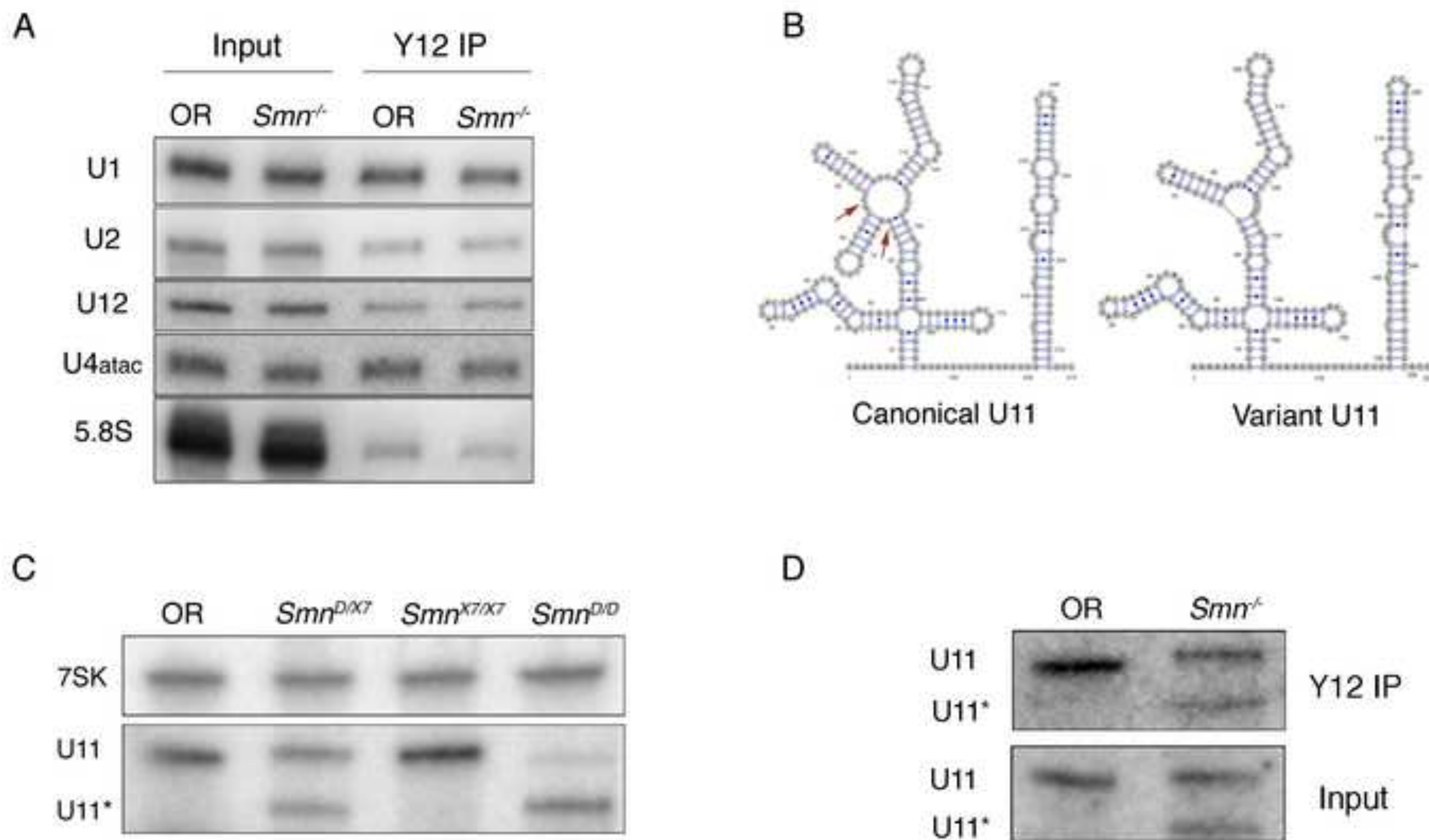
