1 SUPPORTING INFORMATION

- 2 This PDF file includes: 3 4 Supporting Information Figures 1 to 13 5 6 Supporting Information Tables S1 to S4 7 8 Supporting Dataset S1 is uploaded separately as an excel spreadsheet) 9 Supporting Information reference list 10 11 SUPPORTING FIGURES 12
- 13

800 * Biofilm formation (%) 600 400 200 0 Bileertractors Bile extract 0.1% Bile extract 10 eextract 25mg/ml Nucin 0.5% Tenin Somelint D-glucose Nucino.1º10 D-mannose Dimatose Drhannosé Dreellobio

14

Supporting Figure S1. Bile is the only tested environment cue that induces biofilm formation of *B. thetaiotaomicron* VPI-5482. *B. thetaiotaomicron* VPI-5482 after 24h growth in BHIS supplemented with 0.5% D-glucose, D-mannose, D-rhamnose, Dcellobiose and D-maltose, 25 mg/L and 50 mg/L hemin, 0.1% and 0.5% mucin, and

19 0.1%, 0.5%, 1% bovine-ovine bile extract. Min-max boxplot of 3-6 biological replicates

20 for each strain. * p-value<0.05, ** p-value<0.005, Mann-Whitney test.



3 Supporting Figure S2. 0.5% bile extract concentration is non-toxic for *Bacteroides*

4 thetaiotaomicron. A. Flow cytometry of *B. thetaiotaomicron* VPI-5482 cultures grown

5 in presence or absence of bile and dyed with propidium iodide to label live cells. The

6 viable cell count is shown as a min-max boxplot of 6 biological replicates.

7

2

1





1

4 Supporting Figure S3. BT3560-BT3563 operon. RNAseq reads from В. 5 thetaiotaomicron VPI-5482 grown in TYG medium to exponential phase (OD=0.3) in 6 red, mid-exponential phage (OD=2) in green and stationary phase (OD=3.8) in blue are mapped to the BT3560-BT3563 genetic locus. Predicted transcriptional start sites 7 8 (TSS) and terminators (term) are depicted, showing the predicted operon structure of 9 BT3560-BT3563. Data and bioinformatics prediction from Theta-Base database (1) (https://bacteroides.helmholtz-hzi.de/) plotted on JBrowser (2). 10

11



1

2 Supporting Figure S4. **Growth curve**. 24h growth curve of indicated strains in 96-well

3 plate in BHIS+0.5% bile extract. Mean of 6 biological replicates, error bars represent

4 standard error to the mean (SEM).



1

Supporting Figure S5: Quantification of cells in biofilm. 96-well plate biofilm assay after 48h growth in BHIS+0.5% bile extract. Two plates were inoculated. One was used for A. resuspension of the biofilm and colony forming unit (CFU) count, the other was used for B. crystal violet (CV) staining. C. Ratio of CV over CFU count. Min-max boxplot of 8 biological replicates for each strain. * p-value<0.05, ***p-value<0.0005, Mann-Whitney test.



Supporting Figure S6. BT3563 degrades DNA in culture supernatant. Incubation of *B. thetaiotaomicron* genomic DNA (23ng/µL final concentration) with the supernatant of **A.** 24h and **B.** 48h bile-free cultures, as indicated, and loaded immediately on a 1 % agarose gel (t=0h) and after an overnight incubation at 37°C (t=18h). A control using DNase I rather than supernatant is shown in the last lane. pE: empty vector, p3563: vector constitutively expressing BT3563. One replicate of **B.** is shown in Figure 3A.



Supporting Figure S7. BT3563 does not impact protein concentration in the
extracellular matrix. Extracellular protein concentration (ng/µL) in the purified
extracellular matrix of bile-dependent biofilms grown in BHIS+0.5% bile extract for 48h.
Min-max boxplot of 6 biological replicates for each strain. * p-value<0.05, ** p-
value<0.005, Mann-Whitney test.



1

3 Supporting Figure S8. Bile increases eDNA concentration in the supernatant.

4 $\,$ Extracellular DNA concentration (ng/µL) in the supernatant of overnight cultures of

5 indicated strains grown in BHIS or BHIS+0.5% bile extract. Min-max boxplot of 6-11

6 biological replicates for each strain. * p-value<0.05, *** p-value<0.0005, Mann-Whitney

7 test.



