

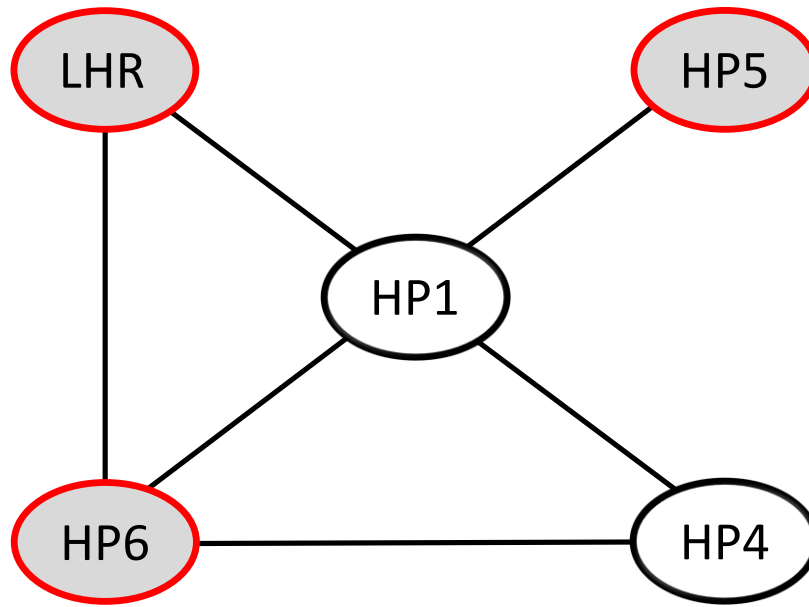


Figure S1 - Diagram of interaction and molecular evolution data of five HPs.

Solid lines connect the proteins that have been reported to interact in previous studies [1-3]. The proteins have been grouped based on data in Table 1 into rapidly evolving ($K_A/K_S > 0.7$; red border, shaded) and non-rapidly evolving ($K_A/K_S < 0.2$; black border, not shaded).

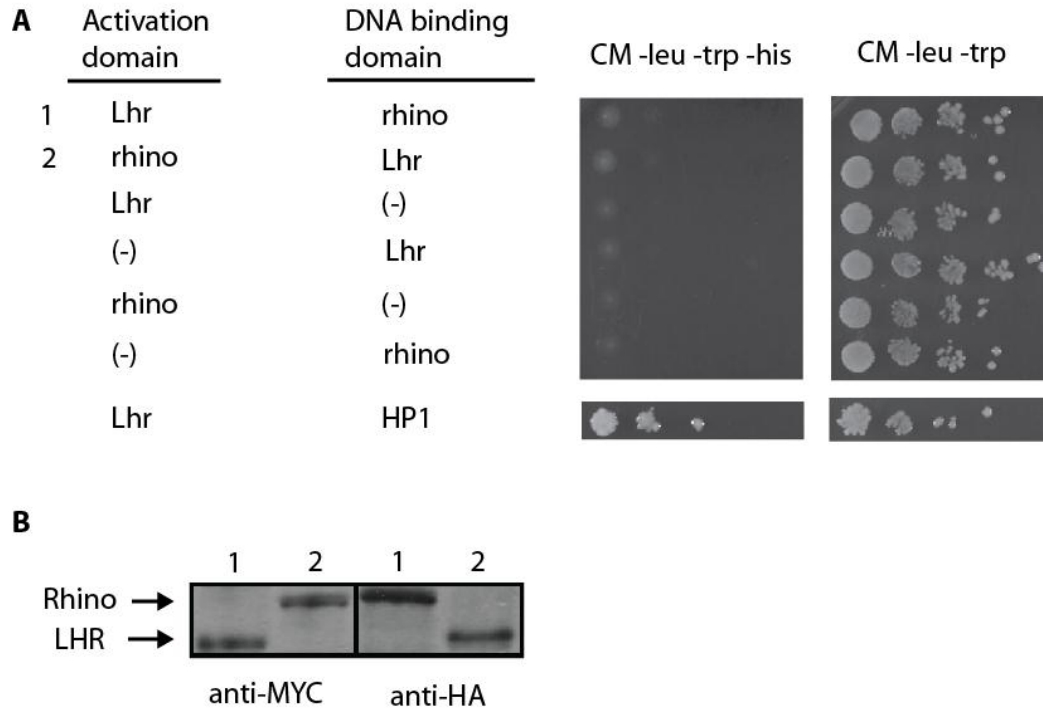


Figure S2 - No interaction was detected between LHR and the HP1 paralog Rhino by yeast two hybrid analysis.

