

Supplementary Figures

Multi-omics characterization of the skin microbiota reveals the anti-aging roles of *Stenotrophomonas maltophilia*

Danni Guo^{1,6}, Yuanyuan Chen^{2,6}, Yahong Wu^{1,6}, Jingmin Cheng¹, Yawen Lin¹, Wenjie Lai³, Wentao Ma¹, Hang Yang¹, Lianyi Han^{4,5}, Lan Ma¹, Haidong Jia^{2,*}, Xiao Liu^{1,*}

¹Shenzhen International Graduate School, Tsinghua University, Shenzhen 518055, China

²Shanghai Jahwa United Co., Ltd., Shanghai 200082, China

³Department of Urology, The Affiliated Guangdong Second Provincial General Hospital of Jinan University, Guangzhou 510317, China

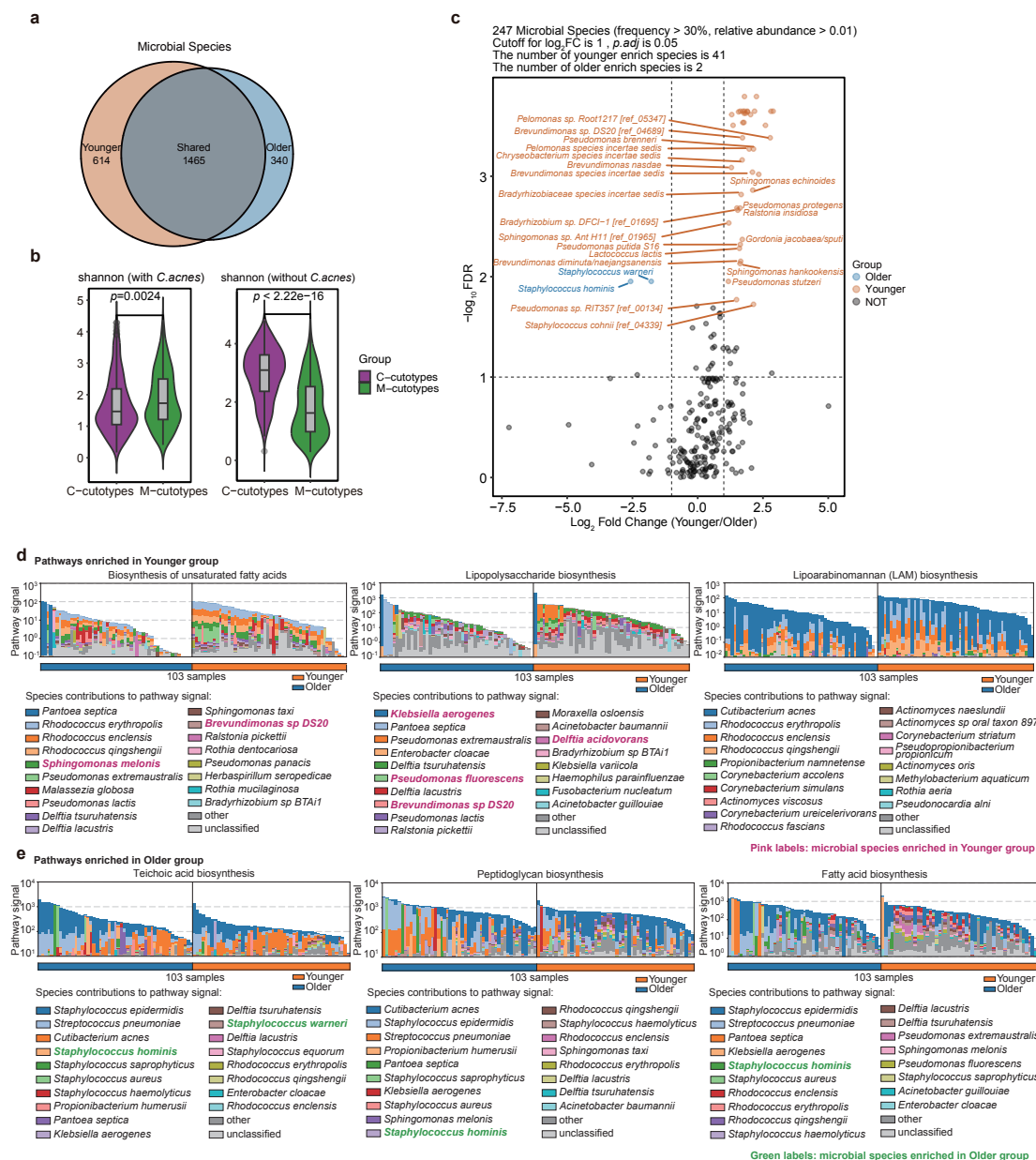
⁴Greater Bay Area Institute of Precision Medicine (Guangzhou), School of Life Sciences, Fudan University, Shanghai 200433, China