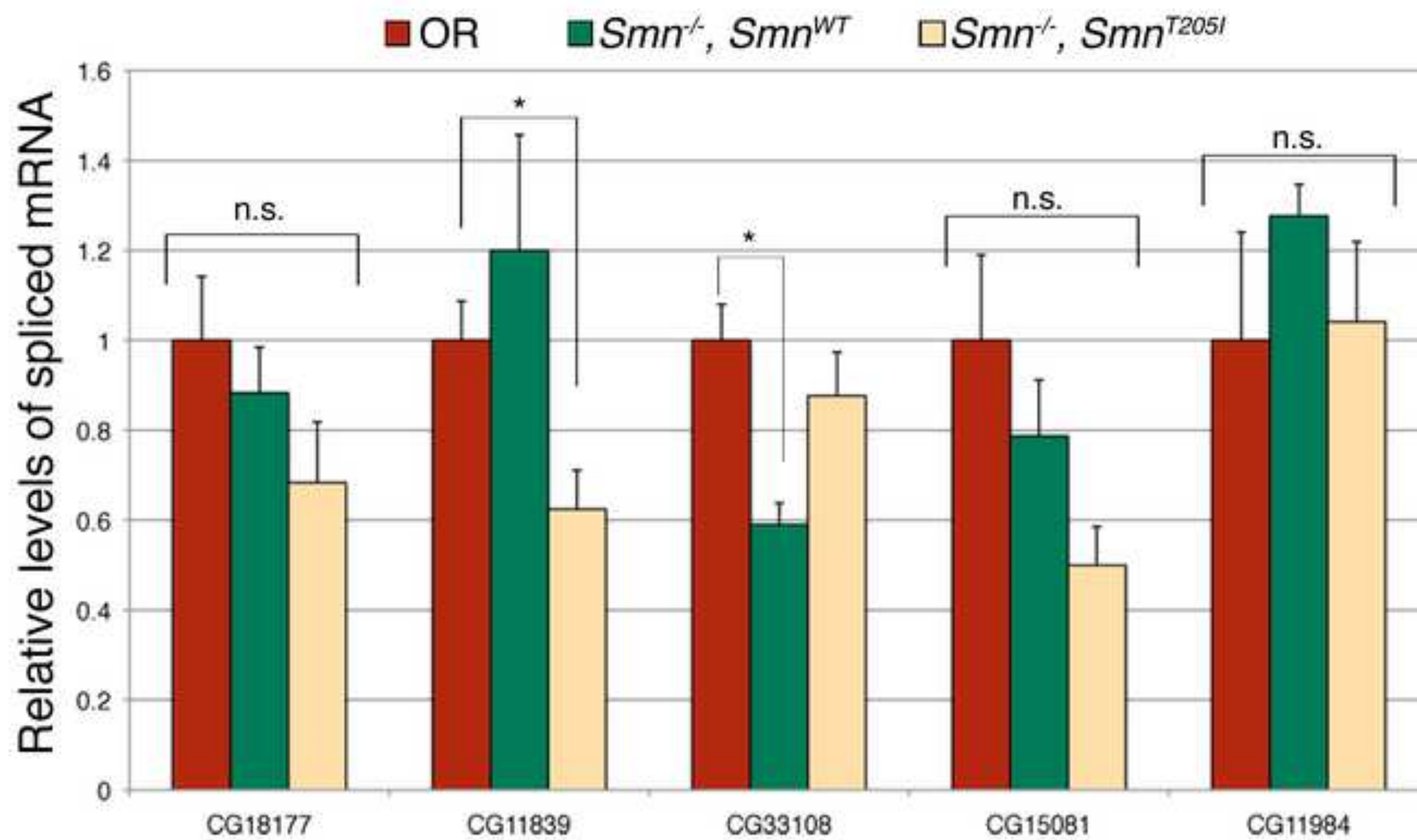
Supplemental Figure S1



