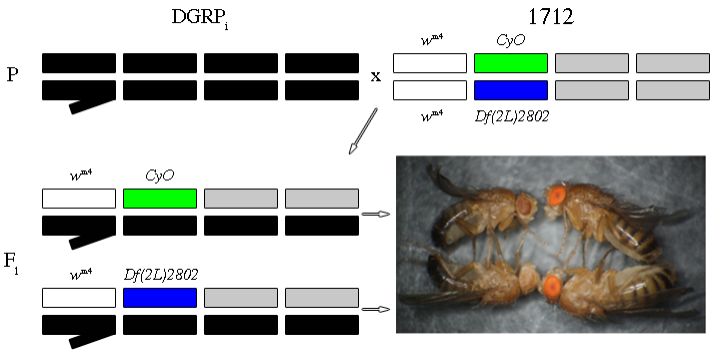

Figure S 1 Experimental cross to generate males with unique DGRP haplotypes, *i*, that also carry w^{m4} and exhibit PEV. F1 males segregate into two variegating classes, those with a *CyO* second chromosome (visible curly wing) and those with *Df(2L)2802* second chromosome (visible straight wing). F1 males were imaged. Females do not display variegation in this cross and were not studied.

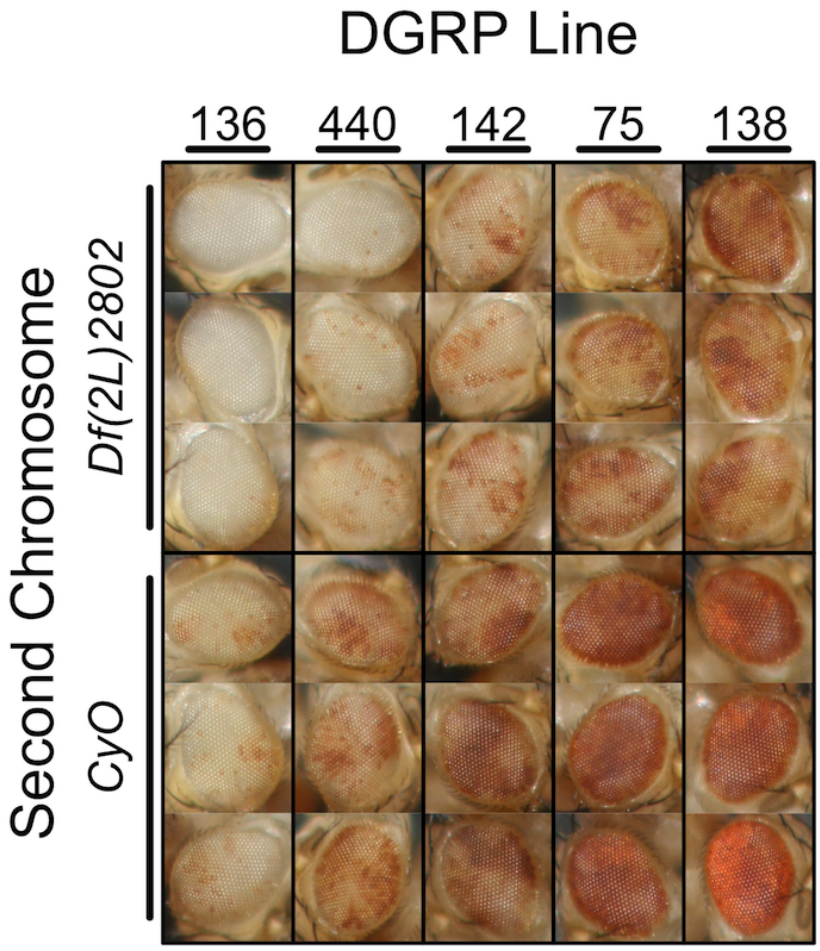

Figure S 2 F1 experimental males show a wide range of visible differences in eye pigmentation, spanning a range that lacks pigmentation (DGRP line 136 with a *Df(2L)2802* second chromosome background) to full pigmentation and near-wild-type eyes (DGRP line 138 with a *CyO* second chromosome background. To demonstrate the range of pigmentation in F1 progeny, three replicates are shown from a subset of DGRP line by second chromosome combinations. Even at a gross level, the PEV phenotype shows greater similarities in pigmentation between replicate individuals than between DGRP line or second chromosome background.

