Supplementary information

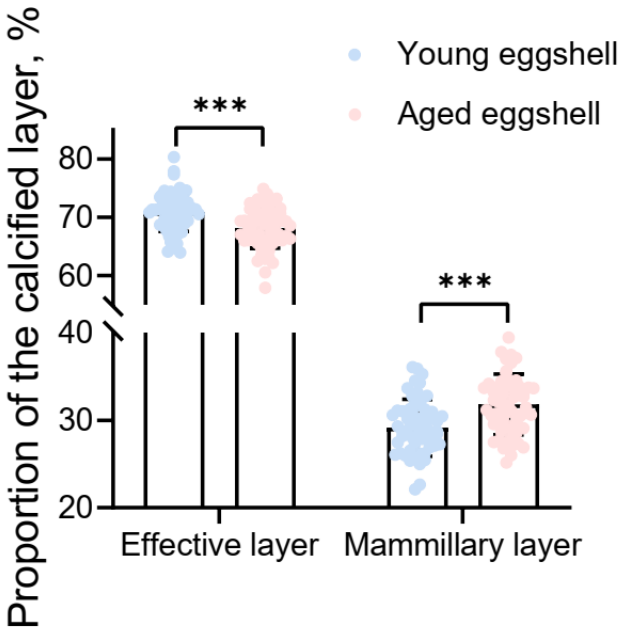

Fig. S1 Proportional distribution of the effective layer and mammillary layer in the eggshell ultrastructure of young and aged laying hens. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

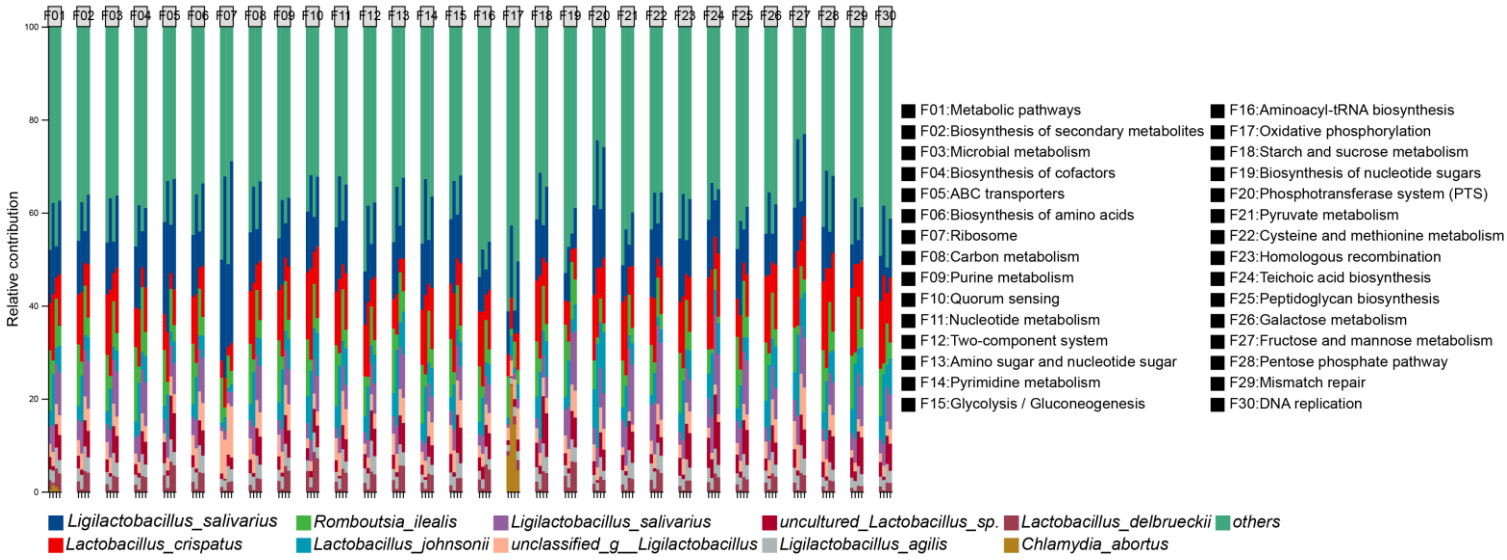

Fig. S2 Contribution analysis of gut microbial taxa to functional profiles identified by metagenomic sequencing in laying hens.

