

***Lactobacillus* (LA-1) and Butyrate inhibit Osteoarthritis by controlling Autophagy and Inflammatory Cell Death of Chondrocytes**

Keun-Hyung Cho^{1,2}, Hyun Sik Na^{1,2}, JooYeon Jhun¹, Jin Seok Woo¹, A Ram Lee^{1,2}, Seung Yoon Lee^{1,2}, Jeong Su Lee^{1,2}, In Gyu Um^{1,2}, Seok Jung Kim³, Sung-Hwan Park⁴, Mi-La Cho^{1,5}

¹Rheumatism Research Center, Catholic Research Institute of Medical Science, Catholic University of Korea, Seoul 06591, Korea

²Department of Biomedicine & Health Sciences, College of Medicine, Catholic University of Korea, Seoul 06591, Korea

³Department of Orthopedic Surgery, College of Medicine, Catholic University of Korea, Seoul 06591, Korea

⁴Division of Rheumatology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, Catholic University of Korea, Seoul 06591, Korea

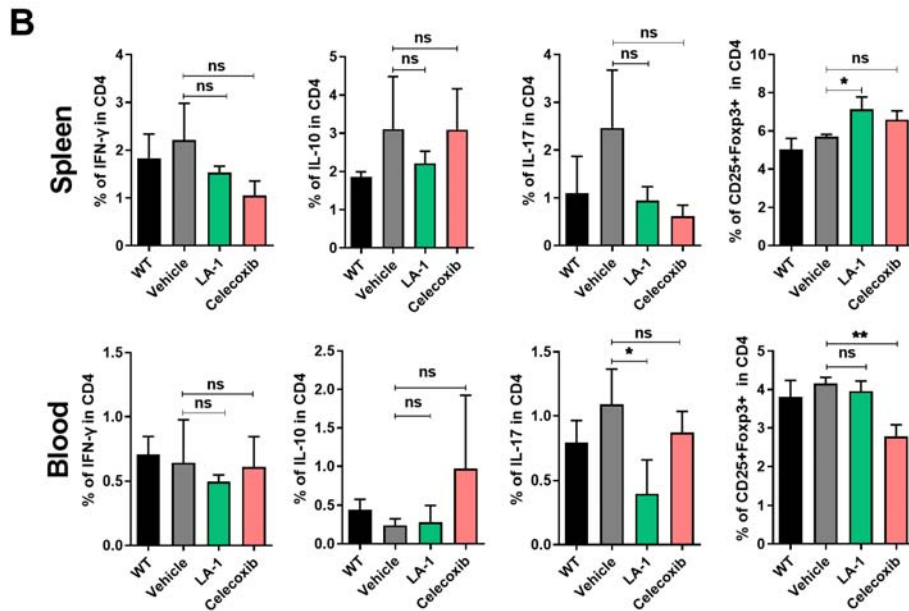
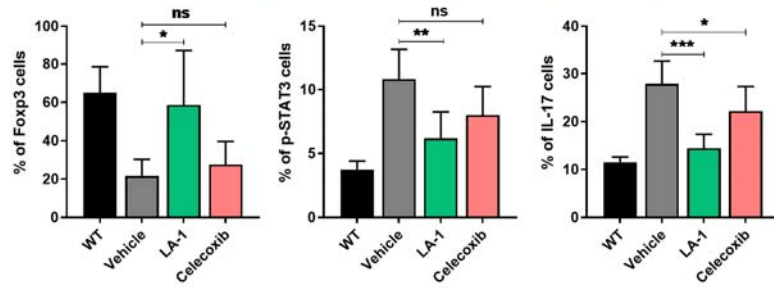
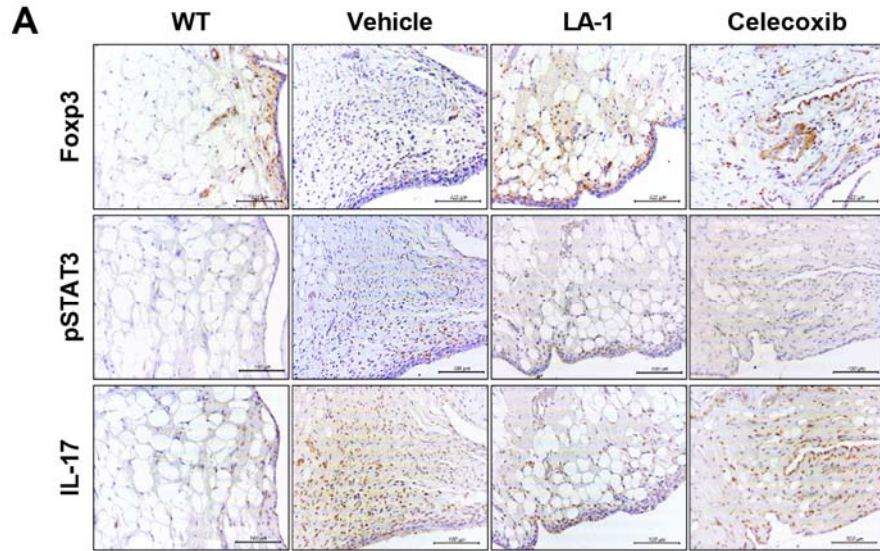
⁵Department of Medical Lifescience, College of Medicine, College of Medicine, Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 06591, Republic of Korea

