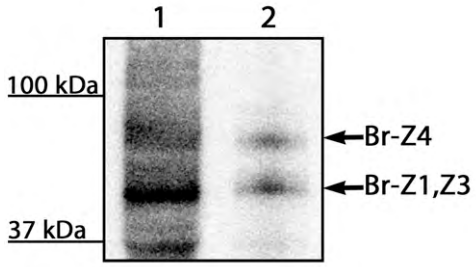

Fig. S1. Expression of Br protein in nuclear extract from S2 cells. Western blot with anti-Br-core show Br isoforms in S2 cell nuclear extract (lane 1) and tissue from third instar w^{1118} wandering larvae (lane 2). The Br-Z4 isoform (~92 kDa) and the Br-Z1/Z3 isoforms (~75 kDa) are present in both S2 cell nuclear extract and in third instar larval tissue.

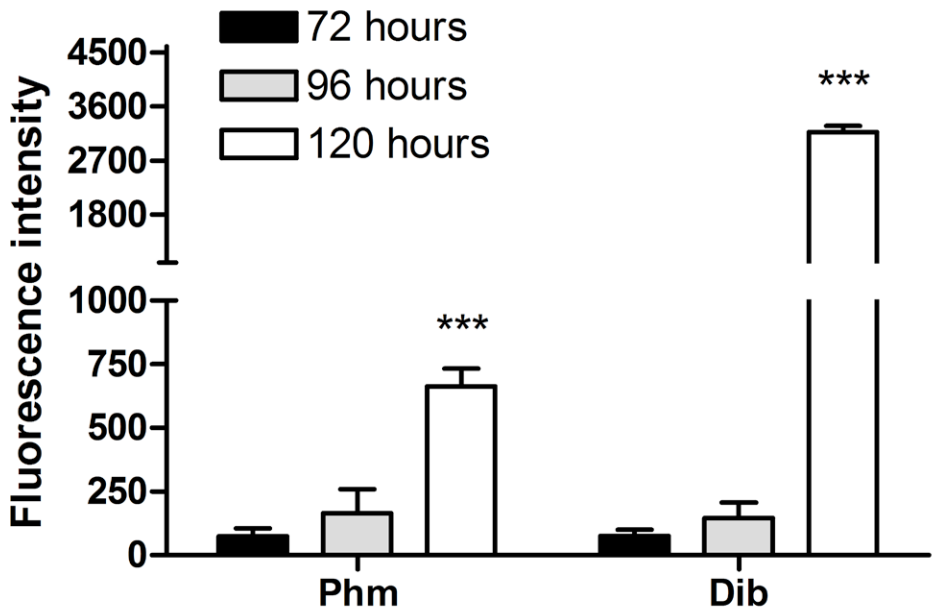

Fig. S2. Quantification of Phm and Dib protein expression during the third instar. Immunohistochemistry images (related to figure 3) of Phm and Dib expression during the third instar at 72, 96 and 120 hours AEL were subjected to image quantification analysis. Fluorescence intensity within the PG was quantified using Image J ($n \geqslant 5$).

