Supplementary Table 1. Comparison of proteins encoded by pBUN24 and pBCPT

pBUN24							% similarity	pBCPT (PvCL04 gene designations)						
gene name	product	motifs	start	stop	bp	aa		gene name	predicted function	motifs	start	stop	bp	aa
repA	repA	PF01051	476	1,453	978	325	92.62%	CS034_04053	repA	PF01051	475	1,452	978	325
CDS_9 (orf2)	hypothetical protein		1,467	1,649	183	60	96.36%	CS034_04054	hypothetical protein		1,466	1,657	192	63
CDS_8 (orf1)	hypothetical protein		1,763	2,284	522	173	94.80%	CS034_04055	hypothetical protein		1,759	2,280	522	173
CDS_7 (orf7)	hypothetical protein		2,314	2,855	542	180		CS034_04056	LuxR-type DNA-binding HTH domain protein	PF00196	2,301	3,938	1,638	545
CDS_6 (orf6)	HTH transcriptional regulator		2,878	3,975	1,098	365		CS034_04057	predicted OMP		4,086	4,658	573	190
CDS_5 (orf5)	putative lipoprotein	PF08450	4,195	5,850	1,656	551		CS034_04058	BcpT (experimental evidence)		4,672	6,171	1,500	499
								CS034_04059	BcpI (experimental evidence)		6,228	6,401	174	57
mobB	MobB		6,405	7,283	879	292	98.29%	CS034_04060	MobB	DUF5712, PF18976	6,579	7,457	879	292
mobA	MobA		7,280	7,885	606	201	99.50%	CS034_04061	MobA	PF01726	7,454	8,059	606	201
CDS_4 (orf4)	hypothetical protein		7,917	8,183	267	88								
CDS_3 (tad)	TA system toxin	PF05973, TIGR03070	8,277	8,624	348	115	98.26%	CS034_04062	HipB-like toxin	PF05973, TIGR03070	8,450	8,797	348	115
CDS_2 (ata)	TA system anti-toxin	PF15731	8,630	8,944	315	104	100.00%	CS034_04063	MqsA-like anti-toxin	PF15731	8,803	9,117	315	104
CDS_1 (orf3)	hypothetical protein		8,938	263	270	89								

a. CDS names are from NCBI GenBank EU818711 file, pBUN24 names are from Fig 1 in **Shkoporov AN, et al.** 2013. Analysis of a novel 8.9kb cryptic plasmid from *Bacteroides uniformis* , its long-term stability and spread within human microbiota. Plasmid. **69(2):**146-59.
Yellow highlighted genes are those unique between each of the two plasmids

