

## Supplementary data

**Table S1 Primers used in this study.**

| Primer name        | Primer sequence (5' to 3')                                                                          |
|--------------------|-----------------------------------------------------------------------------------------------------|
| M-F1               | tagggaattggactagtcgctagcATGACATCGGCAAAAGTAGC                                                        |
| M-R1               | gctgattatgatcctcgagGATCGTGACTTCCTTCTGTCTCGATTACAAAAGCCATTTAC                                        |
| M-F2               | CAGAAGGAAGTCACGATCGAAGCTAATCAGAAGAAAAT                                                              |
| M-R2               | gctgattatgatcctcgagCTATTGAAACGGCGGTGGT                                                              |
| <i>Masc</i> -O-F   | CACTGGCGGCGACAAGAGACGTCATGACATCGGCAAAAGTAGC                                                         |
| <i>Masc</i> -O-R   | GATTATGATCTAGAGTCGGGCCCTATTGAAACGGCGGTGGT                                                           |
| <i>PSI</i> -O-F    | CACTGGCGGCGACAAGAGACGTCATGgattacaaggatgacgacgataaggattacaaggatgacgacgata<br>agATGAGTGATTATTCTTCTATG |
| <i>PSI</i> -O-R    | GATTATGATCTAGAGTCGGGCCCTCATTTTGAGTTCACTGCTGGT                                                       |
| <i>Bmdsx</i> -F-RT | AACCATGCCACCACTGATACCAAC                                                                            |
| <i>Bmdsx</i> -R-RT | GCACAACGAATACTGCTGCAATCG                                                                            |
| <i>Bmrp49</i> -F   | TCAATCGGATCGCTATGACA                                                                                |
| <i>Bmrp49</i> -R   | ATGACGGGTCTTCTTGTGG                                                                                 |
| <i>Masc</i> -Q-F   | ATGACATCGGCAAAAGTAGC                                                                                |
| <i>Masc</i> -Q-R   | ATGATGACGCGGTTATCTTC                                                                                |
| <i>PSI</i> -Q-F    | CCACAACATCAAGACCCTTC                                                                                |
| <i>PSI</i> -Q-R    | GCTCCAGTTTGTCTTGGAG                                                                                 |

**Table S2 Detailed information of 52 common interacting proteins of PSI and Masc.**

| Functional category       | Functional annotation                                                                              | Protein name                                  | UniProt ID                                                                                                                                                                                                                                                             |
|---------------------------|----------------------------------------------------------------------------------------------------|-----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Energy metabolism<br>(10) | Mitochondrial respiratory chain Complex I core subunit (Essential gene; "lethal" mutant phenotype) | Lethal(3)neo18 / NADH dehydrogenase subunit 5 | B5A989                                                                                                                                                                                                                                                                 |
|                           | Mitochondrial respiratory chain Complex IV subunit                                                 | Cytochrome c oxidase subunit 2                | D5LQX6; A0A0U1Z013; A0A0U1YZZ0; A0A0U1Z1Y3; A0A0U1YWU9; Q9MID9; Q9MM50                                                                                                                                                                                                 |
|                           | Glycolysis enzyme                                                                                  | Enolase                                       | A0A1L7P084; A1YQ87                                                                                                                                                                                                                                                     |
| Cytoskeleton (3)          | Major component of microtubules                                                                    | Tubulin beta chain                            | O01676; H9IWQ1                                                                                                                                                                                                                                                         |
|                           | Muscle structure protein                                                                           | Myofilin variant B                            | C3UZ73                                                                                                                                                                                                                                                                 |
| Structure protein (2)     | Structural constituent of cuticle                                                                  | Putative cuticle protein                      | C0H6J4; C0H6L9                                                                                                                                                                                                                                                         |
| Signal transduction (1)   | Cell cycle-related kinase                                                                          | Cdc2-related kinase                           | O17508                                                                                                                                                                                                                                                                 |
| Gene regulation (2)       | Chromatin remodeling complex subunit                                                               | Rsf1                                          | Q1HDZ3; H9J6R3                                                                                                                                                                                                                                                         |
| Unknown function<br>(34)  | Function uncharacterized                                                                           | Uncharacterized protein                       | H9IUV1; H9IWX2; H9J4L6; H9JXH0; H9J2F1; H9J7K1; H9JL27; H9JWS8; H9JVZ2; H9JF91; H9JB82; H9JSI0; H9JSD3; H9JTW5; H9JBE9; H9JR61; H9JUJ8; H9JM71; H9JH96; H9J8J5; H9J6Y6; H9J5Q7; H9J559; H9J1Q1; H9J049; H9IZC6; H9IY97; H9IXZ0; H9IXG9; H9IWX1; H9IVF8; H9ISW3; H9JHG2 |

