Supplementary Materials

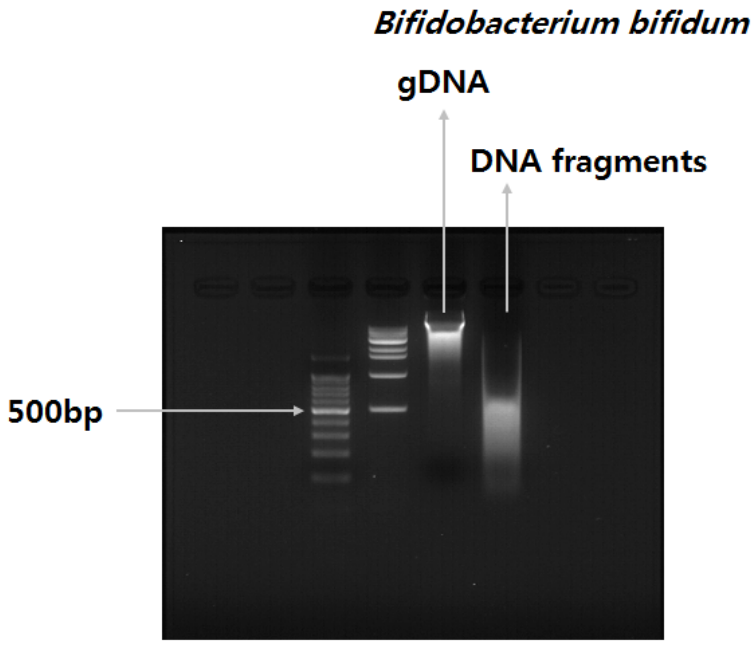

Fig. S1. Preparation of *Bifidobacterium bifidum* CpG ODN. gDNA was extracted from the *Bifidobacterium bifidum* CBT-BF3 and digested with restriction enzyme Sau3AI. The size of the DNA fragments was confirmed by 2% agarose gel electrophoresis, and fragmented gDNA (fgDNA) of less than 500 bp in length was used as CpG OND *B. bifidum.* CpG ODN, cytosine-phosphate-guanosine oligodeoxynucleotide.

Table S1. RT-qPCR primer sequences for analyzing the expression levels of transcription factors and cytokines of Th1, Th2, Th17, Treg cells and *Ga-9*, *FLG*, *TSLP* in the mLNs and spleen

Target gene		Primer	References
GAPDH	Forward	5' CCACCCAGAAGACTGTGGAT 3'	Hwang et al. (2013)
	Reverse	5' CACATTGGGGGTAGGAACAC 3'	
T-bet	Forward	5' TCAACCAGCACCAGACAGAG 3'	van Hamburg et al. (2008)
	Reverse	5' AAACATCCTGTAATGGCTTGTG 3'	
GATA-3	Forward	5' CATTACCACCTATCCGCCCTATG 3'	van Hamburg et al. (2008)
	Reverse	5' CACACACTCCCTGCCTTCTGT 3'	
RORγT	Forward	5' TTCACCCCACCTCCACTG 3'	van Hamburg et al. (2008)
	Reverse	5' TGCAAGGGATCACTTCAATTT 3'	
Foxp3	Forward	5' CCCATCCCCAGGAGTCTTG 3'	Kwon et al. (2010)
	Reverse	5' CCATGACTAGGGGCACTGTA 3'	
IFN-γ	Forward	5' TCAAGTGGCATAGATGTGGAAGAA 3'	Kwon et al. (2010)
	Reverse	5' TGGCTCTGCAGGATTTTCATG 3'	
IL-4	Forward	5' ACAGGAGAAGGGACGCCAT 3'	Kwon et al. (2010)
	Reverse	5' GAAGCCCTACAGACGAGCTCA 3'	
IL-17	Forward	5' TTCATCTGTGTCTCTGATGCT 3'	Kwon et al. (2010)
	Reverse	5' TTGACCTTCACATTCTGGAG 3'	
TGF-β	Forward	5' GAAGGCAGAGTTCAGGGTCTT 3'	Kwon et al. (2010)
	Reverse	5' GGTTCCTGTCTTTGTGGTGAA 3'	
Gal-9	Forward	5' GAGAGGAAGACACACATGCCTTTC 3'	Chabot et al. (2002)
	Reverse	5' GACCACAGCATTCTCATCAAAACG 3'	
FLG	Forward	5' CACTGAGCAAAGAAGAGCTGAA 3'	Shin et al. (2016)
	Reverse	5' CGATGTCTTGGTCATCTGGA 3'	
TSLP	Forward	5' AGAGAAGCCCTCAATGACCAT 3'	Shin et al. (2016)
	Reverse	5' GGACTTCTGTGCCATTTCC 3'	

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; *T-bet*, T-box expressed in T cells; *GATA-3*, GATA binding protein 3; *RORγT*, retinoic acid receptor-related orphan gamma T; *Foxp3*, forkhead box P3; *IFN-γ*, interferon-γ; *IL-4*, interleukin-4; *TGF-β*, transforming growth factor beta; *Gal-9*, galectin-9; *FLG*, filaggrin; *TSLP*, thymic stromal lymphopoietin.

