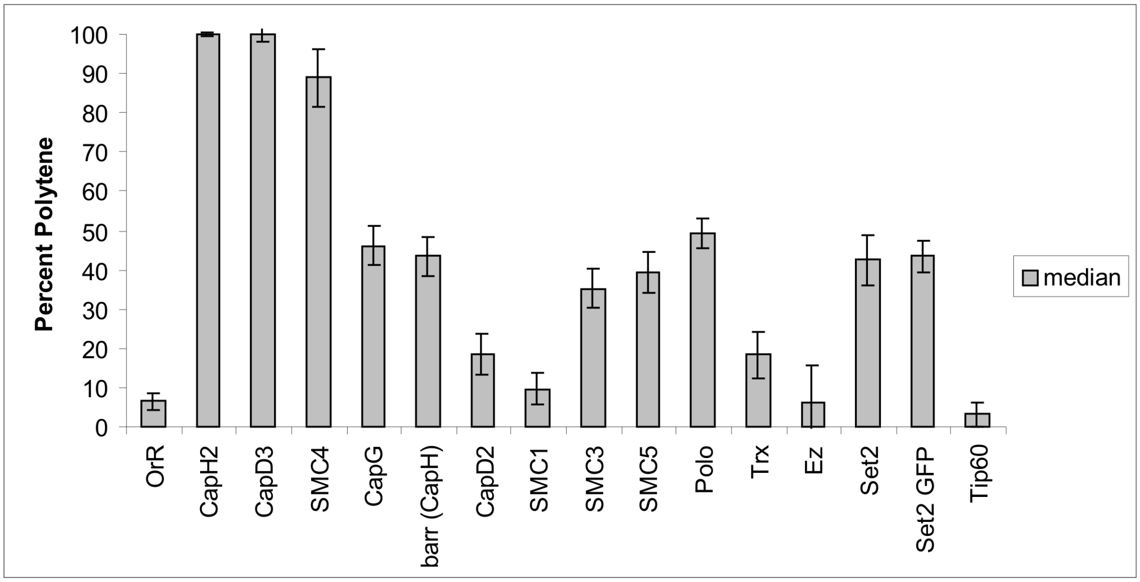
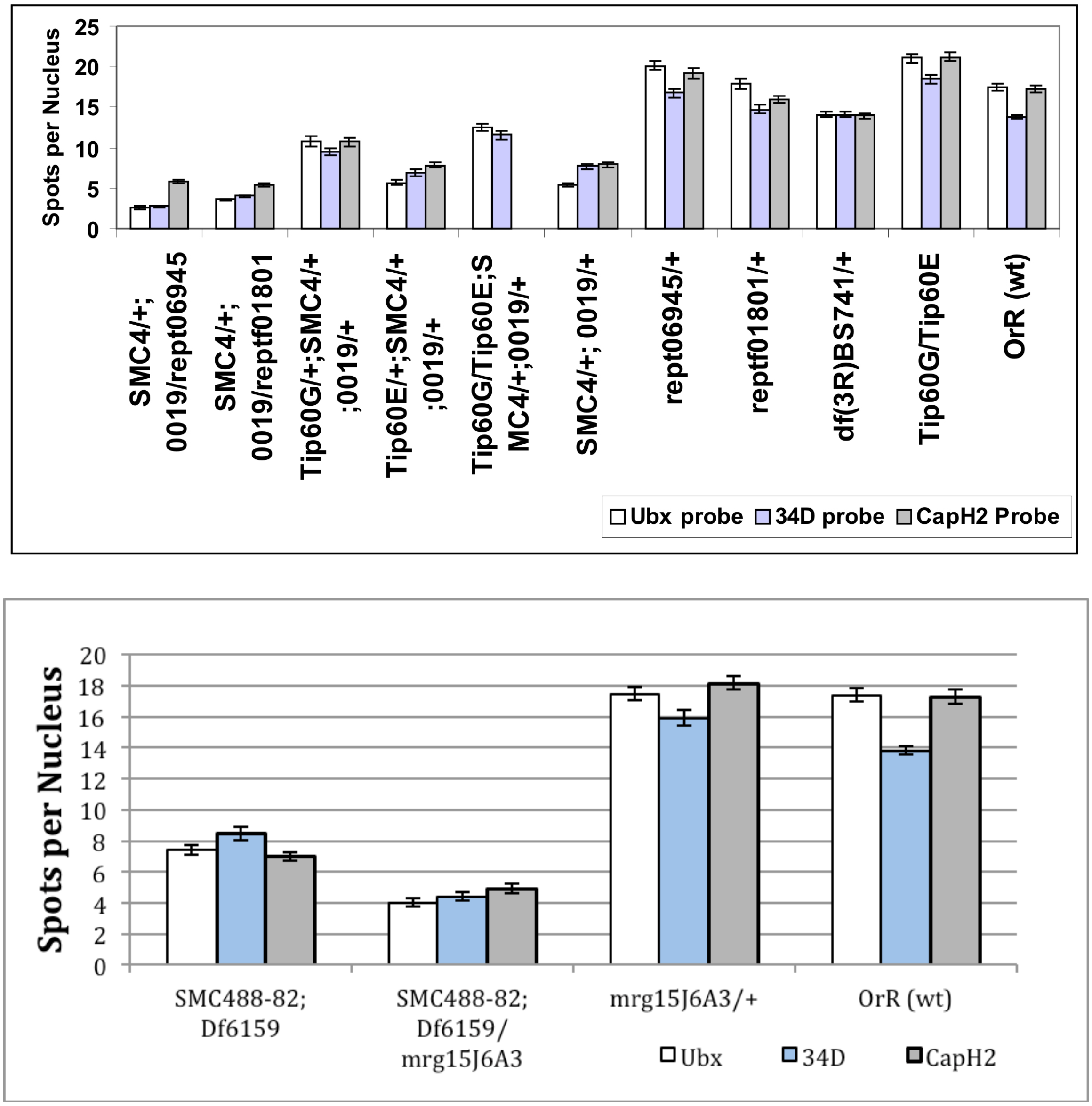
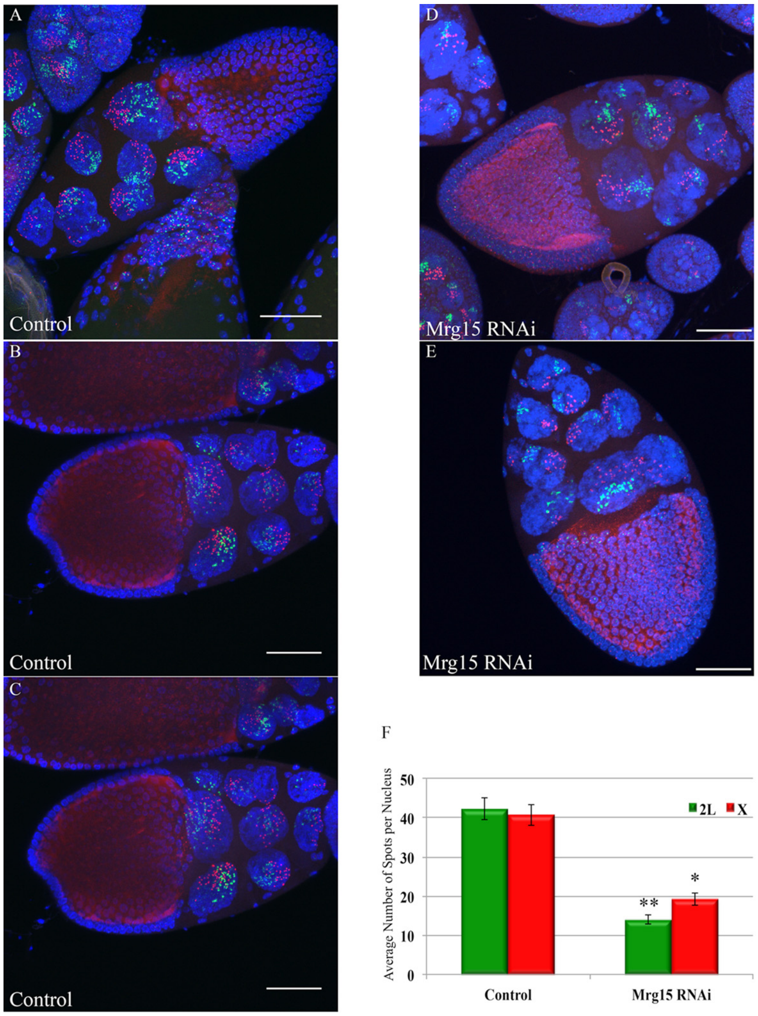


