Immunostimulatory effects of *Bacillus coagulans* SANK70258

Running title: *B. coagulans* SANK70258 as immunostimulant

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Abstract

Specific intestinal bacteria modulate immunoresponses through various pathways, and several probiotic bacteria have been identified as immunostimulants by screening. In the present study, we evaluated the immunomodulating effects of *Bacillus coagulans* SANK70258 (*B. coagulans* SANK70258), a spore-forming and lactic acid-producing bacterium usable as food supplement for human and animals. We found that treatment of mouse splenocytes with γ-ray irradiated *B. coagulans* SANK70258 induced high amount of IFN-γ in comparison with 7 kinds of typical lactic acid bacteria. Further analyses using splenocytes revealed that NK cell is a major source of IFN-γ, and *B. coagulans* SANK70258-induced IFN-γ production was inhibited by neutralization of IL-12 or IL-23, depletion of $CD11c^+$ cells, and inhibition of NFκB. *B. coagulans* SANK70258 also induced release of IFN-γ from activated $CD8^+$ T cells, and increased expression of chemokine receptors in $CD8^+$ T cells. *B. coagulans* SANK70258-treatment induced production of cytokines from bone marrow-derived dendritic cells, which is reduced by knockdown of *Tlr2* and *Nod2*. *B. coagulans* SANK70258-treatment also induced IgA production from Peyer's patch cells with high level among tested lactic bacteria. The oral intake of γ-ray irradiated *B. coagulans* SANK70258 significantly increased intestinal IgA levels and IgA-expressing B cells in the Peyer's patch of mice. Taken together, we conclude that *B. coagulans* SANK70258 possesses high activity as immunostimulant inducing production of IFN-γ and IgA.

Introduction

The intestinal immune homeostasis is in a balance of fight against infectious pathogens and tolerance toward commensal microbiota and food ingredients. Specific intestinal bacteria modulate immunoresponses through various pathways, such as activation of innate immune cells by binding of bacterial cell components to receptors recognizing pathogen-associated molecular patterns, and production of the secondary metabolites including short-chain fatty acids (SCFAs) that regulate gene expression and function of immune-related cells.

Bacillus coagulans is a bacteria species, whose intake alleviates the pathology of intestinal diseases, such as constipation and colitis, and has recently attracted the attention with its usefulness in food processing industry due to the resistance to heat temperature and acidic condition depending on the spore-forming character [1]. *Bacillus coagulans* SANK70258 (*B. coagulans* SANK70258), also termed *Weizmannia coagulans* SANK70258, is a lactic acid bacterium used as probiotics for livestock animals and humans. Oral administration of *B. coagulans* SANK70258 effectively promotes the growth of broiler chickens with conferring the protection ability against *Coccidia* infection [2, 3] and modulates the composition of SCFAs in the intestine [4]. A study using a model culture system of human colonic microbiota revealed that *B. coagulans* SANK70258 increased the concentration of butyrate and number of *Lachnospiraceae* bacteria in the intestine and reduced colonic *Enterobacteriaceae* species [5]. Although these findings support the beneficial effects of *B. coagulans* SANK70258 on the host body, the roles of *B. coagulans* SANK70258 on the host immune systems are still unclear.

In the current study, we investigated the roles of *B. coagulans* SANK70258 as an

64 immunostimulant by using *in vitro* and *in vivo* experiments, and revealed that *B.*
65 *coagulans* SANK70258 cell components exhibit high activity in production of IFN-γ
66 and IgA from immune-related cells.
67

Materials and Methods

Mice and Cells

The present study was approved by the Animal Care and Use Committees of Tokyo University of Science (K22005, K21004, K20005, and K19006), and was conducted in accordance with the guideline of the Institutional Review Board of Tokyo University of Science. The spleen, the Peyer's patch, and the bone marrow were isolated from Balb/c mice (Japan SLC, Hamamatsu, Japan) to obtain splenocytes, Peyer's patch cells, and to generate BMDCs, respectively. BMDCs were developed from BM cells as previously described [6]. To neutralize cytokines, anti-IL-12p40 (clone C17.8, BioLegend), anti-IL-23p19 (clone MMp19B2, BioLegend), anti-IL-6 (clone MP5-20F3, BioLegend), and isotype control (#RTK2071, BioLegend) Abs were used. Depletion of $CD11c^+$ cells from splenocytes was performed by autoMACS Pro Separator (Miltenyi Biotec) with CD11c MicroBeads UltraPure mouse (Miltenyi Biotec). $CD8^+$ T cells were isolated from the spleen using Mojosort Magnetic Separation System CD8 Naïve T cell Isolation Kit (BioLegend). Anti-CD3ε Ab (clone 145-2111C, BioLegend) and anti-CD28 Ab (clone 37.51, TONBO Bioscience) were used to stimulate $CD8^+$ T cells. BAY11-7082, LE540 (#123-04521, Wako), Brefeldin A (#420601, BioLegend) were used as inhibitors.

Preparation of lactic acid bacteria

B. coagulans SANK70258 and 7 kinds of bacteria (*Lactobacillus antri* 15950, *L. sakei subsp. sakei* 1157, *L. buchneri* 1068, *L. gasseri* 1131, *L. plantarum subsp. plantarum* 1149, *Leuconostoc mesenteroides subsp. cremoris* 16943, *L. mesenteroides subsp. cremoris* 6124; all obtained from RIKEN BRC) were cultured in MRS medium for 1

day under a same shaking condition. After washing with saline, harvested bacteria were freeze-dried and were γ-ray irradiated.

