

Supplementary Materials for
**Functional involvement of multiple genes as members of the supergene unit in
the female-limited Batesian mimicry of *Papilio polytes***

Shinya Komata, Shinichi Yoda, Yûsuke KonDo, Souta Shinozaki, Kouki Tamai, Haruhiko
Fujiwara*

*Corresponding author. Email: haruh@edu.k.u-tokyo.ac.jp

This PDF file includes:

Figs. S1 to S15
Tables S1 to S4

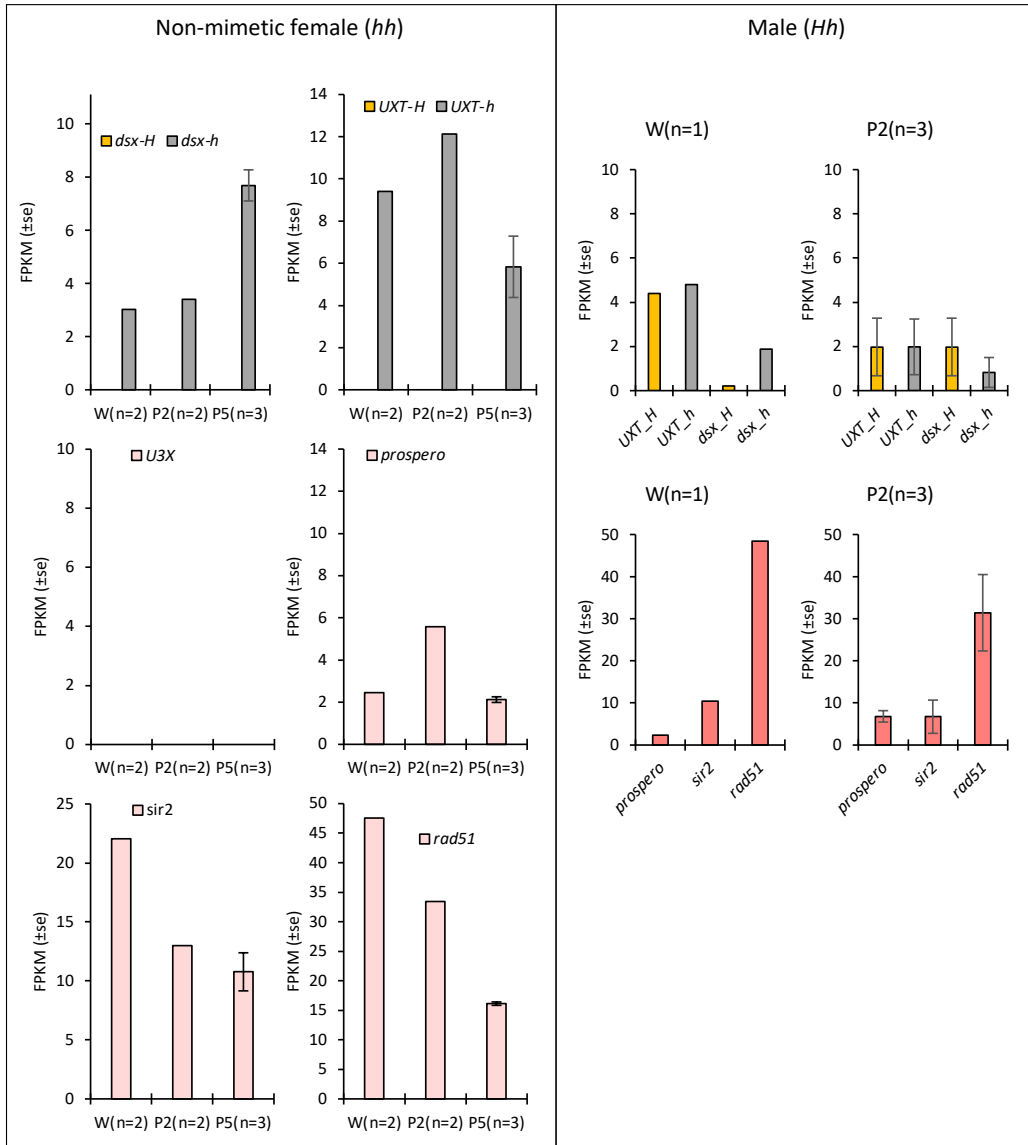


Fig. S1. Expression levels of genes within and flanking the HDR in hindwings of non-mimetic (*hh*) females and males (*Hh*). FPKM values by RNA-seq at the wandering stage (W) of the late last instar larvae, 2 day after pupation (P2) and 5 day after pupation (P5) are shown with SE.

