

Supplementary Information

Table S1 Sequences of primers for qPCR.

Table S2 Down regulated genes in male gonadal cells transfected with *MIER3*-Pex2.

Table S3 Up regulated genes in male gonadal cells transfected with *MIER3*-Pex2.

Table S4 Differentially expressed genes after transcriptome sequencing (see Excel file).

Table S5 The list of GO terms significantly enriched with differentially expressed genes (see Excel file).

Table S6 The list of KEGG pathways significantly enriched with differentially expressed genes (see Excel file).

Figure S1 Some specific sequences in the protein sequence of chicken *MIER3* gene are different. ① is Figure a, ② is Figure b, and ③ is Figure c

Figure S2 Standard curves for detecting the expression level of chicken *MIER3* gene using fluorescence quantitative PCR method. The abscissa is a multiple of 2. *MIER3W*-127 refers to the design of primers for the same sequence of the first, second, and seventh transcripts of the *MIER3*-W gene. *MIER3W*-3, *MIER3W*-45, and *MIER3W*-6 are similar to *MIER3W*-127. *MIER3W* refers to the design of primers for the same sequence of the seven transcripts of the *MIER3* gene, and *HMBS* is an internal reference gene. **Figure S3** Expression levels of different transcripts of *MIER3* in the gonads of E12 chicken embryos. **Figure S4** Evolutionary tree of *MIER3* protein.

Figure S5 Relative mRNA expression of chicken *MIER3*-W, *MIER3*-Z and *MIER3*-ZW in different embryonic tissues in E6 (n=5) and E12 (n=5). The mRNA expression levels were shown as values relative to that of female gonad. SPSS 16.0 (SPSS China, Shanghai, China) was used to perform the Student's t-test for statistical significance of differences between male and female tissues. There was significant difference among different alphabetic columns ($P < 0.05$), * and * * represented $P < 0.05$ and $P < 0.01$,

and the data were mean \pm standard deviation.

Figure S6 The relative expression of *MIER3-Z* in the left gonad of chicken embryo was detected by qPCR.

The mRNA expression levels were shown as values relative to that of female gonad. SPSS 16.0 (SPSS China, Shanghai, China) was used to perform the Student's t-test for statistical significance of differences between male and female tissues. * and ** represented $P < 0.05$ and $P < 0.01$.

Figure S7 Volcano map for differentially expressed genes. Note: The abscissa is $\log_2(\text{Fold Change})$, the ordinate is $-\log_{10}(\text{p-value})$, and the blue dotted line indicates the threshold line of screening criteria for differentially expressed genes. The red and green dots indicate the genes with upregulated and downregulated expression in Chicken embryo male gonad cells transfected with *MIER3* overexpression vector vs. empty vector, respectively.

Figure S8 The histogram showing the results from GO enrichment analysis. Note: The coordination shows GO term on the abscissa and the significance of enrichment on the ordinate. The significance of enrichment is indicated by $-\log_{10}(\text{padj})$. Padj denotes adjusted P value. Different colors represent different categories of GO term, including biological process (BP), cellular component (CC) and molecular function (MF).

