Supplementary Information

Table 1. Key Resources

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Hydroxytyrosol acetate	Santa Cruz Biotech	sc488930
Alizarin red S	Sigma-Aldrich	A5533-25g
Nile Red	Sigma-Aldrich	N3013
Experimental models: Organisms/strains		
Mouse	C57BL/6	Experimental Animal Center of Air Force Medical University
Critical commercial assays		
ELISA kit	mouse TNF-a	Shanghai Enzyme-linked Biotech, ml002095
ELISA kit	mouse IL-6	Shanghai Enzyme-linked Biotech, ml063159
ELISA kit	mouse CRP	Shanghai Enzyme-linked Biotech, ml064282
ELISA kit	mouse ROS	Shanghai Enzyme-linked Biotech, ml009876
Oligonucleotides		
m_*Alpl*_F	5'-GTTGCCAAGCTGGGAAGAACAC-3'	Sangon Biotech
m_*Alpl*_R	5'-CCCACCCCGCTATTCCAAAC-3'	Sangon Biotech
m_*Runx2*_F	5'-CGCCCCTCCCTGAACTCT-3'	Sangon Biotech
m_*Runx2*_R	5'-TGCCTGCCTGGGATCTGTA-3'	Sangon Biotech
m_*Sp7*_F	5'-TCCCTGGATATGACTCATCCCT-3'	Sangon Biotech
m_*Sp7*_R	5'-CCAAGGAGTAGGTGTGTTGCC-3'	Sangon Biotech
m_*Pparg*_F	5'-AAGAGCTGACCCAATGGTTG-3'	Sangon Biotech
m_*Pparg*_R	5'-ACCCTTGCATCCTTCACAAG-3'	Sangon Biotech

REAGENT or RESOURCE	SOURCE	IDENTIFIER
m_*Cebpa*_F	5'-ACCGGGTTTCGGGACTTGA-3'	Sangon Biotech
m_*Cebpa*_R	5'-CCCGCAGGAACATCTTTAAGTGA-3'	Sangon Biotech
m_*Lpl*_F	5'-AACACAACCAGGCCTTCGAGA-3'	Sangon Biotech
m_*Lpl*_R	5'-CCGATGTCCACCTCCGTGTA-3'	Sangon Biotech
m_*Gapdh*_F	5'-TGCACCACCAACTGCTTAG-3'	Sangon Biotech
m_*Gapdh*_R	5'-GGATGCAGGGATGATGTTC-3'	Sangon Biotech
Software and algorithms		
AODB	South China University of Technology	idrugLab
Metascape	Metascape Foundation	N/A
PubChem, PubMed, GEO, Gene, BLAST	National Center for Biotechnology Information	N/A
UniProt	EMBL-EBI, SIB, PIR	N/A
SwissADME, SwissTargetPrediction	Swiss Institute of Bioinformatics	Molecular Modeling Group
Coconut	Friedrich Schiller University Jena	Steinbeck-Lab
SEA	University of California San Francisco	Shoichet Lab
Super-PRED	Charité-University Medicine Berlin	Structural Bioinformatics Group
TargetNet	Central South University	Computational Biology & Drug Design Group
R	R Development Core Team	4.3.2
Rstudio	Posit Software	2024.12.1+563
phyloseq	Stanford University	Paul J. McMurdie
car	John Fox, et al.	3.1-3
emmeans	Russell V. Lenth, et al.	1.11.2-8
effectsize	Mattan S. Ben-Shachar, et al.	1.0.1
outliers	Lukasz Komsta	0.15

REAGENT or RESOURCE	SOURCE	IDENTIFIER
ggplot2	Hadley Wickham, et al.	4.0.0
STAMP	The University of Queensland	Donovan H Parks
MetaboAnalyst 6.0	McGill University	XiaLab
GMrepo v2	Huazhong University of Science and Technology	Wei-Hua Chen group
Image-Pro Plus	Media Cybernetics	6.0
Excel	Microsoft	2501
Graphpad Prism	GraphPad Software	10.1.2(324)
Origin	OriginLab	10.2.0.188
Mimics	Materialise NV	21.0.0.406
Abaqus	Dassault Systemes Simulia Corp.	2023
Photoshop	Adobe	24.5
Illustrator	Adobe	27.5

