Supplementary material to: Wang Z.-L., Li C.-C., Xiao F., Zhu J.-Y., Zhang H.-R., Zhang S.-S., Xia F.-F., Xiao R. & Liu T.-X. 2026: Expression and function of two olfactory-related genes in response to methyl salicylate in the wolf spider *Pardosa pseudoannulata* (Araneae: Lycosidae). — *Eur. J. Entomol.* 123: 233–247.

Figs S1–S11, Tables S1–S4, S6, S7.

Fig. S1. Venn diagram of expressed genes in the four sample groups.

Fig. S2. Transcriptome validation of female samples. Gray bars represent the FPKM results for the transcriptome, while green bars indicate the relative gene expression levels. Each value represents the mean ± SE of three replicates (n = 3).

Fig. S3. Transcriptome validation of male samples. Gray bars represent the FPKM results for the transcriptome, while green bars show the relative gene expression levels. Each value represents the mean ± SE of three replicates (n = 3).

Fig. S4. A volcano plot for differential gene expression. Green represents downregulated expression, red represents upregulated expression, and black represents genes with no significant difference in expression. (A) The results for females, and (B) the results for males.

Fig. S5. COG annotation classification chart of DEGs in *Pardosa pseudoannulata* (A) females and (B) males.

Fig. S6. GO annotation classification chart of DEGs in *Pardosa pseudoannulata* (A) females and (B) males.

Fig. S7. KEGG annotation classification chart of DEGs in *Pardosa pseudoannulata* (A) females and (B) males.

Fig. S8. cDNA sequences and amino acid sequences of *PpseNPC2-7* genes in *Pardosa pseudoannulata.* The red marking indicates the signal peptide, the gray marking indicates the ML (MD-2-related lipid-recognition) domain (122 aa), and the blue marking indicates

the start and stop codons.

Fig. S9. cDNA sequences and amino acid sequences of *PpseSNMP2* in *Pardosa pseudoannulata.* The gray marking indicates the CD36 domain (462 aa) and the blue marking indicates the start and stop codons.

Fig. S10. Electrophysiological responses of female *Pardosa pseudoannulata* to methyl salicylate exposure. Electrophysiological responses of female spider (A) pedipalps, (C) leg I, and (E) leg II to methyl salicylate following ds*GFP* injection, and (B) pedipalps, (D) leg I, and (F) leg II following ds*NPC2-7* injection.

Fig. S11. Electrophysiological responses of male *Pardosa pseudoannulata* to methyl salicylate exposure. Electrophysiological responses of male spider (A) pedipalps, (C) leg I, and (E) leg II to methyl salicylate following ds*GFP* injection, and (B) pedipalps, (D) leg I, and (F) leg II following ds*SNMP2* injection.

Table S1. Primer sequences utilized for quantitative real-time PCR. F, Forward primer; R, Reverse primer.

Table S2. Primer information of *PpseNPC2-7* and *PpseSNMP2*. F, Forward primer; R, Reverse primer.

Table S3. dsRNA primers of olfactory-related genes in *Pardosa pseudoannulata*. The underlined part of the table is the T7 sequence, the upstream primer is F, and the downstream primer is R.

Table S4. Statistical analysis of clean data for each sample from the Illumina sequence.

Table S5. See Supplement S2. Annotation of DEGs in the CK-F vs. MeSA-F and CK-M vs. MeSA-M.

Table S6. Differential expression of olfactory-related genes in male and female spiders.

Table S7. AlphaFold3 prediction scores for olfactory-related proteins.

