Supplemental Material for Jans *et al.*

A condensin-like dosage compensation complex acts at a distance to control expression throughout the genome

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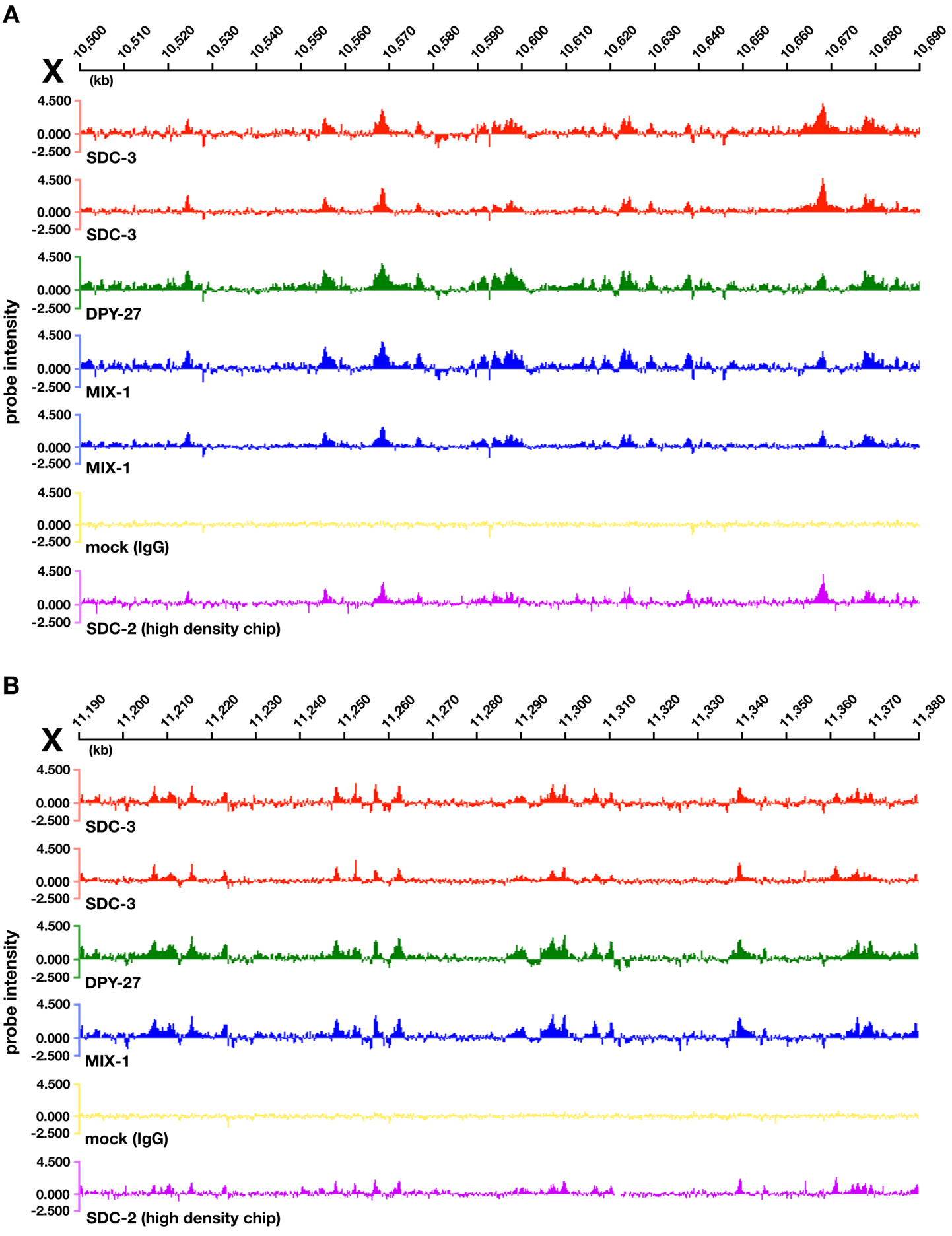
Table of Contents

Supplemental Figure 1



Supplemental Figure 1. ChIP-chip profiles of 4 DCC components. *(A,B)* Representative landscapes of duplicate ChIP-chip profiles for the three dosage compensation components SDC-3, DPY-27, and MIX-1 using the 380,000 feature custom-designed Nimblegen isothermal tiling arrays of predominantly the X chromosome (WS158), and representative landscapes of SDC-2 ChIP-chip profiles using a 2.2 million feature Nimblegen isothermal tiling array of the whole *C. elegans* genome (WS170). The y axis is the probe intensity ratio of fluorescently-labeled DNAs, which were made from chromatin immunoprecipitated by antibodies against different DCC components, to fluorescently-labeled genomic DNA.

Supplemental Figure 2

A

B

C

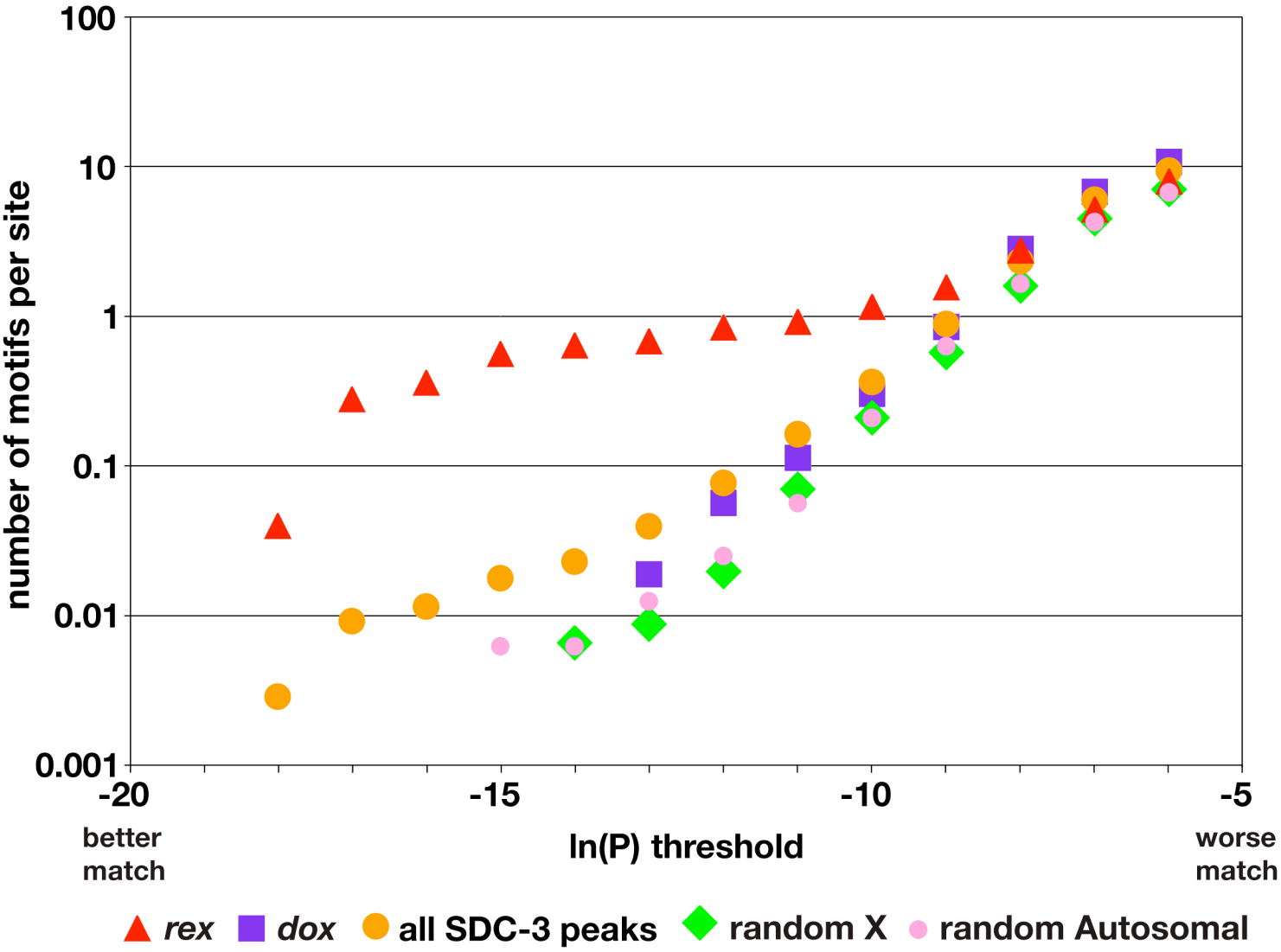
D

Position weight matrix for MEX motif

A	10	2	0	2	0	13	31	5	1	0	21	2
C	0	25	0	29	0	16	0	0	0	0	0	8
G	0	4	31	0	31	2	0	26	30	31	4	21
T	21	0	0	0	0	0	0	0	0	0	6	0

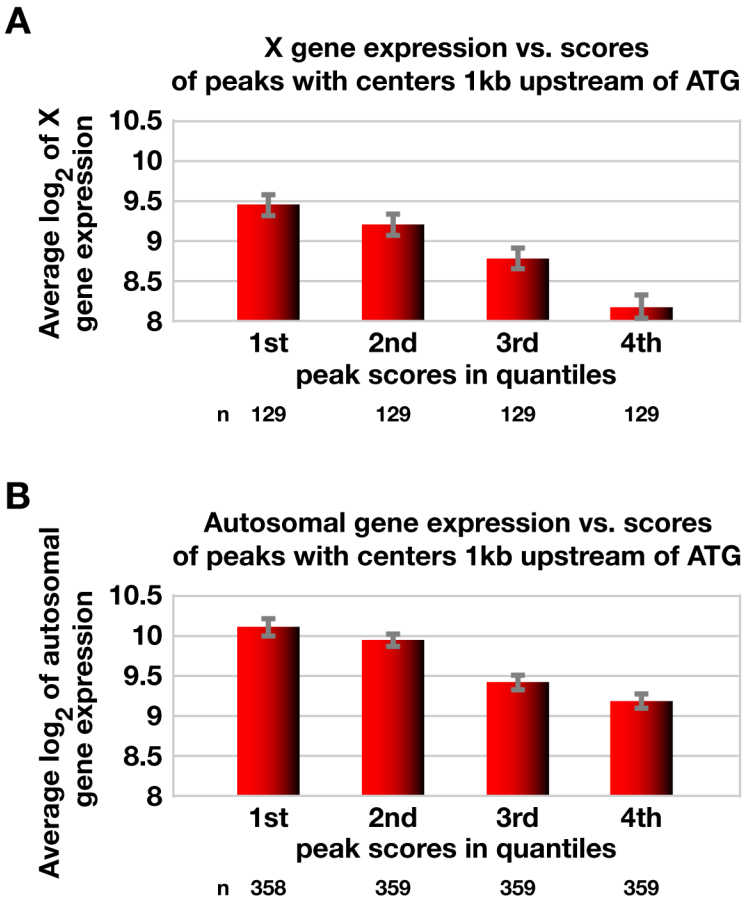
Supplemental Figure 2. Consensus motifs on X.
(A-C) Models describing consensus motifs derived from 3 sources: *rex* sites derived from *(A)* cosmids, *(B)* DCC ChIP-chip peaks shown to recruit in array assays, *(C)* all recruitment sites (*rex* sites from cosmids and DCC peaks). The program wconsensus (Hertz and Stormo 1999) was used for motif finding, except in the case of the motif from the recruiting DCC peaks. BioProspector (Liu et al. 2001) was used to find that motif. *(D)* Position weight matrix for MEX motif.

Supplemental Figure 3



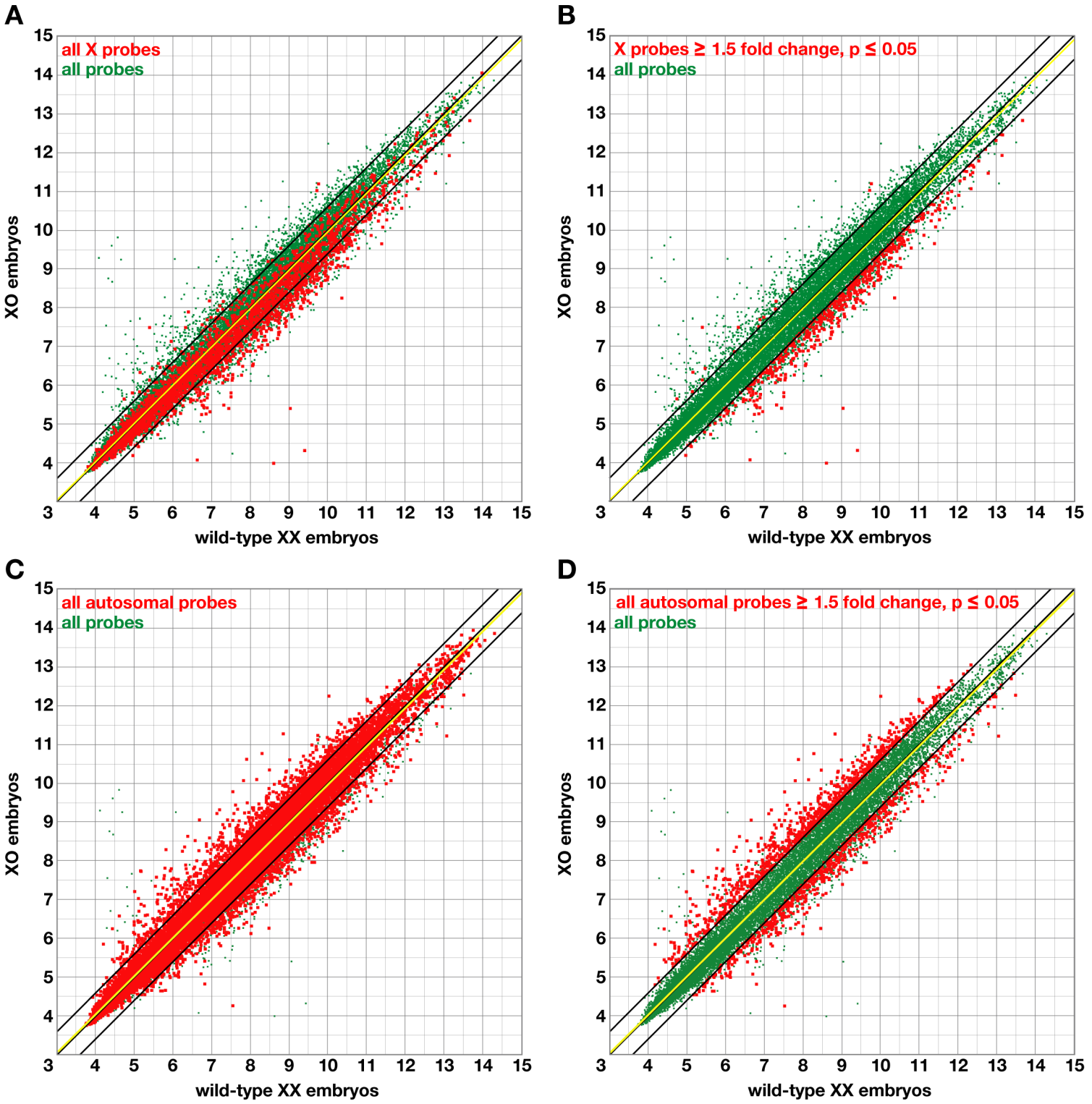
Supplemental Figure 3. The number of MEX motifs discriminates rex sites from dox sites, all X peaks, and random X or autosomal sequences.
ln(P) is the natural log of the probability of finding a 12 mer that matches the MEX matrix as calculated by the Patser program. The plot reflects cumulative scores. For example, number of motifs per site listed at -10 is the number of all 12 mers with ln(P) $\leq$ -10 per site.

Supplemental Figure 4



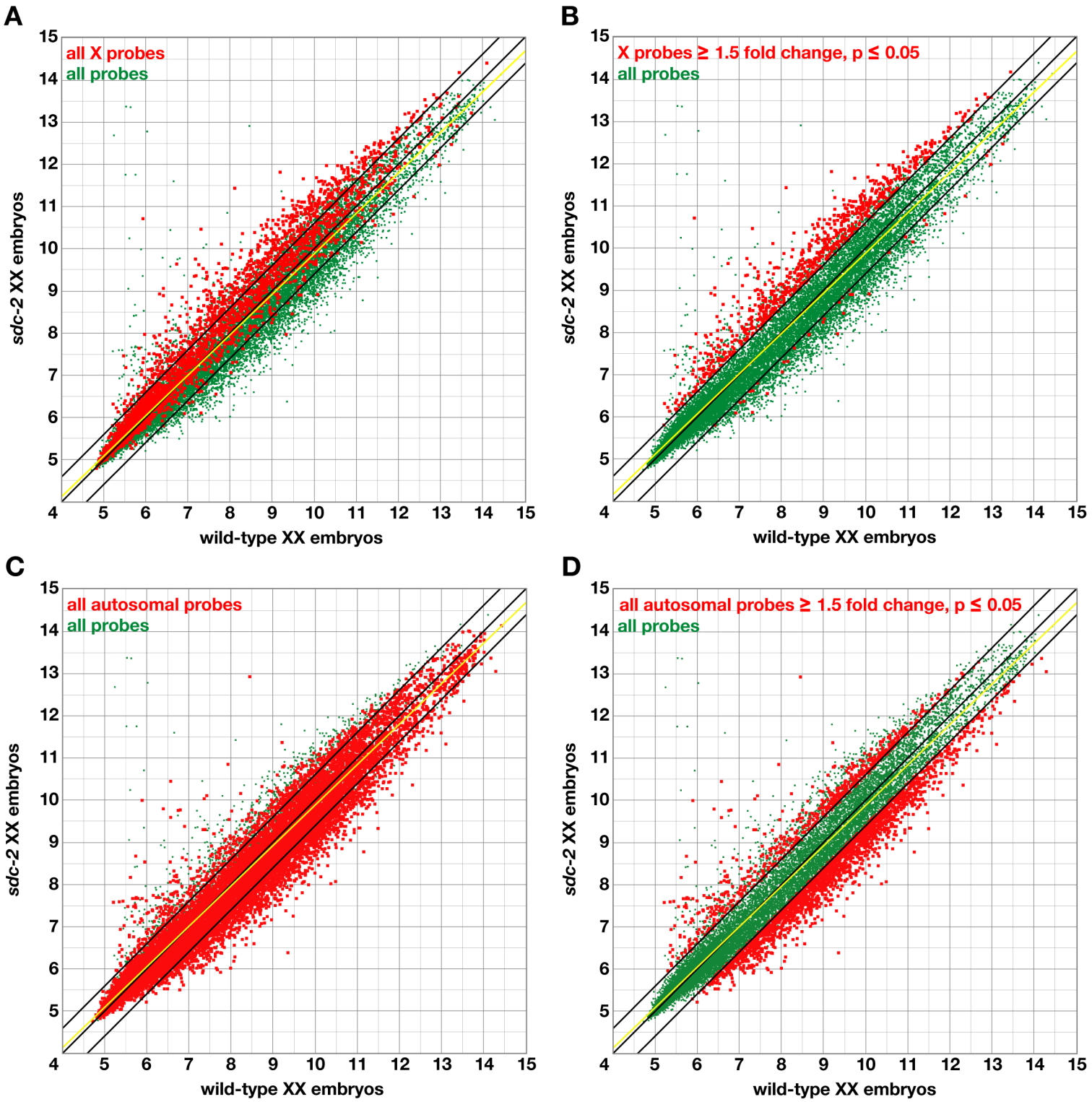
Supplemental Figure 4. Higher DCC peak scores correlate with higher levels of gene expression.
(A,B) Histograms showing average $\log_2$ of X or autosomal gene expression (probe set intensities) versus SDC-3 ChIP-chip peak scores. For this analysis, only peaks with centers within 1 kb upstream of an ATG translation start codon were compared against the expression level of the gene. The peaks were divided into quartiles based on peak scores. The first quartile has the largest peaks. n, number of peaks in each quartile. Error bar, standard error of the mean (SEM).

Supplemental Figure 5



Supplemental Figure 5. Scatter plots comparing X or autosomal gene expression in XO versus XX embryos.
x and y axes are expressed as $\log_2$ of probe set intensity. The yellow line is the actual normalization line. The center black line is the theoretical normalization line, and the upper and lower black lines are 1.5-fold higher or lower, respectively, than the theoretical normalization line. *(A)* Many X probe sets (red) are not significantly different in intensity between XO and XX embryos; the probes sets lie between the upper and lower black lines. *(B)* A significant number of X probe sets are 1.5-fold and $p \leq 0.05$ reduced in XO versus XX embryos, consistent with genes that escape dosage compensation. *(C)* Many autosomal probe sets (red) are not significantly different in intensity between XO and XX embryos; the probe sets lie between the upper and lower black lines. *(D)* A significant number of autosomal probe sets are either over expressed or under expressed by 1.5-fold and $p \leq 0.05$ in XO versus XX embryos. The genotype of XO embryos is *her-1(hv1y101* null*); xol-1(y9* null*) sdc-2(y74* null*) unc-9(e101).* In this strain, the *xol-1* mutation would kill XO animals because the DCC would be activated and assemble onto X. However, the *sdc-2* mutation suppresses the XO lethality by preventing assembly of the DCC onto the single X. The *her-1* mutation transforms the XO embryos into phenotypic hermaphrodite embryos, to avert any complications from sex-specific differences in gene expression. This scheme was designed to maintain a stable population of XO animals that could be grown to high density. These XO hermaphrodites rarely produce XX embryos, and the rare XX embryos are killed by the *sdc-2* mutation.

