Supplemental Material

Co-administration of the *Campylobacter jejuni* N-glycan based vaccine with probiotics improves vaccine performance in broiler chickens

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FIG S1

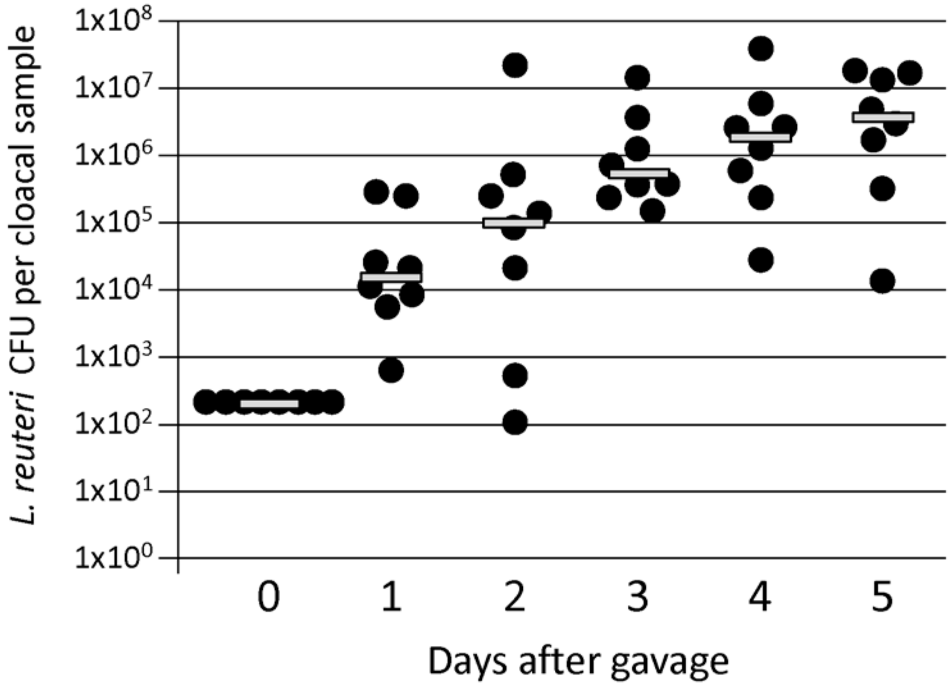

FIG S1 Cloacal swab cell counts on *L. reuteri* isolation medium. Cloacal swabs were collected at day 0 and from days 1 - 5 thereafter from SPF ex-*Lactobacillus*-free leghorn chickens (n= 8) that were gavaged with nine different poultry isolates of *L. reuteri* (S1 Table). Each data point represents a sample from an individual bird; grey bars represent the median for each group.

