Online supplemental material

Three mutations in *Escherichia coli* that generate transformable functional flagella

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Running title: Transformable functional flagella in *E. coli*

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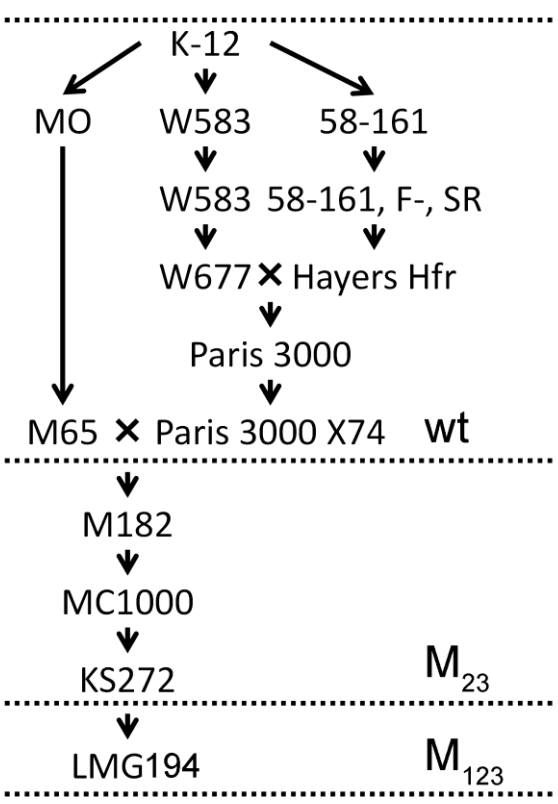

Figure S1. Pedigree of some ancestral strains of LMG194 and their *fli*C alleles.

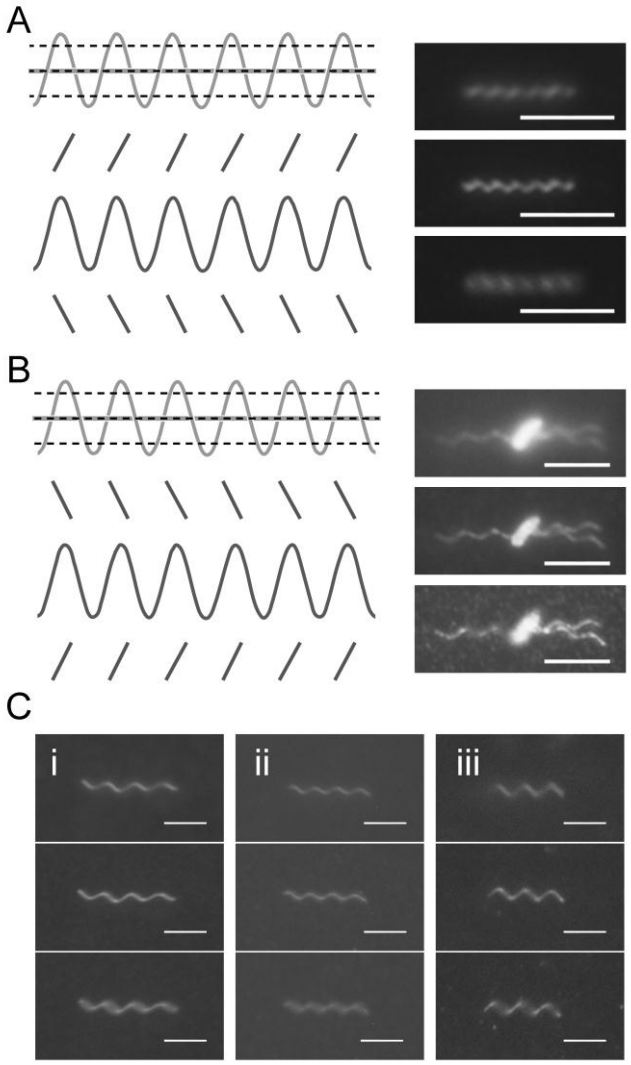

Figure S2. Determination of the helical handedness of flagella. scale bars equal 5 μm. A, The left-handed L-curly flagellum. On the left is a diagram showing the three focal planes and the expected images for a left-handed helix. The real images of a detached M_2 flagellum are on the right. B, The right-handed w-coil flagella on an immobilized cell. Left, a diagram showing the focal planes and the expected images. Right, the images of a M_1 cell. C, Three kinds of right-handed flagella in this study: i, The w-coil helix from the M_1 mutant. ii, The R-normal helix from the M_{23} mutant. iii, The w-coil form the M_{123} mutant at pH 12.0.