Supporting Figure S9. Visualization of *B. thetaiotaomicron* biofilms. IMARIS easy
3D projections from confocal laser scanning (CLSM) microscopy images of *B. thetaiotaomicron* biofilms grown in plates in absence or presence of 0.5% bile extract
("+bile"), RNase I ("+RNase"), or DNase I ("+DNase"). Cells are labelled with SYTO61
dye (left panel, in red). eDNA and dead cells are labelled with TOTO-1 (middle panel,
in green). The merged image is shown on the right. For each image, the virtual shadow
projection of the biofilm is shown on the right. Scale bars represent 40 µm.

WT



WT+ bile +RNase



 $\Delta BT3563$ +bile



 $\Delta BT3563$ +bile +DNase



1

Supporting Figure S10. **3D** reconstruction of *B. thetaiotaomicron* biofilms. 3D reconstruction of *B. thetaiotaomicron* biofilm grown in plates in absence or presence of 0.5% bile extract ("+bile"), RNase I ("+RNase"), or DNase I ("+DNase"). Merged images showing cells labelled with SYTO61 dye in red and extracellular DNA and dead cells labelled with TOTO-1 in green. Scale bar represent 30 µm.

8



WT +bile +DNase



∆*BT3560-3563* +bile





2 Supporting Figure S11. Quantification of confocal microscopy images. Min-Max

- 3 boxplot of **A**. biovolumes of TOTO-1 (μ m³) and **B**. SYTO61 fluorescence (μ m³) and **C**.
- 4 TOTO-1/SYTO61 fluorescence ratio (%). 5-6 biological replicates, each representing
- 5 the mean of 2-3 technical replicates. ** p-value<0.005, Mann-Whitney test.
- 6



- Supporting Figure S12. *BT3555-3564* synteny among the tested Bacteroidales.
 Synteny analysis was performed using the Genome browser tool of the genome analysis
 platform
- 5 (<u>https://mage.genoscope.cns.fr/microscope/mage/viewer.php</u>?) (3) For each genome,
- 6 the first line represents the genes on the chromosome, and the additional lines
- 7 represent homologs of the considered gene within the same genome, when applicable.
- 8 The colored bar represents the length of the homology. Locus tags are indicated for *B*.
- 9 thetaiotaomicron VPI-5482 strain, and the homologs of BT3563 are indicated in a red
- 10 square.
- 11

- 12
- 13







1 2

Query protein sequence:

mrffnrsvvllicctfflvscskddedenkeniafspielpalrngaddiflsptttfnggqvitysmey dkskkharwvafkyynvtgqtnwnrndwkqtewggdpwqsdpnipqadqrvgsdfgkqgydrghicassd rlyskdaneqtfyysnmspqknyfngtkgiwndlegkvrtwgrsstfrdtlyvvkggtidkenqiwtyig gdkskpvpkyyfmallckkgetykaigfwldqsttvkpalsecaktideleeltgldffhnlpdnlenav eskyaisawtglq*

Genomes without synteny:

```
Genomes without synteny:

Dysgonomonas sp HDW5A aal12995551

Proteiniphilum saccharofermentans M3 6 aa9000951351

Paludibacter propionicigenes WB4 aal831351

Fermentimonas caenicola aa9535351

Petrimonas mucosa ING2 E5A aa9000957951

Petrimonas gingivalis A1 aal3142651

Porphyromonas gingivalis A1CC 33277 aal05051

Porphyromonas gingivalis LyG 2 aal81417451

Prevotella denticola KCOM 1525 aa35150451 C1

Prevotella multiformis F0096 aal81279851 C1

Prevotella multiformis F0096 aal81279851 C1

Tannerella forsythia 3313 aal5478751

Tannerella forsythia SC BZ SF2 aa22017951

Draconibacterium orentale FH5 aa6266351

Draconibacterium gallinarum DSM 27622 aal0219751

Chryseobacterium joostei DSM 1627 aa38159751 C1
```

Supporting Figure S13. *BT3563* synteny among diverse Bacteroidetes. Synteny analysis was performed using the web tool SyntTax (4). Homologs of the gene of interest, *BT3563*, are marked in bold. Homologous genes are represented in the same color. Locus tags are annotated on top of each gene. A list of all tested strains for which no *BT3563* homologs was found is present at the bottom.

