

Combining morphology, transcriptomics, and metabolomics to analyze the core regulatory program for regulating coral growth

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ABSTRACT

The coral reef ecosystem is gradually declining and on the verge of extinction. The crux of its continuous degradation lies in the fact that the growth and recovery rate of corals is far outpaced by their loss rate. Currently, our knowledge about the mechanisms governing coral growth and development remains quite limited. In this study, we developed a high-precision morphological technology approach based on photon-counting computed tomography (PCCT-CIPT). Integrated multi-omics analyses with functional experiments, uncovered a host-centered, synchronous developmental mechanism of polyp-skeleton growth that is reproducibly identified across different coral species and habitats. The Wnt-fibroblast growth factor 10 (FGF10)-MAPK/mTOR signaling axis regulates cell division and differentiation processes through *c-Myc* and *Sox9*, effectively promoting the development and maturation of coral polyp morphology. TGF- β signaling activates SMAD5, guiding cells toward differentiation into the biomineralization-associated pathways. As the polyp matures, the AMPK signaling pathway is specifically upregulated, achieving precise regulation of cell proliferation and differentiation by inhibiting the mTOR signaling pathway. During this process, the expression of genes related to biomineralization (including *CA2*, *Lrp5*, and *CLMD*) is gradually upregulated, enabling the transformation of coral skeletons from the construction of primary frameworks to high-density stable structures. In the early stages of coral development, steroid hormones play a key regulatory role in driving the progression of the cell cycle and the initial construction of the skeletal framework. This study preliminarily reveals a host-controlled program regulating coral growth and development, which may offer potential targets for further exploration in coral reef restoration.

KEYWORDS: Scleractinia; Coral growth; Coral reef restoration; Polyp-skeleton

synchronous growth; FGF10 signaling axis

INTRODUCTION

Coral reefs constitute the foundational architectures of marine biodiversity, underpinning the stability and productivity of coastal ecosystems worldwide (Minjie et al., 2020; Morrissey et al., 2023; Putnam et al., 2017). However, these vital ecosystems face an unprecedented crisis, as climate change and anthropogenic activities drive escalating episodes of mass bleaching and disease (De'ath et al., 2009; Travesso et al., 2023; Wepfer et al., 2020). Under current trajectories, many coral reef ecosystems risk functional collapse by the mid-century (Fu et al., 2025; Morrison et al., 2019; Oury et al., 2023). The structural integrity and resilience of coral reef ecosystems fundamentally hinge on the growth of scleractinian corals. The key reason for the continuous decline of coral reef ecosystems lies in the fact that the growth and recovery volume of coral reefs is far lower than their loss volume (Atkinson et al., 1995). In addition, the fragment cultivation technique is the most widely utilized method in the restoration of coral reef ecosystems, primarily relying on the inherent growth capacity of corals (Han et al., 2025). Understanding the intrinsic mechanisms that govern coral growth is therefore not only a fundamental biological question but also an urgent imperative for developing effective conservation strategies (Rinkevich, 2021). Up to the present, research on coral growth has primarily concentrated on the impacts of individual environmental stressors (e.g., elevated temperature or ocean acidification) on the calcification capacity of coral skeletons, as well as elucidating intrinsic skeletal biomineralization mechanisms (Albright et al., 2016; Li et al., 2025). However, the developmental processes of soft tissues like polyps

adhering to the surface of coral skeletons, along with the synergistic interaction mechanisms between the polyp-skeleton, still lack systematic explanation. In fact, the complete development of corals necessitates achieving a dynamic balance between skeletal construction and soft tissue growth. Only through such a harmonious interplay between polyp and skeleton can the normal growth and development of corals be ensured, thereby maintaining the structural stability and functional integrity of coral reef ecosystems.

In contrast, research in vertebrate models has established that signaling pathways such as the Fibroblast Growth Factor (FGF) system are master regulators of tissue polarity, coordinating cell proliferation and differentiation to shape structures (Lemmon & Schlessinger, 2010; Lingfeng et al., 2023). The coordinated actions of FGF signaling, along with various other signaling pathways including Bone morphogenetic proteins (BMP), Transforming growth factor β (TGF- β), parathyroid signaling, Hedgehog (Hh) signaling, and Wntless-type MMTV integration site (Wnt) signaling, collectively regulate the process of biomineralization (Chen et al., 2019; Chijimatsu & Saito, 2019; Duan et al., 2021). Similarly, steroid hormones like glucocorticoids systemically integrate following the loss of their symbiotic zooxanthellae and subsequent separation from the skeletal framework (Simon et al., 2025). Whether these ancient regulatory modules function in the spatial control of growth in cnidarians remains an open question, lacking direct in situ evidence. This multi-pathway coordinated regulatory model ensures precise matching of tissue growth rate, mineralization degree, and structural function during skeletal development in vertebrates. Whether these regulatory modules play a role in the spatial growth regulation of cnidarians such as corals remains to be further investigated.

In our earlier coral domestication experiments, we observed significant heterogeneity in branch-tip elongation rates, as well as in polyp color and morphology. Notably, similar morphological zonation was also observed in diverse coral species from natural marine environments, with distinct differences in polyp color and developmental stage (Supplementary Figure S1). To better detect the dynamic changes in both internal and external morphology during coral growth, this study developed a high-precision morphological technology targeting coral samples based on photon-counting computed tomography (PCCT). By integrating transcriptomics and metabolomic analysis, we determined the dominant regulatory role of the coral host during its growth process. Importantly, we demonstrated the conservation of this growth mechanism across different habitats (corals cultured in the laboratory and those growing in natural marine areas) and different coral species (*Montipore angulata*, *Acropora acuminata*, and *Pocillopora meandrina*). By combining in-situ molecular imaging, receptor–ligand interaction assays, and symbiont functional experiments, we established the first host-centered developmental framework for scleractinian corals. These findings deepen our understanding of growth regulation in reef-building corals and may offer clues for coral artificial propagation and reef restoration.

MATERIALS AND METHODS

Experimental design and coral collection

We defined the regions with relatively high linear extension rates of coral as "growth zones," and the regions where linear extension was nearly arrested as "static zones." Based on the

morphological characteristics of the polyps, the growth zone was further subdivided from apex to base into three subregions: G1 (white polyps with underdeveloped oral discs and tentacles), G2 (transitional pigmentation with fully developed morphology), and G3 (fully developed, uniformly brown polyps). The static zone was designated as S1, in which polyps exhibited fully developed morphology and brown coloration comparable to that of G3.

M. angulata colonies were collected from Sanya, Hainan Province, China (Coordinates: N18°14'00", E109°22'11"). After collection, the samples were grown in an artificial coral tank at the Tropical Marine Institute, measuring 150×60×50cm. The artificial seawater for the tank was prepared using a Haier water purifier and Pure Ocean artificial sea salt, maintaining a salinity range of 33±1‰ (Minjie et al., 2020; Wiedenmann et al., 2023). The artificial seawater was then treated with live rock for two months to remove algae before introducing *M. angulata*, as well as other coral species, clownfish, spotted fish, seahorses, and hermit crabs. The temperature of the tank was kept at a consistent 80°F, with 25% of the seawater being replaced every 1-2 weeks. Lighting for the tank was provided by CoralCare LED (PHILIPS) with a 12-hour on and 12-hour off cycle, running at 60% power between 10:00 and 19:00 daily (Minjie et al., 2020; Wiedenmann et al., 2023). The spatial heterogeneity exhibited during the process of coral artificial cultivation provides a foundation for our research on coral growth mechanisms. For cross-species validation, *A. acuminata* and *P. meandrina* colonies were collected from Fenjiezhou Island, Hainan Province, China (Coordinates: N18°34'51", E110°11'47"). Upon collection, the G1 and S1 zones were immediately dissected, frozen in liquid nitrogen, and stored at -80°C until further processing for transcriptomic and metabolomic analyses.

Development of PCCT-CIPT algorithm

To explore the mechanisms underlying spatial heterogeneity in coral growth, eleven typical coral species were selected, and the PCCT-CIPT method was developed for coral phenotypic detection. The scanning parameters were set as follows: voltages of 15 and 40 kV, a current of 200 mA, and a resolution of 20 μm , ensuring adequate capture of details. To prevent motion artifacts during scanning, the coral samples were fixed on a customized holder and positioned at the center of the scanner. The obtained two-dimensional projection data were reconstructed into a three-dimensional (3D) voxel model using the filtered back-projection algorithm. During the reconstruction process, preprocessing methods such as noise suppression and artifact correction were employed to enhance image quality. The reconstructed voxel data were stored in DICOM format for subsequent analysis and processing. The two-dimensional projection data, once scanned, were reconstructed into a 3D voxel model through the filtered back-projection algorithm. Again, preprocessing techniques, including noise suppression and artifact correction, were applied during this reconstruction phase to improve image quality. The reconstructed voxel data were stored in DICOM format, facilitating further analysis and manipulation.

$$V_p = A_{\text{pixel}} \times \Delta Z \quad (1)$$

$$V_{\text{slice}} = N \times V_p \quad (2)$$

$$V_{\text{total}} = \sum_{i=1}^n V_{\text{slice},i} \quad (3)$$

$$\rho = M/V_{total} \quad (4)$$

V_p : Pixel volume; A_{pixel} : Area per pixel; Δz : Section thickness; V_{slice} : Coral skeleton volume; N : Split the number of pixels in the layer; V_{total} : Coral volume; i : The i -th slice; ρ : The density of coral skeleton; M : The quality of coral skeleton.

Utilizing the reconstructed 3D voxel model, the coral structure was first segmented to remove the background and noise. The Marching Cubes algorithm was adopted to extract the surface mesh model of the coral from the voxel data. The volume was calculated by summing the volumes within the voxels, while the surface area was obtained by summing the areas of the triangular facets of the mesh model. To improve the accuracy of the calculations, the mesh model underwent smoothing treatment, reducing the jagged effects associated with voxelization.

$$A_{triangle} = \frac{1}{2} \times \|(P_2 - P_1) \cdot (P_3 - P_1)\| \quad (5)$$

$$A_{total} = \sum_{j=1}^n A_{triangle,j} \quad (6)$$

$A_{triangle}$: The area of a single triangle; P_1, P_2, P_3 : The coordinates of the three vertices of a triangle are points in 3D space; $\cdot$: Vector dot product; $\|$: Vector modulus; A_{total} : The sum of the surface areas of all triangles; j : The j -th slice.

