[Supplement]

# *Lactobacillus plantarum* and *Bifidobacterium bifidum* alleviate dry eye in mice with exorbital lacrimal gland excision by modulating gut inflammation and microbiota

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Table S1. Ingredients of mouse chow diet used in the present study

| Ingredient | Weight (g) |
|---|---|
| Casein, 80 mesh | 200 |
| L-cysteine | 3 |
| Corn starch | 315 |
| Maltodextrin 10 | 35 |
| Sucrose | 350 |
| Celluose, BW200 | 50 |
| Soybean oil | 25 |
| Lard | 20 |
| Mineral mix S10026 | 10 |
| Dicalcium phosphate | 13 |
| Calcium carbonate | 5.5 |
| Potassium citrate·H2O | 16.5 |
| Vitamin mix V10001 | 10 |
| Choline bitartrate | 2 |
| FD&C yellow dye #5 | 0.05 |
| Total | 1,055.05 |
| Protein (Kcal%) | 19.2 |
| Carbohydrate (Kcal%) | 67.3 |
| Fat (Kcal%) | 4.3 |

Table S2. Food intake and weight gain of mice

| | Group in C57BL/6 mice | | | | | | Group in BALB/c Mice | | |
|---|---|---|---|---|---|---|---|---|---|
| | CON | SHA | DE | LP | BB | LB (3:2) | CON | DE | LB (4:1) |
| Food intake (g/day) | 4.3 ±0.7 | 3.5 ±0.9 | 3.6± 1.0 | 3.7± 0.7 | 3.8 ±0.6 | 3.7± 0.7 | 3.4 ±0.4 | 3.1 ±0.7 | 3.2 ±0.8 |
| Weight gain (g) | 2.1 ±0.4 | 2.2 ±0.6 | 2.0 ±0.2 | 1.8 ±0.5 | 1.9 ±0.3 | 1.8 ±0.5 | 2.4 ±0.1 | 2.6 ±0.6 | 2.0 ±0.3 |

Table S3. Effects of NK151, NK175, and NKM on the gut microbiota composition at the phylum level in mice with ELA-induced dry eye

| Taxon | Mean ± SD | | | | | |
|---|---|---|---|---|---|---|
| | CON[a] | SHA | DE | LP | BB | LB(3:2) |
| Bacteroidetes | 55.2±12.4[b] | 48.9±6.0 | 49.1±9.5 | 57.2±11.5 | 53.9±9.6 | 58.5±7.2 |
| Firmicutes | 39.9±13.9 | 43.8±5.6 | 37.1±11.3 | 34.5±12.8 | 37.0±10.9 | 32.2±7.3 |
| Proteobacteria | 2.9±1.1 | 3.5±2.0 | 5.0±2.6 | 4.3±1.5 | 6.0±2.5 | 5.0±2.1 |
| Verrucomicrobia | 0.9±1.7 | 1.4±2.9 | 5.6±7.7 | 1.4±1.5 | 0.6±1.0 | 2.1±2.8 |
| Cyanobacteria | 0.8±0.8 | 1.7±2.2 | 2.1±2.6 | 0.7±0.5 | 1.5±1.4 | 1.3±1.8 |
| Actinobacteria | 0.1±0.0 | 0.1±0.1 | 0.5±0.4[#] | 0.2±0.5 | 0.2±0.2 | 0.4±0.7 |
| Tenericutes | 0.1±0.2 | 0.2±0.2 | 0.6±0.8 | 1.5±2.3 | 0.6±0.5 | 0.4±0.4 |
| Deferribacteres | ND[c] | 0.3±0.6 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.0±0.0 |

[a)]NK151 (LP), NK175 (BB), and their (3:2) mixture (LB, $5\times10^9$ CFU/mouse/day) orally gavaged daily for 10 days from 25 h after the exposure to ELA (exorbital lacrimal gland excision and 1% atropine/0.1% benzalkonium chloride solution treatment). Normal control mice (CON) and mice exposed with the ELA (DE) were treated with vehicle instead of probiotics.

[b)]Data values were described as mean ± SD (n = 7).

[c)]not detected

[#]$p<0.05$ vs. SHA group. *$p<0.05$ vs. DE group.