To whom correspondence should be addressed to:

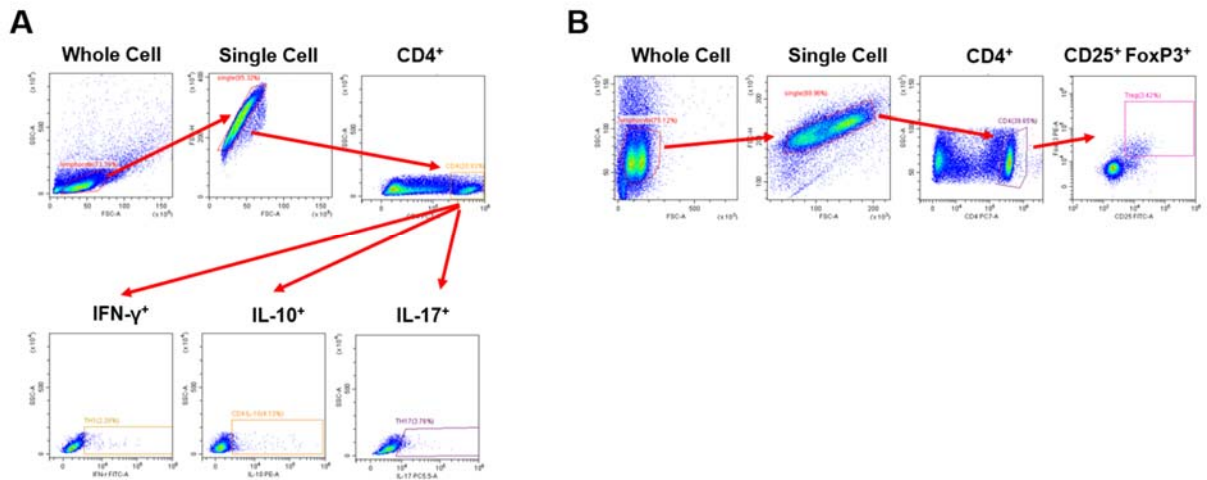
Mi-La Cho, Ph.D., Rheumatism Research Center, Catholic Research Institute of Medical Science, College of Medicine, The Catholic University of Korea, Seoul 06591, Korea (Tel: 82-2-2258-7473, Fax: 82-2-2258-7473, E-mail: iammila@catholic.ac.kr)

