Supplementary Figure S3

Drosophila extracts were incubated with unlabeled let-7 siRNA for 1 hour, then heparin and 5’ end labelled cognate (Hutvagner et al., 2004) (lane 3) and non-cognate (UGG AAA ACU ACC UGU UCC AUG GCC AAC AC U UG) (lane 4) target analog were added to the reactions and incubated for an additional hour, then loaded to the 3.9% acrylamide containing 1×TBE native gel. Drosophila extract incubated with labelled let-7 siRNA was used as a control (lane 2). Star denotes radioactively labeled siRNAs.

Results

To determine if the highest molecular weight complex appearing during siRNA-induced silencing complex formation corresponds to ss siRNA containing holo-RISC, we assayed the ability of this complex to bind to a 2’-O-methyl mRNA analog. Silencing complex formation was induced with unlabeled siRNA for 1 hour, then heparin and the labelled cognate and non-cognate target analog oligos were added to the reactions. Labelled siRNA-induced silencing complexes were used as control. A band was detected in the cognate target analog oligo containing reactions with mobility similar to that of the highest molecular weight complex
found in the control reaction. This band was not detected in the non-cognate target containing sample. This indicates that this complex corresponds to holo-RISC (Supplementary Figure S3, compare lanes 2-4). A complex was detected in the cognate target analog-containing sample with mobility similar to that of RLC, indicating that the RLC complex may bind the target RNA analog. Note that the addition of heparin and/or the extra negative charge provided by the phosphate groups of the target analog RNA slightly changed the migration of both the RISC and RLC complexes in the targeting reactions compared to the labelled siRNA-induced reactions.

References