Distinct chemotactic behavior in the original
 Escherichia coli K-12 depending on forward and-backward swimming, not on run-tumble
 movements

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25 Supplementary Figure 1 Characterization of swimming motility and 26 structural parameters of SHU101

(a) *Left*: Phase-contrast image of the non-flagellated mutant, SHU101. Scale bar, 50 μ m. *Right*: The sequential phase-contrast images with 165-ms intervals were integrated for 5 s with the intermittent color code 'red, \rightarrow yellow, \rightarrow green, \rightarrow cyan, \rightarrow blue.' The processive linear movements could not be seen, indicating that the SHU101 is a nonmotile strain. (b) Motility of SHU101 cells on a 0.25 % (wt/vol) soft-agar plate at 30°C for 7 h. Scale bar, 0.5 cm. (c) Electron micrograph. Scale bar, 2 μ m.

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Supplementary Figure 2 Quantification of rotational rate and structural
 parameters of flagella under total internal reflection fluorescence
 microscope (TIRFM)

39(a) Histograms of the rotation rate and structural parameters during CCW (top) and CW rotation (bottom) in ATCC10798 cells. Solid line represents the Gaussian fitting. The 40 peaks and SDs of rotational rates were 73.3 ± 32.8 Hz in CCW direction (n = 74) and 41 72.4 ± 23.7 Hz in CW direction (n = 39). The flagellar pitches were 1.1 ± 0.1 µm in CCW 42(n =142) and 1.1 \pm 0.1 μ m in CW direction (n = 80). The pitch angles were 38.6 \pm 5.4 43degree in CCW (n = 70) and 40.5 ± 4.5 degree in CW (n = 39). The helix radii were 0.14 44 \pm 0.02 µm in CCW (n = 142) and 0.14 \pm 0.01 µm in CW (n = 80). (b) Histograms of the 45structure and rotation rate of flagella of W3110 during left-handed (top) and right-handed 46 (*bottom*) state. The rotation rates were 87.6 ± 34.0 Hz under a left-handed state (n = 133) 47and 81.5 \pm 22.7 Hz under a right-handed state (n = 42). The flagellar pitches were 2.3 \pm 48 0.2 μ m under a left-handed state (n = 112) and 1.1 \pm 0.1 μ m under a right-handed state (n 4950= 84). The pitch angles were 30.5 ± 4.2 degree under a left-handed state (n = 81); $36.2 \pm$ 4.8 and 53.2 \pm 5.1 degree under a right-handed state (n = 40). The helix radii were 0.21 \pm 51520.03 μ m under a left-handed state (n = 112); 0.13 \pm 0.02 μ m and 0.23 \pm 0.04 μ m under a right-handed state (n = 84). 53



55 Supplementary Figure 3 Real-time imaging of flagellar polymorphism in 56 W3110

Sequential images of the flagellar polymorphism at 2.5-ms intervals. *Left*: The orientation 5758of flagellar filament(s) was from the first quadrant to the third quadrant relative to the major axis of the filament, indicating that the helicity of filaments was left-handed. From 5960 0 to 22.5 ms, the wave of flagella propagated in a direction away from the hook end 61 toward the flagellar tip, indicating that the flagella rotated in CCW direction. The direction of rotation was switched at 30.0 ms, and then their helicity changed from the 62 63 left-handed into right-handed within 50 ms. Taken together that the pitch angle of flagellar filaments was 58 degrees, the filament form was the semi-coiled. Scale bar, 2 64 µm. Right: The orientation of flagellar filament (s) was from the second quadrant to the 65 fourth quadrant relative to the major axis of the filament, indicating that the helicity of 66 filaments was right-handed. We concluded that the flagellar form was the curly state with 67 the fact of the 40°-pitch angle. From 0 to 17.5 ms, the flagellar filament did not move. At 68 69 20 ms, the flagellar filaments gradually moved, suggesting that the flagella started to 70 rotate. The flagellar filaments dynamically twisted at 40 ms, and then the right-handed 71and left-handed flagellar were combined into a single filament at 67.5 and 105.0 ms, which was also seen in another bacterium. Finally, the curry filament(s) transformed into 72the normal state within 100 ms. Scale bar, 2 µm. Data from Supplementary Videos 6 and 737. 74



Supplementary Figure 4 Quantification of flagellar radius at coiled state inW3110

78 (a) The fluorescent micrograph of coiled-state flagellar filaments. Scale bar; 3 μm. (b)

79 Histogram of the radius of coiled flagella. The solid line represents the Gaussian fitting,

- 80 where the peak and SD are $0.78 \pm 0.02 \ \mu m \ (n = 39)$.
- 81



Supplementary Figure 5 Chemotactic response of ATCC10798 [*fliC*(N87K)] and SHU102 [*fliC*(N87K)::*fliC*] cells