Table S 1 Heritability of PEV

	CyO background			*Df(2L)2802* background		
Variable	PC1	PC2	PC3	PC1	PC2	PC3
Line	59.8%	45.3%	44.0%	57.7%	47.8%	55.8%
Vial	< 0.1%	0.5%	< 0.1%	< 0.1%	0.2%	0.2%
Residuals	40.2%	54.3%	56.0%	42.3%	52.0%	44.0%

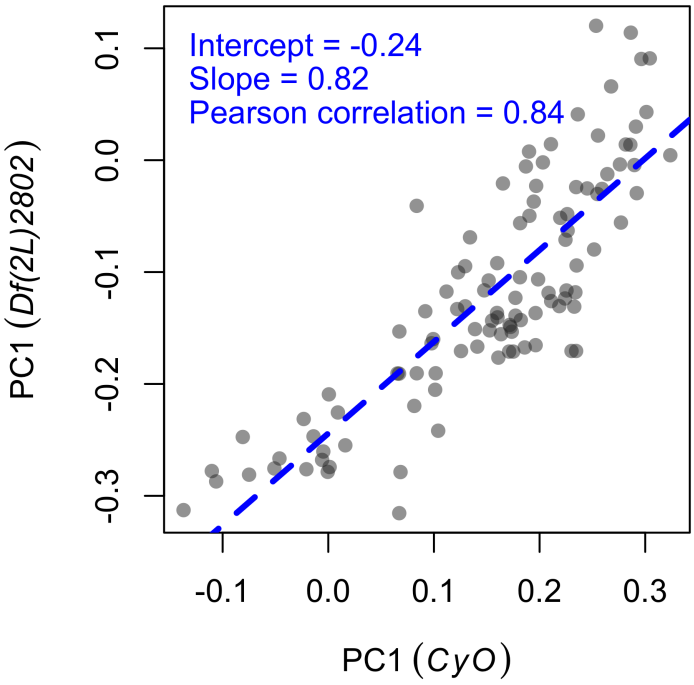

Figure S 3 Comparison between PC1 values of PEV populations with *CyO* and *Df(2L)2802* second chromosome backgrounds. Although there is strong correlation in PEV between the two backgrounds, the *CyO* second chromosome shows less variegation (more red eyes and higher PC1 values) with respect to the *Df(2L)2802* second chromosome background.

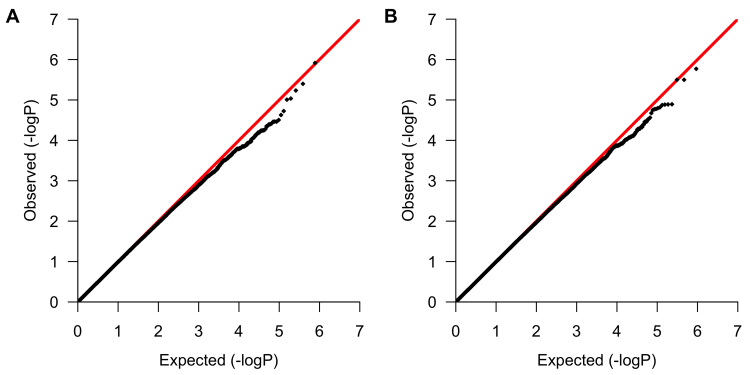

Figure S 4 Q-Q plots of expected (red line) and observed (black points) *P*-values from GWAS. **(A)** Experimental population with *CyO* second background. **(B)** Experimental population with *Df(2L)2802* second chromosome background.

