<u>Supplemental Figures, Movie Legends, and Tables for:</u>

JB00362-17

Joseph J. Johnston, Abhishek Shrivastava, and Mark J. McBride.

Untangling *Flavobacterium johnsoniae* gliding motility and protein secretion

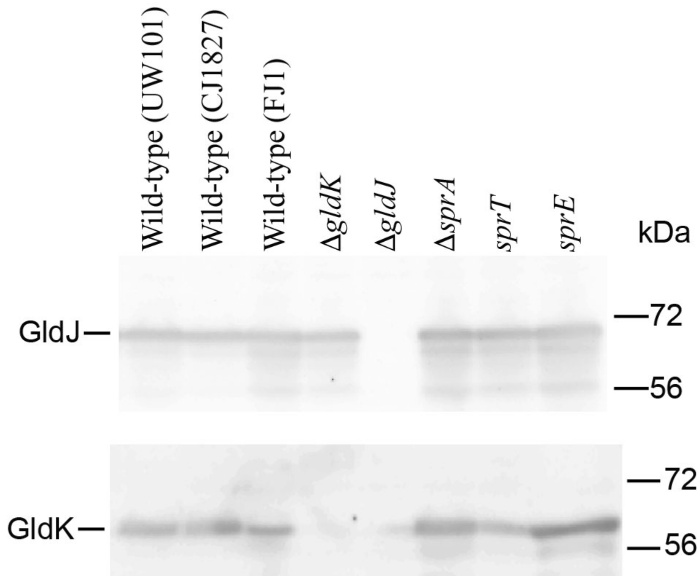

Figure S1. Mutations in *sprA, E, T* had no effect on levels of GldJ and GldK. GldJ and GldK in cells of wild-type or mutant *F. johnsoniae* strains were detected by western blot analyses. Whole cells were analyzed for cultures of wild type *F. johnsoniae* UW101, streptomycin resistant 'wild type' CJ1827, wild type strain FJ1, Δ*gldK* mutant CJ2122, Δ*gldJ* mutant CJ2360, Δ*sprA* mutant CJ2302, *sprT* mutant KDF001, and *sprE* mutant FJ149. Cell samples corresponded to 15 μg protein per lane. Samples were separated by SDS-PAGE and GldJ and GldK were detected using the appropriate antisera (Shrivastava, A, JJ Johnston, JM van Baaren, and MJ McBride. 2013. J. Bacteriol. **195**:3201-3212, and Braun, TF and MJ McBride. 2005. J. Bacteriol. **187**:2628-2637).

GldJ

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                                  10         20         30         40         50         60
F. johnsoniae GldJ          MKVNKIVVLQLMMSMVLMLGTASCSKKS-SSSHA-------SRATGWDVDSQ--NGTAAR
F. psychrophilum GldJ       MKVNKIVTVRVLFSLIVLFGFTSCSKKS-SSKNT-------SSATGWKINDPN-GGFQYN
Ca. ochracea GldJ           --MKRNLLKVPVYMALAGLLLAGCGKKT--DKNT-------SSATGWKINAKE-GGFQYN
Ce. algicola GldJ           MKKQFIKVVLSCAVIAG--GFSSCKNSS-SSKNT-------SRATGWKINAKE-GGFQHN
Ce. lytica GldJ             MKKQFIKVVLSCAVVAG--SITSCKKSS-SSKDV-------SRATGWKINAKE-GGFQHN
Maribacter sp. GldJ         MKKQFIKIVLSCAVIAGGFTVTSCSKKS-SSSKN---V---SRATGWKINAKE-GGFQYN
Ro. biformata GldJ          MKRTRISALLSAAVIAGSLSLQSCKNSG-GGSKN---T---SRATGWKINAKE-GGFQYN
Cr. atlanticus GldJ         MRMKIKLTYRVLAAITLCLGLVSCNNSR-EYRDS-------SRATGWNVNGKD-GGFQLN
G. forsetii GldJ            --MKLNVALKAIIAVVCGVSLLSCNKSN-NYKNT-------SRATGWDINSDE-GGFQYN
Z. profunda GldJ            MRMRKKTAFRAIFTIALGVGLVGCNNSK-NYKNS-------SRATGWSINSKD-GGFQYD
W. virosa GldJ              MKMNKGRIFTLLLTAASMTIFVSCGSGS-GGKKKGGGTKNFTSTSGFKQNDQ--RGWFFN
Ri. anatipestifer GldJ      ---MKKLKLFSLCVLGSSLALVSCGNSNTKKGGGSGKT---TSLTGWKPNDQ--KGWFFS
Cy. hutchinsonii GldJ       MKKMNRVIKHAVVLVAGASLLFSCSKGN-PTSINPGGS---STTTGMAFNEE--NGFQVS
L. byssophila GldJ          ---MKRLILSVPFLALTLFVAASCSSGK-KSSNDPGKR---SRTTGAAYAKKEGEGFRVA
Sp. linguale GldJ           -MIQKYHLQRAAFVLLATAALASCKSKH-PTSVQPGKK---STATGIAYNQK--EGFQVK
D. fermentans GldJ          --MQKMGTRSIALILIVLLAATACSKKK-PTSLKPGKT---SSKTGLAYNGK--DGFEVK
M. tractuosa GldJ           MKKFVFKIQNAMLALAIGL-LFACGSGN-PTAKGPGVL---STTTGLEYNSE--EGFQVA
Pe. heparinus GldJ          ---MKKIYLYLLLSFVAVTALVSCKTKS-EKSDK----------TGMDYNKQEYGGFVVN
Pe. saltans GldJ            MKKNIIAYFIACITVSC--VATSCNKKS-SSGSK---V---SGKTGALYNDKRPGSLQVM
Ch. pinensis GldJ           --MHRLTSVSMLLGASIILSMSACSFGK-KKEK--------SSATGWNYNDPKMGGFSVA
Pal. propionicigenes GldJ   ---MKLKTIAKLATLSLAITLVGCVSKS-TNNGY-------SRVTGWKYNDKTGTGLSVK
O. splanchnicus GldJ        MRNNGFFVFATCCLLV--FSATSCQKIK-GIFGK--KK---SSTTGWTYNDSKNGGIEYN