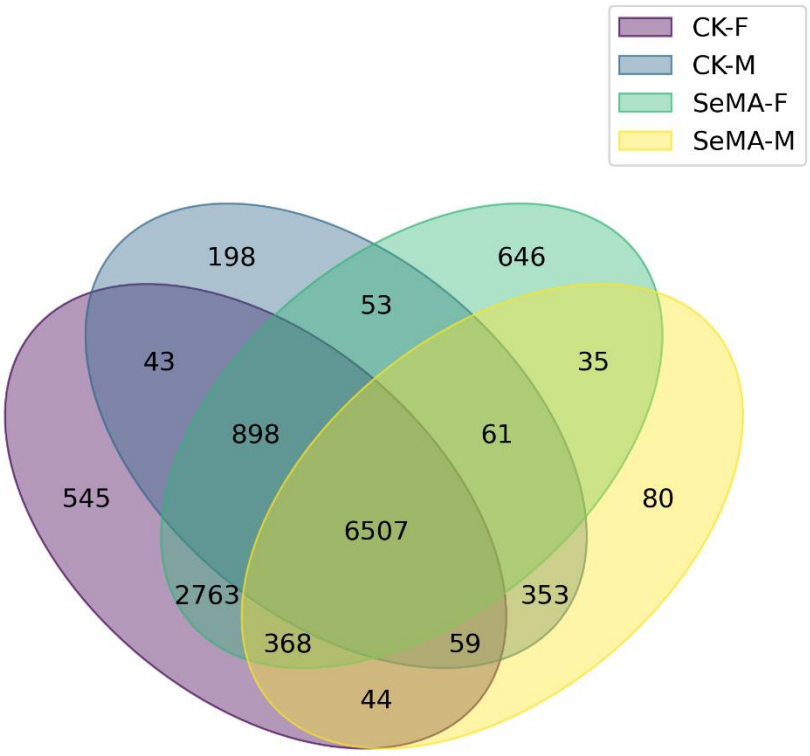

CK-F
CK-M
SeMA-F
SeMA-M
198
646
53
43
35
898
61
545
80
6507
2763
353
368
59
44

Fig. S1

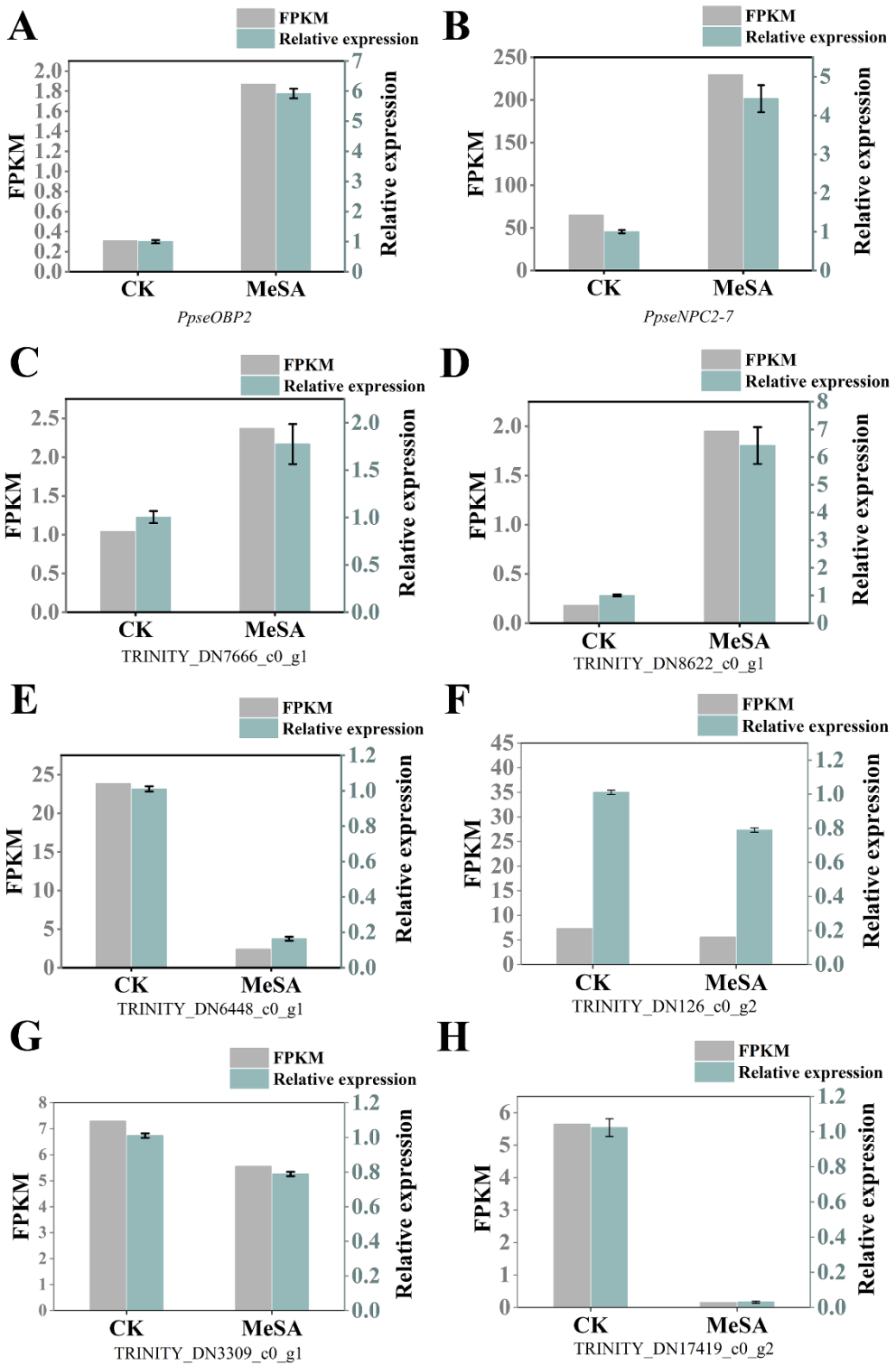

A
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
PpseOBP2
B
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
PpseNPC2-7
C
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
TRINITY_DN7666_c0_g1
D
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
TRINITY_DN8622_c0_g1
E
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
TRINITY_DN6448_c0_g1
F
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
TRINITY_DN126_c0_g2
G
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
TRINITY_DN3309_c0_g1
H
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
TRINITY_DN17419_c0_g2

Fig. S2

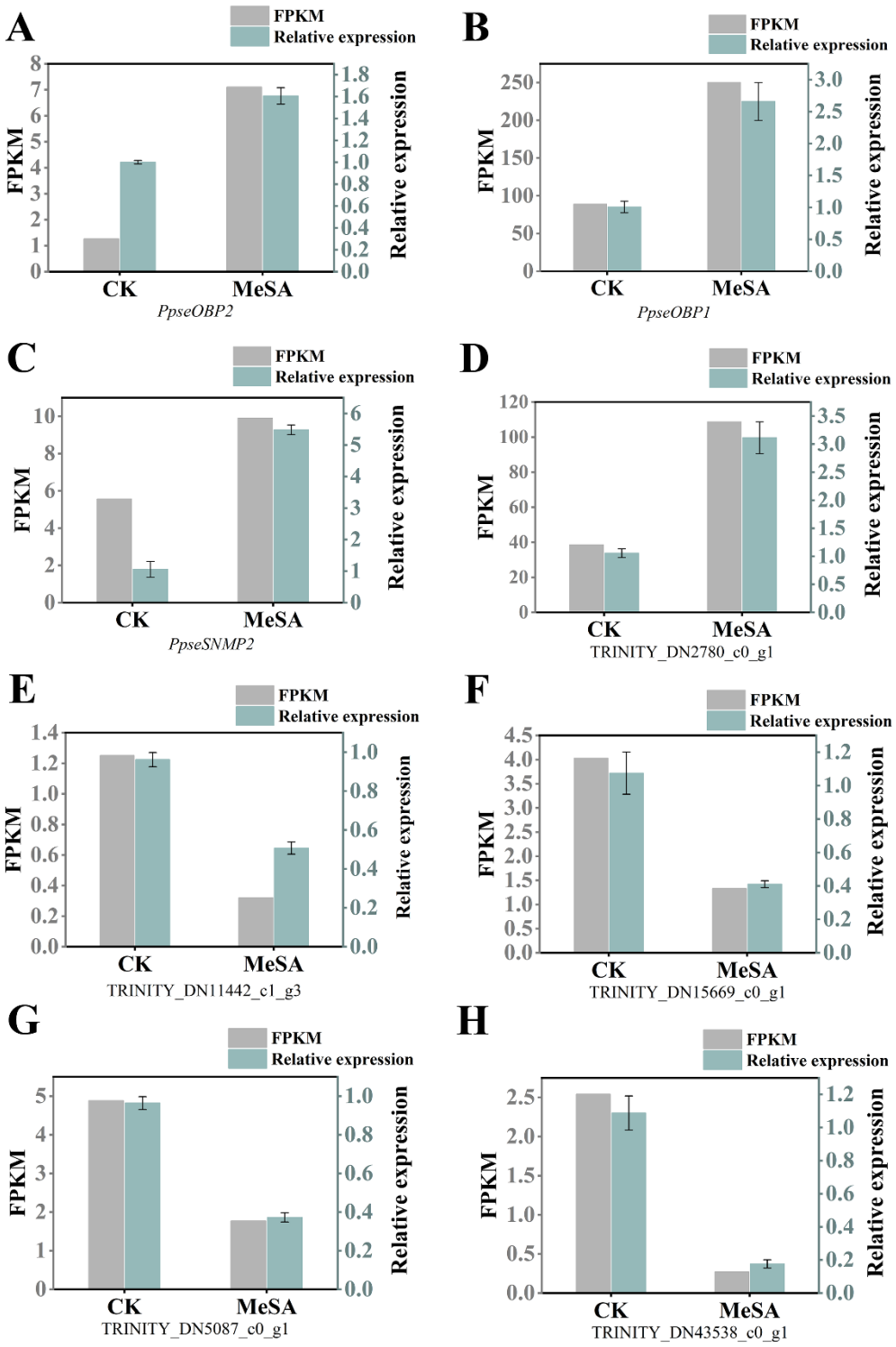

A
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
PpseOBP2
B
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
PpseOBP1
C
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
PpseSNMP2
D
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
TRINITY_DN2780_c0_g1
E
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
TRINITY_DN11442_c1_g3
F
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
TRINITY_DN15669_c0_g1
G
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
TRINITY_DN5087_c0_g1
H
FPKM
Relative expression
FPKM
Relative expression
CK
MeSA
TRINITY_DN43538_c0_g1

Fig. S3

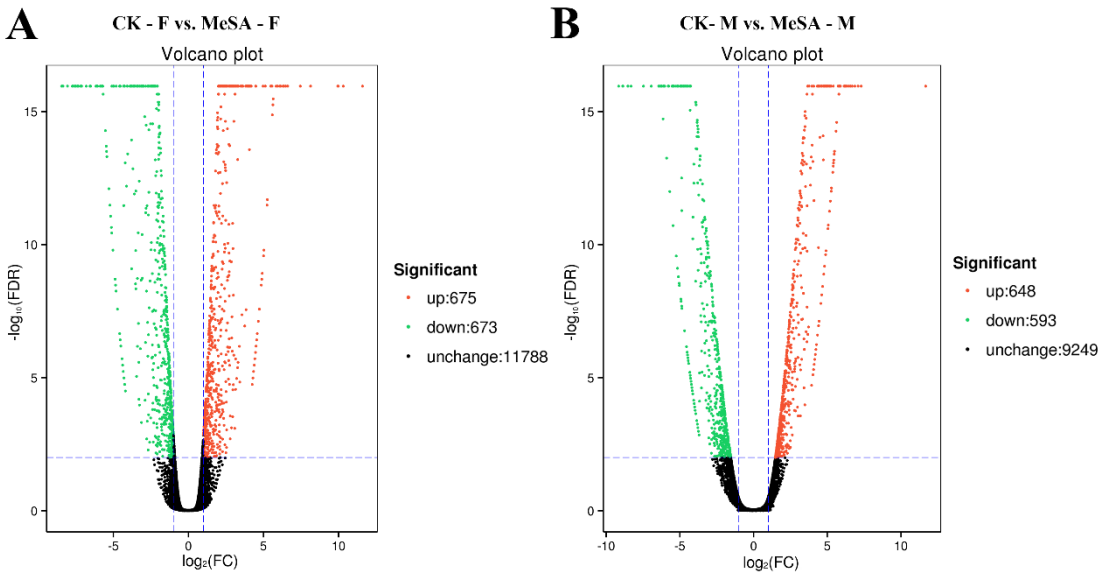
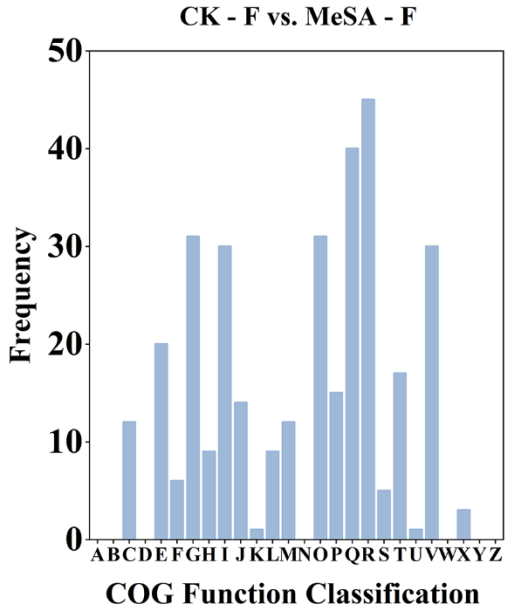

