Cell Host & Microbe, Volume *26*

Supplemental Information

A *Listeria monocytogenes* Bacteriocin Can Target the Commensal *Prevotella copri* and Modulate Intestinal Infection

Nathalie Rolhion, Benoit Chassaing, Marie-Anne Nahori, Jana de Bodt, Alexandra Moura, Marc Lecuit, Olivier Dussurget, Marion Bérard, Massimo Marzorati, Hannah Fehlner-Peach, Dan R. Littman, Andrew T. Gewirtz, Tom Van de Wiele, and Pascale Cossart

Supplemental information

Figure S1. Related to Figure 1. (**A**) Schematic representation of the *lmo2776* genetic region. **(B)** Amino acid alignment of Lmo2776 with members of the Lnc972 family: L82330 from *Lactococcus lactis* subsp. *lactis* Il1403, SP_0109 bacteriocin from *Streptococcus pneumoniae* TIGR4, SAP109 from *Staphylococcus aureus* subsp. *aureus* N315, lcn972 lactococcin 972 from *Lactococcus lactis* subsp. *lactis* and Sil from *Streptococcus iniae* SF. The conserved residues and consensus motif are indicated in color and on the bottom, respectively. **(C)** Cluster analysis based on core genome multilocus sequence typing (cgMLST) profiles of 1,696 genomes (obtained from Moura et al., 2016) and distribution of lmo2774-lmo2775-lmo2776 genes (in blue) across *Lm* phylogenetic lineages. The ten most frequent sublineages (SL) are highlighted. The serogroup and sample source are shown in the first and last columns, respectively, using the color code indicated in the upper right key. **(D)** Relative expression of *lmo2774*, *lmo2775* and *lmo2776* in bacteria grown in stationary phase (white bars) compared to bacteria grown in exponential phase (black bars). The transcripts levels were normalised to the levels of *rpoB*, which were constant under all conditions, and then expressed relative to those of exponential phase. Results are expressed as mean ± SEM of a least 3 independent experiments and P-values were obtained using two-tailed unpaired Student's t-test (*$p<0.05$). **(E)** Relative expression of *lmo2774*, *lmo2775* and *lmo2777* in Δ*lmo2776* bacteria compared to WT bacteria, grown in exponential phase (black bars) or in stationary phase (grey bars). The transcripts levels were normalised to the levels of *rpoB*, which were constant under all conditions. Results are expressed as mean ± SEM of a least 3 independent experiments. **(F)** Growth curves of WT and Δ*lmo2776* bacteria at 37°C with shaking in BHI. **(G)** BALB/c mice were inoculated orally with *Lm* WT (EGDe) or Δ*lmo2776* bacteria. CFUs in the intestinal luminal content were assessed at 24, 48 and 72h pi. **(H)** BALB/c mice were inoculated intravenously with WT or Δ*lmo2776* bacteria. CFUs in the spleen and the liver were assessed at 72h pi. Each dot represents the value for one mouse. Statistically significant differences were evaluated by the Mann–Whitney test. (*$p< 0.05$).

Figure S2. Related to Figure 2. (**A**) Principal coordinates analysis of the weighted Unifrac distance matrix of conventional mice at day 0 (red) and infected with WT strain at day 1 (blue). Permanova P= 0.002. (**B**) Relative abundance of classes in conventional mice at day 0 (left) and infected with WT strain at day 1 (right). LEfSE (**C**) and histogram of the LDA scores (**D**) computed for features differentially abundant between microbiota of mice at day 0 (red) and

infected with WT strain at day 1 (green). (**E**) Firmicutes/Bacteroides ratio in microbiota of mice at day 0 and infected with WT strain at day 1. Each dot represents the value for one mouse.

Figure S3. Related to Figure 3. Levels of butyrate (**A**), isobutyrate (**B**), acetate (**C**) and isovalerate (**D**) in SHIME® vessels infected with WT or Δ*lmo2776* strains or non-infected overtime. Results are expressed as mean ± SEM for 2 to 3 individual vessels. Numbers of *Bs* (**E**) and of different *Lm* strains (**F**) were quantified after 6h of co-culture. Numbers of *Bs* or *E. coli* (**G**) after incubation with supernatant of WT or Δ*lmo2776* strains. Results are expressed as mean ± SEM of a least 3 independent experiments and P-values were obtained using two-tailed unpaired Student's t-test (*$p<0.05$, ***$p<0.005$).