                                      70         80         90        100        110        120
F. johnsoniae GldJ          NAGKKQQAGP--GLVFVEGGTFTM-GKVQDDVMHDWN--NTPTQQHVQSFYMDETEVTNG
F. psychrophilum GldJ       NNFTKQATAP--GLVLVEGGTFTM-GKVQDDVMHDWN--NTPTQQHVQSFYMDETEVTNL
Ca. ochracea GldJ           TDFKDQETGP--GLVLIEGGTFTM-GQVEDDIMHDWN--NTPTQQHVQSFYMDETEVTNK
Ce. algicola GldJ           TDYKEQETAP--GLVFVEGGTFTK-GKVQDDVMHDWN--NTPTSQHVMSFYMDETEVTNS
Ce. lytica GldJ             SDFKEQVTAP--GLVFIEGGTFTK-GKVQDDVMHDWN--NTPTQQHVQSFYMDETEVTNK
Maribacter sp. GldJ         TDFKEQETAP--GLVFVEGGTFTK-GKVQDDVMHDWN--NTPTSQHVQSFYMDETEVTNV
Ro. biformata GldJ          TDFKEQETAP--GLVFVEGGTFTK-GKLQDDVMHDWN--NTPTTQHVQSFYMDETEVTNV
Cr. atlanticus GldJ         TDFQEQETGP--GLVFVEGGTFTM-GQVQDDVMHDWN--NTPNQQHVQSFYMDETEVTNA
G. forsetii GldJ            SDFKEQEAAP--GLVFVEGGTYTM-GRVADDPMHDWN--NTPTQQHVQSFYMDETEVTNK
Z. profunda GldJ            TDYKEQEPAP--GLVFIEGGTFTM-GQVQDDVMHDWN--NTPTQQHIQSFYMDETEVTNR
W. virosa GldJ              GNKTKPNVKPWQNMVFIEGGTFTM-GLVKDDVMHDWN--NTPRRMQVSSFFIGETEVTNY
Ri. anatipestifer GldJ      GKEEKQKGQP--GMVFVQGGTFTM-GLVKDDVMHDWN--NTPRRMQVSSFFIGETEVTNY
Cy. hutchinsonii GldJ       -DYAGQPEGP--NLVYIEGGRTVL-GSFEEDLMKNGD--NVERTVTVASFYMDETEIANI
L. byssophila GldJ          -DFKGQPAGP--NMVFIEGGRFVM-GTLEEDVMYSRD--NIDRTVTVSSFYMDETEIANL
Sp. linguale GldJ           -KFAGQKAGP--NLVFIEGGRFTM-GALEEDVMNSRD--NRERTVSIQSFYMDETEMANV
D. fermentans GldJ          -QYKALPAGP--DLVFIEGGRFTM-GSLEEDVMTRRD--NPKRTVSIQSFYMDQTEVANV
M. tractuosa GldJ           -EFRGQPDGP--NLVYIEGGRTVL-GSFEEDILHTRD--NIERTVTVSSFYMDETEVANI
Pe. heparinus GldJ          KKYR-RGPGP--GLVEIEGGVFVMSGSAINVPGQELSSYNHKRETTVSSFYMDETEVSNT
Pe. saltans GldJ            -PKTKPTPGP--GLVHIEGGTFVMGGSTIDDIGFDHT--SPKKRVTVPTFYMDETEVANV
Ch. pinensis GldJ           -KNKDQQTGP--GLVFVQGGTFAM-GATEQDVMADWN--NIPRRITVSSFYIDETEVANV
Pal. propionicigenes GldJ   KSYTGRIPA---GMVAIEGGSFTM-GEKGEFLTAPRD--NKPRRITVSSFYMDQYEIRNI
O. splanchnicus GldJ        -NVKAQKTGP--GLVFIPGGTFVM-GRTQEDVMGDWN--NTPKRVSIASFYMDETEVRNI

