Supporting Information

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SI Materials and Methods

Experimental Animals and Disease Models. BALB/c mice (6–8 weeks old) and Lewis rats (6–8 weeks old) were purchased from the SLC Co. Do11.10 mice (6–8 weeks old) were purchased from the Jackson Laboratory. Mice were maintained in pathogen-free conditions in the animal facility of Gwangju Institute of Science and Technology (GIST). All animal experiments were approved by the GIST Animal Care and Use Committee.

Determination of Optimal Dosage and Treatment Duration of Probiotics. To determine the optimal dose of probiotics, we tested the protective effect of each probiotic or probiotic combinations in the TNBS-induced colitis model. Probiotics were orally administered with diverse doses (1×10^7, 5×10^7, 1×10^8, 5×10^8, and 1×10^9 cfu/day) dissolved in 100 μL of PBS for 20 days, and colitis was then induced by intrarectal injection of TNBS. A minimum dosage of 1×10^8 cfu/day of probiotics was required to exert a protective effect, and a dosage of 5×10^8 cfu/day of probiotics showed the most potent effects in TNBS-induced colitis. To find the minimum duration of probiotics treatment, 5×10^8 cfu/day of probiotics was differentially administrated for 5, 10, 20, or 30 days and colitis was induced in each group after the final probiotics treatment. Results indicated that more than 10 days of probiotics treatment was required to see protective effects in TNBS-induced colitis.

Disease Models. To test the prophylaxis effects of probiotics, mice were divided into seven groups and fed either PBS or five individual strains or their mixture in combinations of three, four, or five (IRT5) strains (5×10^8 cfu/day) in PBS for 20 days by oral administration with a catheter and experimental colitis was induced. Briefly, to induce experimental colitis, 50 μg/g (mouse weight) of TNBS in 50% (vol/vol) ethanol was injected to slightly anesthetized mice through a catheter inserted into the rectum, and 100 μL of the TNBS-ethanol mixture was carefully poured into the colon. The subsequent course of colitis was evaluated by assessing mortality, body weight decrease, and macroscopic and microscopic observations. After killing mice, lamina propria (LP) and MLN $CD4^+$ T cells or $B220^+$ B cells were isolated for further experiments.

For the induction of experimental AD in BALB/c mice, the ear surface was stripped three times with surgical tape (Nichiban). Thirty minutes after stripping, 20 μL of 1% dinitrochlorobenzene (DNCB; Sigma) dissolved in acetone/olive oil solution (1:3 acetone/olive oil ratio) was painted on each ear surface. Four days after DNCB painting, 20 μL of 10 mg/mL mite extracts (Yonsei University College of Medicine) was repainted after tape stripping. Alternative painting of DNCB or mite antigen was performed. Tape stripping and DNCB/mite extract painting were continued for 4 weeks. Two weeks after AD induction, to confirm the induction of AD, eye breeding was performed to check the serum IgE level. After confirming AD induction, IRT5 or PBS was given daily by oral feeding with a catheter in each group (Pro and Cont) until the end of induction. Ear thickness and clinical scores were measured 24 h after DNCB or mite application. Only tape stripping and painting of PBS were performed in a Cont group of mice.

For the induction of experimental RA, chicken collagen type II (CII; Sigma) was dissolved in 0.1 M acetic acid at a concentration of 4 mg/mL and then emulsified in an equal volume of complete Freund's adjuvant (Difco) containing 4 mg/mL *Mycobacterium tuberculosis* (Difco). One hundred fifty milligrams of this emulsion was intradermally injected in Lewis rats (6–8 weeks old). A booster injection was administered 7 days after the first immunization with 300 mg of CII emulsified in an equal volume of incomplete Freund's adjuvant (Difco). After 7 days of disease induction, rats were fed with PBS or IRT5 up to 70 days. RA clinical scores were checked daily. After killing rats, joint regions from each group were isolated for histological analysis and DLN $CD4^+$ T cells were collected for additional experiments.

Macroscopic and Histological Evaluation. In experimental colitis (IBD) studies, gross intestines were removed from mice and evaluated as previously described (1). For histological scoring, gross intestines were fixed with 4% (vol/vol) paraformaldehyde in PBS and embedded in paraffin. Paraffin sections were stained with H&E and scored as previously described (2). In experimental AD studies, the clinical condition and symptoms of each mouse were evaluated as previously described, with a slight modification (3). The number of total infiltrated cells stained by H&E in the dermis was counted (3). For the clinical evaluation of experimental RA, clinical scores were counted as described previously (4). Each paw was graded, which could result in one rat having a maximum score of 16. Paw swelling was examined by measuring changes in ankle thickness with calipers. To check histological change, joint tissues were prepared at 10–30 mm and stained with H&E. All clinical and histological evaluations were performed in a blinded manner.

