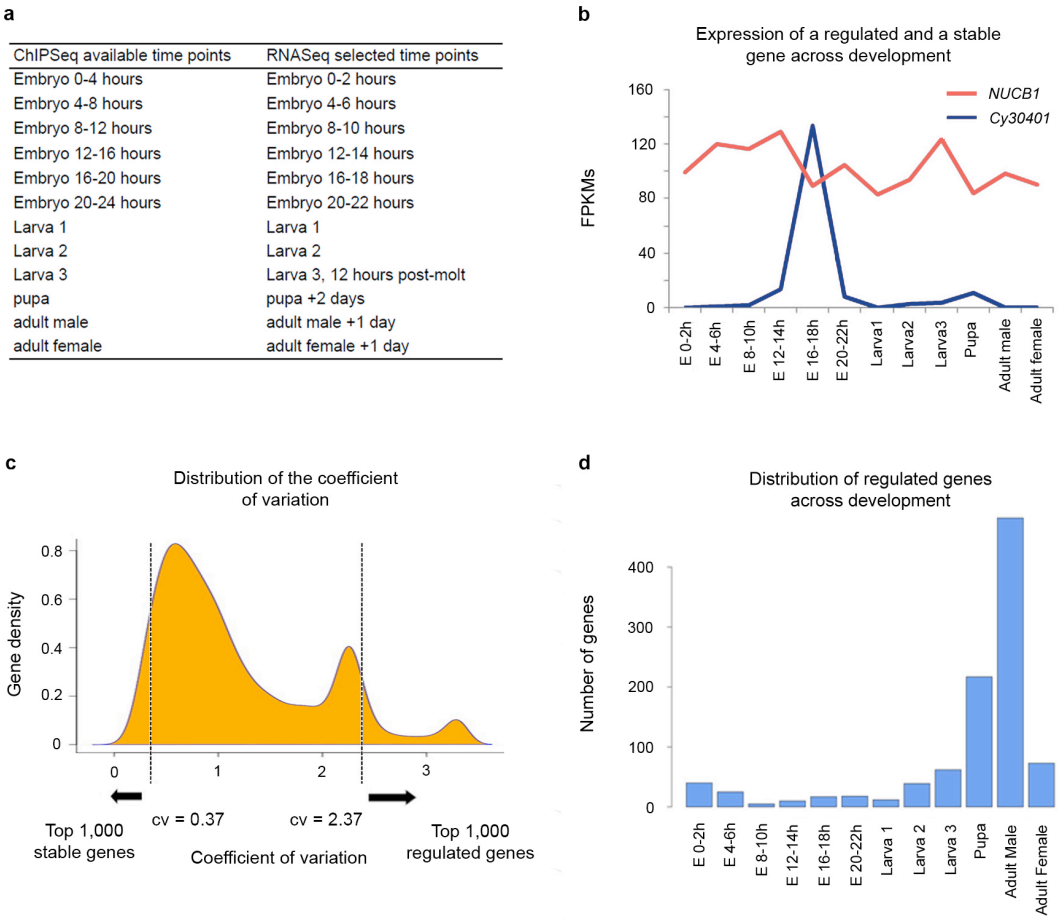
a

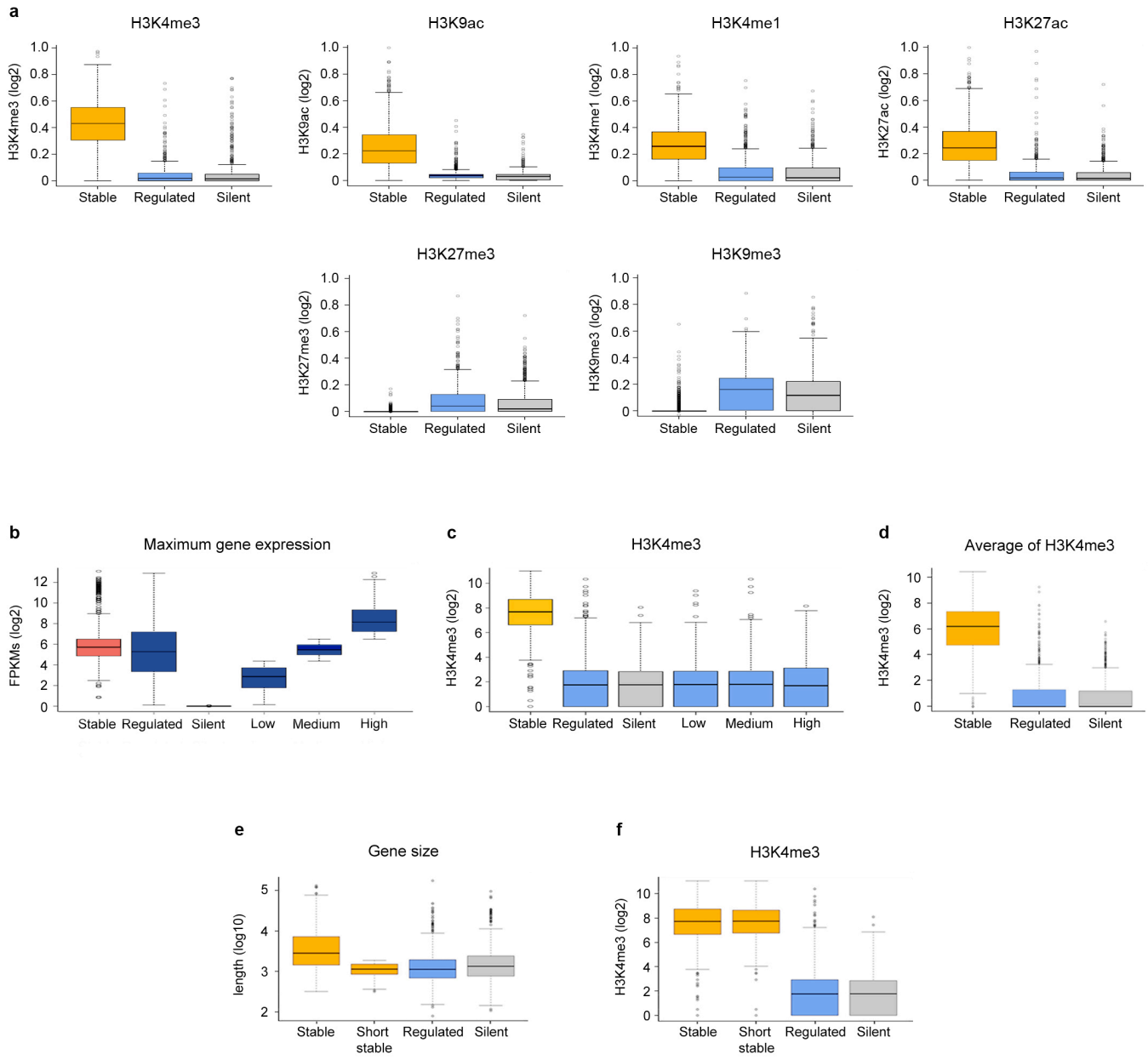
ChIPSeq available time points	RNASeq selected time points
Embryo 0-4 hours	Embryo 0-2 hours
Embryo 4-8 hours	Embryo 4-6 hours
Embryo 8-12 hours	Embryo 8-10 hours
Embryo 12-16 hours	Embryo 12-14 hours
Embryo 16-20 hours	Embryo 16-18 hours
Embryo 20-24 hours	Embryo 20-22 hours
Larva 1	Larva 1
Larva 2	Larva 2
Larva 3	Larva 3, 12 hours post-molt
pupa	pupa +2 days
adult male	adult male +1 day
adult female	adult female +1 day



