

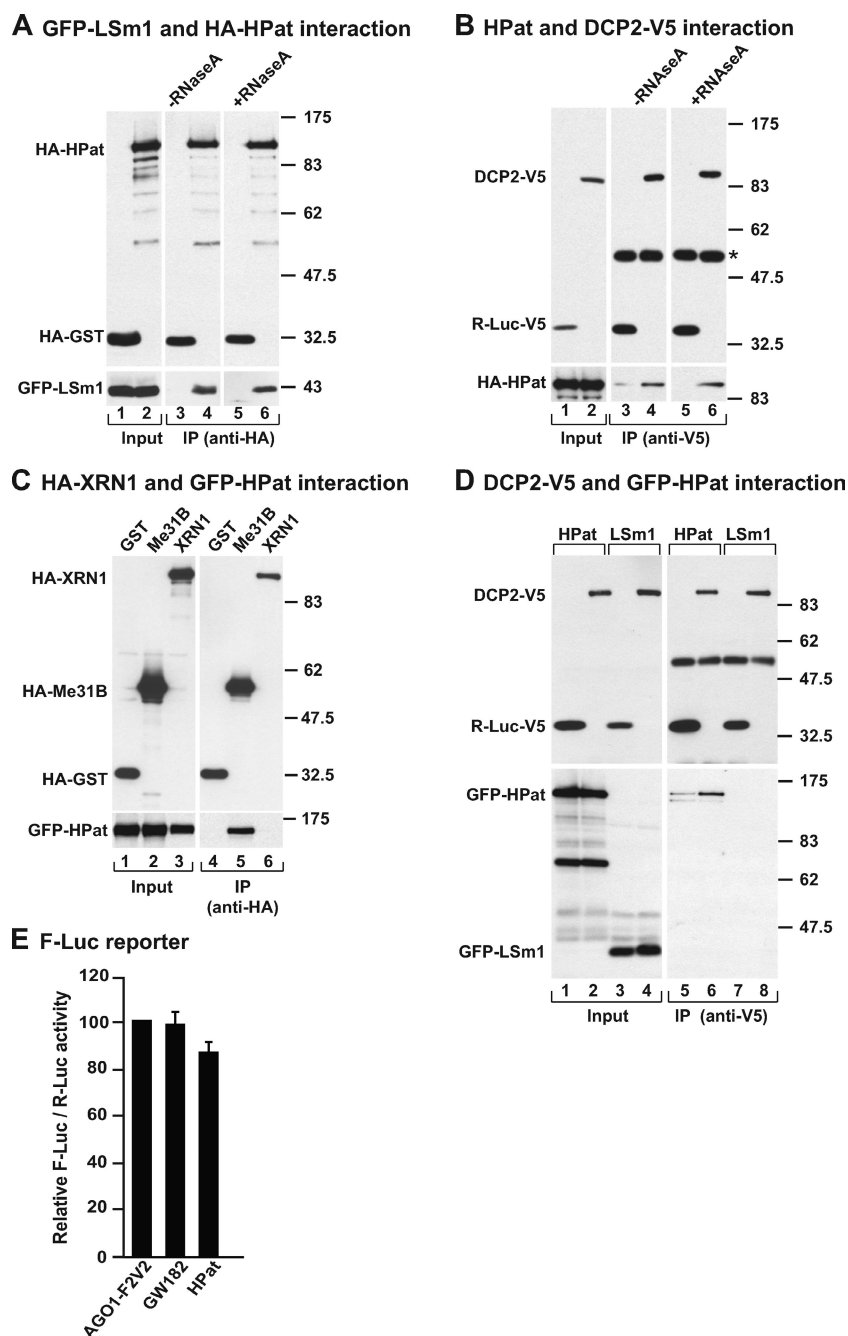
Haas et al., <http://www.jcb.org/cgi/content/full/jcb.200910141/DC1>

