

Supplementary materials

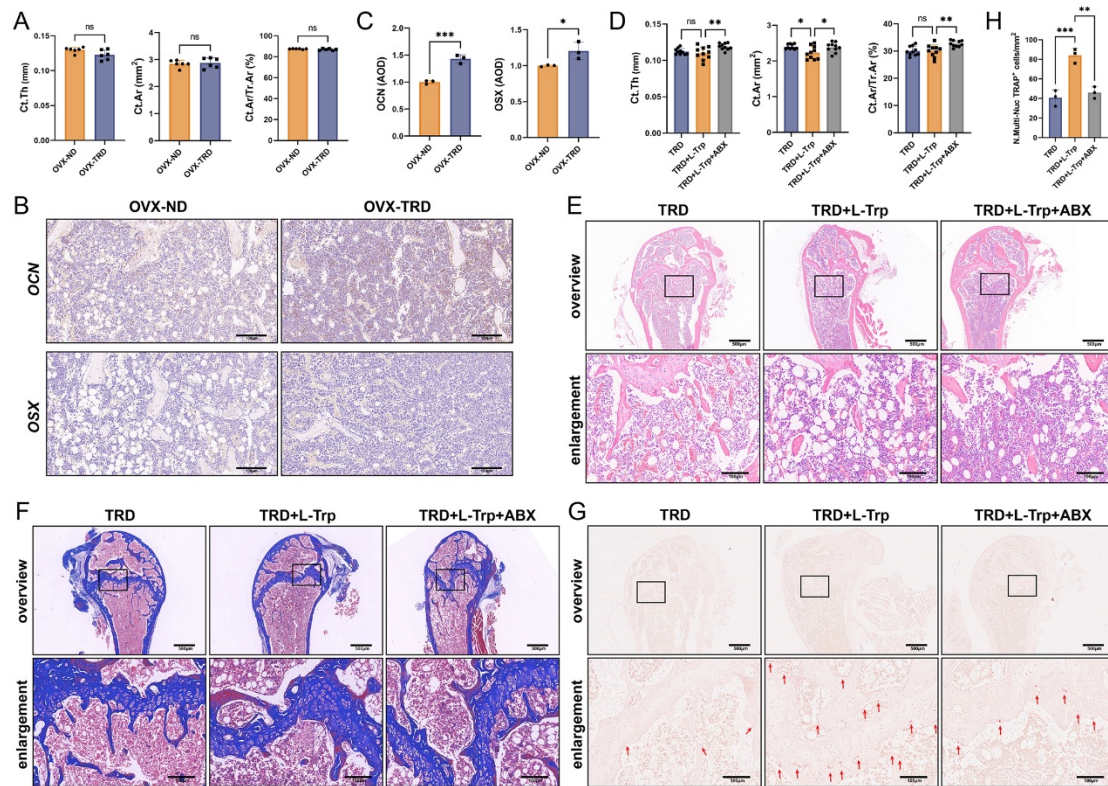


Figure S1: Intermittent tryptophan restriction exerted an osteoprotective effect involved by the gut microbiota. (A) Quantitative analyses of cortical bone histomorphometry indices including Ct.Th, Ct.Ar, and Ct.Ar/Tr.Ar in OVX-ND group and OVX-TRD group. (B) Immunohistochemistry staining of femur sections for OSX and OCN. (C) AOD quantification of OSX and OCN after normalization. (D) Quantitative analyses of cortical bone histomorphometry indices including Ct.Th, Ct.Ar, and Ct.Ar/Tr.Ar among TRD group, TRD + L-Trp group, and TRD + L-Trp + ABX group. (E-G) H&E, Masson, and TARP staining of femur tissue in TRD group, TRD + L-Trp group, and TRD + L-Trp + ABX group. (H) Quantitative analysis of the number of TRAP⁺ cells per mm².