ITS2 sequencing zooxanthellae identification

Total zooxanthellae genomic DNA was extracted from G1, G2, G3, and S1 samples using the E.Z.N.A.[®] DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's instructions. The quality and concentration of DNA were determined by 1.0% agarose gel

electrophoresis and a NanoDrop2000 spectrophotometer (Thermo Scientific, United States) and kept at -80 °C prior to further use. The hypervariable ITS2 region of the Symbiodiniaceae nuclear ribosomal DNA was amplified using the primer pair ITS2-F (5'-GAATTGCAGAACTCCGTG-3') and ITS2-R (5'-GGGATCCATATGCTTAAGTTCAGCGGGT-3') on a T100 Thermal Cycler (Bio-Rad, USA). The PCR reaction mixture including 4 µL 5×Fast Pfu buffer, 2 µL 2.5 mM dNTPs, 0.8 µL each primer (5 µM), 0.4 µL Fast Pfu polymerase, 10 ng of template DNA, and ddH₂O to a final volume of 20 µL. PCR amplification cycling conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s, and single extension at 72 °C for 10 min, and end at 4 °C. The PCR product was extracted from 2% agarose gel and purified using the PCR Clean-Up Kit (YuHua, Shanghai, China) according to manufacturer's instructions and quantified using Qubit 4.0 (Thermo Fisher Scientific, USA). Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina PE300 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Detection of chlorophyll *a* content and zooxanthellae density

To each bag containing a coral sample, 10 mL of filtered seawater (0.22 µm) was added, and the coral tissue was detached using a high-pressure air gun (Anderson-King et al., 2023). This process was repeated three times, and the homogenized tissue was then transferred to a 5 mL centrifuge tube for zooxanthellae density and chlorophyll *a* content analysis (Anderson-King et al., 2023). The remaining homogenized coral tissue was stored at -80°C for future use. The

number of zooxanthellae was counted using a hemocytometer, with each sample being repeated six times. The chlorophyll *a* content of the samples was detected using the Chlorophyll Assay Kit R30353 (Shanghai Yuanye Biotechnology Co., Ltd.). The coral skeleton volume data obtained by PCCT was used to normalize the zooxanthellae density and chlorophyll *a* content of the samples.

Transcriptome Analysis

We conducted transcriptomic analysis on the coral growth zones G1, G2, G3, and the static zone S1, with three independent biological replicates per zone (each replicate derived from a separate coral colony). Total RNA was extracted from each biological sample by TRIzol™ (Thermo Fisher Scientific, USA) on dry ice. RNA concentration and purity was measured using NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE). A total amount of 1 µg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using Hieff NGS Ultima Dual-mode mRNA Library Prep Kit for Illumina (Yeast Biotechnology (Shanghai) Co., Ltd.) and library quality was assessed on the Agilent Bio-analyzer 2100 system. The libraries were sequenced on an Illumina NovaSeq X Plus platform to generate 150 bp paired-end reads. The raw reads were further processed with a bioinformatic pipeline tool, BMKCloud (www.biocloud.net) online platform. The StringTie Reference Annotation Based Transcript (RABT) assembly method was used to construct and identify both known and novel transcripts from Hisat2 alignment results. To ensure data accuracy, we adopted a stepwise annotation strategy. Clean reads were first aligned to reference transcriptome databases of the coral host and zooxanthellae. Unigenes that matched these references were assigned the suffixes _coral or _Symbiodiniaceae,



respectively. The remaining unannotated reads were then aligned to the NCBI non-redundant protein database for animals and plants; Unigenes identified through this step were assigned the suffixes _coral-like or _Symbiodiniaceae-like to indicate their putative origin. To minimize false positives and maintain analytical rigor, we preferentially selected genes with the suffixes _coral and _Symbiodiniaceae for downstream expression analysis and result interpretation, as these represented more confidently assigned transcripts (**Supplementary Data S1, S2**). Gene expression levels were quantified as transcripts per million (TPM). Differential expression analysis of two conditions/groups was performed using the DESeq2. The resulting *P* values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted *P*-value < 0.01 & Fold Change ≥ 2 found by DESeq2 were assigned as differentially expressed. A Venn analysis was performed on the differentially expressed genes (DEGs) of different categories, and KEGG and GO enrichment analyses were performed on the shared DEGs.

Metabolome Analysis

We performed metabolomic analysis on the coral growth zone G1 and the static zone S1, with six independent biological replicates per zone (each replicate derived from a separate coral colony). The LC/MS system for metabolomics analysis is composed of Waters Acquity I-Class PLUS ultra-high performance liquid tandem Waters Xevo G2-XS QToF high resolution mass spectrometer. The column used is purchased from Waters Acquity UPLC HSS T3 column (1.8 μm 2.1 × 100 mm). Negative ion mode: mobile phase A: 0.1% formic acid aqueous solution; mobile phase B: 0.1% formic acid acetonitrile. Injection volume 1 μL. The elution gradient was as follows: 0 min, 98% A/2% B; 0.25 min, 98% A/2% B; 10.0 min, 2%

A/98% B; 13.0 min, 2% A/98% B; 13.1 min, 98% A/2% B; 15.0 min, 98% A/2% B. The parameters of the ESI ion source are as follows: Capillary voltage: 2000V (positive ion mode) or -1500V (negative ion mode); cone voltage: 30V; ion source temperature: 150°C; desolvent gas temperature 500°C; backflush gas flow rate: 50L/ h; Desolventizing gas flow rate: 800L/h. The raw data collected using MassLynx V4.2 is processed by Progenesis QI software for peak extraction, peak alignment and other data processing operations, based on the Progenesis QI software online METLIN database and Biomark's self-built library for identification, and at the same time, theoretical fragment identification and mass deviation all are within 100ppm. After normalizing the original peak area information with the total peak area, the follow-up analysis was performed. Principal component analysis and Spearman correlation analysis were used to judge the repeatability of the samples within group and the quality control samples. The identified compounds are searched for classification and pathway information in KEGG, HMDB and lipidmaps databases. According to the grouping information, calculate and compare the difference multiples, T test was used to calculate the difference significance *P* value of each compound. The R language package ropis was used to perform OPLS-DA modeling, and 200 times permutation tests was performed to verify the reliability of the model. The VIP value of the model was calculated using multiple cross-validation. The method of combining the difference multiple, the *P* value and the VIP value of the OPLS-DA model was adopted to screen the differential metabolites. The screening criteria are $FC > 1$, $P < 0.01$ and $VIP > 1$. The difference metabolites of KEGG pathway enrichment significance were calculated using hypergeometric distribution test.

Integrated Transcriptome and Metabolome Analysis

A comprehensive joint analysis of the transcriptome and metabolome was conducted to uncover the key metabolic pathways involved in the rapid growth process of coral G1, while also providing a detailed discussion on the changes in key metabolites and genes associated with the crucial enzymes in these pathways (**Supplementary Data S3, S4**). The DEGs and DAMs in the steroid hormone biosynthesis between G1 and S1 were normalized and visualized using Omicshare (<https://www.omicshare.com/>), and subsequently illustrated using Ado-be Illustrator 2022 software.

Real-time Quantitative PCR Detecting System (qRT-PCR)

To verify the reliability of the transcriptomic data, we conducted qRT-PCR experiments. Total RNA was extracted from each biological sample by TRIzol™ (Thermo Fisher Scientific, USA) on dry ice. This RNA was subsequently converted into complementary DNA (cDNA) employing the MonScript™ RTIII ALL-in-One Mix with dsDNase (Monad, CHN). For the execution of RT-qPCR, the GS AntiQ qPCR SYBR Green Fast Mix (Universal) (Genesand Biotech Co.,Ltd, CHN). The primers utilized in the qRT-PCR process are detailed in **Supplementary Table S1**. Each reaction mixture comprised 0.4 μL of 10 μM forward and reverse primers, 10 μL of 2×GS AntiQ qPCR SYBR Fast Mix(Universal), 8.2 μL of nuclease-free water, and 1 μL of cDNA template. The RT-qPCR was conducted using a Gene-9660 Real-Time PCR system, with an initial denaturation step at 95°C for 30 seconds, followed by a cycling protocol of 95°C for 10 seconds, 60°C for 10 seconds, and 72°C for 30 seconds, with plate reading after each cycle for a total of 40 cycles. The cycle threshold (Ct) value obtained served as an indicator of gene expression levels. To standardize the expression of target genes, it was normalized to the expression of actin within the same sample (ΔCt).

Further normalization was done relative to the average expression of the respective gene in control samples ($\Delta\Delta C_t$). The final expression level of each gene was computed using the formula $2^{-\Delta\Delta C_t}$.

Yeast two-hybrid (Y2H) assay

To verify the interaction between FGF10 protein and FGFR4 protein, we conducted a Y2H experiment. The *FGF10* gene and the *FGFR4* gene were cloned into the pGBKT7 and pGADT7 vectors, respectively, and then co-transformed into the yeast strain AH109 (Shanghai Xuanya Biotechnology Co., Ltd.) Secondly, an auto-activation assay was conducted. Eight yeast transformants were randomly picked from each of the control groups (pGBKT7-*FGF10* co-transformed with pGADT7 in AH109) and subjected to PCR detection and preservation. From the PCR-confirmed single colonies, three colonies were randomly selected from each group and adjusted to $OD_{600}=0.2$. These colonies were then spotted onto SD-TL, SD-TLH, SD-TLHA, and SD-TLHA+X- α -gal plates and incubated at 30°C for 3-5 days (SD-TL: -trp, -leu; SD-TLH: -trp, -leu, -his; SD-TLHA: -trp, -leu, -his, -ade). To suppress auto-activation, the clones of pGBKT7-*FGF10*+pGADT7, pGBKT7-p53+pGADT7-LargeT, and pGBKT7-LaminC+pGADT7-LargeT were resuspended in 2 mL of sterile ddH₂O, adjusted to $OD_{600}=0.2$, and spotted onto SD-TLH plates containing 0, 2.5, 5, 10, 20, 30, 40, 50, 75, and 100 mM of 3-amino-1,2,4-triazole (3AT). Finally, an interaction verification was performed. The experimental group (pGBKT7-*FGF10*+pGADT7-*FGFR4*), control groups (pGBKT7+pGADT7-*FGFR4* and pGBKT7-*FGF10*+pGADT7), positive control (pGBKT7-p53+pGADT7-largeT), and negative control (pGBKT7-laminC+pGADT7-largeT) were resuspended in 2 mL of sterile ddH₂O, adjusted to $OD_{600}=0.2$, and diluted to

three concentrations of 10^0 , 10^1 , 10^{-2} . Ten microliters of each dilution were spotted onto SD-TL and SD-TLH+2.5 mM 3AT plates for the assay.

Weighted gene co-expression network analysis (WGCNA)

Using the WGCNA package in R software version 4.3.3, we constructed a co-expression network based on the processed transcriptome data (Langfelder & Horvath, 2008). The network was built by calculating the pairwise correlations between gene expression levels and then transforming these correlations into adjacency matrices using a power function. Subsequently, we employed topological overlap measures to cluster genes with similar expression patterns into modules. The resulting gene modules were further analyzed to identify their association with the physiological traits listed in our trait file (**Supplementary Table S2**). The results were visualized using Cytoscape software version 3.7.1 (Shannon et al., 2003).