ELISA

The concentrations of mouse IFN-γ, IL-12p40, IL-6, and IL-10, were determined by ELISA using ELISA MAX Deluxe Sets (BioLegend), and IgA concentration was measured by mouse IgA uncoated ELISA kit (Invitrogen) or mouse IgA ELISA Quantification Set (#E90-103, Bethyl Laboratories).

Flow cytometry

To identify $CD4^+$ T cells, $CD8^+$ T cells, and NK cells in whole splenocytes, cells were stained with anti-mouse CD3ε-PerCP (clone 145-2C11, BioLegend), anti-mouse CD4-FITC (clone GK1.5, BioLegend), anti-CD8-VioGreen (clone 53-6.7, Miltenyi Biotec), anti-NK1.1-PE (clone PK136, BioLegend), and anti-CD49b-APC (clone DX5, BioLegend) Abs. Intracellular IFN-γ was stained with anti-mouse IFN-γ-PE/Cyanine7 (clone XMG1.2, BioLegend) after treatment with Fixation buffer (#420801, BioLegend) and Intracellular staining perm wash buffer (#421002, BioLegend). A MACS Quant analyzer (Miltenyi Biotec) and Flowjo (Tomy Digital Biology, Tokyo, Japan) were used to detect fluorescence and to analyze data, respectively.

Small interfering RNA

Electroporation was performed to introduce siRNA into BMDCs using a mouse dendritic cell nucleofector kit (Lonza, Basel, Switzerland) and a Nucleofector 2b (Lonza). Following siRNAs were purchased from Invitrogen (Carlsbad, CA, USA):

Tlr2 (#MSS216272), *Tlr4* (#MSS211922), *Nod2* (#MSS217440), Stealth RNAi siRNA Negative Control Lo GC (#12935-200) as for negative control of *Tlr4* siRNA, Stealth RNAi siRNA Negative Control Hi GC (#12935-400) for *Tlr2* and *Nod2* siRNAs.

Quantification of mRNA

Complementary DNA was synthesized from total RNA, which was isolated from BMDCs using the ReliaPrep RNA Cell Miniprep System (#Z76012, Promega, Madison, USA), using ReverTra Ace qPCR Master Mix (#FSQ-201, TOYOBO, Osaka, Japan). Quantitative real-time PCR was performed with Thunderbird SYBR qPCR Mix (#QPS-201, TOYOBO) on the StepOne Real-Time PCR System (Applied Biosystems, Kanagawa, Japan). The nucleotide sequences (from 5' to 3') of the primer sets used in qPCR are listed as follows.

Gzmb-F; TGCATTCCCCACCCAGACTA, *Gzmb*-R; TTCAGCTTTAGCAGCATGATGTC, *Prf1*-F; GAGTGTCGCATGTACAGTTTTCG, *Prf1*-R; GCGCCTTTTTGAAGTCAAGGT, *Ccr4*-F; GCAAGGCAGCTCAACTGTTCT, *Ccr4*-R; TGGCATTCATCTTTGGAATCG, *Ccr5*-F; GGCTCTTGCAGGATGGATTTT, *Ccr5*-R; GGTGCTGACATACCATAATCGATGT, *Ccr6*-F; TCTGAATGAATTCCACAGAGTCCTACT, *Ccr6*-R; CCATGGTCTGGAGGAATAGAATAATAC, *Ccr9*-F; GCACTTCCCCTCCTGAAGCT, *Ccr9*-R; CTTGTGAGTTCTGTGGGGCATCA, *Cxcr3*-F; TGCCAAAGGCAGAGAAGCA, *Cxcr3*-R; CATCTAGCACTTGACGTTCACTAACC, *Cxcr6*-F; GAGCACACTTCACTCTGGAACAA, *Cxcr6*-R; CCATCATCCATGGCATCA, *Il1b*-F; AGTTGACGGACCCCAAAGA, *Il1b*-R;

GGACAGCCCAGGTCAAAGG, *Tlr2*-F; GAATTGCATCACCGGTCAGAA, *Tlr2*-R; TCCTCTGAGATTTGACGCTTT, *Tlr4*-F; GCTAAGTGCCGAGTCTGAGTGTAA, *Tlr4*-R; TGCAGCCTTTCAGAAACACATT, *Nod2*-F; CGTGCGCCTGCTCCAT, *Nod2*-R; CACCCTCAGGGACAAGAAGTTC.

Primers for *Il6* [7] and *Aldh1a2* mRNAs [8] were described in our previous reports.

Statistical analysis

A two-tailed Student's t-test was used to compare two samples and a one-way ANOVA followed the Tukey-Kramer multiple comparison test was employed to compare multiple (more than three) samples.