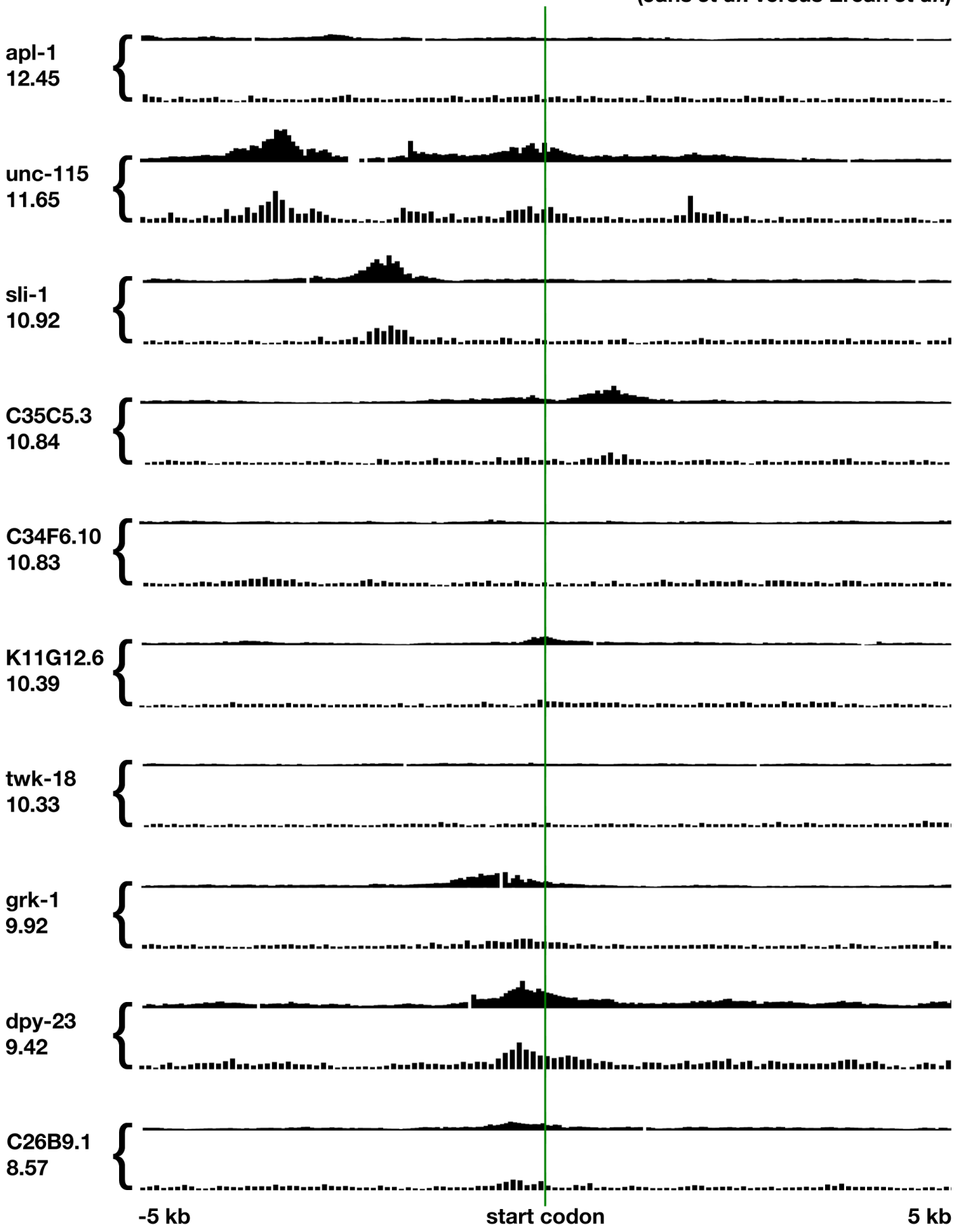
Supplemental Figure 6



Supplemental Figure 6. Scatter plots comparing X or autosomal gene expression in *sdc-2* XX mutant versus wild-type XX embryos.
x and y axes are expressed as $\log_2$ of probe set intensity. The yellow line is the actual normalization line. The center black line is the theoretical normalization line, and the upper and lower black lines are 1.5-fold higher or lower, respectively, than the theoretical normalization line. *(A,C)* Many X and autosomal probe sets are not significantly different in intensity between *sdc-2* mutant versus wild-type XX embryos; these probe sets lie between the upper and lower black lines. *(B)* A significant number of X probe sets are 1.5 fold and $p \leq 0.05$ over expressed in *sdc-2* mutant versus XX embryos, consistent with genes that are dosage compensated. *(D)* A significant number of autosomal probe sets are 1.5 fold and $p \leq 0.05$ under expressed in *sdc-2* mutant versus wild-type XO embryos, consistent with the DCC controlling expression of autosomal genes as well as X genes.

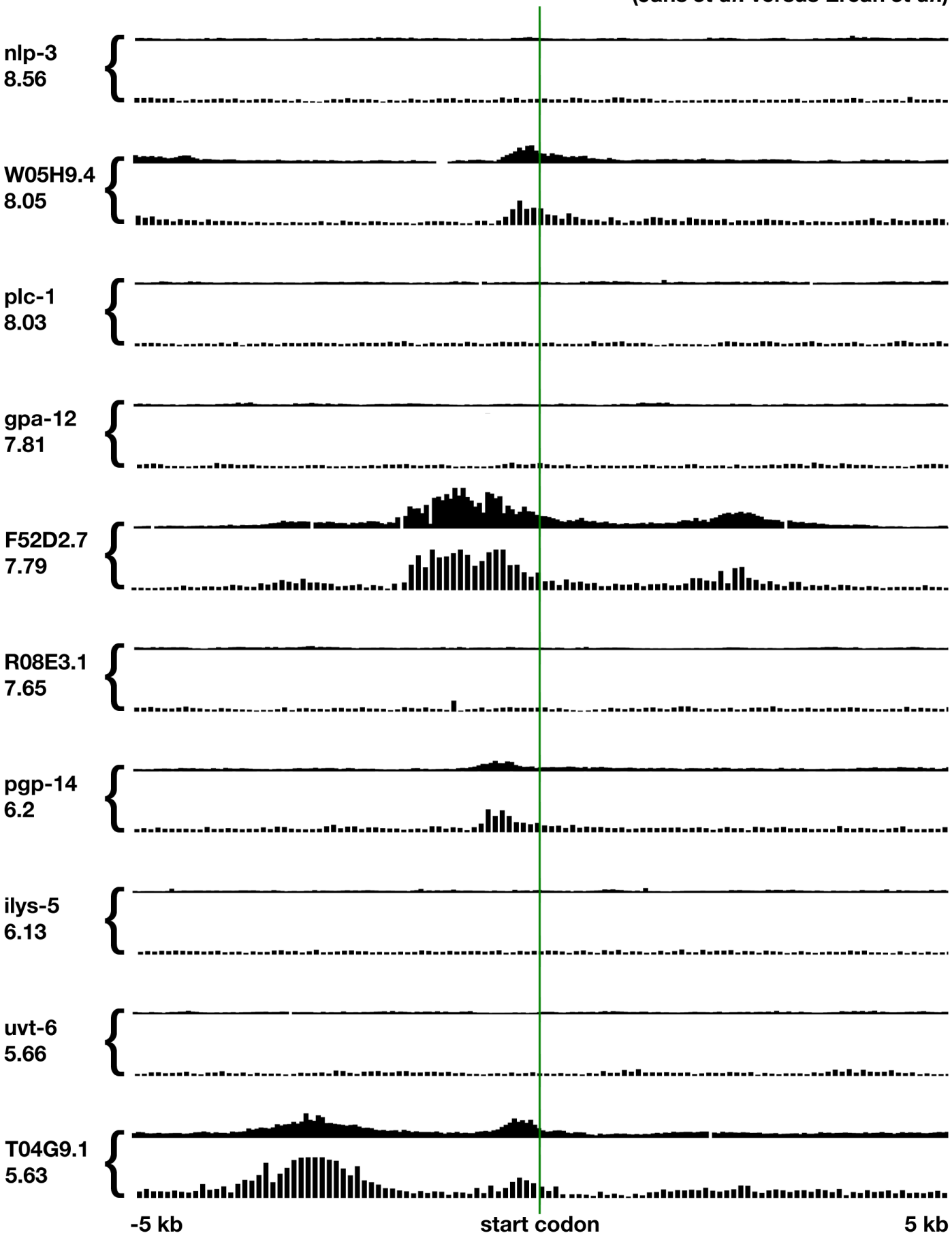
Supplemental Figure 7

SDC-3 Peaks in Dosage-Compensated Genes (Jans *et al*. versus Ercan *et al*.)



Supplemental Figure 7

SDC-3 Peaks in Dosage-Compensated Genes (Jans *et al*. versus Ercan *et al*.)

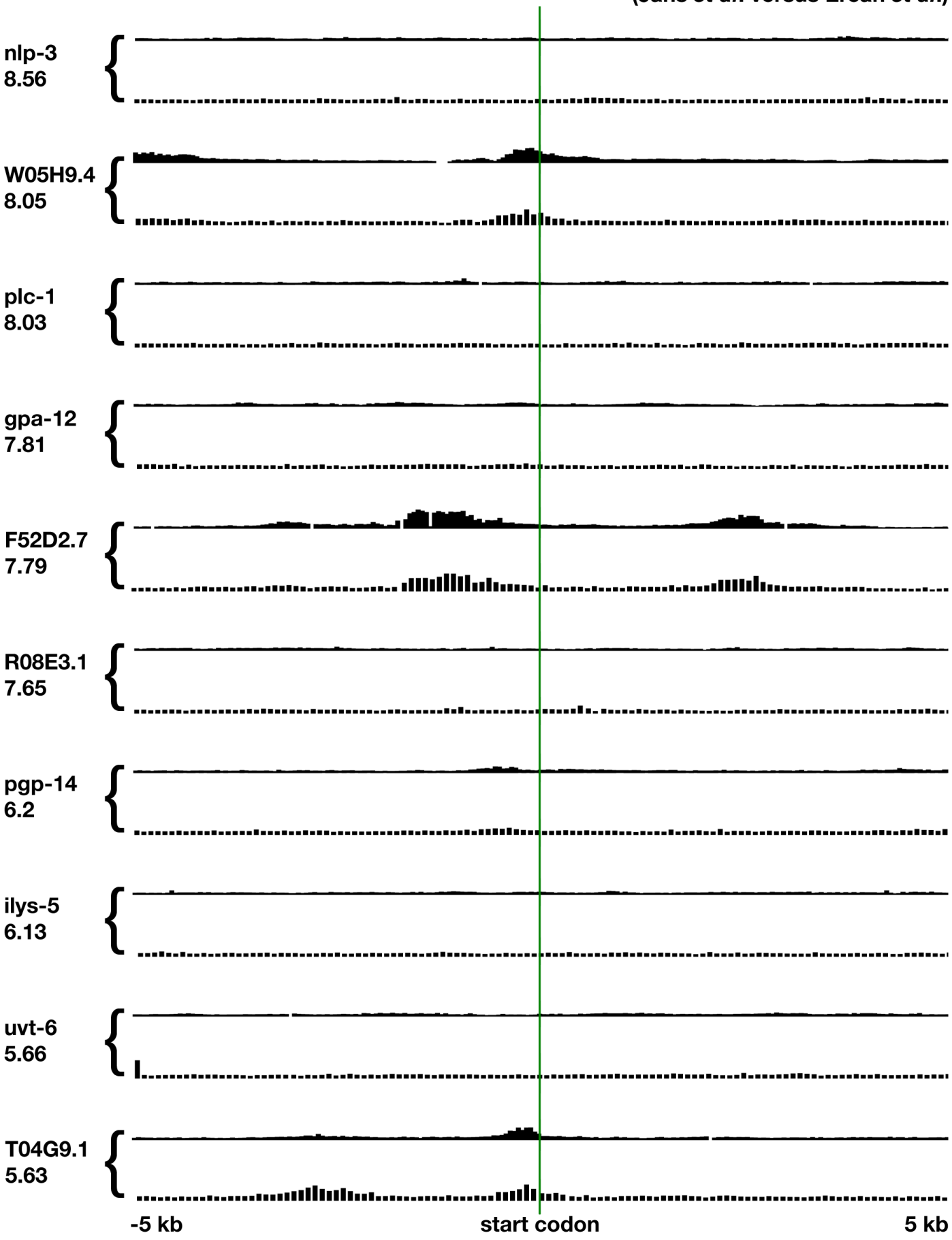


Supplemental Figure 7. Not all dosage-compensated genes have bound SDC-3 in their promoters or coding regions.

Shown is a graphical representation of probe intensity from SDC-3 ChIP-chips along 20 representative dosage compensated genes. Peak landscape is shown from 5 kb upstream to 5 kb downstream of the translation start codon. Shown to the left are gene names and the $\log_2$ value for the gene expression level in wild-type XX embryos. For each gene, our SDC-3 landscape (top) is compared to that of Ercan *et al.* (bottom).

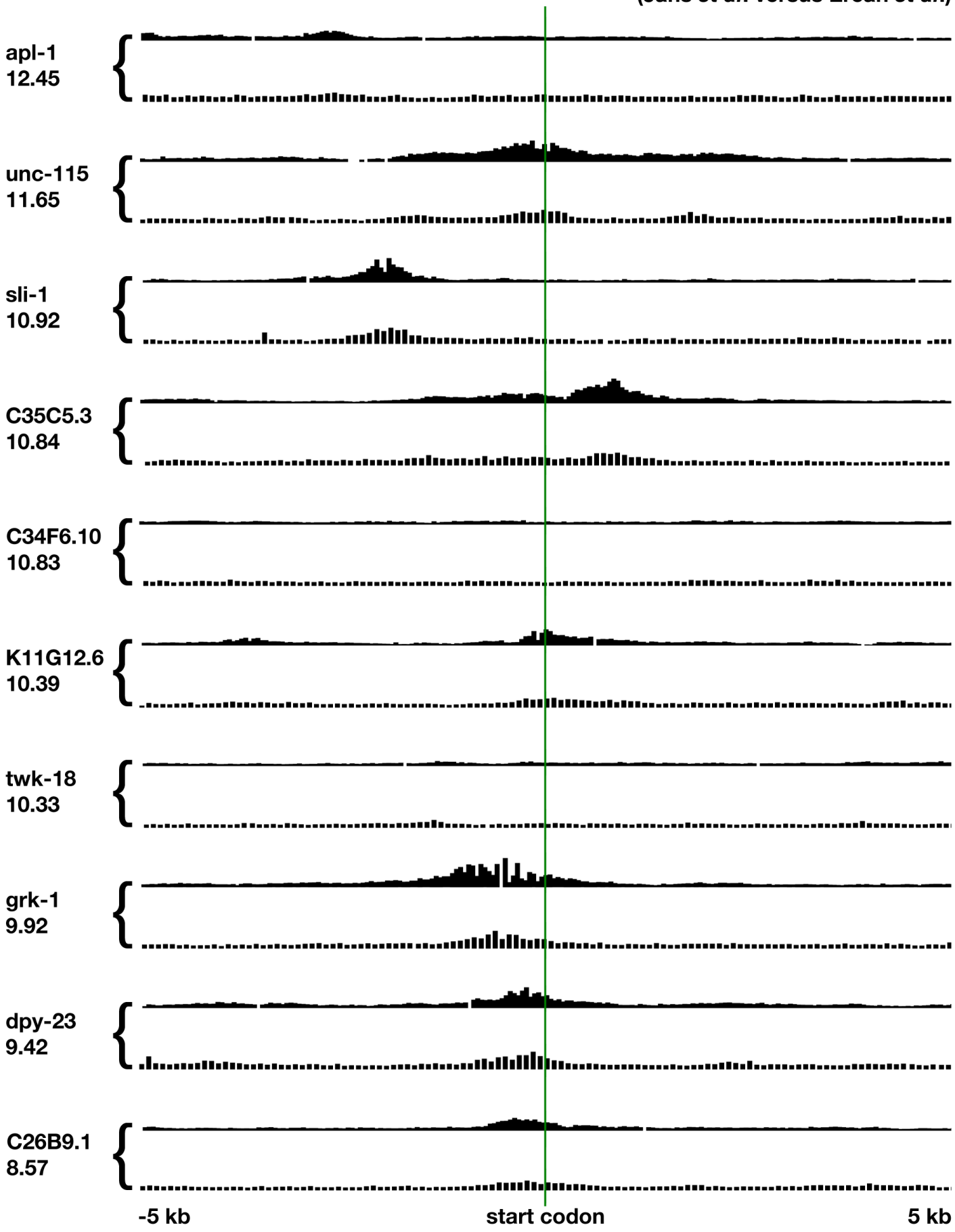
Supplemental Figure 8

DPY-27 Peaks in Dosage-Compensated Genes (Jans *et al*. versus Ercan *et al*.)



Supplemental Figure 8

DPY-27 Peaks in Dosage-Compensated Genes (Jans *et al*. versus Ercan *et al*.)

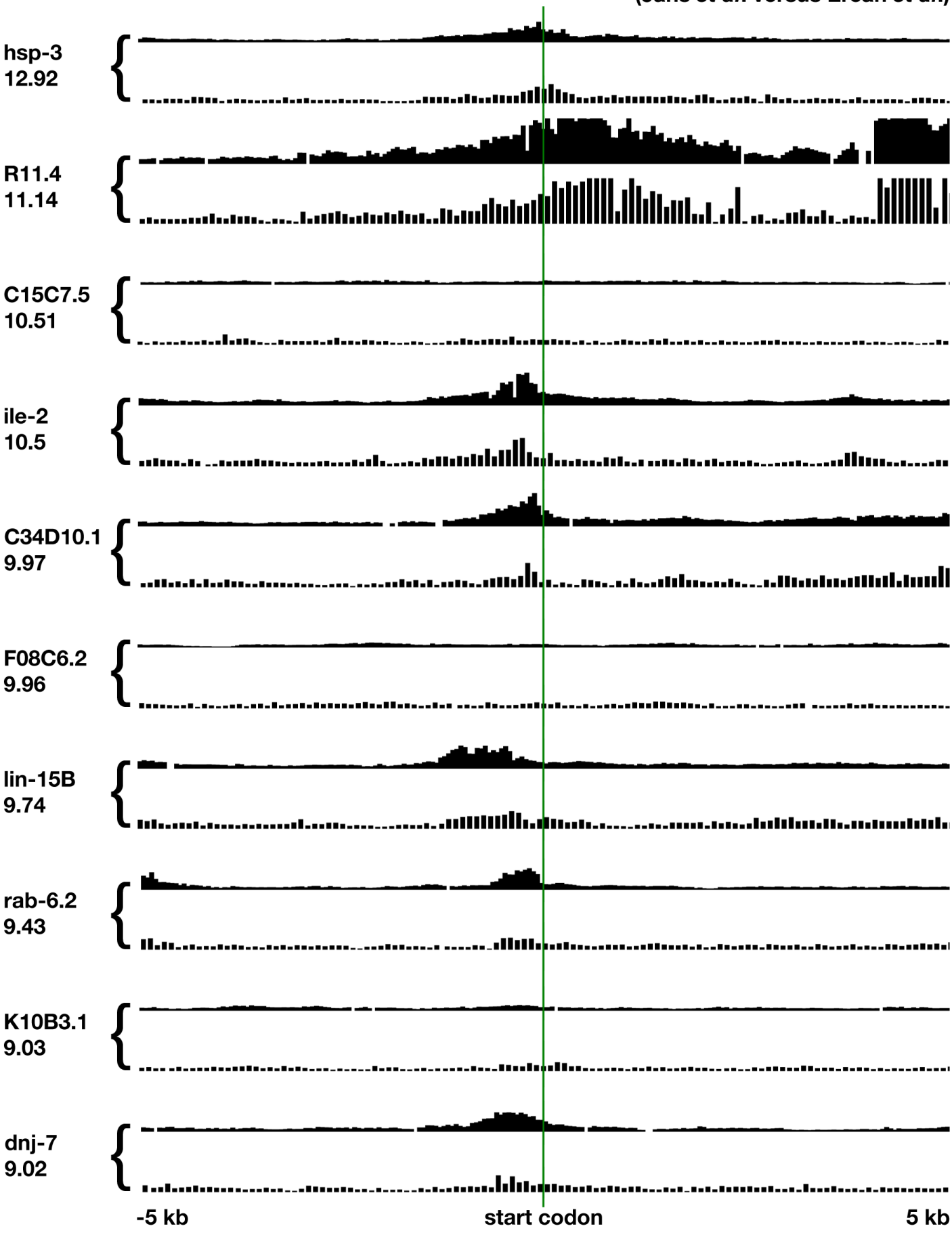


Supplemental Figure 8. Not all dosage-compensated genes have bound DPY-27 in their promoters or coding regions.

Shown is a graphical representation of probe intensity from DPY-27 ChIP-chips along 20 representative dosage compensated genes. Peak landscape is shown from 5 kb upstream to 5 kb downstream of the translation start codon. Shown to the left are gene names and the $\log_2$ value for the gene expression level in wild-type XX embryos. For each gene, our DPY-27 landscape (top) is compared to that of Ercan *et al.* (bottom).