Interactions were analyzed in yeast two-hybrid by activation of HIS3 and growth on complete media lacking histidine (CM -trp -leu -his); growth controls (CM -trp -leu) contain histidine. A) No growth is seen for both LHR-Rhino combinations; indicating that the two proteins do not interact. B) Genotypes 1 and 2 from (A) were tested by Western blot in order to demonstrate that both fusion proteins are expressed in yeast.

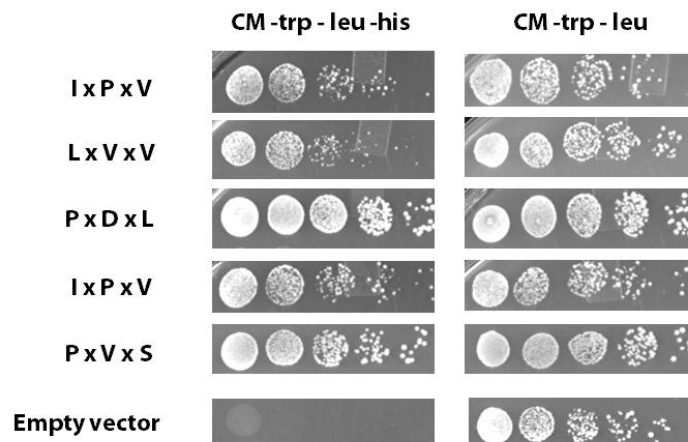


Figure S3 - Testing if five PxVxL variants in LHR mediate the interaction with HP1 in a yeast two-hybrid assay.

Independent mutagenesis of each PxVxL variant in full length LHR failed to abolish interaction between LHR and HP1, indicating none are individually necessary to mediate this interaction.

Growth in the (CM -trp -leu -his) column indicates an interaction, and growth in the (CM -trp - leu) column indicates both the activation domain and DNA binding domain plasmids are present.

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D.melanogaster      GLEAEKILGASDNNGRLLTFLIQFKGVDQAE MVPSSVANEKIPRMVHIFYEERLSWYSDNED
D.simulans          GLEAEKILGASDNNGRLLTFLIQFKGVDQAE MVPSSVANEKIPRMVHIFYEERLSWYSDNED
D.yakuba            GLEAEKILGASDNNGRLLTFLIQFKGVDQAE MVPSSVANQKIPQMVIHIFYEERLSWYSDNED
D.erecta            GLEAEKILGASDNNGRLLTFLIQFKGVDQAE MVPSSVANQKIPQMVIHIFYEERLSWYSDNED
D.ananassae         GLEAEKILGASDNNGRLLTFLIQFKGVDQAE MVPSTVANVKIPQMVIHIFYEERLSWYSDNED
D.pseudoobscura    GQAEKILGASDNNGRLLTFLIQFKGVDQAE MVPSSIANLKIPQMVIHIFYEERLSWYSDNED
D.virilis           GLEAEKILGASDNNGRLLTFLIQFKGVDQAE MVPSTVANVKIPQMVIHIFYEERLSWYSDNED
Clustal Consensus  * *****: ** ***:*** *****