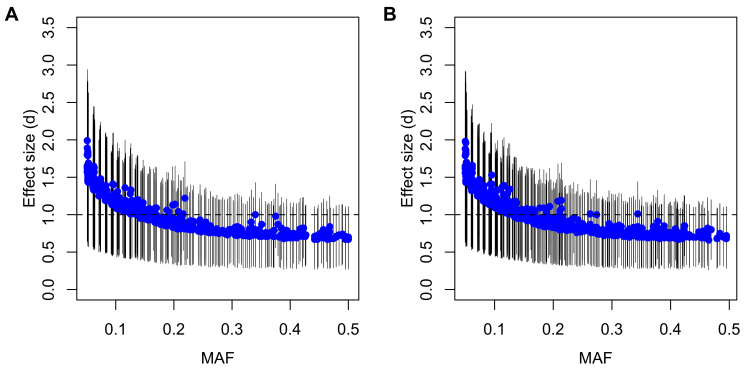

Figure S 5 Effect size (Cohen's *d*) with upper and lower confidence limits of variants having association *P*-values ≤ 0.001. **(A)** Effect sizes of 584 variants from the experimental population with *CyO* second chromosome background. **(B)** Effect sizes of 770 variants from the experimental population with *Df(2L)2802* second chromosome background. 310 and 369 variants from each respective background have effect sizes greater than or equal to 1.

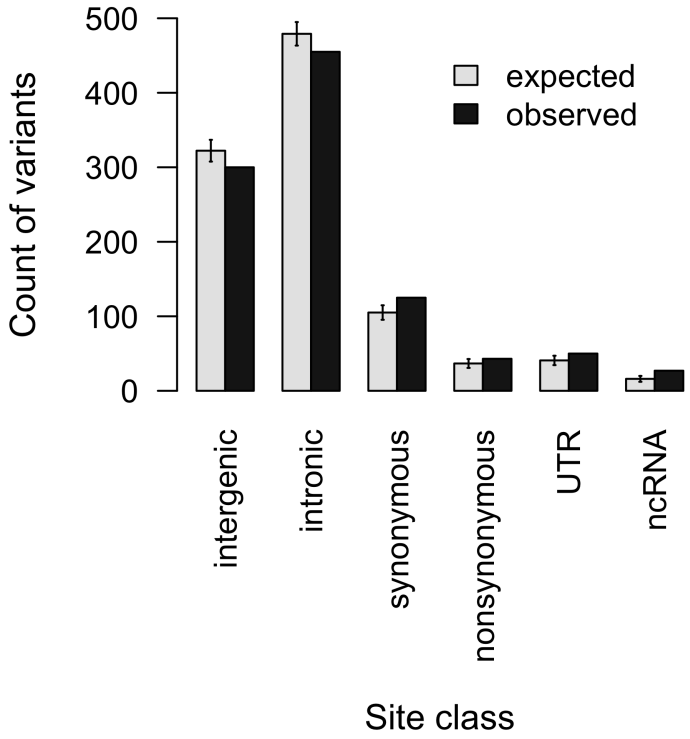

Figure S 6 Distribution of site classes between expected counts (average) and observed counts from the 1000 top associated variants. All observed counts show a significant difference from expected (two-sided, one sample *t*-test, Bonferroni corrected *P*-values all $\leq$ 2.2×10^{-16}. Error bars represent the standard deviation across 10,000 randomly drawn samples.