Supplementary Figures

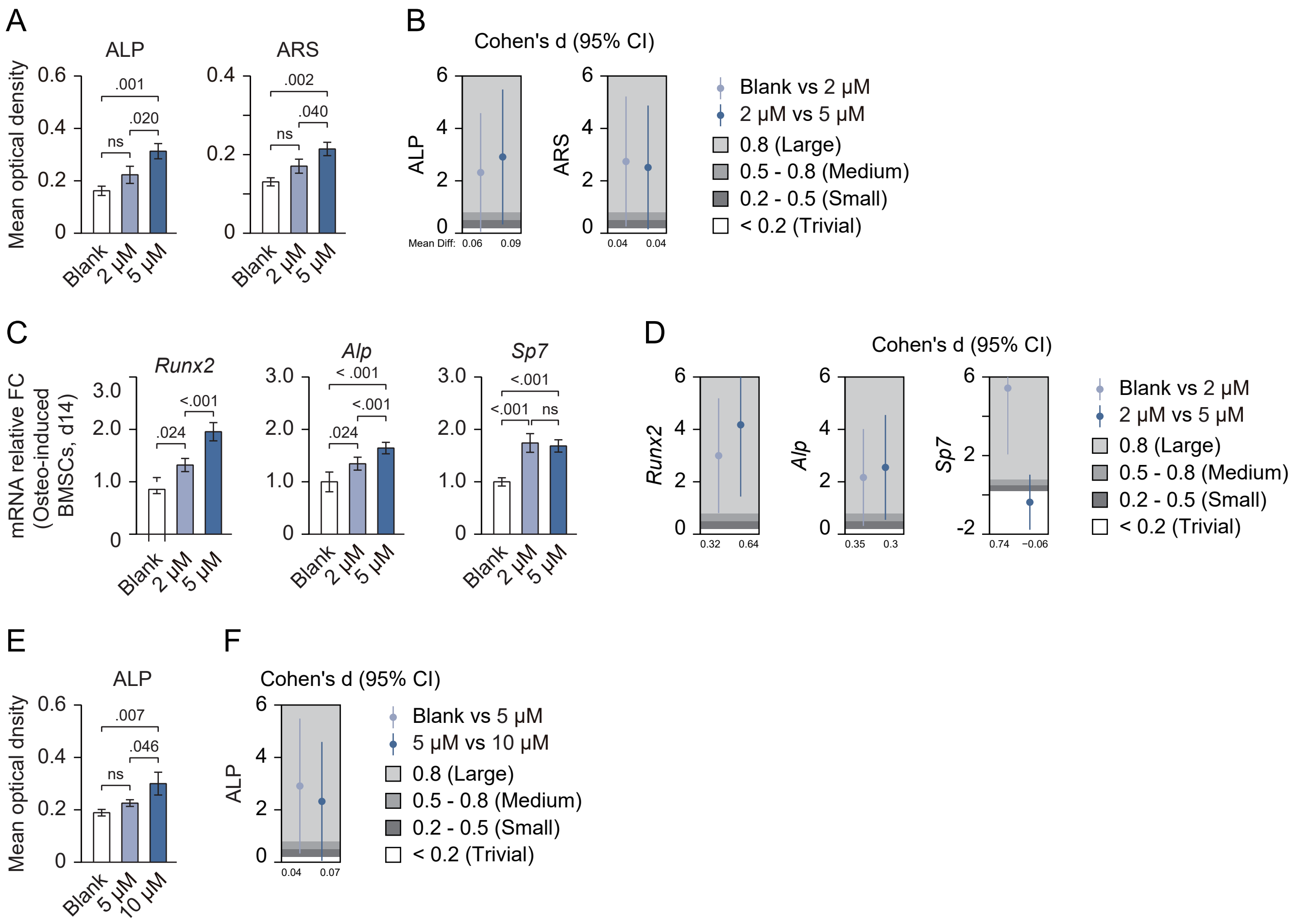

Fig. S1. The effect of HT-ac on osteogenic differentiation of BMSCs and MC3T3-E1 osteoblasts in vitro. (A) Mean optical density of BMSCs after ALP (Alkaline Phosphatase) and ARS (Alizarin Red S) staining. (B) Estimation of effect size for panel A. (C) RT-qPCR analysis of osteogenic genes expression (normalized to Gapdh). (D) Estimation of effect size for panel C. (E) Mean optical density of MC3T3-E1 osteoblasts after ALP (Alkaline Phosphatase) staining. (F) Estimation of effect size for panel E. Statistical significance was determined via One-way ANOVA followed by Bonferroni-corrected post-hoc pairwise comparisons; adj.*p* (denoted as corrected *p*-value in the figure) < 0.05 was considered statistically significant. Effect size (Cohen's d) was compared between adjacent groups, and is classified as Large (≥ 0.8), Medium (0.5 - 0.8), Small (0.2 - 0.5), or Trivial (< 0.2).