A
CK - F vs. MeSA - F
Volcano plot
-log10(FDR)
log2(FC)
Significant
up:675
down:673
unchange:11788
B
CK- M vs. MeSA - M
Volcano plot
-log10(FDR)
log2(FC)
Significant
up:648
down:593
unchange:9249

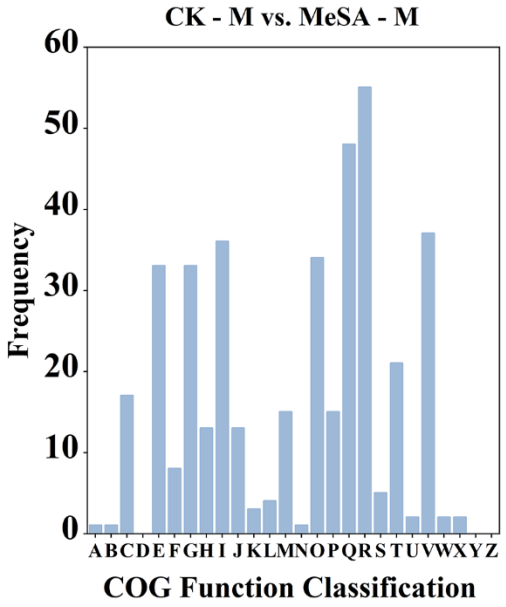
Fig. S4

A



A: RNA processing and modification[0 ~ 0.0%]
B: Chromatin structure and dynamics[0 ~ 0.0%]
C: Energy production and conversion[12 ~ 3.63%]
D: Cell cycle control, cell division, chromosome partitioning[0 ~ 0.0%]
E: Amino acid transport and metabolism[20 ~ 6.04%]
F: Nucleotide transport and metabolism [6 ~ 1.81%]
G: Carbohydrate transport and metabolism[31 ~ 9.37%]
H: Coenzyme transport and metabolism[9 ~ 2.72%]
I: Lipid transport and metabolism[30 ~ 9.06%]
J: Translation, ribosomal structure and biogenesis[14 ~ 4.23%]
K: Transcription[1 ~ 0.3%]
L: Replication, recombination and repair[9 ~ 2.72%]
M: Cell wall/membrane/envelope biogenesis[12 ~ 3.63%]
N: Cell motility[0 ~ 0.0%]
O: Posttranslational modification, protein turnover, chaperones[31 ~ 9.37%]
P: Inorganic ion transport and metabolism[15 ~ 4.53%]
Q: Secondary metabolites biosynthesis, transport and catabolism[40 ~ 12.08%]
R: General function prediction only[45 ~ 13.6%]
S: Function unknown[5 ~ 1.51%]
T: Signal transduction mechanisms[17 ~ 5.14%]
U: Intracellular trafficking, secretion, and vesicular transport[1 ~ 0.3%]
V: Defense mechanisms[30 ~ 9.06%]
W: Extracellular structures[0 ~ 0.0%]
X: Mobilome: prophages, transposons[3 ~ 0.91%]
Y: Nuclear structure[0 ~ 0.0%]
Z: Cytoskeleton[0 ~ 0.0%]

B



A: RNA processing and modification [1 ~ 0.25%]
B: Chromatin structure and dynamics [1 ~ 0.25%]
C: Energy production and conversion [17 ~ 4.26%]
D: Cell cycle control, cell division, chromosome partitioning [0 ~ 0%]
E: Amino acid transport and metabolism [33 ~ 8.27%]
F: Nucleotide transport and metabolism [8 ~ 2.01%]
G: Carbohydrate transport and metabolism [33 ~ 8.27%]
H: Coenzyme transport and metabolism [13 ~ 3.26%]
I: Lipid transport and metabolism [36 ~ 9.02%]
J: Translation, ribosomal structure and biogenesis [13 ~ 3.26%]
K: Transcription [3 ~ 0.75%]
L: Replication, recombination and repair [4 ~ 1%]
M: Cell wall/membrane/envelope biogenesis [15 ~ 3.76%]
N: Cell motility [1~0.25%]
O: Posttranslational modification, protein turnover, chaperones [34 ~ 8.52%]
P: Inorganic ion transport and metabolism [15 ~ 3.76%]
Q: Secondary metabolites biosynthesis, transport and catabolism [48 ~ 12.03%]
R: General function prediction only [55 ~ 13.78%]
S: Function unknown [5 ~ 1.25%]
T: Signal transduction mechanisms [21 ~ 5.26%]
U: Intracellular trafficking, secretion, and vesicular transport [2 ~ 0.5%]
V: Defense mechanisms [37 ~ 9.27%]
W: Extracellular structures [2 ~ 0.5%]
X: Mobilome: prophages, transposons [2 ~ 0.5%]
Y: Nuclear structure [0 ~ 0%]
Z: Cytoskeleton [0 ~ 0%]

Fig. S5

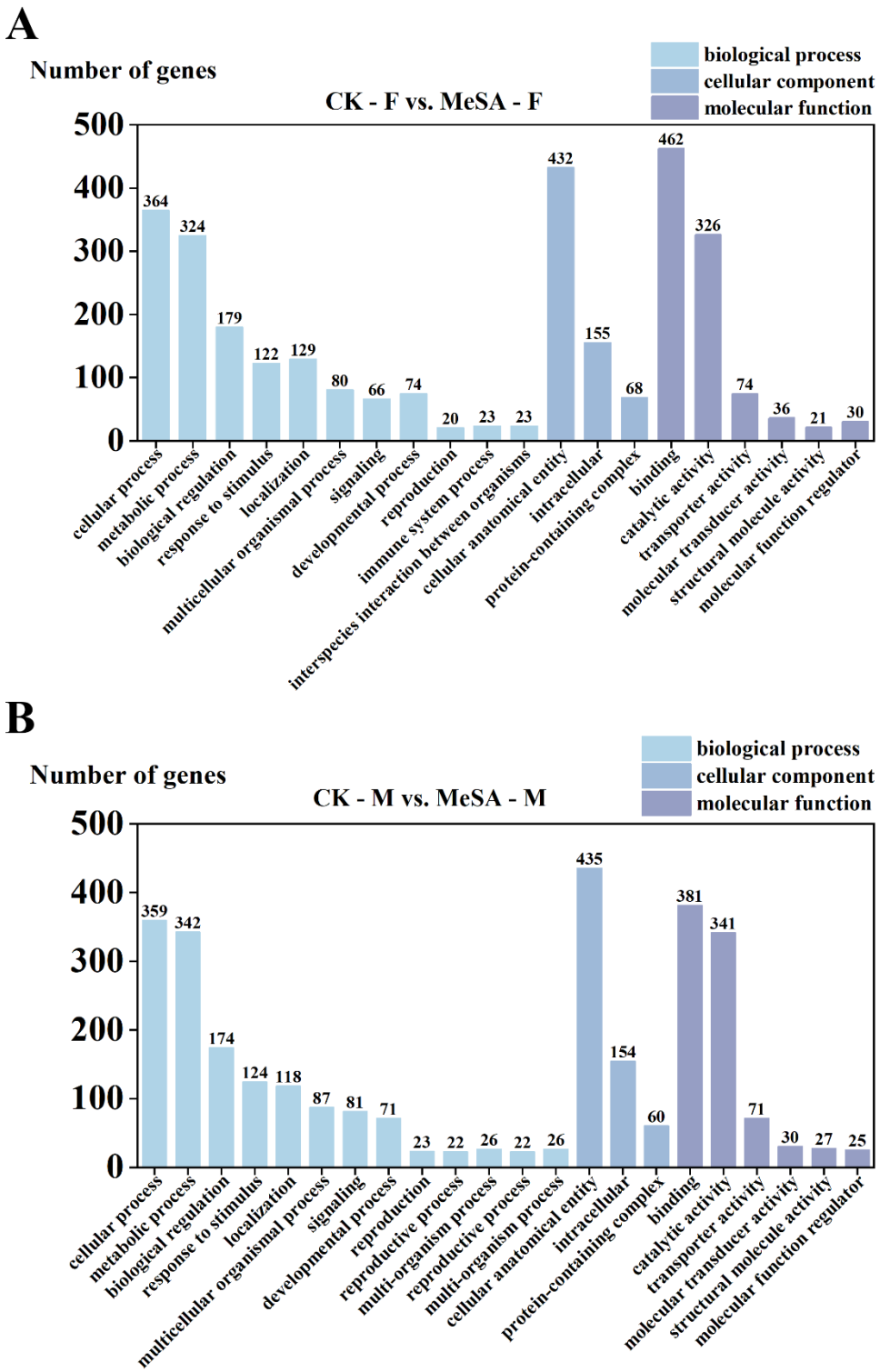
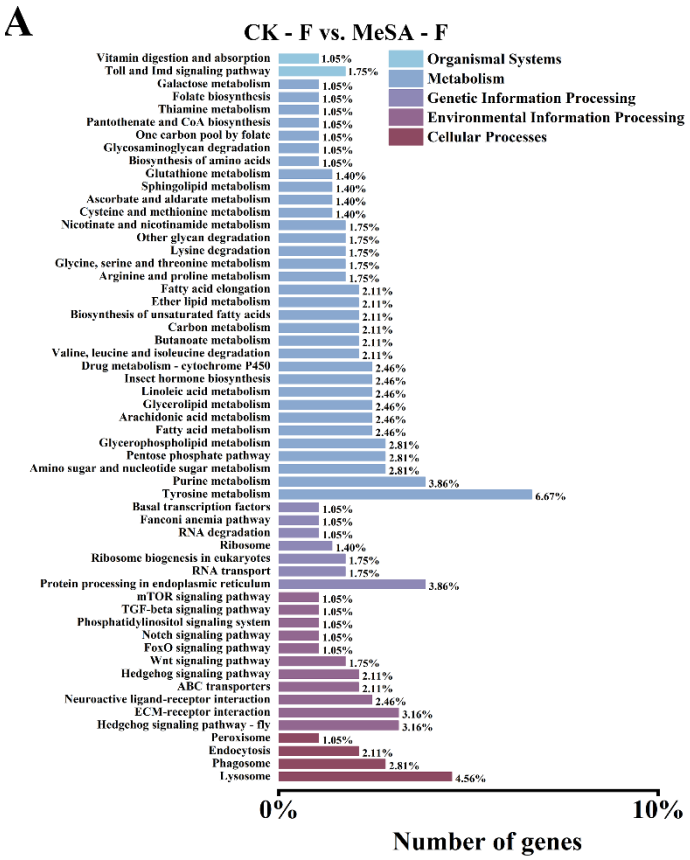

