Table S1 Known SD binding site sequences used to generate position weight matrix to scan the SAM and ORE genomes for predicted SD binding sites.

Gene	Coordinates	Sequence	Source
ct	X:7424390..7424403	TTTGTGAATGAAGT	REDfly TF000140
ct	X:7424422..7424433	TAACATTTAATT	REDfly TF000139
ct	X:7424473..7424487	GATAAACAGCAGTGT	REDfly TF000138
ct	X:7424494..7424520	GCTGTTTTTTTAAATGAATTTTCTCTA	REDfly TF000137
ct	X:7424594..7424610	AAAATTATTGAAATTAC	REDfly TF000136
ct	X:7424692..7424701	GGAATGGGAT	REDfly TF000135
ct	X:7424821..7424843	AATGTAATTCGAAAAATGTCGTC	REDfly TF000134
vg	2R:8782964..8783003	GCTAGTTGGAATGTGCTATGAAATGTCGCCGGAATGCGAT	REDfly TF001715
vg	2R:8783977..8783987	GGAAATATCTT	REDfly TF000458
vg	2R:8784014..8784027	TGGGAATTCCACGG	REDfly TF000459
vg	2R:8784101..8784114	CACGCGGCATGGCA	REDfly TF000460
vg	2R:8784375..8784386	GTTTGGAATGTT	REDfly TF000465
bs	2R:20229832..20229857	TAAGAAATTCCTGGCATAGTTTAAGT	REDfly TF000512
salm	2L:11454576..11454582	TATGCGA	REDfly TF000032
salm	2L:11454656..11454679	AATGGACATTCGTGGGATTCCAGA	REDfly TF001599
salm	2L:11454657..11454680	ATGGACATTCGTGGGATTCCAGAA	REDfly TF000029
salm	2L:11454657..11454680	ATGGACATTCGTGGGATTCCAGAA	REDfly TF000030
kni	3L:20700051..20700068	TACATTTGTCGCATAGTT	REDfly TF000811
kni	3L:20700243..20700251	ATACATACA	REDfly TF000812
kni	3L:20700303..20700311	AAAATGTCG	REDfly TF000813
kni	3L:20700350..20700358	GAAATGCGT	REDfly TF000814
kni	3L:20700420..20700428	AGAAATAGT	REDfly TF000815
diap1		GCATTCCATT	Wu et al. 2008

Table S2 Position weight matrix used to scan the SAM and ORE genomes for SD binding sites, derived from a MEME (Bailey et al. 2009) scan of 23 known SD binding sites.

	A	C	G	T
1	0	5	14	4
2	8	1	14	0
3	18	4	0	1
4	22	0	1	0
5	0	0	0	23
6	3	0	14	6
7	3	3	3	14

Table S3 Detailed sequence pile-up (Li et al. 2009) for *scalloped* binding site regulating *cut* (Halder & Carrol 2001, Guss et al. 2001).

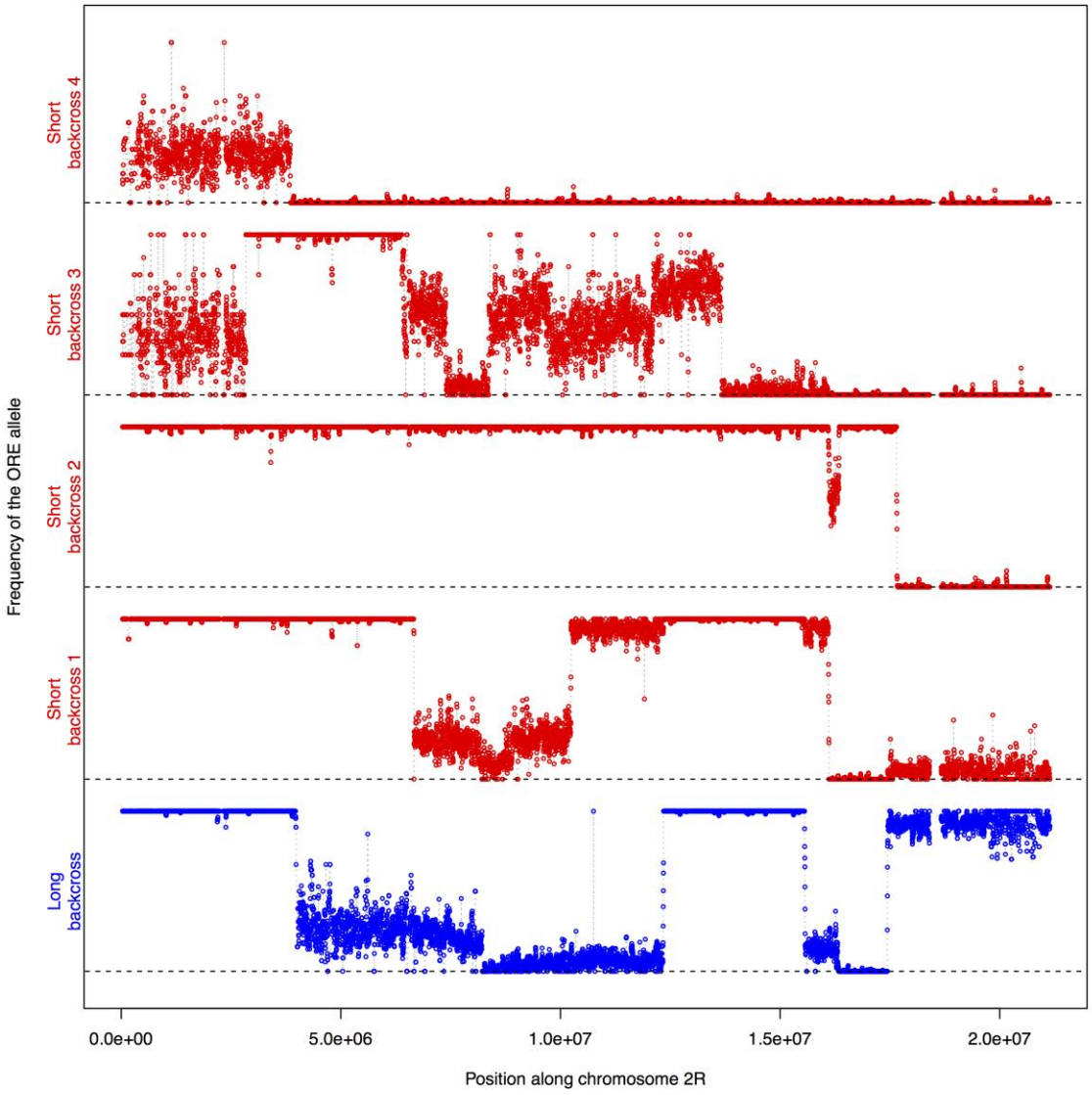
Chrom.	Pos.	Ref.	# reads (SAM)	Read bases (SAM)	Base quality (SAM)	# reads (ORE)	Read bases (ORE)	Base quality (ORE)
X	7424817	T	15	,....,,,,,,...	HFIHIDIBI<GIIII	15	.$,.,,.,.,.,.,.	DIIFIIIIIIIHIII
X	7424818	G	15	,....,,,,,,...	HIHGIEIGIGGIIIG	15	,.,,.,.,.,.,.^],	GIIIIIHGIHIIHIE
X	7424819	T	16	,$....,,,,,,...^].	EHGGICHGIGDIIHGE	15	,$.,,.,.,.,.,.,	EIIIIIIIIIIIII
X	7424820	C	15	....,,,,,,....	IIII4IBIBGIIIGI	16	.,,.,.,.,.,.,^],^],	IGGIIIIIIIIBIIEE
X	7424821	A	15	....,,,,,,..G.	IHIICIBIGGIII*I	18	.,,.,.,.,.,.,,,^],^],	IIIIIIIIBIIIIIIDBB
X	7424822	A	15	....,,,,,,....	FHHIEIGIDGIIGGI	18	.,,.,.,.,.,.,,,,,	IIIIIIHIIIIIIHIIEE
X	7424823	T	15	....,,,,,,....	HIIIDIGICGIIIGI	18	.,,.,.,.,.,.,,,,,	IIIIIIIIHHI@IIIGII
X	7424824	G	15	....,,,,,,....	GIIIDI@IEGIGIGI	18	.,,.,.,.,.,.,,,,,	IIIIIIIIDIIIIHIIIG
X	7424825	T	15	.$...,,,,,,....	EGGG=HDICGIIIGI	18	.,,.,.,.,.,.,,,,,	IIIIIIIIIIGIIEHII
X	7424826	A	14	...,,,,,,....	IHIBIGIDGGIIGI	18	.,,.,.,.,.,.,,,,,	IIIIIIIIIIGIIIIII
X	7424827	A	14	...,,,,,,....	HII:I4IDGIIIGI	19	.,,.,.,.,.,.,,,,,^],	IIIIIIIIIIIIIIHIG>
X	7424828	T	14	...,,,,,,....	III=IEIEGIIIII	22	.,,.,.,.,.,.,,,,,^],^],^],	IHIIIIIHIIIIIIIHIIEBBB
X	7424829	T	14	...,,,,,,....	III?IBICDIIIII	23	.,,.,.,.,.,.,,,,,,,^],	IIHIIIFIIHIIIFIFHICEEED
X	7424830	C	14	...,,,,,,....	IIH?IDIDDIIIII	23	.,,.,.,.,.,.,,,,,,,	IIIIIIHIIIIHIGIIIHBFIHF
X	7424831	G	14	.$..,,,,,,....	EDIDIGICGIIIII	24	.,,.,.,.,.,.,,,,,,,^],	IIIIIIIIHIIIIIIIIGBIHIE
X	7424832	A	14	...,,,,,,....^].	>>DFGI>EIIIII7	25	.,,.,.,.,.,.,,,,,,,^].	IIIHIIIGIIIIIIIHGHIGII>
X	7424833	A	14	.$.,,,,,.....	;;<IGIGHIIIII:	25	.,,.,.,.,.,.,,,,,,,.	IIIIIIIIIIHIIIIIDII>II?
X	7424834	A	13	.$,.,,,,.....	9?IBIGHIIIII5	25	.,,.,.,.,.,.,,,,,,,.	IIIIIIIIIIIIIIEIH>IIHIIA
X	7424835	A	12	,.,,,,.....	BIGIDHIHIII5	25	.,,.,.,.,.,.,,,,,,,.	IIIIIIIIIIIIIIIGIHHIIB
X	7424836	A	12	,.,,,,.....	DIGIDHIIIII2	25	.,,.,.,.,.,.,,,,,,,.	IIIGIIIIIIHIHIEIIGIHBIIE
X	7424837	T	12	,.,,,,.....	@IBGBEFBIII-	26	.,,.,.,.,.,.,,,,,,,.^].	IIIIIIIIII8IHHHI@ADHDEDIE
X	7424838	G	12	,.,,,,.....	GIGHDDIIIII:	26	.,,.,.,.,.,.,,,,,,,.	HIIIIIIIHIIIIIIIIGHHHIIII
X	7424839	T	12	,.,,,,.....	GH@IGGHIFHI4	26	.,,.,.,.,.,.,,,,,,,.	HIIIHIIIHIIGIIIIII3IHHIHII
X	7424840	C	12	,.,,,,.....	GIGI<GIIIII5	26	.,,.,.,.,.,.,,,,,,,.	IIIIIIIIIIIIIIHIG8IHDIHII
X	7424841	G	13	,.,,,,.....^].	:IGIGGIIGII7E	26	.,,.,.,.,.,.,,,,,,,.	IIIIIIIIIIIIHIIIIGIIHIIII

