Legends for Supplementary Figures Johansson et al.

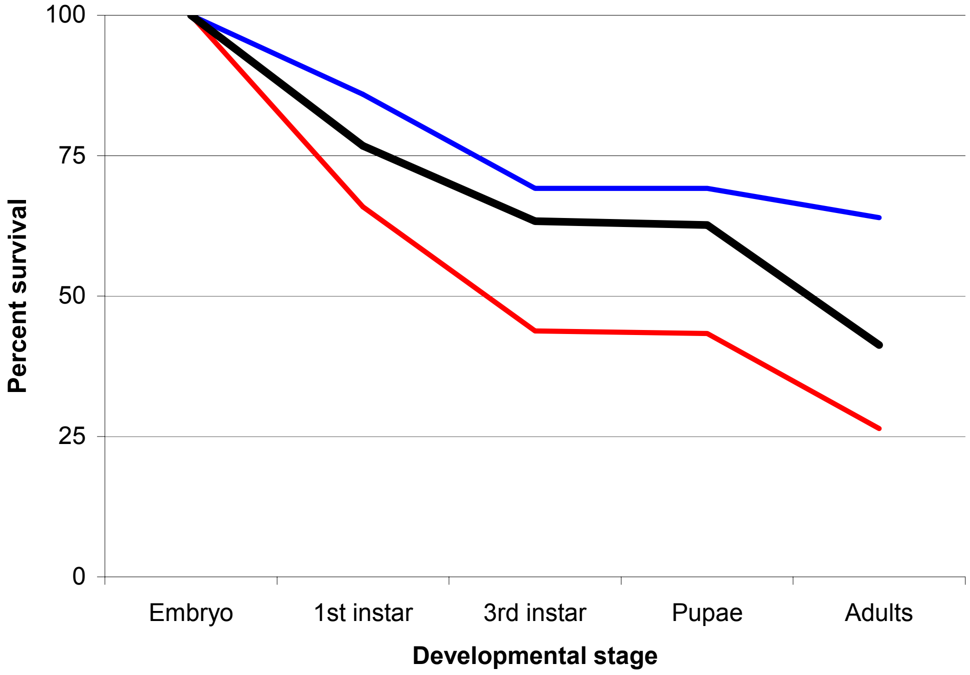
Figure S1. Developmental stage of lethality of Pof^{D140}/Pof^{D140}; $ci^{57g}/0$ offspring. Embryos (n=845) from the cross Pof^{D140}/Pof^{D140}; $C(4)RM\ sv^{spa\text{-}pol}/0$♀♀ × Pof^{D140}/Pof^{D140}; ci^{57g}/ci^{57g}♂♂ were followed through development and counted as embryos, first instar larvae, third instar larvae, pupae and adults. The percent survival is shown in red. As controls embryos (n=645) from the cross Pof^{D140}/Pof^{D140}; $C(4)RM\ sv^{spa\text{-}pol}/0$♀♀ × ci^{57g}/ci^{57g}♂♂ were followed through development and shown in blue. Considering the lethality in the control experiment to be normal for this experiment, the normalized survival of offspring from the cross Pof^{D140}/Pof^{D140}; $C(4)RM\ sv^{spa\text{-}pol}/0$♀♀ × Pof^{D140}/Pof^{D140}; ci^{57g}/ci^{57g}♂♂ is plotted in black. The results suggest that although the majority of the expected 50% Pof^{D140}/Pof^{D140}; $ci^{57g}/0$ offspring die as embryos or early larvae a small proportion survive until pupal stages.

