Supporting Information

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SI Materials and Methods

Bacterial Survival in Simulated Gastric and Small Intestinal Fluids. NCK56 and NCK2025 were inoculated (1%) into de Man, Rogosa, and Sharpe broth (MRS) and propagated for 24 h in microtiter plates. The OD_{600} was recorded every 1 h using a microtiter plate reader (FLOUStar Optima; BMG Technologies). Additionally, simulated gastric and small intestinal fluids were prepared as described previously (1, 2). Simulated gastric fluid was used at pH 2, and small intestinal fluid was used with 3 g/L oxgall. Stationary-phase bacterial cultures (1 mL; grown 16 h in MRS) were pelleted by centrifugation (8,160 × *g* for 1 min, room temperature), washed two times with distilled water, and resuspended in 1 mL distilled water. An aliquot of washed cells (0.2 mL) was added to 1.0 mL small intestinal fluid and 0.3 mL 0.5% NaCl. An aliquot (0.5 mL) was added to 2.5 mL gastric fluid. Suspensions were held at 37 °C, and aliquots were removed every 30 min (gastric fluid) or every 1 h (small intestinal fluid), serially diluted, and plated for enumeration. Samples of the bacterial cells before addition to the fluids were serially diluted and plated to ensure equal addition of cells. NCK56 and NCK2025 were inoculated (1%) for 24 h in MRS for growth curves in microtiter plates. The OD_{600} was recorded every 1 h over a 24-h period using a microtiter plate reader (FLOUStar Optima; BMG Technologies).

Biochemical Analysis of Lipoteichoic Acid. *Lactobacillus acidophilus* NCK56 (5 × 108·cfu·10 mL) and NCK2025 (5 × 108·cfu/·10 mL) were propagated from frozen stocks (−80 °C) in MRS broth (Difco) without erythromycin at 37 °C. Subsequently, the expression of lipoteichoic acid (LTA) in NCK56 and NCK2025 was analyzed as described previously (3). Briefly, the frozen extracts of both strains NCK56 and NCK2025 were dissolved in the citrate buffer (0.05 M) at pH 4.7 followed by syndication for 15 min. The bacteria lysates (30 mL) were mixed with an equal volume of L-butanol under stirring for 20 min at room temperature. Subsequently, centrifugation (17,200 × *g*) for 40 min aqueous phase was collected before the addition of fresh citrate buffer for a second extraction. This reextraction was conducted two times, and three aqueous phases were pooled and lyophilized. After resuspension of the samples in chromatography start buffer (35 mL; 15% n-propanol in 0.1 M ammonium acetate, pH 4.7), all sample were centrifuged (26,900 × *g* for 1 h) and filtered (0.2 μm). Lyophilized material from both bacterial strains was dissolved in 0.7% trifluoracetic acid, and 0.45 mg of extract from each strain were analyzed by HPLC. Chromatographs were obtained by continuously monitoring absorbance at 260 nm.

Methods: Low-Density cDNA Microarray. Colonic distal and proximal regions of each group of mice (n = 5/group) that was treated with 3% dextran sulphate sodium (DSS) alone, NCK56-DSS, or NCK2025-DSS were flushed with PBS and immediately immersed in RNALater (Qiagen) for RNA stabilization. RNA was extracted with the RNeasy Mini Kit (Qiagen) and quality-assessed using Agilent Nanochip Bioanalysis (Agilent). All samples used had RNA Integrity Numbers (RIN) greater than 7. Two mouse inflammatory arrays consisting of 232 genes were performed on each group using at least one independent sample, and in some instances (because of low RNA concentrations), the second array was a pooled sample of the remaining mice. Reverse transcription and hybridization for the microarrays were carried out as described by the manufacturer (DualChip microarray; Eppendorf). Briefly, 6 μg RNA were reverse-transcribed by first incubating the samples with Oligo(dT)12–18 Primer (Invitrogen) at 70 °C for 10 min followed by the addition of the reverse transcriptase mix (Superscript III, dNTPs; Invitrogen), biotin-labeled ATP, and CTP (Perkin-Elmer); then, the sample was incubated at 42 °C for 90 min and 70 °C for 15 min. RNase H was added, and the samples were incubated at 37 °C for 20 min followed by 95 °C for 3 min to terminate the reaction. Resulting cDNA was loaded into the hybridization chamber and incubated overnight at 60 °C, mixing at 349 × *g* in an Eppendorf thermomixer. Slides were washed, and RNA levels were determined by detection of biotin incorporation using the Silverquant detection system as described by the manufacturer (Eppendorf). Analysis was performed using Silverquant analysis software (Eppendorf). Briefly, arrays were scanned, and signal intensities were used to quantify concentrations after a two-step normalization process involving internal standards and housekeeping genes. The average of the gene intensity values of untreated mice was used to determine the change in gene expression in the experimental mice. The distal and proximal regions were analyzed independently.

