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# Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed | |
|---|---|---|
| ☐ | ☒ | The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☒ | ☐ | A description of all covariates tested |
| ☐ | ☒ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☒ | ☐ | For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ | Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | Data were collected using a custom-built confocal Raman microspectroscopy system based on an Olympus BX53 microscope and integrated by SuperVision Medicine Co., Ltd. Raman spectra were acquired with a 532-nm continuous-wave laser, a 40× water-immersion objective, a Horiba iHR 320 spectrograph, and a deep-cooled EMCCD detector. Three-dimensional volumetric imaging was performed by point-by-point XYZ raster scanning at 10 mW and 0.01 s per voxel over a Raman shift range of 0–3850 $cm^{-1}$. Reference spectra were additionally acquired from biomolecular standards and tissue phantoms. Complementary metabolomic data were collected by UHPLC–high-resolution tandem mass spectrometry using a Nexera LC-30A system coupled to a Q Exactive HF-X mass spectrometer in polarity-switching mode, with full-scan MS1 and Top-10 data-dependent HCD-MS2 acquisition. |
| Data analysis | Raman spectral data were processed using custom chemometric routines for cosmic-ray removal, adaptive min–max fluorescence-background subtraction, normalization to the water O–H stretching band, linear spectral unmixing, non-negative least-squares fitting, Bayesian inversion, and concentration calibration. Three-dimensional Raman concentration maps were reconstructed and visualized using ImageJ (Image-Pro Plus 6.0 ). Raw LC–MS/MS data were processed using Progenesis QI (Waters Corporation) for peak alignment, metabolite annotation, QC-based signal-drift correction, and relative quantification; metabolite identities were assigned by comparison with the HMDB and MassBank databases. Statistical and integrative analyses were performed in R using the prcomp function and the factoextra, pheatmap, glmnet, vegan, and linkET packages for principal component analysis, heatmap generation and hierarchical clustering, LASSO feature selection, Spearman correlation analysis, and network visualization, respectively. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The authors declare that all data supporting of results in this study are available within the paper and its supplementary information, or from the corresponding authors upon request. The raw sequencing data generated in this study has been uploaded as Source data.

## Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

| | |
|---|---|
| Reporting on sex and gender | No additional information. |
| Reporting on race, ethnicity, or other socially relevant groupings | No additional information. |
| Population characteristics | No additional information. |
| Recruitment | No additional information. |
| Ethics oversight | No additional information. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Sample size | Raman imaging was performed on one representative osteochondral sample from each group for spatially resolved biochemical visualization; therefore, Raman voxel-level measurements were not treated as independent biological replicates or used alone for group-level statistical inference. Proteomic and metabolomic analyses were performed using six biologically independent samples per group (n = 6). The sample size was determined based on sample availability and the feasibility of high-dimensional multi-omics analysis, and no formal a priori power calculation was performed. |
| Data exclusions | No biological samples or experimental data were excluded from the analyses. |
| Replication | Each group included six biologically independent samples. Sample preparation, Raman spectral acquisition and LC–MS/MS measurements were performed using the same standardized protocols. Where applicable, key measurements were performed in three independent experimental runs to assess technical reproducibility, and consistent results were obtained across replicates. |
| Randomization | Random allocation was not applicable because the samples belonged to predefined age groups or experimental conditions rather than being prospectively assigned to treatment groups. |
| Blinding | Formal blinding was not performed during data collection or analysis. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☐ | ☒ Animals and other organisms |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☒ | ☐ Plants |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

# Antibodies

Antibodies used: Primary antibodies used in this study were: anti-COL I (Servicebio, GB115707, rabbit, 1:500), anti-IL-2 (Servicebio, GB11114, rabbit, 1:200), anti-TNF-α (Servicebio, GB15188, rabbit, 1:500), anti-IL-6 (Servicebio, GB11117, rabbit, 1:200), anti-p16 (Servicebio, GB151143, rabbit, 1:500), anti-p21 (Servicebio, GB155313, rabbit, 1:500), anti-BMP2 (Servicebio, GB15252, rabbit, 1:500), anti-RUNX2 (Servicebio, GB125631, mouse, 1:500), and anti-SPP1 (Servicebio, GB120018, rabbit, 1:500).

Validation: All primary antibodies were commercially obtained from Servicebio and used under the application conditions and dilution ratios specified above.

# Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals: Male C57BL/6J mice were used in this study. Animals were divided into three age groups: 4 weeks (young), 18 weeks (adult), and 21 months (aged), with six mice in each group (n = 6 per group; 18 mice in total). At the designated time points, mice were euthanized following deep anesthesia, and the knee joints were rapidly dissected for Raman imaging, histological, immunofluorescence, metabolomic, and biochemical analyses.

Wild animals: This study did not involve wild animals or animals captured, observed, or collected in the field.

Reporting on sex: Only male mice were included in this study. Sex was considered during study design, and male animals were selected to minimize variability associated with hormonal fluctuations in bone metabolism and osteochondral aging. Therefore, the findings reported here are based exclusively on male mice, and no sex-based comparative analysis was performed.

Field-collected samples: This study did not involve animals or biological samples collected from the field. All samples were obtained from laboratory-maintained C57BL/6J mice

Ethics oversight: All animal procedures were reviewed and approved by the Laboratory Animal Ethics Committee of Shanghai Shengwei Biotechnology Co., Ltd. (approval no. 2026-01-RJYY-CJ-134). All procedures were performed in accordance with relevant institutional and national guidelines for the care and use of laboratory animals

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Plants

Seed stocks: No additional information.

Novel plant genotypes: No additional information.

Authentication: No additional information.