

## Supplementary Material

Supplementary Tables S1 and S2 and Figures S1-S6.

### Supplementary Table S1

PCR primers used in this study.

Gene	Sense primer	Antisense primer
Hippo	GCGAAGGTTCATACGGAAGT	ACGTAAGGACTGTCGCACTG
Sav	TGTCTAGTGGGAGGCTGCTA	ACATGTCCAAACGCAACTTA
Warts	GCTCCGACAAAGGCTCTAAC	GCGGTGAATAATTTCGGACT
Mats	GCACAGAGGAGTCATGTGCT	TGCCCACGTCTCAAATAAT
Yorkie	GGCAGTCCCACAGTCCTATT	CAGTGGAGCCTATGTTGTCG
Sd	GGCCGATAATTCGTTGACT	AATCCGTCTGAGCCTCTGT
Ds	CAACGGAAACGATCAATACG	GTGCGAGCTACGATAACCAA
Fat	CTCTCAAGTCAGCCCACGTA	AGTTCCCGCAGCTGTACTTT
Crb	CATCGGCCTCACCTACTTCT	CAGTTGCTGTCGTTGAGGTT
Ex	ACGCACAAAGGAAGAAATGTG	TTGATCAGGCAAAGAAAGTCG
Mer	GATTGTTCGACCTGGTGTG	CTCTTGCCAACCTCAGCCA
Kibra	ATGTAAGAACCCGTGCATGA	ACCTGAACACTCCGAGCACTCT
Scrib	TAGTCAGCAACTGGAACGC	TTCTAGCCATGCGAATTGAG
Dlg	CTACCTTCGAGAGGATTGCC	GATAGCATGGGTCCACTTT
Lgl	ACTATATTGCCGGTGGCAT	AATCTATAACCCGGCTCGTG
Par3	CCATTCAATGCAAGTTGGTC	CTCGCGCATAAATAGGTTCA
Par6	AGTACGAGTCACACCGAACG	CCAACACTTCATCGTTGACC
aPKC	TGAAATGGGTGGATGAAGAA	AGTGAGTTGGAGTCTCGGT
Rp49	CAGGC GGTTCAAGGGTCAATAC	TGCTGGGCTTTCCACGA
cycE	AGCCTCAACCCTGATGAAC	TGGTTGATCACTGTTCCCAT
Hth	AAGTGTGAGCTAGCGACCTG	TTTGCTGAATACCGCAATGT
IAP	GTCCTCAACGACATCCACAC	TTCAGACGTTCCCTTTCACG
dsEGFP	GGATCCTAATACGACTCACTATAGG GAGAATGGTGAGCAAGGGCG	GGATCCTAATACGACTCACTATAG G GAGACTTGTACAGCTCGTCC
dsYorkie	GGATCCTAATACGACTCACTATAG GAGCCGGACTCAAAGCGAC	GGATCCTAATACGACTCACTATAG G TCCCAGTCGTTTTGT
Full Yorkie	CCGGAATTCATGGCTCTCAACTCGG AC	AAGCGGCCGCTTACAGCCACGT GAGTAC

