

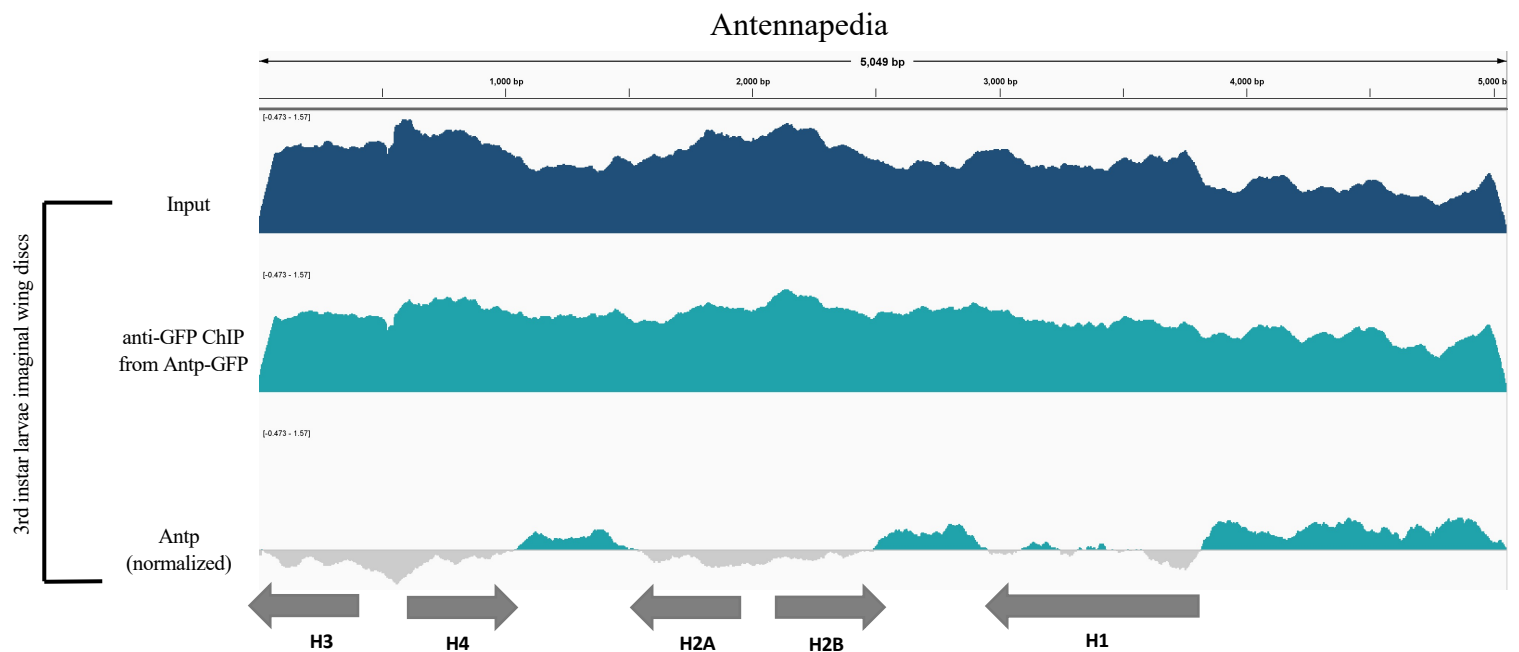
Supplemental Figures

Hodkinson, Smith, *et al.* A bioinformatics screen reveals Hox and chromatin remodeling factors at the *Drosophila* histone locus