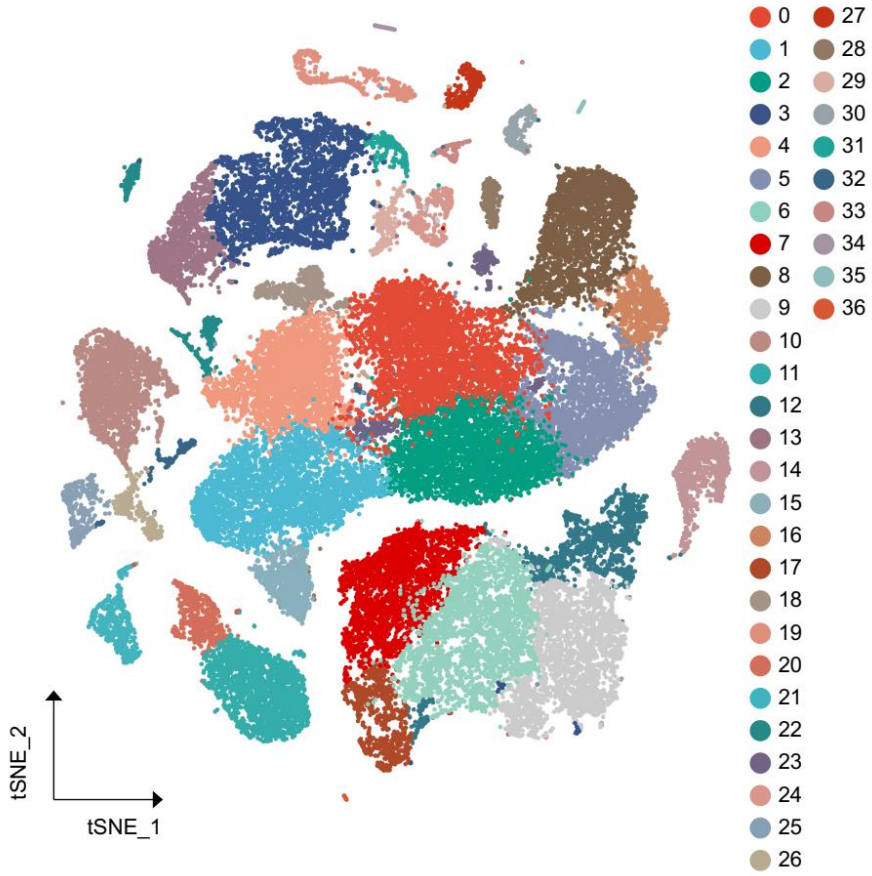

Fig. S3 Clustering analysis of single-cell RNA sequencing data in laying hens.

