

## Supplementary Information:

### **Membrane vesicles of *Lacticaseibacillus rhamnosus* JB-1 contain immunomodulatory lipoteichoic acid and are endocytosed by intestinal epithelial cells**

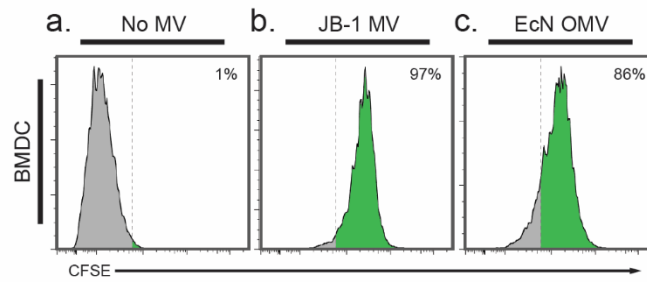
Kevin Champagne-Jorgensen [1,2]\*, M. Firoz Mian [2], Karen-Anne McVey Neufeld [2,3], Andrew M. Stanisiz [2], John Bienenstock [2,3]

[1] Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada

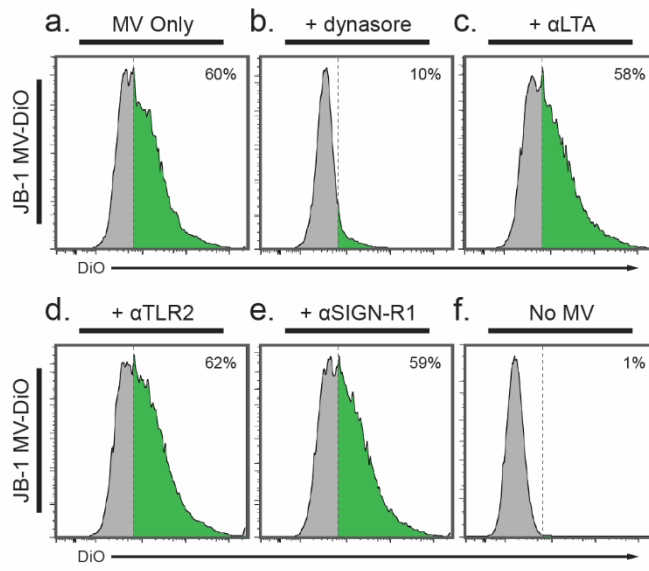
[2] Brain-Body Institute, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada

[3] Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

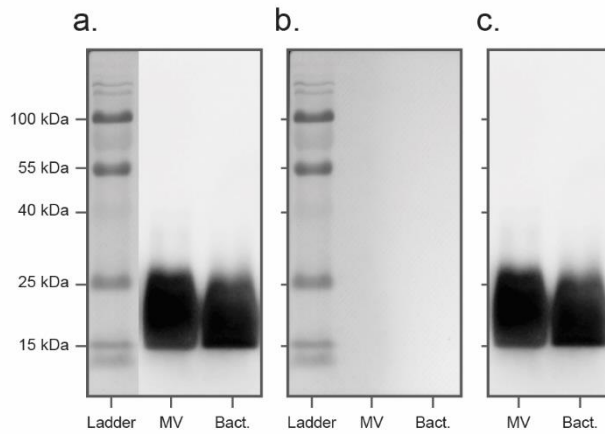
\*Corresponding author: champk1@mcmaster.ca



**Supplementary Figure S1. *L. rhamnosus* JB-1 MV are internalized within 1 hour by bone marrow-derived dendritic cells.** (a) Either PBS vehicle or approx.  $3 \times 10^{10}$  (b) CFSE-labelled JB-1 MV or (c) EcN OMV were incubated at 37°C for 1 h with BMDCs and cell-associated fluorescence was measured by flow cytometry.



**Supplementary Figure S2. *L. rhamnosus* JB-1 MV are endocytosed independent of LTA, TLR2, and SIGN-R1.** (a-e) Approx.  $3 \times 10^{10}$  DiO-labelled JB-1 MV were incubated with MODE-K cells for 2 h after preincubation with (a) nothing added, (b) dynasore, (c) anti-LTA antibody, (d) anti-mouse TLR2 antibody, or (e) anti-SIGN-R1 antibody. (f) Cells were incubated only with PBS vehicle.



**Supplementary Figure S3. *L. rhamnosus* JB-1 MV contain lipoteichoic acid.** (a-c) Full-length western blot used to measure LTA associated with JB-1 MV (MV) or in lysates of whole JB-1 bacteria (Bact.). Images show (a) colorimetric ladder merged with chemiluminescent LTA signal, (b) colorimetric blot alone, or (c) chemiluminescent signal from sample-containing wells.