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Lacticaseibacillus paracasei NK112 mitigates _Escherichia coli_-induced depression and cognitive impairment in mice by regulating IL-6 expression and gut microbiota

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Table S1. *P*-values for the present experiment data.

Figure 1 and S1. Unpaired t-test		
(1A) IL-6 (pg/mg)	NC vs EC	$P=0.025$
	NC vs ENK	$P=0.007$
	EC vs ENK	$P=0.041$
(1B) TNF-α (pg/mg)	NC vs EC	$P<0.0001$
	NC vs ENK	$P<0.0001$
	EC vs ENK	$P=0.006$
(S1A) Intensity p-p65/p65	NC vs EC	$P=0.000$
	NC vs ENK	$P=0.000$
	EC vs ENK	$P=0.033$
(1C) IL-6 (pg/mg)	NC vs EC	$P<0.0001$
	NC vs ENK	$P=0.006$
	EC vs ENK	$P=0.000$
(1D) TNF-α (pg/mg)	NC vs EC	$P<0.0001$
	NC vs ENK	$P<0.0001$
	EC vs ENK	$P=0.005$
(S1B) Intensity p-p65/p65	NC vs EC	$P=0.001$
	NC vs ENK	$P=0.017$
	EC vs ENK	$P=0.016$
Figure 2 and S2. Unpaired t-test		
(2A) Open arm time (%)	NC vs EC	$P<0.001$
	NC vs ENK	$P=0.038$
	EC vs ENK	$P<0.001$
(S2A) Open arm entries (%)	NC vs EC	$P=0.003$
	NC vs ENK	$P=0.563$
	EC vs ENK	$P=0.013$
(2B) Immobility (%)	NC vs EC	$P=0.003$
	NC vs ENK	$P=0.576$
	EC vs ENK	$P=0.002$
(S2B) Immobility (%)	NC vs EC	$P<0.001$
	NC vs ENK	$P=0.008$
	EC vs ENK	$P<0.001$
(2C) Spontaneous alternation (%)	NC vs EC	$P<0.001$
	NC vs ENK	$P=0.816$
	EC vs ENK	$P<0.001$
(2D) IL-6 (pg/mg)	NC vs EC	$P<0.001$
	NC vs ENK	$P<0.001$
	EC vs ENK	$P=0.041$
(S2C) IL-1β (pg/mg)	NC vs EC	$P=0.005$

	NC vs ENK	P=0.098
	EC vs ENK	P=0.044
(S2D) TNF-α (pg/mg)	NC vs EC	P=0.002
	NC vs ENK	P=0.089
	EC vs ENK	P=0.036
(S2E) Intensity p-p65/p65	NC vs EC	P<0.001
	NC vs ENK	P=0.325
	EC vs ENK	P=0.001
(S2E) Intensity p-CREB/CREB	NC vs EC	P=0.005
	NC vs ENK	P=0.048
	EC vs ENK	P=0.031
(S2E) Intensity BDNF/ β-actin	NC vs EC	P=0.018
	NC vs ENK	P=0.043
	EC vs ENK	P=0.735
(2E) CORT (mg/ml)	NC vs EC	P<0.001
	NC vs ENK	P<0.001
	EC vs ENK	P=0.003
(2F) IL-6 (pg/ml)	NC vs EC	P=0.006
	NC vs ENK	P=0.014
	EC vs ENK	P=0.043
Figure 3 and S3. Unpaired t-test		
(3A) Colon length (cm)	NC vs EC	P=0.000
	NC vs ENK	P=0.026
	EC vs ENK	P=0.028
(3B) MPO activity (μunit/mg)	NC vs EC	P<0.001
	NC vs ENK	P<0.0001
	EC vs ENK	P<0.001
(3C) IL-6 (pg/mg)	NC vs EC	P=0.028
	NC vs ENK	P=0.647
	EC vs ENK	P=0.035
(S3A) IL-1β (pg/mg)	NC vs EC	P=0.001
	NC vs ENK	P=0.247
	EC vs ENK	P=0.014
(3D) TNF-α (pg/mg)	NC vs EC	P<0.001
	NC vs ENK	P<0.001
	EC vs ENK	P=0.001
(S3B) Intensity p-p65/p65	NC vs EC	P=0.001
	NC vs ENK	P=0.008
	EC vs ENK	P=0.001

Figure 5 and S6. Unpaired t-test			
(5A) Open arm time (%)			
NC vs EC	P=0.009	EC vs ENS	P=0.001
NC vs EhN	P=0.325	EC vs ENP	P=0.001
NC vs ENS	P=0.983	EhN vs ENS	P=0.266
NC vs ENP	P=0.234	EhN vs ENP	P=0.944
EC vs EhN	P=0.005	ENS vs ENP	P=0.165
(S6A) Open arm entries (%)			
NC vs EC	P=0.004	EC vs ENS	P=0.024
NC vs EhN	P=0.006	EC vs ENP	P=0.005

