

Figure S1

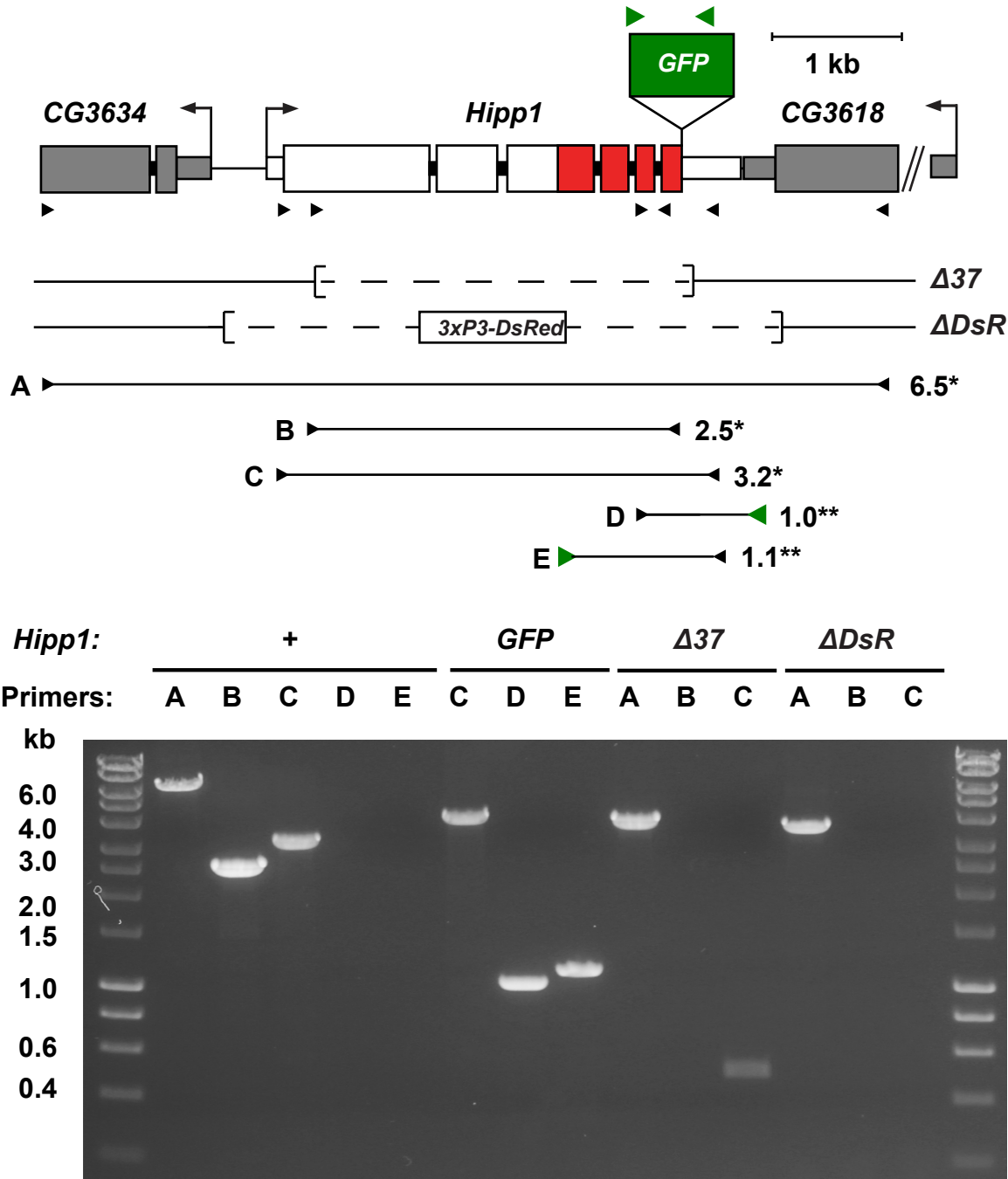


Figure S2

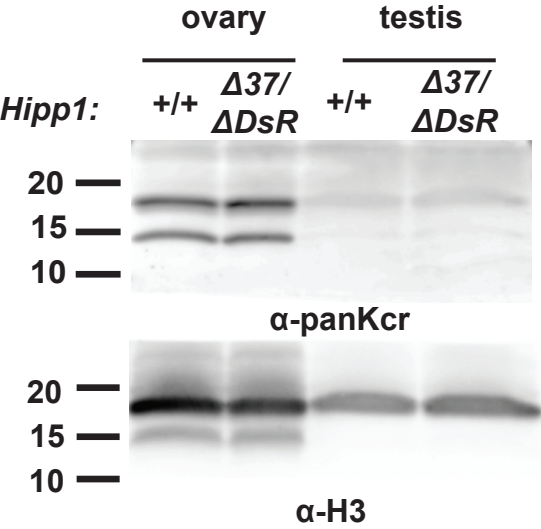


Figure S1. Strategy for analysis of CRISPR generated *Hipp1* alleles

Top: Structure of the *Hipp1* gene and three newly generated alleles, including *Hipp1-GFP* (inverted green triangle) and two deletion alleles ($\Delta 37$ and ΔDsR). Locations of primers used in PCR analyses are indicated as arrowheads. Black arrowheads correspond to endogenous sequences and green arrowheads to GFP sequences. PCR reactions using five primer pairs were completed, using the indicated primer set. Expected sizes (in kilobases) of the corresponding fragments amplified from $*:Hipp1^{+/+}$ and $**::Hipp1^{GFP/GFP}$ are shown at the right. Primers are listed in Table S2. *Bottom:* Ethidium stained agarose gel showing the PCR products generated from indicated PCR reactions. Genomic DNA analyzed was isolated from homozygous animals of the indicated genotype. + corresponds to *Canton S*.

Figure S2. Analysis of Kcr histones in testis and ovary extracts.

