splicing elements can influence transcription (26,28,71).

U1 snRNP, a part of the spliceosome, consists of U1 snRNA, three U1 snRNP specific proteins, and the seven subunit Sm complex (46). Our list of 162 Gro-interacting proteins (Tables S1B and C) includes all three U1 snRNP specific proteins (snRNP-U1C, snRNP-U1-70K, and snRNP-U1-A), as well as two subunits of the Sm complex (Sm-D2 and Sm-D3). We note that we also detected at least four other Sm complex subunits in one of the two replicate screens (Sm-B, Sm-F, Sm-D1, and Sm-G) (Table S1C). Additionally, we showed by co-immunoprecipitation that approximately 13% of U1 snRNA, the RNA component of the U1 snRNP, is associated with Gro in embryonic nuclei. Thus, we have detected essentially the entire U1 snRNP in our proteomic screens for Gro-interacting proteins.

Data from our reporter assay suggests that the U1 snRNP complex is required for optimal Gro mediated repression, as snRNP-U1-C and snRNP-U1-70K knockdown attenuated repression. Consistent with our finding, it has been shown that snRNP-U1-C overexpression can decrease EWS/FLI-activated transcription (30). It is worth noting that the U1 snRNA is known to associate with TFIIH and promote transcriptional initiation *in vitro* (29). Thus, the effect of the U1 snRNP complex in transcription regulation may be context dependent.

Gro recruitment is insufficient for repression–The available S2 cell Gro ChIP-seq data (49) reveals 1242 Gro binding sites in the S2 cell genome associated with 748 genes, while our RNA-seq analysis revealed that only 46 of these 748 genes are differentially expressed in Gro knockdown S2 cells implying that Gro binds to many genes that it does not regulate. The apparent contradiction could be explained by the absence of a required transcriptional activator in S2 cells to activate these genes upon Gro depletion. Regardless of the reason for the finding that Gro binds to many more genes than it regulates, this is a phenomenon that is common to many (perhaps most) eukaryotic gene-specific transcriptional regulators (51,52). Gro ChIP-seq peaks associated with genes differentially expressed upon Gro knockdown are enriched for Su(H) and Brk binding motifs. This is in agreement with the known roles of Su(H) and Brk in the recruit of Gro to target genes in the Notch and Dpp signaling pathways, respectively (72-74).

Genes that are up-regulated in Gro knockdown cells (and which are therefore candidate Gro repression targets) exhibit enrichment in Pol II pausing near the transcriptional start site. This finding is in agreement with the hypothesis that Pol II pausing is one mechanism to repress gene expression (75,76). We note that our proteomic screen revealed the Pol II C-terminal domain (CTD) kinase Cdk12 as a Gro-interacting protein (Table S1). By phosphorylating the CTD on Ser 2, Cdk12 may function to allow release of paused Pol II (77). Consistent with this idea, our reporter assay shows that Cdk12 functions to alleviate Gro-mediated repression (Tables 5 and S2).

Genes that are differentially expressed in Gro and snRNP-U1-C knockdown cells are enriched for H3K36me1 as well as the H3K36 methyltransferase ASH1. While H3K36me is involved in multiple functions including transcriptional regulation, splicing, and DNA repair (78,79), these findings suggest a previously unknown role for this histone mark in Gro mediated repression.

The Gro central region as a regulatory hub of repression activity–In conclusion, our findings reinforce the idea of that the Gro central domains, which are intrinsically disordered, are indispensable for repression (19). Previous studies from our lab and other labs show that the GP domain interacts with the histone deacetylase Rpd3/HDAC1, which may promote local histone deacetylation and alter nucleosome density (16,18). The identification of the ACF chromatin