NC vs ENS	P=0.310	EhN vs ENS	P=0.227
NC vs ENP	P=0.123	EhN vs ENP	P=0.031
EC vs EhN	P=0.004	ENS vs ENP	P=0.097
(5B) Immobility (%)			
NC vs EC	P<0.0001	EC vs ENS	P=0.015
NC vs EhN	P=0.001	EC vs ENP	P=0.001
NC vs ENS	P=0.000	EhN vs ENS	P=0.336
NC vs ENP	P=0.128	EhN vs ENP	P=0.045
EC vs EhN	P=0.003	ENS vs ENP	P=0.007
(S6B) Immobility (%)			
NC vs EC	P=0.000	EC vs ENS	P=0.020
NC vs EhN	P=0.078	EC vs ENP	P=0.002
NC vs ENS	P=0.011	EhN vs ENS	P=0.193
NC vs ENP	P=0.550	EhN vs ENP	P=0.442
EC vs EhN	P=0.001	ENS vs ENP	P=0.105
(5C) Spontaneous alternation (%)			
NC vs EC	P<0.0001	EC vs ENS	P=0.000
NC vs EhN	P=0.021	EC vs ENP	P<0.0001
NC vs ENS	P=0.062	EhN vs ENS	P=0.875
NC vs ENP	P=0.336	EhN vs ENP	P=0.296
EC vs EhN	P<0.0001	ENS vs ENP	P=0.349
(5D) IL-6 (pg/mg)			
NC vs EC	P<0.0001	EC vs ENS	P=0.035
NC vs EhN	P=0.020	EC vs ENP	P=0.000
NC vs ENS	P<0.0001	EhN vs ENS	P=0.292
NC vs ENP	P=0.022	EhN vs ENP	P=0.193
EC vs EhN	P=0.038	ENS vs ENP	P=0.001
(S6C) IL-1β (pg/mg)			
NC vs EC	P=0.001	EC vs ENS	P=0.022
NC vs EhN	P=0.007	EC vs ENP	P=0.002
NC vs ENS	P=0.002	EhN vs ENS	P=0.153
NC vs ENP	P=0.105	EhN vs ENP	P=0.102
EC vs EhN	P=0.005	ENS vs ENP	P=0.013
(S6D) TNF-α (pg/mg)			
NC vs EC	P=0.001	EC vs ENS	P=0.012
NC vs EhN	P<0.0001	EC vs ENP	P=0.000
NC vs ENS	P<0.0001	EhN vs ENS	P=0.731
NC vs ENP	P=0.001	EhN vs ENP	P=0.004
EC vs EhN	P=0.001	ENS vs ENP	P=0.005
(S6E) Intensity BDNF/ β-actin			
NC vs EC	P=0.024	EC vs ENS	P<0.001
NC vs EhN	P=0.002	EC vs ENP	P<0.001
NC vs ENS	P<0.0001	EhN vs ENS	P=0.033
NC vs ENP	P<0.0001	EhN vs ENP	P=0.000
EC vs EhN	P<0.0001	ENS vs ENP	P=0.000
(S6E) Intensity p-CREB/CREB			
NC vs EC	P=0.001	EC vs ENS	P<0.001
NC vs EhN	P=0.003	EC vs ENP	P<0.001
NC vs ENS	P=0.000	EhN vs ENS	P=0.016