A
Number of genes
CK - F vs. MeSA - F
biological process
cellular component
molecular function
500
400
300
200
100
0
364
324
179
122
129
80
66
74
20
23
23
432
155
68
462
326
74
36
21
30
cellular process
metabolic process
biological regulation
response to stimulus
localization
multicellular organismal process
signaling
developmental process
reproduction
immune system process
interspecies interaction between organisms
cellular anatomical entity
intracellular
protein-containing complex
binding
catalytic activity
transporter activity
molecular transducer activity
structural molecule activity
molecular function regulator
B
Number of genes
CK - M vs. MeSA - M
biological process
cellular component
molecular function
500
400
300
200
100
0
359
342
174
124
118
87
81
71
23
22
26
22
26
435
154
60
381
341
71
30
27
25
cellular process
metabolic process
biological regulation
response to stimulus
localization
multicellular organismal process
signaling
developmental process
reproduction
reproductive process
multi-organism process
reproductive process
multi-organism process
cellular anatomical entity
intracellular
protein-containing complex
binding
catalytic activity
transporter activity
molecular transducer activity
structural molecule activity
molecular function regulator

Fig. S6


A
CK - F vs. MeSA - F
Organismal Systems
Metabolism
Genetic Information Processing
Environmental Information Processing
Cellular Processes
Vitamin digestion and absorption 1.05%
Toll and Imd signaling pathway 1.75%
Galactose metabolism 1.05%
Folate biosynthesis 1.05%
Thiamine metabolism 1.05%
Pantothenate and CoA biosynthesis 1.05%
One carbon pool by folate 1.05%
Glycosaminoglycan degradation 1.05%
Biosynthesis of amino acids 1.05%
Glutathione metabolism 1.40%
Sphingolipid metabolism 1.40%
Ascorbate and aldarate metabolism 1.40%
Cysteine and methionine metabolism 1.40%
Nicotinate and nicotinamide metabolism 1.75%
Other glycan degradation 1.75%
Lysine degradation 1.75%
Glycine, serine and threonine metabolism 1.75%
Arginine and proline metabolism 1.75%
Fatty acid elongation 2.11%
Ether lipid metabolism 2.11%
Biosynthesis of unsaturated fatty acids 2.11%
Carbon metabolism 2.11%
Butanoate metabolism 2.11%
Valine, leucine and isoleucine degradation 2.11%
Drug metabolism - cytochrome P450 2.46%
Insect hormone biosynthesis 2.46%
Linoleic acid metabolism 2.46%
Glycerolipid metabolism 2.46%
Arachidonic acid metabolism 2.46%
Fatty acid metabolism 2.46%
Glycerophospholipid metabolism 2.81%
Pentose phosphate pathway 2.81%
Amino sugar and nucleotide sugar metabolism 2.81%
Purine metabolism 3.86%
Tyrosine metabolism 6.67%
Basal transcription factors 1.05%
Fanconi anemia pathway 1.05%
RNA degradation 1.05%
Ribosome 1.40%
Ribosome biogenesis in eukaryotes 1.75%
RNA transport 1.75%
Protein processing in endoplasmic reticulum 3.86%
mTOR signaling pathway 1.05%
TGF-beta signaling pathway 1.05%
Phosphatidylinositol signaling system 1.05%
Notch signaling pathway 1.05%
FoxO signaling pathway 1.05%
Wnt signaling pathway 1.75%
Hedgehog signaling pathway 2.11%
ABC transporters 2.11%
Neuroactive ligand-receptor interaction 2.46%
ECM-receptor interaction 3.16%
Hedgehog signaling pathway - fly 3.16%
Peroxisome 1.05%
Endocytosis 2.11%
Phagosome 2.81%
Lysosome 4.56%
0%
10%
Number of genes