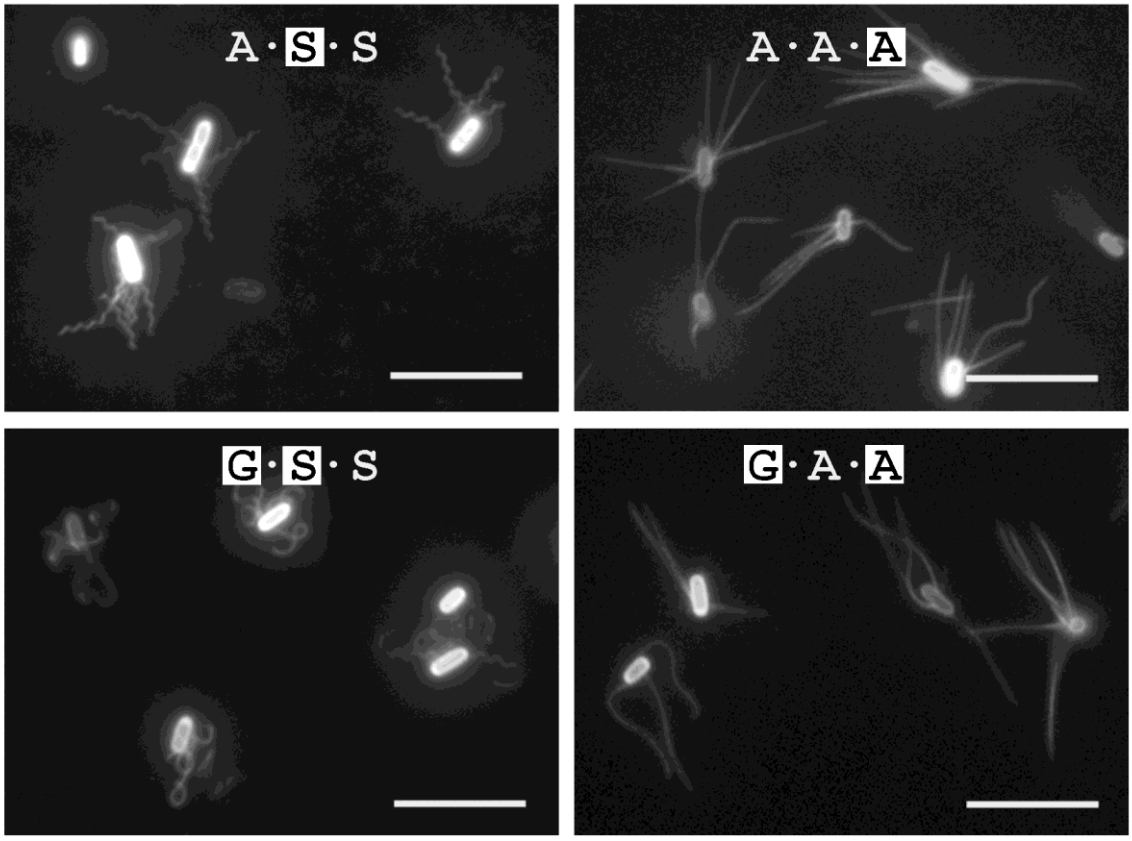

Figure S3. Flagella on the non-motile *E. coli* strains created in this study. Scale bar equals 10 μm. The amino acid sequences in flagellin were shown in each panel: white letter, wild type; black letter in white background, mutation.