Figure S2. Statistical analysis of gene expression in *Pof* mutants and *HP1* RNAi compared to wildtype. (*A*) Microarray analysis showing the median level of mRNA from genes on the 4th chromosome in Pof^{D2}/Pof^{D2} mutant first instar larvae as a function of the median levels in wildtype in log$_2$ scale. Genes for which there were reduced amounts of mRNA (below dotted line) are marked with filled circles and genes for which there were increased amounts with open circles. (*B*) Mean change in gene expression for each chromosome arm, Pof^{D2} – wildtype expression (log$_2$ scale). Squares indicate the mean value and whiskers indicate 95% confidence interval. ANOVA tests for all chromosome arms were performed on log$_2$ differences between median wildtype and median mutant expression. (*C, D* and *E*) A significant ANOVA ($p<0.05$) was followed by Duncan’s multiple range test to pair wise compare all chromosome arms. (*C*) Results from Duncan’s multiple range test, Pof^{D2} – wildtype. Values in red are significant at $p < 0.05$. (*D*) Results from Duncan’s multiple range test, *Pof*^{D119} – wildtype (see Figure 3A, B). (*E*) Results from Duncan's multiple range test, *HP1* RNAi – control (see Figure 3C, D). (*F*) Correlation plot of *Pof*^{D119} and *HP1* RNAi expression change values ($\log_2$ scale). Regression line is indicated in red.

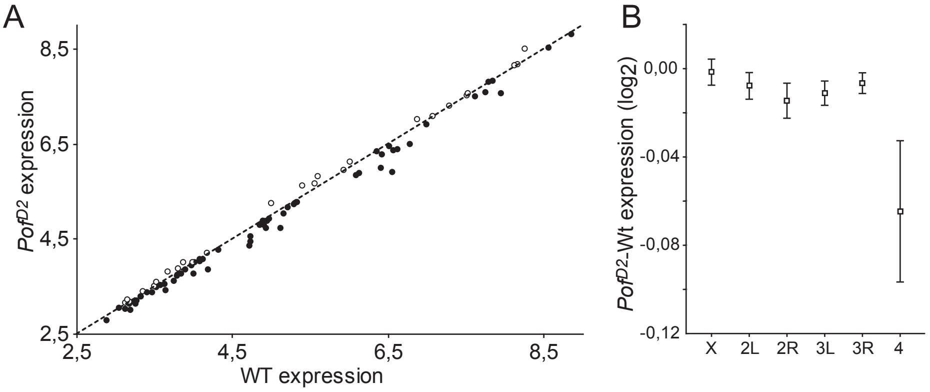
Figure S3. POF binding of chromosome 4 from position 200kb to position 800kb at 10-kb resolution (according to annotation release 4). The Y-axis indicates enrichment plotted as percent of input. Enrichment using a MOCK antisera or no antisera was determined at all positions and never exceeded 0.1%.

Figure S4. POF binding to d4 (distal-4th chromosome) on translocated 4th chromosomes. (*A*) Examples of the four staining classes used in the analysis. The chromosomes were stained with POF (red) and MSL3 (green, to verify the cytology) and counterstained with DAPI (blue). (*B*) Summary of POF binding to different translocations at different conditions.

Johansson_et_al_FigS1



Johansson_et_al_FigS2



C

Pof^{D2}

	X	2L	2R	3L	3R
2L	0,584187				
2R	0,285652	0,554812			
3L	0,421033	0,755880	0,747215		
3R	0,638068	0,907191	0,502378	0,690462	
4	0,000004	0,000003	0,000011	0,000011	0,000004