Table 1. List of primers for real-time PCR in this study

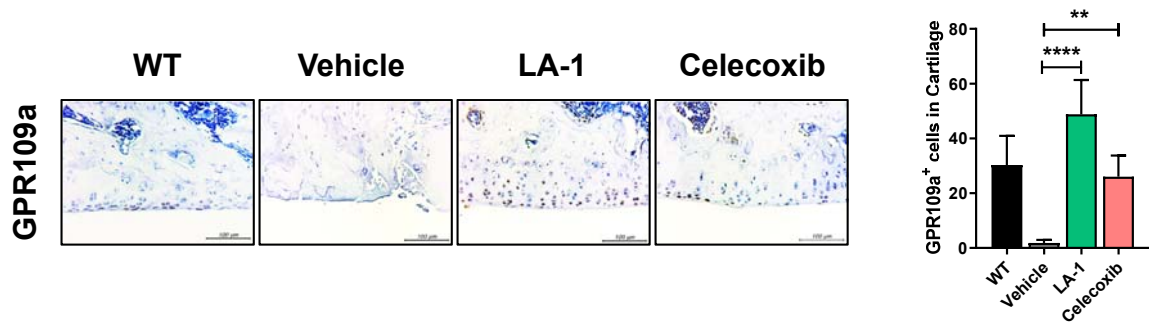
Gene Name	Forward Primer	Reverse Primer
MMP1	CTGAAGGTGATGAAGCAGCC	AGTCCAAGAGAATGGCCGAG
MMP3	CTCACAGACCTGACTCGGTT	CACGCCTGAAGGAAGAGATG
MMP13	CTATGGTCCAGGAGATGAAG	AGAGTCTTGCCTGTATCCTC
TIMP1	AATTCCGACCTCGTCATCAG	TGCAGTTTTCCAGCAATGAG
TIMP3	CTGACAGGTCGCGTCTATGA	GGCGTAGTGTTTGGACTGGT
NFκB	AACAGAGAGGATTTTCGTTTCCG	GGACTTCGAGCAAGAGATGG
iNOS	CCTGAGCTCTTCGAAATCCCA	CCCGAAACCACTCGTATTTGG
RIPK1	GACGAAGCCAACTACCATCTT	TCTCCTTTCCTCCTCTCTGTT
MLKL	GCAAGCTGGTGGCTGTGAAGC	TTCATCCACAGAGGGCCGCA
β-actin	GGACTTCGAGCAAGAGATGG	TGTGTTGGCGTACAGGTCTTTG



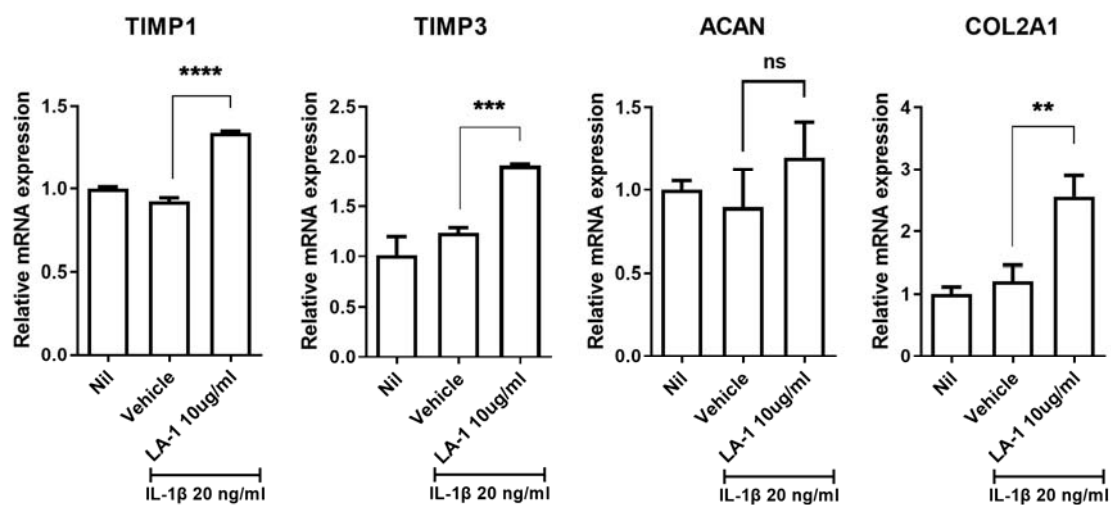
Supplementary Figure 1. Lactobacillus regulates the expression of inflammatory and regulatory T cells. (A) FOXP3, p-STAT3, and IL-17 expression determined immunohistochemically in the synovium of indicated group. (B) Bar graphs show average % of IFN- γ , IL-10, and IL-17 positive CD4⁺ T cells of spleen (top) and blood (bottom) of indicated group. (C) Bar graphs show average % of CD25^{high}FOXP3^{high}CD4⁺ T cells (Treg) of spleen (top) and blood (bottom) of indicated group. Data are means \pm SD (* p < 0.05, ** p < 0.01, **** p < 0.001).



