Dynamic miRNA landscapes unlock sexual plasticity in protogynous orange-spotted grouper under MT-implantation

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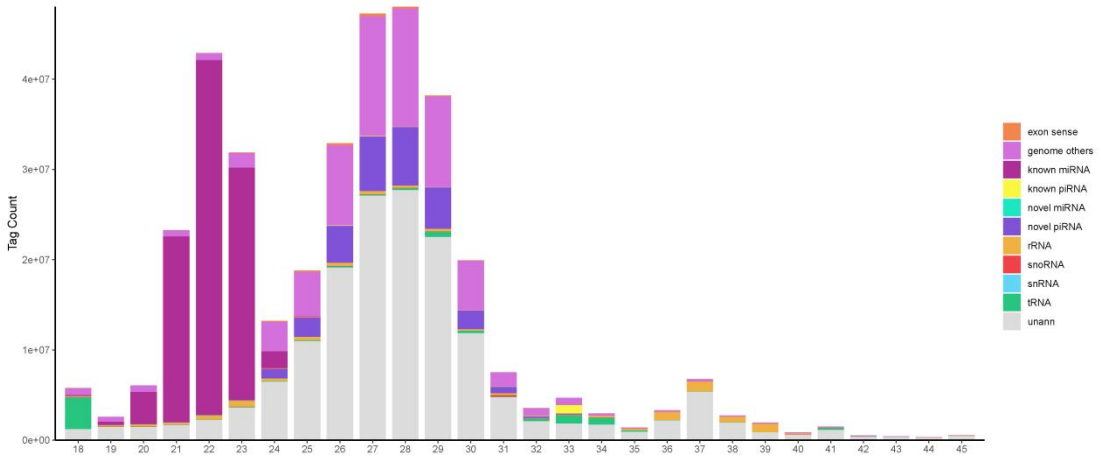
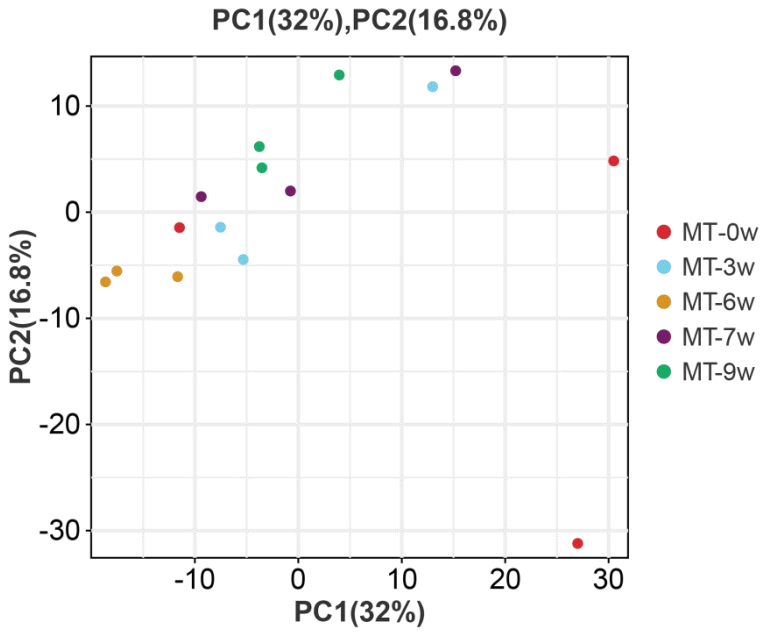
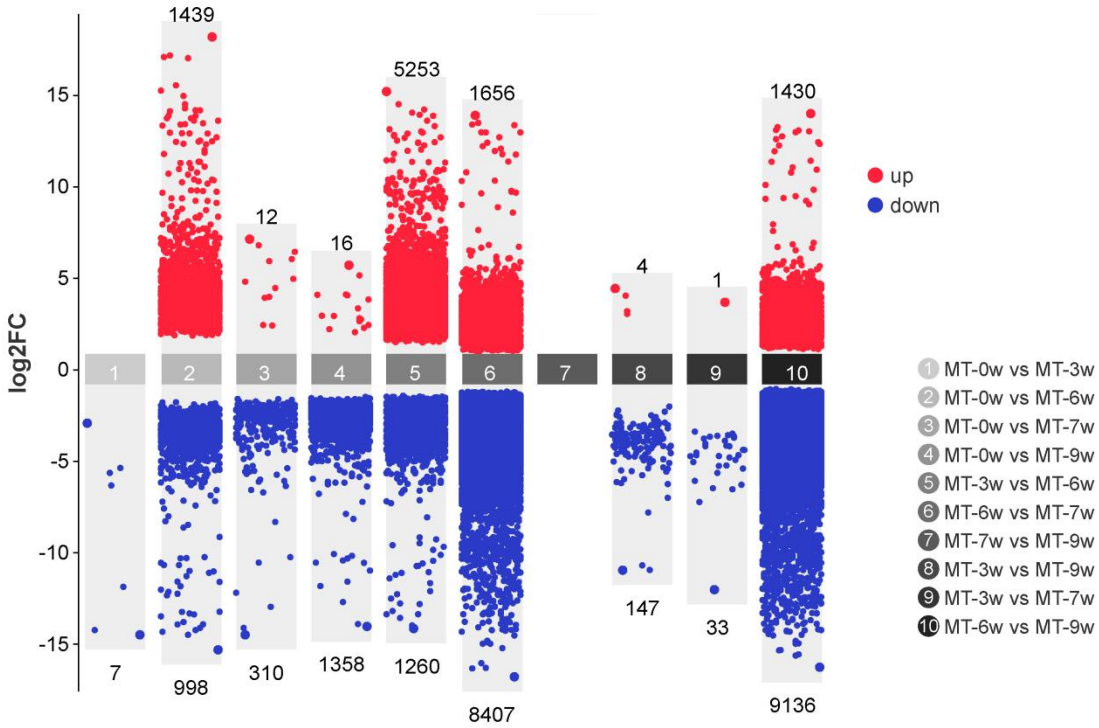


