Materials and Methods

Cell Culture

Raw 264.7 macrophage cells were maintained in RPMI supplemented with 10% fetal bovine serum, penicillin-streptomycin (Penicillin, 100 I.U./ml/Streptomycin, 100μg/ml), and L-glutamine (4.5g/L).

Preparation of LAB Strains and treatment of Raw 264.7 cells

LAB stains (*Lactobacillus paracasei* DKL121 and *Lactobacillus plantarum* DKL 119) were isolated from home-made or commercial Korean Kimichi from different regions in Korea. Established strain *Lactobacillus rhamnosus* (LGG) as well as Kimichi derived LAB were cultured in Lactobacillus MRS broth (Difco, MD) at 37°C overnight. The conditional medium was then collected and filtered using 0.22μm filters. The supernatants were stored in -80°C for use. Cultured Raw 264.7 cells were incubated with or without 10% LGG-CM, 119-CM or 121-CM in culture media for 0.5 h, 1.5h or 3 h then washed with PBS and collected for western blot, RNA extraction and immunofluorescence microscopy.

Immunoblotting

Cultured cells were rinsed twice with ice-cold PBS, lysed in protein loading buffer (50 mM Tris, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10% glycerol), and then sonicated. Equal amounts of protein were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and immunoblotted with primary antibodies. The membrane was incubated with the following primary antibodies: anti-VDR (Santa Cruz Biotechnology Inc., CA), anti-LC3B, (Cell Signal, Beverly, MA) and anti-β-actin (Sigma-Aldrich, Milwaukee, WI, USA) antibodies and

visualized by ECL. Membranes that were probed with more than one antibody were stripped before re-probing.

Real Time quantitative PCR

Total cellular RNA was obtained by TRIzol reagent (Thermo Fisher, USA) according to the manufacturer's protocol. VDR and autophagic markers ATG16L1 and Beclin1 were measured by real-time PCR. The sequences of the primers were shown in **Table 1**. All the gene expression levels were normalized to β-actin through 2-ΔΔCt method.

Immunofluorescence

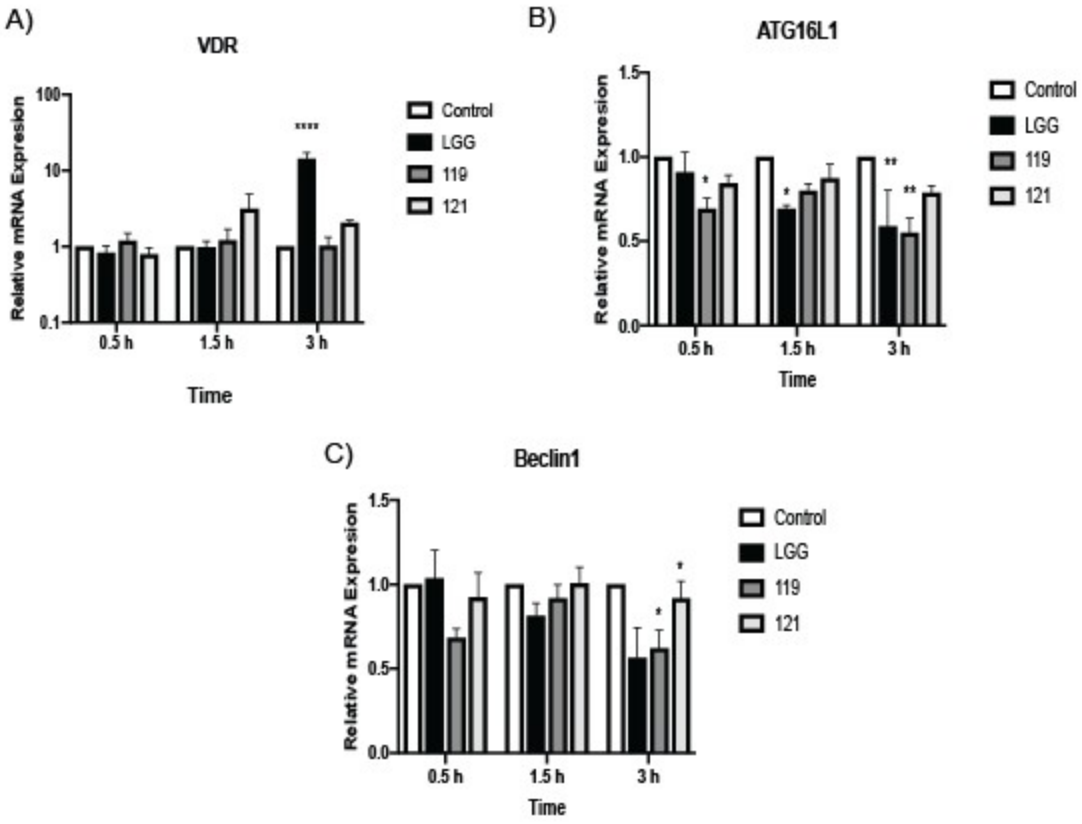
Cells were fixed with 100% Ethanol for 30 min and permeabilized with 0.1% Triton X-100 for 10 min. Normal goat serum was performed to block the non-specific antigen. The primary antibody against LC3B was added and the cells were incubated overnight at 4℃. After incubating with Alexa Fluor 549 anti-Rabbit secondary antibody for 1h, coverslips were mounted by fluoromount-G (SouthernBiotech, USA). Images were photographed using Zeiss laser scanning microscope (LSM 710 (Carl Zeiss Inc., Oberkochen, Germany).