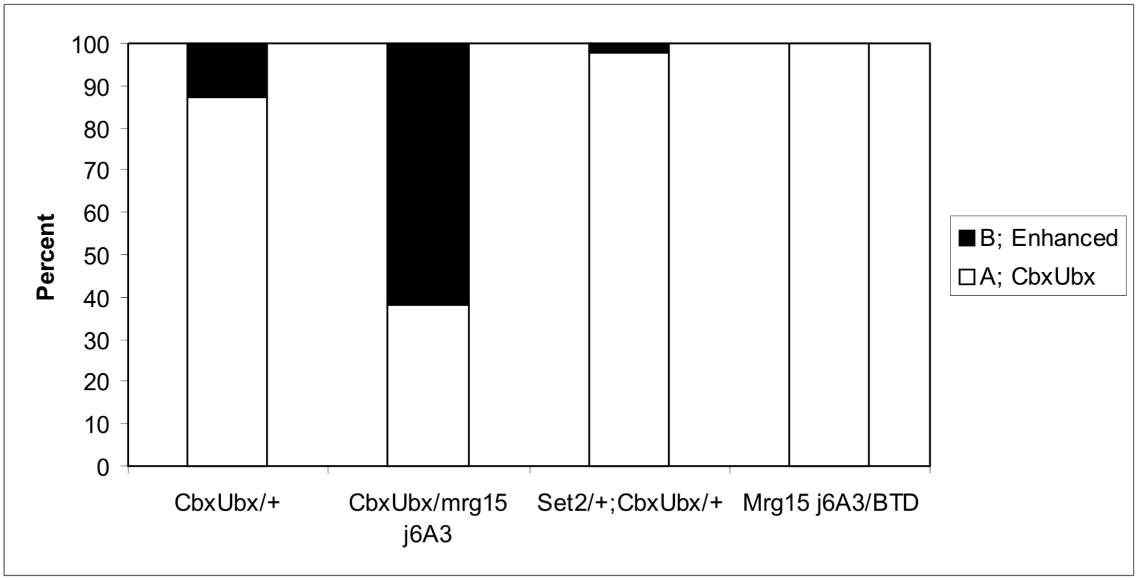
Figure S1 RNAi suppression in salivary glands overexpressing Cap-H2. The graph represents the median value for the various Vienna RNAi lines crossed into the "spots" genotype (LacO 60F, hs83>LacI-GFP; Hsp70>Gal4, *Cap-H2*^{EY09979}), except for the first one, OrR, which is not a RNAi line. The percent polytene was determined by DAPI staining for identification of polytene bands present under 40x magnification. Error bars are shown. See supplemental Table S2 for complete genotype description, number of nuclei and p-values.



