

# **Solitary Living Brings a Decreased Weight and an Increased Agility to the Domestic Silkworm, *Bombyx mori***

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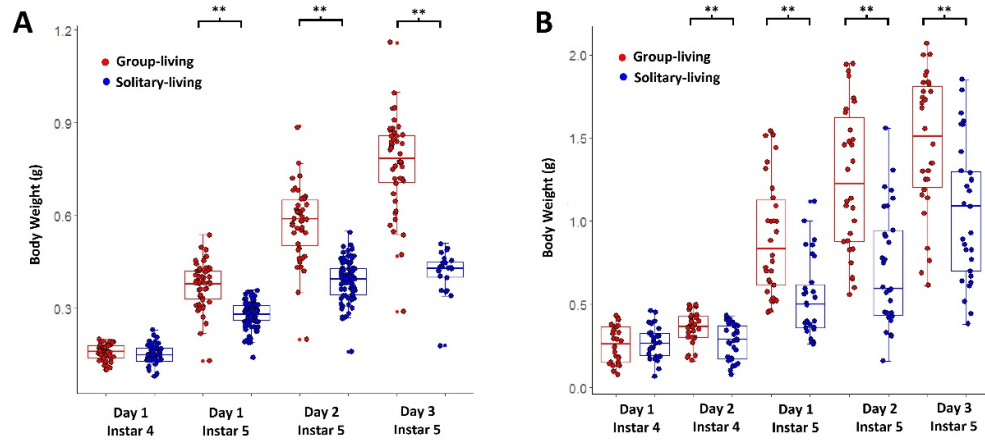


Figure S1. Repeated experiments to compare the body weight between solitary and group-living silkworms from the first day to the 3<sup>rd</sup> day of the 5<sup>th</sup> instar. In the first day of the 4<sup>th</sup> instar, we began to breed solitary silkworms. A and B are two independent results. In B, the data at the 2<sup>nd</sup> day of the 4<sup>th</sup> instar 4 is added. '\*' denotes P-value < 0.05 and '\*\*' denotes P-value < 0.01. Statistics are performed by Wilcox test.

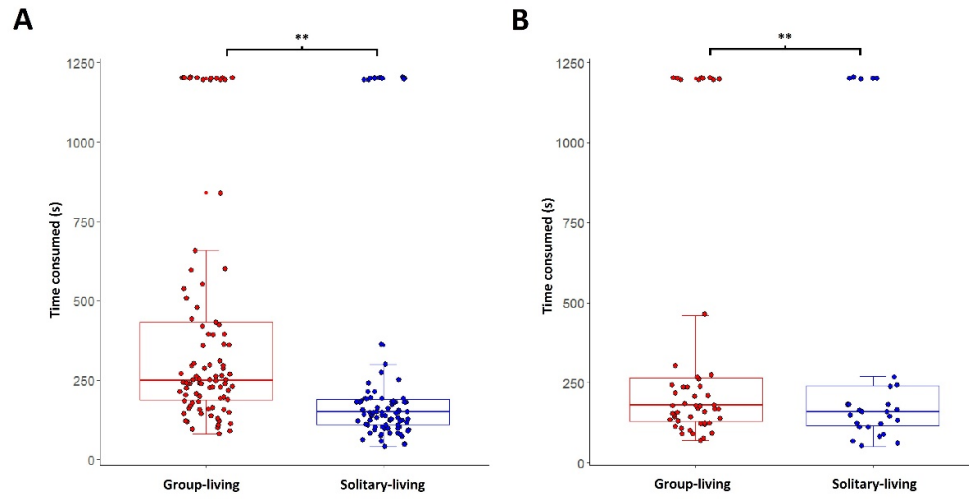


Figure S2. Repeated experiments to compare the time consumed to move under food luring between solitary and group-living silkworms. A and B are two independent results. '\*' denotes P-value < 0.05 and '\*\*' denotes P-value < 0.01. Statistics are performed by Wilcox test.

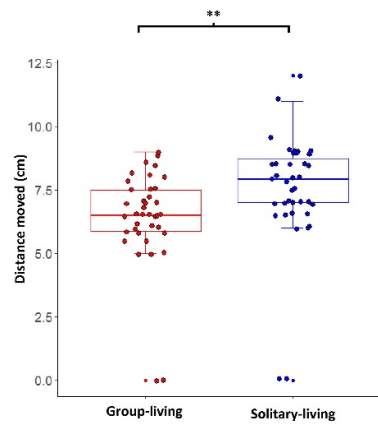
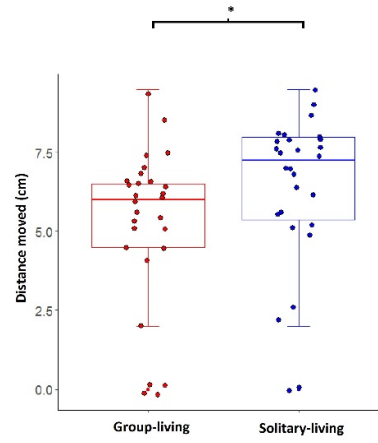
**A****B**

Figure S3. Repeated experiments to compare the distance moved under heat stress between solitary and group-living silkworms. A and B are two independent results. '\*' denotes P-value < 0.05 and '\*\*' denotes P-value < 0.01. Statistics are performed by Wilcoxon test.