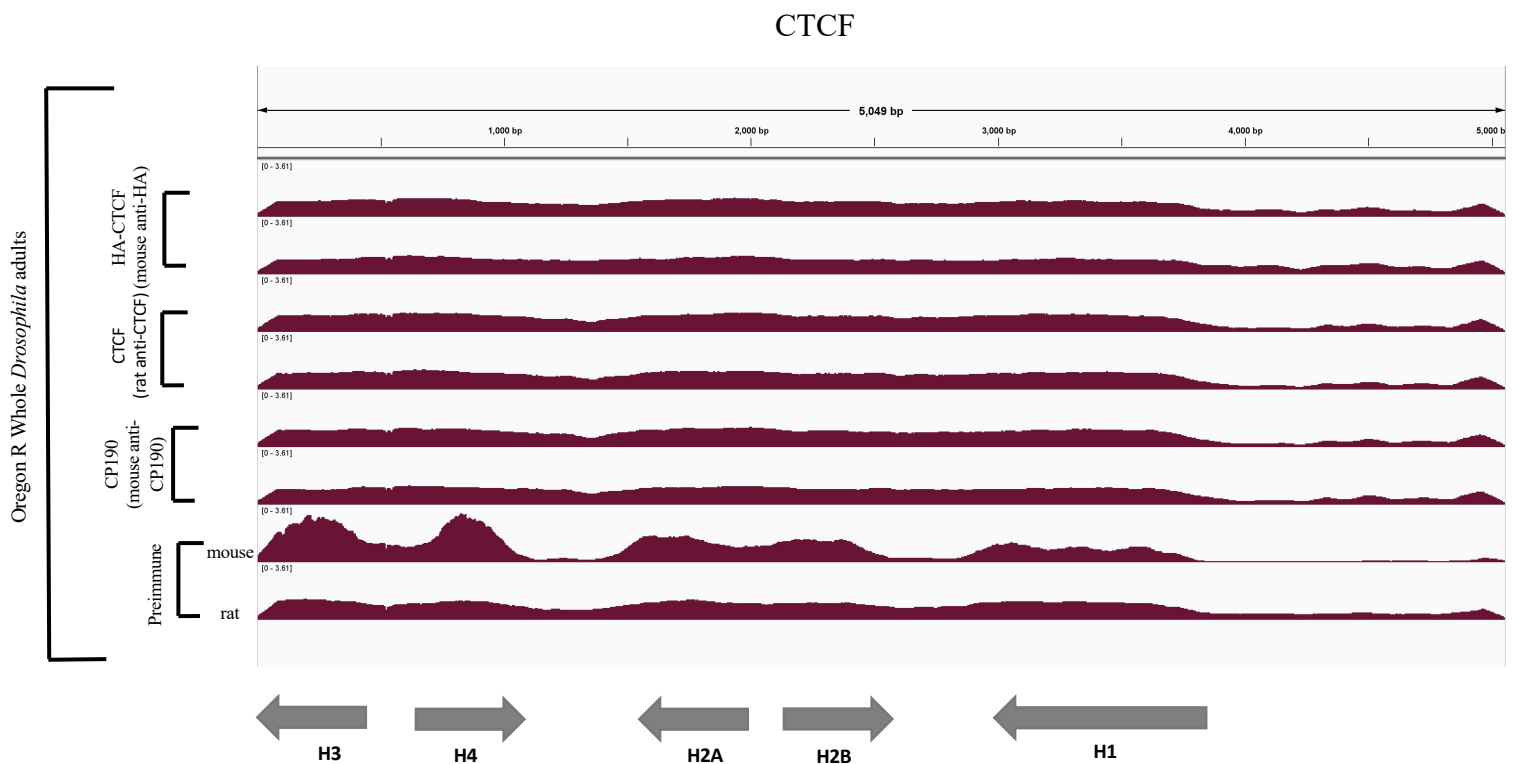


Supplemental Figure 1: Qualitative assessment for scoring candidates as positive or negative.

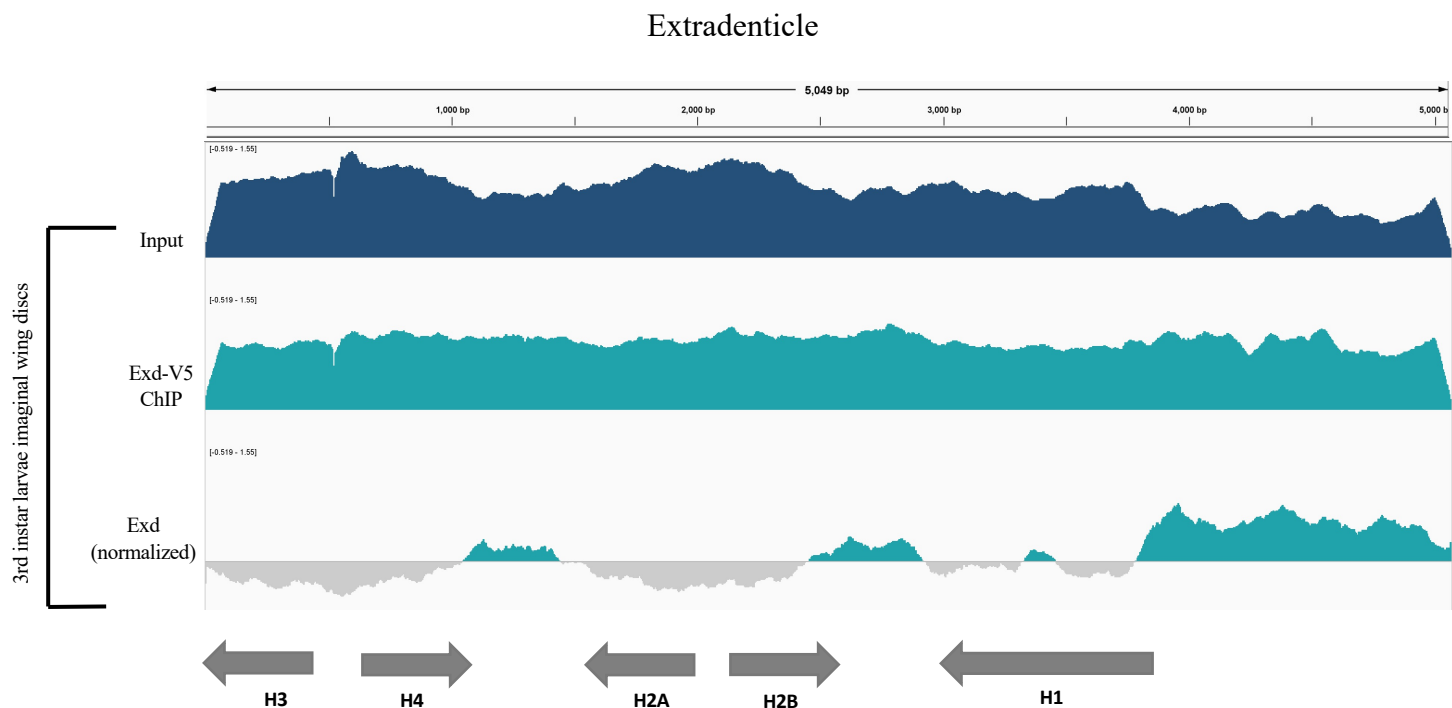
Supplemental Figure 2: Factors considered negative hits.