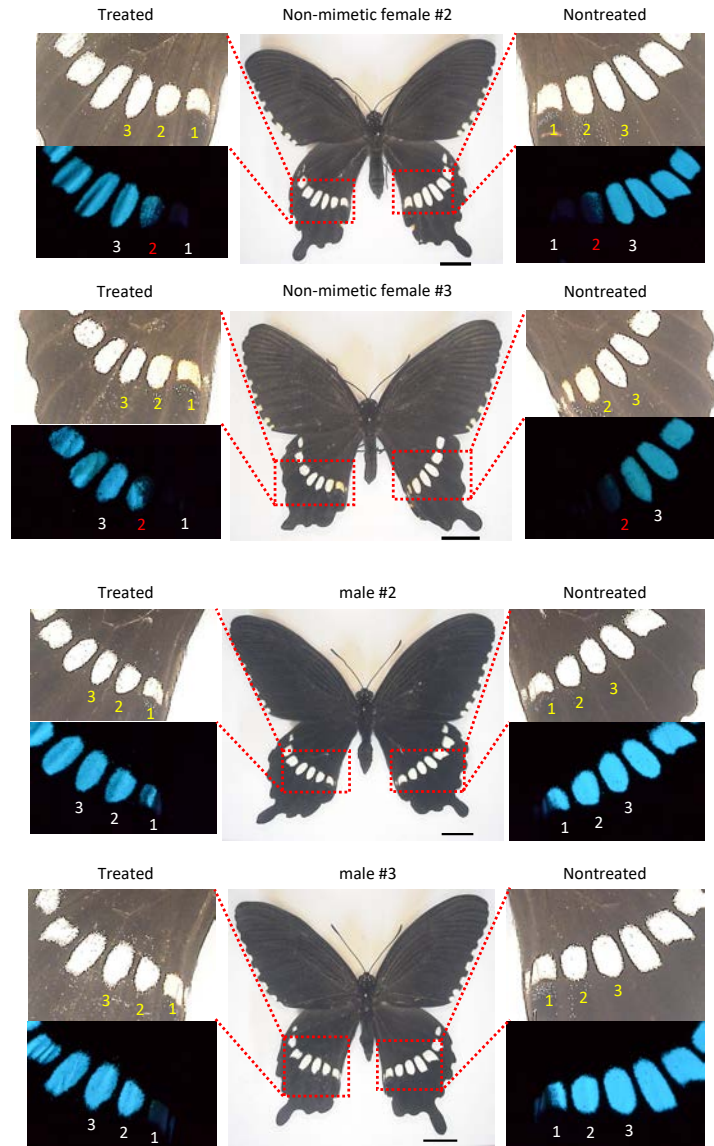


Fig. S2. Knockdown of *dsx* in the left hindwings of non-mimetic (*hh*) females and males of *Papilio polytes*. Other replicates of Fig. 2, A and B.

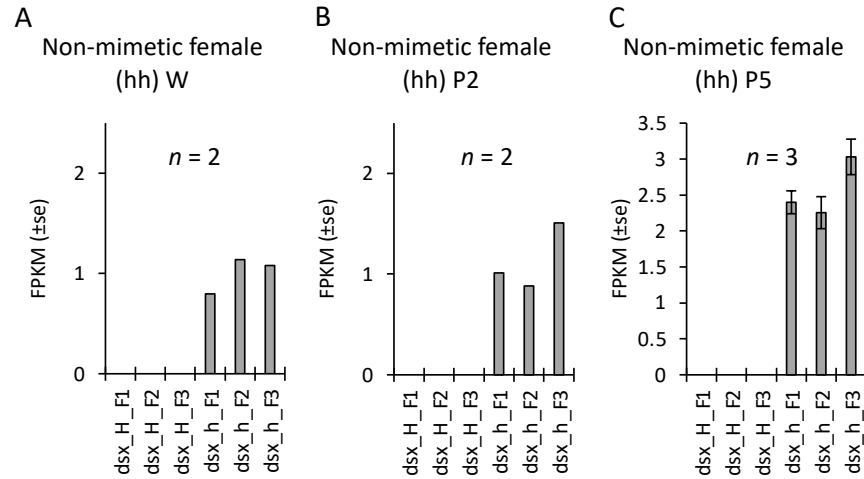


Fig. S3. Gene expression levels of each *dsx* isoforms in non-mimetic (*hh*) females in *Papilio polytes*. FPKM values by RNA-seq at the wandering stage (W) of the late last instar larvae is (A), at 2 day after pupation (P2) is (B) and at 5 day after pupation (P5) is (C). Orange bars indicate the expression levels of *dsx* isoforms from mimetic (*H*) allele and gray bars indicate from non-mimetic (*h*) allele. No expression of *H*-derived isoform (orange bars) here. F1, F2 and F3 means female isoform 1, 2 and 3, respectively. There was no statistically significant difference among isoforms.

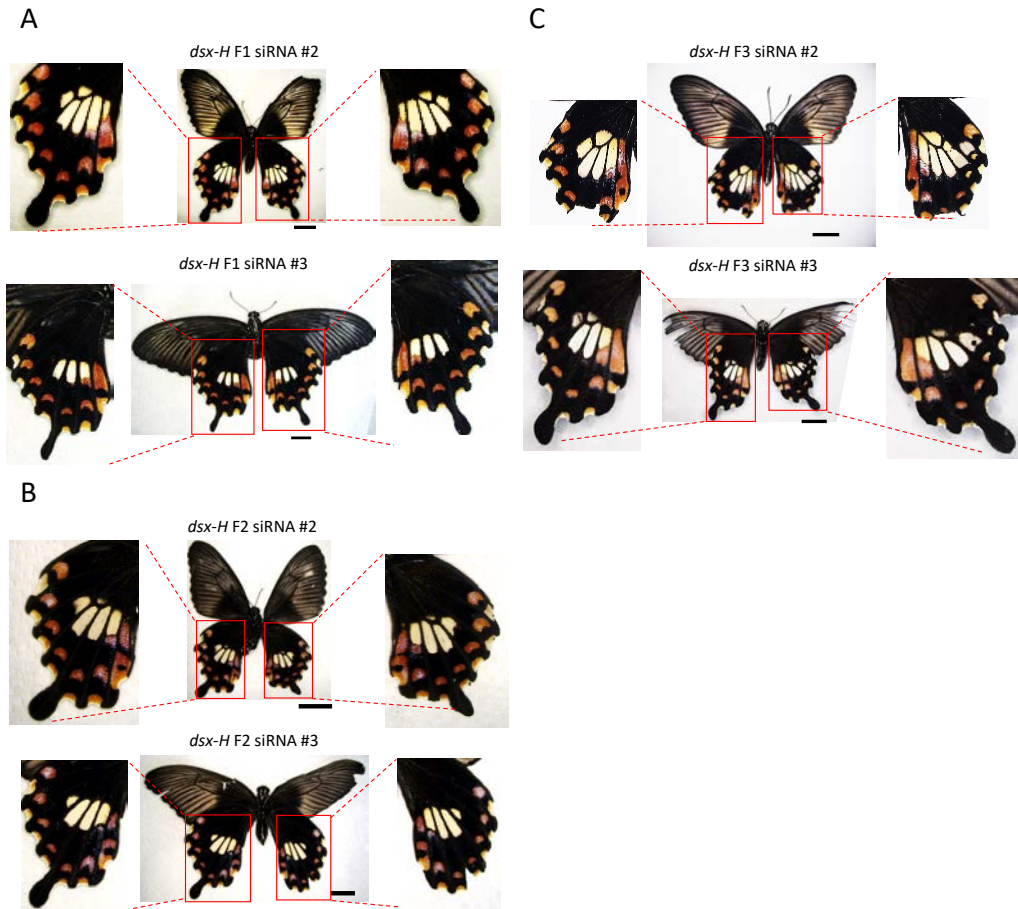


Fig. S4. Knockdown of *dsx* female isoform 1 (A), 2 (B) and 3 (C) in the hindwings of mimetic (*Hh*) females of *Papilio polytes*. Other replicates of Fig. 2, D, E and F.