Table S1 Primer sequences for quantitative PCR analysis

Gene	GenBank Number	Primer Sequence (5'-3')	Product Size
<i>CCL4</i>	NM_204720.3	F:CCTGCAGACTAGAAAGGCC R:AAATGCGCTCCCTACCACTC	135
<i>CDKN2A</i>	NM_204434.1	F:GCGGAAGAAGACACCGCA R:GAATACGGCGAGCATCGGA	235
<i>CDKN2B</i>	NM_204433.2	F:GCGGGGATTAGAGGGATGTG R:TCGGACCGAGGCTTATCAGA	181
<i>CHAC1</i>	NM_001199656.2	F:TCCGGTGCAATACAAAGTGA R:ACCAATGCGAAGGTGAGC	190
<i>CPLX1</i>	XM_046905843.1	F:GAGCCATCGCCATGGATTTT R:TTGGCAGCATCAGGGTCTTT	106
<i>EGR1</i>	NM_204136.3	F:AACACGGCTACCGCCAATTA R:CGGACTGTTCTCACTGGCTT	217
<i>FREM1</i>	XM_004949102.5	F:TTATCATAGGCAGCCCGAAAG M:GGCGACAGTGGGAAAGACG	174
<i>HMBS</i>	XM_025143289.3	F:GCTTTAGCGGTGGAGGTTCG R:TGCAACAGGGACACTACAGC	144
<i>MIER3-W</i>	XM_015300440.3	F:AGGTAGACAGTGGTAATACATCCG R:TGTAGTCCTTCAGGAATTCTGCC	244
<i>MIER3W-127</i>		F:CAGGTTTATACCGCCTCCAC R:TGCGGCCTAGTCCTGCAGACACCTG	96
<i>MIER3W-3</i>		F:GAGGCACCTTGTTCCAGACTA R:TTCCATCATTTCTCTCTTCG	178
<i>MIER3W-45</i>		F:TGCTAGCGCTGTTGCTCAACC R:CCTGCCATAACTGGAATCGTG	289
<i>MIER3W-6</i>		F:GAAGGAGCACTGGGTCAGTC R:TCGAACTCCCAAAGGATGCC	125
<i>MIER3-Z</i>	XM_004937251.5	F:TCTCAAAGGGAGTACCGTGTG R:ATGCAACGATAGCGACCTCC	100
<i>MIER3-ZW</i>		F:AGGAAACATGCCGTTGGAAGA R:TCTGGAAGTTCATCTGCAAGT	117
<i>PTGS2</i>	NM_001167718.2	F:GACTCCGAAGTCAGTGCAGC R:CGAGTTCCACTTGCACGAGA	145
<i>RGS1</i>	XM_422203.8	F:GGAAAGGAGGACTGCAAGCT R:AGGAACTCCCGAAAGACACC	233
<i>TNFRSF13B</i>	NM_001097537.1	F:CACCAGGACAGGCAGACAAA R:CCGATGGAGGAACTGGGAT	190
<i>αGSU</i>	NM_001278021.1	F:ATGCAGGGTTGTCCAGAGTG R:AGCACGTTGCTTCCGATGTA	169
<i>β-Actin</i>	NM_205518.2	F:TATGTGCAAGGCCGGTTTC R:TGTCTTTCTGGCCCATACCAA R:TCGAACTCCCAAAGGATGCC	110

Table S2 Down regulated genes in gonadal cells transfected with *MIER3*-Pex2

Name	Description	<i>p</i> -Value	Fold Change
<i>PTGDS</i>	prostaglandin D2 synthase 21kDa	0.0032	0.29
<i>TGM6</i>	transglutaminase 6	0.0032	0.32
<i>NSUN7</i>	NOP2/Sun RNA methyltransferase family member 7	0.0061	0.48
<i>USHBP1</i>	USH1 protein network component harmonin binding protein 1	0.0084	0.09
<i>EGR1</i>	early growth response 1	0.0111	0.32
<i>HAL</i>	histidine ammonia-lyase	0.0138	0.46
<i>FREMI</i>	FRAS1 related extracellular matrix 1	0.0145	0.39
<i>MSMP</i>	microseminoprotein, prostate associated	0.0165	0.14
<i>TNFRSF13B</i>	TNF receptor superfamily member 13B	0.0249	0.34
<i>TNFRSF18</i>	TNF receptor superfamily member 18	0.0428	0.47
<i>CAMK2A</i>	calcium/calmodulin dependent protein kinase II alpha	0.0474	0.35
<i>ACOT12</i>	acyl-CoA thioesterase 12	0.0495	0.49

Table S3 Up regulated genes in gonadal cells transfected with *MIER3*-Pex2

Name	Description	<i>p</i> -Value	Fold Change
<i>CPLX1</i>	complexin 1	< 0.001	1.68
<i>CCL4</i>	C-C motif chemokine ligand 4	< 0.001	2.7
<i>PTGS2</i>	prostaglandin-endoperoxide synthase 2	< 0.001	1.99
<i>CDKN2A</i>	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	< 0.001	1.91
<i>CDKN2B</i>	cyclin dependent kinase inhibitor 2B	< 0.001	1.85
<i>PCASP2</i>	paracaspase 2	0.0011	11.51
<i>CSF3</i>	colony stimulating factor 3	0.0105	2.04
<i>NFASC</i>	neurofascin	0.0145	2.26
<i>DCAF11</i>	DDB1 and CUL4 associated factor 11	0.0197	5.18
<i>CDH5</i>	cadherin 5	0.0232	2.07
<i>EN2</i>	engrailed homeobox 2	0.0374	9.11
<i>HSPB3</i>	heat shock protein family B (small) member 3	0.0464	2.33