Results and Discussion

B. coagulans *SANK70258 induces IFN-γproduction from NK cells*

To evaluate the effects of *B. coagulans* SANK70258 as immunostimulant, we incubated the mouse splenocytes in the presence of SANK70258 or various lactic acid bacteria, which were sterilized by γ-ray treatment. Determination of IFN-γ concentrations in the culture media of splenocytes after 48 h incubation showed that the SANK70258 component exhibited the highest IFN-γ production ability among tested bacteria components (Fig. 1A), in a dose-dependent manner (Fig. 1B). Then, to identify the IFN-γ-producing cells in whole splenocytes, we performed flow cytometric analysis using Abs against IFN-γ and cell type markers. As shown in Fig. 1C, the SANK70258-treatment markedly increased the frequency of IFN-γ-producing cells in NK population, whereas none and little increase was observed in $CD4^+$ T cells and $CD8^+$ T cells, respectively.

These results indicate that *B. coagulans* SANK70258 effectively induced IFN-γ release from NK cells mainly.

Participant cells and molecules in B. coagulans *SANK70258-induced IFN-γ production*

To reveal the molecular mechanisms by which *B. coagulans* SANK70258-treatment induced IFN-γ production, we examined the effects of cytokine blocking, cells depletion, and intracellular-signaling inhibition. As shown in Fig. 2A, addition of anti-IL-12p40 Ab into culture media of spleen cells completely inhibited *B. coagulans* SANK70258-induced production of IFN-γ, and anti-IL-23p19 Ab also significantly suppressed the IFN-γ release. Then, to confirm the involvement of

IL-12/IL-23-producing cells in the *B. coagulans* SANK70258-induced IFN-γ production, we compared IFN-γ levels between the whole spleen cells and $CD11c^+$-depleted spleen cells, and found that the IFN-γ production following *B. coagulans* SANK70258 treatment was decreased by the depletion of $CD11c^+$ cells (Fig. 2B). To further examine whether the NFκB-signaling activated in *B. coagulans* SANK70258-treated cells participate in IFN-γ production, we stimulated whole splenocyte with *B. coagulans* SANK70258 in the presence or absence of BAY11-7082, an inhibitor of NF-kB. Both production of IFN-γ and IL-12p40 from *B. coagulans* SANK70258-treated splenocyte was completely inhibited by NF-kB inhibition (Fig. 2C). These results demonstrated that *B. coagulans* SANK70258 induced IFN-γ production from NK cells via NF-κB-mediated stimulation of IL-12/23-producible cells including DCs.

In a cytoplasmic-staining experiment (Fig. 1C), slight expression of IFN-γ was detected in $CD8^+$ T cells, which is reported to express TLRs and be able to receive the stimulation by PAMPs [9, 10]. To investigate whether *B. coagulans* SANK70258 directly induces IFN-γ production from $CD8^+$ T cells, isolated splenic $CD8^+$ T cells were incubated in anti-CD3 Ab- and anti-CD28 Ab-coated dishes in the presence or absence of *B. coagulans* SANK70258. IFN-γ in culture supernatant was significantly increased by *B. coagulans* SANK70258 treatment, and mRNA levels of *Gzmb* and *Prf1* were upregulated in *B. coagulans* SANK70258-treated $CD8^+$ T cells (Fig. 2D). In a recent study, CCR6 expression in $CD8^+$ T cells was increased by microbial exopolysaccharide produced by *Lactobacillus*, which contributes the antitumor adjuvant effect of the *Lactobacillus* on immune-checkpoint blockade treatment [11]. Then, we quantified mRNA levels of chemokine receptors in *B. coagulans* SANK70258-treated

199 $CD8^+$ T cells, and observed that mRNAs of several receptors including CCR6 tended to
200 be increased by *B. coagulans* SANK70258 treatment (Fig. 2E).
201
202 ***Roles of NOD2 and TLR2 in* B. coagulans *SANK70258-dependent cytokine***
203 ***production of DC***
204 Above-mentioned results using splenocytes indicated that DC is a candidate source of
205 IL-12, which produces IL-12 following *B. coagulans* SANK70258 treatment. To
206 evaluate the effects of *B. coagulans* SANK70258-treatment on DCs, we determined
207 mRNA levels of cytokines in BMDCs incubated with *B. coagulans* SANK70258.
208 Treatment with *B. coagulans* SANK70258 apparently increased mRNA levels of *Il6*,
209 *Il1b*, and *Aldh1a2* in BMDCs in a dose-dependent manner (Fig. 3A), suggesting that *B.*
210 *coagulans* SANK70258 directly stimulated DCs. To evaluate the roles of PAMP
211 receptors in *B. coagulans* SANK70258-induced stimulation of DCs, we performed a
212 knockdown experiment using siRNAs for *Tlr2*, *Tlr4*, and *Nod2*, which are reported to
213 be receptors for lactic acid bacteria components [12]. Although LPS-induced release of
214 cytokines was decreased in *Tlr4* siRNA-transfected BMDCs, knockdown of TLR4 did
215 not reduce cytokine release from *B. coagulans* SANK70258-stimulated BMDCs (Fig.
216 3B). In contrast, knockdown of TLR2 and NOD2 suppressed the release of IL-6 and
217 IL-12p40 from *B. coagulans* SANK70258-treated BMDCs (Fig. 3C). *B. coagulans*
218 SANK20758 treatment also induced IL-10 release from BMDCs, which is a key
219 character of probiotic bacteria useful for prevention of inflammatory diseases and was
220 markedly reduced by NOD2 knockdown but not by knockdown of TLR2 and TLR4
221 (Fig. 3B and 3C). Based on the finding that reduced expression of PAMP receptors
222 recognizing peptidoglycan-related components suppressed the cytokine release from *B.*

223 *coagulans* SANK70258-treated BMDCs.