(a) The schematic of the tip (capillary) assay. The tip contains a 5-µl buffer containing 85 1 % (wt/vol) agarose, and the other end was sealed with cray to avoid an effect of oxygen 86 on a chemotactic response. (b) Pseudo-colors of phase-contrast images of ATCC10798 87 (top) and SHU102 cells (bottom). In the presence of 1 mM serine, both E.coli strains 88 exhibit a chemotactic response and gather near a tip (orange color). Scale bar, 200 µm. 89 (c) Intensity profiles of b. (d) A phase-contrast image of ATCC10798 cells in the presence 90 of 10 mM serine. In the presence of serine, ATCC10798 cells tend to gather and aggregate 91 92 each other, which was not detected in SHU102 cells. Scale bar, 20 µm.



Supplementary Figure 6 Quantification of rotational rate and structural parameters of flagella of SHU102 under TIRFM

96 Histograms of the structure and rotation rate of flagella of SHU102. The solid line

97 represents the Gaussian fitting. Peaks and SDs of the rotation rate were 78.9 ± 27.5 Hz (n

98 = 33). The flagellar pitch was $2.3 \pm 0.2 \ \mu m$ (n = 30). The pitch angles were 30.2 ± 4.1

99 degrees (n = 30). The helix radius was $0.22 \pm 0.04 \ \mu m \ (n = 30)$.





Supplementary Figure 7 Quantification of rotational rate and structural
 parameters of HCB1336/pYS10 and HCB1336/pSHU61 under TIRFM

104 Histograms of the structural properties and rotation rate of flagella of E. coli HCB1336 105 $(\Delta flic \text{ strain})$ carrying the plasmid encoding wild-type flic (pYS10) and flic(N87K) (pSHU61). Solid line represents the Gaussian fitting. Peaks and SDs of the rotation rate 106 107 were 71.8 \pm 14.1 Hz in HCB1336/pYS10 (top, n = 41) and 98.4 \pm 23.9 Hz in HCB1336/pSHU61 cells (*bottom*, n = 50) and. The flagellar pitches were $2.3 \pm 0.1 \mu m$ in 108109HCB1336/pYS10 cells (top, n = 40) and $1.1 \pm 0.1 \mu m$ in HCB1336/pSHU61 cells (bottom, n = 94). The pitch angles were 30.8 ± 4.1 degree in HCB1336/pYS10 cells (*top*, n = 37) 110and 39.3 ± 5.5 degree in pSHU61/HCB1336 cells (*bottom*, n = 52). The helix radii were 111 $0.22 \pm 0.03 \ \mu\text{m}$ in HCB1336/pYS10 cells (top, n = 40) and $0.14 \pm 0.03 \ \mu\text{m}$ in 112HCB133/pSHU61 cells (*bottom*, n = 94). 113



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115 Supplementary Figure 8 Quantification of motor properties by tethered-cell

- 116 **assay**
- 117 (a) Switching frequency between *E. coli* strains for 10 sec. The average and SD were 1.15
- 118 $\pm 0.60 \text{ s}^{-1}$ in ATCC10798 (n = 99), $1.35 \pm 0.75 \text{ s}^{-1}$ in SHU155 (n = 67), $1.52 \pm 0.84 \text{ s}^{-1}$ in
- 119 W3110 (n = 53), and $1.18 \pm 0.56 \text{ s}^{-1}$ in RP437 (n = 49). (b) CW bias (Time_{CW}/Time_{Total}).
- 120 The average and SD were 0.61 ± 0.21 in ATCC10798 (n = 99) and 0.52 ± 0.30 in SHU155
- 121 (n = 67), 0.48 ± 0.27 in W3110 (n = 53), and 0.42 ± 0.23 in RP437 (n = 49). (c) Rotation
- 122 rates. The average and SD were 7.3 \pm 1.4 Hz in ATCC10798 (n = 99), 7.3 \pm 1.1 Hz in
- 123 SHU155 (n = 67), 7.0 ± 1.3 Hz in W3110 (n = 53), and 7.0 ± 1.2 Hz in RP437 (n = 49).
- 124 ATCC10798 and W3110 data from Figure 3.