                                     130        140        150        160        170        180
F. johnsoniae GldJ          MYLEYLEWLKKVFPPTEENYKNIYEGASPDTLVWRNRLGYNETMTNNYLRHPSYANYPVV
F. psychrophilum GldJ       MYMEYLDWTKRVFPPTEENYKNIYEGALPDTLVWRNRLGYNETMTNNYLRHPAYSQYPVV
Ca. ochracea GldJ           MYMEYLDWLKRVFPPDEEQYRNIYIGALPDTLVWRSPLSANEAMVSNYLRHPAYAEYPVV
Ce. algicola GldJ           MYLEYLDYLKSVYPPEDPRYVNIYTGALPDTLVWRNRLGFNETMTNNYLRHPAYAEYPVV
Ce. lytica GldJ             MYLQFLDYLKSVYPPEDPKYANIYKGALPDTLVWRNRLGFNETMTNNYLRHPGYAEYPVV
Maribacter sp. GldJ         MYLEYLDYLKSVYPPENPKYANIYKGALPDTLVWRNRLGFNETMTNNYLRHPAYAEYPVV
Ro. biformata GldJ          MYLEYLDYLKSVYPPENPQYTAIYEGALPDTLVWRNRLGFNETMTNNYLRHPAYAEYPVV
Cr. atlanticus GldJ         MYLEYLDWLKKVFPPTEESYRNIYNGALPDTLVWRNRLGYNETMTNNYLRHPAYAFYPVV
G. forsetii GldJ            MYLEYLDWLRRVFPPSEENYKNIYDGALPDTLVWRNRLGYNETMVTNYLRHPAYAEYPVV
Z. profunda GldJ            MYLEYLDWLKRVFPPSEQNYRNIYYGALPDTLVWRNRLGYNETMVNNYLRHPAYGEYPVV
W. virosa GldJ              EYREYLTWLKVVFPTEDPAYRDIYEGALPDPAVFSNKLSTDNFSTENYLFSPEFSYYPVV
Ri. anatipestifer GldJ      DYRNYVTWLKFVFPSSDPNFKDIYSGALPDTLVWNNELSRND-YSETYFRSPGFDNYPVV
Cy. hutchinsonii GldJ       HWLEYLFYVQR------DSSQEFYESALPDTTVWASELAYNDPYVDHYFRYPGFRYFPVV
L. byssophila GldJ          DYLEYLYAVSR------DSTREFYAAALPDTTVWAHEMSFNDQYVDQYLRYPGFRSYPVV
Sp. linguale GldJ           HYLEYLNAISR------DSSEEVVKAALPDTTVWANPLSFNDSYVTQYLRYPAFRYYPVV
D. fermentans GldJ          HYLEYLNAVQK------DSSEEFYTKALPDTNVWFNELSFNDSYVTMYLRHPGFRLYPVV
M. tractuosa GldJ           HWLEFLHYVKL------DSSRDYYESVHPDTTVWARKLAYNDPYVDHYLRYPGFRYHPVV
Pe. heparinus GldJ          NWLEYLNWIRTNFP---KDY-EYYYNEMPDTLVWRRPLSYNEPYVDNYLRHPAYQDYPVV
Pe. saltans GldJ            DWLEYLNWIREV--AEDPRW---YYEALPDTLVWRSPLAYNEPYVTNYLRHPAYQDYPVV
Ch. pinensis GldJ           HYREYLYWLRRMY---DESFPDVYRKALPDTLVWRSELAYNEPLVEYYFRHPAYNDYPVV
Pal. propionicigenes GldJ   DWREYTTWMKIVFGHAAPK---LVQDAQPDFKIWREELAYNEPYLENYFTHPAYDQYPIV
O. splanchnicus GldJ        DWLEYLTWLRRVY----TTYPEVYQKALPDTLVWRDQLGYNEPMVKNYLRFPAYAEYPVV
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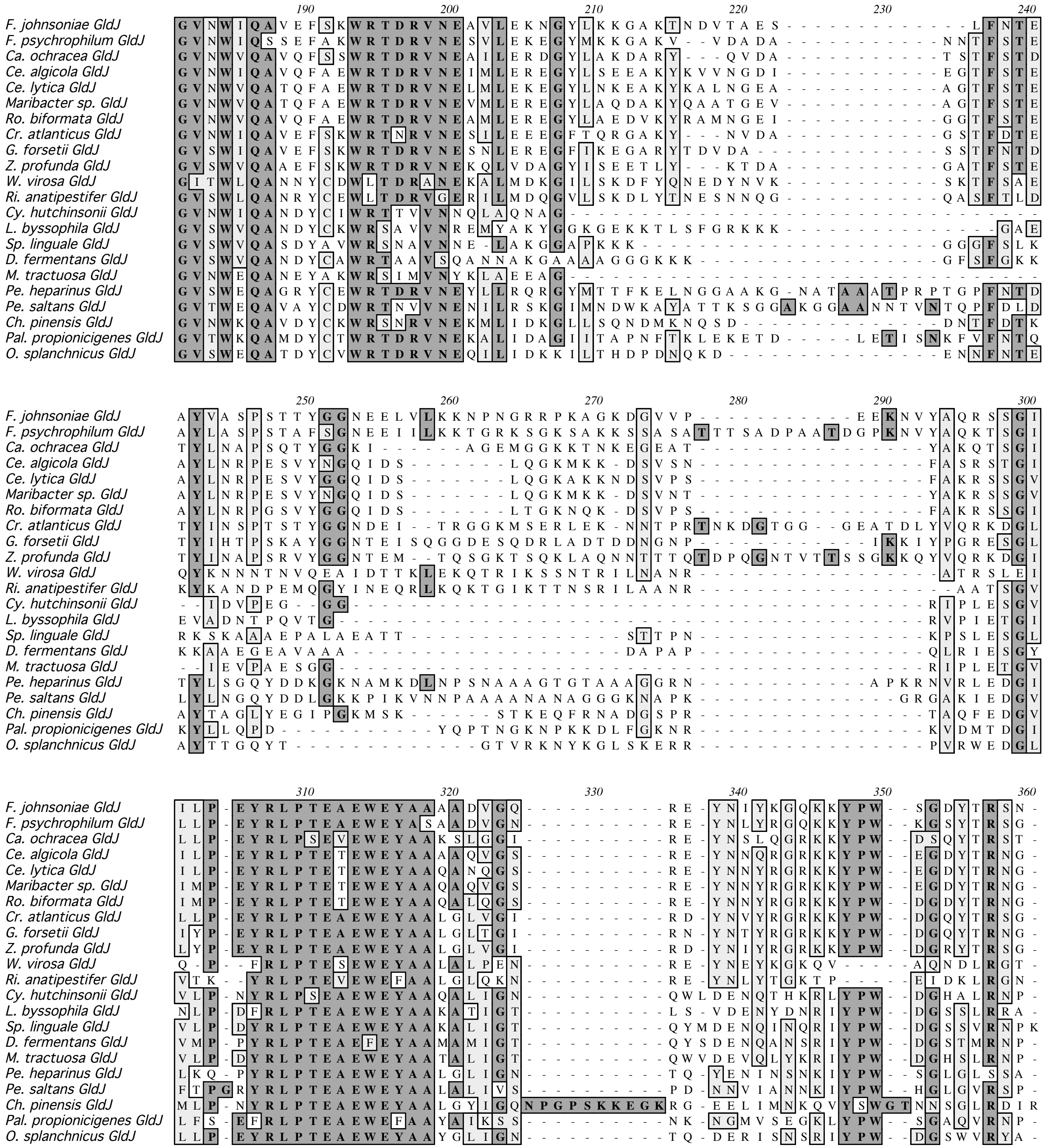
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F. johnsoniae GldJ
F. psychrophilum GldJ
Ca. ochracea GldJ
Ce. algicola GldJ
Ce. lytica GldJ
Maribacter sp. GldJ
Ro. biformata GldJ
Cr. atlanticus GldJ
G. forsetii GldJ
Z. profunda GldJ
W. virosa GldJ
Ri. anatipestifer GldJ
Cy. hutchinsonii GldJ
L. byssophila GldJ
Sp. linguale GldJ
D. fermentans GldJ
M. tractuosa GldJ
Pe. heparinus GldJ
Pe. saltans GldJ
Ch. pinensis GldJ
Pal. propionicigenes GldJ
O. splanchnicus GldJ