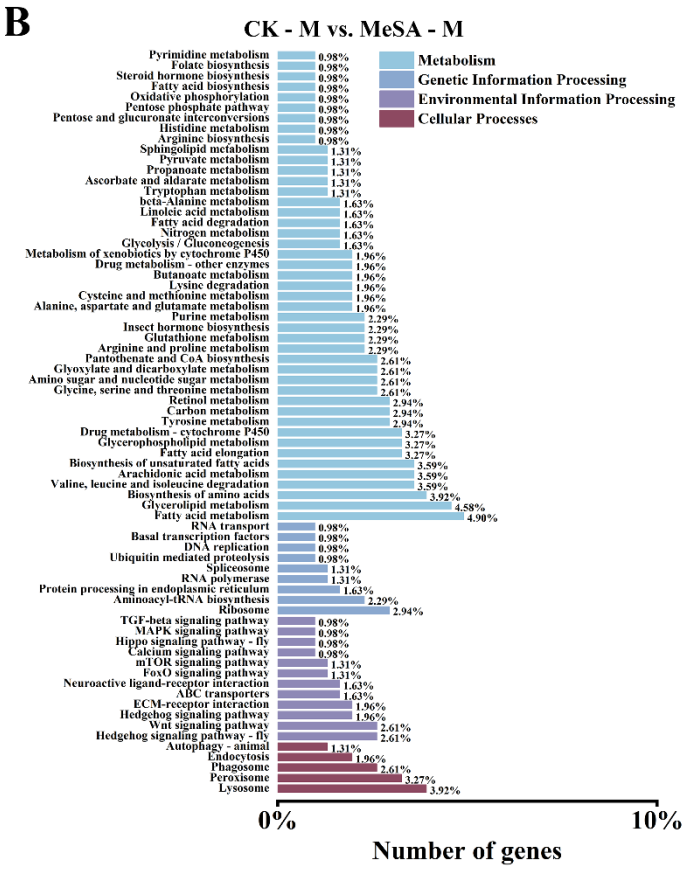
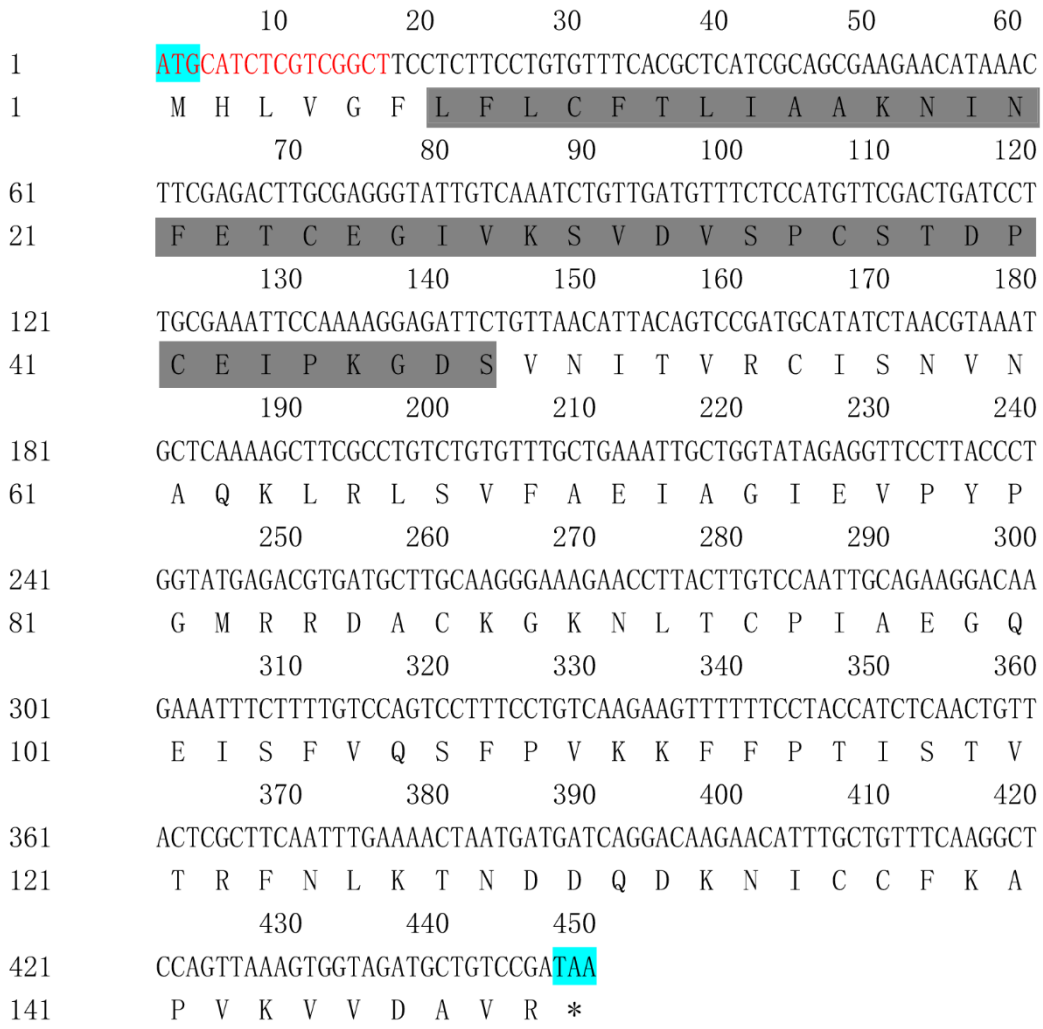

B
CK - M vs. MeSA - M
Metabolism
Genetic Information Processing
Environmental Information Processing
Cellular Processes
Pyrimidine metabolism 0.98%
Folate biosynthesis 0.98%
Steroid hormone biosynthesis 0.98%
Fatty acid biosynthesis 0.98%
Oxidative phosphorylation 0.98%
Pentose phosphate pathway 0.98%
Pentose and glucuronate interconversions 0.98%
Histidine metabolism 0.98%
Arginine biosynthesis 0.98%
Sphingolipid metabolism 1.31%
Pyruvate metabolism 1.31%
Propanoate metabolism 1.31%
Ascorbate and aldarate metabolism 1.31%
Tryptophan metabolism 1.31%
beta-Alanine metabolism 1.63%
Linoleic acid metabolism 1.63%
Fatty acid degradation 1.63%
Nitrogen metabolism 1.63%
Glycolysis / Gluconeogenesis 1.63%
Metabolism of xenobiotics by cytochrome P450 1.96%
Drug metabolism - other enzymes 1.96%
Butanoate metabolism 1.96%
Lysine degradation 1.96%
Cysteine and methionine metabolism 1.96%
Alanine, aspartate and glutamate metabolism 1.96%
Purine metabolism 2.29%
Insect hormone biosynthesis 2.29%
Glutathione metabolism 2.29%
Arginine and proline metabolism 2.29%
Pantothenate and CoA biosynthesis 2.61%
Glyoxylate and dicarboxylate metabolism 2.61%
Amino sugar and nucleotide sugar metabolism 2.61%
Glycine, serine and threonine metabolism 2.61%
Retinol metabolism 2.94%
Carbon metabolism 2.94%
Tyrosine metabolism 2.94%
Drug metabolism - cytochrome P450 3.27%
Glycerophospholipid metabolism 3.27%
Fatty acid elongation 3.27%
Biosynthesis of unsaturated fatty acids 3.59%
Arachidonic acid metabolism 3.59%
Valine, leucine and isoleucine degradation 3.59%
Biosynthesis of amino acids 3.92%
Glycerolipid metabolism 4.58%
Fatty acid metabolism 4.90%
RNA transport 0.98%
Basal transcription factors 0.98%
DNA replication 0.98%
Ubiquitin mediated proteolysis 0.98%
Spliceosome 1.31%
RNA polymerase 1.31%
Protein processing in endoplasmic reticulum 1.63%
Aminoacyl-tRNA biosynthesis 2.29%
Ribosome 2.94%
TGF-beta signaling pathway 0.98%
MAPK signaling pathway 0.98%
Hippo signaling pathway - fly 0.98%
Calcium signaling pathway 0.98%
mTOR signaling pathway 1.31%
FoxO signaling pathway 1.31%
Neuroactive ligand-receptor interaction 1.63%
ABC transporters 1.63%
ECM-receptor interaction 1.96%
Hedgehog signaling pathway 1.96%
Wnt signaling pathway 2.61%
Hedgehog signaling pathway - fly 2.61%
Autophagy - animal 1.31%
Endocytosis 1.96%
Phagosome 2.61%
Peroxisome 3.27%
Lysosome 3.92%
0%
10%
Number of genes

