Figure 4: Genome-wide expression profiling reveals co-regulation of genes by Gro and snRNP-U1-C. (A) Expression of Gro and snRNP-U1-C mRNA after dsRNA treatment. RT-qPCR was performed after extraction of total RNA. Data was normalized to reference gene Rpl32. (B) Comparison of transcriptomes from our wild-type S2 cell RNA-seq data and the modENCODE S2 cell RNA-seq data. (C) Comparison of transcriptomes from our Gro knockdown RNA-seq data and previously published Gro knockdown RNA-seq data (49). The transcripts that were detected at significant levels in only the previously published Gro knockdown study (represented by the points in contact with the vertical axis) correspond primarily to non-polyadenylated transcripts. In B and C, the scale on both axes is $\log_2$(CPM) where CPM is counts per million sequence reads. (D) Based on RNA-seq analysis of wild-type and snRNP-U1-C knockdown cells, genes were categorized as non-differentially expressed upon knockdown (non-DE, 12,028 genes), up-regulated upon knockdown (1,431 genes), and down-regulated upon knockdown (1,691genes). Percent of genes in each category with no introns is shown. Some Drosophila genes lack annotated transcripts and thus it was not possible to determine their intron count. This results in a small numerical discrepancy between the number of differentially expressed genes included in this analysis and the number of snRNP-U1C differentially expressed genes shown in part E of this figure. (E) Venn diagram showing numbers of differentially expressed genes in Gro and snRNP-U1-C knockdown cells and the overlap between these sets. Fisher's exact test indicates that the overlap is highly significant ($p < 2.2 \times 10^{-16}$). (F) Enrichment of Gro/snRNP co-regulated genes for various features. Normalized enrichment scores are calculated using cumulative recovery curves (37). Scores above 2.5 are considered significant.

Figure 5. Gro binding regions in differentially expressed genes. (A) S2 cell ChIP-seq data (49) identified 1242 Gro binding sites, which map to 748 genes, 46 of which were differentially expressed when we knocked-down Gro. Of the 46 differentially expressed genes, 39 were up regulated and 7 were down regulated in response to Gro knock-down. (B) Gro binding regions in the 46 differentially expressed genes are significantly enriched for Su(H) and Brk binding sites.

Figure 6. Gro-repressed genes are enriched for promoter proximal Pol II. Percent of non-differentially expressed genes, and genes that are either up-regulated or down regulated in Gro knockdown cells containing no Pol II bound, Pol II bound, or enriched for promoter proximal Pol II as ascertained by Pol II ChIP-chip analysis (53).