Supporting Information

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SI Text

Materials and Methods

Extraction and Purification of Surface Layer Proteins. One hundred milliliters of overnight *Lactobacillus acidophilus* NCFM and *L. acidophilus* NCK1377-CI were centrifuged (8,000 × *g* for 20 min at 4 °C) followed by washing once with cold distilled water. The pellets were suspended in 5 M LiCl at 4 °C (1 mL of LiCl, 10 mg of biomass) followed by slight stirring for 15 min. Supernatant (≈60 mL) was harvested after centrifugation. Dialysis of the supernatant against distilled water was conducted in a 4 °C cold room with frequent changes of the water during the first 4 h of dialysis for the total duration of dialysis of 24 h. During dialysis, a white precipitate appears that is characteristic of reassociated unsoluble paracrystalline S layers. The precipitate was harvested by centrifugation (40,000 × *g* for 20 min at 4 °C). The pellet of the corresponding S layers was then resuspended and stirred in 1 M LiCl for 15 min at 4 °C (10 mL of LiCl per pellet) followed by centrifugation to 40,000 × *g* for 20 min at 4 °C. Each pellet (pure S layer) was washed by 15 mL of cold sterile distilled water and stored at −20°C before further use.

Dendritic Cell (DC)-Driven Th1/Th2 Differentiation. Immature DCs (iDCs) were cultured from monocytes of healthy donors in Iscove's Modified Dulbecco's Medium (GIBCO), supplemented with 10% FCS (BioWithaker), 500 units/mL IL-4, and 800 units/mL GM-CSF (Biosource). At day 6, DC maturation was induced with varying concentrations of *L. acidophilus* NCFM cells, NCK1377-CI cells, or purified S layer proteins from NCFM (SlpA-dominant) at a concentration 1 μg/mL and both with and without LPS (10 ng/mL). The following positive controls were included in the assay: (*i*) 10 ng/mL *Escherichia coli* LPS (mixed Th1/Th2 response) and (ii) 10 μg/mL PGE2 and 10 ng/mL LPS (Th2). After 2 days, DCs were washed and incubated with autologous $CD45RA^+/CD4^+$ T cells (5×10^3 DC/20×10^3 T cells). In parallel, DCs were analyzed for expression of CD86 maturation marker (BD PharMingen) by flow cytometry. At day 5, recombinant IL-2 (10 units/mL) was added, and the cultures were expanded for the next 7 days. To determine cytokine production by Th cells, at day 12–15 quiescent T cells were restimulated with 10 ng/mL phorbol 12-myristate 13-acetate and 1 μg/mL ionomycin (both Sigma–Aldrich) for 6 h. After 1 h, 10 μg/mL Brefeldin A (Sigma–Aldrich) was added to the T cells. Single-cell production of IL-4 and IFNγ was determined by intracellular flow cytometric analysis. Cells were fixed in 2% paraformaldehyde, permeabilized with 0.5% saponin (Sigma–Aldrich) and stained with anti-human IFNγ–FITC and anti-human IL-4–phycoerythrin (BD PharMingen).

DC Binding by Bacteria. *L. acidophilus* NCFM and NCK1377-CI cells ($\approx 1 \times 10^9$ cfu/mL) were labeled by incubation with FITC (0.5 mg/mL) in PBS for 1 h at room temperature. The FITC-labeled bacteria were washed 3 times to remove unbound FITC. Immature DCs, CHO, or CHO–DC-SIGN cells (5×10^4) were preincubated with neutralizing antibody to DC-SIGN (AZN-D1), or EDTA (10 mM) in TSM buffer [20 mM Tris, 150 mM NaCl, 1 mM $CaCl_2$, and 2 mM $MgCl_2$ (pH 8.0)] plus 0.5% BSA for 10 min at room temperature. Thereafter, the FITC-labeled bacteria (bacteria:cell ratios of 1,000:1 to 1:10) were added and incubated for 45 min at 37 °C. After washing, the cells were analyzed by means of flow cytometry and gated for large cells in the forward scatter/side scatter, excluding unbound bacteria, and using FACScan analysis software (Becton and Dickinson).

