Additional file 1

Additional material related to:

Administration of two probiotic strains during early childhood does not affect the endogenous gut microbiota composition despite probiotic proliferation

by

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Content:

Table S1 – Primers used to detect and quantify BB12 and LGG

Primers	**Sequence (5'-3')**	**Primer length**	**Tm (°C)**	**GC %**	**Product size (bp)**	**Target**
BB12_F	ACTTCAATCGCTCGGCTGATGTA	23	65.3	47.8	154	Type I-U CRIPSR-Cas7 gene [1]
BB12_R	TCGTAGCTCCTGCAAAATACACT	23	60.3	43.5		
LGG_F	GTCAACCATTGCTTGCCCTT	20	62.3	50.0	202	Phage-related gene [2]
LGG_R	AGAAAAGACACCGGGTTTGC	20	61.4	50.0		

Table S2 – Strains used for evaluation of primer specificity

Strain	**Culture Collection**
Lactobacillus rhamnosus GG	ATCC 53103
Lactobacillus rhamnosus	DSM 20021
Lactobacillus paracasei subsp. *paracasei* 85E	DSM 20006
Lactobacillus acidophilus L917	DSM 20079
Lactobacillus johnsonii VPI 7660	DSM 10533
B. animalis subsp. *lactis* UR1	DSM 10140
B. animalis subsp. *lactis* BB-12	Chr. Hansen A/S
B. animalis subsp. *lactis* BL-04	SD5219
B. breve S50	DSM 20091
B. animalis subsp. *animalis*	DSM 20104
B. pseudolongum subsp. *pseudolongum*	DSM 20099

Table S3 – OTUs with crude p-value below 0.05 for differential abundance comparing baseline samples of completers assigned to probiotics (n = 95) vs. placebo (n = 89).

OTU ID	**P-value[a]**	**FDR P-value[b]**	**Mean abundance (%)**		**Green Genes taxonomy**
			Placebo (n = 89)	**Probiotics (n = 95)**	
OTU_76	0.025	0.996	0.035%	0.085%	*Erysipelotrichaceae*
OTU_374	0.026	0.996	0.022%	0.053%	*Moryella*
OTU_916	0.031	0.996	0.140%	0.021%	*Bacteroides caccae*
OTU_106	0.033	0.996	0.048%	0.011%	*Sutterella*
OTU_304	0.040	0.996	0.008%	0.002%	*Erysipelotrichaceae*
OTU_800	0.043	0.996	0.003%	0.005%	*Lachnospiraceae*

[a] P-value according to Mann Whitney test.
[b] False Discovery Rate correction of the P-values.

Table S4 – OTUs with crude p-value below 0.05 for differential abundance comparing samples with BB-12® growth (n = 86) vs. placebo (n = 98).

OTU ID	P-value[a]	FDR P-value[b]	Mean abundance (%)		Green Genes taxonomy
			Placebo (n = 98)	BB-12® growth (n = 86)	
OTU_3	0.009	0.983	7.644%	4.921%	*Bifidobacterium*
OTU_334	0.021	0.983	0.001%	0.051%	*Sutterella*
OTU_60	0.022	0.983	0.117%	0.182%	*[Barnesiellaceae]*
OTU_290	0.031	0.983	0.004%	0.011%	*Butyricimonas*
OTU_6	0.034	0.983	5.504%	3.293%	*Pseudobutyrivibrio*
OTU_575	0.036	0.983	<0.001%	0.062%	*[Eubacterium] biforme*
OTU_181	0.042	0.983	0.015%	0.003%	*Lactococcus*
OTU_25	0.042	0.983	1.457%	0.949%	*Ruminococcus bromii*
OTU_313	0.048	0.983	0.173%	0.143%	*Bacteroides acidifaciens*

[a] P-value according to Mann Whitney test.

[b] False Discovery Rate correction of the P-values.

Table S5 – OTUs with crude p-value below 0.05 for differential abundance comparing samples with LGG® growth (n = 46) vs. placebo (n = 98).

