Supplemental Figures, Tables and Movie Legends for:

JB00218-19

"The carboxy-terminal region of *Flavobacterium johnsoniae* SprB facilitates its secretion by the type IX secretion system and propulsion by the gliding motility machinery."

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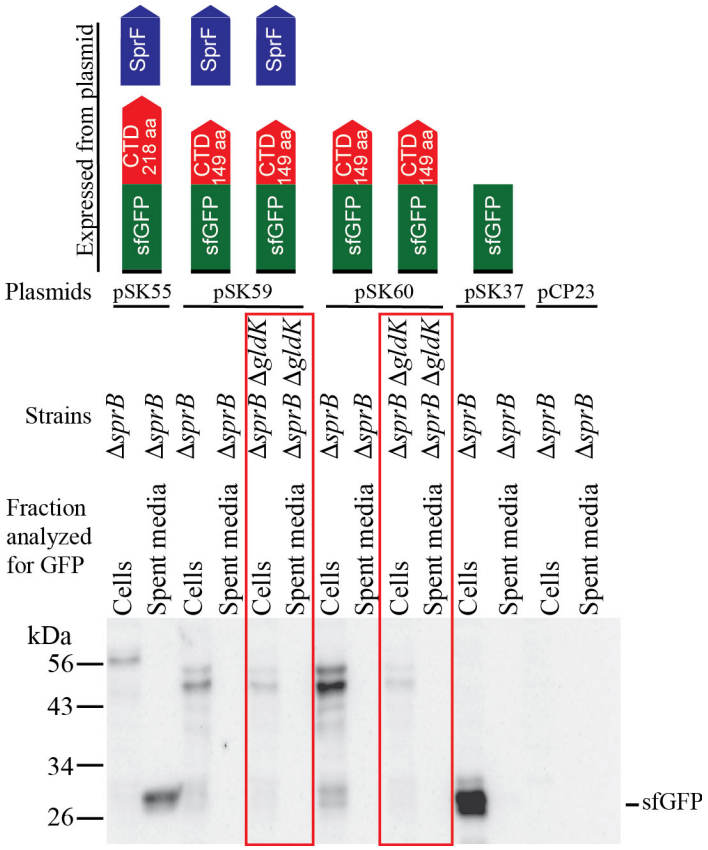

Figure S1. T9SS-mediated secretion of sfGFP fused to the CTD of SprB. (This figure is identical to Fig. 2C in the main text, except that the four lanes boxed in red above that were removed in constructing Fig. 2C are included here. These lanes, which added no useful information, were removed from Fig. 2C to simplify the presentation.) Cultures were incubated in CYE at 25°C with shaking and harvested in the late exponential phase of growth. Cells and spent culture media were separated by centrifugation and analyzed by Western blot using antiserum against GFP. Cell samples corresponded to 10 µg protein per lane and samples from spent media corresponded to the volume of spent medium that contained 10 µg protein before the cells were removed. Plasmids used were pCP23 (Empty vector); pSK37, which expresses sfGFP with N-terminal signal peptide; pSK55, which co-expresses SP-sfGFP- $CTD_{SprB218AA}$ and SprF; pSK60, which expresses SP-sfGFP fused to the 149-amino acid CTD of SprB (SP-sfGFP-$CTD_{SprB149AA}$); or pSK59, which co-expresses SP-sfGFP- $CTD_{SprB149AA}$ and SprF. Cartoons depicting the proteins expressed from the plasmids are indicated above the lanes.

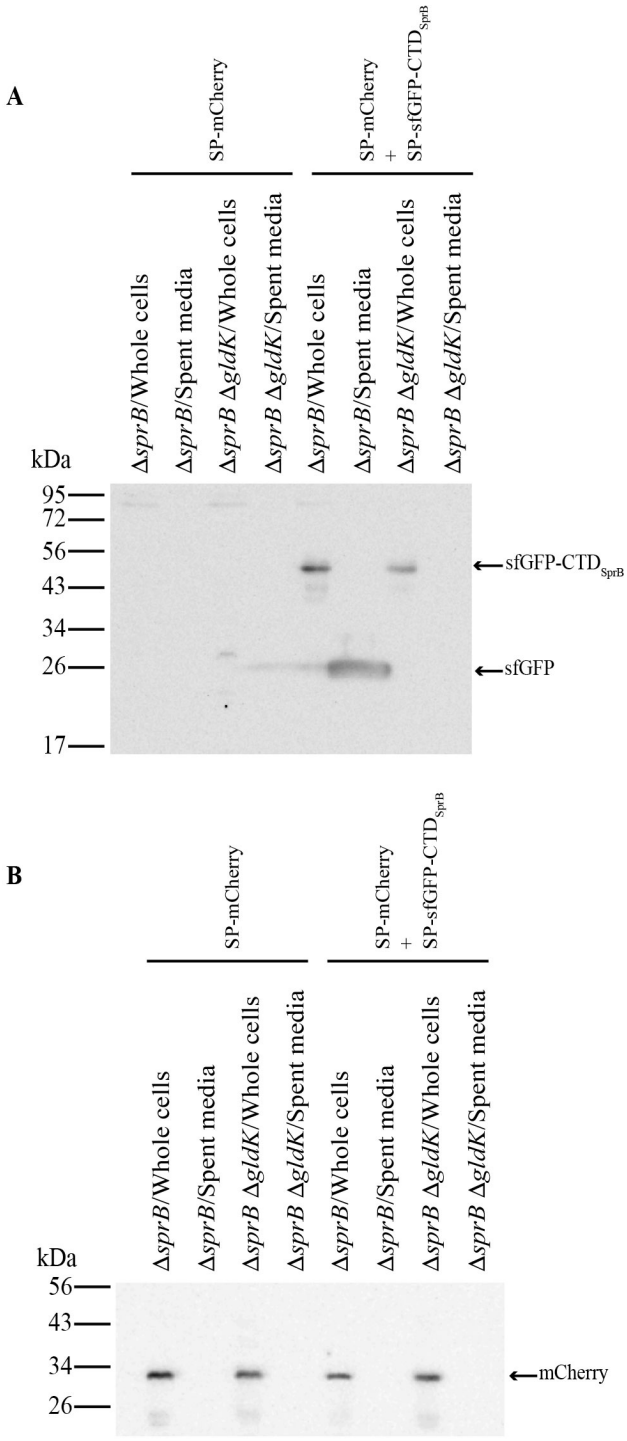

Figure S2. Analysis of cells for leakage of periplasmic mCherry. Cultures of Δ*sprB* mutant or of the T9SS mutant Δ*sprB* Δ*gldK* expressing SP-mCherry (pJJ21), or SP-sfGFP-CTD$_{SprB}$ (pSK55) and SP-mCherry (pJJ21) were incubated in CYE at 25°C with shaking. One ml samples were centrifuged at 22,000 x g for 15 min. The culture supernatant (spent medium) and intact cells were analyzed by SDS-PAGE, followed by western blot using (A) anti-GFP antibodies (to detect secretion of SP-sfGFP-CTD$_{SprB}$) and (B) anti-mCherry antibodies (to detect leakage of periplasmic mCherry). Identical samples were used in panels A and B. Cell samples corresponded to 15 μg protein per lane and samples from spent media corresponded to the volume of spent medium that contained 15 μg protein before the cells were removed. For additional controls regarding specificity of the anti-mCherry antibodies see (1-3).