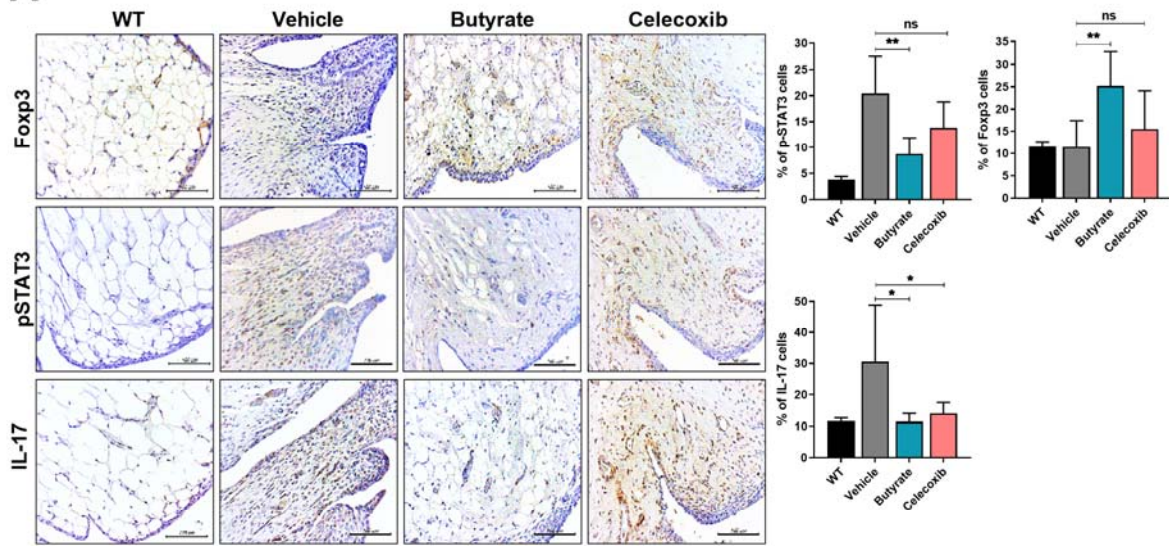
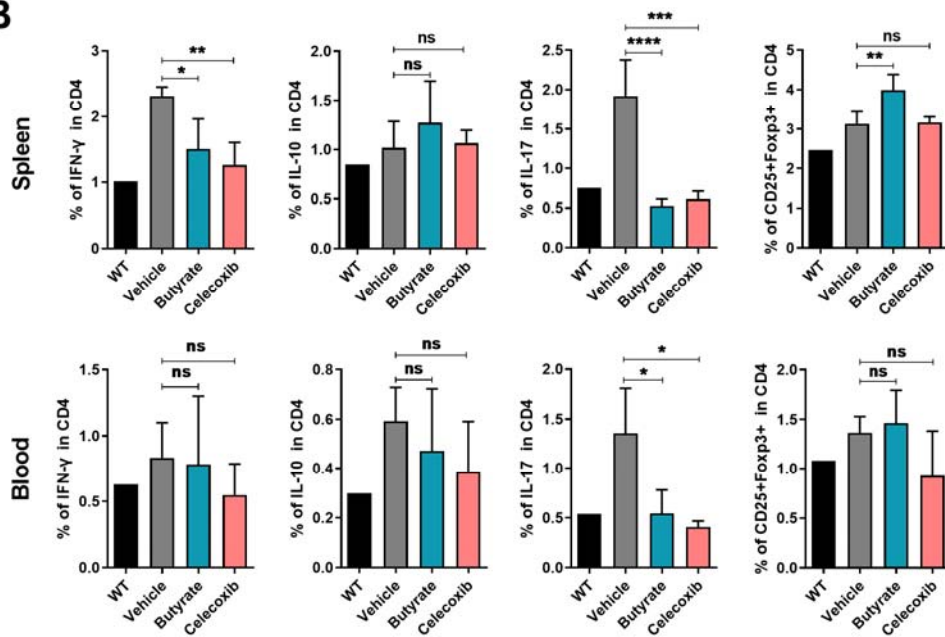
Supplementary Figure 2. An example of gating strategy for flow cytometry. (A) Gating strategy for IFN- γ ⁺, IL-10⁺, and IL-17⁺ CD4⁺ T cells. (B) Gating strategy for CD4⁺CD25⁺FoxP3⁺ cells (Tregs).



