Intestinal microbiota transplantation reveals the role of microbiota in dietary regulation of RegIIIβ and RegIIIγ expression in mouse intestine

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No. of Tables: 4,530

No. of Figures: 4

No. of Supplementary Tables: 2

No. of Supplementary Figures: 2

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Abstract

RegIIIβ and RegIIIγ are antimicrobial peptides expressed in intestinal epithelial cells. Expression of these peptides is reportedly decreased by high-fat diet (HFD) and increased by indigestible oligosaccharides in mice. Clearly, these dietary regimens change the structure of intestinal microbiota. We employed an intestinal microbiota transplantation (IMT) to test whether diet-induced changes in the expression of these peptides are mediated by gut microbiota. C57BL/6J mice were fed either a normal-fat diet (NFD), a HFD, or a NFD supplemented with or without 1-kestose (KES), an indigestible oligosaccharide. Ileal RegIIIβ and RegIIIγ mRNA levels were lower in mice receiving IMT from HFD-fed mice than in those receiving NFD-fed mice and higher in mice receiving IMT from KES-supplemented mice than in those receiving the mice without KES supplementation. Western blot analysis showed that serum RegIIIβ levels changed in parallel with the ileal mRNA levels. We propose that HFD- and KES-induced changes in the ileal RegIIIβ and RegIIIγ expression and in the circulating RegIIIβ levels are mediated, at least in part, by intestinal microbiota.

Keywords: RegIIIβ; RegIIIγ; High-fat diet; 1-Kestose; Intestinal microbiota

1. Introduction

RegIIIβ and RegIIIγ are C-type lectins expressed in the intestinal epithelial cells [1, 2]. These lectins are secreted into the intestinal lumen and exert bactericidal action; thus, RegIIIβ and RegIIIγ are considered to confer protection against the infection with enteropathogenic bacteria. In addition, circulating RegIIIβ has been thought to act as an anti-inflammatory factor [3], and obesity is reportedly associated with higher circulating RegIIIβ levels [4]. These lectins are thus regarded as intestine-derived exocrine and endocrine factors that contribute to the maintenance of homeostasis.

Expression of RegIIIβ and RegIIIγ in the intestinal epithelial cells is influenced by diet, intestinal microbiota and cytokines. RegIIIβ mRNA levels in rat ileum have been shown to be decreased by fasting, followed by recovering by refeeding [5, 6]. Germ-free mice and antibiotics (ABX)-treated mice reportedly showed a diminished intestinal expression of RegIIIβ and RegIIIγ [6-8], and the expression was promoted by bacterial colonization [8]. More specifically in terms of bacterial species, RegIIIγ expression in mouse ileum has been shown to be stimulated by *Bifidobacterium breve* [9]. Furthermore, intestinal expression of RegIIIβ and RegIIIγ has been thought to be dependent on IL-22 released from innate lymphoid cells [2, 10].

Everard *et al.* [11] reported that a high-fat diet (HFD) reduced intestinal RegIIIγ mRNA levels in mice. They also observed that the supplementation with fructooligosaccharides (FOS) increased intestinal RegIIIγ mRNA levels. Clearly, these dietary regimens change the structure of intestinal microbiota. Hildebrandt *et al.* [12] observed that HFD feeding resulted in a decrease in phylum Bacteroidetes and an increase in both phyla Firmicutes and Proteobacteria in mice. In addition, it has been well known that dietary FOS stimulates the growth of bifidobacteria [13]. From these findings, we assumed that intestinal microbiota may mediate diet-induced changes in the intestinal expression of RegIIIβ and RegIIIγ.

Intestinal microbiota transplantation (IMT) is the process of transplanting stool from a healthy

donor to the intestine of a patient for therapeutic purpose. IMT has also been extensively utilized to explore causal relationship between intestinal microbiota and host physiology and pathology. For instance, Turnbaugh *et al.* [14] performed IMT to test whether intestinal microbiota of genetically obese mice contributes to obesity. They showed that the body fat gain was greater in germ-free mice receiving IMT from obese mice than those receiving IMT from lean mice, suggesting intestinal microbiota as a cause of obesity. The present study employed IMT to test whether diet-induced changes in the intestinal expression of RegIIIβ and RegIIIγ are mediated by intestinal microbiota.

2. Materials and methods

2.1. Animals

Five-week-old male specific pathogen-free C57BL/6J mice, which were purchased from Japan SLC (Hamamatsu, Japan), were housed in a temperature-controlled (23 ± 2˚C) room with a dark period from 20:00 to 8:00 h and allowed free access to tap water and AIN-93G purified diet (Research Diets, New Brunswick, NJ). This study was approved by the Hokkaido University Animal Use Committee (approval no. 14-0028), and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

