volume of 250 µl HEMNK buffer overnight at 4˚. 225 µg of Protein A Dynabeads (Invitrogen Cat.# 10001D) were incubated with the samples at 4˚for 1 hour. Samples were then washed with HEMNK buffer three times for 10 minutes each. For RT-qPCR, RNA was eluted in 10 µl water by heating to 80˚ for 2 minutes. Samples were treated with DNase I according to the manufacturer's protocol (Promega Cat.# M6101). Reverse transcription was performed with 300 ng of random primer (Invitrogen Cat.# 48190-011), and qPCR was performed using primers amplifying U1 snRNA (Table 2). Threshold cycle values were converted to percent input values by comparison to a standard curve generated from multiple serial dilutions of RNA isolated by Trizol extraction (Life Technologies, Cat.# 10296010) from the input nuclear extract. Primer specificity was validated by melting curve analysis of the amplification products (data now shown).

For immunoblotting, samples were eluted in SDS-PAGE loading buffer. Proteins were detected with a mixture of mouse anti-Gro (Developmental Studies Hybridoma Bank, 1:650 dilution) and affinity purified rabbit anti-GP domain (1:100 dilution) antibodies. Immunoblots were subsequently probed with goat anti-mouse 680 and goat anti-rabbit 800 IR-dye coupled secondary antibodies (Li-Cor) and imaged with a Li-Cor Odyssey imager.

*Three-reporter luciferase assay*–To guard against off-target effects, each candidate gene was knocked down with three non-overlapping dsRNAs when possible (the complete list of dsRNAs used is available upon request). Each dsRNA was tested in triplicate. dsRNA was synthesized by the Drosophila RNAi Screening Center and re-aliquoted into white flat bottom 96 well plates (USA scientific Cat.# CC7682-7968) with 150 ng/well in 10 µl of water using a Beckman Coulter BioMek FX Workstation.

Transfections were carried out with Effectene reagent (Qiagen Cat.# 301425). 6 µg each of G5DE5-pCBR and DE5G5-pCBG68, 0.6 µg of RpIII128-Rluc, 1 µg of pPac Dl, 0.3 µg of pPac Twi, and 1.2 µg of the pAct Gal4-Gro were suspended in 600 µl buffer EC. 33 µl of this mixture was added to 25 µl of enhancer. After 2-3 minutes, 7.5 µl Effectene was added and mixed by pipetting up and down. 6 µl of this mixture was immediately added into each well of a 96-well plate containing 150 ng of dsRNA. 4-8 minutes later, 100 µl of S2 cells (diluted to 1 x $10^6$ cell/ml) was added to each well. Cells were incubated at 24˚ for 2 days before assaying.

The luminescence signal was measured with a Molecular Devices LJL Analyst HT microplate reader using emission filters ET510/80m and E610LP (Chroma Cat.#S-022658 and #138951). 50 µl of D-luciferin (Chroma-Glo system, Promega Cat.# E2980) was added to each well. Five minutes later the reaction was stopped by the addition of 50 µl of stop buffer containing coelenterazine (Dual-Luciferase system, Promega Cat.# E1960). The luminescence signal was measured immediately without applying a filter.

To address the issue of signal overlap, raw signals were subjected to filter correction. The corrected red luminescence signal R' and green luminescence signal G' were calculated according to the following equations:

$$R' = \frac{Lrf - Lgf \times \left(\frac{Grf}{Ggf}\right)}{\left(\frac{Rrf}{R}\right) - \left(\frac{Rgf}{R}\right) \times \left(\frac{Grf}{Ggf}\right)}$$

$$G' = \frac{Lgf - R' \times \left(\frac{Rgf}{R}\right)}{\left(\frac{Ggf}{G}\right)}$$

Parameters were determined by expressing the individual luciferases and recording the luminescence signals with red and green filters, and without filter (data not shown). The ratio of green signal passed through the red filter, Grf/Ggf, was determined to be 0.0975; the ratio of red signal passed through the red filter, Rrf/R, was determined to be 0.42; the ratio of red signal passed through the green filter, Rgf/R, was determined to be 0; the ratio of green signal passed through the green filter, Ggf/G, was determined to be 0.47. Lrf and Lgf are luminescence signals in