Table S4. Effects of NK151, NK175, and NKM on the gut microbiota composition at the family level in mice with ELA-induced dry eye

| Taxon | Mean ± SD | | | | | |
|---|---|---|---|---|---|---|
| | CON[a] | SHA | DE | LP | BB | LB(3:2) |
| Prevotellaceae | 32.1±10.5[b] | 26.0±9.7 | 22.1±8.3 | 26.7±10.7 | 21.8±4.4 | 18.8±6.0 |
| Lactobacillaceae | 18.1±15.4 | 18.1±9.4 | 6.7±3.9[#] | 9.5±8.2 | 3.8±2.5 | 9.1±9.2 |
| Ruminococcaceae | 12.5±5.5 | 7.9±6.3 | 11.2±3.5 | 8.2±3.4 | 8.6±3.1 | 7.4±3.0 |
| Muribaculaceae | 11.5±3.3 | 12.3±4.3 | 11.6±3.8 | 15.9±3.9 | 19.1±4.0* | 22.4±6.5* |
| Lachnospiraceae | 8.1±3.4 | 16.8±11.9 | 17.9±11.8 | 15.6±6.1 | 23.5±13.1 | 13.1±6.4 |
| Bacteroidaceae | 7.5±4.3 | 6.5±3.8 | 11.2±5.1 | 9.6±6.0 | 7.5±3.1 | 12.1±5.9 |
| Rikenellaceae | 2.2±1.0 | 1.6±0.7 | 2.2±0.9 | 3.1±1.0 | 3.2±0.7* | 3.2±2.0 |
| Sutterellaceae | 1.1±1.1 | 0.6±0.4 | 1.0±0.7 | 0.5±0.3 | 0.5±0.1 | 1.1±0.6 |
| Helicobacteraceae | 1.0±0.7 | 1.4±1.2 | 1.5±1.4 | 1.6±1.2 | 2.4±0.8 | 1.2±1.2 |
| Porphyromonadaceae | 0.9±0.7 | 1.0±0.5 | 0.8±0.5 | 1.2±1.0 | 1.2±0.5 | 1.0±0.7 |
| Akkermansiaceae | 0.9±1.7 | 1.4±2.9 | 5.6±7.7 | 1.4±1.5 | 0.6±1.0 | 2.1±2.8 |
| FR888536_f | 0.8±0.8 | 1.7±2.2 | 2.1±2.6 | 0.7±0.5 | 1.5±1.4 | 1.3±1.8 |
| AC160630_f | 0.7±0.5 | 0.3±0.3 | 1.1±0.8[#] | 0.6±0.4 | 1.0±0.7 | 0.8±1.0 |
| Desulfovibrionaceae | 0.6±0.3 | 1.4±1.0 | 2.3±2.1 | 1.8±0.9 | 2.2±1.6 | 2.0±1.5 |
| Erysipelotrichaceae | 0.5±0.6 | 0.6±0.7 | 0.7±0.7 | 0.6±1.0 | 0.6±0.6 | 1.9±1.9 |
| Odoribacteraceae | 0.2±0.6 | 1.1±2.4 | 0.0±0.1 | 0.1±0.1 | 0.1±0.1 | 0.2±0.3 |
| Clostridiaceae | 0.2±0.2 | 0.0±0.1 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.2±0.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Christensenellaceae | 0.2±0.2 | 0.2±0.1 | 0.2±0.2 | 0.2±0.2 | 0.2±0.2 | 0.3±0.3 |
| Rhodospirillaceae | 0.2±0.3 | 0.1±0.1 | 0.2±0.2 | 0.3±0.4 | 1.0±1.2 | 0.6±0.6 |
| Enterococcaceae | 0.1±0.1 | ND[c] | 0.0±0.1 | ND | ND | 0.1±0.1 |
| Dehalobacterium_f | 0.1±0.1 | 0.2±0.1 | 0.3±0.2 | 0.2±0.1 | 0.2±0.1 | 0.2±0.2 |
| Coriobacteriaceae | 0.1±0.1 | ND | ND | ND | ND | 0.1±0.1* |
| Bacteroidales_uc | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.1±0.0 | ND* |
| PAC000197_f | ND | ND | 0.1±0.0 | ND | 0.1±0.1 | ND |
| Deferribacteraceae | 0.0±0.0 | 0.3±0.6 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | ND |

[a)]NK151 (LP), NK175 (BB), and their (3:2) mixture (LB, $5\times10^9$ CFU/mouse/day) orally gavaged daily for 10 days from 25 h after the exposure to ELA (exorbital lacrimal gland excision and 1% atropine/0.1% benzalkonium chloride solution treatment). Normal control mice (CON) and mice exposed with the ELA (DE) were treated with vehicle instead of probiotics.