⁵Department of Dermatology, Huashan Hospital, Fudan University, Shanghai 200433, China

⁶These authors contributed equally to this work

*Correspondence: liuxiao@sz.tsinghua.edu.cn; jiahaidong@jahwa.com.cn

21 Supplementary figures and figure legends



22

23 **Figure S1. Metagenomic analysis reveals skin aging-related microbial**

24 **dynamics.**

25 (a) Venn diagram displays the distribution of microbial species identified in

26 metagenomic analysis.

27 (b) Violin plots show microbial diversity with and without the influence of C.

28 acnes.

29 (c) Volcano plot illustrates the differential enrichment of 43 microbial species

30 between groups. Volcano plot of microbial species enriched in younger and

older skin microbiomes based on differential abundance analysis using the Wilcoxon rank-sum test. The analysis included 247 microbial species with a frequency >30% and relative abundance >0.01. The analysis used an absolute $\text{Log}_2\text{FoldChange} > 1$ and adjusted $P < 0.05$.

(d) Species-stratified contributions to pathways enriched in the younger group. The y-axis shows pathway signal on a $\log_{10}$ scale. Stacked bars represent species contributions derived from HUMAnN3 stratified outputs (aggregated across pathway KOs). Samples ($n = 103$) are grouped by younger/older; the vertical line separates groups. Species names in pink indicate younger-enriched species.

(e) Species-stratified contributions to pathways enriched in the older group. The y-axis shows pathway signal on a $\log_{10}$ scale. Stacked bars represent species contributions derived from HUMAnN3 stratified outputs (aggregated across pathway KOs). Samples ($n = 103$) are grouped by younger/older; the vertical line separates groups. Species names in green indicate older-enriched species.

