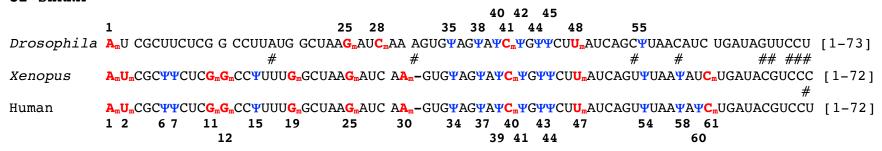
Supplementary Figure 1

U2 snRNA



Alignment of the 5' terminal sequences of U2 snRNA from *Drosophila, Xenopus* and human. The human sequence is identical to that of the mouse and other mammals. Mismatched nucleotides are indicated with a # sign. All known 2'-O-methylated positions (Nm) and pseudouridines (\mathbf{Y}) are depicted. Modifications in *Drosophila*, human and mouse were previously mapped by Deryusheva and Gall 2009, 2017, Deryusheva et al. 2012; the modification pattern of *Xenopus* U2 snRNA was determined in this study. Note that Cm28 is present in *Drosophila* but not in vertebrates, whereas positions 11, 12, 19 30 and 61 (equivalent to position 62 in *Drosophila*) are 2'-O-methylated in vertebrates but not in *Drosophila*.

We previously showed that termination of the reverse transcription reaction at 2'-O-methylated positions depends on the source of the enzyme (Deryusheva at al. 2012). AMV-RT from New England Biolabs, used in this study, is the best choice for testing modifications near C28. However, AMV-RT fails to stop at Gm19 and Cm61 in vertebrate U2. When we injected *in vitro* transcribed *Drosophila* U2 snRNA into *Xenopus* oocytes and looked for modifications, we found that Gm11/12, Gm25, Am30, Cm41 and Um48 were induced by the corresponding *Xenopus* guide RNAs; Gm19 and Cm62 (equivalent to position 61 in vertebrates) even if induced, would not produce stop signals and would not be detected in AMV-RT reactions.

References

Deryusheva, S.; Gall, J.G. Small Cajal Body-specific RNAs of Drosophila Function in the Absence of Cajal Bodies. *Mol. Biol. Cell* **2009**, *20*, 5250–5259. Deryusheva, S.; Choleza, M.; Barbarossa, A.; Gall, J.G.; Bordonne, R. Post-transcriptional modification of spliceosomal RNAs is normal in SMN-deficient cells. *RNA* **2012**, *18*, 31–36.

Deryusheva, S.; Gall, J.G. Dual nature of pseudouridylation in U2 snRNA: Pus1p-dependent and Pus1p- independent activities in yeasts and higher eukaryotes. RNA 2017, 23, 1060–1067.