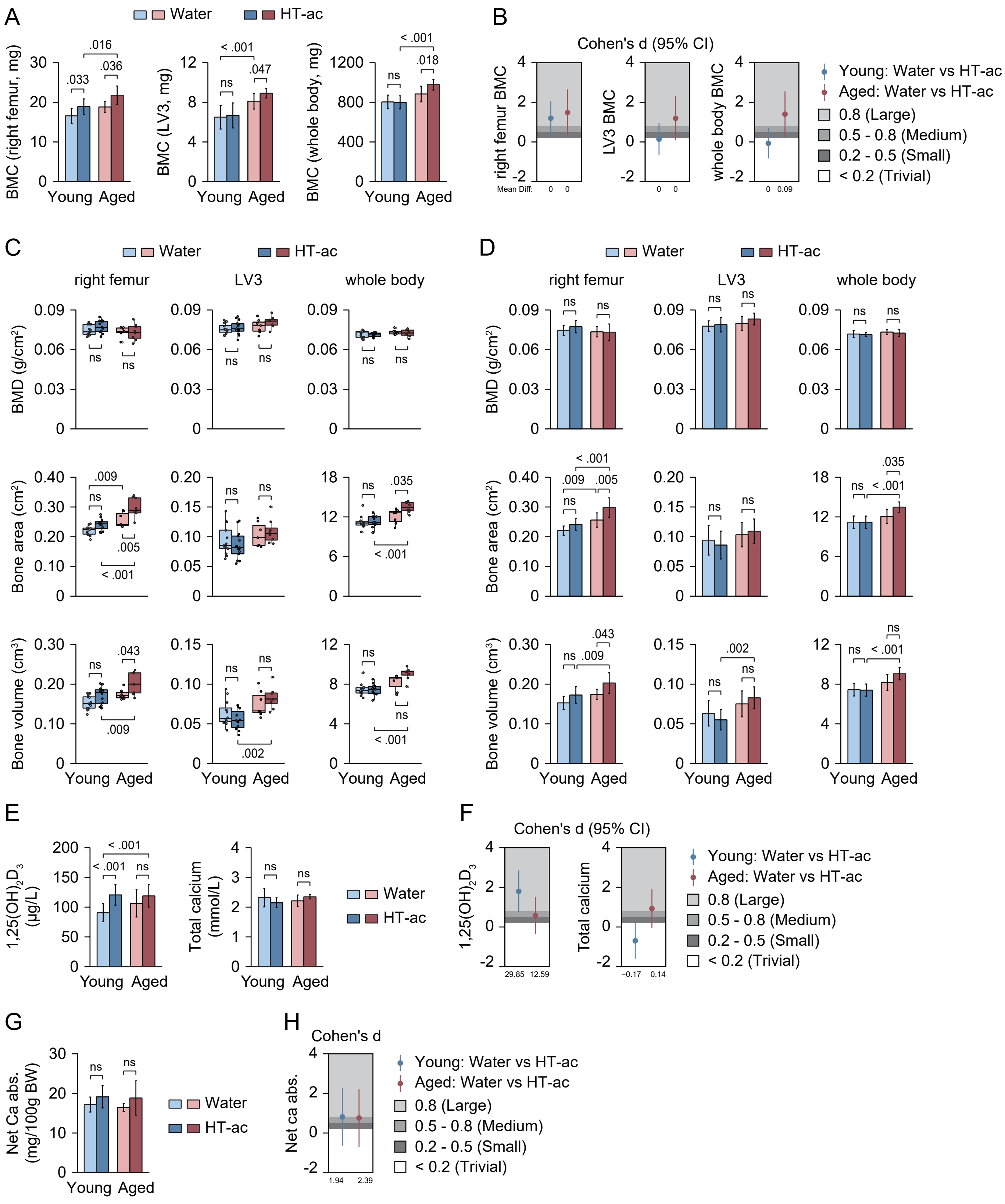

Fig. S2. The effects of HT-ac supplementation on bone properties – DEXA results. (A) Bone mineral content (BMC) of key sites in bar plots. (B) Estimation of effect size for panel A. (C-D) Bone mineral density (BMD), bone area and bone volume of right femur, third lumbar vertebra and whole body, respectively; box plots and bar plots (mean ± SD) are presented simultaneously. (E) Levels of serological factors in bar plots. (F) Estimation of effect size for panel E. (G) Net calcium absorption in bar plots. (H) Estimation of effect size for panel G. Statistical significance was determined via Two-way ANOVA followed by Bonferroni-corrected post-hoc pairwise comparisons; adj.*p* (denoted as corrected *p*-value in the figure) < 0.05 was considered statistically significant. Effect size (Cohen's d) was compared between adjacent groups, and is classified as Large (≥ 0.8), Medium (0.5 - 0.8), Small (0.2 - 0.5), or Trivial (< 0.2).