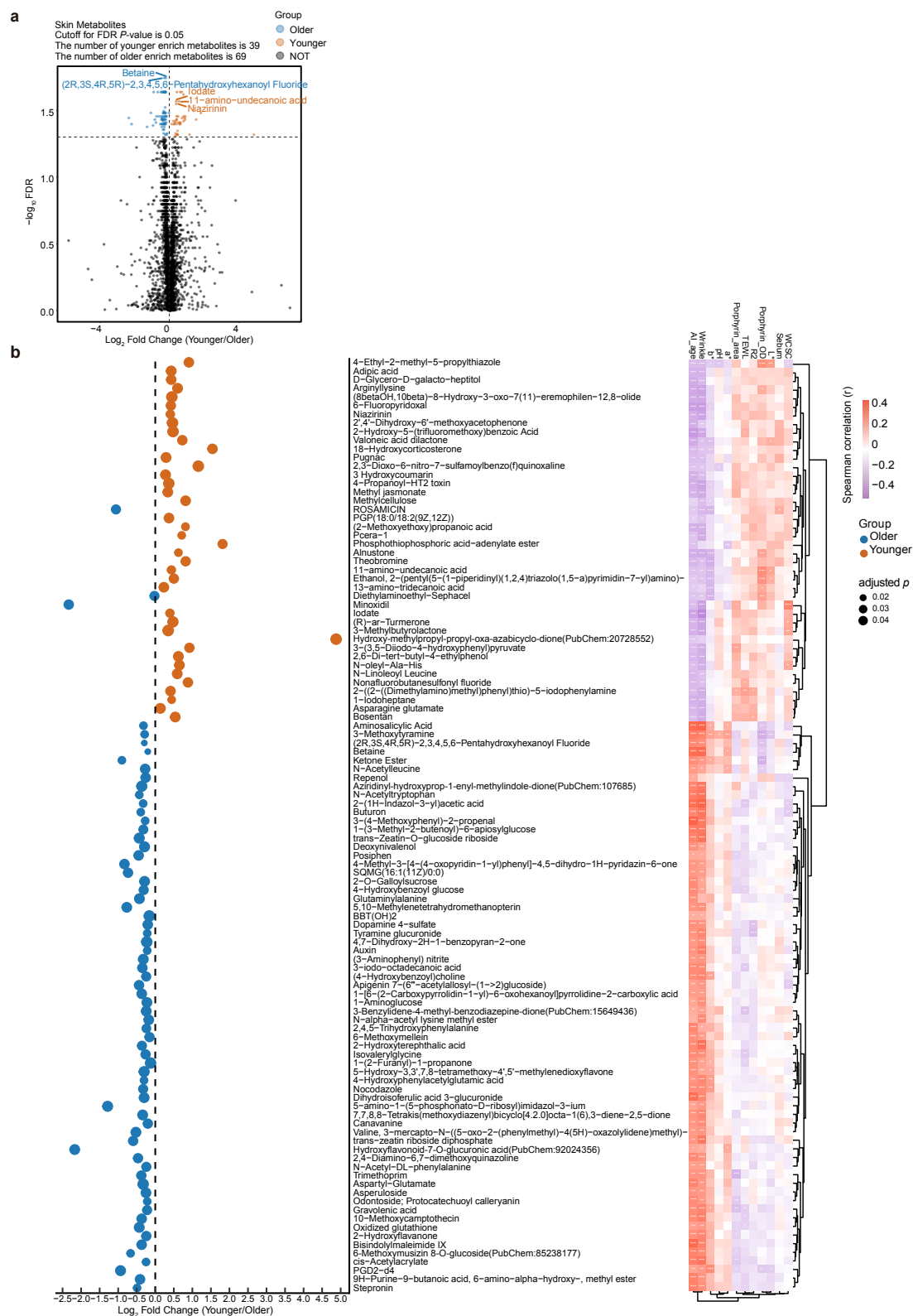


Figure S2. Metabolomic analysis reveals skin-aging-related metabolites.

(a) Volcano plot illustrates the differential enrichment of 108 skin metabolites between groups.

(b) Left: Bubble chart displays all skin metabolites with significant differences between groups (adjusted $P < 0.05$). Right: Heatmap shows the Spearman correlation analysis between all differential skin metabolites and skin phenotypes. Significance levels are indicated as follows: *, adjusted $P < 0.1$; **, adjusted $P < 0.05$; ***, adjusted $P < 0.01$.