NC vs ENP	*P*=0.000	EhN vs ENP	*P*=0.003
EC vs EhN	*P*=0.000	ENS vs ENP	*P*=0.032
(S6E) Intensity p-p65/p65			
NC vs EC	*P*=0.000	EC vs ENS	*P*=0.004
NC vs EhN	*P*=0.003	EC vs ENP	*P*<0.001
NC vs ENS	*P*=0.001	EhN vs ENS	*P*=0.704
NC vs ENP	*P*=0.066	EhN vs ENP	*P*=0.647
EC vs EhN	*P*=0.004	ENS vs ENP	*P*=0.131
(5E) CORT (mg/mL)			
NC vs EC	*P*<0.0001	EC vs ENS	*P*<0.001
NC vs EhN	*P*=0.000	EC vs ENP	*P*<0.001
NC vs ENS	*P*<0.0001	EhN vs ENS	*P*=0.035
NC vs ENP	*P*=0.006	EhN vs ENP	*P*=0.003
EC vs EhN	*P*<0.0001	ENS vs ENP	*P*<0.001
(5F) IL-6 (pg/ml)			
NC vs EC	*P*<0.0001	EC vs ENS	*P*=0.043
NC vs EhN	*P*=0.000	EC vs ENP	*P*=0.001
NC vs ENS	*P*<0.0001	EhN vs ENS	*P*=0.416
NC vs ENP	*P*=0.001	EhN vs ENP	*P*=0.062
EC vs EhN	*P*=0.031	ENS vs ENP	*P*=0.007
Figure 6 and S7. Unpaired t-test			
(6A) Colon length (cm)			
NC vs EC	*P*=0.003	EC vs ENS	*P*=0.163
NC vs EhN	*P*=0.022	EC vs ENP	*P*=0.015
NC vs ENS	*P*=0.005	EhN vs ENS	*P*=0.911
NC vs ENP	*P*=0.166	EhN vs ENP	*P*=0.087
EC vs EhN	*P*=0.198	ENS vs ENP	*P*=0.008
(6B) MPO activity (μunit/mg)			
NC vs EC	*P*=0.001	EC vs ENS	*P*=0.037
NC vs EhN	*P*=0.041	EC vs ENP	*P*=0.003
NC vs ENS	*P*=0.000	EhN vs ENS	*P*=0.005
NC vs ENP	*P*=0.587	EhN vs ENP	*P*=0.452
EC vs EhN	*P*=0.003	ENS vs ENP	*P*=0.018
(6C) IL-6 (pg/mg)			
NC vs EC	*P*<0.0001	EC vs ENS	*P*=0.001
NC vs EhN	*P*=0.001	EC vs ENP	*P*=0.000
NC vs ENS	*P*<0.0001	EhN vs ENS	*P*=0.001
NC vs ENP	*P*=0.024	EhN vs ENP	*P*=0.045
EC vs EhN	*P*<0.0001	ENS vs ENP	*P*<0.0001
(S7A) IL-1β (pg/mg)			
NC vs EC	*P*=0.000	EC vs ENS	*P*=0.010
NC vs EhN	*P*=0.264	EC vs ENP	*P*=0.000
NC vs ENS	*P*=0.006	EhN vs ENS	*P*=0.013
NC vs ENP	*P*=0.281	EhN vs ENP	*P*=0.574
EC vs EhN	*P*=0.000	ENS vs ENP	*P*=0.009
(6D) TNF-α (pg/mg)			
NC vs EC	*P*=0.001	EC vs ENS	*P*=0.009
NC vs EhN	*P*=0.197	EC vs ENP	*P*=0.001
NC vs ENS	*P*=0.047	EhN vs ENS	*P*=0.436

NC vs ENP	P=0.156	EhN vs ENP	P=0.579
EC vs EhN	P=0.003	ENS vs ENP	P=0.141
(S7B) Intensity p-p65/p65			
NC vs EC	P<0.0001	EC vs ENS	P=0.008
NC vs EhN	P=0.008	EC vs ENP	P<0.001
NC vs ENS	P=0.000	EhN vs ENS	P=0.0037
NC vs ENP	P=0.142	EhN vs ENP	P=0.015
EC vs EhN	P=0.025	ENS vs ENP	P=0.034

Table S2. Effect of NK112 on the gut microbiota composition at the family level in EC-treated mice.[1]

Taxon Name	Relative contribution (%)		
	NC	EC	ENK
Bacteroidaceae	15.09±13.84	16.45±7.07	37.17±7.55*
Desulfovibrionaceae	0.67±0.51	1.51±0.99	0.00±0.00*
Helicobacteraceae	3.76±1.63	8.62±7.41	0.01±0.01*
Lachnospiraceae	25.43±10.61	28.85±10.99	13.62±5.48*
Muribaculaceae	20.91±13.14	8.23±3.96#	35.46±5.64*
Odoribacteraceae	2.17±3.77	0.71±1.59	0.00±0.01
Porphyromonadaceae	1.44±1.50	2.16±1.52	0.11±0.04*
Prevotellaceae	10.58±7.75	8.25±4.66	3.49±1.26*
Rikenellaceae	6.66±5.19	9.26±6.38	0.57±0.16*
Ruminococcaceae	8.39±1.73	8.85±2.22	6.86±2.11

[1] Values indicate means ± standard deviation. #P<0.05 vs NC group and *P<0.05 vs EC group.