Supplemental Figure S2

Figure S2



Supplementary information:

Extended Results

Variant forms of snRNAs. In addition to the canonical forms, we also observed expression of U5 and U11 snRNA variants (see Fig. S1). Consistent with a previous report, the variant forms of U5 are predominantly expressed early in larval development (Chen et al., 2005). The *Smn*$^{-/-}$ larvae express a variant form of U11, that contains a 22 bp deletion in stem loop II, a region that is not well conserved among *Drosophilid* species (Fig. S1). The *U11* and *Smn* genes are both on the same chromosome, and we note that this U11 variant tracks with the *Smn*^{D} allele and is not present on the homolog containing *Smn*^{X7}. We also note that the variant U11 snRNA can be immunoprecipitated by anti-Sm antibodies (Fig. S1) and that *Smn*^{D} homozygotes can be rescued by transgenic expression of dSMN (Rajendra et al., 2007, this work). Thus, this variant U11 allele appears to be fully functional.

Figure S1, related to Fig. 1. ***A.*** Total snRNA levels in *Drosophila* larvae reflect snRNP levels. Lysates from ~76 hour OR and $Smn^{X7/D}$ larvae were immunoprecipitated (IP) with anti-Sm antibody, Y12. The bound snRNAs were visualized via northern blotting with probes specific to the indicated snRNAs and 5.8S rRNA, which binds non-specifically to beads and used as an approximate load control. The levels of snRNAs co-precipitated for OR and $Smn^{X7/D}$ were similar to the total level of snRNAs in the input. ***B.*** An illustration of the secondary structures of the canonical and variant forms of U11. The structures are based on those of Schneider et al. (2004) and redrawn using VARNA (Darty et al. 2009). The arrows on the canonical U11 indicate a 22 base stem loop that is deleted in the genomic sequence of the variant allele. ***C.*** Northern blot of RNA from OR, $Smn^{D/X7}$, $Smn^{X7/X7}$ and $Smn^{D/D}$ larvae with U11 specific probes. The asterisk marks the variant U11 that migrates as a shorter band in $Smn^{D/X7}$ and $Smn^{D/D}$, but is absent in OR and $Smn^{X7/X7}$. In $Smn^{D/D}$ larvae, which are homozygous for the U11 variant, remnants of the maternally contributed canonical U11 can be seen. ***D.*** The variant U11 is complexed with Sm proteins. Lysates from OR and $Smn^{D/X7}$ larvae were immunoprecipiated (IP) with anti-Sm antibody, Y12, and RNA was extracted and analyzed by northern blotting with a U11 probe. Both forms of U11 co-purified with the Y12 antibody.

Figure S2, related to Fig. 4. Real-time qRT-PCR analysis of minor-class introns in *Smn* transgenic rescue lines. The four genes showing the greatest reduction in Fig. 2 and a control gene that was not reduced (CG11984) were analyzed. For CG18177 and CG15081, the values among all three genotypes are not significantly different from each other. For CG11839 and CG33108, values are significant to $p \sim 0.05$ level when comparing OR vs. Smn^{T205I} and OR vs. Smn^{WT}, respectively.

Additional details on quantitation in Fig. 4. In Fig. 4, all the Sm-class snRNAs, except for U2, were reduced in *Smn*$^{-/-}$ mutants, with U4, U5, U11, U12 and U4atac ($p \sim 0.001$) showing the greatest reduction. *Smn*^{WT} and *Smn*^{T205I} transgenic animals showed increased levels of U12 compared to *Smn*$^{-/-}$ animals ($p < 0.001$) but did not show a significant difference in the levels of U1, U4, U11 and U4atac ($p > 0.05$). *Smn*^{WT} and *Smn*^{T205I} animals also displayed a slight increase in U5 snRNA levels compared to the *Smn*$^{-/-}$ larvae (p ~0.001 and ~0.045, respectively), although both remained well below those of OR animals.

Extended Experimental Procedures

Immunoprecipitation

Larvae were homogenized in NET (150 mM NaCl, 5 mM EDTA, 50 mM Tris pH 7.5, 0.5% NP40) buffer, clarified by spinning at 10,000xg for 10 min at 4ºC. Immunoprecipitation was performed by incubating lysates with monoclonal Y12 antibody bound beads for 2 hours, washing the beads in NET buffer and then placing them in TRIZOL for RNA extraction.

Supplementary References

Chen, L., Lullo, D.J., Ma, E., Celniker, S.E., Rio, D.C., and Doudna, J.A. (2005). Identification and analysis of U5 snRNA variants in Drosophila. *RNA* **11**: 1473-1477.

Darty, K., Denise, A., and Ponty, Y. (2009). VARNA: Interactive drawing and editing of the RNA secondary structure. *Bioinformatics* **25**: 1974-1975.

Schneider, C., Will, C.L., Brosius, J., Frilander, M.J., and Luhrmann, R. (2004). Identification of an evolutionarily divergent U11 small nuclear ribonucleoprotein particle in Drosophila. *Proc Natl Acad Sci U S A* **101**: 9584-9589.