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D.melanogaster  ---FLLSQLPFFVKSMQAERRHLEVEVLALILEQERQEKATRRKLGPKDLETVQSEYDEF-----LKAIRVKELPA
D.simulans      ---FLLSQLPFFVKSLSPAERRHLEVEVLDIILEQERQEKATRQLGPMELKTVQSEYEED-----LKAIRVKELPA
D.yakuba        -----FLKSLSQSERRRLEVEVLDLILEQERLEKATQELGPKLELTAQSEYDEF-----LKAIRVKELPA
D.erecta        ---FLLSQLNLVKSLSQSGRRRLEVEVLDLILEQERLEKATQKLGPKLELTAQSEYDEF-----LKAIRVKELPA
D.ananassae     -----FLKTIR-----VKELPNNILT-QIIPKVPDSPNKMGLVITQVFSTSS-----KEEIPSTSKAK
D.pseudoobscura FLGSVVRVKELPISALAQLSLDGPPQYKPAQPKIAHTFAIPGPRPKKKRVISSVSGGQDLINIPTATVSIPIQDFKSNVVGPF
D.virilis       -----FLKAIR-----VKELPVQVSSRRSSASSNGSSKPPSSAAARSSNLV-----ISDVRGHSEPO
Clustal Consensus      ::::      :      :      .      .

      90      100      110      120      130      140      150      160
D.melanogaster  DNLLSPAMDRFGISPCHKIRPNVASANKR-----IRYNNACKGSDNVKIKIETAIEPKIKDTTKC-----D
D.simulans      NTLTSPAIDGFRISPRNIRPNLASANKR-----ISYNGVSNESNYVKIKETAIEPKIEDATKF-----D
D.yakuba        DTLIRSTIDGFRISPHSN-PSIINANKRSVGSRRITSDNGISNGSNDVEIKTEKPDPEFNKDNKPF-----D
D.erecta        DTLRLPAMYRFRISPHSK-QNIVNANGRNAGTSKTIISDNVVSNGSNDVEIKTEKADEPVTQCKKKF-----D
D.ananassae     VKPE--PIESPVR-----QKPKEPASGSRSSSRKST-----Q
D.pseudoobscura IDPAPAVSPPLDINVIGAKKEPVECPSPVSPPLDIKTEVEVALAAIELAITEMNVRENKPEEAQQKSPERNGTPEKPPDD
D.virilis       PEPANPPTSPPLVIK-----NEPPDQLEIEQLQPLSKQSP-----D
Clustal Consensus      .      .      .      .      .      .      .      :

      170      180
D.melanogaster  ERPIETPRFVPLKSAKYIYIKRCRI-
D.simulans      ERPIETPRYVPLKSAKYIYIKSRI-
D.yakuba        KRPTETPRYVPLKSAKYIYIKSRI-
D.erecta        DRPTETPRYVPLKSAKYIYIKSRI-
D.ananassae     KTASS-PQFVPIKSAKYIYIKPVRV-
D.pseudoobscura ATLNPNPRYIPIKSAKYITKKLIV-
D.virilis       KEADPRRLRYVPLKSAKYIVKQVVR
Clustal Consensus  :::*:***** *      :

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Alignment of the domains in (A) HP1 and (B) LHR found to mediate the interaction in *D.*

melanogaster, from seven *Drosophila* species.