In situ hybridization (ISH)

Branches were decalcified with Morse's solution (22.5% formic acid, 10% trisodium citrate dihydrate) for 3-5 days. After dehydration with an RNase-free ethanol series and xylene, coral tissue portions were embedded in histoparaffin (Surgipath Paraplast, melting point 56°C) and sectioned at 5 μm thickness. Sections were deparaffinized, hydrated, and prepared for ISH with DIG-labeled RNA probes (*MaFGF10*: 5'-CGTATCCATTCTGACGTAACGTGACG-3') (Zongyan et al., 2024). They were digested with proteinase K (10 $\mu\text{g/mL}$) at 37°C for 15 min, fixed with 4% paraformaldehyde, and pre-hybridized with $2\times\text{SSC}$ and 50% formamide. DIG-labeled RNA probes (500 $\times$ dilution) with

10% dextran sulfate, 2×SSC, 0.02% SDS, and 50% formamide were prepared. *FGF10* was hybridized at 57°C for 10 h. Excess probes were removed with 1% RNase in 2×SSC. Sections were washed in 2×SSC, 1×SSC/50% formamide, 1×SSC, and Tris-buffered saline (Yang et al., 1999). They were then blocked with 10% BSA and maleic acid for 1 h, and DIG-labeled probes detected using anti-DIG alkaline phosphatase antibody (1:1000 dilution) overnight at 4°C. Signals were visualized with BCIP/NBT substrate (Sigma-Aldrich) and observed under a microscope (Zongyan et al., 2024).

Cortisone preparation and administration

To evaluate the effect of cortisone on coral growth rates, three G1 zones per coral colony were injected with the solution. The growth rates of the injection sites were measured, and the mean value was calculated for each colony before and after treatment. A total of six coral colonies were injected. To evaluate the effect of cortisone on coral growth polarity, all S1 zones of each coral colony were injected with the solution. The difference in the number of S1 zones before and after injection was calculated for each colony. A total of six coral colonies were injected.

Cortisone was dissolved in DMSO to prepare a 25.0 mg/mL stock solution. To prepare 1 mL of working solution, 100 µL of the stock solution was mixed with 400 µL of PEG300, followed by the addition of 50 µL of Tween-80 with thorough mixing. The mixture was then brought to a final volume of 1 mL with sterile physiological saline (0.9% NaCl in ddH₂O). The final concentration of cortisone in the working solution was 2.5 mg/mL. Each coral was injected with a volume equivalent to 2 mg/kg body weight into the coral polyp (Mccue et al.,

1978). Cortisone was administered every other day for 15 consecutive days, and coral growth was monitored for 2 months after the final injection. The vehicle-injected control group received the same volume of the solvent mixture (DMSO/PEG300/Tween-80/saline) without cortisone. Physiological saline was prepared by dissolving 0.9 g of NaCl in ddH₂O to a final volume of 100 mL. All solutions were freshly prepared before use.

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism version 8.0.2.263. A one-way ANOVA was performed, followed by the least significant difference (LSD) test for multiple comparisons, with statistical significance set at $P < 0.05$. All reported data represent the mean $\pm$ standard error of three replicate experiments.

RESULTS

Development of a PCCT-based phenotyping approach for corals

To assess the accuracy of conventional paraffin embedding method (PEM) in capturing coral phenotypic metrics (**Figure 1A**), we compared PEM with our newly developed PCCT-based morphological technology (PCCT-CIPT) across 11 coral species (8 scleractinian and 3 soft corals) (**Figure 1B-F**; **Supplementary Figures S2-S10**). Correlation analysis revealed that while the two methods showed moderate correlation in volume measurements ($R^2 = 0.8385$), PEM produced negative volume estimates for smaller coral samples, and its surface area measurements were consistently lower and poorly correlated with PCCT-CIPT ($R^2 = 0.2099$) (**Figure 1G-I**). Bland-Altman analysis confirmed significant systematic underestimation by PEM for both parameters (**Figure 1J**; **Supplementary Figure S11**). These discrepancies

arise from two technical limitations of PEM: uneven coating thickness (**Figure 2C-F**) and incomplete paraffin penetration into the irregular porous skeletal network (**Supplementary Figures S2-S10**). Notably, despite considerable morphological variation among coral species, no significant differences were observed in image segmentation across different coral morphotypes. This can be attributed to the high spatial resolution and sensitivity of PCCT (Schwartz et al., 2025). Therefore, species-specific parameter optimization is not required for coral samples with different morphologies. Given that other phenotypic traits (e.g., skeletal density, zooxanthellae density, chlorophyll content) are derived from volume and surface area, these findings suggest that caution may be warranted when interpreting phenotypic data obtained through PEM.

Holistic assessment of coral growth traits

To delve deeper into the growth characteristics of corals, we conducted a long-term monitoring of growth rates in selected coral growth zones and static zones. Throughout the monitoring period, the overall health of the corals remained stable (**Figure 2A, B**). Growth zones exhibited an average extension rate of $(25.99 \pm 3.94) \times 10^{-2}$ mm/d, significantly higher than static zones (**Figure 2C**). High-magnification observations of the growth and static zones (**Figure 2D**) revealed distinct morphological and pigmentation gradients along the growth axis. The growth zone was subdivided from top to bottom into three regions based on polyp characteristics. In G1, the polyps were completely white, with underdeveloped oral discs and tentacles. In G2, the polyps exhibited a transitional coloration between white and brown, and some were fully developed morphologically. In G3, all polyps were uniformly brown and fully developed (**Figure 2D**). In the static zones S1, S2, and S3, the polyps

showed no significant differences in color or morphology compared to those in G3 (**Figure 2D**). The color of coral polyps primarily originates from zooxanthellae (Buerger et al., 2020; Quigley et al., 2022). ITS2 analysis revealed that zooxanthellae community composition did not differ between growth and static zones, with both dominated by the C15 type (~80%) (**Figure 2E**). The density of zooxanthellae exhibits a similar pattern to that of chlorophyll *a* content, with G1 demonstrating significantly lower values than the other regions (**Figure 2F, G**).

We employed image segmentation-based processing to conduct quantitative analysis on the area of polyps and the abundance of zooxanthellae in the fluorescence sectioning samples (**Figure 2H, I**). The results revealed that polyps in G1 and G2 were morphologically immature, with fewer tentacles and significantly lower zooxanthellae density than in other regions (**Figure 2J, K**). The three-dimensional (3D) model results reconstructed from PCCT-CIPT voxels demonstrate significant morphological differences in the skeletal structures between the growth zones and the static zones of corals (**Figure 2L, M**). In the static zones, there were uniform and regular circular cavity structures. These cavities were not only numerous but also densely distributed, covering almost the entire surface of the coral skeleton (**Figure 2L**). Similar structures were also observed in G2 and G3, albeit with lower densities (**Figure 2L**). In contrast, no circular cavity structures for accommodating coral polyps were found in G1 (**Figure 2L, M**). Furthermore, the longitudinal and transverse sections clearly show that the skeletal chamber walls in the coral static zones were relatively thick, with smaller internal chambers (**Figure 2L**). In contrast, the growth regions exhibited the opposite pattern, especially in G1 and G2, where the skeletal chamber walls were significantly thinner

than those in other areas (**Figure 2L**). To validate these observations, we also conducted precise calculations of the skeletal density in both the growth zones and static zones of corals, with the results aligning with the intuitive observations from the PCCT-CIPT (**Figure 2N**).

Transcriptome analysis of coral host and zooxanthellae

Given the lack of phenotypic differences among S1, S2, and S3 (**Figure 2**), transcriptomic analysis was performed on G1, G2, G3, and S1, with host and zooxanthellae transcriptomes separated. Host transcriptomes showed good reproducibility, with the highest number of DEGs in G1 vs. S1 (**Figure 3A-C**). Pathway enrichment revealed that G1-upregulated genes were enriched in cell cycle (ko04110), MAPK signaling (ko04010), mTOR signaling (ko04150), and steroid biosynthesis (ko00100) (**Figure 3D-F**), whereas genes upregulated in G2, G3, and S1 were enriched in calcium signaling (ko04020), ribosome (ko03010), and AMPK signaling (ko04152) (**Figure 3G-I**). Similar results were also obtained from the GO analysis (**Supplementary Figure S12**).

PCCT-CIPT-derived phenotypic data were used for WGCNA of host transcriptomes (**Figure 4A**). Three key modules (lightyellow, floralwhite, and darkgrey) were identified in the coral host (**Figure 4B, C**). We conducted KEGG pathway analysis on the genes within these three modules. The results showed that the genes in the lightyellow module, which were positively correlated with G1 ($R^2=0.50$), were mainly enriched in pathways such as DNA replication (ko03030), cell cycle (ko04110), and oocyte meiosis (ko04114) (**Figure 4D**). Genes within the floralwhite module, which showed a strong positive correlation with skeletal density ($R^2=0.98$, $P<0.001$), were mainly enriched in pathways such as lysosome (ko04142) (**Figure**

4E). Genes within the darkgrey module exhibit significant enrichment in the phagosome pathway (ko04145) (**Figure 4F**). We conducted an in-depth regulatory network analysis of the genes within the lightyellow module and successfully identified a key transcription factor, *Sox9* (*Unigene0067038_Coral*) (**Figure 4G; Supplementary Figure S13**). The expression level of *Sox9* in G1 is significantly higher than that in S1 (N=3, $P<0.001$) (**Figure 4H**). Similarly, the DEGs in the zooxanthellae transcriptome data were also predominantly found in the comparison of G1 vs. S1 (**Supplementary Figure S14A-C**). The pathways mainly enriched by genes with high expression in G2 and G3 include protein digestion and absorption (ko04974) and ribosome (ko03010) (**Supplementary Figure S14D, E**). The pathway mainly enriched by genes with high expression in S1 is carbon metabolism (ko01200) (**Supplementary Figure S14F**). Among the genes with high expression in G1, the pathways mainly enriched include cancer pathways (ko05200), nitrogen metabolism (ko00910), DNA replication (ko03030), and cell cycle (ko04110) (**Supplementary Figure S14G-I**). Similar results were also obtained from the GO analysis (**Supplementary Figure S15**). Moreover, the zooxanthellae genes associated with photosynthesis and photosynthate translocation, including *RBCL* (Ribulose-1,5-bisphosphate carboxylase/oxygenase, *Unigene0043680_Symbiodinium*), *LHCSR1* (Light-harvesting complex stress-related protein 1, *Unigene0175291_Symbiodinium*), *PETC* (Photosynthetic Electron Transport Chain, *Unigene0042205_Symbiodinium*), *LHCA6* (Photosystem I chlorophyll *a/b*-binding protein 6, *Unigene0040326_Symbiodinium*), and *lhca-P4* (Chlorophyll *a-b* binding protein P4, *Unigene0188174_Symbiodinium*), were significantly downregulated in the G1 phase (**Supplementary Figure S16**). Using WGCNA, we identified two gene modules,

darkmagenta ($R^2=0.90$, $P<0.001$) and lightsteelblue1 ($R^2=0.92$, $P<0.001$), that exhibited a high positive correlation with zooxanthellae density and chlorophyll *a* content (Supplementary Figure S17). The genes within these modules simultaneously showed enrichment in the ribosome (ko03010) pathway (Supplementary Figure S17). Finally, the qRT-PCR expression levels of the 20 key functional genes showed good consistency with the transcriptome sequencing (RNA-Seq) data, which further validated the accuracy and reliability of the RNA-Seq data (Supplementary Figure S18).