Table S3. Effect of NK112 on the gut microbiota composition at the genus level in EC-treated mice.[1]

Taxon name	Relative contribution (%)		
	NC	EC	ENK
Alistipes	5.95±5.02	8.23±5.92	0.57±0.16*
Bacteroides	15.05±13.84	16.44±7.06	35.71±7.00*
Helicobacter	3.75±1.63	8.58±7.32	0.01±0.01*
KE159538_g	4.57±6.54	4.48±3.11	1.60±0.97
LLKB_g	1.17±1.27	3.06±2.48	0.17±0.19
Oscillibacter	1.29±0.47	2.89±1.53#	1.91±0.86
PAC000186_g	4.49±2.60	2.08±1.52	0.03±0.02*
PAC000664_g	6.88±7.43	1.25±0.76	1.65±1.46
PAC001063_g	0.07±0.07	0.11±0.10	23.94±4.47*
PAC001066_g	0.09±0.05	0.10±0.06	3.07±1.65*
PAC001068_g	7.49±7.05	0.77±0.37#	3.53±0.87*
PAC001228_g	1.76±1.86	4.26±4.46	1.11±0.90
Prevotella	1.96±1.04	3.07±1.67	3.36±1.17
*Prevotellaceae*_uc	7.40±6.88	2.03±1.17	0.12±0.09*
Pseudoflavonifractor	2.96±0.84	2.38±1.06	1.54±0.41

[1] Values indicate means ± standard deviation. #$P<0.05$ vs NC group and *$P<0.05$ vs EC group.

Table S4. Effect of NK112 on the gut microbiota composition at the species level in EC-treated mice.[1]

Taxon name	Relative contribution (%)		
	NC	EC	ENK
AB599946_s	10.11±10.75	10.09±3.88	0.01±0.01
AB626924_s	0.03±0.04	2.49±3.86	0.12±0.08
Bacteroides acidifaciens group	1.39±0.85	2.95±1.39	0.95±0.43
*Bacteroides*_uc	1.44±1.35	1.29±0.75	33.36±6.82
EU622763_s group	0.87±0.90	1.77±1.12	0.00±0.00
Helicobacter japonicus	2.75±1.20	3.48±3.32	0.01±0.01
Helicobacter rodentium group	0.85±1.33	4.47±3.17	0.00±0.01
KE159538_g_uc	3.30±6.78	0.17±0.13	0.47±0.32
PAC001063_s group	0.06±0.06	0.10±0.08	13.71±2.59
PAC001065_s group	2.20±1.66	0.61±0.57	0.01±0.01
PAC001066_s	0.08±0.04	0.08±0.05	1.77±0.92
PAC001070_s group	3.64±4.11	0.13±0.10	0.00±0.00
PAC001072_s	1.44±1.32	0.12±0.09	2.00±0.41
PAC001089_s	2.49±3.26	0.01±0.01	0.21±0.29
PAC002027_s	1.49±1.94	0.37±0.53	0.05±0.05
PAC002444_s	1.19±1.06	2.65±3.05	0.00±0.01
PAC002445_s	2.70±2.73	4.06±2.60	0.00±0.01
PAC002481_s	0.55±0.36	0.05±0.11	2.57±0.78
PAC002482_s group	0.95±1.23	1.77±1.21	0.00±0.00
PAC002483_s	0.89±0.96	1.66±1.22	0.00±0.00

[1] Values indicate means ± standard deviation. #$P<0.05$ vs NC group and *$P<0.05$ vs EC group.

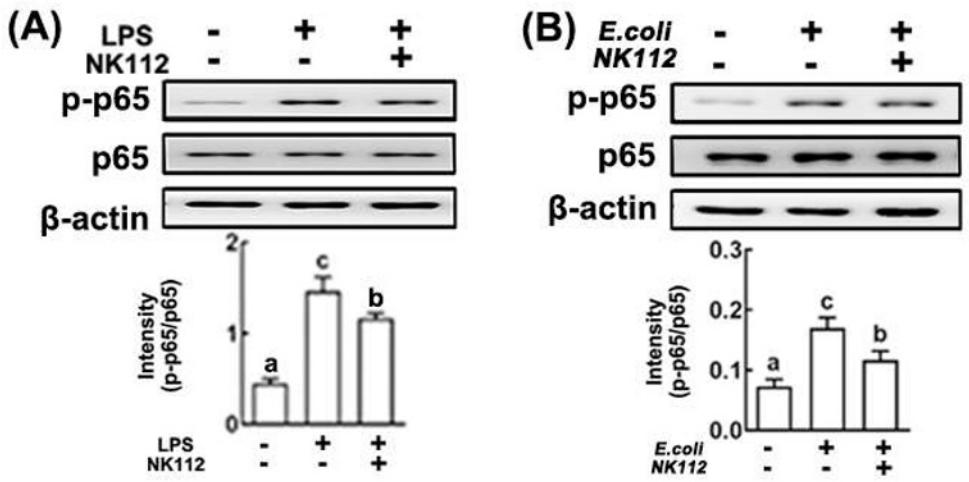

Figure S1. Effects of NK112 on the NF-κB activation in LPS (A) or *Escherichia coli*-stimulated (B) macrophages. Peritoneal macrophage cells (1×10^6/ml) were incubated with NK112 (1×10^5 cfu/ml) in the absence or presence of LPS (0.1 mg/ml) or *E. coli* (1×10^5 cfu/ml). p-p65 and p65 (NF-κB activation) were assayed by immunoblotting. Data values were indicated as mean ± standard deviation (n=4). Means with same letters are not significantly different (P<0.05).