RNA Isolation, cDNA Synthesis, and Quantitative and Standard Real-Time PCR. Total RNA was isolated from the SPs and MLNs of each group using TRIzol reagent (Molecular Research Center). Reverse transcription was performed with reverse transcriptase (Promega) primed with oligo(dT) primer. The synthesized cDNAs were amplified by quantitative real-time PCR (SYBR green; Takara) with CHROMO4 Detector (Biorad) and by standard PCR (SP-Taq, COSMO Genetech; i-Tag, Intron Biotechnology) with an Alpha Unit Block assembly engine system (Biorad) using the following primers: murine L-32 (forward) 5′-GCC CAA GAT CGT CAA AAA GA-3′ and (reverse) 5′-ATT GTG GAC CAG GAA CTT GC-3′, IL-2 (forward) 5′-CCT GAG CAG GAT GGA GAA TTA CA-3′ and (reverse) 5′-TCC AGA ACA TGC CGC AGA G-3′, IL-4 (forward) 5′-ACA GGA GAA GGG ACG CCA T-3′ and (reverse) 5′-GAA GCC CTA CAG ACG AGC TCA-3′, IL-5 (forward) 5′-AGC ACA GTG GTG AAA GAG AC-3′ and (reverse) 5′-TCC AAT GCA TAG CTG GTG ATT T3′, IL-10 (forward) 5′-ATA ACT GCA CCC ACT TCC CA-3′ and (reverse) 5′-TCA TTT CCG ATA AGG CTT GG-3′, IL-12 p40 (forward) 5′-GGA AGC ACG GCA GCA GAA TA-3′ and (reverse) 5′-AAC TTG AGG GAG AAG TAG GAA TGG-3′, IL-13 (forward) 5′-GCA ACA TCA CAC AGG ACC AGA-3′ and (reverse) 5′-GTC AGG GAA TCC AGG GCT AC-3′, IL-17A (forward) 5′-TTC ATC TGT GTC TCT GAT GCT-3′ and (reverse) 5′-TTG ACC TTC ACA TTC TGG AG-3′, IFN-γ (forward) 5′-TCA AGT GGC ATA GAT GTG GAA GAA-3′ and (reverse) 5′-TGG CTC TGC AGG ATT TTC ATG-3′, TNF-α (forward) 5′-CAT CTT CTC AAA ATT CGA GTG ACA A-3′ and (reverse) 5′-TGG GAG TAG ACA AGG TAC AAC CC-3′, TGF-β (forward) 5′-GAA GGC AGA GTT CAG GGT CTT-3′ and (reverse) 5′-GGT TCC TGT CTT TGT GGT GAA-3′, Foxp3 (forward) 5′-CCC ATC CCC AGG AGT CTT G-3′ and (reverse) 5′-CCA TGA CTA GGG GCA CTG TA-3′, hypoxanthine-guanine phosphoribosyltransferase (HPRT) (forward) 5′-TTA TGG ACA GGA CTG AAA

GAC-3′ and (reverse) 5′-GCT TTA ATG TAA TCC AGC AGG T-3′, B7.1 (forward) 5′-ACC CCC AAC ATA ACT GAG TCT-3′ and (reverse) 5′-TTC CAA CCA AGA GAA GCG AGG-3′, B7.2 (forward) 5′-TGT TTC CGT GGA GAC GCA AG-3′ and (reverse) 5′-CAG CTC ACT CAG GCT TAT GTT TT-3′, inducible costimulator ligand (ICOSL) (forward) 5′-GAC TGA AGT CGG TGC AAT GGT-3′ and (reverse) 5′-TGG GTT TTC GAT TTG CCA ATA GA-3′, programmed death-1 ligand (PD1L) (forward) 5′-ATG CTG CCC TTC AGA TCA CAG-3′ and (reverse) 5′-TGG TTG ATT TTG CGG TAT GGG-3′, IDO (forward) 5′-TGG CGT ATG TGT GGA ACC G-3′ and (reverse) 5′-CTG CAT AAG ACA GAA TAG GAG GC-3′, CTLA-4 (forward) 5′-AGA ACC ATG CCC GGA TTC TG-3′ and (reverse) 5′-CAT CTT GCT CAA AGA AAC AGC AG-3′, and GranzymeB (forward) 5′-CTC CTA AAG CTG AAG AGT AAG G-3′ and (reverse) 5′-TTT AAA GTA GGA CTC ACA CTC CC-3′. All the PCR experiments were performed under the same condition as follows: 95°C for 5 min, 95°C for 30 s, 62°C for 30 s, and 72°C for 30 s up to 40 cycles.

Isolation of $CD4^+$ T Cells, $CD4^+CD25^+$ T Cells, $CD4^+CD25^-$ T Cells, $B220^+$ B Cells, and DCs. A single-cell suspension was prepared from the SPs and MLNs of each group. $CD4^+$ T cells were isolated with $CD4^+$ T-cell isolation beads (Miltenyi Biotech; catalog no.130-049-201) and columns (Miltenyi Biotech; catalog no. 130-042-401) or with mouse CD4 beads (Dynal; catalog no.114.45) and DETACHaBEAD (Dynal; catalog no.124.06). To isolate $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells, $CD4^+$ T cells obtained by the latter method were labeled with biotin-conjugated rat anti-mouse CD25 (BD PharMingen; catalog no. 553069) and incubated with streptavidin microbeads (Miltenyi Biotech; catalog no. 130-048-101) in labeling buffer [PBS (pH 7.2), 2 mM EDTA]. Isolation of $CD4^+CD25^+$ Tregs and a $B220^+$ B-cell isolation kit (Miltenyi Biotech; catalog no. 130-091-041) or beads (Miltenyi Biotech; catalog no. 139-049-501) were used, respectively. For the isolation of antigen-presenting cells, an MHC class II-positive population or $CD11c^+$ DCs were obtained from each tissue by MHC II magnetic beads (Miltenyi Biotech; catalog no. 130-052-401) or CD11c microbeads (Miltenyi Biotech; catalog no. 130-052-001), respectively.

Cell Culture and Cell Stimulation. Isolated $CD4^+$ T cells were cultured in T-cell medium containing DMEM (Invitrogen) supplemented with 10% (vol/vol) FBS (HyClone), 3 mM L-glutamine (Sigma), 10 mM Hepes (Sigma), 100 U/mL penicillin (Sigma), 100 U/mL streptomycin (Sigma), and 0.05 mM 2-β-mercaptoethanol (Sigma). For cytokine analysis, cells were stimulated with PMA (20 nM) and ionomycin (2 μM) for 4 h. For proliferation assays, isolated T cells were stimulated with anti-CD3 and anti-CD28 for 72 h. To check intrinsic effects of probiotics for the generation of $Foxp3^+$ Tregs, CFSE-labeled SP $CD4^+$ T cells were stimulated with IRT5 alone without DCs for 3 days and the $Foxp3^+$ population was then measured by FACS.