A

Species/Abbrev	Group Name	A	A	A	G	C	T
1. MIER3-human		T	I	P	V	M	S
2. MIER3-Z1		T	I	P	V	M	S
3. MIER3-Z2		T	I	P	V	M	S
4. MIER3-Z3		T	I	P	V	M	S
5. MIER3-Z4		T	I	P	V	M	S
6. MIER3-Z5		T	I	P	V	M	S
7. MIER3-Z6		T	I	P	V	M	S
8. MIER3-W1		T	I	P	V	M	S
9. MIER3-W2		T	I	P	V	M	S
10. MIER3-W3		T	I	P	V	M	S
11. MIER3-W5		T	I	P	V	M	S
12. MIER3-W4		T	I	P	V	M	S
13. MIER3-W6		T	I	P	V	M	S
14. MIER3-W7		T	I	P	V	M	S

B

[illegible]

C

[illegible]

Figure|S2

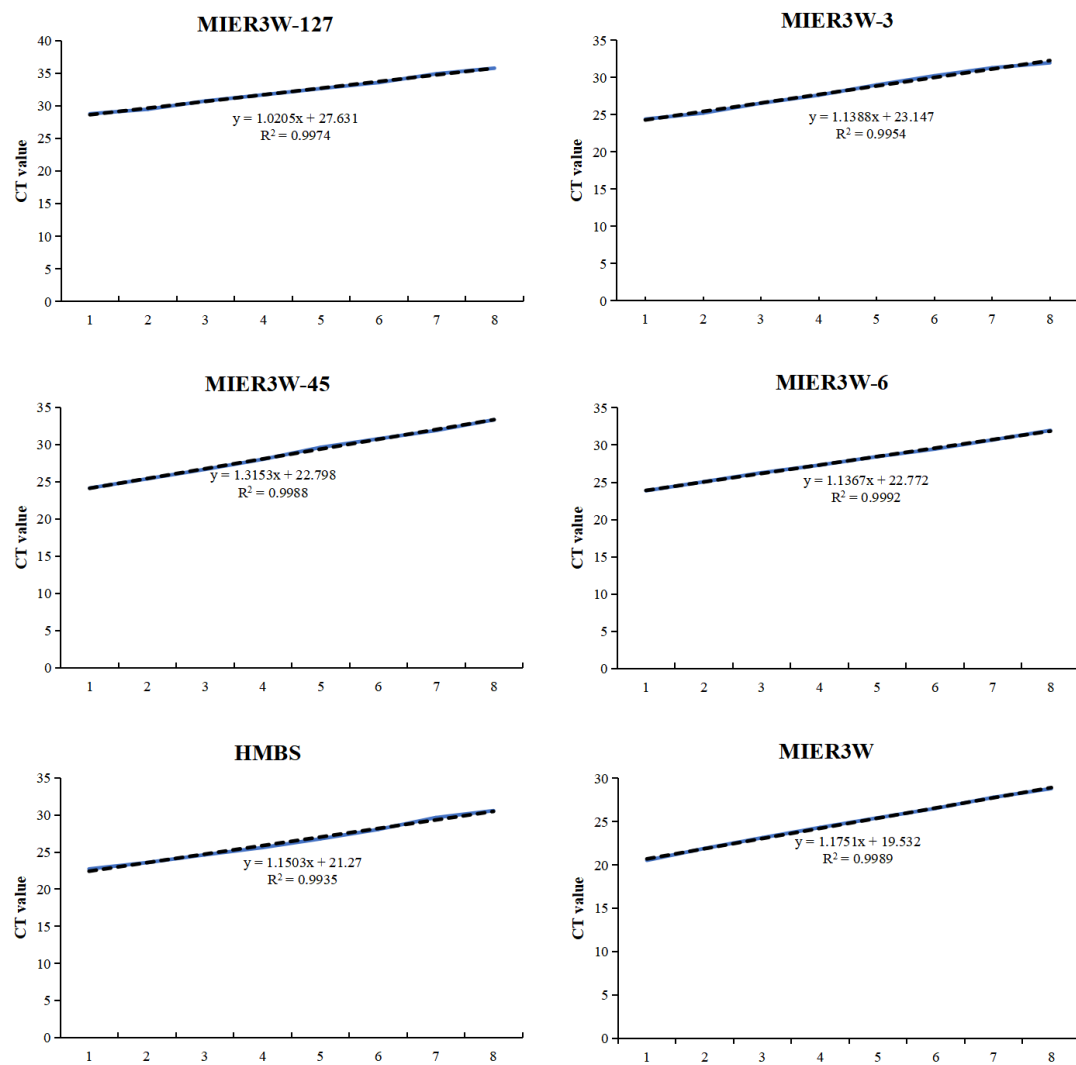


Figure S3

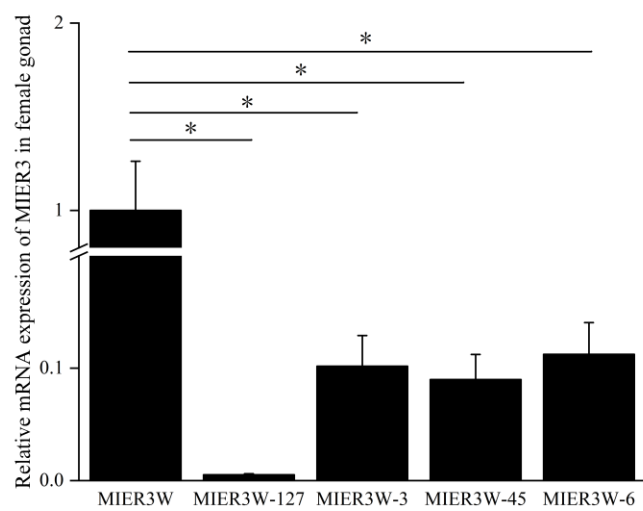


Figure S4

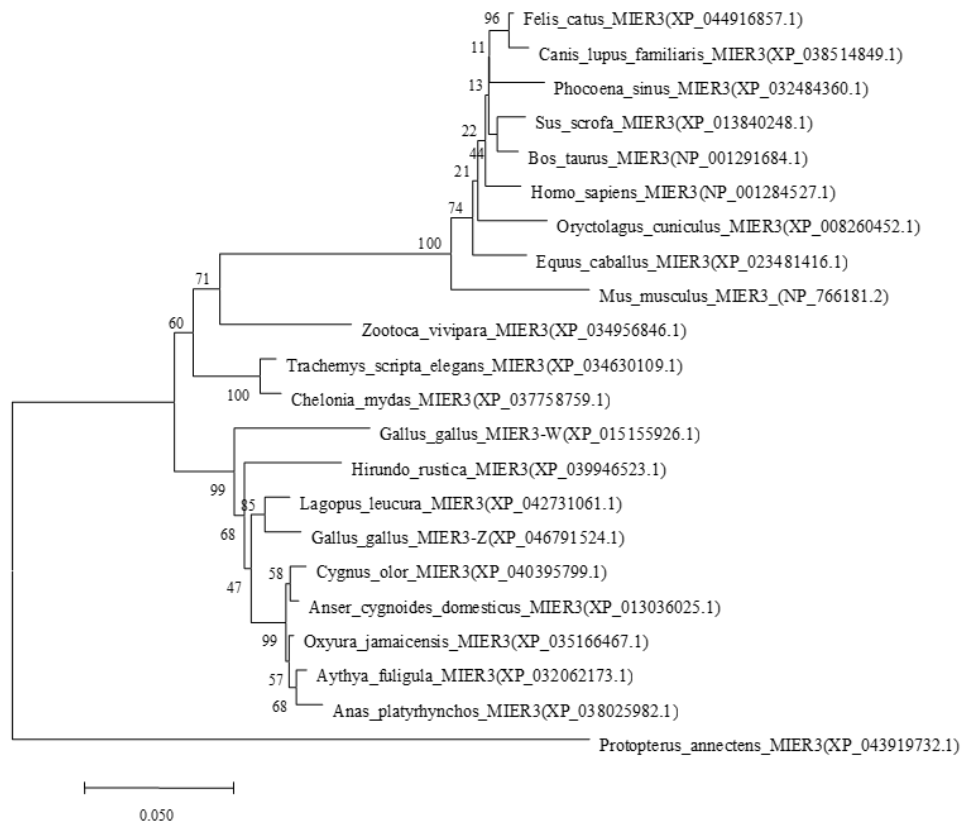
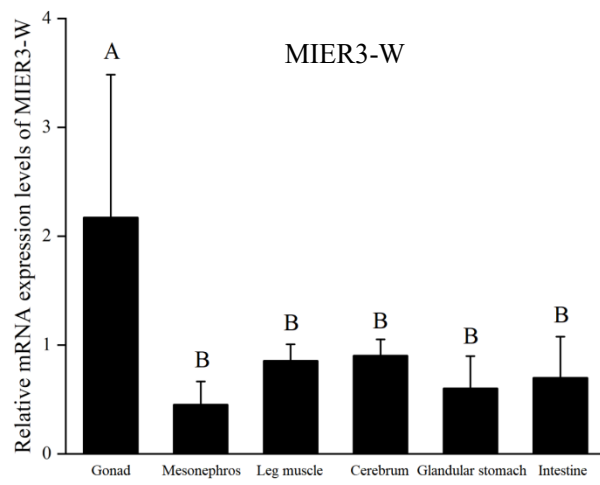
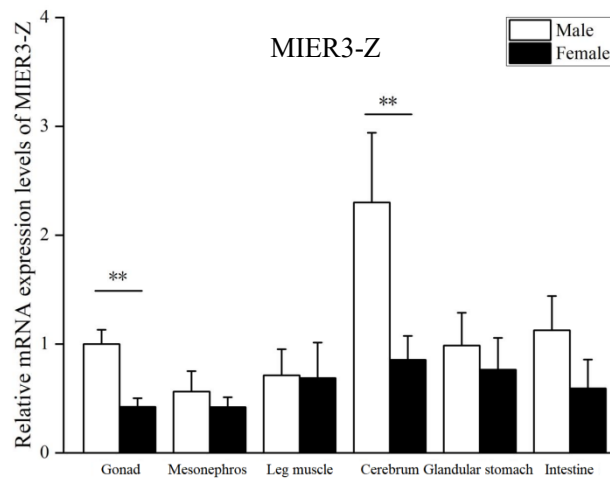


