



**Figure 6 - figure supplement 3: Position-dependent effects on distal enhancer.** To best mimic the endogenous system, we looked at expression driven by the distal enhancer at its endogenous spacing from the promoter for our noise calculations. In this construct we replaced the sequence of the proximal enhancer with sequence of the same length from the lambda phage genome predicted to have low number of *Drosophila* TF binding sites. This increased distance from the promoter had observable effects on the transcriptional dynamics and noise associated with the distal enhancer. **A.** Comparison of total transcriptional expression mediated by the distal enhancer at its endogenous spacing or proximal to the promoter. The distal enhancer at its endogenous spacing, shown as the solid line, produces significantly more total mRNA in the center region of expression than the distal enhancer proximal to the promoter, shown as the dotted line. **B.** Comparison of the average number of transcripts produced per transcriptional burst by each distal enhancer configuration as a function of egg length. **C.** Average burst frequency associated with either distal enhancer configuration as a function of egg length. **D.** Average burst duration associated with either distal enhancer configuration as a function of egg length. **E.** Coefficient of variation of transcriptional activity across nc14 for each distal enhancer configuration as a function of egg length. **F.** Total expression noise associated with either distal enhancer configuration at the AP bin of that construct's peak expression. The total noise distribution for the distal enhancer proximal to the promoter is on the left and that for the distal enhancer at its endogenous spacing from the promoter is on the right. The distal enhancer at its endogenous spacing displays significantly higher total noise ( $p = 0.018$ ) than the distal enhancer proximal to the promoter. Each circle represents the total noise of an individual nucleus and the horizontal bar marks the median total noise value. Y-axis limited to the 75th percentile of the construct with the highest total noise values (distal promoter at endogenous spacing). Error bars in A-E represent 95% confidence intervals.