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                                     370       380       390       400       410       420
F. johnsoniae GldJ            KRKNRGDQLANFKQGNGDYGGIAGWSDDGADITNAVKSYAANDFGLYDMAGNVAEWVADV
F. psychrophilum GldJ         SRSTKGDQLANFKQGKGDYGGIAGWSDDGADITAPVKSYKPNDFGLYDMAGNVAEWVQDV
Ca. ochracea GldJ             KRRTRGDLLANFKQGKGDYGGLAGWSDDKGDITVAVKTYPPNDFGLYDMAGNVAEWVADV
Ce. algicola GldJ             QRLGRGDQLANFKQGKGDYGGIAGWSDDGADITAQVKSYKPNDLGLYEMAGNVAEWVADV
Ce. lytica GldJ               QRVGRGDQLANFKQGKGDYGGIAGWSDDGADITNAVMSYKPNDLGLYDMAGNVSEWVADV
Maribacter sp. GldJ           QRVGRGDQLANFKQGKGDYGGIAGWSDDGADITAEVMSYKPNDLGLYDMAGNVAEWVADV
Ro. biformata GldJ            QRVGRGDQLANFKQGKGDYGGIAGWSDDGADITAEVKSYKPNDLGLYDMAGNVAEWVADV
Cr. atlanticus GldJ           NRRSRGDQLANFKQGDGDYGGIAGWSDDGADITAEIKTYEPNDYGLYDMAGNVAEWVADV
G. forsetii GldJ              DVKKMGDQLANFKQGDGDYGGIAGWSDDGADITAEVKSYESNDFGLYDMAGNVAEWVADV
Z. profunda GldJ              DLRKQGDQLANFKQGAGDYGGIAGWSDDGADITAPVMSYEANDFGLYDMAGNVAEWVADV
W. virosa GldJ                KGKTRGQYLDNFKVGRGDYSGIGGYGNDGSAITNDVRKYQSNDFGLFGMYGNVAEWTADV
Ri. anatipestifer GldJ        KGRNRGMFLENFKYGRGDYSGPAGWNNDGSPTTSDVRQYPSNDLGIYGMYGNVAEWTADV
Cy. hutchinsonii GldJ         YGKQMGMFLANFKRGRGDYAGIAGKLNDGAMITTYIYEFPPNDFGLYCMAGNVNEWVYDV
L. byssophila GldJ            KGKARGEMQANYKRGRGDYAGIAGRLNDKYQITAPVYEYYPNDFGLYNMAGNVNEWVYDL
Sp. linguale GldJ             KGRKQGQMLANFKRGRGDYAGIAGRSNDGAIITAEIYAYPANDFGLYNMAGNVNEWVYDV
D. fermentans GldJ            RGRKQGTMLANFKRGPGDYAGIAGKSNDGAIITQEIRSYPANDNGLYDMAGNVSEWVYDV
M. tractuosa GldJ             YGKEMGYFLANFKRGRGDYGGIAGKLNDGAFITDYIYNFPPNDYGLYNMAGNVNEWVWDL
Pe. heparinus GldJ            KKSTRGLILANFKRTKGDYMGVGGSLNDKGSITVNVRSYTPNDFGLYNMAGNVSEWVADV
Pe. saltans GldJ              KKDTRGMVYANFKQGKGDNMGVAGYLNDGADITAPVRAYVPNDFGLFNMAGNVSEWVYDT
Ch. pinensis GldJ             RGAWQGQFLANFKRGSGDNMGMAGGLNDRASIPGPVIAFFPNTFGIYNMSGNVSEWVLDV
Pal. propionicigenes GldJ     TKKQLGQMQANFVRGKGDMMGTSGTLNDKATITCPVDWYFPNDFGLYNMAGNVNEWVLDV
O. splanchnicus GldJ          EKKHRGRMMANFTRGKGDYMGVAGSANDGWDYTSPVRSFWPNDFGLYDMAGNVNEWVADV

                                       430       440       450       460       470       480
F. johnsoniae GldJ            YRPIIDNEANDFNYYRGNQYAKNKIGKDGKIEIVSTATIKYDTLSNG---------KVVA
F. psychrophilum GldJ         YRPMVDDEANDFNYVRGNVYTKNKIGEDGKIELVTSENIKYDTLSNG---------KVIA
Ca. ochracea GldJ             YRPIVDDEITDFNYYRGNVYMKHAIGEDGKQIVVTPENVKYDTLMNG---------KIIA
Ce. algicola GldJ             YRPIVDDEVSDFNYYRGNIYMKTAIGEDGKVNVI-RDSIVYDTLPNG---------KVVA
Ce. lytica GldJ               YRPIVDDEISDFNYYRGNIYMKTAIGEDGKVNVL-RDSVVYDTLPNG---------KIVA
Maribacter sp. GldJ           YRPIVDDEISDFNYYRGNIYMKTAIGEDGKVNIL-RDSVVYDTLPTG---------KIIA
Ro. biformata GldJ            YRPIVDDEISDFNYFRGNIFMKAAIGEDGKVQVL-RDSIVYDTLPTG---------KVVA
Cr. atlanticus GldJ           YRPIVDDEFNDFNYYRGNVYTKNDINEDGTVKLVTTEDIVYDTLSNG---------KIIA
G. forsetii GldJ              YRPIVDDEFNDFNYYRGNVYTKHAINEDGSVKIVGTDDIVYDTLSNG---------KLVA
Z. profunda GldJ              YRPIVDDEFNDFNYYRGNVYTENAINEDGTVQVVGVDNIKYDTLSTG---------RVVA
W. virosa GldJ                YRPIIDDEANDFNYFRGNVFKTTIDDQNGGFAKYGEDDIEYDTLNNG---------KLVY
Ri. anatipestifer GldJ        YRPIIDDEASDFNYYRGNVNKQMVKNADGTYTKLDGSNITYDTLADG---------RLVY
Cy. hutchinsonii GldJ         YRPLSFQTFDDLNPLRRNGFLD--------------------------------------
L. byssophila GldJ            YRPNSYRDFNDLNPIRRSDYQD--------------------------------------
Sp. linguale GldJ             YRPLSYQDVNDLNPIRRNGYLD--------------------------------------
D. fermentans GldJ            YRPLSNSDVNDLNSFRRDGYQD--------------------------------------
M. tractuosa GldJ             YRPLSFQDFEDLNPVRRDGTLD--------------------------------------
Pe. heparinus GldJ            YRSNTFEAADAFNPYRGNYYQDKKIADPATGKIA-IDKYNKPVMTDA----------LYA
Pe. saltans GldJ              YRQHSFDKVEDFSPFRGNEFLNNEYDNNGKIVTIGKTNIPKKTAATSGRKDAYDLHKEKY
Ch. pinensis GldJ             YRPLNPIDGDDFNYFRGNKFQTTYMNGESEPE----------------------------
Pal. propionicigenes GldJ     YRSTTTDDIAEYNSFRGNVYLTPIAT---GVDELGNKTYKIDSLG-----------RIAT
O. splanchnicus GldJ          YRTLSFQDMAEFNPYRGNVFQSPVLNEDG---------------------------TAAP