1. Charteris WP, Kelly PM, Morelli L, Collins JK (1998) Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic Lactobacillus and Bifidobacterium species in the upper human gastrointestinal tract. *J Appl Microbiol* 84:759–768.
2. Frece J, et al. (2005) Importance of S-layer proteins in probiotic activity of Lactobacillus acidophilus M92. *J Appl Microbiol* 98:285–292.
3. Morath S, Geyer A, Hartung T (2001) Structure-function relationship of cytokine induction by lipoteichoic acid from Staphylococcus aureus. *J Exp Med* 193:393–397.

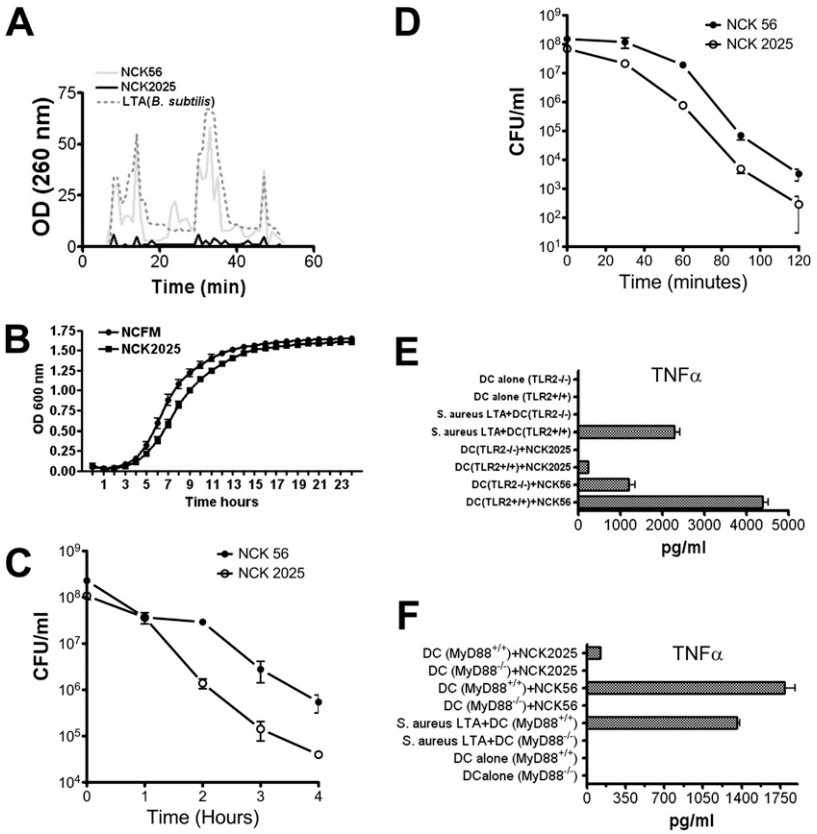

Fig. S1. (*A*) Reverse-phase chromatography of bacterial extracts was performed on NCK56 (gray histogram) and NCK2025 (dark histogram). The elution positions of LTA derived from *Bacillus subtilis* (dotted histogram; Sigma) served as a positive control (dotted histogram). (*B*) Growth of NCK56 and NCK2025 in MRS broth for 24 h. Results shown are the mean ± SD of the mean of quadruplicate samples. This graph is representative of experiments performed three independent times. (*C* and *D*) Survival of NCK56 and NCK2025 in simulated gastric (*C*) and small intestinal (*D*) fluids. Three replicates of these experiments were performed. (*E* and *F*) Induction of TNFα in dendritic cells (DCs). TNFα released in the supernatants of NCK56, NCK2025, or *Staphylococcus aureus* LTA-treated and untreated bone-marrow DCs derived from $TLR^{+/+}$,$TLR2^{-/-}$, $MyD88^{+/+}$, or $MyD88^{-/-}$ mice were assayed by ELISA. Experiments were repeated at least three times with similar results.