[b)]Data values were described as mean ± SD (n = 7).

[c)]not detected

[#]$p<0.05$ vs. SHA group. *$p<0.05$ vs. DE group.

Table S5. Effects of NK151, NK175, and NKM on the gut microbiota composition at the genus level in mice with ELA-induced dry eye

| Taxon | Mean ± SD | | | | | |
|---|---|---|---|---|---|---|
| | CON[a] | SHA | DE | LP | BB | LB(3:2) |
| Lactobacillus | 17.8±14.0[b] | 17.9±8.6 | 6.7±3.6# | 9.4±7.5 | 3.8±2.3 | 9.0±8.4 |
| Prevotellaceae_uc | 12.2±4.7 | 13.5±4.7 | 8.6±3.0# | 10.6±6.0 | 12.0±4.3 | 4.6±4.7 |
| Prevotella | 11.6±5.8 | 7.7±4.8 | 7.7±4.1 | 8.9±5.4 | 7.1±4.4 | 8.0±2.9 |
| Paraprevotella | 7.8±7.7 | 4.5±6.6 | 5.8±4.3 | 6.9±6.4 | 2.7±1.9 | 2.3±2.7 |
| Bacteroides | 7.5±4.0 | 6.5±3.5 | 11.2±4.8 | 9.6±5.6 | 7.5±2.9 | 12.1±5.4 |
| Ruminococcus | 6.4±3.3 | 3.1±3.8 | 3.6±1.8 | 2.0±1.9 | 2.1±2.4 | 1.0±1.0* |
| PAC001068_g | 3.9±0.8 | 5.1±1.8 | 2.9±1.1# | 4.1±1.8 | 4.5±1.4 | 7.8±1.9* |
| Muribaculaceae_uc | 2.6±0.7 | 3.0±1.0 | 3.9±1.6 | 4.5±0.7 | 5.6±0.9* | 6.3±2.2 |
| PAC000661_g | 2.0±1.1 | 1.3±1.5 | 1.8±0.9 | 1.3±1.1 | 1.3±1.5 | 0.6±0.7 |
| Alistipes | 1.9±0.7 | 1.5±0.6 | 1.8±0.9 | 2.6±1.0 | 2.8±0.8 | 3.1±1.9 |
| PAC000186_g | 1.9±0.7 | 2.1±0.9 | 1.6±0.7 | 2.9±0.9* | 3.7±1.4* | 3.4±2.0 |
| PAC000664_g | 1.7±2.3 | 2.7±2.3 | 5.8±3.7 | 1.8±1.2* | 2.8±2.2 | 1.1±1.0* |
| Eubacterium_g23 | 1.6±1.2 | 0.8±0.8 | 1.6±1.2 | 0.4±0.3* | 0.6±0.5 | 1.9±1.5 |
| Parasutterella | 1.0±0.9 | 0.6±0.3 | 0.8±0.6 | 0.5±0.3 | 0.4±0.1 | 0.9±0.6 |
| Helicobacter | 1.0±0.7 | 1.4±1.1 | 1.5±1.3 | 1.6±1.1 | 2.3±0.7 | 1.2±1.1 |
| Pseudoflavonifractor | 0.9±0.3 | 1.2±0.6 | 1.9±0.6 | 1.8±0.5 | 1.8±0.8 | 1.6±0.9 |
| Parabacteroides | 0.9±0.6 | 1.0±0.5 | 0.8±0.4 | 1.2±0.9 | 1.2±0.5 | 1.0±0.7 |
| Akkermansia | 0.9±1.5 | 1.4±2.7 | 5.5±7.0 | 1.4±1.4 | 0.6±0.9 | 2.1±2.6 |
| FR888536_g | 0.8±0.8 | 1.7±2.0 | 2.1±2.4 | 0.7±0.5 | 1.5±1.3 | 1.2±1.6 |
| KE159538_g | 0.8±1.3 | 6.7±8.5 | 0.4±0.4 | 4.9±6.0 | 7.9±11.0 | 3.4±3.4 |
| PAC002482_g | 0.7±0.5 | 0.3±0.3 | 1.1±0.7# | 0.6±0.4 | 1.0±0.7 | 0.8±0.9 |