- 0
- 7

1 SUPPORTING TABLES

Supporting Table S1. *Bacteroides thetaiotaomicron* strains used in this study

Strain	Strain name 2	Origin	From	PATRIC ID	Genbank ID
jmh42	N03576	Perforated ulcer	CHU Limoges	818.1412	JAGURM00000000 0
jmh43	N03525	Blood	CHU Limoges	818.1456	JAGURL000000000
jmh44	N03594	Blood	CHU Limoges	818.1484	JAGWEU00000000 0
jmh47	N03736	Hepatic abscess	CHU Limoges	818.1421	JAGURK000000000
jmh50	Ana-1- p17	Ascitic fluid	CHU Limoges	818.1481	JAGWET000000000
jmh51	Ana2-p19	Blood	CHU Limoges	818.1411	JAGURJ00000000
jmh58	Ana4-p37	Blood	CHU Limoges	818.1450	JAGURI000000000
jmh60	os16-p16	Hip implant	CHU Limoges	818.1415	JAGURH000000000
jmh61	Ana4-p81	Continuous ambulatory peritoneal dialysis (CAPD)	CHU Limoges	818.1480	JAGWES00000000 0
jmh62	10018	Peritoneal fluid	CHU Lille	818.1419	JAGURG000000000
jmh63	10023	Blood	CHU Lille	818.1414	JAGURF000000000
jmh66	10254	Maxilar pus	CHU Lille	818.1416	JAGURE000000000
jmh68	10263	Blood	CHU Lille	818.1451	JAGURD00000000
jmh71	11276	Blood	CHU Lille	818.1482	JAGWER00000000 0
jmh72	11278	Anal abscess	CHU Lille	818.1449	JAGURC000000000
jmh76	11285	Fat tissue	CHU Lille	818.1483	JAGYXC000000000
jmh78	12309	Abdominal abscess	CHU Lille	818.1417	JAGURB000000000
jmh79	11314	Intestinal cyste	CHU Lille	818.1447	JAGURA000000000

		Lengt	ALN		Query	Subject	Hit	Hit		
Genome		h (AA)	Length	Identity	cover	cover	from	to	Score	E value
VPI-5482	fig 226186.12.peg.36 26 (BT_3563)	293	293	100	100	100	1	293	614	0
jmh42	fig 818.1412.peg.329	296	296	87	99	99	4	296	532	0
jmh43	fig 818.1456.peg.161	396	396	34	81	60	149	385	119	8,00E-30
jmh44	fig 818.1484.peg.456	290	290	87	99	100	1	290	525	0
jmh47	fig 818.1421.peg.238 4	290	290	87	99	100	1	290	525	0
jmh50	fig 818.1481.peg.203 3	290	290	87	99	100	1	290	525	0
jmh51	fig 818.1411.peg.850	296	296	87	99	99	4	296	532	0
jmh58	fig 818.1450.peg.210 0	223	223	97	77	100	1	223	457	1,00E-162
jmh60	fig 818.1415.peg.116 1	399	399	34	81	59	152	388	119	1,00E-29
jmh61	fig 818.1480.peg.118 6	291	291	87	100	100	1	291	527	0
jmh62	fig 818.1419.peg.360	399	399	34	81	59	152	388	119	9,00E-30
jmh63	fig 818.1414.peg.287 4	396	396	34	81	60	149	385	119	9,00E-30
jmh66	fig 818.1416.peg.345 4	291	291	87	100	100	1	291	527	0
jmh68	fig 818.1451.peg.696	399	399	34	81	59	152	388	119	1,00E-29
jmh71	fig 818.1482.peg.330 9	399	399	34	81	59	152	388	119	1,00E-29
jmh72	fig 818.1449.peg.296 0	396	396	34	81	60	149	385	119	8,00E-30
jmh76	fig 818.1483.peg.383 8	296	296	85	93	92	26	296	478	1,00E-169
jmh78	fig 818.1417.peg.166 3	293	293	100	100	100	1	293	613	0
jmh79	fig 818.1447.peg.331 0	293	293	100	100	100	1	293	613	0