                                       490       500       510       520       530       540
F. johnsoniae GldJ            RNLPGEIAQVPVDEQETYLRTNFS-TSDNINYRDGDKQSSRYFDFGDSESGS-KADQA--
F. psychrophilum GldJ         RNKPGEIVQVPVNEKETYLRQNFD-KSNEINYRDGDKQSTRFFDYGASEEGEAKKDEKNR
Ca. ochracea GldJ             RNLPGNLATVPIDSNETYLRYNFT-HSDNRSYHDGDKSSTRNYRNQNDLMAE-GSEYTNS
Ce. algicola GldJ             VNLPGELKMVPVDENETYLRTNFS-SSDNRGYRDGEPGSSRFYDRFSDDEDG-DEKKK--
Ce. lytica GldJ               VNLPGEIKMVAVDEEETYLRTNFS-SSDNRGYRDGEPGSTRFYDRFNEADGD-EENKIS-
Maribacter sp. GldJ           VNLPGEIKMVPVDEQETYLRTNFS-SSDNRGYRDGDPSSSRFFDRFSDEDEE-DDTRK--
Ro. biformata GldJ            VNLPGEVKMVPVGEQETYLRTNFS-KSDNRGYRDGDPSSSRFADFYGEDEEK-PA-----
Cr. atlanticus GldJ           RDLPGEIVSVPVDEKETYLRTQFS-TSDNRDFRDGDKRSTRYYTTFGDEEVS-DTKR---
G. forsetii GldJ              RNLPGEIAQVPVTEEDTYMRTNFT-ESDQRDYRDGDKSSSRYYQPFQETEED-PSKR---
Z. profunda GldJ              RNLPGEILQKPITEDETYMRTNFS-KSDNRNFRDGDKSSSRYYQPFQDTDNS-KR-----
W. virosa GldJ                RGLPGSYKKEIEY--------------DYTNYRDGDAMSSLDTRVTTETVAK-TSD----
Ri. anatipestifer GldJ        KGLPGQYKREVVK--------------DKRNFRDGDFMSSLEAGYGRELDSA-AAKEFD-
Cy. hutchinsonii GldJ         ---------------------------EEKGYDKAGFQ----------------------
L. byssophila GldJ            ---------------------------EEKNYDNAN------------------------
Sp. linguale GldJ             ---------------------------DSKNY----------------------------
D. fermentans GldJ            ---------------------------EAKNY----------------------------
M. tractuosa GldJ             ---------------------------PQNNYDNGAR-----------------------
Pe. heparinus GldJ            KKQTWAEKQAAAAKPTDSLKNTYA---DQRGYRDPQSE----------------------
Pe. saltans GldJ              GNVTEESPSTPDAKDPKMTTASPSFSSDQRGYLDKENLD---------------------
Ch. pinensis GldJ             KDSLGHLKMRDVTDEESANRLNYQ-KGDVINYLDGDSLSNVEYGYG--------------
Pal. propionicigenes GldJ     EVAKGD---------------------DVRNFKDGDATSQIDFTLNDSTGLA-SLRN---
O. splanchnicus GldJ          KDSLGRMRYRNQTDAELANRENYR-TADNRNFNDGDLKSRISGDIDWRNTEN-DRGTHD-
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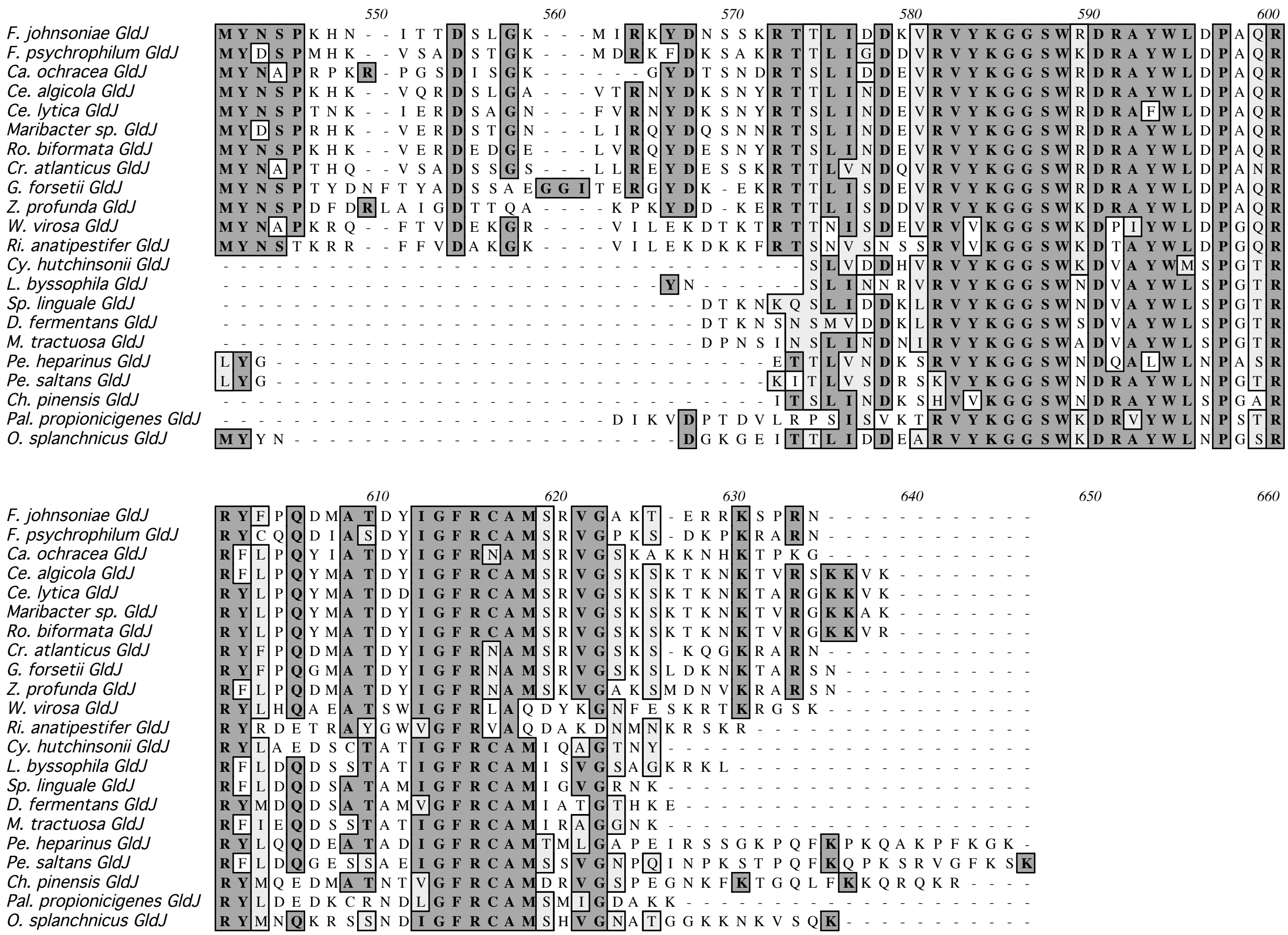

