Supplemental figures

BB0326 is responsible for the formation of periplasmic flagellar collar and the assembly of the stator complex in *Borrelia burgdorferi*

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Figure S1

Figure S1. Amino acid sequence, membrane topology and the domain/motifs of BB0326 (931 aa., accession no. NP_212460.1). Transmembrane helix (aa 329-351) is predicted through TMHMM, potential peptidoglycan-binding site (aa 525-543) (see Fig. 1B) and TPR motifs (repeat 1: aa 736-750, repeat 2: 755-785, repeat 3: 792-820) were determined by analyzing the sequence using NCBI BLASTP.

Figure S2

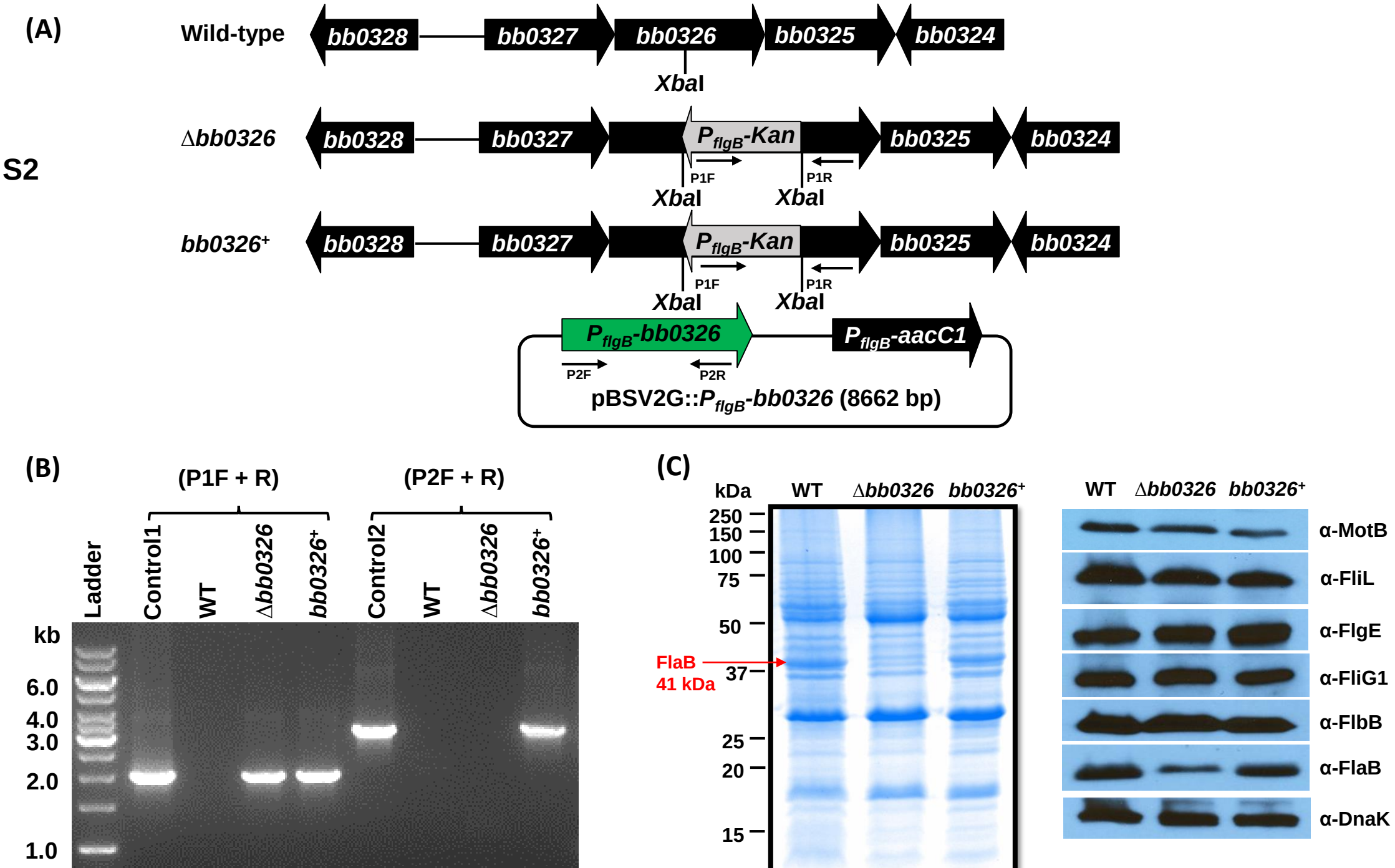
(A)
Wild-type
bb0328
bb0327
bb0326
bb0325
bb0324
XbaI
Δbb0326
P flgB-Kan
P1F
P1R
XbaI
XbaI
bb0326+
P flgB-bb0326
P flgB-aacC1
P2F
P2R
pBSV2G::P flgB-bb0326 (8662 bp)
(B)
(P1F + R)
(P2F + R)
Ladder
Control1
WT
Δbb0326
bb0326+
Control2
WT
Δbb0326
bb0326+
kb
6.0
4.0
3.0
2.0
1.0
(C)
kDa
WT
Δbb0326
bb0326+
250
150
100
75
50
FlaB
41 kDa
37
25
20
15
WT
Δbb0326
bb0326+
α-MotB
α-FliL
α-FlgE
α-FliG1
α-FlbB
α-FlaB
α-DnaK

