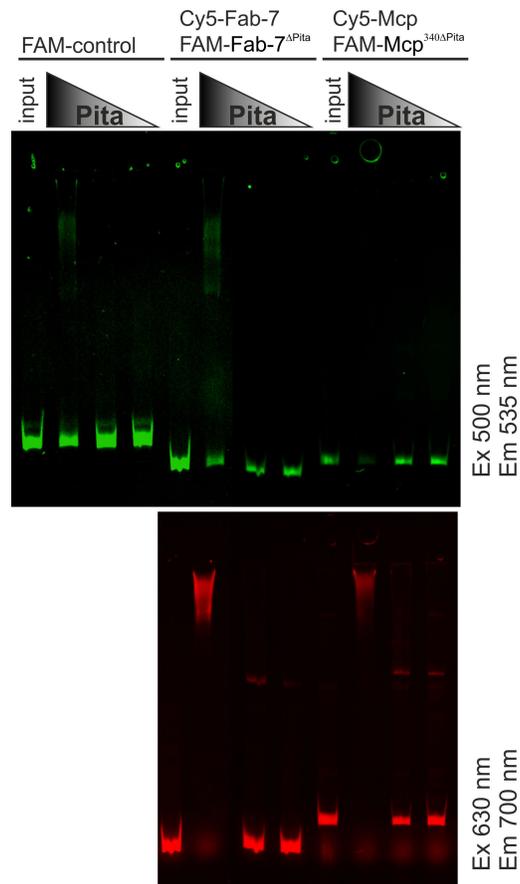
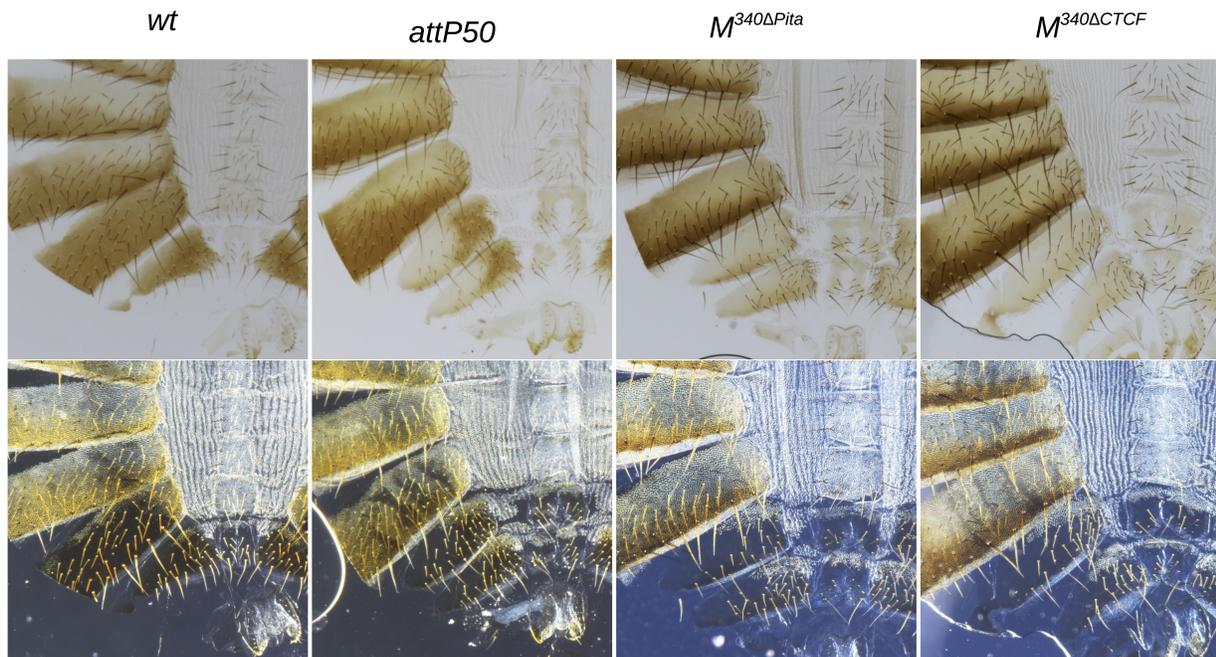


**Fig. S1. Binding of Pita at the boundaries of BX-C in embryos.** Chromatin was isolated from 12-24 h embryos and incubated with antibodies against Pita (red bars). Nonspecific IgG (black bars) was used as a negative control. The results of ChIPs are presented as percentages of input DNA. The  $\gamma$ *Tub37C* (tub) coding region, which is devoid of binding sites for Pita, was used as a negative control. Error bars indicate standard deviations of triplicate PCR measurements from three independent biological samples of chromatin.