| | | | | | | |
|---|---|---|---|---|---|---|
| PAC001092_g | 0.6±0.5 | 0.7±0.6 | 1.0±1.1 | 0.5±0.3 | 0.6±0.3 | 0.6±0.7 |
| LT706945_g | 0.5±0.3 | 1.0±0.7 | 1.8±1.5 | 1.4±1.0 | 1.6±1.1 | 0.7±0.6 |
| Alloprevotella | 0.5±0.6 | 0.2±0.5 | 0.0±0.0 | 0.2±0.3 | ND[c] | 3.9±6.4 |
| PAC000198_g | 0.5±0.2 | 0.8±0.7 | 0.4±0.2 | 0.8±0.2* | 1.2±0.6* | 1.4±0.8* |

[a)]NK151 (LP), NK175 (BB), and their (3:2) mixture (LB, $5\times10^9$ CFU/mouse/day) orally gavaged daily for 10 days from 25 h after the exposure to ELA (exorbital lacrimal gland excision and 1% atropine/0.1% benzalkonium chloride solution treatment). Normal control mice (CON) and mice exposed with the ELA (DE) were treated with vehicle instead of probiotics.

[b)]Data values were described as mean ± SD (n = 7).

[c)]not detected

[#]$p<0.05$ vs. SHA group. *$p<0.05$ vs. DE group.

Table S6. Effects of NK151, NK175, and NKM on the gut microbiota composition at the species level in mice with ELA-induced dry eye