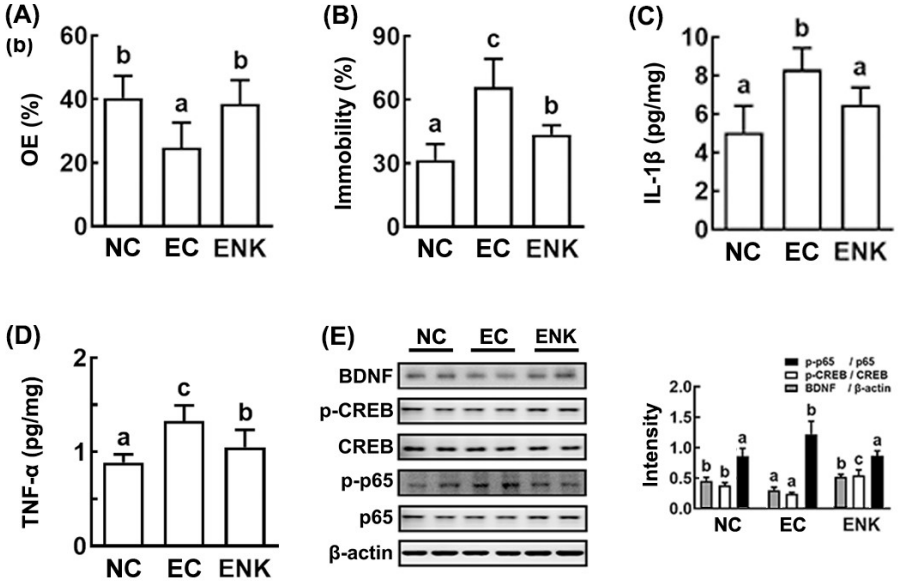

Figure S2. NK112 alleviated *Escherichia coli*-induced anxiety, depression, and cognitive impairment in mice. (A) Effect on the anxiety-like behavior in the EPM task (OE). (B) Effect on the depression-like behaviors in the FST. Effect on the expression of IL-1β (C), and TNF-α levels (D) in the hippocampus. (E) Effect on BDNF expression, CREB phosphorylation, and NF-κB activation in the hippocampus. Mice of EC and ENK groups were exposed to *E. coli* (1×10^9 cfu/mouse/day) daily for 5 days and thereafter treated with NK112 (for ENK, 1×10^9 cfu/mouse/day) or vehicle (for EC) for 5 days. Control group (NC) was treated with vehicle (saline). Data values were indicated as mean ± standard deviation (n=6). Means with same letters are not significantly different ($P<0.05$).

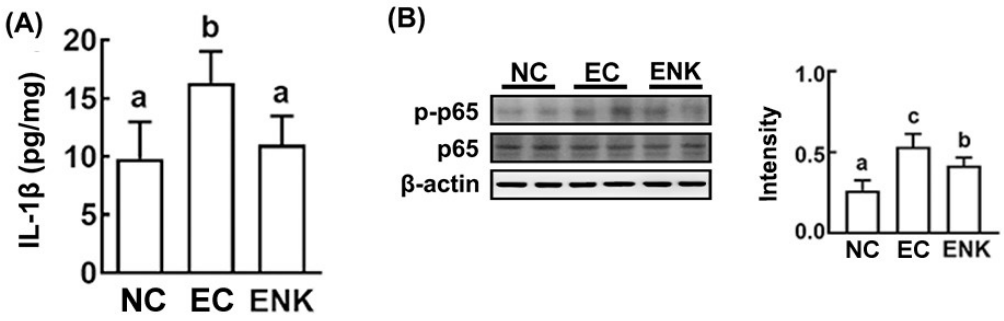

Figure S3. NK112 alleviated *Escherichia coli*-induced colitis in mice. Effects on the IL-1β expression (A) and NF-κB activation (B) in the colon. Mice of EC and ENK groups were exposed to *E. coli* (1×10^9 cfu/mouse/day) daily for 5 days and thereafter treated with NK112 (for ENK, 1×10^9 cfu/mouse/day) or vehicle (for EC) for 5 days. Control group (NC) was treated with vehicle (saline). Data values were indicated as mean ± standard deviation (n=6). Means with same letters are not significantly different ($P<0.05$).