Supplementary Figure 1

Developmentally stable and regulated genes in *D. melanogaster*.

a, Time points selected for the analysis of chromatin marking in genes regulated during fly development. From the available modENCODE RNASeq data, we selected the 12 points for which ChIPSeq experiments on histone modifications were also available. **b**, Expression of one stable (*NUCB1)* and one regulated gene *(Cy30401)*. The value of the coefficient of variation for *NUCB1* is 0.15. *Cy30401* in contrast, shows a peak of expression in one embryonic stage and, consistently, its coefficient of variation is 2.49. **c**, Distribution of the coefficient of variation on fly genes. We calculated the coefficient of variation of expression for the 12,867 genes for which modENCODE has expression data along *Drosophila* development. The coefficient of variation distribution uncovers a large class of genes with low coefficient of variation (constant expression during development), and two other minor classes containing genes whose expression is highly variable during development—often restricted to a limited set of stages. For most of the analysis we arbitrarily considered the top 1,000 genes with the lowest coefficient of variation as stable, and, the top 1,000 genes with the highest coefficient of variation as genes regulated throughout development (Supplementary Fig. 4). **d**, Time point of maximum expression of regulated genes.

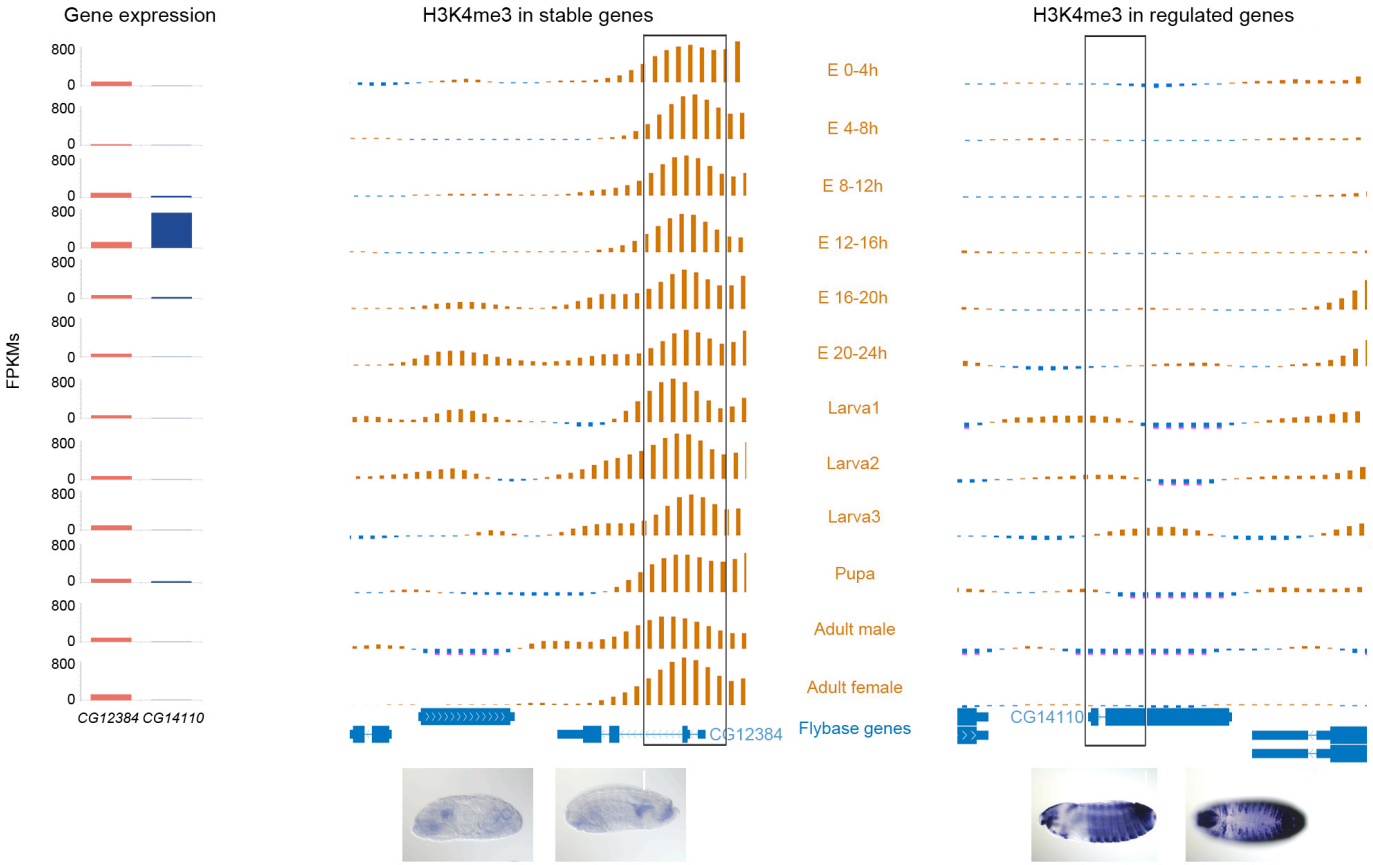


Supplementary Figure 2

Chromatin marking at stable, regulated and silent genes during fly development.