| Taxon | Mean ± SD | | | | | |
|---|---|---|---|---|---|---|
| | CON[a] | SHA | DE | LP | BB | LB(3:2) |
| Lactobacillus murinus group | 13.0±11.1[b] | 7.7±5.3 | 2.4±1.9# | 6.4±6.1 | 3.0±2.2 | 6.3±6.3 |
| EU622763_s group | 9.6±5.7 | 4.7±5.6 | 5.9±3.5 | 7.3±4.6 | 4.2±2.9 | 4.9±2.0 |
| Paraprevotella_uc | 4.4±4.9 | 2.5±4.3 | 3.2±2.6 | 3.8±4.1 | 1.4±1.2 | 1.2±1.6 |
| Lactobacillus_uc | 3.6±4.2 | 3.2±1.7 | 0.5±0.3# | 1.2±1.4 | 0.4±0.3 | 1.5±1.9 |
| Bacteroides acidifaciens group | 3.5±2.0 | 2.4±1.1 | 5.5±3.1 | 1.5±0.5* | 2.6±1.7 | 2.9±1.7 |
| FJ880724_s | 3.4±3.4 | 1.9±2.9 | 2.6±2.1 | 3.1±2.8 | 1.3±0.9 | 1.1±1.3 |
| EF602869_s | 3.3±2.4 | 1.0±2.3 | 2.8±1.5 | 1.1±1.4* | ND[c,]* | 0.1±0.2* |
| PAC001068_g_uc | 2.1±0.5 | 3.0±1.3 | 1.6±0.8# | 2.3±1.3 | 2.6±0.9* | 4.8±1.3* |
| AB606236_s | 1.6±2.2 | 1.2±2.4 | 0.0±0.0 | 0.1±0.2 | 1.1±2.2 | 0.4±1.0 |
| Ruminococcus_uc | 1.4±0.9 | 0.7±1.1 | 0.6±0.5 | 0.5±0.5 | 0.4±0.5 | 0.1±0.1* |
| PAC001982_s | 1.4±1.2 | 0.6±0.8 | 1.4±1.2 | 0.2±0.3* | 0.2±0.3* | 1.2±1.5 |
| AB599946_s | 1.3±1.7 | 2.0±2.0 | 2.3±1.7 | 4.9±3.6 | 2.5±1.5 | 3.7±2.9 |
| PAC000186_g_uc | 1.2±0.5 | 1.3±0.7 | 1.0±0.6 | 1.9±0.8* | 2.7±1.1* | 2.3±1.6 |
| Bacteroides_uc | 1.2±0.8 | 0.8±0.7 | 1.8±1.3 | 1.2±1.3 | 0.9±0.3 | 1.0±0.8 |
| PAC002445_s | 1.1±0.6 | 0.7±0.6 | 0.8±0.6 | 0.7±0.3 | 1.0±0.5 | 0.8±0.9 |
| PAC001081_s group | 1.1±0.8 | 0.7±0.9 | 1.0±0.8 | 0.5±0.5 | 0.4±0.5 | 0.1±0.2* |
| Prevotella_uc | 1.1±0.8 | 0.7±0.7 | 0.4±0.3 | 0.7±0.6 | 0.4±0.4 | 0.5±0.2 |
| PAC002443_s | 0.9±0.7 | 1.1±1.1 | 0.5±0.4 | 1.3±2.0 | 1.1±0.7 | 4.0±4.2* |
| AB606242_s | 0.9±1.7 | 1.2±2.0 | 3.2±3.1 | 0.3±0.6* | 0.1±0.1* | 0.0±0.0* |
| PAC002476_s | 0.8±0.7 | 0.5±0.3 | 0.6±0.6 | 0.4±0.3 | 0.3±0.1 | 0.8±0.5 |
| Akkermansia muciniphila | 0.8±1.3 | 1.2±2.3 | 4.2±5.7 | 1.2±1.2 | 0.5±0.8 | 1.7±2.2 |
| PAC001070_s group | 0.7±0.3 | 1.2±0.4† | 0.4±0.3# | 0.6±0.4 | 0.4±0.3 | 1.8±0.5* |
| Lactobacillus reuteri group | 0.6±0.9 | 2.6±3.9 | 0.9±0.9 | 0.4±0.4 | 0.2±0.1 | 0.7±1.0 |
| Lactobacillus gasseri group | 0.6±0.5 | 4.0±5.9 | 2.5±4.0 | 1.4±1.1 | 0.3±0.2 | 0.5±0.4 |

| | | | | | | |
|---|---|---|---|---|---|---|
| PAC002482_s group | 0.6±0.4 | 0.2±0.2 | 1.0±0.8[#] | 0.5±0.4 | 0.6±0.6 | 0.7±0.9 |

[a)]NK151 (LP), NK175 (BB), and their (3:2) mixture (LB, $5\times10^9$ CFU/mouse/day) orally gavaged daily for 10 days from 25 h after the exposure to ELA (exorbital lacrimal gland excision and 1% atropine/0.1% benzalkonium chloride solution treatment). Normal control mice (CON) and mice exposed with the ELA (DE) were treated with vehicle instead of probiotics.

[b)]Data values were described as mean ± SD (n = 7).

[c)]not detected

[#]p<0.05 vs. SHA group. *p<0.05 vs. DE group.