FigureS2 Condensin II interactors in the ovarian nurse cells. The graphs shows the number of FISH spots per nucleus in stage 6/7 eggchambers for each of the genotypes listed on the x-axis. The white bar represents the Ubx genomic region FISH probe, light blue is the 34D genomic region FISH probe, and grey is the Cap-H2 genomic region FISH probe. 0019 denotes the allelic designations for *Cap-H2*$^{Z3\text{-}0019}$. The *SMC4* allele used in the top graph is *Smc4*^{k08819} and for the bottom graph *Smc4*$^{88\text{-}82}$. The deficiency uncovering the *Mrg15* locus is Df(3R)BSC741. The deficiency uncovering the Cap-H2 locus is Df(3R)Exel6159 abbreviated as "Df6159". The list of complete genotypes is found in supplemental Table S2 along with the number of nurse cells for each probe and p-values.



FigureS3 Germline specific RNAi depletion of Mrg15 leads to polytene unpairing defects. DNA FISH probes to chromosome 2L (green spots) and X-chromosome (red spots) in representative stage 10 eggchambers (DAPI in blue). Note that the unpairing defect was too severe in earlier stages and thus made quantitation of individual spots difficult. Panels A-C are control *Matα4-GAL-VP16* and panels D-E are *Matα4-GAL-VP16, Mrg15-TRiP-GL00128* RNAi depleted tissues (see methods). Panel F, number of FISH spots per nucleus were counted manually in 3D images for 3 different stage 10 eggchambers, and average number of spots is shown with standard error of the mean (two-tailed T-test, assuming unequal variance was calculated using MS Excel $*p<10^{-6}$; $**p<10^{-8}$). For 2L-probes n=17 (control) and n=18 (Mrg15 RNAi) nuclei. For X-probes n=16 (control) and n=16 (Mrg15 RNAi) nuclei. Images acquired with a Nikon laser scanning confocal with a 40x oil immersion lens (see methods), and 2D projections of a limited number of z-optical sections are shown. Scale bars are 50µm in each panel. See Figure 6 of main text for higher magnification images of individual stage 10 nuclei with FISH signals.