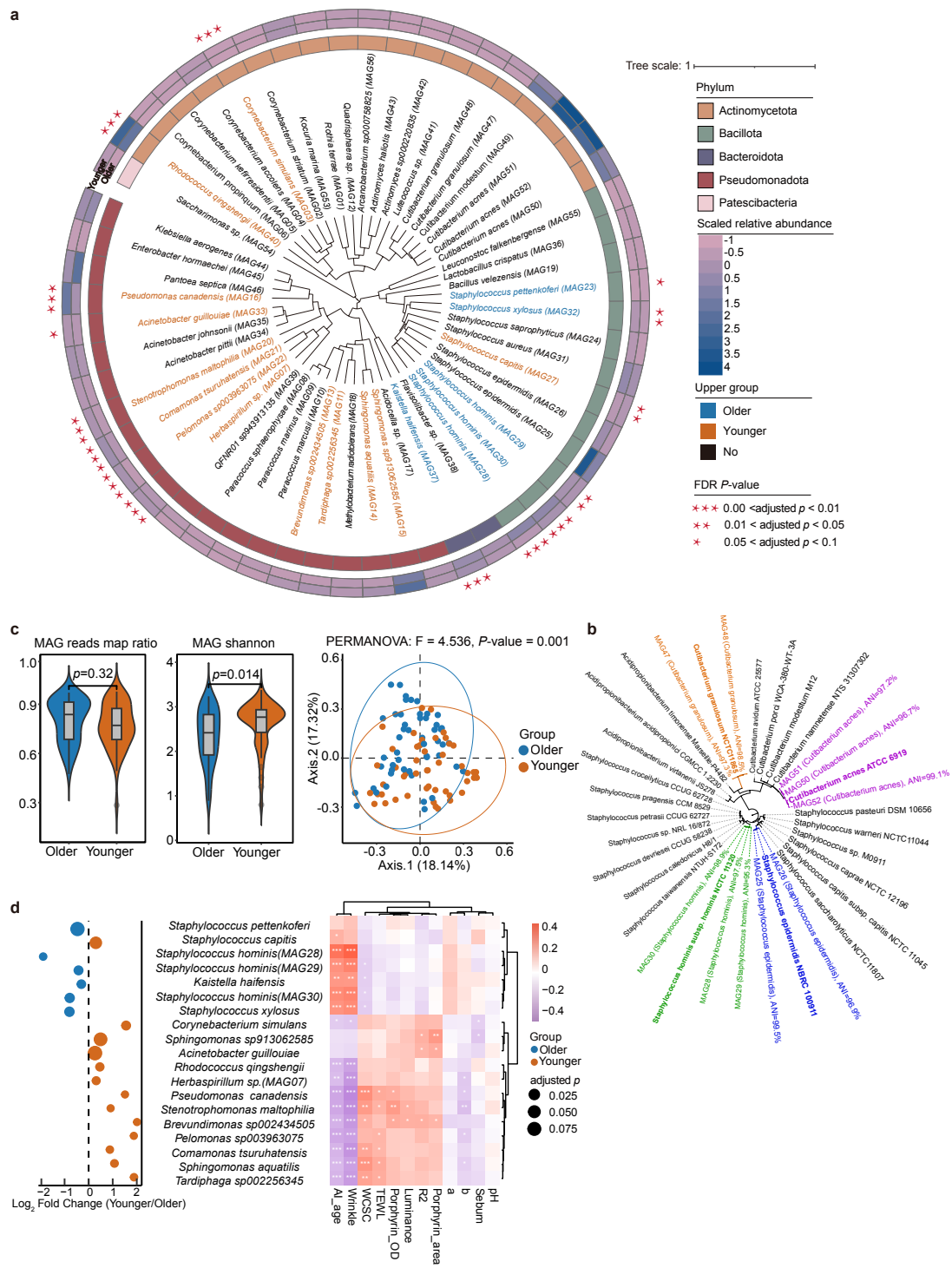


Figure S3. MAGs analysis of age-related differences in skin microbiota.

(a) Phylogenetic tree with heatmap: This visualization combines a phylogenetic tree with a heatmap representing 56 MAGs derived from 103 samples. Microbial names in blue indicate MAGs with higher abundance in the older group, while those in orange represent higher abundance in the younger group. From the center outward, the first circle indicates the phylum, the second circle

shows relative abundance in the older group, the third circle shows relative abundance in the younger group, and the outermost stars indicate significance levels: *, adjusted $P < 0.1$; **, adjusted $P < 0.05$; ***, adjusted $P < 0.01$.