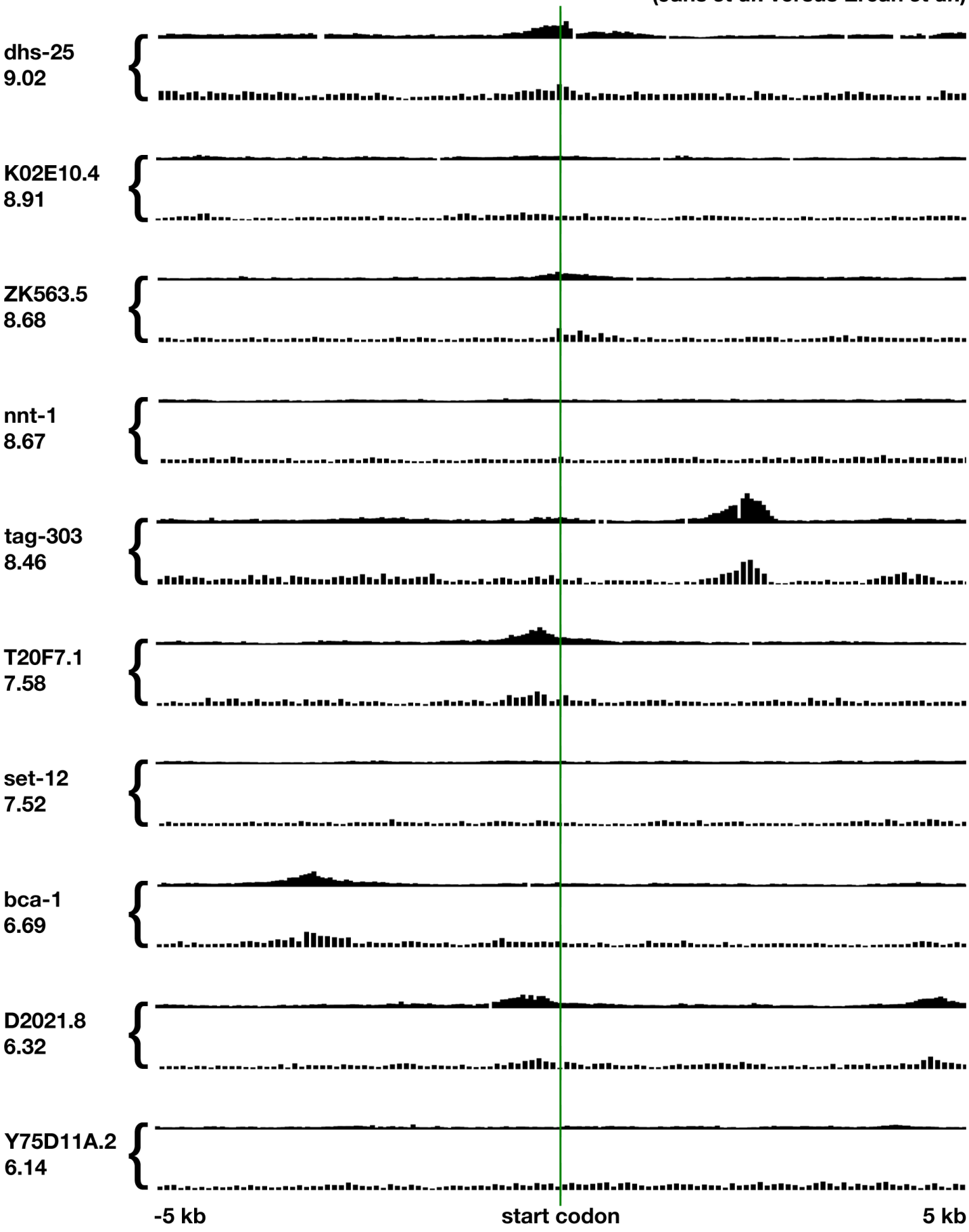
Supplemental Figure 9

SDC-3 Peaks in Non-Dosage-Compensated Genes (Jans *et al*. versus Ercan *et al*.)



Supplemental Figure 9

SDC-3 Peaks in Non-Dosage-Compensated Genes (Jans *et al*. versus Ercan *et al*.)

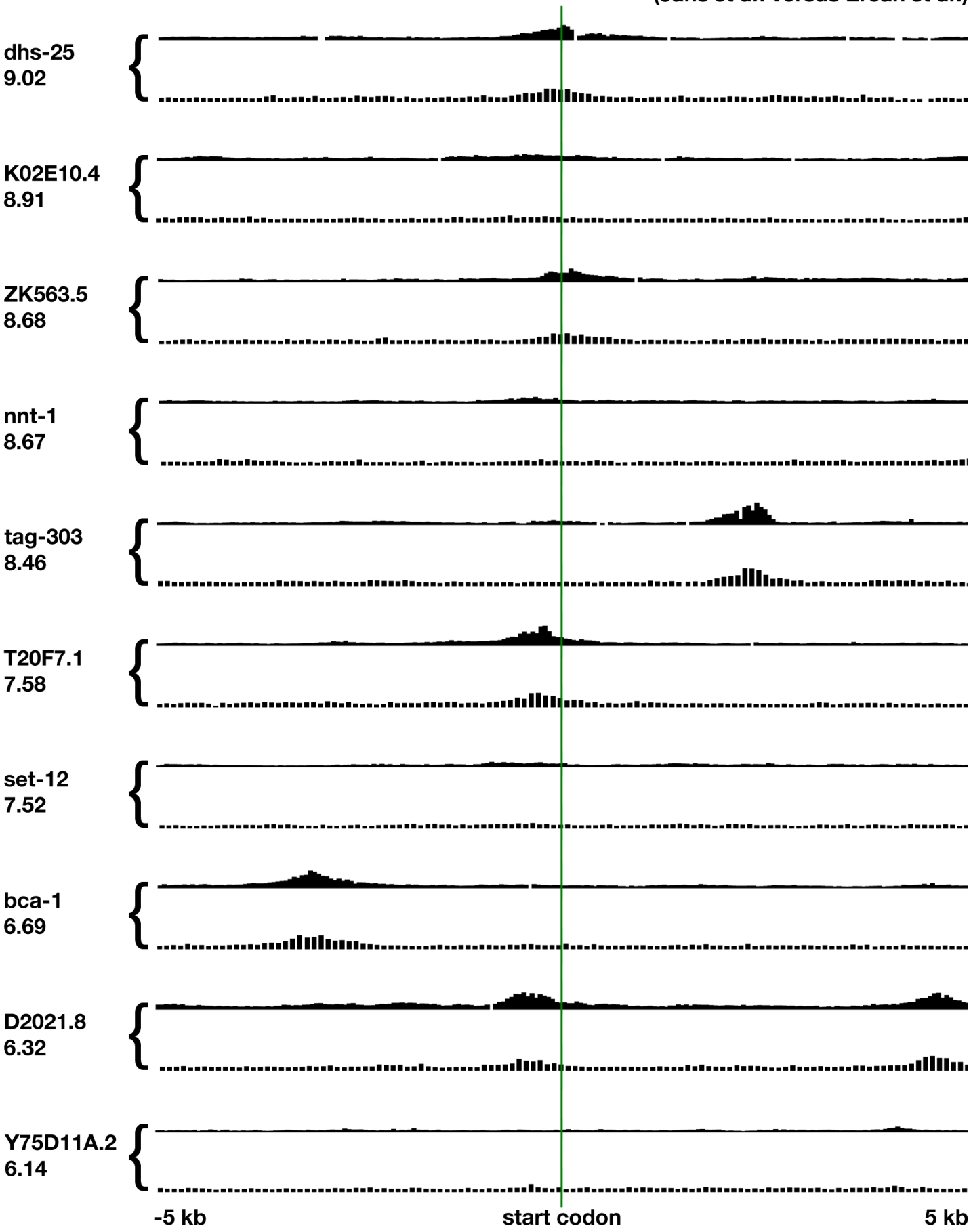


Supplemental Figure 9. Many genes that escape dosage compensation have bound SDC-3 in their promoters or coding regions.

Shown is a graphical representation of probe intensity from SDC-3 ChIP-chips along 20 representative non-compensated genes. Peak landscape is shown from 5 kb upstream to 5 kb downstream of the translation start codon. Shown to the left are gene names and the $\log_2$ value for the gene expression level in wild-type XX embryos. For each gene, our SDC-3 landscape (top) is compared to that of Ercan *et al.* (bottom).

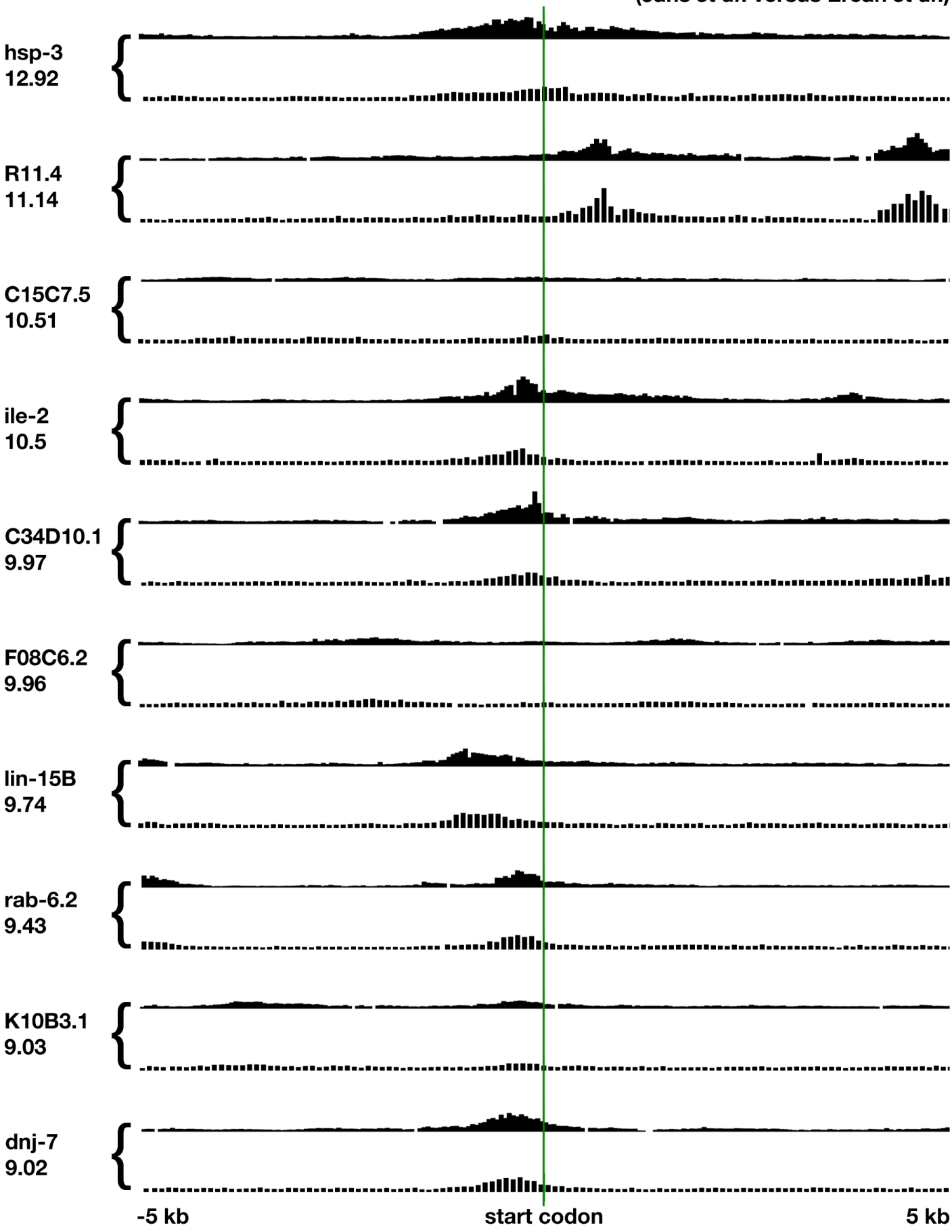
Supplemental Figure 10

DPY-27 Peaks in Non-Dosage-Compensated Genes (Jans *et al*. versus Ercan *et al*.)



Supplemental Figure 10

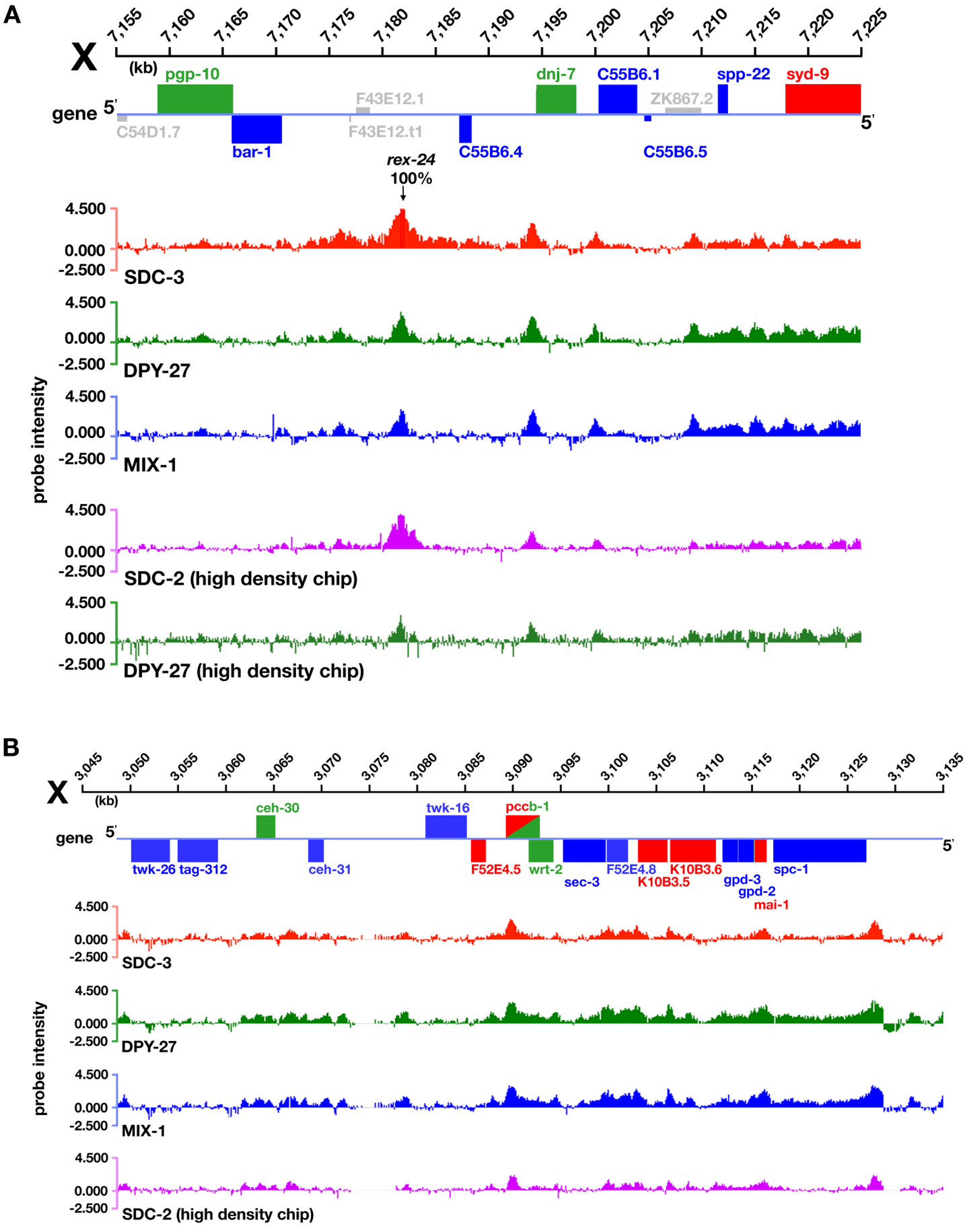
DPY-27 Peaks in Non-Dosage-Compensated Genes (Jans *et al*. versus Ercan *et al*.)



Supplemental Figure 10. Many genes that escape dosage compensation have bound DPY-27 in their promoters or coding regions.

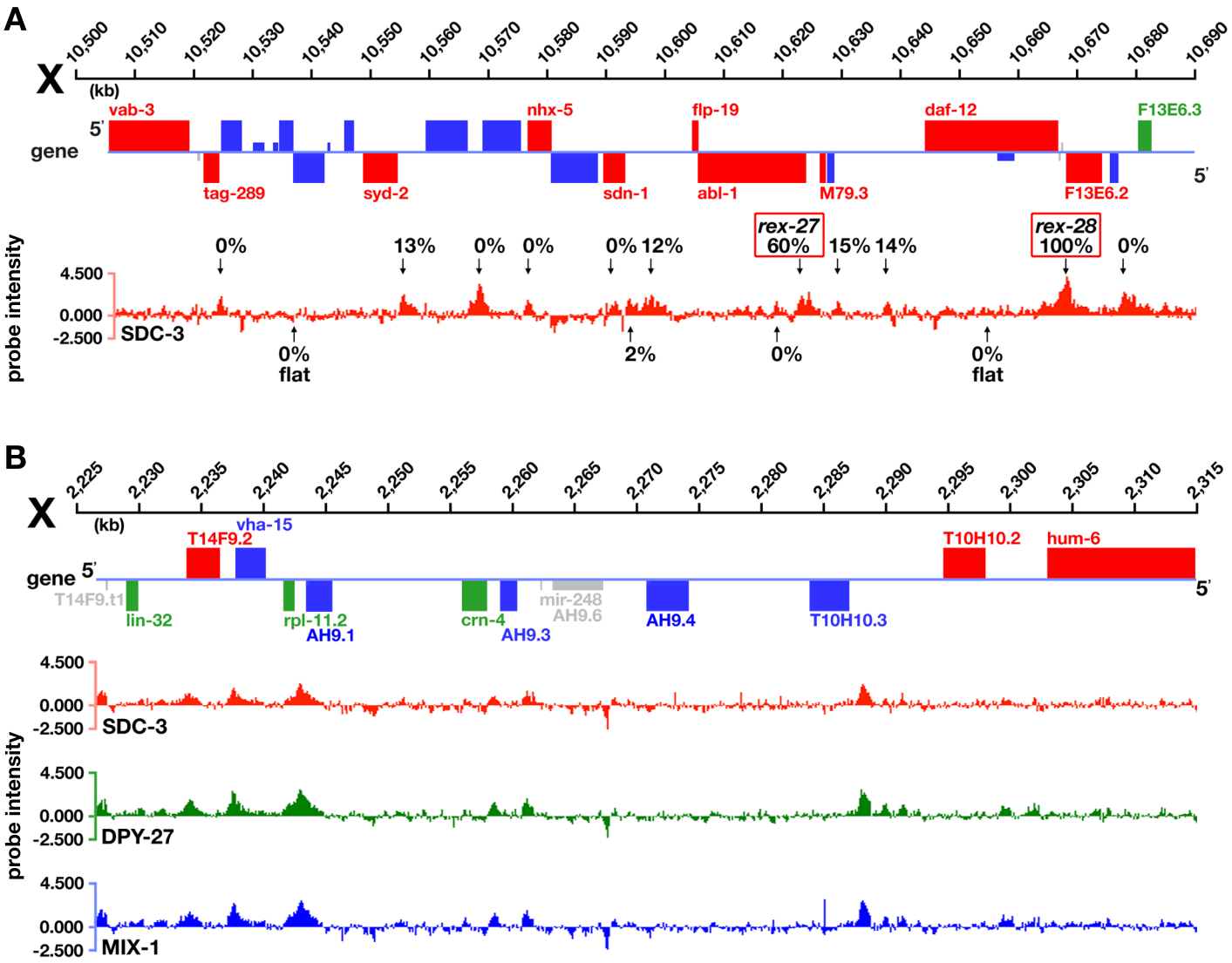
Shown is a graphical representation of probe intensity from DPY-27 ChIP-chips along 20 representative non-compensated genes. Peak landscape is shown from 5 kb upstream to 5 kb downstream of the translation start codon. Shown to the left are gene names and the $\log_2$ value for the gene expression level in wild-type XX embryos. For each gene, our DPY-27 landscape (top) is compared to that of Ercan *et al.* (bottom).

Supplemental Figure 11



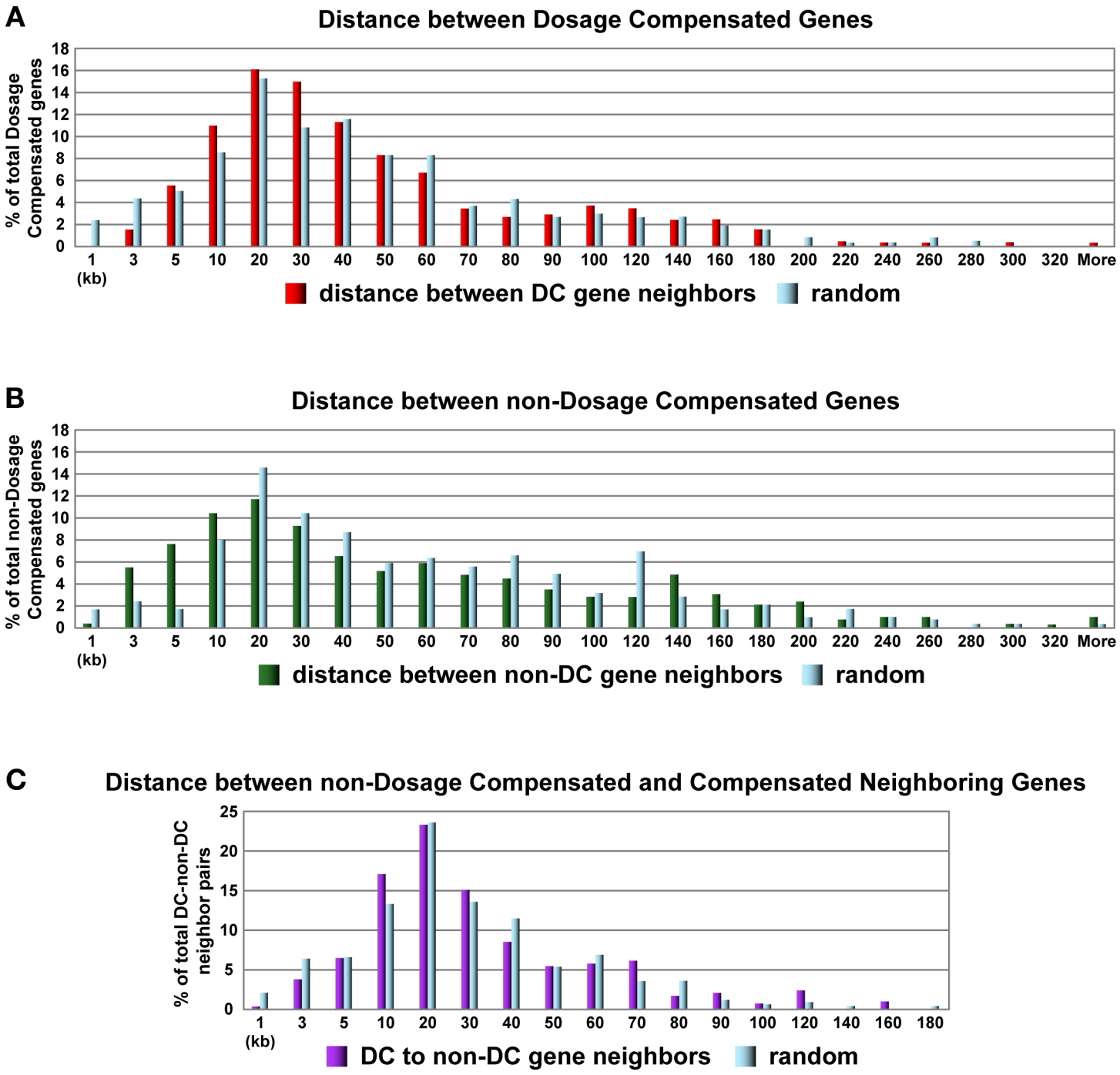
Supplemental Figure 11. Dosage-compensated and non-compensated genes are interspersed.
(A,B) Locations of dosage compensated and non-compensated genes relative to each other and to DCC ChIP-chip probe intensities (high and low density tiling arrays as indicated) from 2 regions of X. The y axis is the probe intensity ratio of fluorescently-labeled DNAs, which were made from chromatin immunoprecipitated by antibodies against different DCC components, to fluorescently-labeled genomic DNA. Peaks are in promoters of genes from both classes. Orientation of genes is left to right above the line and right to left below the line. Red, dosage-compensated gene; green, non-compensated gene; green and red, genes with increased expression in dosage-compensation-defective XX embryos and decreased expression in XO embryos; large blue rectangle, genes not classified; small blue rectangle, genes not expressed in embryos; grey, genes not on the expression array.

Supplemental Figure 12



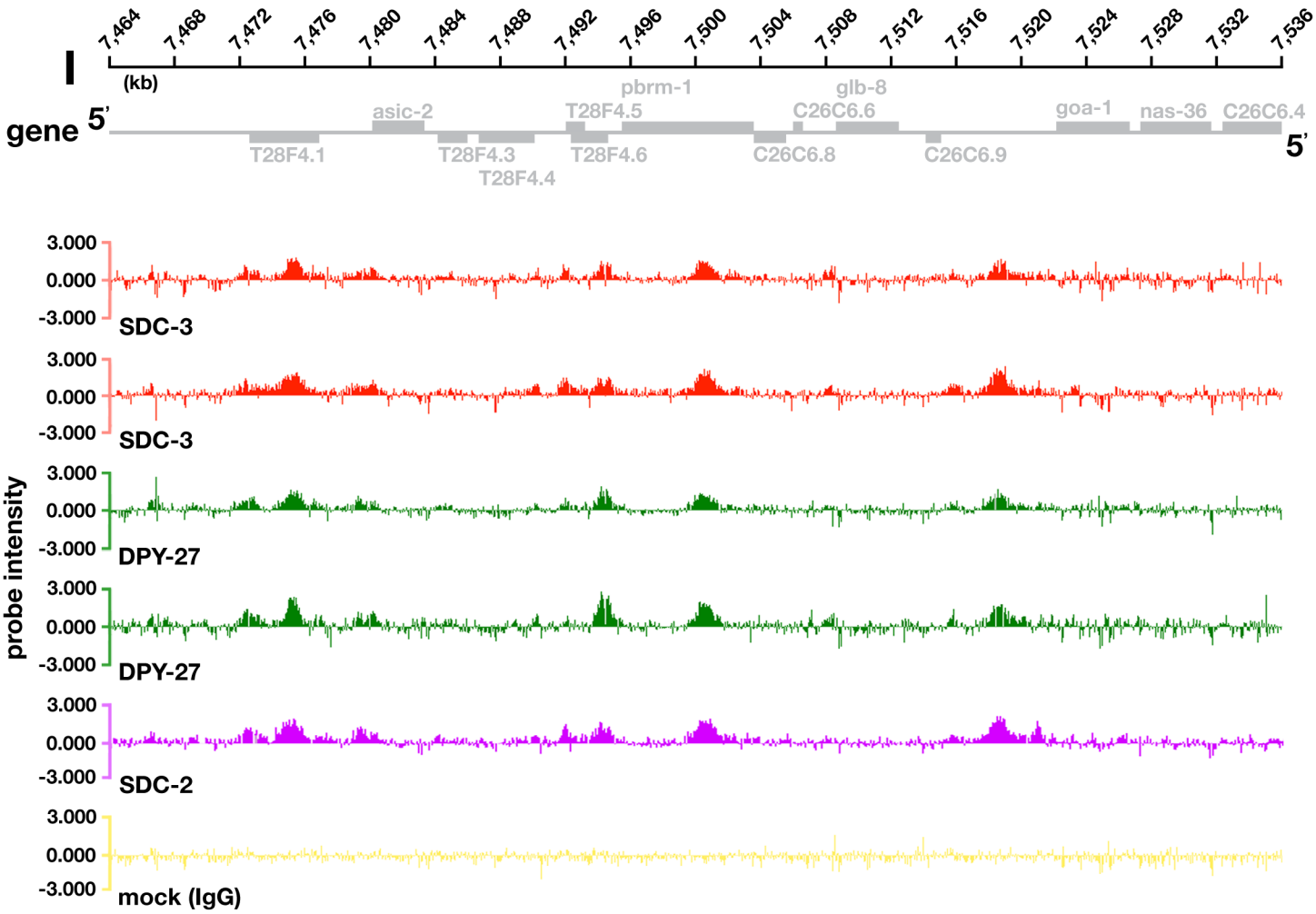
Supplemental Figure 12. Dosage-compensated and non-compensated genes are interspersed, and adjacent genes can belong to either class.
(A,B) Locations of dosage compensated and non-compensated genes relative to each other and to DCC ChIP-chip probe intensities from 2 regions of X. The y axis is the probe intensity ratio of fluorescently-labeled DNAs, which were made from chromatin immunoprecipitated by antibodies against different DCC components, to fluorescently-labeled genomic DNA. Peaks are in promoters of genes from both classes. Orientation of genes is left to right above the line and right to left below the line. In one region *(A)*, locations of dosage compensated and non-compensated genes are shown relative to *rex* and *dox* sites. Red, dosage-compensated gene; green, non-compensated gene; green and red, genes with increased expression in dosage-compensation-defective XX embryos and decreased expression in XO embryos; large blue rectangle, genes not classified; small blue rectangle, genes not expressed in embryos; grey, genes not on the expression array.

Supplemental Figure 13



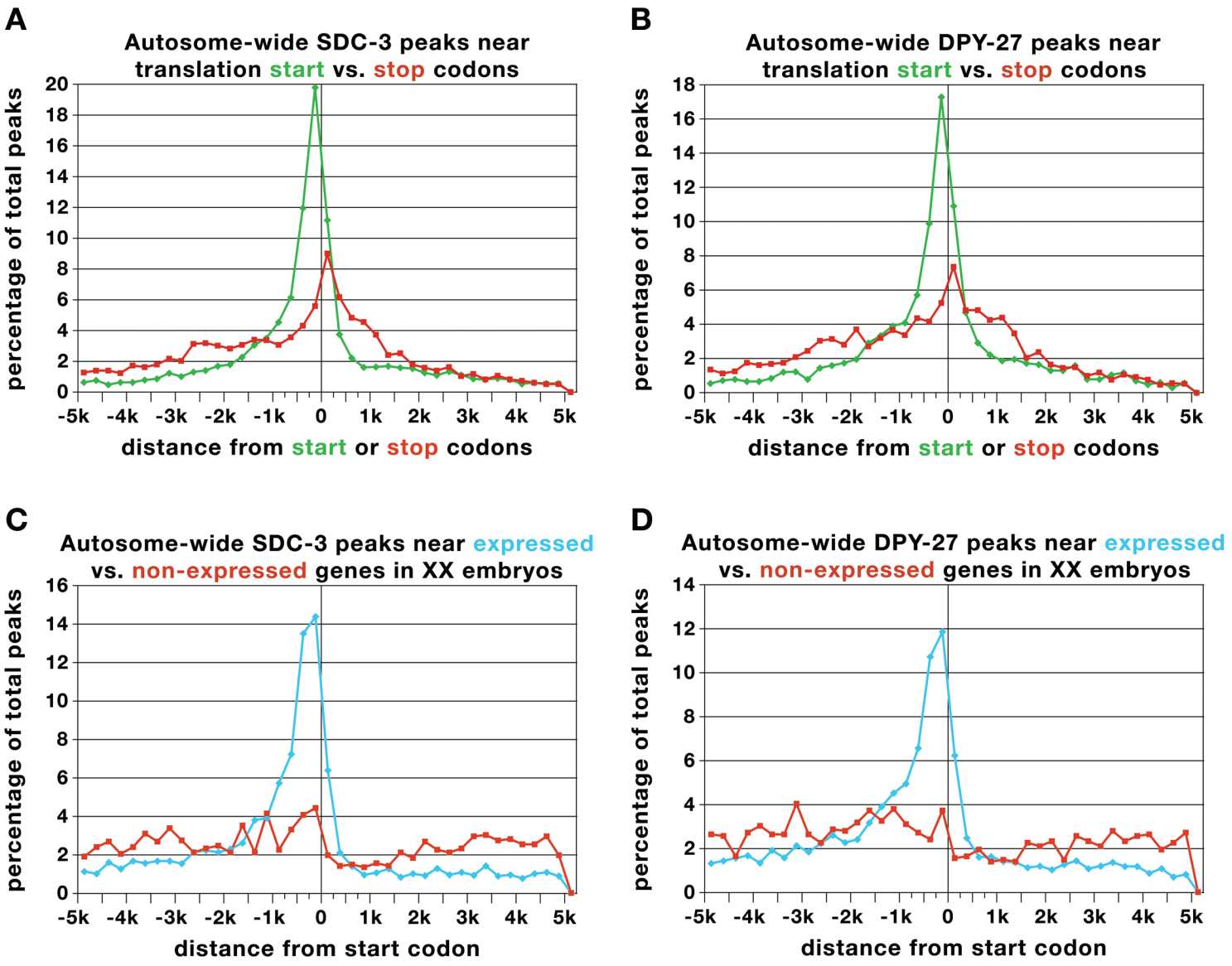
Supplemental Figure 13. The position of a dosage-compensated gene does not influence the location of the next compensated or non-compensated gene. *(A)* Distances between nearest dosage-compensated genes compared to an equivalent number (374) of randomly chosen sequences. Distances between nearest dosage compensated genes were calculated and binned. To generate the random sample, 374 random locations were chosen in the genome, and the distances between nearest neighbors calculated and binned. *(B)* Distances between nearest non-compensated genes compared to an equivalent number (290) of randomly chosen sequences. The distances between nearest non-compensated genes were calculated and binned. To generate the random sample, 290 random locations were chosen in the genome, and the distances between nearest neighbors calculated and binned. *(C)* Distances between dosage compensated and non-compensated genes in closest proximity compared to an equivalent number of randomly chosen sequences. The distances between nearest dosage compensated and non-compensated genes were calculated and binned. For the random sample, 374 random locations were chosen and considered one class. Similarly, 290 random locations were chosen and considered a second class. Distances between class one and class two genes in closest proximity were calculated and binned. *(A-C)* All three comparisons were tested for differences between the experimental and random sets using the Kolmogorov-Smirnov test. In all three cases, the experimental sample was not different from the random sample. For each pairing of genes, three different random samples were analyzed; only one of each is shown. All random samples gave similar results.

Supplementary Figure 14



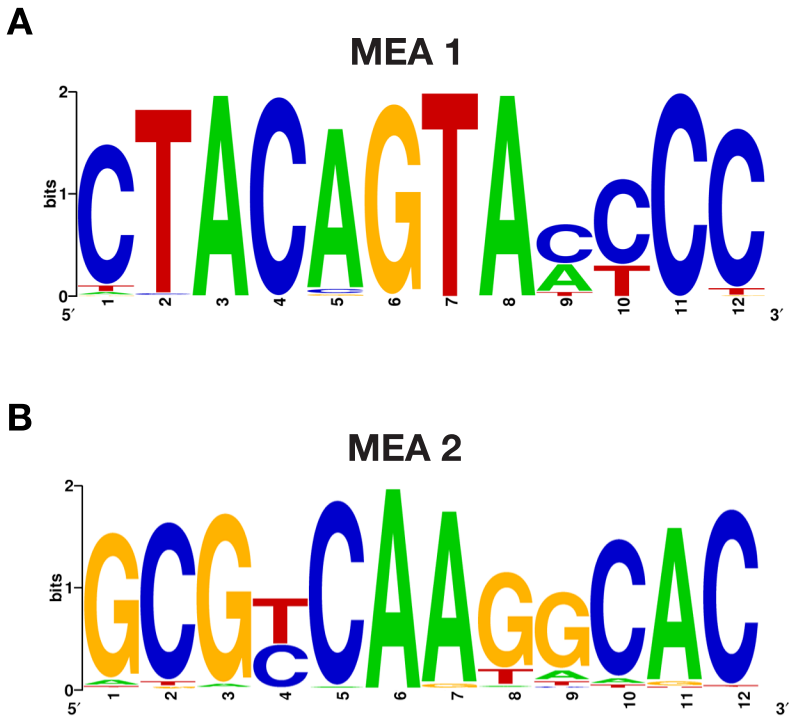
Supplemental Figure 14. The DCC binds to autosomes.
Representative landscapes of SDC-3, DPY-27, and control IgG ChIP-chip experiments on chromosome I compared to gene locations (high density 2.2 million feature tiling arrays). Probe intensity (y axis) is the probe intensity ratio of fluorescently-labeled DNAs made from chromatin immunoprecipitation reactions using different DCC components to fluorescently-labeled genomic DNA. Grey rectangles, genes not changed in expression.

Supplemental Figure 15



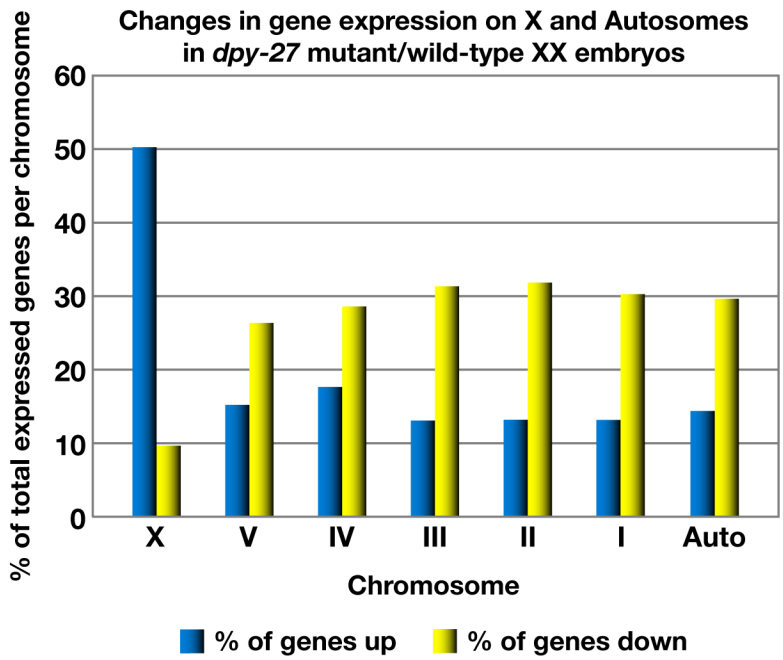
Supplemental Figure 15. DCC peaks reside preferentially in promoters of expressed autosomal genes. *(A,B)* More DCC peaks are near translation start codons than stop codons of autosomal genes. Shown is the distribution of SDC-3 or DPY-27 peaks relative to the translation start (green line) or stop (red line) codons of genes on autosomes. Peaks were mapped to the nearest translation start (green) or stop codon (red) within ± 5 kb interval of the relevant codon. Peaks were then counted in 250 bp bins relative to the start or stop codon. The percentage of peaks in each bin was calculated relative to all peaks within the ± 5 kb interval. *(C,D)* DCC peaks on X are found preferentially in promoters of expressed versus non-expressed genes. Shown is the distribution of DCC peaks relative to distances from the translation start codons of expressed (blue line) or non-expressed (red line) genes. For each expressed or non-expressed gene, DCC peaks with centers within ± 5 kb of the start codon were mapped and counted in 250 bp bins relative to the start codon. The percentage of peaks in each bin was calculated relative to all peaks within the ± 5 kb interval. For SDC-3 peaks, 4767 expressed and 1423 non-expressed genes were analyzed. For DPY-27 peaks, 4232 expressed and 1327 non-expressed genes were analyzed.

Supplemental Figure 16



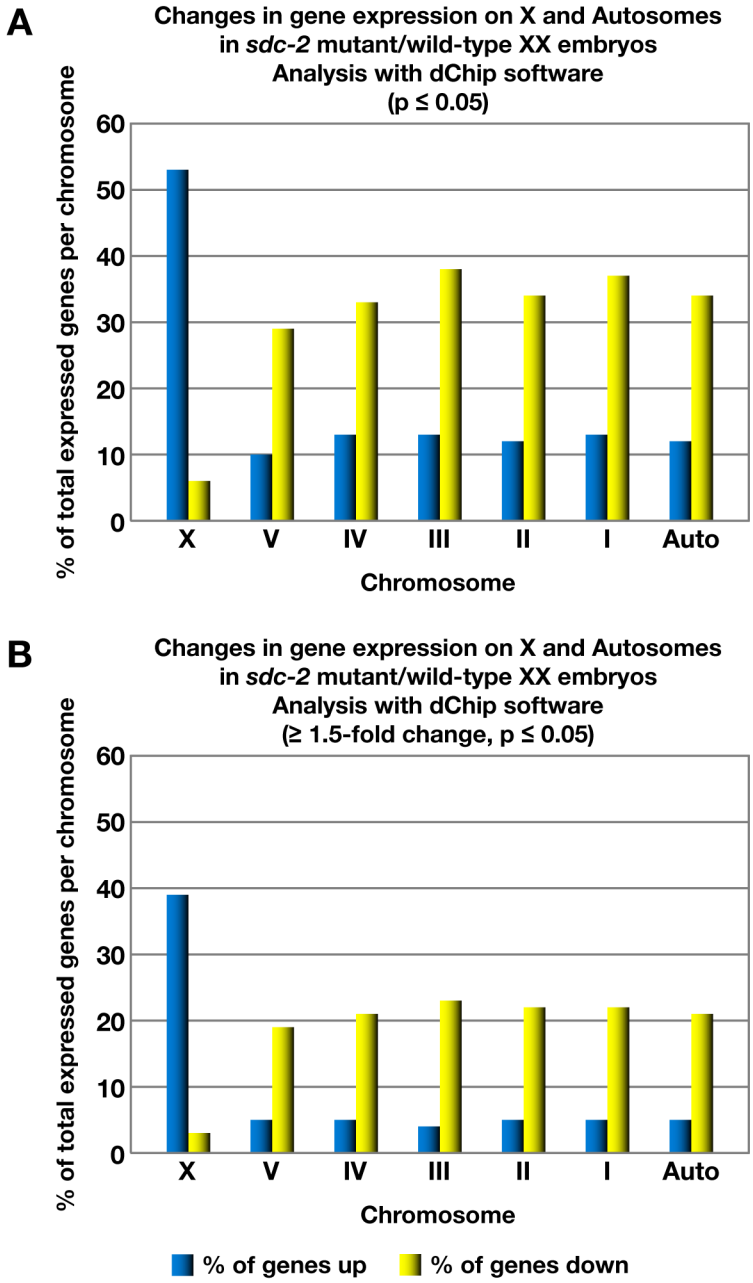
Supplemental Figure 16. Motifs enriched on autosomes. *(A,B)* Models describing the MEA1 and MEA2 motifs from SDC-3 ChIP-chip peaks based on position weight matrices. Motifs were discovered using the wconsensus program. MEA1 is enriched 4-fold on autosomes compared to X. MEA2 is enriched 68-fold on autosomes due to the high incidence on chromosome V. For MEA 1 and MEA 2, respectively, 8.4% or 2.9% of autosomal DCC peaks have the motif, and 20% or 21% of these motifs on autosomes have peaks. In contrast, 2% or 2.9% of X DCC peaks have MEA 1 or MEA 2 motifs, respectively, and 41% or 33% of these motifs on X have DCC peaks. Thus, the prevalence of motifs in autosomal peaks reflects their higher occurrence on autosomes rather than their enhanced ability to attract the DCC when linked to autosomes.

Supplemental Figure 17



Supplemental Figure 17. In XX dosage-compensation-defective embryos, expression of many autosomal genes is reduced, while expression of many X genes is increased.
Histogram showing the percentage of X and autosomal genes changed in expression ($\geq$ 1.5-fold, $p \leq 0.05$) in *dpy-27(y57)* XX mutant vs. wild-type XX embryos. The mutation is a partial-loss-of function allele that only partially disrupts dosage compensation. The number of expressed genes per chromosomes in wild-type XX embryos is the following: X, 1506; V, 1903; IV, 1590; III, 1624; II, 1793; I, 1711.