Western blot of proteins isolated from 1-to 3-day old ovaries and <1 day-old testes from *Hipp1*^{+/+} and *Hipp1* ^{$\Delta 37/\Delta DsR$} animals probed with a pan α -crotonyl-lysine (panKcr) antibody and an antibody against Histone H3 (α -H3). Conditions of western transfer were optimized to capture small proteins by excluding SDS in the transfer buffer and reducing the transfer time and voltage.

Supplemental Table 1. CRISPR generated *Hipp1* mutants

Allele	Position of deletion ¹	Number of inserted nucleotides	Net indel size
<i>1G3</i>	596 to 601	0	-6
<i>1G5</i>	587 to 609	0	-23
<i>2G4</i>	766 to 767	0	-2
<i>3G6</i>	2354	0	-1
<i>3G10</i>	2351 to 2364	4	-10
<i>Δ37</i>	593 to 3260	6	-2662
<i>ΔDsR</i>	-314 to 3912	1350	-2877

1: Position is relative to the *HIPP1* transcription start site.

Supplemental Table 2. Primers used for PCR validation of *Hipp1* alleles

Primer Pair	Forward Primer	Reverse Primer
A	GGTAGTTGATCTCGGCGACCTTTAG	CTTGCCCAGCTCAAACATGAATCTC
B	GATACCAGTCATGATCAGCCTAC	TCCTCATCCAGACGCGGGAATTT
C	GCACTCTGGAGAGGGAGAT	CGTGTAAGCGATAATTTAATTTGCAAAC TTCC
D	CTGTTGCAACGGAAC TTTGGA	CGCTTCTCGTTGGGGTCCTT
E	GGTGGATCTGGAGGTTCCGG	CGTGTAAGCGATAATTTAATTTGCAAAC TTCC