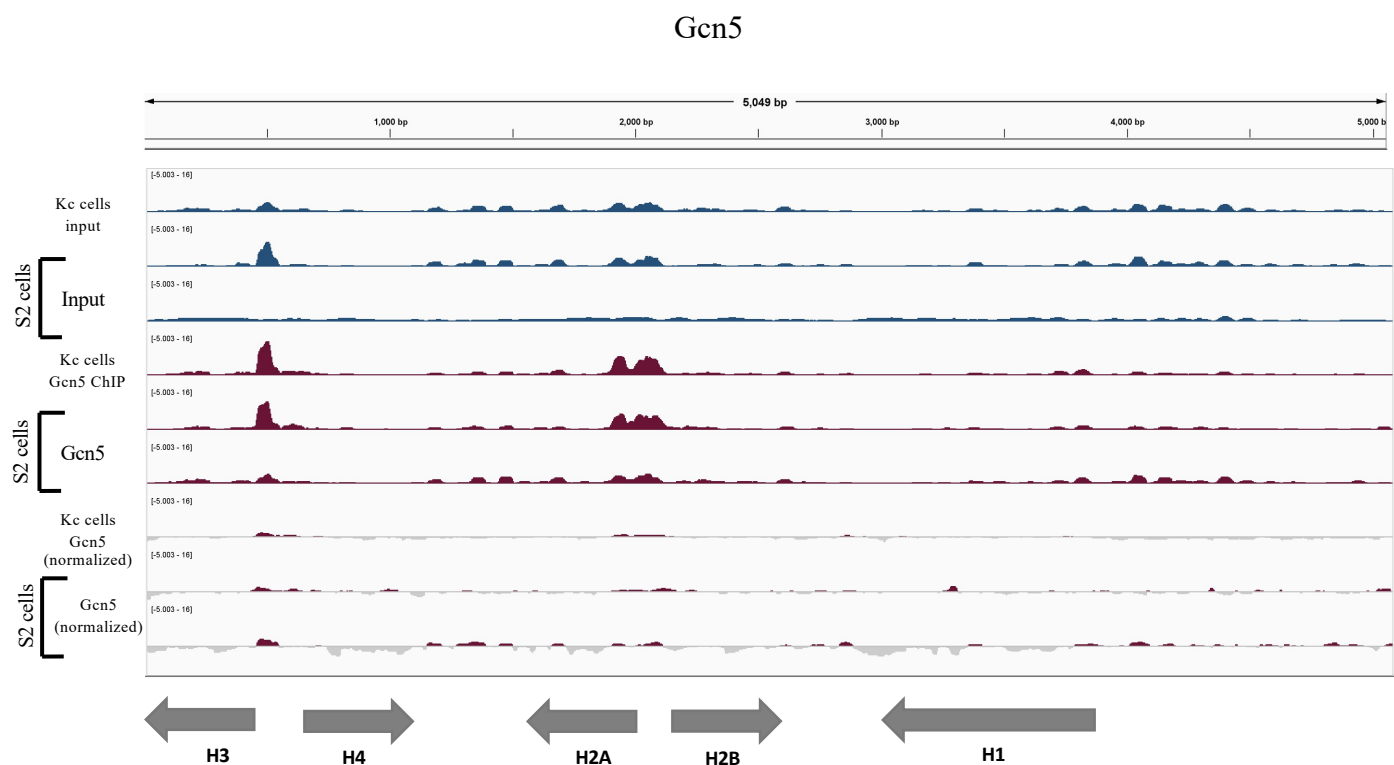
We mapped Antennapedia (Antp) ChIP-seq (cyan) and input (navy) data (Kribelbauer, *et al.* 2020) from 3rd instar larvae imaginal wing discs to the histone gene array. Experiment used an anti-GFP antibody to immunoprecipitate Antp-GFP. Antp does not show convincing localization to the histone gene array when compared to input.



We mapped CTCF-HA ChIP, CP190 ChIP, and preimmune ChIP-seq data (Kyrchanova, *et al.* 2021) from OregonR whole *Drosophila* adults to the histone gene array. CTCF and CP190 do not show convincing localization to the histone gene array when compared to preimmune.