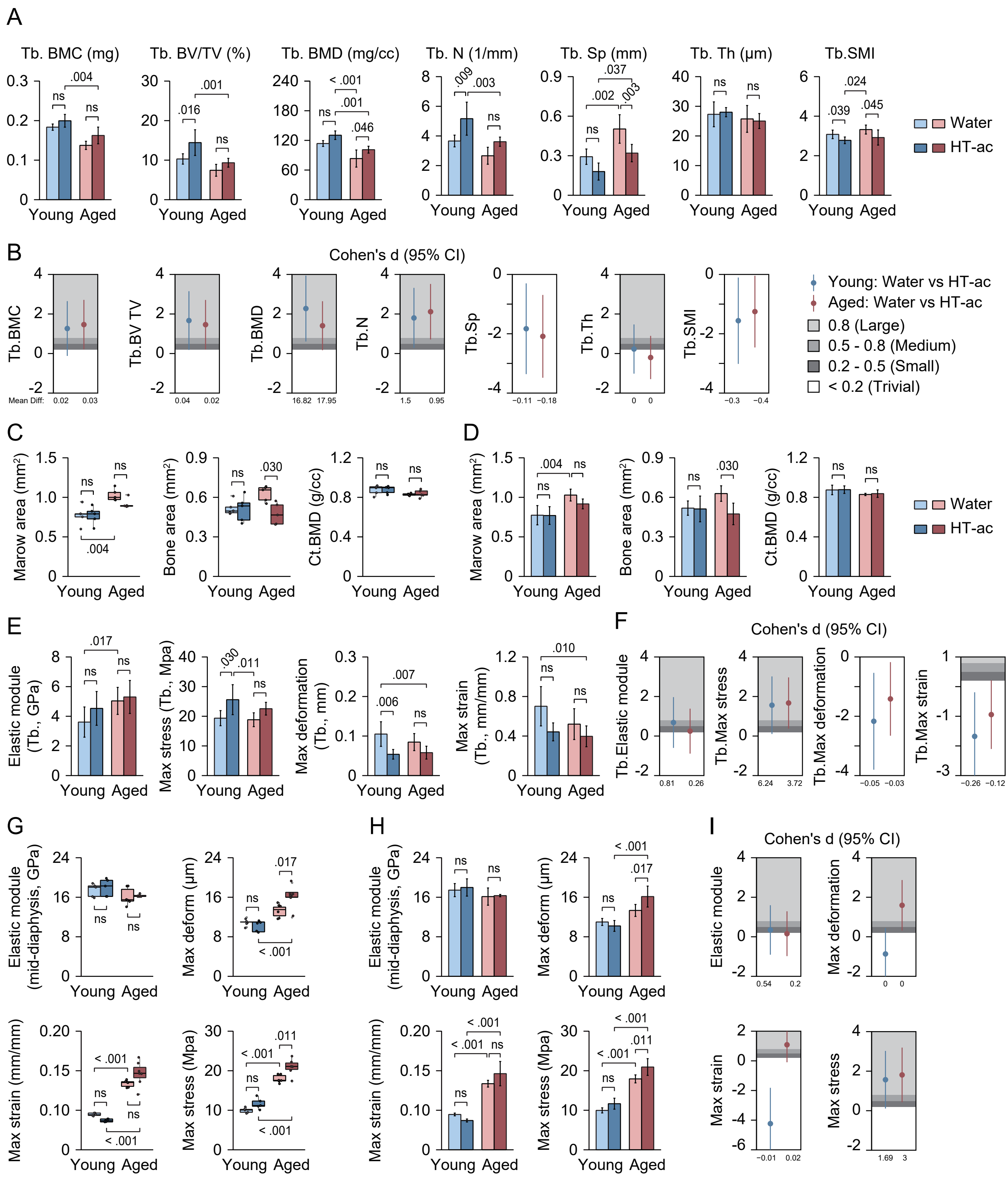

Fig. S3. The effects of HT-ac supplementation on bone properties – microCT results and mechanical indices. (A) Stereological parameters for trabecular bone below the growth plate of femur. BMD, bone mineral density; BV/TV, bone volume fraction; Tb.N, trabecular number; Tb.Sp, trabecular spacing; Tb.Th, trabecular thickness; SMI, structural model index. (B) Estimation of effect size for panel A. (C-D) Cortical bone densitometry parameters in box plots and bar plots. (E) Mechanical properties of trabecular bone below the growth plate of femur. (F) Estimation of effect size for panel E. (G-H) Mechanical properties of the mid-diaphysis in the femur, box plots and bar plots (mean ± SD) are presented simultaneously. (I) Estimation of effect size for panel G/H. Statistical significance was determined via Two-way ANOVA followed by Bonferroni-corrected post-hoc pairwise comparisons; adj.*p* (denoted as corrected *p*-value in the figure) < 0.05 was considered statistically significant. Effect size (Cohen's d) was compared between adjacent groups, and is classified as Large (≥ 0.8), Medium (0.5 - 0.8), Small (0.2 - 0.5), or Trivial (< 0.2).