Supporting Table S2. BT3563 homologs identified by blastp

1	Supporting	Table S3. Bacteria	I strains and place	smids used in this study
---	------------	--------------------	---------------------	--------------------------

Name in this paper	Genotype	Reference
Bacteroides thetaiotaomicro	n	
WT/VPI-5482	VPI-5482 _Δtdk	(5)
jmh42		(6)
jmh43		(6)
jmh44		(6)
jmh47		(6)
jmh50		(6)
jmh51		(6)
jmh58		(6)
jmh60		(6)
jmh61		(6)
jmh62		(6)
jmh63		(6)
jmh66		(6)
jmh68		(6)
jmh71		(6)
jmh72		(6)
jmh76		(6)
jmh78		(6)
jmh79		(6)
3H6	VPI 5482 _ <i>Δtdk-</i> BT3560::Tn	This study
26H1	VPI 5482 _ <i>Δtdk</i> - BT3562::Tn	This study
37G8	VPI 5482 _ <i>Δtdk</i> - BT3560::Tn	This study
46A4	VPI 5482 _ <i>Δtdk</i> - BT3560::Tn	This study
54A8	VPI 5482 _ <i>Δtdk-</i> BT3560::Tn	This study
55B7	VPI 5482 _ <i>Δtdk-</i> BT3560::Tn	This study
59E7	VPI 5482 _ <i>Δtdk</i> - BT3560::Tn	This study
62C12	VPI 5482 _ <i>Δtdk</i> - BT3562::Tn	This study
68H1	VPI 5482 _ <i>Δtdk-</i> BT3560::Tn	This study
∆BT3560-3563	VPI 5482 _∆tdk_∆BT3560-3563	This study
∆BT3560	VPI 5482 _ <i>∆tdk</i> _∆ <i>B</i> T3560	This study
∆BT3561	VPI 5482 _∆tdk_∆BT3561	This study
∆BT3562	VPI 5482 _Δtdk_ΔBT3562	This study
∆BT3563	VPI 5482 _Δtdk_ΔBT3563	This study
WT+pEmpty	VPI 5482 _Δtdk+ pNBU2-bla-ery-p1311	This study
∆ <i>BT3560</i> +pEmpty	VPI 5482 _ <i>∆tdk</i> _∆ <i>BT</i> 3560 + pNBU2-bla-erm-p1311	This study
∆BT3560+p3560	VPI 5482 _ <i>Δtdk_∆BT3560</i> + pNBU2-bla-erm- p1311 <i>BT3560</i>	This study