OTU ID	P-value[a]	FDR P-value[b]	Mean abundance (%)		Green Genes taxonomy
			Placebo (n = 98)	LGG® growth (n = 46)	
OTU_575	0.0194	0.974	<0.001%	0.087%	*[Eubacterium] biforme*
OTU_6	0.0369	0.974	5.504%	2.464%	*Pseudobutyrivibrio*
OTU_217	0.0383	0.974	0.000%	<0.001%	*Clostridiales*
OTU_230	0.0383	0.974	0.000%	0.002%	*Ruminococcaceae*
OTU_189	0.047	0.974	0.014%	0.000%	*Megasphaera*
OTU_575	0.0194	0.974	<0.001%	0.087%	*[Eubacterium] biforme*

[a] P-value according to Mann Whitney test.

[b] False Discovery Rate correction of the P-values.

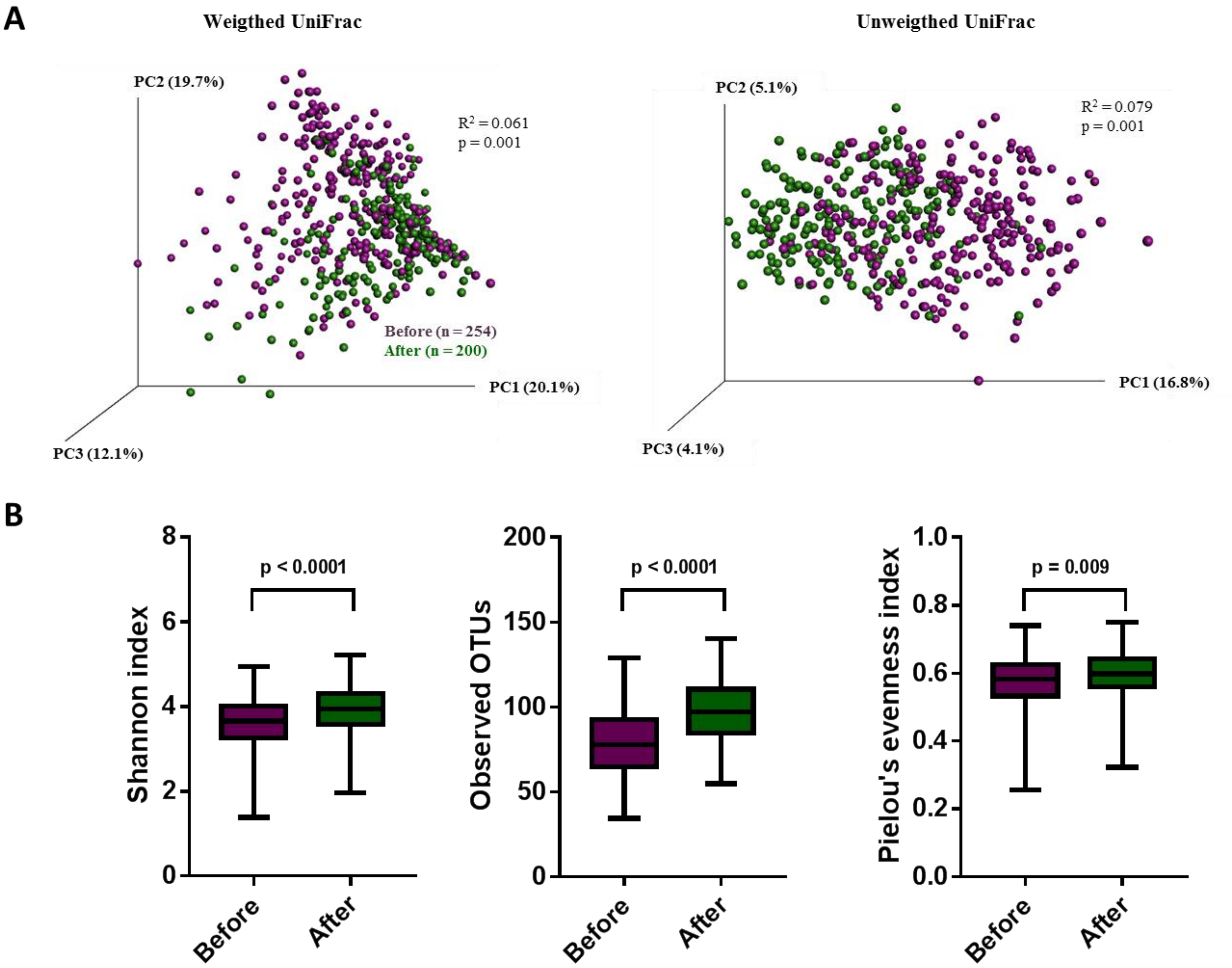

Figure S1 – Gut microbiota changes over the course of the 6 months intervention.