Fluorescent Microscope. *L. acidophilus* NCFM (SlpA-dominant) and NCK1377-CI, the SlpA-knockout mutant (SlpB- dominant), were labeled with FITC as described above. DCs were incubated with the 2 bacteria for 45 min at 37 °C, spotted onto poly(L)-lysine-coated glass slides, and stained in red color by incubation with 4-di-10-ASP (DiA; Molecular Probes). The image analysis was performed on a Nikon Eclipse E800 fluorescent microscope.

Statistics. Data are expressed as means ± SD. Data were analyzed for statistical significance by using ANOVA followed by an unpaired Student's *t* test to compare either different groups of multiple datasets or just 2 sets of data or variables between 2 groups, respectively. A P value of <0.05 was considered to be significant.

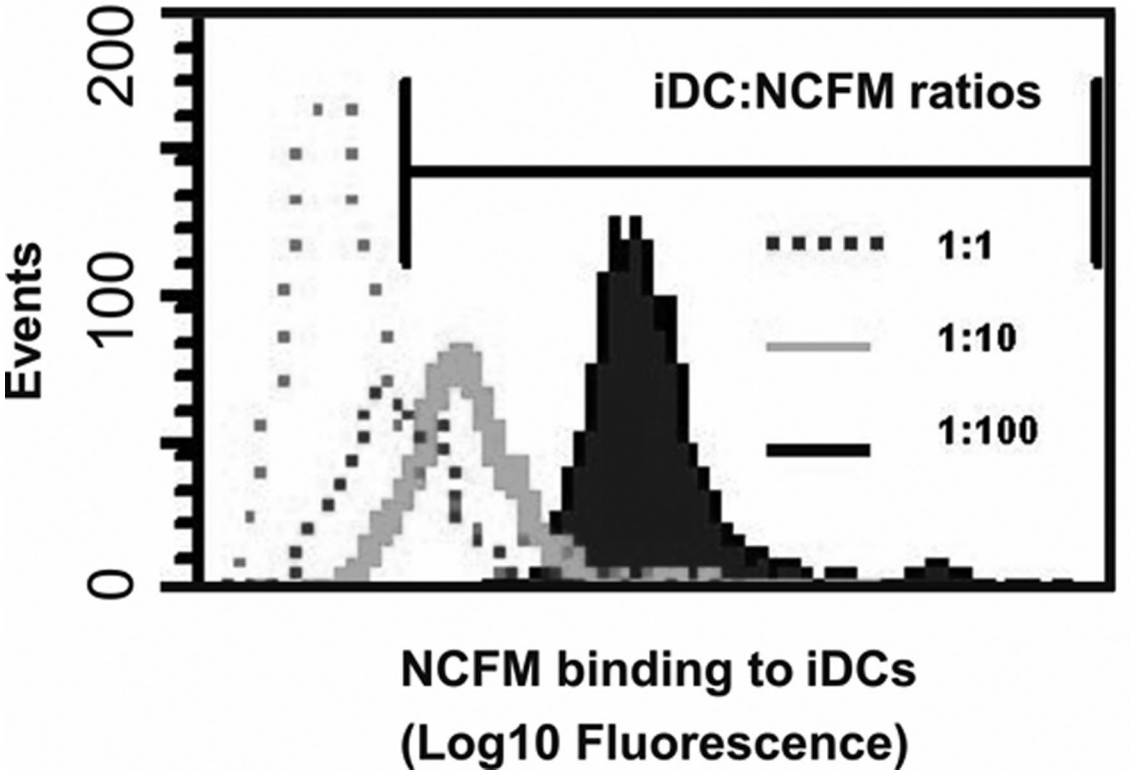

Fig. S1. *L. acidophilus* NCFM binding to iDCs is concentrations-dependent. The ligation of *L. acidophilus* NCFM to iDCs at different ratios was analyzed by FACScan (*SI Materials and Methods*).