Supplemental Figure 18



Supplemental Figure 18. In XX dosage-compensation-defective embryos, expression of many autosomal genes is reduced, while expression of many X genes is increased.
(A) Histogram showing the percentage of X and autosomal genes statistically changed in expression ($p \leq 0.05$) in *sdc-2* XX mutant vs. wild-type XX embryos. The number of expressed genes per chromosomes in wild-type XX embryos is the following: X, 1506; V, 1903; IV, 1590; III, 1624; II, 1793; I, 1711. The normalization of all microarrays was performed with dChip software, which finds and uses an invariant probe set for normalizing all chips.
(B) Histogram showing the percentage of X and autosomal genes statistically changed in expression (fold change ≥ 1.5, $p \leq 0.05$) in *sdc-2* XX mutant vs. wild-type XX embryos. The number of expressed genes per chromosomes in wild-type XX embryos is provided in (A). The normalization of all microarrays was performed with dChip software. Two different array normalization procedures (RMA as in Fig. 7 and dChip as in this figure) indicate that a significant decrease in autosomal gene expression occurs when the X-chromosome dosage compensation process is disrupted.

Supplemental Table 1

Properties of *rex* and *dox* sites

[1]Name	[2]Recruitment Data % (nuclei)	[3]Peak Score SDC-3 IP	[4]MEX Motif P-Values	[5]Site Position		[6]Gene Nearby	[7]Distance to ATG	
				Start	Stop		Promoter Region	Coding Region
rex-1	100% (>50)	3.28	-6.63, **-14.57**, -7.16, -7.7, -6.8	4395434	4395674	none		
rex-2	100% (>50)	3.28	-7.31, -8.28	1908940	1909086	none		
rex-3	100% (>50)	0.91	**-14.43**	11361204	11361318	F42E11.1*		2167
rex-4	100% (>50)	2.70	-8.1, -8.65, -8.37	11521744	11522154	*sdc-2**	-687	
rex-5	100% (>50)	2.52	-8.93	11472531	11472774	T25C12.1		3243
rex-6	100% (>50)	3.28	-7.41, -8.05, -6.75, -8.06, **-13.75**, -6.66, -6.95, -7.14, -7.08, **-17.52**, -7.43, -9.06	12362157	12364129	none		
rex-7	100% (>50)	3.28	-8.12, -6.85, -7.34, -7.86, -9.35, -6.58, **-12.05**, -10.5, -6.94, -8.36	11922233	11924309	F55G7.1	-1112	
rex-8	100% (>50)	3.28	-6.71, -7.05, -6.59, -6.71, -6.85, **-18.85**, -8.36, **-17.29**, -6.69, -10.84, -10.92, -6.72, -6.88	11090336	11095474	none		
rex-9	100% (>50)	NA	-8.36, -6.62, -7.99, -6.83, -6.61, -7.94, -7.26, -6.96, -7.62, -7.09, -7.01, -7.19, -9.19, -7.87, -6.82, -8.3, -7.45, -9.42, -8.21, -7.59, -6.68, -8.04, -7.41, -10.32	11682762	11689913	NA		

[1]Name	[2]Recruitment Data % (nuclei)	[3]Peak Score SDC-3 IP	[4]MEX Motif P-Values	[5]Site Position		[6]Gene Nearby	[7]Distance to ATG	
				Start	Stop		Promoter Region	Coding Region
rex-10	100% (>50)	NA	NA (cosmid)	11293806	11319973	Divergent Promoter F08G12.1* F08G12.4*	 -1090 -404	
rex-11	100% (>50)	NA	-6.59, -7.97, -7.55, -7.55, -7.49, -6.88, -6.63, -6.64, -7.26, -6.71, -9.53, -7.25, -7.46, -7.55, -7.12, -8.05, -6.78, -7.95, -7.6, -6.77, -8.93, -6.81, -7.22, -7.01, -7.87, -7.55, -6.61, **-11.31**	11447281	11454464	NA		
rex-12	100% (>50)	NA	NA (cosmid)	11735385	11778938	NA		
rex-13	100% (>50)	NA	-7.91, -6.92, -7.28, -9.06, -6.9, -8.08, -6.78, -8.31, -8.46, -8.88, -7.01, -8.1, -6.9, -6.97, -8.32, -6.55, -7.86, -8.03, -6.53, -6.66, -6.88, -6.93, -7.71	12162920	12170355	NA		
rex-14	100% (>50)	3.28	**-17.29**, **-15.35**, -8.19	8036153	8037002	Divergent Promoter C18A11.6 C18A11.t1	 -1819 -871	
rex-15	100% (>50)	NA	NA (cosmid)	3171557	3204372	NA		
rex-16	100% (>50)	3.28	-6.89, -9.3, -8.92, **-15.1**	11937383	11938556	none		
rex-17	100% (>50)	2.11	**-15.98**, -7.18	8047824	8050033	none		

[1]Name	[2]Recruitment Data % (nuclei)	[3]Peak Score SDC-3 IP	[4]MEX Motif P-Values	[5]Site Position		[6]Gene Nearby	[7]Distance to ATG	
				Start	Stop		Promoter Region	Coding Region
rex-18	100% (40)	2.73	-10.01, -6.83, -6.67, -7.28, -7.94, -6.69, -7.15, -7.87, -6.69, -6.86, -9.49, -8.59, -6.76	1378811	1381002	F56C3.t1	-237	
rex-19	100% (40)	2.22	-8.23, -6.71, -6.91, **-16.06**, -6.64, **-11.19**, -7.26	1491363	1493563	F09E10.5		1888
rex-20	68% (22)	2.40	-7.19, -7.98, -6.9, -7.23, -8.04, -6.8, -7.41	1681700	1683859	C36C9.1*	-172	
rex-21	100% (>50)	3.28	-6.67, -7.81, -7.19, -7.12, -7.63, -6.74, -6.85	1888103	1889829	none		
rex-22	95% (>50)	1.35	-7.75, -6.81, -7.3, -8.56, -6.8, -9.19, -6.77, **-12.55**, -6.77	4009755	4011626	Divergent Promoter R02E12.2 *hrg-1**	-1517	235
rex-23	100% (46)	3.28	-6.88, **-12.72**, -10.68, -7.37, -7.87, -9.21, -7.09, **-17.29**, **-17.52**, -8.65, -7.98	4208061	4210232	F20B6.7*	-1695	
rex-24	100% (58)	3.28	-7.9, -10.87, -7.22, -9.77, -7.26	7180798	7182818	none		
rex-25	100% (30)	1.68	-6.68, -6.89, -6.98, -6.79, -8.21, -9.93, -6.59	8403257	8405357	F41D9.t3 F41D9.5*	-130	3998
rex-26	100% (41)	2.77	-7.03, -7.09, -10.08, -8.38, -6.83, -8.28, -6.85	10352973	10354960	none		

[1]Name	[2]Recruitment Data % (nuclei)	[3]Peak Score SDC-3 IP	[4]MEX Motif P-Values	[5]Site Position		[6]Gene Nearby	[7]Distance to ATG	
				Start	Stop		Promoter Region	Coding Region
rex-27	60% (47)	1.49	-6.78, -7.37, -6.77, -8.62, -8.85, -7.98, -6.77, -8.14, -8.24, -7.79, -7.43, -8.81, -6.69	10621901	10624108	M79.1*		1108
rex-28	100% (>50)	3.21	-6.6, **-15.66**, -8.37	10667026	10669154	F11A1.t1	-1070	
rex-29	100% (>50)	2.48	-6.64, -7.36, **-11.58**, **-15.92**, -7.51, -6.7	10755366	10757490	Divergent Promoter R07A4.4* R07A4.3*	-1724	1586
rex-30	73% (52)	1.13	-7.6, -6.79, -9.02, -7.55, -7.65, -8.52	11221742	11224062	C34F6.9*	-330	
rex-31	100% (>50)	3.17	-6.84, -7.92, **-12.39**, **-17.52**, -7.62, -6.66, **-16.18**, -6.63, -7.05, -6.57	12729135	12731379	F22E10.1	-1513	
rex-32	98% (60)	3.28	-8.76, -10.86, **-11.09**, -6.74, -7.79, -8.76, **-17.52**, **-18.85**, **-18.85**, -6.61, -6.57, -9.11	2996004	2998096	none		
rex-33	100% (49)	3.28	-7.59, -7.65, **-13.13**, **-15.33**, **-15.35**, -9.4	6295287	6297381	T07H6.2*		3251
rex-34	100% (46)	3.28	-8.84, -7.75, -7.09, **-14.75**, **-15.07**, -8.24, -7.92	5428461	5430561	C41A3.1*		15736
rex-35	98% (51)	3.28	-7.36, -7.31, -8.08, -7.8, **-12.49**, -6.6, **-15.35**	16680887	16683118	none		
rex-36	98% (58)	3.28	-7.64, -8.7, -7.34, -6.77, -9.23, -7.94, -8.19, **-17.29**, -7.5	11898254	11900355	Y62H9A.9	-1242	

[1]Name	[2]Recruitment Data % (nuclei)	[3]Peak Score SDC-3 IP	[4]MEX Motif P-Values	[5]Site Position		[6]Gene Nearby	[7]Distance to ATG	
				Start	Stop		Promoter Region	Coding Region
rex-37	91% (56)	1.60	-6.78, -6.87, -6.62, -6.75, -7.02, -8.47, -7.17, -6.56, -9.71, -6.85, -6.67, -6.79, **-18.16**, -6.65, -6.64, -6.63	8810059	8812187	Divergent Promoter R04E5.2* R04E5.9*	 -972 -150	
rex-38	86% (143)	1.25	-7.22, -7.52, -7.56, -6.74, -6.79, -8.63, -6.61, -7.02, -8.5, -8.59, -7.91, -6.76, -7.97, -7.48, -7.74, -7.89, **-17.29**, -7.82, -9.26, -6.92	5858592	5860766	none		
dox-1	0% (27)	1.49	-6.55, -6.84, -6.58, -8.47, -8.94, -8.1, -7.77, -6.92, -7, -7.58, -7.16, -7.98, -6.99, -8.85, -8.76, -6.94, -7.55, -7.58, -6.53, -7.2, -8.9, -6.72, -6.63, -8.32, -8.98, -7.36	992186	994224	C09E10.2*	-1973	
dox-2	0% (43)	2.51	-6.58, -8.39, -7.74, -7.71, -9.06, -8.06, -6.94, -6.8, -7.35, -9.23, -7.49	1913591	1915698	Divergent Promoter *rgs-9** ZC53.1*	 -615 -1394	
dox-3	0% (29)	1.06	-7.41, -7.39, -7.93, -7.4, -6.56, -8.6, -7, -6.77, -9.04, **-11.44**, -8.18, -6.97, -6.76, -6.7	2115780	2117802	F53B1.6*		1460
dox-4	0% (44)	1.64	-7.07, -8.13, -6.58, -7.46, -10.04	2287187	2289387	T10H10.3*	-1106	
dox-5	0% (41)	1.31	-8.9, -7.28, -6.58, -7.05, -7.04, -6.83, -6.96, -7.33, -6.69, -8.7, -8.97	4253904	4256104	*taf-11.1**	-138	

[1]Name	[2]Recruitment Data % (nuclei)	[3]Peak Score SDC-3 IP	[4]MEX Motif P-Values	[5]Site Position		[6]Gene Nearby	[7]Distance to ATG	
				Start	Stop		Promoter Region	Coding Region
dox-6	0% (33)	1.75	-6.55, -7.42, -8.33, -7.62, -6.87, -6.53, -7.84, -7.16, -8.09	4264788	4266930	C52B9.3*		3
dox-7	0% (51)	2.73	-10.12, -7.53, -7.58, -7.56, -7.22, -6.79, -7.64, -6.68, -6.7, -8.59, -7.68, -8.02, -7.49, -7.22, -8.59, -6.7, -7.32	4388004	4390204	*dpy-23**	-233	
dox-8	0% (23)	2.22	-7.34, -9.02, -6.59, -6.97, -7.28, -8.06, -7.11, -6.71, -8.64, -6.65, -7.4, -6.57	5811217	5813303	none		
dox-9	0% (34)	1.17	-6.98, -6.85, -7.33, -10.01, -7.43	6840022	6842222	Divergent Promoter *asg-2** *glr-6**	 -82 -555	
dox-10	7% (28)	2.59	-9.36, -7.39, -7.14, -6.85	7193086	7195286	*dnj-7**	-327	
dox-11	0% (37)	2.77	-9.29, -7.98, -7.3, -6.92, -8.29, -7.09, -7.4, -6.56, -8.52	8028040	8030207	C34D10.1*	-318	
dox-12	0% (>40)	2.81	-8.48, -8.62, -8.14, -6.62, -8.37, -7.53, -7.44, -8.59	8038417	8041199	C18A11.5*	-1460	
dox-13	0% (>40)	2.50	-8.59, -7.51, -6.68, -6.81, -7.01, -7.36, -8.04, -6.6, -6.61, -6.78, -7.22, -6.66, -8.87, -7.15, -6.55, -7.2, -7.43, -7.36, -7.89, -6.53, -7.97, -6.78	8041039	8047329	C18A11.4 C18A11.5*	-1309	2916

[1]Name	[2]Recruitment Data % (nuclei)	[3]Peak Score SDC-3 IP	[4]MEX Motif P-Values	[5]Site Position		[6]Gene Nearby	[7]Distance to ATG	
				Start	Stop		Promoter Region	Coding Region
dox-14	0% (>40)	1.28	-9.01, -6.6, -9.06, -7.08, -6.68, -6.68, -6.6, -6.55, -7.57, -6.53, -6.63, -7.15, -7.55, -7.87, -6.83, -7.97, -6.65, -7.66, -6.68, -7.41, -7.25, -7.23, -7.78, -8.85	8050059	8053247	C18A11.7*		6520
dox-15	0% (>40)	1.20	-7.66, -6.68, -7.41, -7.25, -7.23, -7.78, -8.85, -7.02, -8.39, -6.82, -6.9, -7.43, -6.75, -7.06, -6.76, -7.29, -8.87, -6.58, -8.41, -8.09, **-11.32**, -7.26	8052696	8059136	C18A11.7*		2257
dox-16	0% (18)	1.35	-6.61, -9.5, -9.9, -10.68, -7.89, -6.62, -6.6, -7.54	8555767	8558014	D2021.1*	-218	
dox-17	0% (29)	1.60	-6.84, -7.01, -9.42, -7.1, -6.6, -7.19	9271062	9273202	B0416.5*	-1348	
dox-18	0% (35)	2.19	-6.69, -6.58, -8.8, -6.61, -6.99, -6.54, -9.13, -9.96, -7.39, -7.15, -6.84, -8.8, -8.73, -10.33, -8.72, -7.01, -6.77, -7.91	9336965	9339038	T20B5.1*		1251
dox-19	0% (26)	1.79	-9.46, -6.67, -8.64, -6.53, -7.03, -9.86, -7.58, -9.58, -7.11	10187476	10189677	F21A10.2		5731
dox-20	0% (31)	1.49	-7.39, -6.91, -8.65, -6.89, -7.27, -6.62, -6.64, -9.71	10523983	10526091	Divergent Promoter *tag-289** *git-1**	-544	211
dox-21	7% (104)	1.89	-6.8, -6.75, -7.78, -7.56, -6.61, -8.03, -8.32	10554557	10556646	*syd-2**	-737	

[1]Name	[2]Recruitment Data % (nuclei)	[3]Peak Score SDC-3 IP	[4]MEX Motif P-Values	[5]Site Position		[6]Gene Nearby	[7]Distance to ATG	
				Start	Stop		Promoter Region	Coding Region
dox-22	0% (26)	2.59	-7.28, -6.54, -8.65, -7.28, -7.21, -7.24, -6.78, -7.37, -9.79, -6.59, -6.83, -8.38, -7.77, -6.68, -9.42, -6.98, -7.43, -6.96	10567613	10569810	F57C7.1*	-490	
dox-23	0% (>50)	1.13	-6.75, -8.91, -7.33	10575531	10577669	*nhx-5**	-288	
dox-24	0% (43)	1.09	-7.89, -6.94, -6.8, -6.53, -8.34, -6.6, -6.76, -6.57, -6.54, -8.14, -6.61, -7.01, -6.86, -7.39	10590425	10592599	F57C7.3*		1750
dox-25	2% (125)	0.73	-6.55, -7.31, -6.96, -6.56, -6.81, -6.92, -7.76, -6.59	10594026	10596099	F57C7.3*	-1800	
dox-26	12% (50)	1.75	**-13.22**, -6.88, -7.13, -6.99, -7.07, -7.85, -6.99, **-11.79**, -8.78, -7.64, -8.31, -6.74, -8.27	10596600	10598678	none		
dox-27	0% (160)	0.95	-7.41, -6.73, -6.97, -7.35, -6.87, -6.8, -8.22, -10.15, -7.2, -7.1, -8.59, -7.31, -6.56, -6.55	10617664	10619901	M79.1*		5330
dox-28	15% (39)	0.95	-6.74, -6.64, -7.33, -6.71	10628303	10630385	M79.2* M79.3*	-428 -1856	
dox-29	14% (22)	1.06	-7.93, -6.71, -7.72, -6.64, -6.76, -8.17, -6.78, -8.34, -6.95, -8.37, -8.29, -7.3, -10.55, -7.07, -6.67, -6.71, -9.1, -6.86, -8.88, -8.97, -6.99, -6.79, -7.05, -8.63, -9.05	10636607	10638723	none		

[1]Name	[2]Recruitment Data % (nuclei)	[3]Peak Score SDC-3 IP	[4]MEX Motif P-Values	[5]Site Position		[6]Gene Nearby	[7]Distance to ATG	
				Start	Stop		Promoter Region	Coding Region
dox-30	0% (40)	1.40	-6.96, -6.6, -6.77, -7.92, -6.98, -7.21, -7.18, -8.03, -7.69, -8.79, -8.01, -7.4	10678264	10680430	F13E6.3*	-1208	
dox-31	0% (34)	1.49	-8.13, -7.62, -7.97	11206026	11208149	*hst-2**	-71	
dox-32	2% (117)	1.06	-8.39, -7.45, -6.62, -6.89, -6.88, -8.7, -6.59, -7.61, -7.31, -6.72, -7.07, -7.41, -8.5, -9.06, -6.67, -8.19	11209673	11211824	C34F6.5* C34F6.7*	-1951	4647
dox-33	0% (32)	1.35	-8.2, -7.8, -6.6, -8.26, -7.32	11214498	11216620	Divergent Promoter C34F6.7* C34F6.8*	 -164 -62	
dox-34	0% (26)	1.60	-6.97, -7.14, -6.62, -7.01, -7.34, -8.3	11247156	11249344	none		
dox-35	35% (31)	0.87	-6.66, -7.36, -7.02, -8.19, -6.61, -7.15, -7.62, -6.55, -8.28, -6.71, -7.87	11251509	11253630	C03A3.t1		18
dox-36	0% (39)	1.46	**-12.57**, -7.66, -8.12, **-12.21**, -7.55, -10.58	11256155	11258230	C03A3.1*	-94	
dox-37	0% (40)	1.57	-7.59, -8.81, -6.83, -8.02, -7.09, -9.06, -8.76, -7.1, -7.14, -6.61, -7.19	11261445	11263583	C03A3.2*	-257	
dox-38	0% (39)	0.69	-7.63, -7.92, -7.33, -6.87, -7.36, -6.87, -6.87, -7.36, -6.87, -6.64, -9.73, -6.52, -8.2, -8.28, -7.52, -7.5, -10.42, -8.13	11289251	11291177	none		
dox-39	35% (34)	1.53	-7.28, -6.8, -6.81, -6.71, -6.91, -8.46, -9.04, -8.39, -7.08, -6.56, -6.74, -7.56	11296087	11298204	*inx-2**	-488	

[1]Name	[2]Recruitment Data % (nuclei)	[3]Peak Score SDC-3 IP	[4]MEX Motif P-Values	[5]Site Position		[6]Gene Nearby	[7]Distance to ATG	
				Start	Stop		Promoter Region	Coding Region
dox-40	4% (101)	1.68	-7.17, -6.86, -6.84, -6.92, -8.27, -6.99, -8.27, -7.66	11298792	11300952	Divergent Promoter F08G12.2* F08G12.3*	 -259 -307	
dox-41	0% (80)	1.09	-6.54, -6.61, -6.83, -7.24, -6.89, -10.02, -7.89	11305724	11307886	Divergent Promoter F08G12.1* *vhl-1**	 -1006 -488	
dox-42	2% (96)	0.70	-7.36, -9.51, -7.24, -6.78, -7.9, -6.83, -6.84, -8.97, -6.8, -6.58, -7.47	11308835	11310973	F08G12.5*		244
dox-43	0% (32)	1.75	-7.26. -6.84, -7.18, -6.7	11338120	11340208	Divergent Promoter *aco-1** ZK455.2	 -634 -1167	
dox-44	10% (31)	1.38	-7.55, -7.96, -6.6, -7.1, -7.34	11364922	11367066	none		
dox-45	1% (75)	0.95	-6.95, -8.04, -7.39, -7.17, -7.51, -8.26, -6.6, -8.37, -6.58, -6.57, -9.09, -6.89	11367106	11369259	F42E11.2*	-463	
dox-46	0% (35)	2.51	-8.85, -9.34, -7.17, -6.71, -6.61, -7.88, -7.06, -7.39, -7.28, -7.35	12392897	12395090	F17E5.2*	-194	
dox-47	0% (36)	1.31	-7.48, -8.34, -6.63, -7.61	12633999	12636190	*tag-147**	-818	

[1]Name	[2]Recruitment Data % (nuclei)	[3]Peak Score SDC-3 IP	[4]MEX Motif P-Values	[5]Site Position		[6]Gene Nearby	[7]Distance to ATG	
				Start	Stop		Promoter Region	Coding Region
dox-48	0% (21)	2.77	-7.97, -7.15, -7.71, -8.03, -6.81, -6.83, -8.76, -7.97, -7.25, -6.98, -6.67, -7.31, -6.68	15724214	15726369	*lin-15B**	-758	
dox-49	0% (35)	2.99	-6.83, -6.93, -6.94, -7.99, -8.37, -8.09, -7.68, -6.6, -6.56	17183351	17185550	B0302.1*	-1844	
NP 1	11% (216)	NA	-8.47, -8.47, -6.6, -6.67, -6.91, -8.16, -6.98, -7.66	11237579	11239630	none		
NP 2	0% (86)	NA	-6.68, -8.76, -7.39, -7.12, -6.85	10536161	10538156	F59F5.3		5156
NP 3	0% (143)	NA	-6.72, -7.51, -7.84, -6.71, -7.16, -7.25, -6.69, -7.11, -6.75, -7.81, -7.53, -9.43, -7.33	10653746	10655785	F11A1.3		10431

[1] *rex* (recruitment element on X), *dox* (dependent on X), NP (no peak)

[2] Percent of array-bearing nuclei with a DCC recruiting extrachromosomal array. Total number of nuclei is in parenthesis. At least 2 independent lines were scored.