In Vitro Lymphocyte Proliferation Assay. $CD4^+$ T cells were stimulated with coated anti-CD3 (1–3 μg/mL; BD PharMingen; catalog no. 553058) alone or together with anti-CD28 (1 μg/mL, BD PharMingen; catalog no. 553294). $B220^+$ B cells were stimulated with 10 μg/mL LPS (Sigma). To compare proliferation capacity of $CD4^+CD25^-$ effector T cells from each group, $CD4^+CD25^-$ T cells were cultured with anti-CD3 (1 μg/mL) alone or together with anti-CD28 (1 μg/mL). Cells were then cultured for 56–72 h in flat-bottomed 96-well plates in T-cell medium containing 10% (vol/vol) heated-inactivated FBS (HyClone). After 56–72 h of culture, 0.5 μCi of [H^3]-thymidine (NEN) was added to each well and the cells were incubated for an additional 16 h. Cells were harvested, and [H^3]-thymidine uptake was measured by liquid scintillation counting. Stimulation indexes were defined by comparison with [H^3]-thymidine incorporation (cpm) by unstimulated cells.

In Vitro T-Cell Suppression Assay. MLN $CD4^+CD25^+$ T cells (Tregs, suppressor cells) were isolated from PBS- or IRT5-fed mice and cocultured with a variable ratio of $CD4^+CD25^-$ T cells (responder cells) isolated from the SP of WT BALB/c mice in the presence of soluble anti-CD3 (1 μg/mL). To test suppression sensitivity of $CD4^+CD25^-$ T cells by $CD4^+CD25^+$ Tregs, WT SP $CD4^+CD25^+$ T cells were cultured with a variable ratio of $CD4^+CD25^-$ T cells from each group in the presence of antigen-presenting cells and soluble anti-CD3 (1 μg/mL). Each sample was triplicated, and culture was maintained up to 72 h. After 56–72 h of culture, 0.5 μCi of [H^3]-thymidine was added to each well and the cells were incubated for an additional 6 h. Cells were harvested, and [H^3]-thymidine uptake was measured by liquid scintillation counting. Stimulation indexes were defined by comparison with [H^3]-thymidine incorporation (cpm) by unstimulated cells.

Flow Cytometric Analysis. To detect apoptotic cells, 1×10^6 $CD4^+$ T cells were resuspended in 1 mL of 1× Annexin V binding buffer (BD Bioscience; catalog no. 556454). After incubating for 15 min with 5 μL of Annexin V-PE and 7-ADD at 25°C in the dark, 400 μL of 1× binding buffer was added to each tube and the cells were immediately analyzed by FACS. To measure the $CD4^+CD25^+$ T cells, $CD4^+$ T cells were stained with anti-CD4-FITC (BD Bioscience) and anti-CD25-PE (BD Bioscience). To measure the $Foxp3^+CD25^+$ and $Foxp3^+CD25^-$ populations, T cells were stained with anti-CD25-FITC (BD Bioscience) and Foxp3-PE (eBioscience). To detect Foxp3, cells were permeabilized with Foxp3 fixation/permeabilization buffer (eBioscience) and stained with anti-Foxp3-PE (eBioscience; catalog no. 12-5773). IgG isotypes were used as a control in all FACS experiments, and the IgG-positive population was shown to be below 0.2%. To analyze the $CFSE^+Foxp3^+$ population converted by rDCs, T cells prelabeled with CFSE (5 μM) were cocultured with DCs in the presence or absence of probiotics for 7 days. Cells were stained with Foxp3 antibody, followed by the Foxp3 detection protocol. The $CFSE^+Foxp3^+$ population was analyzed by FACS.

Isolation of LP and Ear $CD4^+$ T Cells. Intestines (IBD mice) and ears (AD mice) were dissected into 1- to 2-cm segments and placed in Erlenmeyer flasks containing 25 mL of 1.0 mM EDTA/5% (vol/vol) FBS in PBS for 20 min at room temperature, with gentle stirring. The segments were transferred to 50-mL centrifuge tubes containing 15 mL of RPMI medium without serum, minced, and vigorously shaken twice for 15 s. Thereafter, the tissues were transferred to five small vials containing 10 mL of 0.5 mg/mL collagenase type V (Sigma) and incubated for 1 h at 37°C with stirring. After incubation, the suspensions of the cells were washed with ice-cold PBS and used as LP total cells or ear total cells. For further isolation of LP $CD4^+$ T cells, the $CD4^+$ T-cell isolation procedure was used (5).

Analysis of IgG and IgE Levels in Serum. Blood samples were obtained from mice 2 and 4 weeks after AD induction. Total serum IgE level was measured by an IgE ELISA kit (BD Biosciences). Briefly, serum was diluted 200-fold for measuring total IgE and 250-fold for measuring IgE, with 0.1 M sodium carbonate (pH 9.5) used as the primary antibody. Detection antibody was initially diluted 500-fold in assay diluent (PBS containing 10% (vol/vol) FBS), and streptavidin-conjugated HRP was added in detection antibody diluent at a ration of 250:1 for ELISA. For the detection of mite-specific IgE, 100 μL of 10 μg/mL mite extract was coated in a 96-well plate and kept at 4°C overnight. For the detection of serum IgG level, serum was analyzed with a mouse IgG ELISA quantitation kit (BETHYL). Briefly, obtained serum was diluted 2,000- to 100-fold for capturing antibody in 0.05 M carbonate-bicarbonate at pH 9.5 and used as the primary antibody. HRP-conjugated detection antibody was diluted 20,000-fold in assay diluent [50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, 1% BSA (pH 8.0)]. The procedure for evaluation

of mite-specific IgG was similar to that of total IgE detection, and serum was diluted 200-fold for ELISA. After discarding the antigen solution, each well was blocked with blocking solution for 1 h at room temperature and 100 μL of 10-fold diluted samples was added; the remaining procedures were performed according to the manufacturer's protocol. Three wells were prepared for each antigen and serum sample. The concentration and OD value indicated the average of each well. The mean absorbance of an antigen's wells minus the mean absorbance of a nonantigen well was used as the OD value of the antigen.