Figure Legends

Figure 1. mRNA Expression of VDR and autophagy markers in Raw 264.7 cells treated with probiotic-cm. Time course of mRNA levels of VDR (a), ATG16L1 (b) and Beclin1 (c) in Raw 264.7 cells treated with LGG-CM, 119-CM or 121-CM for 0.5h, 1.5h and 3h. LGG-CM and 121-CM induced VDR expression, while neither of the LAB strains induced expression of ATG16L1 or Beclin1. Data is expressed as mean +/- SEM. n=3, two-way ANOVA test, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001 compared to non-treated control group.

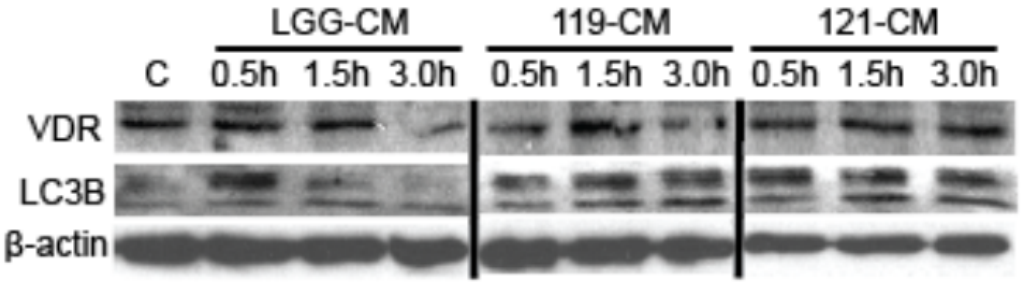
Figure 2. Protein Expression of VDR and autophagy markers in Raw 264.7 cells treated with probiotic-cm. (a) Protein levels of VDR and LC3B in LGG-CM, 119-CM and 121-CM treated Raw 264.7 cells for 0.5h, 1.5h and 3h. (b) Graphical representation of the quantification of VDR and LC3BI/II protein levels. Data are expressed as mean +/- SEM. n=2, two-way ANOVA test, *P<0.05, **P<0.01 and ***P<0.001 compared to non-treated control group. (c) LC3B immunofluorescence of HCT 116 and Raw 264.7 cells after 30-minute treatment with LGG conditioned media. LGG–CM induced LC3B expression (red) in both cell types, while expression was higher in the HCT 116 cells line.

Supplemental Figure 1



Supplemental Figure 2

A



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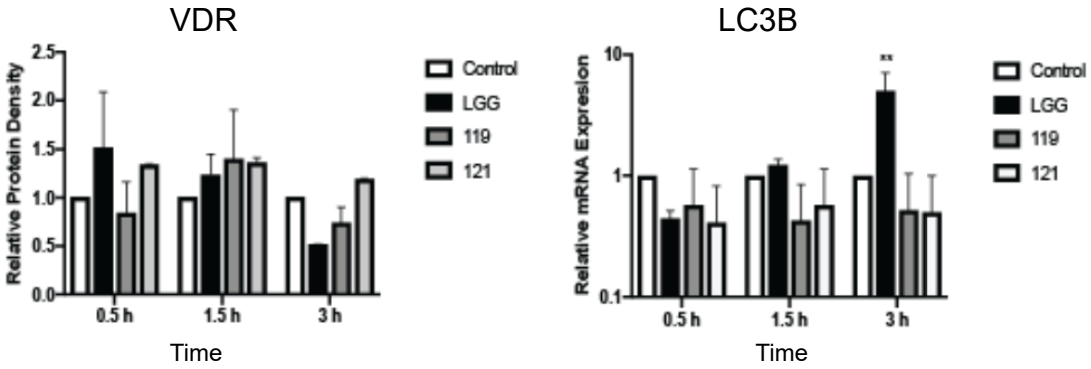

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