**Supplementary Table S2**

**The comparison of amino acid identity of Yorkie proteins in Arthropoda**

Identity(%)	<i>Bombyx mori</i>	<i>Helicoverpa armigera</i>	<i>Papilio polytes</i>	<i>Amyelois transitella</i>	<i>Plutella xylostella</i>	<i>Acyrthosiphon pisum</i>	<i>Pediculus humanus humanus</i>	<i>Apis mellifera</i>	<i>Tribolium castaneum</i>	<i>Drosophila melanogaster</i>	<i>Zootermopsis nevadensis</i>	<i>Timema cristinae</i>	<i>Daphnia pulex</i>	<i>Strigamia maritima</i>	<i>Stegodyphus mimosarum</i>	<i>Ixodes scapularis</i>
<i>Bombyx mori</i>	89	79	82	73	57	57	55	55	55	39	58	51	44	44	37	44
<i>Helicoverpa armigera</i>	89		82	83	74	58	59	55	54	39	59	53	45	45	36	46
<i>Papilio polytes</i>	79	82		78	72	58	57	59	54	41	61	54	46	45	39	44
<i>Amyelois transitella</i>	82	83	78		68	59	58	56	55	38	60	52	47	45	37	45
<i>Plutella xylostella</i>	73	74	72	68		51	54	78	51	41	56	51	42	43	41	42
<i>Acyrthosiphon pisum</i>	57	58	58	59	51		55	56	48	40	59	50	47	41	39	43
<i>Pediculus humanus humanus</i>	57	59	57	58	54	55		59	56	37	64	54	47	43	38	47
<i>Apis mellifera</i>	55	55	59	56	78	56	59		53	39	61	46	45	36	40	39
<i>Tribolium castaneum</i>	55	54	54	55	51	48	56	53		34	55	48	42	41	39	46
<i>Drosophila melanogaster</i>	39	39	41	38	41	40	37	39	34		38	26	44	39	45	36
<i>Zootermopsis nevadensis</i>	58	59	61	60	56	59	64	61	55	38		60	49	49	41	47
<i>Timema cristinae</i>	51	53	54	52	51	50	54	46	48	26	60		44	41	39	40
<i>Daphnia pulex</i>	44	45	46	47	42	47	47	45	42	44	49	44		53	40	47
<i>Strigamia maritima</i>	44	45	45	45	43	41	43	36	41	39	49	41	53		45	52
<i>Stegodyphus mimosarum</i>	37	36	39	37	41	39	38	40	39	45	41	39	40	45		61
<i>Ixodes scapularis</i>	44	46	44	45	42	43	47	39	46	36	47	40	47	52	61	

## **Legends to Supplementary Figures**

**Fig. S1.** A scheme of the potential Hippo pathway in *Bombyx*.

**Fig. S2.** Spatial expression patterns of Hippo pathway genes in multiple tissues on day 3 of the fifth larval instar.

Normalized microarray data (GSE17571) for gene expression in multiple tissues of silkworm larvae on day 3 of the fifth instar was used to survey tissue-specific expression of Hippo pathway genes. Hippo pathway genes were considered to be expressed if their normalized intensity values exceeded 0. The expression levels are illustrated with a seven-grade color scale representing relative expression levels of -3, -2, -1, 0, 1, 2 and 3. Red represents positive; black represents zero; green represents negative. A/MSG, anterior/middle silk gland; PSG, posterior silk gland.

**Fig. S3.** Temporal expression patterns of Hippo pathway genes during the larval-pupal-adult metamorphosis.

Female and male individuals at 19 time points during silkworm metamorphosis were selected for expression profiling using microarray analysis. The sequential time points include V4 (day 4 of the fifth larval instar), V5, V6, V7, W0 (0 h after wandering, just before spinning), W12,W24(PP1), W36, W48 (completion of spinning,PP2), W60 (immediately after pupation,), W72, W96, W120, W144, W168, W192, W216, W240, and adult. V, fifth larval instar; W, wandering.

**Fig. S4.** Developmental expression profiles of the 18 Hippo pathway genes in the wing disc from day 3 of the fourth larval instar to day 2 of the prepupal stage.

4L-3 and 4L-4, day 3 and 4 of the fourth larval instar; 4M, the 4th molting; 5L-1 to 5L-7, day 1 to 7 of the fifth larval instar; W, the wandering stage; PP1 and PP2, day 1 and 2 of the prepupal stage.