224

225 **B. coagulans *SANK70258 accelerates IgA production* in vitro *and* in vivo**

226 *B. coagulans* SANK70258 treatment increased the expression of IL-6 and RALDH2 in
227 DCs, which are known to be accelerator of IgA production [13]. To evaluate the effects
228 of *B. coagulans* SANK70258 on IgA production, we cultured whole cells prepared from
229 the Peyer's patch with *B. coagulans* SANK70258. After 3-7 days cultivation, apparent
230 amounts of IgA accompanied with the production of IL-6 and IFN-γ were detected in *B.*
231 *coagulans* SANK70258-treated Peyer's patch cells (Fig. 4A). We also confirmed that *B.*
232 *coagulans* SANK70258-induced increase of IgA was significantly suppressed in the
233 co-presence of IL-6-neutralizing Ab and an RAR inhibitor (Fig. 4B). When the amounts
234 of IgA in culture media of Peyer's patch-derived cells incubated with or without lactic
235 bacteria were compared, we found that *B. coagulans* SANK70258 exhibited the highest
236 activity of IgA production among the tested bacteria (Fig. 4C).
237 Finally, to investigate the effects of *B. coagulans* SANK70258 *in vivo*, we determined
238 the amount of IgA in feces of mice orally administered *B. coagulans* SANK70258. The
239 intake of *B. coagulans* SANK70258 for 2 weeks significantly increased IgA levels in
240 feces (Fig. 4D), and frequency of IgA-producing B cells in the Peyer's patch (Fig. 4E).
241 In the present study, we found that *B. coagulans* SANK70258 cell components induce
242 the high amount production of IFN-γ and IgA from immune cells. Although we used
243 γ-ray-treated *B. coagulans* SANK70258 focusing on the roles of cell components as an
244 immunostimulant in the present study, considering that *B. coagulans* SANK70258 can
245 reach the intestine alive by forming spore, it is expected that *B. coagulans* SANK70258
246 exhibits further beneficial effects on host body via production of the secondary

metabolites.

Conflicts of Interest

M.A. and R.Y. are employed by the Mitsubishi Chemical Corporation.

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Legends

Figure 1. IFN-γ induction activity of lactic acid bacteria and identification of IFN-γ producing cells in splenocytes stimulated with *B. coagulans* SANK70258

A. and **B.** Concentrations of IFN-γ in culture media of splenocytes. Whole cells prepared from the spleen of mouse (5 x 10^5 cells/500 μL) were incubated in the presence or absence of 10 μg/mL γ-ray irradiated bacteria (**A**) or were incubated with indicated amount of *B. coagulans* SANK70258 (**B**) for 48 h. IFN-γ concentrations of culture media were determined by ELISA. The data represent the mean ± SE of 3 independent experiments performed with triplicate samples (**A** and **B**).

C. Frequencies of IFN-γ producing cells. Whole splenocytes (5 x 10^5 cells/500 μL) cultured with or without 10 μg/mL *B. coagulans* SANK70258 for 12 h were incubated for additional 5 h in the presence of Monensin and Brefeldin A and were then stained with Abs against IFN-γ and cell-type specific markers. The data represent the mean ± SE of 5 independent experiments. Statistical analysis was performed with two-tailed Student's *t*-test. $*p < 0.05$.

Figure 2. Roles of DCs and $CD8^+$ T cells involved in IFN-γ production in *B. coagulans* SANK70258-stimulated splenocytes

A. Effects of neutralizing Abs on *B. coagulans* SANK70258-induced IFN-γ production. Splenocytes were incubated with or without 10 μg/mL *B. coagulans* SANK70258 in the presence of 0.5 μg/mL neutralizing Ab or its control for 48 h.

B. Effects of $CD11c^+$ depletion on *B. coagulans* SANK70258-induced IFN-γ production.

C. Effects of NFκB inhibition on cytokine production in *B. coagulans*

SANK70258-treated splenocytes.

D. *B. coagulans* SANK70258 enhanced IFN-γ production from CD3/CD28-dependently stimulated $CD8^+$ T cells. Splenic $CD8^+$ T cells were incubated in Ab-coated dishes in the presence or absence of 10 μg/mL *B. coagulans* SANK70258 for 48 h, and IFN-γ concentrations in collected culture supernatants and *Gzmb* and *Prf1* mRNA levels in harvested cells were determined.

E. Effects of *B. coagulans* treatment on transactivation of chemokine receptor genes in $CD8^+$ T cells. Splenic $CD8^+$ T cells were cultivated with or without 10 μg/mL *B. coagulans* SANK70258 for 3 h, and then harvested to determine mRNA levels.

The data represent the mean ± SD from three independent assays performed in triplicate. *, $p < 0.05$; **, $p < 0.01$. Tukey-Kramer test (**A**- **D**) and Student's *t*-test (**E** and **F**) were used.