Supplementary Table 2. Primers used in this study

Construction of PvCL04 and PdCL02 Δ*bcpT*	left flank forward	caagggatccataagaaaaaggcacatcgagaac
	left flank reverse	gtcgacgcgttgttacatgaacacaaaatagctg
	right flank forward	atgtacgcgtttgttggtaagccagaaattgta
	right flank reverse	cgaaggatccaagatcttctcctatatcgcaagc
Clone *bcpT* into pFD340	forward	accaggatccaaacacggtataacaaaagaacca
	reverse	aggaggatccactccttccctattttgtttagga
Screening for *bcpT* in unsequenced strains	forward	ggtatatactatggagttgc
	reverse	actccttccctattttgtttagga
Outward primers for circular plasmid confirmation	left junction primer	gtggatatttgttttccaacatga
	right junction primer	aagataaagcgtatggaagcaaag
Construction of His-BcpT for expression in *E. coli*	forward	acatcatatgaacaatgaacttcctattgaaaatgtaca
	reverse	aggaggatccactccttccctattttgtttagga
His-BcpT (R65N)	forward	gataatcctattttaacaaacgcaagttcatcttttg
	reverse	caaaagatgaacttgcgtttgttaaaataggattatc
His-BcpT (R199N)	forward	gggactgaaactctcacaaactccgcaagcgatggaggt
	reverse	acctccatcgcttgcggagtttgtgagagtttcagtccc
Amplify native and site mutants of *bcpT* from His-constructs	forward	gtgttcatgtaacaatgaacttcctattgaaaat
	reverse	attcgagctcggtacccgggactccttccctattttgtttag
Amplify rbs and signal sequence of *bcpT* for construction of *bcpT* site mutants in pFD340	forward	aatcagaattgactctagagcacggtataacaaaagaacc
	reverse	gttcattgttacatgaacacaaaatagctg
Construction of PdCL02 Δ*dpn1* (HMPREF1064_01439) BamHI digested pLGB13	left flank forward	taagattagcattatgagtggcatctaagatagctgac
	left flank reverse	ctttataccagccacatactaataacagaaaac
	right flank forward	agtatgtggctggtataaagatactaatacacattagag
	right flank reverse	cgaattcctgcagcccgggggctaactcagatgctcgc
Construction of PdCL02 Δ*dpn2* (HMPREF1064_00451) BamHI digested pLGB13	left flank forward	taagattagcattatgagtgggtaggctcgttgaacaag
	left flank reverse	cagcccatcccataagaaaaggatcactcttcc
	right flank forward	ttttcttatgggatgggctgaaacggaatc
	right flank reverse	cgaattcctgcagcccggggaagcaatgatggctctttctatc
Complement PdCL02 Δ*dpn2* (in pFD340)	forward	aatcagaattgactctagagtcaagaaaccggaagagtg
	reverse	attcgagctcggtacccgggctggctgcattctccttc
Deletion of PvCL10 M0N98_03761-60 (sigma factor/antisigma factor)	left flank forward	cggtgtaagattagcattatgagtgtccgcttcatggagactc
	left flank reverse	agcaatgacacggtgagcttgcacgtacaaac
	right flank forward	acgtgcaagctcaccgtgtcattgctccttc
	right flank reverse	gatatcgaattcctgcagcccgggggtttcgcggtcactcatc
Clone M0N98_03761 into pFD340	forward	ttctggatcccggatgcagtatttttgcagtctt
	reverse	ctacggatccggttccgaatagccctccatc
Clone M0N98_03760 into pFD340	forward	gatgggatcctacagagccaaaaccttattgatg
	reverse	tcatggatccattttatataagaacagtgaaaaagttcat
Clone M0N98_03761-60 into pFD340	forward	ttctggatcccggatgcagtatttttgcagtctt
	reverse	tcatggatccattttatataagaacagtgaaaaagttcat
Construction of Pv8482 O-ag deletion mutant Bvu_1068-69 cloned into PstI site pLGB13	left flank forward	agtggatcccccgggctgcaccaaaatagggcacgttatatc
	left flank reverse	tctttatgattacctccagtaccggatattc
	right flank forward	actggaggtaatcataaagaaatggatggaagtgatc
	right flank reverse	cttgatatcgaattcctgcagtggatgatccggagctg
Construction of His-BSAP-3 for expression in *E. coli*	forward	taaacatatgtgtacaaatacttatgacgtttgtgaaat
	reverse	ccaccatatgttatggataaacatatcctaaaataccacc
Construction of PvCL04 *ermG* deletion mutant into BamHI site pLGB30	left flank forward	taagattagcattatgagtgcgtaggcaaaaacggcatag
	left flank reverse	ctttgaactacattagtaacttcttacaggtgaatac
	right flank forward	gttactaatgtagttcaaagtcgggtgg
	right flank reverse	cgaattcctgcagcccggggcgacacggttatttgggatag

Construction of pBCPT-*tetQ*

PCR amplify BcCL03T12C61 *tetQ*	forward	ttcgtcgattcttgccagttgaacctac
	reverse	aattctgttcccgtattgccttatagaaatttc
PCR amplify BF638R_1994 transcriptional stop	forward	ggcaatacgggaacagaattggccgaag
	reverse	actgccgagcccaccaattccatattcaag
PCR amplify pBCPT flanks for *tetQ* integration	left flank forward	taagattagcattatgagtgggtcgaacttataacgggc
	left flank reverse	aactggcaagaatcgacgaacaccctcc
	right flank forward	gaattggtgggctcggcagtcgaaagaac
	right flank reverse	cgaattcctgcagcccgggggtgacctcccttgcgctc

Insertion of *ermG* in *B. fragilis* 638RΔT6SS

PCR amplify *ermG* from pNBU2-*ermG*	forward	tcttctaaccgcttgacggaaatcaacaaattttag
	reverse	ttgcactacgcagtttgatttctcaggactttac
PCR amplify flanking regions to insert *ermG*	left flank forward	gctcaacaattgcttgacgggtggagctgatgcgtttc
	left flank reverse	tccgtcaagcggttagaagaattgtggaagtc
	right flank forward	aatcaaactgcgtagtgcaaatatagcg
	right flank reverse	tgcctgttctatttccgaaccgatgtagccattatcgatg
amplify pLGB36 removing plasmid *ermG*	forward	gttcggaaatagaacagg
	reverse	ccgtcaagcaattgttgag
Detect pBCPT in transconjugants	forward	aatcagaattgactctagagcgctccttcccttcacttattg
	reverse	attcgagctcggtacccgggcattggtgatgatgctatttaagatag
forward multiplex primer (for all three species)	MPI forward	gtacacaccgcccgt
multiplex *P. vulgatus* reverse		ctttctctcttccgtatcattac
Multiplex *B. fragilis* reverse	MPI-Bf reverse	gctaatcccccaatcatac
multiplex *B. ovatus* reverse	MPI-Bo reverse	atcaatattgcgtactcgaac
Clone PvCL04 CS034_04056 into pFD340	forward	aatcagaattgactctagagctgagttgtaatcaaacctattg
	reverse	attcgagctcggtacccggggatagttaaggtgcatcac
Clone PvCL04 CS034_04057 into pFD340	forward	aatcagaattgactctagaggtctaactttgcaacgttc
	reverse	attcgagctcggtacccgggttaaaatgaatatccaaaactaatataagg
Clone PvCL04 CS034_04059 into pFD340	forward	aatcagaattgactctagagcgctccttcccttcacttattg
	reverse	attcgagctcggtacccgggcattggtgatgatgctatttaagatag