D

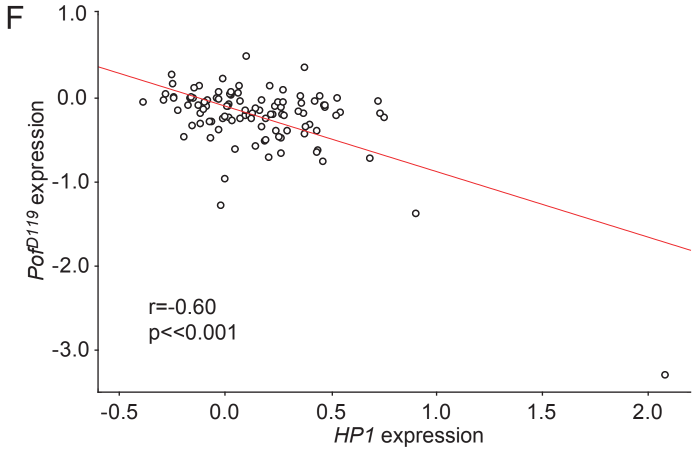
Pof^{D119}

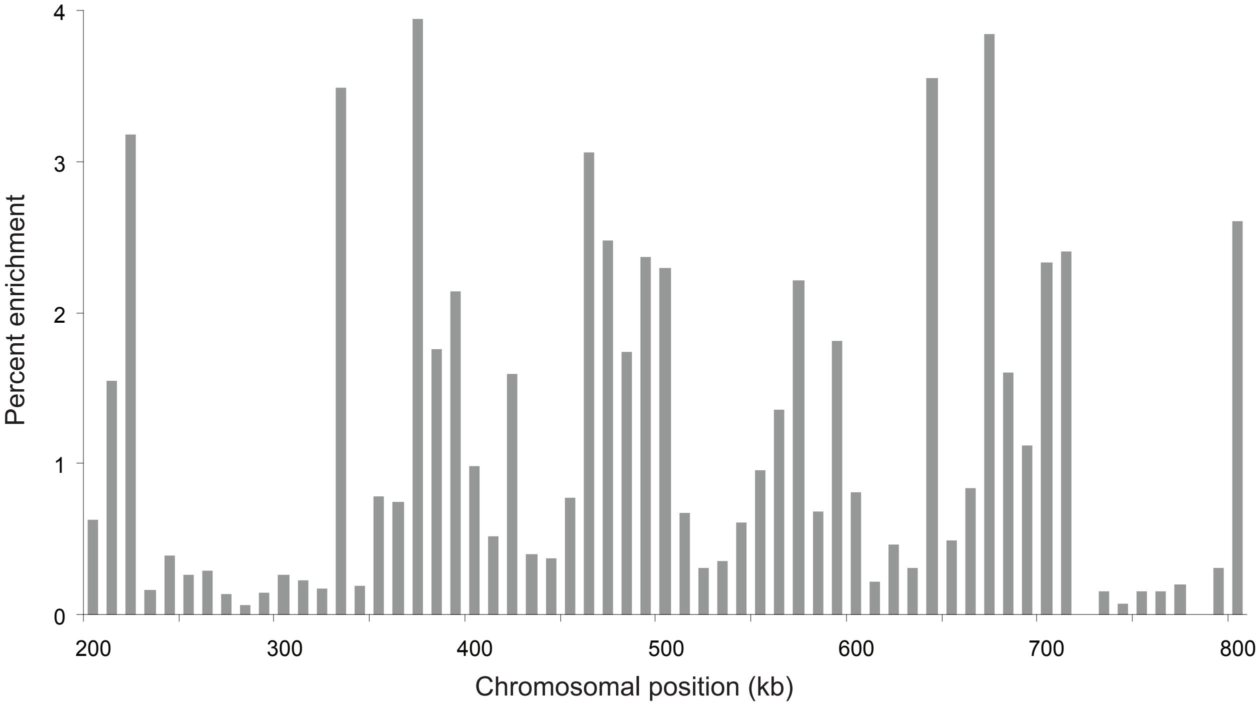
	X	2L	2R	3L	3R
2L	0.129900				
2R	0.927887	0.119411			
3L	0.637170	0.262391	0.600240		
3R	0.479543	0.363060	0.447628	0.775067	
4	0.000011	0.000004	0.000009	0.000003	0.000004