2.2. Experimental design

After acclimatizing for 1-2 weeks, 4 experiments were performed. In experiment (exp.) 1, 12 mice were randomly divided into 2 groups (6 mice in each group) and then fed either a normal-fat diet (NFD, D12450J, 10 kcal% fat, Research Diets) or a HFD (D12492, 60 kcal% fat, Research Diets) for 2 weeks. In exp. 2, 12 mice were randomly divided into 2 groups (6 mice in each group) and then fed the AIN-93G diet and supplemented either with or without 4% w/v 1-kestose (KES, B Food Science Co., Ltd., Chita, Japan), a kind of FOS, in drinking water for 2 weeks. On the last day of the experimental period in exp. 1 and 2, mice were anesthetized with sevoflurane inhalation and then killed by severing the carotid artery. Serum was separated from

the blood samples and stored at -20˚C for western blot analysis. After a laparotomy, a 5-cm segment proximal to the ileo-cecal valve was excised from the small intestine and opened longitudinally, and the contents were collected and stored at -20˚C for DNA isolation. After washing with ice-cold PBS, the mucosa of the segment was scraped off with a glass slide, snap-frozen in liquid nitrogen and stored at -80˚C for total RNA isolation. In exp. 3 (**Supplementary Fig. 1A**), 24 mice were randomly divided into 2 groups of donors and recipients (12 mice in each group) for IMT described below. Donor mice were further divided into 2 groups (6 mice in each group) and then fed either HFD or NFD for 2 weeks. Recipient mice were fed NFD before IMT. After IMT, recipient mice were fed NFD for 1 week. In exp. 4 (**Supplementary Fig. 1B**), 24 mice were randomly divided into 2 groups of donors and recipients (12 mice in each group) for IMT. Donor mice were further divided into 2 groups (6 mice in each group) and then fed the AIN-93G diet and supplemented either with or without 4% w/v KES in drinking water for 2 weeks. Recipient mice were fed the AIN-93G diet and tap water without KES supplementation before IMT. After IMT, recipient mice were fed the AIN-93G diet and tap water for 1 week. Serum samples, ileal contents and ileal mucosal samples in donor and recipient mice were obtained and stored as described above.

2.3. Intestinal microbiota transplantation

Donor mice were anesthetized with sevoflurane inhalation and then killed by severing the carotid artery. After a laparotomy, the distal half of the small intestine was excised, and the luminal contents were collected and pooled in each group, homogenized with PBS, and then immediately subjected to IMT. Prior to receiving IMT, recipient mice were given ABX cocktail in drinking water prepared according to Chevalier *et al.* [15] and intragastrically administered with omeprazole (40 mg/kg/day, Combi-Blocks, San Diego, CA) once a day for 3 days. On the next day of the final omeprazole administration, ABX administration was discontinued, and recipient mice were intragastrically administered with 200 μl luminal contents obtained from donor mice once a day for 2 consecutive days.

2.4 Intestinal organoid experiment

In a separate experiment, effect of recombinant IL-22 (rIL22) supplementation on the mRNA expression of *Reg3b* gene was examined in the intestinal organoids. C57BL/6J mice were anesthetized with sevoflurane inhalation and then killed by cervical dislocation. After a laparotomy, the entire length of the small intestine was excised, and crypts were isolated and cultured to obtain the organoids as previously described [16]. On day 5, rIL-22 (5 ng/ml, Biolegend, San Diego, CA) was added to the culture of organoids for 24 h. The organoids were then harvested for total RNA isolation.

2.5. RNA isolation and analysis

Total RNA was isolated from ileal mucosa and organoid samples using a ReliaPrep RNA Tissue Miniprep System (Promega Japan, Tokyo, Japan) and reverse transcribed to generate first strand cDNA using a ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan) according to the manufacturers' instructions. Murine RegIIIβ, RegIIIγ, IL-22 and β-actin are encoded by *Reg3b*, *Reg3g*, *Il22* and *Actb* genes, respectively. To compare the steady-state levels of these mRNAs, real-time quantitative PCR (RT-qPCR) was performed using a GeneAce SYBR qPCR Mix α No ROX (Nippon Gene, Toyama, Japan) with a Thermal Cycler Dice TP800 (Takara Bio, Otsu, Japan) according to the manufacturers' instructions. Relative mRNA expression levels for each sample were normalized to that of *Actb*. The sequences of primers used for RT-qPCR are described in **Supplementary Table 1**.

2.6. Western blot analysis

Serum samples were separated by 10% SDS–PAGE under reducing conditions. Electrophoresed proteins were electrophoretically transferred to Hybond C nitrocellulose membrane (Amersham Life Science, Amersham, UK). Membranes were then probed with primary antibody against RegIIIβ (MAB5110, R&D systems, Minneapolis, MN), RegIIIγ (REG3G Polyclonal Antibody, Thermo Fisher Scientific, San Jose, CA), and transferrin (F-8, Santa Cruz, Santa Cruz, CA) overnight at 4ºC. Membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at RT. The blots were detected by ECL chemiluminescence (GE Healthcare, Menlo Park, CA) according to the manufacturer's

instructions and visualized with Lumi Vision PRO 400EX (AISIN, Kariya, Japan). The band intensities were quantified by ImageJ software (National Institutes of Health, Bethesda, MD), and relative RegIIIβ and RegIIIγ levels for each sample were normalized to the levels for transferrin.

