

Supplemental Figures for

Functional Analysis of the Zinc Finger Modules of the *S. cerevisiae* Splicing Factor Luc7

Tucker J. Carrocci¹, Samuel Demario^{2,3}, Kevin He^{2,3}, Natalie J. Zeps¹, Cade T. Harkner⁴,
Guillaume F. Chanfreau^{2, 3}, and Aaron A. Hoskins^{1, 4*}

AFFILIATIONS:

¹ Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706, USA

² Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA 90095, USA

³ Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA 90095, USA

⁴ Department of Chemistry, University of Wisconsin-Madison, Madison, WI 53706, USA

*To whom correspondence should be addressed: ahoskins@wisc.edu, (608) 890-3101

Supplemental Figures

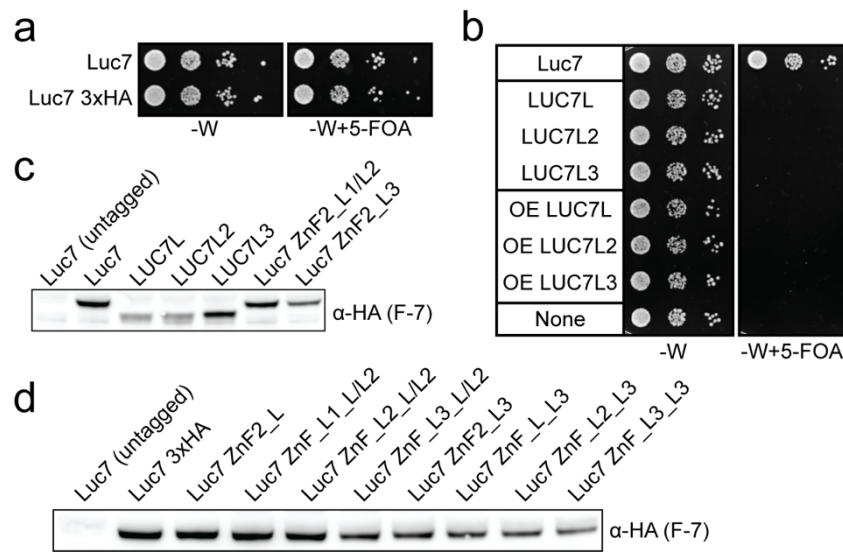


Figure S1. Expression of mutant Luc7 in yeast. (a) C-terminal HA-tagging of Luc7 has no effect on yeast growth compared to the untagged control. (b) Human LUC7L proteins (truncated before the RS domain) cannot support growth in yeast as the sole copy of *LUC7*. Overexpression (OE) of the proteins from a *TDH3* promoter does not rescue growth. (c) Western blotting using the F-7 anti-HA tag antibody shows expression LUC7L, LUC7L2, LUC7L3 and ZnF2 mutants before FOA shuffle in merodiploid strains. (d) Expression levels of ZnF mutant Luc7 proteins in yeast. For these blots, yeast extracts were normalized for total protein content as a loading control and equivalent total amounts of protein were added to each well prior to SDS-PAGE (Eaton et al. 2013).