126 Supplementary Figure 9 Structure and sequence of flagellin

127(a) Subunit structure of L-type flagellar subunit (PDB: 3A5X). The important residues for a curly filament are shown. (b) Side views of L-type flagellar filament. The number 128129of protofilaments is shown. Inset: Top view of filaments. (c) L-type (left, PDB: 3A5X) and R-type (right, PDB: 1UCU) straight filaments of S. typhimurium are shown ^{3,4}. 130 Hydrogen bonds interaction (black) between S0 (green) and S5 subunits (blue). E84, N87, 131E122, T130 of the S0 subunit are colored in yellow, red, orange, and white, respectively; 132133T438 and N439 of the S5 subunit are colored in cyan and pink, respectively. (d) Alignments of the flagellin amino acid sequence from typical bacteria. The red star 134135represents N87 residue; blue stars indicate E84, E122, T130, T438, N439 residues. Images were generated using PyMOL 2.4 (https://pymol.org/2/). 136137



Supplementary Figure 10 Growth curve in *Escherichia coli* K-12 ATCC10798 and W3110

The single colony was scrutinized by the tip, resuspended into 50-ml LB medium, and then cultured at 37°C with shaking. The absorbance of cells at 600 nm were periodically measured with 60-min intervals. During the exponential phase, we fitted the data as the following equation: $f(t) = A \times 2^{\frac{t}{tau}}$, where *A* the constant and *tau* the doubling time. The doubling time was estimated to be ~24 min in ATCC10798 (red) and 30 min in W3110 (blue). The experiments were performed two times.

149 Supplementary Table 1

150 Structural parameters and kinematics of the cell body and flagella in *Escherichia coli*.

- 151 Values were directly measured by either a transmission electron microscopy (EM) or
- 152 optical microscopy (OM).

	EM						OM	
Cells	Body length (µm)	Body width (µm)	Flagellar length (μm)	Flagellar	Flagellar number	Flagellar pitch (µm)	Swimming	Rotation
				helix radius			speed	rate
				(µm)			(µm s-1)	(Hz)
ATCC10798	1.9±0.3	0.70±0.07	4.7±1.1	0.14±0.03	1.8±0.6	1.3±0.2	13.2±4.4	73.3±32.8
	(62)	(62)	(62)	(59)	(61)	(59)	(70)	(74)
W3110	2.1±0.5	0.77±0.13	7.3±1.9	0.22±0.05	6.5±2.3	3.0±0.2	32.5±6.6	87.6±34.0
	(50)	(50)	(48)	(42)	(39)	(41)	(50)	(133)
pYS10/HCB1336	2.1±0.4	0.83±0.17	5.8±1.6	0.22±0.06	3.2±1.3	2.7±0.3	21.7±4.5	71.8±14.1
	(57)	(57)	(57)	(44)	(57)	(42)	(53)	(41)
pSHU61/HCB1336	1.8±0.2	0.73±0.12	4.5±0.9	0.14±0.03	3.1±1.3	1.4±0.1	12.0±4.2	98.4±23.9
	(46)	(46)	(44)	(46)	(46)	(46)	(53)	(50)
SHU101	1.9±0.3	0.74±0.06		n.d.	0	n.d.	n.d.	n.d.
	(21)	(21)	n.a.		(21)			
SHU102	2.1±0.3	0.95±0.20	6.9±1.5	0.20±0.04	5.3±2.4	2.5±0.2	26.3±6.0	78.9±27.5
	(32)	(32)	(29)	(27)	(31)	(27)	(45)	(33)

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$\begin{array}{c} 155 \\ 156 \end{array}$

Supplementary Table 2. Strains and plasmids

Strain/plasmid	Relevant Phenotype/ Genotype	Source of Reference	
Strains			
W3110	Wild type		
ATCC10798	Wild type (<i>fliC</i> N87K)	This study	
RP437	Wild type for chemotaxis	5	
HCB1336	CM735 ΔfliC	H. C. Berg	
SHU101	ATCC10798 fliC(N87K)::tetRA	This study	
SHU102	ATCC10798 fliC(N87K)::fliC	This study	
SHU155	W3110 <i>fliC</i> :: <i>fliC</i> (N87K)	This study	
Plasmids			
pKD46	Red system	6	
pYS10	FliC expression plasmid; pBR322 derivative	7	
pSHU61	FliC(N87K) expression plasmid; pBR322 derivative	This study	