Fig. S7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70 80 90 100 110 120
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21 F E T C E G I V K S V D V S P C S T D P
130 140 150 160 170 180
121 TGCGAAATTCCAAAAGGAGATTCTGTTAACATTACAGTCCGATGCATATCTAACGTAAAT
41 C E I P K G D S V N I T V R C I S N V N
190 200 210 220 230 240
181 GCTCAAAAGCTTCGCCTGTCTGTGTTTGCTGAAATTGCTGGTATAGAGGTTCCTTACCCT
61 A Q K L R L S V F A E I A G I E V P Y P
250 260 270 280 290 300
241 GGTATGAGACGTGATGCTTGCAAGGGAAAGAACCTTACTTGTCCAATTGCAGAAGGACAA
81 G M R R D A C K G K N L T C P I A E G Q
310 320 330 340 350 360
301 GAAATTTCTTTTGTCCAGTCCTTTCCTGTCAAGAAGTTTTTTCCTACCATCTCAACTGTT
101 E I S F V Q S F P V K K F F P T I S T V
370 380 390 400 410 420
361 ACTCGCTTCAATTTGAAAACTAATGATGATCAGGACAAGAACATTTGCTGTTTCAAGGCT
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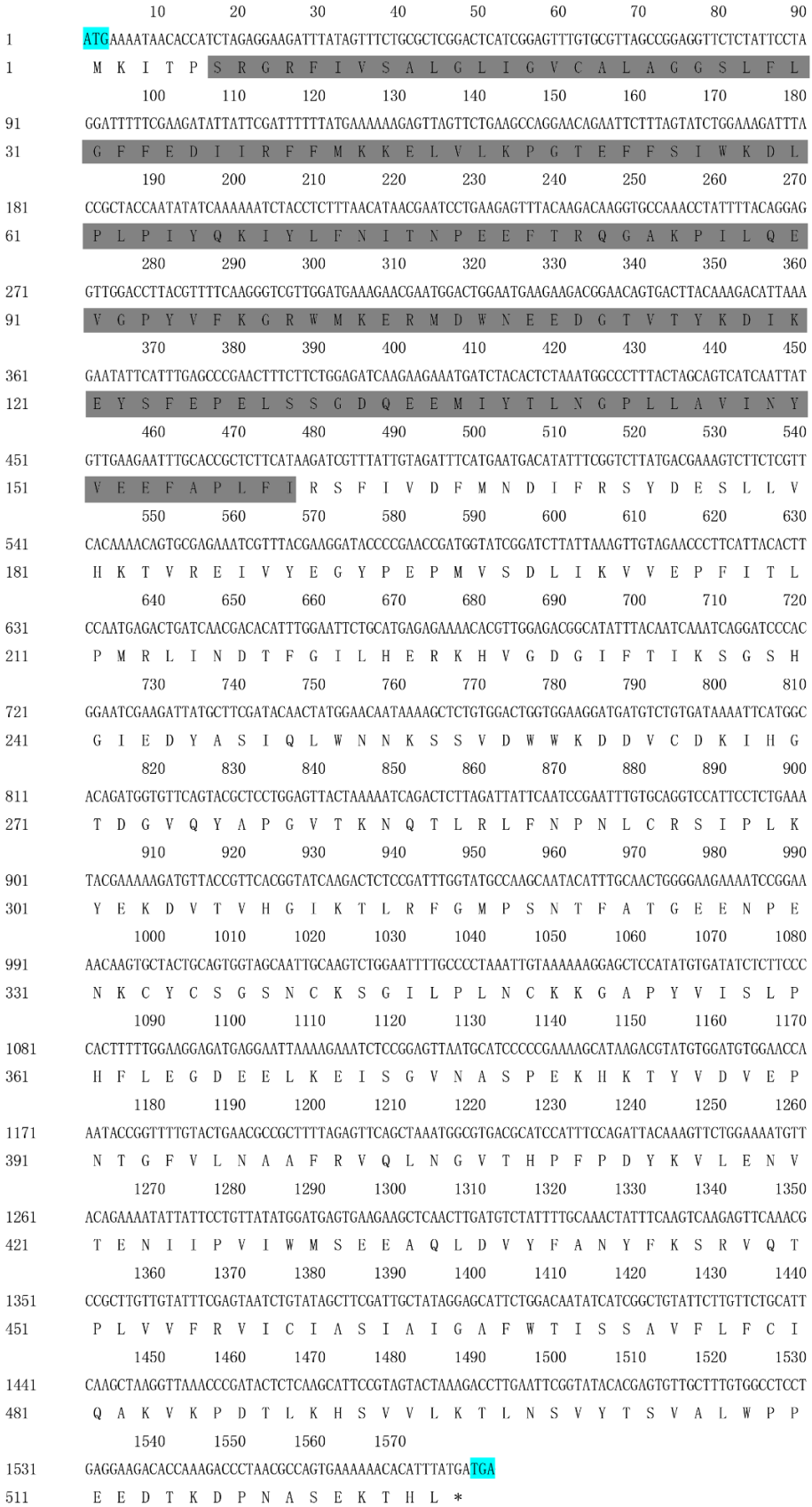
141 P V K V V D A V R *

Fig. S8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100 110 120 130 140 150 160 170 180
91 GGATTTTTCGAAGATATTATTCGATTTTTTATGAAAAAAGAGTTAGTTCTGAAGCCAGGAACAGAATTCTTTAGTATCTGGAAAGATTTA
31 G F F E D I I R F F M K K E L V L K P G T E F F S I W K D L
190 200 210 220 230 240 250 260 270
181 CCGCTACCAATATATCAAAAAATCTACCTCTTTAACATAACGAATCCTGAAGAGTTTACAAGACAAGGTGCCAAACCTATTTTACAGGAG
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280 290 300 310 320 330 340 350 360
271 GTTGGACCTTACGTTTTCAAGGGTCGTTGGATGAAAGAACGAATGGACTGGAATGAAGAAGACGGAACAGTGACTTACAAAGACATTAAA
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370 380 390 400 410 420 430 440 450
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541 CACAAAACAGTGCGAGAAATCGTTTACGAAGGATACCCCGAACCGATGGTATCGGATCTTATTAAAGTTGTAGAACCCTTCATTACACTT
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640 650 660 670 680 690 700 710 720
631 CCAATGAGACTGATCAACGACACATTTGGAATTCTGCATGAGAGAAAACACGTTGGAGACGGCATATTTACAATCAAATCAGGATCCCAC
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910 920 930 940 950 960 970 980 990
901 TACGAAAAAGATGTTACCGTTCACGGTATCAAGACTCTCCGATTTGGTATGCCAAGCAATACATTTGCAACTGGGGAAGAAAATCCGGAA
301 Y E K D V T V H G I K T L R F G M P S N T F A T G E E N P E
1000 1010 1020 1030 1040 1050 1060 1070 1080
991 AACAAGTGCTACTGCAGTGGTAGCAATTGCAAGTCTGGAATTTTGCCCCTAAATTGTAAAAAAGGAGCTCCATATGTGATATCTCTTCCC
331 N K C Y C S G S N C K S G I L P L N C K K G A P Y V I S L P
1090 1100 1110 1120 1130 1140 1150 1160 1170
1081 CACTTTTTGGAAGGAGATGAGGAATTAAAAGAAATCTCCGGAGTTAATGCATCCCCCGAAAAGCATAAGACGTATGTGGATGTGGAACCA
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1180 1190 1200 1210 1220 1230 1240 1250 1260
1171 AATACCGGTTTTGTACTGAACGCCGCTTTTAGAGTTCAGCTAAATGGCGTGACGCATCCATTTCCAGATTACAAAGTTCTGGAAAATGTT
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1270 1280 1290 1300 1310 1320 1330 1340 1350
1261 ACAGAAAATATTATTCCTGTTATATGGATGAGTGAAGAAGCTCAACTTGATGTCTATTTTGCAAACTATTTCAAGTCAAGAGTTCAAACG
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1450 1460 1470 1480 1490 1500 1510 1520 1530
1441 CAAGCTAAGGTTAAACCCGATACTCTCAAGCATTCCGTAGTACTAAAGACCTTGAATTCGGTATACACGAGTGTTGCTTTGTGGCCTCCT
481 Q A K V K P D T L K H S V V L K T L N S V Y T S V A L W P P
1540 1550 1560 1570
1531 GAGGAAGACACCAAAGACCCTAACGCCAGTGAAAAAACACATTTATGATGA
511 E E D T K D P N A S E K T H L *