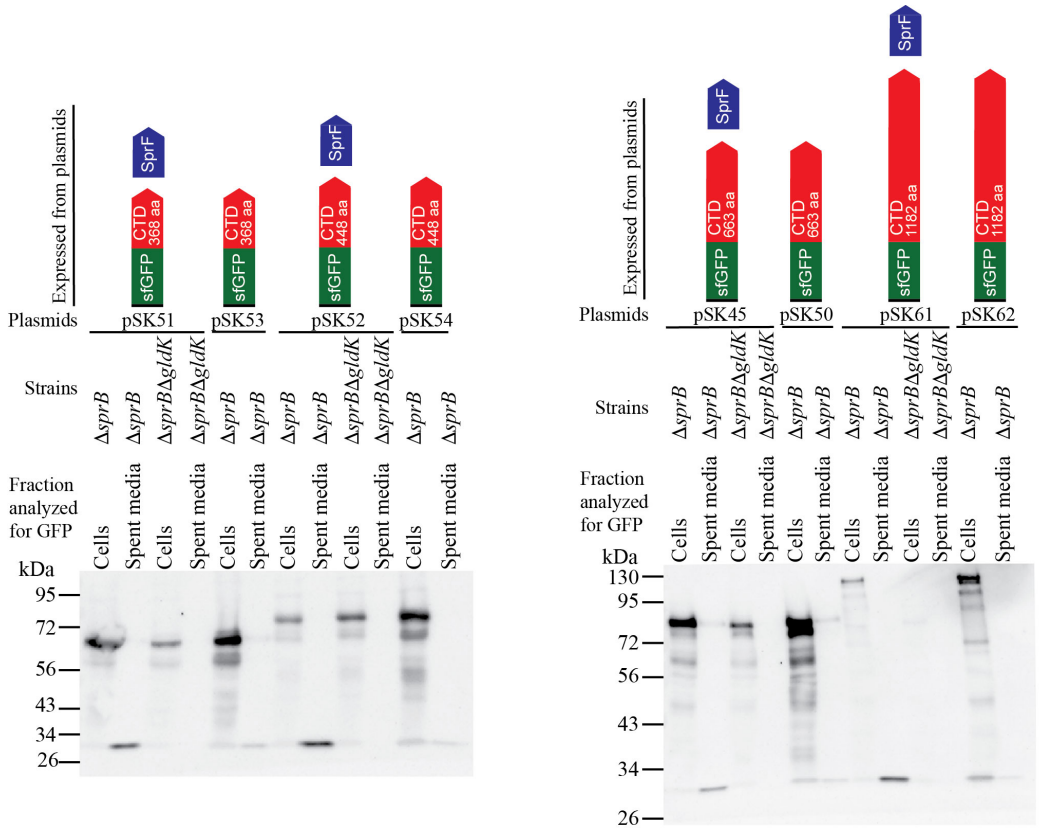

Figure S3. T9SS-mediated secretion of sfGFP fused to long C-terminal regions (CTDs) of SprB, with or without plasmid co-expression of SprF. Cultures carrying the plasmids indicated were incubated in CYE at 25°C with shaking and harvested in the late exponential phase of growth. Cells and spent culture media were separated by centrifugation and analyzed by SDS-PAGE followed by Western blot using antiserum against GFP. Whole cell samples corresponded to 10 µg protein per lane and samples from spent media corresponded to the volume of spent medium that contained 10 µg protein before the cells were removed. All strains carried a deletion of *sprB* to avoid SprF being sequestered by SprB. Some strains carried a *gldK* deletion (Δ*gldK*) to disable the T9SS. All strains were wild type for *sprF* on the chromosome but some expressed additional SprF from plasmid as indicated. Plasmids used were pSK53, which expresses SP-sfGFP fused to the 368-amino acid CTD of SprB (SP-sfGFP-CTD$_{SprB368}$); pSK51, which co-expresses SP-sfGFP-CTD$_{SprB368}$ and SprF; pSK54, which expresses SP-sfGFP fused to the 448 amino acid CTD of SprB (SP-sfGFP-CTD$_{SprB448}$); pSK52, which co-expresses SP-sfGFP-CTD$_{SprB448}$ and SprF; pSK50, which expresses SP-sfGFP fused to the 663 amino acid CTD of SprB (SP-sfGFP-CTD$_{SprB663}$); pSK45 which co-expresses SP-sfGFP-CTD$_{SprB663}$ and SprF; pSK62, which expresses SP-sfGFP fused to the 1182 amino acid CTD of SprB (SP-sfGFP-CTD$_{SprB1182}$); and pSK61 which co-expresses SP-sfGFP-CTD$_{SprB1182}$ and SprF. Cartoons depicting the proteins expressed from the plasmids are indicated above the appropriate lanes.

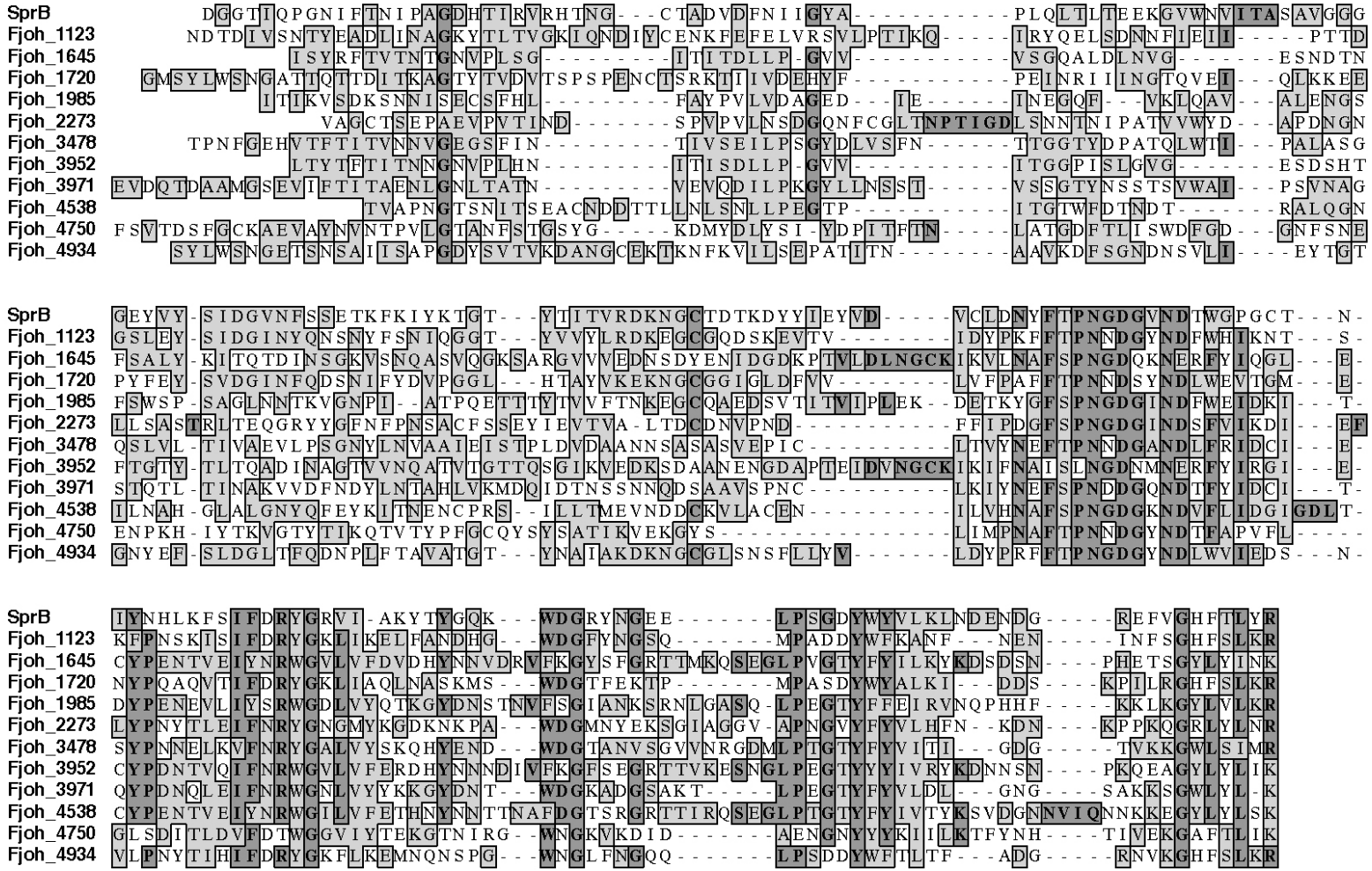

Figure S4. Alignment of the C-terminal 200 amino acids of *F. johnsoniae* proteins that belong to TIGRFAM family TIGR04131 (type-B CTD). Protein sequences were aligned using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. Proteins that were examined experimentally in this study were SprB, Fjoh_3952, and Fjoh_1123.

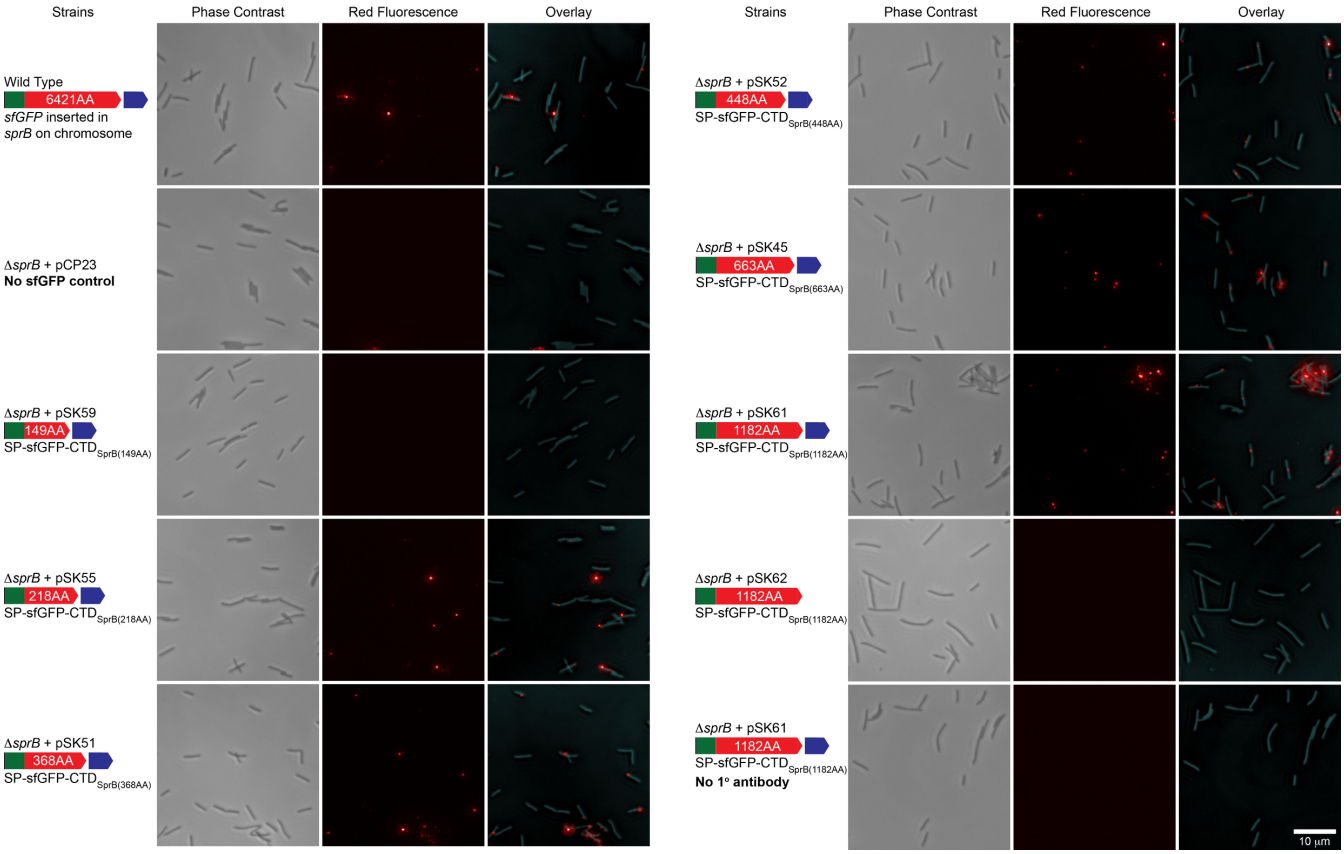

Figure S5. Surface-localization sfGFP by attachment to C-terminal regions of SprB. Cells expressing SP-sfGFP fused to C-terminal regions (CTDs) of SprB ranging from 149 to 6421 amino acids were exposed to anti-GFP and 2° antibody fused to Alexa Fluor 594 and observed by fluorescence microscopy to detect sfGFP exposed on the cell surface. Exposure times for fluorescence images were all 33 msec. Phase contrast images (left column) were superimposed with fluorescence images (middle column) to observe the relationship of the signals to cells (right column). Top left row of three images is wild type cells expressing full length SprB from the chromosome, with sfGFP fused after the signal peptide. All remaining rows of three images are Δ*sprB* mutant cells with, or without, plasmids expressing SP-sfGFP fused to CTD_{SprB} of the lengths indicated. All plasmids except the empty vector pCP23, and pSK62 (right, second row from bottom) also express SprF, to facilitate secretion. Anti-GFP was added to all cells except for those in the bottom right row. For additional controls and experimental results see Figs. S6-S8, and Fig. 3 and 4 in the main text.