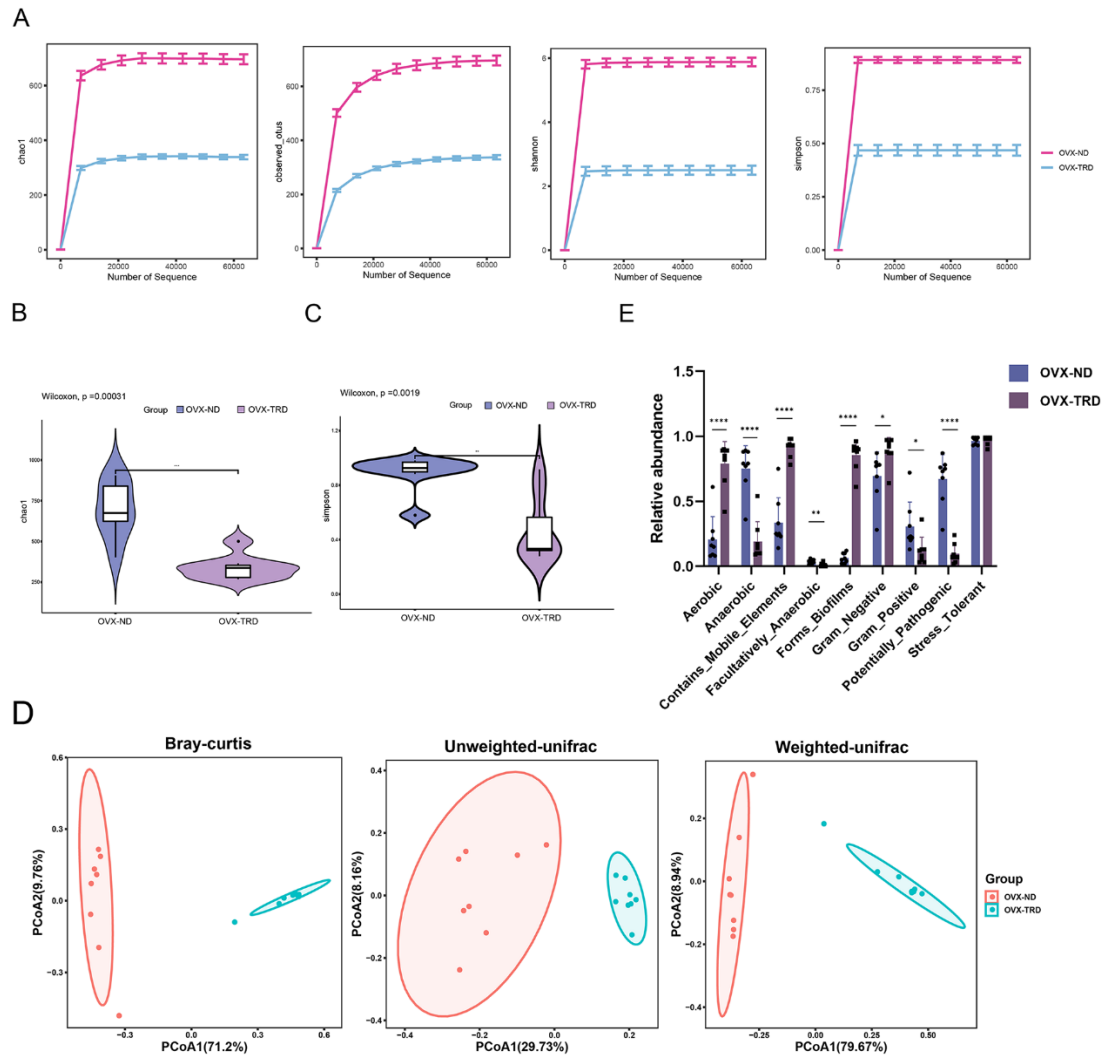


Figure S2: Comprehensive characterization of gut microbiota diversity and composition in the OVX-ND and OVX-TRD groups. (A) Rarefaction curves derived from α -diversity. **(B-C)** Violin plot of related α -diversity consisting of Chao1 and Simpson. **(D)** PCoA based on the Bray-Curtis, Unweighted UniFrac and Weighted UniFrac analyses. **(E)** Bar plot about the abundance of microbiota.