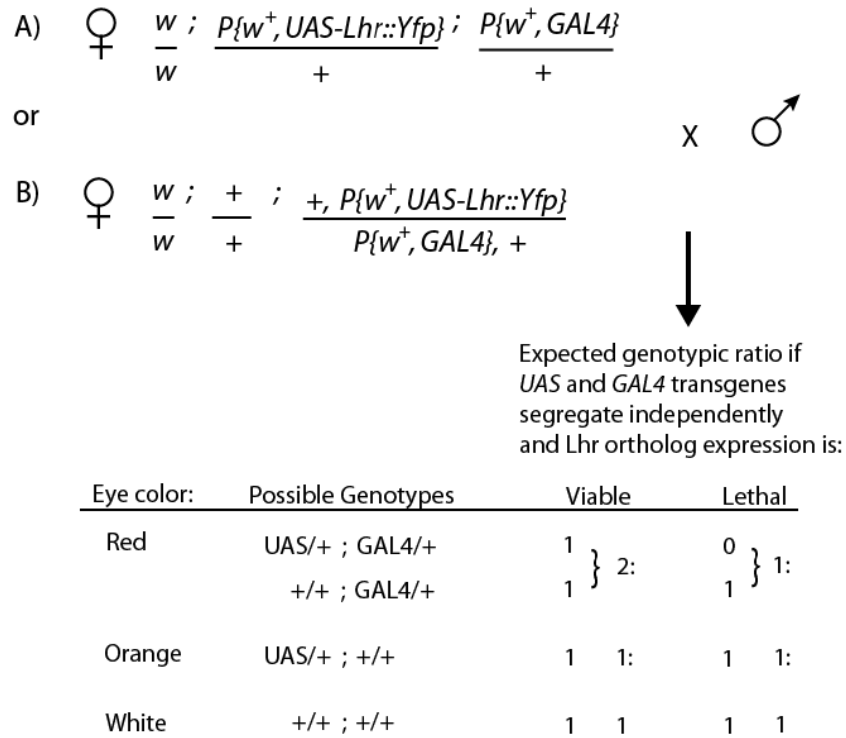


Figure S5 - Complementation crosses to test for suppression of hybrid male rescue.

D. melanogaster female parents heterozygous for *GAL4* and *UAS-Lhr-Yfp* transgenes on different chromosomes (A) or the same chromosome (B), were crossed to *D. melanogaster* (control) or *D. simulans* *Lhr*¹ males (Table 2). The transgene insertions *P{UAS-mel\Lhr::Yfp}1* and *P{UAS-mel\Lhr::Yfp}2* were used in scheme (A). In this scheme the UAS and GAL4 transgenes segregate independently. The *P{UAS-mel\Lhr::Yfp}3* transgene insertion and the *D. simulans*, *D. yakuba*, and *D. virilis* *Lhr-Yfp* transgenes inserted into cytological position 86Fb were used in scheme (B). In this scheme the transgenes will segregate independently if they are unlinked on chromosome 3. Both the *GAL4* and *UAS-Lhr-Yfp* transgenes are marked with *w*⁺, and the *GAL4*-containing transformant has a darker eye color and is epistatic to the lighter *UAS*-containing transformants. Therefore, the red-eye class of progeny could potentially be composed of two different genotypes.

Table S1 - HP mutations do not dominantly suppress hybrid male lethality

Female parent ^a	F1 male progeny		F1 female progeny	
	+/-	+/+	+/-	+/+
HP1 ^b	0	0	151 ^{n.s.}	145
HP4 ^c	0	0	168 ^{n.s.}	176
HP5 ^d	0	0	202 ^{n.s.}	184
HP6 ^e	0	0	191 ^{n.s.}	178

^a – *D. melanogaster* virgin females were crossed to *D. simulans* *w*⁵⁰¹ males and the F1 progeny were scored for the mutant (+/-) or balancer (+/+) chromosome. Crosses were performed at room temperature (~22-24°). Full genotypes of *D. melanogaster* female parents are as follows:

^b *Su(var)2-5*⁰²/*Bc Gla*

^c *y*¹ *w*^{67c23}; *P*{*w*^{+mC} *y*^{+mDint2}=*EPgy2*} *HP4*^{EY01733}/*TM3*, *Sb*¹ *Ser*¹

^d *y*¹ *w*^{67c23}; *P*{*w*^{+mC} *y*^{+mDint2}=*EPgy2*} *HP5*^{EY10901}/*FM7i*, *Act-Gfp*

^e *w*¹¹¹⁸; *HP6*³⁶⁻⁵/*Bc Gla*.

n.s. – The observed number of F1 females inheriting the *HP* mutant chromosome was not significantly different than the expected value by Chi-square analysis ($p > 0.05$).

Table S2 - Primers

attB

F-5'-AAACCCAGGCCTATGCCCCCGGTGACCGTC-3'

R-5'-AAACCCAGGCCTGATGTAGGTCACGGTCTCG-3'

D. melanogaster Lhr

F- 5'-ATGAGTACCGACAGCGCCGAGGAA-3'

R- 5'-TCATGTTCTCAGCGTAGGCCG-3'

D. simulans Lhr

F- 5'-ATGAGTACCGACAGCGCCGAGGAA-3'

R- 5'-TCATGTTCTCAGCGTAGGCCG-3'

D. yakuba Lhr

F- 5'-ATGAGTACCGACAGCGCCGAGGAA-3'