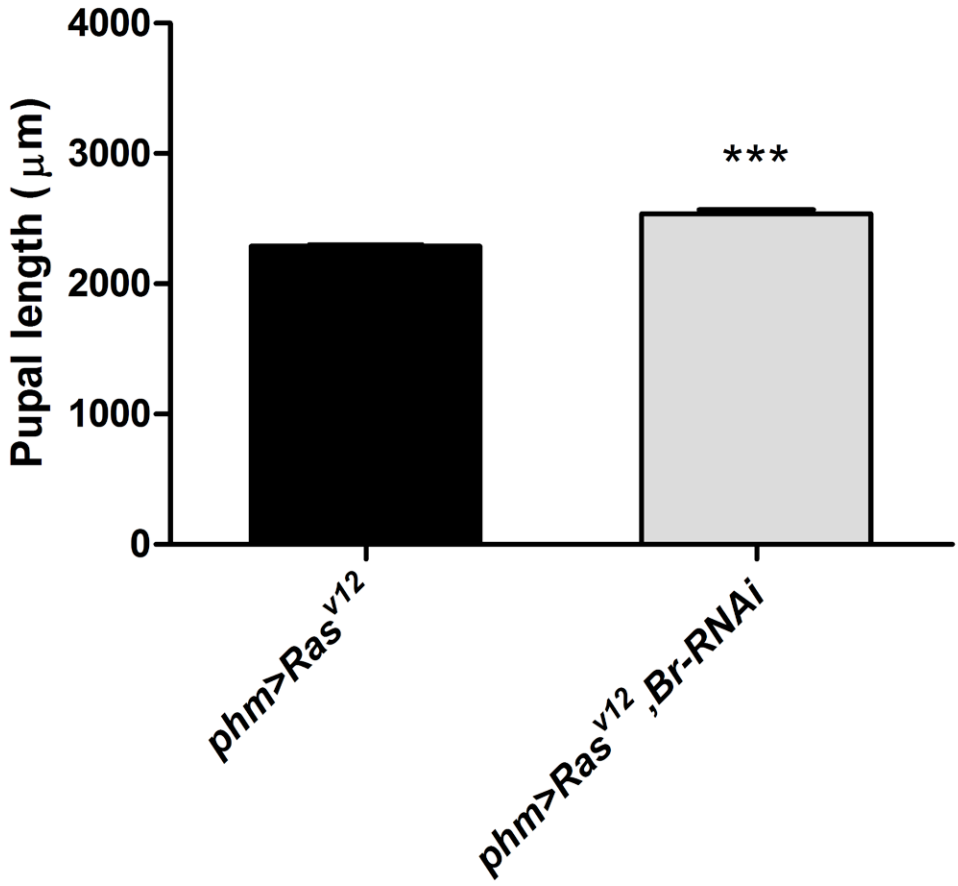

Fig. S3. Reducing *Br* expression causes overgrowth in larvae overexpressing *Ras^{V12}* in PG. Effect on size of *Br-RNAi* in the PG of larvae overexpressing *Ras^{V12}* (n=40-55).