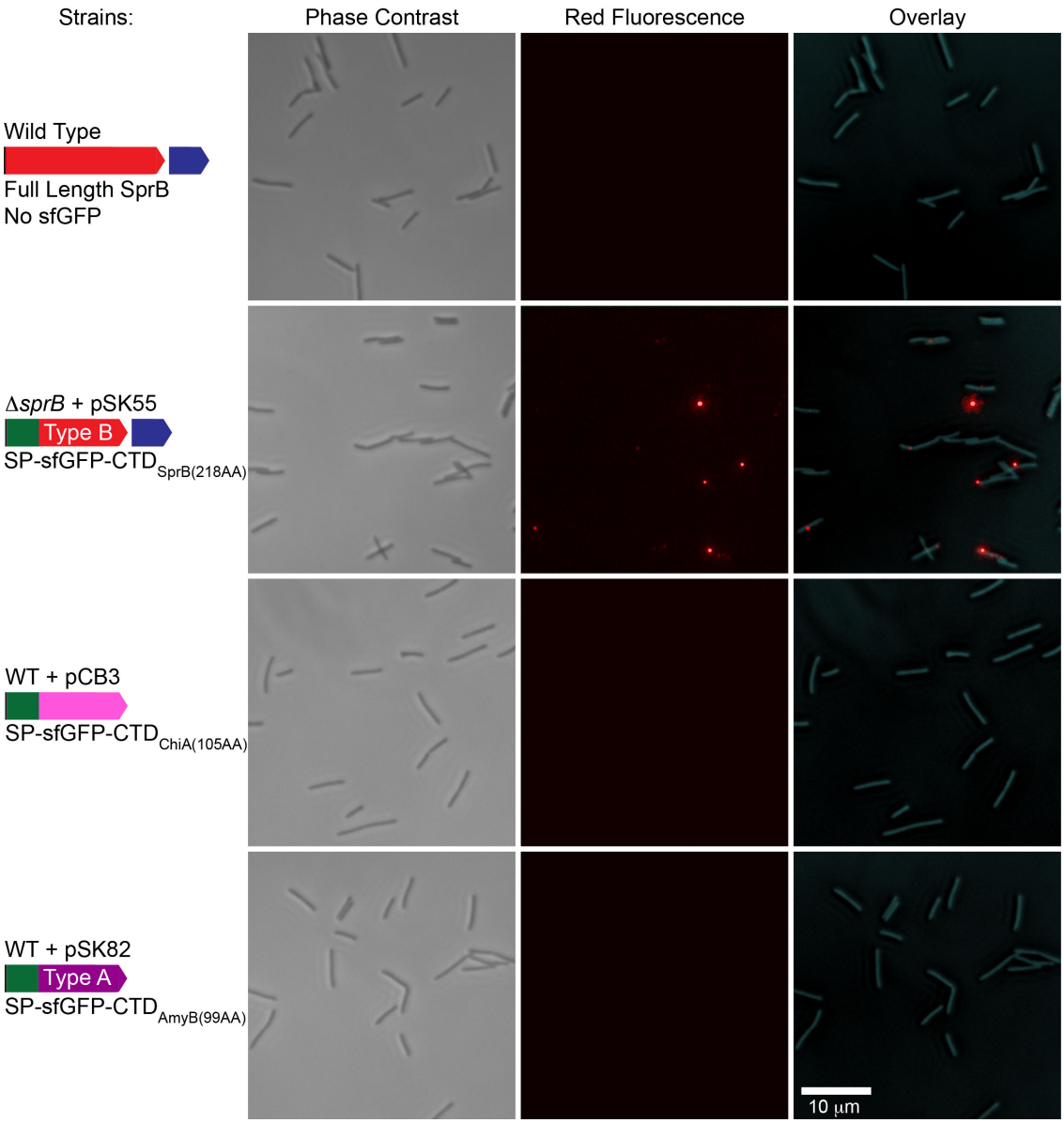

Figure S6. C-terminal regions of the soluble secreted proteins ChiA and AmyB fused to sfGFP do not result in attachment to the cell surface. Cells expressing SP-sfGFP fused to C-terminal regions (CTDs) of SprB, ChiA, and AmyB that are known to facilitate secretion were exposed to anti-GFP and 2º antibody fused to Alexa Fluor 594 and observed by fluorescence microscopy to detect sfGFP exposed on the cell surface. Exposure times for fluorescence images were all 33 msec. Phase contrast images were superimposed with fluorescence images to observe the relationship of the signals to cells. Top row is wild type (WT) cells with no sfGFP. The remaining rows are Δ*sprB* mutant cells or wild type cells with plasmids expressing SP-sfGFP fused to CTD_{SprB}, CTD_{ChiA}, or CTD_{AmyB} as indicated. Blue arrows indicate that SprF is expressed from the chromosome (top row) or from plasmid pSK55 (second row) to facilitate secretion. SprF is not needed to facilitate secretion of SP-sfGFP-CTD_{ChiA} or SP-sfGFP-CTD_{AmyB}, as previously indicated, and these are secreted as soluble proteins, as are full length ChiA and AmyB (1-3). 'Type B' indicates Type B CTD. 'Type A' indicates Type A CTD.

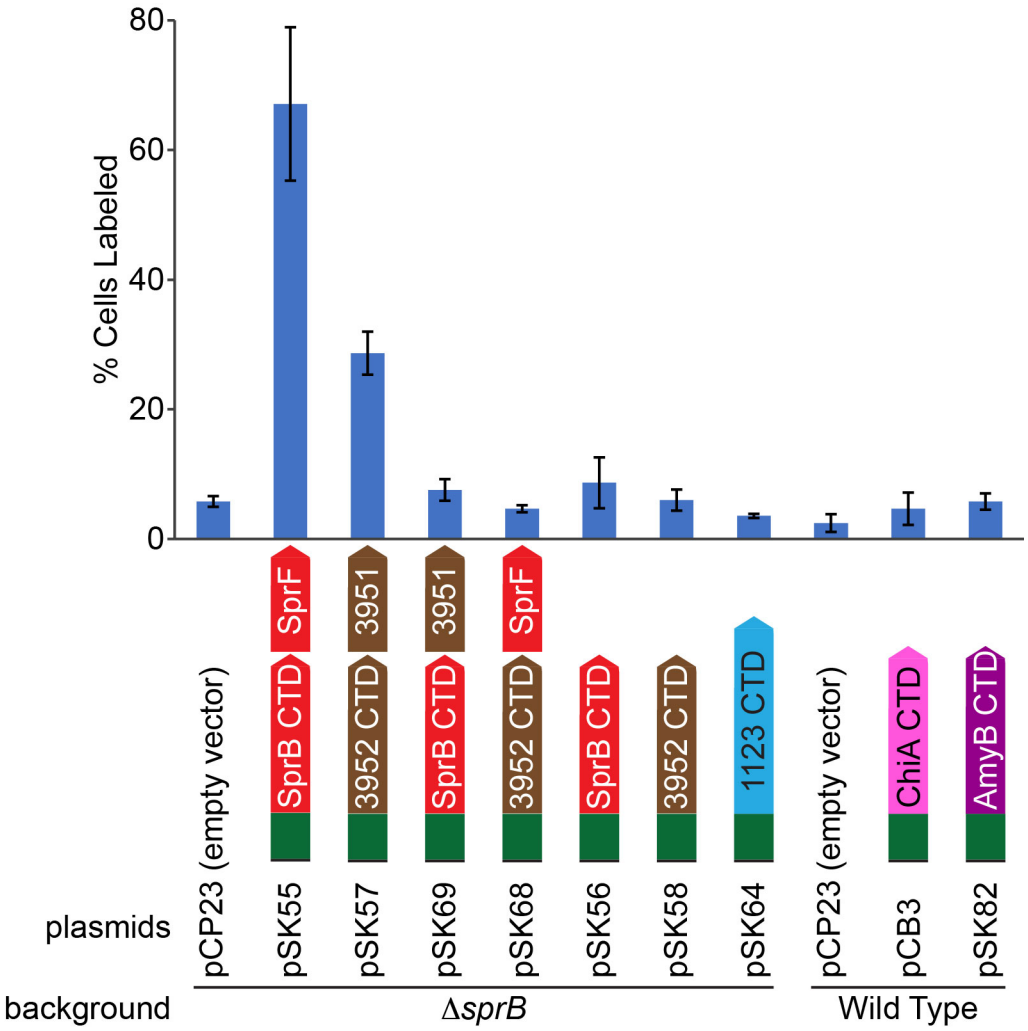

Figure S7. Labeling of cell surface localized sfGFP fused to T9SS CTDs. Cells expressing SP-sfGFP fused to C-terminal regions (CTDs) of SprB (218 AA), Fjoh_3952 (228 AA), Fjoh_1123 (238 AA), ChiA (105 AA) and AmyB (99 AA) were exposed to anti-GFP and secondary antibody fused to Alexa Fluor 594 and observed by fluorescence microscopy to detect sfGFP exposed on the cell surface. Percent of labeled cells was determined by examining three independent samples of 150 cells from each strain. Error bars indicate standard deviations from three measurements. All Type B CTD sfGFP fusions were expressed from their respective plasmids in the Δ*sprB* mutant. The ChiA and AmyB CTD sfGFP fusions were expressed from their respective plasmids in wild type cells.