∆ <i>BT</i> 3562+pEmpty	VPI 5482 _ <i>∆tdk</i> _∆ <i>BT</i> 3562 + pNBU2-bla-erm-p1311	This study
∆BT3562+p3562	VPI 5482 _ <i>∆tdk_∆BT</i> 3560 + pNBU2-bla-erm- p1311_ <i>BT</i> 3562	This study
$\Delta BT3563$ +pEmpty	VPI 5482 _ <i>∆tdk_∆BT3563</i> + pNBU2-bla-tet-p1311	This study
∆BT3563+p3563	VPI 5482 _ <i>∆tdk_∆BT3560</i> + pNBU2-bla-tet- p1311_ <i>BT35</i> 63	This study
∆ <i>BT3560-3563</i> +pEmpty	VPI 5482 _ <i>∆tdk_∆BT3560-3563</i> + pNBU2-bla-tet- p1311	This study
∆BT3560-3563+p3563	VPI 5482 _ <i>Δtdk_∆BT3560</i> + pNBU2-bla-tet- p1311_ <i>BT</i> 3563	This study
jmh61∆ <i>3563</i>	jmh61_ Δ 3563 homolog	This study
jmh43∆3563	jmh43_∆3563 homolog	This study
Bacteroidales		
Bacteroides fragilis 638R		From Laurie Comstock
Bacteroides ovatus CIP 103756T/ATCC 8483		Institut Pasteur, collection
Bacteroides eggerthii CIP 104285T/DSM 20697		Institut Pasteur, collection
Bacteroides vulgatus CIP 103714T/ATCC 8482		Institut Pasteur, collection
Bacteroides uniformis CIP 103695T/ DSM 6597		Institut Pasteur, collection
Parabacteroides distasonis ATCC 8503		From Laurie Comstock
Escherichia coli		
S17λpir_pSAM-bt	S17λpir_pSAM-bt	(7)
S17λpir_pExchange-tdk	S17\pir_pExchange-tdk	(5)
S17λpir_pExchange- <i>BT3560-</i> 3562	S17λpir_pExchange- <i>BT3560-3562</i>	This study
S17λpir_pExchange- <i>BT</i> 3560- 3563	S17λpir_pExchange- <i>BT3560-3563</i>	This study
S17λpir_pExchange- <i>BT</i> 3560	S17λpir_pExchange- <i>BT3560</i>	This study
S17λpir_pExchange- <i>BT</i> 3561	S17λpir_pExchange- <i>BT3561</i>	This study
S17λpir_pExchange- <i>BT</i> 3562	S17λpir_pExchange- <i>BT3562</i>	This study
S17λpir_pExchange- <i>BT</i> 3563	S17λpir_pExchange- <i>BT3563</i>	This study
S17λpir_pLGB13-jmh61_3563	S17λpir_pLGB13- <i>BT35</i> 63 from jmh61	This study
S17λpir_pLGB13-jmh43_3563	S17λpir_pLGB13- <i>BT35</i> 63 from jmh43	This study
S17λpir_pNBU2-bla-erm-p1311	S17λpir_ pNBU2-bla-erm-p1311	This study
S17λpir_pNBU2-bla-erm-p1311- <i>BT</i> 3560	S17λpir_pNBU2-bla-erm-p1311- <i>BT3560</i>	This study
S17λpir_pNBU2-bla-erm-p1311- BT3562	S17λpir_pNBU2-bla-erm-p1311- <i>BT</i> 3562	This study
S17λpir_pNBU2-bla-tet-p1311	S17λpir_pNBU2-bla-tet-p1311	This study
S17λpir_pNBU2-bla-tet-p1311- BT3563	S17λpir_pNBU2-bla-tet-p1311- <i>BT3563</i>	This study
Plasmids	·	·
Name	Description	Reference

pSAM-bt	Random transposon mutagenesis	(6)
pExchange-tdk	Site-directed unmarked gene deletion	(8)
pLGB13	Site-directed unmarked gene deletion in natural isolates	(9)
pExchange-BT3560-3562	Site-directed unmarked gene deletion	This study
pExchange-BT3560-3563	Site-directed unmarked gene deletion	This study
pExchange-BT3560	Site-directed unmarked gene deletion	This study
pExchange-BT3561	Site-directed unmarked gene deletion	This study
pExchange-BT3562	Site-directed unmarked gene deletion	This study
pExchange-BT3563	Site-directed unmarked gene deletion	This study
pLGB13-jmh61_3563	Site-directed unmarked gene deletion	This study
pLGB13-jmh43_3563	Site-directed unmarked gene deletion	This study
pNBU2-bla-erm-p1311	Site-directed unmarked gene deletion	(10)
pNBU2-bla-erm-p1311-BT3560	Complementation	This study
pNBU2-bla-erm-p1311-BT3562	Complementation	This study
pNBU2-bla-tet-p1311	Complementation	This study
pNBU2-bla-tet-p1311-BT3563	Complementation	This study