A) PCoA plots of weighted and unweighted UniFrac distances of the gut microbiota, B) boxplots of gut microbial alpha diversity measures before (purple) and after (green) intervention.

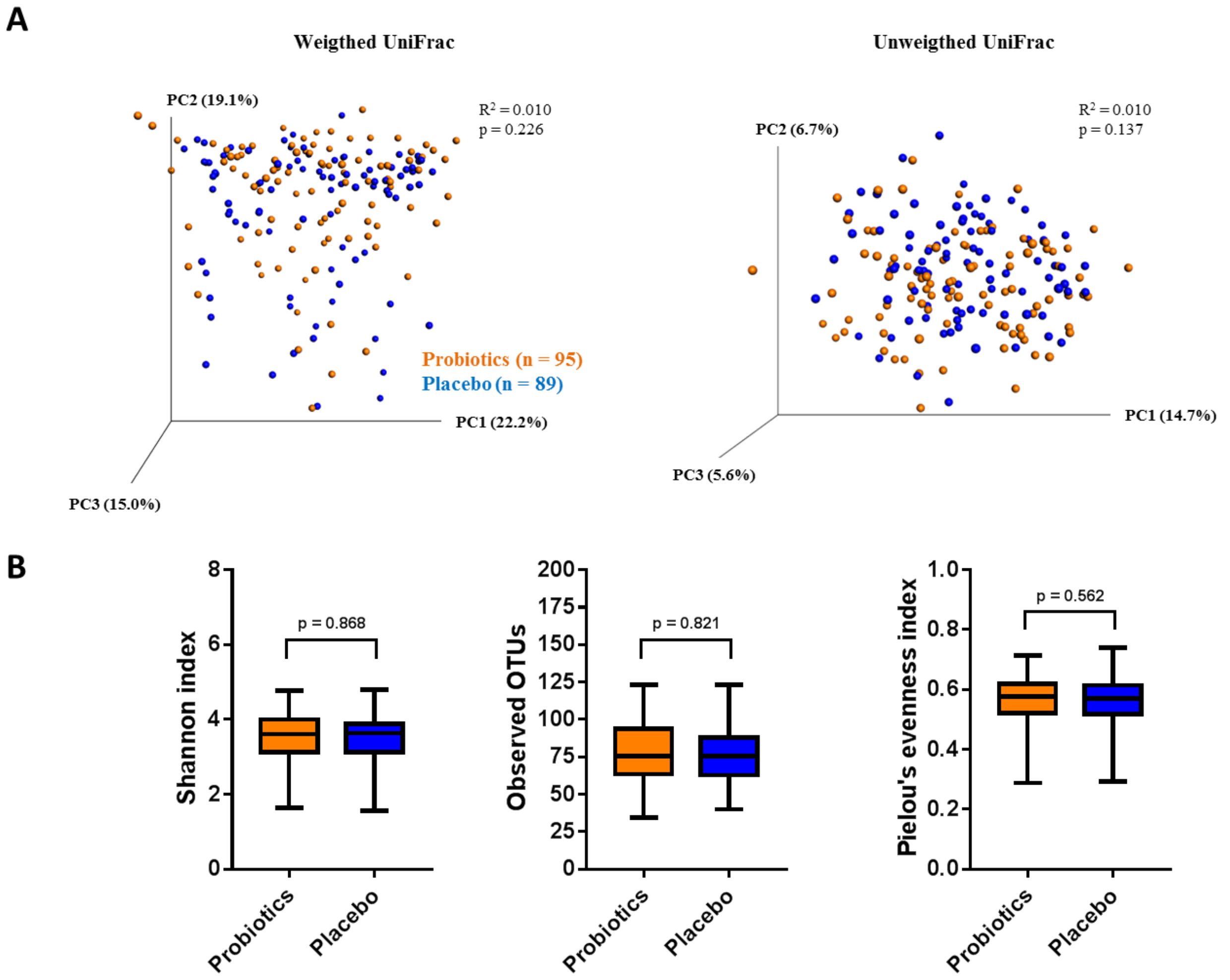

Figure S2 – Gut microbiota at baseline does not differ between probiotics and placebo groups.