Supplemental Table 3. Primers used for RT-qPCR of ovarian RNA

Gene	Forward Primer	Reverse Primer
<i>GAPDH</i>	CGCTGCCCAGAACATCATTC	GAAACGACCTCCTCATCGGT
<i>Ras2</i>	ATTACCAATCGTGGCTCGCA	TGCCGTGGGTGTAATACAGA
<i>B-Tubulin</i>	GACACCAGATCGTTCATGTT	TCCTACTTCGTCGAATGGAT
<i>RpL32</i>	AATCTCCTTGCGCTTCTTG	AAGGGTATCGACAACAGAGT
<i>su(Hw)</i>	GGCGGGCTCGTTAATTTCTA	CAGGGATTTACGCTGCTCTT
<i>Hipp1</i>	AAGAGTTTCCAGTGAAGTGC	CGGAGTGTTGAGTTTCTTT
<i>CG7582</i>	AAACTGGGCGGCGTAGTA	GCCCATCTACTGTTTCCTGA
<i>CG33099</i>	GGAGATGACCTAAACATGACC	CCCGCCGTTTATAAGTATGG
<i>CG6293</i>	GTCAATAGCTCCAGGGTCTT	CTGCCTTGATAATGGTCCTC
<i>l(1)G0148</i>	CGATCATGTCAACGGTGTAT	TGGCATCACTAAAAACGGAA
<i>tipE</i>	CTTTCGAATGTAAAGGCT	GAACGAGGGTATTGGAATGG
<i>mAcR-60C</i>	GTCGAGTTGATGTAGCACAG	TGCTGCCAAGAAGAAAAGA
<i>Mob2</i>	AAATCCGAAGCATAAATCTGGTTATCTCC	CCAACACAACCACAAATCGCACC
<i>Syn2</i>	ATGGA AAAAGTGGTGGACTC	AGCTCCTTGTTTCTATTGC
<i>CG15760</i>	GTGCCAGCTTCTTTGTGAA	CCTCCTGTTCTCATCTTCA
<i>Rbp9</i>	TGGTGCATTTTCGTCTACAA	TGCTTGTTCTTGTTGGTCTT
<i>CG6282</i>	GCATCCGA ACTACTTTGGCG	CTCCACGTAGACAGCAGGAG
<i>Rph</i>	GATGCTTCTCTCGCTCTGCT	CGCTGGTCTTGTGCTTCTTG
<i>CG9813</i>	GGTAATGTTTGCTCAATCGC	TTGGAAGCCTGTAAAATGGG
<i>dpr19</i>	TGAATCCCTCGGTCA GTGTG	AGAGTTACGCCGCTAGTTCC
<i>CG17778</i>	GATGGACGAGATGGAGGTC	ATCCGCTGTTTTAGAGTGTG
<i>nrv3</i>	GCGACTACTATCCCCGAATG	TGACATCTTCCCAACGACTCT
<i>neuroligin 2</i>	ACATCTGAGTCCAAGTTT TAGCC	TGATGCCAGAGAACAGAAACA
<i>fas</i>	ACTTTATGTTTCCACGCCA	TAGTTGCCACGACCAAAAG
<i>CG32017</i>	CATTAAAATGCCAGCAACTGAA	GGTAATCCTCTCAAAAAGCGA