FigureS4 Transvection in *Mrg15*. Wings from the genotypes shown on x-axis were scored as either typical $Ubx^{Cbx\text{-}1}Ubx^{1}$ (class A, white bar) or enhanced (class B, black bar). Note that "typical" class A wings are not wild type and instead have a moderate abnormal morphology as a result of the transvecting $Ubx^{Cbx\text{-}1}$ allele. See Figure 7 of main text for images of typical wings. For simplicity, $Ubx^{Cbx\text{-}1}Ubx^{1}$ is denoted above as “CbxUbx”. The data presented in this Figure S4 for $Mrg15^{j6A3}$ was derived independently of the data in Figure 7 of the main text. As an additional control, the Set^{1}/+ mutation has no significant effect on this transvection system. The last genotype includes the bithorax transvection disruptor (BTD), which is a rearrangement of the $Ubx^{Cbx\text{-}1}Ubx^{1}$ chromosome. N>61 for all genotypes. P-values using chi-squared relative to $Ubx^{Cbx\text{-}1}Ubx^{1}$/++ enhanced class: $Mrg15^{j6A3}$ p<0.001, $Set2^{1}$ p=0.74, $Mrg15^{j6A3}$/BTD *p<0.005*. The $Set2^{1}$ mutation has no significant affect on transvection in this assay and serves as a negative control. Note that since only one *Set2* mutant allele was tested we cannot exclude the possibility that other *Set2* mutations may modify transvecting loci.