Restriction sites are underlined

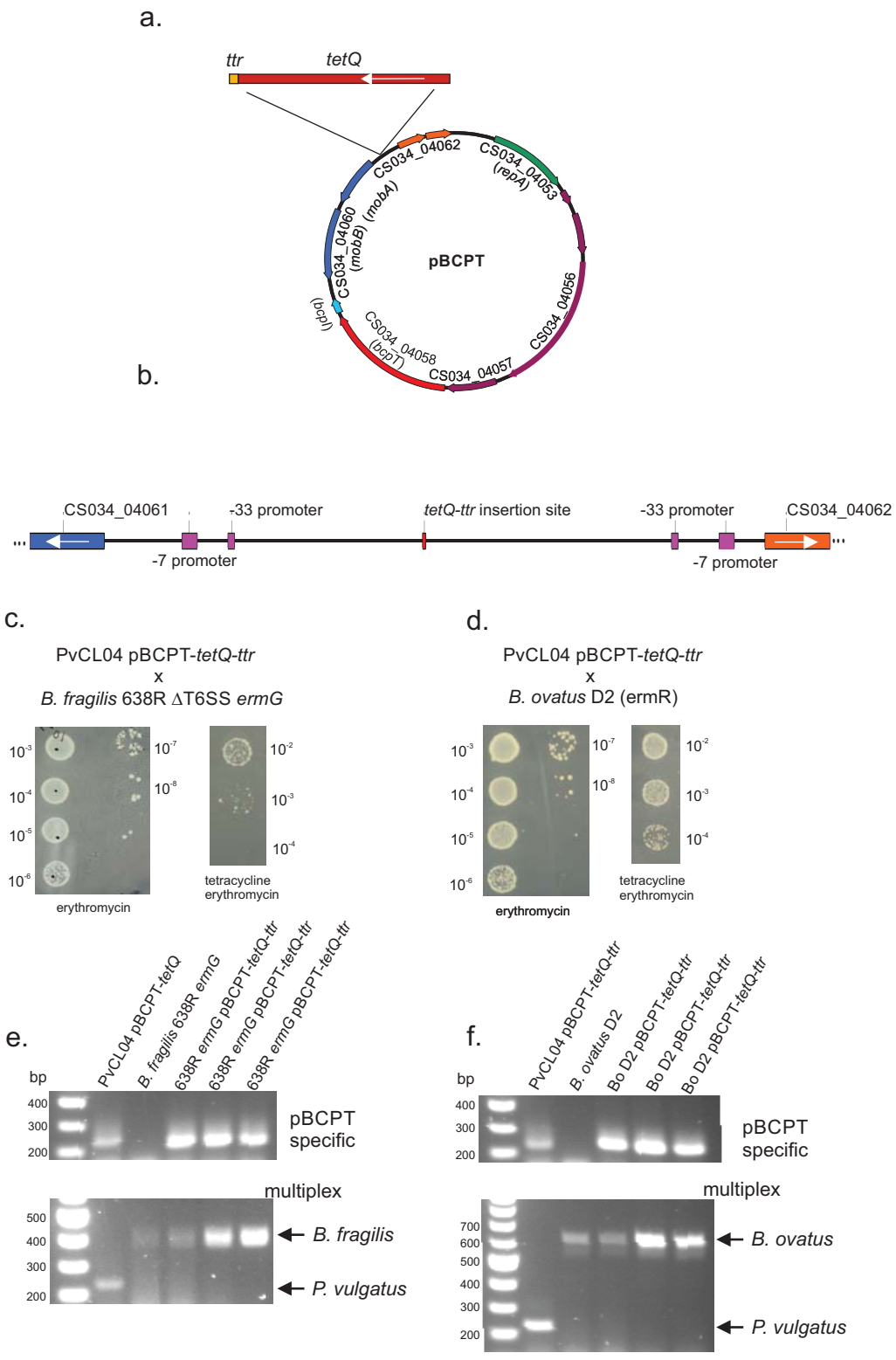
Supplementary Table 3. Strains used in this study	**source**	**toxin phenotype of wild type Pv and Pd strains**
Phocaeicola dorei CL02T00C15	Comstock lab (Zitomersky, 2011)	BcpT
Phocaeicola dorei CL02T00C15 Δ*bcpT* (ΔHMPREF1063_05166)	this study	
Phocaeicola dorei CL02T00C15 Δ*bcpT* p*bcpT*	this study	
Phocaeicola dorei CL02T00C15 Δ*bcpT* p*bcpT* R65N	this study	
Phocaeicola dorei CL02T00C15 Δ*bcpT* p*bcpT* R199N	this study	
Phocaeicola dorei CL02T00C15 Δ*bcpT* p*bcpT* R65N R199N	this study	
Phocaeicola vulgatus CL04T12C01	Comstock lab (Zitomersky, 2011)	BcpT
Phocaeicola vulgatus CL04T12C01 tn10	this study	
Phocaeicola vulgatus CL04T12C01 tn12	this study	
Phocaeicola vulgatus CL04T12C01 Δ*bcpT* (ΔCS034_04058)	this study	
Phocaeicola vulgatus CL04T12C01 Δ*bcpT* p*bcpT*	this study	
Phocaeicola vulgatus CL04T12C01 *Δ ermG* pBCPT-*tetQ-ttr*	this study	
Phocaeicola dorei CL03T12C01	Comstock lab (Zitomersky, 2011)	BSAP-3
Phocaeicola dorei 9_1_42 FAA	BEI	BSAP-3
Phocaeicola dorei 5_1_36/D4	BEI	
Phocaeicola dorei CL01T00C33	Comstock lab (Zitomersky, 2011)	
Phocaeicola dorei CL08T03C21	Comstock lab (Zitomersky, 2011)	
Phocaeicola dorei CL12T00C02	Comstock lab (Zitomersky, 2011)	
Phocaeicola dorei CL15T00C36	Comstock lab (Zitomersky, 2011)	
Phocaeicola vulgatus ATCC 8482	ATCC	
Phocaeicola vulgatus ATCC 8482 Δ*Bvu_1068-69* (O-ag mutant)	this study	
Phocaeicola vulgatus CL05T12C02	Comstock lab (Zitomersky, 2011)	BcpT
Phocaeicola vulgatus CL06T12C01	Comstock lab (Zitomersky, 2011)	
Phocaeicola vulgatus CL07T06C01	Comstock lab (Zitomersky, 2011)	
Phocaeicola vulgatus CL09T03C04	Comstock lab (Zitomersky, 2011)	BSAP-3
Phocaeicola vulgatus CL10T00C06	Comstock lab (Zitomersky, 2011)	
Phocaeicola vulgatus CL10T00C06 Δ*CK234_00400 - 401* (O-ag mutant)	Comstock lab (Laclare McEneany, 2018)	
