### **Supplemental material**

### **1 Supplemental Methods:**

#### 1.1 Isolation of Peyer's patch (PP) -specific Lactobacilli.

The PPs were aseptically removed, and tissue specimens were placed in Betadine antiseptic solution (Seton Healthcare Group plc, Oldham, UK) for 3 min to disinfect the surface. Subsequently, tissues were vortexed in multiple 500-mL aliquots of phosphatebuffered saline to encourage the removal of any bacteria on the tissue surface. Final washes were retained and analyzed by both culture-dependent and culture-independent techniques to determine whether surface decontamination was successful. After homogenization, 1 g of sample was suspended in 10 ml of 0.85% NaCl and diluted by 10<sup>3</sup>, then 1 ml of each dilution was inoculated into 5 mL of Mann Rogosa Sharp (MRS) broth and incubated in a CO<sub>2</sub> saturated anaerobic chamber at 30° C for 24 h. In order to increase specificity, cultures obtained from the more diluted samples were spread onto *Lactobacillus* anaerobic MRS plates with vancomycin and bromocresol green agar (LAMVAB) to isolate *Lactobacillus*. Single colonies randomly taken from the LABVAB were purified by streaking out twice on MRS broths under anaerobic conditions at 37° C. Purity of the isolates was confirmed by repeated streaking and sub-culturing in fresh MRS agar, followed by microscopic examination.

#### 1.2 Amplified fragment length polymorphism (AFLP) typing.

For discrimination of the strains, the AFLP analysis method was performed using chromosomal DNA as described previously<sup>1</sup>. Briefly, total DNA was digested with EcoRI and MseI restriction enzymes and the DNA fragments were ligated to doublestranded restriction site-specific adaptors, EcoRI-adaptors and MseI-adaptors (supplementary table 1). For the pre-selective and selective PCR amplification, primers EcoR1-core/Mse1-core and EcoR1-G/ Mse1- CC were used, respectively. The 5' end of EcoRI primers were labeled with 6-carboxy-fluorescine (FAM). PCR products were analyzed on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) and the AFLP patterns were analyzed and extracted with GeneMapper software v4.0 (Applied Biosystems). Peak height thresholds were set at 200. Bands of the same size in different individuals were assumed to be homologous and to represent the same allele. Bands of different sizes were treated as independent loci and data were exported in a binary format with '1' representing the presence of a band/peak and '0' representing its absence. Data were analyzed using NTSYS-pc software (Exeter Software, Biostatistics, Inc., NY, USA) version 2.1. The similarity coefficient was determined using the similarity program for qualitative data (SIMQUAL) by using the Dice similarity coefficient. Cluster analysis

was performed to construct a tree plot using the unweighted pair-group method with arithmetic averages (UPGMA) in the SAHN program of the NTSYS-pc software.

#### **1.3 Isolation of the epithelium from the crypts.**

Crypt epithelia were isolated from jejunum according to the method described by Flint <sup>2</sup> with minor modification. Briefly, intestines pieces were washed in HBSS with 0.5 mM DTT for 5 min at 4 °C with constant stirring at 200 rpm for 30 min. Detached tissues pellets were transferred to 115 mL of chelating buffer, incubated at 4 °C for 20 min with constant stirring at 100 rpm. Suspension were re-suspended in 25 mL of fresh chelating buffer in a 50 mL centrifuge tube for washing by inverting the tube for 60 times at 2~8 °C. Detached pieces were collected in a new tube. Fresh chelating buffer (20 mL) was added to the wash tube and washed for three times. Detached pieces were collected every time. The purity of crypts was examined by microscopy. Samples were harvested by centrifugation under 1000 rpm for 5 min and stored at -80 °C after addition of 1 mL of Trizol.

#### REFERENCE

- 1 K. Watanabe, J. Fujimoto, Y. Tomii, M. Sasamoto, H. Makino, Y. Kudo and S. Okada, *Int. J. Syst. Evol. Microbiol.*, 2009, **59**, 754–760.
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- 9 C. Corinaldesi, R. Danovaro and A. Dell'Anno, *Appl. Environ. Microbiol.*, 2005, **71**, 46–50.
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# 2 Supplemental Tables:

Oligonucleotide	Sequence (5'-3')	
AFLP adapters		
EcoRI adapter	CTCGTAGACTGCGTACC	
	CATCTGACGCATGGTTAA	
MseI adapter	GACGATGAGTCCTGAG	
	CTACTCAGGACTCAT	
Core sequences of AFLP primers without selective		
bases		
EcoRI core	GACTGCGTACCAATTC	
MseI core	GATGAGTCCTGAGTAA	
Selective amplification		
EcoR1-G	GACTGCGTACCAATTCG	
Mse1-CC	GATGAGTCCTGAGTAACC	