Supporting Table S4: List of primers used in this study

Construct	Name	Sequence (5'>3')
pExchange	pEx-ch-F	TGGGAATTCCCCTCCACCGC
	pEx-ch-R	GGGGAGAGGACGGACAGAAGAT
	pExchange R	CGTCGACTCGAATGTTATCTTC
	pExchangeF	TCTAGAGCGGCCGCCACC
pExchange- BT3560-3562	3560-5Fb	AGATAACATTCGAGTCGACGGTACTTTCCTTCTTTTGGAAC
	3560-5Rb	TACTTTAAAATATGCACCCAAATTTAAATTATTATTAGTGTTAATTTGTAG C
	3562-3Fb	CACTAATAATAATTTAAATTTGGGTGCATATTTTAAAGTACCAGG
	3562-3R	GCGGTGGCGGCCGCTCTAGATTGTGGATGAAGATCAGCAATAGAC
pExchange- BT3560-3563	3560-5Fb	AGATAACATTCGAGTCGACGGTACTTTCCTTCTTTTGGAAC
	3560-5Rb	TATAACAAACAAGTTTTATTAATTTAAATTATTATTAGTGTTAATTTGTAG C
	3563-3Fb	CACTAATAATAATTTAAATTAATAAAACTTGTTTGTTATAGATATAATTAG ACC
	3563-3R	GCGGTGGCGGCCGCTCTAGAAACATATATCTTTGCTGTACAC
	3560-extF	CGCTATACCCATTAAGCCATAC
	3563-extR	GATATTAATGGCAACAACACC
pExchange- BT3560	3560-3F	GATTAGGAATAGTGATTGCAATAAATGGGCAAATTAATACAAGGAAATG
	3560-3R	GCGGTGGCGGCCGCTCTAGACAAGCTTCTGAGTTATCCTGTTTC
	3560-5R	GTATTAATTTGCCCATTTATTGCAATCACTATTCCTAATCTTTGTTTC
	3560-5F	AGATAACATTCGAGTCGACGTTAGCGGATACAGAACAGA
pExchange- BT3561	3561-3F	TAATACAAGGAAATGATATGCAAAGAAATAACTAATAAAATAAAT
	3561-3R	GCGGTGGCGGCCGCTCTAGAAGAAGAGCTAGTACTTGTATATTTAAATC C
	3561-5R	TTTTATTAGTTATTTCTTTGCATATCATTTCCTTGTATTAATTTGCC
	3561-5F	AGATAACATTCGAGTCGACGTTATCAGGGTGATAACGATGGAC
pExchange- BT3562	3562-3F	TGATGTTCCTGCTCCTTATGTGGGTGCATATTTTAAAGTACCAGG
	3562-3R	GCGGTGGCGGCCGCTCTAGATTGTGGATGAAGATCAGCAATAGAC
	3562-5R	GTACTTTAAAATATGCACCCACATAAGGAGCAGGAACATCACTAC
	3562-5F	AGATAACATTCGAGTCGACGTACGAACTTGCGTACTGGCG
pExchange- BT3563	3563-3F	CTTTTTTAACAAAAATGAATTAATAAAACTTGTTTGTTATAGATATAATTA GACC
	3563-3R	GCGGTGGCGGCCGCTCTAGAAACATATATCTTTGCTGTACAC
	3563-5R	CTATAACAAACAAGTTTTATTAATTCATTTTTGTTAAAAAAGGAG
	3563-5F	GAAGATAACATTCGAGTCGACGGCAGATTTGAGTGCTCAAGC
	3563-extF	GATAATACATGGGTATCATTGAC
	3563-extR	GATATTAATGGCAACAACACC
pLGB13	pLGB13- linR	TGATATCGAATTCCTGCAGC
	pLGB13-linF	GCTTATCGATACCGTCGAC
	pLGB13-chF	GGTGTAAGATTAGCATTATGAGTG

