLAG. Relative expression levels are shown below the Western blot and normalized against total protein contain as visualized by Ponceau staining. Expression levels represent the averages from three replicate experiments ±SD.



Supplemental Figure S2. Primer extension analysis of 1st- and 2nd-step splicing efficiencies for ACT1-CUP1 reporters in the presence and absence of ECM2. 1st-step efficiencies were determined as the ratios of (lariat intermediates + mRNA) to total RNA. 2nd-step efficiencies were determined as the ratios of mRNA to total products from the 1st step. Efficiencies were normalized to the WT ACT1-CUP1 reporter in the ECM2 strain. Bar heights represent the averages from three replicate experiments, and error bars represent the standard deviations.



Supplemental Figure S3. Uncropped gel image of a representative primer extension analysis of cryptic 5' SS usage. The region containing the mRNA is magnified in **Fig. 4D**. The primer extension stop located between the pre-mRNA and mRNA is reproducible but has not been attributed to a specific RNA species.



Supplemental Figure S4. A 1st- or 2nd-step allele of U6 (U57C or U57A, respectively) was combined with URA3 plasmids either lacking or coding for Ecm2 variants in *ecm2Δ* yeast. The strains were then tested for suppression or exacerbation of temperature-dependent growth phenotypes. Yeast were plated on -URA dropout media and imaged after 3 (23°C) or 6 (16°C) days of growth.

Supplemental Movie S1 Structural alignment of fragments of yeast (5LJ5.pdb) and human (5YZG.pdb) C complex spliceosomes. The structures were aligned based on the U6 snRNA using Pymol with an average RMSD of 1.941 Å for 646 atoms. The movie was recorded in Pymol and annotated using iMovie (Apple).