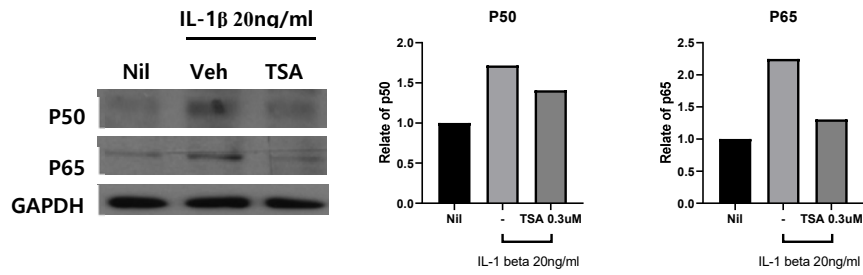
Supplementary Figure 3. Regulation of GPR109a expression by LA-1. Representative images show the expression of GPR109a and bar graph shows GPR109a positive cell number in the indicated group.



Supplementary Figure 4. Lactobacillus regulate the expression of anabolic factors & ECM - related gene. Bar graphs show the mRNA expression level of TIMP1, TIMP3, ACAN, and COL2A1 in the human chondrocyte of indicated group. Data are means \pm SD (** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$).

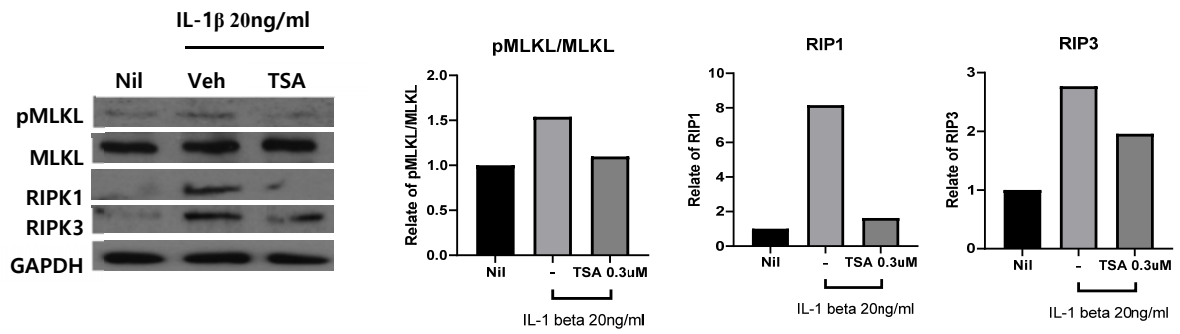
A**B**

Supplementary Figure 5. Butyrate regulates the expression of inflammatory and regulatory T cells. (A) FOXP3, p-STAT3, and IL-17 expression determined immunohistochemically in the synovium of indicated group. (B) Bar graphs show average % of IFN- γ , IL-10, and IL-17 positive CD4⁺ T cells of spleen (top) and blood (bottom) of indicated group. (C) Bar graphs show average % of CD25^{high}FOXP3^{high}CD4⁺ T cells (Treg) of spleen (top) and blood (bottom) of indicated group. Data are means \pm SD (* p < 0.05, ** p < 0.01).



Supplementary Figure 6. Regulatory effect of HDAC inhibitor in the expression of NF-κB.

Representative images show the expression of P50 and P65, and bar graphs show quantification of them in the indicated group.



Supplementary Figure 7. HDAC inhibitor inhibit inflammatory cell death. Representative images show the expression of pMLKL, MLKL, RIPK1, and RIPK3, and bar graphs show quantification of them in the indicated group.