FIG S2

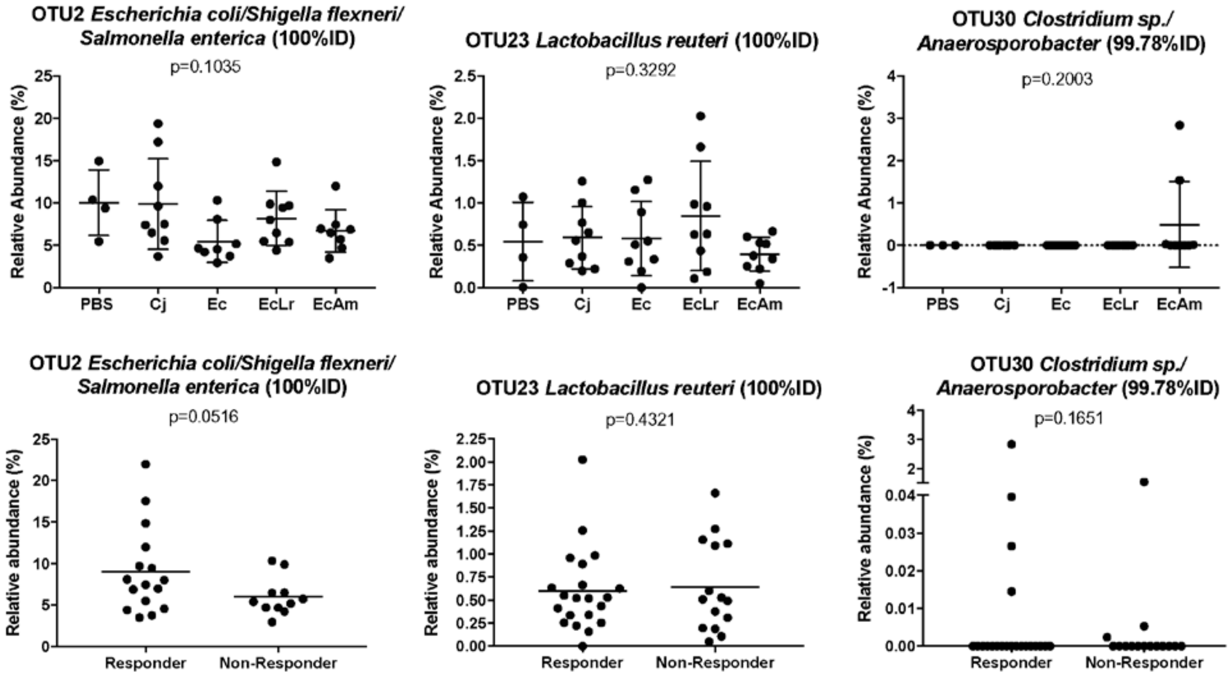

FIG S2 Relative abundance of bacteria of interest for all treatments and for responders and non-responders. Statistics: Grubbs' test for removal of outliers followed by One-way ANOVA with Benjamini and Hochberg FDR correction.