R- 5'-TCATGTTCTTAGCGTAGGCCTTCTG-3'

D. erecta Lhr

F- 5'-ATGAGTACCGACAGCGCCGAGGAA-3'

R- 5'-TCATGTTCTCAGCGTAGGCCGCC-3'

D. ananassae Lhr

F- 5'-ATGAGCGAGGAAGTTGGGGCTCTT-3'

R- 5'-TCAATCTTTTTGTGAATGGCGACT-3'

D. pseudoobscura Lhr

F- 5'-ATGGATACCGAAGCGGGAATGGAC-3'

R- 5'-TTAACGTTTCTTTGGGCGCTTTGC-3'

D. virilis Lhr

F- 5'-ATGGATGGCTTGGAGGATGCATCC-3'

R- 5'-TCAGGAGCTTCTGTTGCGGGTCTT-3'

D. melanogaster Su(var)2-5

F- 5'-ATGGGCAAGAAAATCGACAACC-3'

R- 5'-TTAATCTTCATTATCAGAGTACC-3'

D. melanogaster HP4

F- 5'-ATGTCACCGAAGACTAAAAAAATG-3'

R- 5'-GATTGTCAGATTAGACTCC-3'

D. melanogaster HP5

F- 5'-ATGGATATTTTGTATATGG-3'

R- 5'-CGATTCTAACGCGGTCATTATTTC-3'

D. melanogaster HP6

F- 5'-ATGCCCAGCTCCACTTTGACG-3'

R- 5'-CTAGGCATTTCTGTGATCGTTTCTTC-3'

D. simulans HP6

F- 5'-ATGGATCCGACGAAGTCTAAGACC-3'

R- 5'-CTAGGCACTTGGTGCCCG-3'

D. melanogaster Rhino

F- 5'-ATGTCTCGCAACCATCAGC-3'

R- 5'-T TACTTGGGCACAATGATCCTC-3'

IxPxV #1 to AxAxA

F-5'-GGTATCCCTTCCTTGCCATCGCGCAGGCGGAGCCCCAAAATGGAC-3'

R-5'-GTCCATTTTGGGCTCCGCCTGCGCGATGGCAAGGAAGGGATACC-3'

LxVxV to LxAxA

F-5'-CCGGCATTTAGAAAGCGGAGGCCCTCGCCCTCAT-3'

R-5'-ATGAGGGCGAGGGCCTCCGCTTCTAAATGCCGG-3'

IxPxV #2 to AxAxA

F-5'GCATTAGCCCACACAAAGCAAGAGCCAATGCCGCAAGTGCCAATAAA
AG-3'

R-5'CTTTTATTGGCACTTGCGGCATTGGCTCTTGCTTTGTGTGGGCTAATG
CC-3'

PxDxL to AxAxA

F-5'-GGTGAAAGAGCTCGCCGCAGCCAATGCCCTCAGTCCGGCC-3'

R-5'-GGCCGACTGAGGGCATTGGCTGCGGCGAGCTCTTTCACC-3'

PxVxS to AxAxA

F-5'-CCACACAAAATAAGAGCCAATGCCGCAGCCGCCAATAAAAGAATC-3' R-5'-
GATTCTTTTATTGGCGGCTGCGGCATTGGCTCTTATTTTGTGTGG-3'

Supplementary References

1. Giot L, Bader JS, Brouwer C, Chaudhuri A, Kuang B, Li Y, Hao YL, Ooi CE, Godwin B, Vitols E *et al*: **A protein interaction map of *Drosophila melanogaster***. *Science* 2003, **302**(5651):1727-1736.
2. Brideau NJ, Flores HA, Wang J, Maheshwari S, Wang X, Barbash DA: **Two Dobzhansky-Muller genes interact to cause hybrid lethality in *Drosophila***. *Science* 2006, **314**(5803):1292-1295.
3. Joppich C, Scholz S, Korge G, Schwendemann A: **Umbrea, a chromo shadow domain protein in *Drosophila melanogaster* heterochromatin, interacts with Hip, HP1 and HOAP**. *Chromosome Res* 2009, **17**(1):19-36.