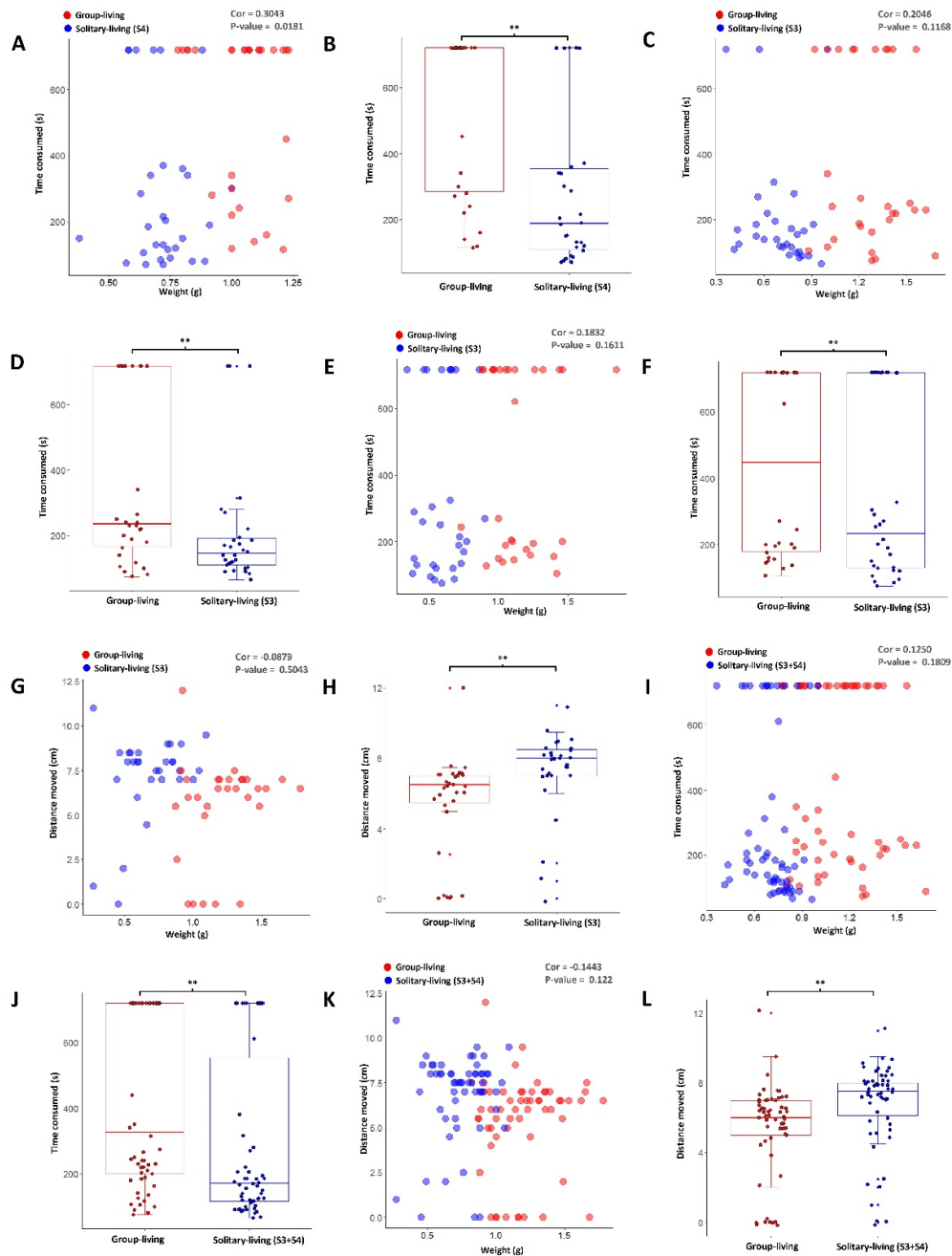


Figure S4. Repeated experiments of the correlation analysis between the weight of solitary/group-living silkworms and the performance under food luring/heat stress. A, C, E and I (mutually independent) are scatterplots showing the correlations of the time consumed to move under food and the body weight, for solitary (blue) and group-living silkworms (red). G and K are scatterplots showing the correlations of the distance moved to evade heat stress and the body weight, for

solitary (blue) and group-living silkworms (red). B, D, F and J are the time consumed to moving under food luring (corresponding to A, C, E and I, respectively) between solitary- and group-living silkworms. H and L are the distances moved (corresponding to G and K, respectively) to evade heat stress between solitary- and group-living silkworms. A and B are comparisons between group- and S4 solitary-living silkworms. C to H are comparisons between group- and S3 solitary-living silkworms. I to L are comparisons between group-living and both types of solitary silkworms, including S3 and S4. I and J are comparisons for the sum of the data in Figure 4A and the data in A to F of this figure. K and L are comparisons for the sum of the data in Figure 4B and the data in G and H of this figure. Other legends follow Figure 4. In B, D, F, H, J and L, ‘\*’ denotes P-value < 0.05 and ‘\*\*’ denotes P-value < 0.01. Statistics are performed by Wilcox test.