IHC for Detection of Foxp3 in Tissue. To analyze the Foxp3$^+$ population in tissues, IHC was performed as described, with a minor modification (6). Briefly, paraffin sections (3 mm) of mouse ear and colon were prepared. IHC was performed with anti-rat Foxp3 (eBioscience; catalog no. 14-5773) or anti-rabbit Foxp3 (Santa Cruz Biotechnology; catalog no. sc-28705) and AlexaFluor488-chicken-anti-rat or rabbit-IgG (Invitrogen). For counterstaining, propidium iodide (Molecular Probes; catalog no. P21493) and Hoeschst 34580 (Molecular Probes; catalog no. H21486) were used. The Foxp3$^+$ propidium iodide-positive population was analyzed using a Fluoview microscope (Olympus).

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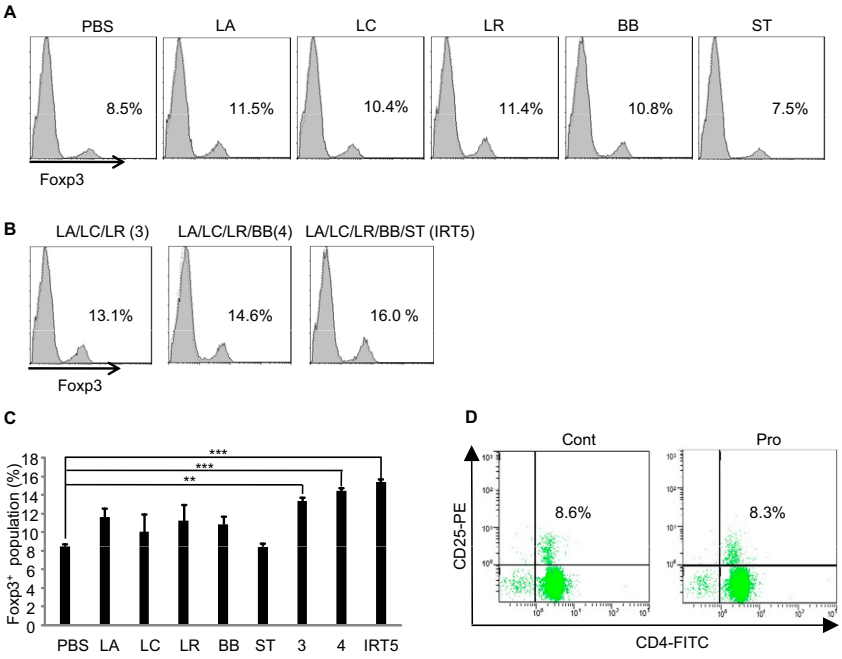

Fig. S1. Probiotics treatment increases CD4$^+$Foxp3$^+$ Tregs. The population of CD4$^+$Foxp3$^+$ Tregs was analyzed by FACS after administration of probiotics such as LA, LC, LR, BB, ST, or a mixture of them. A single strain (*A*) or combination of probiotics as a mixture (*B*) was administered to normal healthy BALB/c mice for 20 days. The probiotics mixture was prepared as follows: a mixture of three [LA/LC/LR(3)], four [LA/LC/LR/BB (4)], or five [LA/LC/LR/BB/ST (IRT5)] strains. (*A* and *B*) Population change of Foxp3$^+$CD4$^+$ Tregs in MLN was monitored by FACS. (*C*) Summary for the changes in the Treg population after probiotics treatment. Data are from 10 mice per group. Error bars indicate SD. Data are representative of three independent experiments. $^{**}P < 0.005$; $^{***}P < 0.001$. (*D*) There was no increase in the population of nTregs by IRT5 administration.

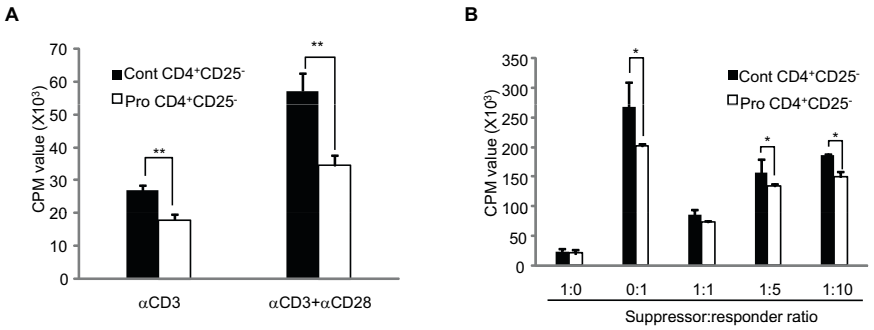

Fig. S2. Probiotics treatment inhibits proliferation of $CD4^+CD25^-$ effector T cells. (*A*) Proliferation capacity of $CD4^+CD25^-$ effector T cells from each group was compared by [H^3]-thymidine incorporation assay with plate-bound anti-CD3 (1 μg/mL) in the absence or presence of soluble anti-CD28 (1 μg/mL). (*B*) Suppression sensitivity of $CD4^+CD25^-$ T cells (responder) by the $CD4^+CD25^+$ Tregs (suppressor) was measured by in vitro suppression assay with various ratios of WT $CD4^+CD25^+$ nTregs. Proliferation rates were confirmed by [H^3]-thymidine incorporation assay. Data are from 10 mice per group; error bars indicate SD. Data are representative of three independent experiments. $*P < 0.05$; $**P < 0.005$.