Table S2. qPCR primer sequences for ten significant gut microbes exhibiting obesity, anti-obesity, butyric acid, and lactic acid-producing traits

Target gene		Primer	References
Universal	Forward	GTGSTGCAYGGYYGTCGTCA	Fuller et al. (2007)
	Reverse	ACGTCRTCCMCNCCTTCCTC	
Bacteroides spp.	Forward	GAAGGTCCCCCACATTG	Bartosch et al. (2004)
	Reverse	CGCKACTTGGCTGGTTCAG	Ramirez-Farias et al. (2009)
Roseburia spp. & *Eubacterium rectale*	Forward	GCGGTRCGGCAAGTCTGA	Walker et al. (2005)
	Reverse	CCTCCGACACTCTAGTMCGAC	Ramirez-Farias et al. (2009)
Faecalibacterium prausnitzii	Forward	GGAGGAAGAAGGTCTTCGG	Wang et al. (1996)
	Reverse	AATTCCGCCTACCTCTGCACT	Ramirez-Farias et al. (2009)
Cluster IV *Ruminococcus* spp.	Forward	GGCGGCYTRCTGGGCTTT	Ramirez-Farias et al. (2009)
	Reverse	CCAGGTGGATWACTTATTGTGTTAA	
Bifidobacterium spp.	Forward	TCGCGTCYGGTGTGAAAG	Rinttilä et al. (2004)
	Reverse	GGTGTTCTTCCCGATATCTACA	Matsuki et al. (2002)
Methanogens	Forward	GGATTAGATACCCSGGTAGT	Hook et al. (2009)
	Reverse	GTTGARTCCAATTAAACCGCA	
Oscillospira spp.	Forward	ACGGTACCCCTTGAATAAGCC	Mackie et al. (2003)
	Reverse	TCCCCGCACACCTAGTATTG	Yanagita et al. (2003)
Leuconostoc mesenteroides	Forward	TGATGCATAGCCGAGTTGAG	Yu et al. (2018)
	Reverse	GAAAGCCTTCATCACACACG	
Leuconostoc citreum	Forward	GGAAACAGATGCTAATACCGAATA	Yu et al. (2018)
	Reverse	TTTACCCCACCAACTAACTAATG	
Weissella cibaria	Forward	GGGAAACCTACCTCTTAGCA	Yu et al. (2018)
	Reverse	GGACCATCTCTTAGTGATAGCA	
Weissella koreensis	Forward	GGGCTACACACGTGCTACAA	Yu et al. (2018)
	Reverse	GATTCCGACTTCGTGTAGGC	
Lactobacillus sakei	Forward	CCATGTGTAGCGGTGAAATG	Yu et al. (2018)
	Reverse	ATCCTGTTTGCTACCCATGC	

Transcription factors and cytokines in Th1, Th2, Th17 and Treg cells in the spleen

Microbiome-derived Toll-like receptor ligands and metabolites act directly on enterocytes and intestinal immune cells. However, they can also travel via systemic circulation to modulate immunity in remote tissues such as the spleen (Shao et al., 2016). The spleen is the most significant secondary lymphoid tissue in the body of animals. It contains various immune cell populations, including CD4+ and CD8+ T cells, essential for anti-infection immune responses (Lewis et al., 2019).