We performed a number of controls to rule out that our observations arise from undetected confounding factors **a,** Normalized levels of H3K4me3, H3K9ac, H3K4me1, H3K27ac, H3K27me3 and H3K9me3 at the time point of maximum expression during development. Because of the differences in heights between modENCODE ChIPSeq tracks, we identified the highest peak of each mark in the genome by checking all expressed genes and used this value to normalize the corresponding profiles. The distributions correspond to the maximum height of the ChIPSeq peak within the gene body for H3K4me3, H3K9ac, H3K4me1 and H3K27ac, and the average height of the ChIPSeq signal over the gene body for H3K27me3 and H3K9me3. Patterns are the same, or even stronger, than those in Figure 1b. The bottom and top of the boxes are the first and third quartiles, and the line within, the median. The whiskers denote the interval within 1.5 times the IQR (Inter Quartile Range) from the median. Outliers are plotted as dots. **b,** Distribution of expression of top 1,000 stable, regulated, and silent genes, and of the set of top 1,000 regulated genes divided into three groups according to expression (low, medium, high) at the time point of maximum expression for each gene. Gene expression was computed as FPKMs by the modENCODE consortium. **c,** Levels of H3K4me3 at the time point of maximum expression for the gene sets defined in b. Values represent the maximum height of the ChIPSeq peak within the gene body. The three sets of regulated genes show similar levels of

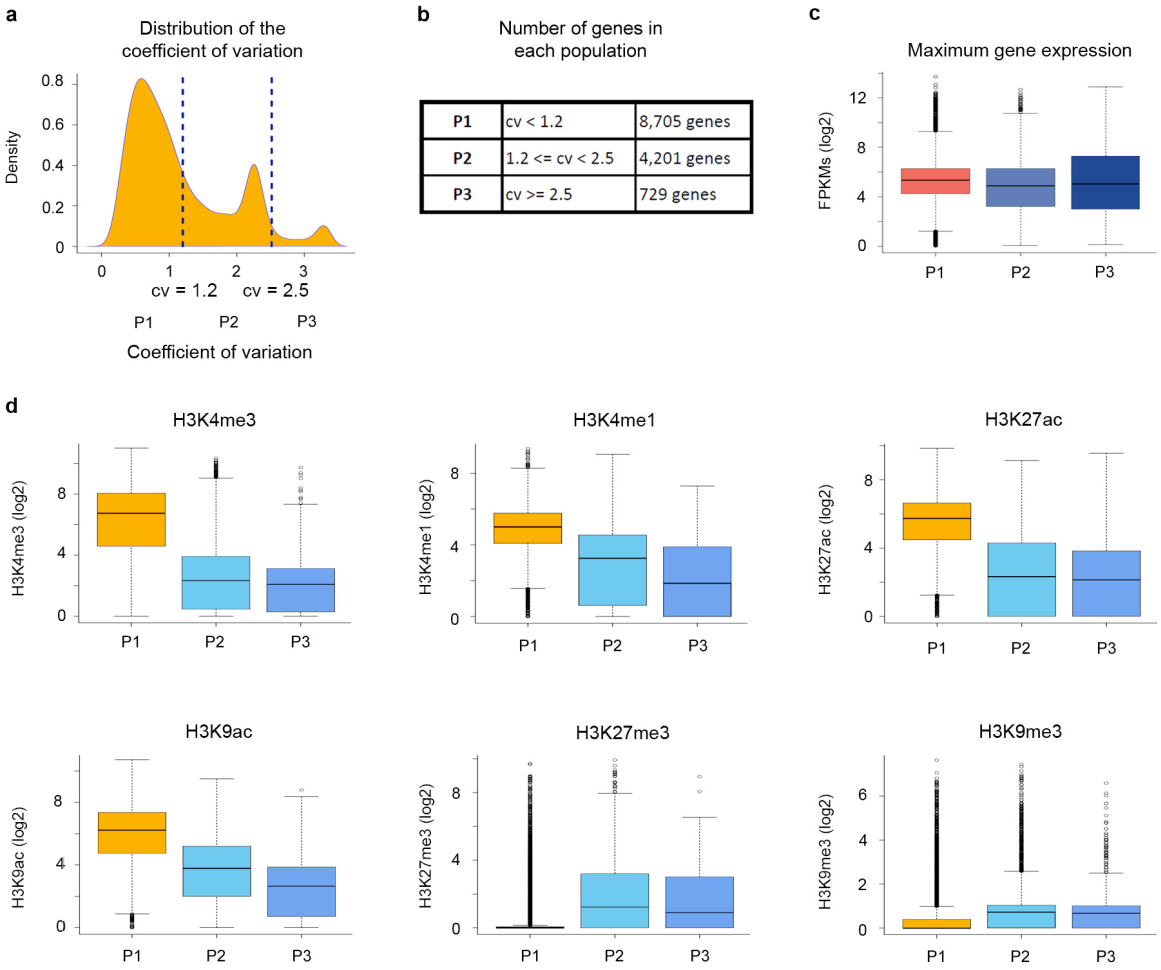
H3K4me3, comparable to silent genes. **d,** Levels of H3K4me3 at the time point of maximum gene expression computed as the average signal over the gene body, instead of as the maximum peak. The pattern is the same than that in Figure 1b. **e**, Length of stable and regulated genes. Regulated genes have a lower number of exons than stable genes (2.6 vs 5.7 on average) and shorter introns (600 bp vs 1,000 bp) and, as a consequence, regulated genes are shorter than stable genes (1,136 bp vs 2,864 bp). To rule out that gene size is a confounding factor, we selected the 520 shortest stable genes. These have an average length (1,188 bp) and number of exons (2.6) very similar to that of variable genes. **f**, H3K4me3 maximum peak at short stable genes is comparable to the peak at the previous set of stable genes, and it is much higher than the H3K4me3 peak at regulated genes. Therefore, there is no effect of gene length and number of exons in our observations.