Phocaeicola vulgatus CL10T00C06 Δ*CK234_00923-924* (Δsig/antisig)	this study	
Phocaeicola vulgatus CL10T00C06 Δ*CK234_00923-924* p*00923*	this study	
Phocaeicola vulgatus CL10T00C06 Δ*CK234_00923-924* p*00924*	this study	
Phocaeicola vulgatus CL10T00C06 pBCPT *gene (CS034_04056)*	this study	
Phocaeicola vulgatus CL10T00C06 pBCPT *gene (CS034_04057)*	this study	
Phocaeicola vulgatus CL10T00C06 pBCPT *gene (CS034_04059)*	this study	
Phocaeicola vulgatus CL11T12C09	Comstock lab (Zitomersky, 2011)	
Phocaeicola vulgatus CL13T00C01	Comstock lab (Zitomersky, 2011)	
Phocaeicola vulgatus CL14T03C19	Comstock lab (Zitomersky, 2011)	bacteroidetocin A, BcpT
Bacteroides thetaiotamicron VPI 5482	lab stock	
Bacteroides thetaiotamicron VPI 5482 pFD340	Comstock lab (Laclare McEneany, 2018)	
Bacteroides thetaiotamicron VPI 5482 p*bcpT*	this study	
Bacteroides ovatus D2	BEI	
Bacteroides ovatus D2 pBCPT-*tetQ-ttr*	this study	
Phocaeicola dorei CL02T12C06	Comstock lab (Zitomersky, 2011)	BcpT
Phocaeicola dorei CL02T12C06 Δ*dpn1*	this study	
Phocaeicola dorei CL02T12C06 Δ*dpn2*	this study	
Phocaeicola dorei CL02T12C06 Δ*dpn1* Δ*dpn2*	this study	
Bacteroides fragilis 638R	lab stock	
Bacteroides fragilis 638RΔT6S*ermG*	this study	
Bacteroides fragilis 638RΔT6S*ermG* pBCPT-*tetQ-ttr*	this study	
Bacteroides ovatus ATCC 8483	ATCC	
Bacteroides uniformis ATCC 8492	ATCC	
Bacteroides finegoldii CL09T03C10	Comstock lab (Zitomersky, 2011)	
Parabacteroiees merdae CL03T12C32	Comstock lab (Zitomersky, 2011)	
Parabacteroides johnsonii CL02T12C29	Comstock lab (Zitomersky, 2011)	
Parabacteroides goldsteinii CL02T12C30	Comstock lab (Zitomersky, 2011)	
E. coli DH5α	lab collection	
E. coli BL21/DE3	lab collection	
E. coli S17 λ pir	E. Martens	
E. coli HB101	lab collection	
E. coli HS	lab collection	
E. coli BL21/DE3 pHis-*bcpT*	this study	
E. coli BL21/DE3 pHis-*bcpT* R65N	this study	