Figure S2. Alignment of GldJ sequences using MUSCLE. GldJ sequences from 22 members of the phylum *Bacteroidetes* were aligned using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. Sequences used in this alignment were from *F. johnsoniae* ATCC 17061^{T}, *Flavobacterium psychrophilum* JIP02/86, *Capnocytophaga ochracea* DSM7271^{T}, *Cellulophaga algicola* DSM 14237^{T}, *Cellulophaga lytica* DSM 7489^{T}, *Maribacter* sp. HTCC2170, *Robiginitalea biformata* HTCC2501^{T}, *Croceibacter atlanticus* HTCC2559^{T}, '*Gramella forsetii*' KT0803, *Zunongwangia profunda* SM-A87^{T}, *Weeksella virosa*, 16922^{T}, *Riemerella anatipestifer* DSM 15868^{T}, *Cytophaga hutchinsonii* ATCC 33406^{T}, *Leadbetterella byssophila* DSM 17132^{T}, *Spirosoma linguale* DSM 74^{T}, *Dyadobacter fermentans* DSM 18053^{T}, *Marivirga tractuosa* DSM 4126^{T}, *Pedobacter heparinus* DSM 2366^{T}, *Pedobacter saltans* DSM 12145^{T}, *Chitinophaga pinensis* DSM 2588^{T}, *Paludibacter propionicigenes* WB4^{T}, and *Odoribacter splanchnicus* DSM 20712^{T}. Sequences were organized with those most similar to *F. johnsoniae* GldJ at the top and those least similar at the bottom.

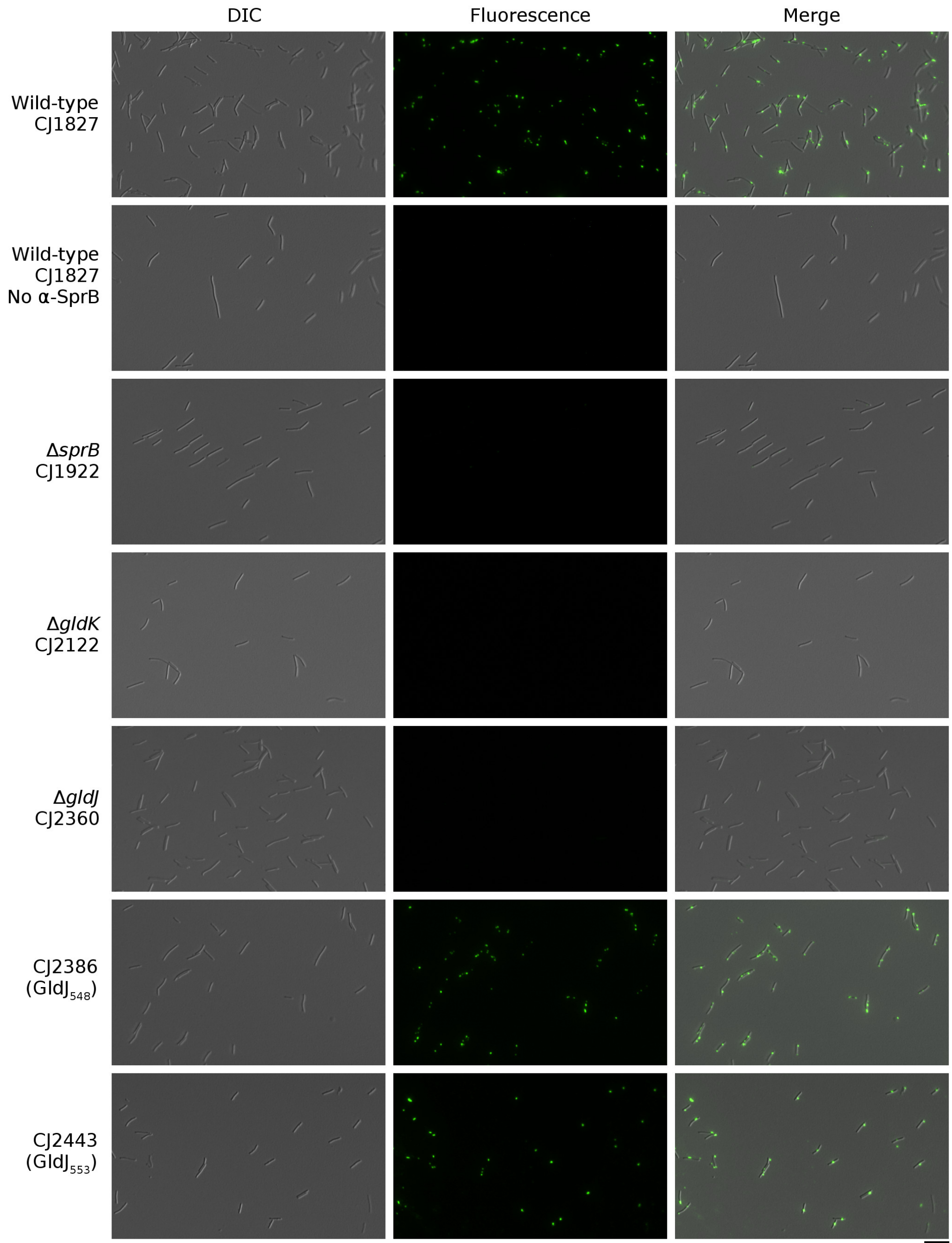

Figure S3. Detection of surface-localized SprB by immunofluorescence labeling. (A) Cells of wild-type and mutant *F. johnsoniae* were exposed to anti-SprB antiserum followed by F(ab') fragment of goat anti-rabbit IgG conjugated to Alexa-488. Cells of mutants CJ2386 and CJ2443 produce truncated versions of GldJ (548 and 553 AA respectively). Images of cells were recorded by differential interference contrast microscopy (DIC; left column) and by immunofluorescence microscopy (center column) and then overlaid (right column). The same exposure time was used for all DIC images, and the same exposure time was used for all fluorescence images. 'No α-SprB' indicates no primary antiserum added to this sample. Bar (lower right) indicates 10 μm and applies to all images. The merged images correspond to those in Fig. 11 of the main text.