Supplementary Figure 1. The length distribution of all obtained small RNAs. Tag refers to the sequence of small RNAs. Tag count means the total abundance of tags. Different colours stand for different types of tags. Abbreviations: exon sense, tags aligned to genome exons, which namely were mRNA degraded fragment; genome others, tags that were aligned to genome without miRNA characteristics, including piRNA and etc.; known miRNA, miRNA aligned to the miRBase; novel miRNA, miRNA with hairpin structure that was aligned to the genome, compensating for the lack of comprehensive data within the miRBase; known piRNA: piRNA aligned to the piRBase; novel piRNA, piRNA predicted by ping-pong cycle; novel piRNA, rRNA, ribosomal RNA; snoRNA, small nucleolar RNA; snRNA, small nuclear RNA; tRNA, transfer RNA; unann, tags that did not match any annotated information.

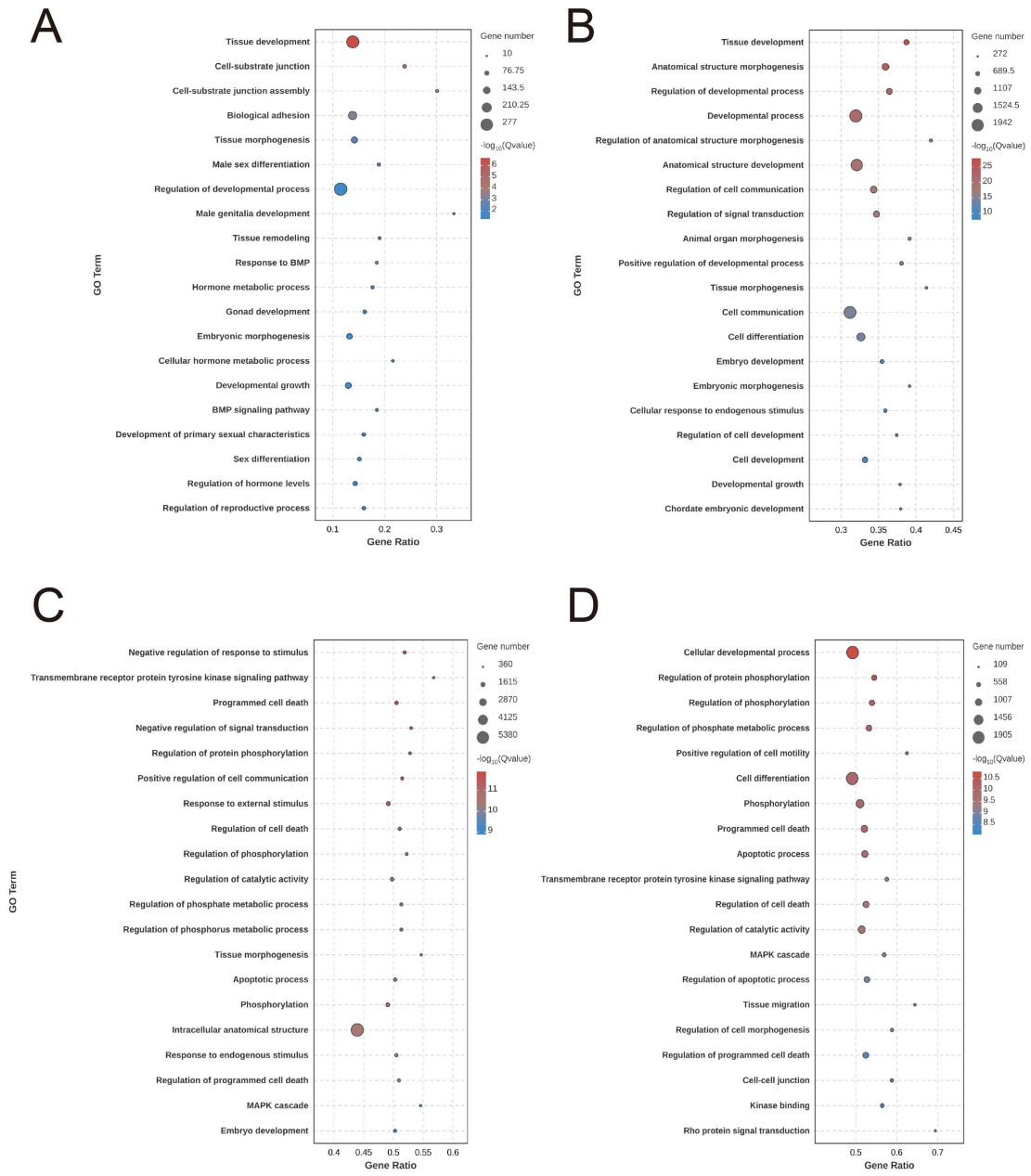


Supplementary Figure 2. Principal component analysis of miRNA sequencing samples. MT-0w represents the gonad samples of untreated *Epinephelus coioides* before implantation. MT-3w, MT-6w, MT-7w, and MT-9w represent gonad samples collected at 3, 6, 7, and 9 weeks after 17α-methyltestosterone implantation (17α-MT). n = 3 biological replicates in all groups.