E. coli BL21/DE3 pHis-*bcpT* R199N	this study	
E. coli BL21/DE3 pHis-*bcpT* R65N R199N	this study	
E. coli BL21/DE3 pHis-*dpn1*	this study	
E. coli BL21/DE3 pHis-*dpn2*	this study	
Salmonella enterica ATCC 14028	ATCC	

Zitomersky NL, Coyne MJ, Comstock LE. Longitudinal analysis of the prevalence, maintenance, and IgA response to species of the order Bacteroidales in the human gut. Infect Immun. 2011 May;79(5):2012-20. doi: 10.1128/IAI.01348-10. Epub 2011 Mar 14. PMID: 21402766; PMCID: PMC3088145.

McEneany VL, Coyne MJ, Chatzidaki-Livanis M, Comstock LE. Acquisition of MACPF domain-encoding genes is the main contributor to LPS glycan diversity in gut Bacteroides species. ISME J. 2018 Dec;12(12):2919-2928. doi: 10.1038/s41396-018-0244-4. Epub 2018 Jul 31. PMID: 30065309; PMCID: PMC6246601.

Roelofs KG, Coyne MJ, Gentyala RR, Chatzidaki-Livanis M, Comstock LE. Bacteroidales Secreted Antimicrobial Proteins Target Surface Molecules Necessary for Gut Colonization and Mediate Competition In Vivo. mBio. 2016 Aug 23;7(4):e01055-16. doi: 10.1128/mBio.01055-16. PMID: 27555309; PMCID: PMC4999547.



Supplementary Figure 1. Analysis of pBCPT plasmid mobilization. **a.** Plasmid map of pBCPT with gene names from PvCL04. The insertion site of *tetQ* and the transcriptional termination region (*ttr*) is shown. **b.** Amplified intergenic region between CS034_04061 and CS034_04061 showing that the insertion does not affect the promoters of either divergently transcribed gene. **c. d.** Plates showing the colonies arising on erythromycin plates (recipient strain) and tetracycline/erythromycin plates (transconjugants) for transfer of the pBCPT-*tetQ* plasmid from PvCL04 to *B. fragilis* 638R*ermG*ΔT6SS (C) and *B. ovatus* D2 (D). **e. f.** Amplicons resulting from PCR with primers specific to the *bcpT* gene (top) and a multiplex of the 16S rRNA gene (bottom) of the transconjugants.