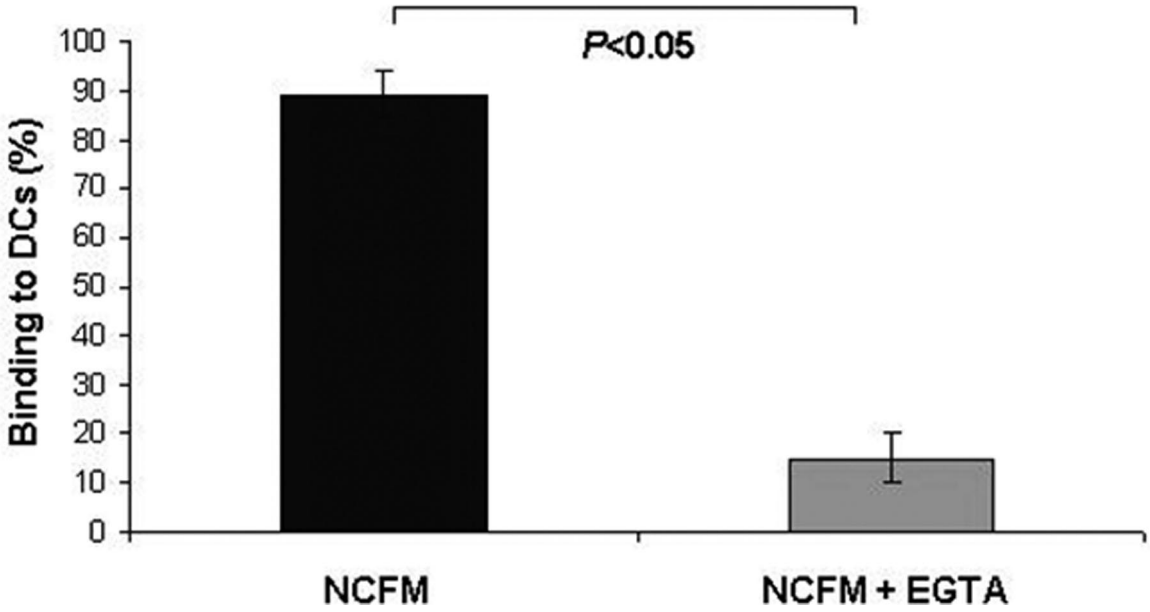

Fig. S2. Involvement of C-type lectin receptors (CLRs) in the binding of *L. acidophilus* NCFM binding to iDCs. iDCs (5×10^4 cells) were treated with FITC-labeled *L. acidophilus* NCFM. The impact of CLRs was determined when iDCs were incubated with *L. acidophilus* NCFM or *L. acidophilus* NCFM in the presence of EGTA and calculating the percentage of the DCs binding to the bacteria by FACs. Experiments were repeated at least 3 times.