Figure 3. Effects of *B. coagulans* SANK70258 treatment on gene expression in BMDCs

A. mRNA levels of *Il6*, *Il1b*, and *Aldh1a2* in BMDCs.

B. Effects of *Tlr4* knockdown on *B. coagulans* SANK70258-induced expression of cytokines in BMDCs.

C. Cytokine production from *Tlr2* or *Nod2* siRNA transfected BMDCs.

D. Cytokine producing activities of *B. coagulans* SANK70258-derived fractions.

To determine mRNA levels in cells and the amounts of cytokines in culture media, cells and culture supernatants were collected 4 h and 24 h after stimulation, respectively. For the stimulation, 10 μg/mL *B. coagulans* SANK70258 or 100 ng/mL LPS was added. BMDCs were cultured for 48 h after siRNA transfection (**B**, **C**).

355 The data represent the mean ± SD from three independent assays performed in triplicate.
356 *, $p < 0.05$; **, $p < 0.01$. Tukey-Kramer test (**A- D**) were used.
357
358 **Figure 4. Induction of IgA production by *B. coagulans* treatment *in vitro* and *in**
359 ***vivo***
360 **A.** The amounts of IgA and cytokine proteins in the Peyer's patch-derived cells. Whole
361 cells (5 x 10^5 cells/500 μL) isolated from the Peyer's patch were incubated with or
362 without indicated concentrations of *B. coagulans* SANK70258 for 3 or 7 days, and
363 concentrations of IgA, IL-6, and IFN-γ in culture supernatant were determined by
364 ELISA.
365 **B.** Effects of IL-6 neutralization and RAR inhibition on IgA production from *B.*
366 *coagulans* SANK70258-stimulated Peyer's patch cells. One μg/mL anti-IL-6 Ab and/or
367 1 μM LE540 were added to culture media of Peyer's patch-derived cells prior to
368 addition of 10 μg/mL *B. coagulans* SANK70258, and the supernatants after 7 days