Figure S5

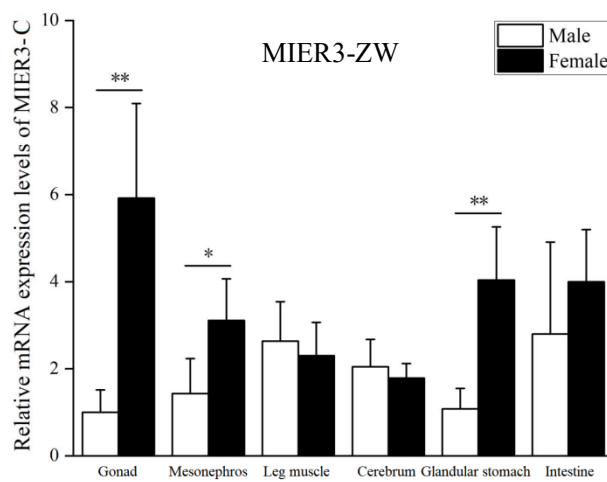
A



B



C



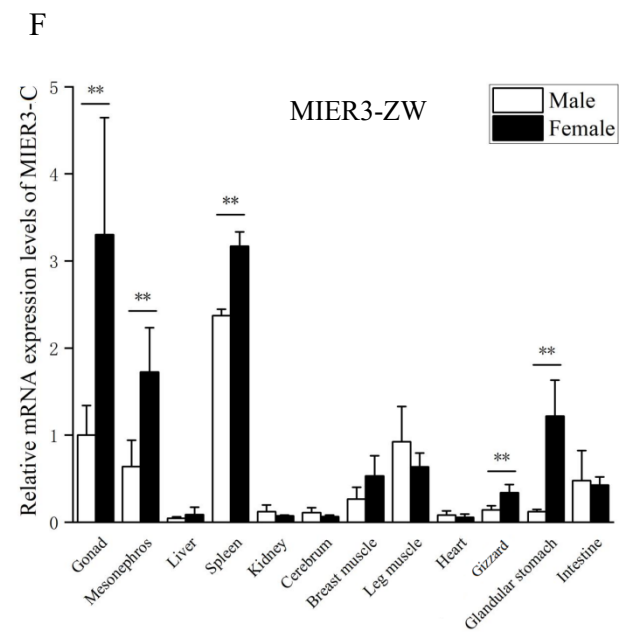
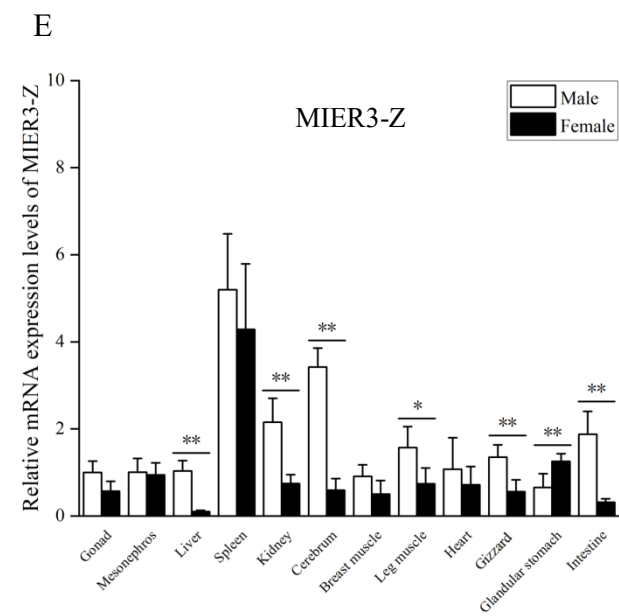
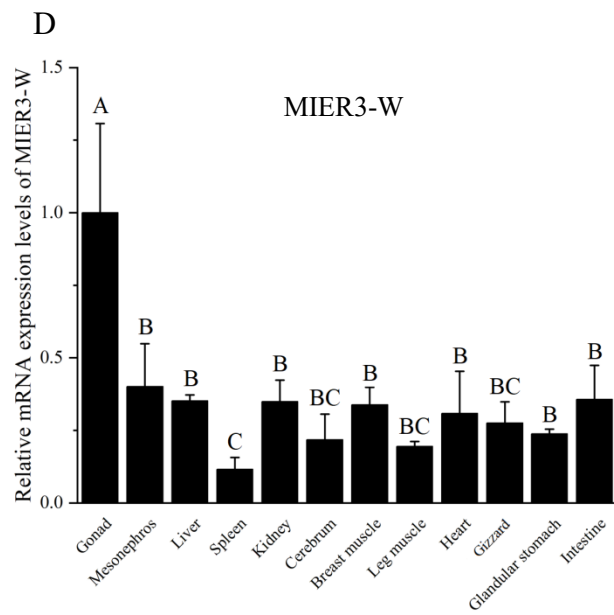