The core signaling networks regulating coral growth

The growth of coral host G1, is centered on the cell cycle (ko04110) as its core pathway and was closely related to the Wnt signaling pathway (ko04310), pathways in cancer (ko05200), mTOR signaling pathways (ko04150), and MAPK signaling pathways (ko04010) (Supplementary Figure S19). Moreover, the starting points of these pathways were all subject to regulation by the FGF-FGFR system. Pathway analysis revealed that the MAPK signaling pathway mediated by FGF10 exhibited upregulated expression in G1. The calcium signaling pathway mediated by PLC γ shows upregulated expression in G2, G3, and S1 (Figure 5A, B). Additionally, the AMPK signaling pathway, which has a counter-regulatory effect on the mTOR signaling pathway, also demonstrated upregulated expression in G2, G3, and S1 (Figure 5C). The qRT-PCR experiments also yielded consistent results (Figure 5C; Supplementary Figure S20A).

Non-targeted metabolomics revealed that DAMs between G1 and S1 were primarily enriched in steroid hormone biosynthesis (ko00140), nucleotide metabolism (ko01232), fatty acid

biosynthesis (ko00061), and vitamin B6 metabolism (ko00750) (**Figure 5E**; **Supplementary Figure S20B-F**), consistent with transcriptomic enrichment of steroid biosynthesis in G1 (**Figure 3D-F**). The primary metabolic pathway of pregnenolone in coral was constructed (**Figure 5F**). Following identification, a total of nine key differential metabolites were found, including 17 α -hydroxyprogesterone, adrenosterone, estradiol-17 β , cortisone, and others (**Supplementary Data S3**). The accumulation levels of these metabolites in the G1 are significantly higher than those in the S1 stage. Notably, the accumulation of cortisone in the G1 was particularly prominent, reaching 24.05 times that in the S1 (**Supplementary Data S3**). Meanwhile, we successfully identified the genes encoding enzymes such as *UGT*, *CYP3A*, *CYP17A*, *HSD17B*, and *AKR* (**Supplementary Data S4**). The expression levels of these genes in G1 were significantly higher than those in the S1 (**Figure 5G**). Interestingly, we found that the natural pigment Rhodoxanthin was highly enriched in G1 (**Figure 5H**).

Similarities of coral growth traits in nature

To exclude species-specific and rearing-related confounding factors, we collected *A. acuminata* and *P. meandrina* from natural habitats for transcriptomic and metabolomic analyses to assess the similarity of growth mechanisms across coral species. G1-upregulated genes in both species were enriched in MAPK, mTOR, Wnt, and steroid biosynthesis pathways (**Figure 6A, B**), consistent with metabolomic results (**Supplementary Figure S21**). FGF10 expression remained consistently high in growth zones across species and habitats (N=3, $P<0.0001$, $P=0.003$) (**Figure 6C**), and cortisone was significantly enriched in G1 across species (N=6, $P<0.0001$ and $P=0.0027$) (**Figure 6D**). Phylogenetic analysis placed coral FGF10 closer to fish FGF10 (**Figure 6E**), with interspecific sequence similarity of

87.5% (**Figure 6F**). Structural comparison between MaFGF10 and human FGF10 showed 97.16% of residues in favored Ramachandran regions (**Figure 6G**) and an RMSD of 0.494 Å (**Figure 6H**), indicating high structural similarity. Together, these results support the accuracy of coral FGF10 annotations.

MaFGF10 (*Unigene0083285_Coral*) showed the highest expression among FGFs, with a G1/S1 ratio of 2.8 (**Supplementary Data S1**). ISH confirmed MaFGF10 upregulation in G1 (**Figure 7A**). Its receptor *MaFGFR4* showed a similar trend (G1/S1=1.58) (**Figure 7B, C**), with a strong positive correlation between them ($R^2=0.85$, $P<0.001$) (**Figure 7D**; **Supplementary Data S1**). Molecular docking studies revealed that the MaFGF10 protein forms stable hydrogen bond interactions with the MaFGFR4 protein through its key residues (e.g., LEU172 and PHE308) (**Supplementary Table S3**). Finally, Y2H assays confirmed an interaction between pGBKT7-MaFGF10 and pGADT7-MaFGFR4 (**Figure 7E**).

Interestingly, cortisone appeared to enhance the growth rate in the growth zones of *M. angulata* and seemed to promote the transition of static zones into growth zones (**Figure 7F, G**). Notably, this positive effect was not confined to a particular species of coral. In our experiment, we selected three different species of corals, namely *M. angulata*, *Acropora hyacinthus*, and *Acropora nana*, as research subjects. Cortisone exerted similar effects on all three species, effectively promoting the growth rate of their growth zones and facilitating the transition of static zones to growth zones (**Figure 7F-K**).

DISCUSSION

The leading position of the host during coral growth

In both experimental coral cultivation conditions and small-scale spatial environments where individual corals survive in the marine environment, environmental factors are close to being homogeneous (Barshis et al., 2010; Rippe et al., 2021). However, the growth of individual coral exhibits spatial heterogeneity, and the causes of this phenomenon may lean more towards intrinsic biological regulation within corals rather than the influence of external environmental factors. Previous studies have shown that under similar environmental conditions, the composition of zooxanthellae types within the same coral species exhibits high stability, and this finding aligns with our experimental results (Coffroth et al., 2023; Johnston et al., 2022; Villela et al., 2024). Zooxanthellae are the primary source of coral coloration (Buerger et al., 2020; Quigley et al., 2022). The energy produced through their photosynthesis serves as a vital energy source for the coral host (Man et al., 2024; Zhuojun et al., 2021). The results regarding zooxanthellae density and chlorophyll *a* content provide a sound explanation for the pronounced color variations in coral polyps across growth zones (**Figure 2D**). Notably, rhodoxanthin accumulated significantly during the G1 phase (**Figure 5H**). This compound is known for its antioxidant and photoprotective properties (Merzlyak et al., 2005). It may help alleviate photodamage in coral polyps that lack zooxanthellae shading at this stage. This interpretation is consistent with the proposed roles of host-derived pigments in coral photoprotection (Ferreira et al., 2023). However, direct functional evidence for rhodoxanthin's role in corals remains limited and awaits further experimental validation.

In the transcriptomic analysis of zooxanthellae, those at the G1 stage achieved rapid self-replication by upregulating key pathways such as DNA replication (ko03030) (Huang et al., 2019) and cell cycle (ko04110) (**Supplementary Figure S14G-I**) (David et al., 2009).

Oxidative phosphorylation (ko00190), as the core pathway for cellular ATP generation (Bournaka et al., 2024), also exhibited an upregulated trend at this stage (**Supplementary Figure S14G**). This suggests that zooxanthellae cells may be actively undergoing physiological regulation, potentially utilizing oxidative phosphorylation to synthesize ATP and thereby preparing for subsequent cell division and proliferation. The upregulated genes in S1 were primarily enriched in the carbon metabolism pathway (ko01200) (**Supplementary Figure S14F**), suggesting that the coral host may have established a relatively stable symbiotic relationship with zooxanthellae (Koch, 2022). Collectively, these results suggest that zooxanthellae proliferation may occur during the coral growth process.

However, these same results fail to account for the inverse relationship between zooxanthellae density/chlorophyll *a* content and coral growth rates (**Figure 2C, G**).

We found that zooxanthellae genes associated with photosynthesis and photosynthate translocation were significantly downregulated in the G1 phase (**Supplementary Figure S16**). Given that photosynthesis of zooxanthellae serves as a vital energy source for the coral host (Nitschke et al., 2024), it is reasonable to speculate that the coral host may possess alternative energy compensation pathways during rapid growth, further suggesting that zooxanthellae may not play a dominant role in the coral growth process. In the G1 zone, key genes involved in fatty acid metabolism in the coral host, including *CPT2* (carnitine palmitoyltransferase 2, *Unigene0097716_Coral*), *ACADVL* (very long-chain acyl-CoA dehydrogenase, *Unigene0038500_Coral*), *ACADM* (medium-chain acyl-CoA dehydrogenase, *Unigene0062438_Coral*), *HADH* (hydroxyacyl-CoA dehydrogenase, *Unigene0047739_Coral*), and *HADHA* (hydroxyacyl-CoA dehydrogenase trifunctional

multienzyme complex subunit alpha, *Unigene0136941_Coral*), were significantly upregulated (**Supplementary Figure S22**). This transcriptional shift further supports the interpretation that, when symbiont-derived photosynthate supply is insufficient, the coral host activates alternative energy compensation pathways, particularly mitochondrial fatty acid oxidation, to sustain rapid growth.

