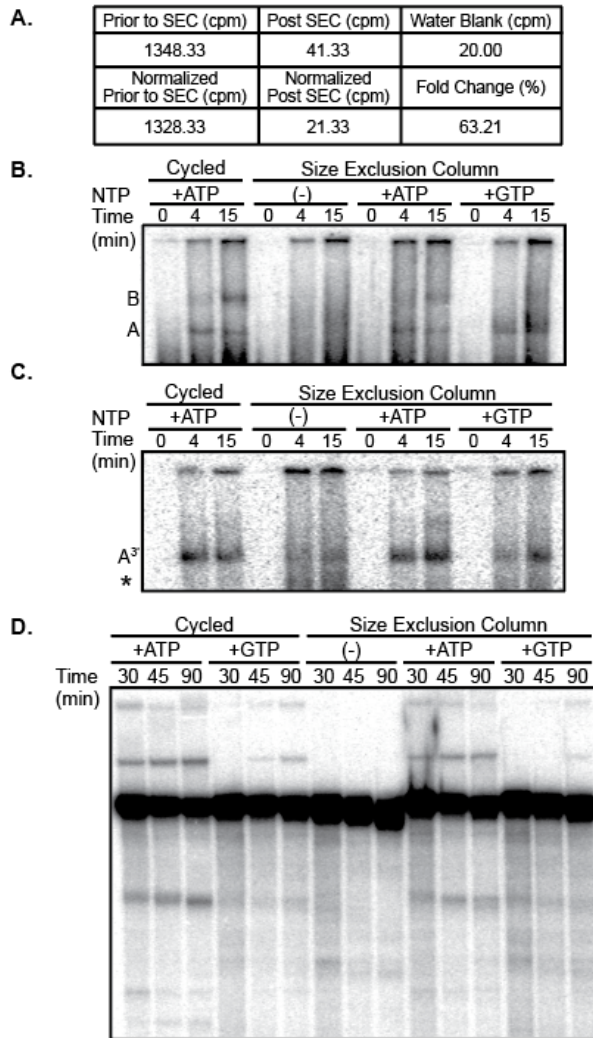
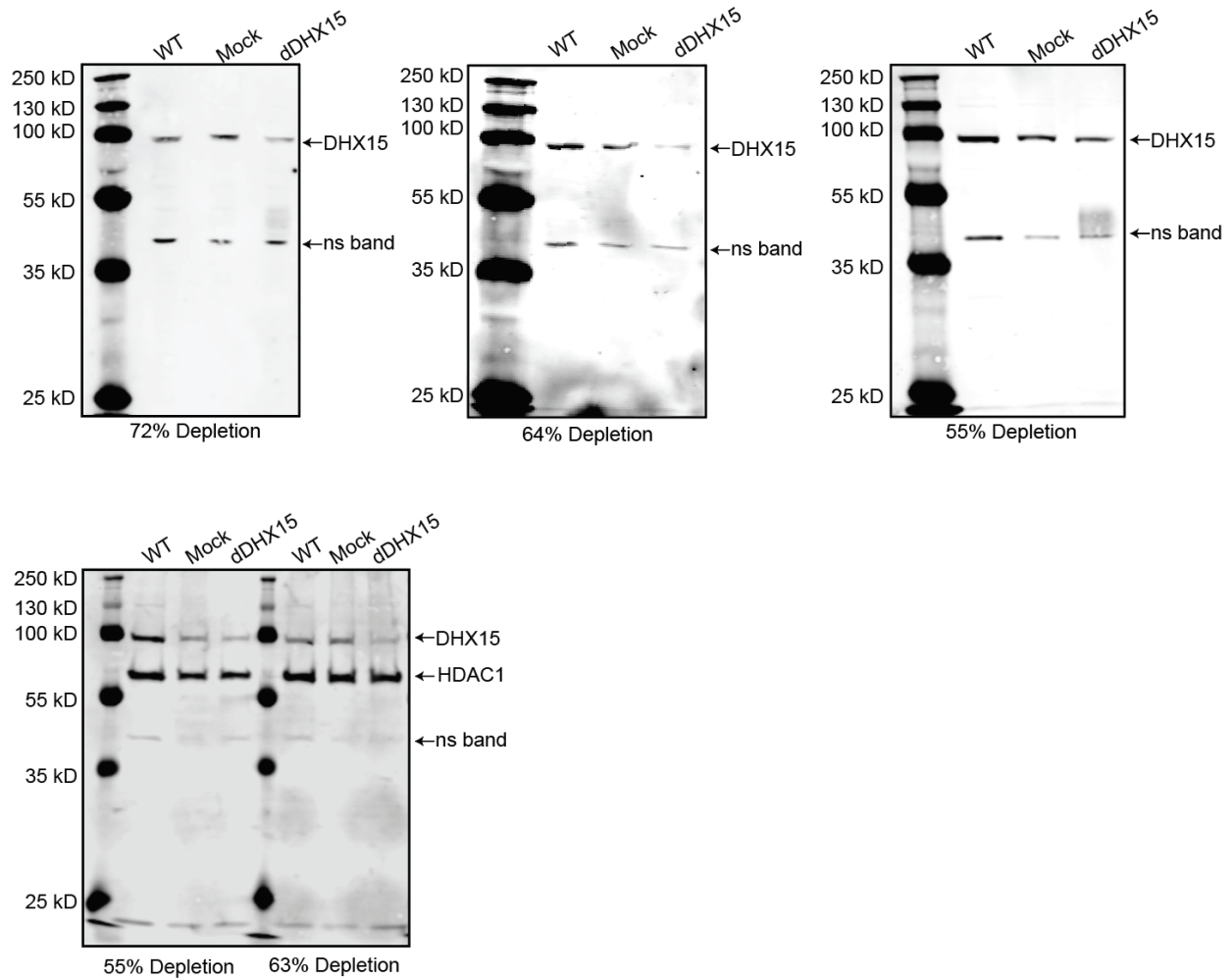