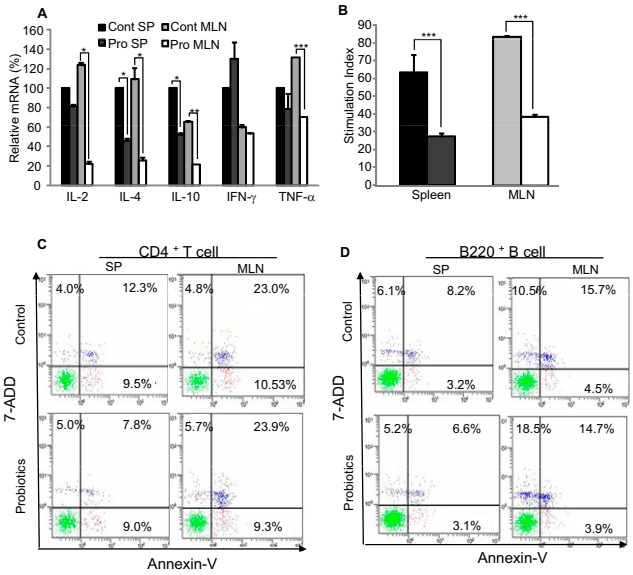

Fig. S3. Probiotics treatment induces lymphocyte hyporesponsiveness without apoptosis induction. (*A*) Levels of cytokine expression in $B220^+$ B cells were analyzed by real-time PCR. Cytokine expression in SP $B220^+$ B cells of Cont group mice was set at 100%. (*B*) Proliferation of $B220^+$ B cells from the Cont or Pro group mice was measured after stimulation with LPS. Stimulation indexes were defined by comparison with [H^3]-thymidine incorporation (cpm) by unstimulated cells. Data are from 10 mice per group; error bars indicate SD. Data are representative of three independent experiments. $*P < 0.05$; $**P < 0.005$; $***P < 0.001$. The apoptotic population (Annexin V^+ and 7-ADD^+) was analyzed by FACS in $CD4^+$ T cells (*C*) and $B220^+$ B cells (*D*) isolated from the SP or MLN of mice fed probiotics (Pro) or PBS (Cont) for 20 days. Data are from 10 mice per group. Data are representative of three independent experiments.

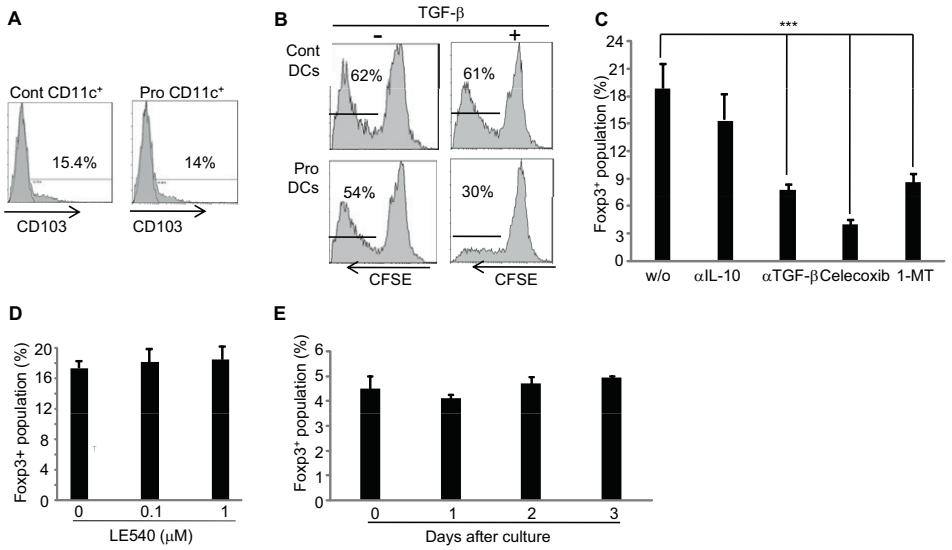

Fig. S4. Characterization of CD11c$^+$ rDCs induced by IRT5 administration. (*A*) No significant changes in the CD103$^+$ DC population by IRT5 administration. CD11c$^+$ DCs were isolated from the mice fed with either PBS (Cont) or IRT5 (Pro), and the CD103$^+$ population was then analyzed by FACS. (*B*) CD11c$^+$ DCs from each group were cocultured with Do11.10 CD4$^+$ T cells labeled with CFSE (5 μM) in the absence (–) or presence (+) of exogenous TGF-β. The degree of CD4$^+$ T-cell proliferation was analyzed by measuring the CFSE signal by FACS. MLN CD11c$^+$ DCs from IRT5-fed mice were cocultured with CFSE-labeled SP CD4$^+$ T cells in the presence of soluble anti-CD3 (1 μg/mL) and various inhibitors, including anti-IL-10 (10 μg/mL), anti-TGF-β (10 μg/mL), celecoxib (50 μM), and 1-MT (20 μM) (*C*) and LE540 (0.1 or 1 μM) (*D*) for 4 days. On day 5, 100 U/mL of IL-2 was added and cultured for 3 more days; the CFSE$^+$Foxp3$^+$ population was then analyzed by FACS. ***$P < 0.001$. (*E*) WT SP CD4$^+$ T cells labeled with CFSE were cultured with IRT5 at a 1:10 ratio for up to 3 days, and the Foxp3$^+$CFSE$^+$ population was measured by FACS. Data are from 10 mice per group; error bars indicate SD. Data are representative of three independent experiments.

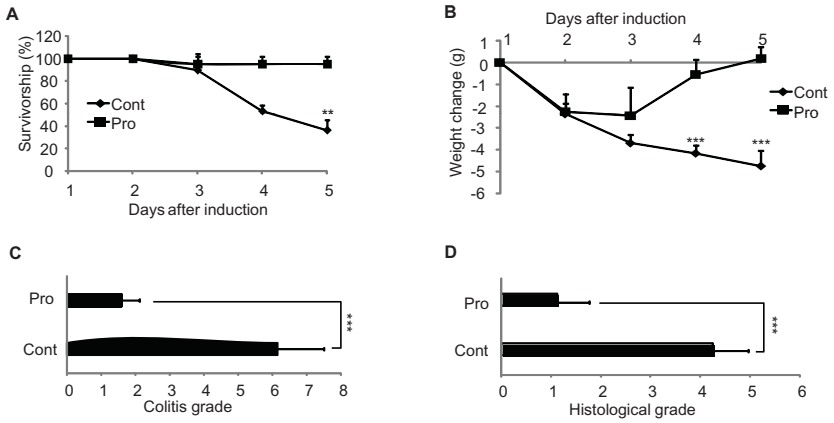

Fig. S5. Probiotics treatment suppresses experimental colitis. Changes in survivorship (*A*), weight loss (*B*), colitis grade (*C*), and histological grade (*D*) were evaluated after H&E staining. Data are from 20 mice per group; error bars indicate SD. Data are representative of three independent experiments. **$P < 0.005$; ***$P < 0.001$.