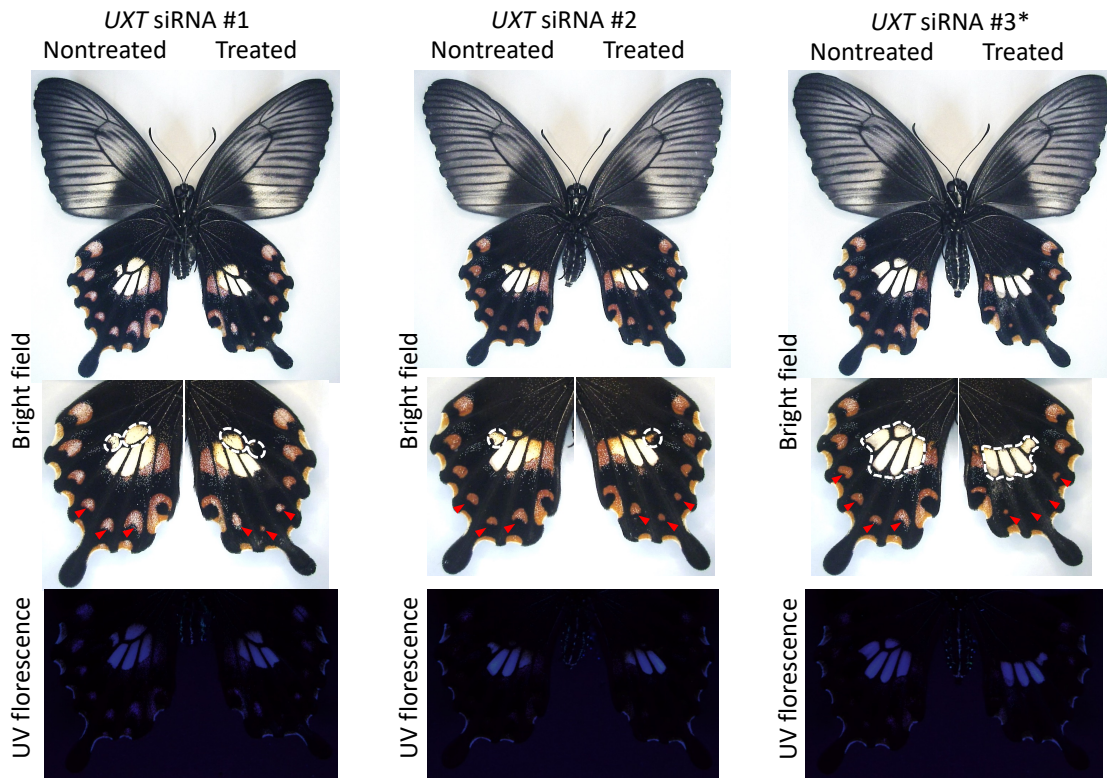
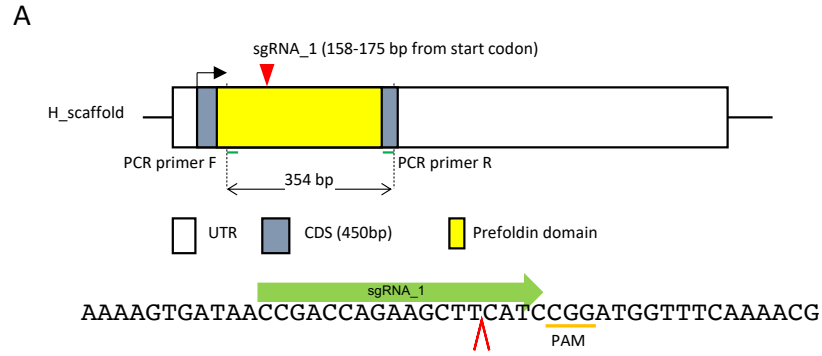


Fig. S5. Knockdown of *UXT* in the hindwings of mimetic (*Hh*) females. Other replicates of Fig. 3A. Red arrowheads represent the changed red regions, and the white dotted line indicates the area where the pale yellow regions has changed. *#3 is the same individual as Fig. 3A.



B

	No. of injecting eggs	No. of hatching eggs	Hatching rates (%)	No. of 1-2 instars	No. of 3-5 instars	No. of pupa	No. of adult	Survival rates from larva to adult
plate1	54	22	40.74	14	8	7	5	22.72
plate2	54	16	29.63	9	4	1	0	0.00
plate3	54	20	37.04	16	7	6	4	20.00
plate4	54	24	44.44	20	6	3	3	12.50
plate5	54	16	29.63	16	8	5	4	25.00
plate6	24	17	70.83	10	7	7	5	29.41
total	294	115	39.12	85	40	29	21*	18.26

* Mimetic female = 8, nonmimetic female = 5, male = 8

Fig. S6. (A) Design of guide RNA in Crispr/Cas9 knockout experiment in *UXT*. PCR primers F and R show the primers for genotyping (Fig. S7). **(B)** Summary of the number of eggs injected and the number of adults obtained in the Crispr/Cas9 experiment.

(Continue to the next page)