2.7. DNA isolation and RT-qPCR for bacterial enumeration

DNA was isolated from ileal contents using a QIAamp Fast DNA Stool Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. DNA samples served as a template for RT-qPCR of the 16S rRNA gene fragment to estimate the number of bacteria belonging to phylum Firmicutes, phylum Bacteroidetes and genus *Bifidobacterium* as previously described [17]. The sequences of primers used for RT-qPCR are described in **Supplementary Table 2**. For RT-qPCR standards, subcloned 16S rRNA gene fragments were prepared from *Blautia coccoides* (JCM 1395^{T}), *Bacteroides fragilis* (JCM11019) and *Bifidobacterium animalis* (JCM 1190^{T}) for phylum Firmicutes, phylum Bacteroidetes and *Bifidobacterium* spp., respectively, as previously described [17].

2.8. Statistical analysis

Results are presented as means ± SEM. To compare the mean values of two groups in exp. 1 and 2, an unpaired *t*-test was used. To compare the mean values of four groups in exp. 3 and 4, two-way analysis of variance (ANOVA) was used. The Tukey-Kramer *post hoc* test was applied when a significant interaction was found by two-way ANOVA. Data were analyzed using GraphPad Prism for Macintosh (version 8, GraphPad Software, San Diego, CA). *P* values of <0.05 were considered to be statistically significant.

3. Results

3.1. HFD and KES alter ileal RegIIIβ and RegIIIγ mRNA levels (exp. 1 and 2)

The mRNA levels of both *Reg3b* and *Reg3g* genes in the ileum were significantly lower in the HFD-fed mice than in the NFD-fed mice (**Fig. 1A**) and significantly higher in the

KES-supplemented mice than in the mice without KES supplementation (**Fig. 1B**). In parallel with ileal mRNA levels, serum RegIIIβ levels estimated by western blot analysis were significantly lower in the HFD-fed mice than in the NFD-fed mice (**Fig. 1C**) and significantly higher in the KES-supplemented mice than in the mice without KES supplementation (**Fig. 1D**). In contrast, HFD and KES failed to alter serum RegIIIγ levels.

3.2. HFD reduction of ileal RegIIIβ and RegIIIγ mRNA levels in donor mice was reproduced in recipient mice (exp. 3)

We employed IMT to test whether HFD-induced reduction of ileal mRNA levels of *Reg3b* and *Reg3g* genes is mediated by intestinal microbiota. We observed that the Firmicutes/Bacteroidetes ratio in the ileal contents was significantly influenced by both HFD and IMT (**Fig. 2A**). The ratio was significantly higher in the HFD-fed mice than in the NFD-fed mice. Likewise, the ratio was significantly higher in the mice receiving IMT from the HFD-fed mice than in the mice receiving IMT from the NFD-fed mice. Also, the ratio was significantly higher in the recipient mice than in the donor mice. The mRNA levels of *Reg3b* and *Reg3g* genes in the ileum (**Fig. 2B** and **2D**, respectively) and serum RegIIIβ levels (**Fig. 2C**) were significantly lower in the HFD-fed mice than in the NFD-fed mice. Similarly, the mRNA levels of *Reg3b* and *Reg3g* genes in the ileum and serum RegIIIβ levels were significantly lower in the mice receiving IMT from the HFD-fed mice than in the mice receiving IMT from the NFD-fed mice. No significant difference was observed in the serum RegIIIγ levels among the groups ((**Fig. 2E**).

3.3. KES increase of ileal RegIIIβ and RegIIIγ mRNA levels in donor mice was reproduced in recipient mice (exp. 4)

Similar to exp. 3, IMT was performed to test whether KES-induced increase of the mRNA levels of *Reg3b* and *Reg3g* genes in the ileum was mediated by intestinal microbiota. We found that the abundance of *Bifidobacterium* spp. in the ileal contents tended to be influenced by both KES supplementation and receiving IMT (**Fig. 3A**). When an unpaired *t*-test was applied, the abundance of *Bifidobacterium* spp. was significantly higher in the KES-supplemented mice

than in the mice without supplementation (p<0.01), whereas the abundance did not differ between the mice receiving IMT from the KES-supplemented mice and the mice receiving IMT from the mice without supplementation. The mRNA levels of *Reg3b* and *Reg3g* genes in the ileum (**Fig. 3B** and **3D**, respectively) and serum RegIIIβ and RegIIIγ levels (**Fig. 3C** and **3E**, respectively) were significantly higher in the KES-supplemented mice than in the mice without supplementation. Similarly, the mRNA levels of *Reg3b* and *Reg3g* genes in the ileum and serum RegIIIβ and RegIIIγ levels were significantly higher in the mice receiving IMT from the KES-supplemented mice than in the mice without supplementation (**Fig. 3B** and **3C**, respectively). IMT significantly decreased the ileal mRNA levels of *Reg3b* and *Reg3g* genes (**Fig. 3B** and **3C**, respectively) and increased the serum RegIIIγ levels (**Fig. 3E**).