369 cultivation were collected to determine IgA concentrations.
370 **C.** IgA concentrations in the culture media of Peyer's patch cells incubated with or
371 without 10 μg/mL lactic acid bacteria for 7 days.
372 **D.** The amount of IgA proteins in feces.
373 **E.** Frequency of IgA^+ B cells in the Peyer's patch.
374 Balb/c mice were fed by 0.2% w/w *B. coagulans* SANK70258-containing or its control
375 diet (**D** and **E**).
376 The data represent the mean ± SEM of individuals (**A**-**E**). *, $p < 0.05$; **, $p < 0.01$.
377 Tukey-Kramer test (**B**) and Student's *t*-test (**D** and **E**) were used.
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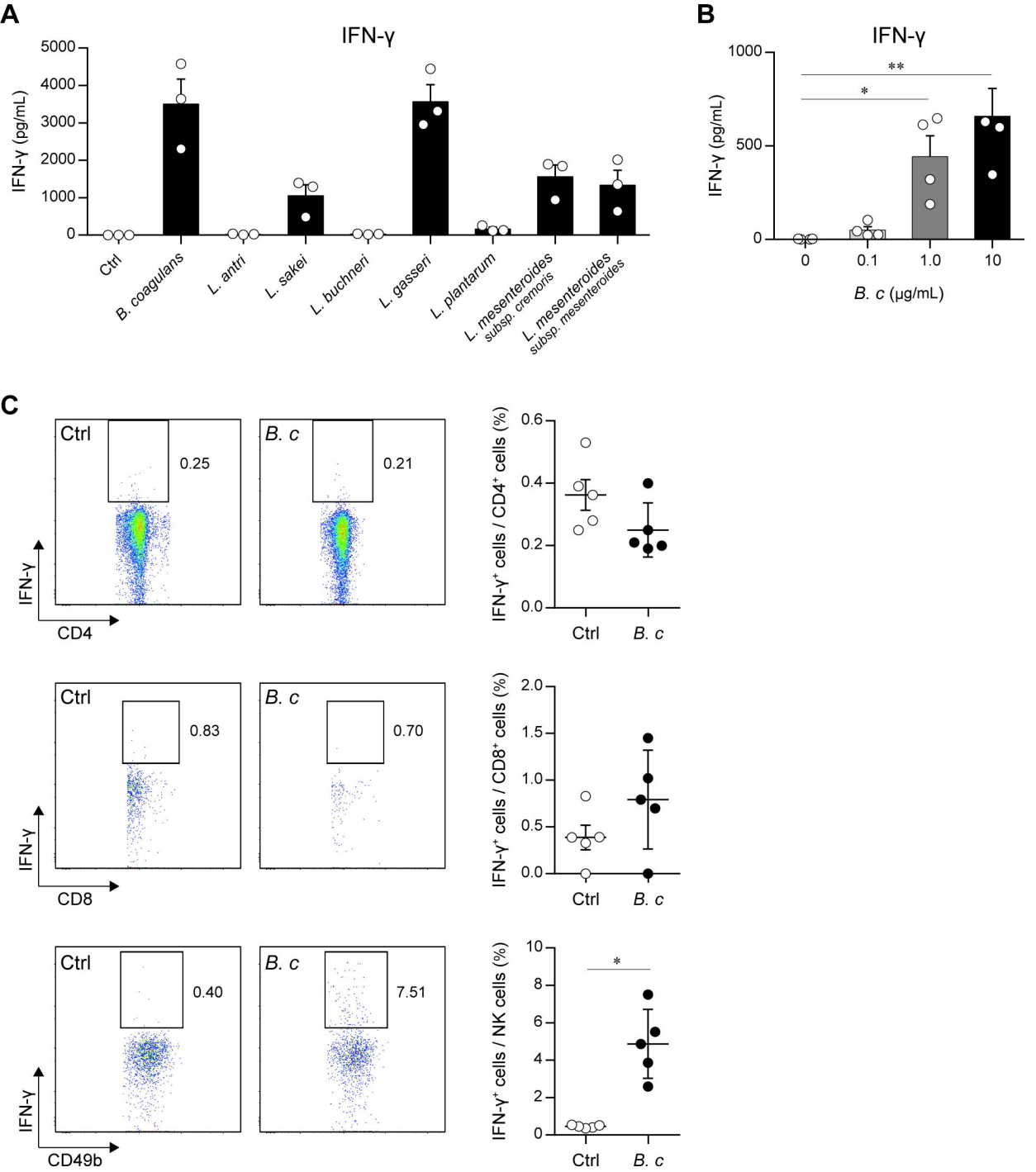
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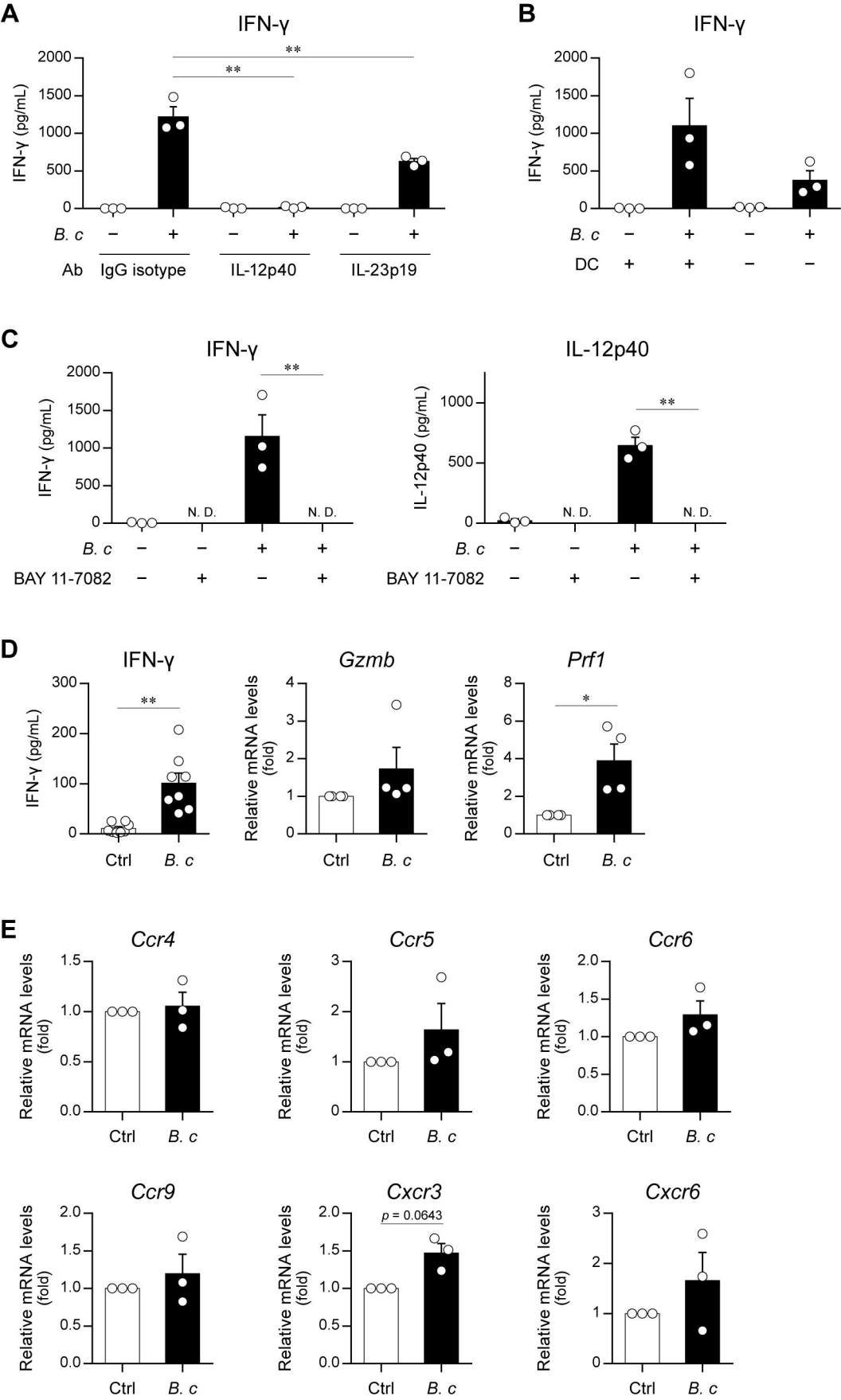
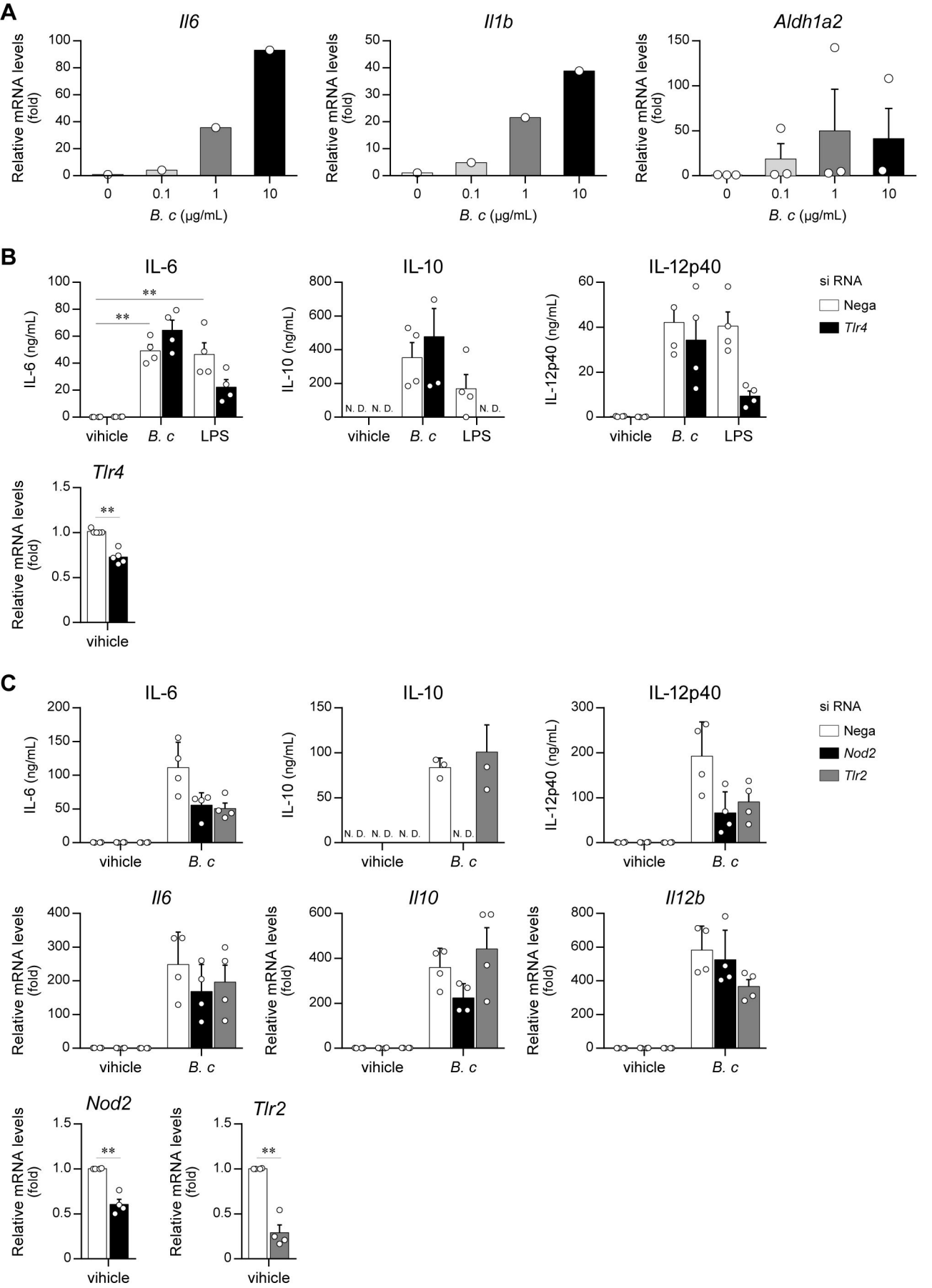
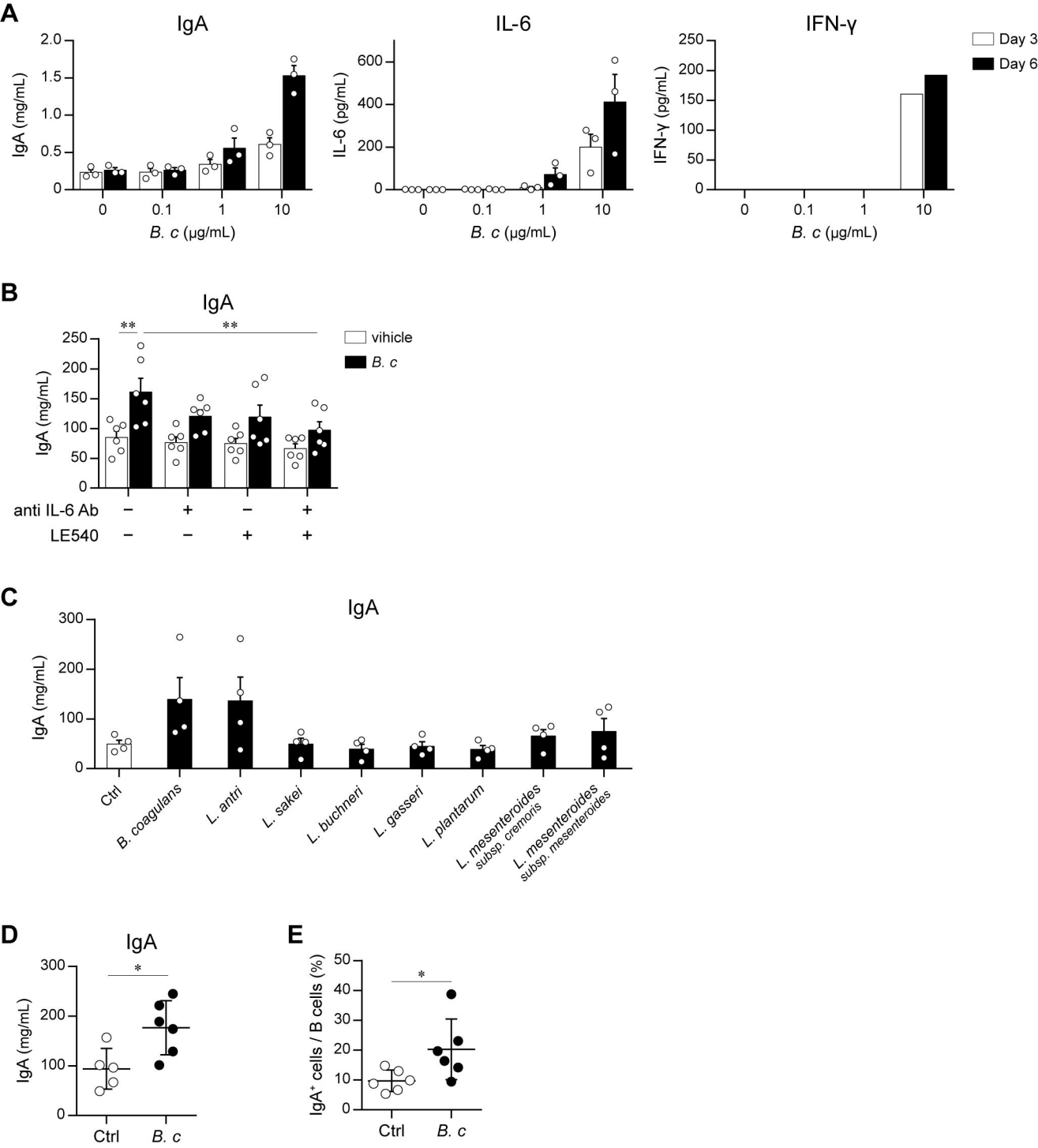