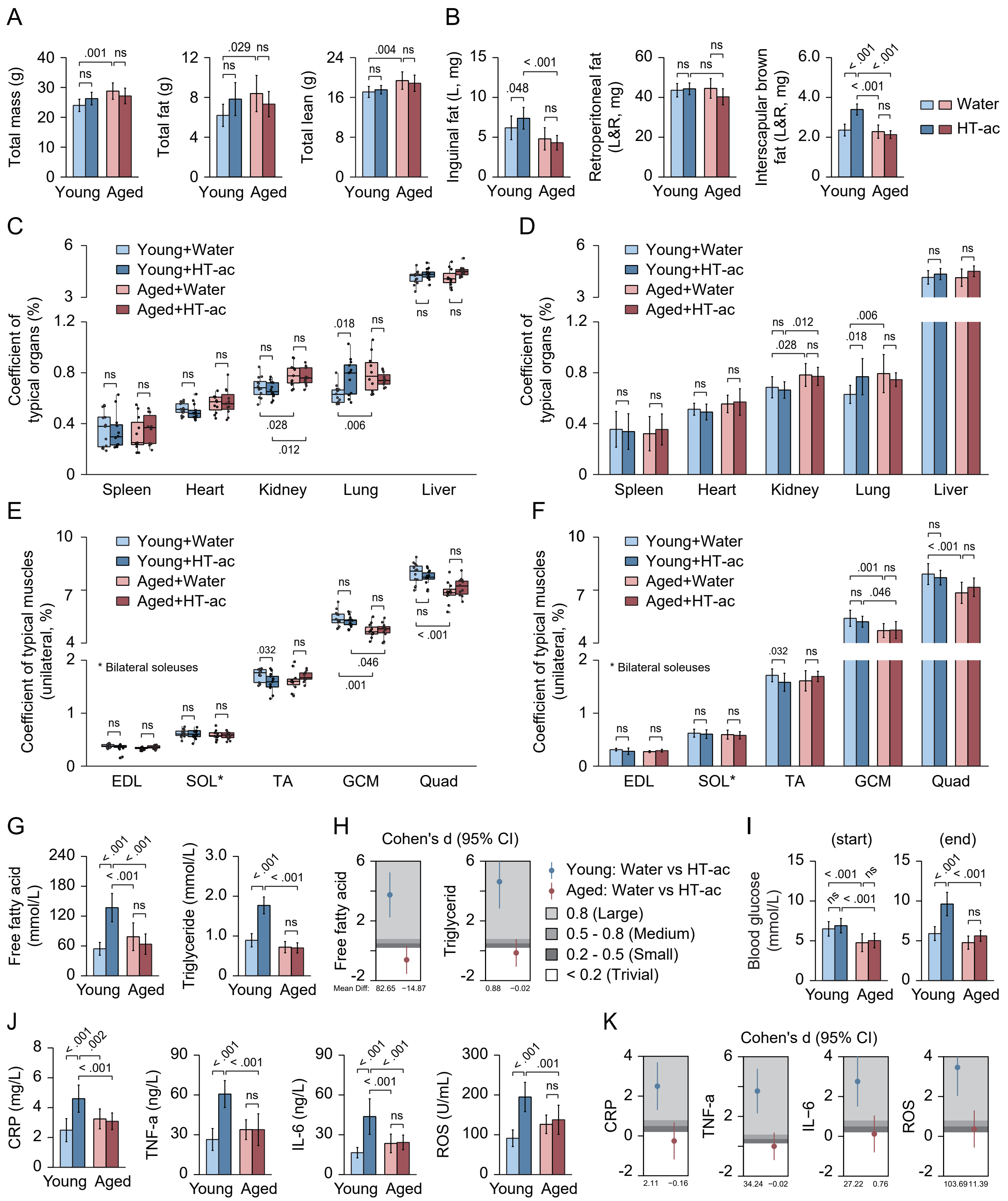

Fig. S4. The effects of HT-ac supplementation on the weight of typical organs and muscle tissues. (A) DEXA-derived indices related to whole body fat and lean. (B) Weights of different adipose tissue at autopsy (mean ± SD). (C-F) Coefficient of typical organs and muscles after the gavage treatment, box plots and bar plots (mean ± SD) are presented simultaneously. (G) Serological factors related to lipid metabolism. (H) Estimation of effect size for panel G. (I) Fasting blood glucose before and after gavage. (J) Serological factors related to inflammation. CRP, C-reactive protein; ROS, reactive oxygen species. (K) Estimation of effect size for panel J. Statistical significance was determined via Two-way ANOVA followed by Bonferroni-corrected post-hoc pairwise comparisons; adj.*p* (denoted as corrected *p*-value in the figure) < 0.05 was considered statistically significant. Effect size (Cohen's d) was compared between adjacent groups, and is classified as Large (≥ 0.8), Medium (0.5 - 0.8), Small (0.2 - 0.5), or Trivial (< 0.2).