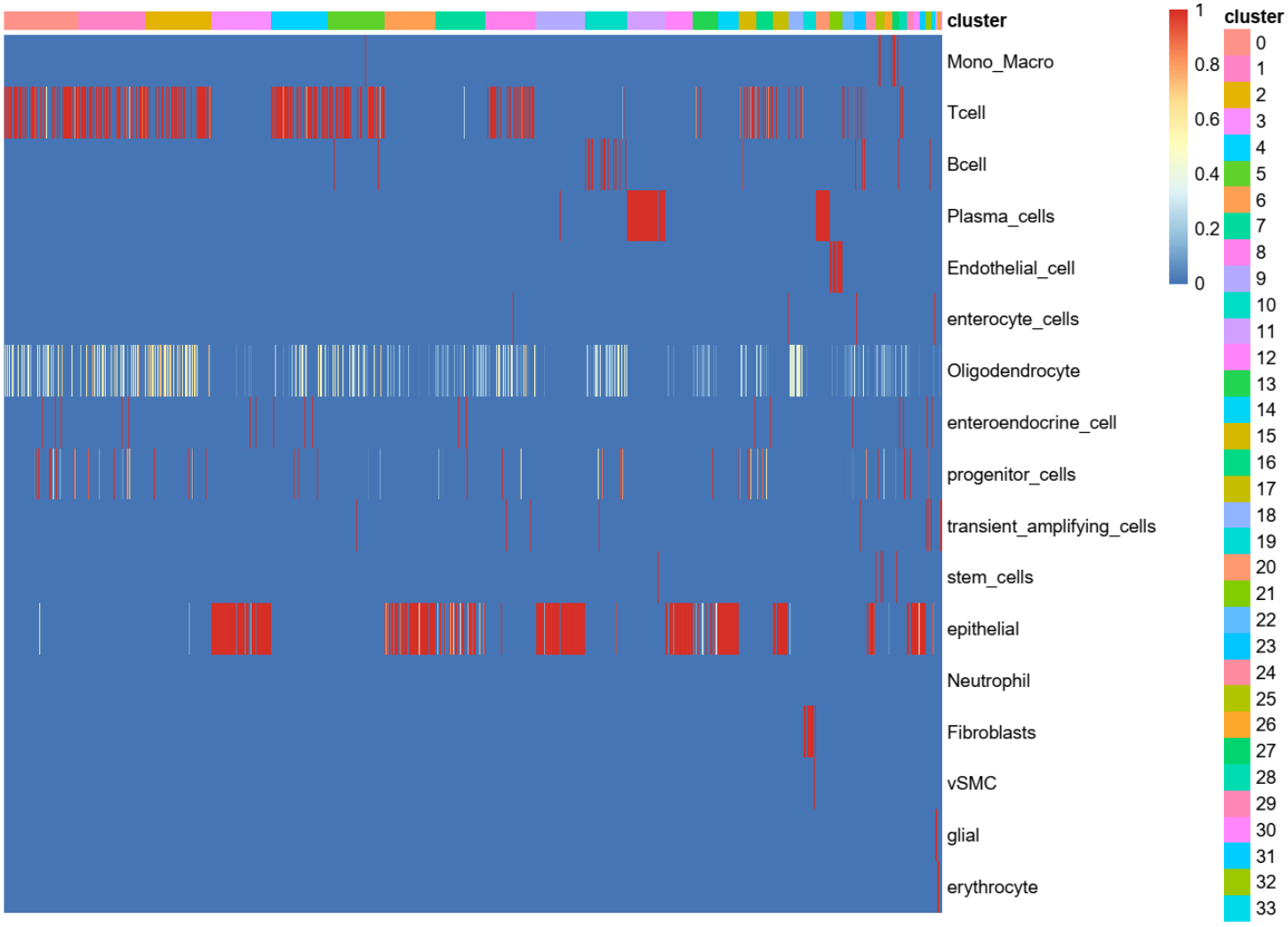

Fig. S4 Cell type annotation based on clustering results from single-cell RNA sequencing in laying hens.