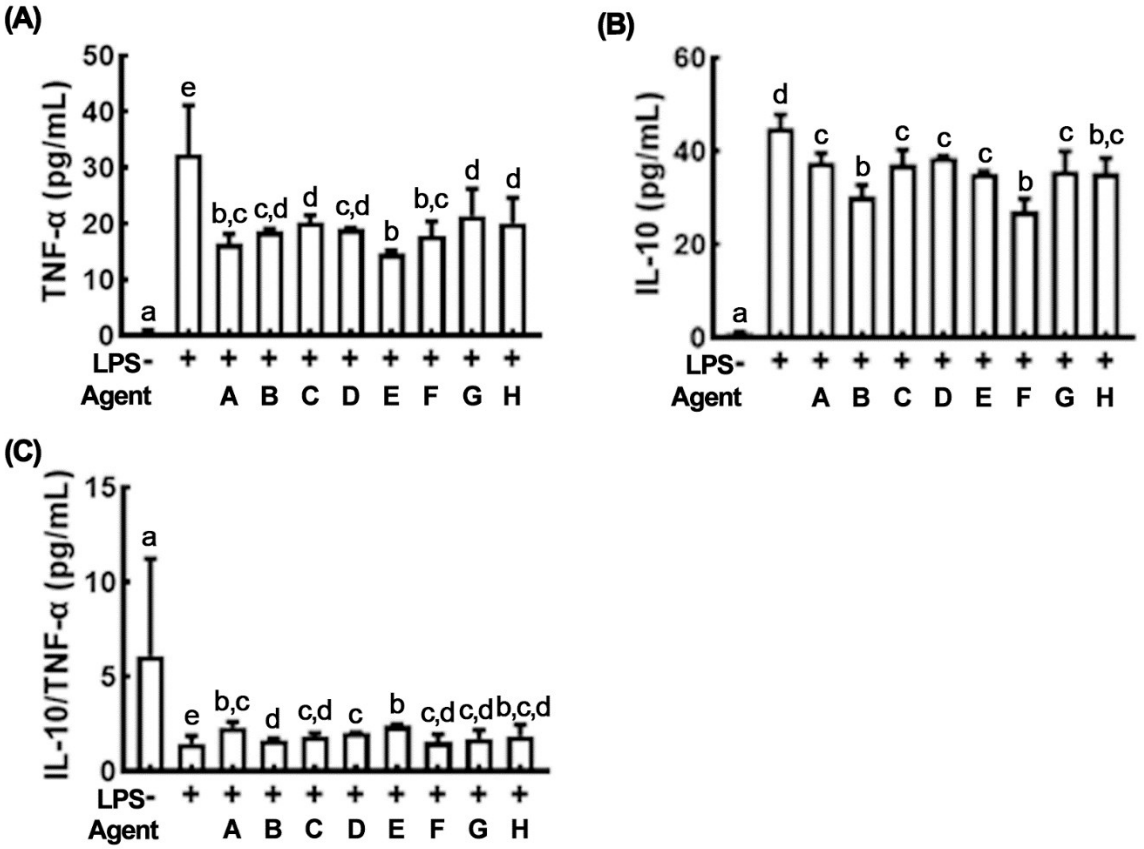


Figure S1. Effects of five gut bacteria isolated from the human fecal bacteria collection on the LPS-induced TNF-α and IL-10 expression in macrophage cells. Effects on TNF-α (A) and IL-10 expression (B) and IL-10 to TNF-α expression ratio (C). Macrophage cells ($1 \times 10^6$/mL) isolated from peritoneal cavity were incubated with gut bacteria ($1 \times 10^5$ CFU/mL) in the absence or presence of LPS. Control group (CON) was treated with saline instead of LPS. A-D (A, NK151) were Lactobacilli and E-H (E, NK175) were Bifdiobacteria. Data values were described as mean ± SD (n = 4). Means with same letters are not significantly different ($p < 0.05$).

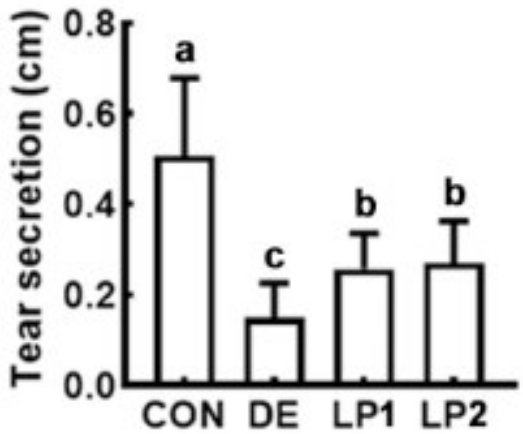


Figure S2. Effect of NK151 on the ELA-induced dry eye in C57BL/6 mice. NK151 (LP1, $5\times10^8$ CFU/mouse/day; LP2, $1\times10^9$ CFU/mouse/day) orally gavaged daily for 10 days from 25 h after the exposure to ELA (exorbital lacrimal gland excision and 1% atropine/0.1% benzalkonium chloride solution treatment). Normal control mice (CON), and mice exposed with the ELA (DE) were treated with vehicle instead of probiotics. Data values were described as mean ± SD (n = 7). Means with same letters are not significantly different ($p < 0.05$).