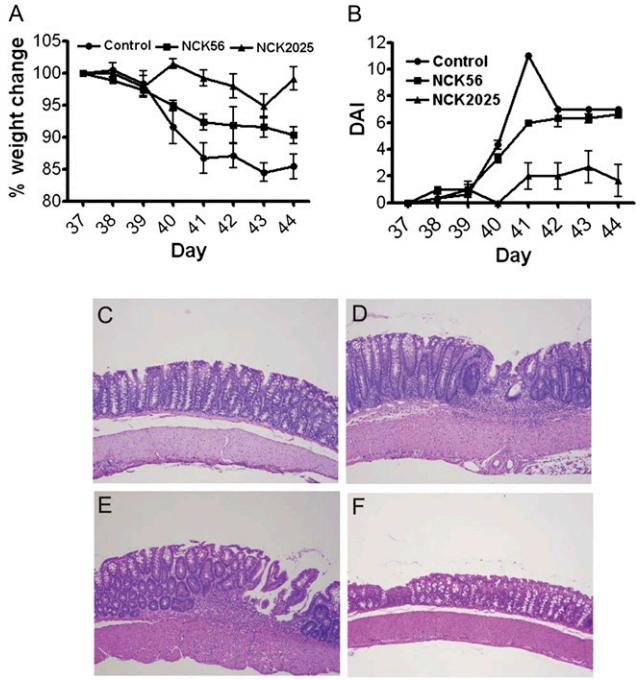

Fig. S2. NCK2025 prevents $CD4^+CD45RB^{high}$-induced colitis. (*A*) Magnetic bead highly enriched $CD4^+CD45RB^{high}$T cells (106/100 μL PBS/mouse; Miltenyi Biotec) were transferred into three groups of C57BL/6 $Rag1^{-/-}$ mice (n = 5/group) by retro-orbital injection in the first and second week. Twenty-four hours later, two groups of mice were treated with either NCK56 or NCK2025 for 4 consecutive d. These mice were then treated one time per week with NCK56 or NCK2025 for the next 6 wk. (*B*) Disease activity indexes (DAI) are presented from day 37 until sacrifice at day 44. (*C*) Colonic sections were stained with H&E. (Magnification: ×10.) (*C*) Control mouse. (*D*) Injection of $CD4^+CD45RB^{high}$T cells alone. (*E*) Injection of $CD4^+CD45RB^{high}$T cells plus NCK56-treated mouse group. (*F*) Injection of $CD4^+CD45RB^{high}$T cells plus NCK2025 treated-mouse group.