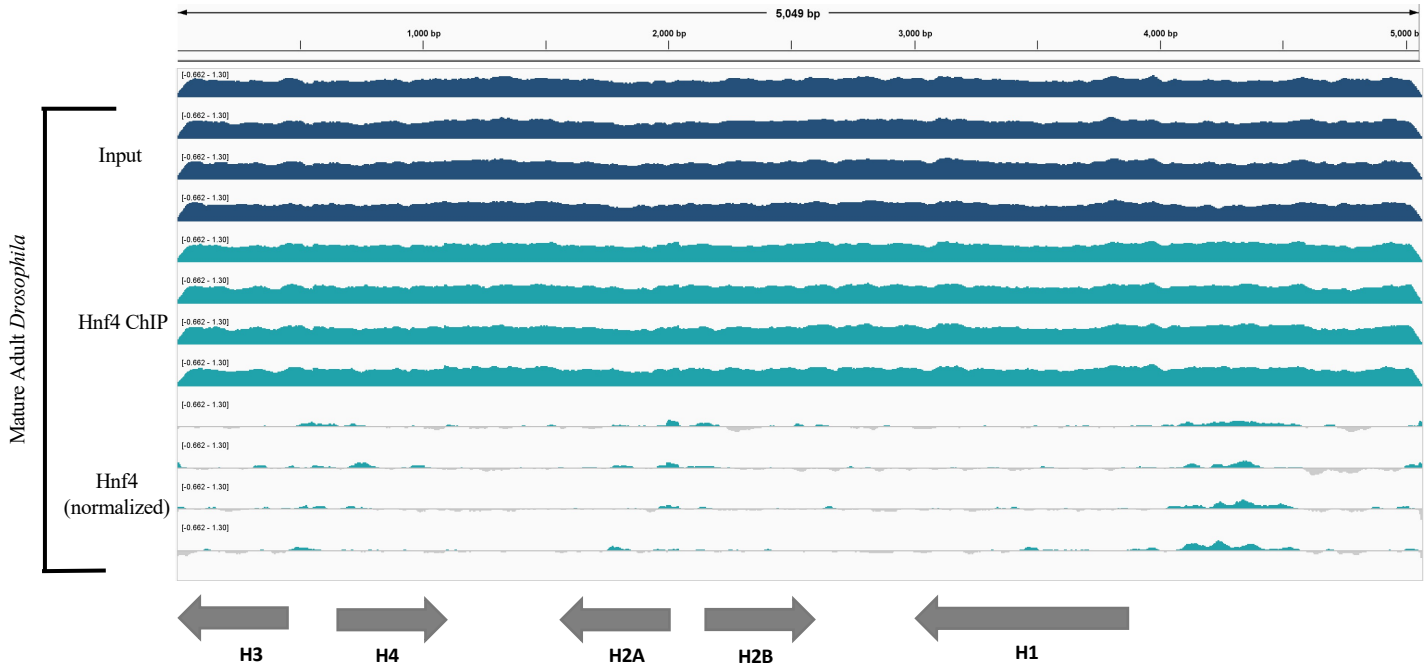


We mapped Extradenticle-V5 (Exd) ChIP-seq (cyan) and input (navy) data (Kribelbauer, *et al.* 2020) from 3rd instar larvae imaginal wing discs to the histone gene array. Experiment used an anti-V5 antibody to immunoprecipitate Exd-GFP. Exd does not show convincing localization to the histone gene array when compared to input.



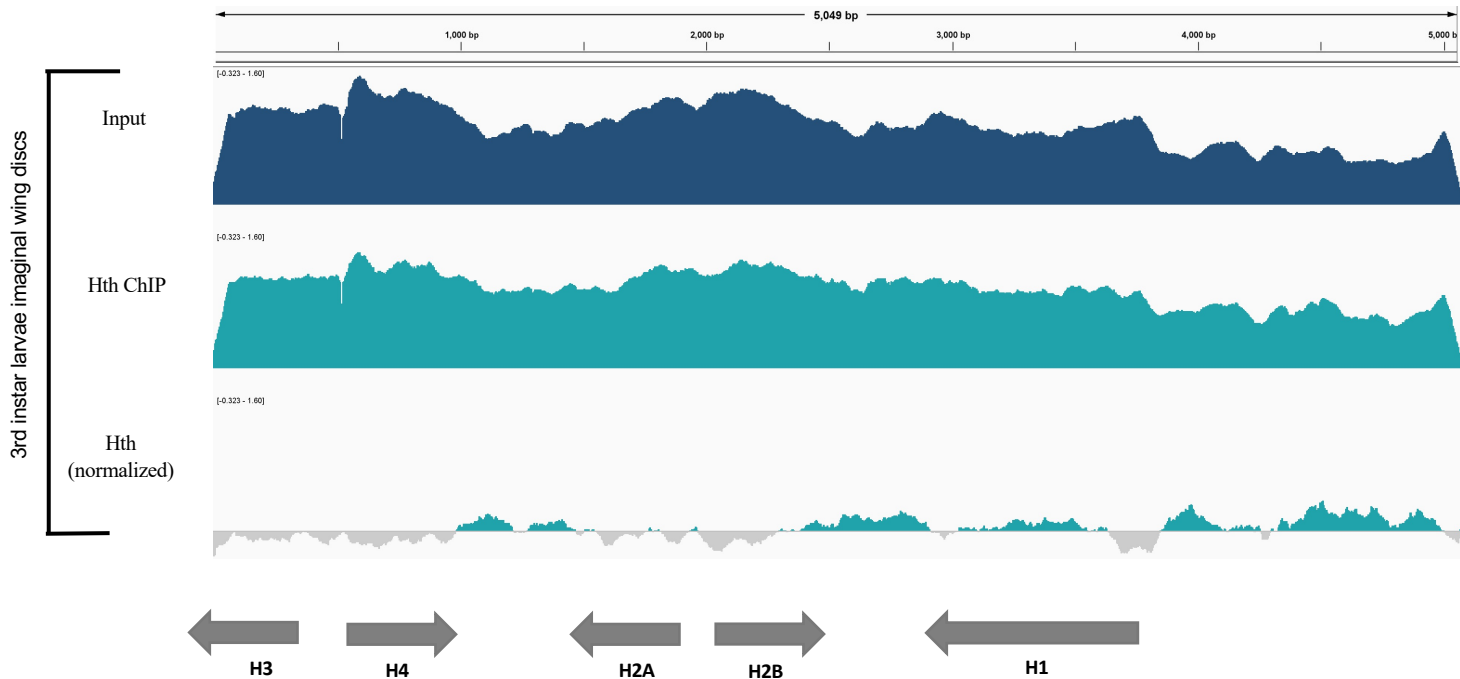
We mapped Gcn5 acetyltransferase (Gcn5) ChIP-seq (maroon) and input (navy) data (Ali, *et al.* 2017) from both Kc cells (one replicate) and S2 cells (two replicates) to the histone gene array. Gcn5 does not show convincing localization to the histone gene array when compared to the corresponding input.

