which cells are co-transfected with both red and green luciferases. Lrf is the signal recorded with red filter, and Lgf is the signal recorded with green filter.

The signal from untransfected cells was then subtracted from the corrected data to eliminate background. Processed data were then normalized to the internal control Renilla luciferase. Finally, data were compared to the signal from cells in the same plate that were treated with control GFP dsRNA. A change in long or short-range repression was considered significant if $p < 0.1$. If multiple dsRNAs were tested for a given gene (as was true in most cases, Table S2), then a change is only listed if $p<0.1$ for at least two separate dsRNAs.

RNA-seq library preparation–Gro dsRNA was generated by PCR amplification of the first 800 nucleotides of the coding sequence using primers containing T7 promoters followed by in vitro transcription with T7 RNA polymerase. snRNP-U1-C dsRNA was generated by PCR and in vitro transcription of the snRNP-U1-C coding sequencing with primers 5'-taatacgactcactatagggtactCAAAGTACTATTGCG ACTACTGC and 5'-taatacgactcactatagggtactCTTGGGTCCGTTCATG ATTCC. Transfection was carried out as previously described (34). RT-qPCR was used to determine the knockdown efficiency prior to RNA-seq library preparation. RT-qPCR primers targeted the 3' UTRs of Gro and snRNP-U1-C. Rpl32 was used as a reference gene. The specificity of all primers was validated by melting curve analysis of the amplification products (data not shown). Sequences of the qPCR primers are listed in Table 2.

Total RNA was extracted with Trizol according to the manufacturer's protocol (Life Technologies, Cat.# 10296010). RNA integrity was determined with an Agilent 2100 Bioanalyzer using the RNA 6000 Nano Kit (Agilent Cat.# 5067-1511). Isolation of mRNA was carried out as follows. Streptavidin magnetic beads (Promega Cat.# Z5481) were prepared in aliquots of 120 μl and 60 μl in 0.5X SSC with 10 mM EDTA. 15 μg of total RNA was mixed with 1.5 μM of biotinylated 15-mer poly(T) oligonucleotide in 0.5X SSC with 10 mM EDTA. Samples were first incubated at 75˚ for 5 minutes, followed by 15˚ for 10 minutes and 10˚ for 10 minutes. Samples were then incubated with 120 μl of magnetic beads at 4˚ for 2 hours, followed by 60 μl of magnetic beads at 4˚ for 30 min. The two aliquots of beads were combined and washed four times with 300 μl of ice cold 0.1X SSC containing 10 mM EDTA. mRNA was first eluted with 100 μl of water followed by 150 μl of water at 37˚ for 10 min each. Samples were precipitated with ethanol and stored at -80˚. Pulldown efficiency of mRNA and depletion efficiency of 18S rRNA were determined by RT-qPCR (data not shown).

The RNA-seq library was prepared according to the manufacturer's protocol (Epicentre, Cat.# SSV21124 and Cat.# RSBC10948). The concentration of the library was determined with Pico Green (Life Technologies, Cat.# Q32851) according to the manufacturer's directions. Fluorescence signal was measured using a TECAN M1000 fluorescent plate reader.

Bioinformatics–Alignment of paired-end reads to the D. melanogaster genome (assembly BDGP 5/dm3) was performed with Tophat2 (v2.0.9) (35) using default parameters. DESeq2 (v1.6.3) (36) was used for gene expression-level normalization and differential expression significance testing. Histone modification and motif enrichment analysis was carried with i-cisTarget (37) using default parameters. Enriched gene ontology analysis was done with Flymine (v31.0) (38) using default parameters.

RESULTS

Identification of Gro interacting proteins–A previous study showed that deletion of the GP or CcN domains in the Gro central region led to a loss of Gro-mediated repression and to lethality, while deletion of the SP domain led to reduced