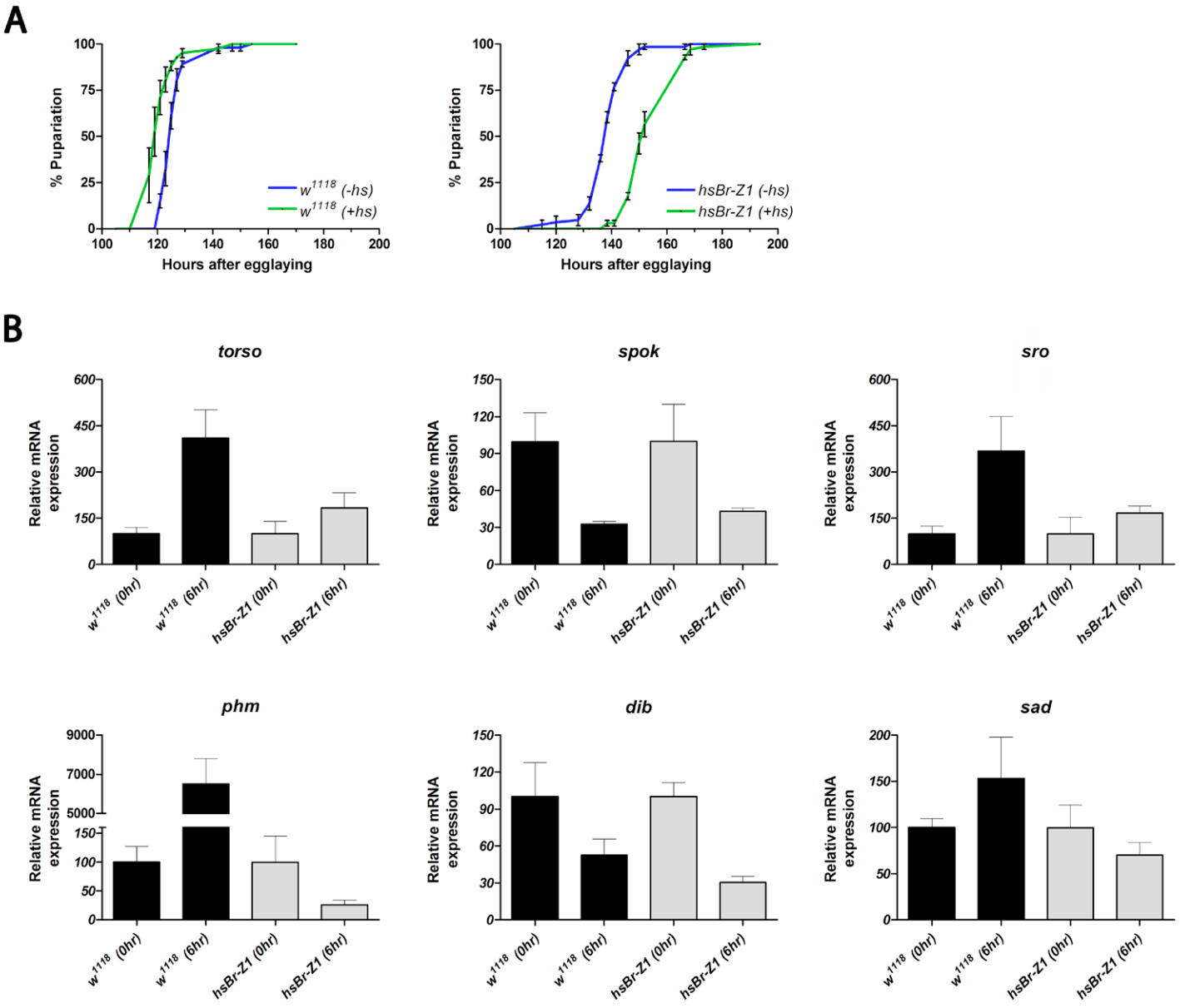

Fig. S4. EcR and Br-Z1 are involved in negative feedback that inhibits PG steroidogenic activity after pupariation. (A) Heat shock at 112 hours AEL (prior to the high level ecdysone pulse) delays pupariation of *hs-Br-Z1* larvae compared to non-heat shocked controls. Heat shock of wild type (w^{1118}) larvae at this time does not delay pupariation (B) Heat shock induced Br-Z1 over-expression 112 hours AEL (T=0 hr) inhibits the developmental increase in expression of genes involved in ecdysone biosynthesis and *torso (tor)* observed 6 hours later (118 hours AEL: T=6 hr) in the *phm*>+ control.

Table S1. Primers for amplification of genomic fragments

Name	5'-3'
phmF1	aatAGATCTcggcatattttatgggtctgc
phmF2	aatAGATCTaggtagacggtcgattgaata
phmF3	aatAGATCTtcgaactgatcccagatttctac
phmF4	aatAGATCTtgggtgtaatgaatgtgcatac
phmF5	aatAGATCTgatgccgaaaccctgtatgct
phmF6	aatAGATCTgtatatggtatatatggtgtggca
phmF7	aatAGATCTgagaatctgggaaaccaagag
phmR1	aataGCTAGCcactttcgatttcctcctgctc
phmR2	aataGCTAGCcttcttggtttcccagattctc
phmR3	aataGCTAGCtgccacaccatatataccatatac
phmR4	aataGCTAGCaaggagagtgcgaaagtaaaatt
dibF1	tctggtccttcccttcggtcacg
dibR1	attcgtattcacgcagcagctga

Capital letters indicate restriction enzyme sites: *Bgl*II in forward (F) primers and *Nhe*I in reverse (R) primers.