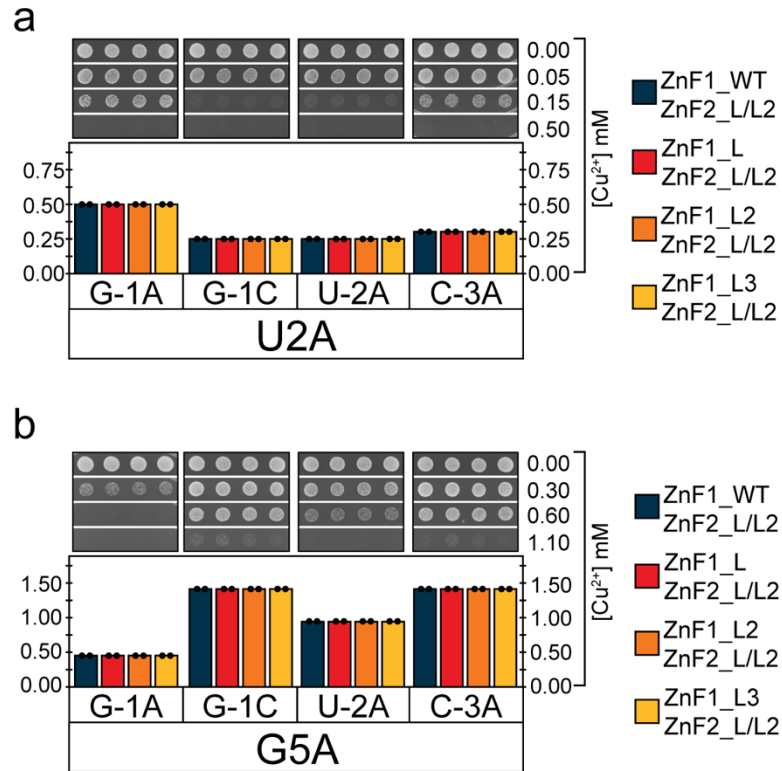


Figure S2. Modifying ZnF1 and ZnF2 simultaneously does not modify 5'ss usage. (a) Growth on Cu^{2+} -containing media of yeast strains with reporters containing substitutions of the U+2 position in addition to the indicated changes at the -1 to -3 sites are unaffected. (b) Same as in (a) except with a substitution at G+5. Dots represent maximum $[Cu^{2+}]$ tolerated in two independent replicates.

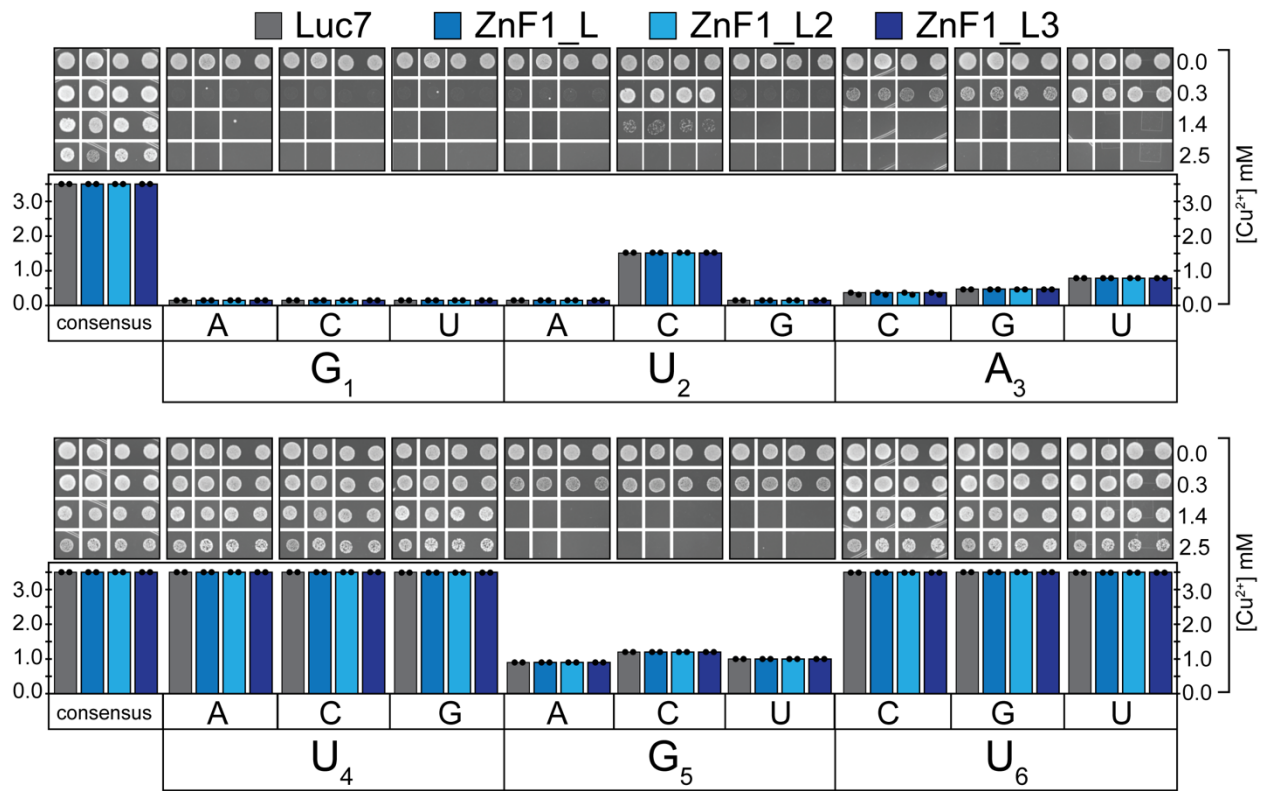


Figure S3. Luc7 ZnF1 mutants do not impact non-consensus 5'ss usage. *ACT1-CUP1* reporters with single substitutions at all positions of the 5'ss have similar Cu^{2+} tolerances with all ZnF1 mutants tested. Dots represent maximum $[\text{Cu}^{2+}]$ tolerated in two independent replicates.