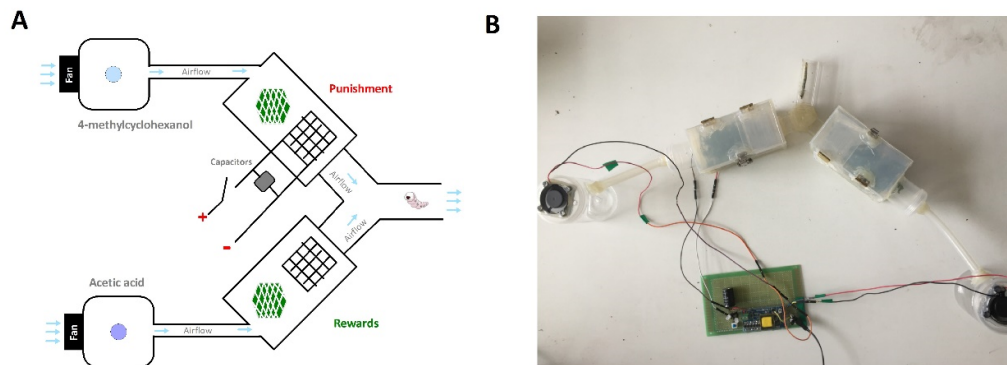


Figure S5. The apparatus used to test the memory strengths of silkworms. A is a sketch showing the details of the apparatus. B is a picture of a real product of A.

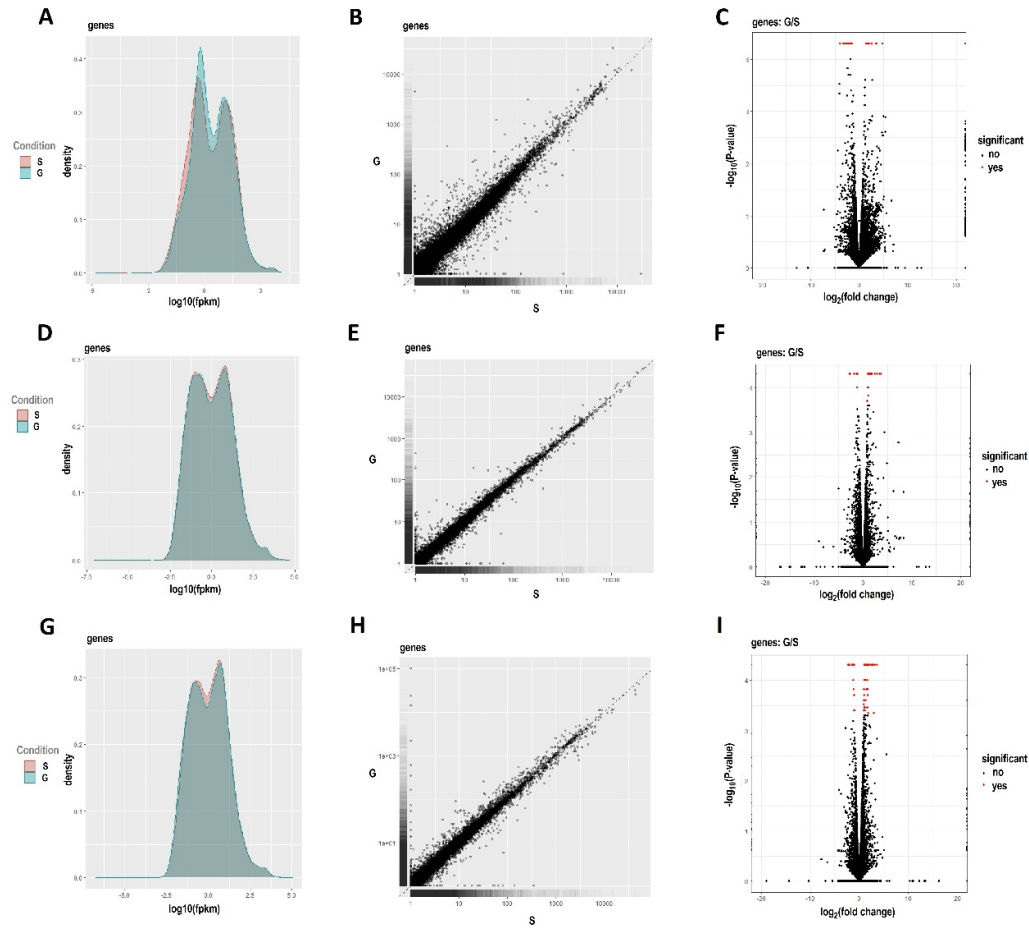


Figure S6. Statistics of RNA-seq data. A, D and G are the density plots for  $\log_{10}$ -transformed FPKM values between solitary (S) and group-living silkworms (G). B, E and H are scatter plots showing the comparison of the FPKM values between solitary (S) and group-living silkworms (G). C, F and I are volcano plots of  $\log$  fold change in expression vs  $-\log(\text{pval})$  for a pair of samples (x,y), solitary (S) and group-living (G) silkworms. A, B and C are the analyses in the brain. D, E and F are the analyses in the midgut. G, H and I are the analyses in the cuticle.