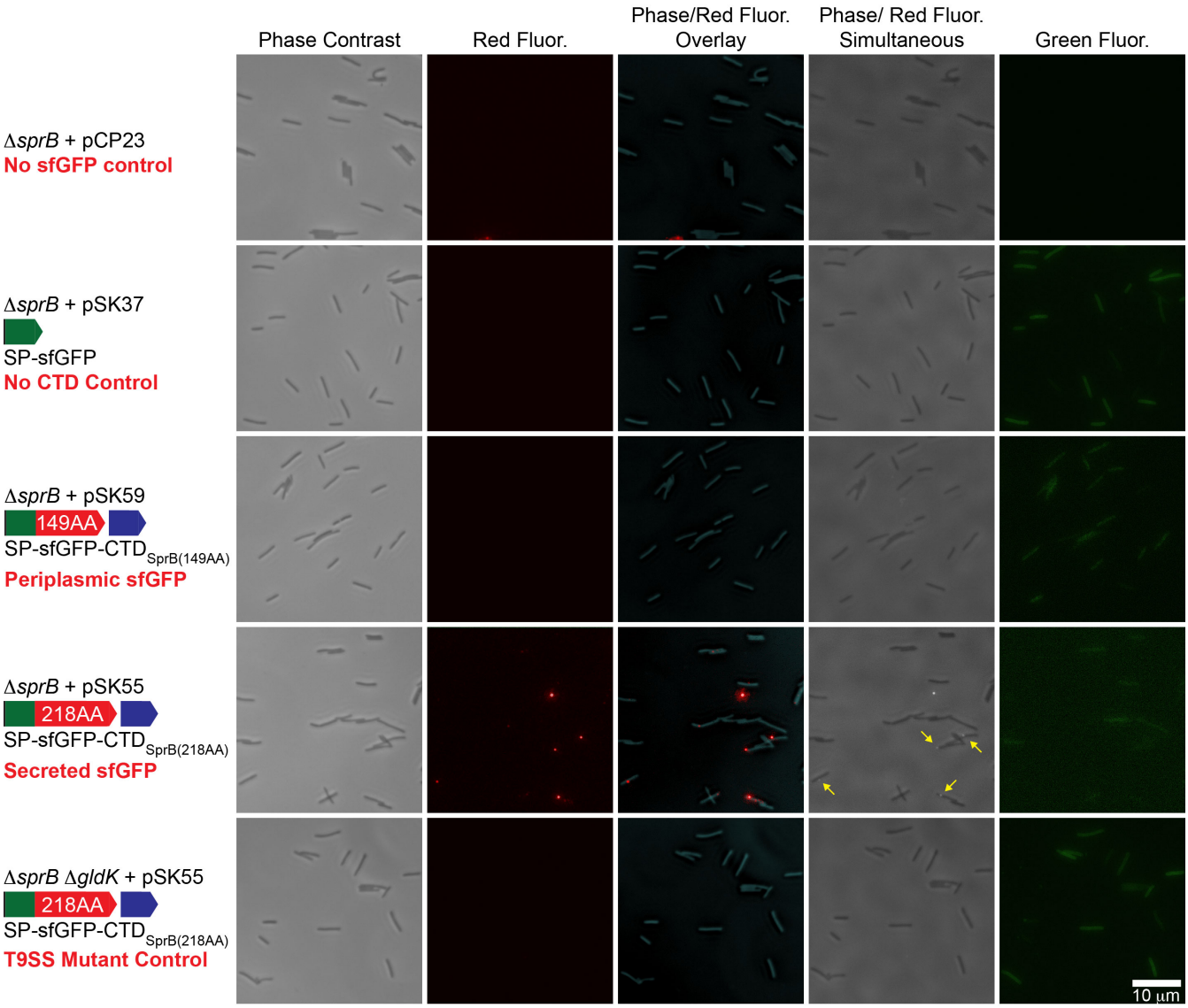

Figure S8. Detection of red and green fluorescence with no overlap, and demonstration of simultaneous phase contrast and fluorescence microscopy. Cells of the Δ*sprB* mutant CJ1922 expressing SP-sfGFP fused to C-terminal regions (CTDs) of SprB were exposed to anti-GFP and secondary antibody fused to Alexa Fluor 594 and observed by fluorescence microscopy to detect sfGFP exposed on the cell surface. Exposure times for fluorescence images were all 33 msec. Phase contrast images (left column) were superimposed with fluorescence images to generate overlay images (third column). The fourth column illustrates detection of cells and fluorescent signals by simultaneous phase contrast and fluorescence microscopy (yellow arrows point to signals), which was used to monitor movement of signals in Movies S1 to S3. Green fluorescence (right column) demonstrated the presence of sfGFP in all cells except those of the 'No sfGFP' control, and demonstrated that surface localized sfGFP (red fluorescence) and total sfGFP (green fluorescence) could be separately detected. All plasmids except pCP23 (empty vector) expressed SP-sfGFP (green), in some cases fused to CTD_{SprB} of different lengths (red), and with or without SprF (blue) as shown by the cartoons. The same region is shown in all five panels of a row. For related experimental results see Figs. S5 and S6, and Fig. 3 in the main text.

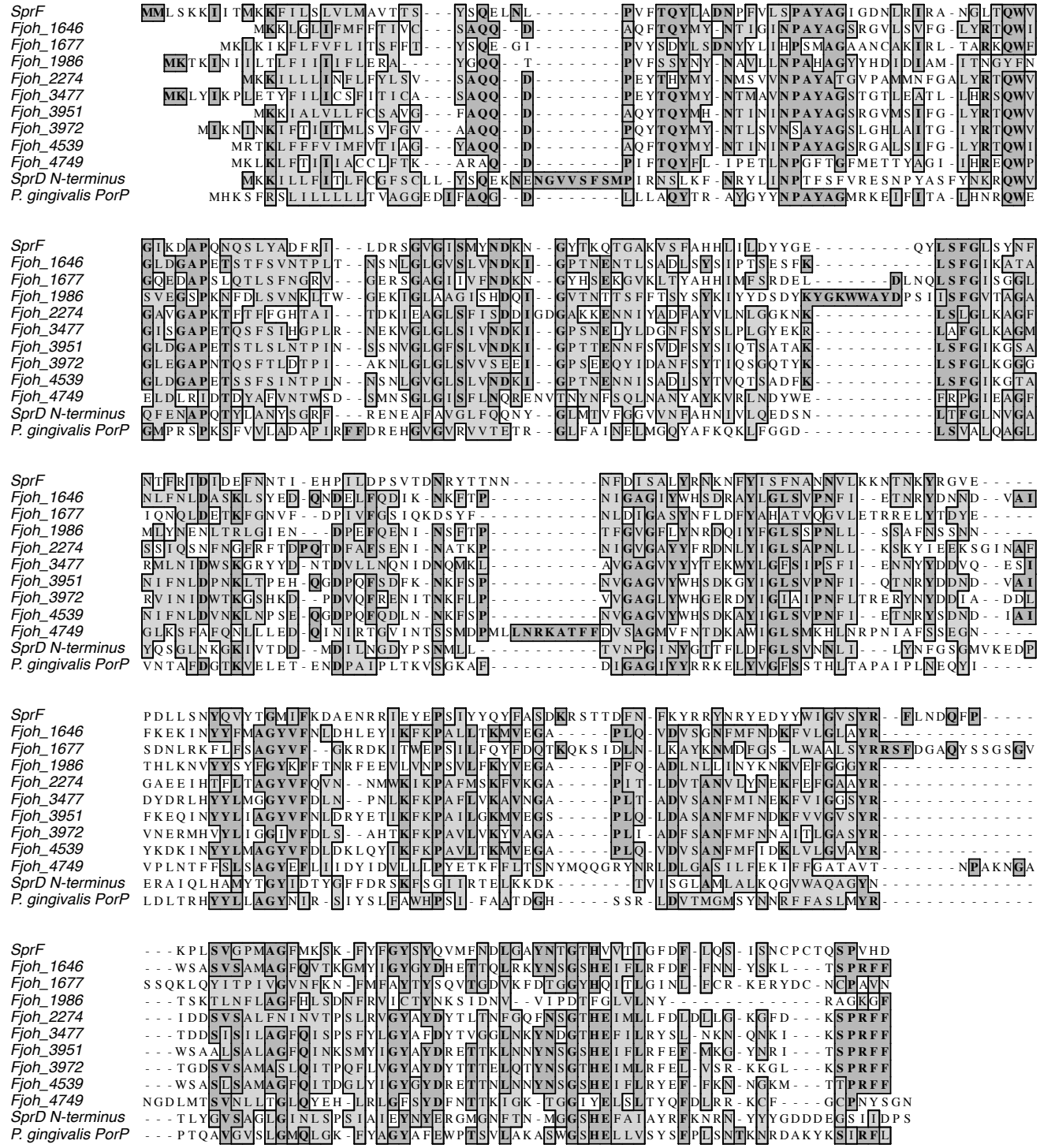

Figure S9. Alignment of *F. johnsoniae* SprF-like proteins and *P. gingivalis* W83 PorP. Protein sequences were aligned using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. Proteins examined experimentally in this study were SprF and Fjoh_3951. The entire protein sequences were used in the alignment, except for SprD for which only the N-terminal 320 amino acids of the 1588 amino acid protein were used.