(b) Phylogenetic tree of strain variation: This tree displays different strains of the same species and their positions within the annotated GTDB database. The Average Nucleotide Identity (ANI) values indicate the genetic similarity of each strain to its nearest annotated counterpart.

(c) Left: Violin plots illustrate the ratio of reads mapped to MAGs versus the total clean reads, suggesting that the MAGs can represent a significant portion of the microbial community in both age groups without significant differences. Middle: Violin plots display microbial diversity calculated using the relative abundance of MAGs. Right: PCA plots highlight significant differences in the compositional structure of the MAGs between the groups, underlining the distinct microbial community structures associated with different age groups.

(d) Left panel: Bubble chart displays all MAGs with significant differences between groups (adjusted $P < 0.05$). Right panel: Heatmap shows the Spearman correlation analysis between all differential MAGs and skin phenotypes. Significance levels are indicated as follows: *, adjusted $P < 0.1$; **, adjusted $P < 0.05$; ***, adjusted $P < 0.01$.

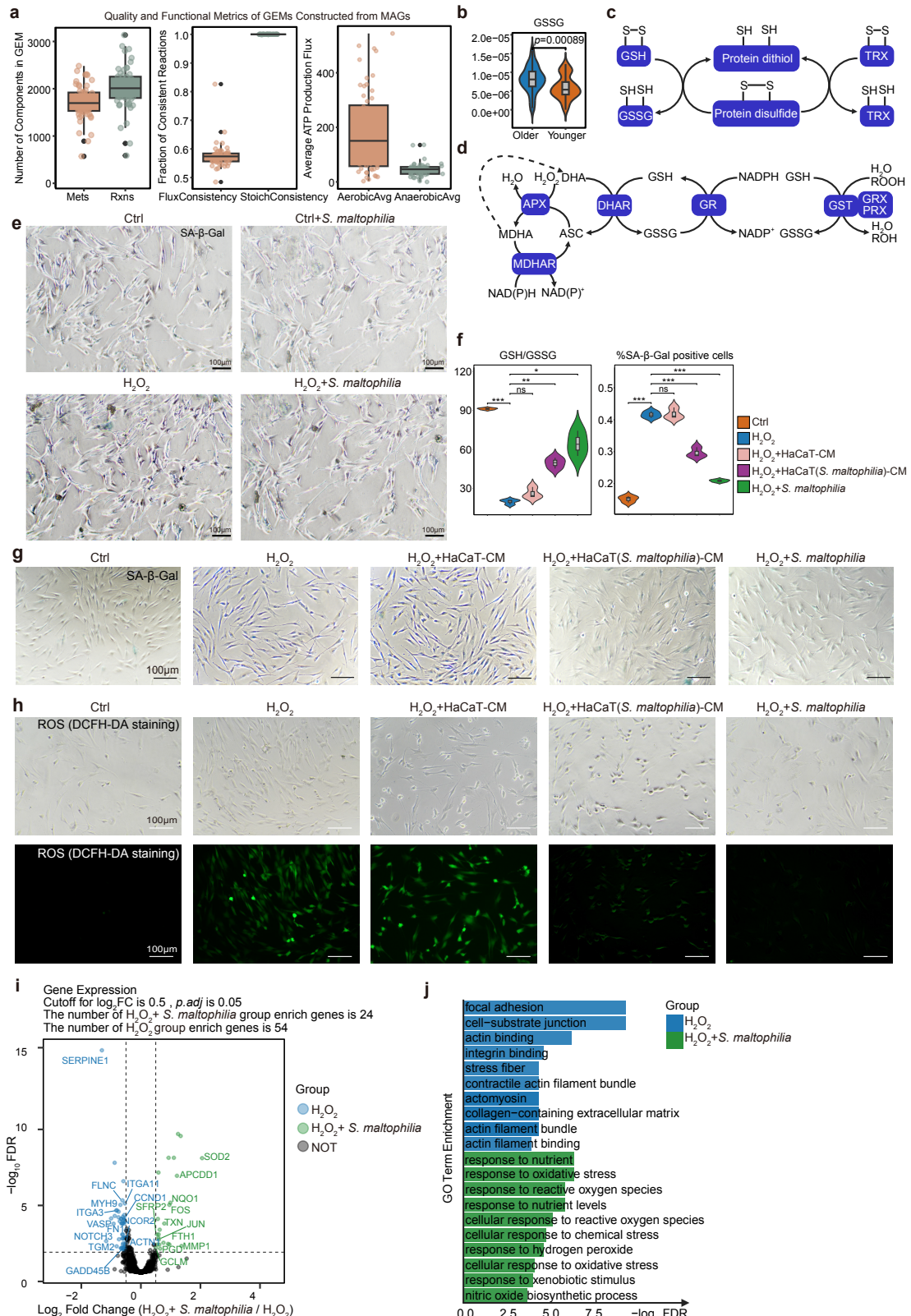


Figure S4. GEM quality assessment and experimental validation reveal antioxidant defense mechanisms.

(a) Reliability assessment of GEMs: Left Box Plot: Displays the number of reactions and metabolites for each MAG derived GEM. This visualization

indicates the complexity and size of each model. Middle Box Plot: Shows the Flux Consistency and Stoichiometric Consistency for each GEM. These metrics evaluate the mathematical and biochemical robustness of the models, ensuring that they accurately represent the metabolic capabilities of the respective microbial communities. Right Box Plot: Compares the average ATP production under aerobic and anaerobic conditions across the GEMs.

(b) Violin plots depicting the significant differences in oxidized glutathione levels between older and younger groups in skin metabolomics. Notable differences are indicated, $P < 0.05$.