**Fig. S2. EMSA of the Pita recombinant zinc finger domains with DNA fragments from the Fab7 and Mcp elements containing Pita binding sites which were used in the replacement experiments.** Zinc finger domains of Pita fused with MBP were incubated with fluorescently labeled DNA fragments; *Fab7* and *Mcp* labeled with Cy5 (*wt*) and corresponding fragments with the mutated Pita binding sites labeled with FAM. Also, a heterologous fragment with no Pita binding sites labeled with FAM was used as a negative control for binding (marked as FAM-control). Signals were detected for the FAM-labeled fragment at the Ex 500 nm / Em 535 nm and for Cy5-labeled fragment at the Ex 630 nm / Em 700 nm. Specificity of interaction was demonstrated by incubation of DNA fragments with varying amounts of Pita protein presented as a series of 2-fold dilutions.



**Fig. S3. Morphology of the abdominal segments of the  $M^{340}$  mutant females.** In  $M^{340\Delta Pita}$  and  $M^{340\Delta CTCF}$  homozygous females A6 is completely transformed into A7 thus demonstrating a clear GOF transformation.



**HS1+2 and HS1+2 $\Delta$ Pita** was amplified without HS3 (*iab-7* PRE)

Designations: F7 HS1+2, GAF binding sites, Pita binding sites, mutated sequence, HS3 (*iab-7* PRE)

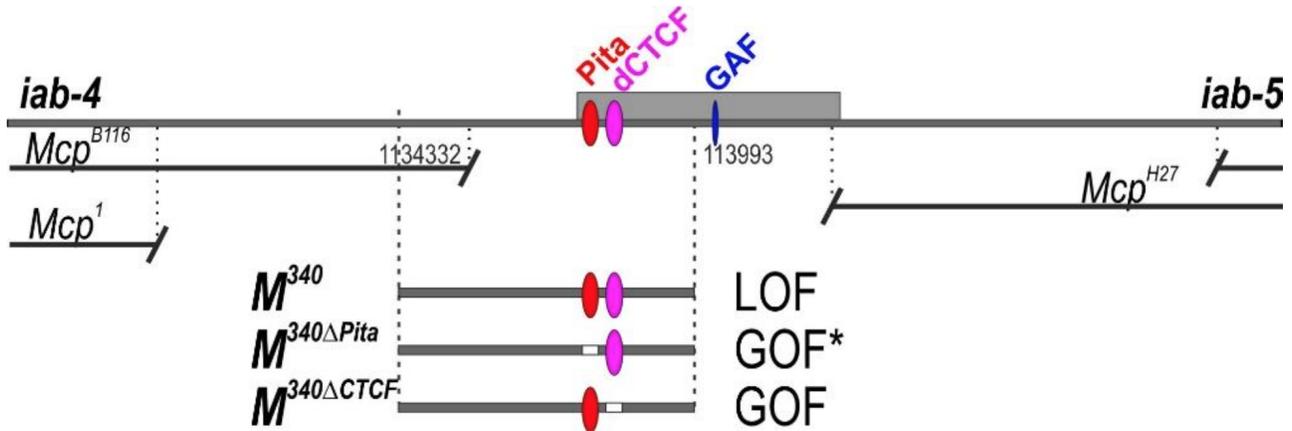
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cgacat

**Mcp**



**M<sub>340</sub>**

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**M<sub>340</sub>  $\Delta$ Pita**