**Fig. S5.** The effects of *Yorkie* RNAi and *Yorkie* overexpression on the wing disc.

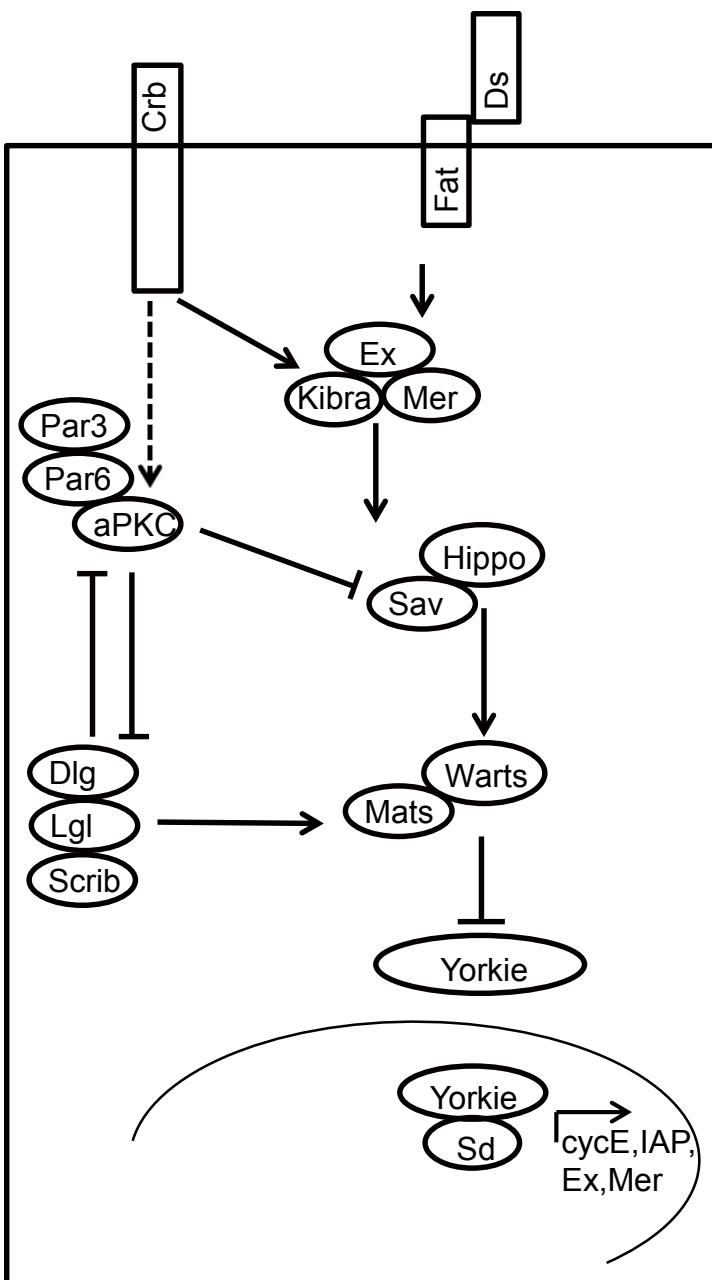
(A and A') Compared to the *EGFP* RNAi control silkworms, the *Yorkie* RNAi silkworms showed reductions in organ size (A) and *Yorkie* expression (A') at 24 h after dsRNA treatment.

(C and C') Compared to the *RFP*-overexpressed control silkworms, the *Yorkie*<sup>CA</sup>-overexpressed silkworms showed increases in the posterior silk gland size (C) and *Yorkie*<sup>CA</sup> expression (C') at 120 h after baculovirus infection.