(c) This Figure demonstrates how glutathione (GSH) maintains proteins in their reduced thiol states within cellular environments. This reaction illustrates how GSH reduces protein disulfides to free thiol groups, thereby maintaining redox homeostasis and protein functionality.

(d) Glutathione-mediated antioxidant defense pathways: Left module: Ascorbate-dependent hydrogen peroxide metabolism: Monodehydroascorbate reductase (MDHAR) uses NAD(P)H to reduce monodehydroascorbate (MDHA) to ascorbate (ASC). Ascorbate peroxidase (APX) then uses ASC as an electron donor to convert H_2O_2 to water (H_2O). Middle module: Glutathione-dependent regeneration of ascorbate: Dehydroascorbate reductase (DHAR) reduces dehydroascorbate (DHA) back to ASC, consuming glutathione (GSH) and producing oxidized glutathione (GSSG). Right module: Glutathione-dependent peroxide metabolism: Glutathione reductase (GR) uses NADPH to convert GSSG back to GSH. Glutathione S-transferase (GST) and Glutaredoxin (GRX)/Peroxiredoxin (PRX) use GSH to reduce hydrogen peroxide (H_2O_2) and organic peroxides (ROOH) to water (H_2O) and alcohols (ROH).

(e) Representative images of skin primary fibroblasts exhibiting SA- β -Gal activity under different treatment conditions. Staining intensity indicates SA- β -Gal activity, with senescent fibroblasts appearing stained. Scale bars represent 100 μm .

(f) Violin plots showing the percentage of GSH/GSSG ratio (left) and the SA- β -Gal-positive cells (right) in primary human fibroblasts under the indicated treatment conditions:

Ctrl: untreated;

H₂O₂: H₂O₂ treatment to induce oxidative stress-associated senescence),

H₂O₂+HaCaT-CM: HaCaT-conditioned medium(CM);

H₂O₂ +HaCaT(*S. maltophilia*)-CM: conditioned medium from HaCaT cells pre-stimulated with *S. maltophilia* supernatant and then cultured in fresh medium after washout; i.e., supernatant-free CM;

H₂O₂ +*S. maltophilia*: *S. maltophilia* culture supernatant.