A

	Distal			Proximal			
	DSS	NCK56	NCK2025	DSS	NCK56	NCK2025	
Immune Regulatory							
Ccl3	13.99	46.26	8.09	2.72	17.83	1.96	> 10
Ccl5	14.44	67.45	10.64	2.14	13.43	3.75	4 - 10
Ccl7	24.43	107.23	22.97	3.97	16.95	2.09	2 - 4
Ccr1	10.14	27.50	5.74	4.05	5.56	1.21	1.41 - 2
Ccr5	7.15	25.51	6.08	3.50	8.71	2.07	1.19 - 1.41
Cx3cr1	1.17	0.99	1.41	4.72	2.55	2.78	0.84 - 1.19
Cxcr4	1.13	1.55	1.47	1.58	1.53	1.01	0.71 - 0.84
Il13	1.28	1.31	1.48	2.01	1.69	1.43	0.5 - 0.71
Il3	0.91	1.06	1.08	2.76	2.17	2.24	0.25 - 0.5
Pla2g7	3.23	10.42	5.50	3.29	4.83	2.12	0.1 - 0.25
Plaa	1.07	1.76	2.41	1.12	1.24	0.96	< 0.1
Srf	0.97	1.41	1.78	1.16	1.11	1.10	
Tgfb1	2.23	4.49	2.62	2.05	2.77	1.50	
Signaling							
Jak2	0.79	0.96	0.95	1.02	0.88	1.07	
Map2k4	1.09	1.03	1.36	1.06	1.20	0.86	
Map3k1	1.07	0.93	1.08	1.09	1.31	1.01	
Mapk9	0.72	0.63	1.06	1.32	1.22	0.93	
Pak1	0.70	0.61	1.33	1.34	1.23	1.05	
Ptprc/CD45	6.59	13.72	4.44	1.95	2.84	1.64	
Raf1	0.99	1.38	1.79	1.21	1.28	1.04	
Socs2	0.74	1.40	1.11	1.84	1.20	1.40	
Sp1	1.01	1.65	1.46	1.38	1.24	1.51	
Stat6	1.06	1.47	1.95	1.88	1.49	1.25	
Timp1	23.09	56.33	27.02	4.74	20.73	1.78	
Tyk2	0.91	1.42	1.91	1.55	1.63	1.16	
Immune Stimulatory							
Ccl11	4.17	10.87	3.31	2.79	6.54	1.76	
Ccl2	28.59	125.23	23.96	3.15	14.98	1.53	
Ccr7	2.19	4.64	2.58	2.32	2.53	1.19	
Cd40	3.83	17.92	4.11	2.49	4.09	1.61	
Cd69	4.36	13.18	1.63	3.18	2.40	0.65	
Cd80	3.06	4.60	3.03	4.41	2.38	1.18	
Cd86	3.49	6.26	2.70	3.08	2.66	1.65	
Il15	0.65	0.61	0.73	1.66	0.83	1.06	
Il1b	66.69	146.65	33.46	4.93	44.98	2.17	
Il23a	0.80	0.68	0.79	2.34	2.16	1.46	
Il6	7.87	28.37	9.26	2.11	12.61	0.34	
Irf3	0.92	0.66	1.08	1.17	1.23	0.98	
Mal	0.90	1.45	4.16	0.57	0.57	1.09	
Nfkb1	1.60	1.62	1.46	0.83	1.15	0.86	
Nfkbib	1.03	1.62	1.73	2.92	1.54	1.38	
Nos2	5.49	21.95	5.72	2.23	3.89	1.41	
P2rx7	0.86	0.86	1.32	3.67	1.27	0.98	
Stat1	5.19	17.39	3.77	1.62	3.15	1.42	
Stat4	3.65	7.27	3.40	2.87	3.57	2.17	
Tnf	3.46	9.28	3.37	1.36	3.80	1.36	
Tnfrsf11a	0.89	1.26	1.70	2.01	1.76	1.78	
Tnfrsf7	0.88	1.79	1.79	3.57	3.35	2.15	
Tnfrsf8	1.05	1.46	1.71	2.36	0.93	0.71	
Tnfsf11	3.42	11.64	3.54	2.71	2.08	0.54	
Traf1	1.57	3.37	2.14	1.40	2.73	2.07	
Traf2	1.16	1.71	2.25	1.03	1.50	1.10	
Traf3	1.31	2.14	1.99	1.38	1.51	1.18	
Traf6	1.10	2.38	2.62	1.44	1.47	0.86	
Proliferation/Apoptosis/Angiogenesis/Adhesion							
Csf1	1.37	2.05	1.94	1.31	1.45	0.89	
Cxcl1	2.82	9.42	5.26	2.45	8.33	0.38	
Cxcl12	1.48	2.29	1.75	1.37	1.94	1.25	
Fasl	1.37	4.03	1.10	2.66	1.53	0.52	
Icam1	4.13	7.71	2.61	1.43	2.86	1.07	
Kitl	0.66	0.77	0.95	0.95	1.09	0.88	
Nrg1	5.77	15.07	4.99	3.36	4.30	1.36	
Plau	0.75	1.78	1.51	3.32	1.54	1.38	
Ptgs2/Cox2	6.81	23.04	5.93	1.50	8.06	1.00	
Sell	5.74	16.52	4.40	1.95	8.78	1.30	
Tnfaip3	1.44	4.03	2.46	1.29	1.74	1.33	
Vcam1	5.24	19.10	4.26	1.49	9.10	1.65	