FIG S3

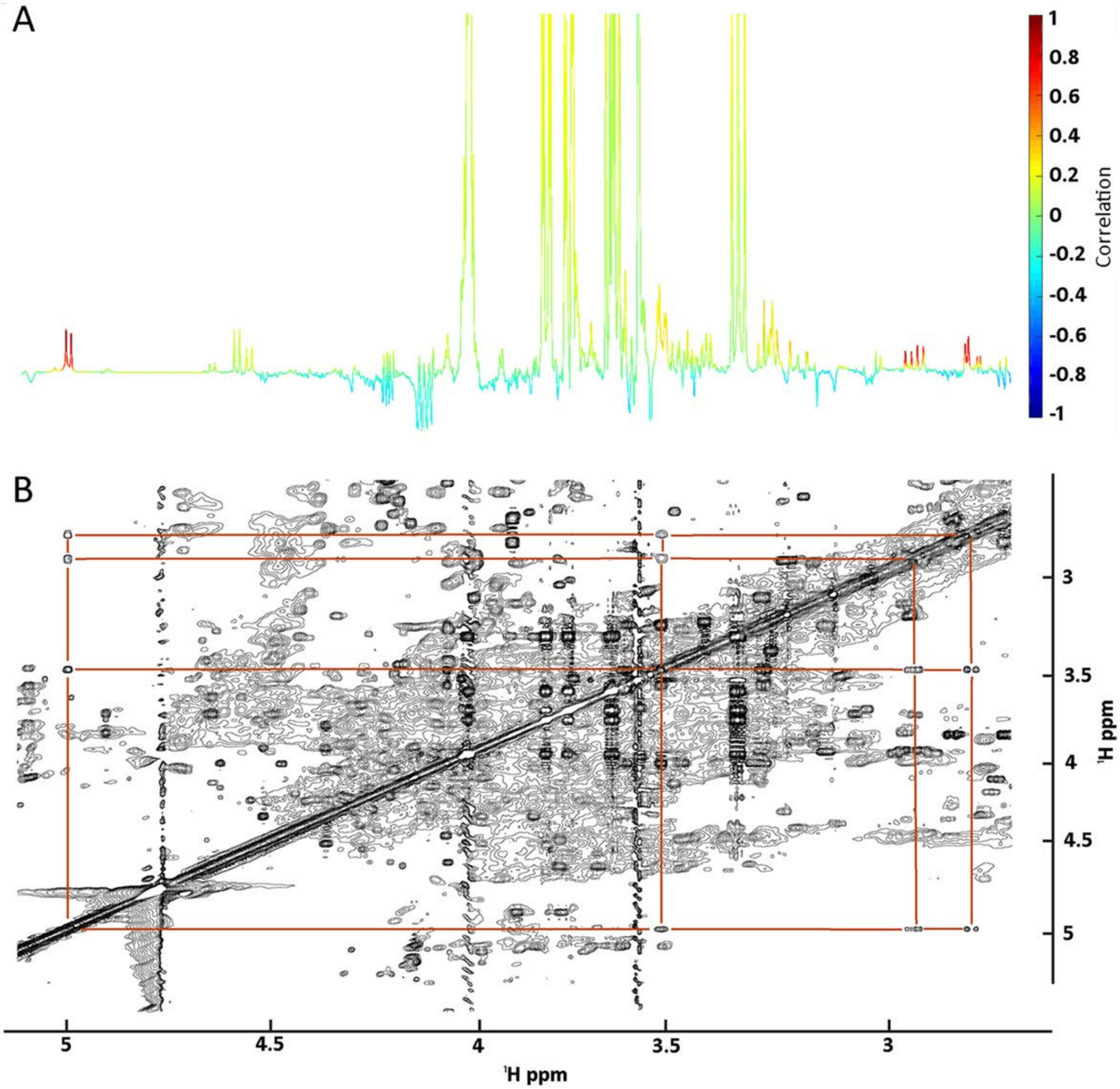

FIG S3 Correlations to the driver peak at 5 ppm. (A) STOCSY output displaying correlation as color and covariance as peak height. Red peaks at 2.7 and 2.9 ppm indicate high correlation to the driver peak at 5 ppm. **(B)** TOCSY experiment shows the lines connecting the correlating the red colored peaks, consistent with the STOCY and an additional peak at 3.5 ppm.

TABLE S1. Strains used in *in vivo* probiotic selection experiments

Strain	**Host of Isolation**(1)	***leuS* allele number**(1)
CSF8	chicken	15
1366	chicken	6
JCM1081	chicken	24
11283	chicken	5
T2	turkey	11
tu160	turkey	38
lk150	chicken	29
hwh3	chicken	22
nck983	chicken	36

Reference:

1. **Oh PL, Benson AK, Peterson DA, Patil PB, Moriyama EN, Roos S, Walter J.** 2010. Diversification of the gut symbiont *Lactobacillus reuteri* as a result of host-driven evolution. ISME J **4:**377-387.

TABLE S2. Weight gain in broilers (raw data for Fig 2D)

		Weight (in grams)		
Group	**Bird**	**Day 8**	**Day 31**	**Day 35**
PBS	**1-1**	222.4	1369	1669
	1-2	208.7	1262	1552
	1-3	211.3	1194	1456
	1-4	198.7	1392	1748
Cj	**2-1**	202.5	1230	1507
	2-2	175.5	1253	1542
	2-3	205.5	1391	1623
	2-4	190	1085	1309
	2-5	237.7	1316	1501
	2-6	193.4	1316	1620
	2-7	200.8	1213	1503
	2-8	170.7	1374	1699
	2-9	211.8	1298	1540
Ec	**3-1**	193.7	1252	1603
	3-2	184.7	1120	1547
	3-3	200.2	1273	1526
	3-4	178.5	1257	1399
	3-5	151.5	1176	1494
	3-6	204.5	1346	1535
	3-7	211.5	994	1200
	3-8	201.2	1423	1733
	3-9	199.5	1125	1412
EcLr	**4-1**	163.8	1351	1653
	4-2	168.2	1275	1544
	4-3	154.1	1396	1588
	4-4	165.4	1315	1652
	4-5	176.2	1338	1609
	4-6	180.1	1358	1694
	4-7	192.5	1395	1665
	4-8	178.1	1407	1752
	4-9	158.9	1238	1553
EcAm	**5-1**	203.7	1348	1635
	5-2	110.9	1204	1490
	5-3	174.1	1285	1549
	5-4	176.4	1361	1638
	5-5	193.2	1303	1590
	5-6	203.4	1461	1596
	5-7	174.5	1186	1477
	5-8	181.9	1340	1632
	5-9	200.3	1430	1721

TABLE S3. Multiplex Primer Mix

Multiplex Primer Mix	**Locus (81-176)**	**Expected Peak Size**	**Control Peak Size**	**Tract Length (Genome)**	**Label**	**Colour**
MIX A	0082	313	312.6	G9	FAM	B
	0590	353	352.3	G10	FAM	B
	1325	253	250.1	G9	FAM	B
	1419	186	181.5	G9	FAM	B
	1429	469	467	G10	FAM	B
MIX B	0086	155	152.8	G9	FAM	B
	0708	130	126.5	G9	FAM	B
	0758	224	223.8	G9	FAM	B
	1160	288	283.2	G10	FAM	B
	1341	392	389.6	G9	FAM	B
MIX C	0646	167	166.2	G9	VIC	G
	1312	161	157.8	G9	NED	Y
	1321	291	290	G9	VIC	G
	1421	262	257.8	G10	VIC	G
	1432	342	339.6	G10	VIC	G
MIX D	0206	287	284.2	G9	NED	Y
	0765	197	194.7	C11	NED	Y
	1327	233	232.3	G9	VIC	G
	1435	321	315.6	G9	NED	Y