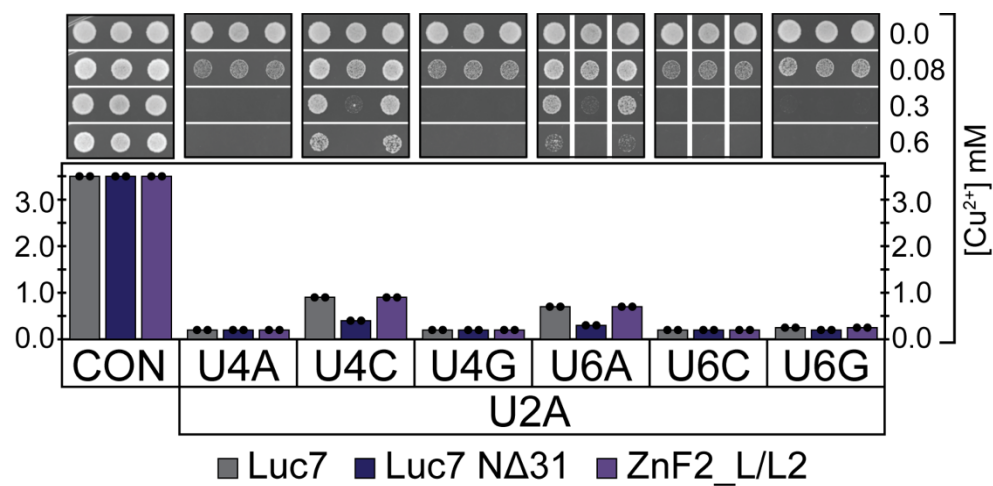


Figure S4. ACT1-CUP1 assays using reporters that combine U2A with substitutions at positions U4 and U6. Dots represent maximum [Cu²⁺] tolerated in two independent replicates.

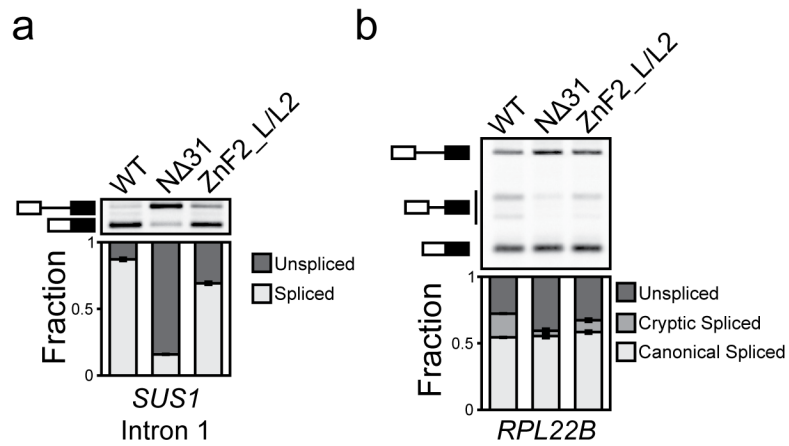


Figure S5. RT-PCR analysis of splicing changes in endogenous transcripts upon mutation of Luc7. (a) Luc7 ZnF2_L/L2 or NΔ31 accumulate unspliced pre-mRNA from the first intron of *SUS1*. (b) *RPL22B* unspliced pre-mRNA accumulates in Luc7 mutants but less cryptic splicing is observed with NΔ31. Error bars represent standard deviation from three independent replicates.