Table S2. Primers for site-directed mutagenesis

Name	5'-3'
phmF mut1	CCCGGGCTGCAGGAATTCAGATCTTGGCATATGTGAATGTGCATACGATAAACGGGCAATTTC
phmR mut1	GAAATTGCCCGTTTATCGTATGCACATTCACATATGCCAAGATCTGAATTCCTGCAGCCCGGG
phmF mut2	GCTGCAGGAATTCAGATCTTGGGTGTAATGAACATATGTACGATAAACGGGCAATTTCATTTTCAAATTTTACTTTCGC
phmR mut2	GCGAAAGTAAAATTTGAAAATGAAATTGCCCGTTTATCGTACATATGTTCATTACACCCAAGATCTGAATTCCTGCAGC
phmF mut3	CAGATCTTGGGTGTAATGAATGTGCATACGCATATGGGGCAATTTCATTTTCAAATTTTACTTTCGCACTC
phmR mut3	GAGTGCGAAAGTAAAATTTGAAAATGAAATTGCCCCATATGCGTATGCACATTCATTACACCCAAGATCTG
phmF mut4	GGTGTAATGAATGTGCATACGATAAACGGGCAACATATGTTTCAAATTTTACTTTCGCACTCTCCTTCTTCG
phmR mut4	CGAAGAAGGAGAGTGCGAAAGTAAAATTTGAAACATATGTTGCCCGTTTATCGTATGCACATTCATTACACC
phmF mut5	GCATACGATAAACGGGCAATTTCATTTTCAAATTCATATGTCGCACTCTCCTTCTTCGATGCCGAAACCC
phmR mut5	GGGTTTCGGCATCGAAGAAGGAGAGTGCGACATATGAATTTGAAAATGAAATTGCCCGTTTATCGTATGC

Table S3. EMSA oligos

Name	Forward, 5'-3'	Reverse, 5'-3'
***phm Br-Z1/Z4*	GCAATTTCATTTTCAAATTTCCCTGTCGC	TGCGACAGGGAAATTTGAAAATGAAATTG
***phm Br-Z1/Z4 mut*	GCAATTTCATCCCTGAATTTCCCTGTCGC	TGCGACAGGGAAATTCAGGGATGAAATTG
dib Br-Z4	TGAGCTAACGGAAACAGATCAGCA	GTGCTGATCTGTTTCCGTTAGCTC
dib Br-Z4 mut	TGAGCTAACCCCTGCAGATCAGCA	GTGCTGATCTGCAGGGGTTAGCTC
****Br-Z4/Z1*	CCTTTTTTTTTTATTTATGAAGTAATT	GAATTACTTCATAAATAAAAAAAAAAG
Non-specific	AACGTAGCTGATCGAATCGGTTAC	AGTAACCGATTCGATCAGCTACGT

Mutations introduced are underlined.
**Oligos have a mutated downstream Br-Z3 site allowing the study of Br-Z1/Z4 site alone.
***Oligos described in Wang et al. (Wang et al., 2009).

Table S4. qPCR primers

Name	Forward, 5'-3'	Reverse, 5'-3'
**rpL23*	GACAACACCGGAGCCAAGAACC	GTTTGCGCTGCCGAATAACCAC
E74A	TTTCTCTGCCGTTGTCGTC	GCACTGAGACCCGCTCAC
E74B	CGCGAGTTCAAAGTGCTCTA	GGAGGGAGAGTGGTGGTGT
E75B	CAACAGCAACAACACCCAGA	CAGATCGGCACATGGCTTT
**phantom* (*phm*)	GGATTTCTTTCGGCGCGATGTG	TGCCTCAGTATCGAAAAGCCGT
**disembodied* (*dib*)	TGCCCTCAATCCCTATCTGGTC	ACAGGGTCTTCACACCCATCTC
**spookier* (*spok*)	TATCTCTTGGGCACACTCGCTG	GCCGAGCTAAATTTCTCCGCTT
**neverland* (*nvd*)	GGAAGCGTTGCTGACGACTGTG	TAAAGCCGTCCACTTCCTGCGA
**shadow* (*sad*)	CCGCATTCAGCAGTCAGTGG	ACCTGCCGTGTACAAGGAGAG
shroud (*sro*)	AGCAGCTGAAGGTCGATAGC	GCGATTCGTGGCAGTAAAC
torso (*tor*)	TGCTTGGATTGGTATCCCTATAA	TGGGTCACAGTAAGATTCTCTGG
Br-Z4	CAAAGGCACACACACACACA	TCGGTCGGTTCTTCTCCTTC
Br-Z1	CCAGACCAACACCCACACAC	ACATTCGCTTCCTTCGTCCT

*Primers described previously (McBrayer et al., 2007).