Supplementary Materials for

apterous A specifies dorsal wing patterns and sexual traits in butterflies

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Figure S1: *ap* mRNA localization in developing wing discs of *Bicyclus anynana*  
A) *apA* mRNA localization (middle) in wildtype 5\(^{th}\) larval instar wing discs with control (right). There is an absence of *apA* expression in future dorsal eyespot centers (arrowhead). Corresponding adult wing is shown (left). 
B) Cross-sectional view of a developing wing disc showing dorsal-specific *apB* expression (left). No staining is seen with control probes for *apB* (middle) and *apA* (right). Scale bar is 20µm  
C) Male (left) and female (right) hindwing discs (28 hours after pupation) showing *apB* mRNA up-regulation in the hair-pencil regions only in males. 
D) Controls for *apB* (left) and *apA* (right) expression in male wings show no staining in the corresponding regions.
Figure S2: CRISPR/Cas9 mosaic wing pattern phenotypes of apB knockout  
A) Top: Region of the apB gene in B. anynana targeted using the CRISPR/Cas9 system  
Bottom: Sequences of the LIM domain region of mutant individuals compared with the wildtype sequence in bold. Blue is the region targeted and the PAM sequence is in red. Deletions are indicated with ‘-‘.  
B) CRISPR/Cas9 apB mosaic phenotypes of B. anynana. B-M9-17: The forewings of a mutant individual showing differences in shape and marginal defects of the right wing as compared to the left. The boxed area is expanded to the right.  
B-M9-15: Mutant with wing pattern changes that do not correspond to mosaic ventral patterns, but appear to indicate disruptions to wing margin development. Boxed area expanded to the right.
Figure S3: A catalog of the different types of CRISPR/Cas9 mosaic wing pattern phenotypes of *apA* homeodomain knockout
Figure S4: A catalog of the different types of CRISPR/Cas9 mosaic wing pattern phenotypes of \textit{apA} LIM domain knockout
Figure S5: A catalog of the different types of CRISPR/Cas9 mosaic wing pattern phenotypes of *apB* LIM domain knockout
**Table S1**: List of primers and guide RNA sequences used in this study

| Gene                  | Primer Name | Primer Sequence                                                                 |
|-----------------------|-------------|---------------------------------------------------------------------------------|
| Apterous A (ApA)      | AM 31, AM 32| Forward 5’ CGGGAGGCCTGTCTTTCTGGC 3’ Reverse 5’ CGTCGGAGCTGGTGATGAGGG 3’         |
| Apterous B (ApB)      | AM 136, AM 137| Forward 5’ CGAACAGTTGAATGCATTG 3’ Reverse 5’ GGGGACTTTCTCTTTCTTGG 3’           |
| ApA Homeodomain       | AM 158      | 5’GAAATTAATACGACTCTATAGGAGCTGGTGATGCTTTAGCAGAATAAGGC 3’                         |
| CRISPR Guide          | AM 235      | 5’GAAATTAATACGACTCTATAGGAGAAGAATAGCGACAAGAATAGC 3’                             |
| ApB LIM domain CRISPR | AM 145      | Guide 5’GAAATTAATACGACTCTATAGGAGGATGGCAGGCCGAGCACGAGAATAGC 3’                   |
| Genotyping            | AM 194, AM 167| Forward 5’ CATTTTTGCGACACGAGCAGTC 3’ Reverse 5’ CTAAATGTCCTCGACTATATG 3’       |
| ApA LIM domain CRISPR | AM 257, AM 258| Forward 5’ GTACAGTAATTAGTTACATCAAC 3’ Reverse 5’ CTTTCAGTTGTTGTCATTTAAG 3’     |
| Genotyping            | AM 385, AM 386| Forward 5’ CACTAGATTAGCCCTAAGGCT 3’ Reverse 5’ CTGTTTTGTAGGAAATAATG 3’         |
Table S2: CRISPR/Cas9 injection concentrations and mutation frequencies

| Guide          | Guide RNA Conc (ng/ul) | Cas9 mRNA Conc (ng/ul) | Eggs injected | Eggs hatched | Hatch ratio | Total adults | Mutant phenotypes |
|----------------|------------------------|------------------------|---------------|--------------|-------------|--------------|-------------------|
| ApA Homeodomain | 360                    | 600                    | 631           | 55           | 8.7%        | 9            | 3 (33%)           |
|                | 450                    | 900                    | 882           | 89           | 10%         | 35           | 9 (25.7%)         |
| ApA LIM Domain | 400                    | 900                    | 266           | n.a          | n.a         | 17           | 6 (35.2%)         |
| ApB LIM Domain | 400                    | 900                    | 228           | 75           | 32.89%      | 45           | 6 (13.3%)         |

* 4 of the 9 mutant individuals were pupae with wings missing from one side as shown in SFigure 3

Table S3: Results from a second set of injections with guides and Cas9 mRNA and Cas9 mRNA alone (Control) to test whether presence of each of the guides impacts hatching ratios. None of the adults that resulted from this experiment showed mutant phenotypes.

| Guide          | Guide RNA Conc (ng/ul) | Cas9 mRNA Conc (ng/ul) | Eggs injected | Eggs hatched | Hatch ratio |
|----------------|------------------------|------------------------|---------------|--------------|-------------|
| Control        | -                      | 900                    | 103           | 53           | 51.4%       |
| ApA Homeodomain | 400                    | 900                    | 113           | 75           | 66.3%       |
| ApA LIM Domain | 400                    | 900                    | 108           | 51           | 47.2%       |
| ApB LIM Domain | 400                    | 900                    | 104           | 53           | 50.9%       |