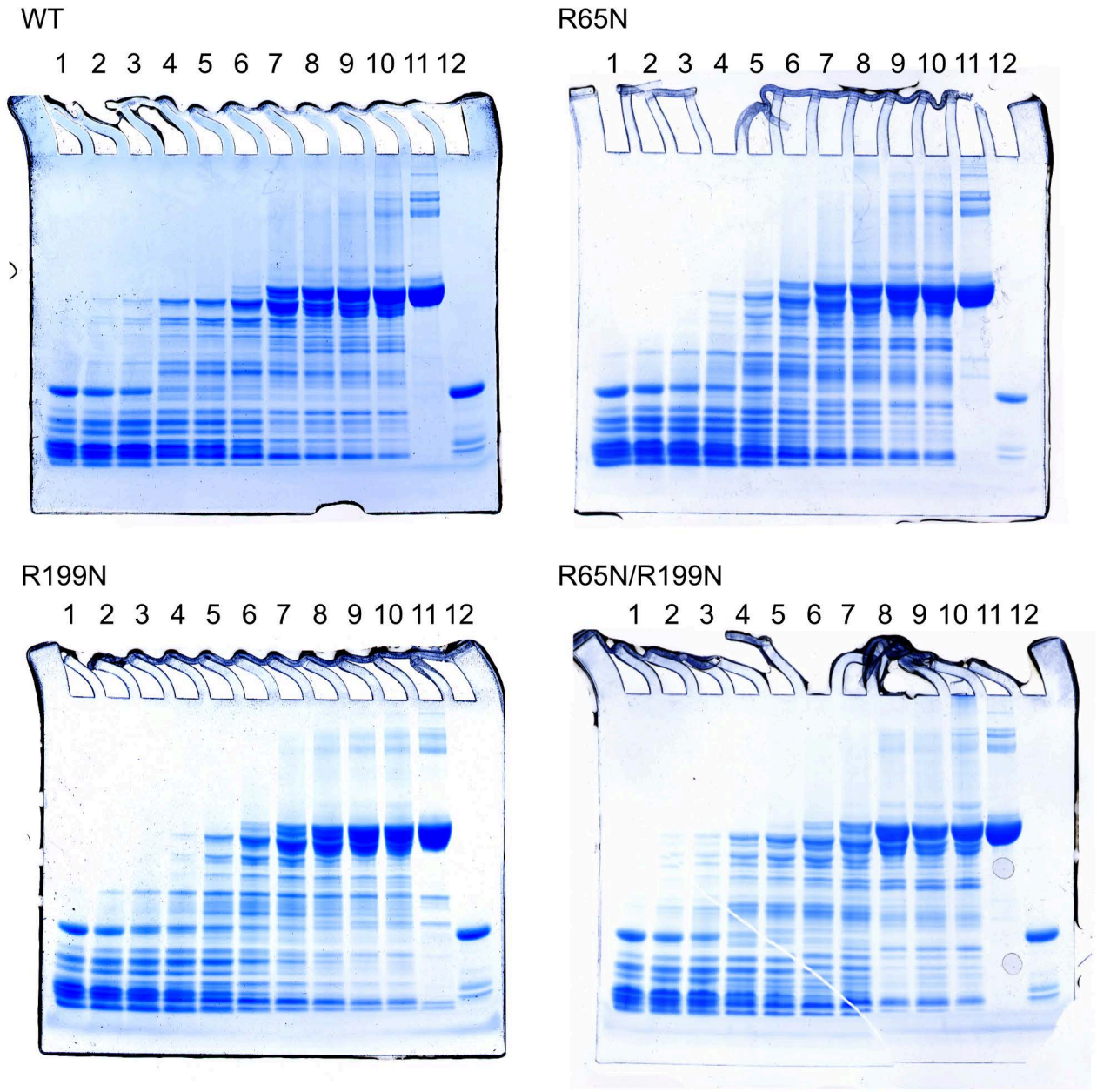


Figure S2. Trypsin digestion of BcpT cleavage site mutants. Purified recombinant wt BcpT and its cleavage site mutants, R65N, R199N and R65N-R199N, were cleaved (5µg in each reaction) with chymotrypsin. Two-fold dilutions of chymotrypsin starting with 2.5 µg of chymotrypsin were generated in HBS and to each dilution 5µg of BcpT was added (final reaction volume of 20µl) and incubated for 30 min at 37°C. The digestion reactions were stopped by the addition of 5µl of SDS-PAGE sample buffer and heating to 95°C for 5 min. The cleavage products were separated on an 8-16% SDS-PAGE: the cleavage products are shown in lanes 1-10. Lane 11 contains uncleaved WT BcpT or its derivatives. Lane 12 contains 2.5 µg of chymotrypsin.