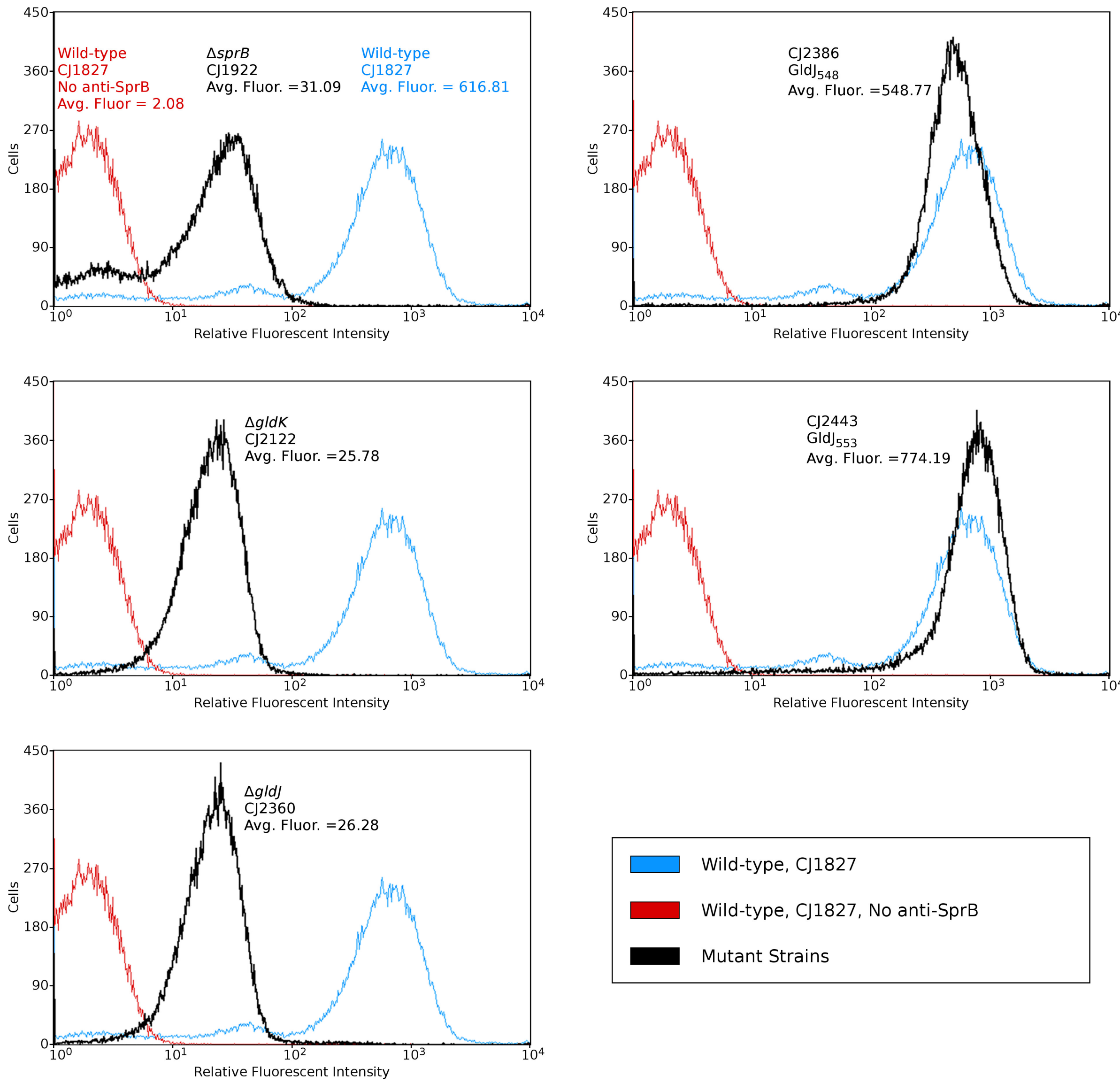

Figure S4. Cell-surface SprB as measured by immuno-labeling and flow cytometry. Cells were exposed to anti-SprB antiserum and F(ab') fragment of goat anti-rabbit IgG conjugated to Alexa-488 and detected by flow cytometry as described in the main text. The relative fluorescence intensities of individual cells (minimum of 49,877 cells per strain) are plotted for each strain and average fluorescence intensities of cells are indicated. Red indicates wild-type cells without anti-SprB antiserum (negative control). Blue indicates wild-type cells with anti-SprB antiserum (positive control). Black indicates individual mutants with anti-SprB antiserum as listed in each panel. The positive and negative controls are repeated in each panel to facilitate comparisons. The Δ*sprB* strain (fails to make SprB) and Δ*gldJ* and Δ*gldK* strains (fail to secrete SprB) each had low levels of background fluorescence presumably because the rabbit anti-SprB antiserum detected other cell-surface antigens besides SprB. Less than 0.6% of cells of strains Δ*sprB*, Δ*gldJ*, and Δ*gldK* exhibited fluorescence above a background cutoff of 10^2 indicating absence of cell-surface SprB for these strains. Greater than 91% of cells of strains CJ2386 (makes truncated $GldJ_{548}$) and CJ2443 (makes truncated $GldJ_{553}$) exhibited fluorescence above this background cutoff indicating that nearly all cells of these mutants had SprB on their surfaces.