In the spleen, the expression levels of Th1 transcription factor *T-bet* and cytokine *IFN-γ* genes did not differ between the C and N groups. However, *T-bet* was higher in the T1 and T2 groups compared to the N group, and *IFN-γ* was higher in the T1 and T3 groups ($p<0.05$; Supplementary Fig. S2A). There was no difference in the expression levels of the Th2 cell transcription factor *GATA3* and cytokine *IL-4* genes between the C and N groups. However, the expression level of the *GATA3* gene was lower in the T1 and T3 groups compared to the N group, and the expression level of the *IL-4* gene was lower in the T3 group ($p<0.05$; Supplementary Fig. S2B). The expression level of the transcription factor *RORγ* gene in Th17 cells did not differ between the C and N groups but decreased in the T2 and T3 groups compared to the N group ($p<0.05$; Supplementary Fig. S2C). The expression level of the cytokine *IL-17* gene increased in the N group compared to the C group and decreased in the T groups compared to the N group ($p<0.05$). The expression level of the transcription factor *TGF-β* gene in Treg cells decreased in the N group compared to the C group and increased in the T1 and T2 groups compared to the N group ($p<0.05$; Supplementary Fig. S2D). The expression level of the cytokine *Foxp3* gene did not differ between the C and N groups but increased in the T1 and T2 groups compared to the N group ($p<0.05$). Treg cell activity was low in the N group but increased in the T1 and T2 groups. Treating AD mice with the three types of *Bifidobacterium bifidum* increased the expression levels of transcription factors or cytokine genes in the Th1 and Treg cells of the spleen and inhibited their expression in Th2 and Th17 cells. In particular, probiotic *B. bifidum* was effective in inducing Th1 activity, postbiotic *B. bifidum* was effective in inducing Treg activity, and CpG ODN *B. bifidum* was effective in suppressing Th2 and Th17 activity.