Table S 2 GWAS top ranked associations by *P*-value

				CyO background			*Df(2L)2802* background		
Position	Alleles	Gene	Class	AF	*P*-value	Rank	AF	*P*-value	Rank
2L 12252172	T/C	*Aret*	Intron	NA	NA	NA	0.095	1.3x10-5	5
2R 11802982	C/T	*Sli*	Intron	NA	NA	NA	0.274	1.6x10-5	9
2R 12408574	T/C	*Sema-2a*	Intron	0.126	1.8x10-5	6	0.124	1.7x10-5	10
2R 18531570	T/C	NA	Intergenic	0.051	3.1x10-5	8	0.05	3.5x10-5	19
3L 1775145	A/G	*CG13933*	5′UTR	0.34	5.8x10-6	3	0.344	3.2x10-6	3
3L 2081047	A/G	*sls*	Synonymous	0.116	2.4x10-5	7	0.113	2.1x10-5	13
3L 2288054	G/T	*DmsR-2*	Intron	0.198	9.8x10-6	5	0.194	1.3x10-5	6
3L 2568884	T/C	*msn*	Intron	0.375	4.0x10-6	2	0.378	1.3x10-5	7
3L 3595434	T/G	*Eip63E*	Intron	0.219	1.2x10-6	1	0.214	1.7x10-6	1
3L 5909455	T/C	*CG13288*	Intron	0.202	9.2x10-6	4	0.208	3.2x10-6	2
3L 5909456	G/C	*CG13288*	Intron	0.208	3.5x10-5	11	0.214	1.3x10-5	4
3L 12852675	C/G	*CG32107*	Nonsyn.	0.333	3.4x10-5	9	0.337	1.2x10-4	78
3L 12852676	T/C	*CG32107*	Nonsyn.	0.333	3.4x10-5	10	0.337	1.2x10-4	79
3R 4014584	C/T	NA	Intergenic	NA	NA	NA	0.116	1.5x10-5	8

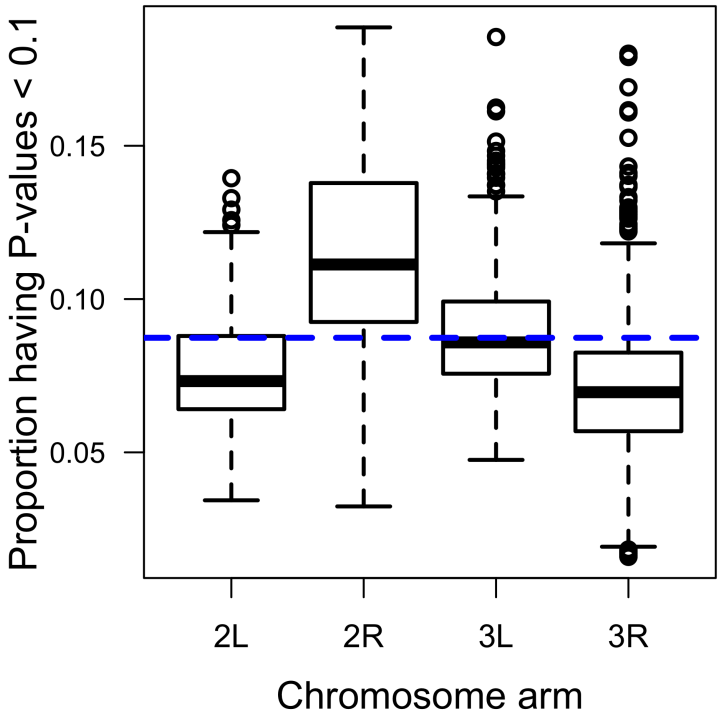

Figure S 7 Proportion of variants (MAF $\geq$ 0.05) within the *Df(2L)2802* deletion (25F2-25F5, 196,021 bps) having a *P*-value less than 0.1. Hemizygous alleles (blue line, proportion = 0.087) show no *P*-value enrichment with respect to similar length (bp) non-hemizygous samplings across the genome. The deficiency regions show an enrichment greater than 73.7%, 17.6%, 53.9% and 81.2% of the respective, 2L, 2R, 3L and 3R chromosome arms, well within 90% of the each distribution mass. Chromosome arms were sampled 1,000 times each in random windows of 196,021 bps.