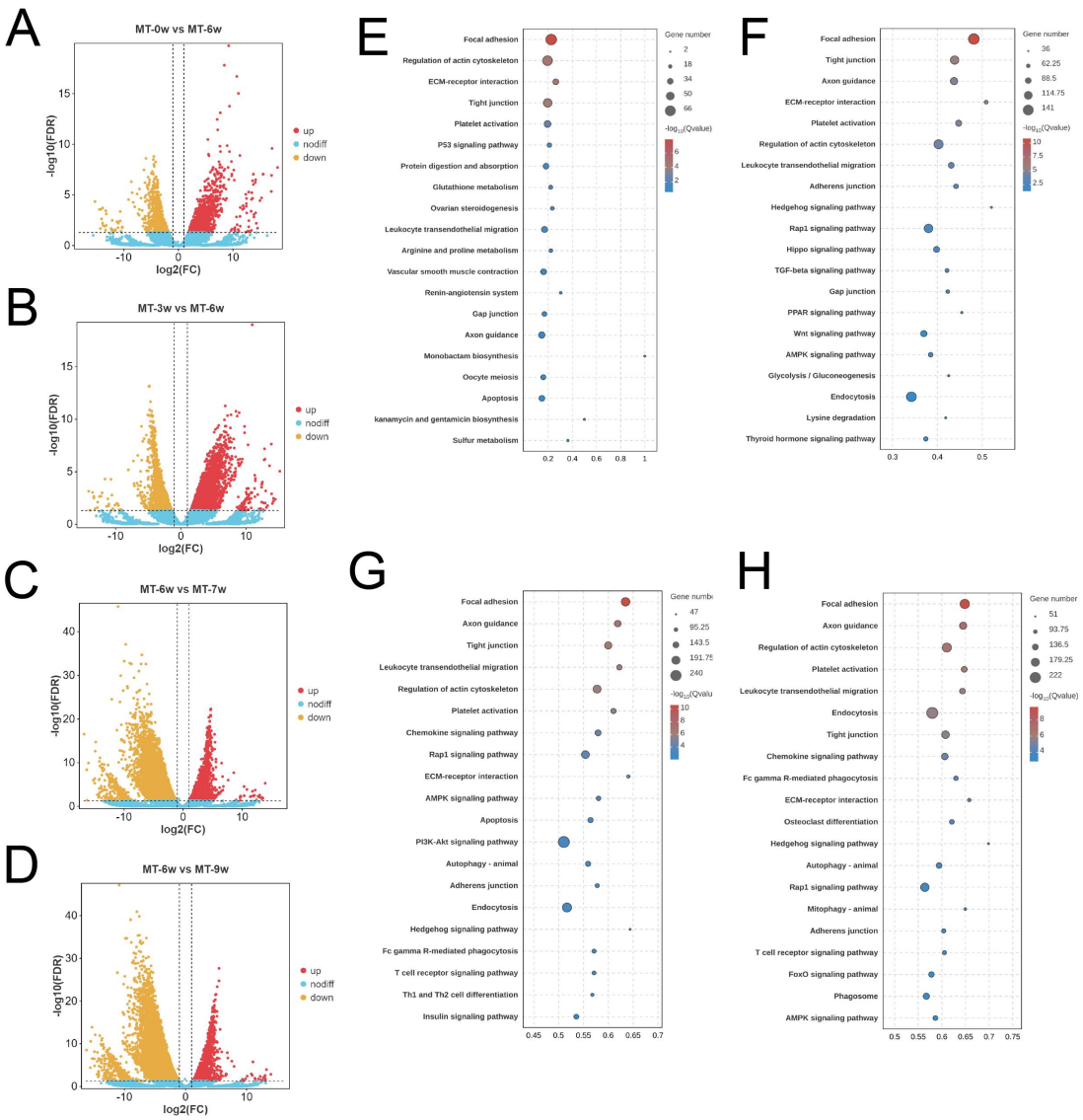


Supplementary Figure 3. Scatter plot of DEGs across multiple groups. In the plot, the y-axis represented the logarithm (base 2) fold change (log_2FC) of inter-group differences, while the x-axis denoted the names of the comparison groups, with 1-10 representing distinct