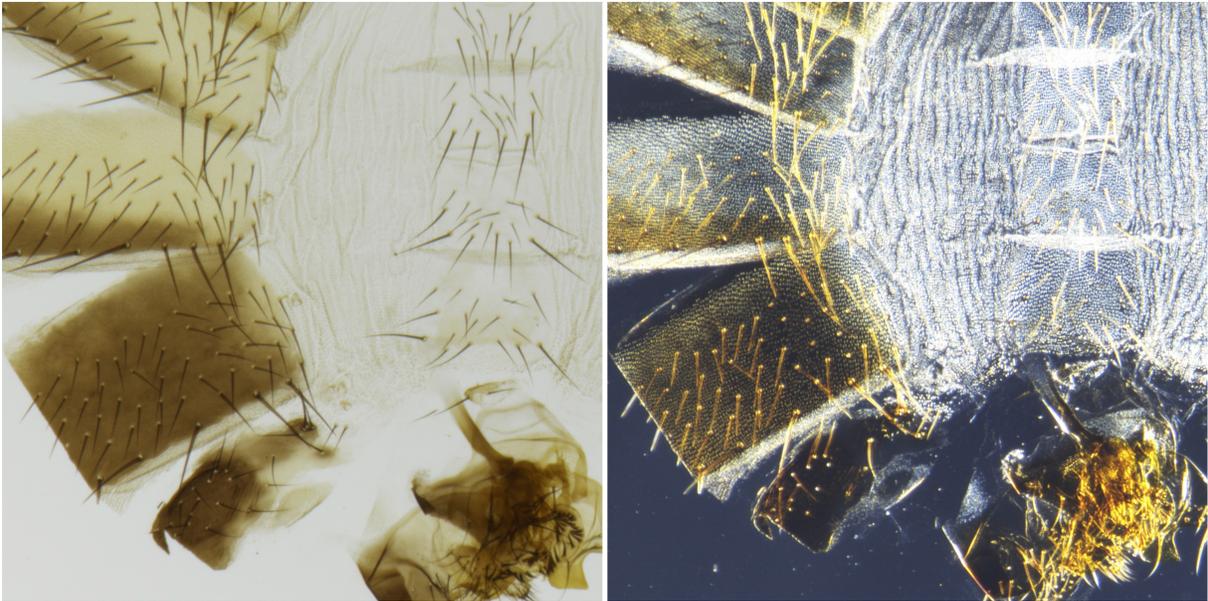
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**M<sub>340</sub>  $\Delta$ CTCF**

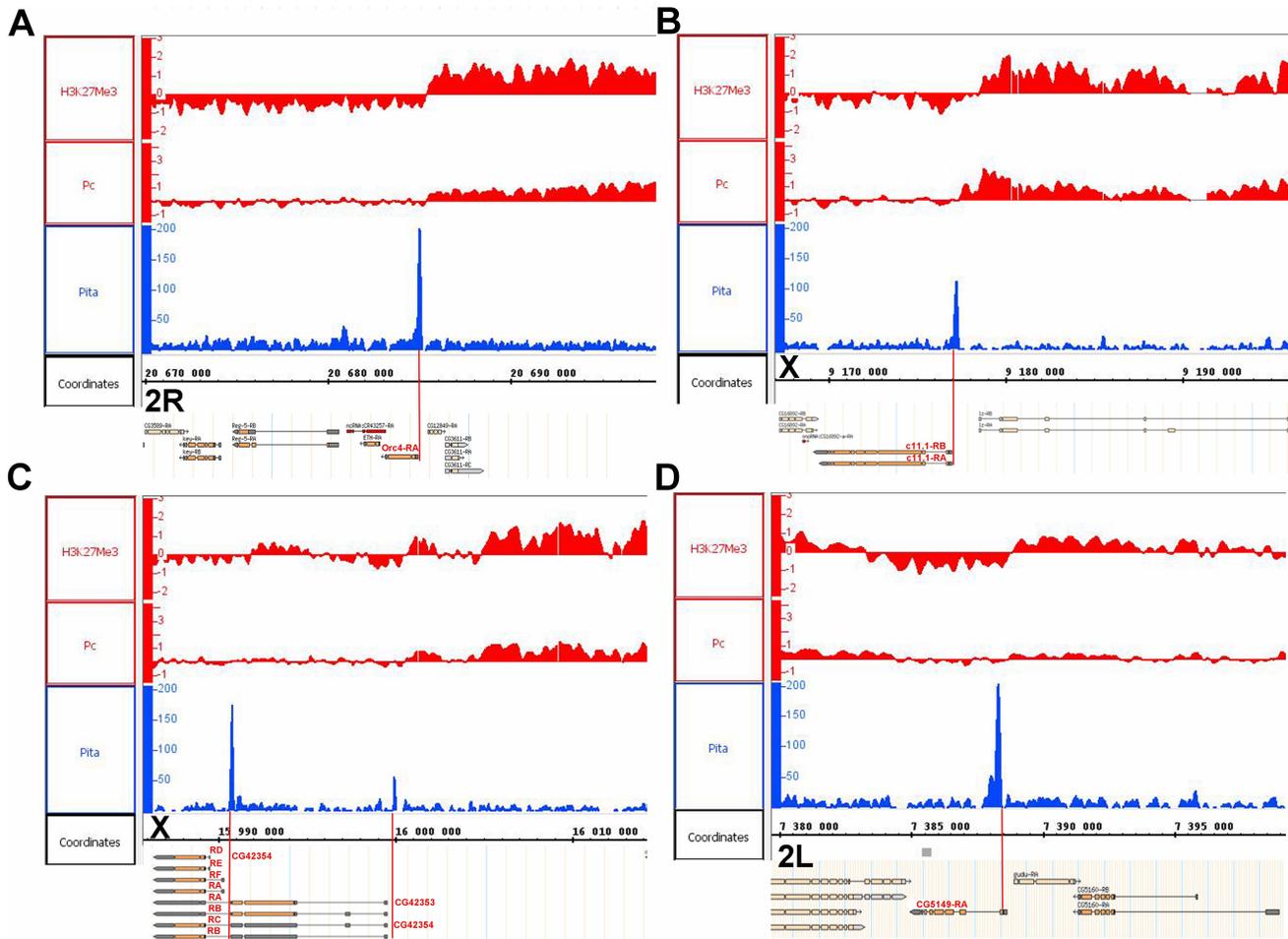
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Designations: **Pita binding sites**, **CTCF binding sites**, *mutated sequence*,