Figure S1. **HPat interacts with LSm1 and DCP2 in an RNA-independent manner.** (A–D) Lysates from S2 cells coexpressing the indicated HA-, V5-, or GFP-tagged proteins were immunoprecipitated using a monoclonal anti-HA or -V5 antibodies. In lanes 5 and 6 of panels A and B, cell lysates were treated with RNase A before IP. Inputs (1%) and immunoprecipitates (10%) were analyzed by Western blotting using anti-GFP, -V5, and -HA antibodies. In B and D, 30% of the immunoprecipitate was loaded. HA-GST, R-Luc-V5, or HA-MBP served as negative controls. The asterisk indicates cross-reactivity of the secondary antibody with the immunoglobulin heavy chain. Molecular mass is indicated in kilodaltons. (E) S2 cells were transfected with a mixture of three plasmids, one expressing the F-Luc reporter without 5BoxB, another expressing R-Luc, and a third expressing λ N-HA-AGO1-F2V2 (negative control) or λ N-HA fusions of GW182 or wild-type HPat, as indicated. F-Luc activity was normalized to that of the *Renilla* and set to 100 in cells expressing λ N-HA-AGO1-F2V2. Mean values \pm standard deviations from three independent experiments are shown.

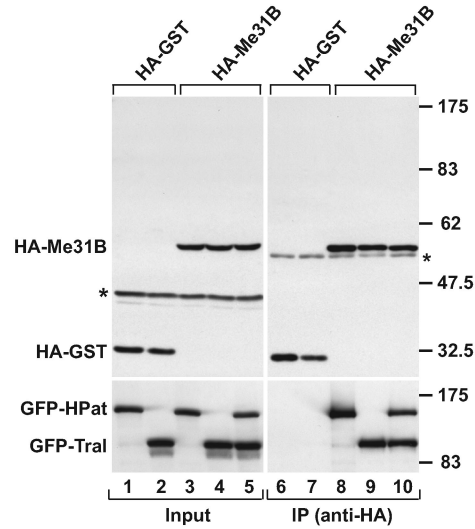
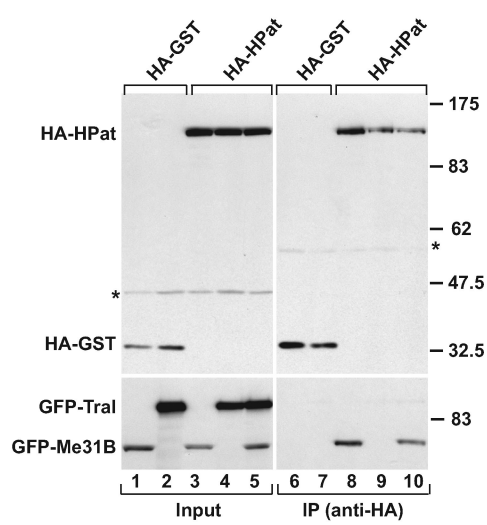
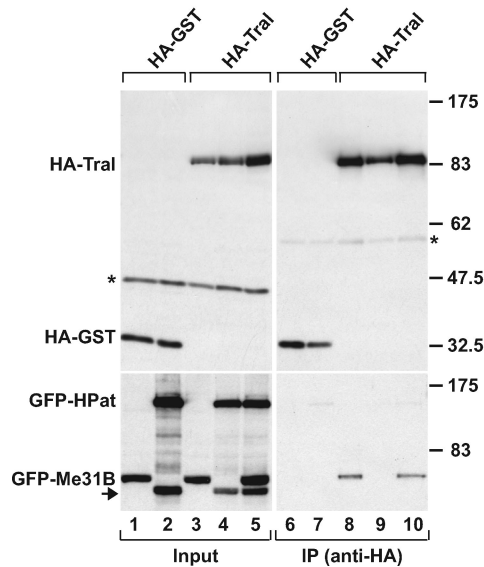
A HA-Me31B / GFP-HPat / GFP-Tral**B HA-HPat / GFP-Me31B / GFP-Tral****C HA-Tral / GFP-Me31B / GFP-HPat**

Figure S2. **HPat and Tral interact with Me31B in a mutually exclusive manner.** (A–C) S2 cells were cotransfected with mixtures of three plasmids. In A, the plasmids encoded HA-Me31B, GFP-HPat, and GFP-Tral; in B, the plasmids encoded HA-HPat, GFP-Me31B, and GFP-Tral; in C, the mixture consisted of HA-Tral, GFP-Me31B, and GFP-HPat. Cell lysates were immunoprecipitated using a monoclonal anti-HA antibody. In all panels, HA-GST served as a negative control. Inputs and immunoprecipitates were analyzed as described in Fig. 1. Asterisks indicate cross-reactivity of the primary antibodies with an endogenous protein (input panels) or of the secondary antibody with the immunoglobulin heavy chain (IP panels). The arrow indicates an HPat protein degradation fragment. Molecular mass is indicated in kilodaltons.