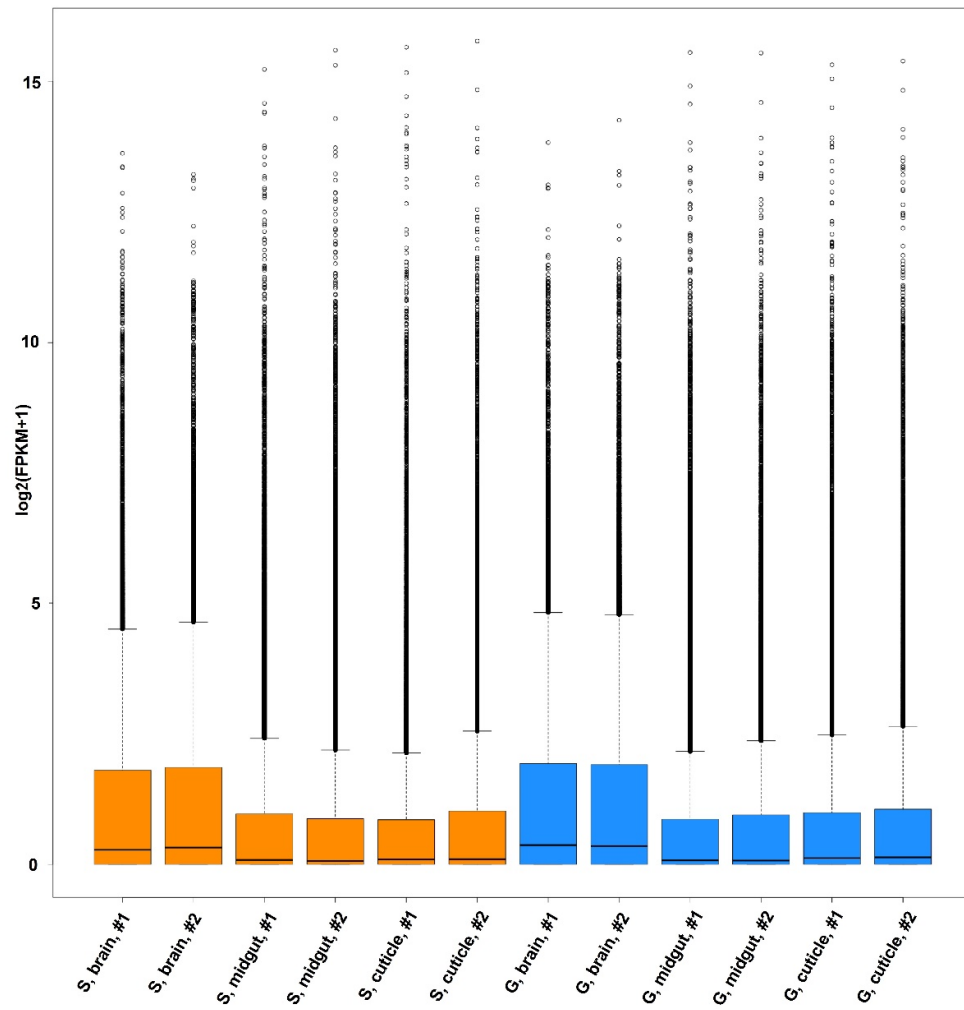


Figure S7. The distribution of genes' express  $\log_2(\text{FKPM}+1)$  in tissues, including brain, midgut and brain. S and G denote solitary and group-living silkworms, respectively. '#1' and '#2' denotes the serial of two repeated sequencing data, respectively.



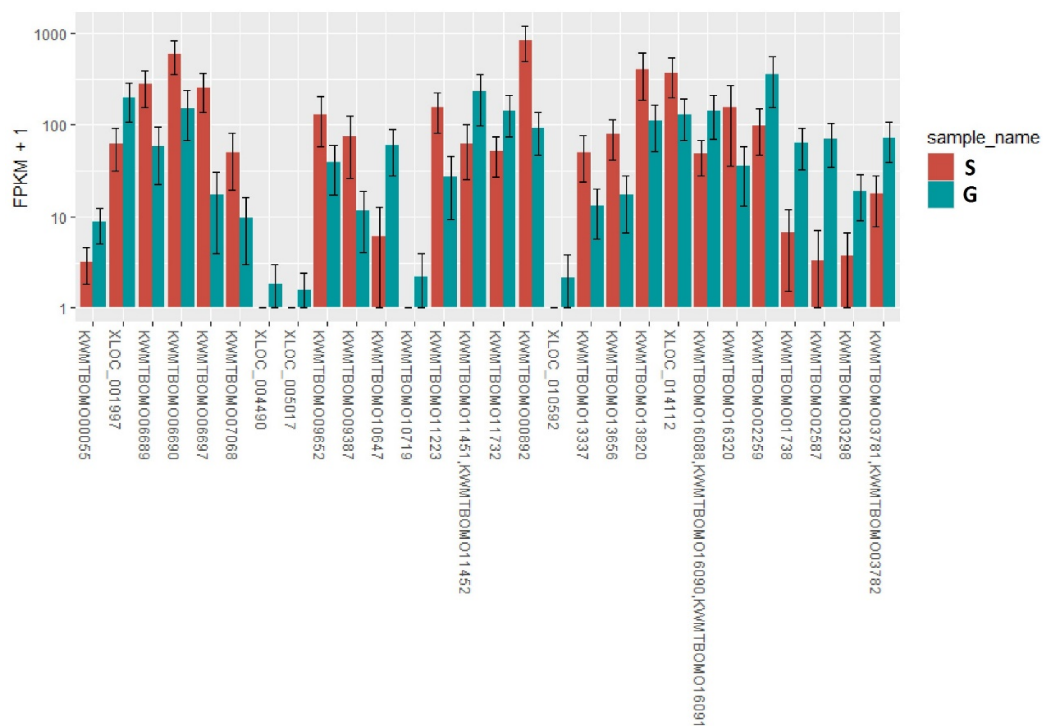


Figure S8. The expression (FKPM) of DEGs in the brain between solitary (S) and group-living (G) silkworms.