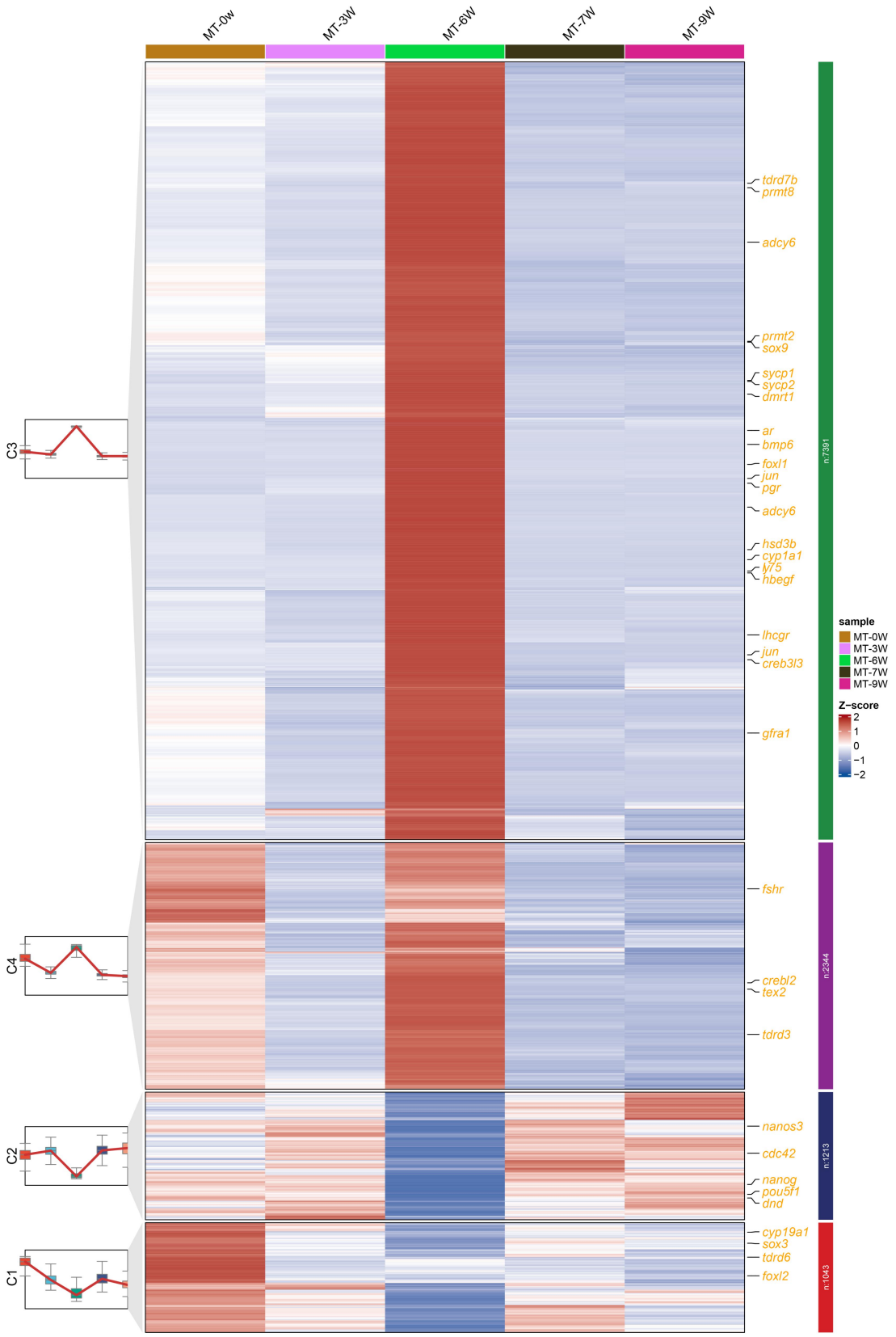
comparison group identifiers. Scatter points positioned above the x-axis indicated up-regulated genes (in red), whereas those below the x-axis represented down-regulated genes (in blue). A fold change greater than 2 and a significance threshold of less than 0.05 were considered.