Figure S4. Related to Figure 4. Assessment of listerial CFUs in the liver of germfree (GF) C57BL/6J mice colonized or not with *Pc*, *Ps* or *Bt* or stably colonized with 12 bacterial species (Oligo-MM^{12}) for 2 weeks and then inoculated with *Lm* WT or Δ*lmo2776* for 72h. Each dot represents one mouse.

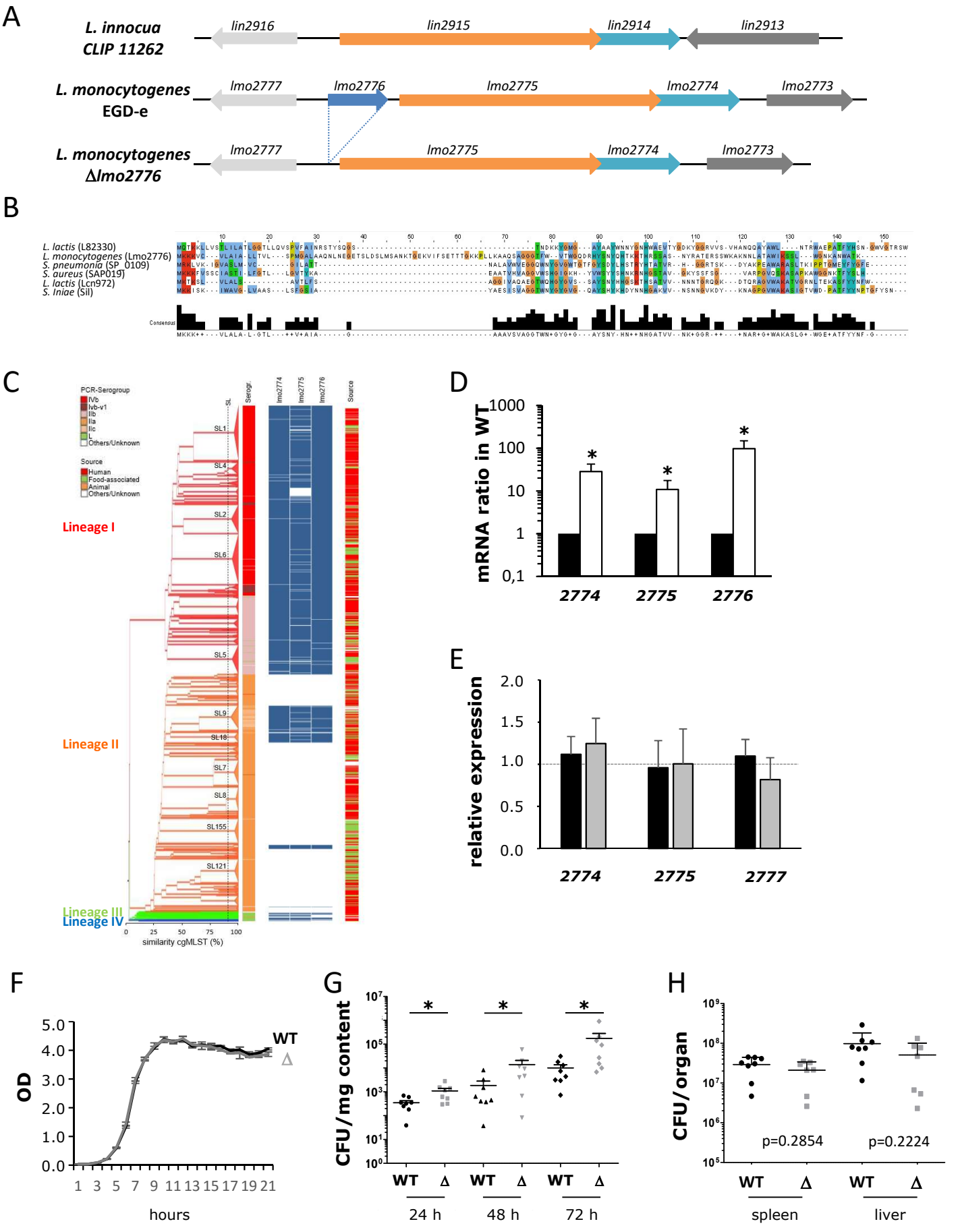
A
L. innocua CLIP 11262
lin2916
lin2915
lin2914
lin2913
L. monocytogenes EGD-e
lmo2777
lmo2776
lmo2775
lmo2774
lmo2773
L. monocytogenes ΔImo2776
lmo2777
lmo2775
lmo2774
lmo2773
B
L. lactis (L82330)
L. monocytogenes (Lmo2776)
S. pneumonia (SP_0109)
S. aureus (SAP019)
L. lactis (Lcn972)
S. Iniae (Sil)
Consensus
C
PCR-Serogroup
IVb
IVb-v1
IIb
IIa
IIc
L
Others/Unknown
Source
Human
Food-associated
Animal
Others/Unknown
Serogr.
lmo2774
lmo2775
lmo2776
Source
Lineage I
Lineage II
Lineage III
Lineage IV
SL1
SL4
SL2
SL6
SL5
SL9
SL18
SL7
SL8
SL155
SL121
similarity cgMLST (%)
D
mRNA ratio in WT
2774
2775
2776
E
relative expression
2774
2775
2777
F
OD
WT
Δ
hours
G
CFU/mg content
WT
Δ
24 h
48 h
72 h
H
CFU/organ
p=0.2854
p=0.2224
spleen
liver

