FIGURE LEGENDS

Figure 1. Purification of Gro-interacting proteins. (A) Schematic representation of Gro. The Q, GP, CcN, and SP domains were tagged with GST. (B) The GST-tagged domains were expressed in E. coli and purified with glutathione agarose beads. They were then resolved by 10% SDS PAGE and visualized by Coomassie Blue staining. These proteins were then used as affinity reagents in the purification of Gro-interacting proteins from embryonic nuclear extracts, which were subsequently identified by MuDPIT (see Tables 4 and S1). (C) Venn diagram showing overlap between the non-ribosomal proteins identified in two replicate sets of affinity purification experiments. Fisher's exact test indicates that the overlap between the two sets is highly significant ($p < 2.2x10^{-16}$).

Figure 2. Validation of the interaction between Gro and U1 snRNP. (A) 0-12 hour Drosophila embryo nuclear extracts were subjected to immunoprecipitation using an affinity purified polyclonal antibody directed against the Gro GP domain, or, as a control, rabbit IgG. To assess immunoprecipitation efficiency and specificity, immunoprecipitates were subjected to SDS-PAGE and immunoblotting. The blot was probed with a mixture of the rabbit anti-GP domain antibody and a mouse monoclonal anti-Gro antibody, and IR-dye labeled secondary antibodies. The signal from the rabbit antibody was detected in the green channel of the IR imager, while the signal from the mouse antibody was detected in the red channel. Rabbit IgG heavy chain (IgG) and Gro bands are indicated with arrows on the right. The orange-yellow color of the Gro band is indicative of the overlap between the red and green signals. Lane 1) Markers labeled in kD; Lane 2) 10% input; Lane 3) Anti-Gro immunoprecipitate; Lane 4) Rabbit IgG immunoprecipitate, Lane 5) Mock anti-Gro immunoprecipitate from which input nuclear extract was omitted. (B) RNA was extracted from immunoprecipitates prepared as described in A. The RNA from the immunoprecipitates as well as the RNA extracted from the input nuclear extracts was analyzed by RT-qPCR as described in Experimental Procedures to determine U1 snRNA levels. Error bars based on two independent biological replicates indicate standard deviation. A two-tailed t-test gives $p = 0.016$.

Figure 3: The three-reporter high throughput luciferase assay. (A) Schematic representation of the three reporters. Constructs are not drawn to scale. In the red luciferase reporter, the Gal4 binding sites (UAS elements) are immediately upstream of the enhancer, while in the green luciferase reporter, the UAS elements are about 2 kb downstream of the transcriptional start site. Expression is induced by the Dorsal and Twist activators and repressed by Gal4-Gro. The Renilla luciferase reporter was used as an internal control for transfection efficiency and cell viability. (B) Flow chart of the reporter assay. (C) Validation of the reporter assay. Co-transfection with Dorsal and Twist (Dl/Twi) encoding plasmids activated both the red and green reporters, while addition of a plasmid encoding the Gal4-Gro fusion resulted in repression of the reporters. Dorsal, Gro (including Gal4-Gro), and the histone deactylase Rpd3, which is partially required for Gro-mediated repression (18), were knocked down by RNAi.. Data are normalized to the red and green signals from the Gro dsRNA sample. Error bars based on triplicate transfection assays represent standard deviation. (D) Representative results of the reporter assay. The luciferase reporter assay was carried out using three non-overlapping dsRNAs from the genes encoding vir, snRNP-U1-C, and SRPK. The result of transfection with each dsRNA was compared to that of transfection with GFP dsRNA. Error bars based on triplicate transfection assays represent standard deviation.