Supplemental materials

Interaction between FliJ and FlhA, components of the bacterial flagellar type III export apparatus

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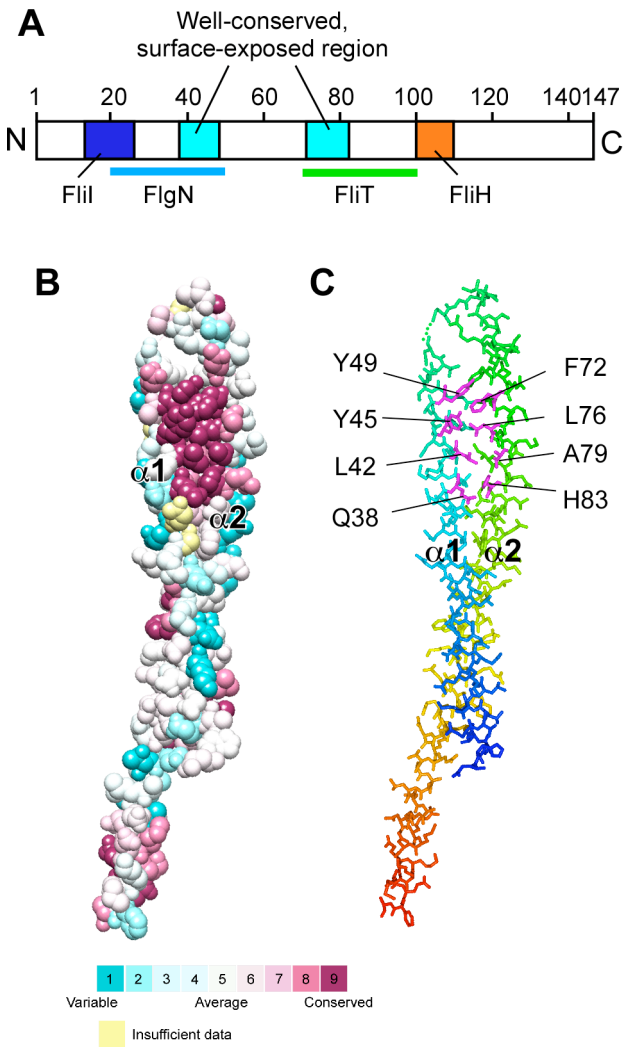

Fig S1. Evolutionary conserved residues of FliJ. (A) The primary structure of *Salmonella enterica* FliJ. FliJ is a small protein with 147 amino acids. The binding regions of FliJ for FliH, FliI, FlgN, and FliT are shown in blue, light green, yellow, and red, respectively. A well-conserved, surface-exposed region, which is formed by residues 38-49 and 72-83, is colored magenta. (B) Space filling drawing of FliJ colored in accordance with evolutionary conservation among 50 different bacterial species. The figure is prepared by ConSurf server (http://consurf.tau.ac.il/). (C) Stick representation of FliJ viewed from the same orientation as (B). The model is colored in rainbow spectrum from the N-terminus (blue) to the C-terminus (red). The eight well-conserved residues are labeled and colored magenta.

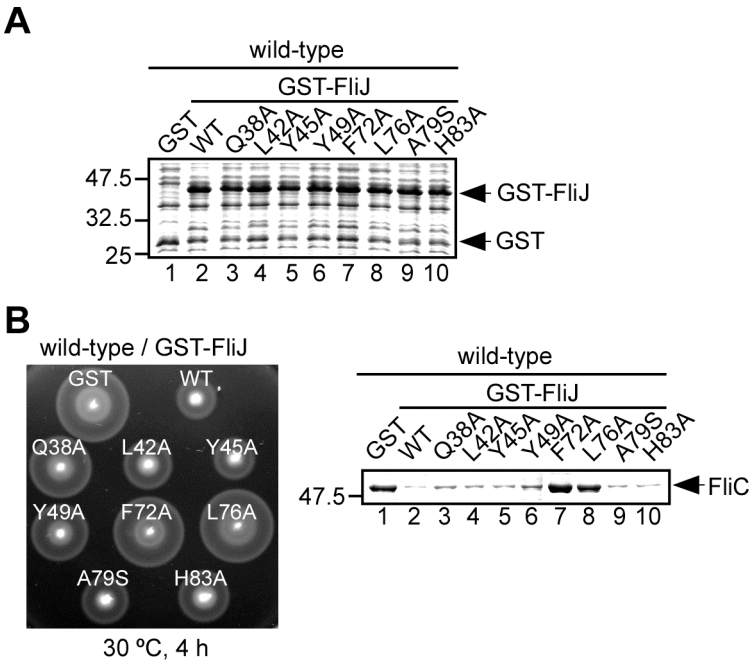

Fig. S2. Dominant negative effect of various point mutant variants of GST-FliJ on motility of and flagellar protein export by wild-type cells. (A) Expression level of various forms of GST-FliJ. Whole cell proteins were prepared from SJW1103 (wild-type) transformed with pGEX-6p-1-based plasmids encoding various forms of GST-FliJ and subjected to SDS-PAGE, followed by Coomassie brilliant blue (CBB) staining. Lane1, GST; lane 2, GST-FliJ (indicated as WT); lane 3, GST-FliJ(Q38A) (indicated as Q38A); lane 4, GST-FliJ(L42A) (indicated as L42A); lane 5, GST-FliJ(Y45A) (indicated as Y45A); lane 6, GST-FliJ(Y49A) (indicated as Y49A); lane 7, GST-FliJ(F72A) (indicated as F72A); lane 8, GST-FliJ(L76A) (indicated as L76A); lane 9, GST-FliJ(A79S) (indicated as A79S); lane 10, GST-FliJ(H83A) (indicated as H83A). The positions of various GST-FliJ variants and GST are indicated by arrows. (B) (Left panel) Motility of the same transformants in soft agar. The plates were incubated at 30ºC for 4 hours. (Right panel) Secretion assays of FliC. Secretion of FliC was analyzed by CBB staining.

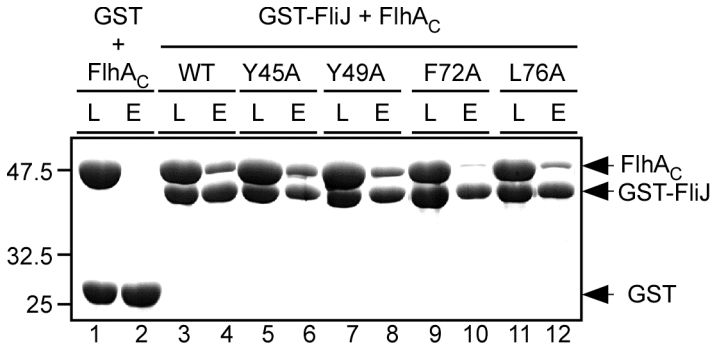

Fig. S3. Effect of FliJ mutations on the interaction of FliJ with $FlhA_C$. Purified His-$FlhA_C$ was mixed with purified GST, GST-FliJ, GST-FliJ(Y45A), GST-FliJ(Y49A), GST-FliJ(F72A) or GST-FliJ(L76A) and then these mixtures were dialyzed overnight against PBS with 2 changes. The mixtures (indicated as L) were loaded onto a GST column. After washing with 5 ml PBS, proteins were eluted with 50 mM Tris-HCl, pH 8.0, 10 mM reduced glutathione. The eluted proteins (E) were analyzed by CBB staining.