Supplementary Figure 3

Profiles of H3K4me3 along fly development for *CG12384*, a stable gene, and *CG14110*, a mid-late embryo-specific gene.

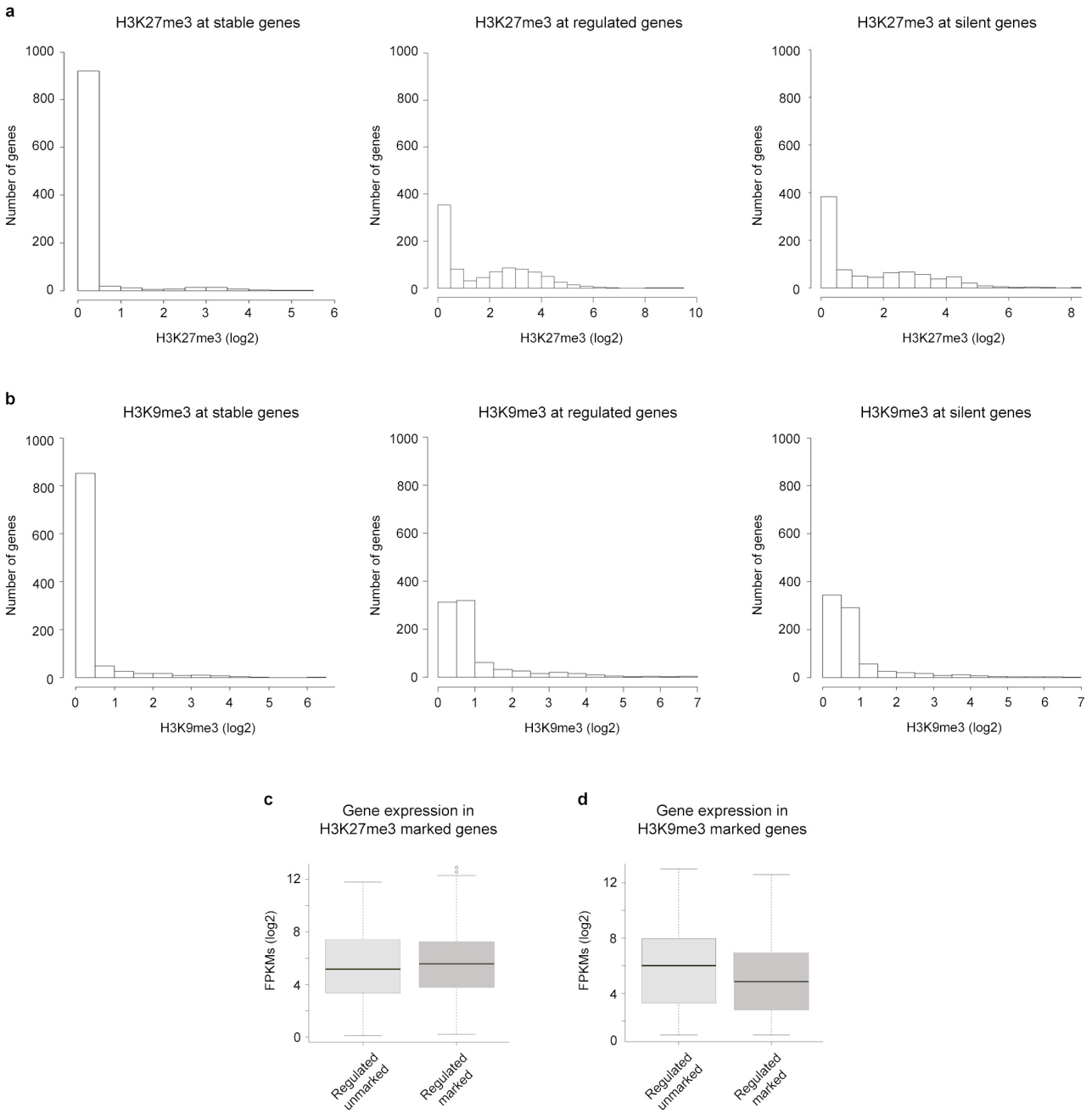
The expression (measured as FPKMs) along these points is given on the left. *CG12384* is expressed throughout development and *CG14110* is highly expressed in E12–16h. *In situ* hybridization images obtained from BDGP[1] correspond to stages 13–16 (9–16h after egg laying) for both genes.



Supplementary Figure 4

Partition of the entire set in stable and regulated genes.