*Mcp<sup>340ΔCTCF</sup>* male

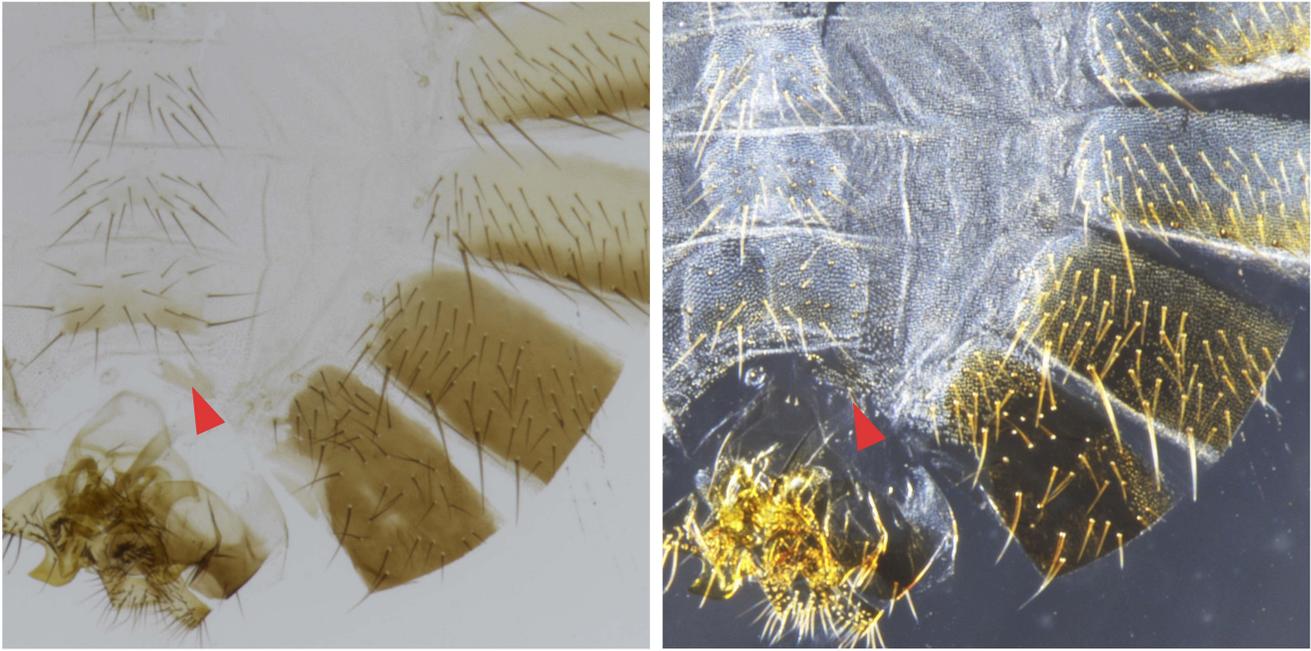


**Fig. S5. Morphology of the abdominal segments of the *M<sup>340ΔCTCF</sup>* mutant males.** About 10% of males of this genotype demonstrate a larger remnant of the A6 tergite, with a mixture of the GOF, LOF, and features resembling wild type: only a part of the tergite surface is covered in trichome hairs, while the rest of the surface is devoid of trichomes, like in a wild type A6 tergite.



**Fig. S6. Chromosomal landscapes at the gene promoters with the Pita binding sites.**

Coverage vectors for Pita (blue track) (UCSC dm3 *Drosophila melanogaster* genome assembly) at the gene promoters with a ChIP-chip tracks for the Polycomb and H3K27me3 (red tracks) binding profiles (downloaded from ModEncode). Coordinates and genes are presented under the coverage histograms: **A.** *Orc4*, **B.** *c11.1*, **C.** *CG9216* a.k.a *CG42353* and *CG42354*, **D.** *CG5149*. Red vertical lines mark coincidences of Pita peaks and gene promoters.



**Fig. S7. Morphology of the abdominal segments in some of the *HSI+2* mutant males.** Though the vast majority of *HSI+2* males lack an A6 sternite, infrequently (~10%) we observe a remaining small patch of sternite tissue (red arrow), which is even more clearly seen in the dark field as a lawn of trichomes.

**Table S1. The list of oligonucleotides used in the study.**

<b>Primer</b>	<b>Sequence (5'----&gt;3')</b>
