**CLAMP regulates Zygotic Genome Activation in *Drosophila* embryos**

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**Supplemental Material:**

**Figure S1. Examples of defect classes for analysis of patterning gene, mitotic, and cytoskeletal phenotypes.** Representative embryos are shown for defect classes of a patterning gene (Btd, A-C), mitosis (pH3, D-F), and cytoskeleton (Nrt, G-I). WT protein patterns are shown in the left column (A,D,G), while the middle column shows “medium” defects (B,E,H) and the right column exhibits “severe” defects (C,F,I). Scale bars represent 10 µm.

**Figure S2. Zygotic knockdown of CLAMP depletes protein levels in early embryonic nuclei.** Control *egfpi* (A,C,E) or *clampi1* (B,D,F) embryos were stained for CLAMP and visualized using DAB. Representative embryos from various nuclear cycles show the efficacy ofzygotic knockdownbeginning at NC 10 (B). Compared to the nuclei from comparably staged embryos (left), *clampi1* embryos have lower and patchy CLAMP protein distribution. Scale bar represents 10 µm.

**Figure S3. Zygotic knockdown of Bicoid depletes protein levels in the anterior of *bcdi* embryos.** Control *egfpi* (A and A’) or *bcdi* (B,B’,C,C’) embryos were stained for Bcd (green) and co-labeled using Hoescht (blue). Bcd levels were compromised zygotically using two different transgenes, and representative embryos are shown from each genotype: *bcdi1* (B and B’) and *bcdi2* (C and C’). Scale bar represents 10 µm. D) Quantification of Bcd knockdown for each genotype. Level of knockdown was classified as none (wildtype gradient of Bcd), medium (detectable but notably compromised Bcd gradient), or severe (very little or no Bcd protein in anterior). n=14-16 per genotype. Significance was determined using Fisher’s exact test (p= 0.001814 and 0.014976 for *bcdi1* and *bcdi2* compared to *egfpi* embryos, respectively).

**Figure S4. RNA-seq reveals more decreased targets in early vs. late datasets.** A-B) Comparison in genes decreased at least 2-fold in 0-1.5 hr (A) and 1.5-3 hr (B) between *clampi1* and *clampi2x* transcriptomes**.** The overlap in genes for each collection was analyzed using hypergeometric tests (p values listed below overlap gene count in diagram). C)Plots of counts per million reads for two pair-rule genes, *eve* and *run* and two gap genes, *btd* and *gt* (as indicated in figure) in 0-1.5 hr **(**Early) and 1.5-3 hr (Late) collections. Significance was determined by FDR (Benjamini-Hochberg) corrected analysis, as stated in Materials and Methods. Significant decreases (pp<0.05) are marked with an asterisk .The effect of CLAMP and/or Zld knockdown on pair-rule and gap gene transcripts is larger in the 0-1.5 hr collection than in the 1.5-3 hr collection; however, the number of reads in the later collection is noticeably increased. Additionally, we compared our decreased zygotic genes (classified according to Lott *et al.* 2011) with previously published genomic datasets examining genes affected by compromising Zld or CLAMP (Table S2).

**Figure S5. Localization of CLAMP and Zld in genomic regions containing the pair-rule genes *eve* and *run* and the gap genes *btd* and *gt*.** Location of ChIP peaks for CLAMP (green) or Zld (red) at NC13. ChIP-seq datasets were obtained from Rieder *et* al. 2017 and Harrison *et al.* 2011, respectively. The raw data were then reanalyzed as described in Materials and Methods. Peaks recovered from our analysis are displayed here, relative to early transcribed genes of interest, as indicated.

**Figure S6. Levels of CLAMP protein levels and patterning gene proteins correlate in *clampi* embryos.** *egfpi* embryos exhibit strong nuclear CLAMP staining (green, A) and an easily discernable band of Btd (red, A’) expression at the anterior. In *clampi1* embryos, the levels of both CLAMP(B) and Btd (B’) proteins are reduced. Scale bar represents 10 µm.