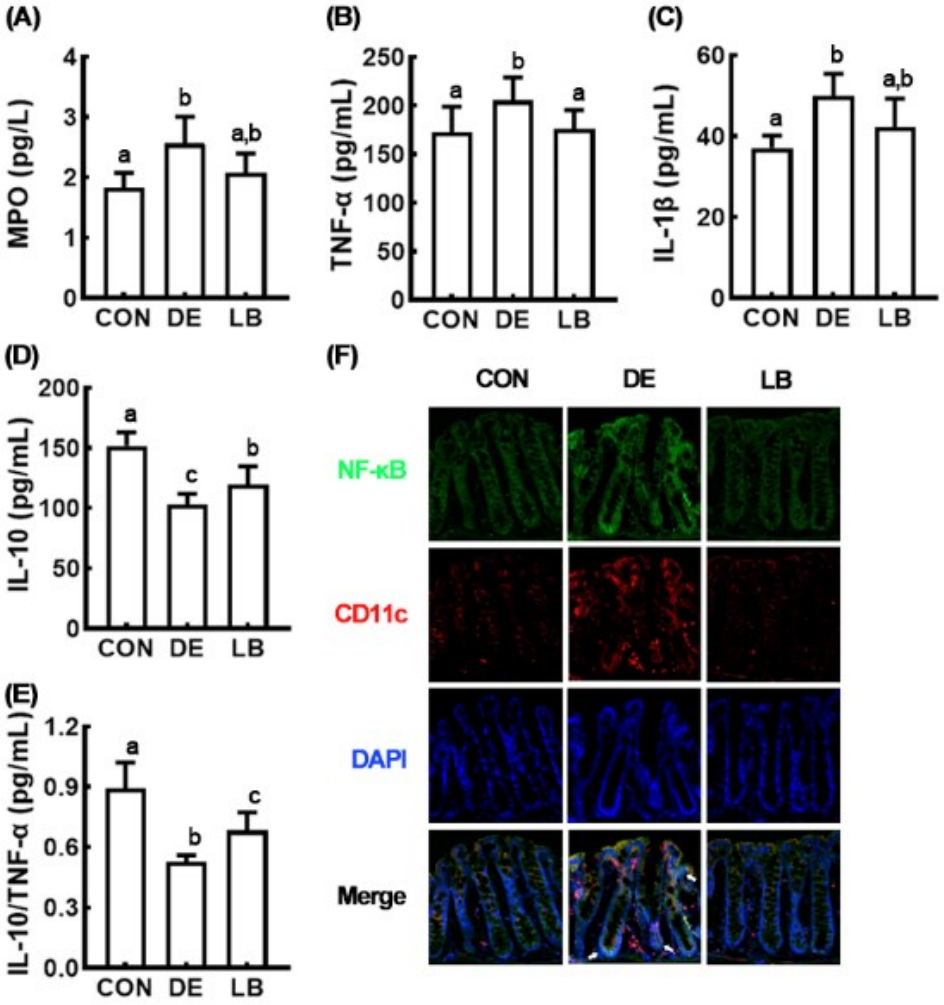


Figure S3. The mixture of NK151 and NK175 (4:1), NKm, alleviated ELA-induced colitis in BALB/c mice. Effects on the myeloperoxidase (MPO) activity (A), TNF-α (B), IL-1β (C), and IL-10 expression (D), and IL-10 to TNF-α expression ratio (E), and NF-κB$^+$/CD11c$^+$ cell population (F) in the colon. NKm (the NK151 and NK175 [4:1] mixture, $5\times10^9$ CFU/mouse/day) orally gavaged daily for 10 days from 25 h after the exposure to ELA (exorbital lacrimal gland excision and 1% atropine/0.1% benzalkonium chloride solution treatment). Normal control mice (CON) and mice exposed with the ELA (DE) were treated with vehicle instead of probiotics. Data values were described as mean ± SD (n = 7). Means with same letters are not significantly different ($p < 0.05$).