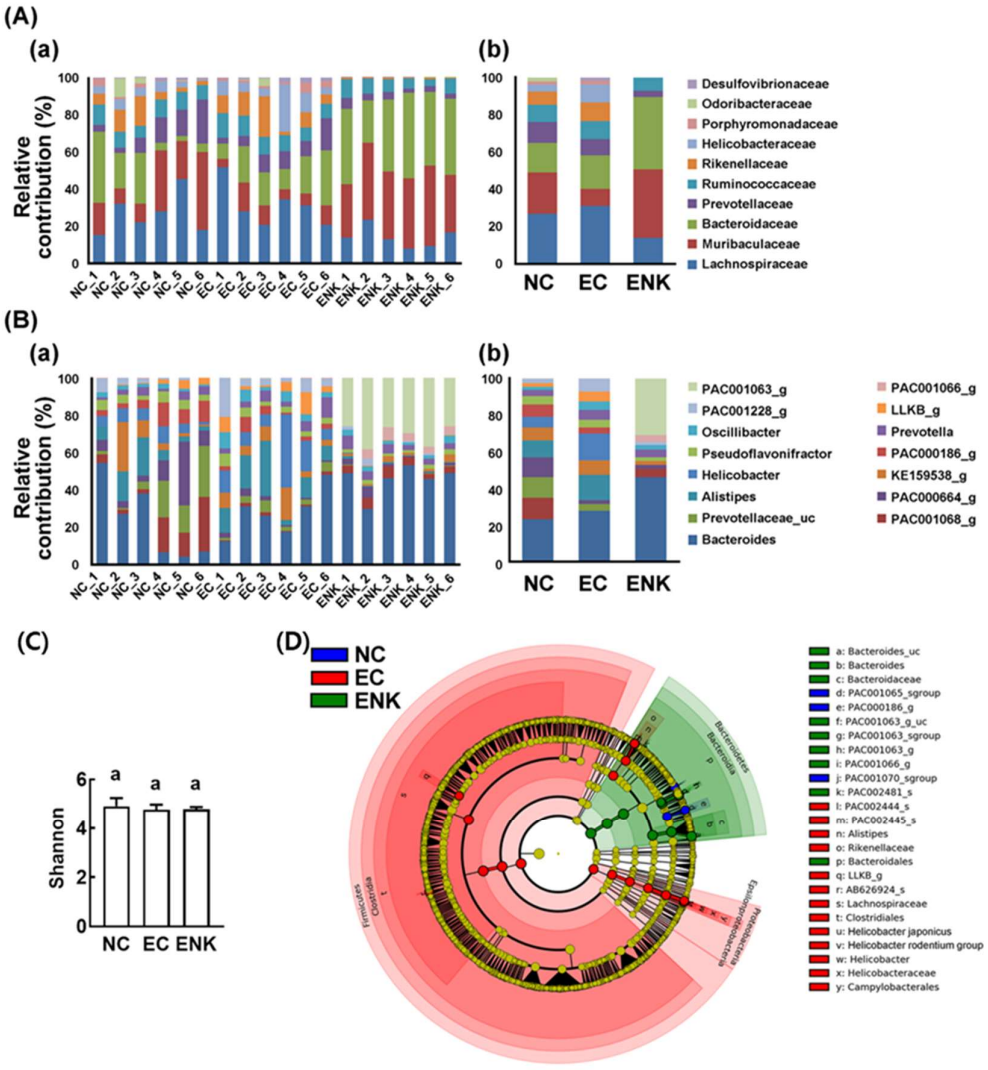

Figure S4. Effect on the composition of gut microbiota. The gut composition at a family level (A: a, the gut composition of individual mice; and b, mean of each group) and at a genus level (B: a, the gut composition of individual mice; and b, mean of each group) was analyzed using Illumina iSeq 100. (C) Effects on α-diversity (Shannon index) and Cladogram (D) generated by LEfSE indicating significant differences in gut microbial abundances among NC (blue), EC (red), and ENK (green) groups. Mice of EC and ENK groups were exposed to *Escherichia coli* (1×10^9 cfu/mouse/day) daily for 5 days and thereafter treated with NK112 (for ENK, 1×10^9 cfu/mouse/day) or vehicle (for EC) for 5 days. Control group (NC) was treated with vehicle (saline) instead of *Escherichia coli* and NK112.