B

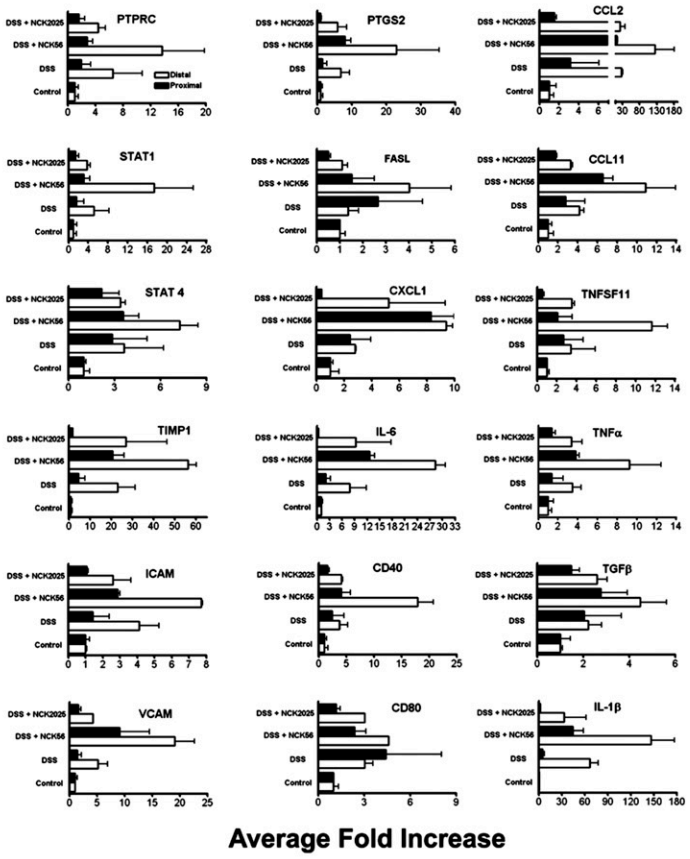

Fig. S3. Regulation of genes in DSS-induced colitis in mice on treatment with NCK56 or NCK2025 or no treatment. (*A*) Mice (n = 5/group) were given NCK56 or NCK2025 or were left untreated for 4 d before DSS induction of colitis. Proximal or distal colonic regions were isolated, and RNA was extracted. cDNA microanalysis reveals differential gene expression patterns in pathways involving immune regulation/stimulation, signaling, proliferation, apoptosis, angiogenesis, and adhesion in the colon of mice. Heat map indicates up-regulated (red) and down-regulated (green) genes compared with control mice. (*B*) Fold increase of selected genes that were regulated in mouse colons on bacterial treatment.

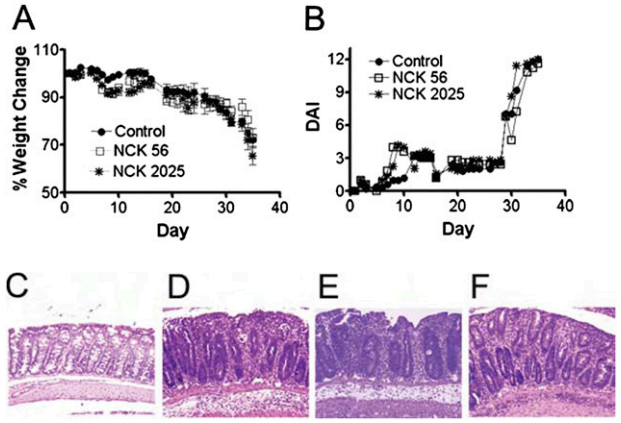

Fig. S4. Critical role of host IL-10 in $CD4^+CD45RB^{high}$T cell-induced colitis. Highly enriched $CD4^+CD45RB^{high}$T cells (106/100 μL PBS/mouse) were transferred into three groups of C57BL/6 *Rag1*$^{-/-}$*IL-10*$^{-/-}$ mice (n = 5/group) by retro-orbital injection in the first and second week. One day later, two groups of mice were treated with either NCK56 or NCK2025 for 4 consecutive d. Subsequently, these mice were treated one time per week with NCK56 or NCK2025 for the next 5 wk. (*A*) Weight loss. (*B*) DAIs are presented from day 0 ($CD4^+CD45RB^{high}$T-cell transfer) until sacrifice at day 35. (*C*–*F*) H&E staining. (*C*) Control mouse. (*D*) Injection of $CD4^+CD45RB^{high}$T cells alone. (*E*) Injection of $CD4^+CD45RB^{high}$T cells + NCK56-treated mouse group. (*F*) Injection of $CD4^+CD45RB^{high}$T cells + NCK2025-treated mouse group.