P values were calculated using T-test. Significance levels are indicated as follows: ns, *P* > 0.05*; *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

(g) Representative images of skin primary fibroblasts exhibiting SA- β -Gal activity under different treatment conditions. Staining intensity indicates SA- β -Gal activity, with senescent fibroblasts appearing stained. Scale bars represent 100 μ m.

(h) Representative images of intracellular reactive oxygen species (ROS) detected by DCFH-DA staining in skin primary fibroblasts under the different treatment conditions (top, bright-field; bottom, DCF fluorescence). Increased fluorescence intensity reflects elevated intracellular ROS levels. Scale bars, 100 μ m.

(i) Volcano plot of differentially expressed genes between the H₂O₂ alone, and H₂O₂ treatment combined with *S. maltophilia* culture supernatant, analyzed using DESeq2. The analysis used an absolute Log₂FoldChange > 0.5 and adjusted *P* < 0.05.

(j) Bar plot shows the top 10 Gene Ontology (GO) terms with the smallest adjusted *P* enriched in the H₂O₂ alone, and H₂O₂ with *S. maltophilia* groups. The GO terms include categories from Biological Process (BP), Molecular Function (MF), and Cellular Component (CC).

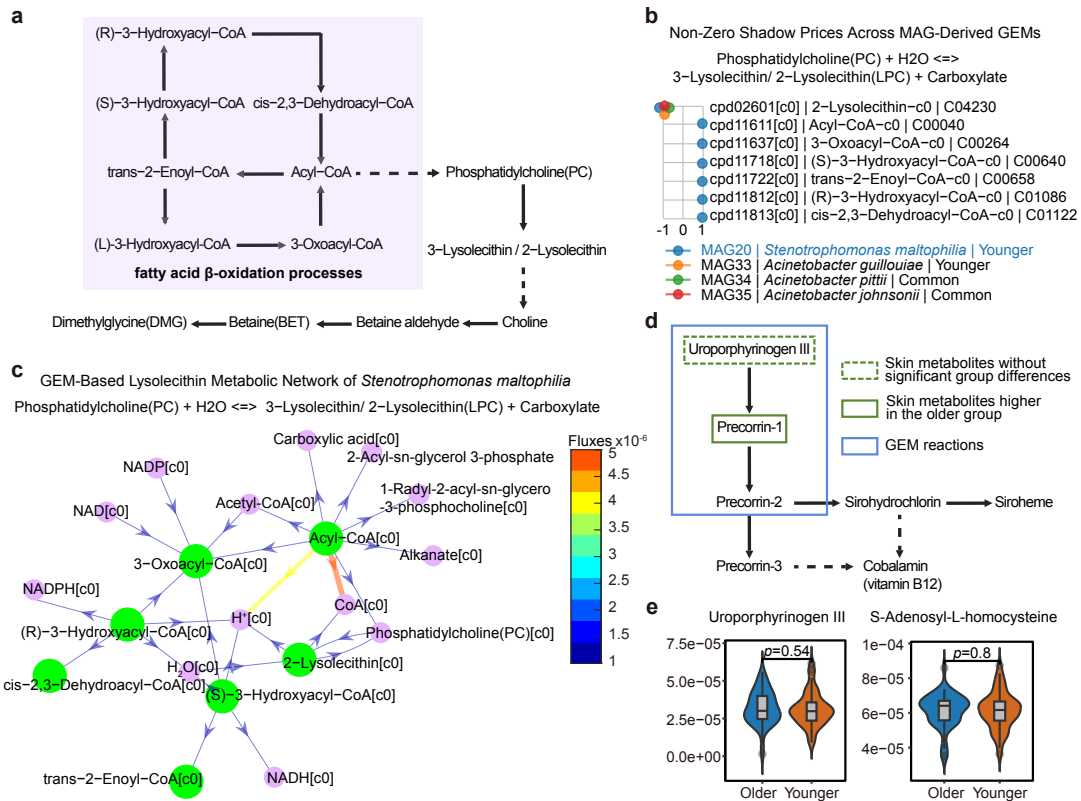


Figure S5. Shadow price analysis from FBA illuminates betaine, lysolecithin synthesis dynamics, and porphyrin metabolism dynamics.