Hepatocyte nuclear factor 4



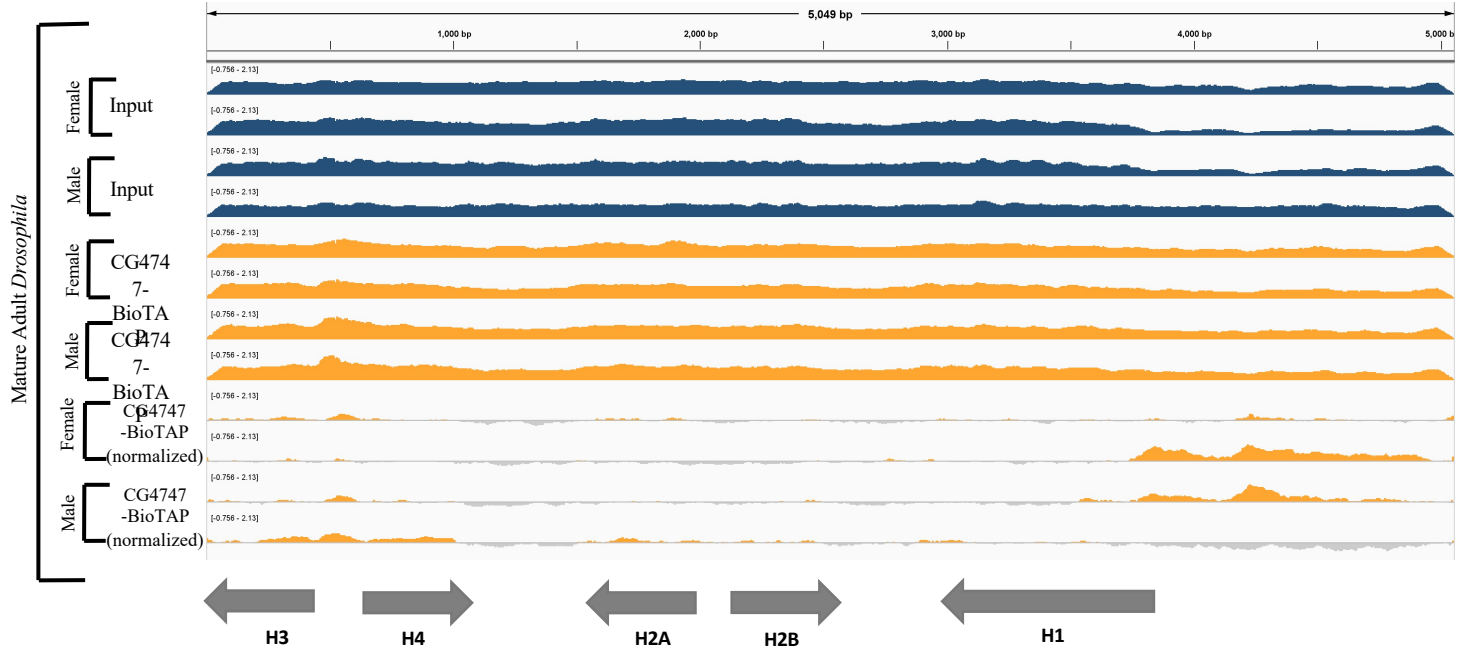
We mapped Hepatocyte nuclear factor 4 (Hnf4) ChIP-seq (cyan) and input (navy) data (Thummel, *et al.* 2015) from whole mature adult *Drosophila* to the histone gene array. Hnf4 does not show convincing localization to the histone gene array when compared to input.

Homothorax



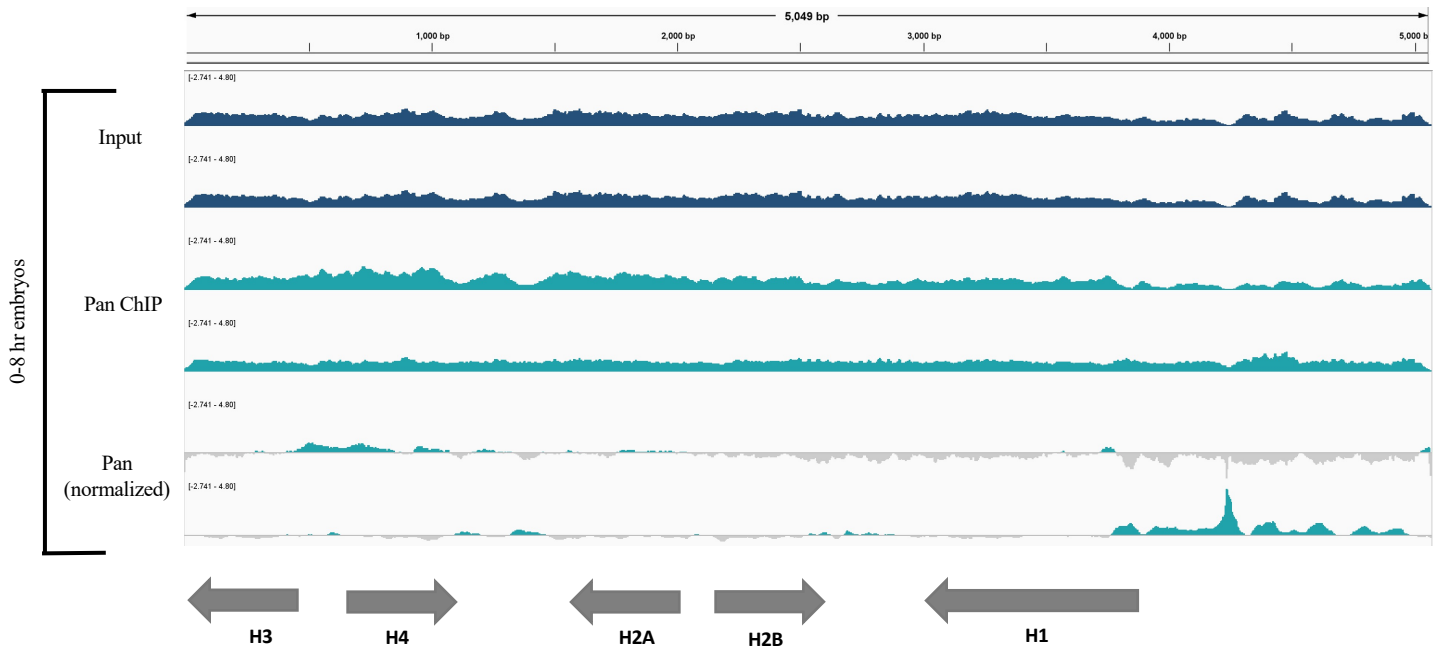
We mapped Homothorax (Hth) ChIP-seq (cyan) and input (navy) data (Kribelbauer, *et al.* 2020) from 3rd instar larvae imaginal wing discs to the histone gene array. Hth does not show convincing localization to the histone gene array when compared to input.