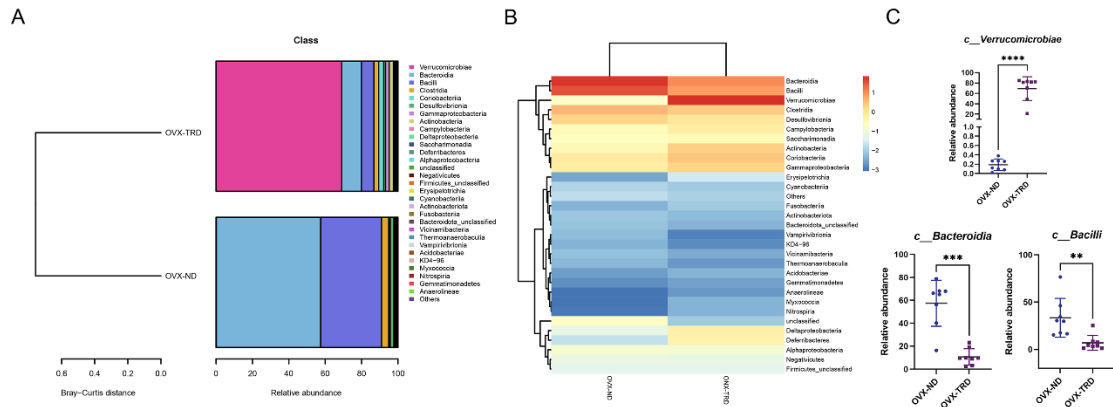


Figure S3: Intermittent tryptophan restriction shifted gut microbiota composition at the class taxonomic level. (A) Bar graph of the bacteria at the class taxonomic level. **(B)** Heatmap depicting different gut microbiota at the class taxonomic level. **(C)** The relative abundance of *c_Verrucomicrobiaea*, *c_Bacteroidia*, and *c_Bacilli* between OVX-ND group and OVX-TRD group.

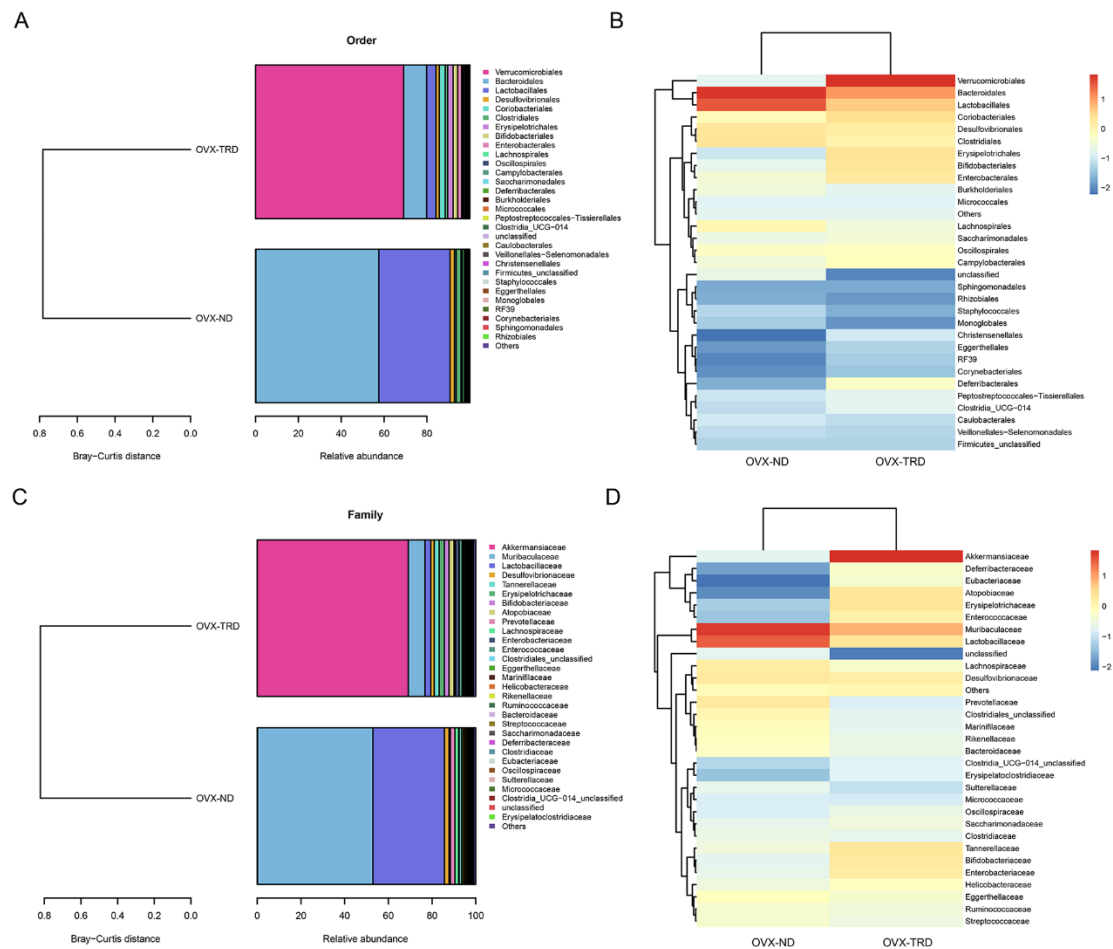


Figure S4: Intermittent tryptophan restriction shifted gut microbiota composition

at the order and family taxonomic level. (A) Bar graph of the bacteria at the order taxonomic level. (B) Heatmap depicting different gut microbiota at the order taxonomic level. (C) Bar graph of the bacteria at the family taxonomic level. (D) Heatmap depicting different gut microbiota at the family taxonomic level.

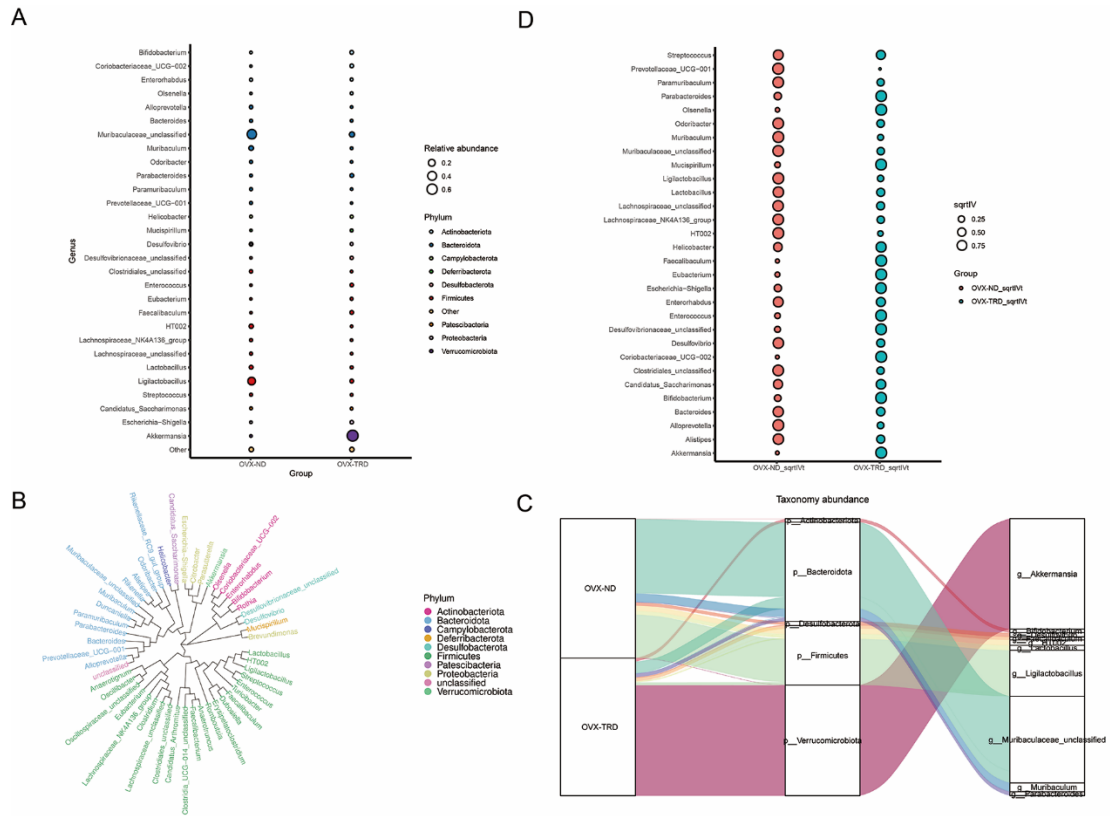


Figure S5: Intermittent tryptophan restriction shifted gut microbiota composition at the genus taxonomic level. (A) Bubble plot of the bacteria at the genus taxonomic level. **(B)** The evolutionary branching tree of the gut microbiota. **(C)** Sankey diagram of genus-level and phylum-level taxonomic shifts between the OVX-ND group and OVX-TRD group. **(D)** Bubble plot highlighting microbiome biomarkers with significant indicator values at the genus taxonomic level.