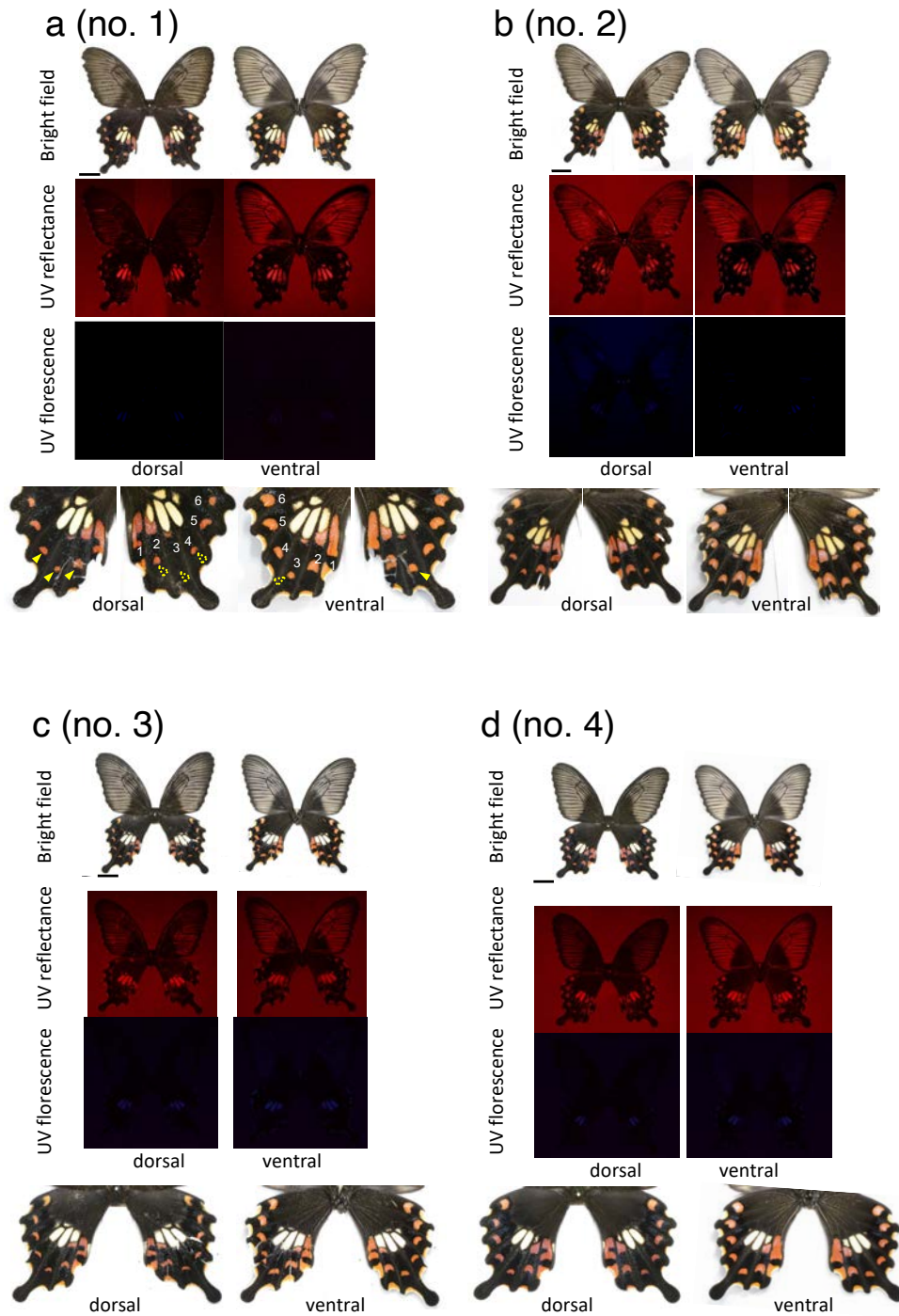


Fig. S8. Mosaic knockout of *UXT* by Crispr/Cas9. Dorsal and ventral views of eight individuals observed are shown. In individual number 1, 5, 6 and 8, arrowheads represent the changed red regions. The individual number 8 is the same individual shown in Fig. 3B. Scale bars, 1 cm. Other replicates of Fig. 3B.

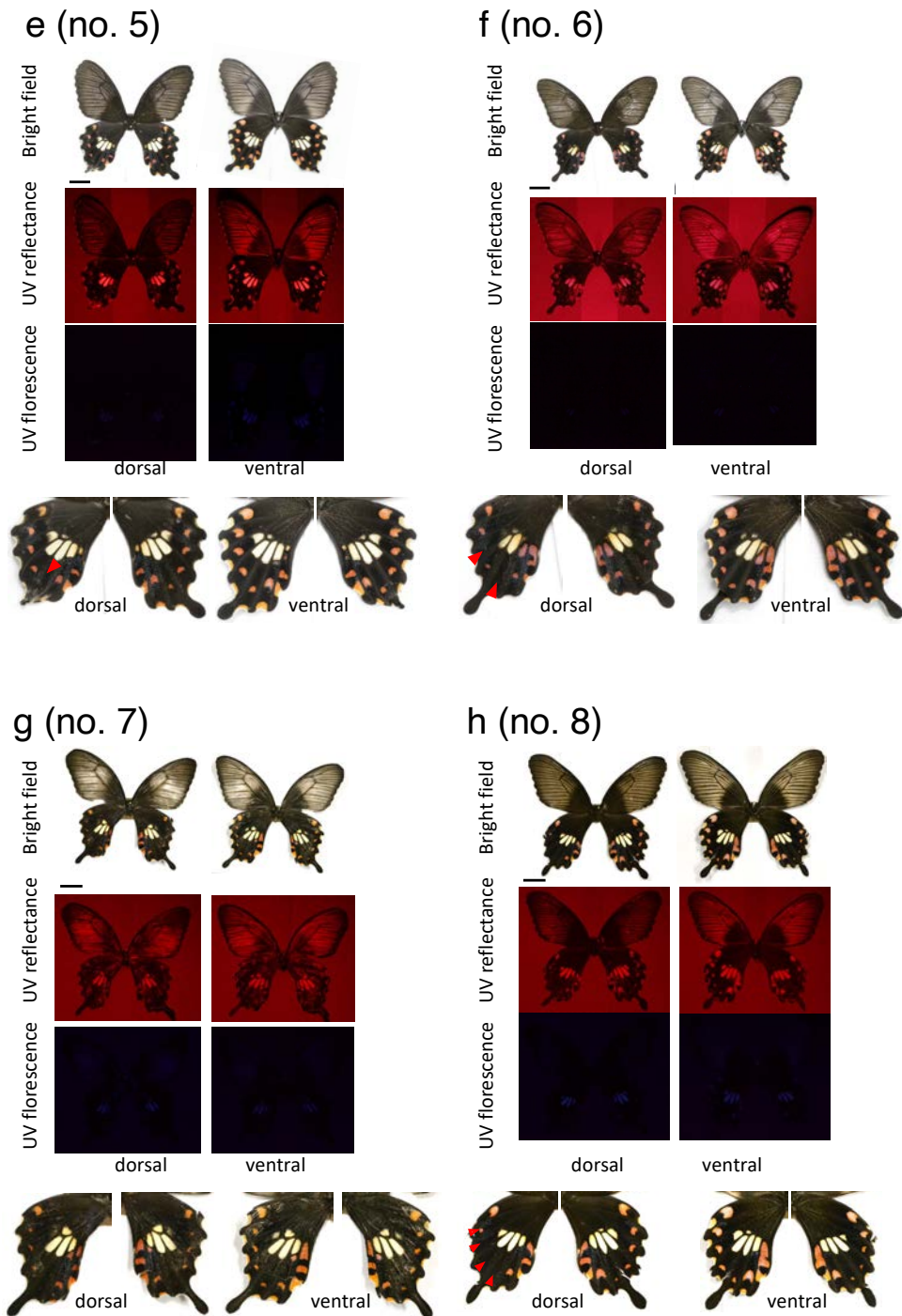


Fig. S8. Mosaic knockout of *UXT* by Crispr/Cas9. Dorsal and ventral views of eight individuals observed are shown. In individual number 1, 5, 6 and 8, arrowheads represent the changed red regions. The individual number 8 is the same individual shown in Fig. 3B. Scale bars, 1 cm. Other replicates of Fig. 3B.