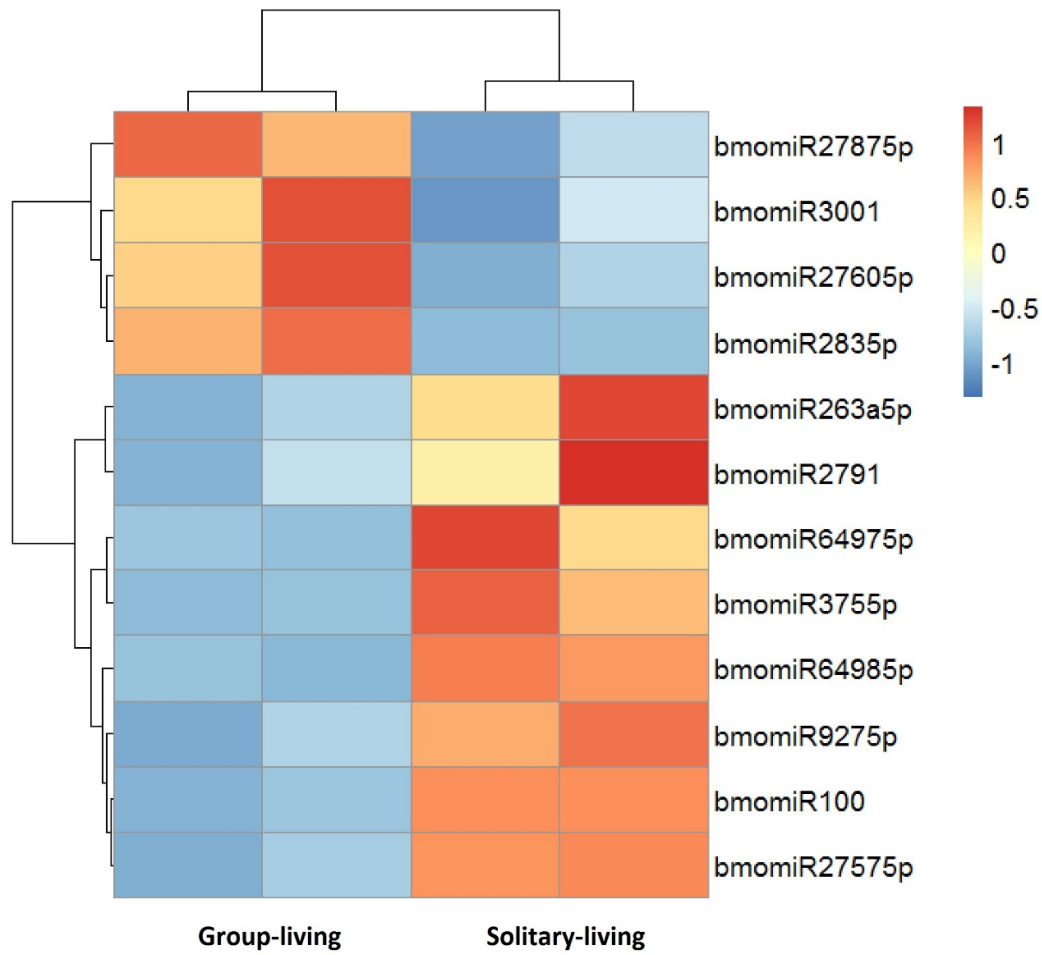
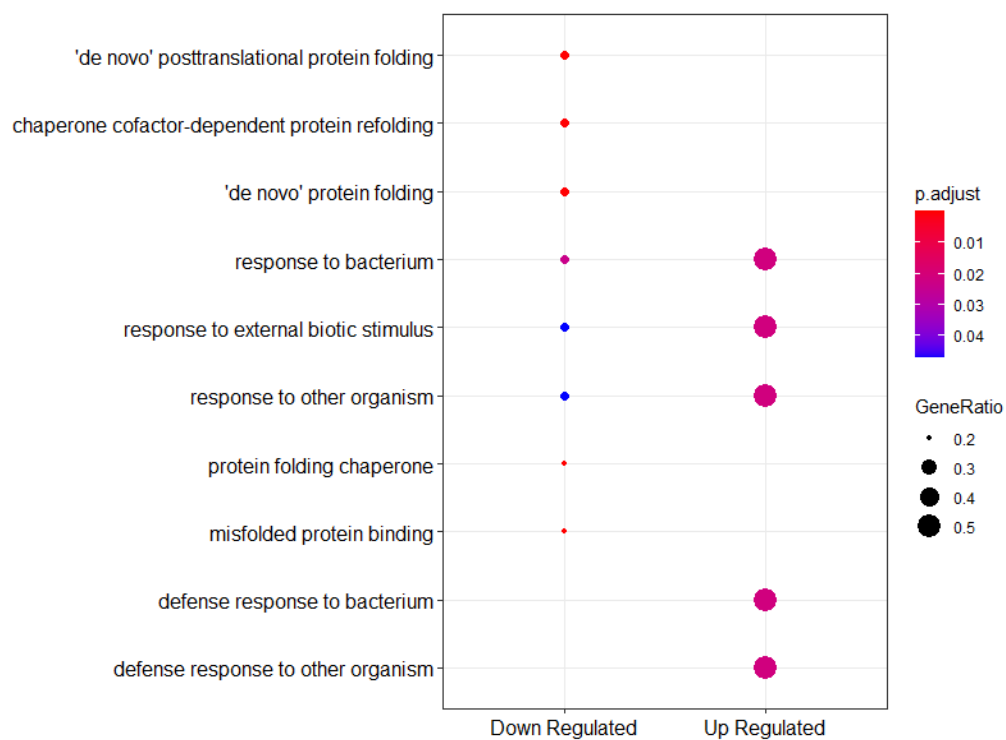
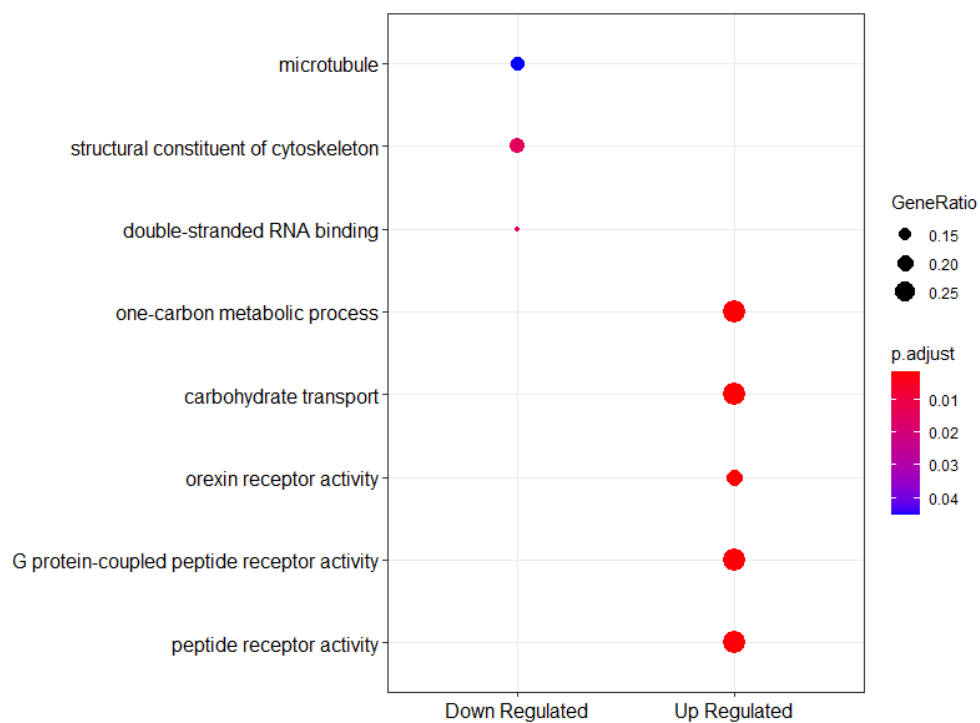