3.4. IL-22 may not be responsible for HFD- and KES-induced alteration of ileal RegIIIβ and RegIIIγ mRNA levels

A mechanistic study for changes in the expression of RegIIIβ and RegIIIγ was carried out. Intestinal expression of RegIIIβ and RegIIIγ has been thought to be dependent on IL-22 [2, 10]. Indeed, we observed that the supplementation of rIL-22 for 24 h increased approximately thousandfold the mRNA levels of *Reg3b* gene in mouse intestinal organoids (**Fig. 4A**). However, the mRNA levels of *Il22* gene in the ileum were the same between the NFD-fed mice and HFD-fed mice in exp. 1 (**Fig. 4B**) and tended to be lower (p = 0.057) in the KES-supplemented mice than in the mice without supplementation in exp. 2 (**Fig. 4C**).

4. Discussion

We observed that the ileal mRNA expression of *Reg3g* gene was reduced by HFD feeding and increased by KES supplementation. These results are in line with Everard *et al.* [11]. We also found that the ileal mRNA expression of *Reg3b* gene was changed in parallel with that of *Reg3g* gene in response to HFD and KES. This seems reasonable since RegIIIβ are usually co-expressed with RegIIIγ in mice [8]. Our findings thus showed that HFD and KES influenced the ileal expression of both *Reg3b* and *Reg3g* genes.

The present study clearly demonstrated that mice receiving IMT reproduced the ileal mRNA expression of *Reg3b* and *Reg3g* genes in the donor mice. Ileal mRNA expression of *Reg3b* and *Reg3g* genes was lower in the mice receiving IMT from the HFD-fed donors and was higher in the mice receiving IMT from the KES-supplemented donors. In the present study, the recipient mice were treated with ABX prior to receiving IMT. We observed that ABX treatment decreased the ileal mRNA expression of *Reg3b* and *Reg3g* genes (**Supplementary Fig. 3**). Thus, ileal mRNA expression of *Reg3b* and *Reg3g* genes in the recipient mice would reflect the recovery of gene expression by receiving IMT. Nevertheless, the present results obtained by IMT experiments suggest that HFD- and KES-induced changes in the ileal mRNA expression of *Reg3b* and *Reg3g* genes are mediated, at least in part, by intestinal microbiota.

The present study investigated serum RegIIIβ and RegIIIγ. The serum RegIIIβ levels changed in parallel with the ileal mRNA levels, whereas inconsistent data were obtained in the serum RegIIIγ levels. These results indicate that circulating RegIIIβ would reflect the ileal expression of *Reg3b* gene. Considering that circulating RegIIIβ is thought to be associated with inflammation [3] and obesity [4], influence of diet and intestinal microbiota on the inflammation and obesity may be mediated, at least in part, through circulating RegIIIβ.

HFD feeding reportedly increases the Firmicutes/Bacteroidetes ratio [12]. In the present study, the Firmicutes/Bacteroidetes ratio in the ileal contents was higher in the HFD-fed mice than in the NFD-mice and also higher in the mice receiving IMT from the HFD-fed donors than those receiving IMT from the NFD-fed donors. It is thus conceivable that the mice receiving IMT reproduced the intestinal microbiota in terms of HFD-induced increase of the Firmicutes/Bacteroidetes ratio. Because IMT itself also increased the Firmicutes/Bacteroidetes ratio, however, we have to consider that the composition of intestinal microbiota in the recipient mice would be not exactly identical to the composition of donors' microbiota. In addition, we observed that the KES supplementation increased the population of *Bifidobacterium* spp. in the

ileal contents, being consistent with our previous studies in rats and dogs [18-20]. However, the recipient mice receiving IMT from the KES-supplemented mice failed to reproduce higher population of *Bifidobacterium* spp. Because Hansen *et al.* [21] showed that dietary indigestible xylooligosaccharide increased both the population of *Bifidobacterium* spp. and the expression of RegIIIγ in mouse ileum, we had initially expected that food ingredients that promote the growth of *Bifidobacterium* spp. could upregulate the ileal expression of *Reg3b* and *Reg3g* genes. From the present results, however, it appears possible that other bacterial groups than *Bifidobacterium* spp. might contribute to KES-induced increase of ileal expression of *Reg3b* and *Reg3g* genes. Further studies are required to identify the bacteria which are involved in the regulation of intestinal expression of *Reg3b* and *Reg3g* genes.