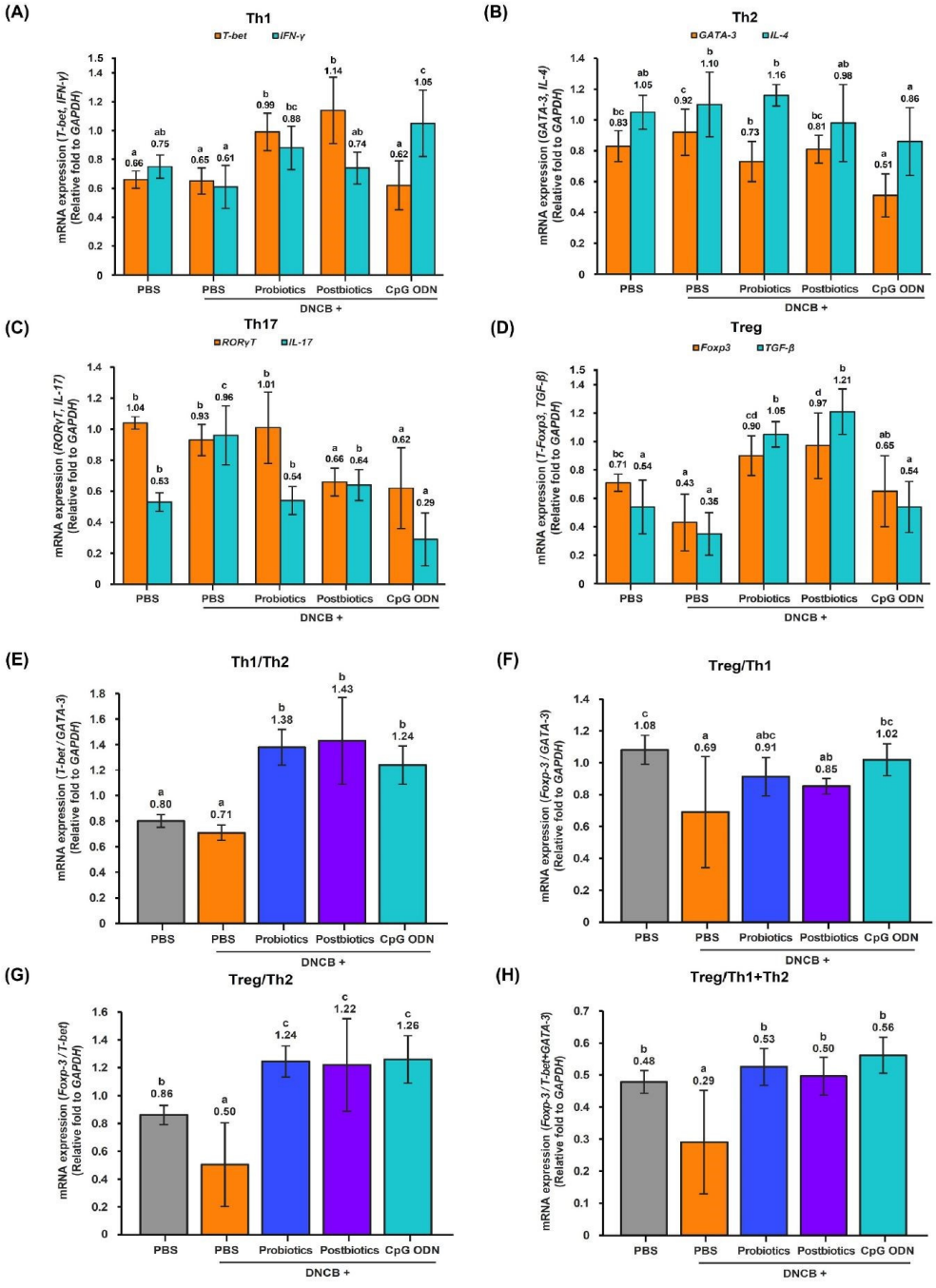

Fig. S2. Effects of dietary probiotic *Bifidobacterium bifidum*, postbiotic *B. bifidum*, and CpG ODN *B. bifidum* on T-cell transcription factor and cytokine expression in the spleen of DNCB-induced AD NC/Nga mice. (A) Th1 (*T-bet*, *IFN*-γ), (B) Th2 (*GATA-3*, *IL-4)*, (C) Th17 (*RORγT*, *IL-17*), (D) *Treg (Foxp3, TGF-β*), (E) Th1/Th2 ratio (*T-bet/GATA-3*), (F) Treg/Th1 ratio (*Foxp3/T-bet*), (G) Treg/Th2 ratio (*Foxp3/GATA-3*), (H) Treg/Th1+Th2 ratio (*Foxp3/T-bet+GATA-3*). C (control), N (negative control, DNCB), T1 (DNCB+probiotic *B. bifidum*), T2 (DNCB+probiotic *B. bifidum*), T3 (DNCB+CpG ODN *B. bifidum*). Oral administration of the three types of *B. bifidum* to AD mice resulted in increased activity of Th1 and Treg cells while suppression of activity of Th2 and Th17 cells. In particular, probiotic *B. bifidum* were effective in inducing Th1 activity, postbiotic *B. bifidum* were effective in inducing Treg activity, and CpG ODN *B. bifidum* was effective in suppressing Th2 and Th17 activity. mRNA levels were normalized to *GAPDH* mRNA levels. $^{a–d}$ Means are significantly different in each group ($p<0.05$). Data represent means±SD of 6 replicates. *T-bet*, T-box expressed in T cells; *IFN-γ*, interferon-gamma; *GATA-3*, GATA binding protein 3; *RORγT*, RAR-related orphan receptor gamma T; *Foxp3*, forkhead box P3; *TGF-β*, transforming growth factor-beta; *IL-4*, interleukin-4; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; DNCB, 2,4-dinitrochlorobenzene; CpG ODN, cytosine-phosphate-guanosine oligodeoxynucleotides.

Th1/Th2, Treg/Th1, Treg/Th2 and Treg/(Th1+Th2) balance in the spleen

Th1/Th2 balance: The expression ratio of Th1/Th2 transcription factors (*T-bet/GATA-3*) did not differ between the C and N groups but increased in the T groups compared to C and N groups ($p<0.05$). Therefore, the balance of Th1/Th2 in the AD mice spleens after treatment with the three types of *Bifidobacterium bifidum* showed predominantly Th1 activation (Supplementary Fig. S2E). Treg/Th1 balance: The expression ratio of Treg/Th1 transcription factors (*Foxp3/T-bet*) decreased in the N group compared to the C group ($p<0.05$). Additionally, it increased in the T3 group compared to the N group ($p<0.05$). In comparing the C and T groups, no significant differences occurred in the T1 and T3 groups. Therefore, the Treg/Th1 balance in the AD mice spleens treated with the three types of *B. bifidum* showed predominantly Treg activation, and postbiotic *B. bifidum* and CpG ODN *B. bifidum* were particularly effective (Supplementary Fig. S2F). Treg/Th2 balance: The expression ratio of Treg/Th2 transcription factors (*Foxp3/GATA-3*) decreased in the N group compared to the C group ($p<0.05$). Additionally, it increased in the T groups compared to the C and N groups ($p<0.05$). Therefore, the Treg/Th2 balance after treatment with the three types of *B. bifidum* in the AD mice spleens showed a preference for Treg activation (Supplementary Fig. S2G). Treg/Th1+Th2 balance: The expression ratio of Treg/Th1+Th2 transcription factors (*Foxp3/T-bet+GATA-3*) decreased in the N group compared to the C group ($p<0.05$). Additionally, it increased in the T groups compared to the N group ($p<0.05$). There was no significant difference between the C and T groups. Therefore, the Treg/(Th1+Th2) balance by the three types of *B. bifidum* treatment in the AD mice spleens showed a preference for Treg activation (Supplementary Fig. S2H).