[3] The peak score is defined by the NimbleScan software.

[4] Listed are the ln (P) values for all MEX motifs within a site at a value ≤ -6.5. ln (P) is the natural log of the probability of finding a 12 mer that matches the MEX consensus motif matrix as calculated by the Patser program. NA indicates the site has not been delimited to a small enough interval to list the motifs. NA (cosmid) means the *rex* site is a cosmid.

[5] Listed are the base pair coordinates of a site on the X chromosome.

[6] Listed are genes, if any, in which the rex or dox site has a center within 2 kb upstream of the translational start codon or in the coding region. Asterisks indicate genes called as present by MAS5 using expression Consol software in Affymetrix microarray experiments, implying expression of the gene in embryos.

[7] Listed is the distance between the site center and the ATG start codon of the nearby gene. (-) is in the promoter. (+) is inside the coding region.

Supplemental Table 2

	SDC-3 X	SDC-3 Autosome	DPY-27 X	DPY-27 Autosome
	% (Peaks)	% (Peaks)	% (Peaks)	% (Peaks)
Promoter[1]	51% (880)	56% (2369)	43% (1086)	57% (2192)
Coding [2]	24% (426)	26% (1118)	34% (862)	26% (1014)
Intergenic	25% (443)	18% (786)	24% (601)	17% (670)

Distribution of SDC-3 or DPY-27 ChIP-chip peak centers with respect to promoters, genes, or intergenic regions on X chromosomes and autosomes. Half of peaks are in promoters.

[1] The promoter is defined as the region from the ATG start codon to 2 kb upstream.

[2] The coding region is defined as the interval between the translational start and stop codons of protein-encoding genes and by the initial transcript of RNA-encoding genes.

Each peak is evaluated for placement; some promoters and genes have more than one peak.

Supplemental Table 3

Gene Class on X	Genes with DCC Peaks -2 kb to Stop Codon		Genes with No DCC Peaks -2 kb to Stop Codon	
	Mean Fold Change ± SEM	No.	Mean Fold Change ± SEM	No.
sdc-2(-) XX	Fold Increase		Fold Increase	
All expressed[a]	1.53 ± 0.024[c]	833	1.46 ± 0.043[c]	749
Overexpressed[b]	1.91 ± 0.027[c]	505	1.92 ± 0.042[c]	370
dpy-27(-) XX	Fold Increase		Fold Increase	
All expressed[a]	1.56 ± 0.027[c]	834	1.56 ± 0.027[c]	749
Overexpressed[b]	2.05 ± 0.031[d]	505	1.90 ± 0.030[d]	560
XO	Fold Decrease		Fold Decrease	
All expressed[a]	1.31 ± 0.014[d]	831	1.23 ± 0.015[d]	749
Underexpressed[b]	1.65 ± 0.027[d]	532	1.55 ± 0.022[d]	343

Correlation between the presence or absence of DCC peaks in X-linked genes and the mean changes in gene expression in dosage-compensation-defective XX embryos or XO embryos compared to wild-type XX embryos. Direct correlation between DCC binding to genes and DCC-mediated repression of gene expression is not evident.

[a] Shown is a comparison of the mean expression changes and standard errors of the mean (SEM) for all active X-linked genes in dosage compensation defective XX embryos or XO embryos compared to wild-type XX embryos. The genes are subdivided into those with or without SDC-3 peak centers in the region from 2 kb upstream of the transcription start codon through the stop codon. No. is the number of genes in each class. The fold increase or fold decrease in expression is relative to the expression level in wild-type XX

embryos. The genes were classified as active in XX embryos through "present calls" using MAS5, as indicated in Materials and methods in the main paper.

[b]Shown is a comparison of the mean expression changes and standard errors of the mean for genes on X judged as over-expressed in *sdc-2* or *dpy-27* XX mutant embryos or under-expressed in XO embryos by the criterion that the expression level be statistically different ($p \leq 0.05$) from that in wild-type XX embryos. The genes are subdivided into those with or without SDC-3 peak centers in the region from 2 kb upstream of the transcription start codon through the stop codon. No. is the number of genes in each class. Genes were classified as over- or under-expressed using RMA normalization, as indicated in Materials and methods in the main paper.

[c]Student's t test shows that the mean increases or decreases in gene expression are not different between genes with and without peaks.

[d]Student's t test shows that the mean increases or decreases in gene expression are different between genes with and without peaks. However the difference in mean values is extremely small and not likely to be biologically relevant.

Supplemental Table 4

Dosage-Compensated Genes

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00001824	*hbl-1*	12.9	1.6	1.9
WBGene00003903	*pab-2*	12.8	1.8	1.8
WBGene00002124	*inx-2*	12.5	1.5	1.5
WBGene00012261	*lpr-3*	12.4	1.7	1.7
WBGene00000149	*apl-1*	12.4	1.8	2.3
WBGene00044068	*syd-9*	12.2	1.6	2.2
WBGene00020216	*trap-2*	12.1	1.5	1.5
WBGene00004276	*rab-14*	12.0	1.9	1.5
WBGene00020921	W01C8.5	12.0	1.8	2.4
WBGene00004788	*sft-4*	11.9	1.9	1.5
WBGene00000899	*daf-3*	11.6	1.8	1.7
WBGene00006839	*unc-115*	11.6	1.8	2.5
WBGene00020848	T27B1.2	11.6	1.5	2.6
WBGene00000482	*chd-3*	11.5	1.6	1.5
WBGene00009793	F46F6.2	11.5	1.9	2.1
WBGene00004682	*rsd-3*	11.5	1.9	2.1
WBGene00004862	*sma-9*	11.5	1.6	2.7
WBGene00001331	*erd-2*	11.4	1.7	2.2
WBGene00006649	*tth-1*	11.3	1.9	1.7
WBGene00015676	C10E2.6	11.2	1.8	2.3
WBGene00004369	*rig-1*	11.2	1.9	2.8
WBGene00003602	*nhr-3*	11.2	1.9	2.9

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00001441	*fkh-9*	11.1	1.8	2.4
WBGene00015494	C05E11.3	11.1	2.0	2.5
WBGene00010701	*ent-2*	11.0	1.7	1.7
WBGene00003087	*lsy-2*	10.9	1.9	1.9
WBGene00004949	*sox-2*	10.9	2.6	2.0
WBGene00006816	*unc-84*	10.9	1.9	2.0
WBGene00004829	*sli-1*	10.9	1.9	2.1
WBGene00009632	F42E11.2	10.8	1.8	1.8
WBGene00007955	C35C5.3	10.8	1.7	1.8
WBGene00010935	M163.1	10.8	2.0	2.0
WBGene00006410	*nck-1*	10.8	2.0	2.3
WBGene00007944	C34F6.10	10.8	1.9	3.3
WBGene00011880	T21B6.3	10.7	2.1	1.7
WBGene00001006	*dlg-1*	10.7	1.6	1.9
WBGene00017447	F14B8.5	10.7	1.9	2.3
WBGene00022500	*lfi-1*	10.7	2.3	2.6
WBGene00022861	*dve-1*	10.7	1.9	2.9
WBGene00016292	C31H2.1	10.7	2.5	3.9
WBGene00004727	*sax-1*	10.6	1.8	1.8
WBGene00011904	T21H8.1	10.6	1.9	2.2
WBGene00015926	C17H11.6	10.6	2.7	2.3
WBGene00044061	*tag-236*	10.5	2.2	2.2
WBGene00022613	ZC449.3	10.5	2.2	2.3
WBGene00002983	*lgx-1*	10.5	1.5	2.8

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00006887	*vav-1*	10.5	2.3	2.9
WBGene00003407	*mrp-1*	10.4	1.5	1.8
WBGene00020732	T23E7.2	10.4	2.3	1.8
WBGene00019657	K11G12.6	10.4	1.6	1.8
WBGene00016067	C24H10.1	10.4	1.6	2.0
WBGene00006588	*tnt-3*	10.4	1.8	2.4
WBGene00003254	*mig-23*	10.4	1.9	2.9
WBGene00017891	*rgl-1*	10.4	1.9	3.0
WBGene00001124	*dyf-8*	10.4	1.9	3.3
WBGene00002123	*inx-1*	10.3	1.9	1.6
WBGene00018657	*acl-4*	10.3	1.9	1.9
WBGene00006757	*unc-18*	10.3	2.5	2.1
WBGene00006672	*twk-18*	10.3	2.1	4.5
WBGene00003600	*nhr-1*	10.2	2.4	2.3
WBGene00011763	T14B1.1	10.2	2.1	2.4
WBGene00004758	*sek-1*	10.2	2.7	2.5
WBGene00015295	*acl-12*	10.2	2.1	3.5
WBGene00017381	*ddr-2*	10.1	1.6	1.6
WBGene00010059	F54E4.3	10.1	1.9	1.7
WBGene00009930	F52D10.2	10.1	1.8	1.9
WBGene00007593	C14H10.3	10.1	1.8	2.3
WBGene00010117	F55F3.3	10.1	3.1	2.3
WBGene00017687	*ets-4*	10.1	2.3	3.2
WBGene00003986	*pes-22*	10.1	2.8	3.3

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00002039	*hum-6*	10.0	1.7	1.9
WBGene00044698	K09F5.6	10.0	2.4	2.4
WBGene00000458	*ceh-37*	10.0	2.1	2.5
WBGene00016617	C43H6.4	10.0	1.8	2.7
WBGene00018607	F48E3.8	10.0	1.5	2.9
WBGene00006942	*wrk-1*	10.0	2.7	3.5
WBGene00001708	*grk-1*	9.9	2.1	1.8
WBGene00007591	C14H10.1	9.9	1.8	2.0
WBGene00021323	Y34B4A.7	9.9	1.5	2.1
WBGene00016490	C36E6.2	9.9	1.7	2.1
WBGene00007935	C34E11.2	9.9	2.0	2.3
WBGene00000803	*csb-1*	9.9	2.0	2.4
WBGene00000393	*cdf-1*	9.9	2.6	2.5
WBGene00006365	*syg-1*	9.9	2.0	3.0
WBGene00008951	F19C6.2	9.9	2.6	3.1
WBGene00008571	F08B12.1	9.9	2.4	3.1
WBGene00001032	*dnj-14*	9.8	2.5	2.2
WBGene00019611	K10B3.5	9.8	2.1	2.4
WBGene00000018	*abl-1*	9.8	2.2	3.0
WBGene00019102	F59C12.3	9.8	2.5	3.0
WBGene00010347	H01A20.2	9.7	1.7	1.6
WBGene00001484	*fox-1*	9.7	2.2	1.6
WBGene00022745	ZK470.2	9.7	2.6	1.9
WBGene00004749	*sdn-1*	9.7	3.8	2.0

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00020673	T22B7.4	9.7	1.8	2.0
WBGene00018857	F55A4.5	9.7	1.7	2.0
WBGene00008977	F20D1.7	9.7	1.6	3.2
WBGene00009380	F34H10.3	9.7	2.5	3.5
WBGene00008924	F17E5.2	9.7	2.2	3.6
WBGene00004370	*rig-3*	9.7	2.5	4.8
WBGene00019365	K03E6.7	9.6	2.4	1.7
WBGene00010221	F57G12.1	9.6	1.6	1.9
WBGene00007666	C18B12.4	9.6	2.3	1.9
WBGene00010700	K09A9.1	9.6	2.4	2.3
WBGene00016106	C25F6.7	9.6	2.3	3.5
WBGene00007174	B0395.2	9.5	1.8	1.7
WBGene00008806	*tag-289*	9.5	2.0	1.8
WBGene00011642	T09B9.1	9.5	1.5	1.8
WBGene00018634	F49E7.2	9.5	1.6	2.0
WBGene00020633	T20F7.6	9.5	2.3	2.1
WBGene00019987	R09F10.3	9.5	2.4	2.1
WBGene00020510	T14G11.1	9.5	3.2	2.4
WBGene00044630	*bus-17*	9.5	2.4	2.5
WBGene00004719	*sad-1*	9.5	1.8	3.0
WBGene00003635	*nhr-45*	9.5	2.7	3.1
WBGene00019729	M02D8.3	9.4	1.7	1.5
WBGene00022199	Y71H10A.1	9.4	2.7	1.6
WBGene00001082	*dpy-23*	9.4	2.2	2.2

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00001821	*ham-2*	9.4	1.7	3.0
WBGene00011444	T04F8.6	9.4	2.0	3.2
WBGene00007520	C11E4.6	9.4	2.5	3.4
WBGene00020919	*set-19*	9.4	1.7	3.7
WBGene00044724	R02E4.3	9.3	3.8	1.5
WBGene00000902	*daf-6*	9.3	1.7	1.6
WBGene00009259	F29G6.3	9.3	2.0	1.9
WBGene00007941	C34F6.7	9.3	1.6	2.1
WBGene00006412	*nlg-1*	9.3	1.8	2.5
WBGene00007266	C03A3.1	9.3	1.7	2.6
WBGene00010776	*pix-1*	9.3	2.5	3.1
WBGene00000277	*cab-1*	9.3	3.1	4.9
WBGene00016423	*tag-275*	9.2	1.7	1.8
WBGene00019780	M60.4	9.2	6.1	2.1
WBGene00020012	R11G1.6	9.2	1.8	2.4
WBGene00012073	T27A8.1	9.2	2.0	3.1
WBGene00009482	F36G3.1	9.1	1.6	1.6
WBGene00015751	C14A11.6	9.1	2.0	1.7
WBGene00006861	*uvt-2*	9.1	2.0	1.7
WBGene00010605	K06G5.1	9.1	1.5	1.8
WBGene00006742	*unc-2*	9.1	2.2	2.7
WBGene00006371	*syn-1*	9.1	2.7	3.1
WBGene00006896	*ver-3*	9.1	2.3	3.2
WBGene00003745	*nlp-7*	9.0	1.8	2.1

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00007524	C11G6.3	9.0	2.3	2.2
WBGene00008940	F18H3.4	9.0	2.6	2.4
WBGene00020920	*set-20*	9.0	2.0	2.4
WBGene00016937	*tag-294*	9.0	2.0	2.4
WBGene00006646	*tsp-20*	9.0	2.1	2.6
WBGene00000103	*akt-2*	9.0	2.8	3.1
WBGene00000526	*clc-5*	9.0	2.2	3.3
WBGene00003245	*mig-13*	9.0	2.3	3.5
WBGene00019872	R04E5.9	8.9	2.4	1.6
WBGene00008749	F13E6.5	8.9	2.0	1.8
WBGene00009801	F47A4.5	8.9	3.4	1.8
WBGene00006637	*tsp-11*	8.9	1.8	1.8
WBGene00021059	W06B11.1	8.9	1.9	2.1
WBGene00006602	*tps-1*	8.9	3.3	2.4
WBGene00018702	F52E4.5	8.9	2.7	2.5
WBGene00020504	T14E8.1	8.9	2.1	2.6
WBGene00000406	*cdk-4*	8.9	2.6	2.8
WBGene00007877	C33A11.1	8.9	3.1	3.1
WBGene00006364	*syd-2*	8.9	1.8	3.3
WBGene00018175	F38B6.6	8.9	1.8	3.4
WBGene00000462	*ceh-41*	8.8	3.2	1.5
WBGene00006635	*tsp-9*	8.8	2.1	1.9
WBGene00016716	*acs-17*	8.8	1.5	2.1
WBGene00015141	*ugt-46*	8.8	1.8	2.5

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00007668	C18B12.6	8.8	1.9	3.1
WBGene00019346	K02G10.5	8.8	1.8	3.4
WBGene00009800	*rrc-1*	8.8	1.9	3.4
WBGene00009552	F39B1.1	8.8	1.6	3.5
WBGene00000282	*cah-4*	8.7	1.6	1.5
WBGene00020158	T02C5.1	8.7	2.5	1.6
WBGene00003616	*nhr-17*	8.7	1.8	1.7
WBGene00020143	T01C8.2	8.7	2.4	1.7
WBGene00016188	*sec-15*	8.7	1.7	2.0
WBGene00044330	*alr-1*	8.7	2.0	2.2
WBGene00003885	*osm-5*	8.7	1.9	2.2
WBGene00001462	*flp-19*	8.7	2.0	2.7
WBGene00009234	F28H6.6	8.7	2.6	3.0
WBGene00018837	F54G2.1	8.7	3.5	3.0
WBGene00001490	*frm-3*	8.7	2.7	3.1
WBGene00002239	*ksr-1*	8.7	2.8	3.4
WBGene00003741	*nlp-3*	8.6	1.9	1.6
WBGene00010702	K09A9.4	8.6	1.7	2.4
WBGene00010553	K04C1.3	8.6	2.1	2.5
WBGene00016131	C26B9.1	8.6	1.6	3.0
WBGene00003623	*nhr-25*	8.6	2.1	3.2
WBGene00000047	*acr-8*	8.6	1.7	3.4
WBGene00018717	F52H2.5	8.5	1.8	1.7
WBGene00012443	Y15E3A.4	8.5	1.8	1.8

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00007592	C14H10.2	8.5	1.6	1.9
WBGene00016102	C25F6.1	8.5	1.6	2.0
WBGene00000908	*daf-12*	8.5	2.6	2.1
WBGene00008206	*set-6*	8.5	1.6	2.1
WBGene00000222	*atf-6*	8.5	1.9	2.8
WBGene00001638	*gly-13*	8.5	2.4	2.9
WBGene00007772	*egrh-1*	8.5	2.7	3.1
WBGene00016898	C53C9.2	8.4	2.2	2.0
WBGene00003561	*ncr-1*	8.4	2.0	1.7
WBGene00006517	*tag-172*	8.4	1.6	2.0
WBGene00004789	*sgk-1*	8.4	1.9	2.0
WBGene00007956	C35C5.6	8.4	1.8	2.4
WBGene00001335	*erp-1*	8.4	2.2	2.5
WBGene00007940	C34F6.6	8.4	1.8	2.6
WBGene00017866	*stn-2*	8.4	1.8	2.6
WBGene00001116	*dyc-1*	8.4	2.3	2.8
WBGene00007943	C34F6.9	8.4	2.0	3.4
WBGene00016661	C45B2.6	8.3	1.7	1.6
WBGene00016620	C43H6.7	8.3	1.5	1.8
WBGene00017513	F16F9.1	8.3	1.7	1.8
WBGene00009617	F41E7.1	8.3	2.1	1.9
WBGene00015177	B0416.1	8.3	1.6	1.9
WBGene00003891	*osm-11*	8.3	3.2	2.1
WBGene00008926	F17H10.2	8.3	2.0	2.2

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00010120	F55G7.2	8.2	2.0	1.5
WBGene00020426	T10H10.2	8.2	1.7	1.6
WBGene00003517	*nac-1*	8.2	1.6	1.6
WBGene00019848	R03G5.7	8.2	2.3	2.0
WBGene00020884	T28B4.1	8.2	2.3	2.2
WBGene00017421	F13B9.6	8.2	1.7	2.6
WBGene00002991	*lin-2*	8.2	2.2	2.8
WBGene00007652	C17G1.5	8.2	1.7	2.9
WBGene00004145	*pqn-62*	8.2	2.9	5.0
WBGene00008956	*nekl-3*	8.1	1.8	1.5
WBGene00006650	*tts-1*	8.1	10.0	1.7
WBGene00004238	*ptr-24*	8.1	1.8	1.8
WBGene00002975	*lev-8*	8.1	1.9	1.9
WBGene00020508	T14F9.2	8.1	1.9	1.9
WBGene00016345	C33E10.1	8.1	2.6	2.0
WBGene00018639	F49E10.1	8.1	1.7	2.3
WBGene00022412	Y102A11A.2	8.1	2.3	2.5
WBGene00020330	T07H6.1	8.1	2.1	2.7
WBGene00014215	*obr-3*	8.1	3.5	2.7
WBGene00009886	F49E2.2	8.1	1.9	3.2
WBGene00000524	clc-3	8.0	2.2	1.9
WBGene00021051	W05H9.4	8.0	1.9	2.1
WBGene00004036	*plc-1*	8.0	2.1	3.1
WBGene00015805	C15H9.11	7.9	1.9	1.6

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00011154	R09A8.5	7.9	1.7	2.1
WBGene00019781	M60.6	7.9	2.3	2.2
WBGene00014294	C53C7.5	7.9	3.1	2.5
WBGene00020294	T06F4.1	7.9	3.0	2.8
WBGene00003368	*mkk-4*	7.8	2.2	1.6
WBGene00017427	F13D11.1	7.8	1.7	1.7
WBGene00006862	*uvt-3*	7.8	1.9	1.8
WBGene00020596	*oga-1*	7.8	2.0	2.1
WBGene00001608	R07B1.8	7.8	1.8	2.1
WBGene00003084	*lst-2*	7.8	2.0	2.2
WBGene00018689	F52D2.7	7.8	5.0	2.4
WBGene00001681	*gpc-1*	7.8	2.2	2.5
WBGene00004033	*pkc-2*	7.8	2.3	2.7
WBGene00017478	F15A8.6	7.8	1.7	2.8
WBGene00001674	*gpa-12*	7.8	2.4	2.9
WBGene00011073	R07A4.2	7.8	2.2	3.3
WBGene00004345	*rgs-2*	7.8	3.2	3.3
WBGene00021320	Y34B4A.4	7.7	1.9	1.7
WBGene00006068	*sto-6*	7.7	1.8	1.6
WBGene00016866	*coel-1*	7.7	2.9	1.6
WBGene00019957	R08E3.1	7.7	1.6	1.8
WBGene00000116	*alh-10*	7.7	1.6	2.3
WBGene00010338	F59F4.3	7.7	2.0	2.9
WBGene00001451	*flp-8*	7.7	1.9	3.0