**Figure S1** Validation of overexpression in cultured cells. (a) RT-PCR analysis of *Bmdsx* splicing patterns in the indicated cell samples. (b, c) Relative expression levels of *BmPSI* (b) and *BmMasc* (c) in the indicated cell samples, as determined by qRT-PCR. Statistical significance: \* $P < 0.05$ , \*\* $P < 0.01$ ; ns, not significant. Abbreviations: Control, empty-vector-transfected cells; Blank, untransfected cells; *BmMasc-wt*, wild-type *Masc* overexpression; *BmMasc-m*, mutant *Masc* overexpression; *BmPSI*, *PSI* overexpression; *BmPSI/Masc-m*, co-overexpression of *PSI* and mutant *Masc*.

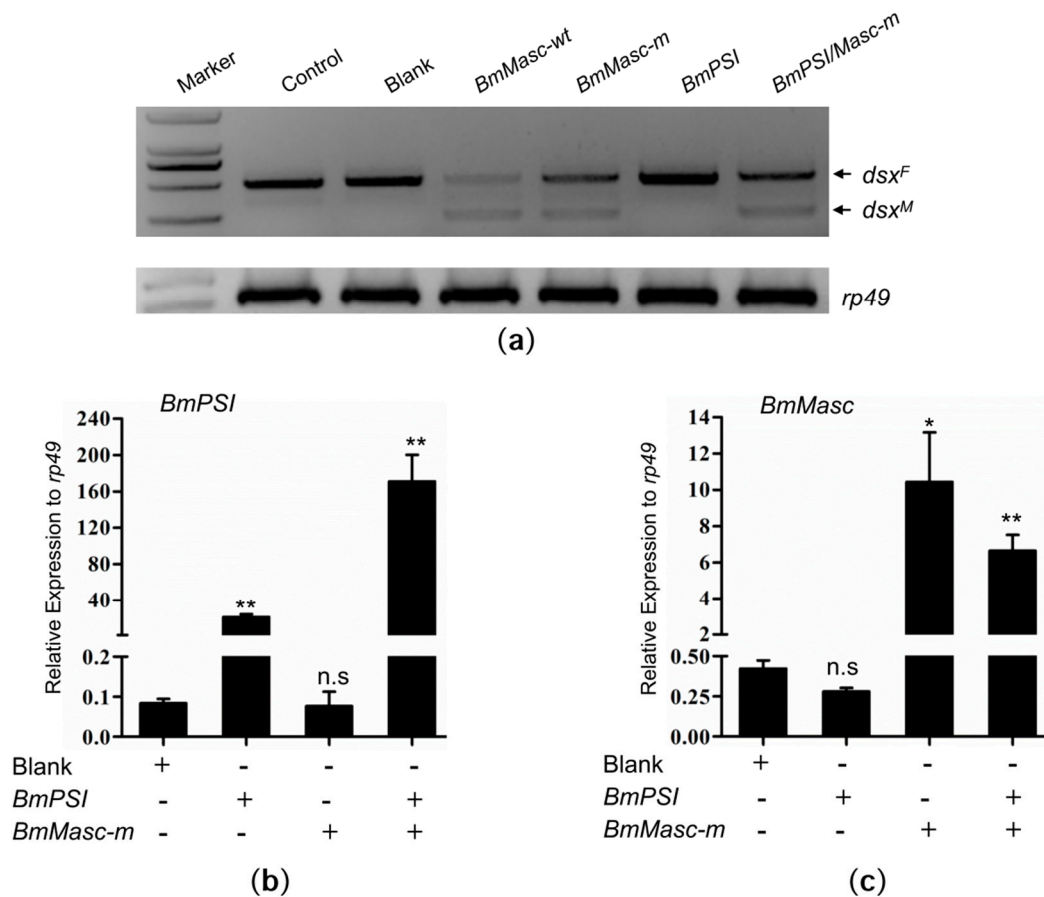


**Figure S2** Functional classification of PSI- and Masc-specific interacting proteins. (a) Venn diagram of proteins identified by FLAG pull-down. (b) Functional classification of PSI-specific interacting proteins. (c) Functional classification of Masc-specific interacting proteins. (d) STRING protein-protein interaction network of the 52 common interactors. Nodes represent proteins and edges represent predicted interactions (combined score  $\geq 0.15$ ). Isolated nodes indicate proteins lacking known interactions, reflecting the current annotation status of the silkworm proteome.