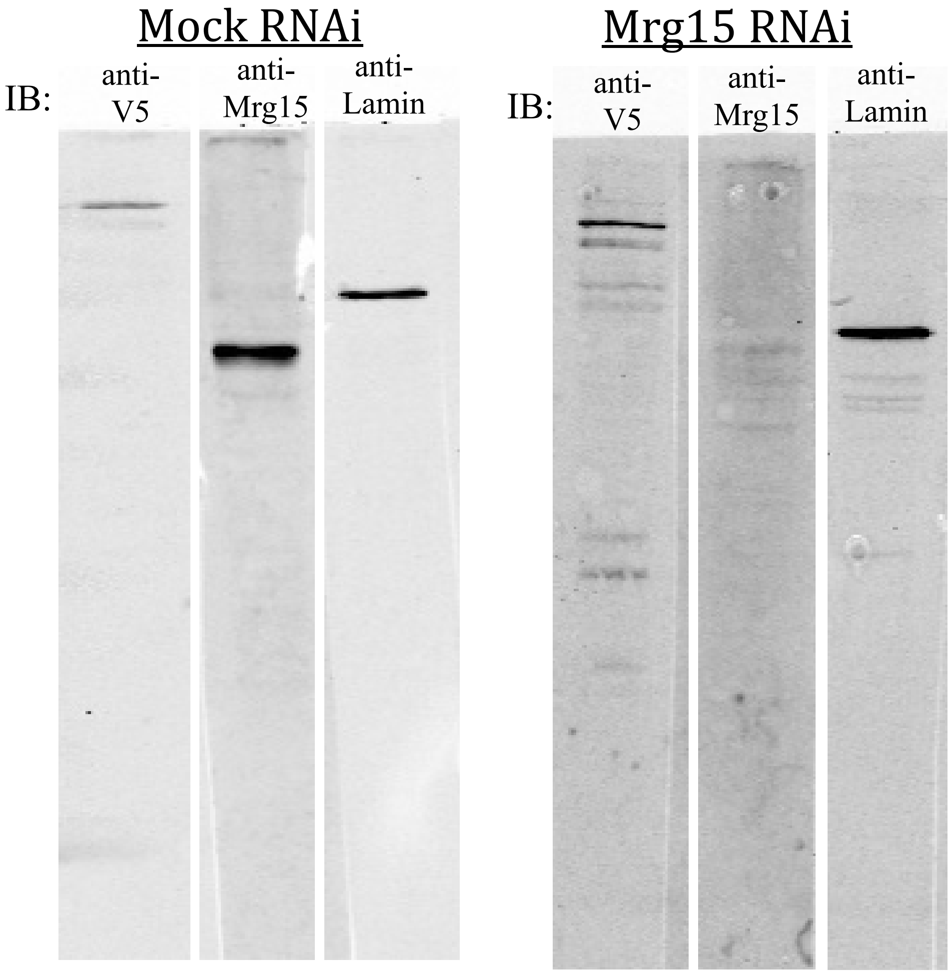


Figure S5 RNAi depletion of Mrg15 protein in S2 cultured cells. Cells were transfected with pMT-Cap-H2-V5 (or Cap-H2-EGFP; only V5 shown here) and were treated with double stranded RNA to pBlueScript SK (control Mock RNAi) or to Mrg15. Whole cell lysates were used for immunoblots (IB) with anti-V5, anti-Mrg15 or anti-Lamin (Dm0, Lamin B). Approximately >75% of Mrg15 protein was observed to be consistently depleted over four biological replicates. Protein depletion was determined from immunoblots exposed to film, scanned and quantitated using ImageJ. Lamin Dm0 bands in the linear range were used as loading controls.

Table S1 List of yeast two-hybrid interactors. The list of clones recovered from the yeast two-hybrid screen. The insert size in base pairs was estimated from PCR products (see main text for methods). The gene descriptor and CG numbers are as given from www.flybase.org based on blast search results from sequenced clones.

clone #	size of insert	gene descriptor	CG-identifier
1CH2-64-1	1000	unknown (eIF-3-p25 domain, Eukaryotic translation initiation factor 3 subunit 12)	CG10306
1CH2-39	500	unknown (methyltransferase-like protein)	CG10584
1CH2-65	400	Programmed cell death 4 ortholog (Pdcd4)	CG10990
1CH2-83-3	200	RpS12	CG11271
1CH2-76-3	250	RpS12	CG11271
1CH2-4	1100	Mitochondrial ribosomal protein S22	CG12261
1CH2-21	200	Pleckstrin-like protein (PH domain-containing)	CG12393
1CH2-32	300	Ribosomal protein L8	CG1263
1CH2-54-1	900	beta-site APP-cleaving enzyme	CG13095
1CH2-7-1	1000	yellow-g2, yellow protein (contain MRJP domain)	CG13804
1CH2-68	900	Chorion protein a at 7F	CG33962
1CH2-83-1	900	lethal (3) 03670 (unknown function)	CG1715
1CH2-51-4	180	RpL5	CG17489
1CH2-14	360	Glutathione S transferase E6	CG17530
1CH2-1	360	Ribosomal protein L31	CG1821
1CH2-28	400	Ribosomal protein S7	CG1883
1CH2-63-1	300	RpS7	CG1883
1CH2-61	800	Translation elongation factor 2b (Ef2b)	CG2238
1CH2-77	550	Translation elongation factor 2b	CG2238
1CH2-2	1000	Yolk protein 1	CG2985
1CH2-73	1350	Yolk protein 1 (Yp1)	CG2985
1CH2-78	120	Yolk protein 1 (Yp1) (same as 1CH2-73)	CG2985
1CH2-45-1	950	Yolk protein 1	CG2985
1CH2-67-1	880	Yolk protein 1	CG2985
1CH2-58	1300	Yolk protein 1	CG2985
1CH2-72-1	900	Yolk protein 1	CG2985
1CH2-45-2	300	RpL41	CG30425
1CH2-72-3	200	RpL41	CG30425
1CH2-64-2	800	14-3-3 epsilon (PAR5, EK3-5, 14-3-3)	CG31196
1CH2-7-2	230	RpL12	CG3195
1CH2-17	360	Pyroglutamyl-peptidase I	CG32147
1CH2-80	250	singed, structural constituent of cytoskeleton	CG32858
1CH2-79-1	780	26S proteasome regulatory complex subunit p42D (Rpt4)	CG3455
1CH2-53	900	unknown (Xylutokinase activity, FGGY-N and FGGY -C domains)	CG3534
1CH2-60	900	bellwether (blw), mitochondrial ATP synthase subunit alpha precursor	CG3612
1CH2-74	700	Mitochondrial ATP synthase alpha subunit	CG3612
1CH2-34	600	Ribosomal protein L23	CG3661
1CH2-19	450	Eukaryotic initiation factor 4E (eIF-4E)	CG4035
1CH2-54-2	500	RpS16	CG4046
1CH2-26	1000	Unknown	CG40460
1CH2-51-1	1150	Unknown	CG40460
1CH2-18	350	Ribosomal protein L35 RpL35	CG4111
1CH2-69	300	Eukaryotic initiation factor 2 (eIF-2 beta)	CG4153
1CH2-59	410	Heat shock protein hsp26	CG4183