Fig. S9

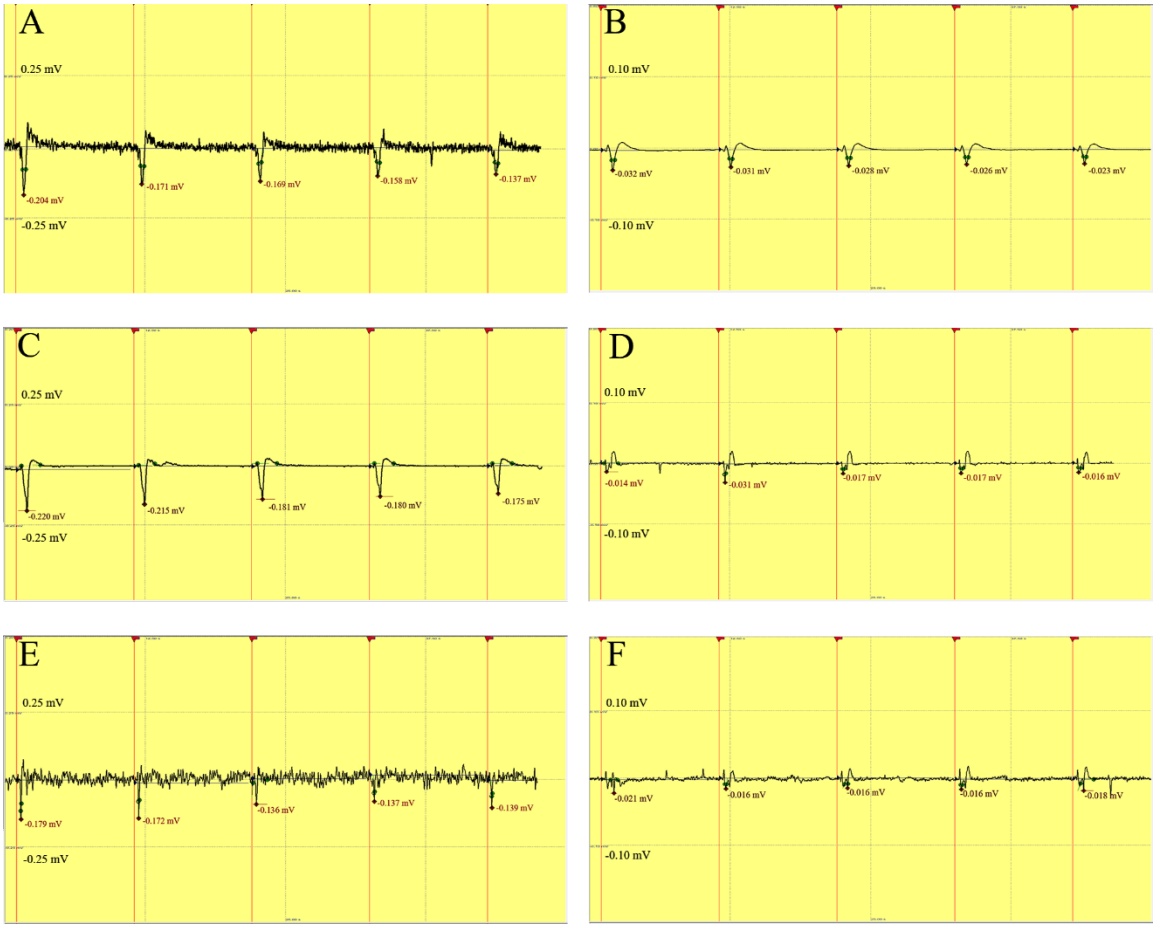
A
0.25 mV
-0.204 mV
-0.171 mV
-0.169 mV
-0.158 mV
-0.137 mV
-0.25 mV
B
0.10 mV
-0.032 mV
-0.031 mV
-0.028 mV
-0.026 mV
-0.023 mV
-0.10 mV
C
0.25 mV
-0.220 mV
-0.215 mV
-0.181 mV
-0.180 mV
-0.175 mV
-0.25 mV
D
0.10 mV
-0.014 mV
-0.031 mV
-0.017 mV
-0.017 mV
-0.016 mV
-0.10 mV
E
0.25 mV
-0.179 mV
-0.172 mV
-0.136 mV
-0.137 mV
-0.139 mV
-0.25 mV
F
0.10 mV
-0.021 mV
-0.016 mV
-0.016 mV
-0.016 mV
-0.018 mV
-0.10 mV

Fig. S10

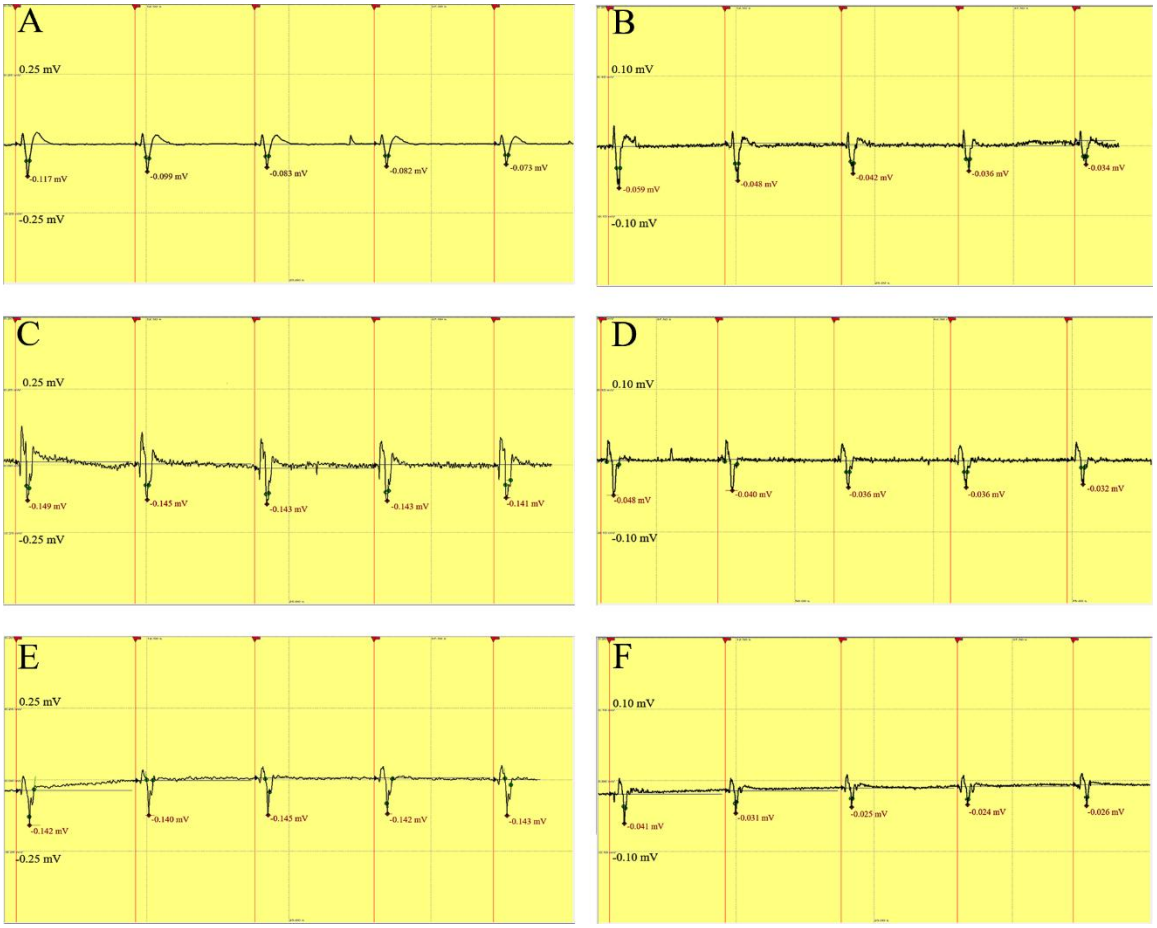
A
0.25 mV
-0.117 mV
-0.099 mV
-0.083 mV
-0.082 mV
-0.073 mV
-0.25 mV
B
0.10 mV
-0.059 mV
-0.048 mV
-0.042 mV
-0.036 mV
-0.034 mV
-0.10 mV
C
0.25 mV
-0.149 mV
-0.145 mV
-0.143 mV
-0.143 mV
-0.141 mV
-0.25 mV
D
0.10 mV
-0.048 mV
-0.040 mV
-0.036 mV
-0.036 mV
-0.032 mV
-0.10 mV
E
0.25 mV
-0.142 mV
-0.140 mV
-0.145 mV
-0.142 mV
-0.143 mV
-0.25 mV
F
0.10 mV
-0.041 mV
-0.031 mV
-0.025 mV
-0.024 mV
-0.026 mV
-0.10 mV