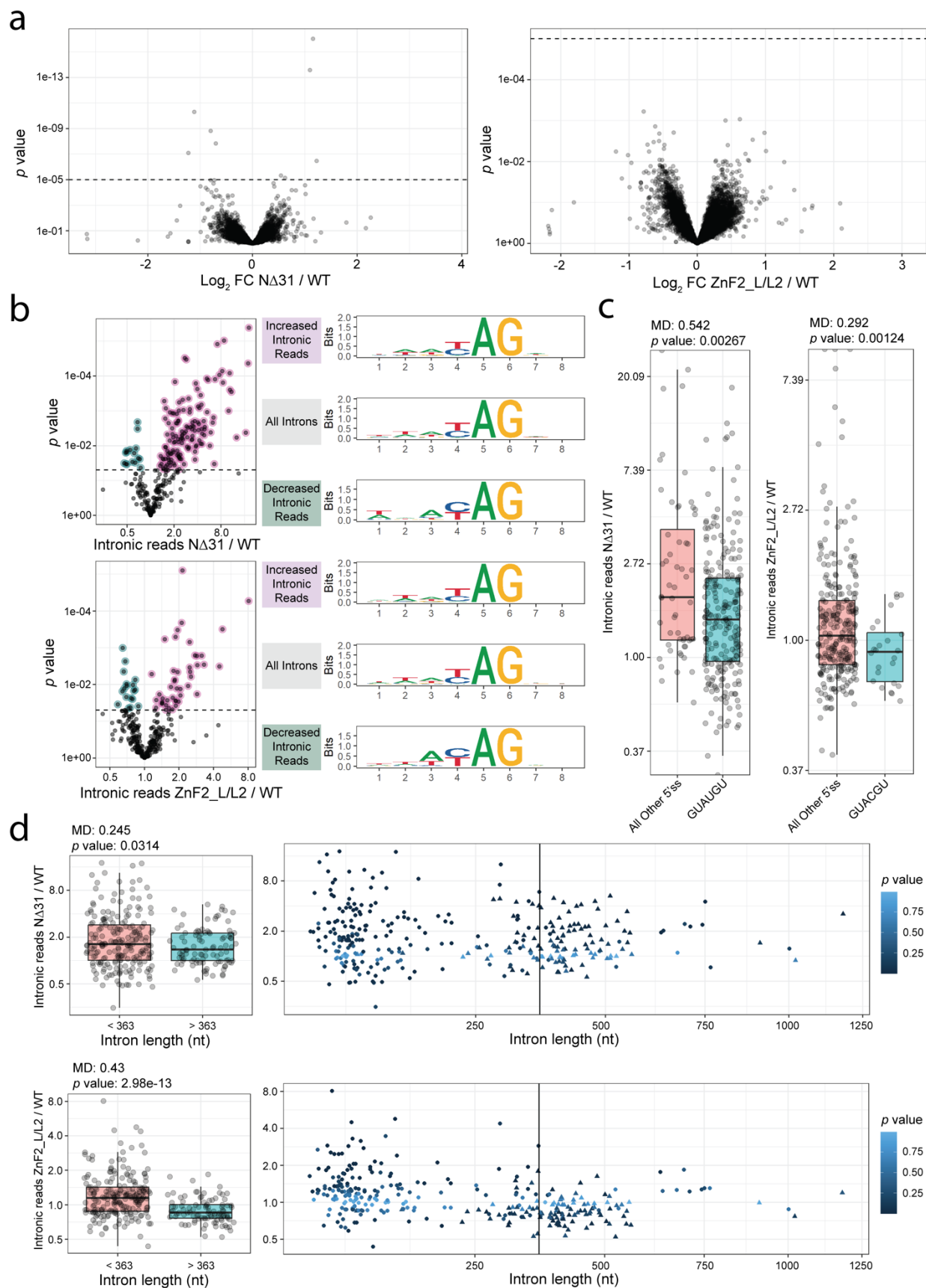


Figure S6. Supplemental analysis of RNA-seq data from cells expressing WT and mutant forms of Luc7. (a) mRNA levels for WT and Luc7 variant strains. mRNA annotation from SGD. Horizontal line indicates p value = $1e-5$. (b) Plots showing change in splicing efficiency for each intron in Luc7 N Δ 31 and Luc7 ZnF2_L/L2. Sequence logos of 3'ss and surrounding bases. (c) Unequal variances t-tests using geometric means were performed for each mutant testing the common 5'ss in yeast ("GUAUGU", "GUAAGU", "GUACGU", "GUAUGA", "GUAUGC") against all other 5'ss. Only 5'ss with p value < 0.05 were reported. (d) Splicing efficiency vs intron length for Luc7 N Δ 31 (Top) and Luc7 ZnF2_L/L2 (Bottom). Intron length threshold was determined by minimizing p values for Luc7 ZnF2_L/L2 along a sliding window of intron lengths between 50 and 500 nt. Data point shape indicates ribosomal protein genes (triangle) or non-ribosomal protein genes (circle). Color indicates p value as in Figure 4C. The x-axis in plots in panels (a) and (b) represent $\log_2$ -fold changes. (MD - Difference of geometric means)