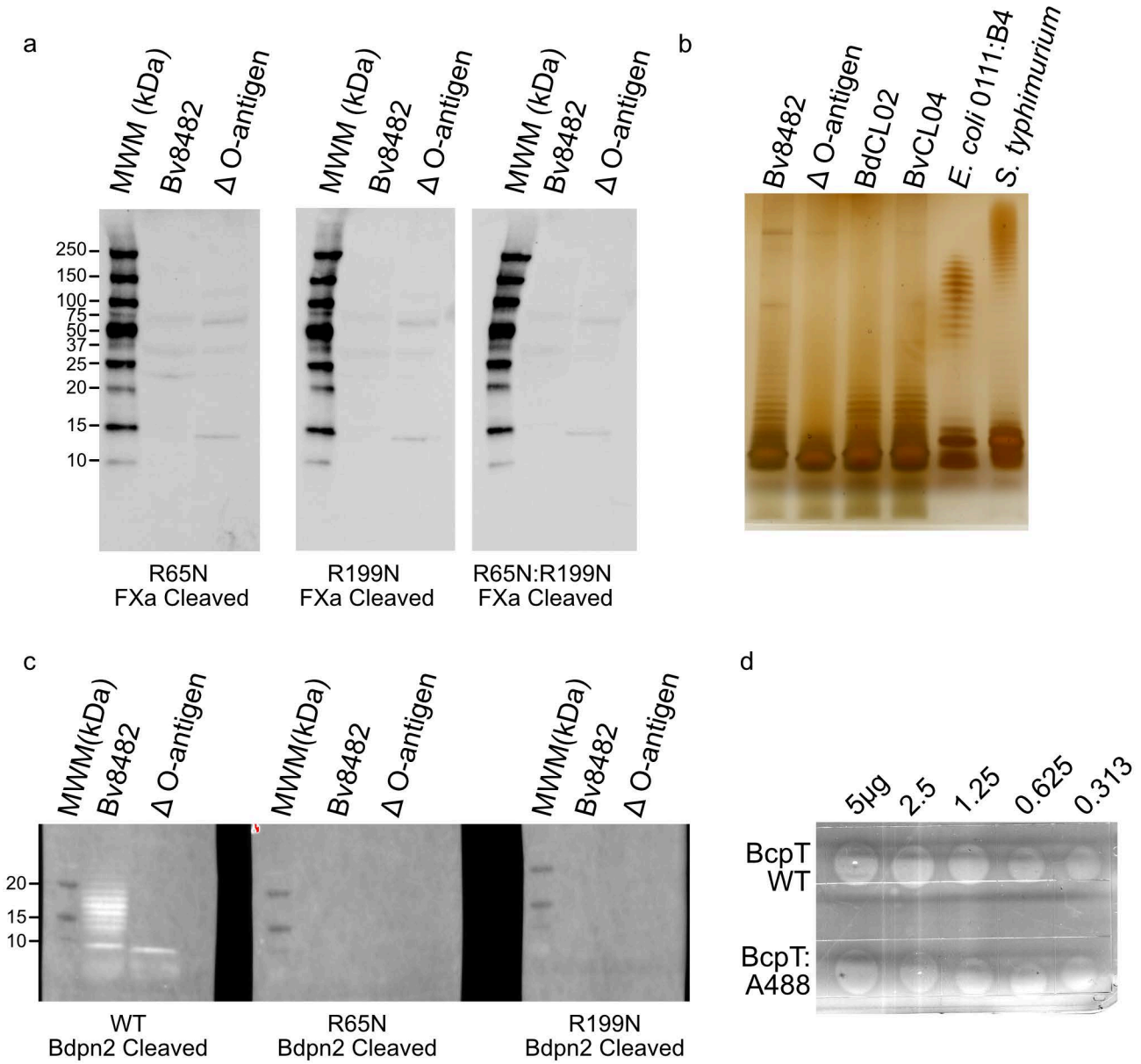


Figure S3. Analysis of BcpT and BcpT site mutants binding to components of membrane fraction and LPS of indicated strains on Western blots. **a**. Receptor blot of membranes from Bv8482 or its O-antigen mutant probed with BcpT site mutants (see Methods for receptor blots details). **b**. Silver-stain analysis of LPS extracts from indicated strains. **c.** Probing of blots containing purified LPS with Alexa-488-labeled BcpT. **d**. Spot tests of the activated WT BcpT and the Alexa-fluor labeled WT BcpT use to probe the lipid blots in panel e and in Fig. 5. **e**. Spot tests of activated BcpT (5µg) on the strains of *E. coli* and *Salmonella enterica*.