Nucleosome-destabilizing factor/CG4747



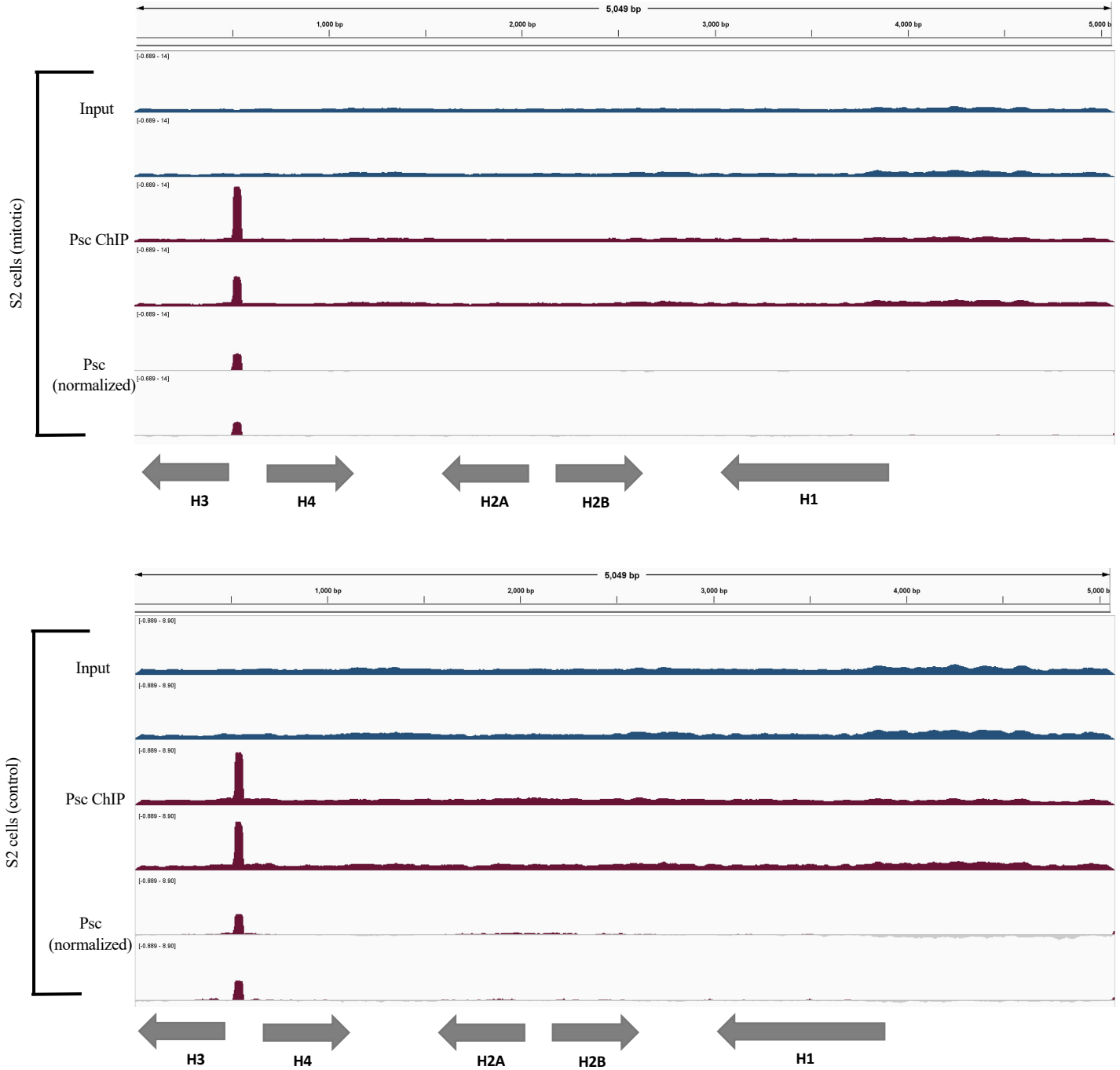
We mapped Nucleosome-destabilizing factor-BioTAP (Ndf/CG4747) ChIP-seq (yellow) and input (navy) data (GSE42025) from whole mature adult *Drosophila* to the histone gene array. Experiment used an anti-Bio-TAP antibody in animals expressing CG47474-BioTAP. Ndf/CG47474 does not show convincing localization to the histone gene array when compared to input.

