Supplementary information

Soluble RANKL expression in *Lactococcus lactis* and investigation for its potential as an oral vaccine adjuvant

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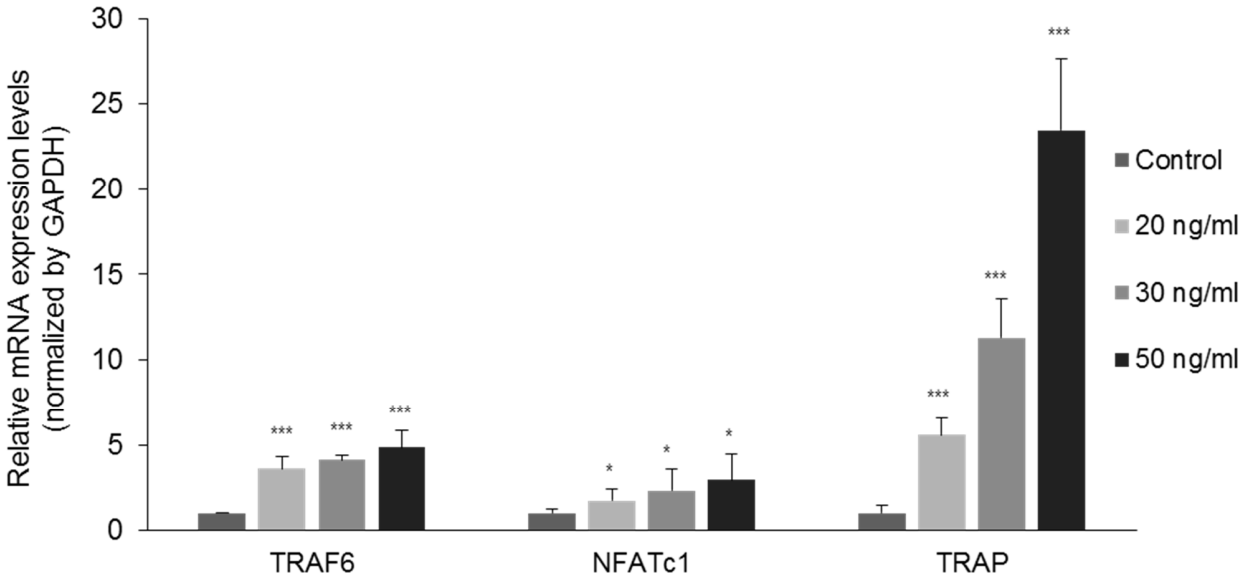

Figure S1. qRT-PCR analysis of RANK-RANKL signaling-related gene expression to optimize the concentration of commercial sRANKL. The mRNA expressions of TRAF6, NFATc1, and TRAP were analyzed at day 6 after exposure of commercial sRANKL (20-50 ng/ml) to RAW 264.7 cells. The mRNA levels were normalized by GAPDH expression, and expressed as relative gene expression compared to control. For significance tests, a one-way analysis of variance (ANOVA) followed by Tukey′s post-hoc test were used, and expressed as follows; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

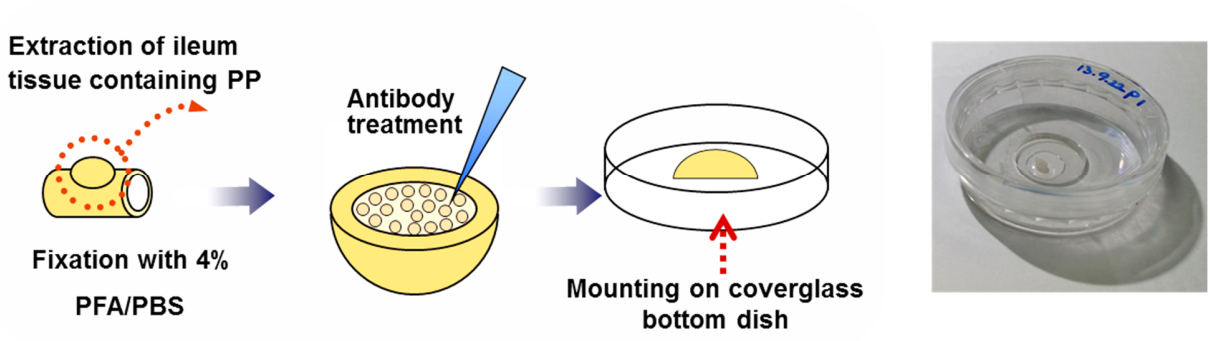

Figure S2. Schematic illustration of IHC. Peyer's patches were isolated from small intestine and fixed with 4% (v/v) paraformaldehyde for 2 h at 4°C. The tissues were blocked with 3% goat serum at RT for 1 h and incubated with Alexa488-labeled GP-2 monoclonal antibody (1:400 dilution) at 4°C overnight. The tissues were mounted on coverglass bottom dishes.