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00006372	*syn-2*	7.7	3.4	3.3
WBGene00019959	R08E3.3	7.6	1.6	1.5
WBGene00043981	T14E8.4	7.6	2.2	1.8
WBGene00022744	ZK470.1	7.6	2.0	1.8
WBGene00003232	*mgl-1*	7.6	1.7	2.2
WBGene00018281	F41D9.1	7.6	1.7	2.2
WBGene00006981	*zig-4*	7.6	1.9	2.3
WBGene00013869	ZC373.4	7.6	1.9	3.2
WBGene00002177	*jkk-1*	7.6	2.1	4.0
WBGene00010329	F59D12.1	7.6	2.4	4.1
WBGene00020196	T03G11.3	7.5	2.1	1.5
WBGene00009932	F52D10.6	7.5	1.8	1.5
WBGene00008093	C44H4.1	7.5	1.8	1.8
WBGene00003394	*mom-1*	7.5	3.7	1.9
WBGene00015948	C18A11.2	7.5	1.8	2.1
WBGene00010903	M79.3	7.5	1.8	3.3
WBGene00000086	*aex-3*	7.5	2.2	3.5
WBGene00019737	M02F4.3	7.5	2.5	3.8
WBGene00003412	*mrp-6*	7.5	2.7	4.0
WBGene00010225	*ttr-31*	7.4	4.0	1.7
WBGene00001149	*bcat-1*	7.4	4.5	2.0
WBGene00000522	*clc-1*	7.4	3.1	2.4
WBGene00007694	C23H4.6	7.4	1.7	2.6
WBGene00006640	*tsp-14*	7.4	3.0	2.7

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00000523	*clc-2*	7.4	2.4	3.3
WBGene00000441	*ceh-18*	7.4	3.6	5.6
WBGene00003733	*nhx-5*	7.3	1.7	1.6
WBGene00010724	K09E9.1	7.3	1.8	1.6
WBGene00007906	C33G3.6	7.3	2.1	1.9
WBGene00019844	*abts-4*	7.3	2.0	1.9
WBGene00018374	*tag-343*	7.3	1.8	2.0
WBGene00006477	*tag-130*	7.3	1.6	2.0
WBGene00020339	T08A9.11	7.3	5.1	2.3
WBGene00014213	ZK1073.1	7.2	4.5	4.1
WBGene00009529	F38B2.2	7.2	1.6	1.7
WBGene00002163	*ist-1*	7.2	2.1	1.8
WBGene00018031	F35B3.4	7.2	5.9	2.0
WBGene00001690	*grd-1*	7.2	1.5	2.0
WBGene00001517	*gar-1*	7.2	1.6	2.1
WBGene00011076	R07B1.3	7.2	1.7	2.1
WBGene00008451	E01G6.3	7.2	1.6	2.2
WBGene00018171	F38B6.1	7.2	1.7	2.2
WBGene00001560	*gei-3*	7.2	2.4	2.6
WBGene00010009	F53H4.4	7.2	4.6	3.1
WBGene00018731	F53A9.8	7.1	4.1	1.6
WBGene00018860	F55A4.8	7.1	1.6	1.7
WBGene00017422	F13C5.1	7.1	2.0	3.4
WBGene00006642	*tsp-16*	7.1	1.8	3.7

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00019749	M03A8.3	7.1	2.9	5.1
WBGene00019518	K08B5.1	7.0	1.7	1.5
WBGene00001450	*flp-7*	7.0	2.4	1.8
WBGene00014140	ZK899.1	7.0	2.7	2.1
WBGene00022668	ZK154.6	7.0	1.7	2.3
WBGene00019871	R04E5.8	7.0	3.4	2.4
WBGene00012225	W03G11.3	7.0	1.7	2.4
WBGene00003166	*mec-2*	6.9	1.6	1.7
WBGene00020844	T27A10.2	6.9	1.6	1.7
WBGene00006750	*unc-10*	6.9	1.9	2.8
WBGene00000035	*ace-1*	6.9	2.0	2.8
WBGene00000072	*add-1*	6.9	1.6	4.2
WBGene00000510	*cka-2*	6.8	1.9	1.6
WBGene00011824	T18D3.7	6.8	1.6	1.7
WBGene00003124	*mai-1*	6.8	3.0	1.8
WBGene00016254	*fbxb-114*	6.8	1.6	2.0
WBGene00016894	C53B7.3	6.7	7.4	1.8
WBGene00001619	*glr-8*	6.7	1.6	1.9
WBGene00019828	R02E12.4	6.7	1.8	1.9
WBGene00010716	*lge-1*	6.7	1.7	2.6
WBGene00006792	*unc-58*	6.6	2.8	2.1
WBGene00021529	Y41G9A.5	6.6	1.7	2.2
WBGene00007813	C29F7.6	6.6	4.1	2.6
WBGene00010334	F59F3.4	6.6	1.9	2.9

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00017176	F02E8.2	6.5	1.9	1.7
WBGene00017861	F27D9.2	6.5	2.1	1.9
WBGene00003408	*mrp-2*	6.5	2.6	2.0
WBGene00022413	Y102A11A.3	6.5	3.7	2.4
WBGene00000083	*adt-2*	6.5	2.2	3.0
WBGene00009819	F47B10.8	6.5	2.9	5.5
WBGene00016438	C35B8.3	6.4	1.6	1.5
WBGene00017400	F12D9.1	6.4	1.9	1.6
WBGene00007751	C26G2.2	6.4	1.6	1.6
WBGene00015921	C17H11.1	6.4	1.5	1.6
WBGene00015294	C01C10.2	6.4	1.6	1.7
WBGene00006980	*zig-3*	6.4	6.2	2.0
WBGene00006870	*vab-3*	6.4	2.0	2.3
WBGene00017149	EGAP4.1	6.3	1.9	1.5
WBGene00011765	T14C1.1	6.3	3.1	1.6
WBGene00016533	C39D10.6	6.3	1.6	1.8
WBGene00021527	Y41G9A.3	6.3	1.9	1.9
WBGene00019345	K02G10.3	6.3	2.2	2.2
WBGene00003530	*nas-11*	6.2	3.2	1.5
WBGene00017898	F28C10.3	6.2	1.6	1.7
WBGene00005129	*srd-51*	6.2	3.3	2.0
WBGene00004008	*pgp-14*	6.2	2.5	2.1
WBGene00019612	K10B3.6	6.2	1.8	2.6
WBGene00004285	*rab-37*	6.2	1.7	2.8

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Increase in *sdc-2* Mutant	[3]Fold Increase in *dpy-27* Mutant
WBGene00017691	*ilys-5*	6.1	1.6	1.6
WBGene00017157	*tyra-2*	6.1	1.8	1.8
WBGene00007958	C35C5.9	6.1	1.9	1.8
WBGene00015822	C16B8.2	6.1	2.1	4.3
WBGene00017108	*cyp-43A1*	6.0	1.6	1.5
WBGene00017449	F14B8.7	6.0	1.7	2.4
WBGene00022200	Y71H10A.2	5.9	2.3	1.6
WBGene00007285	C04A11.1	5.9	2.2	2.1
WBGene00016659	C45B2.2	5.9	27.5	2.9
WBGene00018257	F41B4.1	5.8	3.5	3.0
WBGene00006864	*uvt-6*	5.7	1.7	1.6
WBGene00015947	C18A11.1	5.7	1.7	1.6
WBGene00015182	B0416.7	5.6	1.6	1.7
WBGene00008746	F13E6.2	5.6	2.0	2.6
WBGene00020214	T04G9.1	5.6	1.6	3.1
WBGene00016662	C45B2.8	5.5	4.0	1.8

[1] $\log_2$ of RMA-normalized array probe set intensities (see Methods)

[2] Fold Increase in *sdc-2* Mutant

Calculation: $2^{[\log_2 \text{probe intensity of } sdc\text{-}2 \text{ XX mutant}] - [\log_2 \text{probe intensity of XX wild-type embryo}]}$

Mean 2.2 ± 0.04 SEM; Median 1.9; Minimum 1.5; Maximum 10; Count 374

The outlier gene C45B2.2 (fold increase of 27.5) was not included in the calculation.

[3] Fold Increase in *dpy-27* Mutant

Calculation: $2^{[\log_2 \text{probe intensity of } dpy\text{-}27 \text{ XX mutant}] - [\log_2 \text{probe intensity of XX wild-type embryo}]}$

Mean 2.4 ± 0.04 SEM; Median 2.2; Minimum 1.5; Maximum 5.6; Count 374

Supplemental Table 5

Non-Compensated Genes

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00022219	Y73B3A.18	13.2	1.6
WBGene00004438	*rpl-25.1*	13.1	1.5
WBGene00003963	*pdi-2*	13.1	2.3
WBGene00002007	*hsp-3*	12.8	2.5
WBGene00000503	*cht-1*	12.8	1.5
WBGene00004423	*rpl-11.2*	12.5	1.5
WBGene00018519	F46H5.3	12.4	1.5
WBGene00001828	*hch-1*	12.4	1.6
WBGene00001854	*hil-3*	12.4	1.9
WBGene00002253	*lbp-1*	12.3	1.6
WBGene00000738	*col-165*	12.3	2.4
WBGene00015168	*tag-320*	12.3	1.5
WBGene00020366	T08G2.3	12.2	1.6
WBGene00006494	*hke-4.2*	12.2	1.6
WBGene00020738	T23F2.5	11.9	1.5
WBGene00020812	T25G12.5	11.8	1.8
WBGene00001065	*dpy-3*	11.7	2.2
WBGene00019760	*calu-1*	11.6	1.5
WBGene00006948	*wrt-2*	11.5	1.8
WBGene00001069	*dpy-7*	11.5	2.0
WBGene00006366	*sym-1*	11.5	2.4

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00007350	C05G5.4	11.5	1.9
WBGene00017864	*pcca-1*	11.4	3.3
WBGene00006063	*sto-1*	11.4	1.9
WBGene00012158	*ucr-2.1*	11.3	1.5
WBGene00000210	*asg-2*	11.3	1.9
WBGene00007848	C31E10.7	11.3	1.6
WBGene00015781	C14F11.6	11.3	2.1
WBGene00012255	W04G3.1	11.2	1.7
WBGene00000040	*aco-1*	11.1	1.6
WBGene00011229	R11.4	11.1	2.4
WBGene00006584	*tni-1*	11.1	1.7
WBGene00002280	*let-2*	11.1	1.7
WBGene00008215	C49F8.3	11.0	1.7
WBGene00008975	F20D1.3	10.9	1.5
WBGene00022665	ZK154.1	10.8	2.4
WBGene00000157	*aps-2*	10.8	1.8
WBGene00003495	*mup-2*	10.7	1.6
WBGene00008973	F20D1.1	10.7	2.0
WBGene00008586	F08G12.2	10.7	1.5
WBGene00012257	*lpr-4*	10.6	1.9
WBGene00006321	*sup-12*	10.6	1.9
WBGene00001520	*gas-1*	10.6	2.0
WBGene00009812	F47B10.1	10.6	1.7
WBGene00002255	*lbp-3*	10.6	1.8

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00003828	*nuc-1*	10.6	1.8
WBGene00015474	C05D9.7	10.6	1.5
WBGene00007068	6R55.1	10.6	1.9
WBGene00003149	*mbk-1*	10.5	1.9
WBGene00020194	T03G6.1	10.5	1.8
WBGene00004826	*skr-20*	10.5	2.4
WBGene00003776	*nmy-1*	10.5	1.7
WBGene00009851	F48F7.6	10.5	1.6
WBGene00016103	*dpyd-1*	10.5	1.7
WBGene00015565	C07D8.6	10.5	2.8
WBGene00004148	*pqn-65*	10.4	2.6
WBGene00018656	F49H12.5	10.4	4.3
WBGene00017307	F09F9.2	10.4	1.7
WBGene00000207	*asb-2*	10.4	1.5
WBGene00007129	B0272.3	10.4	1.8
WBGene00004270	*rab-6.2*	10.3	1.6
WBGene00002060	*ife-2*	10.3	2.3
WBGene00019748	*atg-2*	10.3	1.6
WBGene00000929	*dao-3*	10.3	1.7
WBGene00000294	*cas-1*	10.3	1.7
WBGene00017380	F11D5.1	10.3	1.7
WBGene00017244	*apy-1*	10.3	2.4
WBGene00003171	*mec-7*	10.2	1.9
WBGene00012256	W04G3.2	10.2	1.7

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00002071	*ile-2*	10.2	1.6
WBGene00016489	C36E6.1	10.2	2.3
WBGene00007942	C34F6.8	10.2	1.6
WBGene00015791	C15C7.5	10.2	1.7
WBGene00020496	*spat-3*	10.1	1.9
WBGene00020334	*atg-11*	10.1	1.6
WBGene00006890	*vem-1*	10.1	2.0
WBGene00009674	*nucb-1*	10.1	2.1
WBGene00001425	*fis-2*	10.1	1.9
WBGene00011679	*ucr-2.2*	10.0	1.6
WBGene00019272	H42K12.3	10.0	2.3
WBGene00019250	H28G03.2	10.0	2.4
WBGene00018682	F52D1.1	10.0	1.6
WBGene00009574	F40E10.6	10.0	1.5
WBGene00008605	*mlt-9*	10.0	1.9
WBGene00016934	C54G7.2	10.0	1.5
WBGene00000457	*ceh-36*	10.0	2.0
WBGene00002062	*ife-4*	10.0	1.6
WBGene00011104	*cut-5*	9.9	1.6
WBGene00018878	*glit-1*	9.9	2.3
WBGene00009233	F28H6.4	9.9	1.5
WBGene00008260	C52G5.2	9.9	2.0
WBGene00007812	C29F7.3	9.9	1.6
WBGene00022611	ZC449.1	9.8	2.0

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00019656	K11G12.5	9.8	1.5
WBGene00018853	F55A4.1	9.8	1.7
WBGene00018710	F52G3.1	9.8	2.3
WBGene00018485	F46C8.3	9.8	1.8
WBGene00018193	F39C12.1	9.8	1.5
WBGene00017571	*tag-279*	9.8	1.5
WBGene00017419	F13B9.1	9.8	1.7
WBGene00002023	*hsp-25*	9.8	1.6
WBGene00010433	H40L08.1	9.7	1.6
WBGene00004121	*pqn-34*	9.7	1.6
WBGene00015546	C06G1.2	9.7	2.0
WBGene00015529	C06E2.5	9.7	1.7
WBGene00006764	*unc-27*	9.6	1.6
WBGene00011938	T22H6.2	9.6	1.9
WBGene00010835	M03B6.3	9.6	2.2
WBGene00000164	*apm-3*	9.6	1.6
WBGene00008974	F20D1.2	9.6	1.8
WBGene00015561	C07A12.7	9.6	2.0
WBGene00021044	W05H7.1	9.5	2.1
WBGene00012258	W04G3.5	9.5	1.6
WBGene00020199	T03G11.6	9.5	1.5
WBGene00001583	*gfi-3*	9.5	2.0
WBGene00010725	*erv-46*	9.5	1.6
WBGene00023497	*lin-15B*	9.4	2.3

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00010924	M153.1	9.4	2.0
WBGene00001572	*gei-15*	9.4	1.5
WBGene00010115	*aakb-1*	9.4	1.8
WBGene00004745	*sdc-1*	9.4	2.4
WBGene00009531	F38B2.4	9.4	1.6
WBGene00008979	F20D1.9	9.4	1.6
WBGene00008882	F16B12.6	9.4	1.6
WBGene00015778	C14F11.1	9.4	2.2
WBGene00020083	R57.2	9.3	1.9
WBGene00000461	*ceh-40*	9.3	2.5
WBGene00008865	F15G9.1	9.3	1.6
WBGene00017241	F08C6.2	9.3	1.5
WBGene00001435	*fkh-3*	9.3	2.4
WBGene00020765	T24C12.3	9.2	1.9
WBGene00019727	M02D8.1	9.2	2.0
WBGene00010691	K08H2.4	9.2	1.6
WBGene00004769	*sel-12*	9.2	1.7
WBGene00006432	*tag-53*	9.2	1.6
WBGene00002058	*ifd-2*	9.2	1.5
WBGene00000884	*cyn-8*	9.2	1.7
WBGene00016867	C52B9.4	9.2	1.5
WBGene00003778	*nnt-1*	9.2	1.6
WBGene00006393	*taf-11.1*	9.1	2.0
WBGene00004356	*rhi-1*	9.1	1.8

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00002037	*hum-4*	9.1	1.7
WBGene00003990	*pfn-2*	9.1	2.1
WBGene00001025	*dnj-7*	9.1	1.7
WBGene00019938	R07E4.5	9.0	1.9
WBGene00010519	K03A11.1	9.0	2.0
WBGene00016406	C34D10.1	9.0	12.4
WBGene00007811	C29F7.2	9.0	1.8
WBGene00007348	C05G5.2	9.0	1.5
WBGene00007284	C03H12.1	9.0	1.5
WBGene00022504	ZC13.3	8.9	1.6
WBGene00002031	*hst-6*	8.9	1.5
WBGene00020322	T07F12.2	8.9	1.6
WBGene00011226	R11.1	8.9	1.8
WBGene00008691	F11C1.2	8.9	1.7
WBGene00000988	*dhs-25*	8.9	2.0
WBGene00015623	C09B8.4	8.9	2.5
WBGene00015429	*sor-3*	8.9	1.5
WBGene00003681	*nhr-91*	8.8	1.6
WBGene00011107	R07E3.6	8.8	2.6
WBGene00009067	F23A7.1	8.8	1.7
WBGene00006922	*vhl-1*	8.8	1.9
WBGene00007847	C31E10.6	8.8	1.7
WBGene00022769	ZK563.5	8.7	1.5
WBGene00022666	ZK154.4	8.7	1.9

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00021319	Y34B4A.2	8.7	1.5
WBGene00007011	*mdt-1.2*	8.7	1.8
WBGene00011820	T18D3.1	8.7	2.8
WBGene00010981	R03A10.1	8.7	1.9
WBGene00010552	K04C1.2	8.7	1.6
WBGene00000195	*arr-1*	8.7	1.5
WBGene00016643	*vps-45*	8.7	2.1
WBGene00001436	*fkh-4*	8.7	2.7
WBGene00019867	R04B3.2	8.6	1.8
WBGene00010726	K09E9.3	8.6	1.8
WBGene00022667	ZK154.5	8.5	1.7
WBGene00012445	Y16B4A.2	8.5	1.7
WBGene00021046	*sedl-1*	8.5	1.5
WBGene00000245	*bca-1*	8.5	1.8
WBGene00019318	K02E10.4	8.5	2.2
WBGene00016913	*lam-2*	8.5	1.9
WBGene00008207	C49F5.3	8.5	1.7
WBGene00001981	*hnd-1*	8.5	2.7
WBGene00015138	B0310.2	8.5	2.0
WBGene00010834	M03B6.2	8.4	1.7
WBGene00008959	F19H6.4	8.4	2.2
WBGene00008690	F11C1.1	8.4	1.8
WBGene00000391	*cdd-1*	8.4	2.0
WBGene00004335	*ref-2*	8.4	1.7

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00015866	C16E9.2	8.4	1.7
WBGene00022827	ZK816.4	8.3	1.9
WBGene00020571	T19D7.3	8.3	1.7
WBGene00011012	R04D3.4	8.3	2.6
WBGene00010984	*nkat-3*	8.3	1.8
WBGene00019609	K10B3.1	8.3	2.3
WBGene00004377	*rme-6*	8.3	1.6
WBGene00000451	*ceh-30*	8.3	1.5
WBGene00019989	R09F10.8	8.2	2.3
WBGene00010339	*acl-1*	8.2	1.5
WBGene00015548	C06G1.5	8.2	1.5
WBGene00015472	C05D9.3	8.2	1.9
WBGene00022647	ZK54.1	8.1	1.8
WBGene00018236	*fbxb-71*	8.1	2.9
WBGene00016871	*tag-303*	8.1	1.8
WBGene00009483	F36G3.2	8.0	1.5
WBGene00017340	F10D7.3	8.0	1.9
WBGene00012299	W06D11.1	7.9	2.0
WBGene00011139	R08B4.3	7.9	1.7
WBGene00019584	*set-12*	7.9	1.5
WBGene00004127	*pqn-40*	7.9	1.5
WBGene00006535	*tba-9*	7.9	2.1
WBGene00017530	F16H11.1	7.9	1.5
WBGene00016068	C24H10.2	7.9	1.5

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00006496	*tag-150*	7.9	1.5
WBGene00022207	Y73B3A.5	7.9	1.7
WBGene00010066	F54F7.6	7.8	2.9
WBGene00018713	F52G3.5	7.8	1.7
WBGene00000745	*col-172*	7.8	2.0
WBGene00007267	C03A3.2	7.8	2.1
WBGene00021324	Y34B4A.8	7.7	1.5
WBGene00020672	T22B7.3	7.7	1.6
WBGene00011011	R04D3.3	7.7	2.2
WBGene00009820	F47B10.9	7.7	1.6
WBGene00009560	*psa-3*	7.7	1.5
WBGene00008880	F16B12.4	7.7	1.5
WBGene00000075	*adm-4*	7.6	1.5
WBGene00003883	*osm-1*	7.6	1.5
WBGene00010397	H13N06.4	7.6	1.8
WBGene00018077	F35H12.5	7.6	1.5
WBGene00008954	F19C6.5	7.6	1.5
WBGene00011981	T24C2.2	7.5	2.4
WBGene00003943	*pbo-4*	7.5	2.6
WBGene00010689	K08H2.2	7.5	3.3
WBGene00010222	F57G12.2	7.5	1.8
WBGene00003369	*mlc-1*	7.5	1.7
WBGene00006462	*tag-97*	7.5	1.6
WBGene00007286	C04A11.2	7.5	1.7