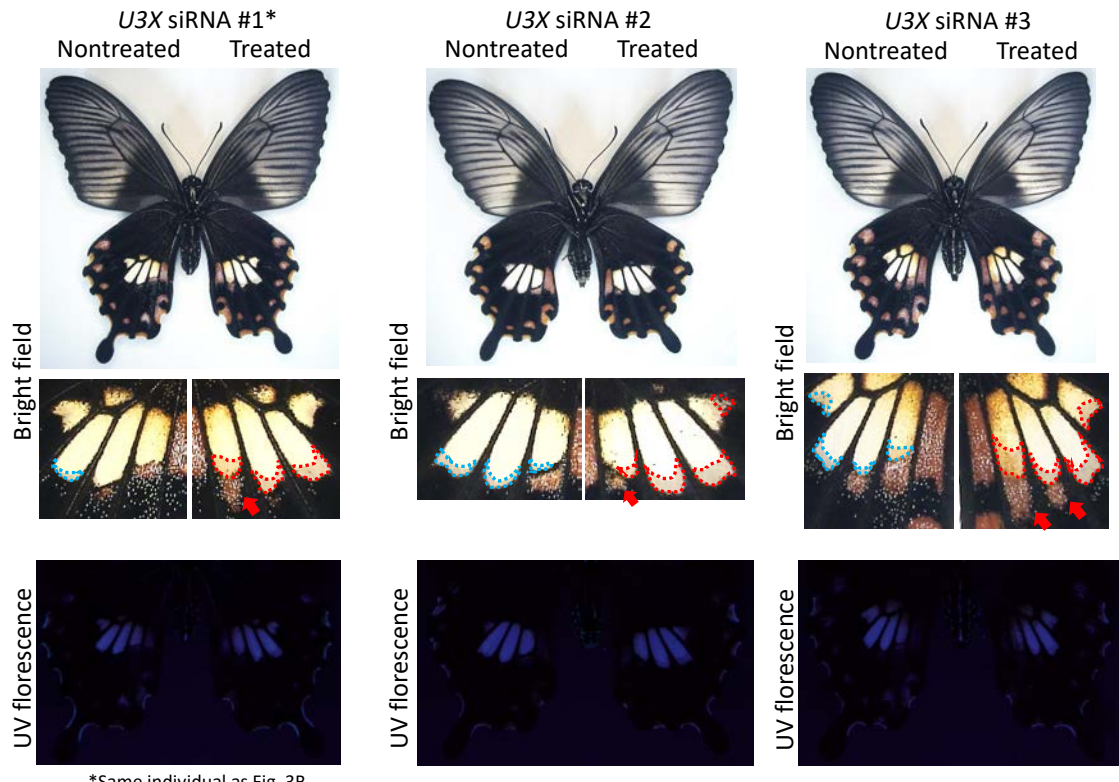


Fig. S9. Knockdown of *U3X* in the hindwings of mimetic (*Hh*) females. In magnified views of the pale-yellow regions, the red arrow indicates the area where the red spot has expanded, and the red dotted line indicates the area where the pale-yellow spot has extended by *U3X* knockdown. Other replicates of Fig. 3B (#1 is the same individual as Fig. 3B.).



Fig. S10. DEGs of transcription factors (A) and signaling factors (B) down-regulated by *dsx-H* knockdown. Sequences of DEGs obtained by RNA-seq were blasted with Uniprot, and transcription factors and signal factors were extracted using GO terms of the top hit Uniprot ID. In case multiple isoforms of the same gene were included, only the isoform with the lowest Log₂FC (Log₂-fold change value = Log₂ siRNA-treated FPKM – Log₂ untreated FPKM) is shown, and Name is the protein name of the top hit Uniprot ID.