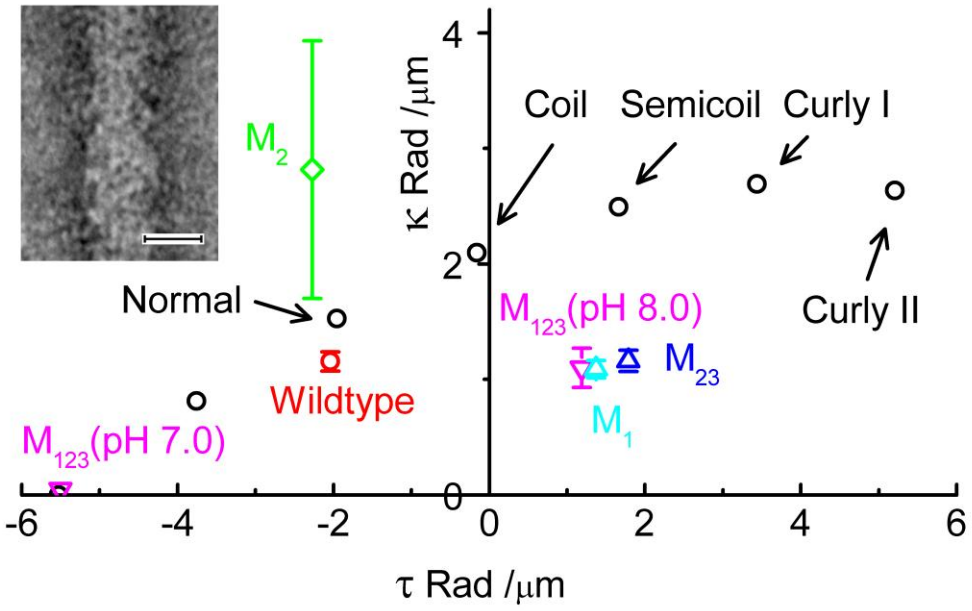

Figure S4. Plot of curvature (κ) against twist (τ) for the major helical forms produced by different mutants. Black open circles are the theoretical values for canonical helices predicted by Calladine's bi-stable model. Both the straight helix of M_{123} flagella (pH 7.0) and w-coil helix (pH 8.0) were plotted. Inset is a TEM image of a piece of the straight M_{123} flagellum at pH 7.0 showing the subunit configuration. Scale bar equals 10 nm.

TABLE S1. Primers used in this study. Mutations are bold and underlined. Restriction sites are italic and underlined.

Name	Sequences 5'-3'	Note
Flic1f	ATGGCACAAGTCATTAATAC	Forward primer for sequencing the entire *fli*C gene
Flic2r	TTAACCCTGCAGCAGAGAC	Reverse primer for sequencing the entire *fli*C gene
Flid01	TGGCGGTCTGGAAAGTCGTC	Forward primer to create plasmids pM1, pM2, pM12, pM13, and pM23
Flic4r	CGATTAACCCTGCAGCAGAGAC	Reverse primer to clone the expression cassette of *fli*C from LMG194 and AW405
Flic5r	TTATCCGTTTCTGCAGGGTTTTTA	Reverse primer to create plasmids pM1, pM2, pM12, pM13, and pM23
Mut01r	CTGACCC**C**CTGCGTCATCC	Reverse primer paring with Flid01 to introduce the mutation at 137 bp in the first-round PCR
Mut01f	GGATGACGCAG**G**GGGTCAG	Forward primer paring with Flic5r to introduce the mutation at 137 bp in the first-round PCR
Mut02r	GAAACGGTTAGCAATCG**A**CTGACC	Reverse primer paring with Flid01 to introduce the mutation at 145 bp in the first-round PCR
Mut02f	GGTCAG**T**CGATTGCTAACCGTTTC	Forward primer paring with Flic5r to introduce the mutation at 145 bp in the first-round PCR
E3flid	TTAAATCCAGACCTGACCCGACTC	Forward primer to create plasmid pM3
Flic2r	TTAACCCTGCAGCAGAGAC	Reverse primer to create plasmid pM3
Mut03r	CCTGGATAG**C**AGACAGATCAGACTCAG	Reverse primer paring with E3flid to introduce the mutation at 331 bp in the first-round PCR
Mut03f	CTGAGTCTGATCTGTCT**G**CTATCCAGG	Forward primer paring with Flic2r to introduce the mutation at 331 bp in the first-round PCR
Kanf	TATAAGGGATTTTGCCGATTTC	Forward primer for amplifying the Kanamycin resistant gene cassette
Kanr	ATAAGGGCGACACGGAAATG	Forward primer for amplifying the Kanamycin resistant gene cassette
Cf1	TACTTGCCATGCGATTTCCTTTTA	Forward primer for amplifying a fragment containing the upstream sequence and first part of *fli*C gene
Cr1	ACCCGCTGCGTCATCCT	Reverse primer for amplifying a fragment containing the upstream sequence and first part of *fli*C gene
KpnCf1	AATT*GGTACC*TACTTGCCATGCGATTTCCTTTTA	Primer for introducing the *Kpn*I restriction site into the PCR product of primers Cf1 and Cr1
SpeCr1	AATT*ACTAGT*ACCCGCTGCGTCATCCT	Primer for introducing the *Spe*I restriction site into the PCR product of primers Cf1 and Cr1
Cf2	CGGATGTGAATGAAACTACTGGTG	Forward primer for amplifying a fragment within the *fli*C gene close to the 3' end
Cr2	CGCTTTCGACATATTGGACACTTC	Forward primer for amplifying a fragment within the *fli*C gene close to the 3' end
XhoCf2	AACA*CTCGAG*CGGATGTGAATGAAACTACTGGTG	Primer for introducing the *Xho*I restriction site into the PCR product of primers Cf2 and Cr2
XbaCr2	AATT*TCTAGA*CGCTTTCGACATATTGGACACTTC	Primer for introducing the *Xba*I restriction site into the PCR product of primers Cf2 and Cr2