A) PCoA plots of weighted and unweighted UniFrac distances of the gut microbiota, B) boxplots of gut microbial alpha diversity measures for individuals assigned to probiotics (orange) and placebo (blue) treatments.

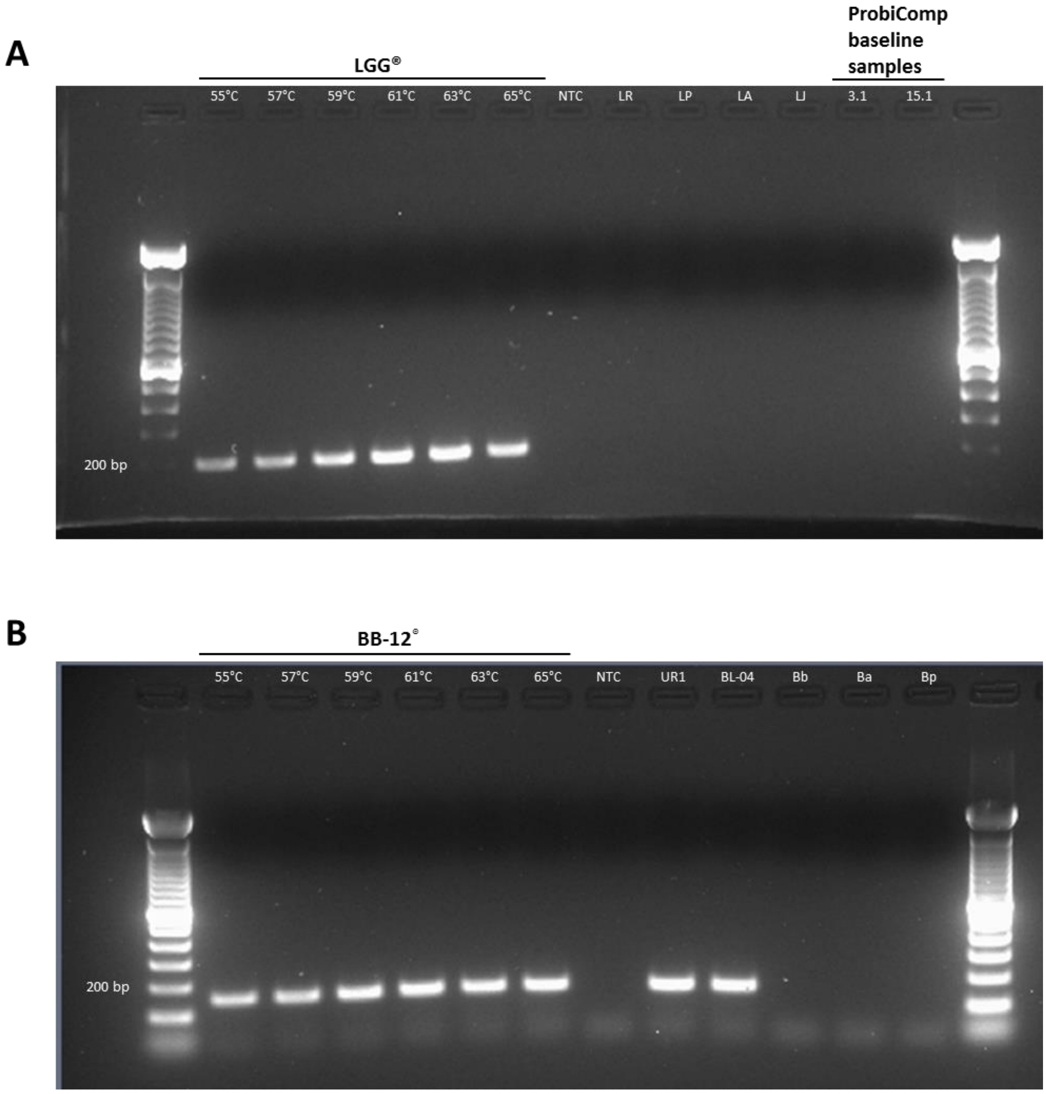

Figure S3 – Evaluation of primer specificity for LGG® and BB-12® primers.