Coral soft tissue growth mediated by the Wnt-FGF10-MAPK/mTOR axis

The FGF signaling pathway exhibits evolutionary conservation (Francesca & Robert, 2023; Jennifer, 2024; Nobuyuki & David, 2010). In vertebrates, FGF-FGFRs regulate cell cycle progression and guide cells to differentiate in different directions by activating the cascade reactions of downstream effector factors, such as the MAPK signaling pathway (Mandal et al., 2021; Wagner & Nebreda, 2009). *FGF10* is primarily expressed in mesenchymal cells, regulating branching morphogenesis, stem cell fate determination, tissue differentiation, and proliferation in vertebrate mice (Francesca & Robert, 2023). FGF2, FGF9, FGF18, and FGF19 can promote the development of cartilage and bone (Haoran et al., 2022). Additionally, studies have demonstrated that the Wnt signaling pathway can upregulate the expression of FGF (Hu et al., 2025). WNT8B is a member of the Wnt family that plays a role in embryonic development by initiating intracellular signaling through interaction with cell surface receptors such as Frizzled and LRP5/6 (Liu et al., 2021). In our study, we observed significant upregulation of *Wnt8B* (*Unigene0089470_Coral*) and its co-receptor *LRP6* (*Unigene0043127_Coral*), as well as the MAPK/mTOR signaling axis activated by FGF10, in the G1 phase of coral hosts (**Supplementary Figure S23, Figure 3D-F, Figure 5A, B**). Moreover, WGCNA analysis identified concurrent upregulation of key effector factors *c-Myc*

and *Sox9* within these pathways (**Figure 4H; Figure 5B**). This signaling axis serves as a core pathway that advances the cell cycle and promotes cell division and tissue differentiation (Dubey et al., 2023; Matthews et al., 2022; Westendorp, 2024). Concurrently, the host cells of corals in the G1 phase exhibit a high demand for DNA replication (**Figure 3D-F**). Given that cell proliferation relies on DNA replication to provide the genetic material basis (Fung-Uceda et al., 2018; Takahashi et al., 2024; Van Den Berg, 2024), these findings collectively suggest that the soft tissue cells of the coral host may be undergoing active cell division and differentiation. In addition, the highly expressed genes in the G1 phase demonstrate significant enrichment in multiple cancer-related pathways, including gastric cancer and breast cancer (**Figure 3D-F**). The pathways in cancer are typically associated with abnormalities in multiple aspects, including cell proliferation, apoptosis, and signal transduction (Matthews et al., 2022; Wilson et al., 2024). Intriguingly, relevant research indicates that coral, under conditions of abnormal growth (such as bosselated, nodular, and exophytic patterns), may exhibit heightened expression of host genes associated with tumorigenesis (Francesco et al., 2022). In our study, the samples selected were coral in normal growth states (**Figure 2A, B**). The enrichment of cancer-related pathways in G1 may reflect the shared use of cell proliferation and survival signaling modules between normal coral growth and pathological hyperplastic states, rather than indicating neoplastic transformation itself. Notably, significant upregulation of the AMPK signaling pathway was observed during stages G2, G3, and S1 (**Figure 3G-I**). Beyond promoting fatty acid biosynthesis, the AMPK pathway negatively regulates the mTOR signaling pathway (Wang et al., 2024). These findings suggest that AMPK may act as a counter-regulatory mechanism

to limit mTOR-driven anabolic activity, potentially facilitating the transition from rapid proliferation to a more quiescent or maturation-oriented state during later stages of coral growth.

Regulatory mechanisms of coral skeletal growth

In vertebrates, the TGF- β signaling pathway activates Smad5 to promote osteoblast differentiation (Duan et al., 2021). Smad5 serves as a pivotal transcriptional regulator within the BMP signaling pathway, participating in the regulation of bone formation and embryonic development (Orlowski, 2017; Wu et al., 2016). Short-term activation of the TGF- β signaling pathway can promote cartilage repair, whereas long-term activation may lead to cartilage degradation (Wu et al., 2016). In our study, we detected the upregulation of the TGF- β signaling pathway and *SMAD5* (*Unigene0171008_Coral*) expression in the G1 phase (**Figure 3F**), suggesting that osteoblast-related cells may be undergoing active differentiation during this stage. The skeletal structure of coral in the G1 phase is loose and exhibits weak mechanical strength (**Figure 2L**), making it difficult for the coral to withstand the mechanical stresses generated by complex ocean currents and wave impacts. Ca^{2+} directly participate in the precipitation reaction of calcium carbonate, serving as a key element in the formation of biomineralized tissues such as coral skeletons (Chandra Rajan et al., 2021). Calmodulin (CLMD) can regulate the activity of calcium pumps, influencing calcium ion concentrations and thereby promoting calcification (Jenna et al., 2023). During the process where the proliferation and differentiation activities of coral cells gradually decline (across the G1, G2, G3, and S1 phases), the expression levels of the Ca^{2+} signaling pathway components, *CLMD*, and *carbonic anhydrase 2* (*CA2*, a key gene closely associated with biological calcification,

Unigene0092875_Coral), instead exhibit a progressive increase (**Figure 3G-I; Figure 5B**).

The expression levels of functional genes related to biological calcification in G1 are lower than those in G2, G3, and S1. This may be attributed to the relatively low degree of biological calcification in G1 due to the incomplete differentiation and relatively small number of calcifying cells at this stage. However, it is noteworthy that CA2 maintains consistently high expression levels throughout the entire growth cycle of coral, even during the G1 phase when calcium-precursor cells are undergoing differentiation. Additionally, studies have shown that mutations enhancing the function of *Lrp5* can lead to a high skeletal density phenotype, whereas *Lrp5* deficiency results in reduced bone mass and an increased risk of fractures (Cui et al., 2011). In coral G2, G3, and S1, *Lrp5* (*Unigene0034376_Coral*) expression is also markedly higher than in G1. In vertebrates, lysosomes regulate bone remodeling via osteoclast-mediated resorption and autophagy-modulated osteoblast function (Erkhembaatar et al., 2017). In this study, lysosomal genes were found to be enriched in a skeletal-density-related module (**Figure 4E**). However, it remains uncertain whether lysosomes directly participate in mineralization during coral growth or exert their effects indirectly via tissue remodeling, and this question requires further experimental investigation.

Signaling network for coordinated growth of coral soft tissues and skeleton

During the initial growth stage of coral, the Wnt-FGF10-MAPK/mTOR signaling axis regulates the processes of cell division and differentiation through effector factors such as *c-Myc* and *Sox9*, effectively promoting the development and maturation of coral polyp morphology. In this context, the TGF- β signaling pathway activates Smad5, guiding cells to differentiate into the osteoblast lineage and triggering the expression of biomineralization-

related genes such as *CA2*, *Lrp5*, and *CLMD*. These genes collectively contribute to the formation of the primary skeletal framework in corals. As the soft tissue cells fully develop and mature, the AMPK signaling pathway is specifically upregulated, which in turn inhibits the cell division and differentiation activities mediated by the mTOR signaling pathway, achieving precise regulation of cellular proliferation and differentiation. Concurrently, the expression levels of calcium-related genes, including *CA2*, *Lrp5*, and *CLMD*, are further significantly upregulated. This not only enhances the calcification density and structural stability of the primary skeletal framework of coral but also markedly improves its resistance to complex marine environmental stresses, effectively reducing the risk of coral fractures caused by environmental factors and providing a solid structural foundation for the long-term survival and reproduction of coral.

Synergistic regulatory effects of steroid hormones on coral growth

Steroid hormones are essential bioactive molecules that regulate a wide range of physiological processes in both animals and plants, including immune response, salt and water balance, glucose metabolism, and developmental sexual maturation (Simon et al., 2025). In vertebrates, endogenous steroid hormones serve as key regulatory factors for osteoblast differentiation (Zhou et al., 2009). They can exert significant influences on the skeletal development process of mice during both embryonic and postnatal stages by modulating the Wnt signaling pathway in osteoblasts (Zhou et al., 2009). In G1, we not only detected a significant accumulation of cortisone, but also observed an upregulated expression of the Wnt signaling pathway (**Figure 3D-F**). The preliminary formation of the coral skeletal framework may be jointly regulated by steroid hormones and the Wnt signaling pathway.

Preliminary results from the cortisone injection experiment indicate that cortisone not only accelerates coral growth but also induces the transition of S1 zones into G1 zones (**Figure 7F-K**). However, we have not examined the residual cortisone or its metabolite distribution in coral tissues after injection, nor have we assessed the expression changes of key genes in the FGF10-MAPK/mTOR pathway. Thus, it remains unclear whether cortisone functions through this signaling axis based on the current data. In addition, a small number of S1 zones were also observed to transition to the G1 phenotype in the vehicle-injected control group during the cortisone injection experiment (**Figure 7G, I, K**). This phenomenon may be related to the local tissue damage and subsequent regenerative response caused by the injection procedure. Therefore, the interplay between cortisone and Wnt signaling, as well as their effects on the FGF10-MAPK/mTOR pathway, will be important directions for future research.

Research significance, limitations, and future prospects

In summary, we have developed the PCCT-CIPT method for detecting coral phenotypes and combined it with functional verifications, including transcriptomics, metabolomics, and in situ molecular imaging (**Figure 7L**). This provides new clues for further understanding the functional similarities of the FGF pathway across different animal lineages. Moreover, we have, for the first time, established a research paradigm for studying the mechanism of coral growth polarity in scleractinian corals, filling a critical gap in understanding coral host-driven developmental mechanisms. These findings not only deepen our understanding of coral reef ecosystems but also offer the possibility of accelerating coral artificial propagation and reef restoration through the regulation of FGF10 and related factors.

At present, corals, as highly complex symbiotic organisms, remain understudied with respect to the mechanisms underlying growth and adaptation, which are still largely at an exploratory stage worldwide. In the field of traditional biology, although gene interference and overexpression techniques have become relatively well-established, a comprehensive experimental system for these techniques in reef-building corals has yet to be developed. In light of this, the current validation of coral functional genes and their associated pathways primarily revolves around gene expression level detection and cross-validation through multi-omics approaches (Levy et al., 2025). Additionally, at this stage, high-quality genomic resources closely related to coral growth and adaptation remain extremely scarce (Bay & Guerrero, 2020). In the future, with the continuous optimization and advancement of gene validation technologies, alternative approaches for functional gene research can be explored, such as leveraging lipid nanoparticles to mediate gene interference, gene editing, and gene overexpression, thereby achieving precise regulation of specific genes in reef-building corals. It should also be noted that functional gain- and loss-of-function experiments for key genes such as *FGF10* and *Sox9* were not performed in the present study, nor were the FGF10-MAPK/mTOR and AMPK pathways directly validated. These represent important avenues for future investigation. In addition, FGF signaling plays an important role in wound healing across different animal lineages (Chen et al., 2022; Son et al., 2023). The Wnt/ β -catenin pathway has been demonstrated to be involved in regeneration in *Hydra* (Vogg et al., 2019). This raises the question of whether the Wnt-FGF10-MAPK/mTOR signaling axis identified in our study may also participate in the regenerative growth of coral fragments. However, direct evidence linking these pathways to coral regeneration remains scarce and awaits

further investigation.

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AUTHOR CONTRIBUTIONS

Y. F.: Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Visualizations; **X. Y.:** Conceptualization, Visualizations, Writing - Review & Editing; **Z. C.:** Methodology; Data Curation, Writing - Original Draft, **K. Y.** and **T. Z.:** Methodology; Data Curation; **Y. L.:** Writing - Review & Editing; **C. F. C.:** Conceptualization, Writing - Review & Editing; **P. W.:** Writing - Review & Editing, Project administration; **L. Y.:** Project administration, Funding acquisition; **H. Z.:** Conceptualization, Writing - Review & Editing, Supervision, Project administration, Funding acquisition. All authors read and approved the final version of the manuscript.

COMPETING INTERESTS

The authors declare no conflicts of interest.