TABLE S4. Distribution of five gene phasotypes between control and vaccinated challenge groups

	Phasotype Groups for a Five Gene Phasotype (0086-1160-1312-1419-1421)[$]		
	Phasogroup 1[*]	Phasogroup 2[*]	Phasogroup 3[*]
Phasotypes	**10010**, **10100**, 10000, 10001, **00100, 01010, 00010**	00101, 00011, **00111, 00110, 01100, 10110**	**01110, 01111**, 10111, **01101**, 10101, **11101**, 11100, 11110, 11111
Gavage	33%	33%	33%
Control	27%	12%	62%
Group 3	34%	41%	25%
Group 4	31%	67%	1%
Group 5	35%	32%	32%
Group 6	34%	14%	53%

[*] Phasotypes in bold were present within the gavage; [$] Numbers in the brackets are the gene numbers for strain 81-176 without the gene prefix CJJ81176.

The five gene phasotypes for five phase-variable genes of the challenge strain (81-176) were evaluated for 24-30 colonies per sample (gavage or caecal contents). The total numbers of colonies analysed were: gavage, 24; Cj, 259 (from nine birds); Ec, 134 (from five birds); EcLr, 89 (from three birds); EcAm, 77, (from three birds).

Phasotypes were arbitrarily distributed into three phasogroups based on the two criteria – numbers of genes expressed and requirement for one switching event between at least one other member of the group. Phasogroup 1 contains phasotypes with one or two expressed genes; phasogroup 2, two or three expressed genes; phasogroup 3, three to five expressed genes. Phasogroup 1 exhibited a similar proportion in isolates from gavage, control and vaccinated birds. Phasogroups 2 and 3 exhibited proportions similar to the gavage while the control exhibited a bias for phasogroup 3 in output colonies.

TABLE S5. Multivariate analysis (PCA and PLS-DA) parameters, list of PCs for PCA (totalling 80% of the variance) and PLS-DA and, classification and fit measurements Q^2 and R^2

Statistical Method	Algorithm	Cross-Validation	Principal Components	% Variance	R^2	Q^2
PCA	Nipals	N/A	**PC1**	25.22	N/A	N/A
			PC2	14.30	N/A	N/A
			PC3	13.02	N/A	N/A
			PC4	8.37	N/A	N/A
			PC5	6.86	N/A	N/A
			PC6	4.70	N/A	N/A
			PC7	3.20	N/A	N/A
			PC8	2.96	N/A	N/A
			PC9	2.20	N/A	N/A
PLS-DA (30 Permutations and 5 components)	SIMPLS	n-fold cross-validation	**PC1**	12.85	0.6023	0.2700
			PC2	6.15	0.7486	0.2366
			PC3	6.98	0.8203	0.1989

TABLE S6. Metabolite database matching using COLMARm and ASSURE NMR

Compound name	COLMARm		Confidence Index
	Matching ratio	Uniqueness	
L-Tryptophan	0.75	6/6	4
L-Isoleucine	0.67	4/4	4
D-Pinitol	1	4/7	4
Alanine	1	4/4	4
Nicotinic acid	1	4/4	4
N-Acetyl-D-glucosamine	1	3/7	4
L-Threonine	1	3/3	4
L-Valine	1	3/4	4
Myo-inositol	1	3/4	4
6-Phosphogluconic acid	0.8	3/4	4
L-Threonine	1	3/3	4
L-Glutamic acid	1	2/3	4
5-Aminopentanoic acid	0.75	2/3	4
DL-alpha-glycerol phosphate	0.75	2/3	4
D-Mannitol	0.75	2/3	4
3-Methyl-2-oxobutinoic acid	1	2/2	4
Lactic acid	1	2/2	4
Taurine	1	2/2	4
Uracil	1	2/2	4
D-Mannose	0.71	3/5	4

Matching ratio and uniqueness are outputs from COLMAR, whereas Confidence Index is our laboratory confidence scale that varies from 1-5 as follows: 1 - putatively characterized compound classes or annotated compounds, 2 – compound match to literature and external databases, 3 - COLMAR 13C-1H HSQC database matching, 4 - COLMAR 13C-1H HSQC validated by 2D 1H-1H TOCSY, 2D TOCSY and 5 - samples are spiked with known amount of a compound(s) to confirm identification.