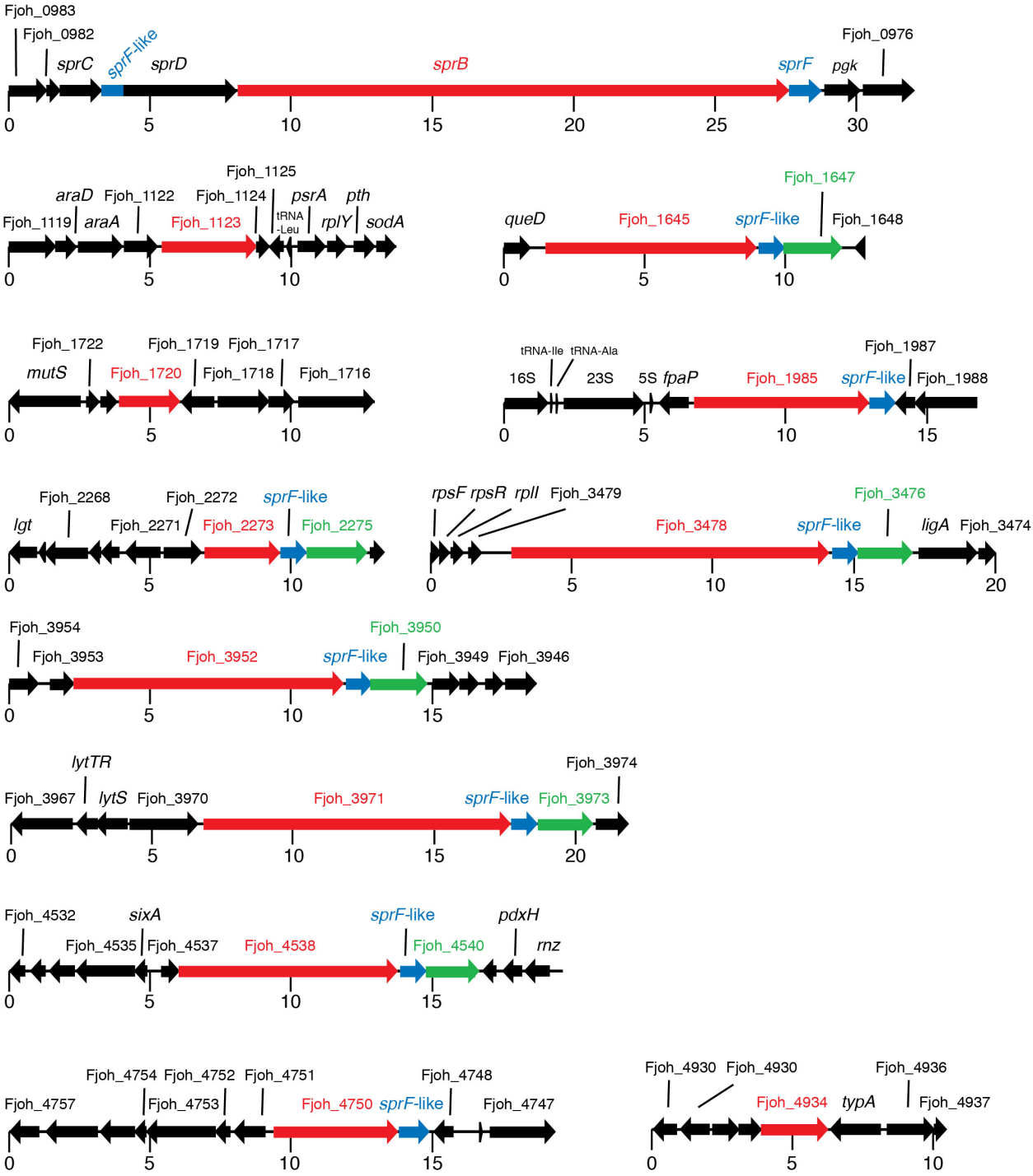

Figure S10. *F. johnsoniae* genes encoding type B CTD-containing proteins (red). *porP*/*sprF*-like genes are shown in blue, and PG1058-like genes are shown in green. One additional *porP*/*sprF*-like gene (Fjoh_1677) is present in the genome but is not shown here because it is not located adjacent to a gene encoding a type B CTD-containing protein.

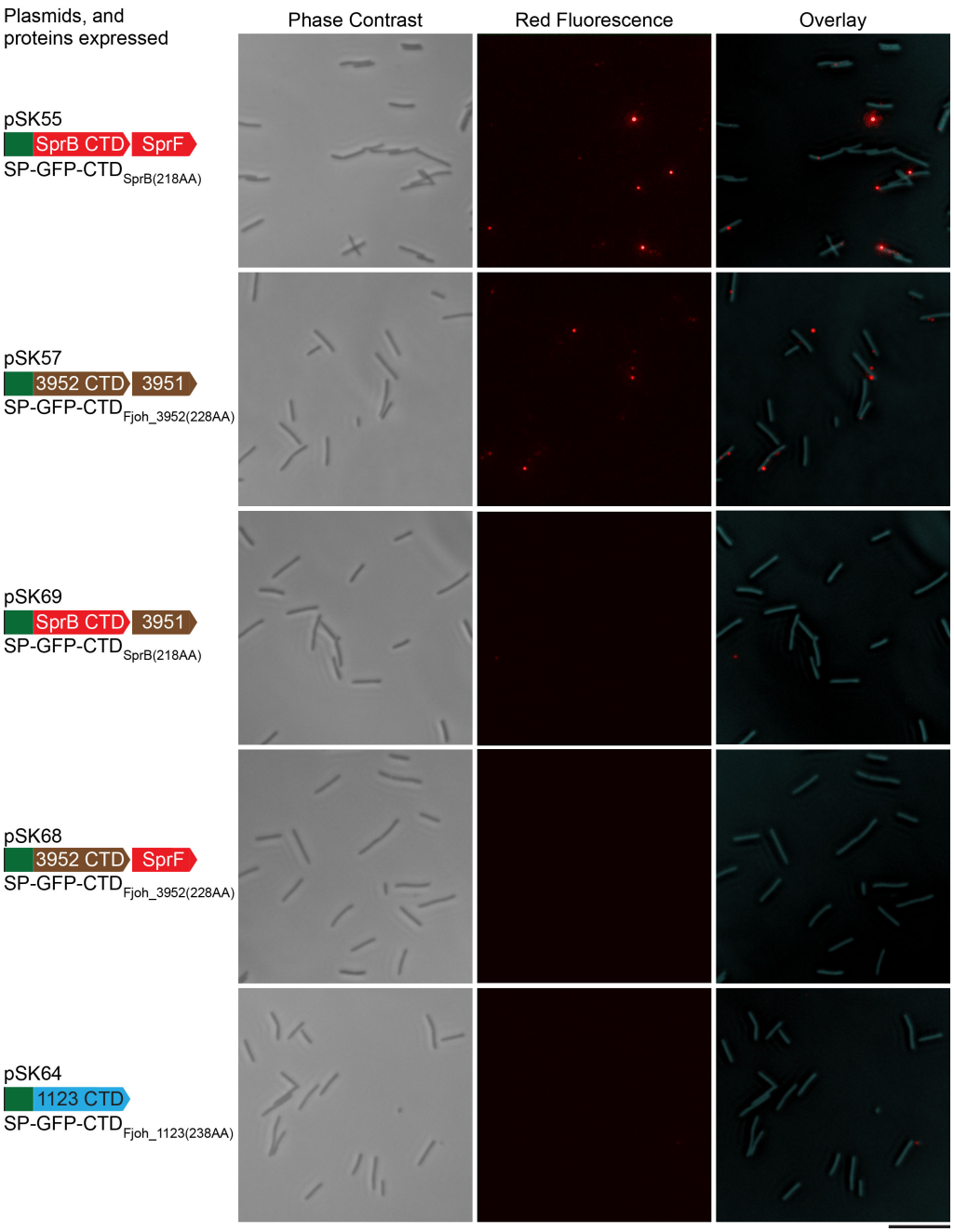

Figure S11. Fusion of the C-terminal region of Fjoh_3952 to sfGFP and co-expression with the cognate SprF-like protein Fjoh_3951 results in attachment of sfGFP to the cell surface. Cells expressing SP-sfGFP fused to C-terminal regions (CTDs) of SprB, Fjoh_3952, or Fjoh_1123 were exposed to anti-GFP and secondary antibody fused to Alexa Fluor 594 and observed by fluorescence microscopy to detect sfGFP exposed on the cell surface. Exposure times for fluorescence images were all 33 msec. Phase contrast images (left column) were superimposed with fluorescence images (middle column) to observe the relationship of the signals to cells (right column). All panels are Δ*sprB* mutant cells with plasmids indicated expressing SP-sfGFP fused to $CTD_{SprB(218AA)}$, $CTD_{Fjoh\_3952(228AA)}$, or $CTD_{Fjoh\_1123(238AA)}$ with or without a PorP/SprF-like protein (either SprF or Fjoh_3951) as shown. Bar indicates 10 μm and applies to all panels.