1CH2-44	200	SUMO/Ubiquitin-like protein SMT3	CG4494
1CH2-8-2	280	RpLP2	CG4918
1CH2-67-2	600	RpS2	CG5920
1CH2-37	650	Vacuolar proton-motive ATPase subunit G VHA13	CG6213
1CH2-41	800	MRG15	CG6363
1CH2-56	1400	MRG15	CG6363
1CH2-8-1	600	MRG15	CG6363
1CH2-52-2	390	Chorion protein 15	CG6519
1CH2-27	130	Chorion protein 19	CG6524
1CH2-66	530	Chorion protein 19	CG6524
1CH2-51-2	310	RpS25	CG6684
1CH2-51-3	280	RpS25	CG6684
1CH2-42	340	Unknown (Nuclear phosphoprotein p8)	CG6770
1CH2-72-2	500	Unknown (Nuclear phosphoprotein p8)	CG6770
1CH2-40-2	270	RpS3	CG6779
1CH2-5	600	Rack1, Receptor of activated protein kinase C1	CG7111
1CH2-15	400	Rack1	CG7111
1CH2-24	600	Rack1	CG7111
1CH2-49	600	Rack1	CG7111
1CH2-71	550	Rack1	CG7111
1CH2-40-1	450	Rack1	CG7111
1CH2-79-2	420	Rack1	CG7111
1CH2-36	300	Ribosomal protein LP0(RpLP0)	CG7490
1CH2-50	400	Ribosomal protein L11 (RpL11)	CG7726
1CH2-43	180	janus A (molecular function unknown, sex differentiation)	CG7933
1CH2-13	510	Unknown (WD40/YVTN repeat-like-containing domain)	CG8001
1CH2-23	350	Cystatin-like protein	CG8050
1CH2-84	160	Ribosomal protein L37A (RpL37A)	CG8527
1CH2-76-2	400	Jon25Biii (elastase activity, trypsin activity)	CG8871
1CH2-12	900	Glyceraldehyde 3 phosphate dehydrogenase 2	CG8893
1CH2-83-2	510	Vitelline membrane 26Aa (Vm26Aa)	CG9048
1CH2-11	360	palisade, TU-1 (vitelline membrane formation)	CG9050
1CH2-25	250	Vitelline membrane 34Ca (Vm34Ca)	CG9271
1CH2-30	900	Glutathione S-transferase-like protein	CG9362
1CH2-52-1	500	Glycoside hydrolase	CG9466
1CH2-76-1	500	Int6 (Translation initiation factor activity)	CG9677
1CH2-57	200	Unknown	CG9350
1CH2-29	60	empty vector	
1CH2-31	70	empty vector	
1CH2-81	40	empty vector	
1CH2-63-2	20	empty vector	

Table S2 Figure S1 supporting information. List of genes, stock numbers, number of glands (nuclei), and p-values for Figure S1. For stock number, BL=Bloomington, v=Vienna Drosophila RNAi Center. The p-value is a two-tailed student t-test in Microsoft Excel and relative to the Oregon-R (OrR-S).

Gene	Stock #	# glands (nuclei)	p-value to OrR
OrR-S	BL4269	29 (1876)	NA
Cap-H2	v24905	15 (1068)	1.14x 10-28
Cap-D3	v9402	11 (584)	1.91x 10-23
SMC4	v10937	14 (882)	8.02x 10-14
Cap-G	v40047	26 (1634)	4.20x 10-11
Cap-H	v26760	23 (1303)	1.55x 10-7
Cap-D2	v33424	22 (1209)	1.01x 10-4
SMC1	v6532	23 (1460)	1.12x 10-2
SMC3	v39205	30 (1654)	3.42x 10-7
SMC5	v38969	37 (1952)	1.67x 10-6
Polo	v20177	27 (1429)	8.16x 10-11
Trithorax	v37715	6 (369)	4.58x 10-5
E(z)	v27646	5 (249)	0.27
Set2	v30707	33 (1467)	1.54x 10-12
Set2-GFP	BL24108	11 (532)	1.21x 10-9
Tip60	v22233	11 (554)	0.931

Table S3 Figure S2 supporting information. “Abbr” denotes abbreviation of genotype used in supplemental Figure S2. List of genotypes, number (n) of nurse cells for each of the three FISH probes, and p-values for supplemental Figure S2. The *Smc4* allele is *SMC4*^{k08819} and the *Cap-H2* allele is *Cap-H2*$^{Z3\text{-}0019}$. The p-value is a two-tailed student T-test in Microsoft Excel and relative to the OrR-S. “nd” denotes not done.