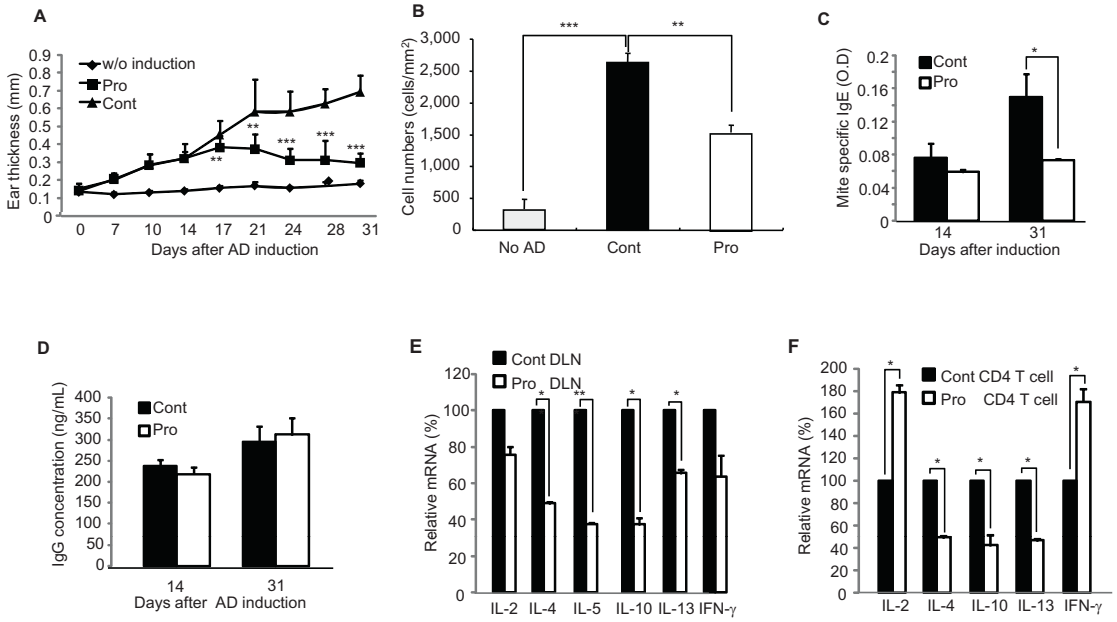

Fig. S6. Probiotics treatment suppresses experimental AD. The therapeutical efficacy of IRT5 was measured by the following criteria for AD progression: ear thickness (*A*), number of infiltrated lymphocytes based on histological analysis (*B*), and mite-specific IgE levels (*C*). (*D*) No effect was observed on IgG levels. Real-time PCR was used to analyze the levels of cytokine transcript in $CD4^+$ T cells of draining nymph nodes (DLNs) (*E*) and in $CD4^+$ T cells isolated from AD ear regions (*F*). Bars are from 10 mice per group; error bars indicate SD. Data are representative of three independent experiments. $*P < 0.05$; $**P < 0.005$; $***P < 0.001$. Interestingly, treatment with IRT5 down-regulated AD-associated cytokine levels in the DLNs (superficial cervical, axillary, and brachial nodes) (*E*), although inducing phenotypic skewing from Th2 to Th1 cytokines in the $CD4^+$ T cells of AD ear (*F*).

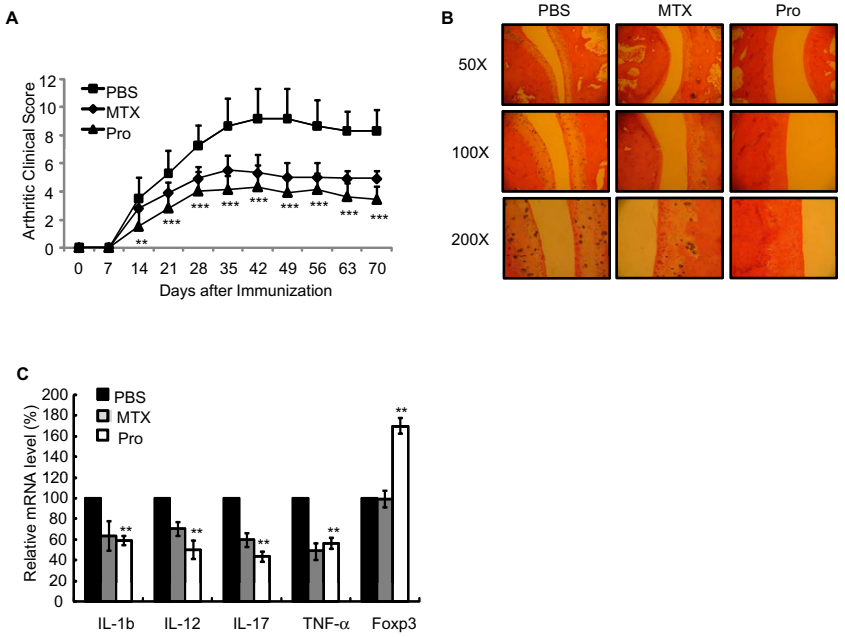

Fig. S7. Probiotics treatment suppresses experimental RA. Experimental RA was induced by immunization of type II collagen with complete Freund's adjuvant. Treatment was started 7 days after disease induction and continued until the end of the experiment. Rats were treated with PBS control (■; negative control), methotrexate (●; MTX; 300 μg/kg twice per week; positive therapeutical control), or IRT5 (▲; Pro; 1×10^9 cfu/day). Pro treatment significantly ($P < 0.01$) protected RA progression, with similar efficacy of MTX, by reducing the clinical score (*A*), lymphocyte infiltration (*B*), and inflammatory cytokines (*C*), through increasing Foxp3 levels (real-time PCR analysis) in draining lymph nodes. Data are representative of three independent experiments. $**P < 0.005$; $***P < 0.001$.