Table S 3 Known autosomal genic modifiers of PEV

Chromosome			
2L	2R	3L	3R
abo	*Asx*	*AGO2*	*Ace*
aub	*Atf-2*	*asf1*	*Acf1*
barr	*BEAF-32*	*ash1*	*Bin1*
CG8677	*bw*	*brm*	*bon*
Chd1	*Cap-G*	*E(z)*	*cav*
chm	*Dcr-2*	*FRG1*	*D1*
dp	*dom*	*HP4*	*Dcr-1*
Etl1	*Dp1*	*Jarid2*	*E(var)3-9*
glu	*E(Pc)*	*JIL-1*	*E2f*
Hel25E	*egg*	*msl-3*	*Fmr1*
kis	*LamC*	*not*	*gpp*
lid	*lat*	*Pc*	*His2Av*
lt	*Nap1*	*pzg*	*Invadolysin*
lwr	*Pcl*	*rept*	*jumu*
Mcm10	*PCNA*	*Rpd3*	*MBD-like*
Mt2	*Psc*	*RpLP0*	*Mekk1*
piwi	*psq*	*Sgf11*	*mod*
Psf2	*SMC2*	*sti*	*mod(mdg4)*
r2d2	*Su(var)2-10*	*stwl*	*mor*
Sam-S	*Su(var)2-HP2*	*Su(var)3-3*	*MRG15*
Sir2	*Su(z)2*	*Su(z)12*	*Orc2*
Sos		*SuUR*	*osa*
Su(var)205		*Trl*	*polybromo*
vig		*Ubp64E*	*Pp1-87B*
			pr-set7
			puc
			Rga
			Sce
			Scm
			Snr1
			spn-E
			Su(var)3-7
			Su(var)3-9
			trx
			vig2
			XNP

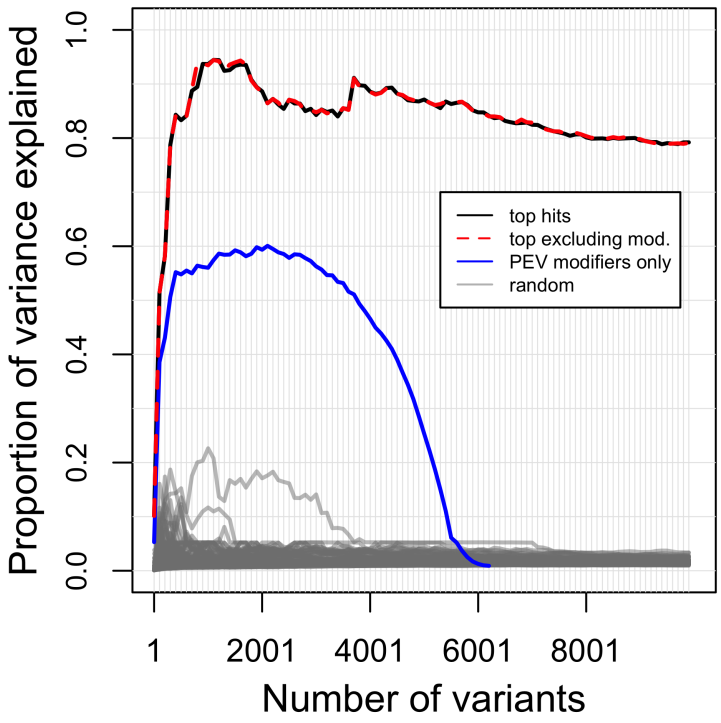

Figure S 8 Proportion of among-line phenotypic variance explained within the *Df(2L)2802* second chromosome population using GCTA. Comparison of SNP groupings include; the most significant GWA variants (black line), top hits excluding variants from known PEV genic modifiers (red line), variants within know PEV genic modifiers only (blue line), and randomly selected autosomal variants (gray line).