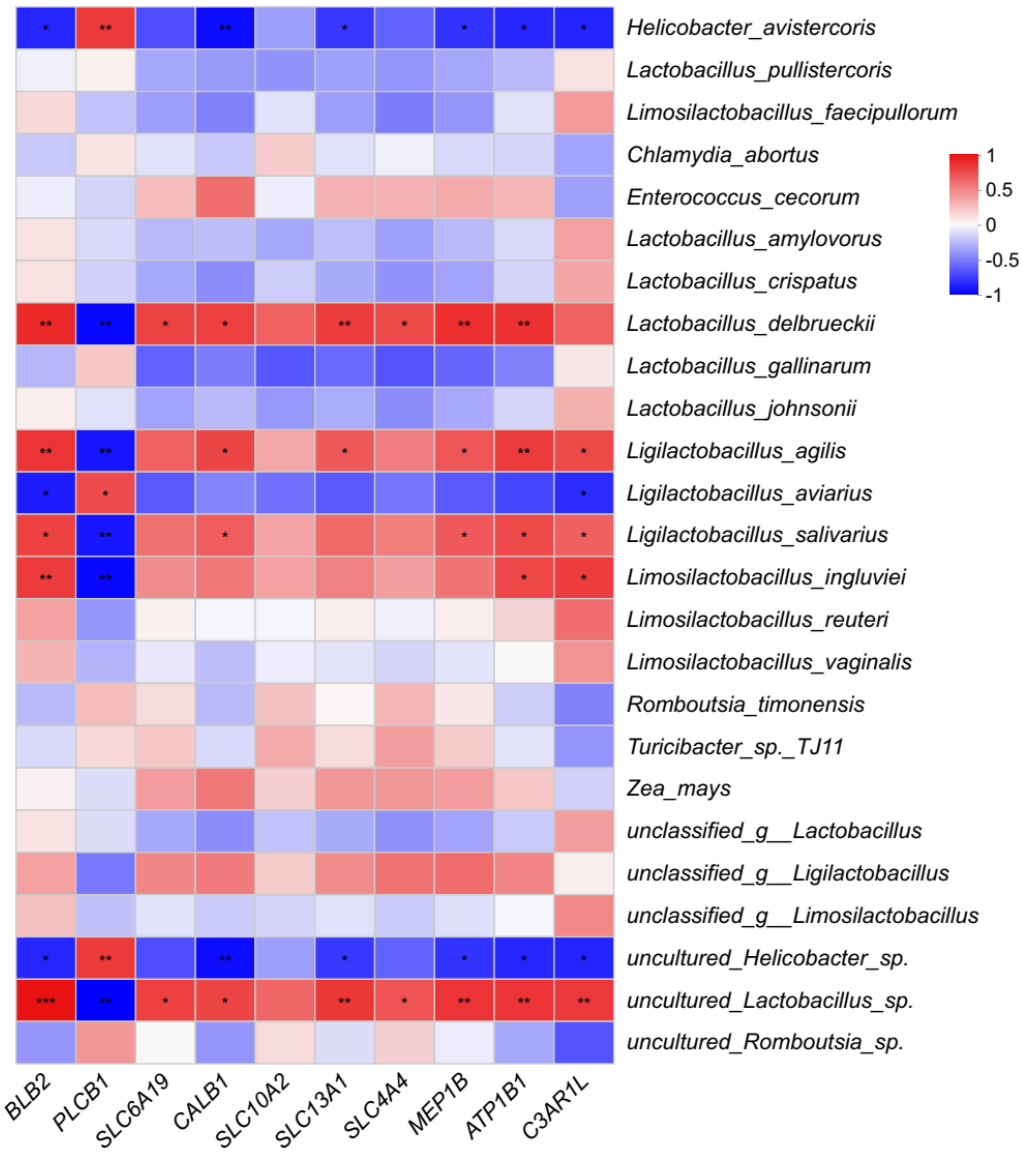

Fig. S5 Correlation analysis between gut microbial taxa and key genes identified by single-cell RNA sequencing in laying hens.