DATA AND MATERIALS AVAILABILITY

The raw transcriptomic and metabolomic sequencing data and Supplementary Data S1-S4

have been deposited and are accessible at <https://pan.baidu.com/s/1w2GDK-Y1vzVcfRaxzE1muA?pwd=xz3t>. All data supporting the findings of this study are available within the paper and its Supplementary Materials.

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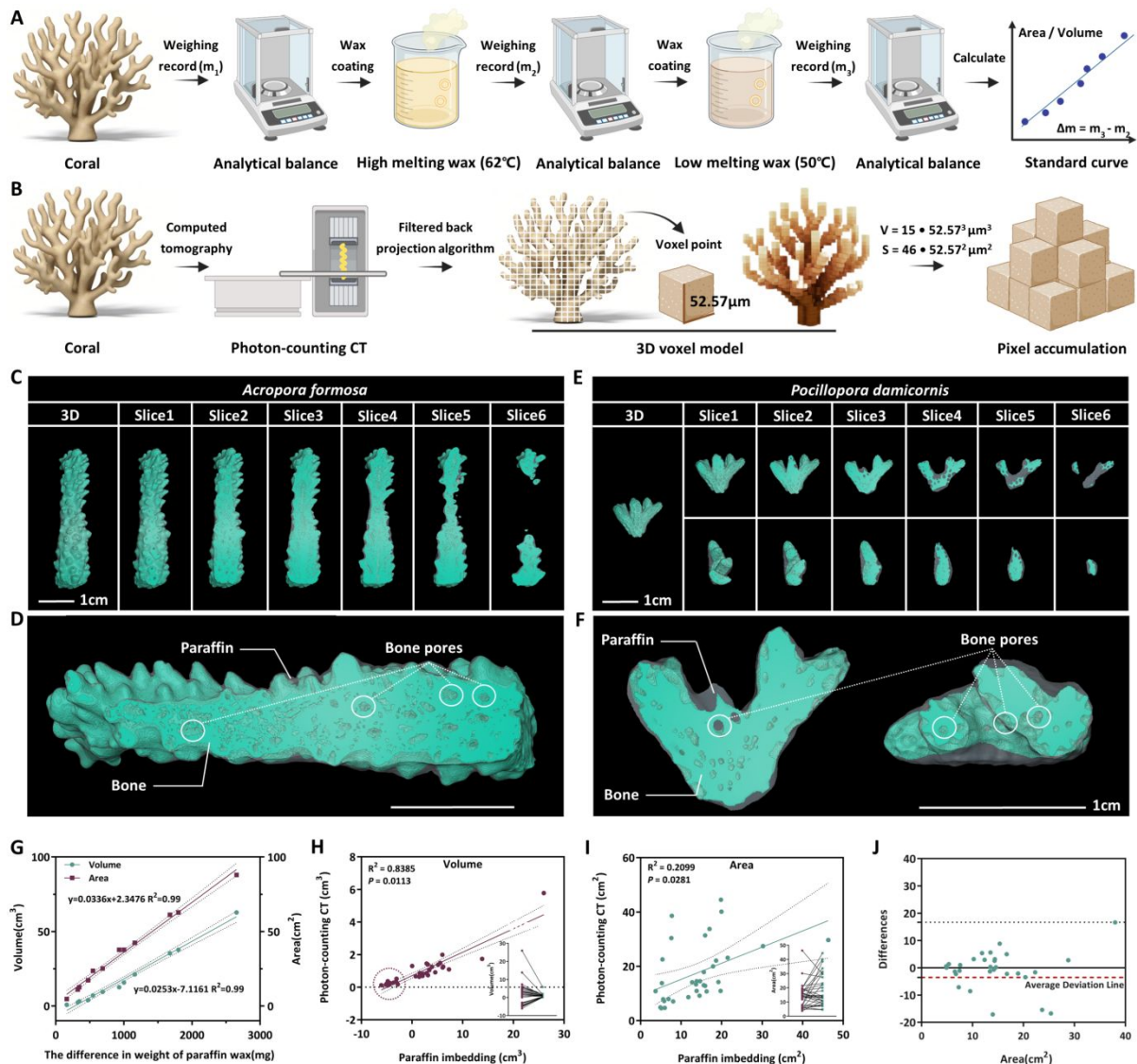
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910

911 **Figure 1. Establishment of a detection method for coral phenotypic indicators based on**

912 **PCCT-CIPT. A: Calculation of coral volume and surface area using PEM. B: Calculation of**

913 **coral volume and surface area using PCCT-CIPT. C: *Acropora formosa* sliced images of**

914 **different layers. D: *A. formosa* layer enlargement diagram. E: *Pocillopora damicornis* sliced**

915 **images of different layers. F: *P. damicornis* layer enlargement diagram. G: The weight**

916 **difference of PEM and the standard curve of coral volume to surface area. H: Correlation**

917 **analysis of coral volume measured by PCCT-CIPT and PEM. I: Correlation analysis of coral**

918 surface area measured by PCCT-CIPT and PEM. J: PCCT-CIPT and PEM for Bland-Altman
919 analysis of coral surface area detection.

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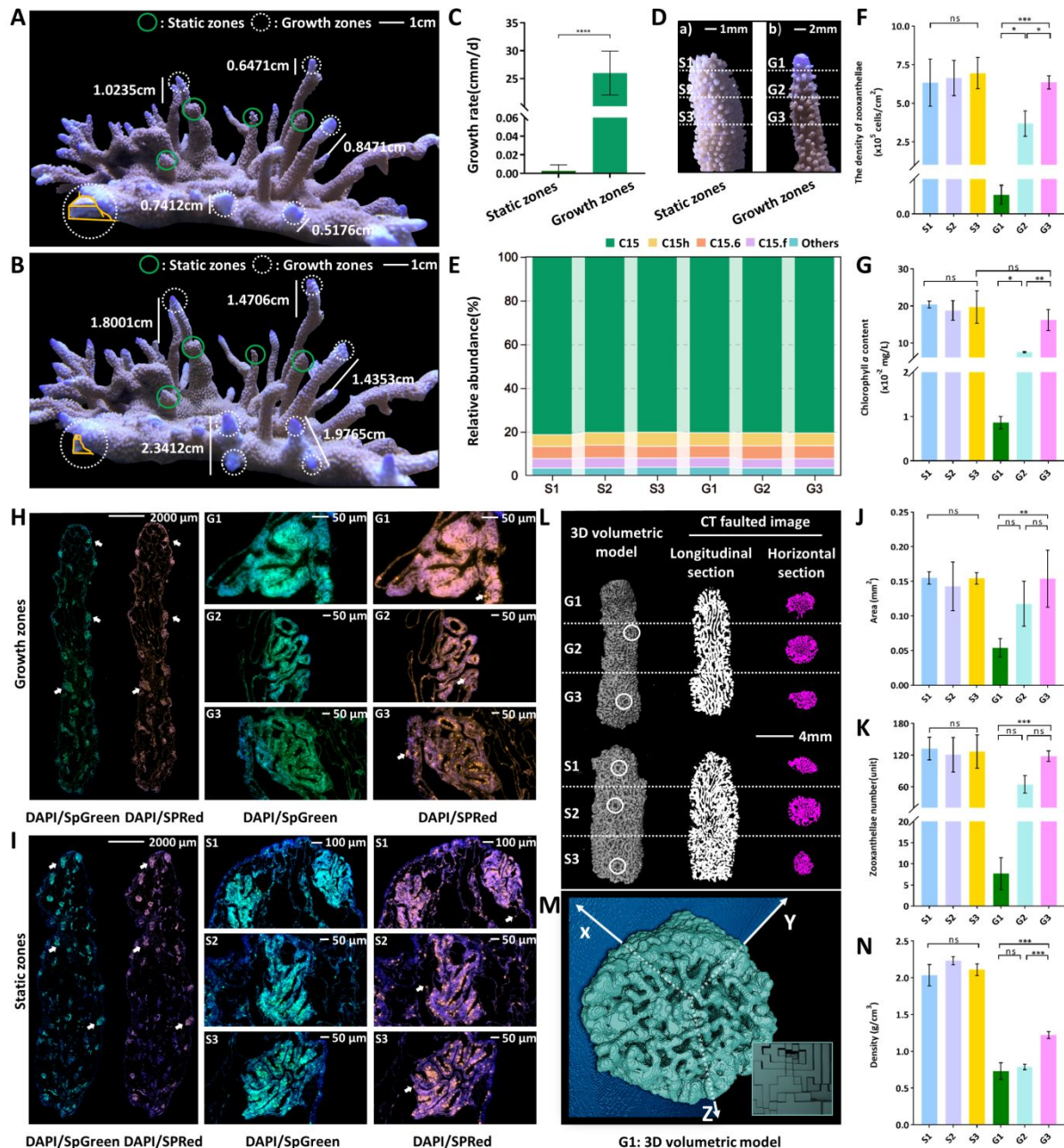


Figure 2. Detecting physiological changes during rapid coral growth through phenomics based on PCCT-CIPT and ITS2 technology. A: Coral growth phenotyping monitoring (with photographs taken at the onset of monitoring). B: Taking photographs at the conclusion of coral growth phenotyping monitoring. C: Average growth rates in growth and static zones of coral (N=10, each derived from the average of at least three branches per coral colony, $P < 0.0001$). D: Localized magnifications of coral growth regions and static regions, with

927 samples classified based on phenotypic differences. E: ITS2 analysis of the composition of
928 zooxanthellae in coral growth zones and static zones. F: Measuring zooxanthellae density in
929 coral growth zones and static zones using PCCT-CIPT. G: Measuring chlorophyll *a* content
930 in coral growth zones and static zones using PCCT-CIPT. H: Carry out fluorescence
931 sectioning experiments in growth zone. I: Carry out fluorescence sectioning experiments in
932 static zone. J: The developmental status of polyps in coral growth zones and static zones was
933 measured using fluorescence sectioning. K: Utilizing fluorescence sectioning to measure the
934 density of zooxanthellae in both coral growth zones and static zones. L: High-fidelity 3D
935 model of coral static zones and growth zones, with extraction of longitudinal and cross-
936 sectional views (where color shading represents skeletal density). M: High-fidelity 3D model
937 diagram of G1. N: Measuring skeletal density in coral growth zones and static zones using
938 PCCT-CIPT. (N=3, ns: Not significant, ***: $P<0.001$).