Supplemental Material



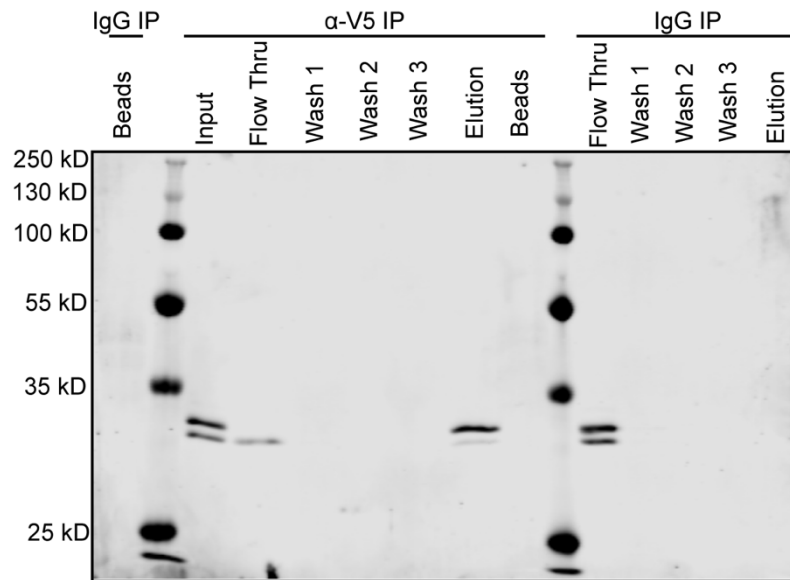
Supplemental Figure 1: Size Exclusion Column Treatment of Nuclear Extract

A. CPM of a sample including radiolabeled UTP prior and post to size exclusion chromatography. B. Representative native gel analysis of *in vitro* spliceosome assembly with a radiolabeled full-length substrate in nuclear extract incubated at 30°C (cycled) or passed through a size exclusion column. A- and B- complex bands positions are labeled. C. Same as (B) except that a A³-substrate was used for spliceosome assembly. D. Representative denaturing gel analysis of radiolabeled RNA isolated at the indicated timepoints from *in vitro* splicing reactions using nuclear extract incubated at 30°C (cycled) or passed through a size exclusion column.



Supplemental Figure 2: Western Blot Analysis of DHX15 Depletion

Full images of western blots with anti-DHX15 to determine depletion efficiency as shown in Figure 2 (top three images). Western blot with both anti-DHX15 and anti-HDAC1 to show that the non-specific (ns) band does not change relative to the house-keeping gene HDAC1.



Supplemental Figure 3: Western blot analysis of anti-V5 and IgG IP's
Full western blot IP's probed with anti-SNRPB2 from Figure 6A.

Full-Length

GAGACCGGCAGATCAGCTTGGCCGCGTCCATCTGGTCATCTAGGATCTGATATCATCGAT
GAATTCGAGCTCGGTACCCCGTTTCGTCCTCACTCTCTTCCGCATCGCTGTCTGCGAGGGC
CAGCGTAAAAGGTGAGTACTCCCTCTCAAAGCGGGCATGACTTCTGCCCTCGAGTTATTA
ACCCTCACTAAAGGCAGTAGTCAAGGGTTTCCTTGAAGCTTTCGTGCTGACCCTGTCCCTT
TTTTTTCCACAGCTGCAGGTCGACGTTGAGGACAACTCTTCGCGGTCTTTCCAGTACTCT
TG

A^{3'}

GTCAAGGGTTTCCTTGAAGCTTTCGTAACCCTTTCCCTTTTTTTTTCCTCTCTCTCTCGA
GCGTACACCATCAGGGTACGAGCTAGCCCATGGCGTACACCATCAGGGTACGACTAGTAG
ATCTCGTACACCATCAGGGTACGGAATTCT

A^{min}

GTAACCCTTTCCCTTTTTTTTTCCTCTCTCTCTGCTCGAGCGTACACCATCAGGGTACGA
GCTAGCCCATGGCGTACACCATCAGGGTACGACTAGTAGATCTCGTACACCATCAGGGTA
CGGAATTCT

Supplemental Figure 4: RNA substrates

DNA template sequences of the RNA substrates utilized in this study.

Name	DNA Sequence
U2 snRNA nt 32-46	AGAACAGATACTACA
U2 snRNA nt 24-38	TACTACACTTGATCT
U2 snRNA nt 12-26	TCTTAGCCAAAAGGC
U2 snRNA nt 1-15	AGGCCGAGAAGCGAT
Control Oligo	CTGATGTCCGCAGACAATGT

Supplemental Figure 5: Sequences of DNA oligonucleotides complementary to the indicated U2 snRNA region for RNase H digestion.