Abbr.	Genotype	T-test to Genotype	Ubx FISH	34D FISH	Cap-H2 FISH	n of Ubx	n of 34D	n of Cap-H2
	SMC4/+;Cap-H2/reptin^P{PZ}06945	SMC4/+;Cap-H2/+	2.07E-15	1.88E-20	3.10E-07	125	37	115
	SMC4/+;Cap-H2/reptin^Pbac{WH}f01801		9.99E-08	2.66E-14	2.63E-08	77	59	63
	SMC4/+;Cap-H2/Mrg15^j6A3		1.58E-12	3.72E-08	3.92E-13	124	19	83
Tip60G	Tip60^P{Mae-UAS.6.11}GG01739/+; SMC4/+;Cap-H2/+		2.13E-12	5.21E-03	1.80E-06	75	49	79
Tip60E	Tip60^Pbac{RB}e02395/+;SMC4/+;Cap-H2/+		4.01E-01	1.63E-01	8.37E-01	89	55	92
	Tip60G/Tip60E; SMC4/+; Cap-H2/+		7.01E-28	2.43E-09	nd	89	81	nd
	SMC4/+;Cap-H2/+	OrR-S	9.36E-53	3.93E-26	1.34E-32	59	61	74
	reptin^P{PZ}06945/+		1.64E-04	2.13E-05	1.28E-02	86	72	63
	reptin^Pbac{WH}f01801/+		4.76E-01	1.64E-01	7.49E-02	63	48	68
Mrg15Df	Df(3R)BSC741/+		2.06E-08	5.51E-01	5.88E-08	63	59	58
	Tip60G/Tip60E		3.49E-07	2.11E-10	1.46E-08	79	57	86
wt	OrR-S					101	86	82

Table S4 Figure 10 supporting information. Supporting data for Figure 10 and pair-wise three-dimensional distance of FISH probes in cultured Kc_{167} cells. Treatment for RNAi depletion or Cap-H2 overexpression (OE), average distance in micrometers (μm), number of nuclei measured (N), standard error of the mean (SEM) and two-tailed, unequal variance T-test p-values, versus (vs) control RNAi samples, are shown. X-chromosome probes X1 is at ~9.4Mb, X2 is at ~7.35Mb and X3 is at ~5.8Mb). Second-Chromosome probes on the left arm (2L) are at ~10.3Mb and ~8.3Mb. All coordinates are taken from the *Drosophila melanogaster* genome version BDGP R5/dm3 (2006) using the UC Santa Cruz genome browser.

Treatment	Average Distance (μm)	N (nuclei)	SEM	P-value vs Control
X1 vs X2 (~2.05Mb apart)				
Control RNAi	0.96	66	0.04	
Cap-H2 RNAi	1.17	63	0.05	0.001
Mrg15 RNAi	1.14	54	0.06	0.009
Cap-H2, Mrg15 (double) RNAi	1.19	27	0.09	0.020
Cap-H2 (OE)	0.71	23	0.05	<0.001
X1 vs X3 (~3.6Mb Apart)				
Control RNAi	1.08	53	0.05	
Cap-H2 RNAi	1.26	58	0.06	0.019
Mrg15 RNAi	1.24	49	0.06	0.028
Cap-H2, Mrg15 (double) RNAi	1.49	39	0.09	<0.001
Cap-H2 (OE)	0.67	22	0.06	<0.001
2L (~2Mb apart)				
Control RNAi	0.81	46	0.04	
Cap-H2 RNAi	1.00	49	0.05	0.005
Mrg15 RNAi	1.06	30	0.06	0.002
Cap-H2, Mrg15 (double) RNAi	1.32	35	0.09	<0.001
Cap-H2 (OE)	0.65	19	0.05	0.002