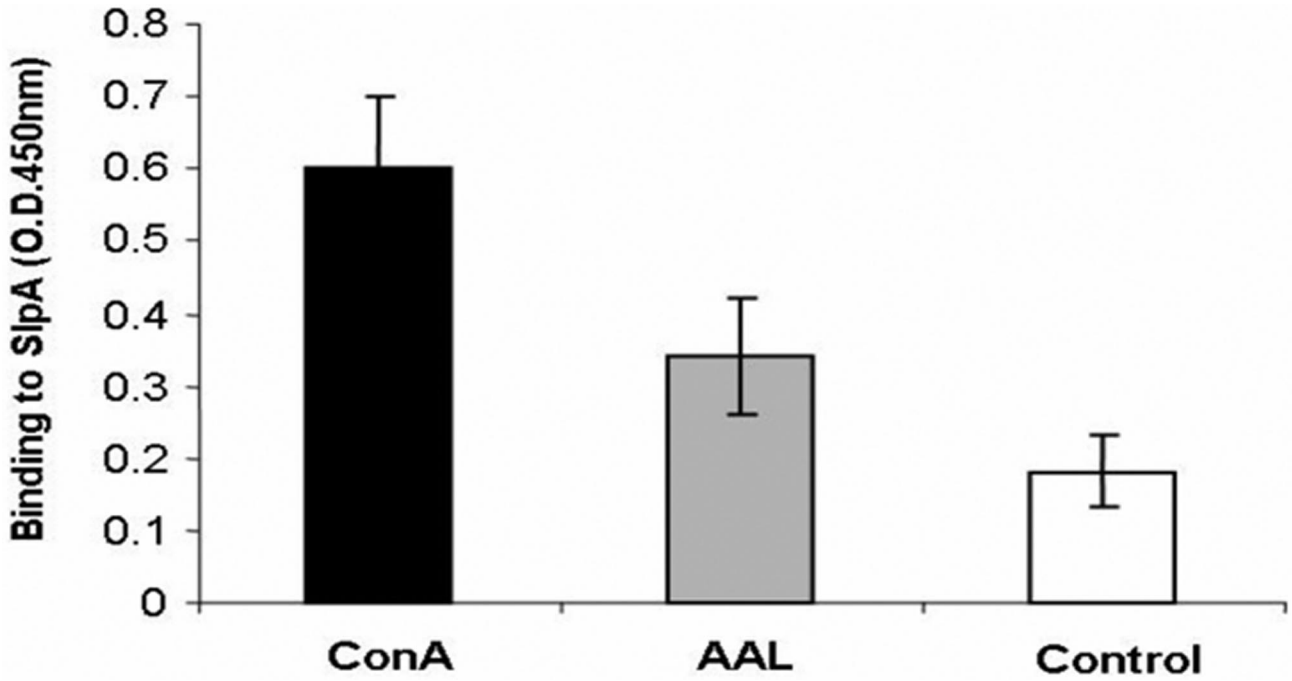

Fig. S3. Putative glycosylation of the purified SlpA demonstrated by plant lectins. Plant lectins, ConA, and aleuria aurantia lectin (AAL), differentially recognize SlpA of *L. acidophilus* NCFM, as assayed by ELISA, mean ± SD.

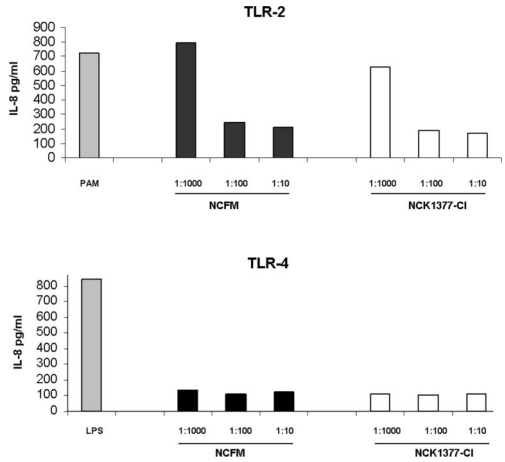

Fig. S4. *L. acidophilus* NCFM (SlpA$^+$) and NCK1377-CI (SlpB$^+$) activate Toll-like receptor (TLR)2 at similar levels. HEK-293 cell line transfected with TLR2 or TLR4 was incubated with TLR2 agonist PAM3CSK (PAM), TLR4 agonist LPS, or with NCFM and NCK1377-CI at different ratios (1:1,000, 100:1, 1:10; HEK-TLR2 or 4:bacteria). Representative experiments for TLR2 or TLR4 activation are shown separately.