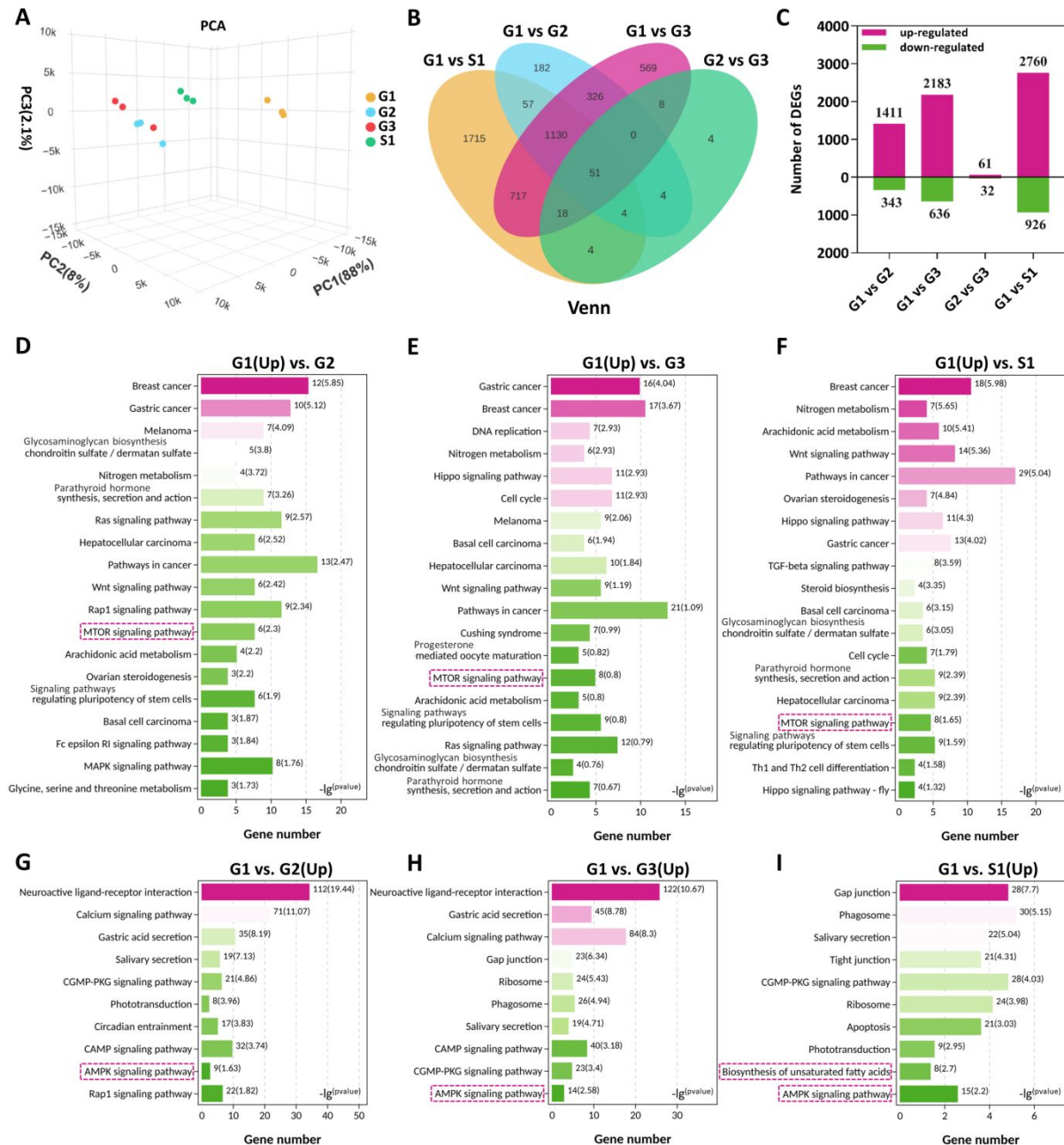


Figure 3. Transcriptome analysis of coral host in growth zones and static zones. A: PCA analysis of transcriptome data from coral host growth zones (G1, G2, and G3) and static zone (S1). B: Venn analysis. C: Changes in DEGs between coral host G1, G2, G3, and S1 (Up- represents upregulated genes, and down-represents downregulated genes). D: KEGG analysis of upregulated genes in G1 vs. G2. E: KEGG analysis of upregulated genes in G1 vs. G3. F: KEGG analysis of upregulated genes in G1 vs. S1. G: KEGG analysis of downregulated

946 genes in G1 vs. G2. H: KEGG analysis of downregulated genes in G1 vs. G3. I: KEGG
947 analysis of downregulated genes in G1 vs. S1.

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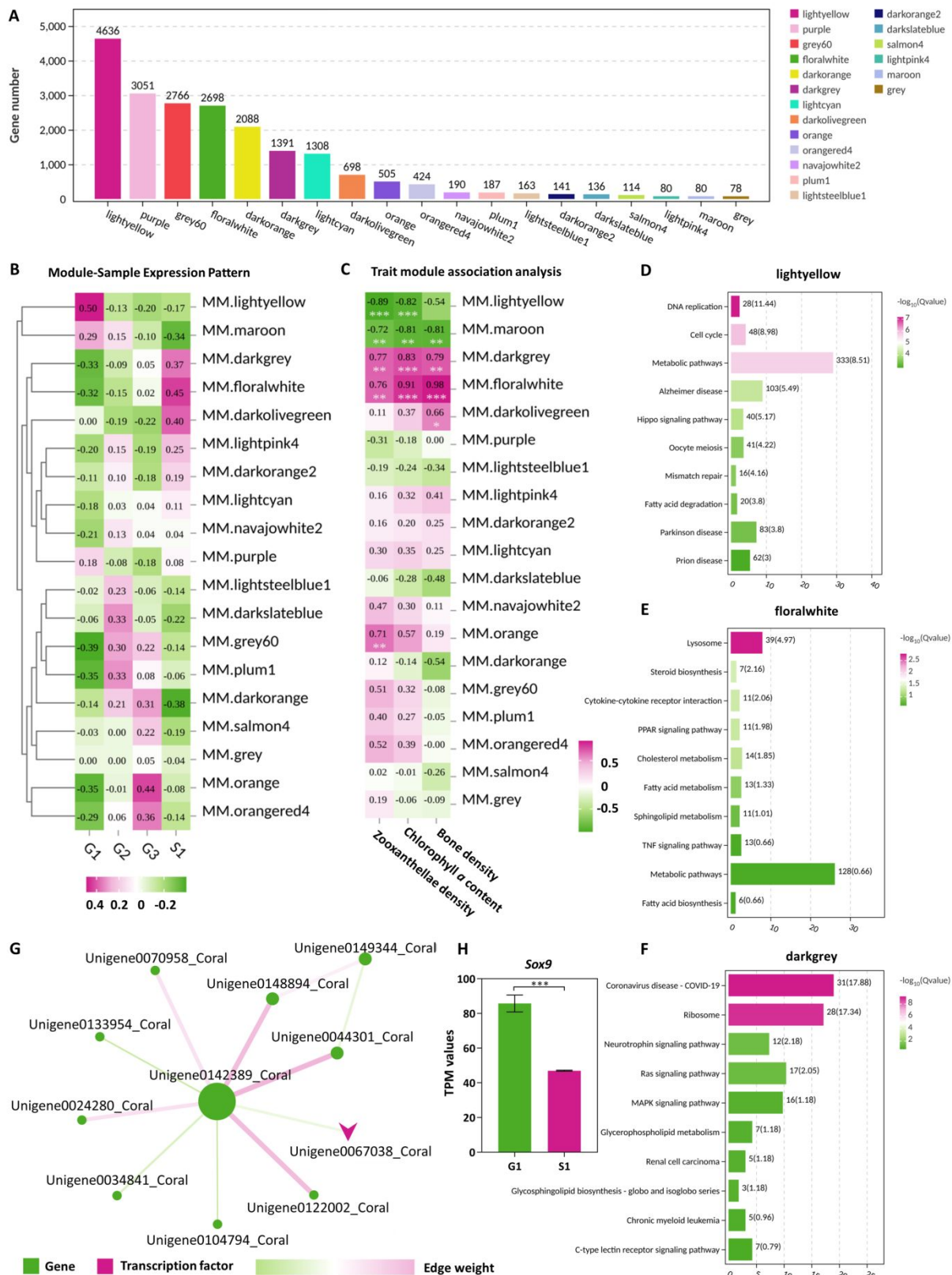


Figure 4. WGCNA analysis of coral host. A: The final results of the module division identified by WGCNA were summarized, and the number of genes contained within each

951 module was displayed. B: Module-sample expression pattern (The values represent the
952 normalized module membership, which is equivalent to a weighted summary of the gene
953 expression levels within each module. The positive or negative sign of these values signifies
954 whether the expression level of the module is relatively upregulated or downregulated,
955 respectively.). C: Correlation analysis of traits and modules (A heatmap is generated based on
956 the Pearson correlation coefficients between the module membership values and trait data. *:
957 $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$). D-F: Genes within the lightyellow, floralwhite, and
958 darkgrey module were mapped to the KEGG database for enrichment categorization, and
959 significantly enriched pathways were identified using the hypergeometric test. G: Analysis of
960 gene regulatory network in floralwhite module (Select all relationship pairs among the top 10
961 genes with the highest module membership). H: The expression levels of the transcription
962 factor *Unigene0067038_Coral (Sox9)* in G1 and S1.

966 expression of key functional genes in the critical signaling pathways mediated by FGFs-
967 FGFRs. C: The negative regulatory effect of the AMPK signaling pathway on the mTOR
968 signaling pathway. D: Validation of the expression of key functional genes involved in the
969 calcium signaling pathway and MAPK signaling pathway in G1 and S1 by qRT-PCR (N=3,
970 *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$). E: Differential metabolite pathway enrichment
971 analysis. F: Differentially abundant metabolites enriched in the steroid hormone biosynthesis
972 pathway. G: Key enzymes in the steroid hormone biosynthesis pathway. H: Key differential
973 metabolite Rhodoxanthin (N=6, ****: $P<0.0001$).