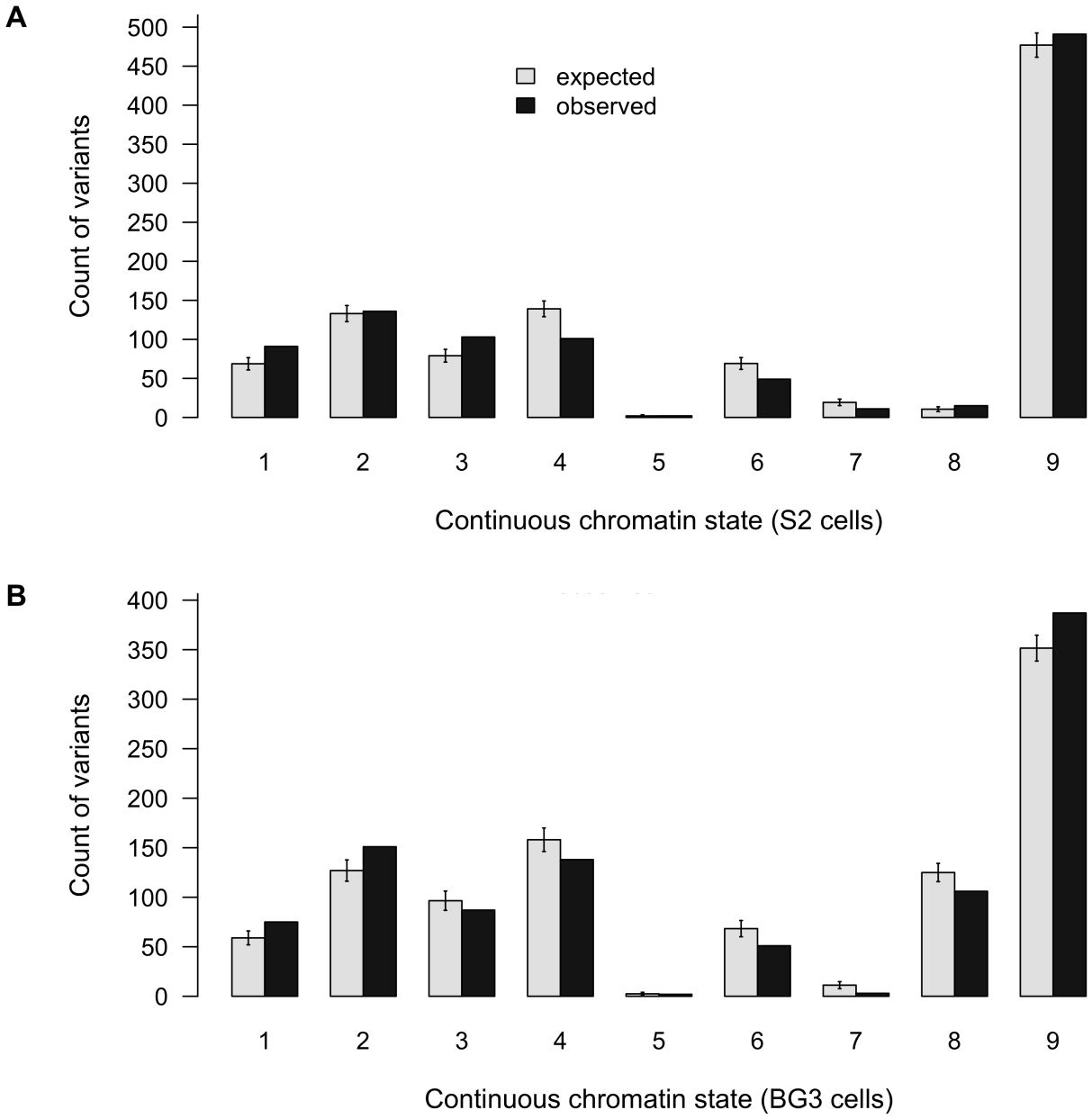

Figure S 9 Counts of continuous 9-state chromatin assignments in randomly select autosomal variants (expected) and the top 1,000 *P*-value ranked SMA variants (observed). **(A)** Counts of chromatin state assignments in S2 cells. **(B)** Counts of chromatin state assignments in BG3 cells.