Figure S9. The heatmap of the expression (normalized) of DE miRNAs (adjusted P-value < 0.05) between solitary and group-living silkworms.

**A**



**B**



C

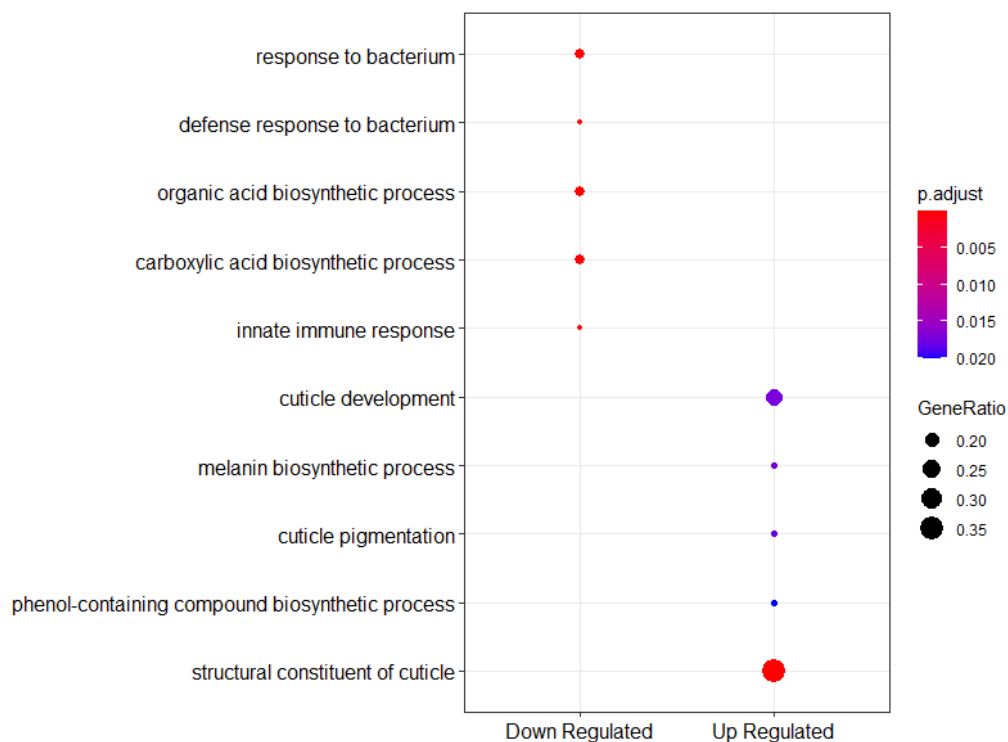


Figure S10. A to C are the GO enrichment results of DEGs in brain, midgut and cuticle, respectively. 'p.adjust' denotes the adjusted P-value of enriched pathways.