	pLGB13- chR	CCATCACTGGAAGATAGGC
pLGB13- jmh61_3563	jmh61-3563- 5R	AACAAACAAGTTTTATTCTATATTGAAAATTAAGGGGAGTATATTAC
	jmh61-3563- 5F	GCTGCAGGAATTCGATATCAACTGAAACTCTTGGTGCG
	jmh61-3563- 3F	ACTCCCCTTAATTTTCAATATAGAATAAAACTTGTTTGTT
	jmh61-3563- 3R	AGTCGACGGTATCGATAAGCGCGCATCAGCGAGATGTAATAAGC
pLGB13- jmh43_3563	jmh43-3563- 5R	TAAGCTAGCTTATTTAAGATATATATTTAGAGACAAGTAAC
	jmh43-3563- 5F	GAAGATAACATTCGAGTCGACGGCAGATTTGAGTGCTCAAGC
	jmh43-3563- 3F	CTCTAAATATATATCTTAAATAAGCTAGCTTATGAAAAAACTGTTATTAG
	jmh43-3563- 3R	GATATTAATGGCAACAACACC
pNBU2-bla	pNBU-chR	GCCAATGCACAAATGCTGTTCC
	pNBU-chF	CAGGTGTATTCCCATCCGG
	pNBU-F	CGACGTCGACTAATTGCC
	pNBU-R	ATGTTAAAAACAGATTTGGAGTGC
pNBU2-bla- erm-p1311- <i>BT3560</i>	NBU-3560-F	AATCTGTTTTTAACATATGAAACAAAGATTAGGAATAGTGATTGC
	NBU-3560- R	TAGGCAATTAGTCGACGTCGTTAGAATCTATATCCAATGTTCAAGAAAG C
pNBU2-bla- erm-p1311- <i>BT3562</i>	NBU-3562-F	AAATCTGTTTTTAACATATGAAAAAGATTCTAAATGCTTTATTCTTG
	NBU-3562- R	TAGGCAATTAGTCGACGTCGTTATTCAACGAACTTATATTCGGTAGG
pNBU2-bla-tet- p1311- <i>BT</i> 3563	NBU-3563- R	TAGGCAATTAGTCGACGTCGCTATTGCAGTCCGGTCC
	NBU-3563-F	TCCAAATCTGTTTTTAACATATGAGATTTTTTAATAGAAGTGTTG

Supporting Dataset S1 (excel spreadsheet). RNAseq analysis: table of A. all genes,

2 B. upregulated, C. downregulated genes in presence of 0.5% bile, D. COG functional

3 categories enrichment.

Supplementary Information Reference list

- 1 2 3 4 D. Ryan, L. Jenniches, S. Reichardt, L. Barquist, A. J. Westermann, A high-resolution 1. transcriptome map identifies small RNA regulation of metabolism in the gut microbe Bacteroides thetaiotaomicron. Nature Communications 11, 1--16 (2020).
- 5 6 2. R. Buels et al., JBrowse: A dynamic web platform for genome visualization and 7 analysis. Genome Biology 17, 1--12 (2016).
- 8 D. Vallenet *et al.*, MicroScope: An integrated platform for the annotation and 3. 9 exploration of microbial gene functions through genomic, pangenomic and metabolic 10 comparative analysis. Nucleic Acids Research 48, D579--D589 (2020).
- 11 4. J. Oberto, SyntTax: A web server linking synteny to prokaryotic taxonomy. BMC 12 Bioinformatics 14 (2013).
- N. M. Koropatkin, E. C. Martens, J. I. Gordon, T. J. Smith, Starch Catabolism by a 13 5. 14 Prominent Human Gut Symbiont Is Directed by the Recognition of Amylose Helices. 15 Structure 16, 1105--1115 (2008).
- 16 J. Mihajlovic et al., A putative type v pilus contributes to bacteroides thetaiotaomicron 6. 17 biofilm formation capacity. 201 (2019).
- 18 A. L. Goodman et al., Identifying genetic determinants needed to establish a human 7. 19 gut symbiont in its habitat. Cell host & microbe 6, 279--289 (2009).
- 20 8. E. C. Martens, H. C. Chiang, J. I. Gordon, Mucosal Glycan Foraging Enhances Fitness and Transmission of a Saccharolytic Human Gut Bacterial Symbiont. Cell Host and 21 22 Microbe 4, 447--457 (2008).
- 23 9. L. Garca-Bayona, L. E. Comstock, Streamlined genetic manipulation of diverse 24 bacteroides and parabacteroides isolates from the human gut microbiota. mBio 10 25 (2019).
- 10. J. Wang, N. B. Shoemaker, G. R. Wang, A. A. Salyers, Characterization of a 26 27 Bacteroides mobilizable transposon, NBU2, which carries a functional lincomycin 28 resistance gene. Journal of Bacteriology 182, 3559--3571 (2000).
- 29 30