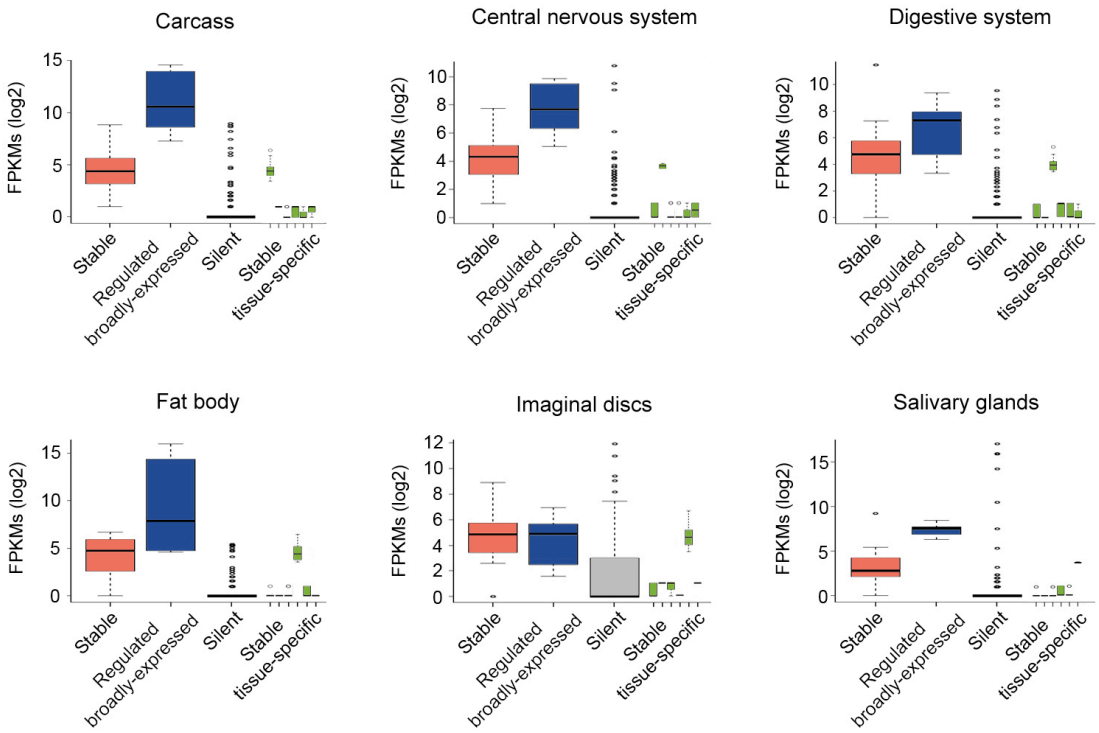
a, Distribution of the coefficient of variation on fly genes. The distribution of the coefficient of variation of gene expression along fly development reveals one major class of stable genes (P1), and two minor classes of regulated genes (P2 and P3). **b,** Number of genes belonging to each class. **c,** Distribution of gene expression levels at the developmental time point of maximum gene expression in each class. Gene expression is measured as FPKM by the modENCODE consortium. The bottom and top of the boxes are the first and third quartiles, and the line within, the median. The whiskers denote the interval within 1.5 times the IQR from the median. Outliers are plotted as dots. **d,** Normalized levels of histone modifications at the time point of maximum gene expression in each gene class.



Supplementary Figure 5

H3K27me3 and H3K9me3 marking in stable, regulated and silent genes.

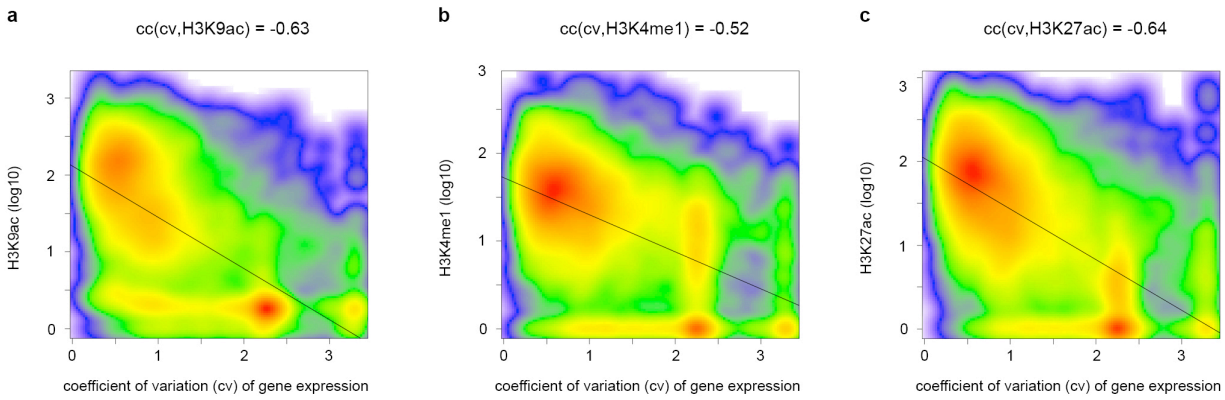
a, Left panel: H3K27me3 in stable genes. As expected, most genes do not show H3K27me3. Middle panel: H3K27me3 in regulated genes. Many genes show either none or very low levels of H3K27me3. Right panel: H3K27me3 in silent genes. The distribution of H3K27me3 marking is very similar to that observed in regulated genes. **b,** Left panel: H3K9me3 in stable genes. Most of genes do not show H3K9me3. Middle panel: H3K9me3 in regulated genes. Many genes show either none or very low levels of H3K9me3. Right panel: H3K9me3 in silent genes. The distribution of H3K9me3 marking is very similar to that observed in regulated genes. **c,** Expression level of regulated genes unmarked and marked with H3K27me3. Marked genes (H3K27me3 (log2) > 0) are expressed at similar levels than unmarked genes. The bottom and top of the boxes are the first and third quartiles, and the line within, the median. The whiskers denote the interval within 1.5 times the IQR from the median. Outliers are plotted as dots. **d,** Expression level of regulated genes unmarked and marked with H3K9me3. Marked genes (H3K9me3 (log2) > 0) are slightly lower expressed than unmarked genes (p-value = 0.001).



Supplementary Figure 6

Expression of stable genes, regulated genes broadly-expressed at L3, silent genes, and stably expressed tissue-specific genes in six different tissues at L3.