Pangolin

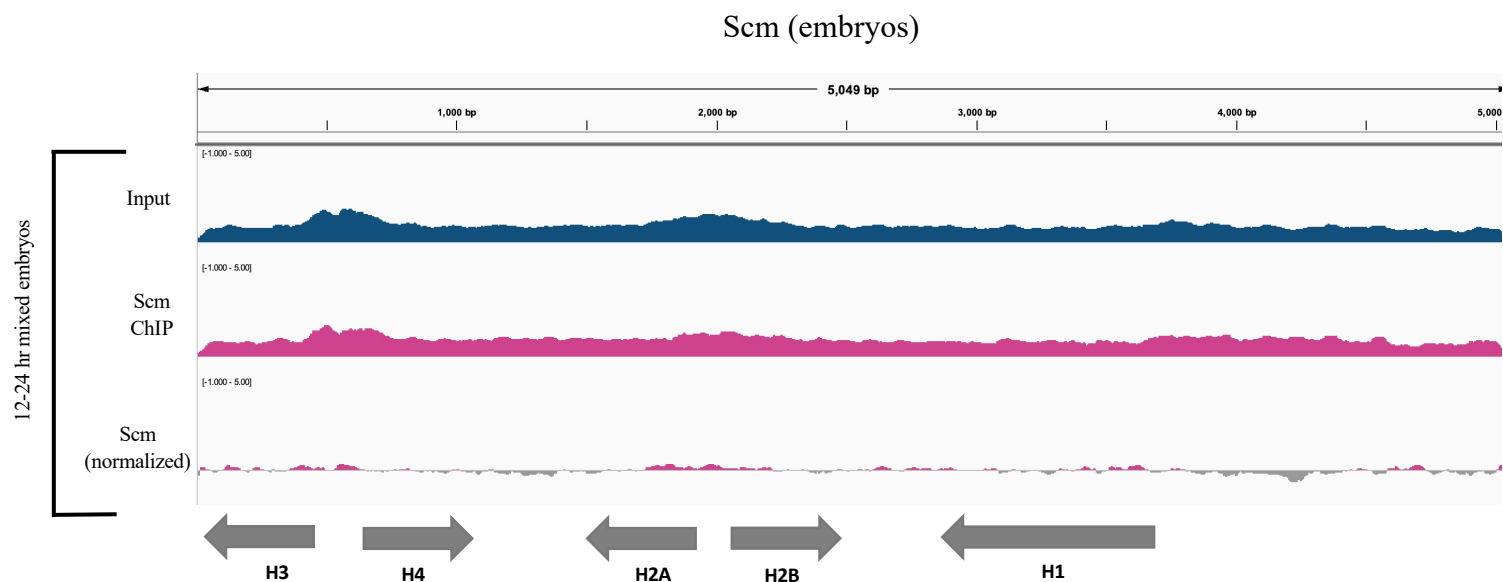


We mapped Pangolin (Pan) ChIP-seq (cyan) and input (navy) data (ModENCODE) from 0-8 hr embryos from the *y,cn,bw,sp* genotype to the histone gene array. Pan does not show convincing localization to the histone gene array when compared to input.

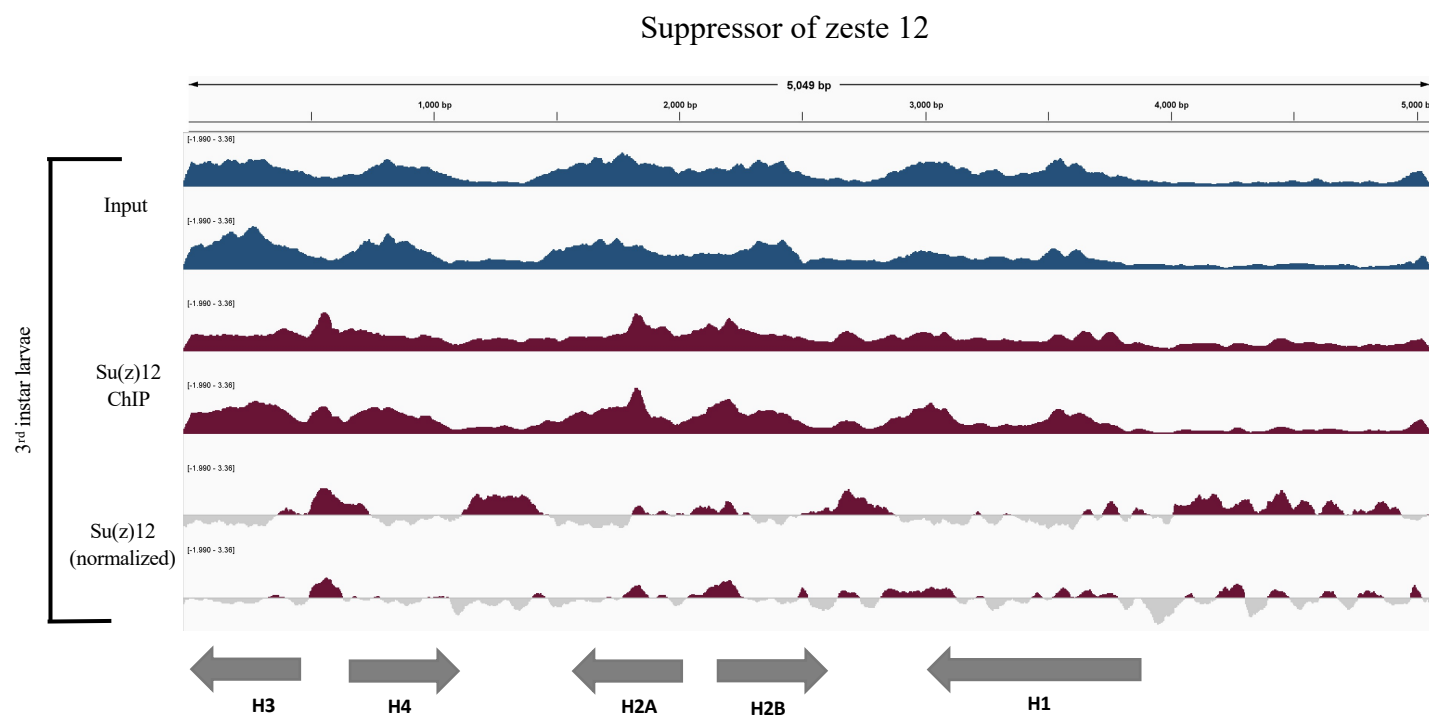
Posterior Sex Combs



We mapped Posterior sex combs (Psc) ChIP-seq (maroon) and input (navy) data (Follmer *et al.*, 2012) from mitotic (top) and control (bottom) S2 cells to the histone gene array. While a sharp peak is apparent in the *H3/H4* promoter, this corresponds to a perfect GA-repeat and is due to the short read length (40 bp) of the datasets. Psc does not show convincing localization to the histone gene array when compared to the corresponding input.



We mapped Scm ChIP-seq (pink) and input (navy) data (Kang H *et al.* 2015) from 12-24 hr mixed population embryos to the histone gene array. Scm does not show convincing localization to the histone gene array when compared to input.