A) UV image of a gel containing PCR products obtained with extracted DNA from *Lactobacillus rhamnosus* (LGG® at different annealing temperatures), none template control (NTC), *L. rhanmosus* DSM 20021 (LR), *L. paracasei* subsp. *paracasei* 85E DSM 20006 (LP), *L. acidophilus* L917 DSM 20079 (LA), *L. johnsonii* VPI 7660 DSM 10533 (LJ) or ProbiComp baseline samples (3.1 and 15.1) using LGG® primers. B) UV image of a gel containing PCR products obtained with extracted DNA from *B. animalis* subsp. *lactis* (BB-12® at different annealing temperatures), none template control (NTC), *B. animalis* subsp. *lactis* UR1 DSM 10140 (UR1), *B. animalis* subsp. *lactis* BL-04 SD5219 (BL-04), *B. breve* S50 DSM 20091 (Bb), *B. animalis* subsp. *animalis* DSM 20104 (Ba) or *B. pseudolongum* subsp. *pseudolongum* DSM 20099 (Bp) using BB-12® primers. A 100 bp DNA ladder (Invitrogen, 15628019) is included in the first and last wells for comparison of basepair size.

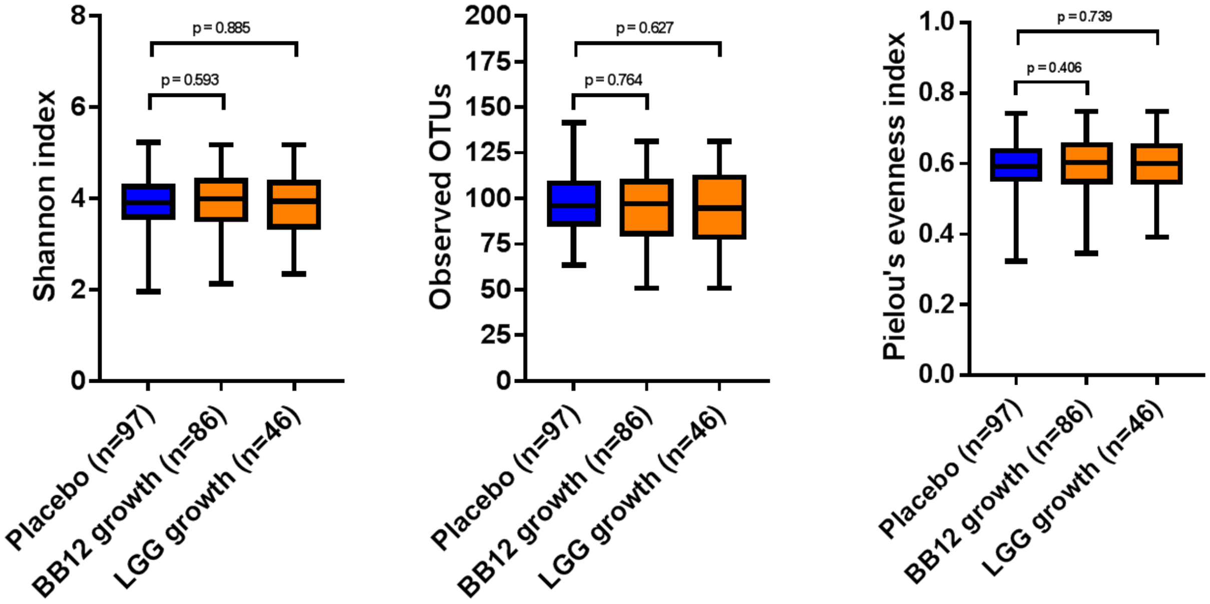

Figure S4 – Gut microbial alpha diversity measures are not affected by active growth of probiotics. Boxplots of gut microbial alpha diversity measures for individuals with detected growth of BB-12® (n = 86) or LGG® (n = 46) as compared to placebo (n = 97).

Additional References

1. Briner AE, Lugli GA, Milani C, Duranti S, Turroni F, Gueimonde M, et al. Occurrence and Diversity of CRISPR-Cas Systems in the Genus Bifidobacterium. Riedel CU, editor. PLoS One [Internet]. Public Library of Science; 2015 [cited 2016 Jul 1];10:e0133661. Available from: http://dx.plos.org/10.1371/journal.pone.0133661

2. Endo A, Aakko J, Salminen S, Ahlroos T, Tynkkynen S, Brandt K, et al. Evaluation of strain-specific primers for identification of Lactobacillus rhamnosus GG. FEMS Microbiol. Lett. [Internet]. The Oxford University Press; 2012 [cited 2016 Jul 1];337:120–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23003331