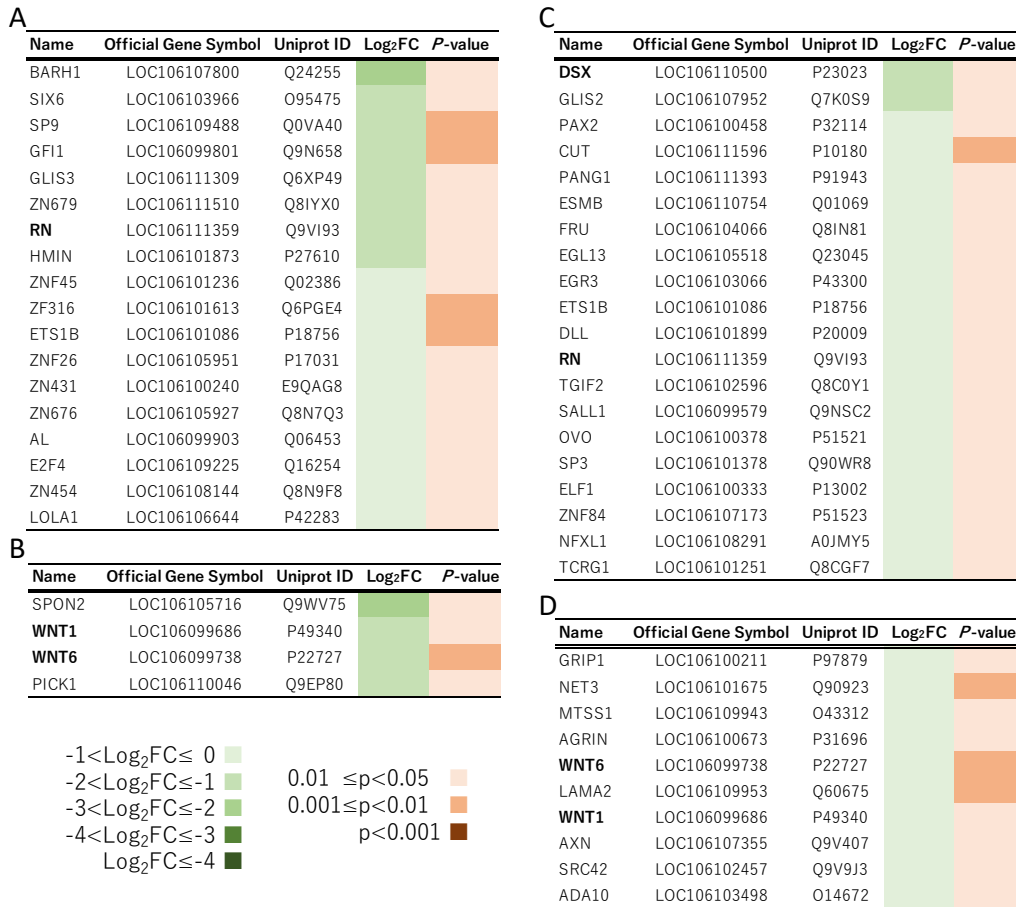


Fig. S11. DEGs of transcription factors and signaling factors down-regulated by *UXT* (A, B) and *U3X* (C, D) knockdown. Among the DEGs obtained by knockdown of *UXT*, transcription factors are shown in (A) and signal factors are shown in (B). Similarly, the transcription factor and signal factor of *U3X* are shown in (C) and (D), respectively.

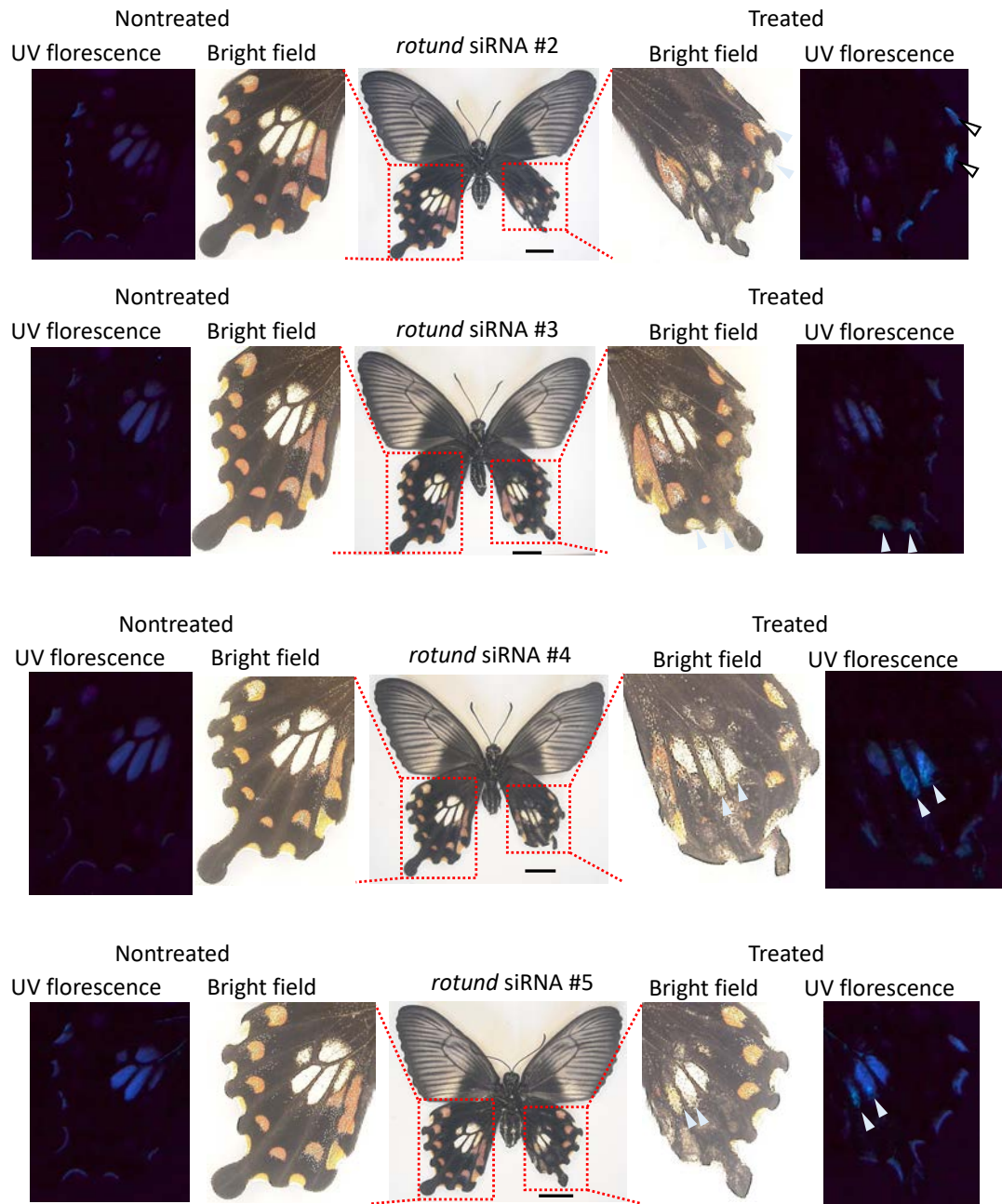


Fig. S12. Knockdown of *rotund* (*rn*) in the hindwings of mimetic (*Hh*) females. Other replicates of Fig. 4D. In UV fluorescence, white arrowheads represent the changed pale-yellow regions by knockdown. Knockdown of *rn* changed the pale-yellow spots to produce UV fluorescence like non-mimetic females and males. Scale bars, 1cm.