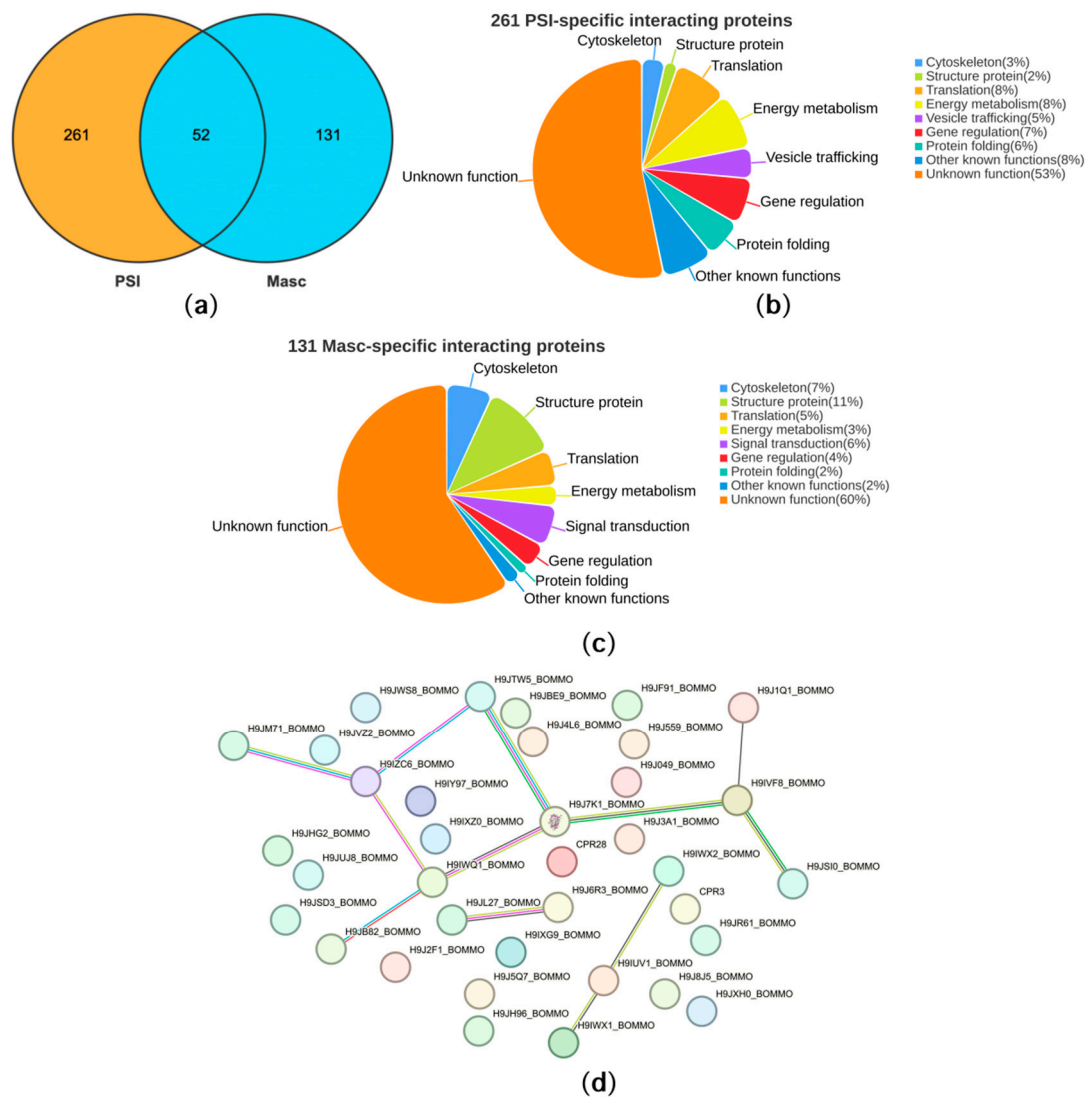


**Figure S3** Transcriptomic analysis of female-sterile *Bmdsx* mutants. (a) Venn diagram of dysregulated genes in  $\Delta dsx^F$ -*F* and  $\Delta dsx^C$ -*F*. (b) Numbers of upregulated and downregulated genes. (c, d) GO enrichment analysis of upregulated (c) and downregulated (d) genes. (e, f) KEGG pathway enrichment analysis of upregulated (e) and downregulated (f) genes.