Figure S2. Inactivation and complementation of *bb0326*. (A) The *bb0326* gene is within an operon consisting of three genes (diagram is not to scale). The *bb0326* gene was inactivated by insertion of P_{flgB}-*Kan* cassette using allelic exchange mutagenesis in B31-A wild-type background. The *bb0326* mutant cells were complemented *in trans* using pBSV2G::P_{flgB}-*bb0326* (8662 bp). **(B)** Confirmation of inactivation and complementation of Δ*bb0326* by PCR. **(C)** Effect of *bb0326* mutation on other flagellar protein syntheses were determined by Western blotting**.** Wild-type, Δ*bb0326*, and *bb0326*$^{+}$ cell lysates (10 µg in each lane) were subjected to SDS-PAGE followed by coomassie staining. 41 kDa FlaB protein band is indicated by a red arrow (left). A gel ran parallelly was transferred to a PVDF membrane for immunoblot analysis (right). Immunoblots were performed with *B. burgdorferi* MotB, FliL, FlgE, FliG1, FlbB, FlaB-specific antibodies. DnaK was used as a loading control.

Figure S3

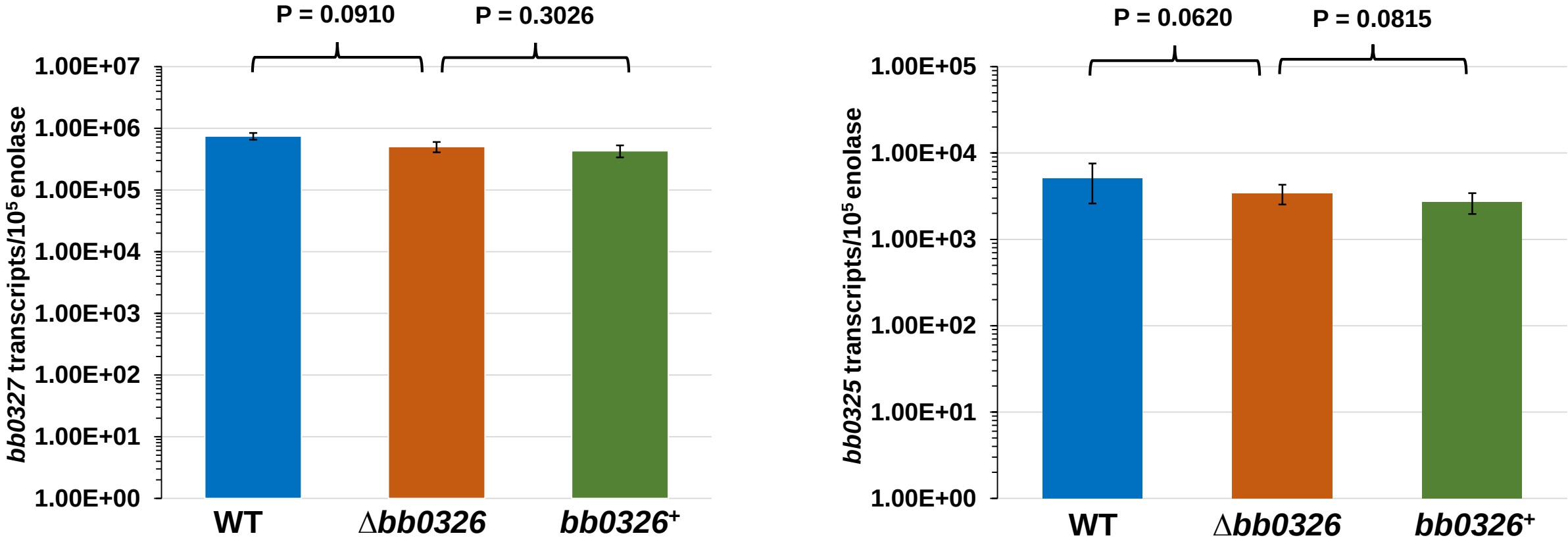

Figure S3. Expression of genes upstream and downstream of *bb0326* were not affected by the targeted mutagenesis of *bb0326*. Quantitative measurement of transcripts in the indicated spirochete clones by qRT-PCR. The relative transcript levels of *bb0327* (left), and *bb0325* (right) were determined in the wild-type, Δ*bb0326* and *bb0326*$^{+}$ cells which were then compared with the expression of *B. burgdorferi enolase* gene. The bars represent mean ± standard deviation of the mean from three independent samples. Statistical analysis was performed by using Student's *t*-test. P-values between samples are shown at the top. A P-value of <0.05 was evaluated as significantly different.