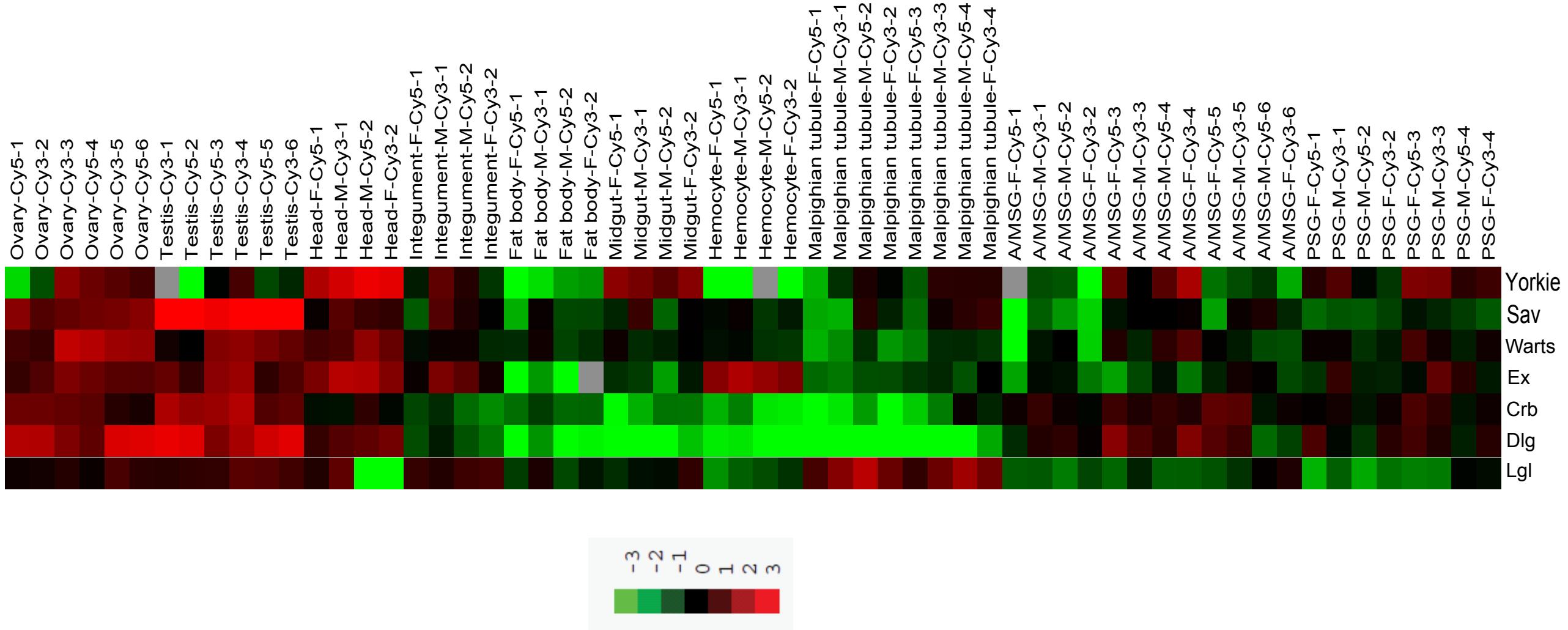
**Fig. S6. The efficiency of *Yorkie* RNAi in different tissues.**

A comparison of the RNAi efficiency of *Yorkie* RNAi in the ovary, wing disc (WD), fat body (FB) and posterior silk gland (PSG) at 24 h after dsRNA treatment.