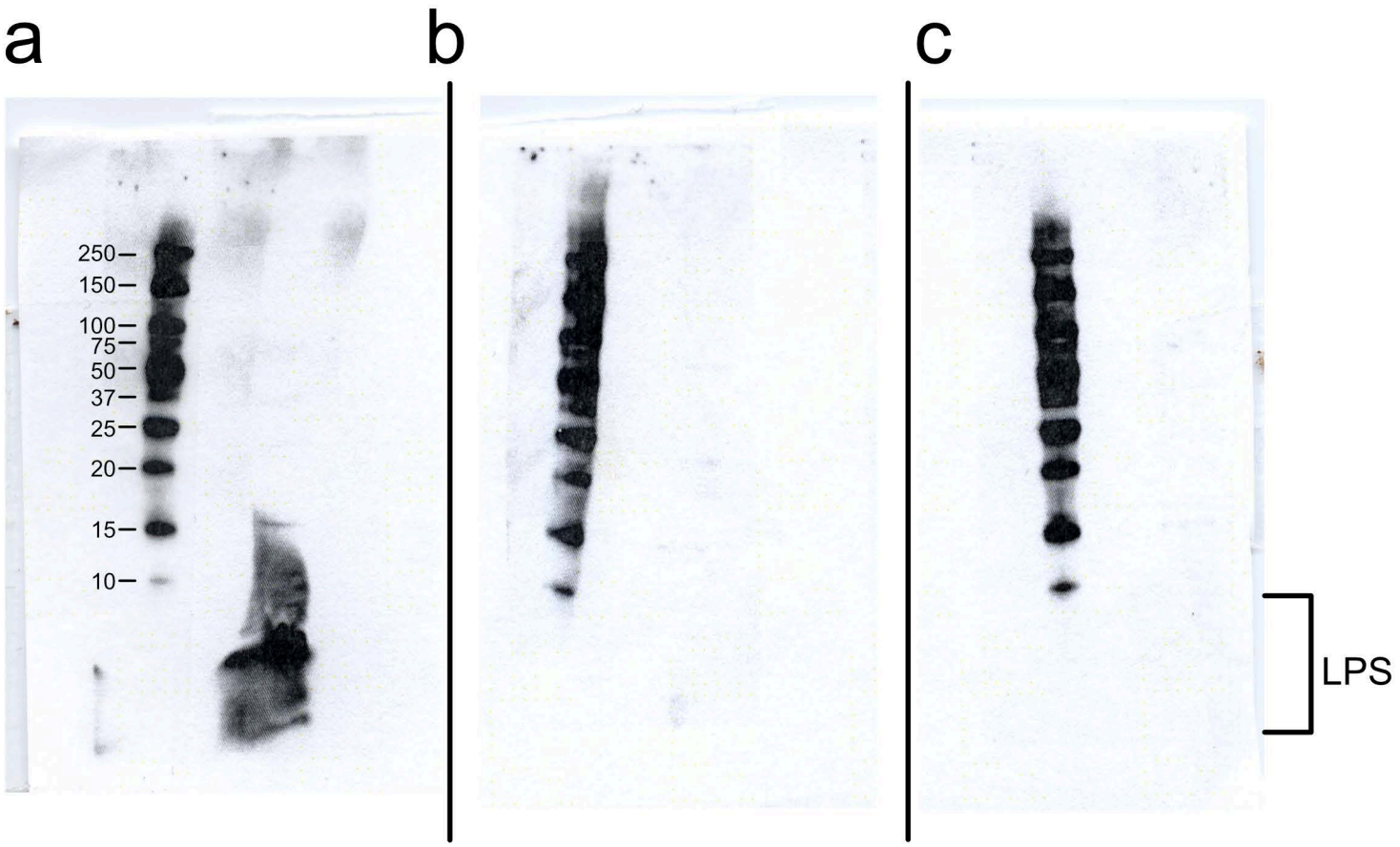


Figure S4. Amino terminal fragments of BcpT block BcpT binding to membrane LPS. a. Receptor blot of Bv8482 membranes probed with FXa-cleaved BcpT (1.5µg/ml) (see Methods for details). **b**. Receptor blot of Bv8482 membranes probed with FXa-cleaved BcpT in the presence of 20-fold molar excess N-terminal BcpT fragment 18-199. **c**. Receptor blot of Bv8482 membranes probed with FXa-cleaved BcpT in the presence of 20-fold molar excess N-terminal BcpT fragment 66-199.

Supplemental references

1) Shkoporov, A. N. *et al.* Analysis of a novel 8.9kb cryptic plasmid from *Bacteroides uniformis*, its long-term stability and spread within human microbiota. *Plasmid* **69**, 146-159, doi:10.1016/j.plasmid.2012.11.002 (2013).

2) Zitomersky NL, Coyne MJ, Comstock LE. Longitudinal analysis of the prevalence, maintenance, and IgA response to species of the order Bacteroidales in the human gut. Infect Immun. 2011 May;79(5):2012-20. doi: 10.1128/IAI.01348-10. Epub 2011 Mar 14. PMID: 21402766; PMCID: PMC3088145.

3) Laclare McEneany, V., Coyne, M. J., Chatzidaki-Livanis, M. & Comstock, L. E. Acquisition of MACPF domain-encoding genes is the main contributor to LPS glycan diversity in gut *Bacteroides* species. *ISME J* **12**, 2919-2928, doi:10.1038/s41396-018-0244-4 (2018).

4) Roelofs, K. G., Coyne, M. J., Gentyala, R. R., Chatzidaki-Livanis, M. & Comstock, L. E. Bacteroidales Secreted Antimicrobial Proteins Target Surface Molecules Necessary for Gut Colonization and Mediate Competition In Vivo. *mBio* **7**, doi:10.1128/mBio.01055-16 (2016).