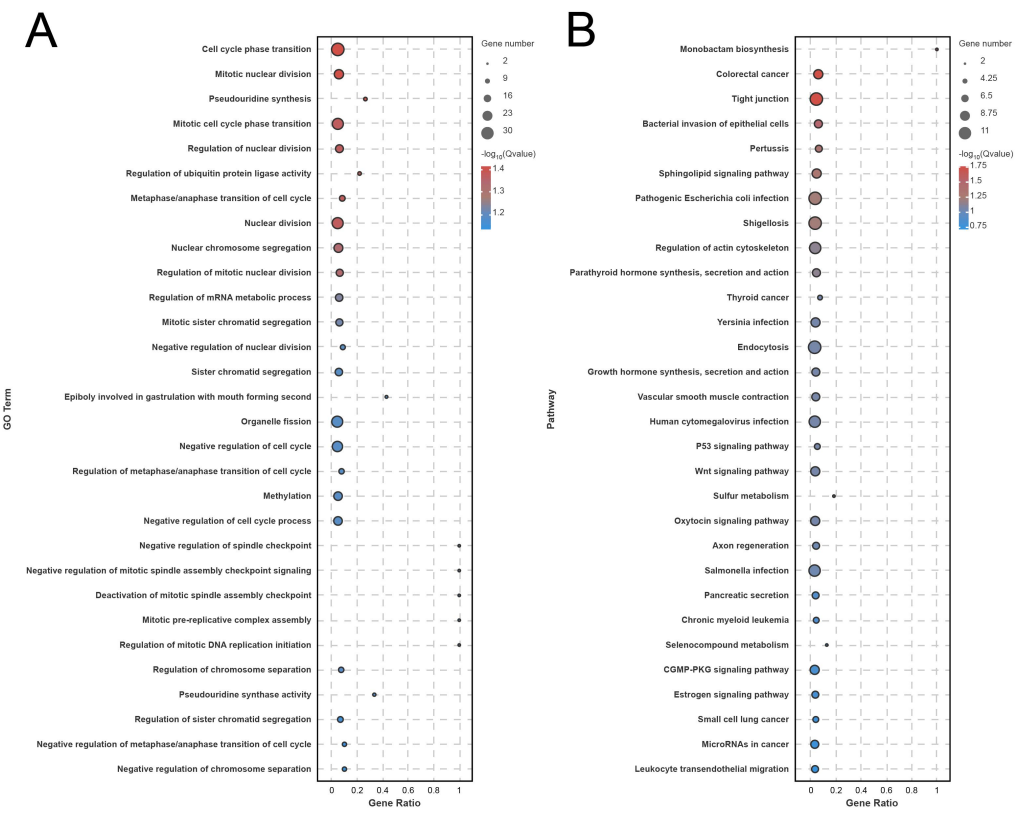
Supplementary Figure 4. Analysis of DEGs across four groups. (A) Top 20 enriched GO terms by DEGs in the 0 week vs 6 week. (B) Top 20 enriched GO terms by DEGs in the 3 week vs 6 week. (C) Top 20 enriched GO terms by DEGs in the 6 week vs 7 week. (D) Top 20 enriched GO terms by DEGs in the 6 week vs 9 week.



Supplementary Figure 5. The overview of DEGs in four compare groups. (A-D) The volcano plot displaying the level of the difference. The x-axis delineated the $\log_2$ of the fold change ratios between the two comparative groups, with genes positioned further towards the extremities of the axis exhibiting a more significant difference. The y-axis corresponded to the $-\log_{10}$ of the False Discovery Rate (FDR), indicative of the statistical significance of the observed differences between groups. The color-coding scheme differentiated between significantly up-regulated genes, denoted in red, down-regulated genes in yellow, and genes with no significant difference in expression, represented in blue. (E-H) Top 20 enriched KEGG terms by DEGs.



Supplementary Figure 6. Composite plot integrating time series clustering and expression trend.



Supplementary Figure 7. Top30 KEGG pathways of 1,145 DEGs which were up-regulated and then down-regulated and 570 DEGs that were down-regulated and then up-regulated during sex reversal.