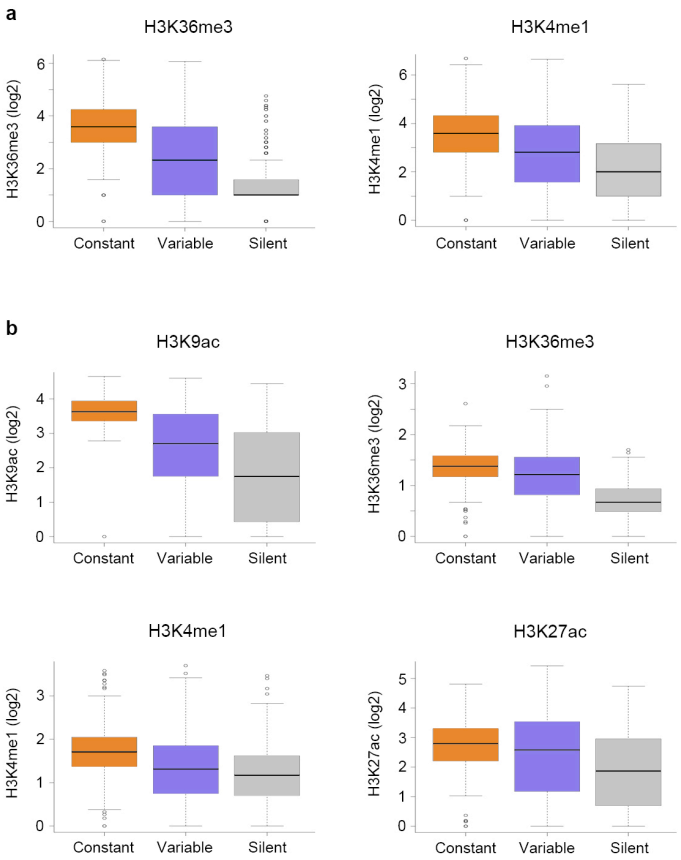
Expression levels, measured as FPKM by the modENCODE consortium, of six different tissues. The expression of stable tissue-specific genes is given for each tissue separately in the following order: carcass, central nervous system, digestive system, fat body, imaginal discs, and salivary glands. Regulated broadly-expressed genes show higher expression than stable tissue-specific genes even in the tissue in which the later are expressed, except in imaginal discs. The bottom and top of the boxes are the first and third quartiles, and the line within, the median. The whiskers denote the interval within 1.5 times the IQR from the median. Outliers are plotted as dots.



Supplementary Figure 7

Correlation between histone modifications and transcription stability in flies.

Scatterplots of H3K9ac (**a**), H3K4me1 (**b**) and H3K27ac (**c**) at the time point of highest expression during fly development and transcriptional stability, measured as the coefficient of variation of gene expression across the 12 developmental time points. The correlation is computed as the partial correlation given gene expression (see Figure 3b).



Supplementary Figure 8

Chromatin marking at genes with constant and variable expression in multiple tissues in mammals.

a, Normalized levels of H3K36me3 and H3K4me1 at the tissue of maximum expression from the 56 human consolidated epigenomes. The bottom and top of the boxes are the first and third quartiles, and the line within, the median. The whiskers denote the interval within 1.5 times the IQR from the median. Outliers are plotted as dots. **b,** Normalized levels of H3K9ac, H3K36me3, H3K4me1 and H3K27ac at the tissue of maximum expression from the ten mouse tissues.

a

	WID cells		EID cells	
Total reads	133,777,031		111,712,574	
Genomic reads	108,639,465	(81.2%)	93,563,103	(83.8%)
Genomic reads. Unique	94,777,137	(70.8%)	86,740,797	(77.6%)
Intronic reads	1,014,566	(0.8%)	1,000,783	(0.9%)
Exonic reads	90,835,551	(67.9%)	83,346,225	(74.6%)
Intergenic read	2,927,020	(2.2%)	2,392,789	(2.14%)
Junction reads	15,057,579	(13.5%)	13,155,357	(11.8%)
Exons (RPKM>0)	50,610	(76.3%)	54,923	(82.8%)
Transcripts (RPKM >0)	17,657	(83.1%)	17,803	(83.8%)
Genes (RPKM >0)	11,507	(81.9%)	12,009	(85.4%)

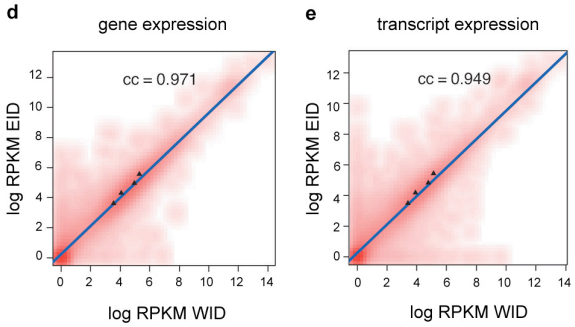
b

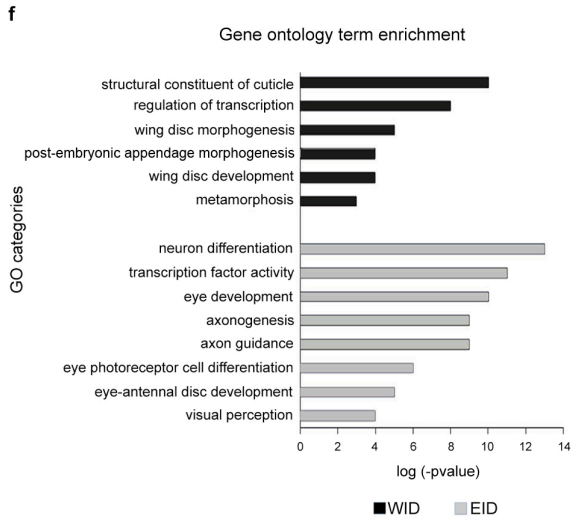
Gene RPKM cutoff	WID	EID	WID & EID
> 0	11,507 (81.9%)	12,009 (85.4%)	11,167 (79.4%)
> 1	7,502 (53.4%)	8,062 (57.3%)	7,373 (52.4%)
> 2	7,098 (50.5%)	7,516 (53.5%)	6,975 (49.6%)
> 4	6,666 (47.4%)	6,945 (49.4%)	6,513 (46.3%)
> 8	5,916 (42.1%)	6,041 (43.0%)	5,667 (40.3%)
> 16	4,425 (31.5%)	4,450 (31.7%)	4,072 (29.0%)
> 32	2,545 (18.1%)	2,465 (17.5%)	2,203 (15.7%)