A
IFN-γ
IFN-γ (pg/mL)
2000
1500
1000
500
0
**
**
B. c
–
+
–
+
–
+
Ab
IgG isotype
IL-12p40
IL-23p19
B
IFN-γ
IFN-γ (pg/mL)
2000
1500
1000
500
0
B. c
–
+
–
+
DC
+
+
–
–
C
IFN-γ
IFN-γ (pg/mL)
2000
1500
1000
500
0
**
N. D.
N. D.
B. c
–
–
+
+
BAY 11-7082
–
+
–
+
IL-12p40
IL-12p40 (pg/mL)
1000
500
0
**
N. D.
N. D.
B. c
–
–
+
+
BAY 11-7082
–
+
–
+
D
IFN-γ
IFN-γ (pg/mL)
300
200
100
0
**
Ctrl
B. c
Gzmb
Relative mRNA levels (fold)
4
3
2
1
0
Ctrl
B. c
Prf1
Relative mRNA levels (fold)
8
6
4
2
0
*
Ctrl
B. c
E
Ccr4
Relative mRNA levels (fold)
1.5
1.0
0.5
0.0
Ctrl
B. c
Ccr5
Relative mRNA levels (fold)
3
2
1
0
Ctrl
B. c
Ccr6
Relative mRNA levels (fold)
2.0
1.5
1.0
0.5
0.0
Ctrl
B. c
Ccr9
Relative mRNA levels (fold)
2.0
1.5
1.0
0.5
0.0
Ctrl
B. c
Cxcr3
p = 0.0643
Relative mRNA levels (fold)
2.0
1.5
1.0
0.5
0.0
Ctrl
B. c
Cxcr6
Relative mRNA levels (fold)
3
2
1
0
Ctrl
B. c


A
Il6
Il1b
Aldh1a2
Relative mRNA levels (fold)
B. c (μg/mL)
0
0.1
1
10
B
IL-6
IL-10
IL-12p40
IL-6 (ng/mL)
IL-10 (ng/mL)
IL-12p40 (ng/mL)
N. D.
vihicle
B. c
LPS
si RNA
Nega
Tlr4
Tlr4
C
Nod2
Tlr2
Il10
Il12b


A
IgA
IL-6
IFN-γ
Day 3
Day 6
IgA (mg/mL)
IL-6 (pg/mL)
IFN-γ (pg/mL)
B. c (μg/mL)
B
IgA
vihicle
B. c
anti IL-6 Ab
LE540
C
IgA
IgA (mg/mL)
Ctrl
B. coagulans
L. antri
L. sakei
L. buchneri
L. gasseri
L. plantarum
L. mesenteroides subsp. cremoris
L. mesenteroides subsp. mesenteroides
D
IgA
IgA (mg/mL)
Ctrl
B. c
E
IgA+ cells / B cells (%)
Ctrl
B. c