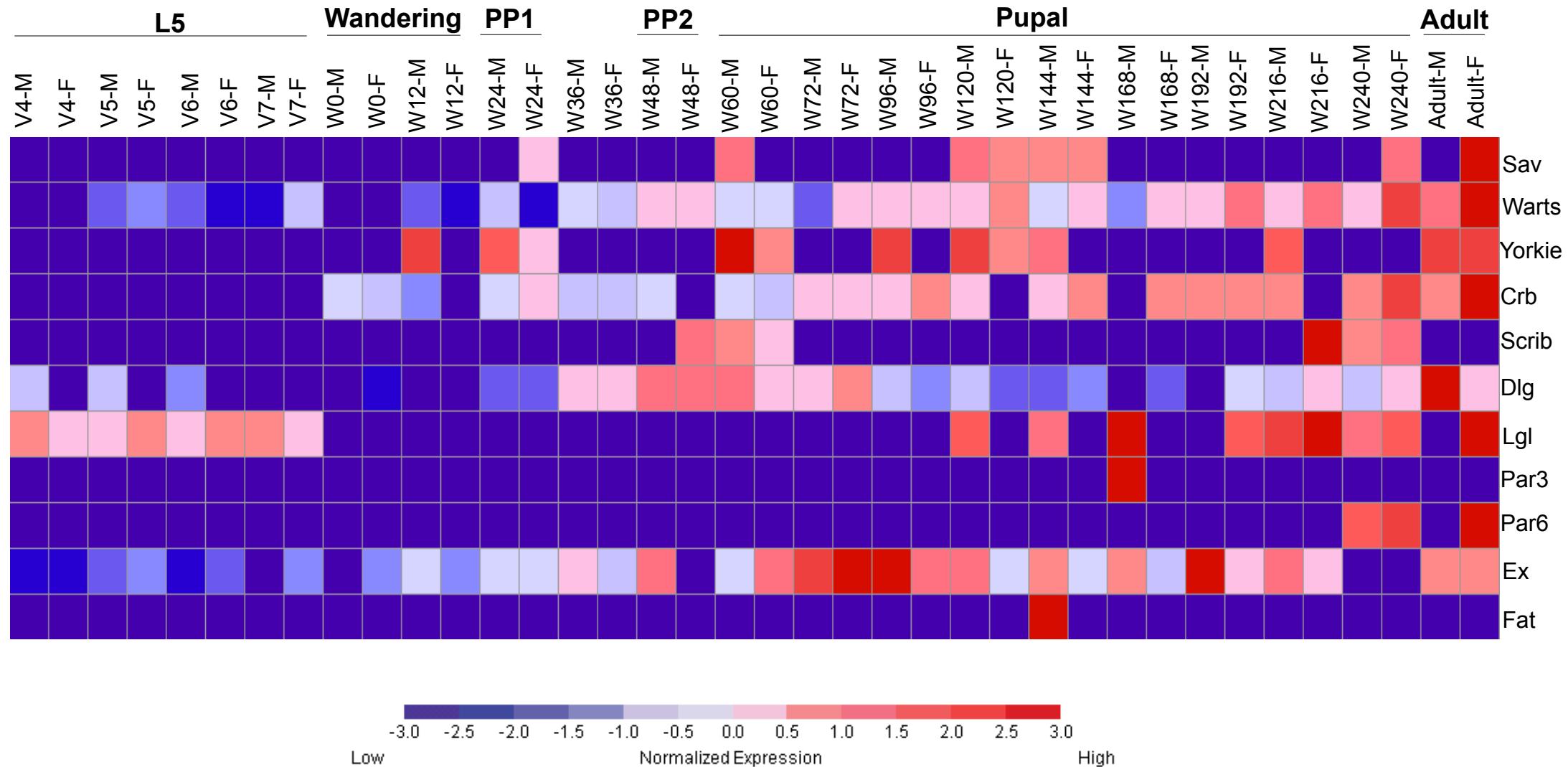
# Figure S1



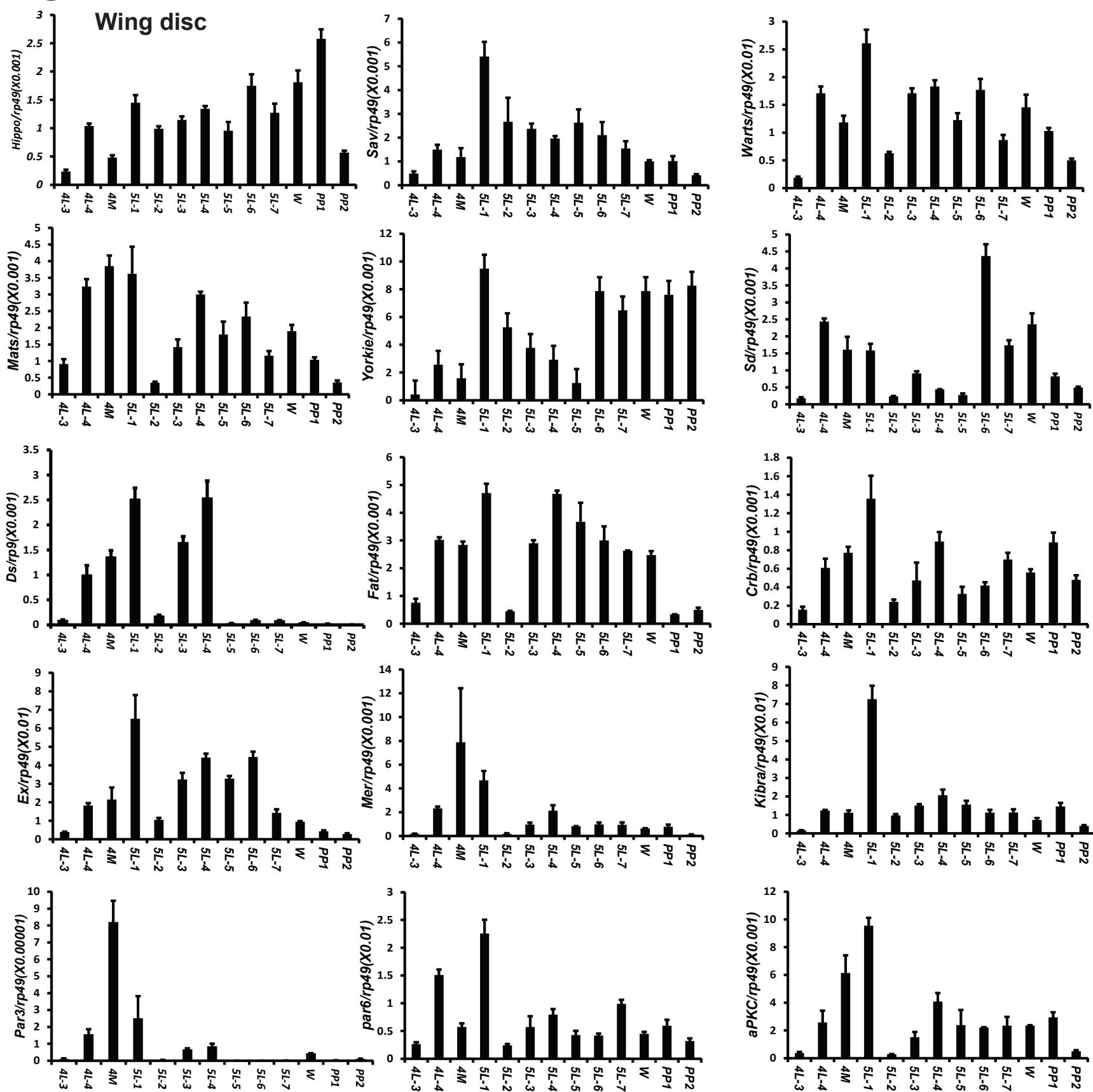
# Figure S2



# Figure S3