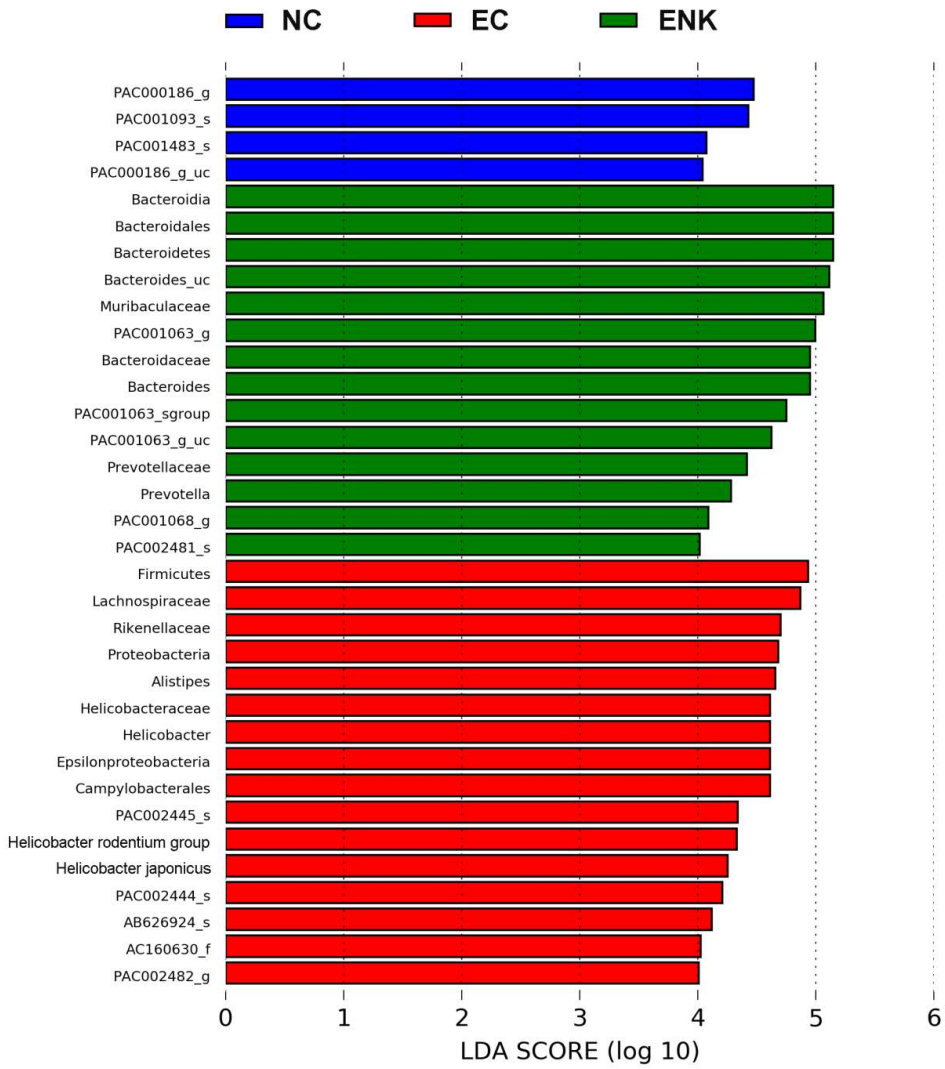

Figure S5. The difference of gut bacteria between NC, EC and ENK group using the linear discriminant analysis (LDA) effect size (LEfSe) analysis. Relative abundance is significant *P*<0.05 and logarithmic LDA score more than 4. Mice of EC and ENK groups were exposed to *Escherichia coli* (1×10^9 cfu/mouse/day) daily for 5 days and thereafter treated with NK112 (for ENK, 1×10^9 cfu/mouse/day) or vehicle (for EC) for 5 days. Control group (NC) was treated with vehicle (saline) instead of *E. coli* and NK112. The described strains (in species) were analyzed to the Linear Discriminant Analysis (LDA) along with effect size measurement (LEfSE) in Galaxy (http://huttenhower.sph.harvard.edu/galaxy/). It was used to discriminate significant differentially strains at each taxon level. The threshold logarithmic score set at 4.0 and ranked. Bacterial strains were described based on 16SrRNA sequencing data (n=6).