Figure S1

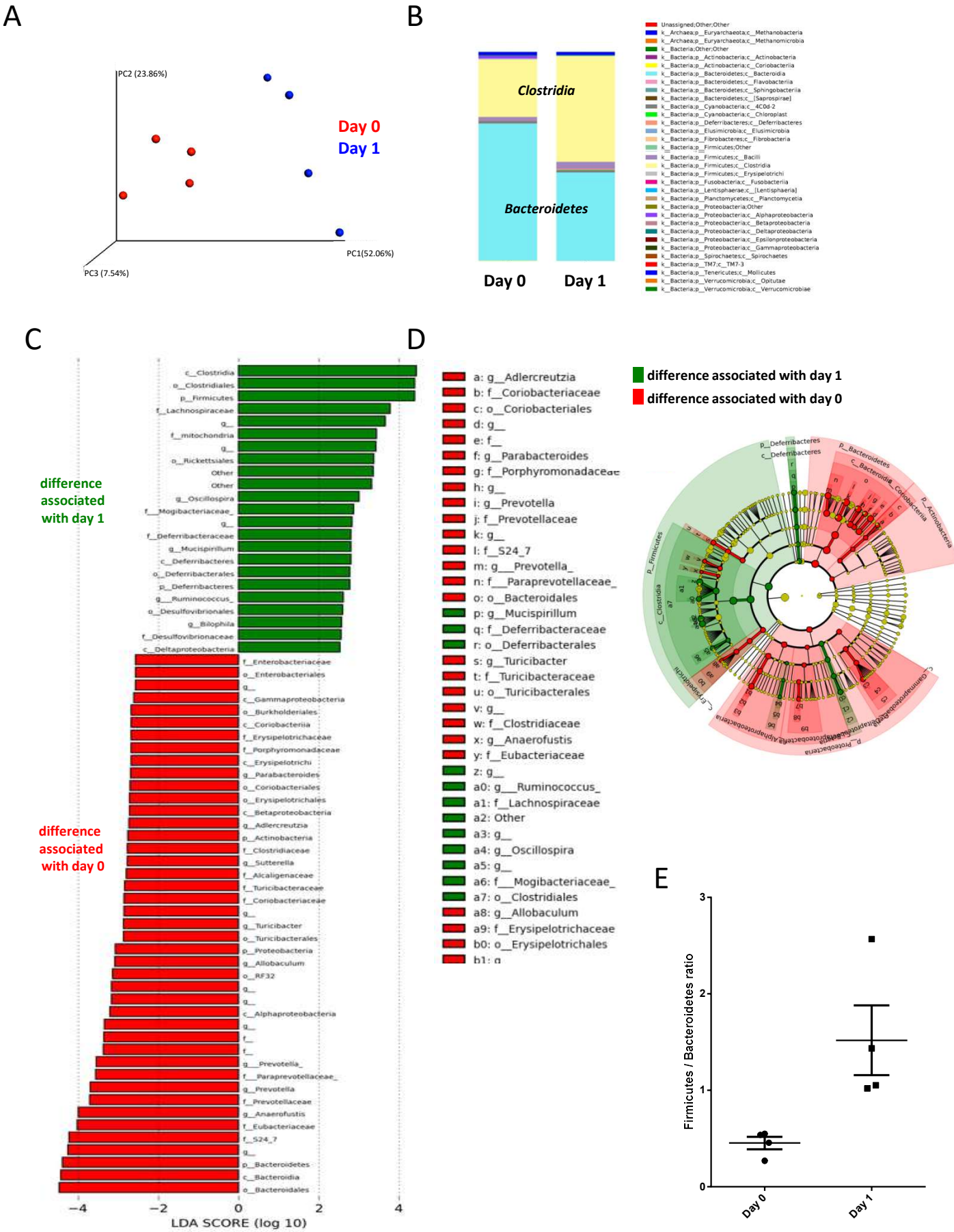
A
PC2 (23.86%)
PC1(52.06%)
PC3 (7.54%)
Day 0
Day 1
B
Clostridia
Bacteroidetes
Day 0
Day 1
C
difference associated with day 1
difference associated with day 0
c__Clostridia
o__Clostridiales
p__Firmicutes
f__Lachnospiraceae
g__
f__mitochondria
g__
o__Rickettsiales
Other
Other
g__Oscillospira
f__Mogibacteriaceae_
g__
f__Deferribacteraceae
g__Mucispirillum
c__Deferribacteres
o__Deferribacterales
p__Deferribacteres
g__Ruminococcus_
o__Desulfovibrionales
g__Bilophila
f__Desulfovibrionaceae
c__Deltaproteobacteria
f__Enterobacteriaceae
o__Enterobacteriales
g__
c__Gammaproteobacteria
o__Burkholderiales
c__Coriobacteriia
f__Erysipelotrichaceae
f__Porphyromonadaceae
c__Erysipelotrichi
g__Parabacteroides
o__Coriobacteriales
o__Erysipelotrichales
c__Betaproteobacteria
g__Adlercreutzia
p__Actinobacteria
f__Clostridiaceae
g__Sutterella
f__Alcaligenaceae
f__Turicibacteraceae
f__Coriobacteriaceae
g__
g__Turicibacter
o__Turicibacterales
p__Proteobacteria
g__Allobaculum
o__RF32
g__
g__