Transcript RPKM cutoff	WID	EID	WID & EID
> 0	17,657 (83.1%)	17,803 (83.8%)	16,683 (78.6%)
> 1	10,227 (48.1%)	11,124 (52.3%)	9,729 (45.8%)
> 2	9,211 (43.3%)	9,930 (46.8%)	8,754 (41.2%)
> 4	8,179 (38.5%)	8,625 (40.6%)	7,729 (36.4%)
> 8	6,664 (31.4%)	6,987 (32.9%)	6,175 (29.0%)
> 16	4,557(21.4%)	4,665 (21.9%)	4,067 (19.1%)
> 32	2,447 (11.5%)	2,455 (11.6%)	2,093 (10.0%)

c

Tissue	Sample	Unambiguously mapped reads	Read length	Fragment length
WID	H3	9,504,688	36	317
	H3K4me3	4,389,032	36	325
	H3K9ac	34,855,962	50	206
	H3K36me3	9,611,956	36	325
	H3K4me1	26,742,570	36	310
	H3K27ac	20,352,560	36	304
EID	H3	16,183,101	36	326
	H3K4me3	27,809,050	36	325
	H3K9ac	44,523,481	50	180
	H3K36me3	10,580,356	36	345
	H3K4me1	11,218,055	40	305
	H3K27ac	18,467,364	36	302

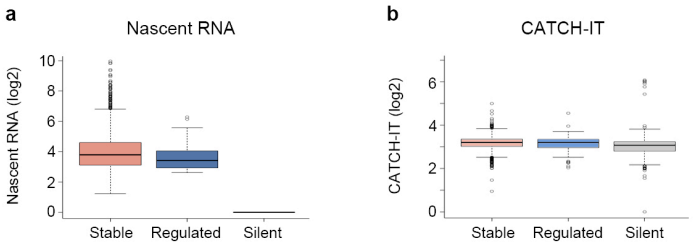




Supplementary Figure 9

RNASeq and ChIPSeq analysis of Wing (WID) and Eye-antenna (EID) imaginal discs.

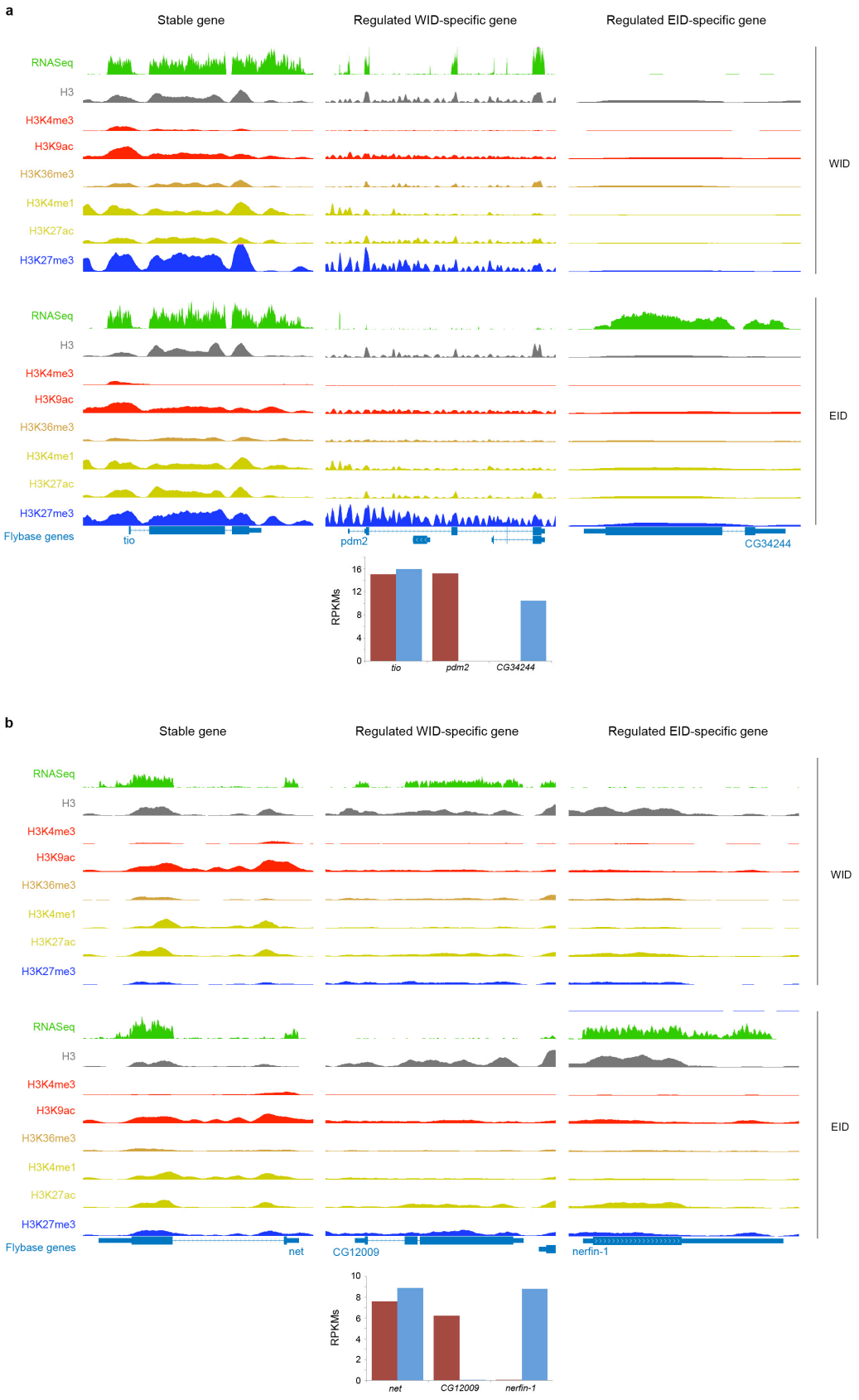
a, RNASeq mapping and quantification statistics. Genomic reads are reads mapping to the genome. Genomic reads mapping uniquely are classified in three classes: intronic reads are reads mapping entirely within a gene, but not entirely within annotated exons; exonic reads are reads mapping entirely within exons; intergenic reads are reads not mapping entirely within genes. Junction reads are reads mapping to splice junctions but not to the genome. **b,** Number of genes and transcripts expressed at different expression cutoffs. **c,** Mapping statistics for the ChIPSeq experiments on histone modifications. The genome-wide Pearson correlation between WID and EID epigenomes is very high: 0.90 for H3, 0.84 for H3K4me3, 0.94 for H3K9ac, 0.96 for H3K36me3, 0.92 for H3K4me1 and 0.92 for H3K27ac when computed on the number of reads mapping onto 1,000 bp long windows. **d, e,** Join distribution in WID and EID of gene and transcript expression. Expression is measured in log RPKM. **f**, Gene Ontology term enrichment of 628 genes preferentially expressed in EID and 184 genes preferentially expressed in WID.