Galectin-9, *filaggrin* and *thymic stromal lymphopoietin* in the spleen

The expression level of the *Gal-9* gene in the spleen did not show a significant difference between the C and N groups but it increased in the T3 group compared to the N group ($p<0.05$; Supplementary Fig. S3A). CpG ODN *B. bifidum* was effective in increasing *Gal-9* gene expression. There was no significant difference in the expression level of the *FLG* gene between the C and N groups, but it increased in the T2 group compared to the N group ($p<0.05$; Supplementary Fig. S3B). Postbiotic *B. bifidum* was the most effective at increasing the expression of the *FLG* gene. There was no significant difference in the *TSLP* cytokine gene expression level between the C and N groups. However, it decreased in the T3 group compared to the N group ($p<0.05$; Supplementary Fig. S3SC). CpG ODN *B. bifidum* was the most effective at suppressing the expression of the *TSLP* cytokine gene. In the spleens of AD mice, CpG ODN *B. bifidum* was effective at inducing the expression of the *Gal-9* gene, postbiotic *B. bifidum* was effective at inducing the expression of the *FLG* gene, and CpG ODN *B. bifidum* was effective at suppressing the expression of *TSLP* cytokine gene.

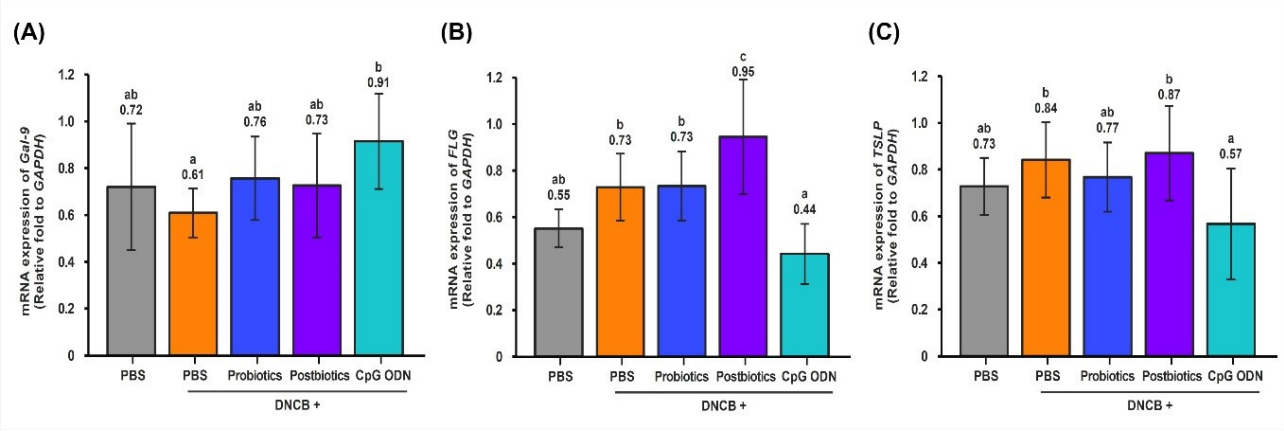

Fig. S3. Effects of dietary probiotic *Bifidobacterium bifidum*, postbiotic *B. bifidum*, and CpG ODN *B. bifidum* on *Gal-9*, *FLG*, and *TSLP* gene expression in spleen of DNCB-induced AD NC/Nga mice. (A) Gal-9, (b) *FLG*, (C) *TSLP*. C (control), N (negative control, AD); T1 (DNCB+probiotic *B. bifidum*), T2 (DNCB+postbiotic *B. bifidum*), T3 (DNCB+CpG ODN *B. bifidum*). In the spleen of AD mice, CpG ODN *B. bifidum* was effective in inducing the expression of the *Gal-9* gene, postbiotic *B. bifidum* was effective in inducing the expression of the *FLG* gene, and CpG ODN *B. bifidum* was effective in suppressing the expression of the *TSLP* gene. mRNA levels were normalized to *GAPDH* mRNA levels. $^{a-c}$ Data represent means±SD of 6 replicates. Means are significantly different in each group ($p<0.05$). AD, atopic dermatitis; DNCB, 2,4-dinitrochlorobenzene; CpG ODN, cytosine-phosphate- guanosine oligodeoxynucleotides; *Gal-9*, galectin-9; *FLG*, *filaggrin*; *TSLP*, thymic stromal lymphopoietin; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenas.