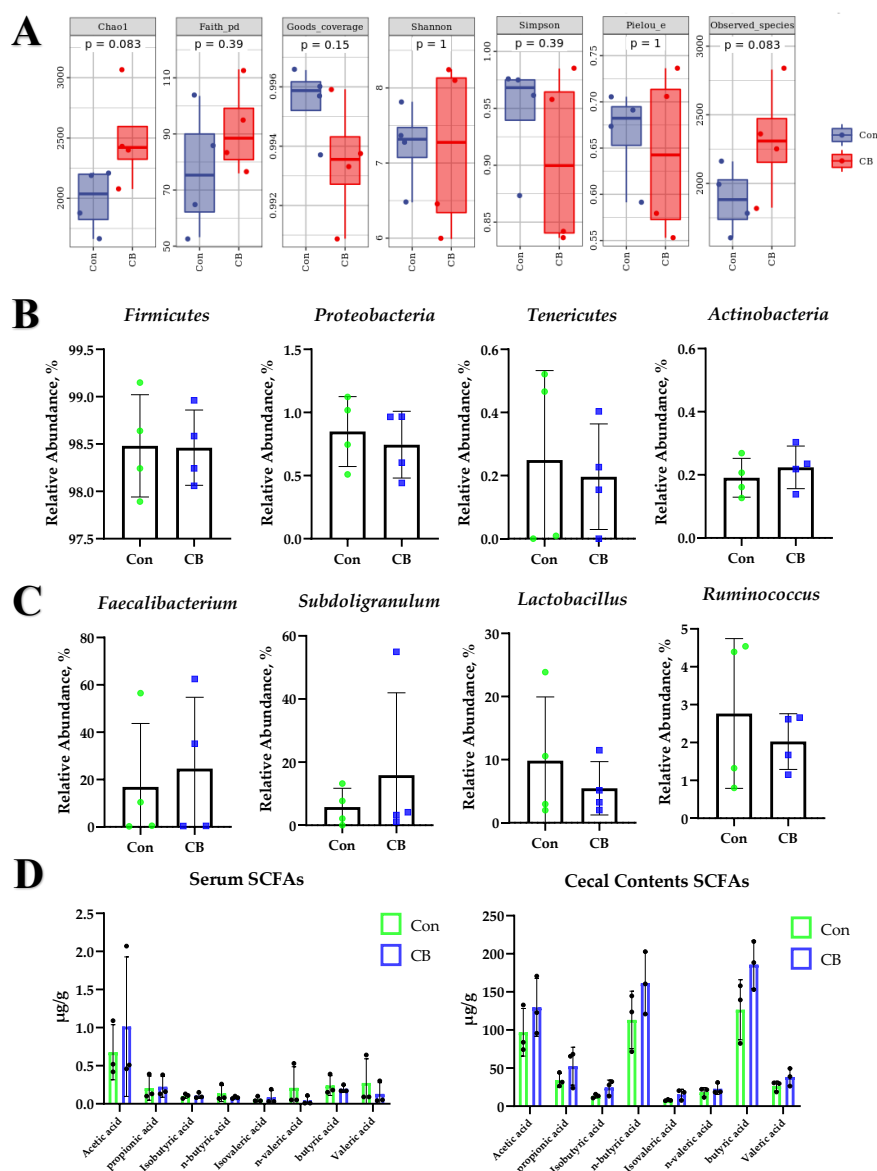
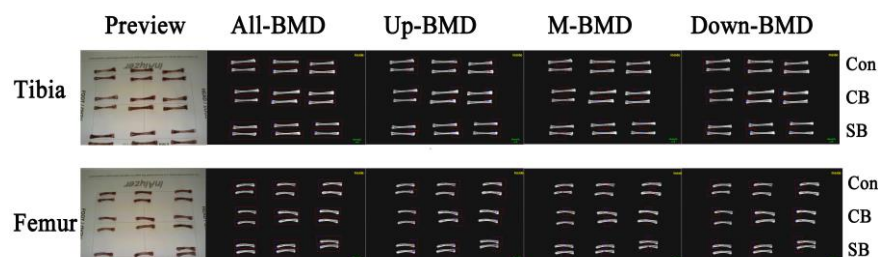


**Figure S1.** Expression of bone metabolism-related cytokines in cecum and femur. Relative mRNA expression levels of *RANKL*, *OPG*, *RUNX2* and *BMP2* in cecum and femur. Con: normal saline; CB: *Clostridium butyricum*. The data are presented as the mean  $\pm$ SD. \*,  $p < 0.05$ .

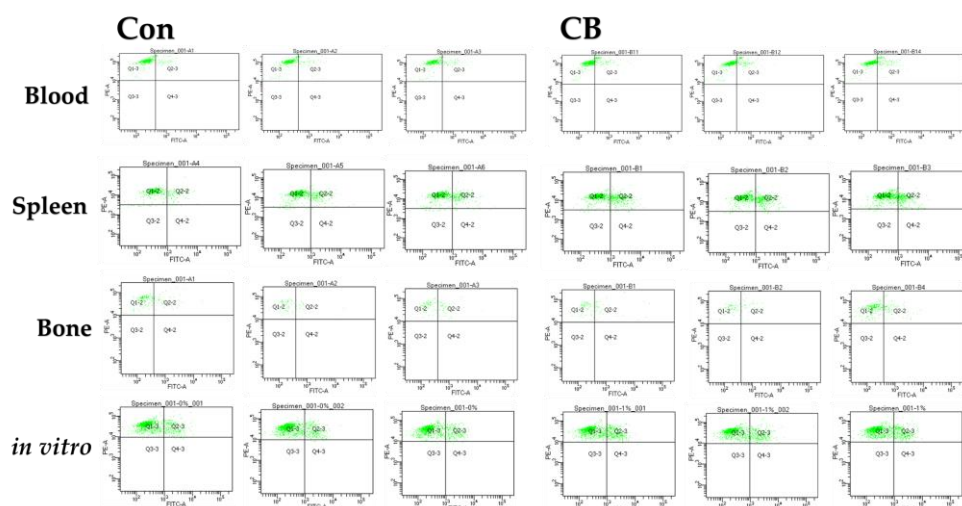


**Figure S2.** The effect of CB on the gut microbiota. (A) Alpha diversity (Ace, Chao1, Shannon and Simpson). (B) Relative abundance of the top four phylum-level abundances of microbial species in

the cecum. (C) Relative abundance of the top four species-level abundances of microbial species in the cecum. (D) The concentrations of short-chain fatty acids (SCFA) in the serum and cecal contents of the layer pullets. Con: normal saline; CB: *Clostridium butyricum*. The data are presented as the mean  $\pm$ SD.



**Figure S3.** Detection strategy for detecting bone density at different locations. The bone density test defines the upper 1/4 of the bone as the Up-BMD, the lower 1/4 as the Down-BMD, and the middle 1/2 as the M-BMD. Con: normal saline; CB: *Clostridium butyricum*.



**Figure S4.** The ratio of CD3<sup>+</sup>CD4<sup>+</sup>CD25<sup>+</sup>T cells in peripheral blood, spleen, bone marrow and lymphocytes cultured *in vitro*. Con: normal saline; CB: *Clostridium butyricum*.