c__Alphaproteobacteria
g__
f__
f__
g__Prevotella_
f__Paraprevotellaceae_
g__Prevotella
f__Prevotellaceae
g__Anaerofustis
f__Eubacteriaceae
f__S24_7
g__
p__Bacteroidetes
c__Bacteroidia
o__Bacteroidales
−4
−2
0
2
4
LDA SCORE (log 10)
D
a: g__Adlercreutzia
b: f__Coriobacteriaceae
c: o__Coriobacteriales
d: g__
e: f__
f: g__Parabacteroides
g: f__Porphyromonadaceae
h: g__
i: g__Prevotella
j: f__Prevotellaceae
k: g__
l: f__S24_7
m: g__Prevotella_
n: f__Paraprevotellaceae_
o: o__Bacteroidales
p: g__Mucispirillum
q: f__Deferribacteraceae
r: o__Deferribacterales
s: g__Turicibacter
t: f__Turicibacteraceae
u: o__Turicibacterales
v: g__
w: f__Clostridiaceae
x: g__Anaerofustis
y: f__Eubacteriaceae
z: g__
a0: g__Ruminococcus_
a1: f__Lachnospiraceae
a2: Other
a3: g__
a4: g__Oscillospira
a5: g__
a6: f__Mogibacteriaceae_
a7: o__Clostridiales
a8: g__Allobaculum
a9: f__Erysipelotrichaceae
b0: o__Erysipelotrichales
b1: g
difference associated with day 1
difference associated with day 0
E
Firmicutes / Bacteroidetes ratio
0
1
2
3
Day 0
Day 1