(a) Schematic of the betaine metabolic pathway. This diagram illustrates the metabolic breakdown of lecithin/lysolecithin into choline, followed by subsequent conversions into betaine aldehyde, betaine (BET), and ultimately dimethylglycine (DMG) as a downstream product. Additionally, the fatty acid β -oxidation pathway is highlighted for its crucial role in generating acyl-CoA intermediates, which contribute to the synthesis and metabolism of lecithin/lysolecithin, thereby linking lipid metabolism with choline production. The dashed lines indicate simplified steps in the metabolic pathway, excluding intermediate metabolites for clarity.

(b) Shadow price analysis from FBA in lysolecithin synthesis reactions across different MAGs. The x-axis, y-axis, and color-coded dots follow a similar format to that in Figure 4a.

(c) Network and flux analysis of lysolecithin synthesis in *S. maltophilia* GEM: This provides a visual representation of the metabolic network involved in

lysolecithin synthesis within *S. maltophilia*, annotated with flux values to indicate the intensity and direction of each metabolic step. The diagram serves to elucidate the complex interaction processes facilitating the synthesis of lysolecithin.

(d) Porphyrin and Vitamin B12 biosynthesis pathway in skin microbiome and host co-metabolism: This diagram illustrates the co-regulated metabolic pathway for porphyrin and Vitamin B12 synthesis involving skin microbiome and host interactions. The pathway initiates with Uroporphyrinogen III, leading to the sequential synthesis of Precorrin 1, Precorrin 2, and further downstream products, culminating in the production of Vitamin B12. Dashed green outlines denote skin metabolites with no significant differences between groups, solid green outlines indicate metabolites with higher abundance in the older group, and blue outlines represent GEM reactions.

(e) Violin plots illustrate the concentrations of critical metabolites, including Uroporphyrinogen III and S-Adenosyl-L-homocysteine, involved in the porphyrin metabolism pathway. The plots compare concentrations between older and younger groups. Despite variations, no statistically significant differences are indicated, p -value > 0.05.



Figure S6. Shadow price analysis from FBA illuminates tyrosine metabolism dynamics.

(a) Violin plots illustrate the distribution of key metabolites involved in tyrosine metabolism within the skin metabolomics data. The plots compare

concentrations between older and younger groups. Despite variations, no statistically significant differences are indicated, p -value > 0.05.

(b) FBA Shadow price analysis for the conversion of DHPAA to DOPAC in tyrosine metabolism: The Figure illustrates the shadow prices derived from FBA. The x-axis represents shadow price values, indicating the influence of each metabolite on this metabolic reaction. Positive values suggest that increasing the metabolite concentration drives the reaction forward. The colors represent different MAGs, highlighting the differential impact across microbial strains.

(c) FBA shadow price analysis for the conversion of HMA to HVA in tyrosine metabolism. The x-axis, y-axis, and color-coded dots follow a similar format to that in Figure S4B.

Supplementary tables

Table S1. Assessment of skin physiological traits, related to Figure 1.

Table S2. Metagenomic analysis of microbial species, diversity, and functional pathways, related to Figure 2.

Table S3. Comprehensive metabolomic analysis, differential core metabolites, and pathway enrichment, related to Figure 3.

Table S4. Analysis of metagenome-assembled genomes (MAGs) across samples, related to Figure S3.

Table S5. Genome-Scale Metabolic Modeling (GEM), experimental validation of *S. maltophilia*'s antioxidant and anti-aging roles, related to Figure 4.

Table S6. Comprehensive shadow price analysis of Betaine, Lysolecithin, Porphyrin, DHPAA, and HMA metabolism across MAGs in skin aging, related to Figures 5 and 6.