<i>CHIP analysis</i>	
$\gamma$ Tub37C_d	GCTTTCCCAAGAAGCTCATACA
$\gamma$ Tub37C_r	GGTTCAGTGCGGTATTATCCAG
RpL32_d	GTTCGATCCGTAACCGATGT
RpL32_r	CCAGTCGGATCGATATGCTAA
hsp70_d	GACAGAGTGAGAGAGCATTAG
hsp70_r	GGTTATTGTGGTAGGTCATTTG
FAB3_d	TAAAGGCCAATGCACAAAGGCGAC
FAB3_r	ACGCTTCAGCGAACGGAATACAGA
FAB4_d	CAATTTGCCAATATTTTCGCAGTCCCT
FAB4_r	CCCTGGCGGGCATATGAGAAA
MCP_d	CGCGGCCATGTATTATGT
MCP_r	TACAACGCTTGGGTTTCTC
FAB6_d	CTGCCAGTGGGAGATACAAAGAT
FAB6_r	AGCTAAACCCGATTTGCTTTGCCG
FAB7_d (=HS2_d)	TAAGCCAACCTGGTTTCCAACCTCT
FAB7_r (=HS2_r)	TTGCCAGGGTAAGTAACGGTAT
FAB8_d	TGTTGGTGAGCAAGCGAAGA
FAB8_r	CGAACATTTTTTACGCGACATGT
AB-I_d	CCAACAACAAGCCAACCTAACA
AB-I_r	ACGAACAAAAACGCTCTCAGAC
HS1_d	CGAGGTAGAATGTCGCTCAAAG
HS1_r	GCGTGCGGTTCTCTTATCAC
i7_d	GTCGCAAGAACTTCACAACAG
i7_r	GCCATCATGGATGTGAAAGA
FUB_d	TCAGCAATTGTCAGGTGTCC
FUB_r	CATGAGCGAGTCCTTGTA
FUB2_d	AATTTTCGTGTTTCGCACTTCC
FUB2_r	GGCAGCACTAGCGTCAAAA
100C_d	CGACAGCATGTAACAGGTATAA
100C_r	CCCGAGGTTTCAACTTTCATA
<i>Primers for EMSA</i>	
EMSA_F_d	FAM-CTCACTATAGGGCGAATTG
EMSA_F_r	FAM-CGCCAAGCTCGAAATTA
EMSA_C_d	Cy5-CTCACTATAGGGCGAATTG
EMSA_C_r	Cy5-CGCCAAGCTCGAAATTA