**Figure S7. Transcription of the pair-rule gene *runt* is disrupted in *clampi1* or *zldi* embryos.** Expression of the pair-rule gene *run* visualized using smFISH in *egfpi* (A), *clampi1* (B), and *zldi* (C) blastoderm stage embryos. The WT seven stripe pattern is decreased and/or disrupted by *clamp* or *zld* knockdown. Scale bar represents 10 µm.

**Figure S8. Zygotic *clamp* knockdown results in variable decrease in Buttonhead protein levels.** A-C) Immunostaining of Btd protein in *egfpi* (A) and *clampi1* (B-C) embryos, which were co-labeled with Hoescht. In WT, Btd protein accumulation within the anterior band is nearly uniform in all nuclei. In *clampi1* embryos, adjacent nuclei show differences in Btd protein levels, giving rise to a patchy appearance (Magnified in panels C and C’). Asterisks mark adjacent nuclei that do not express Btd. Scale bar represents 10 µm. D) Classification of defects for Btd immunostaining. Defect classes were the same as stated for *btd* smFISH experiments (Fig. 5). n= 20-24 per genotype. Significance was determined using Fisher’s exact test (p= 0.00196 for *clampi1* compared to *egfpi* embryos).

**Figure S9. CLAMP and Zelda are required for proper expression of the gap gene *gt*.** A-D) Immunostaining of Gt protein in *egfpi* (A), *clampi1* (B)*, zldi* (C)*,* and *clampi2x* (D) embryos. Embryos were co-stained with Hoescht. Scale bar represents 10 µm. In WT and *egfpi* embryos, Gt protein accumulates in anterior doublet and a broad posterior band (A and A’). This pattern of protein accumulation is altered in *clampi1, zldi,* and *clampi2x* embryos. Different examples of defects are shown in the figure. In the *clampi1* embryo, the dorsal notch in the most anterior doublet is lost, and the expression domains of the anterior doublet and the posterior band are expanded (B and B’). Overall expression levels are also reduced, which is clearer in the *zldi* embryo (C and C’). In the *clampi2x e*mbryo, Gt expression in both the anterior doublet and the posterior band is reduced and patchy (D and D’). E) Classification of defects for Gt immunostaining. Medium defects: modest overall reduction or slight blurring of Gt expression regions. Severe defects: loss of portions of WT Gt expression patterns, absence of patterning specificity, or distinct overall decrease in Gt levels. n=14-17 per genotype. Significance was determined using Fisher’s exact test (p= 0.000677 and 0.000484 for *clampi1* and *zldi* compared to *egfpi* embryos, respectively).

**Figure S10. Levels of Sxl protein are decreased in female compromised for CLAMP activity.** A-B) Sxl protein levels in *white1* (A) or *clampi1* (B) blastoderm stage female embryos. C) Classification of Sxl levels in embryos. In *w1* embryos, the DAB signal for Sxl protein is very high in female embryos (but absent in males). *clampi1* embryos, however, show a distinct and consistent decrease in Sxl levels in female embryos, while males remain devoid of Sxl signal. n>1000 for each genotype. Significance was determined using Fisher’s exact test (p= 0.000).

**Figure S11. CLAMP occupancy shifts during the minor wave of ZGA.** A) Comparison in genes bound by CLAMP at NC0-10 (yellow) and NC11 (green) B) Comparison in genes bound by CLAMP at NC11 and NC13 C) Localization of CLAMP at NC0-10 versus NC 11 in genomic regions containing the pair-rule genes *eve* and *run* and the gap genes *btd* and *gt*.

**Table S1. Decreased targets in both CLAMP knockdown collections at 0-1.5 hr and 1.5-3 hr.**

**Table S2. Comparison of decreased zygotic genes between previously published datasets and our RNA-seq data from zygotic knockdown embryos.**

**Table S3. Maternal/zygotic classification of target genes decreased in 0-1.5 hr RNA-seq datasets.**

**Table S4. CLAMP binding genes near the TSS increases at NC11 and is maintained throughout later nuclear cycles.**

**Table S5. Decreased transcripts (by at least 2-fold) in 0-1.5 hr collections of *clampi1*, *zldi*, and *zld/clampi1* embryos.**