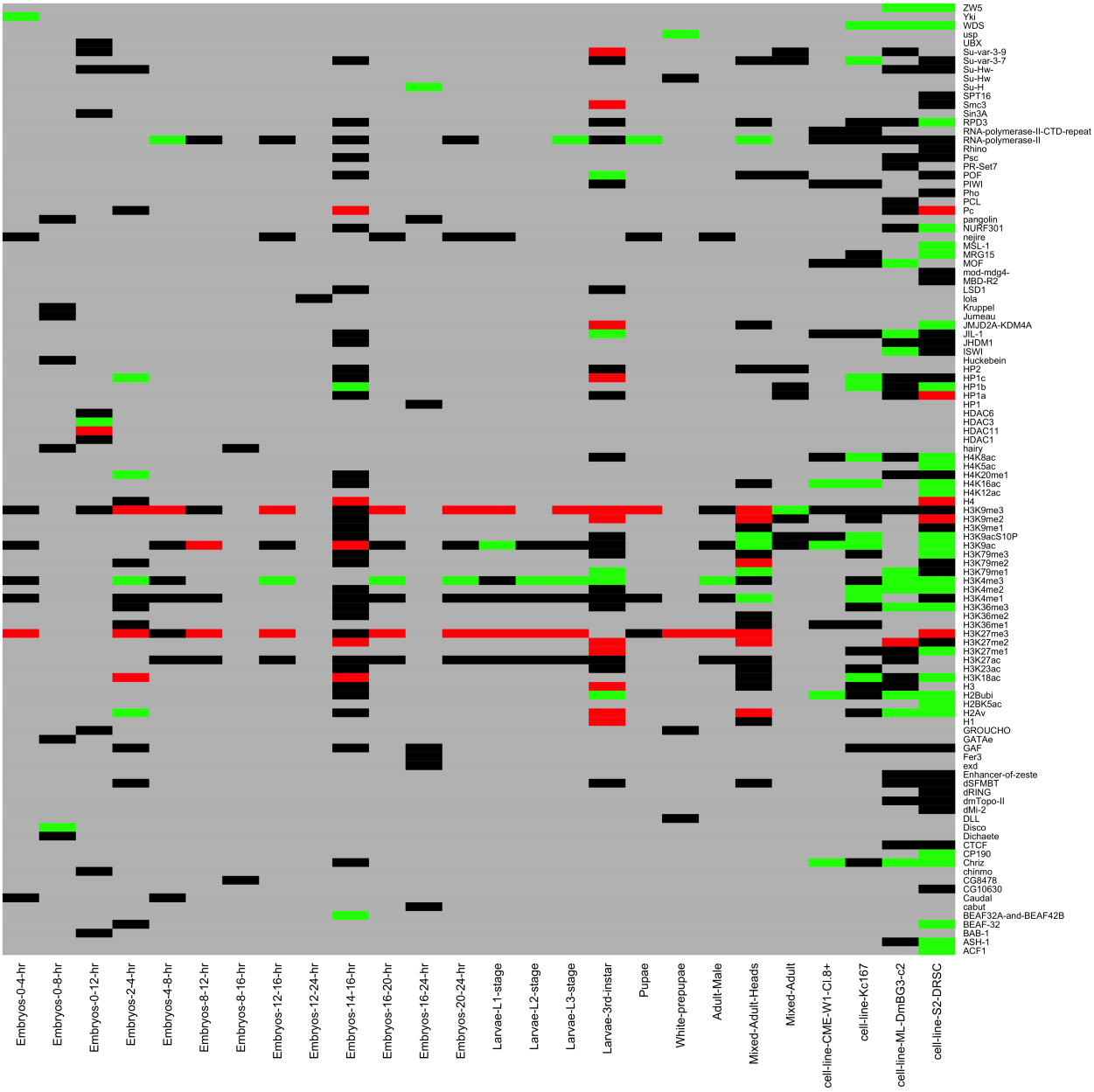

Figure S 10 Expected and observed counts of variants within binding sites of chromatin-associating factors (y-axis) and across various developmental stages (x-axis), as defined through ChIP-chip and ChIP-seq binding assays. For each factor and developmental stage, expected distributions were generated through 10,000 iterations of randomly sampling 1,000 variants from all autosomes, and the number of variants residing within marked binding locations were counted. This expected distribution was then compared to the number of variants residing within marked binding locations from the set of 1,000 top ranked associations. Observed counts with respect the expected distribution are as follows; green represents observed counts that are > 97.5% of the expected distribution, red represents observed counts that are < 2.5% of the expected distribution, black represents observed counts that are within 95% of the expected mass, and gray represents missing sample data.