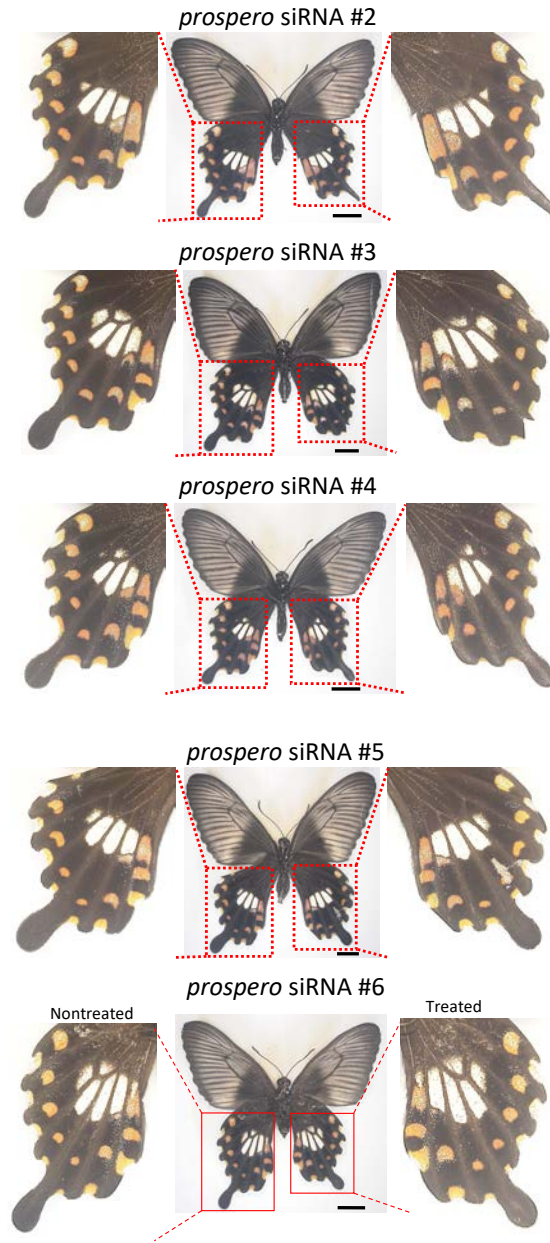


Fig. S13. Knockdown of *prospero* in the hindwings of mimetic (*Hh*) females. Other replicates of Fig. 5A.

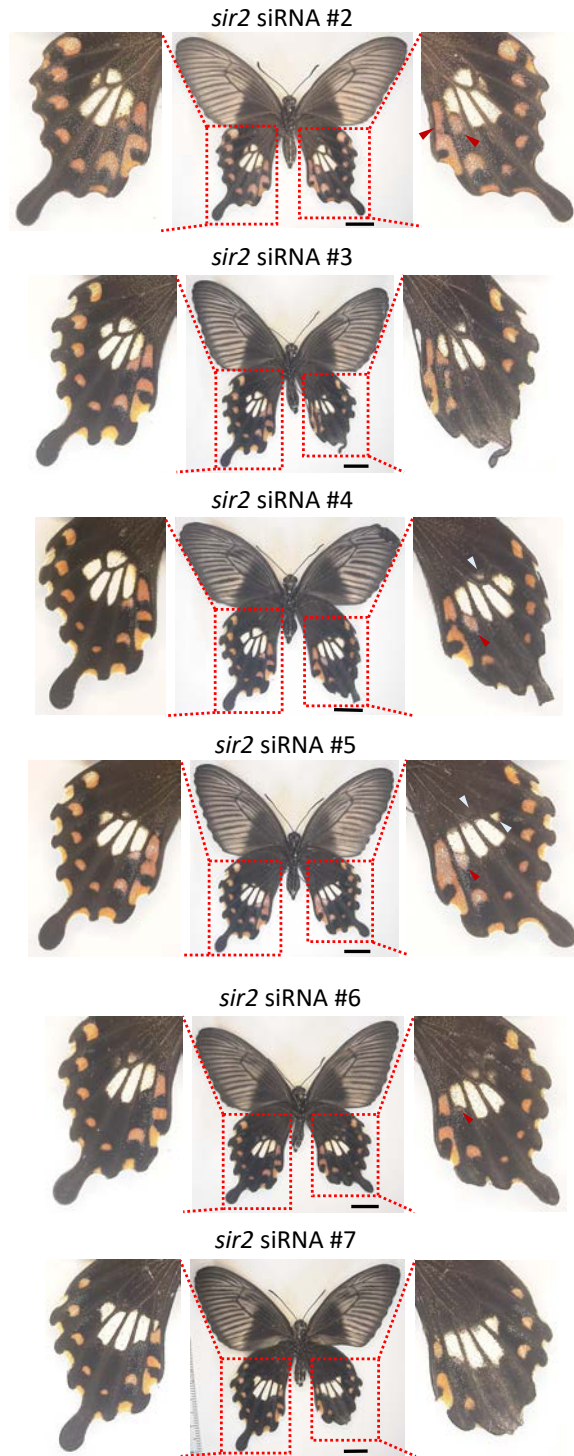


Fig. S14. Knockdown of *sir2* in the hindwings of mimetic (*Hh*) females. Other replicates of Fig. 5B. Red and white arrowheads represent the changed red and pale-yellow regions, respectively.

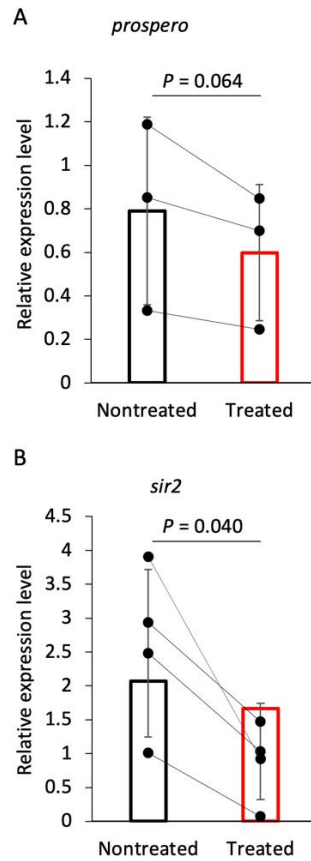


Fig. S15. Measurement of knockdown effect of *prospero* and *sir2* using RT-qPCR. We compared the expression levels of *prospero* (A) and *sir2* (B) between untreated (white bar) and treated hindwings (red bar) by RT-qPCR using *RpL3* as an internal control. Values and error bars denote the mean and standard deviation of three biological replicates. *P* values were obtained by one-tailed paired t-test.