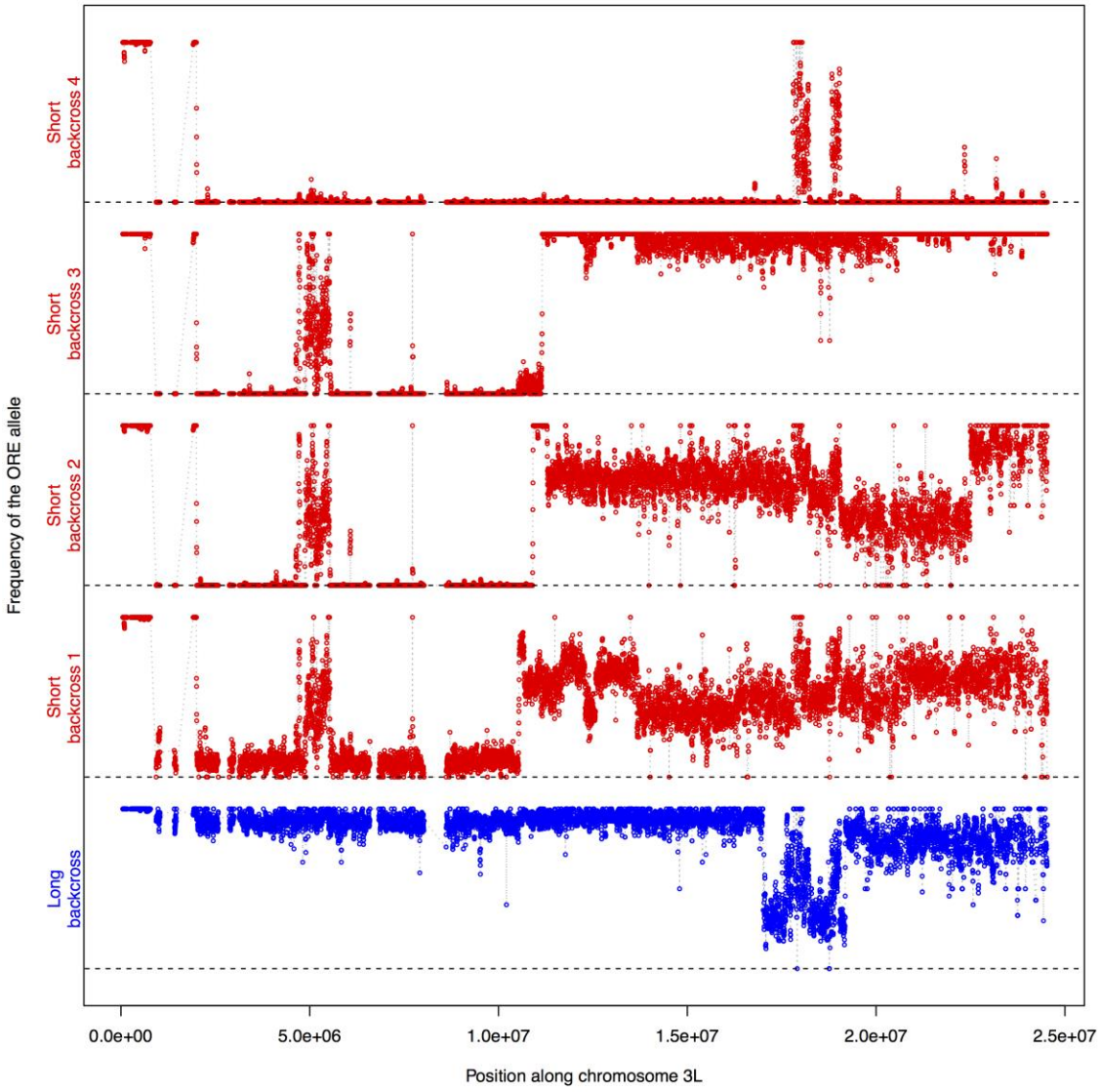
Table S4 Detailed sequence pile-up (Li et al. 2009) for *scalloped* binding site regulating *sal* (Guss et al. 2001).

Chrom.	Pos.	Ref.	# reads (SAM)	Read bases (SAM)	Base quality (SAM)	# reads (ORE)	Read bases (ORE)	Base quality (ORE)
2L	11454659	G	17	,,...,..,,,,.,..,	II+IIIIIGIIIIIHII	17	,,..,,.,.,,.,,,,	77977<97777787777
2L	11454660	G	18	,,...,..,,,,.,..,^],	II3IIIIIGIIIIIEIIE	17	,,..,,.,.,,.,,,,	34644964444454444
2L	11454661	A	18	,,...,..,,,,.,..,,	IH6@HIHIBIIIIIIIHH	17	ggGGggGgGggGGgggg	))644964444454444
2L	11454662	C	18	,$,...,..,,,,.,..,,	EI8IIIIIBHIIIIIIII	18	,$,..,,.,.,,.,,,,^>,	))HFF?IIIIIIIIIIC
2L	11454663	A	17	,...,..,,,,.,..,,	I38IIIIGGHIIIHIII	17	,$..,,.,.,,.,,,,,	)HFFDIHIIIIIIIH8
2L	11454664	T	17	,...,..,,,,.,..,,	IBGIIIIGIIIIHHIII	16	.$.$,$,..,,,.,,,,,	>>>DIGIIIHIIGIIC
2L	11454665	T	18	,...,..,,,,.,..,,^],	IAEIIIIGIIIIIIHIE	13	,$.,.,,,.,,,,,	BIIIHIIIIIII?
2L	11454666	C	18	,$...,.,,,,.,.,,,	EAEIIIIGIHHIIGIIIH	12	.,,,,.,,,,,	5GIIIIIIIHI@
2L	11454667	G	17	...,.,,,,.,.,,,	A@IIIIGIIIIGIIIII	13	.,,,,.,,,,,^],	-8888988888?!
2L	11454668	T	17	.$.$.,.,,,,.,.,,,	?BIIIIDIIIII@IFII	13	CcCccCCcccccc	!44445444442!
2L	11454669	G	15	.,.,,,,.,.,,,	IIIIGIIIIIGIHII	13	.,,,.,,,,,,	!3333433333<!
2L	11454670	G	15	.,.,,,,.,.,,,	IIIIBIIIIHGIIII	13	A$aAaaAAaaaaaa	!1111211111;!
2L	11454671	G	15	.,.,,,,.,.,,,	IIIIEIIIIIGIIII	13	,.,,.,,,,,,^>.	7777877777@0E
2L	11454672	A	15	.,.,,,,.,.,,,	IIIIGIIIIIDIIIH	14	,.,,.,,,,,,.^],	IGIIIIIIII46HE
2L	11454673	T	16	.$,$..,,,,.,.,,,^],	>>IIGHIIIIGIIIFB	15	,.,,.,,,,,,.,^],	GIIIIIIHIH;>HIB
2L	11454674	T	14	.,,,,,.,.,,,	IIGIIIIHGIIHIE	15	,.,,.,,,,,,.,,	HIIIIIIEII,<HIE
2L	11454675	C	14	.$.,,,,.,.,,,	>E@IIIIIGIIIFG	15	,.,,.,,,,a,.,,	EIIHIIIIII,IGII
2L	11454676	C	13	.$,,,,.,.,,,	BGIIIIIIIIII	15	,$.,,.,,,,,.,,	BIIIIIIGII;IHII