Supplementary Figure 10

Nucleosome turnover rates of stable, regulated and silent genes.

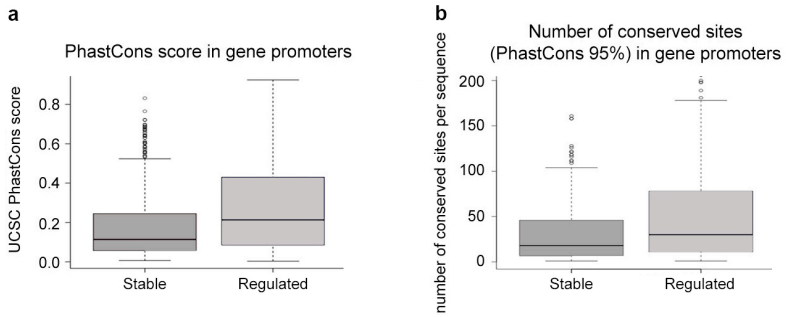
a, Nascent RNA signal computed as the average signal in the gene body for stable, regulated and silent genes in S2 cells[2]. The bottom and top of the boxes are the first and third quartiles, and the line within, the median. The whiskers denote the interval within 1.5 times the IQR from the median. Outliers are plotted as dots. **b,** Nucleosome turnover rate as measured by CATCH-IT[2] average signal in S2 cells for each gene class. Nucleosome turnover rate is not different between regulated and stable genes (p-value = 0.61).


a
Stable gene
Regulated WID-specific gene
Regulated EID-specific gene
RNASeq
H3
H3K4me3
H3K9ac
H3K36me3
H3K4me1
H3K27ac
H3K27me3
Flybase genes
WID
EID
tio
pdm2
CG34244
RPKMs
16
12
8
4
0
tio
pdm2
CG34244
b
Stable gene
Regulated WID-specific gene
Regulated EID-specific gene
RNASeq
H3
H3K4me3
H3K9ac
H3K36me3
H3K4me1
H3K27ac
H3K27me3
Flybase genes
WID
EID
net
CG12009
nerfin-1
RPKMs
10
8
6
4
2
0
net
CG12009
nerfin-1

Supplementary Figure 11

Profiles of RNA expression, H3 and histone modifications in WID and EID-specific genes.

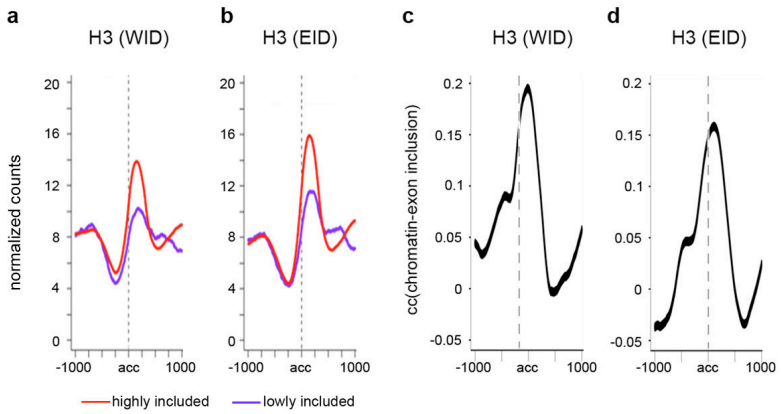
a, Stable gene *tio*, regulated WID-specific gene *pdm2* and regulated EID-specific gene *CG34244* are expressed at the same level (green tracks and bottom panel). Histone modifications typical of gene activation are observed in *tio* whereas the tissue-specific genes lack all of them, even in the tissue in which they are expressed. **b,** Stable gene *net*, regulated WID-specific gene *CG12009* and regulated EID-specific gene *nerfin-1* are expressed at very similar levels (green tracks and bottom panel), but *net* exhibits histone modifications, whereas the tissue-specific genes lack all of them, even in the tissue in which they are expressed.



Supplementary Figure 12

Promoter architecture in stable and developmentally regulated genes.

Conservation of core promoter sequence. **a,** Distribution of PhastCons scores derived from 12 *Drosophila* species in the promoter sequence (defined as 200 bp upstream of the TSS) of stable and regulated genes. Promoters of regulated genes show stronger sequence conservation than those of stable genes: average PhastCons score of 0.27 in regulated genes and of 0.17 in stable genes (p-value < 2.2e–16). The bottom and top of the boxes are the first and third quartiles, and the line within, the median. The whiskers denote the interval within 1.5 times the IQR from the median. Outliers are plotted as dots. **b,** Conservation of transcription factor binding motifs. We identified the predicted binding motifs for Transcription Factors that have a PhastCons score greater than 0.95 in the promoter sequence of stable and variable genes. Boxplots show the distribution of the number of conserved motifs only for promoters that contain at least one prediction (p-value = 2.217e–06). P-values were computed using Wilcoxon test (one-sided).



Supplementary Figure 13

Chromatin structure and splicing.

a-**b**, H3 on highly (red) and lowly (blue) included exons in WID (**a**) and EID (**b**). **c**-**d**, Correlation between exon inclusion and H3 across exon acceptor sites in WID (**c**) and EID (**d**).