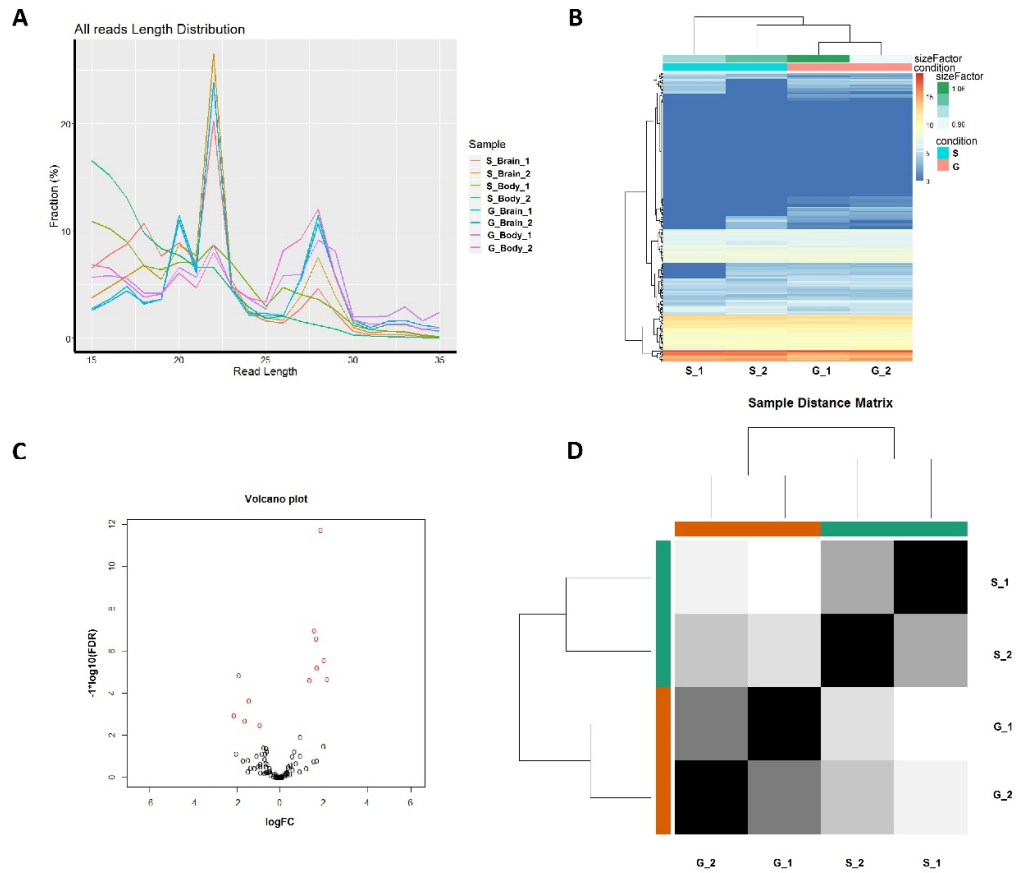


Figure S11. Statistics on microRNA sequencing data. A is all reads length distribution in the brain and the body (with brain excised) of solitary (S) and group-living (G) silkworms. B is the heatmap of all miRNAs with expression signals in the brain of solitary (S) and group-living (G) silkworms. C is a volcano plot of log fold change in expression vs  $-\log(p\text{val})$  for a pair of samples (x,y), the brains of group and solitary-living silkworms. miRNA with  $-1*\log_{10}(\text{FDR}) < 0.01$  are in red. D is the sample distance matrix of the experiments of the brain of solitary (S) and group-living (G) silkworms. In A, B and D, 1 and 2 denote the serials of two repeated experiments.