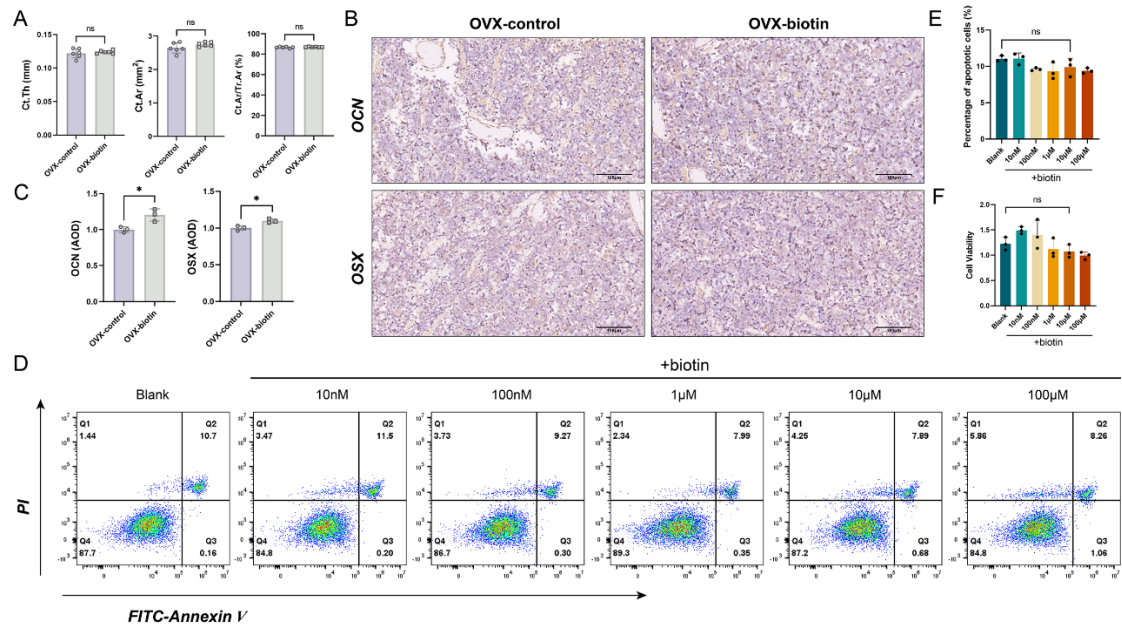


Figure S6: Biotin supplementation promoted osteogenic marker expression. (A) Quantitative analyses of cortical bone histomorphometry indices, including Ct.Th, Ct.Ar, and Ct.Ar/Tr.Ar in the OVX-control group and OVX-biotin group. **(B)** Immunohistochemistry staining of femur sections for OSX and OCN. **(C)** AOD quantification of OSX and OCN after normalization. **(D)** BMDMs were treated with biotin at 10 nM, 100 nM, 1 μM, 10 μM or 100 μM for 24 h, and apoptosis was quantified by Annexin V-FITC/PI flow cytometry. **(E)** Flow-cytometric quantification revealed that 10 μM biotin did not increase the apoptosis of BMDMs ($p>0.05$, ns). **(F)** CCK-8 assay confirmed that 10μM biotin exerted no cytotoxic effect on BMDMs ($p>0.05$, ns).

Table S1: Primer sequence for qPCR.

	Forward Primer (5' → 3')	Reverse Primer (5' → 3')
CD86	TCCTCAAACGTATTGGAAGGAGA	GCTAGGCTGATTCGGCTTCT
TNF- α	ATGAGAAGTTCCCAAATGGC	CTCCACTTGGTGGTTTGCTA
IL-1 β	CACAGCAGCACATCAACAAG	GTGCTCATGTCCATCCTG
Gapdh	GGTTGTCTCCTGCGACTTCA	TGGTCCAGGGTTTCTTACTCC