IL-22 released from innate lymphoid cells has been considered as a regulator of intestinal RegIIIβ and RegIIIγ [2, 10]. Indeed, the present study showed a marked increase of *Reg3b* gene expression by rIL-22 supplementation in the intestinal organoids. Expression of *Il22* gene in the colon was reportedly reduced by HFD feeding [30, 31] and increased by the supplementation with an indigestible fructan inulin [31]; thus, these raise the possibility that the HFD-induced reduction and KES-induced increase of the mRNA expression of *Reg3b* and *Reg3g* genes would be mediated by IL-22. In the present study, however, neither HFD feeding nor KES supplementation influenced the expression of *Il22* gene in the ileum. Thus, it is unlikely that IL-22 is involved in the HFD- and KES-induced changes in the ileal expression of *Reg3b* and *Reg3g* genes. Alternatively, it seems possible that specific bacterial components would stimulate the expression of *Reg3b* and *Reg3g* genes directly in epithelial cells. Indeed, Natividad *et al.* [9] described that *B. breve* upregulates RegIIIγ expression in mouse ileum through TLR-MyD88 signaling in epithelial cells. Another possibility is the contribution of short-chain fatty acids (SCFA), bacterial fermentation products of indigestible saccharides. Zhao *et al.* [22] showed that genetic deletion of G protein-coupled receptor 43 (GPR43), a SCFA receptor, decreased the intestinal RegIIIγ expression in mice. They also found that SCFA supplementation induced RegIIIγ expression in mouse intestine and intestinal organoids in a

GPR43-dependent manner. Considering that intestinal SCFA production is reduced by HFD feeding [23] and increased by KES supplementation [18], SCFA could be involved in the HFD- and KES-induced changes in the ileal expression of *Reg3b* and *Reg3g* genes.

In conclusion, we propose that HFD- and KES-induced changes in the ileal expression of *Reg3b* and *Reg3g* genes and in circulating RegIIIβ levels are mediated, at least in part, by intestinal microbiota.

Acknowledgments

This work was supported in part by grants from the Center of Innovation Program from the Japan Science and Technology Agency, Grant Number JPMJCE1301. The authors have no conflict of interest to disclose.

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Figure legends

Figure 1 Effect of high-fat diet feeding (charts *A* and *C*) and 1-kestose supplementation (charts *B* and *D*) on the RegIIIβ and RegIIIγ mRNA levels estimated by RT-qPCR in the ileum (charts *A* and *B*) and serum RegIIIβ and RegIIIγ levels estimated by western blot analysis (charts *C* and *D*) in C57BL/6J mice (experiments 1 and 2). Data are expressed as means ± SEM (n = 6). NFD, normal-fat diet; HFD, high-fat diet; KES-, without 1-kestose supplementation in drinking water; KES+, 4% 1-kestose supplementation in drinking water. *, $p < 0.05$ *vs.* NFD (charts *A* and *C*) and KES- (charts *B* and *D*). Insets in charts *C* and *D* illustrate the representative western blots.

Figure 2 Effect of high-fat diet feeding and intestinal microbiota transplantation on the Firmicutes/Bacteroidetes ratio estimated by RT-qPCR in the ileal contents (chart *A*), RegIIIβ and RegIIIγ mRNA levels estimated by RT-qPCR in the ileum (charts *B* and *D*) and serum RegIIIβ and RegIIIγ levels estimated by western blot analysis (charts *C* and *E*) in C57BL/6J mice (experiment 3). Data are expressed as means ± SEM (n = 6). NFD, normal-fat diet; HFD, high-fat diet. Two-way ANOVA was used to compare mean values between four groups, and *p* values for the effect of HFD (Diet), intestinal microbiota transplantation (IMT) and interaction effect of HFD and intestinal microbiota transplantation (Diet x IMT) are shown.

Figure 3 Effect of 1-kestose supplementation and intestinal microbiota transplantation on the population of genus *Bifidobacterium* estimated by RT-qPCR in the ileal contents (chart *A*), RegIIIβ and RegIIIγ mRNA levels estimated by RT-qPCR in the ileum (charts *B* and *D*) and serum RegIIIβ and RegIIIγ levels estimated by western blot analysis (charts *C* and *E*) in C57BL/6J mice (experiment 4). Data are expressed as means ± SEM (n = 6). KES-, without 1-kestose supplementation in the drinking water; KES+, 4% 1-kestose supplementation in the drinking water. Two-way ANOVA was used to compare mean values between four groups, and *p* values for the effect of KES (Diet), intestinal microbiota transplantation (IMT) and interaction effect of KES and intestinal microbiota transplantation (Diet x IMT) are shown. In

chart *C*, values not sharing the same letters are significantly different ($p < 0.05$).

Figure 4 Relationship between IL-22 and RegIIIβ and RegIIIγ. Chart *A* shows the effect of recombinant IL-22 (rIL-22) supplementation on the mRNA expression of *Reg3b* gene in murine intestinal organoids. Inset illustrates a representative light-microscopic visualization of intestinal organoids on day 6 of culture, and bar represents 100 μm. Charts *B* and *C* show the effect of high-fat diet feeding and 1-kestose supplementation on the ileal mRNA expression of *Il22* gene in C57BL/6J mice. Data are expressed as means ± SEM (n = 6). NFD, normal-fat diet; HFD, high-fat diet; KES-, without 1-kestose supplementation in drinking water; KES+, 4% 1-kestose supplementation in drinking water.

Supplementary Table 1 Primer sequences for murine genes.