Table S1. Summary of RNA-seq data for analyzing expression levels in hindwing.

sex	genotype	stage*	Total reads	Accession number	Reference
female	Hh (mimetic)	W	32662442	SAMD00018653	Nishikawa et al. 2015
female	Hh (mimetic)	W	43756504	SAMD00018654	Nishikawa et al. 2015
female	Hh (mimetic)	W	32073800	SAMD00018655	Nishikawa et al. 2015
female	Hh (mimetic)	P2	44802896	SAMD00018646	Nishikawa et al. 2015
female	Hh (mimetic)	P2	48917672	SAMD00018647	Nishikawa et al. 2015
female	Hh (mimetic)	P2	34191856	SAMD00018649	Nishikawa et al. 2015
female	Hh (mimetic)	P2	37108690	SAMD00018650	Nishikawa et al. 2015
female	Hh (mimetic)	P5	57845316	xxx	
female	Hh (mimetic)	P5	42352556	xxx	
female	Hh (mimetic)	P5	52407784	xxx	
female	hh (non-mimetic)	W	44210098	SAMD00018651	Nishikawa et al. 2015
female	hh (non-mimetic)	W	49523076	SAMD00018652	Nishikawa et al. 2015
female	hh (non-mimetic)	P2	47905432	xxx	
female	hh (non-mimetic)	P2	46441150	xxx	
female	hh (non-mimetic)	P5	51941714	xxx	
female	hh (non-mimetic)	P5	52537276	xxx	
female	hh (non-mimetic)	P5	48761016	xxx	
male	Hh	W	32114136	SAMD00018657	Nishikawa et al. 2015
male	Hh	P2	54632630	xxx	
male	Hh	P2	47742400	xxx	
male	Hh	P2	52810550	SAMD00018656	Nishikawa et al. 2015

*W: 5th instar larvae (wandering), P2: 2 days after pupation, P5: 5 days after pupation

Table S2. Summary of RNA-seq data for DEGs between control and RNAi hind wings.

Sample type	sex	genotype	stage*	Total reads	Accession number
dsx-H_control1	female	<i>HH</i>	P2	53406060	
dsx-H_knockdown1	female	<i>HH</i>	P2	67393442	
dsx-H_control2	female	<i>Hh</i>	P2	71828378	SAMD00128715
dsx-H_knockdown2	female	<i>Hh</i>	P2	77618118	SAMD00128718
dsx-H_control3	female	<i>Hh</i>	P2	47225584	
dsx-H_knockdown3	female	<i>Hh</i>	P2	42890920	
UXT_control1	female	<i>Hh</i>	P2	53548310	
UXT_knockdown1	female	<i>Hh</i>	P2	56542592	
UXT_control2	female	<i>Hh</i>	P2	47523810	
UXT_knockdown2	female	<i>Hh</i>	P2	56463070	
UXT_control3	female	<i>Hh</i>	P2	59193152	
UXT_knockdown3	female	<i>Hh</i>	P2	63099692	
U3X_control1	female	<i>Hh</i>	P2	46621574	
U3X_knockdown1	female	<i>Hh</i>	P2	64156422	
U3X_control2	female	<i>Hh</i>	P2	57740102	
U3X_knockdown2	female	<i>Hh</i>	P2	52851868	
U3X_control3	female	<i>Hh</i>	P2	52290574	
U3X_knockdown3	female	<i>Hh</i>	P2	59202374	

Table S3. List of qPCR primer

Target gene	qPCR primer name	Formard (5'–3')	Reverse (5'–3')
<i>U3X</i>	Pp_U3X_qPCR	CCTGACATCTACTAAATGCTTCATGG	CAGCACCAGCTCGTGTGCTTG
<i>UXT-H</i>	qPpUXT_mimetic_spec_F	CATTTTCGGAATGATGGATGCGATA	TAGCGGTATCAACATTTGGTAGAGA
<i>UXT-h</i>	qPpUXT_nonmimetic_spec_F	GACGCGATGTTTTAGCTTTTACA	TAGCGGTATCAACATTTGGTAGAGA
<i>dsx-H</i>	Pp_dsx_H_spec_qPCR	gctgcaactcaccacgcagcgcgcaca	ccgcctcggagtcgacggaggt
<i>dsx-h</i>	Pp_dsx_h_spec_qPCR	gctgcaactcaccacgcggcgcaact	ccgagctcgaagtcgacgggggc
<i>sir2</i>	Pp_Sir2_qPCR_1	TTGTAGGAAACAATACTCTTTGGAA	GGCTTGATAATGCCTGGACA
<i>prospero</i>	Pp_prospero_qPCR_1	GAGGTGCCACCCAACCTCAG	CGCGGAAGAATCCCGTAA
<i>RpL3*</i>	Pp_rpl3_qPCR-F2	CACAAAGGGCAAGGGATAC	ACAAGCTACTTTACGCAGAC

*used as an internal control

Table S4. Lists of siRNA

siRNA target gene	siRNA name	siRNA Target sequence	Sense (5'-3')	Antisense (5'-3')
<i>dsx-H</i>	Pp_dsx_mimetic	TTGTCGCAACCACCGGTTGAAGG	GUCGCAACCACCGGUUGAAGG	UUCAACCGGUGGUUGCGACAA
<i>dsx-H&dsx-h</i>	Pp_dsx_common	TTGGTGGAGAACTGTCACAGACT	GGUGGAGAACUGUCACAGACU	UCUGUGACAGUUCUCCACCAA
<i>UXT</i>	Pp_UXT_A	AAGGTGTATGAAGATAAAGCTGA	GGUGUAUGAAGAUAAAGCUGA	AGCUUUUCUUCAUACACCUU
<i>U3X</i>	Pp_U3X_A	AAGAAACAACAAAATTACCATAT	GAAACAACAAAUUACCAUUAU	AUGGUAUUUUUGUUUUUCU
<i>sir2</i>	Pp_Sir2_C	AACCAACAATTTACATTATTTC	CCAACAUUUCACAUUUUUUC	AAUAAUGUGAAAUGUUGGUU
<i>sir2</i>	Pp_Pmem_Sir2_D	TCCGTCATTACACAGAAATATT	CGUCAUUACACAGAAUUAUU	UAUUCUGUGUUAUGACGGA
<i>prospero</i>	Pp_prospero_A	AAGAACAGTTAGCTGAAATGAAA	GAACAGUUAGCUGAAAUGAAA	UCAUUUCAGCUAACUGUUCUU
<i>prospero</i>	Pp_prospero_C	AACAACAACGAGCCTAAATTA	CAACAACGAGCCUAAAUA	UAAUUUAGGCUCGUUGUUGUU
<i>rotund</i>	Pp_ZNF_rotund_A	GAGCACATTCCTAAACACAAAGA	GCACAUUCCUAAACACAAAGA	UUUGUGUUUAGGAAUGUGCUC