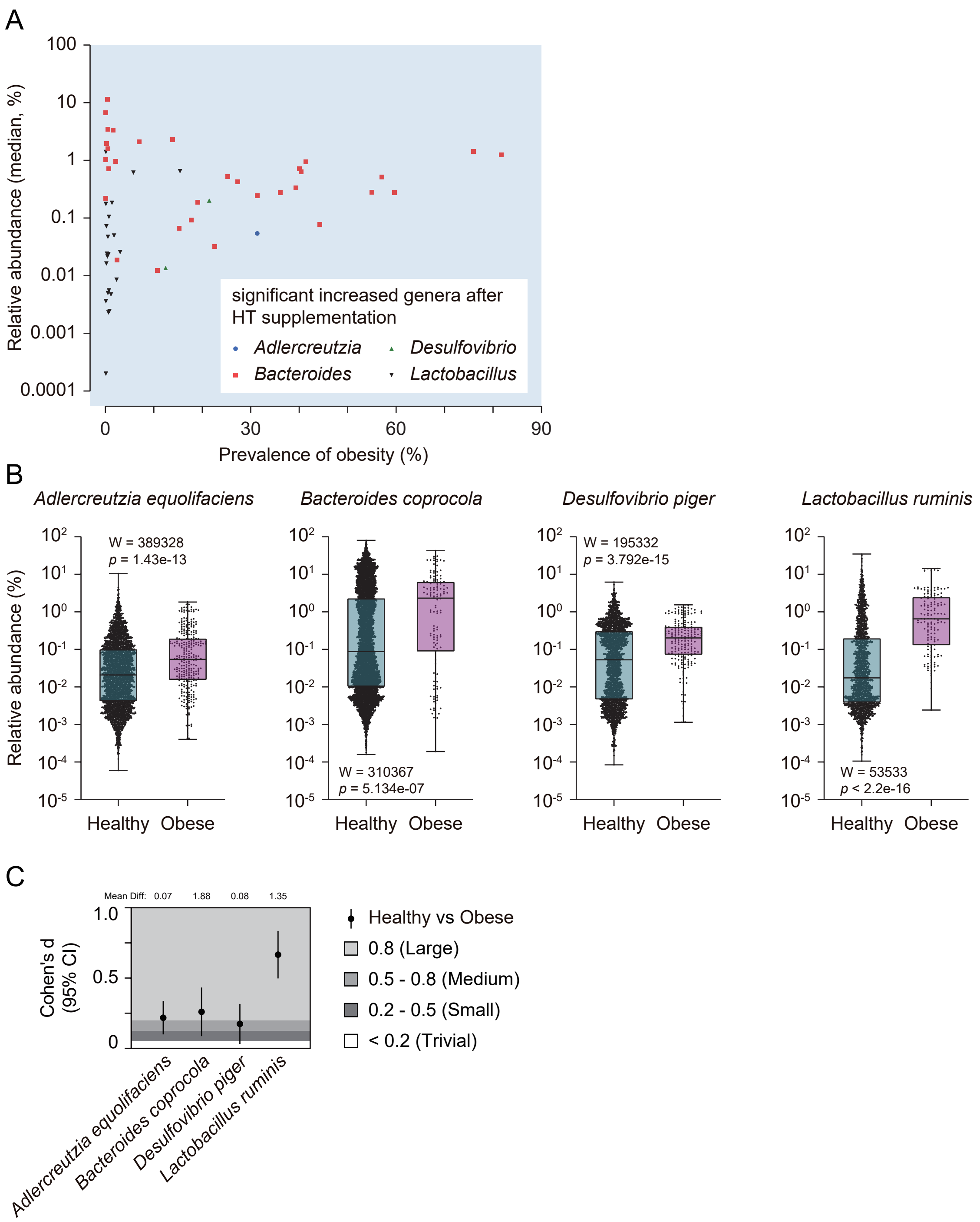

Fig. S5. HT supplementation increased the abundance of gut microbiota associated with obesity. (A) Correlation between the prevalence of obesity and the relative abundance of specific bacterial species that showed a significant increase after HT supplementation. Each point represents a species under the specified genus. (B) Relative abundance of representative species of the specific genus in obese individuals. (C) Estimation of effect size for panel B. Effect size (Cohen's d) was compared between Healthy and Obese group, and is classified as Large (≥ 0.8), Medium (0.5 - 0.8), Small (0.2 - 0.5), or Trivial (< 0.2). Differences between two groups were evaluated using the Mann–Whitney U test.