Supplementary Figure S4

The extract-dependent HC-Pro-siRNA complex contains HC-Pro but is independent of RNA silencing complex formation.

Drosophila extracts were incubated with siRNA (lane 2-5), with HC-Pro (lane 3 and 5), in the absence (lane 2 and 3) or presence of heparin (lane 4 and 5). As a control, purified HC-Pro was incubated with siRNA in the absence (lane 6) or in the presence of heparin (lane 7)

Results

To explore the nature of the siRNA-containing complex we observed in Drosophila extracts in the presence of HC-Pro, we assayed the formation of the new complex in the absence of the RNA silencing complexes. Heparin abolishes assembly of RNA silencing complexes and target cleavage by blocking silencing initiator DCR2-R2D2 formation when added with inducer siRNA at the start of the RNA silencing reaction (Pham et al., 2004; Tomari et al., 2004). We examined the effect of heparin addition on siRNA-containing complexes in the presence of HC-Pro. As expected, heparin inhibited RNA silencing complex formation (Supplementary Figure S4, lane 4), but strikingly, the lack of RNA silencing complexes did not influence the formation of the higher molecular weight HC-Pro complexes
(Supplementary Figure S4, lane 5). Note that the addition of heparin slightly changed the migration of both the new complex and the HC-Pro-siRNA complex we used as a control (compare lane 3 and 5, and lane 6 and 7 in Supplementary Figure S4).

References