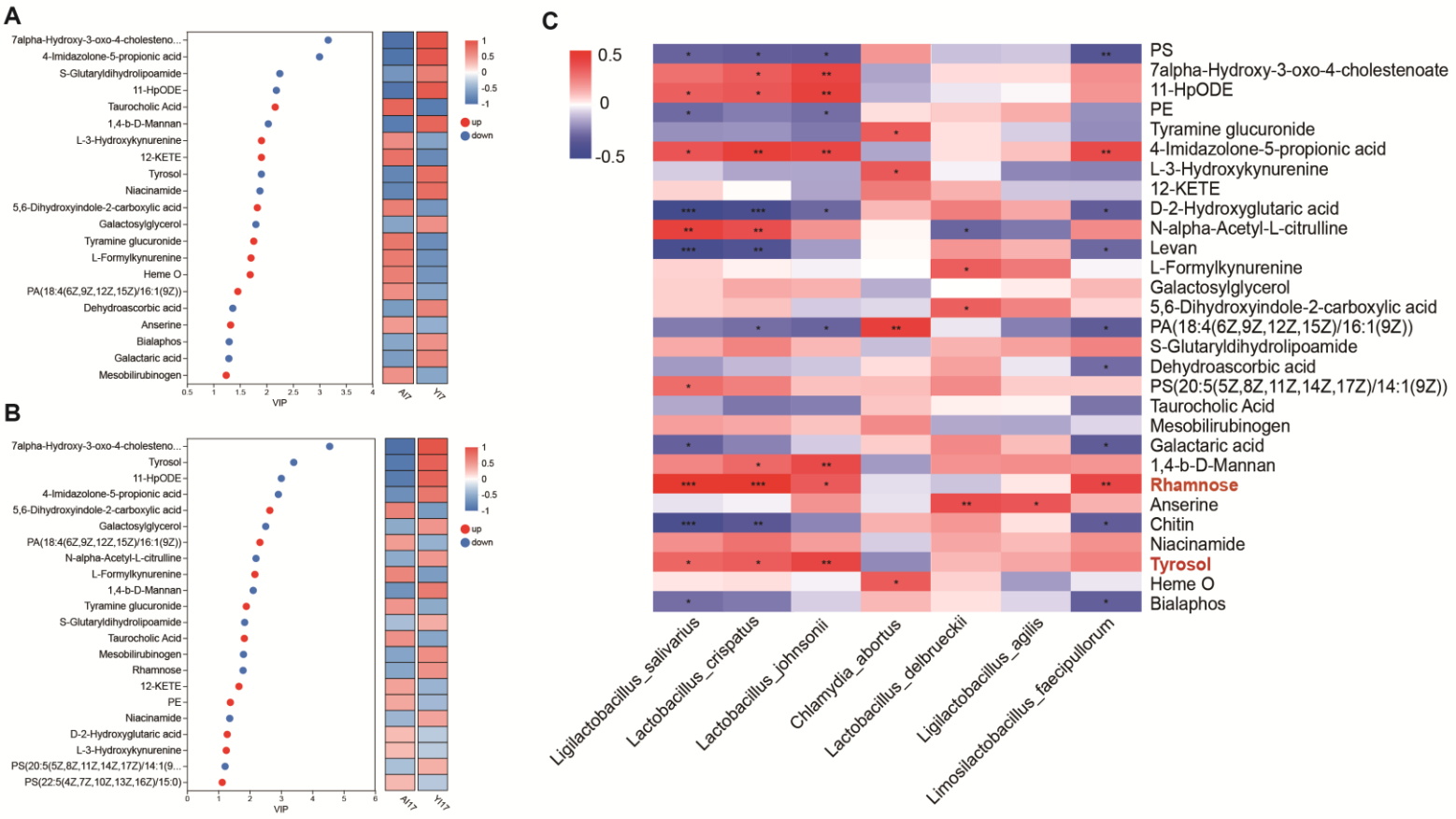

Fig. S6 Importance assessment of gut microbial metabolites and their associations with microbial taxa in laying hens. **A** Differences in metabolite importance during the initial phase of eggshell mineralization between young and aged hens. **B** Differences in metabolite importance during the rapid growth phase of eggshell mineralization between young and aged hens. **C** Correlation analysis between gut microbial taxa and metabolites. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table S1 The composition and nutrient levels of the basal diet (air dry basis)

Ingredients	%	Nutrient levels[2]	%
Corn	61.00	Metabolizable energy, MJ/kg	11.27
Soybean meal	23.86	Crude protein	16.50
Soybean oil	1.20	Calcium	3.47
Wheat bran	3.35	Available phosphorus	0.27
Salt	0.15	Lysine	0.81
Na_2SO_4	0.20	Methionine	0.37
$CaHPO_4$	0.90	Methionine+cystine	0.65
Limestone	8.90		
Premix[1]	0.20		
Choline	0.12		
DL-Methionine	0.12		
Total	100.00		

[1] Provided per kilogram of diet: VA 12,500 IU; VD_3 4,125 IU; VE 15 IU; VK 2 mg; thiamine 1 mg; riboflavin 8.5 mg; calcium pantothenate 50 mg; niacin 32.5 mg; pyridoxine 8 mg; biotin 2 mg; folic acid 5 mg; VB_{12} 5 mg; Zn ($ZnSO_4 \cdot H_2O$) 66 mg; I (KI) 1 mg; Fe ($FeSO_4 \cdot H_2O$) 60 mg; Cu ($CuSO_4 \cdot 5H_2O$) 8 mg; Se (Na_2SeO_3) 0.3 mg.

[2] The nutrient levels were calculated values.