Figure S2

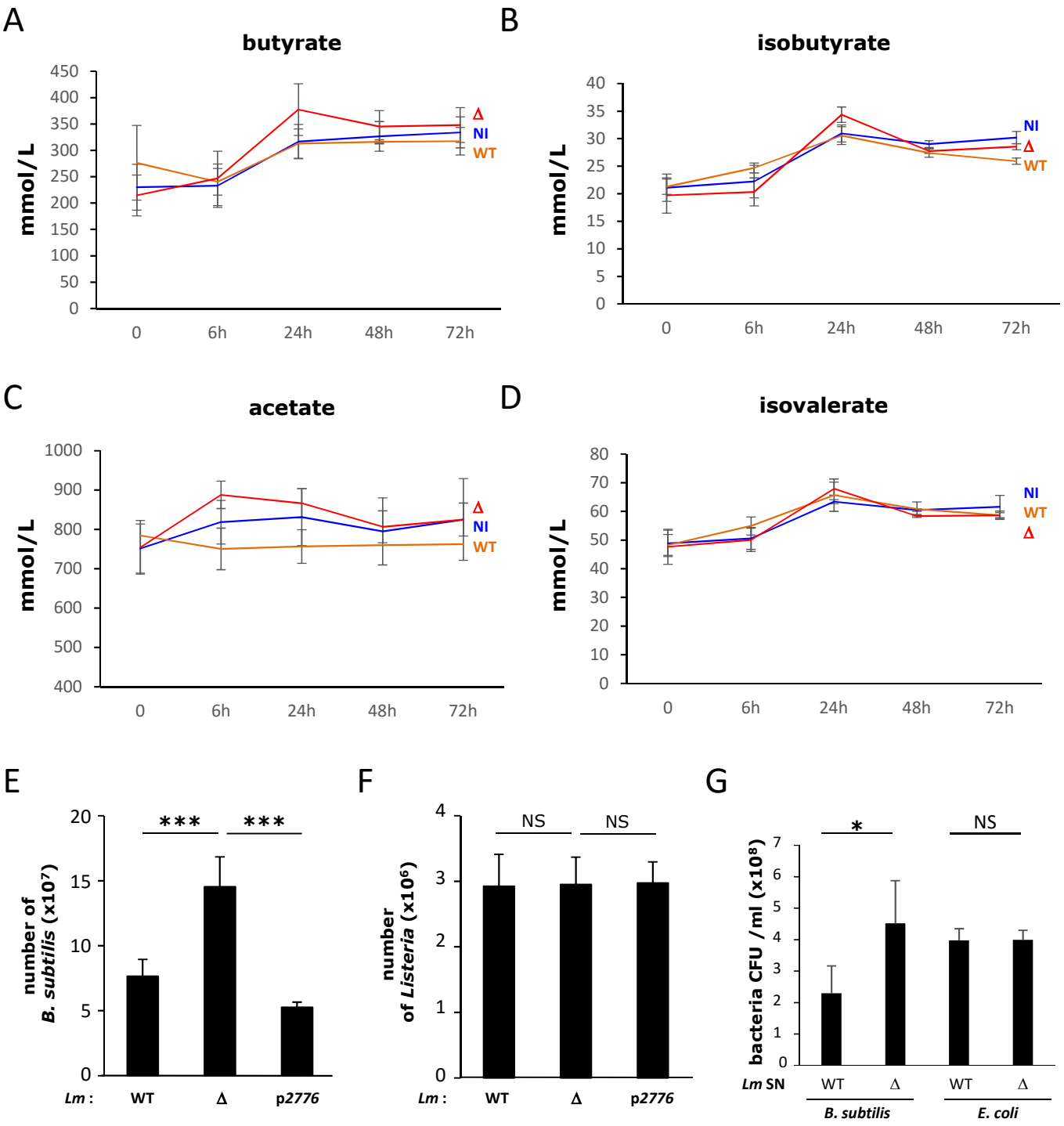
A
butyrate
mmol/L
450
400
350
300
250
200
150
100
50
0
0
6h
24h
48h
72h
Δ
NI
WT
B
isobutyrate
mmol/L
40
35
30
25
20
15
10
5
0
0
6h
24h
48h
72h
NI
Δ
WT
C
acetate
mmol/L
1000
900
800
700
600
500
400
0
6h
24h
48h
72h
Δ
NI
WT
D
isovalerate
mmol/L
80
70
60
50
40
30
20
10
0
0
6h
24h
48h
72h
NI
WT
Δ
E
number of B. subtilis (x10⁷)
20
15
10
5
0
***
***
Lm :
WT
Δ
p2776
F
number of Listeria (x10⁶)
4
3
2
1
0
NS
NS
Lm :
WT
Δ
p2776
G
bacteria CFU /ml (x10⁸)
7
6
5
4
3
2
1
0
*
NS
Lm SN
WT
Δ
WT
Δ
B. subtilis
E. coli

