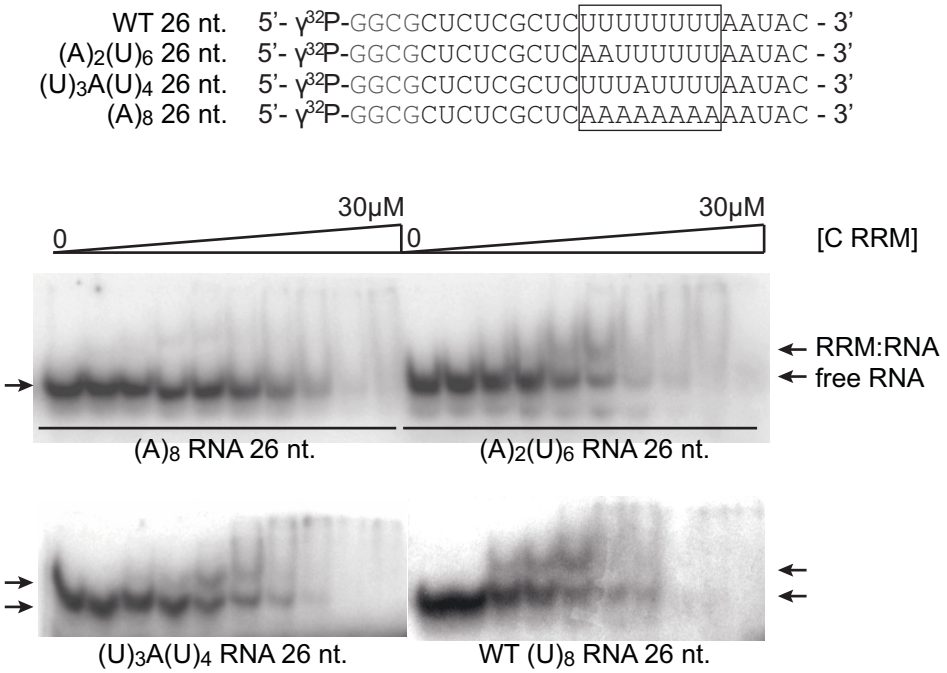

Supplementary Figure S3.

In vitro, both the wild-type and the shortened variants of the unr UC(U)$_8$ tract bind the hnRNP C RRM domain in an equimolar ratio.

To probe in vitro the binding stoichiometry of the hnRNP C RRM to unr UC(U)$_8$ sequence, we transcribed 26 nt.-long fragments of the unr 5' untranslated region containing the uridine-rich site. Four oligomers were designed, analogous to the *in vivo* variants WT, (A)$_2$(U)$_6$, (U)$_3$A(U)$_4$ and (A)$_8$ (Figure 5A). After γ^{32}P 5' end labeling, we performed gel mobility shift assays with these RNA fragments in the presence of increasing concentrations of hnRNP C RRM. We observe only one complex species for the wild-type sequence and the (A)$_2$(U)$_6$ and (U)$_3$A(U)$_4$ variants. The binding affinity of the RRM for these mRNAs is comparable, with estimated apparent dissociation constants (K_D) in the micromolar range. This value being similar to the previously reported K_D of single hnRNP C RRM binding short poly(U) tracts (Cienikova *et al.*, JACS 2014), the observed complex likely corresponds to a 1:1 species. We do not detect any higher stoichiometry complexes – instead, we observe band smearing at high protein concentrations. Since the smearing is present in the negative control mutant (A)$_8$ as well, we suspect that it is due to weak aspecific interactions. Our inability to observe 2:1 species for the WT RNA variant could be connected to random register binding of the hnRNP C RRM on uniform poly-uridine tracts; some of the registers would interfere with the attachment of the second ligand, precluding the formation of higher stoichiometry species. This would lead to an affinity enhancement of the first binding step at the expense of the second step.