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00000993	*dhs-30*	7.4	2.4
WBGene00020665	T22B2.1	7.4	4.1
WBGene00010968	R01E6.2	7.4	1.9
WBGene00019403	K05B2.2	7.4	1.6
WBGene00003410	*mrp-4*	7.4	1.7
WBGene00008094	C44H4.4	7.4	1.8
WBGene00003007	*lin-18*	7.4	1.6
WBGene00004927	*snx-1*	7.4	2.3
WBGene00012301	W06D11.3	7.3	3.5
WBGene00020509	*hex-1*	7.3	1.8
WBGene00000755	*col-182*	7.3	1.9
WBGene00020768	T01B6.1	7.3	2.1
WBGene00020630	T20F7.1	7.2	2.0
WBGene00010971	R01E6.7	7.2	1.8
WBGene00008747	F13E6.3	7.2	1.7
WBGene00016189	C28G1.4	7.2	1.9
WBGene00015363	*dyf-11*	7.2	1.6
WBGene00003018	*lin-32*	7.1	1.5
WBGene00003169	*mec-5*	7.0	1.6
WBGene00014143	ZK899.5	6.9	3.0
WBGene00017473	F14H12.6	6.9	2.2
WBGene00016104	*ddr-1*	6.9	2.3
WBGene00015800	C15H9.4	6.9	1.6
WBGene00007347	C05G5.1	6.9	1.5

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00015180	B0416.4	6.9	2.8
WBGene00012300	W06D11.2	6.8	2.3
WBGene00011081	R07B1.9	6.8	1.7
WBGene00004354	*rgs-11*	6.8	2.0
WBGene00019554	K09C4.10	6.7	1.6
WBGene00009088	F23D12.4	6.7	2.8
WBGene00017298	*toca-1*	6.7	1.8
WBGene00015586	C08A9.6	6.7	1.6
WBGene00000797	*crn-4*	6.7	1.9
WBGene00018522	F46H5.7	6.6	1.6
WBGene00003624	*nhr-28*	6.6	1.8
WBGene00003843	*oct-2*	6.5	1.9
WBGene00010690	K08H2.3	6.5	1.8
WBGene00018026	F35A5.4	6.5	1.5
WBGene00001537	*gcy-11*	6.5	1.9
WBGene00004004	*pgp-10*	6.4	1.5
WBGene00019347	K02H8.1	6.3	1.7
WBGene00017049	D2021.8	6.3	1.5
WBGene00004126	*pqn-39*	6.2	1.9
WBGene00015787	C15B12.6	6.1	1.7
WBGene00022292	Y75D11A.3	6.0	1.7
WBGene00018739	F53B1.8	6.0	1.5
WBGene00008464	E02H4.6	6.0	1.7
WBGene00015713	C12D12.1	6.0	1.6

WormBase Identification	Gene Name	[1]Expression Level in Wild-Type XX Embryos	[2]Fold Decrease in XO Embryos
WBGene00022291	Y75D11A.2	5.7	1.6
WBGene00017728	F22H10.4	5.6	1.9
WBGene00007327	C05C9.3	5.5	1.6
WBGene00018856	F55A4.4	5.2	1.6
WBGene00019576	K09E3.5	5.1	1.5

[1] $\log_2$ of RMA-normalized array probe set intensities (see Methods)

[2] Fold Decrease in XO Embryos

Calculation: $2^{[\log_2 \text{probe intensity of XX embryo}] - [\log_2 \text{probe intensity of XO embryo}]}$

Mean 1.9 ± 0.02 SEM; Median 1.7; Minimum 1.5; Maximum 4.3; Count 290

The outlier gene C34D10.1 (fold decrease 12.4) was not included in the calculation.

Supplemental Table 6

Class	DCC Peak	Gene	Fold Increase in Expression in DC mutant	Fold Decrease in Expression in XO embryo
DC	Yes	*grk-1*	3.10 avg ± 0.29	
DC	Yes	F54B11.3	3.71 avg ± 0.63	
DC	No	C18A11.1	4.65 avg ± 1.01	
DC	No	R08E3.1	1.31 avg ± 0.14	
Control	No	*fasn-1*	1.15 avg ± 0.12	
non-DC	Yes	*pfn-2*		1.72 avg ± 0.15
non-DC	Yes	*ats-11*		2.17 avg ± 0.24
non-DC	No	F45B8.1		4.00 avg ± 0.32
non-DC	No	W06D11.2		4.35 avg ± 0.76
Control	No	*fasn-1*		1.32 avg ± 0.14

qRT-PCR measurements confirm classification of genes as dosage compensated or non-compensated based on expression studies using Affymetrix expression arrays.

Levels of dosage-compensated (DC) or non-compensated (non-DC) gene transcripts were measured by qRT-PCR in *sdc-2* XX mutant embryos or XO embryos, respectively. (See Methods and van Gilst et al. (2005)). Genes with and without DCC peaks in their promoters were chosen. The transcript levels are expressed as the fold change compared to the transcript levels in wild-type XX embryos. For example, an average value of 3.1 means that 3.1-fold as many gene-specific transcripts were measured in mutant versus wild-type XX embryos. All transcript levels were normalized to the levels of the control gene *nhr-64*, an autosomal gene not affected by dosage compensation genes. *fasn-1*, another autosomal gene not affected by dosage compensation, was used as a control to gauge the variability and reliability of

measurements made using qRT-PCR. Experimental error is expressed as standard deviation of the mean. Three independent embryo preparations were measured and averaged for each gene in each genotype.

Supplemental methods

RNA preparation

Gravid adults were bleached and embryos harvested as described (Portman, D.S, Wombook, *Profiling C. elegans gene expression with DNA microarrays*) and 100 µl aliquots snap frozen in liquid nitrogen and stored at -80°C. An aliquot was thawed at 65°C for 10 minutes, resuspended in 1ml Trizol (Invitrogen, Carlsbad, CA), vortexed vigorously to resuspend, and incubated for 20 minutes at room temperature. The sample was then centrifuged for 15 minutes at 12,000xg at 4°C. The aqueous phase was transferred to a new tube and 100 µl of BCP (1-bromo-3-chloropropane) (MRC, Cincinnati, OH) was added, the sample was mixed by vortexing, incubated for 15 minutes at room temperature, and centrifuged for 10 minutes at 12,000xg at 4°C. The aqueous phase was transferred to a new tube and nucleic acid was precipitated by addition of 500 µl of isopropanol. The sample was incubated for 10 minutes at room temperature, centrifuged at 12,000xg for 8 minutes, washed in 70% ethanol and air dried for 5 minutes. Nucleic acid was resuspended in 100 µl nuclease-free water and RNA quantified with a UV spectrophotometer. One hundred micrograms of RNA was then treated with 3 µl RQ1-DNAse (Promega, Madison, WI) and RNA isolated with the RNAeasy kit as per the manufacturer's protocol (Qiagen, Valencia, CA). RNA was again quantified with a UV spectrophotometer and cDNA was prepared from 5 µg RNA using the Protoscript Kit (NEB, Ipswich, MA) and diluted 1:15 in water. Quantitative real time PCR was performed to examine the quality of the isolated RNA. PCR was performed on an Opticon Real-Time PCR Detection System (MJ Research Waltham, MA) using the following conditions: 95°C for 5:00, 94°C for 0:30, 60°C for 0:30, 72°C for 1:00, 78°C for 0:10, plate read, cycling back to step 2 for a total of 40 times.

Chromatin immunoprecipitation from embryo lysates

Embryo isolation and formaldehyde induced protein-DNA cross-linking:

1. Two to five grams of embryos were harvested by bleaching synchronized gravid hermaphrodites (Portman, D.S, Wombook, *Profiling C. elegans gene expression with DNA microarrays*).
2. Embryos were then collected in a 50 ml conical tube by centrifugation at 1000xg for 1 minute. Supernatant was aspirated and the pellet washed three times with 50 ml of M9.
3. Packed embryos were then washed once in 45 ml of 2% (v/v) formaldehyde (ACS grade, EMD Chemicals Inc, San Diego, CA) in M9 (made fresh prior to resuspension). After final centrifugation at 1000xg for 1 minute and aspiration of supernatant, the embryo pellet was resuspended to 50 ml with 2% (v/v) formaldehyde in M9 and rocked at room temperature for 30 minutes.
4. The cross-linked embryos were then washed once with 50 ml of 0.1 M Tris-HCl (pH 7.5) and washed twice with 50 ml of M9.
5. Embryos were then isolated by centrifugation at 1000xg for 1 minute and the supernatant aspirated. Embryos were washed in 50 ml of homogenization buffer. After centrifugation at 1000xg and aspiration of the supernatant, 1 ml of homogenization buffer per gram of packed embryo pellet was added for resuspension and the samples snap frozen in liquid nitrogen. Samples were then stored at -80^{o}C.

Preparation of extract for immunoprecipitation:

6. Five to ten milliliters of cross-linked embryos from step 5 above were thawed on ice and PMSF added to a final concentration of 1 mM.
7. The embryo suspension was sonicated on ice with ten 30 second bursts at 10% power output (Heat System XL2020 Sonicator with a standard 3 mm tapered microtip). A one minute pause between 30 second bursts was allotted to allow the lysate to cool.
8. Embryo debris was isolated by centrifugation at 5000xg for 20 minutes. Supernatant was then transferred to a clean 15 ml conical tube.
9. Sample was sonicated again as described in step 7 to shear the DNA. Debris was isolated by centrifugation at 25,000xg for 20 minutes. The supernatant was collected in 1 ml aliquots.
10. Lysates were incubated with 100 µl IgGsorb (The Enzyme Center, Malden, MA) per ml of lysate, for 30 minutes rocking at 4^{o}C. IgGsorb was removed by centrifugation for 2 minutes at ≤ 500xg. Supernatant was transferred to a new microfuge tube and snap frozen in liquid nitrogen. Samples were then stored at -80^{o}C.

Immunoprecipitation:

11. Embryo lysates from step 10 were thawed on ice and centrifuged at 14,000xg for 10 minutes at 4^{o}C. The supernatant was transferred to a new chilled tube and the DNA concentration was determined using a UV spectrophotometer.
12. Five to 10 µg of affinity purified antibodies were incubated with a volume of embryo lysate containing 2 mg of DNA for 2 hours rocking at 4^{o}C. For mock immunoprecipitation, an identical mass of pre-immune rabbit IgG was used in place of affinity purified antibodies.

    *While incubating, Dynabead Protein A was prepared. For each immunoprecipitation, 25 µl of Dynabead Protein A was aliquoted to a microfuge tube. Each sample was washed four times as per the manufacturer's protocol with 1 ml PBS supplemented with 5 mg/ml BSA (filter sterilized). After the final wash, the Dynabeads were resuspended with an equal volume of PBS+5 mg/ml BSA. The Dynabeads were then left on ice until step 15.
13. Non-specific precipitates were isolated by centrifugation at 14,000xg for 10 minutes at 4^{o}C.
14. The supernatant was transferred to a microfuge tube containing 25 µl of Dynabead Protein A that were prepared in step 12. The tube was then incubated with rocking at 4^{o}C for 30 minutes. Following incubation, samples were centrifuged at 500xg for 2 seconds to recover sample from the lid.
14. The antibody-antigen complex captured on the Dynabeads protein A beads were isolated by placing the tubes on a magnetic rack, as per the manufactures protocol, and the supernatant was then aspirated.
15. The Dynabeads were washed at 4°C eight times using the following buffer changes:

    4 times with 1 ml of ChIP buffer with 100 mM KCl
    2 times with 1 ml of ChIP buffer with 1 M KCl
    2 times with 1 ml of TE.
    The last wash was transferred to a new microfuge tube, the beads captured with the magnetic rack, and the supernatant removed.
16. Two hundred microliters of elution buffer [10 mM Tris-HCl (pH8), 1% (w/v) SDS] was then added and the tube placed on a magnetic rack. The eluate was transferred to a clean

microfuge tube. The elution step was repeated. The two eluates (≈400 µl total vol) were then combined.

*For input samples, 50 µl of embryo supernatant from step 11 was added to 400 µl of elution buffer.

17. Sixteen microliters of 5 M NaCl was added to each input and immunoprecipitated sample and incubated overnight at 65°C to reverse the formaldehyde cross-links.
18. The next morning, 8 µl of 0.5 M EDTA and 16 µl of 1 M Tris-HCl (pH 6.8) were added and mixed. To digest protein, 20 µg of proteinase K (Roche, Indianapolis, IN) was added for 1 hour at 45°C.

Immunoprecipitation cleanup:

19. Immunoprecipitation reactions and input samples from step 18 were extracted twice with 300 µl phenol (equilibrated with TE, pH 8.0), and the aqueous phase retained.
20. Immunoprecipitation reactions and input samples were then extracted once with 300 µl chloroform:isoamyl alcohol (24:1 v/v), and the aqueous phase retained (final volume should be approximately 320 µl).
21. To each tube, 13 µl of 5 M NaCl and 20 µg of glycogen (Ambion, Austin, TX) were added.
22. Seven hundred microliters of 100% ethanol was added and the tube vortexed to mix.
23. Samples were incubated at -80°C for 30 minutes.
24. Samples were then centrifuged at 14,000xg for 15 minutes at 4°C.
25. Supernatant was removed and the pellet washed with 500 µl of ice cold 70% ethanol. Samples were then centrifuged at 14,000xg for 5 minutes at 4°C.
26. Final supernatant was aspirated and centrifuged at 14,000xg for 2 seconds. All liquid was removed with a micropipette and the pellet air dried for 5 minutes.
27. A stock solution of RNase TE was prepared by combining 16.5 µl 10 mg/ml RNase A (Qiagen, Valencia, CA) with 483.5 µl TE. Each sample was resuspended in 30 µl RNase/TE and incubated for 2 hours at 37°C.
28. DNA was purified using a QIAquick PCR purification kit (Qiagen) as per the manufacturer's protocol and eluted in 25 µl of EB elution buffer. The 25 µl eluate was removed from the collection tube and replaced back on the column for a final, second elution.
29. Using a UV spectrophotometer the concentration of DNA of the input samples were determined and the input sample diluted to 20 ng/µl in EB elution buffer. Twenty nanograms of input sample was diluted to 25 µl EB elution buffer and was used in the blunting procedure below.
30. Samples were then stored at -20°C.

Blunting immunoprecipitated DNA ends:

31. Added to each the 25 µl immunoprecipitation sample:
    - 14 µl dH_2O,
    - 10 µl 5x DNA Terminator End Repair Buffer (DNA Terminator End Repair Kit; Lucigen, Middleton, WI)
    - 1 µl DNA Terminator End Repair Enzymes (DNA Terminator End Repair Kit; Lucigen)

    Each sample was mixed and incubated at room temperature for 30 minutes.

32. The DNA was purified using a QIAquick PCR purification kit (Qiagen) as per the manufacture's protocol, and eluted in 25 µl of EB elution buffer. The eluate was removed from the collection tube and replaced back on the column for a final second elution.

Oligonucleotide linker ligation:

33. Ligase mix was prepared on ice:

    5.0 µl 10x T4 ligase buffer (NEB, Ipswich, MA)
    6.7 µl 15uM annealed oligo linkers oJW102+oJW103 (see below)
    0.5 µl T4 DNA ligase (NEB)
    12.8 µl dH_2O
    25.0 µl total per reaction.

34. Twenty five microliters of ligase mix was added to each 25 µl blunted DNA sample on ice.
35. The sample was incubated overnight at 16°C.
36. Twenty micrograms of glycogen (Ambion, Austin, TX) and 6 µl 3M sodium acetate was added and mixed. One hundred and thirty microliters of ethanol was added to each tube and vortexed to mix.
37. Sample was incubated at -80°C for 30 minutes.
38. Sample was centrifuged at 14,000xg for 15 minutes at 4°C.
39. The supernatant was removed and the pellet washed with 500 µl ice cold 70% ethanol. Sample was then vortexed to mix and centrifuged at 14,000xg for 5 minutes at 4°C.
40. The supernatant was aspirated and centrifuged at 14,000xg for 2 seconds. All liquid was removed with a micropipette and the pellet air dried for 5 minutes.
41. The pellet was resuspend in 30 µl dH_2O. Five microliters from each sample was removed for pre-LMPCR qRTPCR quality check (see step 50 below), and the remaining sample stored at -20°C.

Linker mediated PCR:

43. PCR master mix was prepared on ice:

For one reaction	
10x ThermoPol Rxn Buffer (NEB)	4.00 µl
25 mM dNTP	0.50 µl
40 µM oJW102	1.25 µl
dH_2O	9.25 µl
Total volume	15.00 µl

Scale up PCR master mix for the total number of samples being run.

44. Fifteen microliters of PCR mix was added to each sample and mixed.
45. The polymerase master mix was prepared on ice:

For one reaction	
10x ThermoPol Rxn Buffer (NEB)	1.00 µl
Taq 5U/µl (Qiagen)	1.00 µl
Pfu Turbo pol 5U/µl (Stratagene, La Jolla, CA)	0.01 µl
dH_2O	8.00 µl
Total volume	10.00 µl

Scale up PCR master mix for the total number of samples being run.

46. Ten microliters of polymerase master mix were added to each tube in step 44 and mixed.
47. PCR was performed with the following thermocycler program:
    step 1. 55°C for 2'
    step 2. 72°C for 5'
    step 3. 95°C for 2'
    step 4. 95°C for 1'
    step 5. 60°C for 1'
    step 6. 72°C for 2'
    step 7. Go to step 4 for 22 times
    step 8. 72°C for 5'
    step 9. 4°C forever
48. PCR product was purified with a QIAquick PCR purification kit (Qiagen) as per the manufacture's protocol. Sample was eluted with 30 µl of EB elution buffer.
49. Samples were examined on an agarose gel and quality examined with qRT-PCR (step 50 below).

Examining the success of immunoprecipitation by quantitative real-time PCR (qRT-PCR) of known DNA targets.

50. PCR master mixes for 13 reactions x 30 µl for each of your ChIP, mock, and input samples were prepared. In each master mix either 5 µl of the pre-LM-PCR sample or 130 ng of the LM-PCR template were added.
    *Each 27 µl PCR reaction (3 µl primers aliquot separately) should have the following components:

	1x	13x
25 mM dNTP	0.15 µl	2 µl
100x SyberGreen (Invitrogen, Carlsbad, CA)	0.30 µl	4 µl
10x Taq reaction buffer (Invitrogen)	3.00 µl	39 µl
pre-LM-PCR sample		5 µl
OR		
LM-PCR sample		130 ng
Taq polymerase (Invitrogen)	0.15 µl	2 µl
H_20 to a final volume of	27.00 µl	304 µl

After 27 µl aliquots were distributed in a 96 well plate, 3 µl of 3 µM primer sets were added to each reaction, to bring the total volume up to 30 µl. Each primer set was performed in triplicate. The four primer sets *him-1*, *fat-1*, *rex-4*, *her-1* (sequences below) were performed in triplicate yielding 12 PCR reactions per sample.

All quantitative PCR reactions were carried out and analyzed on a DNA Engine Opticon Real-Time PCR Detection System (MJ Research, Waltham, MA).

The following PCR thermocycler program was used for qRT-PCR:

1. 95°C for 5'
2. 94°C for 30''
3. 60°C for 30''

4. 72°C for 1'
5. 78°C for 10''
6. Plate Read
7. Go To Step 2 40 times

Oligonucleotide sequences:
oJW102 GCGGTGACCCGGGAGATCTGAATTC
oJW103 GAATTCAGATC
her-1 forward GAAGTTTCACCGCTAAGTTCG
her-1 reverse CCATTGTCTACGTCATCGTAC
him-1 forward CATCAGGAGCACCGGAAAG
him-1 reverse TTGTGCTCGTGAGCAACGG
rex-4 forward TTCTACGCGACTCAACCCC
rex-4 reverse TCGTTACCGCAGCTCTGAC
fat-1 forward CACTGAAGAGCCACGCATC
fat-1 reverse GTGCCGCAAAGTCTTGCAC

Annealing oligonucleotide linkers
1. 250 µl 1 M Tris, pH 7.9 and 375 µl each of 40 µM oJW102 and oJW103 oligonucleotides were combined in a 1.5 ml microfuge tube.
2. Sample was divided into 100 µl aliquots and place at 95°C for 5 minutes.
3. Tubes were transferred to a 70°C heat block.
4. The block was then removed from the heating unit and let stand to cool to room temperature, 25°C.
5. The samples were then placed at 4°C overnight and stored at -20°C the following day.

Buffer Recipes
M9 buffer: 6 g Na_2HPO_4, 3 g KH_2PO_4, 5 g NaCl, 0.25 g of $MgSO_4 \cdot 7H_2O$ per liter. Autoclave to sterilize.

Homogenization buffer: 50 mM HEPES-KOH, pH 7.6; 1 mM EDTA; 140 mM KCl; 0.5% NP-40; 10% glycerol. Protease inhibitor (Roche), 1 mM Phenylmethylsulfonyl fluoride (PMSF) (Sigma Al) and 5 mM dithiothreitol (DTT) are added fresh].

ChIP buffer: 50 mM HEPES-KOH, pH 7.6; 1 mM EDTA; 0.05% NP-40. Add KCl to the desired concentration. Protease inhibitor (Roche) 1 mM Phenylmethylsulfonyl fluoride (PMSF) (Sigma Al) and 5 mM dithiothreitol (DTT) are added fresh].

Supplemental References

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