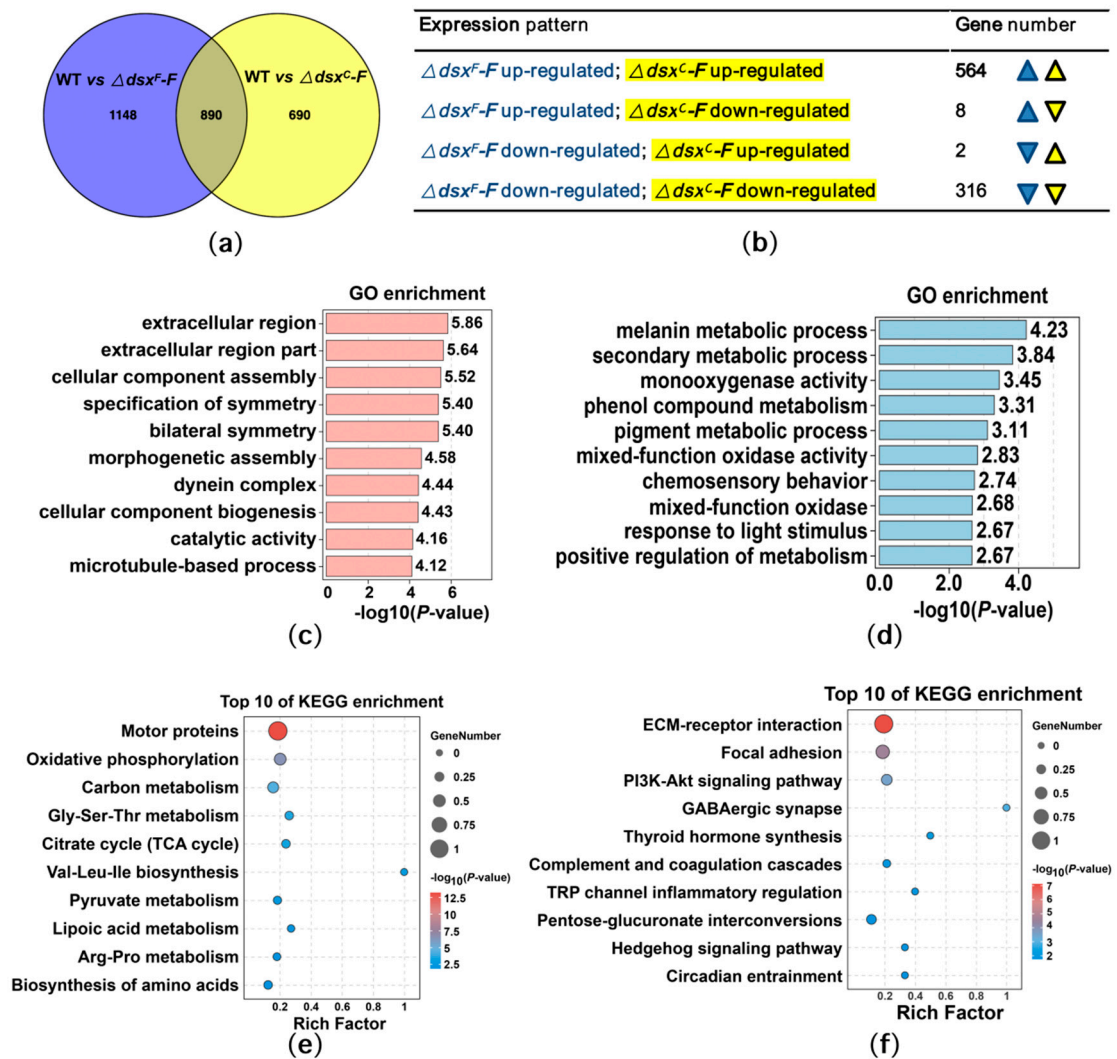


**Figure S4** Transcriptomic analysis of male-sterile *Bmdsx* mutants. (a) Venn diagram of dysregulated genes in  $\Delta dsx^M$ -*M* and  $\Delta dsx^C$ -*M*. (b) Numbers of upregulated and downregulated genes. (c, d) GO enrichment analysis of upregulated (c) and downregulated (d) genes. (e, f) KEGG pathway enrichment analysis of upregulated (e) and downregulated (f) genes.