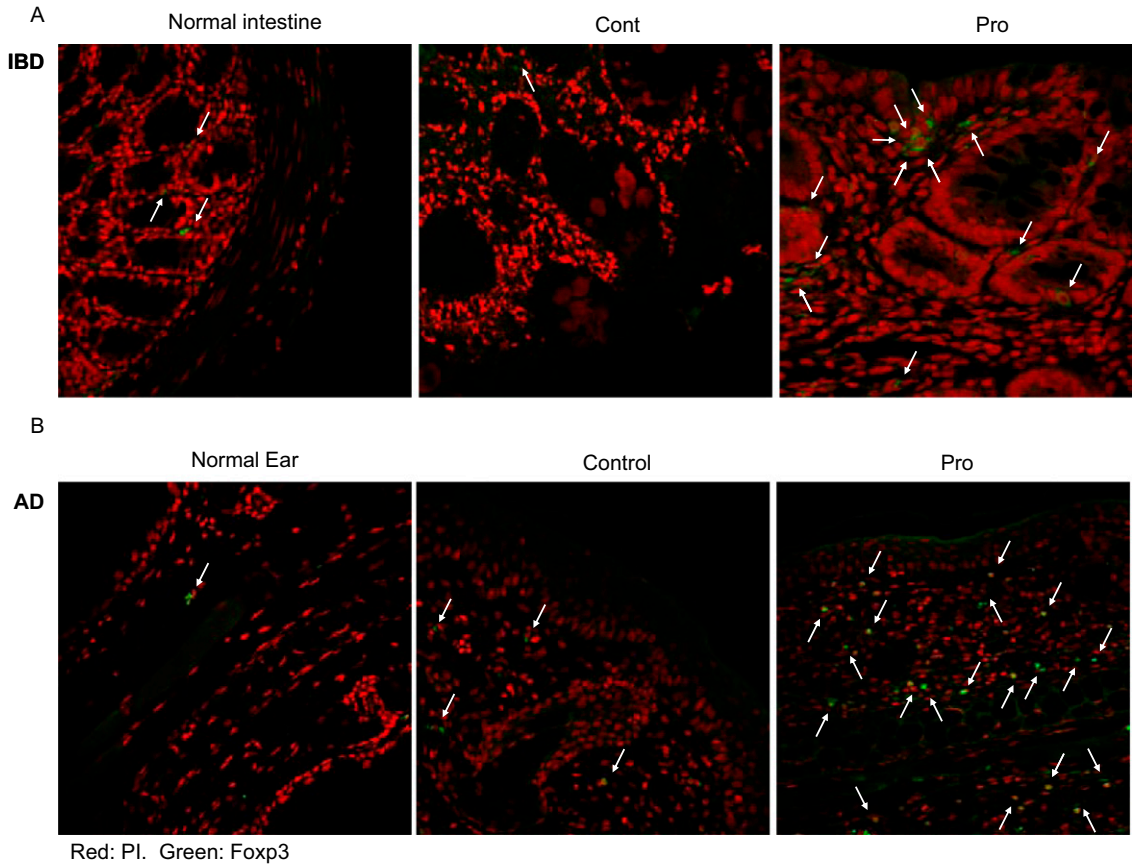

Fig. S8. Enriched $CD4^+Foxp3^+$ Tregs at inflammatory sites are associated with disease suppression. After treatment with probiotics or PBS, the $Foxp3^+$ population from the colon of experimental colitis (*A*) or ear of AD (*B*) mice was analyzed by IHC between the Pro and Cont groups. Arrows indicate $Foxp3^+$ cells. A control reaction was performed with isotype-matched IgG antibodies. Data are from 10 mice per group and are the average of three independent experiments.

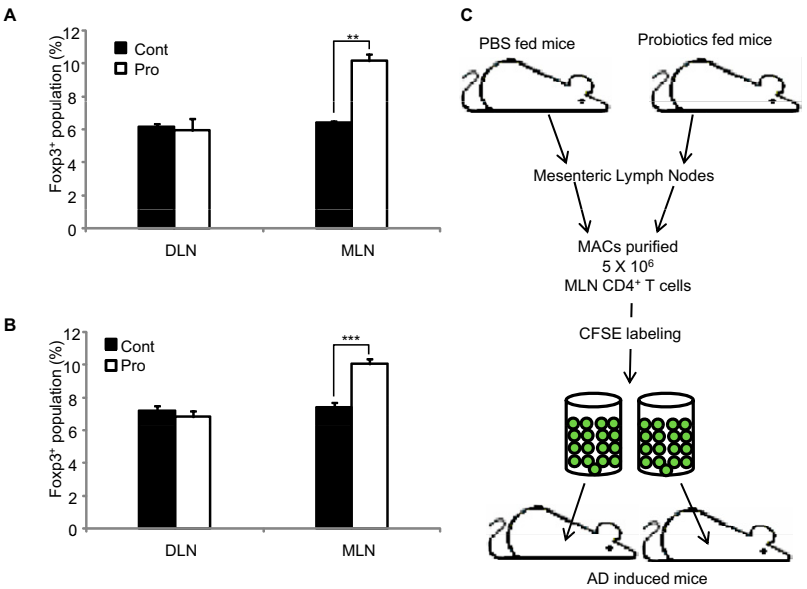

Fig. S9. Administration of probiotics increases $Foxp3^+CD4^+$ Tregs in disease conditions. Draining lymph node (DLN) and MLN were removed from the Cont or Pro group in the IBD (*A*) or AD (*B*) condition, and the $CD4^+Foxp3^+$ Treg population was analyzed by FACS. Data are from 10 mice per group; error bars indicate SD. Data are representative of three independent experiments. $^{**}P < 0.005$; $^{***}P < 0.001$. (C) Schematic flow chart for the adoptive transfer of $CD4^+$ T cells to AD mice. $CD4^+$ T cells (5×10^6) obtained from MLN of the Cont or Pro group were labeled with CFSE (green color) and then i.p. transferred to AD mice. $CFSE^+$ $Foxp3^+$ Tregs were analyzed under fluorescence microscopy (Fig. 7*C*).