Figure S3

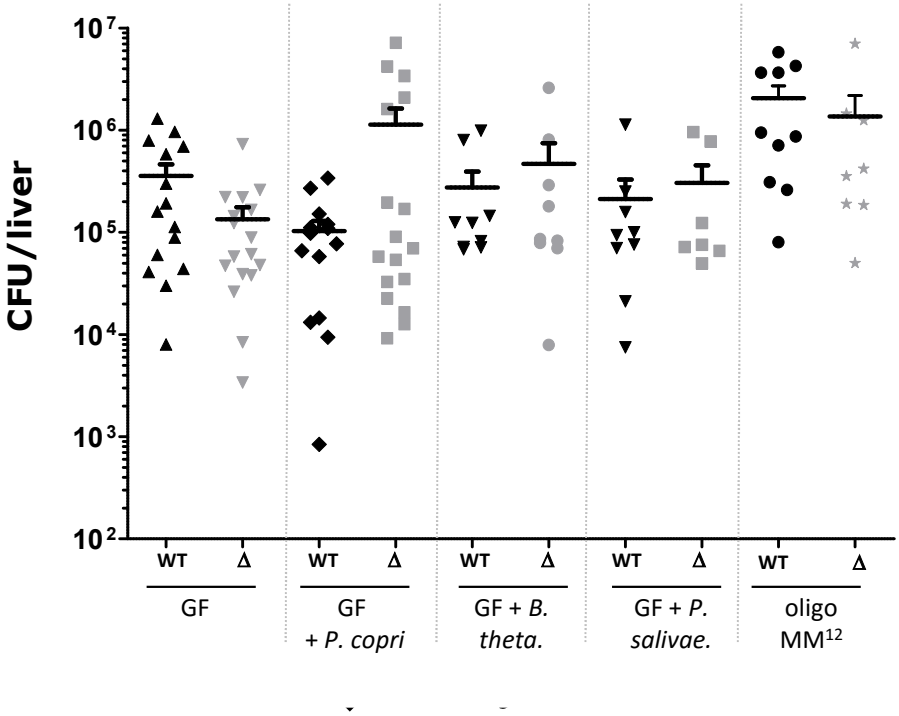
CFU/liver
10^7
10^6
10^5
10^4
10^3
10^2
WT
Δ
GF
GF + *P. copri*
GF + *B. theta.*
GF + *P. salivae.*
oligo MM^{12}

Figure S4

Table S1 Oligonucleotides used in study, related to methods, Figure S1, Figure 2 and Figure 3

Experiment	Primer ID	Sequence	Source
Oligonucleotides used to create deletion mutant	Lmo2776-DelA	GGAAGATCTACATCCTTCACAGGGAAATG	This study
	Lmo2776-DelB	TGTATTCTCCTCTCTTTCAAATTAA	
	Lmo2776-DelC	TTAATTTGAAAGAGAGGAGAATACATTCCTATAAAGCTAAGAAATATTTC	
	Lmo2776-DelD	CGGCCATGGAGCAAAGTCATAAGTAACGGGATAT	
Sequencing insert in pAD-based plasmid	pPL2-Fw	TTCGACCCGGTCGTCGGTTC	Balestrino et al, 2010
	pPL2-Rv	CTTAGACGTCATTAACCCTCAC	
Verification of pAD integration in the *Lm* chromosome	NC16	GTCAAAACATACGCTCTTATC	
	PL95	ACATAATCAGTCCAAAGTAGATGC	
qRT-PCR for gene expression level, related to Figure S1	lmo27741	GTGTTAGAAAACTTATCCATTACAGG	This study
	lmo27742	ATCATCCAAGTTGCCTGTTGGTTCG	
	lmo27751	GGTCTTTAAAGGTTTATGACTTTGG	
	lmo27752	CCCATATACGATTAACTAATAACCG	
	lmo2776 RT1	GTATGTGTTTTAGCGATAGCA	
	lmo2776 RT2	ATAATGTCTATCTTGTCCCC	
	lmo2777 RT1	CTACCACGGAAATGATCGCC	
	lmo2777 RT2	GCACACTAAAGGAGGTAGCG	
	rpoB F	GCGAACATGCAACGTCAAGCAGTA	
	rpoB R	ATGTTTGGCAGTTACAGCAGCACC	
q-PCR for 16S rRNA based bacterial quantification, related to Figure 2 and 3	Prevotella 16S F	CACRGTAAACGATGGATGCC	Scher et al, 2013
	Prevotella 16S R	GGTCGGGTTGCAGACC	
	Universal 16S F	ACTCCTACGGGAGGCAGCAGT	
	Universal 16S R	ATTACCGCGGCTGCTGGC	
	Am_fwd	CAGCACGTGAAGGTGGGGAC	
	Am_rev	CCTTGCGGTTGGCTTCAGAT	