Figure S4

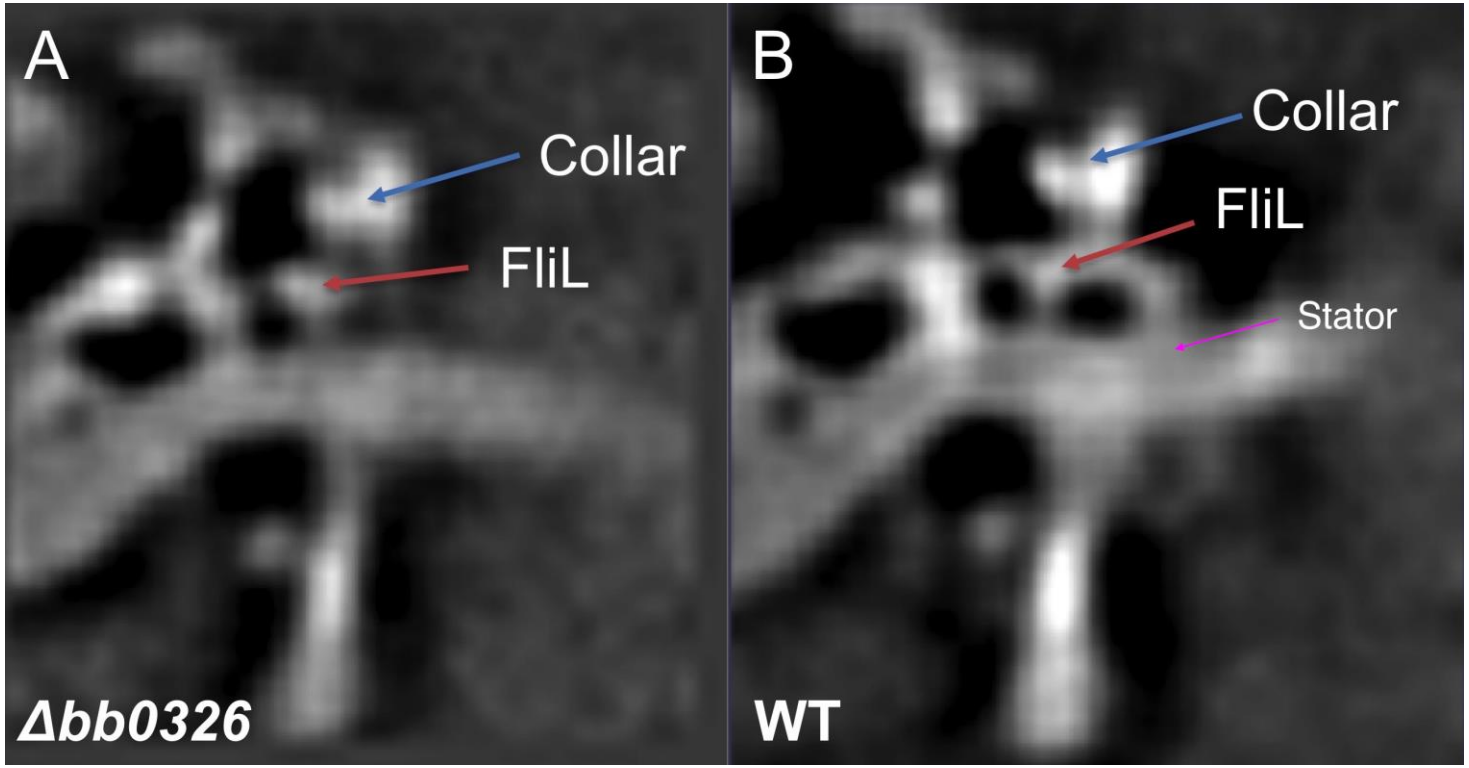

Figure S4. FliL assembly is not affected in the Δ*bb0326*/Δ*flcA* mutant as determined by high-resolution cryo-ET. **(A)** An average structure of the flagellar motor from the Δ*flcA* mutant cells. **(B)** A similar average structure of the flagellar motor from the WT cells. Density around the FliL is shown to explicitly determine collar, FliL and stator structures.