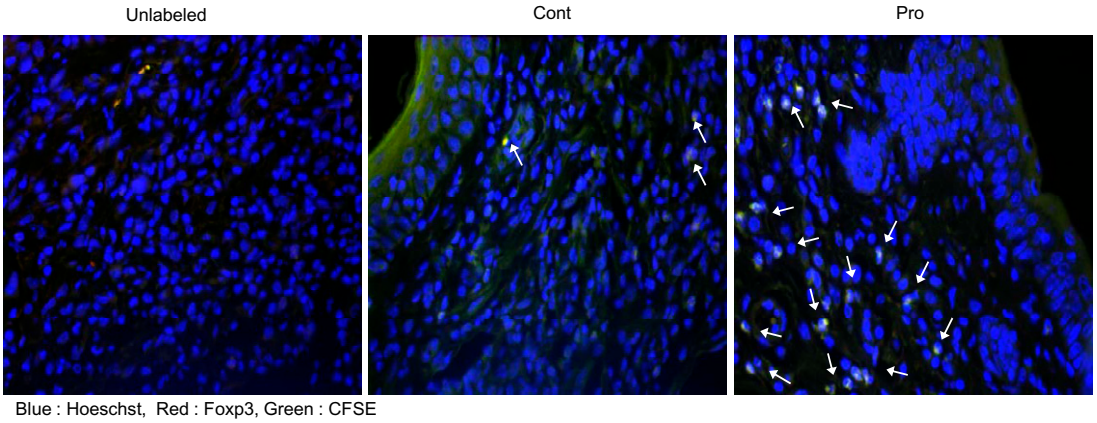

Fig. S10. Migration of MLN CD4$^+$Foxp3$^+$ Tregs to the inflammatory site. CFSE-labeled T cells obtained from MLN of the Cont and Pro groups were adoptively transferred to AD mice. The migration of CFSE$^+$(CD4$^+$)Foxp3$^+$ Tregs to the ear of AD mice was analyzed by IHC. The arrows indicate CFSE$^+$(CD4$^+$)Foxp3$^+$ Tregs.

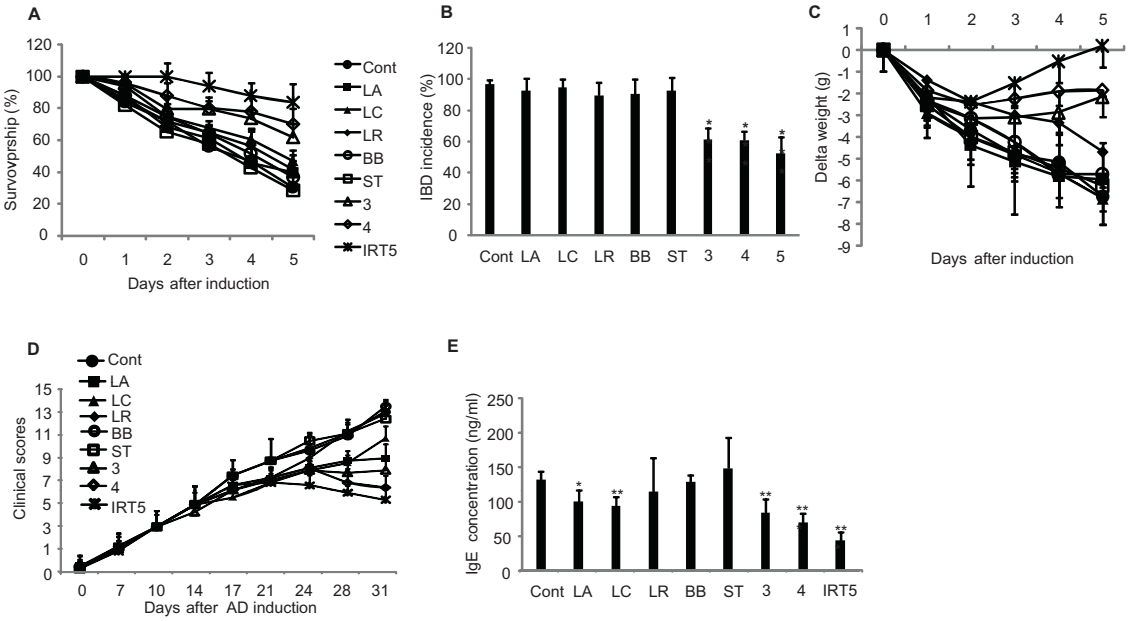

Fig. S11. Mixture of five probiotics (IRT5) shows the most potent protective efficacy compared with a single strain or mixture of probiotics against IBD (*A*–*C*) and AD (*D* and *E*) progression. IRT5 administration increased survivorship (*A*) by reducing IBD incidence (*B*) without a loss in body weight (*C*). In addition, IRT5 administration reduced the AD-related criteria such as clinical score (*D*) and serum IgE levels (*E*). The numbers 3 or 4 and IRT5 indicate the combination of LA/LC/LR, LA/LC/LR/BB, and LA/LC/LR/BB/ST, respectively. Error bars indicate SD. Data are from 20 mice per group. *$P < 0.05$; **$P < 0.005$. Data are representative of three independent experiments.