Table S1. Adapter and primer oligonucleotides used in AFLP analyses

Gene name	Sequence (5'-3')
Tlr2	F:AAAATGTCGTTCAAGGAG
	R:TTGCTGAAGAGGACTGTT
Tlr4	F:GGAACAAACAGCCTGAGACAC
	R:CAAGGGATAAGAACGCTGAGAA
Tlr9	F:GGTGTGGAACATCATTCT
	R:ATACGGTTGGAGATCAAG
Nod2	F:ACAGCACGTCAGGGAACTACCAG
	R:CAGGCAAAGATTCTCCGACCC
Myd88	F:TGGCATGCCTCCATCATAGTTAACC
	R:GTCAGAAACAACCACCACCATGC
Nfkb	F:AGGCTTCTGGGCCTTATGTG
	R:TGCTTCTCTCGCCAGGAATAC
CRS1C	F:TGCTCTTCAAGATGTAGCCCAACG
	R:TGGAGCTTGGGTGGTGATTGCA
CRS4C	F:GCATGGAATCTGGGTCAAGATAAC
	R:AGAAGGAAGAGCAATCAAGGCTAAG
RegIIIγ	F:TTCCTGTCCTCCATGATCAAAA
	R:CATCCACCTCTGTTGGGTTCA
defcr-rs10	F:ATCATCCAGGTGATTCCCAGCCAT
	R:TTCCGGGTCTCCAAAGGAAACAGA
β-actin	F:GGGTCAGAAGGACTCCTATG
	R:GTAACAATGCCATGTTCAAT

TableS2. Primer sequence used for Real-time reverse transcription-PCR for gene expression of crypts

Target	primer	Sequence (5'-3')	Annealing T	reference
bacterial group			(°C)	
All bacteria	F-Eub 338	ACTCCTACGGGAGGCAGCAG	60	3
	R-Eub 518	ATTACCGCGGCTGCTGG		
Firmicutes	Firm934F	GGAGYATGTGGTTTAATTCGAAGC	60	4
		А		
	Firm1060R	AGCTGACGACAACCATGCAC		
Bacteroidetes	Bact934F	GGARCATGTGGTTTAATTCGATGAT	60	4
	Bact1060R	AGCTGACGACAACCATGCAG		
Bacteroides	-	CGATGGATAGGGGTTCTGAGAGGA	60	5
	-	GCTGGCACGGAGTTAGCCGA		
Lactobacillus	Lacl	AGCAGTAGGGAATCTTCCA	55	6
	Lab-0677r	CACCGCTACACATGGAG		

Table S3 Group-Specific Primers Based on 16S rRNA Sequences Used for qRT- PCR

Reference

- 1 K. Watanabe, J. Fujimoto, Y. Tomii, M. Sasamoto, H. Makino, Y. Kudo and S. Okada, *Int. J. Syst. Evol. Microbiol.*, 2009, **59**, 754–760.
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primer	Sequence(5'-3')	Target	Procedure	Reference
27F 534R	AGAGTTTGATCCTGGCTCAG ATTACCGCGGCTGCTGG	v1-v3	96°C, 1min; 55 °C, 1min; 72 °C, 1min	7
27F 907R	AGAGTTTGATCCTGGCTCAG CCGTCAATTCCTTTGAGTTT	v1-v5	94°C, 1min; 55 °C, 1min; 72 °C, 2min	8
27F 1492R	AGAGTTTGATCCTGGCTCAG GGTTACCTTGTTACGACTT	v1-v9	96°C, 1min; 55 °C, 1min; 72 °C, 1min	9
334F 939R	CCAGACTCCTACGGGAGGCAGC CTTGTGCGGGCCCCCGTCAATTC	v3-v5	94°C, 1min; 69 °C, 1min; 72°C, 1.5min	10
968F 1401R	AACGCGAAGAACCTTAC CGGTGTGTACAAGACCC	v6-v8	94 °C, 20s; 54 °C, 20s; 68 °C, 40s	8
530F 1492R	GTGCCAGCMGCCGCGG GGTTACCTTGTTACGACTT	v4-v9	94 °C, 1min; 55 °C, 1min; 72 °C, 2min	8

Table S4. Primers with different amplification regions and its reaction solution.

#### REFERENCE

- 1 K. Watanabe, J. Fujimoto, Y. Tomii, M. Sasamoto, H. Makino, Y. Kudo and S. Okada, *Int. J. Syst. Evol. Microbiol.*, 2009, **59**, 754–760.
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Enzyme	Forward fragment	Reverse fragment
AflIII	119, 134, 348, 363. 474, 507, 511, 524	74, 130, 132, 259, 473, 488, 489,
DdeI	57, 124, 131, 135, 138, 159, 204, 259,	89, 90, 91, 97, 114, 179, 182, 183,
	260, 261, 267, 291, 317, 372, 373, 387,	185, 229, 446
	398, 412, 415, 419, 420, 421, 422, 423	
EcoRI	288, 312, 316, 333, 338, 339, 340	260, 261, 264, 265, 266, 267
DpnII	90	104, 105, 235, 237, 455, 492, 515,
		516, 517, 518, 519, 520, 521
HinfI	29	92, 286, 314
Sau96I	207, 208, 212, 281, 375, 392, 395, 397,	103, 104, 202, 204, 205, 206, 207,
	398, 399, 400	209, 398, 399, 400, 401
AluI	61, 100, 156, 244, 282, 284	65, 78, 79, 451, 454, 500, 503, 504,
		507
MaeII	119, 121, 125, 136, 148, 149	74, 257, 403, 404, 405, 406, 407,
		408, 409, 412, 456, 457, 458, 461

Table S5: The fragment profiles with virtual digestion online.

## **3** Supplemental Figure.

# Figure S1



Figure S1 Gel electrophoresis of PCR products with primers a-f for eDNA amplication. a, 27F/534R; b, 27F/907R; c, 27F/1492R; d, 334F/939R; e, 968F/1401R; f, 530F/1492R.