Table S5 Top 200 genes identified as being differentially expressed between wild-type and sd^{E3} mutant flies by DGE analysis. (See SuppTable5.csv in Dryad package, doi:10.5061/dryad.1375s)

Table S6 Genes showing evidence of allelic imbalance in "hybrid" SAM/ORE flies (i.e., in which one of the two alleles is transcribed at significantly higher levels). (See SuppTable6.csv in Dryad package, doi:10.5061/dryad.1375s)


Short backcross 4
Short backcross 3
Short backcross 2
Short backcross 1
Long backcross
Frequency of the ORE allele
0.0e+00
5.0e+06
1.0e+07
1.5e+07
2.0e+07
Position along chromosome 2R


Short backcross 4
Short backcross 3
Short backcross 2
Short backcross 1
Long backcross
Frequency of the ORE allele
0.0e+00
5.0e+06
1.0e+07
1.5e+07
2.0e+07
2.5e+07
Position along chromosome 3L

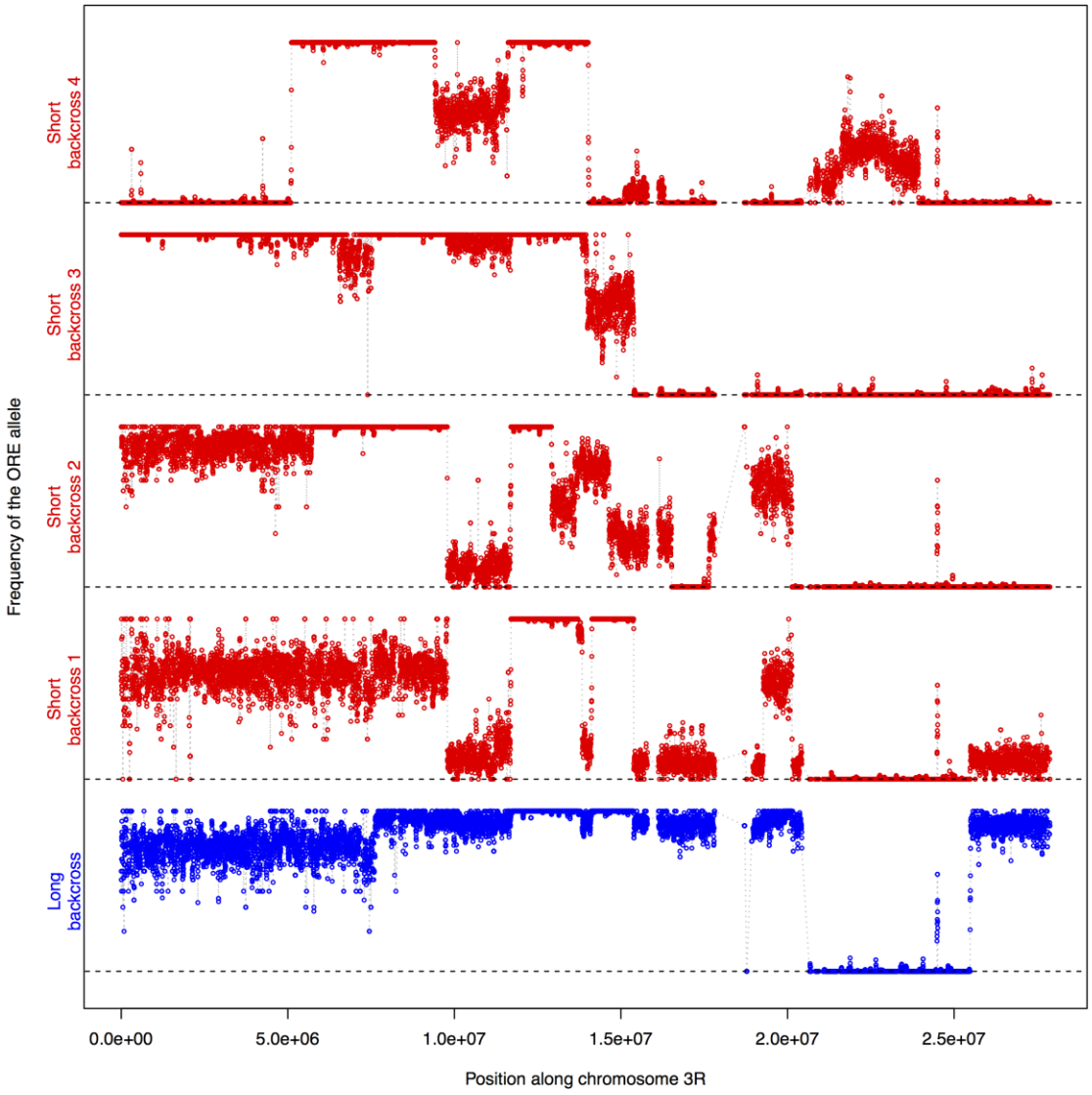


Figure S1 Frequency of the ORE allele along the length of chromosome arms 2R, 3L, and 3R in short- and long-wing *sd*^{E3} backcross lines.

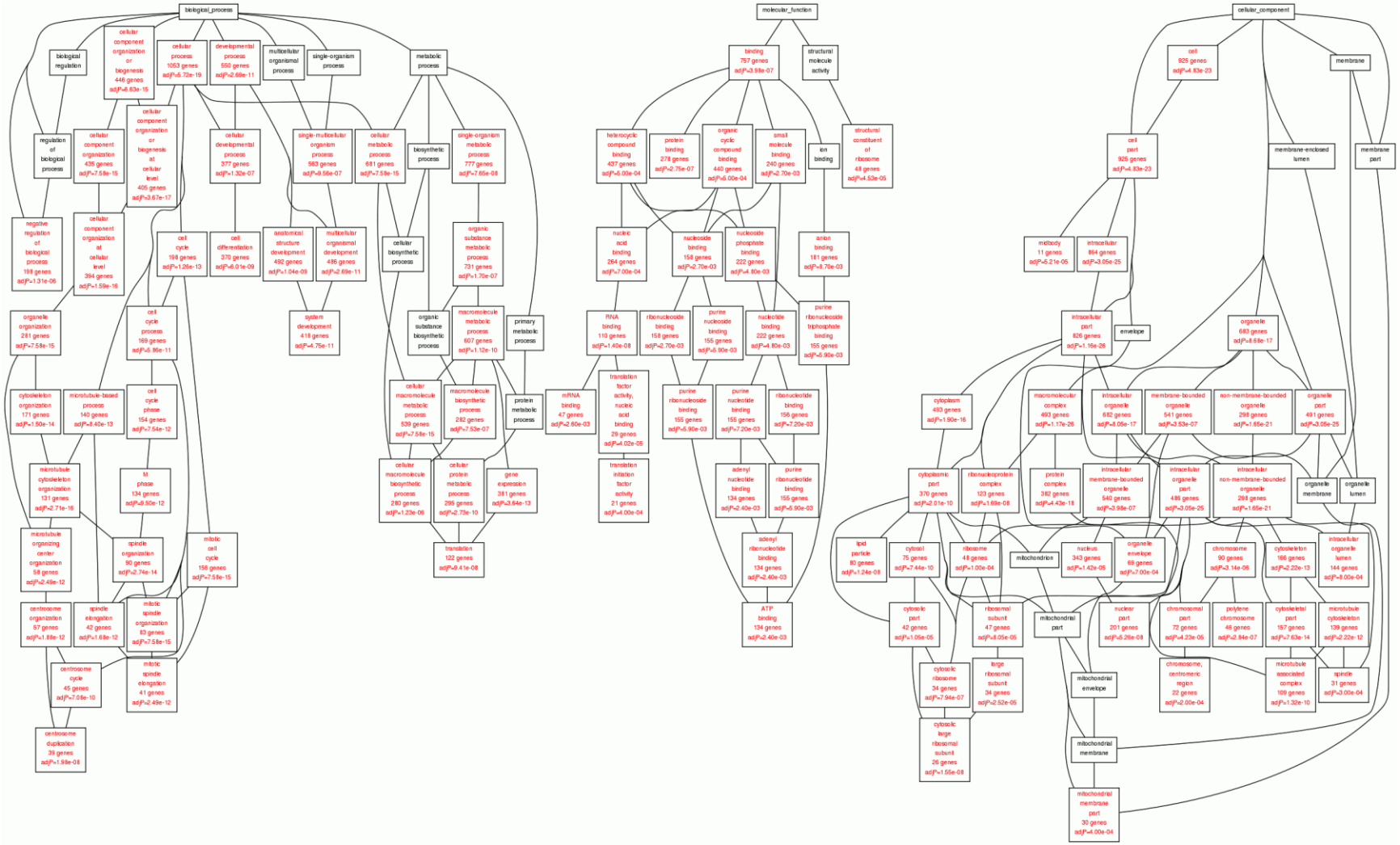


Figure S2 Top hits for GO terms enriched in genes showing evidence of differential expression (q < 0.001) between wild-type and *sd*^{E3} fly wings in the DGE dataset.

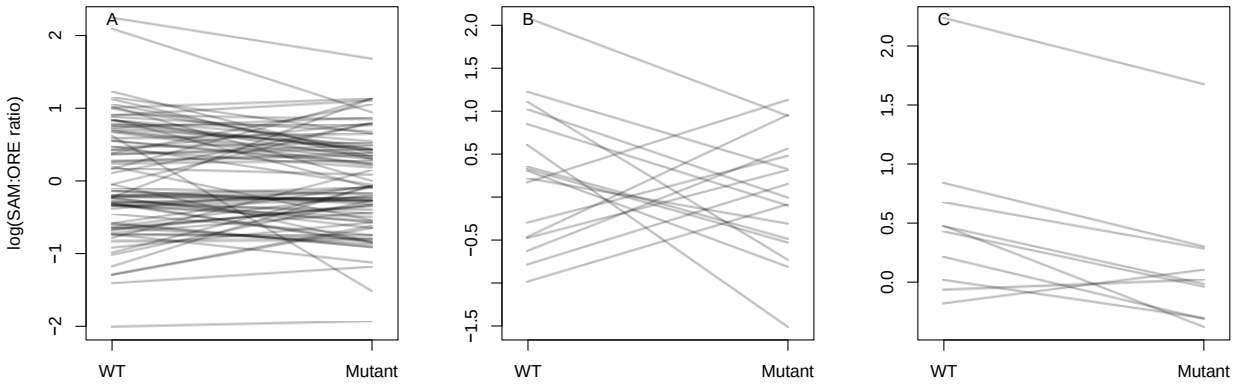


Figure S3 Allelic imbalance in SAM/ORE "hybrid" flies with both wild-type and *sd*^{E3} genotypes. Only sequence tags represented by at least five reads of each allele in all samples are represented in this plot. (A) Genes showing evidence of allelic imbalance ($q < 0.05$). (B) Genes showing evidence of genotype-dependent allelic imbalance ($q < 0.05$). (C) Genes near polymorphic predicted SD binding site ($p < 0.05$).

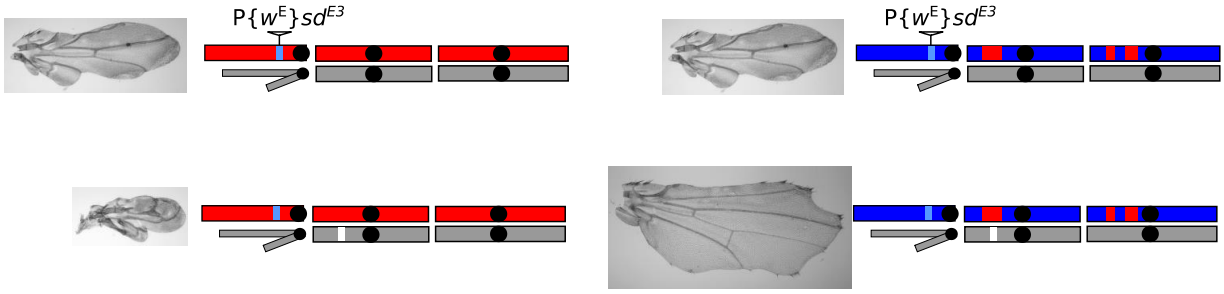
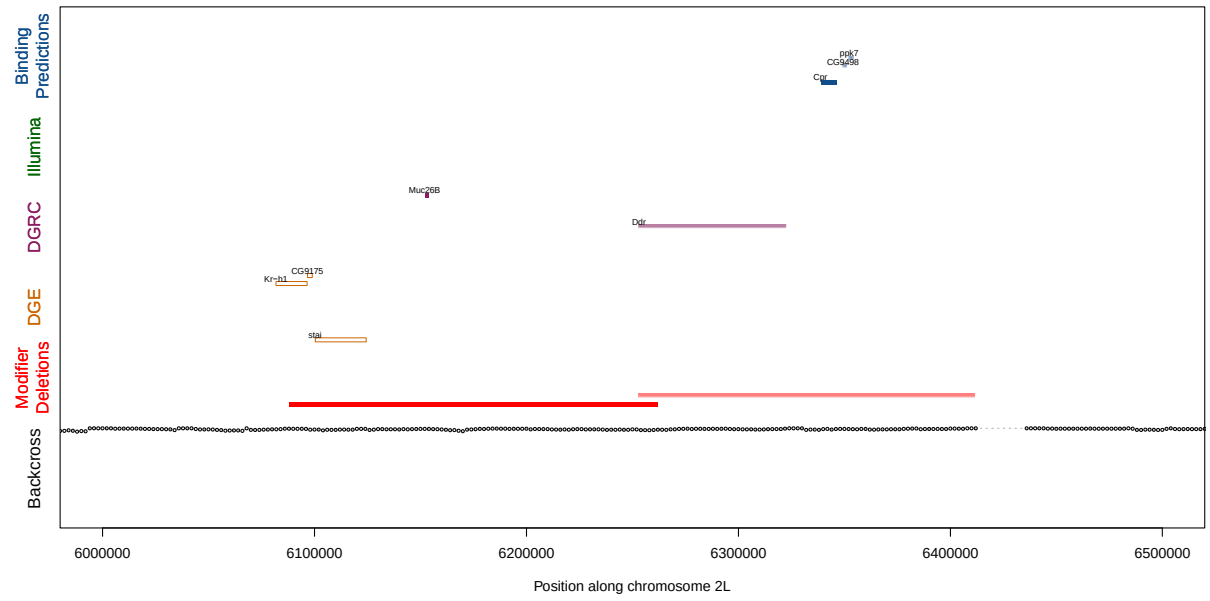
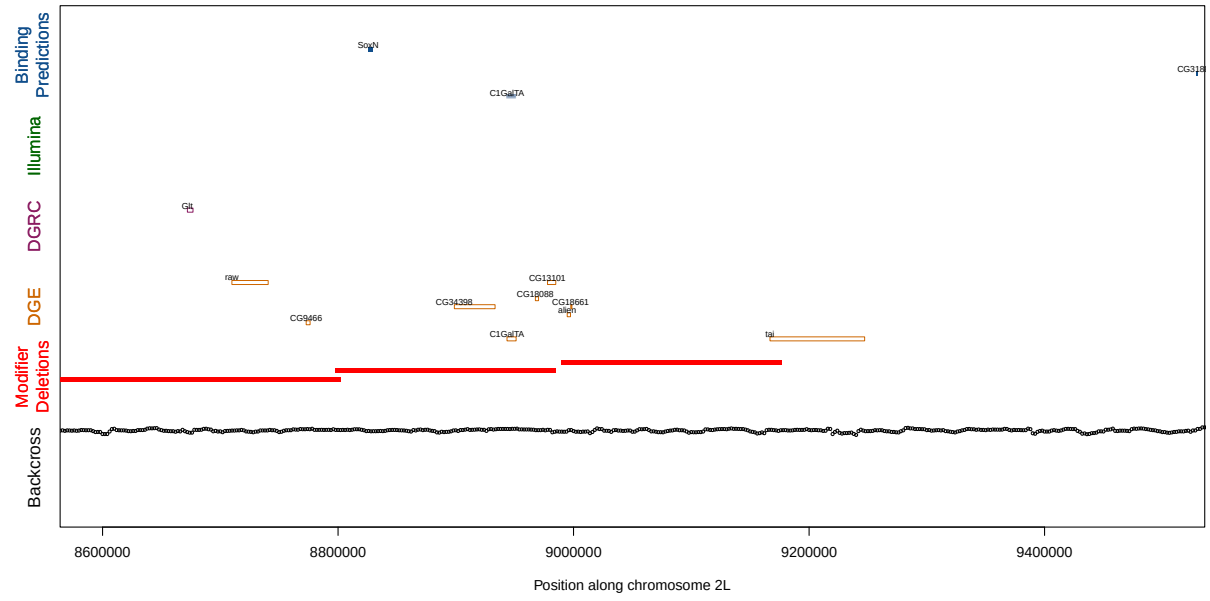
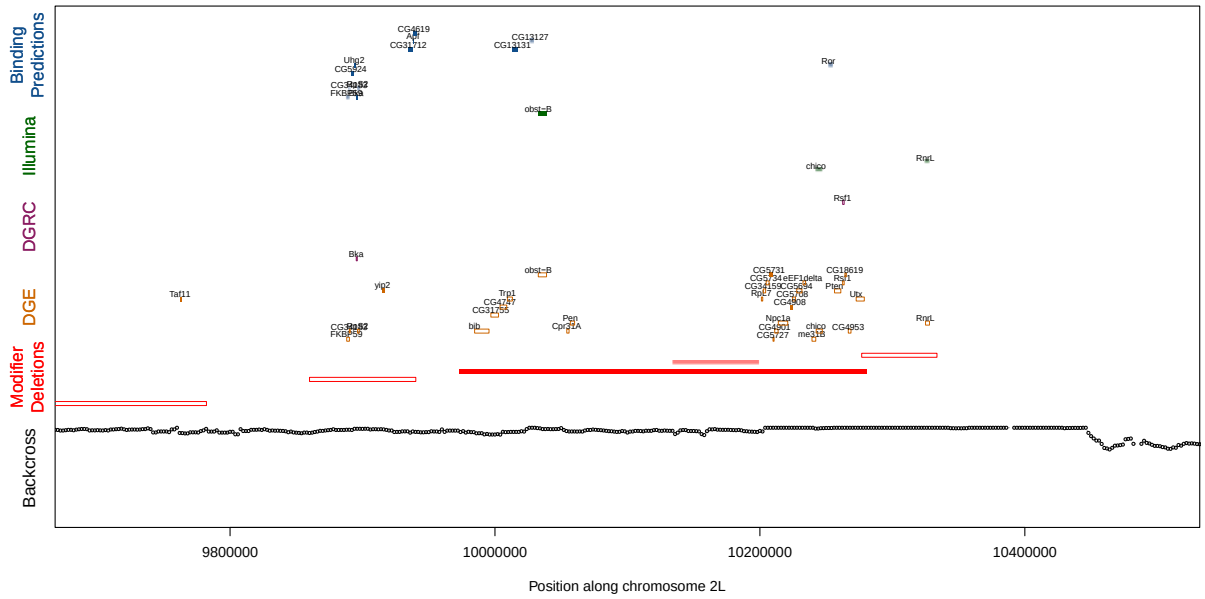
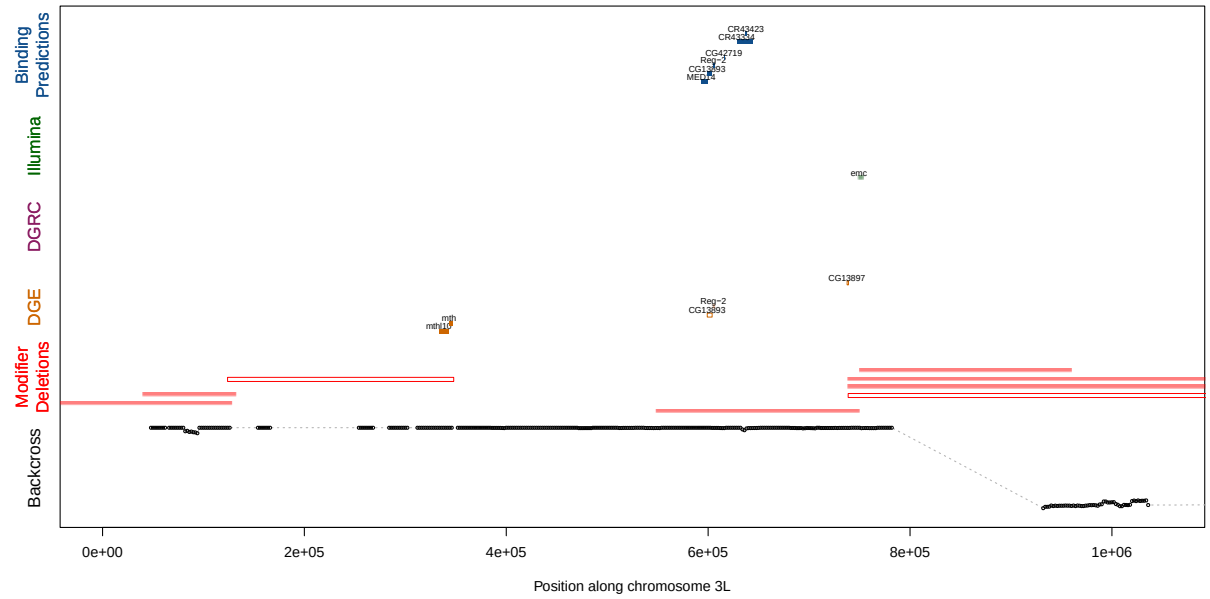


Figure S4 Schematic illustrating tests to distinguish between second- and higher-order epistasis. Red indicates the ORE genetic background; blue indicates the SAM genetic background; grey indicates the genetic background of the deletion strain; white indicates a chromosomal deletion; and the light blue bar on the X chromosome indicates the sd^{E3} allele and the genetic background in which it was originally generated. The genotypes and phenotypes on the left illustrate that this particular deletion enhances the sd^{E3} phenotype in an ORE background, i.e., results in even smaller wings. On the right, however, this deletion suppresses the sd^{E3} phenotype in a short-wing introgression background (i.e., results in larger wings). However, because the short-wing and ORE flies both carry the same genetic background (ORE) opposite the deletion, this background dependence must be due to other loci elsewhere in the genome (in this case, illustrated by the SAM alleles elsewhere in the genome), indicating higher-order epistasis.


Binding Predictions
Illumina
DGRC
DGE
Modifier Deletions
Backcross
ppk7
CG9498
Cpr
Muc26B
Ddr
Kr-h1
CG9175
stai
6000000
6100000
6200000
6300000
6400000
6500000
Position along chromosome 2L


Binding Predictions
Illumina
DGRC
DGE
Modifier Deletions
Backcross
SoxN
C1GalTA
Git
raw
CG13101
CG18088
CG34398
CG18661
allgh
CG9466
C1GalTA
tai
8600000
8800000
9000000
9200000
9400000
Position along chromosome 2L


Binding Predictions
Illumina
DGRC
DGE
Modifier Deletions
Backcross
9800000
10000000
10200000
10400000
Position along chromosome 2L


Binding Predictions
Illumina
DGRC
DGE
Modifier Deletions
Backcross
CR43423
CG42719
Reg−2
CG13893
emc
CG13897
mth
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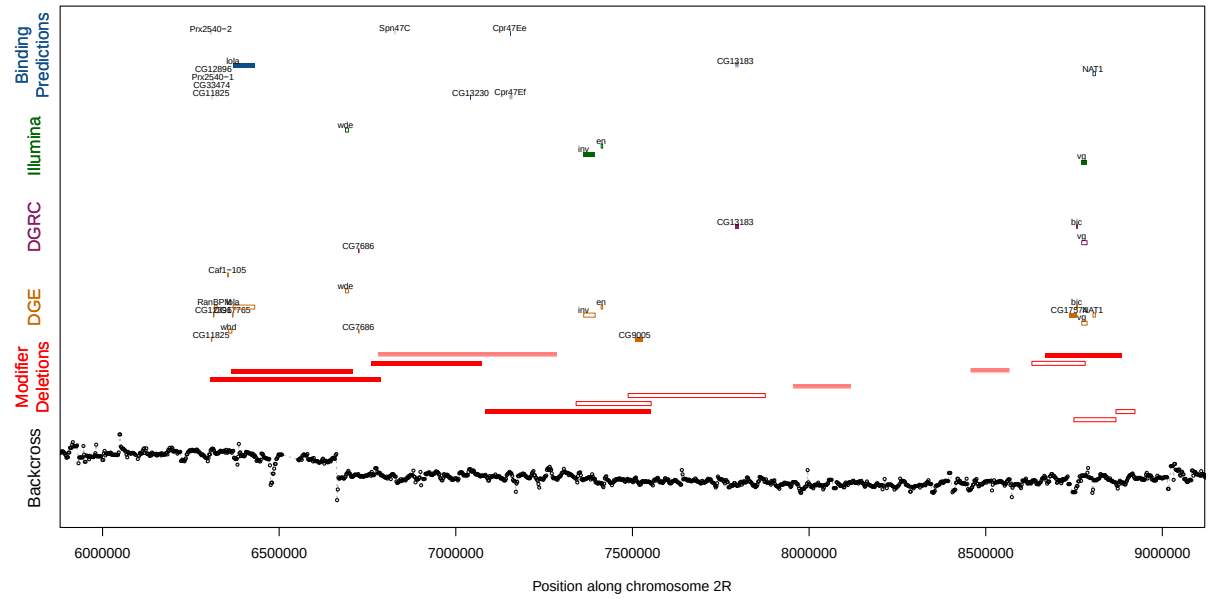
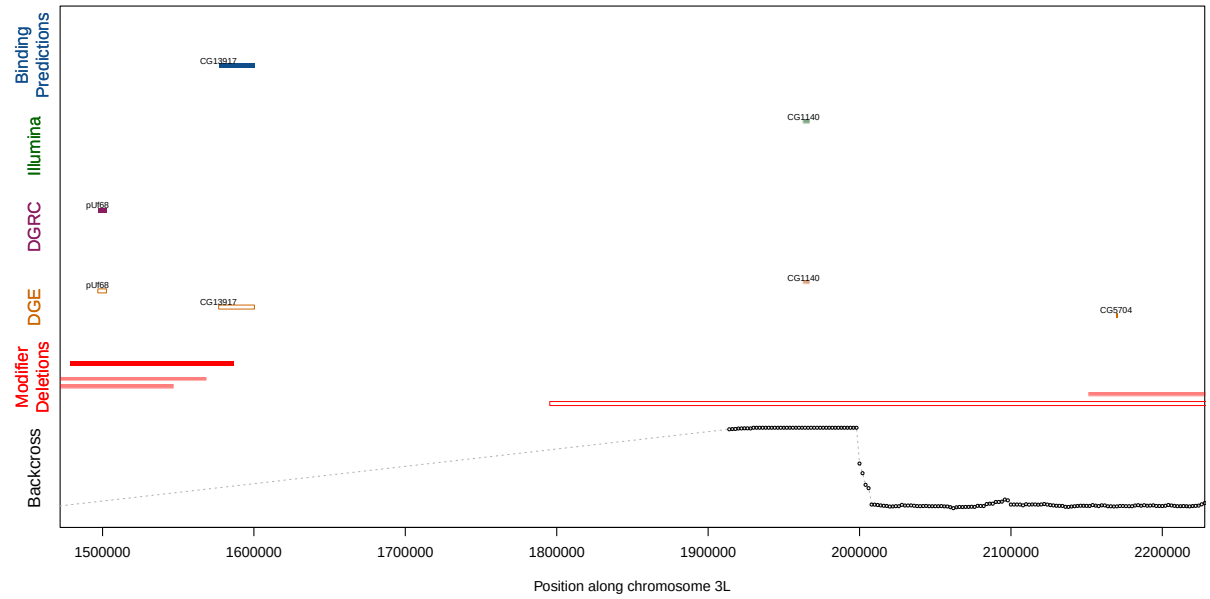
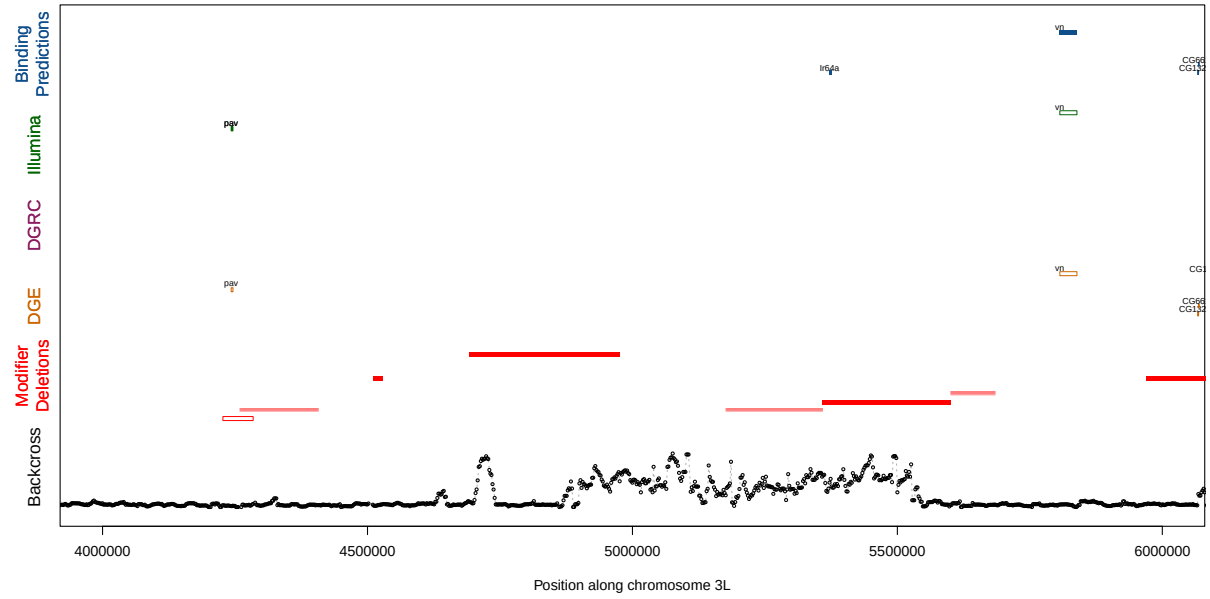
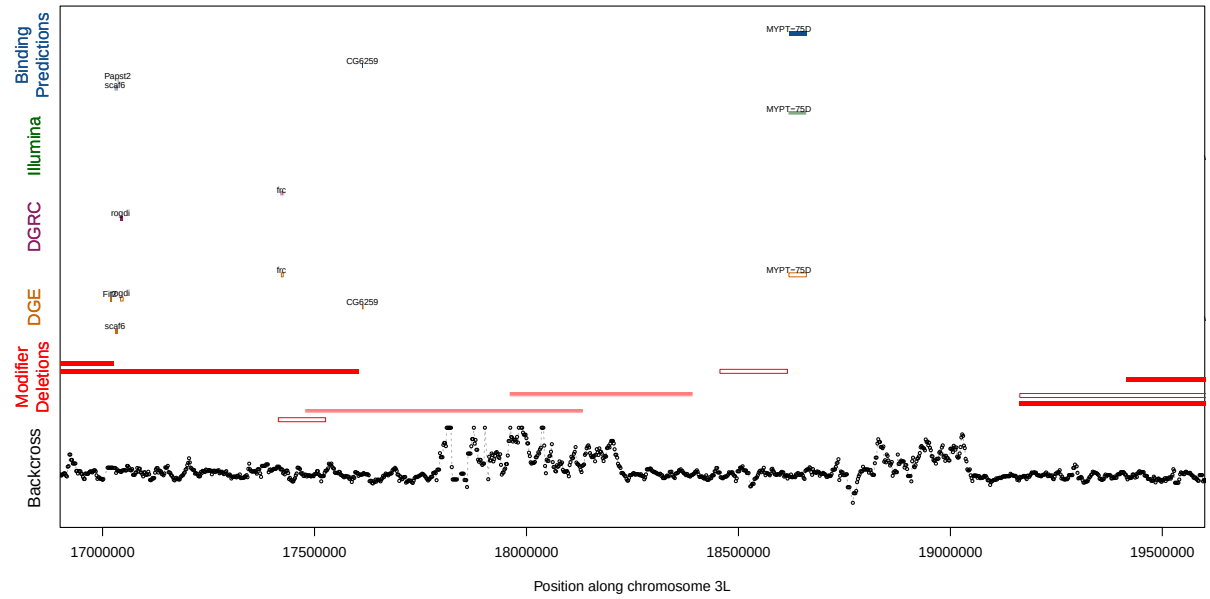
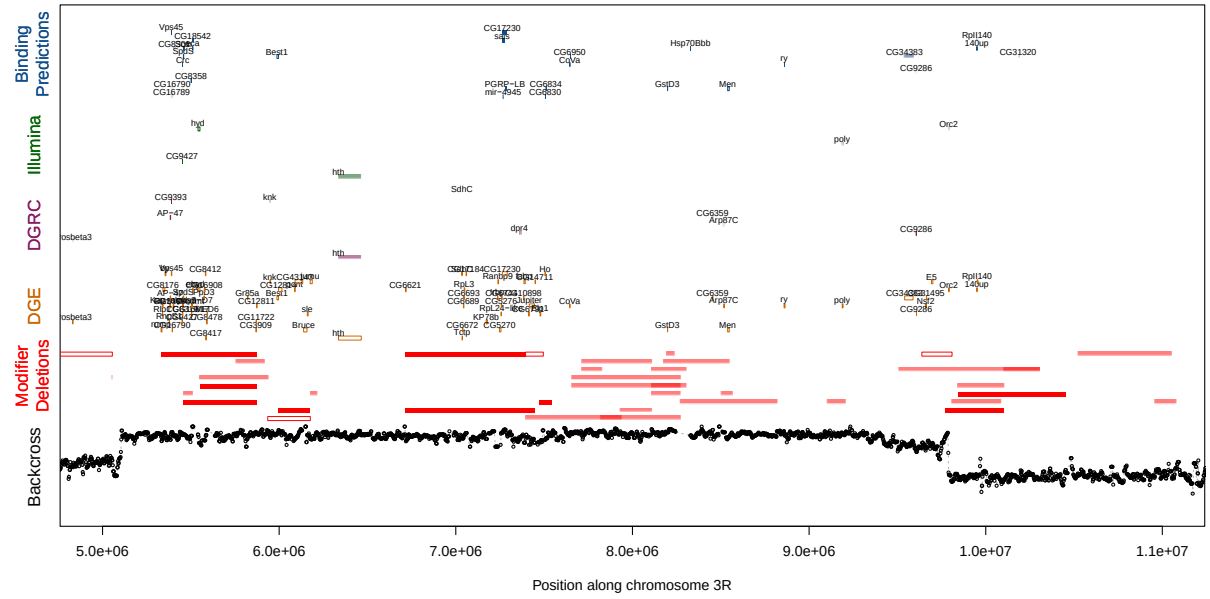
2e+05
4e+05
6e+05
8e+05
1e+06
Position along chromosome 3L


Binding Predictions
Illumina
DGRC
DGE
Modifier Deletions
Backcross
Prx2540−2
Spn47C
Cpr47Ee
lola
Prx2540−1
CG33474
CG11825
CG13230
Cpr47Ef
CG13183
NAT1
wde
inv
en
vg
CG7686
Caf1−105
bic
6000000
6500000
7000000
7500000
8000000
8500000
9000000
Position along chromosome 2R


Binding Predictions
Illumina
DGRC
DGE
Modifier Deletions
Backcross
CG13917
CG1140
pUf68
CG5704
1500000
1600000
1700000
1800000
1900000
2000000
2100000
2200000
Position along chromosome 3L


Binding Predictions
Illumina
DGRC
DGE
Modifier Deletions
Backcross
vn
Ir64a
pav
4000000
4500000
5000000
5500000
6000000
Position along chromosome 3L


Binding Predictions
Illumina
DGRC
DGE
Modifier Deletions
Backcross
MYPT-75D
CG6259
Papst2
scaf6
frc
rogdi
17000000
17500000
18000000
18500000
19000000
19500000
Position along chromosome 3L


Binding Predictions
Illumina
DGRC
DGE
Modifier Deletions
Backcross
Best1
CG6950
CqVa
Hsp70Bbb
GstD3
Men
CG9286
Orp2
poly
CG9427
hth
SdhC
knk
dpr4
CG6359
Arpp87C
CG6621
Ptp
5.0e+06
6.0e+06
7.0e+06
8.0e+06
9.0e+06
1.0e+07
1.1e+07
Position along chromosome 3R

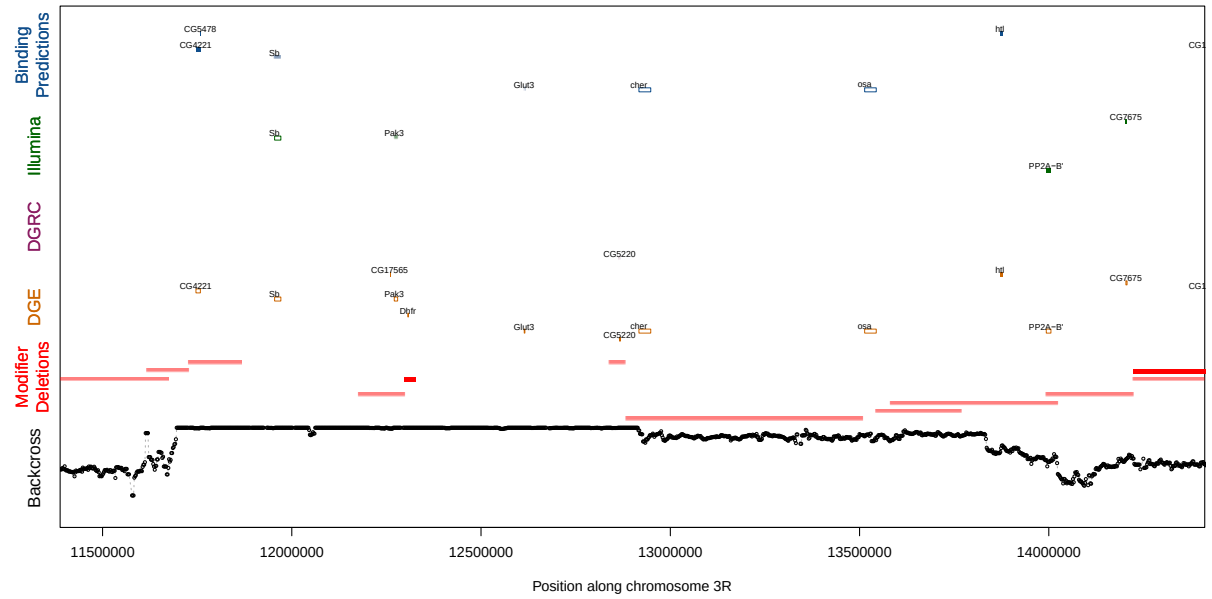
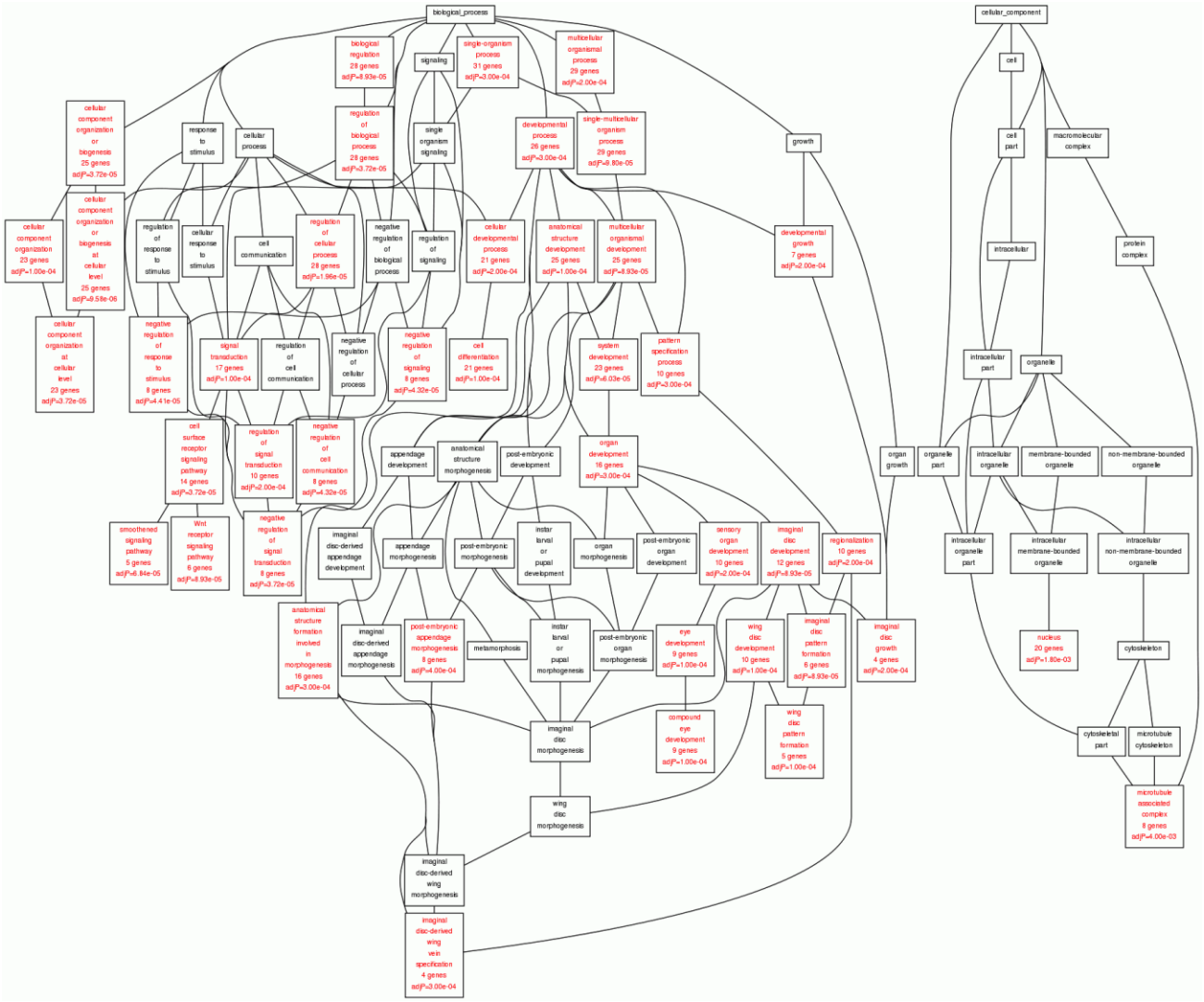


Figure S5 Integrated plots showing results of independent genomic datasets used to investigate the genetic basis of background dependence of the sd^{E3} phenotype. Backcross: Average frequency of the ORE (short-wing) allele across four short-wing introgression lines. Modifier deletions: open bars represent deletions with a significant main effect on the sd^{E3} phenotype; light shaded bars represent deletions with a significant background-dependent effect on the sd^{E3} phenotype; and dark shaded bars represent deletions in which both the main and interaction effects are significant. DGE: open bars represent genes whose transcript counts are influenced by *sd* genotype; light shaded bars represent genes showing evidence of genotype-dependent allelic imbalance; and dark shaded bars represent genes showing evidence of both an overall effect of *sd* genotype and genotype-dependent allelic imbalance. DGRC and Illumina: open bars represent genes showing evidence of an effect of *sd* genotype on expression; light shaded bars represent genes showing evidence of a genotype-by-background interaction effect; and dark shaded bars represent genes showing evidence of both the main and interaction effects. Binding predictions: open bars represent genes predicted to be overall SD binding targets (in at least one of the two genetic backgrounds); light shaded bars represent genes predicted to show differential affinity for SD between the two backgrounds; and dark shaded bars represent genes showing evidence of both overall SD binding and differential affinity between backgrounds. Only genes showing evidence of at least four significant effects across all datasets are shown.


biological_process
biological regulation 28 genes adjP=8.93e-05
signaling
single-organism process 31 genes adjP=3.00e-04
multicellular organismal process 29 genes adjP=2.00e-04
cellular component organization or biogenesis 25 genes adjP=3.72e-05
response to stimulus
cellular process
regulation of biological process 28 genes adjP=3.72e-05
single organism signaling
developmental process 26 genes adjP=3.00e-04
single-multicellular organism process 29 genes adjP=8.80e-05
growth
cellular component organization or biogenesis 23 genes adjP=1.00e-04
cellular component organization or biogenesis at cellular level 25 genes adjP=9.59e-06
regulation of response to stimulus
cellular response to stimulus
cell communication
regulation of cellular process 28 genes adjP=1.98e-05
negative regulation of biological process
regulation of signaling
cellular developmental process 21 genes adjP=2.00e-04
anatomical structure development 25 genes adjP=1.00e-04
multicellular organismal development 25 genes adjP=8.93e-05
developmental growth 7 genes adjP=2.00e-04
cellular component organization at cellular level 23 genes adjP=3.72e-05
negative regulation of response to stimulus 8 genes adjP=4.41e-05
signal transduction 17 genes adjP=1.00e-04
regulation of cell communication
negative regulation of cellular process
negative regulation of signaling 8 genes adjP=4.32e-05
cell differentiation 21 genes adjP=1.00e-04
system development 23 genes adjP=6.03e-05
pattern specification process 10 genes adjP=3.00e-04
cell surface receptor signaling pathway 14 genes adjP=3.72e-05
regulation of signal transduction 10 genes adjP=2.00e-04
negative regulation of cell communication 8 genes adjP=4.32e-05
appendage development
anatomical structure morphogenesis
post-embryonic development
organ development 16 genes adjP=3.00e-04
organ growth
smoothened signaling pathway 5 genes adjP=6.84e-05
Wnt receptor signaling pathway 6 genes adjP=8.93e-05
negative regulation of signal transduction 8 genes adjP=3.72e-05
imaginal disc-derived appendage development
appendage morphogenesis
post-embryonic morphogenesis
instar larval or pupal development
organ morphogenesis
post-embryonic organ development
sensory organ development 10 genes adjP=2.00e-04
imaginal disc development 12 genes adjP=8.93e-05
regionalization 10 genes adjP=2.00e-04
anatomical structure formation involved in morphogenesis 16 genes adjP=3.00e-04
imaginal disc-derived appendage morphogenesis
post-embryonic appendage morphogenesis 8 genes adjP=4.00e-04
metamorphosis
instar larval or pupal morphogenesis
post-embryonic organ morphogenesis
eye development 9 genes adjP=1.00e-04
wing disc development 10 genes adjP=1.00e-04
imaginal disc pattern formation 6 genes adjP=8.93e-05
imaginal disc growth 4 genes adjP=2.00e-04
imaginal disc morphogenesis
compound eye development 9 genes adjP=1.00e-04
wing disc pattern formation 5 genes adjP=1.00e-04
wing disc morphogenesis
imaginal disc-derived wing morphogenesis
imaginal disc-derived wing vein specification 4 genes adjP=3.00e-04
cellular_component
cell
cell part
macromolecular complex
intracellular
protein complex
intracellular part
organelle
organelle part
intracellular organelle
membrane-bounded organelle
non-membrane-bounded organelle
intracellular organelle part
intracellular membrane-bounded organelle
intracellular non-membrane-bounded organelle
nucleus 20 genes adjP=1.80e-03
cytoskeleton
cytoskeletal part
microtubule cytoskeleton
microtubule associated complex 8 genes adjP=4.00e-03

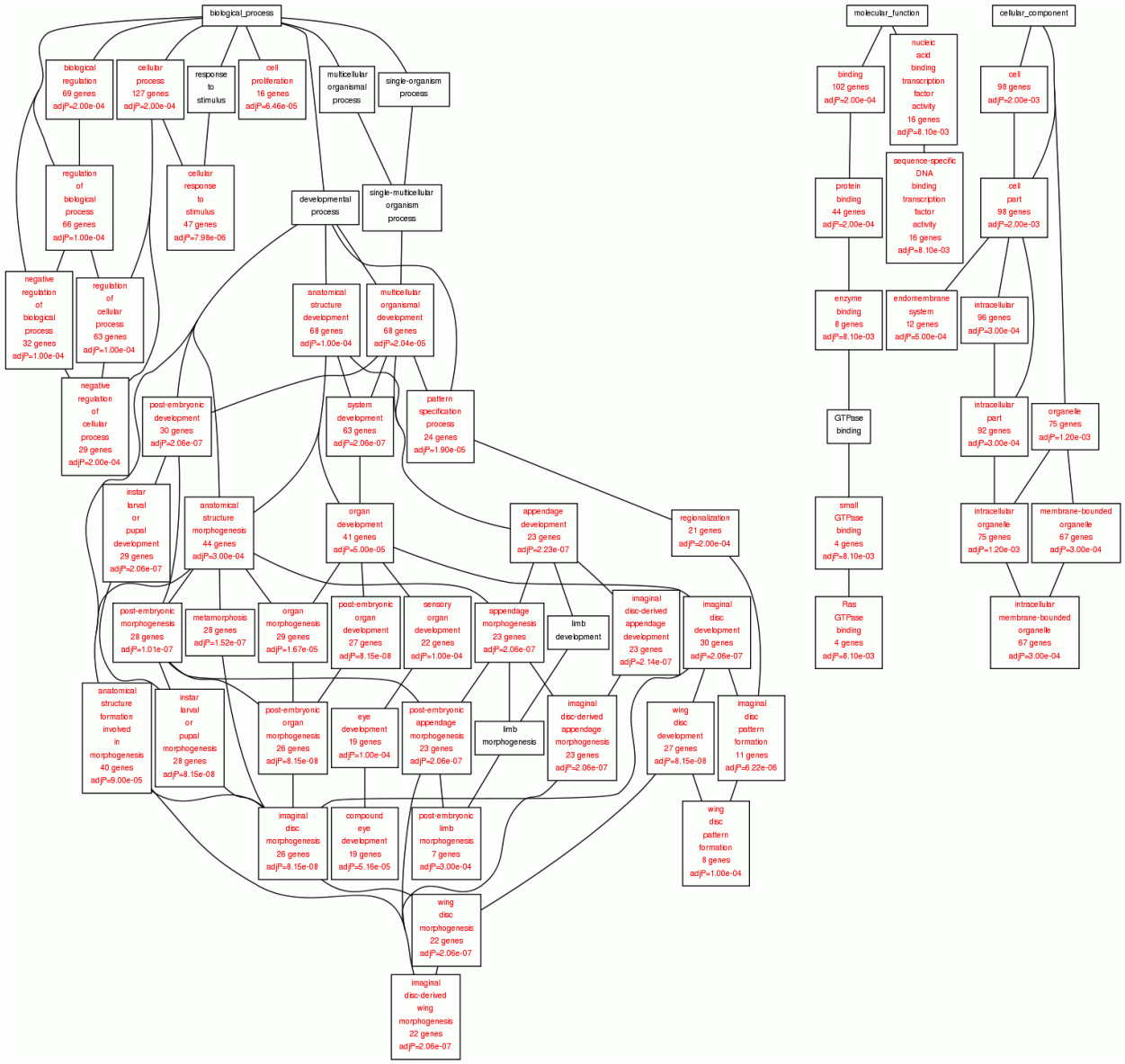


Figure S6 Top scoring GO hits for candidate genes identified by integrated analysis using (A) significant *sd* genotype effects for expression datasets and (B) significant genotype-by-background interaction effects for expression datasets.

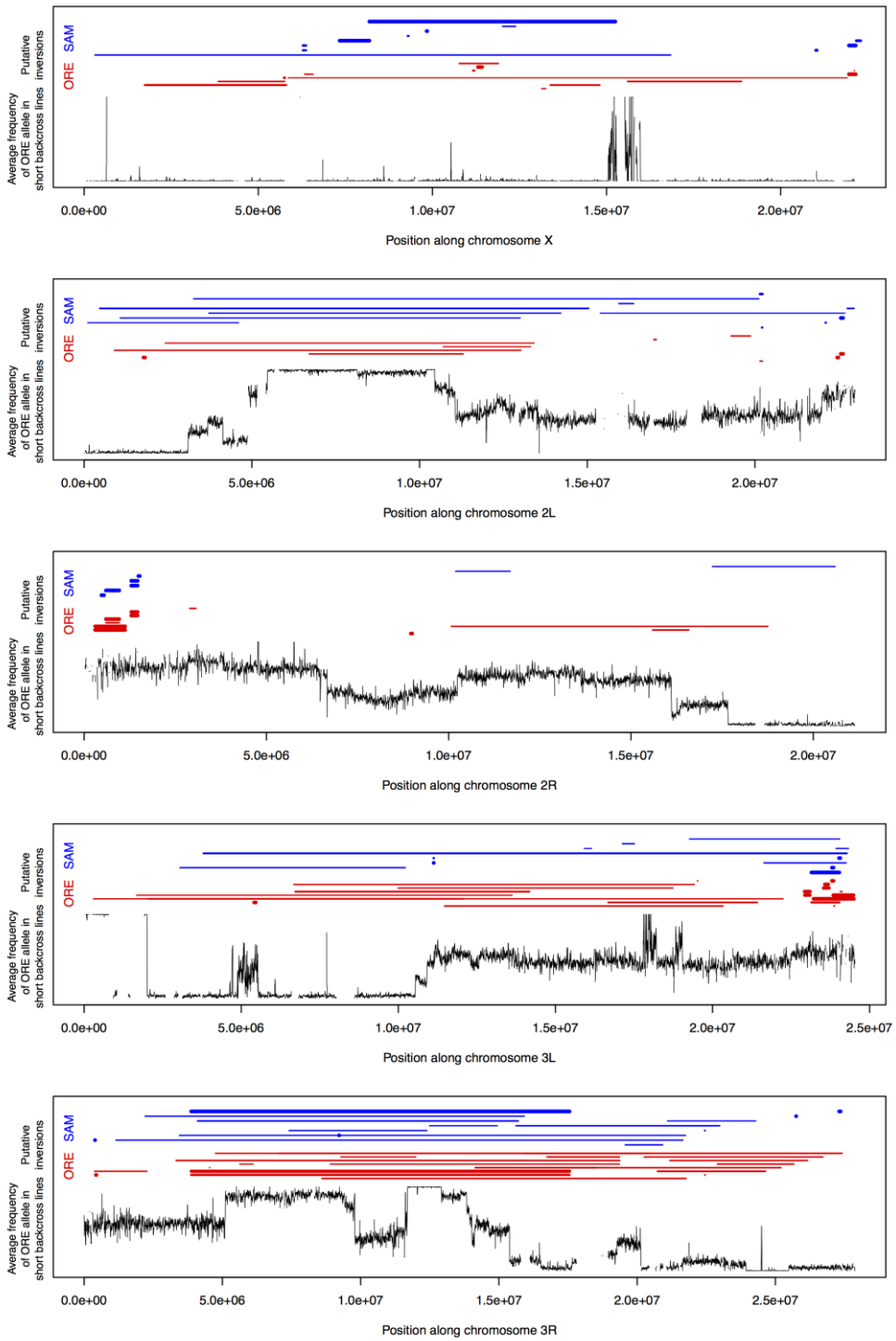


Figure S7 Weak evidence for putative inversions based on paired-end reads mapping to discordant locations in the genome. Thicker bars indicate stronger evidence. Inversions are relative to the *D. melanogaster* reference genome. We searched for inversions using BreakDancer v1.1 (Chen et al. 2009).

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