Figure S6

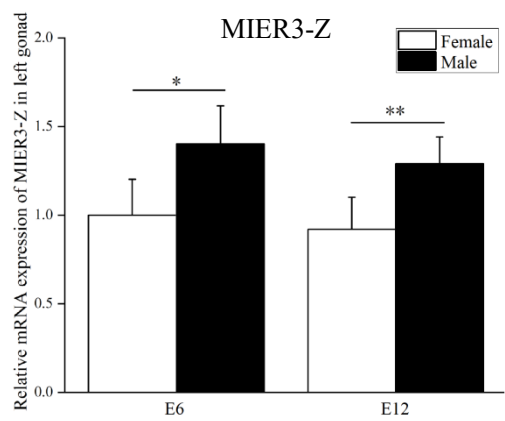
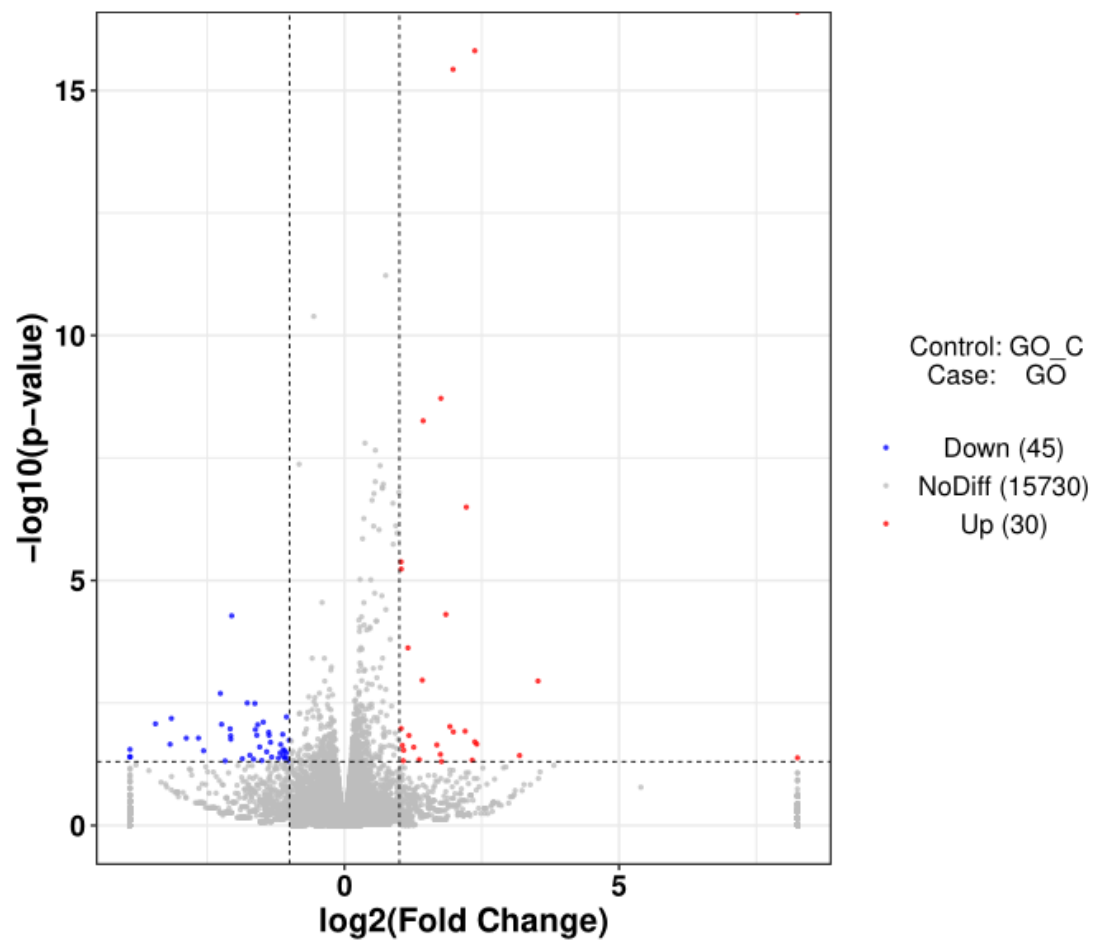
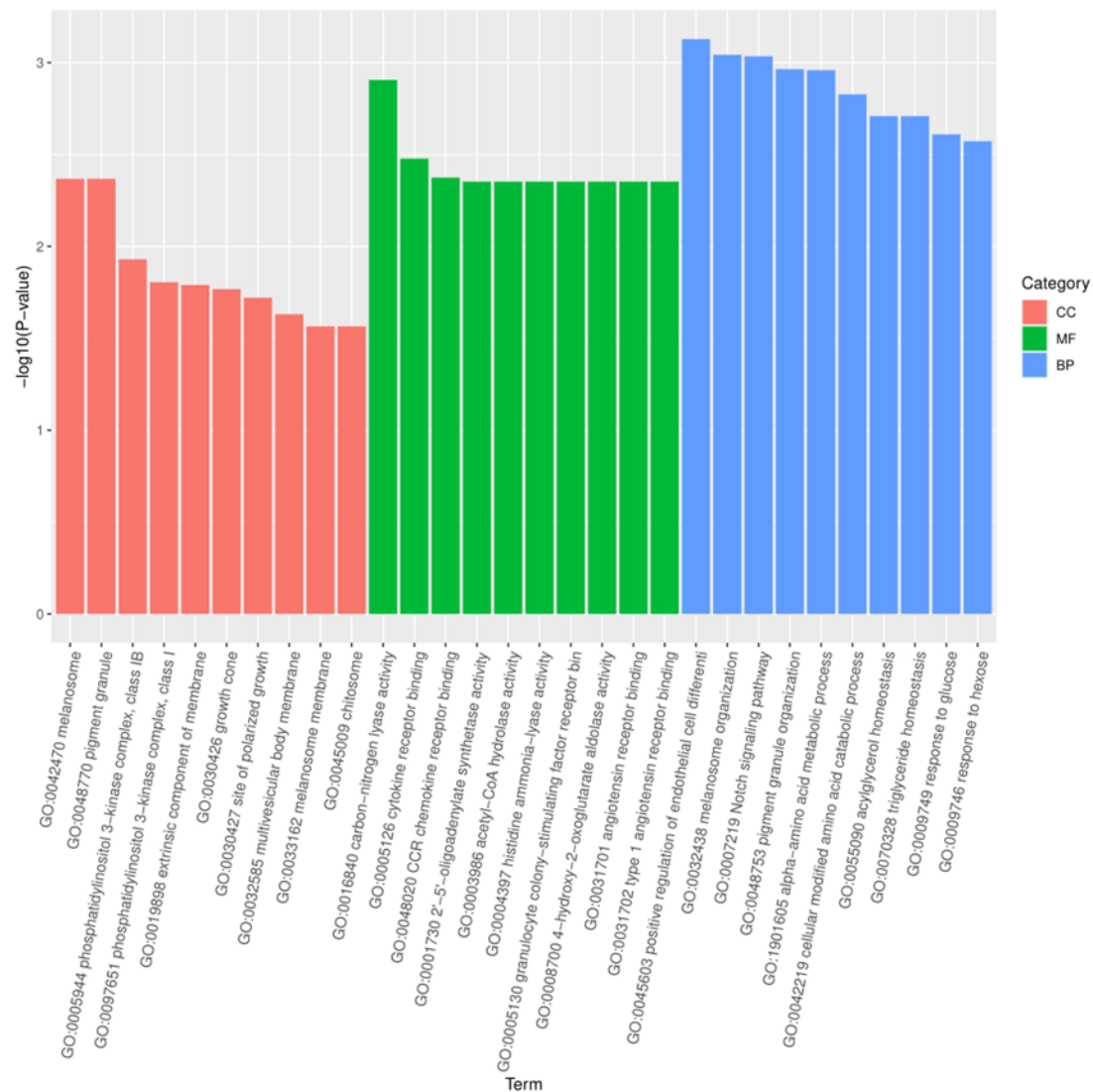


Figure S7



Note: The abscissa is $\log_2(\text{Fold Change})$, the ordinate is $-\log_{10}(P\text{-value})$, and the blue dotted line indicates the threshold line of screening criteria for differentially expressed genes. The red and green dots indicate the genes with upregulated and downregulated expression in Chicken embryo male gonad cells transfected with *MIER3* overexpression vector vs. empty vector, respectively.

Figure S8



Note: The coordination shows GO term on the abscissa and the significance of enrichment on the ordinate.

The significance of enrichment is indicated by $-\log_{10}(\text{padj})$. Padj denotes adjusted p value. Different colors represent different categories of GO term, including biological process (BP), cellular component (CC) and molecular function (MF).