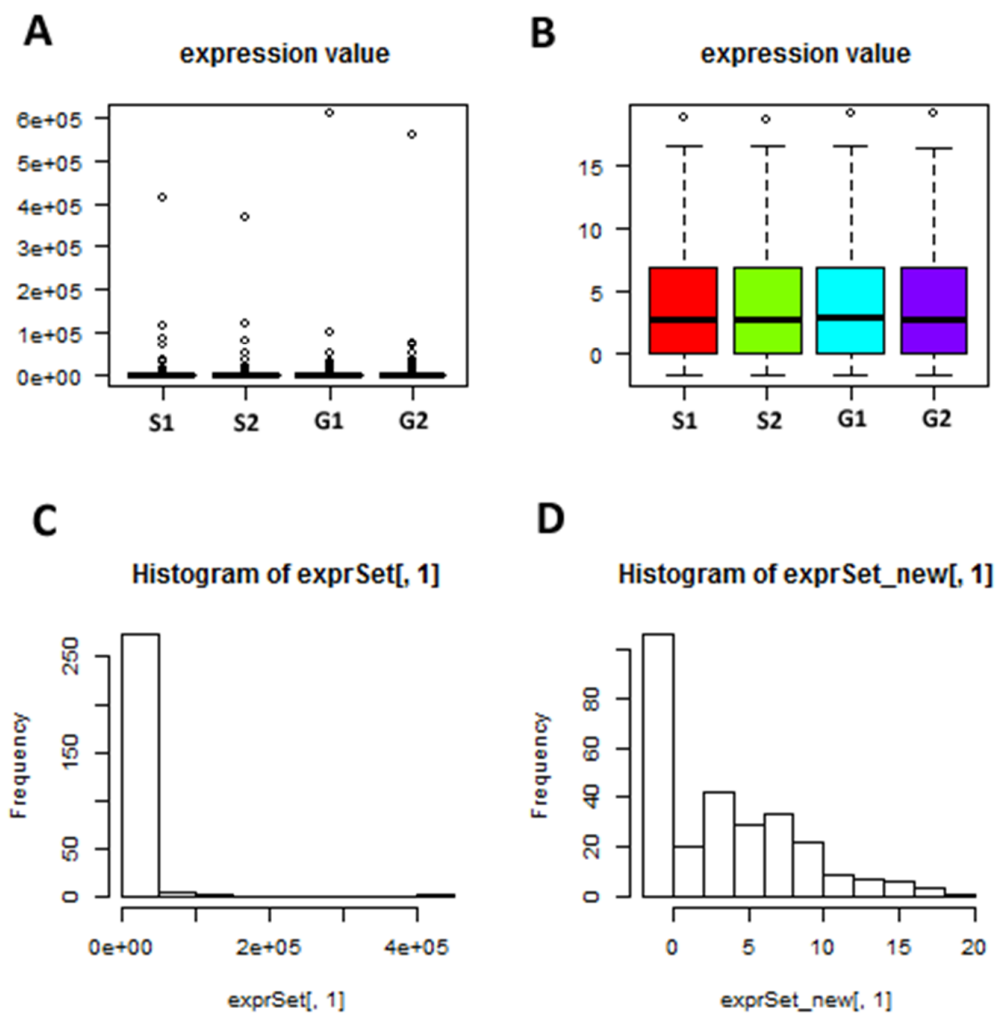


Figure S12. Statistics of the count of reads of microRNA sequencing of the brain. A and B correspond to the distribution of the original (A) and normalized expression (B) values in solitary (S) and group-living (G) silkworms. C is the histogram of the expression of all reads. D is the histogram of the expression normalized of all reads. In A and B, 1 and 2 denote the serials of two repeated experiments.

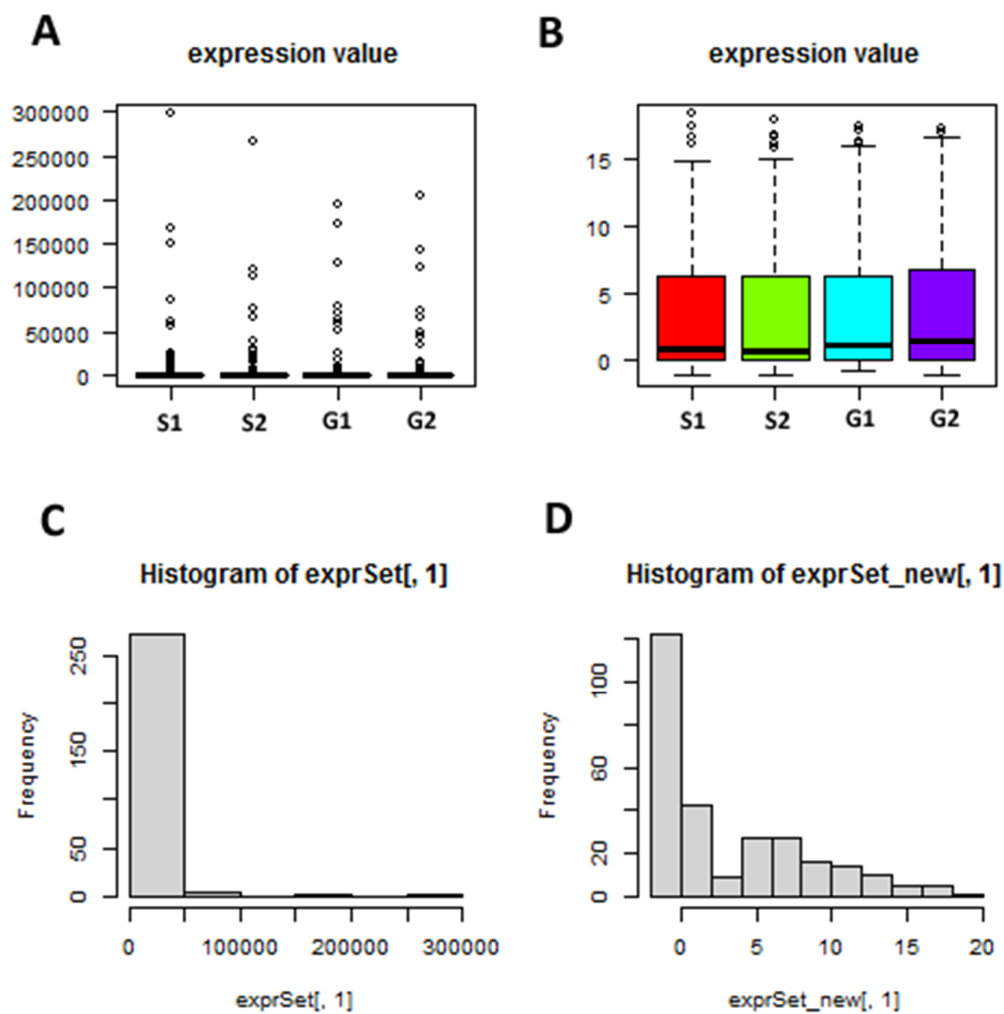


Figure S13. Statistics of the count of reads of microRNA sequencing of the silkworm body with brain excised. The figure convention in A to D follows Figure S9.