E

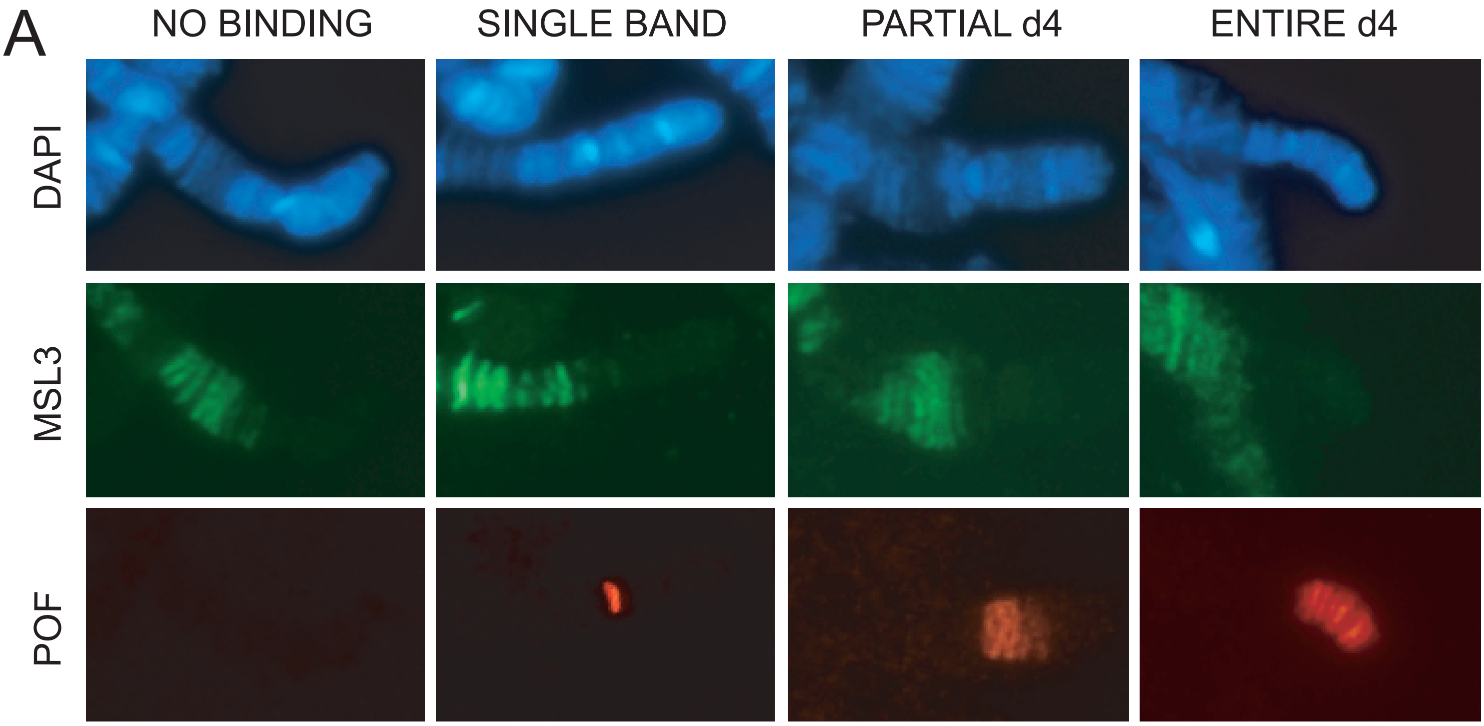
HP1

	X	2L	2R	3L	3R
2L	0.481440				
2R	0.436053	0.913702			
3L	0.678478	0.728928	0.668091		
3R	0.506930	0.939837	0.864148	0.766754	
4	0.000004	0.000011	0.000009	0.000004	0.000003




Percent enrichment
0
1
2
3
4
200
300
400
500
600
700
800
Chromosomal position (kb)

Johansson_et_al_FigS4



B

Translocation	Breakpoints	Y-chr	Temp (ºC)	STAINING CLASSES			
				NO BINDING	SINGLE BAND	PARTIAL d4	ENTIRE d4
T(1;4)w$^{m258\text{-}18}$	3C4-5; h59-h61	0	18	+/-	+	++	+
T(1;4)w$^{m258\text{-}18}$	3C4-5; h59-h61	Y	18	+	++	+	
T(1;4)w$^{m258\text{-}18}$	3C4-5; h59-h61	0	25	+	++	+	
T(1;4)w$^{m258\text{-}18}$	3C4-5; h59-h61	Y	25	++	+		
T(1;4)w^{m5}	3C2; 101F	Y	18	++	+		
T(1;4)w^{m5}	3C2; 101F	Y	25	+++			
T(1;4)e15	13C; >101	Y	18	+++			
T(1;4)B^{S}	16A7-B1;102F	Y	18	+++			
T(1;4)w$^{m258\text{-}21}$	3E5-6; 101F	Y	18	+++			
T(1;4)sc^{H}	1B4;101F1-102D1	Y	18	+++			

+++; all nuclei show this staining class
++; a majority of the nuclei show this staining class
+; a fraction (>5%) of the nuclei show this staining class
+/-; occasional nuclei show this staining class