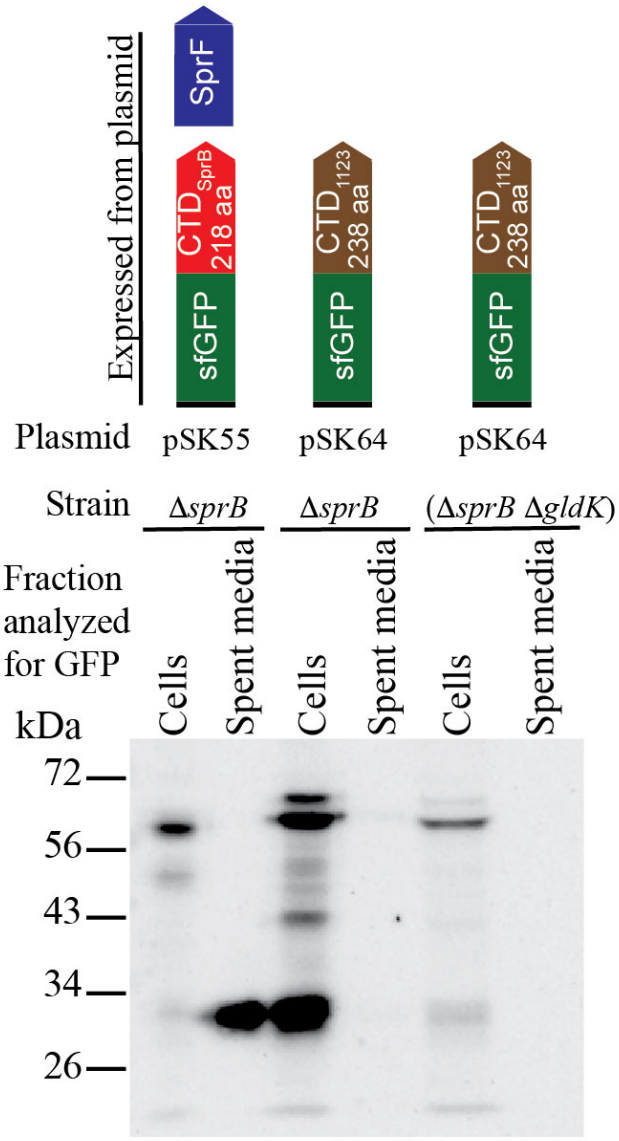

Figure S12. The type B CTD of Fjoh_1123 is not sufficient to target sfGFP for secretion. To determine if Fjoh_1123 CTD can target sfGFP for secretion, cells of Δ*sprB* and of T9SS mutant Δ*sprB* Δ*gldK*, carrying plasmids that expressed SP-sfGFP fused to 238 amino acids of Fjoh_1123 (pSK64) were analyzed. The culture supernatant (spent medium) and intact cells were analyzed for sfGFP by western blot using anti-GFP antiserum. Whole cell samples corresponded to 10 μg protein per lane and samples from spent media corresponded to the volume of spent medium that contained 10 μg protein before the cells were removed.

Figure S13. Alignment of *F. johnsoniae* proteins related to *P. gingivalis* W83 PG1058. Protein sequences were aligned using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. The entire protein sequences were used in the alignments.

Table S1. *F. johnsoniae* T9SS type A CTD-containing proteins[1].

Locus tag	Molecular Mass (kDa)	Gene name	Domains	Known or predicted function
Fjoh_0074	123.1		endonuclease/exonuclease/phosphatase	nuclease/phosphatase
Fjoh_0547	12.6			
Fjoh_0549	59.3		CARDB, 2 copies	adhesin
Fjoh_0707	83.7			
Fjoh_0798	95.7		Metallo-peptidase M12B Proprotein convertase	peptidase
Fjoh_0808	154.0	*remA*	Gal_Lectin	galactose/rhamnose-binding lectin, gliding motility adhesin (4)
Fjoh_0848	54.2		TPR_2; TPR_8; TPR_16	
Fjoh_0886	99.1		Thermolysin, peptidase M4 fn3	peptidase
Fjoh_1022	51.1		GH8	glycoside hydrolase
Fjoh_1188	152.7		LRR_4	
Fjoh_1189	181.4		RCC1 (7 copies) SprB repeat (2 copies) Laminin_G_3	Lectin, Possible motility adhesin
Fjoh_1208	112.5	*amyB*	α amylase GH13 Big_2 (3 copies) CBM26	α amylase (2, 3)
Fjoh_1231	97.8		pectate lyase CBM77	pectate lyase
Fjoh_1269	94.3		SprB repeat (5 copies)	Possible motility adhesin
Fjoh_1408	106.0		α amylase GH13	α amylase
Fjoh_1905	75.1		GH30 RicinB_lectin_2 (2 copies) CBM13	glycoside hydrolase
Fjoh_2150	39.0			
Fjoh_2336	14.0			
Fjoh_2338	12.9		DUF3244	
Fjoh_2339	14.7			
Fjoh_2389	57.7		Peptidase_S8	peptidase
Fjoh_2456	100.1		PL1	polysaccharide lyase
Fjoh_2666	61.2			
Fjoh_3203	104.9		GH87 Big_2 (2 copies)	Glycoside hydrolase
Fjoh_3246	299.4		CARDB	adhesin
Fjoh_3296	65.1			
Fjoh_3324	105.3		Glucose-sorbosone dehydrogenase CBM6 PKD	Glucose-sorbosone dehydrogenase
Fjoh_3421	17.8			
Fjoh_3731	60.5			

Fjoh_3777	128.1		DUF676	Possible lipase/esterase
Fjoh_3855	31.4			
Fjoh_4051	226.7		SprB repeat (13 copies)	Possible motility adhesin
Fjoh_4174	102.5		RicinB_lectin_2 CBM6	carbohydrate binding
Fjoh_4175	57.7		GH18 CBM6	glycoside hydrolase
Fjoh_4176	95.4		GH64 beta gamma crystallin RicinB_lectin_2 CBM13 CBM6	glycoside hydrolase
Fjoh_4177	144.9		GH16 Discoidin	Possible glycoside hydrolase
Fjoh_4242	100.5		Peptidase_S8	peptidase
Fjoh_4436	131.8		GH27 CBM13 RicinB_lectin_2 SusF SusE starch binding (3 copies)	glycoside hydrolase
Fjoh_4721	67.1		endonuclease fn3 LTD	endonuclease
Fjoh_4723	66.0		endonuclease fn3 LTD	endonuclease
Fjoh_4948	34.5			
Average	78.8			
Median	94.3			

[1]The T9SS type A CTD (TIGR04183) is a C-terminal domain that targets a protein for secretion by the T9SS. TIGR04183 is described as 'Por secretion system C-terminal sorting domain' on the J. Craig Venter Institute TIGR website (http://www.jcvi.org/cgi-bin/tigrfams/index.cgi). Each of these proteins contains an N-terminal signal peptide for export across the cytoplasmic membrane, and a T9SS type A C-terminal domain (CTD) for secretion across the outer membrane by the T9SS. In addition, they contain the conserved domains shown:
Carbohydrate active enzymes/proteins as assigned by CAZY: Glycoside hydrolase (GH); polysaccharide lyase (PL); carbohydrate binding (CBM) domains as assigned by CAZY (5). α amylase also assigned based on (pfam00128); pectate lyase also assigned based on (pfam00544).
Other potential carbohydrate active/binding proteins: Fjoh_4177 assigned as GH16 based on relationship to (pfam00722); Galactose/rhamnose-binding lectin (Gal_Lectin, pfam02140); RicinB_lectin_2 (pfam14200); SusF SusE starch binding (pfam16411); Glucose-sorbosone dehydrogenase (pfam07995).
Peptidases: Metallo-peptidase M12B (pfam13583); Proprotein convertase (pfam01483); Thermolysin, peptidase M4 (pfam07504, pfam01447, pfam02868); Peptidase_S8 (pfam00082).
Other Enzymes: Endonuclease/exonuclease/phosphatase family (IPR005135); endonuclease (pfam04231).

Other: Cell adhesion related domain (CARDB, pfam07705); tetratricopeptide repeat domains: TPR_2 (pfam07719), TPR_8 (pfam13181), and TPR_16 (pfam13432); Fibronectin type III domain (fn3; pfam00041); leucine rich repeat 4 (LRR_4, pfam12799); Laminin_G_3 (pfam13385); SprB repeat (pfam13573); Regulator of chromosome condensation (RCC1, pfam00415); bacterial Ig-like domain (Big_2, pfam02368); PKD (pfam00801); beta gamma crystallin (pfam00030)**;** Discoidin (pfam00754); Lamin Tail Domain (LTD, pfam00932); DUF3244 (pfam11589); DUF676 (pfam05057).

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Table S2. *F. johnsoniae* T9SS Type B CTD-containing proteins[1]

Locus tag	Molecular Mass (kDa)	Gene name	Domains	Function or predicted function
Fjoh_0979	672.0	*sprB*	SprB repeat (20 copies)	Gliding motility adhesin (6)
Fjoh_1123	121.9		PKD domain	Possible adhesin
Fjoh_1645	258.1		Hyalin repeat (3 copies) DUF11 (2 copies)	Possible adhesin
Fjoh_1720	78.5		C-type lectin	Predicted carbohydrate-binding adhesin
Fjoh_1985	221.0		Hyalin repeat (8 copies) FG-GAP repeat of integrins (2 copies)	Possible adhesin
Fjoh_2273	93.3			
Fjoh_3478	386.0		DUF11	
Fjoh_3952	330.6		VCBS repeat DUF11 (3 copies)	Possible adhesin
Fjoh_3971	375.3		DUF11 (16 copies)	
Fjoh_4538	271.0		LRR_adjacent (8 copies) VWA_2 Collagen binding protein, Cna	Possible adhesin
Fjoh_4750	158.1		PKD domain (3 copies) SprB repeat (3 copies)	Possible gliding motility adhesin
Fjoh_4934	84.7			
Average	378.4			
Median	239.6			

[1]The T9SS type B CTD (TIGR04131) is a C-terminal domain that targets a protein for secretion by the T9SS. TIGR04131 is described as 'gliding motility-associated C-terminal domain' on the J. Craig Venter Institute TIGR website (http://www.jcvi.org/cgi-bin/tigrfams/index.cgi). Each of these proteins contains an N-terminal signal peptide for export across the cytoplasmic membrane, and a T9SS type B C-terminal domain (CTD) for secretion across the outer membrane by the T9SS. In addition, individual proteins contain the conserved domains shown: SprB repeat (pfam13573); PKD domain (IPR000601); Hyalin repeat (pfam02494); C-type lectin (IPR016186, IPR016187, IPR001304); FG-GAP repeat of integrins (pfam01839); VCBS repeat (TIGR01965); LRR_adjacent (pfam08191); DUF11 (pfam01345); von Willebrand factor type A domain, VWA_2 (pfam13519); Collagen binding protein Cna (IPR008970).