Supplemental Table 4. Primers used for ChIP-qPCR

Site	Forward Primer	Reverse Primer
1A	GTATATCCACATCACCAGACCTCAGG	ACATCCTCGAATCACTATGCAAGTCG
13F	GGCATCCACTCACTTGAAGG	GAAGCTGGAATGATGTTCTGG
64A	AGGAAGTGCTGCCTGATGTAGAAG	TTATATGTTGGCTCCTGCTTCCGC
<i>gypsy</i>	CAGGCGACAAACAGGGTAGT	TTTGCCGAAAATATGCAATG
5C	GCGTTAGATTAGAAATAGGATAATGCCT	AATTGCCCACTGCCACAATCTG
23A	GGAAATCCAAGCCGAAACGGAA	AAGTGCCATAAAACGGATTGCT
61F	GCAGCTTTTGTCTCAGAGGTTGAT	CTTTCAGCCTAAATTCAAGCAACGT
69D	GATCACGCATACGCAACCATTG	GCAGCACGTAATTAACCACATAAGTATG
70C	TTACCCACTGGCATGAATTATTAGT	GCAAAAATGATGGCAGCGAAC
100B	GCGTCAAAAGGGTTTCTTAGCAT	AGCTGCGAGTCAAGCATTTTG
<i>CG6282</i>	TGCGAGGCACAAAAGCATT	CGGACACACAAACCAAAGCC
<i>CG9813</i>	AAAGCAGGCACAGTAAGGCA	GCTCATGCGGCAATTTACT
<i>CG15760</i>	GTGTGAGTCCGACCACAACA	CCTCGTGTGCGATTGTTC
<i>dimm</i>	TGTAAGTCTAGCGGTGGACC	CCTGCACCTGGTCTGTGTGA
<i>Or35a</i>	CCAGCAGTGGCAAAAGTTTATT	TGTATGTGGGTGTTGGAGGC
<i>SSE</i>	AGCCATTTGAAAAAGCGGAAAGA	CGGAATGTTTGTGCTTGAATTTTACT
<i>tipE</i>	TGGAATAATCAATGGCCGCAG	ACACTCGGAGCTAAACATCCT
3D	AATCAAGAGTATGCACTAGATTGCTG	CCGTTCTACACACCTACGCATG
16C1	ATTCCCGTTTAAATGCGAGCTG	ATCATGTAAATGCACTATCGAGTGG
35B	AGGCAATATATCAAGGTGCTAAAGT	CACGTTTAAGAATACGTCCGCTAGA
59F	GGAGCTGAAATTCATCATATAACCAC	GGGCGATGGTAATTTTACATTTGACAT
62D	TTTGGGCTGCACGTATCT	GCGCGGGACAACTTATTT
82E	TGCAGTGCGAAATGAATTGCTTGA	ACTGCATGCTAACTGTAGTGGGC
<i>84B</i>	CTTCGTCAGATTTGACAGCCTG	ACTGACAGTTTCTGACATTCT
<i>Jhe</i>	ACGCATTGAAATCATTTTAAGAGACG	TGCTAAAGAAGTTTATTCATTGTTCC

<i>CG3104</i>	CAGTTGGGAGCTTGCGAATAATATA	GCAGCGACTGGTCACACTT
<i>Mob2</i>	CGATTTGTGGTTGTGTTGGTGT	GCCTGAAAGTATGCACCGAAAA
<i>Rbp9</i>	TTTTCTTTTCGAGTCGAGTTGGGAA	GGTGTAAGTGTAACTGTAACTACATATCCG
<i>16C8</i>	ACCTAGCGGTACATGCTTACATTC	GGCTCCATCTAACTTTAGGCTTATCAA
<i>16D</i>	CATGAAATAATCTAATCAAACAGGCATTTAGTT	GTGAAGTAGATGGCTCTATATGTTTCGATT
<i>44C</i>	ACATCTTACAGACAGTTTACCGTTCT	TAGATGACGCTTTAGTGCAGTCTATG
<i>63E</i>	ACCTTGTAATAATTATTTGGAACTAATGGAG	GTGGCGGCATCTATTTCTGAAGA
<i>78E</i>	AACCTGAGAAGTTACGTGGATTGTA	CACCTATCGGCATAGTCGGTTCA

Supplemental Table 5. Summary of insulator proteins bound to genomic region

Class	Site	IBPs bound ¹										
		Su(Hw)	HIPP1	CP190	Mod	CTCF	IBF1	IBF2	PITA	BEAF-32	GAF	ZIPIC
Negative Controls	1A											
	13F											
	64A											
Su(Hw) only	5C											
	23A											
	61F											
	69D											
	70C											
	100B											
	CG6282 ²											
	CG9813 ²											
	CG15760 ²											
	<i>dimm</i> ³											
	<i>Or35a</i> ³											
	<i>SSE</i> ³											
IBPs + Su(Hw)	3D											
	16C1											
	35B											
	59F											
	62D											
	82E											
	84B											
	<i>Jhe</i> ³											
	CG3104 ³											
	<i>Mob2</i> ²											
	<i>Rbp9</i> ²											
IBPs - Su(Hw)	16C8											
	16D											
	44C											
	63E											

1: Blue indicates that the indicated IBP localizes to the region amplified. Data was derived from the following sources (CELNIKER *et al.* 2009; ROY *et al.* 2010; SOSHNEV *et al.* 2012; CUARTERO *et al.* 2014; MAKSIMENKO *et al.* 2015)

2: Su(Hw) ovary regulated gene (SOSHNEV *et al.* 2013)

3: Su(Hw) testes regulated gene (DUAN AND GEYER 2018)