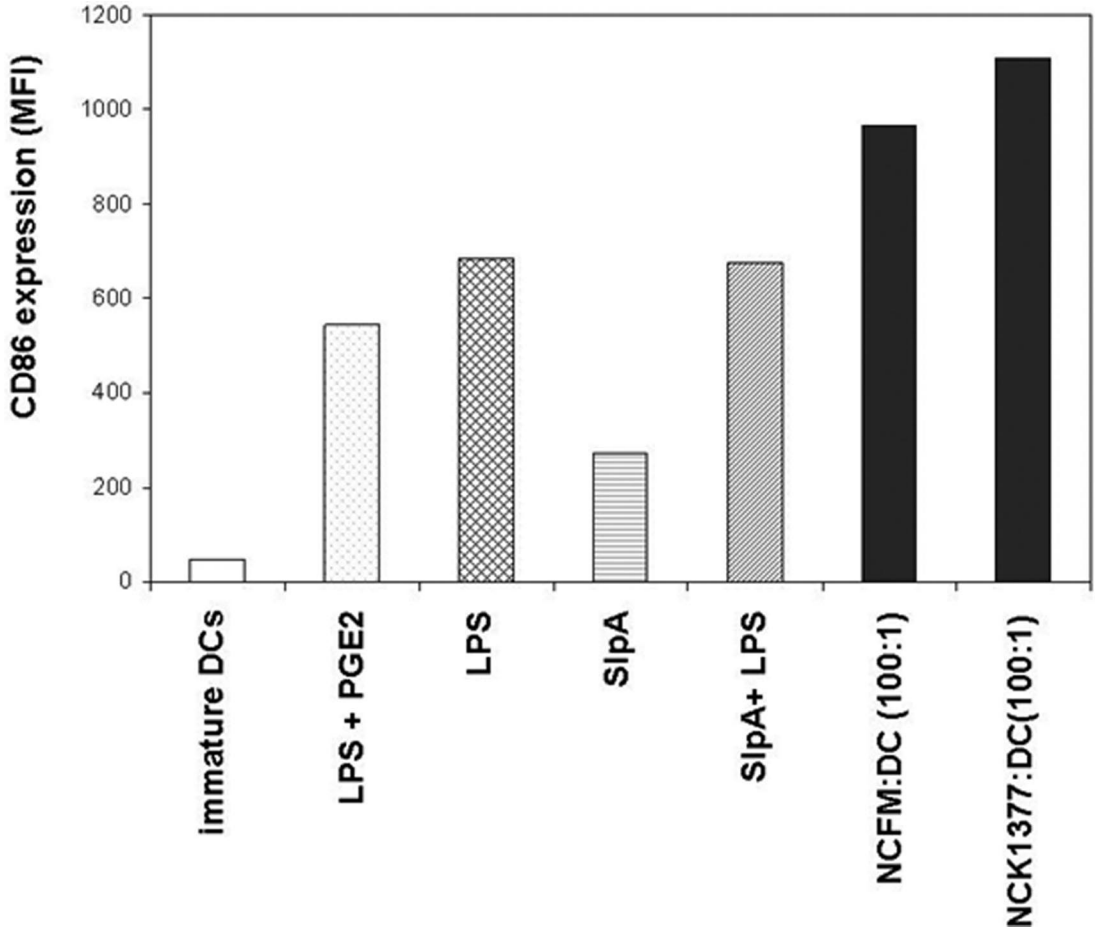

Fig. S5. *L. acidophilus* NCFM (SlpA$^+$) and NCK1377-CI (SlpB$^+$) stimulate maturation of DCs through expression of CD86. FACScan analysis of maturation markers CD86 after *L. acidophilus* NCFM, NCK1377-CI, purified SlpA + LPS or purified SlpA (1 μg/mL) treatment of iDCs is shown. A representative analysis is presented of the iDC maturation after incubation with *L. acidophilus* NCFM cells (100 cfu per iDC), LPS, LPS + PGE_2 or left untreated (immature DCs) for 2 days. Afterward, iDCs were harvested, washed extensively with PBA, stained with specific Abs for 1 h at 4 °C, and analyzed by FACS. Experiments were repeated 3 times, and the values are average ± SD. MFI, mean fluorescence intensity.

Table S1. *L. acidophilus* NCFM strains used in this work

L. acidophilus strains	Origin	Ref.
NCFM	Human intestinal isolate	1
NCK1377-CI	Knockout in the S layer protein A (*slpA*) gene	2
NCK1398	Knockout of *lacL* gene (β-galactosidase)	3
NCK1660	Knockout of Mub, putative mucin-binding protein	2
NCK1661	Knockout of FbpA, putative fibronectin-binding protein	2
NCK166	Knockout of R28, putative adhesion protein	2

1. Sanders ME, Klaenhammer TR (2001) Invited review: The scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. *J Dairy Sci* 84:319–331.
2. Buck BL, Altermann E, Svingerud T, Klaenhammer TR (2005) Functional analysis of putative adhesion factors in *Lactobacillus acidophilus* NCFM. *Appl Environ Microbiol* 71:8344–8351.
3. Russell WM, Klaenhammer TR (2001) Efficient system for directed integration into the *Lactobacillus acidophilus* and *Lactobacillus gasseri* chromosomes via homologous recombination. *Appl Environ Microbiol* 67:4361–4364.