Table S3. Prevalence of T9SS genes, CTD-encoding genes and *porP*/*sprF*-like genes in 104 members of the phylum *Bacteroidetes*[a]

	T9SS components					CTDs			
	GldK	GldL	GldM	GldN	SprA	Type A CTD	Type B CTD	porP/sprF-like genes	porP/sprF-like genes that have type B CTD-encoding genes immediately upstream
Genome	TIGR03525	TIGR03513	TIGR03517	TIGR03523	TIGR04189	TIGR04183	TIGR04131	TIGR03519	
Class Flavobacteriia									
Aequorivita sublithincola DSM 14238	1	1	1	1	1	112	5	4	3
Algibacter alginicilyticus HZ22	1	1	1	1	1	55	10	7	6
Capnocytophaga canimorsus Cc5	1	1	1	1	1	1	10	6	5
Capnocytophaga haemolytica CCUG 32990	1	1	1	1	1	2	6	5	3
Capnocytophaga ochracea DSM 7271	1	1	1	1	1	2	8	5	3
Capnocytophaga sp. F0383	1	1	1	1	1	2	7	5	3
Cellulophaga algicola DSM 14237	1	1	1	1	1	13	16	12	8
Cellulophaga lytica DSM 7489	1	1	1	1	1	14	13	10	6
Chryseobacterium sp. IHB B 17019	1	1	1	1	1	83	18	1	0
Chryseobacterium sp. StRB126	1	1	1	1	1	101	10	1	0
Croceibacter atlanticus HTCC2559	1	1	1	1	1	45	8	4	4
Dokdonia sp. 4H-3-7-5	1	1	1	1	1	15	10	7	5
Dokdonia sp. PRO95	1	1	1	1	1	17	10	7	5
Elizabethkingia meningoseptica FMS-007	0	0	0	0	0	0	0	0	0
Elizabethkingia miricola BM10	0	0	0	0	0	0	0	0	0
Flavobacteriaceae bacterium 3519-10	1	1	1	1	1	55	6	1	0
Flavobacterium branchiophilum FL-15	1	1	1	1	1	37	10	3	3
Flavobacterium columnare ATCC 49512	1	1	1	1	1	35	7	6	4
Flavobacterium indicum GPTSA100-9	1	1	1	1	1	43	16	5	5
Flavobacterium johnsoniae ATCC 17061	1	1	1	2	1	40	12	11	9
Flavobacterium psychrophilum JIP02/86	1	1	1	1	1	38	10	7	5
Fluviicola taffensis DSM 16823	1	1	2	2	1	180	50	15	9
Gramella forsetii KT0803	1	1	1	1	1	11	7	5	4
Lacinutrix sp. 5H-3-7-4	1	1	1	1	1	31	16	8	7
Lutibacter profundi LP1	1	1	1	1	1	19	3	2	1
Maribacter sp. HTCC2170	1	1	1	1	1	10	13	8	3
Muricauda lutaonensis CC-HSB-11	1	1	1	1	1	7	13	9	6
Muricauda ruestringensis DSM 13258	1	1	1	1	1	7	13	13	7
Myroides profundi D25	1	1	1	1	1	7	12	11	10
Myroides sp. A21	1	1	1	1	1	4	7	8	5
Nonlabens dokdonensis DSW-6	1	1	1	1	1	85	17	5	2
Ornithobacterium rhinotracheale DSM 15997	1	1	1	1	1	6	2	1	0
Owenweeksia hongkongensis DSM 17368	1	1	1	1	1	159	26	4	1
Polaribacter sp. MED152	1	1	1	1	1	27	8	5	4
Riemerella anatipestifer DSM 15868	1	1	1	1	1	15	1	1	0
Robiginitalea biformata HTCC2501	1	1	1	1	1	7	12	8	4
Siansivirga zeaxanthinifaciens CC-SAMT-1	1	1	1	1	1	51	10	6	5
Weeksella virosa DSM 16922	1	1	1	1	1	36	3	1	1
Winogradskyella sp. PG-2	1	1	1	1	1	66	17	8	6
Zobellia galactanivorans DsijT	1	1	1	1	1	29	17	14	9
Zunongwangia profunda SM-A87	1	1	1	1	1	7	5	3	2
Class Cytophagia									
Belliella baltica DSM 15883	1	1	1	1	1	11	4	6	3
Bernardetia litoralis DSM 6794	1	1	1	3	1	52	11	5	1
Cyclobacterium amurskyense KCTC 12363	1	1	1	1	1	20	5	7	3
Cyclobacterium marinum DSM 745	1	1	1	1	1	18	7	7	5
Cytophaga hutchinsonii ATCC 33406	1	1	2	2	1	118	27	16	8
Dyadobacter fermentans DSM 18053	1	1	1	1	1	88	11	3	0
Echinicola vietnamensis DSM 17526	1	1	1	1	1	17	9	9	6
Emticicia oligotrophica DSM 17448	1	1	1	1	1	31	10	4	1
Hymenobacter sp. APR13	1	1	1	1	1	83	9	2	0
Hymenobacter sp. DG25A	1	1	1	1	1	54	6	3	1
Hymenobacter sp. PAMC26554	1	1	1	1	1	51	8	3	1
Hymenobacter swuensis DY53	1	1	1	1	1	100	8	3	1
Leadbetterella byssophila DSM 17132	1	1	1	1	1	17	4	4	0
Marivirga tractuosa DSM 4126	1	1	1	1	1	39	11	12	7
Persicobacter sp. JZB09	1	1	1	1	1	32	3	4	1
Pontibacter akesuensis AKS 1T	1	1	1	1	1	47	11	8	5
Pontibacter korlensis X14-1T	1	1	1	1	1	47	11	9	7
Rufibacter sp. DG15C	1	1	1	1	1	49	13	10	7
Rufibacter tibetensis 1351	1	1	1	1	1	56	11	9	6

	T9SS components					CTDs			
	GldK TIGR03525	GldL TIGR03513	GldM TIGR03517	GldN TIGR03523	SprA TIGR04189	Type A CTD TIGR04183	Type B CTD TIGR04131	porP/sprF-like genes TIGR03519	porP/sprF-like genes that have type B CTD-encoding genes immediately upstream
Runella slithyformis DSM 19594	1	1	1	1	1	33	18	5	1
Spirosoma linguale DSM 74	1	1	1	1	1	53	14	4	1
Spirosoma radiotolerans DG5A	1	1	1	1	1	50	15	5	2
Class Sphingobacteriia									
Algoriphagus sp. M8-2	1	1	1	1	1	16	6	8	5
Arachidicoccus sp. BS20	2	1	1	2	1	11	0	0	0
Chitinophaga pinensis DSM 2588	1	1	1	1	1	51	36	17	13
Haliscomenobacter hydrossis DSM 1100	1	1	1	1	1	144	36	14	4
Mucilaginibacter PAMC26640	1	1	1	2	1	7	9	4	3
Niabella soli DSM 19437	0	0	0	0	0	0	0	0	0
Niastella koreensis DSM 17620	1	1	1	1	1	111	31	17	12
Pedobacter cryoconitis PAMC 27485	1	1	1	1	1	3	5	6	4
Pedobacter heparinus DSM 2366	1	1	1	1	1	8	13	10	7
Pedobacter sp. PACM 27299	1	1	1	1	1	1	9	7	6
Pseudopedobacter saltans DSM 12145	1	1	1	1	1	29	10	11	10
Saprospira grandis Lewin	1	1	1	3	2	67	16	10	2
Solitalea canadensis DSM 3403	1	1	1	1	1	6	18	11	8
Sphingobacterium sp. 21	2	1	1	2	1	1	2	1	1
Sphingobacterium sp. ML3W	1	1	1	1	0	1	0	0	0
Class Bacteroidia									
Alistipes finegoldii DSM 17242	0	0	0	0	0	0	0	0	0
Bacteroides cellulosilyticus WH2	1	1	1	1	1	19	1	1	0
Bacteroides dorei CL03T12C01	0	0	0	0	0	0	0	0	0
Bacteroides fragilis NCTC 9343	0	1	1	0	0	0	0	0	0
Bacteroides helcogenes DSM 20613	0	0	0	0	0	0	0	0	0
Bacteroides ovatus ATCC 8483	0	0	0	0	0	0	0	0	0
Bacteroides thetaiotaomicron VPI-5482	0	0	0	0	0	0	0	0	0
Bacteroides vulgatus ATCC 8482	0	0	0	0	0	0	0	0	0
Bacteroides xylanisolvens XB1A	0	0	0	0	0	0	0	0	0
Barnesiella viscericola DSM 18177	1	1	1	1	1	41	1	1	0
Draconibacterium orientale FH5	2	1	1	1	1	23	7	9	4
Odoribacter splanchnicus DSM 20712	0	0	0	0	2	2	1	2	0
Paludibacter propionicigenes DSM 17365	1	1	1	1	1	10	6	6	5
Parabacteroides distasonis ATCC 8503	1	1	1	1	1	7	1	1	0
Porphyromonas asaccharolytica DSM 20707	1	1	1	1	1	29	1	1	0
Porphyromonas gingivalis ATCC 33277	1	1	1	1	1	17	1	1	0
Prevotella dentalis EDSM 3688	1	1	1	1	1	9	1	1	0
Prevotella denticola F0289	1	1	1	1	1	8	1	1	0
Prevotella enoeca F0113	1	1	1	1	1	4	1	1	0
Prevotella fusca W1435	1	1	1	1	1	6	1	1	0
Prevotella intermedia 17-2	1	1	1	1	1	19	1	1	0
Prevotella melaninogenica ATCC 25845	1	1	1	1	1	14	1	1	0
Prevotella ruminicola 23	1	1	1	1	1	1	1	1	0
Prevotella sp. F0039	1	1	1	1	1	13	1	1	0
Rikenellaceae bacterium M3	0	0	0	0	0	0	0	0	0
Tannerella forsythia ATCC 43037	1	1	1	1	1	28	0	1	0
total								527	314

[a]Only members of the *Bacteroidetes* with completed genome sequences were examined and only one member of each species was used. Occurrence of genes encoding T9SS components or of genes encoding proteins with T9SS-associated CTDs are shown. Tan shading indicates the presence of a gene and the number indicates the number of such genes in the genome. Genes were identified using the Integrated Microbial Genomes (IMG version 4.0.1) Function Profile Tool and using the TIGRFAM and pfam terms listed. For TIGRFAM terms the trusted cutoffs set by The Institute for Genomic Research were used as indicated in the Methods section of the main text. These may underrepresent the actual number of proteins secreted by T9SSs. For example, more than 30 proteins are thought to be secreted by the *P. gingivalis* T9SS (7), but only 18 were identified above.