157 **Supplementary Table 3. Primers**

Name	5' > 3'	Note
FliC ATCC FW	GGAAACCCAATACGTAATCA	Forward primer for PCR and sequencing of <i>fliC</i> in ATCC10798
FliC ATCC Rev	CAATTTGGCGTTGCCGTCAGTCTC	Reverse primer for PCR and sequencing of <i>fliC</i> in ATCC10798
1217_fliC(N87K)-f(QC)	CTGTCCGAAATCAACAAGAACTTACAGCGT	Forward primer for mutagenesis on <i>fliC</i>
	GTG	
1218_fliC(N87K)-r(QC)	CACACGCTGTAAGTTCTTGTTGATTTCGGA	Reverse primer for mutagenesis on <i>fliC</i>
	CAG	
0196_fliC-tetRA-F	CAATATAGGATAACGAATCATGGCACAAGT	Forward primer for PCR of tetRA cassette
	CATTAATACCTTAAGACCCACTTTCACATTT	
0197_fliC-tetRA-R	ACCCTGCAGCAGAGACAGAACCTGCTGCG	Reverse primer for PCR of tetRA cassette
	GTACCTGGTTACTAAGCACTTGTCTCCTG	
1232_fliC-F	CAATATAGGATAACGAATCATGGCACAAGT	Forward primer for PCR of wild-type <i>fliC</i> cassette
0199_fliC-R	TTAACCCTGCAGCAGAGACAGA	Reverse primer for PCR of wild-type fliC cassette and sequencing
		of <i>fliC</i>
0210_tetRA-785-R	GGCAAGACTGGCATGATAAGGCC	Reverse primer for Colony PCR
0211_tetRA-1090-F	GTGAAGTGGTTCGGTTGGTTAGGG	Forward primer for Colony PCR
0219_fliC-(-175)-F	ATAGCGGGAATAAGGGGGCAGA	Forward primer for Colony PCR and sequencing of <i>fliC</i>
0220_fliC- (+250)-R	GGTGGCGGGGAAGCACGTTGC	Reverse primer for Colony PCR and sequencing of <i>fliC</i>
0198fliC-F	ATGGCACAAGTCATTAATACC	Forward primer for sequencing of <i>fliC</i>

Captions for Supplementary Videos

- 160 Supplementary Video 1
- 161 Swimming motility of *E. coli* K-12 ATCC10798 (*left*) and *E. coli* K-12 W3110 (*right*)
- 162 observed under a phase-contrast microscope. ATCC10798 cells show 180°-
- reversals against to original swimming direction with the cell body rolling, whereas
- 164 W3110 cells swim with the run and tumble movements. Scale bar, 20 μm.
- 165 Supplementary Video 2
- 166 Swimming motility of ATCC10798 (*left*) and W3110 (*right*) under a conventional
- 167 fluorescent microscope. Scale bar, 20 µm.
- 168 Supplementary Video 3
- 169 High-speed imaging of flagellar rotation of ATCC10798 (*left*) and W3110 (*right*)
- using an EMCCD camera under a fluorescent microscope. Scale bar, 5 µm.
- 171 Supplementary Video 4
- 172 Flagellar rotation of ATCC10798 under TIRFM. Scale bar, 4 μm.
- 173 Supplementary Video 5
- 174 Flagellar rotation of W3110 under TIRFM. Scale bar, 5 μm.
- 175 Supplementary Video 6
- 176 Real-time imaging of a polymorphic flagellar change under TIRFM. A rotational
- direction was changed at 0.11 sec; subsequently, a normal left-handed filament
- was transformed into the right-handed semi-coiled filament. Scale bar, 2 µm.
- 179 Supplementary Video 7
- 180 Real-time imaging of a polymorphic flagellar change under TIRFM. A rotational
- direction was changed at 0.17 sec; subsequently, a right-handed curly filament
- 182 was transformed into the normal left-handed filament. Scale bar, 2 µm.
- 183 Supplementary Video 8
- Swimming motility of SHU102 (*left*), HCB1336/pYS10 (*middle*), and
 HCB1336/pSHU61 cells (*right*) under a phase-contrast microscope. Scale bar,
 50 µm.

- 188 Supplementary Video 9
- 189 Swimming motility of SHU102 cells under a conventional fluorescent microscope.
- 190 **Scale bar, 20 μm**.
- 191 Supplementary Video 10

192 Chemotactic response of ATCC10798 (top) and SHU102 cells (bottom) under a

193 phase-contrast microscope. For the first 10 sec, cells swim in the absence of

serine. For the last 10 sec, both cells swim in the presence of 1 mM serine. Scalebar, 200 µm.

196 Supplementary Video 11

Flagellar rotation of SHU102 (*left*), HCB1336/pYS10 (*middle*), and
 HCB1336/pSHU61 cells (*right*) under TIRFM. Scale bar, 2 μm.

- 199 Supplementary Video 12
- Swimming motility of ATCC10798 (*left*) and W3110 (*right*) on a 0.2 % agarose
- pad. ATCC10798 cells cannot migrate after stuck, whereas W3110 cells keep
- moving with 180°-reversals to escape from a stuck. Scale bar, 50 μm.
- 203 Supplementary Video 13
- Swimming motility of ATCC10798 (*left*) and W3110 (*right*) in the presence of 15 %
- ²⁰⁵ ficoll. Scale bar, 20 μm.

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