Gene	Forward	Reverse
Reg3b	ACAGACAAGATGCTGCCTCC	GAGCCCTTGGGGCAACTAAT
Reg3g	AGCCACAAGCAAGATCCCAA	GGCCATAGTGCACACAGAGT
Il22	CGATCTCTGATGGCTGTCCT	ACGCAAGCATTTCTCAGAGA
Actb	CTGGGACGATATGGAGAAGA	AGAGGCATACAGGGACAACA

Supplementary Table 2 Primer sequences for bacteria.

Taxon	Forward	Reverse
Phylum Firmicutes	GTCAGCTCGTGTCGTGA	CCATTGTAKYACGTGTGT
Phylum Bacteroidetes	AGCAGCCGCGGTAAT	CTAHGCATTTCACCGCTA
Bifidobacterium spp.	TCGCGTCYGGTGTGAAAG	CCACATCCAGCRTCCAC

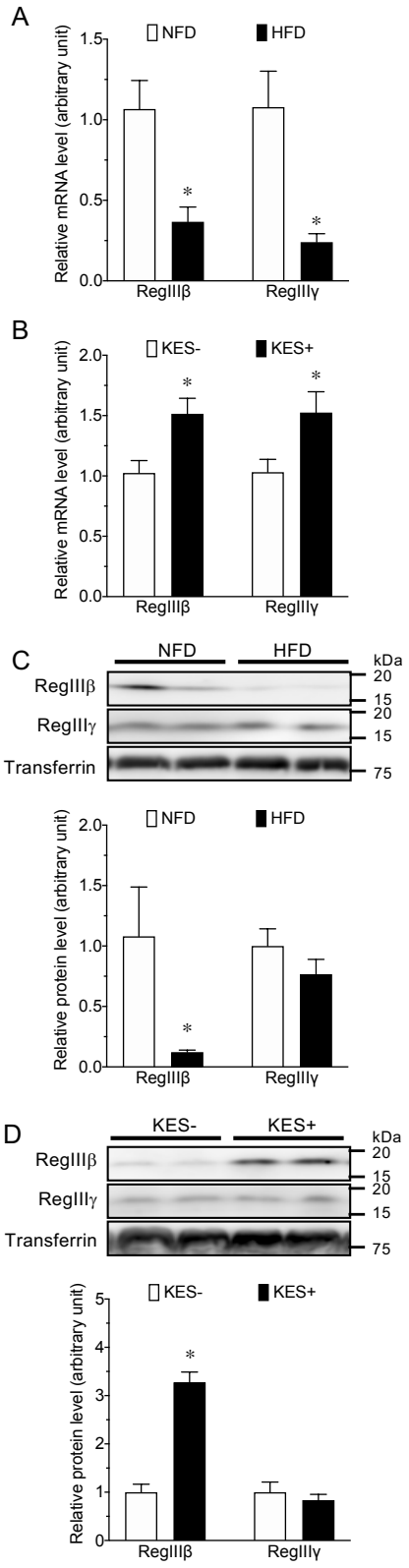


Fig. 1 Udomsopagit *et al.*