Fig. S11

Table S1 Primer sequences of quantitative real-time PCR

	Gene ID	Primers sequences
♀&♂	*β-actin*-F	GTCTGGATTGGTGGCTCTATCT
	β-actin-R	TGCGGTGGACAATGGAAGG
	PpseOBP2-F	GAAGGATGGTTCAACGACGATGGA
	PpseOBP2-R	ATCTTGGCATTCCTGGTGTCTCTTAG
♀	*PpseNPC2-7*-F	GCAAGGATCAGTCGAACATGGAGAA
	PpseNPC2-7-R	CGTCGGCTTCCTCTTCCTGTGT
	DN7666_c0_g1-F	CGGTAATGACAGCAGCAGCAATG
	DN7666_c0_g1-R	GCCACGAGCACATTTCCCACTAT
	DN8622_c0_g1-F	ACAACCACAGCCAGCATCTTCAC
	DN8622_c0_g1-R	GCCGACCTTATCCTCTTCTACATCCT
	DN6448_c0_g1-F	GATGTTGACTTGCTGTGCTGAA
	DN6448_c0_g1-R	TGACAAAGGCAGGGAATATTGC
	DN126_c0_g2-F	AGAACTTCGGATACTCTGAAGACTGTA
	DN126_c0_g2-R	CAAATTCTGTCGCCACTTGAAATCTG
	DN3309_c0_g1-F	CATTAACGCCAACTTGACAACT
	DN3309_c0_g1-R	TTCACGAAAGGAGAACCAGTC
	DN17419_c0_g2-F	GATGAGGATGACGATGAGGCTGAAG
	DN17419_c0_g2-R	CGTTATCGCTACTCTGGTCACTGTC
♂	*PpseOBP1*-F	GGGTGAGGTTCTTGTTCTGGATTTC
	PpseOBP1-R	GGAGAAGTTATTCAAGCCTGTCTGGAA
	PpseSNMP2-F	CACTGGCGTTAGGGTCTTTGGT
	PpseSNMP2-R	TCTGGACAATATCATCGGCTGTATTCT
	DN2780_c0_g1-F	AAGCCGTTCCATTCCAATTCTGAA
	DN2780_c0_g1-R	TGCTGCTCGCTGCCGTATTAG
	DN11442_c1_g3-F	AGACAGACAGCCTGCATACA
	DN11442_c1_g3-R	CAGTTACAGTGAGGCAAGAAGT
	DN15669_c0_g1-F	TCTGCCTTGAATGGTACTGAAT
	DN15669_c0_g1-R	TCGTAGCGGACTTAGGAGAC
	DN5087_c0_g1-F	CTCTGTCTCGGCTGCTGTT
	DN5087_c0_g1-R	CGGTATGTCCTTGAACTCGTAG
	DN43538_c0_g1-F	GTTAATCTGCCACTGCCTTCAA
	DN43538_c0_g1-R	ATAGCCAATCCTGCGTAAGAGA

Note: F, Forward primer; R, Reverse primer.

The reaction system and reaction conditions are as follows.

qPCR reaction system

Reagent	Volume (μL)
2×RealStar Fast SYBR qPCR Mix	10
Forward primer	1
Reverse primer	1
cDNA template	1
ddH_2O	7
Total volume	20

qPCR reaction conditions

Temperature	Time	
95℃	2 min	
95℃	15 s	40 cycles
59℃	30 s	
72℃	30 s	
Melting curve generated at 65-95°C		

Table S2 Primers for validating the transcriptome data by RT-PCR

Gene name	Primers sequences
PpseNPC2-7-F	GCAAGGATCAGTCGAACATGGAGAA
PpseNPC2-7-R	CGTCGGCTTCCTCTTCCTGTGT
PpseSNMP2-F	CACTGGCGTTAGGGTCTTTGGT
PpseSNMP2-R	TCTGGACAATATCATCGGCTGTATTCT

Note: F, Forward primer; R, Reverse primer.

The PCR reaction system is as follows

PCR amplification system

Reagent	Usage amount (μl)
template cDNA	3
Forward Primer	1
Reverse Primer	1
ddH_2O	7.5
2×Taq PCR StarMix (Dye)	12.5
Total volume	20

PCR reaction conditions

Reaction temperature	Reaction time	
Initial Denaturation at 95℃	2 min	
Denaturation at 95℃	30 s	34 cycles
Annealing at Tm	30 s	
Extension at 72℃	1 min	
Final Extension at 72℃	10 min	
Storage at 4℃	∞	

PCR Product Purification

The PCR products were separated by 1.0% agarose gel electrophoresis (130 V, 20 minutes). After identifying the target band under a gel imaging system, the gel was cut out and the PCR products were purified using the FastPure Gel DNA Extraction Mini Kit.

Vector Ligation

The purified PCR products were ligated with the vector (pGEM-T Easy vector) using

the ligation system as shown in the table below. The reaction mixture was gently pipetted to mix thoroughly and then incubated at 4°C overnight for at least 10 hours.

Vector ligation reaction system

Reagent	Usage amount (μL)
2× Rapid Ligation Buffer, T4 DNA ligase	5
pGEM®-T Easy Vector	1
T4 DNA Ligase	1
PCR Product	1
nuclease-free water	2
Total volume	10

Transformation

(1) Add 50 μL of ice-cold E.coli DH5α competent cells to a pre-chilled 1.5 mL centrifuge tube.

(2) Pipette 3 μL of the overnight-incubated ligation product into the competent cells, gently mix, and place on ice for 30 minutes.

(3) Heat shock the mixture in a 42°C metal bath for 50 seconds, then place on ice for 2 minutes to cool.

(4) Add 950 μL of LB liquid medium without ampicillin to the mixture, mix well, and incubate in a 37°C shaker at 160 rpm for 2 hours.

(5) Centrifuge at 5000 × g for 5 minutes, remove 800 μL of the supernatant, leaving the cells concentrated in the remaining 200 μL of medium.

(6) Use a sterile glass spreader to evenly distribute 30 μL of the bacterial suspension, 30 μL of IPTG, and 30 μL of X-Gal onto an LB solid medium containing ampicillin. Seal with parafilm and invert the plate, placing it in a 37°C incubator overnight for 16 hours.

5、Colony Selection, Expansion Culture, and Sequencing

(1) Select a single colony and dissolve it in 30 μL of sterile water.

(2) Use 2 μL of the bacterial suspension as a template and perform PCR amplification using M13 universal primers to verify successful cloning of the target fragment.

138 **Table S3 dsRNA primers of olfactory genes of *P. pseudoannulata***

Primer name	Sequence of primers （5’-3’）
ds*PpseOBP1*-F(♂)	TAATACGACTCACTATAGGGTTTCAAAGCACAGGTGGTG ATGGTT
ds*PpseOBP1*-R(♂)	TAATACGACTCACTATAGGGGCAGTCGTTTACGCCCAAG AGG
ds*PpseOBP2*-F(♀&♂)	TAATACGACTCACTATAGGGCTCCTCCTACAACCGTCCAA CCT
ds*PpseOBP2*-F(♀&♂)	TAATACGACTCACTATAGGGATGTTCTTCTCCACCTCAGC ATCTAAT
ds*PpseNPC2-7*-F♀	TAATACGACTCACTATAGGGTTGAAGCGAGTAACAGTTG AGATGGTA
ds*PpseNPC2-7*-R♀	TAATACGACTCACTATAGGGCGAGACTTGCGAGGGTATT GTCA
ds*PpseSNMP2*-F♂	TAATACGACTCACTATAGGGTCATCCTTCCACCAGTCCAC AGAG
ds*PpseSNMP2*-R♂	TAATACGACTCACTATAGGGTGAAGAAGACGGAACAGTG ACTTACAA
ds*GFP*-F	TAATACGACTCACTATAGGGGCCAACACTTGTCACTACT
ds*GFP*-R	TAATACGACTCACTATAGGGGGAGTATTTTGTTGATAATG GTC

139 Note: The underlined part of the table is the T7 sequence, the upstream primer is F, and the
140 downstream primer is R.
141

142 **Table S4 Statistical analysis of clean data for each sample from Illumina**

Sample name	Read Number	Base Number	GC Content (%)	The percentage of bases with a quality score greater than or equal to 30 (% ≥ Q30)
CK-F	20,076,481	6,005,028,892	40.53	91.45
MeSA-F	20,263,837	6,058,897,988	41.22	92.39
CK-M	20,214,874	6,050,900,658	45.29	92.15
MeSA-M	19,051,472	5,701,559,152	44.68	91.67

143

144 **Table S6 Differential expression of olfactory-related genes in male and female**

Gene ID	Gene name	P-value	log_2FC	regulated
*P. pseudoannulata*_00007583♂	*PpseSNMP2*	0.000198417	1.261729474	up
*P. pseudoannulata*_00021038♂	*PpseOBP1*	4.53E-07	2.376134494	up
*P. pseudoannulata*_00004971♂	*PpseOBP2*	0.002343916	2.493664398	up
*P. pseudoannulata*_00004971♀	*PpseOBP2*	5.17E-10	2.317766525	up
*P. pseudoannulata*_00002169♀	*PpseNPC2-7*	0.00108952	3.186408554	up

145
146

147 **Table S7 AlphaFold3 prediction scores for olfactory-related proteins**

Protein	pLDDT	pTM	Ranking score	Min PAE (Å)	Fraction disordered
PpseOBP1	76.4	0.69	0.84	0.76	0.31
PpseOBP2	72.2	0.63	0.83	0.76	0.40
PpseNPC2-7	90.5	0.83	0.89	0.76	0.12
PpseSNMP2	88.7	0.83	0.86	0.76	0.05

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149