Table S4. Primers used in this study

954	5' GCTAGTCTAGATGGCGAGGAATTACCTTCTGGTGA 3'; forward primer used in construction of pSK45, pSK51, pSK52 and pSK55; XbaI site underlined
955	5' GCTAGGCATGCGGACATTTCGGCTTGTGTTAAATTCG 3'; reverse primer used in construction of pSK59, pSK61 and pSK68; SphI site underlined
1302	5' GCTAGGGATCCCAGAAGCTACAGCGAAAGCAAAAG 3'; forward primer used in construction of pYT108 and pYT296; BamHI site underlined
1305	5' GCTAGGCATGCGAGGGTGTACACCTGACTGATTCTC 3'; reverse primer used in construction of pYT112 and pYT296; SphI site underlined
1356	5' GCTAGTCTAGATACAGGGTTGGGAATGGTAGGTACT 3'; reverse primer used in construction of pYT108 and pYT296; XbaI site underlined
1358	5' GCTAGGTCGACCTTAAGTACGCACAAGGAGAGTATGC 3'; forward primer used in construction of pYT112 and pYT296; SalI site underlined
1366	5' GCTAGGTCGACACCAGAACCACCACCAGAACCACCTTTGTAGAGCT CATCCATGCCGTG 3'; reverse primer used in the construction of pYT296; SalI site underlined; 24 nucleotide linker sequence immediately after Sal site
1400	5' GCTAGGCATGCTTATCTGTATAAAGTGAAATGTCCAAC 3'; reverse primer used in construction of pSK50, pSK53, pSK54 and pSK60; SphI site underlined
1694	5' GCTAGTCTAGACCGGATCCAATTACATTTACAGCAG 3'; forward primer used in construction of pSK45 and pSK50; XbaI site underlined
1749	5' GCTAGTCTAGACTCGAGGGTCCGGCTGGTCTGT 3'; forward primer used in construction of pYT296; XbaI site underlined
1828	5' GCTAGTCTAGA ACAGCTTACGAAGTACCAGGATCTATG 3'; forward primer used in construction of pSK51 and pSK53; XbaI site underlined
1829	5' GCTAGTCTAGAGCAGGTACAGAAATTAGACCGGCA 3'; forward primer used in construction of pSK52 and pSK54; XbaI site underlined
1843	5' GCTAGTCTAGAGTGGTGATTACAATTGATCCAAGC 3'; forward primer used in construction of pSK55; XbaI site underlined
1868	5' GCTAGTCTAGAGTCGAAGTGCCATCGATTACAGTA 3'; forward primer used in construction of pSK57; XbaI site underlined
1869	5' GCTAGGCATGCAACTGCTTTTTGTGCTATTGCGTT 3'; reverse primer used in construction of pSK69; SphI site underlined
1879	5' GCTAGTCTAGAGGTGTTTGGAACGTAATTACAGCT 3'; forward primer used in construction of pSK59 and pSK60; XbaI site underlined
1880	5' GCTAGTCTAGACGTTCTGAAATTACGCTTACTCCG 3'; forward primer used in construction of pSK61; XbaI site underlined
1881	5' GCTAGTCTAGATTCGTAAATGATCTGCCAACAGTA 3'; forward primer used in construction of pSK64; XbaI site underlined
1882	5' GCTAGGCATGCATAAATGTTTGAATGCCATCTCCT 3'; reverse primer used in construction of pSK64; SphI site underlined
1883	5' GCTAGGCATGCTGGCGAGGAATTACCTTCTGGTGA 3'; reverse primer used in construction of pSK68; SphI site underlined
1892	5' GCTAGGCATGCAGTCCAAATCAATAAAATGGCTTA 3'; reverse primer used in construction of pSK57 and pSK69; SphI site underlined
2429	5' GCTAGTCTAGATTCCCCGGTAGAGATAGTTATGGCTAT 3'; forward primer used in construction of pJJ21; XbaI site underlined
2430	5' GCTAGGTCGACTTACTTGTACAGCTCGTCCATGCCG 3'; reverse primer used in construction of pJJ21; SalI site underlined

Supplemental Movie Legends

Movie S1. Movement of fluorescently labeled SprB along the cell surface. Cells of *F. johnsoniae* strains were grown in MM medium at 25°C without shaking until late exponential phase. Cells were labeled with fluorescent antibodies as indicated in Materials and Methods, introduced into tunnel slides, and examined at 25°C. Suspended cells were examined to avoid confusion resulting from the gliding movement of cells over surfaces. The two sequences shown correspond to 15 seconds each. Left) Wild-type *F. johnsoniae* UW101 cells with Alexa Fluor-594-labeled anti-SprB antibodies. Right) Cells of CJ2491, expressing full length SprB from the chromosome, with sfGFP inserted after the signal peptide (SP-sfGFP-$CTD_{SprB(6421AA)}$). Cells in the right panel were labeled with anti-GFP followed by goat anti-rabbit IgG conjugated to Alexa-594. Cells in both panels were examined for movement of red fluorescent signals by simultaneous low-light phase contrast and fluorescence microscopy as indicated in Materials and Methods. Cells were also incubated without primary antibody but failed to fluoresce red as shown in Fig. 3, 4 and S5. The 5 sec sequence shown in Fig. 5 of the main text comes from the left panel of this movie.

Movie S2. Movement of sfGFP fused to the C-terminal 218 amino acids of SprB along the cell surface. Cells of *F. johnsoniae* strains were labeled with fluorescent antibodies as indicated in Materials and Methods, introduced into tunnel slides, and examined at 25°C. Suspended cells were examined to avoid confusion resulting from the gliding movement of cells over surfaces. The two sequences shown correspond to 15 seconds each. Left) Strain CJ1922 (Δ*sprB*) expressing SP-sfGFP-$CTD_{SprB(218AA)}$ and SprF from pSK55. Right) Strain CJ2839 (Δ*sprB gldJ*$_{548}$) expressing SP-sfGFP-$CTD_{SprB(218AA)}$ and SprF from pSK55. CJ2839 produces truncated (548 AA) GldJ lacking its C-terminal 13 amino acids, and is nonmotile for this reason but is functional for secretion. The 5 sec sequence shown in Fig. 5 of the main text comes from the left panel of this movie.

Movie S3. Lack of movement along the cell surface of sfGFP fused to the C-terminal 228 amino acids of Fjoh_3952. Cells of *F. johnsoniae* strain CJ1922 (Δ*sprB*) expressing SP-sfGFP-$CTD_{Fjoh\_3952(228AA)}$ and SprF-like protein Fjoh_3951 from pSK57 were labeled with fluorescent antibodies against sfGFP as indicated in Materials and Methods, introduced into tunnel slides, and examined at 25°C. Suspended cells were examined to avoid confusion resulting from the gliding movement of cells over surfaces. The sequence shown corresponds to 15 seconds.

Movie S4. Effect of expression of SP-sfGFP fused to C-terminal regions of SprB on movement of wild type *F. johnsoniae* cells. Movement of cells of wild type strain CJ1827 carrying control vector pCP23 (top left), pSK59, which expresses SP-GFP-$CTD_{SprB(149AA)}$ and SprF (top right), pSK55, which expresses SP-sfGFP-$CTD_{SprB(218AA)}$ and SprF (bottom left), and pSK61, which expresses SP-sfGFP-$CTD_{SprB(1182AA)}$ and SprF (bottom right). Cells in MM were introduced into tunnel slides, incubated for 5 min, and cells on the cover slip were examined using a Photometrics $CoolSNAP_{cf}^2$ camera mounted on an Olympus BH-2 phase-contrast microscope with a heated stage at 25°C. Four 60-second sequences (speeded up six-fold, resulting in 10-sec playback) are shown. The sequences in this movie correspond to 60 seconds of the 120 sec depicted in the rainbow traces in Fig. 7 of the main text.

Movie S5. Effect of expression of SP-sfGFP fused to C-terminal regions of SprB on movement of *F. johnsoniae* Δ*sprB* mutant cells. Movement of Δ*sprB* mutant cells (strain CJ1922) carrying control vector pCP23 (top left), pSK59, which expresses SP-sfGFP-$CTD_{SprB(149AA)}$ and SprF (top right), pSK55, which expresses SP-sfGFP-$CTD_{SprB(218AA)}$ and SprF (bottom left), and pSK61, which expresses SP-sfGFP-$CTD_{SprB(1182AA)}$ and SprF (bottom right). Cells in MM were introduced into tunnel slides, incubated for 5 min, and cells on the cover slip were examined at 25°C. Four 60-second sequences (speeded up six-fold, resulting in 10-sec playback) are shown. The sequences in this movie correspond to 60 seconds of the 120 sec depicted in the rainbow traces in Fig. 7 of the main text.

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