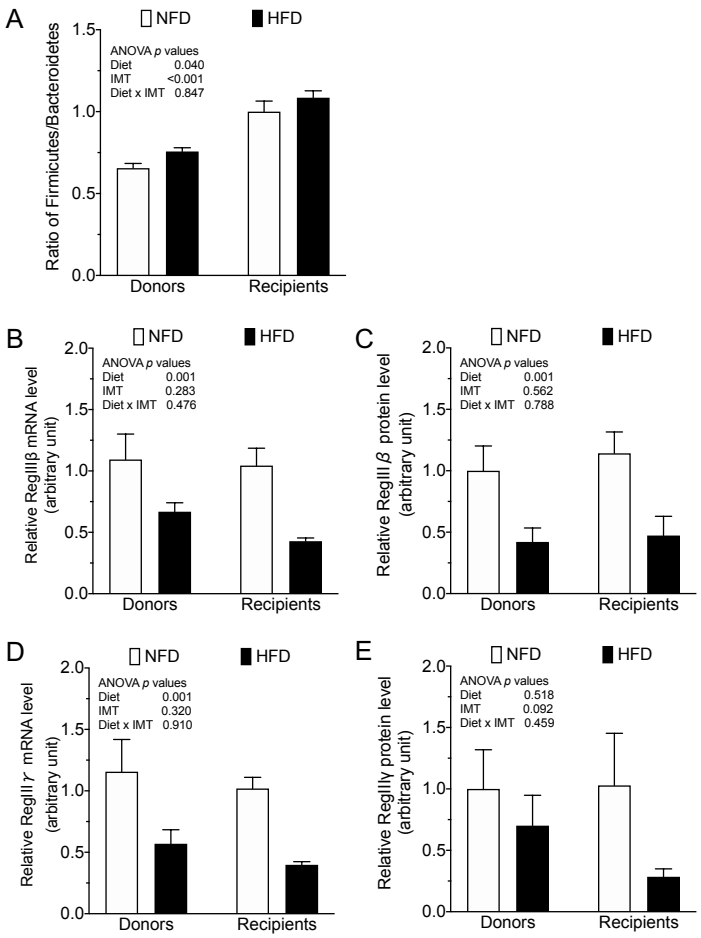


Fig. 2 Udomsopagit *et al.*

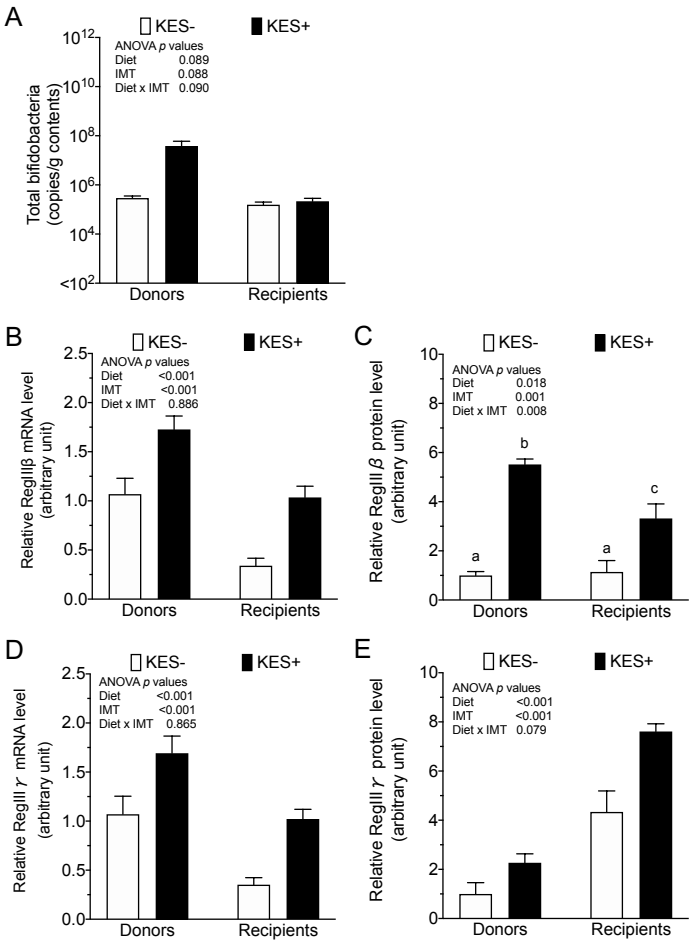


Fig. 3 Udomsopagit *et al.*

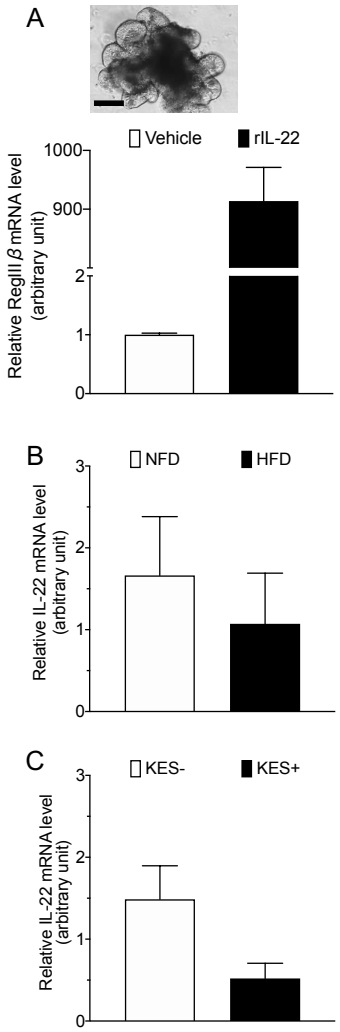
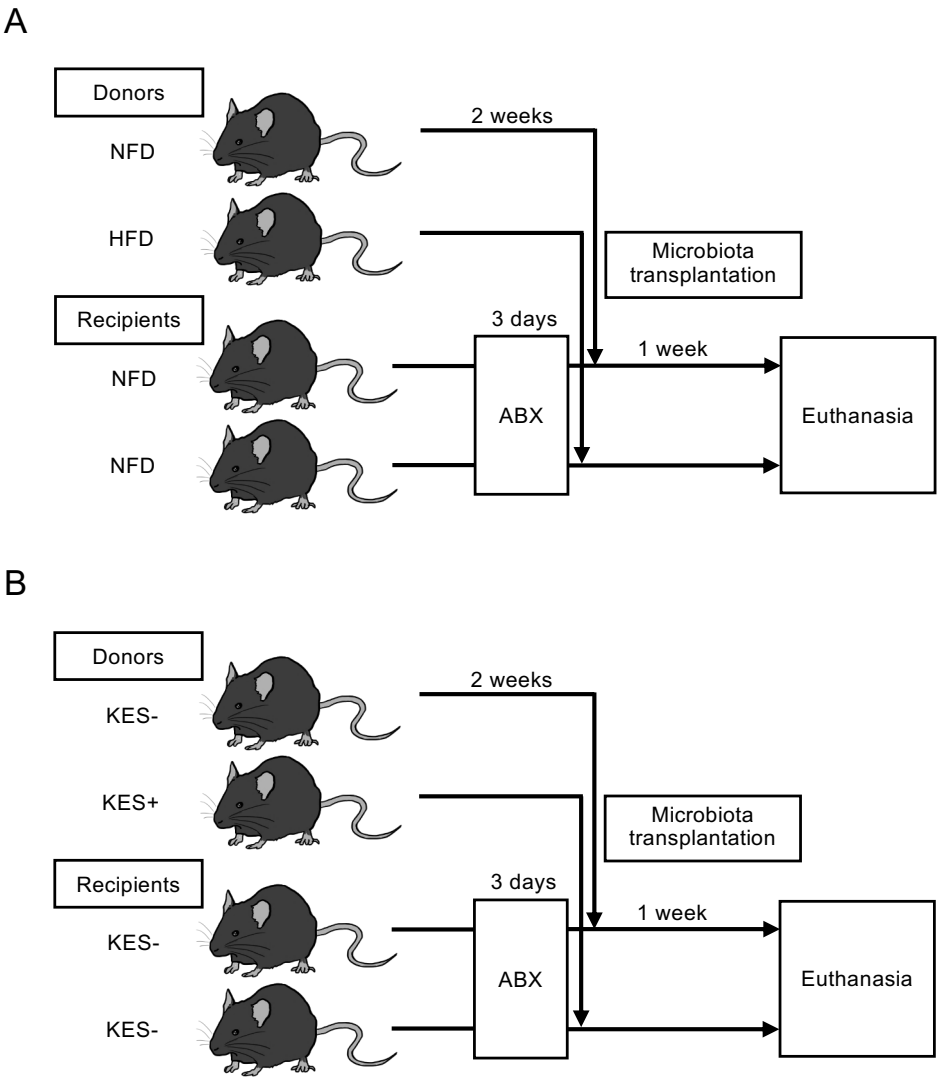
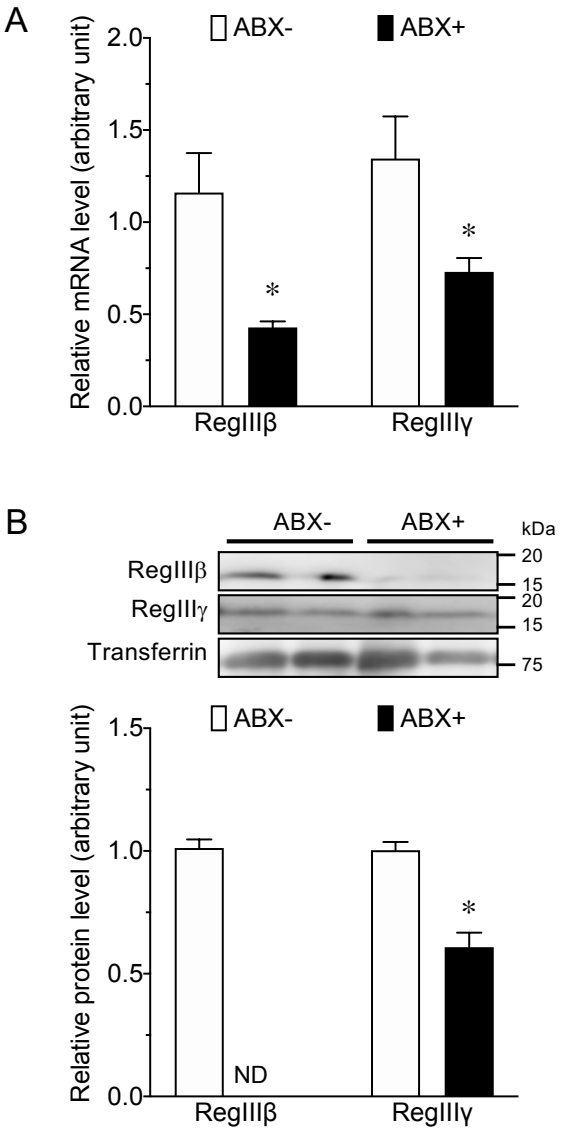


Fig. 4 Udomsopagit *et al.*



Supplementary Fig. 1 Diagram of intestinal microbiota transplantation experiments in C57BL/6J mice. Charts *A* and *B* show the diagram of experiment 3 and 4, respectively. NFD, normal-fat diet; HFD, high-fat diet; ABX, antibiotics; KES-, without 1-kestose supplementation in drinking water; KES+, 4% 1-kestose supplementation in drinking water.



Supplementary Fig. 2 Effect of antibiotics treatment for 3 days on the RegIIIβ and RegIIIγ mRNA levels estimated by RT-qPCR in the ileum (A) and serum RegIIIβ and RegIIIγ levels estimated by western blot analysis (B) in C57BL/6J mice. ABX-, without antibiotics supplementation in drinking water; ABX+, antibiotics supplementation in drinking water; ND, not detectable. *, $p < 0.05$ vs. ABX-. Insets in B illustrate the representative western blots.