We mapped Suppressor of zeste 12 (Su(z)12) ChIP-seq (maroon) and input (navy) data (Herz *et al.*, 2012) from 3rd instar larvae to the histone gene array. Su(z)12 does not show convincing localization to the histone gene array when compared to input.

Supplemental Table 1

Candidate	Category	Rationale	Tissue/Timing	Localization	Region
Abd-A Abdominal A	Early development transcription factor	Continuous expression post 0-2hour developmental stage, with highest RNA expression levels between 2-4hrs (73,79)	Kc cells	Yes	<i>H3/H4</i> promoter
Abd-B abdominal B	Early development transcription factor	Continuous expression post 0-2hour developmental stage, with highest RNA expression levels between 2-4hrs (73,79)	Kc cells	Yes	<i>H3/H4</i> promoter
Antp Antennapedia	Early development transcription factor	Expressed in the early embryo (73,79)	Imaginal wing disk		
CTCF CCCTC-binding factor	Chromatin structure/remodeler	Key chromatin architecture protein, serves as an insulator and allows for distant DNA interacts (42)	Mixed adults		
CP190 Centrosomal protein 190kD	Chromatin structure/remodeler	Insulates centromeric heterochromatin (40)	Kc/S2 cells		
Exd extradenticle	Early development transcription factor	Expressed in the early embryo (73,79)	5-6 days wing		
Fs(1)h female sterile (1) homeotic	Chromatin structure/remodeler	short isoform binds at promoters and enhancers and long isoform binds at chromatin insulators (60)	Kc cells	Yes (long isoform only)	<i>H3/H4</i> promoter <i>H2A/H2B</i> promoter
Gen5	Chromatin structure/remodeler	Acetyltransferase, critical for oogenesis and morphogenesis (41) and associates with insulators (40)	Kc cells		
Hnf4 Hepatocyte nuclear factor 4	Early development transcription factor	Important for major events in embryogenesis, works in cell metabolism pathways (53)	Mixed adults		
Hr78 Hormone-receptor-like in 78	Early development transcription factor	Continuous expression throughout embryogenesis and during metamorphosis (73,79)	8-16h embryos	Yes	<i>H3/H4</i> promoter
Hth homothorax	Early development transcription factor	Expressed in the early embryo (73,79)	Imaginal wing disc		
JIL-1	Dosage compensation/ X-chromosome associated factor	Kinase phosphorylation of H3S10 Enriched on X chromosome for dosage compensation Serine 10 mark prevents heterochromatin spreading (57,58,67)	3 rd instar larvae	Yes	<i>H2A/H2B</i> promoter
M1BP Motif 1 Binding Protein	Chromatin structure/remodeler	Interacts with TRF2 and related to insulator activity by associating with CP190 (52)	Kc/S2 cells		
MSL1 Male specific Lethal 1	Dosage compensation factor	Associates with the known HLB factor CLAMP (46,59)	S2 cells		
Ndf (CG4747) Nucleosome-destabilizing factor	Dosage compensation associated factor	H3K36me3-binding protein that is important for MSLc localization (29)	larval		
Nej Nejire	Hit from previous screen for HLB factors (11)	Acetyltransferase and early developmental transcription factor	2-4 hr embryos S2 cells	Yes (embryos only)	<i>H3/H4</i> promoter <i>H2A/H2B</i> promoter
Opa Odd Paired	Early development transcription factor	Continuous expression post 0-2hour developmental stage, with highest RNA expression levels between 2-4hr when <i>ZGA/</i> histone gene expression is high (73,79)	3h embryo 4h embryo		
Pan Pangolin	Early development transcription factor	Expressed in the early embryo/early in development (73,79)	0-8h embryo		
Pnt Pointed	Hit from previous screen for HLB factors (11)	Early development transcription factor	Stage 11 embryo		
Psc Posterior sex combs	Chromatin structure/remodeler	Polycomb member (43)	S2 cells		

Scm Sex comb on midleg	HLB-associated factor	Genetically interacts with known HLB factor Mxc (34,35)	Embryo 12-24h S2 cells		
su(z)12 suppressor of zeste 12	Chromatin structure remodeler	Polycomb repressive complex member (44) Highest RNA expression levels during 0-2h and 4-8h development stages (73,79)	3rd instar larvae		
TAF1 TBP-associated factor 1	General transcription factor	TATA-box-binding protein known to associate with TBP (30)	S2 cells	Yes	<i>H3/H4</i> promoter
TFIIB Transcription Factor II B	General transcription factor	TATA-box binding protein complex member, known to associate with TBP (31)	OregonR Embryos	Yes	<i>H3/H4</i> promoter <i>H2A/H2B</i> promoter
TFIIF Transcription Factor II F	General transcription factor	TATA-box binding protein complex member, known to associate with TBP (31)	OregonR Embryos	Yes	<i>H3/H4</i> promoter <i>H2A/H2B</i> promoter <i>H1</i> promoter
TRF2 TATA box binding protein-related factor 2	General transcription factor	TATA-less promoter binding activity at <i>H1</i> promoter (8)	S2 cells	Yes	<i>H1</i> promoter
Ubx Ultrabithorax	Early development transcription factor	Continuous expression post 0-2hour developmental stage, with highest RNA expression levels between 2-4hrs (73,79)	Kc cells, imaginal wing disc embryos	Yes (all)	<i>H3/H4</i> promoter

Supplementary Table 1: All candidates categories, function, and tissue details.

Supplemental References:
 79. Gramates LS, Agapite J, Attrill H, Calvi BR, Crosby MA, dos Santos G, et al. FlyBase: a guided tour of highlighted features. Genetics. 2022 Apr 1;220(4):iyac035.