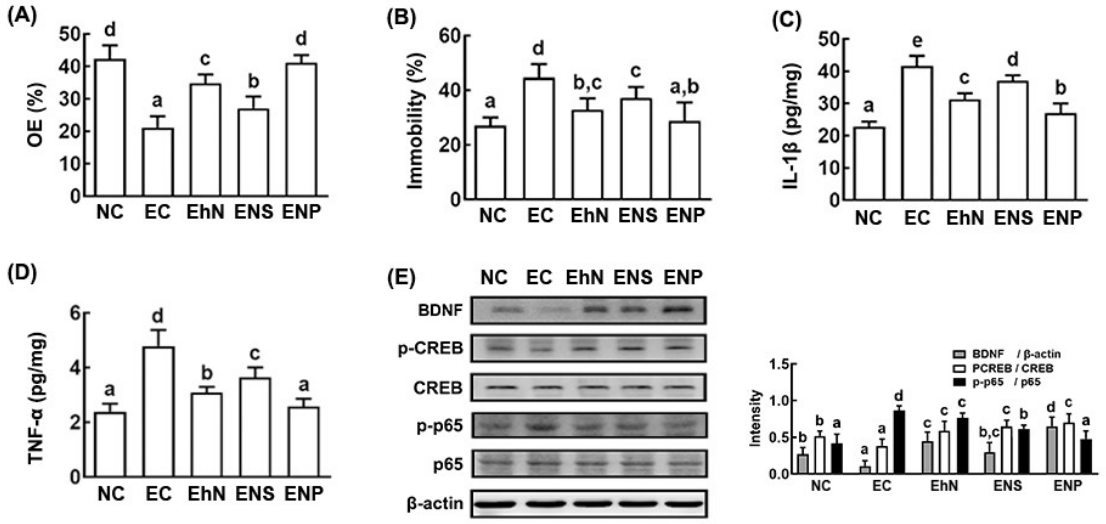

Figure S6. Heat-killed (hNK112) and its lysated supernatant (NS) and precipitate (NP) fractions alleviated *Escherichia coli*-induced depression and cognitive impairment in mice. (A) Effects on the anxiety-like behavior in the EPM task (OE). (B) Effect on the depression-like behaviors in the FST. Effect on the IL-1β (C) and TNF-α levels (D) in the hippocampus. (E) Effect on BDNF expression, CREB phosphorylation, and NF-κB activation in the hippocampus. Mice of EC, EhN, ENS, and ENP groups were exposed to *E. coli* (1×10^9 cfu/mouse/day) daily for 5 days and thereafter treated with test agents (EC, vehicle; EhN, 1×10^9 cfu/mouse/day of hNK112; ENS, supernatant fraction of NK112 (1×10^9 cfu) lysate/mouse/day; ENP, precipitate fraction of NK112 (1×10^9 cfu) lysate/mouse/day daily for 5 days. Normal control group (NC) was treated with saline. Data values were indicated as mean ± standard deviation (n=6). Means with same letters are not significantly different (P<0.05).

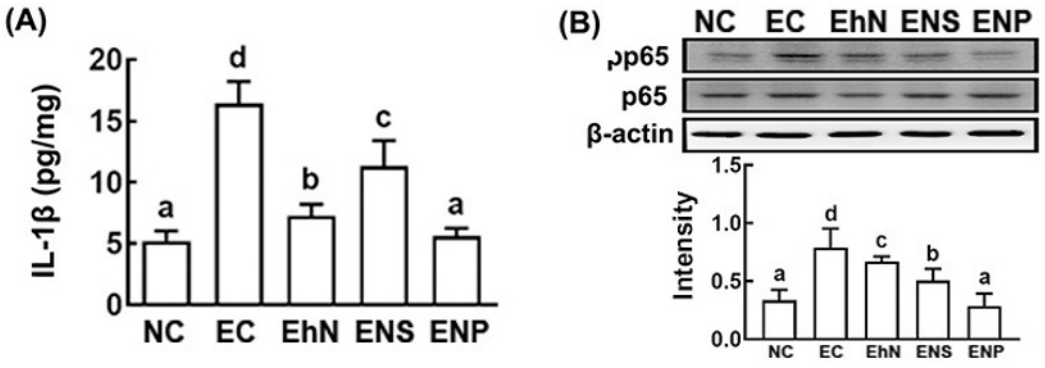

Figure S7. Heat-killed (hNK112) and its lysated supernatant (NS) and precipitate (NP) fraction alleviated *Escherichia coli*-induced colitis in mice. Effects on the IL-1β expression (A) and NF-κB activation (B) in the colon. Mice of EC, EhN, ENS, and ENP groups were exposed to *E. coli* (1×10^9 cfu/mouse/day) daily for 5 days and thereafter treated with test agents (EC, vehicle; EhN, 1×10^9 cfu/mouse/day of hNK112; ENS, supernatant fraction of NK112 (1×10^9 cfu) lysate/mouse/day; ENP, precipitate fraction of NK109 (1×10^9 cfu) lysate/mouse/day daily for 5 days. Normal control group (NC) was treated with saline. Data values were indicated as mean ± standard deviation (n=6). Means with same letters are not significantly different (P<0.05).