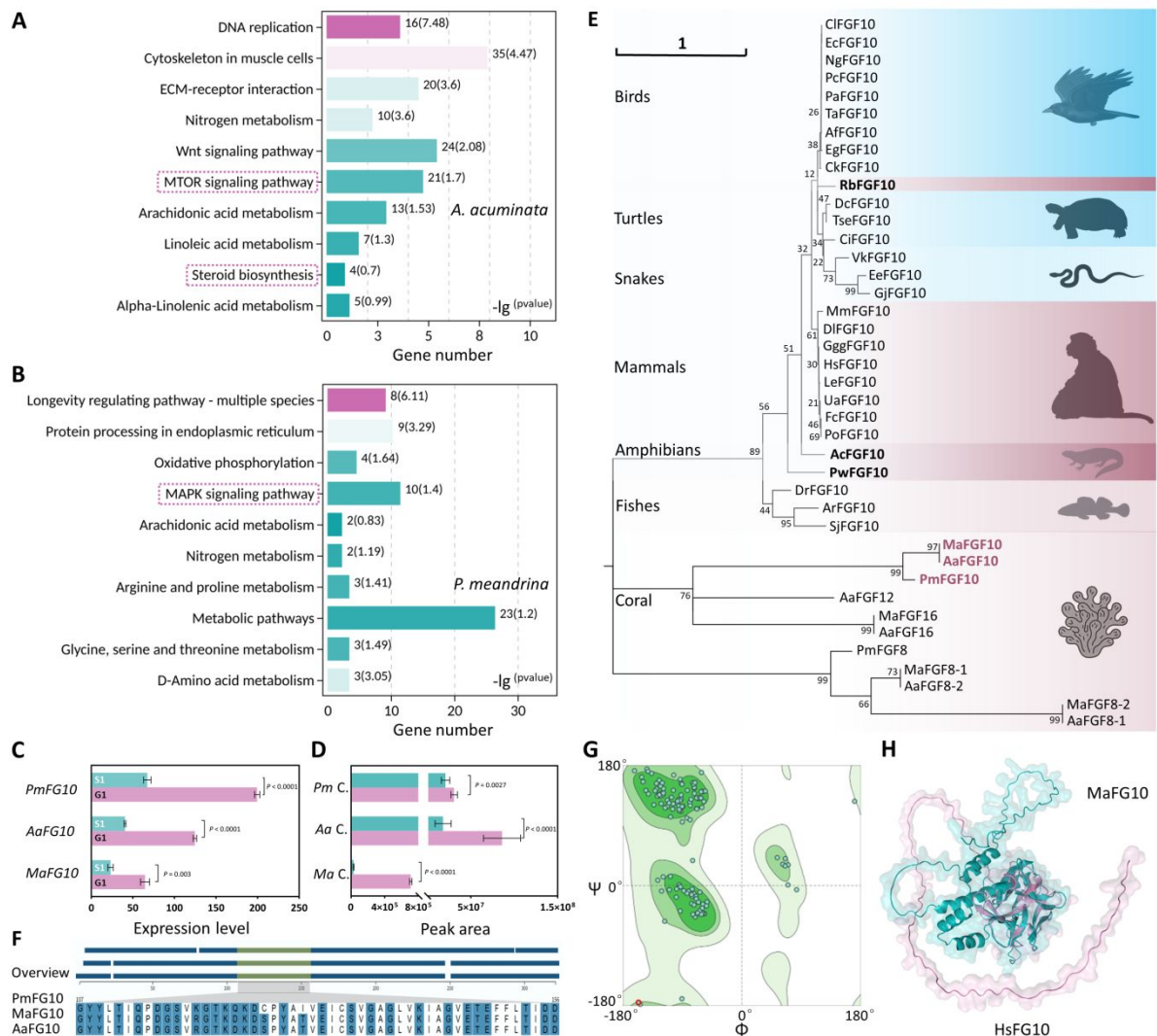


Figure 6. Conservative traits of coral growth in nature. A: KEGG enrichment analysis of upregulated genes in G1 of *A. acuminata*. B: KEGG enrichment analysis of upregulated genes in G1 of *P. meandrina*. C: Expression of *FGF10* in different species of corals (N=3). D: The peak area of cortisone in different species of corals (N=6). E: Evolutionary analysis of *FGF10* in different species. F: Analysis of the conserved domains of *FGF10* in different species of corals. G: Predicted tertiary structure diagram of monomeric MaFGF10. H: Comparative analysis of tertiary structures between MaFGF0 and HsFGF10 proteins.

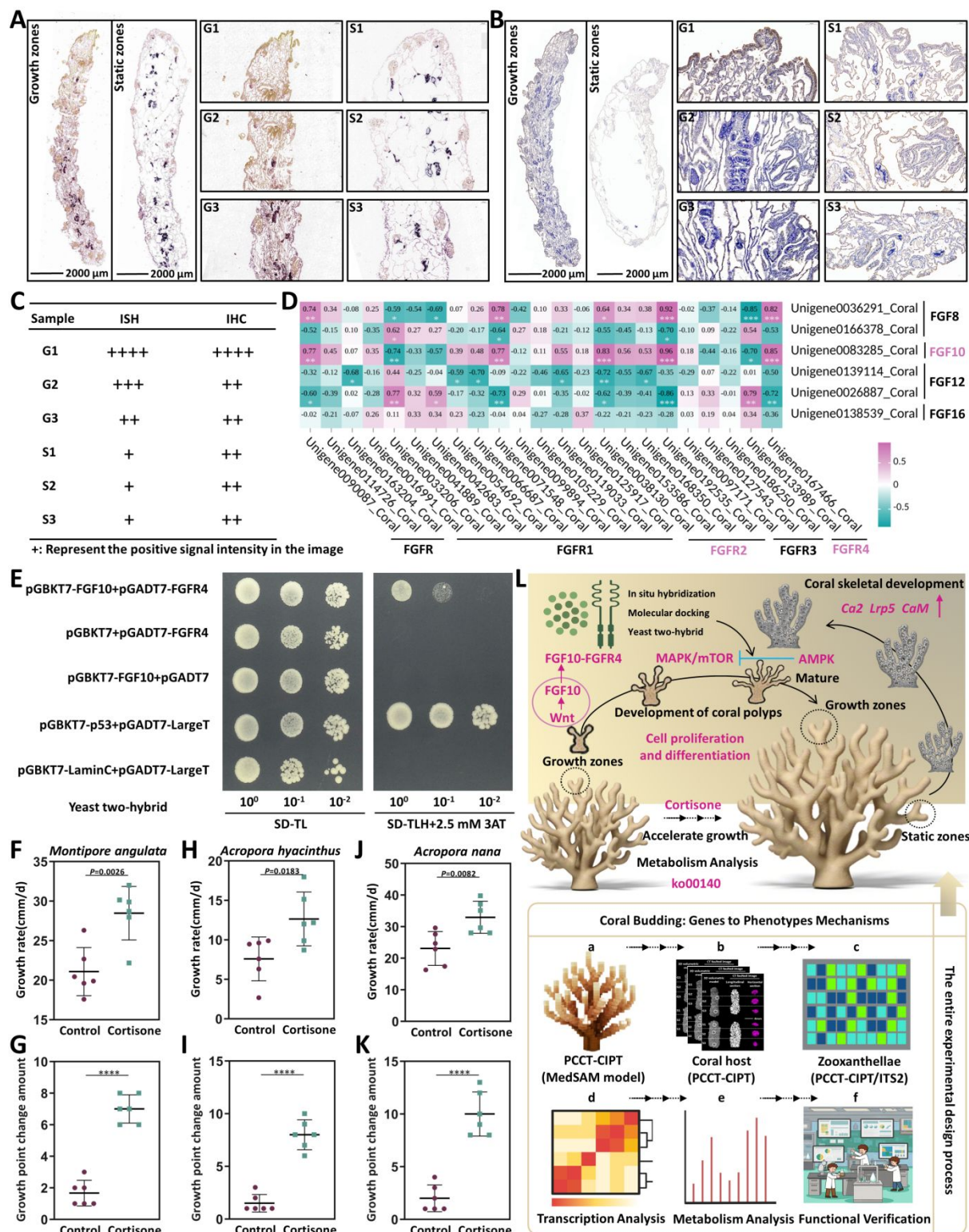


Figure 7. Functional verification of core signaling networks. A: ISH to detect the expression of *MaFGF10* in growth zones and static zones. B: IHC experiment to detect the expression of *MaFGFR4* in growth zones and static zones. C: The positive signal intensity in

986 ISH and IHC images. D: Screening for *FGFRs* that interact with *FGF10* (Generating a
 987 heatmap based on the Pearson correlation coefficients between the expression levels of
 988 *MaFGFs* and *MaFGFRs*, where positive values indicate positive correlations. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$). E: Y2H interaction verification. F: The changes in the growth rate of
 990 *M. angulata* after cortisone treatment (N=6). G: The alterations in growth polarity of *M.*
 991 *angulata* following cortisone treatment (N=6, ****: $P < 0.0001$). H: The changes in the
 992 growth rate of *A. hyacinthus* after cortisone treatment (N=6). I: The alterations in growth
 993 polarity of *A. hyacinthus* following cortisone treatment (N=6, ****: $P < 0.0001$). J: The
 994 changes in the growth rate of *A. nana* after cortisone treatment (N=6). K: The alterations in
 995 growth polarity of *A. nana* following cortisone treatment (N=6, ****: $P < 0.0001$). L: Multi-
 996 omics joint analysis based on PCCT-CIPT to explore the budding growth mechanism of
 997 corals.

998 **Supplementary Materials**

999 This PDF file includes:

1000 Supplementary Figures S1-S22

1001 Supplementary Tables S1-S3

1002 **Supplementary Data**

1003 Data S1: Host gene expression levels of *M. angulata*.

1004 Data S2: Gene expression levels of symbiodiniaceans in *M. angulata*

1005 Data S3: Differentially abundant metabolites enriched in the steroid hormone biosynthesis
1006 pathway.

1007 Data S4: Key enzymes in the steroid hormone biosynthesis pathway.

形态学、转录组学与代谢组学联合解析珊瑚生长核心调控程序

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摘要: 珊瑚礁生态系统正面临持续退化与濒临崩溃的严峻局面。其根本原因在于, 珊瑚生长与自然恢复速率远低于其种群衰退速率。然而, 目前对调控珊瑚生长发育内在机制仍缺乏系统认识。本研究开发了一种基于光子计数 CT 的高精度形态学技术 (PCCT-CIPT), 结合多组学分析与功能实验, 揭示了造礁珊瑚中以宿主为核心、调控水螅体与骨骼协同发育的保守性机制。该机制在不同珊瑚物种及生境中均可被稳定验证。研究发现, Wnt-FGF10-MAPK/mTOR 信号轴通过调控 *c-Myc* 与 *Sox9* 介导的细胞分裂与分化过程, 促进水螅体形态发育与成熟; TGF- β 信号通过激活 SMAD5, 引导细胞向生物矿化



1029 相关方向分化。随着水螅体逐渐成熟，AMPK 信号通路特异性上调，并通过抑制 mTOR
1030 信号通路实现细胞增殖与分化的精确平衡。在此过程中，生物钙化相关基因(包括 *CA2*、
1031 *Lrp5* 和 *CLMD*) 表达逐步升高，驱动骨骼从初级框架构建向高密度稳定结构的逐步转
1032 化。此外，在珊瑚发育早期，类固醇激素在促进细胞周期进程及骨骼框架的初始构建中
1033 发挥关键调控作用。综上所述，本研究初步揭示了一个以宿主为主导的珊瑚生长发育调
1034 控程序，该调控网络的阐明可能为珊瑚礁生态修复提供新的潜在干预靶点。

1035 **关键词：**造礁珊瑚；珊瑚生长；珊瑚礁修复；水螅体-骨骼协同发育；FGF10 信号轴