Movie Legends

Movie S1. Movement of cells of wild-type *F. johnsoniae* UW101 (Left), *gldJ* mutant UW102-81 which produces truncated (542 AAs) GldJ (Center), and UW102-81 complemented with pMM313 that expresses full length (561 AAs) GldJ (Right) on glass cover slips. 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. Three 30-second sequences are shown. Bar indicates 10 μm and applies to all movies. Cells of *gldJ* mutant UW102-81 (Center) failed to attach to glass and the movements seen for the cells in suspension appear to be Brownian motion rather than active motility.

Movie S2. Movement of cells of wild-type *F. johnsoniae* CJ1827 (Left), *gldJ* mutant CJ2386 which produces truncated (548 AAs) GldJ (Center), and CJ2386 complemented with pMM313 that expresses full length (561 AAs) GldJ (Right) on glass cover slips. Cells in MM were introduced into tunnel slides, incubated for 5 min, and cells on the cover slip were examined at 25°C. Three 30-second sequences are shown. Bar indicates 10 μm and applies to all movies.

Movie S3. Movement of cells of wild-type *F. johnsoniae* CJ1827 (Left), *gldJ* mutant CJ2443 which produces truncated (553 AAs) GldJ (Center), and CJ2443 complemented with pMM313 that expresses full length (561 AAs) GldJ (Right) on glass cover slips. Cells in MM were introduced into tunnel slides, incubated for 5 min, and cells on the cover slip were examined at 25°C. Three 30-second sequences are shown. Bar indicates 10 μm and applies to all movies.

Movie S4. Binding and movement of Protein G-coated polystyrene spheres. Protein G-coated 0.5 μm polystyrene spheres were added to cells of *F. johnsoniae* strains in MM plus 0.1% BSA with anti-SprB antibodies. The cells were introduced into tunnel slides and examined at 25°C. The three sequences shown correspond to 30 seconds each. From left to right: 1) Wild-type *F. johnsoniae* UW101 which makes full length GldJ (661 AA). 2) *gldJ* truncation mutant UW102-81 which makes truncated $GldJ_{542}$ (542 AA). 3) UW102-81 complemented with pMM313 that carries wild type *gldJ*. Cells were also incubated without anti-SprB, but failed to bind spheres as demonstrated in Fig. 10 of the main text. Green arrows indicate spheres that move along the length of a cell. No spheres attached to cells expressing $GldJ_{542}$, presumably since there was no SprB on the cell surface (see Fig. 10 in the main text). Bar indicates 10 μm.

Movie S5. Binding and movement of Protein G-coated polystyrene spheres. Protein G-coated 0.5 μm polystyrene spheres were added to cells of *F. johnsoniae* strains in MM plus 0.1% BSA with anti-SprB antibodies. The cells were introduced into tunnel slides and examined at 25°C. The three sequences shown correspond to 30 seconds each. From left to right: 1) Wild-type *F. johnsoniae* CJ1827 which makes full length GldJ (661 AA). 2) *gldJ* truncation mutant CJ2386 which makes truncated $GldJ_{548}$ (548 AA). 3) CJ2386 complemented with pMM313 that carries wild type *gldJ*. Green arrows indicate spheres that move along the length of a cell. Red arrows indicate spheres that attach to a cell but fail to move along the cell. Bar indicates 10 μm.

Movie S6. Binding and movement of Protein G-coated polystyrene spheres. Protein G-coated 0.5 μm polystyrene spheres were added to cells of *F. johnsoniae* strains in MM plus 0.1% BSA with anti-SprB antibodies. The cells were introduced into tunnel slides and examined at 25^{o}C. The three sequences shown correspond to 30 seconds each. From left to right: 1) Wild-type *F. johnsoniae* CJ1827 which makes full length GldJ (661 AA). 2) *gldJ* truncation mutant CJ2443 which makes truncated GldJ$_{553}$ (553 AA). 3) CJ2443 complemented with pMM313 that carries wild type *gldJ*. Green arrows indicate spheres that move along the length of a cell. Red arrows indicate spheres that attach to a cell but fail to move along the cell. Bar indicates 10 μm.

Table S1. **Primers used in this study.**

Primer	Sequence and Description
21	5' ATTCGAGCTCTTCAGAAGTATAACCGATG 3'; used to amplify *gldA*
22	5' ATTATCTAGATGCTTGGCAAATATAACAC 3'; used to amplify *gldA*
1329	5' GCTAGGGATCCCTGAGCCTGTGTATTGTCTGAAACTAA 3'; used to construct pJJ07; BamHI site underlined
1330	5' GCTAG GTCGACCATTGACATCATTAATTGCAAGACTAC 3'; used to construct pJJ07; SalI site underlined
1331	5' GCTAG GTCGACGCAATGTCTAGAGTAGGTGCTAAAACT 3'; used to construct pJJ07; SalI site underlined
1332	5' GCTAGGCATGCCAGGTAGAAGGTTTTGATGAAACG 3'; used to construct pJJ07; SphI site underlined
1360	5' GCTAGGGATCCGAAGCGGAAACCATCTCCTG 3'; used to construct pAB31; BamHI site underlined
1361	5' GCTAGGCATGCTTAGAAACCTATGTAATCAGTTGCCAT 3'; used to construct pAB31; SphI site underlined
1502	5' GCTAGGGATCCCGTGAAGCTCCCATTTATCTGAG 3'; used to construct pJJ10 and pJJ11; BamHI site underlined
1503	5' TAGGTCGACTTATCTAGACATTGCACATCTGAAACC 3'; used to construct pJJ10; SalI site underlined
1504	5 GCTAGGTCGACCATAAAAAATTCCAAATTCCAAAGCTG 3'; used to construct pJJ10 and pJJ11; SalI site underlined
1505	5' GCTAGGCATGCCCAAAATCAGGTACGAACAGCA 3'; used to construct pJJ10 and pJJ11; SphI site underlined
1506	5' CTAGTAATCGGCTGTGGTAAGTC 3'; *gldK* mRNA probe for qPCR
1507	5' AGTTCTTGTTGGAGCATCTTCTA 3'; *gldK* mRNA probe for qPCR
1508	5' ATGGACGCAAGTCTGAACC 3'; 16s rRNA probe for qPCR
1509	5' CGGAGTTAGCCGATCCTTATTC 3'; 16s rRNA probe for qPCR
1555	5' TAGGTCGACTTAAGTTTTAGCACCTACTCTAGACATTG 3'; used to construct pJJ11; SalI site underlined