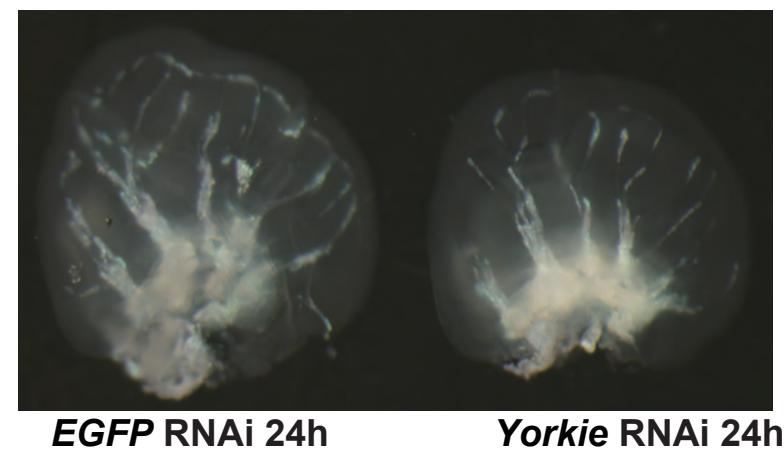
# Figure S4



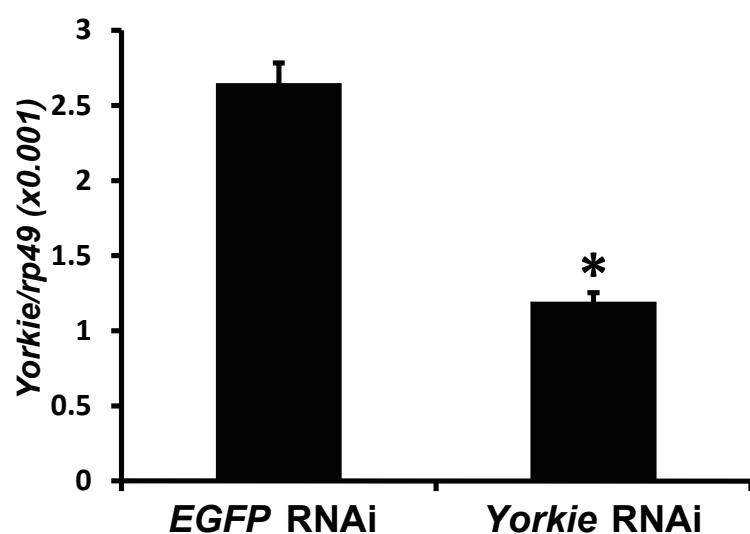
# Figure S5

A

Fore wing

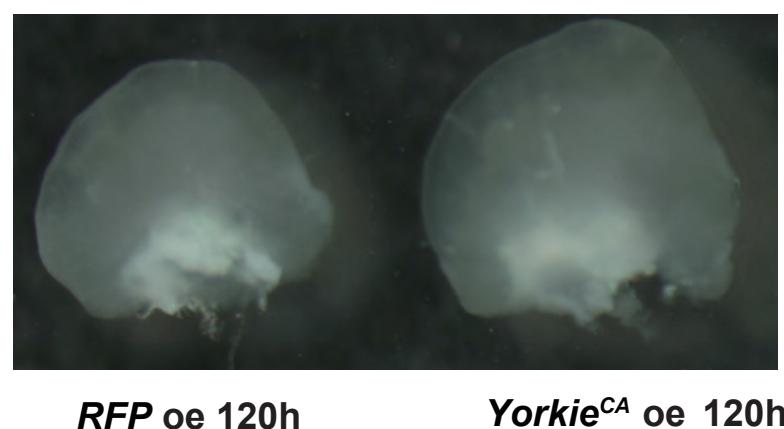


A'

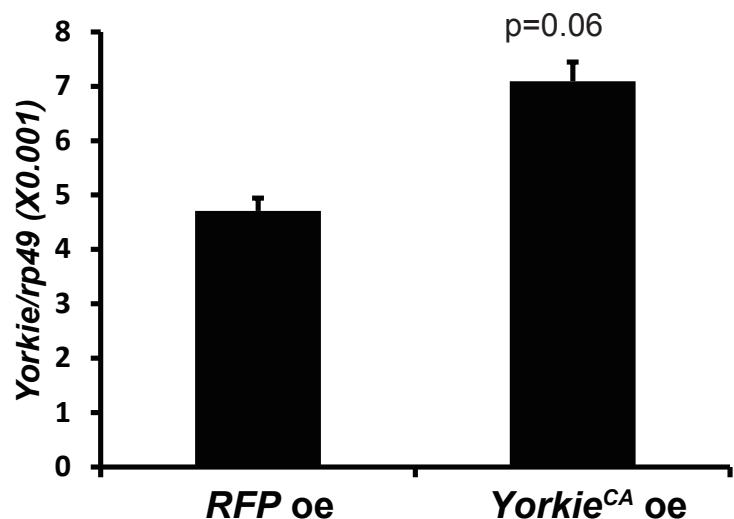


B

Fore wing



B'



# Figure S6

